

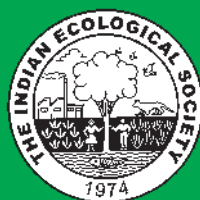
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Water Quality Assessment of Pond Water of Kalanaur Block, Rohtak, Haryana

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Abstract: Various physico-chemical characters of pond water of selected villages of Kalanaur block of Rohtak district in Haryana were compared with WHO and Indian standards. Most of the samples were highly polluted due to higher values of conductivity, turbidity, TDS and dissolved ions and were out of the permissible limits. The results suggested that the water quality of these freshwater bodies is rapidly declining. Regularly updating the data on pond water and appointment of pond managers can help to conserve these freshwater ecosystems.

Keywords: Freshwater, Physico-chemical, Village ponds, Water quality

Water is the basic and most essential requirement for life on earth. All living organisms require water for survival, growth, reproduction and other life processes. About 71% of Earth's surface is covered with water and oceans account about 96.5% of the total water. Earth surface has limited sources of freshwater and it contributes only 2.5% to total water on Earth (Hu et al 2018). It is mainly the freshwater, which is valuable for human beings for drinking and irrigation. Major sources of freshwater are glaciers, rivers, lakes and ponds. From human health to proper functioning of an ecosystem everything depends upon the water (Costanza et al 2017). A pond is a small area of still and fresh water, which holds water for four or more months in a year and is between 1 m² to 20,000 m² in area (Biggs et al 2005). Ponds are the most dynamic and productive freshwater ecosystems with stunning biological diversity. Ponds cover nearly 30% of global water by surface area (Downing et al 2006). Pond ecosystems help in groundwater recharging, supports livestock, prevention of soil erosion, water purification and most importantly in carbon sequestration (Sarma and Saikia 2010, Kumar and Padhy 2015, Nag et al 2019). Ponds provide many ecosystem services free of cost to human beings including social, cultural, economic, scientific, medicinal and aesthetic, etc. (Gupta et al 2012). In spite of having a central role in the life of all organisms, ponds are continuously being degraded due to anthropogenic activities viz. industrialization, urbanization, habitat loss, pollution, etc. (Mishra et al 2014, Chen et al 2019). Due to lack of proper sanitation facilities, most of the people in villages use pond catchment areas for defecation purpose (Adhikary 2016). Increased salinity, acidic content and heavy metal content are some of the consequences of deterioration and

degradation of water quality in these ponds (Nwani et al 2010, Ritchie and Olson 2015, Gerdol et al 2018).

India being an agricultural country has high requirement of freshwater for irrigation purpose. Ponds are mainly exploited by rural people to sustain their life. India has achieved only 71.8% of the target to provide the rural population with safe, adequate and drinking water and is also far behind at achieving its Sustainable Development Goal (SDG) on clean water and sanitation. Thus, conservation of ponds is important in the present scenario to deal with climate change where drought, floods and erratic rainfall are common. Lack of awareness and understanding has somehow hindered the conservation of pond ecosystems. At the national level, various initiatives have been taken by the government of India. National Wetland Conservation Programme (NWCP) was operationalized by the Government of India in close collaboration with concerned State Governments. Recently, Government of India has set up a separate ministry, 'Jal Shakti' to manage and conserve the local water bodies. As a part of the outreach initiative, Maharshi Dayanand University, Rohtak (Haryana) has adopted five villages of Kalanaur block in Rohtak district. Hence, the present study was conducted in these villages to study quality of pond water.

MATERIAL AND METHODS

Study area: Kalanaur block is located in Rohtak district of Haryana, India. The geographical coordinates of the block are 28.8312°N and 76.3956°E. This block receives an average annual rainfall of 44.3cm. The highest temperature recorded is 46°C and the lowest temperature recorded is 4°C. In summers the temperature ranges from 23°C-45°C.

Soil is sandy loam in nature. Bhali Anandpur, Balab, Baniyani, Marodhi Jattan and Marodhi Rangran were the villages selected for physico-chemical analysis of pond water from Kalanaur block of Rohtak district, Haryana (India).

Sample collection: Water samples for analysis were collected from fourteen ponds from five selected villages. Sample collection was carried out from February to April 2018 in morning hours before noon. The bottles were washed with detergent and dilute nitric acid before sampling. Finally, the de-ionized water was used to rinse the sampling bottles and then dried in sunlight. Samples were collected in plastic bottles from each sampling point. After taking the samples the bottles were marked with sampling numbers (Table 1). The samples were collected and preserved in an icebox and were brought to the laboratory of Botany Department, Maharshi Dayanand University, Rohtak, Haryana.

Sample analysis: Physical parameters viz. temperature, pH, conductivity, turbidity and salinity were measured using mercury thermometer, pH meter (ESICO-1615), conductivity meter (ESICO-1615), portable turbidity meter and portable salinity meter, respectively. Sodium and Potassium ion concentration was determined using Flame Photometer (ESICO, Model No.-381) against the standard solutions of NaCl and KCl at different light intensities (Amrutkar 2013). Calcium, Magnesium and total hardness were measured using titration methods. Chloride, carbonate and bicarbonate concentrations were measured using titration methods of Gupta (2000) with minor modifications. The amount of sulphate in the collected samples was measured with little modifications in accordance with (BIS 2003, IS: 3025, Part 24 Reaffirmed 2003).

Statistical Analysis: The data collected was suitably tabulated and analysed. Coefficient of correlation (*r*) among different parameters were also calculated.

RESULTS AND DISCUSSION

The ponds are backbone of rural society. Villagers depend upon village ponds for source of fresh water. In these areas the main occupation is agriculture and animal husbandry. They depend on pond water for cattle drinking, bathing and also for irrigation. But due to continuous decrease in water quality, ponds can no longer be used as a source of water. From the present study, it was observed that most of the village ponds have physical and chemical parameters out of acceptable limit.

Physical Parameters

Colour, odour and temperature: The colour and odour in a natural water body are mainly due to the presence of phytoplankton and zooplankton. Most of the samples were greenish to yellowish in colour. S₁ was blackish and S₂ was

Table 1. Name of villages and ponds selected for sample collection

S. No.	Name of pond	Name of village	Latitude	Longitude
S ₁	Bada johr	Bhali Anandpur	28°52'44"N	76°30'38"E
S ₂	Guhae johr	Bhali Anandpur	28°52'58"N	76°30'31"E
S ₃	Pirali johr	Bhali Anandpur	28°52'50"N	76°30'15"E
S ₄	Tarne wala johr	Baniyani	28°51'04"N	76°30'34"E
S ₅	Budha johr	Baniyani	28°50'54"N	76°30'33"E
S ₆	Thakuron wala johr	Baniyani	28°50'59"N	76°30'46"E
S ₇	Sati wala johr	Marodhi Jattan	28°50'13"N	76°31'24"E
S ₈	Nanu wala johr	Marodhi Jattan	28°50'05"N	76°31'29"E
S ₉	Dalla wala johr	Marodhi Jattan	28°49'58"N	76°31'27"E
S ₁₀	School wala johr	Marodhi Rangran	28°50'01"N	76°31'12"E
S ₁₁	Choudhary wala johr	Marodhi Rangran	28°50'12"N	76°31'15"E
S ₁₂	Mandir wala johr	Balab	28°47'56"N	76°30'30"E
S ₁₃	Badi wali johr	Balab	28°47'59"N	76°30'52"E
S ₁₄	Semali johr	Balab	28°47'53"N	76°30'53"E

turbid. Odour for most of the samples was non-agreeable. The temperature of samples ranged from 19.9°C to 27.6°C and was within permissible limits. The colour and odour are indicators of water quality of fresh water bodies (Dhanalakshmi et al 2013). Further, colour does not affect the aquatic organism directly, but it prevents the light penetration and reduces the growth of aquatic plants (Olopade 2013). The green colour represents higher planktons and light green color represents low planktons level (Das 1997). Some village ponds were covered with cow dung cakes. The black colour of village ponds may be due to dumping of cow dung cakes with rainwater within the ponds. The odour of the water may be due to the organic and inorganic contaminants in water (Table 2).

pH: The value of pH ranged from 7.4-9.0 and was within acceptable limits as specified by Indian standard (BIS 2012). The maximum value of pH was recorded for S₁₄ and minimum for S₂.

Conductivity: The recorded conductivity ranged from 1382-9970 µ-mho cm⁻¹. The maximum conductivity was recorded in S₁₄ (9970 µ-mho cm⁻¹) and minimum in S₃ (1382 µ-mho cm⁻¹). The acceptable limit for conductivity is 600 µ-mho cm⁻¹ (WHO 2018). When compared to WHO standard all the samples were out of the acceptable limit.

Turbidity: Turbidity of water is due to the suspension of particles and these suspension particles make the solution to appear cloudy. Turbidity recorded in the water samples ranged from 04 to 46 Nephelometric Turbidity Units (NTU). The maximum value of turbidity was recorded in S₂ (46 NTU)

and the minimum value of turbidity was recorded in S₅ (4 NTU). In context with turbidity, the results were in concordance with Senthilkumar et al (2017) and Muralidaran et al (2017). The fresh water bodies may get contamination from soil runoff that increases the turbidity (EPA 2000, Schwartz et al 2000).

Total dissolved solids (TDS) and salinity: The TDS ranged from (908 ppm) in S₃ to (6600 ppm) in S₁₄. According to Indian standard (BIS 2012), the acceptable limit of TDS for drinking water is specified at 500 ppm. All the samples were out of acceptable limits. Maximum salinity was reported in S₁₄ (4960 ppm) and minimum in S₃ (680 ppm). The change in salinity might be due to changes in total solids. High level of TDS is an indicator of increased nutrient content which results into eutrophication of aquatic bodies (Singh and Mathur 2005, Verma et al 2012).

Chemical Parameters

Chloride: Chloride anions recorded in pond water samples ranged from 207.5-1614.5 mg l⁻¹ (Table 3). Maximum in S₁₄ (1614.5 mg l⁻¹) and minimum in S₁₁ (207.5 mg l⁻¹). The permissible limit of chloride content is 250 mg/l (BIS 2012). The high chloride content in water samples may be due to the high rate of sedimentation and evaporation. All other samples were out of acceptable limit. The higher concentration of chloride ions in freshwater bodies is an indication of pollution by dumping of domestic sewage. High concentration of chloride ions in natural freshwater bodies is due to

anthropogenic impacts viz. dumping of village waste, cow dung cakes, washing of agricultural tools and tractors, washing of clothes and invasive plants (Singh 2017).

Calcium: Maximum value of calcium cation was recorded in S₁₄ (516.2 mg l⁻¹) and minimum in S₃ (44.1 mg l⁻¹). According to Indian standard (BIS 2012), the acceptable limit of calcium for drinking water is specified at 75 mg l⁻¹. Almost half of the collected samples were out of permissible limits.

Magnesium: Magnesium ions recorded in the pond water samples ranged from 101.5-454.9 mg l⁻¹. Maximum magnesium content was reported in S₁ (454.9 mg l⁻¹) and minimum in S₂ (101.5 mg l⁻¹). According to Indian standard (BIS 2012), the acceptable limit is 30 mg l⁻¹. High concentration magnesium may be due to washing away of nutrients into the ponds with rainwater, from a widespread agricultural area. When the samples were compared with Indian standard, it was reported that all the samples were out of acceptable limit. It may be due to the washing away of nutrients into the ponds with rainwater, from a widespread agricultural area and thus resulting in the increase in level of magnesium ions.

Sulphate: Sulphate is a naturally occurring ion in freshwater bodies. Sample S₁₄ (466.3 mg l⁻¹) was reported to have a maximum value of sulphate and minimum was reported in case of S₉ (18.6 mg l⁻¹). According to Indian standard (BIS 2012), the acceptable limit is 200 mg l⁻¹. The high concentration of sulphate may be due to the extensive growth

Table 2. Physical parameters of the analyzed samples

S. No.	Colour	Odour	Temperature (°C)	pH	Conductivity (μ-mho/cm)	Turbidity (NTU)	TDS (ppm)	Salinity (ppm)
S ₁	Blackish	Non-agreeable	22.1	8.5	4290	18	3290	2330
S ₂	Turbid	Non-agreeable	19.9	7.4	4110	46	2160	2000
S ₃	Light green	Agreeable	24.0	7.8	1382	26	908	680
S ₄	Light green	Non-agreeable	26.7	7.9	2540	10	1790	996
S ₅	Light green	Non-agreeable	26.9	7.5	2980	04	1910	1490
S ₆	Green	Non-agreeable	22.9	7.6	4490	09	2950	2320
S ₇	Yellowish	Non-agreeable	25.1	8.2	3540	08	2300	1720
S ₈	Green	Non-agreeable	23.7	8.7	3560	23	2370	1770
S ₉	Green	Non-agreeable	27.6	8.0	5640	30	3600	2830
S ₁₀	Yellowish	Non-agreeable	26.3	8.4	5140	06	3420	2560
S ₁₁	Yellowish	Non-agreeable	26.6	8.4	3850	34	2660	1900
S ₁₂	Light green	Non-agreeable	24.1	8.1	2410	13	1590	1190
S ₁₃	Green	Non-agreeable	26.5	8.1	6740	16	4490	3380
S ₁₄	Green	Non-agreeable	25.3	9.0	9970	38	6600	4960
Permissible limits								
Range	-	-	-	6.5-9.2	600	1	500	-
Ref.	-	-	-	BIS 2012	WHO 2018	BIS 2012	BIS 2012	-

of water hyacinth (Aneja and Singh 1992). When sulphate samples were compared with Indian standard it was reported that the samples (S_2 , S_3 , S_5 , S_6 , S_7 , S_8 , S_9 , and S_{11}) were within the acceptable limits. The results were in concordance with Senthilkumar et al (2017) and Dubey (2017).

Sodium: Sodium level recorded in the pond samples ranged from 57-460 mg l⁻¹. The maximum sodium concentration was recorded in S_{14} (460 mg l⁻¹) and minimum in S_3 (57 mg l⁻¹). According to (WHO 2006) standard acceptable limit for sodium is 200 mg l⁻¹. Pollution due to human waste disposal in the form of soap solution and detergents might be the cause of high sodium content in ponds. Pond samples viz. S_3 , S_4 , S_5 , S_6 , S_7 , S_{10} and S_{12} were within the acceptable limit. The high concentration of sodium in natural freshwater bodies is mainly due to pollution such as soapy solution, detergent, human and animal waste disposal, precipitation runoff, sewage disposal, mineral deposits and water treatment chemicals such as sodium fluoride, sodium bicarbonate and sodium hypochlorite etc. (Singh 2017).

Potassium: Potassium concentration recorded from water samples ranged from 73-525 mg l⁻¹. The maximum value of potassium was recorded in S_{14} (525 mg l⁻¹) and minimum in S_{12} (73 mg l⁻¹). The acceptable limit of K⁺ for drinking water is specified at 10 mg l⁻¹ (WHO 2018). All water samples were out of acceptable limit. The results also show similarity with the recent studies conducted by Gulia et al (2017) and Akhtar and Rai (2018) for high range of potassium in pond water.

Carbonate: The carbonate anions recorded in pond water samples ranged from 0-120 mg l⁻¹. S_{14} (120 mg l⁻¹) had a maximum value of carbonate ions and most of the other samples had no carbonate content. The carbonate was found nil in more than half of the water samples. The reason for low carbonate was high pH in all the samples because higher pH makes a solution more alkaline and thus less carbonic acid dissociates into carbonate ions.

Bicarbonate: The bicarbonate anions recorded in pond water samples ranged from 414.8-1708 mg l⁻¹. S_{14} (1708 mg l⁻¹) had maximum bicarbonate content and S_5 and S_{12} (414.8 mg l⁻¹) had minimum bicarbonate content. According to Indian standard (BIS 2012), the acceptable limit is 200 mg l⁻¹. Most of the samples had a high concentration of bicarbonate anions.

The dumping of village waste in the ponds is the leading factor for degradation. Most of the villages have very poor drainage system and there is no separate disposal of village waste. Hence, the waste water of most of villages is thrown in ponds. The village waste water contains detergents, cow dung, rotten vegetable waste and many other organic compounds which make the water rich in organic material. There is lack of proper sanitation facilities in most of the houses in villages which results in direct dumping of human excreta in these fresh water bodies. Some religious ponds are also used by villagers for dumping of religious and ceremonial waste i.e. *puja samagri* and garlands in the ponds. Yadav et al (2013) also reported that the ponds surrounded by temple

Table 3. Chemical parameters of analyzed samples

S. No.	Chloride (mg l ⁻¹)	Calcium (mg l ⁻¹)	Magnesium (mg l ⁻¹)	Sulphate (mg l ⁻¹)	Sodium (mg l ⁻¹)	Potassium (ppm)	Carbonate (mg l ⁻¹)	Bicarbonate (mg l ⁻¹)
S_1	925.0	73.8	454.9	345.0	395	187	0	1039
S_2	714.5	230.8	101.5	96.7	256	192	0	1202
S_3	368.4	44.1	175.4	53.0	57	81	0	495.3
S_4	702.9	85.8	350.7	210.1	110	200	0	927.2
S_5	974.1	104.2	311.6	132.5	92	130	0	414.8
S_6	1124.6	393.6	348.1	59.5	131	325	0	1342
S_7	779.6	64.9	319.1	166.4	140	112	0	805.2
S_8	1050.2	359.9	263.7	22.8	260	190	0	1634.8
S_9	869.6	44.9	237.3	18.6	315	210	0	976
S_{10}	707.2	62.5	349.4	299.9	138	275	36	986
S_{11}	207.5	287.0	135.8	111.5	245	300	60	1122.4
S_{12}	620.5	44.9	267.6	238.5	112	73	0	414.8
S_{13}	1028.5	406.4	117.3	329.6	360	230	36	976
S_{14}	1614.5	516.2	261.0	466.3	460	525	120	1708
Permissible limits								
Range	250	75	30	200	200	10	75	200
Ref.	BIS 2012	BIS 2012	BIS 2012	BIS 2012	WHO 2006	WHO 2018	BIS 2012	BIS 2012

Table 4. Correlation among different parameters from analyzed samples

Parameters	pH	EC	Turbidity	TDS	Salinity	Cl ⁻	Ca ²⁺	Mg ²⁺	SO ₄ ²⁻	Na ⁺	K ⁺	CO ₃ ²⁻	HCO ₃ ⁻
pH	1												
EC	0.518	1											
Turbidity	0.154	0.336	1										
TDS	0.591	0.989	0.272	1									
Salinity	0.521	0.996	0.330	0.989	1								
Cl ⁻	0.320	0.729	-0.013	0.726	0.720	1							
Ca ²⁺	0.327	0.656	0.363	0.637	0.651	0.587	1						
Mg ²⁺	0.187	-0.085	-0.636	0.003	-0.065	0.289	-0.497	1					
SO ₄ ²⁻	0.532	0.601	-0.080	0.655	0.596	0.438	0.221	0.254	1				
Na ⁺	0.584	0.798	0.547	0.815	0.814	0.546	0.544	-0.118	0.492	1			
K ⁺	0.488	0.841	0.348	0.843	0.828	0.577	0.742	-0.005	0.454	0.596	1		
CO ₃ ²⁻	0.633	0.785	0.390	0.794	0.766	0.374	0.618	-0.218	0.627	0.563	0.836	1	
HCO ₃ ⁻	0.525	0.638	0.462	0.628	0.632	0.558	0.769	-0.026	0.139	0.643	0.770	0.495	1

receives larger amount of organic and inorganic waste from temples. The pond peripheries are used by villagers for sun drying of cow dung cakes. Gupta et al (2012) conducted study on village ponds of Haryana and also reported the ill impact of cow dung cakes on village ponds.

The EC and TDS, EC and Salinity, TDS and Salinity were closely correlated to each other and value of the r approaches to unity. The correlation between Mg²⁺ and TDS, Mg²⁺ and K⁺ were not significant (Table 4).

CONCLUSIONS

Foul smell from all the samples indicated impurities present in the pond water. Among physical parameters, turbidity and TDS in all the samples were out of permissible limits. According to Indian standard chloride and magnesium, potassium contents were out-off the acceptable limits. Almost half of the samples had more than permissible levels of calcium, sodium and sulphate. So, from the results obtained in the present study, it can be concluded that most of the ponds in these villages were highly polluted and the rest were moderately polluted. Also, due to over-exploitation these water bodies are continuously being deteriorated and degraded. The water from these ponds is unsuitable for human and animal consumption. Assessment and monitoring of pond water should be done on a regular basis. Collective and collaborative efforts by the government and local people can help to conserve and protect these water bodies. Regular monitoring, awareness campaigns and improved regulation of the ponds may help in conservation of these ponds.

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Freshwater Diatoms as Bio-Indicators in Urban Wetlands of Central Gujarat, India

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Abstract: The current study attempts to understand the diatom community structure and their role as bio-indicator in the urban wetlands of central Gujarat. The remarkable variation in the composition and distribution of various diatom species were observed. A total of 26 diatom species distributed among 16 genera, highlights rich biodiversity of the region. Among all, high count of certain diatom species namely *Nitzschia palea*, *N. amphibia*, *N. gracilis*, *Pinnularia biceps*, *Navicula radiosa*, *N. lanceolata*, *N. cryptocephala*, *N. obtusa*, *Gomphonema parvulum*, *G. olivaceum*, *Synedra ulna*, *S. acus*, *Amphora ovalis*, *Eunotia lunaris* and *Stephanodiscus hantzschii* were key indicator diatom species. Only pennate diatoms were present at Manjalpur wetland which is highly polluted and eutrophic in nature. Mesotrophic conditions at Harni wetland and Gotri wetland represented dominance of pollution tolerant species. Bhaily wetland is the least polluted among studied wetlands and showed highest diversity of pollution sensitive diatoms. These results provided insights into the ecological importance of endemic diatoms found in urban ecosystems.

Keywords: Freshwater diatoms, Urban wetlands, Vadodara, Central Gujarat, India

Diatoms are ubiquitous in nature and shows significant morphological diversity in aquatic environment. Their cell size ranges between 5 μm -0.5 mm. They account for approximately 20-25% of oxygen production in the world. They are either planktonic or benthic; occur both in solitary or colonial forms. Community structure of diatoms are specific to each eco-region and significantly varies from pristine to polluted water bodies and this helps in distinguishing oligotrophic from eutrophic water bodies. In the past, the function of diatoms as bio-indicators had been studied in Europe, North America, South Africa and East Asia, but such studies are done in very limited areas of India (Van Dam et al 1994, Blanco et al 2004, Taylor et al 2007a, Cejudo-Figueiras et al 2010, Alakananda et al 2011). Diatom research confirmed their important role in silica cycle and their contribution in other biogeochemical cycles, especially in carbon fixation in the carbon cycle by converting CO_2 into biomass in a large proportion. Diatoms are an important basis of wetland food webs and are referred to as 'algae in glass houses' due to their characteristic siliceous mechanism. They play a key role in ecosystem as primary producer and also support secondary productivity by becoming alternative sustainable source of PUFA (polyunsaturated fatty acid). Hence, they are the major primary producers in all aquatic systems including wetlands (Kale and Karthick 2015).

Urban water bodies exhibit high spatio-temporal variations in temperate and tropical regions that influence the distribution pattern and biodiversity of diatoms. The

wetlands of Central Gujarat are vital ecosystems, providing services such as water for domestic use and moderating atmospheric temperature while balancing biodiversity (Soni and Bhatt 2007, Parikh and Singh 2016). A perusal of literature on central Gujarat shows that earlier work on freshwater diatoms is scanty and mainly focused on marine diatoms (Joshi et al 2019). Notable contribution from this region includes publications of Gandhi (1961, 1967) who had recorded 24 species of freshwater diatoms belonging to 10 genera. However, precise ecological aspects, ionic and nutrient related species variations are yet to be explored in classifying water quality classes for prioritizing more polluted wetlands and identifying indicator species for further environmental management in urban wetlands of Central Gujarat. Vadodara city being the cultural capital of Gujarat harbors great floristic diversity, both in terrestrial and aquatic environments (Rathod et al 2019). But its status quo is urbanized due to rapid development and unplanned anthropogenic activities. Disposal of untreated solid and liquid discharge have amplified pollution level in land and water bodies. Due to high potential of these wetlands as a hotspot of aquatic diversity, this investigation is useful to determine distribution of freshwater diatom flora which will further provide insights on a vast array of ecological and taxonomical studies. Also, help in understanding cosmopolitan- endemism and the relative preference for pristine or polluted waters form the base for developing diatom based indices for water quality monitoring.

MATERIAL AND METHODS

Diatom samples were collected from selected urban wetlands from definite sampling sites during November 2018 – April 2019 following guidelines as per standard protocols (Karthick et al 2010). The slides of cleaned diatoms were prepared using Naphrax mountant (ri, 1.74) and the frustules were counted and identified to species level by referring standard literatures (Round 1990, Gandhi 1998, Taylor et al 2007b). During enumeration the dimensions of diatom valve characteristics, like length, width and striae densities in 10 µm were measured for species level identification. Diatom community counts were calculated using OMNIDIA software version 6.0 (Lecoin et al 1993). This software uses only diatoms for the detection of anthropogenic pollution by calculating Trophic Diatom Index (TDI). The pollution levels of the wetlands have been estimated using TDI using given formula:

$$index = \frac{\sum_{j=1}^n a_j s_j v_j}{\sum_{j=1}^n a_j v_j}$$

Where a_j = abundance or proportion of valves of species j in sample,

s_j = pollution sensitivity (1-5) of species j ;

v_j = indicator value (1-3 ; Values of sensitivity (s))

*A diatom cell wall consists of two valves in total (TDI, a user's manual, 2001)

Study area: Vadodara city with a current population of 2.1 million, according to 2010-11 census is located at 22° 20' 16.5" N latitude, 73° 13' 07.8" E longitude at an average elevation of 129m above sea level. Vadodara features a

semi-arid climate receiving maximum rainfall during monsoons. It comprises of deep black soil which is very fertile. Vadodara's topography includes a mini river Vishwamitri which dries up in summer and banks of Narmada and Mahi rivers. A large number of interconnected water bodies were created during the dynasty of Maharaja Sayaji Rao Gaikwad making provision for the traditional uses of irrigation, drinking, fishing and washing. The selected urban wetlands are located within the periphery of city namely Harni, Gotri, Manjalpur and Bhaily (Table 1). Of these, Harni and Gotri wetland had recently restored under smart city program by Vadodara Municipal Corporation.

RESULTS AND DISCUSSION

Diatom analysis: A total of 26 species belonging to 13 pennate and 3 centric diatoms were observed (Table 2). The dominant genera were *Navicula*, *Gomphonema*, *Nitzschia*, *Synedra*, *Pinnularia*, *Stephanodiscus*, *Amphora* and *Eunotia* in the descending order of relative abundance. Harni wetland was dominated by species of *Navicula*, *Nitzschia* and *Synedra*. In case of Gotri wetland, species of *Pinnularia*, *Stephanodiscus*, *Synedra*, *Navicula*, *Amphora* and *Eunotia* were dominant. While, in the Manjalpur wetland, *Nitzschia*, *Navicula*, *Synedra* and *Gomphonema* were prominent. No centric diatoms were reported here. Bhaily wetland showed a great species diversity of diatoms with the presence of centric diatoms like *Cyclotella*, *Melosira* and *Stephanodiscus*; Pennate diatoms genera such as *Nitzschia*, *Gomphonema*, *Epithemia*, *Cymbella*, *Synedra*, *Fragillaria*, *Navicula*, *Achnanthes*, *Rhoicosphenia* and *Surirella*. Majority of forms are solitary and only few colonial. Also, pennate

Table 1. Morphometric details of the urban wetlands

Characteristics	Harni Wetland	Gotri Wetland	Manjalpur Wetland	Bhaily Wetland
Position	22°20'23.5" N 73°13'12.8" E	22°18'52" N 73°08'08" E	22°16'33" N 73°11'54" E	22° 17' 02" N 73° 07' 35" E
Zone	Residential	Residential	Industrial	Residential
Submergence Area	5.38.73 Hectares	1.69.95 Hectares	0.29.18 Hectares	6.5 Hectares
Status	Perennial	Perennial	Perennial	Annual
Observations	- Restored lake, surrounded by temples, housing societies and state highway - Clear water - Defined boundary for pedestrians walk - Recreational activities like boating and water sports - Lower diversity of birds - No animal entry	- Restored lake with central island - Surrounded by temple and housing societies - Boundary is defined for pedestrian walk area - Water discolored due to many anthropogenic activities - Flowers, garlands, Rubber footwears, Plastic and polythene bags spotted - Harbors good diversity of fishes	- Surrounded by the Industries and slums - Water green in color and eutrophication - Foul smell - Sewage is discharged - No animal activity - Very low diversity of fish and birds	- Forested area with little housing - Water is relatively clear - Few Plastic and Polythene spotted - People washing clothes - Animals drink water - Greater diversity of birds and fishes - Niche of Crocodiles

diatoms had high density as compared to centric diatoms (Singh et al 2010). The Harni wetland is perennial as the water recedes considerably during summers. The lake bed is composed of clay and silica. The absence of sensitive genus *Achnanthes minutissimum* shows the moderate level pollution in the lake. The dominating presence of *Navicula cryptocephala*, *N. obtusa*, *N. radiosa* and *Nitzschia gracilis*, *N. palea*, *N. amphibia* represents excessive organic content in the water. The gradual presence of *Synedra ulna* indicates turbidity of nutrient rich water (Hosmani 2013). Low count of sensitive centric diatoms like *Melosira granulata* and

Cyclotella meneghiniana represents low to moderate level of anthropogenic pollution.

Gotri wetland is also perennial and its lake bed is micronutrient rich. The dominance of *Navicula radiosa*, *Amphora ovalis* and *Stephanodiscus hantzschii* explicit the tolerance of pollution levels in water. The large presence of *Pinnularia biceps*, *Eunotia lunaris* and *Synedra acus* illustrates resistance to heavy metal concentration and ionic strength (Jüttner and Ormerod 2010). This wetland support good diversity of fishes and had relatively clear water after lake restoration. The absence of certain centric diatoms like *Cyclotella* and *Melosira* and pennate diatoms such as *Cymbella*, *Epithemia*, *Surirella* and *Fragillaria* points toward moderate pollution in this wetland (Parmar et al 2016). In Manjalpur wetland, presence of high organic pollution shows abundance of pollution tolerant diatoms such as *Gomphonema parvulum*, *G. olivaceum* and *Nitzschia palea* citing poor oxygenation and anthropogenic eutrophication of wetland (Singh et al 2013). The *Synedra ulna* and *Navicula cryptocephala* were common pollution indicators found attached to stones submerged in the water body. Bhaily wetland is less polluted, moderate degradation with low to moderate organic pollution and anthropogenic eutrophication. Few centric diatoms were reported like *Melosira varians*, *Cyclotella stelligera* and *Stephanodiscus hantzschii*. Pennate diatoms like *Navicula lanceolata*, *Synedra ulna*, *Gomphonema gracile* and *Nitzschia amphibia* were present in large number whereas short occurrence of *Cymbella tumida*, *Fragillaria capucina* and *Surirella linearis* shows low organic pollution (Humane et al 2010). This is further supported by presence of pollution sensitive species of *Achnanthes minutissimum*, *Epithemia turgida* and *Rhoicosphenia curvata* (Parmar et al 2016).

Diatom community structure closely followed the pollution increase gradient with species richness, diversity and equitability generally differing among sampling sites, tending to be higher in relatively unpolluted compared to pollute sites (Alaknanda et al 2013). All studied urban wetlands were polluted. However, they varied on the basis of organic pollution, anthropogenic eutrophication and waste discharge into them. The presence of organic pollution in Manjalpur wetland can be linked to cultural eutrophication due to sewage and is not enough to sustain a diverse community. The electrolytic compounds discharged from nearby industries were responsible for the fairly low diatom count.

All the urban wetlands are polluted, according to the Trophic Diatom Index (Table 3) where 0-20 are clean waters, 21-40 mild pollution, 41-60 bad waters, 61-100 severely polluted (Hattikudur et al 2014). Manjalpur wetland is highly

Table 2. Abundance of diatoms belonging to different genera across the urban wetlands

Diatom	Acronym	HW	GW	MW	BW
<i>Achnanthes minutissimum</i> (Kützing) Czarnecki	AMIN				+
<i>Amphora ovalis</i> Kützing	AOVA		+		
<i>Cyclotella meneghiniana</i> Kützing	CMEN	+			
<i>C. stelligera</i> (Cleve & Grunow) Van Heurck	CSTE				+
<i>Cymbella tumida</i> (Brébisson) Van Heurck	CTUM				+
<i>Epithemia turgida</i> (Ehrenberg) Kützing	ETUR				+
<i>Eunotia lunaris</i> (Ehrenberg) Grunow	ELUN		+		
<i>Fragillaria capucina</i> Désmazieres	FCAP				+
<i>Gomphonema olivaceum</i> (Lyngbye) Kützing	GOLI			+	
<i>G. gracile</i> Ehrenberg	GGRA				+
<i>G. parvulum</i> (Kützing) Cleve	GPAR			+	
<i>Melosira granulata</i> Ehrenberg	MGRA	+			
<i>M. varians</i> Agardh	MVAR				+
<i>Navicula cryptocephala</i> (Grunow) Cleve	NCRY	+		+	
<i>N. radiosa</i> Kützing	NRAD	+	+		
<i>N. obtusa</i> Smith	NOBT	+			
<i>N. lanceolata</i> Ehrenberg	NLAN				+
<i>Nitzschia amphibia</i> Grunow	NAMP	+			+
<i>N. gracilis</i> Hantzsch	NGRA	+			
<i>N. palea</i> (Kützing) Smith	NPAL	+		+	
<i>Pinnularia biceps</i> Patrick and Reimer	PBIC		+		
<i>Rhoicosphenia curvata</i> (Kützing) Grunow	RCUR				+
<i>Stephanodiscus hantzschii</i> (Grunow)	SHAN		+		+
<i>Surirella linearis</i> Smith	SLIN				+
<i>Synedra acus</i> Kützing	SACU		+		
<i>S. ulna</i> (Nitzsch) Ehrenberg	SULN	+		+	+

'+' = Presence of diatom species in particular wetland

HW = Harni wetland; GW = Gotri wetland; MW = Manjalpur wetland; BW = Bhaily wetland

Table 3. Trophic Diatom Index (TDI) values for the urban wetland samples

Sampling stations	TDI	Anthropogenic eutrophication	Anthropogenic pollution indicator
Harni Wetland	42.6	Moderate	NCRY, NRAD, NOBT, NGRA, NPAL, NAMP, SULN
Gotri Wetland	45.5	Moderate	NRAD, AOVA, SHAN, PBIC, SACU, ELUN
Manjalpur Wetland	72.4	High	GPAR, GOLI, NPAL, SULN, NCRY
Bhaily Wetland	37.8	Low	NLAN, NAMP, GGRA, SULN

polluted with deteriorating water quality with increasing temperature. Hence, trophic state of this wetland is eutrophic in comparison to Harni and Gotri wetlands which may be considered as bad waters. The index value of Bhaily wetland falls under the mild polluted category indicating the rate of conversion of organic matter into carbon dioxide and water by the microorganisms was varying between low and moderate. The level of organic pollution was not detected in this lake.

CONCLUSION

The application of total diatom index depicts that Manjalpur wetland is extremely polluted and eutrophic as compared to Harni and Gotri wetland which are mesotrophic in nature. This is possibly a consequence of industrial effluents and untreated sewage discharged in the former and organic waste disposal in the latter. Bhaily wetland was also mesotrophic and mildly polluted due to the raw discharge from the surrounding households. The species richness of following diatoms *Nitzschia*, *Navicula*, *Gomphonema*, *Amphora*, *Stephanodiscus*, *Pinnularia*, *Synedra* and *Eunotia* in studied water bodies has been attributed to obligate nitrogen heterotrophy and enriched nutrient concentration over other sensitive diatoms. The diatom index is derived mainly on the basis of the distribution of diatom species throughout the period of study. Diatom cells often remain undissolved for a longer duration and hence can serve as important indicators of organic as well as anthropogenic pollution. Thus, diatoms not only serve as an important tool to indicate water quality but also depict endemic diversity precisely by using OMNIDIA software.

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Impact Climate Change on Crop Water Requirement of Different Orchard Crops for Agro-Climatic Condition of Udaipur, Rajasthan

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Abstract: The study was carried out to evaluate the impacts of climate change (in term of daily weather parameters like temperature, humidity, wind speed and sunshine hours) on crop water requirement of different perennial orchard crop (citrus, papaya and guava) over period of five year (2014 to 2018) in climatic condition of Udaipur, Rajasthan. The results showed the significant impact of climate change on reference evapo-transpiration and rainfall patterns during year 2014 to 2018. The maximum annual ETo was found for year 2015 with a value of 1410 year⁻¹ whereas, annual rainfall was found to be maximum for year 2016 with a value of 747 mm year⁻¹. The minimum values of annual ETo and annual rainfall were for years 2017 (1259, mm year⁻¹) and 2018 (556 mm year⁻¹) respectively. The uneven trend of ETo and Rainfall for different year due to climate change. The average daily ETo was maximum for May during all year followed by June. The minimum average daily ETo was for December. The average daily ETc for citrus papaya and guava fruit crop varied from 2.8 to 3.2, 2.8 to 3.2 and 2.8 to 3.1 mm day⁻¹ respectively, during year 2014 to 2018. . The maximum crop water requirement for citrus, papaya and guava crop ranges from 27 to 30, 9 to 11 and 34 to 40 litre plant⁻¹ day⁻¹ during all five year. In this area spatial and temporal variability in normal rainfall pattern, atmospheric temperature and reference evapo-transpiration mainly occurs due to overall urbanization, pollution, deforestation, improper use of natural resources and intervention through mining activities, which results improper future planning for establishment of orchard crops.

Keywords: Climate change, Crop evapotranspiration, Crop water requirement, Citrus and papaya

In India establishment of an orchard is basically a long term investment and it require a very critical planning so, it is very essential to know the impact of climate change on different aspect of orchard crop like crop water requirement, growth parameters and fruit yield. Consider impact of climate change in future planning for cultivation of orchard crops in different parts of country. Water is a precious and commonly used natural resource with limited availability. Agriculture sector continues to draw a large share (90%) of available fresh water resources and the demand is likely to increase further (Amarasinghe et al 2008). In present era, fresh water resources are limited and depleted day by day which creates problem of water scarcity. The rainfall trend shows a continuous decrement in average annual rainfall in India. The arid and semi-arid regions of India face the double burden of declining and high erratic rainfall, increment in temperature that surpass threshold limits for major stable orchard crops. In order to minimize all these problems it is very essential to analyze the impact of climate change on agriculture. The selection of orchard crop in an area mainly depends on suitability of existing climatic conditions for better fruit production. The crop water requirement basically depends on the climatic conditions, crop characteristics and evapotranspiration of crops. The crop coefficient of orchard crops varies with season it was found maximum during summer month and minimum during winter season (Doorenbos and Pruitt 1977).

MATERIAL AND METHODS

Study area: Study on impact of climate change on crop water requirement of different orchard crops was carried out for Udaipur district of Rajasthan. The study area is located between 24°35'31.5" latitude 73°44'18.2" longitude with an altitude of 582.17 m above mean sea level (MSL).

Agro-climatic conditions: Udaipur comes under dry, sub-humid agro-climatic region (Fig. 1). The average annual rainfall of Udaipur is 637mm, most of the rain received during the period of July to September. The May is hottest month and December is the coolest of the year. The daily meteorological data such as daily weather parameters like maximum temperature (T_{max}, °C) and minimum temperature (T_{min}, °C), maximum relative humidity (RH_{max}, %) and minimum relative humidity (RH_{min}, %), wind speed (WS, km hr⁻¹) at height of 2.0 m, sun shine hours (SShr, hr), rainfall (R, mm) for five year (2014 to 2018) were collected from meteorological observatory of College of Technology and Engineering, Udaipur.

Reference evapotranspiration (ETo): The modified Penman-Monteith method suggested by (Allen et al 1998) was used to compute reference evapo-transpiration (ETO). The FAO Penman-Monteith method to estimate ETo is given below:

$$ET_0 = \frac{0.408 \Delta (R_n - G) + \gamma \frac{900}{T + 273} U_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34U_2)}$$

where, ET_o = Reference evapotranspiration [mm day^{-1}],
 R_n = Net radiation at the crop surface [$\text{MJ/m}^2\text{day}$],
 T = Air temperature at 2 m height [$^{\circ}\text{C}$],
 e_a = Actual vapour pressure [kPa],
 e_s = Saturation vapour pressure [kPa],
 $e_s - e_a$ = Saturation vapour pressure deficit [kPa],
 G = Soil heat flux density [$\text{MJ/m}^2\text{day}$],
 γ = Psychrometric constant [$\text{kPa}^{\circ}\text{C}^{-1}$],
 u_2 = Wind speed at 2 m height [m s^{-1}],
 Δ = Slope vapour pressure curve [$\text{kPa}^{\circ}\text{C}^{-1}$].

Crop evapo-transpiration: The relationship between crop evapo-transpiration (ET_c) and reference evapo-transpiration (ET_o) can be expressed by the following equation (Allen et al 1998) was used in this study in order to estimate the crop evapotranspiration: $ET_c = ET_o \times K_c$

Where, ET_c = Crop evapo-transpiration. ET_o = Reference evapo-transpiration, K_c = Crop coefficient. The crop coefficient (K_c) for different orchard crops varied in different month based on phenological stages of plant and percentage of the growth shaded by the tree canopy. In the present study the phenological stage wise values of crop coefficient for perennial fruit crops were taken as given by Kisekka et al (2014) (Table 1).

Crop water requirement: Impact of climate change in term of daily weather data on crop water requirement was examined over period of five year. The average daily crop water requirement (litre/day/plant) for different orchard crops during different month was calculated as $CWR = ET_c \times A \times W_p$

Where, CWR = Water requirement of crop, litre/day/plant, ET_c = Crop evapotranspiration, mm/day . A = Area of the crop (m^2) = row to row (m) $\times$ plant to plant spacing (m), K_c = Crop coefficient, W_p = Percentage wetted area, decimal.

Table 1. Typical crop coefficient (K_c) for perennial fruit crops (Kisekka et al 2010)

Month	Citrus	Papaya	Guava
January	0.79	0.86	0.93
February	0.97	1.03	1.05
March	1.05	1.03	1.00
April	0.90	0.87	0.79
May	0.54	0.54	0.54
June	0.71	0.87	0.87
July	0.91	0.91	0.87
August	0.78	0.54	0.54
September	0.80	0.80	0.80
October	0.85	0.90	1.00
November	1.00	1.00	1.00
December	0.85	0.80	0.80

In this study separate value of spacing and wetted area of selected fruits crops were taken which are presented in Table 2.

RESULTS AND DISCUSSION

Impact of climate change on reference evapo-transpiration and rainfall patterns: The significant impact of climate change on reference evapo-transpiration and rainfall patterns during year 2014 to 2018 was observed. The maximum annual ET_o was for year 2015 with a value of 1410 mm/year while, minimum annual rainfall was found for 2016 with a value of 747 mm year^{-1} . The minimum annual ET_o and annual rainfall were for years 2017 (1259 mm year^{-1}) and 2018 (556 mm year^{-1}), respectively. The uneven trend of ET_o and Rainfall was for different year due to climate change. The results reflected because of that the climatic condition of Udaipur region varies year to year due to improper use of natural resources, over industrialization, different kind of pollution and mining interventions, which results improper future planning for establishment of orchard crops.

Impact of climate change on average daily reference evapo-transpiration (mm day^{-1}): The impact of climate change on average daily ET_o during different month for year 2014 to 2018 are presented in Figure 3. The data in Table 3

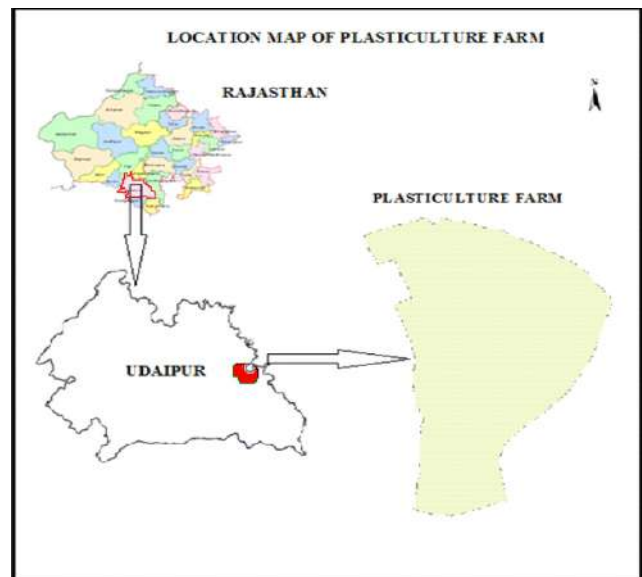


Fig. 1. Location map of the study area

Table 2. Spacing and wetted area of different selected fruits crops

Crop	Spacing ($\text{m} \times \text{m}$)	Wetted area (%)
Citrus	5 $\times$ 5	25
Papaya	2 $\times$ 2	50
Guava	5 $\times$ 5	30

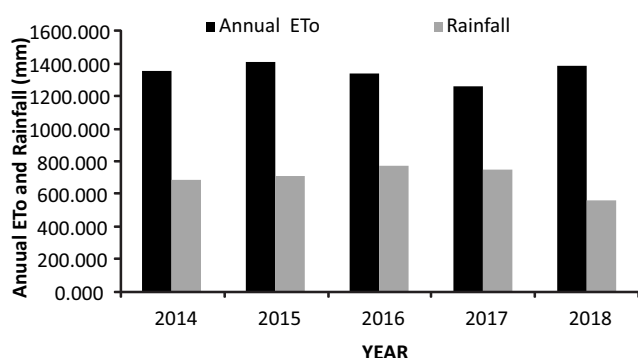


Fig. 2. Impact of climate change on annual ETo and annual rainfall (mm/year) for 2014 to 2018 for Udaipur region

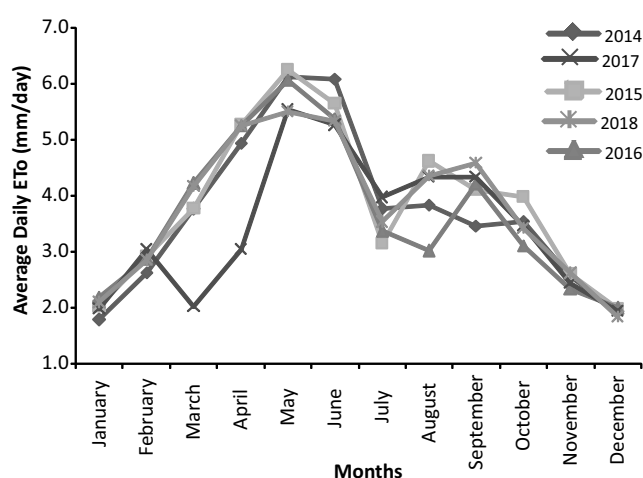


Fig. 3. Average daily ETo (mm day^{-1}) during different month for year 2014 to 2018 under Udaipur region

shows that the maximum average daily ETo was found for May month during all year followed by June month. It is probably because of high atmospheric temperature and more sunshine hours during summer season. The minimum average daily ETo was found for December month during all year followed by January month. But in some cases it varies (in year 2014) due to impact of climate change.

Impact of climate change on ETc: The daily crop evapotranspiration were significant varies with different months and year for all citrus, papaya and guava crop Table 4 to 6. The average daily value of ETc for citrus crop was highest for April month in all year (except 2017). In year 2017, highest ETc was for June month. While average daily ETc for citrus varies from 2.8 to 3.2 mm day^{-1} during year 2014 to 2018. It is probably due to climate change papaya and guava the average daily value of ETc was highest for June in all year. Similar results were reported by (Morgan et al 2017). It shows that peak crop evapotranspiration due to high atmospheric temperature. The average daily value of ETc varies from 2.8

Table 3. Average daily reference evapotranspiration (mm day^{-1}) under climatic condition of Udaipur region

Months	Average daily ETo (mm day^{-1})				
	2014	2015	2016	2017	2018
January	1.8	2.0	2.2	2.0	2.1
February	2.6	2.9	2.9	3.0	2.9
March	3.8	3.8	4.2	2.0	4.2
April	4.9	5.3	5.3	3.0	5.3
May	6.1	6.3	6.1	5.5	5.5
June	6.1	5.7	5.4	5.3	5.3
July	3.8	3.1	3.4	4.0	3.5
August	3.8	4.6	3.0	4.3	4.4
September	3.5	4.1	4.2	4.3	4.6
October	3.6	4.0	3.1	3.5	3.4
November	2.6	2.6	2.3	2.4	2.6
December	1.9	2.0	2.0	1.9	1.8

Table 4. Average daily crop evapotranspiration (mm day^{-1}) of citrus under climatic condition of Udaipur region

Months	Average daily crop evapotranspiration (mm day^{-1})				
	2014	2015	2016	2017	2018
January	1.41	1.60	1.74	1.57	1.66
February	2.56	2.83	2.78	2.95	2.77
March	3.96	3.96	4.43	2.12	4.38
April	4.45	4.74	4.73	2.74	4.74
May	3.31	3.38	3.28	2.99	2.97
June	4.33	4.01	3.82	3.74	3.79
July	3.44	2.86	3.07	3.62	3.22
August	3.00	3.61	2.35	3.38	3.40
September	2.77	3.28	3.37	3.47	3.67
October	3.02	3.38	2.65	2.94	2.90
November	2.56	2.60	2.33	2.44	2.62
December	1.65	1.69	1.69	1.65	1.57

to 3.2 and 2.8 to 3.1 mm day^{-1} for papaya and guava fruit crop respectively, during year 2014 to 2018.

Impact of climate change on crop water requirement (CWR) of orchards crop: The average daily crop water requirement of citrus crop was highest for April during year 2015 and 2018 (30 litre $\text{plant}^{-1} \text{day}^{-1}$). The minimum average daily crop water requirement of citrus crop was for January during year 2014 (8.8 litre $\text{plant}^{-1} \text{day}^{-1}$). The maximum and minimum values of CWR vary randomly with period of time. The similar finding was reported by (Sirgure et al 2014).

In case of papaya and guava crop highest crop water requirement was recorded for June during all year. The maximum values of CWR for papaya and guava crop ranged from 9 to 11 and 34 to 40 litre $\text{plant}^{-1} \text{day}^{-1}$ during all five year.

Table 5. Average daily crop evapotranspiration (mm day⁻¹) of papaya for year 2014 to 2018 under climatic condition of Udaipur region

Months	Average daily crop evapotranspiration (mm day ⁻¹)				
	2014	2015	2016	2017	2018
January	1.53	1.74	1.89	1.70	1.80
February	2.72	3.01	2.95	3.13	2.94
March	3.88	3.89	4.35	2.08	4.30
April	4.30	4.58	4.57	2.65	4.58
May	3.31	3.38	3.28	2.99	2.97
June	5.30	4.92	4.69	4.58	4.65
July	3.44	2.86	3.07	3.62	3.22
August	2.08	2.50	1.63	2.34	2.36
September	2.77	3.28	3.37	3.47	3.67
October	3.20	3.58	2.80	3.11	3.07
November	2.56	2.60	2.33	2.44	2.62
December	1.56	1.59	1.60	1.56	1.48

Table 6. Average daily crop evapotranspiration (mm day⁻¹) of guava for year 2014 to 2018 under climatic condition of Udaipur region

Months	Average daily crop evapotranspiration (mm day ⁻¹)				
	2014	2015	2016	2017	2018
January	1.66	1.88	2.05	1.84	1.95
February	2.77	3.07	3.01	3.20	3.00
March	3.77	3.77	4.22	2.02	4.17
April	3.91	4.16	4.15	2.40	4.16
May	3.31	3.38	3.28	2.99	2.97
June	5.30	4.92	4.69	4.58	4.65
July	3.29	2.74	2.93	3.46	3.08
August	2.08	2.50	1.63	2.34	2.36
September	2.77	3.28	3.37	3.47	3.67
October	3.55	3.98	3.11	3.46	3.42
November	2.56	2.60	2.33	2.44	2.62

Table 7. Average daily crop water requirement (liter plant⁻¹ day⁻¹) of citrus under climatic condition of Udaipur region

Months	Average daily crop water requirement (litre plant ⁻¹ day ⁻¹)				
	2014	2015	2016	2017	2018
January	8.8	10.0	10.9	9.8	10.4
February	16.0	17.7	17.4	18.5	17.3
March	24.7	24.8	27.7	13.2	27.4
April	27.8	29.6	29.5	17.1	29.6
May	20.7	21.1	20.5	18.7	18.6
June	27.0	25.1	23.9	23.4	23.7
July	21.5	17.9	19.2	22.6	20.1
August	18.7	22.5	14.7	21.1	21.3
September	17.3	20.5	21.0	21.7	22.9
October	18.9	21.1	16.5	18.4	18.1
November	16.0	16.2	14.6	15.3	16.4
December	10.3	10.6	10.6	10.3	9.8

Table 8. Average daily Crop Water Requirement (liter plant⁻¹ day⁻¹) of papaya for year 2014 to 2018 under climatic condition of Udaipur region

Months	Average daily crop water requirement (litre plant ⁻¹ day ⁻¹)				
	2014	2015	2016	2017	2018
January	8.8	10.0	10.9	9.8	10.4
February	16.0	17.7	17.4	18.5	17.3
March	24.7	24.8	27.7	13.2	27.4
April	27.8	29.6	29.5	17.1	29.6
May	20.7	21.1	20.5	18.7	18.6
June	27.0	25.1	23.9	23.4	23.7
July	21.5	17.9	19.2	22.6	20.1
August	18.7	22.5	14.7	21.1	21.3
September	17.3	20.5	21.0	21.7	22.9
October	18.9	21.1	16.5	18.4	18.1
November	16.0	16.2	14.6	15.3	16.4
December	10.3	10.6	10.6	10.3	9.8

Table 9. Average daily crop water requirement (liter plant⁻¹ day⁻¹) of guava under climatic condition of Udaipur region

Months	Average daily crop water requirement (litre plant ⁻¹ day ⁻¹)				
	2014	2015	2016	2017	2018
January	8.8	10.0	10.9	9.8	10.4
February	16.0	17.7	17.4	18.5	17.3
March	24.7	24.8	27.7	13.2	27.4
April	27.8	29.6	29.5	17.1	29.6
May	20.7	21.1	20.5	18.7	18.6
June	27.0	25.1	23.9	23.4	23.7
July	21.5	17.9	19.2	22.6	20.1
August	18.7	22.5	14.7	21.1	21.3
September	17.3	20.5	21.0	21.7	22.9
October	18.9	21.1	16.5	18.4	18.1
November	16.0	16.2	14.6	15.3	16.4
December	10.3	10.6	10.6	10.3	9.8

The results shows that the crop water requirement of different orchard crop significantly affected by change in climatic condition over period of time.

CONCLUSIONS

The overall results of this study shows significant impact of climate change on reference evapo-transpiration, rainfall patterns, during year 2014 to 2018. The crop evapo-transpiration and crop water requirement of different orchard crops were also varies with period of time (2014 to 2018). The uneven trend of different parameters for orchard crops was observed due to changing climatic condition in Udaipur

region. In this area spatial and temporal variability in normal rainfall pattern, atmospheric temperature and reference evapotranspiration mainly occurs due to overall urbanization, pollution, deforestation, improper use of natural resources and intervention through mining.

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Impact of Climate Change on the Shift of Landscape and Climate Boundaries

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Abstract: In the article for the territory of the Kabardin-Balkar Republic (the central part of the northern slope of the Greater Caucasus) there are calculations of the displacement of the main landscape and climatic boundaries, the snow line and the upper boundary of the forest-under the influence of climate change. Moreover, based on the analysis of the climate of the Greater Caucasus, three preferable scenarios of climate change are selected, taking into account the anthropogenic impact. In all 3 scenarios, all landscape and climatic boundaries are shifted up to the south-west. The marked increase in the area of forest landscapes will be "smoothed" due to anthropogenic impact. The main anthropogenic load will be transferred from the low-mountain to the high-mountain landscapes. With the general warming of the climate, the snow-avalanche situation will also change. Moreover, the change in the snow-avalanche situation according to these scenarios can complicate the development of high-mountain areas, where the main recreational resources of the Republic are concentrated. This will happen due to the increase of avalanche danger and, as a result, it is create tense and even crisis environmental situations. Therefore, economic activity in this region should be carried out taking into account avalanche activity. This will lead to additional costs of anti-avalanche measures to reduce the impact of avalanches on the natural environment. A specialized GIS-editor was used in the analysis of avalanche danger in the mountainous areas of Kabardino-Balkaria (upper reaches of the Baksan River).

Keywords: Geosystem, Landscape, Transformation of landscapes, Landscape-climatic border, Avalanche activity

Currently, due to global warming in mountain geosystems there is a shift of landscape-climatic boundaries (Kyul, 2004). The geographical location of the object of research is as follows: the mountain system under consideration is the Greater Caucasus (Fig. 1) - located between the Black Sea and the Caspian Sea and together with the Lesser Caucasus forms a mountainous country the Caucasus mountains.

The Greater Caucasus stretches for more than 1150 km from north-west to south-east, from the town of Anapa and the region of Taman to the Apsheon Peninsula on the Caspian coast, near Baku city. It is located on the territory of several countries (Russia, Azerbaijan, Georgia, Armenia) and is a geographical border between the two continents – Europe and Asia.

Based on the physical and geographical features of geosystems, for each mountain country there are several options for climate change scenario. Below there are the most appropriate of them for the study area. These scenarios proposed by M. I. Budyko, M. Ch. Zalikhanov, N. A. Surova and I. I. Borzenkova. They were chosen in the analysis of climate change in the Greater Caucasus to assess avalanche activity (Kyul 2004). The analysis of climate change according to the scenario chosen by the authors,

which allows to estimate the transformation of the landscape structure of the Greater Caucasus, is an urgent and priority task for this mountain region.

The territory of any mountain country is exposed to a range of natural hazards, which makes the mountain landscape very unstable to any outside interference. At the same time, any restructuring of the landscape structure can lead to the activation of natural hazards, which, in turn, increases the level of danger and, as a consequence, the natural risk and damage from natural hazards. The importance of the considered research problem at this time stage increases sharply due to the wide development of high-mountain areas of the Greater Caucasus, namely: the development of recreational complex of the territory with associated infrastructure.

MATERIAL AND METHODS

The analysis of transformation of landscape structure is carried out in several stages. At the preliminary stage, based on the study of climate fluctuations for the study area, the scenario of climate change is selected in the analysis of scientific and stock sources (in this case, the geoecological approach is applied, i.e., both their geographical and environmental components of climate change are

evaluated). Further, the nature of the shift of landscape and climatic boundaries: such as the snow line and the upper boundary of the forest is investigated. The analysis of changes in landscape and climatic boundaries for the Caucasus was carried out by the method proposed by Panova and Khrustalev in 2002. For the snow line, the number of days with snow cover is the main characteristic of snow cover, which determines the level of snow lines. For the Central Caucasus, the calculation of this indicator for different heights is carried out by the formula:

$$N = 0.08 H + 0.024 X = 32.318, (1)$$

where N is the number of days with snow cover, m;

H-height of observation point above sea level, m; X-the amount of precipitation for the cold period of the year (X-IV), mm.

The height of the firn line, H_f , is considered as the average:

$$H_f = (H_c + H_o) / 2, (2)$$

where H_c - the height of the climate line, m; H_o -the height of the orographic line, m.

The relationship between landscape and climate boundaries is linear and is expressed by regression equations (for the Northern slope):

$$H_f = 0.36 H_c + 1007.90; r = 0.55; \delta = 111.80 (3)$$

$$H_f = 0.30 H_f + 1323.46; r = 0.68; \delta = 98.60 (4)$$

$$H_f = 0.24 H_o + 1712.01; r = 0.55; \delta = 111.30, (5)$$

where H_f is the height of the border of the forest, m;

H_c , H_f , H_o -the height of climatic, orographic, firn and snow lines; r-correlation coefficient;

δ —error of the equation.

Calculation of changes in the heights of snow lines is carried out according to the following equations:

$$\Delta H_s = (n_1 - n_2) + 1.042 / 0.084, (6)$$

where ΔH_s —change the height of the snow line, in m; n_1 and n_2 are the number of days with snow cover before and after, respectively climate change.

The correlation coefficient (R) for this equation is 0.85 ± 0.07 .

$$H_f = 90.28 t + 0.49 X + 3570, (7)$$

where H_f is the height of the firn line, in m;

X-the amount of precipitation for the cold period (X-IV), mm; t- the average temperature for the warm period (IV-IX), °C.

The regression equation is used to change the state of glaciers:

$$\Delta S = 1.40 t - 0.01 r + 0.34 S + 1.35, (8)$$

where ΔS is the decrease in the glacial area, km²; t – the average air temperature in June and July, °C; r- precipitation for October-May, mm; S is the area of glaciation at present.

Moreover, these geographical objects are studied

according to the basin principle (within river basins of different orders) in the comparative analysis of different time photos and satellite images. At the field stage during GPS - shooting, conducted with the help of GPS-Navigator "Garmin", the data are refined and supplemented. Characteristics (dimensions, elevation points, coordinates), obtained during their inventory and certification, are reduced to a database, which can serve as the basis for the preparation of special maps of the transformation of the landscape structure.

RESULTS AND DISCUSSION

Climate change scenarios in the Caucasus: According to Panov (2000) and Khrustalev (2002) quantitative characteristics according to the scenarios for the study area (the Greater Caucasus) by 2025 year will be as follows:

1. The temperature in the warm period (IV-IX months) will increase by 1.6-2.0° C, in the cold (X-IV months) - 2-3° C. Precipitation in winter will increase by 7 per cent (in the Central Caucasus may increase to 20-50 %) (Budyko 1980, Zalikhanov et al 1987).
2. Surova (1984) mentioned that the air temperature will increase in the warm period by 0.6°C, the amount of precipitation in the cold-will be reduced by 26 per cent.
3. Borzenkova et al (1987) mentioned that the air temperature in the warm period will change by 1,6°C and the amount of precipitation in the cold period will increase to 7 per cent.

The first and third scenarios take into account the anthropogenic impact on the climate (a sharp increase in the oxidizing role of anthropogenic growth of carbon dioxide in the atmosphere will be due to the combustion of mineral and organic fuels. Paleoclimatic data were used in the development of the scenario by Borzenkova (1987). Surova (1984) concluded on the basis of natural climate change without taking into account the anthropogenic impact, and on it the amount of precipitation does not increase, but decreases. Panov (2000), using long-term data of climatic indicators (1881-1990 years) made the analysis of climate fluctuations. It was found that in the Greater Caucasus in the central part of the northern slope, in the air temperature, the following trends were observed. In the period from 1881 to 1900 years the air temperature did not change. And during 1901 to 1920 years it decreased accordingly, down -0.2 and -0.4°C. However, 1921 to 1990 there is an increase in temperature by + 0, 1 and + 0, 5°C per decade. Since 1990 year, warming has been increasing air temperature will increase by 0.3–2.0°C by 2025 year.

Accordingly, with the temperature changes the amount

of precipitation also changed with some cyclicity. In the course of changing of the amount of precipitation, separated periods of increasing precipitation (1881-1900, 1921-1940 years and 1961-1970 years) by 5-14 per cent; a decrease in precipitation (1901-1920, 1941-1960 and 1971-1980 years) 5-10 per cent; stabilization precipitation (1981-1990 years), when the amount of precipitation has not changed. Moreover, the duration of these cycles, since the middle of the 20th century, decreased from twenty to ten years. Based on the charts of progress in the change of temperature and precipitation, it is possible to identify three periods of correlation, when the value of these two parameters varied the same way: increased in 1901-1921 and 1921-1940 years and decreased in 1961-1970 years. Since 1990 year, by 2025 year, the air temperature will increase by an average of 2, 5°C; precipitation will increase by 7 per cent according to Budyko (1980) and by 20-50 per cent according to Zalikhanov (1987). For the selected research region, namely the northern slope of the central part of the Greater Caucasus, taking into account the analysis of the main climate change scenarios, the authors selected the first scenario proposed by Budyko, as it takes into account both the geographical and environmental components of climate change (Kyul, 2004).

Change in the heights of landscape and climatic boundaries. Two vertical landscape and climatic boundaries are distinguished in the study area snow lines and the upper boundary of the forest. The role of these boundaries in avalanche formation is very high. Since their altitude and spatial position largely depends on climatic factors, climate change will lead to the displacement of these boundaries in space. The analysis of changes in landscape and climatic boundaries for the Caucasus was carried out by Panova together with Khrustalev in 2002. Figures 2-4 show the Kabardin-Balkar Republic with its main geomorphological structures, ridges, and hydrographic network (river basins).

Dynamics of Displacement of Landscape and Climatic Boundaries

Upper limit of the forest (ULF): The spatial position of this boundary depends on a combination of geological and geomorphological, anthropogenic and climatic factors. The "limit of distribution of closed forest communities excluding woodlands" was adopted beyond the upper limit of the forest (Khrustalev et al 2002, Kyul 2012). In the Central Caucasus, the pine and the birch grow on the upper border of the forest. Taking into account the analysis of topographic and special (Razumov et al 2000, 2001) maps of scale 1: 25000-1: 200000 on the main river valleys, the upper limit of the forest passes in the following ranges: 2200 m (the Malka River); 2400-2520 m (the Baksan River); 2420-2440 m (the Chegem

River); 2340-2550 m (the Cherek Bezengiyskiy River); 2340-2420 m (the Cherek Balkarskiy River); 2200 m (the Psygansu river); 2200-2240 m (the Haznidon River). A number of regularities are observed in the distribution of vegetation on the territory of the Republic. In the Central part of the upper border of the forest is higher than in the north-west and south-east, 200-300 m. On the northern slopes it is higher by 100-200 m than in the south. The forest border is 200-300 m higher on the slopes than on the bottoms of river valleys. Lowering the upper limit of the forest, up to its complete destruction, in some river basins (the Malka, Psygansu and Haznidon Rivers) is associated with both the action of avalanches, and with human economic activity.

The average height of the upper boundary of the forest in the study area is 2360 m. Compared to the average height of the Greater Caucasus (2290 m) on the territory of Republic the upper limit of the forest is higher at 170 m. In connection with a possible climate change (increased air temperature and precipitation) will occur a lifting of the upper forest boundary. Landscape-geophysical analysis allowed to determine that this shift will occur at 300 m (the average height of the upper boundary of the forest will increase to 2660 m). Changing the height of the ULF has a significant impact on changing the landscape of the territory of the Republic.

Snow lines: The paper deals with three types of snow lines: snow (climate), orographic and firn. The definitions of these lines are taken from Glaciological dictionary (1984). This included a number of regularities in the distribution of solid precipitation in the Central Caucasus (Khrustalev et al 2002), a reduction in rainfall with a given exposure; the increase in annual precipitation, including the solid, with the height of a particular zone of the maximum (1210 mm at an altitude of 3200 m), redistribution of solid precipitation due to wind on the slopes of the mountain (the Main Caucasian and Lateral ore Watershed) ridges and the presence of zones of unstable, variable and stable snow cover. Within the medium-low mountain (the Pasture ore Cretaceous, the Dzhinalskiy and the Wooded) ridges there is the zone of very unstable snow cover where number of days with a snow cover varies from 50 to 70 days is located. The zone of unstable snow cover includes the mid-mountain the Rocky ridge and the North-Jurassic depression. This zone is characterized by the absence of snowless winters. The number of days with snow cover to 100-340 days starting from the height of 2000 m. The zone variable and permanent snow cover is high mountain ridges (front, lateral and main) and depression (the Kyrtykskaya and the Shtulinskaya). The number of days with snow cover in these zones respectively 340-365 and more than 365 (snow practically does not melt in



Fig. 1. The Greater Caucasus geographical location



Fig. 2. Location on the map of the Kabardin-Balkar Republic

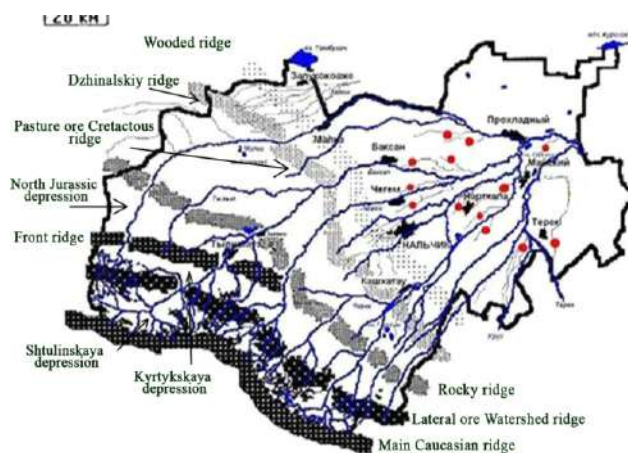


Fig. 3. Major ridges in the Kabardin - Balkar Republic



Fig. 4. Main river basins in the Kabardin-Balkar Republic

the summer). On the northern slope of the Central Caucasus with a height of the number of days with snow cover increases: 45 (1000 m); 100 (1500 m), 155 (2000 m), 210 (2500 m), 265 (3000 m); 320 (3500 m). The altitude of the climatic snow line (CSL) on the northern slope of the Central Caucasus: medium – 3840 m; in the region of Elbrus, the north face – 4860 m, south – 4730 m; the Rocky ridge (the Cherek River) – 3800 m. Along the river valleys the level of CSL ranges from 3860 m on the river Malka to 3760 m on the river Baksan; 3820 and 3800 m level CSL has built on the tributaries of the Baksan River – the Chegem River and the Cherek River. In the basin of the Uruk River (Haznidon) the level CSL is almost the same as in the upper reaches of the Baksan River – 3750 m. The height of the firm snow line (FSL), first of all, depends on snowstorm transport and avalanches that change snow accumulation, as well as on the exposure of glaciers: maximum (3300-3460 m) on the northern, north-eastern and north-western slopes; minimum (3510-3660 m) on the eastern and western (Panova, 2002) for the northern slope of the Greater Caucasus. In the main river basins, the height of the FSL will range from 3720, 3510, 3550 m, respectively, in the rivers Malka and Baksan (upper reaches) and the Chegem River to 3370-3510 m on the Uruk River (Haznidon). Differences in its height are determined by the orographic factor, that is, the height of the ridges (maximum in the area of Elbrus, 3850 m, minimum – in the low mountains, 2410 m). The average height of the FSL in the Central Caucasus on the northern slope is 3530 m and also depends on the exposure of the slopes. On the southern slope it is lower, as the southern slope of the Lateral ridge is in the "rain shadow" and there is less rainfall. Due to global warming recently there is an increase in the height of the firm snow line (an average of 90 m for 1881-1990).

Orographic line (OSL) is least dependent on the climate and is determined primarily by the relief. The lower boundary of permanent snow fields or level of the OSL on the northern slope of the Central Caucasus on the major river basins of the following (Khrustalev 2002): the Malka River, 2640 m; the Baksan River (upper), 2600 m; the Chegem River, 2550 m; the Cherek Bezenziyskiy River, 2580; the Cherek-Balkarskiy River, 2540 m; the Uruk River (Haznidon), 2530 m. The average height of donkeys in the Central Caucasus is 2380 m (the lowest for the northern slope of the Greater Caucasus). The temporal variability of donkey height depends on meteorological factors: it decreases in snowy winters, when the thickness of snow cover increases sharply and due to this, avalanche snowfields are widely developed due to avalanches (Myagkov et al 1992, Kanaev et al 1996).

There is a close connection between the snow lines. The greatest differences between them: CSL-3840, FSL-3530 and OSL-2680 m. The average excess over CSL FSL-290 m above OSL-1160 m. The average excess FSL on OSL-850 m. The lowest here is the orographic line (the height of the lower limit of glaciers – 2760 m). The maximum height difference of snow lines in the Central Caucasus, as well as due to the corresponding size of the glaciation modern oroklimaticheskim conditions, led to a reduction in the area of the glaciation over the past 100 years by 23-37 % (Khrustalev et al 2002). Global warming contributes to the degradation of glaciation, whereby the height of the FSL increases, and the difference between the CSL and the FSL decreases. This should lead in the near future to an improvement in the condition of glaciers. Moreover, due to the warming the height of the CSL also increases.

Boundaries of modern glaciation: There are certain trends

in the distribution of modern glaciation by area on the northern slope of the Central Caucasus: its area decreased by 26 per cent in the period from 1890-1966 and by an average of 6 per cent in 1967-2000 (Panov 2000). Emphasizes, the decrease in the area of glaciation had both positive (increase in forest and meadow zones, decrease in the area of formation of glacial mudflows) and negative (reduction of glacial runoff, increase in the number of glacial and landslide landforms) effect on the natural environment and microclimate of the region. The results of calculations of possible changes in the area of glaciation in 2025 year on the northern slope of the Greater Caucasus scenarios: will include reduction of area 156 km², in year 6. 2 km² (Budyko 1980), decrease in area 196 km² per year, 7. 8 km² (Surova 1984) and air temperature and precipitation will be same as in 1967, the decrease in area, total, will be equal to 123 km², including annually by 4.9 km² (Borzenkova 1987). With all three options, there will be a reduction in the area of modern glaciations and in comparison with other regions of the Caucasus, it is on the northern slope of the Greater Caucasus that it is the largest (32-41%). Even when saving those climatic conditions that occur at the present time, glaciers in this region may disappear completely already to 2025 year. Here are considered the height of the lower border of the glaciers and the height of the ridges glaciation. Taking into account these indicators for the central part of the northern slope of the Greater Caucasus, the reduction of glaciers will stop by 2025 year. With the area of glaciation will be 75-100 km², and the tongues of glaciers will be at altitudes of 4000-4200 m.

Thus, having analyzed the data on the decrease in the area of glaciation for 1890-2025 years, it can be concluded

Table 1. The relationship between snow lines and the upper boundary of the forest along the main river basins according to Yu. P. Khrustalev and S. V. Panova (2002) with the author's additions

Number of river basin (Kyul 2004)	River	Height, m				Excess, m					
		csl	fsl	osl	ulf	csl fsl	csl osl	fsl osl	csl ulf	fsl ulf	osl ulf
13	The Malka	3810	3720	2640	2200	140	220	80	1660	1500	440
14	The Baksan:										
a	riverheads;	3760	3510	2600	2400-2520	250	1100	910	1240-1360	990-1110	80-200
b	The Chegem;	3820	3510	2550	2420-2440	310	1270	960	1400-1570	1070-1120	110-130
v	The Cherek:	3800	3550	2555	2375	250	1265	995	1425	1175	180
14 ¹ v	Bezenziyskiy;	-	-	2580	2340-2550	-	-	-	-	-	-
14 ² v	Balkarskiy	-	-	2540	2340-2420	-	-	-	-	-	-
14 ³ v	The Psygansu	-	-	2530	2200	-	-	-	-	-	-
15a	The Uruk (Haznidon)	3750	3510	2530	2200-2240	240	1220	980	1510-1550	1270-1310	190-210
The average excess: 2003 year ; at 2025 year in 3 scenarios:		3840	3530	2680	2340	310	1160	850	1500	1120	340
1		4040	3440	2360	2640	600	1140	1140	1400	800	-340
2		3960	3450	2340	<2340	510	1620	1110	>1570	>1110	≥ 0
3		4040	3440	2300	2640	600	1140	1140	1400	800	-340

that with the continuing trend of the total reduction in the area of glaciers, its value will decrease from 637 km² in 1890-1967 years to 123-196 km² in 2000-2025 years. This trend will continue only if there is no sharp change in climatic conditions, as in the second scenario. The multiple correlation coefficient of equation 18 is 0.94 ± 0.01 with an average quadratic error of 5.4 km². It is established that on the northern slope of the Greater Caucasus in the central part the area of glaciation will be reduced by 2025 year by 234 km². The average rate of glacial degradation will be 4.03 km²/year versus 5.1 km²/year for the last 70-80 years. On this basis, the rate of reduction of glaciation slowed the Great Caucasus from 36 per cent for the last 70-80 years to 22.4 per cent by 2025 year. It is possible to make the main conclusion, that the complete disappearance of glaciers in the study area in the next 80-100 years, not going to happen. Therefore, the process of deglaciation throughout the Caucasus will gradually level out.

CONCLUSION

The climate change by 2025 year according to several climate scenarios will lead to the displacement of all landscape and climatic boundaries to the south-west. In this case, the 3rd scenario will shift KSL within 170-250 m, FSL-110-120 m, OSL-70-110 m. ULF rise, on average, 300 m. There will be a shift of landscapes up (to the south-west). This will increase the area of forest landscapes. The influence of the anthropogenic factor will lead to the rise of the lower border of the forest and possible "smoothing" in the planned change in the areas of forest landscapes. The main anthropogenic load will be transferred from low-to medium-mountainous to high-mountainous landscapes. The snow-avalanche situation will also change with the general warming of the climate. According to the first and third scenarios, by 2025 year, due to warming and increased solid precipitation, the snow content of winters will increase and their severity will decrease. The number of days with snow cover will be reduced by 21 days, and the avalanche period will decrease. The thickness of the snow cover and snow accumulation will increase. The area of avalanches will increase, but due to the shift of economic activity in the highlands, in general, the degree of avalanche danger in the low and middle mountains will decrease. And only in the highlands there will be a tendency to increase it. This will increase the number of avalanches, including wet, spring snowmelt. The number of catastrophic avalanches will not decrease, and possibly increase. Thus according to the second and fourth scenarios, all snow-avalanche indicators are reduced. Reducing the thickness of the snow cover by 30 cm, the number of days with snow cover for 17 days and an avalanche-prone period for 10-20 days will lead to a reduction

in avalanche-prone areas. With a sharp decrease in avalanche danger, in general, in the highlands, the degree of avalanche danger can even increase due to the reduction of the avalanche danger period and an increase in the area under meadow landscapes, as well as the displacement of economic activity in the area. With a decrease in the total number of avalanches, the number of tray avalanches will increase due to an increase in the continental climate and the settling of landscapes. A decrease in the number of days with snowfalls of 10 mm/day or more for 50 days will lead to a decrease in dry avalanches from fresh snow and the predominance of avalanches of spring snowmelt. At the same time, the change in the snow-avalanche situation according to these scenarios can complicate the development of high-mountain areas, where the main recreational resources of the Republic are concentrated, due to the deterioration of the quality of the natural environment and reducing comfort, creating tense and even crisis environmental situations. Therefore, economic activity in this region should be carried out taking into account avalanche activity. This will lead to additional costs for anti-avalanche measures to reduce the impact of avalanches on the natural environment.

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Assessing Ground Water Quality for the Suitability of Irrigation in Dindigul District, Tamil Nadu, India

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Abstract: An attempt has been made in this paper to assess groundwater quality for irrigation based on various indices, such as sodium absorption ratio (SAR), permeability index (PI), Kelly's ratio (KR), Na%, magnesium ratio (MR) and soluble sodium percentage (SSP) in Dindigul district of Tamil Nadu. The study underwent based on secondary data. Groundwater chemistry in the study area is mixed type and has been validated using Piper, Durov and Gibbs plot. The United States Salinity Laboratory (USSL) plot indicates high salinity and low sodium hazard (C3-S1). Except Kelly's Ratio all other indices values in the groundwater samples are marginally suitable for irrigation purposes. So care should be taken before water gets contamination.

Keywords: Gibbs, Irrigation water quality, PI, Piper diagram, USSL

Groundwater is widely distributed renewable resources of the earth. Generally, groundwater is least polluted but once gets polluted it is difficult to restore to its original quality. In developing country like India, groundwater is considered as a major source of water for domestic supply and it is the most important and reliable source of freshwater (Nishi Kant et al 2018). Groundwater is the important source of water for both industrial and agricultural activities in regions where surface water is scarce (Delgado et al 2010). Depending on the source of water, their evaporation rate, types of rock and reactive to minerals causes differ in water chemistry (Drever 1988, Plummer et al 2003, Arshid et al 2011). The chemical characterization of the aquifer and geology of the area are also the factor which spatially and temporally change the hydro geochemical nature of water (Lakshamanan et al 2003, Ravnborg 2016, Mostafa et al 2017).

Groundwater character, climatic condition, rainfall and soil texture are some of the major components affects the groundwater quality for irrigation (Kumar 2019). Assessing the quality of water is foremost thing to identify the suitability of water for various purposes (Ifatimehin and Musa 2008, Arshid et al 2011). The minerals present in the soil have an impact on plants and soil based on which groundwater quality will determine. The use of wide variety of fertilizers, manure, excessive phosphorus and nitrogen substances during crop production causes degradation of groundwater quality (Getahun and Keefer 2016). The soils get deteriorate due to the use of degraded quality of water for irrigation which results in the decline of agricultural production on such polluted soils. The small amount of soluble salts dissolved in the groundwater depends on the sources for recharge and

strata through which it flows. The crop gets harmful if there is excess amount of soluble salts in the water. The groundwater chemistry should be better understood to evaluate the groundwater quality for irrigation (Karunanidhi et al 2013).

MATERIAL AND METHODS

Study area: Dindigul district lies in between Tiruchirappalli and Madurai districts at 10°05' to 10°09' North latitude and 77°30' to 78°20' East longitude (Fig. 1). District is well-known by its hills and hillocks. Geologically the major portion of the district is occupied by acid intrusive which occupies 56 per cent of the total area mainly found in the northern, eastern and southern part of the district. The major soil type of the district is covered by Alfisol.

To attain the objective of the study, the secondary data collected from the state ground and surface water resources data centre, Tharamani, Chennai for pre monsoon (July) and post monsoon (January) season was used to identify the seasonal behavior of chemical parameters in the groundwater for the year 2016. The samples were collected for post and pre-monsoon season in the same well. A set of 29 common wells were spatially located using ArcGIS software (Table 1). The chemical parameters are attributed to respective wells and geo database were created. Numerous irrigation quality indices are used to define irrigation water quality, such as electrical conductivity (EC), sodium absorption ratio (SAR), Na (%), permeability index (PI), magnesium ratio (MR), Kelly's ratio (KR) and soluble sodium percentage (SSP). The ArcGIS tool has been effectively used to show the map spatially using the interpolation techniques particularly inverse distance weight (IDW). The following

formula has been used to identify the indices values for irrigation quality.

$$\text{SAR} = \text{Na}^+ / \sqrt{\text{Ca}^{2+} + \text{Mg}^{2+} / 2}$$

$$\text{Na \%} = (\text{Na}^+ + \text{K}^+) / (\text{Ca}^{2+} + \text{Mg}^{2+} + \text{Na}^+ + \text{K}^+) * 100$$

$$\text{PI} \{ (\text{Na}^+ + \text{HCO}_3^-) / [\sqrt{(\text{Ca}^{2+} + \text{Mg}^{2+} + \text{Na}^+)}] \} * 100$$

$$\text{MR} = \text{Mg}^{2+} * 100 / (\text{Ca}^{2+} + \text{Mg}^{2+})$$

$$\text{KR} = \text{Na}^+ / (\text{Ca}^{2+} + \text{Mg}^{2+})$$

$$\text{SSP} = \frac{(\text{Na} + \text{K}) \times 100}{\text{Ca} + \text{Mg} + \text{Na} + \text{K}}$$

RESULTS AND DISCUSSION

Mechanisms controlling groundwater chemistry: There are several factors which determine the hydrogeo-chemical compositions and groundwater chemistry within the aquifer. This can be better understood by using Piper tri-linear diagrams, Durov plot and Gibbs plot were widely used by so many researchers (Fig. 2).

The lower right triangle are consider as anions (HCO_3^- , SO_4^{2-} , Cl^-), lower left are consider as cations (Ca^{2+} , Mg^{2+} , Na^+) and the central diamond is a combination of overall character of groundwater. The left side of the triangle is dominated with Na^+ and Mg^{2+} cations. The anion concentrations indicate that Cl^- ions are predominated followed by HCO_3^- ions in both monsoon seasons. The relative concentration of ions are in the order of $\text{Na}^+ > \text{Mg}^{2+} > \text{Ca}^{2+}$ and $\text{Cl}^- > \text{HCO}_3^- > \text{SO}_4^{2-}$. The diamond shape portrays that most of the samples are fallen in the predominance of sodium chloride, mixed Ca^{2+} - Mg^{2+} - Cl^- and calcium bicarbonate (Ca-HCO_3). The mixed water type found in the study area is further validated with the help of Durov diagram. Most of the samples are fallen in the centre of the square indicates mixing of the groundwater (Fig. 3). Gibbs plot indicates that dominant groundwater samples are plot in the rock weathering / rock-water interaction dominance zone, suggesting that the groundwater chemistry in the study area are controlled by rock dominance leading to poor quality of groundwater (Fig. 4).

Sodium absorption ratio (SAR) and electrical conductivity (EC): SAR present in the water ranges from 0.43 to 6.63 epm in post monsoon and 0.33 to 5.64 epm in pre monsoon season with mean of 3.20 and 2.75 epm respectively. All the samples are under excellent class in both the seasons (Table 2). Salinity is one of the primary concerns on irrigation water which affect both the soil structure and crop yield. Most of the soil gets saline mainly due to salts carried in the irrigation water. Wilcox (1948) used United States salinity diagram for rating of irrigation water by plotting the correlation between SAR and electrical conductivity (Fig. 5). The SAR value up to 10 and EC up to 250 define as S1

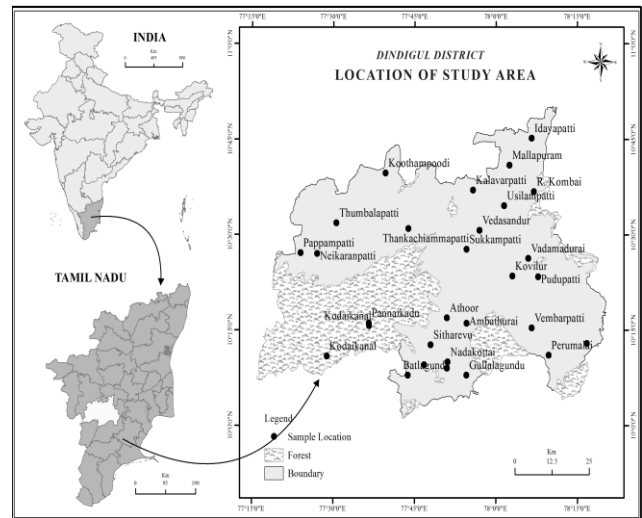


Fig. 1. Location and sampling point of the study area

Table 1. Sampling locations for Irrigation water quality (2016)

Village	Latitude	Longitude	Altitude in (m)
Kovilur	10°37'00"	78°05'00"	254
Pappampatti	10°27'09"	77°24'20"	349
Sukkampatti	10°28'48"	77°55'23"	234
Neikaranpatti	10°27'00"	77°27'45"	327
Thankachiammapatti	10°31'25"	77°44'40"	278
Usilampatti	10°34'20"	78°15'50"	267
Kalavaripatti	10°37'27"	77°56'30"	222
Perumalai	10°51'48"	77°32'53"	242
Ambathurai	10°16'30"	77°54'50"	297
Athoor	10°17'10"	77°51'02"	293
Vembarpatti	10°15'25"	78°07'19"	353
Sitharevu	10°13'36"	77°48'30"	275
Vadamadurai	10°26'30"	78°06'08"	295
Pudupatti	10°18'35"	78°08'31"	364
Thumbalapatti	10°32'02"	77°31'35"	317
Batlagundu	10°08'58"	77°44'34"	240
Nadakottai	10°05'20"	77°49'50"	202
Sithargalnatham	10°06'18"	77°51'15"	203
Samutrapatti	10°12'55"	78°17'24"	209
R. Kombai	10°34'22"	78°05'26"	272
Mallapuram	10°40'55"	78°00'45"	208
Vedasandur	10°31'36"	77°57'18"	218
Koothampoodi	10°40'54"	77°40'50"	298
Kodaikanal	10°11'50"	77°29'54"	1323
Kodaikanal	10°16'50"	77°37'50"	1316
Gullalagundu	10°08'56"	77°54'46"	215
Keelakovilpatti	10°09'34"	77°47'01"	228
Idayapatti	10°45'12"	78°01'42"	199
Pannai	10°16'30"	77°37'33"	1334

water. All the samples are comes under S1 category in both pre and post monsoon season.

The USSSL plot indicates that most of the samples are fall within the C3-S1 category indicating high salinity and low sodium hazard class for both monsoon seasons. Only few samples are fall in C3-S2 category indicates high salinity and medium sodium hazard.

Sodium percentage: The mean percentage of sodium value was 41.88 and 40.08 meq l^{-1} during post and pre monsoon season (Table. 2). The result portrays that 97 per cent of samples in both post and pre monsoon season are under permissible limit (< 60%). The mean value of both the season indicates that sodium percentage is under suitable for irrigation (Fig. 6).

Permeability index: Doneen (1964) has evolved a criterion for assessing the suitability of water for irrigation based on

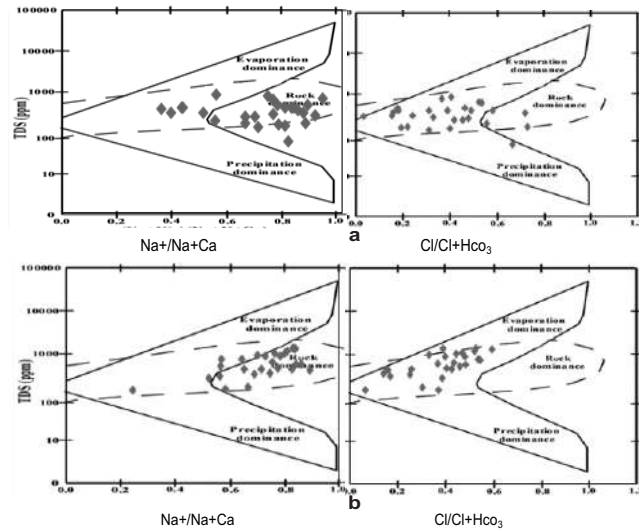


Fig. 4. Gibbs plot for post (a) and pre monsoon season (b)

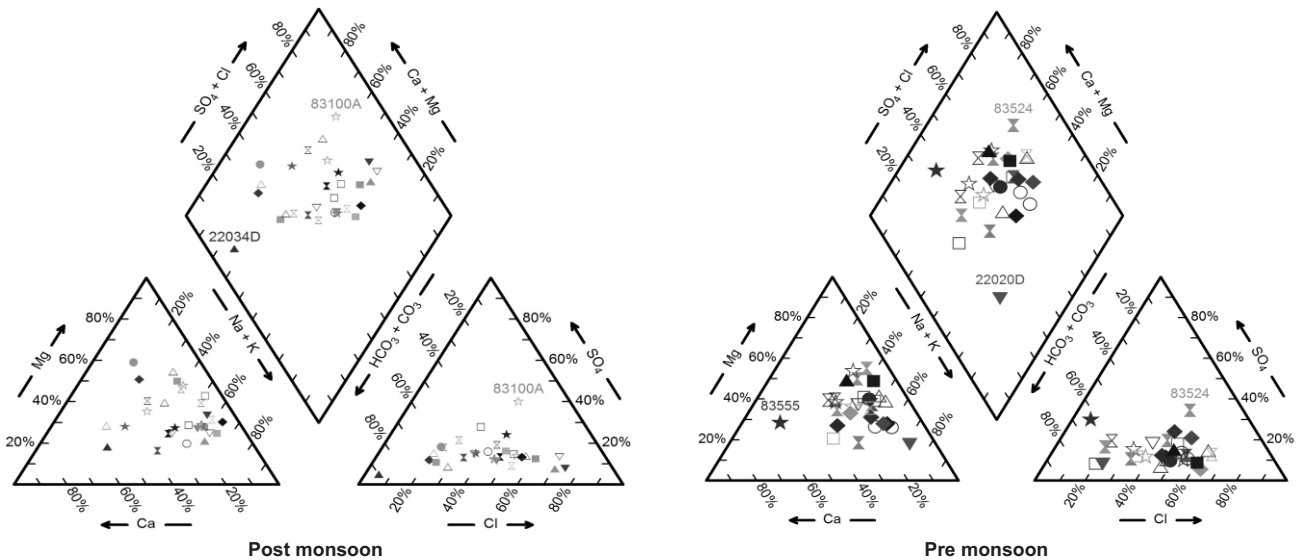


Fig. 2. Piper Diagram for post monsoon and pre monsoon season

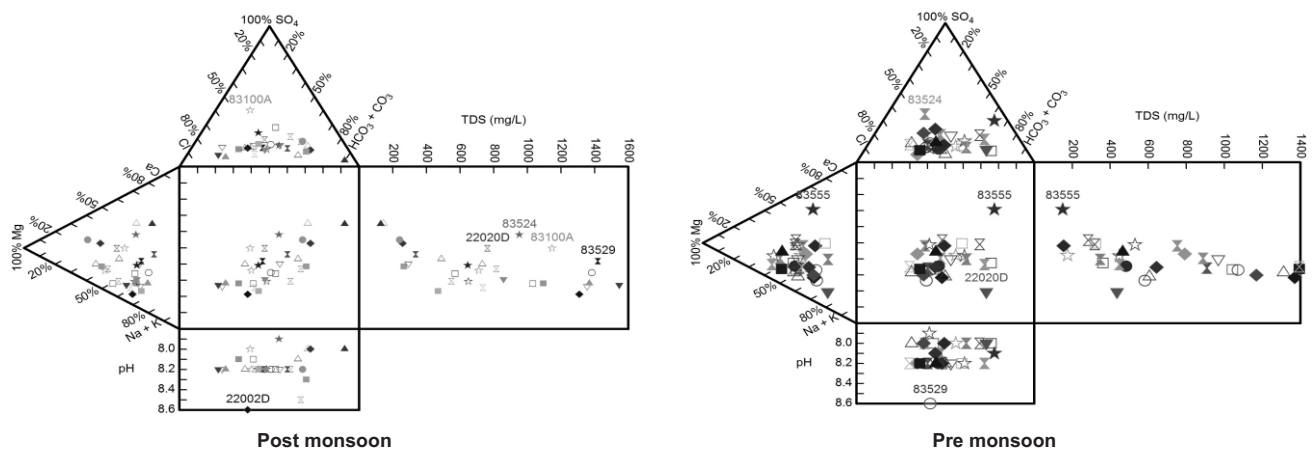
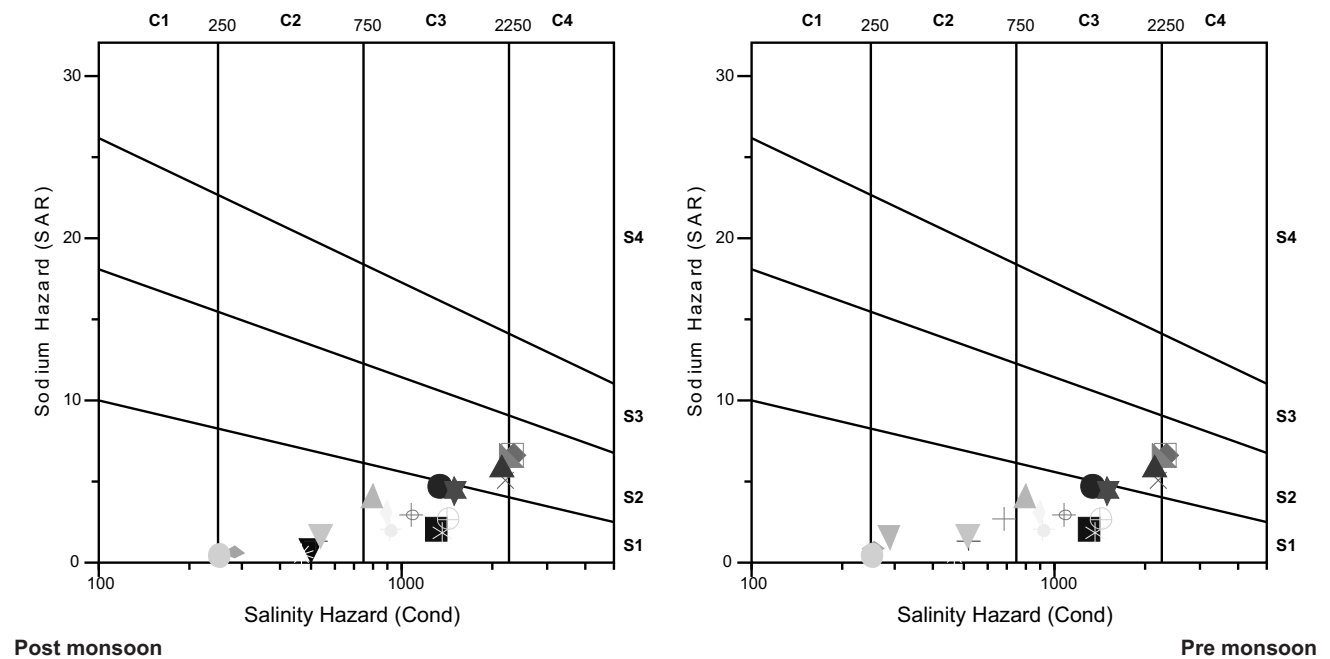


Fig. 3. Durov diagram based on hydro-geochemical for post and pre monsoon

Table 2. Statistical summary and quality indices of groundwater quality for irrigation

Irrigation parameters	Post-monsoon season		Pre-monsoon season		Suitability class		POM		PRM	
	Minimum	Maximum	Minimum	Maximum			Samples	Per cent	Samples	Per cent
SAR (epm)	0.43	6.63	0.33	5.64	Excellent	<10	29	100.00	29	100.00
					Good	10-18		0	-	0
					Doubtful	18-26	-	0	-	0
					Unsuitable	>26	-	0	-	0
EC ($\mu\text{S cm}^{-1}$)	250	2760	240	2550	Excellent	< 250	-	0	1	3.44
					Good	250-750	7	24.13	7	24.13
					Permissible	750-2000	16	55.17	17	58.62
					Desirable	2000-3000	6	20.68	4	13.79
Na % (meq l^{-1})	14.66	60.34	14.08	70.03	Excellent	0-20	3	10.34	1	3.44
					Good	20-40	11	37.91	14	48.27
					Permissible	40-60	14	48.27	13	44.82
					Doubtful	60-80	1	3.44	1	3.44
					Unsuitable	>80	-	0	-	0
PI (meq l^{-1})	38.03	80.71	35.26	89.32	Permissible	<75	26	89.00	27	90.00
					Good	> 75	3	11.00	2	10.00
MR (meq l^{-1})	15.95	80.19	23.29	78.81	Suitable	<50	14	48.27	12	41.37
					Unsuitable	>50	15	51.72	17	58.62
KR (epm)	0.27	2.67	0.21	3.76	Suitable	<1	12	41.37	13	44.82
					Unsuitable	>1	17	58.62	16	55.17
SSP (meq l^{-1})	23.67	76.98	19.77	79.53	Suitable	<200	29	100.00	29	100.00
					Unsuitable	>200	-	0	-	0

**Fig. 5.** Wilcox diagram for post and pre monsoon season

permeability index (PI). PI value has been grouped into two categories. The PI value below 75 per cent is permissible for irrigation and it is grouped as class I. Class II represents the value above 75 per cent which is good for irrigation (Table 2). PI values indicate that about 89 per cent of samples in post and 90 per cent of pre monsoon samples are under class I and 4 per cent in both monsoon are comes under class II. Most of the samples in the study area indicate permissible for

irrigation (Fig. 7).

Magnesium ratio (MR): The groundwater for irrigation is harmful, if the MR value exceeds 50 meq l^{-1} . In the analyzed water samples, the value of MR is ranging from 15.95 to 80.19 meq l^{-1} in post monsoon season (Table 2). In Pre monsoon season the MR varied from 23.29 to 78.81 meq l^{-1} (Fig. 8). The mean MR values in both the season exceeds 50 meq l^{-1} indicates that the groundwater is used for irrigation

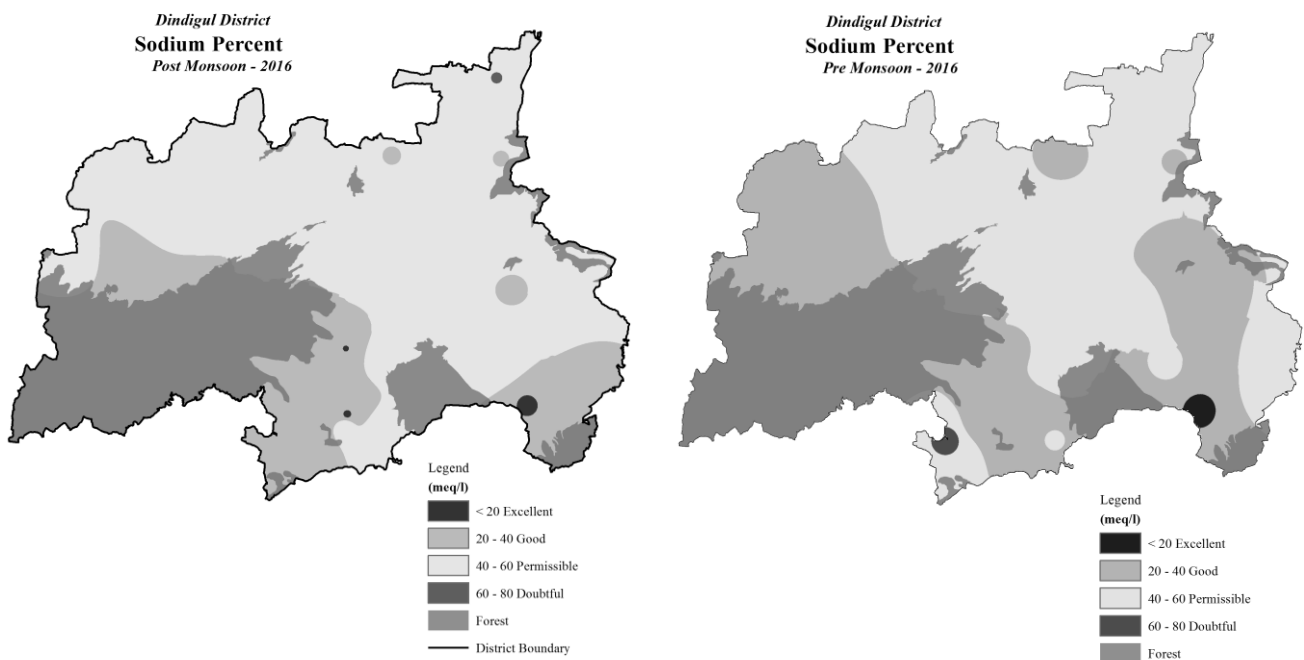


Fig. 6. Spatial distribution of sodium (%)

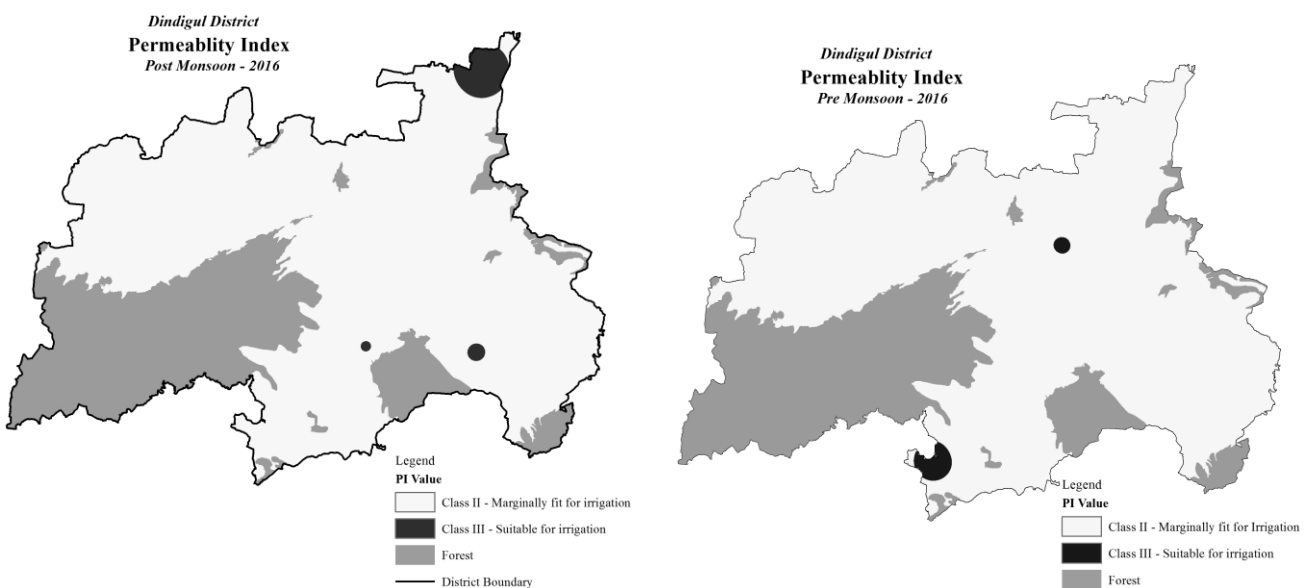


Fig. 7. Spatial distribution of Permeability Index (PI)

along with soil application of organic amendments like farm yard manure, vermicompost, goat/sheep manure will improve the crop production as well as soil health.

Kelly's ratio: Kelly's ratio having the value of above one in groundwater samples is comes under unsuitable for irrigation. Groundwater samples having the Kelly's ratio are ranging from 0.27 to 2.67 epm during post monsoon season with the mean value of 1.33 epm. During pre-monsoon season the range of KR value is from 0.21 to 3.76 epm having

the mean value of 1.20 epm (Table 2). Both the season exceeds the accepted values resulted that most of the groundwater samples are unsuitable for irrigation (Fig. 9).

Soluble sodium percentage (SSP): The SSP value < 200 will indicates suitable for irrigation. In the study region the SSP value ranges from 23.67 to 76.98 meq l⁻¹ during post monsoon season and in pre monsoon season, the SSP values varied from 19.77 to 79.53 meq l⁻¹ (Table 2). The SSP values in all the locations shows suitable for irrigation.

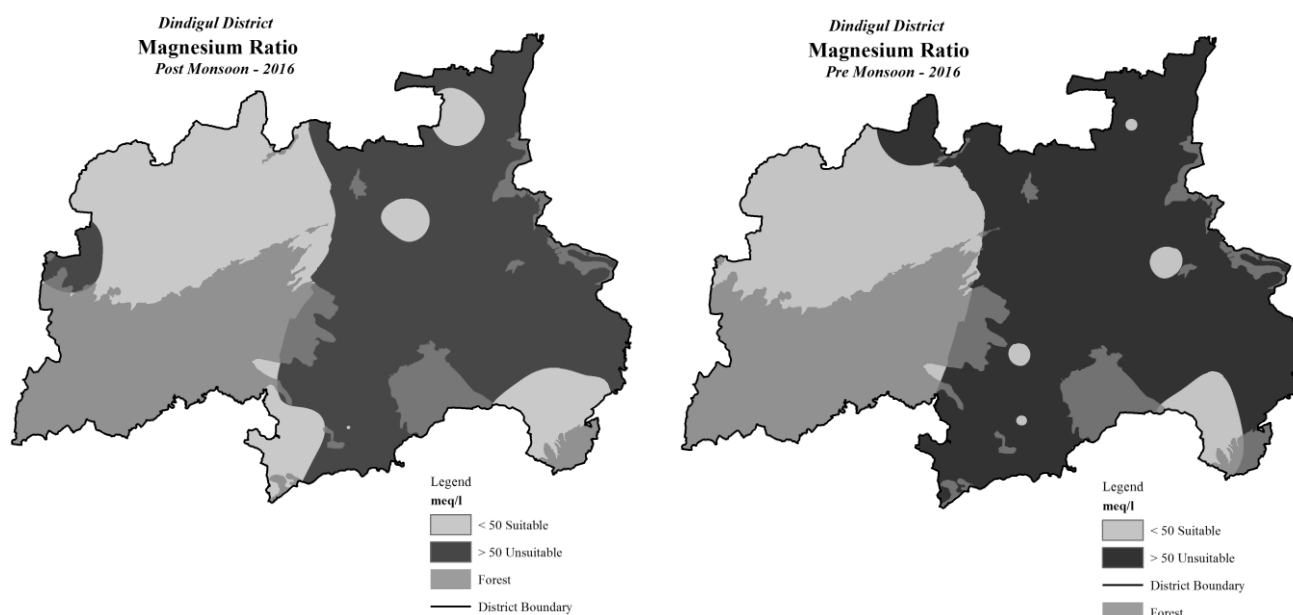


Fig. 8. Spatial distribution of Magnesium Ratio (MR)

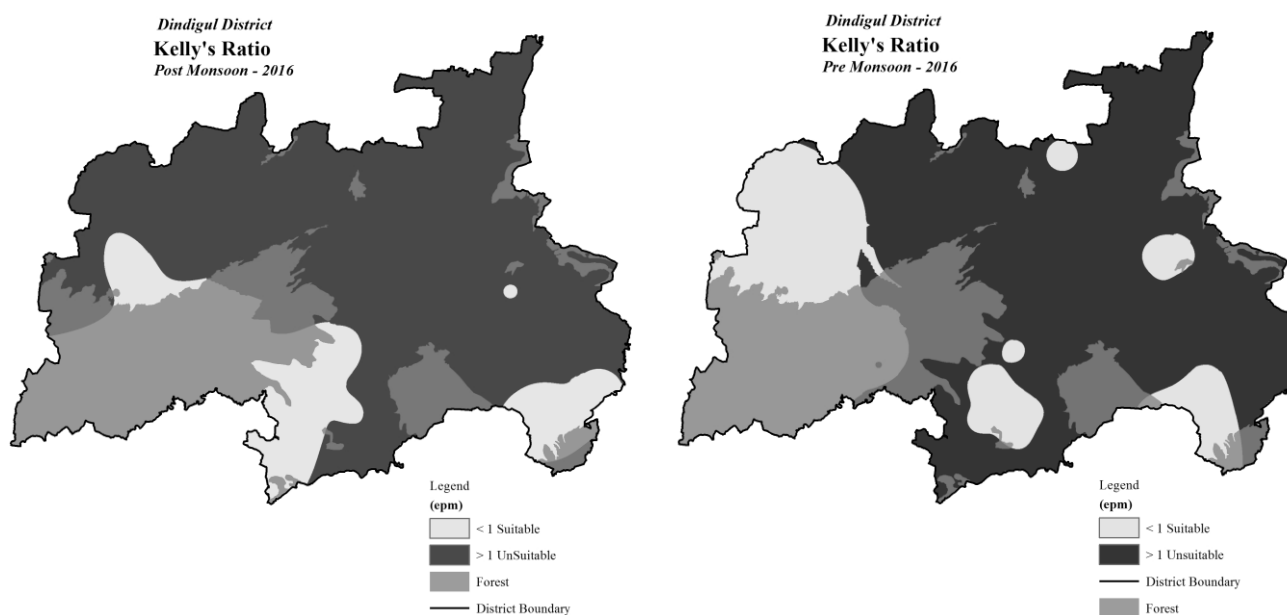


Fig. 9. Spatial distribution of Kelly's Ratio (KR)

CONCLUSION

The groundwater qualities of the study area are highly influenced by types of rocks and mineral's present in the area and anthropogenic activities. Based on Na (%) one sample location and in magnesium ratio (MR) 15 post monsoon and 17 pre monsoon groundwater sample locations are categorized under not suitable for irrigation. The SAR value is under safe for irrigation in all sample locations. The KR analysis of 17 samples in post and 16 samples in pre monsoon seasons are comes under the category unsuitable for irrigation the rest of the samples are comes under suitable for irrigation. The SSP value in both the monsoon seasons comes under the category suitable for irrigation. The western part of Dindigul district is marginally suitable for irrigation in both the monsoon season. The eastern part of the Dindigul district need to be practice soil remedial measures during crop production. The controlled or soil test based application of fertilizer in the crop field will also minimize the degradation of soil quality as well as the leaching of ions from the inorganic fertilizers. The effluents from quarry should be controlled or measures to be taken to protect effluent recharge in aquifers.

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Flood Inundation Mapping of Lower Godavari River Basin using Remote Sensing and GIS

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Abstract: River Godavari is one of the longest rivers in India, which faces flood almost every monsoon. To generate flood inundation map of lower Godavari River, SRTM DEM of 30m resolution, water level and discharge data are used. Hydrodynamic model is calibrated for the year 2010 and, the calibrated model is validated with the years 2011 and 2012. Percentage accuracy of simulated values show minimum of 89% to a maximum of 99% with respect to observed values.

Keywords: Hydrodynamic model, Flood inundation map, River Godavari, HEC RAS

Hydrodynamic modeling is done for Tunisia with the help of flow parameters, topography (Shuttle Radar Topographic Mission Digital Elevation Model (SRTM DEM)–30m resolution), hydrography network, cross section of the river. Different frequency distributions are used to fit the data, GEV shows the best fit (Ben and Saidi 2018). Flood inundation maps are created for two reaches, Strouds Creek of North Carolina and Brazos River of Texas. USGS 30 m, 10 m DEM and Light Detection And Ranging (LiDAR) DEM, and cross section data are utilized to create flood inundation mapping and it is proved that the usage of LiDAR DEM increases the accuracy to generate flood inundation map (Cook and Venkatesh 2009). Simulation of Steady and Unsteady flow analysis in Hydrologic Engineering Center's River Analysis System (HEC RAS) can also be automated with MATLAB scripts (Leon and Christopher 2016). A road network is been proposed to pass the path of flood in Assiut plateau in west bank of Nile valley. Stage-Flow data and DEM 30m are utilized to run steady flow simulation in HEC RAS to identify the flood depth (Ezz 2017). Two-dimensional hydrodynamic model is carried out in Tarland Burn catchment of northeast Scotland using a computational model called TUFLOW and LiDAR DEM is used as an input for the model and flood frequency analysis is performed using a statistical tool called WINFAP-FEH. One in hundred year flood inundation map is created as a result of the work (Logah et al 2017). Hydrodynamic modeling using HEC-RAS is done for a part of Volta River basin to study the impact of dam release. Flood plain geometry and elevation data are main inputs and other inputs are cross section details and sediment data are utilized for the simulation (Mohammadi 2014). Flow data and DEM are used as input for Hydro dynamic model (Moya et al 2016). Flood damage assessment is been done for Neha

River Basin using HEC FDA with the help of the results generated in HEC RAS. HEC-RAS is been used for flood simulation of Llanos de Moxos flood plains in Bolivian Amazonia using flow data and SRTM DEM of 90m resolution. This flood simulation results presented some additional information like flood depth, flood duration and flow velocity (Shrestha et al 2010). Flood from glacial lake of Sun Koshi basin of Hindu Kush of Himalayas region is assessed with HEC RAS flood modeling. Over 30 such scenarios are analyzed and inundation maps are generated in the study. Glacial Lake Outburst Flood risk assessment is also performed using topographic maps, aerial photographs, satellite imageries (Sindhu and Rao 2017).

Hydrologic as well as hydrodynamic modeling is been done for Brahmani-Baitarani Basin using Hydrologic Engineering Center's Hydrologic Modeling System (HEC HMS) and HEC RAS. The input data are CARTOSAT DEM of 30m, soil textural data and gauge discharge data (Wang et al 2018). One of the simulation models of HEC RAS is RVSM (Riparian Vegetation Simulation module). HEC RAS model is also useful for some other applications like – a simulation module to find ripen vegetation using spatial data (i. e) to know the vegetation growth (Wang et al 2018). Hydrodynamic modelling map is created using DEM created using Cartosat 1 stereo data and discharge data for Subarnarekha River Basin. HEC RAS simulation software is used to create Flood Inundation map (Yarrakula et al 2010). This study presents a flood inundation map for Adayar river , Chennai and flood simulation using HEC-RAS is done for three different discharges, which are actually recorded in past flood events and more than 85% of accuracy is achieved (Vasanth and Bhagavanulu 2007). Based on the literature survey undergone, a methodology is introduced to create a

flood inundation map, which is supportive for assessing the flood-affected areas along the downstream of River Godavari from Perur to Polavaram gauging stations, where the flood hits ruthlessly.

MATERIAL AND METHODS

Study area and data used: River Godavari originates from Nashik, an Indian state of Maharashtra and drains into the Bay of Bengal. The study area is downstream of Godavari River, lies within longitude of 79.84° E to 82.00° E and the latitude of 17.00° N to 18.71° N. Lower part of Godavari flows through three states namely Andhra Pradesh, Chhattisgarh and Odisha. Other gauging stations within the extent of the study area are Dummagudem, Bhadrachalam, Kunavaram, Konta, Koida and Polavaram. Konta is a gauging station lies on the River Sabari, which is the tributary of Godavari (Fig. 1) and drains into the lower Godavari. The gauge water quality sediment and discharge data are acquired from central water commission (CWC), Hyderabad, Telangana State (Table 1). To generate the geometric layers such as river, river banks, flow paths, cross section cutline, left over bank, right over bank etc., SRTM DEM of 30m is used (Table 2). Considering hundred days of data from 6th July to 13th October, the present study is carried out to identify the regions that are prone to flood. Very high rainfall is recorded in all corresponding gauging stations for three years such as 2010, 2011 and 2012 between 6th July to 13th October. The water level, discharge data of 2010 is used to calibrate hydrodynamic model and calibrated model is validated with the data of years 2011 and 2012. After removing all the inconsistencies of data, only four gauging station such as Perur, Bhadrachalam, Konta and Polavaram data could be utilized to perform the unsteady flow analysis of hydrodynamic modeling. Hydrodynamic model is employed to study the movement of water along a river, river banks and its flow paths. In this study, a hydrodynamic model is created to generate the flood inundation map in the downstream of Godavari River. Initially, watershed boundary map is created with respect to study area DEM.

Hydrodynamic model: Flow of water in river follows two principle laws, conservation of mass and conservation of momentum, conservation of mass is represented as partial differential equation (Mohammed and Yuvjevich 1975) as equation (1) and (2).

$$\frac{\partial A_c}{\partial t} + \frac{\partial Q}{\partial x} + \frac{\partial S}{\partial t} - q_l = 0 \quad (1)$$

$$\frac{\partial Q}{\partial t} + \frac{\partial(VQ)}{\partial x} + gA \left[\frac{\partial z}{\partial x} + S_f \right] = 0 \quad (2)$$

Where, A is total flow area, Q is the volume of the flow, x is the distance along the channel, q_l is the lateral flow per unit length, t is time, S is storage from non-conveying portion of cross section, $\frac{\partial z}{\partial x}$ is water surface slope, z is water surface elevation, S_f is friction slope, A is total flow area, and V is velocity respectively. There is an exchange of momentum between the channel and floodplain. Using this inter-relationship, the continuity equation can be expressed separately in equation (3) and (4) for channel and floodplain (Brunner 2002).

$$\frac{\partial A_c}{\partial t} + \frac{\partial Q_c}{\partial x_c} = q_f \quad (3)$$

$$\frac{\partial A_f}{\partial t} + \frac{\partial Q_f}{\partial x_f} + \frac{\partial S}{\partial t} = q_c + q_l \quad (4)$$

Similarly, momentum equation is represented in equation (5) and (6).

$$\frac{\partial Q_c}{\partial t} + \frac{\partial(V_c Q_c)}{\partial x_c} + gA_c \left[\frac{\partial z}{\partial x_c} + S_{fc} \right] = M_f \quad (5)$$

$$\frac{\partial Q_f}{\partial t} + \frac{\partial(V_f Q_f)}{\partial x_f} + gA_f \left[\frac{\partial z}{\partial x_f} + S_{ff} \right] = M_c \quad (6)$$

Where c refers channel and f refers floodplain, q_c and q_f are the exchanges of water between the channel and the floodplain, M_c and M_f are momentum fluxes per unit distance exchanged between the channel and floodplain respectively. Digital elevation model of 30 m resolution from SRTM is utilized to extract the elevation values of the study area.

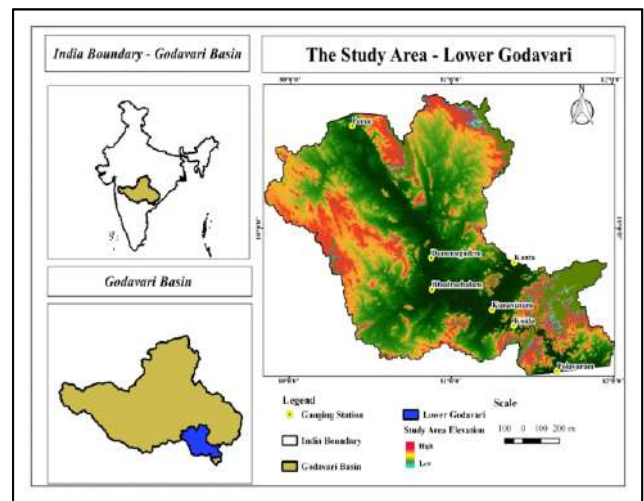


Fig. 1. The geographical location of study area – lower Godavari

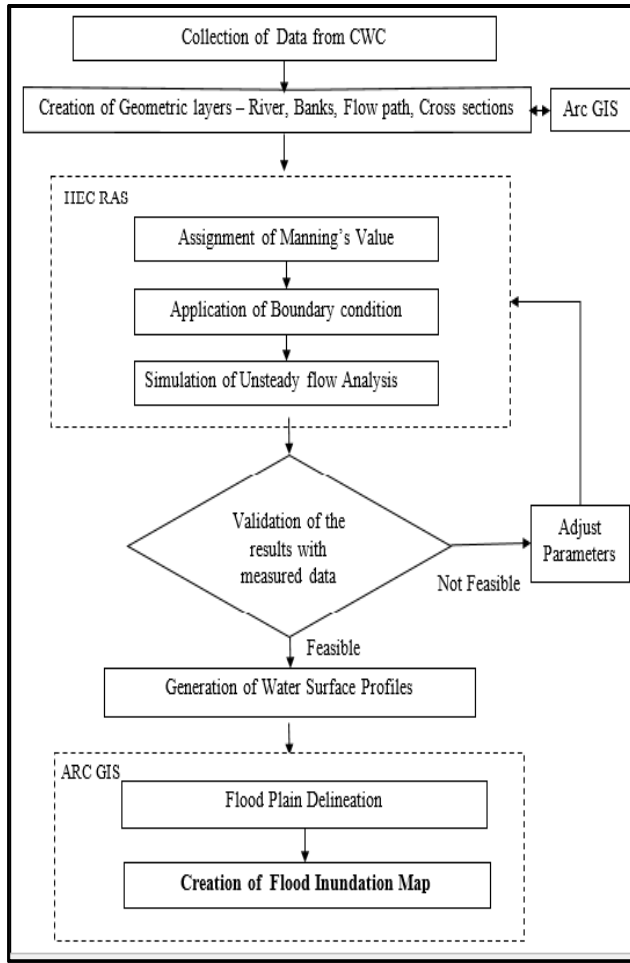


Fig. 2. Work flow of methodology

Geometric layers are created with the help of hydro meteorological data collected from Central Water Commission (CWC) (Fig. 2). Cross sections are extracted using HEC GeoRAS and SRTM DEM. Field measured data are compared to check the accuracy of extracted river cross sections. To simulate Hydrodynamic model, boundary conditions are applied using water level and discharge data to run unsteady flow analysis. Finally, the simulated model is calibrated and validated. The simulated result creates water surface profiles, on make use of it, flood plain extent is extracted. In addition to the inundation map, flood hazard mapping and further management plan can be framed according to affected areas.

The manning's roughness coefficient plays a vital role to calibrate and validate the model. In the present study, for channel and flood plain the manning's roughness coefficient are taken as 0.047 and 0.11 respectively. Equation (7) shows the calculation of discharge using manning's roughness coefficient .After applying boundary conditions, river cross sections adjustments, manning's roughness coefficient

adjustments, the unsteady flow analysis is performed to generate the water surface profiles. Calibration and validation is performed and error metrics are calculated to verify the performance of hydrodynamic model. After completion of all initial boundary conditions, the water surface profiles are exported to GIS platform to generate the flood inundation map. If the cross sections are not enough at the meander of the river, it is essential to interpolate the cross sections at an equal interval and it is necessary to edit cross section profile plots to make the simulation smooth. In the present study area a total of seven gauging stations are identified. Due to data discrepancy, four gauging sites are considered for unsteady flow analysis out of four, three gauging sites i.e. Perur and Konta are taken as inlets and Polavaram is taken as outlet. The analysis is performed for 100 days i.e. from 6th July 2010 to 13th October 2010. The daily data is used to run the simulation. 2010 data is used for the calibration purpose and the validation is carried out for 2011 and 2012 years.

$$Q = \frac{1}{n} AR^{2/3} S^{1/2} \quad (7)$$

Performance evaluation of hydrodynamic model: To assess the performance of hydrodynamic model, four error metrics namely Nash-Sutcliffe coefficient, Index of Agreement, Percentage of deviation in peak and R-Square are selected. Equations (7), (8) (9) and (10) show the calculation method of each of the method respectively.

