

Speciation of Metals and Their Bioaccumulation by Edible Bivalves (*Cassosstrea* Spp. And *Polymesoda* Spp.) in the Aquatic Bodies of Goa, West Coast of India

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Research Article

Keywords: Pollution, Metal Species, Bivalves, Accumulation, Toxicity

Posted Date: June 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-490553/v1>

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Abstract

Grain size, total organic carbon, total metals and their speciation was investigated in sediments from lower Chapora Estuary, middle Zuari Estuary and Moira River in Goa, India. Study aims to understand metal bioavailability and bioaccumulation in two edible bivalves (*Cassosstrea* spp. and *Polymesoda* spp.) in three water bodies of different lengths, catchment areas, hydrodynamics and anthropogenic stress. Hydrodynamics regulated coarser sediment transport and deposition at Chapora and Moira stations, while clay particles deposition at the Zuari Estuary. Considerable difference in total metal level (Mn and/or Zn) from November to December was attributed to their additional input and variation in physicochemical processes. Contamination factor revealed moderate contamination of Zn at Chapora (November), while Zn, Cu and Co were moderately contaminated at Zuari. Presence of metals in considerable or significant amount in bioavailable fractions indicated their bioavailability. Variation in metal accumulation in gills, adductor muscle and digestive gland at three stations and between months was the result of varying hydrodynamics, trophic levels, feeding habits and pollution gradients of metal causes. Interlinkage of metals in water, sediments and bivalves revealed discrepancies in accumulation of metal type. This might be attributed to differential response of species to varying physical and biological factors at study sites. Bivalves showed a selective preference for Zn at three stations highlighting its importance in biological activities. Moreover, metals in bivalves at one or more stations exceeded the standard permissible limit and thus, indicated their toxicity to bivalves and non-suitability for human consumption according to marine pollution index.

Introduction

The rivers/estuaries in Goa over the last two decades are exclusively contaminated with metals as a result of extensive mineral ore mining, industrialization and population expansion (Gadkar et al. 2019). Metal as a pollutant in rivers/estuaries is often introduced through natural rocks weathering and anthropogenic actions such as mining, industries, agricultural waste and house-hold sewage (Nasnodkar and Nayak 2017). Upon reaching the estuarine environment, metals precipitate on to suspended sediments under changing environment. Later, gets deposited in mudflats as being subsequently extracted from the water column (Silva Filho et al. 2009). However, they do not remain permanently adsorbed on the mudflat sediment particles as they are susceptible to frequent changes in pH, salinity, Eh, ionic composition, etc. (Huang et al. 2012). The variation in these physicochemical properties of water can destabilize metals present on sediment surface and causes metal mobilization to water. The metal in the aquatic bodies is present in both mobile and immobile states (Nasnodkar and Nayak 2019). It is the mobile form of the metal that get desorbed from mudflat sediments to water (Sundaray et al. 2011) and is bioavailable to the associated marine biota (Vangronveld and Cunnihgham 1998). Therefore, the determination of different forms of metal viz., mobile (bioavailable) and immobile (residual) is an important criterion to understand the actual concentration of a metal that might cause a potential risk to biota.

Bivalves are often used as a proxy to understand metal toxicity as they possess the ability to accumulate metals from the ambient environment (Martince et al. 1984). The metal bioaccumulation in bivalves often depends on biological and geochemical factors (Boening 1999). Age, size and sex of the species, their geno-pheno-type, feeding mechanism and generative state constitute major biological factors whereas, the geochemical factors include sediment grain size variations (Zodape et al. 2011; Kumar and Weerasooriyagedara 2018), sediment composition, salinity, temperature, pH, Eh, their seasonal fluctuations and different geographical sampling locations. Some of the metals are essential (Fe, Mn, Zn, Cu and Co) to bivalves as they serve major applications in biological activities (Jayaprakash et al. 2015). However, these essential metals can become toxic to bivalves when their level exceeds the threshold concentration (Merciai et al. 2014). The bioaccumulation of metal beyond the threshold limit can directly distress the bivalve behavior and even deliver metal toxicity to humans through food web structure (Burger 2006). Most of the bivalves have rich nutritional value and are consumed on a large scale, particularly by a portion of the human population residing in the coastal regions. The consumption of bivalves contaminated with metals can thus cause serious implications to humans (Sorenson 1991; Turkmen et al. 2005). Considering the environmental risk of metals owing to their long-lasting nature, tendency to bioaccumulate and biomagnify (Uysal et al. 2009, Nasnodkar and Nayak 2018), numerous scientific studies in aquatic regions were undertaken on a global level to classify metal partitioning, transformation and bioaccumulation in marine biota and their various body parts (Wang and Liu 2003; Zhang et al. 2008; Dias and Nayak 2016). These studies facilitated understanding the behavior of metals concerning the geochemical processes, speciation of metals, their retention in the tissues of the marine organisms and their toxic effects.

The current study deals with the understanding of bioaccumulation of essential (Fe, Mn, Zn, Cu and Co) metals in edible bivalves (*Cassosstrea* spp. and *Polymesoda* spp.) from different aquatic bodies subjected to varying natural and anthropogenic stress. Factors viz., length of the river/estuary, catchment area, energy variations and anthropogenic activities do play a central role in the geochemical processes regulating metals deposition in sediments. In turn, such factors influence metal mobilization and bioaccumulation in bivalves that makes this investigation vital. The study will help to generate baseline data on the bioaccumulation of metals in two edible bivalves with respect to bioavailability.

