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## Bioaccumulation of trace elements by *Avicennia marina*

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### PEER REVIEW

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#### Comments

The paper is short, clear and to the point. The title and abstract correspond to the content of the manuscript. Finally, it is a sound and well-written manuscript can be published.

Details on Page 893

### ABSTRACT

**Objective:** To analyze the concentrations of 12 micro-nutrients (Al, B, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, and Zn) in different plant parts of *Avicennia marina* and its rhizosphere soil of the south east coast of India.

**Methods:** The samples were acid digested, then analyzed by using inductively coupled plasma system (ICP-Optical Emission Spectrophotometer).

**Results:** Levels of metals were found in the decreasing order: Cd>Co>Ni>Pb>B>Cr>Zn>Mg>Mn>Cu>Fe>Al. The soil held more levels of metals than plant parts, but within the permissible limits of concentration. Bark and root accumulated higher levels of trace elements in a magnitude of 10–80 folds than other plant parts. The overall bioaccumulation factor in the sampling sites of Vellar, Pichavaram and Cuddalore was 2.88, 1.42 0.47 respectively. Essential elements accumulate high in mature mangroves forest while non-essential elements accumulate high in the industrially polluted mangroves.

**Conclusions:** The ratio between essential and non-essential elements was found higher in young mangrove forest than that in mature mangrove forest and polluted mangrove areas. Thus, the ratio of accumulation can be used as an index of the growth and pollution status of mangroves.

### KEYWORDS

Heavy metals, Accumulation, Mangroves, Remediation

## 1. Introduction

Mangroves are often contaminated with toxic pollution especially heavy metals. However, only limited scientific data are available on the toxic levels of heavy metals in the mangroves[1,2]. A serious question recently addressed on mangrove environments is the cycling of heavy metals, because of their toxicity, bioaccumulation capacity and persistence[3]. In contrast with organic pollutants, heavy metals cannot be biologically or chemically degraded, and thus may either accumulate locally or be transported over long distances[4]. Mangroves, may act as a sink or a source of heavy metals in coastal environments because of their

variable physical and chemical properties[5]. Moreover, many countries have used mangroves in the treatment of sewage effluents. Mangrove plants usually found to cope with low nutrient availability due to the poor aeration in the rooting zone[6], and they have the capability of selective ion transport that may affect uptake, distribution, loading and excretion of micro-nutrients within the plant components[7]. There are very limited studies of estimating the concentrations of heavy metals in different mangrove plant parts[8,9]. But, there are no clear reports on the concentration of heavy metals in plants parts in relation to that in sediments, in industrially polluted mangroves compared to pristine environment. To fill this knowledge gap, the

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present study was undertaken to assess the bioaccumulation of heavy metals in plants parts of the mangrove species, *Avicennia marina* (*A. marina*), growing along the polluted or pristine mangrove habitats.

## 2. Materials and methods

### 2.1. Description of study area

Fresh plant parts (bark, stem, leaf and root) and rhizosphere soil of *A. marina* (Forsk.) Vierh., were collected from three different sampling sites: one industrially contaminated mangroves in Cuddalore and two pristine mangroves along the Vellar–Coleroon estuarine complex (one is naturally formed mature forest in Pichavaram and another one is artificially developed young forest of about 20 years old along the Vellar estuary). Pichavaram mangrove forest with an area of 1400 ha is a natural formation, comprising of about 51 islands with sizes ranging from 10 m<sup>2</sup> to 2 km<sup>2</sup>. About 40% of the area is covered by waterways, 50% by forest, and the rest by mud flats and sandy or salty soils. There are numerous creeks, gullies and canals traversing the forest with a depth ranging from 0.5 to 1.5 m. Another mangrove is an artificially developed one along the Vellar estuary (Latitude 11°46'N, Longitude 79°46' E). Other mangrove is located at the Uppanar estuary situated in Cuddalore (Latitude 11°43'N, Longitude 79°46' E). It is a polluted mangrove habitat as it runs behind the industrial complex of SIPCOT (=State Industrial Promotion Corporation of Tamil Nadu Limited) which comprises many chemical and pharmaceutical industries. The effluents of these industries are released into the estuary. In addition to the industrial wastes, the estuary receives also the municipal wastes and domestic sewage from Cuddalore old town and waste from coconut husk retting.

### 2.2. Sample collection, preparation and analysis of micronutrients

Sampling was made during summer month of May 2011. Fresh plant samples of *A. marina* were collected randomly by using a sharp knife and rhizosphere soil samples (up to 10 cm depth) were also collected simultaneously by using core sampler of 5 cm diameter. The plant samples were washed in distilled water and oven dried at 60 °C for 24 h. The samples were digested in 90% mixture of concentrated nitric acid and perchloric acids, adapting the methods of Watling and Watling<sup>[10]</sup>, and MacFarlane *et al.*<sup>[11]</sup>, and made up to 20 mL volume. Digested samples were stored in labeled acid-washed glass vials. These samples were analyzed in triplicates for 12 trace metals (Al, B, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, and Zn) by using an inductively coupled plasma system (ICP–Optical Emission Spectrophotometer; Optima 2100DV) and quantified against known standards. Results are expressed as µg/g dry tissue. From the data, BAF was calculated for each element as concentration in the plant

part divided by that in respective soil<sup>[12]</sup>.

