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Simultaneous removal of heavy metals from aqueous solutions by *Equisetum diffusum* D. Don

Nguyen Thi Hoang Ha[1*], Vu Thi Thom[2]

[1]*VNU University of Science, Vietnam National University, Hanoi, Vietnam*

[2]*Vietnam Japan University, Luu Huu Phuoc, Hanoi, Vietnam*

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ABSTRACT

Equisetum diffusum D. Don was transplanted into solutions added with different concentrations of Mn, Zn, Cd, Pb, and As for 30 days in the laboratory experiment to assess the removal of these metals and their accumulation in the plant. The highest removal efficiency of Mn, Zn, Cd, Pb, and As from solutions by *E. diffusum* was 99.6, 97.9, 77.5, 85.3, and 61.9%, respectively. The highest daily removal efficiencies of heavy metals were obtained after 1 day of new solution addition. The highest concentrations of Mn, Zn, Cd, Pb, and As in the plant roots were 7230, 1490, 174, 1170, and 274 mg/kg-DW, respectively; those in the shoots were 1960, 566, 33.9, 308, and 108 mg/kg-DW. The bioconcentration factor (BCF) values for Mn, Zn, Cd, Pb, and As were 496, 406, 702, 463, and 191, respectively. The results of this study indicate that *E. diffusum* has the ability to remove simultaneously these metals from water, making it a potential species for phytoremediation of water contaminated with multiple heavy metals.

Keywords: Arsenic; *Equisetum diffusum* D. Don; heavy metals; phytoremediation; removal; wastewater.

1. Introduction

Water pollution by heavy metals is one of the most serious environmental problems that has been attracting considerable attention. Mining and smelting of metalliferous ores, urban sewage, tanneries, landfill leachate, and the textile and chemical industries are the main sources of metal contamination in water (Mishra and Tripathi, 2008; Adriano, 2011; Xiao et al., 2017; Sun et al., 2018). Mn and Zn are known to be essential elements for plant growth (Pahlsson, 1989) but toxic to plant at the concentrations of 300–500 and 100–400 mg/kg, respectively (Kabata-Pendias, 2011). As, Cd, and Pb are unessential elements (Pahlsson, 1989; Selinus et al., 2005) and are toxic to plants at the concentrations of 5–30, 5–20, and 30–300 μg/g, respectively (Kabata-Pendias, 2011). Considering their threats to human and ecosystem, the appropriate treatment of these metals is of great environmental importance. A variety of treatment methods have been developed for the elimination of these metals from water, including coagulation, adsorption, ion exchange, electrocoagulation, and biological processes; however, many of these methods are costly and require major

[*]Corresponding author, Email: hoangha.nt@vnu.edu.vn

investments in equipment and facilities (Wang et al., 2002). Phytoremediation may provide a cost-effective, long-lasting, and environment-friendly technology for the treatment of waters contaminated with heavy metals (Garbisu and Alkorta, 2001).

Aquatic macrophytes have great potential for the phytoremediation of water contaminated with heavy metals (Zayed et al., 1998; Zhu et al., 1999; Wang et al., 2002; Miretzky et al., 2004; Peng et al., 2008). Duckweed (*Lemna minor* L.) (Zayed et al., 1998; Uysal, 2013; Sasmaz et al., 2019), water hyacinth (*Eichhornia crassipes*) (Mishra and Tripathi, 2008; Rezania et al., 2015), water lettuce (*Pistia stratiotes*) (Miretzky et al., 2004), *Potamogeton pectinatus* L. (Peng et al., 2008, and *Phragmites australis* (Vymazal and Březinová, 2016) are among the most potential plant species for phytoremediation of contaminated water. However, the fact that floating macrophytes (e.g., duckweed and water hyacinth) can be easily transported away from water reduces the feasibility of these plant species for phytoremediation of flowing contaminated water. *P. australis* is widely used in constructed wetland for wastewater treatment and showed potential for removal of heavy metals from solutions (Vymazal and Březinová, 2016). Other plant species should be investigated for further selection of suitable candidates for removal of heavy metals from water at contaminated sites. It is important to use native plants for phytoremediation because such plants adapt better to the stress conditions at the site than would plants introduced from other environments (Yoon et al., 2006). *Equisetum diffusum* D. Don was found to grow naturally around the mining area, tolerated with environments seriously contaminated with heavy metals and accumulated them into its biomass (Ha et al., 2011). However, to the best of our knowledge, no previous study has investigated the capacity of this plant species for metal removal from water.

