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Nickel resistant bacterial population in the inner shelf of Bay of Bengal off Chennai

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Original Article

Abstract
Diffused inputs into the marine environment are now recognized as highly significant for some heavy metals. An attempt has been made in the present study to understand the seasonal variation of nickel resistant bacteria diversity in the coastal zone of Chennai, India. A reduction in nickel resistant population was observed with increase in depth when compared to samples collected near the shore. The trend of Nickel resistant bacterial population was found to be Fishing Harbor Traverses > Chennai Harbor Traverses > Cooum River Traverses = Ennore Traverses > Adyar River Traverses. The results of the present study clearly showed the increase in the development of metal resistance among the bacterial population during the study period. The nickel resistant bacterial population in the surface sediment samples studied off Chennai in Bay of Bengal during the three consecutive years varied between 3.3 and 5.3 log10 CFU/g with an average of 4.4 log10 CFU/g of sediment.

Keywords: *Nickel resistant bacteria, microbial population dynamic, anthropogenic impact, bio indicators.*

Introduction

The entry of the pollutants in the marine environment imparts a stress on the pelagic and benthic biota, which thrive in the marine waters and sediments respectively. Sediments are important component of ecosystem in which toxic compounds accumulate through complex physical and chemical adsorption mechanisms depending on the properties of the adsorbed compounds and the nature of the sediment matrix (Leivouri, 1998). Sediment plays an important role in the assessment of metal contamination in natural waters (El Nemr *et al*., 2006). The association of microorganisms with sediment particles is one of the primary complicating factors in assessing microbial fate in aquatic systems. The literature indicates that the majority of enteric bacteria in aquatic systems are associated with sediments and that these associations influence their survival and transport characteristics (Jamieson *et al*., 2005). Resistance to toxic heavy metals and their accumulation by bacteria is a widespread phenomenon that can be exploited for the improvement of the environment. Metal-resistant bacteria have developed very efficient and varying mechanisms for tolerating high levels of toxic metals and thus hold potential for controlling heavy metal pollution (Altug and Balkis, 2009). These organisms develop tolerance to withstand the extreme conditions and to convert the deleterious constituents to less toxic or non-toxic forms can be effectively used to prepare drugs that can cure diseases and also degrade and detoxify the pollutants in environment. The present study aimed to isolate, identify and screening potent nickel resistant marine microbes from marine surface sediments that could be exploited for solving environmental problems. The contaminating effect of heavy metals in the marine environment increased considerably leading to the development of high metal resistance among the marine microbes and also extended the metal tolerance limits of organisms in the marine ecosystems.

Material and methods

Collection of samples

Surface sediment samples were collected from 26 stations along 5 traverses, viz., Ennore Traverse (Korttalaiyar River) (ET), Fishing Harbor Traverse (FHT), Chennai Harbor Traverse (CHT), Cooum River Traverse (CRT) and Adyar River Traverse (ART) in Bay of Bengal off Chennai, India (Fig. 1) during the cruise by CRV (Coastal Research Vessels) Sagar Purvi and Sagar Paschimi, of NIOT (National Institute of Ocean Technology), MOES (Ministry of Earth Sciences), India at >10 m water depth and for sampling shallow waters fiber glass boat was engaged during Pre-monsoon (PRM) (April - June), Monsoon (MON) (July - September) and Post-monsoon (POM) (October - December) seasons of three consecutive years of 2006-07, 2007-08 and 2008-09 using van Veen grab sampler. Position fixing was done using onboard GPS (Global Positioning System). The samples were kept cool in an icebox during transportation to the laboratory.

