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### **Kinetic Speciation and bioavailability of copper and nickel in mangrove sediments**

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#### **ABSTRACT**

An attempt was made to establish a mechanistic linkage between chemical speciation of copper and nickel, and their bioavailability in mangrove ecosystem. Kinetic speciation study was performed to determine the concentrations of labile metal-complexes and their dissociation rate constants in mangrove sediments. Concentration of copper and nickel in the mangrove roots was used as an indicator of bioavailability. It was found that the bioaccumulation of both the metals gradually increased with the increasing concentrations of the labile metal complexes and their dissociation rate constants in the mangrove sediments. This study shows that concentration of labile metal (copper and nickel) complexes and their dissociation rate constants in mangrove sediment can be a good indicator of their bioavailability.

**Key words:** Copper and nickel speciation; mangrove sediment; speciation and bioavailability; kinetic speciation; single extraction method

## 1. Introduction

Mangroves act as natural buffers between the land and sea. Mangrove sediments serve as natural sinks and play an important role in controlling trace/heavy metal distribution, speciation and their bioavailability in coastal environments. Complexations of metal (trace/heavy) with different binding phases in mangrove sediments regulate transport, bioavailability, and toxicity of metals in the system. Important of metals speciation and their bioavailability in mangrove sediments are recognized but poorly understood.

Various experimental methods/techniques are being used to determine speciation (and dynamic/labile complexes) of metals in soils and sediments. The approaches include batch techniques (Beauchemin et al., 2002; Bermond and Varrault, 2004; Yu and Klarup, 1994), flow techniques (Beauchemin et al., 2002; Shiowatana et al., 1999; Silva et al., 2007), kinetic extraction methods (Chakraborty and Chakrabarti, 2006; Chakraborty, 2012; Chakraborty et al., 2013, 2011, 2009; Chakraborty et al., 2012; Chakraborty et al., 2012) , Diffusive Gradients in Thin Films technique (Bermond et al., 1998; Fangueiro et al., 2002; Town et al., 2009). However, the kinetic extraction method has been found to be a simple and sensitive technique (Chakraborty and Chakrabarti, 2006; Chakraborty, 2012).

In this method, trace metals are quantified in an extract (containing a suitable chelating agent) as a function of time until equilibrium is reached. This time dependent metal extraction study from sediments has been reported to be useful to ascertain the potential availability and mobility of metal and its migration in a polluted or naturally contaminated soils/sediments (Beauchemin et al., 2002; Bermond and Varrault, 2004; Fangueiro et al., 2002). This method is capable of determining kinetically distinguishable metal complexes (in soil/sediment) and their corresponding dissociation rate constants in a system.

Concentrations of labile metal complexes and their dissociation rate constant have been obtained from kinetic extraction study. They can therefore be used to estimate bioavailability of metal if a kinetic model can be constructed to represent the process of biological uptake. In this study, a mechanistic linkage between copper and nickel speciation, and their bioavailability in mangrove ecosystem was established. Concentrations of accumulated metals in mangrove roots have been reported to be a good indicator of bioavailability of metals in mangrove system and this observation was related to our values for speciation as a test of bioavailability. The objective of this study was to prove the hypothesis that labile metal complexes from mangrove sediments contribute to the

bioavailable metal flux and increase its bioaccumulation in the mangrove roots. This is the first attempt to establish a linkage between metal (copper and nickel) speciation, and their bioavailability in a mangrove ecosystem.

## **2. Materials and methods**

### **2.1 Study area**

Sediment samples were collected at mangrove forests located at Divar Island in Goa, west coast of India. The adjoining Mandovi estuary is used for transportation of iron ore from mines located upstream. It has been reported that these iron ore beneficiation plants discharged effluents directly into the estuary from the ore processing plants (Fernandes et al., 2012). Sediments were collected from five environmentally relevant sampling sites (**Fig. 1**) at Divar Island, during low tide, in December 2013. Undisturbed sediment cores were collected by using PVC cores (inner diameter 7.5 cm, 20 cm length).

### **2.1 Sediment texture**

Texture of the sediment was analyzed by (after sieving with 63 $\mu$ m sieve size) laser size particle analyzer (LPSA.). Malvern Mastersizer 2000 was used for size particle analysis. The detailed procedure has been described in the literature by Ramaswamy and Rao (2006). The data are presented as weight percentage (wt %) in this study.

### **2.2 Elemental analysis**

Bulk sediment samples were analyzed for total carbon (TC), total inorganic carbon (TIC), total nitrogen (TN) content. TC and TN in sediments were determined using Flash 2000 CHN-elemental analyzer (Thermo Fisher Scientific Incorporation). Precision of the analysis was within  $\pm 5\%$ . Soil NC was used as certified reference material. TIC was determined by coulometry (UIC coulometrics). Anhydrous calcium carbonate was used as standard material. Relative standard deviation of the analysis was within  $\pm 2\%$ . Total organic carbon (TOC) was derived from deducting TIC from TC.

