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Forest Ecosystem Soil Attributes Influence Density of *Pseudomonas fluorescens*

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Abstract**:** *Pseudomonas fluorescens* is a common, multi-flagellated, Gram-negative, rod-shaped bacteria, which are anti-phytopathogenic and plant growth promoting rhizo-bacteria. Study was conducted at the College of Forestry, Sirsi with the objective of measuring the bacterial copiousness in ten different forest and plantation ecosystem *i.e.,* evergreen, semi evergreen, moist deciduous, dry deciduous, myristica swamp, mangroves, scrub forest, teak, Acacia and Eucalyptus plantation; and correlated with different soil parameter *viz.,* soil p^H, electrical conductivity (EC) and soil moisture percentage (SMP). In each ecosystem plots were laid randomly; soil samples and site description were collected. *P. fluorescens* was isolated using Kings B agar as the selective media. Gram's reaction and morphological characterization were used to identify bacterial isolates. Myristica swamp had the highest 99,311.60 CFU/gm (4.997 Log (CFU/gm) bacterial abundance, followed by evergreen (4.937) and semi-evergreen (4.913). Myristica swamps with a p^H of 5.03 showed the highest levels of soil acidity, mangroves had the highest electrical conductivity (0.195 dSm^{-1}). The highest percentage of soil moisture was found in mangrove Forest (142.67%). Soil pH was negatively correlated with *P. fluorescens* abundance (r = -0.376) and soil electrical conductivity was positively correlated (r = 0.238). p^H and bacterial density were inversely correlated; EC, SMP and canopy density were directly related to bacterial density in sequentially sere ecosystems, *viz.,* Dry deciduous, Moist deciduous, Semi-evergreen, and Evergreen forests.

Keywords**:** *Pseudomonas fluorescens*, Density, Soil pH, Electrical conductivity, Soil moisture

Bacteria are common, largely free-living organisms that only have one biological cell. They make up a significant portion of the prokaryotic microbial world and are among the primordial life forms on earth and are typically a few micrometres in length. They can be found in most of its habitats. Gram-negative, rod-shaped *P. fluorescens* is a typical bacterium. *P. fluorescens* is a member of the *Pseudomonas* genus and has multiple flagella. It is abundant in soil and water. According to Deshwal et al (2003, 2011, 2013), plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonise plant roots and promote plant growth by producing a variety of plant growth hormones, P-solubilizing activity, nitrogen fixation, and biological activity. There are just a few strains of well-known PGPRs from genera including *Pseudomonas*, *Azospirillium, Azotobacter, Bacillus, Burkholderia, Enterobacter, Rhizobium, Erwinia,* and *Flavobacterium.* Some *P. fluorescens* strains, such CHA0 or Pf-5, exhibit biocontrol capabilities that shield some plant species roots against parasitic fungi like *Pythium* and *Fusarium* as well as some phytophagous nematodes.

P. fluorescence is a versatile microorganism with a major role in the environment. It is the potent biocontrol agent that protects the crop from various diseases caused by the pathogens (Ganeshan and Manoj 2005) and enhances the crop yield by facilitating the nutrient uptake and inducing systematic resistance (Vleesschauwer et al 2008) in the plants. This bacteria can degrade a wide range of organic pollutants, including hydrocarbons, pesticides, and polychlorinated biphenyls (Gutiérrez et al 2020). This ability makes them useful tools for cleaning up contaminated soil and water. The sensitivity of these bacteria to pollutant and environmental changes makes them good environmental health indicators (Nielsen and Winding 2002), and soil is a significant life support system; healthy soils are crucial for healthy development of the plant (Lehmann et al 2020). Several beneficial microorganisms ensure the soil's health and food security; *Pseudomonas* bacteria is one of them. Hence, the presence of this bacteria in the soil can be associated with the health of the soil. Absence or low abundance of these *P. fluorescences* is associated with degradation of soil health.

