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Identification of metal tolerant plant species in mangrove ecosystem by using community study and multivariate analysis: a case study from Indian Sunderban

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Abstract This study at Indian Sundarbans, identifies metal tolerant mangroves with phytoremediation potential by understanding the affect of metal pollution on the community structure of this estuarine ecosystem. Community study indicates that polluted site has lower relative density of the true mangrove and four metal tolerant species (*Cryptocoryne ciliata*, *Heliotropium currasavicum*, *Hemarthria altissima* and *Avicennia officinalis*) are predominant with 72 % of relative density. Biodiversity indices (Simpson's index of Dominance and Diversity, Shannon-Weiner index) indicate reduction in diversity and increase in dominance of metal tolerance species at the polluted site. The cluster/components originating after cluster and principal component analysis shows that Cr, Pb, Cd and Ni are metal pollutants of anthropogenic origin. Metal accumulation study is conducted on these four species after assessing the status of metal pollution in their rhizospheres using Ecological risk index, Geoaccumulation index, Enrichment factor and Contamination factor. Bioaccumulation factor emphasized that *C. ciliata* has a high potential for extracting Cd, Cr and Pb. Bioconcentration factor of Cr is high for *A. officinalis*, *C. ciliata* (potentially invasive) and *H. altissima* (invasive species) whereas translocation factor indicates Cd, Ni and Zn can be translocated to the aerial part of these plants. In addition, *H. altissima* also transfer Cr and Pb to their aerial parts. This study concluded that *C. ciliata* could be used as phytoextracter for Cd, Cr and Pb in metal contaminated mangrove ecosystem.

Keywords Mangrove · Ecological risk index (ERI) · Bioaccumulation factor (BAF) · Bioconcentration factor (BCF) · Translocation factor (TF) · Biodiversity indices · Heavy metals · Cr, Pb, Cd and Ni pollution

Introduction

Mangroves are coastal forests found in sheltered estuaries, along riverbanks and lagoons in the tropics and subtropics. These are uniquely adapted plant groups showing anatomical, morphological and physiological adaptations to persist in coastal and tidal flats subjected to high salinity stress (Giri et al. 2014). These imitable floral assemblage not only sustain the amphibious ecosystem, but also render numerous economic and environmental services like sequestering carbon (100 tons of CO_2 per hectare), stabilizing the soil particles to control soil erosion, act as a hatchery for aquatic fauna and stands as a first wall of defense against natural disasters like tsunami or cyclones (Donato et al. 2011; Dasgupta and Shaw 2015).

The mangrove forests of South and Southeast Asia are facing escalating risk from habitat modification and fragmentation due to environmental and anthropogenic reasons. In an estimate, it is argued that mangroves are disappearing at an alarming rate of 2 % per year, which is higher than any other endangered ecosystems like coral reefs or tropical rain forest (Duke et al. 2007; Alongi 2008). Transboundary forest of Sundarbans, spread over 1 million Ha of which 60 % is in Bangladesh and 40 % in India, are also at significant risk from exposure to pollution from effluent discharge and diversion of land for

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aquaculture activities (DasGupta and Shaw 2015). It has been apparent from past researches that there is a reduction of 4.38 % of mangrove forest in the Indian part of the Sunderbans between 1999 and 2010 due to these unsustainable anthropogenic practices (Giri et al. 2014).

Mangrove sediment acts as the sink of heavy metal and several researchers are working on the effect of anthropogenic pollutants on the mangrove sediments and halophytes (Defew et al. 2005; Lacerda et al. 1992; Tam and Wong 2000; Natesan et al. 2014). Heavy metal is foremost in imparting deleterious effect to this fragile ecosystem due to their potential to get into the food chain and getting biomagnified in the process (Defew et al. 2005). Higher accumulation of metals in mangroves is due to the unique physico-chemical properties of the asphyxiated mangrove sediment and elevated quantity of organic matter (Harbison 1986; Silva et al. 1990; Tam and Wong 2000; Defew et al. 2005). This is an important reason behind studying metal accumulation in mangroves in order to identify plants with phytoremediation potential. The mangroves that are in close proximity to the urban developmental areas are exposed to high loads of toxic heavy metals (Defew et al. 2005; Tam and Wong 2000). Moreover mangroves are mostly found in coastal/estuarine areas where it remains in close vicinity to the human habitations exposing to the pollutants released due to anthropogenic activities. The presence of elevated concentration of heavy metals and metalloids especially cadmium (Cd) and lead (Pb) have an enzyme disruptive deleterious effect on the native flora as they replace essential metals in pigments and creates reactive oxygen species (Babula et al. 2008). So mangrove patches exposed to heavy metal pollution have a selection pressure that permits only metal tolerants, bioaccumulators or excluders to proliferate in this stressed environment which could affect the community composition of the halophytes and associative flora.

Metal extraction methods and protocols are highly debated in bioaccumulation studies. Particular extraction methods can extract different form of metals in different mineralogical and soil types (Nirel and Morel 1990; Peijnenburg et al. 2007). Generally $CaCl_2$, $Ca(NO_3)$, NH_4Ac, Mg-salts, $BaCl_2$ are used as mild extractants; HCl, Sodium dithionite (hyposulphite), $Na_2S_2O_4$ as reductants; EDTA–DTPA are strong chelating agents; strong acids like H_2SO_4, HNO_3 are also used for metal extraction purposes from sediment matrix but there is no common consensus on the specific metal extraction methodology and reagents in toxicological studies (Peijnenburg et al. 2007). EDTA–DTPA extractable metal is assumed to be the bioavailable part of the total metal and used for some bioaccumulation-translocation studies. Maiti and Jaiswal (2013) have used DTPA, EDTA and *aqua regia* to separately calculate the bioaccumulation/translocation factors in *Typha latifolia*,

Fimbristylis dichotoma, *Amaranthus defluxes*, *Saccharum spontaenum* and *Cynodon dactylon* found near a thermal power plant in India. But both the extractants (EDTA and DTPA) are found most suitable for calcareous soil to extract "plant-available fractions" (Peijnenburg et al. 2007; Das and Maiti 2008).

Total metal is sometime extracted with varied acid mixtures along with HF to break the silicate lattices for the toxicity studies (Bannerjee et al. 2015). Pseudo-total metal concentration is another extraction method popularly used for evaluating metal concentrations and consequent uptake in higher plants where HF is not used to break the plant unavailable silicate lattice bound heavy metals (MacFarlane et al. 2007). Again bioaccumulation and translocation of heavy metals in rice and maize is studied by Kumar et al. (2015), by analyzing the pseudo-total metal concentration in the soil using HNO_3 and $HCLO_4$ in a ratio of 5:1. Pseudo-total metal concentration is also evaluated by Kumar and Maiti (2015), in the cromite-asbestos waste to assess the pollution levels in the area of Chaibasa, India. MacFarlane et al. (2003), have estimated the bioaccumulation of metals in *Avicennia marina* using HNO_3 and H_2O_2 as an extractant. Zhang et al. (2013), have used 6 ml HNO_3 and 1.5 ml $HCLO_4$ to extract pseudo-total metal from 0.2 g of sediment sample to study the bioaccumulation of the metals in the aquatic food chain. MacFarlane et al. (2007) extensively reviewed different methods for metal extraction from sediments and reported that if microwave digester is used (Marchand et al. 2006), method of extraction using HCl + HNO_3 combination gives good result. Marchand et al. (2006) have estimated pseudo-total metal of the sediment samples collected from mangrove patches dominated by *Avicennia germinalis* and associated with other mangroves like *Laguncularia racemosa*, *Acrostichum aureum* and *Rhizophora mangale* at the coastline of French Guiana. Microwave digester is envisaged to be more appropriate for metal extraction studies for better reproducibility and to improve the effectiveness (Ianni et al. 2001; Peijnenburg et al. 2007). This same protocol is adapted in our study to understand the metal contamination in the same ecosystem type for bioaccumulation-translocation studies in mangrove flora of Indian Sundarbans.

In the present study, the areas of Sundurban reserve receiving the discharge of sewage and industrial effluent from Calcutta Metropolitan Corporation (CMC) and tannery industries were selected to screen the tolerant mangrove species according to their degree of heavy metal accumulation. Past researches emphasized that principal component analysis (PCA) and hierarchical cluster analysis (HCA) can be effectively used to identify the polluting metals in the sediment. These methodologies also have been widely used as a tool in interpreting polluting metals

(Ghannem et al. 2014; Haris and Aris 2015). Zhang et al. (2013) studied seasonal and spatial dynamics of trace elements in water and sediment from Pearl River Estuary, South China by using PCA and HCA. Similarly, Ogwueleka (2014) used HCA and PCA to identify pollution sources at Kaduna River in Niger State, Nigeria. Hejabi et al. (2011) studied the metal pollution status in water and sediments in the Kabini River, Karnataka, India. Possible sources of heavy metal contamination in lagoons and canal water in Dhaka, Bangladesh have been elucidated by Bhuiyan et al. (2011). Kumar et al. (2015) have used PCA to identify two factors, of which, one denotes the anthropogenic pollutants (Cu, Cd, Pb and Zn) in the surface sediments of Gulf of Kachchh mangrove ecosystem. Singh et al. (2004) used multivariate statistics to evaluate the temporal and spatial variations in water quality in the Gomti River, India.

HCA identifies the similarity–dissimilarity between variables and group them into distinct classes, which give a recognizable pattern. Factor analysis namely PCA is a powerful and popular technique used to reduce dimensionality of the dataset with a large number of interrelated variables. This reduction is possible by transforming the data into new variables called Principle Components (PCs) by orthogonal rotation to get a meaningful groups.

Pollution indexes like Contamination Factors (C_f), Geoaccumulation Index (I_{geo}) along with multivariate statistics are widely used to evaluate the pollution in soil and water bodies. I_{geo} index compares the concentration of a particular pollutant with the background concentration. An et al. (2009) used I_{geo} index to evaluate heavy metals and polychlorinated biphenyls pollution level in the sediment of Yangtze River estuary (China) and found As and Cd pollution is due to the anthropogenic factors. Manjunatha et al. (2001), used I_{geo} values to shows moderate to high pollution of Hg and Cd in the drainage basin of Karwar, in the south west coast of India.

