

Linkage between speciation of Cd in mangrove sediment and its bioaccumulation in total soft tissue of oyster from the west coast of India

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Abstract

This study established a mechanistic linkage between Cd speciation and bioavailability in mangrove system from the west coast of India. High bioaccumulation of Cd was found in oyster (*Crassostrea* sp.) even at low Cd loading in the bottom sediment. Bioaccumulation of Cd in oyster gradually increased with the increasing concentrations of water soluble, exchangeable, carbonate/bicarbonate forms of Cd in the sediments. Fe/Mn oxyhydroxide phase was found to control Cd bioavailability in the sediment system. Cd-associated with sedimentary organic matter was bioavailable and organic ligands in the sediments were poor chelating agents for Cd. This study suggests that bioaccumulation of Cd in oyster (*Crassostrea* sp.) depends not on the total Cd concentration but on the speciation of Cd in the system.

Keywords: Cd-speciation; Cd-bioavailability; Cd-accumulation; Cd in *Crassostrea* sp; Sequential extraction study

Capsule Abstract:

Bioaccumulation of Cd in oyster (*Crassostrea* sp.) depends not on the total Cd concentration but on the speciation of Cd in the bottom sediments.

1. Introduction

The potential adverse effects of sediment-associated toxic metals is generally assessed by determining their total concentration directly from sediments, organisms on sites, or determined from bioassays of exposed animals (MacDonald et al., 2000; Long et al. 1995; Long and Morgan 1990). However, these determinations are not good indicators of effects in all cases (Chen et al. 2008; Hobbelen, Koolhaas, and Van Gestel 2006). Total metal concentrations do not necessarily correspond with metal bioavailability in natural system. Bioavailability of a metal is a fraction of the total metal that is available for incorporation into biota (bioaccumulation). Bioavailability of metal in sediment depends on its chemical speciation(Chakraborty et al. 2014; Chakraborty et al., 2015). In sediment systems, non-residual metal complexes (water soluble, exchangeable metal complexes, metals bound to carbonates, metals bound to oxyhydroxide of Fe and Mn, and metals bound to organic matter and sulfides) are considered as a good indicator of bioavailability(Chakraborty, et al 2012a; Förstner 1987; Peijnenburg, et al., 2007). Organic matter and oxyhydroxide of Fe and Mn are important binding phases for metals in sediments (Chakraborty et al. 2012a; Chakraborty et al., 2012b; Chakraborty, et al., 2011; Chakraborty 2012) and can significantly influence metal bioavailability in the system. Residual metal complexes (metals within the structure of the sediment and minerals) has been considered as inert and inaccessible to biota(Chakraborty, et al., 2015; Chakraborty, et al. 2014). Metals associate with different binding phases of sediments exhibits different behaviour in terms of chemical interactions, mobility, biological availability and potential toxicity(Singh et al. 2005; Li et al. 2000; Peijnenburg et al.,2007). The sequential extraction schemes are capable of separating non-residual and residual metal complexes in sediment(Chakraborty, et al., 2011; Chakraborty 2012; Chakraborty et al., 2012b; Chakraborty et al., 2012a; Chakraborty et al. 2014; Chakraborty et al. 2012). The sequential extraction protocols proposed by the European Community Bureau of Reference, usually called the BCR method (Ure et al., 2006) has been widely used for metal speciation study in sediments.

It is well known that cadmium (Cd), known to be a toxic metals (Kumar et al., 2008; Lim et al., 2008; Weng et al., 2008) with very little (Lane et al., 2005; Xu et al., 2008) or no nutrient values, is abundant almost everywhere in the nature. Pollution of soils, sediments and waters with Cd cause its incorporation into the food chain, which could result in wide variety of adverse effects in animals and humans, since it is a cumulative contaminant. The consumption of contaminated seafood has been reported as an important route of human exposure to heavy metals in India (Sivaperumal *et al.* 2007; Sankar et al., 2006; Vinodhini and Narayanan, 2008). The possible association between seafood consumption and human exposure to toxic contaminants has not been extensively investigated. Different government agencies (in India) have been creating awareness to people and farmers via organizing meetings, and writing popular articles (<http://www.cmfri.org.in/cmfri-in-media.html>, <http://nfdb.gov.in/html/guidelines.htm>). However, this is a well-recognized but poorly characterized environmental problem in India. The consumption of heavy metal contaminated oyster

can be an important route of human exposure to heavy metals in coastal India. Oysters are known to bioaccumulate contaminants (Jeon et al. 2010; Hobbelen, et al., 2006; Graczyk et al. 2006; Geffard et al. 2007) at water –sediment interface via filter feeding. However, exact mechanism of toxic metal accumulation in oyster is not known. It is known that bioaccumulation of various toxic metals in oyster depends not on the total metal concentrations but on their speciation in the bottom sediments.

