

Unusual rise in mercury resistant bacteria in coastal environs.
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Unusual rise in mercury resistant bacteria in coastal environs

N. Ramaiah* and Jaysankar De

National Institute of Oceanography, Dona Paula, Goa 403 004, India

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*Corresponding author: National Institute of Oceanography, Goa-403004, India.

Fax: 91 832 456702; Tel: 91 832 456700; email: ramaiah@darya.nio.org

Abstract

A sharp rise in mercury resistant bacteria (MRB) capable of tolerating very high concentration of Hg is seen in the last 3-4 years in the coastal environs of India. While none or negligible colony forming units (CFU) of bacteria were counted on seawater nutrient agar with 0.5ppm ($\equiv 2.5 \mu\text{M}$) Hg (II) as HgCl_2 until 1997, over 13 to over 75% of CFU grew on 20 times higher - 10 ppm ($\equiv 50 \mu\text{M}$) - Hg concentrations from almost every recently examined marine samples. Although exceptionally high counts of MRB (96% of CFU) were recorded from samples collected from the polluted zones off Mumbai, the MRB capable of growth on seawater nutrient agar with 10 ppm Hg were quite abundant in most samples collected from many locations with low or no pollution effects. We noticed for the first time the occurrence of aerobic heterotrophic bacterial isolates capable of growth at 50 ppm ($\equiv 250 \mu\text{M}$) Hg. Such MRB grew in much higher concentrations of many toxic xenobiotics than the Hg sensitive ones. Based on the unusual high counts of MRB and some simple experiments, we propose that many marine bacterial species are selected, acquiring plasmids and/or transposable elements and, modifying Hg, which according to recent studies, is on escalation in marine habitats.

Introduction

Leaching naturally [22] and added anthropogenically [18, 29] mercury (Hg) keeps accruing in seawater and marine sediments. Passive uptake of Hg and its compounds by many osmotrophs including bacteria leads to their modification and, in changed forms, they move up the food chain, get bio-magnified and reach human beings where they produce chronic and/or acute ailments [23]. In addition to natural processes of bedrock leaching, Hg and its compounds enter aquatic bodies by washings from soils and atmosphere [34], effluents from chlor-alkali production units [7, 17, 37], chemical laboratories, instrument manufacturing, dentistry and fluorescent light bulb breakage [27] and from sewage treatment facilities [48]. Of these, the atmospheric deposition of Hg emitted by power production is the largest anthropogenic source [29]. Our current understanding is that due to very slow biogeochemical remobilization, toxic heavy metals (e.g., Hg, Cd, As, Cu, Pb) forming complexes with organic components of marine sediments [13, 23, 24, 35] tend to accumulate leading ultimately to deleterious situations to marine life and in turn, human beings. Thus, environmental effects of metal pollution are all encompassing [8].

Substantial changes in physiological responses such as viability [17, 36, 38], metabolism, resting stages and death [1] are caused in bacteria by adverse anthropogenic and natural processes. When compared with other biota, the responses of heterotrophic bacteria to environmental changes are rapid and consistent [40]. Monitoring of bacterial responses is useful for assessing marine microbial heterotrophy [30] and environmental quality [31, 41, 42]. Thus, for a long time now, many investigations have been using bacterial indicators (e.g., coliform groups) to assess effluent discharges in to coastal/ marine environment. Observations on occurrence and distribution of native bacteria capable of metal tolerance are of relevance in microbial ecology not only to understand the extent of metal pollution [8, 9, 26, 43, 44] but also to realize the potential of metal tolerant bacteria to detoxify some of the toxic substances [10, 11, 16]. Induction of Hg lyases, oxidation/reduction, methylation/demethylation are some of the means bacteria adopt to deal with Hg [8, 9, 13, 25].