Study area

The study area included three mudflat sites located at lower Chapora Estuary, middle Zuari Estuary and Moira River in Goa, India (Fig. 1). The catchment area of these three rivers is a part of the Dharwar Craton and is covered largely by the metamorphic rocks of the Archean-Proterozoic age (Nigam et al. 2005). In some areas, a thick layer of laterite covers the Deccan Traps. The region experiences a humid tropical climate with annual average precipitation of around 300–350 cm. During the monsoon, these rivers contain a high concentration of the total suspended matter (Nayak 1993). The Chapora River originates at the Ramghat in the neighbouring state of Maharashtra, enters Goa and flows into the Arabian Sea. It has a length of approximately 21 km (Bhutia 2016) and a catchment area of 255 km² (River Rejuvenation Action Plan 2019). It receives metal waste generated through agricultural, industrial and domestic activities. On the other hand, length of the Zuari River is approximately 70 km and has a catchment area of 550 km² (Rao et al. 2011). River is recipient of mining waste generated from ten major mines proficient of producing tons of reject (Dessai and Nayak 2009). Though mining is currently at a halt, the open cast mining (carried out in past) in the catchment area of the Zuari River is likely to release metal waste. Moira River has a length of 31 km and has around 190 km² of the catchment area and is the tributary of the Mandovi River (DPR-Mapusa-Moide River 2019). It is largely free from mining and industrial input. However, agricultural and domestic waste input does contribute to metals in the Moira River.

Methodology

Sediment, water and biota sampling

Three short mudflat sediment cores of 10 cm length were collected in November and December 2015 using the PVC corer (Fig. 1). The sampling sites include mouth of the Chapora Estuary (C), middle portion of the Zuari Estuary (Z) and Moira River (M). Sub-sampling of the sediment cores was done at 2-cm intervals and packed in clean polythene bags. They were transferred to the laboratory in an icebox. At first, sediments were stowed at 4°C. Later, oven-dried (60°C) to remove the moisture. The water adjacent to the sediment was collected and stored in acid-washed polyethylene bottles (pH fixed at < 4.0 with 1 N HCl).

The bivalves *Cassosstrea* spp. (Chapora and Zuari stations) and *Polymesoda* spp. (Moira station) samples were collected from the sediments. Biota samples were stored in an icebox during transportation to the laboratory and thereafter removed mud particles with the Milli-Q water prior to chemical analysis.

Laboratory analysis

Grain size was evaluated from the oven-dried non-powdered sediment sub-samples following the wet sieving and pipette method (Folk 1974). The oven-dried powdered sediment sub-samples were subjected to chemical analyses to determine Total Organic Carbon (TOC), total metals and speciation. The Walkley-Black method was followed to determine TOC concentration (Gaudette et al. 1974). For total metal analysis, sediment sub-samples were digested with acids (HF, HClO₄ and HNO₃). The metal species in sediments were studied using a revised sequential extraction (five-step) procedure (Dessai and Nayak 2009) proposed by Tessier et al. (1979). Fe, Mn, Zn, Cu and Co concentration was measured using the flame atomic absorption spectrophotometer (Thermo Scientific-SOLAAR M6 AAS model). The accuracy of the methodology adopted was evaluated by acid digesting and analyzing the certified reference sediment standard SGR-1. An average of triplicate samples was reported, and the recoveries were 94 % for Fe, 96 % for Mn and 98 % for Zn, Cu and Co.

The metals in the water samples were extracted following the protocol given by Brooks et al. (1967) and Tsukaijan and Young (1978). Samples were then analyzed using the AAS. The freeze-dried bivalve samples were identified after attaining the room temperature. Shells were opened with knife and soft tissue was extracted (Chiu et al. 2000). It was then dried in an oven at 60°C for 72 h and finely powdered (Ferreira et al. 2004). The soft tissue was digested using the technique described by Yuzerero et al. (2010) and metal (Fe, Mn, Zn, Cu and Co) concentration was measured through AAS.

Contamination status of Chapora, Zuari and Moira stations was assessed through Contamination Factor (CF).

$$CF = C_{\text{metal}}/C_{\text{background}}$$

where, C_{metal} is the metal level in sediment and $C_{\text{background}}$ is the average shale value from Turekian and Wedepohl (1961). Various classes of CF given by Pekey et al. (2004) are low contamination ($CF < 1$); moderate contamination ($1 \leq CF < 3$); considerable contamination ($3 \leq CF < 6$) and very high contamination ($CF \geq 6$).

Moreover, to understand the pollution state in the bivalves the Metal Pollution Index (MPI) was determined. MPI was calculated as follows:

$$MPI = (CF^1 \times CF^2 \dots \dots \dots CF^K)^{1/K},$$

where CF^1 , CF^2 and CF^k are the concentration of first, second and k^{th} metal, respectively. Further, the ability of bivalves to accumulate metals from sediments was studied through Biota-Sediment Accumulation Factor (BSAF) and modified BSAF established by Szefer et al. (1999) and Dias and Nayak (2016), respectively.

$BSAF = \text{Concentration of metal in bivalves} / \text{Total metal concentration in sediment.}$

While, the Modified BSAF = $\text{Concentration of metal in bivalves} / \text{Bioavailable metal concentration in sediment.}$

The BSAF and the modified-BSAF of less than 1, between 1–2 and greater than 2 suggest biota as de-concentrator, micro-concentrator and macro-concentrator of metal, respectively.