MATERIAL AND METHODS

Soil Sample was collected from Evergreen, Semi evergreen, Moist deciduous, Dry deciduous, Myristica swamp, Mangroves, Scrub Forest, Teak, Acacia, and Eucalyptus plantation ecosystems of Karnataka. In each forest ecosystem 3 plots (30m×30m) laid randomly in each ecosystem. Soil sampling was carried out (Parewa et al 2016) and plot descriptions such as canopy density, elevation, litter depth were recorded. Location description of sample collection site was depicted in Table 1. Lab was

Table 1. Descriptive field data of soil sampling plots (* Water depth in mangrove ecosystem)

Forest type	Sample tag	Area	Latitude and longitude	Canopy density (%)	Litter depth (cm)
Evergreen	EGP1	Gerusoppa Range, Honnavara Division, Canara Circle	14° 16' 42.83"N 74° 42' 55.44"E	85.05	1.8
	EGP2	Gerusoppa Range, Honnavara Division Canara Circle	14° 16' 52.71"N 74° 42' 53.42"E	86.5	2
	EGP3	Gerusoppa Range, Honnavara Division Canara Circle	14° 16' 54.55"N 74° 42' 49.81"E	84	2.2
Semi evergreen	SEGP1	Koppa Range, Koppa Division Chikkamagaluru Circle	13° 32' 05.22"N 75° 24' 31.52"E	83.5	1.8
	SEGP2	Siddapura Range, Sirsi Division Canara Circle	14° 16' 01.26"N 74° 48' 44.52"E	84.5	2.2
	SEGP3	Siddapura Range, Sirsi Division Canara Circle	14° 15' 36.82"N 74° 48' 30.04"E	85.5	1.4
Moist deciduous	MDP1	Banavasi Range, Sirsi Division Canara Circle	14° 34' 04.39"N 74° 56' 49.69"E	87.15	2.6
	MDP2	Banavasi Range, Sirsi Division Canara Circle	14° 39' 57.39"N 74° 52' 34.70"E	77.5	1.9
	MDP3	Banavasi Range, Sirsi Division. Canara Circle	14° 42' 19.38"N 74° 56' 51.91"E	84	1.6
Dry deciduous	DDP1	Katur Range, Yellapura Division Canara Circle	14° 45' 56.60"N 75° 01' 18.33"E	75.75	2.5
	DDP2	Katur Range, Yellapura Division Canara Circle	14° 49' 06.01"N 75° 02' 11.44"E	72.5	1.4
	DDP3	Mundgod Range, Yellapura Division Canara Circle	14° 53' 06.65"N 75° 01' 58.36"E	76.25	2.3
Myristica swamp	MYS1	Siddapura Range, Sirsi Division Canara Circle	14° 16' 26.60"N 74° 44' 50.66"E	88.5	2.8
	MYS2	Siddapura Range, Sirsi Division Canara Circle	14° 16' 21.81"N 74° 44' 40.88"E	83.75	3.8
	MYS3	Siddapura Range, Sirsi Division Canara Circle	14° 16' 23.75"N 74° 44' 43.91"E	85.25	3.2
Mangroves	MGP1	Honnavara Range, Honnavara Division Canara Circle	14° 15' 44.12"N 74° 26' 23.71"E	83	8.2*
	MGP2	Kumta Range, Honnavara Division Canara Circle	14° 25' 00.87"N 74° 24' 26.08"E	73	1.25*
	MGP3	Kumta Range, Honnavara Division Canara Circle	14° 27' 47.51"N 74° 23' 22.47"E	69	0.50*
Scrub	SFP1	Sirsi Range, Sirsi Division Canara Circle	14° 35' 44.46"N 74° 50' 38.83"E	0	0
	SFP2	Sirsi Range, Sirsi Division Canara Circle	14° 36' 46.22"N 74° 50' 42.80"E	0	0
	SFP3	Sirsi Range, Sirsi Division Canara Circle	14° 35' 50.50"N 74° 51' 03.93"E	19	0
Teak plantation	TPP1	Katur Range, Yellapura Division Canara Circle	14° 52' 38.59"N 75° 01' 57.09"E	72.5	4
	TPP2	Katur Range, Yellapura Division Canara Circle	14° 52' 28.78"N 75° 02' 03.91"E	77.25	2.4
	TPP3	Katur Range, Yellapura Division Canara Circle	14° 15' 22.72"N 75° 02' 11.95"E	71	0
Acacia plantation	APP1	Sirsi Range Sirsi Division Canara Circle	14° 35' 49.09"N 74° 50' 45.78"E	26.25	1.9
	APP2	Sirsi Range Sirsi Division Canara Circle	14° 35' 53.52"N 74° 50' 58.23"E	53.75	2.8
	APP3	Sirsi Range Sirsi Division Canara Circle	14° 35' 47.10"N 74° 50' 51.60"E	64	3.5
Eucalyptus plantation	NPP1	N R Pura Range, Koppa Division Chikkamagaluru Circle	13° 33' 58.00"N 75° 27' 09.00"E	62.5	2.3
	NPP2	N R Pura Range, Koppa Division Chikkamagaluru Circle	13° 34' 21.00"N 75° 27' 22.20"E	58.25	1.7
	NPP3	N R Pura Range Koppa Division Chikkamagaluru Circle	13° 34' 12.58"N 75° 27' 27.38"E	53.5	2.5

