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Accumulation of Indium and other heavy metals by *Eleocharis acicularis*: An option for phytoremediation and phytomining

Nguyen Thi Hoang Ha [a], Masayuki Sakakibara [a,*], Sakae Sano [b]

[a] Graduate School of Science and Engineering, Ehime University, Matsuyama 790-8577, Japan
[b] Faculty of Education, Ehime University, Matsuyama 790-8577, Japan

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ABSTRACT

Eleocharis acicularis was exposed to different concentrations of In, Ag, Pb, Cu, Cd, and Zn in the laboratory to assess its capability in accumulating these metals. After 15 days, 477 mg/kg dry wt. of In was accumulated by the roots; concentrations of Ag, Pb, Cu, Cd, and Zn in the shoots were 326, 1120, 575, 195, and 213 mg/kg dry wt., respectively. The results indicate that *E. acicularis* has the ability to accumulate these metals from water, making it a good candidate species for phytoremediation and phytomining.

1. Introduction

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; Rai, 2009), and several plant species (i.e., *Alyssum bertolonii*, *Brassica juncea*, *Eichhornia crassipes*, and *Iberis intermedia*) have been considered for phytomining of Ni, Co, Tl, Ag, and Au (Pinto et al., 1987; Robinson et al., 1997; Brooks et al., 1998; Anderson et al., 1999; Boominathan et al., 2004). However, no previous study has investigated the capacity of aquatic plants from water to accumulate In and few studies have been on Ag accumulation (Pinto et al., 1987; Harris and Bali, 2008; Xu et al., 2010), or the use of plants for combined phytoremediation and phytomining (Robinson et al., 1997).

Phytomining has emerged as an environment-friendly technology that employs plants for the uptake of heavy metals (Brooks et al., 1998; Anderson et al., 1999). This technology involves growing plants on appropriate sites, harvesting the metal-accumulating plants, and treating the biomass to recover the metal. Phytomining has the potential to allow economic exploitation of low-grade surface ores or mineralized soils that are too metal-poor for conventional mining (Boominathan et al., 2004).

The aquatic macrophyte *Eleocharis acicularis* (dwarf hair grass) has been reported to accumulate multiple metals from mine tailings, mine drainage, and water, and to be a hyperaccumulator of As, Cu, Zn, and Pb (Sakakibara et al., 2006, 2009; Ha et al., 2009a). The objectives of this study were to investigate the ability of *E. acicularis* to accumulate In, Ag, Pb, Cd, Cu, and Zn and to assess its potential for phytoremediation and phytomining. Indium is of interest because it has found wide-spread application in flat-panel displays such as those used in laptop computers, flat-panel monitors and televisions, cell phones, and digital cameras, which reportedly account for over 70% of global output (Phipps et al., 2008). The high demand for this element could ultimately make phytomining a viable option.

2. Methods

2.1. Experimental plant and growth conditions

E. acicularis used in the present experiment was collected from a clump in an agricultural irrigation ditch in northwestern Shikoku, Japan. The plants were washed thoroughly with tap water followed by Milli-Q water to remove sediments, and placed into beakers filled with 2 l of Milli-Q water, receiving 16 h per day of white fluorescent light (54 μmol/m^2/s). A magnetic stirrer HS-5D (AS ONE Corporation, Japan) was used to make the solution in each beaker homogeneous. Air and water temperature were kept constant (24 ± 1 °C) during the experiment.

* Corresponding author. Address: Graduate School of Science and Engineering, Ehime University, Bunkyo-cho 2–5, Matsuyama 790-8577, Japan. Tel.: +81 89 927 9649; fax: +81 89 927 9640.
E-mail address: sakakiba@sci.ehime-u.ac.jp (M. Sakakibara).

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2.2. Heavy metal preparation

Nitrate salt solutions of In, Ag, Cu, Zn, Cd, and Pb (standard solutions for atomic absorption spectrometry, Kanto Chemical Co. Inc., Japan) were diluted in Milli-Q water to obtain the desired levels. The resulting solutions were adjusted to pH 5.5 with $Ca(OH)_2$. To simulate the nutrient-poor nature of water at most mine sites, no fertilizer was supplied in the experiment solutions of the present study.