The objectives of this study were to test the hypotheses that (a) total metal concentration in sediment may not be a good indicator of its bioavailability (b) non-residual Cd-complexes in sediments may contribute to bioavailable Cd flux in a system. An attempt was also made to establish a linkage between geochemical fractionation of Cd and its bioavailability in the native oyster (*Crassostrea* sp.) and identify the factors that control Cd speciation and its bioavailability in a tropical mangrove system of India.

2. Materials and methods:

2.1 Study area

The sampling site was located in Zuari mangrove estuarine region of Goa (a small coastal state of India, having an area of 3702 sq. kms). Agassaim-Zuar (sampling site) is located at latitude 15°24'54.24 N and longitude 73°54'42.15 E (**Fig. 1**). Monthly sampling was conducted during August to December 2014. Sediment samples were collected meticulously from a fixed station to understand the impact of different environmental variables on Cd bioavailability in the system. In order to understand the impact of river discharge and land run off, sediment sampling was done during monsoon (July to September) and post-monsoon period (October to December). Undisturbed 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. Native oysters *Crassostrea* sp were also collected subsequently from the same station. pH of the overlying water sample was determined with the help of a pH meter (Model-pHep HI96107). Dissolved oxygen of the water sample was estimated by Winkler's Iodometric Method (Trivedy and Goel 1986). Salinity of the sample was estimated with the help of digital ERMA Salinity Refractometer (Model RHS-10 ATC). The sediment sample (from each station) and oyster were stored at -20°C. The top two - four centimetres of sediment cores were separated and used as surface sediments. The samples were oven dried at 30°C. The composite sediments were then subsequently stored at -4° C until needed.

Around 40-60 Oyster (*Crassostrea* sp.) samples attached to the stones/ small rocks from the sampling site were collected during low tide. In the laboratory these samples were deperated and then washed to remove sand, mud attached to its shells. Soft tissues were removed using ultrapure pre washed stainless steel forceps and further rinsed with ultrapure water for complete removal of fine sediments. Samples were then freeze dried until constant weight obtained. The dried soft tissues were ground-milled and homogenized.

2.2 Sediment texture

Texture of the dry sediment sample was analyzed (after sieving with 63 μm sieve size) by Mastersizer 2000 laser diffraction particle size analyzer from Malvern Instruments. Laser diffractometry size analysis is based on the principle that particles of a given size diffract light through a given angle, the angle increasing with decreasing particle size. In this technique, a narrow beam of monochromatic light is passed through a sample cell containing an upward moving suspension of particles. The diffracted light is focused onto detectors, which sense the angular distribution of scattered light intensity from which the size distribution is computed. The detailed description has been given in literature (Ramaswamy and Rao, 2006). The data were presented as weight percentage (wt%) in this study.

2.3 Elemental and isotopic analysis

The 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. Sediment samples were decalcified using 10% suprapur HCl. About 7-10 mg of decalcified dried samples were packed in tin cups and flash combusted in the 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 $\delta^{13}C_{\text{org}}$ and $\delta^{15}N$. Reported ACA values of $\delta^{13}C_{\text{org}}$ and $\delta^{15}N$ are -25.3‰ and 4.6‰ respectively.

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

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

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

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

2.4 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).

The total metals concentrations in the tissues were determined by digesting 0.1 g of ground-milled and homogenized tissue samples with 10.0 cm^3 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 were analyzed by GFAAS.

