Assessment of heavy metal contamination by multivariate statistical methods from the sediment of Ulhas river estuary, Maharashtra, India

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ABSTRACT

Ulhas River estuary is one of the most significant estuarine systems situated western coast of India. This estuary has been polluted by several point and nonpoint source and therefore, the multivariate statistical methods were used to determine sediments parameter concentrations, their distributions, and their relationship. In the present study, sediment samples were collected from five different stations and analyzed eight heavy metals' concentrations with seven other parameters. The multivariate statistical methods (PCA, nMDS, and ANOSIM) were used to determine sediments parameter concentrations, their distributions, and their relationship. The PCA results showed that the concentrations of N%, H%, S%, C/N, C/H, EC, and OC% were significant contributors to PC1 (36%) while the heavy metals such as Cu, Pb, Cd, Ni, Si, and Hg concentration were major contributor to PC2 (20%). Both PCs are indicated anthropogenic pollutant deposition towards the mouth of the estuary. Other results of nMDS showed a high degree of similarities within the stations such as 2, 3, and 4. Moreover, analysis of similarities (ANOSIM) results also support them at a significant level of 0.01% with a global R-value (0.6). The observed level of heavy metals contamination in the sediment samples was in the order of Cr >Pb >Cu > Ni >Zn > Hg >Si>Cd. Industrial discharges within the catchment area may be the potential source of sediment pollution and warrants immediate targeted actions to protect this vital ecosystem and its biodiversity.

Introduction

Coastal zones are rich in natural resources despite being adversely influenced by human activities and ongoing/projected climate impacts (Goudie, 2006). Along the coastal areas, estuaries are ecologically dynamic and fragile ecosystems with unique physico-chemical and biological features (Rathod, 2005). The surface water chemistry of an estuary (at any point) reflects major influences such as the lithology of the catchment, atmospheric inputs, climatic conditions, and anthropogenic inputs (Vase et al., 2018; Bhutiani et al., 2018; Tyagi et al., 2020). Timely identification and critical quantification of these influences are vital for managing land and water resources within the catchment (Bellos et al., 2005). Derived trace metals from the catchment have been shown to accumulate in estuarine sediment and potentially capable of bioaccumulation in aquatic flora and fauna (Diagomanolin et al., 2004). The ulhas river estuary is important in the Arabian Sea region of India. Not only does it support a biodiversity rich mangrove ecosystem, but also acts as a vital nursery ground for many commercially important fish species, which support the livelihoods and food security of local communities (Rathod and Patil, 2009). Nonetheless, the estuary suffers from various anthropogenic stressors within its catchment (Bhosale, 1991; Mirajkar et al., 1995).
This is not surprising as the Ulhas river flows through the industrial (electrical, chemical, and textile) zone of the cities of Thane, Ambernath, and Ulhasnagar, which release their mostly untreated effluent directly into the river (Rathod and Patil, 2009), mainly due to the high cost of the available treatment processes (Dotaniya et al., 2016). As a result, the trace/toxic elements in dissolved and particulate form end up in the estuary (Pavoni et al., 2021) and contribute substantially towards its deterioration. Moreover, Thane creek is more vulnerable to oil spills due to heavy ship transportation of crude oil (Sukhdhane et al., 2013). This creek is connected to the Ulhas river estuary; therefore, oil pollution could be contributing to the estuarine pollution. Past studies from the Ulhas river estuary have reported the presence of heavy metals such as mercury (Hg) from water, sediment, and fish species (Sukhdhane et al., 2013; Raut et al., 2019); lead (Pb) and zinc (Zn) in sediment and polychaete (bristle) worms (Zingde, 1999); nickel (Ni) in sediment (Jha et al., 2000); and mercury (Hg) from the hair samples of the fishermen communities residing near the banks of the Ulhas River (Menon and Mahajan, 2012). This is extremely worrying in terms of the health and wellbeing of the local communities dependent on ecosystem services from this estuary. The present study was conducted on the Ulhas River estuary to evaluate current heavy metal patterns, their distribution, relations, with other nutrient parameters of the sediment by multivariate statistical techniques. This was critical to identify the distribution pattern and their loads on this important coastal environment, recommend remedial strategies to protect and conserve the condition and sustainability of this estuarine ecosystem.

**Material and Methods**

**Study area**

The study was conducted on the Ulhas river estuary (19°29' to 19°18'N, 72°48' to 72°49'E), it is a major estuarine system along the western coast of India. The Ulhas River originates at the western slope of the Sahyadri range in Maharashtra state. It is the largest river of the Konkan coast (a rugged section of India's western coastline) and meets the Arabian Sea. The sampling stations were spread from Ghodbandar (19°16'N, 72°59'E) at the very narrow mouth of the river with a sandy bed, to Dongrichouki (19°18'N, 72°47'E) with the broad mouth of the estuary with muddy banks and mangrove vegetation (Figure 1).