### **2.4 Determination of metals (Cu, Ni, Fe, Mn) in the sediments and mangrove roots**

Multiple sediment sampling was done at each station. The composite sediments were stored at  $-20^{\circ}\text{C}$ , and then freeze dried (Esquire Biotech Pvt. Ltd Model-SK 50L, Chennai India). The composite sediment samples (from each station) were homogenized and ground-milled; the sediment samples

were subsequently stored at 4 °C until needed. Multiple fine nutritive mangrove roots (sp. *Avicennia officinalis*) were collected from each station. The surfaces of the mangrove roots were thoroughly washed with ultrapure water for complete removal of sediments from the roots. All the roots were freeze dried until constant weight obtained. The dried roots were ground-milled and homogenized. The total metals concentrations in the mangrove roots were determined by digesting 0.1 g of ground-milled and homogenized roots samples with 10.0 cm<sup>3</sup> of acid mixtures of HF, HNO<sub>3</sub> and HClO<sub>4</sub> (in 7:3:1 ratio) on hot plate at 200<sup>0</sup>C. The samples were digested and evaporated to dryness. The residues were re-dissolved in 2% HNO<sub>3</sub> and analyzed by GFAAS. Total concentrations of Cu, Ni, Fe, and Mn were determined in the studied sediments. The kinetics speciation of Ni and Cu in the mangrove sediments was also studied.

## 2.5 Kinetic extraction procedure

Two grams (2.0 gm) of sediment was added to 200 cm<sup>3</sup> of 0.05M EDTA solution (at pH 6.0) (Merck Pvt Ltd) in a Teflon beaker (400 cm<sup>3</sup>) and the mixture was continually stirred with a Teflon-coated magnetic stirring bar throughout the experiment. The ratio of the mass of sediment to the volume of EDTA solution (mass/volume) was set at 0.01, as this ratio provided sufficiently high metal concentrations in the extract to be accurately quantified, while requiring a minimum amount of sediment.

A homogeneous suspension was maintained with a constant mass/volume ratio during sampling. Larger mass/volume ratio is undesirable, as they can cause problems with filtration. At set time intervals (0 min, 2 min, 4 min, 6 min, 8 min, 10 min, 15 min, 20 min, 25 min, 30 min, 45 min, 1 h, 2 h, 5 h, 7 h, 10 h, 24 h, 36 h), 2 cm<sup>3</sup> aliquots of the suspension were filtered through a 0.2 μm syringe filter (Millex, Millipore). The initial time for the kinetic measurement (i.e. t=0 s) was taken as the time just before the sediment was added to the EDTA solution. The filtrate samples (1.0 cm<sup>-3</sup>) were then (in 2% ultrapure HNO<sub>3</sub>) analyzed by graphite furnace atomic absorption spectrometer (GFAAS) to monitor the change in concentrations of metals in the extracted solution as a function of time. The kinetic extraction experiments were performed in triplicate for all the samples to ensure repeatability of results. The total metals concentrations in the studied sediments were determined by digesting 0.05 g of sediments samples with 10.0 mL of acid mixtures of HF, HNO<sub>3</sub> and HClO<sub>4</sub> (in 7:2:1 ratio) on hot plate. The sediments were digested and evaporated to dryness. The residues were redissolved in 2% HNO<sub>3</sub> and analyzed by GFAAS.

## 2.6 Graphite Furnace Atomic Absorption Spectrometer (GFAAS)

A GFAAS (Perkin Elmer, PinAAcle 900T) was used for the determination of the total metals concentrations. This instrument was equipped with an AS900 auto sampler. The transversely heated graphite tubes (Perkin-Elmer) were pyrolytically coated and were equipped with integrated L'vov platforms. During the drying, ashing and clean-up cycles, the internal argon gas was passed through the furnace at  $300 \text{ cm}^3 \text{ min}^{-1}$ , but the gas flow was interrupted during the atomization step. The signal was measured in the peak area mode. Concentrations were determined by external calibration. Every fifth sample analysed was a blank. All measurements were done in triplicate. The variation between each replicate samples was less than 2% and the daily variation was less than 2%. MAG-1, a fine grained gray-brown clayey mud with low carbonate content, from the Wilkinson Basin of the Gulf of Maine, was used as certified reference material (CRM) (obtained from USGS).

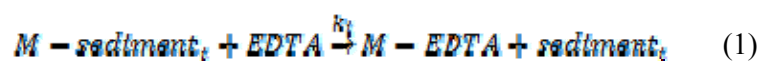
## 2.7 Statistical analysis

The data presented in this study are average of three replicates. Errors are indicated along with the average values. All the data are presented with 95.5% confidence interval. Relationship between concentrations of accumulated metals in the mangrove roots and dynamic metal complexes (obtained from kinetic extraction study) were evaluated with Pearson's correlation coefficient.