As a part of regular coastal environmental impact assessment surveys, mercury resistant bacteria (MRB) were routinely enumerated from many locations along the Indian coast since March 1993. Water and sediment samples were plated on seawater nutrient agar (SWNA)

containing 0.5 ppm ($\equiv 2.5\mu\text{M}$), 1 and 5 ppm Hg (II) as HgCl_2 with a view to observe changes in counts of bacteria capable of growth in the presence of mercury. The MRB were not at all abundant prior to and during 1997 either off Chennai, one of the very polluted regions on the East coast or off Positra, a non-polluted region on the West coast. However, we continued to enumerate their populations from all possible coastal locations for environmental impact assessments and also to collect isolates of bacteria to evaluate their potential for pollution remediation. Upon noticing the unusually high counts of MRB during 1999 in SWNA with 5 ppm Hg, various higher concentrations of Hg (i.e., 20, 30, 50, 75 and 100 ppm) were added to SWNA. Several seawater and sediment samples were examined and the abundance of bacteria with high tolerance for Hg confirmed.

Materials and Methods

Study sites

Various locations sampled for this study are shown in Fig 1. Sampling was carried out from polluted (Mumbai, Chennai, Mangalore, Kulai, Padubidri and Ratnagiri), moderately polluted (Malvan, Karwar, Paradip, Nagapattinam) and non-polluted (Positra, Marmugao, Terekhol and Gopalpur) coastal locations.

The Chennai and Mumbai coastal regions receive a wide variety of industrial, urban and shipping related effluents and are among the most pollution affected zones along the Indian coast. During the last 15 years or so the coastal waters off Mangalore and the nearby Kulai and Padubidri have undergone rapid changes owing to very speedy industrialization, expanded harbor activities and an increasing human settlement (presently from these three places population is >5 million). Varieties of effluents, some after treatment, reach the seas around here and, because of bulk quantities, they affect a large span of coastal water similar to those off Mumbai and Chennai. Both treated and untreated discharges from manufacturing units (e.g., polyvinyl chloride [PVC] pipes and other plastics) and urban effluents from Ratnagiri town (population >1.8 million) reach the coastal waters. The sea off Malvan receives ~ 0.5 million litres of effluents per day from iron-ore mining activities in addition to offshore oil seepage [3]. Although no pollution effect is yet attributable [3], the sea off Terekhol (20 km north of Marmugao) receives ca <0.04 million litres of iron ore mining effluents per day. Sampling site

at Paradip is close to the harbor and is subjected to various perturbations due to relatively active shipping traffic. The coastal zones off Marmugao (Goa) in the West coast and Gopalpur on the East coast are, to a very large extent, less perturbed. The nearshore intertidal region off Positra has a vast expanse of coral reef and is quite pristine with very rich biodiversity.

Enumeration of MRB

During most sampling occasions, water and sediments samples were collected from 9 or 12 stations within 30-35 Km² at each sampling location. At least three water samples and one sediment sample were collected along 5, 10 and 15m depth contours during each collection. During 1993-1997, samples from different locations (Table 1) were plated on to seawater nutrient agar (SWNA with a composition l⁻¹: peptone [Difco] 5.0g, yeast extract [Difco] 3.0g, agar [Difco] 15g, aged seawater 500 ml and deionized water 500 ml and pH 7.5±0.1) containing 0.5, 1 and 5 ppm Hg. Stock solution of HgCl₂ was added to autoclaved media to achieve desired Hg concentrations. Up to 25 ml water samples and 3 ml supernatants of 10 and/or 100 times diluted sediment samples (lower dilutions plated for clayey sediments) were filtered through 0.22 µm membrane filters (Millipore, USA) and these filters placed on SWNA containing Hg for enumerating MRB. As mentioned earlier, upon noticing very high counts for the first time on all plates with 5 ppm Hg in February 1999 from all samples collected off Marmugao (Goa), we increased the concentration of Hg to 10, 20, 30, and 50 ppm. Though the CFU from plates with 50 ppm sometimes exceeded those counted previously from SWNA plates with 5 ppm Hg, to be consistent, we decided to use 10 ppm for MRB enumeration reported here. The extreme tolerance limit of many randomly picked up environmental isolates from the SWNA plates with 10 ppm Hg was checked by growing them on 20, 30, 50, 75, 100 and 200 ppm. A reference strain, *Photobacterium leognathi* (ATCC 25521: PL3) unable to grow in medium containing ≥ 1.0 ppm Hg [41] was included to ascertain the Hg tolerance of environmental isolates.