### 2.3. Statistical analysis

A suite of statistical analysis (SPSS 11.5) was made to find the descriptive statistics mean±standard error, in order to evaluate the significance between variables between the sites or plant parts; ANOVA (Two-way classifications) was applied by computing the general linear regression model. *Post hoc* multiple comparison tests (Tokey's, S–N–K), was also used to identify significantly different combinations and followed by normality test for the descriptive statistics analysis. Correlation matrix was made between all the variables to find out significant correlation between any two variables. Cluster analysis was done to analyze the similarity accumulation of heavy metals in different plant parts.

## 3. Results

The concentrations of each metal in *A. marina* are given in Table 1. They varied significantly between mangrove plants parts or sampling sites. Roots accumulated high levels of heavy metals, while stem did only low levels. The heavy metal accumulation was recorded greater in the polluted area (Cuddalore) than pristine mangrove area (Pichavaram). Among the metals, copper got accumulated maximum in all plant parts and soil, while cadmium was the least accumulated (Table 1).

Copper ranged from 33.00 (leaf) to 84.00 µg/g (bark) in the mature forest, and from 12.00 (leaf) to 196.00 µg/g (soil) in polluted forest, while it varied from 31.00 (bark) to 77.00 µg/g (root) in young forest. Iron ranged from 189.0 (stem) to 2116.0 µg/g (bark and root) in the mature forest, and from 125.0 (bark to 3887.0 µg/g (soil) in polluted forest, while it varied from 93.0 (bark) to 3692.0 µg/g (soil) in young forest.

Magnesium ranged from 531.00 (stem) to 907.00 µg/g (bark) in the mature forest, and from 444.00 (stem) to 1752.00 µg/g (soil) in polluted forest, while it varied from 463.00 (stem) to 1648.00 µg/g (leaves) in young forest. Manganese ranged from 10.00 (root) to 25.00 µg/g (soil) in the mature forest and from 5.00 (leaves) to 41.00 µg/g (soil) in polluted forest, while it varied from 10.00 (stem) to 82.00 µg/g (bark) in young forest.

Zinc ranged from 16.0 (stem) to 37.0 µg/g (bark) in the mature forest, and from 9.0 (leaves) to 65.0 µg/g (soil) in polluted forest, while it varied from 17.0 (leaves) to 81.0 µg/g (soil) in young forest. Boron ranged from 10.2 (stem and leaves) to 20.6 µg/g (soil) in the mature forest, and from 9.0 (leaves) to 19.4 µg/g (soil) in polluted forest, while it varied from 10.2 (stem) to 24.6 µg/g (soil) in young forest.

Cadmium exhibited the high accumulation of 0.40 µg/g in the stem as compared to other plant parts and soil in the mature forest. It showed a range of accumulation from 0.00 (stem) to 0.40 µg/g (root and bark) in polluted forest, while it varied from 0.00 (stem, soil and leaves) to 0.40 µg/g (root) in young forest. Cobalt ranged from 0.04 (leaves) to 3.40 µg/g (soil) in the mature forest and from 0.60 (root, stem and leaves)

**Table 1**

Heavy metal concentrations ( $\mu\text{g/g}$ ) in mangrove plant parts and rhizosphere soil of *A. marina* collected from three different sampling sites (values are average of triplicate samples with standard error; dissimilar alphabets are significant at 5% level).

