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Analyzing the Efficacy of *Salvinia molesta* Mitchell as Phytoremediation Agent for Lead (Pb)

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ABSTRACT

Heavy metals, especially Pb (lead), are generally toxic to living things. Pb can contaminate organisms in the water through the food chain. The purpose of this study is to enhance water quality by using *Salvinia molesta* to phytoremediate Pb-polluted water. This study aims to evaluate the ability of *S. molesta* as a Pb phytoremediator. We evaluated total protein, free amino acids produced by the plant, and plant growth (dry biomass). *S. molesta* was grown in a hydroponic system exposed to Pb at dosages of 0, 5, 10, and 15 ppm for 7 and 14 days. Pb level was analyzed using *Atomic Absorption Spectrophotometer* and amino acids were analyzed using High-Pressure Liquid Chromatography. Data were statistically analyzed using analysis of variance (ANOVA) followed by Tukey's test ($\alpha < 0.05$). Results showed a significant change in Pb content in the roots and leaves of Pb-exposed *S. molesta* Mitch compared to control. In Pb-exposed plants, total protein and amino acids, especially cysteine, were lowered. *S. molesta* could be used as a Pb phytoremediator due to its high potential to survive Pb exposure and its ability to absorb Pb.

INTRODUCTION

Heavy metal pollution, such as Pb, is an environmental issue that endangers living things. The widespread burning of fossil fuels and disposal of industrial waste has led to the accumulation of heavy metals in the soil and water body, which leads to contamination of the food chain (Ergönül et al. 2019, Munzuroglu & Geckil 2002, Shah & Nongkynrih 2007, Wuana & Okieimen 2011, Zeller & Feller 1999). Heavy metals are known to cause changes in the redox balance of cells, resulting in oxidative stress (Sreekanth et al. 2013). Many cellular activities are disrupted by the secondary effects of oxidative stress, such as reduced membrane function due to lipid peroxidation and oxidation of proteins and nucleic acids (Blokhina & Fagerstedt 2010). In addition, Plants demonstrate a short-term response toward heavy metals exposure, resulting in morphological, anatomical, physiological, and biochemical alterations (Rai & Tripathi 2009).

Heavy metals-collecting plants function as biofilters that can effectively reduce heavy metals concentrations in contaminated water (Abhilash et al. 2009, Rai 2011). In addition, phytoremediation has been applied extensively to remediate either contaminated soil or water in several areas (Nouri et al. 2011). Another study by Nouri et al. (2011) also pointed out that original species growing in a contaminated habitat can act as phytoremediators for certain pollutants.

Salvinia molesta is a pteridophyte plant from the Salviniales group, an invasive species of aquatic weed that lives floating on the water surface. *S. molesta* is mainly found in ditches or trenches, rice fields, ponds, lakes, or streams with slow water flow and irrigation channels. Its rapid growth rate allows *S. molesta* to slowly cover the water surface, forming a solid layer with thickness up to 1 m, depending on duration and compaction. This plant is primarily found in various areas of the world, including Indonesia. The community uses it as an ornamental plant, as well as for animal food. However, Sari (2014) found that Salvinia could effectively absorb copper (Cr) from Batik industrial waste. Based on this study, *S. molesta* indicates the "resistance" ability toward heavy metals and states it could be classified as an "accumulator/hyperaccumulator" plant. The plants exhibited a wide range of stress tolerance to all metals and can be used for eco-removal of heavy metals from contaminated water (Rai 2018).

These plants can operate as a bioremediation agent because they produce certain protein-forming free amino acids that are required in responding to heavy metals in their environment. One of the functions of plant amino acid synthesis is to detoxify heavy metals by building complexes with them in the plant (Pilon 2005). Heavy metal deposition in wetland plants has been shown to cause significant physiological and biochemical responses in the root, stem, and leaf growth (Rai & Tripathi 2009, Lyu et al. 2016). After several days

of exposure, biochemical indices such as protein, sugar, and chlorophyll content of plants are generally reduced in plant tissues. In this setting, wetland plants' eco-remediation of hazardous chemicals may manifest as unique physiological and biochemical modifications required to cope with heavy metal stress (Rai & Tripathi 2019). Hence, metal ions must be distributed throughout the plant's organs, from root to shoot, and the plant's ability to adapt to metal exposure from contaminated water must be studied using free amino acid analysis (Kamel 2008).