Growth patterns of MRB

Although all data are not shown, growth of many MRB strains was checked in the presence -and absence- of mercury, DDT and phenol in seawater nutrient broth (SWNB, containing [L⁻¹] 5.0 g peptone; 3.0 g yeast extract, 500 ml aged seawater and 500 ml deionised water). Growth

of these strains was also monitored by supplementing SWNB with 20 ppm Hg, 20 ($\equiv 212.6\mu\text{M}$) ppm phenol and 10 ($\equiv 28.2\mu\text{M}$) ppm DDT. To avoid over or under inoculation, 5 μl of fresh cultures of seven MRB strains were inoculated into SWNB flasks. Standard solutions of HgCl_2 were added to get Hg concentrations of 10 and 50 ppm. All flasks were then incubated on a shaker at 180 rpm at 28 °C and OD_{660} measured every six hours for 120h.

Resistance Induction

An experiment was done to examine if Hg resistance develops in environmental isolates sensitive to Hg (designated as Hg^s) by growing them close proximity (without cell-cell contact) of MRB. This experiment was to test whether slow volatilization of Hg in the medium by the MRB strains in proximity would aid the previously sensitive strains to adjust to toxic *milieu* and acquire Hg resistance. As many as 120 environmental isolates were randomly collected and checked for their ability to grow in the presence of Hg by individually streaking them on to SWNA with 10 ppm Hg. Thirty seven of these 120 isolates did not grow on SWNA with Hg. They were designated as Hg^s . The Hg^s strains were spot inoculated close to MRB strains on SWNA with 10 ppm Hg. Pure cultures of 37 Hg^s as well as three MRB strains were inoculated alternately ca 5-8 mm close to each other onto SWNA plates containing 10 ppm Hg and incubated at $28 \pm 2^\circ\text{C}$ for 72 h. Twenty-five previously sensitive strains grew on SWNA with 10 ppm Hg when MRB were in their close proximity. All these 25 strains were transferred individually onto SWNA with 10, 25 and 50 ppm Hg to confirm their acquiring the ability to grow in SWNA with Hg.

Tolerance of combined xenobiotic toxicity

Ability of MRB to grow in the presence of different toxic xenobiotics was tested by adding DDT, phenol and Hg with four varying combinations with the concentrations mentioned in Table 4. The MRB strains isolated from four samples each collected off Goa and Gopalpur were grown in SWNB containing toxic xenobiotics. All cultures were subjected to aeration by growing them on a rotary at 180 rpm at 28 °C for 14 days before confirming their inability to grow (no visible turbidity) in presence of these toxicants. Absence of growth was ascertained by streaking a loopful of liquid culture on to SWNA from where visible growth (i.e., turbidity) was

not evident or, unable to be discerned. Further, these cultures were also examined under the microscope to ascertain growth inhibition by xenobiotics.

Results and Discussion

Enumeration of MRB

In 1993, bacteria growing on SWNA with 0.5 ppm Hg from water samples collected off Goa (from Marmugao in south to Terekhol in north) ranged from 1.8 to 15.2 cells ml⁻¹ and those from off Mangalore, 1.84 to 21.7 cells ml⁻¹. These CFU accounted for mean percentages of 1.24% of CFU off Goa and 4.86% off Mangalore (Table 1). Furthermore, MRB were none to <3.45% of all CFU in 58 samples — i.e., 20 samples from ca.60 Km Chennai-Kalpakkam stretch off Tamil Nadu coast; 22 from a 120 Km Mangalore-Bhatkal stretch off Karnataka and, 16 samples from 80 Km Talpona -Terekhol off Goa — examined during 1994 (data not shown). In spite of the large volumes of samples examined, there were no colony formations on SWNA with 5 ppm Hg from any of the 214 samples examined until December 1997.