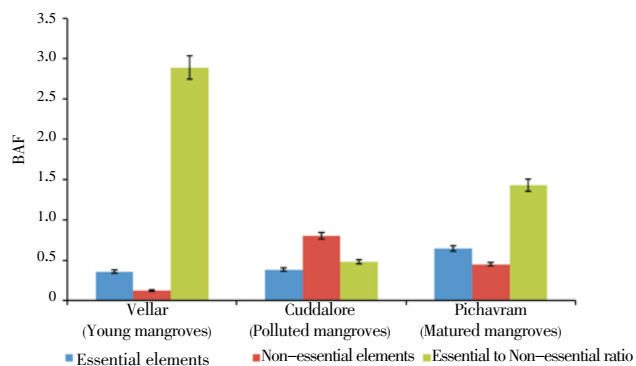
Sampling Station	Sample name	Heavy metals concentration ( $\mu\text{g/g}$ )											
		Essential elements						Non-essential elements					
		Cu	Fe	Mg	Mn	Zn	B	Cd	Co	Cr	Ni	Pb	Al
Mature forest (Pichavaram)	Rhizosphere soil	46.00±0.40 <sup>a</sup>	1770.0±2.9 <sup>b</sup>	816.00±2.30 <sup>b</sup>	25.00±0.30 <sup>b</sup>	25.0±0.4 <sup>b</sup>	20.6±0.2 <sup>b</sup>	0.00±0.10 <sup>b</sup>	3.40±0.20 <sup>b</sup>	17.0±3.5 <sup>a</sup>	9.0±0.1 <sup>b</sup>	8.0±0.2 <sup>b</sup>	2418.0±14.7 <sup>c</sup>
	Root	58.00±0.76 <sup>b</sup>	2116.0±2.3 <sup>c</sup>	616.00±2.30 <sup>b</sup>	10.00±0.30 <sup>a</sup>	18.0±0.1 <sup>a</sup>	13.8±0.2 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.40±0.20 <sup>a</sup>	35.0±3.5 <sup>b</sup>	3.0±0.2 <sup>a</sup>	9.0±0.2 <sup>b</sup>	1740.0±0.2 <sup>b</sup>
	Stem	39.00±0.70 <sup>a</sup>	189.0±2.3 <sup>a</sup>	531.00±2.10 <sup>a</sup>	13.00±0.30 <sup>a</sup>	16.0±0.3 <sup>a</sup>	10.2±0.2 <sup>a</sup>	0.40±0.10 <sup>b</sup>	0.00±0.00 <sup>a</sup>	26.0±3.2 <sup>a</sup>	2.0±0.2 <sup>a</sup>	9.0±0.3 <sup>b</sup>	2771.0±0.2 <sup>c</sup>
	Bark	84.00±0.70 <sup>c</sup>	2116.0±2.6 <sup>c</sup>	907.00±2.50 <sup>b</sup>	22.00±0.30 <sup>b</sup>	37.0±0.4 <sup>c</sup>	13.8±0.2 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.30±0.20 <sup>a</sup>	11.0±3.2 <sup>a</sup>	1.0±0.1 <sup>a</sup>	5.0±0.3 <sup>a</sup>	3542.0±3.3 <sup>c</sup>
	Leaves	33.00±0.70 <sup>a</sup>	525.0±2.7 <sup>a</sup>	799.00±2.50 <sup>b</sup>	17.00±0.30 <sup>a</sup>	18.0±0.2 <sup>a</sup>	10.2±0.2 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.04±0.20 <sup>a</sup>	19.0±3.6 <sup>a</sup>	1.0±0.1 <sup>a</sup>	7.0±0.2 <sup>b</sup>	202.0±13.0 <sup>a</sup>
Polluted forest (Cuddalore)	Rhizosphere soil	196.00±0.70 <sup>c</sup>	3887.0±2.7 <sup>c</sup>	1752.00±2.10 <sup>c</sup>	41.00±0.10 <sup>c</sup>	65.0±0.9 <sup>c</sup>	19.4±0.2 <sup>b</sup>	0.10±0.08 <sup>a</sup>	1.80±0.10 <sup>b</sup>	22.0±3.2 <sup>a</sup>	5.0±0.2 <sup>a</sup>	8.0±0.4 <sup>c</sup>	1894.0±0.2 <sup>b</sup>
	Root	58.00±0.70 <sup>b</sup>	1087.0±2.8 <sup>c</sup>	1662.00±2.40 <sup>c</sup>	21.00±0.30 <sup>b</sup>	21.0±0.8 <sup>a</sup>	18.2±0.2 <sup>b</sup>	0.40±0.12 <sup>b</sup>	0.60±0.10 <sup>a</sup>	16.0±3.1 <sup>a</sup>	3.0±0.2 <sup>a</sup>	7.0±0.4 <sup>b</sup>	389.0±0.2 <sup>a</sup>
	Stem	41.00±0.70 <sup>a</sup>	316.0±2.8 <sup>a</sup>	444.00±2.32 <sup>a</sup>	11.00±0.40 <sup>a</sup>	12.0±0.2 <sup>a</sup>	10.2±0.3 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.60±0.10 <sup>a</sup>	12.0±3.2 <sup>a</sup>	2.0±0.1 <sup>a</sup>	6.0±0.3 <sup>a</sup>	385.0±16.6 <sup>a</sup>
	Bark	61.00±0.70 <sup>b</sup>	125.0±2.7 <sup>a</sup>	1573.00±2.90 <sup>c</sup>	28.00±0.10 <sup>b</sup>	17.0±0.9 <sup>a</sup>	13.8±0.2 <sup>a</sup>	0.40±0.10 <sup>b</sup>	1.80±0.20 <sup>b</sup>	58.0±3.1 <sup>b</sup>	10.0±0.2 <sup>b</sup>	16.0±0.4 <sup>d</sup>	1611.0±0.2 <sup>b</sup>
	Leaves	12.00±0.70 <sup>a</sup>	131.0±2.9 <sup>a</sup>	571.00±2.40 <sup>a</sup>	5.00±0.30 <sup>a</sup>	9.0±0.4 <sup>a</sup>	9.0±0.2 <sup>a</sup>	0.10±0.01 <sup>a</sup>	0.60±0.10 <sup>a</sup>	20.0±3.7 <sup>a</sup>	3.0±0.2 <sup>a</sup>	6.0±0.4 <sup>a</sup>	1093.0±1.9 <sup>a</sup>
Young forest (Vellar developed)	Rhizosphere soil	49.00±0.70 <sup>a</sup>	3692.0±2.1 <sup>c</sup>	1249.00±2.10 <sup>d</sup>	91.00±0.30 <sup>c</sup>	81.0±0.4 <sup>c</sup>	24.6±0.2 <sup>b</sup>	0.00±0.10 <sup>a</sup>	3.40±0.20 <sup>b</sup>	106.0±3.2 <sup>b</sup>	24.0±0.2 <sup>c</sup>	34.0±0.4 <sup>d</sup>	3892.0±0.2 <sup>c</sup>
	Root	67.00±0.70 <sup>b</sup>	410.0±2.3 <sup>a</sup>	845.00±2.30 <sup>b</sup>	15.00±0.30 <sup>a</sup>	25.0±0.9 <sup>b</sup>	13.8±0.2 <sup>a</sup>	0.40±0.10 <sup>b</sup>	1.40±0.20 <sup>b</sup>	28.0±3.2 <sup>b</sup>	8.0±0.2 <sup>b</sup>	8.0±0.4 <sup>b</sup>	1393.0±0.2 <sup>c</sup>
	Stem	77.00±0.70 <sup>c</sup>	118.0±2.7 <sup>a</sup>	463.00±2.10 <sup>a</sup>	10.00±0.30 <sup>a</sup>	22.0±0.2 <sup>a</sup>	10.2±0.2 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.10±0.20 <sup>a</sup>	15.0±3.2 <sup>a</sup>	2.0±0.1 <sup>a</sup>	5.0±0.3 <sup>a</sup>	175.0±0.2 <sup>b</sup>
	Bark	31.00±0.70 <sup>a</sup>	93.0±2.7 <sup>a</sup>	1102.00±2.30 <sup>c</sup>	82.00±0.31 <sup>c</sup>	23.0±0.1 <sup>a</sup>	13.6±0.5 <sup>a</sup>	0.30±0.10 <sup>b</sup>	0.10±0.00 <sup>a</sup>	17.0±3.2 <sup>a</sup>	2.0±0.2 <sup>a</sup>	6.0±0.3 <sup>a</sup>	389.0±0.2 <sup>a</sup>
	Leaves	47.00±0.70 <sup>a</sup>	137.0±2.7 <sup>a</sup>	1648.00±2.50 <sup>c</sup>	26.00±0.30 <sup>b</sup>	17.0±0.6 <sup>a</sup>	12.4±0.5 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.30±0.20 <sup>a</sup>	3.0±3.2 <sup>a</sup>	1.0±0.2 <sup>a</sup>	2.0±0.6 <sup>a</sup>	419.0±0.2 <sup>a</sup>