Results And Discussion

Sediment components

The range and the average of grain size and TOC in sediments for November and December are presented in Table 1.

At the Chapora station, sand was more than silt and clay concentration in sediments for both months. Also, it was dominant (more than 85 %) at the Moira station. However, in the Zuari Estuary, clay was higher than sand and silt in sediments for November and December months. The sand concentration decreased by more than 10 % in the Chapora Estuary and was compensated by an increase in silt from November to December months. In the Moira River, sand showed a slight increase and finer sediments exhibited a slight decrease in concentration from November to December months. In the Zuari Estuary, silt increased from November to December, while a slight decrease in sand and clay was observed. At all three stations, not much change in TOC was observed between the months.

The coarser sediments were predominant near the mouth of the Chapora Estuary. This might be the result of strong currents which act on the bed. It causes resuspension of fine-grained particles and are carried by waves and tides towards middle estuary (Nasnodkar and Nayak 2015). It is well construed that hydrodynamics at the mouth region owing to intense tides and waves is stronger and facilitate the retention of coarser sediments (Siraswar and Nayak 2011). Such estuarine processes seem to have deposited fine (clay) particles at middle region of the Zuari Estuary. In contrast, the sediment core collected from the Moira River was largely composed of sand particles. The presence of coarser sediments is expected at this station, as the energy associated with the river runoff is high to support the deposition of the coarser sediments. Therefore, the sediment transport and deposition at three stations was governed by hydrodynamic.

Among the three stations, the variation in sediment grain size between November and December months was higher at the Chapora station than at Zuari and Moira stations. The observed variation between the months at the Chapora station might be due to the change in energy associated with waves and tides and the disturbance at the surface sediments by the action of fishing trawlers passing via or operating at the mouth region of the Chapora Estuary.

Total metals in sediments

An average value of metals at sampling stations for the months of November and December are presented in Table 2a. A considerable variation in concentration of Mn and Zn was observed between the two months at Chapora and Moira stations. At the Zuari station, Mn level in sediments varied from November to December. The rest of the metals did not show much variation between the months at the three stations. Change in metal concentration in sediments from November to December might be due to the additional input of those metals to estuary or the effect of physicochemical processes which regulate the adsorption and mobilization of metals. The fate (speciation) of mobilized metal is influenced by conditions of the overlying water, especially hydrogen ion concentration, salinity, Eh and number of suspended solids (Li et al. 2013). Further, the metal level in sediments was related to shale value (Turekian and Wedepohl 1961). Zn in November at the Chapora station while, Zn, Cu and Co for both the months at the Zuari station surpassed the shale value (Table 2a) indicating metal input via both natural and anthropogenic sources. Further, CF revealed moderate contamination of Zn at the Chapora Station (November), while at the Zuari Station Zn, Cu and Co showed moderate contamination (Table 2b).

Total metals abundance in sediments

In order to understand the abundance of total metal concentration among three stations isocon diagrams were plotted between the sampling stations (Fig. 2). For November, total Mn, Cu and Co level was high at Zuari while Fe and Zn at Chapora. Later in December, total metals (Fe, Mn, Zn, Cu and Co) were higher in concentration at Zuari than Chapora Station. The comparison of total metals between Zuari and Moira stations for November and December revealed comparatively higher metal concentration at the Zuari Station. The Chapora Station exhibited comparatively higher level of total metals in comparison to Moira Station for November and December, with an exception of slightly higher

total Fe concentration at the Moira Station for December. Among the sampling stations, concentration of total metals was more at the Zuari Station. This is attributed to an enhancing rate of metal input through mining activities at the Zuari Station in comparison to a gradual release of metals via agricultural, industrial and domestic activities at Chapora and Moira stations.

Metal species in sediments

The metals associated with different sediment fractions were studied based on anthropogenic input and their pollution signatures established through total metal analysis and CF. An average of species of Fe, Mn, Zn, Cu and Co in sediments at Chapora, Zuari and Moira stations F1: exchangeable, F2: carbonate, F3: Fe-Mn oxide, F4: organic matter/sulfide bound and F5: residual is graphically illustrated in Fig. 3.

Fe and Cu were significantly present in the residual fraction at Chapora (November and December). Additionally, Cu was around 10 and 16 % in the organic bound fraction in November and December, respectively. In the case of the Zuari station, Fe (88.90 % in November and 91.99 % in December) and Cu (95.49 % in December) were abundant in the residual fraction. Although, Cu was highest in an environmentally dormant fraction (residual), but was also significantly high in the organic/sulphide fraction (27.42 %) in November. Fe also recorded the highest value in an environmentally dormant fraction at the Moira station (70.25 % in November and 72.37 % in December). Amongst the environmentally active fractions (bioavailable), it was significantly held in the Fe-Mn oxide fraction (22.83 % in November and 26.30 % in December) in both months.

Mn was abundantly held in its residual form for November (82.44%), while in December it reported highest value in the carbonate fraction (40.23 %) at Chapora. Also, its concentration was significant in the Fe-Mn oxide fraction (22.85 %) for December. On the other hand, at the Zuari station, its concentration was highest in the Fe-Mn oxide (47.28 % and 56.84 % in November and December, respectively) fraction and was also significantly associated with the exchangeable fraction (21.83 % in November and 12.23 % in December). In Moira, Mn was highest in residual (36.84 %) and Fe-Mn oxide (47.93 %) fractions for November and December, respectively. The exchangeable fraction recorded significant level of Mn for both the months (23.03 % in November and 15.95 % in December), in addition to Fe-Mn oxides (32.31 % for November).

