

Effects of sedimentary heavy metals on meiobenthic community in tropical estuaries along eastern Arabian Sea

M. Nageswar Rao^{1,2}, Gaikwad. S¹, Anirudh Ram¹, U. K. Pradhan^{1*}, Sautya S¹, L. Kumbhar¹, Udayakrishnan PB¹ and V. Siddaiha²

¹CSIR-National Institute of Oceanography, Regional Centre, Mumbai-400053, India.

²Department of Organic Chemistry & Food, Drug and Water, Andhra University, Visakhapatnam- 530003, India

*Corresponding author

Abstract

The central west coast of India comprises the 720 km long coastline of Maharashtra state and houses widespread industrial zones along the Eastern Arabian Sea. Sediments from seven industrial-dominated estuaries along the central west coast were studied for metal enrichment and benthic assemblages to determine sediment quality status and ecological effects in these areas. The suite of geochemical indices highlighted the contamination of sediment in the estuaries concerning heavy metals. Positive correlations of Hg with Co, Zn, Ni, Cr, and Pb, indicated the source similarity and effect of anthropogenic activity. non-Metric Multidimensional Scaling (n- MDS) based on meiofaunal abundance showed a cleared separation of clusters through the gradient of heavy metal concentrations. The Canonical Correspondence Analysis (CCA) results with the Monte Carlo test signified those heavy metals influenced the meiobenthic community. Heavy metals (Cr, Ni, Zn, Cd, Pb, and Hg) were the main drivers shaping the meiofaunal community with a significant ($p < 0.05$) reduction in taxa richness, diversity, and evenness. Dominant meiofaunal assemblages evidence the tolerance of foraminiferans and nematodes. However, these taxa were affected by decreased abundance at impacted sites compared to other fauna. In conclusion, results demonstrated that impairment occurred in the meiofaunal community in most estuaries (except A.B. and K.K.)

Keywords: Sediment; Heavy metals; Benthos; Ecological effects; Estuaries; west coast of India

1. Introduction

Heavy metal enrichment in the aquatic environment is a growing environmental concern. Due to characteristics such as toxicity, resilience to degradation, and bio-magnification, heavy metals present in aquatic sediment and water significantly affect the nutrition and functioning of ecosystems (Islam and Tanaka, 2004; Islam et al., 2015; Zhan et al., 2020). In estuarine and coastal ecosystems, heavy metals balance their natural transport by river discharge and removal by biochemical processes (Tchounwou et al., 2012; Liu et al., 2019). Heavy metals derived through the anthropogenic processes such as wastewater from industries, urban areas, and agricultural soil are dominantly present in the estuaries and coastal region (Demirak et al., 2006; Cevik et al., 2009; Yang et al., 2012; Zhao et al., 2012; Mirza et al., 2019). The anthropogenically sourced heavy metal input into the coastal zones is increased owing to the rapid growth of urbanization and industrialization in the developing countries in the last few years (Islam et al., 2015, Zhang et al., 2019; Liu et al., 2019; Ota et al., 2021). The heavy metals discharged into the estuarine systems get easily incorporated into the sediments, thereby eventually act as an essential source of free ions to the water during organic matter degradation, bioturbation, and change in redox potential (Qiu et al., 2011).

Sediment affected by enriched anthropogenic pollutants disturbs the marine benthic faunal communities by affecting their diversity and abundance (Wu et al., 2014). Benthic communities are good biological indicators of the biotic integrity of aquatic ecosystems (Rakocinski et al., 1997; Blanchet et al., 2004). These organisms are relatively sedentary, responding quickly to ambient conditions (Gray et al., 1992). Although, these organisms cope with changes in environmental variations and interspecific competition through evolution, which is a prolonged process. The significant chemical disturbance in the system causes changes in species composition, abundance, and biomass (Pearson and Rosenberg, 1978). In addition, these community changes may also reflect associated changes or disturbances in ecosystem function, such as the food web (Pearson and Rosenberg, 1978; Reice and Wohlenberg, 1993). Along the Indian continental margins, estuarine and coastal sediments are majorly enriched with metals (Ram et al., 2003; 2009; Rokade, 2009; Yadav et al., 2015; Saravanan et al., 2018). Metal contamination in the sediments from estuarine systems of the eastern Arabian Sea, especially the Thane-Ulhas system, along with Maharashtra, one of the significant industrial provinces of India, has been extensively reported (Jha et al., 2003; Ram et al., 2003; 2009). The effect of such enrichment on the sedentary biotic (benthic faunal) community is essential to understand the further impact on the food web. In general, the benthic community structure in these estuaries is reportedly affected by salinity gradients (Mulik et al., 2020).

In benthic metabolism, the meiofauna contribute five times more than macrofauna (Coull, 1988). Experimentally, it is well known that these small faunas are sensitive to environmental perturbation (Coull and Palmer, 1984); therefore, they can be pollution bioindicators (Austen and Somerfield, 1997; Kennedy et al., 1999; Mitwally and Fleeger, 2016). Quang et al. (2013) have explained the importance of meiobenthos and nematode communities in bio-monitoring programs from the Mekong estuarine area. The composition of meiofauna from the Indian region has been well documented in studies; Ansari et al., 2012, 2014; Baliarsingh et al., 2015; Jayabarathi et al., 2012; Semprucci et al., 2010; Singh and Ingole, 2011 and Varadharajan and Soundarapandian, 2013. The estuarine meiofaunal community and their interactions with pollutants such as metals are crucial for pollution and have less reported in the region. This study studied the interrelationship between sediment heavy metal concentrations and benthic meiofauna assemblage in seven estuarine systems along the salinity gradient. The heavy metals in sediment from these estuaries are hypothesized to be controlled by grain size and allochthonous organic matter (Rao et al., 2019). The objectives of the present study are: (i) to highlight the temporal variation of sediment metal in the estuaries; (ii) to ascertain the controlling factor towards the heavy metal enrichment and (iii) to comprehend the influence of metals on changes in the benthic community structure. The collective result of seven tropical estuaries will provide some valuable information to planners and decision-makers about the distribution of heavy metals and possible future economic damage in urbanized estuaries of India for its proper management.

2. Materials and method

2.1 Description of Study area

Along the Eastern Arabian Sea, nearly 30 small and medium-sized (geographical area: $>10^3$ km²) river-estuary systems have been flowing in Maharashtra. The freshwater discharge to these estuaries is mainly during the southwest monsoon. The estuarine segments of these river basins pass through highly urbanized areas established on their bank. Hence, they receive a substantial amount of domestic as well industrial wastes. The Ulhas Estuary receives 0.5×10^6 m³/day of domestic and 0.05×10^6 m³/day industrial waste, Patalganga estuary receives 0.02×10^6 m³/day industrial waste from several discharges. Amba Estuary receives around 0.07×10^6 m³/day treated effluent, 0.01×10^6 m³/day industrial, and 0.015×10^6 m³/day of domestic waste. In Savitri Estuary, discharge of 0.006×10^6 m³/day industrial and 0.08×10^6 m³/day domestic sewage occurs. Vashishti Estuary receives 0.045×10^6 m³/day industrial and 0.08×10^6 m³/day domestic waste sewage occurs. Vashishti Estuary receives 0.045×10^6 m³/day industrial and 0.08×10^6 m³/day domestic waste daily (CSIR-NIO, 2018).

Briefly, the Amba Estuary receives a higher amount of industrial waste, and the Ulhas Estuary receives a higher amount of domestic waste among all the estuaries of the present study. The average annual rainfall across the region (3322 mm), mainly occur during the Southwest monsoon periods (Sharad et al., 2007). The diurnal tide system influences the estuaries, and the highest tidal elevation is 4.3m (CSIR-NIO, 2009; Rao et al., 2019).

2.2 Collection method and analysis

A total of 110 sediment samples were collected during November-December 2015 (i.e., post-monsoon season) and March-May, 2016 (i.e., pre-monsoon season), along the salinity gradient from seven river-estuarine systems i.e. Ulhas (U.S.), Patalganga (P.T.), Amba (A.B.), Kundalika (K.K.), Savitri (S.T.), Vashishti (V.T.) and Shastri (S.R.) located in the west coast of India, eastern Arabian Sea (Fig. 1). Sediment was collected using a van-Veen grab (0.04 m²), stored in acid-cleaned polythene bottles and stored in icebox. In the laboratory, samples were stored in the refrigerator. After that, sediments were dried in a hot-air oven at 50°C for 72 hours (Ram et al., 2004), powdered homogeneously using an agate mortar before analyzing organic carbon and heavy metals. Grain size analyses of sediment were performed on un-grinded samples using the wet-sieve method (Buchanan, 1984). 25 g of sediment was dispersed in distilled water (200 ml), added sodium hexametaphosphate, and stand overnight. The result was sieved to pass 63 µm sieve. The sediment portions extracted were dried and percentage of sand, silt, and clay were determined with a measurement error of ≤1%.

The organic carbon content was calculated from the difference of total and inorganic carbon, which is measured in a CHNS analyzer (Model: Vario EL CUBE). About 0.2 g of dried and ground sediments were treated with the concentrated acid mixture (H.F. (5mL), HClO₄ (3mL), HCl (5mL), and HNO₃ (5mL)) in Teflon beakers and digested on a Teflon coated hot-plate (Loring and Rantala, 1992). After digestion, remain portion was filtered through Whatman filter paper and samples were diluted to constant volume with high purity water (Milli-Q) and stored in acid-cleaned plastic bottles for metal analysis. Post-digestion steps included evaporation and dilution before their injection into plasma of an Optima 7300DV ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry; Perkin Elmer). Suprapur grade chemicals and acids used throughout the analytical procedures were supplied by Merk, Germany (>99 % purity). Cadmium (Cd) and Lead (Pb) was measured in an Analyst 600 GF-AAS (Graphite Furnace-Atomic Absorption Spectrometry; Perkin Elmer). Mercury (Hg) was measured in FIMS-400 (Flow Injection Mercury System; Perkin Elmer). For Hg, sediments are digested in aqua regia and KMnO₄ (USEPA, 1979). PACS-2 as Standard

Reference Material (National Research Council, Canada) was run with each set of samples to authenticate the analysis results. The limit of detection (LOD) of the instrument was determined from ten replicate measurements of an unprocessed acid blank solution. In this method LOD and LOQ were determined from ten replicate measurements of an un-spiked blank processed in the same manner as samples of different matrices. The limit of detection was calculated as 3σ and LOQ was calculated as 10σ . 2% HNO₃ was used as a calibration blank. The results of method of digestion with a certified amount of the reference material were acceptable in most elements. The results of the recovery range varied within 95% to 100 % of the certified value (Table 1).

For the benthic study, the meiofaunal samples (110) were collected in triplicate using handheld syringe corer (Diameter: 2.9 cm; Height: 10 cm). The cores were pushed through sediment vertically down to 5 cm deep because more than 80% of meiofauna were restricted to 0-5 cm (Chen et al., 1999). The sediment was transferred to a plastic container, and relaxation, staining, and fixation were followed using 7% Magnesium Chloride (MgCl₂), 1% Rose Bengal stain, and 5% formaldehyde respectively, after collection. After that, the samples were shaken to get homogeneity and stored for further analysis. The benthic samples collected from different locations were brought to the laboratory for meiofauna, sediment fraction retained on the 63 μ m sieve was analyzed under a stereomicroscope (Leica). The meiofauna was sorted up to group level and enumerated for its density per m² and 10 cm², respectively.

2.4 Normalization and Pollution indices

Because of the granulometric and mineralogical effects on metal contents in sediment, standardization by a moderate component was preferred (Liu et al., 2003). In order to assess the status of anthropogenic influence, metal such as Al was used for normalization (Datta and Subramanian, 1998; Ram et al., 2003; Milacic et al., 2010). Due to conservative property, the Al was used to separate metals contributed from natural and anthropogenic sources (Ram et al., 2003; 2009). Generally, metal concentrations in surface sediment are higher than their pre- industrialization value because of increased anthropogenic activities in recent years. Therefore, the most suitable method to find the background values is to analyze sediment at the bottom of a core having preserved before industrialization (Angelidis and Aloupi, 1995; Wu et al., 2014). The metal concentrations used as background value for normalization are as follows; Al (7%), Fe (7.2%), Cr (102 μ g/g), Mn (817 μ g/g), Co (44 μ g/g), Ni (36 μ g/g), Cu (86 μ g/g), Zn (84 μ g/g), Cd (0.14 μ g/g), Pb (11.7 μ g/g) and Hg (0.1 μ g/g). These values are the average limits observed in bottom sediment samples with a

calculated age of 118 years in the study region and represent preindustrial values (Ram et al., 2009; Rokade, 2009).

The status of sediment contamination by heavy metals was observed through indices viz. E.F., C.F. and PLI, respectively as enrichment factor, contamination factor and pollution load index previously adopted in various studies (Ram et al., 2003; Feng et al., 2004; Liu et al., 2009) and are calculated as follows;

$$EF = [C_n / C_{Al(\text{sample})}] / [B_n / B_{Al(\text{background})}] \quad [\text{Eq 1}]$$

$C_n / C_{Al(\text{sample})}$ represents the ratio between the concentration of a particular metal and Al in the samples, and $B_n / B_{Al(\text{background})}$ represents the similar ratio of background metals used for normalization. The E.F., which potentially indicated the enrichment of anthropogenic origin of metals, are classified as (i) negligible (E.F. < 2) (ii) moderate (E.F. ≤ 2–5), (iii) Significant (E.F. ≤ 5–20) (iv) high (E.F. ≤ 20–40) and (v) very high (E.F. > 40) (Sutherland, 2000). Similarly;

$$CF = C_{(\text{sample})} / C_{(\text{background})} \quad [\text{Eq 2}]$$

$C_{(\text{sample})}$ represents the average metal concentration in studied samples, and $C_{(\text{background})}$ represents background values of the same metal used for normalization. In this study, the values of C.F. correspond to low (<1), moderate (>1 to 3), considerable (>3 to 6), and very high (>6) metal contamination present in samples (Szefer et al., 1998).

Overall, the PLI was calculated for the enrichment of heavy metals, except Fe and Al, which are most abundant and have the less toxic effect (Tomlinson et al. 1980; Nair et al. 1993). The PLI was computed as follows;

$$PLI = \sqrt[n]{(CF_1 \times CF_2 \times CF_3 \times CF_4 \times \dots \times CF_n)} \quad [\text{Eq 3}]$$

Based on the PLI values, the status of sediment metal pollution is evaluated, such as (i) polluted (PLI > 1) and (ii) non-polluted (PLI < 1) (Chakravarty and Patgiri, 2009; Seshan et al., 2010).