Ecological Risk Index (ERI) has also been a popular method to estimate the level of impact of the polluting heavy metals in the natural ecosystem. Several researchers have used this method to enumerate the impact of metal pollution on the sensitive ecosystem. El-Said and Youssef (2013) have assessed the ecotoxicological impact of heavy metals in the mangrove sediment of the red sea, Egypt. Rahman et al. (2014) have elucidated the potential ecological risk of heavy metal contamination in sediment and water bodies around Dhaka, Bangladesh using ERI.

Identification of metal tolerant species from plant communities affected by metal pollution is a popular method for screening species with phytoremediation potential. Freitas et al. (2004) have identified that *Juncus efesus*, *J. conglomeratus* and *Scirpus holoschoenus* can accumulate Pb and As in high concentration at São Domingos mine in the south east of Portugal, after studying the effect of metal pollution on plant community structure. Hernández and Pastor (2008) have assessed the effect of metal pollution on the grassland biodiversity of abandoned metal mines in the Sierra de Guadarrama, Spain using biodiversity indices, and concluded that Zn has the greatest effect on biodiversity followed by Cd, Cu and Pb. Similar ecological region exposed to varied level of pollution stress may effect the succession pattern, dominance and community structure of the plants favoring pollution tolerant species in stressed areas and normal assemblage in the pollution free area. In this paper it is hypothesized that metal pollution must have selection pressure on a similar community, that would affect the abundance of the species. So, this process could be used to identify/screen pollutant tolerant mangroves by comparing a pollution-affected community with a non-polluted site.

Mangrove plants can bioaccumulate metals from contaminated soils. A plant's ability to accumulate metals from soils is estimated using the Bioconcentration factor (BCF), which is the ratio of metal concentration in the roots to that in soil (Maiti and Nandini 2006; Yoon et al. 2006). A plant's ability to translocate metals from the roots to the shoots is measured using the Translocation factor (TF), which is defined as the ratio of metal concentration in the shoots to the roots (Kumar and Maiti 2014). Different species have shown different potential for metal accumulation. Metal concentration is usually higher in mangrove roots than the aerial parts. But effect of metal pollution on mangrove community structure is rarely dealt in past works and this gap is addressed in this current study. Along with that a screening method is also addressed in this work that identifies plants with metal accumulating/tolerating potential in a dynamic ecosystems using simple community level ecological study.

The main aims of the present work are: (i) to study changes in mangrove community structure due to heavy metal pollution by comparing the community structure of a metal polluted site with a control site, (ii) to ascertain the pollution status of the metal affected part in contrast to a control site using geoaccumulation index (I_{geo}), contamination factor (C_f) and enrichment factor (ErF), (iii) to identify metal pollution and their possible sources by using PCA and HCA, (iv) to identify the species that can tolerate highest degree of ecological risk due to heavy metal pollution by using ERI and plant community study, and (v) bioaccumulation potential of the four metal tolerant species are compared using BCF, BAF (Bioaccumulation factor) and TF to screen out mangroves with phytoextraction potential.

Methodology

Study area

Mangrove community exposed to the municipal sewage and tannery effluent has been selected in the study area to understand the effects of heavy metal pollution on mangrove community structure. The polluted site, Ghushighata (22°31′25.43″N; 88°41′41.71″E) situated in the vicinity of the Kulti River (part of Bidya River) receiving large quantity of domestic sewage and industrial effluent of Calcutta Municipal Corporation (CMC) with the brackish water of Bidya River. Control site was selected amidst the undisturbed mangrove forest (Jharkhali) not influenced by anthropogenic activities (22°00′32.02″N; 88°42′48.23″E) (Fig. 1a–c).

The structure of mangroves community thriving at the high pollution stress zone has a fragmented population of mangrove flora as shown in Fig. 2a. The structure of mangrove community in control site, which is devoid of anthropogenic pollution, is centrally located at the downstream of the Bidya River (Fig. 2b).

Biodiversity assessment

Total 14 quadrate plots (size: 10 m × 10 m) were randomly selected in the natural mangrove patches, out of which, 7 were in polluted site affected by anthropogenic disturbances and 7 plots in control site (devoid of any anthropogenic disturbance). For the assessment of metal accumulation in sediment and their bioaccumulation in mangrove species, 1000 m stretch of Bidya River from the discharge point of effluents was selected. The effluents from CMC and tannery industries at Bantala is released into the Bidya River at Kulti lock gate (up-stream of Bidya River) was taken as the polluted site, affected by the elevated metal pollutions. Bidya River is a tide fed river and it's down-stream has the unpolluted pristine conserved mangrove patches at Jharkhali, taken as a control site for this study. Mangrove propagules float towards up-stream side by tides and established a community at the riverbanks bathed by tidal waters at the vicinity of the Kulti lock gate. So 7 sites were randomly selected at the polluted site to access the condition of biodiversity exposed to pollution. In contrast, the unpolluted Jharkhali site (control site) is
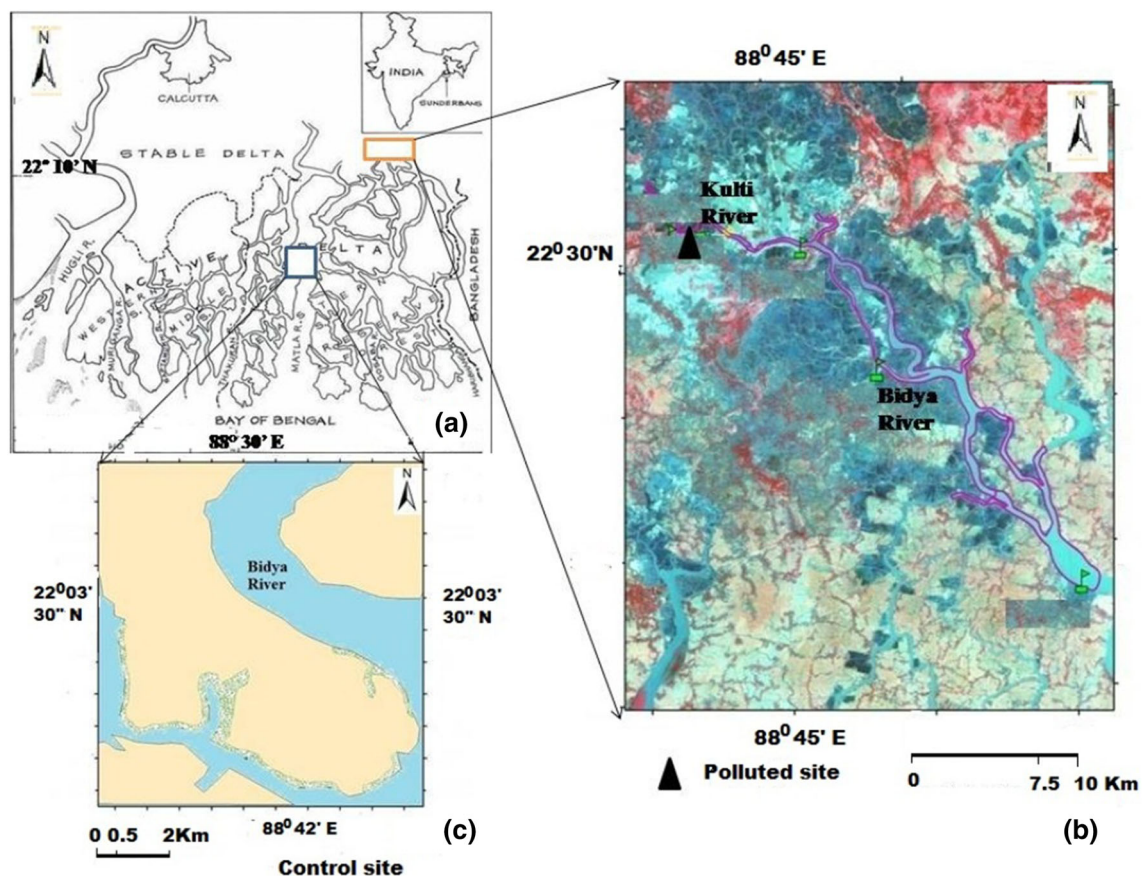

Fig. 1 a Study area with polluted and control site in the Indian Sundarbans, b the polluted site is zoomed out from the whole Sundarban area to show the site effected by CMC effluent and tannery waste, c Jharkhali (the control site) is zoomed out to show the mangrove fragments devoid of pollutions stress
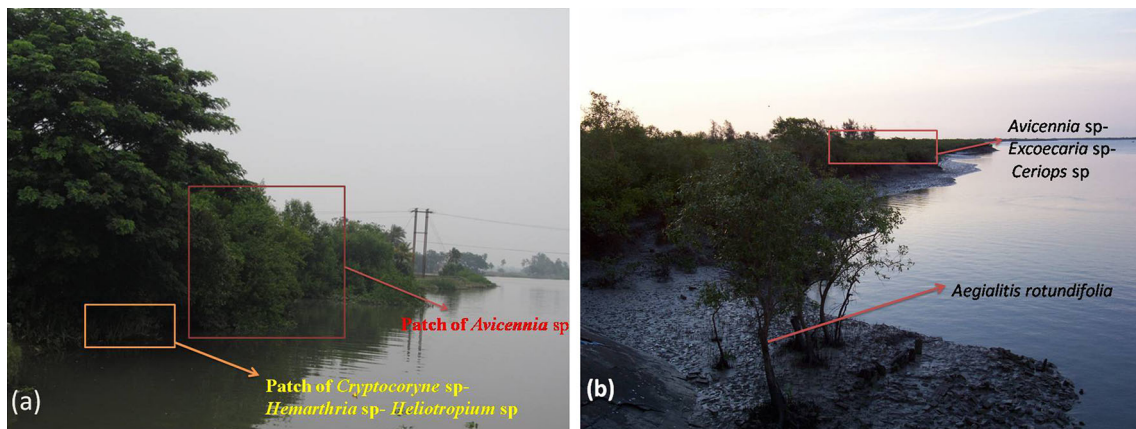
Fig. 2 a Community association at the polluted site (Ghushighata) showing the dominant species in vicinity of the river bank. **b** Community association at the control site (Jharkhali)

selected for assessing mangrove biodiversity not affected by metal pollution by laying 7 random quadrates. Mangrove habitat is said to show a distinct zonation between 0 and 150 m from the river level to High Tide Level (HTL) and plants association responds to soil quality (Ellison and Fransworth 1993; Duke et al. 1998). However research study of Ellison et al. (2000), at Bangladesh part of Sundarbans, have reported the association of plants resulted due to the random colonization rather than any distinct zonation as believed earlier and study showed that there is no actual zonation in mangroves at any scale specially in respect to Sundarbans. Ellison et al. (2000) also opined that the previous concept of mangrove association in the ecosystem has been based on 'a priori' qualitative assumption of zonation without any scientific basis. As the colonization is random and there is no zonation, then the changes in plant abundance would be due to the changes in soil quality parameters and in response to level of pollution in the region, which form the basis of our hypothesis. Still to keep the sampling method unbiased, representative forest community types have been selected along the riverbank between high tide level (HTL) and low tide level (LTL), where the mangrove community lies within 20 m from the riverbank. As the main objective of this study was to screen out mangroves with highest potential of metal tolerance, sites were selected in such a way as to get access to the most polluted part of the ecosystem.

