

## **Operationally defined mercury (Hg) species can delineate Hg bioaccumulation in mangrove sediment systems: A case study**

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**Abstract**

This study presents the linkage between mercury (Hg) speciation in the surficial sediments from mangrove ecosystem of the Zuari Estuary, west coast of India, with Hg bioaccumulation in gastropods collected from the same area. Multiple operationally defined protocols and methods were used for Hg speciation study in the mangrove sediments. Moderately low concentration of Hg was observed in the sediments, ranging from  $37.3 \pm 1.9$  to  $79.6 \pm 4.0$   $\mu\text{g}/\text{kg}$ . Geochemical fractionation study showed that a significant part of sedimentary Hg was present within the structure of the sediment (residual fraction) and not bioavailable. Non-residual Hg was primarily associated with oxidizable (sedimentary organic matter (SOM) or sulfide) binding phase of the sediments and ranged from  $9.2 \pm 0.3$  to  $78.5 \pm 3.9$   $\mu\text{g}/\text{kg}$ . Concentration of methylmercury (MeHg) (a neurotoxin) in the sediments varied from  $1.7 \pm 0.1$  to  $4.4 \pm 0.1$   $\mu\text{g}/\text{kg}$ . L-cysteine, a suitable complexing ligand, extractable Hg concentration in the sediments ranged from  $4.3 \pm 0.1$  to  $15.9 \pm 0.3$   $\mu\text{g}/\text{kg}$ . Statistical analysis suggests that MeHg was adsorbed on Fe/Mn oxyhydroxide phases in the sediments. L-cysteine was found to extract sedimentary MeHg and thermodynamically less stable Hg-SOM complexes from the sediments. Concentration of bioaccumulated Hg in soft tissues of the gastropod, *Pirenella cingulata*, ranged from  $57.6 \pm 4.4$  to  $224.4 \pm 7.2$   $\mu\text{g}/\text{kg}$ . Positive correlations existed between the concentration of bioaccumulated Hg in the gastropods and the concentrations of Hg associated with the oxidizable phase, sedimentary MeHg and L-cysteine extracted Hg in the sediments. This study suggests that operationally defined Hg species can be useful in estimating bioavailable Hg to obligatory deposit feeder in tropical mangrove systems.

**Keywords:** Hg fractionation; sedimentary methylmercury; Organo-Hg compounds; Hg bioaccumulation; gastropod; mangrove sediment

## **Introduction**

Distribution, flux, bioavailability, and toxicity of metals in natural/marine system depend on their chemical speciation (Donnel et al. 1985; Prokop et al. 2003; Ansari et al. 2004; van Leeuwen et al. 2005; Benson et al. 2013, Chakraborty et al., 2010; Chakraborty 2017). Various operationally defined protocols have been widely reported for metal speciation study in natural/marine systems (Liang and Karamanos 1993; Rauret et al. 1999; Ahnstrom and Parker 2001; Bloom et al. 2003; Oyeyiola et al. 2011; Jayachandran et al., 2018). However, there is no direct evidence whether operationally defined metal species really estimate bioavailable fractions of the metal in an environment or not. In this study, different operationally defined protocols were used to speciate sedimentary Hg (one of the high priority pollutants) and its relation to Hg bioaccumulation in a tropical mangrove system.

Several operationally defined protocols have been widely used for Hg speciation study in marine/estuarine sediments (Chakraborty et al 2014a, Padalkar et al 2019). Different geochemical fractionation protocols have been extensively used to quantify distribution of Hg in different binding phases of sediments collected from diverse ecosystems(Chakraborty et al., 2014b, Chakraborty and Babu 2015; Issaro et al., 2009).In general, geochemical fractionation protocol enable us to separate and quantify (a) Hg in water soluble, carbonate/bicarbonate and exchangeable forms;(b) Hg associated with reducible binding phases of sediment; (c) Hg complexed with oxidizing phases of sediment and (d) Hg present within the structure of the sediments. Sedimentary Hg in the (a), (b) and (c) phases are called non-residual Hg and Hg in