disinfected with 4 percent formalin solution at 50^{0}C**.** 42.23 grams of Kings B media (readymade dehydrated media) was mixed with 1000 ml of distilled water and heated to boil. All the glassware and media needed for plating sterilized with autoclave in 121^{0}C at 15 psi pressure.

Preparation of Soil dilutions and spread plates for bacterial culture: 10 g of soil sample added to conical flask containing 90 ml of distilled water. Suspension stirred well and labelled as A. Six 9ml water blank was prepared, before the soil settles, 1 ml of the suspension was removed with a sterile pipette from suspension A and transferred it to a 9-ml distilled water blank. Shaken it well and given label as "B". This dilution was repeated five times, each time with 1 ml of the previous suspension and 9-ml distilled water blank. These Labelled sequentially as tubes C, D, E and F. This results in serial dilutions of 10^{-1} through 10^{-5} grams of soil per ml (Deshwal and Punkaj Kumar 2013) liquefied Kings B media (KBM) poured into petri plate. 0.1 ml of a serial diluted suspension solution pipetted out and spreaded on the petri plate from 10^{-3},$10^{-4}$and 10^{-5} dilution for each ecosystem plot soil and petri plate were sealed with cling film. All these operations carried out inside the laminar air flow under sterile condition. Culture plates were Incubated in biochemical oxygen demand (BOD) incubator for 48 - 72 hours at 28 ±2°C colonies developed in the culture plate were enumerated with digital colony counter through morphological characterization. For Gram's staining clean, grease free slide was taken. Smear of suspension prepared on the clean slide with a loopful of sample and air drying, heat fixing was carried out, Gram's crystal violet was poured and kept for about 2 minutes and rinsed with water. Gram's iodine flooded for 1 minute and washed with water. Then, washed with Gram's decolourizer for about 10-20 seconds and rinsed the slide with water. Safranin, 0.5% w/v was added after 1 minute and washed with water. Air dried and observed under microscope.

Determination of soil pH, EC and Soil moisture percentage: To determine soil p^H and Electrical conductivity (EC) 20gm of soil weighted in a clean 100ml beaker and 50ml of distilled water was added. Suspension was stirred intermittently for 30 min. p^H recorded using p^H meter. Suspension allowed to settle for an hour, EC was measured in the supernatant solution by using EC Bridge. Soil moisture content estimated by using gravimetric method.

