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**Phytofiltration of arsenic and cadmium from the water environment using**

***Micranthemum umbrosum* (J.F. Gmel) S.F. Blake as a hyperaccumulator**

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Running title: Removal of As and Cd by *M. umbrosum*

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## **Abstract**

*Arsenic (As) and cadmium (Cd) pollution in water is an important global issue. Phytofiltration is an eco-friendly technology that helps clean up pollutants using ornamental plants, such as *Micranthemum umbrosum* (J.F. Gmel) S.F. Blake. After a seven-day hydroponic experiment, *M. umbrosum* removed 79.3–89.5% As and 60–73.1% Cd from 0 to 1.0  $\mu\text{g As mL}^{-1}$  and 0.3 to 30.0  $\mu\text{g Cd mL}^{-1}$  solutions, respectively. For As treatment, root to stem and stem to leaf translocation factors greater than 1.0 indicated that accumulation of As in leaves was large compared to that in stem and roots. However, the accumulation of Cd in roots was higher than that in the leaves and stem. In addition, *M. umbrosum* completely removed Cd within three days from 0.38 to around 0  $\mu\text{g mL}^{-1}$  Cd in the solution when the plant was exchanged daily. Bio-concentration factors (2350 for As and 3027 for Cd) for *M. umbrosum* were higher than for other As and Cd phytoremediators. The results show that *M. umbrosum* can be an effective accumulator of Cd and a hyper-accumulator of As, as it can lower As toxicity to a level close to the limit recommended by the World Health Organization (0.01  $\mu\text{g As mL}^{-1}$ ).*

**Key Words:** heavy metals, bio-concentration factor, water environments

## INTRODUCTION

Arsenic (As) and cadmium (Cd) are the most toxic and carcinogenic substances among all of the possible xenobiotics (USEPA-IRIS, 2010), occurring naturally or as a result of anthropogenic influences, and pose a serious threat to environmental and human health worldwide. Contamination in drinking water has been recognized as a serious global problem. For example, As threatened the health of more than 80 million people in Bangladesh (Smith et al. 2000) and West Bengal, India (Nordstrom 2002). Studies have shown that As also enters the food chain via crop uptake from soils contaminated by As-contaminated irrigation water or mining activities (Williams et al. 2006; Zhu et al. 2008). In Bangladesh, concentrations of As in well water were found to be high, ranging from less than  $1 \mu\text{g L}^{-1}$  to more than  $300 \mu\text{g L}^{-1}$  (Smith et al. 2000), whereas the As standard for drinking water is  $10 \mu\text{g L}^{-1}$  (WHO 2011). The International Program on Chemical Safety (IPCS 2001) reported that long-term exposure to As in drinking water increased the risk of cancer in the skin, lung, bladder and kidney, as well as other skin changes such as hyperkeratosis and changes of dermal pigmentation.

Cd and its compounds are used in the steel industry, in plastic and batteries, and are released to the environment through disposal of mining or industrial effluents, wastewater and often from fertilizers. It causes kidney damage, osteomalacia, osteoporosis, and itai itai disease, and has carcinogenic effects (WHO 2011). Langner et al. (2012) that Cd concentration of sediments in the Upper Clark Fork River, Montana, USA showed 4.4 (range

0.6–6.9) mg/kg. In addition, Marine black shale's and slates have frequently been found to contain anomalously high concentrations of Cd (<240 mg/kg) (OECD, 1994). In potable water in Saudi Arabia, 1–26  $\mu\text{g L}^{-1}$  Cd was reported (Mustafa et al. 1988) and the maximum value recorded was 100  $\mu\text{g L}^{-1}$  in the Rio Rimao in Peru (WHO/UNEP 1989), whereas the maximum Cd tolerance level in water is only 3  $\mu\text{g L}^{-1}$  (WHO 2011). As and Cd are classified as Group 1 and Group 2A carcinogenic compounds to humans, respectively (IARC 1987). Therefore, remediation of As and Cd from water and soil is an important global issue. Among various technologies such as precipitation, membrane filtration, adsorption, ion exchange, permeable reactive barriers, biological treatment and phytoremediation (Rahman et al. 2011), phytofiltration is a type of phytoremediation and is an emerging, eco-friendly technology in which green plants are used to remediate or remove metals from contaminated water (Dushenkov et al. 2000). Several studies have focused on phytoremediation of heavy metals from water and soil; however, few plants showed the ability to translocate high amounts of As from root to shoot (Rahman et al. 2011). Raab et al. (2007) studied 46 different plant species in terms of As accumulation, and found that translocation factors of these plants never exceeded 0.9 for As(V). The fact that their translocation factors were less than 1 indicated partial immobilization of As in their roots and a low conveyance of As to the shoots. This immobilization reduces the phytoavailability of contaminants from the environment (Vamerali et al. 2010). *Pteris vittata* L. has shown the highest ability to accumulate and

translocate As from root to shoot (Ma et al. 2001). *Spirodela polyrhiza* L. (Rahman et al. 2007), *Lemna gibba* L. (Mkandawire and Dudel 2005), *Polygonum hydropiper* L. (Robinson et al. 2005), and *Azolla caroliniana* L. (Zhang et al. 2008) were also identified as As accumulators. In addition, *Nymphaea aurora* L. (Schor-fumbarov et al. 2003), *Solanum nigrum* L. (Sun et al. 2007), *Thlaspi caerulescens* J.&C. Presl. (Zhao et al. 2003), and *Arabidopsis halleri* L. (Küpper et al. 2000) were recognized as Cd accumulators. However, these plants have low bio-concentration factors and low root to shoot translocation factors. This indicates the difficulty in employing these plants for As and Cd phytoremediation at a field scale. Therefore, it is necessary to identify plants having high bio-concentration factors and translocation factors (i.e., greater than 1) that can remove As and Cd from contaminated drinking water to levels below the tolerable limit.

