Phytoremediation of heavy metal-contaminated river water by aquatic macrophyte *Eleocharis acicularis* in a mine site, southwestern Japan

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1 Introduction

Environmental contamination is one of the most important factors responsible for the degradation of surface environments, and heavy metals play a dominant role in this degradation. The mining of precious metals, coal, and other commodities is an important part of the economy of many areas and countries. However, mining activities affect human health via the contamination of surface water, groundwater, atmosphere, and soil, depending on the method of extraction.

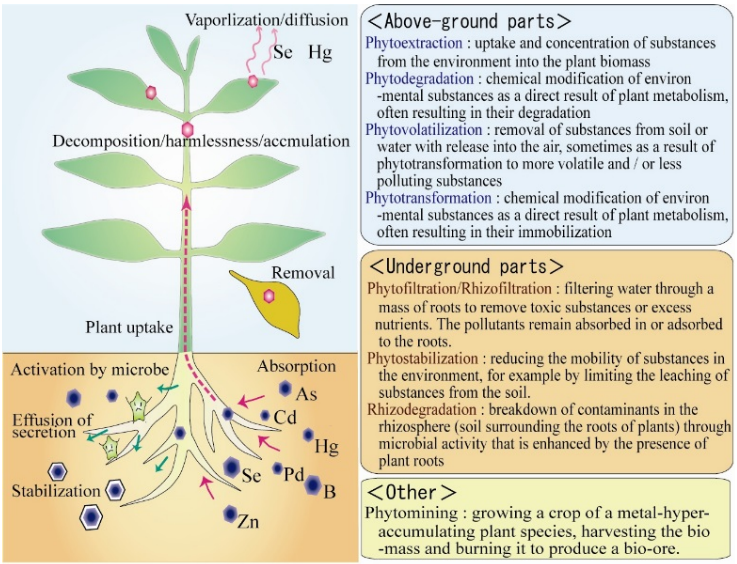
Heavy metals that are released into the environment and that pass through the food chain can have severe toxic effects on living organisms; their impact can be especially large in areas with high population densities. Many heavy metal elements are highly toxic in both their elemental and soluble salt forms. Thus, the presence of heavy metal pollutants in water bodies poses serious risks to the health of humans and ecosystems.

Phytoremediation is a technology that uses plants to remove pollutants from the environment (Fig. 1). In addition to being economic, energy-efficient, and environmentally friendly, phytoremediation can be applied to large areas and is useful for treating a wide variety of contaminants (metals, radionuclides, and organic substances) and growth media (soil, sludge, sediment, and water).

Previous studies have attempted to develop phytoremediation techniques based on laboratory and field trials 1), to elucidate the molecular mechanisms that are the basis for purification processes 2) 3).

The aquatic macrophyte *Eleocharis acicularis*, which belongs to the Cyperaceae family, was reported to be a hyperaccumulator of Cu (based on pot experiments in a greenhouse) 4) 5), a hyperaccumulator of In, Ag, Cd, and Pb (based on a laboratory experiment) 6), and a hyperaccumulator of Pb (based on a field experiment)[7][8]. *E. acicularis* is a strong plant candidate for phytoremediation of water and soils contaminated by heavy metals and metalloids[4][5][6][7][8][9]. However, no previous study has demonstrated its ability to hyperaccumulate heavy metals (i.e., Cu, Zn, Cd, and As) under natural conditions.

The purpose of this study was to design an efficient and feasible method for cultivating *E. acicularis* for the purpose of heavy metal remediation at a mine tailings site in southwestern Japan (Fig. 2).



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Phytoextraction : uptake and concentration of substances from the environment into the plant biomass

Phytodegradation : chemical modification of environmental substances as a direct result of plant metabolism, often resulting in their degradation

Phytovolatilization : removal of substances from soil or water with release into the air, sometimes as a result of phytotransformation to more volatile and / or less polluting substances

Phytotransformation : chemical modification of environmental substances as a direct result of plant metabolism, often resulting in their immobilization

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Phytofiltration/Rhizofiltration : filtering water through a mass of roots to remove toxic substances or excess nutrients. The pollutants remain absorbed in or adsorbed to the roots.

Phytostabilization : reducing the mobility of substances in the environment, for example by limiting the leaching of substances from the soil.

