Potential of *Micranthemum umbrosum* for phytofiltration of organic arsenic species from oxic water environment

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Abstract

Arsenic (As) is a toxic and carcinogenic metalloid that causes various hazards to human health. Phytofiltration is a more eco-friendly and green approach than chemoremediation, or other traditional technologies, for removing As from aquatic environments. Recently, *Micranthemum umbrosum* was shown as a promising candidate for phytofiltration of inorganic As species. This work examines the potential application of M. *umbrosum* to phytofiltration of organic As species, such as monomethylarsonic acid (MMAA, CH5AsO3) and dimethylarsinic acid (DMAA, C2H7AsO2), from oxic water environments. *M. umbrosum* plants were grown in two test concentrations of MMAA and DMAA, or a control, in a hydroponic experiment. After seven days, leaves accumulated 90 ± 3.2 and 48 ± 1.6 µg As g-1 from 1 µg As mL-1 of water added from MMAA and DMAA, respectively. Bio concentration factor values and translocation factor values were always greater than 1.0, indicating that M. umbrosum was a good As accumulator and that leaves accumulated significantly higher amounts of As than stems and roots. Analysis of macro- and micro-nutrient data showed that *M. umbrosum* had higher resistance to

organic As treatments than the control. These results confirm the potential application of *M. umbrosum* for phytofiltration of organic As from contaminated oxic water environments.

Key Words: dimethylarsinic acid; monomethylarsonic acid; phytofiltration; plant; water

1. Introduction

Arsenic in drinking water is one of the most serious environmental health hazards faced by millions of people in many areas of the world, such as Bangladesh, India, USA, China, Taiwan, Mexico, Argentina, Poland, Canada, Hungary, New Zealand, Japan, and Chile (Knobeloch et al., 2006; Mohan and Pittman, 2007; Kim et al., 2011). Arsenic is highly toxic and can lead to a wide range of health problems, being carcinogenic, mutagenic, and teratogenic (NRC, 1999; Smedley and Kinniburgh, 2002). Arsenic exists in the environment in four oxidation states (+V(arsenate), +III(arsenite), 0(arsenic), and −III(arsine)), and in different forms, such as inorganic (arsenous acid, arsenite, arsenic acids or arsenate), organic (MMAA, DMAA, trimethylarsine oxide, etc.), biological (arsenobetaine, arsenocholine, glycerophosphatidylarsenocholine, etc.), and others (Ng, 2005, Rahman and Hasegawa, 2011). Arsenic is very sensitive to mobilizing at pH 6.5–8.5 (typically found in ground water) in both oxidizing and reducing conditions. Inorganic forms of As are mainly found in natural waters as oxyanions of trivalent arsenite (As(III)) or pentavalent arsenate (As(V)), but organic As may be produced by biological activity (bacteria, yeasts, and algae), mostly in surface waters. However, organic forms may also occur where waters are significantly impacted by industrial pollution (Smedley and Kinniburgh, 2003). In oxic seawater, As is typically dominated by As(V), though some As(III) is invariably present and becomes increasingly important in anoxic bottom waters. Ratios of As(V)/As(III) are typically in the range of 10–100 in open seawater (Andreae, 1979; Pettine et al., 1992). Increases in organic As species have also been recorded in these zones as a result of methylation reactions by phytoplankton (Cullen and Reimer, 1989). As such, naturally contaminated surface water contains increasing amounts of organic As species. Hasegawa et al. (1997) reported that DMAA and MMAA were the dominant organic As species in Lake Biwa during summer. Application of As-containing herbicides is another source of organic As in the environment. Giacomino et al. (2010) found that over 40% of As species present in soil were organic, and the remainder were inorganic As(V) and As(III). In the southern United States, both inorganic and organic As are found in rice that is now grown in those fields (Rosen et al., 2008). Arsenite (iAsIII) is

