Phytoremediation Potential of Selected Mangrove Plants for Trace Metal Contamination in Indian Sundarban Wetland

Ranju Chowdhury, Yelena Lyubun, Paulo J.C. Favas, and Santosh Kumar Sarkar

Abstract Uptake, accumulation and distribution pattern of trace metals in mangrove plants organs along with rhizosediment were studied in Indian Sundarban Mangrove Wetland. The mean concentration of metals in rhizosediments was as follows (expressed in mg kg^{-1}) 36.03 ± 24.88 for Cu, 11,097.10 ± 12,880.67 for Fe, 709.04 ± 274.25 for Mn, 14.10 ± 10.88 for Pb, 76.63 ± 77.20 for Cr and 40.42 ± 5.74 for Zn. In the context of geochemical characteristics of the sediment, values of geo-accumulation index (I_{geo}) and pollution load index (PLI) suggest no metal pollution, but enrichment factor (EF) ensures their anthropogenic sources. Concentrations of Cr and Cu were higher than sediment quality guidelines at some sampling sites, implying potential adverse impacts of these metals. In mangrove organs, the concentration of metals showed the following descending order (expressed in mg kg^{-1}): Mn (2298.77) > Fe (1796.47) > Cr (61.30) > Cu (36.51) > Zn (33.13) > Pb (2.55). *Sonneratia apetala* displays a high bioconcentration factor for Fe (10.7) and Mn (5.99) as well as high translocation factor for Mn (31.99), Pb (18.01) and Zn (9.95) and therefore may be employed as a biological indicator to protect this productive environment as the species showed its potential in accumulating metals in its tissues. Pearson's correlation coefficient indicated that a significant positive correlation existed amongst the metals. One-way ANOVA shows that there are significant differences between metal concentrations of mangrove organs in monitored sites.

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1 Introduction

Mangrove forests are diverse communities that commonly thrive in the intertidal zones of tropical and subtropical coastal rivers, estuaries and bays. As one of the most productive ecosystems in the world, mangrove forests provide multiple ecosystem services such as food sources and diverse habitats for large numbers of organisms provide erosion mitigation and stabilization for adjacent coastal landforms. Similar to other estuarine environments, mangrove ecosystems receive large contaminant inputs from catchments derived from run-off, as well as atmospheric and marine inputs. Consequently many of these environments have become important sinks for nutrients, organic and inorganic contaminants including heavy metals. Mangrove sediments have a high capacity to retain heavy metals from tidal water, freshwater rivers and storm water runoff, and they often act as sinks for heavy metals [1, 2–3]. Heavy metals are not biodegradable and persistent in the environment and thus received significant attention due to their long-term effects on the environment especially coastal regions. Therefore, understanding the distribution of heavy metals including the toxic one, and monitoring their potential bioavailability to mangrove plants have become increasingly important [4]. Phytoremediation is described as a natural process carried out by plants and trees in the cleaning up and stabilization of contaminated soils and ground water. It is actually a generic term for several ways in which plants can be used for these purposes. It is characterized by the use of vegetative species for in situ treatment of land areas polluted by a variety of hazardous substances [5]. It is a novel, cost-effective, efficient, environment- and eco-friendly, in situ applicable and solar-driven remediation strategy [6]. Plants are especially useful in the process of bioremediation because they prevent erosion and leaching that can spread the toxic substances to surrounding areas [7]. Plants generally handle the contaminants without affecting topsoil, thus conserving its utility and fertility. They may improve soil fertility with inputs of organic matter [8]. It is suitable for application at very large field sites where other remediation methods are not cost-effective or practicable [9]. Plants dig their roots in soils, sediments and water, and roots can take up organic compounds and inorganic substances; roots can stabilize and bind substances on their external surfaces, and when they interact with microorganisms in the rhizosphere. Uptaken substances may be transported, stored, converted and accumulated in the different cells and tissues of the plant. Finally, aerial parts of the plant may exchange gases with the atmosphere allowing uptake or release of molecules [10].

Presently, there are several types of phytoremediation in practice. One is phytoextraction, which relies on a plant's natural ability to take up certain substances (such as heavy metals) from the environment and sequester them in their cells until the plant can be harvested. Another is phytodegredation in which plants convert organic pollutants into a nontoxic form. Next is phytostabilization, which makes plants release certain chemicals that bind with the contaminant to make it less bioavailable and less mobile in the surrounding environment. Last is phytovolitization, a process through which plants extract pollutants from the soil and then convert them into a gas that can be safely released into the atmosphere [11]. Mangroves are highly productive intertidal forests that interface between marine and terrestrial environments in the tropics and subtropics. These ecosystems generally occur in estuaries, bays and harbours which are areas of rapid urban development. Mangroves include approximately 16 families and 40–50 species (depending on classification). According to Tomlinson [12], the following criteria are required for a species to be designated a "true or strict mangrove": Complete fidelity to the mangrove environment, major role in the structure of the community and has the ability to form pure stands. These plants possess morphological and physiological adaptation to their habitat. They should be isolated taxonomically from terrestrial relatives.

Thus, mangrove is a non-taxonomic term used to describe a diverse group of plants that are all adapted to a wet, saline habitat. Mangrove may typically refer to an individual species. Terms such as mangrove community, mangrove ecosystem, mangrove forest, mangrove swamp and mangal are used interchangeably to describe the entire mangrove community [13]. Anthropogenic impacts from urban growth include metal contamination from sources such as industrial wastes and effluents, mining, sewage treatment plants and runoff [14]. Mangrove forests protect coastal landforms from erosion and act as sediment traps simply by reducing tidal flows and inducing sedimentation at low tides [15]. Mangroves are one of the most productive ecosystems that enrich coastal waters, yield commercial forest products, protect coastlines and even support coastal fisheries and storehouse of numerous endangered faunas. They act as a fragile link between marine and fresh water ecosystems, pollution sink and source of nutrient flux into marine ecosystem.

But, it is surprising to know that such a natural fighter against pollution is constantly being affected by the rising level of pollution [16]. Mangrove plants' special capability of surviving in high-salt and anoxic conditions and high tolerance to trace metal stress [17] contribute to their potential use in preventing dispersion of anthropogenic pollutants into aquatic ecosystems [18]. In spite of their importance, mangrove ecosystems have suffered significant anthropogenic contaminant inputs due to their location close to urban development [19], amongst which the majority are trace metal pollutants [20]. Mangrove plants absorb and store trace metals mainly in roots and still transport a part upward into sensitive tissues: Metal concentrations in shoots appear to be half that of roots or lower [19, 21]. Previous cultivation experiments have proved that excessive essential metals and non-essential metals could affect the growth metabolism activities and cell structure [22] of plants.

The present investigation is an effort to assess the phytoremedial potential of selective mangrove plants growing on metal enriched sediments of Indian Sundarban Wetland. It deals with the absorption, accumulation and dynamics of six trace metals in Indian Sundarban. The aim is to reveal the potential of mangrove plants to accumulate and tolerate the above-mentioned metals, and to find out a potential species for bioindication and phytoremediation.