Rhizodegradation : breakdown of contaminants in the rhizosphere (soil surrounding the roots of plants) through microbial activity that is enhanced by the presence of plant roots

<Other>

Phytomining : growing a crop of a metal-hyper-accumulating plant species, harvesting the biomass and burning it to produce a bio-ore.

Figure 1. Illustration of fundamental phytoremediation pathways.

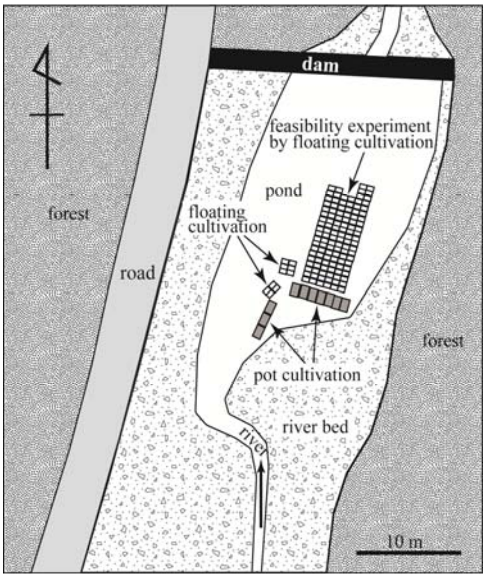

Figure 2. Sketch map of the cultivation site in southwestern Japan.

2 Materials and methods

2.1 Experiment site and cultivation method

The study was conducted on a heavy-metal-contaminated river that flows from the mine tailings area of an abandoned mine, at the location of a pond created by a dam. The site is well suited for phytoremediation experiments using aquatic plants because the flow rate in the river (width from 50 cm to 1 m, depth 10 to 20 cm, flow rate about 10 cm/s) is relatively constant and water levels are relatively unaffected by torrential rains that have plagued many areas in Japan in recent years. The maximum water depth of the pond is approximately 1 m, and argillaceous deposits at the bottom of the pond contain high concentrations of heavy metals.

In this experiment, *E. acicularis* was cultivated in the pond using both the “pot cultivation method” and the “floating cultivation method” (Fig. 3). Samples of *E. acicularis* were collected monthly, and the heavy metal concentrations in the shoots and roots were measured. In addition, the total mass of *E. acicularis* was measured before and after cultivation. The goal of the feasibility experiment was to lower the heavy metal concentrations in the river water using the floating cultivation method.

Pot cultivation method: For the pot cultivation experiments, plastic pots were filled with sand and approximately 50 g of *E. acicularis* was replanted in each pot. The pots were placed in plastic containers and fixed in the marsh at the front edge of the pond, adjacent to the dam. Changes in the biomass of *E.*

acicularis, and the concentrations of heavy metals in *E. acicularis*, were measured monthly. The experiment was conducted from October 2009 to November 2010 (approximately 11 months).