to 1.80  $\mu\text{g/g}$  (soil and bark) in polluted forest, while it varied from 0.10 (stem and bark) to 3.40  $\mu\text{g/g}$  (soil) in young forest.

Chromium ranged from 11.0 (bark) to 35.0  $\mu\text{g/g}$  (root) in the mature forest, and from 12.0 (stem) to 58.0  $\mu\text{g/g}$  (bark) in polluted forest, while it varied from 3.0 (leaves) to 106.0  $\mu\text{g/g}$  (soil) in young forest. Nickel ranged from 1.0 (bark and leaves) to 9.0  $\mu\text{g/g}$  (soil) in the mature forest and from 2.0 (stem) to 10.0  $\mu\text{g/g}$  (bark) in polluted forest, while it varied from 1.0 (leaves) to 24.0  $\mu\text{g/g}$  (soil) in young forest.

Lead ranged from 5.0 (bark) to 9.0  $\mu\text{g/g}$  (root and stem) in the mature forest, and from 6.0 (stem and leaves) to 16.0  $\mu\text{g/g}$  (bark) in polluted forest, while it varied from 2.0 (leaves) to 34.0  $\mu\text{g/g}$  (soil) in young forest. Aluminum ranged from 202.0 (leaves) to 3542.0  $\mu\text{g/g}$  (bark) in the mature forest, and from 385.0 (stem) to 1894.0  $\mu\text{g/g}$  (soil) in polluted forest, while it varied from 175.0 (stem) to 3892.0  $\mu\text{g/g}$  (soil) in young forest.

The average bioaccumulation factor (BAF) for essential elements was high (0.64) in mature forest, intermediate (0.38) in polluted forest and low in 0.35 in young forest. The average BAF for non-essential elements was high (0.80) in polluted forest, intermediate (0.45) in mature forest and least (0.12) in young forest. The ratio of BAFs between essential and non-essential elements was high (2.88) in young forest, followed by mature forest (1.42) than polluted forest (0.47) (Figure 1).