*Micranthemum umbrosum* (J.F. Gmel) S.F. Blake, commonly known as Water fern, Baby's tears, or Pearl grass, belongs to the family Linderniaceae, and it is widely used as an aquarium ornamental plant. In this study, this plant was employed to remediate As and Cd contaminated water for the following reasons: i) the whole plant can be easily removed from a water environment; ii) its growth rate is high and relatively vigorous; iii) it grows under submerged conditions; iv) its light requirement for growth is moderate; and v) It can be used as ornamentation for room in addition to accumulation of As and Cd from water as it is popular as an aquarium plant. There is currently no data regarding phyoremediation of As and

Cd using *M. umbrosum*. In this study, to understand whether *M. umbrosum* would be a good candidate for phytoremediation, metal accumulation pattern in *M. umbrosum* grown in water including As or Cd was investigated. In addition, As and Cd bio-concentration factors and translocation factors of *M. umbrosum* were determined.

## **MATERIALS AND METHODS**

### **Plant and Culture Conditions**

*M. umbrosum* (J.F. Gmel) S.F. Blake, was obtained from Aqua Friend Hokusui (Hokkaido, Japan). Initially, the plants were acclimated for 1 week in water containing plant nutrients (contains some essential trace elements and potassium, Aqua Design Amano, Nigata, Japan) under laboratory conditions, to allow for adaptation prior to the experiment. Then, *M. umbrosum* was grown in glass pots (volume: 765.45 cm<sup>3</sup>) in Milli-Q water (Millipore-Gradient A10, Milli-Q Gradient ZMQG) containing 0.2, 0.45, and 1.0 µg L<sup>-1</sup> As (as NaAsO<sub>2</sub>), or 0.3, 3.0, and 30.0 µg L<sup>-1</sup> Cd solutions (as CdCl<sub>2</sub>.5H<sub>2</sub>O). The exposure experiments were carried out for 7 days under the following conditions: 14:10 h light/dark cycle, 100–125 µmol m<sup>-2</sup> s<sup>-1</sup> light intensity, and 75% humidity at 21±1°C. The pH value of the solutions was maintained at 6.8. After every 24 h, water samples were collected from each pot to measure the As and Cd concentrations in the water. Each experiment was performed thrice.

Milli-Q water containing adequate concentrations of As and Cd was added daily to compensate for the water loss due to plant transpiration and evaporation. After 7 days, the plants were harvested, rinsed four times with Milli-Q water, and then placed on clean absorbent paper for water removal from the plant surface. The plants were then separated into root, stem, and leaf for measurement of the metal accumulation, bio-concentration factors, and translocation factor in each component.

### **Metal Analysis**

After harvesting the plants, the whole plants was washed by milliQ water for three times then the roots, stem and leaves were separated and placed on paper and air dried on the plastic table under room temperature for 24 h. The As treated samples were dried for 48 h in an oven at 65°C (Constant Temperature Oven, DKN602, Yamato Scientific Co. Ltd., Japan) until they reached a constant weight. Dried samples were weighed on a digital balance (A&D Co. Ltd, Japan, HF-200, Max 210 g, d = 0.001 g). After cutting the samples, 20–40 mg samples of root, stem, or leaf were separately placed into 15-mL polyethylene tubes (Thermo Fisher Scientific, NY). Two mL of 65% HNO<sub>3</sub> (Wako Pure Chemical Ind. Ltd., Japan) was added, and the samples were kept under the fume hood for 12 h. Then, the samples were heated on a heating block (TAH-2G, Dry Thermo Unit, Japan) using lids at 95°C for 2 h to digest. After cooling, 1 mL of 30% H<sub>2</sub>O<sub>2</sub> (Wako Pure Chemical Ind. Ltd., Japan) was added,

and the samples were heated again at 105°C for 20 min (Rahman et al. 2007). Digested samples were diluted up to 10 mL with Milli-Q water using 10-mL volumetric flasks (Pyrex, IWAKI Glass), as described by Cai et al. (2000) and Rahman et al. (2007). To digest the wet-weighted Cd-treated samples, they were treated with 2 mL of 68% HNO<sub>3</sub> (Wako Pure Chemical Ind. Ltd., Japan), and subsequently heated at 110°C for 2 h. The digested samples were diluted up to 10 mL with Milli-Q water using 10-mL volumetric flasks. Both sets of diluted samples were then filtered using a 0.45-µm syringe-driven filter unit (Millipore, Billerica, USA) and stored in 15-mL polyethylene bottles. The As and Cd contents were measured using an inductively coupled plasma-mass spectrophotometer (ICP-MS; SPQ 6500, Plasma Quadrupole Mass Analyser, SII-Seiko Instrument, Japan) and a flame-type atomic absorption spectrophotometer (AAS; model 180-80, Hitachi, Japan), respectively. The accuracy of the analysis was checked by the use of certified standard reference material for As (013-15481, Lot ALK 9912, 1000 ppm) and Cd (036-16171, Lot TSP9842, 1000 ppm) obtained from Wako Pure Chemical Ind. Ltd., Japan. The results were expressed as µg g<sup>-1</sup> dry weight for As and µg g<sup>-1</sup> wet weight for Cd in root, stem, and leaf.

### **Bio-concentration Factor (BCF)**

The BCF was determined as an index of the plant's ability to accumulate a metal with respect to the metal concentration in the substrate. The BCF was calculated (L kg<sup>-1</sup>) as

follows (Snyder 2006):

$BCF = \text{As in the plant component (root, stem, or leaf) (mg kg}^{-1}\text{) / As in the substrate water (mg L}^{-1}\text{)}$

### **Translocation Factor (TF)**

The TF was calculated to determine the relative translocation of metals from the water to the various plant components (root, stem, and leaf) (Barman et al. 2000; Gupta et al. 2008).

$TF = \text{Concentration of As in plant tissue (root, stem, or leaf) / Concentration of As in corresponding water or root}$

### **Statistical Analysis**

The mean, standard deviation (SD) and standard error of mean (SEM) were calculated, and TTEST was performed to determine any significant differences among treatments at the 0.1%, 1%, and 5% levels using the Microsoft Excel-2007 program.