Despite the current pollution levels being low, very insignificant or none off Goa (Marmugao, Terekhol), Nagapattinam and Gopalpur, the MRB growing on SWNA with 10 ppm (50µM) Hg were quite high, always over 13% of the CFU during 1999 (Table 2). Apparently, as can be seen from Table 2, their percentages have been high during 2001 and 2002 from most locations where repeat sampling was done. The MRB were also substantial in all samples collected off Karwar, a moderately polluted coastal strip. With a population of over 1.4 million, the township of Karwar discharges substantial amounts of municipal and shipping related wastes and its marine zone is subjected to effects of speedy industrialization and naval dock-building. Off Mumbai, most polluted of all sampled locations, the MRB formed a staggering 96% of water [n=4] and 71.4% of sediment [n=3] CFU during May 2000. Unlike during May 2000 and May 2002, the percentages of MRB in the sediment were lower during November 2001. Irrespective of current levels of pollution at locations where sampling was carried out, the MRB increase was rapid during and post 1999 (Fig 2).

Many previous studies from the European and North American coasts have reported the occurrence of culturable heterotrophic bacteria capable of tolerating ca. 0.5 ppm Hg from

marine locations affected by a variety of anthropogenic activities [13, 43, 45]. Similar to the results we obtained prior to 1999, none to 10% of the CFU have been reported to be growing in general nutrient agar media containing 0.5 ppm Hg. However, none of the earlier studies had examined Hg concentrations tolerated by the environmental heterotrophic bacteria as high as the ones checked out in this study during and after 1999. An increase in the resistant fraction of culturable heterotrophic bacteria in the aquatic ecosystems is due to the growth primarily of mercury-resistant bacteria [12, 35, 43, 44].

It has been observed by many earlier studies that bacteria carrying Hg resistances also are resistant to many antibiotics and other toxic chemicals [10, 11, 16] by virtue of carrying plasmids and/or transposons encoding metal and antibiotic resistances in a genetically linked way. From a compilation of data on mercury concentrations along the Indian coast (Tables 1 and 2 and also see [42]), it is possible to suggest that its increased use for industrial and agricultural practices and the subsequent effluent discharges in to marine regimes continuously increase its concentrations. This might lead to selection of microbial assemblages capable of high tolerance to Hg.

Growth patterns of MRB

Environmental isolates of MRB were identified by an array of physiological and biochemical tests. As many as 30 environmental isolates were thus characterized and these results would be published elsewhere. Representatives of all five MRB groups *viz.*, *Pseudomonas*, *Proteus*, *Aeromonas*, Enterobacteriaceae and *Xanthomonas* from different samples collected from the Indian coasts exhibited prolonged lag phase in the presence of Hg or DDT [19]. Growth curves of only one MRB strain *Pseudomonas* (CH07) and that of the Hg sensitive *P. leiognathi* are shown in Fig. 3. As seen from the growth curves of *Pseudomonas* CH07, during such prolonged lag, the MRB appear to prepare necessary physiological mechanisms including induction of *mer* system. As observed previously by several studies [20, 21, 28], in all the aerobic/facultatively anaerobic heterotrophic cultures examined in this study, the inducible mercury reductase might have enabled them to grow rapidly once adapted to the toxic *milieu*.

One criticism with inoculating bacteria for monitoring growth into media containing Hg or other toxicant(s) is the size of inocula. In that, genetically resistant bacteria have a shorter lag

phase than the genetically sensitive ones. We took care of this by adding identical volumes of inocula into all the flasks. As can be seen from the figure, the growth response in flasks without toxicants, was faster and usual as would happen with most aerobic bacteria. But the prolonged lag phase in *Pseudomonas* CH 07 and in all other six strains we examined implies that the MRB do undergo physiological adjustments before being able to grow in the toxic milieu.

Induction of Mercury Resistance

In all, 37 of the 120 Hg^s isolates tested were found to be extremely sensitive to Hg as discerned by their inability to grow in SWNA with 10 ppm Hg. Of the 37 that did not grow on SWNA with 10 ppm Hg, only 25 were able to grow in the proximity of MRB. All these 25 isolates were confirmed to have acquired mercury resistance as they all grew on SWNA containing 10 and 25 ppm Hg when inoculated individually (*i.e.*; without a known MRB in their proximity). Fifteen of the 25 strains grew even in 50 ppm Hg within 72 h. Rasmussen and Sorensen [43] noticed high levels of self-transmissible Hg resistance plasmids in bacterial communities from a mercury-contaminated site inside the Copenhagen Harbor, which had higher abundance of MRB. These authors reported 62% of the isolates from this location to contain plasmids while about 30% of the isolates had plasmids from a non-contaminated site though there were no differences in Shannon-Weaver diversity indices of the isolates from polluted and non-polluted locations. As seen in this study, about 40% (15 of 37 isolates) of the Hg^s sensitive isolates tolerated 50 ppm Hg when grown in very close proximity of known MRB. Although this is not a direct evidence, as Rasmussen and Sorensen [44] also suggest, it is likely that many species of marine and estuarine bacteria continue acquiring mercury resistance through inter-species horizontal gene transfer.

