

Relationship between the lability of sediment-bound Cd and its bioaccumulation in edible

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Abstract:

A linkage between Cd speciation in sediments and its bioaccumulation in edible oyster (*Crassostrea* sp.) from a tropical estuarine system was established. Bioaccumulation of Cd in edible oyster (*Crassostrea* sp.) increased with the increasing lability and dissociation rate constants of Cd-sediment complexes in the bottom sediments. Total Cd concentration in sediment was not found to be a good indicator of Cd-bioavailability. Increasing trace metals competition in sediments was found to increase lability and bioavailability of Cd in tropical estuarine sediment. Low thermodynamic stability and high bioavailability of Cd in the estuarine sediment was responsible for high bioaccumulation of Cd in edible oysters (3.2-12.2 mg kg⁻¹) even though the total concentration of Cd in the bottom sediment was low (0.17–0.49 mg kg⁻¹).

Keywords: Cd-speciation; lability of Cd-complexes; bioaccumulation of Cd; Cd accumulation in oyster; lability and bioaccumulation

Introduction

Cadmium (Cd), a nonessential trace metal, enters estuarine system mainly via land runoff, river discharge, and aerial deposition (Jayaraju et al., 2011; Hall et al., 1998). Dissolved Cd is quickly scavenged by negatively charged suspended particulate matter (SPM) (like clays, carbonates, oxides and hydroxides of Fe, Mn) as well as dissolved organic carbon (OC) in water column (Jiann et al., 2005; Wen et al., 2008). This toxic metal (after association with different particulate matters) ultimately accumulates in bed sediments (Förstner and Salomons, 1980; Kranck, 1975). Estuarine sediments, therefore, act as important sink for Cd (Salomons et al., 1987). However, mobility and bioavailability of Cd in estuarine sediments does not depend on total Cd concentration but on the physicochemical forms of Cd in the sediments (Tack, and Verloo, 1995).

Cd usually forms weak complexes (labile) with dissolved organic matter (DOM) (Martínez and McBride, 1999; Tuschall Jr. & Brezonik, 1981; Muller 1999; Chakraborty, 2010) or sedimentary

organic matter (SOM) in natural systems (Almås et al., 2000; Chakraborty et al., 2012; Eggleton and Thomas, 2004).

Saar and Weber (1982) have shown that lability of Cd complexes is higher than Cu, Pb complexes with fulvic acid in model test solutions (Chakraborty et al., 2007). Chakraborty et al (2007) have shown that Cd forms thermodynamically less stable complexes with humic substances (Chakraborty and Chakrabarti, 2008). Thermodynamic stability constant (logK) of Cd-humate complexes has been reported to vary from 5.26 ± 0.14 to 5.47 ± 0.08 (Chakraborty et al., 2007). Formation of kinetically labile complexes of Cd in sediments has been reported by Chakraborty et al (2012). It has been found that increase in total Cd loading in coastal and estuarine sediment increases lability and bioavailability of Cd (Chakraborty et al., 2012). In surface sediments, Cd is mobile under oxidising conditions at pH levels below 8 (Reddy and Patrick 1977; Khalid et al., 1981). Mobility of Cd in sediment is likely to be high, especially during of elevated flow, at lower pH. Solubility of Cd is largely unaffected by changes in Eh of the medium (Zia-ur-Rehman et al., 2015; Pascaud et al., 2015). Complexations of Cd with carbonate restrict mobility of Cd in carbonate-rich sediment and soil (Wu et al., 2015; Wang et al., 2015). Sekaly et al (1999) have suggested that zero ligand field stabilization energy (LFSE) of Cd (II) make Cd-DOM complexes thermodynamically weak. The weak stability of Cd–humic complexes has also been attributed to poor effective nuclear charge, polarizability of Cd and the ligand in the system. Thus, stability and lability of Cd complexes in sediment is expected to determine its mobility and bioavailability in sediment. Certain studies have found direct relationships between the metal concentration in sediments and bivalves for various heavy metals (including Cd) (Philip and Yim 1981). Forstner and Wittmann (2012) have observed linear relationship between bioaccumulated Cd and Cu concentrations in oyster and the total concentrations of the same metals in the bottom sediments. However, no simple relationship has been found between metal concentrations in sediments and bivalves (Huanxin et al 2000). It is noteworthy that all these studies aimed to examine the relationship between total metals concentrations and their bioaccumulations in bivalves. However, total Cd concentrations in sediments do not necessarily correspond with Cd bioavailability in the system. Speciation of Cd in sediment is probably the key factor that control Cd bioaccumulation in bivalves.