As most of the plants/saplings were within diameter range of <4 cm and sporadic in nature, individual plant of each species was counted for the assessment biodiversity and abundance. The relative density (%) of mangrove species, biodiversity indices (Simpson's index of Dominance and Diversity, Shannon-Weiner index) were calculated to access the community structure and health of biodiversity along with the heavy metal pollution gradient. Relative density is an estimate of the numerical strength of

a species in relation to all the individuals of all the species defined as;

$$\text{Relative Density}(\%)$$
$$= \frac{\text{Number of individuals of a species}}{\text{Number of individuals of all the species}} \times 100$$

The distribution or dispersion of a individual species is generally estimated as percentage occurrence and defined as;

$$\text{Frequency}(\%)$$
$$= \frac{\text{Number of quadrats the species occured}}{\text{Total number of quadrat studied}} \times 100$$

Both the estimators give an idea of the status of a particular species in relation to the whole community, which is used in this study to understand the changes in distribution of the plants.

Similarity in biodiversity between polluted and control sites can be calculated by Sorenson coefficient of community (CC) (Smith and Smith 2012). This a widely used index to study the similarity of two communities based on the presence/absence of a particular species, calculated as:

$$\text{Sorenson's coefficient of community}(\text{CC}) = \frac{2C}{S1 + S2}$$

where, C is the common species in both the patches (i.e., polluted and control site), $S1$ is the number of species in polluted site, $S2$ is the number of species in control site.

Sediment sampling and analysis

Sediment samples were collected by using the Ekman grab sampler (15 cm × 15 cm). The surface sediment (0–30 cm) was collected from the central part of the grab sample in the rhizospheric zone of the plants, showing highest abundance. Seven rhizospheric sediment samples

were collected for each plant species for the analysis of pesudototal metal concentration. The pH and electrical conductivity (EC) of the sediment were measured in a soil–water suspension (1:1.5; soil:deionised water) using a pH meter (Cyberscan 510) and conductivity meter (EI 601) respectively (Maiti 2013). The collected sediment samples were air-dried, crushed in a porcelain mortar, sieved through a nylon sieve (pore size 0.45 mm) and kept in polypropylene air tight zip bags for further analysis. Organic carbon (OC) was determined by the rapid dichromate oxidation method (Walkley and Black 1934) and total nitrogen by Kjeldhal method (Jackson 1973).

Digestion of sediment for metal analysis

A 10 ml volume of a mixture of HCl (37–40 %; Rankem), HNO$_3$ (69–71 %; Rankem) and H$_2$O (1:1:1) was added to 100 mg of sediment sample (Marchand et al. 2006). For analysis of Pb, similarly 100 mg of sieved sample was digested with 3 % HNO$_3$ acid (Banerjee et al. 2016). The mixture was placed in a Teflon vessels, previously washed with concentrated nitric acid. Samples were digested in a Microwave Digester (Model: ETHOS One, Italy) at 100 % power with pressure set at 120 psi for 25 min (two cycles), and overall digestion time was 50 min. This was used to estimate the pseudo-metal concentration of the sediment samples. Blank acid mixture was digested in the same way. The same process of digestion was used to digest estuarine sediment certified reference material (LGC 6137) and analyzed using a flame atomic absorption spectrophotometer (FAAS-GBC Avanta, Australia) at the most sensitive resonance wavelength, respective to each element. Digested samples were warmed with 1 % HNO$_3$, filtered (Whatman #42 filter, pore size 2.5 1 μm), made up the volume up to 100 ml and stored in polyethylene bottles at 4 °C before analysis at FAAS. Standard Reference Materials (AccuTrace, AccuStandard Inc, USA) were used for the calibration and calibration coefficients were maintained at ≥0.99. The FAAS detection limits of metals are as follows: Cd = 0.0004 mg l^{-1}, Cr = 0.003 mg l^{-1}, Ni = 0.004 mg l^{-1}, Mn = 0.0015 mg l^{-1}, Ni = 0.009 mg l^{-1}, Pb = 0.01 mg l^{-1} and Zn = 0.0005 mg l^{-1}. Accuracy of the measurements were checked by analysis of an estuarine sediment certified reference material (LGC 6137). Analytical results obtained for reference materials differed by less than 12 %.

Metal analysis in plants

For total metal analysis in mangrove plants, whole plants were collected from each sampling sites effected by metal

pollution and that have the highest abundance of the sampled species in the quadrats. Mangrove specimens were incised using acid washed knife into root and shoot, before processing for metal analysis. Samples were washed several times with distilled water and oven dried at 80 °C until a constant weight was achieved. Samples (0.5 g) were digested using HNO$_3$ (69–71 %; RANKEM) and HClO$_4$ (71–73 %; RANKEM) (5:1; v/v) and made up the volume up to 100-ml with 1 % HNO$_3$. Metal concentrations in all the samples were analyzed using flame atomic absorption spectrophotometer (FAAS-GBC Avanta, Australia). Quality assurance (QA) and analytical quality control (AQC) were done using certified reference materials (CRMs): NIST SRM 1547 (peach leaves). Analytical results obtained for reference materials differed by less than 18 %. Inter batch variations were monitored by repeated analysis of selected samples in various analytical batches (less than 10 % relative variation).

Calculation of metal pollution indices

(i) Translocation factor (TF) and bioconcentration factor (BCF) values are used to calculated metal pollution indices. For each species, seven rhizosphere sediment samples were collected (i.e., seven replicates/species). The mean values of these seven replicates are taken for calculation of metal indices. The significant difference between mean values is tested using Duncan's multiple range test $p < 0.05$. Bioconcentration factor is defined as:

$$BCF = \frac{\text{Metal concentration in Root}}{\text{Metal concentration in Soil}}$$
(Maiti and Jaiswal 2008; Alloway 2010)

Translocation Factor (TF) is defined as:

$$TF = \frac{\text{Metal concentration in Shoot}}{\text{Metal concentration in Root}}$$
(Maiti and Jaiswal 2008; Alloway 2010)

Liu et al. (2007) defined Bioaccumulation factor (BAF) as

$$BAF = \frac{\text{Metal concentration in Plant(root + shoot)}}{\text{Metal concentration in Soil}}$$
(Maiti and Jaiswal 2008; Liu et al. 2007; Alloway 2010)

(ii) Degree of Contamination of heavy metals

The contamination factor (C_f^i) is obtained from a ratio between the measured concentration of the heavy metals in rhizospheric sediment and the pre-industrial reference value for the same metal (Hakanson 1980; Rahman et al.

2014). The degree of contamination is defined as the sum of all contamination factors (Table 1).

where C^i is the concentration of heavy metal in sediment, C_n^i is the standard pre-industrial reference level (in mg/kg): The C_f is the ratio of heavy metal concentration in the sediment to the average shell value of earth crust (Hakanson 1980). For background concentration of metals, the average shale values for different metals are used for Pb 20 mg/kg, Mn = 850 mg/kg, Ni 68 mg/kg, Cd 0.3 mg/kg, Cr 90 mg/kg and Zn 95 mg/kg (Turekian and Wedepohl 1961). Hakanson (1980) proposed a method to assess the potential ecological risk for areas under special conservation attention. According to this method, the potential ecological risk factor (E_r^i) of a single element can be assessed with a formulae incorporating the toxic response of each metals as given in Table 1.

C_f^i is contamination factor and T^i is the toxic response factor of ith element which is as follows; Pb = 5, Cd = 30, Cr = 2, Zn = 1, Ni = 5, and Mn = 1 (Hakanson 1980). ERI is the summation of the ecological risk factors (Table 1).

This index shed light into the potential of pollution and used to assess the tolerance of different plants for different ecological risk.

The C_f is the ratio of heavy metal concentration in the sediment to the average shell value of earth crust (Hakanson 1980). For background concentration of metals, the average shale values for different metals are used for Pb 20 mg/kg, Ni 68 mg/kg, Cd 0.3 mg/kg, Cr 90 mg/kg, Zn 95 mg/kg and, Mn 850 mg/kg (Turekian and Wedepohl 1961). Similarly, geoaccumulation index (I_{geo}) is the assessment of contamination by comparing the current and past concentrations originally used with bottom sediment and were determined using Muller's equation as given in Table 1 (Ruilian et al. 2008; Banerjee et al. 2016). Both the factors are single, easy to apply and quantitative index that can be applied without considering the grain size and natural geochemical variability. Enrichment factor (ErF) has also calculated by comparing the value of the polluted soil with the control site, to find out how many times, the polluted site have more metal concentration than the control site (Table 1) (Singh et al. 2010).