Tolerance of combined xenobiotic toxicity

Twenty-one of 120 environmental isolates were capable of growth on SWNA with 50 ppm Hg (Table. 3). Although negative for growing in 75 and 100 ppm Hg, 33 of 34 environmental samples examined had MRB (4.5-7.8% of CFU) capable of growth in 50 ppm. Hg sensitive (Hg^s) reference strain, *P. leognathi* did not grow in any of these conditions.

The facts – that a minimum of 13% of CFU and 69% of 120 isolates from routine but well defined SWNA grew on 10 ppm Hg – are strong indications of significant numbers of native

bacteria to be already resistant to Hg. Such environmental isolates are potentially useful in heavy metal pollution bioremediation [16]. Unlike all the Hg^s strains including the reference one, many strains tolerating 50 ppm Hg were able to grow in seawater nutrient broth with a single or combinations of toxic xenobiotics. In addition, many strains capable of growth in 50 ppm Hg also grew in seawater nutrient broth with several other toxicants added individually. Results of these experiments are published elsewhere [19]. Briefly, 12 of 15 (75%) strains grew in 1000 ppm phenol. Eleven of 17 (ca. 66%) grew in SWNB with 100 ppm Cd, 100 ppm Cu, 100 ppm Zn, 100 ppm Pb and in 100 ppm DDT. Five of 17 (30%) strains grew in 93 ppm penconazole, a new class of fungicide, all 17 grew in 91 ppm profenofos (a pesticide) and in 95 ppm metolachlor (a herbicide). From these observations we suggest that only such MRB, capable of high tolerance for mercury, would bio-transform or completely degrade varieties of toxic residuals in polluted marine environments.

Average concentration of Hg in the Indian Ocean environments has been shown to be on the increase [37]. From the technical publications of NIO during 1990-1999 [2-7], it is discernible that Hg is in the range of 44-1390 ng g⁻¹ dried sediment and 0-2100 ng l⁻¹ seawater along the Indian coast. These maxima are higher than the global ocean averages [24]. In addition, from where ever data are available, an increasing trend in Hg concentration in both water and sediments can be discerned (Tables 1 and 2). The reality that coastal environs of Mumbai, Chennai, Mangalore and Ratnagiri are polluted stems from the substantial volumes of effluent discharges containing both organic and inorganic pollutants from these urban locations. We believe and concur with Hideomi [26] that heavy metal resistant microorganisms do not arise by chance and, that there must be selectional factors like environmental contamination by heavy metals. Ecological implications of increased mercury tolerance we have observed could mean higher rates of biotransformation of toxic heavy metals; their higher mobilization through marine food web and increased levels of Hg⁰ in the atmosphere. As the prokaryotic metabolic pathways dealing with mercury [47] or its many inorganic salts generally lead to production of more toxic forms (e.g., alkylmercury), consequences of enhanced atmospheric Hg⁰ could bring about highly undesirable environmental changes. Since Hg⁰ is oxidized to Hg²⁺ by molecular oxygen [33] in marine environment, it can lead to formation of a variety of mercuric complexes, all of which are deleterious to life systems. In addition, under anaerobic culture conditions, some bacteria are not harmed by mercury as it is changed directly into nontoxic mercuric

sulfide [32]. Also, there are recent evidences that under highly anaerobic environments, many transitional metals including Hg form highly soluble anionic polysulfides that are bioavailable [14, 15, 50, 51]. However, under non-sulfide, low to moderately oxic conditions similar to many low (e.g., Malvan, Karwar, Paradip, Nagapattinam) and no-pollution (Positra, Marmugao, Terekhol and Gopalpur) locations, continued accumulation might lead to drastic reduction of heavy metal-sensitive flora and fauna leading to severely affected natural habitats.