Nash-Sutcliffe coefficient (E_{ns}): Nash-Sutcliffe coefficient is used to assess the performance of hydrologic models and its result ranges 0 to 1. If the predictive model shows '0' then the model is unfit and if it shows '1' then the model is perfectly fit

$$E_{ns} = 1 - \frac{\sum_{i=1}^n (X_{o,i} - X_{s,i})^2}{\sum_{i=1}^n (X_{o,i} - \bar{X}_o)^2} \quad (8)$$

Where, $X_{o,i}$ is observed discharge, $X_{s,i}$ is simulated discharge and the variables i to n carry the values from 1 to 100 which represent instances.

Index of Agreement (d_a): Index of agreement is another measure that shows how the model value is agree with observed value. It is also ranges between 0 and 1. Similar to Nash-Sutcliffe coefficient, values closer to '1' are preferable.

$$d_a = 1 - \frac{\sum_{i=1}^n (X_{o,i} - X_{s,i})^2}{\sum_{i=1}^n (|X_{o,i} - \bar{X}_o| + |X_{s,i} - \bar{X}_o|)^2} \quad (9)$$

Where, $X_{o,i}$ is observed discharge, $X_{s,i}$ is simulated discharge and the variables i to n carry the values from 1 to 100 which represent instances.

Percentage of deviation in peak: Percentage of deviation parameter shows the measure of deviation in peak discharge in percentage. If the percentage is less, then the model is good. It is preferable to get below 25%.

$$\text{Percentage of deviation in peak} = \frac{|X_o^i - X_s^i|}{X_o^i} * 100 \quad (10)$$

Where, $X_{o,i}$ is observed discharge, $X_{s,i}$ is simulated discharge and the variable i carries value from 1 to 100 which represent instances.

Coefficient of determination (R^2): R-Square measure shows how close the predicted value to observed value is. The suggested value is closer to 1.

Where, X_o is observed discharge, X_s is simulated discharge, $\bar{X}_o$ is mean of observed discharge values. The variables i to n carry 1 to 100 which represent instances.

$$R^2 = \frac{n(\sum X_o X_s) - (\sum X_o)(\sum X_s)}{\sqrt{[n\sum X_o^2 - (\sum X_o)^2][n\sum X_s^2 - (\sum X_s)^2]}} \quad (11)$$

RESULTS AND DISCUSSION

The observed and computed values are calibrated for the year 2010 with respect to the corresponding stations Perur, Konta, Bhadrachalam and Polavaram (Fig. 4, 5, 6). Calibrated as well as validated graphs are matched well in peak values. Nash – Sutcliffe coefficient (E_{ns}) gives the values between 0.79 and 0.96, Index of Agreement (d_a) gives the values between 0.92 and 0.99. The percentage variation in peak of the model is 0 to 24% and Coefficient of

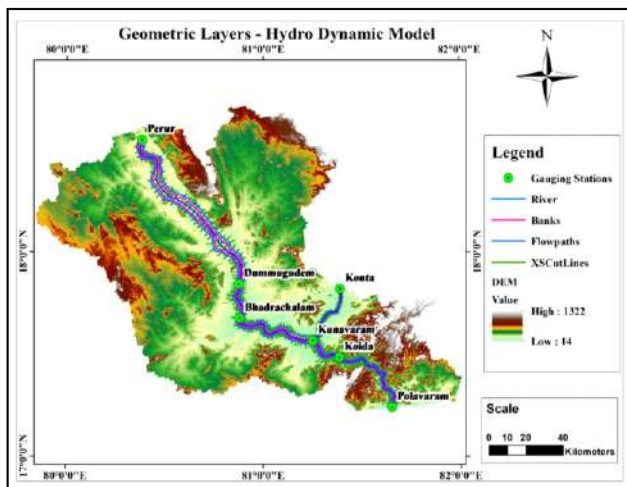


Fig. 3. Geometric layers - Stream central line, bank lines, flow paths and cross section cut lines

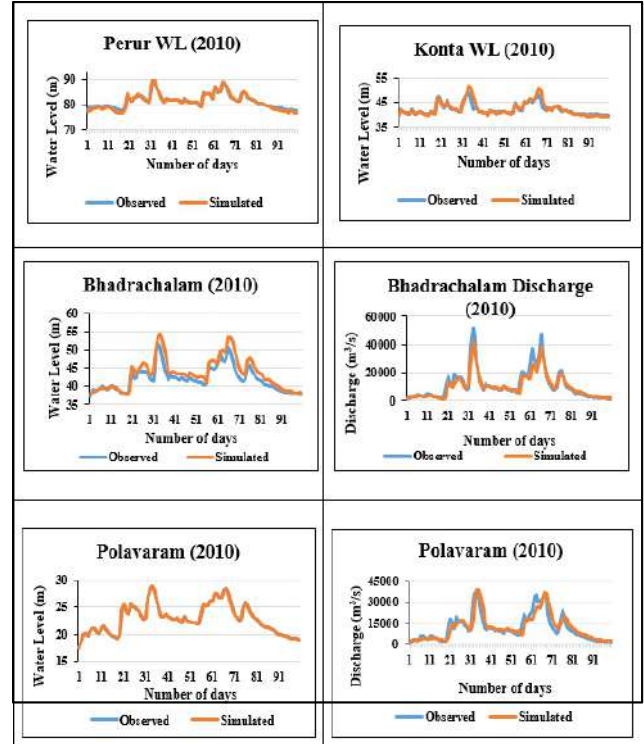


Fig. 4. Water level and discharge of simulated and observed values for the year 2010

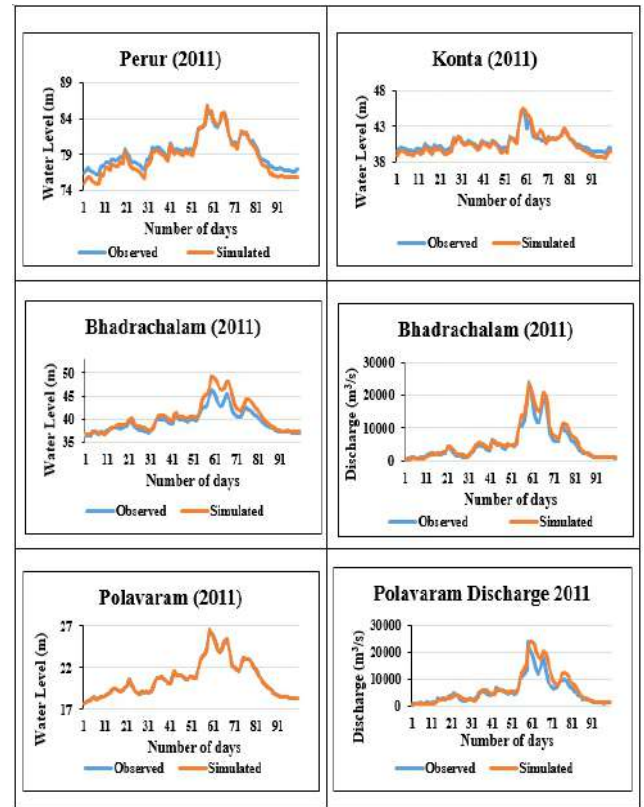


Fig. 5. Water level and discharge of simulated and observed values for the year 2011

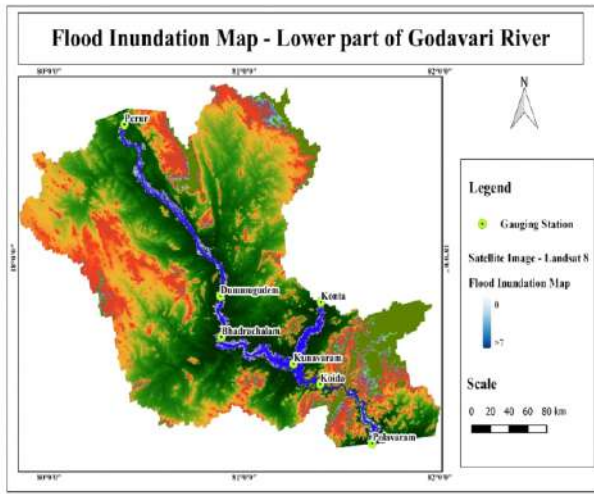


Fig. 7. Flood inundation map of lower Godavari River basin

Table 1. The gauge and discharge data of lower Godavari River

Gauging stations		2010		2011		2012	
		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Perur	Water level (m)	77.45	88.64	76.31	85.78	76.13	86.33
Bhadrachalam	Water level (m)	37.02	51.53	36.43	46.35	35.65	47.675
	Discharge ($\text{m}^3 \text{s}^{-1}$)	875.5	51444	547.795	24065.59	203.1411	28082.01
Konda	Water level (m)	39.72	49.02	39.36	45.39	38.9	47.38
Polavaram	Water level (m)	17.36	28.81	17.7	26.605	17.46	27.04
	Discharge ($\text{m}^3 \text{s}^{-1}$)	685.3991	39046.71	803.9469	23943.02	520.8561	26290.01

Table 3. Error Metrics for Perur, Konda, Polavaram and Bhadrachalam

Errors metrics		Nash-Sutcliffe coefficient	Index of agreement	Average percentage of deviation In peak	R-square
Perur (Water level)	2010	0.967	0.993	0.471	0.993
	2011	0.919	0.984	0.715	0.996
	2012	0.965	0.992	0.448	0.991
Bhadrachalam (Water level)	2010	0.652	0.936	3.398	0.925
	2011	0.643	0.939	2.383	0.976
	2012	0.596	0.927	3.336	0.958
Bhadrachalam (Discharge)	2010	0.917	0.974	16.757	0.957
	2011	0.953	0.989	15.640	0.977
	2012	0.938	0.985	18.002	0.957
Konda (Water level)	2010	0.797	0.96	1.252	0.900
	2011	0.820	0.965	0.998	0.956
	2012	0.859	0.971	1.109	0.946
Polavaram (Water level)	2010	0.999	1	0.008	0.999
	2011	0.999	1	0.009	0.999
	2012	0.999	1	0.009	0.999
Polavaram (Discharge)	2010	0.833	0.955	24.248	0.835
	2011	0.818	0.964	18.158	0.933
	2012	0.860	0.966	21.115	0.896

determination (R^2) value varies from 0.83 to 0.99. On analysing all these values of above error metrics, the results concluded that the presented model is preferred in predicting discharge values and the model is fit to produce flood inundation map.

Flood inundation map: Flood plain delineation process using raster creates flood-inundated extent. The result of the

Table 2. Data used to create geometric layers

Data	Resolution/Scale	Satellite/Source
Toposheets	1:50,000 Scale	Prepared by Survey of India
Multispectral image	30 m	Landsat 8
SRTM DEM	30 m	Shuttle Radar
Water level and discharge	-	CWC

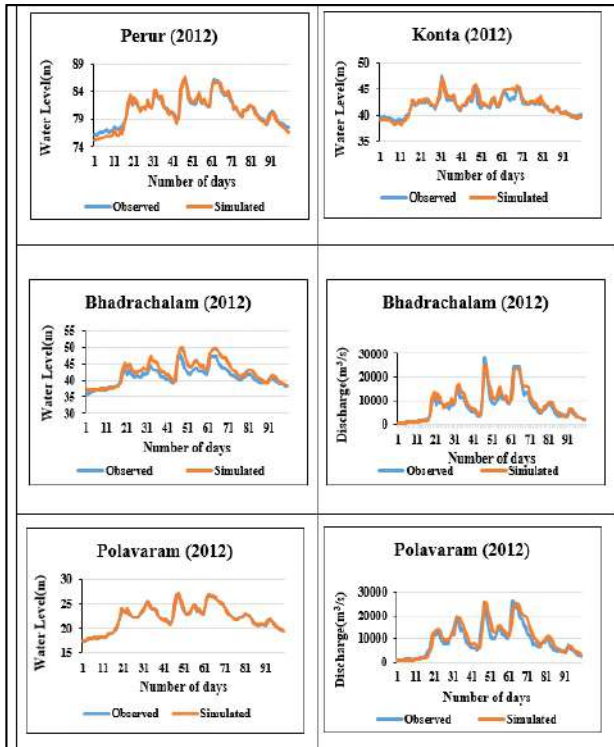


Fig. 6. Water level and discharge of simulated and observed values for the year 2012

simulation shows that the area around 467.20 km² on the banks of Lower Godavari are inundated. Konta is a gauging station located on Sabari, which is a tributary that drains in to Lower Godavari. At the junction where Sabari and main Godavari meets, the flow of water is more when comparing all other places and creates more damage. Especially in the year 2010, more discharge was identified in Bhadrachalam and Polavaram as 51444 m³/s and 390461 m³/s respectively. Therefore, flood inundation map is created for the year 2010 (Fig. 7).

CONCLUSION

The present work is an attempt to generate flood inundation map of lower Godavari basin. Remote Sensing and GIS are viable tools to generate flood inundation map, flood hazard map and to calculate damage assessment for each land use land cover. The error matrix shows maximum of 11% to maximum of 1% error between observed and computed values. Higher resolution of TanDEM,

CARTOSAT-1 DEM, LiDAR DEM can also be used to achieve higher spatial and temporal accuracy. The generated flood inundation map will be used to take right decision for administrators and to provide early warning to the people for reduction of loss of life and property.

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Delineation of Recharge Zones for Panapatti Watershed of Coimbatore District

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Abstract: The present study was undertaken to identify the favorable areas for artificial recharge and suggest suitable recharge structures to augment the aquifer system. The analysis was carried out in Kinathukadavu block of Coimbatore District using Remote Sensing data and GIS techniques. The various thematic maps such as geomorphology, geology, soil, slope, land use, drainage, drainage density, lineament density, runoff isolines, depth to weathered zone, depth to basement, groundwater level fluctuations and water quality were used in the analysis. The above maps were prepared using IRS-1C LISS III satellite data and other collateral information collected from the field and digitized. Criterion tables were generated considering the importance of different themes and necessary ranks and weights were assigned to each theme. Using ARC/INFO GIS software, the above themes have been integrated and the areas suitable for artificial recharge have been identified for Panapatti watershed of Kinathukadavu block of Coimbatore district. From the recharge zone map it was found that the entire area belongs to moderate zone of recharge. Considering the terrain conditions and favourable zonation, the suitable artificial recharge structures such as percolation pond, check dam, recharge pit, recharge shaft and recharge tube wells were recommended.

Keywords: Recharge zones, Thematic layers, Remote Sensing and GIS, Artificial recharge

Groundwater is a dynamic and replenishable natural resource, but in hard rock terrains, availability of groundwater is of limited extent. Occurrence of groundwater in such hard rocks is essentially confined to fractured and weathered horizons. As it is the largest available source of fresh water lying beneath the ground it has become crucial not, only for targeting of ground water potential zones, but also monitoring & conservation of this important resource. An integrated analysis of various parameters provides better estimate of subsurface characteristics from groundwater perspective (Sharmila et al 2017). The factors such as topsoil thickness, weathered zone thickness, anisotropy (formation compactness) and geology are considered, to determine the area most suitable for groundwater recharge in a hard rock aquifer in Vaniyar sub basin by using GIS environment (Kalphana et al 2018). Remote sensing and GIS techniques are increasingly used in ground water exploration and land cover change detection to explore the waterlogged area (Ayyanna et al 2019). The Ground Water Potential Zones in Kangshabati Irrigation Command Area (KICA) was delineated by overlaying the various thematic maps such as slope, relief, soil, geology, geomorphology, drainage, land use by implying weighted overlay methods (Sujit Mondal 2012). The utility and capabilities of a remote sensing data and GIS technique for demarcation of groundwater potable zones in the Madurai district was studied and demonstrated (Chidambaram et al 2010). The present work is an attempt towards this direction. The study focuses on development of

remote sensing and GIS based analysis and methodology in groundwater recharge studies in hard rock terrains. In order to demonstrate the integrated remote sensing and GIS based methodology has been taken.

MATERIAL AND METHODS

The selected area for study is Panapatti village part of Coimbatore district, TamilNadu with the areal extent of 4.79 sq km. This area comprises of 2 villages. The study area lies at 10° 53' to 10° 54'N latitude and 77° 5' to 77° 6'E latitude. The Revenue boundaries of the study area were plotted. The study area contains two villages namely Vadachitur and Panapatti. The total geographical area of the study region is found to be 4.79 sq km. The source maps like village, soil, land use, geology, geomorphology & lineament and watershed maps were collected from Institute of Remote Sensing, Anna University, Chennai, Tamilnadu. Toposheets were obtained from SOI, Guindy, Chennai, Tamilnadu. The source maps were scanned & geo-referenced using ArcGIS 9.2 software.

Soil: The hydrological soil group 'C' with slow infiltration and moderate runoff potential and hydrological soil group 'A' with high infiltration are found in the study area. The infiltration of rainfall depends upon the type and thickness of soil present. Hence, soil is also considered as one of the principle terrain parameters in the groundwater recharge site selection process. Six series of soils are available in the study area.

Land use/land cover: The study area consists of agricultural

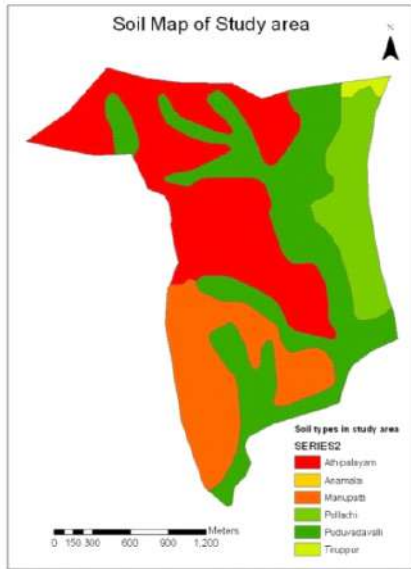


Fig. 1. Soil map

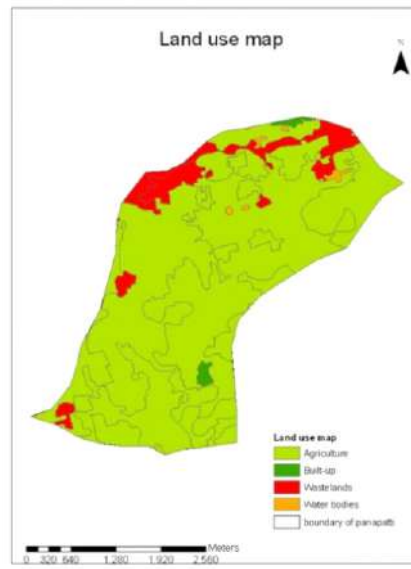


Fig. 2. Land use map

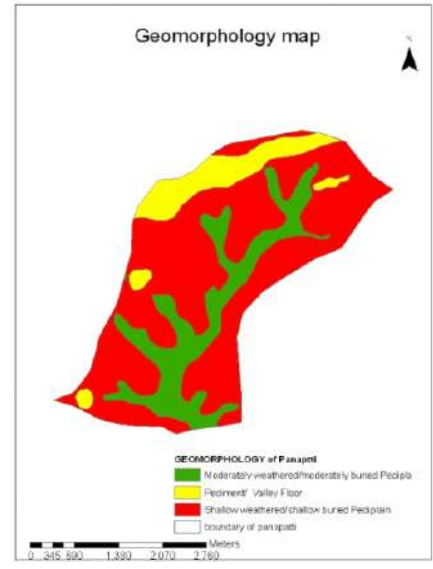


Fig. 3. Geomorphology map

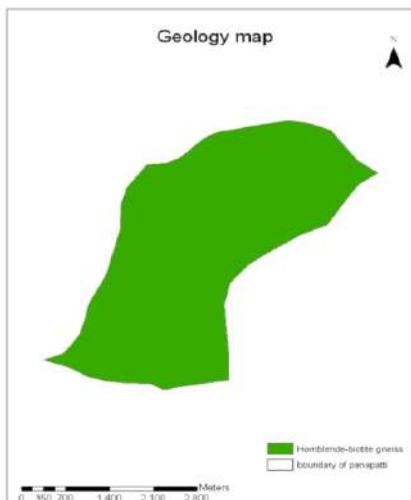


Fig. 4. Geology map



Fig. 5. Drainage map

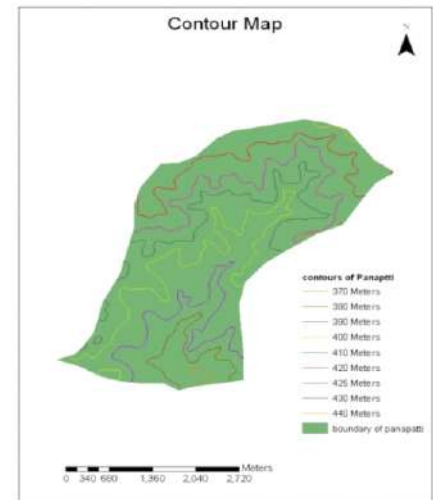


Fig. 6. Contour map

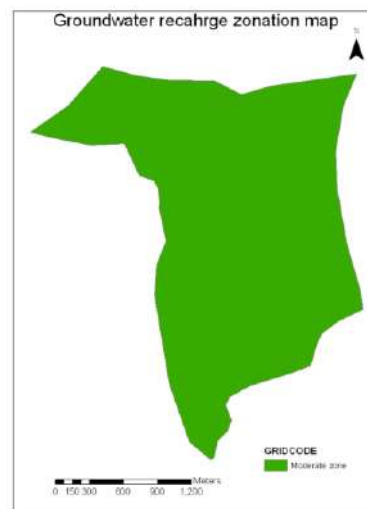


Fig. 7. Recharge Zonation map of Panapatti watershed

land, waste land, wasteland, water bodies and built-up lands. The land use/land cover map was derived from IRS, LISS – IV. Infiltration and runoff are greatly depending on land use/land cover. It is well known that recharge is high in the cultivable land and irrigated land compared to the wasteland and settlements. Hence, this theme is considered as the one of the principle terrain parameters in the recharge site selection. . It was found that 89 percent of the area covers agricultural land and remaining is built up lands and waste lands.

Geomorphology and geology: The predominant geomorphologic features are pediment, buried pediment deep, buried pediment shallow and valley floor. Hornblende Gneiss are the main geological features that are found in this watershed. The predominant geomorphologic features are found to be shallow weathered pediplain, Moderately pediplain and Pediment respectively. The geology features that are found in the entire study area are Biotile.

Drainage distribution pattern: The drainage network tanks were digitized and converted into whole single thematic map. In this region the drainage distribution pattern were identified as 1st, 2nd, 3rd order streams.

DEM and contour map: The contour map (10 m contour interval) was obtained with the contours range of 370 m to 440 m. From the interpolation of contour lines the DEM was obtained as depicted .The highest elevation shows the terrain of the study area. From the DEM, the slope (%) was derived with the range of 0-5%.

RESULTS AND DISCUSSION

The suitable recharge zones for the Panapatti watershed was identified via seven parameters adjoining with the technology of Remote Sensing and GIS. The thematic layers were considered in this study are Soil, Land use, Geology, Geomorphology, slope, lineament and drainage which were prepared using the conventional data.

Weighted overlay analysis: In order to find out the effective recharge zones, the weighted overlay index was applied according to the priority of thematic layers in the study area. Weighted Overlaying Analysis is done by combining the data from the multiple data categories. During the process of overlay, the thematic maps and their attributes were intercepted and a new thematic map with attributes was obtained. The weightages were assigned according to their influencing characters viz., soil=15, Geomorphology = 25, Land use = 10, Slope =15, Lineament = 15, Drainage=15, Geology=5. During the weighted overlay analysis, the rank was assigned in terms of 1, 2, 3 & 4 for each individual parameter of thematic layers to obtain the recharge zones in terms of good, moderate, poor & very poor zones.

Ground water recharge zones: After the weighted overlay

analysis the groundwater recharge zones were obtained in terms of classified zones like good, moderate and poor depending on the potential influencing parameters of ranks and weightages of each thematic layer. The weightages of each criterion was finalized on the basis of the ranges of the maximum and minimum values within each theme. After the weighted overlay analysis the groundwater recharge zones were obtained (Fig. 7). From the recharge zone map it was found that 100 % of the study area falls in zones of moderate category only. There is no good zone and poor zone available in the study area. This composite map has been shown to very useful for selecting the sites of groundwater rechargeable zone. The result supports the prevalence of hard rock terrain, which is covering maximum areal extent of 90% of the study area. Based on this finding the artificial recharge structures were to be identified and implemented to the augment recharge of groundwater. Similar studies conducted in Vaniyar sub basin and the final integration map gives the zones of excellent and moderate zone for recharge and the suitable areas for the construction of artificial recharge structure (Kalphana et al 2018).

CONCLUSIONS

In order to identify the potential recharge zones of Panapatti watershed of Coimbatore district, various maps were obtained from different sources and integrated them into GIS environment for analysis. Thematic maps such as geology, geomorphology, land use/land cover, soil and slope map were prepared using Remote Sensing and GIS. Detail characteristics of each theme were studied in accordance with their response to artificial recharge process. After detail study and criteria, ranking and weightage applied for suitable site selection for artificial recharge process, weighted overlay analysis was carried out. Maximum weightage i.e., importance is given to theme, which is most favorable for groundwater recharge process and low weightage given to theme, which is less favorable for groundwater recharge process. For present study, importance were given in the order of geomorphology > geology > land use/ land cover > Hydrological soil group > slope > lineament > drainage. From the recharge zone map it was found that the entire study area belongs to moderate zone of recharge.

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Impact of Declining Groundwater Levels on River Flows in the Ganga Alluvial Plain-A Case Study of Gomti River, India

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Abstract: This study is carried out on the Gomti River Basin (GRB) in Uttar Pradesh, India – a sub-basin of Ganges, to understand if groundwater abstraction has affected the flow-regimes in the river. Gomti River is a tropical groundwater-fed alluvial river in which base flows form an important component of the overall flow. A large number of sampling points in the basin, i.e. data from 764 groundwater observation wells were computed separately for pre- and post-monsoon periods. The flow data from 1978 to 2015 is used to separate the base flow share from the river discharge. The results of the comparison of changes in groundwater depths and surface flow trends showed a decrease in groundwater levels in past years as well as an overall decrease in river flow. During the lean flow periods, the groundwater usage is highest in the basin, and base flow reduces due to which rivers are not able to meet the minimum flows requirements. This study suggests that river flow can be effectively restored by regulating the indiscriminate abstraction of groundwater in the basin and through promoting conjunctive use of surface and groundwater. The study also recommends that a component of groundwater recharge through water bodies should be included for base flow augmentation to maintain sufficient ecological flows in the river.

Keywords: Groundwater monitoring, Groundwater/surface-water relations, Over abstraction, River flows, Tropical river

Increasing water demands for domestic, agriculture and industrial purposes are resulting in over-abstraction of water from both surface and groundwater reserves. As a result, the overexploitation of freshwater resources is resulting in the drying of the rivers during lean flow seasons, thus converting the perennial rivers into intermittent rivers (Erlewein 2013). There is growing evidence that increased water abstraction for irrigated agriculture and other human uses along with climate change are affecting freshwater ecosystems, which is linked to the greater occurrence, duration, and frequency of river drying (Larned et al. 2010, Döll and Schmied 2012, Datry et al 2014). Urbanization and demographic pressures are resulting in deterioration of the inland water bodies (Vijayan et al 2017) as well as decrease in groundwater quality and course of natural drainage (Singh et al 2013). Intermittent rivers are defined as flowing occasionally and can be dry during the dry season or for several consecutive years; in contrast perennial rivers contain water throughout the year (Niasse et al 2004, Niasse 2005, Chevallier et al 2010). Most of the tropical rivers are perennial rivers, but due to over-abstraction of groundwater and intensive irrigation from canals withdrawing water from these rivers is resulting in diminished river flows and turning them into intermittent rivers (Rushton 2002, Kirk and Herbert 2002). For several rivers in the Ganga Alluvial Plain, base flow provides the majority of flow. During summer month base flow could provide the entire flow in the river. Therefore, understanding these flows is very important in managing overall ecological

flows. The groundwater recharge can be done through managing the traditional water sources such as wells (Kumari et al 2014) as well as through inland water bodies such as lakes and ponds which can augment base flows in lean flow seasons. The case study chosen to illustrate the effects of over-abstraction of groundwater on river flows is of Gomti river, a tributary of Ganges. This study is done to understand the effects of groundwater depletion on the river flows by observing the groundwater trends in the river basin and comparing it with the change in flow-regimes over three decades.

MATERIAL AND METHODS

Area of study: Gomti River, a major tributary of Ganges, is a groundwater-fed perennial river flowing in the interfluvial areas of Ganga and Ghaghra rivers in the northern part of the Indian sub-continent. Gomti River Basin (GRB) experiences a humid subtropical climate with hot summers followed by intense monsoon precipitation and cold winters. Gomti River is 941 Km long, originating from 'Fulhar Jheel' – a swampy area in the Piedmont Zone in district Pilibhit near Puranpur in Uttar Pradesh (Dutta et al 2015). This river flows through 14 districts, originating in Pilibhit district and meeting river Ganges in Ghazipur district near Kathi at 62 meters above mean sea level. The river through its course is continuously fed by freshwater and sediments from various small and major tributaries (Fig. 1). The total basin area drained by the Gomti River is 31009 sq. Km with low-gradient of about

0.10m per kilometer. Gomti River is a well-established channel and is highly sinuous river with a sinuosity index of 1.97. However, during last two years the flow during the summers declined considerably. The basin gets 87 to 125 cm of rainfall during monsoon months (June to September). This river has a sluggish flow round the year except for monsoons, where its flow increases 15 to 50 times due to heavy rainfall in the basin (Dutta et al 2015). For the first time, the upper stretch of the river for about 55 km has dried up (Fig. 2). Many of the tributaries of the river have dried up during past few years and only have water during monsoon and post-monsoon seasons.

Groundwater data collection and analysis: The pre- and post-monsoon groundwater depth data from 1984 to 2015 was obtained from the State Groundwater Department, Government of Uttar Pradesh, India. The data was collected for 764 observatory wells and piezometers distributed randomly throughout the basin for the year 1984, 2005, 2010, and 2015. Cartosat-1 satellite imagery was used to prepare the drainage map of the basin. ERDAS Imagine software was used for geometric rectification and registering the images with the Survey of India topo-sheets on 1:50,000 scale. The groundwater depth data and the satellite images were used to prepare the potential groundwater zone maps. These maps were analyzed to evaluate the groundwater dynamics and trends in past years, and their correlation with the river flows. Based on depth, groundwater levels were categorized into six sub-categories ranging between 0-3mbgl (meter below ground level) to 18-25mbgl from 2005 to 2010. Additionally, these six categories of groundwater depths were re-arranged into four categories and compared with 1984 water levels; to assess the long-term changes in the groundwater levels.

The map preparation and data analysis were done using ArcGIS 10.3 software. Geo-statistical interpolations were done using inverse distance weighted interpolation (IDW) (Xie et al 2011). This method is based upon the assumption that the surface is being driven by the local variation, which can be captured through the neighborhood as well as things that are close to one another that are more alike than those that are farther apart. IDW assumes that each measured point has a local influence that diminishes with distance. It weighs the points closer to the prediction location higher than those farther away, thus named as the inverse distance weighted. The surface calculated using IDW depends on the selection of a power value (p) and the neighborhood search strategy. IDW was chosen to conduct this study due to its simplicity and accuracy in providing accurate interpolation (Xie et al 2011). The data structure of this study suits the requirements of this interpolation method, i.e. well distributed

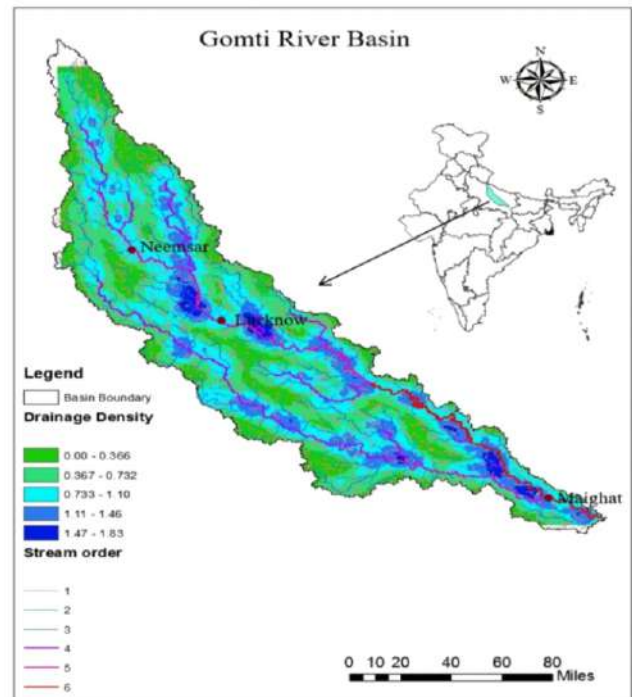


Fig. 1. Study area GRB showing three gauge sites and different stream orders



Fig. 2. Dry channel as a new trend in groundwater-fed rivers - drying of the Gomti river in the upstream as seen during July 2019. The first picture is of 2018 monsoon

and a large number of sampling points (Rabah et al 2011). The analyst model of ArcGIS 10.3 was used to obtain the distribution characteristics of groundwater level parameters. Cross-validation of the interpolation model was also done with the help of a statistical analysis method to verify the accuracy of interpolation model (Dashtpajardi et al 2013, Xiao et al 2016). The R^2 of IDW interpolation was 0.97. The values of pre and post-monsoon groundwater levels observation points for ten years were imposed on vector data of GRB to delineate the potential groundwater zones. The results of these observation points show significant variation between pre and post-monsoon seasons (Rajagopalan and Lall 1998). In case of outliers, the mean error and coefficient of determination of IDW model are dependent on number of samples. A large number of sampling points, i.e. 764 groundwater sampling data, was computed separately for pre- and post-monsoon periods.

Flow data collection and analysis: The river flows data were obtained from Central Water Commission (CWC), Government of India from the year 1978 to 2015 for three gauge sites Neemsar, Lucknow, and Maighat. Using the discharge data the hydrographs were prepared; the duration of the discharge data received for the three sites was divided into four periods, viz. 1978-1988, 1989-1999 to 2000-2015. Further, the hydrographs drawn for selected periods were compared to evaluate the trend in the flow regime of the river during the past years.

The flow data from 1978 to 2015 is used to separate the base flow share from the river discharge. For the computation of base flow, the BFI+ model of hydro-office was used (Gregor 2010). There are multiple methods for the base flow separation, such as graphical separation methods, filtering separation methods, frequency analysis methods, and recession analysis methods (Cheng et al 2014, Thomas et al 2015, Jung et al 2016). For this study, the sliding interval method-a sub-type of filtering separation method was used for the base flow separation. This method is selected among other base flow separation methods as it has the lowest error rate as compared to all of hydrograph base flow separation methods (Mohammadlou and Zeinivand 2018). In this method, the lowest discharge in half of the interval minus one day $[0.5(2N^*-1) \text{ days}]$ is taken before and after the day being considered, which assigns this value to that day (Gregor 2010). These values are connected to give base flow hydrograph as shown in Figure 3.

RESULTS AND DISCUSSION

Groundwater contribution in river flow: The analysis showed that the river has a base flow index of 0.78, 0.76, and 0.69 at Neemsar, Lucknow, and Maighat, respectively. The

base flow contribution evaluated is highest for upstream stretch as compared to the midstream and downstream stretch of the river (Fig. 4).

Groundwater trends in the basin: The seasonal variations were visible in the groundwater zone maps prepared by the interpolation method (Table 1 and Figs. 5, 6, 7). The pre-monsoon data of the year 2010 showed an increase in the basin area having groundwater depth between 0–3 mbgl to 2.03 per cent from 1.96 per cent as observed in 2005, whereas in 2015, this area decreased to 1.54 per cent. In post-monsoon season, a constant decrease is observed from 2005 to 2015, i.e., from 26.05 per cent of the basin area in 2005 to 20.57 per cent in 2010 and finally to 5.49 per cent in 2015. It indicates the loss of shallow groundwater during this period from 2005 to 2015. In 2005 pre-monsoon, 30.57 per cent of the basin area had groundwater between 3–5mbgl, which decreased to 27.44 per cent in 2010, and further, it increased to 34.76 per cent in 2015. Similar trends were observed in post-monsoon of 2010 – a decrease from 2005 levels and an increase in 2015 from 40.67 to 30.42 per cent and further to 45.17 per cent of the basin area, respectively. Overall the basin area having groundwater depth up to 5mbgl declined by 16 per cent between the post-monsoon of 2005 and 2015. Further, moving towards deeper aquifers, a marginal decrease of 1.70 per cent coverage area is observed in 5–8mbgl category between pre-monsoon of 2005 and 2015, and an increase in area by 11.78 per cent is observed during the post-monsoon season between 2005 and 2015. A declining tendency in the basin area having

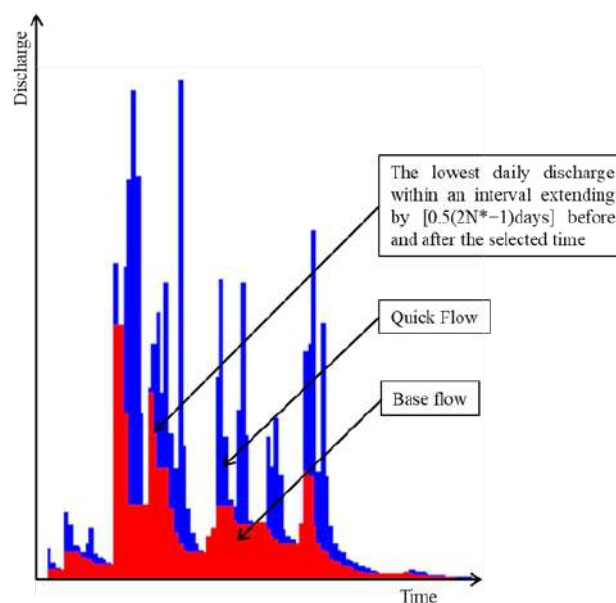


Fig. 3. Schematic diagram of the sliding interval method for base flow separation from hydrograph (adopted from Gregor 2010)

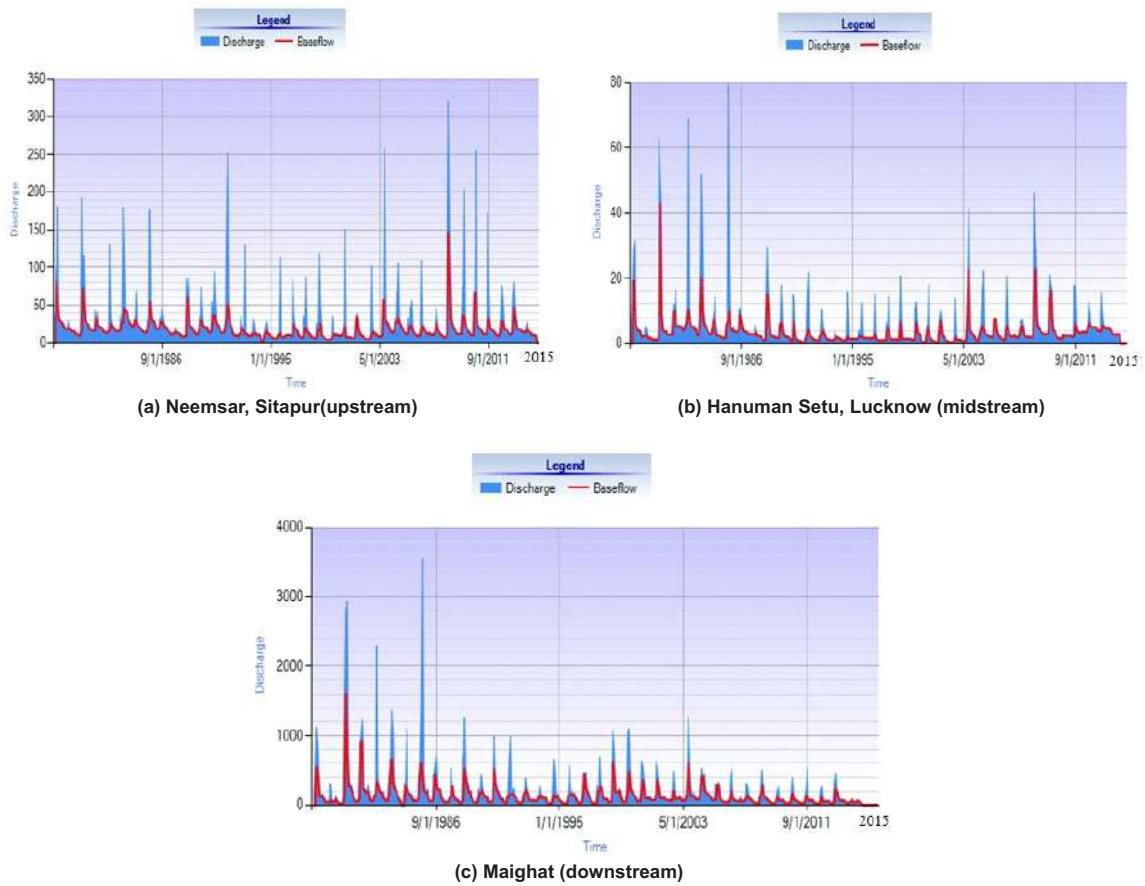


Fig. 4 a,b,c. Graphs showing the base flow contribution at Neemsar, Lucknow, and Maighat respectively

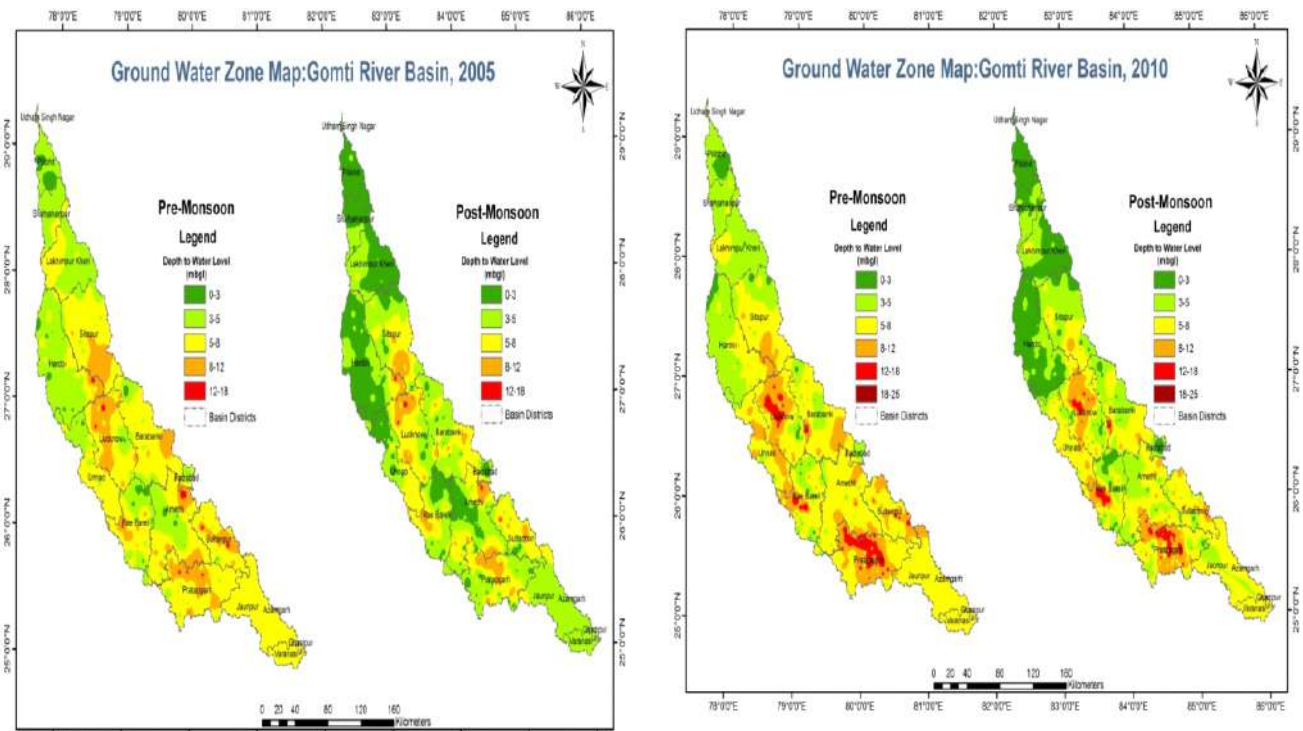


Fig. 5. Groundwater Zone map of GRB in pre- and post-monsoon, 2005

Fig. 6. Groundwater Zone map of GRB in pre- and post-monsoon, 2010

groundwater depth of 8–12mbgl is observed in pre-monsoon, i.e. from 13.35 per cent in 2005 to 8.98 per cent in 2015. The post-monsoon trend in this period shows a marginal gain in coverage area by 2.04 per cent. Further, 0.38 per cent of the area had groundwater depth in the range of 12–18mbgl in pre-monsoon of 2005, which increased to 2.04 per cent in 2015 indicated a declining tendency. The post-monsoon trend also exhibits declining tendency in this range between 2005 and 2015. In 2005, no area was found to have groundwater table more than 18mbgl both in pre-and post-monsoon seasons, however in post-monsoon of 2015, around 4.15 km² of area came under this category, indicating lowering of groundwater tables.

The spatial distribution of groundwater from 1984 to 2015 shows an overall decrease in groundwater level in GRB (Table 2). The probable reason for this decrease is intensive agriculture and the rapid increase in urban and peri-urban areas in the basin. The basin area having a groundwater level above 5mbgl was 57.99 per cent in pre-monsoon of 1984 which decreased to 37.3 per cent in 2015, showing an overall decrease of 35.67 per cent. This shallow water is the most exploited zone of aquifer in the basin. For the same range of water depth above 5mbgl, 90.8 per cent of basin area in 1984 decreased to 50.66 per cent in 2015. It indicates the declining tendency of water table in both pre- and post-monsoon. The deeper water tables are being exploited due to higher

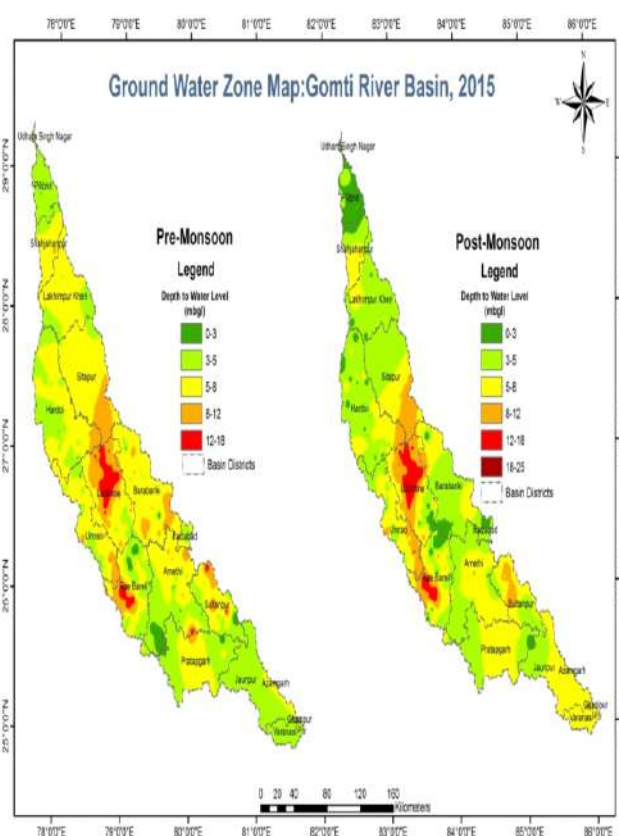


Fig. 7. Groundwater zone map of GRB in pre- and post-monsoon, 2015

Table 1. Trend of groundwater depths in the Gomti River Basin and its coverage in the pre- and post-monsoon of the year 2005, 2010 and 2015

Colour Mbgl code	Pre-Monsoon 2005		Post-Monsoon 2005		Pre-Monsoon 2010		Post-Monsoon 2010		Pre-Monsoon 2015		Post-Monsoon 2015	
	Area (km ²)	Per cent	Area (km ²)	Per cent	Area (km ²)	Per cent	Area (km ²)	Per cent	Area (km ²)	Per cent	Area (km ²)	Per cent
< 3	605.78	1.96	8017.12	26.05	627.46	2.03	6330.807	20.57	475.26	1.54	1690.01	5.49
3-5	9407.39	30.57	12515.55	40.67	8445.29	27.44	9361.741	30.42	10695.88	34.76	13900.15	45.17
5-8	16528.15	53.72	8040.31	26.13	15559.51	50.57	11303.24	36.73	16005.67	52.02	11663.98	37.91
8-12	4107.41	13.35	2137.243	6.94	5180.92	16.83	3195.65	10.38	2962.27	9.62	2765.53	8.98
12-18	118.00	0.38	56.52	0.18	946.67	3.07	571.42	1.85	627.65	2.04	742.90	2.41
18-25	-	-	-	-	6.87	0.02	3.87	0.01	-	-	4.15	0.01
Total	30766.74		30766.74		30766.74		30766.74		30766.74		30766.74	

*mbgl (Meter Below Ground Level)

Table 2. Spatial distribution of groundwater level in the GRB

Water depth range (mbgl)	Pre-monsoon area coverage (%)				Post-monsoon area coverage (%)			
	1984	2005	2010	2015	1984	2005	2010	2015
0-3	5.73	1.96	2.03	1.54	45.35	26.05	20.57	5.49
3-5	52.26	30.57	27.44	34.76	45.45	40.67	30.42	45.17
5-8	38.12	53.72	50.57	52.02	8.92	26.13	36.73	37.91
>8	3.89	13.35	16.83	9.62	0.28	6.94	10.38	8.98

irrigation demand fuelled by diesel and electric pumping sets, and this is resulting in the higher extent of basin area went into deeper groundwater levels. However, the spatial variability of the groundwater levels is not only affected by anthropogenic activities but also by the geo-genic activities, while the nugget effect reflects the spatial association (Ahmadi and Sedghamiz 2007, Xiao et al 2016). Factors such as precipitation, topographic differences, aquifer lithology and different hydro-geological settings, presence of natural recharge structures, and growing demand for drinking, irrigation, and industrial needs affect the local differences in the rate of groundwater decline.

Surface water trends in the basin: The comparison of groups of the hydrograph (Fig. 8) showed a constant decrease in the river flows in the lean period of flow. An increase in the water flow during lean flow periods at Lucknow is observed. The probable reason for this could be the channelization due to riverfront development and impounding of water in the channel due to the rubber dam downstream of the Lucknow city. During 1978-1988, the average peak flow recorded at the three gauging sites in

September was 96.26, 351.10, and 1175.2 cusecs, respectively, which has decreased to 86.88, 132.59, and 618.01 cusecs during 1984-1999. In the past five years, the average peak flow has decreased further to 55.68, 121.41, and 266.81 cusecs at Neemsar, Lucknow, and Maighat, respectively. In May, the average lowest flow recorded at Neemsar, Lucknow, and Maighat was 16.08, 31.99, and 51.07 cusecs during 1978-1988, which was recorded 9.27, 15.33 and 38.18 cusecs during 1989-1999 for the three gauging sites respectively. During the past five years the flow recorded showed a nearly constant flow of 44.45 cusecs during the non-monsoon months for Lucknow site, however for Neemsar and Maighat it was recorded 11.95 and 30.11 cusecs respectively.

Relationship between groundwater abstraction and river flow:

The effects of the increase in pumping within a few hundred meters of a river are visible over a small period, i.e. in a year or two. This phenomenon is composed of two components, an interception and induced recharge (Fig. 9). Interception is groundwater flow that was on its way to the river and is diverted into the well, and the induced recharge is water in the river, which is induced to infiltrate the aquifer

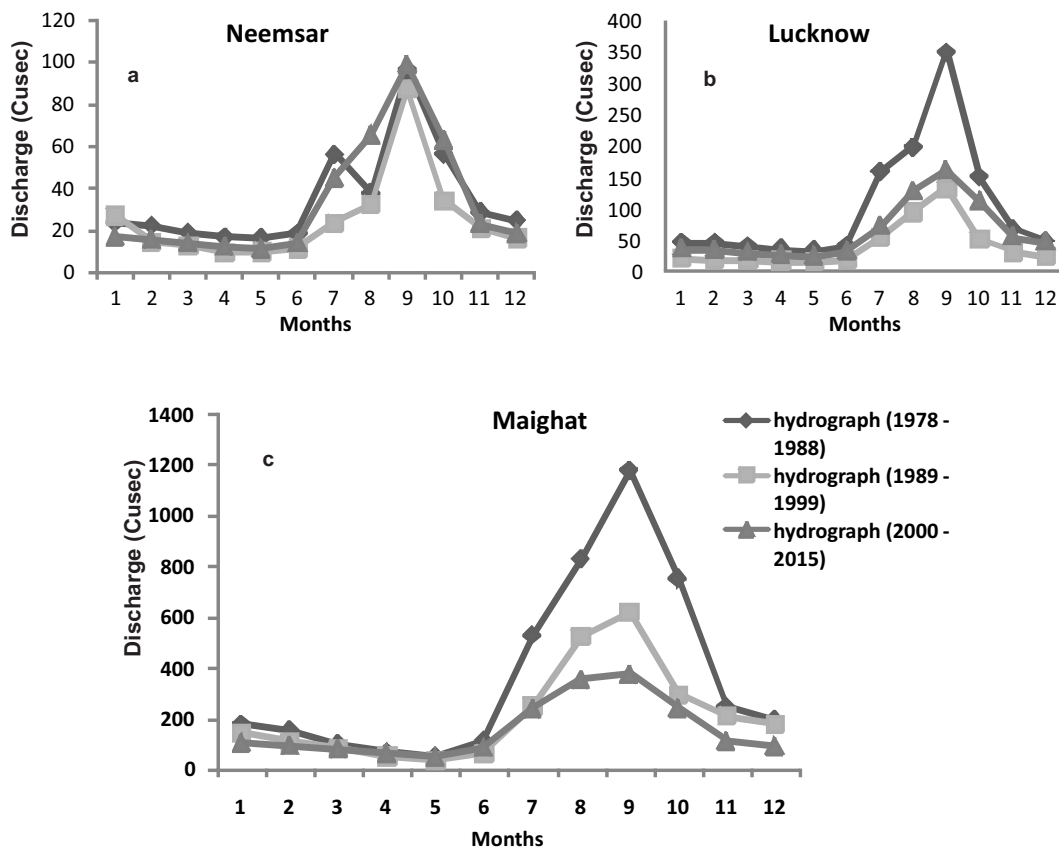


Fig. 8. Hydrograph showing flow trend at the gauged sites, (a) Neemsar, (b) Lucknow, and (c) Maighat

through the riverbed by a hydraulic gradient to the well generated by pumping (Waco and Taylor 2010). Pumping done close to the river will obtain water from combination of these components, whereas, a well further away will obtain its yield only by interception. Pumping from wells situated close to the river diverts the groundwater flow into the well that was previously feeding the river (Butler et al 2001). As a result, the groundwater-fed perennial rivers dry up in low flow seasons when water table is low. Due to this, perennial river turns into an intermittent river or virtually dries up in extreme conditions.

The aquifer transmissivity and specific yield (unconfined aquifer) or storativity (confined aquifer) are the characteristic features of an aquifer over which the aquifer to river-aquifer flows responses depends (Parkin et al 2007). A river might be gaining or losing river depending on the hydraulic connection, river stage elevation, and groundwater head (Fig. 10). The river is a losing river just after the monsoon period, whereas the river is a gaining river in the lean flow seasons when the flow is augmented from the groundwater in the form of base flow. Due to over-abstraction, the

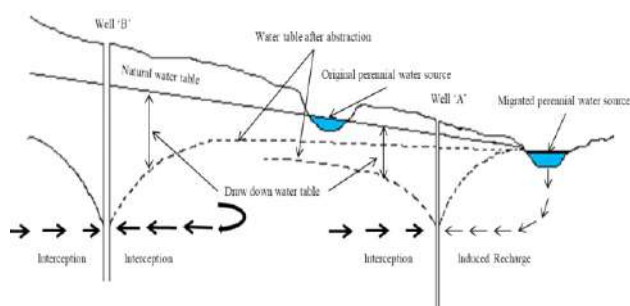


Fig. 9. Schematic cross-section of wells pumping along the groundwater-fed river

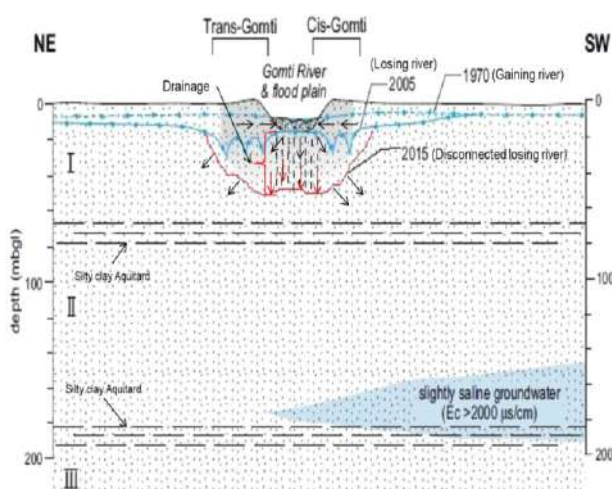


Fig. 10. The aquifer and river-aquifer interactions in Lucknow- middle stretch of GRB

groundwater head falls below the base of the river and the connection between the river and the groundwater breaks. This results in drying of the river as loss of water from the river will not correspond to increase in the groundwater head further.

Upstream region: As per the groundwater trends, there is a constant decrease in the groundwater levels in the district Pilibhit, Shahjahanpur, Lakhimpur, and parts of Sitapur and Hardoi of GRB during the past 30 years. The water level has shifted below 5mbgl in these areas with this subsidence in the groundwater, the surface flow in the stream has also subsided. The average lean period flow in the river has decreased from 16 cusecs from 1978-1988 to 12 cusecs in 2011-2015 for April and May.

Midstream region: The condition of groundwater and surface water is in a bad state in the middle stretch of the river basin as the groundwater table has declined in post-monsoon seasons in the past years. The water level recorded in post-monsoon showed that most of the areas have water level below 3mbgl, which has constantly subsided in the past ten years to 12mbgl or lower in Lucknow and Raibareli districts. Simultaneously the surface flow has also decreased from 198 cusecs and 351 cusecs during 1978 and 1988 to 127 cusecs and 121 cusecs during 2011 and 2015 in August and September. Due to impoundment and construction of water storage structures, a constant flow is observed during the lean flow seasons in the middle stretch of the river.

Downstream region: The records of groundwater availability in this region indicate a sharp decline in the groundwater table from 1984 to 2010. However, the groundwater availability of above five mbgl has increased from 2010 to 2015. It indicates groundwater recharge in the downstream region in the past 5 years.

Gouti River: Perennial to intermittent river: Gouti River is a groundwater-fed river, receiving flow from rainwater in monsoon and base flow from groundwater in lean flow periods. The over-abstraction of groundwater in the GRB is affecting the river's natural flow regime. Gouti River is fed by 26 small and large tributaries, out of which 23 have gone dry in the lean flow season during 2018. Similar effects of over-abstraction of groundwater on the surface flow were presented by Acreman et al (2000) in a study done on rivers and wetlands of the UK. The depletion of groundwater level in the basin, as evident in this study, is a reason that the river is flowing in discontinuous losing phase, similar to the study conducted by Kirk and Herbert (2002) and Yeh et al (2008). An increase in the area of groundwater availability in pre-monsoon under 5mbgl and a decrease in the area of groundwater availability under 5mbgl in post-monsoon was

recorded in the past ten years. A decrease in the area of groundwater availability under 8mbgl was recorded in the pre-monsoon season, whereas there is an increase in the area of groundwater availability under 8mbgl in post-monsoon season in the past 10 years. In comparison to the groundwater levels in 1984 to 2015, a decrease in the area of groundwater level under 5mbgl was recorded in both pre- and post-monsoon season of 21.70 and 40.14 per cent, respectively. An increase in the area of groundwater under 8mbgl was recorded in 2015 in comparison to 1984 in pre- and post-monsoon season of 13.90 per cent and 28.99 per cent, respectively. The decrease in the groundwater table is a result of imbalance between water withdrawal and recharge in the Gomti River basin. As a result, the river is getting dry in the lean flow season. The GRB is a groundwater-fed river with a base flow component of 78%, 76%, and 69 per cent in river discharge at Neemsar, Lucknow, and Maighat, respectively. A study done by Mohammadlou and Zeinivand (2018), concluded that high baseflow component in river discharge is an indicator of porous and has a permeable geological structure in the basin. The higher rates of evapotranspiration and more dependency on groundwater storage for fulfilling the agriculture water demands are also putting stress on groundwater aquifers. The emerging groundwater crisis is also closely related to high governmental subsidies for electricity in the agricultural sector, which leads to over-abstraction. Lucknow city has undergone a new riverfront development plan, which includes construction of 16 m deep diaphragm walls on both sides of the river bank. It would be affecting the surface and ground interactions in the channelized stretch of the river. The river is facing drought-like conditions in its downstream regions (Abeysingha et al 2016). Many aquatic species have low tolerance to drying habitats not well-adapted to dry conditions. If the drying of the channel becomes regular and frequency of intermittency increases, many species may be eliminated from newly intermittent rivers. The habitat fragmentation due to drying channel may limit the recolonization possibilities within the fluvial systems.

CONCLUSION

Flows in the groundwater-fed rivers are getting diminished due to over-abstraction of groundwater as well as surface water abstraction through canals and impoundments. As a result, many of the perennial rivers are becoming intermittent that is affecting freshwater ecosystems. The over-abstraction of groundwater has turned many perennial rivers of Ganga plain into intermittent rivers. This study shows that, Gomti being a groundwater-fed river, has a large proportion of groundwater component in its

overall flow and base flow or the groundwater discharged to the stream constitutes a major source of stream flow during dry periods. During the lean flow periods, the groundwater usage is highest and base flow reduces due to which the river is not able to meet the minimum flows requirements. The base flow contribution is highest for upstream stretch as compared to the midstream and downstream stretch of the river. The study recommends that groundwater and surface water interactions should be managed so that the river continues to provide groundwater recharge in high flow periods and regains its flow in lean flow periods to maintain the sufficient flow throughout the year. The resultant intermittent flow in the river due to excessive groundwater withdrawal may lead to irreversible alterations of aquatic communities and of critical ecosystem functions. The study has shown that pumping of groundwater has a diminishing effect on the flow regimes of the river, with depletion of river flows increasing with an increase in the groundwater abstraction. Base flow is very important in maintaining the variable flow regimes in this perennial river, and the base flow connection should not be disturbed as it has been done in the middle section of the river at Lucknow. This study further stresses the need for restoring natural sites of groundwater recharge specially in the upper and middle part of the basin so that flow in the main channel and its tributaries could be improved. This study also highlights of the need for employing more robust techniques in assessing the interactions between surface and groundwater for developing integrated water management plans. Conjunctive management of surface and groundwater resources should be done to achieve long-term water resource conservation and management. It is important to understand the surface and groundwater interactions for holistic management of the river systems. There is a growing evidence that excessive water abstraction in the catchments are resulting in intermittent flow regimes which may lead to irreversible alterations of aquatic communities and of critical ecosystem functions. The holistic management approaches should be planned keeping the long-term water resource conservation into consideration. The planning system should be incorporated with periodical feedback analysis in the baseline monitoring of the resources.

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Barley Crop Yield Forecasting using Ensemble Method- Adaptive Boosting of Weak Regressors

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Abstract: Prediction and forecasting of crop yields based on various climatic conditions are an intensive process. In the proposed work adaptive boosting prediction model is presented, using the datasets of barley crops along with the climatic features of the states located in Colorado, Wyoming, Idaho and Montana. The study discusses the experiments on weak regression technique like the linear regression, SVR Linear regression and proposes a strong prediction method in terms of regression like the Adaptive Boosting (Adaboost) technology in order to increase the accuracy in the prediction techniques. The Adaboost method combines several weak regressors and presents a weighted sum representing a strong final output. It has been observed from the experiments that, the decision of weak regressors varies due to frequent, inherent attributes of climatic conditions for crop production. The various numerical simulations, results and statistical measures demonstrate the efficiency of the strong Adaboost based regressors when compared to weak linear regressors.

Keywords: AdaBoost, Linear regression, Weak regressors, SVR linear regression, Climatic parameters, Crop yield forecasting

Climate is a highly dynamic variable that differs intermittently. Considering the crop based agrarian framework models, the climate is one amongst the most important and imperative component to be modeled. This component impacts numerous agro-ecological procedures and factors, influencing soil properties farming practices and methods, crop yield, crop production, pest and disease progression, and so on (Sharma et al 2016, Yin et al 2016). As a consequence of this, it is desirable to create and process climatic models with absolute precision which enables to evaluate and assess various climatic factors (Dumont et al 2015) and its variability, its impingement on the agrarian systems or its surrounding environment (Kandiannan et al 2012), in the view of enabling and assisting the decision making capability process in agriculture. Both environmental change and climatic variability could posture enormous difficulties in farming (Dubey et al 2018). Computer researchers and analysts together brought numerous methodologies and approaches to enhance the forecast control (Sellam et al 2016). Regression analysis, one of the methods accessible in statistical examination is the straightforward, normal and essential system used to demonstrate the connection between at least one free or indicator factors and a dependant variable, which we need to forecast (Bagirov et al 2017). At the point when all the indicator factors are ceaseless esteemed then the great determination of expectation strategy is regression analysis. The proposed work looks at different weak regressors like Linear and SVR (Valarmathi et al 2011). Besides proposing strong factors by avoiding the deficiencies of weak

regressors, the work facilitates showing the benefits of adaptive boosting (AdaBoost) to improve the insightful accuracy of product yield (barley). Handling both the learning issues and moreover the computational unpredictability effectively. The consequence of weak regressors may contrast as a result of progressive and inalienable qualities of climatic conditions for crop production.

The vital utilizations of computational estimating models in agriculture incorporates to evaluate the vulnerability of the flood and furthermore, dry season (Dumont et al 2015); to gauge the efficiency capacity of the field (Kandiannan et al 2012) to foresee the few conditions and parameters for crop development (Sharma et al 2016, Yin et al 2016), the computational proficiency in contrast with customary methods for forecast through efficient results (Yadav et al 2015, Sethi et al 2006). The purpose of the proposed research is to develop a support model which builds a strong regressor since the individual regressor can be weak. The computational crop yield or productivity appears in the perspective of LR and SVR and assisted with AdaBoost and are affirmed using available test data for the districts in Colorado, Wyoming, Idaho and Montana states.

MATERIAL AND METHODS

Regression based forecasting models among different procedures make sense of the relationship among an arrangement of illustrative features by utilizing the historical information. Measurable statistical data and regression based strategies have been broadly used to foresee the future patterns in financial information examination and

additionally in the field of harvest creation. The flow of implementation of the weak regression methods like linear regression and SVR regression has been extensively explained in the Figure 1. Initially the weather and crop datasets from the US states are pre-processed and given as input to the weak LR (Baig et al 2017) and SVR regressors (Bieliková 2014). Second, the outcomes got from those have been examined and those occasions which are wrongly forecasted or then again not coordinating to the coveted anticipated forecasting have been helped by utilizing AdaBoost (Sun et al 2016, Dan et al 2016) and the yield of the weak regressors is wound in support of wrongly forecasted instances adaptively delivering strong regressors. At long last, the consequences of weak regressors broke down, contrasted and approved and strong regressors (Liu et al 2017, Hwan et al 2014). The key advantage of this work is to create an arrangement of strong regressors which are appropriate for crop yield estimate by thinking about the climatic influence.

The proposed model forecast regional barley yields in Colorado, Wyoming, Idaho and Montana. The model extracts data from public websites such as the US Department of Agriculture (yield information), and Darksy.net (weather). There are various environmental parameters that affect the crop yield prediction. Weather data is downloaded in advance; the only processing of data was to drop unused columns during the download, in an effort to reduce file size.

Additionally the daily weather data is converted to yearly averages so it can be used with the yield data. The USDA's National Agricultural Statistics Service has an online collection of historical crop yields. This data is automatically collected by the model for cleaning and combining with the weather data. Since Barley was the focus, only the barley data were downloaded. The various climatic parameters considered are cloud cover, dew point, humidity, lat, long, precipitation accumulation, precipitation intensity, precipitation intensity max, precipitation probability, precipitation type, pressure, state, temperature max, temperature min, time, visibility, wind bearing, wind speed.

This system depends on the idea of Vapnik's support vectors. SVR utilizes an indistinguishable standards from the SVM for characterization (Walther et al 2005), with just a couple of minor contrasts. On account of regression, an edge of tolerance (epsilon) is set up in estimation to the SVM which would have been removed from the issue. Other than that, the principle thought is to limit error, individualizing the hyper plane, which amplifies the edge, remembering that part of the error is endured. At the end of the day, exhibiting the arrangement by methods for little subset of preparing focuses gives tremendous computational points of interest. Consequently, utilizing the epsilon intensive loss function we guarantee presence of the generalized least value, in the meantime streamlining of dependable generalization bound.

RESULTS AND DISCUSSION

To improve and enhance the prediction of barley yield an adaboost based strong regression method is involved. The performance measure estimation by means of MAE, MSD and MAD are performed with the following formula:

$$MAE = \frac{1}{N} \sum_{i=1}^N |P_{actual} - P_{predicted}| \quad (1)$$

$$MSE = \frac{1}{N} \sum_{i=1}^N (P_{actual} - P_{predicted})^2 \quad (2)$$

$$MAD = \text{median}(\text{Error}_i - \text{median}(\text{Error})) \quad (3)$$

Here P_{actual} and $P_{predicted}$ depicts the observed power values and error is the difference between the two $|P_{actual} - P_{predicted}|$. The performance indices for the above mentioned measures in case of optimal predictions should be zero. The evaluation is done using the k-fold cross validation where the $k=6$. The entire dataset is split into 6 folds. In them 5 folds are used for training the regression model and one fold is used for testing the model. All the MAE, MSE and MAD measures are low, indicating the better model performance and better insights in the prediction results shown in Table 1 and 2.

Forecasting can be portrayed as the way toward distinguishing a certain parameter based on related things.

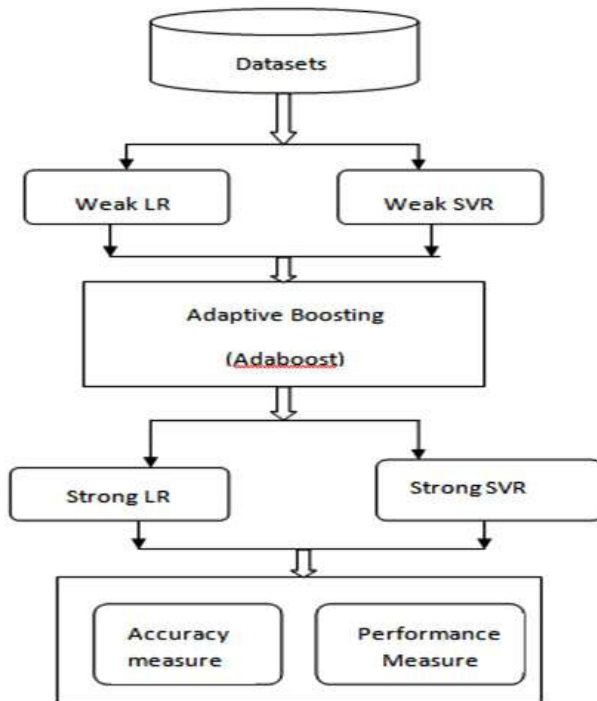


Fig. 1. Graphical representation of the proposed model

For the most part, the forecast can be performed in the field of information mining utilizing regression analysis (Yu et al 2015, Shirani et al 1996, Budka et al 2012). LR is the most broadly utilized factual approach utilized for numerical expectation. This is a procedure of building up relationships between a reliant variable (y) and an arrangement of independent factors (X). For in excess of one independent variable, this procedure is called MLR. High coefficient value suggests the high effect of relating highlight on the expected process. The coefficient diagram demonstrates the significance of various includes by plotting the coefficient values utilized as a part of regression process. The essential highlights of climatic conditions more capable for rice yield estimation utilizing LR. Figure 2 represents a weak linear regression model for crop production.

From the analysis made, it is observed that though weak regression models are giving some prediction results in order to further enhance the prediction we go for strong adaptive boosting regression methods to the LR and SVR. The main aim of adaboost is to convert weak regressor to strong regressor. It uses multiple predicting regressors to build a strong forecasting algorithm. Adaboost chooses a base algorithm for eg. LR and improves it iteratively by accounting for the errors in forecast predictions. Figure 3 shows the prediction results boosted by AdaBoost with different weak estimators, such as: LR and SVR. Among these models SVR

regressor model with AdaBoost gives the best result as compared to others. In order to measure the accuracy of the forecasted prediction various statistical analysis methods like MAE, MSD, MAD for both strong and weak regressors are estimated.

The climatic variables, for instance, precipitation, temperature, solar radiation and wetness have been changing a direct result of an expansive temperature help. It

Table 1. Comparison of MAE, MSE and MAD measures for weak LR and strong LR

States	Weak LR vs strong LR results					
	MAE		MSE		MAD	
	Weak LR	Strong LR	Weak LR	Strong LR	Weak LR	Strong LR
Colorado	126.556	7.834	29537.751	1248.626	83.339	9.594
Wyomin	162.379	2.578	35139.546	136.485	125.892	1.518
Idaho	69.969	5.803	6721.112	208.947	34.644	9.594
Montana	94.148	4.942	9547.34	834.241	59.127	5.348

Table 2. Comparison of MAE, MSE and MAD measures for weak SVR and strong SVR

States	Weak SVR vs strong SVR results					
	MAE		MSE		MAD	
	Weak SVR	Strong SVR	Weak SVR	Strong SVR	Weak SVR	Strong SVR
Colorado	91.307	24.687	81203.881	5200.29	5.689	0.163
Wyomin	1311.695	14.916	216489.7	3033.979	1121.14	0.17
Idaho	45.46	24.37	12488.427	3392.243	45.784	0.542
Montana	67.358	11.523	75325.11	2153.31	2.536	0.115

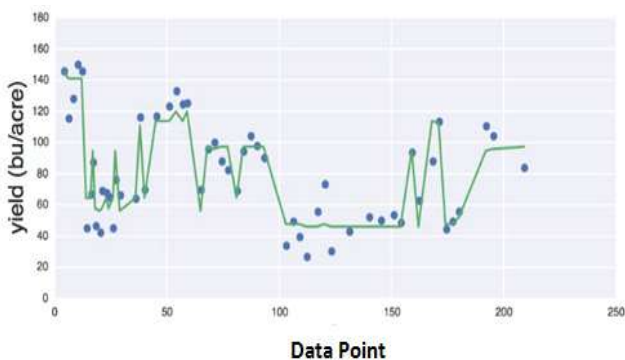


Fig. 2. Barley production prediction results using weak LR

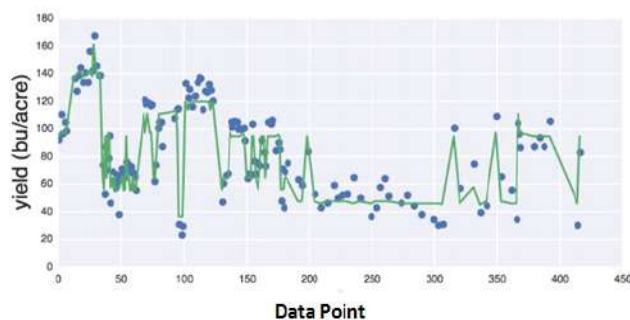


Fig. 3. Represents barley crop yield prediction using strong LR

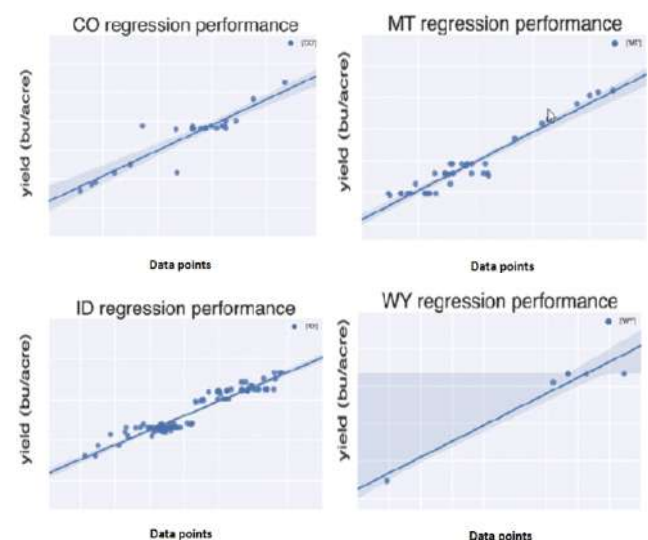


Fig. 4. Regression performance of the four states individually

is foreseen that grains effectiveness will be diminished to 20–50% by 2100 in view of rise in temperature, growing water weight and lessening in the number and dispersal of stormy days. In this condition the desire for future yield benefit is exceptionally anticipated that would propose the agriculturists in time and its examination must be made in keeping to help the farmers for getting most prominent harvest. The results obtained from the Colorado, Wyoming, Idaho and Montana states predicting barley yields for the strong regression method Adaboost for LR has been documented. The lower values of the error measure using Adaboost based strong regressors are in good agreement when compared to the results obtained with the weak regressors.

CONCLUSIONS

The novelty of the work results in proposing a system with strong strategies with reduced errors for regression resulting in the generation of strong predictive forecasts using exploration of adaboost regression methodologies considering climatic factors for barley yield in the four US states Colorado, Wyoming, Idaho and Montana. The most significant advantages of regression measures like, better forecast and stable model made our proposed forecasting system more robust and precise. The learning and observing capacity of the regression methodologies are performing better and can express obviously and viably the current connections between the climatic features and the target variable which is the barley yield. However in future, an intriguing knowledge into the relative significance of the features can be followed and the random or irrelevant variables can be accordingly managed by taking a shot at upgraded systems which can distinguish the highlights of features quickly to foresee the near optimal arrangements.

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Estimation of Rice Crop Biophysical Parameters Using Multi-Temporal Sentinel-2 MSI Sensor Data

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Abstract: The study focuses to efficiently estimate and mapping rice crop biophysical parameters using high temporal and spatial resolution sentinel-2 satellite imagery. Biophysical properties provide a good understanding of physics of the interaction between vegetation and solar radiation. Crop canopy properties can be collected through inversion using nadir reflectance in various bands. NDVI is sensitive to the light, angle of view, sensor after canopy peak is touched. Rice crop biophysical parameters are autonomous from viewing angle and light conditions. The major rice crop biophysical parameters are leaf area index (LAI), Chlorophyll content, fraction of photosynthetically active radiation (FAPAR), canopy water content (CWC), fraction of cover (FCover), and crop biomass respectively. Biophysical parameters are known as mirrors of plant health construction and crop yield. Regression model was developed using chlorophyll, CWC, FAPAR, LAI and FCover with coefficient of determination 0.83 to accurately predict crop biomass. It is concluded that the study is helpful to monitor overall health of rice crop and it is proved that methodology has potential to estimate and monitor biophysical parameters of rice crop. From the study it is known that sentinel-2 has the potential to estimate and map rice crop biophysical parameters precisely.

Keywords: Rice crop, Biophysical parameters, Sentinel-2, Regression model

Worldwide rice '*Oryza sativa*' crop is staple food crop and provides food to around three billion people in the world and growing in 11% of the total crop land worldwide (Mahesh et al 2019b). Accurate and continuous mapping of rice crop biophysical parameters is essential in various applications like precision agricultural and natural resource management, irrigation scheduling, fertilizer application, etc. Rice crop biophysical parameters broadly classified into six types: chlorophyll, leaf area index (LAI), fraction of photosynthetically active radiation (FAPAR), fraction of cover (FCover), canopy water content (CWC) and biomass (Adamala et al 2014, Mahesh et al 2019a). These parameters give insight into fertilizers content and water content and photosynthesis process of crop. Some studies reveal that chlorophyll content could be high interest because it is indication of photosynthesis process in crop. These remote sensing technologies given opportunity to study over large area in less time with precision. The present study uses multispectral sensor data from sentinel-2 provided by European Space Agency ESA. Li et al (2016) performed estimation of rice biophysical parameters using RADARSAT-2 SAR data. Experiment performed in 30 sampling locations over 5 periods. They estimated FAPAR, LAI, CWC, biomass and height from the experiment. The authors analysed backscatter and biophysical parameters and found that these parameters significantly correlated with VV and VH polarizations. Yang et al (2011) estimated rice biophysical parameters using hyperspectral remote sensing images using support vector machine. They analysed four different

reflectance to know their capability to envisage rice biophysical parameters. Depankar et al (2018) estimated rice crop biophysical parameters using sentinel-1 synthetic aperture radar data. Gordana et al (2017) performed estimation of biomass using sentinel-2 satellite images. In the study normalized difference vegetation index (NDVI) was used to estimate biomass. The present study focuses on estimation of rice crop biophysical parameters and spatial mapping using sentinel-2 satellite images.

MATERIAL AND METHODS

Study area: Rice phenology estimation and mapping were performed using experimental data in 20 sampling locations located in Chittoor district of Andhra Pradesh state in India. The study performed in the vicinity of 80°21'39" E to 82°21'48" E longitude and 16°25'50" N to 18°90'0" N latitude. Canal and groundwater were the main irrigation source of agriculture and rice crop is one of the most cultivating crops in the study area. The rice crop is a sole culture in all the parts of the study area. Figure 1 indicate the methodology adopted in the study. The satellite data acquired from 01-01-2019 to 15-04-2019 for the present study. The sentinel-2 satellite sensor provides 10 m resolution with 12 days return period in 12 bands

Biophysical parameters: Biophysical parameters are namely LAI, chlorophyll, FCover, FAPAR and CWC were estimated using biophysical processor embedded Artificial neural network algorithm in SNAP platform. To estimate biophysical parameters red, NIR, near NIR, shortwave IR and red edge bands of sentinel-2 were used.

Biomass experiment: The biomass experiment was conducted in the farmers field for biomass calculation is as follows (Panel et al 1995, Ranjit et al 2018).

$$\text{Biomass} = N_1 \times N_2 \times [W_1 \times W_2]$$

N_1 = No of bunches per m^2 , N_2 = No of stems per bunch, W_1 = Wait of one stem, W_2 = Weight of leaves in one stem.

RESULTS AND DISCUSSION

Leaf Area Index: Leaf area index (LAI) of rice crop in heading stage is 2.6 in the study area, so the rice crop is healthy because healthy rice crop LAI ranges from 2.5 to 3.0. Figure 4 and 5 show trend and classification of LAI in the study area.

Chlorophyll: The chlorophyll content at heading stage of the rice crop is 140 in the study area. This indicates content of the chlorophyll in rice crop is sufficient to do photo synthesis process i.e. converting carbon dioxide to carbohydrates. Figure 4 and 5 show trends of chlorophyll and spatial distribution of chlorophyll in the study area.

FCover: The range of FCover is 0 to 1, and 1 is representing to a situation where the ground is completely covered by vegetation. The FCover of rice crop shows increases trend from 0.1 to 0.77 in the study area (Figure 6, 7).

FAPAR: The FAPAR ranges from 0 to 1, and 1 represent to a photosynthesis fully active. The range of FAPAR for vegetation is 0 to 1, maximum value never achieved because portion of the light reflected back from the canopy. FAPAR depends on illumination of light, i.e. angle of the sun with respect to rice crop. Figure 8 and 9 show FAPAR trend and spatial distribution of FAPAR.

Canopy Water Content (CWC): CWC indices uses reflectance in near infrared and shortwave infrared region of

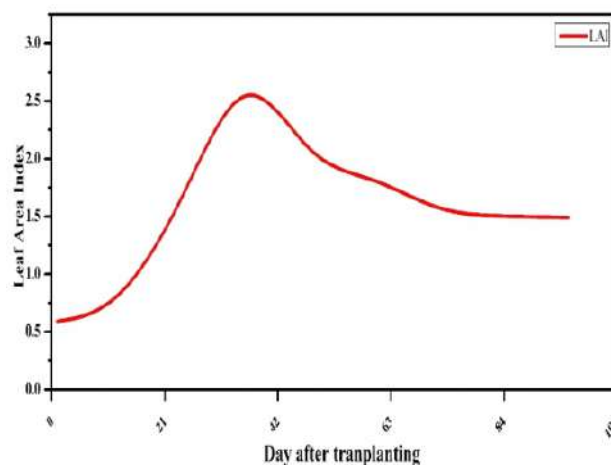


Fig. 2. Trend of leaf area index in the crop cycle period

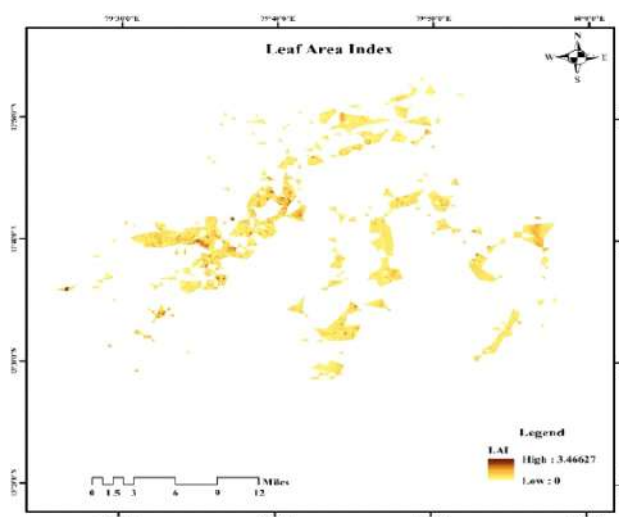


Fig. 3. Spatial map of leaf area index in the study area

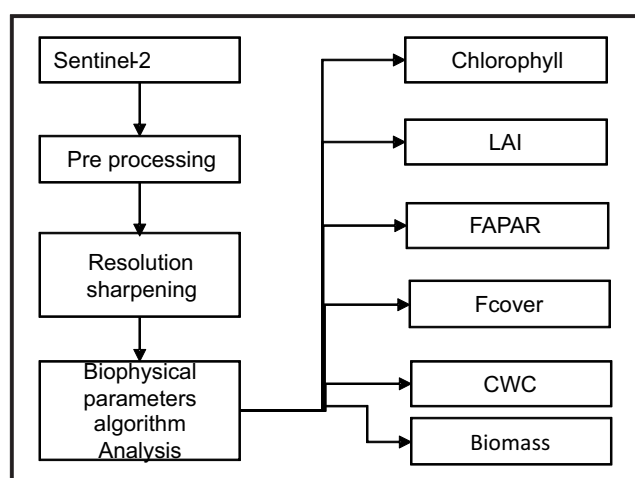


Fig. 1. Methodology flowchart of biophysical parameters estimation

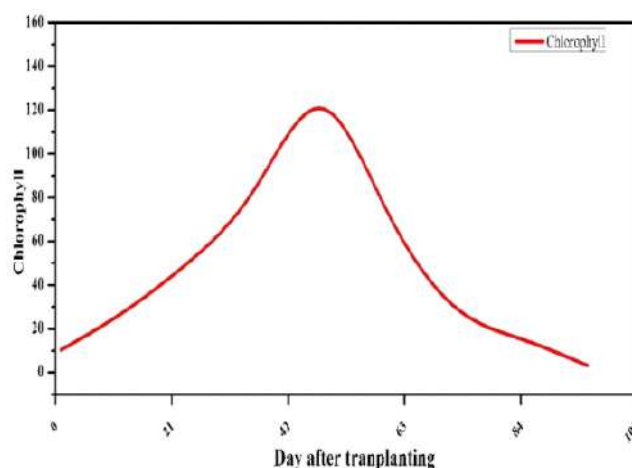


Fig. 4. Trend of plant chlorophyll in crop cycle period

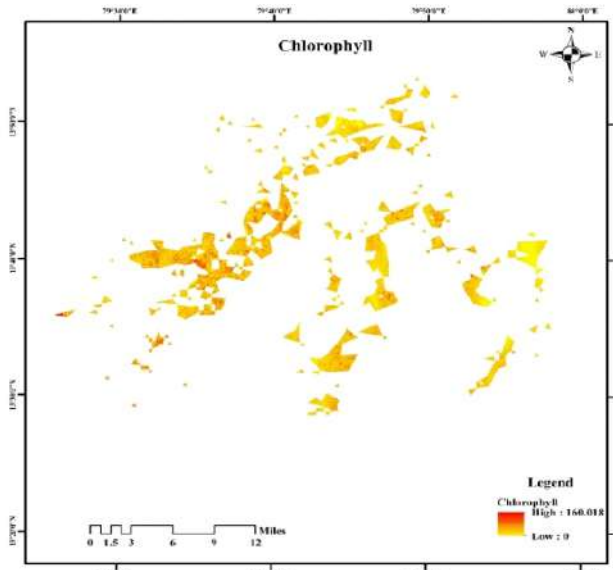


Fig. 5. Spatial map of chlorophyll in the study area

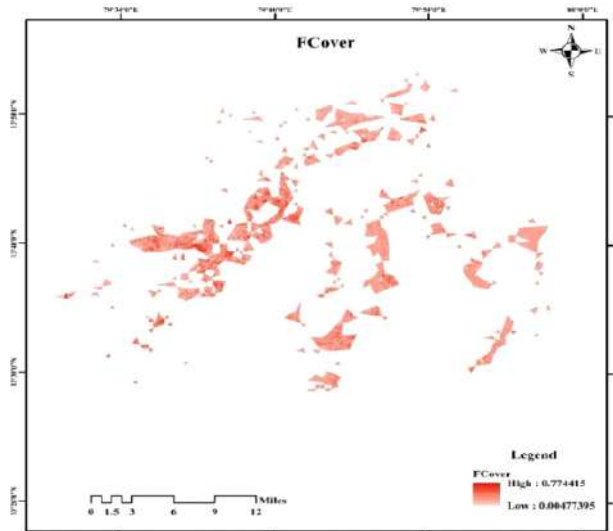


Fig. 6. Spatial map of F Cover in the study area

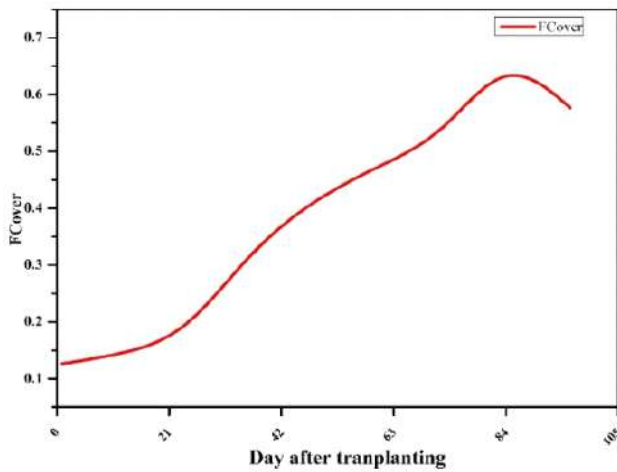


Fig. 7. F Cover trend in crop cycle period

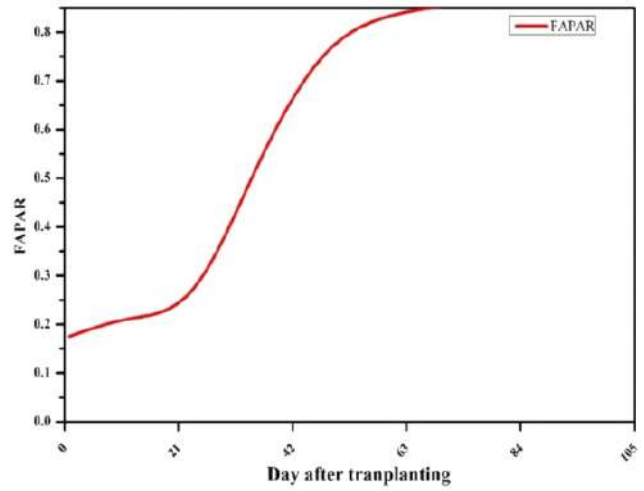


Fig. 8. FAPAR trend in crop cycle period

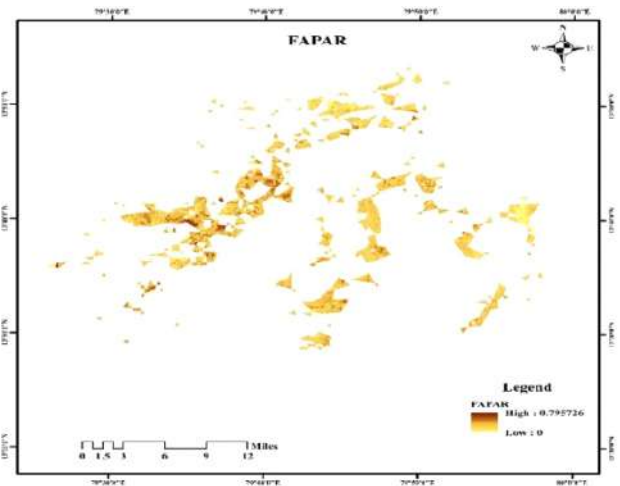


Fig. 9. Spatial map of FAPAR in the study area

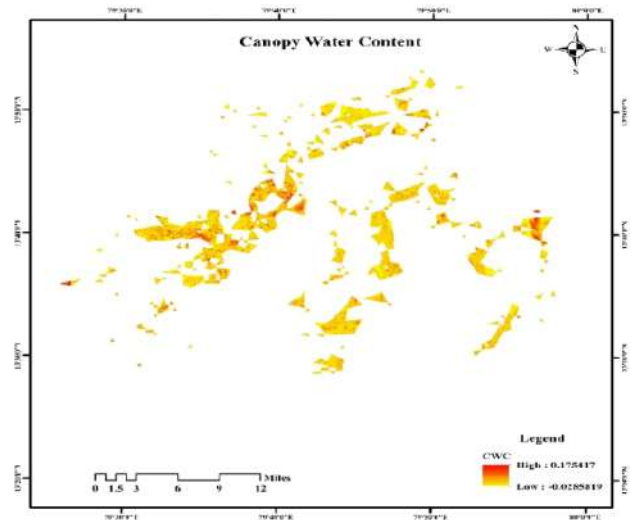


Fig. 10. Spatial map of canopy water content in the study area

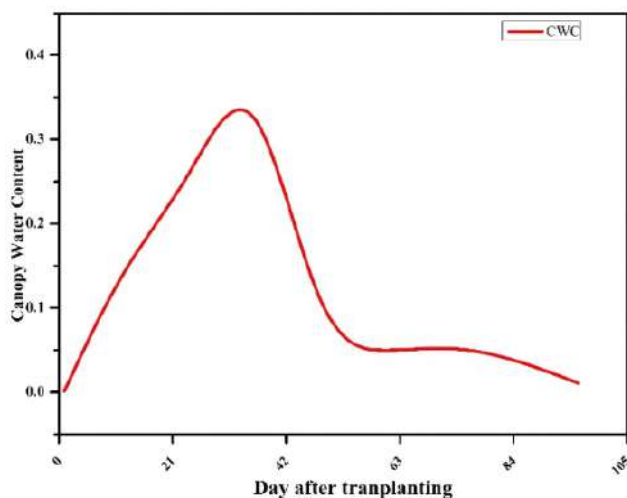


Fig. 11. CWC trend in rice crop cycle

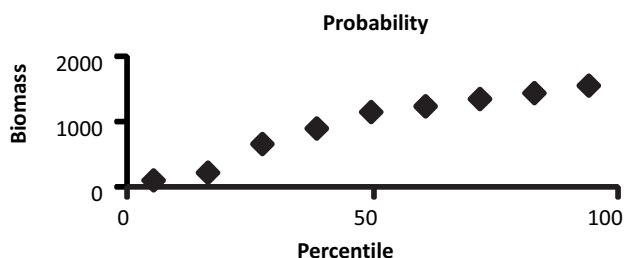


Fig. 12. Biomass probability with respect to percentile

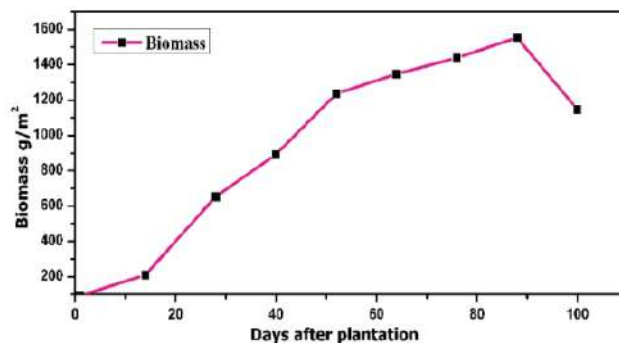


Fig. 13. Biomass trend in crop cycle period

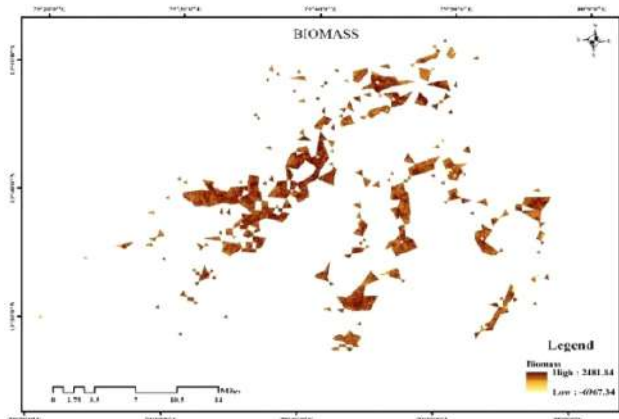


Fig. 14. Spatial distribution of biomass in the study area

Table 2. Biomass ground truth data analysis

Stem	FW (g)		DW (g)		No. of stems	No. of bunches m ⁻¹	Space, cm	days	Biomass (g)
	Bunch	Leaves	Stem	Bunch					
1.01	3.50	0.28	0.15	1.17	37	24	5	100	1146.59
1.30	4.40	0.45	0.49	2.13	37	24	5	88	1554.00
1.20	4.39	0.42	0.48	2.02	37	24	5	76	1438.56
1.15	4.30	0.41	0.47	2.01	36	24	5	64	1347.84
1.08	4.02	0.35	0.46	1.92	36	24	5	52	1235.52
0.95	3.91	0.21	0.43	1.80	32	24	5	40	892.42
0.90	3.16	0.15	0.43	1.16	26	24	5	28	652.70
0.60	1.83	0.13	0.19	0.75	12	24	5	14	210.24
0.39	1.00	0.09	0.11	0.41	8	24	5	1	92.16

Table 3. Statistics of regression model

Regression statistics							
Multiple R	0.91						
R Square	0.83						
Adjusted R Square	0.41						
Standard Error	665.39						
Coefficients	Standard Error	t Stat	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%	
Intercept	0	-	-	-	-	-	-
Chlorophyll	-1.54	13.60	-0.11	-39.30	36.22	-39.30	36.22
CWC	6338.20	4895.15	1.29	-7252.91	19929.32	-7252.91	19929.32
FAPAR	2785.58	2655.46	1.05	-4587.14	10158.31	-4587.14	10158.31
FCover	1740.27	2599.13	0.67	-5476.06	8956.60	-5476.06	8956.60
LAI	-957.60	1234.90	-0.78	-4386.22	2471.02	-4386.22	2471.02

$$\text{Biomass} = 6338.20 (\text{CWC}) - 1.54(\text{chlorophyll}) + 2785.58 (\text{FAPAR}) + 1740.27(\text{FCover}) - 957.60(\text{LAI})$$

EMR spectrum and this is sensitive to increase in CWC. Figure 12 and 13 show trend and spatial map of CWC in the study area.

Biomass : The highest biomass of rice crop was 1580 g/m² in the study area. Chlorophyll, LAI, CWC, FAPAR and FCover parameters influence existence of biomass. Therefore, regression model was developed using Chlorophyll, CWC, LAI, FAPAR and FCover parameters with coefficient of determination (R^2) 0.83 to predict rice crop biomass. Table 2 shows biomass experiment data and Table 3 shows statistics of regression model. Figure 12 and 13 show the biomass percentile and biomass trend in crop cycle period (Figure 14).

CONCLUSIONS

The biophysical parameters estimation was with accuracy of 0.83. This implies the methodology adopted can be implemented for large crop area. The estimation of plant biomass also given satisfactory results. The study helpful to monitor overall health of rice crop and it is proved that methodology has potential to estimate and monitor biophysical parameters of rice crop. The crop biophysical parameters in the study area depicted that the rice crop health is good and growth is steady. The sentinel-2 has the potential to estimate and map rice crop biophysical parameters precisely.

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Vertical Distribution of Nutrients *vis-a-vis* Soil Properties in Different Geomorphic Units of North-Eastern Haryana, India

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Abstract: The profile distribution of primary nutrients (N, P and K) and diethylenetriamine pentaacetic acid (DTPA) extractable micronutrients (Zn, Mn, Cu and Fe) was investigated in soils developed on different geomorphic units of north-eastern Haryana, India. The soils exhibited a gradual fining of texture from Shivalik hills to flood plains. By and large, the soils were slightly acidic to strongly alkaline and calcareous. The macro and micronutrients irrespective of the geomorphic unit were higher in surface horizons than subsurface horizons. However, none of the micronutrients exhibited any systematic pattern of distribution with depth on different geomorphic surfaces. Contents of all micronutrients were higher in fine-textured as compared to coarse-textured soils. Soil pH, organic matter, calcium carbonate and size fractions had strong influence on the distribution of extractable micronutrients. Based on linear coefficients of correlation, DTPA-extractable micronutrients increased with increase in organic carbon and clay content and decreased with increase in pH and CaCO₃ content. Organic carbon was significantly and positively correlated with N, P, K and micronutrients except Zn. The use of principal component analysis (PCA) in the present study effectively reduced fifteen variables to five principal components and described 73.29% of the original variance. Among all the soil properties organic carbon had prominent effect on distribution of micronutrients.

Keywords: Geomorphic unit, Macronutrients, Micronutrients, Calcium carbonate, Principal component analysis

A geomorphic surface or unit is described as the component of the land surface with definite geographic boundaries formed by one or more agencies in a given period of time (Buol et al 2011). Soil is a dynamic body which attains its properties via pedogenic processes that are effective temporally and spatially. The influence of these processes is discernible with reference to morphological and physicochemical characteristics of soils evolved on different landforms (Sharma et al 2013). In order to understand soil landscapes, effectively envisage soil occurrence and predict soil properties there is a need to comprehend the relationships among soils, landscapes and surficial deposits (Wysocki et al 2012). The variability in landscape in association with topographic features influences spatial patterns of water movement on both soil surface and sub surface thereby affecting nutrient availability (Menezes-de-Souza et al 2006). Soil fertility reveals the significance of different soils with respect to the amount and availability of elements indispensable for plant growth (Sarkar et al 2002). Since, nutrient stratification has started when soils began weathering and coming under the influence of terrestrial plants and roots. Therefore, information about the vertical distribution of plant nutrients is imperative because roots of most plants extend beyond the surface layer and obtain part of their nutrient requirements from subsurface layers of the soil (Mishra et al 2015). Plant nutrient management is mainly governed by the level of nutrients in the soil. In natural, uncultivated systems, the availability of macronutrients is not

a limiting factor of growth owing to rapid recycling mechanisms. On the other hand, due to modern agricultural practices macronutrient availability might be a matter of concern as soils often have been over exploited (Kulcheski et al 2015). Nitrogen (N), phosphorus (P) and potassium (K) are the macronutrients which play a pivotal role in growth and development of the plants. The importance of primary nutrients, i.e., nitrogen (N), phosphorous (P) and potassium (K) is well recognized (Singh and Ahuja 1990). Micronutrients are the mineral elements that are needed by plants in small quantities to perform a range of physiological functions (White et al 2012). Micronutrients, by and large, belong to transition elements in the Periodic Table and mostly have high (usually $> 5.0 \text{ mg m}^{-3}$) density (Makoi 2016). In soils, the geographical distribution of micronutrients is intimately correlated to the make-up of the parent materials, in spite of the fact that soils show less variation in micronutrient content compared to parent rocks (Hodgson 1963) and soil forming processes might significantly influence the distribution of trace elements within the soil profile (Sharma et al 2000). The spatial variation of micronutrients in topsoil is by virtue of the differences in parent material, vegetation, topography, climate, fertilizer application and land use type (Wang et al 2008). Accordingly, better understanding of the dynamic variation of the micronutrients and the factors that alter their levels in the soil is imperative (Zhu et al 2016). Sharma et al (2004) studied the distribution of micronutrients in the soil profiles of Inceptisols of Punjab and reported that DTPA-

extractable micronutrients increased with an increase in organic carbon content and CEC and decreased with increasing pH, sand and calcium carbonate content. Devi et al (2015) evinced that soil fertility varies spatially for which availability of nutritional elements is responsible. Soil fertility specialists need well characterized sites with similar soil and other ecological conditions in order to carry out meaningful fertilizer trials. Panwar and Pal (2010) evaluated soil fertility related to altitude, slope and aspect in a hilly micro-watershed of lower Shivaliks. Keeping in view the importance of the area for developing agriculture, the present study was undertaken to determine the vertical distribution of nutrients *vis a vis* soil properties in different geomorphic units of north-eastern Haryana, India. This will enable the farming community and policy makers to identify potential fertility for the production of agricultural crops and design appropriate soil management strategies for sustainable crop production.

MATERIAL AND METHODS

Study area: The study area is situated between 76° 31' to 77° 35' E longitudes and 30° 03' to 30° 57' N latitudes covering districts of Panchkula, Ambala, Yamunanagar and Kurukshetra in Haryana, India. Four major geomorphic units have been recognized in north-eastern Haryana, viz., Shivalik hills, piedmont plains, flood plains and old alluvial plains. The parent material in Shivaliks is predominantly composed of sandstones, sand rocks, clay, conglomerates and limestones mainly belonging to upper tertiary period. The flood plains and alluvial plains are part of the Great Plains of India formed by alluvial sedimental deposition from recent to Pleistocene times. The plains are extraordinarily homogenous with slight variation in relief features. The shifting of river courses in the areas of frequent floods is specific geomorphic process in the plains. The undulating topography south of the Shivaliks is adversely altered by erosion engendered by the seasonal streams locally called as *chos* (Hussain 2008). Two major rivers, Ghaggar and Yamuna, flow through this area. The climate is subtropical, semiarid, continental monsoonal having prolonged hot summer with annual precipitation of 578-1486 mm. The maximum rainfall occurs during monsoon i.e., July to September. The soil moisture regime is ustic and udic and soil temperature regime is hyperthermic. The relative humidity increases from June to September and gradually decreases reaching the minimum during January.

Soil sampling: Field traverses and observations were undertaken in the studied area to identify landform features and associated soil types. For detailed reconnaissance soil survey the topographic maps of India (1:50,000) and aerial photographs were used for site selection and profile

excavation. On the basis of geomorphic-soil relationship established in the area, 13 profiles were exposed and studied in the field (Pedons 1-4 belong to Shivalik hills; 5-7 to piedmont plains; 8-9 to flood plains and 10-13 to alluvial plains). These pedons also form a toposequence, a transect covering from Shivalik hills to lower alluvial plains. The soils chosen were assumed to be representative of the area. For each profile description, associated terrain features including topography, drainage, slope and vegetation were recorded. Sampling of each pedon was done by pedological horizon; 2-3 kg of soil was collected per horizon.

Soil analysis: The soil samples collected from different horizons were air-dried and ground to pass through a 2 mm stainless steel screen for laboratory analysis. Particle size distribution of the soils was determined by International Pipette method (Piper 1950). The pH of the soils was measured with glass electrode using soil suspension of 1:2 (soil: water) and electrical conductivity in supernatant, cation exchange capacity by neutral normal ammonium acetate extraction (Jackson 1973); organic carbon by wet-oxidation method of Walkely and Black (1934), available nitrogen (N) by alkaline permanganate method (Subbaiah and Asija 1956), available P content by extracting the soil samples using 0.5M NaHCO₃ and analysed by spectrophotometer (Olsen et al 1954) and available potassium by using neutral normal ammonium acetate and the content was determined by aspirating the extract into flame photometer (Jackson 1973). Calcium carbonate was determined by rapid titration method (Puri 1930). Ten grams of soil (2-mm sieved) were used for extraction of plant available fractions of Zn, Cu, Mn, and Fe with 20 ml of diethylenetriamine pentaacetic acid (DTPA) extractant (0.005 M DTPA + 0.01 M CaCl₂ + 0.1 M triethanolamine, adjusted to pH 7.3) using atomic absorption spectrometer (Lindsay and Norvell 1978). The weighted means were computed to match the data for each geomorphic unit by the following formula (Sharma et al 2015).

$$\text{Weighted mean (wm)} = \frac{\text{Concentration of each horizon} \times \text{Depth of each horizon}}{\text{Total depth of the profile}}$$

Statistical analysis: The statistical package for the social sciences (SPSS) version 17.0 software (SSPS Inc., Chicago, IL, USA) was used to analyze the statistical characteristics (descriptive statistics, correlation and principal component analysis) of the data.

RESULTS AND DISCUSSION

General soil characteristics: The horizon depth, geomorphic unit and taxonomy of the pedons are given in Table 1. The soils showed wide variation in texture from sand

to clay loam which may be ascribed to variation in parent material, position of soil in the landscape and differential degree of weathering (Table 2). The particle size distribution indicated highest (29.38%) and lowest instead of clay (2.41%) were observed for alluvial plains and Shivalik hills, respectively. The soils exhibited a gradual fining of texture from Shivalik hills to alluvial plains owing to decrease in slope from former to later. However, upper piedmont plains had almost similar clay content as those of pedons on top of Shivalik hills because of the higher soil erosion. The soils were fine textured in subsurface horizons as compared to overlying horizons. The soils in terms of chemical reaction varied from slightly acidic to strongly alkaline (6.45-9.40) (Table 2). The lowest pH was in Shivalik hills and highest in alluvial plains. There was a substantial variation in pH among different geomorphic units which may be due to difference in parent material and organic matter (OM) as the decomposition of OM releases organic acids thereby decreasing pH as evinced in Shivalik hills. The pH increased with depth in all pedons except 4 and 7 which might be ascribed to the loss of bases from the surface horizons.

Table 1. Geomorphic units and soil taxonomy of the pedons

Pedon	Depth (cm)	Geomorphic unit	Soil Taxonomy
P1	0-242	Shivalik hills	Fine loamy, skeletal, Typic Udorthents
P2	0-127	Shivalik hills	Fine loamy, calcareous, Typic Udorthents
P3	0-190	Shivalik hills	Coarse loamy, calcareous, Typic Udipsamments
P4	0-100	Shivalik hills	Loamy skeletal, Fluventic Udorthents
P5	0-241	Piedmont plains	Coarse loamy, Typic Eutrudept
P6	0-75	Piedmont plains	Loamy skeletal, Lithic Udorthents
P7	0-155	Piedmont plains	Coarse loamy, Udic Haplustepts
P8	0-240	Flood plains	Coarse loamy, calcareous, Typic Ustorthents
P9	0-165	Flood plains	Coarse loamy, Typic Ustorthents
P10	0-210	Alluvial plains	Fine loamy, calcareous, Fluventic Haplustepts
P11	0-164	Alluvial plains	Fine loamy, Aquic Haplustept
P12	0-150	Alluvial plains	Fine loamy, calcareous, Aquic Ustorthent
P13	0-250	Alluvial plains	Fine loamy, calcareous, Aquic Ustorthent

Table 2. Range and weighted mean (Wm) of some selected soil properties of major geomorphic units of Haryana

Pedon	Statistical parameter	Sand	Silt	Clay	pH	EC	OC	CEC	CaCO ₃
Pedon 1	Range	52.10-59.21	19.57-21.60	21.22-27.70	8.51-9.05	0.53-1.12	0.11-0.25	11.10-15.24	0-1.47
	Wm	54.33	21.14	24.89	8.97	0.86	0.16	14.13	0.72
Pedon 2	Range	42.27-61.10	18.75-31.73	20.15-26.39	8.25-8.55	0.34-0.44	0.05-0.27	9.61-14.71	0-0.25
	Wm	50.36	25.69	23.49	8.43	0.37	0.13	13.10	0.10
Pedon 3	Range	78.58-92.59	5.00-8.16	2.41-13.26	8.40-8.70	0.28-0.39	0.06-0.19	6.70-9.20	0-0.25
	Wm	84.98	7.01	8.00	8.64	0.30	0.08	7.94	0.11
Pedon 4	Range	50.00-79.43	10.00-27.08	10.57-28.35	6.45-6.85	0.21-0.67	0.34-1.59	6.64-14.83	Nil
	Wm	65.73	18.05	16.07	6.51	0.29	0.72	9.76	Nil
Pedon 5	Range	68.10-80.80	11.20-16.76	8.00-15.14	6.80-7.35	0.21-0.25	0.07-0.60	8.92-12.71	Nil
	Wm	72.75	14.37	12.89	6.99	0.22	0.17	11.00	Nil
Pedon 6	Range	77.22-82.28	6.55-9.97	10.17-12.81	7.00-7.05	0.21-0.24	0.15-0.20	5.21-7.92	Nil
	Wm	80.41	7.94	11.66	7.02	0.23	0.17	7.24	Nil
Pedon 7	Range	50.48-84.25	6.91-28.88	8.84-20.64	7.40-8.20	0.30-0.89	0.03-0.06	5.90-11.02	Nil
	Wm	57.98	23.76	18.26	7.53	0.50	0.03	6.87	Nil
Pedon 8	Range	50.15-86.62	5.88-23.50	7.50-26.35	8.05-8.90	0.44-0.94	0.07-0.51	9.30-15.00	0.87-2.42
	Wm	75.60	11.39	13.01	8.52	0.53	0.15	11.78	1.50
Pedon 9	Range	56.99-95.04	1.80-26.04	3.14-16.97	7.30-8.00	0.26-0.75	0.20-0.42	4.57-12.60	0-3.00
	Wm	83.45	8.49	7.68	7.82	0.37	0.27	7.37	1.07
Pedon 10	Range	46.00-76.00	12.67-24.62	10.92-29.38	8.30-9.40	0.29-0.71	0.02-0.64	11.20-16.40	0.60-2.25
	Wm	57.09	20.54	22.57	9.08	0.43	0.19	14.18	1.47
Pedon 11	Range	38.84-73.80	11.60-27.16	12.80-34.00	7.10-8.30	0.24-0.38	0.12-0.43	8.85-17.00	Nil
	Wm	51.18	22.10	26.73	8.11	0.28	0.16	14.35	Nil
Pedon 12	Range	40.84-67.00	15.00-27.58	18.38-31.58	8.20-9.10	0.44-0.75	0.07-0.53	9.92-16.40	0.57-1.85
	Wm	57.32	20.22	22.73	8.77	0.53	0.24	11.77	0.83
Pedon 13	Range	55.04-68.20	14.30-20.96	18.36-24.00	8.45-9.20	0.23-0.76	0.11-0.49	9.30-11.62	0.17-0.40
	Wm	62.95	16.90	20.50	8.97	0.38	0.20	9.86	0.28

Furthermore, the increasing trend may be due to increase in accumulation of exchangeable sodium and calcium carbonate (Rajeshwar et al 2009). The EC of soil water suspension revealed that soils of all geomorphic units were low in soluble salt content (0.21 to 1.12 dSm⁻¹). The cation exchange capacity varied from 3.10 to 26.80 cmol (p⁺) kg⁻¹ across all the geomorphic units. By and large, CEC was low which may be due to the dominance of illite mineral in these soils. However, higher CEC in some pedons may be ascribed to higher clay content and organic matter. The positively significant correlation (Table 3) between CEC with clay ($r = 0.73$) and silt ($r = 0.63$) may be ascribed to relatively large surface area with proportionately greater number of cation exchange sites compared to sand fraction (Miller and Donahue 1990, Papadopoulos et al 2009). This relationship also suggests that clay contributed to CEC of these soils. The organic carbon was less than one per cent except in surface horizons of forest areas and varied between 0.05 to 1.59, 0.03 to 0.60, 0.07 to 0.51 and 0.02 to 0.64% in Shivalik hills, piedmont plains, flood plains and alluvial plains, respectively. Sharma et al (2004) also reported higher organic carbon in Shivalik soils and ascribed this to high rainfall and comparatively low temperature (udic moisture regime condition). The organic carbon, by and large, decreased with depth which may be ascribed to the low biological activity and prevailing high temperature that is responsible for rapid mineralization of organic matter, as it is expected in subtropical and tropical climates (Bhat et al 2017). Calcium carbonate varied from 0.17 to 3.00% across all the geomorphic units and was absent in pedons 4, 5, 6, 7 and 11.

Absence or low amount of calcium carbonate in piedmont and Shivalik hills was due to high rainfall. Irregular distribution of calcium carbonate was observed which indicated the fluvial nature of these soils. Higher amount of calcium carbonate was observed in soils developed on old alluvial and flood plains as compared to Shivalik hills. Sawhney et al (1996) also reported the higher amount of calcium carbonate in the soils developed on the toe slopes which may be due to finer texture of soils that slow down the infiltration rate of water and/or accretion of carbonates due to lateral subsurface water flow. In general, the results indicated that geomorphic position affects soil properties.

Macronutrients

Available nitrogen: Available nitrogen was low to medium and varied from 152.32 to 313.60, 188.16 to 425.60, 125.44 to 403.2 and 188.16 to 380.80 kg ha⁻¹ in Shivalik hills, piedmont plains, flood plains and alluvial plains, respectively (Fig. 1). The soils of pedons 4 and 11 were medium in available nitrogen whereas rest of the pedons were low to medium. Available nitrogen was higher in surface layers and decreased down the profile which may be ascribed to the decreasing trend of organic carbon with depth and prevailing high temperature. A significant and positive correlation (Table 3) was observed between available nitrogen and organic carbon suggesting that nitrogen is closely associated with organic matter ($r = 0.48$). Hailu et al (2015) ascertained that the trends of total N, in general, followed that of the soil OM showing the strong relationship between OM and total N as indicated by the positive and highly significant ($r = 0.85$) correlation with OM. The positive and significant correlation

Table 3. Correlation among nutrients and physico-chemical properties

Parameter	pH	EC	OC	N	P	K	Fe	Mn	Cu	Zn	CEC	CaCO ₃	Clay	Sand	Silt
pH	1														
EC	0.12	1													
OC	-0.40**	0.27**	1												
N	-0.17	0.21*	0.48**	1											
P	-0.14	0.20*	0.40**	0.42**	1										
K	-0.06	0.39**	0.29**	0.40**	0.37**	1									
Fe	-0.63**	0.03	0.48**	0.45**	0.48**	0.14	1								
Mn	-0.41**	0.04	0.42**	0.26**	0.40**	0.10	0.61**	1							
Cu	-0.15	0.26*	0.52**	0.44**	0.39**	0.30**	0.54**	0.27**	1						
Zn	-0.11	0.20*	0.04	0.27**	0.32**	0.39**	0.29**	0.35**	0.12	1					
CEC	0.39**	0.51**	0.03	0.08	0.08	0.09	-0.23*	0.12	0.16	0.09	1				
CaCO ₃	0.38**	0.33**	0.05	0.10	0.12	0.23*	-0.17	-0.19	0.23*	-0.23*	0.41**	1			
Clay	0.32**	0.28**	0.04	0.09	0.20	0.22*	-0.08	0.22*	0.31**	0.16	0.73**	0.25*	1		
Sand	-0.22*	-0.34**	-0.14	-0.24*	-0.22*	-0.29**	0.07	-0.20	-0.30**	-0.13	-0.73**	-0.25*	-0.91**	1	
Silt	0.10	0.36**	0.20*	0.34**	0.21*	0.32**	-0.06	0.15	0.25*	0.08	0.63**	0.23*	0.71**	-0.93**	1

*, **. Significant at the 0.05 and 0.01 probability level, respectively

between nitrogen and silt ($r = 0.34$) and negative and significant correlation between nitrogen and sand content ($r = -0.24$) indicated that the finer fractions influence the nitrogen than coarser fractions. A positive and significant correlation was observed between N and P ($r = 0.42$) and N and K ($r = 0.40$) which suggests the synergistic effects.

Available phosphorous: The available phosphorous varied between 12.01 to 22.80, 16.80 to 34.34, 8.02 to 56.90 and 8.96 to 71.68 kg ha⁻¹ in Shivalik hills, piedmont plains, flood plains and alluvial plains, respectively (Fig. 2) and was, in general, low to medium with pedon 12 having higher values which may be ascribed to higher organic carbon. The variation in available phosphorus among geomorphic units may be ascribed to differences in parent material, slope gradient, organic matter and soil texture. The higher values of available phosphorus in alluvial plains may be due to addition of phosphatic fertilizers to these soils. Gyawali et al (2016) also reported higher values of available phosphorus in rice growing soils of Haryana. Ordinarily, available phosphorous decreased down the profile which might be attributed to the increment of the clay content that was found to increase with the depth and clay type which can cause fixation of phosphorus. Moreover, higher pH associated with higher calcium and magnesium may cause fixation of phosphorous. A significant positive correlation (Table 3) was observed between available phosphorous and organic carbon ($r = 0.40$) suggesting that higher available P is associated with higher organic matter. Organic matter increases P by anion replacement of $H_2PO_4^-$ ion on adsorption sites thereby increases the quantity of organic P mineralized to inorganic P (Havlin et al 2005, Bhat et al 2017).

Available potassium: The available potassium varied from 67.24 to 430.08, 94.08 to 487.60, 85.12 to 496.22 and 71.68 to 502.96 kg ha⁻¹ in Shivalik hills, piedmont plains, flood plains and alluvial plains, respectively (Fig. 3). Available potassium was higher in alluvial plains which may be ascribed to higher clay content and K bearing minerals like illite in these soils and low to medium in all other pedons because of low organic matter and coarser texture. In general, this nutrient decreased with depth across all the geomorphic units. The higher available K in alluvial plains may be assigned to the build-up of fertilizer potassium as farmers apply potassium fertilizers in higher quantities. The correlation analysis shows that available K had positive and significant correlation with organic carbon ($r = 0.295$), clay ($r = 0.220$), silt ($r = 0.327$) owing to presence of enough cation exchange sites and higher specific surface thereby retaining enough K on the exchange complex (Table 3). Moreover, it indicates the presence of potassium bearing minerals like feldspars, illite, mica in clay and silt fractions (Deka et al

1995, Reza et al 2014). However, available K was significantly and negatively correlated (Table 3) with sand ($r = -0.29$) which may be due to presence of quartz, the dominant mineral in the sand fraction, and does not retain K, therefore, affirmed the results of Reza et al (2014). The significant correlation between OC and N, P and K implies that depletion of soil organic matter would lead to a corresponding depletion of N, P and K.

DTPA-extractable Micronutrients

Iron: The DTPA extractable Fe content varied from 2.41 to 21.012, 3.21 to 20.24, 3.80 to 15.74 and 2.95 to 19.58 mg kg⁻¹ in Shivalik hills, piedmont plains, flood plains and alluvial plains, respectively (Fig. 4). Variation in Fe content among geomorphic units is noteworthy. The higher Fe content in Shivalik hills and piedmont plains as compared to flood and alluvial plains may be due to the higher moisture status because of the udic soil moisture regime. Najafi-Ghiri et al (2013) also reported that Fe content was higher in soils having aquic soil moisture as compared to ustic or aridic soil moisture regime. Considering the critical limit of DTPA extractable Fe for normal plant growth (Table 4), it can be inferred that pedons 1, 2, 3 were deficient, pedons, 7, 8, 9 and 10 were marginal, pedons 5, 11, 12 and 13 were sufficient and pedons 4 and 6 were high in iron content. The high Fe content in some soils may be due to presence of ferromagnesium minerals in parent material. Relatively higher content of available iron was observed in the surface horizons compared to subsurface horizons. This variability in Fe status is the attribution of mobility of Fe in the soil which is governed by its redox potential and various soil properties like pH, organic matter and moisture regimes. Sharma and Singh (2017) also reported that Fe content decreased with increase in depth. Simple correlation studies reveal that available Fe showed significant negative correlation (Table 3) with pH ($r = -0.63$) which might be due to the conversion of Fe^{2+} to Fe^{3+} or may be precipitation of Fe as insoluble $Fe(OH)_2$. Also, the presence of excess phosphates under alkaline conditions may be responsible for the formation of Fe phosphates thereby decreasing the concentration of Fe. A significant positive correlation (Table 3) was observed between Fe and

Table 4. Critical limits of micronutrients in soil (Lindsay and Norvell 1978)

Content	Micronutrients (mg kg ⁻¹)			
	Fe	Mn	Cu	Zn
Deficit	<4.5	<2.5	<0.2	<0.6
Marginal	4.5–9.0	2.5–3.5	0.2–0.4	0.6–1.2
Sufficient	9.0–18.0	3.5–7.0	0.4–0.8	1.2–2.4
High	18–27	>7.0	0.8–1.6	>2.4
Very high	>27		1.6–3.2	

OC ($r = 0.48$) suggesting strong bonding between Fe and OM that may be ascribed to the formation of complexes or chelates called as siderophores which are highly soluble. This explains why Fe is fairly mobile in soils of high pH including calcareous soils (Mengel et al 2001). Najafi-Ghiri et al (2013) attributed this relationship to exchange capacity of organic matter for Fe, chelating ability of organic compounds and acidifying property of organic matter. A negative but non-significant correlation was observed between Fe and clay suggesting that increase in clay content leads to decrease in DTPA extractable Fe. Likewise, significant and negative correlation was observed between Fe and CEC which confirmed the findings of Ibrahim et al (2011). The negative correlations of Fe with pH, clay, CEC, and CaCO_3 contents signifies that organic carbon would be an exclusive source of this nutrient to plants in these soils. Based on these observations, it can be deduced that Fe deficiency will become a serious problem in alkaline calcareous soils. The steady decrease in organic matter in the soil will exacerbate the incidence of Fe deficiency.