The residual fraction showed highest value of Zn for both the months at the Chapora station. In November, a significant concentration of Zn (30.48 %) was reported bound to Fe-Mn oxides and was reduced to almost half a concentration by December (14.71 %). Zn was largely available in its residual form (78.54 % in November and 82.40 % in December) at the Zuari Station and was also considerably held in the Fe-Mn oxide fraction (14.61 % in November and 13.05 % in December). Although, Zn was highest in the residual fraction (46.69 % in November and 59.69 % in December) at the Moira station but, was also significantly held in Fe-Mn oxide form (28.32 % in November and 26.22 % in December).

At the Chapora (63.89 % in November and 59.74 % in December) and the Zuari (57.06 % in November and 56.95 % in December) stations, Co exhibited highest value in the residual fraction. Both the months reported considerable concentration of Co in Fe-Mn oxide (19.97 % in November and 22.26 % in December) and organic/sulphide (10.10 % in November and 10.48 % in December) bound fractions at the Chapora station. On the other hand, it was significantly held in the Fe-Mn oxide (35.36 % in November and 31.53 % in December) fraction at the Zuari station. The Fe-Mn oxide fraction showed highest of Co for November (38.99 %), while for December, the residual fraction (42.60 %) reported maximum concentration in sediments of Moira. However, it was significantly held onto Fe-Mn oxides (29.42 %) in December. Besides, Co was also considerably held in exchangeable (10.14 % in November and 14.35 % in December) and organic/sulphide bound (14.08 % in November and 13.50 % in December) fractions.

The metals viz., Fe and Cu at Chapora, Zuari and Moira stations in November and December, along with Mn (November) at Chapora, Zn (December) at Moira and, Zn and Co at Chapora and Zuari were predominantly high in the residual fraction. In general, metals associated with the residual fraction are immobile as are strongly held in the mineral lattice structure (Wang et al. 2002). The presence of metals in the environmentally dormant fraction (residual) which is relatively stable is recognized as the input derived from natural sources (Sundaray et al. 2011). Therefore, are considered non-bioavailable and have no effects on the environment (Chakraborty et al. 2015; Nasnodkar and Nayak 2017). A metal form that is capable of affecting the environment is one present as the bioavailable fraction, which includes exchangeable, carbonate, Fe-Mn oxides and organic matter/sulfides. Upon fluctuations in pH, salinity, Eh, bioturbation, etc., in estuaries, those metals are likely to mobilize from sediments to the overlying water, thereby become bioavailable and might have significant effects on the environment (Noronha and Nayak 2016). So are called the "effective fraction". In the present study as well, metals (Zn and Co in November, and Mn, Zn, Cu and Co in December at the Chapora station; Mn, Zn, Cu and Co in November and Mn, Zn and Co in December at the Zuari station; Fe, Mn, Zn and Co in both November and December at the Moira station) were considerably or significantly high in certain environmentally active (bioavailable) fractions. Thus, these metals suggested bioavailability to sediment-associated biota and are likely to harm the environment.

Among the bioavailable fractions, the Fe-Mn oxide fraction seemed to have influenced the maximum number of metals (viz., Fe, Mn, Zn and Co) adsorption on sediments at three stations for both months. The processes such as metals adsorption, flocculation, and co-precipitation with Fe-Mn oxyhydroxides perform a chief role in retention of non-residual fraction of metals in sediments (Li et al. 2016). Besides, Cu and Co in November and December (Chapora); Mn and Cu in November (Zuari); and Co in November and December (Moira) were considerably or significantly held onto organic matter/sulphides which was attributed to the complexing nature of organic matter with metals in the estuarine environment (Yu et al. 2010). The metals that are adsorbed onto Fe-Mn oxides and organic matter are able to mobilize from sediments with a change in reduction or oxidation processes (Sundaray et al. 2011). Thus, ultimately increase the bioavailability of metals to the benthic biota. Furthermore, the labile fraction (exchangeable and carbonate) holds the metals less firmly which are susceptible to alteration in ionic composition and pH. The considerable or significant concentration of metals with the exchangeable (Mn in November and December at the Zuari station; Mn and Co in November and December at the Moira station) and carbonate (Mn in December at the Chapora station, Zn in November at the Moira station) fractions indicated their possible mobilization and subsequent bioavailability.

Metal bioaccumulation

The bioaccumulation of metals by *Cassosstrea* spp. showed a difference in retention of metals in different body organs and between the November and the December months at Chapora and Zuari stations (Table 3).

Fe at the Chapora Station was highest in gills for the months November (970 ppm) and December (1860 ppm) as compared to adductor muscle and digestive gland. The concentration of Fe increased from November to December in the gills and digestive gland, while it decreased in the adductor muscle. The Fe level in the bivalve at the Zuari Station was highest in the digestive gland (450 ppm) and adductor muscle (1157 ppm) in November and December months, respectively. It increased from November to December in gills and adductor muscle, while decreased in the digestive gland.

Mn was highest in gills and digestive gland (around 22 ppm) in November at the Chapora Station. In December it was highest in the adductor muscle (37 ppm). It showed a drastic and a gentle decrease from November to December in gills and digestive gland, respectively. However, its concentration doubled from November to December in the adductor muscle. At the Zuari Station, Mn was highest in the digestive gland (26 ppm) and gills (33 ppm) for November and December, respectively. It exhibited an increase from November to December in gills and adductor muscle and showed a slight decrease in the digestive gland.