2 Materials and Methods

2.1 Study Sites

The Indian Sundarban Mangrove Wetland (21°00′–22°30′N and 88°00′–89°28′E) is a tide-dominated anthropocene megadelta belonging to the low-lying coastal zone, formed at the estuarine phase of the Hugli (Ganges) River. It is part of the world's largest delta (80,000 km^2) formed from sediments deposited by three great rivers, the Ganges, Brahmaputra and Meghna, which converge on the Bengal Basin. The whole Sundarban area is intersected by an intricate network of interconnecting waterways, of which the larger channels are often a mile or more in width and run in a north–south direction. A number of southerly flowing rivers, viz., Hugli, Baratala, Saptamukhi, Jamira, Bidyadhari, Matla and Gosaba (as shown in Fig. 1) traverse the wetland from the west to the east [23]. This is one of the most dynamic, complex and vulnerable zones in typical tropical geographical locations in the northeastern part of Bay of Bengal. Geomorphologically, mangrove swamp, tidal marsh, intertidal mudflats, sandy beaches, tidal creeks and inlets characterize the estuarine wetland. The entire mangrove forest extends over 4262 km^2 of which 2320 km^2 is forest and the rest is water [24], and is called Sundarban owing to the dominance of the tree species *Heritiera fomes*, locally known as "Sundari" because of its elegance [25].

Both plant samples and host sediments were collected from three sampling sites of diverse environmental stress located along the east–west gradient of Indian Sundarban and a brief description of each site is furnished below:

Jharkhali (S_1)—This site is characterized with the following features: (a) this is surrounded by Herobhanga Reserve forest. (b) This is the confluence of Bidya and Matla rivers (c) reduced forested area due to severe human pressure and (d) a famous tourist spot where the pollution stress is higher as thousands of people used to gather here. Moreover, this is a wide scale fishery catchment area and mechanized boats are used for fishing which helps to contribute trace metals to water mainly due to complete lack of standard norms and regulation. Rich and diversified luxuriant mangrove vegetation with high diversity of speciesis distinct mainly due to extensive afforestation program.

Gangadharpur (S_2)—It is situated on the western bank of Saptamukhi River, a major tidal inlet in the Hugli–Matla delta complex. Natural mangrove vegetation of mixed type can be seen here. Agricultural runoff, boating and domestic use of water bodies, leaching from domestic garbage dumps are the major sources of metal pollution in this area. Moreover, unawareness of the local people about the mangrove plants and their importance is leading the gradual destruction of this natural habitat. A major section of the natural habitat is already lost due to deforestation by the local people for timber, house making, boat making, etc.

Gangasagar (S_3)—It is an offshore island located open ocean at the extreme southern tip of the estuary mouth, experiencing direct wave and marine influences. The eastern bank of this triangular island faces meso-macrotidal Muriganga River and the western bank faces macrotidal Hugli estuary. In addition to the annual "Sagar

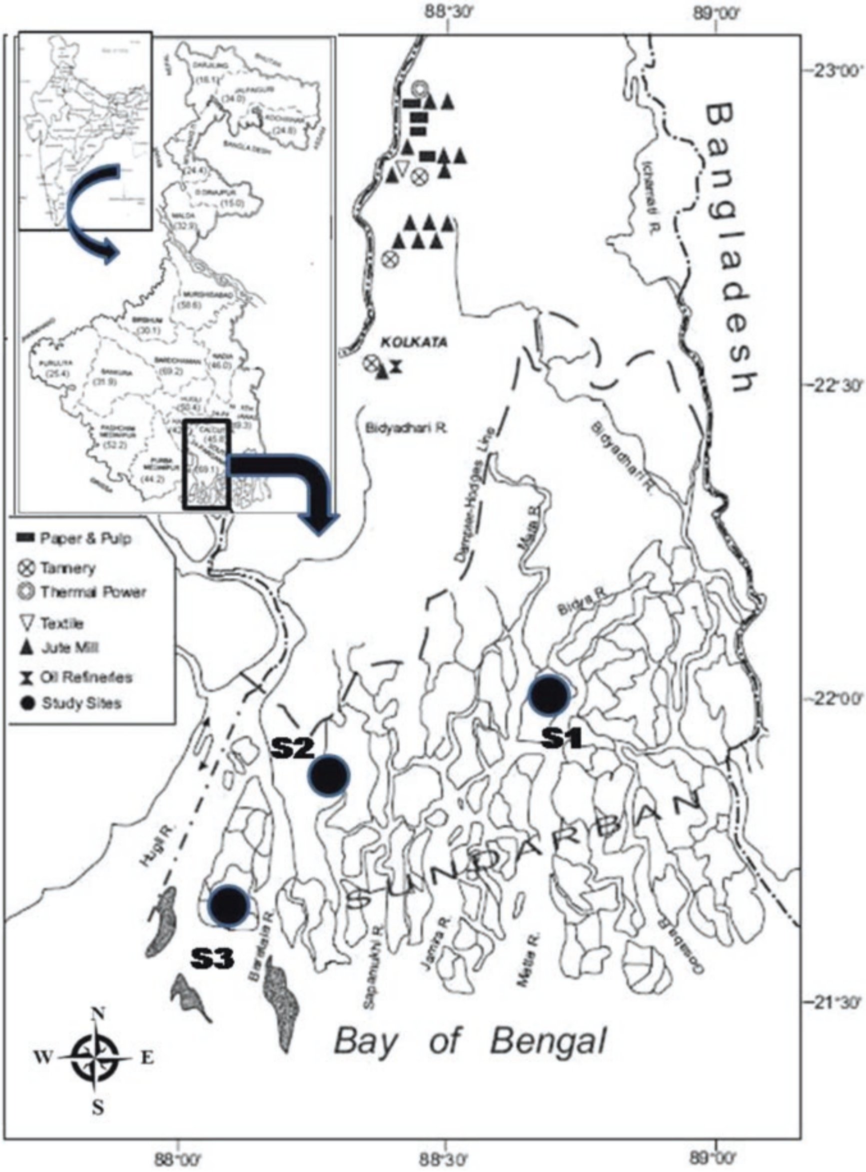

Fig. 1 Map of Indian Sundarban showing the location of the study sites (S_1–S_3). Location of multifarious industries are also shown in the upstream of Hugli river

Mela"—a pilgrim fare of over half a million of people—the area is impacted by anthropogenic stresses arising from rapid growth of settlements, aquaculture practices and tourism throughout the year [26]. Due to which the natural habitat of mangrove plants at this site is degrading though afforestation programme have been initiated by Govt. of India very recently. Two stations (S_1, S_2) are located on the main banks of the River Hugli (Ganges), while the third site (S_3) is located at the southern tip of Sagar Island, the largest delta of Indian Sundarban. The stations maintain a difference in the context of geomorphic and tidal set up that have different wave energy fluxes and distances from the sea (Bay of Bengal) and have diverse human interference with a variable degree of exposure to trace metal contamination.

2.2 Sample Collection and Processing

Surface sediment samples were collected in triplicate from top 0–5 cm at each sampling site covering an area of 1 m × 1 m using a clean, acid-washed fabricated polyvinyl chloride (PVC) scoop. Samples were stored in clean plastic zip lock pouches and transported to the laboratory. Individual sediment samples were placed in a ventilated oven at low temperature (~45 °C) [27] until completely dried, as high temperature may contribute to the alteration of volatile and even non-volatile organics of the sample [28], until they get completely dried. Samples were pulverized using an agate mortar and pestle, sieved through 63 μm mesh for homogenization since this fraction contains more sorbed metals per gram of sediment due to its larger specific surface area. Then, individually transferred into pre-cleaned, inert polypropylene bags and stored at room temperature until subsequent extraction and chemical analyses. Redox potential and pH was measured (T = 25 °C), using a glass electrode (HI 98160, HANNA Instruments, USA, Accuracy: 0.1 pH–0.01 pH, 1 mV (± 2000 mV), 0.1 °C) by inserting the probes directly into the fresh sediment sample. The electrode was calibrated using 4.01, 7.01 and 10.01 buffer solutions (HANNA instruments, USA).