Manganese: The DTPA extractable Mn content varied from 3.27 to 19.95, 4.85 to 21.08, 2.04 to 10.90 and 5.70 to 16.98 mg kg^{-1} in Shivalik hills, piedmont plains, flood plains and alluvial plains, respectively (Fig. 5). Considering the critical limit of DTPA extractable Mn for normal plant growth it is deduced that the soils of pedon 9 were marginal, pedons 1, 2, 3 and 7 sufficient and pedons 4, 5, 6, 8, 10, 11, 12 and 13 were high in manganese content (Table 4). No systematic pattern of depth distribution of DTPA-extractable Mn was observed in these soils. In general, higher DTPA-extractable Mn was observed in surface horizons than the subsurface horizons. A positive and significant correlation (Table 3) was observed between Mn and organic carbon ($r = 0.42$). Sharma et al (2011) proposed that the increase of organic matter in soil enhanced the activity of microbes, which helps in the release of trace elements under specific conditions. Manganese was positively and significantly correlated (Table 3) with clay ($r = 0.22$) suggesting that fine textured soils had ample Mn compared to coarse textured soils which may be due to higher adsorption and retention of Mn by finer fractions. Manganese showed significant negative correlation (Table 3) with pH ($r = -0.41$) which may be attributed to conversion of magnous Mn to manganic oxides which are water insoluble and unavailable to plants (Vijayakumar et al 2011). Sharma et al (2005) also reported negative significant correlation between Mn and pH and evinced that among the various factors associated with DTPA-Mn, pH seemed to be the driving variable of Mn availability. Moreover, Katyal and Sharma (1991) reported that the soils having high pH exhibited lower DTPA-Mn. A

positive and significant correlation between Mn and other micronutrients suggests that these elements are functions of the same pedological factors which confirmed the findings of Verma et al (2007).

Copper: The DTPA extractable Cu content varied from 0.30 to 2.62, 0.20 to 0.92, 0.42 to 2.87 and 0.28 to 3.04 mg kg^{-1} in Shivalik hills, piedmont plains, flood plains and alluvial plains, respectively (Fig. 6). Considering the critical limit of DTPA extractable Cu for normal plant growth (Table 4), it is deduced that soils of pedons 3, 5, 7 were marginal, pedons 6 and 8 were sufficient, pedons 1, 2, 9, 10, 11, 12, and 13 were high and pedon 4 were very high in Cu content. No consistent distribution pattern was observed down the profile in all geomorphic units. The DTPA- Cu was higher in alluvial plains compared to Shivalik hills and piedmont plains which may be ascribed to contribution of associated fluvial parent materials. A significant positive correlation was observed between Cu and clay ($r = 0.31$) and significant negative correlation with sand content ($r = -0.30$) suggesting that the availability of Cu in soils is anticipated to decrease as the texture becomes coarser thereby validating widespread incidence of Cu deficiency in sandy soils (Sharma et al 2004). A positive and significant correlation was observed between Cu and CaCO_3 ($r = 0.23$) which implies that Cu may be fixed in CaCO_3 and is a constituent of clay minerals. However, Sharma et al (2015) observed negative correlation between Cu and CaCO_3 in major Soil Orders in semi-arid soils of Punjab, India. Copper had significant positive correlation with OC ($r = 0.52$) suggesting complex formation between OM and Cu. Moreover, the divalent Cu has a strong affinity to soil organic matter compared with other divalent cations (Mengel et al 2001) as indicated by the correlation coefficient. The significant correlation of Cu with soil texture and organic carbon suggests that greater the sand and organic carbon content, the greater the amount of copper extracted with DTPA as reported by Barona et al (1999).

Zinc: The DTPA extractable Zn content varied from 0.80 to 1.80, 1.20 to 4.92, 0.11 to 1.20 and 1.00 to 2.80 mg kg^{-1} in Shivalik hills, piedmont plains, flood plains and alluvial plains, respectively (Fig. 7). The higher content of Zn was observed in piedmont plains as compared to Shivalik hills and alluvial plains which may be due to the specific geographic location and land use change in the study area. Also, the higher calcium carbonate in flood and alluvial plains exhibits higher capacity for total Zn adsorption and low capacity for exchangeable Zn. Najafi-Ghiri et al (2013) also reported that alluvial soils had lowest content of Zn compared to piedmont plains and plateaus. Considering the critical limit of DTPA extractable Zn for normal plant growth (Table 4), it is deduced that almost all soils of pedons 8 and 9 were deficit, pedons 1,

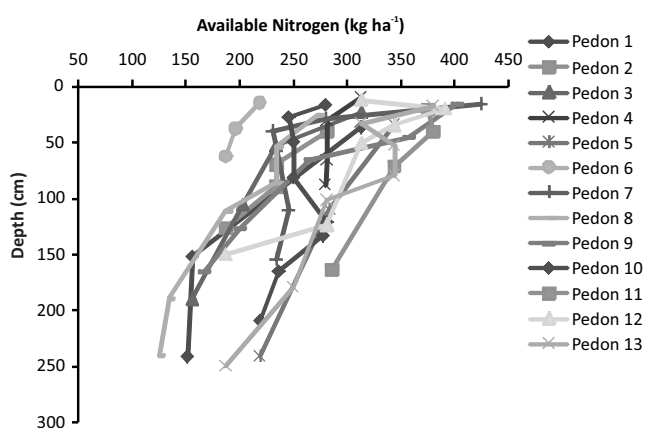


Fig. 1. Vertical distribution of nitrogen in soils of different geomorphic units of Haryana

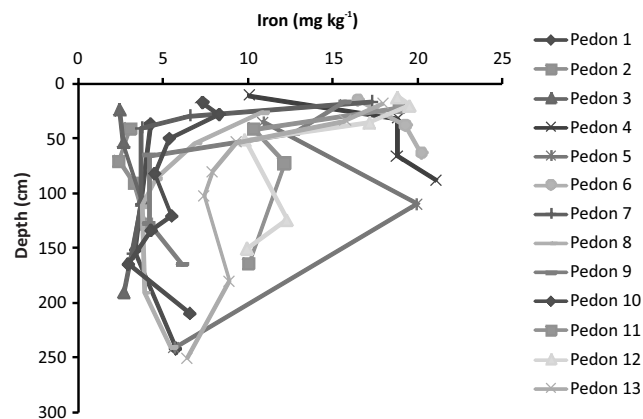


Fig. 4. Vertical distribution of iron in soils of different geomorphic units of Haryana

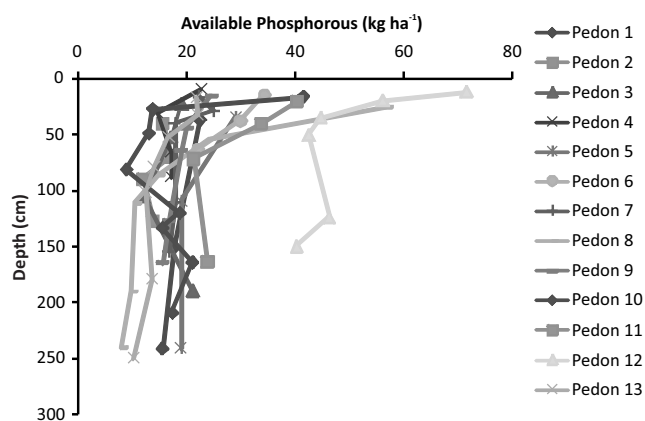


Fig. 2. Vertical distribution of phosphorous in soils of different geomorphic units of Haryana

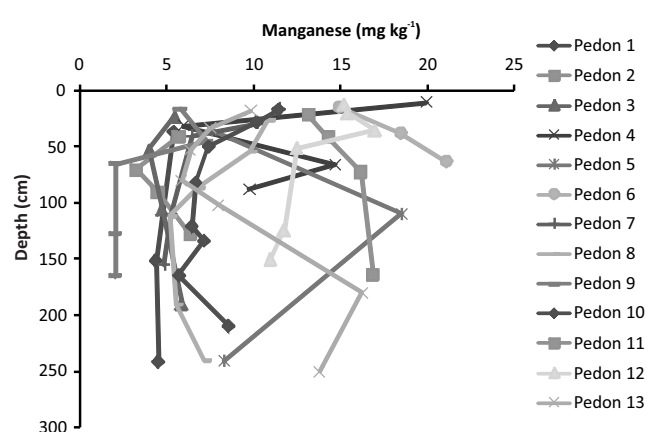


Fig. 5. Vertical distribution of manganese in soils of different geomorphic units of Haryana

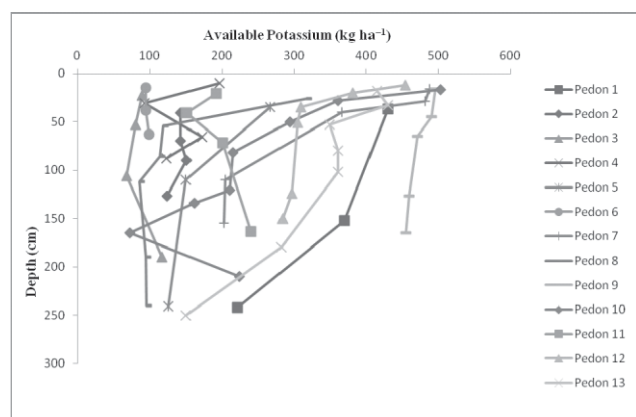


Fig. 3. Vertical distribution of phosphorous in soils of different geomorphic units of Haryana

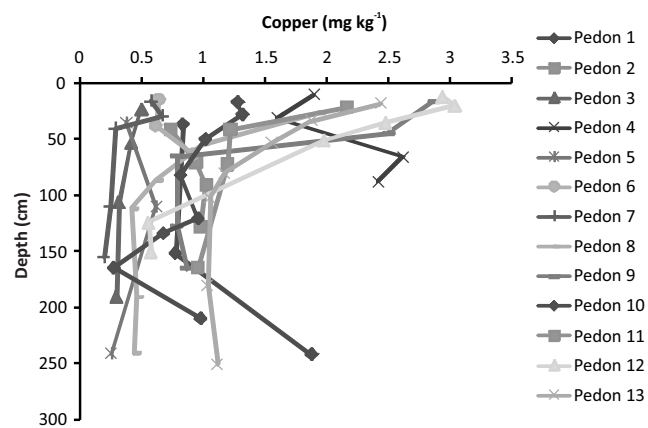


Fig. 6. Vertical distribution of manganese in soils of different geomorphic units of Haryana

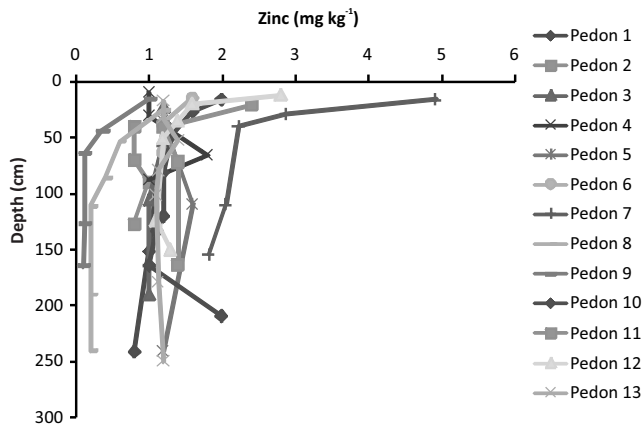


Fig. 7. Vertical distribution of zinc in soils of different geomorphic units of Haryana

2, 3, 13 were marginal and pedons 4, 5, 6, 7, 10, 11 and 12 were sufficient in Zn content. Zinc showed irregular trend with increase in depth. However, surface horizons have higher Zn content which could result from biomineralization and turnover by plant residues. Similar results were reported by Sharma et al (2005) in some Alfisols of Punjab. Available zinc showed significant negative correlation (Table 3) with calcium carbonate ($r = -0.24$) which may be attributed to adsorption of Zn by CO_3^{2-} , precipitation as ZnCO_3 or formation of insoluble calcium zincate thereby lowering availability of zinc (Rajput et al 2015). This relationship suggests that CaCO_3 is the most influential soil characteristic that predicts Zn availability. The positive and significant correlation between Zn and P indicates a relationship between application of phosphatic fertilizers and sources of zinc. The positive and significant correlation between NPK and micronutrients suggests that the levels of NPK can affect the availability and distribution of micronutrients. The variation of micronutrients among the geomorphic units may be ascribed to differences in parent material, topography, climate, biochemical and geochemical processes in the soil. In general, the values of extractable micronutrients in the soils decreased irregularly down the profile which may be assigned to leaching effect on micronutrients and then pumping effect from the underlying soil. Moreover, the decrease with depth confirms that these nutrients have been phytomined and redeposited on the surface with organic matter.

Principal component analysis

The principal component analysis limits the variables and extracts smaller number of independent factors (principal components) for deducing the association among observed variables. Principal component analysis was performed by varimax rotation with Kaiser Normalization. Varimax rotation was employed because orthogonal rotation

Table 5. Principal component analysis of soil parameters of major geomorphic units of Haryana

Parameter	PC1	PC2	PC3	PC4	PC5
N	0.088	0.631	0.326	0.008	0.065
P	0.087	0.635	0.214	-0.156	0.291
K	0.04	0.252	0.828	-0.081	0.286
Fe	-0.184	0.823	0.007	-0.301	-0.127
Mn	0.204	0.613	-0.075	-0.499	-0.078
Cu	0.186	0.749	0.131	0.205	0.121
Zn	0.092	0.126	0.44	-0.69	0.123
OC	0.055	0.744	0.217	0.148	-0.283
pH	0.305	-0.491	-0.024	0.321	0.613
EC	0.426	0.064	0.638	0.164	-0.158
CaCO_3	0.263	0.058	0.287	0.723	0.251
Sand	-0.90	-0.17	-0.093	0.04	-0.191
Silt	0.804	0.194	0.165	0.008	0.058
Clay	0.865	0.115	0.003	-0.087	0.316
CEC	0.924	-0.076	0.164	0.128	0.011
Eigen values	5.545	3.868	1.81	1.433	1.27
Variance (%)	29.18	20.36	9.53	7.54	6.68
Cumulative variance (%)	29.18	49.54	59.07	66.61	73.29

minimizes the number of variables with a high loading on each component and therefore facilitates the interpretation of PCA results. The number of significant principal components was selected in view of the Kaiser criterion with eigenvalue greater than 1 (Kaiser 1960). Five principal components (PC1, PC2, PC3, PC4 and PC5) with eigenvalues greater than 1 were extracted (Table 5). PCA led to reduction of the initial dimension of the dataset to five components which explains 73.29% of the total dataset variance. The PC1 is the most important component and explains 29.18% of total variance having a strong positive loading of silt, clay and CEC whereas negative loading of sand thereby can be defined as the soil texture component. This hypothesis can be explained by the correlation between CEC and soil texture (positive with clay and silt and negative with sand). Papadopoulos et al (2009) also reported high factor loadings for soil texture in soils of western Macedonia. PC1 may be explained as the lithogenic factor indicating that these parameters are governed by parent material. PC2 has positive loadings of N, P, Fe, Mn, Cu and OC and accounted for 20.36% of the total variance thereby represents soil fertility in terms of nutrients. This component may be deemed as influenced by organic matter since positive and significant correlation was observed between these variables and organic carbon. PC3 exhibited positive loadings of available K and EC and explained 9.52% of the total variance. This component

seems to be arisen from a different source such as fertilizers. The PC4 showed positive loading for CaCO_3 and negative loading of Zn and can be related to geochemical weathering. This component accounted for 7.54% of the total variance. The PC5 exhibited positive loadings of pH suggesting sodicity factor since high pH is associated with high exchangeable sodium percentage levels as found in low lying old alluvial plains. The variance explained by PC5 was 6.68% and it also indicates the translocation of Na^+ in the pedons. Principal component loadings describe the amount of variance of a given variable accounted by principal components (Lattin et al 2003). Although these PC factors do not account for the total variation of the whole set of the variables, PCA is deemed as rational dimension reduction method and PC factors can be properly interpreted.

CONCLUSION

The profiles high in the landscape are generally impoverished while those in depressions are enriched. By and large, DTPA-extractable micronutrient content of surface horizons was higher compared to lower horizons because of the accrual of organic matter in surface horizons by biological processes linked with natural vegetation and crop production seems to have ensued in relatively higher extractable values. The elemental distribution in the soils is a function of geochemical and pedogenic processes acting over the parent material. A positive and significant correlation was observed among the micronutrients except Zn and Cu suggesting that these elements are functions of the same pedological factors. Principal component analysis was shown to be a major tool to extract information from a complex dataset. In this case, it revealed that five principal components accounted for 73.29% of the total variance.

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Preparation of Osmo-dried Peel Sticks From Galgal

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Abstract: An experiment was conducted at Division of Food Science and Technology, SKUAST-Jammu during the year 2017-2018 to prepare osmo-dried peel sticks from galgal peel. The osmo-dried galgal peel sticks were made by using different treatment combinations *i.e.* dipping concentration of sugar and jaggery solution of 30, 40 and 50°Brix. The osmo-dried galgal peel sticks were stored for 3 months and the chemical and organoleptic changes were recorded at an interval of one month. The maximum values of TSS and ascorbic acid of 54.02°Brix and 120.05mg/100g. However, with an advancement of storage period an increasing trend was observed in TSS but decreasing trend in titratable acidity, moisture, ascorbic acid and carotenoids during three month of storage. Treatment 50° Brix with sugar showed maximum value (8.19) of overall acceptability during storage. After completion of three months storage period, treatment T₃: 50° Brix with Sugar was more acceptable in comparison to the other treatments.

Keywords: Galgal, Peel, Value addition

Citrus fruits are one of the world's most important fruit crops, and are known for their nutritive values and special aroma. Citrus is mainly consumed as fresh fruit or juice. In India, citrus crop is cultivated on an area of about 1003 m ha with a production of about 12546 MT (Anonymous 2018). In Jammu and Kashmir State, citrus crop is grown on an area of about 2565 m ha with a production of 7337 MTs (Anonymous 2019). Lemon fruit is only utilized for pickle making, culinary purposes and for blending with other fruit juices for squash preparation at small scale. Left over pomace and peel of fruit after juice extraction is discarded as waste. For citrus processing industry, disposal of peel and other residual material is a big problem. Peel of citrus 25-35% of fruit weight and is quite nutritious but it goes waste and do not fetch any economical price to the industry or grower (Attri and Maini 1996). The other useful option is to utilize waste peel for preparation of peel candy. Hence, citrus processing industry can easily opt for making candied peel which finds a ready market in confectionery. The candied citrus peel is used in the baking industry in the preparation of cakes, cookies and fruit breads. The judicious processing of waste peel into candy will not only add to the income of processing industry but it will provide remunerative price to growers as well (Mehta et al 2005). Considering the importance of nutrition value of citrus peel, this experiment was planned with the objectives of utilize kandi lemon (Galgal) waste into value added product and study the effect of storage on quality of finished product.

MATERIAL AND METHODS

The lab study was conducted at Main Campus Chatha, SKUAST-Jammu; J&K. Galgal fruit was procured from local market. Juice was used for making a squash and the waste of

galgal peel were used for making osmo-dried galgal peel sticks. The dried and bruised peels were thoroughly sorted out manually. Peels of almost same size were selected for processing. The graded peels were washed thoroughly under running tap water to remove dust and dirt from the surface. The peels were cut uniformly about 2 to 3 cm length and 0.5 to 0.8 cm breadth. Osmo-dried galgal peel sticks were prepared by six treatments *i.e.* 30, 40% and 50% with sugar and jaggery syrup, respectively. Galgal peels were then blanched for 2 minutes followed by steeping in sugar and jaggery syrup for 20 h as per the treatment combinations. After completion of steeping time, sugar and jaggery syrup was drained and peel sticks were spread out on trays. Then osmo-dried peel sticks were kept for drying in cabinet dryer for 5-6 h at 60°C temperature. The dried samples were collected and packed in LDPE bags for storage purpose. Galgal peel sticks were stored at room temperature for evaluating changes in chemical constituents and organoleptic quality at monthly interval during three months storage. Products were analyzed for TSS, acidity, moisture, ascorbic acid and carotenoids as per the procedure of (Ranganna 2006). Organoleptic evaluation was carried out by semi-trained panelists on 9-point hedonic rating scale (Amerine et al 1965). The data obtained was statistically analyzed using CRD factorial.

RESULTS AND DISCUSSION

Total soluble solids (TSS): Effect of different treatments on total soluble solids of osmo-dried galgal peel sticks was significant during storage (Table 1) and indicated a slight increase in the total soluble solids content with the advancement of storage. The mean value total soluble solids

Titrateable acidity: Titrateable acidity of osmo-dried galgal peel sticks indicated a slight decrease in titrateable acidity during storage (Table 1). The acidity of osmo-dried galgal peel sticks decreased to 0.53 per cent after 3 months of storage from the initial value of 0.57 per cent. The significantly higher titrateable acidity value (0.57%) was in T_4 which was followed by T_5 , T_1 and T_2 , whereas the statistically lower acidity (0.51%) was in T_3 . Statistically

Moisture: A significant decrease in moisture content of osmo-dried galgal peel sticks was observed during storage (Table 2). The overall effect of treatments and storage period on moisture of osmo-dried galgal peel sticks was significant. The moisture content decreased to 13.34% at 3 months from an initial value of 14.85%. After three months of storage higher moisture content (15.19%) was in T_1 whereas the initial value significantly was 15.82% at 0 month of storage. The mean maximum moisture content was in T_1 treatment followed by T_4 and T_2 . Similar trend of decreasing moisture content during storage were observed by Devi (2014) in dehydrated wild pear halves, Chavan et al (2010) in osmo-

Treatments		TSS (°Brix)					Acidity (%)				
		Storage period (Months)					Storage period (Months)				
		0	1	2	3	Mean	0	1	2	3	Mean
T ₁ : 30° Brix with Sugar		34.44	36.00	38.00	39.00	36.86	0.59	0.58	0.55	0.53	0.56
T ₂ : 40° Brix with Sugar		45.33	47.66	48.00	49.55	47.64	0.57	0.56	0.56	0.55	0.56
T ₃ : 50° Brix with Sugar		51.66	52.90	54.84	56.67	54.02	0.54	0.52	0.50	0.48	0.51
T ₄ : 30° Brix with Jaggery		34.00	35.95	37.92	38.90	36.69	0.60	0.58	0.56	0.54	0.57
T ₅ : 40° Brix with Jaggery		45.10	47.00	47.80	49.00	47.23	0.58	0.57	0.56	0.54	0.56
T ₆ : 50° Brix with Jaggery		51.00	52.70	54.00	56.00	53.43	0.55	0.54	0.53	0.52	0.54
Mean		43.59	45.37	46.76	48.19		0.57	0.56	0.54	0.53	
Factors	CD (5%)	CD (5%)									
Treatments	0.65	0.008									
Storage	0.53	0.006									
Treatments x Storage	1.29	0.02									

Treatments		Moisture (%)					Ascorbic acid (mg 100 g ⁻¹)				
		Storage period (Months)					Storage period (Months)				
		0	1	2	3	Mean	0	1	2	3	Mean
T ₁ : 30° Brix with Sugar		15.82	15.28	15.00	14.67	15.19	122.40	115.34	113.67	111.60	115.75
T ₂ : 40° Brix with Sugar		14.91	14.70	13.98	13.26	14.21	123.60	119.60	117.20	114.30	118.68
T ₃ : 50° Brix with Sugar		13.98	13.20	12.99	12.60	13.19	125.80	120.56	118.62	115.20	120.05
T ₄ : 30° Brix with Jaggery		15.80	15.20	14.92	14.52	15.11	122.00	114.96	112.20	110.90	115.02
T ₅ : 40° Brix with Jaggery		14.82	14.19	13.03	13.00	13.76	123.50	119.00	117.14	114.32	118.49
T ₆ : 50° Brix with Jaggery		13.78	13.10	12.70	12.00	12.90	125.70	118.82	116.20	114.96	118.92
Mean		14.85	14.28	13.77	13.34		123.83	118.05	115.84	113.55	
Factors	CD (5%)	CD (5%)									
Treatments	0.19	1.39									
Storage	0.16	1.14									
Treatments x Storage	0.38	2.78									

Table 3. Effect of treatments and storage periods on carotenoids and overall acceptability quality of galgal peel sticks

Treatments	Carotenoids (mg 100 g ⁻¹)					Organoleptic quality				
	Storage period (Months)					Storage period (months)				
	0	1	2	3	Mean	0	1	2	3	Mean
T ₁ : 30° Brix with Sugar	26.0	24.0	22.0	21.0	23.3	6.65	6.50	6.00	5.85	6.25
T ₂ : 40° Brix with Sugar	24.0	23.0	21.0	20.0	22.0	7.28	7.08	7.00	5.95	6.83
T ₃ : 50° Brix with Sugar	23.0	22.0	20.0	19.0	21.0	8.63	8.36	8.00	7.76	8.19
T ₄ : 30° Brix with Jaggery	25.0	23.0	21.0	20.0	22.3	6.60	6.42	5.78	5.70	6.13
T ₅ : 40° Brix with Jaggery	23.0	21.0	20.0	19.0	20.8	7.00	7.02	6.84	5.84	6.68
T ₆ : 50° Brix with Jaggery	22.0	20.0	18.0	18.0	19.5	8.52	8.16	7.88	7.50	8.02
Mean	23.8	22.2	20.3	19.5		7.45	7.26	6.92	6.43	
Factors	CD (5%)	CD (5%)								
Treatments	0.32	0.10								
Storage	0.26	0.08								
Treatments x Storage	0.64	0.20								

dried banana slices and Amitabh et al (2000) in osmotically dehydrated mango slices.

Ascorbic acid: The mean level of ascorbic acid in osmo-dried galgal peel sticks was found to be 113.55 mg 100 g⁻¹ against its initial value of 123.83 mg 100 g⁻¹ after three months storage period (Table 2). However, the mean values of ascorbic acid at the end of each storage (i.e 0, 1, 2 and 3 month) differed significantly from each other. The significantly highest ascorbic acid content (120.05) was in T₃ which was statistically at par with T₆ and T₂. In ascorbic acid T₂, T₅ and T₆ were at par with each other. Statistically significant differences were among the interaction between storage intervals and treatments. Similar results of decrease in ascorbic acid content during storage. The degradation of ascorbic acid upon storage has been implicated partially to its oxidation to 2, 3-diketogulonic acid and finally to furfural compounds and partially to its involvement in browning reactions in the presence of high acidic environment. Similar results were also noticed by Sharma et al (2006) in dehydrated apple rings.

Carotenoids: Different osmotic treatments have a significant effect on carotenoids content of osmo-dried galgal peel sticks. Maximum carotenoids content was in T₁ (23.3 mg 100 g⁻¹) which was significantly different from rest of the samples. However, the carotenoids in T₂ and T₄ were statistically at par with each other. Among the osmotic treatments, lowest value (19.50 mg 100 g⁻¹) of carotenoids was in T₆. Different osmotic treatments significantly affected the carotenoids content after three months of storage and it decreased to 19.5 mg 100 g⁻¹ from the initial value of 23.8 mg 100 g⁻¹. The carotenoids content in osmo-dried galgal peel sticks decreased during the subsequent storage period in comparison to initial content (Table 3). The loss of carotenoids content in processed samples was mainly due to

leaching in syrup, oxidation as well as thermal degradation. These results are also inconformity with the findings of Kumar et al (2008) and Sra et al (2011).

Overall acceptability: The treatments had significant decrease on overall acceptability of osmo-dried galgal peel sticks during storage period (Table 3). Osmo-dried peel sticks showed significant decrease in overall acceptability during storage period and the mean value decreased from 7.45 to 6.43. Osmo-dried galgal peel sticks showed maximum score during zero day for treatment T₃ and minimum score with T₁ and during the different storage periods same results were observed. The interaction between treatment and storage period was also significant. Treatment combination T₃ remained the most acceptable during the storage period due to the high sugar concentration (50%). Similar results were also reported by Patil et al (2014) in sensory quality and economics of preparation of karonda candy and Gupta and Kaul (2013) during study of sugar concentration and time interval on quality and storability of berchuhara.

CONCLUSION

The osmo-dried Galgal peel sticks prepared from 50°Brix with sugar is organoleptically the best for the commercialization purpose. However, it also aids for utilization of Galgal peel waste to enhance the economic status of the farmer.

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Epicotyl Grafting: A New Method for Propagation in Walnut (*Juglans regia* L.)

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Abstract: Walnut is very difficult to propagate and its multiplication is mainly done in the open field conditions, which generally takes two to three years for production of grafted nursery plants. For rapid multiplication of walnut plants, the present study was conducted in polyhouse conditions to standardize the method and time of epicotyl grafting with three methods of epicotyl grafting viz. cleft, wedge and tongue performed on 15, 30 and 45-days-old epicotyls. The cleft grafting done on 15-days-old epicotyls gave highest percentage of bud-sprouting (94.66 %), followed by wedge grafting which resulted in 90 per cent success. The highest total linear growth, scion girth and leaf area were produced by wedge method performed on 15-days-old epicotyls. On the basis of two years study, it is concluded that wedge method of grafting on 15-days-old epicotyls is recommended for commercial multiplication of nursery plants of walnut in a year under protective conditions.

Keywords: Epicotyls, Walnut, Bud-sprouting percentage, Grafting

The Persian walnut (*Juglans regia* L.) known as English walnut is one of the important nut fruit of the world. It is grown extensively in USA, China, France, Italy, Turkey, Poland, Yugoslavia, Rumania and Iran. In India, it is grown mainly in the states of Jammu and Kashmir, Himachal Pradesh, Uttarakhand and Arunachal Pradesh in an area of 1,25,210 hectares with an annual production of 2,05,730 MT (Anonymous 2015). Although there is tremendous scope for its cultivation in entire Himalayan region between elevations of 1200 to 2500 m amsl, yet its commercial has remained in a state of neglect particularly in Himachal Pradesh and Uttarakhand. At present, the walnut is grown as scattered trees and not in the form of well laid out regular plantation or orchard. The majorities of trees are of seedling origin, which has a long gestation period and are highly variable producing nuts of inferior quality with low shelling. The non-availability of grafted plants of superior cultivars due to lack of suitable propagation technique(s) is the major constraints in extending the area under walnut cultivation in the state as well as in the country. For a long time, propagation through seed was only method available for walnut multiplication, though this practice gives rise to highly variable plants. Some efforts have been made on the standardization of propagation techniques using grafting, budding and hot-callusing technique and these methods have been reported to give varying degree of success under different environmental conditions to which the plants are subjected before and after propagation. The traditional production of grafted walnut plants in the nursery needs 2-3 years, which is long-term and labour consuming. Hence, a study was

undertaken to evaluate the feasibility of using epicotyl grafting for propagation of walnut and secondly to reduce the nursery production period from two years to one year.

MATERIAL AND METHODS

The experiment was carried out in 2015 and 2016 at Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan. The seeds of common walnut (*Juglans regia* L.) selected for obtaining rootstocks, were immersed in water at the middle of December. After 3 days, they were taken out of water and put for stratification in alternate layers of moist sand in plastic bags at a temperature of 3-4 °C for 70-80 days. After the seeds cracked open, they were sown in black polythene bags (45.72×22.86 cm) containing mixture of soil, FYM and sand (2: 1: 1) and polybags were kept in a non-heated polyhouse. The optimum moisture for seed germination is maintained in the growing media of polybags by regular irrigation. After seed germination, 15, 30 and 45-days-old epicotyls were grafted with cleft, wedge and tongue methods using a dormant scion. The scions of cultivar Prialara were collected from mother trees during dormancy and covered with a moist sphagnum moss grass, wrapped with polythene and then stored in cold storage at 3-4°C until the time of grafting. Day first, when the plumule just begin to sprout was taken as reference date for counting total number of days and called as age of rootstocks. Terminal portion of scions 6-7 cm long was used for epicotyl grafting. The grafting was performed on 15 (1st week of April), 30 (last week of April) and 45 (1st week of May) –days-old epicotyls with cleft, wedge and tongue (Fig. 1-3) methods of grafting.

During grafting the vascular cambium of scion and rootstock were accorded with each other and the graft joint was carefully wrapped with polythene strip.

The experiment was laid-out in a completely randomized design with three methods of grafting and each treatment was replicated thrice having 50 seedlings per replication. The bud sprouting was recorded by counting the number of plants sprouted after grafting and graft-take success was recorded after 60 days of grafting by counting the survival of grafted plants. The total linear growth, scion girth and saleable plants were observed after the cessation of growth in December. The number of leaves and leaf area were recorded in August. Ten fully expanded leaves were detached from the plant and leaf area was measured with the help of Licor-3100 leaf area meter. For rapid multiplication with increased graft-take success rate of walnut plants, the present study was conducted in polyhouse conditions. Suitable temperature and high relative humidity during and after grafting had a positive effect on graft-take success under nursery conditions. Highest graft-take percentage was determined under conditions with 27°C and 80 % relative humidity.

RESULTS AND DISCUSSION

Epicotyl grafting methods and rootstock age significantly influenced the bud-sprouting (Fig. 4) and graft-take success (Fig. 5) during both the years. Among the three methods of epicotyl grafting, cleft method showed maximum bud-sprouting (85.34 and 87.33 %) and graft-take success (79.32 and 81.27 %) during 2015 and 2016, respectively, followed by wedge grafting. The comparatively higher percentage of bud-sprouting and graft-take success in cleft and wedge methods of epicotyl grafting may be due to more intimate contact and lining up of cambial layers of rootstock and scion. Results are in accordance with Gandev (2009). Similarly, Suk-In et al (2006) recorded higher graft-take success with cleft method of grafting in walnut. The epicotyls grafted with tongue method gave lowest bud-sprouting (62.76 and 65.55%) and graft-take success during 2015 and 2016, respectively (Table 1).

The juvenile epicotyls produced early bud-sprouting and higher bud-sprouting and graft-take success, which decreased with the age of rootstock. The higher percentage of bud-sprouting (86.02 and 88.33) was on 15 days old

Table 1. Effect of different methods of epicotyl grafting and age of rootstocks on bud-sprouting (%), linear growth (cm) and scion girth (cm) in walnut

Methods of grafting	Age of rootstocks						Mean	
	15 days		30 days		45 days			
	2015	2016	2015	2016	2015	2016	2015	2016
Bud-sprouting (%)								
Cleft	92.51 (74.12)	94.66 (76.62)	87.08 (68.93)	89.00 (70.61)	76.42 (60.93)	78.33 (62.23)	85.34 (67.99)	87.33 (69.82)
Wedge	88.28 (69.96)	90.00 (71.55)	86.61 (68.52)	88.00 (69.71)	74.26 (59.49)	76.00 (60.64)	83.05 (65.99)	84.66 (67.30)
Tongue	77.28 (61.51)	80.33 (63.65)	57.76 (49.44)	60.00 (50.74)	53.25 (46.84)	56.33 (48.61)	62.76 (52.60)	65.55 (54.33)
Mean	86.02 (68.53)	88.33 (70.61)	77.15 (62.30)	79.00 (63.69)	67.97 (55.75)	70.22 (57.16)		
Linear growth (cm)								
Cleft	22.49	24.96	21.62	23.67	19.90	21.92	21.34	23.52
Wedge	33.04	35.14	28.07	30.68	26.89	28.34	29.33	31.38
Tongue	29.60	31.19	25.75	27.75	21.65	24.45	25.67	27.80
Mean	28.38	30.43	25.15	27.37	22.81	24.90		
Scion girth (cm)								
Cleft	0.87	0.90	0.76	0.79	0.82	0.85	0.81	0.85
Wedge	1.21	1.23	1.14	1.17	0.80	0.83	1.05	1.08
Tongue	0.91	0.93	0.86	0.89	0.77	0.80	0.84	0.87
Mean	0.99	1.02	0.92	0.95	0.79	0.83		
CD (0.05)	Bud-sprouting		Linear growth		Scion girth			
	2015	2016	2015	2016	2015	2016		
Methods of grafting	0.97	0.60	1.11	0.38	0.009	0.08		
Age of rootstocks	0.97	0.60	1.11	0.38	0.009	0.08		
Method x age	1.69	1.04	1.93	0.66	0.016	0.14		



Fig. 1. Cleft grafting



Fig. 2. Wedge grafting



Fig. 3. Tongue grafting



Fig. 4. Bud-sprouting in wedge grafting



Fig. 5. Graft-take success in wedge grafting

epicotyls, which decreased with the age of rootstock and lowest bud-sprouting and graft-take success was on 45-days-old epicotyls (Table 1). The early sprouting in wedge grafting on 15-days-old epicotyls may be attributed to the better graft union due to more cambial contact of stock and scion and initiation of subsequent scion growth. These results are in line with those of Upadhyaya et al (2014) and Kumar and Thakur (2016), who reported that epicotyl grafting performed with wedge method on 10-days-old rootstocks showed early sprouting than those grafted on 20-days-old rootstocks.

The higher activity of meristematic cells in young or juvenile rootstock resulted in faster callus formation and quick healing of graft union may accounted for better graft-take success on 15-days-old rootstock. The phenolic content increases with the increase in age of rootstocks, which interfere in graft healing process and adversely affect on graft-take success. It is observed that after 20-days of seed germination the accumulation of phenolic compound like 4-hydroxynaphthoquinone was more, which resulted in poor callus formation and graft-take success in walnut. The more accumulation of phenolic compound in 45-days-old rootstock may account for poor callus formation and graft take success in the present study. Scion wood provides the hormones (auxins and gibberellins) necessary for cambial activity and for the differentiation of callus tissue formed to bridge the connection between scion and rootstock. Higher

concentration of phenols alters the activity of IAA oxidase and transport of IAA. Phenol oxidation accompanied by the protein deposition to produce in soluble polyphenols to form dark necrotic layer when, grafting, which affected the grafting survival by cutting off the inter change between the scion and stock of the graft union. Results are in accordance with Qureshi et al (2014), who reported comparatively highest graft-take success and linear growth in epicotyl grafting with cleft method. Gandev (2009) and Suk-In et al (2006) observed higher bud sprouting percentage on epicotyls with cleft method performed on juvenile rootstock. Epicotyl grafting success declined in interaction indicates that minimum graft-take was obtained with tongue method on 45-days-old rootstocks. The vegetative growth was significantly influenced by methods of epicotyl grafting and age of the rootstocks (Tables 1 and 2). The highest linear growth (29.33 and 31.38 cm), scion girth (1.05 and 1.08 cm), number of leaves (11.47 and 12.01) and leaf area (290.51 and 294.73 cm²) during 2015 and 2016, respectively was in wedge grafting, which was significantly higher than tongue and cleft methods. Among the age of rootstocks, grafting performed on 15-days-old rootstock attained maximum vegetative growth. Islam et al (2003) recorded maximum number of leaves on 14-days-old rootstocks than 21-days-old seedling rootstocks in epicotyl grafts of jackfruit. The results pertaining to maximum leaf area are in line with findings of Jha and Brahmachari (2002), who reported the higher values for leaf area in epicotyl grafting on

Table 2. Effect of different methods of epicotyl grafting and age of rootstocks on number of leaves and leaf area (cm²) in walnut

Methods of grafting	Age of rootstocks						Mean	
	15 days		30 days		45 days			
	2015	2016	2015	2016	2015	2016	2015	2016
Number of leaves								
Cleft	10.57	10.64	9.69	9.88	9.57	9.62	9.94	10.04
Wedge	13.47	13.89	10.90	11.39	10.04	10.74	11.47	12.01
Tongue	12.96	13.62	10.82	11.44	10.23	10.87	11.34	11.98
Mean	12.33	12.72	10.47	10.90	9.95	10.41		
Leaf area (cm ²)								
Cleft	295.60	297.27	249.22	251.89	303.60	309.60	282.81	286.25
Wedge	256.22	257.22	339.83	349.16	275.48	277.81	290.51	294.73
Tongue	368.55	372.88	238.88	240.52	263.53	276.20	290.32	293.53
Mean	306.79	309.12	275.97	280.52	280.87	284.87		
CD (0.05)								
	Number of leaves		Leaf area					
	2015	2016	2015	2016				
Methods of grafting	0.62	0.46	1.24	0.84				
Age of rootstocks	0.62	0.46	1.24	0.84				
Method x age	1.07	0.79	1.15	1.45				

one-week-old seedling rootstocks than two-weeks-old rootstocks. There was significant effect of interaction on vegetative growth parameters. The highest linear growth, scion girth, leaf area and per cent saleable plants were observed with wedge grafting on 15-days-old rootstock. The lowest linear growth, scion girth, leaf area and saleable plants were found in cleft grafting on 45-days-old epicotyls. The higher growth parameters in wedge grafted plants on 15-days-old epicotyls may be due to fast healing of graft union which accounted for early bud-sprouting and scion growth.

CONCLUSION

The cleft and wedge grafting performed on 15 days old rootstocks gave highest bud sprouting percentage, however growth was better in wedge grafting and more than 94 per cent plants attain a saleable size in a year. The wedge method of grafting on 15 days old epicotyls is recommended for commercial multiplication of nursery plants of walnut in a year under protective conditions.

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Quality Evaluation and Storage Stability of Spinach Puree Enriched Instant Noodles

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Abstract: Instant noodles were prepared by adding spinach puree at 0,10,20,30,40 and 50gm puree per 100 gm flour. The noodles were evaluated for its various quality characteristics viz. cooking quality, proximate constituents and sensorial characteristics; followed by evaluation of its storage quality during the storage of 12 months. The cooking time was reduced drastically with addition of spinach puree in noodles. The cooking loss and swelling index decreased progressively with increase in level of puree in composite flour. Cooking weight increased linearly, protein and fat remained unchanged, whereas ash and fiber increased, and carbohydrates were decreased with increase in level of spinach puree in noodles. The sensory quality with respect to colour, texture and overall acceptability of noodles were improved. Noodles with 40gm puree per 100 gm flour scored highest. The results pertaining to storage studies revealed that moisture content increased significantly and progressively during storage of 12 months in LDPE and HDPE. However, increase in moisture content was more pronounced after 6 months. Peroxide value and free fatty acids were increased marginally during storage in both LDPE and HDPE and the values were within the prescribed limits. There was no yeast and mould growth during 6 months of storage in both the packages. The total plate count increased with increase in the moisture content and it was more pronounced in LDPE as compared to HDPE pouches. The microbial load was well within the tolerable limits during storage.

Keywords: Instant noodles, Spinach puree, Storage study, Quality

Normally noodles are rich in starch but depleted in dietary fiber, vitamins, minerals and other bioactive compounds. Children generally do not prefer vegetables through their diet but they prefer to consume the vegetables through their liking food items such as noodles. Hence, efforts have been made to incorporate some green leafy vegetables which are rich in vitamins, minerals, chlorophyll, carotenoids and other bioactive compounds. The bioactivity of various minerals from leafy vegetables was found to be 25 % more as compared to non-green vegetables (Gupta et al 2006). Spinach particularly contains flavonoids, vitamin C, vitamin E and phenolic compounds. Hence, it is also an excellent source of antioxidant to control free radical formation in body (Bergquist 2006). Therefore, present investigation was aimed to prepare the composite noodles by incorporating spinach puree and study its effect on the sensorial attributes, cooking quality, proximate composition and storage stability.

MATERIAL AND METHODS

Preparation of spinach puree: The fresh spinach leaves were sorted and made free from petioles. The leaves were washed in running tap water and were steamed for about 3 min. and passed through grinder without adding any water. Smooth paste obtained was quizzed through muslin cloth and fine puree was obtained (Fig.1).

Preparation of noodles: Blends were prepared by adding

spinach puree at 0, 10, 20, 30, 40 and 50 gm puree per 100 gm flour (Table 1). The dry ingredients, flour and salt were added in mixing compartment of automatic Kent noodle maker. Dry mixing of ingredients was carried out for 5 minutes and water, puree and whole egg were added. Mixing and forming cohesive dough was performed and machine automatically switches to extrusion mode to extrude the noodles. Extruded noodles were cut to a length of 10 cm. The wet noodles were steamed for 5 min. and were dried at 60±5°C in a cabinet drier for 3 hrs. The noodles (100 gm) were properly packed in low density polyethylene (LDPE) (50um thickness) and high density polyethylene (HDPE) (200um thickness) pouches and were stored at ambient temperature.

Proximate composition: Moisture, fat, protein, ash peroxide value and free fatty acids and fiber content were determined using standard AOAC (2004) methods. The total carbohydrates were calculated by subtracting the moisture, fat, protein and ash from 100 (Srivastava and Sanjeev 2002).

Sensory evaluation: Sensory evaluation of spinach noodles was carried using 20 semi trained panel members by hedonic rating on a 9 point scale with 9= liked extremely to 1=disliked extremely. The sensory evaluation was carried with respect to colour, flavour, taste, texture, mouthfeel and overall acceptability.

Cooking Quality

Optimum cooking time: Optimum cooking time was

determined by cooking 30 gm of noodles in 300 ml. distilled water on a thermostatically controlled heater. For every 30 sec one strand was removed from boiling water and squeezed in between glass plates. The process was repeated till central white core disappears. The time at which central white core disappears was taken as optimum cooking time.

Cooking loss: Cooking loss is amount of solids lost in cooked water. After determining the cooking time the remaining water was drained and dried in oven to a constant weight. The residue was weighed and reported as percent cooking loss.

$$\text{Cooking loss (\%)} = \frac{\text{Dry matters in cooked water}}{\text{Weight of noodles}} \times 100$$

Cooked weight: Cooking weight was determined according to the method of AACC (2010).

$$\text{Cooked weight} = \frac{\text{Weight of cooked noodles (g)}}{\text{Weight of noodles (g)}} \times 100$$

Water absorption and swelling index: Water absorption and swelling index was determined by AACC (2010).

$$\text{Water absorption (\%)} = \frac{\text{Weight of cooked noodles (g)} - \text{Weight of noodle (g)}}{\text{Weight of noodles (g)}} \times 100$$

$$\text{Swelling index (ml gm}^{-1}\text{)} = \frac{\text{Weight of cooked noodles} - \text{Weight of noodles after drying}}{\text{Weight of noodles after drying}}$$

Storage stability: Storage stability of the noodles was evaluated in LDPE and HDPE pouches by storing 100 gm sample in each pouch. The product was evaluated for its moisture content, peroxide value, free fatty acid content and microbial analysis at every month and results were reported at 3 months interval. Microbial analysis for bacteria, yeast and mould count was performed. From each stored bag 1 gm of sample was homogenized in 9 ml sterile water. Serial dilution was performed up to 10^{-2} for total plate count and upto 10^{-1} for yeast and mould count. Total plate count was performed on nutrient agar and yeast and mould count on potato dextrose agar using pour plate technique.

Statistical analysis: The data obtained was analyzed statistically to determine statistical significance of treatments. Completely Randomized Design (CRD) was used to test the significance of results. P value < 0.05 was considered significant. Data from storage stability was also subjected to post-hoc Tukey HSD test and t-test.

RESULTS AND DISCUSSION

Proximate composition: The moisture content significantly and progressively increased with addition of spinach puree in the composite flour and varied from (6.00) control to (7.50) P50 (Table 2). The increase in moisture content may be due to increase in fiber in the composite flour. Fiber has great affinity for water and hence water might be binding to fiber molecules in bound form which retain in fiber during drying. Similar results were reported by Eko nurchaya (2011). The results pertaining to fat and protein content were non-significant. There was marginal decrease in fat and protein content with increase in level of puree in combination. This might be due to dilution of wheat gluten by addition of spinach puree in flour. Ash and fiber content of resultant noodles increased progressively with addition of spinach puree in flour. The increase in ash might be due to presence of more minerals in added spinach puree in the flour. The carbohydrates were significantly decreased with increase in level of spinach puree in the formulation. Similar results for

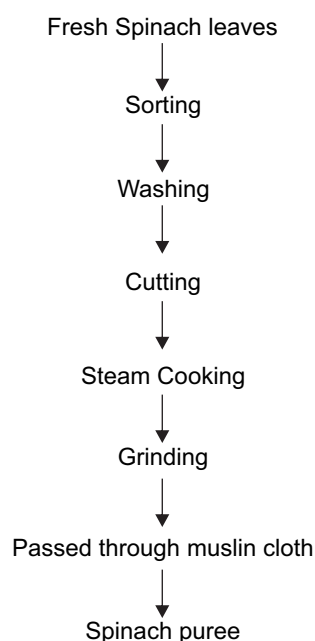


Fig. 1. Flow sheet for production of spinach puree

Table 1. Formulation of instant noodles with spinach puree

Ingredients (g)	P ₀	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀
Wheat flour	100	100	100	100	100	100
Spinach puree	-	10	20	30	40	50
Whole egg	8.0	8.0	8.0	8.0	8.0	8.0
Water	30	30	30	30	25	20

*P₀: 100% wheat flour noodles; P₁₀:10g Spinach puree in 100g flour; P₂₀:20 g Spinach puree in 100g flour; P₃₀:30 g Spinach puree in 100g flour; P₄₀:40 g Spinach puree in 100g flour; P₅₀:50 g Spinach puree in 100g flour

improvement in fibre and ash content and decrease in the carbohydrate content was also reported by Sahni and Shere (2016,2017) for incorporation of vegetable pomace powders. From above results of higher fiber and lower carbohydrates content, it can be inferred that such type of noodles will confer health benefits by offering a high fiber and low carbohydrate formulation.

Sensory attributes: Colour is one of crucial sensory attribute that tends to provide product which has better acceptability by the consumer and, thus influence their purchase decision (Sahni and Aneja 2018). The sensory score (Fig. 2) reveals that colour of the product improved progressively and significantly with increase in level of spinach puree in the noodles from 7.00 (control) to 9.00 (P40), followed by slight decrease in the colour score 8.2 (P50). Due to addition of spinach puree which was with strong green colour was liked by most of the panelist. Similar results were obtained for taste and flavor were the acceptability of the noodles increased due to peculiar taste and flavor of spinach. Texture and mouth feel scores of the noodles slightly decreased with the increase in the level of incorporation of puree; however, it was acceptable upto P40. Kamble et al (2018) reported score of 6.41 and 8.28 in drumstick leaf powder noodles and Taneya et al (2014) 6.50-8.0 in sweet potato flour noodles. Thus, sample P30 and P40 was better in comparison to control. Hence, the sample with 40 gm spinach puree /100 gm was used for storage and

further investigation.

Cooking quality: There was noticeable effect of adding spinach puree on cooking time of noodles (Table 3). The optimum cooking time decreased progressively and significantly from 6.24 min (P0) to 5.15 min (P50). Addition of spinach puree might be disturbing the gluten protein network of noodles causing dilution of these compounds and thus reduce the optimum cooking time (Brenan et al 2002). At P40 and P50 the effect was much more pronounced. Rekha et al (2013) also reported decrease in cooking time of noodles with spinach puree. Cooking loss is the amount of solids that are lost during cooking and may be an indicator of noodle structure integrity during cooking. Li et al (2015) reported that cooking loss is supposed to be one of the important parameter in deciding the noodle quality and integrity. It can be seen from table that cooking loss decreased progressively with increase in spinach puree in noodles from 14.35 % (control) to 6.8 % (P50). This indicates that addition of spinach puree in flour enhanced the binding capacity of noodle strands in a cohesive form. Due to this cohesiveness the solids were not much lost in cooking water. Rekha et al (2013) also reported decrease in gruel loss in vegetable incorporated pasta. However, Tudorica (2002) reported increase in cooking loss in dietary fiber enriched pasta. The cooked weight of noodles increased linearly and significantly with increase in spinach puree level in formulation. The cooked weight increased from 210.31% (control) to 262.26%

Table 2. Proximate composition of spinach puree enriched instant noodles

Treatments	Moisture (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Fiber (%)	Ash (%)
P ₀	6.00	4.50	12.90	75.00	1.75	1.60
P ₁₀	6.10	4.40	12.80	74.90	2.10	2.10
P ₂₀	6.50	4.35	12.78	74.39	2.60	2.60
P ₃₀	6.80	4.10	12.75	74.25	2.85	2.85
P ₄₀	7.10	4.00	12.68	73.82	3.10	3.10
P ₅₀	7.50	3.95	12.60	73.35	3.50	3.50
CD (p=0.05)	1.34	NS	NS	2.39	0.74	0.84

*Each value is average of 3 determinations

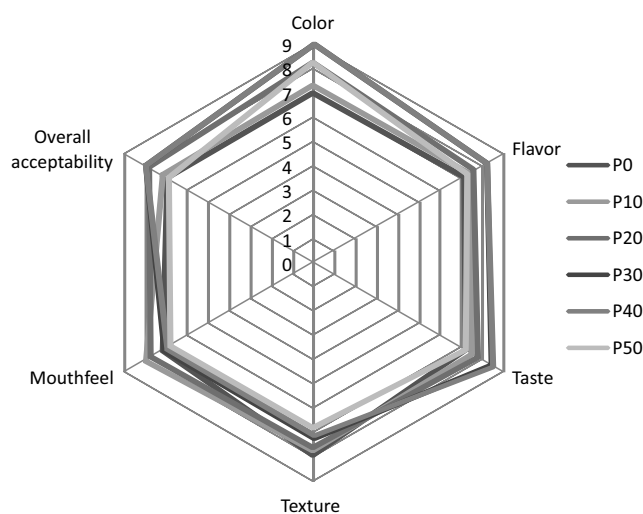
Table 3. Cooking quality of spinach puree enriched instant noodles

Treatments	Cooking time (min)	Cook weight (%)	Cooking loss (%)	Swelling index (ml g ⁻¹)	Water absorption (%)
P ₀	6.24	210.31	14.33	1.85	150.27
P ₁₀	5.45	222.29	8.5	1.82	152.2
P ₂₀	5.25	234.45	7.65	1.82	154.4
P ₃₀	5.20	250.65	7.60	1.80	155.6
P ₄₀	5.18	254.75	7.40	1.79	160.7
P ₅₀	5.15	262.26	6.8	1.78	162.6
CD (p=0.05)	0.43	5.97	1.01	0.32	3.72

See Table 1 for details

(P50). The increase in cooked weight might be due to more absorption of water by added fiber particles through spinach puree. The fiber particles might be getting hydrated more during cooking resulting in higher cooked weight. Dhull and Sandhu (2018) also reported increase in cooked weight in wheat-fenugreek composite flour noodles. The swelling index the noodle sample with spinach puree decreased with increase in level of spinach puree from 1.85 (control) to 1.78 (P50). The slight decrease in swelling index was a result of competition between starch molecules and added fiber particles through spinach puree. The fiber molecules might be absorbing more water as compared to starch granules resulting in decrease in swelling index. However, water absorption properties were increased with increase in spinach puree level in noodles.

Storage stability: The moisture content increased significantly and progressively during storage in LDPE and HDPE pouches (Table 4). The moisture content remained quite stable during first 6 months storage in both the packages. However, after 6 month storage the moisture was increased but moisture content of all the samples remained well within standards. As per the codex standards maximum permissible limit is 10% moisture. The intensity of increasing moisture in noodles was higher in HDPE as compared to LDPE pouches. Peroxide value marginally increased during storage from 5.20 (control) to 12.70 in LDPE pouches and to 10.50 miliequivalent (12th month) in HDPE pouches. The peroxide value is important in deciding the quality of noodles. The peroxide value increases with increase in oxidation of fat and oxidized fats and oils are toxic to consumer. The peroxide values in both types of pouches were well within the BIS limits (<10 miliequivalent kg⁻¹ fat). Thus, the noodles stored in both the pouches were well within standards up 9 months. Marginal increase in free fatty acids was in both packaging materials. The intensity of increase in fatty acids was more in LDPE pouches as compared to HDPE pouches.



*P₀: 100% wheat flour noodles; P₁₀:10g Spinach puree in 100g flour; P₂₀:20 g Spinach puree in 100g flour; P₃₀:30 g Spinach puree in 100g flour; P₄₀:40 g Spinach puree in 100g flour; P₅₀:50 g Spinach puree in 100g flour
*Each value is average of 20 determinations

Fig. 2. Sensory evaluation of Spinach puree enriched instant noodles

The free fatty acid was within range even after 12 month of storage. Noodles are low moisture foods and long storage life food, hence it is necessary to study the microbial load during storage. There was no yeast and mould count up to 6 months of storage. However, after 6 months storage few colonies were visible in both the packages used. The total plate count increased with increase in moisture and storage period. The plate count in LDPE higher as compared to HDPE pouches. The plate count increased from 0.21×10^2 (control) to 2.4×10^2 in 6 month storage in LDPE pouches and 2.6×10^3 in 12 months storage. Where as in HDPE plate count increased from 0.21×10^2 (control) to 2.1×10^2 (6 month) and to 2.5×10^3 (12 month). Thus the microbial load of noodles was well within the acceptable limits in both the stored packaging materials.

Table 4. Storage quality of spinach noodles at ambient temperature in low density polyethylene (LDPE) high density polyethylene (HDPE) pouches

Storage period (months)	Moisture (%)		Peroxide value (Mili eqv. kg ⁻¹)		Free fatty acids (%)		Yeast and mould count gm ⁻¹		Total plate count gm ⁻¹	
	LDPE	HDPE	LDPE	HDPE	LDPE	HDPE	LDPE	HDPE	LDPE	HDPE
0	8.10 ^{dA}	8.10 ^{dA}	5.20 ^{eA}	5.20 ^{eA}	0.80 ^{dA}	0.80 ^{dA}	Nil	Nil	0.21x10 ^{2eA}	0.21x10 ^{2eA}
3	8.10 ^{dA}	8.10 ^{dA}	6.10 ^{dA}	5.50 ^{dB}	0.80 ^{dA}	0.80 ^{dA}	Nil	Nil	2.2x10 ^{2dA}	0.25x10 ^{2dA}
6	9.30 ^{cA}	8.30 ^{CB}	8.50 ^{cA}	5.75 ^{CB}	0.86 ^{cA}	0.82 ^{CB}	Nil	Nil	2.4x10 ^{2cA}	2.1x10 ^{2cB}
9	9.95 ^{bA}	9.10 ^{bB}	9.80 ^{bA}	6.75 ^{bB}	0.94 ^{bA}	0.86 ^{bB}	0.2x10 ^{bB}	0.3x10 ^{bA}	2.6x10 ^{2bA}	2.3x10 ^{2bB}
12	10.75 ^{aA}	9.25 ^{aB}	12.70 ^{aA}	10.50 ^{aB}	1.00 ^{aA}	0.88 ^{aB}	0.3x10 ^{aB}	0.4x10 ^{aA}	2.6x10 ^{3aA}	2.5x10 ^{3aB}
CD (p=0.05)	0.6411	0.6414	0.7455	0.4512	0.0329	0.0260	-	-	48.352	12.449

*a,b,c,d,e The means within the column followed by different superscripts are significantly different at p < 0.05 by Tukey's test

*A,B The means within row followed by different superscripts are significantly different at p < 0.05 by t-test

CONCLUSION

Spinach puree can be incorporated in instant noodles at 40g per 100g of refined wheat flour and resulted in enhancement of sensorial attributes along with improvement in the nutritional profile of the noodles. Particularly, profound increase in the fiber and ash content was observed on incremental increase in the spinach puree. Therefore, such product will provide high fiber and low carbohydrate formulation for consumption. Moreover, the positive influence was also observed on the cooking quality of noodles with incorporation of spinach puree where cooking time was significantly reduced along with reduction in gruel loss. As aforesaid, the sensorial properties were also enhanced with the level of incorporation, particularly the colour, taste and flavor. However, the slight reduction in the texture and mouth feel score offers scope of intervention of improvement of texture and mouth feel noodles by incorporation of hydrocolloids. Therefore, noodles can be utilized as effective vehicle for the incorporation of spinach puree at level of replacement of 40 g per 100 g of refined wheat flour to deliver healthy and nutritious product without compromising on the quality and sensorial attributes.

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Investigating Green Synthesis of Silver Nanoparticles from Orange Peel (*Citrus sinensis*) and the Effects of Chitosan, Sylamol, Nanosilver on *Rhizopus stolonifer* in Tomato (*Solanum lycopersicum*)

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Abstract: This research was carried out in order to synthesize green nanoparticles of orange peel extract and its effect on *Rhizopus stolonifer* control in tomato at Islamic University of Tehran Research and science branch. Silver nanoparticles were successfully prepared from orange peel. Size 8-21 and spherical shape was determined by using TEM and XRD. The effect of chitosan, sylamol, aqueous extract and silver nanoparticles was investigated with different concentrations in the response of tomatoes inoculated with *Rhizoctonia stolonifer*. This experiment was conducted in a completely randomized block design with three levels of chitosan, sylamol, aqueous extract and silver nanoparticles (10, 20 and 30 mg/L) and with three replications. Measurement of traits was carried out at 4, 1, 3, 7, and 11 days. The growth of *Rhizopus stolonifer* was affected by increasing treatments levels. Results indicated that 30 mg l⁻¹ of silver nanoparticles had the highest inhibitory effect.

Keywords: Nanoparticles, *Rhizopus stolonifer*, TEM, XRD, Chitosan, Sylamol

Chemical methods to control pests and plant diseases threaten the health of the environment. Although these agricultural pesticides have been effective in combating disease in the short term, their harmful effects on humans, living organisms and the environment have gradually evolved. Unconscious using of the pesticides conflicts with ecological principles and can be the source of many problems, such as the resistant strains of pests, the prevalence of pests, adverse effects on non-target organisms such as parasitoids, pesticides residues in agricultural products, and it is a direct source of toxicity for the consumer (Esnard et al 1994). Orange' peel is composed of outer and inner peel, which is considered an appropriate source of antioxidants, which has a high level of Flavonoid (Mazaheri Tehrani et al 2007). Also reported Orange peel mesocarp has the most antioxidant properties among the various orange in north of Iran. Tomato is the second most important vegetable in the world in terms of its economic value. This plant accounts for 28.7 per cent of the total vegetable crop area in Iran. Iran, however, has only about 3.4 percent of global tomato production, with 14 percent of global cultivation. Low yield along with the inappropriateness harvesting, packing and storage conditions is one of the most

important factors in reducing production and high level of losses of this product in this country (Vaidyanathan et al 2009). Because of the strong antimicrobial characters, silver nanoparticles are used in a variety of applications to control the growth of microorganisms. Silver nanoparticles are a potentially fatal agent against a wide range of gram negative and positive bacteria, even antibiotic-resistant strains. The extracted metal nanoparticles are more absorbed by cells than nanoparticles coated with chemical stabilizers (polymers), due to this fact that the herbal extracts stabilized by nanoparticles contain various proteins and fibers on their surface. In this case, the probability of pending to the cells is greater. Chitosan is a biodegradable compound derived from crustacean shells such as crabs and shrimp, the main feature of which is its polycotin content. Chitosan has been shown to control multiple diseases before and post harvesting horticultural products (Kustrzeba-Wójcicka et al 2014). Sylamol, with changes in the structure of leaf, stem and root plants, results in complete photosynthesis, resistance to pests and aerobic and soil pathogens (Adhikari et al 2017). The most important post-harvest pathogens reported from tomato included by *Rhizopus stolonifer* and *A. Alternata*. The soft rot on the succulent tissues of vegetables, fruits and

ornamentals caused by *Rhizopus* sp. occurs throughout the world. The disease mainly occurs during sale, transports, market shelf and storage after harvest. *Rhizopus stolanifer* is omnipresent as a saprophyte and sometimes as a weak parasite on stored organs of plants. When the epidermal cells are collapsed, the fungus emerges through the wounds and produces aerial sporangiophores, sporangia, stolons, and rhizoids, the latter capable of piercing the softened epidermis. The infected parts of cracks on matured fruits appeared water soaked at first, and then became softer. Gray hyphae grew from the sites where the fungus invaded primarily and covered the affected portions by producing tuft whisker like gray sporangiophores and sporangia. The infected tissues finally broke down and disintegrated in watery rot (Montealegre et al 2003). Considering that the tomato plant is strongly infected by fungal diseases and because of its post-harvest life, the results of this study can provide new methods for increasing the post-harvest life of tomatoes by controlling fungal diseases.

MATERIAL AND METHODS

In this study, the extract of the orange mesocarp peel containing flavonoids was used to extract the silver ion. The extracts were isolated by filter paper (Munktell Grade 338). The total flavonoid content in the extract was determined (Mazhari and Behniafar 2003). The pH, plant extract concentration, size and shape of nanosilver, were measured. pH is one of the important factor in the bio-formation of metallic nanoparticles and used pH 4, 7, 8, 10 for green synthesis of silver nanoparticles using orange peel extract. Silver nanoparticles were characterized by UV-Vis spectra were recorded over the 300–800 nm range with a UV 1650 PC Shimadzu B UV-Vis spectrophotometer. The XRD (X-ray powder diffraction) studies were done by a Siefert XRD 3003 PD (Germany). Morphology of Ag nanoparticles was performed using transmission electron microscopy (TEM) image by means of a Philips EM 208 instrument with 100 kV acceleration voltages. In this study used 3 concentrations of orange's peel extract (50:50, 40:60 and 20:80) for green synthesis of silver nanoparticles using orange's peel extract. Silver nanoparticles and orange peel extracts were prepared at the Chemistry Lab of Islamic Azad University. Experiments were carried out in laboratory with a temperature of 21 ± 1 °C and relative humidity in the range of 60-65%.

Rhizopus stolanifer were prepared from the collections of the Plant Pathology Lab at the Research Branch, Islamic Azad University, Tehran, Iran. The fungi was cultured on the PDA and, the parts of the medium, were grown and stored in the -70 (Shankar et al 2004). Silver nanoparticles, orange peel extract, chitosan and syalamol were dissolved

separately into 700 ml of distilled water. Then, it was placed on a heater. After it was dissolved, the pH was adjusted to 7, then added to the PDA. In order to determine the inhibitory effect of treatments on growth of *Rhizopus stolanifer*, different concentrations of silver nanoparticles and extracts of orange, syalamol and chitosan was used in completely randomized design with three replications (Table 1). Tomatoes from a greenhouse in Pakdasht were transferred to Tehran. The fruits were immersed in 1 per cent sodium hypochlorite solution for 2 minutes, then rinsed with distilled water and dried to air. Sterile scarper scratched each fruit in the small scar on 5×5 mm under laminar hood. 20 μ L of each treatment were injected on the scratch wound and after 10 minutes 10 μ L of spore suspension was injected subcutaneously at a concentration of 5×10^4 . A control with 3 replicates was injected with sterile sterilized water. Another control was prepared by injecting 10 μ L spore suspension at a concentration of 5×10^4 with 3 replicates.

RESULTS AND DISCUSSION

Change the color of silver nanoparticles: Since Orange's peel extracts was used, color change was occurred due to Ag^+ reduce into Ag-NPs. Orange's peel suspension was yellow but after adding $AgNO_3$ (10 minutes) it change to dark brown. Surface Plasmon resonance phenomenon might be corresponding to its reason (Fig. 1).

pH: the pH 7 were more effective than other pH of silver nanoparticles (Fig. 2).

Different concentrations of Orange's peel extract: UV-VIS activity of various concentrations of Orange's peel extract of synthesized AgNPs is showed that the best concentration is 4ml (Fig. 3).

X-ray Diffraction analysis (XRD): XRD confirmed the presence of silver nanoparticles (Fig. 4).

Transmission electron microscopy (TEM): AgNPs size was conducted to be 50-65 nm (TEM) which was according to XRD analysis (Fig. 5).

The different treated fruits were classified into 4 groups according to severity of contamination: Group 0 without infection, group 1 with low infection rate, group 2 with moderate infection, and group 3 with high contamination. Treated fruits with 10 $mg\ l^{-1}$ silver nanoparticles, had a second-degree infection. In the treatment of 20 $mg\ l^{-1}$ silver nanoparticles, each sample had a degree of infection. In the treatment of 30 $mg\ l^{-1}$ of silver nanoparticles, all three replicates were grade 0 (no infection). Treated fruits with orange peel extract, the severity of the infection and the mean inhibitory level were low. In the treatment of 10 $mg\ l^{-1}$ orange extract, the treated fruits had three ones of type 2. In the treatment of 20 $mg\ l^{-1}$ of aqueous extract of orange peel,

Table 1. Inhibitory effects of treatments on growth of *Rhizopus stolonifer* on fruit

Treatment	Radial Growth of <i>Rhizopus stolonifer</i> on fruit (mm)
Control	3
Silver nanoparticle solution 10	2
Silver nanoparticle solution 20	1
Silver nanoparticle solution 30	0
Orange peel extract 10	2
Orange peel extract 20	1
Orange peel extract 30	1
chitosan solution 0.1%	3
chitosan solution 0.3%	2
chitosan solution 0.5%	1
Sylamol 1/1000	3
Sylamol 2/1000	2
Sylamol 3/1000	2

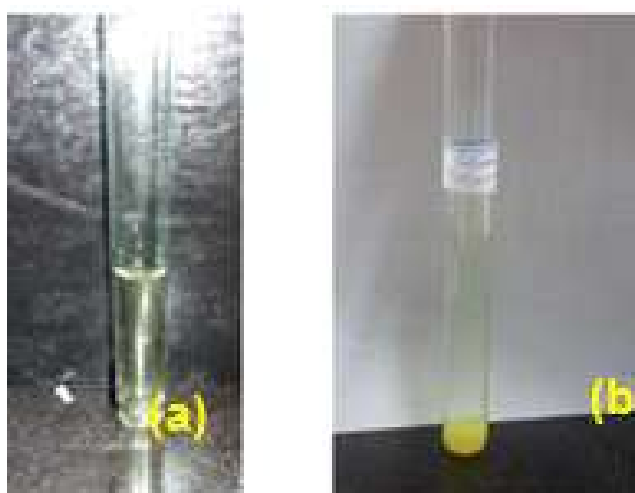
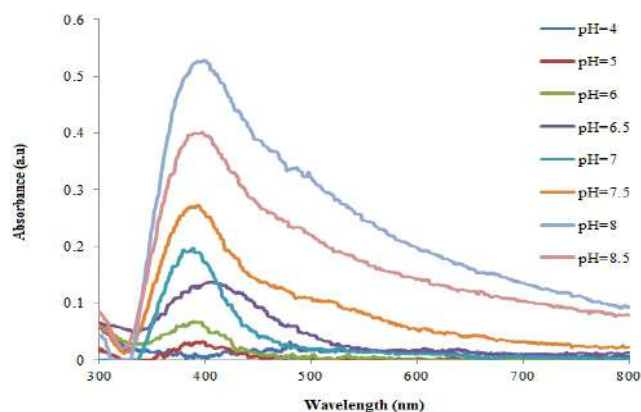
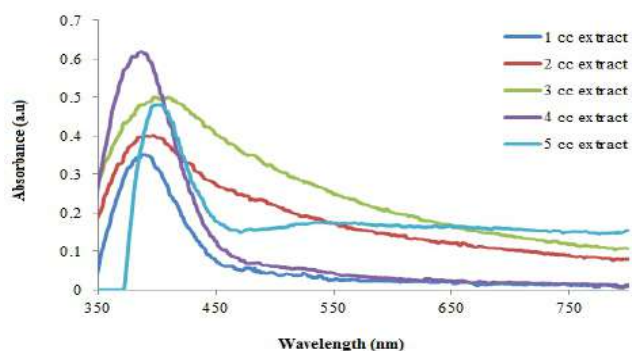
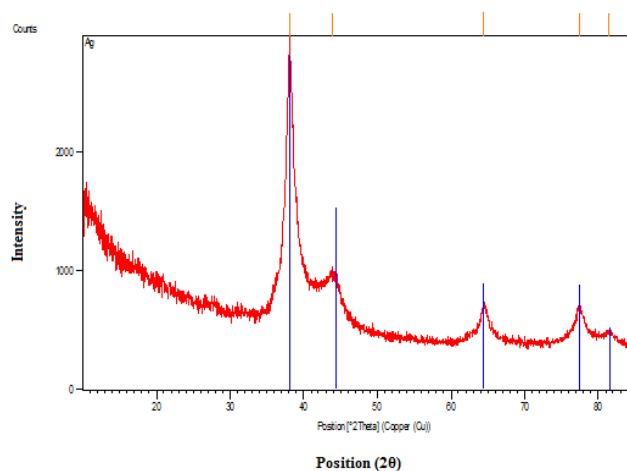
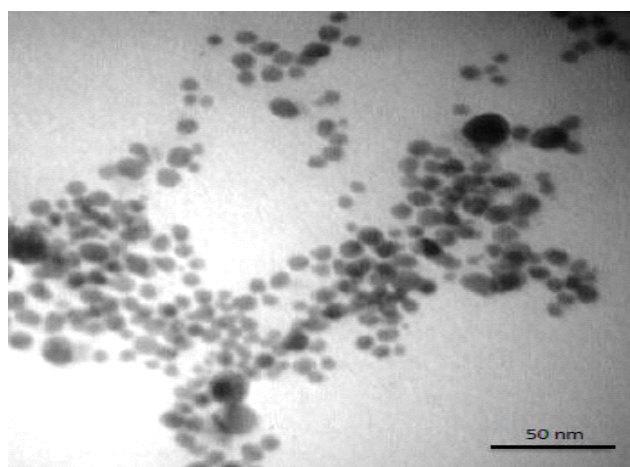
**Fig. 1.** Silver nitrate solution, synthesis of silver nanoparticles**Fig. 2.** Effect of different pH for green synthesis of silver nanoparticles**Fig. 3.** Effect of different concentrations for green synthesis of silver nanoparticles**Fig. 4.** XRD pattern of Ag nanoparticles using Oranges peel extract**Fig. 5.** TEM images of silver nanoparticles synthesized by using Orange's peel extract



Fig. 6. The inhibitory effect of the treatments on growth of *Rhizopus stolonifer*: (a) not treated (b) treated

the type 1 infection was observed, the same result was shown in treatment of 30 mg l⁻¹ orange peel extract. In normal conditions for chitosan-treated fruits were not similar for different concentrations. At concentrations of 0.1 and 0.2 per cent, infection was 3 and 2, respectively. However, grade 1 was observed in all three replicates of 0.3 per cent chitosan. In treatment 1/ 1000 sylamol, observed three types of infection. At concentrations of 2/1000 and 3 / 1000 sylamol, all repeats had second grade.

In this study, nanoparticle production and particle evaluation methods were investigated using aqueous extract of orange peel, chitosan, sylamol, and its effect on tomato post-harvesting. The flavonoid extracts of orange peel has a high potential for reduction of the silver nanoparticles and it is suitable for production silver nanoparticles. It was relieved that the size of nanoparticle was 8-12 nm and the average size was determined 55.02 nm with shape of spherical. This result is consistent with Krishnaraj et al (2010) research on silver nanoparticles derived from pomegranate leaf extract; synthesis of Nanoparticles from *Aloe vera* (Ahamed et al 2011), *Acalypha indica* (Ankanna et al 2010) and *Boswellia ovalifoliolata* plant (Rai and Yadav 2009).

There was a significant difference between treated tomatoes with its control for decay caused by the growth of *Rhizopus* fungi. As in treated tomatoes, the decay radius was less than non-infected. The observation of the number of first, second, and third tomatoes in terms of the development and growth of fungal pathogens has also shown that the superiority of sprayed tomatoes with silver nanoparticles compared to others reduced decay. Reducing symptoms and thereby increasing the number of healthy tomato fruits as a result of the application of these treatments can indicate the ability of these substances to reduce the development of fungal pathogens and hence the importance of using these agents. Antimicrobial effects of different types of metal nanoparticles such as copper, zinc, titanium, magnesium, gold and silver have been studied. However, silver

nanoparticles have shown the most recommended because of their antimicrobial effect against bacteria, viruses and other eukaryotic microorganisms. The increasing nanosilver concentration did not increase fungal growth, which is consistent with research Sahar and Ouda (2014), which indicates that nanosilver reduced the growth of microorganisms. In addition, it is according to Faghihi et al (2017); a significantly higher percentage of disease damage was appeared in control plant, while symptoms were observed in treated plants with nanosilver.

CONCLUSION

In conclusion, silver nanoparticles were successfully prepared from orange peel. The effect of chitosan, sylamol, aqueous extract and silver nanoparticles was investigated with different concentrations. The growth of *Rhizopus stolonifer* was affected by increasing treatments levels. Results indicated that nanosilver has better antifungal activity against *Rhizopus stolonifer* in comparison with others.

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Foliar Nutrition with Calcium Nitrate in Strawberries (*Fragaria × ananassa* Duch.): Effect on Fruit Quality and Yield

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Abstract: Study investigates the effect of pre-harvest calcium nitrate sprays on the qualitative and quantitative characteristics of 'Winter Dawn' strawberries (*Fragaria × ananassa* Duch.). Strawberry fruits were sprayed with different doses of Calcium nitrate (0.2%, 0.4%, 0.6% and 0.8%) under field conditions. In comparison to the control, foliar nutrition with $\text{Ca}(\text{NO}_3)_2$ significantly improved fruit quality and yield related parameters. Calcium nitrate (0.4%) registered the highest total soluble solids (TSS), total sugars, reducing sugars, non-reducing sugars and ascorbic acid content over the other treatments and the control. Moreover, maximum fruit size, weight and yield plant⁻¹ was also obtained with $\text{Ca}(\text{NO}_3)_2$ at 0.4 per cent. Supplementary calcium feeding played a significant role in enhancing fruit firmness by imparting strength and thickness to fruit cell wall. Therefore, it is intended that foliar feeding $\text{Ca}(\text{NO}_3)_2$ at 0.4% is the most effective to extend the qualitative and quantitative characteristics of 'Winter Dawn' strawberries.

Keywords: Pre-harvest, $\text{Ca}(\text{NO}_3)_2$, Physico-chemical attributes, Yield

Calcium plays a prominent role in fruit production and enhancing the shelf life of fruits. The rationale behind the practice of pre-harvest calcium (Ca) application is based on its physiological activity in membrane stability, strengthening and thickening of the cell wall by cross-linking the pectin chains of middle lamella (Demarty et al 1984, Glenn et al 1988). Calcium is concerned with the formation and enhancement of proteins inside the mitochondria, therefore, associating it with aerobic respiration and absorbing nutrients. As such, it can be said that foliar spraying of calcium at the start and middle of harvesting improves fruit yield and quality (Dastjerdy et al 2014). It also enhances fruit firmness and delays the ripening process, while preventing various calcium-related disorders (Ferguson and Drobak 1988, Zocchi and Mignani 1995). In addition, deficiency of calcium causes several physiological disorders in fruit and vegetable crops (Bonomelli and Ruiz 2010, Shear 1975). There are as many as 35 types of disorders found in different fruits and vegetables, which are mainly associated with an inadequate concentration of Ca in the soil. Particularly in strawberries due to localised deficiency of Ca, rapidly growing leaves may suffer tip burn (Mason and Guttridge 1974), and fruit may develop a hard texture and become acidic in taste (Ulrich et al 1980).

Foliar feeding of certain nutrient elements like Ca, Mn, B, Fe and Zn is considered better than soil application (Dastjerdy et al 2014). Ca is not easily mobilised from older plant tissues and its redistribution occurs through the phloem, but the instant rate at which Ca supplies in the xylem further depends on the unidirectional stream of transpiration (White and Broadley 2003). Moreover, the uptake of Ca^{2+} is

also mainly limited to the root tips and its absorption by older roots is comparatively low (Mengel and Kirkby 2001). Thus, Ca is immobile nutrient, meaning its application through soil is not very effective. Foliar nutrition is the rapid and efficient method as it supplies nutrients directly to a place wherever, it is required (Murtić et al 2017). On the contrary, Lanauskas et al (2012) found that foliar Ca application in apples was not very effective because the mobility of Ca^{2+} through aerial plant organs (especially leaves and fruits) is limited.

Strawberries are highly sensitive plants. Almost all of its vegetative and reproductive plant parts are affected by biotic and abiotic stresses, which resulting in the reduction of production potential and also enhance the rate of mortality. Due to its highly delicate nature, fruit quality parameters of strawberries such as fruit firmness, soluble solids, sugars and acid content are the prime governing factors in maintaining prolonged post-harvest life and fruit nutritive values. However, Ca nutrition helps in delaying the fungal decay and loss of fruit firmness in strawberries (Hernández-Muñoz et al 2006). Experiments carried out with foliar application of $\text{Ca}(\text{NO}_3)_2$ in strawberry showed that it improved vegetative growth parameters and nutrients uptake in plants (Sidhu et al 2018). Thus, considering gaps in the literature concerning the role of foliar nutrition with Ca in fruit plants, this study was aimed to analyse the function of Ca ions in the form of calcium nitrate on the qualitative and quantitative parameters of 'Winter Dawn' strawberries.

MATERIAL AND METHODS

The experiment was carried out at Precision Farming Development Centre (PFDC) at Punjab Agricultural

University in Ludhiana, India. Fruit quality was estimated at PG laboratory, Department of Fruit Science, PAU Ludhiana. The objective was to determine the effect of different concentrations of $\text{Ca}(\text{NO}_3)_2$ as pre-harvest foliar sprays at 60, 75 and 90 days after transplantation on various qualitative and quantitative characteristics in 'Winter Dawn' strawberries. The trial was conceived with a randomised block design and contained five treatments, each replicated four times. The plants were sprayed with $\text{Ca}(\text{NO}_3)_2$ at 0.2%, 0.4%, 0.6% and 0.8% and the control plants sprayed with water. The raised beds, each spaced by 40 cm, were prepared and transplanting was completed in IInd fortnight at 30 cm $\times$ 30 cm at spacing. The soil texture of the experimental block was sandy loam with pH 7.5.

Assessment of fruit quantitative parameters: Fruits were randomly harvested at optimum maturity (at different intervals during the season) from all four different treatments. Diseased and misshape fruits were sorted out and healthy and uniform-sized fruits used for physico-chemical analysis. Fruit size was estimated in terms of length and breadth. Fruit size of ten randomly selected fruits from each replication were measured with digital vernier's caliper and expressed in millimetre (mm). Fruits were also used for recording fruit weight with digital weighing balance and expressed in grams. From the selected plants, the number of fruits plant⁻¹ were counted as expressed in g plant⁻¹.

Assessment of fruit qualitative parameters: Among the various qualitative parameters, total soluble solids (TSS) was recorded using a digital refractometer (Atago PAL-1, Model 3810, Japan) at room temperature by making subsequent corrections at 20°C, expressed as °Brix. Acidity was determined by titrating 2 ml of juice with 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator. Total reducing and non-reducing sugars and vitamin C content was estimated according to standard procedures (AOAC 2005). The Color Flex spectrophotometer (Hunter Lab, Reston, USA) was used to determine the colour of fruits. The results were expressed as Hunter L^* a^* b^* values, where L^* denotes the luminance or lightness component, which ranged from 0 to 100

(black to white), and a^* (from green to red) and b^* (from blue to yellow) denote the chromatic components. Fruit firmness was estimated at the equatorial portion of each strawberry fruit using a texture analyser with a 5 mm stainless steel convex tip probe. The results were expressed in Newton (N).

Statistical analysis: The data for fruit quality assessments were collected at the end of each harvesting period. The pooled data were analysed using the statistical software package CPCS-1 for ANOVA and the least significant difference (LSD) between mean values to forge pair-wise comparisons at $P \leq 0.05$.

RESULTS AND DISCUSSION

Qualitative fruit parameters: The results in Table 1 show that pre-harvest foliar application of different doses of $\text{Ca}(\text{NO}_3)_2$ in strawberry significantly improved the nutritional quality of fruits in terms of TSS and TSS/acid ratio, titratable acidity, juice pH, vitamin C content, and sugars (total, reducing and non-reducing). The highest TSS (9.9 °Brix), total sugars (6.52%), reducing sugars (4.12%), non-reducing sugars (2.28%) and ascorbic acid content (74.95 mg 100 g⁻¹) were attained in T₂ ($\text{Ca}(\text{NO}_3)_2$ 0.4%), while the lowest values of 7.6 °Brix, 5.02%, 2.85%, 2.17% and 61.23 mg 100 g⁻¹, respectively were observed under the control. These results are corroborated with the findings of Asrey et al (2004) in Chandler strawberries, Ganai (2006) and Raese and Drake (1993) in apple and pear fruits, and Robson et al (1989) in peaches. As such, higher TSS and sugar concentrations may be attributed to a reduction in respiration due to Ca applications, leading to less utilization of sugars in various metabolic processes (Gupta et al 1980).

In addition, fruit titratable acid content exhibited a progressive decline, or negative correlations, with higher concentrations of $\text{Ca}(\text{NO}_3)_2$ (Fig. 1). The plants supplied with 0.8% dose of $\text{Ca}(\text{NO}_3)_2$ experienced minimum (0.48%) acidity whereas the maximum (0.59%) was registered in the control. Similar results were also reported by Ganai (2006) in apples and Bakshi et al (2013) in strawberries. The decrease in acidity may be directly related to the increased sugars

Table 1. Effect of pre-harvest calcium nitrate sprays on fruit qualitative characteristics

Treatments	TSS (°Brix)	TSS/acid ratio	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Ascorbic acid (mg 100 g ⁻¹)
$\text{Ca}(\text{NO}_3)_2$ 0.2% (T ₁)	8.5 ^b	14.8 ^c	6.05 ^b	3.86 ^b	2.19 ^{bc}	66.33 ^b
$\text{Ca}(\text{NO}_3)_2$ 0.4% (T ₂)	9.9 ^a	18.6 ^a	6.52 ^a	4.12 ^a	2.28 ^a	74.95 ^a
$\text{Ca}(\text{NO}_3)_2$ 0.6% (T ₃)	8.5 ^b	16.9 ^b	5.88 ^c	3.55 ^c	2.21 ^b	66.15 ^b
$\text{Ca}(\text{NO}_3)_2$ 0.8% (T ₄)	8.1 ^b	16.8 ^b	5.36 ^d	2.99 ^d	2.25 ^a	64.07 ^b
Control (Water spray)	7.6 ^c	12.9 ^{cd}	5.02 ^e	2.85 ^e	2.17 ^c	61.23 ^c
LSD ($p \leq 0.05$)	0.46	0.94	0.07	0.08	0.03	2.55

Mean values with the same letter are not significantly affected by LSD ($P \leq 0.05$)

content of strawberries due to higher Ca application. Conversely, the juice pH significantly positively correlated with increasing $\text{Ca}(\text{NO}_3)_2$ concentrations (Fig. 2), with the maximum juice pH (4.77) measured at T_4 (calcium nitrate at 0.8%) and the minimum (4.25) at T_5 (control). These findings confirm the results in Ganai (2006) in apple cv. Red Delicious.

In terms of the TSS/acid ratio, the highest (18.6) was found in the plants sprayed with 0.4% $\text{Ca}(\text{NO}_3)_2$ followed by 0.6%, 0.8% and 0.2% treatments; conversely, the lowest (12.9) value was found in the control (Table 1). Significantly higher TSS/acid ratios under 0.4% $\text{Ca}(\text{NO}_3)_2$ and lower under the control may be attributed to higher TSS and lower acidity in fruits applied with 0.4% $\text{Ca}(\text{NO}_3)_2$ and low TSS and high acidity in the fruits under the control.

The higher fruit brightness is found in ripened fruits is due to loss of chlorophyll content and induction of yellow and red colours which is mainly associated with an increase in the level of carotenoids, anthocyanins and other pigments (Yahia

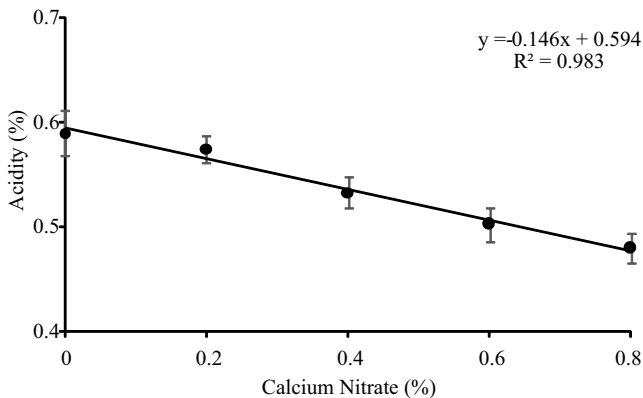


Fig. 1. Relationship between calcium nitrate and titratable acidity (Error bars represent confidence interval at 5% level of significance)

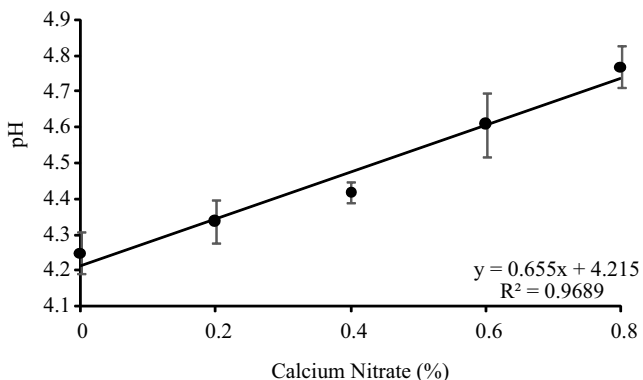


Fig. 2. Relationship between calcium nitrate and juice pH (Error bars represent confidence interval at 5% level of significance)

Table 2. Effect of pre-harvest calcium nitrate sprays on fruit qualitative characteristics

Treatment	L^*	a^*	b^*	Hue angle	Chroma
$\text{Ca}(\text{NO}_3)_2$ 0.2% (T_1)	29.7 ^{ab}	32.6 ^a	17.1 ^b	36.8 ^b	27.6 ^c
$\text{Ca}(\text{NO}_3)_2$ 0.4% (T_2)	29.9 ^{ab}	32.1 ^a	14.3 ^c	35.2 ^c	23.9 ^d
$\text{Ca}(\text{NO}_3)_2$ 0.6% (T_3)	31.9 ^a	32.4 ^a	18.2 ^b	37.2 ^b	29.3 ^b
$\text{Ca}(\text{NO}_3)_2$ 0.8% (T_4)	25.2 ^c	27.6 ^b	12.3 ^d	30.2 ^d	24.1 ^d
Control (Water spray)	28.3 ^b	28.4 ^b	29.1 ^a	45.7 ^a	40.6 ^a
LSD ($P \leq 0.05$)	2.39	2.93	1.56	0.85	1.18

Mean values with the same letter are not significantly affected by LSD ($P \leq 0.05$)

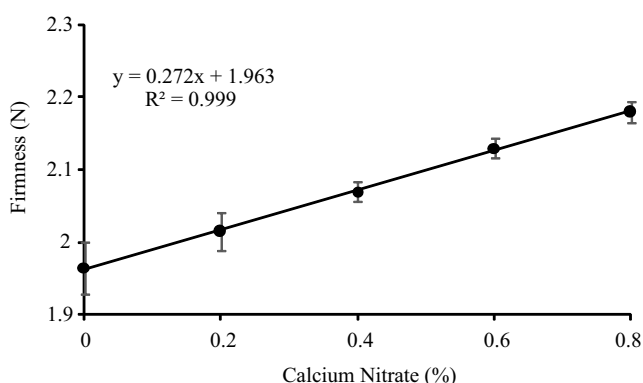
and Ornelas-Paz 2009). Table 2 depicts the fruit colour data. Among the various components of colour, the maximum value of L^* (31.9) was recorded in fruits sprayed with $\text{Ca}(\text{NO}_3)_2$ at 0.6% (T_3), whereas the minimum ($L^* = 25.2$) was obtained using 0.8% $\text{Ca}(\text{NO}_3)_2$. The a^* value was highest (32.6) under 0.2% $\text{Ca}(\text{NO}_3)_2$ and decreased gradually at higher concentrations, while the lowest value of 27.6 was observed under 0.8% $\text{Ca}(\text{NO}_3)_2$. Further, b^* value peaked at 29.1 in the control and dipped to 12.3 in fruits from plants under 0.8% $\text{Ca}(\text{NO}_3)_2$. In terms of hue, the highest recorded redness (lowest value mean, 30.2) in fruits was recorded under 0.8% dose of $\text{Ca}(\text{NO}_3)_2$ and the lowest (highest value mean, 45.7) was observed under the control. The chroma also reached a maximum value of 40.6 under the control, but dipped (24.1) at 0.4% $\text{Ca}(\text{NO}_3)_2$. Evidently, the results show that different treatments of $\text{Ca}(\text{NO}_3)_2$ significantly improved the fruit colour over the control. Similar outcomes were also recorded by Mangat (1989), in that the pre-harvest spray of Ca improved the colour in peach cv. Flordasun. This may be associated with the increased formation of anthocyanin pigment in the fruit pericarp due to higher presence of calcium. As such, pre-harvest foliar application of Ca stimulates the expression and structural genes of anthocyanin, and further enhances the accumulation of anthocyanin in strawberry fruits (Xu et al 2014).

Calcium plays a central role in imparting strength and thickness to the plant cell wall, confirming its importance in terms of fruit firmness (Easterwood 2002). Fruit firmness progressively increased with greater concentrations of calcium nitrate, and, thus, significantly positively correlated with increasing calcium concentrations (Fig. 3). Therefore, the maximum firmness (1.18 N) was observed under 0.8% of $\text{Ca}(\text{NO}_3)_2$ followed by 0.6% and 0.4% doses, whereas the minimum value (2.02) of fruit firmness was observed at 0.2% treatment which was statistically on par with the control. Similar results were also noted in Luna and Zanta (Bieniasz et al 2010), Chandler (Singh et al 2007) and Elsanta (Martinsson et al 2006) strawberry cultivars. The progressive improvement in fruit firmness may be attributed to thickening

Table 3. Effect of pre-harvest calcium nitrate sprays on fruit quantitative characteristics

Treatment	Fruit length (mm)	Fruit breadth (mm)	Fruit weight (g)	No. of fruits plant ⁻¹	Fruit yieldplant ⁻¹ (g)
Ca(NO ₃) ₂ 0.2% (T ₁)	39.3cd	30.5bc	19.3c	16.0a	308.6c
Ca(NO ₃) ₂ 0.4% (T ₂)	46.8a	35.0a	22.7a	15.0a	341.1a
Ca(NO ₃) ₂ 0.6% (T ₃)	43.9b	34.7a	21.2b	15.3a	324.0b
Ca(NO ₃) ₂ 0.8% (T ₄)	41.4bc	33.2ab	21.0b	15.2a	318.9b
Control (Water spray)	38.5d	28.3c	17.0d	14.4a	246.2d
LSD (P ≤ 0.05)	2.85	2.88	0.71	NS	8.65

Mean values with the same letter are not significantly affected by LSD (P ≤ 0.05). 'NS' denotes non-significant

**Fig. 3.** Relationship between calcium nitrate and fruit firmness (Error bars represent confidence interval at 5% level of significance)

of the middle lamella of the cell wall as a result of increased calcium pectate formation and deposition (Gupta et al 1984, Singh et al 2007).

Quantitative fruit parameters: Table 3 shows the effect of various foliar spray concentrations of Ca(NO₃)₂ on fruit size, fruit weight, number of fruits plant⁻¹ and fruit yield plant⁻¹. The maximum significant size, in terms of fruit length (46.8 mm) and breadth (35.0 mm), was observed under 0.4% Ca(NO₃)₂ (T₂), whereas minimum fruit size was observed under the control. In the literature, Koul and Muthoo (1999) also found that Ca(NO₃)₂ improved the fruit size in peach cv. Flordasun. Further, fruit weight and yield plant⁻¹ were also highest (22.7 g and 341.1 g, respectively) at 0.4% Ca(NO₃)₂ treatment (T₂) and lowest (17.0 g and 246.2 g, respectively) at T₅ (control). However, the difference between the number of fruits plant⁻¹ was not significant among the treatments. Similarly, Rani and Brahmachari (2004) reported that calcium salts used as pre-harvest spray significantly improved fruit weight and yield plant⁻¹ in mango cv. Amrapali. On the contrary, the results obtained did not support Bieniasz et al (2010), who found no significant improvement in fruit weight and yield using calcium in Luna and Zanta strawberry cultivars.

CONCLUSION

This study revealed the effect of pre-harvest Ca(NO₃)₂

sprays on qualitative and quantitative characteristics of 'Winter Dawn' strawberries. As found, Ca(NO₃)₂ significantly affected the fruit quality and yield characteristics of strawberry fruits compared to the control. The results showed that foliar nutrition with Ca(NO₃)₂ at 0.4% retained the highest TSS, total sugars, reducing and non-reducing sugars, and ascorbic acid content. Moreover, fruit size, weight and yield plant⁻¹ also peaked using Ca(NO₃)₂ treatment at 0.4%. Overall, it can be surmised that pre-harvest treatment of Ca(NO₃)₂ is an effective method for improving the qualitative and quantitative characteristics of 'Winter Dawn' strawberries.

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Effect of Rhizobacterium on Growth, Yield and Quality of Strawberry

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Abstract: The present investigation was carried out to study the co-inoculation effect of effective rhizospheric bacteria on growth, yield and quality of strawberry cv. Chandler during the year 2017-18. The strawberry plants were treated with rhizospheric bacteria i.e. *Pseudomonas* strains namely, CP109 and CPS67 and *Bacillus* strains namely HCA61, RCA3 and SYB101, whereas untreated soil served as control. The growth, yield and quality of fruits were significantly influenced by rhizospheric bacteria. Among different treatment, treatment T₄ (CP109 + HCA61) recorded the significantly highest fruit yield per plant (257.92 g). The growth parameters, viz. plant height (14.11 cm), number of leaves per plant (12.34), crown diameter (13.21 mm), fresh weight (45.89 g) and dry weight (13.11 g) of plant maximum in treatment combination CP109+ HCA61, whereas the bacterial inoculations did not affect plant spread, fruit weight, fruit size and moisture content % in strawberry. However, with respect to TSS (%), Ascorbic acid and anthocyanin content (mg 100 g⁻¹) of fruits *Bacillus* HCA61+ *Pseudomonas* CP109 was found best. The co-inoculation with *Bacillus* and *Pseudomonas* strains could be an ecofriendly and cost effective technology for improving the growth, yield and quality of strawberry.

Keywords: *Bacillus*, *Pseudomonas*, strawberry, growth

Strawberry is a shallow rooted herbaceous plant, of which, 50-90% of the root system is confined to upper 15 cm soil (Galletta and Bringham 1990). Therefore, fertility, moisture, drainage and microbial status in upper soil layer have a great impact on growth, development, fruit yield and quality. The modern strawberry cultivation that warrants high yield and quality requires extensive use of chemical fertilizers, which not only disrupt the balance of nature but also reduce economic efficiency. The chemical fertilizers play a key role by contributing 50-60% increase in productivity (Sindhu et al 2010) but continuous use of chemical fertilizers not only affects soil health and environment adversely but also reduces the productivity of crops. This situation emphasized the need for the development of alternate production systems, which are environment friendly and is more judicious to manage the soil health. Use of natural products like biofertilizers in crop cultivation will help in safeguarding the soil health and the quality of crop (Choudhary and Trivedi 2008). More recently, the use of plant growth promoting rhizobacteria has been exploited in fruit crops and they have proved to be a major tool in fruit production. Plant growth promoting rhizobacteria have gained worldwide importance and acceptance for horticultural benefits. These are probably potential tools to a sustainable horticulture and trend for the future. The plant rhizosphere bacteria belonging to the genera *Pseudomonas* and *Bacillus* have been recognized as early root colonizers, which enhance plant growth by increasing seed emergence,

plant weight and crop yield. Growth promoting substances are likely to be produced in large quantity by rhizosphere microorganisms that influence indirectly the overall morphology of plants. Some of the plant bio-regulators are synthesized endogenously but occasionally needed to be supplemented exogenously for additional stimulus in short duration crops like strawberry that require quick response for increased growth, fruit set and yield. Keeping this in view, the present investigation was undertaken with the objective effect of co-inoculation of effective rhizospheric bacteria on growth, yield and disease incidence in strawberry.

MATERIAL AND METHODS

The study was conducted on "Chandler" strawberry plants during the (2017-18) in greenhouse at CCS Haryana Agriculture University, Hisar (29°10' N latitude and 75° 46' E longitudes). The experiment was replicated twice in completely randomized design. The treatments contained five fertilizer types, *Pseudomonas* strains namely, CP109 and CPS67, *Bacillus* strains namely HCA61, RCA3 and SYB101, whereas untreated soil served as control. Total 60 plants with four replications were inoculated with plant growth promoting rhizobacteria. The planting was done in the last week of October during the both years. Single uniform runner was planted in each pot after treating with carbendazim (0.1%). Each pot was filled with 4 kg of soil. Basal dose of fertilizers was added to soil in each pot along according to per plant requirement as per basal dose. The seventy five per

cent fertilizers viz., urea, liquid NPK (19:19:19), sulphate of potash and murate of potash were given at weekly interval as per the recommendation. Five PGPR strains HCA61, RCA3, SYB101, CP109 and CPS67 were obtained from Department of Microbiology. These bacteria were reported as plant growth promoting bacteria and potential bio-control agents against a wide range of bacterial and fungal pathogens for different cereal and oil seed crops. Bacterial cultures were maintained on Luria Bertani (LB) medium (Sambrook et al 1989). Growth parameters such as height of plant was measured individually and plant spread was calculated by measuring the canopy of plant in East-West and North-South direction with the scale, number of leaves per plant were counted from the time of transfer to the end of growing season (November-March) at fortnightly interval and the average number of leaves per plant, crown diameter of plant was measured [with the help of fruit dimensions (mm) (length and breadth) were also determined in the samples by 'Inox' vernier scale (± 0.05 mm accuracy). Fresh weight of plant recorded at after harvesting of fruits and plants taken for fresh weight were first dried at room temperature and there after dried in oven at 45°C for five to six days until the reduction in weight became constant.

Yield parameter such as fresh weight of ten fruits in each replication were randomly selected to determine average fruit weight. Fruit dimensions (mm) (length and breadth) were also determined in the samples by 'Inox' vernier scale. Quality traits, viz., TSS, acidity, ascorbic acid, anthocyanin content and moisture content of fruits were measured at commercial maturity stages. Total soluble solids (%) was determined using hand refractometer having a range of 0 - 32 (ERMA) by putting a drop of juice and taking the readings. The titratable acidity (%) and ascorbic acid (ml/ 100ml) was determined as per the method suggested by Association of Official Analytical Chemist AOAC (1990) and anthocyanin content (ml 100 ml⁻¹) was determined using pH differential method. The moisture content (%) of fruits was taken for

fresh weight. Then fruits were first dried in oven at 55°C up to 15 days until the reduction in weight became constant.

RESULTS AND DISCUSSION

Plant height and crown diameter were affected significantly with the application of rhizobacteria (Table 1). The maximum plant height and crown diameter was with the Co- inoculation of CP109 strain of *Pseudomonas* and HCA61 strain of *Bacillus*. The increase in plant height and diameter might be due to the solubilization of phosphate caused by the strains, which further increased the nutrients uptake. Number of leaves per plant and fresh weight of plants were influenced significantly by inoculation of *Bacillus* and *Pseudomonas* strains. It might be due to the fact that *Bacillus* and *Pseudomonas* inoculation enhanced the plant growth by different mechanisms including increased solubilization of insoluble nutrients, increased iron availability in the rhizosphere by producing siderophores and production of phytohormones including auxins and cytokinins (Sindhu et al 2010). The dry weight of plant was recorded maximum with T₄ treatment (Table 1). The increase in root dry weight might be attributed to the activity of free living bacteria of *Bacillus megatherium* found in the rhizosphere of roots as phosphate dissolving bacteria, which saved the available phosphorus. Rhizobacteria promote plant growth through phosphate solubilization, indole and siderophore production and therefore, have a potential for use as inoculants to improve crops. IAA like compounds are the most common growth regulators produced by PGPR and is known to enhance plant growth (Glick 2012). Varying level of IAA production was recorded in *Bacillus* and *Pseudomonas* strains (Sharma et al 2018). Bacteria belonging to genera *Bacillus* and *Pseudomonas* are reported to solubilize the insoluble phosphate compounds and aid in plant growth promotion, and siderophores suppress the growth of phytopathogenic fungi and function as stress factor in inducing host resistance (Ahmad et al 2008). Lowering of plant ethylene by

Table 1. Effect of co-inoculation of effective rhizospheric bacteria on growth of strawberry plants

Treatments/observations	Plant height (cm)	Plant spread (cm)	Number of leaves per plant	Crown diameter (mm)	Fresh weight of plant (g)	Dry weight of plant (g)
CPS67+SYB101 (T ₀)	13.27	23.59	11.84	12.89	44.13	12.81
CPS67+HCA61 (T ₁)	13.69	23.84	12.17	13.14	45.10	12.97
CPS67+RCA3 (T ₂)	12.81	23.26	11.51	12.71	43.07	12.19
CP109+SYB101 (T ₃)	13.89	23.79	12.11	13.10	44.76	12.89
CP109+HCA61 (T ₄)	14.11	23.93	12.34	13.21	45.89	13.11
CP109+RCA3 (T ₅)	13.12	23.41	11.87	12.83	43.81	12.51
Control	11.8	21.44	9.87	11.44	40.11	10.71
CD (p=0.05)	0.23	NS	0.13	0.11	0.56	0.14

*NS= Non significant

Table 2. Effect of co-inoculation of effective rhizospheric bacteria on yield of strawberry

Treatments/observations	Number of fruits per plant	Average weight of fruit (g)	Length of fruit (mm)	Breadth of fruit (mm)	Yield per plant (g)
CPS67+SYB101 (T ₀)	21.50	11	40.89	29.31	236.45
CPS67+HCA61 (T ₁)	22.50	11.15	41.15	29.44	250.92
CPS67+RCA3 (T ₂)	21.00	10.85	40.67	29.14	217.01
CP109+SYB101 (T ₃)	22.00	11.17	41.18	29.45	245.83
CP109+HCA61 (T ₄)	23.00	11.21	41.33	29.63	257.92
CP109+RCA3 (T ₅)	21.00	10.92	40.75	29.21	229.40
Control	19.25	9.86	38.89	27.81	187.91
CD (p=0.05)	0.17	NS	NS	NS	3.26

*NS= Non significant

Table 3. Effect of co-inoculation of effective rhizospheric bacteria on quality of strawberry fruits

Treatments/observations	TSS (%)	Acidity (%)	Ascorbic acid (mg 100 ml ⁻¹)	Anthocyanin content (mg 100 ml ⁻¹)	Moisture content in fruit (%)
CPS67+SYB101 (T ₀)	7.40	0.77	44.23	45.36	92.51
CPS67+HCA61 (T ₁)	7.43	0.82	44.50	46.21	92.33
CPS67+RCA3 (T ₂)	7.30	0.76	42.37	44.21	92.72
CP109+SYB101 (T ₃)	7.46	0.81	44.80	45.81	92.44
CP109+HCA61 (T ₄)	7.60	0.83	45	46.78	92.21
CP109+RCA3 (T ₅)	6.80	0.78	43.89	44.52	92.61
Control	6.31	0.84	38.84	40.43	94.00
CD (p=0.05)	0.08	NS	1.12	1.36	NS

*NS= Non significant

deamination of its immediate precursor 1-aminocyclopropane-carboxylate (ACC) is key trait found in many rhizobacteria. Many other studies have also shown positive effect of inoculation with PGPR strains, especially *Bacillus* and *Pseudomonas* sp. on growth of fruit crops (Yu et al 2012) which could promote plant growth through different mechanisms such as production of IAA and siderophore and solubilization of phosphate.

The root inoculation of strawberry plants with *Bacillus* and *Pseudomonas* in combinations showed significant effect on number of fruits per plant and yield of fruits (Table 2). The maximum number of fruits per plant and yield of fruits was obtained from the plants inoculated with CP109 strain of *Pseudomonas* and HCA61 strain of *Bacillus*. It which might be due to the fact that strains had potential for greater solubilization of insoluble phosphorus and some other factors such as release of growth promoting substances, control of plant pathogens and proliferation of beneficial organisms in the rhizosphere. Similar results were reported by Pandey et al (2013) in gladiolus, Singh (2015) in strawberry, Karishma et al (2013) in gerbera and Barman et al (2003) in tuberose.

The total soluble solids in fruits of strawberry were significantly affected by different strains of *Bacillus* and

Pseudomonas (Table 3). The maximum TSS was recorded with the application of *Pseudomonas* strain CP109 and *Bacillus* strain HCA61. The maximum ascorbic acid content and anthocyanin content were recorded in fruits harvested from the plants inoculated with treatment T₄. Karakurt et al (2011) also reported that increased in TSS of sour cherry fruits harvested from the plants inoculated with rhizobacteria had more vegetative growth, which had positive correlation with quality of fruits, which might be due to that more vegetative growth could provide more photosynthesis activity and more synthesis of soluble matter, such as sugars, which affected the fruit quality.

CONCLUSION

The rhizospheric bacteria in association with 75% RDF have great potential for use as PGPR to increase the production of strawberry. Further studies are required under field conditions for application of these PGPR as biofertilizers and biopesticides for sustainable agriculture.

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Geo-Statistical Analysis to Understand Nature of Forest Patch Shape Complexity in Panchet Forest Division under Bankura District, West Bengal

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Abstract: Habitat quality depends on both functional and structural pattern of landscape. Structural pattern of the habitat address fragmentation, complexity, configuration etc. Therefore, nature of forest habitat shape complexity becomes a new subject matter in biodiversity management. The present study also addresses this issue considered 36 forest patches in Panchet Forest Division under Bankura district for shape quality assessment. Landscape ecological indices like Shape Index, Fractal Dimension, and Perimeter Area Ratio are calculated using ArcGIS 10.1 and FragState 4.2 version software. From these indices, it is found that the larger forest patches are more structurally fragmented and also more complex than small patches. Not only that study also compared these indices values with a standard shape i.e. circle by regression method. The comparative results present that the larger forest patches are significantly more complex from standard shape than small patches in Panchet Forest Division.

Keywords: Biodiversity, Corridor, Fragmentation, Habitat, Landscape ecology, Species migration

Habitat patch shape and size are the key factors which control species inter patch movement, colonization, and conflict (Farina 2006, Ewers et al 2007). Its nature i.e. linear, curvilinear or circular is a vital concept in forest management (Cushman et al 2010). In a human altered landscape, forest areas are randomly distributed. Similar pattern is found in PFD. Isolated pattern of forest patches create landscape complexity. Every patch has a unique shape and size but its nature depends on its perimeter, curvature and size (McGarigal and Cushman 2005, Hanski 2015). Therefore, habitat patch size and shape compromise different situation functioning in the landscape (Forman and Godron 1986). Diversified landscape exposes several kinds of opportunity to wildlife animal community for their ecological survival (Forman 2006) where each and every land cover plays a significant role. But in such condition, habitat is an important criterion to continue their existence. Forest cover is a spatial unit, which is the most important part of habitat entire the inhomogeneous landscape. Another concept is size of the habitat. It is common perception that patch size has an impact on population size, colonization rate, immigration level, edge contrast, etc. in a region (Bowman et al 2002, Connor et al 2000, Prugh et al 2008). Larger habitat patches in a landscape play an important role than isolated small patches (Steenweg 2016, Carvajal et al 2018) in accordance with habitat dominance and dependency. So the fragmentation quality of larger patches is a vital consideration in landscape quality. High fragmented large patch address low habitat quality in the question of

species colonization, frequent movement and disturbance. Not only larger patches but also structural and geographical fragmentation pattern of entire landscape is another very important consideration for understanding the regional ecological process. Both fragmentations are shape dependent (Collinge and Forman 1998, Fahrig et al 1988, Ewers and Didham 2006). The trend is significantly linear in between geographical and habitat structural fragmentation (Cushman 2006). In response, high fragmented or complex landscape does not acquire sustainable biodiversity as well as balance number of animal population (Dramstad et al 1996, Fahrig 2013). Finally, the level of habitat shape complexity or forest fragmentation is very essential issue in landscape management (Crooks et al 2017). Above literatures present that quantification of shape complexity is very urgent work for biodiversity management through landscape analysis.

The forest landscape under Panchet Forest Division (PFD) is very fragmented in nature. Therefore, the present study quantifies forest shape complexity and compare with a standard shape to understand, which patches are structurally poor in condition, which opens an approach to manage forest landscape in the study area. Patch area, perimeter, curvature and edge are the basic elements for understanding landscape surface pattern (Fahrig 2013). Individual patch structural complexity depends on these facts. Depending on these facts, study tries to address the structural configuration of forest patches for understanding which patches are more complex in nature under PFD considering.

MATERIAL AND METHODS

Study area: PFD located at 22°53'N to 23°12'N and 87°03'E to 87°42'E. Total geographical area of the division is 1355.62 km². This division adjoined with Paschim Medinipur forest division in south and Darakeswar River in north. The percentage of forest cover had been increasing from last few decades (Sudhakar et al 1994) but patchy and adjacent with agricultural land (Mandal et al 2018). The study area is under tropical dry deciduous forest matrix. According to State Forest Report 2010, Sal (*Shorea robusta*) trees dominance is common in every forest patch with indigenous scrubs and herbs. Due to agricultural expansion, encroachment of forest is a common fact in this region. Study considered 36 forest patches under PFD, with area greater than 100 ha. Less than 100 ha forest patches were not considered because of its less ecological influence in the landscape.

The study area was masked from P6 satellite image 2015 from ISRO (Indian Space Research Organisation) departmental website. To demarcate forest patch, a well classified map was prepared from this image using ERDAS 9.3 and ArcGIS 10.1 version software through unsupervised classification method. After classification 36 forest patches were demarcated. Surface pattern matrices like SI, FD, and PAR were calculated by using FragState interface tool for selected 36 forest patch (Fig. 1). In same way, these same indices were calculated for manually created 36 different size circles (Fig. 2) because it holds unique value to understand shape characters. After quantification, a comparison analysis was prepared to understand the shape complexity between forest patches and standard circle shape. Here linear correlation regression method was also applied to find the trend between patch size and shape.

Perimeter Area Ratio (PAR) (McGarigal and Marks 1994)

$$PAR = \frac{\sum p_{ij}}{\sum a_{ij}}$$

P_{ij} is the perimeter (m) of j focal patch in i class and a_{ij} is the area (ha) of j focal patch in i class. PAR value m ha⁻¹ increasing approaches indicate higher complexity of the patch shape. It is the simplest measure of shape complexity.

Shape Index (SI) (McGarigal and Marks 1994)

$$SI = \frac{P_{ij}}{\sqrt{a_{ij}}} \text{ Adjusted by circle}$$

P_{ij} is the perimeter (m) of j focal patch in i class and a_{ij} is the area (ha) of j focal patch in i class. N is the total number of focal patch. MSI is equal to 1 when all patches are circular (for polygons) and it increases with increasing patch shape irregularity. SI (single patch) is broadly applicable in landscape ecological study (Forman and Godron 1986).

Fractal Dimension (FD) (McGarigal and Marks 1994)

$$FD = \frac{2 \ln p_{ij}}{\ln a_{ij}} \text{ adjust by circle standard}$$

P_{ij} is the perimeter (m) of focal patch and a_{ij} is the area (ha) of the focal patch. N is the total number of focal patch. Mean patch fractal dimension (FD) is another measure of shape complexity. It nears 1 when patch shapes are 'simple', such as circles and it approaches 2 as patch shape perimeter complexity increases.

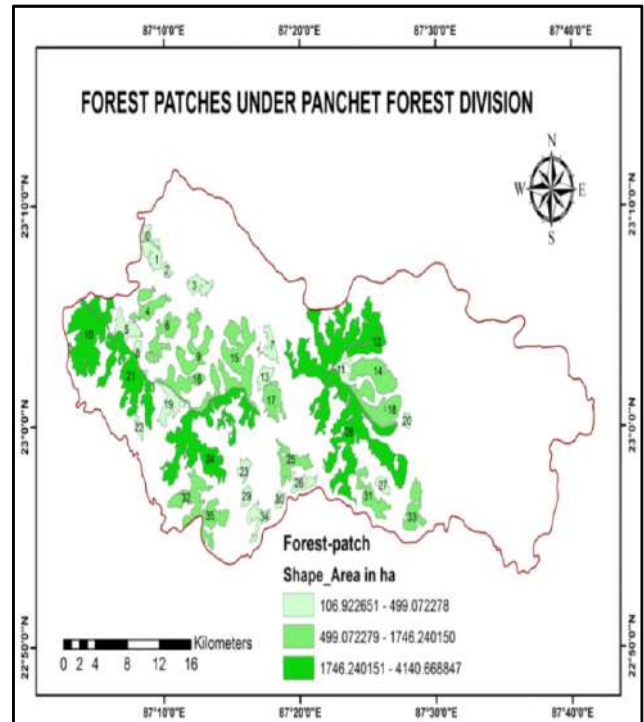


Fig.1. Considered 36 forest patches with ID under PFD

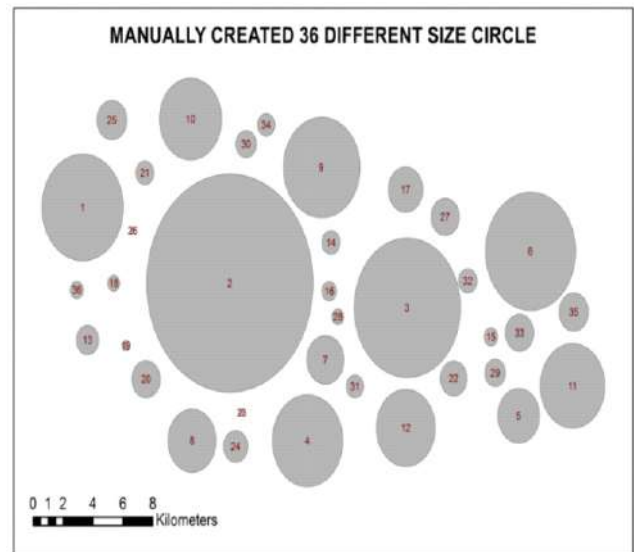


Fig. 2. 36 different size circles with ID

RESULTS AND DISCUSSION

Individual forest patch matrix addresses different shape complexity under PFD. 36 selected forest patches were not in same size and shape. The largest patch was ID-28 with 4144.25 ha and lowest patch was ID-2 with 107.69 ha area (Table 1). Generally, a linear positive relation was common between patch areas and perimeter and the relation was just negative in case of PAR when patch shape was standard. This situation addresses that largest patch hold minimum edge per patch area than small patches. Same trend was also found in selected 36 forest patches under PFD but in case of larger patches the decreasing trend line was not follow the same direction (Fig. 4). It follows the parallel direction (red line in Fig. 4), which indicates the larger patches hold substantial perimeter length. It was possible when patch becomes more curvilinear or complex.

PAR is the simple measure of shape size relationship. Actually, shape complexity depends on fractal nature and amount of curvature of a particular patch. Therefore, SI and FD are another two most important measures to understand this circumstance (Andren 1994, Fahrig 2013). These two landscape ecological indices value implies same trend that the larger patches hold high shape complexity (Fig. 5 and 6) in PFD. Usually larger patch has a capacity to hold high

number of population (Dramstad et al 1996, Villard et al 2014). Therefore, this is a sound full controversial issue in biodiversity management that is larger patch holds high number of animals with high structural complexity. This condition creates more man animal conflict. The larger patches in the study area hold high SI and FD value. The largest forest patch in the study area i.e. ID-28 grips (SI-5.55 and FD-1.34) at that time PAR value is minimum (30.60 m ha⁻¹). The smallest patch ID-2 under study area holds (SI-1.61

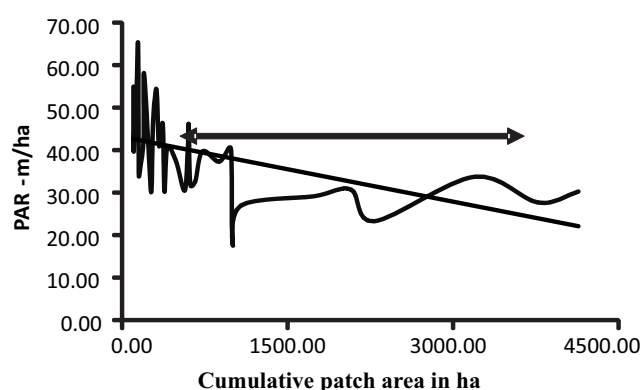


Fig. 4. Relation between patch area and PAR among 36 forest patches under PFD

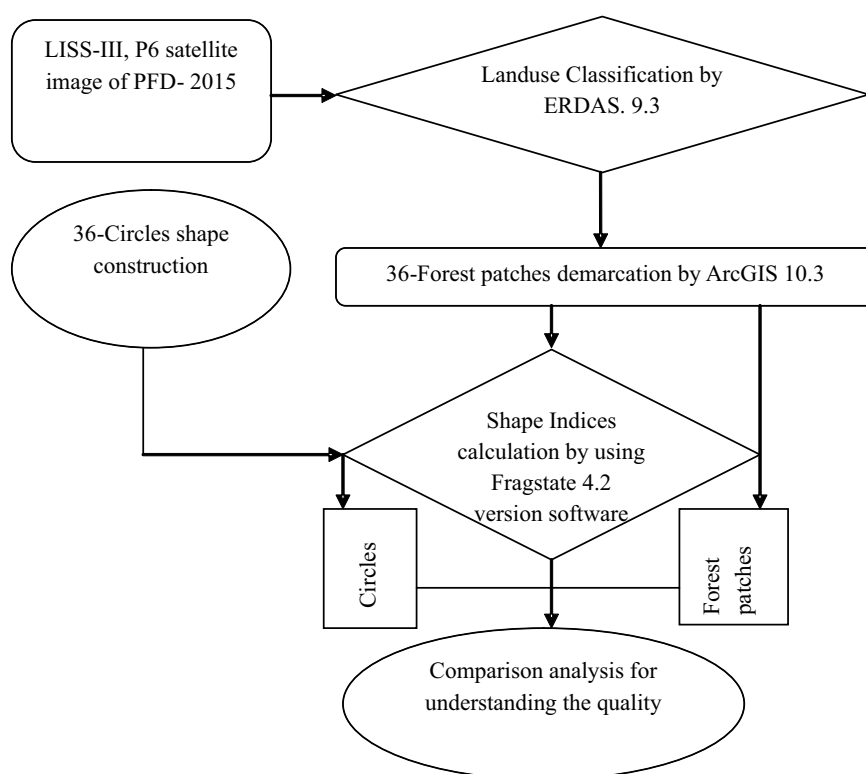


Fig. 3. Research steps for complete objectives of the study

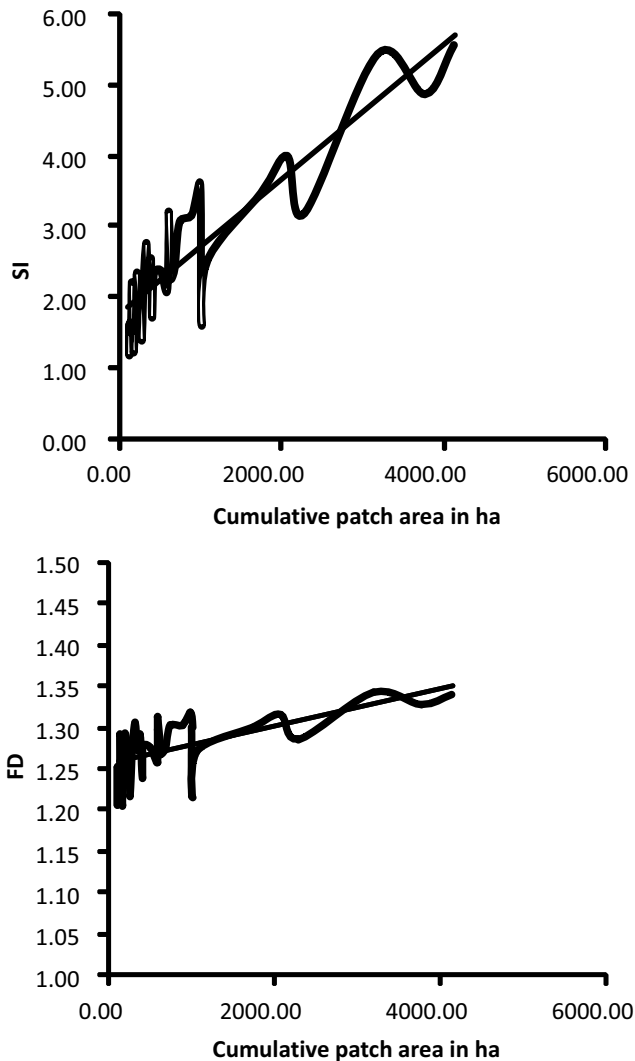


Fig. 5 and 6. Relation between patch area and shape indices (SI,FD) in 36 forest patches under PFD

and FD-1.25) with higher PAR (55.30 m ha⁻¹). In the next section, correlation and regression analysis has been made to describe main two issue. First is the comparison between circle shape with forest patches shape to understand the complexity differences. Next one is trend analysis between forest area (independent variable) and other selected parameters for both circle and forest patches under PFD.