2.5 Sequential extraction protocol

The BCR sequential extraction protocol was modified (water soluble metal complexes in the sediments were further separated in this modified protocols) (Chakraborty et al., 2015) and used to understand the distribution of Cd in the different phases of the mangrove sediments. This protocol allows us to determine the concentrations of water soluble (Fr. 1) ion-exchangeable, and carbonate/bicarbonate form of metals (Fr. 2): metals bound to Fe–Mn oxides i.e., reducible fraction of metals (Fr. 3); concentrations of metals associated with organic phases, i.e., oxidizable fraction of metals (Fr. 4) and residual metal fraction (Fr. 5) in sediment. The protocol has been vividly described in literature (Chakraborty et al 2014, 2015). Several extracting reagents were used sequentially to separate metals associated with different binding phases in the studied sediments. Concentrations of metals leached in different extracting reagents were analyzed by GFAAS. Sequential extraction experiments were performed in triplicate for all the samples to ensure repeatability of the results.

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 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., 2015). 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%.

2.7 Statistical analysis

The data presented in this study are an average of three replicates. Errors are indicated along with the average values. All the data are presented with 95.5% confidence interval. Relationship between bioaccumulated metals in oyster (*Crassostrea* sp.) and the different non-residual Cd complexes (obtained from sequential extraction study) were evaluated with Pearson's correlation coefficient and regression analysis.

2.8 Quality assurance and quality control

Quality control and assurance measures were implemented in this study. In addition to blanks and replicates, a certified reference material (PACS-3 Marine Sediment Reference Material for Trace Metals) from National Research Council Canada was used to evaluate the effectiveness of the sediment digestion procedure. The certified reference concentration of Cd in PACS-3 is 2.23 ± 0.16 mg/kg. The Recovery of Cd from the CRM was 2.2 ± 0.1 mg/kg. The analytical precision of replicate analyses of selected samples (n=5) was <3% for Cd.

The reliability of sequential extraction protocols (modified BCR) was checked by using certified reference material BCR-701 (from Community Bureau of Reference, the former reference materials programme of the European Commission, marketed by Sigma Aldrich). DORM 4 (Fish protein certified reference material for trace metals analysis from National Research Council Canada) was used as a certified reference material to check the metal recoveries from the soft tissues of the oysters. Lower limit of detection, of GFAAS (Pinaacle 900T) has been reported to be 0.002 $\mu\text{g/L}$.

3. Results and discussion

3.1 Physical and chemical composition of sediments

Variation in particle size distribution, concentrations of sedimentary organic carbon (SOC), total nitrogen (TN), trace metals concentrations in the studied sediments during the monsoon and post monsoon periods are shown in Table 1. 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 ERL (effects range low)/ERM (effects range median) analysis was performed to understand estuarine sediment quality. Metal concentrations below the ERL value are not expected to elicit adverse effects, while levels above the ERM value are likely to be very toxic. A station is rated “good” if the concentrations of all the metals are below the ERL limit. An “intermediate” rating applies if any metal exceeds an ERL limit, and a “poor” rating for a metal indicates that its ERM limit has been exceeded. The studied site was rated poor. The concentrations of Ni in all the sediments were higher than the ERM value (Long et al. 1995; MacDonald et al., 2000).

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 recommended by sediment quality guideline. 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 metal concentrations do not necessarily correspond with metal bioavailability in natural system. Thus, geochemical fractionation of Cd in the mangrove sediments was further studied by using a modified BCR sequential extraction protocol (Chakraborty et al., 2015). Table 2 shows the average concentrations of Cd reported in the coastal sediments from the different parts of India compared with the concentrations of Cd found in the studied sediments (from the coastal part of Goa, west coast of India). The concentrations of Cd in the studied sediment were found to be much lower than the concentrations recommended by the United State

Environmental Protection Agency and the Canadian Council of Ministers of the Environment for the protection of aquatic life.

3.2 C/Nmolar ratios, $\delta^{13}\text{C}_{\text{org}}$, $\delta^{15}\text{N}$ as source indicators of organic matter provenance in sediment from the coastal mangrove system

C_{org} and TN contents in the sediments were found to vary from 0.8%-3.2% and 0.08%-0.29% 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). The variation of TN against TOC showed linear relation in the studied sediments with a regression of $R^2= 0.94$. The straight line was also found to pass through the origin (**Fig. 2**). Thus, the $\text{C:N}_{\text{molar}}$ ratio reported here is strictly $\text{C}_{\text{org}}/\text{total-N}$, they can be taken to approximate $\text{C}_{\text{org}}/\text{N}_{\text{org}}$ ratio.