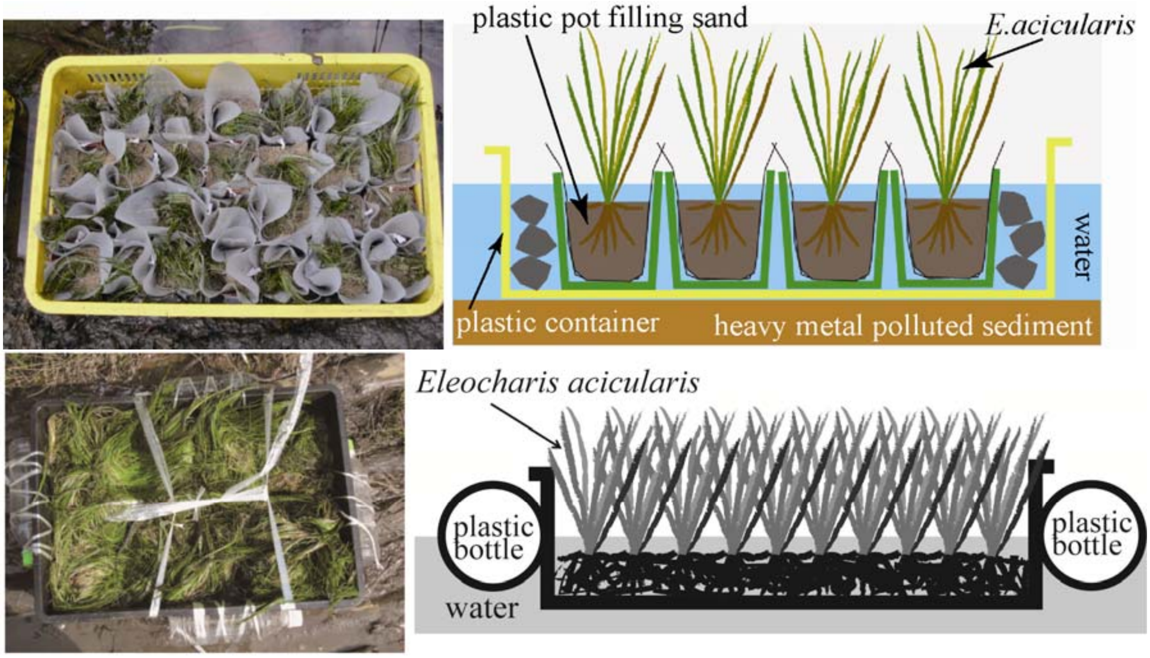

Figure 3. Illustration and photograph of the pot and floating cultivation methods of *E. acicularis*. a) pot caltivation method, b) floating cultivation method.

Floating cultivation method: For the floating cultivation experiments, plastic baskets (length 36 cm x width 50 cm x height 11 cm) were filled with approximately 4 kg of *E. acicularis*; the baskets were mounted on plastic bottles such that they floated in the pond. The experiment was conducted from April to November 2010 (approximately 7 months).

The feasibility experiment was conducted in the pond using the floating cultivation method (Fig. 4). The floating plastic baskets were each filled with approximately 3 kg of *E. acicularis*. Approximately 180 kg of *E. acicularis* was replanted in a first replanting at September 2010, and the same amount was replanted in a second replanting at October 2010. In addition, water samples were collected 2 months before and 1 month before the cultivating from the river upstream of the pond, from the pond, and from the river downstream of the pond. The heavy metal concentrations in the water samples were also measured. In addition, *E. acicularis* samples were collected in November 2010. The *E. acicularis* used in the experiment was found growing naturally in a river on the Matsuyama Plain, Ehime Prefecture, Japan.

Water samples were collected in polyethylene bottles at three locations (upper stream, pond, and lower stream) in the pond. Sample bottles and other containers were soaked in 5% nitric acid and rinsed with deionized water several times prior to use. After collection, water samples were filtered through membrane filters (pore diameter of 0.45mm). The filtered samples were preserved by acidification with nitric acid and were stored in polyethylene bottles.

Figure 4. Photograph of the feasibility experiment in the dam pond.

2.2 Analytical methods

Metal concentrations in the water were determined using inductively coupled plasma–mass spectrometry (ICP–MS) (ELAN 6000, Perkin Elmer) at the Integrated Center for Sciences, Ehime University, Japan. The quality of analyses was verified using reference materials (NIST 1643e) from the National Institute of Standards and Technology and appropriate replicates. Plant samples (30 mg) were digested using 1 mL of HNO3, and were heated in a microwave (150 W) for particle-induced X-ray emission (PIXE) analysis. Concentrations of heavy metals and silicon in the plant tissues were determined by PIXE analysis at the Cyclotron Center of Iwate Medical University, Japan. The analytical accuracy and precision of analyses were verified using environmental sample NIES CRM No. 1. The results were accurate and indicated a high level of reproducibility 5).

Soil samples (3.1 g) were finely ground and homogenized for analysis by energy-dispersive fluorescent X-ray spectrometry (Epsilon 5) at the Integrated Center for Sciences, Ehime University.

2.3 Statistical Analysis, Quality Assurance, and Quality Control

The chemical analyses were run in triplicate to evaluate experimental reproducibility. Statistical parameters were determined using Minitab, v. 14". The 95% confidence level ($P<0.05$) of each parameter in a given sample was used to estimate the margin of error. A certified standard reference plant material (NIES CRM No. 1) was used for calibration and quality assurance for each analytical batch.

Reagent blanks and analytical triplicates were also used where appropriate to ensure the accuracy and precision of the analyses. The recovery rates were 90%–99% for all of the metals and metalloids in the reference materials.

