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Biological Activity and Distribution of Microbes as Influenced by Salinity in Coastal Rice Growing Soils of Guntur District, Andhra Pradesh

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Abstract: Research was conducted to study the biological activity and and isolate the dominant fungi and bacteria in coastal saline soils. Soil samples having different salinity levels *viz.* 0.60, 1.95, 3.59, 5.50, 7.50 and 9.35 dS m^{-1} were collected from coastal soils of Guntur district, AP. The soil enzymatic activity (dehydrogenase and phosphatase) and microbial population (fungi, bacteria, actinomycetes) were assayed by following standard procedures. The dominant fungal and bacterial isolates were identified using standard procedures. The data were analyzed statically. Biological assay of soil samples collected at different salinity levels revealed that the soil enzymatic activity and microbial population were significantly influenced by soil salinity. The soil biological activity was the highest at the low levels of salinity and vice-versa. A total of seven fungal and 18 bacterial isolates were observed in the coastal rice growing soils of Guntur district, AP. This study clearly indicated that the soil salinity significantly influenced the number and distribution of microbial population and enzyme activity.

Keywords: Enzyme activity, Isolates, Microbial populations, Bacteria, Fungi, Actinomycetes, Soil salinity, Biological activity

Andhra Pradesh has a vast stretch of 974 km coast line covering nine districts of the state. The major cropping systems of the coastal system are rice-rice, rice-pulse and rice-maize/jowar. Soil salinity is a major problem especially in the rice growing soils which are mostly subjected to irrigation, leading to secondary salinization affecting 20 per cent of irrigated land (Glick et al 2007). Soil salinization is a process of accumulation of excess salts comprising of chloride and sulphate ions. Soil salinity, a concern of the natural ecosystem in arid and semi-arid regions where, precipitation is insufficient to leach ions from the soil profile, is increasing day by day in agricultural soils (Shrivastava and Kumar 2015). Sometimes migration of salts upward in the soil from shallow groundwater or overuse of fertilizers can also be the contributors for soil salinity. In Andhra Pradesh, 7.48 per cent of coastal soils are affected by salinity of which 0.42 per cent is confined to Guntur district (Mandal et al 2018). Global soil salinization was estimated to continue spreading at a rate of up to 2 Mha yr^{-1} (Abbas et al 2013). Salinization is recognized as the main threat to environmental resources and human health in many countries which have resulted in serious consequences to global natural resources and diminished microbial activity/diversity (Patel et al 2011). Salinization effects in a long-term scenario, the biodiversity (Church et al 2013) and normal functioning of the soil ecosystems by affecting soil organisms, which participate in fundamental ecological processes like organic matter decomposition, nutrient cycling and maintenance of soil structure (Lavelle et al 2006). Soil microorganisms constitute less than 0.5 per cent (w/w) of the soil mass, but they play a key role in soil processes (Yan et al 2015), mediate numerous chemical reactions involved in soil nutrient cycling; transformation of plant and microbe debris; mineralization and transformation of organic matter within the carbon cycle, transformation and degradation of potentially hazardous pollutants etc., thus contributing to the restoration and remediation of polluted soils. Due to salinity, the existence of high osmotic pressure and ion toxicity in rice growing soils of coastal regions are serious constraints to many organisms (Rietz and Haynes, 2003).

Dehydrogenase activity is a good indicator of soil microbial activity, as this enzyme group occurs only within the living cells, unlike other enzymes which can occur in extracellular state (Kumar et al 2013) and play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to accepters. Phosphorus (P) in soil is mainly in the organic form. Phosphatase enzymes (alkaline and acid) catalase the

hydrolysis of both esters and anhydrides of phosphoric acid, increasing the availability of phosphorus in soils (Wang et al 2021). However, but as salinity increased, the metabolic quotient (respiration per unit biomass) is increased. The sensitivity of soil enzyme activities to salinity varies; Wang (2013) reported that the activities of dehydrogenase and phosphatase are strongly inhibited by salinity while, Rietz and Haynes (2003) stated that soil respiration is not significantly correlated with salinity. Isolation of different bacteria and fungi in saline environment provides us the knowledge of their dominance or diversity under high salt conditions. The isolates with high salt tolerance capacity are very much useful for biotechnological applications in terms of bio-remediation and bio-mineralization (Das et al 2011). Keeping this in view the study was carried out to find the soil enzymatic activity (dehydrogenase, phosphatase), microbial population (fungi, bacteria, actinomycetes) and to identify dominant micro-organisms (fungi and bacteria) in the coastal rice growing soils of Guntur district, Andhra Pradesh.

