Bacterial contribution to mitigation of iron and manganese in mangrove sediments

K.P. Krishnan a,b, Sheryl Oliveira Fernandes a, G.S. Chandan a,c, P.A. Loka Bharathi a,*

aNational Institute of Oceanography, Dona Paula, Goa, India
bNational Centre for Antarctic and Ocean Research, Vasco da Gama, Goa, India
cDepartment of Applied Geology, ERSM, Jnanasahyadri, Kuvempu University, Karnataka, India

Abstract

The Mandovi and Chapora are two tropical estuaries lying in close geographic proximity on the west coast of India. Seasonal changes in down core variation of Fe, Mn and Total Organic Carbon (TOC) in the mangrove sediments adjoining these estuaries were studied to assess their influence on some of the representative benthic bacteria belonging to heterotrophic and autotrophic groups. Heterotrophic bacteria (HB) cultured on different nutrient concentrations (0.01%, 0.1% and 25%) together with nitrifiers (NtB; representing autotroph) were chosen to assess the influence of the above-mentioned abiotic parameters on the former. The experimental site located along the Mandovi is under the influence of extensive ferromanganese ore mining, while the control site at Chapora is relatively free from such influences. Geoaccumulation index computed for Mandovi showed that sediments (0–10 cm) were ‘uncontaminated to moderately contaminated’ by Fe during the pre monsoon and monsoon seasons, while in the post monsoon season the 4–10 cm fraction was almost completely restored from contamination. Similar computations for Mn showed that in pre monsoon, sediments fell in the ‘moderately contaminated’ and ‘moderately to strongly contaminated’ categories, while in the monsoon and post monsoon seasons all the sections were ‘Uncontaminated’. The difference observed in correlation between Fe and Mn with the various fractions of heterotrophs and nitrifiers indicated that though these two elements shared a similar chemistry in the environment, microbes involved in biogeochemical processes might prefer them differentially. The relationship between TOC and HB enumerated on 0.01% dilute nutrient agar remained at r = 0.50, p < 0.05 throughout the year. Hence, it could be apparently linked to their preferred concentration of organic carbon requirement. A relationship of r = 0.61, p < 0.01 between manganese concentration and heterotrophs recovered on different strengths of nutrient agar is suggestive of their response to the metal enrichment. They could thus contribute towards maintaining the level of Mn at par with reference levels at Chapora. A positive correlation between Mn with NtB (n = 10, p < 0.05, r = 0.58) at the experimental site during the non-monsoon months is suggestive of the latter’s contribution to regulation of the metal concentration in the sediment probably through anaerobic nitrification at the expense of manganese. The study therefore supports our hypothesis that both autochthonous auto trophs and heterotrophs work in tandem to mitigate concentration of Mn and related metals in mangrove sediments.

Keywords: Iron; Manganese; Mangroves; Heterotrophs; Nitrifiers; Geoaccumulation
1. Introduction

The Mandovi and Chapora are two tropical estuaries lying in close geographic proximity on the west coast of India but the latter is relatively distanced from pollution. The hydrological characteristics of these two estuarine systems are governed by the monsoon regime. The physical characteristics of the Mandovi and Chapora estuaries have been described earlier (Varma and Rao, 1975; Varma and Cherian, 1977; Murthy et al., 1976). The estuarine channel of the Mandovi is used to transport large quantities of ferromanganese ores from mines located upstream to the Marmagao harbour (Arabian Sea), while the Chapora is free from movement of ferromanganese ore bearing barges. Lush mangrove vegetation fringe both the estuarine systems. Though the impacts of iron ore processing on the surface sediments of the Mandovi estuary have been documented earlier by Alagarsamy (2006), its influence on the benthic bacterial flora is sparsely understood.

In this study, an attempt has been made to understand the downcore distributory patterns of heterotrophic and autotrophic bacteria together with total Fe and Mn during monsoon and non-monsoon periods. Retrievable heterotrophic bacteria were enumerated on various nutrient strengths to estimate the various fractions requiring different carbon and nitrogen requirements. Nitrifiers were enumerated as a representative autotrophic community as they are one of the most important physiological groups in this ecosystem responsible for converting ammonia to its oxidized forms.