3 Results and discussion

3.1 Cultivation experiments

Results of the pot cultivation method: The first growth shoots of *E. acicularis* replanted in the pot cultivation method were withered on account of heavy metal toxicity attributed to the sediments in the pond; the next leaves, however, grew to approximately 10 cm long. The maximum heavy metal concentrations in the shoots were as follows (all units in mg/kg-dry weight, DW): Cu, 1050; Zn, 2800; As, 831; Cd, 153; and Pb, 78.0. The heavy metal concentrations in the roots were as follows (in mg/kg-DW): Cu, 605; Zn, 2510; As, 3700; Cd, 44.0; and Pb, 133. The As concentrations exceeded the concentration level for hyperaccumulator plants. However, during the experiment, the shoots of *E. acicularis* were covered with sediment when the water levels in the river and pond increased during torrential rainfall, thus delaying their growth.

Results of the floating cultivation method: The biomass of *E. acicularis* cultivated in the floating cultivation experiment was approximately 1.7 times greater at the end of the experiment than at the beginning of the experiment. The maximum heavy metal concentrations were as follows (in mg/kg-DW): Cu, 1420; Zn, 2860; As, 121; Cd, 31.9; and Pb, 111 for the shoots; and Cu, 1130; Zn, 2560; As, 1470; Cd, 52.3; and Pb, 76.2 for the roots. Cu and As exceeded the minimum concentrations (>1,000 mg/kg) for hyperaccumulator plants. In particular, the floating cultivation method allowed for stable, uninterrupted growth, despite the torrential rainfall.

As a result of the experiments, it was concluded that phytoremediation using the floating cultivation method was more practical and more effective than phytoremediation using the pot cultivation method, when considering effects such as water flow increases and water level changes due to torrential rains. The *E. acicularis* cultivated using the pot cultivation method was covered in clay from the bottom of the pond, on account of increased water levels caused by rain, because the containers were fixed to the bottom of the pond; as a result, the plants were covered by sediment and their growth was compromised. Using the floating cultivation method, *E. acicularis* was able to grow in an essentially stable environment, as the baskets were floating and were linked to prevent scattering.

3.2 Bioconcentration Factor (BCF) of *E. acicularis*

Bioconcentration Factor (BCF) is a more important measure than the actual concentration of a heavy metal in a shoot, when considering the potential of a given biological species for phytoextraction. BCF was calculated from the initial concentrations of an element in the culture medium, as follows:

$$\mathrm{BCF_W} = \mathrm{C_{shoots}} / \mathrm{C_{water}}$$

where $\mathrm{C_{shoots}}$ denotes the trace element concentration (mg/kg) in the plant shoots at harvest, $\mathrm{C_{water}}$ is the initial concentration of the element in water (mg/L), and $\mathrm{BCF_W}$ is a dimensionless parameter related to the phytoremediation capacity (higher values reflect higher capacities). In metal-excluder plants, $\mathrm{BCF_W}$ is typically less than 100, whereas in metal-accumulator species it is generally greater than 100; $\mathrm{BCF_W}$ values greater than 100 have been used as a guideline for indicating the positive potential of a plant species for phytoremediation. Figure 5 shows the relationship between the $\mathrm{BCF_W}$ value of *E. acicularis* and the concentrations of total Mn, Cu, Zn, As, Cd, and Pb in the water. *E. acicularis* shows maximum $\mathrm{BCF_W}$ values for Mn (26,000), Cu (86,800), Zn (4820), As (4370), Cd (8420), and Pb (1,490,000). Many samples of *E. acicularis* in the present field experiment yielded $\mathrm{BCF_W}$ values greater than 100 (Mn, Cu, Zn, As, Cd, and Pb). Based on these high $\mathrm{BCF_W}$ values, it was concluded that *E. acicularis* has the potential to phytoextract Mn, As, Cu, and Pb (Fig. 5).

BCFw values tend to decrease with increasing concentrations of heavy metals in the water. The relationship between BCFW and metal concentration, which is negative and log-linear, indicates a diminishing efficiency of Mn, Cu, Zn, As, Cd, and Pb accumulation with increasing of heavy metal concentrations of water. This decreasing trend may be due to the toxic effects of Mn, Cu, Zn, As, Cd, and Pb on *E. acicularis*.

