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The efficacy of *Salvinia molesta* Mitch. and *Marsilea crenata* Presl. as phytoremediators of lead pollution

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Abstract

This study focused on the absorption of lead (Pb) by two species of ferns, *Salvinea molesta* Mitch and *Marsilea crenata* Presl. The aim of this research was to describe the accumulation of lead in *S. molesta* and *M. crenata* roots and leaves, and in addition, to find whether these are accumulators or excluders. Both of these ferns were grown in a hydroponic system to which there was added Pb at 0, 5, 10, and 15 ppm concentration, exposed for 7 and 14 days. After the treatment, determination of the lead amounts in the roots and leaves used the AAS method and the RGR calculation. The data were analyzed by ANOVA using SPSS Program 16 edition. The results showed that the accumulation of lead was higher in the roots than the leaves. Both of the ferns had a bioconcentration factor of more than 1 and the factor of translocation was low, *i.e.*, less than 1. It is concluded that *S. molesta* and *M. crenata* are appropriate species for phytoremediation, especially as excluders.

Key words: *Salvinea molesta, Marsilea crenata,* lead, phytoremediation

Introduction

Water pollution by heavy metals is an emerging issue (Garbisu and Alkorta, 2003; Kachenko *et al.*, 2007b). One of the causes of heavy metal pollution is human activity, including the use of chemical fertilizers in the agriculture sector and industrial emissions. According to the United States Environmental Protection Agency (EPA), lead (Pb) is one of 'the big three' heavy metal pollutants, along with mercury (Hg) and cadmium (Cd), these having the highest level of danger to human health because of their very high level of toxicity even at low concentration levels. Lead can cause teratogenicity effects. In addition, the lead may also inhibit the synthesis of hemoglobin, causing kidney malfunction and affect the reproductive system and the cardiovascular system. It also may cause acute and chronic damage to the central nervous system and peripheral nervous system (Duruibe *et al.*, 2007). Lead is not essential for metabolism and may cause cancer in humans. Therefore, there should be an effort to decrease lead contamination in water.

One method that has been used to reduce the levels of heavy metals is through chemical physics and remediation that is expensive and may degrade the environment in its own right. Another method that can be used is phytoremediation. Phytoremediation is an environmentally friendly and economically feasible technology for mitigating water/soil contamination using plants (Cheng *et al.*, 2015; Ghosh & Singh, 2005; Gonzaga *et al.*, 2008). The ability of plants to reduce the metal content in the water can be calculated as the bioaccumulation factor (BAF = metal in shoots/metal in media) and the bioconcentration factor (BCF = metal in roots/metal in media), while the ability of plants to move metal from roots to shoots is defined as the translocation factor (TF = metal in shoot/metal in root) (Yoon *et al.*, 2006). Basically, from the values derived from these parameters, plants that extract metal are known as excluders or accumulators. Excluders, with TF<1, and BAF and BCF>1 can absorb metals in the roots, termed as phytostabilization (Cornara *et al.*, 2007). Accumulators (TF>1), also known as hyper accumulators, move the metal from the roots to shoots. Hyper accumulators can accumulate more than 10,000 mg/kg in parts of the plant above the surface without causing any adverse effects.

Few ferns, including *Pteridium* sp are hyper accumulators alongwith some higher plants (Ghosh *et al.*, 2005). A few ferns have been researched in terms of their absorption capacity; however, the potential of water ferns *Marsilea crenata* (water clover) and *Salvinia molesta* (salvinia) as hyper accumulators or excluders in the tropical waters has not been reported.

The results of an initial study showed that *S. molesta,* from Porong, Sidoarjo, East Java, growing in polluted water contained lead concentrations of 0.5 ppm and *M. crenata,* from Kendung, Sidoarjo, East Java, lead concentrations of 0.8 ppm. Each of these species showed good growth and did not show any adverse signs growing in the polluted water. In this study, an exploration of the mechanisms of lead accumulation into both of these species was conducted. The presence of lead in both of these species could be observed by the color changes of the leaves. Based on this, the aim of this study was to describe the distribution of the lead in the roots and shoots of these water ferns; in addition, the study was also intended to evaluate the possibility of using them for phytoremediation of polluted waters in tropical areas.