the (d) is considered as residual Hg. A part of non-residual sedimentary Hg represents bioavailable Hg pool in sediment systems (Baldi and Bargagli 1982; John and Leventhal 1995; Adekola et al., 2010; Chakraborty et al., 2014a). However, residual Hg has been recognized as inert and non-bioavailable Hg-pool (Baldi and Bargagli 1982; John and Leventhal 1995; Adekola et al., 2010; Chakraborty et al., 2014a). Boszke et al., (2006) have assessed mobility and bioavailability of sedimentary Hg by geochemical fractionation study in sediments from the Tsunami inundated coastal zone of Thailand. They have also used fractionation study to assess mobility and bioavailability of Hg in soil from contaminated areas Warsaw, Poland (Boszke et al., 2008). They have identified that water-soluble Hg and acid-soluble Hg (exchangeable mercury) can easily enter aquatic system and accumulate in living organisms. Yu et al., (2012) and Chakraborty et al (2015) have reported that SOM controls concentration, distribution, lability of Hg in sediments. Bioavailability of Hg has also been reported to control by MeHg concentration (Ravichandran 2004; Bergquist and Blum 2007). Wang et al (1998) have reported that assimilation efficiencies (AE) for MeHg in biological system is much higher than Hg(II). MeHg displayed comparable AEs for both oxic and anoxic sediment. The uptake rate of MeHg is much higher than Hg(II) species in marine environment. There are several techniques and protocols have been widely used in literature to quantify MeHg in estuarine/marine sediments (Vazquez et al., 1997; Kehrig et al., 2003; Hammerschmidt and Fitzgerald 2004). An independent solvent extraction protocol is also widely used for sedimentary MeHg speciation (Maggi, et al. 2009). L-cysteine has been reported as an effective binding ligand for Hg in biological systems (Aschner and Aschner 1990; Giles et al. 2003; Rooney 2007). However, there are very few data available in the literature that describe operationally defined sedimentary Hg species and its relation to bioaccumulation in benthic organism in tropical estuarine systems. In this study, Hg species in sediments were quantified by following three different operationally

defined extraction protocols viz., (1) geochemical fraction study, (2) extraction of sedimentary MeHg, and (3) L-cysteine extractable sedimentary Hg. The information was used to assess bioavailable Hg concentration in mangrove sediments.

Gastropod species was chosen as a bio-indicator for estimating Hg-bioaccumulation in this study. Cardoso et al (2013) have reported that gastropod is a tolerant species to Hg. Berto et al. (2006) have reported that bioaccumulation of Hg in *Nassarius reticulatus* (*Gastropoda*, *Prosobranchia*) gradually increases with increasing sedimentary Hg concentration in the southern Venice Lagoon. Aquatic gastropod has been identified as a good bioindicator of Hg pollution in Pantanal of Poconé region (Mato Grosso, Brasil; Callil and Junk, 2001). *Pirenella cingulata* (Family *Potamididae*), a common and widely available gastropod, was chosen as a bioindicator in this study. *Pirenella cingulata* has been reported to be abundantly found in India, different parts of Southeast Asia, Vietnam (Zvonareva & Kantor, 2016), Indonesia (Reid and Ozawa, 2016). It is also a dominant species in the intertidal and sub-tidal zones of estuarine mangrove systems of Goa coast, India (Sarkar 1999). However, there is no data available that establish the linkage between Hg speciation and Hg-bioaccumulation in tropical estuarine systems. In this work, *Pirenella cingulata* was used as bio-indicator.

The objective of this study was to test the hypothesis that, Hg speciation (determined by operationally defined protocols) in sediments can be useful in estimating bioavailability and bioaccumulation in mangrove ecosystem. This study also aimed to identify the factors that control Hg fractionation, speciation and its bioavailability in surface mangrove sediments.