To complete initial objective that is forest patches complexity assessment, several linear regression analysis is taken to understand the quality differences between circles shape and existing 36 forest patches under PFD. A comparative analysis was made keeping patch area as independent variable both for circles and forest patches. The deviation value of r in leaner relation suggests that the nature of forest patch shape complexity was high than standard circle shape and it was very general situation in the

Table 1. Selected shape indices of 36 forest patches (ID-0 to 35) under PFD

ID	Area	SI	FD	PAR	ID	Area	SI	FD	PAR
0	203.28	2.39	1.12	58.50	18	1081.74	2.63	1.12	27.30
1	389.62	1.73	1.07	30.60	19	493.68	1.73	1.06	37.80
2	107.69	1.43	1.05	55.30	20	110.11	1.21	1.03	40.00
3	340.01	2.00	1.09	41.40	21	2081.20	4.11	1.17	31.10
4	608.63	3.10	1.14	46.40	22	123.42	1.68	1.07	53.40
5	415.03	2.48	1.12	40.90	23	200.86	1.67	1.07	43.00
6	733.26	3.05	1.14	40.10	24	3202.87	4.01	1.17	34.10
7	317.02	1.42	1.05	54.70	25	602.58	2.85	1.13	41.20
8	150.04	2.11	1.11	65.60	26	286.77	2.24	1.11	47.00
9	999.46	3.03	1.14	35.50	27	171.82	1.43	1.05	36.40
10	2290.53	2.93	1.13	23.60	28	4144.25	2.57	1.07	30.60
11	245.63	2.00	1.09	40.80	29	157.30	1.36	1.04	34.40
12	3778.83	4.76	1.18	28.00	30	130.68	1.44	1.05	47.10
13	271.04	1.47	1.05	30.50	31	627.99	2.63	1.12	31.90
14	1009.14	1.71	1.07	18.00	32	883.30	3.28	1.15	37.60
15	1744.82	3.54	1.15	29.50	33	618.31	2.30	1.11	35.50
16	986.15	3.75	1.16	41.00	34	373.89	2.76	1.13	46.50
17	573.54	2.18	1.10	30.80	35	667.92	2.55	1.12	32.90

landscape. In case of standard shape (circle) SI, r value was almost 0 that is insignificant relation between size and shape (Fig. 7). But in case of 36 forest patch in PFD, the linear regression value was positive (Fig. 7) and r value is + 0.889 (FD) and + 0.639 (SI). These trend address that shape complexity will be high when larger the forest patch. Negative exponential trend was found in 36 different size circle PAR. The r value is - 0.389 (Fig. 7), which shows circle area when increased area per perimeter will decrease. That means shape complexity will decrease when circle area is increased. Same exponential trend was not found rather it become more haphazard among 36 forest patches in PFD the r value is - 0.504 (Fig. 7), which was lower and less significant than standard circle shape. This decreasing r value suggests minimum correlation between forest patch PAR and patch area.

After analyze the ecological indices, it has been cleared that shape complexity is a common landscape surface pattern. The study area almost geographically and structurally fragmented (Mandal et al 2018), from these methods it has been understood clearly that how much complex the individual forest patch under PFD compare to standard shape. Another perspective is also cleared that which patches are more complex in shape. From this analysis, it is furnish that larger patches are more prone to high fragments than small patches in the study area. In the

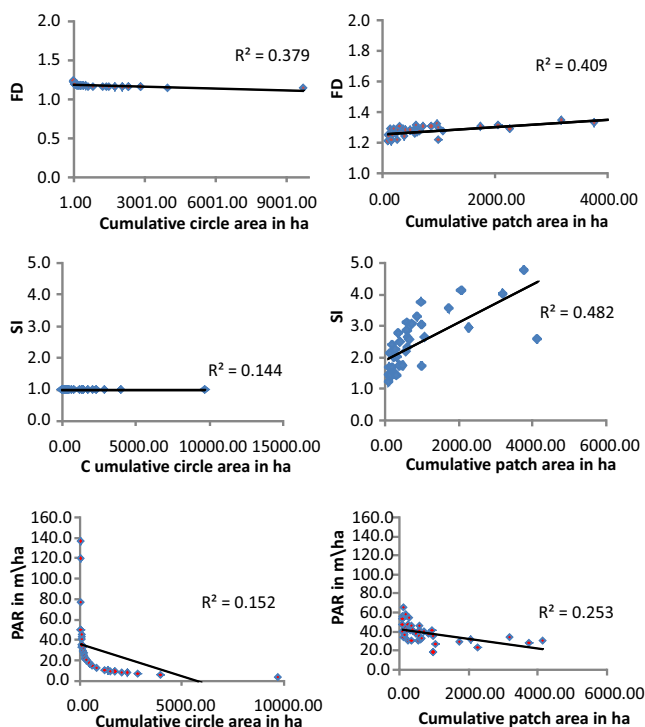


Fig. 7. Comparative correlation matrix between standard 36 circles shape and 36 forest patches shape under PFD

Table 2. Regression value differences between circle (standard shape) and forest patches (present landscape) shape complexity measurement indices

Both circle and forest patch area is independent variable		
Dependent variables	Circles (r)	Forest patches (r)
SI	0.000	+ 0.889
FD	- 0.616	+ 0.639
PAR	- 0.389	- 0.504

same way larger forest patches in a landscape are playing very vital role in biodiversity function (MacArthur et al 1967). This situation makes more man animal conflict (Chatterjee 2016) due to high edge density, extinction, haphazard movement (Desai 2010), and other societal problems (Taylor 1993). Therefore, management approach always should be focused on larger forest patches to manage biodiversity in a region (Cullotta et al 2014). In the study area, the larger patches like ID-10, 12, 14, 18, 28, 24, 21, and 15 will have high man animal conflict.

CONCLUSION

Larger forest patches under PFD are more complex or fragmented but these are ecologically significant. As a result of it maximum number of ecological incident occurred on that forest patches under PFD reported by Forest Department

2017. Therefore, shape structural management of these forest patches may be the best way to decrease shape complexity which balance the ecological situation.

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Effect of Pre-Sowing Treatments on the Germination of Five Legume Species and Their Tolerance to Desiccation

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Abstract: Two Experiments were conducted to understand the suitable pre-treatment methods to know the germination behaviour of five selected legume species (*Albizia thompsonii*, *Calopogonium mucunoides*, *Crotalaria micans*, *Tephrosia candida*, *Albizia procera*) and their desiccation tolerance. Pre-treatments given were control (no treatment), mechanical scarification (seed coat cutting), H₂SO₄ (97%) for different durations (30 sec, 5 min, 15 min and 30 min) and heat scarification 60°C (9, 19 and 24 hours) and 80°C (4, 19 and 24 hours). Out of the 11 pre-treatments tested, conc. H₂SO₄ and mechanical scarification provided significant results in all the five species. Mechanical scarification provided maximum germination (96.25%) of *C. micans* while, conc. H₂SO₄ treatment for 30 min provided maximum germination of *T. candida* (97.50%). Seeds of all species were dried down to the target moisture contents (TMC) of 9, 7, 5, 3 and 2.5 per cent using silica gel for the second experiment to study the effect of seed desiccation on viability of these seeds. The viability of *C. mucunoides* reduced to 80 per cent at 3 per cent TMC while for *T. candida*, it was 75 per cent at 5 per cent TMC. In addition, the viability of both the species reduced greatly when dried below 3 per cent TMC. Both species i.e. *C. mucunoides* and *T. candida* took 37 days to reach the set TMC of 2.5 per cent using silica gel drying.

Keywords: Pre-sowing treatment, Seed Dormancy, Seed Desiccation, Seed viability

The family Fabaceae or Leguminosae is considered to be the third-largest family after Asteraceae and Orchidaceae which consists of 751 genera and 19,500 species (Christenhusz and Byng 2016). The member of this family extends in all terrestrial climates except Antarctica and the high arctic (Stevens 2017) and the trees were found often in tropical regions, while outside the tropics the herbaceous plants and shrubs are predominated (Watson and Dallwitz 2007). The distinctive floral structures, the formation of pod and nodule formation in the root are the distinctive features of this family. Despite the great importance one of the major problems in successful seed germination of legumes is the presence of hard seed coat (Jayasuriya et al 2013) which causes delayed or decreased seedling emergence. Therefore, it is necessary to lessen the hard seed content before sowing of the seeds. This problem can be overcome by application of one of the several pre-treatments available. Many researchers (Kumar 2016, Kumar et al 2017, Maharana et al 2018) suggested that the pre-sowing treatments significantly influenced the germination parameters. Important methods of seed treatment include mechanical scarification (Pandurangi et al 2003) and acid treatment (Can et al 2009). Heat scarification is also one of the most common methods because it is simple and easy to use, but appropriate treatment time and temperature determine the effectiveness on cracking of hard seed coat and improvement on germination (Rutar et al 2001). *Albizia*

procera (Roxb.) Benth. is a tall tree up to 30 m with an open canopy and trunk of 35 cm in diameter and it is planted in soil erosion degraded land due to its soil binding capacity. While *Albizia thompsonii* Brandis is a large deciduous tree and is classified as 'Near Threatened' in the IUCN Red List of Threatened Species (Chadburn 2012). The tree is recognised for its high ecological, economic and medicinal value. *Tephrosia candida* (Roxb.) DC. on the other hand is a shrub or small tree up to 3.5 m tall, with straight branches from the base and is suitable for rehabilitation of degraded lands as it forms root nodules with Bradyrhizobium and fixes atmospheric nitrogen. *Crotalaria micans* Link is a shrub up to 4 m tall and also form root nodules with Bradyrhizobium spp. and fixes nitrogen (Orwa et al 2009). *Calopogonium mucunoides* Desv. is a vigorous, short-lived perennial legume and provides soil protection from erosion and also improves the fertility of the soil. In this study, two experiments were conducted. In the first experiment; we examined the effect of pre-sowing treatments on germination behaviour of the selected legume species. Another experiment was to understand the time taken to reduce the seed moisture content to the desired level and its effect on seed viability.

MATERIAL AND METHODS

Seed materials: Seeds of *Albizia thompsonii*, *Calopogonium mucunoides*, *Crotalaria micans*, *Tephrosia candida* and *Albizia procera* were collected from Mizoram University

campus. Seeds of *A. thompsonii* were collected in January; *A. procera* seeds in March, while *T. candida*, *C. micans* and *C. mucunoides* during February to March by direct extraction of pods from the plant. The seeds were extracted manually from the pods and initial moisture content, viability and germination were determined. Experiments were done in the Department of Forestry, Mizoram University.

Determination of moisture content and viability: The Moisture Content (MC) was determined by using the method described in the International Rules for Seed Testing (ISTA 2007).

$$MC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

The viability of seeds was determined using Tetrazolium assay test (Verma and Manoj 2013). Four replicates of 30 seeds were used for viability assessment and the Viability percentage was calculated as the average of the four replicates.

Germination test: A random sample of 80 seeds was taken out from the seed lot and placed in 4 Petri dishes i.e. 20 seeds in one petri dish. The filter paper was used as a substrate in Petri dishes. The germination experiment lasted for 21 days and the numbers of germinated seeds were counted daily. Seeds were considered germinated when the radicle appeared for at least 2 mm (ISTA 1999). The germination percentage was calculated as the average of the four replicates.

Experimental details: Eleven dormancy breaking treatments were applied viz. control (no treatment), mechanical scarification (cutting of hard seed coat), concentrated sulphuric acid scarification (30 sec, 5 min, 15 min and 30 min) and heat scarification at different temperature and durations 60°C (9, 19 and 24 hours) and 80°C for (4, 19 and 24 hours). Mechanical treatment was given to the seeds by cutting a small portion of the seed coat in order to enhance the imbibition of water. Deep cuts were avoided in order to prevent damage to the embryo.

Seed desiccation: Seed samples were dried to target moisture contents of 9, 7, 5, 3 and 2.5 %. The seeds were put inside the empty tea bags. One sample bag consists of eighty seeds. The tea bags were placed in a desiccator and mixed with an equivalent weight of silica gel and held at 20°C. After every 48 hours, the silica gel was changed and the weight of the seeds was taken to compare with target weight. The formula developed by ISTA 2007 was used to calculate the target weight that corresponded to each target moisture contents.

$$\text{Weight of seed (g) at target} = \frac{100 - \text{IMC}}{100 - \text{TMC}} \times \text{initial seed weight (g)}$$

Where IMC=initial seed moisture content

TMC=target seed moisture content

When the target moisture content of seed was achieved, a viability test was conducted using the tetrazolium assay test (Verma and Manoj 2013). Twenty seeds of four replicates were used for viability estimation.

Parameters associated with germination

- i) Germination Percentage (GP) was calculated as per ISTA 1999

$$GP = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

- ii) Germination Energy (GE): The percentage of seed germination obtained at maximum daily germination speed.

- iii) Mean Germination Time (MGT) was calculated by the following formula (Ellis and Roberts 1981)

$$MGT = \frac{(n_1 \times d_1) + (n_2 \times d_2) + (n_3 \times d_3) + \dots}{\text{Total number of days}}$$

- iv) Germination Value (GV) was calculated as per Czabator 1962, i.e.,

$$GV = PV \times MDG$$

Where, Peak Value (PV) was as follows

$$PV = \frac{\text{Highest seed germinated}}{\text{Number of days}}$$

and, Mean Daily Germination (MDG) was as follows

$$MDG = \frac{\text{Total number of germinated seeds}}{\text{Total number of days}}$$

- v) Germination Index (GI) was calculated by dividing the total number of seeds germinated at the end of the experiment with the time taken for 50 per cent germination.

RESULTS AND DISCUSSION

Prior to the experiment the moisture content, viability and germination of all the five species were recorded. *Crotalaria micans* have the highest initial moisture content (14.99%) as compared to other species. All the five species possessed a good initial seed viability percentage (ranging between 90 to 97%). The initial germination ranged from 0.00% for *Albizia thompsonii* to 42.50% for *Crotalaria micans* (Table 1).

Effect of the pre-sowing treatments on seed germination: *Albizia thompsonii* showed significant GP (95%) when treated with conc. H_2SO_4 for 30 min., which was at par with that of mechanical scarification (93.75%). The maximum germination energy was observed in conc. H_2SO_4 treatment for 30 minutes (Table 2). The seeds failed to

germinate without any treatment. The highest germination value (GV) was in the mechanical scarification (11.57) followed by conc. H_2SO_4 treatment for 30 min. The germination index was also highest in this treatment. The significantly highest GP for *Albizia procera* seeds was observed for heat treatment of 60°C for 24 hrs (92.53%) which was at par with the conc. H_2SO_4 treatment for 30sec (92.50%) (Table 3). The germination energy (90.02%) and mean germination time (16.05) were also highest for the heat treated seeds at 60°C for 24 hrs. The present study carried out on *Albizia* spp. (*A. procera* and *A. thompsonii*) showed that both the species gave maximum germination when pre-treated with conc. H_2SO_4 at different duration (30sec to 30

min), *A. thompsonii* responded well with an increase in the germination percentage with the increase in immersion duration from 30 sec to 30 min (i.e. 57% to 95%), but in case of *A. procera* the opposite trend was observed. The increase in duration showed a decline in the GP from 92.5 per cent (30 sec) to 42.50 per cent (30 min). Kheloufi et al (2017) in their study also suggested an improvement in the germination percentage for *A. cyanophylla* and *A. farnesiana* while increasing the duration of H_2SO_4 (from 60 to 120 min) on the contrary, increasing the duration had a negative effect on the seed germination of *A. decurrens* from 97 per cent (60 min) to 43 per cent (120 min). It was seen from the present study that seeds untreated (control treatment) gave the least

Table 1. Initial seed parameters, seed dimensions and number of seeds of selected species

Species	Initial parameter (%)			Seed dimension (mm)			Number of seeds kg ⁻¹
	Moisture content	Viability	Germination	Length	Width	Thickness	
<i>A. thompsonii</i>	9.62	97.50	0.00	5.69±0.29	4.51±0.33	1.43±0.14	42,000
<i>C. mucunoides</i>	11.12	95.00	2.50	3.31±0.25	2.47±0.17	1.96±0.16	73,000
<i>T. candida</i>	9.53	97.50	35.00	5.22±0.25	3.96±0.23	1.80±0.16	45,000
<i>C. micans</i>	14.99	90.00	42.50	4.90±0.39	3.81±0.27	1.49±0.11	43,000
<i>A. procera</i>	10.19	95.00	30.00	6.44±0.31	4.90±0.30	2.42±0.37	23,000

Table 2. Effect of pre-sowing treatments on germinability of *Albizia thompsonii*

Treatment	GP (%)	GE (%)	MGT	GV	GI
Control	0.00 (0.00)	0.00 (0.00)	0.00	0.00	0.00
Mechanical scarification	93.75 (75.91)	70.00 (56.80)	17.55	11.57	1.10
H_2SO_4 (30sec)	57.51 (49.31)	55.00 (47.85)	10.90	1.84	0.65
H_2SO_4 (5min)	90.05 (71.75)	72.50 (58.39)	15.43	4.97	1.08
H_2SO_4 (15min)	85.00 (67.26)	70.00 (56.81)	14.81	4.52	1.04
H_2SO_4 (30min)	95.00 (77.38)	92.44 (74.37)	14.29	8.38	1.18
Heat (60°C, 9hrs)	5.17 (13.01)	5.00 (12.81)	20.00	0.01	0.02
Heat (60°C, 19hrs)	20.00 (26.53)	20.00 (26.52)	16.88	0.16	0.12
Heat (60°C, 24hrs)	67.50 (55.25)	67.50 (55.24)	16.81	1.83	0.40
Heat (80°C, 4hrs)	32.51 (34.74)	32.54 (34.77)	14.47	0.38	0.24
Heat (80°C, 19hrs)	55.00 (47.86)	55.00 (47.86)	14.45	1.28	0.38
Heat (80°C, 24hrs)	32.50 (34.72)	32.50 (34.72)	15.62	0.42	0.22
CD (p=0.05)	3.44	3.10	2.06	0.46	0.22

Figures in parentheses are arc sine transformed values
GP : Germination percent, GE : Germination energy, MGT : Mean germination time, GV : Germination value and GI : Germination index

Table 3. Effect of pre-sowing treatments on germinability of *Albizia procera*

Treatment	GP (%)	GE (%)	MGT	GV	GI
Control	30.01 (33.20)	21.25 (27.43)	8.86	0.32	0.23
Mechanical scarification	83.75 (66.25)	62.73 (52.35)	13.19	10.85	1.39
H_2SO_4 (30 sec)	92.50 (74.26)	77.53 (61.70)	12.29	9.11	1.44
H_2SO_4 (5 min)	72.55 (58.41)	53.75 (47.13)	9.90	5.07	1.09
H_2SO_4 (15 min)	60.00 (50.75)	50.01 (44.99)	8.07	3.81	1.00
H_2SO_4 (30 min)	42.69 (40.78)	31.64 (34.21)	5.90	1.76	0.70
Heat (60°C, 9 hrs)	76.44 (60.94)	68.35 (55.75)	13.80	2.40	0.59
Heat (60°C, 19 hrs)	85.02 (67.3)	80.00 (63.45)	14.77	3.06	0.58
Heat (60°C, 24 hrs)	92.53 (74.23)	90.02 (71.58)	16.05	3.36	0.57
Heat (80°C, 4 hrs)	80.09 (63.48)	80.16 (63.54)	14.98	2.58	0.61
Heat (80°C, 19 hrs)	60.01 (50.75)	60.00 (50.75)	13.92	1.31	0.53
Heat (80°C, 24 hrs)	72.52 (58.37)	72.02 (58.05)	14.14	2.11	0.51
CD (p=0.05)	2.51	1.59	0.92	0.75	0.33

See Table 2 for details

germination percentage, indicating that the seeds have the typical hard, impermeable seed coat of the leguminous species (Kheloufi et al 2017). As a result of the hard seed coat, the non-scarified seeds of the leguminous species failed to show any germination or imbibitions as reported by Chauhan and Johnson (2009). Kheloufi (2017) had demonstrated the efficiency of H_2SO_4 to enhance integumentary inhibition.

For *Calopogonium mucunoides* conc. H_2SO_4 provided highest GP of 95.01 per cent with MGT of 21.00 and GI of 1.00 when immersed for 30 min (Table 4). The least GP (2.50%) was in control. The highest GV (7.80) was for mechanical scarification. In a study conducted by Wycherley (1960) untreated seed of *C. mucunoides* often gave low germination percentage and establishment is uneven and slow, and scarification, mechanically by cutting and by conc. sulphuric acid promotes germination in this species. The H_2SO_4 pre-treatment showed significant results for *Tephrosia candida* seeds (Table 5). The highest GP (96.57%) was recorded for 30 min. conc. H_2SO_4 with maximum GE of 97.27 and MGT of 19.76. Mechanical scarification also gave a good GP (81.25%) as compared to the other treatments. Even though the 30 minutes conc. H_2SO_4 treatment has the highest GP, the highest germination value (8.63) was in mechanical scarification. The mechanical scarification gave the maximum germination index with 1.22 (Table 5). *Tephrosia candida* possesses seed physical dormancy due to the hardness of testa (Bawar 2015). In the present study, H_2SO_4 (5-30 min) were found to be the suitable pre-treatments. Similarly, mechanical scarification also provided good results in enhancing the germination. According to Bawar (2015) germination can be enhanced from 22.77 per cent (control treatment) to 90.14 per cent by mechanical scarification.

The seeds of *Crotalaria micans* responded well to mechanical scarification giving the highest GP (96.25%) as compared other treatments (Table 6). The highest germination energy was recorded in mechanical scarification with 90.01 per cent with the MGT of 12.19. The least GP (10%) was observed when seeds of *C. micans* were heat treated for 24 hrs at 80°C. Mechanical scarification gave the highest germination value as well as the germination index (15.53 and 1.76 respectively). From the present study it was found that the *C. micans* possess physical dormancy and hence it shows best germination results when mechanically scarified but significant results were also seen under conc. H_2SO_4 (30 min) treatment. According to the study carried out by Okonwu and Eboth (2017) significant germination resulted when *Crotalaria verrucosa* was treated with H_2SO_4 (20 min).

Effect of desiccation on seed viability: In *Tephrosia*

Table 4. Effect of pre-sowing treatments on germinability of *Calopogonium mucunoides*

Treatment	GP (%)	GE (%)	MGT	GV	GI
Control	2.50 (9.09)	2.51 (9.10)	8.28	0.00	0.01
Mechanical scarification	76.27 (60.86)	63.40 (52.75)	15.85	7.82	0.86
H_2SO_4 (30sec)	35.00 (36.25)	32.50 (34.74)	7.71	0.58	0.35
H_2SO_4 (5min)	51.53 (45.86)	35.00 (36.26)	15.91	0.93	0.39
H_2SO_4 (15min)	82.50 (65.29)	80.00 (63.45)	19.37	3.34	0.67
H_2SO_4 (30min)	95.01 (77.34)	72.51 (58.37)	21.00	4.02	1.00
Heat (60°C, 9hrs)	12.50 (20.69)	2.50 (9.1)	12.20	0.12	0.30
Heat (60°C, 19hrs)	10.01 (18.43)	10.01 (18.43)	12.25	0.05	0.09
Heat (60°C, 24hrs)	22.50 (28.3)	20.01 (26.56)	10.57	0.24	0.26
Heat (80°C, 4hrs)	35.01 (36.26)	27.53 (31.64)	13.29	0.60	0.29
Heat (80°C, 19hrs)	15.01 (22.78)	15.00 (22.78)	10.00	0.16	0.59
Heat (80°C, 24hrs)	35.01 (36.26)	35.01 (36.26)	13.50	0.49	0.34
CD (p=0.05)	2.05	1.22	1.36	0.16	0.39

See Table 2 for details

Table 5. Effect of pre-sowing treatments on germinability of *Tephrosia candida*

Treatment	GP (%)	GE (%)	MGT	GV	GI
Control	35.02 (36.26)	35.05 (36.28)	9.66	0.52	0.25
Mechanical scarification	81.25 (64.38)	66.25 (54.47)	14.75	8.63	1.22
H_2SO_4 (30 sec)	42.51 (40.67)	35.01 (36.26)	9.14	0.81	0.41
H_2SO_4 (5 min)	80.03 (63.46)	54.98 (47.84)	15.80	3.72	0.89
H_2SO_4 (15 min)	80.01 (63.44)	80.02 (63.53)	16.18	3.76	0.64
H_2SO_4 (30 min)	96.57 (79.61)	97.27 (80.64)	19.76	5.18	0.82
Heat (60°C, 9 hrs)	52.52 (46.42)	35.03 (36.27)	10.19	1.74	0.57
Heat (60°C, 19 hrs)	65.03 (53.74)	60.1 (50.81)	8.92	2.70	0.77
Heat (60°C, 24 hrs)	57.55 (49.33)	47.5 (43.55)	10.56	1.89	0.58
Heat (80°C, 4 hrs)	17.53 (24.73)	17.52 (24.73)	15.87	0.13	0.11
Heat (80°C, 19 hrs)	30.04 (33.22)	30.01 (33.20)	10.77	0.53	0.28
Heat (80°C, 24 hrs)	52.57 (46.46)	52.5 (46.41)	15.02	1.00	0.34
CD (p=0.05)	2.50	2.23	0.70	0.10	0.34

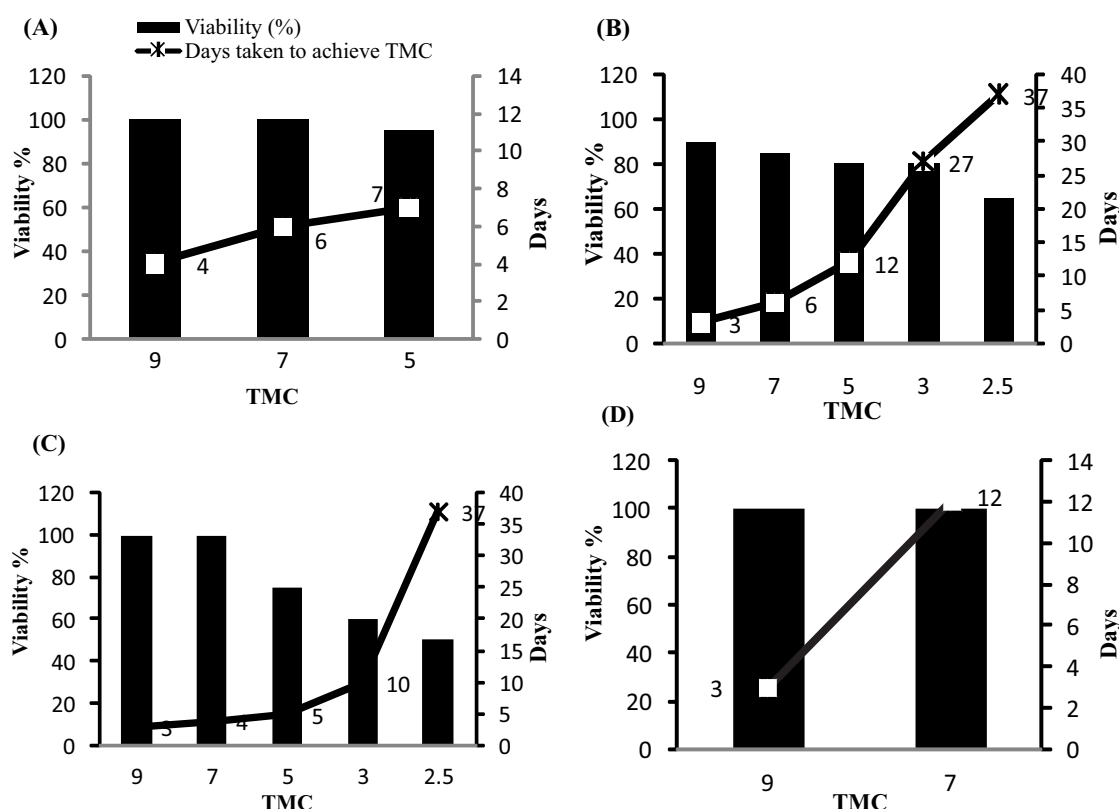
See Table 2 for details

Table 6. Effect of pre-sowing treatments on germinability of *Crotalaria micans*

Treatment	GP (%)	GE (%)	MGT	GV	GI
Control	42.50 (40.64)	37.51 (37.74)	5.14	2.04	0.70
Mechanical scarification	96.25 (78.92)	90.01 (71.77)	12.19	15.53	1.76
H ₂ SO ₄ (30sec)	42.50 (40.67)	40.01 (39.22)	4.52	2.16	0.81
H ₂ SO ₄ (5min)	62.52 (52.24)	50.01 (44.99)	7.52	3.96	1.31
H ₂ SO ₄ (15min)	57.52 (49.31)	50.01 (44.99)	6.19	3.67	1.21
H ₂ SO ₄ (30min)	75.00 (60.01)	57.50 (49.3)	10.38	5.48	1.25
Heat (60°C, 9hrs)	30.03 (33.21)	27.56 (31.64)	5.01	1.26	0.62
Heat (60°C, 19hrs)	32.51 (34.74)	30.01 (33.2)	10.91	0.57	0.31
Heat (60°C, 24hrs)	32.50 (34.74)	17.53 (24.73)	10.08	0.72	0.41
Heat (80°C, 4hrs)	12.51 (20.7)	12.50 (20.7)	17.40	0.07	0.07
Heat (80°C, 19hrs)	17.51 (24.72)	17.50 (24.72)	16.36	0.13	0.10
Heat (80°C, 24hrs)	10.00 (18.43)	10.01 (18.44)	14.75	0.04	0.06
CD (p=0.05)	2.21	2.08	0.62	0.32	0.37

See Table 2 for details

candida and *Calopogonium mucunoides* seeds moisture content can be reduced to 2.5 per cent using silica gel for long term storage while sacrificing some percentage of viability and germination. Other members of Fabaceae like *Bauhinia purpurea* and *Pongamia pinnata* tolerate desiccation without loss of viability to moisture content less than or equal to 5 per cent (Kumar et al 2007). We also tried to bring down the moisture content below 2.5 per cent using silica gel but were not possible. It is also found that *Albizia thompsonii* takes 4 days to achieve TMC 9 while *Albizia procera*, *Calopogonium mucunoides* and *Tephrosia candida* took 3 days to achieve the same. For 7 per cent moisture content *Tephrosia candida* took the least number of days 4 days while *Albizia procera* took the longest duration of 12 days. For 5 per cent moisture content *Tephrosia candida* takes least number of days (5 days) while *Calopogonium mucunoides* took 12 days. For 3 per cent moisture content *Calopogonium mucunoides* took 27 days and *Tephrosia candida* took 10 days. Seeds of *Albizia thompsonii* tolerated desiccation in silica gel upto 7 per cent moisture content (MC) maintaining its initial viability (Fig. 1 A). When MC of these seeds was further reduced to 5 per cent the viability of the seeds decreased to 95 per cent. The set target moisture content of 3 per cent and 2.5 per cent was not achieved till the end of the experiment (45 days). The

**Fig. 1.** Number of days taken to achieve the target seed moisture content (TMC) of *Albizia thompsonii* (A), *Calopogonium mucunoides* (B), *Tephrosia candida* (C), *Albizia procera* (D) and their seed viability

set TMC 9 per cent was achieved on the fourth day, TMC 7 per cent was achieved on the sixth day and TMC 5 per cent was achieved on the seventh day for this species.

The viability of *Calopogonium mucunoides* seeds reduced to 85 per cent when the seeds were dried down to 7 per cent MC (Fig. 1B). It was observed that when the seeds were dried to 5 per cent, 3 per cent and 2.5 per cent MC the viability was reduced at 80 per cent, 80 per cent and 65 per cent respectively. It was found that the TMC 9 per cent was achieved on the third day and the TMC 2.5 per cent was achieved on the thirty-seventh day of the experiment. Tolerance to desiccation was also seen in *Tephrosia candida* at 9 per cent MC maintaining the initial viability (i.e. 97%). But, when the seeds were dried to 2.5 per cent MC the viability of the seeds were reduced 50 per cent. The set TMC 9 per cent was achieved on the third day and TMC 2.5 per cent was achieved on the thirty-seventh day of the experiment (Fig. 1C). Similarly, the seeds of *Albizia procera* tolerated desiccation up to 7 per cent MC maintaining the initial viability (i.e. 95%) (Fig. 1D). The set TMC of 5, 3 and 2.5 was not achieved in the species with silica gel till the end of the experiment (45 days). The TMC 9 per cent was achieved on the third day of the experiment and the TMC 7 per cent was achieved on the twelve-day of the experiment. On the other hand, the seeds of *Crotalaria micans* did not achieve any of the set TMC in the silica gel during the experiment.

All the selected five species belongs to the Fabaceae family and 97 per cent of the seeds which belong to it are known to possess orthodox nature (Liu 2008). Out of five species selected only two species achieve all the set TMCs i.e. *Calopogonium mucunoides* and *Tephrosia candida*. *Crotalaria micans* did not achieve any of the set TMC. The reason may be due to the presence of a hard impermeable seed coat as many species of Fabaceae and Malvaceae possess hard-seedcoatness (Michael et al 2007). The seed coat protects the seeds from the external factors and increases the storability without getting damaged. Desiccation seems to increase the seed hardness and the viability of the seeds tend to reduce when the moisture content is brought down up to 3 per cent and 2.5 per cent. In *Tephrosia candida* viability was seen to be reduced to 50 per cent at 2.5 per cent moisture content.

CONCLUSION

Dormancy in the legume species is mainly due to their hard seed coat covering. The acid and mechanical scarification were effective in enhancing the germination of all the 5 species. Mechanical scarification can be used to achieve maximum germination for the seeds of *Crotalaria micans*. While, conc. H_2SO_4 (30 min) enhances the

germination of the *Calopogonium mucunoides*, *Tephrosia candida* and *Albizia thompsonii* seeds. However, both mechanical scarification and conc. H_2SO_4 (30 min) were equally effective for *Albizia procera*. Seed desiccation is important to identify how seeds of different species can be stored. Seeds of many species tend to reduce their viability after desiccation at low moisture content. It was observed that after reducing the moisture content of the freshly collected seeds, seeds viability of these species reduces to some extent.

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Ecosystem Services in Mountainous Area: A Case Study of Nghe an, Vietnam

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Abstract: A study was carried out in the mountainous Western area of Nghe An province, Vietnam, which is a forested area inhabited by ethnic minorities for payment for forest environment services with focus on watershed protection and carbon sequestration services. Remote sensing - GIS methods were used to map forest area for biomass assessment. In addition, the method of determining the level of payment for forest environmental services was used to calculate the Pu Hoat natural reserve. The study shows that there is great potential for the development of forest ecosystem services in the study area, which can contribute significantly to improve the livelihoods of indigenous people besides protecting/enriching forest capital. A number of practical solutions to improve the good implementation of forest environmental services have been proposed for the study area, which can also be applied in other areas. This research has highlighted the practical benefits of the Forest (environmental) Payment Service (FPS) and pointed out the shortcomings that need to be addressed.

Keywords: Forest Payment Service (FPS), Carbon absorption capacity, Nghe an province, Vietnam

A payment for Ecosystem or Environmental Services (PES) is an innovative approach for the nature conservation. Ecosystem services are the benefits that humans derive from natural systems. PES encourages the maintenance of natural ecosystems through environmentally friendly practices. People seek many services from ecosystems of forests and thus perceive the condition of an ecosystem in relation to its ability to provide desired services (Burkhard et al 2012). Ecosystem services include provisioning services obtained directly from the ecosystem such as food provision, regulating services such as water regulation, habitat, air quality, and water quality, and cultural services, which the people obtain through tourism, aesthetic values, spiritual enrichment, and sense of place (Perrings et al 2011, Wainger et al 2011). The ecosystem services approach is useful for decision-making in conservation and natural resource management because it assigns value to nature by translating ecosystem properties into human needs (Castro et al 2013). This is a rather complex issue, including ecosystems, natural resources, economic, social and human values in space, so the integration of both natural and social science perspectives is needed (Helfenstein and Kienast 2013). Ecosystem services provide a valuable framework for

analyzing and acting on the linkages between people and their environment (Gomiero et al 2003). PES is becoming increasingly popular in recent years as a market-based approach to conserve and manage ecosystems that uses economic incentives to improve the livelihoods of environmental-service providers.

In developing countries like Vietnam, poverty households control much of the ecologically sensitive land, so the potentials to gain benefit from PES is eligible at the household or community level, in different forms (Pagiola et al 2005, 2008, Phuc et al 2012). The potential for PES to alleviate rural poverty by 2030 in developing countries was quantified, which indicated that markets for biodiversity conservation could benefit 10-15 million, carbon markets could benefit 25-50 million, markets for watershed protection could benefit 80-100 million, and markets for landscape beauty and recreation could benefit 5-8 million (Wunder and Dung 2005, Milder et al 2010). In Vietnam, pilot project of PES schemes has been implemented in Vietnam with partial success associated with some problems such as insecure land tenure, high transaction costs, and potential risk for livelihood of households (Chiramba et al 2011, Turner and Pham 2015). These issues are also challenges of the

mountains of Nghe An, province of Vietnam that selected as study area. Our present research aim is to address the questions of how is the service potential to pay for forest environment? how has the locality made payment?, how do people perceive and assess this issue?, what solutions can be applied to make PES better? and answer to these questions requires detailed information of Land Cover (LC), which may change with the population growth and area development. The, vegetation may differ in pools of carbon capacity and their sequestration rate with time (Vitousek et al 1997; Gomiero et al 2000, Chauhan et al 2016, Sharma et al 2016). LC change leads to the change of forest function. Thus, it is required to update forest status and accordingly change the value of payment for forest environmental services for unit areas. This will encourage upland farmers to protect and plant more forests.

MATERIAL AND METHODS

Methodology adopted in this study mainly include generation of land cover map of the study area, determination of carbon absorption capacity and ethnographic interviews.

Study area: This study was conducted for the Western Nghe An province, located in the middle of Vietnam (Fig. 1), where has steeply sloping terrain, upstream of rivers and diverse tropical forest ecosystems. Same as tropical forest areas in Vietnam (about 15 per cent of the Vietnamese population and 30 per cent of the country's poorest people) (Nguyen and Masuda 2015), there are mainly inhabited by ethnic minorities (Thai). The livelihoods of local people depend mainly on forests. In Nghe An, more than 80 per cent of Thai ethnic depends on the agricultural production and rely on traditional agriculture. Que Phong district is located in the high mountains of Nghe An province, belong to Pu Hoat Nature Reserve. The steep sloping terrain and large flow are favorable conditions for this district to build four hydroelectric plants. The majority living by practicing their own traditional knowledge of agriculture by protecting biodiversity in the buffer zone of Pu Hoat Nature Reserve. This knowledge has led to the creation of a strong relationship between the local culture and natural resources, which has helped in managing and protecting biodiversity in this area. People from ethnic minorities are normally subsistence farmers depending on the natural resources (Brandon 2014). When the land they cleared belongs to the managed. Traditional land use rights of these peoples are not guaranteed they clear land in the Pu Hoat nature reserve.

Generation of land cover (LC) map: The LC map was generated from Landsat 8 images of the United States Geological Survey (USGS) using remote sensing method

(Dorren et al 2003, Dwivedi et al 2005). Normalized Difference Vegetation Index (NDVI) was applied for the development of LC map by using the formula $NDVI = (NIR - VIS) / (NIR + VIS)$ (Afirah Taufik et al 2019, Xu et al 2014).

NDVI values at a point (position) on the image ranges from (-1) to (+1). Areas of barren rock, sand, or snow usually show very low NDVI values (0.1 or less). Sparse vegetation (shrubs and grasslands) may result in moderate NDVI values (approximately 0.2 to 0.5). High NDVI values (approximately 0.6 to 0.9) correspond to dense vegetation. Higher values of NDVI indicate stronger photosynthetic especially in temperate and tropical forests or crops at their peak growth stage. The NDVI values can be used in the identification of vegetation including forest types. Maps of the study area were generated using NDVI indices, Google Earth Images and GPS survey data in GIS environment (Vieira et al 2012).

Determination of carbon absorption capacity: The carbon absorption assessment and commercial value of carbon accumulated can be evaluated by determining biomass of forests. In this study, we used the remote sensing method in conjunction with field survey for mapping biomass to determine the carbon absorption capacity of forest vegetation, which would be useful for carbon trade. In the study area, we selected 09 Typical Standard Cells (TSC), of 9 m x 9m dimension representing forest types (Table 3, 4 and Fig. 5). In each TSC, species name, density, spacing between trees, height, Diameter at Breast Height (DBH) and basal area of the tree were measured. This information was verified and improved by field survey and interviewing forest owners (Questionnaire in standard format). In order to determine the value of biomass, measurement values of TSC were converted by the formula $Y = 0.11 * r * D1.32 + c$;

Where: D1 is tree biomass (kg tree⁻¹); r is wood density (for forests re-afforested naturally: $r = 0.5 \text{ g cm}^{-3}$); c is the extrapolation factor (for forests re-afforested naturally: $c = 0.62$).

The amount of carbon absorption was calculated as:

$$WC (\text{ton C ha}^{-1}) = 0.46 * \text{Mass}.$$

The value of carbon trading: $C (\text{USD}) = WC * 5 (\text{USD})$.

Ethnographic interviews: Socio-economic ecosystem service survey of forest area was conducted at six case sites located in Pu Hoat area. Each survey site corresponds to six different villages. The survey was conducted in different villages (the Na Sai, Pa Com village in Hanh Dich commune, Que Phong district; the Phuong Tien, Dan village in Tien Phong commune, Que Phong district; the Muong Phu, Muong Phiet villages in Thong Thu commune, Que Phong district) to ensure the reliability of the data collection for the analysis (Fig. 4). In total 300 persons were interviewed through questionnaires. The population samples were

selected to cover a wide range of ecosystem service beneficiaries such as local inhabitants and environmental technical experts. In each village, 50 representative households (16.67%) were surveyed under the guidance of local officials. After doing survey in the field, data were analyzed to judge perceptions of ecosystem services to explored status of payments for forest environment services in western Nghe An including advantages and disadvantages; the level of willingness of the payer to pay, and the level of satisfaction of the beneficiary .

RESULTS AND DISCUSSIONS

Land cover (LC) map: Generation of LC map (Fig. 4) of the study area was done to analyse and evaluate status of the vegetation and carbon sequestration in this area. The NDVI of the area was determined using ENVI 5.0 software as shown in Table 1. Results showed that the NDVI index for the different classes of vegetation in the study area is generally high (0.1 to >0.7; NDVI mean = 0.280330), which represent dense vegetation.

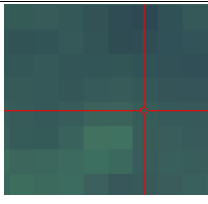
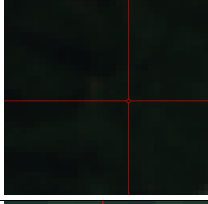
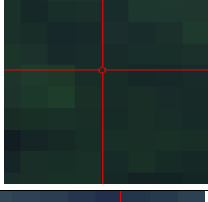
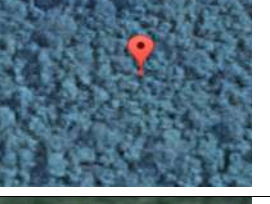
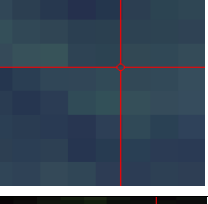
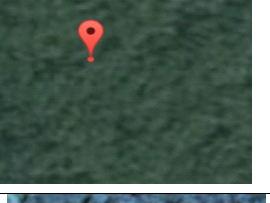
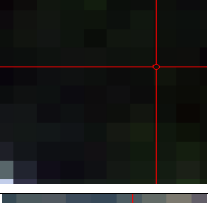
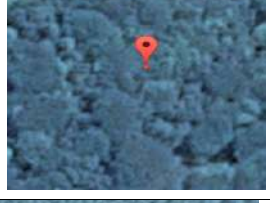
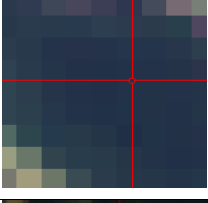
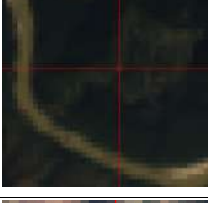
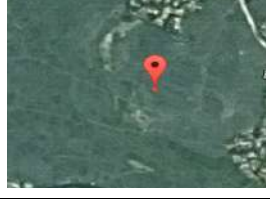
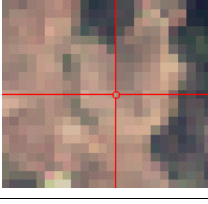
Potential of Carbon Storage and Sequestration

Value of biomass and commercial carbon: The location of the standard plots was selected, representing forest types. Coordinates of locations were determined from Google Earth Images, and verified by GPS survey, besides layout of roads and traffic conditions (Table 2). The result of field survey shows that most of vacant forest in the research area comprises of barren land, shrubs interspersed with trees (trees regenerate 10% coverage). The quality of poor natural tropical forest is low, trees are small: the diameter of average tree⁻¹ ranges from 9.71 to 10.41cm, the sum of horizontal section (area) of trees ranges from 4.13 to 4.52 m², the destiny ranges from 451 to 510 tree ha⁻¹ and the average height ranges from 9.59 to 10.52 m. The native forest regeneration is of large trees with complex species. The average diameter of trees is greater than 10 cm, whereas, the average height ranges from 10.5 to 12.65 m of rich and medium natural tropical forest.

Table 1. NDVI index of forest area

Order	NDVI	Objects	Area (ha)
1	< 0.1	Vacant land; Other land cover	696
2	0.1 – 0.3	Agricultural crop	127
3	0.3 – 0.5	Native forest regeneration; Poor natural tropical forest; Planted forest	31189
4	0.5 – 0.7	Young forest restoration; Tropical mixed forest	31181
5	> 0.7	Rich nature forests, Medium nature forests	44791
Sum			107984

Table 2. Position of standard plots

Coordinates (GPS- ArcGIS)	Location in Google Map	Sample interpretation key
19°33'33.10"N105°3' 28.26"E		
		
19°25'24.15"N104°5' 38.35"E		
19°35'59.88" 105°1'2.57"E		
19°24'49.03"N 104°49'4.67"E		
19°33'39.32"N10 5°4'25.08"E		
19°33'15.91"N 105°12'40.20"E		
19°34'43.59"N 105°2'10.01"E		

The average of biomass determined at each TSC is 79.418 (ton ha⁻¹). Total biomass of naturally re-afforested forest area in Que Phong district is 10587497.916 ton. The quantity of Carbon accumulated in biomass of naturally re-afforested forest area in Que Phong district is 2812985.5 ton. The commercial value of forest carbon cover in Que Phong district is C = 14064927.8 USD (Tables 3, 4).

Potential of payment for forest ecosystem services: The study area was divided into three types of forest (special use forest, protection forest and planted forest) and there were two types of allocation schemes, in which special use forest and protection forest are grouped into one type. The division ensures feasibility and scientifically determining the coefficient 'K', which is required to determine the relative value of different forest areas based on an average of four factors: forest type (KLR) (protection category/special use/production); quality of forest (KCLR) (rich/moderate/poor/secondary); origin of forest (KNGR) (natural forest/planted forest); and human impact (KTD) (near road or residential areas, remote forest area) (Chiramba et al 2011). The national pilot policy on PFES provided guidance for applying a coefficient (the 'K coefficient') to determine the relative value of different forest areas based on an average of four factors, that is coefficient $K = (KLR + KCLR + KNGR + KTD)$. Currently, in Nghe An province, only four hydroelectric plants built earlier were the sole payer for local forest services (Table 5). Vietnam Government stipulates that the level of payment for environmental services applied to hydropower generation facilities is 0.001585 USD/kWh (Decree No. 147/2016/ND-CP dated 2 November 2016, <https://thuvienphapluat.vn/van-ban/tai-nguyen-moi-truong>). For the total area 139723.08 ha, the total amount collected from the ecosystem services is 1965954.75 USD. The average payment per hectare of forest (P) at Pu Hoat Nature Reserve is 14.07 USD (Table 5).

The amounts of money, which service users (hydropower plants), have to pay for forest protection and management of the region: The amount of money that Pu Hoat nature reserve received is 1207488.41 USD. Population in the Western Nghe An currently is estimated to be 1109.052 people. Had this payment been made, local people would had the opportunity to receive more money.

The amount of additional money would be 567498.35 USD (Table 6). Similarly, each year from 2018 to 2020, amount at Pu Hoat NR to be received for services would be 1208184.77 USD, 1208741.85 USD and 1211062.99 USD, respectively.

Actual benefit of local people from forest environmental services: In Nghe An only payments was made for watershed protection services. In this case, estimated value for people services which they would have received for one ha of forest is VND 287,621.34. However, in reality, the payment value is much lower than the estimation (in Pu Hoat NR is now VND 197,000 ha⁻¹ year⁻¹). Moreover, Western Nghe An forests, still had great potential for the CO₂ absorption service. Results of the natural forest area (for protection forests) analysis indicated that in Que Phong district each hectare of forest was worth 4,961,000 VND. Had both services been implemented (watershed protection and carbon sequestration), the income for the protection of forest would had increased significantly. This will improve livelihood of people, and thus they will be motivated to protect and develop forests.

Local perception of ecosystem services of tropical forests: Survey of the study area revealed that most people were aware about payments for the ecosystem services

Table 3. The results of trees density and size analysis in the TSC

	Density N/0,81 ha (1 tree)	D _{1,3} (cm)	H _{vn} (m)	Basal area G (m ² ha ⁻¹)
TSC1	458	10.03	11.55	5.06
TSC2	451	9.82	10.43	4.52
TSC3	510	10.15	10.32	5.99
TSC4	405	10.07	12.77	5.14
TSC5	335	12.20	11.93	6.12
TSC6	370	11.82	12.65	5.63
TSC7	385	10.41	10.52	4.13
TSC8	459	9.71	9.59	4.25
TSC9	258	8.3	11.31	4.14

Table 4. Results of biomass determined at the sample plots

TT	TSC1	TSC2	TSC3	TSC4	TSC5	TSC6	TSC7	TSC8	TSC9
Biomass	83.55	71.22	85.13	82.34	93.39	102.67	68.91	65.16	37.14

Table 5. The amount of money the hydropower plants have to pay

Payer	Calculation unit	Production	Unit price	Total amount
Hydropower plant Sao Va	KWh	13450,00	0.001585	21318.25
Hydropower plant Ban Coc	KWh	80000000	0.001585	126800
Hydropower plant Hua Na	KWh	716900000	0.001585	1136286.5
Hydropower plant Cua Dat	KWh	430000000	0.001585	681550
Total				1965954.75



Fig. 1. The location of study area

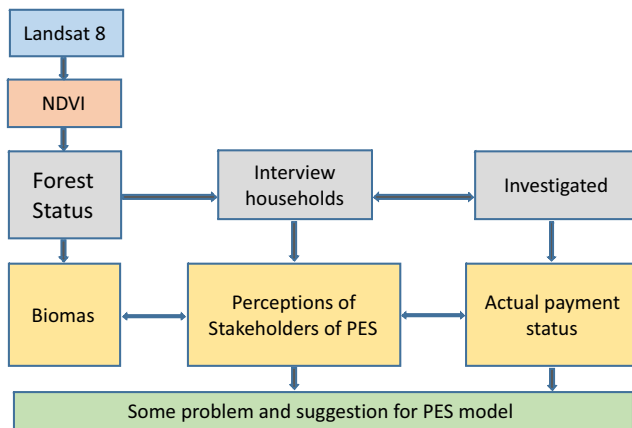


Fig. 2. Flow chart of study

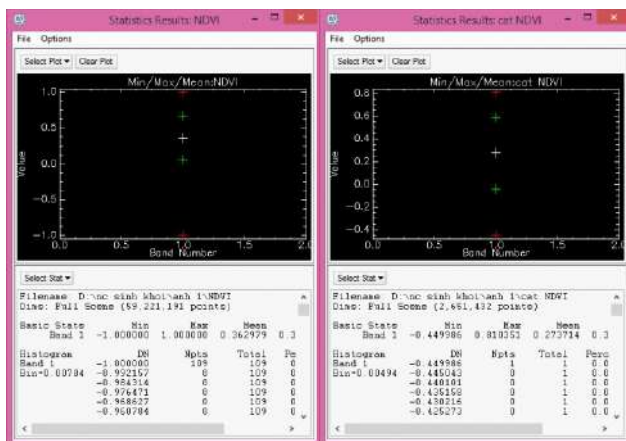
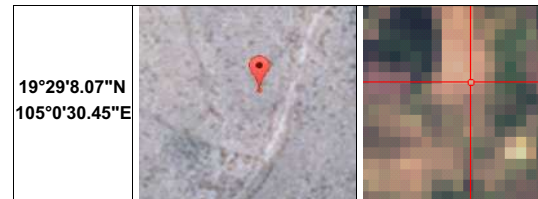


Fig. 3. The statistics of NDVI



Fig. 4. Forest map developed from NDVI index



MAP OF VEGETATION AND SAMPLING LOCATIONS IN QUE PHONG DISTRICT



Fig. 5. Location of TSC

(96.67%). Out of the 300 respondents, 157 (52.33%) answered the purpose of the payment is to protect watershed, remaining 143 (41.33%) answered that it is for protecting the forest as well as to ensure livelihoods of forest owners (Table 7). People were also asked about the main responsibility for implementing the forest environment payment service, virtually all of those who responded said that it was hydropower plants. In total, 219 people (73%) opined that the payment was for hydropower plant service (Table 8). The payment for the forest environment and the method of payment determination were relatively simple, which only apply for watershed protection services. Payment levels were not too high, suitable for the process of operation as well as revenue/expenditure of the payers. Methodology of payment service was not much clear to the respondent, so most of them expressed their dissatisfaction.

The level of satisfaction of the beneficiary: In the study area, local people were aware of the importance of forest payment services. Out of the 300 house inhabitants interviewed, 294 people (98%) said that payment for forest environment was very necessary (Table 9). During interviews, most of the respondents said that the amount of money they received from the services contributed significantly in improving their living condition. Analysis of survey data indicated that for their daily living, 58.33% people expressed satisfaction with the amount they received, whereas 32.34% people were unsatisfied (Table 10). This study reveals that the payment for the forest environment services is still inadequate. Therefore, it is required to be revised and enhanced to improve further quality of their living condition.

Suggestions of Management to Improve PES Effectiveness in Western Nghe An

Orientation of the carbon exchange market model: The carbon exchange market model include the suppliers and enterprises for PES as below (Fig. 6):

Suppliers: Carbon credits are provided by many people and agencies (local people, protected area management boards, forest protection stations, and those directly involved in the protection and replanting of forests). Parties of verification and calculation of services include foreign-invested joint

Table 6. The amount of money, which the hydropower plants were required to pay

Forest area	Average price	Coefficient K	Area (ha)	Total amount (USD)
Special purpose forests				
i. Natural forest	14.07	1.3	30929.43	565730.20
ii. Planted forest	14.07	1.1	0	0
Protective forest				
i. Natural forest	14.07	1.3	42210.43	772070.98
ii. Planted forest	14.07	1.1	1.01	15.63
Planted forest				
i. Natural forest	14.07	0.9	319.56	4046.59
ii. Planted forest	14.07	0.7	0	0
Total			73460.43	1207488.41

Table 8. Payment responsibility to ecosystem services

Payment responsibility	Number of people	Portion (%)
Authorities	4	12
Hydropower plants	25	73
Tourism enterprises	2	3
Others	4	12
Total	35	100

Table 9. Evaluate the necessity of forest environmental payment services

Levels of evaluation	Number of vote (person)	Scale (%)
Unnecessary	2	0.67
Normal	4	1.33
Necessary	102	34.0
Very necessary	192	64.0
Total	300	100

Table 10. Assess the degree of satisfaction with the amount

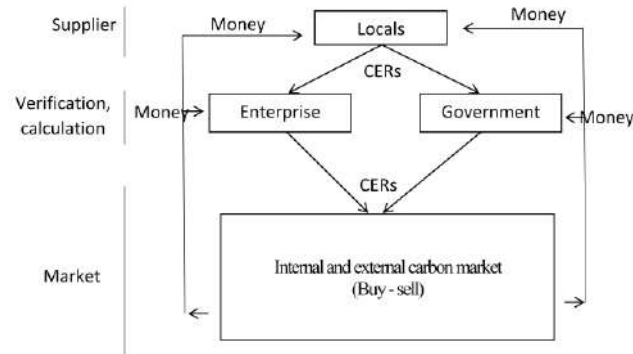
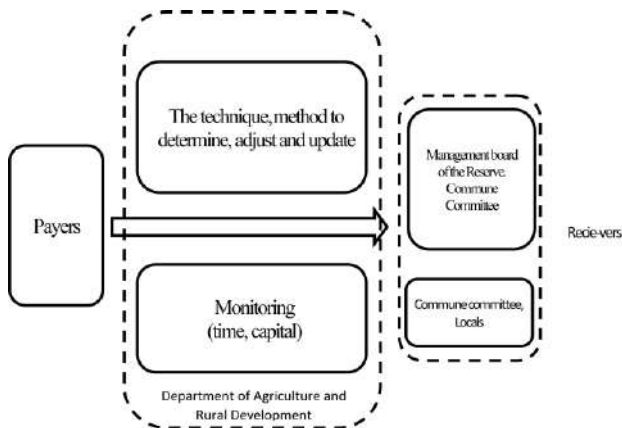
Result (n=300)	Levels of assessment		
	Very satisfied	Satisfied	Unsatisfied
The number of vote	28	175	97.0
Portion (%)	9.33	58.33	32.34

Table 7. Local perception of ecosystem services of tropical forests

Levels of knowing about the service	Number of people	Portion (%)	Payment purposes	Number of people	Portion (%)
Yes	32	91.45	Pay for forest owners to ensure livelihoods	124	41.33
No	0	0	Pay for the state	4	1.33
Unknown	1	2.85	Pay for watershed protection	157	52.33
No idea	2	5.7	Others	15	5.01
Total	35	100	Total	300	100

Table 11. Assess the time of payment

Result (n=300)	Levels of assessment			
	Unreasonable	Reasonable	No comment	Suggestion
The number of vote	156	50	94	Payback regulators
Scale (%)	52	16.67	31.33	

**Fig. 6.** Flow diagram of the carbon exchange market model in Nghe an province**Fig. 7.** Direct payment model applied for West Nghe An

ventures, private companies, or government agencies, which calculate the carbon sequestration from the forests.

Enterprises: Enterprises who can participate in this market are forestry companies, hydropower plants companies such as Ban Coc, Khe Bo.

Building a model of direct payment: Payers (hydropower plants, water plants) in the province will pay directly to the receivers (forest guards, mainly forest owners and management staffs of nature reserves, national parks). Funding would be provided by the management boards of nature reserves, national parks/authorities. In order to make payments directly, it is necessary to have an agency to calculate and determine the level of payment reasonably and logically. Technical and professional department under the

Department of Natural Resources and Environment. Supervision (including monitoring the implementation of calculation and distribution, implementation of payments) are also required to be strengthened. This will help in direct cash transfer directly from "seller" to "buyer". This will ensure timely payment and more transparency by avoiding intermediaries (Fig. 7).

CONCLUSIONS

The mountains West Nghe An province, Vietnam has enriching forest capital for the development PES. This potential to become very valuable mechanisms for generating new revenues and transfer of money to concerned people for sustainable development. The benefits of the Forest (environmental) Payment Service (FPS) the shortcomings that need to be addressed. This method would also help in building capacity of development in poor and indigenous communities, especially ethnic minorities. The implementation of forest environmental services have been proposed for the study area, which can also be applied in other areas. Payments for environmental services (PES) model help in the better protection of forest and in the implementation of policies for the forest area development. With this model local people realized the purpose and value of forest protection, thus, they agreed to participate in the conservation of forest and development of the area. Government is willing to pay for tree planting and protecting forest activities in the western Nghe An area. The carbon exchange market in the times to come for the western region of Nghe An. This model was applied for the limited period in the study area, which is recommended to be continued for long term assessment of its effect on the ethnic population.

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Impact of Fly Ash on Germination and Initial Seedling Growth of *Leucaena leucocephala* (Lam.) De Wit

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Abstract: Impact of fly ash (FA) on germination and initial seedling growth performance of *Leucaena leucocephala* (Lam.) de Wit was studied during 2016-2017. Substrate was prepared by admixing FA to forest soil (S) at five concentrations viz. 20%, 40%, 60%, 80% and 100% (w/w). The experimental design was CRD with six treatment and three replications. Eighteen hundred freshly collected seeds were treated with hot water and sown at 0.5-1.0 cm depth in germination trays containing substrates of different treatments. Significant ($P < 0.05$) variation in germination (G.) period, G. rate, G. capacity and G. Index with respect to FA concentration in growing media was observed ($n=100$). G. period was reduced by 5.67 days in 20% fly ash admixed growing media (FAAGM) over control (S). Maximum G. rate (88.67%), G. value (18.47) and G. index (1.58) were found in 20% FAAGM after 30 days of sowing. Observations pertaining to growth dynamics were recorded at 90 days after transplanting (DAT). Significant difference ($P > 0.05$) in seedling survival rate, plant height, diameter growth, nodules per plant and seedling quality index were observed. The survival rate (91.83%) and root nodule number was (7.20) was maximum at 20% FAAGM. But plant height (51.68 cm), diameter growth (3.96 cm) and seedling quality index (0.706) were attained maximum at 40% FAAGM. It is concluded from the present investigation that FA can be admixed @ 20% (w/w) in forest nurseries for improving germination and @40% (w/w) for promoting seedling growth and quality improvement.

Keywords: Forest nursery, Fly ash, Germination catalyst, Pollution control, Seedling quality index, Solid waste management

Natural resources including air, water, soil, forest, fossil fuel etc. are vital for existence of life on earth. Carbon is a principal element in every resource and acts as a source of energy for functionality of every system. For getting requisite energy, human beings are constantly exploiting assimilated carbon of plants. Carbon stored in fossil fuels are used for generating needed energy (electricity) for running combustion engines in industry and coal fired thermal power plants holds a principal share of this (Cainenga et al 2016). Fly ash (FA) is one of the major noxious wastes generated from coal based thermal power plants (Behera et al 2018). It is ultrafine in nature and contains a number of toxic metals such as arsenic (As), barium (Ba), mercury (Hg), chromium (Cr), nickel (Ni), vanadium (V), lead (Pb), zinc (Zn), etc. depending upon the source of coal (Dwivedi and Jain 2014). India ranks fourth in the world in the production of coal ash as by-product waste after USSR, USA and China, in that order (Senapati 2011). National production of fly ash was 176.74 MT during the year 2015-16 (Central Electricity Authority 2015-16) and the annual production is expected to cross 300 MT by the year 2025. Proper disposal and management of such a huge quantity of FA possessing potential threats of air, water and soil pollution is a great challenge (Rawat et al 2018).

Utilization of FA for a particular purpose depends up on its elemental content which is primarily controlled by type of coal and its source. Fly ash consists of practically all the

elements present in soil except nitrogen. Hence, it possesses a vast potential for use in agriculture as an amendment agent. Many researchers have explored the potential of fly ash in agriculture (Kishor et al 2010). It has been found beneficial for the growth of many plants. Crop plants of the families *Brassicaceae*, *Chenopodiaceae*, *Fabiaceae*, *Leguminosae* and *Poaceae* are most tolerant to FA (Cheung et al 2000). Apart from agriculture FA is used in a array of ways like manufacturing cement, concrete, bricks, wood substitute products, in road construction, waste land development, filling of underground mine spoils etc. (Kaur and Goyal 2015). Still then a large quantity of FA is being dumped up in ash ponds and lagoons. Hence the danger of contamination of terrestrial as well as aquatic food chain with elemental toxicity present in FA cannot be over ruled. Some of the fly-ash contains deadliest toxic metals like arsenic, mercury, cadmium, chromium and selenium. These toxic metals along with other toxicants can cause cancer and neurological damages in humans. They can also harm and kill wildlife, especially fish and other water-dwelling species (Ahmad et al 2014). The current status of utilization of FA in India is only 55-61% (Central Electricity Authority-2015), providing a wide scope for searching new avenues. One of the most potential areas of utilization is in forestry sector where it can be consumed either in nursery or for tree plantation activities. This will help in locking the toxic heavy metals in the wood biomass for longer period of time. Use of

fly ash in forest nursery is not a new concept. Goyal et al (2002) reported its use in nursery but commercial use is scanty or absent. Hence attempt was made to know its practical use for quality planting material production. *Leucaena leucocephala* was selected because of its nitrogen fixing ability and multiplicity of products harvested from it. *L. Leucocephala* is widely distributed around the world and often preferred for pasture land development, energy plantation, reclamation of waste lands, mine spoils especially in arid and semiarid regions (Brewbaker 1987, Chaturvedi et al 2014).

Studies on regeneration and development of established tree species on land fill site is also necessary for understanding the ecological balance in man-made ecosystem and restoration of different biogeochemical cycles. Information on limiting dose of FA for seed germination will be helpful for self-sustenance of a habitat. Perusal of literature reveals that such kind of information is scanty. Keeping this in view the present study was carried to know the impact of fly ash (at different concentrations) on seed germination and initial seedling growth attributes of *L. leucocephala*.

MATERIAL AND METHODS

The experiment was conducted in the experimental field and laboratory of College of Forestry, Orissa University of Agriculture and Technology, Bhubaneswar in India situated at 20° 15' N 85° 52' E at an altitude of 25.9 m amsl. Fly ash (FA) was collected from one of the silages of Indian Metals and Ferro Alloys (IMFA) Limited, situated at Choudwar in Cuttack district of Odisha (20°31'50"N 85°54'26"E). The mean monthly maximum & minimum temperature, relative humidity and rainfall of the experimental site given in Figure 1.

Preparation of substrate: Substrate (growing media) was prepared by admixing FA to forest soil (S) at concentrations 20%, 40%, 60%, 80% and 100% (w/w). There were six treatments (T₁-20% FA+ S, T₂-40% FA+ S, T₃-60% FA+ S, T₄-80% FA+ S, T₅-100% FA) including control (T₆-S). Substrate was analysed for different physical and chemical properties. Bulk density (BD) and water holding capacity was determined by using the protocol given by Piper (1966). pH and electrical conductivity (EC) were measured as per the protocol given by Jackson (1967), organic carbon was estimated as per Walkley and Black (1934). Available nitrogen, phosphorus and ammonium acetate extractable potassium were estimated as per the procedure given by Subbiah and Asija (1956), Olsen et al (1954) and Merwin and Peech (1951) respectively. Freshly collected seeds were given hot water treatment prior to sowing. Seeds were

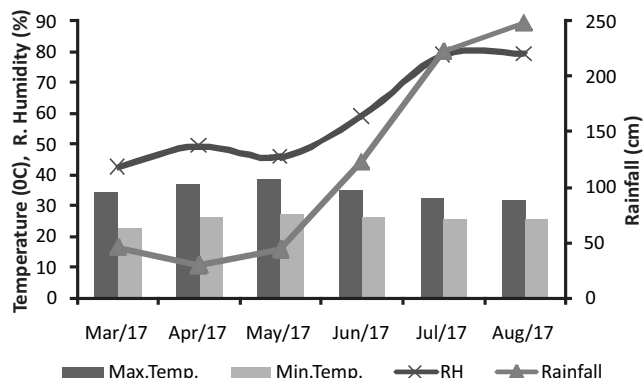


Fig. 1. Climatic parameters of the experimental site

soaked in boiled water at 70°C for 20 minutes followed by cold water treatment (the volume of water was five times the volume of seed) for 24 hours (Tadros et al 2011). Eighteen germination trays having dimension 90 cm (L) X 45 cm (B) X 15 cm (H) were filled with above six mentioned substrates to the brim leaving 3.0 cm. Hundred seeds per replication (totaling 300 seeds per treatment) were sown at a depth of 0.5-1.0 cm in germination trays. The surface of the germination trays were covered with paddy straw and kept at open nursery condition. Manual watering to the field saturation level once a day during morning hours was made. Observations pertaining to germination parameters were recorded daily up to 30 days after sowing. Germination period was determined by observing the day taken for first germination (DTFG) to 30th day when about 80-85% seeds have germinated. Based on the number of seeds germinated the following parameters were calculated as per the standards given by Czabatore (1962) and AOSA (1983).

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

$$\text{Germination capacity} = \frac{\text{Total seeds germinated} + \text{viable seeds}}{\text{Total No of seeds sown in all replications}} \times 100$$

$$\text{Germination Value} = \text{PV} \times \text{MDG}$$

Where, PV = Peak value of Germination

MDG = Mean daily Germination

$$\text{Germination Index} = \frac{\text{No. of Germinated seed}}{\text{Days of first count}} + \dots + \frac{\text{No. of Germinated seed}}{\text{Days of final count}}$$

After completion of germination study, seedlings were transplanted into poly pots containing substrates of above mentioned treatment combinations. Growth parameters such as shoot length, collar diameter and number of leaves were assessed monthly after 30 days of transplanting for 3

months. Total shoot length was measured by using ruler (taken from the apical bud of the plant to the base of the shoot) and stem diameter by using electronic digital calliper (6"/150 mm, accuracy ± 0.01 mm, Mitutoyo- CD-6"ASX:500-196-30).

For recording the quantitative parameters pertaining to root growth, the entire seedling was dipped in a bucket of water at 90 days to remove adhering soil from it. It was then carefully washed so that no damage was made to root system. Length of roots (starting from collar region to the end point) and number of root nodules were recorded. Thoroughly washed seedlings (without damage to root and shoot) were dried under sun for 30 minutes. The shoot and root portions were separated for each seedlings. The shoot was cut from the collar portion. Fresh weight of the shoot and root were recorded by a digital balance. Then the root and shoot sample were put in paper bags separately and were oven dried at 80°C for 48 hours. The dry weight were recorded and expressed in gram. Growth observation was based on 45 numbers of randomly selected plants from each treatment. The seedling quality index (SQI) was calculated by using the formula as described by Dickson et al (1960).

$$SQI = \text{Seedling Dry Wt. (gm)} \left[\frac{\text{Height (cm)}}{\text{Diameter (mm)}} + \frac{\text{Shoot dry Wt. (gm)}}{\text{Root dry Wt. (gm)}} \right]$$

The experiment was completely randomized design with three replications. The collected data were analysed with a general linear model using SPSS software version 20 for windows operating system. Means were analysed according to the Duncan Multiple Range Test (DMRT) at $P < 0.05$.

RESULTS AND DISCUSSION

There exists a vast scope for utilizing FA in forestry

sector particularly as a growing media for quality seedling production and rhizosphere improvement material at difficult sites prior to plantation. But the matrix of application depends upon the elemental composition of FA to be used, tolerance limit of plant species selected and physiochemical property of plantation site soil or growing media in which FA need to be added. Research finding says that FA bears the potential to improve fertility status of poor degraded waste lands and problematic soils in a varying agro-climatic situation (Dash et al 2017). Enhancement in crop yield and vegetative growth of woody perennials have been reported by many workers when applied judiciously (Kumar et al 2002, Sinha et al 2005, Ramesh et al 2008, Chaudhary et al 2009, and Krzaklewski et al 2012)

Properties of FA substrate: FA used in the study was alkaline (8.12) in nature with 58.2% water holding capacity (WHC). Growing media (forest soil) was acidic (pH-6.41) in nature with low in available nitrogen ($< 280 \text{ kg ha}^{-1}$), high in available phosphorus ($> 25 \text{ kg ha}^{-1}$) and potassium ($> 280 \text{ kg ha}^{-1}$) content. Notable change on a dose dependent manner in physical and chemical properties of the substrate was observed (Table 1). The pH of FA admixed media was increased significantly ($P < 0.05$) from 6.67 in control to 7.45 in 80% FA. EC value increased ($P < 0.05$) with increasing concentration of FA from 20% to 80%. The maximum EC (1.28 dS m^{-1}) was found in 80% FA level while in the control it was 0.21 dS m^{-1} . The same trend was observed in WHC demonstrating a marked increase of 5.5%, 11.8%, 18.8% & 32.5% at 20%, 40% 60% and 80% FA, respectively. The BD of growth media without FA (forest soil) was highest at 1.48 gm cm^{-3} which significantly ($P < 0.05$) decreased up to 0.76 gm cm^{-3} in 80% FA mixing. The organic carbon content of FA admixed media was decreased as compared to normal soil. Available N and P decreased significantly ($P < 0.05$) with increased level of FA. T_5 had the lowest N (37.5 kg ha^{-1}) & P

Table 1. Initial physicochemical properties of flyash, forest soil and flyash substratum

Property	Fly ash (FA)	Forest soil (S)	FA substrate (Forest soil + FA % (w/w))			
			S+20% FA*	S +40% FA*	S +60% FA*	S+80% FA*
pH	8.12e	6.41a	7.06b	7.26c	7.39c	7.65d
EC(dS m^{-1})	0.68b	0.21a	0.62b	0.93c	1.11d	1.28e
N (kg ha^{-1})	0.002f	125.50a	106.15b	92.67c	79.17d	37.50e
P (kg ha^{-1})	6.70e	56.13a	48.23b	33.59c	18.91d	14.69d
K (kg ha^{-1})	146.43e	474.36d	496.41c	506.12c	539.53b	613.15a
OC (%)	0.005f	0.570a	0.519b	0.464c	0.382d	0.261e
BD(g cm^{-3})	0.69f	1.48a	1.38b	1.21c	0.95d	0.76e
Pore space (%)	49.52a	32.8c	43.2b	44.4b	46.25ab	49.35a
WHC (%)	58.2a	40.67f	43.25e	45.84d	48.72c	54.33b

FA- fly ash; S- Forest Soil, EC- Mean values followed by the same letter are not statistically different at $P < 0.05$

(14.69 kg ha⁻¹) and the lowest levels were found in control (N = 125.5 kg ha⁻¹ and P = 56.13 kg ha⁻¹). Available K content increased significantly (P<0.05) with FA concentration with highest level found in T₅ (613.15 kg ha⁻¹) and the lowest in control (474.36 kg ha⁻¹). The pore space per cent increased positively with FA levels. The change in physicochemical properties of media was in accordance with the earlier findings (Sharma and Karla 2006, Tomar et al 2015, Mishra et al 2017). The increase in pH was might be due to neutralization of H⁺ by alkali salts or precipitation of soluble cautions in the FA admixed growing media. Increase in EC value because of enhanced availability of metal elements as a result of FA addition (Qurratul et al 2013). The reduced BD in FA mixed media, improved total pore space and water retention capacity as well as better its workability.

Effect of substrate on seed germination: A number of environmental and endogenous factors control seed germination process. Suitable seed pre-treatment schedule may reduce unfavourable endogen window. In a controlled environment the exogenous variables can be successfully managed to achieve maximum germination rate. Substrate property especially pH and water retention capacity have a marked impact on germination. pH affects germination either by increasing the osmotic pressure of the media to a plant that will retard or prevent the intake of water or by causing toxicity to the embryo (Rashid 2004). FA had significant effects on seed germination parameters like g. period, g. rate, g. capacity and g. index, however did not have any impact on the number of days taken for first germination and germination value (Table 2). The germination period in substrate having 20% FA (8.0 days) was reduced by 5.67

days over control (13.67 days). The delayed germination process in a dose-dependent manner was due to an increased pH towards alkalinity. Similar type of observations was reported by Rashid et al (2004). The highest seed germination rate (88.67%) was observed in substrate having 20% fly ash (T₁) and was significantly (P>0.05) higher than other treatments. The germination rate of 66.33 and 52.00% in T₂ and T₆ (control) respectively are statistically at par with each other. Minimum germination rate (30.67%) was observed in substrate having 100% FA (T₅). The increased germination rate (88.67%) in 20% FA admixed growing media was attributed to the improvement in the physicochemical condition of germinating media over control (52.0%). The reduction in germination rate beyond 40% FA addition (w/w) in media (other than T₁) was due to enhanced pH, elemental toxicity of metals like Cu²⁺, Zn²⁺ at higher EC to embryo and reduced biological activity (Pitchel and Hayes 1990, Gupta et al 2000 and Rashid et al 2004). There existed negative relationship between FA rate with DTFG, germination percentage, germination capacity, germinative value and germination index but it was positive with germination period.

Effect of substrate on seedling growth: After 90 days of transplanting significant difference (P>0.05) in survival rate, plant height, diameter growth, nodules per plant and seedling quality index of *L. leucocephala* was observed. But no significant difference in number of leaves and mean root length were recorded (Table 3). Highest seedling survival rate (91.83%) was in substrate containing 20% FA (T₁) and was statistically at par with control (91.27%). The maximum survival rate (91.83%) of seedlings in substrate containing 20% FA (T₁) was due to the improved aeration and water

Table 2. Effects of substrates on germination of *L. leucocephala* seeds at 30 days after sowing

Parameters→ Treatments↓	DTFG	G. Period	GP (%)	GC	GV	GI
T1	5.33a	8.00a	88.67a	94.67a	18.47a	1.58a
T2	6.33ab	12.53b	66.33b	94.67a	13.98b	1.31a
T3	6.33ab	13.17bc	48.00c	81.33b	8.28cd	0.98b
T4	6.21ab	14.55c	37.33cd	70.67bc	5.07de	0.80bc
T5	6.00ab	20.83d	30.67d	65.33c	4.10e	0.57c
T6	7.33b	13.67bc	52.00bc	94.33a	10.29bc	0.87bc
Statistical analysis						
P (0.05)	0.35	0.005	0.044	0.035	0.22	0.035
SE	0.569	0.180	4.92	3.97	0.428	0.103
F	3.53	6.11	3.25	2.71	2.27	3.51
Linear Regression analysis (y = concerned parameter, x = FA rate)						
y=	6.55-0.01x	9.78+ 0.08x	73.75-0.4x	95.56-0.27x	15.52-0.11x	1.31-0.01x

Treatments T₁=(20% FA+S), T₂=(40% FA+S), T₃=(60% FA+S), T₄=(80% FA+S), T₅=(100% FA), T₆=(Soil/Control), FA- fly ash, S- Forest Soil, DTFG- Days taken for first germination, GP- Germination Percentage, GC- Germination Capacity, GV- Germination Value, GI- Germination index, Mean values followed by same letter are not statistically significant.

Table 3. Effects of substrate on growth and quality of *Leucaena leucocephala* seedlings at 90 DAT

Parameters→ Treatments↓	Survival (%)	Plant height (cm)	Collar diameter (cm)	Number of leaves	Mean root length (cm)	Nodules/ Plant (No.)	SQI
T1	91.83 a	45.85 b	3.46 b	9.20 a	21.25 ab	7.20 a	0.480 b
T2	83.24 b	51.68 a	3.96 a	9.27 a	24.00 a	5.33 b	0.706 a
T3	73.14 c	41.63 c	3.24 c	9.80 a	24.63 a	3.07 c	0.364 c
T4	59.30 d	32.30 d	2.59 d	8.93 a	13.42 bc	1.70 cd	0.299 d
T5	51.90 d	22.73 e	1.97 e	5.4 b	12.20 c	1.33 d	0.110 e
T6	91.27 a	44.47 b	3.23 c	8.73 a	19.15 abc	6.33 ab	0.319 d
Statistical analysis							
P (0.05)	0.009	0.013	0.013	NS	NS	0.020	0.03
SE	2.57	0.64	0.051	1.90	2.69	0.525	0.008
F	5.21	4.71	4.69	0.615	2.68	4.137	3.58
Linear Regression analysis (y = concerned parameter, x = FA rate)							
y =	96.86-0.44x	51.16-0.23x	3.76-0.02x	-	-	7.29-0.06x	0.52-0.01x

See Table 2 for details.

retention capacity of substrate. The survival rate decreased linearly with increased concentration of FA up to minimum 51.90% in growth media having 100% FA. The maximum height (51.68 cm) and diameter growth (3.96 cm) of seedlings was found in substrates having 40% FA. Both T₁ and control/soil had statistically at par number of root nodules per plant. A similar trend in growth of seedlings with respect to FA concentration was also reported by Khajuria et al (2000), Gupta et al (2000) and Pandey et al (1996). The vigour in seedling height, diameter and root growth of this species at 40% FA was due to neutral pH, improvement in availability of nutrients in ionic form at rhizosphere solum and improved nitrogen fixation rate (Table 2), and reduced or no attack of nursery insect and pest. Goyal et al (2002) observed 10% increase in the growth of *Eucalyptus tereticornis*, *Acacia auriculiformis* and *Casuarina equisetifolia* during early 6 months, grown in FA amended soils (ESP FA@18–24% (v/v)). Better root nodulation per plants (7.20) in substrates having 20 % FA could be attributed to uptake of optimum amount of metals by the roots. However, the nodulation rate decreased after 20% FA linearly up to 100% FA which may be due to the reduced ability of nitrogen fixing bacteria with increasing stress level (Faizan and Kaushar 2010). The depressive nodulation effect was substantiated by reduced plant height, collar diameter growth, and seedling quality index. Further the plants grown in 20% FA were observed to be very healthy. Better seedling quality index in T₂ (0.706) was obviously due to the improved availability of micronutrients that supported higher biomass production and shoot: root ratio (Wong and Wong 1989, Gupta et al 2000).

CONCLUSION

The results recommend FA should be admixed at 20% (w/w) level in nursery beds for early sprouting and better

germination of *L. leucocephala*, however, for improving its seedling growth and for raising quality of planting stock the growing media should be admixed with FA @ 40% w/w. Further study is necessary to quantify the economic benefit or net profit gain from utilizing FA in forest nursery.

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Validation of Carbon Contents in Different Traits and Components of Herbaceous Species from Tropical Grassland

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Abstract: Because of global climate change events; the quantification of carbon (C) present in different species from diverse ecosystems are urgently needed. Study evaluated the performances of different methods used for the C estimation in different plant components and traits by comparing with harvest method. For this, 117 herbaceous species just-before flowering were harvested from the campus of Banaras Hindu University, Varanasi. Across the components, the mean C contents (g plant^{-1}) varied between 0.33 for roots of procumbent and 7.93 for shoots of native trait categories. On g m^{-2} basis, it ranged from 29 (roots) to 85 (shoots). Similarly across the components, the mean C: B ratio ranged between 0.41 (roots) and 0.44 (shoots). The linear relationship between C and B had high correlation coefficient, low standard error of estimate together with low discrepancy in extrapolation compared to the other models. Hence, compared to other models, the linear equation could be used in C estimation. Further among all the estimators; at the species, component and functional group levels, the mean C: B (0.43) ratio of the entire herbaceous species resulted as an accurate estimator of the C. Because of its simplicity and low discrepancy, the mean C: B (0.43) ratio seems to be reasonable to estimate the C content based on the known biomass. Stems of perennials, erects, leguminous and native plants had greater C than the others. Thus, the perennial, erect, leguminous and native herbaceous species could be an alternative for reducing the atmospheric C in the tropical grasslands.

Keywords: Carbon estimator, C:B ratio, Climate change, Herbaceous species, Non-destructive, Regression, Tropical grassland

Currently, the average Carbon dioxide (CO_2) in atmosphere is around 385 ppm which is increasing at more than the average rate of 2 ppm yr^{-1} and by 2100 its concentration in atmosphere would be around 800-1000 ppm (IPCC 2014, Kaur et al 2018). Among the greenhouse gases; CO_2 alone has the potential to contribute a total of 60 % of the global warming (Singh 2017) and predictions showed that the temperature could rise by 4°C by the end of the year 2100 (IPCC 2014, Singh 2017). As a consequence, it is further expected tremendous spatiotemporal changes in rainfall patterns, extreme weather and shifting of seasons due to increasing level of atmospheric CO_2 . Hence, the global researches felt to reduce the rate of CO_2 increase by storing in potential C sinks, viz; soil, above and below ground plant biomass, dead wood and litters (Chauhan and Chauhan 2011, IPCC 2014, Lu et al 2015, UNFCCC 2015, Odiwe et al 2016, Jithila and Prasad 2018, Nandal et al 2019). Given that C stored in plants is determined by biomass inputs through photosynthesis and outputs to senescence, mortality (Friend et al 2014), rates of decomposition of dead plant materials (Wang et al 2013) and C residence time (Friend et al 2014). The plant C residence time is dependent on the plant components, its functional traits (Friend et al 2014) and decomposition rates (Ontl and Schulte 2012). Once, tropical forests were major terrestrial C sink (San Jose et al 1998). Now, their declining C storage capacity (Dunne 2017, Ma et al 2017) warranted to move on

herbaceous species which stores roughly 34% of the global terrestrial C pool (White et al 2000). However, they show high decomposition rate (Schlesinger 1990), less C residence time and limitations in estimating the total C than the woody species (Sankey et al 2013, Wang et al 2013). The regression equations, C: B and root (R): shoot (S) ratios are commonly used non-destructive methods for C computation in different woody vegetations (UNFCCC 2015, Sainju et al 2017). Unfortunately, it is totally lacking for the herbaceous species. The use of the IPCC default R: S for estimating the root C of grasslands locating in different ecological conditions may increase the discrepancy in the prediction of total C (Barbosa et al 2012), while the use of the vegetation-specific R: S ratios could improve the accuracy in estimating the root C as a part of global C accounting (Mokany et al 2006). Thus, it is urgent to identify the novel traits for developing the strategies to mitigate the elevated CO_2 and to determine the accuracy of different methods used for the estimation of missing C sink (Sims and Bradford 2001, De Deyn et al 2008). Looking into the above account; study was framed in a tropical grassland with the objectives to quantify the C content of herbaceous plants at species, plant component and functional group levels and to assess the performances of the different methods by estimating the % discrepancy of estimation, and finally to suggest the traits which could be helpful for the policy and planning makers in curbing the rate of increasing atmospheric CO_2 .

MATERIAL AND METHODS

Study area: The study was conducted on the campus of Banaras Hindu University (24°18' N and 83°03' E and 80.71 m above sea level), Varanasi, India, during January 2014 - December 2015. The climate is a tropical monsoon with distinct cold winter (November to February), hot summer (April to June) and warm rainy (July to September). The October and March are transitional months between rainy and winter, and between winter and summer seasons, respectively. During the study period, the mean maximum temperature was 30.19°C (range 8-44.6°C) while the mean minimum temperature was 20.27°C (range 6.4-30.8°C). The soil is alluvial, well-drained, pale brown, silty loam and inceptisol. In general, it is moderately fertile being low in available nitrogen and medium in available phosphorus and potassium with neutral to alkaline soil pH.

Experimental design: The mature herbaceous species (just before the onset of the flowering stage) were collected from the entire campus of the University. For each species; 3-10 healthy individuals (depending upon the availability) were harvested. Each harvested plants were separated into root, leaf, and stem components. For our convenience, the term 'stem' is used throughout the ms instead of 'culm'. The plant fractions were oven-dried at 80°C to constant weighed. The oven dried plant material was used to determine the ash content. Ash content was measured after combustion of the sample in a muffle furnace at 550°C for 6 h (Singh et al 2011). C content was determined by using the loss on ignition method (Mcbrayer and Cromack 1980) which is approximately 50 % of ash-free weight (van Soest 1963). The plant functional attributes were studied at life form (forbs, grasses, legumes and sedges), growth form (erect, prostrate, procumbent and decumbent), lifespan (annual, biennial and perennial), and origin or distribution (native, non-native and cosmopolitan) levels (Verma et al 2015). These traits were determined with the help of Flora of Raipur, Durg and Rajnandangaon (Verma et al 2015) and Flora of the upper Gangetic plain (Duthie 1903). Different linear regression equations between C and oven dried B for each plant component were developed considering total species as data points to estimate the C content for a given B. The C content of each component was estimated on the basis of mean C to B ratio (C: B ratio) of the entire 117 species. Additionally, the root C was estimated using R: S C ratio. The estimated C contents using these methods were compared with that of experimentally measured C content by computing the percent discrepancy: % discrepancy = $100 \times (\text{measured C} - \text{estimated C}) / \text{measured C}$ (Chave et al 2005). The estimated C based on the linear equation, C: B ratio for each plant component in addition to estimated root C using R:S

ratio, were validated at species, functional group and site levels. For site level validation, three sites differing in anthropogenic perturbations (less/site-1, medium/site-2, and high/site-3) were selected on the basis of a rigorous survey of the entire campus of Banaras Hindu University. For each site; 15, 1×1 m plots were used for the sampling of plants for their B and C measurement. Hence, a total of 45, 1×1 m plots (3 sites × 15 replicate) were used for the validation of C content. For each 1 × 1 m plot, above ground peak, live B of all species was clipped at the soil surface in mid-October of the sampling year. All samples were oven-dried at 80°C to constant mass and weight. This oven dried B was used for the determination and validation of C contents. The root, as well as total C contents, was estimated by using dry B of easily available plant components (leaf, stem and shoot) in the regression equations.

RESULTS AND DISCUSSION

Measured C contents: Among components, the mean C:B ratio varied between 0.41 and 0.45 (Fig. 1). Similarly, the mean C expressed in g per plant ranged from 0.89 to 4.95. The values of C: B ratio and C (g plant⁻¹) contents were low for roots and high for stems (Fig. 2). Across the species and components, the measured C content using C:B ratio (% of dry biomass) varied between 36 and 50 with a mean value of 42.70. Davies et al (2011) reported 42.02% C of the dry biomass for the herbaceous species. Tumuluru (2015) suggested 42.08 and 43.92% C, respectively for the switch grasses and corn stover. Moreover, majority of local, regional, and global studies showed 35 to 65 % plant C based on the plant dry weight (Lewis 2009, Fang et al 2001, Blanc 2009, Redondo-Brenes 2007, Thomas Martin 2012) and as a mean it has been reported around 45% (Wang et al 2013, Farrar et al 2014). Thus, 36 to 50% variation in C estimation based on C:B ratio could be comparable to these studies. Evidently, the statistical analysis also suggested variation in the experimentally measured plant C due to species and plant components. The C content of each plant component significantly varied among the plant functional groups, except for leaf, stem and shoot components of lifespan and root component of life form functional groups as suggested by 'F'-test. The measured C contents expressed either in g plant⁻¹ (Table 1) or gm⁻² (Table 2) was maximum in perennials, legumes and erects plant trait categories than the others. The greater C storage by perennials compared to the annuals and biennials may be due to the excess accumulation of photosynthates in form of carbohydrates, lipids and other chemical compounds for their respiration and maintenance during the dormant season (Dickson 1989) particularly in winter (Farrar et al 2014). Among all life form categories; the

legumes exhibited relatively greater C compared to others which could be linked to the greater nitrogen availability through the process of nitrogen fixation used as a resource during the C production (Diekmann and Falkengren-Grerup 2002, Neufeld and Young 2003). The higher C exhibited by the erect plant trait than the other traits could be argued because of greater availability of light to capture the atmospheric C for their biomass build-up as a result, plants having such traits out shade the prostrate and short-statured species (Diekmann and Falkengren-Grerup 2002, Cong et al 2014, Verma et al 2015, Sagar et al 2017). Therefore, perennial plants having leguminous and erect traits could be considered as a key driver for C storage. The R:S ratio (a measure of above ground and below ground C partitioning) also differed substantially due to differences in species, lifespan, nativity and growth forms. The analysis of average C partitioning into the different plant components showed five

times greater C in shoots than the roots (Table 2), which is also supported by Irving (2015). Such pattern signified the dependency of the plant growth on the supply of C from the shoots, and the nutrients and water from the roots. The ecological research on C portioning in different plant components is not only important for the assessment of competitive fitness, reproduction and growth of the plants against the climate change (Dickson 1989) but also precisely contribute to the global total C estimation and prediction (Mokany et al 2006, Russell et al 2015, Panwar et al 2017). Similarly, the plant functional trait could be a key component of the global C cycle by regulating the storage and fluxes of soil C. The fast-growing plants are easily decomposable than the slow-growing and long-lived native plants and high root: shoot ratio suggests the suitability of plant to sequester the C in high amount (Schlesinger 1990).

Relationships between B and C: Among the tested models

Table 1. Mean herbaceous carbon; C (g plant⁻¹), C to B ratios and percent discrepancy in prediction based on linear regression equations and mean C:B ratio (0.43) for different plant trait categories

Parameters	Annual	Biennial	Perennial	Native	Non-native	Cosmopolitan	Forbs	Grasses	Legumes	Erect	Prostrate	Procumbent
Leaf-C	1.94	2.01	2.43	3.36	1.82	1.73	1.94	3.42	2.07	2.45	1.65	0.87
C to B ratio	0.43	0.41	0.43	0.43	0.43	0.43	0.42	0.44	0.45	0.43	0.43	0.42
C to B ratio (0.43) based discrepancy	0	5	-1	-1	0	1	1	-2	-4	0	0	4
Equation based discrepancy	0	5	0	1	0	1	2	-1	0	0	0	1
Stem-C	2.27	2.77	3.31	4.56	2.27	1.11	2.70	4.12	2.81	3.43	1.08	0.88
C to B ratio	0.46	0.45	0.47	0.46	0.46	0.45	0.46	0.47	0.46	0.47	0.45	0.42
C to B ratio (0.43) based discrepancy	-6	-4	-8	-8	-7	-5	-7	-9	-9	-8	-4	2
Equation based discrepancy	0	3	-2	0	-1	-2	0	-2	-3	-1	0	5
Shoot carbon	4.22	4.78	5.75	7.93	4.09	2.84	4.64	7.54	4.88	5.87	2.73	1.75
C to B ratio	0.45	0.44	0.45	0.45	0.46	0.44	0.45	0.45	0.46	0.45	0.44	0.42
C to B ratio (0.43) based discrepancy	-4	-0	-5	-5	-4	-1	-3	-6	-7	-5	-2	3
Equation based discrepancy	00	4	2	0	0	1	1	0	-3	0	1	3
Root-C	0.56	0.74	1.22	1.75	0.57	1.68	0.80	1.29	0.78	0.99	0.74	0.33
C:B ratio	0.40	0.40	0.41	0.42	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.40
C to B ratio (0.43) based discrepancy	6	7	4	3	6	4	5	3	5	4	6	8
Equation based discrepancy	0	1	0	0	0	1	0	-1	0	-1	0	0
R:S C ratio based discrepancy	202	158	86	81	188	-32	135	134	150	137	47	112
Total- C	4.78	5.52	6.97	9.68	4.66	4.51	5.44	8.82	5.65	6.86	3.47	2.08
C to B ratio	0.44	0.43	0.44	0.45	0.44	0.43	0.44	0.45	0.45	0.44	0.43	0.41
C to B ratio (0.43) based discrepancy	-3	1	-3	-3	-3	1	-2	-5	-5	-3	0	4
Equation based discrepancy	1	5	1	1	1	5	2	0	-1	1	4	8

Foot note: '-' sign represents underestimation.

(linear, exponential, logarithmic, power and second order polynomial); the linear equation between the B and C for each plant component exhibited comparatively higher correlation coefficient and lower standard error of estimate than the other models. Thus, the fitted linear equation between the B and C for each plant component presented in Figure 2 is used for further analysis. Statistically; the higher correlation coefficient, lower standard error of estimate, least discrepancy in prediction (Sagar et al 2003, SYSTAT 2009) wide adaptability with minimum time and cost epitomize the performance and utility the of model (Xie et al 2009). Interestingly, the t -test showed insignificant difference between the means of measured and estimated (using biomass data in the respective fitted linear regression equation) C contents (t ranged from -0.37 to 1.86 and p values varied between 0.07 and 0.77) for each plant component (Fig. 2).

Performance of the models used in C prediction: The prediction and validation of C content in different plant parts for each species using mean C:B ratio (0.43) suggested that 84% of the total recorded species exhibited less than ten percent discrepancy in C estimation for root component, while leaf had 95%, stem had 98% and more interestingly at total plant level 100 % species yielded less than ten percent discrepancy in C estimation. At the functional group levels, the percent discrepancy in C estimation using mean C:B ratio and respective fitted linear regression equations across the

plant component and traits varied from 0 to 9 (Table 2). Similarly, the maximum percentage discrepancy in prediction of C using mean C:B ratio and linear equations for different plant components across the sites ranged between 9 and 10 (Table 2). On the other hand, the percent discrepancy in estimation of root C with the help of R:S C ratio at functional group and site levels ranged between -55 and 202 (Table 2). Thus, the discrepancies in C prediction based on the R:S ratio are several fold greater than the prediction based on the C:B ratio and linear equations. The maximum of 10% discrepancy has been advocated for selecting a model in

Table 2. Mean value of herbaceous carbon content (g m^{-2}), and discrepancy in prediction (%) based on linear regression equation, C:B ratio of total plant species, and R:S C ratio at three locations in tropical grasslands differing in disturbance intensity

Vegetation parameters	Less (Site-1)	Medium (Site-2)	High (Site-3)
Leaf-C	43	25	21
C to B ratio based discrepancy	3	3	2
Equation based discrepancy	5	5	4
Stem-C	42	38	31
C to B ratio based discrepancy	-5	-4	-4
Equation based discrepancy	5	6	6
Shoot-C	85	63	51
C to B ratio based discrepancy	1	-1	-1
Equation based discrepancy	6	6	6
Root-C	75	43	29
C to B ratio based discrepancy	6	10	8
Equation based discrepancy	3	7	6
R:S C ratio (0.40) based discrepancy	-55	-42	-30
Total-C	160	106	81
C to B based discrepancy	2	3	2
Equation based discrepancy	7	9	8

Foot note: '-' sign represents underestimation

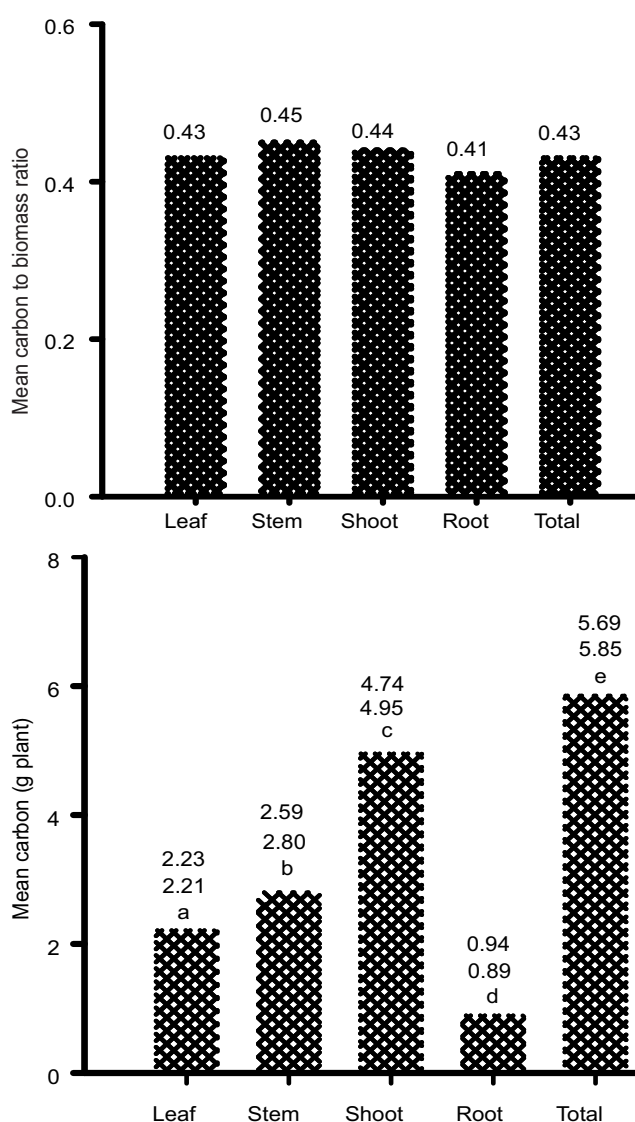


Fig. 1. Mean carbon (C) content (g plant^{-1}) and carbon to biomass ratio based on 117 herbaceous species. Bars affixed with different letters within each component are significantly different from each other. The value on the bar of each component indicates the measured C and italic values indicate the estimated C

many computational and statistical analyses (NRC 2012, Wang et al 2013). Based on these assumptions, the mean C:B ratio (0.43) and the best fitted linear regression equations could be used for the C estimation in the tropical grassland. Moreover, at species, components and functional group levels, the mean C: B ratio exhibited less than ten percent discrepancy in C prediction. The fitted linear regression equations also showed less than ten percent discrepancy in C estimation at the selected plant components, and functional groups levels. On the other hand, the R:S C ratio exhibited disproportionately very high discrepancy in the estimation of root C at the functional group and site levels. Due to inherent simplicity, greater prediction accuracy and consistent results, the C:B ratio could be used for the C estimation at large-scale tropical grassland.

CONCLUSION

The stems of perennial, erect, leguminous and native

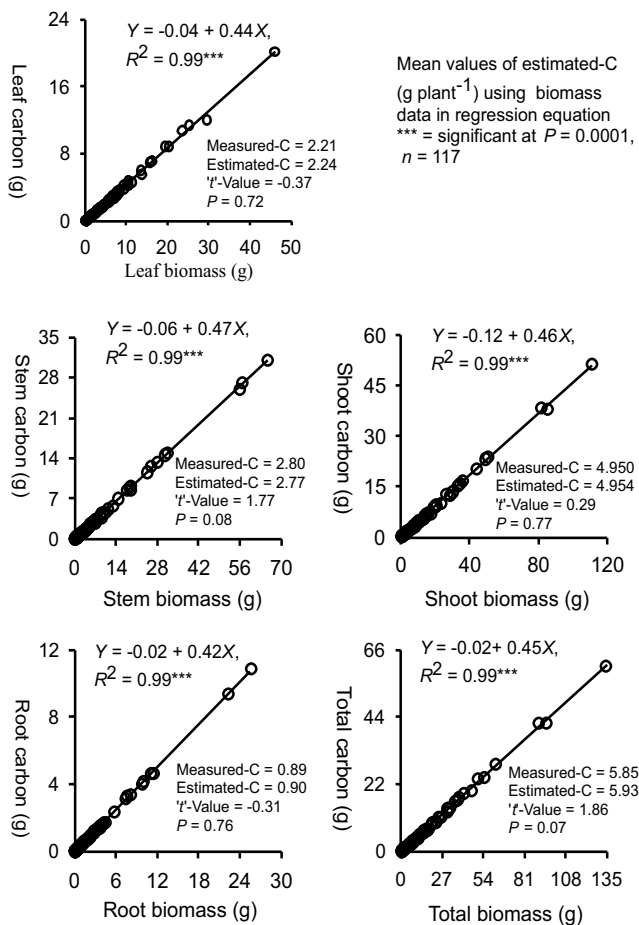


Fig. 2. Linear relationships between dry weight (X) and carbon content (Y) for different plant components and differences in measured and estimated carbon based on 't'-test'. P = level of significance, *** P at ≤ 0.0001

plants have high ability to reduce the atmospheric CO₂ by capturing it and then converting its significant amount into the B. The mean C: B ratio (0.43) of the entire herbaceous species yielded less discrepancy for the estimation of C in different components of the tropical grassland. The determination of the ash and C contents using loss on ignition method may give precise data on C, but time-consuming and costly to cover at large scale, hence, mean C:B ratio reported in present study could be helpful for the quantification of the missing C using biomass data at the large-scale tropical grassland. The perennial, erect, leguminous and native plants could be helpful for the policy makers to curb the rising atmospheric-C for better human well being against the global climate change problem.

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Socio-economic Evaluation of Agroforestry Systems

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Abstract: Majority of the farmers practiced bund (38.89 %) and boundary planting (38.89 %) followed by scattered planting (22.22 %) and density of trees per hectare varied in the range of 20 to 35. However, Chi-square test found significant association between the tree density and farmers preference of growing trees to size of land holding, net income, energy source and holding of livestock population ($P < 0.05$). Further, farmers preferred trees for productive value such fuel wood, timber and fodder which make them self reliant to meet their basic needs than the protective value. Further, the majority of the respondent expressed competition with crop followed by lack of irrigation facility and small land holding size were the major constraints for growing trees on the farm land. Understanding the farmer's immediate need, preference and perception should be effectively addressed while designing agroforestry systems.

Keywords: Agroforestry systems, Boundary planting, Fuel wood, Productive value, Tree density

In recent past agroforestry is gaining greater attention in the globe as sustainable land use system particularly in the tropics for its economical and ecological advantage. Indeed the practice of retaining trees on the farm land is an age old practice where farmers deliberately retain and/or plant specific perennial trees and shrubs to get food, fodder, timber, fuel wood and to obtain shade, shelter, combat erosion, soil fertility improvement and improve aesthetic value of landscape (Chittapur and Patil 2017). However, in spite of high ecological value these traditional tree based land use system are disappearing due to low economic significance as compared to conventional agriculture (Nerlich et al 2013, Moreno et al 2017). The low economic value of these land use system may be due to lack of sustainable management of agroforestry systems and as well as difficult to quantify its ecological benefits in terms of monetary value. Further, it is evident that the practice of conventional agriculture resulted in irrevocable change in function and structure of ecosystem. Whereas, agroforestry systems known to counteract the negative impacts of intensively managed agricultural system thus agroforestry systems are considered to be as dynamic, ecological based natural resource management system that diversifies and sustain the production for increased socio-economic and environmental benefits (Verchote et al 2007, Dhillon et al 2010). However, the structure and function of agroforestry systems are influenced by heterogeneous factors such as ecological condition of the location, socioeconomic status of the farmer and economic utility of tree species (Giller et al 2006, Chauhan et al 2009). Further, few studies also reported

that socioeconomic and ecological factors such as land holding size, size of household, number of livestock, income sources, abundance of trees species in the wild and altitude of the place affect the tree diversity and density in agroforestry systems (Nanda et al 2001, Acharya 2006, Bellow et al 2008). Hence, tree based land use system need to be studied with multidimensional approach. However, most of the studies assessed agroforestry systems in terms biophysical aspects and gave little importance on socio-economic aspects which substantially influence on the adoption of agroforestry systems (Chauhan et al 2012). It is evident that the people perception and attitudes plays major role in decision making and adoption of these long rotation crops on the farm land. Keeping above facts in view the present investigation on socio-economic analysis of agroforestry systems was undertaken under rainfed agro-ecosystem of north-eastern part of Karnataka.

MATERIAL AND METHODS

The study was under taken under rainfed agro-ecosystem in north eastern part of Karnataka, India. The study area lies within the geographical region of North maiden; it spreads between 14° 60' to 18° 30' Northern latitude and 75° 60', to 77° 70' Eastern longitude. The average annual rainfall ranges from 600 to 900mm and mean elevation ranges from 350-680 m MSL. The soils are deep to very deep black soils and medium black soils in majority areas while sandy loam and light textured soils and late rite soils also found in some pockets. The most of this region is characterized by semi arid to arid climatic condition and

covers nearly 23 per cent of total geographical area of the state. The major crops grown are pigeonpea, greengram, bengalgram, groundnut, soybean, sunflower, safflower, sorghum, and pearl millet. Multistage purposive randomized sampling technique was used to select the samples for the study by selecting districts as unit (6 districts) and in each district two taluks were identified, in each taluk one village was identified and in each village 6 respondents of 2 each in small farmer (<2 ha), medium farmer (2 to 4 ha) and large farmer (>4ha) were selected randomly among the list of the farmers who have already practicing agroforestry systems and in all the total sample size of the study was 72 farmers. The information on the socio-economic characteristics viz., land holding, income, literacy, farm income, number of ruminants, energy consumption pattern, house-hold status, and perception of agroforestry farmers were collected by interviewing the farmers with structured questionnaire prepared for the study. Further, The kind of agroforestry system practiced by the farmers in the study area were identified by visiting the field and classified based on the nature of the component and the pattern of tree planting on the farm land and the number of farmers practicing specific agroforestry systems were recorded and expressed in percentage out of the total farmers surveyed. Species richness, species density and tree density in the existing agroforestry systems were recorded with plot size of one hectare representing the total farm land of each individual farmer. The data collected were analyzed using descriptive statistics and Chi-square test (goodness of fit) was used at 5 per cent level of significance to test the nature of association between the agroforestry systems, number of trees per hectare, purpose of tree retention to size of land holding, age, level of education, size of household, holding of livestock population, source of energy and net income by using SPSS (Statistical Package for Social Science).

RESULTS AND DISCUSSION

It was recorded that three prominent agroforestry systems were practiced by the farmers and the majority of the farmers practiced bund planting (38.89%) and boundary planting (38.89%) followed by scattered planting (22.22%) system. This indicates that the majority of the farmer retained the trees more on bund and boundary of the farm land rather going for the scattered planting as the retention of the trees on bund and boundary planting will have lesser limiting effect on crop performance compared to in- field scattering. Further, boundary planting helps to protect the farm from stray cattle menace, erosive and desiccating wind, conserve soil from erosion besides serving as a demarcation of the farm. Behera and Dhir (2013) also reported that bund planting was the

major practice followed by the farmers in rainfed condition (50.4%) in Bouda district of Odisha. However, Chi-square test indicated there was no significant association between agroforestry systems practices and independent variables of respondents such as size of land holding, age, level of education, size of household, energy source, holding of livestock population, net income ($P>0.05$) (Table 1). The density of trees ranged from 20 to 35 trees per hectare in the existing systems. Further, the Chi- square test indicated that there was significant association between number of trees per hectare to size of land holding, net income, energy source and holding of livestock population of the farmer (Table 1). Whereas, age, level of education, household size did not influenced on the number of trees per hectare P . This suggests that higher net income and larger land holding farmers accommodate more of number of tree per hectare. Further, farmers retain more number of trees on the land to meet out their basic needs such as fuel wood and fodder. The results are in agreement with Bucagu et al (2013) in their study on assessment of tree diversity in different categories of farmers and reported higher density of tree species in wealthier farmer than poor farmer. Further, they opined that the rich farmers had large farms and which makes them flexibility to accommodate more number of trees on their farm.

In the present study respondents were asked economical and ecological intention of retention of tree species on farm land. The majority of the respondents retained trees for fuel wood purpose (58.33%) followed by timber (52.78%) and fodder (51.39%). Whereas, for ecological purpose, majority retained for protection (36.83%) followed by erosion control (28.61%) and shade (23.61%) (Table 2). This indicates that more priority was given to productive value of trees than the protective values. This could be to meet out the basic needs of the rural people such as fuel wood, fodder, timber and sustains the livelihood of the rural people. Earlier workers (Kareemulla et al 2009, Bijalwan et al 2011, Jamala et al 2013) also conferred that farmers preferred tree for productive value than intangible benefits. Majority of the rural people use fuel wood as major energy source for heating and cooking, timber for construction, agricultural implements, furniture and fodder requirement for livestock is also met from trees especially in the off season, therefore they prioritized accordingly. Further, significant association was observed between the economical retention of tree species with size of land holding, energy source, livestock population and net income of the farmer P (Table 2). However, the level of education, age and size of household did not significantly associated with economical intention of trees by the respondents (Table 2). The higher land holding farmers preferred timber species, whereas, the farmers with smaller land holding preferred fuel and fodder.

Table 1. Different agroforestry systems practiced by the farmers and mean no. of trees per hectare

Independent variables	Agroforestry systems			No. of trees ha ⁻¹		
	Bund	Boundary	Scattered planting	20-25	26-30	31-35
Farm holding size						
Small (n=26)	12	08	06	09	16	01
Medium (n=27)	09	11	07	04	10	13
Large (n=19)	07	09	03	00	05	14
Total (N=72)	28 (38.89)	28 (38.89)	16 (22.22)	13 (18.06)	31 (43.05)	28 (38.89)
P Value	0.748	0.748	0.748	0.000*	0.000*	0.000*
Education						
Illiterate (n=10)	05	03	02	04	03	03
Primary (n=42)	16	16	10	08	19	15
Secondary (n=15)	03	08	04	01	06	08
Tertiary (n=05)	04	01	00	00	03	02
Total (N=72)	28 (38.89)	28 (38.89)	16 (22.22)	13 (18.06)	31 (43.05)	28 (38.89)
P Value	0.353	0.353	0.353	0.360	0.360	0.360
Age						
30-40 (n=19)	07	08	04	05	08	06
41-50 (n=35)	13	13	09	07	13	15
51-60 (n=18)	08	07	03	01	10	07
Total (N=72)	28 (38.89)	28 (38.89)	16 (22.22)	13 (18.06)	31 (43.05)	28 (38.89)
P Value	0.949	0.949	0.949	0.449	0.449	0.449
House Hold						
1-5 (n=27)	10	11	06	04	11	12
6-10 (n=39)	17	15	07	08	16	15
11-15 (n=06)	01	02	03	01	04	01
Total (N=72)	28 (38.89)	28 (38.89)	16 (22.22)	13 (18.06)	31 (43.05)	28 (38.89)
P Value	0.480	0.480	0.480	0.714	0.714	0.714
Energy Source						
Fuel wood (n=27)	11	07	09	05	18	04
Fuel wood +crop residue (n=30)	11	14	05	08	08	14
Fuel wood + LPG (n=15)	06	07	02	00	05	10
Total (N=72)	28 (38.89)	28 (38.89)	16 (22.22)	13 (18.06)	31 (43.05)	28 (38.89)
P Value	0.357	0.357	0.357	0.002*	0.002*	0.002*
Livestock holding						
0 (n=2)	04	06	03	01	03	09
1-3 (n=23)	11	04	06	08	10	03
4-6 (n=47)	13	18	07	04	18	16
Total (N=72)	28 (38.89)	28 (38.89)	16 (22.22)	13 (18.06)	31 (43.05)	28 (38.89)
P Value	0.282	0.282	0.282	0.006*	0.006*	0.006*
Net Income						
>1 Lakh (n=7)	04	03	00	01	04	02
1-1.5 lakh (n=25)	08	08	09	09	11	05
1.5-2 lakh (n=20)	10	06	04	03	10	07
< 2 lakh (n=20)	06	11	03	00	06	14
Total (N=72)	28 (38.89)	28 (38.89)	16 (22.22)	13 (18.06)	31 (43.05)	28 (38.89)
P Value	0.222	0.222	0.222	0.009*	0.009*	0.009*

The values in parenthesis indicates percentage, *Indicates the significant association at 0.05 P level of Chi square test

Table 2. Purpose of trees retained on farm land as expressed by the respondents (frequency)

Independent variables	Rainfed agro-ecosystem (N=72)						
	Timber	Fruit	Fuel wood	Fodder	Shade	Erosion Control	Protection
Farm holding size							
Small (n=26)	7	12	21	15	08	11	20
Medium (n=27)	15	11	18	18	07	16	18
Large (n=19)	16	04	03	4	02	08	13
Total (N=72)	38 (52.78)	27 (37.50)	42 (58.33)	37 (51.39)	17 (23.61)	35 (48.61)	51 (70.83)
P Value	0.001*	0.208	0.000*	0.007*	0.269	0.375	0.688
Education							
Illiterate (n=10)	04	05	09	07	03	06	06
Primary (n=42)	20	15	26	21	09	20	32
Secondary (n=15)	10	05	06	09	05	07	08
Tertiary (n=05)	04	02	01	00	00	02	05
Total (N=72)	38 (52.78)	27 (37.50)	42 (58.33)	37 (51.39)	17 (23.61)	35 (48.61)	51 (70.83)
P Value	0.290	0.838	0.024*	0.067	0.446	0.872	0.143
Age							
30-40 (n=19)	11	08	09	10	09	09	14
41-50 (n=35)	18	13	22	17	04	19	25
51-60 (n=18)	09	06	11	10	04	07	12
Total (N=72)	38 (52.78)	27 (37.50)	42 (58.33)	37 (51.39)	17 (23.61)	35 (48.61)	51 (70.83)
P Value	0.869	0.858	0.524	0.883	0.012*	0.564	0.890
House Hold							
1-5 (n=27)	10	13	15	19	07	16	15
6-10 (n=39)	24	11	25	16	09	19	32
11-15 (n=06)	04	03	02	02	01	00	04
Total (N=72)	38 (52.78)	27 (37.50)	42 (58.33)	37 (51.39)	17 (23.61)	35 (48.61)	51 (70.83)
P Value	0.114	0.208	0.339	0.042*	0.884	0.032*	0.065
Energy Source							
Fuel wood (n=27)	10	11	19	18	04	11	21
Fuel wood +crop residue	16	14	20	16	11	19	22
Fuel wood + LPG (n=15)	12	02	03	03	2	05	08
Total (N=72)	38 (52.78)	27 (37.50)	42 (58.33)	37 (51.39)	17 (23.61)	35 (48.61)	51 (70.83)
P Value	0.028*	0.085	0.003*	0.014*	0.088	0.097	0.229
Livestock holding							
0 (n=2)	11	3	1	1	1	6	11
1-3 (n=23)	08	11	16	15	8	8	13
4-6 (n=47)	19	13	25	21	8	21	27
Total (N=72)	38 (52.78)	27 (37.50)	42 (58.33)	37 (51.39)	17 (23.61)	35 (48.61)	51 (70.83)
P Value	0.027*	0.191	0.000*	0.001*	0.110	0.442	0.367
Net Income							
>1 Lakh (n=7)	01	04	06	04	02	03	04
1-1.5 lakh (n=25)	09	11	20	16	08	12	19
1.5-2 lakh (n=20)	13	06	10	12	06	11	12
< 2 lakh (n=20)	15	06	06	05	01	09	16
Total (N=72)	38 (52.78)	27 (37.50)	42 (58.33)	37 (51.39)	17 (23.61)	35 (48.61)	51 (70.83)
P Value	0.007*	0.464	0.003*	0.049*	0.147	0.913	0.406

Dependent variables are of multiple responses. The values in parenthesis is percentage of total sample

*Indicates the significant association at 0.05 P level of Chi square test

Table 3. The major constraints of growing trees as expressed by respondents

Major constraints	Percentage of respondents
Competition with crops	54.17
Fear of theft	12.50
Small land holding	36.11
Long gestation period	25.00
Non availability of seedlings	15.28
Lack of irrigation facility	41.67
Stray cattle menace	25.00
Lack of marketing facility	11.11
Shortage of labor	18.06

The above statements are multiple responses

The respondents were also interacted through semi structured questionnaire on constraints of growing trees on farm land. The majority respondents expressed that the competition with the field crop (54.17 %) was the major constraint followed by lack of irrigation and small land holding (Table 3). However, few other constraints were also mentioned by the respondents such as long gestation period, lack of non-availability of seedlings, stray cattle menace, shortage of labour, lack of marketing and fear of theft. However, competition with crops was considered to be major constraints this implies that trees significantly affect the crop yield and further, although farmers interested to grow trees on farm land but the resources like water and land could be the other major deterrents in growing trees under rainfed agro-ecosystem. The results are in line with Devarnavadagi et al (2010) who reported competition with crops was the major constraints both in rainfed and irrigated agro-ecological situations by 56.8 and 59 % respectively followed by long gestation period in northern dry tract of Karnataka.

CONCLUSION

The majority of farmers practiced bund and boundary planting followed by scattered planting. A significant association between density of trees and farmers preference for trees on farm land to land holding size, net income, energy source and livestock population was observed. Farmers highly preferred trees for productive values than the protective values. Further, the tree density and preferences were directly proportional to size of land holding, net income, energy source and livestock holding. Farmers also expressed competition with crops and lack of irrigation facility were the major constraints for growing of trees.

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Effect of Seed Priming on Germination of *Acacia nilotica*

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Abstract: The effects of season and seed priming with chemicals on seed germination of *Acacia nilotica* was assessed with ten treatments of 3 primers and 3 concentrations and one control (soaking in water). The result shows that germination percentage, mean daily germination, peak value germination value and germination energy was significantly influence by sowing seasons, different chemical primers and concentrations as well. The maximum germination percentage and germination peak value was observed in KCl 10 Mmole, which was at par with KCl 5 Mmole and KCl 15 Mmole, CaCl_2 5 Mmole, CaCl_2 10 Mmole, CaCl_2 15 Mmole, hydro-priming and minimum for NaCl 5 Mmole (51%) irrespective of seasons, which was statistically inferior to other treatments.

Keywords: *Acacia nilotica*, Seed priming, Germination, CaCl_2 , KCl, NaCl

The priming treatments, which enhance seed germination and seedling growth, include hydro priming, priming with plant growth promoters, halo-priming and osmo-priming (Afzal et al 2006, Chauhan et al 2017). Priming allows seed hydration to initiate the early events of germination, but not permit redical emergence, followed by drying to initial moisture (McDonald 2000, Vanangamudi et al 2000, Ashraf and Foolad 2005). The hydropriming significantly improve germination rate of seed (Ghassemi-Golezanik et al 2008). Hydro-priming is a simple low-cost method of seed priming that requires no sophisticated equipment and gives good results (Foti et al 2008). *Acacia nilotica* (babool/kikar) is a multipurpose tree, well accepted and extensively planted under afforestation and a variety of agroforestry systems. With its excellent timber values, one of the highly significant attributes of this tree species is its exceptional capacity to tolerate drought conditions. *Acacia nilotica*, indeed is a xerophytic tree, which is evident from the leaf structures and other modifications. In recent past, this tree species has registered severe decline in North India. The dying trees exhibit various symptoms including reduction in leaf size, change in foliage colour and then a sort of die-back of the twigs from top downwards. It has wider distribution covering large tracts in natural landscapes of sub -tropical and tropical parts of the country. It grows on a variety of soils under different fertility regimes, surviving on inhospitable sites also. In spite of this in recent past at some locations the mortality up to 80 per cent has been recorded (Chauhan et al 2006). This species has low natural regeneration capacity and low levels of germination of seeds. Hydropriming has been used to increase the speed and uniformity of germination and improved final stand. The effect of both

hydro and chemical priming on seed germination of *Acacia nilotica* species has been studied.

MATERIAL AND METHODS

The seeds were subjected to ten treatments with three primers and their three concentrations [NaCl (5,10,15 Mmol l^{-1}), KCl (5,10,15 Mmol l^{-1}), and CaCl_2 (5, 10, 15 Mmol l^{-1}) and a control (soaking in fresh water at room temperature) in randomised block design (factorial) with three replications. Seeds were sown on 17th July, 2017 (rainy season) and 7th February, 2018 (spring season) with 84 seed plot⁻¹. Seeds were sown by using dibbling method up to 1cm deep and weeding was done manually as and when required. During the process of germination, the seeds were observed for days to first germination and based on the germination observations taken on daily germination. After 25 days of germination, seedlings were evaluated for germination based on normal seedling characters and the results were reported in percentage. The mean daily germination, peak value, germination value and germination energy were calculated by using the formula given by Czabator (1962).

RESULTS AND DISCUSSION

Germination percentage, mean daily germination, peak value germination value and germination energy were significantly influence by sowing seasons and different chemical primers and their variable concentrations (Table 1). Significantly more germination percentage was recorded in rainy season (77.3%) than rainy season (49.7%) irrespective of chemical concentrations. The percentage change recorded in germination between rainy and spring season was 35.7%. The maximum germination percentage and

germination peak value was observed in KCl 10 Mmole, which was at par with KCl 5 Mmole and KCl 15 Mmole, CaCl₂ 5 Mmole, CaCl₂ 10 Mmole, CaCl₂ 15 Mmole, hydro priming and minimum for NaCl 5 Mmole (51%) irrespective of seasons. Among interactions the maximum germination percentage was in KCl 10 Mmole (87.9%) in rainy season, whereas minimum was recorded for NaCl 15 Mmole (66.0%) during rainy season. During spring season, the maximum germination percentage was observed for KCl 15 Mmole and CaCl₂ 10 Mmole, CaCl₂ 15 Mmole (57.9%), while minimum was for NaCl 5 Mmole (34.8 %). However, interaction effect due of season, chemical primer and their concentration was non-significant for all the germination parameters. Zaman et al (2012) reported that Na/K ratio was higher in unprimed seeds of maize. The higher uptake of water in primed seed increases the germination percentage. Priming allows some of the metabolic processes necessary for germination to occur without germination take place. In priming, seeds are soaked in different solutions with high osmotic potential. This prevents the seeds from absorbing in enough water for radical protrusion, thus suspending the seeds in the lag phase (Taylor et al 1997). The higher seed germination due to seed prime with KCl and CaCl₂ might be due non toxic effect of K and Ca ion accumulation in embryo. Beneficially effect of CaCl₂ may be due to its role in cell membrane integrity. Nutrient solution application of K decreases the Absciscic acid (ABA) concentration due to reduced ABA levels germination is enhanced. The mechanism is inhibition of germination and seedling growth by NaCl might be due to low absorption of water or toxicity of NaCl on the embryo which might have reduced germination percentage or delay emergence and further growth of plant. Present results are in accordance with Rafiq et al (2006) where in pre sowing treatment like hydro priming and CaCl₂.2H₂O etc. in wheat seeds increased germination.