## **RESULTS AND DISCUSSION**

### **Phytofiltration of As from Water**

As concentration in the solution decreased with increasing time, and *M. umbrosum* significantly removed (when compared to the previous day) As up to the third, sixth, and

fourth day from the 0.2, 0.45, and 1  $\mu\text{g As mL}^{-1}$  solutions, respectively (Figure 1). For the 0.2 and 0.45  $\mu\text{g mL}^{-1}$  As solutions, the water contained only 0.041 (Figure 1a) and 0.047  $\mu\text{g mL}^{-1}$  As (Figure 1b), respectively, after seven days of growing *M. umbrosum*. However, an As concentration of 0.207  $\mu\text{g mL}^{-1}$  was observed in the water when the initial As concentration was 1.006  $\mu\text{g mL}^{-1}$  (Figure 1c). In addition, As concentration remained constant in the control treatment without plants (data not shown). Therefore, at lower initial concentrations (0.2 and 0.45  $\mu\text{g As mL}^{-1}$ ), *M. umbrosum* removed As from the water to achieve a final concentration below the maximum level (0.05  $\mu\text{g mL}^{-1}$ ) prescribed by the Bangladesh Government (World Bank 2005). As listed in Table 1, the plant removed As from the water solution to differing extents as the As concentration was increased (80.5, 89.6, and 79.3% As were removed from water containing 0.2, 0.45, and 1.0  $\mu\text{g As mL}^{-1}$ , respectively). This tendency might be due to As inhibiting growth of the plant at a concentration of 1  $\mu\text{g As mL}^{-1}$  since at 1.8  $\mu\text{g mL}^{-1}$  As, the plant died (data not shown). Growth of *Wolffia globosa* was also significantly inhibited ( $P < 0.001$ ) by arsenate at more than 30  $\mu\text{M}$  concentration and by arsenite at more than 10  $\mu\text{M}$  concentration, and it decreased total As concentration in the solution from 200 to 116  $\mu\text{g L}^{-1}$  within 48 h (Zhang et al. 2009).

### **As Accumulation in Plant Material**

As accumulation patterns in the root, stem, and leaf of *M. umbrosum* 7 days after

incubation are shown in Figure 2. The leaf component took up significantly ( $P < 0.001$  and  $0.005$ ) higher amounts of As than the corresponding stem and root components. The As accretion patterns from contaminated water to root, root to stem, and stem to leaf showed high accumulation for each treatment (Figure 2). Leaf and stem contained  $1179.3 \pm 11.6$  and  $1001 \pm 16.5 \mu\text{g As g}^{-1}$  (dry wt. basis) at the  $1 \mu\text{g As L}^{-1}$  dose, whereas  $802 \pm 18.7$  and  $470 \pm 14.5 \mu\text{g As g}^{-1}$  was accumulated in leaves at the  $0.45$  and  $0.2 \mu\text{g As mL}^{-1}$  doses, respectively (Figure 2). These results are consistent with studies of Zhang et al. (2009) who reported that *Wolffia globosa* accumulated  $1057 \pm 61 \text{ mg As kg}^{-1}$  dry weight after 7 days growth in  $15\text{-}\mu\text{M As}$  solution. Rahman et al. (2007) also showed that *Spirodela polyrhiza* took up  $0.353 \pm 0.003 \mu\text{mol As g}^{-1}$  dry weight 6 days after exposure to  $4 \mu\text{M As}$ . However, compared to these previous studies, the plant used in this study took up much more As from the As-contaminated water. Therefore, *M. umbrosum* has a high potential for As remediation from contaminated drinking water.

### **Phytofiltration of Cd from Water**

Cd concentrations in the water were detected according to the time-dependent manner in which plants were grown (Figure 3). The Cd concentrations in the water gradually decreased day by day. The pattern of Cd decrease was similar across the  $0.3$ ,  $3$ , and  $30 \mu\text{g Cd mL}^{-1}$  treatments (Figure 3). The rate of Cd concentration decrease was observed to be largest on the

first day with a strongly significant difference observed in the 0.3- $\mu\text{g Cd mL}^{-1}$  treatment ( $P < 0.05$ ) (Figure 3a). The rate of decrease exponentially declined day by day. As shown in Figure 3, at 0.3, 3, and 30  $\mu\text{g Cd mL}^{-1}$  concentrations, *M. umbrosum* could not completely remove Cd from the solution. Therefore, the plants were replaced with new ones each day and Cd concentration in the water was measured. Under these conditions, it was observed that when initial Cd concentration in the water was 0.38  $\mu\text{g mL}^{-1}$ , Cd in the water was completely remediated after three days (data not shown). Abhilash et al. (2009) conducted an experiment using *Limnocharis flava L.* grown in 0.5, 1, 2, and 4  $\text{mg Cd L}^{-1}$  solutions, and found that after 30 days, more than 93% of the Cd was removed. However, here, *M. umbrosum* can remove around 100% of the Cd within 3 days by replacing the plants with new ones each day. When the plants were not replaced, 70.4, 73.1, and 60% Cd were removed after 7 days from the 0.3, 3.0, and 30  $\mu\text{g Cd mL}^{-1}$  solutions, respectively (Figure 3).