2.3. Experimental setup and sampling

The laboratory experiment was carried out over 15 days. Fifty gram (fresh weight) of *E. acicularis* were placed in 2 l of water containing 0 (control), 0.1 (A), 0.2 (B) and 0.4 mg/l (C) of In and Ag, and 0 (control), 1.0 (A), 2.0 (B) and 4.0 mg/l (C) of Cu, Zn, Cd, and Pb. The plants were exposed to the solutions for 3 days, then the solutions were decanted and replaced with fresh metal solutions. This process was repeated five times. Metals were also added simultaneously to the beakers to yield concentrations of In, Ag, Cu, Zn, Cd, and Pb as per experiments (A), (B), and (C) above to evaluate the effects of water evaporation and precipitation of heavy metals during the experiment.

Water samples for metal analysis were collected before incubation and 1, 2, and 3 days after each change of solution. Plant samples were collected at the start of the experiment and again after 15 days.

2.4. Analytical methods

Harvested *E. acicularis* was washed well, thoroughly rinsed with deionized water, and dried in a ventilated oven at 80 °C for 48 h. The dried samples were ground into fine powder using a mortar mill. Plant samples (20 mg) were digested with a mixture (H_2O_2:HF:HNO_3 = 2:5:10) for analysis by inductively coupled plasma–mass spectrometer (ICP–MS). Elemental analyses of plant and water samples were performed by ICP–MS (Varian 820-MS) at the Integrated Center for Sciences, Ehime University, Japan.

Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in the ICP–MS analyses of elements. Certified reference materials NIES CRM No. 1 (National Institute for Environmental Studies, Japan) and SRM 1643e (National Institute of Standards Technology, USA) were used for quality control of the analytical procedure employed for plant and water samples, respectively, and the recoveries of elements ranged from 91% to 101% of the certified values.

2.5. Bioconcentration 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).

2.6. Statistical analysis

All of the analyses were repeated in triplicate. Statistical analyses of experimental data were performed using the SPSS 15.0 package. All data were tested for goodness of fit to a normal distribution, using a Kolmogorov–Smirnow one-sample test. Student's *t* tests were used to detect significant differences in the concentrations of heavy metals between the plant roots and shoots. Evaluation of significant differences among different treatments was performed using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ indicating statistical significance.

3. Results and discussion

All metal treatments had toxic effects on *E. acicularis*. Growth rate showed a progressive decrease when the plants were exposed to heavy metals (Fig. 1). The plants in contaminated water showed abnormal darkening of shoots. The extent of chlorosis was more severe in plants treated with higher concentrations of metals, as follows: (C) > (B) > (A) > control, but growth of new shoots and roots of *E. acicularis* in (C) was slightly faster than that in (A), (B), and control (data not shown).

The concentrations of each heavy metal within control beakers showed a gradual increase (1.48 ± 0.13%/day) due to the combined effects of water evaporation and metal precipitation. Accordingly, all concentration data presented in this study were recalculated to eliminate these effects.

3.1. Heavy metal removal from water and accumulation in E. acicularis

3.1.1. Indium

The concentrations of In in solution showed a rapid decline each time the plants were exposed to the fresh metal solution (Fig. 2). The highest removal rate of In from water was obtained after 1 day of incubation (Table 1) and little In was removed on the second and third days of incubation. Concentrations of In in plant roots were significantly higher than those in the shoots ($p < 0.05$), with the exception of the 0.1 mg In/l supply (Table 2). The lower rate of In accumulation in shoots compared with roots may reflect the poorer capacity for translocating In compared with the other heavy metals. Concentrations of In in *E. acicularis* were significantly higher than the initial concentration ($p < 0.001$) by factors of approximately 20,500, 41,900, and 59,300 for plants growing in 0.1, 0.2, and 0.4 mg/l supplies, respectively. We observed significant differences ($p < 0.001$) among concentrations of In in the three treatments. The highest In accumulation was 477 mg/kg dry wt. in the roots, and 353 mg/kg dry wt. in the shoots at 0.4 mg In/l (Table 2). Metal uptake increased with increasing metal concentration in the substrate and increasing exposure time (Fig. 2 and Table 2). Of the total In removed from the water, the percentages accumulated by *E. acicularis* were 83%, 84%, and 86% at 0.1, 0.2, and 0.4 mg/l, respectively. The remain In may have been removed during washing of the plants and precipitation from solutions due to chemical change after transplanting *E. acicularis*. For levels of 0.1, 0.2, and 0.4 mg In/l, BCF values for *E. acicularis* were 1170, 1310, and 1040, respectively.

