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PHYTOREMEDIATION OF HEAVY METALS BY THE DOMINANT MANGROVE ASSOCIATE SPECIES OF INDIAN SUNDARBANS

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Highlights

- *Suaeda maritima* and *Salicornia brachiata* showed bioaccumulation of considerable levels of heavy metals (HMs) like zinc (Zn), copper (Cu), and lead (Pb) within their vegetative tissues from the surrounding soil with the highest degree of enrichment in the case of Zn, followed by Cu and Pb.
- Interestingly, the selected HMs displayed high organ-specificity for both these mangrove associate species with the highest enrichment in the roots (below-ground biomass), followed by the shoots and leaves (above-ground biomass). Hence, these halophytic species may be used as potential agents of HM phytoremediation and their large-scale farming in the supralittoral zone of Indian Sundarbans would be effective in the ecorestoration of this deltaic complex in context to conservative pollutants.
- Enhanced enrichment of selected HMs in the roots, and their reduced translocation to the shoots and leaves provide further scope for the use of aerial plant parts in food preparation (owing to their high nutritional values and pleasing taste) as means of nonconventional alternative livelihood for upgrading the economic profile of the people of Indian Sundarbans.
- However, the translocation factor (TF) of HMs in the leafy parts must be considered with priority before preparing food items from these halophytes as it is an indicator of HM level in the leaves of these halophytic species, which are currently used as ingredients of snacks and other food items.
- Halophyte farming at a macro level in relatively low HM-polluted zones of Indian Sundarbans may expand the horizon of innovative livelihood opportunities, provided it is linked with proper branding and marketing strategies through well-defined supply chain management (SCM) systems.

Abstract. The present study aims to investigate the phytoremediation potential of zinc (Zn), copper (Cu), and lead (Pb) by two dominant mangrove associate species, *Suaeda maritima*, and *Salicornia brachiata*, found in the high saline supralittoral zone of Indian Sundarbans in four stations of the Hooghly-Matla estuarine complex during the premonsoon season (May 2019). We found that concentrations of biologically available heavy metals (HMs) in the ambient soil and bioaccumulated HMs within the vegetative plant parts occurred as per the order: Sagar South > Bakkhali > Jharkhali > Bali Island. The order of biologically available and bioaccumulated HMs was Zn > Cu > Pb. Interestingly, the selected HMs display high organ-specificity for both species with the highest enrichment in roots, followed by stems and leaves. We propose that these halophytes could be used as agents of phytoremediation and their farming would be effective in the ecorestoration of this deltaic complex in context to conservative pollutants.

Keywords: Indian Sundarbans, heavy metals, bioaccumulation, mangrove associate species, phytoremediation.

Introduction

The Indian Sundarbans with an estimated area of ~9,630 sq. km is located between 21°32′–22°40′ N and between 88°85′–89°00′ E, within the North and South 24 Parganas district of the maritime state of West Bengal. The region is surrounded by the Bay of Bengal to the south, the River Hooghly to the west, the Dampier-Hodges line to the north, and the Harinbhanga-Raimangal River along the international boundary with Bangladesh to the east (Naskar & Mandal, 1999). The deltaic complex of Indian

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Sundarbans at the tip of the Bay of Bengal comprises one of the most taxonomically diverse and biologically prolific mangrove ecosystems in the Indian subcontinent with highly dynamic physicochemical parameters. It covers 9,630 sq. km of Biosphere Reserve area and houses around 102 islands, out of which 48 are inhabited, while 54 remain uninhabited (Mitra, 2000). The mangrove-dominated Indian Sundarbans sustains around 34 species of true mangrove floral plants and 69 mangrove associate species widely distributed in the intertidal mudflats, among which the genera *Suaeda* and *Salicornia* are the most dominant and are found in the high saline supralittoral zone of this deltaic lobe where they provide various ecosystem services, thereby forming a unique ecological niche.

Suaeda maritima represents a salt marsh mangrove associate plant species, which grows in the supralittoral zone where the daily tides seldom reach. Its young leaves and seeds can be used as fresh vegetables or cooked. The young shoots can be pickled in vinegar or consumed raw as a garnish in salads in small quantities due to their pleasant salty flavor (Hedrick, 1972; Tanaka, 1976; Kunkel, 1984; Facciola, 1990). This halophytic species is nutritionally rich as it contains considerable levels of sodium, potassium, calcium, and other minerals. This species of halophytes is commonly known as Sea Blite, herbaceous seepweed, and Rich's seepweed (or Giria shak in the local language of Indian Sundarbans) and belongs to the Chenopodiaceae family. The plants are annual, growing to about 30–40 cm in height and the stems develop several lateral branches with greenish-red fleshy leaves. A study of salt marshes of Sussex (a county in Southern England) revealed that this species thrives at the extreme high tide mark, and upon growth on sheltered banks (without the interference from other species) they usually reach a height of more than 30 cm with several lateral branches (Wetson et al., 2012). Naskar and Mandal (1999) observed that *S. maritima* is distributed all over the coastal regions and in the salt marshes in India and Southeast Asia. The plant prefers light sandy and medium loamy soils with pH ranging between neutral and basic (alkaline) and shows growth in high saline and alkaline soils. However, the species fails to grow in shades. It prefers moist soil and can withstand maritime exposure.

Like *Suaeda*, *Salicornia* is also a halophytic mangrove associate species, which belongs to the Amaranthaceae family. It is commonly known as glasswort, pickleweed, sea asparagus, sea beans, samphire, and crow's foot greens (Singh et al., 2014). In fact, the name *Salicornia* has been derived from the Latin word, which means "salt". Studies show that some species of this genus (such as *Salicornia europaea*) display tolerance towards salinity (i.e., up to 3% NaCl) (Yamamoto et al., 2009). This fleshy plant species is generally found at the edges of marshes, wetlands, seashores, and mudflats (in fact on most alkaline flats) (Smillie, 2015). *S. brachiata* has been historically used for making glass as the species is a source of soda (sodium carbonate). The species has unique medicinal value and is used to combat oxidative stress, diabetes, inflammation, asthma, hepatitis, and gastroenteritis (Essaidi et al., 2013). In recent times, both *Suaeda* and *Salicornia* have been widely used to prepare food products like samosa and kachori, which are among the most common and delicious Indian snacks (Mitra, 2020).