The concentration of Zn was highest in the gills for both November (790 ppm and 723 ppm at Chapora and Zuari Stations, respectively) and December (923 ppm and 1262 ppm at Chapora and Zuari Stations, respectively) months in comparison to adductor muscle and digestive gland. Gills and digestive gland showed an increase in the level of Zn, while revealed a drastic decrease from November to December at the Chapora Station. On the other hand, an increase in Zn level was observed from November to December in all the body parts at the Zuari Station. The metals viz., Cu and Co exhibited low concentration in gills, adductor muscle and digestive gland at Chapora and Zuari Stations. At both stations, Cu was highest in the gills for November (8 ppm and 5 ppm at Chapora and Zuari Stations, respectively) and December (7 ppm and 16 ppm at Chapora and Zuari Stations, respectively) months. The change in Cu level from November to December was not so significant at the Chapora Station, while a slight increase was noted in gills from November to December at the Zuari Station. The level of Co was negligible for November in all body parts and reported the highest concentration of 8 ppm in gills for December at the Chapora Station.

The bivalve *Polymesoda* spp. collected from the Moira Station showed the highest concentration of metals for November (2719 ppm, 5 ppm, 425 ppm, 18 ppm and 4 ppm of Fe, Mn, Zn, Cu and Co respectively) and December (1680 ppm, 3 ppm, 35419 ppm, 1.45 ppm and 3 ppm of Fe, Mn, Zn, Cu and Co respectively) in gills than that in adductor muscle and digestive gland. Overall, the concentration of Fe (all studied body tissues) and Zn (gills) was significantly high in the bivalve.

The concentration pattern of metals in tissues of *Cassosstrea* spp. and *Polymesoda* spp. showed discrepancies in metal accumulation at three stations. The feeding habits of species, their trophic levels and the contamination gradients of metal sources might be the reasons for such variations in metal level (Jayaprakash et al. 2015). High concentration of Fe in gills, adductor muscle and digestive gland revealed organic matter increase and metal released through the anthropogenic activities (Satheesh-Kumar and Kumar 2011). The significant Fe concentration in digestive gland of both the species at three stations indicated an enhanced rate of feeding and metabolic activity (Filipovic-Marijic and Raspor 2014). Next to Fe, concentration of Zn was considerably high in the gills of the bivalves. Zn is rapidly assimilated through food as a vital element in bivalves (Dallinger et al. 1987). Fe, Mn, Zn and Cu were higher in gills (at most stations) than adductor muscle and digestive gland. This was attributed to (i) their ion exchange from water through the chief route (gills) due to large surface area facilitating swift metal diffusion (Dhaneesh et al. 2012). (ii) Presence of a mucous layer assists quick metal accumulation (Sarkar 2018). (iii) The amount of water filtered by gills than adductor muscle and digestive gland. (iii) Free interaction with the adjacent water leading to rapid ingestion and bioaccumulation (Pringle et al. 1968).

Metals in bivalves varied at Chapora, Zuari and Moira stations. The variation in metal accumulation in bivalves at three stations might be due to the existence of different hydrodynamic conditions at these stations. The sampling station at the Chapora was close to the mouth region, while was at the middle estuarine and upper regions of Zuari and Moira, respectively. These stations experience different energy conditions and physicochemical factors governing the processes associated with the retention of metals and their bioavailability. Ultimately, regulate the ecological conditions which perhaps differed at the three sampling stations. Additionally, the differential source of metals at three stations might be another factor that regulated the metal accumulation in bivalves. For instance, the Zuari Estuary is reported to have received a significant concentration of metals through the catchment area operated ferromanganese mining activities (Noronha and Nayak 2016; Gadkar et al. 2019). On the contrary, the rest of the two water bodies receive metal input through industrial, agricultural and domestic activities.

Interrelating metal bioavailability and bioaccumulation

In order to understand the relation between metal bioavailability and total metal bioaccumulation isocon diagrams were plotted between the sampling stations (Fig. 4 and 5). In November, bioavailable Fe, Mn and Co were more at Zuari, whereas bioavailable Zn and Cu were slightly higher at Chapora. Fe and Zn in an edible biota was more at the Chapora station than the Zuari Station. Rest of the metals namely, Mn, Cu and Co were also slightly more in concentration at the Chapora station. During December, bioavailable Fe, Mn, Zn and Co were higher at the Zuari Station, while Cu was relatively more at the Chapora Station. Edible biota retained more of Fe, Zn and Co in tissues at the Chapora Station, whereas bioaccumulation of Mn and Cu was slightly higher at the Zuari Station. All metals exhibited higher bioavailability at the Zuari Station than the Moira Station for November and December. However, there was a change in metal bioaccumulation pattern between the two months. Zuari Station revealed higher bioaccumulation of Mn and Zn during November, while Mn and Cu were relative more accumulation in an edible biota during December. At Moira Station, bioaccumulation of Fe, Cu and Co was more during November, while an edible biota retained more of Fe, Zn and Co during December. The Chapora station showed enhanced bioavailability of metals in comparison to the Moira Station during November and December. As far as bioaccumulation is concerned, Mn and Zn were more during November, whereas Mn, Cu and Co were higher during December at the Chapora Station than the Moira Station. During November, an edible biota showed higher bioaccumulation of Fe, Cu and Co at the Moira Station. Further, bioaccumulation of Fe and Zn was more at the Moira Station for December.