Hyperaccumulators are defined as plants with leaves able to accumulate at least 100 mg/kg dry wt. Cd, 1000 mg/kg dry wt. Cu and Pb, or 10,000 mg/kg dry wt. Zn (Reeves and Baker, 2000). Indium and Ag occur naturally in plants at concentrations of

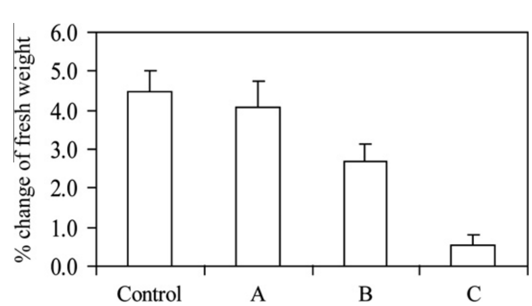

Fig. 1. Percent change of fresh weight of *Eleocharis acicularis* at control and at (A) Ag, In 0.1 mg/l; Cu, Zn, Cd, Pb 1.0 mg/l, (B) Ag, In 0.2 mg/l; Cu, Zn, Cd, Pb 2.0 mg/l, and (C) Ag, In 0.4 mg/l; Cu, Zn, Cd, Pb 4.0 mg/l. Error bars on columns are standard deviations ($n = 3$).