Electrode were inserted for several minutes in the mud until stable values were reached, it was thoroughly washed and subsequently rubbed with fine tissue paper after each measurement in order to prevent the poisoning of electrodes by sulphide [29]. Organic carbon (C_{org}) content of the sediments was determined following a rapid titration method [30]. All the experiments were repeated three times with triplicate samples. The sand fraction was separated by wet sieving using a 63-μm mesh sieve. The silt (4–63 μm) and clay (<4 μm) fractions were determined using the pipette method [31] in which a sample suspension is prepared using sodium hexametaphosphate as the dispersing agent, and aliquots are pipetted at different time intervals from different depths, dried and weighed for mass determination. Statistical computation of textural parameters was done by using the formulae of Folk and Ward [32] following standards of Friedman and Sanders [33].

In each study site, mature mangrove trees of similar size and health condition selected for sampling. Live plant parts (young, mature and yellow leaves, bark, root/pneumatophore) were collected, from ten different mangrove plant species, namely, *Avicennia alba Blume., Avicennia officinalis L., Avicennia marina Forssk.* (Avicenniaceae), *Aegialitis rotundifolia Roxb.* (Plumbaginaceae), *Aeigeceros corniculatum L.* (Myrsinaceae), *Bruguiera gymnorrhiza L., Ceriops decandra Griff.* (Rhizophoraceae), *Exocaria agallocha L.* (Euphorbiaceae), *Sonneratia apetala*

Buch.- Ham. (Lythraceae) and *Xylocarpus mekongensis Pierre* (Meliaceae). A young leaf was selected as the leaf most proximal to the shoot apical meristem. The largest fully expanded leaf immediately distal to the shoot apical meristem was designated as mature [34]. Yellow leaves which are ready to fall from trees were also picked [35]. A sterilized knife was used to remove the bark from the tree trunks. Around the sampled trees, we excavated root system of the trees during low tide and collected pneumatophore/root as applicable. Samples were washed by deionised water in the laboratory thoroughly to remove any adhering dirt or dust particles. These were then grinded and oven-dried to constant weight under 50 °C till they became completely dry and subsequently homogenized adopting the methods performed by MacFarlane et al. [36].

2.3 Plant Description

The term mangroves collectively refers to woody halophytic angiosperm trees inhabiting in the intertidal zone of coastal estuarine regions in the tropics and subtropics, especially between 25°N and 25°S where the winter water temperature remains not less than 20 °C. Mangrove has a worldwide circumtropical distribution, the highest concentration being located in the IndoPacific region. The mangroves dominate almost 1/4th of world's tropical coastline. The total mangrove area which spans 30 countries including various island nations is about 100,000 km^2 [37]. The ten mangrove plants in consideration are thoroughly distributed in Indian Sundarban and form a mangrove bioassemblage in this sector. The most dominant plant *Avicennia* has a wide geographical distribution, with members found in intertidal estuaries along many of the world's tropical and warm temperate coasts. *Avicennia alba* and *A. officinalis*, distinctive genus in eastern tropics, are woody, possess stilt roots and are provided with pencil-like pneumatophores for aerial respiration [38].

Avicennia marina, which is a facultative halophyte that has various adaptations for hypersaline environments [39, 40], is a widely distributed species [36]. *Bruguiera gymnorhiza* was selected for investigation because it is an evergreen mangrove tree widely distributed in intertidal areas of tropical and subtropical coastlines of Asian, southern and eastern Africa, and northern Australia [41]. *Sonneratia apetala* is naturally distributed in India (the Bengal region) as a dominant species in local mangrove communities [42]. It is highly adaptable, fast growing and is used as a pioneer species in ecological succession in many degenerated mangrove forests [43]. Due to its high adaptability and seed production capacity, it has been utilized for restoration purposes in many other places besides its original locations [44, 45]. *Ceriops decandra* is an evergreen small, much branched tree which is very common in Indian Sundarban. *Aegialitis rotundifolia* is a characteristic mangrove associate but does not itself occur within closed mangrove communities, since it prefers or even requires exposed sites.

It is a low growing treelet having distinctive features like anomalous secondary thickening, abundant sclereids and incipiently viviparous seeds. *Aegiceras corniculatum* (Black mangrove), one of the most common and dominant mangrove plants, is usually 1–3 m tall. It often grows together in the intertidal habitat to form *A. corniculatum* communities in the wetland [46]. *Excoecaria agallocha* (Milky mangrove), belonging to family Euphorbiaceae [47], is found near the bank of tidal rivers in brackish water and almost all the places in the above study area of Sundarban. *Xylocarpus mecongenesis* is a woody, perennial, deciduous tree distributed throughout the mangrove habitats of Indian coasts, deltas and Andaman and Nicobar Islands [38].

2.4 Chemical Analysis

Plant and sediment samples were digested by using a microwave system (MARS Xpress, CEM Corp., USA) in automatic mode, with constant control of temperature and pressure. Sediment or dry plant material (200 mg) was quantitatively transferred to Teflon containers for mineralization, after which 8 mL of 10 M HNO_3 (Suprapur®, Merck) and 3 mL of H_2O_2 (30 %, analytical grade) were added. The containers were left to stand for 15 min to achieve preliminary acid digestion and then were placed in a microwave oven for mineralization. The digests were reconstituted with ultrapure deionized water to 20 mL for subsequent analyses of total metals. The element concentration in the solutions was determined by atomic absorption spectrometry (Thermo Scientific ICE 3500). For preparing calibration standards, certified reference AAS element standards solution (TraceCERT®, Sigma-Aldrich) was used.

2.5 Mangrove Microstructure Analysis

SEM analysis was performed to study the morphological characteristics of salt glands formed on the upper surface of the mangrove leaves. For studying the surface morphology of the leaves, scanning electron microscopy Model EVO 18 special edition (Carl Zeiss, Inc., Germany) was used. Samples of dried leaves were placed on double-sided carbon adhesive tape, which had previously been secured to aluminium-alloy stubs. These were metalized with gold coating with a sputter coater and analysed at 10 kV acceleration voltage, and the photomicrographs were taken at suitable magnifications.

2.6 Assessment of Sediment Contamination

In order to assess the level of contamination and the possible anthropogenic impact in the sediment samples, the contamination factor (CF), pollution load index (PLI) geoaccumulation index (I_{geo}) and enrichment factors (EFs) were estimated for some selected potentially hazardous trace metal evaluated in this study.

2.6.1 Contamination Factor (CF)

Metal concentration in a given environment is controlled by varied parameters like nature of substrate, physico-chemical conditions controlling the dissolution and precipitation of metals, and closeness to the pollution sites. Sediment has the capability to record the history and indicate the degree of pollution [48]. Different metals have synergetic and antagonistic effects on the prevailing environment. Concentration factor is considered to be an effective tool in monitoring the pollution over a period of time. The CF is the ratio obtained by dividing the concentration of each metal in the sediment by the baseline or background value (concentration in unpolluted sediment):

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

The contamination levels may be classified based on their intensities on a scale ranging from 1 to 6 (0 = none, 1 = none to medium, 2 = moderate, 3 = moderately to strong, 4 = strongly polluted, 5 = strong to very strong, 6 = very strong) [49]. The highest number indicates that the metal concentration is 100 times greater than what would be expected in the crust [50].

2.6.2 Pollution Load Index (PLI)

For the entire sampling site, PLI has been determined as the *n*th root of the product of the *n*th CF:

$$\mathrm{PLI} = \left(\mathrm{CF}_1 \times \mathrm{CF}_2 \times \mathrm{CF}_3 \times \cdots \times \mathrm{CF}_n\right)^{1/n}$$

This empirical index provides a simple, comparative means for assessing the level of trace metal pollution. When $\mathrm{PLI} > 1$, it means existence of pollution; in contrast, $\mathrm{PLI} < 1$ indicates metal pollution [51].