## 2.8 Theory

The kinetic model proposed by Olson and Shuman(1985) was adapted (Chakraborty, 2012; Chakraborty et al., 2013, 2011; Chakraborty et al., 2012; Chakraborty et al., 2012) to estimate kinetically distinguishable forms of Cu and Ni in the mangrove sediments. It is based on the assumption that each sediment sample consists of  $n$  different components, in which each component,  $M$ -sediment <sub>$i$</sub> , exists in equilibrium with its dissociation products:  $M^{2+}$  or extractable  $M$  complexes, a naturally occurring heterogeneous complexant, sediment <sub>$i$</sub> . The subscript,  $i$ , represents different binding sites of the naturally-occurring heterogeneous complexant.

The extraction of metals from the sediment using EDTA is represented by the following reactions (charges are omitted for simplicity):



Where  $M - \text{sediment}_i$  and  $M - \text{EDTA}$  represent the metal ion M, bound to a sediment binding site,  $\text{sediment}_i$ , and EDTA, respectively. If EDTA is added in large excess, the M is extracted from the original sediment binding site with a rate constant  $k_i$ . The change in concentration of  $M - \text{sediment}_i$  with time is given by the following pseudo first-order rate law.

$$-\frac{dC_{M-\text{sediment}_i}}{dt} = k_i C_{M-\text{sediment}_i} \quad (2)$$

The integrated rate law derived from equation 2, expressed in terms of the concentration of M-EDTA, shows that the concentration of metal extracted,  $C_{M-\text{EDTA}}$ , rises exponentially over time to a limiting value, as shown in Eq. 3. Unfortunately, it is impossible to study the individual binding sites separately, or to a priori know the number of discrete binding sites, and there may be a nearly continuous distribution of sediment binding sites. Hence, Eq. 3 is written as a summation of exponentials.

$$C_{M-\text{EDTA}}(t) = \sum_{i=1}^n C_{M-\text{sediment}_i} (1 - e^{-k_i t}) \quad (3)$$

This system described by equation 3 can be approximated by a two -component first-order reaction model.  $C_{M-\text{EDTA}}(t) = c_1(1 - e^{-k_1 t}) + c_2(1 - e^{-k_2 t})$  (4)

Where,  $C_{M-\text{EDTA}}(t)$  is the concentration of M (Cu or Ni) extracted by EDTA at time  $t$ ,  $c_1, c_2$  are the concentrations of EDTA-extractable M initially bound to labile (quickly extracted), or stable (with respect to the time scale of measurement) sediment binding sites, respectively, and  $k_1, k_2$  are the corresponding dissociation rate constants.

### 3. Results and Discussions

The texture, total organic carbon (TOC), nitrogen (TN), and concentrations of metals in the mangrove sediments are presented in **Table 1**. The average total concentrations of Cu in the sediments varied from 41.3 to 58.4 mg. kg<sup>-1</sup>. The concentration of Ni in the studied sediments was found to vary from 52.6 to 76.5mg.kg<sup>-1</sup>. The sediments were found to have high content of Fe (8.4 to 11.5%). This high content of Fe was probably because of the adjoining Mondovi estuary which is heavily used for transportating Fe ore from the mines located upstream. Certified reference material (MAG-1) was used to validate method recovery. A good recovery of both the metals (more than 99%) was found from the MAG-1.

A significant positive correlation coefficient were found between the total Cu, Ni, and Pb concentrations and the fine (silt, clay) particles content in the bulk sediments ( $R^2=0.95$ ,  $0.91$ , and  $0.95$ ,  $p < 0.001$ ). Phenomena like sorption, co-precipitation, complexation with trace metals are more prevalent with finer particles with more surface area (Chakraborty et al., 2014a, 2014b). This may contribute to the higher metals concentrations in the finer particles of the mangrove sediments. Strong positive correlation between the fine particles (silt, clay) TOC and TN ( $R^2=0.99$  and  $0.98$ ) content in the mangrove sediments indicate that there was high sorption and affinity of organic carbon on finer particles in aquatic system (Hedges and Keil, 1995). A strong positive correlation ( $R^2=1.0$ ) was obtained between TOC and TN content in the sediments and probably indicates that a major part of TN was associated with OC and can be considered as TON (Gireeshkumar et al., 2013, Paropkari et al., 1987).

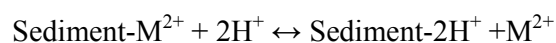
It is well known that the total concentration of metals in sediments is inadequate to predict of its speciation and their bioavailability in the system. Further kinetic extraction study was carried out to understand Ni and Cu-speciation in the mangrove sediments by using a published protocol (Chakraborty et al 2014b).

### 3.1 Optimization of Experimental Parameters

In order to obtain optimal experimental conditions, a systematic investigation was performed to determine the influences of pH and EDTA concentrations on the efficiency of Ni and Cu extraction from the mangroves sediments. These are the two most important parameters to be considered for metal/sediment extractions study (Bermond et al., 1998; Fanguero et al., 2002). The optimization experiments were performed in one sample, as the representative of all the sediment samples.

### 3.2 Optimization of pH

The pH dependence of metal extraction by EDTA is due to competitive extraction by  $H^+$  (Bermond et al., 1998; Fanguero et al., 2002), as shown in the reaction below.