The deltaic complex of Indian Sundarbans has coastal characteristics encompassing estuaries, dunes, beaches, creeks, inlets, mudflats, mangrove swamps, etc. The rivers are the supporting system of biodiversity in this deltaic complex. In Indian Sundarbans, about 2,069 sq. km area is covered by tidal rivers or estuaries, all of which are finally emptying in the Bay of Bengal. The seven important estuaries networking the Indian Sundarbans from west to east include Hugli, Muriganga, Saptamukhi, Thakuran, Matla, Gosaba, and Harinbhanga (Chaudhuri & Choudhury, 1994).

The deltaic complex of Indian Sundarbans experiences a subtropical monsoon climate with an annual rainfall of approximately 1,600–1,800 mm along with several moderate to strong/severe cyclonic storms (Gopal & Chauhan, 2006). The Hooghly-Matla estuarine complex generally experiences a hot and humid climate during most part of the year. The climate of this region is chiefly governed by the monsoon with an active monsoon prevailing for almost four months of the year (i.e., from June to September) and the mean rainfall in this area is approximately 1,700 mm (Nath et al., 2004). The region is exposed to frequent cyclonic depressions like Aila (a severe cyclonic depression that hit the region on 25th May 2009), Amphan (on 20th May 2020), etc. that caused massive devastation like loss of lives, livestock, uprooting of mangroves, breaching of embankments, intrusion of seawater in the agricultural fields, etc.

The Indian Sundarbans, a UNESCO World Heritage Site and a proposed Ramsar site, is the home of the famous Royal Bengal tiger (*Panthera tigris tigris*) and is among the largest mangrove ecosystems in the world. In the recent past, due to unplanned urbanization, rapid industrialization, mushrooming of tourism units in and around the Indian Sundarbans as well as several other anthropogenic factors (like promotion of shrimp and crab culture farms, brick kilns, etc.), the mangrove ecosystem is under severe ecological threat of heavy metal (HM) pollution. Apart from the various industrial effluents that finally reach the Sundarbans estuary, the numerous fishing trawlers, and vessels that glide in this deltaic region add considerable amounts of HMs, primarily owing to the HM-rich antifouling paints that are used to coat the bottom of these vessels contain zinc (Zn), copper (Cu), and lead (Pb) (Bighiu et al., 2017). However, the region has continued to serve as a natural sink over the years because of the rich and diverse indigenous flora and fauna, a large proportion of which represents the mangrove biota (Figure 1).

The Indian Sundarbans can be demarcated into three sectors based on salinity. The western sector is hyposaline, the central sector is hypersaline, and the eastern sector is moderately saline (Trivedi et al., 2016). Out of these three sectors, the western sector experiences severe

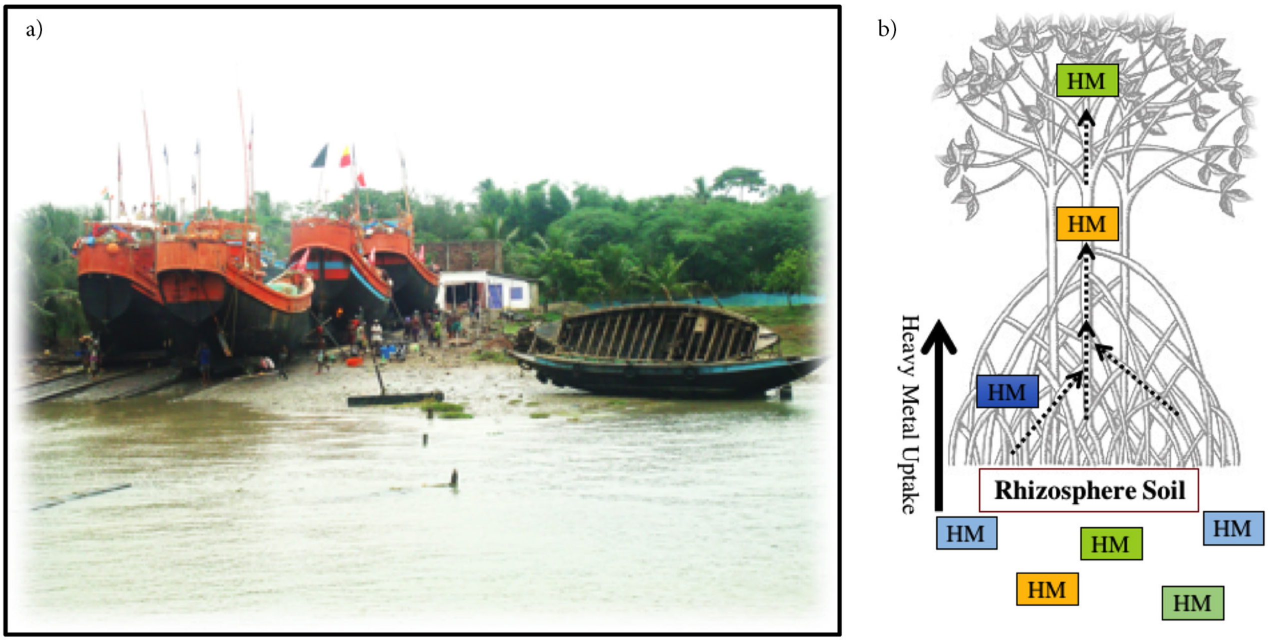

Figure 1. A potential point source of heavy metal (HM) pollution and its natural attenuation process in Indian Sundarbans: a) HM-rich antifouling paints that are used to coat the bottom of the fishing vessels and trawlers, which glide in the Hooghly-Matla estuarine complex of Indian Sundarbans; b) Mechanism of natural attenuation of HMs through phytoremediation by the mangroves and mangrove associate species of Indian Sundarbans. The black dotted arrows indicate the direction of HM transport

anthropogenic pressure due to the presence of a chain of industries and factories along the western bank of the River Hooghly (Mitra, 1998, 2013b; Mitra & Zaman, 2014, 2015, 2016). The central sector also experiences the threat of pollution as the municipal and urban wastes from the megacity of Kolkata reach the region through Dry Weather Flow (DWF) and Storm Weather Flow (SWF) canals. The eastern sector of Indian Sundarbans is devoid of anthropogenic activities and is noted for its wilderness as most of the portion of this region falls under the reserve forest.