## **Materials and methods:**

### **Study area and sample collection**

Sediment and biological samples were simultaneously collected from intertidal mangrove region of the Zuari Estuary (Sancoale), Goa, West coast of India. Twelve sampling locations are presented in **Figure 1**. Sampling was done in two different seasons. The sampling was done in ST-1, ST-3, ST-5, ST-7, ST-9, and ST-11 during monsoon season (19<sup>th</sup> August 2015). The sediment and biological samples were also collected from ST-2, ST-4, ST-6, ST-8, ST-10, and ST-12 during post-monsoon period (04<sup>th</sup> November 2017). These sampling was purposefully done in two different seasons to check whether operationally defined Hg speciation in mangrove sediments can delineate Hg bioaccumulation in sessile organisms or not. Coordinates of the sampling locations are presented in the supporting documents (**Table 1**).

At low tide conditions, triplicate sediment cores were collected at each station by using acid cleaned PVC pipe with a diameter of 7.5 cm. The sediment cores were brought back to the laboratory and stored at a temperature of -20°C. Surface sediments (0-1 cm) of the three cores from each station were sub sectioned and composite surface sediment was made by mixing these three surface sediments. Composite sediment samples were then freeze dried, subsequently ground milled, homogenized, and stored in zip-lock bags at 4°C. pH of overlying water column in the sampling stations were determined by using a portable multiparameters meter (Eutech cyberscan PCD 650 with ECFC7252203B pH electrode, Thermo Scientific). A large number of marine gastropods (*Pirenella cingulata*) of similar size (length  $17.2 \pm 2.1$  mm and width  $4.7 \pm 0.8$  mm) were collected from the same locations during the sediment sampling. Gastropods were then depurated in natural seawater filled aquaria at room temperature for 48 hours in the laboratory. Soft tissues were then taken out by using Teflon coated forceps without breaking the shell. Soft tissues were further washed homogenized, freeze dried and preserved in zip-lock bags at -4°C.

**Texture analysis**

A particle size analyzer (LPSA, Malvern Mastersizer 2000) was used to determine the grain size distribution in the studied sediments. Frozen sediment samples were dried at 50°C in a hot air oven and wet sieved through <62 µm to separate sand fractions from the bulk sediment. Silt and clay fraction were quantitatively determined by using LPSA. The detail description of the procedure is presented in the literature (Chakraborty et al., 2015; Ramaswamy and Rao, 2006). Standard particles of 0.2-62 µm sizes (viz., Duke's latex and glass sphere standards) were used to calibrate the instrument. Each sample was analyzed three times and the average value was considered.

**TOC/TIC/TN analysis**

Concentrations of total carbon (TC), and total nitrogen (TN) in the sediment were determined by using Flash 2000 CHN-Elemental Analyzer (Thermo Fisher Scientific Incorporation) with a precision of ± 5%. Soil NC from Elemental Microanalysis Ltd, UK (Cat No B2051) was used as certified reference material. Total inorganic carbon (TIC) was determined by using coulometer (UIC Coulometrics). Anhydrous calcium carbonate was used as certified reference material (99.5% pure, Merck, Germany). Sedimentary organic matter (SOM) was calculated by subtracting TIC from TC.

**Spectrophotometric characterization of SOM**

Molecular characterization of SOM was done by using UV-Visible Spectrophotometer (Merck, Spectroquant Pharo 300). SOM was extracted from the sediments by following the protocol described by He, et.al. (2016). An approximate amount of 0.5 g of powdered sediment sample was mixed with 20 mL of 0.05 N sodium hydroxide (NaOH) solution and pH of the slurry adjusted to 10. The mixture was vertically shaken at 50 rpm for 12hrs to extract SOM from the

sediments. The mixture was then centrifuged at ~3000 rpm for 10mins. SOM containing alkali extract was then separated by slow decantation and used for spectrophotometric characterization of SOM. Absorbance of SOM was measured at wavelengths ranging from 200-700 nm. Spectral absorbance at 250, 364, 465 and 665 nm were used to calculate the  $E_2/E_3$ ,  $E_4/E_6$  ratios as described in the literature (Chen et.al., 1977; Helms et.al., 2008). Accuracy and precision of the spectrophotometer was repeatedly checked by using NIST-grey glass filters SRM 1930 and Holmiumoxide Solution (Merck reference material for Spectroquant® PhotoCheck) (Travis, et al., 2005).