It is therefore, necessary to determine at what pH this competition is minimized. The optimum pH must also minimize two undesirable effects at low and high pH conditions. At very low pH, the complexation efficiency of EDTA is reduced due to protonation of the EDTA molecule. At very high

pH conditions, there is an increasing tendency for the metal ions to hydrolyse to form slightly soluble metal hydroxides.

**Figures 2 a) and b)** show the change in Ni and Cu concentrations, respectively, as a function of pH. The results indicate that within a pH range of approximately 5 - 9, there was no significant change in the concentration of metal extracted, suggesting that the metals were predominantly extracted by EDTA and not by  $H^+$ . No significant pH changes were observed after 24 h of extraction for any of the pH values studied. The optimal pH was chosen to be pH 6 due to the fact that this value is acidic enough to prevent precipitation of metal hydroxides, and was demonstrated to be alkaline enough to minimize competitive extraction by  $H^+$ . pH 6 also optimizes EDTA buffering capacity, as  $pK_{a,3}$  of EDTA is 6.16, which ensures minimal variations in pH throughout the kinetic extraction experiments.

### 3.3 Optimization of concentration of EDTA

EDTA is a non-specific complexing agent; it extracts a wide variety of cations from sediments. Hence, an important parameter to consider is the ratio,  $R$ , between the EDTA concentration and the total concentration of all extracted cations.

$$R = \frac{[EDTA]}{\sum[M_i^{n+}]}$$

where  $M_i^{n+}$  is any major cation (e.g.  $Fe^{2+}$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $K^+$ , etc.). When  $R \ll 1$ , there is a lack of EDTA, and increasing concentrations of EDTA will extract greater concentrations of cations. However, when  $R \gg 1$ , EDTA is in excess and has attained maximum extraction efficiency; hence, any further increases in EDTA concentration will not increase extracted cation concentrations (Bermond et al., 1998; Fangueiro et al., 2002). An excess of EDTA is a necessary condition in order for the kinetic model to be valid, as it minimizes suppression of metal solubilization due to competition by other major cations for complexation by EDTA (Ghestem and Bermond, 1998).

The results for the EDTA concentration optimization experiments indicate that the concentration of Ni and Cu (extracted by EDTA) increased with the increasing concentrations of EDTA. The concentrations of both the metals were found to reach a plateau in presence of  $0.05 \text{ mol dm}^{-3}$  of EDTA. This finding, suggest that  $0.05 \text{ mol dm}^{-3}$  of EDTA represents an excess of EDTA. Similar concentration has also been recommended for the harmonization of extraction techniques under the auspices of the Community Bureau of Reference (BCR) (Ure et al., 2006).



### 3.4 Kinetic speciation of Ni and Cu in the mangrove sediments

Figures 3a and b show the changes in concentrations of extracted Ni and Cu in 0.05M EDTA solution as a function of time. Each curve displays an exponential increase in the metal concentrations within the extracting EDTA solution as a function of time. The curved solid lines represent the fitted data from the non-linear regression analysis. The corresponding numerical results that describe the fitted data are presented in **Table 2**. Each kinetically-distinguishable component is expressed as a fraction of the total EDTA-extractable metal (Ni or Cu) concentrations in the sediments (i.e. as a percentage of the total dynamic Ni or Cu concentration).

The experimental data from the kinetic extraction curves were fitted to a two component model because it was the simplest model that gave an adequate statistical and visual fit to the data. Although use of more components in the model often statistically fit the data better, the additional components were often of negligible concentration or had a high degree of uncertainty. The two-component system should not be considered to assume that there are only two discrete sediment binding sites, but that it allows for diversity among sites, where the calculated parameters can be thought of as representing average values over a distribution of similar sites with closely-spaced rate constants.

#### 3.4.1 Kinetic speciation of Ni

Each curve in **Fig. 3a** shows two distinguishable features: a quickly rising section that represents the rapid dissociation rate ( $k_{d1}$ ) of weak Ni-sediment complexes ( $c_1$ ), and another part that is almost parallel to x-axis which corresponds to relatively stronger Ni complexes ( $c_2$ ) with slower dissociation rate constant ( $k_{d2}$ ). The first part of all the curves in **Fig. 3a** are almost indistinguishable from one another, suggesting that they represent dissociation of one or more Ni complexes having very similar fast dissociation rate constants ( $k_{d1}$ ); probably, they are all of Ni complexes with low thermodynamic stability and are dynamic (within the time scale of the measurement) in nature. The dissociation rate constants of Ni-sediment complexes are presented in **Table 2**

The percentage of weak Ni-sediment complexes ( $c_1$ ) with low thermodynamic stability were found vary from 33.8 to 55.8 % (of the total dynamic extractable Ni) in the mangrove sediments. The dissociation rate constants ( $k_{d1}$ ) of Ni-sediments complexes were found to vary from value of  $\sim 1.0 \times 10^{-3} \text{ s}^{-1}$  to  $8.1 \times 10^{-5} \text{ s}^{-1}$ . This weak Ni-sediment complex ( $c_1$ ) with low thermodynamic stability can be considered as a good representative of bioavailable Ni in the mangrove sediment.