Molar C/N ratios of sediments are widely used for source identification of organic matter (Perdue and Koprivnjak 2007; Hélie and Hillaire-Marcel 2006). Terrestrial plants have a higher C/N ratio (>20) as compared to plankton (6-7) and bacteria (2-4) (Lee and Fuhrman, 1987; Hedges et al., 1997). Microbial degradation may also alter the C/N ratio in sediment. While the C/N ratios of higher plants get depleted on mineralisation, the C/N ratios of planktonic origin increase upon diagenesis. The C/N ratios in the studied sediments were in the range between 11 and 20 throughout the study period (Table 3). This clearly indicates that the sedimentary organic carbon was influenced by marine and terrestrial derived OM.

Stable carbon and nitrogen isotopes have been known to be excellent diagnostic tools for source identification of organic matter (Carriera et., 2002). With the exception of October, $\delta^{13}\text{C}_{\text{org}}$ ranged narrowly between -24.6‰ and -23.9‰. The $\delta^{15}\text{N}$ in those months varied from 4.2‰ to 5.5 ‰. October recorded the most depleted $\delta^{13}\text{C}_{\text{org}}$ (-26.8‰) and $\delta^{15}\text{N}$ (2.96‰) values corresponding with the highest C/N value (19.2) (Table 3). This indicates that the influence of terrestrial OM in the sedimentary organic carbon was highest in the month of October. Bardhan et al., (2015) have also reported seasonal variation of terrigenous and marine organic matter input in coastal sediments. This study indicates the inputs of both terrigenous and marine sources of OM to the sedimentary OM at the sampling site.

3.3 Geochemical fractionation of Cd in the mangrove sediments

Figure3 shows that Cd had different affinities for different binding-phases of the sediments. The concentrations of water soluble Cd complexes (Fr. 1) were found to vary from 0.8- 8.2% of the total Cd in the sediments. However, the concentrations of Cd as carbonate and bicarbonate and exchangeable complexes (Fr. 2) were high in all the studied sediments and varied from 6.6 – 38.6% of the total Cd. The high concentrations of labile Cd complexes (Fr. 1 and Fr. 2) suggest that the mobility and bioavailability of Cd in the studied sediments was probably high. Association of Cd

with Fe–Mn oxyhydroxide phases (Fr. 3) was found to be the highest in all the sediments. The concentration of Cd associated with Fe/Mn oxyhydroxide was found to vary from ~13.9–27.0% of the total Cd content in the sediments. It was found that ~4.5–17.9% of the total Cd was complexed with sedimentary organic phase (Fr. 4). Major fractions of the total Cd (ranging from 35.6 to 60.2%) were present as residual fraction (Fr. 5). The residual fraction of Cd (with an average absolute concentrations $118.3 \mu\text{g kg}^{-1}$) was expected to be non-bioavailable.

Figure 4 shows the change in distribution of Cd in different binding phases with varying Cd loading in the sediments. The total concentrations of water soluble (Fr.1); exchangeable carbonate and bicarbonate forms (Fr.2) of Cd (Fr.1+ Fr.2) was found to gradually increase (~10.0 to 39.2% of the total Cd) with the increasing total Cd concentrations in the sediments (as shown in **Figure 4a**). The similar observation was reported by (Chakraborty et al., 2012a) in the coastal sediment from the west coast of India. This study suggests that Cd preferred to undergo complexation reaction with strong binding sites (in sediments) at low Cd loading. However, this toxic metal starts to form water soluble, exchangeable and carbonate and bicarbonate forms of Cd (after saturating relatively stronger binding sites) with increasing Cd loading in the sediment. This observation suggests that mobility and bioavailability of Cd may increase with the increasing Cd loading in the study area.

All the data are presented in (Table 4). Water soluble (Fr.1); exchangeable, carbonate and bicarbonate (Fr.2) forms of Cd (a good representative of bioavailable Cd) were found to be the highest forms in the sediments. The reliability of sequential extraction protocols (modified BCR) was checked by using certified reference material BCR-701 (from Community Bureau of Reference of the European Commission, marketed by Sigma Aldrich). Metal recovery from different geochemical phases of certified reference sediment (BCR1) is shown in Table 5. The data suggest that the method was reliable.