MATERIAL AND METHODS

Soil samples (60) up to a depth of 25 cm collected from rice growing areas representing the coastal region of Guntur district were processed and analyzed for soil electrical conductivity (1: 2.5 soil: water) (Jackson 1973). Based on the soil conductivity, a total of six salinity levels of approximately 0.60, 1.95, 3.59, 5.50, 7.50 and 9.35 were considered and for each level four samples were collected. Hence, a total of 24 fresh soil samples were collected and assayed for biological activity. The moisture content of the soils was estimated by gravimetric method (Gupta 2009) and the biological activity was expressed on dry weight basis**.** The method described by Casida et al (1964) was used to determine dehydrogenase activity whereas; phosphatase activity was estimated using the protocol given by Tabatabai and Bremner (1969). Enumeration of microbial population for bacteria, fungi and actinomycetes was carried out as per the procedures outlined by Paroda (2007) in fresh soil samples by following serial dilution plate count technique (Dhingra and Sinclair 1985) and spread plate method using nutrient agar for bacteria, Martins Rose Bengal Agar for fungi and Ken-Knight and Munaier's medium for actinomycetes and expressed on dry basis as colony forming units (CFU) g^{-1} soil. The data obtained were analysed statistically using completely randomized design. The fungal colonies obtained during enumeration were categorized based on colony morphology and further isolated, purified and preserved for studying their morphological features and to identify up to genus level. The fungal isolates were observed under microscope following wet mount method to distinguish characteristic shape and spore arrangement (Bartholomew and Mittwar 1950). Identification and characterization of bacterial isolates was done by recording morphological features microscopically (Claus 1992) and biochemical tests such as catalase test, Voges-Proskauer test, methyl red test, indole production test, citrate utilization test, starch hydrolysis, acid and gas production from glucose broth and comparing these tests with Bergey's Manual of Determinative Bacteriology.

RESULTS AND DISCUSSION

Enzyme activity: Enzymatic activity was maximum at the lowest electrical conductivity of 0.60 dS m^{-1} and decreased with increasing salinity (Table 1). The decrease in dehydrogenase activity was 65.95 per cent with increase in salinity from 0.60 to 9.35 dS m^{-1}. The dehydrogenase activity at salinity level 7.50 and 9.35 dS m^{-1} were at par with each other while, other levels were significantly differing among one another. Similarly, phosphatase enzyme activity (acid and alkaline) vary significantly with different salinity levels. The maximum acid (161 µg PNP g^{-1} $soil^{-1}$ hr^{-1}) and alkaline (275 µg PNP g^{-1} $soil^{-1}$ hr^{-1}) phosphatase activities were at the lowest EC (0.60 dS m^{-1}). The decline in activity of acid and alkaline phosphatase enzymes was 59.00 and 71.63 per cent, respectively with increase in conductivity from 0.60 to 9.35 dS m^{-1}. The enzymes generally originate from microorganisms and the decrease in microbial population reduces enzymatic activity. Yan et al (2015) reported that increase in soil salinity reduced the soil respiration, which ultimately reduced the enzyme activity. The decline in enzyme activities might also be due to the change in osmotic potential of soil–water phase. The salting-out effect, which modifies the ionic conformation of the protein-enzyme active site and specific ionic toxicity cause a nutritional imbalance

Table 1. Soil enzymes activity at different levels of electrical conductivity in soils of Khajipalem revenue village

Electrical conductivity (dS m^{-1})	Enzyme Activity		
	Dehydrogenase (µg TPF g^{-1} soil day^{-1})	Acid Phosphatase (µg PNP g^{-1} soil hr^{-1})	Alkaline Phosphatase (µg PNP g^{-1} soil hr^{-1})
0.60	139.50	161	275
1.95	97.50	150	220
3.59	72.50	113	145
5.50	65.00	103	116
7.50	50.00	74	86
9.35	47.50	66	78
CD @ 0.05	4.40	5.10	6.91
CV (%)	3.77	3.09	3.04

for microbial growth and subsequent enzyme synthesis (Silva and Fay 2012). The osmotic desiccation of microbial cells and limitation of carbonaceous substrates also contribute to the reduction in enzyme activity (Siddikee et al 2011).

