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Effect of heavy metals (Hg and Zn) on the growth and phosphate solubilising activity in halophilic phosphobacteria isolated from Manakudi mangrove

S.Ravikumar*, G.Prakash Williams, S. Shanthy,
N. Anitha Anantha Gracelin, S. Babu and P.S. Parimala

Division of Marine Microbiology and Medicine, Centre for Marine Science and Technology
Manonmaniam Sundaranar University, Rajakkamangalam - 629 502, India

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Abstract: *The diversity of phosphobacteria in Manakudi mangrove ecosystem of Tamil Nadu was carried out in root and rhizosphere soil samples. The counts of phosphobacteria were found higher in root samples than in soil samples particularly in Hymenachene acutigluma. The abundance of phosphobacterial diversity in Manakudi mangrove showed high degree of positive correlation with the content of phosphate in rhizosphere soil of all the mangrove and associated plant species. Nine phosphobacterial species belonging to 7 genera were reported from Manakudi mangrove ecosystem. All the identified bacterial species are sensitive to both the heavy metals (mercury and zinc) in terms of growth and physiology even at lower concentrations. The content of protein and total sugars were increased by the higher concentrations of heavy metals whereas decreased trend was noticed in lower concentrations of heavy metals.*

Key words: *Heavy metal, Halophilic phosphobacteria, Phosphate solubilising activity, Mangrove*

Introduction

Mangroves are woody plants that grow at the interface between land and sea in tropical and subtropical latitudes (Chapman, 1984). These plants and the associated microbes, fungi, plants and animals constitute the mangrove forest community or mangal (Spalding, 1997). Recently, Kathiresan (2002) described the causes for degradation of mangrove habitat, which is due to poor microbial counts. Microorganisms in mangroves play an important role in the recycling of nutrients and establishment of mangrove seedlings through the mineralization and also by the production of phytohormones (Ravikumar *et al.,* 2002a and b; Ravikumar *et al.,* 2004). Reduction in the total counts of microorganisms in mangroves affects the growth of the mangrove fauna and flora (Kathiresan, 2001). Among the variety of microorganisms, phosphate solubilising bacteria plays a vital role in the regeneration of phosphates through solubilisation of unavailable inorganic phosphorous to available form. However, this group of bacteria has reported to have low in number even in organic rich mangrove sediments and also high concentrations of inorganic form of phosphorous. This might be due to the various noxious chemicals including heavy metals. Unlike other pollutants, heavy metals due to their recalcitrant nature, tend to accumulate in various forms of life and enters the food chain posing a serious threat to the environment, animals and human (Laxman and Rao, 2001). The effect of most of the heavy metals on heterotrophic bacteria, isolated form the environments and grown in defined media under laboratory conditions were studied (Talburt and Johnson, 1967; Sobek and Talburt, 1968; Ramamoorthy and Kushner, 1975, Ravikumar *et al.,* 2002 c). But such studies are lacking with the halotolerant aquatic marine habitat microorganisms. Hence, the present study has been undertaken to find out the effect of heavy metals on the growth and phosphate solubilising activity of halophilic phosphobacteria from Manakudi mangrove habitat.

Materials and Methods

a) Collection of samples:

To isolate the halophilic phosphobacteria, root and rhizosphere soil samples were collected from two mangrove species *viz., Avicennia officinalis* L., *Rhizophora mucronata* Poir. and three mangrove associated species *viz., Panicum repens* L., *Hymenachene acutigluma*, *Acrostichum aureum* L., from Manakudi mangrove environment, South West coast of India (Lat.8°05'36''N ; Long.77°29'E) which has an area of about 150 ha, is situated about 8 kilometers North West of Cape Comorin. Root and rhizosphere soil samples were obtained by carefully pulling out the root with a lump of soil intact.

b) Isolation of phosphate solubilizing bacteria:

Collected samples were serially diluted using sterile water blank and plated on Pikovskya's medium (Holt *et al.,* 1994) by following pour plate technique and incubated at 37ºC for 2 days. After incubation, the colonies appeared on dilution plates with surrounding solubilisation zone were counted and morphologically different colonies were repeatedly streaked on to the same agar plates for purification. The isolated strains were identified by following Bergey's manual of determinative bacteriology (Holt *et al.,* 1994).

