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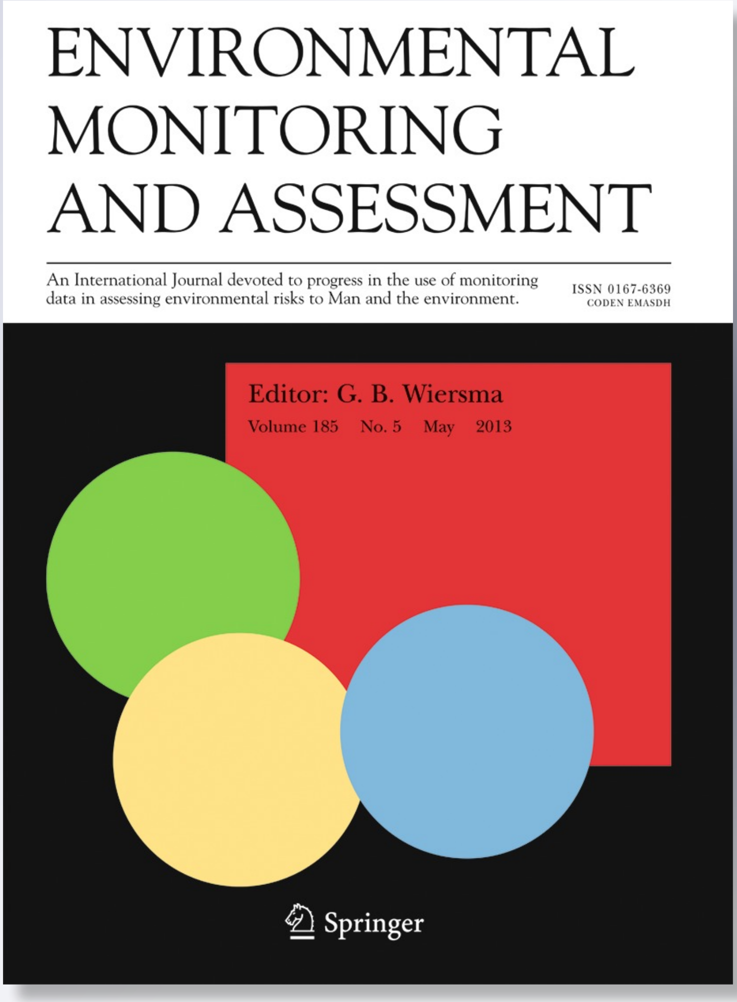

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Bacterial response to dynamic metal concentrations in the surface sediments of a solar saltern (Goa, India)

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Abstract The Ribandar solar saltern, situated adjacent to the Mandovi estuary is influenced by the barge transport of ferromanganese ore to the Mormugao harbour (Arabian Sea). The current study focuses on the distribution of metals and related heterotrophic bacterial populations in the surface sediments (0–10 cm) of the Ribandar salterns (Goa, India) during the salt-making (January to May) and non salt-making seasons (August and November). The concentrations of heavy metals in the sediments ranged from 17.2±2.8 to 26.3±6.7 % Fe; 0.6±0.2 to 0.9±0.2 % Mn; 27.6±7.3 to 51±8.3 ppm Ni; 28.4±8.9 to 35.2±10.6 ppm Co; 44±21.6 to 62.8±23.6 ppm Zn; 0.1±0.01 ppm Cd and 1.7±0.1 to 2.6±0.7 ppm Pb and were much higher than those reported at the same site in a previous study by Kerkar (2004). Hg concentrations were below detection limits. In general, computation of "geoaccumulation index" revealed the sediments as 'uncontaminated to moderately contaminated' with Fe, Mn, Ni, Co, Pb and Zn during the salt-making season. The abundance of metal-tolerant bacteria was comparatively restricted to the salt-making season and was higher than the non salt-making season. Fe-, Mn-, Ni-, Co- and Pb- (200 ppm) tolerant bacteria were retrieved and restricted to the surface sediments (0–5 cm), Cd and Fe being the two most regulatory elements governing bacterial populations in the non salt-making season. However, during the salt-making season, the concentration of Zn was found to be pivotal in regulating the counts of Fe-, Mn- and Ni-tolerant bacteria. In general, the strength of correlation of metals and microbes was higher in the non salt-making season as compared to the salt-making season. This would probably indicate metal-induced limitations in microbial populations in the non salt-making season and the absence of this effect during the salt-making season. In this study, we test the hypothesis that solar salterns behave as ecological sinks with a potential to transform native bacterial populations to metal-resistant strains, in relation to the dynamic changes in the surrounding metal concentrations.

Keywords Solar saltern · Sediment · Metals · Bacteria

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Introduction

Goa is an important mineral producing area on the west coast of India and its economy is dependent mainly on iron ore mining and its export. Two thirds of the volume of ores in Goa comes from the mines located along the Mandovi and Zuari estuaries, and almost 90 % is transported through these riverine waterways (Maheswari 1994). The increase in transport of ferromanganese ores in Goa has led to an increase in the deposits of metals in the estuarine coastal systems (Attri and Kerkar 2011). Salterns consist of a series of linked pans where gradients of salinity occur due to evaporation of seawater. Brine gets concentrated sequentially and is subjected to daily salt harvesting leading to perturbation in surface sedimentation. The saltern is demarcated into primary, secondary, tertiary, and finally, the crystallizer pond. Salinity in these ponds ranges from 10 to 350 psu during the salt-producing season (Kerkar and Loka Bharathi 2007). In the Ribandar saltern, seawater from the Mandovi estuary enters the creek through a sluice gate at high tide, and during low tide water from the creek is drained out into the estuary. Water heats up and evaporates in the sun, and gets condensed and saturated and eventually the salt crystallises. The salt production is at a maximum in the pre-monsoon season. Studies on various physico-chemical, biological and mineralogical aspects of the Mandovi estuary have been carried out earlier (Varma and Rao 1975; Murthy et al. 1976; Qasim and Sen Gupta 1981; Upadhyay and Sen Gupta 1995; de Sousa 1999) but limited data is available on the distribution and variation of metal concentrations in the sediments during the salt-making and non salt-making season of Ribandar salterns. A study of metal concentrations from sediment cores of the Ribandar saltern was carried out in 2001 by Kerkar (2004). However, the concentrations of metals seem to have increased manifold over the last 10 years.

