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HEAVY METAL ACCUMULATION AS PHYTOREMEDICATION POTENTIAL OF AQUATIC MACROPHYTE, MONOCHORIA VAGINALIS (BURM.F.)K.PRESL EX KUNTH

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Abstract
Bioaccumulation potential of six ecotypes, collected from six different industrial zones of lower Indo-Gangetic basin of West Bengal, India, of Monochoria vaginalis, commonly known as oval-leafed pondweed has been investigated based on chromium (Cr), cadmium (Cd) and Copper (Cu) accumulation pattern in different plant organs. Bioaccumulation potential was assessed by bioaccumulation factors (BFs-leaves metal concentration/soil metal concentration), bioconcentration factors (BCFs- roots metal/soil metal), transfer factors (TFs-leaves + rhizomes/roots) and enrichment factors (EFs-metals in edible parts/soil metal). Accumulation pattern significantly differed among ecotypes, and accumulation in plant organs was highly metal-specific. BFs for Cr and Cd were >>1 in most of the ecotypes while high TFs (>>1) were noticed in six ecotypes for Cr and Cu. BCFs was >>1 in all the ecotypes for Cd accumulation only. EFs values for the three metals hovered around 1 but it was > 1.0 for Cu in all the six ecotypes. The results suggested that Cr and Cu predominantly accumulated in leaves and rhizomes while Cd was predominantly sequestered in roots of M. vaginalis ecotypes. Cu, a redox active metal, showed higher capability than Cd and Cr to accumulate in edible parts. In the present study, potential plant parts in M. vaginalis have been identified as bioaccumulation organs without any apparent symptoms of toxicity which can be used as phytoremediation of heavy metal contamination in aquatic ecosystems of lower Indo-Gangetic basin of India.

Key words: Heavy metal; Bioaccumulation potential; Gangetic basin; Monochoria vaginalis

Introduction
Monochoria vaginalis, commonly known as pickerel-weed and belonging to the angiosperm family Pontederiaceae, is an aquatic herbaceous plant with its native range in temperate and tropical Asia. The plant has a short rhizome with dimorphic leaves, few to numerous blue flowers arranged in a raceme and capsule fruits, and has been enlisted as an invasive weed in rice fields (Juraimi et al., 2012; Bhuyan et al., 2014; Buragohain and Yasmin, 2014). Phytochemical work of the alcoholic extract of roots showed the presence of glycosides, flavonoids and tannins (Gupta et al., 2008) and essential amino acids and minerals in leaves and flowers (Chandran and Parimelazhagan, 2012). The plant is medicinally useful as edible vegetables, antioxidant and analgesic while root stock and leaves have cooling, aromatic, alterant and diuretic properties. The leaf juice is used to treat cough and fever and that of roots isused to treat stomach and liver problems, boil, hepatitis, gastrosis, bronchosis, asthma, toothache and haemorrhages(Chandran and Parimelazhagan, 2012).

Heavy metal pollution originating from increased industrialization and urbanization and its contamination in aquatic ecosystems due to discharge of industrial effluents pose a serious threat to human health through contaminated water and water-soil-plant chain (Ozdilek et al., 2007; Roy et al., 2010). In recent years, metal/metalloid contamination in major crop plants like cereals, legumes and vegetables has become a major ecological and economic problem throughout the world (Rai, 2008; Talukdar, 2012, 2013a, d; Talukdar and Talukdar, 2013) and water pollution by metal (loids) have been identified the major threat to ecosystems in developing and underdeveloped countries (Shrestha et al., 2014). Metal polluted aquatic ecosystems can decrease the quality of water, aquatic biodiversity and its associated terrestrial species, and can cause many species extinct. Instead of using costly and conventional physical and chemical technologies which may also have adverse effects on aquatic ecosystems, phytoremediation of metals by aquatic macrophytes and bacteria is economical and eco-friendly technology. This is based on the use of specially selected metal-accumulating plants to remove toxic metals from soils and water. Wetland plants are important tools for heavy metal removal (Rai, 2008; Mukherjee et al., 2014).
Metal uptake by plant has three patterns: a) True exclusion in which metals are restricted from entering the plant, b) Shoot exclusion in which metals are accumulated in the root but translocation to the shoot is restricted, and c) Accumulation where metals are concentrated in the plant parts. Primary evidence pointed out that Monochoria vaginalis can accumulate substantial amount of lead (Pb), arsenic (As), and cadmium (Cd) in its plant parts (Liu et al., 2007; Mahmud et al., 2008; Hariyadi et al., 2013).