Isolation and screening of microorganisms

Sediment samples (1g) were serially diluted and 10^{-4}, 10^{-5} and 10^{-6} dilutions were used for screening nickel resistant bacterial (NiRB) populations. The dilutions were plated on nickel amended SWNA (sea water nutrient agar) medium. The sea water nutrient medium was prepared by mixing Peptone - 5.0 g, Beef extract - 3.0 g, NaCl - 5.0 g and Aged sea water - 1000 mL at a pH of 7.0 $\pm$ 0.2. Based on the previous studies from Pulicat Lake sediments, near Chennai coast (Kamalakannan *et al.*, 2006), basic concentration of 2 mM of $NiCl_2$ (Nickel chloride) was selected for the isolation of NiRB from sediment samples. Inoculated petri plates were incubated at 35°C for 48 h and the colonies developed were counted. Morphologically distinguished colonies were purified by repeated quadrant streaking on solid SWNA plates. The purified colonies were stored in slants as well as in glycerol stocks (25% v/v) at -40ºC for further studies. The cultures were maintained by sub-culturing at regular interval of one month. The selected bacterial strains were characterized by subjecting to routine microbiological and biochemical tests (Cappuccino and Sherman, 1999) and identified using the key provided by Manual of Determinative Bacteriology (Bergey, 1994).

Determination of minimum inhibitory concentrations

The growth and metal tolerance ability of the test strains were evaluated on minimal salts agar medium amended with metals. After initial screening for nickel resistance, the

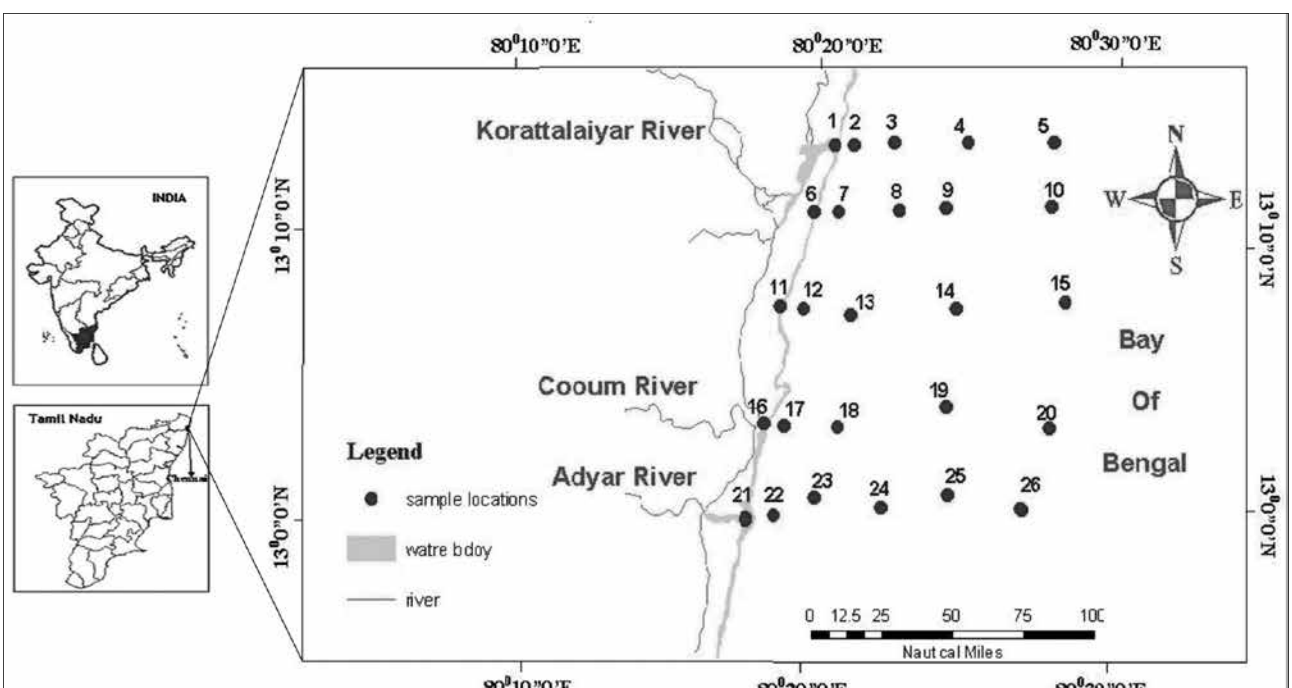

Fig. 1. Study area showing sample locations in the innershelf of Bay of Bengal Off Chennai, Tamilnadu, India

isolates were subsequently transferred to the next higher concentrations like 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 mM of $NiCl_2$. The test isolates were streaked and incubated at 35ºC for 24 h and the tolerance was observed based on the growth of the culture on respective metal amended plates.