This study was conducted to determine (1) the simultaneous removal of Mn, Zn, Cd, Pb, and As from water by *E. diffusum*; and (2) the potential use of this plant species in phytoremediation of wastewater contaminated with heavy metals.

2. Materials and methods

2.1. Experimental plant, growth condition, and heavy metal preparation

E. diffusum of approximately 30–35 cm high was collected at the area which was supposedly uncontaminated and washed thoroughly with tap water followed by Milli-Q water to remove sediments. The plant was placed into 30 L experimental tanks (56.4 cm × 37.9 cm × 20.5 cm) filled with 10 L of Milli-Q water, where it received 16 h of white fluorescent light per day. The air and water temperatures were kept constant (24±1°C) during the experiment. *E. diffusum* was transplanted in the tanks during 1 month for stabilization before the experiments.

Five metals (Pb, Zn, Mn, Cd, and As) were added as standard solutions ($Pb(NO_3)_2$, $Zn(NO_3)_2$, $Mn(NO_3)_2$, Cd $(NO_3)_2$, and Na_2HAsO_4). The reagents were dissolved in Milli-Q water to obtain the desired contamination levels which were set following the real concentrations of these metals in wastewater at different outlets from a Pb-Zn mine, northern Vietnam (Table 1). The resulting solutions were adjusted to pH=7 using NaOH and HNO_3.

2.2. Experimental design and sampling

The experiment to examine the removal efficiency of heavy metals by *E. diffusum* was carried out during 30 days. Five tanks were prepared including plant and metal control tanks (Table 1). All laboratory experiments were repeated in duplicated. Limestone (14% calcite and 86% ankerite) with grain-size of 2–3 cm was used as substrate to keep the plant in the experimental tanks.

Table 1. Experimental design

Tank	*E. diffusum* (fresh weight)	Concentrations of metals (mg/L)	Note
A	200 g	Milli-Q water, no metal was added	Plant control
B	200 g	Mn (20.0), Zn (6.0), Cd (0.5), Pb (20.0), and As (1.0)	
C	200 g	Mn (4.0), Zn (1.5), Cd (0.1), Pb (0.6), and As (0.4)	
D	None	Mn (20.0), Zn (6.0), Cd (0.5), Pb (20.0), and As (1.0)	Metal control
E	None	Mn (4.0), Zn (1.5), Cd (0.1), Pb (0.6), and As (0.4)	Metal control

The plants were exposed to the solutions for 10 days, then the solutions were removed and replaced with fresh metal solutions. This process was repeated three times. Water samples for metal analysis were collected before and at 1 day, 2 days, 4 days, 7 days, and 10 days of each incubation. Plant samples were collected at the start and the end of the experiment.

In addition to the above experiments, the effect of pH (5, 7, and 9) on the growth of *E. diffusum* was also investigated during 2 weeks by designing 3 tanks with 200 g fresh weight of the plant. No metal was added in this experiment.

2.3. Sample preparation and analysis

Plant samples were well rinsed with deionized water and dried in a ventilated oven at 80°C for 48 hours. The dried samples were ground into fine powder using a mortar mill. Plant samples (100 mg per each) were digested with mixture (H_2O_2 : HNO_3 = 1 : 5) before elemental analysis. The concentrations of heavy metals in the plant and water samples were performed by the Atomic Absorption Spectrometer (AAS, Agilent 240FS with hydride generation accessory VGA77) at the Key Laboratory of Geoenvironment and Climate Change Response (GEO-CRE), VNU University of Science, Vietnam National University, Hanoi, Vietnam. Reagent blanks, internal standards, and duplicated samples were used where appropriate to ensure accuracy and precision in the analyses. The detectable limit of Pb, Zn, Mn, Cd, and As by the AAS was 0.01, 0.001, 0.002, 0.002, and 0.001 mg/L, respectively.

2.4. Statistical analysis

Statistical analyses of experimental data were performed using SPSS 20.0. Evaluation of significant differences among treatments was performed using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ indicating statistical significance.