It is well known that microbial processes may be important and even dominating factors in the distribution and fate of specific metals. Though some of the metals like Cu, Fe, Mn, Ni and Zn are essential as micronutrients for life processes, they are proved detrimental beyond a certain limit (Marschner 1995; Bruins et al. 2000), which is low for some elements like Cd (0.01 mg/L), Pb (0.10 mg/L) and Cu (0.050 mg/L). Sediments act as indicators of the burden of heavy metals in a coastal environment, as they manifest as principal reservoirs (Fitchko and Hutchinson 1975). The measurement of trace metal concentrations and distribution in the hypersaline salterns is therefore important for understanding the changes induced by them in the adjoining water bodies.

Solar salterns could facilitate natural enrichment of metals which otherwise in surface sediments would get flushed out of the estuary during the high tides. The solar salterns could also serve as avenues for transformation of native bacterial flora, into potentially heavy metal-resistant strains. During non salt-making seasons, the metals along with the metal-tolerant microbes could be mobilized into the riverine system. The impact of these altered metal-tolerant microbes on the environment is not known. In the present study, we attempt to evaluate the magnitude of enrichment of metals in the Ribandar saltern sediments through various indices and also correlate the findings with metal-tolerant bacterial load.

Materials and methods

Study area and sampling site

The study site was the Ribandar saltern (15° 30.166 N and 73° 51.245 E) Goa, India and is situated along the Mandovi estuary. The total area of this saltern is 12,329.12 m^2 (Fig. 1). This area is under the influence of semidiurnal tides and the surrounding marshy land supports rich mangrove vegetation. The site is subjected to heavy annual rains (125 cm) during the monsoons. The climate on an average is generally warm and humid, fluctuating from a minimum of 20 °C in the month of December to 42 °C in May. Sediment cores (0–5 and 5–10 cm) from the saltern of Ribandar were collected in the months of January–May 2008 (salt-making season); August and November (representative of the non salt-making season) during low tide in triplicates using 1.5-in.-diameter graduated PVC hand-held corers. The corers were sealed at both ends with sterile core caps to prevent direct contact with air and transported to the laboratory in an icebox for further physico-chemical and microbiological analysis.

Physico-chemical parameters

Portions of the sediments were analysed for temperature record on site. Eh and pH were measured by Thermo Orion model 420A, USA as described in Orion instruction manual. Sub-samples for metal analysis were dried at 60(±2)°C for 48 h and disaggregated in an agate mortar before chemical treatment for