2.5 Statistical analysis

The statistical significance of the analyzed data (grain size, TOC, and metals) was tested using a Permutational multivariate analysis of variance (Permanova). The results presented were with a 95% confidence level. The n-MDS (non-Metric Multidimensional Scaling) used to understand the classification of the study area by dissimilarity of all studied metals. Interrelationship among the metal and other geochemical parameters, and the significance levels were determined through

Pearson's correlation. Principal component analysis (PCA) and cluster analysis (C.A.) was performed to understand the factors influencing the metal contamination and their association.

Benthic indices were calculated by using the density of the groups as an initial step in PRIMER v6. The indices are Shannon-Wiener diversity index (H' (log2)) (Shannon and Weaver, 1949), Pielou's evenness index (J') (Pielou, 1969), and Margalef species richness index (d) (Margalef, 1968). The benthic parameters such as total species number (S) per station, the total density of all organisms (N), and indices were extracted for meiofauna separately. All these parameters were used to perform the n-MDS for calcification of benthic community groups and CCA to understand the relationship between chemical and benthic parameters and distinguish the biological impacts caused via pollution. This is subjected to assuming all estuaries have different geomorphologic changes. PRIMER v6 (Clarke and Gorley, 2006), STATISTICA v7, and CANOCO v4.5 (ter Braak and Šmilauer, 2002) were used for all the statistical analyses.

3. Results and discussion

3.1 Sedimentary grain size and TOC

The average limits of sediment grain size found during pre- and post-monsoon seasons in all the studied estuaries were presented in Table 2. Significant variations in the sand, silt, and clay compositions were noticed across the salinity gradient during both seasons in all the estuaries of this study. The changes in the distribution of sediment grain size in estuaries located from the south through the north have a close resemblance to the differences in catchment rock type and the magnitude of their riverine sediment supply to the estuaries. This allows the coarser particle to quickly settle down in the estuarine region owing to weaker riverine discharge during the pre and post-monsoon (Rao et al., 2019). The average range of TOC was 1.3-2.4% (mean $1.6 \pm 1.0\%$) and 1.0-2.2% (mean $1.8 \pm 1.0\%$) during pre- and post-monsoon seasons, respectively, in all the estuaries (Table 2). Higher TOC in the middle zone of most estuaries was attributed to organic matter, mainly from the organic wastes through anthropogenic sources (Rao et al., 2019). Furthermore, the TOC content positively varied with clay and silt content ($p \leq 0.05$), negatively with sand, together with suggestive towards a robust control of grain size over TOC in these monsoonal estuaries. This means the high absorptive capacity of fine particles for organic matter and rapid sediment deposition rates (Rao et al., 2019).

3.2 Sedimentary heavy metals

The distribution of sedimentary heavy metals measured in this study during the post and pre-monsoon seasons were shown in Figs S1-S3, and their comparison with the regions around the globe was presented in Table 3. The enrichment of the heavy metals in the sediment samples collected from the estuaries is broadly in the following order Fe (3.7 – 20.9 %; av. 9.6 ± 2.8 %) > Al (4.0 – 11.0 %; av. 6.9 ± 1.2 %) > Mn (183 – 2663 $\mu\text{g/g}$; av. 1278.4 ± 465.2 $\mu\text{g/g}$) > Cr (95.0 – 691.0 $\mu\text{g/g}$; av. 233.2 ± 85.0 $\mu\text{g/g}$) > Cu (34.0 – 468.0 $\mu\text{g/g}$; av. 157.1 ± 93.1 $\mu\text{g/g}$) > Zn (10.0 – 777.0 $\mu\text{g/g}$; av. 130.9 ± 71.6 $\mu\text{g/g}$) > Ni (26.0 – 218.0 $\mu\text{g/g}$; av. 83.3 ± 21.7 $\mu\text{g/g}$) > Co (25.0 – 940.0 $\mu\text{g/g}$; av. 78.0 ± 100.8 $\mu\text{g/g}$) > Pb (2.7 – 76.5 $\mu\text{g/g}$; av. 14.2 ± 7.2 $\mu\text{g/g}$) > Cd (0.06 – 239.18 $\mu\text{g/g}$; av. 0.3 ± 0.2 $\mu\text{g/g}$) > Hg (0.01 – 3.6 $\mu\text{g/g}$; av. 0.2 ± 0.4 $\mu\text{g/g}$).

Higher concentrations of Al (11%) and Fe (16.2%) were recorded in the middle and lower zones of the S.R. and V.T. estuaries during the post-monsoon. The middle reaches of the U.S. showed higher Cr (589 $\mu\text{g/g}$). Mn (2312 $\mu\text{g/g}$ and 2663 $\mu\text{g/g}$) were found to be higher in the lower and upper parts of most of the estuaries. Higher Co (604 $\mu\text{g/g}$), Ni (158 $\mu\text{g/g}$), and Cu (377 $\mu\text{g/g}$) was observed in the middle zone of the P.T. estuary. The maximum concentrations of Zn (706 $\mu\text{g/g}$) and Pb (76.5 $\mu\text{g/g}$) were recorded in the middle part of the U.S. estuary. The maximum concentrations of Cd (1.8 and 0.77 $\mu\text{g/g}$) were in the middle part of the K.K. and U.S. estuaries, respectively. The maximum concentration of Hg (2.6 $\mu\text{g/g}$) was recorded in the middle part of the P.T. estuary.

Higher Al (8.9 and 8.8%) was recorded in the upper and middle parts of the S.T. and S.R., respectively, whereas Fe (20.9%) was recorded in the upper parts of the S.R. during the pre-monsoon. The maximum concentration of Cr (691 $\mu\text{g/g}$) was observed in the middle part of the U.S. Higher Mn (2371 $\mu\text{g/g}$) was noticed in the upper part of the S.T. estuary, and the maximum concentration of Co (940 $\mu\text{g/g}$), Ni (218 $\mu\text{g/g}$), and Cu (468 $\mu\text{g/g}$) observed in the middle P.T. estuary. The maximum concentration of Zn (777 $\mu\text{g/g}$) and Pb (68.6 $\mu\text{g/g}$) were recorded in the middle part of the U.S. estuary. The maximum concentrations of Cd (1.8 $\mu\text{g/g}$) and Hg (3.6 $\mu\text{g/g}$) were observed in the middle part of the P.T. estuary. All heavy metals showed significant variability between the estuaries ($p < 0.05$; Permanova) but did not show seasonal variability in all estuaries. It may be due to the fluctuation of the large quantity of sewage and industrial waste discharged into the study area

From the concentration patterns, it might be seen that in the middle part of the U.S., P.T. and K.K. estuaries were recorded with higher concentrations of the metals Co, Ni, Cu, Cr, Zn, Cd, Pb,

and Hg than other studied estuaries (Fig S1-S3). Such higher concentrations are attributed to the discharge of a large quantity of sewage and industrial waste in these estuaries (Ram et al., 2003 and 2009; CSIR-NIO, 2018). During both seasons, higher concentration of Al and Fe were observed, indicating that enrichment was more noticeable in the middle and upper parts of the S.R. than the other parts of estuaries. A higher concentration of Al and Fe may be attributed to the mineral richness of the soils of the area (Rokade, 2009). Furthermore, n-MDS analysis was performed on the distribution of heavy metals in this study (Fig 2). Group-I estuarine sediments have enriched concentrations of metals (Co, Ni, Cu, Cr, Zn, Cd, and Hg) in the U.S. and P.T. (northern segment) as compared to the Group-II (A.B. and K.K.; middle segment) and Group-III (S.T., VT, and S.R.; southern segment).

The Enrichment Factor (E.F.) and Contamination Factor (C.F.) were calculated for Cr, Mn, Co, Ni, Cu, Zn, Cd, Hg, and Pb, which were presented in Table 4. Higher Cr was observed in the middle part of the U.S. and P.T. estuaries, showing significant enrichment (E.F.>5) and very high contamination (C.F.>6) than in the other parts of studied estuaries. Such enrichment of the metal is attributed to the domestic and industrial waste discharged in the middle segments of these estuaries, as explained above. The highest concentrations of Co, Ni, Cu, Cd, and Hg were observed in the middle part of the P.T. estuary, showing significant enrichment (E.F.>5) and high contamination (C.F.>6) than the other part of the studied estuaries. These results indicate the impact of sewage and industrial effluents discharged to the estuary (CSIR-NIO, 2011). Cd was high in middle segments of the K.K., U.S., and P.T. estuaries. The highest concentrations of Zn, Pb, and Hg showed significant enrichment (E.F.>5) and very high contamination (C.F.>6) in these estuaries, indicated anthropogenic sources (Yadav et al., 2015). Thus, the present study results indicate that the highest concentration of most of the heavy metals was in the sediments of the middle segments of the studied estuaries during post and pre-monsoon. The minor concentration of heavy metals was recorded in the open coast of all estuaries, demonstrating that a significant fraction of the heavy metals discharged to the aquatic environment gets adsorbed on particulate matter and settles within the sediment near the source (Gonzalez and Brugmann, 1991; Ram et al., 2009).

In summary, Cr, Cd, Pb, and Hg showed a significant enrichment and contamination observed in the estuaries of U.S. and P.T. sediments than in the K.K., AB, S.T., VT, and S.R. during post and pre-monsoon seasons (Fig 2). These patterns are consistent with the results of the n- MDS analysis representation, forming three main groups of heavy metals, as previously described. The average values of PLI varied from 0.9 to 4.8 during both seasons in all the studied estuaries (Fig 3). Higher

values were recorded in the middle part of all the studied estuaries ($PLI > 1$) during both seasons, with the highest (> 4) being in the middle part of the P.T. estuary. The result of the PLI calculation indicates an alarming situation in the middle segments of the U.S. and P.T. estuaries, indicating the anthropogenic impact in these regions. The average value of pollution load indices in the studied estuaries was calculated and found in the following order: PT (2.1) > US (2.0) > SR (1.7) > VT (1.6) > ST (1.5) > KK (1.4) > AB (1.3). As per the above grade, the sediments in the study area are significantly contaminated, suggesting an overload of anthropogenic activities occurred in these regions.

3.3 Heavy metals contamination source identification

Correlation analysis (Table 5) revealed that sedimentary Mn and Fe showed a significant negative correlation with silt and clay, while a positive correlation with sand ($p < 0.01$). Sedimentary Cd and Pb showed a significant positive correlation with clay and TOC contents ($p < 0.01$). Which indicates finer particles of sediment and organic matter might act as significant sinks for heavy metals caused by their strong assimilation ability and expanded surface area (Zhang et al., 2001; Laing et al., 2008). Sedimentary Hg exhibits significant association with Co, Zn, Ni, Cr, and Pb ($p < 0.01$), indicating the same point source for these metals as influenced by human induced activities (Zhang et al., 2018; Fang et al., 2019). The good connection of organic matter with heavy metals suggests the formation of organic complexes with heavy metals as a ligand by flocculation influences and consequently impacts their distributions, owing to their large surface area (Marchand et al., 2006; Zourarah et al., 2008). The good association of Fe with Mn ($p < 0.01$), suggests both metals have similar chemical characteristics, which exist partly in oxide form and partly in hydroxide form (Chapman et al., 1998). A significant relationship exists between Co, Cr, Ni, Cu, and Zn that indicates their resemblance and identical behavior.

Additionally, the stage of contamination using heavy metals from lithogenic activity and anthropogenic sources were detected by PCA (Yi et al., 2011; Chabukdhara and Nema, 2012). Based on the results of PCA analysis (Fig 4a) two main factors, accounting for 57.6% of cumulative percentage, were detected as controlling factors for the geochemistry of heavy metals with organic carbon. The strong loading of Ni, Zn, Co, Cr, and Hg in the factor 1 accounted for 35.9 % of the total variance. High loading of silt, clay, TOC along with Pb and Cd in the factor-2 accounted for 21.7 % of the total variance (Fig 4a). Factor -1&2 describes that the same point source for these metals, dependent on organic matter and grain size purely from anthropogenic sources i.e. industrial, domestic effluents, and other human activities in the U.S. and P.T. estuaries compared to other part

of studied estuaries (Veerasingam et al., 2013). Similar relations were identified in the cluster analysis (C.A.) (Fig. 4b).

3.4 Meiobenthic invertebrate community structure

In total, 25 taxa of the meiofaunal community were recorded from the seven west-flowing estuaries. They were subordinate to 14 phyla, including Protozoa (foraminiferans), Cnidaria, Mollusca, Arthropoda (crustacean), Annelida (polychaetes; oligochaetes), Platyhelminthes, Echiura, Nemertea, and other phyla (Nematoda, Gastrotricha, Gnathostomulida, Phoronida, Kinorhyncha, and Tardigrada). Concerning taxa, the meiofaunal community was dominated by Nematoda (77.47%). Foraminiferans (6.82%) were the second most dominant, followed by nematodes. The meiofaunal communities did not differ significantly between the seasons (Table S1). At the same time, it differed significantly between segments (north, middle, and south) and seven estuaries (Pair-wise tests in Permanova; $p < 0.05$). The meiofaunal communities differed pair-wise with each segment (Table S2). The n-MDS and cluster results showed meiofaunal communities distributed within four major groups (m, n, o, and r; containing at least five sampling occasions) at 55% similarity (Fig 5). The classification of groups was characterized by heavy metal concentrations, productivity (food availability), and sediment grain size. The groups differentiated based on cluster and n-MDS differed significantly regarding the meiofaunal community ($p < 0.05$; Table S1).

Based on meiofaunal abundance, group-o was the largest cluster (34 sampling occasions) formed, including sites mostly from the southern and middle segment estuaries (K.K., S.T., VT, and S.R.; Table S1). Group-o was featured with Fe, Cu, clay, and silt (cumulative 43.6%; Table S3). The second group was group-n, comprising sites from the northern and a few from the southern segment (U.S., P.T., and S.T.). The levels of Cd, Mn, clay, Al, Cr, and TOC were high in the sediments of group-n contributing 57.0%, suggesting moderate perturbation. The middle sites from the U.S. estuary have clustered together to form group-m and feature high Cd values, clay, TOC, Pb, Cu, and Zn. The group-r includes a few middle sites from the U.S., PT KK, and V.T. The high values of Pb, Cr, Zn, Al, sand, and Mn were reported from group-r. Nematodes and foraminiferans were the dominant fauna among meiofauna in all the groups presented (Table S4). However, the abundance of these taxa was found to have decreased in group-o due to copepods, polychaetes, and nauplii contributing significantly to the community (34.8%). The group-o and r were found largely dissimilar, with 64.8% relatively. The nematode depicted changes in the average abundance by decreasing in the order of group-o > group-n > group-m > group-r. This decreasing trend may have led to the enrichment of heavy metals. Copepods, polychaetes nauplii, and ostracods indicated a

decrease in average abundance concerning the heavy metal gradient. The univariate indices were calculated for the groups separated based on the n-MDS plot were shown in Fig 6. The group-o was characterized by high mean taxon richness, number of individuals, and diversity except for evenness (Fig 6). Meiofaunal evenness was highest at group- r. The group- n and r were found at the lowest mean taxon richness, number of individuals, and diversity.