Statistical analysis

Statistical analyses were carried out to interpret the result by using Data Analysis Package of MS Excel 2007 and SPSS 16 (SPSS Inc. Chicago, USA). Descriptive statistics like one way ANOVA was used to access the variance

Table 1 Contamination indices to assess the level of contamination

Factor/index	Formula	Notations	Threshold values
Contamination factor (C_f)	$C_f = [C]_{sediment}/[C]_{shale}$	$[C]_{sediment}$ = metal concentration in sediment; $[C]_{shale}$ = average shell value of earth crust	$C_f = 0$ none; $C_f = 1$ none to medium; $C_f = 2$ moderate; $C_f = 3$ moderate to strong; $C_f = 4$ strongly polluted; $C_f = 5$ strong to very strong; $C_f = 6$ very strong
Geoaccumulation index (I_{geo})	$I_{geo} = \log_2 \left(\frac{C_{sediment}}{1.5\ B_n} \right)$	$C_{sediment}$ = metal concentration in sediment; B_n = geochemical background metal concentration; 1.5 = correction factor for the background matrix due to lithogenic effects	$I_{geo} \leq 0$ practically uncontaminated; $0 < I_{geo} < 1$ uncontaminated to moderately contaminated; $1 < I_{geo} < 2$ moderately contaminated; $2 < I_{geo} < 3$ moderately to heavily contaminated; $3 < I_{geo} < 4$ heavily contaminated; $4 < I_{geo} < 5$ heavily to extremely contaminated; $5 < I_{geo}$ extremely contaminated
Enrichment factor	$ErF = \frac{P\ site}{C\ site}$	P site = polluted site, C site = control site	Varies, it is the ratio between the concentration in polluted to control site
Ecological risk factor (E_r^i)	$E_r^i = T^i \times C_f^i$	C_f = contamination factor, T^i = toxic response factor of ith element	$E_r^i < 40$ = low ecological risk, $40 < E_r^i < 80$ = moderate potential ecological risk, $80 < E_r^i < 160$ = considerable potential ecological risk, $160 < E_r^i < 320$ = high potential ecological risk, $E_r^i > 320$ = very high ecological risk
Ecological risk index (ERI)	$E_r^i = \sum_{i=1}^{n} E_r^i$	E_r^i = ecological risk factor	$150 < ERI$ = low ecological risk, $150 < ERI < 300$ = moderate ecological risk, $300 < ERI < 600$ = considerable ecological risk, $ERI > 600$ = very high ecological risk

between the means of a analyzed parameter, between the sampling sites and where significant F value is observed, difference between individual means were tested using DMRT (Duncan's Multiple Range Test) at the 5 % level of significance. ANOVA is conducted between the rhizospheres of polluted sites (4 species) and the control sites with $n = 7$ and degree of freedom = 4. This help us to test whether the means of the analyzed parameters are same or different. Metal associations were tested with one tailed Pearson test to get a matrix of correlations between the parameters. HCA was performed on normalized dataset of heavy metals to group similar metal clusters using Pearson coefficient as distance and between group by linkage methods. PCA was applied on the metal concentrations in the sediment to obtain groups of similar metals (Bhuiyan et al. 2011; Rahman et al. 2014). Normalized variables (original variables) were transformed into the rotated components to extract significant principal components (PC) by suppressing the contribution of variables with minor significance, after Kaiser–Meyer–Olken (KMO) test that determines the appropriateness of data reduction through PCA analysis. Furthermore, these PC's were subjected to varimax rotation with loading coefficients (>0.1) to generate PC factors/groups. There is always a debate between the uses of orthogonal versus oblique rotation methods. Orthogonal rotation classifies the components/factors with the assumption that they are unrelated to each other. But in case of metal clusters unrelated components are easy to read, so varimax orthogonal rotation was used in the current study. Out of several methods, multivariate statistical analyses with PCA, Factor Analysis (FA) and unsupervised pattern recognition (i.e., HCA) are popularly used in source identification of the pollutant (Srivastava et al. 2011; Magesh et al. 2011; Bhuiyan et al. 2011; Ogwueleka 2014; Rahman et al. 2014).

Result and discussion

Effect of anthropogenic pollution on community composition

A total of 24 species were found in polluted and control sites, out of which 33 % are mangrove associates, only one is an invasive saline grass and rest were true mangrove varieties (Table 2). In the polluted sites only 16 species were found, of which relative density of three species, namely *C. ciliata* (26.2 %), *H. altissima* (21.83 %) and *H. currasavicum* (20.58 %) accounts for 69 % of the total recorded species. In contrast, the control site has 24 species and none have a relative density more than 10 % which is also evident from the relative density distribution graph of species between control and polluted

site (Fig. 3). True mangrove and endangered species of rhizophoraceae family like *B. cylindrica*, *B. sexangula*, *R. mucronata* and endangered mangrove timber species of Meliaceae family like *X. granatum* and *X. molluccensis* was totally absent in the polluted sites (Fig. 3).

Xylocarpus spp, generally grows in areas with balanced flow of fresh water, so it is a possibility that the pollutant laden effluent is counterproductive for the proliferation of the species in the polluted site (Aston and Macintosh 2002). Again the edaphic sub-climax mangrove palm (*P. paludosa*) was also not recorded in the polluted site. Similarly, salinity tolerant pioneer species *S. maritima* was also absent in polluted site. The true mangrove varieties recorded in the polluted site also have a low frequency and relative density in contrast with the control site. True mangrove varieties like *Bruguiera gymnorrhiza* shows 14 % frequency in the polluted site in contrast to 100 % in the control. Same is also true for other Rhizophoraceae (true mangroves) members like *Ceriops tagal* and *Ceriops decandra* shows 43 % frequency in polluted site, where as 100 % in the control site. The control site showed predominance of true mangrove varieties having maximum abundance of *P. coarctata*–*S. maritima* (pioneer species). Tides prominently effect the colonization of the river banks bringing propagules and nourishing the seedlings, so metal pollution would act as a stressor in this succession process excluding the stress sensitive species. There is a maximum distribution of the seedlings (<1 m height) and saplings (>1 m height) in the river bank showing a high diversity. *Avicennia* spp, is the only tree species with considerable girth (15–35 cm) and height (10–20 m) was found in the metal polluted areas. Other tree species encountered in the site were *E. agallocha*, *A. marina* and *S. caseolaris* but their girth range between 10 and 20 cm.

Many hypothesis are used to understand the changes in forest community structure in response to environmental factors that include geomorphological factors, climatic variability, physio-anatomical variations due to changing abiotic parameters, biotic factors such as herbivory, predation, parasitism and animal plant interactions (Aston and Macintosh 2002; Smith and Smith 2012). Two mangrove associates (*C. ciliata* and *H. currasavicum*) and invasive saline red swamp grass (*H. altissima*) have proliferated growth and dominance in the pollution effected meta-populations, whereas healthy patches shows a decreased dominance of these species. *C. ciliata* is also potentially invasive for mangrove ecosystem (Biswas et al. 2007). *H. altissima* has been one of the foremost invaders that replace the coastal mangroves patches subjected to reduced tidal inundation (McCartney 2009). But as this species was observed to be proliferating in the riverbank, there could be a possibility that it has adapted to metal pollution stress.

Table 2 Occurrence of mangrove species in polluted and control site their relative density, frequency

Botanical name (family)	Site	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Relative density	Frequency (%)
Acanthus illicifolius+ (Acanthaceae)	P	8	8	6	14	4	12	14	7	100
	C	10	8	10	12	14	4	12	9	100
Aegialitis rotundifolia (Plumbagineaceae)	P	4	0	2	0	2	0	2	1	57
	C	10	8	10	6	10	8	8	6	100
Aegiceras corniculatum (Myrsinaceae)	P	2	0	0	0	0	2	2	1	43
	C	14	12	14	10	14	14	10	10	100
Avicennia marina (Avicenniaceae)	P	8	4	8	4	6	8	6	5	100
	C	8	4	10	4	8	2	8	5	100
A. officinalis (Avicenniaceae)	P	4	6	4	2	4	0	6	3	86
	C	2	0	4	2	0	0	2	1	57
Bruguiera cylindrica (Rhizophoraceae)	P	0	0	0	0	0	0	0	0	0
	C	0	2	0	2	0	4	4	1	57
Bruguiera gymnorhiza (Rhizophoraceae)	P	0	0	0	0	0	2	0	1	14
	C	10	8	10	4	10	12	10	7	100
Bruguiera sexangula (Rhizophoraceae)	P	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	2	2	2	1	43
Ceriops decandra (Rhizophoraceae)	P	0	0	2	0	2	8	0	1	43
	C	8	10	8	4	8	2	8	5	100
Ceriops tagal (Rhizophoraceae)	P	6	0	0	0	2	0	2	1	43
	C	4	2	8	10	8	12	8	6	100
Clerodendron inerme (Verbenaceae)	P	4	2	0	0	0	0	2	1	43
	C	4	2	8	2	0	0	2	2	71
Cryptocoryne ciliata + (Araceae)	P	36	36	40	36	30	38	36	26	100
	C	6	4	2	0	0	2	4	2	71
Derris trifoliata +++ (Fabaceae)	P								0	0
	C	4	6	8	4	8	4	8	5	100
Excoecaria agallocha (Euphorbiaceae)	P	4	2	4	2	2	0	2	2	86
	C	4	6	4	2	4	6	6	3	100
Heliotropium currasavicum+ (Boraginaceae)	P	30	30	24	28	24	30	32	21	100
	C	2	0	4	2	0	10	6	3	71
Hemarthria altissima++ (Poaceae)	P	30	36	24	30	36	24	30	22	100
	C	2	0	6	4	8	12	6	4	86
Lumnitzera racemosa+ (Combretaceae)	P	0	0	0	0	0	6	4	1	29
	C	4	2	2	0	6	2	8	3	86
Phoenix paludosa (Arecaceae)	P	0	0	0	0	0	0	0	0	0
	C	4	6	6	8	4	4	8	4	100
Porteresia coarctata+ (Poaceae)	P	10	10	24	10	8	12	12	9	100
	C	8	16	18	16	12	16	12	11	100
Rhizophora mucronata (Rhizophoraceae)	P	0	0	0	0	0	0	0	0	0
	C	4	2	0	4	2	4	4	2	86
Sonneretia caseolaris (Sonneretiaceae)	P	2	2	0	0	0	0	2	1	43
	C	4	2	6	4	0	2	2	2	86
Suaeda maritima+ (Amaranthaceae)	P	0	0	0	0	0	0	0	0	0
	C	6	8	16	14	20	4	16	9	100
Xylocarpus granatum (Meliaceae)	P	0	0	0	0	0	0	0	0	0
	C	2	0	0	0	4	2	2	1	57

Table 2 continued

Botanical name (family)	Site	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Relative density	Frequency (%)
X. moluccensis (Meliaceae)	P	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	2	0	0	0.22	14

Q1–Q7 quadrat from 1 to 7, + mangrove associate; ++(SG) saline grass (non mangrove) and invasive species; +++ mangrove associate and invasive species, P polluted site, C control site
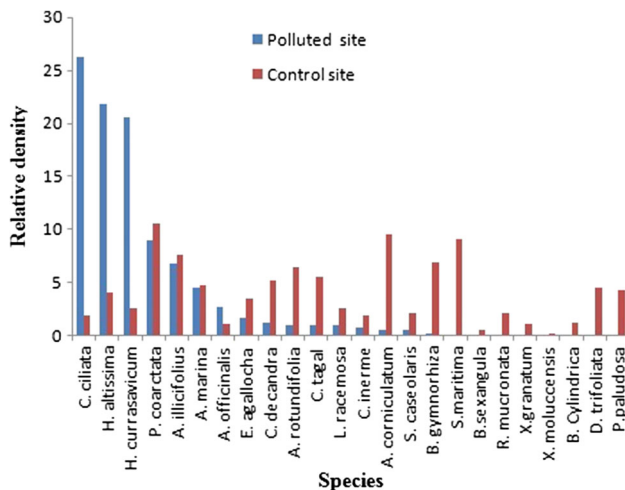

Fig. 3 Relative density distribution of the mangrove plants in control and polluted sites

Avicennia spp have tolerance to different concentrations of heavy metal pollution. *A. marina* roots and leaves have the potential for bioaccumulation of Pb, Zn, Cu and it's roots could be used as metal pollution indicator in soil (MacFarlane et al. 2003). *A. officinalis* was also found in the polluted site with higher relative density than the control site and have a frequency of 86 % in polluted area unlike 57 % in the control patch. So we analyzed the metal accumulation of the mangrove tree along with three abundant herbs at polluted site. Growth of *A. officinalis* in close proximity of the bank is shown in Fig. 2a. We identified four species that may have metal tolerant trait, which were the three herb namely *C. ciliata*, *H. altissima*, *H. currasavicum* and one true mangrove tree species of *A. officinalis*.