Multiple metal resistant spectrums

After screening of MIC, the high $NiCl_2$ tolerant isolates were subsequently transferred to the plates containing higher concentrations with multiple metals like 15.0, 20.0 and 25.0 mM of $CuSO_4$ (Copper Sulphate), 6.0, 8.0 and 10.0 mM of $CdSO_4$ (Cadmium Sulphate), 6.0, 8.0 and 10.0 mM of $CoSO_4$ (Cobalt Sulphate), 6.0, 8.0 and 10.0 mM of $PbNO_3$ (Lead Nitrate), 1.5, 2.0 and 2.5 mM of $HgCl_2$ (Mercury Chloride), 6.0, 8.0 and 10.0 mM of $K2Cr2O_7$ (Potassium dichromate), 6.0, 8.0 and 10.0 mM of $FeSO_4$ (Ferrous sulfate), 6.0, 8.0 and 10.0 mM of $MnSO_4$ (Manganese Sulphate) and 6.0, 8.0 and 10.0 mM of $ZnSO_4$ (Zinc Sulphate) respectively. Similarly the multiple metal resistant spectra of other metal resistant isolates were also recorded.

Results and discussion

Population dynamics of nickel resistant bacteria (NiRB) in surface sediments

Bacteria isolated from natural habitats show metal tolerance and multi metal resistance, which provide them selective advantages. It may arise from non-specific mechanisms, such as impermeability of the cell, or it may be the result of specific resistance transfer factors (Sterrit and Lester, 1980). The nickel resistant bacterial population in the surface sediment samples studied in Bay of Bengal, off Chennai during the three consecutive years varied between 3.3 and 5.3 $\log_{10}$ CFU/g with an average of 4.4 $\log_{10}$ CFU/g of sediment. Samples collected near the shore showed more nickel resistant population and showed reduction with the increase in depth. The trend of NiRB population was found to be Fishing Harbor Traverses > Chennai Harbor Traverses > Cooum River Traverses = Ennore Traverses > Adyar River Traverses (Table 1). Bezverbnaya *et al.* (2003) explained that colony-forming heterotrophic bacteria isolated from several locations differed in extent and character of heavy metal pollution in the coastal waters of Primorye, Russia. The population of NiRB was almost similar during the three consecutive years with slight changes. Highest NiRB populations were recorded in sediment samples of harbor regions (5.3 ± 0.13 Log_{10} of CFU/g) when compared to the river mouth traverses like ART, CRT and ET (4.9 ± 0.24 Log_{10} of CFU/g) (Korttalaiyar River), which scored lesser number of NiRB. Similar condition was reported in harbour region of Mar Piccolo of Taranto (Ionian Sea, Italy) (Cavallo *et al.*, 1999) and in Eastern Harbour of Alexandria, Egypt (Gouda, 2006).