2.6.3 Geoaccumulation Index (I_{geo})

The geoaccumulation index (I_{geo}) [52] was used to evaluate the degree of elemental pollution in the sediments from the study area. Mathematically, I_{geo} is given as:

$$I_{\text{geo}} = \log_2 [(C_n) / 1.5(B_n)]$$

where C_n is the concentration of metals examined in sediment samples, and B_n is the geochemical background concentration of the metal (n). Factor 1.5 is the background matrix correction factor introduced to account for possible differences in the background values due to lithospheric effects. The geoaccumulation index consists of seven classes [52] Class 0 (practically unpolluted): $I_{geo} \leq 0$; Class 1 (unpolluted to moderately polluted): $0 < I_{geo} < 1$; Class 2 (moderately polluted): $1 < I_{geo} < 2$; Class 3 (moderately to heavily polluted): $2 < I_{geo} < 3$; Class 4 (heavily polluted): $3 < I_{geo} < 4$; Class 5 (heavily to extremely polluted): $4 < I_{geo} < 5$; Class 6 (extremely polluted): $5 > I_{geo}$ [53].

2.6.4 Enrichment Factor

The behaviour of a given element in the sediment (i.e. the determination of its accumulation or leaching) may be established by comparing concentrations of a metal with a reference element. The result obtained has been described as enrichment factor (EF), which was calculated using the following equation:

$$\mathrm{EF} = \left(C_n / C_{\mathrm{ref}}\right) / \left(B_n / B_{\mathrm{ref}}\right)$$

In which C_n is the content of the examined element in the sediment, and C_{ref} is the content of the examined element in earth crust. B_n is the content of the reference element in the sediment, and B_{ref} is the content of the reference element in earth crust. In the present study, Fe was used as reference element because of the following reasons (a) Fe is associated with fine solid surfaces; (b) its geochemistry is similar to that of many trace metals and (c) its natural concentration tends to be uniform [54]. The world average elemental concentrations reported by Turekian and Wedepohl [55] in the Earth's crust were used as reference in this study because regional geochemical background values for these elements are not available. EF values less than 5.0 are not considered significant because such small enrichments may arise from differences in the composition of local sediment material and reference sediment used in EF calculations [56]. However, there is no accepted pollution ranking system or categorization of degree of pollution on the enrichment ratio and/or factor methodology. Five contamination categories are recognized on the basis of the enrichment factor: EF < 2 states deficiency to minimal enrichment, EF = 2–5 moderate enrichment, EF = 5–20 significant enrichment, EF = 20–40 very high enrichment and EF > 40 extremely high enrichment [57]. EF can easily be used to differentiate between elemental concentrations from anthropogenic source and those from natural origin. EF values between 0 and 1.5 indicate the metal is entirely from crustal materials or natural origin, while EF > 1.5 suggests that the sources are more likely to be anthropogenic. EFs greater than 10 are considered to be non-crusted source [58].

2.6.5 Potential Ecological Risk Index

The potential ecological risk index (PER) was also introduced to assess the contamination degree of trace metals in the studied sediments. The equations for calculating the PER were proposed by Hakanson [59] as follows:

$$E = \mathrm{TC}$$

$$C = C_a / C_b$$

$$\mathrm{PER} = \pounds E = \pounds\,\mathrm{TC}$$

where C is the single element pollution factor, C_a is the content of the element in the samples and C_b is the reference value of the element. The sum of C for all the metals examined represents the integrated pollution degree (C) of the environment. E is the potential ecological risk factor of an individual element and T is the biological toxic factor of an individual element, which is set at Cu = Pb = 5, Zn = 1, Cr = 2 and [59]. PER is a comprehensive potential ecological index, which equals the sum of E. It represents the sensitivity of a biological community to toxic substances and illustrates the potential ecological risk caused by contamination.

2.6.6 Sediment Quality Guidelines

Sediment quality guidelines (SQGs) are very useful to screen sediment contamination by comparing sediment contaminant concentration with the corresponding quality guideline [60]. These guidelines evaluate the degree to which the sediment-associated chemical status might adversely affect aquatic organisms and are designed to assist in the interpretation of sediment quality. Such SQGs have been used in numerous applications, including designing monitoring programmes, interpreting historical data, evaluating the need for detailed sediment quality assessments, assessing the quality of prospective dredged materials, conducting remedial investigations and ecological risk assessments and developing sediment quality remediation objectives [60]. The consensus-based sediment quality guidelines (SQGs) were used in this study to assess possible risk arises from the trace metal contamination in sediments of the study area. The SQGs were developed from the published freshwater sediment quality guidelines that have been derived from a variety of approaches [60]. These synthesized guidelines consist of a threshold effect level (TEL) below which adverse effects are not expected to occur and a probable effect level (PEL) above which adverse effects are expected to occur more often than not. Long et al. [61] also identified two guideline values: the effects range-low (ER-L) and the effects range-median (ER-M). Concentrations below the ER-L value were rarely associated with biological effects. Concentrations in the range between ER-L and ER-M were found to occasionally co-occur with biological effects. Biological effects were also often found to co-occur with concentrations above the ER-M value.

2.7 Bioaccumulation Indices for Hyperaccumulation

Three internationally recognized hyperaccumulator indices were used to evaluate the hyperaccumulator species listed as follows:

2.7.1 Translocation factor (TF)

$TF_{leaf} = C_{leaf}/C_{root}$, where C_{leaf} and C_{root} are the trace metal concentrations in the leaf and root, respectively [62, 63]. A translocation factor greater than 1 indicates preferential partitioning of metals to the shoots [64].

2.7.2 Extraction Coefficient (EF)

$$EF = C_{shoot} / C_{sediment}$$

It evaluates the ability of the plant to accumulate heavy metals in shoot biomass [64] and extraction coefficient more than 1 is one of the criteria for identifying hyperaccumulator plants [65].

2.7.3 Bioaccumulation Factor (BCF)

$$BCF_{leaf} = C_{leaf} / C_{sediment}; BCF_{bark} = C_{bark} / C_{sediment}; BCF_{root} = C_{root} / C_{sediment}$$

where C_{leaf}, C_{bark} and C_{root} are the trace metal concentrations in the leaf, bark and root, respectively, and $C_{sediment}$ is the extractable concentration of trace metal concentration in the sediment. It is used for quantitative expression of accumulation [64].

2.8 Statistical Analysis

To identify the relationship amongst trace metals in sediments, Pearson's correlation coefficient analysis and cluster analysis (CA) were performed using the commercial statistics software MINITAB version 13 for Windows. The correlation coefficient measures the strength of interrelationship between two trace metals. Data were analysed using student's test (*t*-test) and a one-way analysis of variance (*F*-test). Independent variables examined with exponential accumulation relationships were log transformed ln $(x+1)$, prior to statistical calculation. The logarithm-transformed data were applied to eliminate the influence of different units of variance and give each determined variable an equal weight [66].

Cluster analysis classifies a set of observations into two or more mutually exclusive unknown groups based on a combination of internal variables. This is often coupled with PCA to check results and to group individual parameters and variables [67]. The purpose of CA is to discover a system of organizing observations, where a number of groups/variables share observed properties. Dendrogram is the most commonly used method of summarizing hierarchical clustering. In the current study, CA was used to evaluate the sources similarities of trace metals in sediment samples.