Geoaccumulation index ($I_{geo}$, Müller, 1979) was used to assess the impact of mining on the mangrove sediments. 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 for more appropriate results. In this study, $I_{geo}$ has been
calculated using regional metal concentrations in Chapora mangrove sediments. A comparison between the Mandovi and Chapora mangrove sediments was therefore made to assess the probable influence of mining on bacterial groups in this region. We hypothesize that bacterial population in the mangrove sediments could play an important role in maintaining the concentration of the metal species from accumulating.

2. Materials and methods

2.1. Site description and sampling procedure

Sediment cores were collected from fringing mangrove forests along the Chapora and Mandovi estuaries. The control site in the Chapora estuary was located at Tuvem whereas the Divar mangrove ecosystem was selected as the experimental site along the Mandovi estuary (Fig. 1). The experimental site is under the influence of ferromanganese ore mining. Samples were collected during the months of April (Pre Monsoon), July (Monsoon) and October (Post Monsoon) of 2005 using a PVC hand-held sediment corer. Sub samples were taken at 2cm intervals from surface to 10cm by carefully sectioning the core in the lab.

2.2. Chemical analyses

Organic carbon was determined by wet oxidation method with a precision of 0.01% (El Wakeel and Riley, 1957). Sub samples for metal analyses were dried at 60(±2)°C for 48 hours and disaggregated in an agate mortar, before chemical treatment for Fe and Mn analysis. For each sample, a known quantity (~0.2 g) of sediment was digested in a teflon vessel with a solution (10ml) of concentrated HF (48% GR; Merck), HNO₃ (69% GR;
Merck) & HClO₄ (35% GR; Merck) in a ratio of 7:3:1. The sediment was then dried on a hot plate in a fume hood chamber.

An aliquot of 5ml of the above acid mixture was added and dried on the hot plate for one hour. Further, 2ml of concentrated HCl (35% GR; Merck) was added followed by 10ml of HNO₃ (69% GR; Merck). The residue was warmed and then transferred to a clean, dry standard flask to make up final volume to 100ml with double distilled water. Detailed procedure of sediment digestion is given in Balaram et al. (1995). Trace metal concentrations (Fe and Mn) were measured using a flame atomic absorption spectrophotometer (AAS, PerkinElmer Model 5000). The accuracy of the analytical procedures was assessed using the certified reference material MAG-1 (USGS) that yielded results within the reference value range (Flanagan, 1967 and 1976).

2.3. Microbial analyses

Sediment core was sectioned at 2 cm intervals in sterile conditions to obtain representative samples at 0-2, 2-4, 4-6, 6-8 and 8-10 cms depth. Sub-samples of approximately 5 gm wet weight sediment were sampled using sterile syringe cores. The sub samples were transferred to 45ml of full strength sterile seawater (10⁻¹ dilution). Tween80 (50µL) was added and the mixture was sonicated at 40 mHz for 10 secs. Serial dilutions of the sediment samples were performed in autoclaved seawater to yield dilutions from 10⁻¹-10⁻⁶/⁷.

Medium for the isolation of heterotrophic bacteria (HB) was prepared using various nutrient strengths of 0.01, 0.1 and 25% which correspond to 0.01%, 0.1% and 25% nutrient broth + 2% agar respectively. A concentration of 100% corresponds to 8 g nutrient broth (HiMedia Laboratories Pvt Ltd., Bombay, India) per 1000 ml seawater. About 100µL from
10^{-2} dilution was spread plated onto the medium. The plates were incubated for 15 days at room temperature. 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. Dry weight of the sediment used for the dilutions was determined by drying the filtered sediment in an oven at 60°C for 48 hours.