Generally, enzyme activity is low in summer due to lesser enzyme secretion by the surviving soil microorganisms as most of the microorganisms must have died as a result of rise in salt concentration in soil (Tripathi et al 2007). The higher amount of alkaline phosphatase than acid phosphatase activity might be due to predominance of neutral or alkaline soils in the study area. Similar observations were also reported by Kirankumar and Lakshmi (2015) in paddy growing areas of West Godavari district; Purvi et al (2016) in coastal ecosystems of Gujarat; Laxminayarana and Naik (2016) in the coastal saline soils of Orissa. Tripathi et al (2007) attributed the decrease in enzyme activity with soil salinity in coastal soils of West Bengal to the prevailing semi-arid conditions wherein, many of the enzymes are extracellular and form complexes with the organics and mineral colloids.

Microbial populations: The data pertaining to population of fungi, bacteria and actinomycetes of the coastal rice growing soils at different electrical conductivity levels indicated a significant effect of salinity on soil microbial population (Table 2). The population of all groups was maximum at the lowest EC level of 0.60 dS m^{-1} and decreased with increasing EC level up to the tested highest EC of 9.35 dS m^{-1}. The maximum fungi, bacteria and actinomycetes population of 18.00 x 10^3, 67.50 x 10^5 and 100.25 x 10^3 CFU g^{-1} soil, respectively, was at the lowest EC level (0.60 dS m^{-1}) whereas, minimum was observed at 9.35 dS m^{-1}. A significant reduction in microbial population was observed with increment in salinity level. The decrease in population with change in salinity from 0.60 to 9.35 dS m^{-1} was to a tune of 81.94 in fungi, while bacteria and actinomycetes declined up to 94.07 and 96.00 per cent, respectively. Bacterial population at salinity levels 1.95 and 3.59 were comparable with each other as there was no significant difference. The study indicated that salinity has a negative effect on soil organisms and this might be due to the low osmotic potential of the soil solution and ion toxicity or imbalanced ion uptake. The osmotic potential of soil water as a result of salinity removes water from microbial cells through plasmolysis, which leads to death of micro-organisms (Ibekwe et al 2010). Among different microbial populations, actinomycetes were more sensitive to salinity followed by bacteria and fungi. The reason for decline in actinomycetes population is the inability to release cell wall deficient (CWD) cells, which on prolonged exposure converted to L- forms, an adaptation strategy in actinomycetes (Ramijan et al 2018). The results were similar to Adilakshmi et al. (2018) where she reported biological activity reduced at high salinity (12 dS m^{-1}) when compared to low salinity level (1.5 dS m^{-1}). Presence of microbial population at higher salinity level indicates the adaption to low osmotic potential by accumulating osmolytes in the cell to counteract the increase in osmotic pressure and production of organic compounds which antagonize the concentration gradient between the soil solution and the cell cytoplasm (Hagemann 2011). Sensitive microbial cells are damaged by the low osmotic potential as soon as exposed to salinity while, some microorganisms get adapted by accumulating osmolytes that help to retain water. Synthesis of osmolytes requires large amounts of energy resulting in reduced proliferation of microorganisms (Yan et al 2015).

Table 2. Microbial populations at different levels of electrical conductivity in soils of Khajipalem revenue village

Electrical conductivity (dS m^{-1})	Microbial population (CFU g^{-1} soil)		
	Fungi (x 10^3)	Bacteria (x 10^5)	Actinomycetes (x 10^3)
0.60	18.00	67.50	100.25
1.95	14.50	54.50	57.00
3.59	10.75	52.75	41.75
5.50	8.50	22.50	36.50
7.50	7.50	14.00	15.75
9.35	3.25	4.00	4.00
CD @ 0.05	0.74	2.57	2.74
CV (%)	4.80	4.82	4.34

The high salt concentrations in saline soils have high bio-energetic taxation, since microorganisms need to maintain osmotic equilibrium between the cytoplasm and the surrounding medium, excluding sodium ions from inside the cell. However, the energy required for osmo-adaptation is lacking due to salinity stress (Silva and Fay 2012). Hence increased salinity becomes detrimental to the microbial community. Laxminarayan and Naik (2016) reported that increased concentration of soluble salts diminishes the multiplication of microbes in coastal saline soils of Eastern India and the same had been revealed in the present study where in the soil micro-fauna showed negative relationship with salinity. Similar reduction in soil population with increasing salinity was also observed by Jasmine et al (2020) in saline soils of Uppugunduru region of Andhra Pradesh.