3 Results and Discussion

3.1 Sediment Geochemistry

Physical properties of coastal sediments are important variables in order to understand geological events in coastal environments [68]. Sediment grain size distribution was generally homogenous in the rhizosediments, which ranged between 58.76–60.00 %, 15.10–41.40 % and 0.40–26.14 %, respectively, for the proportion of clay (<2 μm), silt (2–63 μm) and fine sand (63–250 μm) with slightly basic pH varying between 7.22 and 7.66 which is the characteristic of coastal sediments suffering from marine influence and limited buffer capacity. The highest percentage of organic carbon (0.95 %) was obtained in station Gangasagar (S_3) and the lowest (0.50 %) was found in Jharkhali (S_1). These low values of C_{org} are probably related to the poor absorbability of organics on negatively charged quartz grains, which predominate in the rhizosediments of this estuarine environment [23, 69]. The prevailing pH and organic carbon (C_{org}) content in the rhizosediments affect the availability and mobility of trace metals [70]. Since mangrove sediments are generally anoxic and waterlogged, trace metals are precipitated as insoluble sulphides [71]. The redox potential (E_h) values ranged between −7.6 mV and −33.5 mV. These negative potentials indicate the natural Eh oscillation [72]. The oxidation/reduction state (redox potential, Eh) of sediment is an important parameter affecting As transformation. Sediment redox levels can greatly affect toxic metals uptake by plants [73]. However, there is little information on redox chemistry of metals in rhizosediment from West Bengal (Table 1).

3.2 Metals in Sediment

The average concentrations of trace metals ($n = 3$) in mangrove sediments are summarized in Table 2 along with a comparative account in selective mangrove wetlands around the world. Concentration of majority of the trace metals (Cr, Pb and Zn) was very much similar to Yellow Sea, China [74], but the value for Cr was slightly higher than N. America [75], Korean Coast, Korea [76] and Pichavaram mangrove forest, India [77]. Metal concentration was found lower than the study carried out by Suresh et al., 2015 [78] in Kerala, India and Chakraborty et al. [79] at Cochin Estuary, India but higher (except Cu) than the study of Kathiresan [77] at

Table 1 Descriptive statistics of the studied trace metals in mangrove plant organs (values expressed in mg kg^{-1})

Organs		Cr	Cu	Fe	Mn	Pb	Zn
Young leaf	max	27.4	21.09	1130.85	2089.84	2.55	32.3
	min	1.04	4.008	106.99	25.58	0.02	4.87
	median	5.78	10.81	286.68	86.97	0.31	16.98
	mean	8.42	12.27	437.08	239.78	0.64	17.59
	SD	± 7.06	± 5.22	± 338.12	± 519.03	± 0.81	± 8.26
Mature leaf	max	22.9	16.46	1610.13	2298.77	4.98	30.19
	min	2.45	4.53	80.41	17.07	0.09	5.70
	median	8.78	9.49	328.03	114.96	0.46	12.50
	mean	9.34	9.75	484.52	323.61	0.92	16.82
	SD	± 5.55	± 3.59	± 454.57	± 585.67	± 1.20	± 8.95
Bark	max	61.3	36.51	1796.47	436.53	2.49	33.13
	min	2.6	3.35	188.42	9.58	0.13	4.43
	median	8.85	9.93	463.72	67.39	0.46	12.73
	mean	16.1	13.29	748.17	115.96	0.83	13.88
	SD	± 16.7	± 9.79	± 563.11	± 127.92	± 0.87	± 8.12
Root/pneumatophore	max	25.6	14.72	1380.64	137.20	1.46	16.55
	min	2.59	4.66	257.02	19.12	0.03	3.29
	median	8.36	8.82	628.27	42.31	0.23	6.84
	mean	9.61	9.87	750.24	62.37	0.39	9.56
	SD	± 7.27	± 3.32	± 407.57	± 44.91	± 0.43	± 5.34

Pichavaram mangrove forest, India. In the present study, values of Cu and Pb were found similar with the results of Hawaii Beach, Malaysia [80] but higher than Saudi coastline, Saudi Arabia [81].

The concentration of most of the metals is greater than the concentration of plant organs (see Fig. 2), as mangrove sediment is rich in sulphide or due to the effect of chelating substances such as humic acids [82]. They therefore favour the retention of waterborne trace metals [2], and the subsequent oxidation of sulphides between tides allows element mobilization and bioavailability [83]. The maximum concentrations of majority of trace metals were recorded at Gangadharpur (S_2) resulting deposition of metals from intensive human activities like agriculture practice, aquaculture practice, use of antifouling paints wood polishing work, etc. throughout the year. The average total contents of trace metals were in the following descending order of Fe (11,097.11 mg kg^{-1}) > Mn (709.04 mg kg^{-1}) >> Cr (76.63 mg kg^{-1}) > Ni (45.89 mg kg^{-1}) > Zn (40.42 mg kg^{-1}) > Cu (36.03 mg kg^{-1}) > Pb (14.09 mg kg^{-1}) > As (9.45 mg kg^{-1}) > Co (7.25 mg kg^{-1}). The observed high concentration of Fe might be a result of the textural and mineralogical characteristic of marine sediments [84].

In the present study, the concentration of Fe (11,097.11 mg kg^{-1}) at Gangasagar (S_3) is maximum and shows higher concentration in sediment than mangrove organ. High concentrations of Fe might be due to the precipitation of Fe as iron sulphide which is common in mangrove ecosystems. Iron is generally described as the principal metal

Table 2 The minimum, maximum and average concentrations of trace elements (mg kg^{-1}) in the rhizosediment of the present study and selective mangrove wetlands around the world

Location	Cr	Cu	Mn	Fe	Pb	Zn	References
Gulf of Guayaquil (N. America)	48.36	139.46	359.06	13,431.1	37.66	331.31	Fernandez-Cadena et al. [75]
Yellow Sea. China	–	15.1	410	1.33	12.3	47.3	Jiang et al. [74]
Hawaii Beach, Malaysia	254	32.24	–	–	18.6	18.7	Nagarajan et al. [80]
Saudi coastline, Saudi Arabia	295	7.39	–	–	9.51	36.5	Al-Trabulsy et al. [81]
Korean Coast, Korea	58.3	36.5	–	–	35	122	Ra et al. [76]
Kerala, southern part India	80.94	76.73	–	–	189.64	127.6	Suresh et al. [78]
Pichavaram mangrove forest, south eastern India	17	46	25	1770	8	25	Kathiresan et al. [77]
Kochi Estuary, south west India	131.9	43.4	–	–	39.8	422.7	Chakraborty et al. [79]
Sundarban Wetland, India	76.63	36.03	709.06	11,097	14.1	40.42	Present study

that precipitates with sulphidic compounds in anaerobic sediments [85], and these sulphides form a major sink for metals in the mangrove area. According to Badr et al. [86], rhizosediment was enriched with some trace metals such as Mn mainly due to discharge of untreated industrial and sewage wastes. The use of gasoline may be considered as a possible reason for the Pb contamination 4.98 mg kg^{-1} in mature leaf of *X. mecongenesis* at Jharkhali (S_1) [87]. Several researchers have previously measured elevated concentrations of trace metals in mangrove sediments over the world, reflecting the long-term pollution caused by human activities [2, 88]. Elements of natural origin reach coastal areas from rivers in the form of particulate material. These elements are mainly chemically bound to aluminosilicates and are therefore lowly bioavailable. On the other hand, anthropogenic elements are more loosely bound to the sediments and may be released back to the aqueous phase with the change of physical and chemical characteristics (E_h, pH, salinity and the content of organic chelators) [89].

