

Immobilization of manganese, cobalt and nickel by deep-sea-sediment-microbial communities

Sujith P.P, Anindita Das, Babu Shashikant Mourya and Loka Bharathi P.A*

Microbiology Laboratory, Biological Oceanography Division,
National Institute of Oceanography, Council of Scientific and Industrial Research,
Dona Paula, Goa-403 004, India

Ph:091-0832-2450281

Fax: 091-0832-2450606

*Corresponding author: loka@nio.org

Running title: Metal immobilization in deep-sea sediments

ABSTRACT

To test the hypothesis that the autochthonous microbial communities from polymetallic nodule rich Central Indian Basin sediments could actively participate in immobilizing metal ions, box core samples BC26 and BC36 from geologically different settings were examined. The bottom water dissolved oxygen concentration in the northern siliceous ooze BC26 was reported to be 4.2 to 4.3 ml l⁻¹ and in the southern pelagic red clay BC36 to be 4.1 to 4.2 ml l⁻¹; the sedimentation rates of these regions were 0.834 and 0.041cm kyr⁻¹ respectively. The onboard experiment conducted under oxic and sub-oxic conditions with 100 μmol concentrations of Mn, Co and Ni showed that the microbial immobilization under sub-oxic condition was higher than azide treated controls in BC26 for Mn, Co and Ni at 30, 2 and 4cm below sea floor (bsf) respectively after 45d. The immobilization trend was BC26>BC36, Co>Mn> Ni under oxic and Mn>Co>Ni under sub-oxic conditions. The depth of maximum immobilization of Co in BC26 under sub-oxic condition coincided with the yield of cultured Co tolerant bacteria and Ni only with organic carbon at 4cm bsf. This study demonstrates that organic carbon content and the concentration of the bioavailable metals in sediments regulate microbial participation in metal immobilization.

Keywords: metals; microbes; immobilization; sediment; Central Indian Basin

1. Introduction

Sediments are complex mixtures of Fe and Mn oxide mineral phases, detrital organic matter and several other organic and inorganic phases. They support different groups of metal oxidizing and reducing microorganisms [1]. Microbes in sediments can interact with dilute metal ions in solution, including sequestration and aiding the concentration of these ions into the sediment. The ions remain either complexed in sediments as bio-metal coordinates, or undergo a series of microbial or chemical transformations. This could lead to the recycling of metals into the overlying water column or to their immobilization in sediments as authigenic mineral phases [2].

Microbial immobilization of metals can be either catalytic or non-catalytic; it precedes through four different mechanisms namely biosorption, bioaccumulation, redox reaction and complex formation [3]. Immobilization may be through cellular sequestration and accumulation, or through extra-cellular precipitation [4-5]. The marine bacteria produce large amounts of organic surface material that interact with the metal ions. They subsequently involved in the precipitation of the soluble metal cations and formation of oxide species on bacterial walls that can chemically react with the residual metals in solution [6-8].

The significant portion of the metals and their various organic derivatives in nature are contained in the living biomass. For example, Razzell and Trussell (1963) [9] addressed the interaction of *Thiobacillus* spp with sulfide bearing ores and their role in dissolution of oxides and release of valuable metals. Likewise, Fortin et al (1994), Basnakova and Macaskie (1996) [10-11] presented the contribution of bacteria in the formation of sulfide and phosphide minerals by chemical complexing with available constituent sulfide and phosphide groups. A few studies also explain the intracellular sequestration [12-13] and extracellular precipitation [14-15] of metals by a group of metal resistant bacteria each affecting the metal mobility in the environment.

The deposition of organic carbon and the availability of oxygen from the bottom waters are primary factors that affect the geochemical and biological processes in the sediment water system [16]. Availability of nutrients and oxygen therefore determine the sensitivity and adaptation of an organism to metals. The sediments being the ultimate repositories for environmental particles, the microbial processes within the sediment can also control the chemical changes with response to metal exposure. Earlier experiments have shown the immobilization of metals in sterile sediments

by individual isolates [17-18]. The work presented here aimed to investigate the immobilization of metals by autochthonous microbial communities.

The present study area covers two regions of the Central Indian Basin (CIB) (Figure 1 modified [19]). The region for Box Core (BC) 26 is located at 10°S latitude and 75.5°E longitude in the northern region of the ocean. The site of sampling has a water depth of 5339 m and is relatively less oxidizing [20] and receives more clay supplies from rivers [21&22]. BC36 located at the southern region of the basin is at 16°S latitude and 75.5°E longitude. The region has a water depth of 5042m, the environment is more oxidizing [20] and is known for the slower rate of sediment deposition [21]. Formation and dissolution of authigenic (oxyhydr)oxides of Fe and Mn influence the cycling of trace metals in oxic/sub-oxic surface sediments [23]. In view of that, we performed the experiment under oxic and sub-oxic incubation conditions to test the hypothesis that the autochthonous microbial communities from CIB sediments could actively participate in metal immobilization. This study assesses the contribution of redox condition, microbial abundance and activity, to immobilization of Mn, Co and Ni.

2. Materials and Methods

2.1 Onboard sampling

The sediment cores were retrieved using a 50 × 50 × 50 cm box corer. The sediment core BC26 was collected from a siliceous ooze area and BC36 from a pelagic red clay region. The box cores were sub-sampled by inserting acrylic core liners of 6.3 cm diameter into the sediment. The sediment cores were further sub-sectioned at 2 cm intervals down to a depth of 10 cm and thereafter at 5 cm intervals to a depth of 35 cm. The sediment samples were collected in sterile polyethylene bags and were stored at 4 °C until further investigations.

2.2 Total bacterial counts

The sediment dilution for total bacterial counts (TBC) was prepared by suspending about 2 g of homogenized sediment in 9 ml of sterile seawater. A small portion of the above dilution was fixed with buffered formalin to a final concentration of 2% and was stored at 4 °C until analysis [24]. The samples were sonicated at 15 Hz for 15 s, allowed slurry to settle for 30 s and 1 ml of the supernatant was filtered through a 0.22 μ black Millipore polycarbonate filter. Samples were then stained with 0.01% acridine orange for 3 min prior to microscopic observation. About 10-15 microscopic fields

were counted for each sample at 1500X magnification using a Nikon 80i epifluorescence microscope (Nikon, Tokyo, Japan). The counts were normalized per gram of dry sediment.

2.3 Plate counts

The bacteria resistant to Mn, Co, Ni and heterotrophic bacteria were enumerated in triplicates by spread plate method from the above dilution. The heterotrophs were grown on 20% ZoBell marine agar (ZMA) media. Metal resistant bacteria were cultured on seawater agar (SWA) medium (1.5% bacto agar in natural seawater with no other additional nutrients) amended with 100 μmol concentrations of metal salts $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (Sd. fine Chem. Ltd). For isolation of bacteria resistant to more than one metal, 100 μmol concentrations of each metal was added to SWA medium of the above composition. The inoculated plates were incubated at 3 ± 1 °C for 4-10 d and the colony forming units (CFU) were counted and normalized for gram dry weight of sediment.

2.4 Eh measurements

The Eh measurements were made at the beginning and end of the experiments. Before measurements, the Eh electrode was rinsed with distilled water and calibrated with oxidation reduction potential (ORP) standard solution to ensure accurate readings. For measurements, the electrode was slowly dipped into the sediment slurry in tubes and allowed to stabilize for a period of 60 s. The direct reading obtained by mV meter was taken in triplicate and the mean values were determined. The Eh was measured following the user's guide (Redox / ORP Electrodes) Thermo Electron Corporation (2005).

2.5 Determination of total organic carbon (TOC)

Total inorganic carbon was analyzed using UIC CM 5014 Coulometer with CaCO_3 as the standard and total carbon with NCS 2500 elemental analyzer [25], cross-checked with UIC CM 5014 Coulometer. Total organic carbon was determined indirectly by subtracting the values of total inorganic carbon from total carbon [26].

2.6 Solid phase concentration of metal ions in sediments

The concentration of metal ions in the sediment was determined by voltammetry after total decomposition of 50 mg of lyophilized sediments by the closed vessel digestion method [27]. The

Mn concentration in aqueous samples was determined following the method of Colombini and Fuocco (1983) [28] with 2 standard additions of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (4 mg/l). The concentration of Ni and Co in samples was determined by the modified method of Herrera-Melian et al (1997) [29]. The analysis of the metals was carried out using a voltammeter in interface with 797VA computrace (Metrohm, Switzerland). The potential was measured against an Ag/AgCl reference electrode and a platinum rod as a counter electrode. The Ni and Co determination was carried out with HMDE (Hanging mercury drop electrode) in the differential pulse mode. The determination was carried out at pH 9 in a measuring cell containing 10 ml of supporting electrolyte (Ammonia buffer, pH 9.5) and 100 μl of dimethylglyoxime (0.1 mol) in ethanol. Sample volume (1 ml), depending on the concentration, was appropriately diluted with Milli-Q water (18.2 Ω resistance) prior to analysis. The sample was directly added to the measuring vessel containing the electrolyte and was degassed with 99.9995% Nitrogen (Medgas and Equipments) for 300 s prior to deposition. The analysis was carried out with deposition time 30 s, equilibration time 5 s and voltage step time of 0.3 s. The potential was scanned in replications of 3 from -0.699 to -1.2 V with sweep rate of 6mVs^{-1} . The analysis was quantified with 2 standard stock additions of Ni^{2+} ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 1 mg/l) and Co^{2+} ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 1 mg/l).