2.5. Bioaccumulation factor

The bioconcentration factor (BCF), which can be used to estimate the potential of a plant for phytoremediation, is defined as the ratio of the total concentration of element in the whole plant to that in the growing solution (Zhu et al., 1999).

3. Results and discussion

3.1. Removal of heavy metals from solutions

Concentrations of heavy metals within control tanks showed a gradual increase (2.41±0.23%/day) due to evaporation of water during the experiment. Accordingly, all concentration data presented in this study were recalculated to eliminate the evaporation effect. The concentrations of heavy metals showed a rapid decline over the period of the experiment (Fig. 1) for every initial concentration and 3 times (10 days per each) of replaced solutions. The initial concentrations of Mn, Pb, Zn, As, and Cd in tank B were 20.0, 20.0, 6.0, 1.0, and 0.5 mg/L, respectively; which decreased to 11.9, 13.5, 4.0, 0.85, and 0.35 mg/L after 1 day of incubation and 3.9, 4.5, 1.3, 0.40, and 0.15 mg/L at the 10^{th} day of the experiment. The initial concentrations of Mn, Pb, Zn, As, and Cd in tank C were 4.0, 0.6, 1.5, 0.4, and 0.10 mg/L, respectively; which decreased to 1.3, 0.41, 0.64, 0.33, and 0.07 mg/L after 1 day of incubation and 0.02, 0.09, 0.03, 0.19, and 0.02 mg/L at the 10th day of the experiment. At higher levels of heavy metals (Tank B), the concentrations of all studied metals were higher than the allowable limits for wastewater (QCVN 40:2011/BTNMT) with the exception of Zn. By contrast, the concentrations of Mn, Pb, and Cd in the water met the allowable limits-type B (QCVN 40:2011/BTNMT) after the 2nd day of the experiment. Whereas, the concentrations of As in water in tank C exceeded the allowable limit-type B (QCVN 40:2011/BTNMT) during the experiment. These results indicated that *E. diffusum* demonstrated effective removal capacity with medium concentrations of heavy metals in water. In addition, limestone substrate for the plant growth in the experiment should be replaced by As-adsorbents such as laterite (Maji et al., 2008; Thao et al., 2016), red mud (Hülya et al., 2003; Pepper et al., 2018) or δ-FeOOH (Faria et al., 2014) to improve the removal efficiency of As from water by the system.

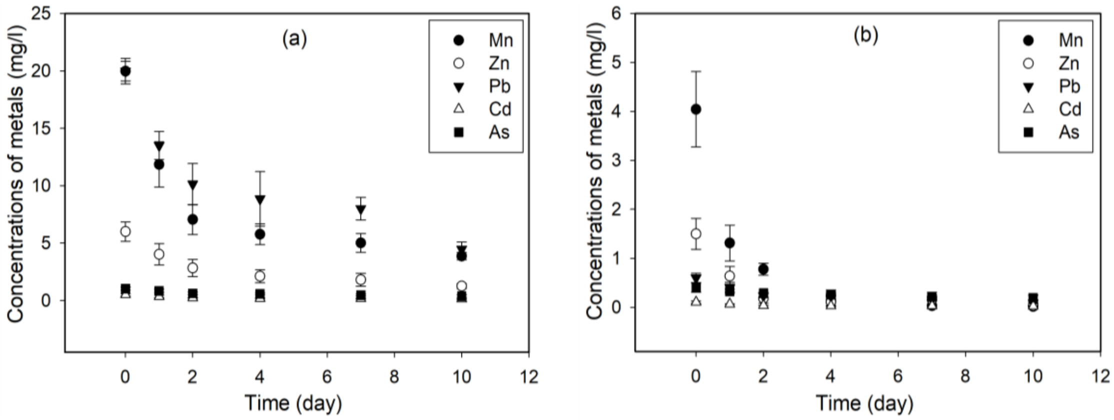

Figure 1. Concentrations of heavy metals in water in tank B (a) and tank C (b)