### **Determination of Total mercury ( $Hg_T$ ) in the sediments and biological samples**

Concentrations of Hg in sediments and biological samples were determined by using direct mercury analyzer (Tri-Cell DMA-80, Milestone, Italy). The working principle of DMA-80 is based on following steps: (a) thermal decomposition of sample, (b) catalytic conversion and removal of interfering species, (c) pre-concentration of Hg in gold by amalgamation and (d) determination of Hg by atomic absorption spectrophotometry. Oxygen was used as the carrier gas in DMA-80. MESS-3 and PACS-2 were the certified reference materials (CRM) used to check the sensitivity and recovery of the instrument. The analysis of certified Hg concentrations in the MESS-3 and PACS-2 were found to be  $91.0 \pm 9.0$  and  $3020.0 \pm 100$   $\mu\text{g}/\text{kg}$ , respectively. DORM-4 (fish protein, a CRM used for trace metal analysis, from National Research Council Canada) was used to check Hg recoveries from the soft tissues of the gastropod. The certified concentration of Hg in DORM-4 was  $412.0 \pm 36.0$   $\mu\text{g}/\text{kg}$ . Total recovery percentage of Hg from the CRM's were more than 99%. The data are expressed as average  $\pm$  standard deviation ( $n=3$ ) for Hg. Detection limit of DMA-80 is 0.02 ng of Hg.

### **Sequential extraction protocol for geochemical fractionation of sedimentary Hg**



The sequential extraction protocol suggested by the European Community Bureau of Reference (Rauret et al. 1999) was applied to quantify Hg distribution in different binding phases of the studied sediments. The sequential extraction protocol helped to estimate the concentrations of (1) water soluble and carbonate/bicarbonate form of Hg (Fr1); (2) Hg associated with Fe-Mn oxyhydroxides (Fr2), (3) Hg associated with organic matter and sulfide (Fr3) and (4) Hg within the structure of the sediment (Fr4). The detail description of the protocol is presented as supporting material **Figure 1SM**. The non-residual Hg fractions (Fr1+ Fr2+ Fr3) are grossly considered as labile Hg complexes in sediments. It is important to note that the total sedimentary Hg concentration and the sum of the concentration of Hg associated with different binding phases were compared to evaluate the quality of our data. BCR-701 was used as a CRM to assess the accuracy of extraction protocol. Many other protocols has also been used for sedimentary Hg fractionation in the literature (Bloom et al. 2003; Lechler et al. 1997; Renneberg and Dudas, 2001; Wallschlager et al. 1996). However, the BCR protocol had been successfully used in our previous studies and therefore, further applied in this study.

#### **Determination of sedimentary methylmercury (MeHg)**

The protocol, used for methylmercury (MeHg) extraction from sediment in this study, has been vividly described in the literature (Chakraborty et al., 2016, Maggi et al., 2009). The flow chart of the protocol is presented as supporting material **Figure 2SM**. The certified reference material of MeHg from Sigma-Aldrich (SQC1238) was used to check the sensitivity of the extraction protocol. The certified value of MeHg was  $10.00 \pm 0.35$   $\mu\text{g}/\text{kg}$ . The recovery of MeHg from the CRM was more than 97%.

#### **Determination of L- cysteine extractable sedimentary Hg**

L-cysteine, an essential amino acid, present in different types of organisms, in natural and marine systems, has been used as a suitable complexing ligand for Hg (Emteborg et al., 1996; Maggi et

al., 2009; Schaefer and Morel 2009; Ndu et.al. 2012) in natural system. In this study, L-cysteine was used as an extractant to release leachable Hg (i.e. thermodynamically weak Hg complexes) from the sediments. This extraction was done to mimic the ligand exchange reaction which probably occurs in biological systems. The detail description of the extraction protocol is presented as supporting material **Figure 3SM**.