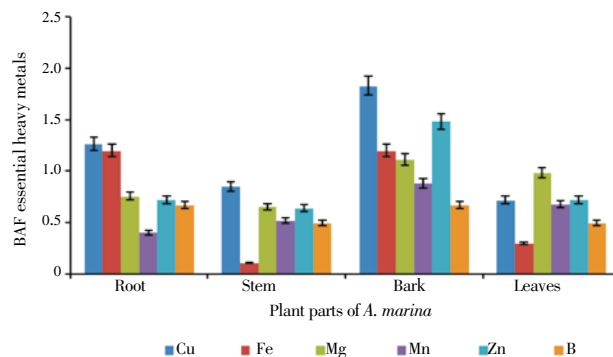


**Figure 1.** Average BAFs for essential and non essential elements in *A. marina* growing in three mangrove areas.

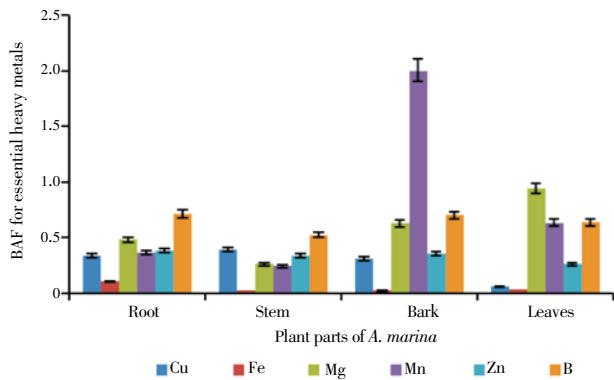
Thus *A. marina* in the mature forest concentrated high amount of essential elements in the plant parts: 1.82 fold and 1.68 fold higher than young forest and polluted forest respectively. The mangrove species in the polluted forest concentrated high quantity of non-essential elements in the plant parts: 1.9 fold and 6.7 fold in mature and young forests respectively. The ratio of BAFs between essential and non-essential elements was high in young forest to the magnitude of 2 fold and 6.1 fold as compared to the mature and polluted forests respectively.

The maximum essential heavy metals got more accumulated in non-polluted area than that in polluted mangrove habitats, while the non-essential heavy metal (BAF) was found higher in the polluted area than that in non-polluted mangrove habitats. The BAF ratio in different sampling site 2.88:1.42:0.47 at Vellar, Pichavaram and Cuddalore respectively indicating that essential elements accumulated high in mature mangroves forest while non-essential elements did in the industrially polluted mangroves habitat.

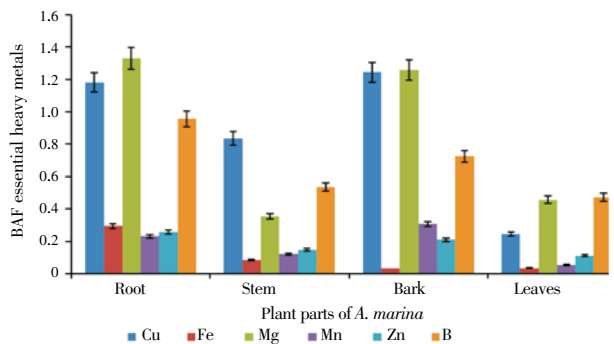
The BAF of Mn was higher in bark than the other plant parts in Pichavaram and Cuddalore respectively (Figures 1 and 2). Similarly it was minimum in the leaves than in other plant parts (Figures 3 and 4). However, it was significant between sampling site ( $F=118.38$ ;  $df=2$ ) and plant parts ( $F=242.96$ ;  $df=4$ ).



**Figure 2.** BAF for essential elements in plant parts of mangrove (*A. marina*) in Pichavaram.



**Figure 3.** BAF for essential elements in plant parts of mangrove (*A. marina*) in Cuddalore.

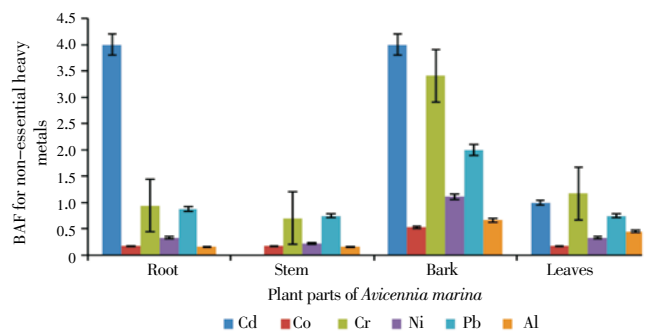


**Figure 4.** BAF for essential elements in plant parts of mangrove (*A. marina*) in Vellar.

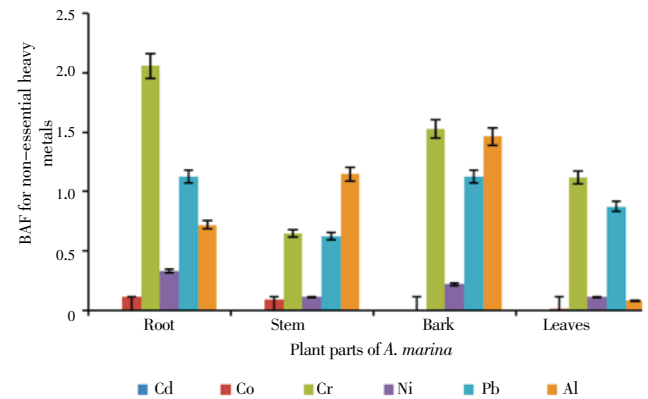
The BAF of Mn was higher in the bark than that in other plant parts in Pichavaram (Figure 2), Cuddalore (Figure 3) and Vellar (Figure 4). It was significant between sampling sites ( $F=102.43$ ;  $df=2$ ), and between plant parts ( $F=761.20$ ;  $df=4$ ). Level of Zn was higher in the bark than that in other plant parts in the Pichavaram (Figure 2), Cuddalore (Figure 3) and Vellar (Figure 4), However it was significant between sampling sites ( $F=883.14$ ;  $df=2$ ) and between plant parts ( $F=461.69$ ;  $df=4$ ).