CFU per g of soil

$$\frac{\text{No of colonies}}{\text{Wg of soil sample}} \times \text{Dilution factor} \times \frac{1}{\text{Aliquot}}$$

Soil moisture percentage

$$\text{Soil moisture (\%)} = \frac{\text{Wg of wet soil} - \text{Wg of dry soil}}{\text{Wg of dry soil}}$$

RESULTS AND DISCUSSION

Variation of canopy density (Fig. 1) and litter depth (Fig. 2) across different study plots shows wide fluctuation. The canopy density varied from zero to 88.5 per cent where in Scrub land (SFP1 and SFP2) records zero canopy density and Myristica swamps records highest canopy density (88.5 %). The litter depth varied from zero to 8.2 cm wherein Scrub land (SFP1, SFP2 and SFP3) records zero litter depth and mangroves (MGP1) records highest litter depth of 8.2 cm.

The variation of *P. fluorescence* bacterial density in ten different forest and plantation ecosystems and its relation with soil pH, electrical conductivity and moisture percentage is presented in Table 2. The highest abundance of *P. fluorescens* observed in Myristica Swamp (99,311.60 CFU/gm and 4.997 Log CFU/gm) followed by Evergreen, Semi-evergreen, Moist deciduous, Dry deciduous, Eucalyptus plantation, Mangroves, Acacia plantation, Scrub Forest and Lowest was observed in Teak plantation (5,128.61 CFU/gm and 3.710 Log CFU/gm) (Fig. 3). Highest soil p^H recorded in Mangroves (6.497) and lowest was in Myristica Swamp (5.030) (Fig. 4). Highest electrical conductivity was in Mangroves (0.195dSm^{-1}) and lowest was observed in Scrub Forest (0.044dSm^{-1}) along with Teak plantation (0.044dSm^{-1}) (Fig. 5). Maximum soil moisture percentage was observed in Mangroves (142.673%) and lowest was observed in Scrub Forest (Fig. 6).

Study revealed that Myristica swamp ecosystem having lowest p^H(5.030) with highest bacterial abundance 4.667 Log CFU/gm of soil and Mangrove Forest having Highest p^H comprising bacterial abundance 4.047 Log CFU/gm. Soil p^H was negatively correlated (Rousk et al 2009) with *P. fluorescens* bacterial abundance (r = -0.376) between the P^h range of 5.030 to 6.497 and 14.13% of variation in bacterial density is due to variation in soil p^H (Coefficient of Determination r^2 = 0.141) and the correlation was statistically significant. Mangrove Forest having Highest EC (0.195dSm^{-1}) comprising bacterial abundance 4.047 Log CFU/gm. Scrub and teak plantation having lowest soil EC (0.044 dSm^{-1}) with bacterial abundance 3.710 and 3.913 log CFU/gm respectively. Soil electrical conductivity was positively

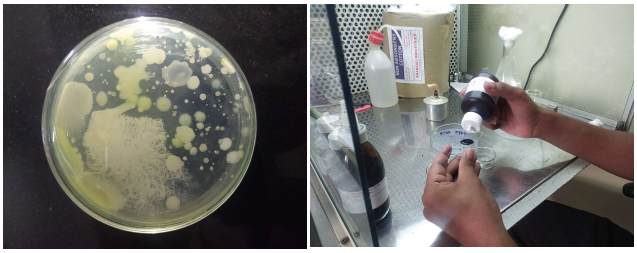

Plate 1. *Pseudomonas fluorescence* bacterial growth in KBM culture plates and Gram's staining

correlated with bacterial abundance (r = 0.238) with 5.66% of variation in bacterial density is due to variation in soil electrical conductivity (r^2 = 0.0566) and the correlation was statistically significant. Mangrove ecosystem having highest 142.67 SMP with bacterial abundance 4.047 Log CFU/gm and scrub forest contains lowest 15.22 SMP with lowest bacterial abundance 3.933 Log CFU/gm. In the monocultured plantations have lower bacterial population than in natural forest (Liu et al 2018) even its established in midst of natural forest. In sequential sere ecosystem *i.e.,* Dry