### **Quality Control/Quality Assurance (QA/QC)**

USEPA (QA/QC) protocols (Barron et al., 1995) were closely followed during analyzing sediment and biological samples, running triplicate samples and blanks. The direct mercury analysis follows USEPA Method 7473.

### **Results And Discussion:**

Variation in pH (ranging from  $7.30 \pm 0.05$  to  $8.51 \pm 0.05$ ) and salinity (ranging from 13.8 to 30.8 PSU) of overlying water in the sediment sampling stations represents the two sampling seasons (Table 1). Freshwater dominated monsoon was reflected by the low pH and low salinity, and post-monsoon season represented by high pH and high salinity. Sediments samples were dominated by finer particles (>95%) at all the twelve stations in both the seasons. Concentration of SOM varied from 2.0 to 4.1% (wt. %). Total sedimentary nitrogen (TN) concentration ranged from 0.14 to 0.22 (wt. %). A positive correlation ( $r^2=0.6$ ,  $p < 0.05$ ,  $n=12$ ) between the SOM and TN (as shown in supporting material **Figure SM4**) suggests that sedimentary TN were primarily associated with the sedimentary organic matter. C/N ratio of the SOM varied from 14.7 to 26.6,

indicating of their terrestrial origin and non-labile nature (Chakraborty et al., 2015; Chakraborty et al., 2016) during the sampling times.

UV-Visible spectrophotometer was used to qualitatively characterize the SOM. Spectral slope coefficient of the SOM between 275 nm and 295 nm ( $S_{275-295}$ ) and ratio of the spectral slope coefficients ( $S_R = S_{275-295} / S_{350-400}$ ) provided qualitative information about molecular weight (MW) of the SOM in the studied sediments.

**Table 1** show that  $S_{275-295}$  and  $S_R$  of the SOM varied from 0.008-0.041 and 0.9-4.1 respectively.

Both  $S_{275-295}$  and  $S_R$  values are inversely related with the MW of the SOM. The MW of the SOM was found to depend on the pH and salinity of the overlying bottom water (**Figure SM5**).

**Figure SM5** shows that  $S_R$  value of the SOM exponentially decreased with increasing pH and salinity of the overlying water column and indicates increase in MW of SOM with pH and salinity. However, it is difficult to correlate the MW of SOM with pH and salinity as there are many other factors (such as supply of terrestrial organic matter, land runoff, bacterial activity, and local hydrodynamics) can convolute the system. It is just suggested that the MW of SOM changed in two different sampling seasons.

Sedimentary Hg concentrations varied from  $37.3 \pm 1.9$  to  $79.6 \pm 4.0$   $\mu\text{g}/\text{kg}$ . Based on the sediment quality guidelines developed for the National Status and Trends Program, this sediment samples can be classified as uncontaminated sediment with respect to total Hg concentration and safe for environment and human health (NOAA, 1999).

Positive linear correlation ( $r^2 = 0.5$ ,  $p < 0.05$ ,  $n = 12$ ) existed between the total Hg and SOM concentrations (Figure SM 6) in the sediments at two different seasons. This observation indicates that SOM in the sediments probably acted as a host for Hg. It has been reported in the literature that SOM act as a host for Hg in estuarine and mangrove sediments (Chakraborty et al.,

2014a). However, no significant relationship was observed between varying C/N ratio (ranging from 14.7 to 26.6) of SOM and total Hg concentration ( $r^2 = 0.0$ ,  $p > 0.05$ ,  $n = 12$ ) in the sediments. This observation suggests that variation in non-lability (represented by changing C/N ratio) did not have any impact in hosting Hg. In order to understand distribution, fate and bioavailability of sedimentary Hg, three independent operationally defined extraction protocols were carried out in the studied sediments.