3.5 Relationships between the heavy metals and benthic meioinvertebrates

The overall results suggested that the benthic community was highly influenced by the enrichment of heavy metals followed by TOC. The northern and middle segment sites were primarily impacted, along with a few sites from the southern segment. The impact was evident by high metal contaminations, TOC, and characterized by low diversity indices with perturbation in some taxa. Furthermore, the predominance of nematodes and foraminiferans in meiofauna revealed the susceptibility of the benthic ecosystem. In upstream stations of the northern and middle segment, the direct influence of industrial discharge resulted in high metal concentrations, which driven the benthic assemblages.

The result of Canonical Correspondence Analysis (CCA) between benthic assemblages (meiofauna) and environmental parameters was concluded a significant negative impact. Before CCA analysis, an automatic selection procedure with the Monte Carlo permutation test was used to understand the significance of each environmental variable on each assemblage. Based on meiofauna abundance, CCA clearly showed that metals (Hg, Co, Cu, Zn, and Al) had influenced the distribution of meiofaunal assemblages (Fig 7) more significantly than any other variable in the surveyed area (Table S5). Group-n, m, and r were the most impacted clusters, and the sites within them were found with the highest contribution, more than 60% of nematodes and foraminiferans. Some nematodes (*Ptycholaimellus ponticus*, *Sabatieria pulchra*, *Molgolaimus demani*, and *Axonolaimus paraspinosus*) are known for their tolerance to metal concentrations (Sommerfield et al., 1994). Foraminiferans did not show significant changes in metal levels but did show a decrease of the abundance in impacted sites (group-n, m, and r). A similar result was demonstrated in the lowering of the growth rate, but not fatal by Ben-Eliahu et al. (2020). However, some foraminifera, such as *Criboelphidium magellanicum* and *Protelphidium tuberculatum* showed perfect tolerance to metals (Guo et al., 2020). At the same time, group-o showed a 55.6% contribution by nematodes and foraminiferans, which was lower than the impacted group. Copepods, polychaetes, and nauplii also contributed significantly to group-o. We need to consider the fact that toxicity is a result of the combined consequence of all present metals, and also that these are pollution inputs to the ecosystem and

undoubtedly linked with discharges of domestic and industrial wastewater (Guagliardi et al., 2013; Huang et al., 2015).

3.6 Assimilation of sedimentary heavy metals, toxicity and benthic faunal changes

Threshold Effect Level (TEL) and Probable Effect Level (PEL) guideline were usually used in the sediment quality assessment (MacDonald et al., 1996). According to these criteria, our study average results showed (Table S6; Table 6) the concentrations of Cr, Ni, Cu, Zn, Pb, and Hg in most of the estuaries were higher than the TEL value, while lower than the PEL value, except for Cr, Ni, Cu in most of the estuaries and Hg in the U.S., P.T., and S.R. Therefore, elevated concentrations of these metals may lead to more toxicity in the study area (MacDonald et al., 1996; Wu et al., 2014). The integration of heavy metals and benthic fauna showed a comprehensive response. In the present study, the mean of univariate indices (richness, evenness, and diversity) was used to understand the overall significant differences relative to the reference sites. The reference sites were selected based on the n-MDS partitioning, which was located away from the impacted sites. The sites were selected from group-o as reference sites for meiofaunal univariate indices. The estuaries were assessed for impairment based on the low mean indices values of the estuaries as compared to the mean value of reference sites. Richness, evenness, and diversity were observed lower in most estuaries than the mean of sites clustered in group-o (Table 6). Therefore, sediment quality showed that all the estuaries were impaired in terms of heavy metals. The benthic assessment correspondingly supported similar impairment results except for the A.B. and K.K. estuaries.

4. Conclusions

The present examination results provide essential details about the accumulation of heavy metals, contamination sources, and connecting these metals to change in the benthic community structure in urbanized estuaries along the eastern Arabian Sea as follows:

1. The distribution of all heavy metals highlighted significant spatial variability but no seasonal variability in all the estuaries due to the fluctuation of the magnitude and type of sewage and industrial waste delivery into the estuary.
2. A suite of indices such as E.F., C.F., and PLI indicated that studied estuaries are polluted owing to excess anthropogenic loadings of metals, especially in the middle part of U.S. and P.T. estuaries.
3. According to the correlation, Cd and Pb showed a positive correlation with clay and TOC fraction due to their strong assimilation capacity and increased surface areas. Moreover, positive correlations of Hg with Co, Zn, Ni, Cr, and Pb indicate the common source.

4. Results from PCA and C.A. demonstrated the unique source of heavy metals in most of the estuaries during both seasons. Heavy metals characteristics varied with sediment grain size and TOC owing to coarser sediments in the estuaries.
5. The meiofauna community responded to the heavy metal concentrations with changes in the taxon richness, evenness, and diversity measures and an extremely high and low number of individuals mainly associated with the dominance of single tolerant taxonomic taxa or species.
6. The results of CCA showed that the heavy metals were significantly correlated to the changes in benthic faunal structure. Meiofaunal taxa were observed located at a distance from most of the heavy metals.
7. The heavy metals and meiofauna integration demonstrated that impairment occurred in the estuaries (except A.B. and K.K.).

Anthropogenic heavy metals in the urbanized estuaries along the west coast of India still need further long-term monitoring due to increasing urbanization and industrialization. Hence, anthropogenic heavy metals dominated the present study, and it is essential to understand how anthropogenic metals affect the sediment ingesting organisms both now and in the future. The results from this study can be used for reasonable management practices to overcome the challenges of pollution control and the improvement of biodiversity of the study area.

Acknowledgments

The authors would like to thank the Director, CSIR-National Institute of Oceanography (CSIR-NIO), and the Scientist-in-Charge, CSIR-NIO, Regional Centre, Mumbai, for providing all necessary facilities and encouraging the work. The authors are very glad and grateful to the Maharashtra Pollution Control Board (MPCB) for financial support. We also would like thank the other members and entire team CSIR-NIO for physical support during field collections.

Conflict of Interest: The authors declare that they have no conflict of interest.

Data availability: The data that support the findings of this study are available on request from the corresponding author, [U.K. Pradhan]. The data are not publicly available due to [restrictions e.g. their containing information that could compromise the privacy of research participants]

Author contribution: All authors contributed to the study conception and design. **M. Nageswar**

Rao: Sample collection, analysis and writing, **Gaikwad. S:** Collection, analysis and writing,

Anirudh Ram: Supervision, **U. K. Pradhan:** Editing, **Sautya S:** Review, **L. Kumbhar:**

Sample collection, **Udayakrishnan PB**: analysis, **V. Siddaiha**: Supervision.

Animal Research: This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Consent to publish: I (U. K. Pradhan), give my consent for the publication of identifiable details, which can include figures, tables and details within the text (all authors declare that they have no objection) to be published in this Journal.

References

- Angelidis, M.O., Aloupi, M. 1995. Metals in sediments of Rhodes Harbour, Greece. *Mar. Pollut. Bull.* 31, 273–276.
- Badr NBE, El-Fiky AA, Mostafa AR, Al-Mur BA .2009. Metal pollution records in core sediments of some Red Sea coastal areas, Kingdom of Saudi Arabia. *Environ. Monit. Assess.* 155:509–526.
- Brown, A.C., Nordstrom, K., McLachlan, A., Jackson, N.L., Sherman, D.J., 2008. Sandy shores of the near future. In: Polunin, N.V.C. (Ed.), *Aquatic Ecosystems*. Cambridge University Press, Cambridge, UK, pp. 263-280.
- Buchanan, J.B., 1984. Sediment analysis. In *Methods for study of marine benthos* (second edition), ed. N.A. Holme and A.D. McIntyre, 1–65. Oxford: Blackwell Scientific publications.
- Cevik, F., Goksu, M. Z. L., Derici, O. B., Findik, O. 2009. An assessment of metal pollution in surface sediments of Seyhan dam by using enrichment factor, geoaccumulation index and statistical analyses, *Environ. Monit. Assess.* 152, 309–317.
- Chabukdhara, M. and Nema, A.K., 2012. Assessment of heavy metal contamination in Hindon River sediments: a chemometric and geochemical approach. *Chemosphere*, 87(8), pp.945-953.
- Chakravarty, I.M., Patgiri, A.D. 2009. Metal pollution assessment in sediments of the Dikrong River, N.E. India. *J. Hum. Ecol.* 27 (1), 63–67.
- Chapman, P.M. and Wang, F., 2001. Assessing sediment contamination in estuaries. *Environmental Toxicology and Chemistry: An International Journal*, 20(1), pp.3-22.
- Chapman, P.M., Wang, F., Janssen, C., Persoone, G., Allen, H.E. 1998. Ecotoxicology of metals in aquatic sediments: Binding and release, bioavailability, risk assessment, and remediation. *Can. J. Fish. Aquat. Sci.* 55, 2221–2243.
- Cotano, U., Villate, F. 2006. Anthropogenic influence on the organic fraction of sediments in two contrasting estuaries: a biochemical approach. *Mar. Pollut. Bull.* 52, 404-414.

- CSIR-National Institute of Oceanography (CSIR-NIO) technical report. 2009. Monitoring of Coastal marine and estuarine ecology of Maharashtra-Phase I. Part A (Main report).
- CSIR-National Institute of Oceanography Technical report., 2009. Monitoring of Coastal marine and estuarine ecology of Maharashtra-Phase I. Part A (Main report).
- CSIR-NIO technical report, 2009. Monitoring of Coastal marine and estuarine ecology of Maharashtra-Phase I. Part A (Main report).
- CSIR-NIO technical report, 2011. Release of treated effluent from M/s. Lenzing Modi in Patalganga Estuary.
- CSIR-NIO technical report, 2018. Assessment of impact of release of effluents on ecology of inshore and coastal areas of Maharashtra and their management.
- Datta, D.K., Gupta, L.P., Subramanian, V. 1999. Distributions of C, N and P in the sediments of the Ganges Brahmaputra–Meghna river system in the Bengal basin. *Org. Geochem.* 30, 75–82.
- Datta, D.K., Subramanian, V. 1998. Distribution and fractionation of heavy metals in the surface sediments of the Ganges-Brahmaputra-Meghna river system in the Bengal basin. *Environ. Geol.* 36 (1–2), 93–101.
- Demirak, A., Yilmaz, F., Tuna, A. L., Ozdemir, N. 2006. Heavy metals in water, sediment and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey. *Chemosphere*, 63, 1451–1458.
- Du Laing, G., Rinklebe, J., Vandecasteele, B., Meers, E. and Tack, F.M., 2009. Trace metal behaviour in estuarine and riverine floodplain soils and sediments: a review. *Science of the total environment*, 407(13), pp.3972-3985.
- Feng, H., Han, X., Zhang, W. and Yu, L., 2004. A preliminary study of heavy metal contamination in Yangtze River intertidal zone due to urbanization. *Mar. Pollut. Bull.*, 49(11-12), pp.910-915.
- Gireeshkumar, T.R., Deepulal, P.M., Chandramohanakumar, N. 2012. Distribution and sources of sedimentary organic matter in a tropical estuary, south west coast of India (Cochin estuary): A baseline study. *Mar. Pollut. Bull.* 66, 239-245.
- González, H., Brüggemann, L. 1991. Heavy metals in littoral deposits off Havana City, Cuba. *Chem. Ecol.* 5, 171-179.
- Islam, M.S., Ahmed, M.K., Raknuzzaman, M., Habibullah-Al-Mamun, M., Masunaga, S., 2015. Assessment of trace metals in fish species of urban rivers in Bangladesh and health implications. *Environ. Toxicol. Pharmacol.* 3, 347-357.
- Islam, M.S., Tanaka, M., 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. *Mar. Poll. Bull.* 48, 624-649.
- Jha, S.K., Chavan, S.B., Pandit, G.G., Sadasivan, S. 2003. Geochronology of Pb and Hg pollution in a coastal marine environment using global fallout. *J. Environ. Radioact.* 69, 145–157.

- Kumar, S.P., Edward, P. 2009. Assessment of metal concentration in the sediment cores of Manakudi estuary, south west coast of India. *Indian J. Mar. Sci.* 38 (2), 235–248.
- Laing, G.D.; Rinklebe, J.; Vandecasteele, B.; Meers, E.; Tack, F.M.G. 2008. Trace metal behaviour in estuarine and riverine floodplain soils and sediments: A review. *Sci. Total Environ.* 2008, 407, 3972–3985.
- Liu, C., Xu, J., Liu, C., Zhang, P. and Dai, M., 2009. Heavy metals in the surface sediments in Lanzhou Reach of Yellow River, China. *Bulletin of environmental contamination and toxicology*, 82(1), pp.26-30.
- Liu, C., Xu, J., Liu, C., Zhang, P., Dai, M. 2009. Heavy metals in the surface sediments in Lanzhou Reach of Yellow River China. *Bulletin. Environ. Contam. Toxicol.* 82, 26–30.
- Liu, M., Hou, L.J., Xu, S.Y., Ou, D.N., Yang, Y., Yu, J. and Wang, Q., 2006. Organic carbon and nitrogen stable isotopes in the intertidal sediments from the Yangtze Estuary, China. *Mar. Pollut. Bull.* 52(12), pp.1625-1633.
- Liu, W. X., Li, X. D., Shen, Z. G., Wang, D. C., Wai, O. W. H., Li, Y. S. 2003. Multivariate statistical study of heavy metal enrichment in sediments of the Pearl River Estuary. *Environ. Pollut.* 121, 377–388.
- Loring, D.H., Rantala, R.T.T. 1992. Manual for the geochemical analyses of marine sediments and suspended particulate matter. *Earth Sci. Rev.* 32, 235–283.
- MacFarlane, G.B., Burchett, M.D., 2000. Cellular distribution of Cu, Pb, and Zn in the Grey mangrove *Avicennia marina* (Forsk.). *Vierh Aquat. Bot.* 68, 45-59.
- Marchand, C., Lallier-Vergés, E., Baltzer, F., Albéric, P., Cossa, D., Baillif, P. 2006. Heavy metals distribution in mangrove sediments along the mobile coastline of French Guiana. *Mar. Chem.* 98(1–2), 1–17.
- Milacic, R., Scancar, J., Murko, S., Kocman, D., Horvat, M. 2010. A complex investigation of the extent of pollution in sediments of the Sava River. Part 1: selected elements. *Environ. Monit. Assess.* 163, 263–275.
- Nair, C.K., Balachand, A.N., Chacko, Jacob. 1993. Sediment characteristics in relation to changing hydrography of Cochin estuary. *Indian. J. Mar. Sci.* 22, 33–36.
- Pradhan, U.K., Wu, Y., Shirodkar, P.V., Zhang, J., Zhang, G. 2014. Sources and distribution of organic matter in thirty five tropical estuaries along the west coast of India-a preliminary assessment. *Estuar. Coast.Shelf. Sci.* 151, 21-33.
- Qiu, Y.-W., Lin, D., Liu, J.-Q., Zeng, E. Y. 2011. Bioaccumulation of trace metals in farmed fish from South China and potential risk assessment. *Ecotoxicol. Environ. Saf.* 74, 284-293.
- Ram, A., Rokade, M.A., Zingade, M.D. 2003. Mercury in sediments of Ulhas estuary. *Mar. Pollut. Bull.* 46, 846–857.