The primed seed of *Acacia nilotica* during rainy season (2.09) recorded significantly higher germination peak value in than autumn season (1.18). The percentage change recorded in germination peak value between rainy and autumn season was 43.54. Data revealed that seed priming with different chemical concentrations also influenced germination peak value, mean daily germination and germination value. The maximum germination peak value was recorded in KCl 10 Mmole (2.83), whereas, minimum was recorded for NaCl 5 Mmole (1.33) during rainy season. During autumn season, the maximum germination peak value was observed for CaCl₂ 15 Mmole (1.57), while minimum was for NaCl 5 Mmole (0.68). Peak germination value indicates the highest seed germinated per number of days. Higher peak germination was recorded with application

of KCl and CaCl₂ as compared to NaCl seed priming treatment. The higher seed germination due to seed priming with KCl and CaCl₂ might be due to non-toxic effect of K and Ca ion accumulation in embryo. On the other hand, germination was inhibited by NaCl, this might be owing to reduced water absorption or toxic effects of NaCl on the embryo which leads to delay emergence and reduced growth of plant. Similarly, *S. bicolor* seeds soaked in CaCl₂ or KNO₃ solution, activity of total amylase, α -amylase and proteases were increased in germinating seeds under salt stress Kadiri and Hussaini (1999).

Mean daily germination was recorded significantly higher in rainy season (4.73) than autumn season (2.76). The percentage (%) change in mean daily germination between rainy and autumn season was 41.64. The interaction of season $\times$ chemical concentration had no significant influence on mean daily germination. The maximum mean daily germination was observed for KCl 10 Mmole (4.72) which was statistically at par with KCl 5 Mmole, KCl 15 Mmole, CaCl₂ 15 Mmole and minimum for NaCl 5 Mmole irrespective of seasons. The maximum mean daily germination was in KCl 10 Mmole (6.31), whereas minimum was for NaCl 15 Mmole (3.43) during rainy season. During autumn season, the maximum mean daily germination was observed for KCl 15 Mmole (3.49), while minimum was for NaCl 5 Mmole (1.60). Mean daily germination is total number of seeds germinated divided by total number of days. The higher mean daily germination in seeds primed with KCl and CaCl₂ might be due to enhanced germination in seed priming treatments and less time required to reach final population while NaCl resulted in slower germination rate and less germination percentage resulting less mean daily germination. The germination was inhibited by NaCl, might be due to reduction in water absorbed or toxicity of NaCl on the embryo which might have reduced mean daily germination. Results are similar with Srivastava et al (2010) where different priming treatments such as water (hydro-priming), calcium chloride (CaCl₂) (chemo-priming), and abscisic acid (ABA) (hormonal priming) showed increased rate of germination as compared to non-primed seeds of Indian mustard (*B. juncea* L.). The germination value of *A. nilotica* is presented in Table 4.4. Germination value was significantly influenced by planting seasons and different chemical concentrations as well. Significantly more germination value was recorded in rainy season (10.41) than autumn season (3.50) irrespective of chemical concentrations. The percentage (%) change recorded in germination value between rainy and autumn season 66.37. The seed priming with different chemical concentrations also influenced germination value. The maximum germination

value was observed for KCl 10 Mmole (11.05), which was statistically at par with KCl 15 Mmole, CaCl₂ 5 Mmole, CaCl₂ 15 Mmole and minimum for NaCl 5 Mmole irrespective of seasons. The maximum germination value was recorded in KCl 10 Mmole (17.83), whereas minimum was recorded for NaCl 15 Mmole (4.81) during rainy season. During autumn season, the maximum germination value was observed for CaCl₂ 15 Mmole (5.26), while minimum was for NaCl 5 Mmole (1.13).

Germination value can be defined as product of peak germination value and mean daily germination. Since, both peak germination value and mean daily germination are higher in seed treatment with CaCl₂ and KCl while lower in seed priming with NaCl thus lower germination value was recorded in NaCl as compared to hydro priming and seed priming with NaCl and CaCl₂. The higher uptake of water in primed seed increases the germination rate and germination percentage. During seed priming expansion of embryo takes place and compresses the endosperm which results in hydrolytic activities in the cell. Due to hydrolytic activities the formation of tissue takes place upon dehydration. It produces empty space and facilitate radical emergence. The higher seed germination due to seed primed with KCl and CaCl₂ might be due non toxic effect of K and Ca ion accumulation in embryo. Beneficially effect CaCl₂ may be due to its role in cell membrane integrity. Nutrient solution application of K decreases the ABA concentration due to reduced ABA levels germination is enhanced. The mechanism is inhibition of germination and seedling growth by NaCl might be due to low absorption of water or toxicity of NaCl on the embryo which may reduce germination percentage or delay emergence and further growth of plant. Results are in accordance with the findings of Palani et al (1995) who reported that significantly higher germination in primed seeds as compared to unprimed seeds of *A. nilotica*.

Germination energy was significantly influenced by sowing seasons and different chemical concentrations as well. Significantly higher germination energy was recorded in rainy season (0.53) than autumn season (0.39) irrespective to chemical concentrations. The percentage (%) change recorded in germination energy between rainy and autumn season was 26.41. It was found that seed priming with different chemical concentrations also influenced germination energy. The maximum germination energy was observed for potassium chloride 15 Mmole (0.60) which was statistically at par with KCl 5 Mmole, KCl 10 Mmole, calcium chloride 5 Mmole, CaCl₂ 10 Mmole, CaCl₂ 15 Mmole and minimum for NaCl 5 Mmole irrespective to seasons. The maximum germination energy was recorded in KCl 5 Mmole, KCl 10 Mmole, potassium chloride 15 Mmole (0.64), whereas

Table 1. Effect of seed priming treatments on germination percentage (%), peak germination and mean daily germination, germination value and germination energy of *Acacia nilotica* in seeds sown in nursery

Treatments	Germination percentage (%)			Germination value			Germination peak value			Mean daily germination			Germination energy		
	Summer	Autumn	Mean	Summer	Autumn	Mean	Summer	Autumn	Mean	Summer	Autumn	Mean	Summer	Autumn	Mean
KCl 5 Mmole	82.1	51.9	67.0	11.19	3.71	7.45	2.07	1.23	1.65	5.32	3.00	4.16	0.64	0.38	0.51
KCl 10 Mmole	87.9	54.0	71.0	17.83	4.26	11.05	2.83	1.36	2.10	6.31	3.13	4.72	0.64	0.32	0.48
KCl 15 Mmole	83.9	57.9	70.9	13.14	4.99	9.07	2.36	1.43	1.89	5.33	3.49	4.41	0.64	0.56	0.60
NaCl 5 Mmole	67.2	34.8	51.0	4.88	1.13	3.00	1.33	0.68	1.01	3.61	1.60	2.61	0.44	0.18	0.31
NaCl 10 Mmole	71.8	37.9	54.9	6.20	1.28	3.74	1.60	0.72	1.16	3.76	1.78	2.77	0.34	0.17	0.25
NaCl 15 Mmole	66.0	40.5	53.2	4.81	1.38	3.09	1.38	0.74	1.06	3.43	1.87	2.65	0.46	0.24	0.35
CaCl ₂ 5 Mmole	76.4	53.9	65.2	12.18	4.56	8.37	2.51	1.38	1.94	4.83	3.26	4.04	0.56	0.52	0.54
CaCl ₂ 10 Mmole	78.0	57.9	68.0	9.27	5.21	7.24	1.95	1.50	1.73	4.68	3.46	4.07	0.60	0.57	0.58
CaCl ₂ 15 Mmole	75.9	57.9	66.9	12.39	5.26	8.83	2.33	1.57	1.95	5.20	3.35	4.27	0.56	0.55	0.55
Control	83.8	49.9	66.9	12.20	3.19	7.70	2.52	1.16	1.84	4.83	2.65	3.74	0.39	0.44	0.42
Average	77.3	49.7		10.41	3.50		2.09	1.18		4.73	2.76		0.53	0.39	
CD (p=0.05) Season		4.584			1.389			0.192			0.284			0.07	
CD (p=0.05) Chemical conc.		10.251			3.105			0.429			0.634			0.16	
CD (p=0.05) Interaction		NS			NS			NS			NS			NS	

minimum was recorded for NaCl 10 Mmole (0.34) during rainy season. However, during autumn season, the maximum germination energy was observed for CaCl₂ 10 Mmole (0.56), while minimum was for sodium chloride 10 Mmole (0.16). The germination energy depends upon germination rate and germination percentage. It indicates synchronization in germination process. Higher germination energy in CaCl₂ and KCl seed priming treatments might be attributed to better germination parameters germination percentage germination peak value, mean daily germination, germination value and germination energy than NaCl (Table 1).

CONCLUSION

CaCl₂ plays an important role in cell membrane integrity. Potassium causes reduction the ABA levels and enhances germination. The mechanism is inhibition of germination and seedling growth by NaCl might be due to low absorption of water or toxicity of NaCl on the embryo which may reduce germination percentage or delay emergence and further growth of plant. Our results are in line with Rafiq et al (2006), who reported that pre sowing treatment with primers increased the germination energy.

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Tree Diversity and Ecological Status of *Madhuca latifolia* (Roxb.) J.F. Macbr in Forests of Odisha

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Abstract: The present paper aims to study the tree diversity in *Madhuca latifolia* (Roxb.) J.F. Macbr. (Mahua) dominated forests of Odisha situated in 10 different agroecological zones. A total of 94 tree species belonging to 29 families were recorded. The most dominant family was Fabaceae. The Shannon-Wiener index (H') for all the natural forests studied under different agroclimatic zones ranges from 1.592–2.560 with maximum in AEZ-9 (2.560) and minimum in (AEZs) (1.592). The quantitative features such as density and importance value index of different species varied significantly between the forests in different agroecological zones. The tree density varied from 343 stems ha^{-1} (in AEZ-4-Nayagarh Baispalli-Buguda-Banigochha Forest) to 790 stems ha^{-1} (in AEZ-2-Mayurbhanj Similipal Reserve Forest, Chandbil), and the majority of the tree species were found in intermediate girth class. *M. latifolia* recorded for maximum stem density in AEZ-2 (137.5 stems ha^{-1}) and minimum in AEZ-8 (30 stems ha^{-1}). There was a lot of variation in tree size of this species between the forests and the tree size heterogeneity of this species was clearly related to stand density; the stands with larger trees were less heterogeneous in tree size.

Keywords: Species composition, Stand structure, Association index, *Madhuca latifolia*, Tropical forests

Madhuca latifolia (Roxb.) J.F. Macbr is a species of much ecological and economic importance to the people of Odisha, and therefore this species is subjected to much human pressure for extraction of its flower and seeds which in turn may lead to change in vegetation structure of this species. The data on characteristics, classification, relationship and distribution of plant communities are useful to describe the population dynamics of each species and how they relate to the other species in the same community. The ecological status of a species and its distribution pattern can well be described by studying various ecological attributes and this information are vital for proper planning and management. Quantitative study of vegetation is important in understanding the ecosystem dynamics as it describes the vegetation in terms of floristic composition, diversity and phytomass, explains or predicts its pattern and helps in classifying it in a meaningful way. To determine the biodiversity of an area ecologists have devised a number of formulae, indices and models to estimate species richness in a community and to explain the distribution and abundance of the species (Kunin et al 2000). Simpson (1949) successfully formulated an index known as Simpson's index of dominance. The value of Simpson's index increases as the index of general diversity decreases. The most widely accepted tool for measuring diversity is the Shannon and Weiner's (1949) index commonly known as the Shannon's index of general diversity. Trees are important component of vegetation and therefore their diversity must be constantly monitored to for maintaining species and habitat diversity (Attua and Pabi 2013). Stand

tree density has an important implication not only on stand growth and yield but also on the forest health and subsequently on forest ecosystems (Wassihum et al 2019, Zhang et al 2019). Stand density index is often calculated to relate to the tree-size heterogeneity to stand heterogeneity (Zeller et al 2018). Several studies have been carried out in on plant diversity in tropical deciduous forests of Eastern Ghats (Reddy et al 2011, Panda et al 2013) wet evergreen forests of Western Ghats (Parthasarathy 2001), Andaman and Nicobar islands (Rasingam and Parthasarathy 2009). However, there are limited studies on different forest reserves of Odisha with special reference to the stands that are dominated with Mahua trees. The objectives of the paper are to study the role of disturbance/human pressure in vegetation structure and to assess tree species diversity and composition in different forest under 10 agroecological zones of Odisha, with special reference of Mahua tree.

MATERIAL AND METHODS

Study area: The study was conducted in forests located in 10 agroecological zones (AEZs) of Odisha (Fig. 1). These were AEZ-1- Sundargarh (Ramkata Reserve Forest), AEZ-2- Mayurbhanj (Similipal Reserve Forest, Chandbil), AEZ-3- Balasore (Tinkosia Naranpur Gobipalla Forest), AEZ-4- Nayagarh (Baispalli-Buguda-Banigochha Forest), AEZ-5- Kandhamal (Siringi Reserve Forest, Balliguda), AEZ-6- Nabarangpur (Ranigarh-Papadahandi-Mahendra Forest Area), AEZ-7- Malkangiri (Haldikund-Ramgiri Forest Area), AEZ-8- Nuapada (Sunabeda Wildlife Reserve), AEZ-9-

Bolangir (Khaprakhol-Harishankar Reserve Forest), AEZ-10-Angul (Bambro forest Area). In this study of tree associates in natural forests and the ecological status of *M. latifolia* was studied.

Tree vegetation sampling: For studying twenty five random quadrats/plots each measuring 20 m x 20 m were selected during 2016-17. All the trees having ≥ 30 cm and girth at breast height (gbh- above 137 cm from the ground) that were present in the quadrats/plots were identified, recorded and evaluated for the phytosociological attributes. The area of each sampled plots were measured using a tape along the boundary and permanent study plots were laid within and marked with paint for reference. Geographical co-ordinates at the centre of these plots were taken using a hand held GPS. All trees present in each sampled plot were identified and recorded by their botanical name, or by local name and later confirmed from published books. Flora of Presidency of Madras (Gamble and Fischer 1915-1935) and Flora of Orissa (Saxena and Brahmam 1990) were consulted. The stand structures were measured in terms of species composition, frequency, basal area, importance value index and association index.

Tree species composition, diversity and community indices: Data recorded in 2016 were pooled by different plot categories to estimate density (D), frequency (F), abundance (A), total basal area as dominance, and the relative values respectively (Misra 1968, Muller-Dombois and Ellenberg 1974). Importance value index (IVI) was calculated as sum of relative values of density (RD), frequency (RF), and dominance (RD) (Curtis, 1959). Tree species diversity index, H' ; Concentration of dominance of trees, Cd ; Tree species richness index, SRI and evenness index, E was calculated using the formula as given in the references below:
Shannon–Weiner diversity index, H' (Shannon and Weiner 1963):

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

Where, H' is the Shannon–Weiner diversity index, p_i is the proportion of individuals in the i^{th} species i.e. (n_i/N).

Simpson Index of Dominance, Cd (Simpson 1949):

$$Cd = - \sum_{i=1}^n p_i^2$$

Where, p_i = proportion of individual in the i^{th} species.

Marglef richness index, SRI (Marglef 1958):

$$SRI = \frac{S-1}{\ln(N)}$$

Where, S is the total number of species, N the number of individuals

Evenness index, E (Pielou 1969):

$$E = \frac{H'}{\ln S}$$

Where, H' is the Shannon–Weiner diversity index, S is the total number of species. Sorensen's similarity index (Sorensen 1948):

$$S = \frac{2C}{A+B}$$

Where, S = Sorensen's similarity coefficient, A = Number of species present in sample A, B = Number of species present in sample B, C = Number of species present in both samples

Dispersion pattern of a species is given by its abundance to frequency ratio (A/F) and the ranges of values for determining dispersion pattern are presented by Cottam and Curtis (1956) as regular (< 0.025), random ($0.025 - 0.05$) and contiguous (> 0.05).

Association Index: The inter-specific association was evaluated by association index which helps in determining the main associates of a important species in the forest. It was estimated by the equation

$$\text{Association Index of a Species (A)} = \frac{\text{Total no. of quadrants in which species a occurs along with another species (B) in the stand}}{\text{Total no. of quadrant in which species a occurs in that stand}} \times 100$$

Each tree species recorded in the forest plots in different AEZs were classified by family and genera.

RESULTS AND DISCUSSION

Taxonomic diversity and species richness: Tree species richness in forests of different AEZs varied to a great extent. The forests of AEZ-8 (Nuapada) had maximum number of tree species (29) followed by AEZ-6 (Nabarangpur) 26 species, AEZ-9 (Bolangir) and AEZ-10 (Bambro forest under Angul) 23 each, AEZ-2 (Mayurbhanj) 22 species, AEZ-5 (Kandhamal) 20 species, AEZ-1 (Sundargarh) 19 species, AEZ-4 (Nayagarh) 18 species, AEZ-3 (Balasore) 17 species and the lowest 15 species in the forests under AEZ-7 (Malkangiri) (Table 1). The family-wise distribution of tree species in different AEZs is given in Table 2. The 54 tree species belonging to 29 families were encountered from the forests of different AEZs. Fabaceae was the most dominant family contributing 9 species, followed by Rubiaceae which contributed 6 species. Combretaceae, Moraceae, Anacardiaceae each contributed 4 species while Phyllanthaceae, Meliaceae; Myrtaceae was represented by 2 species each. The other families which were represented in the tree flora included Rubiaceae, Mimosoideae, Bombacaceae, Lecythidaceae, Arecaceae, ceasalpinaceae, Poaceae, Burseraceae, Boraginaceae, Detariodeae, Apocynaceae, Lythraceae, Sapotaceae, Euphorbiaceae, Annonaceae, Oleaceae, Sapindaceae, Dipterocarpaceae,

Table 1. Phytosociological and diversity attributes of tree species in the forest stands in the study area

Parameters	AEZ-1	AEZ-2	AEZ-3	AEZ-4	AEZ-5	AEZ-6	AEZ-7	AEZ-8	AEZ-9	AEZ-10
No. of species	19	22	17	18	20	26	15	29	23	23
No. of families	14	13	11	13	11	19	11	17	14	17
Density (individuals ha ⁻¹)	425	790	710	343	460	740	480	663	478	415
Basal area (m ² ha ⁻¹)	44.12	107.04	93.46	33.14	26.54	87.40	100.48	37.19	77.39	36.67
Shannon Diversity Index (H')	2.2713	2.0760	1.6846	2.3009	1.5923	2.0760	2.0306	2.5254	2.5604	1.8035
Pielou Evenness Index (J')	0.771	0.662	0.595	0.768	0.532	0.787	0.750	0.750	0.817	0.567
Simpson Dominance Index (Cd)	0.1520	0.2285	0.2266	0.1691	0.3352	0.1251	0.1892	0.1503	0.1039	0.0343
Margalef Species Richness Index (D _{mg})	3.664	4.193	2.784	3.862	3.374	4.741	3.067	5.415	4.368	4.984

AEZ-1- Sundargarh (Ramlata Reserve Forest), AEZ-2-Mayurbhanj (Similipal Reserve Forest, Chandbil), AEZ-3-Balasore (Tinkosia Naranpur Gobipalla Forest), AEZ-4-Nayagarh (Baispalli--Buguda-Banigochha Forest), AEZ-5-Kandhamal (Siringi Reserve Forest, Balliguda), AEZ-6-Nabarangpur (Ranigarh-Papadahandi-Mahendra Forest Area), AEZ-7-Malkangiri (Haldikund-Ramgiri Forest Area), AEZ-8-Nuapada (Sunabeda Wildlife Reserve), AEZ-9-Bolangir (Khaprakhol-Harishankar Reserve Forest), AEZ-10-Angul (Bambro Forest Area)

Table 2. Family-wise tree distribution in the forest stands in different AEZs

Family	AEZ-1	AEZ-2	AEZ-3	AEZ-4	AEZ-5	AEZ-6	AEZ-7	AEZ-8	AEZ-9	AEZ-10
Anacardiaceae	3	3	3	3	3	3	2	3	3	4
Annonaceae	-	-	-	-	-	1	-	-	-	-
Apocynaceae	1	-	-	-	-	1	-	1	-	1
Arecaceae	-	-	-	-	1	-	-	-	-	-
Bignoniaceae	-	-	-	-	-	-	1	-	-	-
Bombacaceae	1	1	-	-	-	1	-	-	-	1
Boraginaceae	-	-	-	1	-	1	-	1	1	1
Burseraceae	-	1	-	-	-	-	-	-	-	-
Caesalpiniaceae	1	-	-	-	1	1	-	1	1	1
Combretaceae	2	5	4	2	4	4	2	4	4	4
Detarioideae	-	-	-	-	-	1	-	1	-	-
Dipterocarpaceae	1	1	1	1	1	1	1	-	-	1
Ebenaceae	1	1	1	1	-	1	1	-	1	1
Euphorbiaceae	1	-	-	-	-	-	-	-	-	-
Fabaceae	1	3	1	2	2	2	2	6	4	1
Lecythidaceae	1	-	1	-	-	1	-	1	1	-
Lythraceae	1	-	-	1	-	1	1	1	1	1
Meliaceae	-	-	-	-	1	-	-	1	-	1
Mimosoideae	-	-	1	1	-	-	-	-	-	-
Moraceae	-	-	-	-	4	1	-	-	-	-
Myrtaceae	-	1	1	1	1	2	2	-	-	1
Oleaceae	-	-	-	-	-	-	-	1	-	1
Phyllanthaceae	2	2	1	2	-	1	-	1	2	2
Poaceae	-	-	-	-	1	-	-	1	-	1
Rhamnaceae	-	-	-	-	-	-	-	-	1	-
Rubiaceae	-	2	2	3	-	1	2	3	1	1
Rutaceae	1	1	-	-	-	-	-	1	1	-
Sapindaceae	-	1	-	1	-	1	1	1	1	-
Sapotaceae	1	1	1	1	1	1	1	1	1	1

Key as in Table 1

Bignoniaceae, Ebenaceae and Rhamnaceae, each of which contributed one species each. The number of families contributing to tree diversity also varied widely among the forests of AEZs. Anacardiaceae was the most dominating family followed by Combetaceae, Fabaceae, Phyllanthaceae and Ruubiaceae in contributing to tree taxonomic diversity in the study area.

Ecological diversity and association index: The Shannon diversity index of trees varied from 1.684 in forests under AEZ-3 to 2.5604 in AEZ-9. Pileu's evenness index varied from 0.532 (AEZ-05) to 0.817 (AEZ-9). Simpson's dominance index varied from 0.03425 (AEZ-10) to 0.33516 (AEZ-5) and Margalef species richness varied from 2.784 (AEZ-3) to 5.415 (AEZ-8). The Shannon-Wiener index (H') for all the natural forests studied under different agroclimatic zones ranges from 1.592–2.560 with maximum in AEZ-9 (2.560) and minimum in AEZ-5 (1.592). These values indicate that the present dry deciduous and moist deciduous forests studied are having species diverse system. The diversity values for Indian forests were reported by many researchers in the range of 0.83 to 4.1 (Parthasarthy et al 1992, Visalakshi 1995, Rasingam and Parthasarathy 2009, Shukla 2009). Thus, the diversity values of tree species found in this present study is well within the earlier reported range of Indian tropical forests. However, the values do not figure closer to the higher side of the reported range (4.1) which may be due to the higher anthropogenic interferences in these forests for collections of mahua flowers, seeds, other NTFPs and fuel wood directly or indirectly affecting soil erosions, soil moisture, plant growth and regeneration of species. The Simpson's index in the present study was lower but within the range reported in other forests (Visalakshi 1995, Lalfakawma et al 2009).

Similarity: Sorensen's index of qualitative similarity of trees indicated maximum similarity (60.61) between the forest

stands under AEZ-4 and AEZ-7, followed by AEZ-6 and AEZ-7 (53.66) and AEZ-3 and AEZ-4 (51.43) and AEZ-4 and AEZ-6 (50.0) and AEZ-3 and AEZ-7 (Table 3). The least similarity in tree species was found between AEZ-5 and AEZ-9 (18.60).

Associate index and spatial distribution pattern: The association index of species for *M. indica* varied widely (Table 4) and the most important associates were *S. robusta*, *T. bellerica*, *B. lanzana* and *L. parviflora*. Majority of the tree species showed clumped or contiguous distribution. This was true in all AEZs studied except AEZ-7 (Malkangiri) and AEZ-9 (Bolangir) forests (Table 5). Regular distribution pattern was observed in very few trees growing in the AEZs. The tree species which showed regular distribution in AEZs included *M. latifolia*, *T. bellerica* and *L. parviflora* in AEZ-1, *T. tomentosa* in AEZ-2, *S. cumuni* in AEZ-3, *M. latifolia*, *S. cumuni* and *T. tomentosa* in AEZ-4, *M. indica* and *S. anacardium* in AEZ-5, *S. cumuni*, *M. latifolia* and *F. religiosa* in AEZ-6, *M. latifolia*, *P. marsupium* and *D. melanoxylon* in AEZ-7, *D. strictus* and *M. latifolia* in AEZ-8, *D. paniculata* and *M. latifolia* in AEZ-9 and *B. lanzan*, *B. retusa* and *M. latifolia* in AEZ-10. The per cent of tree species showing contiguous or clumped distribution in different AEZs were AEZ-2 (73%) > AEZ-5 (70%) > AEZ-1 (68%) > AEZ-7 (67%) > AEZ-8 (66%) > AEZ-6 (62%) > AEZ-10 (61%) > AEZ-4 (44%). Higher number of tree species, however, was observed with random distribution in AEZ-7 (67%) and AEZ-9 (57%). *M. latifolia* showed random dispersal pattern in most of the agroclimatic zones and contiguous dispersal pattern in few agroclimatic zones. This may be due to the degraded edaphic conditions of the forest created by anthropogenic activities which donot favour the growth and establishment of the mahua seedlings and saplings though the species is has a a very high germination rate. In most of the agroclimatic zone, *S. robusta*, *M. latifolia* and *B.lanzan* showed higher IVI values. The tree species with higher IVI values indicate good

Table 3. Sorensen's similarity index of trees between forest stands under different AEZs

	AEZ-1	AEZ-2	AEZ-3	AEZ-4	AEZ-5	AEZ-6	AEZ-7	AEZ-8	AEZ-9	AEZ-10
AEZ-1	100.00	29.27	38.89	27.03	25.64	35.56	35.29	29.17	33.33	47.62
AEZ-2		100.00	46.15	45.0	38.10	41.67	48.65	31.37	35.56	40.0
AEZ-3			100.00	51.43	43.24	41.86	50.0	30.43	40.00	45.00
AEZ-4				100.00	31.50	50.0	60.61	33.33	43.90	43.90
AEZ-5					100.00	34.78	40.00	24.49	18.60	37.21
AEZ-6						100.00	53.66	43.64	40.82	44.90
AEZ-7							100.00	31.82	42.11	42.11
AEZ-8								100.00	38.46	46.15
AEZ-9									100.00	47.83
AEZ-10										100.00

Key as in Table 1

Table 4. Association index (AI) of tree species in *M. latifolia* stands in the forests of different agroecological zones (AEZs) studied

Tree species	Family	Species code	AEZ-1	AEZ-2	AEZ-3	AEZ-4	AEZ-5	AEZ-6	AEZ-7	AEZ-8	AEZ-9	AEZ-10
<i>Lagerstroemia parviflora</i> Roxb.	Lythraceae	LAP	0.88	-	-	0.10	-	0.11	0.80	0.30	0.30	0.33
<i>Lannea coromandelica</i> (Houtt.) Merr	Anacardiaceae	LAC	0.13	0.16	0.36	0.20	-	0.22	-	0.20	0.40	0.33
<i>Madhuca Latifolia</i> (Roxb.) J. F. Macbr,	Sapotaceae	MAL	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>Mallotus philippines</i> (Lamarck) Müller	Euphorbiaceae	MAP	0.13	-	-	-	-	-	-	-	-	-
<i>Mangifera indica</i> L.	Anacardiaceae	MAI	-	0.16	-	-	0.60	-	-	-	-	0.16
<i>Milusa tomentosa</i> (Roxb.) J. Sinclair	Annonaceae	MIT	-	-	-	-	-	0.33	-	-	-	-
<i>Mitragyna parviflora</i> (Roxb.) Korth	Rubiaceae	MIP	-	-	-	-	-	-	-	-	0.10	0.16
<i>Morinda tinctoria</i> Roxb.	Rubiaceae	MOT	-	-	0.09	0.10	-	-	-	-	-	-
<i>Nyctanthus arborescens</i> L.	Oleaceae	NYA	-	-	-	-	-	-	-	0.10	-	0.33
<i>Pterocarpus marsupium</i> Roxb.	Fabaceae	PTM	-	0.33	0.09	0.10	0.06	0.44	0.40	0.30	0.30	0.33
<i>Schleichera oleosa</i> (Lour.) Oken	Sapindaceae	SCO	-	0.33	-	0.40	-	0.33	0.20	0.80	0.30	-
<i>Semecarpus anacardium</i> Linn.	Anacardiaceae	SEA	0.13	-	0.09	0.30	0.46	0.11	0.20	0.10	0.30	0.16
<i>Shorea robusta</i> Gaertn	Dipterocarpaceae	SHR	0.88	0.66	1.00	1.00	0.80	0.55	0.80	0.10	-	0.83
<i>Soymida febrifuga</i> (Roxb.) Juss	Meliaceae	SOF	-	-	-	-	-	-	-	-	-	0.16
<i>Stereospermum suaveolens</i> DC.(Syn. S. chelonoides)	Bignoniaceae	STS	-	-	-	-	-	-	0.20	-	-	-
<i>Syzigium cerasoides</i> (Roxb.) Raiz.	Myrtaceae	SYC	-	-	-	-	-	0.22	0.80	-	-	0.16
<i>Syzigium cumuni</i> L. Skeels.	Myrtaceae	SYCU	-	0.33	0.54	0.50	0.06	0.88	0.80	-	-	-
<i>Tamarindus indica</i> L.	Fabaceae	TAI	-	-	-	-	0.06	-	-	-	-	-
<i>Terminalia bellerica</i> (Gaertn.) Roxb.	Combretaceae	TEB	0.63	0.16	0.36	-	0.20	0.11	0.20	0.10	0.30	0.66
<i>Terminalia chebula</i> Retz.	Combretaceae	TEC	0.38	0.33	0.09	-	0.26	0.33	-	0.10	0.10	0.16
<i>Terminalia tomentosa</i> (Roxb.) Wight & Arn.	Combretaceae	TET	-	0.66	0.72	0.50	0.13	0.55	0.20	0.60	0.30	0.16
<i>Toona ciliata</i> M. Roem		TOC	-	-	-	-	0.06	-	-	-	-	-
<i>Zizyphus mauritiana</i> (L.) Gaertn.	Rhamnaceae	ZIM	-	-	-	-	-	-	-	-	0.50	-

Key as in Table 1.

Table 5. Density (D) and importance value index (IVI) of tree species in the forests of different agroecological zones (AEZs) studied

Species	AEZ-1		AEZ-2		AEZ-3		AEZ-4		AEZ-5		AEZ-6		AEZ-7		AEZ-8		AEZ-9		AEZ-10	
	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI
ACL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.50	2.07	-	-
ACP	-	-	4.0	2.89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ADC	-	-	8.25	4.07	2.25	1.78	27.50	16.79	-	-	2.75	4.33	5.0	4.04	10.00	7.23	-	-	-	-
AGM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.50	9.59	-	-
ALP	-	-	-	-	2.25	2.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANL	3.12	3.05	4.0	2.78	11.25	6.99	5.0	5.12	3.25	2.14	13.75	6.05	-	-	10.00	7.23	12.50	8.84	4.0	2.50
ARH	-	-	-	-	-	-	-	-	3.25	3.10	-	-	-	-	-	-	-	-	-	-
BAR	6.25	5.03					5.0	3.74							2.50	2.27				
BOC	3.12	3.05	4.0	3.72	-	-	-	-	-	-	2.75	3.26	-	-	-	-	-	-	4.00	5.05
BRR	3.12	2.84	4.0	2.95	2.25	1.80	10.0	12.55	-	-	2.75	1.98	-	-	-	-	10.00	8.33	20.75	20.59
BUL	50.0	27.93	70.75	22.61	145.25	45.90	35.0	20.86	18.25	11.17	52.75	19.43	5.0	4.03	45.00	25.48	70.0	34.25	50.0	20.14
BUM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.00	2.87	70.00	34.25	-	-
CAA	6.25	3.71	-	-	6.75	5.98	-	-	-	-	13.75	12.0	-	-	2.50	2.27	5.0	4.17	-	-
CAU	-	-	-	-	-	-	-	-	6.5	4.81	-	-	-	-	-	-	-	-	-	-
CAF	3.12	2.83	4.0	2.78	-	-	-	-	5.0	3.90	2.75	1.90	-	-	10.00	6.63	2.50	2.04	4.0	2.67
CLS	3.12	2.89	12.50	3.98	-	-	-	-	-	-	-	-	-	-	25.00	11.42	-	-	-	-
CLC	46.87	16.80	29.0	10.51	-	-	-	-	-	-	-	-	-	-	150.00	52.63	32.50	15.96	20.75	8.09
DAL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.50	6.73	-	-	-	-
DAP	-	-	4.0	3.11	-	-	-	-	-	-	2.75	2.15	-	-	45.00	25.48	60.00	71.22	-	-
DES	-	-	-	-	-	-	-	-	1.5	5.11	-	-	-	-	30.00	78.55	-	-	8.25	10.24
DIM	21.87	12.10	37.50	4.73	2.25	1.78	7.50	5.84	-	-	19.25	12.70	25.0	15.05	-	-	32.50	32.30	20.75	8.33
ERS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.50	2.27	-	-	-	-
FIB	-	-	-	-	-	-	-	-	5.0	5.50	-	-	-	-	-	-	-	-	-	-
FIG	-	-	-	-	-	-	-	-	1.5	7.54	-	-	-	-	-	-	-	-	-	-
FIR	-	-	-	-	-	-	-	-	1.5	6.84	44.25	30.64	-	-	-	-	-	-	-	-
GAL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25.00	11.42	-	-	-	-
GAP	-	-	8.25	3.99	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GRT	-	-	-	-	-	-	2.50	2.34	-	-	2.75	2.15	-	-	2.50	2.17	2.50	2.13	4.0	2.77
HAB	-	-	-	-	-	-	-	-	-	-	2.75	1.93	-	-	10.00	6.63	-	-	-	-
HOA	3.12	3.14	-	-	-	-	-	-	-	-	155.50	92.03	-	-	5.00	2.87	-	-	4.0	3.17
IXR	-	-	37.50	9.89	-	-	2.50	2.29	-	-	19.25	10.56	5.0	4.0	2.50	5.11	-	-	-	-
																				Cont...

Cont...

Table 5. Density (D) and importance value index (IVI) of tree species in the forests of different agroecological zones (AEZs) studied

Species	AEZ-1			AEZ-2			AEZ-3			AEZ-4			AEZ-5			AEZ-6			AEZ-7			AEZ-8			AEZ-9			AEZ-10		
	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI	D	IVI
LAP	28.12	22.16	-	-	-	-	2.34	-	2.50	2.34	-	2.75	1.93	65.0	24.48	10.00	7.08	7.50	6.21	16.50	6.69									
LAC	6.25	4.54	4.0	3.02	13.50	9.04	10.0	6.69	-	-	-	8.25	4.51	-	-	12.50	6.73	12.50	9.59	12.50	7.37									
MAL	56.25	54.77	137.50	76.74	127.25	49.29	40.0	34.80	123.25	84.27	35.0	30.80	30.64	35.0	30.80	30.00	78.55	60.0	71.22	37.50	28.25									
MAP	3.12	3.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-									
MAI	-	-	4.0	4.61	-	-	-	21.50	29.11	-	-	-	-	-	-	-	-	-	-	8.25	6.50									
MIT	-	-	-	-	-	-	-	-	-	-	13.75	7.27	-	-	-	-	-	-	-	-	-									
MIP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.50	2.13	8.25	5.15									
MOT	-	-	-	-	2.25	1.89	2.50	2.34	-	-	-	-	-	-	-	-	-	-	-	-	-									
NYA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.50	2.51	-	-	12.50	7.73									
PTM	-	-	8.25	5.62	2.25	1.89	2.50	2.46	1.5	1.87	-	-	-	10.00	9.11	10.00	7.08	7.50	6.86	16.50	14.07									
SCO	-	-	12.50	7.73	-	-	17.50	20.35	-	-	11.00	6.82	5.0	4.65	150.00	52.63	7.50	6.21	-	-	-									
SEA	9.37	4.98	-	-	2.25	1.81	7.50	7.81	15.0	14.11	5.50	2.81	5.0	4.08	7.50	3.74	12.50	7.61	4.0	5.64										
SHR	131.25	96.04	337.50	77.95	306.75	125.89	1222.50	116.80	236.50	87.02	155.50	92.03	170.0	128.89	-	-	-	-	112.50	58.23										
SOF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.50	2.27	-	-	4.0	3.02									
STS	-	-	-	-	-	-	-	-	-	-	-	-	-	5.0	4.23	-	-	-	-	-	-									
SYC	-	-	-	-	-	-	-	-	-	-	33.25	10.50	75.0	31.29	-	-	-	-	-	-	-									
SYCU	-	-	16.50	7.37	13.50	11.11	12.50	12.47	1.50	1.79	58.25	28.88	50.00	23.47	-	-	-	-	-	-	-									
TAI	-	-	-	-	-	-	-	-	1.5	2.58	-	-	-	-	-	-	-	-	-	-	-									
TEB	21.87	19.21	8.25	4.43	18.0	9.19	-	-	5.0	5.61	2.75	4.33	5.0	5.0	2.50	2.51	7.5	6.86	25.0	17.39										
TEC	18.75	11.55	8.25	5.81	2.25	1.83	-	-	6.50	7.36	13.75	7.27	-	-	2.50	5.11	2.50	2.07	4.0	2.67										
TET	-	-	25.0	19.85	50.0	21.52	17.50	13.90	3.25	4.30	52.75	19.43	15.0	6.83	37.50	22.98	12.50	8.84	4.0	3.02										
TOC	-	-	-	-	-	-	-	-	1.5	1.80	-	-	-	-	-	-	-	-	-	-	-									
ZIM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32.50	15.96	-	-									

Key as in Table 1, Species code as in Table 4

regenerating capability, more adaptability and wide ecological amplitude. In the present study *S. robusta*, *M. latifolia* and *B. lanzan* showed wider adaptability and good regenerating ability in different forests under various agroclimatic zones. The IVI of *M. latifolia* was in the order of AEZ-5 (84.27) > AEZ-8 (78.55) > AEZ-2 > (76.74) > AEZ-9 (71.22) > AEZ-1 (54.77) which indicates its adaptability, regenerating ability and establishment in the respective forests.

Density: The density of tree species varied widely between and within the forest stands across the AEZs (Table 4). In AEZ-5, *Shorea robusta* had highest density (131.25 individuals ha^{-1}), followed by *M. latifolia* (*B. lanzan*, *C. collinus* (*C. swietenia*) had lower number of individuals ha^{-1} . *S. robusta* invariably, was the most dominant tree species in majority of the AEZs. In addition the other most common and notable species in terms of their tree density, in the study area were *M. latifolia*, *L. parviflora*, *C. collinus*, *L. coromandelica*, *T. bellerica*, *T. chebula* and *S. anacardium*. Some tree species had a very restricted distribution, *A. heterophyllum* and *F. bengalensis* were found only in AEZ-5 (Kandhamal forest areas). Similarly, *M. tomentosa* was restricted to AEZ-6 (Nabarangpur forests), *N. arbortristis* to AEZ-10 (Bamro forests under Angul), *S. suaveolens* to AEZ-6 (Nabarangpur forests) and *Z. mauritiana* to AEZ-9 (Bolangir forests). Under AEZ-3 *S. robusta* had maximum tree density (306.75 ha^{-1}), followed by *. lanzan* (), *M. latifolia* (), *T. tomentosa* (1), where as the minimum density (2.25 ha^{-1}) were recorded for *T. chebula*, *D. melanoxylon*, *B. retusa*, *B. ceiba*, *C. fistula*, *S. anacardium*, *P. marsupium*, *A. cordifolia*, *A. procera* and *M. tinctoria*. In AEZ-4, also *S. robusta* had maximum density (1) followed by *M. latifolia* (1), *B. lanzan* (1). In AEZ-5 *S. robusta* (236.50 ha^{-1}) and *M. latifolia* (123.25 ha^{-1}), showed higher tree density. In AEZ-6, the maximum density of *S. robusta* and *H. antidysenterica* (155.5 ha^{-1}) followed by *S. cumini* (1), *Buchnanian lanzan* and *Terminalia tomentosa* (1), *M. latifolia* and *F. Religiosa*. The minimum density (2.75 ha^{-1}) was of *T. bellerica*, *L. parviflora*, *B. ceiba*, *C. fistula*, *B. retusa*, *G. tilifolia*, *D. panniculata*, *A. cordifolia* and *H. binnata*. In AEZ-7, 15 major tree species were recorded with maximum density, under AEZ- maximum density (150 ha^{-1}) was of *C. collinus* and *S. oleosa* and in case of AEZ-9, maximum density for *B. lanzan* and *monosperma* (70 ha^{-1}) were observed. *S. robusta* (112.5 ha^{-1}) was the most dominant species in AEZ-10,

The density of trees (>30 cm dbh) in different forests under various AEZs varied from 342.5 to 789.5 stems ha^{-1} with maximum number encountered in AEZ-2 and minimum in AEZ-4. Reddy et al (2011) reported 527-665 stems ha^{-1} in tree diversity study in tropical forest of Similipal Biosphere Reserve, Odisha, whereas, Sahu et al (2010) observed s

density as 671 ha^{-1} in Site-I and 565 ha^{-1} in Site-II as the arboreal taxa diversity of tropical forests of Gandhamardan Hill Range in Odisha. In tropical forests of south Western Ghats density of trees in various tropical evergreen forests ranged from 852 to 965 stems ha^{-1} in the medium elevation of forests of Kalakad (Parthasarathy 2001) 435 to 767 trees ha^{-1} in Eastern Ghats of Andhra Pradesh (Naidu and Kumar 2016). The range of density of tree species in our study falls within the range of 276-905 stems/ha reported for trees ≥ 15 cm gbh in other tropical forests (Bhadra et al 2010). The density of tree species reported in other tropical sites across the world also shows similar trend as in Costa Rica from 448 to 617 ha^{-1} (Heaney and Proctor 1990); in Brazil from 420 to 777 ha^{-1} (Campbell et al 1992) and in Malaysia 250 to 500 ha^{-1} (Primack and Hall 1992). Higher stand tree density nevertheless is desirable for optimum forest growth and yield. However, stand density is also important in explaining the degree to which the growing space is available for tree growth (Reyes-Herandez and Comeau 2015) and changes in stand density as a result of tree mortality (owing to various anthropogenic or natural factors) may adversely affect individual tree sizes. *M. latifolia* showed higher density in AEZ-2 (137.5 stems ha^{-1}), AEZ-3 (127.25 stems ha^{-1}) and AEZ-5 (123.25 stems ha^{-1}) and contributes alone 17.41, 17.91 and 26.63% respectively to the total tree density in these AEZs. This indicates these three zones are more favourable for proper establishment, growth and development of *M. latifolia*.

Dominance: In AEZ-1, based on the IVI value the most dominant species was *S. robusta* (96.04) followed by *M. latifolia* (54.22) and the lowest IVI was recorded for *C. fistula* (2.83). IVI was highest in AEZ-2 for *S. robusta* (77.95) followed by *M. latifolia* (76.74), and lowest were *A. latifolia* and *C. fistula* (2.78). Highest IVI for *S. robusta* (125.89) was in agroclimatic zone 3 which was followed by *M. latifolia* (49.29), *B. lanzan* (45.9) and lowest was recorded for *D. melanoxylon* and *A. cordifolia* (1.78). For AEZ-4 5 and 6 and in AEZ-7 also the highest was for *S. robusta* (128.87) followed by *S. cerasoides* (31.29) and lowest IVI was for *I. arborea* (4.00). For AEZ-8, the highest IVI was for *M. latifolia* and *D. strictus* (78.55) followed by *C. collinus* and *S. oleosa* (52.63) and lowest was for *E. suberosa* and *G. tilifolia* (2.17). IVI were observed highest for both *M. latifolia* and *D. panniculata* (71.22) in AEZ-9 followed by *B. lanzan* and *B. monosperma*, *D. melanoxylon* (The highest IVI was for *D. robusta* (58.23) and for *D. melanoxylon* (2.5).

Dominance-diversity curve and distribution of basal area in different girth class: The distribution of basal area in the study sites revealed that majority of species were in intermediate girth class barring sites AEZ-8 and AEZ-10 (Fig. 3). In the later two stands, the frequency distribution of tree

size in the forest exhibited somewhat reverse J-shaped (a clear trend in AEZ-10) which revealed that a majority of the tree population in these stands were younger ones. Selection removal of poles and collection of fuelwood, forage and non-timber forest products were observed during the field visit which might have also affected the girth-class distribution of tree species in forests of different AEZs. The contribution of *M. latifolia* to the stand basal area varied widely from as low as 8.06% (in AEZ-4) to as high as 60.01% (in AEZ-6). The other stands where basal area (%) of *M. latifolia* was significantly higher were AEZ-2 (46.7), AEZ-5 (37.04), AEZ-8 (35.36), AEZ-9 (32.85) and AEZ-1 (26.09). Many studies have reported decreasing species richness with increasing girth class of trees in various tropical forests (Panda et al 2013).

Ecological Status of *M. latifolia*: The density of *M. latifolia* in most of the forests under respective agroclimatic zones was next to the density of *S. robusta* except AEZ-6 and 7 where the position of *M. latifolia* is 3rd in decreasing order of density (Table 5). In case of agroclimatic zone 10 with *S. Robusta* at highest density, *M. latifolia* recorded at 5th position. However, the in AEZ-8, maximum density was of *C.*

collinus and *M. latifolia* in 3rd position and in AEZ-9 *B. lanzan* was with highest density followed by *M. latifolia*. Among all the AEZs, *M. latifolia* recorded for maximum density in AEZ-2 (137.5 ha^{-1}) and minimum for AEZ-8 (30 ha^{-1}), however, the relative density was found maximum in case of AEZ-5 (26.52) and minimum for AEZ-10 (4.73). The frequency of occurrence of *M. latifolia* was be 100% in all the forests under respective agroclimatic zones; however, the relative frequency was d maximum in forest under AEZ-5 (and minimum in forest under AEZ-10 ()). Within the agroclimatic zone, abundance was second highest incase of *M. latifolia* next to *S. robusta* in AEZ- 2, 3 and 5. However, among the agroclimatic zones maximum abundance was for *M. latifolia* in agroclimatic zone 2 (5.50) and minimum for agroclimatic zone 8 (1.20). Relative dominance was observed highest incase of *M. latifolia* in AEZ- 5, 8 and 9, however, observed

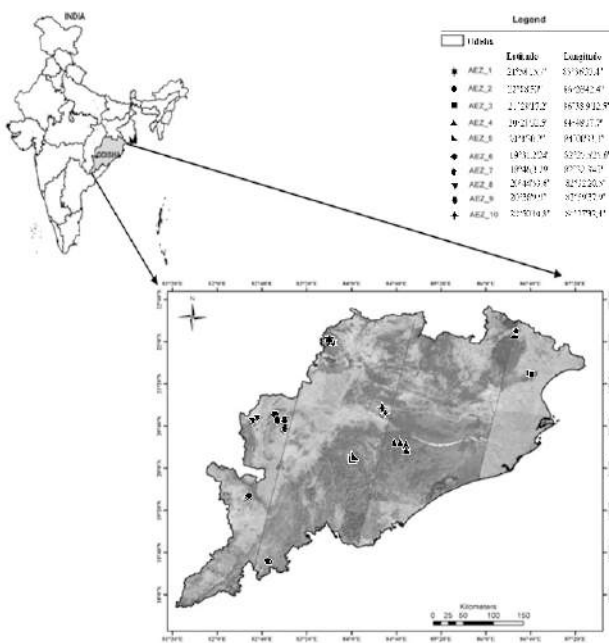
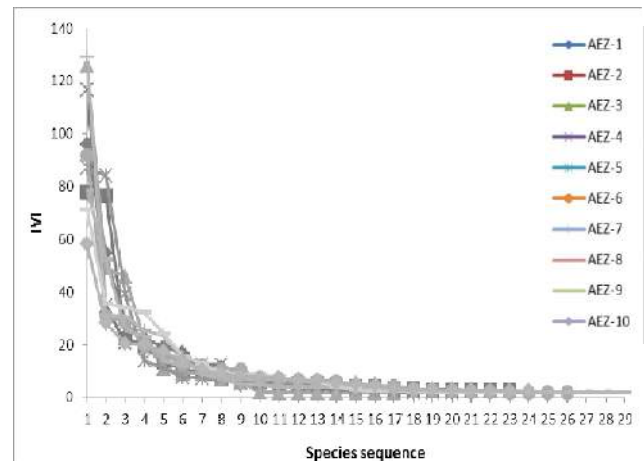
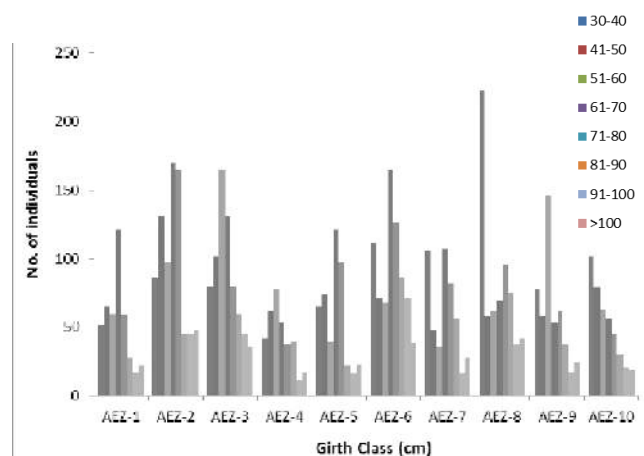


Fig. 1. Map showing the location of study area. AEZ-1- Sundargarh (Ramlata Reserve Forest), AEZ-2-Mayurbhanj (Similipal Reserve Forest, Chandbil), AEZ-3-Balasore (Tinkosia Naranpur Gobipalla Forest), AEZ-4-Nayagarh (Baipalli--Buguda-Banigochha Forest), AEZ-5-Kandhamal (Siringi Reserve Forest, Balliguda), AEZ-6-Nabarangpur (Ranigarh-Papadahandi-Mahendra Forest Area), AEZ-7-Malkangiri (Haldikund-Ramgiri Forest Area), AEZ-8-Nuapada (Sunabeda Wildlife Reserve), AEZ-9-Bolangir (Khaprakhol-Harishankar Reserve Forest), AEZ-10-Angul (Bambro forest Area)



Key as in Figure 1

Fig. 2. Dominance-diversity curve for trees in different forests



Key as in Figure 1

Fig. 3. Density-diameter distribution of tree species in different forest of Odisha

Table 6. Ecological Status of *M. latifolia* in natural forests under different agroclimatic zones of Odisha

Site	Density ha ⁻¹	Relative density	Relative frequency	Abundance of species	Total basal area (cm ² ha ⁻¹)	Relative dominance	Dispersal pattern	IVI
AEZ-1	56.25±3.2	13.23	15.68	2.25	11.51	25.85	0.02	54.77
AEZ-2	137.50±11.7	17.36	12.76	5.50	50.04	46.60	0.05	76.74
AEZ-3	127.25±13.3	17.89	15.94	5.09	14.46	15.46	0.05	49.29
AEZ-4	40.00±3.6	11.67	15.15	1.60	2.67	8.03	0.01	34.8
AEZ-5	123.25±19.5	26.52	20.83	4.93	9.83	36.91	0.04	84.27
AEZ-6	44.25±4.6	8.20	12.32	1.77	5.25	10.11	0.01	30.64
AEZ-7	35.00±3.2	7.29	14.70	1.40	8.76	8.80	0.01	30.80
AEZ-8	30.00±2.6	6.81	13.8	1.20	13.15	57.85	0.01	78.55
AEZ-9	60.00±5.4	15.58	13.69	2.40	25.42	41.94	0.02	71.22
AEZ-10	37.50±4.2	4.73	11.53	1.50	4.40	11.97	0.01	28.25

Key as in Table 1.

second highest for *M. latifolia* after *S. robusta* in agroclimatic zones 1, 2, 3, 6, 7 and 10. Among the AEZs, relative dominance value of *M. latifolia* was recorded maximum for AEZ-8 (57.85), whereas, minimum value was recorded for AEZ-4. Within each agroclimatic zone, Importance Value Index (IVI) was highest for *M. latifolia* under AEZ-8 and 9, whereas, IVI was second highest for *M. latifolia* after *Shorea robusta* under agroclimatic zone 1, 2, 3, 4, 5 and 6. Among the agroclimatic zones, maximum value of IVI for *M. latifolia* was observed in AEZ-5 (84.27) and minimum value of IVI for *Madhuca latifolia* was d for AEZ-10 (28.25). The tree size heterogeneity of this species was clearly related to stand density and the stands with larger trees were less heterogenous in tree size.

CONCLUSIONS

Our results indicate that the forests of Odisha have high tree species richness and diversity (both ecological and taxonomic). The better tree co-existence observed could have been attributed to the differential forest architecture, disturbances and interactions and essential to conservation of biodiversity and ecosystem services. The dominance of *M. latifolia* in most forest ecosystems in the present study further emphasizes its role in the overall forest productivity and carbon services. Further, our results provide baseline information for conservation of biodiversity and management in the state of Odisha.

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Population Structure and Diversity of Trees in Amarkutir, A Tropical Dry Deciduous Forest of West Bengal, India

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Abstract: Present study analyzed the population structure and species diversity of tree layer in Amarkutir, a tropical dry deciduous forest of Sriniketan-Santiniketan lateritic patch in Birbhum district of West Bengal. A total of 295 individuals belonging to 14 species were recorded, out of which 221 individuals had diameter ranging between 1-10 cm indicating that the forest is in under-mature condition. *Acacia auriculiformis* was the dominant tree species and *Shorea robusta* present co-dominantly. All the tree species showed clumped distribution. Shannon's diversity index of the tree species was 2.46. Simpson's index indicated absence of dominance by single or few species. Highest family index value (FIV) was exhibited by Mimosaceae (104.73) followed by Dipterocarpaceae (46.4), whereas Flacourtiaceae (7.62) was the least represented family.

Keywords: Diameter class, Dry deciduous forest, Lateritic patches, Species diversity, Species richness

Tropical forests, which cover even less than 10% of the total land area and harbour nearly 50-90% of the known terrestrial plant and animal species (Seppala et al 2009, FAO 2010), provide most of the forest ecosystem services (MEA 2005). Tropical dry forest constitutes tree communities growing in warm to hot climates with a pronounced seasonality in rainfall and 2–6 months of drought each year during which the ratio of potential evaporation to rainfall (E/R) is greater than one. Waeber et al (2012) observed that dry forests constitute up to 60% of Indian forests, of which about 38.2% are tropical dry deciduous forests (India: State of Forest Report 2009, Blackie et al 2014, Shankar 2001) stressed on the conservation of tropical dry forests in India because they are the most used and threatened ecosystems. Extensive anthropogenic disturbances such as grazing, fuel wood extraction and NTFP collection, result from the livelihood dependency of forest dwelling populations, barring not even protected forests, in most of the developing countries, including India (Hegde and Enters 2000, Pattanayak et al 2003). In the last quarter of the twentieth century, efforts of the international community to sustainably manage forests and increase biodiversity have resulted in net gains in forest area in Europe and Asia and in 13% of the total forest area being brought under formal protection (UNEP 2011). Although the global rate of net forest cover loss has slowed, forest biodiversity loss has continued to occur disproportionately. Sahu et al (2012) reported on importance of studies on population structure, species diversity and distribution pattern of tropical forests in forest management besides its ecological significance. In this study population structure, distribution and species diversity of tree layer in the

selected site was analyzed. It is expected that the results will help in better management of the forest flora and structure by plantation.

MATERIAL AND METHODS

Study area: The study was conducted in Sriniketan-Santiniketan Lateritic patch near Amarkutir located in the Birbhum district of West Bengal, extending between 23°40' N latitude and 87°39' E longitude. It is included within the jurisdiction of Bolpur Range under Birbhum Forest Division of South East circle (Ganguli et al 2016b). This forest is actually the extension part of Ballavpur Wild Life Sanctuary (BWLS) created in between 1953 and 1999 and declared as Ballavpur Wild Life Sanctuary in 1977 (Department of Forests 2008). Average annual rainfall is 1300 mm and temperature ranges from 6°C (minimum) to 45°C (maximum). Winter, summer and rainy seasons are distinctly present in this area.

Vegetation sampling: Ten random quadrats of 10m x 10m each were laid during January to August 2017 for collecting vegetation data. All the trees (dbh ≥ 1cm) were identified and their numbers counted in each quadrat. Diameter at breast height (dbh) or girth at breast height (gbh) of all the tree species was recorded. Regional floras were consulted for species identification of the collected plant specimens (GuhaBakshi 1990, Sanyal 1994). Families were arranged according to *Flora of Bilaspur* by Panigrahi and Murti (1989) and the scientific names of plants were enlisted by following The Plant List (www.theplantlist.org).

Data analysis: For each tree species different phyto-sociological characters like frequency, density, abundance, basal area, distribution pattern and importance value index

(IVI) were calculated following Misra (1968). Relative values of frequency, density and dominance (basal area) were summed up to calculate IVI. For estimating family importance value (FIV), the relative values of diversity, density and dominance of a family were summed up (Ganesh et al 1996). Species dispersion pattern was estimated from the ratio of abundance to frequency (A/F) (Curtis and Cottom 1956). Number of individuals in different diameter classes was determined to study population structure of tree species, starting from 1–10 cm to 40–60 cm diameter classes. Four indices of species diversity were estimated- Shannon – Wiener's Heterogeneity (H') (Shannon and Weaver 1949), concentration of dominance (Cd), known as Simpson's index (Simpson 1949), Margalef's index of species richness (M) (Margalef 1968) and evenness (E) (Pielou 1966).

$$H' = \sum_{i=1}^S p_i \ln p_i$$

$$Cd = \sum (p_i)^2$$

$$E = H'/H'_{\max}; H'_{\max} = \ln(S)$$

$$M = (S-1)/\ln N$$

Where, S = total no of species, $p_i = n_i/N$, n_i = total no of individual of "ith" species, N = total no of individual of all species, $\ln$ = natural log.

RESULTS AND DISCUSSION

Fourteen tree species under 10 families having 295 individuals were recorded (Table 1). The minimum and maximum numbers of individuals for various species were 1

and 95, respectively. Highest number of individuals (184) were of family Mimosaceae followed by Dipterocarpaceae (55). A single species having single individual represented the family Flacourtiaceae. In a quadrat the number of species ranged from 2 to 7 and number of individuals from 12 to 105. Vegetation analysis of Sriniketan-Santiniketan Lateritic Patch revealed relatively lower species richness as compared to Garhjungle sacred forest, West Bengal (Ganguli et al 2016a) and Ballavpur Wildlife Sanctuary, West Bengal (Ganguli et al 2016b). The differences in species richness are due to the disturbance and the plantation of exotic species *Acacia auriculiformis* and *Acacia holosericea*. *A. auriculiformis* exhibited the highest IVI value (71.61) and was the dominant tree species whereas *Glochidion ramiflorum* showed the lowest IVI value (2.52). *Shorea robusta* having IVI value of 60.99 was a co-dominant species. Highest family index value (FIV) was exhibited by Mimosaceae (104.73) followed by Dipterocarpaceae (46.4). This observation of *A. auriculiformis* and Mimosaceae as the most dominant species and family, respectively, is different from the previous studies carried out by Gupta Joshi (2012) and Ganguli et al (2016b) in similar adjacent forests. In the sampled plot (0.1 ha) the tree species occupied total basal area of 23.21 m² ha⁻¹ which was higher compared to the earlier studies of Ganguli et al (2016b) in Ballavpur Wildlife Sanctuary, West Bengal (22.49 m² ha⁻¹) and Sagar and Singh (2006) in a disturbed dry tropical forest of northern India (1.31 to 13.78 m² ha⁻¹).

Values of various diversity indices are shown in Table 3. Shannon-Wiener Index (H') was 2.46. High evenness

Table 1. Phyto-sociological characteristics of tree species in Sriniketan-Santiniketan lateritic patch

Tree species	Family	Total No.	Density (N ha ⁻¹)	Abundance	Frequency (%)	Basal area (m ² ha ⁻¹)	IVI
<i>Acacia auriculiformis</i> Benth	Mimosaceae	95	950	10.56	90	4.604	71.61
<i>Acacia holosericea</i> G.Don	Mimosaceae	88	880	29.33	30	0.167	37.07
<i>Albizia lebbeck</i> (L.) Benth.	Mimosaceae	1	10	1	10	0.087	2.89
<i>Buchanania cochinchinensis</i> (Lour.) M.R.Almeida	Anacardiaceae	12	120	2.4	50	1.008	19.28
<i>Casearia tomentosa</i> Roxb.	Flacourtiaceae	1	10	1	10	0.032	2.65
<i>Casuarina equisetifolia</i> L.	Casuarinaceae	3	30	1.5	20	4.465	24.6
<i>Eucalyptus tereticornis</i> Sm.	Myrtaceae	9	90	2.25	40	2.905	24.26
<i>Glochidion ramiflorum</i> J.R.Forst. & G.Forst.	Euphorbiaceae	1	10	1	10	0.001	2.52
<i>Madhuca longifolia</i> var. <i>latifolia</i> (Roxb.) A.Chev	Sapotaceae	21	210	4.2	50	4.577	37.71
<i>Shorea robusta</i> Gaertn.	Dipterocarpaceae	55	550	5.5	100	4.783	60.99
<i>Suregada multiflora</i> (A.Juss.) Baill.	Euphorbiaceae	1	10	1	10	0.003	2.53
<i>Syzygium cumini</i> (L.) Skeels	Myrtaceae	6	60	3	20	0.171	7.12
<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Combretaceae	1	10	1	10	0.238	3.54
<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Combretaceae	1	10	1	10	0.165	3.22
	Individuals	295	2950		460	23.206	
	Species	Total	Total density		Total frequency	Sum of basal area	

resulted in lower concentration of dominance. Lower value of Simpson's index (0.24) indicates that the area was not dominated by a single or few species. The values of Shannon-Wiener diversity index and Simpson's dominance index in the present study are within the reported range for tropical forests. Previous studies have reported a diversity (H') range of 0.83 - 4.1 for various Sal forests (Krishnamurthy et al 2010, Sahu et al 2012, Ganguli et al 2016) and the dominance (C_d) range of 0.10-1 for other tropical dry forests

(Kumar et al 2010, Sahu et al 2012). Recently diversity (H') range of 0.75-4.47 and concentration of dominance (C_d) range of 0.04-0.22 has been reported by Ifo et al (2016) for tropical rainforests.

Diameter class 1 to 10 cm was the most preferred class showing the highest density of individuals (Fig. 1). Four out of fourteen species-*Acacia holosericea*, *Casearia tomentosa*, *Glochidion ramiflorum* and *Suregada multiflora* were restricted to 1 to 10 cm diameter class. Diameter classes of

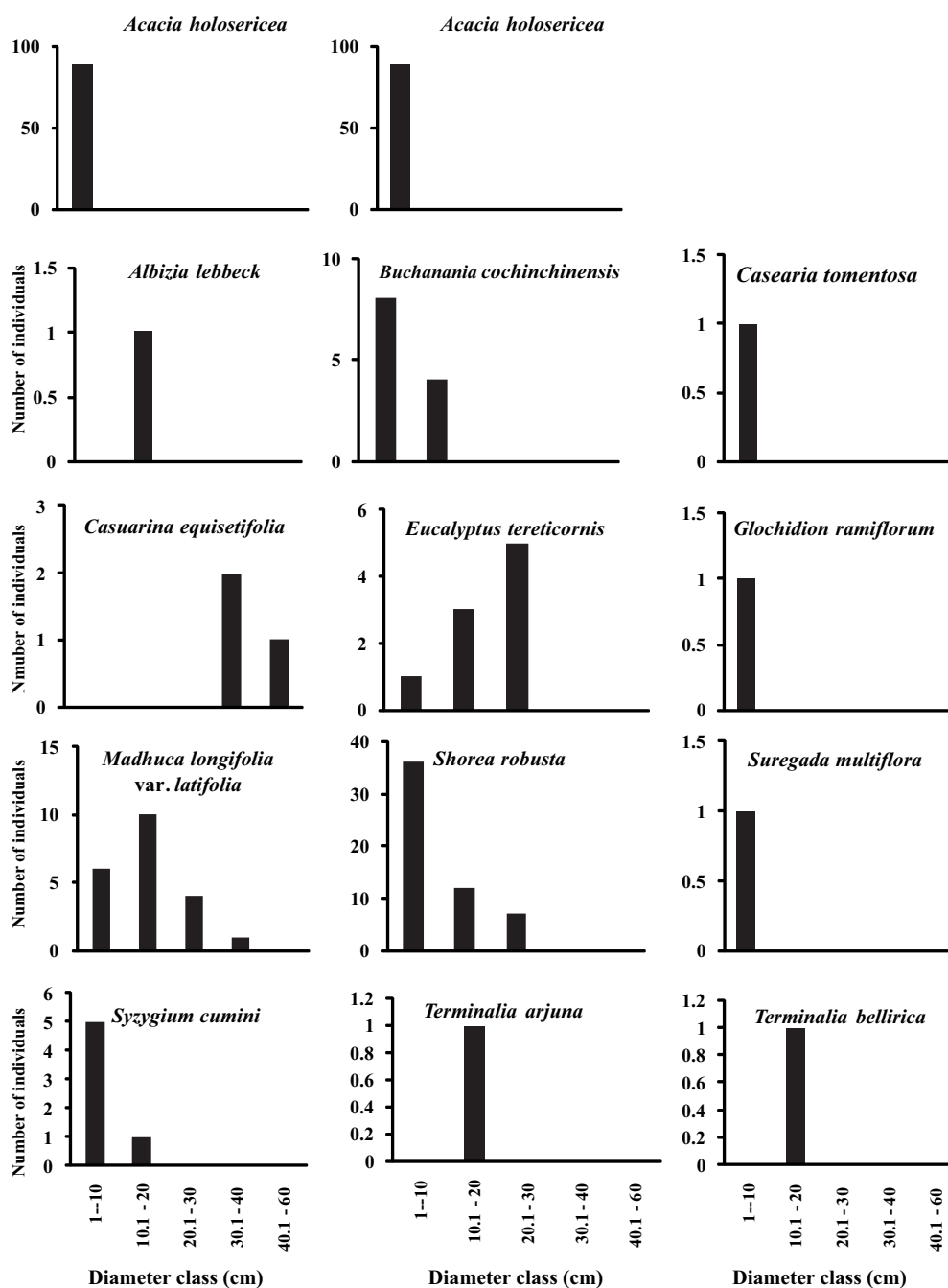


Fig. 1. Diameter Class Distribution of Tree Species in Sriniketan-Santiniketan Lateritic Patch

Table 2. Family importance value (FIV) based on number of species, density and basal area under different families

Family	Genus	Species	Trees	Relative density	Relative diversity	Relative dominance	FIV
Anacardiaceae	1	1	12	4.07	7.14	4.34	15.55
Casuarinaceae	1	1	3	1.02	7.14	19.24	27.4
Combretaceae	1	2	2	0.68	14.28	1.73	16.7
Dipterocarpaceae	1	1	55	18.64	7.14	20.61	46.4
Euphorbiaceae	2	2	2	0.68	14.28	0.133	15.11
Flacourtiaceae	1	1	1	0.34	7.14	0.14	7.62
Mimosaceae	2	3	184	62.37	21.42	20.93	104.73
Myrtaceae	2	2	15	5.08	14.28	13.25	32.63
Sapotaceae	1	1	21	7.12	7.14	19.72	33.98
Total	12	14	295	100	100	100	300

Table 3. Diversity Values of tree layer in Sriniketan-Santiniketan lateritic patch

Diversity indices	Value
Simpson's Dominance index (Cd)	0.24
Shannon – Wiener's Heterogeneity (H')	2.46
Margalef's species richness (M)	2.29
Pielou's evenness (E)	0.93

Eucalyptus tereticornis and *Shorea robusta* ranged from 1 to 30 cm. *Albizia lebbek* and both the species of *Terminalia* showed the diameter class from 10.1 to 20 cm. Two species, *Acacia auriculiformis* and *Madhuca longifolia* var. *latifolia* exceeded the diameter class 40 cm and only one species *Casuarina equisetifolia* exceeded the diameter class 50cm. Highest diameter recorded was 58.43 cm for *Casuarina equisetifolia*. Dominance of 1-10 cm diameter class, restriction of four species within 1-10 cm diameter class and only single species exceeding 50cm dbh indicates the young and regenerating forest. All the species had clumped distribution or dispersion (A/F >0.5) in the present study. According to Odum (1971) clumped dispersion is the most common dispersion pattern in nature.

CONCLUSION

A young or regenerating population of tree species was present in Amarkutir area. Percentage of occurrence for exotic species *Acacia auriculiformis* and *Acacia holosericea* was more than 60 whereas some species were represented by only a single individual. Therefore, further conservation efforts are immediately needed especially for those species. Further study is also needed to analyze if there is some allelopathic effect of *Acacia auriculiformis* and *Acacia holosericea* on other species present in the forest.

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Renewable Biomass Growing in Low Fertile Land and Forest-Steppe of Ukraine

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Abstract: For research of using the low fertile land in the Polissya and Forest-steppe zone of Ukraine, the structural-logical scheme of agricultural lands evaluation was developed. The key tool in the scheme is the procedure of environmental audit to assess the state and trends of agricultural land transformation in order to optimize its future use. The cost of straw and grain in comparison with the cost of energy willow in one year was determined. The identified trends confirm the importance of taking into account the socio-ecological and economic effects in determining the optimal use of low-yielding agricultural land. The maximum effect was obtained from the cultivation of willow for energy from Polissya zone and crop cultivation from more fertile soils from forest-steppe zone.

Keywords: Efficiency of land use, Energy raw materials, Low fertile agricultural land, Naturally restored forest stands

The use of non-traditional and renewable energy sources (NPDEs) today is one of the most promising ways of solving the growing problems of energy supply in all countries of the world. So, in most countries of the world and in the European Union, programs have been introduced and initiatives are being taken to increase the use of non-traditional sources of energy. The growth rates of energy production in NPDE nowadays exceed considerably the same for traditional types of energy. Thus, over the past 10 years, the share of renewable sources for electricity and heat production has almost doubled in the EU and biomass covers 1/5 of electricity production from NPDE and 9/10 of heat production from NPDE (Geletukha et al 2013). Bioenergy is considered one of the most up-to-date suppliers of renewable energy sources, which provides about 60% of renewable energy from biomass and waste in the countries of the Organization for Economic Co-operation and Development (OECD). Such policy will stimulate further growth of the bioenergy sector (EEA 2010). Bioenergy is also being rolled out in many areas, such as the world's leading bioenergy program by 2030, which is expected to reduce to 120 million tons of biological waste in landfills, reduce 47 billion tons of CO₂ emissions into the atmosphere, and so on. At the same time, global demand for biomass can double to 108 EJ by 2030. The share of renewable electricity will increase from 19% in 2009 to 50-60% in 2050 (Panoutsou 2007).

The EU is actively developing a biomass cultivation direction. For example, in Denmark, energy willow is grown for only 500 hectares of agricultural land, while in Sweden -

more than 20,000 hectares (Brussaard et al 2010). Therefore, in Sweden, the share of bioenergy from total energy consumption in 2007 was 28% (Dauber 2012). At the same time, the total sustainable world energy potential of biomass is about 100 EJ (wood biomass is 41.6 EJ), which is about 30% of total energy consumption today. Approximately 40 EJs of available biomass are used for energy. About 60% of this biomass is used only in Asia. In most of the countries, the current use of biomass is clearly lower than existing capacities. Only in Asia the current use exceeds the existing potential. Therefore, it is possible to increase use of biomass, for example, for the production of biofuels in most countries. Most of the biomass is refined on pellets. In 2016, the world produced 28.9 million tons of pellets. The EU-28, as a world leader, has produced 14.0 million tons of pellets providing 65% of its own demand. The pellets market in Ukraine is a relatively young market, which demonstrates a generally positive development dynamic. Launched in 2002 by the production of wood pellets, today the pellets market accounts for the amount of biofuels produced up to 10% of the total biomass market in Ukraine. At the moment, the market is experiencing a period of reorientation from exports to the domestic market, partly thanks to a change in the priorities of Ukraine's energy policy. Given the environmental trends in the world and the EU, it should be noted that biomass for the production of pellets must be certified according to sustainability standards - grown in permitted places and / or forestry waste (Diachuk et al 2017, Arulkumar et al 2019).

In Ukraine, energy consumption is increasing, while its own production is decreasing. Consumption of energy

resources in our country is oriented, for the most part, to traditional non-renewable energy sources: natural gas 41-43%, oil and coal by 19%, uranium - 17%. Non-traditional renewable energy sources in Ukraine make up only 4% of the total energy supply balance. In the structure of final consumption among the main sources of energy, the share of natural gas remains the largest (31.5%), the second is electricity (20.1%) and biomass (2.5%). In recent years, the share of NPV in gross final energy consumption is 3%, including biomass -2%, which is 63% of all NPDE (Beringer et al 2011, Lakyda et al 2011).

An important issue at the same time at the level of the Food and Agriculture Organization (FAO) of the United Nations is the use of agricultural-free lands (land that is not in use). So in particular, in 2015, the European Union (EU-28) used 106.9 million hectares of arable land. The total agricultural area amounted to 177.1 million hectares. The amount of reserved land that can be used for bioenergy in the EU is not shown by statistics, because it is not measured as a certain class. Abandoned land in the EU is classified under permanent pasture or steam (Khanna and Zilberman 2017). Consequently, in the context of the implementation of the energy strategy in Ukraine and as a supplier of pellets to the EU, it is necessary to justify the use of low-yielding agricultural land in the north of Ukraine, to develop sustainability criteria for biomass.

MATERIAL AND METHODS

Getting biomass for various purposes is associated with the land resources use, including agricultural. The growing demand for bioenergy produces conflicts in land use for growing biomass or food. Such conflicts can be solved by continuing food production on productive agricultural and growing biomass on low-yielding ("superfluous", not used because of low fertility) lands. In this case, there is uncertainty due to environmental, economic and social constraints on the use of land resources. The Polissya area of Ukraine occupies 113,000 km² (19% of the territory of Ukraine) and is located in the northern part of Volyn, Rivne, Zhytomyr, Kyiv, Chernihiv and Sumy regions. For such a territory, degradation processes of land resources are characterized by the economic problems - reduction in incorporation of organic and mineral fertilizers, the actual suspension of liming of acid soils, the introduction of anti-erosion and other measures (Lambin 2010). Therefore, on the territory of the Polissya Zone, there are ecological processes on the restoration of the natural state in relation to the inoculation and afforestation of agricultural land.

Soils characteristic of the Polissya area are not resistant to erosion and are less fertile. Such soils require the constant

addition of organic and mineral fertilizers, liming, compliance with crop rotation, and so on. Forest-steppe is a transition zone between mixed and broad-leaved forests and the steppe occupies 25% of the territory of Ukraine. It covers the entire central part of Ukraine with an average width of 110 km from the Polissya area. The forest-steppe is dominated by fertile black soil, which is predominantly used in agriculture. The present research was conducted on the territory of the Rivne region, which is located in two natural zones- north of the region in the Polissya area and south in the forest-steppe zone. In the Rivne region there are 123 thousand hectares of degraded land that could potentially be used for biomass production. An analysis of the current state of land resources in the Rivne region indicates an increase in the areas of eroded lands, a decrease in the potential fertility of the land. As a result of degradation processes increased and the fertile soils in the Polissya area were virtually unused and overgrown with forests. In general, it is necessary to create more than 2 million hectares of new forests to achieve the optimal forests of Ukraine (20%). This is potentially possible at the expense of land withdrawn from agricultural, which is about 8 million hectares.

The FAO database FAOSTAT, European Biomass Association (AEBIOM), Report by Global CCS Institute, European Biomass Industry Association (EuBiA), statistical-reports EUROSTAT and other public actions are used for gathering the data. At early evaluation low fertile agricultural land, we conducted an expert assessment of land using a comparison of sales prices of similar land, capitalization of net income from the use of land and pricing for components of agricultural land. Three approaches were followed to assess the differences i.e., growing of cereals for the purpose of obtaining grain and straw for the average yield over the last 5 years; growing of grain crops for the purpose of obtaining grain and straw by the area of land (4 t ha⁻¹ for Polissya and 6 t ha⁻¹ for the forest-steppe, taking into account the type of soil) and growing the energy willow at a yield of 15 t ha⁻¹ year, harvesting after 3 years.

An important issue with regard to the use of low-yielding agricultural land is determination of the final product rent, based on the methodological approaches of balance method for land. The rent was determined as the difference between the current costs of capitalized net operating income (or rental income) from the improved area and the cost of land improvements (the cost of straw, the cost of increasing humus content in soils, etc.). To determine the costs of fertility restoration of low fertile lands, the data from agrochemical surveys of the Rivne region lands for many years of observation of the Rivne branch of the State Enterprise "Hruntohorona", on the example of the restoration of humus

content by using the organic fertilizers (biohumus) was available. The result of the evaluation is to determine the option that is optimal for all users.

RESULTS AND DISCUSSION

Analysis of the current condition of land resources in Rivne region testifies to their deep degradation with significant increase in the area of eroded lands (387.6 thousand ha), which is 41% of all farmland. Over the past 30 years, the area of land has increased by 15%. Land degradation and sometimes their complete exclusion from agricultural use has been recorded. But in the Rivne region, in general, and in the area of Polissya, there are large areas of fertile agricultural land what was not used for a long period of time, and as a result are covered by forest. Therefore, the issue of growing ecologically viable and economically beneficial crop is important. According to our research, these lands are environmentally appropriate and economically advantageous to rehabilitate forests' natural regeneration. Scientific researchers have shown that 22% of agro-industrial groups of soils in Rivne region produce damage

even under the most favorable conditions. This represents 8% of the total agricultural land area – 45396 ha. These lands can be transferred in forestry, meadow, etc. for growing biomass. For agricultural production systems analysis, data of agrochemical certification and monitoring of agricultural land was used according to the State Enterprise "Hruntohorona". Therefore, the research of using the low fertile agricultural land in the Polissya and Forest-steppe area of Ukraine should be carried out in accordance with the developed structural and logical scheme for the evaluation of agricultural land (Fig. 1). The key tool in the scheme is an environmental audit procedure to assess the state and trends of agricultural land transformation in order to optimize its use (afforestation, ecological and economic rehabilitation, energy crops growing, heritage, green tourism, conservation, and foraging).

Based on the world experience in assessing the natural environment, the standards of DSTU (National Standard of Ukraine), ISO 14000 "Environmental Management", we have developed an environmental audit to justify the use of small fertile agricultural land (Fig. 2).

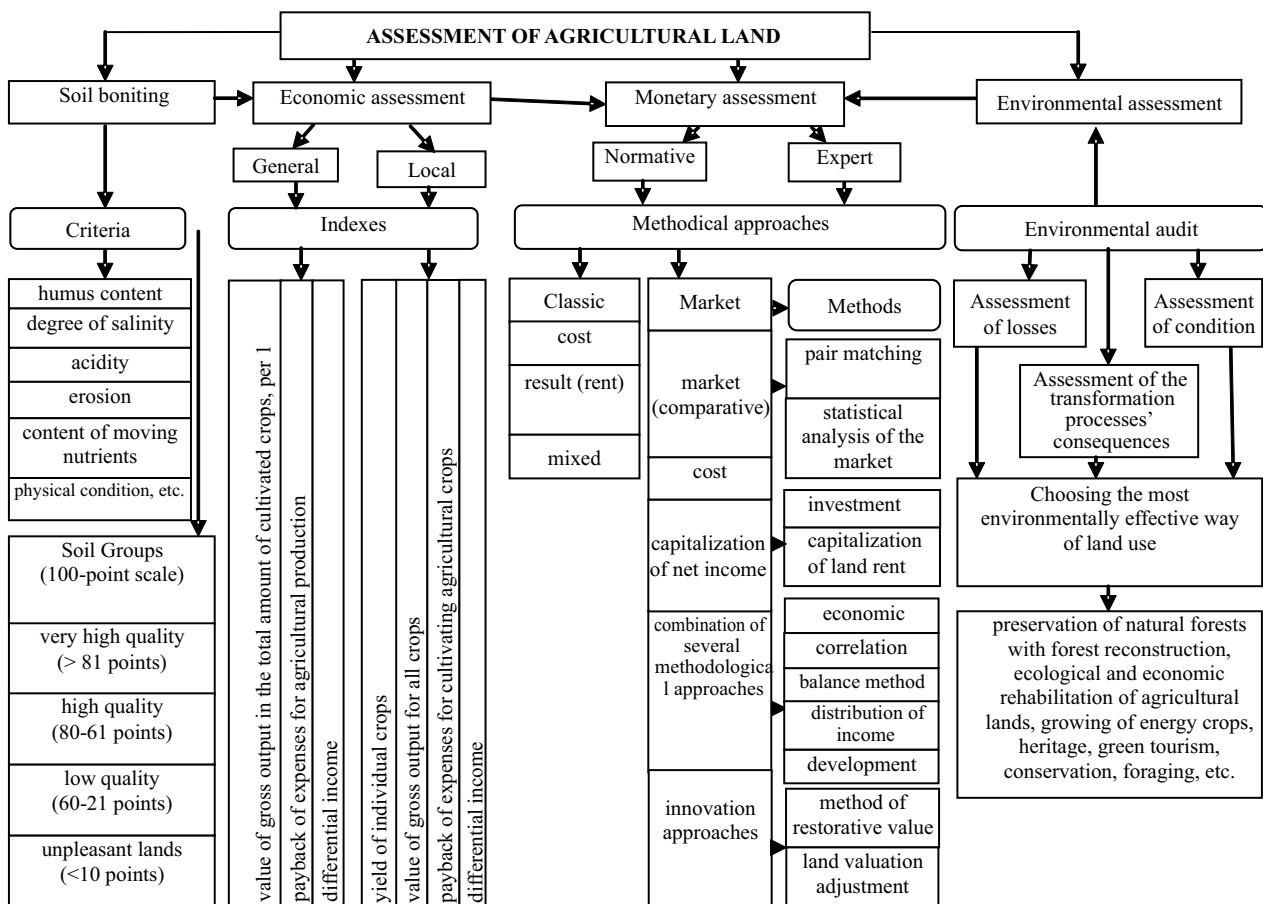


Fig. 1. Components of the structural and logical scheme for the evaluation of agricultural land

For certificated products producing, like biomass, produced on lands that have experienced reforestation in the natural way, it is appropriate to use a provision of certification standards: International Sustainability and Carbon Certification (ISCC), REDcert – certifications according to with the provisions of EU directives and NTA-8081 [Certification sustainable biomass 2012, NTA8081 - 2012]. In Ukraine, for these purposes, you can use ISO 14000 for products life cycle assessment. The sustainable biomass certification process due to ISCC standards requires registration of the relevant documents by all parties, as well as, coordination and compliance with all of the trading chain. The implementation of the sustainability certification scheme should be used by filling in a form (declaration), which shall include the main characteristics of biomass production. This arrangement simplifies and reduces the cost of the certification process (enterprise suppliers of biomass to be selective inspection). Such a declaration is submitted to the original purchaser and is used later in confirmation of compliance with sustainability. In particular, it is required to specify, which land is used for cultivation and it's earlier status (i.e. no change of use). However, development of such provisions should be against the background of macroeconomic stability, implementation of best practice developed countries, reform of the institutional system, as evidenced in the Rozelle's and Swinnen's researches (Lakyda et al 2011). Formation of the agricultural land market

requires efficiency consideration of its use and consideration of the transformation processes of agricultural environmental management, problem analysis using degraded, low fertile, forested lands in a natural way especially in the area of Polissya (Soloviy 2017). The volumes of growing energy raw materials are given in Table 1. The costs of various types of products are shown in Table 2.

A good response may be obtained by growing energy willow graft. The total increase in 3 years will be 37305 thousand USD and 12435 thousand USD per year. The effect of growing cereal straw and grain is 1.6 less in Polissya zone and 1.4 times greater for the forest-steppe zone as there are less forested areas and good grain and straw yield due to higher soil fertility (Table 3). An important issue regarding the use of agricultural land taking into account the transformation processes is the determination of the final product rent, based on methodological approach of balance method. In market conditions, the theory of rent is the basis of the state economic policy. In the current legislation on the natural resources use, there are no provisions related to the regulation of rental relations. Therefore, a significant part of rental income due to the lack of special legislative regulation does not enter the budget system. The rent is defined as the difference between the current cost of capitalized net operating income (or rental income) from the improved area and the cost of land improvements (the cost of straw, the cost of increasing the content of humus in soils, etc.). Taking into

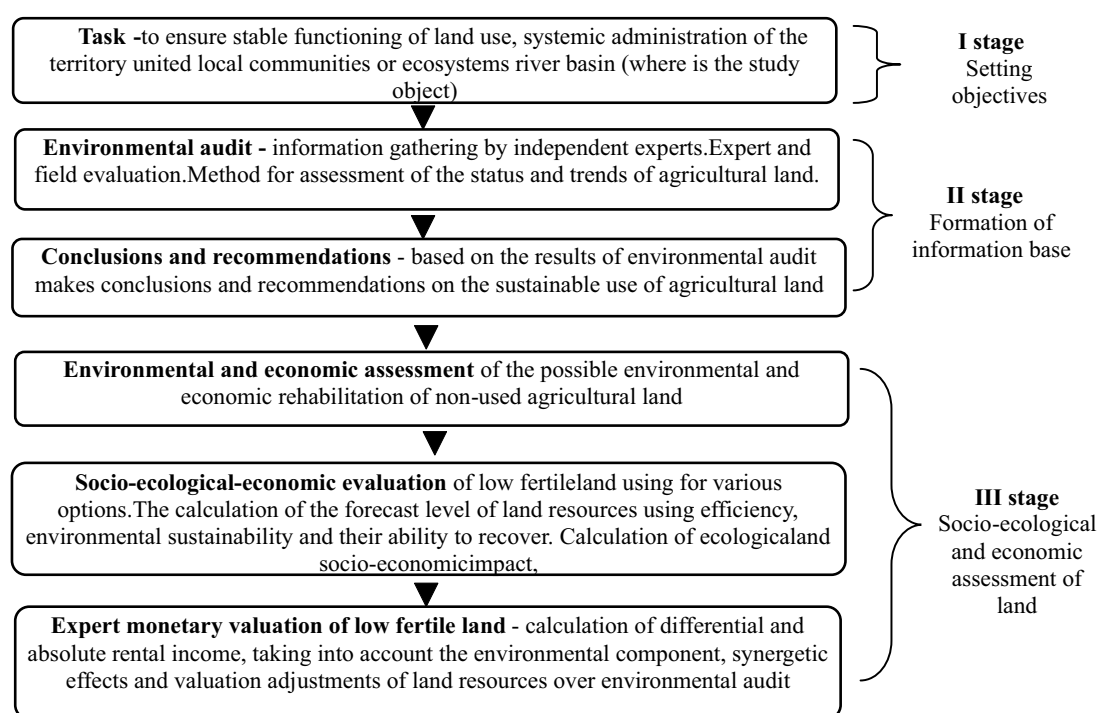


Fig. 2. Algorithm of environmental and economic assessment of transformed agricultural land

Table 1. Growing the raw materials

Administrative areas (Polissya zone in italics)	The area of not used land (ha)	Option 1. Number of received grain straw (t)*	Option 2. Number of straw cereals (t)	Option 3. Number of energy willow graft (t)**)
<i>Berezne</i>	12795	27957	51180	191 925 (63975)
<i>Volodymyrets</i>	7278	13391	29112	109 170 (36390)
Goshcha	4462	9749	26772	66930 (22310)
Demidov	1849	7229	11094	27735 (9245)
Dubno	9738	26876	58428	146070 (48690)
<i>Dubrovitsya</i>	11963	24763	47852	179445 (59815)
<i>Zarichne</i>	6195	12111	24780	92925 (30975)
Zdolbuniv	7202	23046	43212	108030 (36010)
Korets	12779	52 905	76674	191685 (63895)
<i>Kostopol</i>	15471	26687	61884	232065 (77355)
Mlyniv	8083	34393	48498	121245 (40415)
Ostrog	4451	18427	27706	66765 (22255)
Radivilov	1434	6266	8604	21510 (7170)
Rivne	9178	36941	55068	137670 (45890)
<i>Rokytno</i>	8812	24321	35248	132180 (44060)
<i>Sarny</i>	7122	14724	28488	106830 (35610)
For Polissya area	69 636	143 954	278 544	1 044 540 (348 180)
For the steppe zone	59 176	215 832	356 056	887 640 (295 880)
For the region	128812	359786	634600	1 932 180 (644060)

*Yields are calculated on the basis of statistical data for each area separately.

** Increase per year

Table 2. Cost of different kind of products

Administrative areas (Polissya zone in italics)	Option 1. The effect of straw and grain (in parentheses) thousand USD*	Option 2: The effect of straw and grain (in parentheses) thousand USD**	Option 3: The effect of growing willow graft (total increase for 3 years (in parentheses for one year), thousand USD***
<i>Berezne</i>	499 (1439)	914 (1854)	6854 (2284)
<i>Volodymyrets</i>	239 (702)	520 (982)	3898 (1299)
Goshcha	174 (766)	478 (1069)	2390 (797)
Demidov	129 (567)	198 (439)	991 (330)
Dubno	480 (2111)	1043 (1631)	5216 (1739)
<i>Dubrovitsya</i>	442 (1326)	854 (1738)	6408 (2136)
<i>Zarichne</i>	216 (648)	442 (875)	3318 (1106)
Zdolbuniv	412 (1810)	772 (2171)	3858 (1286)
Korets	945 (3012)	1369 (3966)	6845 (2281)
<i>Kostopol</i>	476 (1429)	1105 (2058)	8288 (2762)
Mlyniv	614 (1906)	866 (2771)	4330 (1443)
Ostrog	329 (1447)	494 (1613)	23844 (7948)
Radivilov	111 (492)	154 (534)	768 (256)
Rivne	659 (2902)	983 (3226)	4916 (1638)
<i>Rokytno</i>	434 (1302)	629 (1498)	4720 (1573)
<i>Sarny</i>	262 (788)	508 (1034)	3815 (1271)
For Polissya area	2570(7637)	4974(10041)	37305 (12435)
For the steppe zone	3854(15286)	6358(17790)	31701 (10567)
For the region	6424(22924)	11332(27831)	69006 (23002)

*Attraction areas that are not used for crops with a complete cycle of agricultural production (making all kinds of fertilizers for harvest in 2016) and at the cost of straw bulk 20 USD t⁻¹, the value of the grain grown in the area Polissya–60 USDt⁻¹ and 88 USDt⁻¹ in the forest-steppe zone, prices for 2016 (the cost of grain include its low quality)

**Grain cost is similar to option 1

***with yield of 15 t ha⁻¹ year⁻¹ (harvest after 3 years), the average cost of graft is 40 USD t⁻¹, prices for 2016

account the provisions of the effectiveness of restoring primary products (K) on an alternative basis is that the "price" of environmental interest should not be lower than the price of economic interest of nature use (Park 2014):

$$R = \frac{P \times r}{1 + r} \times K_1 \times K_2 \quad (1)$$

R - rent of the unit of final product of nature use, USD; P - price of the final product of nature use, USD; r - coefficient of efficiency (profitability) of products of nature use, 0.3; K_1 - coefficient of combination of ecological and economic interests of nature use (0.9), depending on functional zones (regulated nature use, recreation, etc.), it can vary from 0.6 to 1 (the maximum is characteristic for the protected area); K_2 - output of the final product of nature use from the unit of natural resource, fractions of units (0.85), for different ecosystems has different significance, based on practice of nature management, its value for forest ecosystems is 0.9 and for other ecosystems is 0.8. Determination of the rent has been held for the final product of nature use by its cost for the above-mentioned variants (Table 3).

For the Polissya area, the rent from the straw grown grain and grain in option 1 and option 2 is in 1.6 and 1.2 times lower compared to the rent for the cost of growing willow graft

in one year. The less value of rent for option 1 is due to the potentially lower yield of grain and hence the amount of income from its cultivation. For the Forest-steppe zone, the rent from the cost of straw grown grain and grain in option 1 and 2 in 1.8 and 17.4 times higher than the rent from the cost of energy willow graft in one year. The higher value of rent compared to the Polissya zone in all variants is due to the potentially higher yield of grain and its quality, and hence the amount of income from its cultivation. The identified trends confirm the importance of taking into account the socio-ecological and economic effects in determining the optimal use of low-yielding agricultural land. According to the of environmental and economic assessment of transformed agricultural land and analysis of the rent for straw grown grain and grain, energy willow it has been established that its low values are due to the low humus content. In the agricultural land market, the procedure of environmental audit will include: control of humus content, expert valuation of low fertile land, calculations of differential and absolute rent income taking into account the environmental component, synergistic effects and adjusting the valuation of land resources. One of the recommendations of the environmental audit is the humus content restoring. Taking

Table 3. Rent* of the final products of nature use on low fertile lands that are not used

Administrative areas (Polissya zone in italics)	Option 1: Rent of straw and grain (in parentheses) thousand USD	Option 2: Rent of straw and grain (in parentheses) thousand USD	Option 3: Rent of growing the energy willow graft (total increase for 3 years (in parentheses for one year), thousand USD
<i>Berezne</i>	74,53 (215)	136 (277)	1024 (341)
<i>Volodymyrets</i>	35,7 (105)	78 (147)	583 (194)
Goshcha	4,6 (22)	71 (160)	357 (119)
Demidov	3,4 (16,6)	30 (66)	148 (49)
Dubno	12,7 (62)	156 (244)	779 (260)
<i>Dubrovitsya</i>	11,7 (42,2)	128 (260)	957 (319)
<i>Zarichne</i>	32,3 (97)	66 (131)	496 (165)
Zdolbuniv	61,5 (270)	115 (324)	576 (192)
Korets	141 (450)	204 (592)	1022 (341)
<i>Kostopol</i>	71 (213)	165 (192)	1237 (413)
Mlyniv	92 (285)	129 (414)	647 (215)
Ostrog	49 (216)	74 (241)	3561 (1187)
Radivilov	17 (74)	23 (80)	115 (38)
Rivne	99 (433)	147 (482)	734 (245)
<i>Rokytno</i>	65 (195)	94 (224)	705 (235)
<i>Sarny</i>	39 (118)	76 (154)	570 (190)
For Polissya area	384 (1141)	743 (1499)	5571 (1857)
For the steppe zone	576 (2283)	949 (26567)	4734 (1578)
For the region	959 (3423)	1692 (4156)	10305 (34350)

*Calculated by authors

into account the data of agrochemical surveys of the Rivne branch of the State Enterprise "Hruntohorona", the calculation has been carried out of necessary funds for ecological and economic rehabilitation of the quality of transformed agricultural land - the restoration of humus content to the optimum for typical soils of administrative districts of the Rivne region by using organic fertilizers. Using the method of determining the size of the costs for the restoration of soil fertility, we define norms of making the litter manure for restoring the humus content to the level of the early 1990's (by the 1990s, the humus content in the soils of the region had optimal values) (Shpak 2015):

$$D = \frac{2500 \times P \times (C_1 - C_2)}{100} \times H \quad (2)$$

D - the norm of litter manure, necessary for the restoration of humus content, t ha⁻¹; 2500 - the volume of an arable layer of soil on 1 hectare, m³; P - soil density t m⁻³; C₁ - the humus content in the early 90's, %; C₂ - the humus content currently, %; H - norm of humus restoration in the soil of one ton of litter manure, tons. Restoration of the soils' structure and fertility by making manure on a large area is a labor-intensive and very expensive. In addition, in the conditions of a total decline in livestock production and, accordingly, a significant reduction in the production of organic fertilizers (manure), increasing the dose of its making

is impossible. It is more expedient and cheaper to use elements of alternative agriculture – side rates and bio-humus for the restoration of soil fertility. By its properties, bio-humus is much better than organic fertilizer, so 1 ton of bio-humus contains 350 kg of humus, 3 kg of phosphorus and 1 kg of potassium in an active substance. In regions where farming is intensive, and it is mostly Forest-steppe zone of the Rivne region, the humus loss compared to the 1990's beginning is quite significant. Polissya zone is characterized by a positive trend of increasing the humus content in the soil due to the use of meadow and not enough fertile land (there is natural reproduction of soil fertility). The cost of bio-humus is 3000 UAH t⁻¹. Calculating the cost of restoring the previous level of humus with the aim of ecological and economic rehabilitation of quality of the transformed agricultural land in Rivne region has been shown in Table 4.

According to the calculation results, it was found that the greatest costs for the restoration of humus content for Mlyniv, Rivne, Zdolbuniv, Demidiv and Dubno districts where intensive farming is conducted. In the Polissya region, the natural processes of land renaturalization have taken place: in the first stage of grazing (up to 1-4 years), gradual afforestation mainly by pine and birch (in our time the plantation of these trees in some cases is up to 24 years old), continuous overgrown with forest (mainly areas near the

Table 4. Calculating* the cost of restoring the previous level of humus with the aim of ecological and economic rehabilitation of quality of the transformed agricultural land in Rivne region

Administrative areas (Polissya zone in italics)	Average weighted index of humus content in arable land of the area (%)		Weight of arable layer of soil hectare ⁻¹ (t)	The humus demand, t ha ⁻¹	Demand for 1 t ha ⁻¹ , in terms of biohumus, (1 ton of bio-humus = 0.35 t of humus)	Costs of purchasing and making the bio- humus, thousand USD ha ⁻¹
	Original content	After using				
<i>Berezne</i>	1,81	2,1	3750	-	-	-
<i>Volodymyrets</i>	1,95	1,9	3750	1,9	5,36	0,27
<i>Goshcha</i>	2,41	2,31	3250	3,3	9,29	0,31
<i>Demidov</i>	2,42	2,2	3500	7,7	22,0	0,37
<i>Dubno</i>	2,39	2,1	3500	10,2	29,0	0,37
<i>Dubrovitsya</i>	1,77	2,05	3750	-	-	-
<i>Zarichne</i>	1,81	2,06	3750	-	-	-
<i>Zdolbuniv</i>	2,36	2,2	3250	5,2	14,86	0,35
<i>Korets</i>	2,16	2,23	3250	-	-	-
<i>Kostopol</i>	1,96	1,95	3750	0,4	1,07	0,11
<i>Mlyniv</i>	2,54	2,11	3500	15,1	43,0	0,36
<i>Ostrog</i>	2,44	2,5	3250	-	-	-
<i>Radivilov</i>	2,52	2,7	3500	-	-	-
<i>Rivne</i>	2,37	2,1	3250	8,8	25,1	0,36
<i>Rokytno</i>	1,89	1,95	3750	-	-	-
<i>Sarny</i>	2,03	2,1	3750	-	-	-

*Calculated by authors using data of "Hruntohorona" in Rivne region; *in some areas the humus content has increased due to non-use of agricultural land

forest up to 500 meters). Stabilization and gradual increase of humus content is mainly due to becoming meadows of: pasture, hayfields, abandoned land, etc. In the Forest-steppe zone, such lands are much smaller and most of them are located far from settlements (up to 10 kilometers from settlements). However, in the last 3 years there has been a re-plowing and exploitation of these lands, mainly by agricultural holdings under grain crops grown using the intensive technologies. Therefore, in the procedure of environmental audit in order to formulate recommendations for the further use of fertile soils, it is necessary to take into account that for getting planned grain yields, significant costs for the restoration of fertility in time are required. In order to substantiate such decisions, the calculation of the profitability of products cultivation was carried out to determine the best options for land using under the conditions of planning different profitability of its cultivation (Table 5).

Analysis of variants of using the low fertile agricultural lands has shown that for the Polissya zone, under the conditions of different profitability of growing grain and energy willow, the profit obtained from the sale of straw and grain at a profitability of 25% is actually equal to the profit from the sale of graft with a profitability of 15%. For the Forest-steppe zone, there is a tendency for more profitability of grain growing, taking into account potentially higher yields, better grain quality and, consequently, grain prices than in the Polissya zone. It is also established that for the Polissya region, the profitability of grain crops is 1.6 times lower than the willow cultivation with a profitability of 25%. For the Forest-steppe zone, the profitability of grain crops is 1.4 times higher than the willow cultivation with a profitability of 25%. Such facts are explained by that the grain yield in the Forest-steppe zone on potentially more fertile soils is higher, the quality of grain corresponds to the food grade, and therefore in such a zone it is expedient to grow crops. If we compare the overall effects of the straw grown grain and grain and the effects of growing the energy willow graft

obtained in one year for Rivne region, then we can say that they are actually identical. This tendency is explained by the fact that Rivne region was actually divided in half divided by the area of Polissya and Forest-Steppe. The calculations confirm the opinion that the most environmentally and economically advantageous option will be the cultivation of energy willow primarily for the Polissya area.

The procedure and conclusions of the environmental audit also take into account the current legislation of Ukraine. For example, the use of low fertile lands does not solve the problem of the Law of Ukraine "On the Basic Principles (Strategy) of the State Environmental Policy of Ukraine for the Period up to 2020", which stipulates that the rate of plowing can be foreseen from 71% to 50%. These indicators are not fulfilled currently, but in reality there is natural deforestation and flooding at the expense of the use of poorly degraded, degraded, acidified, contaminated, and other agricultural land. The Land Code of Ukraine provides that some of the lands of Ukraine which are degraded or unproductive may become the subject to conservation. Therefore, the conservation of few fertile agricultural lands is a conservation measure and is primarily due to the suitability of plots for arable farming (Bitev 2019).

Consequently, the changes in the agricultural land use of the Polissya area of Ukraine, the aspects of reforming the agrarian economy and the introduction of the agricultural land market in the nearest future require using the algorithm and tools of environmental and economic assessment of transformed agricultural land. The conclusions of the environmental audit can also provide recommendations that take into account the analysis of "costs - ecosystem benefits" in relation to the cultivation of grain or energy willow, the quality of agricultural land, the conditions for raw materials and food products depending on the ecological state of land resources, the market price of land, encouragement for quality improvement land, taking into account synergetic effects for the state. Therefore, the ecological and economic

Table 5. Variants of using the low fertile lands

Indexes	Polissya zone	Forest-steppe zone	Rivne region
Option 1. Effect of straw grown grain and grain, thousand USD:	7129	14267	21396
Profits at the profitability of grain crops 15%	1069	2140	3209
Profits at the profitability of grain crops 20%	1426	2853	4279
Profits at the profitability of grain crops 25%	1782	3567	5349
Option 3. Effect of growing the energy willow graft in parentheses for one year, thousand USD	11606	9862	21468
Profit at the profitability of growing energy willow 15%	1740	1479	3219
Profit at the profitability of growing energy willow 20%	2312	1972	4284
Profit at the profitability of growing energy willow 25%	2902	2466	5368

justification of using the low-yielding land should be based on the costs of agricultural production and / or the cultivation of biomass, natural forage and afforestation, and the formation of an ecologically optimal land use structure.

CONCLUSION

Analyzing socio-environmental and economic efficiency for the various versions of fertile agricultural land was found that the effect of growing during one year of grain and straw cereals (for option 1) is 1.2 times lower than for similar products for option 2, obtained from not used lands in Rivne region. The effect of straw grown grain and grain (option 1) and the effect of energy willow graft are actually the same. However, obtaining the necessary grain yield significant cost of environmental and economic rehabilitation for 3-10 years or more and for growing energy willow necessary additional costs of preparing and fertilizing the land. An important innovation tool in assessing the use of low fertile agricultural land is the rent, that confirm degradation, ecological, economic and other land use processes. Thus, for example, the cost and quality of the received grain on the low fertile land is much lower because of its low quality, low yields and, in some cases, loss-making cultivation. It is substantiated that the most suitable way of preserving some fertile land in the Polissya area is the afforestation or the energy willow cultivation.

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Seasonal Dynamics of Soil Microbial Biomass Carbon under Different Forests of North Western Himalaya, India

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Abstract: Present research was conducted to study the seasonal variation of soil microbial biomass carbon (MBC) in relation to soil depth under different forest based ecosystems of western Himalaya, India. Soil samples were collected randomly from each of the five land uses, in different seasons (i.e., rainy, winter and summer). Soil samples were collected in triplicate, from four different depths (0-15, 15-30, 30-45 and 45-60 cm), in every season. The chloroform fumigation extraction method was used to determine soil microbial biomass carbon. Irrespective of land uses, the highest value of soil microbial biomass carbon was observed during rainy season, followed by summer and minimum in winter. Microbial biomass carbon showed a strong positive correlation with soil organic carbon and soil moisture. MBC was highest in surface soil (0-15cm) of *Quercus* (572.9 $\mu\text{g g}^{-1}$ soil) forest and lowest in agricultural lands (262.2 $\mu\text{g g}^{-1}$ soil) at 45-60 cm depth. The effect of forest based ecosystems on soil microbial carbon was most prominent in surface soil and decreased as the soil depth increased.

Keywords: Himalaya, Microbial biomass carbon, Land use, Seasonal dynamics, Soil depth

Soil microorganisms play a significant role in ecosystem functioning, which has led an increased interest in assessing microbial biomass in soil (Azam et al 2003). Soil microbial biomass carbon (MBC) constitutes about 1-5% of total organic carbon in soil (Nsabimana et al 2004, Gil-Sotres et al 2005). Soil microbial biomass, being a living component of soil organic matter, acts as a transformation matrix of organic matter and also serves as labile reservoir and responsible for releasing nutrients from organic matter for use by plants. Because of its relatively faster turnover rate, MBC responds more rapidly to changes of soil environment (degradation, erosion) than most other soil C pools in soils and thus microbial biomass can be considered as a sensitive indicator of soil quality and has a direct influence in ecosystem stability and fertility (Bending et al 2004). Climatic conditions alter soil moisture and temperature and thus have a direct impact on soil microbial communities (Chattopadhyay et al 2012, Ullah et al 2012), and climatic parameters through interaction with vegetation, topography and landscape, affect soil microbial biomass indirectly (Myers et al 2001). *Quercus*, *Pinus*, *Deodar* are dominant forest forming species in Indian western Himalayan region and the Himalayan ecosystems are subjected to a marked seasonality. Soil microbial biomass being highly sensitive to climatic fluctuations, also act as sensitive bio-indicator to ongoing climate change (Schindlbacher et al 2011). Forest types exerts great impact on soil microbial biomass and SOC dynamics by controlling the amount and chemical composition of organic inputs returned to the soil (Jin et al 2010), which in turn influences

the pool size and activity of the soil microbial biomass (Kasel and Bennett 2007). Research in north east India reported a 16% decrease in microbial biomass within just four years after the conversion from grassland into an agro-ecosystem (Singh and Yadava 2006). Other than forest types, seasonal variations of rainfall, temperature, soil moisture and litter fall also have great influences on microbial biomass in soil (Chen et al 2005, Devi and Yadava 2006, Chauhan et al 2018).

A number of earlier research works have provided information on soil microbial biomass carbon in different forest ecosystems (Devi and Yadava 2006, Patel et al 2010) but information on seasonal variation of MBC in an annual cycle in forest ecosystem is limited (Diaz-Ravina et al 1995, Arunachalam and Arunachalam 2000). The research on temporal variation of SMB in temperate region indicated close association of MBC with changes in hydrological regime and temperature (Wardle 1998). Therefore, information on seasonal variations in soil microbial biomass in relation to soil moisture and other soil properties is needed to have a in depth understanding of soil nutrient transformation and availability. Present study aimed to investigate variation in soil microbial biomass carbon and soil characteristics in selected western Himalayan forests, and understand relationships between microbial biomass C and soil characteristics.

MATERIAL AND METHODS

Study area: This study was located in Shivalik region of western Himalaya, in Shimla district of Himachal Pradesh, India (30° 45' N latitude, 70° 45' E longitude, and 1773 m

above mean sea level). Four forest types viz., Oak (*Quercus leucotricophora*), Chir-pine (*Pinus roxburghii*), Deodar (*Cedrus deodara*), mixed and one agriculture land use were selected.

Climate: The climate of the area is temperate with prolonged winter season, often with snow and with a summer of short duration. There are three distinct seasons in the study area which consists of short duration summer (April to June) with low rainfall, followed by monsoon (July to September) and chilling and snowy winter (December-February). The mean monthly maximum temperature varied from 11°C to 26°C and mean monthly minimum temperature varied from 4°C to 17°C. The average annual rainfall of the area was 2347mm and nearly 70 to 75% rain occurs during the months of mid-June to mid-September). The average monthly minimum temperature is reached in January (2.5°C) and average monthly maximum temperature is (24.1°C in June), (based on analysis of last 45 years data) (Fig. 1).

Soil sampling and analysis: Soil is sandy loam and well drained in the study site. Soil samples were collected randomly from each of the five land uses, in different seasons (i.e., rainy, winter and summer). Soil samples were collected in triplicate, from four different depths (0-15, 15-30, 30-45 and 45-60 cm), in every season. Composite soil sample was prepared from each of the land uses by thorough mixing and sieved (< 2mm). The coarse materials such as stones, litters and roots were removed manually. A combined glass-calomel electrode was used to determine the pH of aqueous suspensions (1:2.5 soil/solution ratios). Electrical conductivity (EC; dS m⁻¹) was measured in the supernatant liquid of soil/water suspension (1:2) with conductivity bridge (Richards 1954). The soil organic carbon (Walkley and Black 1934), available nitrogen (Subbiah and Asija 1956) and potassium (Rich 1965) were estimated by standard methods. Chloroform fumigation extraction method (Vance et al 1987) was used to determine soil microbial biomass C. Chloroform fumigated and non-fumigated soils were extracted by 0.5 mol L⁻¹ K₂SO₄ at a 1:4 soil to extractant ratio. The C concentration was estimated by titration (Anderson and Ingram 1993). The difference in C concentration between the fumigated and nonfumigated soils was calculated, and then this value was multiplied by 2.64 to obtain the MBC concentration as suggested by Vance et al (1987).

Statistical Analysis: For statistical analysis of data, Microsoft Excel (Microsoft Corporation, Pullman, Wash.) and MSTATC packages (MSTATC, East Lansing, Mich.) were used.

RESULTS AND DISCUSSION

Soil characteristics: The soil was sandy loamy in texture

with soil pH from 5.80 to 6.25, soil organic carbon from 1.4 to 2.5 %, soil available phosphorus from 29 to 62 Kg ha⁻¹ and potassium from 180 to 360 Kg ha⁻¹ (Table 1).

Soil organic carbon and available nutrients: The lowest SOC among land use was in agriculture (0.95 to 1.44%) and the highest in Quercus (1.79 to 2.54%), followed by mixed forest (Table 2). The SOC also varied significantly among different depth in all the land uses. The surface soil (0-15 cm) revealed maximum SOC, followed by subsurface soil (15-30 cm). The land use impact on SOC was inconsistent at lower depth. With increase in soil depth, the variation among land uses with respect to SOC decreased. Soil organic carbon varied significantly among land uses and soil depth. Higher SOC was in Quercus (2.54%), followed by mixed, Deodar, Chir and least in agriculture. A decrease in SOC with increase in soil depth upto 30 cm was observed but beyond 30 cm, results were inconsistent. The soil available nitrogen was in medium range under different land uses. Agricultural land had lower range of soil available nitrogen (236.2 to 285.4 Kg ha⁻¹) which was significantly lower than Quercus. Among forest land uses, Deodar had the lowest soil available nitrogen, at surface soil. Soil nitrogen showed significant variation among land uses upto 30 cm depth, whereas, the variation was inconsistent at lower depths. Soil available potassium ranged between 186.3 to 317.3 Kg ha⁻¹, in surface soil. The higher potassium was available in Quercus which was 70.4% higher than agriculture. The available potassium decreased with increase in depth, but depth effect was non-significant.

Soil microbial biomass carbon under different land uses: MBC in monsoon was higher in Quercus (572.9 µg g⁻¹ soil) which was statistically higher from all other land uses. The lowest MBC was in agricultural lands (260.2 µg g⁻¹) in surface soil, which was 120% lower than Quercus. There was a consistent trend of decrease in MBC with increase in soil depth. Among all depths and land use, the lowest MBC (91.3 µg g⁻¹ soil) was in agricultural land at 45 to 60 cm soil depth. Following the trend of MBC in monsoon, summer as well as winter season MBC were higher in surface soil of Quercus landuse. The lowest MBC was in lower depths of agriculture land in summer in winter. The effect of land use decreased as the soil depth increased and this effect was more prominent during winter. Results in this study indicated that the four forest types showed significant variation with respect to soil microbial biomass carbon, soil organic carbon and available nutrients. The soil properties across three seasons and four soil depths for all the forest types revealed that oak forest was the richest in terms of soil organic carbon, available nutrients and microbial biomass carbon, followed by mixed forest and least was in agricultural land. The range of MBC reported in

the present study ($572.9 \mu\text{g g}^{-1}$ to $37.9 \mu\text{g g}^{-1}$) is well within the range reported by Vance et al (1987) for various temperate and tropical forest soils ($61 \mu\text{g g}^{-1}$ to $2000 \mu\text{g g}^{-1}$, Devi and Yadava (2006) for the mixed oak forest ecosystem ($71 \mu\text{g g}^{-1}$ to $1412 \mu\text{g g}^{-1}$) and Ravindran and Yang (2015) for subalpine mountain forest soils ($140 \mu\text{g g}^{-1}$ to $1320 \mu\text{g g}^{-1}$).

The MBC values were significantly higher in oak forest,

Table 1. Physico-chemical properties of experimental soil

Parameter	Range of values
pH	5.80- 6.25
EC (dS m^{-1})	0.06 – 0.39
Organic carbon (%)	1.4 – 2.5
P_2O_5 (kg ha^{-1})	29 - 62
K_2O (kg ha^{-1})	180 - 360
Available Cu (ppm)	3.50 – 11.5
Available Mn (ppm)	4.60 – 19.1
Available Fe (ppm)	5.7 -24.7
Available Zn (ppm)	0.3 – 1.25

Table 2. Effect of land-use systems on soil organic carbon

Land use		0-15	15-30	30-45	45-60
<i>Quercus leucotricophora</i>	T1	2.54	2.03	1.79	1.81
<i>Pinus roxburghii</i>	T2	1.86	1.55	1.47	1.48
<i>Cedrus deodara</i>	T3	1.88	1.46	1.48	1.52
Mixed forest	T4	2.16	1.85	1.50	1.48
Agriculture	T5	1.44	1.16	1.07	0.95
CD (0.05)		Land use 0.32		Soil depth 0.21	

Table 3. Effect of land-use system on soil available nitrogen and potassium (Kg ha^{-1})

Land uses	0-15	15-30	30-45	45-60
Soil available nitrogen (Kg ha ⁻¹)				
<i>Quercus leucotricophora</i>	349.1	311.5	289.6	265.5
<i>Pinus roxburghii</i>	304.2	271.8	280.1	272.8
<i>Cedrus deodara</i>	302.1	258.2	231.0	245.7
Mixed forest	325.1	283.3	260.3	239.4
Agriculture	285.4	241.5	236.2	255.1
CD (p=0.05)	Land use 28.70		Soil depth 33.1	
Soil available potassium (Kg ha ⁻¹)				
<i>Quercus leucotricophora</i>	317.3	290.5	266.2	255.4
<i>Pinus roxburghii</i>	268.4	177.0	192.6	196.4
<i>Cedrus deodara</i>	285.6	198.6	199.7	201.2
Mixed forest	354.3	339.4	278.9	255.0
Agriculture	186.3	191.9	168.4	174.3
CD (p=0.05)	Land use 32.70		Soil depth NS	

Table 4. Effect of land-use system on soil microbial biomass carbon

Land use		0-15	15-30	30-45	45-60
Monsoon					
<i>Quercus leucotricophora</i>	T1	572.9	437.1	301.4	112.8
<i>Pinus roxburghii</i>	T2	316.3	238.4	224.1	127.9
<i>Cedrus deodara</i>	T3	351.7	306.9	185.4	119.9
Mixed forest	T4	446.6	328.6	272.2	97.3
Agriculture	T5	260.2	191.1	125.1	91.3
CD (p=0.05)		Land use 87.50		Soil depth 58.68	
Summer					
<i>Quercus leucotricophora</i>	T1	404.2	335.8	200.9	97.7
<i>Pinus roxburghii</i>	T2	270.6	220.8	150.7	104.0
<i>Cedrus deodara</i>	T3	288.4	234.4	140.6	101.0
Mixed forest	T4	341.1	245.4	238.5	114.1
Agriculture	T5	193.4	133.7	95.4	87.6
CD (p=0.05)		Land use 107.98		Soil depth 85.33	
Winter					
<i>Quercus leucotricophora</i>	T1	270.6	151.3	56.6	37.9
<i>Pinus roxburghii</i>	T2	151.2	140.2	74.3	44.8
<i>Cedrus deodara</i>	T3	179.4	143.7	66.7	44.8
Mixed forest	T4	211.0	150.8	62.5	47.4
Agriculture	T5	84.4	135.9	75.8	37.9
CD (p=0.05)		Land use 81.10		Soil depth 66.44	

followed by mixed forest, followed by deodar, pine and least in agriculture, in all the seasons. Microbial biomass carbon was found to be higher in the surface soil layer (0-15cm) and decreased as the soil depth increased. The MBC results are also in line of the results of Sharma et al. (2004) from Sikkim (219 to $864 \mu\text{g g}^{-1}$) and Gosai et al (2010) for a rainfed agricultural field in north east India (275.70 – $498.17 \mu\text{g g}^{-1}$). However, the present values of MBC was on lower side in comparison to the report by reported by Arunachalam and Arunachalam (2000) for subtropical forests ($978 \mu\text{g g}^{-1}$ to $2088 \mu\text{g g}^{-1}$). However, the microbial biomass carbon in present study is higher than the reports of Arunachalam and Pandey (2003) from north eastern India (175.21 – $200.04 \mu\text{g g}^{-1}$) and Mahmood et al (2005) for Faisalabad, Pakistan (11 – $169 \mu\text{g g}^{-1}$). The differences in soil microbial biomass C in present study may be attributed to differences in vegetation, soil moisture, types of land use and management, as well as seasonal effect (Murrieta et al 2007). Differences in the quantities and qualities of organic litter input to the soil caused by different species of plants alters nutrient cycling processes which is consequently reflected in variation in soil microbial community (Grierson and Adorns 2000).

Seasonal variation of MBC: Dry and rainy seasons are two

extreme climatic conditions, that significantly affects productivity, nutrient cycling and soil microbial biomass. Several studies have earlier reported seasonal variability of microbial biomass, with soil microbial biomass in both dry (Montaño et al 2007) and wet seasons (Devi and Yadava 2006). The microbial biomass carbon was significantly higher

during the rainy season and lower in winter season, irrespective of land uses. This may be due to higher immobilization of nutrients by the microbes from the decomposing litters as microbial activities are maximum during rainy season. Low value of microbial biomass carbon in dry and cool winter season may be due to low activities of

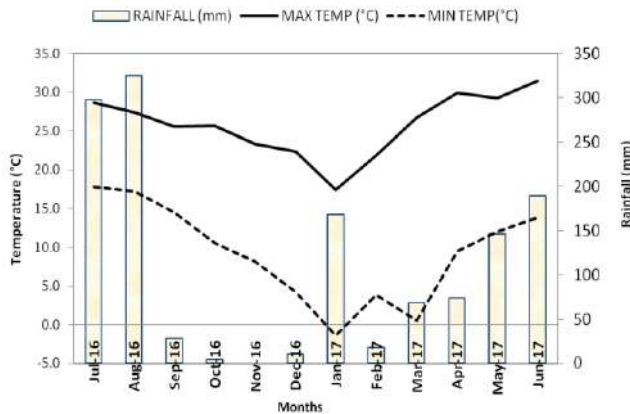


Fig. 1. Meteorological parameters during the study period

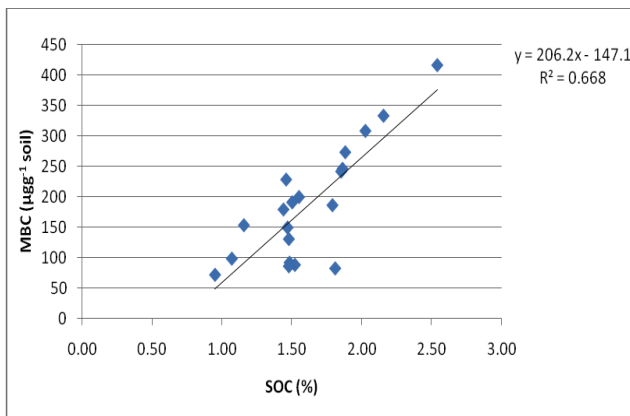


Fig. 2. Relationship between soil microbial biomass carbon with soil organic carbon

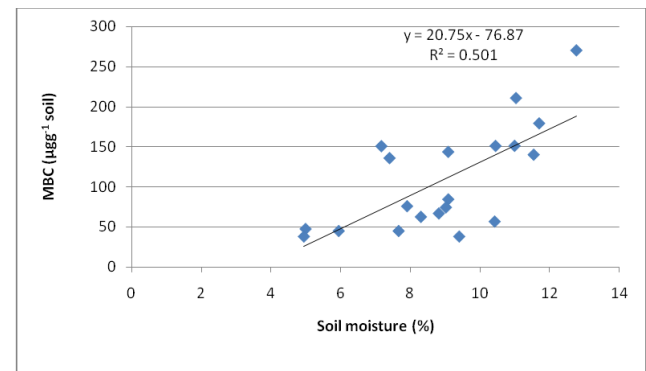


Fig. 3c. Relationship between soil microbial biomass carbon with soil moisture in winter

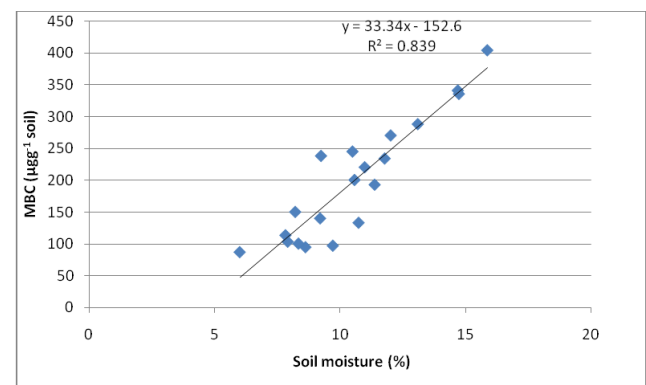


Fig. 3d. Relationship between soil microbial biomass carbon with soil moisture in summer

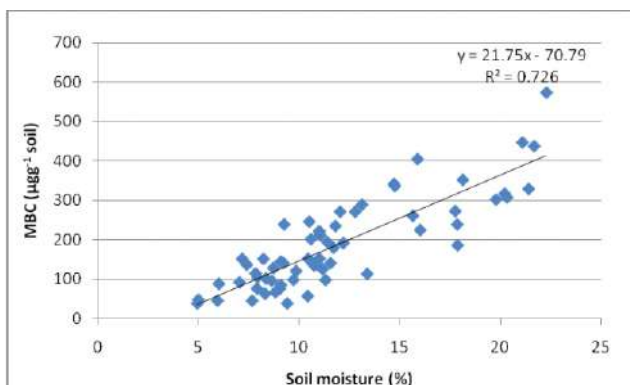


Fig. 3a. Relationship between soil microbial biomass carbon with soil moisture (averaged over 3 seasons)

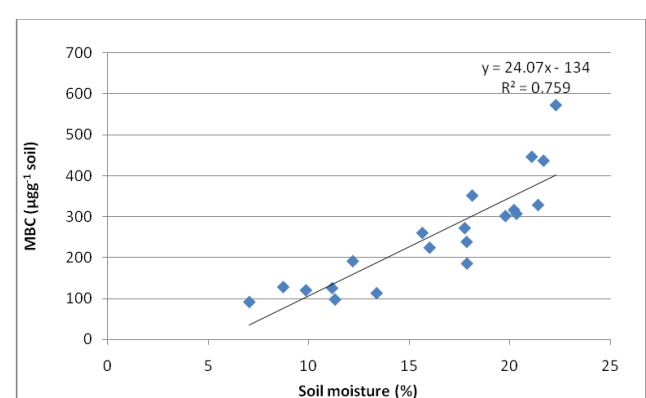


Fig. 3b. Relationship between soil microbial biomass carbon with soil moisture in rainy season

microorganisms and slow rate of decomposition of litters. Diaz- Ravina et al (1995) reported that water scarcity is a major limiting factor for microbial biomass, as compared to temperature since lower microbial biomass contents were observed in dry than in wet period. For a deciduous forest in north-east India, Devi and Yadava (2006), reported the highest contents of MBC during the rainy season and the lowest contents during the dry winter period. In subtropical soils of China, more MBC was present in the hot-humid than in the cold-dry season (Iqbal et al 2010). In contrary to the present study, in tropical dry deciduous forest, savanna and temperate pastures peak values were reported during early spring or summer (Saratchandra et al 1984) and in subtropical humid forest maximum value was observed in winter season (Arunachalam and Arunachalam 2000), which may be due to differences in quality of litter and rainfall pattern. The wide variability of microbial biomass under a range of forest and climatic scenarios suggest that seasonal variations in soil microbial biomass probably depend not only on the specific forest ecosystems, climatic factors but also on soil type, land-use, vegetation, topography.