The aquatic subsystem in the study area is highly dynamic with low salinity in the western part of Indian Sundarbans, where stations Sagar South (station 1) and Bakkhali (station 2) are situated. On the contrary, Jharkhali (station 3) and Bali Island (station 4) (located in the central Indian Sundarbans) are hypersaline; this is due to complete blockage of the freshwater discharge as a result of massive siltation in the Bidyadhari River that used to supply fresh water in the 15th century (Chaudhuri & Choudhury, 1994; Mitra, 2013b, 2019; Mitra & Zaman, 2015, 2016).

The main drivers of mangrove growth in the present study area are soil texture, soil salinity, organic carbon, nitrate-nitrogen, and phosphate-phosphorus content of the soil. In the domain of soil texture, significant variations have been observed for sand and clay between sectors, but not between stations. The silt is uniformly distributed in all the stations of western, central, and eastern Indian Sundarbans. The soil texture in the present study area has a uniform trend with the highest sand percentage, followed by silt and clay. The stations in the lowermost part of Indian Sundarbans, which are proximal to the Bay of Bengal (like stations 1 and 2), have high sand percentages owing to the influence of marine water that carries sand during tidal influx. The Soil Organic Carbon (SOC) in the mangrove ecosystem is mainly contributed by the mangrove litter and detritus and other factors like the waste generation from shrimp farms and industries. These may be the reason for relatively higher SOC in the intertidal mudflats of the stations in western Indian Sundarbans. The chain of factories situated in the western bank of the River Hooghly is one of the important factors contributing organic matter and carbon to the soil (Mitra & Choudhury, 1993; Mitra, 1998, 2013b; Banerjee et al., 2018). Soil nitrate-nitrogen and phosphate-phosphorus play major roles in the growth and survival of the mangrove flora (Kathiresan et al., 1994). The anthropogenic influences play a critical role in regulating the soil parameters on the surface layers. Primarily, such influences come from the adjacent agricultural lands, shrimp farms, fish landing stations, and effluents from the highly urbanized townships along the banks of the Hooghly River, which bring with them several pollutants, including HMs.

Mangroves, like other plants, possess an inherent phytoremediation potential and are capable of HM uptake from the ambient water and sediment, and accumulating them within their vegetative tissues, including the roots, stems, and leaves (Mitra, 2013b, 2019). The process of phytoremediation of HMs could occur through one of the three phytoremediation techniques or phytotechnologies, namely a) phytoextraction, b) phytostabilization, and 3) phytovolatilization. In phytoextraction, HM transport and concentration occur in the above-ground biomass (i.e., stems and leaves) from the ambient soil.

In phytostabilization, accumulation of HMs by the roots or their precipitation within the rhizosphere takes place, thereby minimizing metal mobility in the contaminated soil. In phytovolatilization, soil HMs are converted into volatile chemical species, which eventually escape into the atmosphere through the leaves during the transpiration of plants (Nascimento & Xing, 2006). Interestingly, the potential of phytoremediation of HMs by mangrove plants varies greatly within genera. The mangrove community binds and stabilizes soil/sediments, ions/nutrients, and persistent pollutants, including HMs (Mitra, 2013a; Chakraborty et al., 2014a, 2014b), and therefore may aid in improving the overall water quality. A large number of research studies have been carried out in the present geographical locale on the pollution status of the estuarine water and sediments (Mitra & Choudhury, 1992; Mitra et al., 1994, 2011; Mitra, 1998; Banerjee et al., 2012; Mitra & Ghosh, 2014; Chakraborty et al., 2014a, 2016; Mitra & Zaman, 2015), but very scanty works have been undertaken on the bioaccumulation of HMs within the vegetative parts of mangrove associate species. On this background, we took an attempt to monitor the ambient environmental quality as well as the bioaccumulation of selected HMs within the roots, stems, and leaves of the dominant mangrove associate species of Indian Sundarbans from the surrounding soil. Several studies were conducted on HM bioaccumulation in mangroves and mangrove associate species of Indian Sundarbans (Mitra et al., 2014; Chowdhury & Maiti, 2016; Banerjee et al., 2018). However, the merit of the present work lies in the fact that metal concentrations in the ambient soil have also been measured to determine the potential of the species as HM bioaccumulators, and therefore, as agents of HM phytoremediation. The present study aims to investigate the phytoremediation potential of Zn, Cu, and Pb by the two thriving mangrove associate species of Indian Sundarbans, *S. maritima* and *S. brachiata* in four stations located in the Hooghly-Matla estuarine complex, namely Sagar South (station 1), Bakkhali (station 2), Jharkhali (station 3), and Bali Island (station 4) during the month of May 2019 (i.e., the premonsoon season in the study area).

1. Materials and methods

The entire methodology of the present research work (carried out during May 2019) consists of four distinct phases, namely 1) selection of study area and sampling sites, 2) sampling of ambient soil and plant species, 3) analyses of selected HMs, and 4) determination of phytoremediation potency of selected mangrove associate species.