- Ram, A., Rokade, M.A., Zingde, M.D., Borole, D.V. 2009. Post-depositional memory record of mercury in sediment near the effluent disposal site of a chlor-alkali plant in Thane Creek-Mumbai Harbour, India. *Environ. Technol.* 30 (8), 765–783.
- Rao, M.N., Ram, A., Pradhan, U.K. and Siddaiah, V., 2019. Factors controlling organic matter composition and trophic state in seven tropical estuaries along the west coast of India. *Environ. Geochem. Health.* 1-18.
- Rokade, M.A. 2009. Heavy Metal Burden in Coastal Marine Sediments of North-west Coast of India in Relation to Pollution A Ph.D. thesis submitted to Mumbai University, pp. 1–162.
- Seshan, B. R. R.; Natesan, U.; Deepthi, K. 2010. Geochemical and statistical approach for evaluation of heavy metal pollution in core sediments in southeast coast of India. *Int. J. Environ. Sci. Tech.* 7 (2), 291-306.
- Sharad, K.J., Pushpendra, K., and Agarwal, V.P.S., 2007. *Hydrology and Water Resources of India*, Springer, 743.
- Sutherland, R.A. 2000. Bed sediment-associated trace metals in an urban stream Oahu, Hawaii. *Environ. Geol.* 39, 611–627.
- Szefer, P., Glasby, G.P., Kusak, A., Szefer, K., Jankowska, H., Wolowicz, M., Ali, A.A. 1998. Evaluation of anthropogenic flux of metallic pollutants into Puck Bay, Southern Baltic. *Appl. Geochem.* 13, 293–304.
- Tchounwou, P., Yedjou, C., Patlolla, A., Sutton, D., 2012. Heavy metal toxicity and the environment. *Mol. Clin. Environ. Toxicol.* 101, 133-164.
- Tomlinson, D. L., Wilson, J. G., Harris, C. R., Jeffrey, D. W. 1980. Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. *Helgol. Meeresunters.* 33, 566–575.
- Valdés, J., Vargas, G., Sifeddine, A., Ortlieb, L. and Guinez, M., 2005. Distribution and enrichment evaluation of heavy metals in Mejillones Bay (23 S), Northern Chile: geochemical and statistical approach. *Mar. Pollut. Bull.* 50(12), pp.1558-1568.
- Veerasingam, S., Venkatachalapathy, R. and Ramkumar, T., 2014. Historical environmental pollution trend and ecological risk assessment of trace metals in marine sediments off Adyar estuary, Bay of Bengal, India. *Environ. Earth. Sci.* 71(9), pp.3963-3975.
- Wu, B, Song, J, Li, X. 2014. Linking the toxic metals to benthic community alteration: A case study of ecological status in the Bohai Bay, *Mar. Pollut. Bull.* 83, 116–126.
- Yadav, A., Ram, A., Majithiya, D., Salvi, S., Sonavane, S., Kamble, A., Ghadigaonkar, S., Jaiswar, J.R.M., Gajbhiye, S.N. 2015. Effect of heavy metals on the carbon and nitrogen ratio in *Avicennia marina* from polluted and unpolluted regions. *Mar. Pollut. Bull.* 101 (1), 359-365.
- Yang, Y., Chen, F., Zhang, L., Liu, J., Wu, S., Kang, M. 2012. Comprehensive assessment of heavy metal contamination in sediment of the Pearl River Estuary and adjacent shelf. *Mar. Pollut. Bull.* 64, 1947-1955.

- Zhang, W., Yu, L., Hutchinson, S.M., Xu, S., Chen, Z. and Gao, X., 2001. China's Yangtze Estuary: I. Geomorphic influence on heavy metal accumulation in intertidal sediments. *Geomorphology*, 41(2-3), pp.195-205.
- Zhang, W., Yu, L., Hutchinson, S.M., Xu, S., Chen, Z., Gao, X. 2001. China's Yangtze Estuary: I. Geomorphic influence on heavy metal accumulation in intertidal sediments. *Geomorphology*. 41, 195–205.
- Zhao, H., Xia, B., Fan, C., Zhao, P., Shen, S. 2012. Human health risk from soil heavy metal contamination under different land uses near Dabaoshan Mine, Southern China. *Sci. Total Environ.* 417, 45-54.
- Yi, Y., Yang, Z. and Zhang, S., 2011. Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. *Environ. Pollut.* 159(10), pp.2575-2585.
- Zoumis, T., Schmidt, A., Grigorova, L., Calmano, W. 2001. Contaminants in sediments: remobilisation and demobilisation. *Sci. Total Environ.* 266, 195-202.
- Zourarah, B., Maanan, M., Robin, M., Carruesco, C. 2008. Sedimentary records of anthropogenic contribution to heavy metal content in OumErBia estuary (Morocco). *Environ. Chem. Lett.* 7(1), 67–78.

Legend to figures:

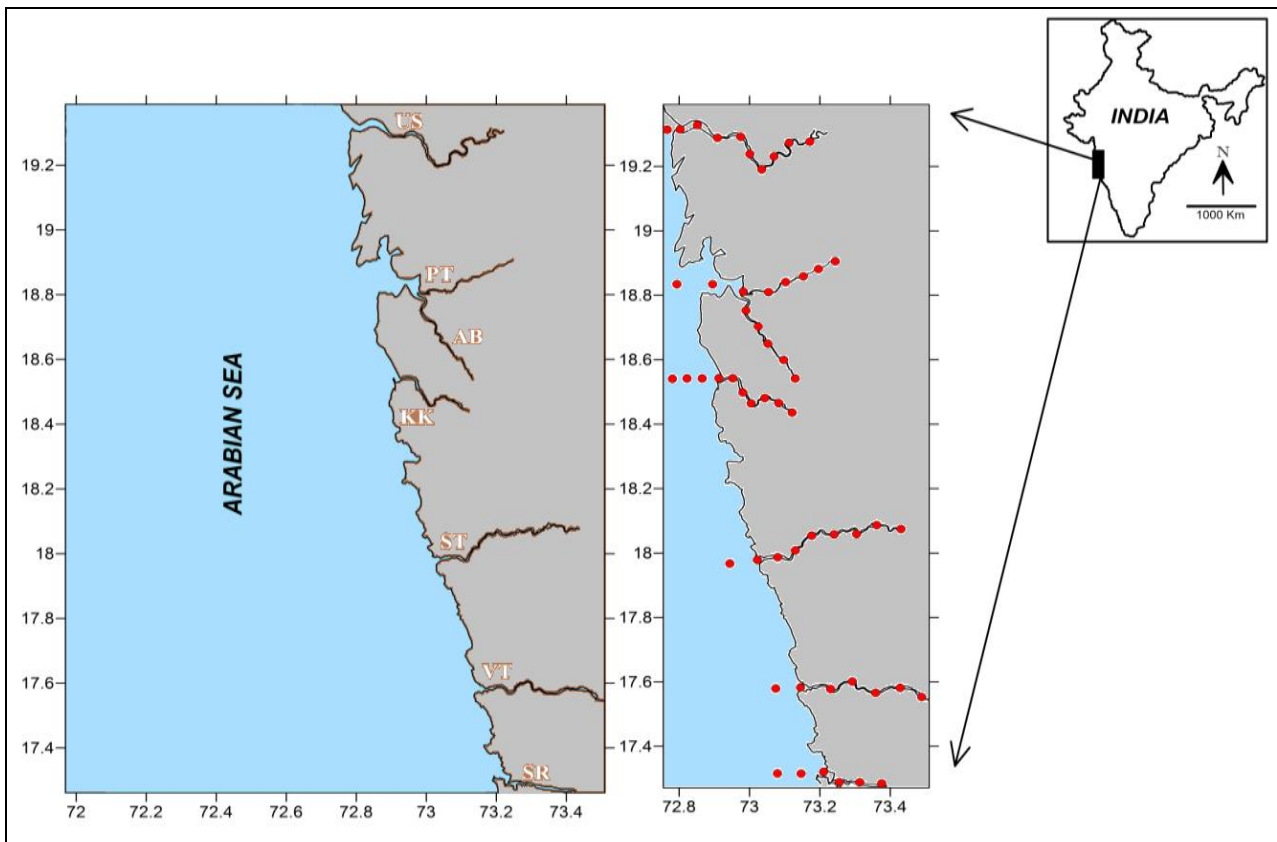


Fig 1. The map of sampling strategy and locations of collection sites along the seven tropical estuaries along the eastern Arabian Sea.

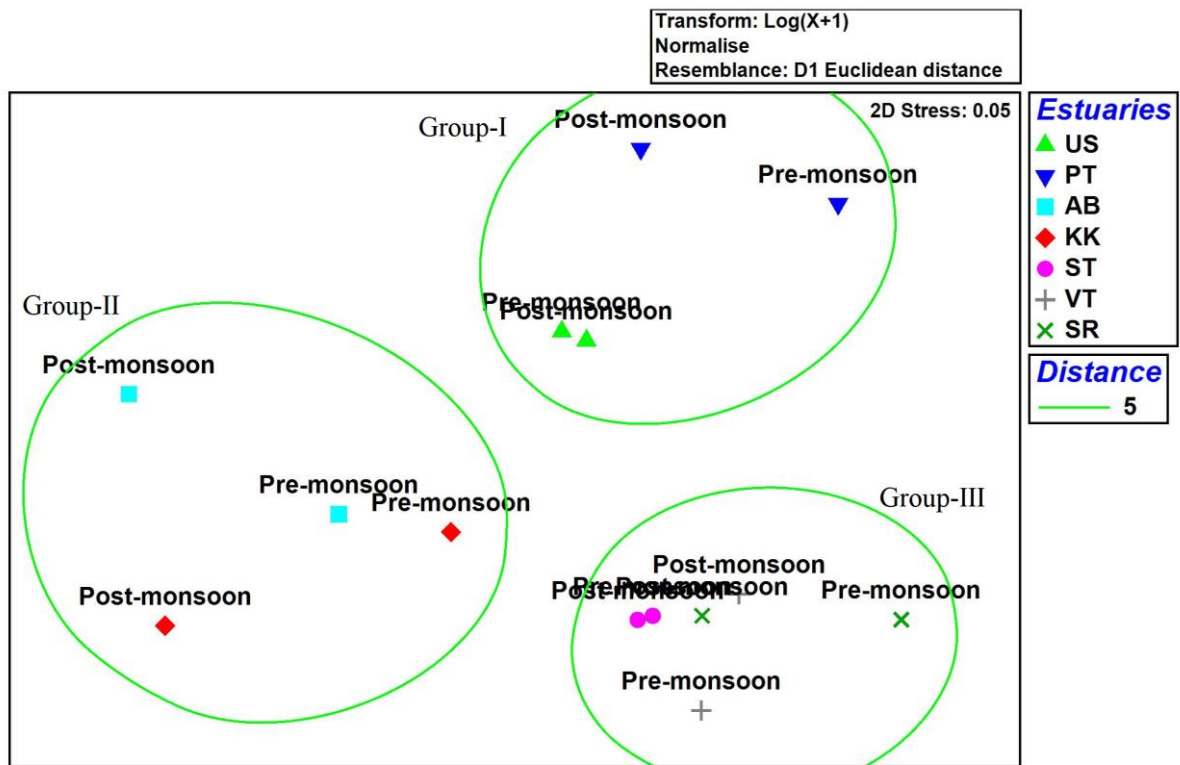


Fig 2. The non-Metric Multidimensional Scaling (n-MDS) based on the concentration of all studied metals showing separate spatial patterns in the study area.

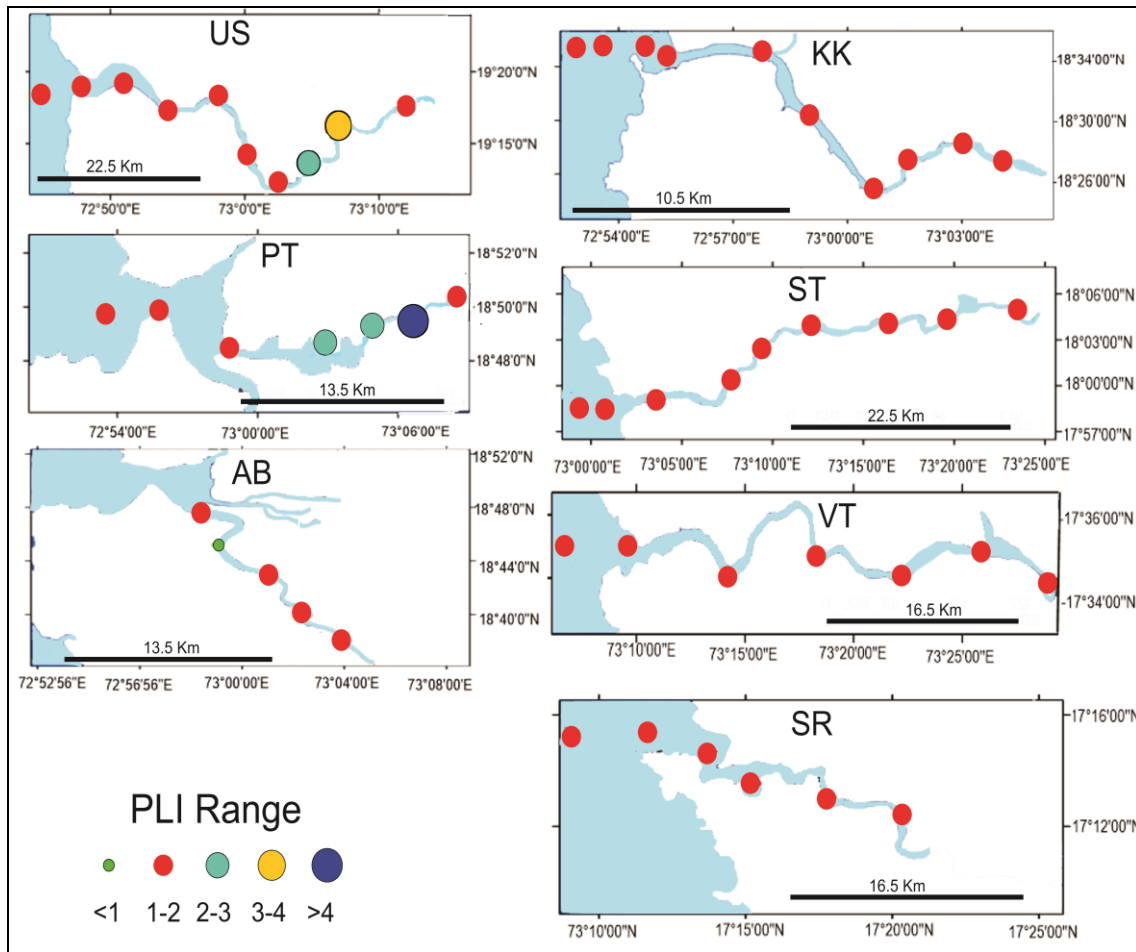


Fig 3. Map of the study area showing the mean Pollution Load Index (PLI) in the urbanized estuaries along the west coast of India.