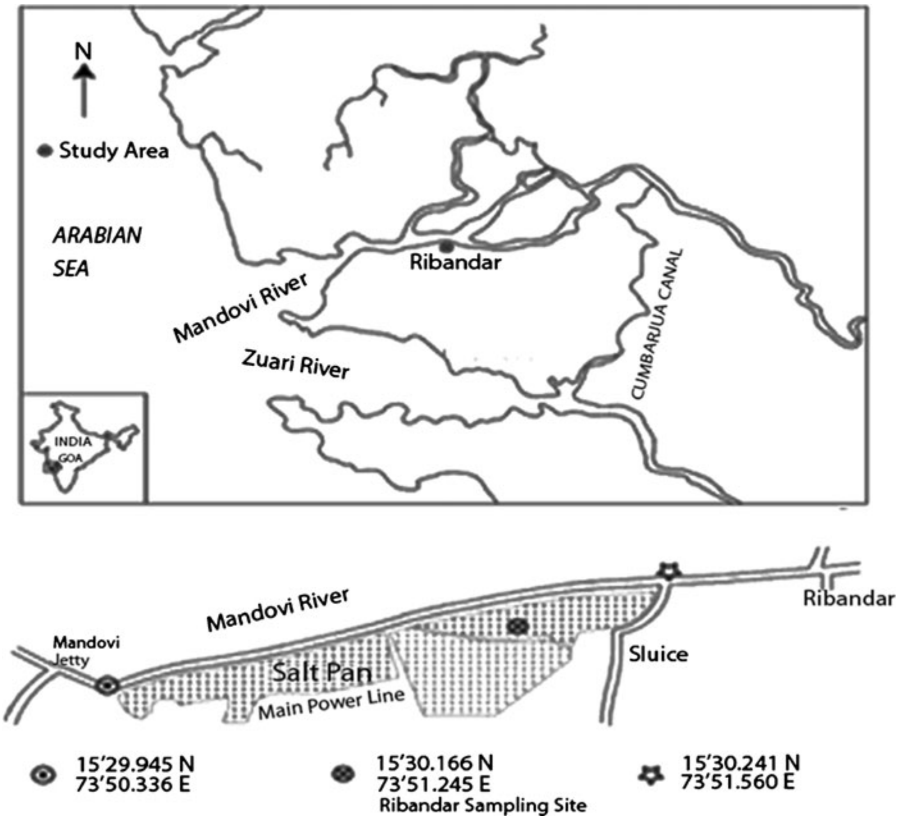

Fig. 1 Sampling area and site

the measurement of Fe, Mn, Ni, Co, Pb, Zn, Cd and Hg following sediment digestion methods as described by Balaram et al. (1995). Briefly, a known quantity (0.2 g) of sediment was digested in a Teflon vessel with a solution (10 ml) of concentrated HF (48 % GR; Merck), HNO_3 (69 % GR; Merck) and $HClO_4$ (35 % GR; Merck) in the ratio 7:3:1. The mixture was digested on a hot plate in a fume hood chamber at 70 °C for 4–6 h. The procedure was repeated with 5 ml of acid mixture. A further 2 ml of concentrated HCl (35 % GR; Merck) was added followed by 10 ml of HNO_3 (69 % GR; Merck). The residue was warmed and transferred to a clean, dry standard flask to make a final volume of 50 ml with double distilled water. The concentration of the metals was analysed on an atomic absorption spectrophotometer (AAS; GBC 932AA model). Blank corrections were applied wherever necessary and the accuracy was tested using standard reference material MAG-1 (United Geological Survey). The particle size analysis was carried out by the wet sieving method for sand and the pipette method for silt and clay as reported by Day (1965) and Carver (1971).

Sediment quality assessment

Geoaccumulation index

The geoaccumulation index (I_{geo}) formulated by Müller (1969) was used to determine the intensity of metal contamination. Although I_{geo} was originally devised for use with global standard shale values as background metal levels, Rubio et al. (2000) have shown the use of regional background values to give more appropriate results. In this study, I_{geo} has been calculated using background values (B_n) for median metal concentrations recorded from the saltern sediments during the monsoon season. A comparison between the salt-making and the non salt-making season was therefore made to assess the probable influence of mining.

Geoaccumulation index can be expressed as: $I_{geo}=\log_2 (C_n/1.5\ B_n)$.

Where C_n=measured concentration of metal 'n' in the sediment

B_n=the background values for the metal 'n'.

The Factor 1.5 is a value intended to offset potential oscillations in background data resulting from lithological variations.

Contamination factor

The level of contamination of soil by metal is expressed in terms of a contamination factor (CF) calculated as: $CF = C_m\text{Sample}/C_m\text{Background}$.

Where CF=contamination factor, C_mSample=metal concentration in polluted sediments and C_mBackground=background value of the metal under consideration. A contamination factor $CF<1$ refers to low contamination; $1 \le CF < 3$ means moderate contamination; $3 \le CF \le 6$ indicates considerable contamination and $CF>6$ indicates very high contamination.

Pollution load index

The site was evaluated for the extent of metal pollution by employing the method based on the pollution load index (PLI) developed by Thomilson et al. (1980), as follows:

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n)^{1/n}$$

In the above equation, n is the number of metals studied (eight in this study) and CF is the contamination factor calculated as described above. The PLI provides simple but comparative means for assessing a site quality, where a value of $PLI<1$ denotes perfection; $PLI=1$ presents that only baseline levels of pollutants are present and $PLI>1$ would indicate deterioration of site quality (Thomilson et al.1980).

Microbial analyses

Sediment core was sectioned at 5 cm intervals in sterile conditions to obtain representative samples at 0–5 and 5–10 cm. Sub-samples of ca. 5 g wet weight sediment were sampled using sterile syringe cores. The sub-samples were transferred to 45 ml of full strength sterile seawater (10^{-1} dilution). Serial dilutions of the sediment samples were performed in autoclaved seawater to yield dilutions from 10^{-1} to 10^{-6}. Medium for the isolation of metal-tolerant heterotrophic bacteria was prepared using 25 % nutrient broth+ 2 % agar and amended with different metals at concentrations of both 200 and 400 ppm for Fe, Mn, Ni, Co, Pb, Zn, Cd and Hg respectively. A concentration of 100 % nutrient broth corresponds to 13 g nutrient broth (Hi Media Laboratories Pvt. Ltd., Bombay, India) per 1,000 ml seawater. Except for Fe where a sulphate salt was used and Pb where a nitrate salt was used, the rest were all metal chloride salts (Merck). About 100 μL from10^{-5} dilution was spread plated onto the metal amended media. Bacterial counts in the form of colony-forming units (CFU) formed on the medium were recorded after a 15-day incubation period at 28(±1)°C.

Statistical analysis

Statistical analysis was carried out using Pearson's correlation coefficient and analysis of variance, in order to explore the possible associations and variances existing between/within different variables. The analysis was done using Microsoft Excel 2000. The bacterial parameters were given a log transformation before analyses.

Results and discussion

Physico-chemical parameters

A comparison of the seasonal variations in environmental parameters of the Ribandar saltern during the salt-making and the non salt-making season is presented in Table 1. The pH of the saltern sediments during the salt-making season was mostly acidic in the range of 5.7–7±0.1. The lowest pH value was encountered in the month of May, after an unexpectedly heavy shower. However, the pH was basic soon after the salt-making season began. At higher salt concentration, i.e. in April during the peak salt-making season, the pH of the overlying water was lower (6.8± 0.3). A similar observation was also made by Attri and Kerkar (2011) in the adjoining mangroves and has been attributed to the high organic carbon content in the mangrove sediments. Besides, the oxidation of sulphide pyrite which releases the dissolved ferrous iron is also known to be responsible for a shift towards more acidic conditions (Stumm and Morgan 1996).

