Biosorption Mechanism of Wild Celery, *Vallisneria natans* towards Lead and its Effect on the Chlorophyll Content

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ABSTRACT

Vallisneria natans is a variety of the *Vallisneria americana* species and is commonly found in freshwaters. In this study, *Vallisneria natans* (wild celery) was used as an accumulator in water solutions containing high levels of lead. *Vallisneria natans* also called *Vallisneria americana Var. natans*, is a genus within the family of frog bite plants *hydrocharitaceae.* The *Vallisneria natans* samples were exposed to different lead concentrations: 100 ppm, 300 ppm, 500 ppm, and 0 ppm for 15 days without prior acclimatization. The uptake of the plant in different concentrations was determined by AAS analysis. The uptake mechanism of lead on *V. natans* was also investigated through the determination of the different lead ion concentrations adsorbed (supernatant), and absorbed (residue) by the macrophyte. The extent of *V. natans* to phytoremediate lead was also assessed by determining the percentage of lead ions accumulated by the plant. Furthermore, examination of its photosynthetic activity at different lead concentrations was also done by double-beam UV-Vis spectrophotometry to determine the general effect of lead ions to *V. natans*. The concentration of chlorophyll a and b was also determined to compare the effect of lead contamination on chlorophyll a and chlorophyll b. The percentage of loss in chlorophyll a and b in varying lead concentrations was also measured for clearer depiction of the effect of lead on chlorophyll content of *V. natans*.

Results have shown that *V. natans* favors adsorption in biosorbing lead ions and that *V. natans* proved to be effective in adsorbing high levels of lead even in treatments without acclimatization. Results have also shown that the photosynthetic activity of *V. natans* was appreciably affected by the presence of lead ions. Thus, the use of *V. natans* as a biosorbent in the field of bioremediation was proven to be a promising and efficient approach for the removal of lead ions up to 500 ppm as observed in this study. In immediate bioremediation, *V. natans* still proved to be effective as it is capable of adsorbing high levels of lead even in treatments without acclimatizing the plant. The percentage uptake of residue samples have decreased as the concentration of lead is increased which means that the ability of *V. natans* to absorb lead ions is weakened by the increasing presence of lead ions. Also, comparison of the mean lead uptake of *V. natans* in supernatant and residue samples at all lead treatments has shown that the macrophyte is more capable of sequestering lead ions via adsorption. Results obtained have also shown that the photosynthetic activity of *V. natans* was appreciably affected by the presence of lead ions. Chlorophyll a content of the macrophyte has decreased as lead concentration was increased. Chlorophyll b content has increased as lead concentration was increased from 0 ppm to 100 ppm and then decreased as lead concentration was increased from 100 ppm to 300 ppm, and then to 500 ppm.

KEYWORDS: Biosorption mechanism, *Vallisneria natans,* Lead, Chlorophyll content

1. INTRODUCTION

Lead is considered one of the most toxic heavy metals and has been recognized for its harmful influence on the environment where it is accumulated via food chain having a serious threat to human health, animals and plants[1]. Therefore, it is very crucial to treat lead from wastewaters properly. Recently, there has been a great interest in developing an effective and environment-friendly technology involving the removal of heavy metals from wastewaters.

Phytoremediation is the utilization of plant to remove and accumulate contaminants from the environment. Aquatic macrophytes have been widely used to remove, transform or stabilize heavy metals in water and sediments[2] because they accumulate heavy metals effectively and help restore the aquatic system. Phytoremediation has several advantages and is the most significant one in study of sub-lethal levels of bioaccumulated contaminants within the tissues/ components of organisms, which indicate the net amount of pollutants integrated over a period of time [3].

Organic molecules that are not biodegradable can still be removed from the wastewater by the microbial biomass via the process of biosorption. Biosorption is the passive uptake of pollutants from aqueous solutions by the use of non-growing or non-living microbial mass, thus allowing the recovery and/ or environmentally acceptable disposal of the pollutants, could also be considered[4]. The main attractions of biosorption are high selectivity and efficiency, cost effectiveness and good removal performance; raw materials which are either abundant (aquatic plants) or wastes from industrial operations can be used as biosorbents.

Macrophytes are considered as important component of the aquatic ecosystem not only as food source for aquatic invertebrates, but also act as an efficient accumulator of heavy metals[5].

The mechanisms of biosorption may be classified as being: extracellular accumulation, cell surface sorption, and intracellular accumulation[6]. This can occur by complexation, co-ordination, chelation of metals, ion exchange, adsorption, and micro precipitation[7]. The bioremoval process using aquatic plants contains two uptake processes: (i) passive adsorption – an initial fast, reversible, metal-binding process (biosorption), the efficiency of which is dependent on cell wall structural organization and metal solution chemistry, and (ii) a slow, energy dependent, irreversible, ion-sequestration step (bioaccumulation).