Among the sampling stations, concentration of bioavailable metals was more at the Zuari Station. This was attributed to higher mobility of metals with respect to varying salinity and pH with higher tidal influence in Zuari Estuary compared to Chapora and Moira stations. It is interesting to understand relation between total metal bioaccumulation and bioavailable metals in sediments as it is ultimate factor for bioaccumulation in an edible biota. However, present study to a larger extent revealed non-linear relationship between bioavailable metals in sediments and total metal bioaccumulation indicating selective preference of metals by the different species of edible biota.

The sampling at November and December months showed a variation in net metal concentration in the bivalves (Table 3). The changes in pH, Eh, salinity, ionic composition are most frequent in the aquatic bodies where there is a mixing of seawater with the river water. The variations in these factors within the aquatic bodies can trigger the adsorption and desorption of metals between the sediment and the water. Such processes might have regulated the change in metal concentration between November and December months in the bivalves. Further, the study revealed different combinations of metal concerning dissolved ion (Table 4), its bioavailability (Table 5a) and net bioaccumulation (Table 3). For instance, first, the metals such as Fe (Zuari), Mn (Zuari) and Zn (Moira) exhibited a decrease in bioavailability (sediments) and dissolved concentration (water) from November to December, while registered an increase in the net bioaccumulation. Secondly, Fe (Chapora) and Co (Chapora) showed an increase in bioavailability and net bioaccumulation, although not much variation was observed between the months in the dissolved concentration. Third, Fe (Moira) and Mn (Chapora and Moira) exhibited an increase in bioavailability from November to December, while registered a decrease in net bioaccumulation and dissolved level. Fourth, Zn (Chapora) indicated an increase in dissolved concentration, while there was a decrease in its bioavailability and net bioaccumulation from November to December. Fifth, Cu (Chapora) revealed an increase in dissolved level, bioavailability and so the net bioaccumulation of metals from November to December. Sixth, Co (Moira) exhibited not much variation in dissolved level and bioavailability from November to December, whereas showed a slight decrease in the net bioaccumulation. Seventh, Co (Zuari) exhibited not much variation in dissolved level and net bioaccumulation and reported an increase in the bioavailability from November to December. Eighth, Zn (Zuari) showed an increase in dissolved level and net bioaccumulation, while indicated a decrease in its bioavailability from November to December. Ninth, Cu (Zuari) exhibited not much difference in dissolved level and showed a decrease and increase in bioavailability and net bioaccumulation from November to December, respectively. In general, the interlinkage of dissolved metal ion (water), bioavailability (sediments) and bioaccumulation (bivalves) suggested that mere metal bioavailability does not cause net bioaccumulation of metals. The physiology of the bivalves (trophic location, life span, size of the body, mode of ingestion, gender, reproducing status of species) affecting their differential response to different metals in the aquatic system might have regulated the net metal bioaccumulation (Mustafa and Guluzar 2003). The BSAF and modified BSAF construed the same (Table 5b). Although metals viz., Fe and Mn were present in the significant quantity in sediments, the bivalves showed the selective preference to Zn

and/or Cu and/or Co at the studied stations, except for Fe at the Moira station. The BSAF and the modified BSAF revealed significant preference of bivalves for Zn as the macro-concentrator for both November and December months at Chapora, Zuari and Moira stations attributed to its application in biological activities. Moreover, as per the BSAF, the bivalve species at the Moira station was also the macro-concentrator (November) and micro-concentrator (December) of Fe, and micro-concentrator of Co (November and December). According to the modified BSAF, at the Chapora Station bivalve was macro-concentrator and micro-concentrator of Cu (November and December) and Co (December) respectively, at the Zuari Station bivalve was micro-concentrator (November) and macro-concentrator (December) of Cu, while at the Moira Station bivalve species was macro-concentrator (November) and micro-concentrator (December) of Co.

Comparison of metal concentration in bivalves with the standard values

The net (sum of gills, adductor muscle and digestive gland) metal concentration in bivalves was compared with the standard metal limit in marine biota prescribed by FAO/WHO (2004), WHO (1989) and presented by Charbonneau and Nash (1993) to understand metal contamination and bivalves suitability for human consumption (Table 3). Fe, Mn and Zn (all three sampling sites), while Cu (Zuari-December) and Co (Chapora-December) in bivalves exceeded the standard limit value. Furthermore, MPI for Chapora (77.68 for November and 132.93 for December), Zuari (42.96 for November and 97.84 for December) and Moira (72.09 for November and 96.84 for December) stations was higher and thus, indicated metal contamination with toxic effects on the bivalves and their non-suitability for the human consumption.

Conclusion

The higher hydrodynamics retained coarser sediments at the lower Chapora Estuary and the Moira River, while relatively lesser hydrodynamics facilitated retention of finer sediments at middle Zuari Estuary. The total metals, especially Mn and Zn, showed variations between November and December as a result of a change in physicochemical factors and additional metal sources. Zn at Chapora (November), and Zn, Cu and Co at Zuari were moderately contaminated in sediments as indicated by CF. The considerable or significant bioavailable Zn and Co in November, and Mn, Zn, Cu and Co in December at Chapora; Mn, Zn, Cu and Co in November and Mn, Zn and Co in December at Zuari; Fe, Mn, Zn and Co in November and December at Moira revealed their possible bioavailability to the bivalves. In bivalves, representing all three stations and November-December months, the metal concentration varied in gills, adductor muscle and digestive glands which was attributed to energy conditions that regulated metal bioavailability and bioaccumulation in addition to feeding behaviour of species, their trophic location and pollution gradients of metal sources. High metal accumulation in gills than adductor muscle and digestive glands was result of ion exchange process, presence of mucous film and a huge volume of water filtration by gills. Further, the interrelation of metal concentration in water, sediments and bivalves indicated a role of physical and biological factors in metal bioaccumulation. The selective preference for Zn by bivalves suggested its significance in the biological activities and growth factors. Upon comparison with the standard permissible limit, the metals in bivalves were higher at one or more stations. Therefore, signaled their toxicity to bivalves and humans which was also construed through the marine pollution index.