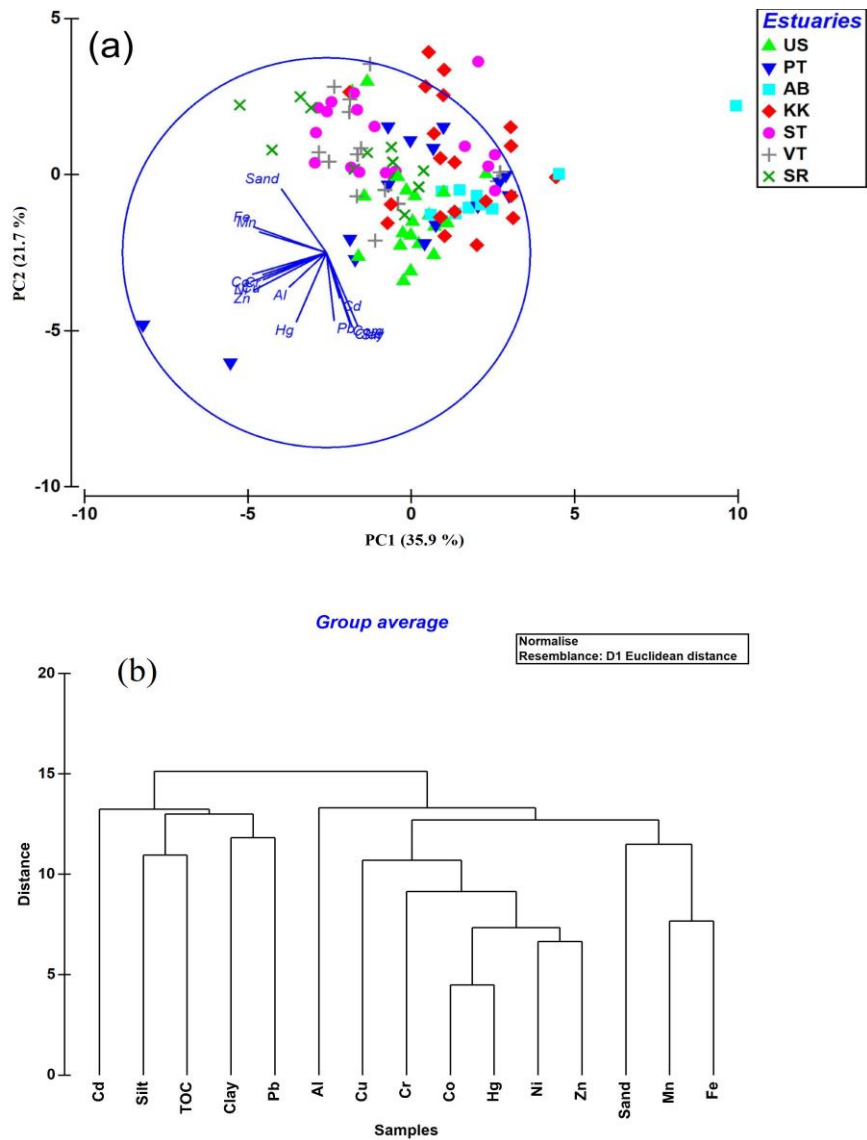


Fig 4. (a). Principal component analysis (PCA) representing loading pattern of grain size, TOC and various heavy metals in the sediments of study area. (b). Cluster Analysis (dendrogram) showing relationships among grain size, TOC and various heavy elements within the study area.

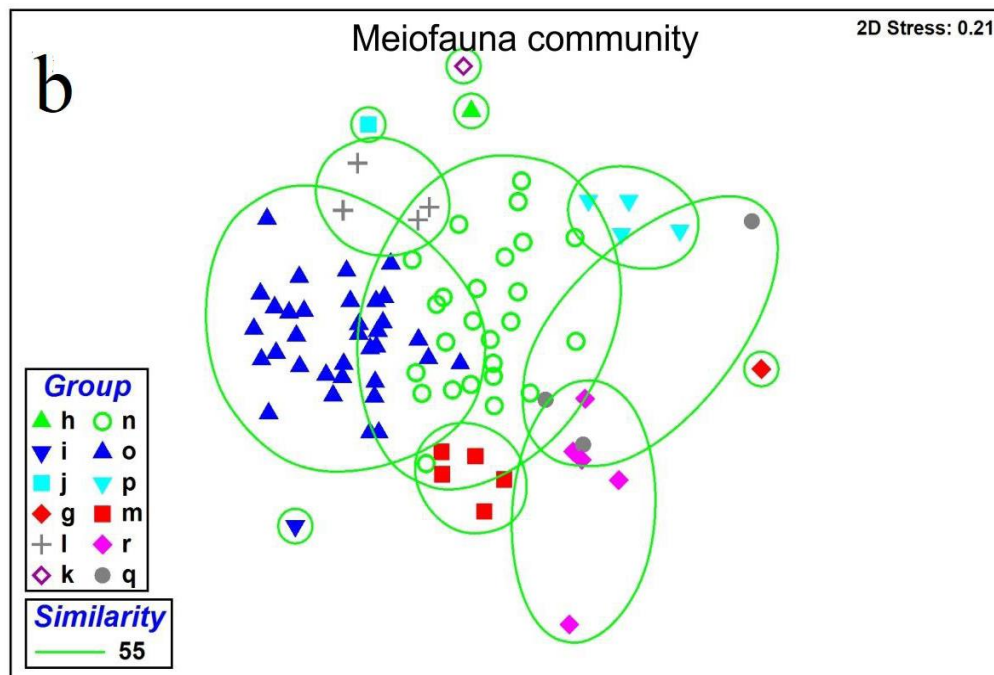
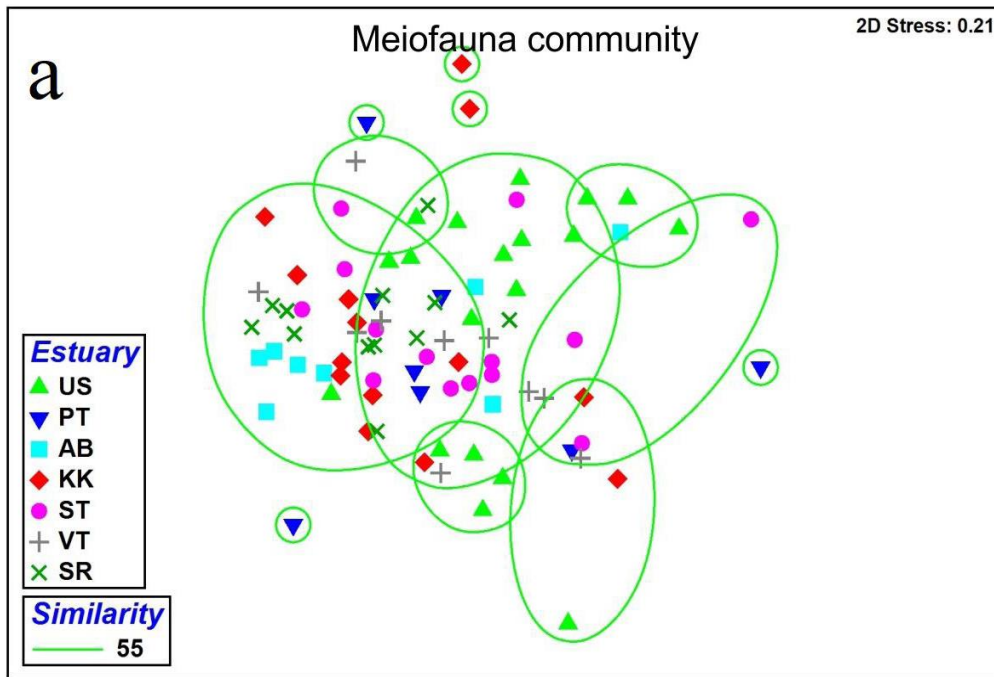


Fig 5. non-metric multidimensional scaling (n-MDS) ordination plot based on meiofaunal community data of seven estuaries along west coast of India. (a) Estuary wise differentiation; (b) group wise differentiation.

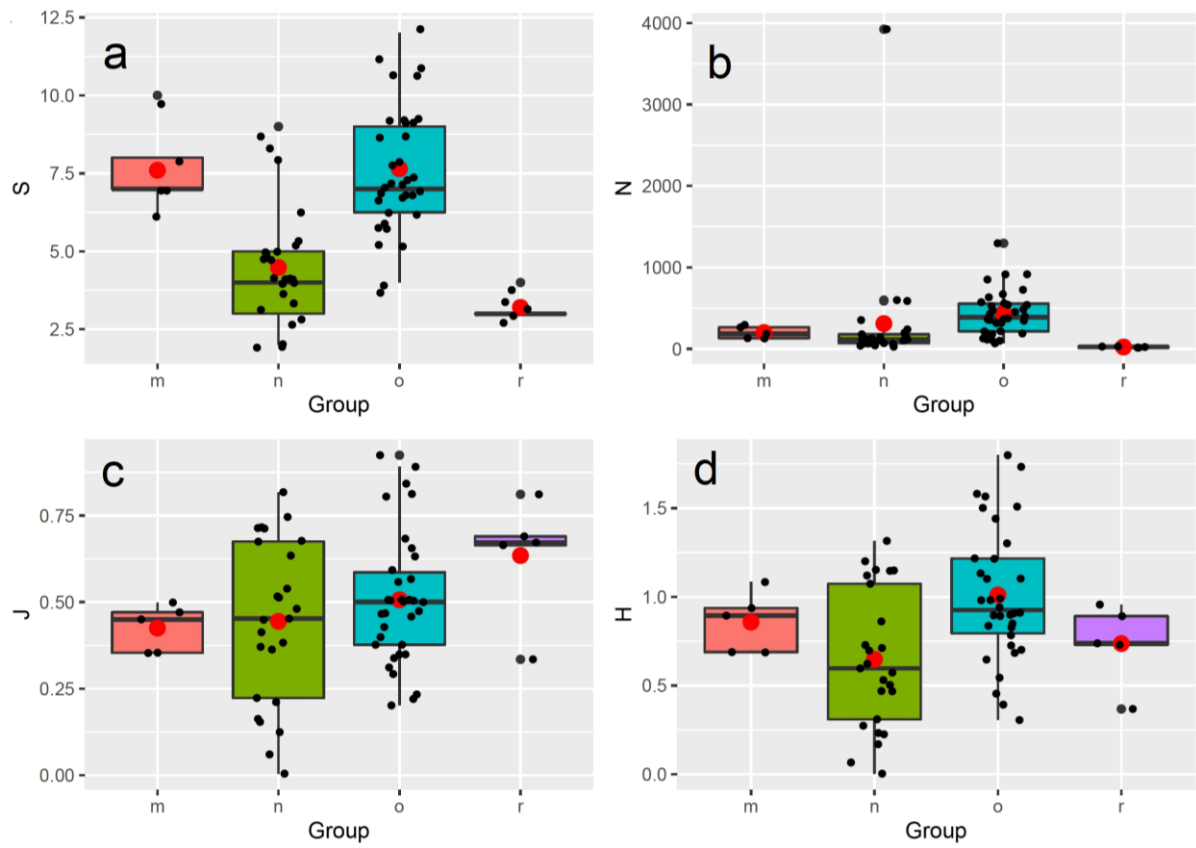


Fig 6. Box plot of univariate indices of major groups formed by n-MDS based on meiofaunal community data of seven estuaries along west coast of India. (a) S – taxa richness, (b) N – abundance, (c) J – evenness and (d) H - diversity.

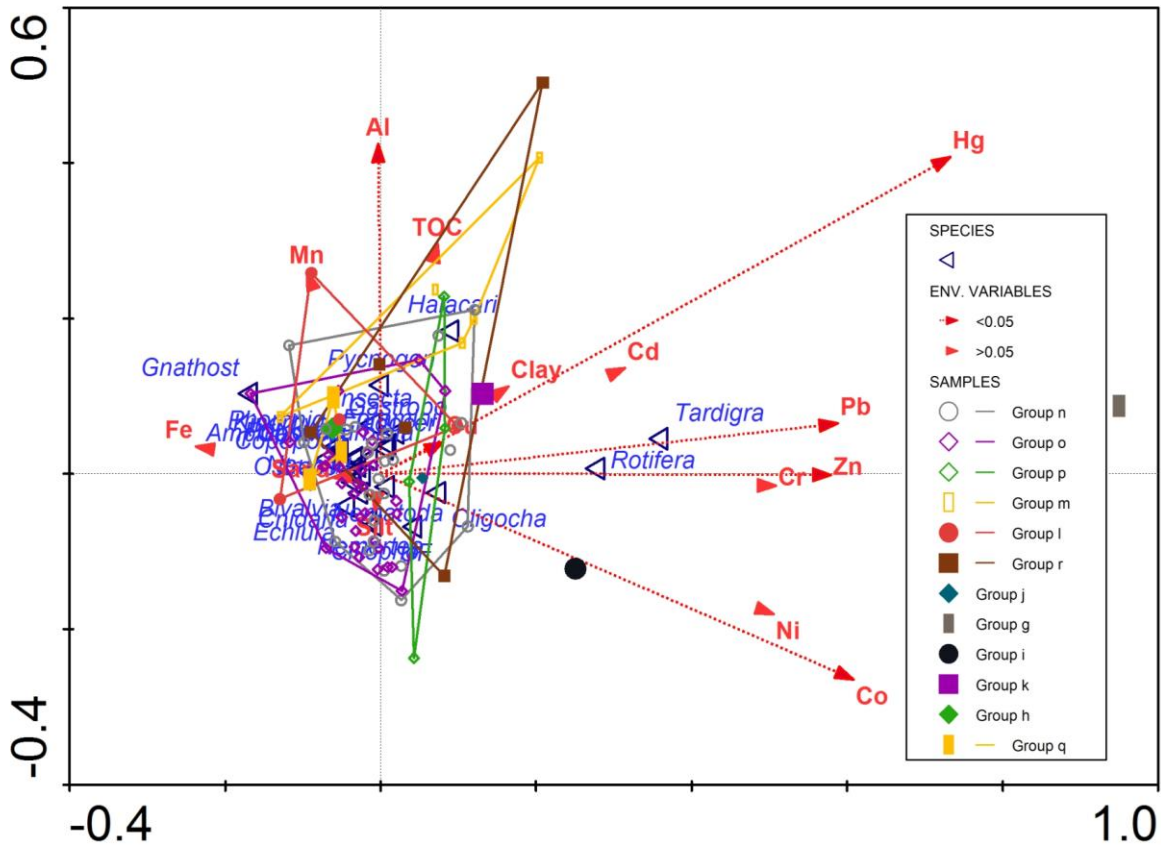


Fig 7. Ordination plot based on canonical correspondence analysis (CCA) of possible relationships between meiofauna and environmental variables of seven estuaries along west coast of India. The variables marked with the complete arrow were significant ($p < 0.05$) based on the Monte Carlo test. Samples were grouped based on the separation made in n-MDS plot.