This study aims to determine how effective *S. molesta* is as a Pb phytoremediator based on total protein, free amino acids produced by the plant, and plant growth (dry biomass) data, as explained above. This study examines if *S. molesta* can be employed as a heavy metals Pb phytoremediator and assesses its ability to produce free amino acids.

MATERIALS AND METHODS

S. molesta Growth and Pb-Exposure

This study was designed as a completely randomized block design with three replications. Two treatment factors were applied: Pb concentration (K1: 0 $mg.L^{-1}$, K2: 5 $mg.L^{-1}$. K3: 10 $mg.L^{-1}$, K4: 15 $mg.L^{-1}$); and exposure time (seven and 14 days). Each factor combination was carried out for three replications.

S. molesta was collected from the Porong wetlands in the Sidoarjo region, East Java, Indonesia. The experiment was initiated by acclimatizing plants and reducing contaminant levels in the plants by growing *S. molesta* in a plastic chamber filled with 20 L Hoagland's medium in a greenhouse for seven days. After that, *S. molesta* was sorted at 90 g for the respective treatment of the plant. Finally, each plant was maintained for ten days in a different plastic container filled with 20 L distilled water supplemented with Hoagland's solution (Göthberg et al. 2004).

Acclimatized plants were rinsed using distilled water and moved into a 40×30×35 cm glass aquarium filled with 5 L distilled water and Hoagland's solution with Pb level set according to the respective treatments. Each aquarium was filled with 100 g of Salvinia. The initial pH and at the end of the experiment were recorded. Plants were adjusted to 12:12 hours light-dark circle daily with 389-candles photon flux density. Plant biomass was recorded after all plant samples were harvested according to the exposure time set (seven and 14 days).

Pb Level Measurement in *S. molesta*

After *S. molesta* was harvested, its phytoremediation ability was determined by measuring Pb absorption in roots and leaves using the extraction method (Göthberg et al. 2004). First, harvested plants were separated into roots and leaves. Each plant portion was dried in an oven at 80°C for 48 hours before being weighed dry. Following that, 5 g of each plant organ sample was pulverized in a mill. Next, 5 g of each plant organ sample was taken and ground using a mill. After that, 0.5 g of each powdered plant sample was diluted into 5 mL HNO_3 and 50 mL deionized double distilled water. Fifty milliliters of respective diluted sample and medium were analyzed using atomic absorption spectrophotometer to record its Pb level. Total accumulation and partitioning of heavy metals by the plants were calculated.

Free Amino Acids Identification and Level Measurement

Using the HPLC method with hydrolysis processes and derivatization, the protein-mapping pattern of Pb-exposed *S. molesta* was also analyzed from free amino acids and the total protein content of plant roots (Waters 2017). The reagent kit (AccQ-FluorTM Reagent Kit for hydrolysate amino acid Analysis) was prepared first by heating it at 55°C. Next, AcrQ fluo reagent powder was heated for 2-3 minutes. Then, 1 mL of AccQ fluorine reagent diluent was put into vial 2A, followed by heating and mixing until all the powder was spread evenly. Next, the solvent was prepared by diluting 19 g sodium acetate and 2.27 g TEA into 1 L distilled water. Forty percent phosphoric acid (+ 6 mL or above) was added until pH reached 5.1 followed by 5 mL acetonitrile and distilled water.

The hydrolysis of the sample was performed on a 100 mg sample. The sample was placed into a tube and combined with 5 mL 6 N HCl. The sample was dried using nitrogen or argon. The respective tube was covered and placed in an oven at 112°C for 22 h. The sample (100 mL) was then filtered using 0.45 pm filter paper and dissolved into 5mL MiliQ water.

For derivatization, a 50 pL diluted sample was mixed into 350 pL AccQ derivatization buffer and 100 pL AccQ fluor reagent. The mixture was shaken briefly and put into heated water at 55°C for 10 min. Finally, the sample was injected into the HPLC instrument (Waters 2017).

Data Analysis

The parameters observed in this study were: (1) dry weight; (2) Pb level in roots and leaves; (3) free amino acids level in roots; (4) bacteria species in the roots. Qualitative data for dry weight and Pb level was analyzed using one-way ANOVA, followed by Tukey's test at 95% confidence level using SPSS 21st edition. Free amino acids were analyzed descriptively based on the percentage and total protein.