2.7 Demonstration of metal immobilization

Box core sediments collected from two distinct regions of CIB were used in the present study to set up the laboratory experiments on-board. Portions of sediments from each sub-section of the cores were inoculated using sterile spatula into 15 ml screw-capped tubes containing 100 μmol concentrations of metal chloride salts dissolved in sterile seawater. Suitable poisoned controls sodium azide (10 mmol) to check for metal adsorption and autoclaved natural seawater amended with metal salts without any inocula (sterile controls) for abiotic precipitation were maintained. The incubation was carried out in triplicates at 3 ± 1 °C (to simulate *in situ* temperature) under oxic and sub-oxic conditions. The oxic incubation was assured by directly inoculating 1.5 ± 0.5 g wet sediment in half-filled tubes and sub-oxic in completely filled tubes.

2.8 Metal analysis

The supernatant (1 ml) from inoculated and uninoculated tubes after centrifugation (8000 rpm for 10 min at 4 °C) were analyzed on day 0 and at the end of 45 days for determining the change in metal concentration. The residual concentration of metals in each tube was analyzed by spectrophotometric

(Multiskan Thermo Spectrum) method. Any samples that had delay in analysis was acidified with 1N HCl and stored at 4 °C. The Mn concentration in the sample was determined with 1-(2-pyridylazo)-2-naphthol method at 560 nm [30]. The determination of Ni with dimethylglyoxime and Co with nitroso-R-salt was done according to the scheme of Chester and Hughes (1968) [31] at 460 and 500 nm respectively. Mean and standard deviation were calculated for microbially (biotic) and non-microbially (abiotic) promoted metal immobilization. The values were corrected for chemical precipitation in both the experimental setups. One-way analysis of variance (ANOVA) and correlation analysis including the levels of significance were done for data points n =10 using Statistica (2005).

2.9 Determination of sediment dry weight

The sediment slurry from each tube was mixed and poured onto a preweighed filter positioned in a filtration setup at the end of analysis. The filter with sediment was dried at 105 °C and reweighed until constant. The filter weight was subtracted from the sediment weight to derive the actual dry weight of sediment. The concentration of the immobilized metal was deduced and normalized for gram dry weight sediment after correcting for corresponding controls.

3. Results and Discussion

3.1 Characteristics of the two sampling stations

Two sediment cores BC26 and BC36 were collected along the north south transect of the CIB and were analyzed for immobilization of Mn, Co and Ni by the sediment microbial communities. The sediment slurry was found to support growth of both heterotrophic and metal resistant bacteria. The yield of cultured heterotrophic bacteria varied from below detectable levels (BDL) to $5.4 \pm 0.73 \times 10^3$ (CFU) per gram dry sediment in BC26 (Table 1) and $8.0 \pm 0.18 \times 10^4$ to $2.4 \pm 0.37 \times 10^5$ CFU/g sediment in BC36 with maximum culturability at 8 cm depth (Table 2). The counts of metal resistant bacteria ranged from below detectable levels to $9.0 \pm 0.11 \times 10^2$ for Ni at 10 cm depth, $4.3 \pm 0.31 \times 10^4$ for Co and Mn at 2 and 20 cm depth respectively. In mixtures of f Mn, Co, and Ni (100 μ mol each), culturability of bacteria was $1.4 \pm 0.33 \times 10^3$ CFU/g at 10 cm depth in BC26 (Table 1). In parallel, with BC36 it was $2.0 \pm 0.1 \times 10^4$ to $2.8 \pm 0.04 \times 10^5$ CFU/g for Co with maximum culturability at 10 cm depth, $3.9 \pm 0.06 \times 10^4$ to $2.4 \pm 0.31 \times 10^5$ for Ni, 1.2 ± 0.24 to $3.9 \pm 0.02 \times 10^5$ for Mn and $5.0 \pm 0.02 \times 10^4$ to $2.2 \pm 0.18 \times 10^5$ CFU/g for metals in combination with maximum culturability at 4 cm depth (Table 2).

Though total bacterial abundance did not show a definite trend with depth of the cores (Figure 2A, B), the distribution of organic carbon did. The organic carbon in sediments varied from 0.175 to 0.485% in BC26 (Figure 2C) and 0.09 to 0.19% sediment dry weights in BC36 (Figure 2D) with maximum carbon concentration at 4 and 2 cm depth respectively. The total organic carbon content in seawater was 21.78 in BC26 and 9.18 mg C/l in BC36. The abiotic precipitation of metal ions in uninoculated sterile controls was negligible. The Eh as a measure of oxidizing and reducing conditions showed that BC26 could support sub-oxic and BC36 the oxic processes better (Figure 2E, F). The Eh in BC26 experimental tubes declined from +80.2 mV to +11.98 mV (± 2.6 , $n = 3$) in the oxic and +6.24 mV (± 3.2 , $n = 3$) in the sub-oxic incubations at 35 and 30 cm bsf respectively. The Eh in BC36 on the other hand decreased from +79.96 mV to +28.36 mV (± 2.6 , $n = 3$) in the oxic and +25.52 mV (± 3.2 , $n = 3$) in the sub-oxic incubations at 2 and 10 cm respectively for Mn (Table 3).

The maximum solid phase concentration of Mn (14.93) and Ni (4.55 $\mu\text{mol/g}$) in BC26 was observed at 8 cm depth and Co (6.24 $\mu\text{mol/g}$) at 30 cm depth. The concentration of Mn in BC36 sediment core was maximum (265.8 $\mu\text{mol/g}$) at 10 cm depth and Co (12.29 $\mu\text{mol/g}$) and Ni (17.52 $\mu\text{mol/g}$) at 35 cm depth (Figure 3). The concentration of the above metal ions was found unevenly distributed in the sediment cores. The maximum concentration of all the three metals was observed towards the surface, decreased with depth and showed a secondary maximum towards the subsurface in both the sediment cores. Our results agree with the report of Zhang et al (2002 & references therein [32]) on the double maxima for solute profiles.

3.2 Immobilization under oxic condition in BC26.

3.2.1 Biotic immobilization

The results showed higher Mn immobilization in the subsurface sediment (Figure 4A). The immobilization of the metal was $34.4 \pm 0.053 \mu\text{mol/g}$ dry sediment at 35 cm below sea floor (bsf). Manganese immobilization increased with depth ($r = 0.852$, $p < 0.001$). The yield of cultured heterotrophic bacteria co-varied with TBC at an r value of 0.602 ($p < 0.05$) suggesting that the variation in the former is responsible for 36% of the variation in the latter. The immobilization maximum observed at the deeper depth suggests that oxidized Mn is stable at this depth and is less prone to dissolution. The subsurface manganese maximum observed at 30-35 cm depth [33-35] in 13 different sediment cores collected from CIB substantiates the present finding.

In contrast to Mn, higher Co immobilization ($35 \pm 0.328 \mu\text{mol/g}$) was recorded at the sediment surface (Figure 4B). Interestingly, though the immobilization of this metal was maximum at the surface and coincided with the maximum yield of cultured Co tolerant bacteria (Table 3), this process showed a negative relationship with the yield of cultured Co tolerant bacteria by r value of 0.745 ($p < 0.01$). This probably suggests that immobilization of Co is mediated by other metal immobilizing bacteria in the sediments. The association of Co with Mn in the surface sediment is one process where Mn oxidizing bacteria indirectly participate in Co immobilization. The results agree with the observations of Sundby et al (1986) and Glasby (2006) [36 &37] on Co enrichment in the surface sediments. Our observations also support the earlier findings [38 &39] on the co-oxidation of Co with Mn in the aquatic systems. Likewise, immobilization of Co is known to co-occur with iron reduction in bacteria [40].

Like Mn, higher immobilization of Ni ($6.3 \pm 0.055 \mu\text{mol/g}$) was observed at the deeper 25 cm layer. The immobilization of Ni negatively related with the yield of Mn tolerant bacteria by r value of 0.643 ($p < 0.05$). This relation suggests that Ni immobilization may not be catalyzed by Mn oxidizing bacteria but more by bacteria resistant to Ni or Co. Further it is also suggested that in certain microorganisms, the resistance to one metal can also confer resistance to the other metals depending on the bioavailability and the combination of metals. Interestingly, the study of Stoppel and Schlegel (1995) [41] has shown that the bacteria tolerant to Ni are also tolerant to Co and possess determinants that resemble each other.

3.2.2 Abiotic immobilization

The abiotic immobilization was comparatively less than the biotic as only the passive process could be involved. The difference could be as much as 3 - 4 times in the case of Mn and 4 - 6 times in the case of Co and 6 times in the case of Ni. The maximum immobilization of Mn (11.61 ± 0.011) and Co ($8.45 \pm 0.06 \mu\text{mol/g}$) occurred at 30 - 35 cm bsf and Ni ($0.98 \pm 0.08 \mu\text{mol/g}$) at 25 cm bsf (Figure 5). The Mn immobilization showed depth dependence with r value of 0.928 ($p < 0.001$) and also related to biotic Mn oxidation with r value of 0.930 ($p < 0.001$) suggesting that 86% of the variation of biotic Mn oxidation could be due to the variation in abiotic. The BC26 sediments are composed of siliceous ooze and are low in sediment concentration of metal ions (Figure 3A, B, C). Our results shows that abiotic metal immobilization proceeds slowly and the rate depends upon the oxygen concentration, redox condition prevailing in the sediments, and the rate of the reverse activity ie dissolution. The results agree with the reports of Muller et al (1988) [42] and Glasby (2006) [37] on

surficial diagenesis and regeneration rate of Mn in the sediments with that of accretion rate of the metal in the associated polymetallic nodules. The passive sorption of metal ions on sediment and mineral particles occurs by physical attraction and / or chemical precipitation.