The BAF of B was higher in the bark than that in other plant parts in Pichavaram (Figure 2), similarly it was higher in the root than that in other plant parts in Cuddalore (Figure 3). However it was significant between sampling sites ( $F=33.33$ ;  $df=2$ ), and between plant parts ( $F=106.97$ ;  $df=4$ ). Fe was also higher in the bark and root than that in other plant parts in Pichavaram (Figure 2), and Cuddalore (Figure 3). It was significant between sampling sites ( $F=787.88$ ;  $df=2$ ) and also between plant parts ( $F=573.43$ ;  $df=4$ ). Mg was higher in bark and lower in stem in Pichavaram (Figure 2). Similar results were observed in the other two stations. It was significant between sampling sites ( $F=443.80$ ;  $df=2$ ) and between plant parts ( $F=559.56$ ;  $df=4$ ).

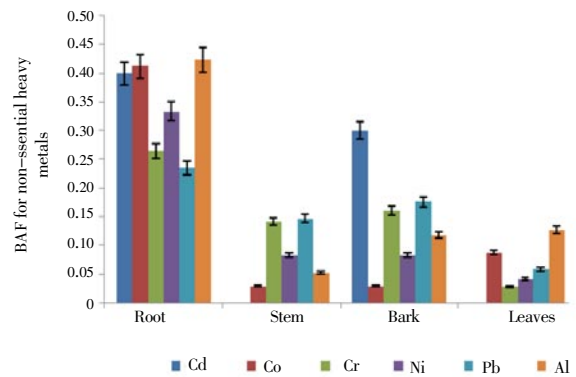
The BAF of Cd was higher in the bark in Pichavaram (Figure 5), and Vellar (Figure 7). Variation was significant between the sampling sites ( $F=5.16$ ;  $df=2$ ), and between plant parts ( $F=8.75$ ;  $df=4$ ). Co was higher in the bark than that in other plants parts in Pichavaram (Figure 5), and it was higher in the bark and root than that in the other plants parts in cuddalore( Figure 6). Similarly it was higher in the root than the other plant parts in Vellar (Figure 7). The values were significant between sampling sites ( $F=3.76$ ;  $df=2$ ), or between plant parts ( $F=77.44$ ;  $df=4$ ).



**Figure 5.** BAF for non essential elements in plant parts of mangrove (*A. marina*) in Pichavaram.



**Figure 6.** BAF for non-essential elements in plant parts of mangrove (*A. marina*) in Cuddalore.

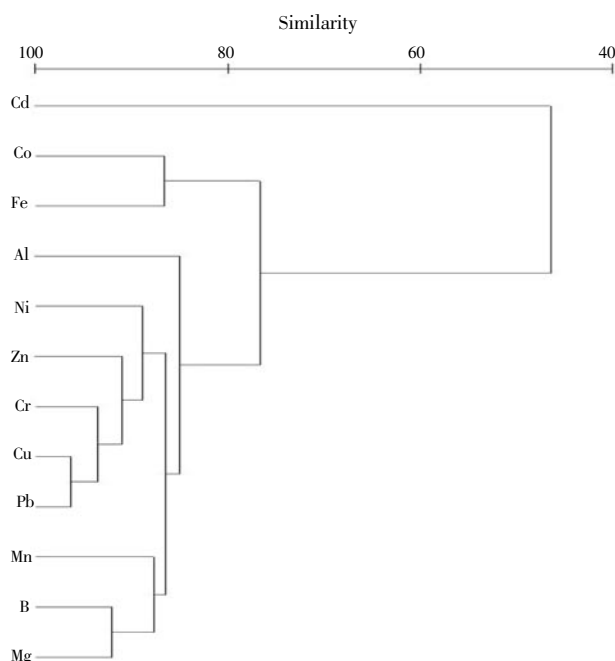


**Figure 7.** BAF for non essential elements in plant parts of mangrove (*A. marina*) in Vellar.

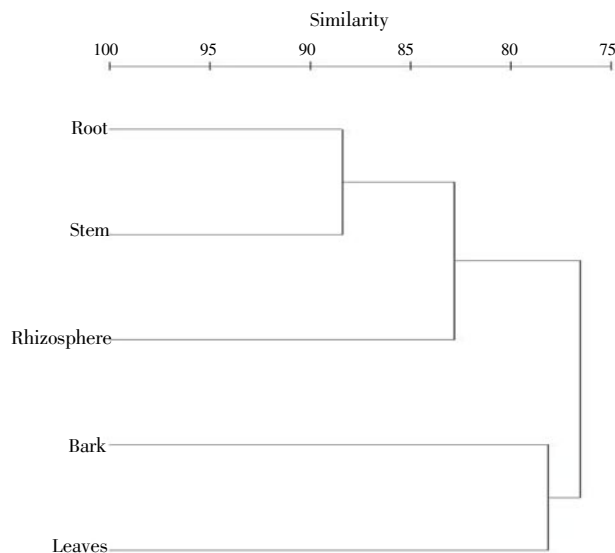
The concentration of Cr was higher in bark in Pichavaram (Figure 5) and root at Cuddalore (Figure 6) and root in Vellar (Figure 7) than that in other plant parts. It was significant between sampling sites ( $F=13.42$ ;  $df=2$ ) and between plant parts ( $F=32.60$ ;  $df=4$ ). Ni was higher in bark than that in other plant parts in Pichavaram (Figure 5), and it was also more in bark than other plant parts in Cuddalore (Figure 6), and in root at Vellar (Figure 7). It was significant between sampling sites ( $F=327.26$ ;  $df=2$ ) and between plant parts ( $F=835.80$ ;  $df=4$ ). In case of Pb, it was higher in the bark than that in other plant parts in Pichavaram (Figure 5), Cuddalore (Figure 6) and in root at Vellar (Figure 7), it was significant among sampling sites ( $F=80.22$ ;  $df=2$ ) or plant parts ( $F=310.03$ ;  $df=4$ ). The accumulation of Al was maximum recorded in the bark and minimum in the stem in Pichavaram (Figure 5), and Cuddalore (Figure 6) and root in Vellar (Figure 7). However, the variance of Fe, Mg, and Al was significant between sampling sites