usually more toxic than arsenate (iAsV). Recent studies have shown that MMA(III) and DMA(III) were more acutely toxic and more genotoxic than their parent compounds (Petrick et al., 2000; Mass et al., 2001). These trivalent arsenicals are more toxic than iAs(V), MMA(V), and DMA(V) in vitro (Styblo et al., 2000; Mass et al., 2001). Recently, LC50 values for human cells were calculated as 571, 843, 5.49, and 2.16 µM for iAsV, DMA(V), iAs(III), and DMA(III), respectively (Naranmandura et al., 2007). This study also showed that dimethylmonothioarsenic (DMMTA(V)) was much more toxic than other pentavalent nonthiolated arsenicals (Naranmandura et al., 2007). Therefore, the daily accumulation of organic As species in oxic water, soil, and plants, as well as food products, is of particular concern. There are several remediation processes of contaminants from the environment. Among them phytoremediation is a well-known ecologically friendly technology and inexpensive alternative, to remediate contaminants from water environment. Uptake and accumulation of inorganic As species by aquatic macrophytes have been studied extensively (Mkandawire and Dudel, 2005); however, few studies have examined the uptake of organic As species. Moreover, arsenate and DMAA are the major As species in oxic aquatic systems (Hasegawa et al., 1999). In our previous study, we found that *M. umbrosum* could uptake more than 1000 µg g^{-1} inorganic As (added from sodium arsenite) from the water environment (Islam et al., 2015). Current research focuses on the potential of *M. umbrosum* to phytofiltrate organic As species from oxic water environments.

2. Materials and Methods

2.1 Plant culture

Approximately 3 g (fresh weight) of *M. umbrosum* plants was grown in hydroponic cultures in laboratory conditions for 7 days in a plant growth chamber under controlled environment (14:10 light/dark cycle, 100–125 µM•m^{-2}•s^{-1} light intensity, 75% humidity, and 21 ± 1 °C temperature). The hydroponic medium contained 200 and 1000 µg As L^{-1} [from MMAA (CH_5AsO_3) and DMAA ($C_2H_7AsO_2$)] Milli-Q water (Millipore-Gradient A10, Milli-Q Gradient ZMQG), with 500 mL Hoagland nutrient solution (Hoagland and Arnon, 1950) as a nutrient source. Both pH and redox potential impose important controls on arsenic speciation in the natural environment (Ferguson and Gavis, 1972). Therefore, the solution was maintained at pH 7.0 by adding KOH or HCl, to retain equal concentrations of $AsO_2(OH)^{2-}$ and $AsO_3(OH)^{2-}$. MMA and DMA are diprotic and monoprotic acids, respectively (Cox and Ghosh, 1994). At a neutral pH, the

major species of MMA(V) is $CH3AsO2(OH)^{-}$, although the minor species, $CH3AsO3^{2-}$, will also be present. For DMA(V), both (CH3)2AsO(OH) and $(CH3)2AsO^{2-}$ exist at pH 7.0 (Sharma and Sohn, 2009). All treatments were replicated three times, plus and a control was maintained both for the As and the plant.

2.2 Sample collection, preparation, and chemical analysis

Arsenic status in water samples from each pot was recorded at 24 h intervals. After 7 days, whole plants were harvested and rinsed with Milli-Q water three times. Whole plants were separated into leaves, stems, and roots for analysis of total As, calcium (Ca), magnesium (Mg), manganese (Mn), and zinc (Zn) content. Leaf, stem, and root samples were air dried on absorbent paper for 24 h at room temperature. Samples were then oven dried at 65 °C (Constant Temperature Oven, DKN602, Yamato Scientific Co. Ltd., Japan) for at least 48 h until they reached a constant weight measured by a digital balance (, HF-200, Max 210 g, d = 0.001 g; A&D Co. Ltd, Japan). After grinding the samples, 25-40 mg samples of leaves, stems, or roots were placed individually into 15 mL polyethylene tubes (Thermo Fisher Scientific, NY, USA). Two mL of 65% HNO3 (Wako Pure Chemical Ind. Ltd., Japan) were added, and the samples kept under the fume hood for 12 h. Samples were then covered and digested on a heating block (TAH-2G, Dry Thermo Unit, Japan) at 95 °C for 2 h. After cooling, 1 mL of 30% H2O2 (Wako Pure Chemical Ind. Ltd., Japan) was added and the samples were covered and heated again at 105 °C for 20 min (Rahman et al., 2007). Digested samples were diluted to 10 mL with Milli-Q water using 10 mL volumetric flasks (Pyrex®, IWAKI Glass), as described by Cai et al. (2000) and Islam et al. (2013). The diluted samples were then filtered using a 0.45-µm syringe-driven filter unit (Millipore, Billerica, USA) and stored in 15 mL polyethylene bottles. As, Mg, Mn, and Zn contents were measured using an inductively coupled plasma-mass spectrophotometer (ICP-MS; Agilent G1820 Model), whereas Ca contents were measured by a flame-type atomic absorption spectrophotometer (AAS; Model 180–80, Hitachi, Japan). The accuracy of the analysis was checked using certified standard reference materials for As (013–15481, Lot ALK 9912, 1000 mg L^{-1}), Mn (133-12131, Lot KWR 2425, 1004 mg L^{-1}), Zn (264-01421, Lot KWQ 4136, 1005 mg L^{-1}), and Mg (136-12121, Lot KWR 2871, 1001 mg L^{-1}) obtained from Wako Pure Chemical Ind. Ltd., Japan. Bio-concentration factor (BCF) and root-to-stem and stem-to-leaf translocation factors (TF) were