Bacteria species isolated from *S. molesta* roots were also analyzed descriptively.

RESULTS AND DISCUSSION

Growth of Plants Exposed to Pb

The current study found a significant difference in the growth of plants given various Pb concentrations. In contrast, no differences in growth were found based on exposure duration (seven days vs. 14 days) (Fig. 1). In addition, *S. molesta* dry weight was affected by Pb concentration in the growth medium (Table 1). Medium pH was also found to change, from 5.4-6.9 at the beginning of the experiment to 6.5-7.0 at the end of the experiment (Table 2).

According to biomass weighted after treatment, *S. molesta* was able to grow effectively in the Pb-contained medium. The increasing Pb concentration in the medium, on the other hand, induces a decrease in Salvinia biomass. Phetsombat et al. (2006) reported that the *S. molesta* plant group exposed to Pb at concentrations of 10, 20, 30, and 40 mg.L^{-1} with exposure durations of 2, 4, 6, and 8 days, respectively, also demonstrated decreasing growth. According to Hardiani et al. (2011), the plants adapted slowly to a high-grade medium containing heavy metals. Other studies, on the other hand, such as those by Göthberg et al. (2004), grew Pb-exposed water spinach supplemented with various concentrations of Hoagland nutrients. Abhilash et al. (2009) studied Yellow velvetleaf exposed to Cd with concentrations of up to 2 mg.L^{-1}. Rachmadiarti et al. (2012) grew Yellow velvetleaf exposed to Pb at a concentration up to 15 mg.L^{-2}, and Rachmadiarti & Trimulyono (2019) examined water clover exposed to Pb and found that various plant species could grow even when there is heavy metal contamination.

Xin et al. (2010) found that Pb concentration in the medium is in line with the Pb level concentrated in the roots. As the exposure period increases, the amount of Cu absorbed increases (up to 14 days). Meanwhile, Zou et al. (2011) discovered a relationship between Pb content in herbaceous dicotyledonous plants (*Arpesium abrotanoides, Conyza canadensis, Anemone vitifolia,* Monocotyledoneae *Juncus effussus*, and pteridophytes *Ahyrium wardii*, and *Pseudocyclosorus subochthodes*) and the concentration of Pb in the medium and the duration of exposure. Another research noted that the final Pb removal in *Lemna gibba* L. after 21 days of exposure was up to 94% (Bokhari et al. 2019).

Distribution of Pb in Plant Organs

During the plant-growing process, Pb was removed from the growth media. Pb was absorbed by plants and translocated to various plant organs as a result. The distribution of Pb in

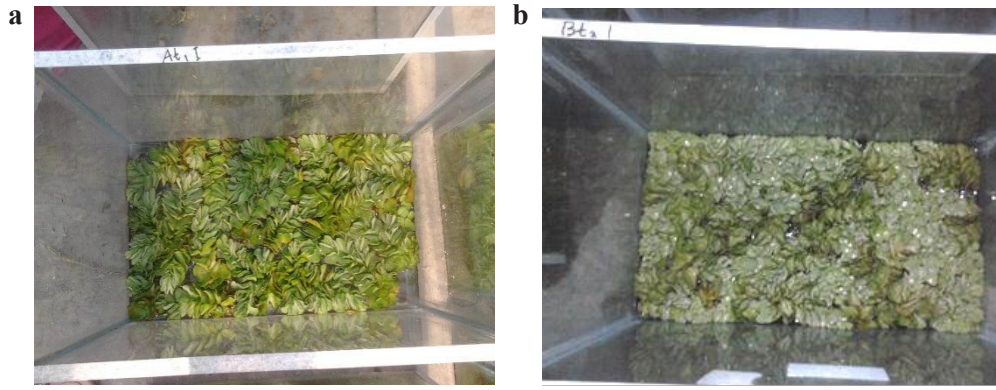

Fig. 1: *S. molesta* plant from (a) control and (b) experiment group.

Table 1: Salvinia dry weight (g) after Pb exposure for 7 and 14 days.