c) Analysis of chemical characteristics in rhizosphere soil:

All the rhizosphere soil samples were subjected for the analysis of chemical parameters *viz*., pH, electrical conductivity,

Corresponding author: E-Mail: ravibiotech201320@yahoo.com, Tel:+91-4652-261320(Home), 253078 (Work), Fax: +91-462-2322973

nitrogen, phosphorus, potassium, iron, copper, zinc and manganese by following the method of APHA (1985).

d) Effect of Heavy metal on phosphobacteria :

To find out effect of heavy metals (Hg and Zn) on the growth and phosphate solubilizing activity of nine phosphobacterial species *viz.*, *Bacillus subtilis, Micrococcus roseus, Bacillus megaterium*, *Micrococcus luteus*, *Arthrobacter ilicis Pseudomonas aeruginosa, Citrobacter freundii, Escherichia coli* and *Enterobacter aerogenes* sterilized Pikovsky's broth was prepared with different concentrations of heavy metals (0,1,10, 50, 100 ppm) in a 250 ml conical flask. To this, one ml of 12 hr old liquid bacterial cultures were added into each conical flask and kept for incubation at 28 ± 2° C in a thermostat shaker for 24 hrs. After incubation, the growth of phosphobacterial species was measured at 600 nm by using UV spectrophotometer (Shimadzu, Japan)and also the phosphate solubilizing activity of each species grown in different concentrations of heavy metals was measured by following standard method. Simultaneously, the effect of heavy metals on the content of protein (Lowry *et al.*, 1951) and total sugars (Dubois *et al.,* 1956) were also measured by following standard methods.

Results and Discussion

Phosphate solubilising bacteria in rhizosphere soil and root samples from five mangrove and mangrove associated plant species reveals that, the phosphobacterial counts were found maximum in root samples than in rhizosphere soil particularly in *Hymenachene acutigluma* (Fig.1). Ravikumar *et al*., (2002 a,b,c) reported that, the counts of *Azotobacter, Azospirillum* and phosphobacteria were reported to found maximum in root samples of mangrove plants in Pichavaram mangrove ecosystem. Five species of gram positive phosphobacteria *viz.*, *Bacillus subtilis, Micrococcus roseus, Bacillus megaterium*, *Micrococcus luteus* and *Arthrobacter ilicis* and four species of gram negative phosphobacteria *viz., Pseudomonas aeruginosa, Citrobacter freundii, Escherichia coli, Enterobacter aerogenes* were identified from both the root and rhizosphere soil samples (Table.1) in Manakudi mangrove environment. Ravikumar *et al.,* (2002 b) isolated three species of phosphobacteria, which are belonging to the same genera (*Bacillus*) in Pichavaram mangrove environment along South East coast of India. Among the bacterial species, *Escherichia coli* and *Bacillus megaterium* were found in all the rhizosphere soil samples of mangroves and mangrove associates whereas *Pseudomonas aeruginosa , Arthrobacter ilicis* and *Micrococcus luteus* were reported in all the root samples (Table 2). However, the species diversity was found maximum in the roots of mangrove associates and rhizosphere soil of mangrove plant species. This is due to the secretions of

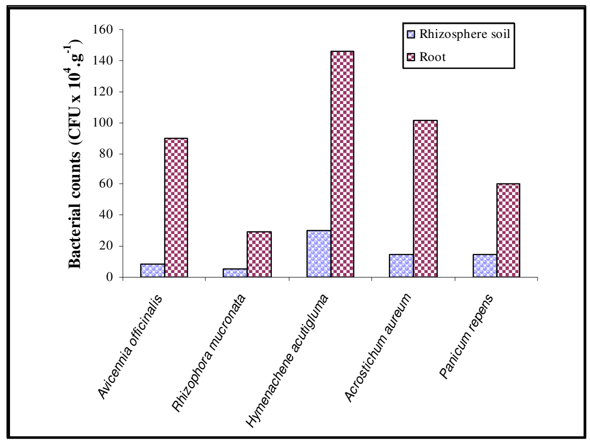

Fig. 1: Counts of halophilic phosphobacteria in rhizosphere soil and root of associated plant species