3.3 Immobilization under sub-oxic condition in BC26

3.3.1 Biotic immobilization

As mentioned above the biotic immobilization is definitely more than abiotic. The sub-oxic immobilization is 2.4 times more with Mn and 1.3 times more with Co than oxic.

Under sub-oxic condition, the microbial immobilization of Mn ($85.6 \pm 0.047 \mu\text{mol/g}$) was maximum at 30 cm bsf. Maximum Co ($46.3 \pm 0.29 \mu\text{mol/g}$) immobilization was at 2-4 cm bsf and it synchronized with the maximum yield of cultured Co tolerant bacteria ($4.3 \pm 0.31 \times 10^4 \text{ CFU/g}$) and organic carbon (0.485%) suggesting bacterial participation. The present results agree with previous studies [43-47] which reported positive correlation between heavy metal concentrations and the percentage of bacteria resistant to metals in different soils and sediments. However, maximum Ni immobilization ($6 \pm 0.08 \mu\text{mol/g}$) was evident at 4 cm depth (Figure 4C) and it synchronized with the organic carbon concentration (0.485%) in sediment, suggesting an energy dependent low affinity uptake process. The results are in agreement with Jasper and Silver (1977) and Bryson and Drake (1988) [48&49] on energy dependent Ni transport in bacteria and the chemoorganotrophic mode of nutrition in microorganisms in the presence of nutrients.

3.3.2 Abiotic immobilization

It is emphasized again that abiotic immobilization is less than the biotic especially under sub-oxic condition. The immobilization profile of Mn was similar to that occurring under oxic condition with the maximum of $22.5 \pm 0.047 \mu\text{mol/g}$ at 35 cm bsf (Figure 5A). The peaks of Co and Ni immobilization of 6.97 ± 0.027 and $0.65 \pm 0.012 \mu\text{mol/g}$ were at 10 and 4 cm bsf respectively (Figure 5B, C). The distribution of Mn and Co did not match with the organic carbon content of the sediment. The immobilization detected at low levels in poisoned sediments suggests passive immobilization on Mn oxide phase. Our results agree with earlier reports on such trapping of metal ions by manganese oxides [50&51]. However, the immobilization of Ni was less than Co and it corresponded with the organic carbon (0.485%) content of the sediment. Thus organic carbon in sediments could also complex with Ni and contributes to its immobilization at a much lower rate

than under biotic condition. Such association of Ni with organic carbon in sediments has been previously observed by Turner et al (1998) and Xue et al (2001) [52&53].

3.4 Immobilization under oxic condition in BC36

3.4.1 Biotic immobilization

In this core biotic immobilization is greater than abiotic except in the case of Mn where it is vice versa. Under oxic condition, the immobilization of all the three metals was maximum at the sediment surface (2 cm bsf) and was lower than at BC26. The trend in the immobilization was Co (45 ± 0.028) > Mn (19.8 ± 0.41) > Ni ($7 \pm 0.09 \mu\text{mol/g}$) as shown in Figure 4D - F. The solid phase concentrations of metal ions were Mn > Ni > Co. Thus, the depth at which maximum immobilization occurred did not match with the depth that showed maximum for solid phase concentration of metal ions. This suggests that the gradient of the metal concentration was more optimal for immobilization at the surface than at the subsurface. The organic carbon content of the core was maximum (0.19 %) at the surface and it related with the immobilization of Mn with r value of 0.835 ($p < 0.01$) suggesting mixotrophic mode of nutrition. The culturability of bacteria tolerant to Mn - Co - Ni was maximum (1.9 to 2.2×10^5 CFU/g) within the 0 - 4 cm depth in multiple metal amended plates. These observations suggest that microbially mediated immobilization of the above metals in BC36 sediments are driven by the nutritive status of the sediment and the predominance of metal tolerant bacteria. It is consistent with the observation of Dean-Ross and Mills (1989) [54], that groups of microbial communities that are relatively abundant in the sediment can utilize one or more aromatic compounds and are resistant to one or more heavy metals.

The microbial immobilization of Co synchronized with the maximum in Mn immobilization. The yield of cultured Co tolerant bacteria positively related with the TOC and yield of Ni tolerant bacteria by r values of 0.766 ($p < 0.01$) and 0.606 ($p < 0.05$) respectively. These relations suggest that Ni and Co immobilization are mediated by the same microbes to a certain extent (36%) and 58% variation in both is dependent on the variation in TOC. The ability of Ni resistant bacteria to immobilize Co is evident from previous experimental records [55]. However, Co and Ni immobilization can be independent of Mn immobilization and could occur by process where Ni immobilizing bacteria catalyze Co immobilization and vice versa.

The immobilization of Ni was comparatively lower than the other metals. However, the yield of cultured Ni tolerant bacteria could correlate with the yield of Co tolerant bacteria ($r = 0.606$, $p <$

0.05) suggesting that the same microbial communities are capable of immobilizing both the metals. These results agree with our recent reports [56 & 4] on Ni and Co immobilization by the Mn oxidizing bacterial isolates from the Indian Ridge system. TBC correlated positively and TOC negatively with depth ($r = 0.868$, $p < 0.001$ and $r = 0.666$, $p < 0.05$) respectively. These observations suggest that microbial abundance, the diversity of their metabolic activities and the number of metal resistant bacteria in sediment direct the immobilization of Ni in the BC36 sediments. Nonetheless, TOC in sediments were also found to influence the bioavailability of the metal in sediments. It is clear from the report of D'Hondt et al (2004) [57] that organisms that depend on electron-accepting pathways with higher standard free energies may have higher energy requirements than organisms that depend on pathways with lower standard free energy yields.

3.4.2 Abiotic immobilization

Although the prevailing environmental conditions in these sediments are appropriate for microbial immobilization of Mn, the abiotic immobilization of the metal was higher than the biotic. It is suggested that processes that occur in these sediments could be more passive than active. The maximum immobilization of Mn ($25.33 \pm 0.04 \mu\text{mol/g}$) was at 6 cm depth (Figure 5D). Co and Ni immobilization continued to remain higher under biotic condition and showed only maximum abiotic immobilization of $6.43 \pm 0.011 \mu\text{mol/g}$ for Co and $0.95 \pm 0.009 \mu\text{mol/g}$ for Ni at 8 and 25 cm depth respectively (Figure 5E, F). The immobilization of metal ions did not relate with organic carbon or the natural concentration of the metal ions in sediments. The results suggest that immobilization by abiotic process is directed to changes with concentration of the metal and the availability of charged particle binding sites in sediments. Our results agree with the report [58] on trace metal binding to reactive sediment surfaces.

3.5 Immobilization under sub-oxic condition in BC36

3.5.1 Biotic immobilization

Unlike under oxic condition, the immobilization of Mn was maximum ($13.5 \pm 0.035 \mu\text{mol/g}$) at deeper depth of 10 cm bsf. Co and Ni continued to show maximum immobilization of 27.5 ± 0.54 and $6.6 \pm 0.075 \mu\text{mol/g}$ at shallower depths of 6 and 8 cm bsf respectively (Figure 4E-F). Thereafter a gradual decrease in immobilization was observed towards the bottom of the core. Earlier investigations [54] on bacterial community composition and function along a heavy metal gradient provide explanation that bacteria are not exposed to the same concentrations of heavy metal *in situ* as

in laboratory-prepared media. However, the present study does not use laboratory prepared organic media. Moreover, it simulates deep-sea conditions by avoiding any organic amendments and maintaining the temperature at $3\pm 1^\circ\text{C}$. Therefore, it is suggested that the results could reflect *in situ* trend to a certain degree. The maximum immobilization of Mn and Ni coincided with peak culturability of heterotrophic bacteria and the solid phase sediment concentration of metal ions.

The immobilization of Mn showed a negative relationship with the yield of cultured Mn tolerant bacteria ($r = 0.609$, $p < 0.05$). It suggests that the background concentration along with the amended Mn could be beyond the tolerance limit. The toxicity of excess Mn is evident from the earlier studies [59 & 14]. In contrast, the depth of highest Co immobilization synchronized with maximum culturability of multiple metal tolerant bacteria and organic carbon content of sediments. The immobilization of Co showed correlation with the yield of cultured Mn tolerant bacteria ($r = 0.677$, $p < 0.05$). The association of Co with Mn and its co-oxidation by Mn oxidizing microorganisms result in immobilization. Our results agree with the report of Lienemann et al (1997) [60] on the oxidation of Co in association with Mn in the aquatic waters. It is therefore inferred that metal concentration in the sediment, its organic carbon content and the microorganisms present could govern the active immobilization of metal ions.