Depth variation of microbial biomass carbon parameter in different land use: The highest amount of microbial biomass carbon (MBC) was observed in upper layers and it decreased with increase in soil depth in all the seasons. The relatively greater accumulation of litter in upper soil layers may facilitate the growth of microbial populations and the accumulation of C in microbial biomass. This is common in many ecosystems because much of the organic input is localized on soil surface. Earlier reports revealed that in Senegal, MBC in a savannah ecosystem was most abundant in upper soil surface and near Acacia trees (Fall et al 2012). Blume et al (2002) observed that upper soil layers of 0–20 cm in semiarid forest soils of Spain showed a much higher microbial population than lower layers. For humid subtropical climate in southern Brazil, Babujia et al (2010) reported more MBC in surface than in sub surface soil. A similar trend of microbial abundance had been reported in the profiles of agricultural fields (Taylor et al 2002) and Fushan forest soils (Tsai et al 2007).

Relationship between soil microbial biomass carbon and soil characteristics: The significant positive relationship between the soil microbial biomass C with soil organic C and soil moisture was observed. A linear regression equation was fitted to see the relationship between SOC and mean MBC. The two parameters are interdependent on each other, with R^2 of 0.67. Thus with increase in SOC the microbial biomass carbon increased. Gravimetric soil moisture was also plotted against MBC which showed that as the moisture content increased, soil

microbial activity also increased (Fig. 3). The strong positive relationship ($R^2 = 0.726$) was between the two parameters. The MBC was strongly related to soil organic carbon and soil moisture as evident from significant positive correlation between the parameters over all the seasons. These findings agrees with earlier reports Jia et al (2005) and Wang and Wang (2011) who reported strong positive correlation between soil organic carbon and soil microbial biomass carbon. The microbial biomass dynamics may be attributed to differences in quantity and quality of organic carbon input and labile soil organic matter (Smolander and Kituen 2002, Steenwerth et al 2002). Positive significant correlation has been found between MBC and SOC in the present study which establishes that soil organic C strongly affects the size and activity of soil microbial biomass. Similar relationships between these soil properties and microbial biomass have been observed in several forest based land uses by Arunachalam and Arunachalam (2000). Chen et al (2006) reported that organic matter acts as a substrate for soil microbial biomass; therefore, any reduction in soil organic carbon results into decrease in soil microbial biomass. The availability of carbonaceous inputs and substrates from the decomposing litter fall and root decay are important factors for supplying energy for microbial populations. Poor litter quantity and quality, such as pine litter being rich in recalcitrant compounds, lead to a slower rate of decomposition and consequently slower nutrient (Leckie et al 2004), which may results into lower microbial biomass carbon in pine forest. The seasonal dynamics of soil water content coincided with the seasonal variation in rainfall of the study area. The seasonal variations in soil moisture may be responsible for the variation in MBC in all the land uses, as soil water content was significantly related to MBC concentrations. Chen et al (2003) also found similar relationships between soil water content and MBC in pine plantations in the subtropical zone.

CONCLUSION

The soil microbial biomass is strongly influenced by seasonal variation. Soil moisture and soil organic carbon exhibits strong positive correlation with soil microbial biomass carbon. The results also indicate that different forest based ecosystems affect growth and activity of microbial population due to variation in quality and quantity of litter produced. High microbial biomass carbon during rainy season may be considered as a nutrient conservation strategy. The seasonal variation in climatic conditions changes soil moisture dynamics and thus leads to variation in MBC in different ecosystems.

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Diversity and Antibacterial Activity of Endophytic Fungi In *Memecylon umbellatum* Burm. F.- A Medicinal Plant in the Western Ghats of Karnataka, India

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Abstract: Endophytic fungi in *Memecylon umbellatum* were isolated by potato dextrose agar (PDA), malt extract agar or moist blotter methods, and studied for species richness, diversity and evenness. Sixty-three species of 22 genera of fungi and 10 morphotype isolates were documented. Certain morphotypes were characterized as *Endomelanconiopsis endophytica*, *Lasiodiplodia iranensis*, *L. theobromae* and *Phyllosticta capitalensis* by ITS regions of rDNA. *Aspergillus niger*, *Cochlibolus lunatus* and *Pestalotiopsis guepinii* were dominant species in leaves, bark and fruits. Winter season supported the high fungal incidence of species of *Cochlibolus*, *Bipolaris* and *Khuskia*. PDA supported high fungal expression in foliages during winter. *Cochlibolus affinis* and *P. guepinii* expressed high antibacterial activity against clinical bacterial strains. The study suggested rich endophytic fungal diversity in *M. umbellatum* which depended on isolation methods, seasons and plant parts, and some of them could be the future source of novel compounds with biological activities.

Keywords: Fungal diversity, *Pestalotiopsis*, *Endomelanconiopsis*, *Phyllosticta*, Season-specific, Incubation method

Endophytes, an important component of fungal diversity, reside in plant tissues at some stage in their life cycle without causing apparent damage to their host plant species (Bacon and White 2000). Endophytes are diverse species and ubiquitous with wide host range (Arnold et al 2000). Being capable of living under extreme and inhospitable conditions, endophytes occupy distinct biological niches and are known to produce secondary metabolites similar to that of the host (Strobel and Daisy 2003, Strobel et al 2004). The ability of endophytic fungi to stimulate plant growth, increase disease and pest resistance in host plants, improve plant's ability to withstand environmental stress and recycle nutrients have been attributed to the secondary metabolites produced by them, in return for space and nutrition provided by plants (Strobel and Daisy 2003). The endophytic fungal secondary metabolites are vested with antimicrobial, anticancer, neuroprotective, antioxidant or anti-parasitic activities (Gunatilaka 2006, Huang et al 2007, Lin et al 2008, Martinez-Luis et al 2008, Dai et al 2009). Consequently, plants with medicinal properties are being increasingly studied for their endophytic fungal association and the beneficial effects produced in such plants (Deshmuk et al 2009, Kaul et al 2012, Nisa et al 2015). The Western Ghats of India is rich in biodiversity and is a hotspot to valuable endemic and endangered medicinal plants and several undescribed plant species. The medicinal plants in this area have been studied for their endophytic assemblages and the ability of endophytic fungi to produce secondary metabolites with

bioactive potentialities have been recorded (Mani et al 2015, Shweta et al 2015). Suryanarayanan et al (2011) studied endophytic fungal association in 33 dicotyledonous tropical trees in Nilgiri Biosphere Reserve of the Western Ghats including *Memecylon malabaricum* of melastomataceae. A thorough literature survey indicated that *M. umbellatum* has not been studied for its endophytic fungal assemblages and their ability to produce bioactive compounds. *M. umbellatum* (common name - Ironwood), an endangered medicinal plant in the Western Ghat of India was selected for the study.

MATERIAL AND METHODS

Selection of study site: The plant species *M. umbellatum* growing abundantly in semi-evergreen forests of Sringeri (13°38'54" N 75°10'32" E) and Thirthahalli (13°25'30" 75°15'36" E) regions of the Western Ghats in Karnataka were identified for the collection of samples during rainy (June-September, winter (October-January) and summer (February - May) seasons of 2016–2018.

Collection of plant samples, incubation and fungal identification: Six apparently healthy trees of *M. umbellatum* (in triplicate, each with two individuals), having an approximate age of 30 years in each study region, were labeled for the collection of samples. Samples (leaves, bark, flowers and fruits) from each candidate tree were collected in sterilized polypropylene bags contained in cool containers and brought to the laboratory and subjected to a regime of surface disinfectant treatments with hydrogen peroxide

(0.2%, 2 min), ethanol (70%, 2 min) and sodium hypochlorite (0.3%, 2 min) and the effectiveness of surface disinfection was confirmed. The samples were segmented (1-2-cm segments) and were incubated by potato dextrose agar (PDA) and malt extract agar (MEA) amended with antibiotic ciprofloxacin (500 mg l⁻¹) or moist-blotter (MB) methods (Arnold et al 2000, Achar and Shivanna 2013, Rekha et al 2014) under 12/12h cycle of light/nUv light regime at 21±2°C for 5 to 12 days. The fungal species emerging from the incubated segments were identified based on the morphological characteristics (Subramanian 1971, Ellis 1976, Barnett and Hunter 1998). The nomenclature of fungal species was confirmed by visiting Index Fungorum (www.indexfungorum.org). The fungal units of non-sporulating fungi were observed for the presence of septa, croziers or clamp-connections. The fungal species that failed to sporulate even after prolonged incubation on media with or without nutrients under nUV light were considered as morphotypes.

Fermentation in culture broth and secondary metabolites extraction: Pure isolates of fungi were cultured on PDA separately and culture discs (5-mm-dia.) from 5-day old colony culture were inoculated into PD broth (pH 5.6) and incubated in dark at 21±2°C for 8-11 days with alternate shaking. The culture broth was filtered (three-layered muslin cloth) to separate out the mycelial mat (MM). The culture filtrate (CF) was filtered (three-layered Whatman no.1 filter paper discs) and the filtrate added with an equal volume of ethyl acetate (Himedia, Mumbai), mixed well and kept undisturbed (5 min.) to obtain two clear immiscible layers. The upper layer of ethyl acetate containing the compounds was extracted thrice and pooled. The MM was dried in an oven (34°C, 24h) and ground into a fine powder, which was suspended in methanol for 24h and the filtrate collected. The CF and MM extracts were evaporated to dryness at room temperature.

Antibacterial activity: The antibacterial activity was carried out by agar well diffusion technique (Atalla et al 2008, Yadav et al 2010) against nine bacterial species-human pathogens (*Escherichia coli*, MTCC-1599), (*Enterococcus faecalis*, MTCC-439), (*Klebsiella pneumoniae*, MTCC-7028), (*Pseudomonas aeruginosa*, MTCC-1934), (*Salmonella enteric*, MTCC-738), (*Salmonella typhi*, MTCC-734), (*Staphylococcus aureus*, MTCC-4734) and plant pathogens – (*Pseudomonas syringae*, MTCC-1604) and (*Xanthomonas campestris*, MTCC-228). The respective wells were loaded with 20 µL (1 mg ml⁻¹ of extract) of the sample in dimethyl sulfoxide (DMSO) and reference antimicrobial drug (amoxicillin, ciprofloxacin chloramphenicol and tetracycline) or DMSO was used as

positive and negative controls, respectively. The experiment was carried out in triplicate. The plates were incubated at 37°C overnight and the zone of inhibition (mm) was recorded (Valgas et al 2007)

Fungal DNA extraction, amplification and sequencing:

The fungal DNA was extracted from young fungal colony culture; the mycelium was ground in liquid nitrogen to obtain mycelium powder, which was extracted with lysis buffer (100 mM Tris HCl [pH 8.0], 50 mM EDTA, 3% SDS). The fungal tissue homogenate was added with RNAase (10mg mL⁻¹), incubated at 37°C for 15 min. and then with an equal volume of phenol: chloroform: Isoamyl alcohol (25:24:1) and centrifuged at 13,000 rpm for 10 min. To the upper aqueous layer equal volume of ethanol (100%) was added to precipitate at -20°C and centrifuged at 12,000 rpm to obtain the DNA pellet. The DNA pellets were air-dried and dissolved in 1× TE buffer (Doyle and Doyle 1987).

The ITS region of the ribosomal DNA was amplified by PCR using the primers ITS1-F (5'-CTTGGTCATTTAGAGG AAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR amplifications were done in a final volume of 50 µL, containing 2 µL of DNA, 0.5 mM of each primer, 150 mM of dNTP, 1U of *Taq* DNA polymerase (Promega), and PCR reaction buffer. Amplification was carried out in a thermal cycler with an initial denaturation of three min. at 94°C, followed by 35 cycles of one min. at 94°C, one min. at 50°C, one min. at 72°C, and a final extension of 10 min. at 72°C (White et al 1990). The PCR product was sequenced by Sanger' dideoxynucleotide chain termination method which was carried out in Chromous Biotech Pvt. Ltd. Bangalore, India. The sequences were submitted to Gen Bank, NCBI (<http://www.ncbi.nlm>). Sequences obtained in this study were compared with reference deposited sequences in the Gen Bank database, using BLAST.

Phylogenetic analyses: The ITS rDNA sequence was constructed using molecular evolutionary genetic analysis (MEGA) ver. 7.1. The sequence with >98% similarity were considered as closely related species, depending on percentage they were assigned to a taxon with closest BLAST match. The multiple sequences were aligned by CLUSTAL W (Thomson et al 1994) and the maximum likelihood method (ML) phylogenetic tree was constructed (Tamura et al 2011).

Statistical analyses: The data, of two-year trials following homogeneity (ANOVA, P =0.004), were averaged and subjected to the statistical analyses. The colonization frequency of endophytic fungi was determined by the method of Suryanaryana et al (2000). Species diversity, evenness, and richness were determined by Shannon's diversity and Simpson's evenness indices (bootstrap 999, 95%

confidence interval). The other analyses carried out included rarefaction curves, principal component analysis (PCA) and cluster analysis (PAST ver. 3).

RESULTS AND DISCUSSION

The incubation of plant segments by three different methods – PDA, MEA and MB yielded 1240 fungal isolates from 2200 segments of *M. umbellatum* collected during rainy, winter and summer seasons. The literature review indicated that more than one isolation method was employed for determining the endophytic fungal communities in medicinal plants (Kusari et al 2013, Manganyi et al 2018), using large number of samples (Gond et al 2007, Sahani and Hemalatha et al 2018). The fungal isolates were characterized into 63 species of 22 genera, in addition to 10 isolates of morphotypes. Further, they were grouped into 38 anamorphic and 26 teleomorphic fungi. Among the endophytic fungi, anamorphic fungal forms were frequently documented by different incubation methods (Rekha et al 2014, Singh et al 2017).

Incubation media and fungal communities: The occurrence of endophytic fungal species in different plant segments varied depending on the incubation methods. The colonization frequency of endophytes was 44% in PDA followed by 33% in MEA and 22% in MB methods (Table 1). The PDA supported the high incidences of *Pestalotiopsis guepinii* (86.3), followed by *Cladosporium herbarum* (40.27) and *Aspergillus niger* (39.07). The MEA method supported the high incidences of *Cochliobolus lunatus* (37.21), *Penicillium commune* (16.12) and *Fusarium chlamydosporum* (7.45), while MB supported *Alternaria alternata* (26.37), *Khushkia oryzae* (14.12) and *Chaetomium globosum* (8.54). Other than *P. guepinii*, species of *P. versicolor* and *P. neglecta* have also been found to be influenced by PDA (Kumaran et al 2010). In this study, certain species such as *C. reflexum*, *Graphium ulmi* and *A. ochraceus* occurred only on PDA, while species such as *Beltrina rhombica*, *Bipolaris specifera* and *Monodictys fluctuata* were recorded only on MB Method. The PDA media has been shown to support the high fungal occurrence of anamorphic and teleomorphic fungi as compared to other incubation media (Sandhu et al 2014, Singh et al 2017). On the other hand, the MEA medium has been also reported to be suitable for the expression of certain endophytic fungi (Rekha et al 2014, Chowdhary et al 2015). The PDA was shown to be detrimental to the slow-growing fungal species since they are often suppressed by the fast-growing ones (Sharma et al 2010, Hase et al 2017). As compared to the above methods, the MB is also found to be suitable in this study for certain fungi, since it induces stress and promotes

the sporulation of the culture (Achar and Shivanna 2013, Toma et al 2013).

Plant parts and endophytic fungal communities:

Different plant parts of *M. umbellatum* showed diverse endophytic fungal communities. The leaf supported high colonization frequency (46%) followed by bark, fruit and flower. Among the fungal species, *P. guepinii* (84.6) followed by *A. niger* and *C. herbarum* were isolated in the high frequency in the leaf region. Certain species like *A. alternata* (31.2) occurred frequently in the bark. The incubation of flowers yielded *P. rubrum* (7.8), *C. trifolii* (11.1) and *C. gracile* (6.29), while that of the fruit yielded *P. guepinii* (12.3), *K. oryzae* (8.9) and *P. commune* (6.6). Literature survey indicated that it is the foliage that were colonized predominantly by fungal endophytes (Kharwar et al 2008, Suryanarayanan et al 2011, Unterseher et al 2013). Earlier studies also documented the occurrence of species of *Pestalotiopsis* as the most common species in several plant taxa (Toofanee and Dulyamode, 2002; Wei et al 2007). The bark has been the next most studied plant region for endophytic fungal occurrence in *Azadirachta indica*, *Bauhinia forficata*, *Canavalia maritime* and *Pinus tabulaeform* (Puri, et al 2008, Verma et al 2011, Bezerra et al 2015). Examples of fungal species in the bark documented in this study included *M. fluctuata*, *F. solani*, *Purpureocillium liliacinum*, and *Verticillium* sp. In contrast to the documentation of endophytic fungi in the above plant parts, flowers of plants are the most neglected part for documenting the fungal endophytes. In this study, *Cochliobolus* sp. was detected in flowers of *M. umbellatum*. Similarly, endophytic fungi associated with fruits also find less mention in the literature (Verma et al 2011). Fruits of *M. umbellatum* were associated with *Lasiodiplodia theobromae* in addition to *P. guepinii* and latter species was also found associated with leaves and stem of *M. umbellatum*. This suggested that *P. guepinii* is an aggressive colonizer of foliage, bark and fruit. The prominently occurring fungi - *P. guepinii* and species of *Phomopsis* in this study have also been the source of antitumor and antimicrobial compounds (Wang et al 2012). Among the fungal species that occurred in *M. umbellatum*, *Aspergillus* species have been reported to produce secondary metabolites with ability to exhibit antitumor and anti-leishmanial activities (Sun et al 2008, Martinez-Luis et al 2012). Similarly, *Colletotrichum gloeosporioides* was isolated from *Piper nigrum* with ability to produce piperine which is known for the antimicrobial, antidepressant, anti-inflammatory and anticancer activities (Chithra et al 2014). The observations indicate possible utilization of endophytic fungal species of *M. umbellatum* in pharmaceutical industries.

In the present study, species of *Aspergillus*, *Penicillium*, *Cladosporium* and *Trichoderma* were also isolated in different frequencies. The above fungal species are commonly referred to as saprophytes rather than as

endophytes (Promputtha et al 2007, Rampadarath et al 2018). However, their occurrence on incubated tissues of *M. umbellatum* even after stringent surface disinfection regimes suggested their endophytic association. Some of the above

Table 1. Occurrence of endophytic fungal species in *M. umbellatum* by potato dextrose agar (PDA), malt extract agar (MEA) and moist blotter (MB) methods

Endophytic fungal isolates	Frequency of occurrence(%) ^{1/} incubation methods		
	PDA	MEA	MB
Anamorphic ascomycetes			
<i>Alternaria</i> spp. ²	1.29	0.76	0.038
<i>Aspergillus</i> spp. ³	5.34	3.55	2.064
<i>Diaporthe</i> spp.	0	0	0.060
<i>Fusarium</i> spp. ⁴	0.31	0.81	0.674
<i>Gliocladium roseum</i>	0	0.04	0.025
<i>Graphium</i> spp. ⁵	0.02	0	0.129
<i>Lasiodiplodia theobromae</i>	0	0	0.720
<i>Monodictys fluctuata</i>	0	0	0.11
<i>Myrothecium roridum</i>	0.37	0.15	0
<i>Khuskia oryzae</i>	0.67	1.14	1.139
<i>Penicillium</i> spp. ⁶	5.25	4.27	2.87
<i>Pestalotiopsis</i> spp. ⁷	13.6	10.1	5.96
<i>Purpleocillium lilacinum</i>	0	0	0.121
<i>Torula herbarum</i>	0	0.03	0
<i>Trichoderma viride</i>	0.28	0	0.072
<i>Verticillium</i> sp.	0	0.22	0
Total frequency	27.2	21.2	14.0
Teleomorphic ascomycetes			
<i>Beltrania rhombica</i>	0	0	0.319
<i>Bipolaris</i> spp. ⁸	0.08	0.10	0.075
<i>Chaetomium</i> spp. ⁹	0.63	1.12	0.68
<i>Cladosporium</i> spp. ¹⁰	5.76	3.96	1.14
<i>Colletotrichum</i> spp. ¹¹	1.56	0.33	0.47
<i>Cochliobolus</i> spp. ¹²	4.19	4.43	2.26
<i>Phoma</i> spp. ¹³	1.33	0.08	0.33
Total frequency	12.8	8.82	4.22
Morphotypes ¹	3.13	2.02	0.40

Note: ¹Fungal endophyte occurrence was calculated based on the number of segments colonized by each fungus over the total number of segment studied and represented as percentage, Data is an average of 3 replicates with 270 segments in each replicate, PDA = potato dextrose agar; MEA= Malt extract agar; MB= Moist blotter method; ²*Alternaria* spp.=*A. alternata* (0.96, 0.32 and 2.1), *A. tenuissima* (1.2, 0.7 and 0.03); ³*Aspergillus* spp. = *A. awamori* (0.016, 0 and 0.06), *A. flavus* (0.33, 0.19 and 0.15), *A. fumigatus* (0.92, 0.58 and 0.025), *A. nidulans* (0.5, 0.02 and 0), *A. niger* (3.1, 2.68 and 1.56), *A. ochraceus* (0.03, 0 and 0), *A. rubrum* (0.016, 0 and 0), *A. stellatus* (0.1, 0.09 and 0), *A. versicolor* (0.122, 0 and 0); ⁴*Bipolaris* spp. = *B. halodes* (0.08, 0.10 and 0.07), *B. specifera* (0, 0 and 0.02); ⁵*Chaetomium* spp. = *C. globosum* (0.22, 0.06 and 0.68), *C. reflexum* (0.09, 0 and 0), *C. robustum* (0.06, 0 and 0); ⁶*Cladosporium* spp. = *C. cladosporioides* (2.4, 0.5 and 0.5), *C. herbarum* (3.08, 3 and 0.11), *C. oxysporum* (0, 0.08 and 0), *C. sphaerospermum* (0.07, 0.17 and 0.02); ⁷*Colletotrichum* spp. = *C. acutatum* (0.63, 0.11 and 0.06); *C. boninense* (0, 0.2 and 0), *C. gloeosporioides* (0.09, 0.03 and 0.35); *C. lentis* (0.3, 0.2 and 0.3), *Colletotrichum* sp. 2 (0, 0.20 and 0); ⁸*Cochliobolus* spp. = *C. affinis* (0.2, 0.6 and 0.64), *C. eragrostidis* (0.7, 0.6 and 0.7), *C. geniculata* (0.08, 0.03 and 0), *C. lunatus* (2.6, 3.0 and 0.83), *C. trifolii* (0.49, 0.12 and 0.21); ⁹*Fusarium* spp. = *F. chlamydosporum* (0.07, 0.06 and 0.5), *F. oxysporum* (0.24, 0.21 and 0.06), *F. solani* (0, 0.05 and 0); ¹⁰*Graphium* spp. = *G. ulmi* (0.038, 0.024 and 0.02); *G. penicilloides* (0, 0 and 0.34); Morphotypes/Non sporulating fungi: Morphotype 1 (1.03, 0.99 and 0.2), Morphotype 2 (0, 0.26 and 0), Morphotype 3 (0.51, 0.10 and 0), Morphotype 4 (1.08, 0.2 and 0), Morphotype 5 (0.3, 0.2 and 0.12), Morphotype 6 (0.4, 0 and 0), Morphotype 7 (0, 0.02 and 0.1), Morphotype 8 (0.05, 0.07 and 0), Morphotype 9 (0.04, 0 and 0), Morphotype 10 (0.04, 0 and 0); ¹¹*Penicillium* spp. = *P. aurenticulum* (0.36, 1.6 and 0.2), *P. chrysogenum* (0.77, 1.2 and 0.6); *P. citrinum* (2.4, 0.9 and 1.9), *P. commune* (1.3, 1.2 and 0.4), *P. glabrum* (0, 0 and 0.03), *P. rubrum* (0, 0 and 0.03), *Penicillium* sp.2 (0.007, 0 and 0); *Penicillium* sp.3 (0, 0 and 0.03); *Penicillium* sp.4 (0, 0 and 0.03); ¹²*Pestalotiopsis* spp. = *P. glandicola* (2.1, 2.0 and 0.5), *P. guepinii* (5.8, 4.09 and 2.704); *P. microspora* (2.9, 3.6 and 2.3); ¹³*Phoma* spp. = *P. eupyrena* (3.74, 0.95 and 0.9); *P. glomerata* (0.5, 0 and 0.08); *P. humicola* (0, 0.7 and 0.011).

species were also studied for their endophytic association in certain plant species (Kasim 2011, Kusari et al 2013).

Seasons and endophytic fungal communities: The seasonal variation influenced the occurrence of endophytic fungi in *M. umbellatum*. The colonization frequency of endophytic fungi was high during the winter season (42%) followed by rainy and summer. Fungi like *C. herbarum* (24.06), *C. lunatus* (62.6) and morphotype isolate 1 (120.4) occurred predominantly during winter, however *P. guepinii* (81.8), *C. gloeosporioides* (12.8) and *F. chlamydosporum* (3.22) occurred frequently during the rainy season, while *A. alternata* (14.32), *B. halodes* and *C. robustum* (2.66) were dominant during summer. The high level of colonization was also reported, by endophytic fungi, in shrubby medicinal plants and perennial grass species during the winter season (Naik et al 2008, Vasanthakumari and Shivanna 2011). *Bipolaris specifera*, *C. gracile*, *Monodictys fluctuata*, *K. oryzae* and *Diaporthe* sp. were recorded only during the winter season. There are also contrasting reports of high frequencies of fungal colonization during monsoon in *Cinnamomum bejolghota*, *Nyctanthes arbor-tristis* and *Tectona grandis* (Murali et al 2007, Suwannarach et al 2012, Nalini et al 2014, Singh et al 2017). In the present study, certain fungal species were season-specific; for example, *C. orbiculare*, *G. roseum* and *G. penicillioides* occurred during rainy while, *A. versicolor* and *Penicillium* spp. during the summer season. Guo et al (2008) documented the lowest colonization and isolation rates of endophytic fungi in *Pinus tabulaeformis* during summer. This was attributed to the newly growing needles and twigs in the summer season when the endophytic infections were lowest. The precipitations occurring prior to the winter solubilized mineral nutrients in the soil that supported the growth of plants as well as the endophytic fungi. This could be the reason behind the enhanced frequency of endophytic fungal occurrence in winter. On the other hand, the water deficit condition in certain plants might lead to the accumulation of non-structural carbohydrates leading to the build-up of carbon-based defenses such as tannins. The endophyte distribution within the plant is controlled by genes of both the plant and endophyte and modulated by the environmental factors, which could be attributed to the role of seasonal influence on endophytic expression in a host plant (Moricca et al 2008).

Diversity indices: Diversity indices of fungal endophytes varied depending on the incubation method employed, plant regions as well as seasons. In the present study, high species richness (55) was recorded in PDA than in MEA (49) and MB (44). This indicated that PDA supported the high expression of many fungal species, while MEA supported only the specific fungal species, as discussed previously. Shannon

diversity (H') and Shannon evenness (J') indices, as well as Simpson's diversity (D') and Simpson's evenness (E') indices, were again high on PDA when compared to MEA and MB methods (Table 2). Rekha et al (2014) also supported the present study. The number of taxa is more in the leaf (55) and bark (50) than in flower (11) and fruit (16). This is supported by Simpson's diversity and Shannon's evenness indices which were high for leaves when compared to bark, flower, and fruit. Similar observations of high fungal diversity in the leaf region were also documented in certain medicinal plants (Kharwar et al 2008, Suryanarayanan et al 2011, Verma et al 2011). In the present study, the number of fungal species occurring in the winter season was 47, while it was 41 in rainy and 38 in summer seasons, respectively (Fig. 1, Table 2). This is indicative of the fact that seasons play an important role in the supporting the endophytic fungal diversity in *M. umbellatum*, as suggested by previous workers (Naik et al 2008, Vasanthakumari and Shivanna 2011).

Rarefaction index indicated that the number of fungal species always increased with an increase in the number of isolations from different plant parts, particularly from leaf and bark in all seasons. However, in the case of flowers and fruits, the increase in endophytic fungal occurrence was season-specific (Fig. 2) since the flowering and fruiting ended by June and August, respectively. Rarefaction index has used to explain the increase in fungal frequency with the number of isolation (Seena and Sridhar 2004, Nalini et al 2014, Li et al 2016). In the present study, the bi-plot generated by PCA suggested the season specificity of endophytic fungi in *M. umbellatum*. The plot showed 87.7% of the variance in three different seasons. The incidence of species of *Cochliobolus*, *Bipolaris*, and *K. oryzae* highly correlated with the winter season, while species of *Pestalotiopsis*, *Aspergillus* and *Penicillium* showed a strong relationship with the rainy season and *Alternaria* spp. with the summer season (Fig. 3).

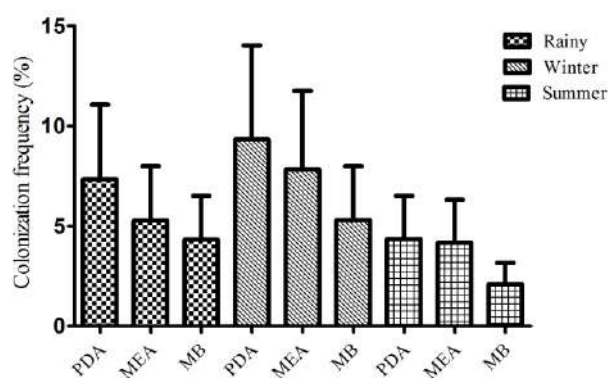


Fig. 1. Occurrence of endophytic fungal species in *M. umbellatum* by three incubation methods during different seasons

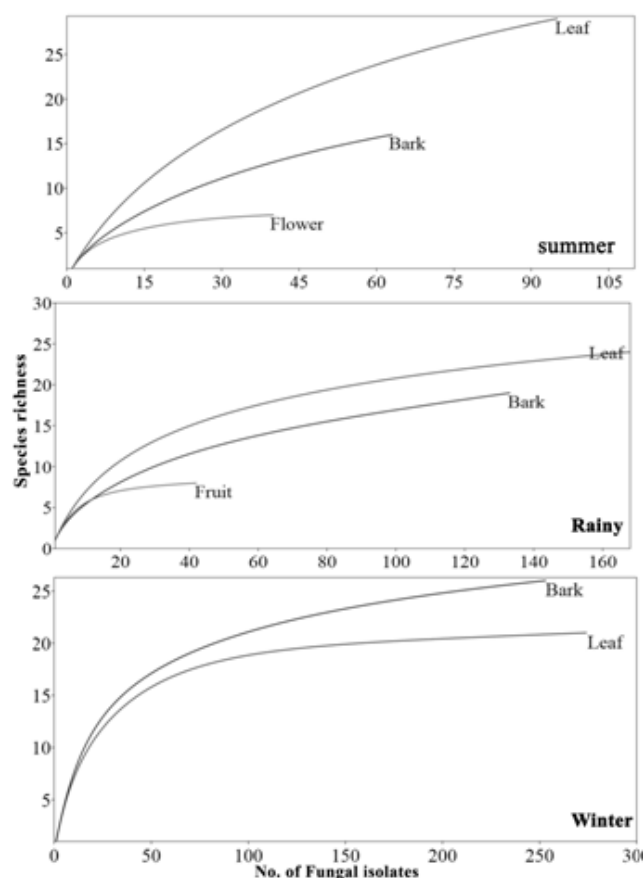


Fig. 2. Rarefaction curve of endophytic fungal species occurring in different plant parts of *M. umbellatum* during summer, rainy and winter seasons

Similar observations were made also in case of *Cinnamomum bejolghota*, *Tectona grandis* and *Taxus globosa* in relation to season-specific fungal correlations of endophytic fungi (Rivera-Orduña et al 2011, Suwannarach et al 2012, Singh et al 2017).

Phylogenetic analysis: Certain morphotypes frequently occurring in PDA/MEA media and that failed to sporulate even after prolonged exposure to nUV light were characterized based on ITS regions of rDNA (Table 3). The ITS sequences of six endophytic fungi were compared with the closest sequence (98% identity) obtained during BLAST

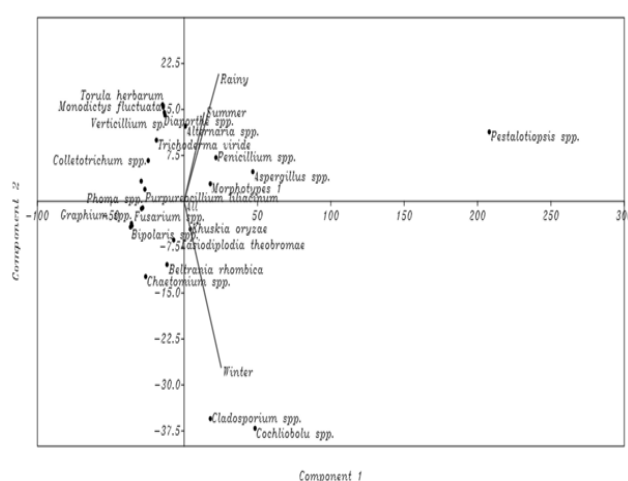


Fig. 3. Principal component analysis (PCA) of fungal endophytes of *M. umbellatum* occurring in different seasons

Table 2. Species richness, diversity and evenness indices of endophytic fungal communities occurring in different locations, seasons, plants parts and incubation methods*

Sample units	Species richness	Diversity indices		Evenness indices		Total no. of isolates
		Shannon diversity (H')	Simpson's diversity (D)	Shannon evenness (J)	Simpson's evenness (E)	
Loc 1	58	3.09	15.2	0.77	0.069	637
Loc 2	48	2.95	13.7	0.76	0.08	420
PDA	54	3.21	14.8	0.8	0.06	531
MEA	49	3.14	14.9	0.77	0.06	401
MB	48	2.95	12.9	0.77	0.041	251
Leaf	54	3.08	14.9	0.76	0.069	531
Bark	50	2.90	14.1	0.75	0.057	468
Flower	11	2.21	3.75	0.92	0.093	61
Fruit	16	2.63	5.26	0.95	0.078	97
Summer	38	2.82	12.7	0.047	0.73	238
Rainy	41	2.80	11.6	0.762	0.085	420
Winter	47	2.93	12.7	0.08	0.73	506

*Data is an average of three replicates, each with 270 samples

and based on the similarity of sequences, the phylogenetic tree was constructed (Table 3 Fig. 4). *Colletotrichum* species have been isolated from several tropical plants are well-

known plant pathogens and which cause anthracnose (James et al 2014). Pandi et al (2011) reported that *Lasiodiplodia theobromae*, an endophytic in *Morinda citrifolia*

Table 3. Fungal endophytes obtained from *M. umbellatum* with the Gen-bank accession number and the closest match retrieved from NCBI

Strain number	Plant parts	Gen bank accession number	Morphological identification	Closest NCBI match	Similarity	Reference accession number
G3	Leaf	MK816966	<i>Colletotrichum gloeosporioides</i>	<i>Colletotrichum gloeosporioides</i>	99.81%	DQ453993.1
G7	Leaf	MK816978	<i>Colletotrichum</i> sp. 2	<i>Colletotrichum siamense</i>	100.00%	MG642980.1
G5	Leaf	MK621278	Morphotype 5	<i>Endomelanconiopsis endophytica</i>	100.00%	KU997686.1
G6	Leaf	MK826035	Morphotype 6	<i>Lasiodiplodia iranensis</i>	100.00%	MK529998.1
G2	Fruit	MK816958	Morphotype 2	<i>Lasiodiplodia theobromae</i>	99.42%	MH265980.1
G8	Leaf	MK621278	Morphotype 8	<i>Phyllosticta capitalensis</i>	99.83%	MF595520.1

Table 4. Antibacterial activities of endophytic fungi isolated from *M. umbellatum*

Fungal species	Con.mg m L ⁻¹	Zone of inhibition by the test bacterial strain (mm)						
		EC	PA	PS	SA	SE	ST	XC
Ethyl acetate extract of culture filtrate								
<i>Cochliobolus</i> sp.	100	12±0.152	19±0	19±0.57	21±0	-	20±0	-
	50	4±0.23	10±0	9±0.1	13±0.19	-	9.6±0.05	-
<i>Pestalotiopsis microspora</i>	100	17±0.2	-	-	-	-	-	18±0.1
	50	5±0.1	-	-	-	-	-	8±0.1
<i>Pestalotiopsis guepinii</i>	100	19±0.15	-	-	-	16±0.06	-	18±0
	50	8±0.26	-	-	-	6±0.15	-	9±0.05
<i>Khuskia oryzae</i>	100	-	-	-	10±0.15	-	-	16±0.06
	50	-	-	-	4±0.12	-	-	6±0.15
Methanol extract of mycelia mat								
<i>Cochliobolus</i> sp.	100	12±0.1	15.6±0.57	16±0.057	17±0.20	-	15±0.05	-
	50	9±0	8.6±0.057	5±0	9±0.05	-	9±0.1	-
<i>Pestalotiopsis microspora</i>	100	15±0.15	-	-	-	-	-	-
	50	4±0.05	-	-	-	-	-	-
<i>Pestalotiopsis guepinii</i>	100	12±0	-	-	-	14±0.1	-	14±0.1
	50	4±0.05	-	-	-	3±0.06	-	3±0.06
<i>Cochliobolus</i> sp.	100	12±0.1	15.6±0.57	16±0.057	17±0.20	-	15±0.05	-
	50	9±0	8.6±0.057	5±0	9±0.05	-	9±0.1	-
<i>Pestalotiopsis microspora</i>	100	15±0.15	-	-	-	-	-	-
	50	4±0.05	-	-	-	-	-	-
<i>Khuskia oryzae</i>	100	-	-	-	15±0.11	-	-	14±0.1
	50	-	-	-	7±0.1	-	-	3±0.06
Amoxicillin	100	20±0.05	20±0.05	19±0.26	20±0.11	18±0.11	19±0.01	18±0.26
Chloramphenicol	100	25±0.1	21±0.01	21±0.11	20±0.05	25±0.01	20±0.01	20±0.11
Ciprofloxacin	100	29±0.05	30±0.05	30±0.26	29±0.26	29±0.26	29±0.0	29±0.05
Tetracycline	100	15±0.02	18±0.11	17±0.05	19±0.02	19±0.11	20±0.01	19±0.05
Control (DMSO)		-	-	-	-	-	-	-

EC= *Escherichia coli*; PA= *Pseudomonas aeruginosa*; PS = *Pseudomonas syringae*; SA = *Staphylococcus aureus*; SE = *Salmonella enterica*; ST = *Salmonella typhi*; XC = *Xanthomonas campestris*, 25mg/mL⁻¹ showed less activity; All the fungal species showed no activity against EF = *Enterococcus faecalis* and KP = *Klebsiella pneumoniae*

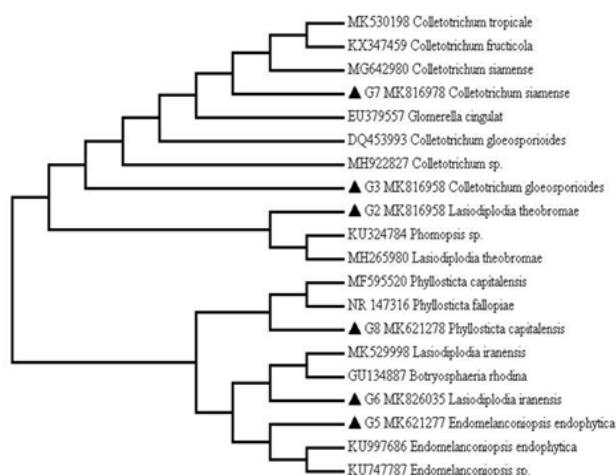


Fig. 4. Phylogenetic tree showing relationship between endophytic fungi isolated from *M. umbellatum* and other fungal species sequence sourced from gene bank. Black triangle represents the sequence obtained in the present study. The tree was constructed based on ITS1-5.8-ITS4 of rDNA by using maximum likelihood method. The bootstrap analysis was performed with 1000 replications

produces the anti-cancerous drug taxol. This is the first report of the occurrence of *Lasiodiplodia iranensis* as leaf endophytic fungi from *M. umbellatum*. Another endophyte *Phyllosticta capitalensis* distributed world-wide is documented as an endophyte and weak plant pathogen (Wikee et al 2017). Yet another fungus *Endomelanconiopsis endophytica* was also documented in *Ficus hirta* a medicine plant in Chinese folk medicine (Sun et al 2016). The above observations indicated that *M. umbellatum* is associated with a number of sporulating and morphotype endophytic fungi; some of them are being reported for the first time. These fungal species could be screened for their ability to produce secondary metabolites and their potential biological activities.

Antibacterial activity: The ethyl acetate and methanol extracts of four endophytic fungi exhibited potential antibacterial activity against test bacterial strains. The present study revealed that ethyl acetate extract of *Cochliobolus affinis* showed strong antibacterial activity against *P. aeruginosa*, *P. syringae*, *S. aureus*, and *S. typhi*, when compared to that of standard antibiotic compounds amoxicillin, ciprofloxacin and chloramphenicol. Based on the mode of action of the above antibiotics, the ethyl acetate extract of *C. affinis* could be suggested with the activity to inhibit colony growth of test bacteria due to the inhibitory effect on the synthesis of DNA gyrase, protein, and cell wall and cell division (Table 4). In the previous study, curvularin produced by a *Cochliobolus* species was shown with good

antimicrobial activity (Ataides et al 2018). In this study, ethyl acetate extract of CF of another fungal species *Pestalotiopsis guepinii* also produced a high zone of inhibition against *Escherichia coli*, *Salmonella enterica* and *Xanthomonas campestris*. Similarly, ethyl acetate extract of *P. microspora* also showed antibacterial activity against the two former bacterial pathogens. In this study, *Pestalotiopsis* species were isolated from all plant parts; *Pestalotiopsis* was described as a highly creative genus capable of producing a plethora of novel secondary metabolites including Paclitaxel (Strobel et al 1997), ambuic acid (Li et al 2001), pestalachloride A (Li et al 2008) and pestalotiopyrones A–I (Xu et al 2011) with antibacterial activity.

CONCLUSION

The present study indicated the occurrence of a large number of endophytic fungal diversity in *M. umbellatum*. The plant part, season and incubation method significantly influenced the colonization frequency of endophytic fungi. Potential endophytic fungal species documented are species of *Cochliobolus*, and *Pestalotiopsis*, which showed high antibacterial activity. These hidden treasures of plant microbes can be helpful for mankind.

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Extraction and Purification of α -amylase from Gastrointestinal Tract of *Cyprinus carpio*

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Abstract: This study included the extraction and purification of α -amylase from the gastrointestinal tract of fish using several stages involved, raw extract (crude), sedimentation with ammonium sulphate with different saturation rates, dewatering and gel filtration by the Sephadex G-100 gel. These processes were conducted under various conditions to find optimum pH and temperature of activity and enzymatic stability as well as determination of kinetic constants. The study also included estimation the molecular weight of the extracted enzyme according to the standard curve of the known molecular weight proteins. Results showed that the specific efficiency, enzymatic yield and the molecular weight of the enzyme were 1166.6 enzymatic units mg^{-1} of extracted solution, 12.3% and 52.8 kg Dalton respectively. The ideal temperature was 70 C° and the optimum pH ranged from 6.5 to 7.5. The maximum activity of the enzymatic interaction was 75.78 micromole min^{-1} respectively. The study aimed to purify and identify the alpha-amylase from the residual fish's intestines, as an available source to use it for preparing food and medical substances to improve human health.

Keywords: α -amylase, Enzymatic interaction, Enzymatic extraction, Enzymatic activity

Enzymes are recognized that their properties still constant during its chemical reactions so they have reduced energy activation through reactions. Amylase enzyme is one of useful hydrolyzed enzymes were used in industrial and food purposes. Each of enzymes is created for a specific task at specific time to achieve a specific role, no vital cells survived without its specific enzymes (Tallapragada et al 2017). There are two types of amylase enzyme: first one called alpha-amylase (Endo-hydrolase), its optimum pH is 7, which destroys glycoside bonds inside starch molecule, second one called beta amylase (Exo-hydrolase) that act at pH between 4-5, attacks starch molecule from final to inside part (Majid et al 2011).

Amylase enzyme is one of hydrolysis enzymes, its common form is alpha-amylase found in humans and other organisms, and is excreted from the salivary glands into mouth called amylase saliva. It is also extracted from pancreas, called pancreatic amylase, and is found in plants such as barley, rice and isolated from microorganisms bioactivities, its amount in plasma between 17-60 units ml^{-1} , the concentration may be increased when there are some cases of pancreatitis inflammation (Souza and Magalhaes 2010). This enzyme destroys glycoside bonds (1-4) of starch molecule at random sites to produce maltose and dextrin. External conditions affect enzyme activity like pH, temperature and banded metals to enzyme structure (Sufi 2010).

There are many plants, animals and microorganisms sources used to produce amylase. This study comprised

purification and defining alpha-amylase properties that extracted from intestines pancreas, liver of *Cyprinus carpio* fish and finding optimal conditions to obtain the highest enzyme activity and stability for using it in different useful applications like food and medicals preparation.

MATERIAL AND METHODS

Preparation of buffer solution for enzymatic extraction :

Phosphate buffer solution was prepared at concentration 0.2 M which included NaH_2PO_4 and Na_2HPO_4 at pH 6. This solution was used for amylase extracting (Tallapragada et al 2017).

Extraction method: Enzymatic extraction process was done according to method of Annamalai et al (2011) with some modifications. First stage included mixing of 100 g: 400 ml (intestinal materials: extracted solution) and this continued for 2 minutes by magnetic stirrer device then stored to next day at 4 C°, filtrated solution was isolated by using centrifugation at 5000 ppm for 30 min and filtrated solutions was used for estimating amylase enzymatic activity, protein concentration and extracted volume of enzyme. The second stage had included enzymatic purification using 100 ml of extraction, it comprised precipitation by ammonium sulphate in three grades saturation were 30, 60 and 80, starting at 30%, added 18.1 g of salt to obtain 60%, then added 12.9 g of salt to obtained 80%. Precipitated residual material of extraction was preserved at 4C°.

Third stage of purification process comprised dissolving

precipitated residual material by little amount of distilled water up to 20 ml, the ammonium sulphate was removed when sample was putted in to dialysis tube which surrounded by buffer solution then completed with another phase of dialysis to remove water of the sample by adding sugar surrounded dialysis tube, after 24 hours the volume became 10 ml.

Fourth stage comprised estimating the molecular weight of extracted enzyme (Fake 2013), the Blue dextrin solution had molecular weight attained 2,000,000 Dalton, was prepared by 1 mg material per 1 ml buffer solution and passed through gel (Sephadex G-100 prepared from granule materials dissolved in 0.2 M of buffer), degassing gel was done before pouring gelin to column 1: 30 cm (width: height), the blue dextrin solution was made to calculate size of gapsin side column, collected volume of this solution reached 21 ml (V_o) at highest photo absorbance 600 nm then washed with buffer solution. Standard curve of different proteins solutions had gradual molecular weight included BSA, Ovalbumin, Pepsin and Trypsin, with molecular weights were 67000, 43000, 35000 and 23800 Dalton respectively, prepared and passed separately through the gel filtration, This gel contained pores of diameters allow passing molecular weights between 4000 -100000. Buffer solution was poured at level of 1 cm above gel surface during the process. The flow rate was arranged to be 0.5 ml min⁻¹ or 3 ml per test tube.

Collected volumes of each protein (V_e) were recorded at 280 nm. Equation between (V_e/V_o) was calculated and converted to logarithm value. Molecular weight of amylase enzyme was calculated through passing it into column then applied same equation. This stage was getting another performance by giving highest concentration of protein at 280 nm.

After each stage the amylase enzymatic activity, protein concentration and volume of enzyme were estimated for processing the enzyme purification stages and definition of enzyme properties.

Determination of enzymatic activity: Enzymatic efficiency was measured according Miller method to estimate absorbance at wavelength 540 nm by Shimadzu spectrophotometer. The standard curve of numeral concentrations for maltose sugar solution stock at 2 mg ml⁻¹, using 3,5-dinitrosalicylic acid (DSAA) was prepared 1g of DNSA in 50 ml distilled water. Enzyme activity unit (micromole ml⁻¹) was measured through incubation solution contained extracted enzyme, buffer and starch solution then compared with maltose sugar standard curve. The process included putting 0.1ml of raw extracted enzyme in 0.9 ml of starch solution 0.5% which prepared of 500 mg starch in 25 ml of buffer solution, then added 50 ml of boiled buffer

solution and completed with distilled water to 100 ml.

It was placed in water bath for 10 minutes at 45 C°, putted 1 ml of DNSA dye, added 20 ml of sodium hydroxide (2 M), then added gradually 30g of Rochelle salt for increase melting solution, completed volume to 100 ml into dark vial. Sample was placed in boiling water for 3 minutes to stop the reaction, then cooled and add 10 ml of distilled water, measured absorption at 540 nm, using prepared standard curve to estimate enzymatic units that analyzes 0.1 mg of substance during 10 min, same process occurred for control treatment to measure enzymatic unit that contained only starch and buffer solution for comparison. Same process was used to measure enzymatic efficiency for other stages (Mawahib and Yagoub 2010).

Determination of protein concentration: The protein concentration was estimated at absorption 280 nm (Lowry et al 1951). The standard curve with stock of bovine serum albumin (BSA) protein at 2 mg ml⁻¹. Absorption of protein solutions extended between 0-0.6 mg ml⁻¹ at 595 nm using drops of Kumasi blue dye was prepared by dissolving 100 mg of material in 50 ml (96%) ethanol with 10 ml of phosphoric acid then completed with buffer solution to 1 L. Protein standard curve was used to measure protein concentration of all purification process of enzyme in experiment work.

Exam of optimal pH of enzymatic activity and stability: Optimal pH was pointed according to method of Fake (2013) with some modification. The optimal pH for enzymatic activity was conducted by adding 0.1 ml of extracted enzyme in 0.9 ml of starch solution into each tube which used particular pH. Enzymatic activity of each tube was measured.

Stability of enzymatic activity was measured by putting into each tube 0.2 ml of buffer solution, placed 0.2 ml of extracted enzyme alone without starch solution then incubated at 37 C° for half hour, enzymatic activity was estimated after added equal volumes of starch solution into each tube.

Determination of optimal temperature for enzymatic activity and stability: The optimal temperature for enzymatic efficiency and stability was estimated (Fake 2013) with some modifications. Temperature between 20-90°C during 10 minutes for each tube as well extracted enzyme and starch solution at optimal pH to detect optimal temperature indicated highest enzymatic activity. enzymatic stability was done by putting 0.2 ml of buffer solution at optimal pH and individual temperature for each tube during 5 minutes, adding 0.2 ml of extracted enzyme, staying the temperature through 5 minutes, then 0.3ml from each tube was removed, so added 0.9 ml of starch solution for evaluating the enzymatic activity during 5 minutes to detect optimum temperature that gave highest stability of enzymatic

activity. The highest enzymatic stability represented as percentage of 100% then calculated the ratios of other enzymatic stability proportionality according the gradual temperatures.

Determination of kinetic constants of enzyme: Kinetic constants as Mikales constant (K_m) and maximum velocity of enzymatic reaction (V_{max}) were detecting by method of Majid et al (2011) with some modifications. Enzymatic activity was estimated using various concentrations of starch solution extended between 0.1-0.9% with buffer solution and 0.1 ml of extracted enzyme whose were done into test tubes.

RESULTS AND DISCUSSION

The enzymatic efficiency of extracted solution during four stages included raw extracted solution, salt sedimentation, dialysis and gel filtration were estimated according to maltose standard curve through starch degradation which was extended between 0-600 micromole ml^{-1} at photo absorbance between 0-37.76 (Fig. 1), enzymatic activity of four stages were 340, 290, 430 and 350 (micromole/ml), respectively (Table. 1).

The relationship between photo absorbance and concentration of diluted protein solutions was used for standard curve of bovine serum albumin in protein, those were between 0-0.6 $mg\ ml^{-1}$ with photo absorbance ranged between 0-1.5. Protein solutions concentrations of four stages were 0.7, 0.5, 0.5 and 0.3 $mg\ ml^{-1}$ respectively (Fig. 2).

Results of alpha-amylase enzyme purification for four stages of raw extracted solution (crude), salts sedimentation, dialysis and gel filtration indicated that the first stage achieved 100% of yield because there was no losing of enzyme during process, as well highest volume and activity of total extracted enzyme whose were 100 ml and 34000 unit ml^{-1} respectively.

Second stage comprised using salt through sedimentation process. The highest amount of extracted enzyme was detected at 80% saturation degree and pH 6. Solubility and efficiency of enzyme were lowest for separated filtrate. Various results of enzymatic efficiency, volumes, protein concentration, total efficiency and yield were observed during process. Crude's volume was 100 ml then reduced to 12 ml at final stage during experiment included

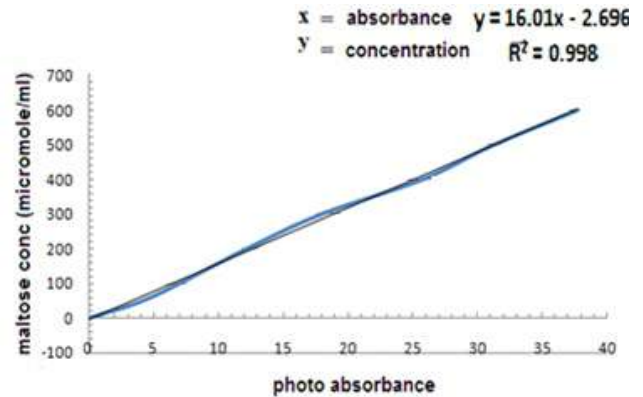


Fig. 1. Relationship between photo absorbance and maltose sugar concentration during amylase enzyme activity determination

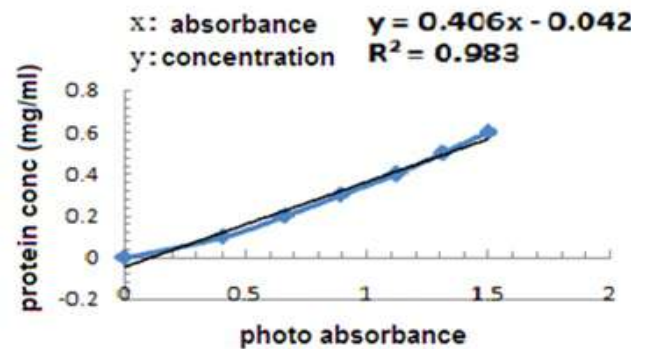


Fig. 2. Relationship between photo absorbance and various concentrations of bovine serum albumin protein solutions

sedimentation and water removing during dialysis process, while there was gradual increase in enzyme specific activity, as well decreasing of protein concentration, total enzyme activity with increasing in number of purification times and yield at advanced stages, that indicate increasing of enzyme purification and converted it from partial to absolute purified status. Mawahib and Yagoub (2010) indicated that the yield of crude was between 12.3- 100%. Gel filtration stage was useful for two aim, first one to increase purification of enzyme, so another to estimate enzyme molecular weight.

The relationship of ratio V_e/V_o with the logarithm of proteins molecular weight were shown in (Fig. 3). The

Table 1. Extracted amylase enzyme stages during purification process using intestinal of *Cyprinus carpio* fish

Treatment	Yield (%)	Number of purification times	Total activity (unit)	Specific activity (unit mg^{-1})	Protein conc (mg ml^{-1})	Volume (ml)	Enzymatic activity (unit ml^{-1})
Crude	100	1	34000	485.7	0.7	100	340
Salts Sedimentation	17.1	1.2	5800	580	0.5	20	290
Dialysis	13	1.8	4300	860	0.5	10	430
Gel filtration	12.3	2.4	4200	1166.6	0.3	12	350

Molecular weight of enzyme was 52.8 kd. Ceci and Lozano (2002) observed that the extracted amylase of apple juice contained highest protein concentration and enzymatic activity to achieve huge ability for enzyme purification. The temperature affects stability and activity of alpha-amylase enzyme, those were in gradual increase till 70°C, but there were decreasing at others temperatures.

Activity of enzymes varies depending environment surrounding organisms. Ghavidel and Davoodi (2011) observed that activity of alpha-amylase enzyme increased when temperature increase to 65°C, but it had decreased with rising temperature. Majid et al (2011) noticed that optimized temperature for alpha-amylase enzyme activity in white maize was 70°C, as well enzymatic efficiency remained at highest value with same temperature through 10 min.

The different behavior of enzymatic reactions during variable temperature invert the enzyme potential and kinetic energy toward substances. High temperature act to reduce enzymatic activity and damage enzyme's structure with changing its characteristics, the relationship between temperature and photo absorbance to show enzyme's activity and stability of enzymes (Fig. 4).

Stability of enzyme's activity at highest photo absorbance was 0.09 and 70°C (100%). Stability at temperatures 40, 50, 60, 80, 90°C were 60, 79, 82, 78, 56% respectively. The pH between 6.5 to 7.5 achieved highest photo absorbance of enzymatic activity

Ceci and Lozano (2002) also observed the highest efficiency of purified alpha-amylase of white maize at pH between 4-8, Egwin and Oloyed (2006) indicated that the optimal pH of alpha-amylase enzyme activity is between 5-7 (Fig. 5). The gradual increasing of enzymatic activity with increase of substance concentration was observed that gave linear curve between them at beginning then there was reduction with increase of substance concentration till 0.9 %. Maximum velocity attained 75.78 micromole min⁻¹ (Fig. 6). Reaction continued for 10 minutes at optimal Reaction continued for 10 minutes at optimal conditions to measure enzyme activity and then enzyme activity was calculated per 1 minute by dividing total value of enzyme activity on 10 minutes, that represented velocity of reaction (V) and was 0.32. Km is meaning to attract enzyme toward substance, its lower value lead to high activity of enzyme (Fig. 6).

Tallapragada et al (2017) showed that Km and Vmax values of alpha-amylase which extracted from *Monascus sanguineus* yeast were 22.07 micromole min⁻¹ and 0.055 respectively, this suggested that extracted enzyme of yeast is more active toward substance than extracted enzyme at current study because there was contrast at Km value between them, so enzymatic activity has grew up when

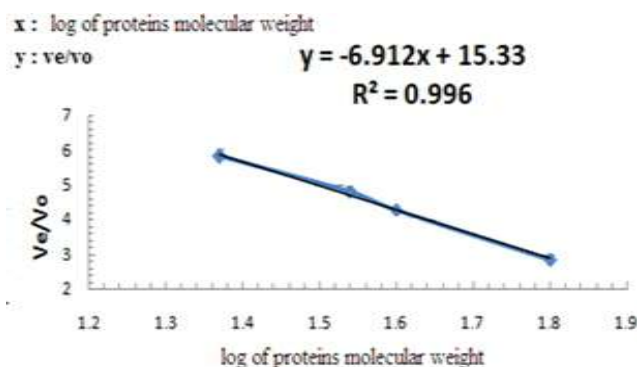


Fig. 3. Relationship between logarithm of proteins molecular weight and V_e/V_o values during filtration process to calculate enzyme molecular weight

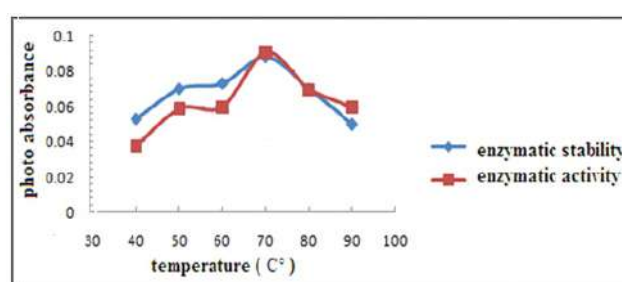


Fig. 4. Relationship between temperature and activity of extracted alpha-amylase enzyme of intestinal carp fish

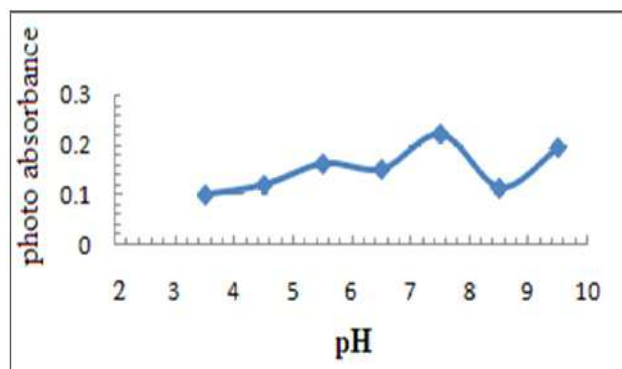


Fig. 5. Relationship between pH values and enzymatic activity according its photo absorbance

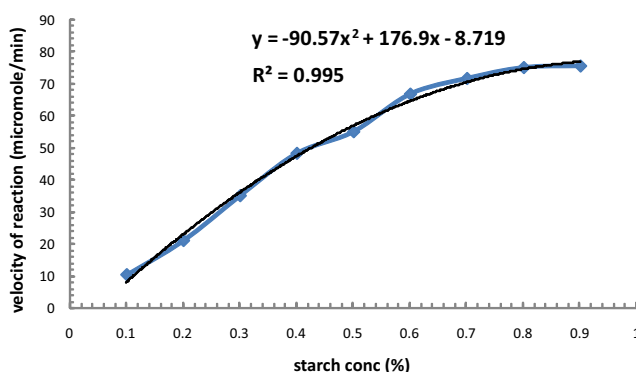


Fig. 6. Relationship between velocity reaction an increasing of substance concentration at remaining enzyme concentration

increasing of substance dose then reach at stable level with rising of substance concentration to attain at maximum velocity through reaction. Majid et al (2011) found that Km value for purified enzyme alpha-amylase of local malt was 0.55, which was near of Km that calculated during current study, while maximum enzymatic activity was 255.75 micromole min⁻¹.

CONCLUSION

Alpha-amylase enzyme from available source of residual carp fish gastrointestinal tract could be extracted at several stages of purification forgetting activated enzyme can be used for different purposes like food technology to enhance human health as well as may be used to prepare medical materials.

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Efficacy Leaf Litters as Substrate on Reproductive Potential of Epigeic Earthworm *Eudrilus eugeniae*

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Abstract: The study was aimed to analyze the vermicomposting ability and reproductive potential of *Eudrilus eugeniae* in different leaf litters such as *Arthrocarpus heterophyllus*, *Mangifera indica*, *Coffea arabica*, *Psidium guajava*, *Morus alba*, *Erythrina veriegata*, *Gliricidia sepia*, *Lawsonia inermis*, *Musa paradisica*, *Cinnamon zeylanicum* and *Ficus religiosa* with native Vechur breed cow dung as the reference sample. *E. eugeniae* showed highest reproductive potential in *F. religiosa*, *C. arabica*, *M. alba*, *M. indica*, *A. heterophyllus*, *P. guajava* when compared to control samples. Whereas, lowest reproductive potential observed in culture medium with *G. sepia*, *M. paradisica*, *C. zeylanicum*, *L. inermis*. The phytochemical components of leaf litters can directly effects the microbial activities during decomposition process. The results, indicates that leaf litters can be converted into value added manure for sustainable soil fertility management program.

Keywords: *Eudrilus eugeniae*, Leaf litters, Reproductive potential, Vermicomposting

Leaf litters are potential energy resource if they are naturally and biologically converted into organic matter. The vermicomposting is an easy and efficacious cost effective way to recycle the leaf litter along with bioconversion of organic waste materials into nutritious vermicompost by the activity of earthworm (Mall et al 2005) and it is an eco-biotechnological process that converts energy rich and complex organic substances into a stabilized humus-like product (Benitez et al 2000) and nutrient rich, microbiologically active organic amendment generated as a result of the mutual interactions between earthworms and microorganisms in breaking down of organic matters (Lazcano and Dominguez 2010). Earthworms have great economic value to mankind because they improve the soil quality by their action. The earthworms act as physically an aerator, crusher and mixer, chemically as a degrader and biologically a stimulator in the decomposition subsystem. In tropical and sub-tropical conditions earthworm, *E. eugeniae* observed as a best vermicomposting species and are highly adaptable in wide range of environmental conditions, fast-growing and highly voracious feeder which converts all type of organic waste into nutrient rich substances; they were ideally using animal feed proteins as well as for rapid organic waste conversions (Jorge Dominguez et al 2001). Leaf litters are a type of organic waste which can be processed using earthworm to transform into nutrient rich vermicompost and used with the biofertilizers on cultivating crops. In addition of leaf litters the composting medium of dung materials enhances the humification process (Huang et al 2001). *Eudrilus eugeniae* were widely used for vermicomposting because of high voracity, high reproductive capacity and their

ability to adapt adverse conditions (Gajalakshmi and Abbasi 2004). The present study was conducted to assess the reproductive potential ability of epigeic earthworm *E. eugeniae* in different leaf litters medium.

MATERIAL AND METHODS

Earthworm samples and leaf litters collection: The vermicomposting epigeic earthworm *Eudrilus eugeniae* were collected from vermicomposting unit of M. S. Swaminathan Research Foundation, Kalpetta, Wayanad district, Kerala. The leaf litters were collected in and around the research foundation. The leaf litters of *A. heterophyllus*, *M. indica*, *C. arabica*, *P. guajava*, *M. alba*, *E. veriegata*, *G. sepia*, *L. inermis*, *M. paradisica*, *C. zeylanicum* and *F. religiosa* were collected (Fig. 1). Then the leaf litter wastes were introduced in 14 days pre-composted Native vechur breed cowdung in 1:1 ratio (leaf litter 1 kg: cow dung 1 kg). Due to the increase in temperature and methane production during pre-composting is very essential to avoid the death of the worms (Gunadi et al 2003).

Preparation of vermibed: Vermibed were prepared in round tubs with 45 cm height and 35 cm diameter. Triplicate samples were kept as experimental setup with different leaf litters. Chopped leaf litters were added with native Vechur breed cow dung (*Bos taurus indicus*) in 1: 1 ratios. Vermicomposting were carried out in room temperature and moisture content was maintained around 70-80 per cent water. In each tub containing vermibed medium with substrate were inoculated with 10 earthworms and Native Vechur breed cow dung alone with introducing earthworms were kept as reference medium.

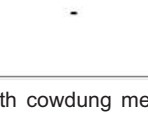
	LEAF LITTERS	VERMICOMPOST MEDIUM	VERMICOMPOST AFTER 45 DAYS	TOTAL BIOMASS AFTER SHORT TERM ANALYSIS			
				ADULTS	SUB- ADULTS	JUVANILES	COCOONS
A							
B							
C							
D							
E							
F							
G							
H							
I							
J							
K							
L	Native Vechur breed cowdung (Control)						

Fig. 1. Different vermibeds and total biomass produced in different leaf litters with cowdung medium in (1:1 ratio). Leaf litters of (A) *Arthrocarpus heterophyllus* (B) *Mangifera indica* (C) *Coffea arabica* (D) *Psidium guajava* (E) *Morus alba* (F) *Erythrina variegata* (G) *Gliricidia sepium* (H) *Lawsonia inermis* (I) *Musa paradisiaca* (J) *Cinnamon zeylanicum* (K) *Ficus religiosa* are experimental samples and (L) Native Vechur breed (*Bos taurus indicus*) cow dung as control sample.

Reproductive potential analysis: The reproductive potential was examined by hand sorting and picking from culture media after 45 days of vermicomposting, in all the triplicates samples. The total biomass were studied by counting the number of adult, sub-adult, juveniles and cocoons.

Statistical analysis: Duncan multiple ranged were performed using IBM SPSS 20 Software to determine the significant difference between treatments ($P < 0.05$).

RESULTS AND DISCUSSION

Initially, 10 adult earthworms with total body mass of ~ 2.04 gm were introduced in each pre-composted vermibed medium. The total biomass and reproduction were maximum with *Ficus religiosa* leaf litter and least in *Lawsonia inermis* this confirms that the nutritional importance of the bulking agents. *E. eugeniae* attained maximum total biomass and body weight in leaf litters of *Ficus religiosa* when compared to other experimental and control samples followed by the leaf litters of *Coffea arabica*, *Erythrina variegata*, *Morus alba*, *Mangifera indica*. *Arthocarpus heterophyllus* enhanced the growth of earthworm population when compared to the reference samples. The culture medium *Lawsonia inermis* showed the least biomass production followed by *Cinnamom zeylanicum*, *Musa paradisica*. The reproductive potential highly diminishes when compared with the reference sample medium. The reference medium containing 100% of native Vechur breed coudung showed a medium level of reproduction and while addition of substrates *Ficus religiosa*, *Coffea arabica*, *Erythrina variegata*, *Morus alba*, *Mangifera indica* enhanced the reproductive potential and body mass of earthworms. Supportively, Gajalakshmi et al (2005) showed that the earthworms can able to survive, grow and breed in the vermireactors with composted *Mangifera indica* tree leaves. Curry (2007) explained that the mostly all kinds of animal dung are highly attractive and nutritious food sources for earthworms and coudung forms the important part of the worm-diet. The cattle dung can able to increase the stability of the material to be converted into vermicompost as feed for both microbes and earthworms (Kaur 2010). The vermicompost is a product of mesophilic biodegradation of organic materials through earthworm-microbial interactions (Lazcano and Dominguez 2010). In addition to the animal dung some leaf litter substrates the reproductive potential and biomass got enhanced but in some leaf litter reproductive potential got reduced and leading to the death of earthworms. This is because due to the variation in chemical contents of different leaf litters it may affect the health and reproductive potential. Akanksha and Manoranjan (2012) observed that highest population density of *P. sansibaricus* in

Table 1. Total body mass of *Eudriluseugeniae* in different leaf litters as substrates with coudung as bedding material

Plant substrates	Total body mass of earthworms (g)	Individual adult worm weight (g) (Initial weight - 0.20)
<i>A. heterophyllus</i>	20.66 ^d	2.00 ^a
<i>C. zeylanicum</i>	7.33 ⁱ	1.44 ⁱ
<i>C. arabica</i>	13.33 ^a	1.97 ⁱ
<i>E. variegata</i>	19.33 ^d	1.54 ^b
<i>F. religiosa</i>	109.33 ^a	3.82 ^a
<i>G. sepia</i>	12.66 ^a	1.46 ^k
<i>L. inermis</i>	5.00 ^g	1.84 ^g
<i>M. indica</i>	31.66 ^c	2.61 ^c
<i>M. alba</i>	66.66 ^b	2.81 ^b
<i>M.paradisica</i>	12.33 ^a	1.48 ⁱ
<i>P. guajava</i>	30.33 ^c	2.05 ^d
Native cow dung (Control)	30.66 ^c	1.50 ⁱ
CD (p=0.05)	1.3472	0.00

mango leaf litter culture. They concluded that in mango litter, mangiferin (xanthone), a natural pigment favored the earthworms to increase their biomass. The rate of cocoon production and hatchability rate of earthworms during vermicomposting were significantly related to the quality of culture medium provided.

CONCLUSIONS

The medium of native Vechur breed cattle manure provide a better environment for the *Eudrilus eugeniae* to grow and while adding the leaf litters as substrates, earthworm population results on the hatchability of cocoon, body mass and the survival of earthworms. The reproductive potential and body mass of earthworms were enhanced in the medium with leaf litters of *Ficus religiosa*, *Coffea Arabica*, *Erythrina variegata*, *Morus alba*, *Mangifera indica* and in some substrate the reproductive rate and growth rate get diminished. The growth and reproductive potential of earthworms are highly influenced by the chemical constituents and quality of the feed provided.

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Survey of Molluscs in Killai Backwaters of South India

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Abstract: Assess the distribution of molluscs in mangrove forest of Killai backwaters in Tamil Nadu, south India. Three different sampling sites based on salinity variation were selected. During the study period eighteen species of molluscs including nine species of gastropods and 9 species bivalves were observed. The gastropod *Cerithidea cingulata* and the bivalve *Crossostrea madrasensis* was absent in fresh water zone, but rich in marine zone. The gastropod (*Pythia plicata*) was absent in marine zone, but rich in freshwater zone in arboreal habitat was found only few collections. The *Pythia plicata* found in few mangrove plants (*Avicennia marina*, *Excoecaria agallocha*, *Avicennia officinalis* and *Acanthus ilicifolius*). Maximum number of individuals (52) of *Pythia plicata* were observed in *Avicennia officinalis* at 225 cm height and 21 number of individuals were found at 125 – 150 cm height. The another gastropods *Nassarius stolatus* were also found from *Avicennia marina* and *Acanthus ilicifolius* plants at the height of 0 – 25 cm in mangrove rich zone. The maximum number of *Cerithidea cingulata* (97 numbers per 25 square centimeters) was reported in marine zone.

Keywords: Gastropod, Mangrove, Bivalve, Arboreal, Diversity

Mangroves are intertidal plants contribute rich nutrients, an ideal nursery and breeding grounds to most of the marine and brackish water fish and shell fishes (Sakthivel et al 2014). Molluscs are the second largest invertebrate group on earth, next to insects (Bouchet 1992). Mangrove molluscs found in all habitats as herbivores, predators, detritivores and filter feeders with horizontal and vertical distribution (Cannicci et al 2008). The gastropods and bivalves constitute 98% of the total population of molluscs live in land, fresh and marine water. The molluscs used for food, ornamental purpose, pearls, lime, cement, calcium resources, poultry feed and medicine. Monitoring of heavy metals pollution, molluscs have been used as a tool. Accumulations of heavy metals are more in molluscs, because of their sedentary nature. Molluscs are very good source of medicine for pharmacological products with antibacterial, antiviral and antitumor properties. The family belongs to bivalvia molluscs, Teredinidae and Pholadidae cause substantial damage to underwater wooden construction, floating timber and wooden sailing crafts particularly in the tropical conditions. In the fishing industry depends mainly fishing wooden boats which suffer heavily as a result of borer damage.

The number of molluscs species varies from 80,000 to 135,000 (Abbott 1989). There are about 31,000-100,000 species recorded in marine environment, 14,000-35,000 in terrestrial and 5,000 species in freshwater (Seddon 2000). In India, 5,070 species of Mollusca have been recorded, 3,370 species are from marine environment (Venkataraman and Wafar 2005). There are 611 families of gastropods, of which 202 families are extinct, being found only in the fossil record. In Mudasal Odai and Nagapattinam coast of south India

molluscs were reported (Sakthivel & Antony Fernando 2002; 2013; 2014). Marine gastropods diversity carried out in Uran coast of Navi Mumbai, west coast of India (Pawar and Al-Tawaha 2017). Molluscan diversity studied in Mumbai mangrove ecosystem (Kantharajan et al 2017) and Mangrove ecosystem of Bhatye, Ratnegiri (Kulkarni and Mukadam 2015). Few species of molluscs were endemic in mangrove forest and molluscs survey in mangrove are also very less, the preset survey may give some information about molluscs from mangroves of Killai backwaters.

MATERIAL AND METHODS

The molluscs collection has been made from Killai backwaters station I fresh water zone (Lat.11°29'N; Long. 79°46' E), station II mangrove rich zone (Lat.11°29'N; Long. 79°46' E) and station III marine water zone (Lat.11°29'N; Long. 79°46' E). Three different sampling sites were chosen with 3 kms distance between any two stations. The first station, fresh water zone is located 2 km west of Tamil Nadu Tourist harbor, and receives water carrying wastes from adjacent agriculture lands and also from aquaculture practice in addition to domestic waste. The second station is characterized by thick mangrove forest. The third station receives neritic waters from the adjacent Bay of Bengal through the mouth of Chinnavaikal. Samples were brought to laboratory, cleaned with brush and identified using appropriate reference (Satyamurthy 1952, Kasinathan 1987, Fred Pinn 1990).

RESULTS AND DISCUSSION

Arboreal gastropod (*Pythia plicata*) was observed only

three collections during on 30th October 2015, 30th December 2015, and 13th January 2016 from the first station of freshwater zone, the number of individuals varied (69, 21, 9) first, second and third collection respectively. The molluscs *Pythia plicata* was found only in four mangrove plants (*Avicennia marina*, *Excoecaria agallocha*, *Avicennia officinalis* and *Acanthus ilicifolius*) and number of individuals (52, 23) were observed in *Avicennia officinalis* and *Excoecaria agallocha* respectively. It was found up to 225 cm height, and the number of individuals were gradually reduced to 21, 19, 18, and 16 in the plants (125-150 cm; 50-75 cm, 75-100 cm; 100-125 cm) different height (Table 1-3). The arboreal molluscs *Pythia plicata* and *Nassarius stolatus*

were reported 0 – 25 cm height in *Avicennia marina* and *Acanthus ilicifolius* plants at mangrove rich zone on 29th January 2016 (Table 4 & 5).

Totally 18 species of molluscs including 9 species of gastropods and 9 species bivalves were from 10 collections from three different collection sites. Two molluscs species one from gastropod (*Cerithidea cingulata*) and another from bivalve (*Crossostrea madrasensis*) were observed in all collection at Marine zone as dominant species. The gastropod *Cerithidea cingulata* was totally absent in fresh water zone and mangrove rich zone. The bivalve *Crossostrea madrasensis* was absent in fresh water zone. Two gastropods one from fresh water zone (*Pythia plicata*)

Table 1. Arboreal molluscs *Pythia plicata* from fresh water zone (30th October 2015)

0-25 cm	25-50 cm	50-75 cm	75-100 cm	100-125 cm	125-150 cm	150-175 cm	175-200 cm	200-225 cm	Total
<i>Avicennia marina</i>									
0	0	4	0	0	0	0	0	0	4
<i>Excoecaria agallocha</i>									
0	0	2	0	0	0	0	0	0	2
0	0	3	0	0	0	0	0	0	3
0	1	0	2	0	0	0	0	0	3
0	0	0	0	2	3	2	0	0	7
<i>Avicennia officinalis</i>									
0	0	0	2	3	0	0	0	0	5
1	0	0	2	0	0	0	0	0	3
2	3	4	2	3	2	2	2	1	21
0	1	1	0	1	1	0	0	0	4
1	2	5	6	3	0	0	0	0	17
4	7	19	14	12	6	4	2	1	69

Table 2. Arboreal molluscs *Pythia plicata* from fresh water zone (30th December 2015)

0-25 cm	25-50 cm	50-75 cm	75-100 cm	100-125 cm	125-150 cm	150-175 cm	175-200 cm	200-225 cm	Total
<i>Avicennia marina</i>									
0	0	0	0	0	2	0	3	0	5
<i>Excoecaria agallocha</i>									
0	0	0	0	1	0	0	0	0	1
0	0	0	0	1	2	0	0	0	3
<i>Avicennia officinalis</i>									
0	0	0	0	0	1	0	0	1	2
0	0	0	1	0	0	0	0	0	1
0	0	0	0	1	1	0	0	0	2
0	0	0	0	1	1	0	1	1	4
0	0	0	0	0	1	0	0	0	1
0	0	0	0	0	2	0	0	0	2
0	0	0	1	4	10	0	4	2	21

[illegible]

Table 6. Molluscs recorded in three different stations of Pichavaram mangroves

Species	Freshwater zone	Mangrove rich zone	Marine zone	Total
Gastropods				
<i>Pythia plicata</i>	+++	++	-	2
<i>Cassidula nucleus</i>	+	++	-	2
Ellobiidae				
<i>Melampus cylonicus</i>	+	++	-	2
<i>Dostia crepidularia</i>	+	+	-	2
Littorinidae				
<i>Littorina scabra</i>	+	+	-	2
<i>Telescopium telescopium</i>	+	+++	-	2
Cerithiidae				
<i>Cerithidea cingulata</i>	-	-	++++	1
Nassariidae				
<i>Nassarius stolatus</i>	-	+	-	1
Neritidae				
<i>Clithon oualaniensis</i>	+	+	-	2
Total	7	8	1	
Bivalves				
<i>Crassostrea madrasensis</i>	-	+	++++	2
<i>Saccostrea cucullata</i>	-	-	++	1
<i>Meretrix meretrix</i>	-	-	+	1
<i>Meretrix casta</i>	-	-	++	1
<i>Marcia opima</i>	-	-	+	1
Mytilidae				
<i>Modiolus metcalfei</i>	-	-	+	1
<i>Perna viridis</i>	-	-	+	1
Donacidae				
<i>Donax scortum</i>	-	-	+	1
<i>Donax faba</i>	-	-	+	1
Total	7	9	10	

-: Not recorded; +: Present (1-3 collection); ++: Co Abundant (4-6collection); +++: Abundant (7-9 collection); Dominant (above 9collection)

gastropod *Cerethidea cingulata* is found to variety of habitats from well sheltered soft muddy to deforested hard substrate and open mudflats without mangrove vegetation. In the present study the *Cerethidea cingulata* was found more in number (5 - 97 per 25 square centimeters) (Table 7). The *Telescopium telescopium* distributed in muddy habitat either low desiccation tolerance or preferred muddy habitat has rich in food. In the present study it was abundant species in mangrove rich zone areas (Table 6). The bivalve *Crassostrea madrasensis* are attached in the pneumatophores of mangrove trees (Ziaullah 2018). The gastropods *Telescopium telescopium* and *Cerithidea cingulata*, members of Potamididae family can stay out of water for a long period of time, they were reported as a most dominant species from Mangrove Forests of Mojo village, Ulujami district, Pemalang Regency, Indonesia (Puryono and Suryanti 2019) and found in all collection with a more number of individuals from the Mangrove Forests of Pakistan (Nazim et al 2015) as well as in Bhavanapadu Mangroves, Northeast Coast of Andhra Pradesh (Ranjan and Babu 2014). In marine zone area the *Crassostrea madrasensis* was found as dominant species (Table 6).

One hundred and twenty-five gastropod and 43 bivalve species were recorded from Darvel Bay off southeast Sabah, Borneo (Shabdin et al 1998). About 170 molluscan species have been identified from Christmas Island (Indian Ocean) (Tomlin, 1934). Three hundred and nine gastropod and 118 bivalve species were recognized from Hainan Island, South China Sea (Hasegawa et al 2001). More than 350 species of shelled molluscs were recorded from Pulau Seribu and Jakarta Bay (Roberts et al 1982). Some 895 prosobranch and 485 opisthobranch gastropods and 339 bivalve species have been recorded from Guam (Carlson and Hoff 2003, Paulay 2003, Smith 2003) and 1,031 gastropod species were identified by Fukuda (1993, 1994, 1995) from the Ogasawara (Bonin) Islands. Fifty-two gastropods were recorded in

Table 7. Population studies of *Cerithidea cingulata* at III station (Marine zone) numbers/25 cm²

Sites	Collection Dates									
	15.10.15	30.10.15	30.12.15	13.1.16	29.1.16	12.2.16	24.2.16	11.03.16	11.4.16	23.9.16
1	45	75	52	21	14	06	14	11	39	15
2	25	43	32	18	10	13	28	15	27	77
3	37	27	15	35	11	18	25	05	23	97
4	23	48	18	17	13	13	21	07	13	17
5	39	95	43	28	08	21	08	12	37	45
6	18	53	11	36	06	11	32	27	36	22
7	13	46	26	21	21	15	47	34	21	28
8	28	21	31	13	27	09	43	31	25	33
9	35	34	18	19	55	18	31	15	35	16

Table 8. Diversity analysis of *Cerithidea cingulata* at III station (Marine zone)

Season	H' (log2)	d richness	J' evenness	Brillouin	Fisher	Simpson
Monsoon	3.09	1.39	0.97	2.09	1.72	0.88
Postmonsoon	3.13	1.53	0.99	2.07	1.97	0.89
Summer	3.10	1.50	0.98	2.04	1.92	0.88
Premonsoon	2.85	1.37	0.89	1.92	1.69	0.84

planted mangroves areas in Central Vietnam (Zvonareva et al 2015). In mangroves of Abtan river on Bohol Island, Philippines 151 gastropods were found (Lozouet and Plaziat 2008). In Malay peninsula 50 species found (Ashton et al 2003), In the Indian Sundarbans mangrove 56 species (Dey 2006), on the west coast of Thailand 30 gastropods (Macintosh et al 2002), In south Thailand 32 species were recorded (Sri-aroon et al 2005). Sixty one species of molluscs including 46 gastropods, 14 bivalves and one polyplacophora species were recorded in Mumbai mangroves (Kantharajan et al 2017) and 60 species of gastropods were reported in Uran coast, Navi Mumbai, west coast of India (Pawar and Al-Tawaha 2017). In Mudasal Odai coast, and Nagapattinam coast in south India 33 and 31 species bivalves reported respectively (Sakthivel & Antony Fernando, 2002; 2013). Gastropods 75 species in Mudasal Odai coast and 67 species in Nagapattinam coast have been report (Sakthivel & Antony Fernando 2014). Thirty seven species of molluscs including 21 gastropods and 16 bivalves have been recorded from the Pondicherry mangrove areas (Satheeskumar and Khan 2012). Fifty-two gastropod were found in three mangrove areas of central Vietnam (Zvonareva et al 2015). In the present study 18 species of molluscs including 9 gastropod and 9 bivalve species obtained from this survey (Table 6). Saravanakumar et al (2007) recorded 17 species of gastropods and 16 species of bivalves in the Arid Zone Mangroves of Gulf of Kachchh. Saravanan et al (2008) had recorded 9 species of gastropods and 4 species of bivalves in an earlier study in the same mangrove. Twenty three species of gastropods and 6 species of bivalves has reported in the mangrove swamps of Myeik areas, Taninthayi region, Myanmar (Htwe and Naung 2019). Forty four species of Gastropods, 23 species of Bivalves and 3 species of Cephalopods were documented in Andhra Pradesh coast (Monolisha and Patterson Edward 2015). Forty four species of gastropods were identified in Mangrove forest of Govater, Gulin Sistan & Baluchestan (Moradi et al 2019). Gastropods 34 species and Bivalve 17 species were recorded in Pulicat Lagoon (Ramanibai and Govindan, 2018). Nineteen species of molluscs have been recorded Bhavanapadu Mangroves, Northeast Coast of Andhra Pradesh, India (Joseph Uday

Ranjan and Ramesh Babu 2014). The bivalve species *Meretrix meretrix*, *Meretrix casta*, *Marcia opima* were recorded only in the third marine zone collection sites (Table 6). Sandy bottom is one of the favorable conditions for filter feeding organisms especially bivalves (Kalyanasundaram 1982, Jayabal 1984, Balasubramanian 1984).

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Sex-Specific Extra Floral Feeding Preferences among Subtropical Butterfly Fauna of Terai Arc Landscape, Southern Himalaya, India

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Abstract: The knowledge on specialized feeding requirements of adult butterfly species with the exception of dietary floral sources is crucial for the proper management and conservation of butterflies. Present study conducted from September 2016 to August 2017 under the natural wild conditions of Nandhaur Wildlife Sanctuary (NWS) located in the central position of Terai Arc Landscape, India, aimed to observe extra floral feeding preferences, sex-specific and seasonal puddling patterns among butterfly communities. Out of the total 497 individuals recorded, 85.92% males and 14.08% females of 22 species belonging to four families were recorded from the different puddling sites in NWS. Observations revealed that sex-specifically, 80.09% of individuals of males of 22 species that were probed feeding at different substrates such as puddles, moist soil, mammalian dung and algal mats, showed rich puddling behavior in comparison to the females of butterflies belonging to seven species that were recorded feeding particularly on puddles and soil. Across the different seasons, maximum number of species and individuals of butterflies were recorded in puddling activity during the summer season, followed by rainy and winter seasons, respectively. It is suggested that habitats providing puddling grounds in and around the NWS should be earmarked for conservational and ecological research aspects of butterflies.

Keywords: Behavior, Butterfly families, Mud puddles, Sanctuary, Seasons

In the behavioral ecology of Lepidoptera, the term 'puddling' includes feeding by adult butterflies and moths either singly or in large congregations at mud puddles and on various excrements and secretions of vertebrates and carrion. Puddling behavior has been probably evolved either from the drinking of water as in the dry habitats water may be a prime reason for visiting puddles (Adler 1982 and Larsen 1991) or due to the shortage of minerals in the larval diets of many Lepidoptera (Adler and Pearson 1982). Although this behavior is known from tropical as well as temperate habitats, it is far more prevalent in tropical regions (Beck et al 1999). The list of adult Lepidopteran dietary sources, other than floral nectar is extensive, yet poorly understood (Downes 1968 and Hall and Willmott 2000). Foraging effort and puddling intensity within species vary among age classes and between the sexes (Adler 1982, Adler and Pearson 1982 and Boggs and Dau 2004). In many Lepidoptera, puddling behavior is considered to be exhibited typically by young males, whereas females rarely puddle (Adler 1982, Adler and Pearson 1982, Collenette 1934 and, Sculley and Boggs 1996), although not exclusively carried on by males (Boggs and Jackson 1991). Moreover, identification of mud puddling sites of butterflies is strategically important in conservation of local flora and to elucidate information on nectar food plants and migrations of adult butterflies (Basumatari et al 2016).

Among butterflies, puddling behavior has been reported

in several species. The members of families Papilionidae and Pieridae often visit soil puddles (Sreekumar and Balakrishnan 2001) and members of families Nymphalidae and Lycaenidae get attracted towards carrion, decaying fruits and animal excreta (Hamer et al 2006). Studies concerning the puddling behavior of different species of butterflies and moths have been carried out by various workers in different parts of the world (Downes 1974, Adler 1982 and Boggs and Jackson 1991, Launer et al 1993, Sculley and Boggs 1996, Beck et al 1999, Frey et al 2002, Otis et al 2006, Molleman 2010, Sidhu 2010, Ahmed et al 2016 and Fernando et al 2017). However, no such attempts have so far been made from the foothills of Indian Himalayan region. Therefore, our present study aimed primarily to observe extra floral feeding preferences, sex-specific and seasonal puddling patterns of subtropical butterfly communities in and around the Nandhaur Wildlife Sanctuary, Uttarakhand, India.

MATERIAL AND METHODS

Study area: The Terai-Bhabar region in the southern slopes of Shiwalik Himalaya and the foothills landscape is popularized as Terai Arc Landscape (TAL), flanked by the River Yamuna in India towards west to the River Bhagmati in Nepal towards east (Irengbam et al 2017). Occupying a central position within the eastern part of TAL, Nandhaur Wildlife Sanctuary (29°09'00" N Latitude and 79°80'00" E Longitude) covering an area of 269.95 km² is the sub-land

scape bounded by Rivers Gola and Ladhya in the west and River Sharda in the east and intersected by the River Nandhaur which flows east to west in the northern area (Fig. 1 & 2). Encompassing three forest divisions of Uttarakhand viz. Haldwani, Terai East and Champawat, Nandhaur Wildlife Sanctuary (NWS) provides a crucial link for wildlife populations to move across the eastern forests of TAL like Brahmadev and Sukhlaphata Wildlife Reserve of Nepal and western forests of Ramnagar and Terai Central Forest Division in India (Verma 2011). The sanctuary was crafted in 2012 and due to its varied topography, geology and climate; it is a cradle of valuable biodiversity harbouring a marvelous diversity of flora and fauna. The forest is generally dominated by moist deciduous and subtropical broad-leaved plants including more than 100 species of trees, 30 species of shrubs and 20 species of climbers and grasses. The region is predominately covered by Sal (*Shorea robusta*) forest covering approximately 70% of its total area. Besides this, common trees include species such as *Dalbergia sisso*, *Tectona grandis*, *Pinus roxburghii*, *Adina cordifolia*, *Acacia catechu*, *Holoptelea integrifolia*, *Aegle marmelos*, *Anogeissus latifolia*, *Butea monosperma*, *Emblica officinalis*, *Azadirachta indica*, *Acacia nilotica*, *Mallotus philippensis*. While the ground vegetation is generally covered with *Colebrookea oppositifolia*, *Clerodendrum infortunatum*, *Calotropis procera*, *Lantana camara*, *Justicia adhatoda*, *Murraya koenigii*, *Glycosmis pentaphylla*, *Ageratum conizyoides*, *Woodfordia fruticosa*, *Cynodon dactylon* and other species of shrubs and herbs (Verma 2011). The sanctuary is bestowed with rich mammalian assemblages of 32 species remarkably noted for high leopard and ungulates densities i.e., 9.57/100 sq km and 7.04 per sq km, respectively (Mann et al. 2013). Apart from this, 250 species of birds, 15 species of reptiles and 20 species of fishes have also been reported from different locations of the NWS.

For the present study which was conducted from September 2016 to August 2017, the Nandhaur Range covering an area of 131.30 km² of Haldwani Forest Division and the Dogadi Range (103.30 km²) of Champawat Forest Division were selected for the sampling of butterflies (Fig. 2). The climate of the study area is typically subtropical with an average rainfall of 1,400 mm, characterized by hot summer season (March-June), moist and wet rainy season (July-October) and cold winter season (November-February). In the last decade, the mean temperature of the sanctuary ranged between 28°C in January to 37°C in May (Sanwal et al 2017).

Sampling of butterflies: Representative samples of microhabitats like moist areas along stream sides, banks of the river and forest edges were visited from 10:00 am to

04:00 pm at a 30 days interval in order to observe puddling behavior of butterflies in and around the selected forest ranges of NWS. Images 1 & 2 provide a view of present study sites that were characterized by forest edges, small puddles and damp patches created by rivers, streams and other sources. Butterflies were rarely captured by adopting the net sweeping method (Gadagkar et al 1990), followed by photography using digital camera CANON 750D. Identification of the species was made by using butterfly field guides (Haribal 1992, Kumar 2008, Kehimkar 2014, Singh 2017 and Sondhi and Kunte 2018).

Extra floral feeding preferences and sex-specific puddling behavior of butterflies: In order to observe the puddling behavior only those individuals with their proboscis extended to the puddles and substrates were counted (Adler 1982). At the selected study sites, each specimen was observed puddling for an average of 2-5 minutes. In general, puddling time for butterflies was observed between 11:30 am to 03:00 pm. The age of individuals was estimated from the conditions of wing and wing coloration. Most of the specimens of males were young while few specimens of females were old. Sexes of butterflies were determined on the basis of conspicuous sexual dimorphism in wing pattern (Beck et al 1999). The sex of representative species of butterflies which were difficult to identify through sexual dimorphism were caught then brought to the laboratory and later identified. Sex-specific puddling proportion for different species of butterflies was calculated by adopting the method used by Boggs and Jackson (1991) and Sculley and Boggs (1996).

Statistical analysis: Using software PAST 3.04 (Hammer et al 2014), the values of Shannon diversity index (H) for species diversity (Shannon & Wiener 1949), Simpson's dominance index (D) for species dominance (Simpson 1949) and Equitability or Pielou's Evenness index (J) for species evenness (Pielou 1969) were calculated for seasonally occurring butterfly communities in the NWS.

RESULTS AND DISCUSSION

Species composition and extra floral feeding preferences of butterflies: The species wise summary of relative abundance, sex-specific puddling proportions and extra floral feeding preferences among butterfly fauna of the NWS is provided in Table 1. A total of 497 individuals belonging to 22 species and four families of butterflies were sampled from the different locations (Table 1). Figure 3 shows the photographs of total butterflies in the puddling activity during the study period. The maximum number of genera and species of butterflies belonged to the family Nymphalidae (10 genera and 10 species), followed by

Pieridae (3 genera and 5 species), Papilionidae (2 genera and 5 species) and Lycaenidae (2 genera and 2 species), respectively (Fig. 4). Individual abundance varied significantly among different families of butterflies. Family

Pieridae was the most dominant averaging 40.05% of the total sampled butterflies, followed by Nymphalidae (33.20%), Papilionidae (25.55%) and Lycaenidae (1.20%), respectively. Species namely, *Catopsilia pomona* (Fabricius)

Table 1. Family wise species composition, sex-specific puddling proportions and extra-floral feeding preferences of the butterflies recorded in the NWS

Species name	Common name	Capture at puddle/ Total capture		Feeding preferences	Relative abundance
		Males	Females		
Family: Nymphalidae					
<i>Danaus genutia</i> (Cramer)	Striped Tiger	5/9 (0.55) [1.46%]	0/3 (0.00)	P & S	2.42
<i>Euploea core</i> (Cramer)	Common Crow	17/28 (0.60) [4.97%]	0/4 (0.00)	AM	6.44
<i>Euthalia aconthea</i> (Cramer)	Common Baron	10/14 (0.71) [2.92%]	0/3 (0.00)	P& S	3.42
<i>Junonia lemonias</i> (Linnaeus)	Lemon Pansy	9/15 (0.60) [2.63%]	-	P& S	3.02
<i>Melanitis leda</i> (Linnaeus)	Common Evening Brown	1/4 (0.25) [0.29%]	-	AM	0.80
<i>Neptis sankara</i> (Kollar)	Broad Banded Sailor	23/30 (0.76) [6.73%]	0/3 (0.00)	P& S	6.64
<i>Parantica aglea</i> (Stoll)	Glassy Tiger	13/21 (0.61) [3.80%]	0/2 (0.00)	P &S	4.63
<i>Phalanta phalantha</i> (Drury)	Common Leopard	14/19 (0.73) [4.09%]	1/7 (0.14) [8.33%]	P & S	5.23
<i>Polyura athamas</i> (Drury)	Common Nawab	1/1 (1.00) (0.29%)	-	P &S	0.20
<i>Vanessa indica</i> (Herbst)	Indian Red Admiral	1/2 (0.50) [0.29%]	-	MD	0.40
Family: Pieridae					
<i>Catopsilia pomona</i> (Fabricius)	Common Emigrant	71/78 (0.91) [20.76%]	2/8 (0.25) [16.67%]	P& S	17.30
<i>Catopsilia pyranthe</i> (Linnaeus)	Mottled Emigrant	28/32 (0.87) [8.19%]	2/10 (0.20) [16.67%]	P& S	8.45
<i>Colias erate</i> (Esper)	Pale Clouded Yellow	5/12 (0.41) [1.46%]	-	P & S	2.42
<i>Eurema andersoni</i> (Moore)	One Spot Grass Yellow	14/16 (0.87) [4.13%]	1/6 (0.16) [8.33%]	P & S	4.44
<i>Eurema hecabe</i> (Linnaeus)	Common Grass Yellow	25/28 (0.89) [7.31%]	2/9 (0.22) [16.67%]	P &S	7.45
Family: Papilionidae					
<i>Graphium nomius</i> (Esper)	Spot Swordtail	1/1 (1.00) [0.29%]	-	P& S	0.20
<i>Graphium sarpedon</i> (Linnaeus)	Common Blue Bottle	2/2 (1.00) [0.58%]	-	P &S	0.40
<i>Papilio clytia</i> (Linnaeus)	Common Mime	1/1 (1.00) [0.29%]	-	P & S	0.20
<i>Papilio demoleus</i> Linnaeus	Lime Butterfly	29/37 (0.78) [8.48%]	1/7 (0.14) [8.33%]	P &S	8.85
<i>Papilio polytes</i> Cramer	Common Mormon	68/71 (0.95) [19.88%]	3/8 (0.37) [25%]	P& S	15.89
Family: Lycaenidae					
<i>Arhopala amantes</i> (Hewitson)	Large Oakblue	3/4 (0.75) [0.87%]	-	P& S	0.80
<i>Flos asoka</i> (de Niceville)	Spangled Plushblue	1/2 (0.50) [0.29%]	-	AM	0.40

Abbreviations used: S = Soil, P = Puddle, AM = Algal Mat, MD = Mammalian Dung

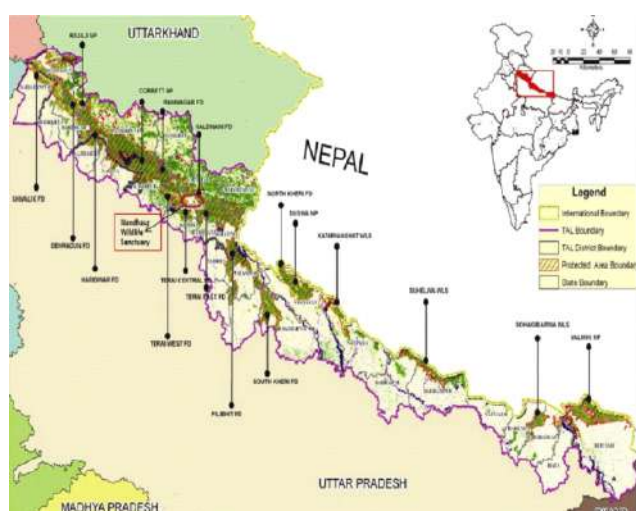
Symbols used: Puddling proportions and percent contributions are given in parentheses and brackets

was the most abundant with relative abundance of 17.30, followed by *Papilio polytes* Cramer (15.89), *Papilio demoleus* Linnaeus (8.85), and *Catopsilia pyranthe* (Linnaeus) (8.45), whereas, *Graphium nomius* (Esper), *Papilio clytia* (Linnaeus) and *Polyura athamas* (Drury) were reported observed as least abundant species during the study period. Feeding preferences varied significantly among the total reported species of butterflies and they were observed feeding on a variety of extra floral substrates i.e., puddles, soil, mammalian dung and algal mats. Interestingly, majority of species of butterflies were recorded feeding at puddles and

moist soils (81.82%). However, four species of butterflies showed anomalous feeding preferences viz. *Flos asoka* (de Niceville), *Euploea core* (Cramer) and *Melanitis leda* (Linnaeus) which were observed feeding on algal mats, while *Vanessa indica* (Herbst) was observed feeding at mammalian dung during the study period.

Species wise sex-specific puddling patterns:

Sex-specifically, a total of 427 male individuals were recorded from all the species of butterflies, while only 70 individuals of females belonging to 12 species were sampled. No female individual of the family Lycaenidae was recorded.



Source: Irengbam et al (2017)

Fig. 1. Location map of the NWS bordering TAL of the Himalaya



Modified: Verma (2011)

Fig. 2. Geographical position of study areas in the protected Nandhaur landscape, Uttarakhand

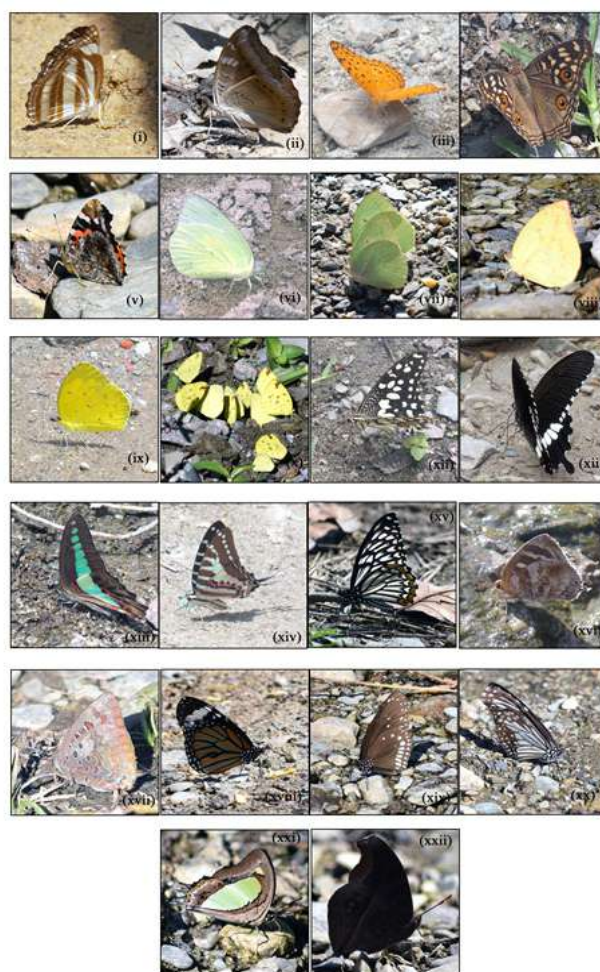


Fig. 3. Photographs of recorded butterfly species on different feeding substrates in the NWS (i) *Neptis sankara*, (ii) *Euthalia aconthea*, (iii) *Phalanta phalantha*, (iv) *Junonia lemonias*, (v) *Vanessa indica*, (vi) *Catopsilia pomona*, (vii) *Catopsilia pyranthe*, (viii) *Colias erate*, (ix) *Eurema hecabe*, (x) *Eurema andersoni*, (xi) *Papilio demoleus*, (xii) *Papilio polytes*, (xiii) *Graphium sarpedon*, (xiv) *Graphium nomius*, (xv) *Papilio clytia*, (xvi) *Flos asoka*, (xvii) *Arhopala amantes*, (xviii) *Danaus genutia*, (xix) *Euploea core*, (xx) *Parantica aglea*, (xxi) *Polyura athamas*, (xxii) *Melanitis leda*

Observations revealed that 80.09% of the total male individuals of butterflies showed rich puddling behavior which were probed visiting at moist soils, puddles, mammalian dung and algal mats for feeding. Similarly, 12 individuals of female butterflies belonging to seven species were recorded in puddling activity and constituted 17.14% of the total female individuals of butterflies. The maximum mud puddling proportion of males and females was recorded for species namely, *Papilio polytes* (0.95 males: 0.37 females), followed by *Catopsilia pomona* (0.91 males: 0.25 females), *Eurema hecabe* (0.89 males: 0.22 females) and *Catopsilia pyranthe* (0.87 males: 0.20 females), respectively (Table 1). The individuals of these species were also found often in large aggregations at the puddling sites (Image 3). On the other hand, puddling proportion of female individuals was recorded least for species *Eurema andersoni* (Moore) (0.16), *Papilio demoleus* Linnaeus (0.14) and *Phalanta phalantha* (Drury) (0.14). Further, no female individual for species *Danaus genutia* (Cramer), *Euploea core* (Cramer), *Euthalia aconthea* (Cramer), *Neptis sankara* (Kollar) and *Parantica aglea* (Stoll) were recorded in the puddling activity.

Family wise sex-specific puddling patterns: Data on proportion and percent contribution of total numbers of individuals of male and female butterflies of different families has been presented in Table 2. In all, 354 individuals of male and female butterflies representing four families were caught from different puddling substrates during the study period. Family Pieridae was represented by maximum number of male and female individuals engaged in mud puddling i.e., 41.81% of total individuals of males and 58.34% of total individuals of females, followed by Papilionidae (29.53% males and 33.33% females), Nymphalidae (27.49% males and 8.33% females) and Lycaenidae (1.17% males), respectively. Furthermore, the maximum value of sex-specific puddling proportions of butterflies was reported for family Papilionidae (0.90 males: 0.26 females), followed by Pieridae (0.86 males: 0.21 females), Nymphalidae (0.65 males: 0.04 females) and Lycaenidae (0.66 males), respectively.

Seasonal variation in puddling patterns: During summer season the maximum number of species and individuals of butterflies were recorded i.e., 18 species and 171 individuals, followed by rainy (17 species and 143 individuals) and winter (12 species and 40 individuals), respectively (Fig. 5). The various measures of diversity indices were calculated in order to understand seasonal puddling patterns among butterflies (Table 3). Rainy season showed higher values for computed diversity indices as compared to the other seasons. Table 4 depicts seasonal patterns in puddling behavior of total number of species and individuals of



Image 1. Open forest habitat during summer season along stream side of the River Gola in Nandhaur Range

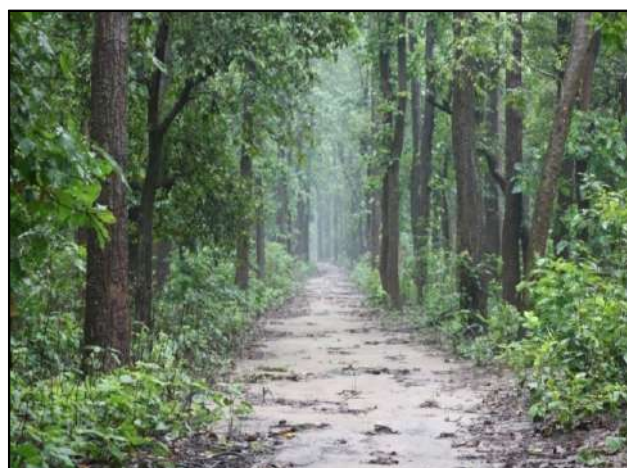


Image 2. Forest edge habitat during monsoon season nearby the River Sharda in Dogadi Range



Image 3. Individuals of few species of butterflies (*Catopsilia pomona* and *Papilio polytes*) found often in large aggregations at the puddling sites

different families of butterflies recorded during the study period. Nymphalidae was the most dominant family in terms of species richness i.e., 31.81% of total recorded butterflies during the summer and rainy seasons (Table 4). However, during winter season the numbers of species were found to be similar for the families Nymphalidae and Pieridae (5 species each). Pieridae being the dominant family showed much pronounced puddling behavior across the different seasons when compared with the individuals of species belonging to other families.

The present study that was aimed primarily to study the ecological and behavioral aspects of butterflies was first of its own type in the NWS as well as in the TAL region. Previously, Arya and Dayakrishna (2017) recorded 36 species of butterflies from the NWS, wherein 18 species belonged to family Nymphalidae and rest to the others. Similarly, Sanwal et al (2017) reported 35 species majority of which belonged to the family Nymphalidae (16 species) from the NWS. Many adult butterfly species exhibit specialized feeding preferences and can be observed to feed on fluids such as

Table 2. Proportion and percent contribution of total number of individuals of males and females butterflies of different families recorded in puddling activity

Family	Capture at puddles/Total capture		
	Males	Females	Total
Nymphalidae	94/143 (0.65) [27.49%]	1/22 (0.04) [8.33%]	95/165 (0.57) [26.84%]
Pieridae	143/166 (0.86) [41.81%]	7/33 (0.21) [58.34%]	150/199 (0.75) [42.37%]
Papilionidae	101/112 (0.90) [29.53%]	4/15 (0.26) [33.33%]	105/127 (0.82) [29.66%]
Lycaenidae	4/6 (0.66) [1.17%]	0/0	4/6 (0.66) [1.13%]

moist soils, human sweat, blood, tears, animal dung and urine, rotting fruits, tree sap, honeydew, fungi, alkaloids from the withered leaves and juices of carrion (Krenn 2008). During the present study, most of the species of butterflies were probed feeding at puddles and moist soils. On the other hand, four species of butterflies were caught feeding at algal mats floating on the surface of the water and mammalian dung, respectively. In comparison, Adler (1982) has also reported a similar pattern of feeding preferences with slight contradiction for different species of moths (Lepidoptera) from the Pennsylvania. Hall and Willmott (2000) recorded male individuals of 89 species feeding on rotting carrion of fish which belonged to the butterfly family, Riodinidae. Pola and Garcia-Paris (2005) observed males of *Papilio polytes* Linnaeus drinking seawater at low tide while standing on green algal mats or on exposed coral structures located at the southeast coast of the island of Guam, USA. Sidhu (2010) recorded butterfly species such as *Papilio machaon ladakensis* and *Agrynnis adippe pallid* puddling at the edges of high altitude brackish water lakes in cold desert of Ladakh, Jammu and Kashmir, India. Ahmed et al (2016) documented 35 species of butterflies exhibiting puddling behavior, three species feeding on dung of wild animals and two species on rotten fruits from the protected areas of the Gir region in Saurashtra peninsula of Gujarat, India. Fernando et al (2017) reported different species of butterflies puddling on Asian elephant (*Elephas maximus*) dung from the south-western part of Wasgamuwa National Park, Sri Lanka.

The results of present findings emphasize that the puddling is exclusively a male behavior in most of the recorded butterflies. The substantial numbers of male butterflies fed proportionately more at the puddles rather than the females. Such patterns of observations agree well with findings that have also been reported earlier for different species of the Lepidoptera (Collenette 1934, Norris 1936, Downes 1974, Adler 1982, Boggs and Jackson 1991, Sculley and Boggs 1996, Otis et al 2006, Sidhu 2010). However, our findings appear slight contradictory to the previous

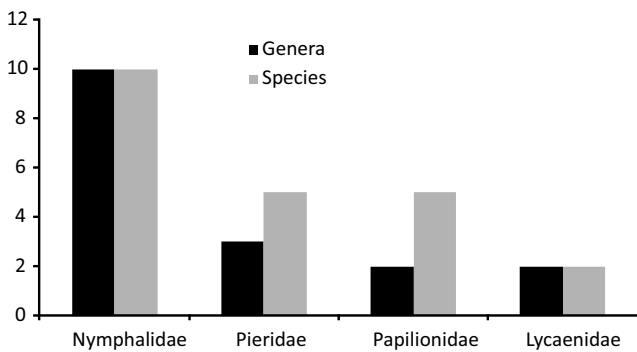


Fig. 4. Family wise total number of genera and species of butterflies recorded in puddling activity during the study period

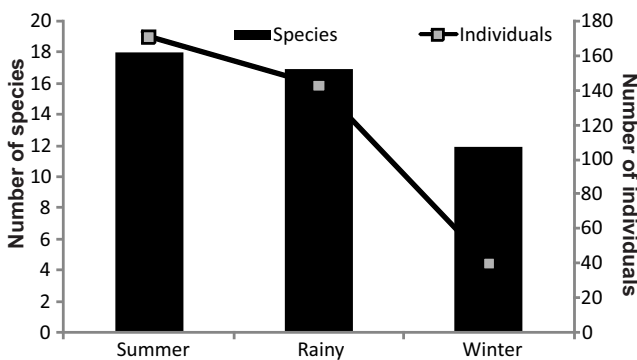


Fig. 5. Seasonal variation in number of species and individuals during the study period

observations made by Launer et al (1993), wherein large number of Californian Bay checker spot butterflies (*Euphydryas edithabayensis*) and females in particular, were observed exhibiting puddling behavior. During the present study period, family Lycaenidae was represented by only male individuals and no female individual was recorded. This may be either due to the rarity of observations or the fact that least number of individuals of this family was sampled during the study period.

There is no single explanation with regard to the wide occurrence of sexual differences in the puddling vigor of adult butterflies. Boggs and Jackson (1991) found that older females of species *Speyeria mormonia* (Nymphalidae) puddle to a greater extent than females of the other well studied species. In our present investigations, out of the total recorded species, four species of Pieridae, two species of Papilionidae and one species of Nymphalidae differed significantly in sex-specific mud puddling proportions at the puddling sites. Perusal of literature reveals that as nutritional requirement vary with age and sex, the mating and puddling patterns may co-vary among different species within the same taxonomic entity (Karlsson 1995, 1996, Sculley and Boggs 1996). It was suggested that, if female mates several times, it get infused by multiple units of puddle nutrients through spermatophores which can lead to a reduction in their frequency of puddling, thus young males needed to puddle more frequently. On the other hand, where females mate only once is more likely to assemble at the puddles

when old to replenish their sodium reserves that had been depleted (Sculley and Boggs 1996 and Boggs and Dau 2004). In the present study, the lack of constancy in puddling individuals of butterflies across the seasons and scarcity of data on the longevity and mating frequency of butterflies demerits us to empirically evaluate this hypothesis for inter-specific variance in the sex ratios at puddles. An alternative explanation for sex-specific puddling patterns involves the use of sodium for neuromuscular activity by both sexes correlated with their flight morphology, at least as in the case of Riodinidae (Hall and Willmott 2000). Hence, puddling behavior of butterflies is not a simple matter as it might be further governed by several possible underlying mechanisms such as mate choice and mating frequency of a given species, the intra-sexual competitive abilities, current nutrient status of the individuals and several ecological pressures as well as the availability of resources at the puddling sites (Boggs and Jackson 1991 and Molleman et al 2005).

In general, members of several species of butterfly either from the same or different families often aggregate in groups at the puddles (Larsen 1991). In order to explain this behavior, the concept of local enhancement, a form of social facilitation against predation has been described as phenomenon in which an individual is attracted to a specific location by the presence or actions of other organisms performing the same behavior (Clayton 1978 and Beck et al 1999). Butterflies of pierids and swallowtails, often provide

Table 3. Various measures of diversity indices computed for butterfly assemblages across different seasons

Diversity Indices	Diversity among individuals recorded in puddling activity			Diversity among individuals recorded in entire study period		
	Summer	Rainy	Winter	Summer	Rainy	Winter
Shannon H	2.336	2.415	2.028	2.427	2.547	2.159
Simpson D	0.873	0.891	0.818	0.894	0.909	0.851
Equitability	0.808	0.871	0.816	0.839	0.899	0.868

Table 4. Percent contribution for species and individuals recorded from different families of butterflies in puddling activity across different seasons

Family	Number of species (No. of puddling species/total species)			Number of individuals (No. of puddling individuals/total individuals)		
	Summer	Rainy	Winter	Summer	Rainy	Winter
Nymphalidae	7/10 [31.81%]	7/10 [31.81%]	5/10 [22.73%]	46/77 [26.90%]	42/71 [29.38%]	7/17 [17.50%]
Pieridae	4/5 [18.18%]	5/5 [22.73%]	5/5 [22.73%]	67/86 [39.18%]	61/77 [42.65%]	22/36 [55.00%]
Papilionidae	5/5 [22.73%]	3/5 [13.64%]	1/5 [4.55%]	56/67 [32.75%]	39/46 [27.27%]	10/14 [25.00%]
Lycaenidae	2/2 [9.09%]	2/2 [9.09%]	1/2 [4.55%]	2/3 [1.17%]	1/2 [0.70%]	1/1 [2.5%]

visual stimulus to other members by fluttering their wings at times when puddling, thus embracing intra-specific as well as inter-specific attraction for those seeking to locate such minerals rich resources which are usually rare and moderately disturbed (Opler and Krizek 1984, Beck et al 1999, Otis et al 2006). Similarly, in the present study, such behavior has been recorded among species such as *Catopsilia pomona* (Fabricius), *Catopsilia pyranthe* (Linnaeus), *Eurema hecabe* Linnaeus, *Papilio demoleus* Linnaeus and *Papilio polytes* Cramer at the mud puddles located in the NWS. Studies suggest that this sort of puddling behavior by multi-species in dense congregations can cause a dilution effect, reducing the high risk of predation to individual butterflies, particularly in the tropics where predation intensity is relatively higher on butterflies (Clayton 1978 and Burger and Gochfeld 2001), and thus can be accounted as a strong selective force for the local enhancement at the mud puddles (Srygley and Chai 1990, Tyler et al 1994, Beck et al 1999).

Seasonal puddling activity among the different species as well as individuals was observed maximum during summer season when the climate in the study area remains extremely dry. The availability of nectar may act as a determining factor which often get reduced due to extreme weather conditions (Tiple et al 2006) and thus, altered the many species of butterfly to the water absorbing sources such as mud puddles and moist soils present in the vicinity of NWS. In addition, it is possible that the increased rate of sodium consumption affected by drought could lead to forced puddling activity (Launer et al 1993). Moreover, overall species diversity was much higher in the rainy season, followed by summer and winter seasons which support the earlier findings of Arya and Dayakrishna (2017) from the NWS.

In spite of being a protected area, the NWS is under tremendous pressure due to anthropogenic activities such as forest fragmentation and degradation, over-exploitation of forest resources, agriculture extension, negative interactions between human and wildlife, unregulated and unsustainable riverbed mining (Irengbam et al 2017). Recent studies by Basumatari et al (2016) on palynological data from the mud puddling locations of butterflies suggests critical and indicative role of these sites in revealing ecological attributes such as type of forests, status of the current vegetation, habitat degradation, presence or absence of human activities and the recovered diversity of pollen taxa from such puddling sites reflects valuable information on adult nectar sources and migrations of butterflies. Thus, our study recommends that such habitats should be identified and must be prioritized for the formulation of conservation planning of butterflies and

other flora and fauna in the NWS as well as in the TAL region.

CONCLUSION

The present study which was conducted in the natural wild conditions of NWS provides support for sex-specific feeding patterns of puddling behaviour among different species of butterflies. Mostly young males of butterflies which must be in order to replenish water or essential nutrients expended during mating, gamete production or general metabolism were seen at the puddles. Moreover, for a better understanding of sex-specific puddling patterns, an integrated theoretical as well as experimental approach is required to elucidate nature and the role of adult feeding in butterflies. It is also suggested that sites providing puddling resources play a vital role in sustaining the populations of butterfly communities during harsh climatic conditions, such areas within or adjacent to the NWS should be earmarked for the conservation and research on these beautiful creatures.

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Avifauna of Ghatigaon Wildlife Sanctuary of Gwalior Madhya Pradesh, India

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Abstract: A total of 73 bird species belonging to 15 orders, 42 families and 67 genera were observed. Family ardeidae, accipitridae, phasianidae and columbidae having 5 species each with 6.85 per cent share, followed by cuculidae, alcedinidae, sturnidae and muscipidae with 3 species each with 4.11 per cent share, psittacidae, strigiidae, picidae, alaudidae, dicuridae, corvidae and campephagidae having 2 species each with 2.74 per cent share, and phalacrocoracidae, ciconiidae, anatidae, gruidae, rallidae, jacanidae, recurvirostridae, charadriidae, caprimulgidae, apodidae, meropidae, coraciidae, upupidae, blucerotidae, ramphastidae, lanidae, oriolidae, pycnonotidae, timaliidae, monarchidae, rhipiduridae, cisticolidae, motacillidae, nectariniidae, passeridae, ploceidae and astrildidae having 1 species each with 1.37 per cent share. From 73 species of birds 12, 26, 17 and 18 species fall in a category of very common, common, uncommon and rare. The highest bird population was at Woodland, followed by Scrubland, while as lowest bird population was at Grassland and Tighra Reservoir. Among 73 species of birds one is critically endangered (*Ardeotis nigriceps*) and is included in CITES Appendix I, one is near-threatened (*Mycteria leucocephala*).

Keywords: CITES, Habitat, Common, Uncommon and rare

In the Gwalior-Chambal Region of Northern Madhya Pradesh four Wildlife Sanctuaries and one National Park are present. Among them Ghatigaon in Gwalior district is declared as the Wildlife Sanctuary for the protection of endangered species Great Indian Bustard (GIB). Species diversity and their conservation issues in the Ghatigaon Bustard Sanctuary had not been studied scientifically in detail. Due to lack of scientific information proper management of the Sanctuary is not taking place. The Great Indian Bustards form the chief attraction of the sanctuary and this Sanctuary also given an umbrella protection to many other endangered and rare species. Understanding the diversity and structure of bird communities is essential to delineate the importance of regional or local landscapes for avian conservation (Kattan and Franco 2004). Various reports are available on the avifauna of the state. Whitehead (1911) noted the birds of Sehore. Osmaston (1922) and Bates (1927) listed the birds of Panchmarhi. Roonwal (1942) collected the birds of Hoshangabad. Various workers had worked on the ecology, habitat use, threats, and population estimation and conservation status of Great Indian Bustard (Baker 1929), (Rahmani and Soni 1997), (Khan et al 2008), (Munjpara et al 2011), (Kasambe et al 2007), Newton et al (1986) made survey of birds of Kanha National Park. Malhotra et al (2005) reported 32 species of waterfowls from Sirpur tank, Indore. Rao (2008) recorded 120 species of wetland birds from the Gwalior Chambal region of North Madhya Pradesh. The present study deals with the avian diversity of the Ghatigaon Bustard Sanctuary.

MATERIAL AND METHODS

Study area: Ghatigaon Bustard Sanctuary (77° 45' to 78° 08'E and 26° 40' to 26° 15' N) is gently sloping with rolling grassy meadows laid with boulders in Madhya Pradesh. Total area of the Sanctuary is 512 sq. km. To preserve and propagate Indian Bustard the Madhya Pradesh Government has declared Ghatigaon Wildlife Sanctuary vide Gazette Notification No. 15-16-75/Das/2 Wildlife Protection Act, 1972 dated 21st May 1981. The vegetation in the sanctuary is dominated by thorny forest. The forests in the sanctuary are mainly dry deciduous type intermingled with low bushes of *Zizyphus* spp. Climate is generally hot and dry. The average temperature in winter and summer months varies from 18.89°C to 37.47°C. The normal range of annual rainfall is 700 mm. For the present study four study sites were selected on the basis of different habitats such as: Aquatic, Grassland, Scrubland and Woodland habitat.

Study Sites

Site I: Tighra reservoir, geographically it lies on 78° 01' 24" E longitudes and 26° 12' 59" N latitude, constructed on Sank River in the year 1917 near Tighra village (Fig. 1).

Site II: Pawta Military Grass Bir, geographically it lies on 77° 57' 14" E longitudes and 26° 10' 37" N latitude (Fig. 2).

Site III: It is located near Ghatigaon and geographically lies on 77° 57' 41" E longitudes and 26° 9' 5" N latitude (Fig. 3).

Site IV: It is located near Lakan Pura village and geographically lies on 77° 54' 37" E longitudes and 26° 12' 9" N latitude (Fig. 4).