The abundance and distribution of sediment components revealed that the saltern sediment was clayey in nature, being highest during the salt-making season (64±8.96 %). During the non salt-making season, there was a shift in the distribution with sand being the dominant component (48.36±25.12 %). This shift

Table 1 Seasonal variations in environmental parameters of the Ribandar saltern during the salt-making and the non salt-making season

Depth in cm	Jan 0–5	Jan 5–10	Feb 0–5	Feb 5–10	Mar 0–5	Mar 5–10	Apr 0–5	Apr 5–10	May 0–5	May 5–10	Aug 0–5	Aug 5–10	Nov 0–5	Nov 5–10
pH	7.0±0.1	6.7±0.04	6.4±0.03	6.3±0.07	6.5±0.02	6.5±0.04	6.5±0.07	6.2±0.13	6.5±0.04	5.7±0.01	6.5±0.13	6.6±0.02	7.4±0.11	7.2±0.04
Eh	28.1±10.8	27.9±10.8	−10.3±10.2	−10.7±2.6	23.7±14.6	20.3±11.1	−14.2±10.3	−24.5±10.0	−26.4±15.4	−29.5±4.7	27.4±15.9	27.2±10.5	72.4±16	45.6±10.4
Sand	28.3±5.1	24.7±4.5	19.8±2.5	36.0±5.4	16.0±9.7	14.6±5.1	16.4±3.2	29.45±6.9	19.2±9.6	19.7±5.5	10.6±2.4	17.4±0.1	63.2±11.6	77.8±12.8
Silt	7.0±0.2	19.6±0.5	15.6±0.9	15.3±1.5	14.9±1.6	16.4±0.4	16.5±0.3	17.±0.3	14.6±0.3	16.4±0.7	16.3±0.1	17.7±0.5	8.5±0.4	3.9±0.3
Clay	64.6±4.2	56.4±2.0	64.7±3.9	48.6±0.9	69.1±0.4	69±2.3	67.1±0.5	53.6±2.0	66.3±1.9	63.9±1.6	73.1±0.8	64.9±2.2	28.3±0.7	18.3±0.3

Sand, silt and clay content has been expressed as %

could be attributed to the pre-dominance of terrestrial over tidal sediments (Ukpong 1997). It is generally recorded that metals are associated with smaller grain size particles (Martincic et al. 1990; Biksham et al. 1991)

Distribution of metals in the sediments

Figure 2 compares the depth-compromised average values of metals in the saltern for both the seasons with that obtained in 2001 by Kerkar (2004) at the same site. In our study, the mean values of the concentrations for all the seasons in general resulted in 17.2±2.8 to 26.3±6.7 % Fe; 0.60±0.2 to 0.9±0.2 % Mn; 27.6±7.3 to 51±8.3 ppm Ni; 28.4±8.9 to 35.2±10.6 ppm Co; 44.0±21.6 to 62.8±23.6 ppm Zn; 0.06±0.01 ppm Cd; 1.7±1.0 to 2.6±0.7 ppm Pb and below detection limit Hg. Though there were no significant variations in the concentration of metals between the depth intervals studied, there existed significant variations between the salt-making and non salt-making seasons. Metal concentrations were higher in the salt-making season by 52 % for Fe, 42 % for Mn, 85 % for Ni, 23 % for Co, 42 % for Zn and 47 % for Pb, compared to the non salt-making season. Evaluating the concentrations of metals obtained in this study with a study of metals from sediment cores (0–10 cm) of the Ribandar saltern observed in 2001 by Kerkar (2004) shows an escalation in the concentration of almost all the metals over the last 10 years. Fe obtained then was 0.97–3.04 %, whereas presently it showed an 8–17-fold increase. Similarly Mn showed a

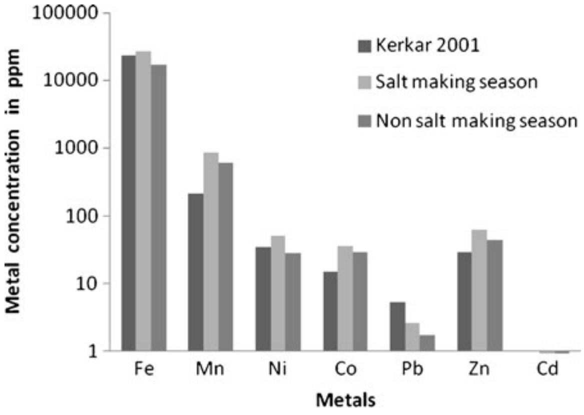

Fig. 2 Depth-compromised average distribution of metals for both the seasons (salt-making and non salt-making) compared with that of Ribandar saltern in 2001