### **Cd Accumulation in the Plant**

Cd accumulation in the leaves, stem, and roots of *M. umbrosum* is shown in Figure 4. The amount of Cd accumulation in the plant components was in the following order: roots>leaves>stems. Cd accumulation in each component was significantly increased by the increase in Cd levels in the hydroponic solution (Figure 4). In the case of 30  $\mu\text{g Cd mL}^{-1}$  exposure, the Cd contents in the roots ( $13296.2 \pm 1962.6 \mu\text{g g}^{-1}$  wet weight) were higher than

those in the corresponding stems ( $3377.7 \pm 208.0 \mu\text{g g}^{-1}$  wet weight) and leaves ( $4491.4 \pm 300.3 \mu\text{g g}^{-1}$  wet weight). The accumulation of Cd in the various parts of aquatic macrophytes under laboratory conditions has been reported in several species of aquatic plants such as *Limnocharis flava* (Abhilash et al. 2009), *Ipomea aquatica* (Wang et al. 2008), *Potamogeton natans* (Fritioff and Greger 2006), *Lemna minor* (Hou et al. 2007), and *Elodea canadensis* (Fritioff and Greger 2007). Cd concentrations were reported to be higher in the roots in most of these studies. The high Cd concentrations in the roots of *M. umbrosum* were because of the numerous fibrous roots of this plant, as mentioned by Abhilash et al. (2009) for *L. flava*. Similarly, with 3 and  $0.3 \mu\text{g Cd mL}^{-1}$  exposure, Cd contents in the root were also slightly higher than those in the stem and leaf (Figure 4). However, in the case of treatment with different As concentrations, roots contained lower amounts of As as compared with stems and leaves. The reason for differing accumulation of Cd and As in the plant components is still unclear; a possible reason could be the usage of different uptake and translocation mechanisms for As and Cd (Schiorup and Larsen 1981).

### **BCF of As and Cd in *M. umbrosum***

BCF is defined as the ratio of metal concentration in the plant to the initial concentration of metal in the feed solutions. Higher values of BCF indicate the ability of plants to concentrate metals in their tissues (Abhilash et al. 2009). The BCF values for different

components (root, stem, and leaf) of *M. umbrosum* for As and Cd at different exposure levels were calculated (Tables 1 and 2). The highest BCF value was obtained after exposure to 0.2  $\mu\text{g As mL}^{-1}$  ( $2350\pm 72.3$  for leaf) and 0.3  $\mu\text{g Cd mL}^{-1}$  ( $3026.91\pm 1389.12$  for root), and the lowest BCF value was found for both As and Cd at the highest concentration treatment in the experiments (Tables 1 and 2). It was observed that the plant was a good accumulator of As and Cd if the water contained concentrations 50 times (up to  $500 \mu\text{g L}^{-1}$ ) and 100 times (up to  $300 \mu\text{g L}^{-1}$ ) the maximum levels of As ( $10 \mu\text{g L}^{-1}$ ) and Cd ( $3 \mu\text{g L}^{-1}$ ) recommended by the World Health Organization, respectively (WHO 2011). From the point of view of phytoremediation, a good accumulator has been defined as having the ability to concentrate the heavy metal in its tissues. In general, a plant with a BCF of more than 1000 is considered a hyperaccumulator. A plant with a BCF of 1 to less than 1000 is considered an accumulator, and with a BCF of less than 1 as an excluder (Zayed et al. 1998). In addition, A plant is defined to be a hyper-accumulator if it can concentrate the pollutants in any above ground tissue of dry weight; which varies according to the pollutant involved:  $>1000 \text{ mg/kg}$  for Ni, Cu, Co, Cr or Pb;  $>10,000 \text{ mg/kg}$  for Zn or Mn (Morel et al. 2006). In this study, as the BCF value of *M. umbrosum* was shown to be higher than 1000 in the leaf, stem, and root in the 0.2  $\mu\text{g As mL}^{-1}$  and 0.3  $\mu\text{g Cd mL}^{-1}$  treatments, and leaf and stem in the 0.45 and 1.0  $\mu\text{g As mL}^{-1}$  treatments (Tables 1 and 2), the plant can be recognized as a hyperaccumulator for As and Cd. Some plant species have shown similar or higher accumulation of As and Cd. For example,

BCF values of *W. globosa* were 940 and 476 for 15  $\mu\text{M}$  arsenite and 30  $\mu\text{M}$  arsenate, respectively (Zhang et al. 2009). Abhilash et al. (2009) reported Cd BCF values of more than 934 in *L. flava*. In addition, Sela et al. (1989) reported markedly high BCF values (24000) for Cd in the roots of *Azolla filiculoids*. However, some other plant species were shown to have lower accumulation of As and Cd, and low BCF values. Anwar et al. (2006) assessed the exposure and bioavailability of As using *Pteridium aquilinum*, *Erica australis*, *Juncus effuses*, *Phalaris caerulea*, and *Spergula arvensis* plant species in contaminated soils from the La Parrilla mine, Spain. They reported BCF values of 2.1 to 593.9 for the As contaminated site. Brix et al. (1983) found a BCF value of 6 for *Zostera marina* grown in a Cd-contaminated site.

#### **TF of As and Cd in *M. umbrosum***

TF values of the various As and Cd treatments for root to stem and stem to leaf transfers are given in Tables 1 and 2. All TF values for the As treatments, and TF values of stem to leaf for the Cd treatments, were greater than 1.0. It was indicated that As was readily translocated from root to stem and stem to leaf. Abhilash et al. (2009) reported that TF values for *L. flava* were from 0.90 to 4.41 for 0.5, 1, 2, and 4 mg Cd L<sup>-1</sup> treatments after 3, 7, 21, and 30 days. Rabb et al. (2007) studied 46 plant species to determine translocation into the shoots for arsenate, methyl arsonate, and dimethylarsinate. They found, for arsenate (V), that none of the

plant species had a TF of more than 0.9 for shoot to root transfer. In this study, high TF values (>1) of root to stem and stem to leaf for As, and stem to leaf for Cd, revealed that *M. umbrosum* is a good phytofiltrator as compared with other species.

## CONCLUSION

Water pollution by heavy metals such as As and Cd is a serious problem for humans and aquatic organisms. One approach to remedy this pollution, is to develop cost effective, practically applicable, novel, and eco-friendly phytoremediation technologies. Although many studies have already been conducted using plants to remediate contaminants from water bodies, the lack of suitable plants is still limiting the effectiveness of phytoremediation. In the present study, we used *M. umbrosum* in a hydroponic environment to evaluate its phytofiltration potential for two noxious metals, As and Cd. It was revealed that *M. umbrosum* was a suitable plant for the phytofiltration of low-level As and Cd contamination in water because of i) a high removal rate (79.3–89.5% As and 60–73.1% Cd), ii) an enough BCF (2350 for As and 3026.91 for Cd), iii) a TF value of more than 1, and iv) ease of culturing and harvesting. Therefore, the proposed plant is a candidate as a good phytoremediator for As- and/or Cd-contamination. Further investigation will be needed to clarify the mechanism of metal accumulation in *M. umbrosum* in order to use it as an effective phytofilter for As and Cd removal from drinking water.