Hyperaccumulators can uptake, translocate and tolerate high levels of certain heavy metals that would be toxic to most other organisms. Plants whose leaves may contain >100 mg kg⁻¹ of Cd and Cr, and >1000 mg kg⁻²of Cu when grown in metal rich medium are considered as hyperaccumulators for these metals. Soils and water of lower Indo-Gangetic basin is heavily contaminated with different types of heavy metals/metalloid but the potential of Monochoria vaginalis in accumulation and tolerance of metals has been poorly understood in the region. The plant is widely found in the region as aquatic, semi-aquatic and marshy plant and grows well in heavy-contaminated industrial belts. Six ecotypes of Monochoria vaginalis have been collected from six heavy polluted industrial areas of West Bengal, India and their bioaccumulation as well as phytoremediation potential was assessed in the present investigation.

Materials and Methods

Plant materials and soil samples

Plant parts (leaves, rhizomes, roots and fruits) of six ecotypes (Accession 1-6) were collected from six different industrial belts of Gangetic West Bengal, India, namely as Acc-1 (Chakdaha, Nadia), Acc-2 (Kalyani, Nadia), Acc-3 (Khardaha, North 24 pgs), Acc-4 (Ichhapur, North 24 pgs), Acc-5 (Rishra, Hooghly), and Acc-6 (Kolkata). These sites are located within heavy-polluted Hooghly Industrial belt of Gangetic West Bengal (Biswas et al., 2015), and thus were selected for the present study. Voucher specimens were identified, authenticated, tagged and deposited in herbaria of Department of Botany and has been digitized in departmental on-line phyto-informatics data bases (http://www.rpmcdigital phytoinformatics.com/). Soil samples from respective areas (S1 to S6) were also collected and analyzed. Four replicates for each of the soil and plant samples were maintained.

Analysis of heavy metals in plants and soil

The plant samples were washed thoroughly in heavy metal free water to remove adhered soils and dusts, then rinsed in de-ionized water, immersed for 10 min in 10×10⁻³ M KH₂PO₄solution (pH 6.0) to remove external metal contaminants from the root surface and blotted dry. The washing of the plant samples were finished as fast as possible to avoid any possible leakage of absorbed metals.
Statistical analysis
The results presented here are the mean values ± standard errors (SE) of at least four replicates. Multiple comparisons of means were performed by ANOVA (SPSS Inc. v. 10), and the means were separated by Duncan’s Multiple Range Test considering significant differences at $P < 0.05$.