![Figure 1: Locations of the study sites (Station 1 – Station 5) on the Ulhas river estuary.](image)

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Sample collection and analysis
Sediment samples were collected along the estuary from the selected sites (S1 – S5) monthly during the post-monsoon season (September 2017 to February 2018). The collected sediment samples were airdried, ground to a fine powder in a glass mortar, and separately stored in new polythene bags. The sediment pH and the electrical conductivity (EC) was measured by portable conductivity meter (Eutech Instruments, India). Total nitrogen (N), total sulphur (S), and total hydrogen (H) were analyzed using vario Micro cube CHNS Elemental analyzer, Germany. The available phosphorus (AP) in the sediment samples was estimated according to Olsen’s (Olsen et al., 1954) and the oxidizable organic carbon (OC) by Walkley-Black method (Walkley and Black, 1934). The analysis of trace metals was conducted by digesting the collected samples using supra pure concentrated acids in a microwave-based digestion system (microwave 3000, Anton Parr, USA). About 0.5 g of the sediment sample was taken in the microwave digestion vessel, to which 5 ml concentrated nitric acid, 2 ml hydrochloric acid and 1 ml hydrofluoric acid were added. The vessel was capped and heated in the microwave unit at 1200 W to a temperature of 190°C for 30 minutes at a pressure of 25 bars. The digested sample was diluted to 50 ml and used for the analysis of metals, cadmium (Cd), copper (Cu), chromium (Cr), zinc (Zn), nickel (Ni), silicon (Si) and lead (Pb) by atomic absorption spectrophotometer using flame atomization (Analyst 800, Perkin Elmer, USA). The cold vapour atomic absorbance method with flow injection for atomic spectroscopy (FIAS) was used to measure the concentration of mercury (Hg) (APHA, 2005). The analytical method and instrumentation efficiency was verified by the analysis of the reference materials obtained from the National Research Council of Canada for estuarine water (SLEW-3) and lobster hepatopancreas (TORT-2) in five replicates.

Results and Discussion
Nutrient concentration of sediment
The spatial distribution of various characteristics of the sediment values of pH, EC, OC, AP, N, H, S, C/N ratio and C/H ratio are presented in the box plot (Figure 2). Water salinity in the estuary varied from 10 to 30 % during the sampling period, minimum value 10-25 % was reported at stations 1 and 2 during September and October. The station-wise concentration range of EC (0.30 to 4.18 mS/cm) and pH (7.60 to 8.50) of sediment were recorded. The organic carbon contained in the sediment was measured between 0.38 to 2.49%. Higher mean OC% was found in the middle portion of the estuary, and lower mean value at the narrow belt of the estuary (Station 1). Other sediment nutrients like AP were reported between 0.09 to 1.60 g/kg, higher at station 3. The percentage range of N (0.31 to 0.63) and S (0.03 to 0.43) had similar distribution except station 1. This similarity range distribution is also shown in the ratio of C/N (5.57 to 0.85) and C/H (0.49 to 2.27).

Metal concentration of Sediment
The distribution of heavy metals (Cr, Cu, Zn, Pb, Cd, Ni, Si, and Hg) in the sediment represented on the boxplot (Figure 2). The average concentration of metal during the sampling period of the five-station ranged between 31.28 and 872.8 mg/kg for Cr; between 61.73 and 418.20 mg/kg for Pb; between 56.3 and 149 mg/kg for Cu; between 50.93 and 119.70 mg/kg for Zn; between 0.08 and 30 mg/kg for Hg; between 0.98 to 4.73 mg/kg for Si; between 0.14 to 3.46 mg/kg for Cd. Higher concentration levels of metal were observed between the station 3 and 5. Based on the average concentration of the metal, the following sequential order viz., Cr > Pb > Cu > Ni > Zn > Hg > Si > Cd was observed. There is no significant association in the spatial and temporal distribution of the estuary sediment's nutritional parameter. In disparity, metal concentrations in sediment were showed an increasing trend towards the mouth of the estuary and correlated to each other. Therefore, the correlation coefficient for metal concentration in sediment was performed (p< 0.05), Among the metal concentration in sediment, Hg has strongly correlated with Cd, Pb and Si (r² = 0.93, 0.96; p < 0.001 respectively) whereas Cr has strongly correlation with Ni, Cd and Pb, (r² = 0.88, 0.87; p< 0.05) (Figure 3).