The concentrations of relatively strong Ni-sediment complexes (represented by  $c_2$ ) with slow dissociation rate constants ( $k_{d2}$ ) are presented in **Table 2**. The percentage of relatively strong Ni-sediment complexes ( $c_2$ ) with higher thermodynamic stability were found vary from 44.2 to 66.3 % (of the total extractable Ni) in the studied mangrove sediments. The dissociation rate constants ( $k_{d2}$ ) of Ni-sediment complexes ( $c_2$ ) were found to vary from  $2.3 \times 10^{-5}$  to  $3.2 \times 10^{-5} \text{ s}^{-1}$  in the studied sediments.

### 3.4.2 Kinetic speciation of Cu

Each curve in **Fig. 3b** also shows similar two distinguishable features as found for Ni: a quickly rising section (representing the rapid dissociation rate ( $k_{d1}$ ) of weak Cu–Sediment complexes ( $c_1$ ), and the last part of the curve that lies almost parallel to x-axis (representing slow dissociation ( $k_{d2}$ ) of relatively strong Cu–sediment complexes ( $c_2$ )). The first part of all curves in **Fig. 3b** are almost indistinguishable from one another, suggesting that they represent dissociation of one or more Cu complexes having very similar fast dissociation rate constants. They were all probably of Cu complexes with low thermodynamic stability and were dynamic in nature (within the time scale of the measurement). The dissociation rate constants and dynamic fractions of Cu–sediment complexes are presented in **Table 2**. Fractions of weak Cu-sediment complexes ( $c_1$ ) with low thermodynamic stability were found to vary from 33.4 to 54.9%. The  $k_{d1}$  value of fast dissociating Cu-sediment complexes was found to vary from  $\sim 2.0 \times 10^{-3}$  and  $5 \times 10^{-3} \text{ s}^{-1}$  respectively. The variations in dynamic Cu complexes are presented in **Table 2**.

Slow dissociating Cu complexes ( $c_2$ %) were found to vary in the studied sediment from  $\sim 47$ -66%. However, dynamic fast dissociating Cu-sediment complexes ( $c_1$ ) can be considered as a good representative of bioavailable Cu. Further investigation was carried out to determine the accumulation of Ni and Cu in mangrove roots from the same study areas.

### 3.5 Accumulation of Ni and Cu in mangrove roots

Measuring the content of metals in living organisms is of great interest to assess the actual bioavailability of metals in the same systems. Mangrove roots have been shown to be efficient accumulation indicators of sediment contamination by metals.

In this study, the concentrations of Cu and Ni were measured in the mangrove roots. **Table 2** shows the concentrations of Cu and Ni in the mangrove roots collected from the five different stations.

The extent of assimilation of metals in mangrove roots from sediments depends on metal speciation in the sediments. **Figs. 4a and b** show the variation of Cu accumulation in the roots against the dynamic Cu complexes ( $c_1$ ) and their dissociation rate constant ( $k_{d1}$ ) in the sediments. It shows that the accumulation of Cu in mangrove roots gradually increased with the increasing concentrations of labile Cu-sediment complexes ( $c_1$ ) and their dissociation rate constants ( $k_{d1}$ ). The variation of Ni accumulation in the roots against the labile Ni complexes ( $c_1$ ) and their dissociation rate constant ( $k_{d1}$ ) in the sediments are shown in **Figs. 4c and d**. It also shows that the accumulation of Ni in mangrove roots gradually increased with the increasing concentrations of labile Ni-sediment complexes ( $c_1$ ) and their dissociation rate constants ( $k_{d1}$ ) in the studied sediments. This indicates that labile metal-sediment complexes (determined by kinetic speciation study) can be a good indicator of bioavailability in mangrove system.

Dissociation of M-sediment complexes is a fundamental process in natural systems. The mechanism of metal uptake by mangrove roots from metal-sediment complexes may follow two different pathways; 1) disjunctive and 2) adjunctive pathways (Kraemer et al. 2006). In disjunctive pathways, dissociation of the metal-sediment complexes takes place. The dissociated metal ion comes into pore water and it further taken up by roots. However, in adjunctive pathway, direct attack by the binding ligands (from mangrove roots) takes place to form ternary complex (root-metal-sediment) with the metal and followed by loss of the sediment (as ligand) (as shown below).



We assumed that the dissociated metal ion may desorb into pore water of the sediment and further can be taken up by mangrove roots. The concentrations of Cu and Ni in pore waters of these mangrove sediments were analyzed to provide information for the proposed metal uptake mechanism by mangrove roots from the sediments. The concentration of these metals in pore water was found to depend on the lability of both the metals. Concentrations of Cu and Ni in pore water were found to increase with the increasing lability of the metal-sediment complexes and their dissociation rate constants. Similarly distinct increases in metal accumulation were also found in the roots with the increasing lability and dissociation rate of metal-sediment complexes. Thus, a plausible pathway of metal uptake by mangrove roots from sediment is proposed below (**Fig. 5**). It is suggested that adjunctive pathway is one of the most plausible mechanisms for metal uptake by mangrove roots.