3.3 Potential Risk Assessment

On the basis of their average geoaccumulation Index (I_{geo}) values, The trace metals can be arranged in the following sequence Fe > Zn > Pb > Cr > Cu > Mn. In the present work, I_{geo} showed very high values except lead at two stations indicating that

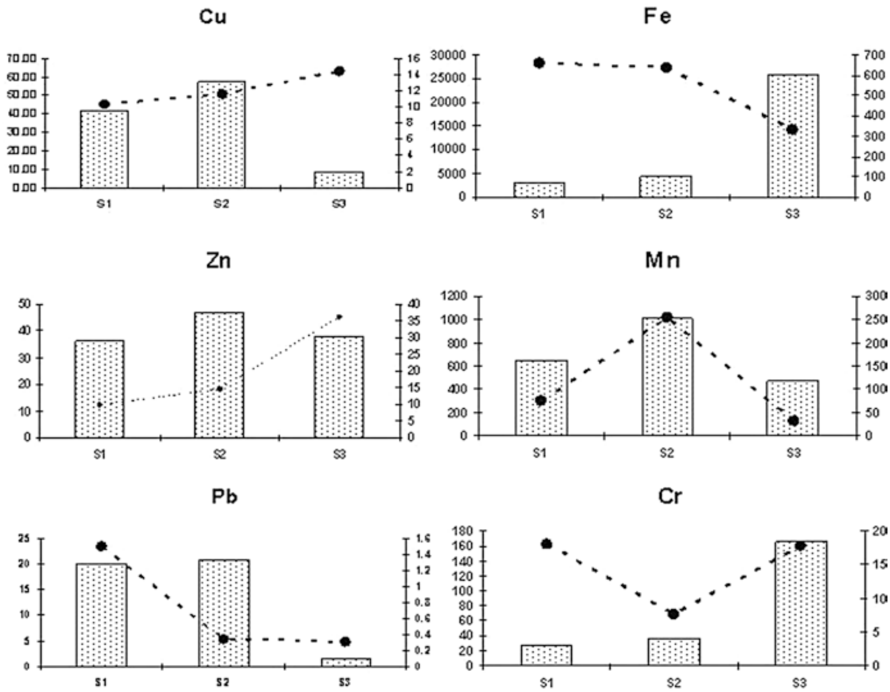

Fig. 2 Pooled mean value (expressed in mg kg^{-1}) of trace metals in rhizosediment (Y axis, column) and plant organs (X axis, discontinuous line) concentrations found in rhizosediments (mg kg^{-1}, columns and *Y* axis) and the average metal concentrations measured in plants considering pooled mean values of all studied plants collected at each site (mg kg^{-1}, discontinuous line and *Y* axis)

sediments are strongly polluted [53]. The results from EF (as shown in Fig. 3) indicate that the highest EFs values (>10) for Cu, Mn and Pb were obtained in Jharkhali (S_1) and Gangadharpur (S_2). The high EF values for these metals in sampling sites suggests the presence of contaminated sediments derived from various sources like domestic sewage, power-plant operation, major storm events, or dumping of dredged sediments dredging along the international shipping zones [90]. The highest CF values for most of the metals (Cu, Mn, Pb) studied were found at Gangadharpur (S_2), which receives a huge amount of agricultural and domestic discharge in regular basis along with aerial particulate Pb [91] from nearby road. The CF values for these trace metals were $1 < CF < 3$ and indicate moderate contamination in sediments. Effect range-low (ER-L) and threshold effect level (TEL) values were exceeded by Cr and Cu implying that adverse consequences to biota may occasionally occur (as shown in Fig. 4). Chromium comes from the untreated industrial effluents from steel and tannery industries [91]. The potential sources of Cu in this coastal region might be due to antifouling paints [92] and extensive use of fertilizers and pesticides for agricultural needs. However, exceedance of SQGs

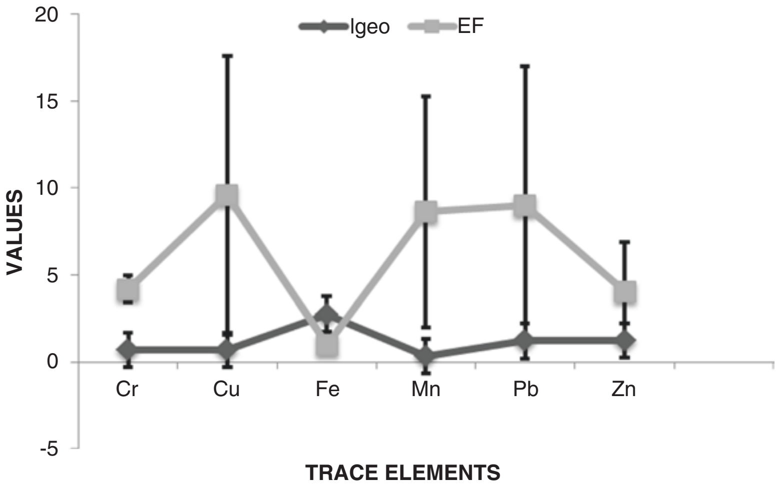

Fig. 3 Pooled mean values of Index of Geoaccumulation (I_{geo}) and Enrichment Factor (EF) considering three study sites of Sundarban (Average ± SD)

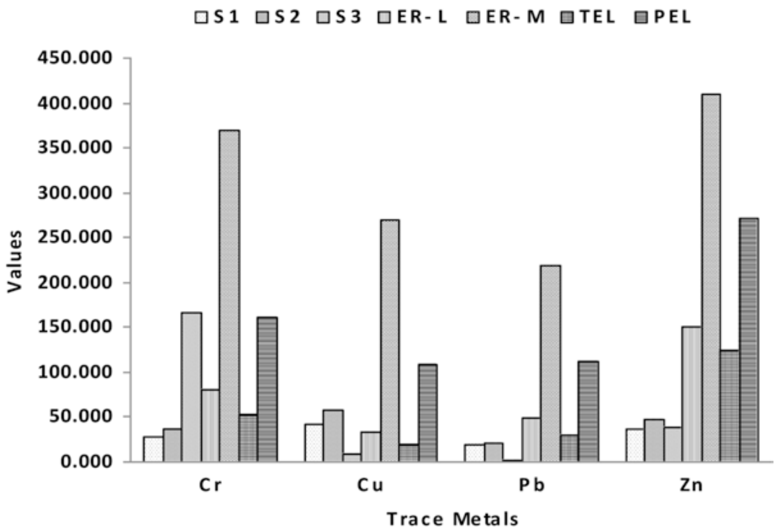

Fig. 4 Distribution of studied trace metals, ER-L, ER-M, TEL and PEL (SQGs) in rhizosediment

is not necessarily due to human stress and may be inherit from the local geological background and depositional settings [93].

Potential ecological risk was used to evaluate the potential risk of one metal or a combination of multiple metals. According to Hakanson [59], the potential ecological risk that trace metals pose in coastal sediments can be classified into the following categories: Low risk: E<40, PER<150. Moderate risk: 40≤E<80, 150≤PER<300. Considerable risk: 80≤E<160, 300≤PER<600. High risk: 160 < E<320, PER≥600. Very high risk: E≥320. It was found that the single risk factors (E) of trace metals were ranked in the order of Cu>Pb>Cr>Zn. The average ecological risk (E) for all metals in most surface sediments was less than 40, indicating a low risk to the local ecosystem [94].

3.4 Metals in Mangroves

There exists wide range of variations for trace metal uptake and distribution in three aerial tissues, and this might be due to complex physiological mechanisms involving cell wall immobilization, complexes with humic substances and presence of barrier at the root epidermis [95] (see Fig. 5). The trend of accumulation of trace metal maintained the following descending order (average for all four study sites): Fe (656.01 mg kg^{-1})>Mn (193.28 mg kg^{-1})>Zn (14.54 mg kg^{-1})>Cr (11.12 mg kg^{-1})>Cu (11.07 mg kg^{-1})>Pb (0.68 mg kg^{-1})>Co≥Ni≥As~BDL.