### **Geochemical fractionation of Hg in mangrove sediments**

**Table 2** shows that Hg had different affinity towards different binding phases of the sediments. Concentration of water soluble, exchangeable, carbonate and bicarbonate forms (Fr1) of Hg was not detectable ( $< \text{LOD}$  of DMA-80) in the sediments. Hg concentration in reducible phase (i.e. with the Fe/Mn-oxyhydroxy phase, Fr2) of the sediments varied from very low (not detectable) to  $27.3 \pm 1.4 \mu\text{g/kg}$  ( $\sim 0\text{-}27\%$  of the total sedimentary Hg concentration). Concentrations of Hg associated with oxidizable phase (i.e. with SOM and sulphide binding phases in the sediments, Fr3) of the sediment ranged from  $9.2 \pm 0.3$  to  $78.5 \pm 3.9 \mu\text{g/kg}$  ( $\sim 9.2\%$  to  $75.6\%$  of the total Hg content in the sediments). A major part of the total sedimentary Hg ( $17.9\text{-}84.7\%$  of the total Hg) was present within the structure (residual fraction, Fr4) of the sediments. Concentration of Hg in the residual fraction of the sediments is considered as inert and non-bioavailable Hg. Bar diagram of Hg distribution in different binding phases of the sediments is presented in **Figure 2a**.

A negative correlation ( $r^2 = 0.6$ ,  $p < 0.05$ ,  $n = 12$ ) was observed between the concentration of Hg associated with the oxidizable phase and total sedimentary Hg concentration in the sediments (**Figure 2b**). This negative relation can be explained by considering the complexation and reduction reactions of Hg with SOM in the sediments. Complexation (i.e., association of Hg with

oxidizable phase) probably predominated over Hg reduction reaction at low Hg loading in the sediments but reduction processes gradually increased with increasing Hg loading in the sediment. It has been reported in the literature that Hg-dissolved organic carbon (DOC) complexation predominate at lower Hg loading but reduction reaction predominates at higher Hg concentration (Chakraborty et al., 2016,). Phenolic-OH and -COOH groups in DOC have been reported to interact with Hg(II) and favor photoreduction of Hg(II) (Fantozzi et al., 2007). The geochemical fractionation study suggests that increasing Hg concentrations in the sediments decreased Hg association with SOM and probably increased Hg reduction process and Hg concentration in the reducible phase of the sediments (**Figure 2b**). However, weak correlation was observed between the Hg loading and Hg association with the reducible phase. Geochemical fractionation data of Hg suggests that organomercury compound was predominating non-residual Hg in the sediments. Therefore, further operationally defined protocol was used to estimate sedimentary MeHg concentration.

### **Methylmercury (MeHg) in mangrove sediments**

The concentrations of MeHg in the studied sediment varied from  $1.7 \pm 0.1$  to  $4.4 \pm 0.1$   $\mu\text{g}/\text{kg}$ . **Figure 3a** shows that a negative correlation existed between the concentrations of MeHg and SOM in the sediments ( $r^2 = 0.69$ ,  $p < 0.05$ ,  $n = 12$ ). This observation can be justified by considering the C/N ratio of the SOM. It has been reported that labile SOM (C/N = 4-10) increases MeHg formation in marine sediments (Chakraborty et al. 2015; Schartup et al. 2015). The presence of inert SOM (with C/N > 11) in the studied sediment probably increased reduction/complexation of Hg(II) and decreased MeHg formation. C/N ratio of the SOM in the studied sediments indicated their terrestrial origin and non-labile nature and, therefore, didn't promote MeHg formation in the system.

**Figure 3b** also shows that negative correlation existed between total sedimentary Hg concentrations and MeHg concentrations ( $r^2= 0.5$ ,  $p <0.05$ ,  $n=12$ ) in the sediments. Both these observations indicate that SOM plays important role in complexing Hg and controlling MeHg formation in the sediment system. It is proposed that Hg reduction (at higher Hg loading in the sediment) probably decreased MeHg formation in presence of non-labile SOM (Chakraborty et al., 2015; 2016; Vudamala et al., 2017). Thermodynamically weak Hg-sediment complexes can be useful in providing better understanding fate, mobility and bioavailability of Hg in the sediments. Thus, another operationally define protocol used to estimate loosely bound Hg in the sediments.