Table 1. Concentration of heavy metals ($\mu\text{g/g}$) with $\pm\text{SD}$ in CRM-PACS-2 of the studied sediment.

Element	PACS 2 (Certified value)	Observe value	Recovery (%)
Al (%)	6.62 ± 0.32	6.4 ± 0.25	97.0
Cr ($\mu\text{g/g}$)	90.7 ± 4.6	86.3 ± 2.1	95.2
Mn ($\mu\text{g/g}$)	440 ± 19	427.0 ± 10	97.0
Fe (%)	4.09 ± 0.06	4.0 ± 0.02	96.8
Co ($\mu\text{g/g}$)	11.5 ± 0.3	11.7 ± 0.1	101.4
Ni ($\mu\text{g/g}$)	39.5 ± 2.3	38.8 ± 2.0	98.1
Cu ($\mu\text{g/g}$)	310 ± 12	297.1 ± 10	95.8
Zn ($\mu\text{g/g}$)	364 ± 23	346.9 ± 15	95.3
Pb ($\mu\text{g/g}$)	183 ± 8	175 ± 5	95.6
Cd ($\mu\text{g/g}$)	2.11 ± 0.15	2.0 ± 0.1	95.3
Hg ($\mu\text{g/g}$)	3.04 ± 0.20	3.0 ± 0.1	98.7

Table 2. Mean with standard deviation (\pm) of sand, silt, clay and C_{org} during post-monsoon and pre-monsoon seasons (n =110) along the seven studied estuaries (Rao et al., 2019).

Content	US		PT		AB		KK		ST		VT		SR	
	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre
Sand (%)	38.3 \pm 27.2	17.6 \pm 10.4	19.5 \pm 15.7	36.8 \pm 32.8	20.4 \pm 18.3	2.4 \pm 1.1	38.4 \pm 32.7	42.8 \pm 39.2	50.8 \pm 39.8	49.9 \pm 44.4	24.5 \pm 29.1	51.0 \pm 46.2	41.1 \pm 33.0	46.0 \pm 38.6
Silt (%)	47.4 \pm 27.7	51.6 \pm 16.5	63.0 \pm 18.4	36.0 \pm 28.8	57.9 \pm 23.1	68.9 \pm 13.3	52.8 \pm 33.2	45.0 \pm 34.8	42.4 \pm 36.4	42.4 \pm 39.6	61.5 \pm 25.4	43.8 \pm 43.4	49.6 \pm 32.4	45.1 \pm 36.3
Clay (%)	25.3 \pm 20.0	30.8 \pm 13.1	17.5 \pm 11.4	30.8 \pm 27.2	23.8 \pm 13.0	28.7 \pm 13.4	14.5 \pm 11.6	12.2 \pm 10.1	6.7 \pm 4.2	7.7 \pm 6.1	13.9 \pm 7.8	5.8 \pm 5.2	9.3 \pm 6.2	9.0 \pm 2.8
TOC (%)	2.4 \pm 1.0	2.2 \pm 0.8	2.3 \pm 1.1	1.8 \pm 1.2	2.2 \pm 0.3	2.0 \pm 0.3	1.7 \pm 1.1	1.6 \pm 1.0	1.5 \pm 1.1	1.5 \pm 1.0	1.5 \pm 1.1	1.2 \pm 1.0	1.3 \pm 0.9	1.0 \pm 0.9

Table 3. Comparison of the present study (n= 110) with heavy metals ($\mu\text{g/g}$) from different estuaries in the world.

Study area	Al (%)	Cr	Mn	Fe (%)	Co	Ni	Cu	Zn	Cd	Pb	Hg	References
Havana Bay	-	-	-	0.83	-	-	297	234	1.2	340	7.2	Gonza'lezandBru'gmann (1991)
Azerbaijan	-	-	-	3.71	-	-	31.9	83.2	0.14	19.6	0.15	Mora et al .(2004)
Pearl River Estuary, China	-	89	-	-	-	41.7	46.2	150	-	59.3	-	Zhou et al. (2004)
Bay of Bengal, South East Coast of India	-	506.2	373	2.72	-	38.61	506.2	126.83	6.58	32.36	-	Rajand Jayaprakash (2007)
Scheldt estuary, Europe	-	41.6-161.4	-	-	-	10.8-45.5	18.6-118.4	-	1.51-10.24	-	-	Laing et al., 2009
KafrainDam,Jordan Vellar and Coleroon Estuary	-	160	730	-	60	100	-	120	-	-	-	Ghrefat et al. (2011)
South East Coast of India	-	12.12-77.76	30.12-455	1493-11804 (ppm)	-	2.64-25.16	14.52-30.6	8.56-44.72	0.2-1.8	6.46-30.12	-	Sundaramanickam et al. (2016)
Poombuhar to Karaikal, East Coast, of Tamilnadu	41,725 (ppm)	383	1641	66216 (ppm)	23	42	-	90	-	50	-	Devanesan et al. (2017)
Korean coast	-	N.D.	-	-	-	ND	0.18-104	-	0-1.34	0.04-85.4	-	Hwang et al. (2019)
Yangtze Estuary, Chaina	-	69.5-103	-	-	-	18.8-38.9	14.3-32.1	-	0.037-0.212	13.7-23	-	Wang et al. (2020)
West coast of India	2.3-11	95-691	183-2663	3.7-20.9	25-940	26-218	34-468	10-777	0.06-1.8	2.7-76.5	ND-3.64	Present study

Table 4. Minimum, Maximum and mean with standard deviation (\pm) of E.F. and C.F. during post-monsoon and pre-monsoon seasons along the seven studied estuaries (n =110).

EF	US		PT		AB		KK		ST		VT		SR	
	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre
Cr	1.3-6.0 (3.5 \pm 1.5)	2.0-7.0 (3.4 \pm 1.8)	2.1-6.2 (3.7 \pm 1.7)	1.6-4.2 (2.8 \pm 1.2)	1.7-3.5 (2.3 \pm 0.8)	1.5-1.8 (1.6 \pm 0.1)	1.2-4.4 (2.0 \pm 0.9)	1.5-3.1 (2.3 \pm 0.5)	1.5-3.9 (2.3 \pm 0.8)	1.5-2.8 (2.0 \pm 0.5)	1.5-3.1 (1.9 \pm 0.6)	1.3-2.2 (1.8 \pm 0.3)	1.2-3.4 (2.2 \pm 0.7)	1.4-4.4 (2.7 \pm 1.2)
Mn	1.0-3.3 (1.6 \pm 0.8)	1.0-1.7 (1.4 \pm 0.2)	1.1-3.3 (1.8 \pm 0.8)	0.9-2.1 (1.4 \pm 0.4)	0.7-1.4 (1.0 \pm 0.3)	0.9-1.6 (1.3 \pm 0.3)	0.9-3.0 (1.5 \pm 0.6)	0.8-2.4 (1.6 \pm 0.5)	0.9-3.3 (2.0 \pm 0.8)	0.8-2.0 (1.5 \pm 0.5)	0.9-3.3 (1.7 \pm 0.9)	0.8-2.9 (1.9 \pm 0.8)	1.1-3.0 (1.8 \pm 0.6)	1.2-3.8 (2.4 \pm 1.1)
Co	0.7-2.1 (1.4 \pm 0.5)	1.1-2.1 (1.4 \pm 0.3)	0.8-12.8 (3.2 \pm 4.3)	1.3-18.0 (4.1 \pm 6.2)	0.8-1.7 (1.3 \pm 0.3)	0.9-1.3 (1.1 \pm 0.2)	0.8-1.8 (1.1 \pm 0.3)	1.1-2.1 (1.6 \pm 0.3)	0.8-2.3 (1.4 \pm 0.5)	1.1-2.8 (1.7 \pm 0.5)	0.9-2.7 (1.6 \pm 0.6)	1.1-2.4 (1.8 \pm 0.5)	0.8-2.5 (1.7 \pm 0.7)	1.3-4.5 (2.6 \pm 1.5)
Ni	0.7-1.8 (1.2 \pm 0.3)	1.0-1.7 (1.2 \pm 0.2)	1.8-4.1 (2.8 \pm 1.0)	2.0-5.1 (2.8 \pm 1.1)	2.2-3.3 (2.5 \pm 0.5)	2.0-2.6 (2.2 \pm 0.2)	1.5-4.1 (2.1 \pm 0.7)	1.9-3.4 (2.4 \pm 0.4)	0.8-1.5 (1.0 \pm 0.2)	0.9-1.2 (1.0 \pm 0.1)	1.7-2.8 (2.0 \pm 0.4)	1.9-3.1 (2.5 \pm 0.4)	1.6-3.0 (2.3 \pm 0.5)	2.0-4.7 (3.0 \pm 1.2)
Cu	0.6-2.6 (1.4 \pm 0.5)	1.3-2.7 (1.6 \pm 0.4)	0.6-5.6 (2.4 \pm 1.9)	1.1-5.0 (2.7 \pm 1.8)	1.0-1.3 (1.2 \pm 0.1)	1.0-1.4 (1.2 \pm 0.1)	0.9-1.7 (1.2 \pm 0.2)	1.1-1.6 (1.3 \pm 0.2)	0.8-2.6 (1.6 \pm 0.7)	1.2-3.4 (2.0 \pm 0.7)	1.0-4.2 (2.5 \pm 1.0)	1.3-3.4 (2.5 \pm 0.7)	1.5-4.3 (2.5 \pm 1.0)	1.8-6.4 (3.5 \pm 1.9)
Zn	1.2-8.8 (2.7 \pm 2.2)	1.3-9.3 (2.7 \pm 2.4)	1.2-4.3 (2.0 \pm 1.1)	0.9-5.9 (2.0 \pm 1.8)	0.4-1.2 (1.0 \pm 0.3)	0.9-1.3 (1.0 \pm 0.2)	1.1-1.7 (1.3 \pm 0.2)	0.9-1.3 (1.1 \pm 0.1)	1.0-2.6 (1.5 \pm 0.5)	0.9-2.0 (1.3 \pm 0.4)	1.0-2.8 (1.6 \pm 0.6)	1.0-2.2 (1.7 \pm 0.4)	0.9-2.7 (1.6 \pm 0.6)	1.0-4.6 (2.5 \pm 1.4)
Cd	1.0-5.7 (2.5 \pm 1.4)	1.3-3.7 (2.0 \pm 0.8)	3.5-5.6 (4.4 \pm 0.7)	1.6-12.3 (3.5 \pm 3.9)	1.1-3.7 (1.9 \pm 1.1)	1.4-2.7 (1.9 \pm 0.5)	1.4-14.7 (5.3 \pm 4.4)	0.7-3.6 (2.0 \pm 0.9)	0.9-1.8 (1.2 \pm 0.3)	1.0-2.0 (1.3 \pm 0.3)	0.6-1.5 (1.0 \pm 0.3)	1.1-1.7 (1.3 \pm 0.3)	0.4-2.0 (1.0 \pm 0.5)	1.0-1.8 (1.3 \pm 0.3)
Pb	0.4-8.5 (1.8 \pm 1.8)	0.9-5.4 (2.0 \pm 1.4)	1.1-2.5 (1.9 \pm 0.6)	0.9-2.4 (1.7 \pm 0.5)	0.6-1.8 (2.3 \pm 0.4)	0.8-1.5 (1.2 \pm 0.2)	1.2-5.2 (1.2 \pm 0.5)	0.2-1.1 (1.7 \pm 0.6)	0.4-2.7 (0.8 \pm 0.3)	0.8-1.8 (0.7 \pm 0.4)	0.7-3.2 (0.9 \pm 0.2)	0.7-4.0 (0.6 \pm 0.4)	0.9-2.0 (0.9 \pm 0.3)	0.9-3.2 (0.9 \pm 0.1)
Hg	0.6-6.8 (3.2 \pm 2.6)	0.8-5.9 (3.9 \pm 1.4)	0.2-24.3 (4.9 \pm 8.7)	5.1 \pm 11.3 ()	1.8-2.7 (1.3 \pm 0.5)	1.0-1.5 (1.1 \pm 0.3)	0.4-2.3 (3.0 \pm 1.6)	0.8-2.5 (0.7 \pm 0.3)	0.3-1.4 (1.3 \pm 0.8)	0.2-1.4 (1.2 \pm 0.3)	0.6-1.3 (1.6 \pm 0.8)	0.3-1.4 (1.6 \pm 1.1)	0.5-1.2 (1.4 \pm 0.4)	0.9-1.0 (2.2 \pm 0.9)
CF														
Cr	1.5-5.7 (3.4 \pm 1.2)	1.9-6.8 (3.3 \pm 1.8)	1.7-5.1 (3.1 \pm 1.4)	1.5-5.0 (2.8 \pm 1.2)	0.9-3.0 (1.8 \pm 0.8)	1.1-2.1 (1.6 \pm 0.4)	1.0-2.5 (1.6 \pm 0.4)	1.6-2.7 (2.1 \pm 0.4)	1.4-3.6 (2.3 \pm 0.7)	1.5-2.9 (2.2 \pm 0.5)	1.5-2.8 (2.1 \pm 0.4)	1.2-1.9 (1.7 \pm 0.2)	1.8-2.9 (2.2 \pm 0.4)	1.7-3.3 (2.4 \pm 0.6)
Mn	1.1-2.7 (1.6 \pm 0.6)	1.0-1.6 (1.3 \pm 0.2)	0.9-2.6 (1.4 \pm 0.6)	0.8-1.9 (1.8 \pm 0.3)	0.2-1.3 (0.8 \pm 0.4)	0.9-1.7 (1.2 \pm 0.3)	0.7-1.9 (1.3 \pm 0.4)	0.9-2.2 (1.5 \pm 0.4)	0.9-3.3 (2.0 \pm 0.8)	0.8-2.1 (1.6 \pm 0.5)	0.9-3.0 (1.8 \pm 0.7)	0.7-2.6 (1.8 \pm 0.7)	1.4-2.3 (1.7 \pm 0.3)	1.5-2.9 (2.0 \pm 0.6)
Co	0.8-2.2 (1.3 \pm 0.4)	1.1-1.9 (1.4 \pm 0.2)	0.7-13.7 (3.0 \pm 4.8)	1.2-21.4 (4.5 \pm 7.5)	0.6-1.2 (1.0 \pm 0.3)	0.8-1.4 (1.1 \pm 0.2)	0.7-1.1 (0.9 \pm 0.1)	1.2-1.9 (1.5 \pm 0.2)	0.8-2.2 (1.5 \pm 0.5)	1.1-2.3 (1.8 \pm 0.4)	0.9-2.4 (1.8 \pm 0.5)	1.0-2.2 (1.8 \pm 0.4)	1.2-2.3 (1.7 \pm 0.4)	1.3-3.3 (2.2 \pm 0.9)
Ni	1.7-3.2 (2.5 \pm 0.5)	2.1-3.6 (2.5 \pm 0.4)	1.5-4.4 (2.4 \pm 1.0)	1.9-6.1 (2.8 \pm 1.5)	0.7-2.9 (2.0 \pm 0.8)	1.6-2.4 (2.2 \pm 0.4)	1.4-2.4 (1.7 \pm 0.3)	1.9-2.8 (2.3 \pm 0.3)	1.4-2.9 (2.2 \pm 0.5)	1.9-2.8 (2.3 \pm 0.3)	1.7-2.5 (2.2 \pm 0.3)	1.9-2.7 (2.4 \pm 0.3)	2.1-2.8 (2.3 \pm 0.3)	1.9-3.6 (2.6 \pm 0.6)
Cu	0.5-2.4 (1.4 \pm 0.6)	1.1-2.7 (1.6 \pm 0.4)	0.5-4.4 (2.1 \pm 1.6)	1.0-5.7 (2.9 \pm 2.2)	0.4-1.2 (0.9 \pm 0.3)	0.7-1.6 (1.2 \pm 0.3)	0.6-1.8 (1.0 \pm 0.3)	0.7-1.8 (1.3 \pm 0.3)	0.7-3.2 (1.7 \pm 0.8)	1.2-2.7 (2.1 \pm 0.6)	1.0-5.4 (2.9 \pm 1.3)	1.2-3.5 (2.4 \pm 0.7)	1.8-3.3 (2.4 \pm 0.6)	2.0-4.8 (3.0 \pm 1.2)
Zn	1.3-8.4 (2.7 \pm 2.1)	1.1-9.3 (2.7 \pm 2.4)	1.0-4.6 (1.8 \pm 1.3)	0.9-7.0 (2.1 \pm 2.2)	0.1-1.1 (0.8 \pm 0.4)	0.6-1.2 (1.0 \pm 0.3)	0.9-1.4 (1.1 \pm 0.2)	0.7-1.4 (1.0 \pm 0.2)	1.0-2.4 (1.5 \pm 0.5)	0.9-1.6 (1.3 \pm 0.3)	1.1-2.5 (1.8 \pm 0.5)	1.0-1.9 (1.6 \pm 0.3)	1.3-2.1 (1.6 \pm 0.3)	1.2-3.5 (2.1 \pm 0.9)
Cd	1.1-5.5 (2.5 \pm 1.4)	1.2-3.7 (1.9 \pm 0.8)	2.6-4.4 (3.7 \pm 0.7)	1.2-12.9 (3.6 \pm 4.1)	1.1-1.6 (1.3 \pm 0.2)	1.6-2.1 (1.8 \pm 0.2)	1.4-12.9 (4.6 \pm 4.2)	0.8-4.1 (1.9 \pm 0.9)	0.8-1.7 (1.2 \pm 0.3)	1.1-1.8 (1.4 \pm 0.2)	0.6-1.9 (1.2 \pm 0.5)	1.0-1.7 (1.3 \pm 0.2)	0.4-1.6 (1.0 \pm 0.4)	0.9-1.4 (1.1 \pm 0.2)
Pb	0.7-6.5 (1.8 \pm 1.7)	0.8-5.9 (2.0 \pm 1.5)	0.9-2.7 (1.6 \pm 0.6)	0.8-2.9 (1.7 \pm 0.6)	0.9-2.4 (1.8 \pm 0.6)	0.7-1.5 (1.2 \pm 0.3)	0.4-2.4 (1.0 \pm 0.5)	0.7-2.7 (1.6 \pm 0.6)	0.3-1.2 (0.8 \pm 0.3)	0.2-1.4 (0.8 \pm 0.4)	0.7-1.7 (1.0 \pm 0.3)	0.3-1.4 (0.6 \pm 0.4)	0.6-1.1 (0.9 \pm 0.2)	0.6-1.1 (0.8 \pm 0.2)
Hg	0.4-9.1 (3.3 \pm 2.8)	0.8-5.8 (3.8 \pm 1.4)	0.1-26.0 (4.9 \pm 9.4)	5.9 \pm 13.5 ()	0.6-1.5 (1.0 \pm 0.4)	0.8-1.1 (1.0 \pm 0.1)	1.1-5.0 (2.5 \pm 1.5)	0.2-1.2 (0.7 \pm 0.3)	0.4-2.6 (1.3 \pm 0.8)	1.0-1.8 (1.3 \pm 0.3)	0.6-4.1 (1.9 \pm 1.1)	0.6-4.1 (1.6 \pm 1.2)	1.0-1.8 (1.4 \pm 0.3)	0.7-3.0 (2.2 \pm 1.1)