3.5.2 Abiotic immobilization

At this sampling site, abiotic immobilization of metal ions is higher than the biotic. The maximum immobilization of Mn ($22.89 \pm 0.03 \mu\text{mol/g}$) was at 6 cm, Co ($7.18 \pm 0.024 \mu\text{mol/g}$) at 8 cm and Ni ($0.70 \pm 0.01 \mu\text{mol/g}$) at 4 cm bsf (Figure 5). The depth of maximum immobilization of Co matched with that of maximum culturability of heterotrophic bacteria from the biotic samples. Besides, the passive rates with the azide treated sediments were much lower for Co and Ni than untreated samples. Similarly, depth of immobilization of Ni coincided with the yield of cultured Ni tolerant and Mn - Co - Ni tolerant bacterial numbers in biotic samples. Results suggest that azide when used as a poison restricts the active process of metal immobilization by bacteria but does not inhibit the passive process of metal ion binding to the bacterial cell surfaces. Our results agree with the report [61] that surface adsorption is possible even in non-viable cells albeit at low levels. Heavy metal can be immobilized by metabolism-independent process on cell surface components and charged particles in sediment depending upon the milieu.

3.6 Immobilization under oxic vs sub-oxic conditions

The microbial immobilization of metals under sub-oxic condition was significantly higher ($p < 0.001$) in BC26 than in BC36 and was higher than azide treated controls. The trend in immobilization was $\text{Co} (35 \pm 0.328) > \text{Mn} (34.4 \pm 0.053) > \text{Ni} (6.3 \pm 0.055 \mu\text{mol/g})$ under oxic and $\text{Mn} (85.6 \pm 0.047) > \text{Co} (46.3 \pm 0.29) > \text{Ni} (6 \pm 0.08 \mu\text{mol/g})$ under sub-oxic conditions. In BC36, the metal immobilization was higher under oxic condition. The trend in immobilization was $\text{Co} (45 \pm 0.028) > \text{Mn} (19.8 \pm 0.41) > \text{Ni} (7 \pm 0.09 \mu\text{mol/g})$ under oxic and $\text{Co} (27.5 \pm 0.54) > \text{Mn} (13.44 \pm 0.035) > \text{Ni} (6.6 \pm 0.075 \mu\text{mol/g})$ under sub-oxic condition. The immobilization rates of metals in these sediments were mostly $\text{Mn} > \text{Co} > \text{Ni}$. Generally, the immobilizing rates of Mn and Co were greater at BC26 than at BC36 with the rates of sub-oxic conditions being higher than oxic conditions. Such discernible differences were not evinced in the case of Ni.

4. Summary and Conclusions

From the present study it is clear that the higher immobilization of metals under sub-oxic condition in the subsurface sediment was not greatly influenced by organic carbon but by higher availability of reduced metal ions. The significant difference in metal immobilization between the experiment and poisoned controls probably indicate the involvement of both active and passive process of metal immobilization in experiment and only latter process in the case of control. This study demonstrates that organic carbon content and the concentration of the bioavailable metals in sediments regulate microbial participation in metal immobilization.

Acknowledgement

We are grateful to the Director, National Institute of Oceanography- Goa, for his support and encouragement. The Project Leader, Dr. R. Sharma and team members of the Polymetallic Nodules Environmental Impact Assessment Programme are thanked for the assistance and full support for this work. The Chief Scientist, Dr. N. H. Khadge, Captain, and Crew onboard *Akademic Boris Petrov* cruise no 26 are greatly acknowledged for their help and cooperation. SPP is grateful to Dr. V. K. Banakar for permitting him to participate and execute this work onboard. The lead author wish to thank Dr. N.T. Manoj and Mr. Pavaskar for the help in generating the map and Dr. B.N. Nath and Mascarenhas-Pereira for their valuable suggestions. SPP and AD are indebted to all labmates for their positive support. The authors thank Ministry of Earth Sciences (Government of India), for funding. AD and SPP acknowledges the Council of Scientific and Industrial Research, New Delhi-

India, for the award of Senior Research Fellowship. Comments from anonymous reviewers greatly improved the contents. This manuscript has NIO contribution number XXXX.

References

- [1] U. Forstner, *Inorganic sediment chemistry and elemental speciation*, in *Sediments: Chemistry and toxicity of in-place pollutants*, R. Baudo, J. Giesy, and H. Muntau, eds., Lewis Publishers, 1990, pp. 61-73.
- [2] T.J. Beveridge, J.D. Meloche, W.S. Fyfe, and R.G.E. Murray, *Diagenesis of metals chemically complexed to bacteria: Laboratory formation of metal phosphates, sulfides, and organic condensates in artificial sediments*, *Appl. Environ. Microbiol.* 45 (1983), pp. 1094-1108.
- [3] H. Brandl, and M.A. Faramarzi, *Microbe-metal-interactions for the biotechnological treatment of metal-containing solid waste*, *China Particuology* 4 (2006), pp. 93-97.
- [4] P.P. Sujith, V.D. Khedekar, A.P. Girish, and P.A. Loka Bharathi, *Immobilization of Nickel by Bacterial Isolates from the Indian Ridge System and the Chemical Nature of the Accumulated Metal*, *Geomicrobiol. J.* 27 (2010), pp. 1-11.
- [5] H.L. Ehrlich, *Microbes and metals*, *Appl. Microbiol. Biotechnol.* 48 (1997), pp. 687-692.
- [6] W.A. Corpe, *Metal-binding properties of surface materials from marine bacteria*, *Dev. Ind. Microbiol.* 16 (1975), pp. 249-255.
- [7] F.G. Ferris, W.S. Fyfe, and T.J. Beveridge, *Bacteria as nucleation sites for authigenic minerals in a metal-contaminated lake sediment*, *Chem. Geol.* 63 (1987), pp. 225-232.
- [8] D. Fortin, B. Davis, G. Southam, and T.J. Beveridge, *Biogeochemical phenomena induced by bacteria within sulfidic mine tailings*, *J. Ind. Microbiol. Biotechnol.* 14 (1995), pp. 178-185.
- [9] W.E. Razzell, and P.C. Trussell, *Microbiological leaching of metallic sulfides*, *Appl. Environ. Microbiol.* 11 (1963), pp. 105-110.
- [10] D. Fortin, G. Southam, and T.J. Beveridge, *Nickel sulfide, iron-nickel sulfide and iron sulfide precipitation by a newly isolated *Desulfotomaculum* species and its relation to nickel resistance*, *FEMS Microbiol. Ecol.* 14 (1994), pp. 121-132.
- [11] G. Basnakova, and L.E. Macaskie, *Bioaccumulation of nickel by microbially-enhanced chemisorption into polycrystalline hydrogen uranyl phosphate*, *Biotechnol. Lett.* 18 (1996), pp. 257-262.
- [12] S. Glasauer, S. Langley, and T.J. Beveridge, *Intracellular manganese granules formed by a subsurface bacterium*, *Environ. Microbiol.* 6 (2004), pp. 1042-1048.
- [13] Z. Shao, and F. Sun, *Intracellular sequestration of manganese and phosphorus in a metal-resistant fungus *Cladosporium cladosporioides* from deep-sea sediment*, *Extremophiles* 11 (2007), pp. 435-443.

- [14] S.O. Fernandes, K.P. Krishnan, V.D. Khedekar, and P.A. Loka Bharathi, *Manganese oxidation by bacterial isolates from the Indian ridge system*, *Biometals*. 18 (2005), pp. 483-492.
- [15] K.W. Mandernack, J. Post, and B.M. Tebo, *Manganese mineral formation by bacterial spores of the marine Bacillus, strain SG-1: Evidence for the direct oxidation of Mn(II) to Mn(IV)*, *Geochim. Cosmochim. Acta*, 59 (1995), pp. 4393-4408.
- [16] M.T. Schaanning, K. Hylland, D.O. Eriksen, T.D. Bergani, J.S. Gunnarson, and J. Skei, *Interactions between eutrophication and contaminants. II. Mobilization and bioaccumulation of Hg and Cd from marine sediments*, *Mar. Pollut. Bull.* 33 (1996), pp. 71-79.
- [17] H.L. Ehrlich, *Conditions for bacterial participation in the initiation of Mn deposition around marine sediment particles*, in *Environmental biogeochemistry and geomicrobiology*, W.E. Krumbein, ed., Ann Arbor Science Publishers, 1978, pp. 839-845.
- [18] H.L. Ehrlich, *Enhanced removal of Mn²⁺ from seawater by marine sediments and clay minerals in the presence of bacteria*, *Can. J. Microbiol.* 28 (1982), pp. 1389-1395.
- [19] M.B.L. Mascarenhas-Pereira, B.N. Nath, D.V. Borole, and S.M. Gupta, *Nature, source and composition of volcanic ash in sediments from a fracture zone trace of Rodriguez Triple Junction in the Central Indian Basin*, *Mar. Geol.* 229 (2006), pp. 79-90.
- [20] B.N. Nath, I. Roelandts, M. Sudhakar, and W.I. Plüger, *Rare earth element patterns of the Central Indian Basin sediments related to their lithology*, *Geophys. Res. Lett.* 19 (1992), pp. 1197-1200.
- [21] Das A, C.E.G. Fernandes, S.S. Naik, B.N. Nath, I. Suresh, M.B.L. Mascarenhas-Pereira, S.M. Gupta, N.H. Khadge, C. Prakash Babu, D.V. Borole, P.P. Sujith, A.B. Valsangkar, B.S. Mourya, S.U. Biche, R. Sharma, and P.A. Loka Bharathi, *Bacterial response to contrasting sediment geochemistry in the Central Indian Basin*, *Sedimentology* DOI: 10.1111/j.1365-3091.2010.01183.x.
- [22] V.K. Banakar, G. Parthiban, J.N. Pattan, and P. Jauhari, *Chemistry of surface sediment along a north-south transect across the equator in the Central Indian Basin: an assessment of biogenic and detrital influences on elemental burial on the seafloor*, *Chem. Geol.* 147 (1998), pp. 217-232.
- [23] A. Stockdale, W. Davison, H. Zhang, and J. Hamilton-Taylor, *The association of cobalt with iron and manganese (oxyhydr)oxides in marine sediment*, *Aquat. Geochem.* 16 (2010), pp. 575-585.
- [24] J.E. Hobbie, R.J. Daley, and S. Jasper, *Use of nucleopore filters for counting bacteria by fluorescent microscopy*, *Appl. Environ. Microbiol.* 3 (1977), pp. 1225-1228.
- [25] R.L. Patience, C.J. Clayton, A.T. Kearsley, S.J. Rowland, A.N. Bishop, A.W.G. Rees, K.G. Bibby, and A.C. Happer, *An integrated biochemical, geochemical and sedimentological study of organic diagenesis in sediments from ODP LEG 112*, *Proc.ODP Sci. Repts*, 1990.