Low thermodynamic stability and high bioavailability of Cd-sediment complexes is expected to increase Cd accumulation in benthic organisms and aquatic biota, with consequent problems of toxicity. Humans may also get exposed to Cd by consuming Cd-contaminated seafood. Long term exposure of Cd may cause cancer (Satarug and Moore, 2004; WHO 1996). The maximum tolerable intake of Cd is regarded as $7 \mu\text{g kg}^{-1}$ of body weight (WHO 1996).

Unfortunately, the baseline data for Cd and their speciation in tropical estuarine sediments is scarce. In this study an effort was made to understand the stability and lability of Cd complexes in estuarine sediments and its relation with Cd-bioaccumulation in edible oysters from the west coast of India. Different other factors were also identified that control Cd-speciation in sediments and its bioaccumulation in oyster.

Materials and methods:

Study area

The sediment samples were collected from the Zuari mangrove estuarine region of Goa (a small coastal state of India). Agassaim-Zuar (sampling site) is located at latitude 15°24'54.24 N and longitude 73°54'42.15 E (**Figure 1**). Surface sediment samples were collected by hand using PVC pipes (inner diameter 7.5 cm, 20 cm length). Multiple sampling was done at each station. The composite sediment sample (from each station) and oyster were stored at -20°C. The samples were oven dried at 30°C. The sediments were then subsequently stored at -4° C until needed. Multiple oyster (*Crassostrea* sp.), were collected from the sampling stations, and depurated, washed with ultrapure water for complete removal of sediments from the oyster. The soft tissues were taken out and freeze dried until constant weight obtained. The dried soft tissues were ground-milled and homogenized. This is an edible oyster and part in the diet of many tropical islanders. Oyster (*Crassostrea* sp.) is also consumed by the local people. Native oysters (*Crassostrea* sp.) were also collected subsequently from the same station. pH of the overlying water sample was determined by using a pH meter (Model-pHep HI96107). Dissolved oxygen of the water sample was estimated by Winkler's iodometric titration method (Trivedy and Goel 1986). Salinity of the sample was estimated with the help of digital ERMA Salinity refractometer (Model RHS-10 ATC).

Sediment texture

Texture of the dry sediment sample was analyzed (after sieving with 63 µm sieve size) by size particle analyzer (LPSA, Malvern Mastersizer 2000). The data were presented as weight percentage (wt%) in this study.

Elemental and isotopic analysis

Sedimentary organic C (C_{org}) and total N (TN) contents along with the stable C and N isotopic compositions were measured using an Eurovector EURO3000 elemental analyser connected to a Thermo DELTA V PLUS isotope-ratio mass spectrometer in a continuous-flow mode. About 7-10 mg of decalcified dried samples was packed in tin cups and flash combusted in the elemental analyzer (EA) at 1050°C. A laboratory standard, ε-Amino-n-Caprioic acid, ($C_6H_{15}NO_2$; ACA) was

used for calibration of elemental data as well as $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$. Reported ACA values of $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ are -25.3‰ and 4.6‰ respectively.

Carbon and nitrogen isotopic ratios of sedimentary organic matter (SOM) are expressed as δ values ($\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$) with reference to V-PDB and atmospheric N_2 , respectively.

$$\delta^{13}\text{C}_{\text{org}} \text{ and } \delta^{15}\text{N} = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000 \dots (1)$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ and ${}^{15}\text{N}/{}^{14}\text{N}$.

The analytical precision of replicate analyses of selected samples (n=3) was $<2\%$ for C_{org} and TN and $<0.17\text{‰}$ for $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$.

Determination of metals (Cu, Ni, Pb, and Cd) in the sediments and oyster

The dried composite sediment samples (from each station) were homogenized and ground-milled. The total metals concentrations in the sediments were determined by digesting 0.05 g of sediments samples with 10.0 mL of acid mixtures of HF, HNO_3 and HClO_4 (in 7:3:1 ratio) on hot plate at 200°C . The sediments were digested and evaporated to dryness. The residues were re-dissolved in 2% HNO_3 and analyzed by graphite furnace atomic absorption spectrometer (GFAAS).