The highest removal efficiency of heavy metals from water was obtained after 1 day of incubation (for every time the metal solutions was replaced) (Fig. 2), indicating the optimal retention time for further design of constructed wetland using *E. diffusum*. The decreasing trend of metal removal from the first to the end of the experiment was also observed (Fig. 2). The removal efficiency of Mn, Zn, Cd, Pb, and As from tank B solution during 10 days of the experiment varied within 40.5-80.6, 32.9–79.0, 30.7–70.7, 32.4–77.6, 18.9–61.9%, respectively; that from tank C was 67.6–99.6, 57.5–97.9, 37.3–77.5, 31.3–85.3, and 19.3–51.7%. Arsenic was gradually removed from water solutions during the experiment. In contrast, considerably high amounts of heavy metals were removed from solutions after 2 days of incubation, indicating the sufficient time period for constructed wetland design. The result of metal control (Tanks D and E) demonstrated that the percentage of As, Pb, Cd, Zn, and Mn removed by the plant compared to total removal (Tanks B and C) fluctuated within 70.2–85.6, 16.1–22.2, 23.4–51.1, 50.5–71.0, and 28.7–58.3%, respectively. This result indicated that other amount of metal removal from solutions was possibly due to natural precipitation and adsorption into limestone substrates in tanks.

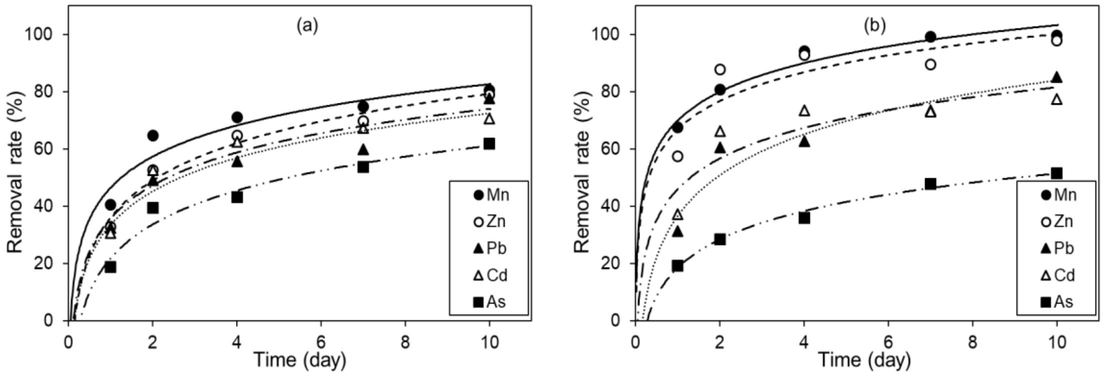

Figure 2. Cumulative removal efficiency of heavy metals from water in tank B (a) and tank C (b)

3.2. Accumulation of heavy metals in E. diffusum

E. diffusum was observed to adapt well at high concentrations of heavy metals with the growth of new shoots during 30 days of the experiment. The highest concentrations of Mn, Zn, Cd, Pb, and As in the plant roots were 7230, 1490, 174, 1170, and 274 mg/kg-DW, respectively; those in the shoots were 1960, 566, 33.9, 308, and 108 mg/kg-DW (Table 2). No significant difference of metal concentrations in the plant before and after the experiment at control tank (Tank A) was found. The concentrations of heavy metals in *E. diffusum* growing in tanks B and C were significantly higher than the initial concentrations ($p < 0.05$). In addition, concentrations of heavy metals in the plant roots were significantly higher than those in the shoots ($p < 0.05$) (Table 2). The concentrations of Mn and Zn in *E. diffusum* were higher than those of Cd, Pb, and As, reflecting the biological essential of Mn and Zn in the plant (Pahlsson, 1989). The lower rate of heavy metals accumulation in shoots compared with roots may reflect the poorer capacity of *E. diffusum* for translocating these elements. This result is consistent with previous studies on higher accumulation of metal in the root than that in the shoot (Conesa et al., 2006; Ghaderian and Ravandi, 2012; Monterroso et al., 2013).