Effect of metal pollution on mangrove biodiversity

The effect of metal pollution on mangrove biodiversity was studied by using Simpson's index of dominance, Shannon-Weiner index and Similarity index. Species diversity is reciprocal to the dominance, so increase in dominance reduces the diversity. While comparing the two patches, it was observed that the dominance is more in the polluted patches and so have lesser diversity (Table 3). The polluted patches have larger dominances ($D = 0.16$) because only

three species (*C. ciliata*, *H. altissima* and *H. currasavicum*) contributed 69 % of dominance value, and Simspon diversity was found lower (0.84) compared to the control site (0.953). Similarly, Shannon-Winner value for polluted site was found lesser (2.03) compared to control site (2.7). Dominant species generally achieve their majority in the ecosystem at the expense of the non-competitive species (Smith and Smith 2012). Sorenson similarity index shows a similar mangrove patch with a value of 0.8 between the two sampling points (polluted and control). As Sundarbans is a prograding delta and heavily influenced by tidal regime, succession is dynamic in this delta (Comeaux et al. 2012).

Physiochemical characteristics of the rhizospheric sediment

Metal and associated parameters of the rhizospheric sediments from vegetation plots with maximum species abundance of the concerned flora are given in Table 4. All the analyzed parameters show a significant difference in means when subjected to ANOVA, between rhizosphere of different plants and control site. pH is lower in all the polluted rhizospheric samples unlike the control site. Low soil EC value of the rhizospheres is due to its distance from the sea, and more influx of fresh water. Organic carbon (OC) and plant available N (Av N), is also lower in the polluted sites indicating a stressed ecosystem with lesser activity of the detritus food chain. Amongst the four plants, *A. officinalis* rhizospheric sediment has the highest OC and Av N may be because of the decay of its leaves from higher canopy. Cr was found in elevated concentration in all the rhizospheric soil and it was observed that a concentration of 75–100 mg/kg of soil can have toxic effects on plants and in case of Pb the permissible limit was reported for the uncontaminated soil is <20 mg/kg (Kabata-Pendias and Pendias 2001; Alloway 2013). Cd concentration generally ranges between 0.1 and 1.0 mg/kg in unpolluted soil, whereas the polluted rhizospheres have concentration above this limit unlike the control site (Alloway 2013). Mean metal concentrations in the *C. ciliata* rhizospheric soil follow a order of Mn > Cr > Zn > Ni > Pb > Cd. Mean metal concentrations in rhizospheric soil of *A. officinalis*, *H. altissima* and *H. currasavicum* follows an order

Table 3 Comparison between the biodiversity status of the control and polluted sites

Indices	Formulae	Ghusighata (P)	Jharkhali (C)	References
Simpson's index of dominance	$D = \sum \frac{n(n-1)}{N(N-1)}$	0.16	0.047	Simpson (1949) and Laude (1996)
Simpson's index of diversity	$L = 1 - D$	0.84	0.953	Simpson (1949) and Laude (1996)
Shannon-Weiner index	$H' = -\sum \text{pi} Ln\text{pi}$	2.03	2.724	Shannon (1948) and Laude (1996)

D Simpson's index of dominance, L Simpson's index of diversity, H' Shannon's index, n number of individuals of each species, N total number of individuals of all the species, pi total number of individuals in each species/total number of individuals in all the species, P polluted site, C control site

of Cr > Mn > Zn > Ni > Pb > Cd. Jharkhali, the control site devoid of anthropogenic pollutants shows Mn > Zn > Ni > Cr > Pb > Cd order of mean metal concentration. Control site have lower concentration of metals except Mn, which is showing similarity with *Heliotropium* sp and *Avicennia* sp rhizosphere in polluted site. The reason can be a anthropogenic independent cause of the presence of these two metals in the ecosystem. Zn have a similar trend with other rhizospheres because of it's geogenic origin, which would be clearly understood in later statistical treatment of the data using PCA and HCA. EC values are higher in control site because of its proximity to Bay of Bengal and the tidal currents bring saline water from the seas.

The metal concentrations of the four polluted rhizospheres and the control site are subjected to Pearson correlation test to find out relations between analyzed parameters elucidated in Table 5. Variables are ten in number and number of samples are 70 ($n = 7 \times 10 = 70$). This gives an insight into the correlation of the parameters in the total environment combining both the polluted and control sites. This will also indicates whether the association is anthropogenic or geogenic ones. Here Cd shows weak correlation with Ni, while strong correlation with Cr and Pb could indicates a similarity of the source of these metals. Reduction in pH increases the mobility of Cd ions hence Cd shows a negative significant correlation with pH (Alloway 2013). Cd also inhibits the microbial activity due to its cytotoxicity so negative significant correlation was observed in the matrix. Organic carbon (OC) has negative correlation with most of the metals (Cd, Cr—significant high correlation, Ni—significant low correlation). OC has an inverse proportionality with metal ions as it binds/adsorb the mobile cations making it less available and mobile (Alloway 2013). Cd, Cr, Mn, Pb, Ni have a negative correlation trend with pH and EC mainly because acidic pH increase the mobility of metal ions, whereas increase in light metals (Na, K) can competitively exclude the availability of metal ions in the soil-capillary water phase. Positive significant correlation of Pb with Ni, Cd and Cr indicates a similar origin of the metals in the sediment. The

reason is that at lower pH the metallic cations are more mobile and shows more activity. EC shows a negative significant correlation with Cr. Metals have an increased adsorption rate as pH rises, because of reduced competition with H^+ ions and increase in negative surface potential (Alloway 2013). As pH rises there is a hydrolysis to $CrOH^{2+}$, indicating a negative correlation of Cr-pH in the Pearson matrix (Fischer et al. 2007). As microbial activity is negatively affected by decreasing pH as well as in increasing pH, thus OC and N showed a positive significant correlation.

Identifying the anthropogenic pollutants (heavy metal)

The paradox of locating the source of pollution and to ascertain whether the metal pollution is geogenic or anthropogenic in origin, can be assertained by the component and cluster analysis (Ogwueleka 2014). The dendrogram (between groups) obtained by using HCA with Pearson correlation distance, shows the pattern of metals present in the polluted rhizospheres of the four investigates species (Fig. 4).

There are distinct clusters of Cr, Pb and Cd in one joined by Ni and Mn–Zn in another cluster. The former is showing anthropogenic origin, while the later is geogenic origin. The other soil parameters like pH, EC, OC, N were in a separate cluster.

The Kaiser–Meyer–Olkin (KMO) test is used to determine the appropriateness of data reduction through PCA analysis. The KMO test also measures of sampling adequacy while Bartlett's test of spericity indicates the strength of correlation among variables. In this study the KMO test result was found above 0.78 and significant Bartlett's test (significance = 0.001) showed that data reduction can be achieved through PCA. Therefore, PCA test was performed with varimax orthogonal rotation. Here ten variables (pH, EC, OC, N, Cd, Cr, Mn, Ni, Pb and Zn) from four polluted rhizosphres and one-control sites were analyzed through PCA. Seven samples ($n = 7$) of each variable are taken from each

Table 4 Analysis of pseudo-total metal concentration, organic carbon, available N, EC ($\mu S\ cm^{-1}$) and pH of the rhizopheric soil (0–30 cm)

Site	Plant rhizosphere	Descriptive statistics	pH (1:1.5, soil:water)	EC (1:1.5, soil:water)	OC (%)	Avail N (mg kg⁻¹)	Concentration (mg kg⁻¹)					
							Cd	Cr	Mn	Ni	Pb	Zn
P	Cryptocoryne ciliata (n = 7)	Mean	6.34ᵃ	1434.57ᵃ	0.94ᵃ,ᵇ	42.45ᵃ	1.49ᵃ	170.7ᵃ	176.79ᵃ	44.7ᵃ	22.4ᵃ	94.64ᵃ
		Standard deviation	0.08	72.35	0.06	9.22	1.7	3.5	8	2.7	1.5	1.7
		CV (%)	1	5	7	22	5	2	4	6	7	2
P	Hemarthria altissima (n = 7)	Mean	6.4ᵃ	1280ᵃ,ᵇ	0.82ᵃ,ᵇ	41.16ᵃ	1.95ᵇ	172.78ᵃ	163ᵇ	45.5ᵃ	26.36ᵇ	93.43ᵃ
		Standard deviation	0.13	128	0.06	0.09	0.35	4.8	3.6	1.6	0.9	1.7
		CV (%)	2	10	7	20	20	3	2	4	3	2
P	Avicennia officinalis (n = 7)	Mean	6.2ᵇ	1244ᵇ	1.01ᵇ	67.09ᵇ	2.44ᶜ	173.8ᵃ	159.29ᵇ,ᶜ	45.07ᵃ	26.7ᵇ	93.43ᵃ
		Standard deviation	0.1	109	0.21	7.8	0.36	4.8	1.9	1.24	1.1	1.7
		CV (%)	2	9	21	12	15	3	1	3	4	2
P	Heliotropium currasavicum (n = 7)	Mean	6.2ᵇ	1347ᵃ,ᵇ	0.7ᵃ	39.21ᵃ	2.86ᵈ	173.8ᵃ	159.29ᵇ,ᶜ	44.79ᵃ	26.4ᵇ	93.64ᵃ
		Standard deviation	0.2	124.3	0.06	9.7	0.25	4.8	1.9	1.22	0.9	1.7
		CV (%)	2	9	8	20	9	3	1	3	3	1
C	Jharkhali (n = 7)	Mean	7.3ᶜ	3694.2ᶜ	1.8ᶜ	84.27ᶜ	0.5ᵉ	22.35ᵇ	156.4ᶜ	40.7ᵇ	12.8ᶜ	93.6ᵃ
		Standard deviation	0.1	261	0.2	8.8	0.4	1.4	2.4	4.5	1.07	2.4
		CV (%)	2	7	13	10	80	6	3	10	8	3
ANOVA(one way)—between rhizospheric soils, df = 4			F* = 60.023	F* = 98.33	F* = 336.52	F* = 27.04	F* = 33.5	F* = 3.96	F* = 1908	F* = 25.31	F* = 0.517	F* = 201.19

df degree of freedom, *P* = polluted site, *C* = control site, n = number of replicates