1.1. Selection of study sites

Four stations were selected in the Hooghly-Matla estuarine complex of Indian Sundarbans based on the differential anthropogenic activities. Sagar South (station 1; 21°31′4.68″ N, 88°01′47.28″ E) and Bakkhali (station 2; 21°34′22.80″ N, 88°17′52.10″ E) are located in the western sector, while Jharkhali (station 3; 22°05′52.82″ N, 88°41′47.25″ E) and Bali Island (station 4; 22°04′35.17″ N, 88°44′55.70″ E) are located in the central sector of Indian Sundarbans (Figure 2).

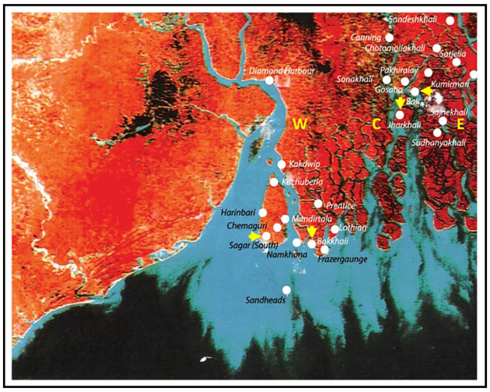

Figure 2. A satellite image of the study area (Hooghly-Matla estuarine complex of Indian Sundarbans) pointing to the selected study sites. The map shows three sectors of Indian Sundarbans, namely the western (W) (hyposaline), central (C) (hypersaline), and eastern (E) (hyposaline) sectors with four selected stations (Sagar South, Bakkhali, Jharkhali, and Bali Island) indicated with bold yellow arrowheads (source: Modified after Mitra, 2013b)

Sagar South and Bakkhali are noted for extremely high tourism activities. The Kapil Muni temple in Sagar South attracts about 10 lakhs devotees every year during Makar Sankranti (in mid-January) for taking a holy bath in the estuarine water (Mitra & Zaman, 2020). The picturesque beach in Bakkhali is a favorite weekend getaway destination primarily for the people of West Bengal. Jharkhali and Bali Island are relatively less exposed to anthropogenic activities except for occasional tourism pressure during the postmonsoon season.

1.2. Sample collection and processing

For the present study, two thriving mangrove associate species of Indian Sundarbans, namely *S. maritima* and *S. brachiata* were selected for the estimation of HM bioaccumulation from the ambient soil (Figure 3). The ambient soil of *S. maritima* and *S. brachiata* (5–10 cm depth) from four different stations were collected and transferred to a clean polyethylene bag with the help of a clean plastic spatula, sealed immediately, and brought to the laboratory. The samples were then freed from visible shells and shell fragments, dried in an oven for 5–6 h at 105 °C, ground to homogeneous powder in a mortar, and stored in clean acid-washed polythene containers at 4 °C for further analysis.

The halophyte samples from each of the four different stations were uprooted, put inside sterile bags, and brought to the laboratory. The roots, stems, and leaves for each plant type were then carefully separated, and the segregated vegetative tissues were gently washed thrice with deionized water and allowed to air dry. Air-dried samples were further dried in an oven at 60 °C for 24 h; then, these

a)

b)


Figure 3. Representative images of the two thriving mangrove associate species growing in the high saline supralittoral zone of Indian Sundarbans: a) *Suaeda maritima*, a member of the Chenopodiaceae family; b) *Salicornia brachiata*, a member of the Amaranthaceae family

oven-dried samples were homogenized using mortar and pestle as described by MacFarlane (2002) and stored in clean acid-washed polythene containers at 4 °C for further analysis.

1.3. Heavy metal analysis using Atomic Absorption Spectroscopy

The biologically available Zn, Cu, and Pb (in ppm) present in the ambient soil were measured simultaneously, and then, their respective concentrations within the plant vegetative parts (in ppm dry weight) were assessed by acid digestion of the oven-dried soil and plant tissue samples using atomic absorption spectroscopy.

Analysis of biologically available HMs in the ambient soil samples was carried out by taking 1 gm of soil and digesting it with a weak acid (0.5 N HCl) based on the standard procedure described by Malo (1977).

For the analysis of bioaccumulated HMs within the plant tissues, 1 gm of the powdered root, shoot, and leaf samples of *S. maritima* and *S. brachiata* collected from each station were digested with a concentrated mixture of HNO_3 and H_2O_2 under fume hood following the procedure as outlined by Krishnamurthy et al. (1976) and MacFarlane (2002).

The acid-digested samples were filtered, made up to a final volume of 25 ml and the resulting solutions were then kept aside in clean containers for the determination of respective HM ion concentrations. Analysis of each selected HM (Zn, Cu, and Pb) was carried out using a flame Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer, Type 3030) equipped with a deuterium background corrector. The reagent blank lacks any detectable amount of trace metals. Concentrations of HMs were calculated from the absorbance values and expressed in ppm and ppm dry weight for ambient soil and mangrove associate species samples, respectively.

1.4. Phytoremediation potency of selected mangrove associate species

1.4.1. Estimation of bioaccumulation factor (BAF)

For bioaccumulation of HMs within the roots, shoots, and leaves of the selected mangrove associate species from ambient soil, bioaccumulation factor (BAF) values (i.e., amount of HMs present within the plant tissues relative to the ambient soil) for each selected HM in the plant root, shoot, and leaf samples collected from each of the four stations were mathematically calculated using Eqs (1–3) as described in Mukherjee et al. (2019) with modifications:

For root tissues,

$$\mathrm{BAF} = Cr \div Cas. \tag{1}$$

For shoot tissues,

$$\mathrm{BAF} = Cs \div Cas. \tag{2}$$

For leaf tissues,

$$\mathrm{BAF} = Cl \div Cas, \tag{3}$$

where: *Cr*, *Cs*, and *Cl* represent HM concentrations in the roots, shoots, and leaves of the mangrove associate species under study, respectively, and *Cas* represents HM concentrations in the ambient soil of the mangrove associate species under study.