also calculated (Snyder, 2006; Gupta et al., 2008) to evaluate phytofiltration ability of *M. umbrosum* plants.

2.3 Statistical analysis

Results were expressed as the means ± standard error (S.E.) of the three replicates. The degree of significance was calculated using a t-test and curve fitting was applied using Microsoft Excel (Microsoft Office 2007 Professional).

3. Results and discussion

3.1 Phytofiltration of As from organic sources

Organic As concentration in the nutrient solution decreased over time. The decreasing trend was significant on the 2nd and 5th days for both MMAA (Fig. 1) and DMAA (only for 0.2 µg mL^{-1} treatment, Fig. 2b), as *M. umbrosum* plants can grow when submerged. Whole plants acted as an active site for As absorption as there was no evidence for physiochemical adsorption by the plants or glass pot as described by Robinson et al. (2006). After 7 days of hydroponic culture, *M. umbrosum* was able to remove approximately 60-75% As from MMAA (Table 1) and 50-61% As from DMAA (Table 2). At a lower initial concentration (0.2 µg mL^{-1}), *M. umbrosum* removed As from the solution to a final concentration (53 µg L^{-1}, Fig. 1b) near the maximum level (50 µg L^{-1}) approved by the Bangladesh and China Government Standard (World Bank, 2005). The rate of organic As uptake is lower than inorganic As in the same plant species. Islam et al. (2015) found that *M. umbrosum* can uptake more than 1000 µg As g^{-1} from inorganic sodium arsenite. Furthermore, it removed approximately 79.3% to 89.5% inorganic As from the solution (Islam et al., 2013). Trivalent methylated arsenic species may be more toxic than inorganic As, as they are more efficient at causing DNA breakdown and lower physiological activity of plants (Vaclavikova et al., 2008).

3.2 Phytoaccumulation of As within *M. umbrosum*

The As phytoaccumulation pattern of *M. umbrosum* is depicted in Fig. 3. *M. umbrosum* leaves contain significantly ($P < 0.001$) higher amounts of As compared to stems and roots for 1 µg As L^{-1} as MMAA or DMAA. However, there were no significant differences between the stems and leaves in As accumulation

for the 0.2 µg As L^{-1} treatment (Fig. 3). However, larger amounts of As accumulation were observed in leaves (90 µg g^{-1}) and stems (68 µg g^{-1}) treated with MMAA (1 µg As mL^{-1}) compared with leaves (48 µg g^{-1}) and stems (28 µg g^{-1}) treated with DMAA (1 µg As mL^{-1}) (Fig. 3). These results are consistent with a study by Rahman et al., (2007) that reported accumulation of only 7.65 ± 0.27 nM As g^{-1} dry weight in Spirodela polyrhiza exposed to 4.0 µM As from DMAA. Furthermore, S. polyrhiza can bioaccumulate 79% more As from inorganic arsenate than from DMAA. *M. umbrosum* can also accumulate 10-fold more inorganic As than MMAA and 20-fold more than DMAA (Islam et al., 2015). One reason may be that DMAA has a poor affinity for –SH groups, so that it has an easier transport route to shoots and grain (Meharg et al., 2008), whereas inorganic As uptake mechanisms in *M. umbrosum* appear to involve –SH groups or protein containing –SH groups.