Characterization and Distribution of the Microbial Isolates (Fungi and Bacteria)

Fungi: The colonies of isolates 1, 3, 4, 5 and 6 are circular while 2 and 7 were dendroid and irregular in shape, respectively (Table 3, Fig. 1). The colony margins of different

isolates were entire (isolates 1, 3, 5 and 6), filiform (isolates 2 and 4) and undulate (isolate 7). All the isolates were flat in elevation except 2 and 6, which have raised and crateriform elevation, respectively. Colour varied among different isolates. Isolate 1 was black in colour, while isolate 2 was yellow coloured. White colour was observed in the isolates 3, 4 and 5 whereas, isolates 6 and 7 were orange and olivaceous green in colour, respectively. The distribution of different species varied with soil salinity (Table 4). The genera *Cladosporium, Aspergillus, Pencillium, Fusarium* and *Mucor* among fungi were dominant in the study area. There was a decrease in population abundance as the salinity increased from 0.60 to 9.35 dS m^{-1}. At the lower EC levels of 0.6 and 1.95 dSm^{-1}, fungal species of *Cladosporium, Aspergillus* and *Fusarium* were dominant, whereas *Pencillium* and *Mucor* along with *Fusarium* were dominant at 3.59 and 7.50 dS m^{-1}, respectively. At 5.50 dS m^{-1}, *Cladosporium* and *Fusarium* were observed whereas, at the highest salinity level (9.35 dS m^{-1}) only *Fusarium* prevailed. The study indicated that *Fusarium* is the only fungi that can survive and proliferate at all salinity levels due to certain salt tolerance mechanism. In fungi, low osmotic potential due to salt stress decreases spore germination, the growth of hyphae and also cause changes in the morphology and gene expression resulting in the formation of spores with thick walls (Mandeel 2006). The results are corroborating with those of Yang and Sun (2020) where Cladosporium fungal species in saline soils of Yellow river delta, China; Ashok et al. (2015) in coastal soils of Tamil Nadu with EC 2.95 dS m^{-1}; Rajpal et al. (2016) in the saline soils (0.4 to 15.0 dS m^{-1}) of Kutch Gujarat (dominance of Aspergilli, *Fusarium* and *Penicillium)*. Nayak et al (2019) isolated the fungal genera like *Fusarium sp., Penicillium sp. and Trichoderma* from coastal sandy soils of Orissa.

Bacteria: A total of eighteen bacterial isolates were identified on the basis of colony and cell morphology (Tables 5, 6). The colonies of the isolates 1, 2, 4, 8, 9, 10, 12, 14, 15, 16 and 17 were circular; colonies of 3, 6, 7 and 18 were irregular; colony 13 was rhizoid whereas the colonies of isolates 5 and 11 were filamentous in form. The isolates 3, 5, 7, 8, 10, 11 and 13 were white in colour whereas, isolates 6, 9 and 18 recorded light brown colour. Yellow colony colour was in isolate no 4, 15 and

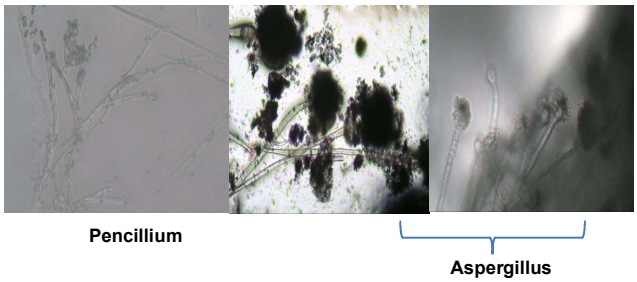

Fig. 1. Microscopic view of some of fungal isolates

Table 3. Characterization fungal isolates

Isolate No.	Form	Margin of the colony	Elevation	Colour	Tentative micro organism
1	Circular	Entire	Flat	Black	*Cladosporium sp.*
2	Dendroid	Filiform	Raised	Yellow	*Aspergillus-sp.*
3	Circular	Entire	Flat	White with pink	*Fusarium sp.*
4	Circular	Filiform	Flat	White with black	*Aspergillus sp.*
5	Circular	Entire	Flat	White	*Fusarium sp.*
6	Circular	Entire	Crateriform	Orange	*Mucor sp.*
7	Irregular	Undulate	Flat	Olivaceous greenish	*Pencillium sp.*