Multiple native oyster *Crassostrea* sp., collected from the sampling stations, and were depurated, washed with ultrapure water for complete removal of sediments from the oyster. The soft tissues were taken out and freeze dried until constant weight obtained. The dried soft tissues were ground-milled and homogenized. The total metals concentrations in the tissues were determined by digesting 0.1 g of ground-milled and homogenized tissue samples with 10.0 mL of acid mixtures of HF, HNO_3 and HClO_4 (in 7:3:1 ratio) on hot plate at 200°C . The samples were digested and evaporated to dryness. The residues were re-dissolved in 2% HNO_3 . Concentrations of metals in soft tissues of oysters were analyzed by GFAAS.

Kinetic extraction procedure

Two grams (2.0 gm) of sediment was added to 200 mL of 0.05M EDTA solution (at pH 6.0) (Merck Pvt Ltd) in a Teflon beaker (400 mL) 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 cm³ 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 cm³) were then (in 2% ultrapure HNO₃) 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, HNO₃ and HClO₄ (in 7:3:1 ratio) on hot plate. The sediments were digested and evaporated to dryness. The residues were redissolved in 2% HNO₃ and analyzed by GFAAS. MAG-1, a fine grained gray-brown clay mud with low carbonate content, from the Wilkinson Basin of the Gulf of Maine, was used as certified reference material (CRM) (obtained from USGS).

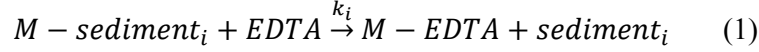
Graphite furnace atomic absorption spectrometer (GFAAS)

A GFAAS (Perkin Elmer, PinAAcle 900T) was used for the determination of the total metals concentrations. This instrument was equipped with an AS900 autosampler. The transversely heated graphite tube (pyrolytically coated and equipped with integrated L'vov platforms) was used in this study. During the drying, ashing and clean-up cycles, the internal argon gas was passed through the furnace at 300 mL min⁻¹, but the gas flow was interrupted during the atomization step. The instrumental parameters of drying temperature, ashing temperature and atomization temperature have been described in literature (Chakraborty et al., 2014a, b). The signal was measured in the peak area mode. Every fifth sample analyzed 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%.

Theory

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

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



Where $M - sediment_i$ and $M - EDTA$ represent the metal ion M, bound to a sediment binding site, $sediment_i$, 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 $M - sediment_i$ with time is given by the following pseudo first-order rate law.

$$-\frac{dc_{M-sediment_i}}{dt} = k_i c_{M-sediment_i} \quad (2)$$

The integrated rate law derived from equation 2, expressed in terms of the concentration of M-EDTA, shows that the concentration of metal extracted in solution, c_{M-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.

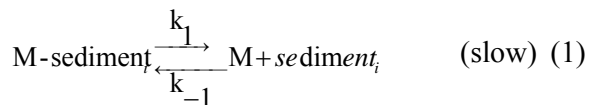
$$c_{M-EDTA}(t) = \sum_{i=1}^n c_{M-sediment_i}(1 - e^{-k_i t}) \quad (3)$$

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

Where, $c_{M-EDTA}(t)$ 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.

The lability criteria in CLE method is given below.

The concept of *lability* describes the ability of complexes to maintain equilibrium with the free metal ion, M, within the context of an ongoing interfacial process in which a particular species, usually M, is consumed. Consider sediment of n components, in which each component, designated $M-sediment_i$, exists in equilibrium with its dissociation products:



Where, where k_1 and k_{-1} are the rate constants for complex association and dissociation respectively and the thermodynamic stability constant, K , equals k_1/k_{-1} .

Under conditions of sufficient excess ligand (L) over metal (M), the association reaction is quasi-monomolecular with rate constant $k'_1 = k_1 c_L$. On time scales, t , much larger than the characteristic lifetimes of M ($1/k'_1$) and M-sediment_i ($1/k_{-1}$), a given metal ion undergoes frequent interchange between M and M-sediment_i. The complex system is then sufficiently dynamic to maintain bulk equilibrium and obeys the double condition (Gaabass et al., 2009)

$$K'_1 t, k_{-1} t \gg 1 \quad (2)$$

In the usual situation of practical interest, $K' (= K c_{\text{sediment}}) k'_1/k_{-1} > 1$, so Eq. 2 reduces to $k_{-1} t \gg 1$.