However, association of Cd with Fe/Mn-oxyhydroxide and organic phases also gradually increased with the increasing Cd loading in the sediments (**Fig.4b**). It was found that Fe/Mn oxyhydroxide phase was one of the major binding phases for Cd in the studied sediments (~ 14.0 -27.0% of the total Cd). The third highest concentration of Cd was found to associate with organic binding phases (Fr.4) of the sediments (**Fig.4c**). It is interesting to note that there was increase in Cd concentrations in non-residual and residual (**Fig.4d**) phases of the sediments with the increasing total Cd loading. This observation suggests that Cd-complexes (associated with different phases) were probably of similar nature with low thermodynamic stability.

It is well known that metals prefer to associate with organic binding phase in sediment. However, the low concentration of Cd associated with organic binding phases of the studied sediments can be rationalized by considering the hard soft acid base (HSAB) theory. According to HSAB theory, Cd^{2+} ion is a soft acid and would prefer a softer base; S- and N-containing groups in organic binding phases to form stable complexes with higher conditional stability constant values. It has been

estimated that in humic substances, 60–90% of the acid groups are carboxylic and the remainder are phenolic (Chakraborty 2007), and ~1–10% of the total binding sites are strong binding sites that bind metals to form strong complexes. Since the concentrations of soft base ligands are very little (they are minor sites) in organic binding phases, they cannot bind all the Cd; the remaining Cd binds with major sites, such as –COOH and –OH groups, giving a low conditional stability constant (Chakraborty and Chakrabarti 2008). It has been reported that Cd forms relatively weak complexes because of the competition of stronger competing metals like Cu, Ni, Fe, Al for the binding sites of organic phases in sediments. These weak organic-Cd complexes may become bioavailable in the system along with the labile Cd complexes (Fr. 1 and Fr. 2) in the sediments. In addition to that cadmium intends to exist as various chloro-complexes rather than free Cd²⁺. Hence Salinity variations in the study region could had some control over the observed chemical associations for Cd. However, it was difficult to identify the bioavailable Cd complexes in the sediments. An attempt was made to identify the Cd complexes which were bioavailable in the mangrove sediments system and contributing in bioaccumulation of Cd in *benthic organisms* (in this study, an oyster).

3.4 Accumulation of Cd in oyster

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 (Long and Morgan 1990; Paul et al. 1992; Long et al. 1995; Neff 2002; Peijnenburg, et al., 2007). 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 area was expected to depend on Cd speciation in the sediments. The concentrations of Cd in the oyster were found to vary from 3.2 to 12.2 mg kg⁻¹ (Table 4). The concentration of Cd was low during monsoon period. However, high concentration of bioaccumulated Cd (in *Crassostrea* sp.) was found in post monsoon period. The acceptable level of Cd in Oysters for human consumption is shown in Table 6. 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. Table 7 shows the concentration of Cd in edible oysters reported in literature from the different mangrove areas of India. The reliability of digestion procedure of the soft tissues was checked by using certified reference material DORM-4 (from from National Research Council Canada). Metal recovery from DORM-4 is shown in Table 8.

3.5 Bioaccumulation of Cd at varying salinity and temperature

Filtration rate, metabolic rate, assimilation efficiency and growth rates of adult oyster (*Crassostrea* sp.) has been reported to be significantly affected by changing temperature and salinity of the

overlying water column (Atkinson et al., 2007; Lawrence and Mason 2001; Apeti et al., 2005; Lekhi et al. 2008). Bioaccumulation of Cd in *Oyster (Crassostrea sp.)* is expected to depend on salinity and temperature, pH and dissolved oxygen of the overlying water column.

Figure 5a, b, and c show that the concentrations of bioaccumulated Cd increased with the increasing salinity, temperature, and dissolved oxygen of the overlying water column.