3.3 BCF and TF of MMAA and DMAA in *M. umbrosum*

BCF provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate (Snyder, 2006), whereas TF, or mobilization ratio, determines the relative translocation of metals from the substrate (water or soil) to other parts (root-to-stem-to-leaf) of the plant (Barman et al., 2000; Gupta et al., 2008). BCF, root-to-stem, and stem-to-leaf TF values for various treatments of MMAA and DMAA are given in Tables 1 and 2. BCF values for MMAA and DMAA treatments for different compartments (root, stem, and leaf) of the plant ranged from 21-90 (Table 1) and 9-55 (Table 2), respectively. Higher BCF values indicate the ability of plants to concentrate metal in their tissues (Abhilash et al., 2009). According to Zayed et al. (1998), a plant with a BCF of more than 1000 is considered a hyperaccumulator, a BCF of 1 to less than 1000 an accumulator, and a BCF of less than 1 an excluder. Data from this study show that *M. umbrosum* is an accumulator for this organic As treatment as all BCF values ranged from 9 to 90 (Tables 1 and 2). Some plant species have shown similar or higher accumulation of inorganic As. Anwar et al. (2006) assessed the exposure and bioavailability of inorganic As using Pteridium aquilinum, Erica australis, Juncus effuses, Phalaris caerulescens, 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. Root-to-stem and stem-to-leaf TF values (Tables 1 and 2) were greater than 1 in all cases. As from MMAA and DMAA was readily translocated from root-to-stem-to-leaf in oxic aquatic systems, similar to inorganic As species.

3.4 Effects of MMAA and DMAA on macro- and micronutrients in *M. umbrosum*

Essential plant macro- (Ca and Mg) and micro- (Mn and Zn) nutrient statuses were determined in both MMAA and DMAA treated roots, stems, and leaves after 7 days of the hydroponic experiment (Table 3). Ca content significantly ($P < 0.01$ and 0.05) decreased in leaves and roots, whereas Mg content increased ($P < 0.01$) in stems for the 1000 µg As L^{-1} (added from MMAA) treatment compared to the control (Table 3). There were no significant changes in macronutrient status in the DMAA treatment. Significant ($P < 0.01$ and 0.05) increases in micro-nutrients were observed for MMAA treated roots, stems, and leaves. However, As from DMAA negatively influenced the accumulation of Zn ($P < 0.05$) within the plant body, compared with the control (Table 3). In a previous study, we found that Mn content was negatively correlated with added inorganic As; however, the present study showed that *M. umbrosum* contained increasing amounts of Mn for both MMAA and DMAA treatments. Therefore, there might be different As uptake mechanisms from inorganic and organic sources, as reported by Rahman et al. (2007).

4. Conclusion

M. umbrosum was shown as a good As accumulator that can uptake significant amounts of As added from organic sources in oxic aquatic environments. This aquatic plant can reduce As concentration from 750 to 300 µg L^{-1} and 200 to 53 µg L^{-1}, over 7 days in hydroponic conditions, although decreases were slightly lower in the DMAA treatment (400 and 71 µg L^{-1}). This phytofiltrated As-free water can be used for irrigation to prevent As deposition in agricultural crops, as well as for drinking water to reduce As contamination in humans. A previous study showed that *M. umbrosum* is a hyperaccumulator of inorganic As, and is, therefore, a good phytofiltrator for both inorganic and organic As contamination. This plant species can be used as a phytofilter of As contaminated water in small scale rural and urban areas by cultivating it in an aquarium or dish. Further investigations are required to clarify the mechanisms and speciation of As in *M. umbrosum* for this plant to be used as an effective phytofilter of As from contaminated water.

5. Conflict of Interest

The authors declare that there are no conflicts of interest.

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Captions of Figures

Fig. 1. As (µg L^{-1}) remaining in water in which *M. umbrosum* grown with **(a)** 1.0 and **(b)** 0.2 µg As mL^{-1} added from MMAA. Error bar indicates mean ± S.E. (n=3). ** and * denotes significantly different at P<0.01 and 0.05, respectively, against for previous days.