34–54-fold increase from 0.01–0.03 to 0.6–0.9 %; Co increased from 4.8–24.8 to 28.4–35.2 ppm, which was a 1–6-fold increase; Ni increased from 15–46 to 27.6–51 ppm which is a 1–2-fold increase; Zn increased from 14–38 to 44–62.8 ppm again showing a 1–3-fold increase and Cd which was previously not detectable showed 0.06 ppm, thereby suggesting an increase in the input of these metals in the estuary, which probably could be attributed to anthropogenic sources and ferromanganese mining. Comparatively, the concentration of Pb showed a 0.4–0.7-fold decrease. In a recent study, Attri and Kerkar (2011) have demonstrated anthropogenic influence in the sediments of Divar, a mangrove swamp in the Mandovi estuary in the vicinity of our saltern by comparing it with another pristine mangrove area free from human influence. The Divar swamp is geochemically similar to the Ribandar saltern and hence could serve as a negative control for our study. According to Attri and Kerkar (2011) the annual average concentration of Fe in Divar was 18.3±1.9 %, Mn was 0.19±0.002 %, Co was 36.2 ±4.2 ppm and Zn was 102.3±9.8 ppm. These values obtained in the Mandovi riverine body were lesser than the metal concentrations encountered in the present study in the Ribandar saltern during the salt-making season except in the case of Zn. The annual average metal concentrations reported in the Ribandar saltern in the present study were 21.7±4.7 % for Fe, 0.72±0.16 % for Mn, 31.8±9.7 ppm for Co and 53.4±22.6 ppm for Zn. Lower values of Zn in the saltern could be explained by the high level of positive correlation between Zn metal and Ni-, Fe- and Mn-tolerant bacteria (Table 5). Studies by Alagarsamy (2006) showed that the concentrations of Fe varied from 2.2 to 49.7 % in the surface sediments of Mandovi estuary, while the concentration of Mn ranged below detection limits to 1.61 %. These high values of Fe in the saltern sediment of Ribandar could be attributed to the precipitation of the respective metal sulphide compounds in anaerobic sediments (Howarth 1979). The high concentrations of Mn and Fe could be explained by the strong association of the geochemical matrix between the two elements ($r=0.503$, $p<0.1$, $n=10$) during the salt-making season, as well as the non salt-making season ($r=0.762$, $p<0.1$, $n=4$). The statistical analysis of intermetallic relationship revealed a high degree of correlation between Fe, Mn and Ni during both the salt-making as well as the non salt-making seasons indicating an identical behaviour of these elements during their transport in the estuarine environment. Significant inter-relationships existed between the concentrations of both Fe and Mn metals, suggesting similar sources for and/or similar geochemical processes controlling these metals. Similar observations have been made by several authors, e.g. Abu-Hilal and Badran (1990); El-Sayed (1980). Variations in trace metal data provide new information on the surface sediment geochemistry in the salterns. The overall variation in concentration of metals in the saltern could be due to the differential discharge of untreated effluents originating from industries and agriculture as well as from domestic sewage along with the fishing and boating activities.

Sediment quality assessment

The sediment quality of the saltern was assessed using various indices such as geoaccumulation index, contamination factor and pollution load index.

Geoaccumulation index (I_{geo})

To enhance the data inventory for the region and to understand the influence of anthropogenic activities and the monsoon on biogeochemical process in the saltern ecosystem, the I_{geo} (Müller 1979) was determined. The choice of the background value plays an important role in the interpretation of geological data. I_{geo} has been widely utilised as a measure of pollution in freshwater (Müller 1980; Singh et al. 1997; Kralik 1999) and marine sediments (Stoffers et al. 1986; Bryan and Langston 1992; Dickinson et al. 1996). I_{geo} for all the metals under study in the salt-making season (with metal values from the non salt-making season as background values) were computed based on Müller (1979) for both the seasons. The background values (B_{n}) used for computing I_{geo} for each metal at a depth of 0–5 and 5–10 cm, respectively, were 15.2 and 14.5 % for Fe, 0.6 and 0.4 % for Mn, 19.6 and 25.3 ppm for Ni, 21 ppm for Co at both depths, 28.5 and 76 ppm for Zn, 0.073 and 0.055 ppm for Cd and 0.78 and 1.11 ppm for Pb. With respect to the I_{geo} classifications (Table 2), it could be inferred that in most of the cases the sediments fell in the 'uncontaminated to moderately contaminated' category. The sediments at 0–5 cm interval were moderately contaminated by Pb, while it was 'uncontaminated to moderately contaminated' with Fe, Ni, Co and Zn.

There was no contamination with Mn and Cd (Fig. 3a). At 5–10 cm, the impact of Fe, Mn, Ni, Co and Pb resulted in the sediments being classified as 'uncontaminated to moderately contaminated', while they were uncontaminated with Cd and Zn (Fig. 3b). Hence, it is obvious that the impact of ferromanganese mining has substantial impact on the sediments of the salterns adjoining the Mandovi estuary.

Contamination factor and pollution load index

The CF was greater than one for Fe, Mn, Ni, Co, Zn and Pb during the salt-making season, indicative of 'moderate contamination' while there was very low contamination with Cd. A similar situation was encountered in the non salt-making season except for Zn which was found to have no contamination effect. It could also be observed that though in general, the magnitude of CFs decreased, the CF of Pb increased considerably. A detailed account of the contamination factor for each metal is given in Table 3.

The PLI is aimed at providing a measure of the degree of overall contamination at a sampling site. According to the PLI, lower values imply no appreciable input from anthropogenic sources (Singh et al. 1997). Our study revealed that the pollution index varied from 1.1 to 1.6 (Fig. 4). Relatively high PLI values suggest input from anthropogenic sources attributed to increased human activities.