1.4.2. Estimation of translocation factor (TF)

For translocation of HMs from the roots to the shoots in the selected mangrove associate species, translocation factor (TF) values (i.e., amount of HMs present within the plant shoot tissues relative to the root tissues) for each selected HM in the plant shoot samples collected from each of the four stations were measured using Eq. (4) as described in Mukherjee et al. (2019) with modification:

$$\mathrm{TF} = Cs \div Cr, \tag{4}$$

where: *Cs* represents HM concentrations in the shoots of the mangrove associate species under study and *Cr* represents HM concentrations in the roots of the mangrove associate species under study.

For translocation of HMs from the shoots to the leaves in the selected mangrove associate species, TF values (i.e., amount of HMs present within the plant leaf tissues relative to the shoot tissues) for each selected HM in the plant leaf samples collected from each of the four stations were measured using Eq. (5):

$$TF = Cl \div Cs, \tag{5}$$

where: Cl represents HM concentrations in the leaves of the mangrove associate species under study and Cs represents HM concentrations in the shoots of the mangrove associate species under study.

1.5. Statistical analysis

All experimental analyses were done in triplicates. Statistical analysis was performed using two-way analysis of variance (ANOVA) to determine whether the concentrations of selected HMs varied significantly between stations and between mangrove associate species. P-value < 0.01 is considered to be statistically significant. All graphs were prepared using GraphPad Prism Software 8.1.2 (227) (San Diego, California, USA). SPSS 21.0 (for Windows) was used for the statistical analysis.

2. Results and discussion

We observed a clear trend in our results as listed and discussed below.

2.1. Biologically available and bioaccumulated heavy metals

All the three selected HMs (including Zn, Cu, and Pb) in the ambient soil and the vegetative (i.e., root, shoot, and leaf) portions of the two selected mangrove associate species of Indian Sundarbans (viz. *S. maritima* and *S. brachiata*) exhibit a common sequence with considerable high HM concentrations at Sagar South and Bakkhali (in the hyposaline western Indian Sundarbans), followed by Jharkhali and Bali Island (in the hypersaline central Indian Sundarbans). Biologically available and bioaccumulated HM concentrations in the ambient soil and the two plant samples, respectively, in the four selected stations, were found as per the order: Sagar South (station 1) > Bakkhali (station 2) > Jharkhali (station 3) > Bali Island (station 4). The order of biologically available HMs in the ambient soil as well as bioaccumulated HMs within the plant tissues was found to be Zn > Cu > Pb (Figures 4–6).

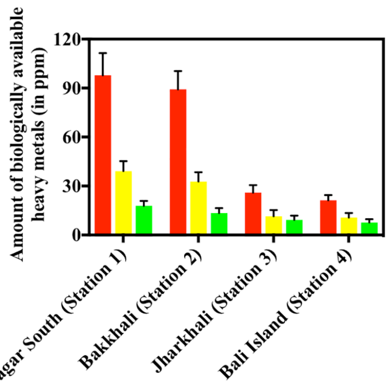

Figure 4. Biologically available heavy metals (HMs) present in the ambient soil of the selected mangrove associate species sampled from four different study sites in the Hooghly-Matla estuarine complex of Indian Sundarbans. Error bars indicate standard deviation from the mean of triplicate values. Two-way ANOVA shows that the p-value is < 0.1 between stations and between HMs

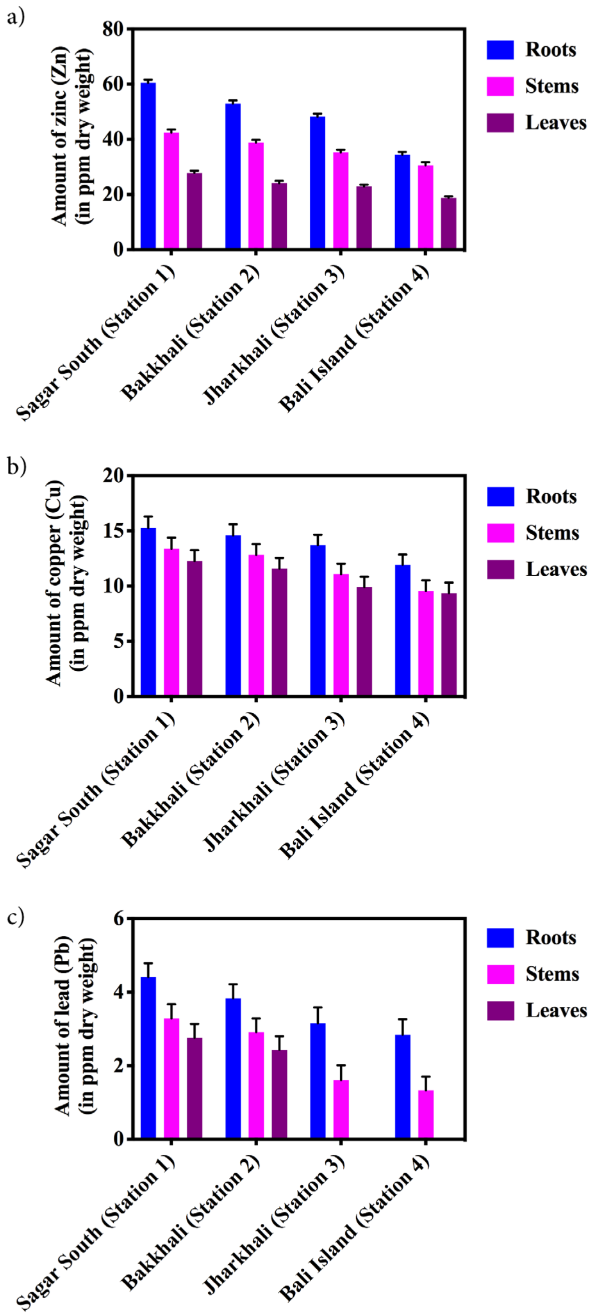

Figure 5. Heavy metal bioaccumulation within the vegetative tissues of *Suaeda maritima* sampled from the selected study sites in the Hooghly-Matla estuarine complex of Indian Sundarbans. Error bars indicate standard deviation from the mean of triplicate values

2.2. Organ specificity of bioaccumulated heavy metals

Additionally, we noticed that all the selected HMs have high organ specificity for both selected halophytic species. The accumulation was highest in the roots, followed by the stems and leaves (Figures 5 and 6). This observation is consistent with previous reports on mangroves and mangrove associate species of Indian Sundarbans (Mitra et al., 2014; Chowdhury & Maiti, 2016; Banerjee et al., 2018).