Table 4. Soil fungi at different EC levels

Isolate no.	Micro organism	Electrical conductivity (dS m^{-1})					
		0.60	1.95	3.59	5.50	7.50	9.35
1	*Cladosporium sp.*	✓			✓		
2	*Aspergillus-sp.*	✓	✓				
3	*Fusarium sp.*	✓	✓				✓
4	*Aspergillus sp.*	✓					
5	*Fusarium sp.*	✓	✓	✓	✓	✓	
6	*Mucor sp.*					✓	
7	*Pencillium sp.*			✓			

Table 5. Characterization of bacterial isolates

Isolate No.	Colony morphology				Cell morphology		Biochemical tests							Tentative micro organism
	Form	Colour	Margin of the colony	Elevation	Cell shape	Gram reaction	Catalase test	VP test	MR test	Indole test	Citrate test	*Starch hydrolysis*	Acid and Gas	
1	Circular	Orange	Entire	Raised	Cocci	+ve	+	+	+	-	+	+	-	*Micrococci sp.*
2	Circular	Light orange	Undulate	Flat	Cocci	+ve	+	+	-	-	-	-	+	*Staphylococci sp.*
3	Irregular	White	Undulate	Umbonate	Cocci	+ve	+	-	-	-	-	+	+	*Diplococci sp.*
4	Circular	Yellow	Entire	Flat	Cocci	+ve	+	-	-	-	-	+	+	*Diplococci sp.*
5	Filamentous	White	Filiform	Flat	Spiral	+ve	+	-	-	-	+	+	+	*Azospirillum sp.*
6	Irregular	Light brown	Undulate	Flat	Cocci	+ve	+	+	-	-	+	+	+	*Serratia sp.*
7	Irregular	White	Entire	Flat	Short Rods	+ve	+	-	-			-	+	*Diplococci sp.*
8	Circular	White	Entire	Convex	Spiral	-ve	+	-	-	-	+	+	+	*Azospirillum sp.*
9	Circular	Light brown	Entire	Raised	Short rods	-ve	-	-	-	-	+	-	+	*Pseudomonas sp.*
10	Circular	Dull White	Entire	Flat	Short rods	-ve	-	-	-	-	+	-	+	*Pseudomonas sp.*
11	Filamentous	White	Filiform	Flat	Short Rods	-ve	+	-	-	-	+	+	+	*Azospirillum sp.*
12	Circular	Pink	Entire	Raised	Rods	+ve	+	-	-	-	+	+	-	*Bacillus sp.*
13	Rhizoid	White	Filiform	Flat	Short Rods	+ve	+	-	-	-	+	-	+	*Pseudomonas sp.*
14	Circular	Light orange	Entire	Raised	Cocci	-ve	+	-	-	-	+	+	+	*Xanthomonas sp.*
15	Circular	Yellow	Entire	Flat	Cocci	+ve	+	+	+	-	+	+	-	*Micrococci sp.*
16	Circular	Light pink	Entire	Raised	Short Rods	-ve	+	-	-	-	+	-	+	*Pseudomonas sp.*
17	Circular	Light yellow	Entire	Flat	Short Rods	-ve	+	-	-	-	+	-	+	*Pseudomonas sp.*
18	Irregular	Light brown	Undulate	Spiral	Short Rods	-ve	+	-	-	-	+	-	+	*Pseudomonas sp.*