Declarations

Acknowledgment

The authors wish to thank Prof. Ramola Antao for correcting the English grammar and usage of the draft of the manuscript. One of the authors (Prof. G. N. Nayak) thanks CSIR, New Delhi for awarding the CSIR Emeritus Scientist position.

Funding: Not Applicable

Conflict of interest: The authors declare that they have no conflicts of interest related to this research.

Ethics approval: Not applicable.

Consent for publication: All the authors agree with the present submission of this paper.

Availability of data and material: Raw data will be made available on request.

Code Availability: Not applicable.

Author contribution:

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Tables

Table 1: Range and average concentration of sand, silt, clay and total organic carbon.

Stations	Sand (%)		Silt (%)		Clay (%)		Total Organic Carbon (%)	
	Range	Average	Range	Average	Range	Average	Range	Average
Chapora_November	62.71 - 83.21	76.44	10.30 - 28.64	15.95	6.48 - 8.64	7.60	0.57 - 0.82	0.72
Chapora_December	40.94 - 78.66	61.10	16.85 - 55.09	34.40	3.96 - 5.04	4.49	0.40 - 1.08	0.73
Zuari_November	5.68 - 30.53	18.36	28.98 - 33.25	31.41	40.48 - 61.44	50.22	1.55 - 2.85	2.15
Zuari_December	8.60 - 38.56	15.50	12.11 - 65.96	37.21	22.56 - 56.28	47.28	1.64 - 2.94	2.56
Moira_November	90.63 - 95.43	92.98	0.44 - 4.44	2.19	3.0 - 8.92	4.81	0.28 - 0.34	0.30
Moira_December	83.81 - 94.70	88.25	2.10 - 14.1	6.49	2.08 - 8.60	5.24	0.71 - 0.51	0.36

Table 2a: The concentration of total metals in sediments.

Stations	Fe (%)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Co (ppm)
Chapora_November	1.90	518	134	32	13
Chapora_December	1.18	500	84	34	18
Zuari_November	1.83	547	110	58	23
Zuari_December	1.83	529	104	57	23
Moira_November	0.17	86	37	ND	5
Moira_December	0.19	101	19	ND	4
Average shale value	4.72	850	95	45	19
Turekian and Wedepohl (1961)					

Table 2b: Contamination factor (CF) for metals.

Stations	CF				
	Fe	Mn	Zn	Cu	Co
Chapora_November	0.40	0.60	1.41	0.71	0.68
Chapora_December	0.25	0.58	0.88	0.75	0.94
Zuari_November	0.38	0.64	1.15	1.28	1.21
Zuari_December	0.38	0.62	1.09	1.26	1.21
Moira_November	0.03	0.10	0.38	ND	0.26
Moira_December	0.04	0.11	0.20	ND	0.21

Table 3: The concentration of metals in different body parts of bivalves.

Metals (ppm)	Chapora				Zuari				Moira			
	Gills	Adductor muscle	Digestive gland	Total	Gills	Adductor muscle	Digestive gland	Total	Gills	Adductor muscle	Digestive gland	Total
Fe_Nov	970	714	640	2324	138	60	450	648	2719	400	1472	4591
Fe_Dec	1860	377	704	2942	679	1157	334	2170	1680	299	1027	3006
Mn_Nov	22	17	22	61	9	3	26	38	5	1	3	9
Mn_Dec	2	36	18	56	33	23	20	76	3	1	1	5
Zn_Nov	780	302	780	1872	723	12	16	751	425	2	22	449
Zn_Dec	923	19	808	1750	1262	38	22	1322	35419	4	0.83	35423
Cu_Nov	8	1	4	13	5	2	4	11	18	1	2	21
Cu_Dec	7	6	5	18	16	6	12	34	2	1	1	4
Co_Nov	0.27	0.12	0.45	0.84	0.02	0.20	0.48	0.72	4	0.43	1	5
Co_Dec	8	0.21	0.02	8	0.61	0.10	0.50	1	3	0.50	0.77	4

Standard metal value in marine biota

Fe = 0.0034 - 0.0107 % Charbonneau and Nash (1993)

Mn = 0.01 ppm, Zn = 40 ppm WHO (1989)

Cu = 30 ppm, Co = 0.1 – 5.0 ppm FAO/WHO (2004)

Table 4: The average concentration of metals in the water.

Stations	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Co (ppm)
Chapora_November	37.71	2.62	9.12	3.22	0.70
Chapora_December	37.55	1.27	12.62	4.36	0.19
Zuari_November	32.85	3.57	9.59	2.72	0.73
Zuari_December	16.47	0.43	13.24	1.96	0.99
Moira_November	64.77	8.10	13.2	2.84	0.6
Moira_December	11.97	1.29	2.45	2.18	1.03

Table 5a: The bioavailable concentration of metals (Sum of F1, F2, F3 and F4) in the sediments.