Fig. 2 As (µg L^{-1}) remaining in water in which *M. umbrosum* grown with **(a)** 1.0 and **(b)** 0.2 µg As mL^{-1} added from DMAA. Error bar indicates mean ± S.E. (n=3). ** and * denotes significantly different at P<0.01 and 0.05, respectively, against for previous days.

Fig. 3. As accumulation (µg g^{-1}) pattern in root, stem and leaf of *M. umbrosum* after seven days exposure to 0.2 and 1.0 µg As mL^{-1} water as **(a)** MMAA and **(b)** DMAA. Error bars indicates mean ± S.E. (n=3). ** and *denotes significantly different at P<0.001 and 0.005, respectively, against for As from water to root, root to stem and stem to leaf.

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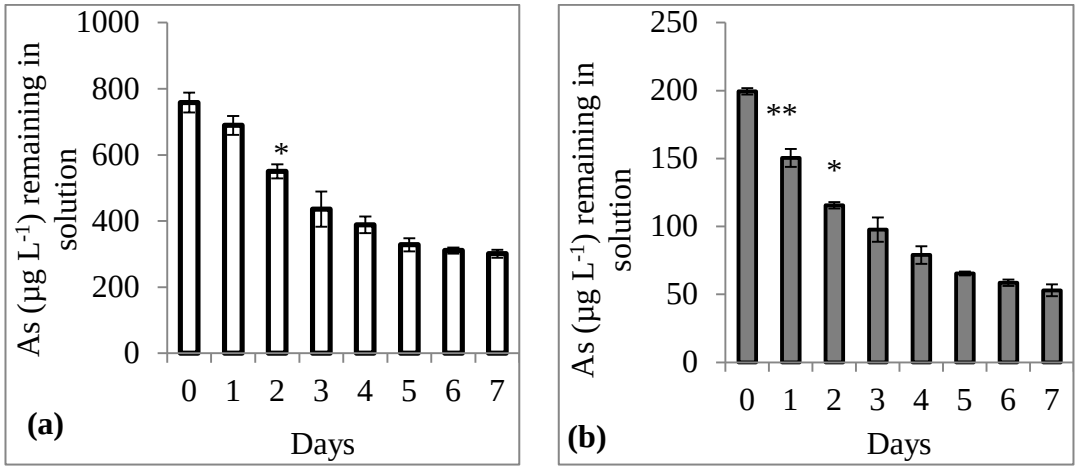

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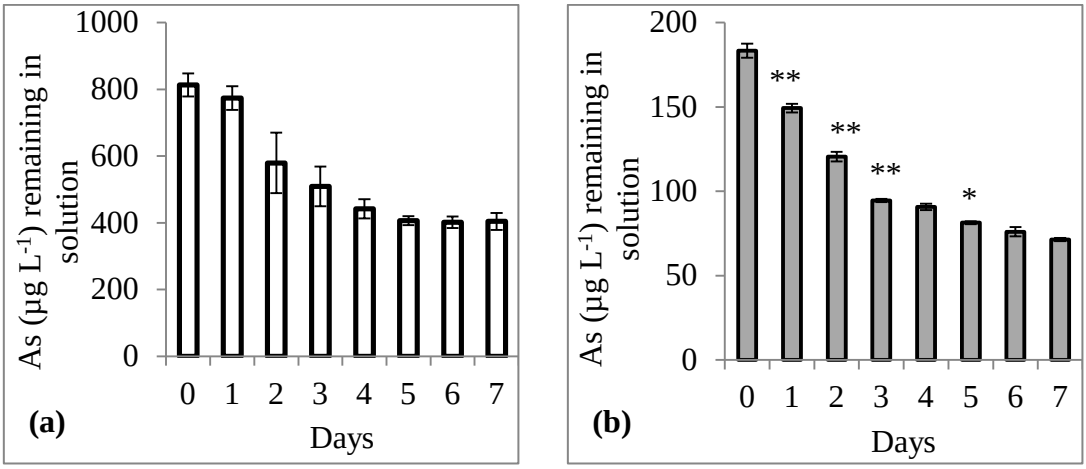