Nitrifiers (NtB) were enumerated by the Most Probable Number (MPN) method of Alexander and Clark (1965). Nitrifying media (seawater ammended with NH₄Cl of 2mM final concentration) was distributed in 5 ml quantities in 15 ml screw capped tubes. From each dilution ranging from 10^{-1}-10^{-6/7}, 500µl was inoculated in triplicate to the nitrifying media till the inoculation from the highest dilution into culture tubes yielded negative results. The culture tubes were incubated in dark for a period ~60 days at 28(±1)°C. After incubation, the tubes were tested for the presence of NO₂⁻ and/or NO₃⁻. The combinations of positive and negative tubes were scored and MPN assessed from McCready’s table (Rodina, 1972).

Statistical analysis was carried out using Pearson’s Correlation Coefficient in order to explore the possible associations existing between different variables. The analysis was done using Microsoft Excel 2000. The bacterial parameters were normalized by log transformation before analyses.

4. Results and Discussion

4.1. Down core variation and Index of geo accumulation for Iron and Manganese

Figures 2a,b and 3a,b shows the down core variation of Fe and Mn during pre monsoon, monsoon and post monsoon seasons. Overall range in sedimentary Fe values ranged
between 8 and 12.6% at the experimental site while it ranged between 4.7 and 9.3% at the control site. The Mn values varied from 0.06 to 0.52% at the experimental site and 0.05-0.48% at the control site. The values reported in the present study are comparatively higher to those reported by Ray et al. (2006) from the Godavari estuarine mangrove ecosystem on the eastern coastline of India. These authors reported that the average sedimentary Fe and Mn values were 0.004% and 0.001% respectively. Studies by Alagarsamy (2006) showed that the concentrations of Fe varied from 2.2 to 49.7% on the surface sediments of Mandovi estuary, while the concentration of Mn ranged below detection limit to 1.61%. Though reports from the Mandovi estuary (Alagarsamy, 2006) showed that metal concentrations were generally low during monsoon, compared to the pre and post monsoon, at the adjoining mangrove sediments they were found to vary. In the present study, the highest accumulation of Fe was observed during pre monsoon, while the values in the monsoon and post monsoon were lower and comparable. The variation of Mn values was similar to observations made by Alagarsamy (2006) in the surface sediments of the adjoining Mandovi estuary.

Irrespective of the depth, the experimental site showed an enrichment of Fe >100% of the control site during pre monsoon. Percentage of enrichment has been calculated using the formula:

If a>b:

Percentage of enrichment = \( \frac{(a-b)}{b} \times 100 \)

If b>a under washout conditions:

Percentage of enrichment = \( \frac{(b-a)}{a} \times 100 \)

where,
a = concentration of Fe/Mn at experimental site
b = concentration of Fe/Mn at control site

In general, enrichment increases with depth to reach values >165% at 8-10 cm interval during pre monsoon. During monsoon, the general enrichment pattern is reversed with the highest enrichment (85.6%) at 0-2 cm. Moreover, observations from statistical analysis indicate that there is no significant correlation between Fe and Mn in the monsoon months neither at the control nor at the experimental site. However, a direction is suggested in the relationship. It is negative at the experimental site and positive at the control site perhaps suggesting that under lower concentration of iron, manganese concentration tends to increase. With higher concentration of iron up to 12-13%, this trend changes suggesting that the increase of both the elements do not get coupled after a threshold.

During post monsoon, it could be observed that though the trend in down core variation fairly resembled the monsoon season, the magnitude fell sharply to show negative enrichment. It was interesting to note that though the control site was free from the influence of mining, the experimental site showed negative Mn enrichment in all sections during monsoon and post monsoon. This could be due to enhanced fresh water supply to the Mandovi compared to Chapora. These observations are contrasted by very high enrichment (393-773%) of Mn at the experimental site during pre monsoon season especially in the depth range of 4-6 cm. A positive relation between Fe and Mn during the non-monsoon months ($r=0.64$, $p<0.02$, $n=10$) at the control site and absence of such a relation at the experimental site showed that though the chemistry of Fe and Mn are closely related, they could be differentially preferred by organisms which in turn is influenced by
the prevailing environment. Alternatively, the concentration of iron is too high to warrant any bacterial dependence.