Higher NiRB populations were recorded at shoreline of all traverses (4.7 to 5.3 Log10 of CFU/g) and the density of the populations decreased with the distance away from the shore (3.5 to 4.4 Log_{10} of CFU/g). Viable bacterial populations in water and sediments were higher in nearshore areas than offshore areas of Beaufort Sea (Kaneko *et al.*, 1976). The association of microorganisms with sediment particles is one of the primary complicating factors in assessing microbial fate in aquatic systems. Majority of microbes in aquatic systems are associated with sediments and that these associations influence their survival and transport characteristics (Jamieson *et al.*, 2005; Gouda, 2006; Karthikeyan *et al.*, 2007). The samples collected at less than 10 m depths were rich in heavy metal resistant populations. Wollast (1991) reported that the coastal and shelf sediments play a significant role in the demineralization of organic matter which supports the growth of microbes. Anon (1997) also reported the higher bacterial population density in the sediments than water, which is generally due to the rich organic content of the former and the lesser residence time of the microorganisms in the water column than the sediments. Horizontal and vertical distribution of bacterial populations in sediments is influenced by various factors, such as the physicochemical nature of sediments and the presence of high organic matter concentrations. In aquatic ecosystems, the flux of organic matter to the bottom sediments depends on primary productivity at the ocean surface and on water depth.

Isolation and identification of nickel resistant bacteria

Total number of NiRB isolates

Totally 208 Ni^{2+} resistant bacterial isolates were randomly scored from the initial screening. Out of 51 Ni^{2+} resistant isolates scored during 2006-07, 26 isolates were selected from the samples of PRM and 25 from POM. Among the 61 isolates recorded in 2007-08, 31 isolates were from the PRM samples and 30 from the samples of POM. During 2008-09, 96 Ni^{2+} resistant isolates were exhibited of which 33 isolates were selected from PRM samples and 31 and 32 isolates were selected from MON and POM samples respectively (Table 2). One clear indication from this study is the observation of steady increase in nickel resistant isolates during the study period, since the nickel resistant bacteria was higher during 2008-09 when compared to 2006-07 and 2007-08. In the present study, maximum number of nickel resistant bacterial populations available during three seasons of three consecutive years were scored during PRM season when compared to MON and POM seasons and this observation was supported by a previous study on metal resistant population on Uppanar estuary in TamilNadu, India (Karthikeyan *et al.*, 2007). Sathyamurthy *et al.* (1990) isolated organisms from Pichavaram mangroves and reported that the higher metal resistant population was attributed to industrial discharge and terrigenous materials through land runoff.

Table 1. Population dynamics of Nickel resistant bacteria (Log10 of CFU/g) in the surface sediments from the inner shelf of Bay of Bengal, Chennai