Abundance of metal-tolerant microbes in the saltern

Retrievable counts of metal-tolerant bacteria in various metal amended media are given in Fig. 5a and b for the salt-making and non salt-making seasons, respectively. In general, the counts of Zn- and Cd-tolerant bacteria ranged from 10^{4-5} CFU g^{-1} sediment. Growth of Zn- and Cd-tolerant bacteria was observed only on 200 ppm amendments and mostly restricted to salt-making season. During non salt-making season, the counts of Pb-tolerant bacteria on both 200 and 400 ppm were in the order of 10^6 CFU g^{-1} sediment;

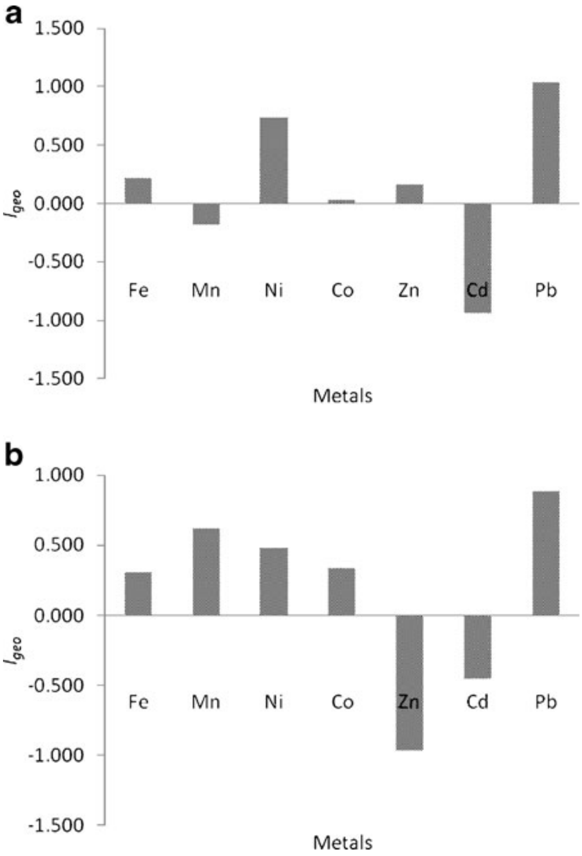

Fig. 3 **a** Geo accumulation index for metals during salt-making season at a depth of 0–5 cm. **b** Geo accumulation index for metals during salt-making season at a depth of 5–10 cm

Table 2 I_{geo} classification

I_{geo} value	I_{geo} class	Designation of soil quality
>5	6	Extremely contaminated
4–5	5	Strongly to extremely contaminated
3–4	4	Strongly contaminated
2–3	3	Moderately to strongly contaminated
1–2	2	Moderately contaminated
0–1	1	Uncontaminated to moderately contaminated
0	0	Uncontaminated

Table 3 Seasonal variation in contamination factor for various metals

Metals	Non salt-making season	Salt-making season
Fe	1.2	1.8
Mn	1.3	1.9
Ni	1.2	2.3
Co	1.1	1.7
Zn	0.8	1.3
Cd	0.9	0.9
Pb	1.9	2.7

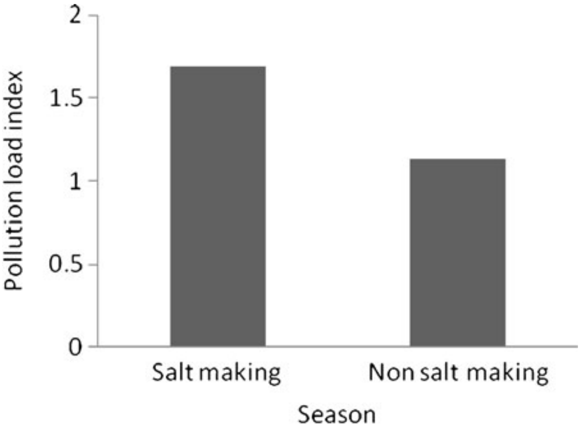

Fig. 4 Seasonal variation in pollution load index

however, in the salt-making season, it ranged from 10^{4-7} CFU g^{-1} sediment, with higher counts observed at 5–10 cm in both the concentrations tested. The counts of Co-tolerant bacteria during both non salt-making season and salt-making season at 200 ppm were in the order of 10^6 CFU g^{-1} sediment. Though Co-tolerant bacteria were not detected at 400 ppm in non salt-making season, during salt-making season it occurred at ~10^5 CFU g^{-1} sediment. At both the depth intervals, the counts of Ni-tolerant bacteria in non salt-making season at 400 ppm were lower by an order compared to 200 ppm (~10^7 CFU g^{-1} sediment). The counts of Ni-tolerant bacteria on 400 ppm amendment in salt-making season in both the depths were uniform