## REFERENCES

- Abhilash PC, Vimal CP, Srivastava P, Rakesh PS, Chandran S, Singh N, Thomas AP. 2009. Phytofiltration of cadmium from water by *Limnocharis flava* (L.) Buchenau grown in free-floating culture system. *J Hazard Mater.* 170(2-3):791-797.
- Anawar HM, Sanchez AC, Murciego A, Buyolo T. 2006. Exposure and bioavailability of arsenic in contaminated soils from the La Parrilla mine, Spain. *Environ Geol.* 50(2): 170-179.
- Barman SC, Sahu RK, Bhargava SK, Chatterjee C. 2000. Distribution of heavy metals in wheat, mustard and weed grains irrigated with industrial effluents. *Bull Environ Contamin Toxicol.* 64(4):489-496.
- Brix H, Lyngby JE, Schierup HH. 1983. Eelgrass (*Zostera marina* L.) as an indicator of trace metals in the Limfjord, Denmark. *Mar Environ Res.* 8(3):165-181.
- Cai Y, Georgiadis M, Fourqurean JW. 2000. Determination of arsenic in seagrass using inductively coupled plasma mass spectrometry. *Spectrochimica Acta Part B.* 55(9):1411-1422.
- Dushenkov S, Kapulnik Y. 2000. Phytoremediation of Toxic Metals. In: Raskin I, Ensley BD. eds. *Using Plants to Clean Up the Environment.* New York: John Wiley & Sons. p.

89-106.

Fritioff A, Greger M. 2007. Fate of cadmium in *Elodea canadensis*. *Chemosphere*.

67(2):365–375.

Fritioff A, Greger M. 2006. Uptake and distribution of Zn, Cu, Cd, and Pb in an aquatic plant

*Potamogeton natans*. *Chemosphere*. 63(2):220–227.

Gupta S, Nayek S, Saha RN, Satpati S. 2008. Assessment of heavy metal accumulation in

macrophyte, agricultural soil and crop plants adjacent to discharge zone of sponge iron

factory. *Environ Earth Sci*. 55(4):731-739.

Hou W, Chen X, Song G, Wang Q, Chang CC. 2007. Effects of copper and cadmium on

heavy metal polluted waterbody restoration by duckweed (*Lemna minor*) *Plant Physiol*

*Biochem*. 45(1):62-69.

IARC. 1987. Overall evaluations of carcinogenicity: An updating of IARC Monographs

Volumes1–42. Lyon, International Agency for Research on Cancer. p 100–106 (IARC

Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7).

IPCS. 2001. Arsenic and arsenic compounds. Geneva: World Health Organization,

International Programme on Chemical Safety (Environmental Health Criteria 224;

<http://www.inchem.org/documents/ehc/ehc/ehc224.htm>).

Küpper H, Lombi E, Zhao FJ, McGrath SP. 2000. Cellular compartmentation of cadmium and

zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta*

212(1):75–84.

Langner HW, Greene E, Domenech R, Staats MF. 2012. Mercury and other mining-related contaminants in ospreys along the Upper Clark Fork River, Montana, USA. *Arch Environ Contam Toxicol.* 62(4):681-695.

Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED. 2001. A fern that hyperaccumulates arsenic. *Nature.* 409:579.

Mkandawire M, Dudel EG. 2005. Accumulation of arsenic in *Lemna gibba* L. (duckweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany. *Sci Total Environ.* 336(1-3):81–89.

Morel J, Echevarria G, Goncharova N (2006). *Phytoremediation of Metal Contaminated Soils.* Springer, Netherlands.

Mustafa HT, Hassan HM, Abo-Melha A, Rihan TI. 1988. Cadmium and zinc concentrations in the potable water of the eastern provinces of Saudi Arabia. *Bull Environ Contam Toxicol.* 40(3):462–467.

Nordstrom DK. 2002. Public health–worldwide occurrences of arsenic in ground water. *Science.* 296(5576): 2143–2145.

OECD. 1994. OECD environment monograph series No. 104: Risk Reduction Monograph No. 5: Cadmium. Background and National Experience with Reducing Risk. Environmental Directorate. Organisation for Economic Co-operation and Development,

- Paris 1995 (cancels and replaces the same document distributed on 15 Nov. 1994). p.33.
- Raab A, Williams NP, Meharg A, Feldmann J. 2007. Uptake and translocation of inorganic and methylated arsenic species by plants. *Environ Chem.* 4(3):197-203.
- Rahman MA, Hasegawa H. 2011. Review on Aquatic arsenic: Phytoremediation using floating macrophytes. *Chemosphere.* 83(5):633-646.
- Rahman MA, Hasegawa H, Ueda K, Maki T, Okumura C, Rahman MM. 2007. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): A good option for phytoremediation. *Chemosphere.* 69(3):493–499.
- Robinson B, Marchetti M, Moni C, Schroeter L, van den Dijssel C, Milne G, Bolan N, Mahimairaja S. 2005. Arsenic accumulation by aquatic and terrestrial plants. In: Naidu R, Smith E, Owens G, Bhattacharya P, Nadebaum P, eds. *Managing Arsenic in the Environment: From Soil to Human Health.* Collingwood, Victoria: CSIRO. p. 235–247.
- Schiorup HH, Larsen VJ. 1981. Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. 1. Availability, uptake and translocation of heavy metals in *Phragmites australis* (Cav.) Trin. *Aquat Bot.* 11:197-210.
- Schor-Fumbarov T, Keilin Z, Tel-Or E. 2003. Characterization of cadmium uptake by the water lily *Nymphaea aurora*. *Int J Phytorem.* 5(2):169–179.
- Sela M, Garty J, Tel-Or E. 1989. The accumulation and the effect of heavy metals on the water fern *Azolla filiculoides*, *New Phytol.* 112(1):7-12.