The maximum concentration of Fe in mangrove tissue are associated with the highest concentrations in the surrounding sediments which may be related to the precipitation of iron as iron sulphides in these mangrove sediments which might act as the potential source of this enrichment. Iron is an essential micronutrient and constituent of cytochromes and of nonheme iron proteins involved in photosynthesis, nitrogen fixation and respiration. Wide range of variations (from 53.78 mg kg^{-1} in bark of *A. alba* to 1796.47 mg kg^{-1} in bark of *E. agallocha* at S_2) of Fe was observed in the present study. Manganese, an essential element showed a wide range of variations (from 24.32 mg kg^{-1} in bark of *A. rotundifolia* at Gangasagar (S_3) to 2298.77 mg kg^{-1} in mature leaf of *S. apetala* at Gangadharpur (S_2)). Generally, Mn^{+2} is taken up by root/pneumatophore of the plant and mostly required in leaf for photosynthesis and nitrogen and carbohydrate metabolism [96]. Also, precipitation of authigenic Mn carbonate in coastal sediments acts as a potential source of Mn [97].

Trace metals can be absorbed by plants using their roots, or via stems and leaves, and stored into different plant parts. Moreover, the distribution and accumulation of trace metals in the plants depend on plant species, metal sources as well as metal concentration in sediments [98]. The maximum values of essential metals like Cu (24.17 mg kg^{-1} in *S. apetala* at Jharkhali (S_1)), Fe (1796.47 mg kg^{-1} in *E. agallocha* at Gangadharpur (S_2)) as well as non-essential metal Cr (61.26 mg kg^{-1} in *A. rotundifolia* at Gangasagar (S_3)) were recorded in trunk bark. Trunk bark is lipophilic in nature and readily adsorbs and collects metals as an excellent passive atmospheric sampler as endorsed by Fu et al., 2014. Previous reports also support the phytoextraction capacity of bark in mangrove plants in other Indian estuaries (Kathiresan et al. [77] at Cuddalore and Pichavaram estuary, southern part of India and Chowdhury et al. [3] from Indian Sundarban). Copper is required in chloroplast reactions, enzyme systems, protein synthesis, growth hormones and carbohydrate metabolism [99]. It is also required in various redox reactions in photosynthesis and

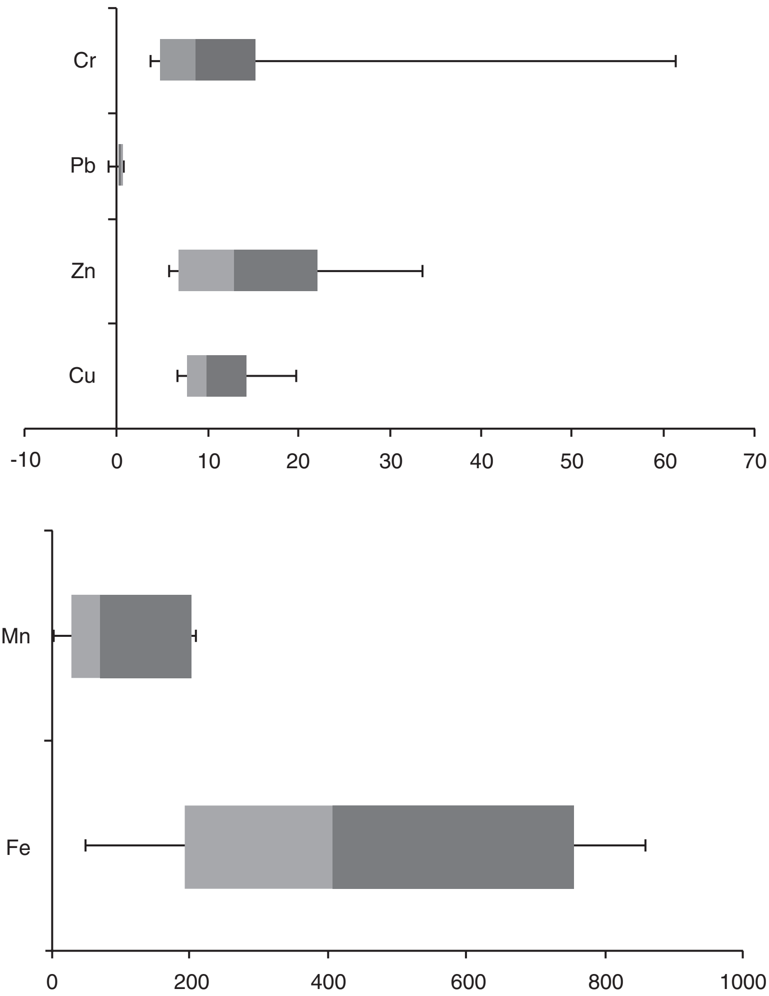

Fig. 5 Box-Whisker plots of metal concentration found in mangrove organs. All the boxes show the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest coefficients, while the line inside the boxes expresses the median

respiration [100]. Chromium is toxic to plant growth and also easily taken up and translocated [101]. The high concentration of Cr inhibits the growth of plants causing chlorosis and necrosis [102]. However, no apparent adverse effects were detected in this study, which may be due to mangrove's high tolerance to Cr stress.

Another essential metal Zn (55.80 mg kg^{-1} in *A. rotundifolia* at Gangasagar (S_3)) and Mn (2298.77 mg kg^{-1} in *S. apetala* at Gangadharpur (S_2)) along with toxic metal Pb (4.98 mg kg^{-1} in *X. mekongenesis* at Jharkhali (S_1)) showed a common tendency of accumulation in leaves, which may be attributed to acropetal movement of elements through translocation [103]. Mangrove plants are known to accumulate considerable amount of metals in leaves and other vegetative parts [77]. Nonetheless, it might indicate that the leaves of mangroves are able to take up and store certain trace metals. Moreover, the sampled leaves did not show any sign of injury in cases where concentrations were high. This suggests that leaves were tolerant to the trace metals by imparting minimal physiological effects to the leaves [104]. According to Verkleij and Schat [105], the translocation of excessive metals into mature leaves shortly before their shedding can also be considered as a tolerance mechanism, as can the increase in metal-binding capacity of the cell wall [106]. With the development of leaves from young to old, the changes in concentrations of metals in leaves indicated that Zn, Mn and Pb were apt to be accumulated in older leaves. Higher concentrations of these essential metals in leaf tissue may be because they were translocated to above ground parts and reused in plant system. It has been reported that some essential metals were transferred and reutilized in many plant species before defoliation, while toxic materials were accumulated in older leaves and then removed via defoliation [107].

In our study, *S. apetala* exhibited its capacity to absorb Cu in its bark (24.17 mg kg^{-1}) at Jharkhali (S_1) and Mn in mature leaf (2249.77 mg kg^{-1}) at Gangadharpur (S_2) S_2. According to the studies on leaf anatomy [108], different leaf morphology features were observed in *S. apetala* [109]. Epidermal trichomes were located outside *S. apetala* upper and lower epidermis while they were not observed on other mangrove species; stomatas distributed in both the upper and lower epidermis of *S. apetala* while only in the lower epidermis of other species. Such features might affect metal uptake and maintain process [22]. Chua and Hashim [110] also reported foliar absorption of certain elements especially in polluted industrial area. It was seen that only *S. apetala* absorbed higher magnitude of Fe and Mn than other mangroves in all the cases. For both the elements, the concentration varied more than ten times. For translocation factor, *S. apetala* exhibited highest TF values for Mn (31.99) and Pb (18.01) at Gangadharpur (S_2) and 9.95 for Zn at Jharkhali (S_1), respectively, where the highest value for translocation for other plant was 8.00 for Cr in case of *A. corniculatum*. Similar results were found by Sinegani and Ebrahimi [111] who observed significant metals mobilization between the plant parts above and below the surface of the sediment with translocation factor (TF) > 1. This indicates that the plant translocates elements effectively from root to the shoot and hence they could be labelled as accumulators of pollution as described earlier [112]. The prevalent trend justifies in considering the species as an effective indicator of trace metal contamination which was also endorsed by Nazli and Hashim [113] from Peninsular Malaysia.