As Silver and Phung [46] propose, toxic heavy metal resistance determinants might be preexistent to human activities. In all likelihood, varieties of natural prokaryote assemblages possessing resistance mechanisms would get 'selected' as a result of human pollution [49] in recent centuries. Such assemblages are of continued relevance in ecosystem stability. It is therefore, of pertinence to take note of adaptive responses by native microflora and decipher their involvement in biogeochemical cycling of mercury on a global scale [15]. Our long-term monitoring is thus useful to realize that already a substantial fraction of CFU and, diverse groups of marine bacteria, are capable of high tolerance for mercury and that they are rapidly adapting physiological and/or genetic strategies for coping up with changing marine ecosystems. High counts of MRB from every examined location after 1999 are strong proofs of the possibility of natural selection of mutants with an advantage to deal with adversities.

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Table 1. Total viable counts (TVC), counts of mercury resistant bacteria (MRB) capable of growth on seawater nutrient agar with 0.5 ppm Hg and, *in situ* concentrations of Hg from different locations along the Indian coast during 1993-1997

Location	Year (month)	Sample (n)	TVC range; (mean±SD)	MRB range; (mean±SD)	% of MRB in TVC	Hg conc. ¹
Chennai	1993 (Feb)	water (18)	40.5-220 ² ; (122.05±75.94)	0-0.6; (0.23±0.3)	0.19	0.62-0.68
		sed (18)	0.6-12.45; (6.62±4.23)	0-0.03; (0.0003±0.00015)	0.7	NA ³
	1996 (May)	water (9)	51.5-250.5; (130±80.63)	0-0.5; (0.28±0.26)	0.23	42-145
		sed (9)	1.32-13.41; (6.68±4.22)	0-0.05; (0.0007±0.0001)	0.006	16-44
	1997 (Apr)	water (10)	228.67-920; (539±251.39)	6-55; (29.85±19.67)	7.9	42-145
		sed (9)	8.98-513.1; (141.93±177.05)	0.08-0.48; (0.28±0.14)	8.48	0-118
Mangalore	1993 (May)	water (18)	51-400; (290.16±150.54)	1.84-21.76; (11.37±5.86)	4.86	NA
	1996 (June)	water (18)	47-848; (273.95±198.02)	0-10.53; (2.63±0.81)	0.96	10-112
		sed (18)	0.002-2.08; (0.52±0.36)	0.0002-0.02; (0.005±0.0006)	0.96	NA
	1997 (March)	water (20)	575-5500; (2046.02±1410.47)	0.2-11; (2.28±2.99)	0.09	17-177
		sed (20)	47.59-784.12; 226.17±172.87	0.015-1.18; (0.28±0.26)	1.6	18-118
	Mormugao	1993 (Feb)	water (6)	17-203; (79.75±64.25)	1.8-45.2; (6.62±5.08)	1.24
1996 (May)		water (12)	27-405.5; (92.24±78.06)	0-22.5; (7.12±3.8)	0.89	32-62
		sed (12)	40.46-2070.06; (508±492.6)	0.0003-0.005; (0.002±0.0007)	1.03	NA
Positra	1996 (Sep)	water (14)	98-300.67; (165.55±54.85)	0-0.5; (0.09±0.14)	0.06	0-102.2
		sed (5)	2.77-8.37; (4.72±2.28)	0-0.01; (0.0004±0.0005)	0.14	22-101
	1997 (Feb)	water (14)	60-326; (160.87±71.82)	0-2.2; (0.26±0.58)	0.22	5-102
		sed (5)	14.97-41.49; (24.23±11.47)	0.009-0.03; (0.02±0.009)	0.65	22-71

¹range of mercury concentration in water (ng/l) and sediment (ng/gm dry wt.) ²TVC no ml⁻¹ water and no x 10⁴ g⁻¹ dried sediment (sed); ³data not available

Table 2. Total viable counts (TVC), counts of mercury resistant bacteria (MRB) capable of growth on seawater nutrient agar with 10 ppm Hg and, *in situ* concentrations of Hg from different locations along the Indian coast during 1999-2002