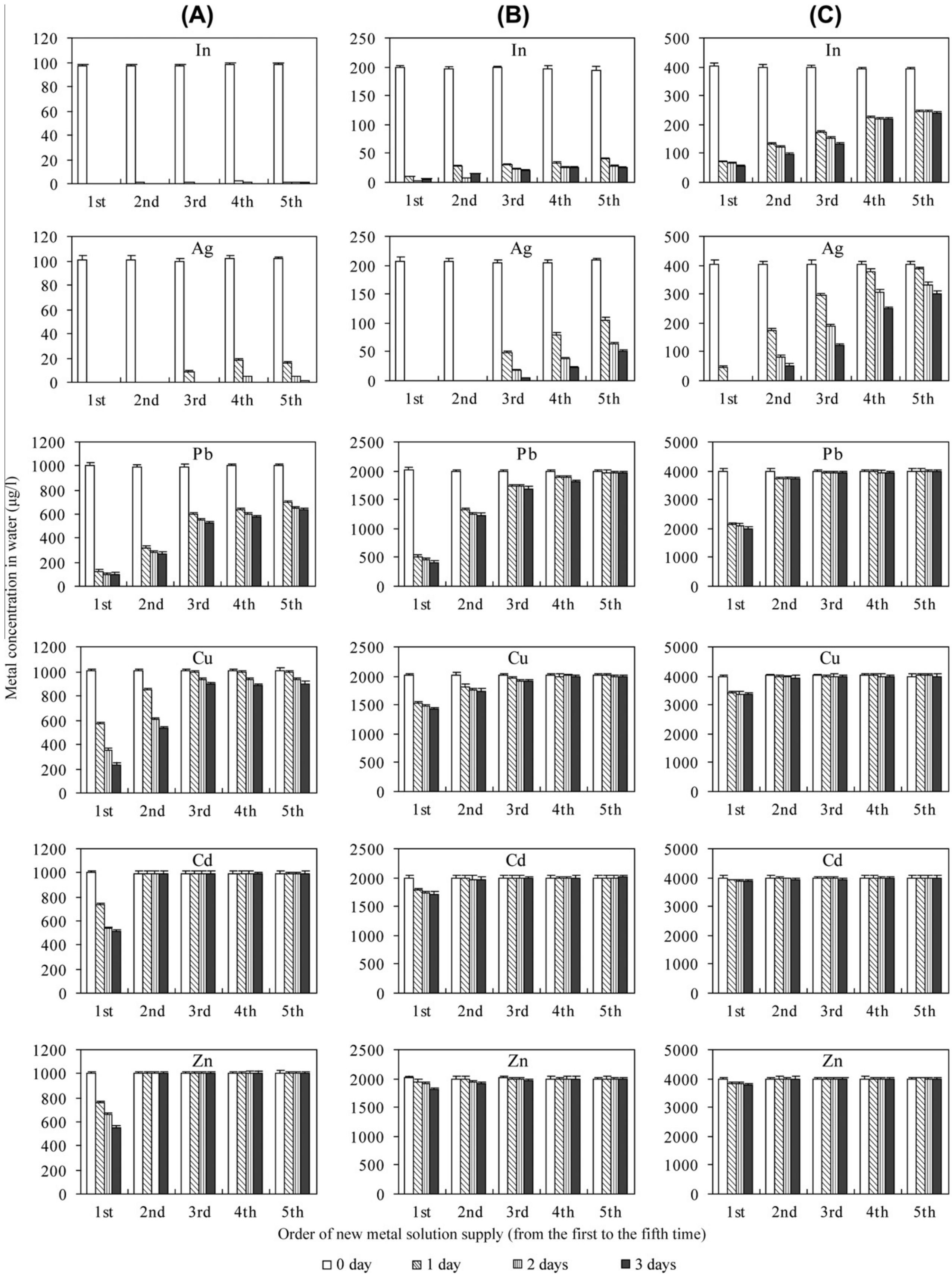

Fig. 2. Concentrations of heavy metals in water during 15 days of the laboratory experiment at different initial supply levels (A) Ag, In 0.1 mg/l; Cu, Zn, Cd, Pb 1.0 mg/l, (B) Ag, In 0.2 mg/l; Cu, Zn, Cd, Pb 2.0 mg/l, and (C) Ag, In 0.4 mg/l; Cu, Zn, Cd, Pb 4.0 mg/l. Metal solutions were changed regularly every 3 days. The time was again counted starting with day 1. Error bars on columns are standard deviations ($n = 3$).

Table 1
Heavy metal removal rates (μg/l/day) by *Eleocharis acicularis*.

Heavy metal	A (mg/l) In, Ag (0.1); Cu, Zn, Cd, Pb (1.0)		B (mg/l) In, Ag (0.2); Cu, Zn, Cd, Pb (2.0)		C (mg/l) In, Ag (0.4); Cu, Zn, Cd, Pb (4.0)	
	Average	1st day	Average	1st day	Average	1st day
In	32.3 ± 1.3*	96.8 ± 1.8	63.4 ± 2.9	190 ± 5	82.5 ± 6.4	331 ± 13
Ag	35.2 ± 2.1	103 ± 4	63.4 ± 3.6	206 ± 9	82.5 ± 7.6	356 ± 14
Pb	193 ± 11	880 ± 25	207 ± 13	1490 ± 35	166 ± 19	1850 ± 61
Cu	105 ± 9	441 ± 17	72.3 ± 9.9	485 ± 43	49.4 ± 8.0	595 ± 46
Cd	33.0 ± 6.9	258 ± 15	22.0 ± 6.4	215 ± 54	15.9 ± 4.5	91.0 ± 18.2
Zn	31.3 ± 8.5	241 ± 13	22.6 ± 5.1	54.0 ± 12.2	15.3 ± 3.9	149 ± 32

* Means ± standard deviations ($n = 3$).

Table 2
Concentrations of heavy metals (mg/kg dry wt.) in *Eleocharis acicularis* before the experiment (initial) and after 15 days (final)

Heavy metal	Concentrations of heavy metals				
	Initial	Final (Control)	Final (A)	Final (B)	Final (C)
Root					
In	0.011 ± 0.002^{a,*}	0.023 ± 0.003a	123 ± 12^{b}	300 ± 27^{c}	477 ± 24^{d}
Ag	0.093 ± 0.095^{a}	0.387 ± 0.115^{a}	161 ± 24^{b}	215 ± 84^{b}	208 ± 57^{b}
Pb	3.61 ± 0.29^{a}	4.39 ± 0.32^{a}	663 ± 47^{b}	820 ± 51^{c}	723 ± 64^{b,c}
Cu	25.1 ± 5.2^{a}	25.1 ± 6.2^{a}	463 ± 39^{d}	342 ± 15^{c}	282 ± 10^{b}
Cd	1.05 ± 0.10^{a}	0.953 ± 0.120^{a}	94.7 ± 6.5^{d}	75.8 ± 4.1^{c}	60.8 ± 2.3^{b}
Zn	84.9 ± 7.5^{a}	66.1 ± 6.7^{a}	95.2 ± 3.6^{b}	84.0 ± 9.4^{b}	67.5 ± 2.91^{a}
Shoot					
In	0.004 ± 0.001^{a}	0.025 ± 0.012^{a}	110 ± 10^{b}	226 ± 12^{c}	353 ± 26^{d}
Ag	0.047 ± 0.069^{a}	0.243 ± 0.189^{a}	88.7 ± 26.3^{b}	256 ± 84^{b,c}	326 ± 63^{c}
Pb	1.33 ± 0.15^{a}	2.65 ± 0.29^{a}	1020 ± 60^{b}	1120 ± 44^{b}	1030 ± 49^{b}
Cu	13.6 ± 5.5^{a}	17.8 ± 2.5^{a}	575 ± 33^{d}	433 ± 24^{c}	286 ± 12^{b}
Cd	0.201 ± 0.105^{a}	0.907 ± 0.213^{a}	195 ± 14^{d}	139 ± 13^{c}	100 ± 11^{b}
Zn	80.5 ± 6.6^{a}	75.9 ± 6.6^{a}	213 ± 23^{d}	150 ± 17^{c}	109 ± 11^{b}

Figures followed by different letters (a, b, c, and d) in the same row are significantly different at $p < 0.05$.
A: 0.1 mg/l In, Ag; 1.0 mg/l Cu, Zn, Cd, Pb; B: 0.2 mg/l In, 2.0 mg/l Ag; Cu, Zn, Cd, Pb; C: 0.4 mg/l In, Ag; 4.0 mg/l Cu, Zn, Cd, Pb.
* Means ± standard deviations ($n = 3$).