Thus, metal complexes in sediment will be called labile if their $k_1 t \gg 1$.

Results and discussion

Geochemical characteristics of sediments

Variation in particle size distribution, concentrations of trace/ metals in the studied sediments, pH, salinity, dissolved oxygen and temperature of the overlying water column during the monsoon and post monsoon periods are presented in Table 1. The particle size distribution analysis suggests that clay particles in the sediments dominated in all the month except the month of October. The pH of the overlying water column was found to vary from 7.4 - 8.2. Lower pH and salinity during the monsoon season was due to the influence of fresh water influx in the estuary. The salinity of the overlying water column was found to vary from 10-30PSU. There was not much variation in temperature and DO level of the overlying water column during the study period. The concentrations of metals (Cu, Ni, Pb, Cd) in the sediments were comparatively low (except Pb) during the monsoon period (August to October) compared to the post monsoon period (November–January). The decrease in river discharge (i.e., decrease in dilution effect) during the post monsoon period was probably responsible for the increase in total metals content in the sediment during the post monsoon period. This change in metals concentrations suggests that there was probably point source of Cu, Ni, and Cd. However, river discharge and land runoff were probably the major sources of Pb in the sediments.

The total concentration of Cd in the sediments was in the range of 0.17–0.49 mg kg⁻¹. The concentration range of Cd in the sediments was much lower than the ERL value (1.2 mg/kg) recommended by sediment quality guideline (Burton, 2002). The highest concentration of Cd in the studied sediments was found during the month of November (0.49 mg kg⁻¹) and the lowest

concentration was found in the month of August (0.17 mg.kg^{-1}). However, total Cd concentrations do not necessarily correspond with metal bioavailability in natural system.

Low thermodynamic stability (Chakraborty et al, 2010) and high bioavailability of Cd-sediment complexes is expected to increase Cd accumulation in benthic organisms and aquatic biota, with consequent problems of toxicity. Humans may also get exposed to Cd by consuming Cd-contaminated seafood (Reeves and Chaney, 2008). Thus, kinetic speciation of Cd in the estuarine sediments was further studied to determine the concentration of labile Cd-complexes and their dissociation rate constant.

It is well documented that quality and quantity of sedimentary organic matter determines metals speciation, bioavailability and their mobility in a sediment system. Nature of organic matter in the studied sediments was determined before the speciation study of Cd was conducted in the sediments.

Nature of sedimentary organic matter

Table 2 shows the variation in organic carbon (C_{org}), total nitrogen (TN), stable carbon and nitrogen isotopes in the studied sediments. C_{org} and TN contents in the sediments were found to vary from $\sim 0.8\%$ - 3.1% and $\sim 0.08\%$ - 0.30% respectively. The highest values for both C_{org} and TN were observed in the month of December. The C_{org} and TN contents show a strong positive correlation with clay ($r^2 = 0.4$ for C_{org} and 0.8 for TN) and a weak correlation with sand ($r^2 = 0.3$ for C_{org} and 0.6 for TN) particles of the sediments. Stable carbon and nitrogen isotopes were used for source identification of organic matter. The value of $\delta^{13}\text{C}_{\text{org}}$ ranged narrowly between -26.8‰ and -22.4‰ . The $\delta^{15}\text{N}$ in those months varied from 3.0 to 5.5‰ in the sediments. Sediment collected in the month of October recorded the most depleted $\delta^{13}\text{C}_{\text{org}}$ (-26.8‰) and $\delta^{15}\text{N}$ (2.9‰) values corresponding with the highest C/N ratio (19.2). This indicates that the influence of terrestrial OM in the sedimentary organic carbon was highest in the month of October. This study indicates there was input from both terrigenous and marine sources of OM to the sedimentary OM at the sampling site.

Kinetic speciation of Cd

Figure 2 shows the changes in concentrations of extracted Cd from the sediments by EDTA (complexing agent) as a function of time. Each curve displays an exponential increase in the Cd concentration in the extracting EDTA solution with respect to time. The curved solid lines represent the results from the non-linear regression analysis, and the corresponding numerical results that describe the fitted data are presented in Table 3.