* Significant difference of mean at significance level ≤0.05, same letters indicate no significant difference at ∝ = 0.05 according to DMRT post hoc test

**Table 5** Pearson correlation between analyzed parameters of rhizosphere of contaminated soil

	Cd	Ni	Zn	Mn	Cr	OC	Pb	N	pH	EC
Cd	1									
Ni	0.430**	1								
Zn	−0.042	0.069	1							
Mn	−0.062	0.317	0.181	1						
Cr	**0.783****	0.563**	0.059	0.388*	1					
OC	**−0.710****	−0.436**	−0.13	−0.276	**−0.855****	1				
Pb	**0.857****	**0.615****	−0.108	0.16	**0.936****	**−0.800****	1			
N	−0.538**	−0.314	0.082	−0.378*	**−0.738****	**0.704****	**−0.645****	1		
pH	**−0.774****	−0.492**	−0.018	−0.271	**−0.944****	**0.813****	**−0.885****	**0.641****	1	
EC	**−0.780****	−0.578**	−0.013	−0.349*	**−0.985****	**0.853****	**−0.944****	**0.714****	**0.945****	1

* Correlation is significant at the 0.05 level (2-tailed); the bold letters indicate values above 0.6

** Correlation is significant at the 0.01 level (2-tailed)

Fig. 4 Dendrogram (HCA) with Pearson correlation as distance showing clusters of analyzed parameters of rhizospheres of the metal tolerant species
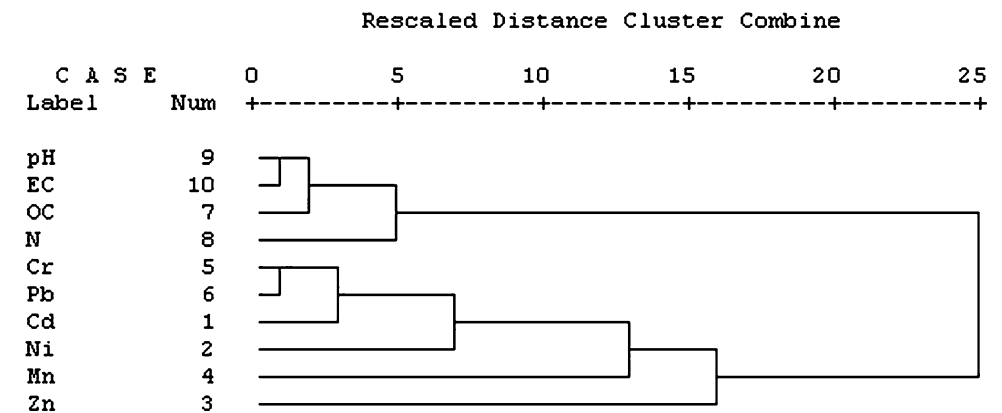
sites (rhizospheres and control site). So in total there are 350 cases. Here four rhizospheric sediment data and that of the control site are taken together for PCA analysis. The reason is that in this study the aggregation of the metals in both the polluted and control sites are considered together to understand their association in same ecosystem (mangrove) with two different stress levels (metal contaminated and uncontaminated). From this it would be evident that the metals with geogenic origin has same trends in both the sites so would come together in the components after orthogonal rotation and same is true for anthropogenic pollutants. In this multivariate analysis identifying the origin of the metals in the ecosystem is the foremost objective. The rotated explanation of the total variations clearly indicate that two components can be solved out of this data reduction and exploratory methods as they cumulatively explained a variation of 75.6 % of the total variance (Table 6). The rotated solution in accordance with the

dendogram clusters, which showed three clear groups: Pb, Cr, Cd and Ni in one group, Zn and Mn in another group, whereas other associative soil parameters like OC, pH, EC and available N in the third group (Fig. 5).

The former group was of anthropogenic origin. Major anthropogenic sources are industrial sources like tannery industry accounts for the Cr (used for tanning hides), Pb (present in low quality diesel/petrol fossil fuels) and Cd–Ni (battery, industrial sources, tannery effluent) (Alloway 2013; Mwinyihija 2010). Zn has a geogenic origin in Sundarban waters and as Mn is associated with Zn in the dendrogram and PCA components it could be presumed that both are of the same origin (Guhathakurta and Kaviraj 2000).

Contamination indices

Contamination factor (C_f), shows that there is medium contamination of Cd in rhizospheres of *Cryptocoryne* sp

Table 6 Total variance explained and extracted components of PCA

Total variance explained

| Component	Initial eigen values			Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	% of variance	Cumulative (%)	Total	% of variance	Cumulative (%)	Total	% of variance	Cumulative (%)
1	6.279	62.791	62.791	6.279	62.791	62.791	6.195	61.947	61.947
2	1.282	12.822	75.614	1.282	12.822	75.614	1.367	13.667	75.614
3	0.912	9.122	84.735						
4	0.732	7.319	92.054						
5	0.335	3.350	95.404						
6	0.197	1.965	97.369						
7	0.161	1.614	98.983						
8	0.075	0.753	99.736						
9	0.018	0.177	99.913						
10	0.009	0.087	100						

Extraction method: Principal Component Analysis
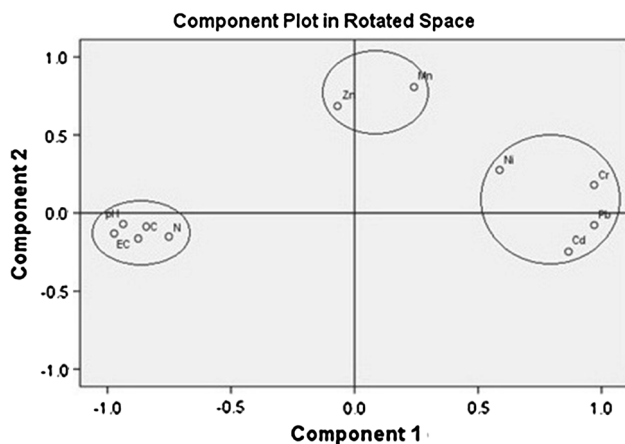
Fig. 5 Resultant groups of the Principal Components Analysis (PCA) based on the analyzed parameters and after varimax rotation

(1.5) and *Hemarthria altissima* (2.0) as shown in Fig. 6a. Cd concentration is moderate in rhizospheres of *A. officinalis* and *H. currasavicum*. Cr pollution is medium in all the investigated rhizospheres (1.8–2.0) at the polluted site. Rest of the metals (Ni, Zn, Mn and Pb) has no pollution pressure in all the sites. Control site of Jharkhali shows no pollution so substantiated its selection as a reference site in this current study.

I_{geo} is the geoaccumulation index that compares the contamination of a particular metal against a background value normalized by a factor of 1.5 to minimize the lithographic effect and the I_{geo} values of the metals for the five sites are given in Fig. 6b. The *C. ciliata* rhizosphere is uncontaminated to moderately contaminate with Cd (1.7), whereas other rhizospheres are moderately contaminated (2.1–2.7). Cr also shows an uncontaminated to moderately

contamination in the four polluted rhizospheres (0.3). Control site shows no contamination for all of these metals.

Enrichment factor (ErF), compares the contamination level of the pollutants with a reference control site. Here the Jharkhali site is selected as a control site owing to it's pristine conserved mangrove patches, lower level of metals in the rhizospheres and as evident from previous indices like I_{geo} and C_f. The comparison between the four polluted rhizosphere shows that *C. ciliata* have Cd level three times of the control, almost four times for both *H. altissima* and *A. officinalis* and highest five time for *H. currasavicum*. Cr contamination in all the rhizospheres ranges around eight times to the control soil and Pb ranges around two times. These three anthropogenic metals (Pb, Cd and Cr) are heavily polluting the Ghushighara site as evident from the enrichment factors.

The assessment of the potential ecological risk of the heavy metal contamination was used as a diagnostic index for water pollution control purposes as a result of the increasing content of heavy metals in sediments that could threaten ecological health (Hakanson 1980; Rahman et al. 2014). The Ecological risk factor for individual metals and ERI is given in Table 7. ERI of rhizospheres show a descending trend of: *H. currasavicum* > *A. officinalis* > *H. altissima* > *C. ciliata* > control site (Jharkhali). But individual Ecological risk factor (E_r) highlights the tolerance of the plants to individual metal. In respect Cr all the species have near equal tolerance. *A. officinalis* showed a higher tolerance to Pb and *H. currasavicum* to Cd. The E_r value of Cd range between 149 and 286 in polluted sites whereas the control site has a value of 50. Here *C. ciliata* shows Cd pollution in "Considerable Potential Ecological Risk" category and rhizosphere of *A. officinalis*, *H. currasavicum*