- [26] S.M. Gupta, and P. Jauhari, *Radiolarian abundance and geochemistry of the surface-sediments from the Central Indian Basin: Inferences to Antarctic bottom water current*, Curr. Sci. 66 (1994), pp. 659-663.
- [27] P. Roy, V. Balaram, A. Kumar, M. Satyanarayanan, and T.G. Rao, *New REE and Trace Element Data on Two Kimberlitic Reference Materials by ICP-MS*, Geostand. Geoanal. Res. 31 (2007), pp. 261-273.
- [28] M. P. Colombini, and R. Fuoco, *Determination of manganese at ng/ml levels in natural waters by differential pulse polarography*, Talanta 30 (1983), pp. 901-905.
- [29] J.A. Herrera-Melian, J.M. Dona-Rodriguez, J. Hernandez-Brito, and J. P. Pena, *Voltammetric determination of Ni and Co in water samples*, J Chem. Edu. 74 (1997), pp. 1444-1445.
- [30] C.S. Chin, K.S. Johnson, and K.H. Coale, *Spectrophotometric determination of dissolved manganese in natural waters with 1-(2-pyridylazo)-2-naphthol: Application to analysis in situ in hydrothermal plumes*, Mar. Chem. 37 (1992), pp. 65-82.
- [31] R. Chester, and M.J. Hughes, *Scheme for the spectrophotometric determination of Cu, Pb, Ni, V and Co in marine sediments: Applied Earth Science*, Trans. Inst. Min and Metallurgy, 77 (1968), pp. 37-41.
- [32] H. Zhang, W. Davison, R.J.G. Mortimer, M.D. Krom, P.J. Hayes, and I.M. Davies, *Localised remobilization of metals in a marine sediment*, Sci. Total. Environ. 296 (2002), pp. 175-187.
- [33] J. N. Pattan, and A.V. Mudholkar, *The oxidation state of manganese in ferromanganese nodules and deep-sea sediments from the Central Indian Ocean*, Chem. Geol. 85 (1990), pp. 171-181.
- [34] V.K. Banakar, S.M. Gupta, and V.K. Padmavathi, *Abyssal sediment erosion in the Central Indian Basin: Evidence from radiochemical and radiolarian studies*, Mar. Geol. 96 (1991), pp. 167-173.
- [35] A.V. Mudholkar, J. N. Pattan, and G. Parthibhan, *Geochemistry of deep sea sediment cores from the Central Indian Ocean Basin*, Ind. J. Mar. Sci. 22 (1993), pp. 241-246.
- [36] B. Sundby, L.G. Anderson, P.O.J. Hall, and A. Iverfeldt, *The effect of oxygen on release and uptake of cobalt, manganese, iron and phosphate at the sediment-water interface*, Geochim. Cosmochim. Acta, 50 (1986), pp. 1281-1288.
- [37] G.P. Glasby, *Manganese: predominant role of nodules and crusts*, in *Marine Geochemistry*, H.D. Schulz, and M. Zabel, eds., Springer Berlin Heidelberg, 2006, pp. 371-427.
- [38] Y. Lee, and B.M. Tebo, *Cobalt(II) oxidation by the marine manganese(II)-oxidizing Bacillus sp. Strain SG-1*, Appl. Environ. Microbiol. 60 (1994), pp. 2949-2957.
- [39] J.W. Moffett, and J. Ho, *Oxidation of cobalt and manganese in seawater via a common microbially catalyzed pathway*, Geochim. Cosmochim. Acta. 60 (1996), pp. 3415-3424.
- [40] M.J. McInerney, *Energetics and kinetics of anaerobic aromatic and fatty acid degradation*, Report prepared for the US department of Energy, Oklahoma, 1993.

- [41] R.D. Stoppel, and H.G. Schlegel, *Nickel-resistant bacteria from anthropogenically nickel-polluted and naturally nickel-percolated ecosystems*, Appl. Environ. Microbiol. 61 (1995), pp. 2276-2285.
- [42] P.J. Muller, M. Hartmann, and E. Suess, *The chemical environment of pelagic sediments*, in *The manganese nodule belt of the Pacific Ocean, Geological environment, nodule formation, and mining aspects*, P. Halbach, G. Friedrich, and U.von Stackelberg, eds., Enke Verlag, Stuttgart, 1988, pp.70-99.
- [43] A.L. Mills, and R.R. Colwell, *Microbiological effects of metal ions in Chesapeake Bay water and sediment*, Bull. Environ. Contam. Toxicol. 18 (1977), pp. 99-103.
- [44] J.F. Timoney, J. Port, J. Giles, and J. Spanier, *Heavy metal and antibiotic resistance in the bacterial flora of sediments of New York Bight*, Appl. Environ. Microbiol., 36 (1978), pp. 465-472.
- [45] C. Houba, and J. Remacle, *Composition of the saprophytic bacterial communities in freshwater systems contaminated by heavy metals*, Microb. Ecol. 6 (1980), 55-69.
- [46] T. Duxbury, and B. Bicknell, *Metal-tolerant bacterial populations from natural and metal-polluted soils*, Soil Biol. Biochem. 15 (1983), pp. 243-250.
- [47] S.G. Hornor, and B.A. Hilt, *Distribution of zinc-tolerant bacteria in stream sediments*, Hydrobiologia. 128 (1985), pp. 155-160.
- [48] P. Jasper, and S. Silver, *Magnesium transport in microorganisms*, in *Microorganisms and minerals*, E. D. Weinberg, ed., Marcel Dekker, 1977, pp. 7-47.
- [49] M.F. Bryson, and H.L. Drake, *Energy-dependent transport of nickel by Clostridium pasteurianum*, J. Bacteriol. 170 (1988), pp. 234-238.
- [50] P. N. Froelich, G. P. Klinkhammer, M. L. Bender, G. R. Heath, D. Cullin, P. Dauphin, D. Hammond, B. Hartman, and V. Maynard, *Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic; Suboxic diagenesis*, Geochim.Cosmochim. Acta 43 (1979), pp. 1075-1090.
- [51] T. Shaw, J. Gieskes, and R. Jahnke, *Early diagenesis in differing depositional environments: the response of transition metals in pore water*, Geochim Cosmochim Acta 54 (1990), pp. 1233-1246.
- [52] A. Turner, M. Nimmo, and K. A. Thuresson, *Speciation and sorptive behaviour of nickel in an organic-rich estuary (Beaulieu, UK)*, Marine Chemistry 63 (1998), pp. 105-118.
- [53] H.B. Xue, S. Jansen, A. Prasad, and L. Sigg, *Nickel Speciation and Complexation kinetics in freshwater by ligand exchange and DPCSV*, Environ. Sci. Technol. 35 (2001), pp. 539-546.
- [54] D. Dean-Ross, and A.L. Mills, *Bacterial community structure and function along a heavy metal gradient*, Appl. Environ. Microbiol. 55 (1989), pp. 2002-2009.

- [55] T. Schmidt, and H.G. Schlegel, *Nickel and cobalt resistance of various bacteria isolated from soil and highly polluted domestic and industrial wastes*, FEMS Microbiol. Ecol. 62 (1989), pp. 315-328.
- [56] R. Antony, P.P. Sujith, S.O. Fernandes, P. Verma, V.D. Khedekar, and P.A. Loka Bharathi, *Cobalt immobilization by manganese oxidizing bacteria from the Indian Ridge system*, Curr. Microbiol (2010), doi: 10.1007/s00284-010-9784-1.
- [57] S. D'Hondt, B.B. Jørgensen, D.J. Miller, A. Batzke, R. Blake, B.A. Cragg, H. Cypionka, G.R. Dickens, T. Ferdelman, K. Hinrichs, N.G. Holm, R. Mitterer, A. Spivack, G. Wang, B. Bekins, B. Engelen, K. Ford, G. Gettemy, S.D. Rutherford, H. Sass, C.G. Skilbeck, I.W. Aiello, G. Guerin, C.H. House, F. Inagaki, P. Meister, T. Naehr, S. Niitsuma, R.J. Parkes, A. Schippers, D.C. Smith, A. Teske, J. Wiegel, C.N. Padilla, and J.L.S. Acosta, *Distributions of microbial activities in deep seafloor sediments*, Science 306 (2004), pp. 2216-2221.
- [58] B.T. Hart, *Uptake of trace metals by sediments and suspended particulates: a review*, Hydrobiologia 91 (1982), pp. 299-313.
- [59] H.Y. Cheung, L. Vitkovic, and M.R.W. Brown, *Toxic effect of manganese on growth and sporulation of Bacillus stearothermophilus*, J. Gen. Microbiol. 128 (1982), pp. 2345-2402.
- [60] C.P. Lienemann, M. Taillefert, D. Perret, and J.F. Gaillard, *Association of cobalt and manganese in aquatic systems: Chemical and microscopic evidence*, Geochim. Cosmochim. Acta. 61 (1997), pp. 1437-1446.
- [61] K. Kuroda, and M. Ueda, *Bioadsorption of cadmium ion by cell surface-engineered, yeasts displaying metallothionein and hexa-His*, Appl. Microbiol. Biotechnol. 63 (2003), pp. 182-186.