## Conclusions

Bioaccumulation of Cu and Ni in mangrove roots increases with increasing concentrations of labile complexes of Cu and Ni in mangrove sediments. Dissociation rate constants of metal-sediment complexes in mangrove sediments influence metal accumulation in mangrove roots. This study shows that concentrations of labile metal-complexes and their dissociation rate constants can be a good indicator of their bioavailability in a mangrove sediment system.

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## CAPTIONS AND LEGENDS

**Figure 1:** Map of the sampling areas in the mangrove areas located in Divar Island (west coast of India). The filled circles are the locations of the five sampling sites.

**Figure 2:** Optimization of pH for a) Ni, b) Cu. Error bars represents one standard deviation of the mean.

**Figure 3:** Release of extractable dynamic (a)Ni, b)Cu complexes from the mangrove sediments as a function of time in presence of 0.05M EDTA at pH 6.

**Figure 4:** Variation of Cu accumulation in the roots against (a) the concentration of dynamic Cu complexes ( $c_1$ ) and (b) their dissociation rate constant ( $\log k_{d1}$ ) in the sediments. Variation of Ni accumulation in the roots is presented against (c) the concentrations of dynamic Ni complexes ( $c_1$ ) and (b) their dissociation rate constant ( $\log k_{d1}$ ) in the sediments.