The study was carried out from May to October 2012 by making regular field visits. The number of birds was estimated by direct observation and point counting method. The other most important aspect kept in consideration was the activity of birds. Since the peak activity in most birds lasts for 1 or 2 hours after sunrise or before sunset, so monitoring of transect was done either in early morning or late evening hours Thakur (2008) and survey was conducted mostly in morning hours between 6.00 a.m. to 10.00 a.m. and late afternoon during 3.00 p.m. to 7.00 p.m. Birds were observed and counted with aid of field Binocular (8x40X). Photographs of birds were obtained with the help of Nikon Digital Camera (D60, 70-300 mm). Field identification was carried out with the help of various field guides such as Grimmet et al (1999) and Ali (2002). Data collected by direct counting methods (Chhetry 2006) and counting flock method were done separately to calculate the bird species diversity. The data recorded in each survey was analyzed for relative abundance on the basis of relative frequency scale of occurrence depending upon the number of sightings, as followed by McKinnon and Philips (1993) as: very common (VC)- sighted more than 10 times, common (C) - sighted seven to nine times, uncommon (UC) - sighted three to six

times and rare (Rr) - sighted only once or twice.

RESULTS AND DISCUSSION

Seventy three bird species (Table 1) belonging to 15 orders, 42 families and 67 genera were observed. Great Indian Bustard were reported in the Sanctuary, but it is not seen during the present study. The family wise proportion of species richness of birds varied from 1.37%-6.85 per cent. Family Ardeidae, Accipitridae, Phasinidae and Columbidae having 5 species each (6.85%), followed by Cuculidae, Alcedinidae, Sturnidae and Muscicapidae represented by 3 species, Psittacidae, Strigiidae, Picidae, Alaudidae, Dicruridae, Corvidae and Campephagidae having 2 species each, and Phalacrocoracidae, Ciconiidae, Anatidae, Gruidae, Rallidae, Jacanidae, Recurvirostridae, Charadriidae, Caprimulgidae, Apodidae, Meropidae, Coraciidae, Upupidae, Blucerotidae, Ramphastidae, Lanidae, Oriolidae, Pycnonotidae, Tamaliidae, Monarchidae, Rhipiduridae, Cisticolidae, Motacillidae, Nectariniidae, Passeridae, Ploceidae and Astrildidae having 1 species (Table 2). Mahabal (2000) recorded 61 species of birds from Talra Wildlife Sanctuary district Shimla, Himachal Pradesh, belonging to 19 families. Out of the total birds recorded, 31



Fig. 1. Tighra Reservoir (Site I)



Fig. 2. Grassland (Site II)



Fig. 3. Scrubland (Site III)



Fig. 4. Woodland (Site IV)

Table 1. Checklist of birds in Ghatigaon Bustard Sanctuary

Order	Family	Common name	Zoological name	IUCN status	Sight frequency
Pelecaniformes	Phalacrocoracidae	Little Cormorant	<i>Phalacrocorax niger</i>	LC	C
	Ardeidae	Grey Heron	<i>Ardea cinerea</i>	LC	C
		India Pond Heron	<i>Ardeola grayii</i>	LC	VC
		Cattle Egret	<i>Bubulcus ibis</i>	LC	C
		Little Egret	<i>Egretta garzetta</i>	LC	C
		Large Egret	<i>Casmerodius albus</i>	LC	C
	Ciconiidae	Painted Stork	<i>Mycteria leucocephala</i>	NT	Rr
Anseriformes	Anatidae	Comb Duck	<i>Sarkidiornis melauotos</i>	LC	C
Falconiformes	Accipitridae	Black Winged Kite	<i>Elanus caeruleus</i>	LC	UC
		Black Kite	<i>Milvus migrans</i>	LC	UC
		Shikra	<i>Accipiter badius</i>	LC	UC
		Crested Serpent Eagle	<i>Spilornis cheela</i>	LC	Rr
		Tawny Eagle	<i>Aquila rapax</i>	LC	Rr
Galliformes	Phasinidae	Grey Francolin	<i>Francolinus pondicerianus</i>	LC	VC
		Black Francolin	<i>Francolinus francolinus</i>	LC	Rr
		Jungle Bush Quail	<i>Perdica asiatica</i>	LC	Rr
		Red Spur Fowl	<i>Gallus spadicea</i>	LC	Rr
		India Peafowl	<i>Pavo cristatus</i>	LC	VC
Gruiformes	Gruidae	Indian Bustard	<i>Ardeotis nigriceps</i>	CR	Rr
	Rallidae	Moorhen	<i>Gallinula chloropus</i>	LC	UC
Charadriiformes	Jacaniidae	Black Winged Jacana	<i>Metopidius indicus</i>	LC	Rr
	Recurvirostridae	Black-winged Stilt	<i>Himantopus himantopus</i>	LC	VC
	Charadriidae	Red-wattled Lapwing	<i>Vanellus indicus</i>	LC	VC
Columbiformes	Columbidae	Yellow Footed Green Pigeon	<i>Treron phoenicopterus</i>	LC	UC
		Rock Pigeon	<i>Columba livia</i>	LC	VC
		Collared Dove	<i>Streptopelia decaocto</i>	LC	C
		Spotted Dove	<i>Stigmatopelia chinensis</i>	LC	UC
		Laughing Dove	<i>Stigmatopelia senegalensis</i>	LC	C
Psittaciformes	Psittacidae	Rose-ringed Parakeet	<i>Psittacula krameri</i>	LC	VC
		Blossom-headed Parakeet	<i>Psittacula roseate</i>	LC	UC
Cuculiformes	Cuculidae	Common Hawk Cuckoo	<i>Cuculus varius</i>	LC	Rr
		Asian Koel	<i>Eudynamis scolopaceus</i>	LC	C
		Greater Coucal	<i>Centropus sinensis</i>	LC	C
Strigiformes	Strigidae	Eurasian Eagle Owl	<i>Bubo bubo</i>	LC	UC
		Jungle Owlet	<i>Glaucidium radiatum</i>	LC	Rr
Caprimulgiformes	Caprimulgidae	Indian Nightjar	<i>Caprimulgus asiaticus</i>	LC	C
Apodiformes	Apodidae	Little Swift	<i>Apus affinis</i>	LC	C
Coraciiformes	Alcedinidae	Pied Kingfisher	<i>Ceryle rudis</i>	LC	UC
		White-breasted Kingfisher	<i>Halcyon smyrnensis</i>	LC	C
		Common Kingfisher	<i>Alcedo atthis</i>	LC	C
	Meropidae	Little Green Bee-eater	<i>Merops orientalis</i>	LC	C
	Coraciidae	Indian Roller	<i>Coracias benghalensis</i>	LC	C
	Upupidae	Eurasian Hoopoe	<i>Upupa epops</i>	LC	UC
	Bucerotidae	Indian Grey Hornbill	<i>Ocyrceros birostris</i>	LC	UC
	Ramphastidae	Coppersmith Barbet	<i>Megalaima haemacephala</i>	LC	UC
Piciformes	Picidae	Black-rumped Flame Back	<i>Dinopium benghalensis</i>	LC	UC
		Yellow Crowned Woodpecker	<i>Dendrocopos mahrattensis</i>	LC	Rr

Cont...

Passeriformes	Alaudidae	Ashy-crowned Sparrow Lark	<i>Eremopterix griseus</i>	LC	UC
		Rufous Tailed Lark	<i>Ammomanes phoenicura</i>	LC	Rr
	Laniidae	Long Tailed Shrike	<i>Lanius schach</i>	LC	Rr
	Oriolidae	Golden Oriole	<i>Oriolus oriolus</i>	LC	C
	Dicruridae	Black Drongo	<i>Dicrurus macrocercus</i>	LC	VC
		White Bellied Drongo	<i>Dicrurus caerulescens</i>	LC	Rr
	Sturnidae	Brahminy Starling	<i>Sturnus pagodarum</i>	LC	C
		Common Myna	<i>Acridotheres tristis</i>	LC	C
		Bank Myna	<i>Acridotheres fuscus</i>	LC	C
	Corvidae	House Crow	<i>Corvus splendens</i>	LC	C
		Rufous Tree pie	<i>Dendrocitta vagabunda</i>	LC	C
	Campephagidae	Small Minivet	<i>Pericrocotus cinnamomeus</i>	LC	Rr
		Large Cuckoo Shrike	<i>Coracina macei</i>	LC	UC
	Muscicapidae	Tickell's Blue Flycatcher	<i>Cyornis tickelliae</i>	LC	Rr
		Oriental Magpie Robin	<i>Copsychus saularis</i>	LC	C
		India Robin	<i>Saxicoloides fulicatus</i>	LC	VC
	Pycnonotidae	Red-vented Bulbul	<i>Pycnonotus cafer</i>	LC	C
	Timaliidae	Jungle Babbler	<i>Turdoides striata</i>	LC	Rr
	Monarchidae	Asian Paradise Flycatcher	<i>Terpsiphone paradise</i>	LC	C
	Rhipiduridae	White-browed Fantail	<i>Rhipidura aureola</i>	LC	VC
	Cisticolidae	Ashy Prinia	<i>Prinia socialis</i>	LC	VC
	Motacillidae	White-browed Wagtail	<i>Motacilla madaraspatensis</i>	LC	UC
	Nectariniidae	Purple Sunbird	<i>Nectarinia asiatica</i>	LC	C
	Passeridae	House Sparrow	<i>Passer domesticus</i>	LC	VC
	Ploceidae	Baya Weaver	<i>Ploceus philippinus</i>	LC	UC
	Estrildidae	Red Avadavat	<i>Amandava amandava</i>	LC	Rr

LC = Least concern, NT = Near-threatened, CR = Critically Endangered, VC = Very Common, C = Common, UC = Uncommon and Rr = Rare

species were found commonly in the sanctuary area and 28 species were rarely seen, only two species of birds viz. Spotted Nutcracker and Grey-winged Blackbird were found to be abundant. A total of 62 species of birds belonging to 7 orders, 20 families and 43 genera from the Kaza area of Lahul and Spiti district of Himachal Pradesh were recorded, out of 62 species 25 were common, 20 were uncommon, 15 were very common and 2 were rare (Thakur and Mattu 2011). Major study of endangered 'Great Indian Bustard' was conducted at Karera Bustard Sanctuary by Bombay Natural History Society (Ali 1982-84). Malhotra et al (2005) reported 32 species of waterfowls from Sirpur tank, Indore, belonging to 7 orders and 10 families. Rao (2008) recorded 120 species of wetland birds from the Gwalior Chambal region of North Madhya Pradesh. Nair (2009) surveyed National Chambal Sanctuary and during the survey 118 bird species were sighted of which 16 were migratory.

The categorization of birds into relative abundance categories revealed that 26 species are common, 17 species are uncommon, 12 species are very common and 18 species are rare (Table 1). Among 73 species of birds one is critically endangered (*Ardeotis nigriceps*) and is included in CITES Appendix I, one is near-threatened (*Mycteria leucocephala*)

and rest 71 species are least concern. The population of birds at different sites varies due to difference in habitat qualities, highest bird population was found at site IV, followed by site III, while as lowest bird population was found at sites I and site II (Table 3). The species diversity index fluctuates from 1.63 (Site IV) to 1.32 (Site II). The highest diversity was shown in the Site IV, while the Site II has lowest diversity. Apart from diversity, species evenness has shown variation in the first four sites with values of 0.96 (Site I), 0.94 (Site II), 0.93 (Site IV) and 0.92 (Site III) with respect to each other (Table 3).

CONCLUSIONS

The Great Indian Bustard were reported in the Sanctuary but during the present study this majestic bird is not found. One of the reasons may be that this bird which is facing extinction through anthropogenic activities, like habitat destruction, poaching for meat etc. For the protection of this bird, management measures like habitat evaluation of Great Indian Bustard (GIB) in the Sanctuary should be carried out. Potential habitats should be restored and maintained and reintroduction of these birds may be an option for the Sanctuary managers. One of the most important measures to be adopted in preserving this Sanctuary is to create

Table 2. Total number and percentage of bird species of different families

Family	No. of genera	No. of species	Percentage contribution of families
Phalacrocoracidae	1	1	1.37
Ardeidae	5	5	6.85
Ciconiidae	1	1	1.37
Anatidae	1	1	1.37
Accipitridae	5	5	6.85
Phasinidae	4	5	6.85
Gruidae	1	1	1.37
Rallidae	1	1	1.37
Jacaniidae	1	1	1.37
Recurvirostridae	1	1	1.37
Charadriidae	1	1	1.37
Columbidae	4	5	6.85
Psittacidae	1	2	2.74
Cuculidae	3	3	4.11
Strigidae	2	2	2.74
Caprimulgidae	1	1	1.37
Apodidae	1	1	1.37
Alcedinidae	3	3	4.11
Meropidae	1	1	1.37
Coraciidae	1	1	1.37
Upupidae	1	1	1.37
Blucerotidae	1	1	1.37
Ramphastidae	1	1	1.37
Picidae	2	2	2.74
Alaudidae	2	2	2.74
Laniidae	1	1	1.37
Oriolidae	1	1	1.37
Dicruridae	1	2	2.74
Sturnidae	2	3	4.11
Corvidae	2	2	2.74
Campephagidae	2	2	2.74
Muscicapidae	2	3	4.11
Pycnonotidae	1	1	1.37
Timaliidae	1	1	1.37
Monarchidae	1	1	1.37
Rhipiduridae	1	1	1.37
Cisticolidae	1	1	1.37
Motacillidae	1	1	1.37
Nectariniidae	1	1	1.37
Passeridae	1	1	1.37
Ploceidae	1	1	1.37
Estrildidae	1	1	1.37
Total	67	73	

Table 3. Population of birds at different sites in Ghatigaon Bustard Sanctuary

Common Name	Site I	Site II	Site III	Site IV	Total
Little Cormorant	18	-	-	-	18
Grey Heron	14	-	-	-	14
India Pond Heron	22	-	-	-	22
Cattle Egret	20	18	-	-	38
Little Egret	18	14	-	-	32
Large Egret	16	10	-	-	26
Painted Stork	4	-	-	-	4
Comb Duck	12	-	-	-	12
Black Winged Kite	-	4	6	9	19
Black Kite	-	1	3	4	8
Shikra	-	-	-	12	12
Crested Serpent Eagle	-	2	1	2	5
Tawny Eagle	-	1	2	1	4
Grey Francolin	-	-	24	10	34
Black Francolin	-	-	4	-	4
Jungle Bush Quail	-	-	4	2	6
Red Spur Fowl	-	-	4	2	6
India Peafowl	-	4	14	28	46
Indian Bustard	-	-	-	-	0
Moorhen	8	-	-	-	8
Black Winged Jacana	2	-	-	-	2
Black-winged Stilt	30	-	-	-	30
Red-wattled Lapwing	32	18	12	-	62
Yellow Footed Green Pigeon	-	-	-	10	10
Rock Pigeon	16	18	22	20	76
Collared Dove	8	10	14	16	48
Spotted Dove	4	6	10	14	34
Laughing Dove	16	14	19	22	71
Rose-ringed Parakeet	-	-	-	32	32
Blossom-headed Parakeet	-	-	-	12	12
Common Hawk Cuckoo	-	-	2	2	4
Asian Koel	-	-	14	18	32
Greater Coucal	-	-	12	16	28
Eurasian Eagle Owl	-	-	2	7	9
Jungle Owlet	-	-	-	2	2
Indian Nightjar	-	10	16	14	40
Little Swift	-	12	14	20	46
Pied Kingfisher	8	-	-	-	8
White-breasted Kingfisher	14	-	-	-	14
Common Kingfisher	12	-	-	-	12
Little Green Bee-eater	-	-	8	24	32
Indian Roller	6	16	12	8	42
Eurasian Hoopoe	-	-	4	12	16

Cont...

Indian Grey Hornbill	-	-	-	13	13
Coppersmith Barbet	-	-	-	8	8
Black-rumped Flame Back	-	-	-	10	10
Yellow Crowned Woodpecker	-	-	-	2	2
Ashy-crowned Sparrow Lark	-	-	4	8	12
Rufous Tailed Lark	-	-	1	2	3
Long Tailed Shrike	-	-	-	2	2
Golden Oriole	-	-	-	16	16
Black Drongo	-	-	20	38	58
White Bellied Drongo	-	-	-	2	2
Brahminy Starling	10	14	15	18	57
Common Myna	13	16	12	18	59
Bank Myna	24	10	12	6	52
Rufous Treepie	-	-	-	18	18
House Crow	18	14	20	24	76
Small Minivet	-	-	4	2	6
Large Cuckoo Shrike	-	-	-	8	8
Tickell's Blue Flycatcher	-	-	-	2	2
Red-vented Bulbul	-	12	18	22	52
Jungle Babbler	-	24	28	42	94
Asian Paradise Flycatcher	-	-	-	13	13
White-browed Fantail	-	-	-	2	2
Ashy Prinia	-	-	8	14	22
Oriental Magpie Robin	8	19	22	18	67
India Robin	6	24	28	17	75
White-browed Wagtail	8	-	-	-	8
Purple Sunbird	-	-	-	18	18
House Sparrow	18	32	40	27	117
Baya Weaver	-	-	-	10	10
Red Avadavat	1	2	-	-	3
Total	386	325	455	699	1865
Shannon-Weiner Diversity Index	-1.3879	-1.3248	-1.4516	-1.6129	
Evenness Index	-0.9490	-0.9362	-0.9256	-0.9310	

consciousness among the people particularly local inhabitants to realize the importance of this endangered (Great Indian Bustard) bird.

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Ultrastructure of Coccidian *Eimeria isabellae* Lom and Dyková (Eimeriidae, Protozoa) in *Conger conger* L.

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Abstract: Life cycle stages of *Eimeria isabellae* in the naturally-infected teleost fish, *Conger conger* L., from the French Mediterranean coastal region of Languedoc-Roussillon (Banyuls-sur-mer, Grau du Roi and Sète) was studied by light and electron microscopy. Eighteen infected individual fish were examined and found with developmental stages of the parasite in the intestine epithelium. Merogonial, gamogonial and sporogonial stages were found both localized intracellularly and epicellularly in the microvillus region of enterocytes. They were separated from the intestinal lumen by closely spaced enterocyte and parasitophore vacuole membranes with several zones of attachment to the host cell. First generation merozoites were formed by ectomerogony. Macrogamonts contained lipid inclusions, amylopectin and dense granules. The study revealed the presence of *Eimeria isabellae* coccidia in the epithelium of the midgut with an infestation rate of 18 European conger (*Conger conger*) out of 31 fish sampled. Ultrastructure study of *Eimeria isabellae* has demonstrated the intra-cytoplasmic nature of the infestation and confirmed the coccidia belonged to the Eimeriidae group. Of particular interest were the relationships between the different intracellular stages and their host cell. Formation of a parasitophorous vacuole and various histopathological and biological aspects are discussed in this study.

Keywords: *Eimeria isabellae*, Coccidian, Parasite, Ultrastructure, Life cycle

Freshwater and marine fishes are parasitized by a wide spectrum of morphologically different species including apicomplexan coccidial parasites (Molnar 1989, Lukes 1990). In the family Eimeriidae, there are a number of species in the genus *Eimeria* (Landsberg and Paperna 1987, Paperna and Landsberg 1987, Benajiba et al 1994, Molnár and Székely 1917), one of which considerably extends the range of morphological types within the genus. Members of two other genera (*Goussia* and *Cryptosporidium*) also have been found in the microvillous zone of fish intestine (Molnar and Hanek 1974, Daoudi et al 1986; 1987, Lukes 1990, Lukes 1992, Diouf and Toguebaye 1994, Molnar et al 2006, Székely et al 2013, Lovy and Friend 2015). Dykova and Lom (1981) define the genus *Eimeria* as characterized by epi-cellular development of schizogony and gamogonia; sporogony being intracytoplasmic. The genus is cosmopolitan and is represented by several species i.e. *Eimeria anguillae* (Leger and Holland 1922) and *Eimeria isabellae* Lom and Dykova 1982 and 1995 (Daoudi et al 1987, Radujkovic and Raibut 1989a, b).

Sub-epithelial stages of the coccidia of *Eimeria isabellae* parasites of the midgut of European conger (*Conger conger* L.) are described in fish collected from the French Mediterranean in order to elucidate the life cycle in host cells. The ultrastructure of the mode of implantation, the relationship with the feeder cell and the nature of the

development cycle of these coccidia provide the best criteria for placing them within the Eimeriidae (Duszynski et al 2013). Their affinities and differences with cryptosporidia and coccidia Eimeriidae are described and discussed in this study.

MATERIAL AND METHODS

Thirty one European conger (*Conger conger* L.) specimens from the French Mediterranean coast of Languedoc-Roussillon (Banyuls-sur-mer, Grau du Roi and Sète), were studied. Fresh mucosa of the anterior, central and posterior parts of the intestine and gut contents were examined. Infection was confirmed by examination of excrement for oocysts. Pieces of the infected tissue from the anterior gut were fixed in Karnowski's solution for 24 hours at 4°C, rinsed repeatedly in Cacodylate buffer, 0.1 M, pH 7.4 and post-fixed in 1 per cent osmium tetroxide using the same buffer for 1 hour. After rinsing, the material was dehydrated in an ascending ethanol series and embedded in Epon. Thin sections, cut by a LKB III ultratome with diamond knives, were stained on the grid with uranyl acetate and lead citrate and examined with a Jeol 100 CX TEM (Venable and Coggeshall 1965).

RESULTS AND DISCUSSION

Of the 31 European conger specimens, 16 were infected

for *Eimeria isabellae*. In most cases, the infection was massive and developmental stages were found mostly in the posterior part of the intestine. Coccidia are detected in the border of the midgut epithelium. The species is attributed to the genus *Eimeria* because of its epi-cellular development of the merogonic and gamogonic stages and for its intraepithelial sporogony.

The parasite protrudes into the intestinal lumen, enclosed in a wall that connects it to the basal part of the host cell, hence the extracellular appearance in photonic microscopy. Merozoites and micromeres are free whereas all other stages of merogony are surrounded by a parasitophorous wall formed by two simple membranes (Fig. 1-7, 9, 16, 31). Parasite/host cell ratios are best observed by the young trophozoites and gamonts stages in host cells. The infesting germ cells (sporozoite or merozoite) develop in the host cell cytoplasm isolated by the parasitophorous membrane. The intracytoplasmic stage projects into the intestinal lumen and remains in contact with the feeder cell only through its basal region (Fig. 1-7). The membrane of intestinal microvilli is applied intimately to the parasitophorous membrane except where it is in contact with the cytoplasm of the host cell. The parasitophorous membrane produces finger-tip like extensions, 0.2-0.5 μm long, inside the cytoplasm of the host cell (Fig. 1, 4-5, 32). These extensions are internally consolidated by numerous osmiophilic granules conforming to the shape of the digitations (Fig. 5, 6, 32); the mature stages (meronts and gamonts) are often released in the intestinal lumen and become embedded in the parasitophorous wall. The coccidian stages are intracellular and ectoplasmic stages of the parasite.

Merogony and Morphology of Merozoites: The initiator stages of merogony are intracellular and the ectoplasmic trophozoites are spherical (3.5 μm in diameter) or ovoid (2 x 3.8 μm). Their cytoplasm is limited by a film of 2 membranes lacking microspores (Fig. 1-7). Granules, similar to those of the micronemes are present in the apical part of the young meronte (Fig. 7) and are probably remnants of the merozoite or original sporozoite. About ten mitochondria, small and elongated, are located at the periphery of the young meronte. The cytoplasm, rich in ribosomes, contains, among other things, amylopectin grains, endoplasmic reticulum and a central nucleus. During meronte development, the two internal components of the typical cell wall fragment then disappear. The wall of the meronte is then shown with a single unit membrane, devoid of microspores. The nucleus, increases in volume and undergoes 3 or 4 divisions. The resulting wire core spindles are spherical with a diameter of 1.6 μm . During nuclear divisions, the cytoplasm is enriched

in mitochondria of rather large size, sometimes bifurcated, in the endoplasmic reticulum. Differentiation of merozoites is by peripheral ectomerogony: evaginations are formed in the meronte membrane, each of which penetrates a nucleus, mitochondrion or the cytoplasm (Fig. 8). By strangulation, the young merozoites then become free in the parasitophorous vacuole.

Mature meronts are spherical to sub-spherical (Fig. 9) but the number of merozoites produced is variable (8-12). They are usually elongated, averaging 4.65 μm long by 1.35 μm wide. They are asymmetrical being tapered at one pole, defined as anterior, and the other more rounded pole as the posterior. The cell wall of the merozoite consists of two unitary membranes, the most external drift from the cell wall of the original meronte and is c. 12 nm thick. The free merozoites in the parasitophorous vacuole have characteristic organelles of the Eimeriidae, such as: conoid, rhoptries, micronemes, Golgi apparatus, granular endoplasmic reticulum, and mitochondria. At the anterior end of the merozoite there is a set of organelles characteristic of the sporozoites: includes the apical complex, constituted by the conoid, apical rings, rhoptries and micronemes.

The conoid is located in the center of the apical ring; it is in the form of a truncated cone oriented along the longitudinal axis of the cell (Fig. 11). Its length is about 250 nm, its anterior diameter is 180 nm and its diameter at the base is 370 nm. It consists of several microtubules arranged obliquely in a helical winding. From the center of the conoid, at least two tubular-shaped peduncles, 60 nm in diameter, start to extend to the middle part of the merozoite where they end in a larger diameter (250 nm) saccule (Fig. 3, 10, 11), these are the rhoptries and have a high osmiophilic content. Many micronemes, whose structure is close to that of rhoptries, are found in the anterior half of the merozoite.

Under the membrane complex of the merozoite, at the apical pole and encircling the conoid, dense annular formations are observed, from which the tubular fibers originate (Fig. 11). These microtubules are regularly placed under the cell wall, all around the circumference of the merozoite. They travel the entire cell longitudinally, exceeding twenty in number. The middle part of the merozoite is occupied by a nucleolar nucleus (1.45-1.7 μm in diameter) surrounded by several small tubular type mitochondria with finger-like and pedunculated ridges. The remainder of the cytoplasm contains the various cellular and reserve organelles: Golgi, ribosomal grains, endoplasmic reticulum, and some amylopectin grains. At maturity, the meronts are detached from the host epithelial cell and fall into the intestinal lumen (Fig. 9). The release of merozoites occurs after rupture of the parasitophorous wall.

Gamogonia, gamete formation and fertilization: The male and female gamonts, like the merogony stages, are also contained in the ectoplasmic intracellular position. At their base they are in contact with the cytoplasm of the host cell and, on the opposite side, they project into the intestinal lumen. These stages are enclosed in the parasitophorous vacuole.

Micro-gametogenesis: The first identifiable stages of male sexual development are globular or slightly oval cells, measuring on average (5-8.5) x (7-10) μm . They are already

plurinucleated (Fig. 12-13) and show some micronemes in the apical part with remnants of merozoite of origin. These microgonts are delimited by a single membrane (Fig. 13), lacking micropores. Their cytoplasm is rich in ribosome grains and peripheral mitochondria. Some trabeculae of endoplasmic reticulum and platelets of amylopectin are observed inside the cytoplasm (Fig. 12-13). The chromatin of mitotic division nuclei takes on a characteristic cap-like appearance. The wire cores spindles are located at the periphery of the microgamonte (Fig. 12-13) and undergo one or two additional divisions. During these transformations, the cytoplasm is enriched with lipid and polysaccharide reserve grains arranged in the center of the microgamonte. The mitochondria are located on the periphery. Before the differentiation of microgamontes, the mass of the gametocyte is formed of two zones; a central zone containing reserve grains and some cytoplasmic organelles and a peripheral zone or nuclear zone containing the nuclei of future micromeres (Fig. 14) aligned in a single layer under the membrane of the microgametocyte. These nuclei are often associated with mitochondria and are directed towards the microgametocyte membrane by a centriolar structure. The nuclei are then subdivided into two distinct zones: a peripheral zone, very osmophilic and rich in cap-shaped chromatin, and a second, clearer zone; differentiation of microgametes occurs on the surface of microgametocysts (Fig. 15-16). The flagella grow from the centriolar structures in the most osmophilic nuclear zone and penetrate into a membrane evagination on the surface of the microgametocyst (Fig. 17), accompanied by mitochondria. The microgametes are arranged tangentially on the surface of the gamonte (Fig. 15-18) to which they are connected by a pedicle. Strangulation rupture of the latter results in the release of the microgamete into the parasitophorous vacuole. At the end of this process, there remains, in the center of the microgametocyst, a small residue consisting of cytoplasm and nuclear material (Fig. 33).

The microgamete of *Eimeria isabellae* is an elongated cell (Fig. 15, 16, 17, 18) in the cell body it measures approximately 4 μm long by 0.5 μm wide. Its wall consists of a unitary membrane enveloping the cell body and the flagella (Fig. 20). The flagella develop from the apex of the microgamete (Fig. 19). The microgamete then becomes rapidly independent of the cell body and assumes the classical structure of the coccidia. In cross section, each flagellum, 0.22 μm in diameter, shows 9 pairs of peripheral tubules surrounding a central pair (Fig. 20). The cell body contains little cytoplasm; it is essentially filled with nuclear material. The nucleus, very dense and elongated (Fig. 17), has in its anterior part, a gutter where a tubular

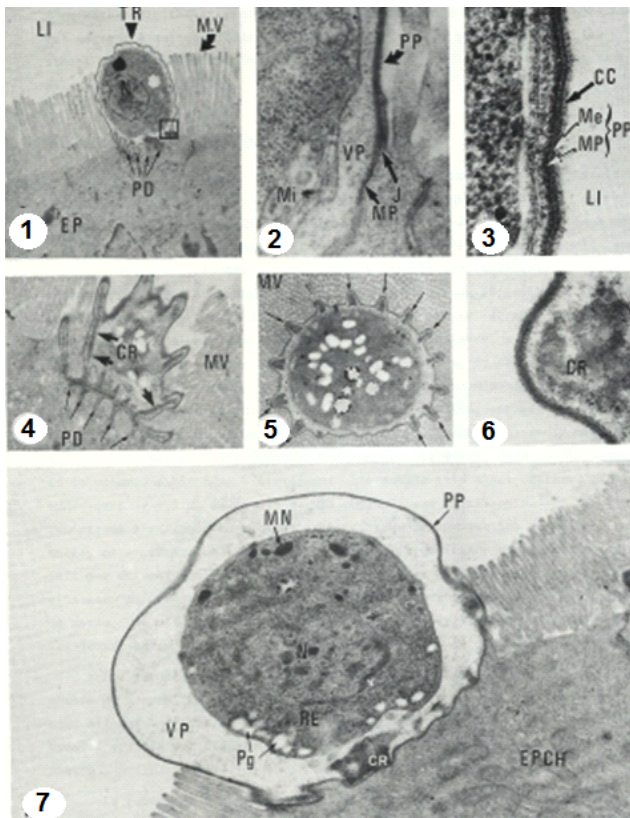


Plate 1. Cell structure of *Eimeria isabellae*

Fig. 1. The young trophozoite (TR)

Fig. 2. Detail of the junction (J) between the parasitophorous vacuole (VP) and the cytoplasmic membrane of the host cell (= intestinal microvilli membrane) (x 50,000)

Fig. 3. Structure of the parasitophorous wall (PP) composed of an outer membrane (Me) covered with a cell-coat (CC) and an inner membrane (MP); the parasitophorous membrane itself (x 120,000)

Fig. 4. The parasitophorous membrane sends several finger-like prolongations (PD) to the cytoplasm of the host cell. These finger-like projections contain filling bodies (CR) consisting of dense granules (x 10,000)

Fig. 5. Cross-section showing the radiating arrangement of the fences (arrows) and their filling material (x 7,200)

Fig. 6. Granular nature of the filling body (CR) (x 70,000)

Fig. 7. Uninucleate Meront (N) in its parasitophorous vacuole (VP). Note the presence of micronemes (MN) (x 16,300)

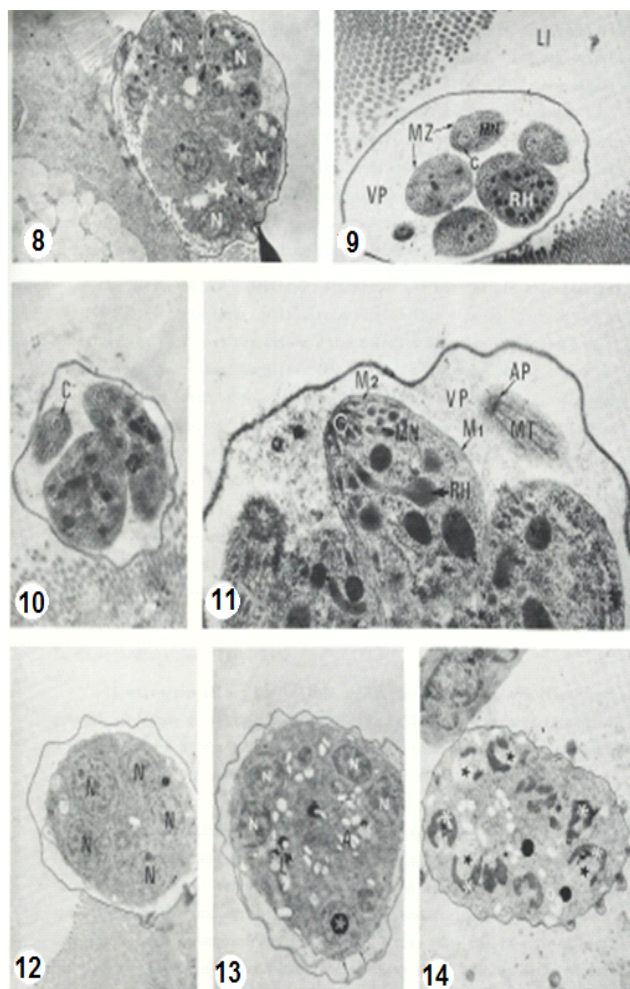


Plate 2. Microgametogenesis in *Eimeria isabellae*

- Fig. 8.** The meronte stage of budding. Each evagination contains a nucleus (N), micronemes, one or more mitochondria and polysaccharide granules (x 7,100)
- Fig. 9.** Section of a mature meronte passing through 6 free merozoites (MZ) in the parasitophorous vacuole (VP) (x 13,300)
- Fig. 10.** Three mature merozoites. Circular structure of the conoid (C) in cross section. Peduncles of the rhoptries are observed inside the conoid (x 11,600)
- Fig. 11.** Structure of the anterior merozoite region showing the truncated cone appearance of the conoid (C). Showing presence of the microtubules under the pellicle (MT) originate from the polar ring (AP) and the arrangement of rhoptries (RH) and micronemes (MN). (x 28,000).
- Fig. 12.** Young multinucleated microgametocyte (x 6,650)
- Fig. 13.** Older microgametocyte. The nuclei are arranged on the periphery with centriolar structures (arrows) when they are divided. Note the considerable increase of cytoplasmic reserve grains: lipids (L); amylopectin (A). (x 6,650)
- Fig. 14.** Microgametocyte after condensation of chromatin. Each nucleus consists of a dense peripheral zone of a cap (clear star) and a low osmiophilic inner zone (dark star) (x 7,100)

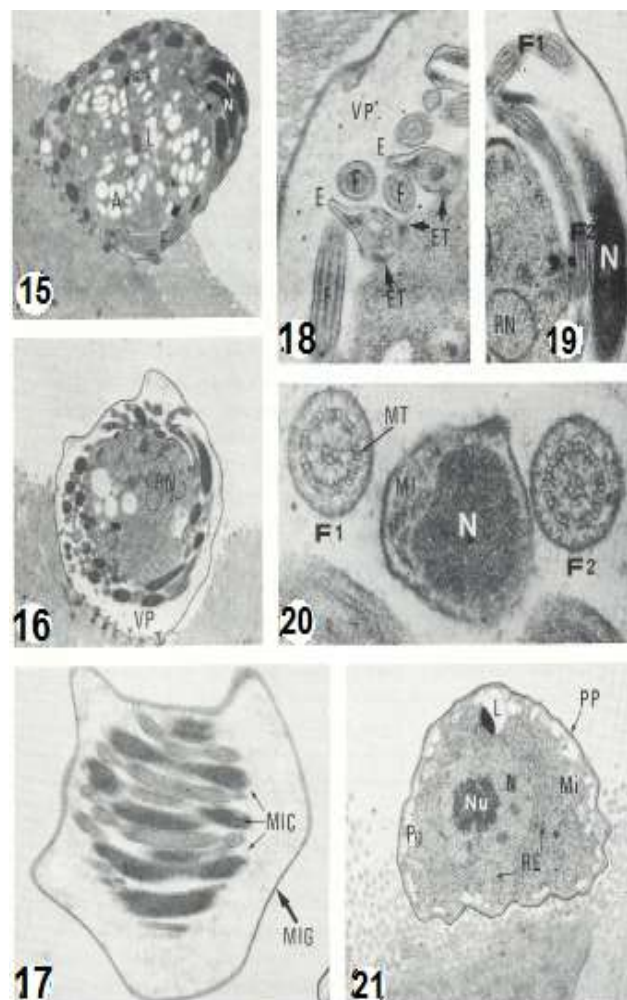


Plate 3. Microgametocyst of *Eimeria isabellae*

- Fig. 15.** Microgametocyte showing early beadwork (x 6,700); (A) amylopectin, (F) flagella; (L) lipids; (N) nucleus;
- Fig. 16.** Other gametocytes showing differentiation of microgametes inside the parasitophorous vacuole (VP). Numerous nuclear residues (RN) in the cytoplasm corresponding to the sparse zone of the nucleus not incorporated in the microgamete (x 5200)
- Fig. 17.** Free mature microgamonte in the intestinal lumen (MIG). The microgametes (MIC) are wrapped around each other, in a ball, in the parasitophorous vacuole (x 13,600)
- Fig. 18.** Phase of the development of microgametes in evaginations (E) of the cell membrane. Dizziness of these evaginations (ET) (x 27,000)
- Fig. 19.** Longitudinal section of a microgamete showing the two flagella (F1-F2) and the nucleus (N) very dense (x 18,600)
- Fig. 20.** Cross section of a microgamete with the two typical flagella (F1-F2), with 9 pairs of peripheral microtubules (MT) surrounding a central pair and the cell body with tubular mitochondria (MT) nested in the dense nucleus (N) (x 94,000)
- Fig. 21.** A young microgamonte surrounded by the parasitophorous wall (PP). Note the volume occupied by the nucleus central nucleus (Nu) (x 11,700)

mitochondrion is housed (Fig. 20).

Macrogametogenesis: The macrogamonte is a spherical cell at the beginning of its development, taking an elliptical form at maturity. Its size gradually changes from 5 μm in diameter to 10 $\times$ 12 μm . Remarkable changes occur during the evolution of macrogamonts and concern mainly the cytoplasmic constituents. In the young gamont, the central nucleus has a large nucleolus, its cytoplasm is very rich in ribosomes, in endoplasmic reticulum with numerous peripheral mitochondria (Fig. 21). The cell wall is formed of a unit membrane sporadically lined by another membrane which corresponds to the remnants of the inner membrane of the original merozoite, and even the conoid, as well as some micronemas, persist in fairly advanced stages of macrogametogenesis (Fig. 23). The macrogamonte is

transformed into a macrogamete by quantitative and qualitative modifications of the cytoplasmic organelles. Among the quantitative changes is an increase in the number of polysaccharide granules (amylopectin), to the point that in mature macrogametes they occupy the majority of the cytoplasm around the central nucleus (Fig. 24, 25). The

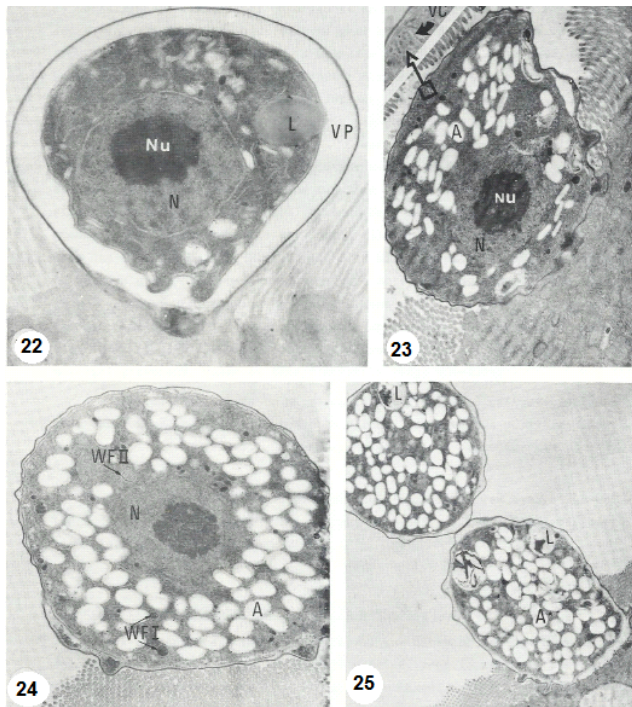


Plate 4. Macrogametogenesis in *Eimeria isabellae*

- Fig. 26.** Epicellular macrogamonte in the parasitophorous vacuole (VP). Roughing of amylopectin grains in the anterior region (x 16,400) Nu and N
- Fig. 27.** Macrogamonte at a more advanced stage of evolution. Significant increase in amylopectin (A) grains and peripheral mitochondria (x 9,700). The triangular box shows the conoid, remnant of initial merozoites (x 18,700)
- Fig. 28.** Mature macrogamete showing the increase in the number of amylopectin granules and the appearance of dense bodies (WF I) and labyrinthine bodies (WF II) (x 10,000)
- Fig. 29.** Two mature macrogametes; the reserves in lipid inclusions (L) and polysaccharides (A) are very important (x 5,400)

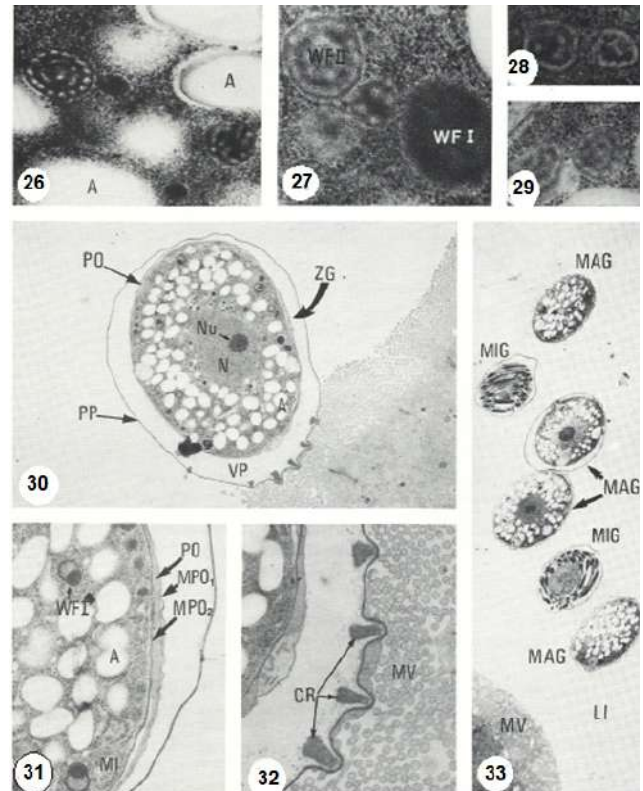


Plate 5. Macrogametogenesis in *Eimeria isabellae*

- Fig. 26.** Structure of labyrinthine bodies with concentric dispositions showing many gaps, A (x 49,000)
- Fig. 27.** Morphology of a dense body (WF I) and a labyrinthine body (WF II) (x 73,000)
- Fig. 28.** Labyrinthine bodies with their limiting membrane (x 54,000)
- Fig. 29.** Peripheral disposition of labyrinthine bodies (x 50,000)
- Fig. 30.** Zygote (ZG) still in epicellular position with early formation of thick wall (PO) (x 5,400)
- Fig. 31.** Detail of the cortical zone of the zygote. The wall of the oocyst (PO) is limited on both sides by two membranes (MP01 - MP02). Only dense bodies (WF I) in disintegration are still observed in the cytoplasm of the zygote (x 14,300)
- Fig. 32.** Zygote-host cell contact zone. The finger-like extensions persist as well as their filling bodies (CR) (x 14,300)
- Fig. 33.** Six gamontes: 4 microgamonts (MAG) and 2 microgamonts (MIG) surrounded by the parasitophorous wall, detached from their host cell and free in the intestinal lumen (LI). Note the disappearance of fingerlings as well as dense granulations that form the filling body (x 2,500).

cytoplasm is also enriched with mitochondria, endoplasmic reticulum, and lipid inclusions (Fig. 22, 23, 24). A striking fact in the maturation of macrogonts, is the progressive appearance of a globular structure opaque to electrons, with a dense or lacunous appearance. These structures can be assimilated into the dense bodies (wall forming bodies I: WF I) and the labyrinthine bodies (WF II) which enter the formation of the oocystic hull in coccidia of higher vertebrates. The dense bodies (WF I) in *Eimeria isabellae* (Fig 24, 26, 29) are spherical with a diameter of 0.32 to 0.55 μm . They have a homogeneous and dense appearance. Other formations specific to macrogametes are lacunous or labyrinthine bodies (WF II) (Fig. 24, 26, 28). They are rounded masses of 0.27 to 0.45 μm in diameter, moderately dense, hollowed out by numerous small clear gaps in an irregular network, limited by a simple membrane inside a clear cavity of c. 36 nm in thickness (Fig. 26, 28).

The macrogamete is often an elliptical (9-10.8) x (11.5 - 12.7) μm cell (Fig. 24, 25, 33), with a nucleus, central or slightly eccentric (3.4 μm in diameter) (Fig. 23-24). This nucleus is surrounded by numerous polysaccharide, spherical (0.5-0.75 μm) or oval (0.44-0.7) x (0.8-1.1) μm platelets. (Fig. 24-25). One or two oval lipid inclusions (2 x 1.5 μm) are present in the macrogamete section (Fig. 25, 33). The first sign marking development of the macrogamete in the zygote, are differentiation and organization of a thick wall, resembling that of terrestrial *Eimeria*. Directly below the unit membrane of the macrogamete a slightly granular and slightly osmiophilic material is deposited which is organized into a layer of variable thickness (50-100 nm) (Fig. 30, 31, 32). A second neoformed membrane internally covers this layer; it appears to come from the cytoplasmic membrane stock of the zygote (Fig. 31, 32, 33).

The cytoplasm of the zygote still contains dense bodies (WF I), smaller than those described in macrogametes but in larger numbers (Fig. 30, 31). They will probably participate in the formation of a second layer below the neoformed unit membrane. At maturity, the anchoring system of macrogametes (zygotes) seems to be resorbed; they are detached from the top of host epithelial cells, developed by the parasitophorous wall and fall into the intestinal lumen (Fig. 33). Only tetrasporous oocysts at different stages of maturity are found within the intestinal epithelium and at no time was an oocinet (mobile zygote) observed in the intraepithelial position.

CONCLUSION

This ultrastructure study of *Eimeria isabellae* and its epicellular stages demonstrates the intracytoplasmic localizations and confirms the typical Eimerian nature of

these life cycle stages. These observations are contrary to the interpretation of Levine (1984) which brings these stages closer to the cryptosporidia. In contrast to the merogonic and gamogonic stages, sporogony of *E. isabellae* is intracytoplasmic and occurs in the epithelium of the midgut; only oocysts at the end of maturation could be observed. The oocyst wall is a thin structure represented by two unitary membranes. The sporozoites begin their differentiation within the sporocysts and show the presence of micronemes, rhoptries, amylopectin grains and a large globule probably marking the origin of retractile globules in the sporozoites. Young sporocysts show a filamentous ornamentation. Dense bands appear on the periphery of each sporocyst which forms on top of each other to make the spore wall. At the end of development, the sporocyst is well stratified with a thick spore wall of 250 nm. The membrane layer beneath is 10 nm thick and the inner (240 nm) layer is compact and slightly osmiophilic. Intestinal coccidiosis appears to have little effect on their host and is not strongly pathogenic compared to extra intestinal coccidiosis. The boom in farming of various species of fish for economic purposes often encourages parasitism; careful monitoring of coccidiosis and their modes of transmission is clearly needed in order to intervene effectively on parasite expansion and to minimize control their pathogenicity.

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Mammalian Feces Used a Non-Invasive Tool to Indicate Heavy Metal Pollution in Galtaji, Jaipur, India

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Abstract: This study investigated feces metal concentration as a non-invasive tool of indicator of environmental health with the *Macaca Mulatta* (Rhesus Monkey), *Capra aegagrus hircus* (Goats) and *Bos Taurus* (Cow) of Galtaji, Jaipur. This technique is used to study gross exposure of metal pollution. Varying concentrations of Zinc (Zn), Copper (Cu) and Lead (Pb) were found in feces samples. Zinc concentration was in range of 151.33 (*Macaca mulatta*) to 96.91 (*Capra aegagrus*) ppm, Copper was in between 174.66 (*M. mulatta*) to 140.83 (*C. aegagrus*) ppm and lead was in range of 271.33 (*M. mulatta*) to 87.41 (*C. aegagrus*) ppm which was maximum among the metals. The differences were in the heavy metal occurrence in the feces of monkey, goat and Among these soils showed significantly high concentration of metals as well as the results also reflected a close correlation between metal concentrations in the living environment and in the feces of mammals.

Keywords: Bioindicator, Non-invasive, Feces, Heavy metals pollution, Mammals

Jaipur has to facing serious environmental challenges in the process of industrialization and modernization of the country. Driven by urbanization, industrialization and the over-exploitation of minerals, a large amount of waste containing heavy metals is discharged into the environment. In addition, Jaipur on top ten location with respect to RSPM in industrial area: 238 $\mu\text{g}/\text{m}^3$ (Central pollution control board, 2009). Among the pollutants, heavy metals are the subject which is the most of attention because they accumulate through the food chain and create environmental problems affect to the environment and human health (Paul et al 2015). The presence of heavy metals and high concentrations of heavy metals often pose a serious threat to biota and the environment (Cheng et al 2003). Heavy metals such as Cd, Cr, and Pb are main threats to human health through bioaccumulation. Heavy metals can survive for long periods in the environment and accumulate to toxic levels. The main pathway of absorption of animals and plants are the eating process, heavy metals are absorbed in food and water (Sharma et al 2007).

Urbanization, industrial and population growth are the source of pollutants into the ecosystem and daily basis, the amount of waste have been increasing worldwide. These anthropogenic activities have generated important transformations in aquatic and soil during the last few decades. Persistent pollutants containing heavy metals are distributed globally in long-term through the process of bioaccumulation and biomagnifications by food chain (Costa et al 2013). The water and soil pollution due to heavy metals is one of the major concerns in most of the metropolitan cities of developing countries. Naturally, these heavy metals are not easily degradable so accumulate in the animal as well as

human bodies in a very high toxic amount which cause undesirable effects beyond a certain limit.

In Jaipur, the management of solid waste is poor and backward. The system of primary waste collection is not focused. Especially waste does not process and classify at source. People invariably dispose of their solid waste on the streets, open space, and in drains, which has resulted in unhygienic conditions in the city (Jawaid et al 2017). According to MSME (2016) there are 48 industrial parks covering more than 37,000 industrial units located in Jaipur, of which the popular are mineral based. These industrial wastes are not desirable byproducts. When improperly handled and disposed, industrial wastes imperil both human health and the environment (Mathur 2005). Hence, indicator and monitoring of heavy metal becomes an important responsibility of all the stakeholders generating waste and authorities managing human habitat and environment. In recent environmental conservation practices biological monitoring has limitation to sacrificing an animal to monitor environmental contamination. To overcome these problem feces of mammals can be used as non-invasive tool to monitor the health of environment (Gupta 2013).

Galtaji temple is an ancient Hindu pilgrimage and it is famous temple in Jaipur (Fig. 1). Annually, there are many tourists and pilgrims at this temple who bathe in water tanks for luckily. In the temple there are many mammals such as rhesus monkeys, goats and cows. They usually drink water from the water tank in the temple and pick up food from the roadside or receive from the pilgrims. Fecal matter of these mammals was used to indicate metal contamination in Galtaji, Jaipur.

MATERIAL AND METHODS

Study site: Galtaji GPS coordinates are 26.9172° N, 75.8581° E (Fig. 2). The Galtaji temple is famous for the many troops of monkeys that dwell in this area. Pilgrims always taking a dip in the natural springs of Galtaji is considered very auspicious and is said to cleanse a person of his sins. In addition, can witness a large family of monkeys flocking towards the temple tank, for a bath in sunset.

Sample site: Random sampling was done at seven sites (an area where many pilgrims cross) (Fig. 3) (Table 1, 2) and feces of three commonly occurring mammal species *Macaca Mulatta* (Rhesus Monkey), *Capra aegagrus hircus* (Goats) and *Bos Taurus* (Cow) as well food, vegetation, soil and water were collected from Galtaji.

Sample digestion: Collected samples were dried in an oven at 50°C for 15h and then chopped. Feces, food, soil, vegetable were weighed at 0.1g and water was taken 2ml. Concentrated nitric and perchloric acid were added into 4: 1 ratio to each test tube for digestion. Then samples were boiled at 100°C in water bath upto 26 hours for fecal samples and 18h for food, water, soil, vegetable samples until they were completely digested and became clear. Samples were never allowed to dry completely. When sample was cooled down, add 5-6 drops of H₂O₂ (70%) and continue to boil for 1 hour to neutralize. After completion of the digestion process, the samples were filtered and diluted to 20 ml with de-ionized water. Samples were stored at room temperature before analysis.

Sample analysis: Concentration of heavy metals (Pb, Cu, Zn) in feces of *Macaca Mulatta* (Rhesus Monkey), *Capra aegagrus hircus* (Goats) and *Bos Taurus* (Cow) by atomic absorption spectrophotometer (Varian AA240FS) at



Fig. 1. Galtaji temple in Jaipur, India



Fig. 2. GPS location of Galtaji

wavelength of 213.9 nm for Zinc, 324.7 nm for copper and 217 nm for lead respectively with 6mA current.

The concentration of the heavy metals is determined by the formula:

Metal concentration = AAS reading (Abs)

$$\times \frac{\text{Dilution factor}}{\text{Weight of sample (ppm)}}$$

The statistical analysis of samples collected from different sites of Galtaji of Jaipur was done by using GraphPad prism 8.0.1.

RESULTS AND DISCUSSION

The feces sample analysis shows the presence of zinc (Zn), copper (Cu) and lead (Pb) in varying concentrations (Table 3). Zinc concentration was observed in range of 155 (*Macaca Mulatta*) to 96.91 (*Capra aegagrus*) ppm, Copper was in between 187.33 (*Macaca Mulatta*) to 140.83 (*Capra aegagrus*) ppm and lead was in range of 271.33 (*Macaca Mulatta*) to 87.41 (*Capra aegagrus*) ppm in feces of mammals of Galtaji, Jaipur. Metal concentration was in descending order as follows in different mammals *Macaca Mulatta* > *Bos Taurus* > *Capra aegagrus* (Table 3). As well among the metal following descending order was observed Copper > Zinc > Lead (Table 3). Food, vegetable, water and soil samples collected from different sites also showed good concentration of zinc, copper and lead (Table 4). In food, zinc (76.33ppm), copper (111.33ppm) and lead (36.67) were detected. In vegetable zinc (50.67ppm), copper (99.33ppm) and lead (43.33 ppm) was analyzed. Concentration of Zinc (116ppm), Copper (162ppm) and Lead (184ppm) were measured in soil whereas, in water Zinc (54ppm), Copper (128ppm) and lead (43.33ppm) were also detected. Concentration of metals following descending order was observed soil > water > food > vegetable (Table 4). Central pollution control board (2018) also quantified the ambient air quality status which was 177

Table 1. Sampling sites of mammalian feces in Galtaji, Jaipur

Sample	Characteristics
GM1, GM2, GM3	<i>Macaca mullata</i> fecal matter in road site near Galtaji Park
GM4, GM5, GM6	<i>M. mullata</i> fecal matter in Baba Cave near Payohan Gufa
GM7, GM8, GM9	<i>M. mullata</i> fecal matter in stairs near the middle water tank
GM10, GM11, GM12	<i>M. mullata</i> fecal matter near Galtaji Kund (the below water tank)
GG1, GG2, GG3	<i>Capra Aegagrus</i> fecal matter near Tea Point
GG4, GG5, GG6	<i>C. aegagrus</i> fecal matter in road site near Hanuman Ji Temple
GC1, GC2, GC3	<i>Bos Taurus</i> fecal matter near the top water tank

Table 2. Sampling sites of food, vegetable, soil, water in Galtaji, Jaipur

Sample	Characteristics
GF1, GF2	Food – Chickpeas near the top water tank
GF3, GF4	Food - Chapati cake in Baba Cave near Payohan Gufa
GF5, GF6	Food – Pear near Galtaji Kund (the below water tank)
GV1, GV2, GV3	Vegetable in road site near Hanuman Ji Temple
GS1, GS2, GS3	Soil in between the top water tank and the middle water tank
GW1, GW2, GW3	Water in Galtaji Kund

Table 3. Mean concentrations (in ppm) of zinc, copper and lead measured in the feces of mammals of Galtaji, Jaipur (Mean $\pm$ S.D.)

Mammal	Zinc (Zn)	Copper (Cu)	Lead (Pb)
<i>B. Taurus</i>	151.33 $\pm$ 14.19#	174.66 $\pm$ 26.02	94.16 $\pm$ 2.94
<i>C. aegagrus</i>	96.91 $\pm$ 11.7*	140.83 $\pm$ 48.39*	87.41 $\pm$ 7.00*
<i>M. mullata</i>	155 $\pm$ 38.91	187.33 $\pm$ 31.32#	271.33 $\pm$ 14.27#

Table 4. Mean concentrations (in ppm) of zinc, copper and lead measured in the food, vegetable, soil, water sample of Galtaji, Jaipur (Mean $\pm$ S.D.)

Item	Zinc (Zn)	Copper (Cu)	Lead (Pb)
Food	76.33 $\pm$ 5.59	111.33 $\pm$ 51.58	36.67 $\pm$ 12.63
Vegetable	50.67 $\pm$ 9.02	99.33 $\pm$ 38.28	43.33 $\pm$ 5.03
Soil	116 $\pm$ 5.29	162 $\pm$ 36.39	184 $\pm$ 36.72
Water	54 $\pm$ 12.17	128 $\pm$ 10.39	43.33 $\pm$ 5.77

$\mu\text{g}/\text{m}^3$ much greater than the permissible limit ($60 \mu\text{g}/\text{m}^3$) of repairable suspended particulate matter (RSPM) or PM_{10} . In environment, heavy metal emission by wide range of processes and pathways to the air, surface waters and to the soil (Järup 2003).

Few heavy metals are excreted in the bile, i.e. As, Pb and Hg. A substance excreted by liver into bile from where excreted in intestinal tract, further eliminated from the body in the feces, or it may be reabsorbed. Since most of the substances excreted in the bile are water-soluble, they are

not likely to be reabsorbed as such. For instance, there is 99.5 per cent of total ingested lead that is excreted through feces of which 90 per cent were removed from the body and 9.5 per cent after being absorbed and metabolized leaving only 0.5 per cent to be deposited in various body tissues (Gupta 2013). Excess metal will cause an imbalance in the ecosystem as it accumulates in the organism, causing negative impacts on biota and can lead to death of living organisms (Bindu 2014).

CONCLUSION

This study established the value of feces analysis as bio-indicator of metal contamination. Analysis of feces as bioindicator has advantage that it indicates gross exposure, does not involve disturbing and killing the animals and monitoring of exposure to contamination at 24 hours intervals. The feces of mammals which is a non-invasive tool to monitoring metal pollution.

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Characterization of Grain Amaranth Genetic Resources for Agro-Morphological Traits

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Abstract: A study to assess the variation among 125 grain amaranth accessions using six morphological characters was conducted. Based on *per se* performance, the promising genotypes of grain amaranth viz., KBGA10, KBGA11, Durga, VLC 44, SKGPA 63, IC 32186, IC35642, SKGPA65, SKGPA 64, Durga, SKGPA 81, BGA2, VLC 44 and KBGA 10 were found superior over the checks under comparison (suvarna and annapurna) for yield and yield contributing traits. Multivariate analysis of phenotypic characters showed the first three principal components contributed 72.74% of observed variability amongst the 125 lines with the eigenvalue >1 and PC1 accounted for 35.84% of the total morphological variation for the traits. Seed volume weight had negative values for the three components (PC 3, 4 and 6) but contributes to the V^m component with highest positive value (0.724). The results will help researchers and breeders to understand, utilize, conserve, and manage the collection for more efficient contribution to amaranth research and cultivation.

Keywords: Amaranthus germplasm, Principal component analysis, Mean performance, Genetic resources, Morphological traits

Amaranth is a self-pollinated crop, but wide variation in genotypes existed due to varying amounts of outcrossing and frequent interspecific and inter-varietal hybridization (Ray and Roy 2009 and Mosyakin and Robertson 1996). Amaranths also exhibit tremendous diversity related to their wide adaptability to different eco-geographic situations (Lee et al 2008). Genotype identification in amaranth had been a long term challenge (Wax 1995) due to the close relationships that exist within the genus. *Amaranthus* L. is a genus of Amaranthaceae family with around 70 different species distributed worldwide including pigweeds, water hemsps and grain amaranths (Sauerbeck 2002). There are two types of Amaranth species, grain type (*A. caudatus*, *A. cruentus* and *A. hypochondriacus*) and vegetable type (*A. dubius*, *A. tricolor* and *A. cruentus*). Grain Amaranth species are of new world origin, *A. caudatus* from Andean Peru and Ecuador, *A. cruentus* and *A. hypochondriacus* from Mexico and Central America. This has been classified under the group of crops called pseudo cereals, it displays wide morphological diversity, which have unique qualities, nutritional importance, drought tolerance, disease and pest resistance, high yield in production, and increasing rate of consumption have made this crop more attractive for cultivation. Nowadays, the grain amaranths are cultivated from the temperate to tropical zone. Compared with other crops, this pseudo cereal is rich in protein (17–19% of dry weight) with double the amount of essential amino acids than wheat grain protein (Bressani et al 1987).

Despite being a self-pollinated crop, varying amounts of outcrossing and frequent inter-specific and inter varietal

hybridization have brought wide variation in amaranth genotypes. However, selection is the major breeding methodology followed in Amaranth improvement in India. Although inter-varietal crosses have been found to be successful, little progress has been made due to minute flowers, which are difficult to emasculate and un-availability of precise crossing.

Knowledge of genetic diversity and trait variations in populations is useful in plant breeding and for developing *ex situ* conservation strategies of plant genetic resources (Stoilova and Pereira 2013). Plant phenotype is commonly used for estimating genetic diversity as it provides a simple way of quantifying genotypic variation. Grain amaranth remains a largely underexploited crop for grain purposes in Tamil Nadu despite the potential as a hardy field crop with generally excellent nutritional qualities. A number of accessions of amaranth have been introduced that have acclimatized well in the region, but evaluation of agronomic and nutritional traits in the peculiar agro-ecologies of the region has not been conducted. The availability of genetic variation among and within the different accessions for these traits provides great scope for crop improvement through selection and other breeding methods to develop desired genotypes. The objective of this study was to characterize and evaluate the agronomic performance of some grain amaranthus accessions. This would provide information that would serve for their selection according to the importance of the traits they may carry to assist the grain amaranthus breeding program.

MATERIAL AND METHODS

Experimental site: The experiment was conducted in 2017 at the experimental farm of Department of Forest Biology and Tree Improvement, Forest College and Research Institute (FC&RI), Tamil Nadu Agricultural University, Mettupalayam, Tamilnadu. The institute is perched at an altitude of 300 m with a longitude of 11.9° N, latitude of 77.56° E and an annual rainfall temperature are 32.2° and 23.2° c respectively. Because of the limited quantity of seeds, the experiment was raised with 125 grain amaranthus germplasm in augmented block design (Federer 1991) in two rows along with five checks viz., Suvarna, Annapurna, GA-2, BGA-2 and RMA-7. Plants were spaced 45 cm apart between and within rows, with an inter-plot spacing of 15 cm. Phenotypic traits viz., days to 50 per cent flowering, days to maturity, plant height (cm), ear head height (cm), grain yield (gm) and seed volume weight (gm) were assessed on five randomly tagged plants in each plot. Cultural practices were as suggested by Van Sloten (1981). Neither chemicals nor fertilizers were used

during the experiment and manual weed control was followed as and when required.

Principal component analysis (PCA) was computed from correlation matrices using SAS Procedure PRINCOMP in order to assess the patterns of phenotypic trait variation considering all variables simultaneously.

RESULTS AND DISCUSSION

The summary of descriptive statistics for all the quantitative traits is given in Table 2. There were significant differences ($p < 0.05$) among the amaranth accessions for morphological traits as some exhibited broad variability.

The First order Statistical measures i.e. maximum, minimum, mean and standard deviation (SD) for the measured traits are presented in Table 2. The genotype GAG 115 (KBGA- 1) has taken the longest days (54 days) for days to 50 per cent flowering and GAG 107 (Durga) had short duration of 50 per cent flowering and maximum ear head length (101 cm). The overall mean for flowering for the

Table 1. List of grain amaranthus germplasm used (GAG-Grain Amaranthus Germplasm)

AC No.	Genotype	AC No.	Genotype	AC No.	Genotype	AC No.	Genotype	AC No.	Genotype
GAG 1	SKGPA 61	GAG 26	SKGPA 86	GAG 51	IC21803A	GAG 76	IC 35742	GAG 101	IC 120690
GAG 2	SKGPA 62	GAG 27	SKGPA 87	GAG 52	IC 21937	GAG 77	IC81698B	GAG 102	BGA2
GAG 3	SKGPA 63	GAG 28	SKGPA 88	GAG 53	IC 21938	GAG 78	IC 94654	GAG 103	RMA7
GAG 4	SKGPA 64	GAG 29	SKGPA 89	GAG 54	IC 32186	GAG 79	IC 94661	GAG 104	KBGA-10
GAG 5	SKGPA 65	GAG 30	SKGPA 90	GAG 55	IC 32190	GAG 80	IC 95204	GAG 105	KBGA -11
GAG 6	SKGPA 66	GAG 31	SKGPA 91	GAG 56	IC 32193	GAG 81	IC 95244	GAG 106	Durga
GAG 7	SKGPA 67	GAG 32	SKGPA 92	GAG 57	IC 32195	GAG 82	IC 95248	GAG 107	VLC-44
GAG 8	SKGPA 68	GAG 33	SKGPA 93	GAG 58	IC 35404	GAG 83	IC 95251	GAG 108	SKGPA – 74
GAG 9	SKGPA 69	GAG 34	SKGPA 94	GAG 59	IC 35415	GAG 84	IC 95371	GAG 109	MGA – 15
GAG 10	SKGPA 70	GAG 35	SKGPA 95	GAG 60	IC 35615	GAG 85	IC 95382	GAG 110	KBGA -1
GAG 11	SKGPA 71	GAG 36	SKGPA 96	GAG 61	IC 35633	GAG 86	IC 95383	GAG 111	KBGA-4
GAG 12	SKGPA 72	GAG 37	SKGPA 97	GAG 62	IC 35635	GAG 87	IC 95389	GAG 112	BGA -38
GAG 13	SKGPA 73	GAG 38	SKGPA 98	GAG 63	IC 35638	GAG 88	IC 95391	GAG 113	BGA43
GAG 14	SKGPA 74	GAG 39	SKGPA 99	GAG 64	IC 35642	GAG 89	IC 95406	GAG 114	KBGA-12
GAG 15	SKGPA 75	GAG 40	SKGPA 100	GAG 65	IC 35651	GAG 90	IC 95430	GAG 115	KBGA-13
GAG 16	SKGPA 76	GAG 41	SKGPA 101	GAG 66	IC 35661	GAG 91	IC 95498	GAG 116	KBGA-14
GAG 17	SKGPA 77	GAG 42	SKGPA 102	GAG 67	IC 35665	GAG 92	IC 95510	GAG 117	RHGA-13-2
GAG 18	SKGPA 78	GAG 43	SKGPA 103	GAG 68	IC 35701	GAG 93	IC95516	GAG 118	KBGA-10
GAG 19	SKGPA 79	GAG 44	SKGPA 104	GAG 69	IC 35702	GAG 94	IC 95556	GAG 119	BGA-7-1 (E)
GAG 20	SKGPA 80	GAG 45	SKGPA 105	GAG 70	IC 35711	GAG 95	IC 120621	GAG 120	BGA-29
GAG 21	SKGPA 81	GAG 46	SKGPA 106	GAG 71	IC 35713	GAG 96	IC 120649	GAG 121	SUVARNA
GAG 22	SKGPA 82	GAG 47	SKGPA 107	GAG 72	IC 35716	GAG 97	IC 120668	GAG 122	Annapurna
GAG 23	SKGPA 83	GAG 48	SKGPA 108	GAG 73	IC 35717	GAG 98	IC 120670	GAG 123	GA – 2
GAG 24	SKGPA 84	GAG 49	SKGPA 109	GAG 74	IC 35719	GAG 99	IC 120689	GAG 124	BGA – 2
GAG 25	SKGPA 85	GAG 50	SKGPA 110	GAG 75	IC 35735	GAG 100	IC 432086	GAG 125	RMA – 7

germplasm accessions was 47 days. The mean for days to maturity of 125 germplasm lines were 85 days with the short duration and short stature genotype GAG 122 (RHGA-13-2) and long duration of 58 days in the in the genotype GAG 104 (RMA-7).

Mean for ear head height was 52.95 with the minimum of 36.00 cm and maximum value of 101.00 cm in the genotype GAG 02 (SKGPA 62) and in the genotype GAG 107 (Durga) and the GAG 4 (SKGPA 64) showed maximum single plant yield (15.6 g). The germplasm GAG 30 (SKGPA 90) showed minimum number of seed volume weight. Maximum seed volume weight (7.80 g) recorded by the genotype KBGA-11 (GAG 106) with the mean value of 7.18. The salient features of the promising genotypes grain amaranth viz., KBGA10, KBGA11, Durga, VLC 44, SKGPA 63, IC 32186, IC35642,

SKGPA65, SKGPA 64, Durga, SKGPA 81, BGA 2, VLC 44 and KBGA 10, which were superior for yield and yield contributing traits over the checks under comparison (Suvarna and Annapoorna) are presented in Table 3.

Principal Component Analysis

In order to assess the patterns of variation, principal component analysis (PCA) was done by simultaneously considering all the variables. The first three principal components (PCs) accounted for 72.74 per cent of the variability amongst the 125 lines with the eigenvalue >1 under study (Table 4 and Fig. 1a-c). Pamela et al (2016) also observed the first four principal components (PCs) contributed 57.53 per cent of the variability present among the 29 accessions for the 27 traits studied. The higher the coefficients, regardless of the sign, the more effective they

Table 2. Descriptive statistics of quantitative traits of 125 accessions of amaranth

Characters	Germplasmname	Minimum	Germplasmname	Maximum	Mean	SD
DFF	GAG 107	42.00	GAG 115	54.00	47.08	1.88
DM	GAG 122	58.00	GAG 103	104.00	85.11	5.77
PH	GAG 122	105.00	GAG 117	224.00	167.25	22.60
EHH	GAG02	36.00	GAG 107	101.00	52.95	11.87
YP	GAG 94	05.90	GAG04	15.60	9.41	73.24
SVW	GAG 30	6.80	GAG 106	7.80	7.18	0.19

DFF-Days to fifty % flowering; DM-Days to maturity; PH- Plant height (cm); EHH; Ear head height(cm); YP-Yield per plant (g); SVW-Seed Volume weight (g)

Table 3. Top five superior germplasm lines over checks for grain amaranth production for yield and yield contributing characters

Character	Superior germplasm lines					Checks	
						Suvarna	Annapoorna
Ear head height (cm)	KBGA 10 (98 cm)	KBGA 11 (100 cm)	Durga (98 cm)	VLC 44 (100)	-	48 cm	82 cm
Yield plant ⁻¹ (g)	SKGPA 63 (15.6)	IC 32186 (14.2)	IC35642 (14.1)	SKGPA65 (14.1)	SKGPA 64 (13.2)	12.2 g	8.3 g
Seed volume weight (g)	Durga	SKGPA 81 (7.6)	BGA 2 (7.6)	VLC 44 (7.6)	KBGA 10 (7.6)	7.6 g	7.5 g

Table 4. Principal component analysis of the different agro-morphological traits

Particulars	Eigenvectors					
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	2.150	1.272	1.014	0.717	0.568	0.352
Variability percentage	35.84	21.21	15.69	11.94	09.46	05.86
Cumulative percentage	35.84	57.05	72.74	84.68	94.14	100.00
Traits	Co-efficient of variates					
Days to fifty per cent flowering	-0.337	0.477	-0.250	0.729	0.128	0.221
Days to maturity (days)	-0.373	0.512	-0.056	-0.652	0.005	0.413
Plant height (cm)	0.392	0.542	-0.116	-0.001	-0.659	-0.323
Ear head height (cm)	0.569	-0.147	-0.267	0.058	-0.105	0.754
Yield plant ⁻¹ (g)	0.232	0.286	-0.888	0.144	0.118	0.201
Seed volume weight (g)	0.464	0.388	-0.245	-0.139	0.724	-0.259

will be in discriminating among accessions. There are no inferential tests to prove significance of proper values and the coefficients. From the principal component analysis, PC1 accounted for 35.84 per cent of the total morphological variation for the traits with Eigenvalue >1 among total genotypic variability for six quantitative characters of 125germplasm including checks.

The first PC has ear head height, seed volume weight, plant height and yield plant⁻¹ as the traits with the largest coefficients, all with positive sign. Therefore, first component distinguished tall lines with thick ear head and more seed volume weight. The first PC seems to be more related to yield and yield contributing traits as PC1 had high positive values for these traits. This means that, the listed descriptors are the most effective characters for distinguishing among the eighteen traditional cowpea varieties sampled and PC1 is one of the most important features in the selection of these characters. Thus, it could be a useful tool to help breeder for selection. This is in accordance with Khosro Mafaheri and Mohammad Reza Bihamta (2017) in cowpea. Ogechi and Joseph (2017) studied sixteen amaranthus genotype for morphological characterization and observed fourteen principal component axes. Prin. 1, which constituted the highest accounted for 0.3376 of the total proportion with eigen value of 4.7269, while Prin. 14 was the least with proportion of 0.0003 and eigenvalue of 0.0038. The second component accounted for 21.21 per cent of the variance and was more related to days to fifty per cent flowering, days to maturity, plant height and seed volume weight with high coefficients but having positive signs. Seed volume weight had negative values for the three components (PC 3, 4 and 6) but contributes to the Vth component with highest positive value (0.724). The third component accounted for 72.74 per

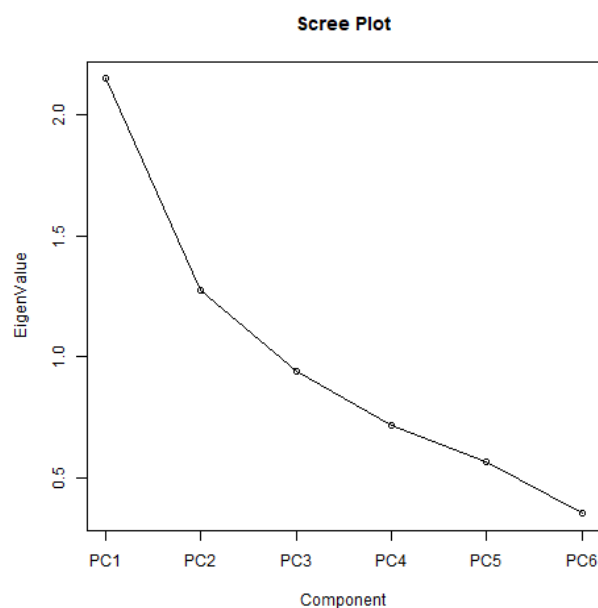


Fig. 2. Scree plot showing Eigen value variation

cent of the cumulative and having negative sign to all the characters. PC 4 is related to yield plant⁻¹ and ear head height with positive sign and PC 5 was related seed volume weight (0.724) and days to 50 per cent flowering (0.128).

Screen plot: Screen plot explained the percentage of variance associated with each principal component obtained by drawing a graph between eigenvalues and principal component numbers. PC1 showed 35.84% variability with eigen value 2.15, which then declined gradually herewith Haizheng Xiong et al (2016) also got highest variability in PC1 with eigen value more than 1.0. Semi curve line is obtained after three PC tended to become straight with little variance observed in each PC. From the graph, it is clear that

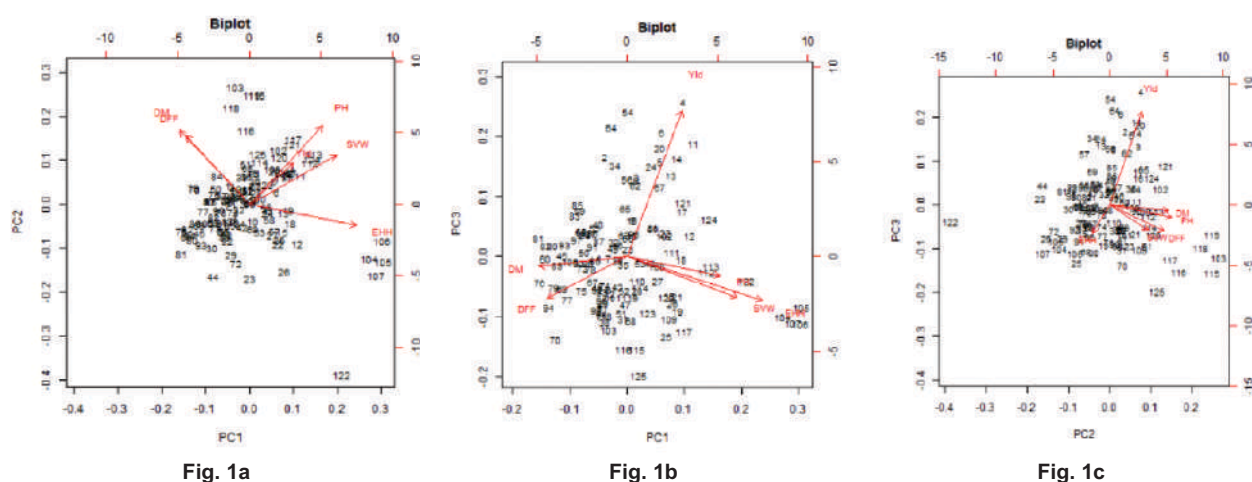


Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1. Distribution of genotypes across three components

the maximum variation was observed in PC1 in comparison to other PCs. So, selection of lines from this PC will be useful (Fig. 2). Those principal components having more than one eigen value that showed more variation among the grain amaranth genotypes for the selection of the diverse parents.

The knowledge of genetic relationships among genotypes provides useful information to address breeding programs and germplasm resource management (Roldán-Ruiz et al 2001). In that, principal component analysis revealed that six quantitative characters viz., days to 50% flowering, days to maturity, plant height, ear head height, yield per plant⁻¹ and seed volume weight, significantly influenced the variation in these cultivars. This is in accordance with the reports of Nwangburuka et al (2012) and Olawuyi et al (2014) who considered genetic variability as essential in crop breeding. The first three components in the PCA analysis with eigenvalues >1 contributed 72.74 per cent of the variability among genotypes evaluated for different agro-morphological traits. Other PCs (4 - 6) had eigenvalues less than 1. Selections based on this characters and the genetic diversity inherent in the plants could thus improve productivity considerably. These performances shown by *Amaranthus* hybridization breeding procedure for crop improvement with desired traits in the parents line (Ogechi and Joseph 2017). Thus, the prominent characters coming together in a particular principal component by contributing towards variability has the tendency to hang together offer opportunity for its utilization in crop breeding.

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Management Practices for Biodiversity Conservation through Community Conserved Areas in Meghalaya, India

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Abstract: Community Conserved Areas are described as natural or modified ecosystems which contain significantly high levels of biodiversity, provide ecological services, and preserve local cultural values (Upadhaya et al 2003). The purpose of this study is to document the traditional management practices for preserving biodiversity in their community conserved areas. Four villages in Khasi Hills District have been studied to understand the strategies used for managing their Community Conserved Areas. The study is based on observations of the ecological values of the groves, questionnaires and open ended interviews with key informants of the traditional management practices related to sacred groves and Other Effective (area based) Conservation Measures in these villages. Data are store and analysed in Excel and represented as graphs. The conservation efforts by communities include continuation of traditional management methods that lead to sustainable use practices, or where these traditions have been recently revived. The local people have been developed through long-term trial and experiments, an elaborate, functional and generally democratic system of conservation and management of forests and associated natural ecosystems.

Keywords: Management of community conserved areas, Traditional practices, Sacred groves, Meghalaya

India is one of the key countries in Asia in terms of the wealth of traditional knowledge systems which are related to the utilization of plant and animal species. Traditional communities have retained some of their diverse heritage of cultural traditions related to conservation of flora fauna and varied forests types (Singh et al 2017). The two classes of forest management systems included "reserved" forests which cannot be used by the local populations in any manner other than specified and "protected" forests in which local people are permitted to forage or collect resources for specified uses (Khan and Tripathi 2008). Forests are an important source of consumptive resources such as food, fuel wood, fibre, freshwater and construction materials for subsistence, as well as cash based productive uses for their livelihood. Both are closely associated with forage-based tribal people. Forests act as a 'safety net' in times of hardship (Tiwari 2015). The diverse natural habitats across the Himalayan and North-eastern region of India are repositories of plant and animal diversity that are utilized for a variety of purposes which include nutritional support, medicine, fuel wood, spices, dyes, housing, making agricultural implements etc. A large number of wild edible plants and traditionally cultivated flora are widely used as food and in local traditional systems of medicine. Traditional local indigenous crop varieties are used for nutritional value and wild medicinal plants are used locally by village folk as well as through specialized Traditional Knowledge Systems of medicine men. These resources are collected by forager communities and sold through market places to be manufactured through

formal Ayurvedic Pharma companies, or used for products such as cosmetics, dyes etc manufactured in several industries. About 43% of the total flowering plants are reported to be of medicinal value (Goswami et al 2014). The present study was conducted to understand the differences in traditional practice used by local people for conserving biology diversity in community conserved areas and to document resources dependency on forests in the study area in relation to local folklore and sacredness.

MATERIAL AND METHODS

Study area: Meghalaya includes the South Garo Hills, West Garo Hills, East Garo Hills, West Khasi Hills, East Khasi Hills, Ribhoi and Jaintia Hills Districts lying between 25°47' to 26°10' N latitude and 89°45' to 92°45' E longitude. The state covers an area of 22,429 sq. km. It is bordered on the north, east and west by Assam and on the south by Bangladesh. The elevation ranges from 50 m to 1960 m. The forest covers 17,146 sq. km of the state. Forests are present in 76.45 percent of the total geographic area (Forest Survey of India 2017). In terms of forest canopy density classes, the state has 453 sq. km under very dense forest, 9,386 sq. km under moderately dense forest and 7,307 sq. km under open forest. Out of the total forests, the Reserved Forest covers only 981 sq. km (4.37 % of the total area of the state). This is managed by the State Forest Department. Unclassed forest area covers 8503 sq. km (37.91 % of the total area of the state). In Meghalaya forests have traditionally belonged to the local communities, individuals, or District Councils. Meghalaya

has nearly 40 percent of its land under shifting cultivation (Tiwari 2015), which is carried out in forested hill slopes.

The Khasi people in Meghalaya have a way of life, which is largely influenced by the natural environment of the hills. The Khasi had several local administrative layers for protection of their forests. Through their customary laws, the Khasi manage the forest wealth by categorizing forests in terms of their local management systems and thus are able to manage them for specific needs. These categories include i) forests kept aside for religious needs (Law-Kyntang), ii) private forests owned and managed by families (Law kur), and iii) forests for villages and clan groups (Law Raid). Each of these has their distinctive traditional management practices, and is governed by locally applicable rules and regulations. They also have clearly defined hierarchical governance systems.

Meghalaya is inhabited by three main tribal groups, the Khasi (42%), the Jaintia (12%) and the Garo (32%), which together comprise 86% of the state's total population of 2.3 million. A high percentage of the population is engaged in occupations related to utilisation of forest resources as hunter-gatherer (foragers), or through collection of Non Timber Forest Products, timber harvesting and charcoal making which is a reflection of the livelihood dependency on the forest resources. A total of 120 interviews were conducted with the people in four villages which have community conserved areas viz. Mawpat community conserved areas (Raid); Mawpdang Community Conserved Areas (Raid); Nongkseh sacred groves (Law-kyntang); Mawphlang sacred grove (Law-kyntang). These local terms are related to varied rules and regulations, and define how they are to be governed and under which local authority they are administered.

A semi-structured questionnaire using both open and closed-ended questions was used to elicit the view of the 120 respondents. Interviews were randomly conducted. The age of respondents of those who were interviewed ranged from 20-90 years. They were residents in the villages for more than 30 years. They included elders, farmers, paid labourers,

and teachers. The chiefs of different traditional institutions and local forest managers (Lyngdoh, Basan, Myntri, Sardar and Rangbah shnong) provided expert inputs into management regimes of the forest. The open ended interview on management was done by visiting the chief, the elders, and the villagers starting by asking about their names and how many years they have live in the village and how they conserved the forest. The relevant questions include :- Who put forth the proposal of creating the Community Conserved Areas, community support to conserve the forest, women participation in the conservation of the community conserved forest, improvement of the community conserved forest compare to 10 years ago and the scheme for conserving the forest.

During the study four Community Conserved areas in Mawpat, Mawpdang, Nongkseh, and Mawphlang villages were studied. Two were identified as Community Conserved forests and are locally called as the "Khlaw Raid" in Mawpat and Mawphlang village. These forests are considered the common property of the village and are within the village boundaries. They are usually set aside to meet the genuine mainly consumptive needs of the local villagers. The forest is under the control and management of the village council called "Hima Myllem or Hima Khyrim". This is expected to ensure the sustainability of the forests. The two communities that have conserved forests referred to them as Community Conserved Forest which includes the key sacred grove.

RESULTS AND DISCUSSION

The 120 respondents reported that the Community Conserved Areas have been there for many generations and they are under the control and management by the different traditional institutions (Hima Myllem, Hima Khyrim, and Hima Lyngdoh). The Chief are the heads of these institutions and work with other members such as the (Myntries, Basan and Rangbah shnong). These forests are conserved and protected through local management systems for magnifying resource use. This benefits all the villagers through the

Table 1. Profile of villages in the study area

Name	Size of village and forest area	Population of villages	Forest classification based on local governance
Mawpat village	Forest: 91.9 hectare	Approximately 3000	Mawpat village is under the traditional institution called "Hima Myllem". It consists of a large patch of forest known as the "Law Syiem or Law Raid".
Nongkseh village	Forest: 3.64 hectare	Approximately 2500	The village has a proud history because of the preservation of the sacred forest, which the local people call "Law-Lyngdoh".
Mawpdang village	Village area 3237.48 hectare	Approximately 1614 people	The village includes a patch of forest which is locally called as "Raid", which is under the management and control of the head of the Raid, who is called a "Basan Raid"
Mawphlang village	Sacred groves 76.8 hectares	Approximately 3000 people	Mawphlang has a sacred forest which is known as Law Lyngdoh, it is preserved by the local people since ancient times.

community governance processes at village level. They can also use the Community Conserved Areas for agriculture, and grazing cattle without paying for the land or the resource. They can collect wild edible plants and fruits from the forest. Some of the wild edible plants and fruits they collect from the forest are also sold in the local markets. In the Mawpat Community Conserved Area (Raid) the respondents reported that the community conserved area is under the control and management of the “Hima Mylliem”. This institution is managed by the chief (Syiem) who is the head of the village and works along with other members (Lyngdohs, Myntries, and Basan and Rangbah shnong). Each of these officials has specific tasks in the administration of the forest. The respondents reported that they conserved the forest in sacred groves because of their belief in the taboo which is an integral part of their traditions and culture. In the two village i.e., Mawpat and Mawpdang village, the villagers are however permitted to collect natural resources at a controlled (sustainable) limit without destroying the forest. Mawphlang Sacred Grove consists of a dense forest patch. It is surrounded by undulating grassy hillocks which reveal a rather sharp contrasting picture to the well demarcated edge of the grove. These grassy hillocks are erstwhile degraded forests exposed to constant human interference and animal grazing. The fringe of the sacred grove is surrounded by a dense growth of *Castanopsis kurzii* trees forming a protective hedge, and preventing the intrusion of *Pinus* trees which are present here and there in the forest edges. The sacred grove consists of a thick and continuous dense mosaic of trees, shrubs, herbaceous plants, orchids, epiphytes and rich fern flora. The orchids are found in abundance in the less dense part of the forest where the entry of sunlight is greater. Mixed evergreen trees which are found in this grove include *Quercus*, *Rhododendrons* and species of *Schima* and *Cinnamomum*. Within the decaying wood, the recycling of nutrients is a part of the dynamics of the natural ecosystem. The stratification within the forest is layered. The trees form tiers in the natural vertical zonation of the forest structure.

The canopy of the forest forms a nearly continuous layer. It is composed of tall trees, a mid-canopy layer which is dominated by medium sized trees. The understory layer is dominated by regenerating saplings and shrubs, and the ground vegetation is dominated by herbs, shrubs, ferns, mosses, root parasites etc. The woody climbers and lianas, orchids, ferns, mosses, lichens exist in the middle and upper strata of the forest. The Community Conserved Forest in Mawpat, Mawpdang and Nongkse forests are surrounded by a dense growth of *Pinus*, *Quercus*, *Castanopsis* and *Rhododendron* trees, forming a live protective hedge for the forests. The vegetation in the forest is dominated by herbs

and shrubs with a few gymnosperm trees as a result of anthropogenic activities. The vegetation Forest is conserved for the benefit of local people. Where the villagers collect fuel wood, wild plants or practice shifting agriculture. There is a variable degree of degradation of the forest. The canopy is opened up and the ground layer vegetation is patchy.

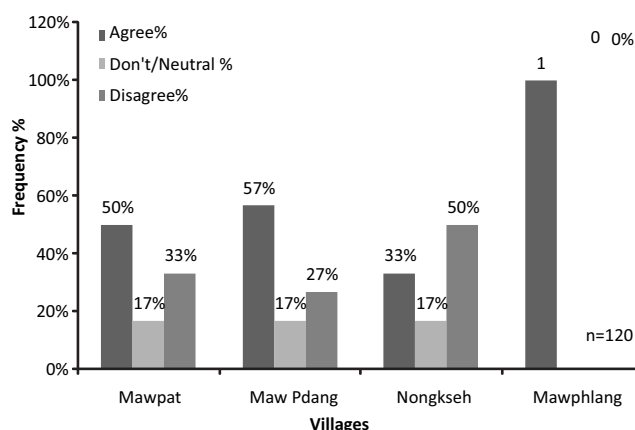


Fig. 1. Status of forest conservation

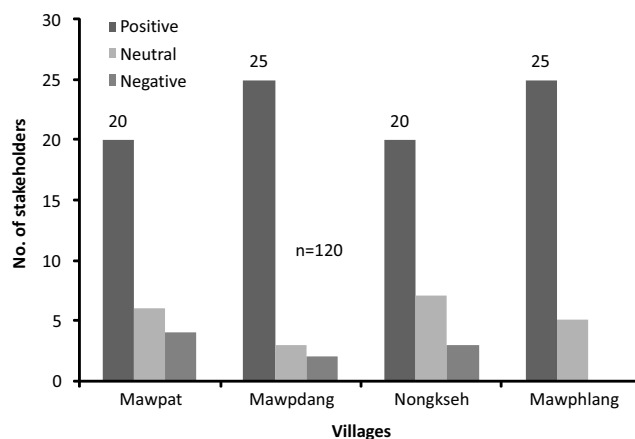


Fig. 2. Attitude of the local people to presentation of forests

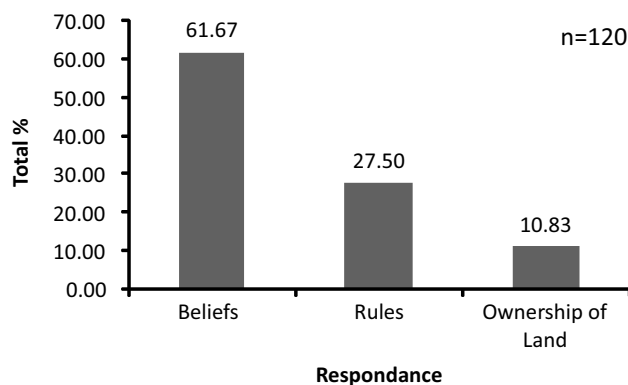


Fig. 3. Triggers for forest conservation

Respondents from Mawphlang said that the sacred grove has improved during the last 10 years because the village folk look at the forest as a sacred site. The forest is preserved as an outcome of the rites and rituals which are still performed by the local community. This has resulted in its pristine nature. The sentiment is increasing because the village has a strong leader (Lyngdoh) and through half of the villagers practice Hinduism, many still follow their ancestral traditional culture and religious rites in the grove. In the Nongkseh the sacred forest is fairly well preserved. 50% of the respondents said that the forest is now being degraded because the local community has lost their belief in the ancient rites and rituals. An important finding is that the small clan which once protected the forest does not exist in the village anymore. Thus the protective sentiment which is not adhered to by other clans has led to its degradation. Therefore the sacred forest has been converted into a Community Conserved Forest. This has led to a decline in the importance given to protecting the forest through the local original traditional governance system. As a sacred grove there were strict taboos for collecting resources. However, as a Community Conserved Areas these norms have been relaxed. This has led to a loss of the 'natural' ecological status of the forest. In Mawpat 50% of respondents claimed that the forest is being gradually degraded in the last 10 years because most of the local people in the community are converted to Christianity. Most young people are now educated in the modern system. They have thus lost their traditional beliefs in their ancient religion and do not practice their original cultural ceremonies. They have lost the knowledge required to perform rites and rituals which were performed in earlier times. Their way of worshiping of deities and the spiritual customs linked to their rites have been homogenized into a new hybrid system. The resources collected from the forest include fuel wood, orchids, wild edible plants, ferns, fruits, and mushrooms. Bamboo is used locally and is sold for construction of houses and making other utility items which is sold in the local market.

A review of all the four villages showed that a major proportion of the respondents expressed a positive attitude towards the conservation of forests.

The reasons for preservation of forests included:

- The local people from all the four villages depend mainly on the forest for natural resources such as fuel wood, foraging for food, orchids, and bamboos.
- A fair proposition of responders still retains a strong belief in the ancient rites and rituals in Mawphlang and Mawpdang villages. The respondents from Mawphlang show the most overt and strong beliefs in their ancient traditional religion and tribal culture. This is extended to

the protection of their famous Sacred Grove. The grove has strong support from local leadership.

- 25% of the respondents from Mawpdang and Mawphlang villages reported a positive attitude towards conservation of the forests because of the strong power and control by traditional institutional management

According to the respondents, a large proportion of the local people (61%) stated that they still have a strong belief in their culture and traditions. This is through their belief in the local deities, despite the fact that the majority are now of the Christian faith. The rules set up by the local traditional institutions have still survived. Thus several of the forest patches are still protected they were as in ancient times. Local people conserve the forests because of the strong influence of the traditional institutional mechanisms which is present over several generations. Mawphlang Sacred Forest has developed over the last 10 years. The forest is well preserved through rites and rituals by the local community. It is thus still pristine in nature. Collection and removal of any material from this sacred grove is prohibited and is strictly ensured by the local management regime prescribed for the Mawphlang sacred grove. This strong positive attitude of the local managers, which is supported by the local people, is responsible for the high level of conservation of the forest (Chandrashekara and Sankar, 1998). Religious and traditional beliefs and cultural practices play a crucial role in the conservation of its environment and biodiversity (Singh et al 2017). The Nongkseh sacred forest lacks protection of the forest at a sustainable level, because the local community has lost their strong belief in the traditional rites and rituals. The specific clan Thangkhiew and Rumnong clans which were responsible for the protection of the forest through sacred rites which were present in the past were small community. This has ceased to exist in the village. The sacred forest is thus converted to a Community Conserved Forest with new more relaxed rules for its management. This has led to a decline of community conservation action. The changing attitude of the local people has led to the erosion of traditional beliefs, and human impacts have caused degradation of this sacred grove in Nongkseh village. Conservation of forests in Meghalaya is not possible without the active participation of the local people. The Mawphlang village is 27 km from the local market, while the Nongkseh village is only 7.2 km away from the town. Proximity to the urban market is leads to a higher level of extraction of the forest products. This is also linked to the lower economic status of the local people.

The stakeholder's opinion for conservation of the sacred groves differs significantly. This is because, in the case of Nongkseh, the local people strongly feel that they are

enjoying benefits from the grove. Thus, they have a commitment towards conservation. About 90% of the Nongkseh villagers are Christian. This is linked to the loss of the religious traditional beliefs and the loss of their close association with the deity of the forest. The Nongkseh “Law-Lyngdoh” was a sacred forest. It was believed by the people in the village that the Titular deity called U Rynkew, or U Basa resides in it. The deity is supposed to have dwelt in this forest. It was believed that the deity protected the villagers. Earlier, a goat was used as a sacrifice to worship the deity. This was done once a year. This practice has now been stopped, since the later half of the 1960s. The Lyngdoh Nongkseh clan is not present any more in the village to carry out the sacrifice. The initiation of Christianity is one of the reasons that have led to a loss of traditional beliefs of the local tribal people. This has led to the conversion of a Sacred Forest to a Community Conserved Forest. Flora and fauna was not collected from the sacred forest in the past. Now the old trees and the fallen trees are usually auctioned.

Traditional knowledge systems: A vast knowledge on medicinal plants exists as an oral traditional knowledge system among the folk people and traditional medicine men in these villages. A large number of medicinal herbs are found growing wild (Hynniewta and Kumar 2008). The study shows that the local people have retained some of the knowledge in their own rural traditional folk healers. They use herbal medicines which they collect from the indigenous plants found in the forest. This is said to cure a number of ailments. The villagers in Mawpdang and Mawphlang village are primarily agriculturists. Because Mawpdang is a village forest (Khlaw Raid) the villagers can access resources freely in the surrounding forest for farming. They depend entirely on the forest for their basic necessities such as collection of firewood, wild vegetables, fruits, indigenous medicinal plants, etc. Traditional Knowledge holders have developed the most appropriate and useable tools to make household goods for their daily use (Bharucha 2017). The forests are protected and controlled by local people through institutional arrangements developed to benefit the community as a whole. The classification, protection, regeneration and extraction procedures practiced for management of these forests are among the best examples of traditional ecological knowledge (Tiwari 2015). The Mawpat and Nongkseh villages have a relatively lower commitment toward conservation of forest resources because the clan “Lyngdoh” who earlier performed the worship of the deity during the rituals have left the area. Thus the practice of ritual worship in the forest has been stopped and there is no transfer of this indigenous ritual to the present generation. Nongkseh is presently the only village of the Thangkhiew clan and the

Rumnong clan remaining in the Raid Sawkur Nongkseh. The Nengnong clan has migrated out from the Raid Nongkseh and the Lyngdoh Nongkseh clan is now not present in the village. The other reason for recent degradation is that Nongkseh village is near the local market. Thus local residents collect natural resources from the forest such as wild fruits and wild vegetables and sell the resources in the nearby market. In previous years, animals and birds were hunted in this forest. This led to the loss of biodiversity even in the adjoining Sacred Forest.

The governance of Forests in most parts of India is under the Forest Departments in the States. This limits resource use by people without taking into account several complex localized issues especially of forest for ager communities. Thus in Protected Areas and Reserved forests conflicts with local people are a serious concern. To understand the current traditional governance, a total of 120 interviews were conducted with the people in four different villages which include Community Conserved Areas viz. Mawpat Community Conserved Area (Raid), Mawpdang Community Conserved Area (Raid), Nongkseh sacred grove (Law-kyntang), Mawphlang sacred groves (Law-kyntang). The information regarding the status of the forests and sacred groves elicited from stakeholder and the local knowledge of resources associated with a specific deity has a relationship to the nearest human habitation. Free access to resources where permitted shows that the status of floral and faunal diversity of some groves is gradually being degraded. However, other researchers in the past have pointed out that the community managed forests presented lower and less variable annual deforestation rates than government Protected Forests (Poffenberger 2015). They consider that a more resilient and robust forest conservation strategy must consider the regional aspects with different land use types in

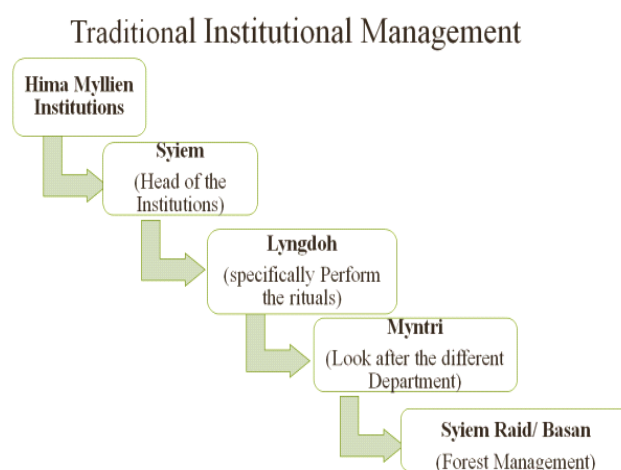


Fig. 4. Hierarchy of Traditional Institutional

which social and economic needs of local people, as well as tenure rights and local capacities, are recognized (Porter-Bolland et al 2012). Our findings have demonstrated that management effectiveness of the community forests by the local people controls and prevents severe levels of deforestation. From the four Community Conserved Areas, we found that the local people use dead branches of trees for firewood and consume a small amount of timber from the village forest. Currently the traditional institutions along with the village committee (Dorbar) arrange a programme of afforestation every year. The forests are controlled and managed by the traditional institutions which they have set up with locale specific rules and regulations. Anyone who violates the rules, are made to pay a penalty. Thus the forest is conserved and is used at a sustainable level.

Previous researchers have found that the tribal communities of Meghalaya live in natural forests. The village habitations are near water sources, or on steep slopes in ecologically sensitive lands (Jaiswal 2010). The Khas is a distinctive tribal community with very strong bonds to their sacred groves as well as their religious practices and rituals (Bharucha 2016). Their forest conservation system can be seen prominently in sacred groves. However village restricted forests, village supply forests, clan forests, and other managed forests, constitute about 90% of Meghalaya's total forest area. These forests are protected and managed by the community through different institutional arrangements developed to benefit the community as a whole. The identity given to different types of forest, the specific rules of protection and the restriction for extraction procedures are practiced for management of the forests. This is an example of traditional ecological knowledge (Tynsong, Lapasam and Sharma 2013). We also found that the different categories of forests are managed by using several traditional institutional arrangements.

Law Kyntang, Law Lyngdoh, Law Niam (Sacred Groves):

These forests are set apart for religious purposes and are believed to be inhabited by deities such as "Ryngkew, Basa, and Labasa". These are some of the many ancient deities to whom these groves are dedicated. Nothing in this category of forests can be used or destroyed by anyone irrespective of his religion. Their ancestors from the village have performed their traditional worship in these sacred groves over several generations. Two sacred groves were studied i.e., the well-known Mawphlang sacred grove and the Nongkseh sacred grove, which are managed by different traditional institutions i.e., Hima Lyngdoh and Hima Myllem. This shows that there are local cultural differences in the way these forests are managed.

Law raid (Community forest): Each "kur" forms a basic

social unit, an independent religious institution, a primary political institution, and an independent economy within a community. This is referred to as the "Raid". Two community conserved forests in Mawpat and Mawpdang villages were studied. It was found that these forests are under the management and control of the head of the Raid. However, every member of the area has the right to use forest products. The traditional management practices have helped in conserving the resource, as is evident from the presence of large patches of well-preserved forests. These systems if retained in future will enhance sustainable use. The forest would also as a 'resource' for the people of the communities for emergency use. This practice suggests that these adaptation measures could be effective in the presence of climate change.

Each traditional forest type provides a different facility to the people in the village. The sacred forest is the home of a deity which is believed to protect the village from natural calamities, death, and diseases. Other forms of village forests give firewood, wild edible plants, and poles for house construction and repairs. Previous studies have reported that the sacred grove which has been protected by the local people for centuries is dedicated to the deities that are said to reside in them and protect the villagers from different calamities (Tiwari 2015). In the current context the link between local communities in terms of biodiversity, culture, religious and ethnic heritage is still linked to sacred groves. In Mawphlang sacred grove (Law-kyntang), it was reported by the local people that the traditional institution called the "Hima Lyngdoh" protects forests through local management regimes. They control and manage the forests which have been protected for centuries. This is a historical cultural ethic and a religious belief which has strong taboos. These are believed to be dictated by the deities whose side in them. The Four villages have several similarities in term of managing the Community Conserved Areas. However, they have their own traditional local institutions which are actively functioning at present.

The Community Conserved Forest in Mawphlang, Mawpat, and Mawpdang village has a dense forest cover. This indicates that the traditional institutions are effectively managing to control the utilization of resource and are limiting the use of community forests at a sustainable level. The traditional institutions have their own hierarchal structure in the four villages which are managed and controlled by different chiefs or heads of institutions. We recorded two types of Community Conserved Forests viz. Village forest (Raid) and Sacred Groves (Law-Kyntang). The local people through long term trial and experimentation (which are often based on their folk stories and beliefs) have led to developing

their functional democratic system of conservation and management of forests for the benefit of the community and the associated ecosystem. A number of studies suggest that the cultural significance of sacred forests provides an important protection for the conservation of biological diversity (Rutte, 2018). This includes medicinal plants which are highly prized. From this study, Mawphlang Sacred Groves has been a highly successful in conservation of the area, where the local people have a strong belief in their culture and religion. The deity "Labasa" is said to dwell in the forest which they consider as an important Sacred Grove. Previous researchers have suggested that the community based natural resource management helps improve rural livelihood (Suich 2010). Similarly, from this study, the Community Conserved Areas in all four villages has improved the livelihood of local people. In the Community Conserved Areas in Mawpat and Mawpdang the villagers can freely enter the forest for collecting wild edible plants, fuel wood, and other resources. The respondents of Mawpdang village said that the villagers can freely access any land in the Village Forest (Raid) for agricultural practices.

Forest resource use: A review of forest resources used and their significance as a part of Traditional Knowledge Systems reported by the respondents shows that they depend extensively on the forests for their varied needs such as timber and non-timber forest products, wild edible plants, etc.

which are available in the forest in different seasons. The respondents report that their community prefers to use traditional health care that is based on folk medicines and traditions. They are knowledgeable about wild medicinal plants and depend on several herbal products for the treatment of most of their common ailments and diseases. Community Conserved Forests are still considered a treasure house of a number of medicinal plants utilized in the local and regional health care systems. The Mawphlang Sacred Grove (Law Kyntang) includes many medicinal plants. Since resource collection is prohibited from this forest, they serve as a repository of germplasm of medicinal plants. The respondents indicate that their common belief in folk medicine influences a majority of other local people. Use of such knowledge and herbal ingredients in the treatment of ailments and in some instance even major disease, or chronic ailments, cuts across social and economic strata (Ormsby and Bhagwat 2010). Even well to do urbanites visit the herbal healers in these villages. Future strategies and actions required for sacred groves in Meghalaya must use the successful management models observed in these villages. It is evident that they are different levels of sustainability in groves that have been traditionally preserved in Meghalaya.

Intact sacred groves: There are groves where the traditional cultural, religious or spiritual aspects are still

Table 2. Parameters for management strategies

Village	Strength of local	Collection of resources	Presence of degradation	Level of intactness	Precautionary/ Non-interference
Raid Mawpat (Mawpat Community Conserved Areas)	The strong rules and regulations of the traditional Institution "Hima Myliem" and the village council "Dorbar" strengthen the local sentiments of the people.	Resources like wild edible plants such as mushrooms, <i>Centella asiatica</i> , <i>Houttuynia cordata</i> (Jamyrdoh), fuel woods and bamboos.	The forests are disturbed due human interference like forest fires, extraction of wild edible plant and animal grazing.	Moderate	A strong communication, education, public awareness (CEPA) action plan for the local people to conserved the traditional cultures.
Raid Mawpdang (Mawpdang Community Conserved)	The strong power of the traditional Institution of "Hima Khyriem" and the village council "Dorbar" is strengthened by the local sentiment of the people.	Resources like fuel wood, wild edible plants and fruits, bamboos, and land for agriculture	The upper canopy and sub canopy of the forest get degraded due to human interference like forest fires, shifting agriculture, and extraction of wild edible plants.	High	Good governance is fundamental for achieving sustainable use and conservation of biodiversity.
Nongkseh Law-kyntang (Nongkseh Sacred Groves)	The strong power of the Traditional Institutional "Hima Myliem" and the village council "Dorbar" strengthen the attitude of the local people.	Resources like wild edible plants and fruits, mushrooms, fuels wood, and orchids.	The ground vegetation dominated by herbs, shrubs, ferns, mosses, root parasites etc are degraded due to human interference.	High	Conservation under the Forest Department of the state should strengthen the powers of the Traditional Institutions.
Mawphlang Law Kyntang (Mawphlang Sacred Grove)	The Traditional Institution of "Hima Lyngdoh" and the Village Council "Dorbar" and sentiments of the local people protect the deity in the sacred grove.	No resource is allowed to collect any resources.	The forest is degraded due to natural calamities like heavy rainfall and earthquake.	Low	Conservation by Forest Department as a community Reserve or Conservation Reserve under the Wildlife Protection Act

strong enough to prevent their degradation. This is indicated by the local people's views on the ecological intactness canopy cover, minimal extraction of resource use of herbs, presence of wild food, medicinal plants, absence of lopping for fuelwood, etc. The strategy for such groves should only be to regularly monitor the status of conservation and no interventions should be done except for using the precautionary principle. A strong communication, education, public awareness (CEPA) action plan should be carried out, to convince local people that their sentiments are not only traditionally appropriate, but are scientifically tenable, as there is now a critically important role of preserving sacred groves as 'hot-specks' of biological diversity.

Semi intact groves with signs of loss of 'naturalness':

There are groves which have begun to show a loss of sentiments due to a demographic or religious alteration in recent times. Such groves show a loss of canopy, signs of lopping as a result of moderate to high level of resource extraction such as wild food plants-ferns mushrooms etc, and loss of faunal abundance such as avifauna and butterflies. These groves are likely to degrade further, as local sentiments are being lost. Here a support intervention through Communication Education and Public Awareness (CEPA) may be sufficient to prevent further degradation. Convincing local people of the scientific value may be sufficient to prevent further degradation and restore previous levels of naturalness. Therefore use of a precautionary principle approach and monitoring for positive or negative change at regular short intervals is however essential.

Severely degraded groves undergoing serious loss of biodiversity and 'naturalness': These groves require a new management approach through the Forest Department as a Community Reserve or Conservation Reserve under the Wildlife Protection Act. This would require funds for scientific management. Reforestation must use only local species, withdrawal of threats by providing alternatives and a very intensive CEPA program for multiple sectors of society is an essential measure.

CONCLUSION

The effectiveness of these unique and highly locale specific strategies must be accessed through a Rapid Assessment Tool to gauge the status of each of the important sacred groves in Meghalaya. This model is based on the review of a few representative groves done during this study. Forests in Meghalaya have similar conservation issues with specific management strategies across Meghalaya. These groves irrespective of their current biodiversity status are extremely valuable natural assets and are part of the natural capital and spiritual value and the economic benefits that are

prevalent across the state of Meghalaya (Trivedi, Bharucha and Mungikar 2018). Thus using the Precautionary Principle to manage these ancient forests is of prime importance for the state. The four Community Conserved Forest patterns have their own ways of functioning for conserving and sustainably using resources. In one of the Sacred Groves (Mawphlang), it is observed that the implementation and functions of traditional management have conserved the forest since ancient times and is still being carefully conserved. Results from this study indicate that each of the Community Conserved Forest practices is based on highly localized traditional knowledge, of local people. The access to forest resources in the Khasi traditional way of governance resonates with the Access and Benefit Sharing mechanism in the Biodiversity Act 2002. Access is provided only to local village folk and not to any outsider. Community lands in Meghalaya have rules and regulations by which some form of controlled use is possible by taking official permissions. The survey has revealed that landless, poor or disaster affected people are given preference on a need based approach. This resonates with equitability in the societal pillar of sustainability as an important aspect other than economic exploitative use, or environmental concerns.

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Epipelion Community Structure in Tigris River within Baghdad City, Iraq

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Abstract: Five sites were selected along the river within Baghdad city during November 2019 to July 2019, to study the qualitative and quantitative of epipelion diatoms. A total number of 186 species of epipelion diatoms belong to 59 genera were identified. These genera are belong Bacillariophyceae with relative abundance of (79-85.8%), followed by Fragilariophyceae (8.61-12.7%) and Coscinodiscophyceae (3.5-8.9%). Temporal and spatial variations were observed. The lowest total cell number of diatoms ($270 \text{ cells} \times 10^4/\text{cm}^3$) was at site 2 for Coscinodiscophyceae and the highest number ($315 \times 10^4/\text{cm}^3$) at site 5 for Bacillariophyceae. *Achnanthes minutissima*, *Cocconeis placentula*, *Gomphonema parvulum*, *Aulacoseira granulata*, *Nitzschia frustulum*, *Navicula radiosa* and *Ulnaria ulnare* were recorded in a high cell number throughout the study period, Most abundant species belonged to Bacillariophyceae which reflect the impact of pollution on water quality of the river.

Keywords: Lotic ecosystems, Algae, Benthic, Diversity, Pollution

The Tigris River is the unique water resource in Baghdad for different purposes, drinking, industrial irrigation, fishing, tourism and other purpose. This source needs to monitor regularly (Hassan and Mahmood 2018). Ecological indicators are fundamental to bioassessment and are measurable structural or functional features of ecosystems, including biological circumstances (USEPA 2002). Algae are one of important bio indicators and because of their broad geographical distribution, well-studied ecology reacts rapidly to environmental changes to physical, chemical or biological change in water, diatoms are an invaluable instrument in the water quality assessment and monitoring (Chen et al 2016). Benthic diatoms situated at the start of the ecosystem food chain are significant regulatory systems for absorbing, adsorbing, oxidizing and decomposing, precipitating and storing nutrients in fresh water (Tang et al 2017). Benthic diatoms have become an essential component of water ecological surveillance over the past decade in Europe (Jakovljević et al 2014). Diatoms as bioindicators are more specific in their preference and tolerance of environmental conditions than most other aquatic biota, the first group of biota used for detecting organic pollution (Both drought and flooding effects on diatomic community in river as a climate change impact and cause alteration water quality (ven Vilet and Zwolsman 2008, Hassan and Shaawiat 2016, Abbas and Hassan 2018). Hassan and Abdulameer (2014) reported that Bacillariophyceae is represented as a dominant epipelion algae (88.31%) with a high number of pennate in contrast with the number of centric diatoms. Al-Jobouri et al (2018) found that epipelion diatoms were the dominant species in

Diyala River (Iraq). The present study aimed to illustrate the species composition, density and bio-diversity of the (Epipelion algae) in Tigris River within Baghdad city, and to know if there is an effect of this city on the benthic algal species composition of the river.

MATERIAL AND METHODS

Study area: Tigris River originate from the eastern mountains of Turkey. This river enters Iraqi territory from the northwestern border and passing throughout many cities before entering the capital city at Baghdad and continue its water course until converge with Euphrates River in Karmat Ali (north of Basrah City). Five sites were selected along the Tigris River within Baghdad city from November 2018 to June 2019. The site 1 is the location where the river entering the Baghdad City, and its bank of river characterized by lack of rocks, a dense growth of aquatic plants (Reed, water hyacinth and *Ceratophyllum*) and its sediment are silty-clay loam. The three other sites (2, 3 and 4) are located in the middle segments of the river in the center of Baghdad City and affected by urban activities. Their sediment ranged from clay loam to as silty-clay loam. Also, some aquatic plants noticed at these sites (Reed and *Ceratophyllum*). The site 5 is located at the confluence of the river with its tributaries Diyala River. This site is characterized as an agriculture region on both banks and its sediment is clay-loam. The Global Positioning System (GPS) was used to determine the positions of the study sites (Fig. 1 and Table 1).

Sampling Collection: Monthly mud samples were collected by scrapping off the clay (randomly) using a spatula from

area (50 m²) and (3-5 mm) from the upper (0.5-1cm) surface of the sediment were collected. Lens tissue were used to trap the epipellic algae as according to Eaton and Moss methods (Salman et al 2017). Tissues for cell count and identification of algae were placed in vials containing 10 ml of the distilled water with a few drops of Lugol's solution. While tissues used for estimation of chlorophyll-a were placed in a glass Homogenizer (Parsons et al 1984). The diatoms were counted following the Microtransect method (Hassan et al 2012), and the identified after cleaning with hot concentrated nitric acid (Round et al 1990). The classification in this study was followed Round et al (1990).

RESULTS AND DISCUSSION

A total of 186 species belonging to 59 genera were

recorded (Table 2). Bacillariophyceae diatoms (%) were evidently dominant and represented as 84.1, 81.3, 85.8, 82.9, and 79 from the total epipellic diatoms at sites 1-5, respectively, and there were no clear monthly variation in their total cell number and found in all study periods (Fig. 2, Table 3). While The Fragilariophyceae varied from as 8.61(S1) to 12.7% (Site 5), with monthly spatial and temporal variation (Table 4). The Coscinodiscophyceae varied from 3.5 to 8.9 (Table 4). All studies on Iraqi aquatic ecosystems noticed these results of dominance of pennate diatoms (Bacillariophyceae and Fragilariophyceae) in algal community (Hassan and Abdulameer 2014, Al-Jibouri et al 2018).

Some species presented for the most period of the study such as *Brebissonia lanceolata* which appear in first months of study and disappear from April to July. Other species appear in all sites and months (*Cocconeis placentula* and *Cocconeis placentula* var. *euglypta*. *Caloneis bacillum*, *Thalassiosira lacustris* and *Achnanthes brevipes* were observed once only during February, April, July), respectively. Other species were observed in the middle period of study in March, April, May (*Epithemia adnata*, *Epithemia sorex*, *Eunotia pectinalis*, *Frustulia vulgaris*). The *Hippodonta hungarica* (Grunow) Lange-Bertalot, Metzeltin & Witkowski appeared only in last three months of the study

Table 1. Geographical positions (GPS) of the five study sites

Symbol	Area	Coordinate	
		North	East
S1	Al-Muthanna Bridge	33°25'41.85"	44°20'49.63"
S2	Al-Sarafiya Bridge	33°21'12.99"	44°22'28.77"
S3	Al-Shuhadaa Bridge	33°20'19.99"	44°23'19.91"
S4	Al-Jadriya	33°16'58.35"	44°22'31.87"
S5	Al-Zafraniya	33°17'25.44"	44°26'58.23"

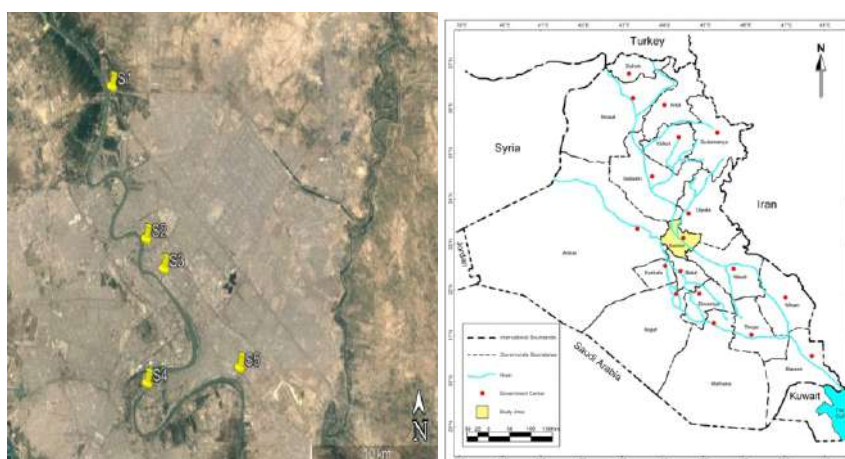


Fig. 1. Map of study areas (Source: <https://earth.google.com>)

Table 2. Numbers and percentages of epipellic diatoms during the study period

Classes/Sites	Bacillariophyceae			Fragilariophyceae			Coscinodiscophyceae		
	Genus	Species	RA%	Genus	Species	RA (%)	Genus	Species	RA (%)
Site 1	51	135	84.1	7	18	8.61	7	11	7.3
Site 2	49	124	81.3	6	13	9.7	8	10	8.9
Site 3	40	131	85.8	6	15	10.7	6	9	3.5
Site 4	48	138	82.9	7	16	10.7	4	7	6.2
Site 5	40	125	79.0	6	16	12.7	6	9	8.5

RA: Relative abundance

Table 3. List of identifying Bacillariophyceae during the study period in Tigris River

Class 1: Bacillariophyceae			
<i>Achnanthes brevipes</i> C. Agardh	<i>Gomphonema acuminatum</i> Ehrenberg	<i>N. gregaria</i> Donkin	<i>N. perminuta</i> (Grunow) M. Peragallo
<i>A. hungarica</i> (Grunow) Grunow	<i>G. angustatum</i> (Kützing) Rabenhorst	<i>N. halophila</i> (Grunow) Cleve	<i>N. perpusilla</i> Rabenhorst
<i>Achnantheidium affine</i> (Grunow) Czarnecki	<i>G. constrictum</i> Ehrenberg	<i>N. lanceolata</i> Ehrenberg	<i>N. pusilla</i> Grunow
<i>A. delicatula</i> (Kützing) Grunow (A. delicclata)	<i>G. constrictum</i> var. capitatum (Ehrenberg) Grunow	<i>N. mourmei</i> R.M. Patrick	<i>N. recta</i> Hantzsch ex Rabenhorst
<i>A. exiguum</i> (Grunow) Czarnecki A. lanceolata	<i>G. gracile</i> Ehrenberg	<i>N. oblonga</i> (Kützing) Kützing	<i>N. sigma</i> (Kützing) W. Smith
<i>A. minutissima</i>	<i>G. intricatum</i> Kützing	<i>N. phyllepta</i> Kützing	<i>N. sigmoidea</i> (Nitzsch) W. Smith
<i>A. pyrenaicum</i> (Hustedt) H. Kobayasi	<i>G. lanceolatum</i> f. turris (Hustedt) Hustedt	<i>N. pseudohalophila</i> Chohnoky	<i>N. subcapitellata</i> Hustedt
<i>Amphora coffeiformis</i> (C. Agardh) Kützing	<i>G. longiceps</i> Ehrenberg	<i>N. pseudolanceolata</i> Lange- Beralot	<i>N. supralitorea</i> Lange-Beralot
<i>A. ovalis</i> (Kützing) Kützing	<i>G. olivaceum</i> (Hornemann) Brébisson	<i>N. pygmaea</i> (Kützing) Pantocsek	<i>N. umbonata</i> (Ehrenberg) Lange-Beralot
<i>A. pediculus</i> Kützing Grunow	<i>G. parvulum</i> (Kützing) Kützing	<i>N. radiosa</i> Kützing	<i>N. vermicularis</i> (Kützing) Hantzsch
<i>A. tenuis</i> (Hustedt) H. Kobayasi	<i>G. pumilum</i> (Grunow) E. Reichardt & Lange-Beralot	<i>N. radiosa</i> var. tenella (Brébisson ex Kützing) Van Heurck	<i>Peronia fibula</i> (Brébisson ex Kützing) R. Ross
<i>Bacillaria paxillifera</i> (O.F. Müller) T. Marsson	<i>G. sparsistriatum</i> f. maculatum K.E. Camburn	<i>N. rhyncocephala</i> Kützing	<i>P. biceps</i> W. Gregory
<i>Brachysira vitrea</i> (Grunow) R. Ross	<i>G. vibrio</i> Ehrenberg	<i>N. schroeteri</i> F. Meister	<i>P. borealis</i> Ehrenberg
<i>Brebissonia lanceolata</i> ©. Agardh) R.K. Mahoney & Reime	<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	<i>N. tripunctata</i> (O.F. Müller) Bory	<i>P. leptosoma</i> (Grunow) Cleve
<i>Caloneis</i> sp.	<i>G. attenuatum</i> (Kützing) Rabenhorst	<i>N. trivialis</i> Lange-Beralot	<i>P. parva</i> G. Gregory
<i>C. bacillum</i> (Grunow) Cleve	<i>G. distortum</i> (W. Smith) Griffith & Henfrey	<i>N. viridula</i> (Kützing) Ehrenberg	<i>Placoneis clementis</i> (Grunow) E.J. Cox
<i>C. hustedtii</i> Aleem	<i>G. peisonis</i> (Grunow) Hustedt	<i>Nitzschia amphibia</i> Grunow	<i>Planothidium lanceolatum</i> (Brébisson ex Kützing) Lange-Beralot
<i>Cocconeis pediculus</i> Ehrenberg	<i>G. scalproides</i> (Rabenhorst) Cleve	<i>N. angustata</i> (W. Smith) Grunow	<i>Platessa conspicua</i> (Ant. Mayer) Lange-Beralot in Krammer & Lange-Beralot
<i>Cocconeis pediculus</i> Ehrenberg	<i>Halamphora normanii</i> (Rabenhorst) Levkov	<i>N. apiculata</i> (W. Gregory) Grunow	<i>P. oblongella</i> (Østrup) C.E. Wetzel, Lange-Beralot & Ector
<i>C. placentula</i> Ehrenberg	<i>H. venata</i> (Kützing Levkov)	<i>N. clausii</i> Hantzsch	<i>P. salinarum</i> (Grunow) Lange-Beralot
<i>C. placentula</i> var. euglypta (Ehrenberg) Grunow	<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	<i>N. commutata</i> Grunow	<i>Pleurosigma elongatum</i> W. Smith
<i>Cymatopleura solea</i> .	<i>Hippodonta hungarica</i> (Grunow) Lange-Beralot, Metzeltin & Witkowski	<i>N. dissipata</i> (Kützing) Rabenhorst	<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-Beralot
<i>Cymbella affinis</i> Kützing C. microcephala	<i>Karayevia ploenensis</i> (Hustedt) Bukhtiyarova	<i>N. dubia</i> W. Smith	<i>Rhopalodia gibba</i> (Ehrenberg) O. Müller
<i>C. aspera</i> (Ehrenberg) Cleve	<i>Iconella capronii</i> (Brébisson & Kitton)	<i>N. fasciculata</i> (Grunow) Grunow	<i>R. musculus</i> (Kützing) O. Müller
<i>C. cistula</i> (Ehrenberg) O. Kirchner	<i>Lemnicola hungarica</i> (Grunow) Round & Basson	<i>N. filiformis</i> (W. Smith) Van Heurck	<i>Staurosira construens</i> f. venter (Ehrenberg) Bukhtiyarova
<i>C. helvetica</i> Kützing	<i>Luticola mutica</i> (Kützing) D.G. Mann	<i>N. fonticola</i> (Grunow)	<i>Stenopterobia intermedia</i> (F.W. Lewis) Van Heurck ex Hanna
<i>C. lanceolata</i> (C. Agardh) C. Agardh	<i>Mastogloia smithii</i> Thwaites. Ex W. Smith	<i>N. frustulum</i> (Kützing) Grunow	<i>Surirella angusta</i> f. ovate Skvortzov
<i>C. obtusiuscula</i> Kützing	<i>Navicula angelica</i> Ralfs	<i>N. frustulum</i> var. minuta Pantocsek	<i>S. minuta</i> Brébisson & Kützing
<i>C. tumida</i> f. rostrata P. Rivera	<i>N. bottnica</i> Grunow		<i>S. ovalis</i> Brébisson
<i>C. ventricosa</i> Kützing			Cont..