Principle component analysis (PCA) and nonmetric multidimensional scaling (nMDS)
Multivariate statistical techniques are constructed by using various methods for viewing and analyzing complex data (Olkin and Sampson, 2001).
Figure 2: Box plots of nutrients parameter and heavy metals of the Ulhas river estuary sediment (the whisker shows the minimum and maximum values and the line of each plot is the median value).

Figure 3: The bivariate scatter plots of heavy metals (Cr, Cu, Zn, Pb, Cd, Ni, Si, and Hg) (bottom) and the value of the correlation plus the significance level (top), symbol: p-values (0, 0.001, 0.01, 0.05, 0.1, 1) symbols (****, ***, **, *, ., --)
Figure 4: Plots of PC1 vs. PC2 showing all sampling station and sediment parameters.

Figure 5: Ordination plot from nMDS (Euclidean distance) showing the relationship between monthly sampling site.
Principle component analysis (PCA) and nonmetric multidimensional scaling (nMDS) are two visualized methods that allow us to assume how particular variables are distributed, correlated, and determine their association (Clarke and Gorley, 2015). Therefore, euclidean distance-based PCA and nMDS were calculated and visualized by using PRIMER-e package. PCA estimates the variable's correlation structure by finding a new principal component (PC) that accounts for the variance (or correlation) in a multidimensional data set. These new variables are a linear combination of the original variable (Grimnes and Martinsen, 2015). This method assists us in identifying groups of variables based on the loadings and groups of samples based on the scores (Zakir et al., 2021). Each sample has a score and each component, which shows the sample's location to detect sample patterns, groupings, similarities, or differences (Facchinelli et al., 2001; Han et al., 2006). All selected sediment variables are analyzed by the PCA model (Figure 4). To understand the association of sediment samples from the five areas in six months. This implies normalizing all variables using their mean subtracted and are divided by their standard deviations. The eigenvalues measure the variance accounted for by the corresponding eigenvectors (Jolliffe and Cadima, 2016). From the eigenvalues of the PCA model, the first three PCs are adequate to explain 70% of the variation. The eigenvectors of the sediment parameter vectors lie along the PC1 axis, showing their larger values towards stations 2, 3, and 4 compared to stations 1 and 5. They all have significant positive coefficients in the PC1 but negligible in the PC2. The concentrations of N%, H%, S%, C/N, C/H, EC, and OC% were significant contributors to PC1 (36%) while the heavy metals such as Cu, Pb, Cd, Ni, Si, and Hg concentration were major contributor to PC2 (20%). The nMDS method is used for grouping variables or samples based on resemblance matrix and stress value. The results of nMDS at stress (0.15) showed that monthly sampling stations were clustered into three groups viz., station 1, station 5, and included stations 2, 3, and 4 (Figure 5). The stress value for nMDS indicated a strong representation of the degree of similarity of monthly sampling station
Assessment of heavy metal contamination

Ulhas river estuary has been laid with urban pollution for more than four decades and has been recognized as a hotspot of metal contamination. The present study reports on the spatial distribution of nutrient and metals concentration from the bottom sediment along the estuary. The average depth of the sampling site was varied between 3.8 m to 7.7 m during the low tide period. The minimum depth ranges were reported at the broad mouth of the estuary (Stations 3 to 5), and maximum depth ranges were reported at the narrow mouth (Stations 1 to 2). The sediment texture of the Ulhas river estuary was found sandy loamy with an average of 60% sand, 24% silt, and 14% clay (Raut et al., 2019). Sampling sites 1 and 2 are located adjoining the industrial discharges point of metropolitan cities like Dombiwali, Thane, and Bhayandar. The sediment texture in the sampling site 1 and 2 was sandy (>80%) at the average depth between 5 to 7m. this estuary has carried a large amount of untreated sewage and effluents towards the coastal area. However, the initial station (i.e., stations 1 and 2) has been reported less concentration of nutrients and heavy metals; it may be occurred due to the presence of >80% sand in the soil texture. Therefore, the concentration range of sediment parameters and heavy metals was increased from stations 3 to 5. The observed concentrations of heavy metals were higher in sediment samples at the mouth of an estuary, which could be due to nutrients availability and the high sedimentation rate here. The shallow depth of the river mouth has been reported to favor the re-suspension of sediment matters that contain a significant amount of Pb, Cu, Cr, Ni, and Zn; hence, it gets easily scavenged at the sediments-water interface (Dauby et al., 1994). Additionally, automobile exhausts (a significant source of lead pollution) from urban run-off and the fishing boats could be contributing to the high level of lead. The considerable concentration of Si, Cd, and Hg was also reported in the sediment sample. It could be due to domestic discharges, and industrial effluents around the catchment sediments serve as sorbents/concentrators for various inorganic and organic chemicals. Thus, the strong binding affinity of the heavy metals has the potential to result in low concentration in water and high concentration in sediments (Salomons, 1982). The higher concentration of metals also supported during the post-monsoon months might be attributed to the heavy rainfall and subsequent river runoff, bringing much industrial and land-derived material along with domestic, municipal, and agriculture waste (Karthikeyan et al., 2007; Sankar et al., 2010; Ruhela et al., 2019). Moreover, sediment particles moved downstream and were deposited at the river's mouth, thus facilitating the increasing accumulation of Hg, Pb, Cr, and Cd in the sediment. The metals were significantly correlated with each other and moderately correlated with the nutrient composition of the estuarine sediment, suggesting a common origin in the area. The most untreated wastewater discharges from industries into the surrounding drainage (which runs into the estuary) could be responsible for this (Bhosale, 1991; Ram et al., 2003). Therefore, a significant concentration between the metals Cd, Pb, Si, Hg, Cr, and Ni in the sediment is attributed to the spatial distribution in the estuary.