The geoaccumulation index ($I_{geo}$) was originally defined by Müllner (1979) for metal concentrations in the <2μ fraction and developed for global standard shale values, which is expressed as follows:

$$I_{geo} = \log_2 \left( \frac{C_n}{1.5B_n} \right)$$

where,

$C_n$ = Measured concentration of metal 'n' in the sediment

$B_n$ = Background value for metal 'n'

The factor 1.5 is used for the possible variations of the background data due to lithological variations.

The choice of the background value plays an important role in the interpretation of geological data. $I_{geo}$ has been widely utilized as a measure of pollution in freshwater (e.g. Müllner, 1980; Singh et al., 1997; Kralik, 1999) and marine sediments (e.g. Stoffers et al., 1986; Bryan and Langston, 1992; Dickinson et al., 1996). Geoaccumulation index ($I_{geo}$) of Fe and Mn in the experimental site (control site metal values were taken as reference values) was computed based on Müllner (1979) for all the depths and seasons. The results are plotted in Figures 4a and b.

According to the $I_{geo}$ classification (Table 1), it could be inferred that the sediments in the depth range 0-10 cm fall in the 'uncontaminated to moderately contaminated by iron' category during the pre monsoon and monsoon season. While, in the post monsoon season,
though the 0-4 cm still remains 'uncontaminated to moderately contaminated by iron', the 4-
10 cm layer has recovered from Fe contamination and could be termed as 'Uncontaminated'. The contamination due to Mn is more acute than Fe during the pre monsoon season. During the pre monsoon the 0-8 cm section falls under the 'Moderately to strongly contaminated' category while 8-10 cm section falls under the 'Moderately contaminated category'. All the depths fall in the 'Uncontaminated' group during the monsoon and post monsoon and hence could be assessed as free from the Mn pollution. These observations could again suggest that though the elements are closely related, the biogeochemical cycling of Mn could be more efficient and rapid when compared to Fe in the mangrove sediments. Moreover, the mangrove ecosystems play a buffering role by reducing the enrichment levels of Fe and Mn in the sediments. The overall assessment could be that though the Mandovi estuary is under the influence of ferro-manganese ore mining with significant impact on the estuarine sediments, the sediments of the adjoining mangroves are comparatively less contaminated, but when strongly contaminated could be self-regulatory and recover in the time scale tested.

4.2. Total Organic Carbon (TOC) and Bacterial Trophic Structure

Down core variability in TOC for the different seasons are illustrated in figures 5 a and b. TOC varied from 0.02-1.8% in the control site, where as at the experimental site it varied from 0.12-2.74%. Lowest TOC values were recorded during the post monsoon season at the control site and the highest values were recorded during the pre monsoon at the experimental site.
TOC varied with different fractions of retrievable heterotrophic bacteria (Figs 6 and 7). In pre monsoon season, TOC and HB retrieved on all three strengths of nutrient agar showed a positive relationship at the control site. However, only on 0.01% HB the correlation was significant at $r=0.80$, $p<0.05$. Despite the season, it could be observed that at the control site the abundance of HB retrieved on 0.01% dNA is limited ($r=0.50$, $p<0.05$, $n=5$) by the availability of organic carbon. The extent of limitation due to carbon availability for HB retrieved on 0.01% ($r=0.89$, $p<0.01$, $n=5$) and 0.1% dNA ($r=0.96$, $p<0.001$, $n=5$) is maximum during the monsoon. Though there is a heavy organic flux into the estuary during the monsoon, the higher dependency of HB on TOC could be due to the lower lability of the organic carbon. Reduced residence time during monsoon could be responsible for the delayed degradation of complex organic matter into simpler labile forms. Short residence time could also be due to the large seasonal influx of freshwater into the estuary, with unchanged tidal amplitude over large distances (Unnikrishnan et al., 1997). As in the control site, at the experimental site too, TOC showed a positive relation with 0.01% HB ($r=0.72$, $p<0.1$) during pre monsoon. There does not exist any significant relation between 25 and 0.1% fraction of HB with TOC at the experimental site during pre monsoon and monsoon season.