Table - 1: Phosphobacterial species reported in rhizosphere soil of two mangroves and three mangrove associates

Bacterial species	Plant species				
	Rhizophora mucronata	***Avicennia officinalis***	***Acrostichum aureum***	***Panicum repens***	***Hymenachene acutigluma***
Bacillus subtilis	+	+	+	+	+
Bacillus megaterium	+	+	+	+	+
Micrococcus roseus	+	+	+	-	+
Micrococcus luteus	-	+	+	+	-
Pseudomonas aeruginosa	+	-	+	+	-
Citrobacter freundii	+	+	-	-	-
Escherichia coli	+	+	+	+	+
Arthrobacter ilicis	+	-	+	-	-
Enterobacter aerogenes	-	+	-	-	+

(+) Presence (-) Absence

carbohydrates, sugars and amino acids from root exudates of plants that enhance the growth and multiplication of bacterial species (Ravikumar *et al.,* 2004; Kathiresan and Ravikumar, 1995; 1993). The abundance and distribution of phosphobacterial species were found to be influenced by various chemical characteristics of the soil. For instance, the content of phosphorus in rhizosphere soil showed high deree of positive correlation with the counts of phosphobacteria in the rhizosphere soil of mangrove and associated plant species. However, other factors such as potassium, iron, nitrogen, salinity also influenced the phosphobacterial diversity in rhizosphere soil and root with the type of plant species (Table 3). Studies carried out by several authors (Meybeck, 1982 and Fox, 1983) indicate that the phosphorus seems to be the major limiting factor for phytoplanktonic growth in sea water. Agasimani *et al*., (1995) recorded significantly higher population of phosphate solubilisation at higher concentration of inorganic phosphorus. Moreover, the growth and activity of certain bacteria were reported to influence by the pollutants in the mangrove environment (Kathiresan and Bingham, 2001). Hence, it is imperative to know the species abundance and the effect of heavy metals on the growth, phosphate solubilizing activity and macromolecular content of phosphobacterial species isolated from the Manakudy mangrove environment (South West coast of India).

Table - 2: Phosphobacterial species reported in the root of two mangroves and three mangrove associates

Bacterial species	**Plant species**				
	Rhizophora mucronata	***Avicennia officinalis***	***Acrostichum aureum***	***Panicum repens***	***Hymenachene acutigluma***
Bacillus subtilis	-	-	+	+	+
Bacillus megaterium	+	-	+	+	-
Micrococcus roseus	-	-	+	+	+
Micrococcus luteus	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+
Citrobacter freundii	-	-	-	+	-
Escherichia coli	-	-	-	-	+
Arthrobacter ilicis	+	+	+	+	+
Enterobacter aerogenes	+	-	-	-	+

(+) Presence (-) Absence

Table - 3: Chemical parameters in rhizosphere soil samples of mangrove and associated plants

Name of the plant species	**Samples**	**Chemical parameters**								
		pH	**EC ($ds.m^{-1}$)**	**N ($\mu g.g^{-1}$)**	**P ($\mu g.g^{-1}$)**	**K ($\mu g.g^{-1}$)**	**Fe (ppm)**	**Zn (ppm)**	**Cu (ppm)**	**Mn (ppm)**
Avicennia officinalis	Rhizosphere soil	3.92	5.54	42.04	1.75*	21.82	13.45	2.09	1.1	9.49
Rhizophora mucronata	Rhizosphere soil	4.62	4.58	42.05	1.93*	26.78	14.22^{+}	2.3	1.18	10.96
Hymenachene acutigluma	Rhizosphere soil	4.66	5.06	40.65*	2.19*	28.1*	14.68	2.39	1.02	10.40
Acrostichum aureum	Rhizosphere soil	4.68	3.76*	28.19	1.4*	23.63	12.87	2.6	1.09	12.42
Panicum repens	Rhizosphere soil	4.93	4.1^{+}*	28.45	2.46*	21.05	11.24	1.48	2.59	10.62

* indicates positive correlation with rhizosphere soil phosphobacteria
$^{+}$ indicates positive correlation with root phosphobacteria