Location	Year (month)	Sample (n)	TVC range; (mean±SD)	MRB range; (mean±SD)	% of MRB in TVC	Hg conc. ¹
Paradip	2002 (Feb)	water (6)	310-2670 ² ; (1213.33±846.21)	262.5-1712.5; (747.08±543.59)	83.8	NA ³
		sed (6)	5.72-1084.07; (207.56±429.87)	0.28-102.9; (34.77±46.77)	49.13	NA
Gopalpur	1999 (Mar)	water (16)	34-1350; (573.45±492.07)	1.4-660; 148.41±197.75	34.86	2-117
		sed (8)	94.12-5566.36; (2341.48±2297.12)	28.08-783.37; (260.72-243.48)	40.30	72-128
	2000 (Mar)	water (8)	150-8856; (2072.75±3057.69)	36-120; (75.5±24.97)	13.40	NA ³
		sed (8)	674.28-2531.43; (1427.86±728.21)	54.29-91.43; (74.86±13.82)	6.60	NA
Chennai	1999 (Nov)	water (5)	848-25386.68; (13492.27±9542.08)	200-480; (364±113.50)	12.65	100-2100
		sed (3)	9.93-224.6; (178.32±68.71)	6.1-78.20; (38.78±36.52)	32.20	237-338
Nagapattinam	2001 (Dec)	water (13)	12-2208; (590.77±610.28)	12.5-2200; (406.15±592.15)	95.99	0-788
		sed (11)	476.43-2279.92; (1150.49±577.58)	96.86-522.43; (234.44±141.28)	23.46	NA
Mangalore	1999 (Mar)	water (6)	2160-16320; (7408.88±5531.99)	990-5440; (2693.33±1475.80)	48.87	NA
		sed (3)	7165.21-10747.05; (9169.93±1828.80)	50.46-80.49; (70.32±17.20)	8.10	NA
	2000 (Mar)	water (8)	166.67-9813.33; (2513.33±3412.28)	27.5-1940; (558.44±640.41)	46.66	45-1088
		sed (8)	5840.94-31691.88; (19684.17±10948.2)	45.63-7690.25; (3227.25±3208.40)	13.95	133-172
Karwar	2000 (Feb)	water (4)	493.3-6400 (2583.33±2697.99)	13.3-366.7 (138.33±158.54)	15.4	NA
		sed (4)	3.82-11.04 (7.43±5.12)	1.1-1.14 (1.12±.029)	20.0	NA
Mormugao	1999 (Feb)	water (38)	65-38826.67; (4721.89±7592.84)	70-10240; (1815.26±2593.19)	44.82	152-456 ⁴
		sed (22)	7.87-16542.16; (2529.67±4048.03)	0.001-408.99; (103.24±119.89)	9.32	53-194
	2000 (Mar)	water (10)	26.67-17386.67; (1957.33±2423.51)	25-5020; (704.45±1530.14)	92.39	NA
		sed (5)	33.48-3141.83; (1230.67±1430.28)	0-2987.43; (1156.85±1242.68)	84.76	NA