( $F=636.08$ ,  $658.23$  and  $356.23$ ;  $df=2$ ,  $2$  and  $2$ ) and plant parts ( $F=869.57$ ,  $425.32$  and  $23.5$ ;  $df=4$ ,  $4$  and  $4$ ).

Cluster analysis was tested on the data set using average linkage between groups (rescaled distance cluster). Although not substantially different from factor of metal accumulation, cluster analysis can be used as a substitute method to confirm the results of factor of metal accumulation. The results are illustrated in the dendrograms on hierarchical cluster analysis between the heavy metals (Al, B, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, and Zn) accumulation at different sampling stations (Figure 8), and at different plant parts (bark, stem, leaves, root and rhizosphere soil) level of similarity of metal accumulation (Figure 9). Cluster analysis was made between all of the variables heavy metals (Figures 8 and 9) and it was significant with each other, which revealed that metals accumulation in plant parts was dependent on trace metals.



**Figure 8.** Dendrogram showing hierarchical cluster analysis for similarity between the trace elements at different sampling stations.



**Figure 9.** Dendrogram showing hierarchical cluster analysis for similarity between the different plant parts in trace elements accumulation.

## 4. Discussion

In general, the bioaccumulation of environmental pollutants is approached with the assumption that organisms achieve a chemical equilibrium with respect to a particular medium or route of exposure<sup>[12]</sup>. This approach is used to estimate bioaccumulation of chemical residues in plants from measured concentrations in the appropriate reference media. The BAF is defined as the ratio between  $C_{\text{biota}}/C_{\text{soil}}$ , where  $C_{\text{biota}}$  and  $C_{\text{soil}}$  are the total metal concentrations in taxa and soil, respectively.

Previous studies analyzed the heavy metal concentrations (Fe, Cu, Mn, Zn) in the leaves of the five mangrove species *Rhizophora mucronata*, *Avicennia officinalis*, *Bruguiera cylindrica*, *Ceriops decandra* and *Xylocarpus granatum* from the Bhitarkanika, Orissa, east coast of India. The study has found that *Avicennia officinalis* accumulates high concentrations of metals analyzed<sup>[13]</sup>. The order of abundance in concentration of heavy metals falls as  $\text{Fe} > \text{Mn} > \text{Cu} > \text{Zn}$ . Concentration and accumulation of heavy metals in the sediments is closely related to the frequency and duration of tidal flood and river pollution. The uptake of elements by the plant parts varies with each element. Our present results are in accordance with the previous results. In addition, the present work found an interesting trend of accumulation between essential and non-essential metals in the mangrove species in relation to pollution and growth status of mangrove forests. In general, essential heavy metals were in higher quantities in mangrove plant parts than non-essential metals except Al. Bioaccumulation of essential heavy metals was more in *A. marina* of mature forest than that in polluted or young forests. While, the bioaccumulation of non-essential heavy metals was greater in *A. marina* of polluted forest than that in mature or young forests. The ratio between essential and non-essential heavy metals was high in *A. marina* of young forest than that in mature or polluted forests. In general, bark was found to exhibit higher bioaccumulation of heavy metals than other plant parts. In *A. marina* of mature forest, bioaccumulation of essential heavy metals was highest in bark and least in stem, while a similar trend seen with non-essential elements. In *A. marina* of polluted forest, bark and root were higher in bioaccumulation of essential heavy metals than stem and leaf, while bark exhibited highest bioaccumulation of non-essential elements. In *A. marina* of young forest, bark was the highest in bioaccumulation of essential heavy metals, whereas root was the highest in bioaccumulation of nonessential metals followed by bark. The essential metal accumulation showed relation with maturity of mangrove forest and this may be attributed to the fact that the essential metals such as iron, manganese, copper, zinc, vanadium, cobalt and molybdenum are known to influence primary production.