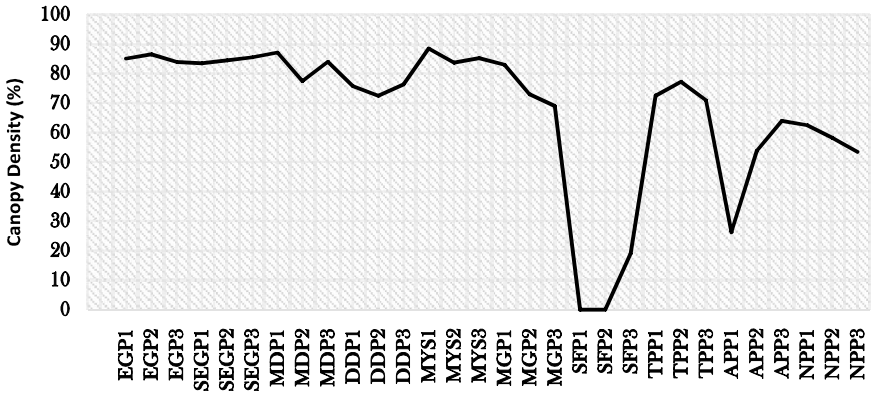

Fig. 1. Variation of canopy density across different sample plots

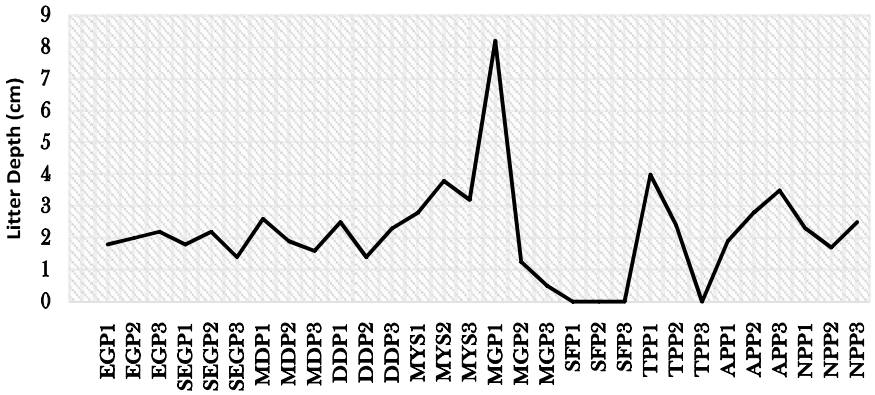

Fig. 2. Variation of Litter depth across different sample plot (Water depth in mangrove ecosystem *i.e.,* MGP1, MGP2 and MGP3)

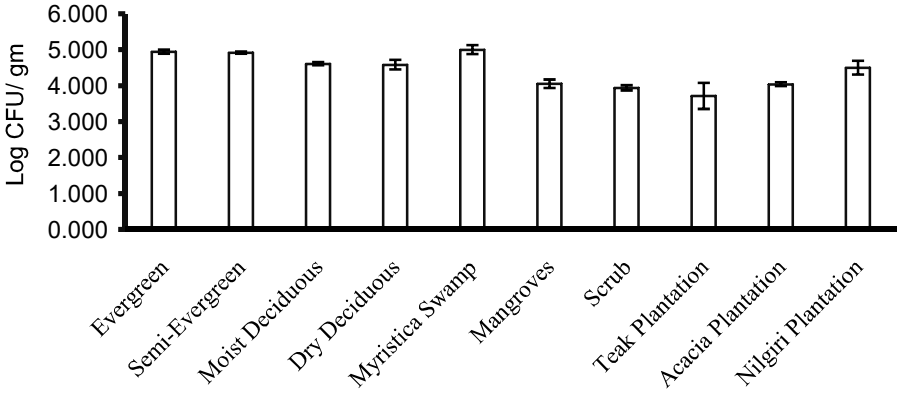

Fig. 3. Variation in mean bacterial density across different forest and plantation ecosystem (Error bars indicate 5% Standard error)