The effect of mercury and zinc on the growth of phosphobacterial species reveals that, the growth was decreased with the increasing concentration of heavy metals from 0.1ppm to 100 ppm levels (Figs.2 and 3). The effect of mercury on the phosphate solubilizing activity reveals that, the activity was completely arrested even with the 1 ppm concentration and extended upto 100ppm concentration of mercury in all the bacterial species except *Citrobacter freundii* and *Arthrobacter ilicis* (Table 4). However, the phosphate solubilising activity was completely arrested in all bacterial species grown at different concentrations of zinc (Table 5). The fold of decrease on the phosphate solubilising activity was increased with the increasing concentrations of zinc and mercury. Earlier findings reported that, the growth of the free living nitrogen fixing bacteria of *Azotobacter vinelandii* and *Azotobacter chroococcum* isolated from the Pichavaram mangrove forest was inhibited at higher concentrations of mercury and zinc not at the lower heavy metal

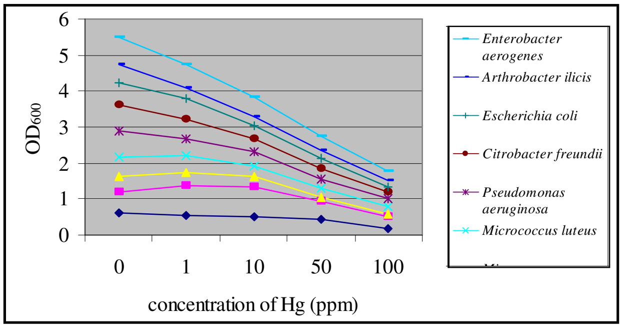

Fig. 2: Effect of mercury on the growth of halophilic phosphobacteria

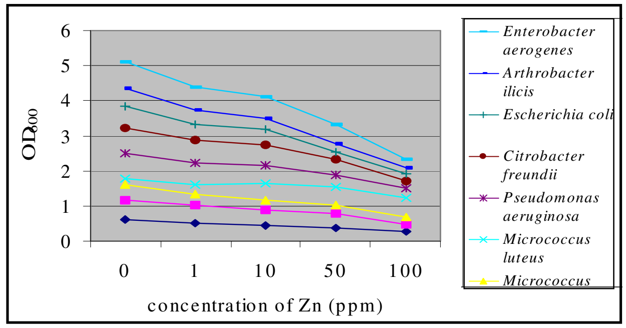

Fig. 3: Effect of zinc on the growth of halophilic phosphobacteria

Table - 4: Effect of mercury on the phosphate solubilizing activity of halophilic phosphobacteria

Bacterial species	Concentrations of Hg (ppm)				
	0	1	10	50	100
Bacillus subtilis	0.14	0.07 (-99)	0.04 (-250)	0.013 (-976)	0.012 (-1066)
Bacillus megaterium	0.125	0.06 (-108)	0.052 (-140)	0.059 (-111)	0.028 (-421)
Micrococcus roseus	0.12	0.08 (-49)	0.078 (-53)	0.016 (-649)	0.013 (-823)
Micrococcus luteus	0.09	0.048 (-87.5)	0.024 (-275)	0.019 (-373)	0.019 (-373)
Pseudomonas aeruginosa	0.144	0.1 (-45)	0.079 (-82)	0.02 (-619)	0.015 (-859)
'Citrobacter freundii	0.16	0.11 (-145)	0.05 (-220)	0.031 (-416)	0.016 (-899)
Escherichia coli	0.148	0.121 (-22.5)	0.021 (-604)	0.015 (-886)	0.014 (-957)
Arthrobacter ilicis	0.096	0.02 (-380)	0.017 (-464)	0.013 (-638)	0.013 (-638)
Enterobacter aerogenes	0.106	0.041 (-158)	0.018 (-488)	0.016 (-562)	0.012 (-783)

Values in parentheses show percentage decrease over control

Table - 5: Effect of zinc on the phosphate solubilizing activity of halophilic phosphobacteria