**Figure 5:** A plausible pathway of metal uptake by mangrove roots from mangrove sediment



Figure 1

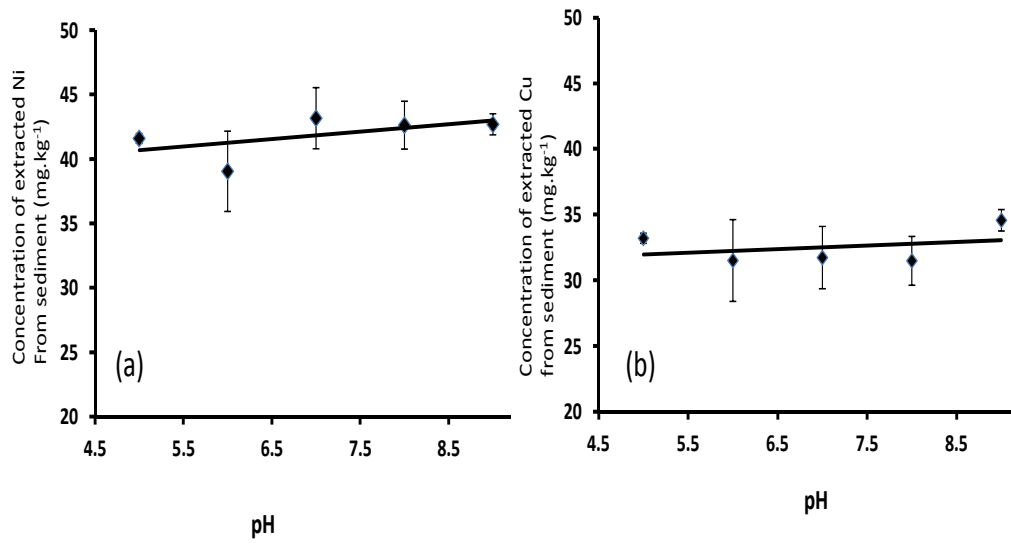


Figure 2



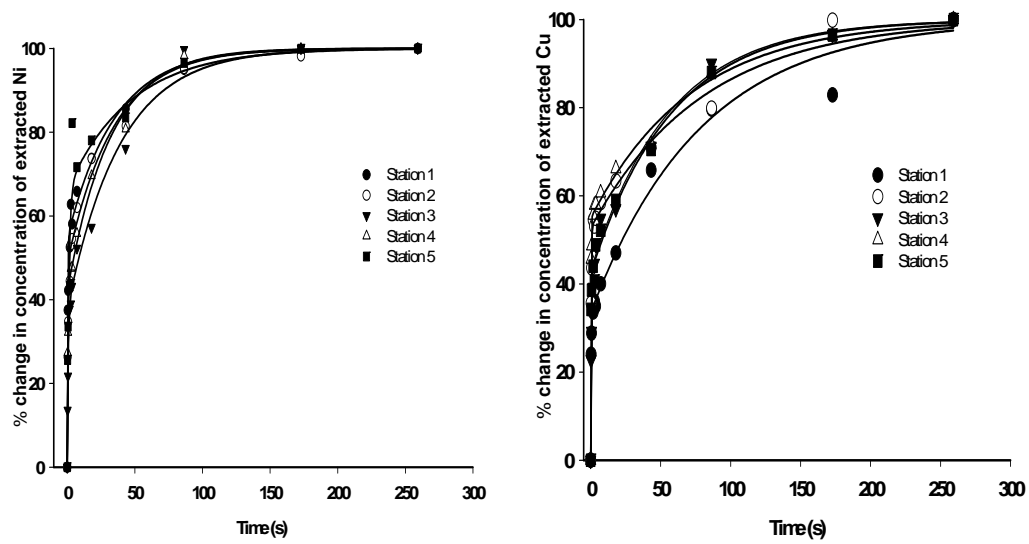


Figure 3

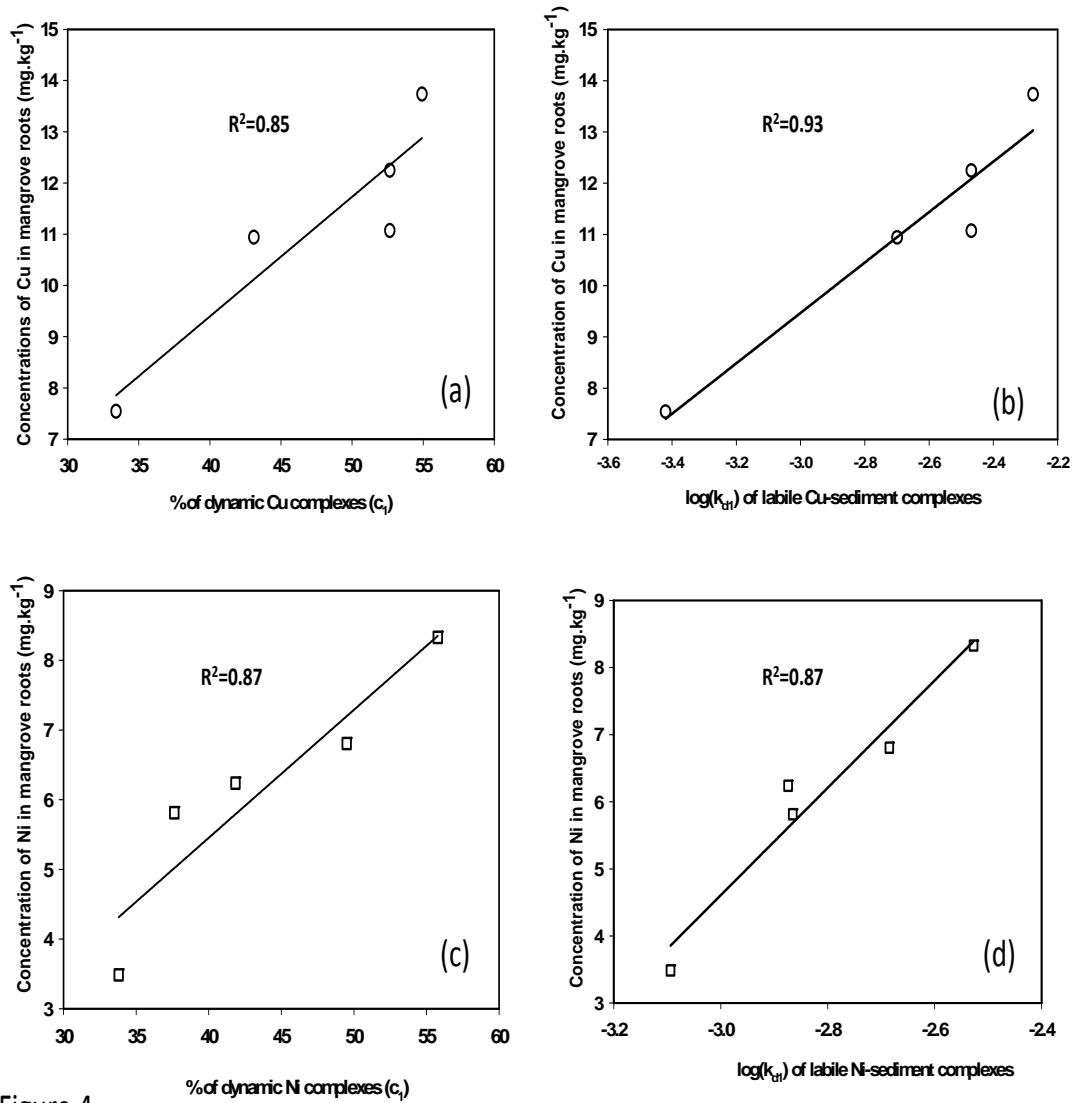


Figure 4

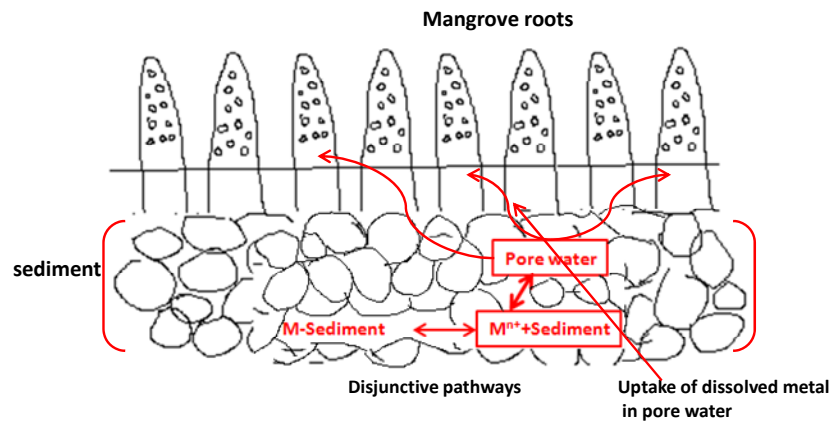


Figure 5

**Table 1.** Texture, total organic carbon, total nitrogen, total concentrations of metals and their variation in the mangrove sediments collected at five locations from