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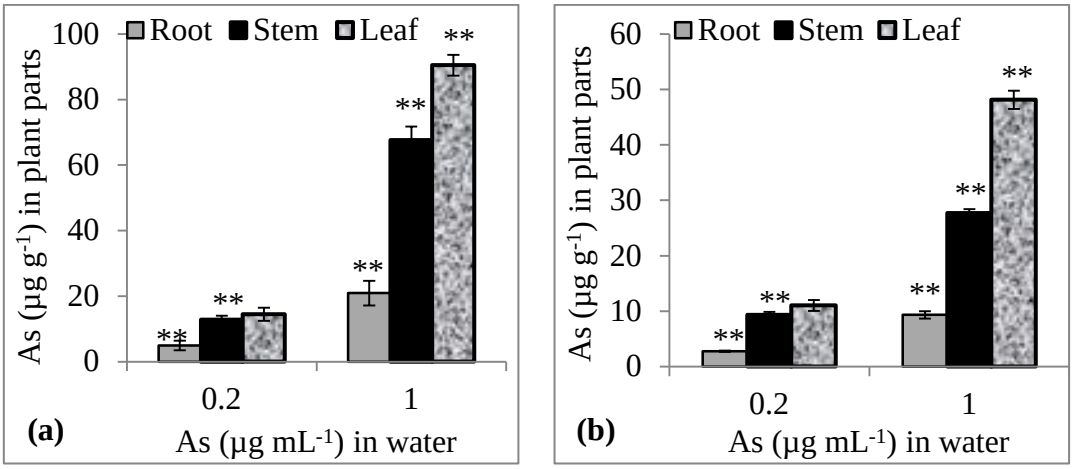

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Table 1. Bio-concentration factor (BCF), root to stem and stem to leaf TF (translocation factor) values and As removal efficiency (%) of *M. umbrosum* (n=3) for MMAA treatment.

Conc. of As ($\mu g\ mL^{-1}$)	Plant parts	BCF [Mean ± S.E.]	TF	% Removed
0.2	Root	25 ± 7.2		75
	Stem	64 ± 5.8	2.6	
	Leaf	72 ± 9.8	1.1	
1	Root	21 ± 3.8		60
	Stem	68 ± 4.1	3.2	
	Leaf	90 ± 3.2	1.3	

Table 2. Bio-concentration factor (BCF), root to stem and stem to leaf TF (translocation factor) values and As removal efficiency (%) of *M. umbrosum* (n=3) for DMAA treatment.

Conc. of As ($\mu g\ mL^{-1}$)	Plant parts	BCF [Mean ± S.E.]	TF	% Removed
0.2	Root	14 ± 0.7		61
	Stem	47 ± 2.7	3.4	
	Leaf	55 ± 5.0	1.2	
1	Root	09 ± 0.7		50
	Stem	28 ± 0.7	3.0	
	Leaf	48 ± 1.7	1.7	

Table 3. Composition of nutrient elements (oven dry basis) of *M. umbrosum* plant parts grown in 1000 µg L^{-1} As (from MMAA and DMAA) tainted water

Source of As	Dose (µg L^{-1})	Plant parts	Ca (mg g^{-1})	Mg (µg g^{-1})	Mn (µg g^{-1})	Zn (µg g^{-1})
Control	0	Leaf	3.54±0.10	22.66±0.37	225.09±8.32	82.57±1.46
		Stem	2.89±0.15	18.03±1.11	120.05±3.94	39.26±1.88
		Root	3.94±0.08	4.87±1.62	93.98±5.65	37.94±1.19
MMAA	1000	Leaf	*2.99±0.09	30.66 ±2.61	*269.14±6.00	*123.34±5.23
		Stem	3.16±0.20	**26.95±1.07	**184.97±3.91	**81.26±5.29
		Root	**2.81±0.19	4.99±0.97	**278.65±4.56	**125.12±4.28
DMAA	1000	Leaf	4.01±0.27	31.76±2.64	**121.32±5.34	*57.59±3.94
		Stem	3.32±0.26	22.72±0.69	105.01±4.21	28.30±3.71
		Root	3.98±0.91	2.66±0.36	**289.54±9.29	*23.17±3.62

Each value indicated as Mean ± S.E. (n=3); ** and * showed significantly difference against control or 0 (µg L^{-1}) at $p < 0.01$ and 0.05, respectively.