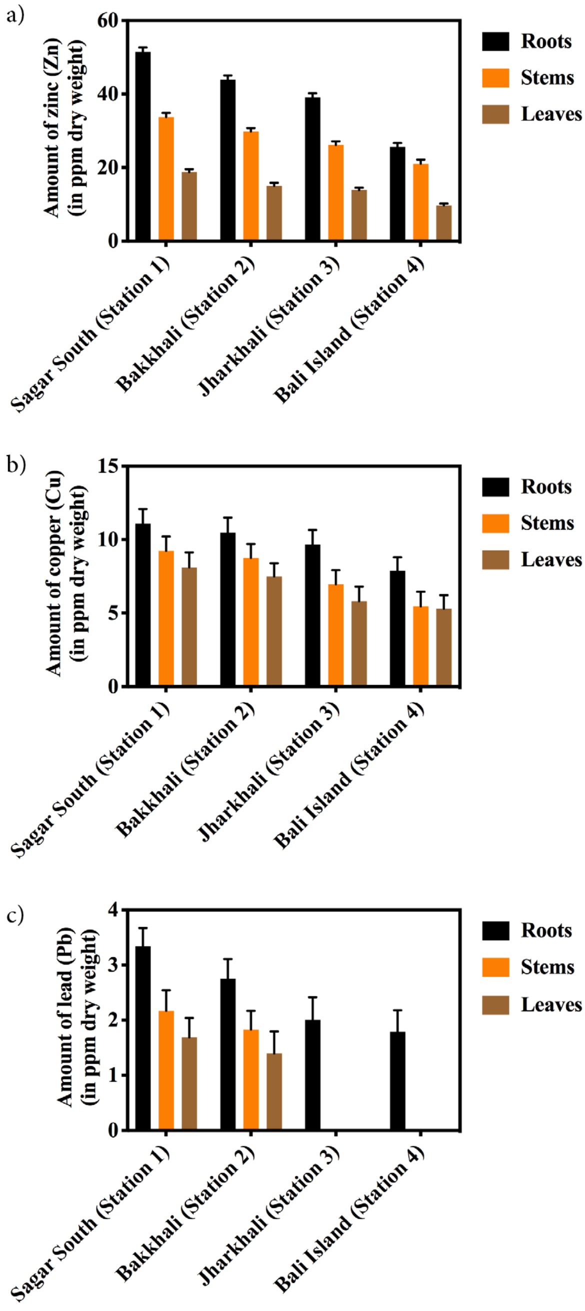

Figure 6. Heavy metal bioaccumulation within the vegetative tissues of *Salicornia brachiata* sampled from the selected study sites in the Hooghly-Matla estuarine complex of Indian Sundarbans. Error bars indicate standard deviation from the mean of triplicate values

2.3. Bioaccumulation of heavy metals within roots, shoots, and leaves from ambient soil

Table 1 shows the BAF values of selected HMs in the vegetative tissues (viz. roots, shoots, and leaves) of *S. maritima* and *S. brachiata* collected from the selected four stations of Hooghly-Matla estuarine complex. In context to the roots and ambient soil of *S. maritima* and *S. brachiata*, the order of BAF for Zn is Jharkhali > Bali Island > Sagar South > Bakkhali, the order of BAF for Cu is Jharkhali > Bali Island > Bakkhali > Sagar South, and the order of BAF for Pb is Bali Island > Jharkhali > Bakkhali > Sagar South (Table 1). For both selected species from all four selected stations, the BAF values are highest in the roots, followed by shoots, and are lowest in the leaves with maximum enrichment of Zn, followed by Cu and minimum enrichment of Pb in all the plant tissues (Table 1). This is consistent with the biologically available HM concentrations of the ambient soil of both these mangrove associate species in all the selected stations under study.

2.4. Translocation of heavy metals from roots to shoots

In context to the shoots and roots of *S. maritima* and *S. brachiata* collected from the four stations, the order of TF for Zn is Bali Island > Bakkhali > Jharkhali > Sagar South, the order of TF for Cu is Bakkhali > Sagar South > Jharkhali > Bali Island, and the order of TF for Pb is Bakkhali > Sagar South > Jharkhali > Bali Island (Table 2). Here, the average maximum enrichment is seen in the case of Cu, followed by Zn, and minimum enrichment of Pb is observed in both plant species (Table 2).

2.5. Translocation of heavy metals from shoots to leaves

In context to the leaves and shoots of *S. maritima* and *S. brachiata* collected from the four stations, the order of TF for Zn is Sagar South > Jharkhali > Bakkhali > Bali Island, the order of TF for Cu is Bali Island > Sagar South > Bakkhali > Jharkhali, and the order of TF for Pb is Sagar South > Bakkhali > Jharkhali and Bali Island (Table 3). Here, the average maximum enrichment is seen in the case of Cu, followed by Pb, and minimum enrichment of Zn is observed in both plant species (Table 3).

2.6. ANOVA of heavy metals in the roots, stems, and leaves between stations and between species

ANOVA results exhibit significant variations of HMs (Zn, Cu, and Pb) in the roots, stems, and leaves between stations and between species except for Pb in leaves between stations and between species ($p < 0.03$ and $p > 0.1$, respectively). The different activities to which the selected stations of Indian Sundarbans are exposed may have attributed to the spatial variation in their pollution status (Tables 4–6).