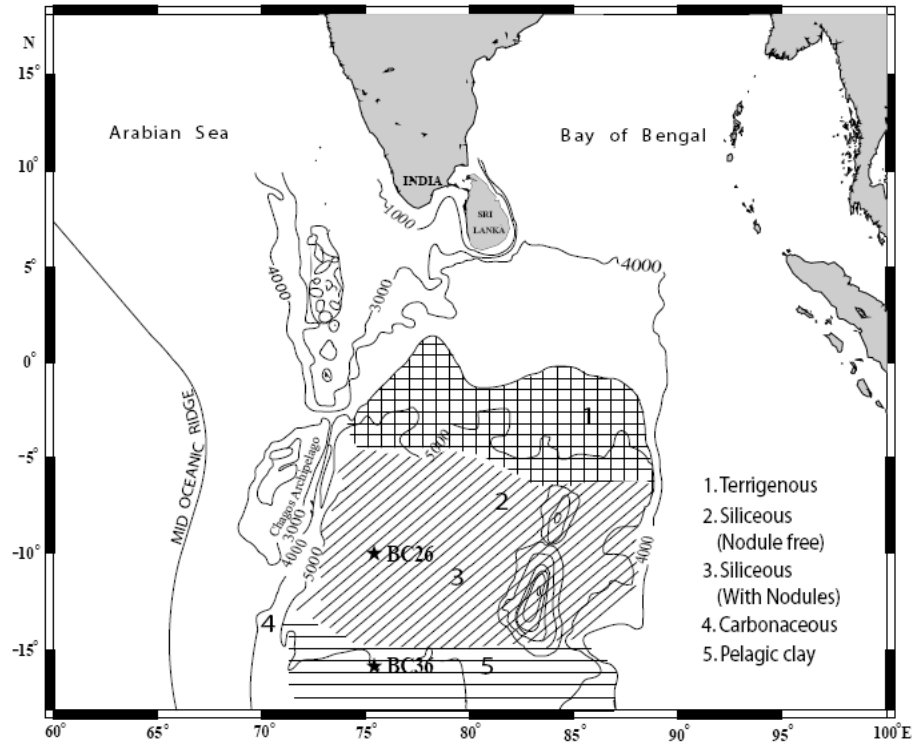


Figure 1. Area map shows sediment types and sampling locations for BC26 and BC36. It is modified from Mascarenhas-Pereira et al (2006).

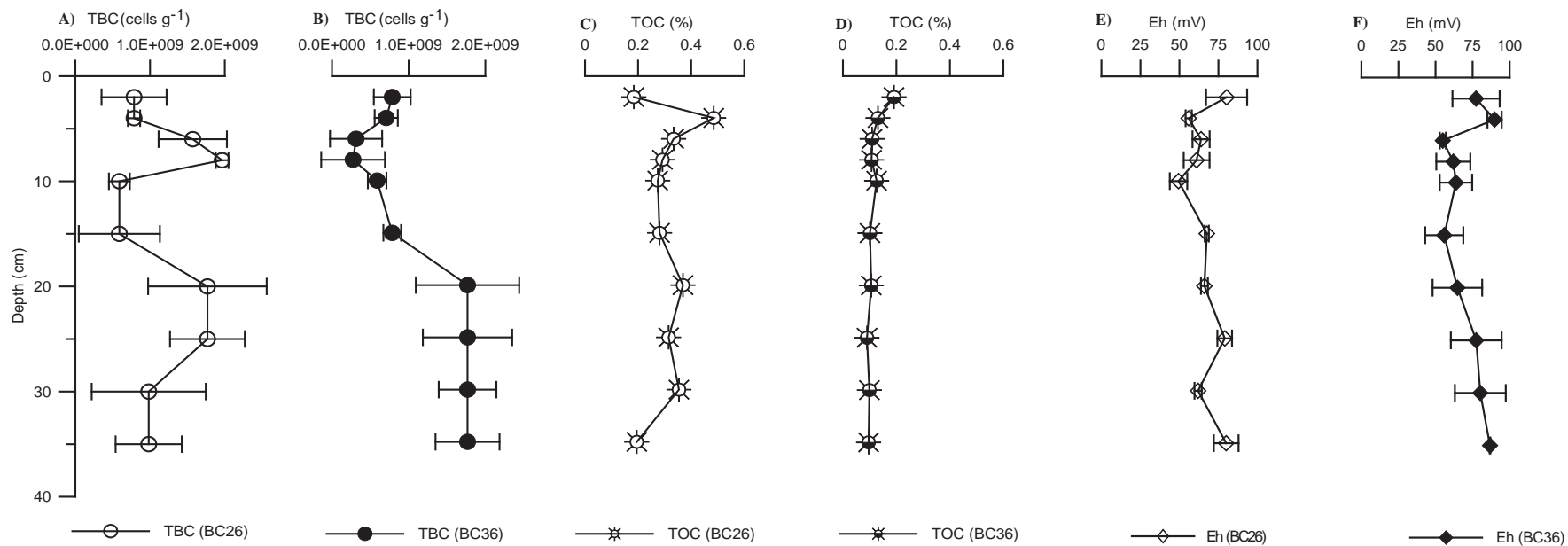


Figure 2. Depth profile of Total Bacterial Count (TBC), Total Organic Carbon (TOC) and Eh in sediment cores BC26 and BC36 (mean \pm SD, n = 10 for TBC and n = 3 for Eh).

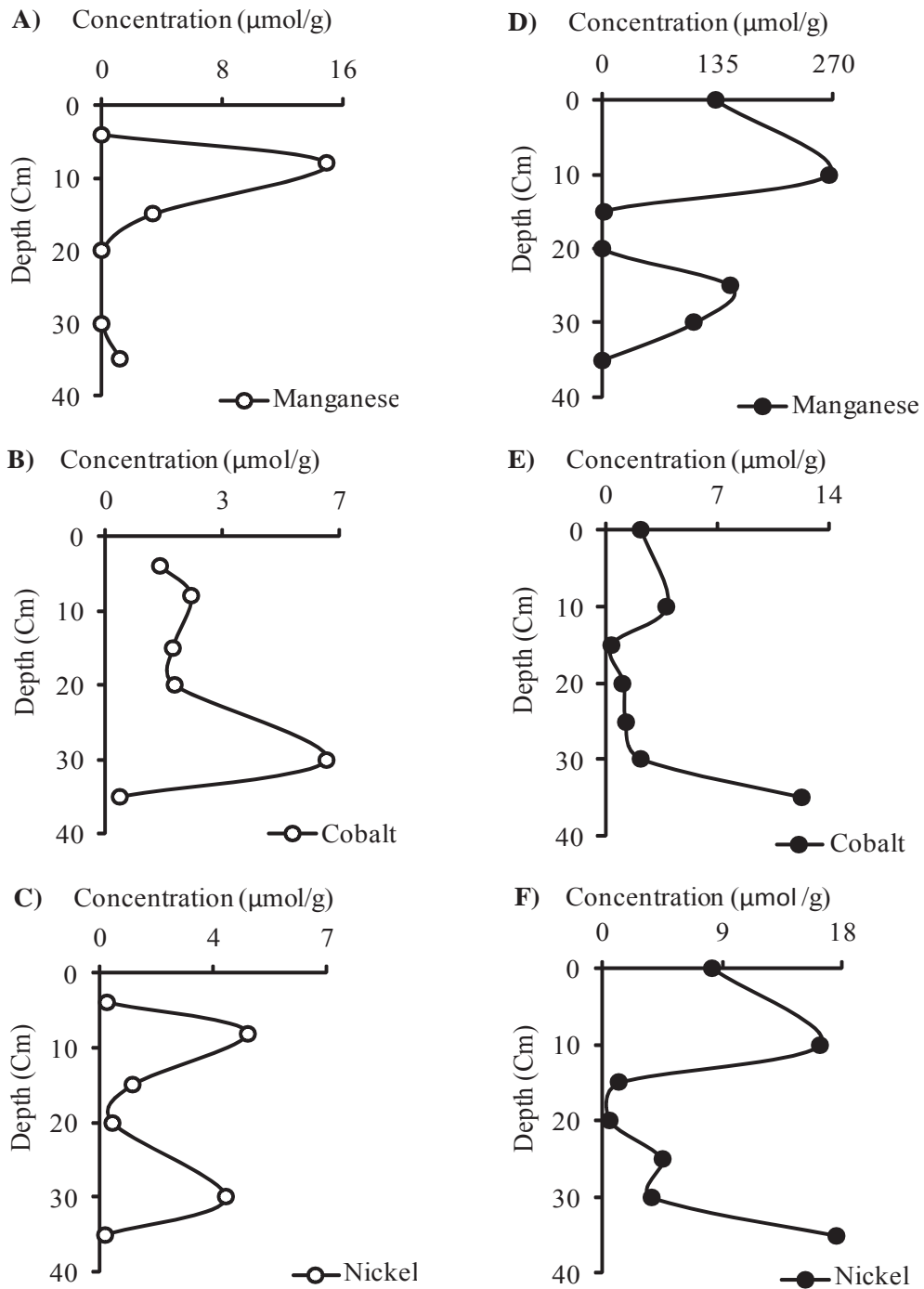


Figure 3. Solid phase concentrations (single measurements) of Mn, Co and Ni in sediment cores collected from Central Indian Basin. Figures A - C represents metal concentrations in BC26 and D - F in BC36.