		Population dynamics of Nickel resistant bacteria						
T	D(m)	2006-07	2007-08	2008-09				
		PRM	POM	PRM	POM	PRM	MON	POM
ET	Shore	5.0±0.24a	5.0±0.12a	4.9±0.12a	4.9±0.12a	4.8±0.12a	4.8±0.12a	4.8±0.12a
	4m	4.9±0.12a	4.7±0.23b	4.4±0.21b	4.5±0.22b	4.6±0.22b	4.6±0.22b	4.6±0.23b
	9m	4.6±0.11b	4.3±0.11c	4.4±0.11b	4.4±0.11b	4.4±0.11bc	4.4±0.11c	4.4±0.11c
	29m	4.3±0.21c	4.3±0.21c	4.3±0.21bc	4.3±0.21b	4.1±0.20d	4.1±0.20d	4.3±0.21c
	62m	4.0±0.10d	3.7±0.09d	3.8±0.10d	3.6±0.09c	3.6±0.09e	3.5±0.09e	3.7±0.09d
FHT	Shore	5.1±0.24a	5.0±0.24a	5.0±0.24a	5.0±0.24a	5.0±0.24a	5.0±0.24a	5.1±0.24a
	5m	5.0±0.12a	5.1±0.12a	4.9±0.12a	4.8±0.12ab	4.9±0.12a	4.7±0.11b	4.8±0.12b
	13m	4.7±0.23b	4.5±0.22b	4.6±0.22b	4.6±0.22bc	4.6±0.22b	4.3±0.21c	4.4±0.22c
	31m	4.4±0.11c	4.0±0.10c	4.4±0.11c	4.4±0.11c	4.4±0.11c	4.1±0.10d	4.3±0.11c
	60m	4.4±0.22c	3.5±0.18d	4.3±0.21c	4.1±0.21d	4.1±0.21d	4.0±0.20d	4.0±0.20d
CHT	Shore	5.2±0.12a	5.2±0.12a	5.0±0.12a	4.9±0.12a	5.0±0.12a	5.2±0.12a	5.3±0.13a
	5m	5.0±0.24ab	4.7±0.23b	5.0±0.24a	4.5±0.22b	5.0±0.24a	4.9±0.24b	5.0±0.24b
	17m	4.8±0.12b	4.6±0.11b	4.8±0.12b	4.4±0.11bc	4.7±0.11b	4.3±0.11c	4.4±0.11c
	30m	4.3±0.21c	3.8±0.19c	4.4±0.22c	4.3±0.21c	4.4±0.22c	4.1±0.20c	4.3±0.21c
	64m	3.8±0.10d	3.3±0.09c	4.2±0.10d	3.6±0.09d	4.1±0.10d	3.7±0.09d	4.0±0.10d
CRT	Shore	4.7±0.22a	4.8±0.23a	4.7±0.22a	4.7±0.23a	4.7±0.23a	4.7±0.23a	4.8±0.23a
	8m	4.6±0.11a	4.6±0.11ab	4.6±0.11a,b	4.6±0.11ab	4.7±0.11a	4.5±0.11b	4.6±0.11b
	19m	4.6±0.22a	4.4±0.21b	4.5±0.22b	4.5±0.22bc	4.6±0.22a	4.5±0.22b	4.6±0.22b
	34m	4.3±0.11b	4.0±0.10c	4.3±0.11c	4.4±0.11c	4.3±0.11b	4.3±0.11c	4.4±0.11c
	72m	3.8±0.19c	3.6±0.18d	4.0±0.20d	4.1±0.20d	4.0±0.20c	4.1±0.20d	4.1±0.21d
ART	Shore	4.9±0.12a	4.9±0.11a	4.6±0.11a	4.7±0.11a	4.7±0.11a	4.7±0.11a	4.8±0.12a
	6m	4.9±0.24a	4.7±0.24a	4.6±0.22a	4.7±0.23a	4.6±0.22a	4.7±0.23a	4.7±0.23ab
	18m	4.6±0.11b	4.3±0.11b	4.4±0.11b	4.5±0.11b	4.4±0.11b	4.4±0.11b	4.6±0.11b
	20m	4.3±0.21c	4.3±0.21b	4.2±0.21c	4.3±0.21c	4.2±0.21c	4.1±0.21c	4.3±0.21c
	45m	4.0±0.10d	3.5±0.09c	4.0±0.10d	4.1±0.10d	4.1±0.10c	4.0±0.10c	4.2±0.10c
	65m	3.8±0.19d	-	4.0±0.20d	3.9±0.20e	3.8±0.19d	3.6±0.18d	3.9±0.20d
AVG	4.5	4.3	4.5	4.4	4.4	4.4	4.5	
MAX	5.2±0.12	5.2±0.12	5.0±0.24	5.0±0.24	5.0±0.24	5.2±0.12	5.3±0.13	
MIN	3.8±0.10	3.3±0.09	3.8±0.10	3.6±0.09	3.6±0.09	3.5±0.09	3.7±0.09	

T- Traverses; Values are mean of three replicates with SD. Values in a table with same letter are not statistically significant ($p < 0.05$) according to the Duncan's multiple range test.

Identification of NiRB strains isolated based on morphological and biochemical tests

Vibrio spp. (45) was found to be the most dominant genus resistant to $NiCl_2$ represented from all traverses of the study area. The second dominant position was held by genus *Bacillus* spp. (23) which were also isolated from all traverses. This was followed by *Halomonas* spp. (21), *Marinobacter* spp. (20), *Flavobacterium* spp. & *Micrococcus* spp. (17), *Alteromonas* spp. (14), *Alcaligenes* spp. (13), *Pseudomonas* spp. (12) and *Marinomonas* spp. & *Acinetobacter* spp. (7). Nearly 12 NiRB isolates could not be identified by biochemical methods (Table 3). Alavandi (1989) observed the abundance of *Vibrio* sp. in coastal waters of Cochin. Parvathi *et al*. (2009) reported *Bacillus* strains as dominant group in the coastal environment of Cochin, India. Promod and Dhevendaran (1987) & DeSouza *et al*. (2000) reported *Bacillus* sp. as the dominant group in the inshore areas of west coast of India.