The experimental data (obtained from the kinetic extraction studies) were fitted to two component model. Each curve in Figure 2 shows two distinguishable features: a quickly rising section that

represents the rapid dissociation rate (k_1) of weak Cd–Sediment complexes (c_1), and the last part of the curve that lies almost parallel to x-axis, which can be attributed to slow dissociation (k_2) of strong Cd–sediment complexes (c_2).

The first part of all curves in Figure 2 is almost indistinguishable from one another, suggesting that dissociation of one or more Cd complexes having very similar fast dissociation rate constants; they were Cd complexes with low thermodynamic stability and were labile (within the time scale of the measurement). The dissociation rate constants and labile fractions of Cd–sediment complexes are presented in Table 3, shows that the k_1 of Cd-sediment complexes were fast and almost very similar in all the sediments collected at five different months from the same station.

Leaching of weak Cd-sediment complexes (c_1) with low thermodynamic stability were found vary from 36.4 to 68.3% of the total Cd in the studied sediments collected at different months. The dissociation rate constant (k_1) of labile Cd-sediment complexes were found to vary from $\sim 8 \times 10^{-3}$ to $3.2 \times 10^{-3} \text{ s}^{-1}$. The highest fraction of labile Cd-sediment complexes was found in the sediment collected in the month of November ($\sim 68.3\%$ of the total Cd present in the sediment with k_1 value of $\sim 7.3 \times 10^{-3} \text{ s}^{-1}$) (Table 3). The lowest fraction of labile Cd complexes was found in the month of September ($\sim 36.4\%$ of the total Cd present in the sediment with k_1 value of $\sim 3.2 \times 10^{-3} \text{ s}^{-1}$). The concentration of labile Cd-sediment complexes was found to depend on the total Cd loading of the system.

This study reveals that the concentrations of weak complexes of Cd (c_1) (which is probably a good indicators of its bioavailability in a system) in estuarine sediments were having fast dissociation constant and were labile (based on lability criterion). High concentration of labile Cd-sediment complexes may alter the delicate balance between the speciation, bioavailability and toxicity of this metal which is essential to sustain life in the sediments and their overlaying water column. Zero ligand field stabilization energy (LFSE) of Cd^{2+} was probably responsible for forming thermodynamically weak Cd-sediment complexes. The weak stability of Cd–humate complexes has also been attributed to poor effective nuclear charge, polarizability of Cd and the ligand in the system. However, competition of other trace metals with Cd^{2+} for different binding sites (cation exchange sites and nonionic sorption sites) of sediments may increase the lability of Cd-sediment complexes. The last part of the curve that lies almost parallel to x-axis, which was attributed to slow dissociation of inert Cd-sediment complexes (represented by c_2) are presented in Table 3 with slow dissociation rate constants (k_{d3}). These Cd-complexes were not considered as bioavailable.

Trace metal competitions and Cd speciation in sediment

Competition of Cu and Ni with Cd ion for different binding sites of the studied sediments at environmental condition was investigated. Change in kinetic speciation data (concentrations of dynamic complexes and their dissociation rate constants) with varying concentrations of other trace metal was examined. **Figure 3a and b** shows that the increasing concentrations of Cu(II) and Ni(II) loading in the sediment increased the concentrations of labile Cd-sediment complexes in the studied sediments. The total concentrations of Cu and Ni present in the sediments were ~3 orders of magnitude higher than those of Cd. It has also been reported that Cu and Ni form thermodynamically more stable complexes than Cd(II) in sediments. It is expected that Cu and Ni can outcompete Cd from undergoing complexation reactions with different binding sites present in the sediments and resulting Cd(II) forming weak complexes that are labile. A related fact is that the high concentrations of major cations (such as Na⁺ and Ca⁺⁺) in seawater should release Cd⁺⁺ from cation exchange sites of sediments. The significance is that metal (trace and major) competition may increase Cd bioavailability in sediments. Increase in Cd bioavailability may increase Cd accumulation in benthic organisms such as oyster. However, further study will be carried out to understand the distribution of Cd in different binding phases of sediments.