Fig. 6 Comparison of metal pollution between control and polluted site. **a** I_{geo} is compared between the polluted plant species and control site, **b** contamination factor compared between control and polluted site, **c** enrichment factor is compared between the polluted and control site
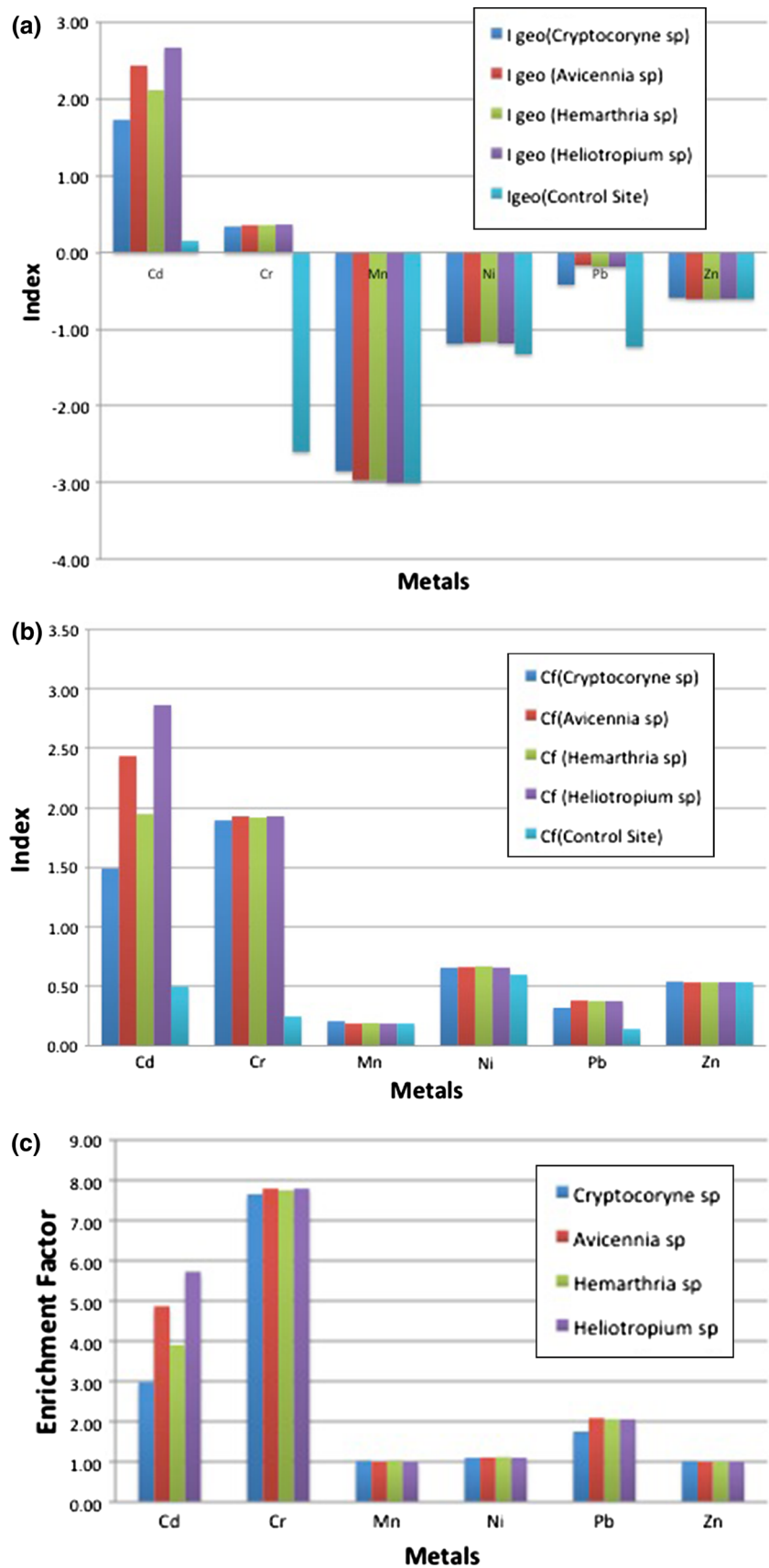
and *H. altissima* falls under "High Potential Ecological Risk" as per E_r values. The Cd pollution in the polluted site may be due to industrial pollution from tannery industry (Mwinyihija 2010). *A. officinalis* shows highest Pb in their rhizosphere followed by *H. altissima* and *H. currasavicum*. But all these anthropogenic pollutants showed higher Ecological risk in the polluted site than the control site indicating anthropogenic impact. In control site ERI value falls under the category of "Low Ecological Risk" whereas *H. currasavicum* shows "Considerable Ecological Risk" and rest of the polluted sites are in "Moderate Ecological Risk" category.

Several reasons are there for the bioavailability of the naturally derived metals in the sediment core and foremost amongst is the deposition rate, which is dependent on the hydrology of the river (Fernandes et al. 2014). Metal pollution have a far reaching effect on the fragile biodiversity of the region, as metals have a potential to enter into the food chain and get biomagnified (Zhang et al. 2014; Bannerjee et al. 2015). Pb is known to cause toxicity in the people especially children (Alloway 2013). Again Cd have a high bioavailability potential and owing to it's lesser phytotoxicity, is accumulated in higher levels in plant tissues, which can come into the terrestrial food chain through the process of grazing. Cr has toxic properties to flora and fauna alike. Toxicity of Cr is expressed as xeromorphic foliage, development of large root system, dwarfism, plagiotropism, glabrescence or pubescence, glaucescence and erythrism in plants (Alloway 2013).

Bioaccumulation of metals in the plants

Numerous researches shed light into the fact that the root system of the plants can impart change in their rhizospheric soil by changing pH or exuding H_2CO_3 or any other means that can affect the bioavailability of the metals. The metal concentration in root and shoot/leaves components of the four plants namely *A. officinalis*, *C. ciliata*, *H. currasavicum* and *H. altissima* is shown in Fig. 7. None of the species are hyperaccumulators of any metals (Alloway 2013). Hyperacumulators are generally accumulate more than 100 mg kg^{-1} of Cd, 1000 mg kg^{-1} of Co, Cu, Ni, Pb and 10,000 mg kg^{-1} of Mn, Zn in their aboveground body parts (KabataPendias and Pendias 2001).

Root portions of *C. ciliata* have the highest Cr concentration than any other plants followed by *A. officinalis*. But the ability of these plants to tolerate heavy metal pollutants could be useful for phytostabilization. Both bioconcentration factors (BCF) and translocation factors (TF) have a wide use to estimate a plant's potential for phytoremediation purpose (Yoon et al. 2006). Enrichment occurs when a contaminant taken up by a plant is accumulated in the plant instead of getting degraded. For phytoextraction, the translocation of heavy metals to the easily harvestable plant parts, i.e. shoot is required. By comparing BAF, BCF and TF, the ability of different plants of taking up metals from soils and translocating them to the shoots, can be compared (Fig. 8). Tolerant plants restrict the soil–root and root–shoot transfers, and therefore have much less metal concentration in their biomass, while hyperaccumulators actively take up and translocate metals into their aboveground biomass (Yoon et al. 2006; Maiti and Jaiswal 2008).

The most important sources of Cd pollution are metal industry, plastics, tannery industry and sewers (Aksoy et al. 2005; Mwinyihija 2010). In this study it was found that Cd showing high bioconcentration in all the investigated plant species except *H. currasavicum*. The later species can tolerate highest Ecological risk in respect to Cd but is showing limited transmission to root. Elevated CO_2 can also induce more uptake of Cd by increasing the root growth and hence capture more Cd in the root part as observed in poplars and willows (Wang et al. 2012). Glutathione linked phytocheletins are generally responsible for Cd tolerance in many plant species and yeast (Clemens et al. 2002). So deficiency in the same can restricts the inactivation of Cd and sensitivity to this metal. But *H. currasavicum* was seen to proliferate in the rhizosphere with highest Cd risk yet shows low bioconcentration factor (<1) so can be an excluder for this metal. Cd can easily taken up by the plants through apoplastic and symplastic transport to higher plant parts via vascular tissues and have low toxicity to macrophytes than other pollutants (Alloway 2013; Clemens et al. 2002). *A. officinalis* and *H. currasavicum* show a low TF for Cd substantiating the exclusion of this metal in the root parts. Ma et al. (2011), reported a high bioaccumulation (bioenrichment) of Cd in the aerial in the aquatic

Table 7 Potential ecological risk factor (E_r) and ecological risk index of different rhizospheres of the polluted site and control site

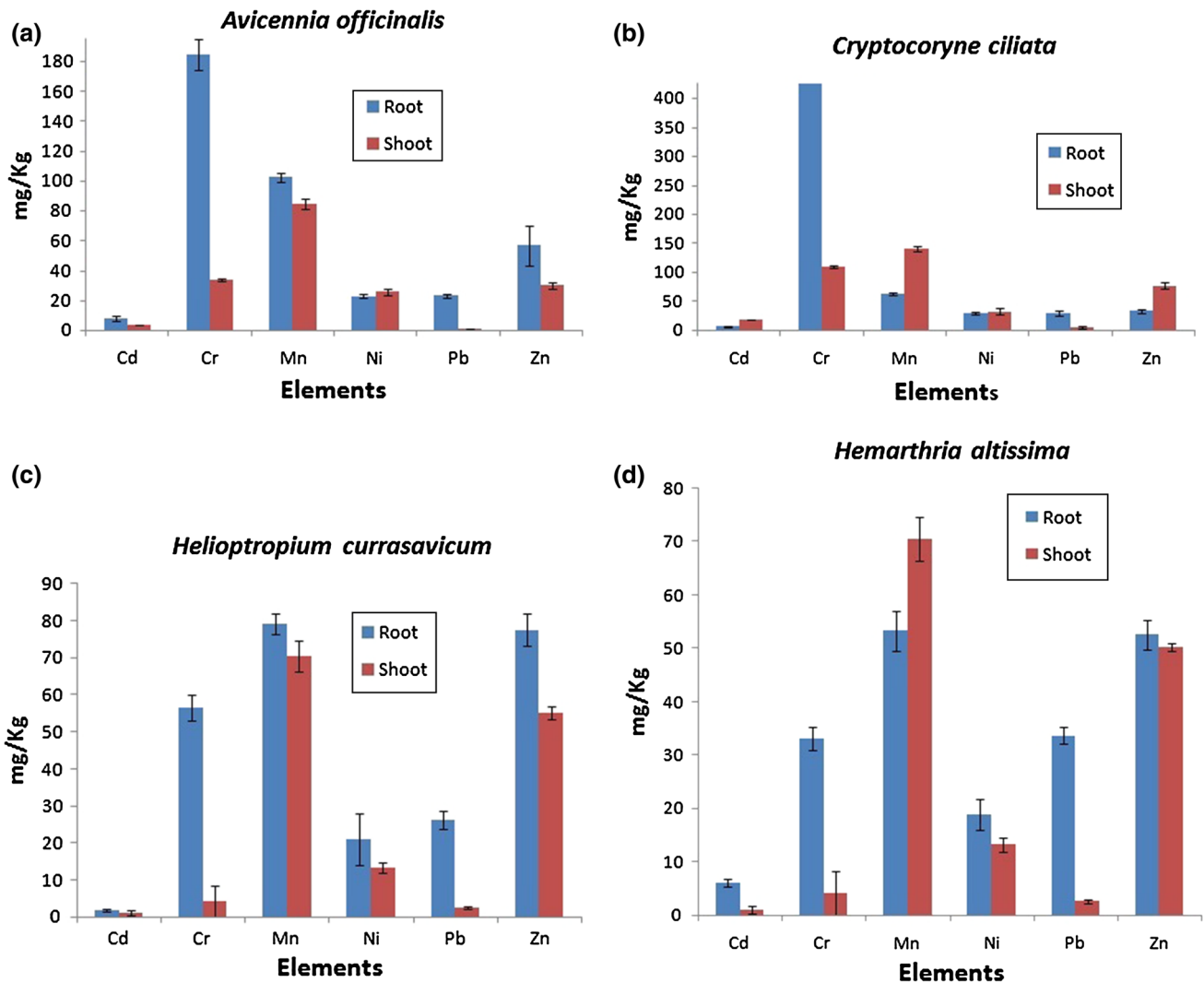| Species	E_r (Cd)	E_r (Ni)	E_r (Zn)	E_r (Mn)	E_r (Cr)	E_r (Pb)	ERI
Cryptocoryne ciliata	149.3	3.3	1.0	0.2	3.8	5.6	163.2
Hemarthria altissima	195.0	3.35	0.98	0.19	3.84	6.59	209.95
Avicennia officinalis	243.57	3.31	0.98	0.19	3.86	6.68	258.60
Heliotropium currasavicum	286.43	3.29	0.99	0.19	3.86	6.59	301.35
Control site (Jharkhali)	50.00	2.99	0.98	0.18	0.5	3.20	57.86