Materials and methods

To study the potency of *S. molesta* and *M. crenata* as the phytoremediation agents for the lead, the plants were grown in a medium containing Pb solution. To the growth medium Pb solution of various concentrations was added, *i.e.*: (1) 0 ppm of Pb as the control; and (2) 5 ppm; (3) 10 ppm; (4) 15 ppm of $Pb(NO_3)_2$ solution. These treatments were arranged in a randomized block design through three replications.

S. molesta was collected from Porong, Sidoarjo, East Java, and *M. crenata* from Kendung, Surabaya, East Java. Both of these ferns were adapted and grown in a hydroponic system by using a batch system in Hoagland's solution during seven days. After adapting them to the procedure, the plants were cleaned with distilled water, and then put in glass aquarium of 40 cm length, 30 cm width and 35 cm depth containing 5 L distilled water and Hoagland's solution or the plants were moved into an aquarium containing 5 L of lead/Hoagland's solution (0, 5, 10 and 15 ppm Pb) and exposed for 7 and 14 days. The ferns were grown under a 12-hour light-dark cycle. The plant samples from each aquarium were harvested after either 7 and 14 days. The pH of the medium varied from 5.4-6.7 at the beginning of the experiment, to 6.5 -7.0 at the end of the experiment.

Overall biomass yield and metal content were measured as specified below. Harvested plants were divided into two parts: roots and leaves. Plant biomass yield was measured on dry weight basis. Each part was oven-dried for 2 hours at 80 ^{o}C on successive days and weighed to determine dry weight. Dried plant tissues were milled into a powder. Powdered samples weighing 0.5 g were digested with 5 mL of HNO_3 and diluted to 50 mL with de-ionized double distilled water. The lead analysis was conducted through an extraction method. Both media samples (50 mL) and digested plant samples were analyzed for lead by using an atomic absorption spectrophotometer. The total accumulation and partitioning of the metals by the plants were calculated.

Relative growth rate (RGR) is stated in grams per kilogram per day, and it was calculated as follows:

$$RGR = \frac{\text{(Final dry – Initial dry weight)}}{\text{Initial weight x days}} \quad (1).$$

The bioconcentration factor (BCF) for the lead of the root system and the upper parts (stem/peduncles leaves) were calculated according to the formula as proposed by Wang *et al.* (2007), *i.e.*:

$$BCF = C_{roots}/C_{medium} \quad (2)$$

$$BAF = C_{shoots}/C_{medium} \quad (3)$$

Where, C_{roots} = concentration of lead in the roots (mg Pb kg^{-1} dry weight)

C_{shoots} = total of the concentration of lead in stems or peduncles (mg Pb kg^{-1} dry weight)

C_{medium} = concentration of lead in growth medium (mg Pb L^{-1}).

The translocation factor (TF) was calculated to evaluate the translocation of Pb from the *M. crenata* and *S. molesta* roots to shoots (stem/petiole and leaves) by using equations (2) and (3) as follows:

$$TF = BAF_{shoots}/BCF_{roots} \quad (4)$$

Analysis of variance (ANOVA) was performed using SPSS 16 software package to analyze the data. If there were significant differences, the Tukey test (P=0.05) was used.

Results and discussion

There were no significant differences in growth between fern species or the duration of exposure to lead (7 days *vs.* 14 days) among treatments. The growth of *S. molesta* and *M. crenata* was influenced by the concentration of the lead in the growth media (Table 1). The growth of both water fern species at days 7 and 14 was characterized by a positive value of RGR, generally equal to or higher than 0, although the RGR values of the treatment groups tended to be smaller than the control group.

Table 1. RGR of *S. molesta* and *M. crenata* treatments after 7 and 14 days planted (P=0.05)

Species	Concentration Pb (ppm)	RGR	
		7 days	14 days
Salvinea molesta	0	0.02±0.00b	0.04±0.00b
	5	0.00±0.00a	0.00±0.00a
	10	0.00±0.00a	0.00±0.00a
	15	-0.02±0.04a	0.00±0.00a
Marcilea crenata	0	0.03±0.00b	0.01±0.0b
	5	0.01±0.00a	0.00±0.00a
	10	0.01±0.00a	0.00±0.00a
	15	0.01±0.00a	0.00±0.00a

Table 2. Percentage removal of Pb by *S. molesta* and *M. crenata,* with Pb concentration (ppm) (P=0.05)