It has been reported that *Oyster (Crassostrea madrasensis* and *C. gryphoides*) are euryhaline. *Crassostrea madrasensis* and *Crassostrea gryphoides* occur throughout the Indian coast whereas and *Crassostrea rivularis* are restricted to the north-west coastal regions (Mahadevan and Nagappan Nayar, 1987). Survival and behavior of oysters in water of varying salinities at Ratnagiri on the west coast of India has already been studied. Increasing salinity of the overlying water column increases the filtration rate, metabolic rate, assimilation efficiency of the *Crassostrea* sp. These species have preference for more saline conditions and low salinity results in a cessation of feeding (Mane, U.H., 1978). The presence of weak (bioavailable) Cd complexes (Fr.1+ Fr.2 + Fr.4) in the sediment probably increased Cd accumulation in the soft tissues of these native oysters with increasing filter feeding at higher salinity (Figure5a).

The filtration rate, metabolic rate, assimilation efficiency and growth rates of the species has been also reported to depend on temperature of the overlying water column. Figure 5b shows the accumulation of Cd increased with the increasing temperature of the overlying water column.

No information is available regarding the tolerance of oysters to changes in oxygen concentration from tropical mangrove areas. Reduced oxygen levels may be problematic if combined with reduced salinity where the valves are kept closed as much as possible limiting respiration. Figure 5c shows that the concentration of Cd increased with the increasing DO concentrations in the overlying water column.

Fig. 6 shows the variation in bioaccumulation of Cd in the oyster as a function of Cd concentration associated with different geochemical binding phases of the sediments. Bioaccumulation of Cd in *Oyster (Crassostrea sp.)* gradually increased with the increasing total Cd concentrations (**Fig.6a**). Increasing concentrations of water soluble (Fr.1); exchangeable, carbonate and bicarbonate forms (Fr.2) of Cd (Fr 1+ Fr 2) in the sediments increased Cd accumulation in the oyster(**Fig 6b**). It suggests that water soluble, exchangeable and carbonate and bicarbonate forms of Cd can be a good indicator of Cd bioavailability in a tropical coastal sediment system. However, a poor correlation was found between the concentrations of Cd associated with Fe/Mn-oxyhydroxide binding phase (Fr 3) and the accumulated Cd concentrations in the oyster. It was found that the increasing accumulation of Cd in Fe/Mn oxyhydroxide phases decreased Cd bioaccumulation in the *Oyster (Crassostrea sp.)* by decreasing Cd bioavailability (**Fig 6c**). The accumulation of Cd in oyster was found to increase (exponentially and reaching a maximum) with the increasing association of Cd with the organic phases (Fr 4) of the sediment (**Fig.6d**). This indicates that a part of Cd associated with organic

phases, were bioavailable. Speciation study showed that the Cd formed relatively weak complexes with the binding sites of organic phases in the sediments and thus, contributed to the bioavailable Cd flux in this system.

This is first attempt to establish a mechanistic linkage between Cd-speciation and its bioavailability in tropical mangrove sediments.

This study reveals that total concentration of Cd in sediment cannot be considered as an indicator of bioavailability. High bioaccumulation of Cd in edible oyster may occur even at low Cd loading in sediment. This study related geochemical fractionation of Cd in sediments to their bioavailability in the system. It was found that water soluble, exchangeable and carbonate and bicarbonate forms of Cd and Cd associated with organic phases in the sediments were bioavailable in the system. Fe/Mn-oxyhydroxy binding phase was found to be the controlling factors for Cd speciation and its bioavailability in the system. This study suggests that increasing sediment particle uptake and Cd-speciation in the sediment control Cd accumulation in the oyster.

Acknowledgements:

Authors are thankful to the Director, CSIR-NIO, Goa for his encouragement and support. DR is thankful to CSIR for providing the senior research fellowship. This work is a part of the Council of Scientific and Industrial Research (CSIR) supported GEOSINKS (PSC0106) project. This article bears NIO contribution number XXXX.

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Captions and legends for figures

Figure 1: Sampling Location at Zuari estuary, east coast of India

Figure 2: Variation of total nitrogen and total organic carbon in the sediments

Figure 3: Geochemical fractionation of Cd in mangrove sediments

Figure 4: Variation of Cd in different geochemical phases of the sediments as a function of total Cd loading

Figure 5: Variation of Cd accumulation in oyster under varying environmental condition of the overlying water column

Figure 6: Bioaccumulation of Cd in oyster as a function of total Cd concentration and concentrations of Cd in different geochemical phases of the sediments.