Bioaccumulation of Cd in oysters

Determination of total metal concentrations in benthic organisms in coastal/estuarine system is of great interest to assess the actual bioavailability of metals in a sediment system (Neff 2002;; Peijnenburg, et al., 2007; Long and Morgan 1990; Long et al. 1995). Benthic organisms are efficient accumulation indicators of sediment contamination by trace/heavy metals (Pinheiro et al., 2012; Prasannakumari et al., 2014). The extent of assimilation of Cd in oyster *Crassostrea* sp. collected from the same station was expected to depend on Cd speciation in the sediments. The concentrations of Cd in the oyster (in *Crassostrea* sp.) were found to vary from 3.2 to 12.2 mg kg⁻¹ (Table 3). The lowest concentration of Cd was found during the month of September (3.2 ± 0.3 mg.kg⁻¹). However, highest concentration of bioaccumulated Cd was found in the month of November (11.8± 0.3) mg.kg⁻¹ post monsoon period (Table 3). It has been reported that the permitted maximum limits for Cd in edible oyster are 3–4 mg·g⁻¹ in the U.S., 2 mg·g⁻¹ in Hong Kong and Australia, and 1 mg·g⁻¹ in New Zealand (Brotten 1998; Kruzynski 2002). From the human public health point of view, comparison between the mean concentrations of the Cd in the soft tissue of oyster and global guidelines clearly indicates that nearly in all cases concentration of Cd were higher than the permissible amounts for human consumption. This study suggests that the total concentration of bioaccumulated Cd in oysters can be high even if the total concentration of Cd in the bottom

sediment is low. It is strongly believe that speciation of Cd-sediment complexes probably responsible for the bioaccumulation of Cd in the soft tissues of the oysters.

Relation between kinetic speciation of Cd in sediments and its impact on Cd bioaccumulation in oyster

Soft tissues in oysters have been shown to be efficient accumulation indicators of sediment contamination by metals. In this study, the concentration of Cd was measured in the soft tissues of oysters. Table 3 shows the variation in concentrations of Cd in the oysters collected at four different months from the same sampling location. The extent of incorporation of Cd in soft tissues of the oysters from sediments depends on metal speciation in the sediments. Oysters are known to bioaccumulate contaminants (; Graczyk et al. 2006;; Gagnaire, Thomas-Guyon, and Renault 2004; Frazier 1979) at water –sediment interface via filter feeding. Figures 4a and b shows the variation of Cd accumulation in the soft tissues of the oysters with varying concentrations of labile Cd complexes (c_1) and their dissociation rate constant (k_{d1}) in the sediments respectively. It shows that the accumulation of Cd in oysters gradually increased with the increasing concentrations of labile Cd-sediment complexes (c_1) and their dissociation rate constants (k_{d1}). The variation of Cd accumulation in the soft tissues of the oysters increased with the increasing total concentrations of Cu and Ni in the sediments (Figures 5a and b). This again indicates that increasing concentrations of Cu and Ni in the sediment increased lability of Cd–sediment complexes (by trace metal competition) in the system (determined by kinetic speciation study) and thus, increased Cd accumulation in the oysters.

The most plausible pathway of Cd bioaccumulation by oyster is presented in Figure 6. Oysters (filter-feeder) filter in and out the surrounding water for food. Finer sediment particles (suspended) in water enter inside the organism during filter feeding. Cd associated with finer sediment particles also enters inside the oysters. Bioaccumulation of Cd in oyster increases with increasing concentrations of labile Cd-complexes in the bottom sediments. The concentration of Cd in oyster depends on the dissociation rate constant of Cd-sediment complexes. Thermodynamically stable (inert) Cd-sediment complexes do not participate in Cd bioaccumulation process in oyster.

Conclusion

This is the first study that established a linkage between Cd speciation in sediments and bioaccumulation in edible oyster from a tropical estuarine system. This study suggests that bioaccumulation of Cd in oyster increases with increasing concentrations of labile complexes of Cd in the bottom sediments. Dissociation rate constants of Cd-sediment complexes in estuarine sediments influence Cd accumulation in oysters. Low thermodynamic stability and high

bioavailability of Cd in the estuarine sediment was responsible for high bioaccumulation of Cd in edible oysters (3.2-12.2 mg kg⁻¹) even though the total concentration of Cd in the bottom sediment was low (0.17–0.49 mg kg⁻¹). This study shows that concentrations of labile Cd-complexes and their dissociation rate constants can be a good indicator of their bioavailability in an estuarine sediment system.