- Smith AH, Lingas EO, Rahman M. 2000. Contamination of drinking water by arsenic in Bangladesh: a public health emergency. *Bull World Health Organ.* 78:1093-1103.
- Snyder KVV. 2006. Removal of Arsenic from Drinking Water by Water Hyacinths (*Eichhornia crassipes*). *J U S S J W P.* 1: 41-58.
- Sun RL, Zhoua QX, Sun FH, Jin CX. 2007. Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, *Solanum nigrum* L. *Environ Exp Botany.* 60(3):468–476.
- USEPA-IRIS. 2010. Toxicological Review of Inorganic Arsenic (Cancer)-External Review Draft. U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-10/001, 2010.
- Vamerali T, Bandiera M, Mosca G. 2010. Field crops for phytoremediation of metal-contaminated land: A review. *Environ Chem Lett.* 8(1):1–17.
- Wang KS, Huang LC, Lee HS, Chen PY, Chang SH. 2008. Phytoextraction of cadmium by *Ipomoea aquatica* (water spinach) in hydroponic solution: Effects of cadmium speciation. *Chemosphere.* 72(4):666-672.
- WHO. 2011. Guidelines for drinking-water quality, 4th ed. Chemical fact sheets-chemical contaminants in drinking water. Geneva: World Health Organization.
- WHO/UNEP. 1989. GEM-Global fresh water quality. Published on behalf of the World Health Organization/United Nations Environment Programme. Oxford: Blackwell
- Reference.

- Williams PN, Islam MR, Adomako EE, Raab A, Hossain SA, Zhu YG, Feldmann J, Meharg AA. 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. *Environ Sci Technol.* 40(16):4903–4908.
- World Bank. 2005. Environment and Social Unit-South Asia Region. Towards a More Effective Operational Response. Arsenic Contamination of Groundwater in South and East Asian Countries Volume II Technical Report. Environmental Study.
- Zayed A, Gowthaman S, Terry N. 1998. Phytoaccumulation of trace elements by wetland plants: 1. Duckweed. *Environ Qual.* 27(3):715–721.
- Zhang X, Zhao FJ, Huang Q, Williams PN, Sun GX, Zhu YG. 2009. Arsenic uptake and speciation in the rootless duckweed *Wolffia globosa*. *New Phytologist* 182(2):421-428.
- Zhang X, Lin AJ, Zhao FJ, Xu GZ, Duan GL, Zhu YG. 2008. Arsenic accumulation by the aquatic fern *Azolla*: Comparison of arsenate uptake, speciation and efflux by *Azolla caroliniana* and *Azolla filiculoides*. *Environ Pollut.* 156(3):1149–1155.
- Zhao FJ, Lombi E, McGrath SP. 2003. Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. *Plant Soil.* 249(1):37-43.
- Zhu YG, Sun GX, Lei M, Teng M, Liu YX, Chen NC, Wang LH, Carey AM, Deacon C, Raab A, Meharg AA, Williams PN. 2008. High percentage inorganic arsenic content of mining impacted and non-impacted Chinese rice. *Environ Sci Technol.* 42(13):5008–5013.

Figure 1: Remaining As ( $\mu\text{g mL}^{-1}$ ) in water in which *M. umbrosum* was grown with 0.2 (a), 0.45 (b), and 1.0 (c)  $\mu\text{g As mL}^{-1}$ . Error bar indicates mean $\pm$ S.E.M. (n = 3). \*\* and \* denote significant differences at  $P < 0.01$  and  $0.05$ , respectively, compared to previous days

Figure 2: As accumulation in root, stem and leaf of *M. umbrosum* seven days after exposure to 0.2, 0.45, and 1.0  $\mu\text{g As mL}^{-1}$  water. Error bars indicate mean $\pm$ S.E.M. (n = 3). \*\* and \* denote significant differences at  $P < 0.001$  and  $0.005$ , respectively, compared to As from water to root, root to stem, and stem to leaf

Figure 3: Remaining Cd in water in which *M. umbrosum* was grown with 0.3 (a), 3.0 (b), and 30 (c)  $\mu\text{g Cd mL}^{-1}$ . Error bar indicates mean $\pm$ S.E.M. (n = 3). \* denotes significant differences at  $P < 0.05$ , compared to day 0

Figure 4: Cd accumulation in leaf, stem and root of *M. umbrosum* seven days after exposure to 30, 3.0, and 0.3  $\mu\text{g Cd mL}^{-1}$  water. Error bars indicate mean $\pm$ S.E.M. (n = 3). \* denotes significant differences at  $P < 0.05$ , compared to Cd from root to stem