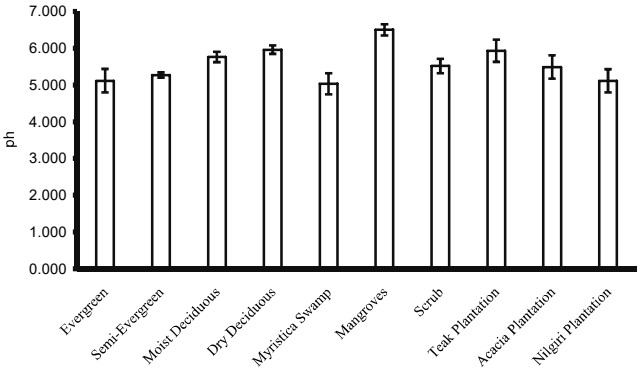

Fig. 4. Variation in mean soil pH across different forest and plantation ecosystem (Error bars indicate 5% Standard error)

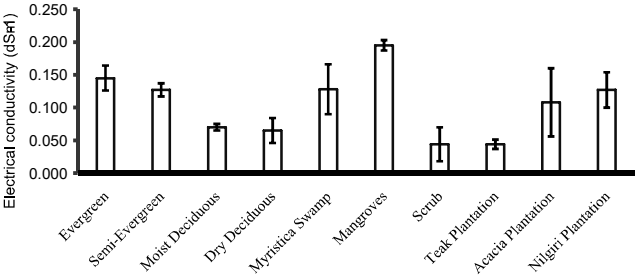

Fig. 5. Variation in mean soil electrical conductivity (dSm^{-1}) across different forest and plantation ecosystem (Error bars indicate 5% Standard error)

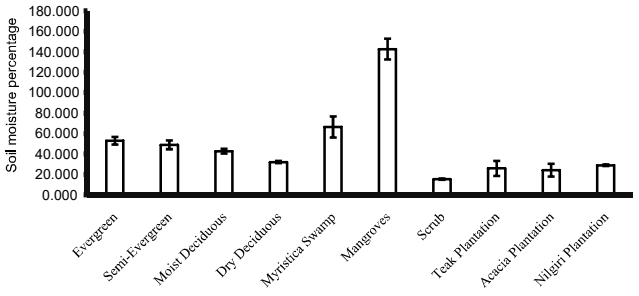

Fig. 6. Variation in mean soil moisture percentage across different forest and plantation ecosystem (Error bars indicate 5% Standard error)

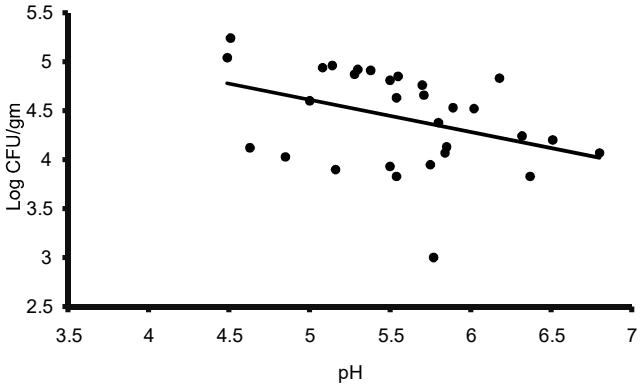

Fig. 7. Variation in bacterial abundance (Log CFU/gm) across different forest soil pH($r = -0.376, r^2 = 0.141, p = < 0.01$)

Table 2. Variation of *P. fluorescence* bacterial density across different forest ecosystems with soil pH, electrical conductivity and moisture percentage