#### **Extraction of sedimentary Hg by L-cysteine**

L-cysteine was used as a suitable competing ligand (with respect to the other natural ligands) to leach out thermodynamically less stable Hg complexes (with respect to the stability of Hg-L-cysteine complexes) to estimate an approximate amount of bioavailable Hg in the sediments. The concentration of L-cysteine varied from 0.25% to 5% solution. The concentration of extracted Hg was found to increase with increasing L-cysteine concentration in the solution. The concentration of extracted Hg was found to increase with increasing L-cysteine concentration in the solution. The highest concentration of L-cysteine (5%) was used in this study. However, further experiments are required to quantify optimized L-cysteine concentration to extract bioavailable Hg (for a specific indicator species) from estuarine systems. The extracted Hg concentrations, in the sediments varied from  $4.3 \pm 0.1$  to  $15.9 \pm 0.3$   $\mu\text{g}/\text{kg}$  as shown in Table 2.

Combination of speciation data obtained from the above three different operationally defined protocols suggests that Hg formed strong complexes with SOM at low Hg loading but reduction predominated at higher Hg loading. There was a positive relationship existed between the Hg associated with the reducible fractions ( $r^2=0.6$ ,  $p<0.05$ ,  $n=9$ ) and the MeHg concentrations in the sediments (Figure 4a). This observation indicates that a fraction of MeHg was probably strongly adsorbed on the Fe/Mn oxyhydroxide phases in the sediments. It has been reported in the literature that Hg and MeHg strongly adsorb onto iron oxyhydroxide over the pH range of 3.2–7.1 (Rytuba 2000). It has been reported that iron oxide exhibits a strong adsorption and co-precipitation reaction with MeHg (Feyte et al., 2010). Concentration of L-cysteine extractable Hg showed a positive relationship with MeHg concentration ( $r^2=0.6$ ,  $p<0.05$ ,  $n=12$ ) in the sediments (Figure 4b). This observation suggests that L-cysteine extracted sedimentary MeHg from the sediment. It is interesting to note that concentration of L-cysteine extractable Hg was higher than MeHg concentrations in the sediments. This observation suggests that L-cysteine extracted not only MeHg from the sediments but also thermodynamically less stable Hg-SOM complexes (with respect to the stability of Hg-L-cysteine complexes) from the sediments. The operationally defined Hg-speciation data were further correlated with bioaccumulated Hg in the mangrove system.

### **Bioaccumulated Hg in gastropods (*Pirenella cingulata*) and its relationship with operationally defined sedimentary Hg species**

Concentrations of bio-accumulated Hg in soft tissues of the gastropods (*Pirenella cingulata*) varied from  $57.6\pm 4.4$  to  $224.4\pm 7.2$   $\mu\text{g}/\text{kg}$ . Variation in concentration of bioaccumulated Hg in the gastropods is presented in Table 2. Bioaccumulated Hg concentration in this region was very

similar to the bioaccumulated Hg concentrations reported from different unpolluted regions of the world (57.6–224.4  $\mu\text{g}/\text{kg}$ ) (Levasseur 1977; Metcalfe-Smith et al., 1996).