In PCA, eigenvectors of all sediment parameters and heavy metal concentration of PC1 and PC2 lie towards stations 3, 4, and 5 (Figure 4). Factor loadings are classified into three categories i.e., strong (>0.75), moderated (0.75 to 0.5) and weak (0.5 to 0.3) (Liu et al., 2003). PC1 dataset is explain 36% of the total variance and is strongly positive load with EC, OC%, H%, N%, S%, C/N, and C/H ratio. It indicated high nutrient availability toward the mouth of the estuary by the content of silt and clayey texture of soil (Chaston et al., 2008). All the nutrients availability may have come from various sewage discharges from the metropolitan cities of the river catchment area (Singare et al., 2011). PC2 dataset is explain 20% of the variance. It is strongly positively loaded with pH, Cr, Cu, Pb, Cd, Si, and Hg, indicating dispersion of heavy metals. It may originate from industrial effluents and infrastructure activities surrounding the catchment area (Singare et al., 2012). Some nutrient parameters like salinity, pH, OC% are moderately correlated with the heavy metal concentration in the sediment’s particles (Najamuddin and Surahman, 2017). PC3 represents

and distributed nutrient concentrations; however, hypothesis test and analysis of similarities (ANOSIM) results also support them at a significant level of 0.01% with a global R-value (0.6) (Figure 6).
14% of the variance and is positively loaded with EC, AP, Zn, and Ni, indicating anthropogenic sources. Zn is dispersed within the aquatic environment may from high traffic density (tire wear particles) (Callender and Rice, 2000), and Ni is the primary source from the use of liquid and solid fuels, as well as municipal and industrial waste (Genchi et al., 2020). They were settled as sediment at the estuarine bottom, and water currents and tidal fluctuation have carried out the dispersion of heavy metals towards the estuarine mouth (Dias et al., 2013). The non-metric multidimensional scaling (nMDS) method is described the degree of similarity among the sampling station of each month based on sediment parameter concentrations. Moreover, cluster analysis analyzed groups of similarity by using Euclidean distance. Here, the group of station 1 of each month has separated, indicating less concentration of nutrient and heavy metal parameters. This station is situated at a narrow part of the estuary. There was less silt%, clay%, and organic carbon% was recorded; therefore, maybe it was separated from the remaining station (Figure 5). Another cluster of station five is situated at the front of the estuary. More exposure to seawater and salinity at this station may dilute some nutrients and heavy metals parameters like N%, H%, AP%, Cr, and Zn. Therefore, they are reported less in concentration. The third-largest cluster of stations 2, 3, and 4 indicates that the middle portion of estuarine sediment quality is similar (Figure 5). Moreover, in the last month of the post-monsoon season’s station, five also support them. In this cluster, the physical properties like depth and soil texture were also recorded similarly in that area.

**Conclusion**

The gradual increase in heavy metal levels (compared to previous studies in the catchment area) has the potential for bioaccumulation in aquatic species, and eventually in humans, through the food web. Aquatic species such as fish form an important component contributing towards the food and livelihood security of the dependent communities in the catchment area. Therefore, the current findings contribute to the strong information base for the estuary and, more importantly, stress the need for long-term monitoring of pollution sources. The findings also warrant the formulation of targeted policies for establishing, developing, and improving wastewater treatment plants in the riverine catchment area, mangrove plantation on the estuarine margin area and increased attention on the rich biodiversity supported by the ecosystem.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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