0.3–0.7 and <0.2 mg/kg dry wt., respectively (Kabata-Pendias and Pendias, 1992). A plant is considered to be a hyperaccumulator if it contains concentrations of the element that are about 100 times greater than those expected in non-accumulating plants growing in the same substrate (Brooks, 1998). Therefore, we suggest 100 mg/kg dry wt. In or Ag in plant matter would indicate that the species is a hyperaccumulator of these metals. To the best of our knowledge, no previous study has reported the hyperaccumulation of In in any plant species; therefore, *E. acicularis* is the first known hyperaccumulator of In, and it could be used for phytomining, possibly representing a new future supply of In.

3.1.2. Silver

Silver in water at 0.1, 0.2, and 0.4 mg/l also showed a rapid decline over time for all five sets of fresh water (Fig. 2). This may indicate the great ability of *E. acicularis* for cumulative accumulation of Ag by the plant. Like In, the highest removal rate of Ag from water was obtained after 1 day of incubation, for all initial concentrations (Table 1). Silver concentrations in *E. acicularis* were significantly higher (by factors of approximately 1780, 3360, and 3820) than the initial concentration for plants growing in 0.1, 0.2, and 0.4 mg/l, respectively ($p < 0.05$) (Table 2). The highest Ag accumulations in plants were 326 mg/kg dry wt. in the shoots at 0.4 mg/l (Table 2). Therefore, *E. acicularis* is a hyperaccumulator of Ag. Silver accumulation in plants showed an increasing trend with increasing metal concentration in the water. Of the total Ag removed from the water, the percentages accumulated by *E. acicularis* were 66%, 63%, and 53% at 0.1, 0.2, and 0.4 mg/l, respectively. For levels of 0.1, 0.2, and 0.4 mg Ag/l, BCF values for *E. acicularis* were 1250, 1180, and 670, respectively.

Xu et al. (2010) showed that *Potamogeton crispus* L. accumulated 29.3 μg/g of Ag when exposed to a concentration of 20 μM for 5 days. This concentration is lower than that achieved by *E. acicularis*; however, other wetland plants have been shown to accumulate higher amounts of Ag. Harris and Bali (2008) investigated the uptake of Ag by two common plants, *B. juncea* and *Medicago sativa*. *B. juncea* accumulated up to 12.4 wt.% Ag when exposed to an aqueous substrate containing 1000 ppm $AgNO_3$ for 72 h. A high amount of Ag (up to 13.6 wt.%) was accumulated in *M. sativa* when exposed to an aqueous substrate containing 10,000 ppm $AgNO_3$ for 24 h. However, the Ag BCF values attained by *B. juncea* and *M. sativa* were 6–67 and 10–124, respectively, much lower than those attained by *E. acicularis* (670–1250), even the competition effect among the six heavy metals in the present study.

3.1.3. Lead

The concentrations of Pb in water at an initial concentration of 1.0 mg/l showed a slight decline over time for all five sets of water (Fig. 2). However, the decrease in concentrations in the experiments with initial concentrations of 2.0 and 4.0 mg/l occurred mainly during the third and first sets of fresh water, respectively. This may reflect that *E. acicularis* is saturated with Pb at these supply levels. During 15 days of the experiment, the highest removals of Pb from the water were obtained after 1 day of incubation, for all concentrations (Table 1). Lead concentrations in *E. acicularis* were approximately 340-, 387-, and 356-times higher than the initial concentrations ($p < 0.001$) for plants growing at 1.0, 2.0, and 4.0 mg/l, respectively. We found no significant differences in the concentrations of Pb in the plant shoots among the three treatments (Table 2). The highest concentration of Pb (1120 mg/kg dry wt.) was obtained in shoots of *E. acicularis* growing in the 2.0 mg/l water (Table 2). Of the total Pb removed from the water, the percentages accumulated by *E. acicularis* were 81–90%. The highest BCF value for *E. acicularis* for Pb (840) was obtained at the 1.0 mg/l supply level.