<b>Sampling station</b>	<b>Cu (mg/kg)</b>	<b>Ni (mg/kg)</b>	<b>Mn (%)</b>	<b>Fe (%)</b>	<b>TOC (%)</b>	<b>N (%)</b>	<b>Clay (%)</b>	<b>Silt (%)</b>	<b>Sand (%)</b>
<b>Mang 1</b>	57.6±4.0	76.5±4.9	0.40±0.01	9.9±0.1	8.8	0.42	31.8	48.7	19.6
<b>Mang 2</b>	58.7±0.4	74.8±0.6	0.50±0.01	11.5±0.1	7.5	0.39	32.1	50.2	17.7
<b>Mang 3</b>	43.6±1.8	59.2±2.1	0.26±0.01	9.3±0.1	1.9	0.13	10.8	17.6	71.7
<b>Mang 4</b>	41.3±0.3	52.6±2.3	0.28±0.01	10.6±0.1	2.1	0.13	11.8	20.9	67.3
<b>Mang 5</b>	45.6±0.2	57.0±1.4	0.03±0.0	8.4±0.1	5.0	0.26	21.0	35.0	44.0
<b>MAG-1*</b>	<b>29.2±0.8</b>	<b>53.8±0.3</b>	<b>-</b>	<b>6.7±0.9</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>

\* MAG-1 is a fine grained gray-brown clayey mud with low carbonate content, from the Wilkinson Basin of the Gulf of Maine. This certified reference material (CRM) was obtained from USGS.

**Table 2.** Kinetically distinguishable components of Cu and Ni complexes in sediments and their associated dissociation rate constants.

Element	Sampling stations	Total dynamic fraction (mg/kg)	c <sub>1</sub> (%)	k <sub>d1</sub> (s <sup>-1</sup> )	c <sub>2</sub> (%)	k <sub>d2</sub> (s <sup>-1</sup> )	Concentration in mangrove roots (mg./kg)
Ni	Mang 1	33.8± 0.8	55.8 ± 3.0	$(3.0 \pm 0.7) \times 10^{-3}$	44.2 ± 3.8	$(2.5 \pm 0.7) \times 10^{-5}$	8.3 ± 0.1
	Mang 2	33.4± 2.2	49.5 ± 4.6	$(2.1 \pm 0.6) \times 10^{-3}$	50.5 ± 3.5	$(2.7 \pm 0.6) \times 10^{-5}$	6.8 ± 0.4
	Mang 3	15.6± 1.8	37.6 ± 2.7	$(1.4 \pm 0.3) \times 10^{-3}$	62.4 ± 3.1	$(2.6 \pm 0.4) \times 10^{-5}$	5.8 ± 0.2
	Mang 4	16.8± 1.9	41.9 ± 1.9	$(1.3 \pm 0.5) \times 10^{-3}$	58.2 ± 2.3	$(3.2 \pm 0.4) \times 10^{-5}$	6.2 ± 0.1
	Mang 5	25.2± 3.1	33.8 ± 1.1	$(8.1 \pm 1.2) \times 10^{-4}$	66.3 ± 1.1	$(2.3 \pm 0.2) \times 10^{-5}$	3.5 ± 0.2
Cu	Mang 1	29.1± 1.3	33.4 ± 2.2	$(3.8 \pm 0.2) \times 10^{-4}$	66.6 ± 4.7	$(1.3 \pm 0.2) \times 10^{-5}$	7.5 ± 0.2
	Mang 2	26.2± 2.7	52.7 ± 1.6	$(3.4 \pm 0.3) \times 10^{-3}$	47.4 ± 3.4	$(1.2 \pm 0.2) \times 10^{-5}$	12.2 ± 0.4
	Mang 3	13.2± 0.9	43.1 ± 2.0	$(2.0 \pm 0.2) \times 10^{-3}$	56.9 ± 3.0	$(1.8 \pm 0.2) \times 10^{-5}$	10.9 ± 0.1
	Mang 4	14.3± 1.1	54.9 ± 1.2	$(5.3 \pm 0.4) \times 10^{-3}$	45.1 ± 2.5	$(1.4 \pm 0.2) \times 10^{-5}$	13.7 ± 0.2
	Mang 5	17.8± 1.3	52.7 ± 1.6	$(3.4 \pm 0.4) \times 10^{-3}$	47.4 ± 3.4	$(1.2 \pm 0.3) \times 10^{-5}$	11.1 ± 0.3