The phenotypic characterization of NiRB exhibited the dominance of Gram-negative phenotype when compared to Gram-positive ones in the study area of Bay of Bengal, off

Table 2. Total number of nickel resistant bacterial isolates scored in the study area

T	Depth (m)	2006-07		2007-08		2008-09			T. wise	Near shore	Inner shelf
		PRM	POM	PRM	POM	PRM	MON	POM			
ET	Shore	1	2	3	2	2	2	2	43	28	15
	4m	2	2	2	2	2	2	2			
	9m	2	1	1	1	1	1	2			
	29m	1	0	1	1	1	1	1			
	62m	0	0	0	0	0	0	0			
FHT	Shore	2	2	2	2	3	2	2	38	27	11
	5m	1	1	2	2	2	2	2			
	13m	1	1	1	1	2	1	1			
	31m	0	0	1	1	1	0	0			
	60m	0	0	0	0	0	0	0			
CHT	Shore	2	2	2	2	2	2	2	38	27	11
	5m	2	1	2	2	2	2	2			
	17m	1	1	1	1	1	1	1			
	30m	1	0	0	1	1	0	1			
	64m	0	0	0	0	0	0	0			
CRT	Shore	1	2	3	2	2	3	2	45	27	18
	8m	1	1	2	2	2	2	2			
	19m	1	1	1	1	2	1	2			
	34m	1	1	1	1	1	1	1			
	72m	0	1	0	0	1	0	0			
ART	Shore	2	2	2	2	2	3	2	44	27	17
	6m	2	1	2	2	1	2	2			
	18m	1	1	1	1	1	1	2			
	20m	1	1	1	1	1	1	1			
	45m	0	1	0	0	0	1	0			
	65m	0	0	0	0	0	0	0			
Season wise	26	25	31	30	33	31	32				
Year wise	51		61			96					
Total				208							

T- Traverses; m- Meters

Chennai (Table 3). This observation was in agreement with Moriarty and Hayward (1982) who reported that the majority of bacteria in marine environments are Gram-negative. Tolerance to heavy metals was also reported to be well pronounced in Gram-negative bacteria (Nair *et al.*, 1993; Duxbury, 1986). The majority of the isolates belong to the genera *Pseudomonas* sp., *Vibrio* sp. and *Flavobacterium* sp. Beja *et al*. (2002) also suggested that considerable functional diversity might exist even in bacteria having similar taxonomic identity even at the molecular level. The diversity of the Gram-negative species was more evident in sediment samples. All the water environments in the coastal region showed a tendency to natural bacterial communities dominated generally by Gram-negative bacterial populations (Saida *et al.*, 2001).

MIC for NiRB in the study area

After initial screening (2.0 mM $NiCl_2$) NiRB isolates were transferred subsequently to the higher concentrations of Nickel viz., 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 mM. Many of these isolates were able to resist $NiCl_2$ upto 25.0 mM concentrations (Table 4). Out of 208 NiRB isolates selected surface sediment samples, 93 were able to grow in 5.0 mM, 46 isolates in 10.0 mM, 26 isolates in 15.0 mM, 8 isolates in

Table 3. Total number of isolated NiRB strains from the innershelf of Bay of Bengal, Chennai