Table 5. Pearson correlation between sedimentary parameters in the study area (n =110). Significance level, Bold value p<0.01 and *p<0.05.

	Sand	Silt	Clay	TOC	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	Hg	
Sand	1.00															
Silt	-0.90	1.00														
Clay	-0.51	0.09	1.00													
TOC	-0.52	0.44	0.31	1.00												
Al	-0.02	0.01	0.02	0.19	1.00											
Cr	0.20*	-0.19*	-0.08	0.04	0.03	1.00										
Mn	0.40	-0.32	-0.29	-0.21*	0.16	0.39	1.00									
Fe	0.38	-0.25	-0.38	-0.32	0.14	0.36	0.73	1.00								
Co	0.11	-0.08	-0.09	-0.02	0.16	0.52	0.17	0.26	1.00							
Ni	0.22*	-0.20*	-0.12	-0.02	0.31	0.76	0.39	0.47	0.79	1.00						
Cu	0.09	-0.02	-0.17	0.04	0.37	0.32	0.29	0.60	0.49	0.58	1.00					
Zn	0.08	-0.09	0.00	0.00	0.24	0.68	0.34	0.43	0.78	0.79	0.59	1.00				
Cd	-0.19	0.12	0.19*	0.25	-0.03	0.04	-0.20	-0.23	0.04	-0.04	0.04	0.02	1.00			
Pb	-0.26	0.12	0.35	0.33	0.11	0.30	-0.25	-0.32	0.30	0.30	0.06	0.32	0.18	1.00		
Hg	-0.08	0.05	0.09	0.15	0.19	0.44	0.01	0.02	0.91	0.66	0.34	0.77	0.17	0.42	1.00	

Table 6: Integration of sediment quality with benthic faunal univariate indices used for assessment in the study area (n=110).

Name of Estuary	Heavy metals	Overall assessment	Meiofauna	Overall assessment
U.S.	●	Impaired	●	Impaired
P.T.	●	Impaired	●	Impaired
AB	●	Impaired	○	Unimpaired
K.K.	●	Impaired	○	Unimpaired
ST	●	Impaired	●	Impaired
VT	●	Impaired	●	Impaired
SR	●	Impaired	●	Impaired

Heavy metals: “●” indicates more than one heavy metals concentration in all estuaries are higher than TEL. Benthic fauna (i.e. meiofauna): “●” indicates significant reduction in benthic community richness and diversity compared to mean of reference sites; “○” no reduction in benthic community indices (more than one) compared to mean of reference sites.

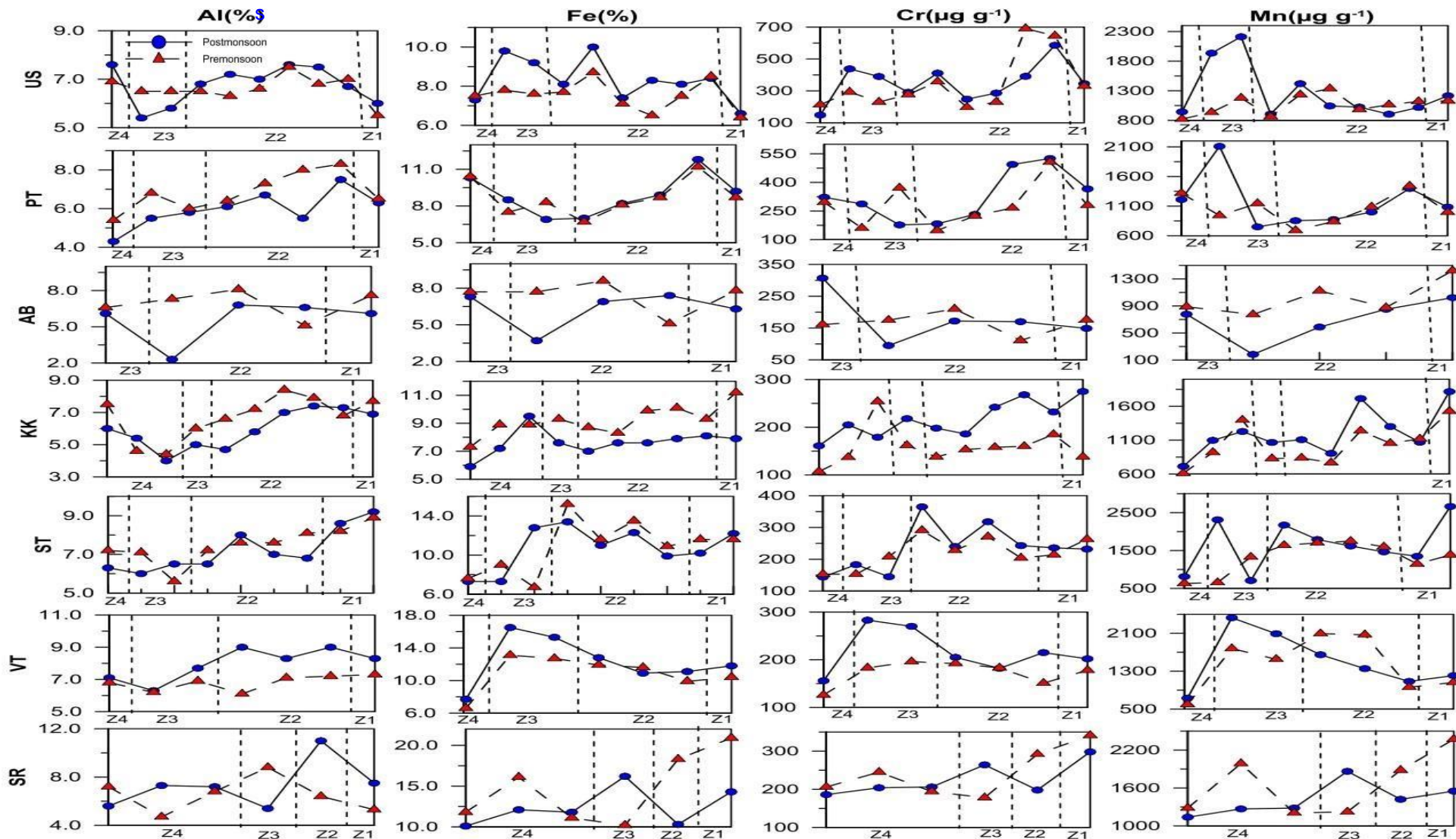


Fig S1.

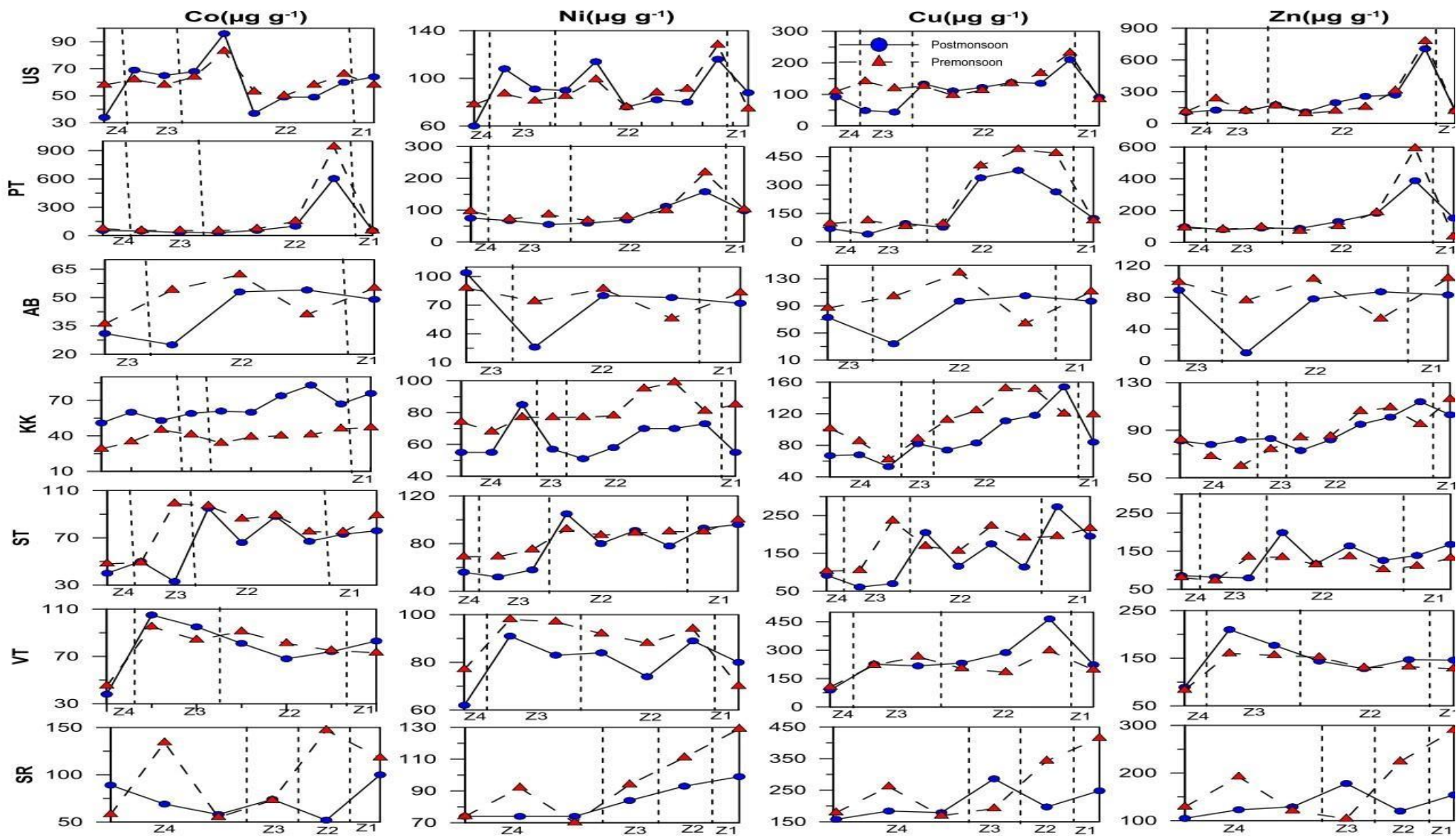


Fig S2.