Table 1. Bioaccumulation factor (BAF) values of heavy metals in the vegetative tissues of *S. maritima* and *S. brachiata*

Sl. no.	Stations	BAF values of heavy metals in the vegetative plant tissues																	
		S. maritima									*S. brachiata*								
		Roots			Stems			Leaves			Roots			Stems			Leaves		
		Zn	Cu	Pb	Zn	Cu	Pb	Zn	Cu	Pb	Zn	Cu	Pb	Zn	Cu	Pb	Zn	Cu	Pb
1.	Sagar South (Stn.1)	0.62	0.39	0.25	0.43	0.34	0.18	0.28	0.31	0.15	0.53	0.28	0.19	0.34	0.24	0.12	0.19	0.21	0.09
2.	Bakkhali (Stn.2)	0.59	0.45	0.28	0.44	0.39	0.22	0.27	0.35	0.18	0.49	0.32	0.20	0.33	0.27	0.14	0.17	0.23	0.10
3.	Jharkhali (Stn.3)	1.86	1.20	0.34	1.36	0.97	0.17	0.88	0.87	BDL	1.51	0.85	0.22	1.01	0.61	BDL	0.54	0.51	BDL
4.	Bali Island (Stn.4)	1.62	1.12	0.37	1.44	0.90	0.17	0.88	0.88	BDL	1.20	0.74	0.23	0.99	0.51	BDL	0.46	0.50	BDL

Note: Each value is the mean of triplicate data. Stn. denotes station and BDL denotes below detectable level.

Table 2. Translocation factor (TF) values of heavy metals in the shoot tissues of *S. maritima* and *S. brachiata*

Sl. no.	Name	TF values of heavy metals in the shoot tissues					
		S. maritima			*S. brachiata*		
		Zn	Cu	Pb	Zn	Cu	Pb
1.	Sagar South (Stn.1)	0.70	0.88	0.74	0.65	0.83	0.65
2.	Bakkhali (Stn.2)	0.73	0.88	0.76	0.68	0.84	0.67
3.	Jharkhali (Stn.3)	0.73	0.81	0.51	0.67	0.72	BDL
4.	Bali Island (Stn.4)	0.89	0.80	0.47	0.82	0.69	BDL

Note: Each value is the mean of triplicate data. Stn. denotes station and BDL denotes below detectable level.

Table 3. Translocation factor (TF) values of heavy metals in the leaf tissues of *S. maritima* and *S. brachiata*

Sl. no.	Name	TF values of heavy metals in the leaf tissues					
		S. maritima			*S. brachiata*		
		Zn	Cu	Pb	Zn	Cu	Pb
1.	Sagar South (Stn.1)	0.66	0.92	0.84	0.56	0.88	0.78
2.	Bakkhali (Stn.2)	0.62	0.90	0.84	0.50	0.85	0.77
3.	Jharkhali (Stn.3)	0.65	0.89	BDL	0.53	0.83	BDL
4.	Bali Island (Stn.4)	0.61	0.98	BDL	0.46	0.97	BDL

Note: Each value is the mean of triplicate data. Stn. denotes station and BDL denotes below detectable level.

Table 4. ANOVA of heavy metal concentrations in the root samples between stations and between species

Factor	Variable	F_{cal}	F_{crit}	P-value
Tissue Zn (root)	Between stations	22251.605661	9.276628	$p < 0.0001$
	Between species	14983.622722	10.127964	$p < 0.0001$
Tissue Cu (root)	Between stations	1811.679104	9.276628	$p < 0.0001$
	Between species	14980.388060	10.127964	$p < 0.0001$
Tissue Pb (root)	Between stations	1328.399999	9.276628	$p < 0.0001$
	Between species	3139.266667	10.127964	$p < 0.0001$

Table 5. ANOVA of heavy metal concentrations in the shoot samples between stations and between species

Factor	Variable	F_{cal}	F_{crit}	P-value
Tissue Zn (shoot)	Between stations	919.023056	9.276628	p < 0.0001
	Between species	2748.213333	10.127964	p < 0.0001
Tissue Cu (shoot)	Between stations	7255.480000	9.276628	p < 0.0001
	Between species	40541.040000	10.127964	p < 0.0001
Tissue Pb (shoot)	Between stations	74.507697	9.276628	p = 0.0026*
	Between species	109.486479	10.127964	p = 0.0019**

Note: *p < 0.003 = Significant; **p < 0.002 = Significant.

Table 6. ANOVA of heavy metal concentrations in the leaf samples between stations and between species

Factor	Variable	F_{cal}	F_{crit}	P-value
Tissue Zn (leaf)	Between stations	88893.586665	9.276628	p < 0.0001
	Between species	524465.639989	10.127964	p < 0.0001
Tissue Cu (leaf)	Between stations	6706.893130	9.276628	p < 0.0001
	Between species	61970.038168	10.127964	p < 0.0001
Tissue Pb (leaf)	Between stations	15.709055	9.276628	p = 0.0244*
	Between species	2.997825	10.127964	p = 0.1818**

Note: *p < 0.03 = Not significant; **p > 0.1 = Not significant.

The Indian Sundarbans is one of the most biologically diverse and taxonomically rich ecosystems of the tropics, and is a unique gene pool where mangroves and tigers share the same environment. However, the wilderness of this deltaic complex is under stress due to the negative impacts posed by the chain of industries and urban centers concentrated in the western region. The wastes released from the megacity of Kolkata and Haldia port-cum-industrial complex reach the western sector through the Hooghly estuary (Mitra & Choudhury, 1993; Trivedi et al., 1995; Mitra, 2013b) due to which the HM level in Sagar South (station 1) was highest compared to the other three stations. In addition to the industrial discharges and municipal wastes, the antifouling paints used for the conditioning of passenger vessels, fishing vessels, trawlers, tankers, floating barges, jetties, etc. also contribute an appreciable amount of HMs (primarily Zn, Cu, and Pb) to the ambient environment of Sundarbans. Bakkhali (station 2) is noted for heavy tourism pressure due to the scenic beach, hotels, restaurants, and fish landing activities. The antifouling paints used in the fishing boats and trawlers are another major contributor of HMs in this region. Jharkhali (station 3) is the gateway of the forest area of Indian Sundarbans and hence experiences a tourist inflow preferably during the winter season. This has made the area moderately contaminated with the HMs. Bali Island (station 4) in the central Indian Sundarbans is practically devoid of anthropogenic pressure and is only exposed to some fishing-related activities due to which Zn, Cu, and Pb have leached in the ambient soil.