Table 3. Cont...

<i>Delicatophycus delicatulus</i> (Kützing) M.J. Wynne	<i>N. cari</i> Ehrenberg	<i>N. gracilis</i> Hantzsch <i>N. heufferiana</i> Grunow	<i>S. robusta</i> Ehrenberg
<i>Diploneis ovalis</i> (Hilse) Cleve	<i>N. capitatoradiata</i> H. Germain	<i>N. inconspicua</i> Grunow	<i>Tryblionella hantzschiana</i> Grunow
<i>Encyonema lacustre</i> (C. Agardh) Pantocsek	<i>N. cincta</i> Ehrenberg Ralfs	<i>N. intermedia</i> Hantzsch	<i>T. hungarica</i> (Grunow) Frenguelli
<i>Encyonopsis cesatii</i> (Rabenhorst) Krammer	<i>N. cryptocephala</i> Kützing	<i>N. linearis</i> W. Smith	
<i>E. microcephala</i> (Grunow) Krammer	<i>N. cuspidata</i> (Kützing) Kützing	<i>N. longissima</i> (Brébisson) Ralfs	
<i>Epithemia adnata</i> (Kützing) Brébisson	<i>N. cymbula</i> Donkin	<i>N. microcephala</i> Grunow	
<i>E. sorex</i> Kützing	<i>N. digitoradiata</i> (W. Gregory) Ralfs	<i>N. minutula</i> Grunow	
<i>Eunotia pectinalis</i> (Kützing) Rabenhorst	<i>N. exigua</i> (W. Gregory) Grunow	<i>N. obtusa</i> W. Smith	
<i>Frustulia vulgaris</i> (Thwaites) De Toni	<i>N. graciloides</i> A. Mayer	<i>N. palea</i> (Kützing) W. Smith	

Table 4. List of identifying Fragilariophyceae and Coscinodiscophyceae during the study period in Tigris River

Class 2: Fragilariophyceae		Class 3: Coscinodiscophyceae
<i>Belonastrum berolinense</i> (Lemmermann) Round & Maidana	<i>F. radians</i> (Kützing) D. M. Williams & Round	<i>Aulacoseira granulata</i> (Ehrenberg) Simonen
<i>Diademesis laevisissima</i> (Cleve) D.G. Mann	<i>F. rumpens</i> Kützing G.W.F. Carlson	<i>A. italica</i> (Ehrenberg) Simonen
<i>Diatoma elongata</i> (Lyngbya) C. Agardh	<i>Fragilaria</i> Sp.	<i>Coscinodiscus curvatulus</i> Grunow
<i>D. Moniliformis</i> sub sp. ovalis (<i>F. Fricke</i>) Lange-Bertalot, Rumrich & G. Hofmann	<i>Pseudostaurosira brevistriata</i> (Grunow) D.M. Williams & Round	<i>C. lacustris</i>
<i>D. tenuis</i> C. Agardh	<i>Synedra nana</i> F. Meister	<i>Cyclotella meneghiniana</i> Kützing
<i>D. vulgaris</i> Bory	<i>S. pulchella</i> f. major Grunow	<i>C. ocellata</i>
<i>Fallacia enigmatica</i> (H. Germain) Lange- Bertalot & Werum	<i>S. tabulata</i> (C. Agardh) Kützing	<i>C. straita</i>
<i>Fragilaria acus</i> (Kützing) Lange- Bertalot	<i>Tabularia fasciculata</i> (C. Agardh) D.M. Williams & Round	<i>Lindavia compta</i> (Kützing) Nakov, Gullory, Julius, Theriot & Alverson
<i>F. amphicephaloides</i> Lange-Bertalot	<i>Ulnaria biceps</i> (Kützing) Compère	<i>Melosira varians</i> C. Agardh
<i>F. capucina</i> Desmazières	<i>U. capitata</i> (Ehrenberg)	<i>Pantocsekiella ocellata</i> (Pantocsek) K.T. Kiss & E. Ács
<i>F. intermedia</i> (Grunow) Grunow	<i>U. ulna</i> (Nitzsch)	<i>Stephanodiscus astraia</i> (Kützing)
<i>F. intermedia</i> var. continua	<i>U. danica</i> (Kützing) Compère & Bukhtiyarova	<i>Thalassiosira lacustris</i> (Grunow) G.R. Hasie
<i>F. pygmaea</i> (Kützing) Stickle & D.G. Mann	<i>U. oxyrhynchus</i> (Kützing) Aboal	

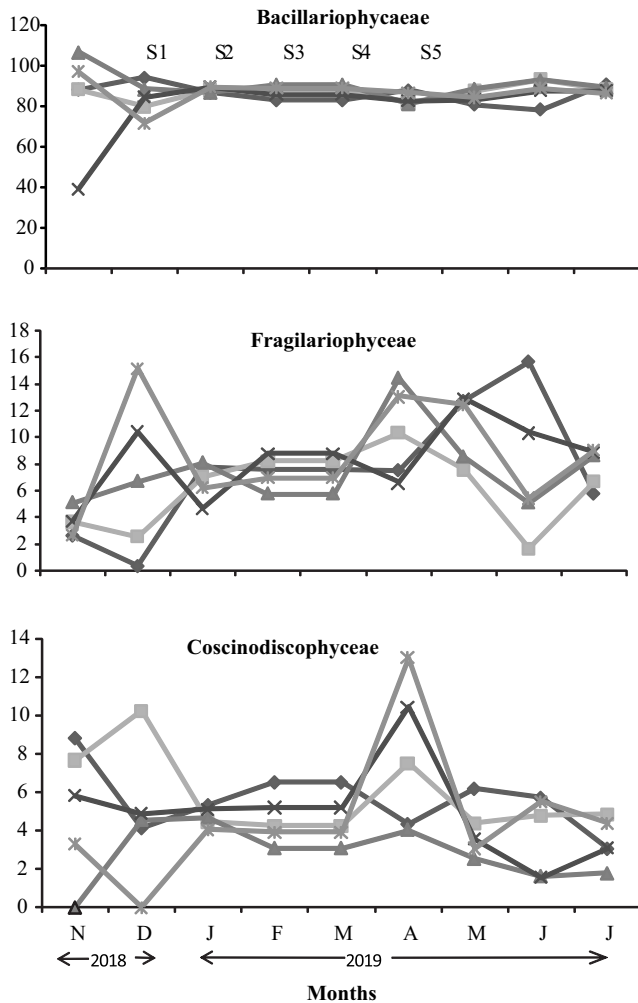
period. The spatial variation in relative abundance among the study sites might be related to the impact of different activities of human and pollution (Lianso 2002).

The highest cell number of species was *Achnantheidium minutissima*, *Cocconies placentula*, *Gomphonema parvulum*, *Aulacoseira granulata*, *Nitzschia frustulum*, *Navicula radiosa*, *Ulnaria ulna*. *Achnantheidium minutissima* were in well oxygenated, fresh waters. *Cocconies placentula* in meso- eutrophic flowing and standing waters. *Navicula radiosa*, a cosmopolitan species occurring in a wide variety of waters ranging from humic, weakly acidic, oligotrophic, electrolyte poor waters to strongly alkaline, eutrophic, calcareous waters. This species is, however, very sensitive

to organic pollution. *Aulacoseira granulata* was observed in both the benthos and plankton of eutrophic rivers and lakes. The *Ulnaria ulna* was in the benthos of rivers and lakes and is easily suspended in the plankton due to its relatively large surface area, and were often found in meso-eutrophic, alkaline waters. *Gomphonema parvulum* is very widespread in a range of aquatic systems, from small pools to lakes and rivers and generally considered to be tolerant of extremely polluted conditions. *Nitzschia frustulum* is found in electrolyte-rich and brackish waters. Also this species has tolerant of fluctuations in osmotic pressure and of critical levels of pollution (Taylor et al 2007). The *Nitzschia* genus is considered a biological monitoring that indicator for organic

Table 5. Total number and percentages of each three classes and the total number of diatoms (cell $\times 10^4/\text{cm}^3$) for each site during the study period on the Tigris River (-) = not found, S = site, Per cent= Abundance percentages

Class	S1	Per cent	S2	Per cent	S3	Per cent	S4	Per cent	S5	Per cent
Bacillariophyceae	231	84	220	81	234	86	237	83	249	79
Fragilariophyceae	24	9	26	10	29	11	31	11	40	13
Coscinodiscophyceae	20	7	24	9	10	4	18	6	27	8
Total (Cell $\times 10^4/\text{cm}^3$)	275	100	270	100	273	100	286	100	315	100

**Fig. 2.** Temporal and spatial variation of Epipelion diatom classes during the study period in Tigris River

pollutant and high pollutant contaminants in sediments (Wehr and Sheath 2003). Many environmental factors lead to be dominant species also their cells characteristics such as mucose membrane which help these organisms to be in appropriate environments (Poulič kova.2008). Moreover, their ability to resist the harsh circumstances of water flow speed, nutrient availability, microbial activities and water quality (Leghari et al 2002, Al-Nashai 2012, Nahar et al 2012). The presence or absence of certain species may be

due to quality of water, velocity of flow and enhanced water discharge in the river (Kadhim et al 2013). Site exposure to pollution that impacts the particular structure of the diatoms connected to the sediments as sediments are a repository of natural and human operations products (Hassan and Shaawat 2016, Ali et al 2018). Site 2 and 3 were impacted by different pollution (Al-Ani et al 2019) so two classes (Fragilariophyceae and Coscinodiscophyceae) of epipelion diatoms were more affected by pollution rather than Bacillariophyceae. The quantitative study was revealed that the lowest total number of cells ($270 \times 10^4/\text{cm}^3$) was for Coscinodiscophyceae at site 2 and the highest total number of epipelion was ($315 \times 10^4/\text{cm}^3$) of Bacillariophyceae at site 5 (Table 5).

Epipelion diatoms are used as indications of pollution in the water environment because they are impacted in many respects. The pollution prevented their growth as a result of their lack of sunlight or to prevent development and reproduction by altering physical and chemical variables. The resistance species can continue growing and be dominant and less frequent, leading to a rise or decline in the complete algal aggregates in the water system. This has caused the five sites to differ (Salman et al 2013). Diatoms are more efficient and resistant to water currents than other algae on the surface. They adhere to submersible surfaces and are more stable on benthic sediments and more adaptable to the fundamental environmental circumstances.

CONCLUSION

The most abundant species of diatoms belongs to Bacillariophyceae and Fragilariophyceae in the Tigris River. There were spatial and temporal variation in epipelion diatoms in Tigris River. Epipelion diatoms are a good bioindicators and reflected the status of the environment which affected by pollution. In this study, the two classes of epipelion diatom (Fragilariophyceae and Coscinodiscophyceae) were more affected by pollution rather than Bacillariophyceae. That indicated the ability of species belonged to Bacillariophyceae more resistance to pollution. Hence some genera were considered as pollution resistance such *Cocconies*, *Nitzschia*, *Navicula*, *Gomphonema*. The

qualitative and quantitative study revealed that Bacillariophyceae are more stable in the term of species number and total number of cells throughout the study period than other classes.

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Yield and Quality Analysis of Paneer from Cow Milk and Buffalo Milk

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Abstract: Paneer is an acid and heat coagulated product but not a fermented product. In the present study different combinations of cow and buffalo milk was taken to prepare paneer. At first milk combination was heated to coagulation temperature of 75°C, 80°C and 85°C and was coagulated with 1% citric acid. Chemical analysis of paneer was done to evaluate its moisture content, protein content and fat content. The maximum yield of 163.955g paneer was observed for 700: 300 (buffalo: cow), 85°C coagulating temperature and 1% citric acid solution with a desirability value of 0.781. The maximum fat content of 61.0863% from paneer was observed for 740: 260 (buffalo: cow), 83.88°C coagulating temperature and 1% citric acid solution with a desirability value of 0.794. The maximum protein content of 18.7148% from paneer was observed for 700:300 (buffalo: cow), 85°C coagulating temperature and 1% citric acid solution with a desirability value of 0.908. The study concluded that 700: 300 (buffalo milk: cow milk) combination along with 80.73°C coagulating temperature and with 1% citric acid solution as coagulating agent given maximum yield of 165.642g paneer with 18.28% protein and 58.21% fat content.

Keywords: Chhana, Coagulating temperature, Coagulating agent, Paneer

Paneer is not a fermented product but it is prepared from heat and acid coagulation of milk. During heat and acid coagulation of milk, large structural aggregates of milk proteins are formed, in which milk fat and a small fractions of whey (soluble milk solids) are integrated while the major portions of the whey (watery liquid) is separated from the coagulates. India has escorted to appear as the largest milk producer country in the world, exceeding a record level of 155.5 million metric tonnes in 2016 accounting for 15% of the world's total milk production (NDDB 2016). An estimated 5% of milk produced in India is transmuted into paneer (Khan and Pal 2011). Without any fermentation and renneting, modified process of direct acidification has a number of advantages over conventional processes of cheese manufacturing, as cost of milk coagulating enzymes are eliminated and the use of hydrochloric acid or citric acid as an acid source is less expensive than to buy or propagate starter cultures. The prime advantage of this process is that it avoids the tough texture of low fat cheese, as rennet is not used. Biological value of protein in paneer is in the range of 80 to 86 (Shrivastava and Goyal 2007). Paneer is highly nutritious since it contains about 90% fat and protein, 50% minerals and 10% lactose of the original milk. During heating of milk, interaction of whey with casein micelles at natural pH may yield hydrophobicity of micellar surface and lowers down the hydration barrier against aggregation, thus allowing aggregation to occur. At normal pH of milk (6.8), casein micelles are stabilized by hydration and steric repulsion due to their negative charge. When milk is acidified by acidic

medium, colloidal calcium phosphate in casein micelles solubilises and micelles become unstable as a result of charge neutralisation, isoelectric point (pH-4.6) is approached and thus results into aggregation of casein micelles and formation of three dimensional networks. Fat globules are also entrapped in this network.

MATERIAL AND METHODS

Milk: The buffalo milk and cow milk was procured from a local dairy farm by properly cleaned bottle. It was analyzed for quality attributes like moisture content, fat content, protein content, and solid not fat content before use in experiments. Different combinations of cow milk and buffalo milk which are shown in Table 1(a) were taken for the experimentation.

Chemical analysis of milk: The milk samples were analyzed for quality attributes like total solid content, fat content, and protein content before making of paneer.

Total solid content: Total solid content of milk was measured by gravimetric method. Milk sample was heated slowly to 35°C to 40°C on a water bath having a careful mixing to incorporate any cream adhering to the sample. Then milk was cooled to room temperature. A dish alongside with its lid was heated in the drying oven for at least 1 hour. 5 ml of prepared milk sample was added in the dish and lid was placed and it was weighed. The dish was placed without the lid on the vigorously boiling water bath in such a way that the bottom of the dish was directly heated by the steam of 100°C. The heating was continued till most of the water was removed. Then it was weighed. The operation was repeated

again until the difference in the two consecutive weights does not exceed 1 mg. The lowest mass was recorded.

$$\text{Total solid} = \frac{d_2 - d_0}{d_1 - d_0} \times 100\%$$

Where, d_2 = mass in g of dish + lid + dried test portion,

d_1 = mass in g of dish + lid + test portion,

d_0 = mass in g of dish + lid.

Protein content: Protein content of milk was estimated by pynes method or formal titration method. 10 ml of milk sample with 1 ml of phenolphthalein indicator and 0.4 ml of saturated potassium oxalate was taken and was set aside for 2 minutes. The content was neutralized with 0.1 N NaOH solutions to a pink coloured end point. 2 ml of neutralized formalin solution was added and the same was titrated with 0.1 N NaOH to the same pink colour. Volume of the NaOH used was measured.

$$\text{Protein\%} = \text{ml of NaOH used} \times 1.75$$

Fat content: Fat content of milk was measured by gerber centrifuge method. The milk was mixed with gerber acid (H_2SO_4) and iso-amyl alcohol in a special gerber tube called milk butyrometer, permitting complete dissolution milk protein and release of fat. The sulphuric acid helps in complete dissolution of milk protein and helps in making of fat globules free, whereas amyl alcohol because of partition coefficient helps in separating fatty layer from the aqueous layer. 10 ml of sulphuric acid was measured into a butyrometer tube, preferably by use of an automatic dispenser without wetting the neck of the tube. The milk sample was preheated to 65°C. Then 10.75 ml of milk sample was poured into the butyrometer tube along the side wall without wetting the neck of the butyrometer tube and left to drain for 3 seconds and touch the pipette's tip against the base of neck of the butyrometer tube. 1 ml of iso-amyl alcohol was added and it was closed with lock stopper. Then it was centrifuged for 4 minute at 1000 rpm. The tubes were put into

centrifuge so as to conform radial symmetry and as evenly spaced as possible in order to protect the bearing of the centrifuge. Then the butyrometer tubes were removed and the percentage of the fat was measured after adjusting height in the tube as necessary by the movements of the lock stopper with the key. The reading was taken corresponding to the lowest point of the fat meniscus and the surface of the separation of the fat and the acid.

Preparation of Paneer: Cow milk and buffalo milk samples were procured from local dairy farm in completely cleaned bottles. Then the chemical analysis of milk was carried out to evaluate its moisture content, protein content, and fat content after preheating of milk to a temperature of 65°C. Then the combination of cow milk and buffalo milk was taken as per Table 1(a). Firstly milk was heated to a temperature of about 90°C then it was cooled to coagulating temperature in an oven. As per Table 1(b) experiments were carried out. Then the coagulating agent of 1 % citric acid was taken by adding 2 g citric acid with 200 ml water. Then 1 % citric acid was heated to coagulating temperature by heater. Different combination of coagulating temperature taken were 75°C, 80°C and 85°C for the experiment as per Table 1(b). At this coagulating temperature citric acid was added to combination of cow milk and buffalo milk. Then during this coagulation stirring was retarded to form coagulant lumps, after that immediate straining was done with muslin cloth and it was checked that the temperature does not fall down below 63°C. The coagulant lump or chhana was pressed in a cylindrical hoop (Fig. 1) for 20 minutes by manually and 0.05 to 0.1 kg cm⁻² pressure was exerted. Then paneer was chilled for 45 minutes by water having temperature of 4-5°C.

Yield evaluation and quality analysis of paneer: After the preparation of paneer, yield of paneer and its quality were measured

Yield of paneer: Yield of paneer was measured by weighing balance after chilling of paneer. At the same time whey

Table 1(a). Different combinations of cow milk and buffalo milk

Cow milk (ml)	Buffalo milk (ml)
100	900
200	800
300	700
400	600
500	500
600	400
700	300
800	200
900	100



Fig. 1. Cylindrical hoop (Haryana Agro Industries)

Table 1(b). Experiments as per different treatments

Cow milk (ml)	Buffalo milk (ml)	Coagulating Temperature (°C)	Citric acid (%)
100	900	75°C	1
200	800	75°C	1
300	700	75°C	1
400	600	75°C	1
500	500	75°C	1
600	400	75°C	1
700	300	75°C	1
800	200	75°C	1
900	100	75°C	1
100	900	80°C	1
200	800	80°C	1
300	700	80°C	1
400	600	80°C	1
500	500	80°C	1
600	400	80°C	1
700	300	80°C	1
800	200	80°C	1
900	100	80°C	1
100	900	85°C	1
200	800	85°C	1
300	700	85°C	1
400	600	85°C	1
500	500	85°C	1
600	400	85°C	1
700	300	85°C	1
800	200	85°C	1
900	100	85°C	1

removal was also measured to get the information about solid loss through whey using weighing balance.

Moisture content: Moisture content of paneer was measured by gravimetric method. Empty dish with its lid was weighed after properly cleaned and then it was dried in oven dryer. Small fragment of paneer was taken into the dish and the lid was placed above it and its weight was measured. Then it was kept in oven dryer at a temperature of 101°C for 18 hours to make sure that no water is remained until the difference between two consecutive weight become same. Then weight of paneer sample with dish was measured.

$$\text{Wet basis moisture content} = \frac{(m_1 - m_0) - (m_2 - m_0)}{(m_1 - m_0)} \times 100\%$$

Where, m_0 = mass in g of empty dish,

m_1 = mass in g of dish + initial weight,

m_2 = mass in g of dish + final weight after drying.

Protein content: Protein content of paneer was estimated by pyrenes method.

Fat content: Fat content of paneer was measured by soxhlet extraction method. 5 gm of protein sample was dried in order to remove moisture because moisture restricts the penetration of solvent and thus retard the process of oil extraction. Then small fragment paneer sample was placed in a thimble. Thimble was kept in a soxhlet extractor and 30 ml of hexane was added in the thimble by the beaker and the whole setting was then heated. The extraction process is continued for 6 hours. In this process hexane penetrates into dried paneer sample and extracts oil from paneer sample and then gets released with oil keeping only sample without oil in the thimble. And hexane again recovered by condensation. Condensing unit was removed from extraction unit and solvent was collected after distillation. Sample was weighed until the difference between two consecutive weight becomes same.

$$\text{Fat \%} = \frac{w_2 - w_1}{p} \times 100\%$$

Where, w_2 = mass in g of thimble with sample,

w_1 = mass in g of empty thimble, p = mass in g of sample.

Experimental design: The study is based on the hypothesis that yield and moisture content, protein content, fat content are functionally related to process variables (Coagulating temperature and Combination of cow milk and buffalo milk), and thus attempts to fit a multiple regression equation describing quality and yield responses. Care was taken to ensure independent operation of the variables, since an error in the choice of variables could lead to an indeterminate solution. The design depended upon the symmetrical selection of variation increments about the central composition retortable design. These levels of variation were chosen to be within the reasonable range, and the increments were carefully selected, since interpretation of the result was valid only within the experimental limits. Process variables were coded for solution of the multiple regression equation. A CCRD (Central Composite Rotatable Design) was adopted and it is given in Table 2 and response surface methodology (rsm) was performed. In this design, experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors.

Analysis of data: The mapping of the fitted responses was achieved using Stat-Ease software (Stat-Ease, version 6.01 2001 2021, Minn.). The response surfaces and contour plots for these models were plotted as a function of two variables.

RESULTS AND DISCUSSION

Experimental results with respect to the processing

conditions like combinations of cow milk and buffalo milk, different coagulating temperature and coagulating strength were observed to find chemical quality of paneer. Yield content, moisture content, fat content, protein content of paneer was studied at different processing conditions. Chemical analysis consisted of moisture content, protein content, fat content of paneer helped to find out the effect of different processing conditions. It was seen that yield values were very poor from 600: 400, 500: 500, 400: 600, 300: 700, 200: 800, 100: 900 (buffalo: cow milk) combinations. Decreasing of buffalo milk caused decreasing of its fat content in milk combination and also produced soft body of paneer and thus those experiments were rejected.

Quality of milk: Quality of cow milk and buffalo milk was measured after preheating the sample to 65°C.

Total solid content: Total solid content of both cow milk and buffalo milk was measured. Buffalo milk contained average 13.32% total solid and cow milk contained average 12.17% total solid.

Fat content: Similarly fat content of both cow milk and buffalo milk was measured. Buffalo milk contained average 5.7% fat and cow milk contained average 3.6% fat.

Protein content: Buffalo milk contained average 2.98% protein and cow milk contained average 2.55% protein.

Quality analysis of paneer: Physical and chemical properties of different paneer samples were observed using different combinations of cow milk and buffalo milk and coagulating temperature.

Yield of paneer: The yield of all paneer samples were measured and are given in Table 3(a). From the results it is clear that the yield varied from 176.7 g to 147.3 g. The highest yield of the paneer sample is observed with 900: 100 (buffalo:

Table 3(a). Results of yield for different paneer samples

Buffalo: Cow milk	Coagulating temperature (°C)	Yield (g)
900: 100	75	170.5
800: 200	75	163.3
700: 300	75	147.3
900: 100	80	176.7
800: 200	80	169.3
700: 300	80	175.4
900: 100	85	167.8
800: 200	85	163.5
700: 300	85	161.2

$$\text{Yield} = -1211.92564 - 521.85337 \times \text{buffalo: cow} + 36.25225 \times \text{coagulating temp} + 5.25598 \times \text{buffalo: cow} \times \text{coagulating temp} + 121.793 \times \text{buffalo: cow}^2 - .2347 \times \text{coagulating temp}^2$$

cow milk) ratio, at 80°C coagulating temperature and 1% citric acid solution.

The increase in yield of paneer is shown in Figure 2(a) with increase of buffalo: cow milk because buffalo milk contains more fat and the fat globules present in this milk is larger than the cow milk. The increase in coagulating temperature increased the yield of paneer because at high temperature complete denaturation of protein occurs but further increase of temp causes reduction of yield because moisture expulsion occurs more at high temp. The effects of all the independent variables were analysed through the software Design Expert 7.0.0 to optimize the values for maximum yield. The maximum yield of 163.955g paneer was observed for 700: 300 (buffalo: cow) ratio, at 85°C coagulating temperature and 1% citric acid solution with a desirability value of 0.781.

Moisture content: The moisture content of all paneer

Table 2. Central composite rotatable design and responses

Std	Run	Block	Factor 1 A: Buffalo: Cow milk	Factor 2 B: Coagulating temperature deg celsius	Response 1 yield (g)	Response 2 moisture content (%)	Response 3 fat content (%)	Response 4 protein content (%)
7	1	Block 1	0.27	72.93	161.32	57.32	55.19	17.65
11	2	Block 1	0.27	80.00	167.63	56.21	65.32	17.27
4	3	Block 1	0.43	85.00	161.3	51.24	55.6	19.2
12	4	Block 1	0.27	80.00	167.63	56.21	65.32	17.27
9	5	Block 1	0.27	80.00	167.63	56.21	65.32	17.27
3	6	Block 1	0.11	85.00	167.8	53.3	59.11	18.23
13	7	Block 1	0.27	80.00	167.63	56.21	65.32	17.27
2	8	Block 1	0.43	75.00	147.3	51.59	56.75	18.56
5	9	Block 1	0.05	80.00	182.2	51.43	50.68	18.4
8	10	Block 1	0.27	87.07	156.6	54.23	55.78	17.86
6	11	Block 1	0.49	80.00	171.47	51.32	51.165	18.62
1	12	Block 1	0.11	75.00	170.5	61.81	48.39	17.37
10	13	Block 1	0.27	80.00	167.53	56.21	65.32	17.27

samples were measured and are given in Table 3(b). From the results it is clear that the value of moisture content varied from 61.81 to 51.24%. The highest value of moisture content of the paneer sample was observed with 900: 100 (buffalo: cow milk) ratio, at 75°C coagulating temperature and 1% citric acid solution.

In Figure 2(b) the moisture content increased with increase of cow milk because cow milk contains more moisture than the buffalo milk but after that moisture content reduced with decrease in buffalo milk. The increase in coagulating temperature continuously decreased the moisture content of paneer linearly because high temperature caused moisture expulsion from paneer. The effects of all the independent variables were analysed through the software Design Expert 7.0.0 to optimize the values for maximum moisture content. The maximum

moisture content of 54.179% paneer was observed for 265: 735 (cow: buffalo) ratio, at 85°C coagulating temperature and 1% citric acid solution with a desirability value of 0.599.

Fat content: The fat content of all paneer samples were measured and are given in Table 3(c). From the results it is clear that fat content of paneer varied from 62.03 to 48.39%. The highest fat content of the paneer sample was observed with 800: 200 (buffalo: cow milk) ratio, at 80°C coagulating temperature and 1% citric acid solution.

The increase in fat content of paneer is shown in Figure 2(c) with increase of buffalo: cow milk because buffalo milk contains more fat and the fat globules present in this milk is larger than those in cow milk. The increase in coagulating temperature increases the fat content of paneer because at high temperature complete denaturation of protein occurs and at this high temperature a three dimensional network forms where fat globules are also entrapped with casein micelles. The effects of all the independent variables were

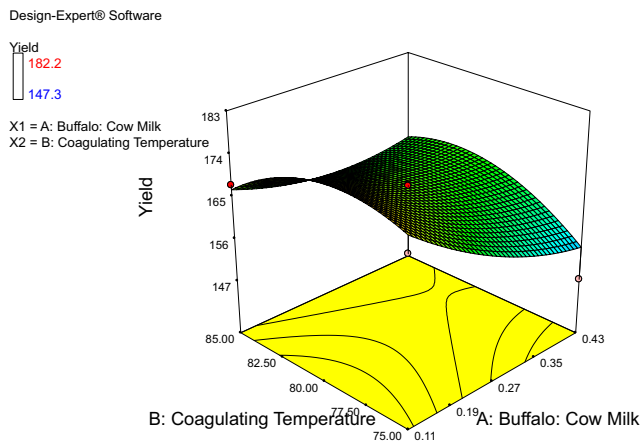


Fig 2(a). Response surface plot of yield as a function of buffalo: cow milk and coagulating temperature for preparation of paneer

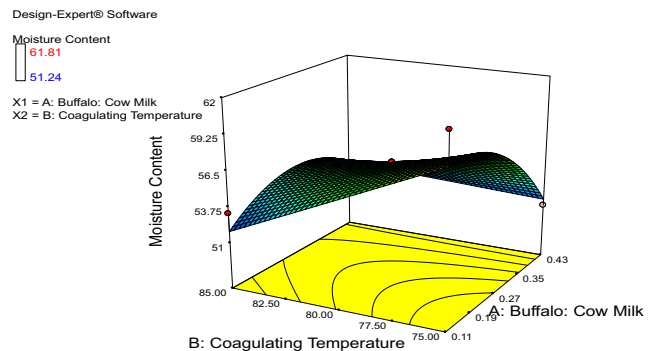


Fig 2(b). Response surface plot of moisture content as a function of buffalo: cow milk and coagulating temperature for preparation of paneer

Table 3(b). Results of moisture content for different paneer samples

Buffalo: Cow milk	Coagulating temperature (°C)	Moisture content (%)
900: 100	75	61.81
800: 200	75	56.91
700: 300	75	51.59
900: 100	80	53.42
800: 200	80	56.06
700: 300	80	51.32
900: 100	85	53.32
800: 200	85	55.04
700: 300	85	51.24

Moisture content = $137.027 - 168.498 \times \text{buffalo: cow} - 1.088 \times \text{coagulating temp} + 2.57 \times \text{buffalo: cow} \times \text{coagulating temp} - 86.899 \times \text{buffalo: cow}^2 - 4 \times 10^{-4} \times \text{coagulating temp}^2$

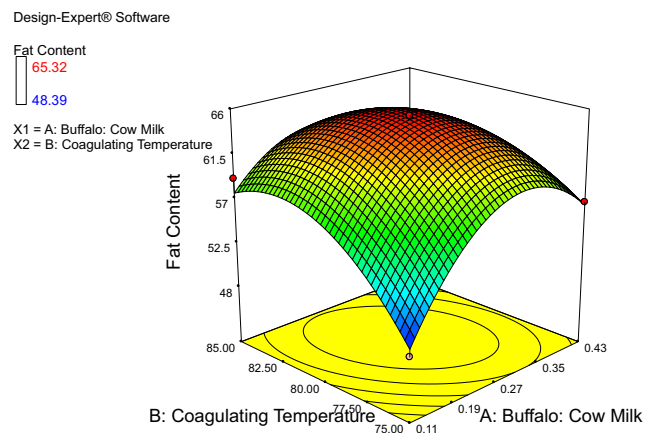


Fig 2(c). Response surface plot of fat content as a function of buffalo: cow milk and coagulating temperature for preparation of paneer

analysed through the software Design Expert 7.0.0 to optimize the values for maximum fat content of paneer. The maximum fat content of 61.0863% from paneer was observed for 740: 260 (buffalo: cow) ratio, at 83.88°C coagulating temperature and 1% citric acid solution with a desirability value of 0.794.

Protein content: The protein content of all paneer samples were measured and are given in Table 3(d). From the results it is clear that protein content of paneer varied from 19.20 to 16.53%. The highest protein in the paneer sample was observed with 800: 200 (buffalo: cow milk) ratio, at 85°C coagulating temperature and 1% citric acid solution.

The increase in protein content of paneer is shown in Figure 2(d) with increase of buffalo: cow milk because buffalo milk contains more protein. Again with the increase of cow milk the protein content increased. The increase in coagulating temperature increased the protein content of paneer because at high temperature complete denaturation of protein occurs and at this high temperature a three

Table 3(c). Results of fat content for different paneer samples

Buffalo: Cow milk	Coagulating temperature (°C)	Fat content (%)
900: 100	75	48.39
800: 200	75	56.15
700: 300	75	56.75
900: 100	80	50.62
800: 200	80	62.03
700: 300	80	51.17
900: 100	85	51.105
800: 200	85	57.91
700: 300	85	55.20

$$\text{Fat content} = -1203.223 + 448.193 \times \text{buffalo: cow} + 29.926 \times \text{coagulating temp} - 3.738 \times \text{buffalo: cow} \times \text{coagulating temp} - 268.20 \times \text{buffalo: cow}^2 - 0.179 \times \text{coagulating temp}^2$$

Table 3(d). Results of protein content for different paneer samples

Buffalo: Cow milk	Coagulating temperature (°C)	Protein content (%)
900: 100	75	17.37
800: 200	75	17.59
700: 300	75	18.56
900: 100	80	17.50
800: 200	80	16.53
700: 300	80	18.62
900: 100	85	18.23
800: 200	85	19.20
700: 300	85	17.88

$$\text{Protein content} = 88.956 - 6.899 \times \text{buffalo:cow} - 1.820 \times \text{coagulating temp} - 0.069 \times \text{buffalo:cow} \times \text{coagulating temp} + 26.66 \times \text{buffalo:cow}^2 + 0.0117 \times \text{coagulating temp}^2$$

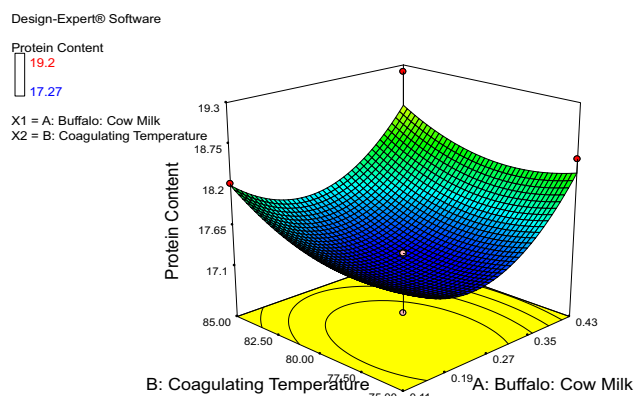


Fig. 2(d). Response surface plot of protein content as a function of buffalo: cow milk and coagulating temperature for preparation of paneer

dimensional network forms, thus resulting in increase of protein content. The effects of all the independent variables were analysed through the software Design Expert 7.0.0 to optimize the values for maximum protein content of paneer. The maximum protein content of 18.7148% from paneer was observed for 700:300 (buffalo: cow) ratio, at 85°C coagulating temperature and 1% citric acid solution with a desirability value of 0.908.

CONCLUSIONS

The highest yield of the paneer sample is observed with 900: 100 (buffalo: cow milk), 80°C coagulating temperature and 1% citric acid solution. The yield of paneer is a very important factor during paneer preparation. Increase of buffalo milk quantity causes increase of yield of paneer. The highest fat content of the paneer sample is observed with 800: 200 (buffalo: cow milk), 80°C coagulating temperature and 1% citric acid solution. Increase of buffalo milk causes increase of fat content of paneer because buffalo milk contains more fat and fat globules are bigger size here than cow milk. The highest protein content of the paneer sample is observed with 800: 200 (buffalo: cow milk), 85°C coagulating temperature and 1% citric acid solution.

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Climate Change Vulnerability and Perception of Different Occupational Groups of Household in the Hill Regions of Darjeeling District, West Bengal

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Abstract: The present paper attempts to measure climate change vulnerability of different occupational groups of households like tea garden labourers, casual labourers, and workers in the informal sector, petty businessmen, tourist guide cum driver and workers in the formal sector in the hill regions of Darjeeling district of West Bengal. In addition, the present paper examines the socio-economic conditions of such occupational groups of households to understand the nature of vulnerability. Besides, the paper tries to measure the perception index of such occupational group of households and to investigate the relationship with the vulnerability. The Livelihood Vulnerability Index (LVI) of Hahn et al (2009) and modified LVI-IPCC index have been used to measure such vulnerability. The paper is based on primary data collected from 150 households from four villages in the hill regions of Darjeeling district of West Bengal, India, in 2018 with the help of a structured questionnaire. The results of the paper revealed that on the basis of such indices values (both measures of LVI) the tea garden labour, casual labour and workers in the informal sector are more vulnerable than that of other occupational group like tourist guide cum driver, workers in the formal sector and petty businessmen. The climate change perception index for tea garden labour, casual labour and workers in the informal sector are very low compared to other occupational groups of households like petty businessmen and workers in the formal sector. This indicates that the occupational groups of households who have lower perception of climate change are more vulnerable and vice versa. This paper has an important policy implication for enhancement of adaptive capacity as well as betterment in the perception for vulnerability reduction measures.

Keywords: Livelihood vulnerability index, Perception index, Tea garden labour, Informal sector, Socio-economic conditions

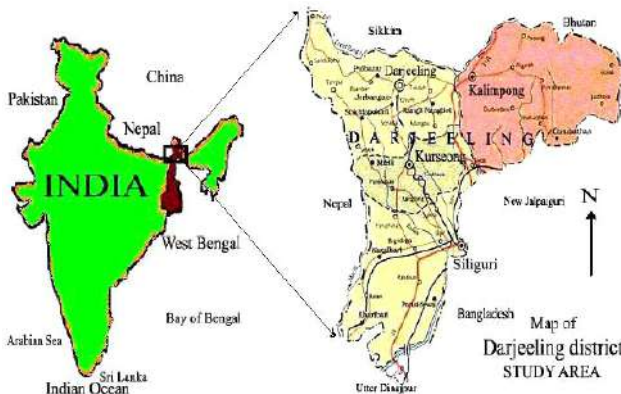
Mountain ecosystem services focuses on international and national developmental goals like poverty reduction, urbanization and economic development in India, Nepal and Bhutan (Aryal et al 2014, Barua et al 2014, Maraseni 2012). About 24% of the total terrestrial areas of the world are under mountain cover and about 26% of total world populations are living in the mountain regions. In addition, about 40% of the total world population is dependent on the ecosystem services of mountain regions. The mountains are one of the most fragile socio-ecological systems and sensitive to the climate change (Schild 2008). The Himalayan region is warming more than the global average rate (Liu and Chen 2000, Shrestha et al 1999). Analysis of the impact of climate change on poor and non-poor households in the mountain regions are relatively rare and hardly have been addressed in the earlier climate change research in mountain regions for developing policy (IPCC 2001, Parry 2007b, Salick and Ross 2009). It therefore seems imperative to investigate communities' perception in coping with climate vulnerabilities (Gippner et al 2012, Picketts et al 2013).

This paper is relevant because it seeks to study the impact of climate change on different occupational groups of households like tea garden labour, casual labour, petty businessmen and workers in the informal sector and tourist

guide cum drivers and workers in the formal sector etc at the local or household level. In addition, the paper attempts to investigate the relation between climate change perceptions of different occupational groups and their livelihood vulnerability. There are two main methodologies developed for the measurement of vulnerability. First is the indicator or index based methodology and other is econometrics based. Given the above backdrop, the main objectives of the paper are three fold. First, to quantify the degree of vulnerability of different occupational groups of households like tea garden labour, casual labour, worker in the informal sector, petty businessmen, tourist guide cum driver and workers in the formal sector etc. in the hill regions of Darjeeling district in West Bengal. Second, is to examine the socio-economic conditions of different occupational groups in the hill regions of Darjeeling district. Third, is to measure climate change perception index of the different occupational groups of households and to investigate the relationship between climate perception and vulnerability. The present paper has utilized index based vulnerability measurement. The first one is livelihood vulnerability index (LVI) of Hahn et al (2009) and the second is the modified livelihood vulnerability index which is known as LVI-IPCC which includes exposure, sensitivity and adaptive capacity as three major factors of vulnerability.

MATERIAL AND METHODS

Study area: The study area is the district of Darjeeling which is a part of the eastern Himalayas bounded by Sikkim in the north, Bhutan in the northeast, Nepal in the West and Bangladesh in the southeast. Tea garden, forestry and horticulture, tea industry and tourism are the major sources of livelihood of the people in the district. The economy of Darjeeling is basically dependent on tourism and tea production. The major proportions of the people are Lepchas, Limbus, Gorkhas, Sherpas and Bhutias. Total Literacy rate of the district is 79.6% which is above the state literacy rate. The trend in rainfall of this district over 100 years (1901-2002) is increasing (Fig. 1). It would be early average minimum temperature, yearly average maximum temperature and yearly average temperature over 100 years (1901-2002) show increasing trend (Fig. 2-4). Data was plotted on the scatter diagram and using software the trend line was drawn. The regression equations are shown in the diagram on the basis of the best fit of different curves measured by highest value of R^2 . Darjeeling Hill area has been witnessing increased number of landslides at different locations (Ghosh et al 2012). Forest of Darjeeling district consists of sal, oak, semi-evergreen; temperate and alpine forests. This district also faces acute water shortage and is thickly populated. Map of the district is shown below



Source: Mukherjee 2013

Data collection: The present paper is based on primary data collected from four villages namely, Dupka Gaon, Lamahata, Banekburn Tea Estate and Manebhanjangan in the district of Darjeeling, West Bengal, India during 2018 with the help of a structured questionnaire and group discussions. 10% of households from each village have been selected as sample households. Thus, total number of sample households is 150. The sample households are classified by major occupational groups into tea garden labour, tourist guide-cum-driver, workers in the informal sector, petty businessmen, casual labour, and workers in the formal sector. Data consist of socio-economic data like age, sex,

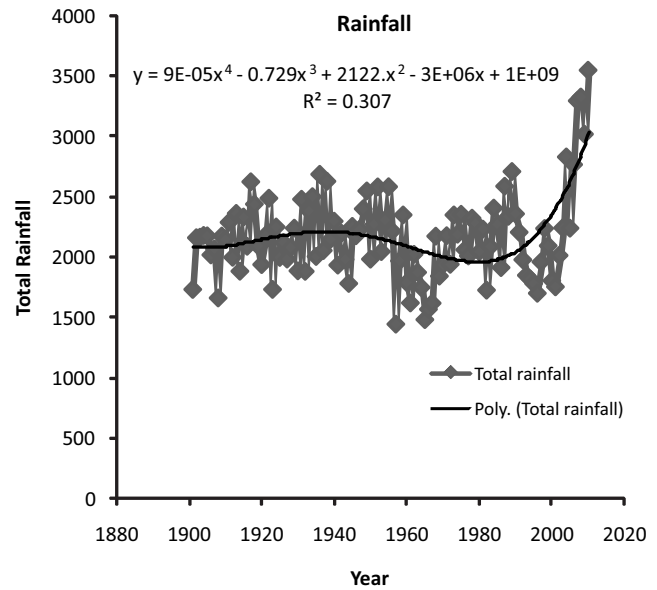


Fig. 1. Trends in rainfall (mm)

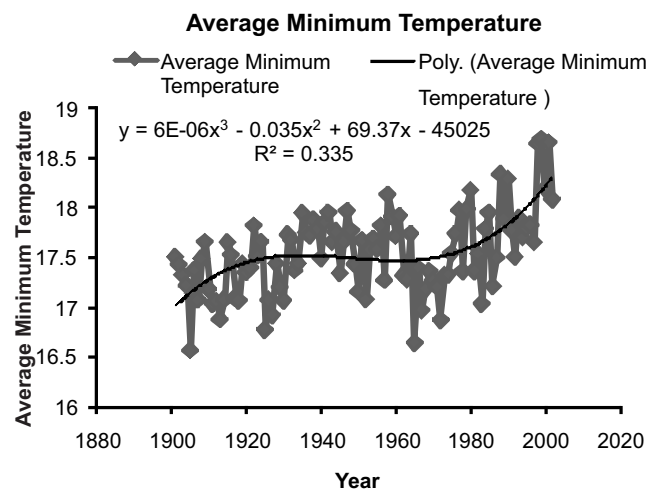


Fig. 2. Trends in minimum temperature

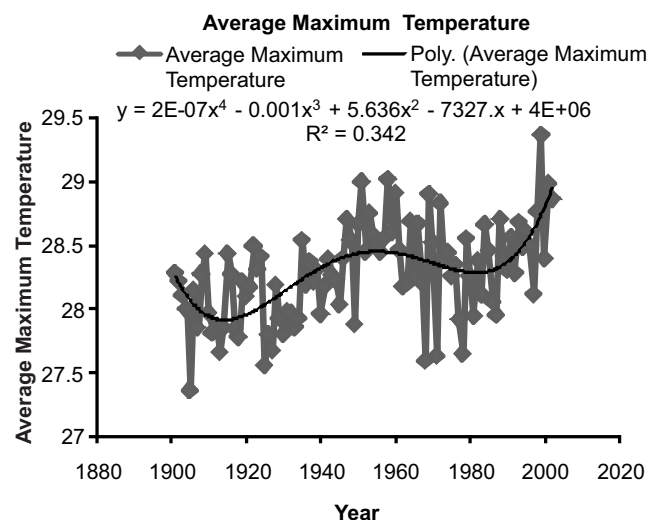


Fig. 3. Trends in maximum temperature

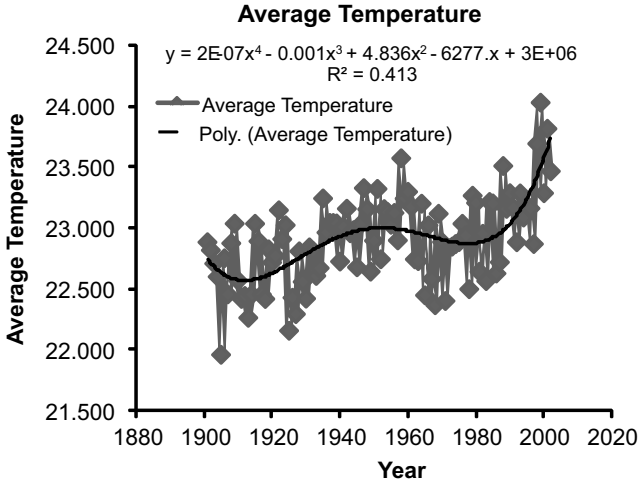


Fig. 4. Trends in average temperature

education, marital status, landholdings, housing, sanitation, assets, social capital, food access, water access, health access and climate perceptions etc.

Vulnerability index: In the calculation of livelihood vulnerability index (LVI) eight sub-components of vulnerability like socio demographic profile (SDP), livelihood strategies (LS), food, social network (SN), natural capital (NC), water, health and climatic issues are considered. Each of the sub-components index is again built with several indicators. The modified livelihood vulnerability index considers three contributory factors like exposure; sensitivity and adaptive capacity of vulnerability. In order to formulate index we take normalize value of each indicator. The normalize value lies between 0 to 1. "0" shows minimum and "1" shows maximum values. This normalization procedure was followed by the methodology of Human Development Index (UNDP 2006).

Some indicators show positive relationships with vulnerability and others have negative relationship with vulnerability. For positive relationship with vulnerability we have used the following formula.

$$Y_{ij} = \frac{y_{ij} - \text{Min}(y_{ij})}{\text{Max}(y_{ij}) - \text{Min}(y_{ij})} \quad (1)$$

Min (y_{ij}) and Max (y_{ij}) are the minimum and maximum values of the j indicator reflecting low and high vulnerability.

On the other hand for negative relationship with vulnerability, the formula is given by

$$Y_{ij} = \frac{\text{Max}(y_{ij}) - y_{ij}}{\text{Max}(y_{ij}) - \text{Min}(y_{ij})} \quad (2)$$

The weighted vulnerability index is constituted by following method of (Iyenger and Sudarshan 1982).

$$\bar{W}_i = \frac{\sum_{j=1}^k k_j Y_{ij}}{\sum_{j=1}^k k_j} \quad (3)$$

Where W_i is the vulnerability Index of the i th subcomponent, Y_{ij} is the normalized score of j th indicator. k_j is the weights of j th indicators such that ($0 < k < 1$ and $\sum_{j=1}^k k_j = 1$). $k_j Y_{ij}$ is the weighted value of j th indicator of i th sub component.

Since $\sum_{j=1}^n k_j = 1$ equation (3) can be rewritten as

$$\bar{W}_i = \sum_{j=1}^n k_j Y_{ij} \quad (4)$$

The weight is determined by

$$k_j = \frac{a}{\sqrt{\text{var}_i(y_{ij})}} \quad (5)$$

Where 'a' is constant such that

$$a = \left[\sum_{j=1}^{j=n} \frac{1}{\sqrt{\text{var}_i(y_{ij})}} \right]^{-1} \quad (6)$$

After calculating 8 sub components, livelihood vulnerability index (LVI) is computed by weighted mean as follows:

$$LVI = \frac{\sum_{i=1}^8 k_i \bar{W}_i}{\sum_{i=1}^8 k_i} \quad (7)$$

Since weight (k_i) of i th sub component is 1, ($\therefore k_i = \sum_{j=1}^n k_j = 1$) equation (7) can be simplified as

$$LVI = \frac{\sum_{i=1}^8 \bar{W}_i}{8} \quad (8)$$

The livelihood vulnerability index varies from 0 (lowest value) to 1 (highest Value) (Pandey and Jha 2012).

LVI-IPCC index: The contributory factors of vulnerability are exposure, sensitivity and adaptive capacity. Adaptive capacity is constructed with four sub components (SDP), Livelihood Strategies (LS), and (SN).

$$\text{Adaptive capacity Index} = \frac{\sum_{i=1}^4 k_i \bar{Y}_i}{\sum_{i=1}^4 k_i} = \frac{\sum_{i=1}^4 \bar{W}_i}{4} \quad (9)$$

$$(\therefore k_i = 1) = \frac{\bar{Y}_{sdp} + \bar{Y}_{ls} + \bar{Y}_{food} + \bar{Y}_{sn}}{4}$$

The sensitivity index is also based on three sub components like Natural Capital, Water and Health.

$$\text{Sensitivity Index} = \frac{\sum_{i=1}^3 k_i \bar{W}_i}{\sum_{i=1}^3 k_i} = \frac{\sum_{i=1}^3 \bar{W}_i}{3} \quad (10)$$

$$(\therefore k_i = 1) = \frac{\bar{Y}_{nc} + \bar{Y}_{water} + \bar{Y}_{health}}{3}$$

$$\text{Exposure Index} = \text{Index of climate variable} = \frac{\sum_{j=1}^n k_j y_j}{\sum_{j=1}^n k_j} \quad (11)$$

Lastly, LVI_IPCC index is then calculated as an average of exposure, sensitivity and adaptive capacity. Symbolically,

$$LVI_IPCC = \frac{Exposure + Sensitivity + Adaptive Capacity}{3} \quad (12)$$

RESULTS AND DISCUSSION

The key socio-economic and demographic features of sample households are presented in Table 1. Majority of them were workers in the formal sector (31%) followed by tourist guide cum driver to petty businessmen (Fig. 5). The majority of the households belong to scheduled tribes and other backward categories. More than 90 percent of households constitute landless, marginal and small farmers. Most of the households have sufficient education. Average size of family members is more than 4 and more than 50 percent of households are living below the poverty line in terms of holding BPL card. The livestock asset and physical asset for different occupational groups of households are presented in Table 2 and Table 3, respectively.

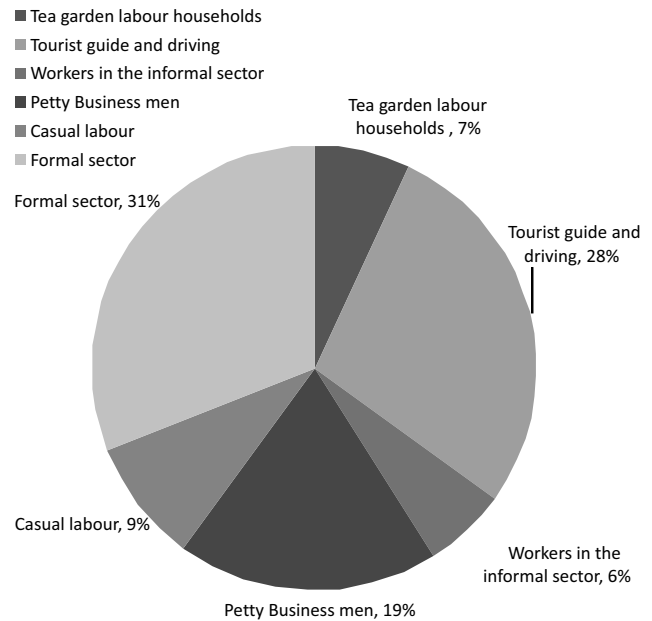


Fig. 5. Classification of different occupational groups of households

Table 1. Socio-economic conditions of different occupation groups in hill region of Darjeeling district of West Bengal, India

Parameters	Driver cumtourist guide	Petty business	Workers in informal sector)	Workers in formal sector	Casual labour	Teagarden labour	Total (150)
Social status							
ST	33 (78.57)	17 (60.71)	8 (88.89)	34 (72.34)	12 (85.71)	5 (50)	109 (72.67)
General	3 (7.14)	3 (10.71)	1 (11.11)	6 (12.77)	1 (7.14)	4 (40)	18 (12)
OBC	6 (14.29)	8 (28.57)	--	7 (14.89)	1 (7.14)	1 (10)	23 (15.33)
Gender							
Female	20 (47.62)	15 (53.57)	6 (66.67)	21 (44.68)	6 (42.86)	7 (70)	75 (50)
Male	22 (52.38)	13 (46.43)	3 (33.33)	26 (55.32)	8 (57.14)	3 (30)	75 (50)
Age distribution of head of households							
21-40 years	16 (38.1)	11 (39.29)	2 (22.22)	16 (34.04)	5 (35.71)	2 (20)	52(34.67)
41-60 years	16 (38.1)	10 (35.71)	6 (66.67)	18 (38.3)	5 (35.71)	5 (50)	60(40)
above 60years	9 (21.43)	6 (21.43)	1 (11.11)	11 (23.4)	4 (28.57)	2 (20)	33(22)
Education							
Illiterate	9 (21.43)	4 (14.29)	4 (44.44)	7 (14.89)	5 (35.71)	3 (30)	32 (21.33)
Primary	10 (23.81)	5 (17.86)	0--	5 (10.64)	2 (14.29)	2 (20)	24 (16)
Secondary	17 (40.48)	12 (42.86)	3 (33.33)	16 (34.04)	6 (42.86)	5 (50)	59 (39.33)
Above secondary	6 (14.29)	7 (25)	2 (22.22)	19 (40.43)	1 (7.14)	0--	35 (23.33)
Average of family size	4.26	4.64	4.33	4.64	3.57	3.40	4.33
Economic status							
BPL	21 (50)	17 (60.71)	5 (55.56)	27 (57.45)	6 (42.86)	4 (40)	80 (53.33)
APL	21 (50)	11 (39.29)	4 (44.44)	20 (42.55)	8 (57.14)	6 (60)	68 (45.33)
Land holding (acre)							
Land less	40 (95.24)	23 (82.14)	9 (100)	41 (87.23)	13 (92.86)	9 (90)	135 (90)
<1 Acre	2 (4.76)	5 (17.86)	0--	6 (12.77)	1 (7.14)	1 (10)	15 (10)

Source: Field Survey, 2018; **Note:** Figures in the parentheses represent percentage of the respective number of occupational group of households

Vulnerability of different occupation groups of the sample households in the hill region of Darjeeling district:

The livelihood vulnerability index of different occupational groups varies from 0.5339 to 0.6275 (Table 4).

The tea garden labourers in the Darjeeling district are most

vulnerable (0.6275) followed by the casual labour, the workers in the informal sector and the petty businessmen. The workers in formal sector are least vulnerable (0.5339).

The tea garden labourers are more vulnerable than other occupational groups due to higher values of food index,

Table 2. Livestock assets of different categories of occupation groups in hill region of Darjeeling district, West Bengal, India

Livestock assets per household	Driver/tourist guide	Petty business	Workers in informal sectors	Workers in formal sectors	Casual labour	Tea garden labour	All (150)
Cow per household	0.26	0.89	0.20	0.38	0.20	0.25	0.41
Goat per household	0.00	0.00	0.00	0.19	0.00	0.20	0.07
Hen per household	8.53	10.36	12.00	5.68	8.46	12.60	8.51
Pig per household	3.76	2.57	0.78	1.68	5.25	1.20	2.61

Source: Field Survey, 2018

Table 3. Physical assets possessed by different categories of occupational groups in the hill region of Darjeeling district of West Bengal

Physical assets	Driver/Tourist guide (No. of HH =42)	Petty business (No. of HH =28)	Workers in informal sectors (No. of HH =9)	Workers in formal sectors (No. of HH =47)	Casual labour (No. of HH =14)	Teagarden labour (No. of HH =10)	All (No. of HH =150)
Radio	27 (64.29)	17 (60.71)	5 (55.56)	27 (57.45)	10 (71.43)	10 (100)	96 (64)
Television	42 (100)	28 (100)	9 (100)	46 (97.87)	14 (100)	10 (100)	149 (99.33)
Mobile phone	42 (100)	28 (100)	8 (88.89)	47 (100)	13 (92.86)	8 (80)	146 (97.33)
Motorbike	8 (19.05)	2 (7.14)	--	12 (25.53)	1 (7.14)	5 (50)	28 (18.67)
Bicycle	2 (4.76)	--	--	1 (2.13)	--	--	3 (2)
Car	5 (11.9)	1 (3.57)	1 (11.11)	2 (4.26)	--	--	9 (6)
Refrigerator	17 (40.48)	8 (28.57)	3 (33.33)	25 (53.19)	1 (7.14)	1 (10)	55 (36.67)
LPG connection	42 (100)	26 (92.86)	8 (88.89)	47 (100)	14 (100)	8 (80)	145 (96.67)
Stove	36 (85.71)	29 (103.57)	8 (88.89)	42 (89.36)	14 (100)	8 (80)	137 (91.33)
Almirah	42 (100)	28 (100)	9 (100)	46 (97.87)	14 (100)	10 (100)	149 (99.33)
Other furniture	30 (71.43)	14 (50)	6 (66.67)	29 (61.7)	9 (64.29)	7 (70)	95 (63.33)

Source: Field Survey, 2018; Note: Figures in the parentheses represent percentage

Table 4. Indices of sub components & vulnerability indices for different livelihood groups in hill region of Darjeeling district, West Bengal

Symbol of Indicators	Tourist guide cum driver N ₁ =42	Petty business men N ₂ =28	Workers in informal sectors N ₃ =9	Workers in formal sectors N ₄ =47	Casual labour N ₅ =14	Tea garden labour N ₆ =10
Socio-demographic profile index	0.2739	0.3173	0.3644	0.2494	0.3705	0.4498
Livelihood strategy index	0.7294	0.7461	0.8533	0.6048	0.7296	0.4460
Food index	0.9774	0.9352	0.9944	0.9795	0.9911	0.9860
Social network index	0.7462	0.4571	0.5952	0.6117	0.4430	0.4738
Natural capital index	0.7140	0.6215	0.4551	0.6274	0.6488	0.9669
Water index	0.3234	0.6224	0.3814	0.3288	0.3948	0.4001
Health index	0.2269	0.2933	0.3452	0.2334	0.3190	0.5467
Climate variable index	0.5664	0.5925	0.6476	0.6361	0.7673	0.7506
Livelihood vulnerability index (by Hahn et al 2009)	0.5697(5)	0.5732(4)	0.5796(3)	0.5339(6)	0.5830(2)	0.6275(1)

Source: Computed by Author from primary data; Figures in the parenthesis represent rank

natural capital, and climate index (Table 4). The high dependency of natural capital, inaccessibility to food and less perception about climatic events are responsible for higher vulnerability. The high value of food index is due to low value of crop diversity (less than 1). The dependency of food crop is very high and workers in the informal sector and petty businessmen are less affected by socio-demographic variable, natural capital health and water. The low vulnerability of the workers in the formal sector is due to that fact that they are less affected by socio-demographic variables, natural capital, water, health and climate variable (Table 4). The LVI of different occupational groups are shown in the Radar diagram of Figure 6. Radar diagram is the diagrammatic representation of multivariate data in the form of two or more dimensional quantitative variables.

The modified livelihood vulnerability indices (LVI-IPCC) of tea garden labourers is highest (0.6591) and lowest for workers in the informal sector (0.5480). The more vulnerable communities are tea garden labourers followed by casual labourers. The tea garden labourers are facing with higher exposure and sensitivity compared to the other occupational group of households (Table 5). The Radar diagram for modified livelihood vulnerability indices of different sub-components for different occupational groups of households are in Figure 7.

Climate change perception of livelihood groups: In the hill region of Darjeeling district selected eight indicators for measuring climate change perception of different occupational groups of households. The indicators selected are like decrease in rainfall, increase in rainfall, landslides, storm, number of hot days, and number of cold days, rainy days and earthquake. The most of occupational groups of households have expressed their response in favour of increased number of hot days, cold days, rainy days and earthquake (Table 6). The increase in rainfall, decrease in rainfall, increase in landslide and increase in storm, most of the households in different occupational groups have responded “No” and “Don't know” against these climate events.

The perception index of each occupational group of households is constructed by taking average of normalized

score of the above eight perception indicators. The perception index of different occupational group of households is in Figure 8. The perception index is highest for tourist –cum- driver (0.420) followed by petty businessmen, workers in formal sectors, casual labourer and lowest

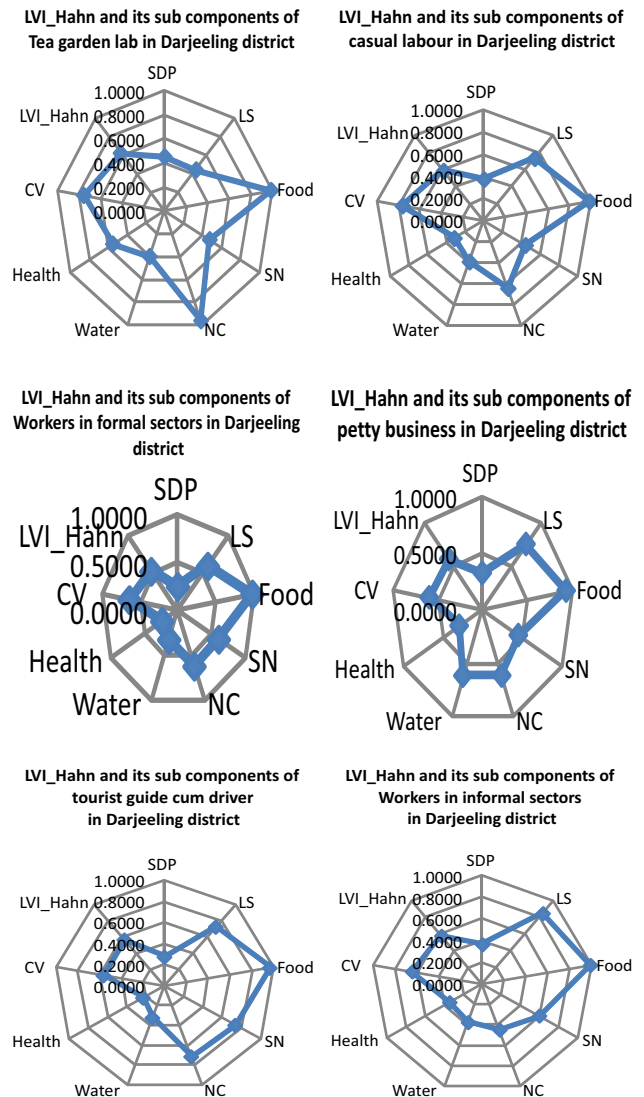


Fig. 6. Radardiagram for LVI (Hahn et al. 2009) for different occupational groups in the Darjeeling district of West Bengal

Table 5. Modified vulnerability indices (LVI-IPCC) for different livelihood groups in hill region of Darjeeling district, West Bengal

	Tourist guide cum driver	Petty business men	Workers in informal sectors	Workers in formal sectors	Casual labour	Tea garden labour
Exposure index	0.5664	0.5925	0.6476	0.6361	0.7673	0.7506
Sensitivity index	0.4214	0.5124	0.3939	0.3965	0.4542	0.6379
Adaptive index	0.6817	0.6139	0.7018	0.6114	0.6335	0.5889
LVI_IPCC	0.5565 (5)	0.5729 (4)	0.5811 (3)	0.5480 (6)	0.6184 (2)	0.6591 (1)

Source: Computed by Author from primary data; Figures in the parenthesis represent rank

perception index was in the workers in the informal sector (0.319).

Relationship between Climate change perception and livelihood vulnerability: The overall correlation coefficient value is negative and significant at 10% level. This means that the households who have higher vulnerability, lower perception and vice versa. The negative and significant value of correlation coefficient holds across different occupational groups of households except tea garden labourers (Table 7).

Climate variables and non-climatic factors such as social, physical and cultural structure of the communities in the mountain regions are found to be responsible for the causes of vulnerability of the people and communities (Adger et al 2013, Thwaites et al 2014). The poorest and non-poorest communities are at the same climate risk in the mountain regions. The present study has utilized the indicator based methodology that consistent with the other studies based on indicator based methodology (Tonmoy et al 2014, Binita et al 2015, Rod et al 2015) or a ranking of regions

or countries (Brooks et al 2005) and other researchers are with the help of econometrics based methodology (Hoddinott and Quisumbing 2003, Christiaensen and Subbarao 2004). Most of these studies have focused on the measurement of vulnerability at the global or regional level but no

Table 7. Correlation between livelihood vulnerability index and perception index by different livelihood groups in Darjeeling district

Occupational group of households in Darjeeling district	Correlation between LVI and perception index
Tourist guide cum driver	-0.3642**
Petty business men	-0.1111*
Workers In informal sector	-0.1741*
Workers in formal sector	-0.3051*
Tea garden labour	0.0864+
Casual labour	-0.1198*
Over all	-0.1766*

*10% level of significance, ** 5% level of significance; + insignificant

Table 6. Climate change perceptions of different livelihood groups in the hilly Darjeeling district

Perception indicators		Tourist guide cum driver	Petty business men	Workers in informal sectors	Workers in formal sectors	Casual labour	Tea garden labour
Increase in rainfall	Yes	10 (23.81)	3 (10.71)	1 (11.11)	10 (21.28)	2 (14.29)	1 (10)
	No	15 (35.71)	12 (42.86)	6 (66.67)	21 (44.68)	5 (35.71)	4 (40)
	Don't know	17 (40.48)	13 (46.43)	2 (22.22)	16 (34.04)	7 (50)	5 (50)
Decrease in rainfall	Yes	10 (23.81)	5 (17.86)	1 (11.11)	9 (19.15)	1 (7.14)	1 (10)
	No	12 (28.57)	10 (35.71)	2 (22.22)	20 (42.55)	7 (50)	3 (30)
	Don't know	20 (47.62)	13 (46.43)	6 (66.67)	18 (38.3)	6 (42.86)	6 (60)
Increase in Landslide	Yes	16 (38.1)	12 (42.86)	2 (22.22)	14 (29.79)	6 (42.86)	2 (20)
	No	12 (28.57)	8 (28.57)	5 (55.56)	15 (31.91)	5 (35.71)	5 (50)
	Don't know	14 (33.33)	8 (28.57)	2 (22.22)	18 (38.3)	3 (21.43)	3 (30)
Increase in storm	Yes	15 (35.71)	11 (39.29)	3 (33.33)	15 (31.91)	4 (28.57)	3 (30)
	No	12 (28.57)	8 (28.57)	5 (55.56)	14 (29.79)	4 (28.57)	4 (40)
	Don't know	15 (35.71)	9 (32.14)	1 (11.11)	18 (38.3)	6 (42.86)	3 (30)
Number of hot days increase	Yes	22 (52.38)	14 (50)	6 (66.67)	23 (48.94)	6 (42.86)	3 (30)
	No	14 (33.33)	6 (21.43)	0	8 (17.02)	3 (21.43)	3 (30)
	Don't know	6 (14.29)	8 (28.57)	3 (33.33)	16 (34.04)	5 (35.71)	4 (40)
Number of cold days increase	Yes	25 (59.52)	19 (67.86)	4 (44.44)	22 (46.81)	7 (50)	4 (40)
	No	13 (30.95)	6 (21.43)	3 (33.33)	13 (27.66)	4 (28.57)	6 (60)
	Don't know	4 (9.52)	3 (10.71)	2 (22.22)	12 (25.53)	3 (21.43)	0
Number of rainy days increase	Yes	24 (57.14)	13 (46.43)	2 (22.22)	21 (44.68)	6 (42.86)	8 (80)
	No	10 (23.81)	9 (32.14)	5 (55.56)	13 (27.66)	4 (28.57)	1 (10)
	Don't know	8 (19.05)	6 (21.43)	2 (22.22)	13 (27.66)	4 (28.57)	1 (10)
Increase in earthquake	Yes	19 (45.24)	12 (42.86)	4 (44.44)	27 (57.45)	7 (50)	6 (60)
	No	15 (35.71)	11 (39.29)	1 (11.11)	9 (19.15)	7 (50)	3 (30)
	Don't know	8 (19.05)	5 (17.86)	4 (44.44)	11 (23.4)	0	1 (10)

Source: Field Survey, 2018. Figures in the parentheses represent percentage

Table 8. Indicators of livelihood vulnerability of occupational group of households in the Darjeeling district

Sub component	Symbol of indicators
Socio-demographic profile	Percentage of dependent people <20 years, >60years
	Percentage of female headed households
	Percentage of illiteracy among households head
	Percentage of female family earner
	SDP Index
Livelihood strategies	Percentage of households with family member working outside local area
	Percentage of households change sowing and cropping schedule
	Livelihood diversification index
	Average livestock diversification index
	Monthly per capita income (Rs)
Food	LS Index
	Average crop diversity index = household has the capacity to grow at least 1 additional crop such as vegetables or pulse along with traditional crop
Food	Food Index
Social network	Percentage of households not having access to communication media (like TV)
	Percentage of households not associated with any organization (cooperative/ group) i.e. in Self Help Group (SHG)
	Percentage of households being non member of MGNREGA
	SN Index
Natural capital	Adaptive Index
	Percentage of households using only forest based energy for cooking
	Percentage of marginal and small farmer households
Water	NC Index
	Percentage of households not having regular drinking water supply
	Percentage of households that utilize natural water source for drinking water
	Percentage of households go at least 1 km to fetch water
Health	Water Index
	Percentage of households with family member suffering from chronic illness
	Percentage of households do not receiving treatment in local health centre
	Percentage of households do not have toilet facility
	Health Index
Climate variable	Sensitivity Index
	Percentage of households realize reduction in rainfall in past 5 years
	Percentage of households about the perception of increased landslide in last 5 years
	Percentage of households about the perception of increased storm in last 5 years
	Percentage of households who did not receive warning about natural disasters

Table 9. Indicators of LVI-IPCC

Livelihood Vulnerability Index_IPCC		
Exposure	Sensitivity	Adaptive capacity
Climate variable	Natural capital	Socio-demographic profile
	Water	Livelihood strategies
	Health	Food
		Social network

Table 10. LVI of different occupational group of households in Darjeeling district of West Bengal

Sub component	Symbol of indicators	LVI Driver/tourist guide	LVI Petty business	LVI workers in informal sector	LVI workers in formal sector	LVI wage labour	LVI tea garden labour
Socio-demographic profile	Percentage of dependent people <20 years, >60years	0.12	0.15	0.09	0.11	0.12	0.15
	Percentage of female headed households	0.06	0.07	0.09	0.06	0.08	0.13
	Percentage of illiteracy among households head	0.03	0.04	0.07	0.02	0.06	0.05
	Percentage of female family earner	0.04	0.05	0.10	0.04	0.09	0.10
	SDP Index	0.27	0.31	0.36	0.24	0.37	0.44
Livelihood strategies	Percentage of households with family member working outside local area	0.01	0.01	0.02	0.02	0.01	0.01
	Percentage of households change sowing and cropping schedule	0.03	0.02	0.02	0.03	0.02	0.01
	Livelihood diversification index	0.07	0.06	0.03	0.11	0.14	0.08
	Average livestock diversification index	0.06	0.03	0.11	0.13	0.07	0.10
	Monthly per capita income (Rs)	0.54	0.61	0.64	0.29	0.47	0.23
Food	LS Index	0.72	0.74	0.84	0.60	0.72	0.44
	Average crop diversity index = household has the capacity to grow at least 1 additional crop such as vegetables or pulse along with traditional crop	0.97	0.93	0.99	0.97	0.99	0.98
Food	Food Index	0.97	0.93	0.99	0.97	0.99	0.98
Social network	Percentage of households not having access to communication media (like TV)	0.45	0.23	0.22	0.25	0.27	0.15
	Percentage of households not associated with any organization (cooperative/ group) i.e. in Self Help Group (SHG)	0.12	0.06	0.22	0.14	0.13	0.25
	Percentage of households with non-member of MGNREGA	0.16	0.15	0.14	0.21	0.03	0.06
	SN Index	0.74	0.45	0.59	0.61	0.44	0.47
	Adaptive Index	0.68	0.61	0.70	0.61	0.63	0.58
Natural capital	Percentage of households using only forest based energy for cooking	0.19	0.27	0.01	0.23	0.21	0.01
	Percentage of marginal and small farmer households	0.51	0.34	0.44	0.39	0.43	0.94
	NC Index	0.71	0.62	0.44	0.62	0.64	0.96
Water	Percentage of households not having regular drinking water supply	0.20	0.46	0.19	0.18	0.27	0.23
	Percentage of households that utilize natural water source for drinking water	0.07	0.07	0.09	0.07	0.08	0.05
	Percentage of households go at least 1 km to fetch water	0.04	0.07	0.08	0.06	0.03	0.11
	Water Index	0.32	0.62	0.38	0.32	0.39	0.40
Health	Percentage of households with family member suffering from chronic illness	0.13	0.17	0.23	0.13	0.16	0.37
	Percentage of households do not receiving treatment in local health center	0.05	0.06	0.06	0.06	0.07	0.08
	Percentage of households do not have toilet facility	0.03	0.05	0.04	0.03	0.07	0.08
	Health Index	0.22	0.29	0.34	0.23	0.31	0.54
	Sensitivity Index	0.42	0.51	0.39	0.39	0.45	0.63
Climate variable	Percentage of households realize reduction in rainfall in past 5 years	0.14	0.19	0.20	0.17	0.24	0.21
	Percentage of households about the perception of lands lide increased in last 5 years	0.16	0.15	0.15	0.17	0.15	0.16
	Percentage of households about the perception of storm increased in last 5 years	0.15	0.13	0.20	0.18	0.11	0.21
	Percentage of households that did not receive warning about natural disasters	0.10	0.11	0.07	0.11	0.24	0.16
	CV Index	0.56	0.59	0.64	0.63	0.76	0.75
	Exposure	0.56	0.59	0.64	0.63	0.76	0.75
	LVI_Hahn	0.56	0.57	0.57	0.53	0.58	0.62
	LVI_IPCC	0.55	0.57	0.58	0.54	0.61	0.65

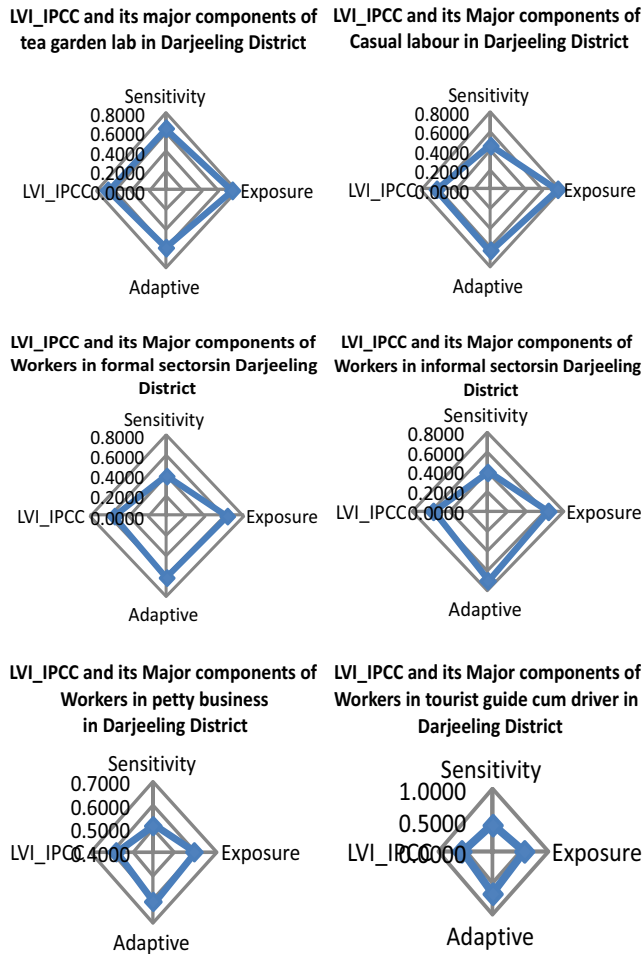


Fig. 7. Radardigram for LVI_IPCC for different occupation groups in hill region of Darjeeling district

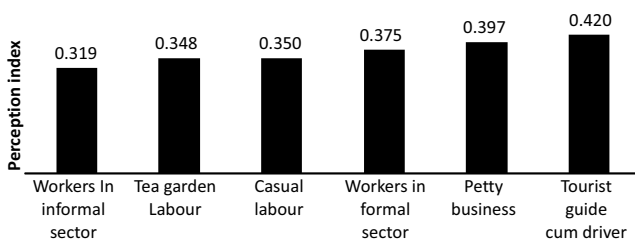


Fig. 8. Perception index of different occupational groups of households

standardized vulnerability assessment methods are applied at the household or local scale (Preston et al 2011, Holsten and Kropp 2012, Costa and Kropp 2013).

CONCLUSIONS

Most of households are landless and belong to below poverty line and dependent on livestock assets. They are educated and use information communication technology like mobile phone and television. In terms of LVI and modified

LVI-IPCC, the tea garden labourers are most vulnerable while the workers in the formal sector are least vulnerable. Tea garden labourers are most vulnerable because they are more dependent on natural capital, inaccessibility of food and low perception of climate events. The perception index is found to be highest for tourist- cum- driver followed by petty businessmen, workers in formal sectors, casual labourer and tea garden labourers. There is a negative and significant relation between vulnerability index and perception index of the households as indicated by the value correlation coefficient. Priority should be given on National Food Security Act, MGNREGA, rain water harvesting, renovation of local water bodies and agro-forestry for the reduction of vulnerability.

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Income Inequalities in Different Regions of Rural Punjab

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Abstract: Regional disparities have been increasing over time in both rural as well as urban areas. In rural areas, inequality related to land ownership has also increased. The study is based upon the primary data, collected from four districts situated in three different regions (Malwa, Doaba and Majha) of the Punjab state. The main source of income in all the regions is farm business income, followed by income from non-farm employment and casual agricultural labour. Malwa has recorded the highest share of farm business income. The second significant source of income in the case of an average rural household is non-farm wage employment. Doaba has recorded the highest income from non-farm wage employment. The per capita income is also the highest in Malwa and the lowest in Doaba for an average rural household. A relatively better distribution of per household income is observed in the case of small farmers. When we compare household income of land owning households and landless households in different regions, inequality seems wider in land owning households than the landless households in three regions.

Keywords: Doaba, Economics, Employment, Income, Inequalities, Majha, Malwa, Rural Punjab

Regional disparities have increased during 1990's. Southern region and western regions of India have grown on a better pace but northern and eastern regions lagged behind. Disparities between the rural and urban areas too increased in the same period (Deaton and Dreze 2002). Real agricultural wages have grown at a lower rate as compared to the wages of public sector employees. Since agricultural labourers and public sector employees typically live in rural and urban areas, respectively, this also increased the economic inequalities between the rural and urban areas. In rural areas, inequality related to land ownership, assets has increased (Toor et al 2018, Toor et al 2019). Punjab, Haryana, Andhra Pradesh and Tamil Nadu are the states which have highest inequality related to land ownership (Rawal 2008). Inequality related to assets also increases income inequality as agriculture is the main source of income for rural people. The income inequalities widens among the different sections of farming population due to the new technologies. The main objective of the present study is to examine the inequality in income among different regions, i.e. Malwa, Majha and Doaba of rural Punjab.

MATERIAL AND METHODS

The analysis is based upon the primary data, collected through a detailed schedule, of 591 sampled households involved from the 23 villages belonging to four districts (Mansa, SAS Nagar Mohali, SBS Nagar Nawanshahr and Amritsar) situated in three different regions (Malwa, Doaba and Majha) of the Punjab state. These 23 villages include, four villages (Chadiala, Rasanheri, Sialba, Khijergarh) from SAS Nagar (Mohali), five villages (Bir khurd, Bhadra,

Chhapian wali, Aspal, Rajrana) from Mansa district, five villages (Bharo majara, Bana, Anokharwal, Behloor kalan, Phirni majra) from SBS Nagar (Nawanshahr) and nine villages (Kotli kazian, Bhakna khurd, Padhri, Chetanpura, Khela, Budha theh, Thothian, Bathuchak, Loharka khurd) from Amritsar district. Standard statistical tools like mean values and proportions have been used while carrying out the tabular analysis. Gini co-efficient have been used to support the findings. The pattern of income distribution among families and population of land owning and landless households has been worked out by taking cumulative percentage of per household income for each decile group after arranging the same in an ascending order. Gini ratios have also been calculated to justify the pattern of distribution.

RESULTS AND DISCUSSION

Region-wise average value of household income: The variations in the income levels of households across the different regions in Punjab were observed (Table 1). The average rural household in Malwa earns Rs. 159122, in Majha and Doaba are Rs. 147391 and Rs. 107040, respectively. The average household income of marginal farm households is the highest in Doaba (Rs. 112083), whereas it is the lowest in Malwa (Rs. 96808). The main sources of income in all the regions are farm business income, followed by income from non-farm employment and casual agricultural labour. Income from government job salary appears at the fourth place in Doaba and Majha, but it ranks at the second positioning Malwa. Income from private job salary ranks at the fifth position in Doaba and Majha. The average household income of small farm households is the

Table 1. Region-wise average household income (Mean values in Rs. per annum)

Sources of income	Marginal farm households			Small farm households			Medium farm households			Large farm households		
	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha
Farm business income	84808	88901	68356	121598	135951	136838	279603	228359	259229	1776777	1140400	771366
Rent from land leased out	0	0	0	0	0	0	0	0	0	0	0	30000
Leasing out labour on contractual basis in agricultural sector	0	0	0	0	0	0	0	0	0	0	0	0
Leasing out labour on casual basis in agricultural sector	0	0	0	0	0	0	0	0	0	0	0	0
Livestock	8000	11545	14152	17629	27429	16524	26640	26400	15176	29500	40000	17300
Government job	1600	0	0	0	0	9143	0	0	7059	0	0	0
Private job	1200	0	6364	1371	0	6000	0	0	7059	0	0	3600
Manufacturing and processing	0	0	1818	0	0	1143	0	0	0	0	0	0
Artisanwork	0	1818	0	0	0	0	0	0	0	0	0	0
Non-farm wage employment	1000	0	1091	0	0	0	0	0	0	0	0	0
Pensions	200	1636	9818	0	0	10286	4080	14400	3529	0	0	0
Scholarships	0	0	0	0	0	0	0	0	0	0	0	0
Remittances	0	0	0	0	0	0	0	0	0	0	0	0
From MGNREGS	0	0	0	0	0	0	0	0	0	0	0	0
Others	0	8182	1091	0	0	2571	0	0	2118	0	0	0
Total	96808	112083	102690	140598	163380	182505	310323	269159	294170	1806277	1180400	822266

Table 1 (Contd...)

Sources of income	Agricultural labour households			Non-agricultural labour households			Artisan households			Other households			All sampled households		
	Malwa	Doaba	Majha	Malwa	Doab	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha
Farm business income	0	0	0	0	0	0	0	0	0	0	0	0	99394	35098	76666
Rent from land leased out	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1333
Leasing out labour on contractual basis in agricultural sector	0	0	5000	0	0	0	0	0	0	0	0	0	0	0	1511
Leasing out labour on casual basis in agricultural sector	37928	43294	45904	6234	417	5514	8500	3750	0	2407	2105	828	8078	6842	15058
Livestock	128	0	0	0	0	0	0	0	0	0	0	0	6931	4092	5533
Government job	0	0	0	1149	1333	1091	0	2000	0	55130	37895	65517	12516	6700	10044
Private job	769	0	1765	2809	0	1500	0	0	0	24667	37368	37655	6415	5917	7867
Manufacturing and processing	0	0	0	0	0	818	0	0	0	5111	6947	18207	1122	1100	2880
Artisanwork	128	0	88	638	444	545	71000	67000	106667	2556	0	0	3589	3717	1556
Non-farm wage employment	1615	15294	8485	55234	72074	63023	0	7500	20000	3778	15000	6621	11760	37350	16169
Pensions	1692	1765	1235	1277	0	1636	1800	0	0	11222	10547	5793	3488	2670	4107
Scholarships	74	0	95	53	0	45	0	0	0	0	0	103	22	0	51
Remittances	0	0	221	0	0	0	0	3333	0	0	0	4138	0	167	600
From MGNREGS	2310	531	388	1140	233	0	965	0	0	427	0	0	717	180	117
Others	462	882	1191	1404	0	0	0	0	0	21630	14737	23103	5089	3208	3898
Total	45107	61766	64373	69938	74502	74173	82265	83583	126667	126927	124600	161966	159122	107040	147391

Source: Field Survey, 2015-16

highest in Majha and is the lowest in Malwa because of the higher operational costs and less job opportunities. Average household income of this category in Doaba is Rs. 163380.

The per household income of medium farm households is the highest in Malwa and the lowest in Doaba. Apart from the farm business income, income from livestock ranks at the second place in all the three regions. The per household income of large farm households is again the highest (Rs.1806277) in Malwa due to large size of farm land and the lowest (Rs.822266) in Majha. Income from livestock and private job salary are the other important sources of income after farm business income in all the three regions for large farm households. There are some variations in the income levels of agricultural labour households. Agricultural labour households of Majha earn on an average Rs. 64373, whereas, the corresponding figures for Doaba and Malwa are Rs. 61766 and Rs.45107, respectively. The average household income of non-agricultural labour households is the highest in Doaba and the lowest in Malwa. Artisan household earnings from artisanal work was highest (Rs. 126667) in Majha, followed by Doaba and Malwa. The average income of 'Other' households is the highest in Majha; and the lowest in Doaba. Income from non-farm wage work ranks at the second place in all the regions except Malwa. This is the highest in Doaba and the lowest in Malwa. Income from casual agricultural labour work ranks at the third place in Majha and Doaba. The analysis indicates that average household income for all the household categories is highest in Malwa and the lowest in Doaba.

Region-wise pattern of household income: The main source of income is farm business income for a rural household (Table 2). However, there are variations in the relative share from this source across the three selected regions. Malwa has recorded the highest share of farm business income (62.46%). The second important source of income for average rural household is non-farm wage employment except for Majha, where income from government job salary is at the second rank (7.87%). An average rural household in Doaba was 34.89% income from non-farm wage employment, followed by Majha and Malwa. Income from casual agricultural labour is at third place in Majha and Doaba. In marginal farm households, the relative share of farm business income in the total income is the highest (87.60%) in Malwa followed, by Doaba and Majha. The share of income from livestock is the highest (13.78%) for marginal farm households in Majha followed by Doaba and Malwa. In small farm households, the relative share of farm business income in the total income is the highest (86.49%) in Malwa, followed by Doaba, and Majha. The share of income from livestock is the highest (16.79%) for

small farm households in Doaba, followed by Malwa, and Majha. For large farm households and the medium farm households, the main source of income is farm business income. However, there are variations in the relative shares from this source across the regions. Malwa has recorded the highest share of farm business income i.e., 90.10 and 98.37 per cent for medium and large farm households, respectively; and for Majha are 88.12 and 93.81 per cent.

In agricultural labour households, the relative share of casual agricultural labour income in the total income is the highest (84.08%) in Malwa, followed by Majha and Doaba. The share of income from non-farm wage employment is the highest in the total income of agricultural labour households in Doaba followed, by Majha and Malwa. Another important source of income for agricultural labour households is income from MGNREGS. The proportional share of this source is the highest in Malwa followed, by Doaba and Majha. For non-agricultural labour households, the main source of income is non-farm wage employment. However, there are variations in the relative shares from this source across the regions. Doaba has recorded the highest share of non-farm wage employment i.e., 96.74 per cent for this category. In the case of artisan households, the relative share of artisanal work income in the total income is the highest (86.31%) in Malwa, followed by Majha and Doaba. The share of income from casual agricultural labour is the highest (10.33%) in the total income of artisan households in Malwa. The average household income of artisan households of Majha is 1.54 times more than that of average household income of artisan households of Malwa. All other households categories have almost the same average household income.

The second significant source of income in the case of an average rural household is non-farm wage employment. Doaba has recorded the highest income from non-farm wage employment. The other components of income such as casual agricultural labour, government job salary and private job salary account for a very low proportion of the total income in all the three regions. The analysis indicates that rural households in all the three regions supplement their income with different activities.

Region-wise per capita income: The average household income, per capita income is also the highest in Malwa (Rs. 32674) and the lowest (Rs. 21668) in Doaba for an average rural household (Table 3) and is Rs. 25813 for Majha. Marginal farm households earn the highest per capita annually in Doaba and the lowest in Majha. For small farm households, the per capita income is the highest (Rs. 31962) in Majha, whereas the corresponding figures for Malwa and Doaba are Rs. 30042 and Rs. 27230, respectively. For

Table 2. Region-wise structure of household income (percentage)

Sources of income	Marginal farm households			Small farm households			Medium farm households			Large farm households		
	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha
Farm business income	87.60	79.32	66.57	86.49	83.21	74.98	90.10	84.84	88.12	98.37	96.61	93.81
Rent from land leased out	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.65
Leasing out labour on contractual basis in agricultural sector	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leasing out labour on casual basis in agricultural sector	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Livestock	8.26	10.30	13.78	12.54	16.79	9.05	8.58	9.81	5.16	1.63	3.39	2.10
Government job	1.65	0.00	0.00	0.00	0.00	5.01	0.00	0.00	2.40	0.00	0.00	0.00
Private job	1.24	0.00	6.20	0.98	0.00	3.29	0.00	0.00	2.40	0.00	0.00	0.44
Manufacturing and processing	0.00	0.00	1.77	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00
Artisanwork	0.00	1.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Non-farm wage employment	1.03	0.00	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pensions	0.21	1.46	9.56	0.00	0.00	5.64	1.31	5.35	1.20	0.00	0.00	0.00
Scholarships	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Remittances	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Others	0.00	7.30	1.06	0.00	0.00	1.41	0.00	0.00	0.72	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 2 (Contd...)

Sources of income	Agricultural labour households			Non-agricultural labour households			Artisan households			Other households			All sampled households		
	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha
Farm business income	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	62.46	32.79	52.02
Rent from land leased out	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90
Leasing out labour on contractual basis in agricultural sector	0.00	0.00	7.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.03
Leasing out labour on casual basis in agricultural sector	84.08	70.09	71.31	8.91	0.56	7.43	10.33	4.49	0.00	1.90	1.69	0.51	5.08	6.39	10.22
Livestock	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.36	3.82	3.75
Government job	0.00	0.00	0.00	1.64	1.79	1.47	0.00	2.39	0.00	43.43	30.41	40.45	7.87	6.26	6.81
Private job	1.71	0.00	2.74	4.02	0.00	2.02	0.00	0.00	0.00	19.43	29.99	23.25	4.03	5.53	5.34
Manufacturing and processing	0.00	0.00	0.00	0.00	0.00	1.10	0.00	0.00	0.00	4.03	5.58	11.24	0.71	1.03	1.95
Artisanwork	0.28	0.00	0.14	0.91	0.60	0.74	86.31	80.16	84.21	2.01	0.00	0.00	2.26	3.47	1.06
Non-farm wage employment	3.58	24.76	13.18	78.98	96.74	84.97	0.00	8.97	15.79	2.98	12.04	4.09	7.39	34.89	10.97
Pensions	3.75	2.86	1.92	1.83	0.00	2.21	2.19	0.00	0.00	8.84	8.46	3.58	2.19	2.49	2.79
Scholarships	0.16	0.00	0.15	0.08	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.06	0.01	0.00	0.03
Remittances	0.00	0.00	0.34	0.00	0.00	0.00	0.00	3.99	0.00	0.00	0.00	2.55	0.00	0.16	0.41
From MGNREGS	5.12	0.86	0.60	1.63	0.31	0.00	1.17	0.00	0.00	0.34	0.00	0.00	0.45	0.17	0.08
Others	1.02	1.43	1.85	2.01	0.00	0.00	0.00	0.00	0.00	17.04	11.83	14.26	3.20	3.00	2.64
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.0	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Source: Field Survey, 2015-16; **Note:** Income from MGNREGS is zero for farm households

Table 3. Region-wise per-capita value of household income (Rs. per annum)

Sources of income	Marginal farm			Small farm households			Medium farm households			Large farm households		
	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha
Farm business income	16962	21736	12406	25983	22659	23965	53770	35681	24227	247807	76027	77137
Rent from land leased out	0	0	0	0	0	0	0	0	0	0	0	3000
Leasing out labour on contractual basis in agricultural sector	0	0	0	0	0	0	0	0	0	0	0	0
Leasing out labour on casual basis in agricultural sector	0	0	0	0	0	0	0	0	0	0	0	0
Livestock	1600	2823	2568	3767	4571	2894	5123	4125	1418	4114	2667	1730
Government job	320	0	0	0	0	1601	0	0	660	0	0	0
Private job	240	0	1155	293	0	1051	0	0	660	0	0	360
Manufacturing and processing	0	0	330	0	0	200	0	0	0	0	0	0
Artisanwork	0	445	0	0	0	0	0	0	0	0	0	0
Non-farm wage employment	200	0	198	0	0	0	0	0	0	0	0	0
Pensions	40	400	1782	0	0	1801	785	2250	330	0	0	0
Scholarships	0	0	0	0	0	0	0	0	0	0	0	0
Remittances	0	0	0	0	0	0	0	0	0	0	0	0
From MGNREGS	0	0	0	0	0	0	0	0	0	0	0	0
Others	0	2000	198	0	0	450	0	0	198	0	0	0
Total	19362	27404	18637	30042	27230	31962	59677	42056	27493	251921	78693	82227

Table 3 (Contd...)

Sources of income	Agricultural labour households			Non-agricultural labour households			Artisan households			Other households			All sampled households		
	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha
Farm business	0	0	0	0	0	0	0	0	0	0	0	0	20409	7105	13427
Rent from land leased out	0	0	0	0	0	0	0	0	0	0	0	0	0	0	234
Leasing out labour on contractual basis in agricultural sector	0	0	992	0	0	0	0	0	0	0	0	0	0	0	265
Leasing out labour on casual basis in agricultural sector	8053	9311	9108	1176	87	1054	1635	643	0	792	471	148	1659	1385	2637
Livestock	27	0	0	0	0	0	0	0	0	0	0	0	1423	828	969
Government job	0	0	0	217	277	209	0	343	0	18135	8478	11741	2570	1356	1759
Private job	163	0	350	530	0	287	0	0	0	8114	8360	6748	1317	1198	1378
Manufacturing and processing	0	0	0	0	0	156	0	0	0	1681	1554	3263	230	223	504
Artisanwork	27	0	18	120	92	104	13654	11492	13907	841	0	0	737	752	272
Non-farm wage employment	343	3289	1684	10422	14984	12050	0	1286	2608	1243	3356	1187	2415	7561	2832
Pensions	359	380	245	241	0	313	346	0	0	3692	2360	1038	716	540	719
Scholarships	16	0	19	10	0	9	0	0	0	0	0	19	5	0	9
Remittances	0	0	44	0	0	0	0	572	0	0	0	742	0	34	105
From MGNREGS	490	114	77	215	49	0	186	0	0	140	0	0	147	36	21
Others	98	190	236	265	0	0	0	0	0	7115	3297	4140	1045	649	683
Total	9577	13283	12772	13196	15489	14182	15820	14337	16515	41752	27875	29026	32674	21668	25813

Source: Field Survey, 2015-16

medium farm households, the per capita income is the highest (Rs. 59677) in Malwa and the lowest (Rs. 27493) in Majha. For large farm households, per capita income is the highest (Rs. 251921) in Malwa and the lowest (Rs. 78693) in Doaba. The per capita income for agricultural labour households is the highest (Rs. 13283) in Doaba and the lowest (Rs. 9577) in Malwa. Non-agricultural labour households earn the highest (Rs. 15489) per capita annually in Doaba and the lowest (Rs. 13196) in Malwa. For artisan households, the per capita income is the highest (Rs. 16515) in Majha, whereas the corresponding figures for Doaba and Malwa are Rs. 15820 and Rs. 14337, respectively. The per capita income of 'Other' households is the highest in Malwa (Rs. 41752) and the lowest (Rs. 27875) in Doaba for an average rural household.

Region-wise distribution of income: The household income of land owning and landless households in the different regions, inequality seems wider in land owning households than the landless households in the three regions (Table 4). The bottom 10 per cent land owning households share only 1.95 per cent of the total income earned by all the land owning households together, whereas the top 10 per cent households share 48.79 per cent of the income of all the land owning households in Malwa. This is 25.02 times the income shared by the bottom 10 per cent land owning households. However, the corresponding figures for Doaba are 2.34, 32.77 and Majha are 1.40, 35.29 per cent, respectively. The bottom 50 per cent land owning households account only for 17.42 per cent of the total income, whereas the top 50 per cent households account for 82.58 per cent of the total income earned by all the land owning households taken together in Malwa. Almost a similar pattern can be seen

in the other two regions. In all the three regions inequality among land owning households is higher in Malwa, as compared to Majha and Doaba. Around 50 per cent of the total land owning household income in Malwa is earned by the lower ninety per cent households, whereas the remaining half goes to the upper ten per cent households only. The corresponding figures for Doaba are 80 and 20 per cent, indicating that the lower 80 per cent land owning households earn 50 per cent of the total income earned by land owning households and the remaining 50 per cent income is earned by 20 per cent of land owning households. The corresponding figures for Majha are 80 and 20 per cent, respectively.

The bottom 10 per cent of the landless households claim 3.43 per cent of total landless household income in Malwa. However, the corresponding figures for Doaba and Majha stand at 2.83 and 5.39 per cent, respectively. On the other hand, the top 10 percent landless households appropriate 30.79, 10.46 and 13.59 per cent in Malwa, Doaba and Majha, respectively. Slightly higher than the one-fourth i.e., 27.14 per cent of the total household income is shared by the bottom 50 per cent of landless households in Malwa. The remaining around three-fourths i.e. 72.86 of the total household income is shared by the upper 50 per cent of the landless households in Malwa. The bottom 50 per cent of the landless households claim 39.90 per cent of total household income in Doaba. However, the corresponding figures for Majha stands at 40.20 per cent. On the other hand, the top 50 per cent households appropriate 72.14, 60.10 and 59.80 per cent for Malwa, Doaba and Majha, respectively. In all the three regions inequality among landless households is higher in Malwa, as compared to Doaba region and Majha.

Table 4. Region-wise distribution of income

Cumulative percentage of households	Cumulative percentage of household income								
	Land owning households			Landless households			All sampled households		
	Malwa	Doaba	Majha	Malwa	Doaba	Majha	Malwa	Doaba	Majha
10	1.95	2.34	1.40	3.43	2.83	5.39	2.08	2.09	3.49
20	4.52	8.33	3.79	8.24	11.90	12.73	5.24	5.00	8.77
30	8.07	12.70	7.43	13.99	19.35	21.29	8.67	8.50	14.56
40	12.02	20.77	12.06	20.30	31.39	30.43	12.85	12.26	20.92
50	17.42	27.00	18.01	27.14	39.90	40.20	17.79	17.02	27.64
60	22.75	33.32	25.91	34.59	48.97	51.50	24.00	22.98	34.85
70	29.61	43.27	34.03	43.50	63.45	62.40	31.39	30.25	43.76
80	38.97	50.54	45.15	54.61	73.84	74.09	42.08	40.09	54.26
90	51.21	67.23	64.71	69.21	89.54	86.41	56.34	53.44	67.88
100	100	100	100	100	100	100	100	100	100
Gini coefficient	0.53	0.37	0.48	0.35	0.14	0.13	0.5	0.52	0.35

Source: Field Survey, 2015-16

As many as 80 per cent of the total landless households earn 50 per cent of the total income earned by landless households in Malwa. The remaining 50 per cent of income is earned by only 20 per cent of all the landless households in Malwa. However, the corresponding figures for Doaba and Majha stand at 60 and 40 per cent, respectively. The bottom 10 per cent of all the rural households share only 2.08 per cent of the total income whereas the top 10 per cent households share 56.34 per cent in Malwa. The bottom 50 per cent of all the rural households account only for 17.79 per cent of the total income, whereas the top 50 per cent households account for 82.21 per cent of the total income in Malwa. Almost a similar pattern can be seen in Doaba. In Majha, the bottom 50 per cent of all rural households account only for 27.64 per cent of the total income, whereas the top 50 per cent households account for 72.36 per cent of the total income. The income concentration among the land owning households is greater than the landless households. The values of Gini coefficient also support this evidence. These values are 0.53, 0.48 and 0.37 for the land owning households in Malwa, Majha and Doaba respectively. A relatively better distribution of per household income is observed in the case of landless households. In their case, the concentration coefficients are 0.35 in Malwa, 0.14 in Doaba and 0.13 in Majha.

CONCLUSIONS

The present study, focusing on the inequality in income of the rural households across the different regions of Punjab, concludes that income concentration among small farm, medium farm, agricultural labour, non-agricultural labour and artisan households is greater than marginal farm, large farm and 'Other' households. A relatively better distribution of per household income is observed in the case of small farmers. Inequality seems wider in land owning households than the landless households in all the three regions. The main source of income in all the regions is farm business income, followed by income from non-farm wage employment. The other

components of income such as casual agricultural labour, government job salary and private job salary account for a very low proportion of the total income in all the regions, clearly indicating that rural households in all the regions supplement their income with different activities. Income generated from farm activities and subsidiary occupations is too small to satisfy farm household's needs. The income level of poor farm households can be raised by developing subsidiary occupations like dairying, poultry, fisheries, repairing activities and small trading. Income of labour households depends on the work they get, which they are not getting in sufficient numbers. Lack of gainful employment opportunities within the accessible areas is adding to the woes of such households. Equally important is the need to promote rural non-farm sector so that burden on the agricultural sector is reduced. Therefore, proper and effective implementation of government employment generation programmes is necessary to improve the condition of these poor rural households.

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Design and Development of Walking Type Multi-Crop Power Weeder

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Abstract: Designed walking type multi-crop power weeder consisted of 1.8 kW petrol engine, worm gear reduction box, spring steel cutting blades, flanges, rotor shaft, ground wheels, frame and handle. Provision was given to alter its working width in the range of 150 to 450 mm by attachable shafts to rotor shaft serving it to be useful in multiple crops. Rotor shaft diameter was calculated to be 18 mm by considering the maximum tangential force of 75.63 kg acting on it at maximum tangential speed of blade of 2.51 m/s at 200 rpm of rotor shaft. The operational speed observed with 6 blades per flange arrangement was faster in okra crop (0.39 m s^{-1}) than in chilli crop (0.38 m s^{-1}). The operational speed observed in maize crop with 6 blades per flange arrangement was slower (0.36 m s^{-1}) than 4 blades per flange arrangement (0.38 m s^{-1}). The field efficiency determined with 6 blades per flange arrangement was higher in okra crop (82.5%) than in chilli crop (80.7%). The field efficiency in maize crop determined with 6 blades per flange arrangement was lower (74.9%) than 4 blades per flange arrangement (77.6%). The 6 blades per flange performed better weeding operation than 4 blades per flange arrangement.

Keywords: Power weeder, Multi crop weeder, Cutting blades, Field efficiency, Operational speed, Weeding

In India, weeds are one of the major biological constraints that limit crop productivity as they compete with crops for water, light, nutrients and space. They can serve as hosts for diseases and provide shelter for insects and may also interfere with harvesting operation. Few weeds produce chemical substances that are toxic to crops, animals or people. Weeds decrease crop yields from 15 to 50 per cent depending on the species, density and weeding time through competition with main crop for light, water and nutrition (Hasanuzzaman et al 2009). Weeding is an important but equally labour intensive agricultural operation. One third of the cost of cultivation is spent on weeding alone when carried out with the manual labour. Manual weeding requires huge labour force and accounts for about 25 per cent of the total labour requirement i.e., 900-1200 man-hours/hectare (Yadav and Pund 2007). In India, weeding operation is mostly performed manually with the use of traditional hand tools in bending posture causing back pain for majority of labour. Manual weeding requires higher labour force and is also a very tedious and time-consuming operation. Farmers mainly follow the hand weeding though chemical weeding is slowly becoming popular but will have residual effect on the soil quality.

In Rajasthan, the percentage of small and marginal landholdings is 58.39 per cent of total land holdings (Anonymous 2015). The rotary power weeders are particularly aimed for this class of farmers as they are economical when compared with other weed management practices and power weeder stirs the soil accurately, strokes the weed roots and displaces them from soil. In addition, this aids in keeping the soil in loose condition for proper aeration.

The primary advantage of power weeder is that the power used for operation requires less draft which results in less wheel slip and enhanced field performance. The power weeders available are only for fixed row crop spacing and there is no provision for altering the working width of machine according to different crops varied row spacing. The cost of weeding by engine operated weeder is about one-third of weeding by manual labour (Tajuddin 2006). To design an effective and economic power weeder with higher weeding efficiency, it should be targeted for multiple crops. Therefore, there is a need to develop a weeder, which could be used for variable row crop spacing by designing attachable shafts with cutting blades and a suitable power transmission to accomplish the weeding operation.

MATERIAL AND METHODS

Structural design of walking type multi-crop power weeder: The main components of the power weeder such as rotor shaft and cutting blades were designed from strength considerations. For designing these components, data of depth of operation, soil resistance, rotational speed of blades and total tangential load sustained by rotor shaft information was required. Soil resistance is an important parameter to determine the power requirement of weeder. It gives a force per square centimeter required for cone penetrometer to move into soil. The readings of soil resistance were taken by cone penetrometer in the field during weeding stage of crop in sandy loam soil of CTAE Instructional Farm.

Power requirement: Speed of operation was assumed as 1.5 km h^{-1} as it is a walking type power weeder. Maximum working width of operation was decided as 450 mm based on

the cutting blade and flange dimensions. As the depth of root growth of weeds is not more than 50 mm, depth of operation was taken as 50 mm. The soil resistance of sandy loam soils was assumed as 0.15 N/mm² based on the experiment conducted.

The power required by the weeder to perform weeding operation (Sahay 2013);

$$P = \frac{S \times w \times d \times S_r}{1000} = \frac{1.5 \times 1000 \times 450 \times 50 \times 0.15}{1000} = 1.4 \text{ kW}$$

Where,

P= Power requirement, kW, S= Speed of operation, m/s, w= width of operation, mm d =depth of operation, mm, S_r= Soil resistance, N/mm²

Therefore, an engine of 1.8 kW power was selected as power source for working of power weeder from the different power ranges of engines available in the market.

Design of rotor shaft: To design rotor shaft, maximum tangential force which can be sustained by the rotor shaft was considered. Maximum tangential force occurs at minimum cutting blades tangential speed for available power and was calculated by following equation (Bernacki et al 1972).

$$K_s = \frac{75 C_s N_c M_c M_z}{2 \pi \pi N} = \frac{75 \times 1.5 \times 2.4 \times 0.9 \times 0.75}{2.51} = 72.51 \text{ kg}$$

Where,

K_s= Maximum tangential force sustained by the rotor shaft, kg

C_s= Reliability factor 1.5 for non-rocky soils and 2 for rocky soils

N_c= Power of engine = 2.4 hp

M_c= Traction efficiency value for forward rotation of the rotor shaft is 0.9,

M_z= Coefficient of reservation of engine power is in the range of 0.7-0.8,

N = Revolutions of rotor shaft = 200 rpm, R = Tangential distance of rotor = 120 mm

The maximum moment on the rotor shaft (M_s) is calculated by following equation (Mott 1985):

$$M_s = K_s \times R = 72.51 \times 120 = 8701.2 \text{ kg-mm}$$

Where,

R = Rotor radius = 120 mm

Rolled steel material was opted for rotor shaft as it withstands heavier loads. The rotor shaft made from rolled steel material had yield stress of 53.02 kg/mm². The allowable stress on rotor shaft (τ_{all}) was calculated using the following equation (Mott 1985).

$$\tau_{all} = \frac{0.577 k \sigma_y}{f} = \frac{0.577 \times 0.75 \times 53.02}{2} = 11.47 \text{ kg/mm}^2$$

Where,

k= Coefficient of stress concentration= 0.75, f=

Coefficient of safety = 2, σ_y= Yield Stress = 53.02 kg/mm²

The required diameter of the rotor shaft (d) can be calculated by equation (Mott 1985):

$$d = \sqrt[3]{\frac{16 M_s}{\pi \tau_{all}}} = \sqrt[3]{\frac{16 \times 8701.02}{\pi \times 11.47}} = 15.69 \text{ mm}$$

Hence, a rotor shaft of diameter 18 mm was chosen as the worm gear box selected had provision of 18 mm diameter hole to fit in it.

Design of cutting blades: The design of cutting blades of weeder has a considerable effect on the weeding operation to be performed as they are responsible for uprooting and displacing the weeds from soil. Cutting blades are the actual working elements interacting with the soil, so they were carefully designed from the knowledge obtained from study regarding strength, durability and economic aspects. There are different shapes of blades used in weeders such as Flat blade, C-shape, L-shape, J-shape etc. Of all the cutting blade shapes in use, L-shaped blades are the most primarily used cutting blades for fields with crop residue and had shown satisfactory results in removal of weeds when compared with C-shaped and J-shaped blades (Ahmad 2009). In power weeder, one-fourth of cutting blades act simultaneously on the soil. The total power of machine would be distributed between the cutting blades. The number of flanges that support the cutting blades are obtained by the following formula:

$$i = \frac{b}{b_i} = \frac{400}{100} = 4$$

Where, i = Number of flanges, b = Actual working width = 400 mm, b_i= Distance between the flanges = 100 mm

Therefore, 4 flanges were considered for fixing the cutting blades with both 6 blade and 4 blade arrangement as part of performance evaluation of weeder. The total power from the engine is disbursed to cutting blades through worm gear, rotor shaft and flanges to accomplish the weeding operation. Total soil force acting on each of the cutting blades (K_e) was calculated by using the following equation (Bernacki et al 1972):

$$K_e = \frac{4 K_s C_p}{i Z_c} = \frac{4 \times 72.51 \times 1.5}{4 \times 6} = 18.12 \text{ kg}$$

Where,

K_s= Maximum tangential force sustained by the rotor = 72.51 kg

C_p= Coefficient of the tangential force for non-rocky soils = 1.5

i = Numbers of flanges, Z_c= Number of cutting blades on the flange

Performance evaluation of machine: The performance evaluation of developed walking type multi-crop power weeder was conducted at Rajasthan College of Agriculture

(RCA), Maharana Pratap University of Agriculture and Technology (MPUAT), Udaipur. For 250 mm working width of machine, chilli and okra crops were chosen to test in which their row to row spacing was 300 mm. Similarly for 450 mm working width of machine, maize crop was selected in which row to row spacing was 900 mm. Three replications were taken with each blade arrangement of 4 and 6 cutting blades to flanges in chilli, okra and maize crops.

RESULTS AND DISCUSSION:

Working of weeder: The walking type multi-crop power weeder consists of an engine which is the primary power source and the engine output shaft revolutions are fed to the worm gear box. The worm gear box reduces the revolutions of engine to low rpm of rotor shaft to enable a tilling action for weeding operation. The rotor shaft is fixed with flanges and cutting blades. The weeding action is performed by the rotation imparted to cutting blades from the path of engine to cutting blades through worm gear, rotor shaft and flanges.

Soil resistance: Soil resistance increased with depth and it varied in the range of 11.2 to 15 N/cm². As the root growth of weeds is upto 5 cm depth, the soil resistance readings with digital cone penetrometer were taken upto 5 cm depth. The maximum soil resistance of 15 N/cm² was used for determining the engine power used for the machine.

Engine: Engine is the source of power input for the developed power weeder. As per the design calculations, 1.8 kW engine power was obtained to furnish the weeding operation. Therefore, a single cylinder, 2-stroke, air cooled petrol engine with 52 cc displacement and 3600 rpm was chosen to serve the purpose of weeding. Power unit contained various components like clutch, fuel system, ignition system, throttle lever and recoil starter.

Worm gear box: A light weight worm gear box was used to reduce the engine rpm to desired level of rpm of rotor shaft. The power coming from the engine was fed to worm gear reduction box which has rotor shaft fixed in it. The worm gear box chosen was reducing the engine rpm to rotor shaft in the speed ratio of 18:1 and its weight was 2.5 kg. The worm gear box reduces the revolutions of engine from 3600 rpm to 200

Table 1. Specifications of engine

Particulars	Specifications
Number of strokes	2 strokes
Number of cycles	2 cycle
Type of cooling system	Air-cooling system
Displacement (cc)	52
Maximum output (kW @ rpm)	1.8 @3600
Carburetor	Float-type
Method of start	Recoil type
Fuel type	Petrol mixed with lubrication oil (25:1)
Fuel tank capacity	1 litre
Weight	13 kg



Fig. 1. Engine



Fig. 2. Worm gear box fixed with rotor shaft

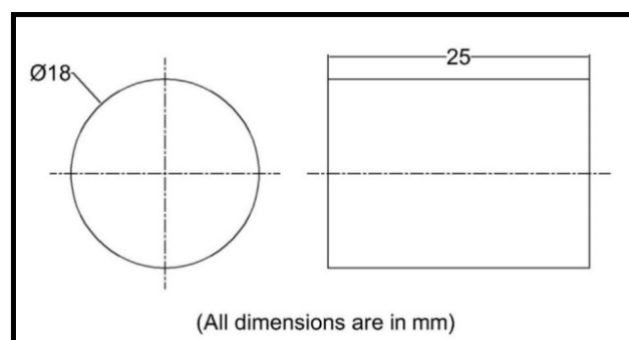
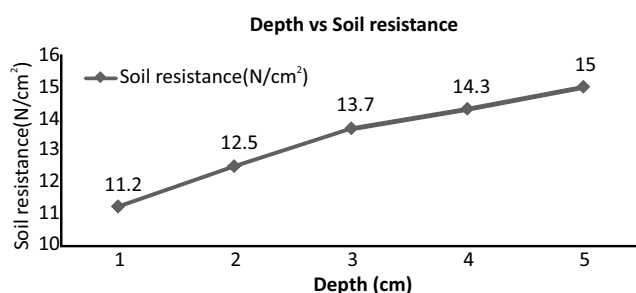


Fig. 3. Diagram of rotor shaft

rpm of rotor shaft to enable a tilling action for weeding operation.

Rotor shaft: The rotor shaft gets rotational motion from engine output shaft through worm gear. The soil working elements of flanges with L-shaped cutting blades are made to fix with rotor shaft by nuts and bolts. Rotor shaft was made from material of rolled steel. The dimensions of rotor shaft such as diameter and length of rotor shaft were 18 mm and 250 mm respectively.

Cutting blades: L-shaped cutting blades are most commonly used for fields with crop residue to remove weeds (Khodabakhshi et al 2013). The material chosen for cutting

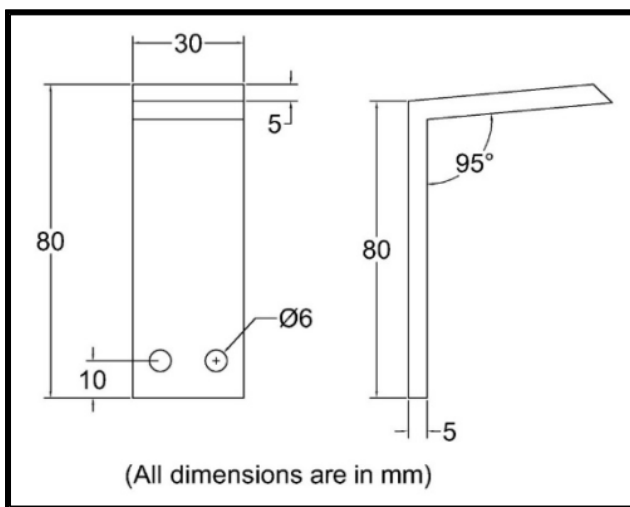


Fig. 4. Diagram of cutting blade



Fig. 5. Cutting blade



Fig. 6. Walking type multi-crop power weeder

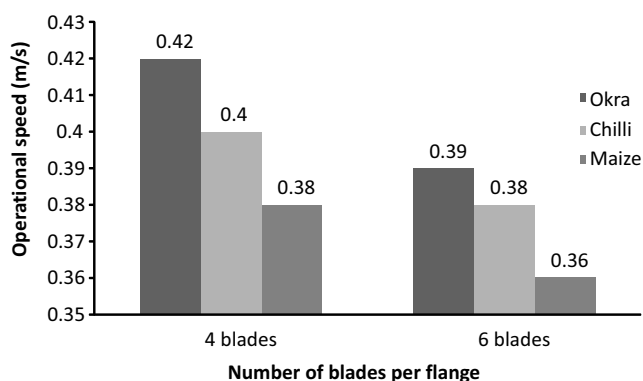
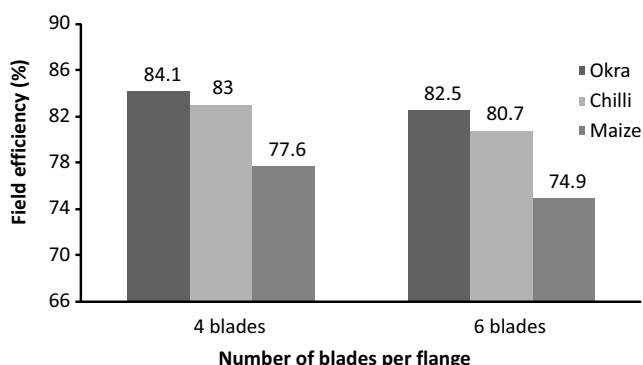
blades was spring steel as it was the hardest material available in the market with a hardness of 45 HRC fulfilling the requirement of Indian standard code. Forty spring steel flats of 130 mm length and 30 mm width were cut by hydraulic shearing machine and then they were marked for 50 mm length from one end to bend into L-shape form by the heat of gas cutting machine. A bend angle of 95° for L-blade was suggested as optimum for power weeder by Heman et al (2016). The L-shaped blades made were sharpened on edges of both sides to acquire a good cutting action. These blades were attached to the flanges mounted on the rotor shaft by nuts and bolts. Two cutting blade arrangements of 4 and 6 blades were used for analyzing the performance of the weeder.

Walking type multi-crop power weeder: The machine was designed for weeding in variable row spaced crops. Power requirement of weeder was mainly affected by soil resistance, working width, depth of weeding and operational speed. The width of cut and depth of weeding was assumed as 450 mm and 50 mm respectively as the depth of penetration of weed roots is not more than 50 mm into soil. Power requirement was determined to be 1.8 kW. The rotor shaft diameter was calculated to be 18 mm by considering the maximum tangential force of 72.51 kg acting on it at minimum tangential speed of blade of 2.51 m s^{-1} at 200 rpm of rotor shaft. There was a provision given to attach and detach the rotor shafts to enable a low working width of 150 mm and high working width of 450 mm.

L-shaped blades were the most efficient blade types in

Table 2. Specifications of cutting blade

Particulars	Specifications
Shape	'L' type
Width of cut	50 mm
Depth of cut	50 mm
Thickness of blade	5 mm
Width of blade	30 mm
No. of blades per flange	4 & 6
Total no of blades	40

**Fig. 7.** Effect of number of blades per flange on working speed**Fig. 8.** Effect of number of blades per flange on field efficiency

removing the weeds effectively as per the review. The total number of blades in 6 blades set was 24, while in 4 blades it was 16. Total soil force acting on each of the cutting blades was 18.12 kg.

Operational speed: The operational speed observed with 6 blades per flange arrangement was faster in okra crop (0.39 m s^{-1}) than in chilli crop (0.38 m s^{-1}). The operational speed observed with 4 blades per flange arrangement was faster in okra crop (0.42 m s^{-1}) than in chilli crop (0.4 m s^{-1}). The data presented above on operational speed revealed that slower working speed was obtained with 6 blades per flange arrangement in chilli crop as the soil had more moisture which created halts while faster working speed was obtained with 4 blades per flange arrangement in okra crop as it had

drier soil which did not allow a higher depth of cut aiding in faster forward speed.

The operational speed in maize crop with 6 blades per flange arrangement was slower (0.36 m s^{-1}) than 4 blades per flange arrangement (0.38 m s^{-1}). The data presented above on operational speed revealed that slower working speed was obtained with 6 blades per flange arrangement as the depth of cut was more than 4 blades per flange arrangement. Also the working speed in maize crop was slower than okra and chilli crops as the working width of machine was more in maize crop.

Field efficiency: The field efficiency determined with 6 blades per flange arrangement was higher in okra crop (82.5%) than in chilli crop (80.7%). The field efficiency determined with 4 blades per flange arrangement was higher in okra crop (84.1%) than in chilli crop (83%). The data presented suggested that field efficiency obtained with 4 blades per flange arrangement was higher than 6 blades per flange arrangement. The field efficiency in maize crop determined with 6 blades per flange arrangement was lower (74.9%) than 4 blades per flange arrangement (77.6%). The field efficiency in maize crop was lower than okra and chilli crops as the 450 mm working width machine was operated for two runs in the row spacing of 900 mm in maize crop.

CONCLUSION

The soil resistance greatly influenced power requirement of the machine for weeding operation. It was observed that with increase in depth of cut, operational speed of the machine decreased. Also, the operational speed of machine was influenced by number of blades per flange and field condition. The field efficiency of machine was affected by effective field capacity i.e., machine field coverage per unit time. The 6 blades per flange performed better weeding operation than 4 blades per flange arrangement. The developed walking type multi-crop power weeder performed weeding operation satisfactorily and can be recommended for operation in multiple crops with varied crop row spacing.

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