Bacterial species	Concentrations of Zn (ppm)				
	0	1	10	50	100
Bacillus subtilis	0.14	0.11 (-27.2)	0.09 (-55.5)	0.064 (-118)	0.046 (-204)
Bacillus megaterium	0.125	0.121 (-3.3)	0.1 (-25)	0.07 (-78.5)	0.056 (-123)
Micrococcus roseus	0.12	0.1 (-19)	0.089 (-34.8)	0.0195 (-373.6)	0.019 (-531.5)
Micrococcus luteus	0.09	0.079 (-13.9)	0.02 (-349)	0.019 (-515.3)	0.017 (-429)
Pseudomonas aeruginosa	0.144	0.11 (-30.9)	0.04 (-259)	0.018 (-699)	0.01 (-1339)
Citrobacter freundii	0.16	0.162 (1.23)	0.06 (-166.6)	0.031 (-416)	0.022 (-627)
Escherichia coli	0.148	0.119 (-24.3)	0.04 (-269)	0.015 (-886.6)	0.01 (-1379)
Arthrobacter ilicis	0.096	0.1 (+4)	0.08 (-19)	0.07 (-37.1)	0.04 (-139)
Enterobacter aerogenes	0.106	0.102 (-3.9)	0.087 (-21.8)	0.046 (-130.4)	0.014 (-657)

Values in parentheses show percentage increase or decrease over control concentrations and also they observed that the complete arrest of growth was reported at 100ppm concentrations of heavy metals. Comparatively, the bacterial species from Manakudi mangrove ecosystem along south west coast of India were more tolerant to toxic heavy metals than the phosphobacterial species isolated from the south east coast of India (Pichavaram mangrove). Moreover, the decreased trend on the phosphate solubilizing activity even at lower concentrations of heavy metals might be due to the loss of capacity to synthesize phosphatase enzyme by the halophilic phosphobacterial species due to the inhibition of protein synthesis.

The effect of heavy metals on the content of protein and total sugars in the cell supernatant of nine phosphobacterial species indicates that, the protein and carbohydrate contents were increasing with the increasing concentration of heavy metals. However, the increment of concentration of macromolecules at varied concentrations of heavy metals was found varied with the bacterial species (Fig.4-7). This might be due to the over production of certain carbohydrates and proteins at heavy metal stressed conditions for their better survival (Ravikumar *et al*., 2002 c) except phosphatase enzyme. Evaluation of the potential influence of the various abiotic factors

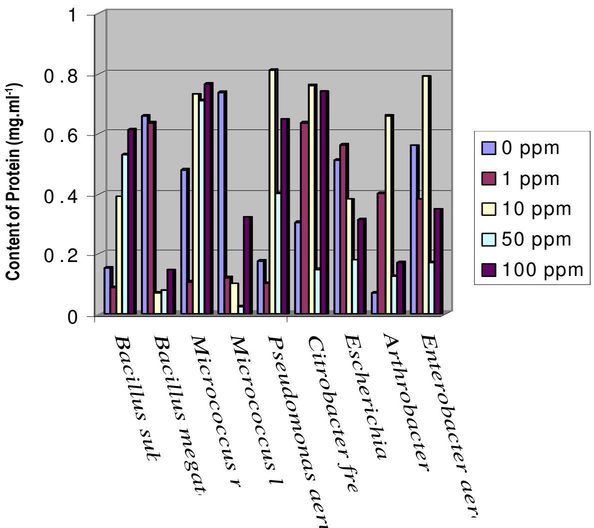

Fig. 4: Effect of mercury on the content of protein in halophilic phosphobacteria