Stations	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Co (ppm)
Chapora_November	2883	91	46	3	5
Chapora_December	3986	244	22	6	7
Zuari_November	6670	1782	30	10	6
Zuari_December	5108	1084	28	1	9
Moira_November	801	60	20	ND	2
Moira_December	1927	83	16	ND	2

Table 5b: The biota sediment accumulation factor (BSAF) and the modified BSAF.

Stations	Fe		Mn		Zn		Cu		Co	
	BSAF	Modified BSAF	BSAF	Modified BSAF	BSAF	Modified BSAF	BSAF	Modified BSAF	BSAF	Modified BSAF
Chapora_November	0.12	0.80	0.11	0.67	13.97	40.61	0.40	4.33	0.06	0.16
Chapora_December	0.24	0.73	0.11	0.22	20.83	79.54	0.52	3.00	0.44	1.14
Zuari_November	0.03	0.09	0.06	0.02	6.82	25.03	0.18	1.10	0.03	0.12
Zuari_December	0.11	0.42	0.14	0.07	12.71	47.21	0.59	34.00	0.05	0.13
Moira_November	2.70	5.73	0.10	0.15	12.13	22.45	ND	ND	1.00	2.50
Moira_December	1.58	1.55	0.04	0.06	1864.36	2213.93	ND	ND	1.00	2.00

Figures



Figure 1

Map showing the sampling locations. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

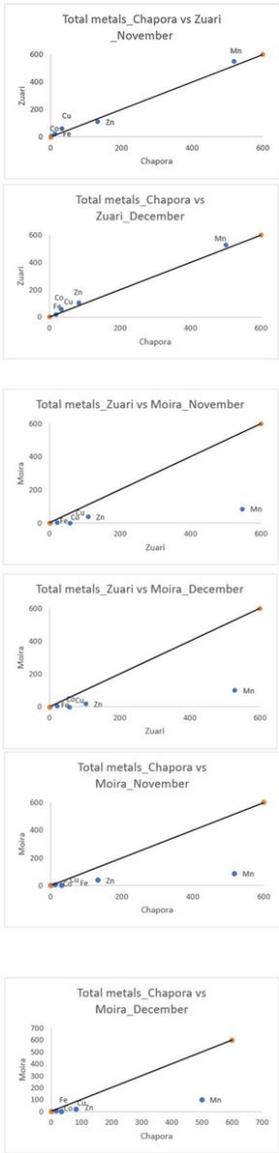


Figure 2

Isocon plots comparing total metal concentration between sampling stations.



Figure 3

Speciation of metals in exchangeable (F1), carbonate (F2), Fe-Mn oxide (F3), organic matter/sulfide bound (F4) and residual (F5) fractions in sediment cores of Chapora, Zuari and Moira stations.

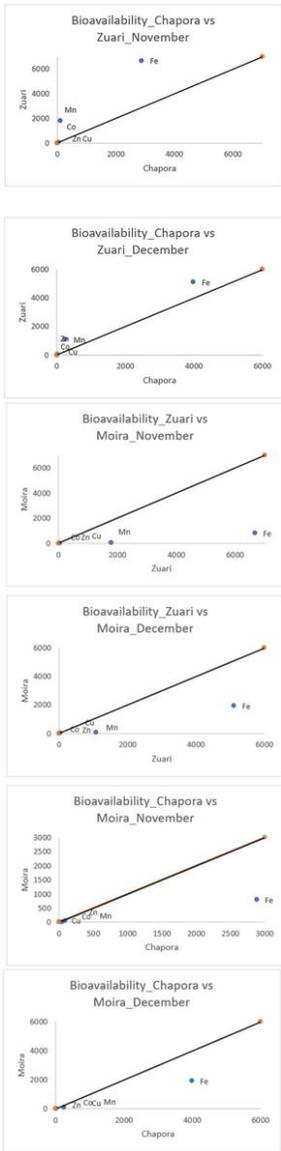


Figure 4

Isocon plots comparing bioavailable metal concentration between sampling stations.

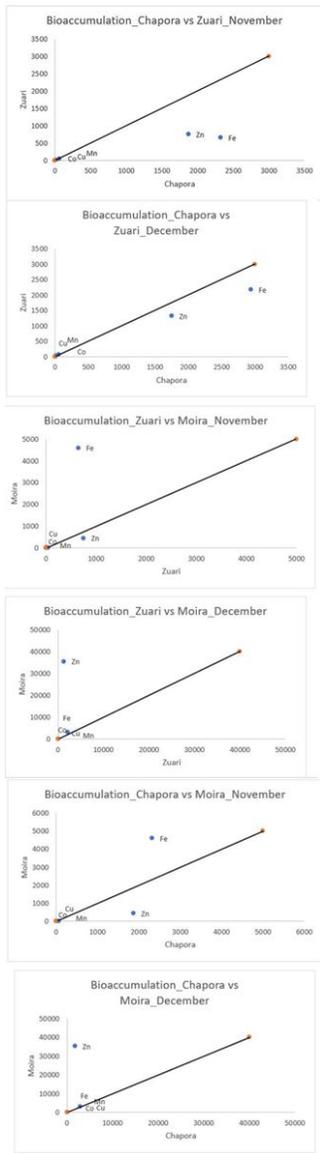


Figure 5

Isocon plots comparing total metal bioaccumulation between sampling stations.