Strains	2006-07		2007-08		2008-09			ST
	PRM	POM	PRM	POM	PRM	MON	POM	
Gram-Negative								
Acinetobacter spp.	1	1	1	1	1	1	1	7
Alcaligenes spp.	2	2	2	1	2	2	2	13
Alteromonas spp.	2	2	2	2	2	2	2	14
Flavobacterium spp.	1	1	3	3	3	3	3	17
Halomonas spp.	3	3	3	3	3	3	3	21
Marinobacter spp.	4	3	2	2	4	2	3	20
Marinomonas spp.	1	1	1	1	1	1	1	7
Pseudomonas spp.	1	1	2	2	2	2	2	12
Vibrio spp.	5	5	7	7	7	7	7	45
	20	19	23	22	25	23	24	156
			Gram-positive					
Bacillus spp.	3	3	3	4	3	4	3	23
Micrococcus spp.	2	2	3	2	3	2	3	17
Unidentified	1	1	2	2	2	2	2	12
	6	6	8	8	8	8	8	52
Total of G+& G-	26	25	31	30	33	31	32	
Grand Total					208			

ST; Species wise Total

20.0 mM and 4 isolates in 25.0 mM of $CuSO_4$. Most of the higher MIC isolates were scored from river mouth region of the study area. Patel *et al*. (2006) reported *Pseudomonas fragi* resistant to nickel at concentrations of 2.5 mM from effluent samples. Lee *et al*. (2001) reported a strain of *P. putida* strain 06909 that exhibited resistance at 11.5 mM of zinc and 1.0 mM of nickel. Several nickel resistant strains have been identified as *Ralstoniaeutropha* (*Alcaligeneseutrophus*) isolated from different biotopes heavily polluted with heavy metals (Lelie *et al*., 2001) and thus some of the isolates of the present study were highly resistant to Ni^{2+}, indicating the stress of Ni2+ on the bacterial population. Chisholm *et al*. (1998) reported 15 isolates of *ThioBacillus ferrooxidans* recovered from the tailings fields of a Cu-Ni mine using filtration of water samples, which showed inhibitory concentrations of Cu2+ and Ni2+ with a range of 80.0 mM to more than 320.0 mM to both the metals. Cultivable heterotrophic bacterial communities represent only a small fraction of taxa present in the environment; nevertheless, some authors consider the fraction of the cultivable bacteria to be an useful indicator for measuring shifts on bacterial communities (Stephan *et al*., 1999). Several authors consider estimating the number of cultivable heavy metal-tolerant bacteria as more effective in establishing the toxic effects of heavy metals instead of the number of total cultivable bacteria (Doelman *et al*., 1994; Huysman *et al*., 1994; Viti and Giovannetti, 2001).

Multiple metal ions resistance in NiRB isolates

Based on the resistance to Ni^{2+} at 20.0 mM and above, 8 of the Ni^{2+} resistant isolates were further selected for identification and multi metal resistance studies, which were labeled as VKRKNi6 to VKRKNi13. Out of 8 Ni^{2+} resistant isolates selected, 4 of them showed resistance to Cu^{2+}upto 15.0 mM, Cd^{2+} and Co^{3+}upto 10.0 mM was tolerated by 2 isolates each, 1 isolate resisted Pb^{2+} at 10.0 mM, 1 isolate resisted Hg^{2+}upto 2.0 mM, 7 isolates showed growth with Cr^{6+} upto 6.0 mM, 1 isolate resisted Fe^{3+} at 8.0 mM, Mn^{2+} was tolerated by 2 isolates and 6 isolates resisted Zn^{2+} upto 6.0 mM (Table 5). Every group of the metal resistant isolates of the present study exhibited various levels of resistance against a wide spectrum of heavy metal ions and this observation has been very well recognized by many workers (Yilmaz, 2003; Kamalakannan *et al*., 2006; Adarsh *et al*., 2007; Roy and Nair, 2007; Abskharon *et al*., 2008; Altug and Balkis, 2009). Multiple tolerances occur only to toxic compounds that have similar mechanisms underlying their toxicity. Since heavy metals are all similar in their toxic mechanism, multiple tolerances are common phenomena among heavy metal resistant bacteria.