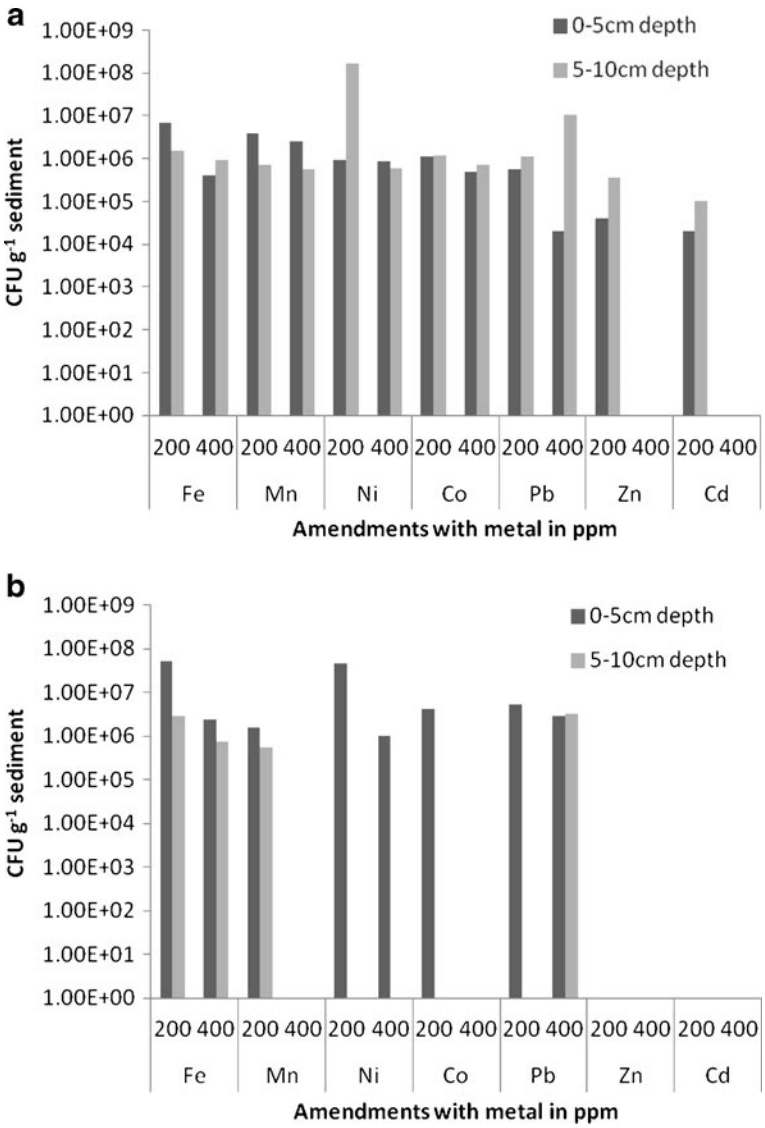

Fig. 5 **a** Seasonal variation in the microbial population during salt-making season. **b** Seasonal variation in the microbial population during non salt-making season

(10^5 CFU g^{-1} sediment). However, the counts were higher by three orders on 200 ppm amendment in the depth interval of 5–10 cm. At 0–5 and 5–10 cm, irrespective of the amendments and the season, the counts of Mn-tolerant bacteria were mostly in the order of 10^6 and 10^5 CFU g^{-1} sediment, respectively. In 200 ppm amendment, the count of Fe-tolerant bacteria was highest (10^7 CFU g^{-1} sediment) in 0–5 cm during non salt-making season while for rest of the observations the counts were less by one order. A similar trend was also observed in 400 ppm amendment but less by an order.

Metal microbe interactions

Tables 4, 5 and 6 illustrate the significant correlations between the various groups of metal-tolerant bacteria and metals. When inter-relationships between metals and microbes were analysed as a whole (Table 4), irrespective of the season, it was seen that Ni (n=14, r=−0.531, p<0.05) had a negative correlation with the levels of Fe-tolerant bacteria whereas Cd was responsible for a 34 % variation in the number of Fe-tolerant bacteria (n=14, r=0.580, p<0.02). The concentration of Zn was weakly associated with the abundance of Pb-tolerant bacteria (n=14, r=−0.499, p<0.05) whereas it had a strong correlation with the Mn-tolerant bacterial population (n=14, r=0.465, p<0.1 and r=0.500, p<0.05). On the other hand, Co had a positive correlation with Pb-tolerant bacteria (n=14, r=0.483, p<0.1). Since Pb-tolerant bacteria were related to Co and Zn, any variation in these elements could affect the population numbers of these bacteria. Subsequently, any increase or decrease in the population of Pb-tolerant bacteria could be related to the levels of Co and Zn, respectively, in the surrounding environment.

Table 5 Table for correlation coefficients (r) obtained for the data which includes abundance of microbes isolated at metal concentrations of 200 and 400 ppm and metal concentration in surface sediments of Ribandar saltern for the salt-making season (n=10, significant correlations are given in italics)

Metal-tolerant bacteria	Metals	
	Fe	Zn
Fe isolates (200 ppm)	0.184	*0.530 (p< 0.1)*
Mn isolates (200 ppm)	0.180	*0.569 (p< 0.1)*
Mn isolates (400 ppm)	0.152	*0.539 (p< 0.1)*
Ni isolates (400 ppm)	0.287	*0.617 (p< 0.05)*
Co isolates (400 ppm)	*0.674 (p< 0.02)*	0.317

An analysis of the salt-making season (Table 5) indicated a positive correlation between Zn concentration in the sediments and Fe- (n=10, r=0.530, p<0.1), Mn- (n=10, r=0.569 and 0.539, p<0.1) and Ni-tolerant bacteria (n=10, r=0.617, p<0.05). This could mean that Zn could be a key element and a limiting nutrient for Fe-, Mn- and Ni-tolerant bacteria and therefore any increase in its concentration would immediately reflect on the counts of Fe-, Mn- and Ni-tolerant bacteria. The relation of Zn to Mn is so strong that its effect is not subdued even when the data for all seasons was considered. It was also observed that Fe had a positive effect on the Co-tolerant bacterial population (n=10, r=0.674, p<0.02) during salt-making season.