Heavy metals are distributed more uniformly among the plant parts<sup>[14]</sup>, and this statement is supported by the narrow range of the heavy metals between plant parts of *A. marina* at different sampling stations. However,



the bark accumulation was high as 10% (Cd) or 80% (Fe) as compared to other plant parts. A similar observation has been made in the case of another mangrove species: *Rhizophora mangle* exhibited 222 times greater accumulation of iron in roots than in other parts in Panama<sup>[15]</sup>, and showed 52 fold higher in roots than in leaves in Sepetiba Bay, Brazil<sup>[16]</sup>. Importance of Fe is well-known in the formation of chlorophyll, protein synthesis and root growth<sup>[17]</sup>, and hence, iron accumulated in the plant parts in particular roots. Moreover, oxygen released by the roots of mangrove plant creates an oxidant geochemical microenvironment<sup>[16]</sup>, which helps to oxidize soluble Fe<sup>2+</sup> and Mn<sup>2+</sup> to insoluble Fe(OH)<sub>3</sub> and MnO<sub>2</sub>. After oxidization, these oxide-hydroxides of Fe and Mn strongly co-precipitates with other metals<sup>[18]</sup>, and the formation of iron plaques on root surfaces is common in the mangrove environment<sup>[1]</sup>. Higher content of iron in the roots could be possibly due to the oxidant geochemical reaction occurring in the mangrove soil.

According to Braune *et al.*<sup>[19]</sup>, Pb accumulates in plants primarily from the atmosphere. The concentrations of Pb among five plant parts reported in this study were higher than the normal range of Pb concentration (5.0–10.0 mg/g) of plant materials reported by Alloway<sup>[20]</sup>, and Bodin *N et al.*<sup>[21]</sup>, which showed obvious signs of environmental contamination. Similarly, mangrove plants are known to accumulate considerable amount of heavy metal in roots and leaves<sup>[22]</sup>. Also, vegetables grown near toxic waste dump sites are reportedly to contain high concentrations of heavy metals including Pb<sup>[23]</sup>.

Fluctuation in climatic condition (rainfall and temperature), soil edaphic factors, available nutrients in the substrate and concentration of other nutrients can affect the nutrients concentration considerably in plant components as well as mineral metabolism and uptake of nutrients by the roots<sup>[14,24–26]</sup>. The fluctuation in environmental parameters and soil physiochemical characteristics may affect the uptake of Al, B, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn in different plant parts of *A. marina* as well sediment. Moreover, metal content in plant components may be influenced by its physical and chemical characteristics. Plant species, types of plant components, physiological age of tissue and seasons also influences in metal accumulation<sup>[24]</sup>.

In this study, all the heavy metals showed variations in relation to metals, sampling sites and plant parts of *A. marina*. This finds support of previous reports. The seedlings of *Rhizophora mucronata* are reported to show high accumulation of copper and zinc in the roots followed by leaves and stems in Sepang Lukut mangrove forest, Malaysia<sup>[8,9]</sup>. The seedlings of *Rhizophora stylosa* at Shankou Mangrove Reserve are reportedly accumulating higher copper and zince in stems followed by roots and leaves<sup>[27]</sup>. According to Kabata and Pendias<sup>[14]</sup>, and MacFarlane GR and Burchett MD<sup>[27]</sup>, the rate of nutrients uptake by plants is positively related with the nutrients level in soil. Similarly, Al, B, Cd, Co, Cr, Cu, Fe, Mg,

Mn, Ni, Pb, and Zn in different components of *A. marina* showed positive similarity with the soil nutrient levels. Thomas G and Fernandez TV<sup>[28]</sup> have observed a positive correlation between Fe and Cu concentrations in leaves of *Avicennia officinalis* and their respective concentration in soil. On the contrary, Cu, Fe and Zn concentrations in leaves of *Acanthus ilicifolius*, *Bruguiera gymnorhiza*, *Sonneratia caseolaris* and *Bruguiera racemosa* show a negative correlation with their respective concentrations in soil<sup>[29]</sup>. In another study, Srivastava *et al.*<sup>[30]</sup>, fail to obtain a significant correlation between Fe in leaves of *Rhizophora apiculata* and soil. However, the studies of Srivastava *et al.*<sup>[30]</sup>, and Thomas and Fernandez<sup>[28]</sup>, have performed only one time sampling and they do not consider the seasonal fluctuation of Cu, Fe and Zn in soil and plant components. The present investigation found that the BAF for metals was recorded high in plant parts of mangroves growing in polluted area of Cuddalore and this might be because of industrail discharges that are finding way in to the Uppanar estuary. In general, the mangrove habitat, especially of *A. marina* was found efficient to act as a sink for the heavy metals and it is suggested that a massive planting of the polluted estuarine coastal environs with of the mangrove *A. marina* would help to reduce the heavy metal toxicity.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### Comments

#### Background

This paper systematically analyses the metal concentration accumulated by mangrove plant *A. marina* present in Pichavaram mangrove forest. Author conferred the results for analyzing the growth and pollution status of mangroves. Very few studies have been reported in this regard, which is almost a need of the hour.

#### Research frontiers

This work's main conclusion is that the ratio between essential and non-essential elements was found higher in young mangrove forest than that in mature mangrove forest and polluted mangrove areas. That makes a lot more hypothesis to do research on it.

#### Related reports

The information provided in this manuscript is

interesting; the results accurately reflect the stated objectives and flow from the methods. The introduction and discussion seem informative with apt literature, which are absolutely supports the manuscript.

#### Innovations and breakthroughs

Analyzing the metals in the complete plants parts makes more sense than other reported work.

#### Applications

As the author suggested, the work could be used as an index of the growth and pollution status of mangroves.

#### Peer review

The paper is short, clear and to the point. The title and abstract correspond to the content of the manuscript. Finally, it is a sound and well-written manuscript can be published.

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