Formation of ferric oxide/hydroxide precipitates or iron plaques (IPs) near the root zone enveloping the roots is common in wetland plants and is accountable for the selective sequestration of various mineral/HM ions, and eventually their uptake, and transport. IP may either act as a buffer or a barrier and, therefore, may increase or decrease the uptake of HMs by plants (Tripathi et al., 2014). The presence of root IPs may, in turn, stabilize/immobilize HM species through co-precipitation (Hansel et al., 2001) or enhance their bioavailability via sequestration (Blute et al., 2004). Although root Fe/Mn plaque formation has been reported in *Suaeda* and/or *Salicornia* species (Caçador et al., 2009; Zhao et al., 2019), root IPs have not been observed to play a significant role in the uptake of HMs, at least in *Salicornia* species (Smillie, 2015).

The BAF values for all the HMs in the roots, stems, and leaves reflect the potential of the mangrove associate species in absorbing HMs from the surrounding media (Table 1). The level of accumulation of selected HM ions in the plant tissues with maximum level of enrichment in the roots (below-ground biomass) confirms that these mangrove associate species can be used as agents of phytoremediation of HMs at the macro level. Moreover, hyperaccumulation of Zn and Cu in the roots of *S. maritima* and *S. brachiata* (collected from Jharkhali and Bali Island) indicates the potential of the selected halophytic species for phytoremediation of these HMs through phytostabilization (Table 1). The reduced translocation of HMs to the aerial vegetative parts of these mangrove associates from the roots (with the least amount of translocation to the leafy parts) as observed from the TF values (Tables 2 and 3) gives promise for large-scale farming of these halophytes in the supralittoral zone for HM phytoremediation, and subsequently for their use in food preparation (as these species are extremely rich in nutrients and have a palatable taste) only when grown in regions with low anthropogenic footprints and HM stress, which will

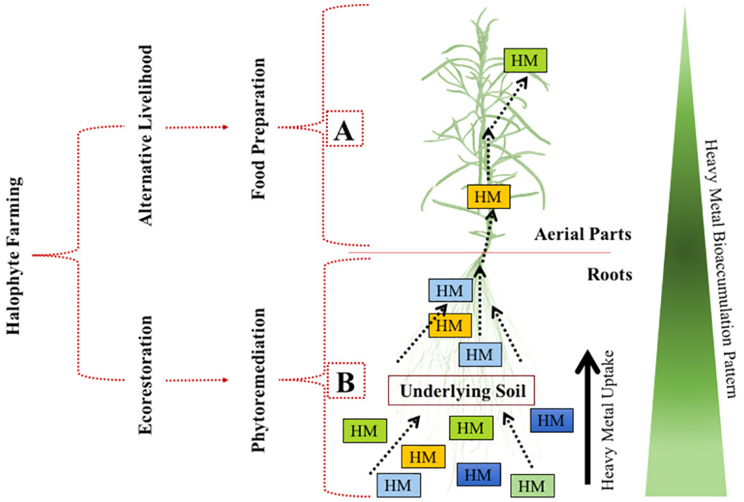

Figure 7. Proposed mechanism of phytoremediation of heavy metals (HMs) by the mangrove associate species of Indian Sundarbans and their application in ecorestoration and alternative livelihood generation through large-scale farming. A schematic diagram of a representative mangrove associate species shows enhanced HM enrichment in the below-ground biomass (i.e., roots) and reduced HM translocation to the above-ground biomass (i.e., shoots and leaves). The red dotted bracket "A" indicates the portion of the plant (i.e., aerial parts) that could be used for food preparation after farming of these halophytes since maximum metal enrichment is seen in the roots with the lowest metal translocation to leaves, followed by shoots in the present study. The red dotted bracket "B" indicates the portion of the plant (i.e., roots) that primarily assists in phytoremediation through the phytostabilization process. The black dotted arrows denote the direction of metal uptake/transfer. The base of the green-colored triangle denotes the highest HM concentration, while its apex indicates the lowest metal concentration

eventually generate alternative (nonconventional) livelihood scopes for the underprivileged island dwellers of Indian Sundarbans (Figure 7).

To sum up, it can be suggested that farming of these halophytes in the supralittoral zone might be effective in maintaining the overall health of the ecosystem in context to conservative pollutants and could also be beneficial for alternative livelihood generation in the mangrove-dominated Indian Sundarbans Delta.

Conclusions

The entire research leads to a few important core findings as highlighted here.

1. The order of HM accumulation in both the mangrove associate species (*S. maritima* and *S. brachiata*) is Zn > Cu > Pb.

2. The biologically available HMs in the ambient soil followed the sequence Zn > Cu > Pb, which is similar to that accumulated in the case of the mangrove associate floral species.

3. Since both these selected plant species are rich in nutrients and have a pleasant salty taste, their leaves can be used for preparing snacks and other food items as means of alternative livelihood for upgrading the economic profile of the people of Indian Sundarbans. In this context, it is important to monitor the HMs in the plant parts from the human health point of view. It is to be noted that the species with less TF should be selected from stations for preparing the food items as it indicates less bioavailability of the HMs to the leafy parts.

4. The species can also be used for biomonitoring of HMs in the ambient soil/sediments, preferably as indicator species of HM pollution as suggested by Smillie (2015).

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Conflict of interest

The authors declare no conflict of interest.

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