VP - Voges-Proskauer, MR - Methyl red test, IND - Indole production

light yellow was in isolate 17. An orange shade was observed in isolates 1, 2 and 14 while, pink shades were found in isolates 12 and 16. The isolates 1, 4, 7, 8, 9, 10, 12, 14, 15, 16 and 17 were having entire margin. Undulate margin was observed in isolates 2, 3, 6, 18 and filiform margin was observed in the isolates and 5, 11, 13. Isolates 2, 4, 5, 6, 7, 10, 11, 13, 15 and 17 were having flat elevation while raised elevation was recorded in isolates 1, 9, 12, 14 and 16. Likewise umbonate, convex and spiral elevations were reported in isolates 3, 8 and 18, respectively. Cell morphology includes cell shape and gram reaction of bacterial colonies where observing under microscope with the help of MICAPS-Micro View. The bacterial isolates 1, 2, 3, 4, 6, 14 and 15 were cocci while, isolates 7, 9, 10, 11, 12, 13, 16, 17 and 18 were short rods in shape. Isolates 5 and 8 were spiral in shape when observed under microscope. Among eighteen bacterial isolates, only 10 isolates *viz.* 1, 2, 3, 5, 4, 6, 7, 12, 13 and 15 were gram positive bacteria and remaining isolates were gram negative.

Among all salinity levels, dominance of *Micrococci, Staphylococci, Diplococci, Azospirillum, Serratia, Pseudomonas and Xanthomonas* bacterial species were observed, whereas at the higher salinity levels of 7.50 and 9.35 dS m^{-1} only *Azospirillum, Diplococci* and *Pseudomonas sp.* survived (Table 6). Different species of *Micrococci, Diplococci, Azospirillum* and *Pseudomonas* were dominant at 1.95 dS m^{-1} salinity level. *Diplococci, Azospirillum and Pseudomonas* bacterial species were dominant at 3.59 and 5.50 dS m^{-1} salinity levels. Among all, *Pseudomonas sp.* survived at all soil salinity levels.

Presence of bacteria under high saline conditions indicates the salt tolerance mechanism which require both energy and carbon for the synthesis of compatible osmolytes (Kakumanu and Williams 2014). Decreased growth of bacterial isolates with high salt concentration may be due to the detrimental effect of salts on bacterial populations through direct toxicity as well as osmotic stress. Corroborative findings were reported in the rice soils of the coastal region of the Gangetic delta of West Bengal (Barua et al 2011) and Orissa (Dangar et al 2017) where dominance of Bacillus bacterial species was observed. Bhatt et al (2015) reported the prevalence of *Pseudomonas, Serratia, Bacillus* species in the coastal soils of Jamnagar, Gujarat. Azmi and Chatterjee (2016) reported that, the microbial population of coastal belt of West Bengal comprised of aerobic heterotrophic, gram negative and spore forming bacteria with dominant *Pseudomonas* species. A total of 47 bacterial strains of *Psuedomonas sp.* were isolated from coastal sandy soils of Chennai as reported by Nayak et al (2019).

Table 6. Soil bacteria at different EC levels

Isolate no.	Tentative micro organism	Electrical conductivity (dS m^{-1})					
		0.60	1.95	3.59	5.50	7.50	9.35
1	*Micrococci sp.*	✓					
2	*Staphylococci sp.*	✓					
3	*Diplococci sp.*			✓	✓	✓	
4	*Diplococci sp.*		✓			✓	
5	*Azospirillum sp.*						✓
6	*Serratia sp.*	✓					
7	*Diplococci sp.*	✓				✓	
8	*Azospirillum sp.*	✓	✓	✓	✓		✓
9	*Pseudomonas sp.*	✓	✓				
10	*Pseudomonas sp.*	✓	✓				
11	*Azospirillum sp.*			✓			
12	*Bacillus sp.*	✓					
13	*Pseudomonas sp.*						✓
14	*Xanthomonas sp.*	✓					
15	*Micrococci sp.*	✓	✓				
16	*Pseudomonas sp.*	✓					
17	*Pseudomonas sp.*	✓	✓	✓	✓		
18	*Pseudomonas sp.*		✓				

CONCLUSION

The study elucidated the variable effect of salt concentration in soil on proliferation and distribution of different microbial species and soil enzyme activity. At high salinity level only, few organisms *viz*. *Fusarium sp.* among fungi and *Azospirillum sp., Pseudomonas sp.* among bacteria survived. The salt tolerance capacity of the organisms surviving at high salinity levels need to be further explored and these microorganisms are very useful for biotechnological applications in terms of bioremediation and bio mineralization.

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