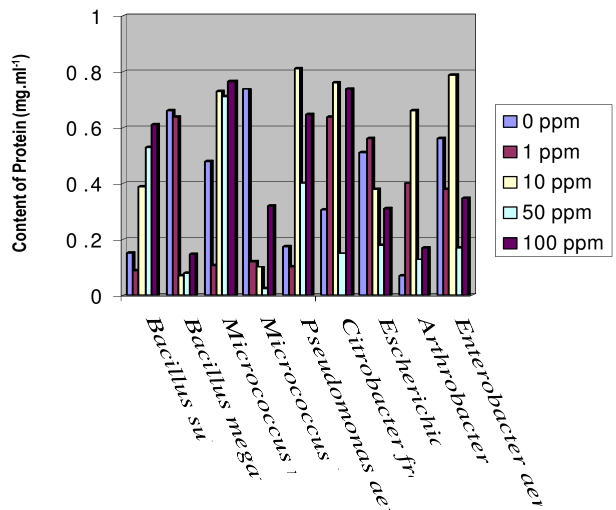

Fig. 5: Effect of zinc on the content of protein in halophilic phosphobacteria

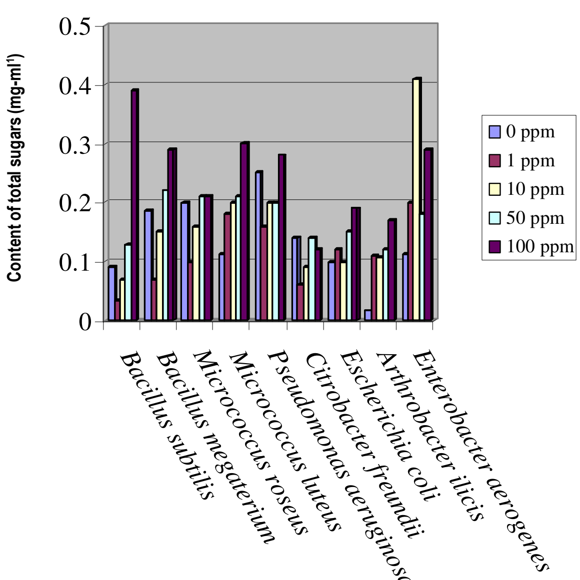

Fig. 6: Effect of mercury on the content of total sugars in halophilic phosphobacteria

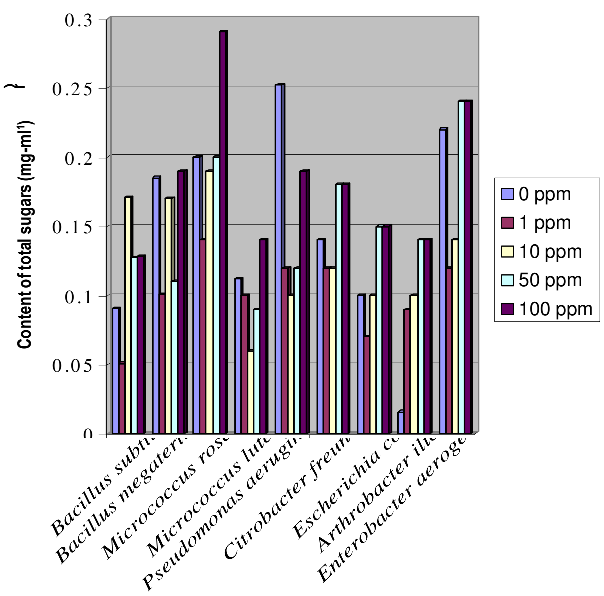

Fig. 7: Effect of zinc on the content of total sugars in halophilic phosphobacteria

upon the heavy metal pollutant and the impact of the heavy metal pollutant under such conditions upon microorganisms would be useful for clear understanding of the interaction of heavy metals on biogeochemical cycles. The present study concluded that, the phosphobacterial species isolated from Manakudy mangrove environment are found to be tolerant form to a certain extent of heavy metal concentrations than the strains from other marine environments however, the growth of mangroves could be affected with the depletion of nutrients through the biological conversion of minerals due to inhibition of phosphatase enzyme activity. It is also inferred from the present study that, immediate steps has to be undertaken to assess the toxic heavy metals in Manakudy mangrove ecosystem as a part of conservation of mangroves in south west coast of India.

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References

Agasimani, C.A., G. Ravishankar,G.D. Radder and M.N. Sreenivasa: Influence of phosphate solubilising microorganisms and sources of phosphate on growth and yield of groundnut. Proc. Natl. Symp. Frontiers in Appl. Environ. Microbiol., 11-13 December, SES,CUSAT, Cochin, pp.161-163 (1995)

APHA.: Standard methods for the examination of water and waste water. 15th Edn.: APHA, AWWA, WPCF. Washington D.C. pp 327-354 (1985).