The isolation of heavy metal resistant isolates (Ni^{2+}), which could tolerate such high levels of metal ions, has provided

Table 4. Minimum inhibitory concentration (MIC) for NiRB isolates from the innershelf of Bay of Bengal, Chennai

Seasons	T	MIC						
		2*	5	10	15	20	25	30
2006-07 PRM	ET	6	4	2				
	FHT	4	2	1				
	CHT	6	3	1				
	CRT	4	2					
	ART	6	3	2	1	1		
2006-07 POM	ET	5	2	1				
	FHT	4	2	1	1			
	CHT	4	1					
	CRT	6	3	1				
	ART	6	3	1	1			
2007-08 PRM	ET	7	4	2	2			
	FHT	6	2	1				
	CHT	5	2					
	CRT	7	3	2	1			
	ART	6	3	2	2	1	1	
2007-08 POM	ET	6	2	1				
	FHT	6	1					
	CHT	6	2					
	CRT	6	3	2	1			
	ART	6	2	1				
2008-09 PRM	ET	8	4	2	1			
	FHT	6	3	1				
	CHT	8	4	2	1			
	CRT	5	3	2	2	1	1	
	ART	6	2	1				
2008-09 MON	ET	6	2					
	FHT	5	1					
	CHT	5	3	2	1			
	CRT	7	3	2	1			
	ART	8	4	3	3	2	1	
2008-09 POM	ET	7	3	2	2	2	1	
	FHT	5	3	1				
	CHT	6	3	2	1			
	CRT	7	3	3	3	1		
	ART	7	3	2	2			
Total		208	93	46	26	8	4	Nil

*-Initially screened concentration; T- Traverses.

an opportunity to infer that these organisms were under constant metal stress exhibited in the study area due to anthropogenic input. The use of bacteria for rehabilitation of polluted environments may provide an ecologically sound method for abatement of pollution and a natural solution for recovery of contaminated sediment and water (Gupta and Ali, 2004). In addition to the human settlement along the banks, the small industries may add to the metal pollution in this zone. Furthermore, for many years, heavy wastes from nearby streams have affected this region. In urban areas, runoff from yards, sewage overflows, and sewage discharges during rain events may increase the total number of microbes at the beach (Sampson *et al*., 2006). The maximum metal accumulation was attributed to higher heavy metal discharge mainly through anthropogenic inputs, industrial effluent or land run-off (Karthikeyan *et al*., 2007). The frequency of tolerant microorganisms may increase with an increase in toxic metal levels (Huysman *et al*., 1994; Kunito *et al*., 1997). This can lead to a decrease in species diversity and therefore a shift in microbiota composition (Pennanen *et al*., 1996). Shakibaie and Harati (2004) suggested that the incidence of a high metal resistant population resulted from increased environmental pollution. Thus, the continuous stress of heavy metal ions in the study area is the cause for less diversity. Hence, there is an urgent need for controlling the input of the metal ions in the coastal zone of Chennai.

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Table 5. Metal resistance spectrum of NiRB isolates from the innershelf of Bay of Bengal, Chennai (Conc. in mM)

Isolates	MIC	Cu			Cd			Co			Pb			Hg			Cr			Fe			Mn			Zn		
code		15	20	25	6	8	10	6	8	10	6	8	10	1.5	2	2.5	6	8	10	6	8	10	6	8	10	6	8	10
VKRKNi6	20	-	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
VKRKNi7	20	+	-	-	+	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-
VKRKNi8	20	+	-	-	+	+	-	+	+	+	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
VKRKNi9	20	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VKRKNi10	25	+	-	-	+	-	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	-	-
VKRKNi11	25	+	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-
VKRKNi12	25	-	-	-	+	-	-	+	+	+	+	+	-	+	+	-	+	-	-	+	+	-	+	+	-	+	-	-
VKRKNi13	25	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

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