A comparison of the two seasons indicated a number of positive correlations between metals and microbes during the non salt-making season

Table 4 Table for correlation coefficients (r) obtained for the data which includes abundance of microbes isolated at metal concentrations of 200 and 400 ppm and metal concentration in surface sediments of Ribandar saltern for the entire year (n=14, significant correlations are given in italics)

Metal-tolerant bacteria	Metals			
	Ni	Co	Zn	Cd
Fe isolates (200 ppm)	*−0.531 (p< 0.05)*	−0.359	−0.110	*0.580 (p< 0.02)*
Mn isolates (200 ppm)	0.017	−0.107	*0.465 (p< 0.1)*	0.079
Mn isolates (400 ppm)	0.004	−0.139	*0.500 (p< 0.05)*	0.150
Pb isolates (200 ppm)	−0.337	0.185	*−0.499 (p< 0.05)*	0.106
Pb isolates (400 ppm)	0.089	*0.483 (p< 0.1)*	−0.382	0.161

Table 6 Table for correlation coefficients (*r*) obtained for the data which includes abundance of microbes isolated at metal concentrations of 200 and 400 ppm and metals in surface sediments of Ribandar saltern for the non salt-making season (*n*=4, significant correlations are given in italics)

Metal-tolerant bacteria	Metals						
	Fe	Mn	Ni	Co	Pb	Zn	Cd
Fe isolates (200 ppm)	−0.449	0.098	−0.708	−0.520	−0.636	−0.224	*0.896 (p< 0.02)*
Mn isolates (200 ppm)	*0.809 (p< 0.1)*	0.341	0.345	0.618	0.628	−0.644	−0.336
Mn isolates (400 ppm)	−0.496	0.056	−0.725	−0.557	−0.670	−0.171	*0.902 (p< 0.01)*
Ni isolates (400 ppm)	0.653	*0.885 (p< 0.02)*	0.202	0.557	0.440	*−0.976 (p< 0.01)*	0.097
Co isolates (200 ppm)	*0.996 (p< 0.001)*	0.724	0.800	*0.965 (p< 0.01)*	*0.959 (p< 0.01)*	−0.705	−0.690
Co isolates (400 ppm)	0.560	0.734	*0.874 (p< 0.05)*	0.771	0.750	−0.287	−0.708
Pb isolates (400 ppm)	*0.923 (p< 0.02)*	*0.927 (p< 0.02)*	*0.818 (p< 0.1)*	*0.964 (p< 0.01)*	*0.918 (p< 0.02)*	−0.762	−0.605
Zn isolates (200 ppm)	−0.496	0.056	−0.725	−0.557	−0.670	−0.171	*0.902 (p< 0.01)*

(Table 6). This could imply that the substrate dependence or coupling of the metal-tolerant bacteria with metals was not very significant during the salt-making season, probably because the heterotrophic bacteria were not limited by the availability of metals. In the non salt-making season generally the metal concentrations were low due to the dilution effect of the monsoons and a stronger coupling existed as indicated by numerous and stronger correlations. Similarly, when the seasons were analysed together (Table 4), a well-defined pattern of correlation was not observed; indicating that metal limitation was not a critical aspect for the abundance of microorganisms. A visual assessment of the number of correlations during the non salt-making season indicated that Cd and Fe were positively correlated to three different types of metal-tolerant bacterial populations, closely followed by Pb, Ni, Co and Mn which had a positive correlation with two types of metal-tolerant bacterial populations. Zn was found to be correlated only to the abundance of Ni-tolerant bacterial population. This is in contrast to salt-making season where Zn was the key element and the most significant nutrient for Fe-, Mn- and Ni-tolerant bacteria. Similarly Cd-tolerant bacteria were not correlated to the metals studied, whereas the population of Pb-tolerant bacteria showed a high degree of correlation to five metals. This could indicate that the population of Pb-tolerant bacteria may not only be affected by Pb but also by the presence of other metals in the environment. An increase in concentration of these metals in the sediments also resulted in an increase in Pb-tolerant bacteria. In the present study it was observed that Co was strongly correlated to Pb-tolerant bacteria followed by Pb, Mn and Fe respectively. Relation between Fe-tolerant bacteria and Cd metal, and the Pb-tolerant bacteria and Co metal which was seen in non salt-making season was also observed when the entire spectrum of data was analysed irrespective of the season reiterating that these relations could be highly relevant and critical. Fe had a positive correlation with the level of Mn-tolerant bacteria (n=4, r=0.809, p<0.1), Pb-tolerant bacteria (n=4, r=0.923, p<0.02) and Co-tolerant bacteria (n=4, r=0.996, p<0.001) during the non salt-making season. Positive influence of

Fe has also been observed on Co-tolerant bacteria during the salt-making season. The obvious correlation between Fe with Mn- and Pb-tolerant bacteria during the non salt-making season, along with its absence during the salt-making season, further supports our observation that Fe concentrations could be one of the critical parameters that could serve as an index of the ecological balance in salterns. Heavy metal pollution could exert a selective pressure on microbial community leading to the emergence of resistant strains (Silver and Misra 1984; McGrath et al. 2001; Lasat 2002; Li et al. 2006; Souza et al. 2006; Wang et al. 2007). Similar studies have been carried out by Haristha et al. (2002) and Kerkar (2004) on Pb and Hg tolerance by hypersaline SRB in the same saltern. Hence, it is obvious that solar salterns could serve as avenues for transformation of native bacterial flora to strains with increased heavy metal tolerance, acting as sinks for bacteria—"potential bioremediators of heavy metals".

Conclusion

Sediment quality indicates that the saltern sediments were 'uncontaminated to moderately contaminated' by Fe, Mn, Ni, Co, Pb and Zn during the salt-making seasons which promote the proliferation of metal-tolerant bacteria. Zn was found to affect the counts of Fe-, Mn- and Ni-tolerant bacteria in the salt-making season while Cd and Fe influenced metal-tolerant bacteria in the non salt-making season. The strength of metal microbe interactions in terms of correlation was higher during the non salt-making season compared to the salt-making season probably indicating the absence of metal-induced-limitation during the latter. Hence, solar salterns act as ecological sinks with a potential to transform native bacterial populations to metal resistant strains corresponding to the dynamic changes in the surrounding metal concentrations.

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