Species	Concentration Pb (ppm)	Pb removal	
		7 days	14 days
Salvinea molesta	0	0.00±0.00a	0.00±0.00a
	5	97.33±0.12b	97.33±0.12b
	10	98.50±0.00c	98.57±0.12c
	15	98.96±0.04b	98.89±0.13b
Marcilea crenata	0	0.00±0.00a	0.00±0.0a
	5	94.93±2.55b	86.67±1.14b
	10	96.27±0.47c	91.47±1.50c
	15	97.22±0.0.65b	80.07±5.5b

Based on the results presented in Table 1, it can be concluded that both water fern species were able to grow adequately well in the presence of Pb. The favorable growth of these plants was accompanied by the removal of lead metal from the media (Table 2).

The concentration of the lead in the root tissues of both species was relatively constant in accord with the age of the plants. In root tissues of *S. molesta* and *M. crenata*, the concentration of lead increased from 5 to 15 ppm at 7 and 14 days after planting. Corroborating the data presented in Table 3, it seems that the increase of Pb occurred at each treatment concentration. For both species, on the other hand, the concentration of the lead in the stem and leave tissues increased as the plants growing older. The results presented in Table 4 show that until the 14th day, TF of both *S. molesta* and *M. crenata* were less than 1.0, showing that the metal ion was largely retained in the roots. The results support previous data which show that the highest concentration of the lead was in the root tissue.

S. molesta and *M. crenata* were able to grow in a solution of up to 15 ppm Pb, but the ability to displace lead from media was at best at concentrations of 10 ppm. The removal of lead by both species at concentrations of 5 and 15 ppm were not significantly different. From data presented in Table 2, both of these plants were able to remove lead metal from media up to 98 %. More

Table 3. Mean and Sd of Pb content in roots and leaves (ppm) of *S. molesta* and *M. crenata* in different Pb treatments after 7 and14 days of planting

Species	Concentration Pb (ppm)	7 days		14 days	
		Roots	Leaves	Roots	Leaves
Salvinea molesta	0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	5	0.59±0.02b	0.29±0.00b	0.64±0.00b	0.39±0.00b
	10	0.63±0.00c	0.33±0.01c	0.67±0.02c	0.43±0.00c
	15	0.64±0.00d	0.38±0.01d	0.73±0.01d	0.47±0.01d
Marsilea crenata	0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	5	0.68±0.14b	0.18±0.01b	0.94±0.06b	0.22±0.07b
	10	1.45±0.08c	0.19±0.02e	1.69±0.11c	0.58±0.05c
	15	1.63±0.35d	0.78±0.02d	2.92±0.43d	0.41±0.01d

lead in the media resulted in an increasingly higher concentration of lead in both *S. molesta* and *M. crenata,* both in tissues of roots and leaves. However, the concentration of lead in the roots was significantly higher than in the leaves.

In order to help characterize the ability of the plants to concentrate lead from the media, the bioconcentration factor (BCF), bioaccumulation factor (BAF) and translocation factor (TF) were calculated. The results are presented in Table 4, which shows the higher the concentrations of lead in the media, the higher the TF value; the value of TF at 14 days, however, was very low and less than 1.0. Similarly, for the BCF and BAF, the higher concentrations of the treatment, the higher value of the BCF and BAF. Table 4 shows that the value of BCF for the roots is greater than the value of BAF for the shoots.

The greatest growth of *S. molesta* and *M. crenata* occurred in the control group, in which the results showed that the biomass increased rapidly in the treatment for 7 and 14 days compared with the treatment group. With increased concentrations of lead in the growing media, growth was slightly slower; both of the species had a positive growth rate even up to concentrations of lead up to 15 ppm in the growth media. Positive values of RGR indicated that both species were able to have adapted morphologically to the pressure of lead present in the media. The adaptation could be exhibited by these plants by establishing specific amino acids.

There was an increased displacement of lead from the growing media, which seemed to be caused by the ability of the roots of both species to absorb Pb ions from the growing media, and then translocate them to other parts of the plant, like the shoot. Roots of both species are fibrous and have a modified layer form of epidermal layer similar to root hairs that have a high ability to absorb nutrients and other substances. The higher concentration of lead may decrease the level of absorption significantly from the growing media, with an optimum concentration of 10 ppm. At concentrations of 15 ppm, the roots of plants may have been in a saturated condition such that the displacement of metals from the growing media was decreased.