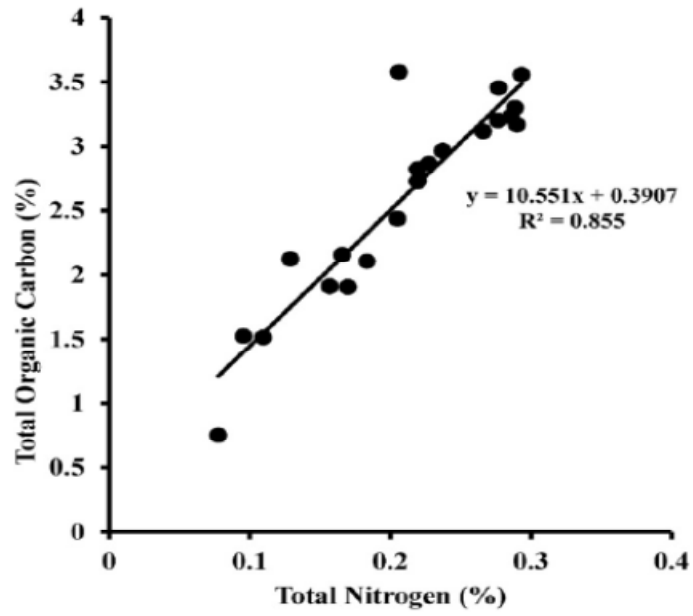


Figure 2

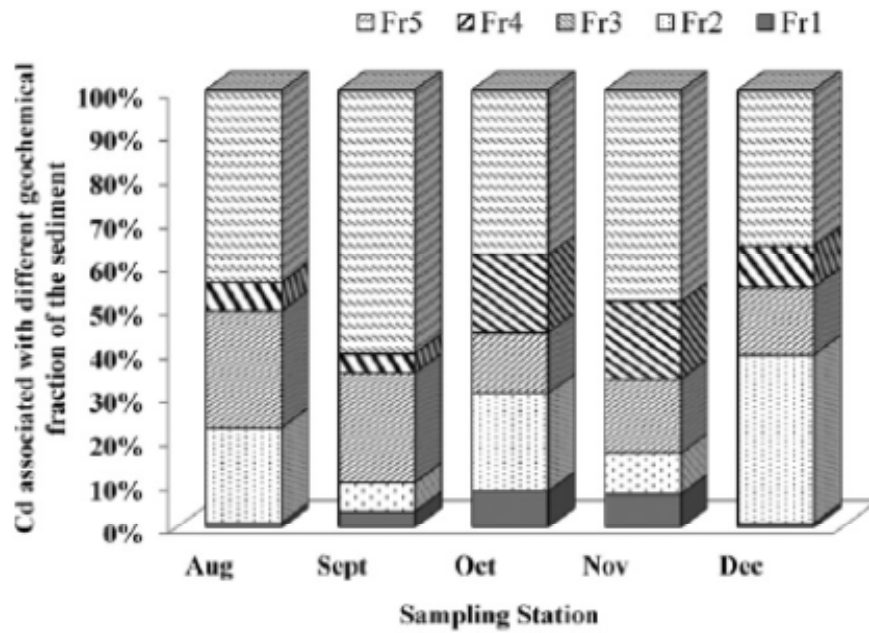


Figure 3

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

Month of sampling	Silt (%)	Clay (%)	Sand (%)	pH	Water Temperature (°C)	Salinity (PSU)	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
August	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
September	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
October	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
November	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
December	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. Cd concentration in estuarine sediments from different parts of India

Location	Cd in sediment (µg/g)	References
Pichavaram Mangrove	6.96	Ramanathan et al. (1999)
Pichavaram Mangrove (post tsunami)	34.74	Ranjan et al. (2008)
Tamiraparani estuary	11.13	Magesh et al. (2011)
Muthupettai Mangroves	10.74-48.53	Thilagavathi et al. (2011)
Pondicherry	7.31	Govindasamy et al. (1997)
Sundarbans Mangroves	1.88	Banerjee et al. (2012)
Sundarban Wetland	0.21	Chowdhury et al. (2015)
Vellar estuary	9.15	Palpandi and Kesavan (2012)
Vellar estuary	0.135	Ponnusamy et al.(2014)
Zuari estuary	0.17-0.49	This study

Table 3: 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 sediments.