Fig. 7 Metal concentrations with ±1 SD in root and shoot of plants

plant *Erigeron bonariensis* growing in the Nanjing section of Changjiang River, China.

Cryptocoryne ciliata have the high BCF in respect to Cd, Cr, Pb whereas have high TF value for Cd, Zn, Mn, Pb. Research studies have proved that many heavy metals/ metalloids are transported to aerial plant parts by binding ligands, especially sulphur ligands and organic acids (Babula et al. 2008). Otherwise metals are restricted to the vacuolar parts hence restricting its upward transport. Cd has an affinity for Ni–Zn enzyme, therefore very mobile in higher plants as evident from our study. Past researches have revealed that glandular tissues of halophytes can secrete toxic metals, such as Cd, Zn, Pb which can accumulate in their salt glands or trichomes (Manousaki and Kalogerakis 2011). Many salt tolerant extreme halophytic species like *Salicornia brachiata*, can bioaccumulate Cd and the catalase activity was markedly increased due to contamination of Cd and Ni (Sharma et al. 2010).

Cr is a toxic metal that is available in elevated concentration in all the rhizosphere of polluted sites. Accumulation of Cr has been observed in some aquatic and floating plants, namely *Eichhornia crassipes* (Babula et al. 2008). Cr rarely gets transported to the leaves and leaves vascular bundles (Alloway 2013), so above ground biomass of the investigated plants shows lesser translocation of Cr. Cr toxicity resulted in stunted growth, poorly developed root system, discolored leaves, leaf chlorosis and it is observed that in uncontaminated soil Cr concentration in the plant tissues rarely exceeds 0.2 mg kg^{-1} due to it's relative immobility in soil, water and plant (Alloway 2013). But none of the investigated flora shows such defects in their morphology. Only *H. altissima* (red swamp grass, an invasive species) is an exception showing TF factor >1. This tolerance of *H. altissima* to the Cr toxicity may be responsible for proliferation of invasive grass in the mangrove patches exposed to metal pollution. Though Pb
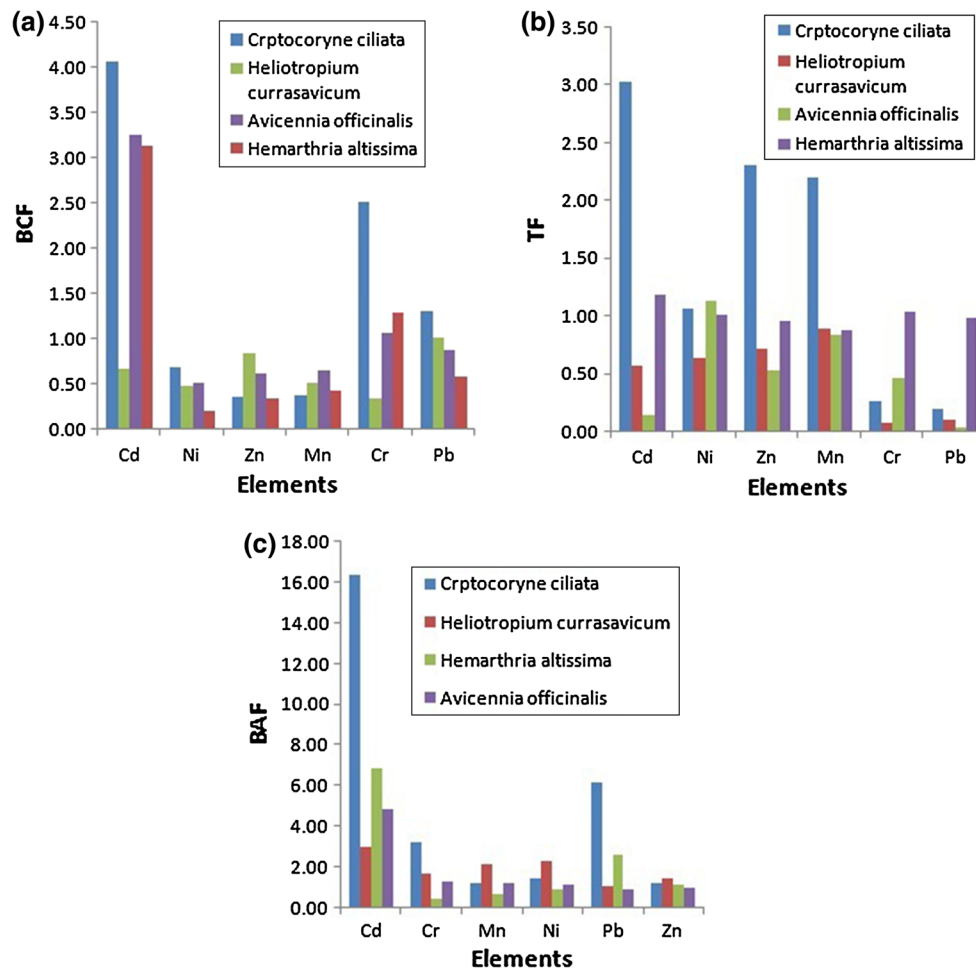
Fig. 8 a Bioconcentration factor (BCF), b translocation factor (TF) and c bioaccumulation factor (BAF) of analyzed elements in selected macrophytes

is rarely mobile in soil–water interface due to its strong affinity to soil particles hence shows low bioavailability, however, it is bioconcentrated in *C. ciliata*. Mangroves like *A. corniculatum* also have BCF values >1, but previous researches reported translocation to higher plant parts is rare (Zhou et al. 2011).

Bioaccumulation factor (BAF) shows that *C. ciliata* have potential to be used as phytoextractors for Cd (BAF >16), Pb (BAF >5) and Cr (BAF >2). Ni and Mn also have BCF >1 for *H. currasavicum*. *H. altissima* shows higher BCF values for Pb and Cd. TF of Ni is higher than 1 in *A. officinalis*, *C. ciliata* and *H. altissima*. Cr is slightly soluble in soil–water phase and is not generally taken up by the macrophytes like Pb which is also strongly bound with the soil particles. Cd and Zn are more mobile in plants and has a greater affinity to be transported to the vegetative parts (Kabata-Pendias and Pendias 2001). Ni and Zn also less toxic owing to its presence in several key plant enzymes. Generally Ni is easily translocated to the aerial parts of the plants owing to its affinity in many biochemical pathways of the plant. Hydrogenases, carbon mono-oxide dehydrogenase, methyl coenzyme M reductase are few key plant enzymes dependent on Ni for their proper functioning (Alloway 2013). *C. ciliata* (Araceae amphibious member with waxy leaves) has highest tolerance to most of the pollutants. Anatomical and physiological process of the plant may be responsible for the same. As seen in *E. crassipes*, the presence of few unique anatomical adaptations of the aquatic plant like presence of aerenchyma, is responsible for elevated tolerance, which can also be the case for *C. ciliata*.

Conclusion

This study shows that three anthropogenic heavy metal pollutants namely Cd, Pb and Cr affect the mangrove site exposed to industrial, municipal and tannery effluent. Here the concentration of Cr coming from tannery industries is above the permissible limit and warrants

immediate attention. I_{geo}, C_f and ErI shows that control site is devoid of pollution pressure. ERI and E_r values indicate that all the four species (*H. currasavicum*, *A. officinalis*, *H. altissima* and *C. ciliata*) have equal tollerence in respect to Cr stress, whereas *A. officinalis* is tolerent to Cd. Control site (Jharkhali) is devoid of pollution pressure, having a mangrove community structure different from the stressed region. True mangrove varieties are replaced by stress tolerent halophytes and invasive flora in the polluted site. Diversity is also lesser in the polluted site than the pristine mangrove patches of the control site with higher dominance of stress tolerent species. Three herbs namely *C. ciliata*, *H. altissima* and *H. currasavicum* were seen to proliferate in these metal stressed polluted mangrove fragments. *A. officinalis*, a true mangrove tree species, was also present in higher abundance at that polluted patch. Here *C. ciliata* (Araceae family) shows high metal extraction potential in respect to Cr, Cd and Pb. Other plants have different levels of tolerance to the pollutants. *H. currasavicum* have the potential to tolerate highest Ecological risk but shows low uptake level for the metals. It is also observed that metal tolerant mangrove associates and invasive flora was excluding the true mangrove members as evident from low diversity and high dominance in the stressed mangrove community in respect to the control site that is away from the anthropogenic industrial activities. This work also highlights that by understanding the affect of pollution on fragile ecosystem and studying the plant community structure, metal tolerant species with phytoremediation potential could be screened out or identified. So this methodology can be employed in similar habitats in other parts of the globe to find out pollutant extracting/tolerating plant species.

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