Chapman, V.J.: Botanical surveys in mangrove communities. *In* :The Mangrove Ecosystem Research Methods. (*Eds*: S.C. Snedaker, J.C.Snedaker). UNESCO, Paris, pp. 53 – 80 (1984).

Dubois, M., K.A.Gills, J.K.Hamilton, P.A.Reser and F. Smith: Colorimetric method for determination of sugars and related substances. *Anal. Chem.,* **28**, 350 – 356 (1956).

Fox,L.X: The chemistry of aquatic phosphorus inorganic processes in rivers. *Hydrobiologia*, 253,1-6 (1983).

Holt, J.G., N.R. Krieg, P.H.A.Sheath, J.T. Staley and T.Williams: Bergey's manual of determinative bacteriology. Williams and Wilkins, Baltimore (1994).

Kathiresan, K.: Why are mangroves degrading? *Cur. Sci.*, **83**,1246-1249 (2002)

Kathiresan, K. and B.L. Bingham: Biology of mangroves and mangrove ecosystems. *Advances in Marine Biology,* **40 ,** 81 – 251(2001).

Kathiresan, K. and S. Ravikumar: Influence of tannius, aminoacids and sugars on fungi of marine halophytes. *Mahasagar*, **26(1)**, 21-25 (1993).

Kathiresan, K. and S. Ravikumar: Influence of tannins, sugars and amino acids on bacterial load of marine halophytes. *Environ. Ecol.,* **13**(**1**), 94–96(1995).

Laxman, L.S. and B.S. Rao: Biosorption / Bioremediation of heavy metals. *In* : Role of microbes in the management of environmental pollution. APH publishing corporation, New Delhi. pp . **89** (2001).

Lowry, O.H., N.J. Rostbrough, A.L.Fan and R.J. Randal: Protein measurement with the Folin-phenol reagent, *J. Biol. Chem.,* **193**, 265 – 275(1951).

Meybeck,M.: Carbon, nitrogen and phosphorus transport by world rivers. *Am.J.Sci.*, **282**, 401-450 (1982).

Ramamoorthy, S. and D.K.J. Kushner: Binding of mercuric and other heavy metal ions by microbial growth media. *Micro Ecol.,* **2** ,162-176(1975).

Ravikumar,S., G. Ramanathan, N. Suba, L.Jeyaseeli and M. Sukumaran: Quantification of halophilic *Azospirillum* from mangroves. *Ind. J. Mar. Sci.*,**31**(**2**),157-160(2002 a).

Ravikumar,S., G. Ramanathan, M.Babuselvam and S.Prakash: Quantification of halophilic phosphobacteria from Pichavaram mangroves (South east coast) and their potential application to crop culture, *Proc.Nat.Sem. Creeks, Estuaries and Mangroves* (*Ed*: G.Quadios). Pollution and conservation, Thane, India. Vidya Prasarak Mandal-S.B.N. Bandodkar College of Science, pp.301-303 (2002 b).

Ravikumar, S. and K. Kathiresan: Influence of tannins, aminoacids and sugars on fungi of marine halophytes. *Mahasagar*,**26**(**1**),21-25(1993).

Ravikumar, S., A.NuralShiefa and E.R. Nishalekshmi: Heavy metal toxicity on extreme halophilic *Phormidium* sps. *J. Environ Res.*, **12**(**1**), 14-23(2002 c).

Ravikumar, S., K. Kathiresan, S. Thadedus Maria Ignatimmal, M. Babuselvam and S. Shanthy: Nitrogen fixing azotobacters from mangrove habitat and their utility as marine biofertilizers. *J.Exp.Mar. Biol.Ecol.*,**312**(**1**), 5-17(2004).

Sobek, J.M. and D.E. Talburt: Effect of the rare earth cerium on *Escherichia coli. J. Bacteriol.,* **95** , 47-51(1968).

Spalding,M.: The global distribution and status of mangrove ecosystems. International News letter of Coastal Management-Interlock Network, Special Edition,**1**,20-21(1997).

Talburt, D.E. and G.T. Johnson: Some effects of rare earth elements and yttrium on microbial growth. *Mycologia,* **59** , 492-503(1967).