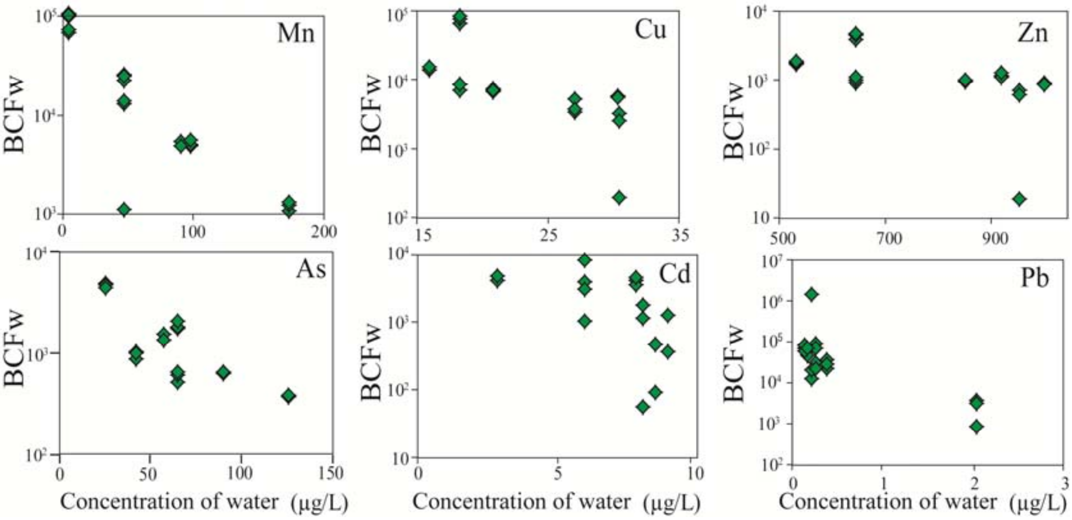

Figure 5. Relationship between BCFW values and heavy metal concentration of the river water.

3.3 Evaluation of phytoremediation by *E. acicularis* based on the feasibility experiment

By the end of the feasibility experiment, the Cu concentration in the river downstream of the pond dropped to approximately half the level at the start of the experiment, and the concentration of As dropped to approximately one-quarter of the original level, despite fluctuations in the concentrations of Cu and As in the river upstream of the pond. Furthermore, the cultivation by the floating cultivation method are simple and its costs are much lower than those of other environmental remediation techniques. Thus, it was concluded that the floating cultivation method using *E. acicularis* is effective for phytoremediation of water contaminated by heavy metals. The current field feasibility experiment showed that concentrations of Cu and As were reduced in the water downstream of the pond by 5 months after the start of the experiment; this reduction can be explained by the absorption and accumulation of Cu and As by *E. acicularis*, based on the net effects of material balances between (1) water volume, flow rate, and heavy metal concentrations in the river, and (2) the biomass, heavy metal concentrations, and heavy metal absorption rates of *E. acicularis*. Even though from October the amount of precipitation was low and the heavy metal concentration of Cu in the river water located upstream of the pond was high, it can be inferred that phytoremediation using *E. acicularis* was functioning in the open system of this research site, based on the fact that the heavy metal concentrations downstream of the pond were reduced by more than half during the course of the experiment. (Fig. 6)