A weak correlation was found between the total sedimentary Hg concentration and the bioaccumulated Hg in the *Pirenella cingulata* species (**Table 2**). Weak relationship between total sedimentary Hg concentrations and bioaccumulated Hg concentration, as found in this study, suggests that total sedimentary Hg concentration did not provide any information about bioavailability in the sediment systems. However, positive correlation between total sedimentary Hg concentrations and bioaccumulated Hg in the benthic organisms has been reported in the literature (Hornung et al., 1981). Berto et al. (2006) have reported that a positive correlation between the total Hg concentration in sediments and bioaccumulated Hg in the gastropod (*N. reticulatus*) from the southern Venice Lagoon. Taylor et al., (2012) have also observed an increase in Hg-bioaccumulation with increasing total sedimentary Hg concentration in a southern New England estuary. Further, speciation parameters of sedimentary Hg were correlated with the bioaccumulated Hg concentration in the sediment system. **Figure 5a** shows that there was a positive correlation ( $r^2=0.5$ ,  $p < 0.05$ ,  $n=11$ ) existed between the fraction of total sedimentary Hg associated with the oxidizable phase and the concentration of bio-accumulated Hg. This study suggests that a fraction of Hg associated with oxidizable phase in the sediments were probably responsible for bioaccumulation of Hg in soft tissues of the gastropods.

**Figure 5b** shows that bioaccumulation of Hg in the gastropods positively correlated ( $r^2=0.64$ ,  $p < 0.05$ ,  $n=12$ ) with the fraction of MeHg (with respect to total sedimentary Hg concentration) present in the sediments. Increasing concentration of sedimentary MeHg (one of the highly toxic and bioavailable forms of Hg) increased bioaccumulation of Hg in the gastropods. A similar relationship has been reported in the literature (Desy et al., 2000). **Figure 5c** also shows that a



positive correlation ( $r^2=0.7$ ,  $p < 0.05$ ,  $n=12$ ) existed between the fraction of total Hg extracted by L-cysteine and the bioaccumulated Hg in the mangrove system. This observation suggest that concentrations of Hg associated with oxidizable phase, sedimentary MeHg and L-cysteine extractable Hg (from mangrove sediment) can be a good representative of bioavailable Hg in mangrove sediment systems.

Overall, this study suggests that Hg associated with SOM, sedimentary MeHg and L-cysteine extractable Hg in sediments were bioavailable for obligatory deposit feeder in the tropical mangrove systems in different seasons. This is a first study that established the linkage between sedimentary Hg speciation (by applying operationally defined protocols) and its bioaccumulation in sessile organisms in a tropical mangrove system. This study establishes a linkage between Hg speciation (in sediment) and Hg bioaccumulation by using only one indicator species (*Pirenella cingulate*) in a mangrove system. However, it is not claimed that this biological species can be used as an indicator to reflect the status or predicts the condition(s) (with respect to Hg) of mangrove environments. It is important to note that establishing a relationship between sedimentary Hg speciation with bioaccumulation in a single population rarely reflects the complexity of an environment. Therefore, extensive further research is needed to prove whether operationally defined Hg speciation in sediments can be holistically useful in delineating Hg bioaccumulation in different biological species from tropical estuarine systems or not.

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### **Captions and legends of Figures**

[Figure 1:](#) Map of sampling location in the estuarine mangrove system of the Zuari River.

[Figure 2:](#) (a) Distribution of Hg (%) in different binding phases of the studied sediments. (b) Variation in the percentage of Hg associated with oxidizable phase with respect to total sedimentary mercury concentration ( $\mu\text{g}/\text{kg}$ )

[Figure 3:](#) Variation in MeHg concentration ( $\mu\text{g}/\text{kg}$ ) as a function of varying a) SOM (%) and b)  $\text{Hg}_T$  ( $\mu\text{g}/\text{kg}$ ) in the studied mangrove sediments.

[Figure 4:](#) a) Variation in concentration of reducible Hg ( $\mu\text{g}/\text{kg}$ ) fraction in the sediment with varying sedimentary MeHg concentration (with respect to the  $\text{Hg}_T$ ) and b) Variation of sedimentary MeHg concentration with L-cysteine extractable Hg of the sediment.

[Figure 5:](#) Variation in bioaccumulated Hg ( $\mu\text{g}/\text{kg}$ ) concentrations in gastropod with varying a) Percentage of Hg associated within the organic fraction of the sediment b) percentage of

sedimentary MeHg (with respect to the total Hg) and (c) concentration of Hg leached by 5% solution of L-Cysteine.