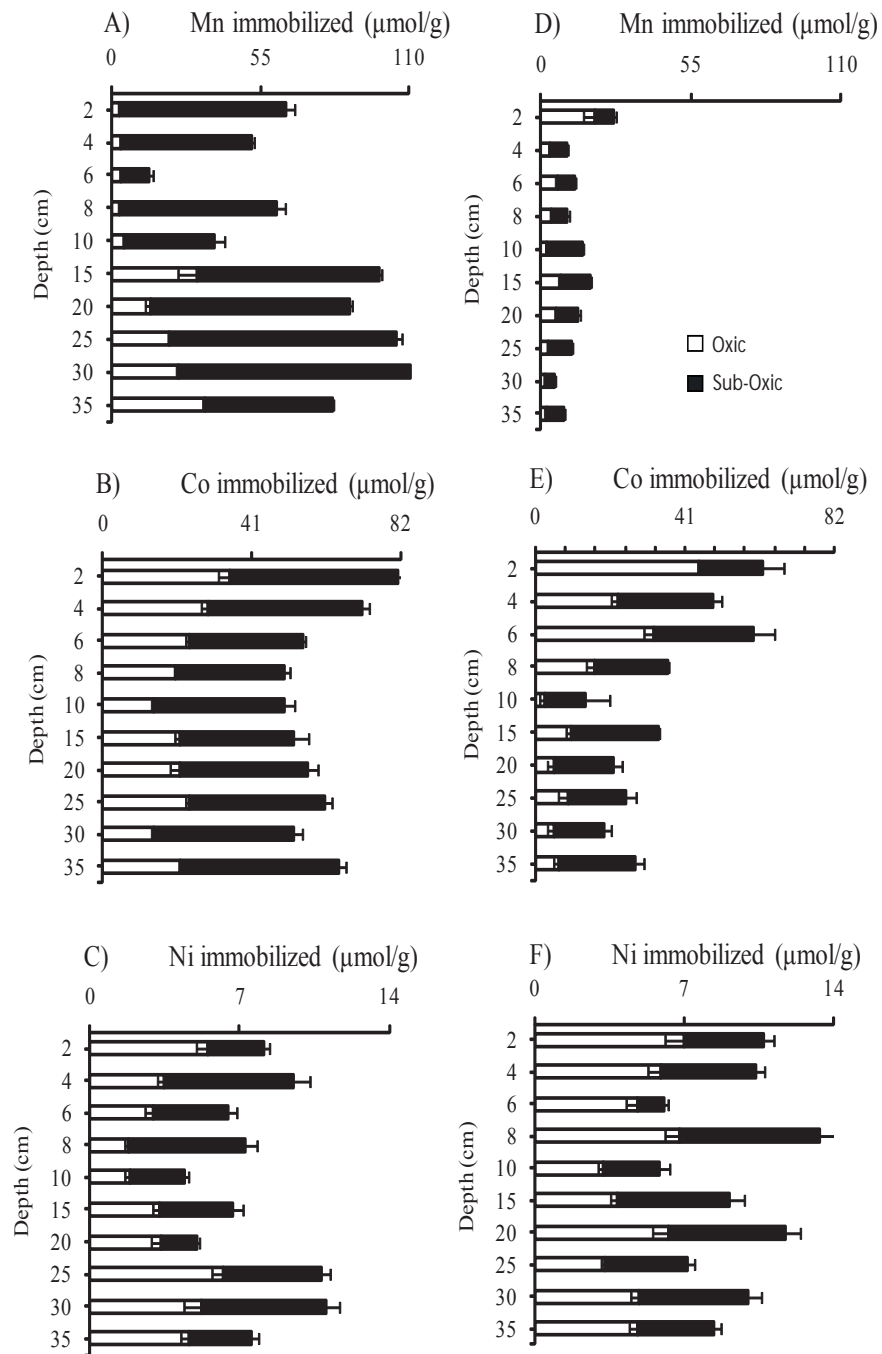


Figure 4. Microbially promoted immobilization of metals ($\mu\text{mol/g}$ dry weight, mean \pm SD, n = 3) in experiments as a function of depth. Figures A - C represents the immobilization of metals in BC26 and figures D - F in BC36. The error bars are shown on the left hand side for oxic and on the right hand side for suboxic incubation.

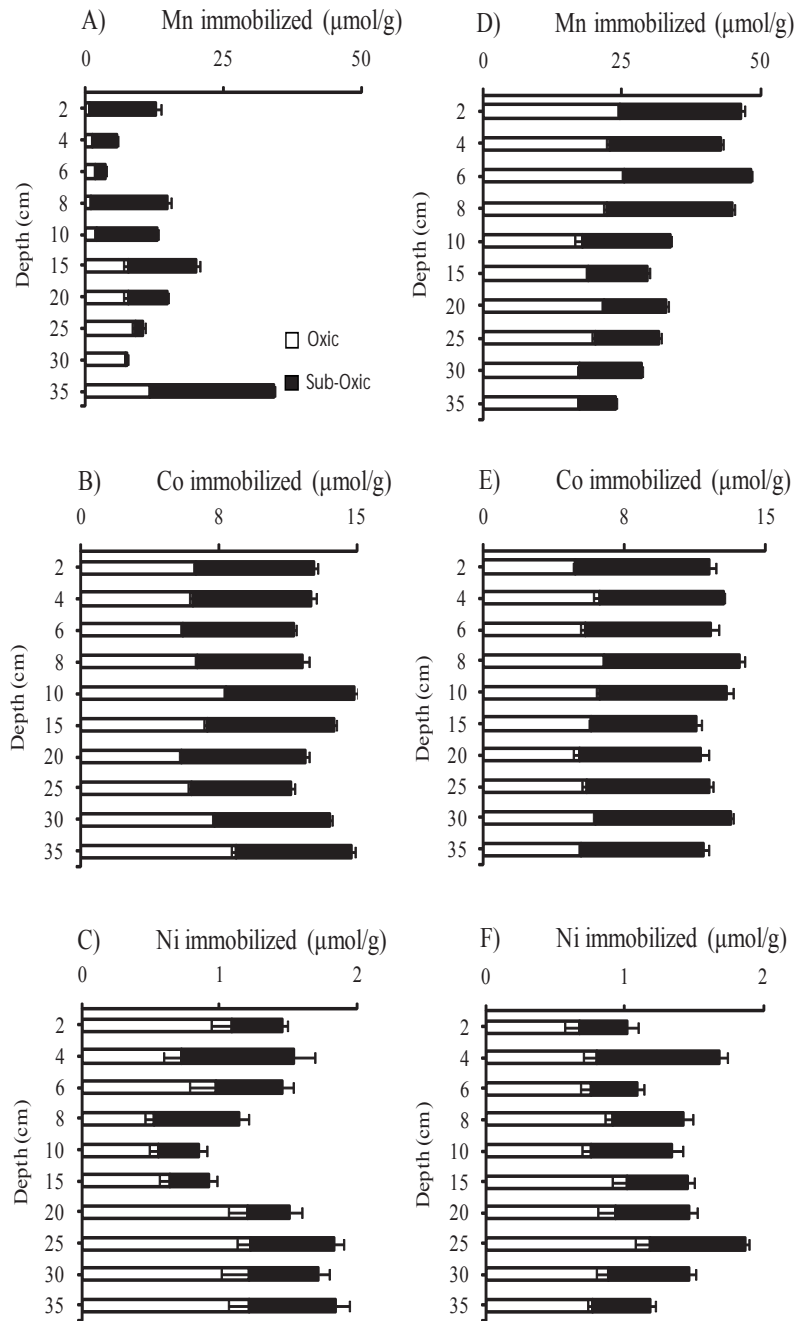


Figure 5. Abiotic immobilization of metals ($\mu\text{mol/g}$ dry weight, mean \pm SD, $n = 3$) in azide (10 mmol) treated sediments as a function of depth. Figures A-C represents the adsorption of metals in BC26 and figures D - F in BC36. The error bars are shown on the left hand side for oxic and on the right hand side for suboxic incubation.

Tables:

Table 1. Plate counts of heterotrophic and metal resistant bacteria in 1.5 % bacto agar amended seawater with and without metal chlorides (100 μmol) in BC26. All the values in the table represent CFU (Colony Forming Unit) per gram dry weight of sediment. The data represent mean values \pm SD, n=3. *ZMA= ZoBell Marine Agar, SWA = Sea Water Agar, bsf= below sea floor and BDL=Below Detection Level. For details, see text (section 2.3).