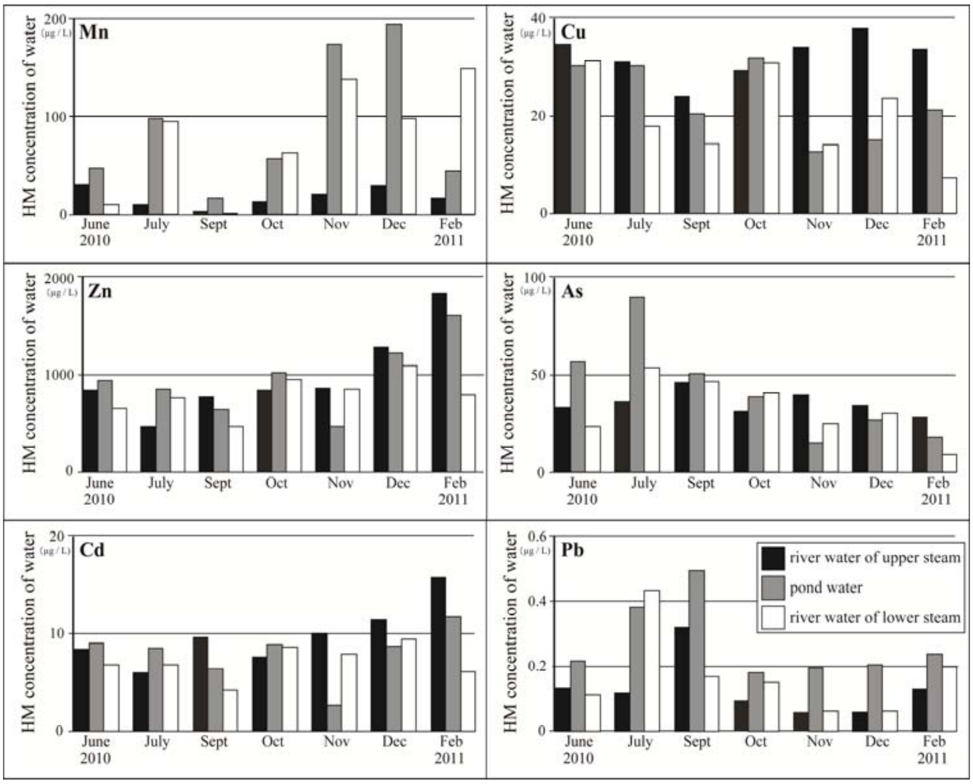

Figure 6. Heavy metal concentrations of the river and pond water during the feasibility experiment.

4 Conclusions

In this study, the practicality of two cultivation methods, pot cultivation and floating cultivation, was evaluated on the basis of small-scale field experiments. The floating cultivation method is more suitable than pot cultivation for phytoremediation of heavy metal-polluted river water by *E. acicularis*. The *E. acicularis* in the present field experiment yielded BCFw values of greater than 100 for Mn, Cu, Zn, As, Cd, and Pb. During the feasibility experiments using *E. acicularis*, a marked percentage reduction in the concentrations of metals was recorded. *E. acicularis* shows great potential for use in the phytoremediation of waters (marshes, rivers, paddy fields, lakes, ponds, etc.) contaminated by heavy metals and metalloids at mining sites in humid regions of Asia.

Acknowledgment

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References

(1) Rai, P. K. 2009. Heavy Metal Phytoremediation from Aquatic Ecosystems with Special Reference to Macrophytes, *Crit. Rev. Environ. Sci. Technol.* 39: 697–753.

(2) Shao, H. B., Chu, L. Y., Ruan, C. J., Li, H., D. G. Guo & Li, W. X. 2010. Understanding Molecular Mechanisms for Improving Phytoremediation of Heavy Metal-Contaminated Soils, *Crit. Rev. Biotechnol.* 30: 23–30.

(3) Wu, G., Kang, H. B., Zhang, X. Y., Shao, H. B., Chu, L. & Ruan, C. J. 2010. A Critical Review on the Bio-removal of Hazardous Heavy Metals from Contaminated Soils: Issues, Progress, Eco-environmental Concerns and Opportunities, *J. Hazard. Mater.* 174: 1–8.

(4) Sakakibara, M., Harada, A., Sano, S., Hori, R. S. & Inouhe, M. 2006. Phytoremediation of heavy metals contaminated by *Eleocharis acicularis* (in Japanese). The 12nd Symposium on Soil and Groundwater Contamination and Remediation: 545-548. Kyoto, Japan.

(5) Sakakibara, M., Harada, A., Sano, S. & Hori, R. S. 2009. Heavy metals tolerance and accumulation in *Eleocharis acicularis*, a heavy metal hyperaccumulating aquatic plant species. *Geo-pollution Science, Medical Geology and Urban Geology* 5: 1-9.

(6) Ha, N. T. H., Sakakibara, M. & Sano, S. 2011. Accumulation of Indium and other Heavy Metals by *Eleocharis acicularis*: An Option for Phytoremediation and Phytomining. *Bioresour. Technol.* 102: 2228–2234.

(7) Ha, N. T. H., Sakakibara, M., Sano, S., Hori, R. S. & Sera, K. 2009a. The potential of *Eleocharis acicularis* for phytormediation: case study at an abandoned mine site. *Clean: Soil, Air, and Water* 37: 203-208.

(8) Ha, N. T. H., Sakakibara, M. & Sano. S. 2009b. Phytoremediation of Sb, As, Cu, and Zn from contaminated water by the aquatic macrophyte *Eleocharis acicularis*. *Clean: Soil, Air, and Water* 37: 720-725.

(9) Sakakibara, M., Ohmori, Y., Ha, N. T. H., Sano, S. & Sera, K. 2011. Phytoremediation of heavy metal-contaminated water and sediment by *Eleocharis acicularis*. *Clean: Soil, Air, and Water* 39(8): 735-741.