Table 2. Concentrations of heavy metals in *E. diffusum* (mg/kg-DW)

Metal	Plant part	Initial	Tank A	Tank B	Tank C
Mn	Root	162±45	159±32	7230±710	3140±220
	Shoot	91.1±10.9	94.4±14.5	1960±350	829±118
Zn	Root	53.2±12.7	54.9±18.2	1490±270	985±174
	Shoot	67.3±13.2	72.7±21.2	566±107	234±74
Cd	Root	0.12±0.05	0.15±0.02	174±41	120±23
	Shoot	0.06±0.02	0.06±0.01	33.9±10.6	20.1±2.5
Pb	Root	62.5±17.6	64.9±20.0	1170±330	404±84
	Shoot	28.1±5.7	30.3±8.0	308±101	151±51
As	Root	12.9±2.3	13.4±1.8	274±64	80.0±13.7
	Shoot	10.4±2.9	11.9±2.3	108±23	57.0±5.7

Values present means ± standard deviations (N=3–6)

Previous study also reported high concentrations of heavy metals in *E. diffusum* when grown naturally in and around the Cho Don Pb-Zn mine (Ha et al., 2011). Although the concentrations of Mn, Pb, and As in the roots of *E. diffusum* in this laboratory experiment were lower than those reported by Ha et al. (2011), higher concentrations of all studied metals in the shoots in the present study were obtained. This result is possibly due to experimental design in this study with high concentrations of bioavailable metals in growing solutions which support the uptake and translocation to the shoots.

3.3. Potential of E. diffusum for phytoremediation

Being a hyperaccumulator is commonly mentioned as one of the most important criteria for a plant being used for phytoremediation (Reeves and Brooks, 1983; Brooks, 1998; Baker et al., 2000; Garbisu and Alkorta, 2001). Hyperaccumulators are defined as plants with leaves able to accumulate at least 100 mg/kg of Cd; 1000 mg/kg of As, Cu, Pb, Ni, Co, Se, or Cr; or 10,000 mg/kg of Mn and Zn (dry wt.) when grown in a metal-rich environment (Baker et al., 2000). In addition, high and fast-growing biomass, wide distribution, easy cultivation and harvesting are the common characteristics for potential plants being used for phytoremediation (Garbisu and Alkorta, 2001; Ma et al., 2001). Moreover, plants able to concentrate metals within the whole plant at concentrations 100 times higher than those in the growing solution (BCF higher than 100) should be considered good accumulators. The TF value should not be considered an important criterion regarding plant performance in the phytoremediation of water because both the roots and shoots of plant species are easily taken up after the remediation procedure.

The highest concentrations of Mn, Zn, Cd, Pb, and As in the shoots of *E. diffusum* were lower than the hyperaccumulating levels (Table 2) (Baker et al., 2000). The short incubation time in the present study may affect the maximum accumulation of these metals by the plant. It is noted that the highest concentrations of Mn, Zn, Cd, Pb, and As in the *E. diffusum* were higher than the upper limits reported by Kabata-Pendias (2011), indicating the capacity of this plant species to accumulate heavy metals.

The highest BCF values for Mn, Zn, Cd, Pb, and As for *E. diffusum* were 496, 406, 702, 463, and 191, respectively, implying the great capacity of the plant for removal of these metals from growing solutions.

Although *E. diffusum* has medium biomass, it can be adapted with heavily contaminated environment and grows naturally along the stream system near mining areas (Ha et al., 2011). Therefore, this plant species can be considered potential candidate for phytoremediation of water contaminated with heavy metals. However, the plant should be combined with proper substrates to improve the removal efficiency. In addition, the results on the effects of pH on the growth of *E. diffusum* showed that among the experimental pH values (5, 7, and 9), the plant growth best at pH=5, following by pH=7. By contrast, the plant demonstrated slow growth rate (Table 3) and abnormal darkening of shoots at pH=9, implying the poor adaptation of this plant at base environment.

Table 3. Effects of pH solutions on the growth of *E. diffusum*

Plant growth	Initial	pH=5	pH=7	pH=9
Height (cm)	35–40	38–44	37–42	35–40
Biomass (g)	200	237	216	188

4. Conclusions

E. diffusum is a potential species for phytoremediation of water contaminated with multi-metals, given its good adaptation, high tolerance, and accumulation of heavy metals, rapid growth, medium biomass, and high BCF values. Slight acid to neutral pH is a favorable for plant growth. This plant species should be grown in good As-adsorbent substrates to increase removal of As from solutions. Further studies should be conducted to understand the effect of different substrates on metal removal as well as assessment of phytoremediation feasibility of this plant at larger scales using real wastewater.

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