Pb Concentration	Dry weight [g]	
	7 days	14 days
0	6.50±0.65^{c}	6.00±0.15^{c}
5	6.76±0.65^{c}	6.26±0.15^{c}
10	5.06±0.27^{b}	4.56±0.27^{a}
15	4.41±0.37^{a}	3.91±0.37^{a}

*Different letters indicate statistical differences based on the Tukey test (p = 0.05)

Table 2: Initial and final medium pH as a function of Pb concentration on days 7 and 14.

Pb concentration (mg.L^{-1})	Day	Initial pH	Final pH
0	7	7.0±0,0	7.0±0,0
5		6.9±0,0	6.9±0,0
10		6.4±0,0	6.8±0,1
15		5.9±0,0	6.7±0,1
0	14	7.0±0,0	7.0±0,0
5		6.9±0,0	7.0±0,0
10		6.4±0,0	7.0±0,0
15		5.9±0,0	7.0±0,0

the roots and leaves of *S. molesta* plants was studied in this research (Fig. 2). The Pb content in the roots of *S. molesta* showed a significantly higher concentration of Pb absorbed over the same period, with 5 ppm as the best result.

The ability of S. molesta to accumulate a higher amount of Pb in roots than in leaves was linked to its ability to immobilize toxic ions in the planting media by collecting and adsorbing the pollutant at the root zone. The roots also functioned as a rhizofilter, absorbing Pb toxic ions after phytostabilization. Therefore, the increase in Pb level in the roots is caused by the Pb accumulation process in the roots (Mangkoedihardjo & Samudro 2010).

When Pb and other heavy metals are present, this heavily loaded environment caused regulatory proteins in this plant to create sulfide bonds at the tip of sulfur in cysteine, further promoting the synthesis of complex molecules. Lead and other heavy metals would be transported to various plant tissues as a result.

There was no difference in Pb levels in the *S. molesta* leaves when different Pb concentrations were used for varied exposure times. However, Pb content was higher in roots than in leaves (Pourrut et al. 2011). Its function includes adsorbing the water surface and precipitating and accumulating pollutants in the root zone (Lyu et al. 2016, Salakinkop & Hunshal 2014). Rhizodegradation, the enhanced breakdown of a contaminant by increasing the bioactivity using the plant rhizosphere environment to stimulate the microbial populations (mostly Rhizosphere bacteria), is then carried out, with the products being dispersed into the root zone.

Plants Biochemical Response

Table 3 gives *S. molesta* protein mapping patterns based on

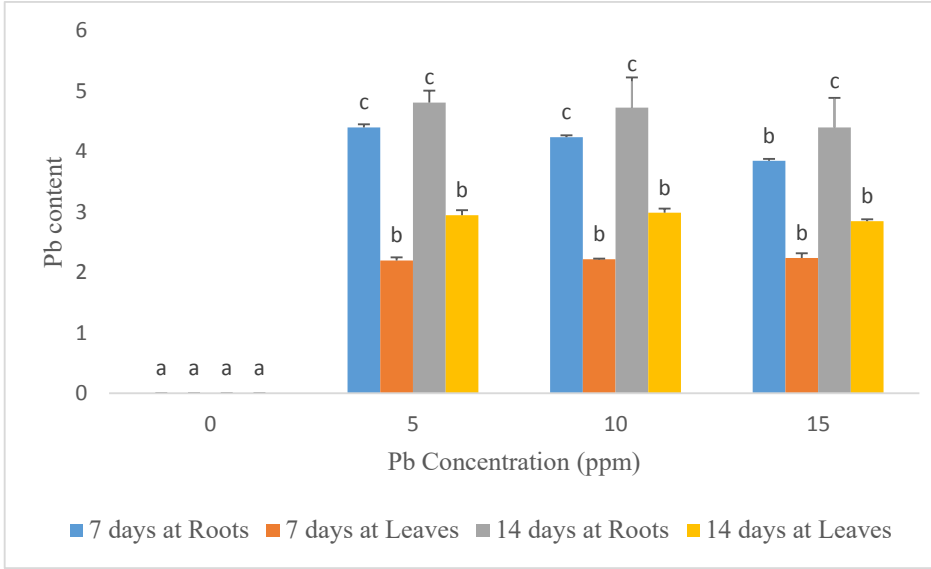

Fig 2: Pb content in roots and leaves of *S. molesta* plant after 7 and 14 days exposure.