The scenario in post monsoon is very different from the preceding seasons at both the control and experimental sites. It was observed that the 0.01% dNA fraction of HB had a positive relationship of $r=0.889$ ($p<0.02$) and $r=0.79$ ($p<0.05$) respectively with TOC. The other fractions bore an insignificant, negative relationship. However, it is suggestive of enhanced utilization compared to production of organic carbon. Though the estuarine environment is eutrophic with considerable organic loading, a persistent relation between
TOC and 0.01% DNA fraction of HB could be due to a dominant fraction of heterotrophs which constantly require an optimal organic carbon concentration of 0.01%. Similar observations have been made earlier by Goltekar et al. (2006) in the Mandovi estuary which indicate that bacteria from relatively more dynamic estuarine systems seemed less resilient compared to the coastal and offshore populations, as they were best retrieved only on 1% nutrient strength.

4.3 Relation of sediment geochemistry with bacterial trophic status

It was interesting to note that TOC related positively to Fe ($r=0.67$, $p<0.01$, $n=15$) and Mn ($r=0.92$, $p<0.001$, $n=15$) at the experimental site where as the relationships were negative at the control site ($r=-0.72$, $p<0.001$, $n=15$ for Fe and $r=-0.51$, $p<0.05$, $n=15$ for Mn). These relationships could imply that at the experimental site there is considerable extraneous input of TOC favoring the accumulation of Fe and Mn. Wangersky (1988) has reported that coatings of organic matter prevalent in fine-grained sediments bind a variety of trace elements. Enrichment of Mn in the pre monsoon and its subsequent removal in the monsoon indicate that Mn turnover time could be very less as it is actively removed both by physical processes and biogeochemical sequestration. It could be observed that at the experimental site NtB are higher at the depth ranges 2-4cm and 8-10 cm. Irrespective of the season, a positive correlation between Mn and NtB ($r=0.61$, $p<0.01$, $n=15$) at the experimental site suggests that Mn could be used actively as a co factor for ammonia monooxygenase enzyme. More importantly, it could also serve as an alternate terminal electron acceptor in anaerobic respiration (Hulth et al. 1999). This relationship disappeared when the Mn levels decreased during the monsoon season and get re-established during the
post monsoon season ($r=0.62$, $p<0.02$ $n=10$) when the Mn levels increased. Absence of a significant relation between Fe with various fractions of heterotrophs and nitrifiers at both the control and experimental sites, suggested that Fe is present in excess and perhaps unlimiting. Moreover Mn (IV) reduction preceeds that of Fe (III) because of reduction energetics of the solid phases (Burdige et al., 1992).

The relationship between Mn and various fractions of HB indicate that the latter has a considerable influence in regulating the levels of Mn. At the experimental site, HB cultured on 0.01% DNA related to Mn during pre monsoon ($r=0.826$, $p<0.05$, $n=5$) and further strengthened during the monsoon ($r=0.968$, $p<0.001$, $n=5$). HB on 0.1% DNA also showed a relationship to Mn concentrations ($r=0.951$, $p<0.001$, $n=5$) during the monsoon. However, during post monsoon, the variation in HB on a higher strength of 25% nutrient agar affected the variation in Mn concentration up to 90% ($r=0.949$, $p<0.001$, $n=5$). All these observations show that the different fractions of HB could respond in sequence to the Mn enrichment-taking place in the pre monsoon season. These relationships demonstrate that HB are actively involved in maintaining the level of Mn at par with reference levels at Chapora estuary.