Result and Discussions

Cr accumulation in plant parts
Significant ($P < 0.05$) differences were observed between ecotypes regarding transport and bioaccumulation of Cr in different plant parts of *Monochoria vaginalis*. Considering all the six ecotypes, Cr accumulated in the highest amount in leaves, followed by rhizome, fruits and then roots. The leaves of Acc-5 the highest concentration of Cr, and it was distantly followed by Acc-4, Acc-3 and Acc-6 (Table 1). In rhizomes, highest accumulation was estimated in Acc-3, and it was followed by Acc-2, Acc-5, Acc-6, Acc-1 and Acc-4 (Table 1). In roots, highest Cr level was estimated in Acc-1, followed by Acc-3, Acc-5 and Acc-6. Lowest concentration was recorded in Acc-4 (Table 1). Root Cr level in Acc-2 was very close to Acc-6 and did not change significantly with Acc-5, also. Among the ecotypes, Cr accumulation in fruits was the highest in Acc-3 and the lowest in Acc-6 (Table 1). Rest of the ecotypes contained Cr level in between Acc-2 and Acc-1 (Table 1). Barring Acc-1, BFIs were $> 1.0$ in rest of the five ecotypes with highest magnitude ($>3.0$) in Acc-4 and Acc-5, indicating significant bioaccumulation potential of Cr in photosynthetic parts of five ecotypes (Table 2). Contrastingly, BCFs value in all the six ecotypes with $< 1.0$ suggested little capability of roots to retain the Cr which was promptly transferred to leaves and rhizomes. This fact was further substantiated by high TFs ($>1.0$) of Cr in all the six ecotypes specially, Acc-4 and Acc-5 in which TFs values hovered between 20.0 and 30.0 (Table 2). Accumulation of Cr in edible parts was screened by EFs which was $>1.0$ in Acc-2 and Acc-3 but was $<1.0$ in rest four ecotypes. The results strongly indicated significant ecotype differences in accumulation pattern of Cr in different plant organs. Whereas roots were least capable to accumulate Cr, leaves and rhizomes were major storage organs of Cr. *Monochoria* leaves and rhizomes have been used as vegetables in many countries (Chandran and Parimelazhagan, 2012). However, High EFs in the present study indicated non-suitability of edible parts to be taken as vegetables grown in Cr-contaminated soil/water. Conflicting reports are available in Cr accumulation pattern in different parts of plants. Cr, the 17th most abundant element in the Earth’s mantle and a non-essential element in plant growth, is reportedly accumulate predominantly in roots, followed by stems, leaves and roots in legumes and vegetables (Panda and Choudhury, 2005; Deyet et al., 2009; Gopal et al., 2009) while higher root accumulation has been reported in *Lolium perenne* (Verney, 2007). Among aquatic plants, Cr accumulation has been reported in *Salvinia minima*, *Vallisneria spiralis*, *Hydrilla verticillata*, *Pistia*, *Eichhornia*, and *Lemma* (Mishra et al., 2009; Gupta et al., 2010; Prado et al., 2010). In the present study, leaves of Acc-3, Acc-4, Acc-5 and Acc-6 accumulated $Cr > 100$ mg kg$^{-1}$, and therefore, primarily screened as Cr-hyperaccumulator.

Cd accumulation in plant parts
Cd accumulation was significantly ($P < 0.05$) higher in roots of the ecotypes than the other plant parts. Lowest accumulation was noticed in fruits. Cd accumulation in roots was the highest in Acc-5 which did not change significantly in Acc-4 but significantly higher than Acc-1, Acc-2 and Acc-3. Lowest level was observed in Acc-6 (Table 1). In the leaves, highest Cd accumulation was recorded in Acc-5 (and the value did not change significantly ($P > 0.05$) in Acc-4. In comparison, Cd accumulation in roots was significantly lower in rest of the four ecotypes with lowest accumulation was recorded in Acc-2 (10.39 mg kg$^{-1}$) and the values did not change significantly in Acc-1, Acc-3 and Acc-6 (Table 1). On the other hand, significant differences were observed among the ecotypes in Cd accumulation in rhizomes. Cd accumulation in rhizome was the highest in Acc-1 and it was distantly followed by Acc-5, Acc-4, Acc-6, Acc-3 and Acc-2 (Table 1). Cd concentration in fruits was the highest in Acc-1 but the lowest in Acc-4 and varied significantly among the six ecotypes (Table 1). BFIs were $>1.0$ in Acc-1, Acc-2, Acc-3 and Acc-6 and crossed 3.0 mark in Acc-4 and Acc-5 while BCFs were $>1.0$ in Acc-1, Acc-2, Acc-3 and Acc-6 but considerably enhanced ($>8.0$) in Acc-4 and crossed 10.0 mark in Acc-5 (Table 2). The results pointed out major sequestration of soil Cd in roots and leaves of *Monochoria* ecotypes which prevented Cd to be further uploaded in edible parts. The fact was supported by the $<1.0$ TFs as well as EFs in all the six ecotypes (Table 2). The EFs $=1.0$ in Acc-5 was due to significantly higher accumulation of Cd in its rhizomatous part and also in fruits compared to other ecotypes (Table 1, 2). Cd is a non-essential non-redox-active metal element for plant metabolism even though it is rapidly taken up by plant roots and can be loaded into the xylem for its transport into the leaves (Mobin and Khan, 2007; Talukdar, 2014b). The ecotypes differed significantly ($P< 0.05$) in all the four bioaccumulation factors considered here (Table 2). The toxicity of Cd accumulation is manifested in Cd-sensitive plants by poor growth, leaf injury and low biomass accumulation through interference in several metabolic processes (Ortega-Villasante et al., 2005; Hasan et al., 2009; Siddiqui et al., 2012; Talukdar, 2014b). No external injury was visible in any of the ecotypes of present *Monochoria* sp, indicating apparent tolerance and phytoremediation potential of *Monochoria vaginalis* in Cd-contaminated soils and water.
Table 1: Chromium (Cr), cadmium (Cd) and copper (Cu) concentrations (mg kg⁻¹) in six ecotypes of Monochoria vaginalis