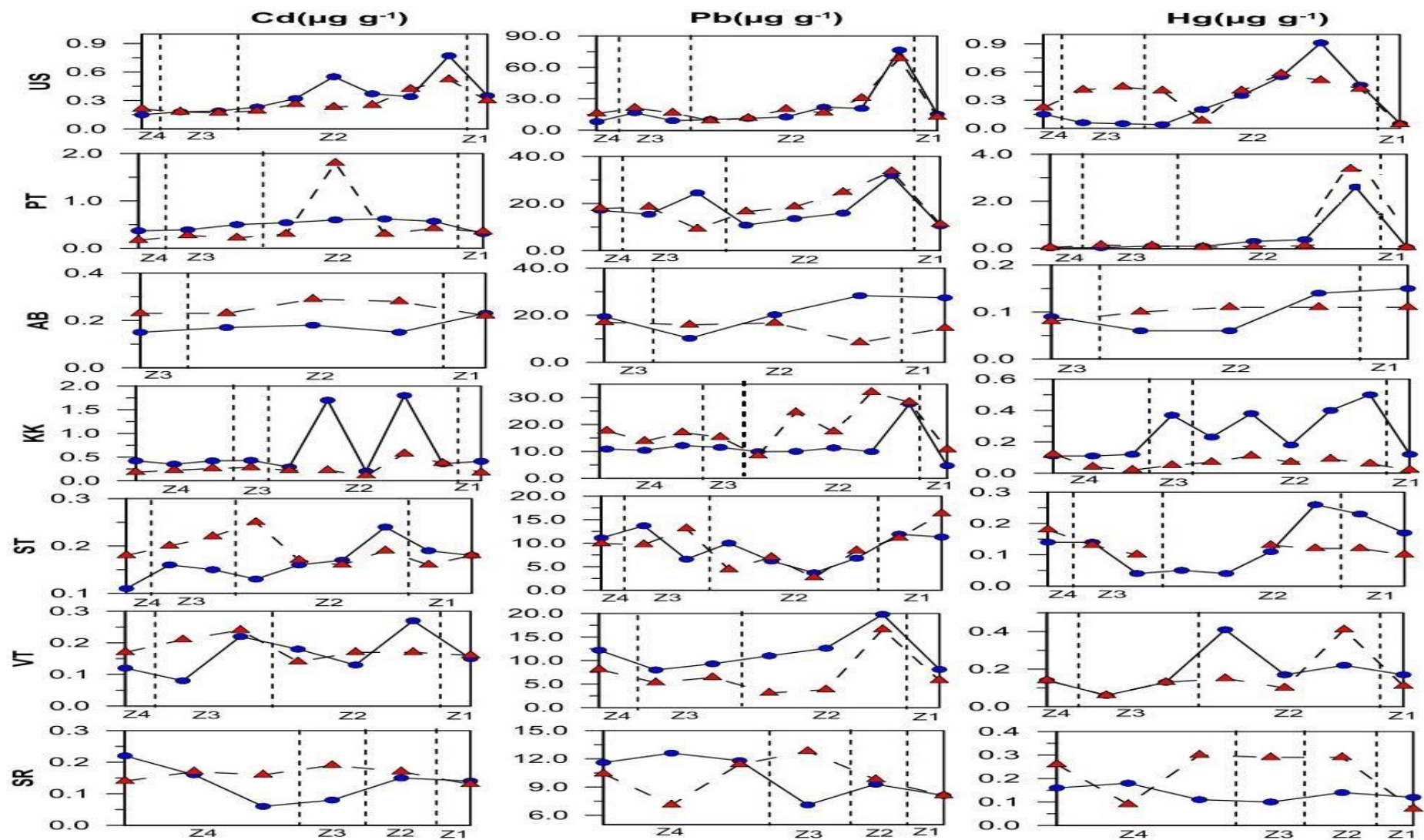


Fig S3

Table S1.

Factors	df	SS	MS	PseudoF	P(perm)	Unique perms	P(MC)
Seasons	1	1506.1	1506.1	1.0861	0.353	998	0.34
Estuaries	6	17209	2868.1	2.2507	0.001	999	0.001
Segments	2	9405.2	4702.6	3.5971	0.001	999	0.002
Groups (nMDS based)	11	64856	5896	8.3171	0.001	996	0.001

Table S2.

Factors	t	P(perm)	Unique perms	P(MC)
Estuaries				
US, PT	1.4298	0.054	999	0.076
US, AB	1.8483	0.006	998	0.013
US, KK	1.7926	0.009	998	0.008
US, ST	1.2447	0.161	999	0.158
US, VT	1.5738	0.029	999	0.04
US, SR	2.3227	0.001	999	0.001
PT, AB	1.0576	0.384	921	0.371
PT, KK	0.98826	0.461	998	0.423
PT, ST	1.2343	0.157	998	0.205
PT, VT	1.2349	0.154	989	0.157
PT, SR	1.8473	0.002	995	0.003
AB, KK	0.75003	0.774	997	0.73
AB, ST	1.4257	0.075	999	0.086
AB, VT	1.418	0.069	991	0.093
AB, SR	1.307	0.144	992	0.128
KK, ST	1.5492	0.034	998	0.04
KK, VT	1.4485	0.063	999	0.078
KK, SR	1.5988	0.018	998	0.029
ST, VT	0.78905	0.692	997	0.655
ST, SR	1.6612	0.02	997	0.027
VT, SR	1.6099	0.021	997	0.038
Segments				
North, middle	1.8655	0.002	997	0.003
North, south	1.9304	0.002	999	0.002
Middle, south	1.888	0.002	999	0.005
Groups (nMDS based)				
n, o	4.2268	0.001	999	0.001
n, m	2.5947	0.001	997	0.001
n, r	3.3943	0.001	994	0.001
o, m	3.1656	0.001	997	0.001
o, r	4.4568	0.001	999	0.001
m, r	3.0989	0.008	126	0.001

Table S3.

Group n					
Average squared distance = 10.08					
Species	Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
Co	-0.18	0.04	0.49	0.36	0.36
Hg	-0.06	0.212	0.5	2.1	2.46
Zn	-0.24	0.24	0.37	2.38	4.84
Ni	-0.13	0.458	0.5	4.54	9.38
Sand	-0.18	0.616	0.42	6.12	15.5
Fe	-0.21	0.653	0.38	6.48	21.99
Pb	-0.05	0.681	0.48	6.76	28.74
Silt	0.16	0.684	0.48	6.79	35.53
Cu	-0.18	0.75	0.37	7.45	42.98
TOC	0.18	0.752	0.46	7.47	50.45
Cr	-0.06	0.791	0.46	7.85	58.3
Al	0.02	0.869	0.33	8.63	66.93
Clay	0.10	0.873	0.52	8.66	75.59
Mn	-0.21	0.977	0.41	9.7	85.29
Cd	0.00	1.48	0.23	14.71	100
Group o					
Average squared distance = 11.29					
Species	Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
Co	-0.11	0.06	0.42	0.55	0.55
Hg	-0.29	0.128	0.37	1.13	1.68
Cd	-0.23	0.13	0.38	1.15	2.83
Zn	-0.21	0.437	0.39	3.87	6.7
Cr	-0.26	0.483	0.45	4.28	10.98
Ni	-0.11	0.545	0.45	4.83	15.81
TOC	-0.27	0.801	0.5	7.09	22.9
Pb	-0.06	0.807	0.42	7.15	30.05
Al	-0.12	0.855	0.47	7.57	37.62
Mn	0.00	1.04	0.45	9.2	46.82
Sand	-0.01	1.08	0.52	9.59	56.41
Cu	0.01	1.12	0.39	9.96	66.37
Clay	-0.08	1.13	0.28	9.98	76.35
Silt	0.05	1.18	0.54	10.43	86.78
Fe	0.21	1.49	0.41	13.22	100

Group m
Average squared distance = 6.10

Species	Av.Value	Av.Sq.Distance	Sq.Distance/SD	Contrib%	Cum.%
Co	-0.22	0.01	0.6	0.12	0.12
Sand	-0.83	0.01	0.52	0.16	0.28
Mn	0.31	0.117	0.59	1.92	2.19
Al	0.77	0.141	0.6	2.32	4.51
Ni	-0.03	0.169	0.62	2.77	7.29
Fe	-0.28	0.189	0.52	3.1	10.39
Silt	0.47	0.196	0.58	3.21	13.6
Cr	0.25	0.324	0.57	5.32	18.92
Hg	0.80	0.333	0.52	5.46	24.38
Zn	0.63	0.468	0.52	7.67	32.05
Cu	-0.01	0.604	0.49	9.91	41.96
Pb	0.68	0.726	0.53	11.9	53.86
TOC	1.28	0.752	0.59	12.34	66.2
Clay	0.96	0.966	0.59	15.85	82.05
Cd	0.44	1.09	0.51	17.95	100

Group r
Average squared distance = 13.82

Species	Av.Value	Av.Sq.Distance	Sq.Distance/SD	Contrib%	Cum.%
Co	-0.20	0.02	0.53	0.12	0.12
Cd	0.31	0.218	0.52	1.58	1.7
Fe	-0.15	0.295	0.55	2.14	3.83
Cu	-0.40	0.337	0.6	2.43	6.27
Hg	-0.14	0.375	0.49	2.72	8.98
Silt	-1.07	0.438	0.56	3.17	12.15
Clay	-0.50	0.44	0.51	3.18	15.33
TOC	-0.59	0.581	0.62	4.2	19.54
Ni	0.08	0.594	0.52	4.3	23.83
Mn	0.44	0.7	0.54	5.07	28.9
Sand	1.15	0.726	0.53	5.25	34.15
Al	-0.48	1.07	0.5	7.71	41.87
Zn	0.36	1.89	0.5	13.68	55.55
Cr	0.56	2.32	0.59	16.77	72.31
Pb	0.31	3.83	0.61	27.69	100

Table S4.

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Nematoda	4.64	40.61	4.39	65.11	65.11
Foraminifera	2.05	10.96	1.08	17.58	82.68
Copepoda	1.09	5.92	0.93	9.5	92.18
Group o: Average similarity: 62.52					
Nematoda	5.29	22.82	3.71	36.51	36.51
Foraminifera	2.95	11.94	2.61	19.1	55.6
Copepoda	2.97	11.64	2.24	18.62	74.23
Polychaeta	2.23	6.38	1.34	10.2	84.42
Nauplii	1.64	3.75	0.79	6	90.42
Group m: Average similarity: 65.49					
Foraminifera	4.79	30.8	14.19	47.04	47.04
Nematoda	3.25	18.36	6.85	28.04	75.08
Halacaridae	1.55	6.31	1.12	9.64	84.71
Copepoda	0.94	4.91	2.56	7.49	92.2
Group r: Average similarity: 72.80					
Foraminifera	2.6	45.14	5.21	62	62
Nematoda	1.95	24.94	2.08	34.26	96.26

Table 5.

Variable	Marginal Effects		Conditional Effects		
	Var.N	Lambda1	LambdaA	P	F
Hg	10	0.08	0.08	0.002	4.55
Co	5	0.06	0.04	0.05	2.78
Cu	7	0.04	0.06	0.01	3.18
Zn	8	0.05	0.04	0.002	2.88
Al	1	0.04	0.04	0.034	2.03
Pb	11	0.05	0.02	0.04	1.79
Fe	4	0.03	0.02	0.25	1.19
Sand	13	0.02	0.03	0.058	1.61
Ni	6	0.05	0.02	0.122	1.51
TOC	12	0.02	0.02	0.298	1.15
Silt	14	0.02	0.01	0.568	0.84
Mn	3	0.02	0.01	0.67	0.75
Cr	2	0.05	0.01	0.984	0.44
Cd	9	0.02	0	0.972	0.36
Clay	15	0.03	0.01	1	0.03

Table S6.

Overall	Estuary	Toxic metals							Metals	assessment
		Cr	Ni	Cu	Zn	Cd	Pb	Hg		
	Ulhas	288.1	87.1	115.5	163.7	0.3	17.3	0.3	●	Impaired
	Patalganga	302.8	94.3	194.2	159.8	0.5	20.3	0.5	●	Impaired
	Amba	177.0	75.8	93.0	79.0	0.2	18.2	0.1	●	Impaired
	Kundalika	187.8	72.0	100.4	88.6	0.4	15.2	0.2	●	Impaired
	Savitri	227.3	81.7	160.8	121.1	0.2	9.1	0.1	●	Impaired
	Vashishthi	194.4	84.2	229.2	141.6	0.2	9.3	0.2	●	Impaired
	Shastri	234.3	89.0	234.3	155.8	0.1	10.0	0.2	●	Impaired
	TELa	37.3	35.0	35.7	123.0	0.6	18.0	0.2		
	PELa	90.0	91.3	197.0	315.0	3.5	36.0	0.5		
			Me-S	Ma-J	Me-H			Meiofauna		Overall assessment
	Ulhas		5.105263158	0.38	0.68530769			Impaired		●
	Patalganga		7.125	0.46	0.766129289			Impaired		●
	Amba		7.75	0.56	0.98875202			Unimpaired		○
	Kundalika		6.285714286	0.61	1.134632595			Unimpaired		○
	Savitri		4.642857143	0.56	0.908663863			Impaired		●
	Vashishthi		5.7	0.5	0.958461991			Impaired		●
	Shastri		6.75	0.38	0.737030327			Impaired		●

Ref site 7.647058824 0.51 1.010233322