The highest concentration of Pb in *E. acicularis* in the present study exceeds the hyperaccumulation level set for Pb. Moreover, the amount of Pb removed from water and accumulated in *E. acicularis* remained constant during the experiment; therefore, this plant showed high tolerance to Pb toxicity. However, the greatest amount of Pb accumulated by *E. acicularis* in the present study was lower than that accumulated by other wetland plants. Muramoto and Oki (1983) reported that water hyacinth (*E. crassipes*) accumulated 25.8 g Pb/kg when supplied with 8 mg Pb/l. Peng et al. (2008) reported maximum values of 3030 and 2550 mg Pb/kg dry wt. in *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq., respectively, after 2 h of hydroponic treatment.

Other wetland plants accumulate lower amounts of Pb than did *E. acicularis*. Zayed et al. (1998) reported a Pb concentration of 0.63 g Pb/kg in duckweed (*Lemna minor* L.) for a supply level of 10 mg Pb/l after 8 days. Liu et al. (2007) reported Pb concentrations in whole plants ranging from 4.89 (*Phragmites communis* Trin.) to 148 mg/kg dry wt. (*Monochoria vaginalis* (Burm. f.) Presl) after 60 days. Miretzky et al. (2004) reported maximum values of 227 and 78 mg Pb/kg in *Pistia stratiotes* and *Spirodela intermedia* after 15 days of incubation in 4.0 and 1.0 mg Pb/l, respectively.

3.1.4. Copper

The concentrations of Cu in water at an initial concentration of 1.0 mg/l showed a slight decline over time for all five sets of water (Fig. 2). However, the decrease in concentrations in the experiments with initial concentrations of 2.0 and 4.0 mg/l occurred mainly during the third and first sets of fresh water, respectively. This may reflect that *E. acicularis* is saturated with Cu at these supply levels. During the 15 days of the experiment, the highest removals of Cu from the water were obtained after 1 day of incubation, for all concentrations (Table 1). Copper concentrations in *E. acicularis* were 27-, 20-, and 15-times higher than the initial concentrations ($p < 0.001$) for plants growing at 1.0, 2.0, and 4.0 mg/l, respectively. We found significant differences ($p < 0.05$) in the concentrations of Cu among the three treatments (Table 2). The highest concentration of Cu (575 mg/kg dry wt.) was obtained in shoots of *E. acicularis* growing in the 1.0 mg/l water (Table 2). Of the total Cu removed from the water, the percentages accumulated by *E. acicularis* were 94–98%, respectively. The highest BCF value for *E. acicularis* for Cu (520) was obtained at the 1.0 mg/l supply level.

L. minor L. is reported to be a good accumulator of Cu, with concentrations of 16 g/kg dry wt. after 4 days of incubation (Bassi and Sharma, 1993). Zayed et al. (1998) measured a maximum of 3.36 g/kg dry wt. Cu in *L. minor* L. growing in 10 mg Cu/l for 8 days. Zhu et al. (1999) assessed the phytoaccumulation of Cu from water containing 10 mg Cu/l, reporting maximum concentrations in shoots and roots of 130 and 2800 mg/kg dry wt., respectively, after 14 days. In another study, Mishra and Tripathi (2008) reported that the highest concentrations of Cu in three aquatic macrophytes (*P. stratiotes* L., *Spirodela polyrhiza*, and *E. crassipes*) were 0.875, 0.186, and 2.75 mg/g dry wt., respectively, over 15 days. Peng et al. (2008) reported maximum Cu concentrations of 1130 and 945 mg/kg dry wt. in *P. pectinatus* L. and *P. malaianus* Miq., respectively, after 2 h of hydroponic treatment. Kamal et al. (2004) observed maximum Cu concentrations of 304, 848, and 314 mg/kg dry wt. in parrot feather (*Myriophylhum aquaticum*), creeping primrose (*Ludwigina palustris*), and water mint (*Mentha aquatic*), respectively, after 21 days of incubation. In a study of *E. crassipes*, Miretzky et al. (2004) reported maximum values of 0.965 and 0.431 mg Cu/g in *P. stratiotes* and *S. intermedia* after 15 days of incubation in 4.0 and 1.0 mg Cu/l, respectively.