| Sample | Cr  | Cd   | Cu       |
|--------|-----|------|----------|
| S1     | 79.32 ± 0.21a | 9.48 ± 0.17a | 30.86 ± 0.14a |
| S2     | 48.28 ± 0.17c | 6.95 ± 0.09c | 27.31 ± 0.12a |
| S3     | 64.05 ± 0.26b | 8.61 ± 0.07b | 27.68 ± 0.13a |
| S4     | 56.07 ± 0.19c | 6.50 ± 0.06c | 23.51 ± 0.10a |
| S5     | 50.67 ± 0.20c | 5.43 ± 0.02d | 25.96 ± 0.13a |
| S6     | 62.34 ± 0.22b | 9.63 ± 0.06a | 22.92 ± 0.18a |

Leaves
- Acc-1 74.74 ± 0.18e 11.34 ± 0.06b 13.40 ± 0.10b
- Acc-2 82.90 ± 0.21d 10.39 ± 0.05b 11.68 ± 0.03c
- Acc-3 108.19 ± 0.24c 11.25 ± 0.04b 19.07 ± 0.08b
- Acc-4 169.23 ± 0.27b 23.85 ± 0.11a 35.27 ± 0.10a
- Acc-5 205.56 ± 0.31a 25.28 ± 0.12a 47.64 ± 0.16a
- Acc-6 104.93 ± 0.22c 11.93 ± 0.07b 14.12 ± 0.08c

Rhizome
- Acc-1 15.23 ± 0.09d 7.48 ± 0.07a 31.44 ± 0.12c
- Acc-2 42.70 ± 0.09b 0.39 ± 0.01e 43.22 ± 0.18b
- Acc-3 54.19 ± 0.12a 1.31 ± 0.04d 29.17 ± 0.11c
- Acc-4 9.89 ± 0.07e 3.18 ± 0.11c 41.19 ± 0.14b
- Acc-5 29.19 ± 0.13c 5.37 ± 0.12b 58.64 ± 0.22a
- Acc-6 17.22 ± 0.10d 1.93 ± 0.07d 28.12 ± 0.18c

Roots
- Acc-1 20.48 ± 0.16a 29.11 ± 0.07b 10.41 ± 0.09a
- Acc-2 8.19 ± 0.09b 32.21 ± 0.08b 3.51 ± 0.11c
- Acc-3 18.19 ± 0.13a 31.23 ± 0.14b 3.35 ± 0.07c
- Acc-4 5.81 ± 0.05c 53.85 ± 0.18a 6.18 ± 0.08b
- Acc-5 10.98 ± 0.08b 55.28 ± 0.19a 0.37 ± 0.02e
- Acc-6 8.88 ± 0.27b 19.26 ± 0.07c 1.17 ± 0.07d