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Caption and legends for Figures

Figure 1. Map of the sampling site in the Zuari mangrove estuarine region of Goa (west coast of India). The filled circle is the locations of the sampling site.

Figure 2. Release of extractable dynamic Cd complexes from the studied sediments as a function of time in presence of 0.05 M EDTA solution at pH 6.

Figure 3. Impact of trace metals, Cu (a) Ni (b), competition on the concentrations of labile Cd-complexes in the studied sediments.

Figure 4. Variation of Cd accumulation in the soft tissues of the oysters with varying concentrations of labile Cd complexes (c_1) (a) and their dissociation rate constant (k_{d1}) (b) in the sediments.

Figure 5. Variation in Cd accumulation in the soft tissues of the oysters with increasing total concentrations of Cu (a) and Ni (b) in the sediments.

Figure 6. A plausible pathway of Cd uptake by oysters in mangrove sediment.

Table 1: Environmental parameters and total metal concentration of Cd, Cu, Ni and Pb in estuarine sediments

Sampling station	Silt (%)	Clay (%)	Sand (%)	Water			Dissolved Oxygen (ml.l ⁻¹)	Cd _T (mg.kg ⁻¹) Sediment	Cu _T (mg.kg ⁻¹) Sediment	Ni _T (mg.kg ⁻¹) Sediment	Pb _T (mg.kg ⁻¹) Sediment
				pH	Temperature (°C)	Salinity (PSU)					
Aug St-1	18.7	56.7	25.3	7.4	31.7	11	4.6	0.17 ± 0.01	69.4 ± 1.7	93.7 ± 2	69.6 ± 1.5
Sep St-1	17.3	41.4	41.3	7.6	31.8	10	4.8	0.26 ± 0.01	38.2 ± 0.02	63.5 ± 1.5	43.5 ± 0.6
Oct St-1	7	30.9	62	7.9	32	28	6.2	0.17 ± 0.01	37.9 ± 0.3	59.6 ± 1.2	39.8 ± 0.5
Nov St-1	14.7	55.6	29.7	8.1	32	28	5.8	0.49 ± 0.06	67.4 ± 0.5	104.5 ± 2.3	13.7 ± 0.1
Dec St-1	15.6	74	10.3	8.2	32.2	30	7.3	0.32 ± 0.11	88.3 ± 0.7	149.6 ± 0.9	19.2 ± 0.02

Table 2: Total Organic Carbon, total nitrogen, C/N ratio, and $\delta^{15}\text{N}_{\text{TN}}$, and $\delta^{13}\text{C}_{\text{org}}$ in the mangrove sediment

Station	TOC %	TN%	C/N(molar)	$\delta^{15}\text{N}_{\text{TN}}$	$\delta^{13}\text{C}_{\text{org}}$
Aug St-1	2.4	0.20	13.9	5.2	-24.6
Sep St-1	0.75	0.08	11.3	4.2	-22.4
Oct St-1	2.1	0.13	19.2	3.0	-26.8
Nov St-1	1.9	0.17	13.1	4.3	-24.2
Dec St-1	3.1	0.30	12.7	5.5	-24.0

Table 3: Kinetically distinguishable components of Cd complexes in mangrove sediment and their associated dissociation rate constants.

Stations	C_1 (%)	k_{d1} (s^{-1})	C_2 (%)	k_{d2} (s^{-1})	Total Cd ($\text{mg}\cdot\text{kg}^{-1}$)	Cd in oyster ($\text{mg}\cdot\text{kg}^{-1}$)
Aug St-1	49.8	5.7×10^{-3}	50.2	9.31×10^{-7}	0.17 ± 0.01	7.3 ± 0.4
Sep St-1	36.4	3.2×10^{-3}	63.6	7.6×10^{-7}	0.26 ± 0.01	3.2 ± 0.3
Oct St-1	56.5	8.2×10^{-3}	43.5	1.0×10^{-6}	0.17 ± 0.01	8.9 ± 0.5
Nov St-1	68.3	7.3×10^{-3}	31.7	2.0×10^{-6}	0.49 ± 0.06	11.8 ± 0.3