In the present work, *E. acicularis* appears to be a poor accumulator of Cu. However, in a previous study, Sakakibara et al. (2009) found that *E. acicularis* accumulated 20,200 mg/kg dry wt. of Cu after 2 months of transplanting at an abandoned mine drainage site in Japan. In addition, BCF values for Cu in *E. acicularis* have been reported to be 21,500 and 31,300 in a laboratory experiment and field trial at a mine site, respectively (Ha et al., 2009b). These BCF values are much higher than those reported for other aquatic plants; e.g., 3000 (Bassi and Sharma, 1993) and 200–800 (Zayed et al., 1998) for *L. minor* L.

3.1.5. Cadmium

The concentrations of Cd in water with initial metal concentrations of 1.0, 2.0, and 4.0 mg/l showed a slight decline during the first 3 days of incubation, especially for 1.0 mg/l (Fig. 2). For the second to the fifth sets of fresh water, Cd concentrations in the water remained constant. This may reflect that the plant is saturated with Cd for all initial supply levels in this study. The highest removals of Cd from the water were obtained after 1 day of incubation, for all concentrations (Table 1). Concentrations of Cd were significantly higher in plant shoots than in roots ($p < 0.01$) (Table 2). Cadmium concentrations in *E. acicularis* were significantly higher than the initial concentration ($p < 0.001$), by factors of approximately 230, 171, and 128 for plants growing in the 1.0, 2.0, and 4.0 mg/l experiments, respectively. The highest concentration of Cd was 195 mg/kg dry wt. respectively, in shoots of *E. acicularis* growing in 1.0 mg/l. Cadmium accumulation in the plants showed a declining trend with increasing metal concentration in the water. This result indicates that solutions with concentrations of Cd exceeding 1.0 mg/l may inhibit plant growth and metal uptake. This is supported by the darkening of shoots and a slight reduction in growth over time (Fig. 1). Of the total Cd removed from the water, the percentages accumulated by *E. acicularis* were 82–89%, respectively. The highest BCF value for *E. acicularis* for Cd (145) was obtained at the 1.0 mg/l level.

The highest concentration of Cd in *E. acicularis* in the present study exceeds the level proposed to define hyperaccumulation. Results of Cd accumulation and removal have also been reported in previous studies. The greatest amount of Cd accumulated by a wetland plant species is 36 g/kg, by *E. crassipes* (Muramoto and Oki, 1983). Zayed et al. (1998) reported high concentrations of Cd (13 g/kg) in *L. minor* L. when supplied with 10 mg Cd/l. Zhu et al. (1999) reported that *E. crassipes* accumulated 6103 and 371 mg/kg dry wt. of Cd in roots and shoots, respectively when 10 mg Cd/l was supplied. Mishra and Tripathi (2008) found that the highest concentrations of Cd in three aquatic macrophytes (*P. stratiotes* L., *S. polyrhiza*, and *E. crassipes*) were 0.39, 0.25, and 0.31 mg/g dry wt., respectively, over 15 days. Sivaci et al. (2004) reported maximum concentrations of 80 and 150 mg Cd/kg dry wt. in the shoots of *Myriophyllum spicatum* L. and *Myriophyllum triphyllum*, respectively after being exposed to 16 mg Cd/l for 4 days. Peng et al. (2008) obtained maximum concentrations of 202 and 178 mg Cd/kg dry wt. in *P. pectinatus* L. and *P. malaianus* Miq., respectively, after 2 h of hydroponic treatment. Finally, Liu et al. (2007) reported low Cd concentrations in whole plants, ranging from 4.98 (*P. communis* Trin.) to 36.3 mg/kg dry wt. (*M. vaginalis* (Burm. f.) Presl) after 60 days.

3.1.6. Zinc

The concentrations of Zn in water with initial metal concentrations of 1.0, 2.0, and 4.0 mg/l showed a slight decline during the first 3 days of incubation, especially for 1.0 mg/l (Fig. 2). For the second to the fifth sets of fresh water, Zn concentrations in the water remained constant. This may reflect that the plant is saturated with Zn for all initial supply levels in this study. Like other heavy metals in the present study, the highest removals of Zn from the water were obtained after 1 day of incubation, for all concentrations (Table 1). This is in line with the finding of rapid adsorption kinetics of *E. acicularis* reported by Miretzky et al. (2010). This factor has significant practical importance in estimating the amount of plant and growing system for a given project, and makes *E. acicularis* a promising plant species for the phytoremediation and phytomining of contaminated sites marked by flowing water.