Table 3: Amino acid content of Pb-exposed *Salvinia* plant.

Amino acid	Content [%] in Pb-exposed plant		
	0 ppm	5 ppm	15 ppm
Histidine	0.42±0.02	0.26±0.02	0.25±0.02
Threonine	0.92±0.00	045±0.00	0.49±0.02
Proline	0.71±0.02	0.48±0.02	0.41±0.02
Tyrosine	0.53±0.02	0.27±0.02	0.30±0.02
Leucin	1.39±0.02	0.91±0.02	0.77±0.02
Aspartic acid	1.16±0.02	0.79±0.02	0.74±0.02
Lysin HCl	0.56±0.02	0.40±0.02	0.37±0.02
Glycine	1.09±0.02	0.66±0.02	0.62±0.02
Arginine	0.91±0.02	0.55±0.02	0.50±0.02
Alanine	0.84±0.02	0.59±0.02	0.50±0.02
Valin	0.77±0.01	0.50±0.01	0.42±0.01
Isoleucine	0.63±0.01	0.41±0.01	0.35±0.01
Phenylalanine	1.13±0.01	0.61±0.01	0.59±0.01
Glutamic acid	1.49±0.02	1.04±0.02	0.91±0.02
Serine	0.90±0.00	0.47±0.00	0.49±0.00
Methionine	0.23±0.02	0.11±0.02	0.10±0.02
Cystine	0.01±0.00	0.00±0.00	0.00±0.00
Total	13.69	8.51	7.83

free amino acids and total proteins. Plant responses to environmental changes were defined by varying protein levels. Changes in the levels of particular amino acids (proline and cysteine) indicated protein resistance and degradation as a result of changes in environmental quality.

Because the ability to absorb Pb in the treatments of 5 and 10 was similar, the amino analysis was performed for 0, 5, 10, and 15 ppm; consequently, the 10 ppm did not include total protein analysis. The control group (0 ppm Pb) had a greater total protein content (13.69%) than the plants treated to 5 ppm Pb (8.51%) and 15 ppm Pb (7.83%) (Table 2). Several amino acids, including proline, glycine, arginine, histidine, glutamic acid, and cysteine, were found to be reduced following exposure to Pb.

At 14 days of exposure, the total percentage of amino acids in S. molesta decreased in tandem with the increase in Pb level. The lower the total percentage of protein from plants, the higher the Pb levels in the medium. According to studies on algae, the total protein content of *Chlorella vulgaris* was found to decrease in artificial media exposed to metals (Afkar et al. 2020). Another study found that co-exposed *Chlorella vulgaris* cells had no change in total protein content. This suggests that storing low concentrations of heavy metals in proteins or enhancing their respiration by using carbohydrates with the advantage of protein accumulation could be one strategy for organisms to mitigate their harmful effects (Akmukhanova et al. 2018, Osman et al. 2004). According to Andra et al. (2010), the role of phytochelatin in inducing Pb-tolerance in vetiver grew in the contaminated medium.

Six amino acids have been identified as being significant in the regulation of osmotic plants based on the amino acid analysis (Mansour 2000). Arginine, proline, leucine, valine, serine, and glycine are the six amino acids. In *S. molesta*, the concentration of proline tended to decrease. The roots were able to absorb Pb from the medium and adapt to the new environment by generating amino acids. Arginine was also discovered in the plant. Arginine is required for the synthesis of polyamine, which acts as an antioxidative agent (Sharma & Dietz 2006) and signaling molecule (molecule signal). This means that plants can withstand stress by producing antioxidants.

CONCLUSION

In conclusion, *Salvinia molesta* was found to have potential as a phytoremediator for Pb-contaminated water, indicated by its ability to grow adequately in a Pb-contained medium and absorb Pb from the environment into its body. The op-

timal treatment is by 5 ppm. Pb content in plants was found to be higher in roots compared to leaves. Total proteins and many amino acids were reduced in Pb-exposed plants, demonstrating a method of plant response to environmental change. It is vital to investigate further the phytoremediation capabilities of S. molesta by increasing heavy metals levels in the medium in a shorter time frame and the remediation mechanism occurring within the plant body. The findings of this study can be transferred to further research involving the use of S. molesta as a phytoremediation agent for domestic or industrial wastewater.

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