3.5 Biological Risk Assessment

Hyperaccumulator plants can accumulate concentrations of trace metals in their aerial tissues far in excess of normal physiological requirements and above the levels found in most plant species [114]. An ideal plant for metal phytoextraction has to be tolerant to high levels of the metal and must accumulate high metal concentrations in its organs. Additional favourable traits are fast growth, easy propagation and a profuse root system [115, 116]. Translocation factor is considered as a potential tool for the determination of hyperaccumulator plants. A translocation factor greater than 1 indicates preferential partitioning of metals to the shoots [117–119]. Translocation factor values of the present work shows that *S. apetala* exhibited high values for Mn (4.48 and 31.99), Zn (9.95, 3.25) and Cu (3.42, 3.47) and Pb (1.84, 18.01) for Jharkhali (S_1) and Gangadharpur (S_2), respectively. *Aegiceros corniculatam* recorded high TF values for Cr (1.67, 8.00) and Pb (6.68, 6.25) at Jharkhali (S_1) and Gangadharpur (S_2), respectively. Members of family Avicenniaceae, *A. Alba* and *A. officinalis* showed high values for Fe (4.36, 2.78), Mn (9.53, 26.10), Pb (5.28, 5.93), and Cu (2.18, 2.23) at Gangadharpur (S_2). Bioconcentration factor, which is also considered as a tool for hyperaccumulation indicator, presented high values for *S. apetala* at Jharkhali (S_1) (5.99 for Mn and 10.7 for Fe in bark) and Gangadharpur (S_2) (2.28 for Mn in leaf). *Aegialitis rotundifolia* also showed high values for Mn (1.94 in bark), Cu (1.77 in leaf) and Zn (1.68 in bark) at Gangasagar (S_3). As stated earlier, extraction coefficient (EF) reflects the ability of plant shoot to accumulate metals and our study shows that *S. apetala* recorded the highest value for Mn (4.92) at Gangadharpur (S_2) and for Cu (1.73) and for Cr (3.01) at Jharkhali (S_1). *Aegialitis rotundifolia* recorded high value for Cu (6.51) at Gangasagar (S_3). Highest value of EF for Cr (4.22) was recorded in *X. mecongenesis* at Jharkhali (S_1). Thus, in the present study, *S. apetala* could be considered as hyperaccumulators as it fulfils most required criteria and is suitable for phytoextraction of metal-contaminated soils.

3.6 Result of Statistical Analysis

Pearson's correlation coefficient gives an idea about the possible relationships between metals: common origin, uniform distribution, similar behaviors and relationships amongst metals. The linear correlation coefficients calculated for metals in the mangrove organ samples indicated that a significant positive correlation existed amongst the metals. Significant correlation of Cu-Fe was found in case of all organs (Jharkhali (S_1): young leaf: $r = 0.899, p < 0.05$; mature leaf: $r = 0.931, p < 0.05$, Gangadharpur (S_2): mature leaf: $r = 0.790, p < 0.05$; root: $r = 0.763, p < 0.05$). Copper also showed significantly positive correlation with Manganese at Jharkhali (S_1) (young leaf: $r = 0.873, p < 0.05$; mature leaf: $r = 0.939, p < 0.05$). All mangrove plants showed significant differences between element concentrations in monitored plots (One-Way ANOVA: $-\mathrm{df} = 5$; $F = 20.26$; $P < 0.01$).

Table 3 reflects the factor loadings, variance percentages and cumulative percentages corresponding to principal components after varimax rotation was performed to secure increased environmental significance. The analysis resulted in the explanation of 81.1 % of variances in the data. The first factor (factor 1) explains 26.5 % of total variance and is related to the variables Mn, while the parameters Pb,

Table 3 Results of factor analysis (after Varimax rotation) considering different organs of all the mangroves

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Cu	–0.048	0.717	0.516	0.167
Fe	–0.029	–0.326	0.737	–0.554
Zn	0.414	0.733	–0.027	–0.143
Mn	0.514	0.114	–0.379	–0.67
Pb	–0.758	0.2	–0.311	–0.184
Cr	–0.758	0.244	–0.021	–0.338
% variance	26.5	21.2	17.5	15.9
Cumulative var %	26.5	47.7	65.2	81.1

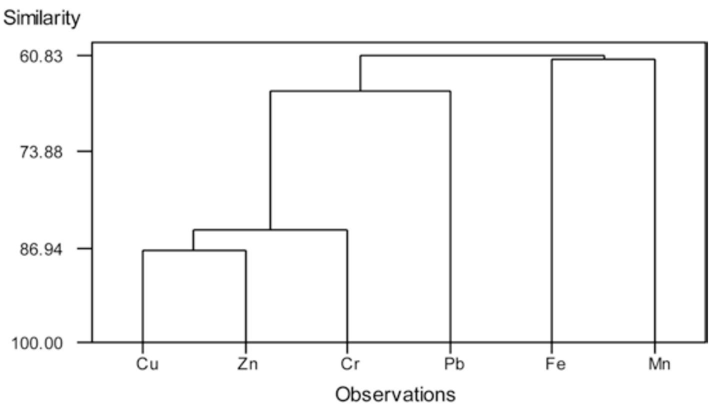

Fig. 6 Dendrogram showing the relationship between sediment samples in terms of trace metals at three study sites

Cr are negatively loaded with this factor. This may be due to very low or below detection limit of the element concentration in different plant organs of the studied mangroves. Factor 2 represents 21.2 % of the total variance and is positively loaded with Cu and Zn. Factor 3 explains 17.5 % of the total variance and is loaded with Cu and Fe. On the other hand, factor 4 represents 15.9 % of the total variance and is negatively loaded with Fe and Mn.

A hierarchical cluster analysis was carried out to identify any anomalous behaviour pattern in the mangrove plant organs. As shown in Fig. 6, which could be grouped into two clusters of Cu-Zn and Fe-Mn have been identified explaining that they are mainly generated from natural sources such as surface runoff and the presence of some metal bearing minerals in different locations of the study area. The Euclidean distance of the standardized data was chosen as dissimilarity measurement.

4 Conclusion

The present study has demonstrated the efficient role of *S. apetala* in accumulating the trace metals especially in pneumatophores and barks from a highly stressed estuarine mangrove system. This was mainly done through phytoextraction by adopting complex and cohesive processes and mechanisms. This dominant true mangrove species acts as both physical and biogeochemical barriers to trace metal mobility and hence has the potential to protect Sundarban ecosystem. Trace metal concentration in rhizospheric sediment are mainly controlled by the presence of finer particle sizes as well as organic carbon. In plants metal contamination is mainly concerned in root/ pneumatophore which is due to the formation of iron plaques on root surfaces. This tropical mangrove region is getting critically polluted due to severe anthropogenic stresses, and an extensive study is required to understand the role of rhizosphere processes in accumulation of trace metals in potential mangrove plants such as *S. apetala, A. alba and A. officinalis*.

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