The experimental results presented in Table 3 show that the highest lead concentrations in *S. molesta* and *M. crenata* was found in the root tissues. The mechanism behind the higher lead concentration in the roots may include the binding process of the positively charged heavy metal ions to negative charges in the cell walls (Grill *et al.*, 1995). Gothberg *et al.* (2002) suggested that in most plants, a larger proportion of metal is retained in the roots, and thereby it may be prevented from interfering with sensitive metabolic reactions in the shoots. The results are similar to other species *S. minima* (Alvarado et *al.,* 2016). This is probably an internal mechanism to avoid toxic metal concentrations in the shoots.

Both, *S. molesta* and *M. crenata* could be categorized as accumulator plants. In addition, Yoon *et al.* (2006) stated that the plants can be classified as excluders and accumulators if the value of TF<1 and BCF>1. The higher values of BCF and TF of the tested species may enable them to accumulate large amount hazardous metals in the harvested parts. The proper disposal of the harvested plant parts is the final and most important step in any kind of plant-based remediation technology. If it is not disposed off properly, the accumulated heavy metals may return to the system or may enter into the food chain. However, bioaccumulation of the trace metals in *M. crenata* tissues was not a concern because the measured levels were lower than the permissible levels set by FAO and WHO (0.5 mg kg^{-1}) for consumption.

The results of this study showed that both *S. molesta* and *M. crenata* have relatively high values of BCF and relatively low value of TF. Patterns of accumulation of both ferns were in accord

Table 4. Mean and Sd of translocation factors (TF), bioconcentration factors (BCF), bioaccumulation factor (BAF) for the different Pb treatments after 7 and14 days of planting

0	Concentration Pb	7 days			14 days		
	(ppm)	BCF	BAF	TF	BCF	BAF	TF
Salvinea molesta	0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	5	4.40±0.05b	2.20±0.05b	0.50±0.01b	4.81±0.20b	2.95±0.08b	0.61±0.01b
	10	4.24±0.03c	2.22±0.08b	0.52±0.02b	4.73±0.50c	2.99±0.07b	0.62±0.01b
	15	3.85±0.03b	2.24±0.08b	0.62±0.01c	4.40±0.49b	2.85±0.03b	0.63±0.01c
Marsilea crenata	0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	5	3.71±0.87b	0.77±0.05b	0.00±0.00b	1.85±0.02b	0.35±0.04b	0.00±0.0b
	10	6.13±0.54c	0.53±0.06b	0.00±0.00b	3.24±0.06c	0.68±0.07b	0.00±0.00b
	15	6.56±0.56b	0.51±0.08b	0.09±0.15c	1.77±0.04b	0.01±0.00b	0.13±0.00c

with previous research that ferns have high value of BCF and low value of TF and adapted to high concentrations of metal by immobilizing metals and metalloids in the root system, reducing the translocation of these metals to the shoots (Kachenko *et al.*, 2007a). This strategy seems to occur in ferns generally, as sequestration in the root system of various metals and metalloids were also observed in some species in Australia (Kachenko *et al.*, 2007b). Tolerant mechanism of the metal acumulation is a result of continuous flow through phloem tissue and resulting saturation sequesteration in root/rhizome of plants. This is a collector-type system in these plants (metal excluders), contrasting with the mechanism that has been reported in the genus *Pteridium,* which is capable of transferring metal from root to shoot tissue. The bioconcentration factor of lead in the root system/rhizome has been also found in angiosperms such as water spinach and yellow velvetleaf. This is supported by investigations, which show that many wetland plants can collect heavy metals from polluted waters, and are found to tolerate a wide range of heavy metals (Pb, Zn, Cu, and Cd), and have demonstrated their possible use in phytoremediation (Leguizamo *et al.*, 2017). Further research is required to increase our understanding of the potential of local plant species in wetlands as phytoremediators.

The results of this study show that the water ferns *S. molesta* and *M. crenata* primarily accumulated lead in their roots rather than leaves. Both ferns have shown a bioconcentration factor greater than 1.0, and a low translocation factor, less than 1.0. In short, *S. molesta* and *M. crenata* are suitable as excluder phytoremediators in heavy metal–polluted waters, as these ferns were able to regulate the accumulation of Pb and limit the metal transport to the shoots of the plant, allowing for continued growth and thus easier harvest at the water surface, in order to reduce overall heavy metal levels in a polluted water body.

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