Fruits
- Acc-1 11.23 ± 0.07a 0.48 ± 0.01a 0.33 ± 0.01c
- Acc-2 9.37 ± 0.03b 0.41 ± 0.01a 0.67 ± 0.07a
- Acc-3 14.19 ± 0.12a 0.29 ± 0.04b 0.19 ± 0.04d
- Acc-4 9.91 ± 0.07b 0.18 ± 0.01c 0.22 ± 0.05d
- Acc-5 13.10 ± 0.03a 0.37 ± 0.02a 0.43 ± 0.04b
- Acc-6 7.22 ± 0.10b 0.21 ± 0.07e 0.38 ± 0.09b

Data are means ± SE of four replicates. Means followed by different lower case letters in each column indicate significant differences by ANOVA followed by Duncan’s Multiple Range Test at P < 0.05

Table 2: Bioaccumulation factors (BFs), bioconcentration factors-BCFs, transfer factors-TFs, and enrichment factors –EFs in six ecotypes of Monochoria vaginalis

| Traits | Acc-1 | Acc-2 | Acc-3 | Acc-4 | Acc-5 | Acc-6 |
|--------|-------|-------|-------|-------|-------|-------|
| Cr     | 0.94 ± 0.03d | 1.72 ± 0.06c | 1.69 ± 0.08c | 3.02 ± 0.10b | 4.06 ± 0.15a | 1.68 ± 0.05c |
| BCFs   | 0.26 ± 0.01a | 0.17 ± 0.01b | 0.28 ± 0.02a | 0.10 ± 0.01c | 0.22 ± 0.01a | 0.14 ± 0.01b |
| TFs    | 4.40 ± 0.17c | 15.33 ± 0.29c | 8.93 ± 0.15d | 30.83 ± 0.38a | 21.38 ± 0.20b | 13.80 ± 0.23c |
| EFs    | 0.33 ± 0.02c | 1.08 ± 0.61a | 1.07 ± 0.81a | 0.35 ± 0.09c | 0.83 ± 0.07b | 0.39 ± 0.04c |
| Cd     | 1.20 ± 0.02d | 1.49 ± 0.02c | 1.31 ± 0.01d | 3.68 ± 0.09b | 4.66 ± 0.18a | 1.24 ± 0.02d |
| BCFs   | 3.07 ± 0.10c | 4.63 ± 0.18b | 3.63 ± 0.11c | 8.30 ± 0.18a | 10.18 ± 0.20a | 2.00 ± 0.04d |
| TFs    | 0.65 ± 0.09a | 0.33 ± 0.03c | 0.40 ± 0.04c | 0.50 ± 0.06b | 0.55 ± 0.07b | 0.72 ± 0.17a |
| EFs    | 0.84 ± 0.07b | 0.12 ± 0.03e | 0.20 ± 0.02d | 0.52 ± 0.07c | 1.06 ± 0.09a | 0.22 ± 0.02d |
| Cu     | 0.43 ± 0.01c | 0.43 ± 0.01c | 0.70 ± 0.04b | 1.50 ± 0.07a | 1.84 ± 0.04a | 0.62 ± 0.01b |
| BCFs   | 0.34 ± 0.01a | 0.13 ± 0.01b | 0.12 ± 0.01b | 0.27 ± 0.02a | 0.01 ± 0.00c | 0.05 ± 0.00c |
| TFs    | 4.31 ± 0.16d | 15.64 ± 0.29c | 14.40 ± 0.17c | 12.37 ± 0.21c | 287.24 ± 0.78a | 36.10 ± 0.33b |
| EFs    | 1.03 ± 0.08c | 1.61 ± 0.06b | 1.06 ± 0.06c | 1.76 ± 0.07b | 2.28 ± 0.10a | 1.24 ± 0.06c |