Ratnagiri	2001 (Mar)	water (29)	6.67-35840; (11783±11058.93)	0-10575; (3588.28±3488.60)	73.48	NA
		sed (11)	37.88-20000; (4800.72±6662.64)	0-1303.05; (545.49±460.87)	74.67	NA
	2002 (Mar)	water (32)	2-1200; (247.87±336.64)	0-300; (97.95±103.67)	65.81	NA
		sed (2)	26611.23-27906.48; (27259.1±916.23)	8684.65-9069.77; (8859.21±217.78)	32.50	NA
	1999 (Mar)	water (6)	6613.33-17280; (12088.89±3713.88)	1420-8960; (4441.67±2831.78)	40.61	22-103 ⁴
		sed (6)	116.55-6588.23; (2951.30±3309.57)	60.6-204.68; (111.96±80.45)	20.86	44-208
	2000 (Mar)	water (10)	20-4026.67; (1113.33±1262.49)	12.5-2270; (747±663.06)	92.34	NA
		sed (5)	1398.89-8187.63; (5181.5±2438.02)	0-1373.96; (576.84±602.57)	25.42	NA
	2001 (Mar)	water (16)	6.67-7573.33; (1967.5±2361.32)	20-9350; (1553.91±2603.26)	92.40	NA
		sed (8)	8.11-14557.06; (3224.24±4961.79)	0-913.88; (207.06±332.15)	15.14	NA
Mumbai	2000 (May)	water (4)	16540-16660; (16040.5±16010)	16020-16057.5; (16040.5±16035)	97.00	180-440 ⁵
		sed (3)	2375.84-2790.36; (2583.09±293.11)	97.31-800.58; (448.95±497.29)	16.39	210-1390
	2001 (Nov)	water (13)	270-6192; (1822.61±1517.24)	100-2410; (930.09±686.43)	58.14	NA
		sed (11)	2.65-2360; (468.52±663.12)	0-152.4; (22.4±43.93)	3.28	NA
	2002 (May)	water (3)	382.5-3785000; (1262224±2184788)	255-2380000; (793722.5±1373757)	66.76	NA
		sed (9)	12.33-482.04; (175.67±132.77)	9.02-452.37; (138.66±133.89)	70.80	NA

¹range of mercury concentration in water (ng. l⁻¹) and sediment (no. g⁻¹ dry wt.) ²TVC no. ml⁻¹ water and no x 10⁴ g⁻¹ dried sediment (sed); ³data not available; ⁴data for 1997 [5]; ⁵data for 1998 [6]

Table 3. Bacterial isolates originally isolated from seawater nutrient agar (SWNA) without Hg and checked for growth on SWNA with different concentrations of Hg

no. of isolates*	Hg (ppm) in SWNA	number positive for growth	Major genera identified and no of isolates
85 from water 35 from sediment	10	water (60) sediment (23)	Not checked
85 from water 35 from sediment	20	water (35) sediment (13)	PM ¹ 20; XN ² 9; AM ³ 4; AT ⁴ 4; <i>Proteus</i> 8; EN ⁵ 2; UN ⁶ 1
35 from water 13 from sediment	50	water (16) sediment (5)	PM 9; XN 4; AM 1; AT 1; <i>Proteus</i> 4; EN 1; UN 1

*Bacterial isolates were from water and sediment samples collected off Marmugao, Chennai and Gopalpur; ¹*Pseudomonas*, ²*Xanthomonas*, ³*Aeromonas*, ⁴*Alteromonas*, ⁵Enterobacteriaceae, ⁶Unidentifiable.

LEGEND TO FIGURES:

Fig 1: Sampling locations. Approximate human population of the nearby coastal towns/cities in millions given in parentheses

Fig 2: Mean *percent* of mercury resistant bacteria (in total viable counts of bacteria) in water and sediment samples. The MRB abundances pooled from no-pollution (Positra, Marmugao, Terekhol and Gopalpur) low pollution (Malvan, Karwar, Paradip, Nagapattinam) and high pollution (Mumbai, Chennai, Mangalore, Kulai, Padubidri and Ratnagiri) coastal locations to depict their *percent* increases during 1993-2002. See Tables 1 and 2 for complete data.

Fig 3: Growth curves of a mercury resistant strain of *Pseudomonas* (CH07) in seawater nutrient broth (SWNB) with (a) no added Hg (circles); 10 ppm (\equiv 50 μ M) Hg (open triangles) and 50 ppm (or 250 μ M) Hg (squares). (b) Growth curves of the same strain shown for SWNB with no added toxicant (circles) and for a combination of 10 ppm (or 28.2 μ M) DDT, 20 ppm (or 212.6 μ M) phenol and 20 ppm (or 100 μ M) Hg (filled triangles). Over seventeen generations occurred in control flasks (SWNB alone) before the first generation could occur in flasks with 10 or 50 ppm Hg and, in flasks with DDT + Hg + phenol combination. (c) Growth curves of *Photobacterium leiognathi*, a Hg sensitive strain with 1.00 ppm Hg (closed circle) and without Hg (open circles). Optical densities (OD_{660}) from a minimum of three replicates from each growing condition were measured and used for calculating cell numbers. One unit of OD_{660} corresponded to 9.98×10^9 cells ml^{-1} .