Concentrations of Zn were significantly higher in plant shoots than in roots ($p < 0.01$) (Table 2). Zinc concentrations in *E. acicularis* showed a slight increase for plants in the 2.0 and 4.0 mg/l experiments, and were two times higher than the initial concentration for plants growing in the 1.0 mg/l experiment. The highest concentration of Zn was 213 mg/kg dry wt. respectively, in shoots of *E. acicularis* growing in 1.0 mg/l. Zinc accumulation in the plants showed a declining trend with increasing metal concentration in the water. This result indicates that solutions with concentrations of Zn exceeding 1.0 mg/l may inhibit plant growth and metal uptake. Of the total Zn removed from the water, the percentages accumulated by *E. acicularis* were 92–98%, respectively. The highest BCF value for *E. acicularis* for Zn (154) was obtained at the 1.0 mg/l level.

Zinc accumulation has been reported in previous studies. The highest concentrations of Zn in duckweed are as much as 30 g/kg dry wt. when grown in medium containing 10 mg Zn/l (Bassi and Sharma, 1993). Kamal et al. (2004) reported that the highest concentrations of Zn reached 549, 1243, and 1498 mg/kg dry wt. for parrot feather (*M. aquaticum*), creeping primrose (*L. palustris*), and water mint (*M. aquatic*), respectively, after 21 days of incubation. Mishra and Tripathi (2008) reported maximum concentrations of Zn in three aquatic macrophytes (*P. stratiotes* L., *S. polyrhiza*, and *E. crassipes*) of 0.98, 1.5, and 6.51 mg/g dry wt., respectively, over 15 days. Peng et al. (2008) measured maximum values of 1320 and 1230 mg Zn/kg dry wt. in *P. pectinatus* L. and *P. malaianus* Miq., respectively, after 2 h of hydroponic treatment. Liu et al. (2007) reported Zn concentrations in whole plants ranging from 47.3 (*P. communis* Trin.) to 349 mg/kg dry wt. (*Isachne globosa* (Thunb.) Kuntze) after 60 days of experiment. Miretzky et al. (2004) found maximum concentrations of 1.214 and 0.479 mg Zn/g in *P. stratiotes* and *S. intermedia*, respectively after 15 days of incubation in 4.0 and 1.0 mg Zn/l, respectively. Finally, Ha et al. (2009b) obtained a maximum value of 20.5 mg/kg dry wt. in *E. acicularis* after 10 days of exposure to 3.08 μg Zn/l.

In the present study, *E. acicularis* appears to be a poor accumulator of Zn; however, it has been reported that *E. acicularis* accumulated 13,700 mg/kg dry wt. of Zn after 2 months of transplanting at an abandoned mine drainage site in Japan (Sakakibara et al., 2009). In addition, BCF values for Zn in *E. acicularis* are reported to be 23,900 and 90,400, as obtained in a laboratory experiment and in a field trial at a mine site, respectively (Ha et al., 2009b). Lower BCF values for Zn and Cu accumulated in the plants in the present study may reflect the effect of toxicity when the plant is exposed to high concentrations of these metals in solution (1.0, 2.0, and 4.0 mg/l), a lack of fertilizer supply, and uptake competition among the six metals in solution (Cu, Zn, Cd, Pb, Ag, and In).

3.2. Potential of E. acicularis for phytoremediation and phytomining

An ideal plant for phytoremediation would have shoots with a high capacity to accumulate heavy metals/metalloids (hyperaccumulator), have a high biomass and rapid growth, and BCF and TF (translocation factor) values higher than 1 (Garbisu and Alkorta, 2001). Of these characteristics, the concentration-based criterion identifies those plants with potential for phytoremediation (Reeves, 2006). In addition, plants able to concentrate metals within the whole plant at concentrations 100 times higher than that in the growing solution (BCF) should be considered good accumulators. The TF value should not be considered an important criterion regarding phytoremediation of water because both roots and shoots can be harvested easily. The data presented in this study indicate that *E. acicularis* might be a good candidate species for phytoremediation of water contaminated with multiple heavy metals (Pb, Cd, Cu, and Zn), preferably at metal concentrations less than 1.0 mg/l.

The In and Ag accumulation levels observed in this study also suggest that *E. acicularis* has potential for phytomining. Since phytomining alone might currently not be an economically viable process, its combination with phytoremediation could make the process more cost-effective; however, studies on the accumulation of rare metals such as Ag and In will have to be carried out under field conditions, and the use of fertilizers and chelators to optimize uptake will have to be explored.

4. Conclusions

E. acicularis, which is easily cultivated and controlled and is well adapted to contaminated environments, hyper-accumulated In and Ag, and is thus a potential candidate for phytomining. Its accumulation of Cu, Zn, Cd, and Pb also suggests its potential for phytoremediation of water contaminated with these heavy metals at concentrations of less than 1.0 mg/l.

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