Station	TOC %	TN%	C/N(molar)	$\delta^{15}\text{N}_{\text{TN}}$	$\delta^{13}\text{C}_{\text{Org}}$
August	2.4	0.20	13.9	5.2	-24.6
September	0.75	0.08	11.3	4.2	-22.4
October	2.1	0.13	19.2	3.0	-26.8
November	1.9	0.17	13.1	4.3	-24.2
December	3.1	0.30	12.7	5.5	-24.0

Table 4: Sequentially extracted non-residual and residual fraction of Cd in the mangrove sediments

Station	Fr.1 (%)	Fr.2 (%)	Fr.3 (%)	Fr.4 (%)	Fr.5 (%)	Total Cd (mg.kg ⁻¹)	Cd Oyster (mg.kg ⁻¹)
August	1.0 ± 0.1	21.7 ± 0.03	26.8 ± 0.02	6.5 ± 0.02	44.1 ± 0.1	0.17 ± 0.01	7.3 ± 0.4
September	3.6 ± 0.1	6.6 ± 0.1	25.1 ± 0.03	4.5 ± 0.02	60.2 ± 0.1	0.26 ± 0.01	3.2 ± 0.03
October	8.2 ± 0.1	22.4 ± 0.1	13.9 ± 0.02	17.9 ± 0.03	37.6 ± 0.1	0.17 ± 0.01	8.9 ± 0.05
November	7.7 ± 0.1	9.3 ± 0.1	17.1 ± 0.06	17.6 ± 0.1	48.4 ± 0.1	0.49 ± 0.06	11.8 ± 0.3
December	0.8 ± 0.1	38.6 ± 0.08	15.4 ± 0.1	9.6 ± 0.1	35.6 ± 0.1	0.32 ± 0.11	12.2 ± 0.2

Table 5 Recovery of Cd ($\mu\text{g/g}$) from different geochemical phases of the sediment with respect to BCR-701.

	BCR Certified Value	Observed Concentration	Percent recovery
Fraction 1	7.34 ± 0.35	7.21 ± 0.02	98.22
Fraction 2	3.77 ± 0.28	3.59 ± 0.04	95.22
Fraction 3	0.27 ± 0.06	0.25 ± 0.04	92.52
Fraction 4	0.13 ± 0.08	0.12 ± 0.01	92.32

Table 6. Permissible limits ($\mu\text{g/g}$) for Cd in oysters recommended by various organizations

Authorities	Cd (maximum permissible limit)
Malaysian Food act (1983)	1
European Commission	1
FDA, (2007)	4
Australia (2011)	2
New Zealand (2011)	2
Codex Alimentarius Commission (2006)	2
FSSAI (2012)	1.5
Present Study	3.2-11.8

FDA-Food and Drug Administration, FSSAI- Food Safety and Standard Authority of India

Table 7. Cd concentration in Oyster *Crassostrea* sp from different regions.

Oyster	Cd ($\mu\text{g/g}$) in tissue	Location	References
<i>Crassostrea madrasensis</i>	0.062	Vellar estuary	Kesavan et al. (2010)
<i>C. cingulata</i>	0.086	Vellar estuary	Kesavan et al. (2010)
<i>C. madrasensis</i>	0.84	Pulicat lake	Laxmi priya et al. (2011)
<i>C. madrasensis</i>	10.4	Gosthani estuary, East coast of India	Gawade et al. (2013)
<i>Crassostera</i> sp	3.2-11.8	Zuari Estuary	Present study

Table 8. Metal recovery of various trace metals ($\mu\text{g/g}$) in Oyster samples with respect to DORM-4

Element	DORM-4	Observed Concentration	Percentage recovery
Cu	15.9 ± 0.9	15.4 ± 0.6	96.8
Ni	1.36 ± 0.22	1.3 ± 0.1	95.5
Pb	0.416 ± 0.053	0.35 ± 0.02	85.3
Cd	0.306 ± 0.015	0.30 ± 0.03	98.0