Ecosystem	Champion and Seth Forest Classification (Khanna 2015)	CFU/ gm	Log (CFU/ gm)	p^H	EC (dS/m)	Moisture percentage (%)
Evergreen	Southern Tropical Wet Evergreen Forest (1A)	86,496.79	4.937	5.113	0.145	52.883
Semi-Evergreen	Southern Tropical Semi-Evergreen Forest (2A)	81,846.48	4.913	5.267	0.127	48.937
Moist deciduous	Southern Tropical Moist Deciduous Forest (3A)	40,086.67	4.603	5.757	0.070	42.657
Dry deciduous	Southern Tropical Dry Deciduous Forest (5A)	38,018.94	4.580	5.957	0.065	32.107
Myristica Swamp	Myristica Swamp Forest ($4C/FS_1$)	99,311.60	4.997	5.030	0.128	66.320
Mangroves	Mangrove Forest ($4C/TS_2$)	11,142.95	4.047	6.497	0.195	142.673
Scrub	Southern Tropical thorn Forest (6A)	8,570.38	3.933	5.513	0.044	15.223
Teak plantation	-	5,128.61	3.710	5.927	0.044	25.770
Acacia plantation	-	10,889.30	4.037	5.483	0.108	24.157
Eucalyptus plantation	-	31,117.16	4.493	5.110	0.127	29.040
Mean (±SE)	-	-	4.425 ± 0.152	5.565 ± 0.24	0.105 ± 0.026	47.9767 ± 5.842
C.V.	-	-	5.94	7.463	42.053	21.09
C.D	-	-	0.451	0.712	0.076	17.355
SE(d)	-	-	0.215	0.339	0.036	8.261

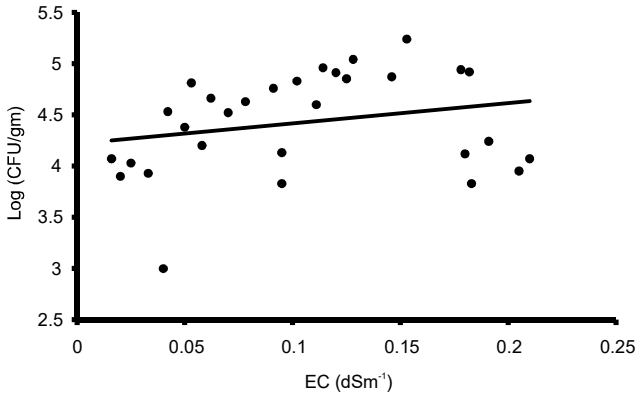

Fig. 8. Variation in bacterial abundance (Log CFU/gm) across different forest Soil electrical conductivity (dSm^{-1}) ($r = 0.238$, $r^2 = 0.0566$, $p = < 0.01$)

deciduous, Moist deciduous, Semi-evergreen and Evergreen Forest bacterial density inversely related with pH and directly related with Electrical conductivity, soil moisture percentage and canopy density. These soil properties are known to influence soil flora directly or indirectly, fauna, tree species (Rodrigues et al 2018) abundance and diversity in respective niche and ecosystems.

There are billions of soil microorganisms resides in soil. Biodegradation of pollutants, maintenance of soil structure, and circulation of biogenic elements which makes nutrients available to plants are all services supported by bacteria's (Furtak and Gajda 2018). Some are even PGPR as well as anti-phyto-pathogenic in nature. Loss of soil biodiversity is major problem due to excessive use of inorganic fertilizers, weedicides, fungicides *etc.,* (Bishtand Chauhan 2020). As the forest soils are having least anthropogenic intrusion when compared to agriculture and industrial soils. Based on studies on beneficial microbial abundance in these ecosystems minimum typical benchmark bacterial abundance can be determined and which can be retained as yardstick to measure the microbial abundance to decide health of soil. The presence of microorganisms in soil depends on their chemical composition, moisture, p^H, and structure. Many factors *viz.,* chemicals secretion, secondary metabolites (litter), decomposition, insolation *etc.,* might have influenced on bacterial density and p^H (Furtak and Gajda 2018).

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CONCLUSION

Conservation of soil health is major concern of the century and microorganisms are integral part of soil many beneficial microorganisms ensure the soil health and food security. *P. fluorescens* is one such among beneficial bacteria. *P. fluorescens* bacterial profusion varies from 99,311.60 to 5,128.61 Log CFU/gm of different forest soils. Based on present study minimum representative bacterial population can be determined which can be retained as standard to measure soil biological health status of soil.

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