Depth bsf (cm)	ZMA	SWA				
		No metal	Ni	Co	Mn	Ni-Co-Mn
2	$1.5 \pm 0.18 \times 10^2$	BDL	BDL	$4.3 \pm 0.31 \times 10^4$	$2.5 \pm 0.22 \times 10^4$	BDL
4	$2.0 \pm 0.40 \times 10^2$	BDL	BDL	$2.7 \pm 0.15 \times 10^4$	$1.5 \pm 0.25 \times 10^4$	BDL
6	BDL	BDL	BDL	$1.5 \pm 0.10 \times 10^3$	$6.0 \pm 0.05 \times 10^2$	BDL
8	$5.4 \pm 0.73 \times 10^3$	BDL	BDL	$1.7 \pm 0.32 \times 10^4$	$4.1 \pm 0.61 \times 10^4$	BDL
10	$5.0 \pm 0.28 \times 10^1$	$1.5 \pm 0.57 \times 10^2$	$9.0 \pm 0.11 \times 10^2$	$6.6 \pm 0.60 \times 10^3$	$3.8 \pm 0.72 \times 10^4$	$1.4 \pm 0.33 \times 10^3$
15	BDL	BDL	BDL	$1.7 \pm 0.13 \times 10^4$	$3.9 \pm 0.23 \times 10^4$	BDL
20	BDL	BDL	BDL	$1.2 \pm 0.24 \times 10^4$	$4.3 \pm 0.45 \times 10^4$	BDL
25	$1.4 \pm 0.11 \times 10^3$	BDL	BDL	BDL	BDL	BDL
30	$5.0 \pm 0.02 \times 10^1$	BDL	BDL	BDL	BDL	BDL
35	BDL	BDL	BDL	BDL	BDL	BDL

Table 2. Plate counts of heterotrophic and metal resistant bacteria in 1.5 % bacto agar amended seawater with and without metal chlorides (100 μmol) in BC36. All the values in the table represent CFU (Colony Forming Unit) per gram dry weight of sediment.

The data represent mean values \pm SD, n=3. *ZMA= ZoBell Marine Agar, SWA = Sea Water Agar and bsf= below sea floor. For details, see text (section 2.3).

Depth bsf (cm)	ZMA	SWA				
		No metal	Ni	Co	Mn	Ni-Co-Mn
2	$8.0 \pm 0.18 \times 10^4$	$6.8 \pm 0.70 \times 10^4$	$1.6 \pm 0.02 \times 10^5$	$2.6 \pm 0.23 \times 10^5$	$1.8 \pm 0.10 \times 10^5$	$1.9 \pm 0.13 \times 10^5$
4	$1.0 \pm 0.11 \times 10^5$	$6.1 \pm 0.06 \times 10^4$	$2.4 \pm 0.31 \times 10^5$	$1.7 \pm 0.07 \times 10^5$	$3.9 \pm 0.02 \times 10^5$	$2.2 \pm 0.18 \times 10^5$
6	$8.8 \pm 0.09 \times 10^4$	$5.4 \pm 0.42 \times 10^4$	$9.1 \pm 0.46 \times 10^4$	$1.3 \pm 0.35 \times 10^5$	$3.3 \pm 0.21 \times 10^5$	$1.6 \pm 0.09 \times 10^5$
8	$2.4 \pm 0.37 \times 10^5$	$3.5 \pm 0.51 \times 10^4$	$2.1 \pm 0.10 \times 10^5$	$1.5 \pm 0.16 \times 10^5$	$2.4 \pm 0.03 \times 10^5$	$6.8 \pm 0.42 \times 10^4$
10	$1.6 \pm 0.24 \times 10^5$	$5.0 \pm 0.02 \times 10^4$	$1.6 \pm 0.05 \times 10^5$	$2.8 \pm 0.04 \times 10^5$	$1.3 \pm 0.09 \times 10^5$	$5.0 \pm 0.02 \times 10^4$
15	$1.2 \pm 0.08 \times 10^5$	$1.2 \pm 0.07 \times 10^4$	$3.9 \pm 0.06 \times 10^4$	$1.4 \pm 0.05 \times 10^5$	$1.2 \pm 0.24 \times 10^5$	$6.2 \pm 0.18 \times 10^4$
20	$1.4 \pm 0.15 \times 10^5$	$7.1 \pm 0.15 \times 10^4$	$5.1 \pm 0.31 \times 10^4$	$6.6 \pm 0.02 \times 10^4$	$1.7 \pm 0.11 \times 10^5$	$1.6 \pm 0.31 \times 10^5$
25	$1.6 \pm 0.26 \times 10^5$	$2.5 \pm 0.11 \times 10^4$	$4.2 \pm 0.17 \times 10^4$	$2.0 \pm 0.10 \times 10^4$	$1.7 \pm 0.07 \times 10^5$	$7.9 \pm 0.01 \times 10^4$
30	$2.0 \pm 0.24 \times 10^5$	$3.1 \pm 0.32 \times 10^4$	$9.9 \pm 0.06 \times 10^4$	$1.0 \pm 0.08 \times 10^5$	$2.2 \pm 0.05 \times 10^5$	$1.5 \pm 0.11 \times 10^5$
35	$1.7 \pm 0.09 \times 10^5$	$3.0 \pm 0.13 \times 10^4$	$9.5 \pm 0.22 \times 10^4$	$4.8 \pm 0.15 \times 10^4$	$2.4 \pm 0.21 \times 10^5$	$7.2 \pm 0.45 \times 10^4$

Table 3: Maximum immobilization of metals at various depths below sea floor (bsf) compared with maximum in plate counts CFU (Colony Forming Unit). All the values in the table correspond to gram dry weight of sediment. The data in the table are mean values \pm SD, n=3. For details, see text (sections 2 and 3).

Metal	Parameters	Core BC26								Core BC36								Inference
		Biotic				Abiotic				Biotic				Abiotic				
		Oxic ^a		Sub-oxic ^b		Oxic ^c		Sub-oxic ^d		Oxic ^e		Sub-oxic ^f		Oxic ^g		Sub-oxic ^h		
		Value	Depth (cm)	Value	Depth (cm)	Value	Depth (cm)	Value	Depth (cm)	Value	Depth (cm)	Value	Depth (cm)	Value	Depth (cm)	Value	Depth (cm)	
Mn	Immobilization (μ mol)	34.4 \pm .053	35	85.6 \pm .047	30	11.6 \pm .011	35	22.5 \pm .047	35	19.76 \pm .41	2	13.44 \pm .04	10	25.33 \pm .04	6	22.89 \pm .03	6	<p>a. Suggests that oxidized Mn is less prone to dissolution at this depth. b. Suggests bacterial participation c. Suggests that 86% of the variation in biotic oxidation could be due to the variation in abiotic. d. Suggests passive immobilization on Mn-oxide phase e. Suggests mixotrophic mode of nutrition f. Suggests that high background Mn concentration along with the amended Mn could be toxic g. Could be regulated by concentration of the metal and the availability of particle binding sites in sediments h. Active process of immobilization is inhibited by azide and not the passive.</p>
	Plate counts (CFU) i) Seawater agar	-		-		-		-		6.8 \times 10 ⁴		-		-		-		
Co	Immobilization (μ mol)	35 \pm .33	2	46.3 \pm .29	2	8.45 \pm .055	35	6.97 \pm .027	10	45 \pm .028	2	27.5 \pm .537	6	6.43 \pm .011	8	7.18 \pm .024	8	<p>a. Immobilization of Co by other metal tolerant bacteria in the sediments. b. Suggests bacterial participation c. Only passive process could be involved d. Suggests passive immobilization on Mn oxide phase e. Could be by process where Ni immobilizing bacteria catalyze Co immobilization and vice versa f. Suggests co-oxidation of Co by Mn oxidizing microorganisms g. Could be regulated by concentration of the metal and the availability of particle binding sites in sediments h. Could be by metabolism independent process like cell surface adsorption/precipitation</p>
	Plate counts (CFU) i) Seawater agar ii) Co	- 4.3 \times 10 ⁴		- 4.3 \times 10 ⁴		-		-		6.8 \times 10 ⁴		-		-		-		
Ni	Immobilization (μ mol)	6.3 \pm .055	25	6 \pm .08	4	0.98 \pm .008	25	0.65 \pm .012	4	7.03 \pm .09	2	6.6 \pm .075	8	0.95 \pm .009	25	0.703 \pm .01	4	<p>a. Ni immobilization may be catalyzed more by bacteria resistant to Ni or Co. b. Energy dependent low affinity uptake process c. Complexation of Ni with organic carbon in sediments d. Complexation of Ni with organic carbon in sediments could contribute to its immobilization at a much lower rate e. Suggests that the same microbial communities are capable of immobilizing both the metals f. Suggests that the same microbial communities are capable of immobilizing both the metals g. Could be regulated by concentration of the metal and the availability of particle binding sites in sediments h. Could be by metabolism independent process like cell surface adsorption/precipitation</p>
	Plate counts (CFU) i) Seawater agar ii) ZoBell marine agar	- -		- -		-		-		6.8 \times 10 ⁴		- 2.4 \times 10 ⁵		-		-		