Data are means ± SE of four replicates. Means followed by different lower case letters indicate significant differences by ANOVA followed by Duncan’s Multiple Range Test at P < 0.05

Pattern of Cu accumulation in plant parts
Cu accumulated in highest amount in rhizomes, followed by leaves, roots and fruits and significantly varied among ecotypes (Table 1). Cu accumulation in rhizomes was the highest in Acc-5 and it differed significantly with accumulation in rest of the ecotypes. Acc-2 and Acc-4 both accumulated significantly lower Cu accumulation than Acc-5 but in significantly higher amount than Acc-1, Acc-3 and Acc-6 (Table 1). In the leaves of six ecotypes, Cu level was the maximum in Acc-5 but was minimum in Acc-2 (Table 1). Intermediate level was found in rest of the ecotypes but
they differed significantly with each other in leaf Cu level. Highest Cu accumulation in roots was recorded in Acc-1, and it was distantly followed by Acc-4 (Table 1). Significantly lower Cu level than Acc-4 was observed in Acc-2 and Acc-3, and the lowest value was measured in Acc-5. In fruits, Cu level varied between Acc-2 and Acc-3 and the ecotypes differed significantly for Cu level of fruits (Table 1). The BFVs were > 1.0 in Acc-4 and Acc-5 but plummeted below 1.0 in rest of the ecotypes. BCFs, in contrast, were <1.0 in all the six ecotypes with extremely low accumulation factor value (0.01-0.05) in Acc-5 and Acc-6 (Table 2). The TFs varied greatly among ecotypes, ranging between 4.31 (Acc-1) and 287.24 (Acc-5) while EFs > 1.0 in all the six ecotypes. The results pointed out incapability of roots to retain soil Cu which was promptly transferred to photosynthetic as well as to edible parts. Significantly enough, Acc-4 and Acc-5 stored substantial amount of Cu not only in their leaves but also in rhizomes and fruits. In other ecotypes, major Cu accumulation occurred in rhizomes, instead of leaves, resulting in lowering of BFVs but incited high TFs and EFs in Acc-1, Acc-2, Acc-3 and Acc-6 (Table 2). The results also suggested strong differences among ecotypes in Cu accumulation pattern in their organs. Cu is a redox active essential metal in plant growth but its high bioavailability causes major upset in redox homeostasis as accumulation in plant parts, as observed in Canna indica plant, a major partner plant of Monochoria in contaminated soil and water (Talukdar, 2013b), and in other aquatic macrophytes and crop plants (Umebese and Motajo, 2008; Kousar and Puttaiah, 2009; Kumar et al., 2012; Talukdar, 2014c). Absence of any visible injury of chlorosis and/or necrosis in any plant organ suggested tolerance and possible phytoremediation and pollution biomonitoring potential of Monochoria but at the same time toxicity test is necessary to introduce M. vaginalis as a potential food supplement in future.

In the present study, bioaccumulation potential of six ecotypes in Monochoria vaginalis has been tested based on Cr, Cd and Cu accumulation pattern in different plant organs. Accumulation pattern greatly differed among ecotypes, primarily indicating genotypic roles in determining heavy metal accumulation by this aggressive aquatic/semi-aquatic macrophyte. Furthermore, it is evident from the present study that accumulation pattern in plant organs is greatly metal-specific. For example, Cr and Cu predominantly accumulated in aboveground parts and rhizomes while Cd was sequestered in roots. The ecological differences in any tropical plants often change over multiple spatial scales (Jayakumar and Nair, 2012; Joshi et al., 2015). The environment and its heterogeneity, and environment and genotypic interaction, like its spreading and tolerance level to different environmental factors are some of the factors influencing diversity of ecotypes (Aravind et al., 2010). In the present study, potential plant parts in M. vaginalis has been identified as bioaccumulation organ without any apparent symptoms of toxicity in lower Indo-Gangetic basin of India. However, to what extent these ecotypes can be used in phytoremediation process and how genotypes are determining factors in defining bioaccumulation potential, further study is needed to decipher it.

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