Very strong positive correlation between NtB and TOC in the pre monsoon season ($r=0.987$, $p<0.001$, $n=5$) showed that heterotrophic nitrification could be a dominant process controlling the cycling of ammonia in sediments. Potential of heterotrophic nitrification in sediments has been previously reported by Schimel et al. (1984). Further, the absence of a relation in monsoon and a negative relationship in post monsoon ($r=-0.673$, $p<0.1$, $n=5$) could indicate a shift from a relatively heterotrophic to relatively autotrophic mode of nitrification.
5. Conclusion

The present study shows that the heterotrophs respond to the Mn enrichment and could be active in maintaining its level and the other associated metal concentrations like that of Fe at par with reference site. A positive correlation between Mn with NtB at the experimental site during the non-monsoon months is indicative of the latter’s contribution to regulation of the metal concentration especially of Mn in the sediment probably through its use as a terminal electron acceptor in respiration at the expense of manganese oxide. The study therefore lends support to our hypothesis that both autochthonous autotrophs and heterotrophs work in tandem to mitigate manganese and perhaps related metals like iron in mangrove swamps. Though the Mandovi estuary is under the influence of ferro- manganese ore mining with relatively higher sediment metal concentrations, the adjoining mangroves are comparatively less contaminated due to the self-regulation facilitated by the native bacterial flora.

Acknowledgements

We are thankful to the Dr. S.R. Shetye, Director, NIO, for providing facilities to carry out this work. We also acknowledge the help rendered by Dr. Maheshwari Nair, Mr.Vineesh and Mr. Martin in trace metal analysis. KPK and SOF record their gratefulness to UGC and CSIR (Government of India) for the award of Senior Research Fellowship. This is NIO contribution no. 4246.
References:

Alagarsamy, R. 2006. Distribution and seasonal variation of trace metals in surface sediments of the Mandovi estuary, west coast of India. Estuarine, Coastal and Shelf Science 67, 333-339.


**Figure legends:**

Fig. 1. Location of sampling sites in the Chapora and Mandovi estuary.

Figs 2 and 3: Down core variation in Fe (2a&b) and Mn (3a&b). ‘a’ refers to the control site while ‘b’ refers to experimental site. (○ Pre Monsoon, + Monsoon and ■ Post Monsoon).

Fig 4a. and 4b represent $I_{geo}$ values of Fe and Mn respectively for the experimental site at Divar. The Y-axis scale and legend to Fig.4b is same as that of Fig.4a.

Fig 5 a (Control site) and 5 b (Experimental site) show the down core variation in Total Organic Carbon (TOC) in sediments. . (○ – Pre Monsoon, + - Monsoon and ■ - Post Monsoon).

Figures 6 (Control site) and 7 (Experimental site) show the down core variation of bacterial groups with different nutritional requirements. (○ – Pre Monsoon, + - Monsoon and ■ - Post Monsoon).

**Legend for Table:**

Table 1. Description of sediment quality by geoaccumulation index (Müller,1979).
Fig. 1.
Geo-accumulation index of iron at experimental site

![Geo-accumulation index of iron at experimental site](image)

Fig. 4a

Geo-accumulation index of manganese at experimental site

![Geo-accumulation index of manganese at experimental site](image)

Fig. 4b
Bacterial contribution to mitigation of iron and manganese in mangrove sediments
Table 1.

<table>
<thead>
<tr>
<th>$I_{geo}$ Value</th>
<th>$I_{geo}$ Class</th>
<th>Description of sediment quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;5</td>
<td>6</td>
<td>Extremely contaminated</td>
</tr>
<tr>
<td>4--5</td>
<td>5</td>
<td>Strongly to extremely strongly contaminated</td>
</tr>
<tr>
<td>3--4</td>
<td>4</td>
<td>Strongly contaminated</td>
</tr>
<tr>
<td>2--3</td>
<td>3</td>
<td>Moderately to strongly contaminated</td>
</tr>
<tr>
<td>1--2</td>
<td>2</td>
<td>Moderately contaminated</td>
</tr>
<tr>
<td>0--1</td>
<td>1</td>
<td>Uncontaminated to moderately contaminated</td>
</tr>
<tr>
<td>&lt;0</td>
<td>0</td>
<td>Uncontaminated</td>
</tr>
</tbody>
</table>