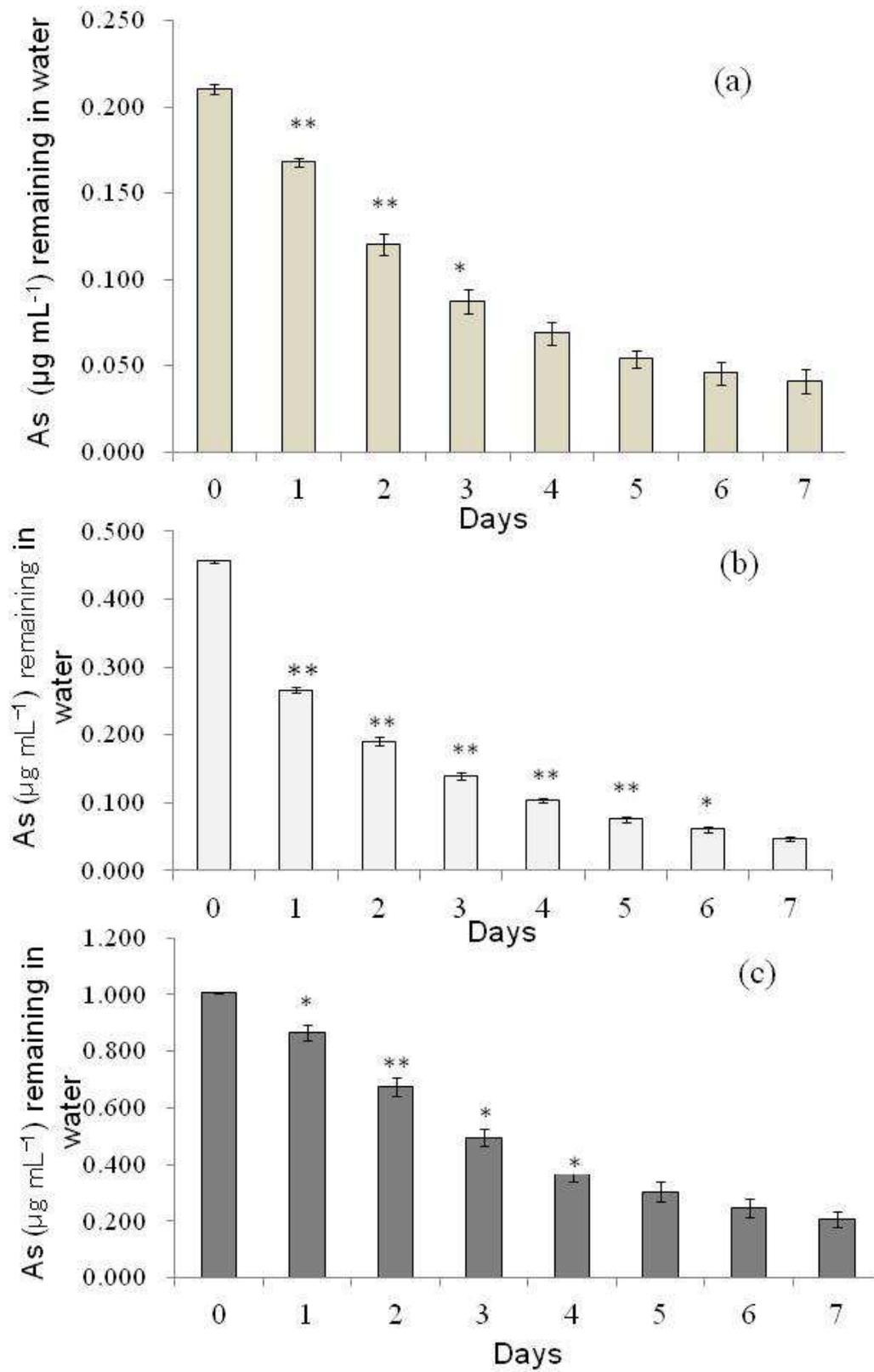


Fig. 1

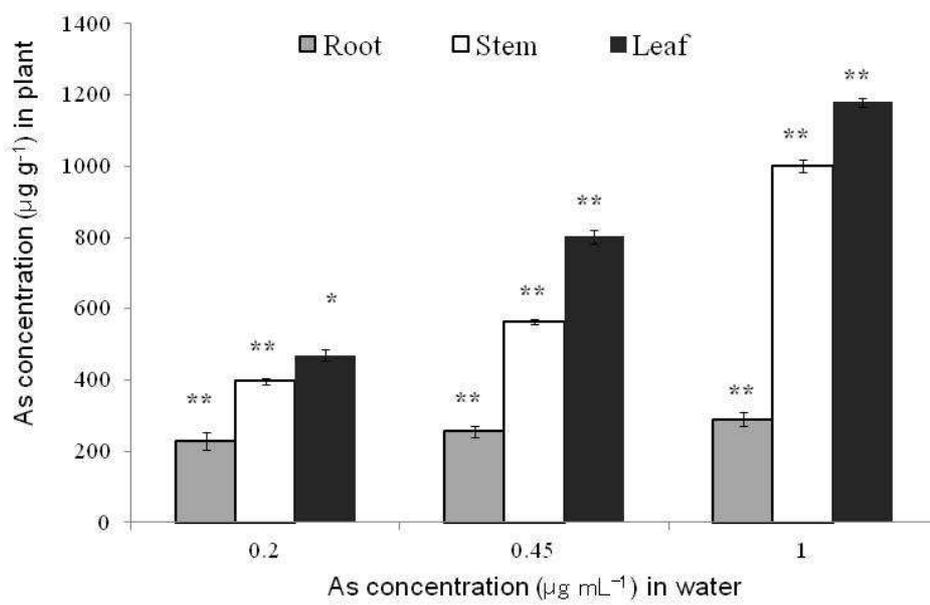


Fig. 2

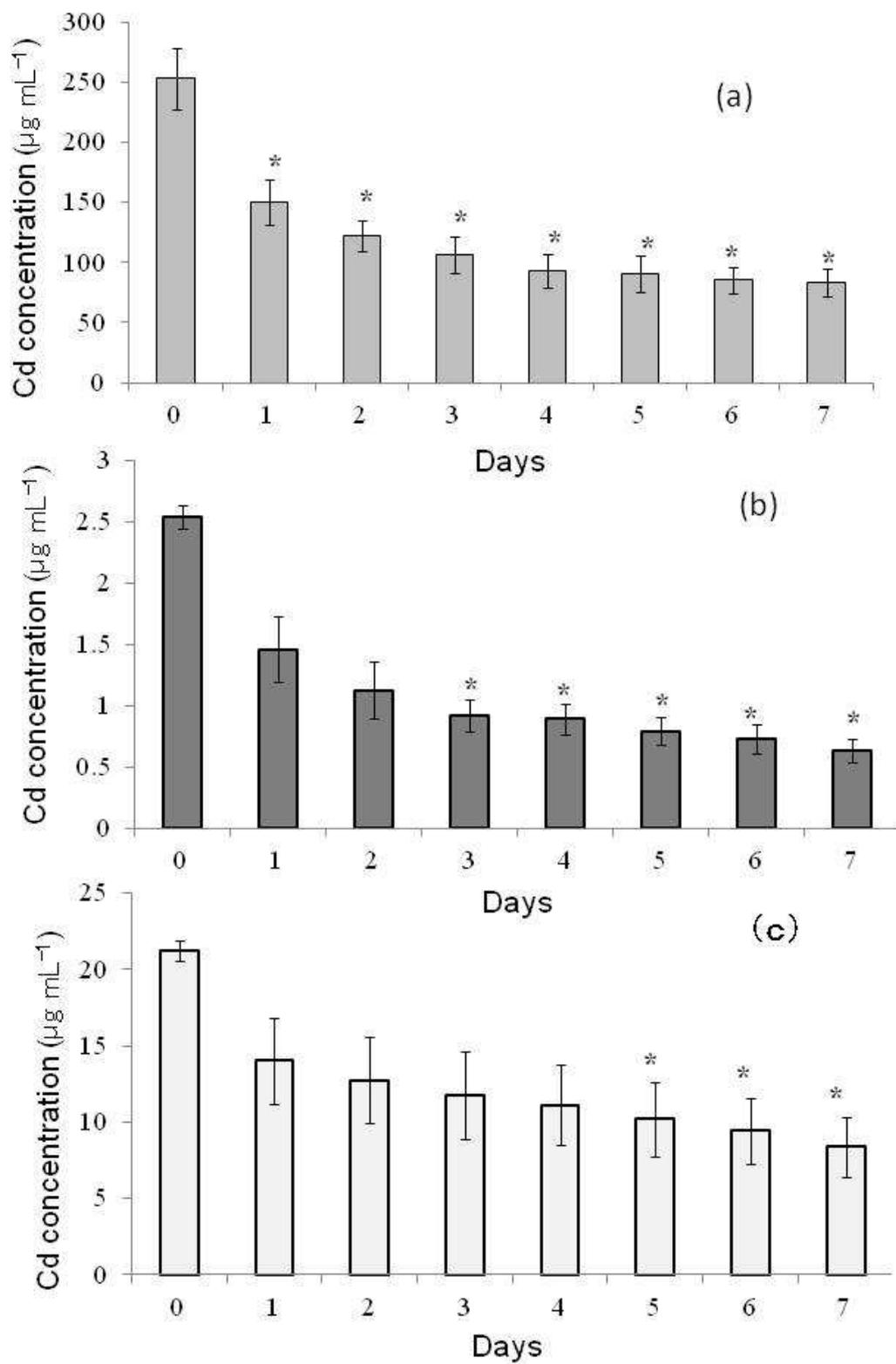


Fig. 3

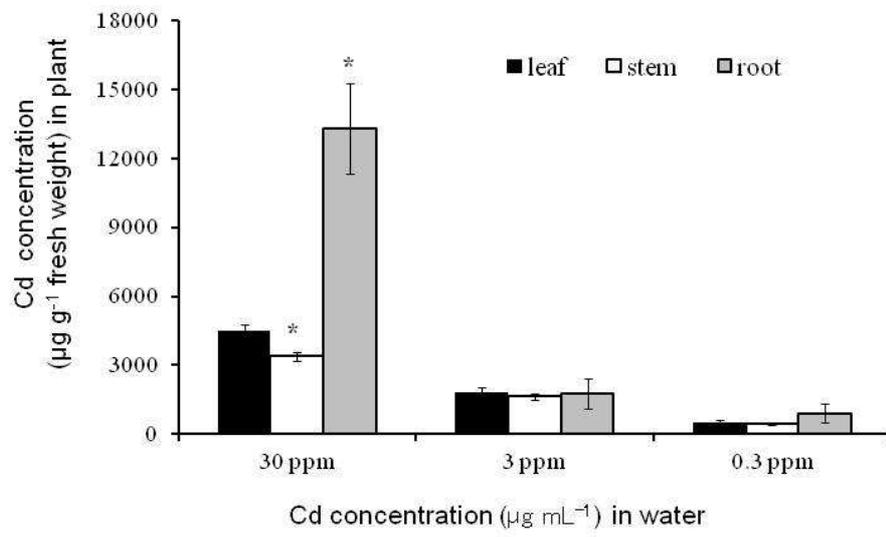


Fig. 4

**Table 1:** BCF values (dry weight basis), root to stem and stem to leaf TF values, and As removal efficiency (%) of *M. umbrosum*. (n = 3)

Conc. of As ( $\mu\text{g mL}^{-1}$ )	Plant parts	BCF [Mean $\pm$ SEM]	TF	% Removed
0.2	Root	1140 $\pm$ 121.4		
	Stem	1983 $\pm$ 38.4	1.74	80.48
	Leaf	2350 $\pm$ 72.3	1.18	
0.45	Root	567.4 $\pm$ 32.9		
	Stem	1253.3 $\pm$ 17.3	2.21	89.56
	Leaf	1782.2 $\pm$ 41.5	1.42	
1	Root	289.3 $\pm$ 19.2		
	Stem	1001 $\pm$ 16.5	3.46	79.3
	Leaf	1179.3 $\pm$ 11.6	1.27	

**Table 2:** BCF values (fresh weight basis), root to stem and stem to leaf TF values, and Cd removal efficiency (%) of *M. umbrosum*. (n = 3)

Conc. of Cd ( $\mu\text{g mL}^{-1}$ )	Plant parts	BCF [Mean $\pm$ SEM]	TF	% Removed
0.3	Root	3026.91 $\pm$ 1389.12		
	Stem	1473.91 $\pm$ 219.02	0.49	70.4
	Leaf	1686.56 $\pm$ 277.22	1.14	
3.0	Root	585.14 $\pm$ 215.21		
	Stem	542.97 $\pm$ 39.18	0.93	73.1
	Leaf	596.49 $\pm$ 86.06	1.10	
30	Root	443.21 $\pm$ 65.42		
	Stem	112.59 $\pm$ 6.93	0.25	60
	Leaf	149.71 $\pm$ 10.01	1.33	