Heavy metal transport across cell membranes may be mediated by the same mechanism used to convey metabolically important ions such as potassium, magnesium, and sodium. Physical adsorption takes place with the help of van der Waal's forces[8].

In this study, wild celery, *Vallisneria natans* was used as an accumulator in water solutions containing high levels of lead. *Vallisneria natans* also called *Vallisneria americana var. natans*, is a genus within the family of frog bite plants *Hydrocharitaceae.* It reproduces asexually by runners and can spread to occupy large areas[9,10]. The common name for *V. natans* is commonly called sintas-sintasan and is commonly found in shallow lakes and streams[11].

V. natans is a variety of the *Vallisneria americana* species. It is commonly found in freshwaters in China[12] but is also native in the Philippines and now cultured by aquascaping hobbyists here in the Philippines.

2. MATERIALS AND METHODS

2.1 Preparation of samples

Vallisneria natans samples were obtained from three (3) separate freshwater tubs at Brgy. Baybay-Tinagsa, Brgy. Riverside, and Brgy. Poblacion, Kolambugan, Lanao del Norte. The plants were separated into individual portions, and rinsed with distilled H_2O. From the gross sample, twenty-four (24) laboratory samples were chosen and weighed.

2.2 Lead treatments of *V. natans*

A stock solution of lead with a concentration of 1000 ppm was prepared and from this concentration, 1000 mL of 100 ppm, 300 ppm, and 500 ppm solutions of lead in three (3) replicates were prepared using distilled water as solvent. Three (3) replicates of 1000 mL distilled water were also prepared and used as control. Each of these solutions was placed separately in previously acid washed 1 L containers containing red sand substrate of about 2 inches deep. The previously weighed samples were then planted in the containers separately and aerated for 10 minutes daily for 15 days to allow homogenization of the media. About 0.1 mL of ISTA liquid fertilizer was applied to each of the 1 L containers containing the sample every 5 days.

2.3 Determination of lead in *V. natans*

After 15 days of exposure, the samples were removed from the containers and their wet weights were obtained. The samples were then soaked in 20 mL of 0.05 M EDTA in 150 mL Erlenmeyer flasks for 48 hours.

The solutions were filtered using Whatman #1 filter paper and the filtrate was labeled as supernatant. This solution contains lead ions that were adsorbed by *Vallisneria natans.* The plant samples were placed in 250 mL Erlenmeyer flasks and labeled as residue.

2.3.1 Digestion of supernatant samples

The collected supernatant samples were placed over a hot plate and digested under a fume hood with 2.5 mL concentrated HNO_3 and evaporated to dryness.

The samples were then treated with 3 mL of concentrated HNO_3 and evaporated again to dryness. The digested samples were removed from the hot plate and the resulting residue or precipitate were dissolved in 3 mL concentrated HCl and 3 mL deionized water. The samples were filtered and diluted to 50 mL with deionized water and were stored in pre-labeled acid-washed containers. The resulting solutions were then subjected to the atomic absorption spectrophotometer at the Philsaga Mining Corporation to determine the amount of lead ions adsorbed by *V. natans*.

2.3.2 Preparation and digestion of residue samples

The residue samples were placed in pre-constant-weighed crucibles and heated to 600°C in a furnace for an hour then placed in a desiccator to cool and then was weighed. This procedure was repeated until a constant weight is obtained.

After achieving a constant weight, the dried ash in the crucible was washed with 15 mL concentrated HNO_3 and transferred to 250 mL Erlenmeyer flask. Another 15 mL concentrated HNO_3 was added to the flask. The flasks were placed on a hot plate under a fume hood and were evaporated to about 15-20 mL making sure not to boil them. The samples were removed from the hot plate and set aside for cooling.

Three mL of 1N HNO_3 and three mL of deionized water were then added to the cooled samples and placed back on top of the hot plate to continue the digestion. The addition of 1N HNO_3 and deionized water were repeated until the color of the obtained digest is pale yellow or clear. The samples were evaporated to near dryness and three mL of 1:1 v/v concentrated HNO_3 and deionized water was added to dissolve any precipitate or residue that would have resulted during the evaporation.

The beaker walls as well as the watch glass covering were washed down with deionized water and the washings were filtered using a Whatman #1 filter paper to get rid of the silicates and other insoluble materials. Lastly, the volumes of the filtrates were diluted to 50 mL with deionized water and were stored in pre-labeled acid-washed containers. The resulting solutions were then subjected to atomic absorption spectrophotometer at the Philsaga Mining Corporation to determine the amount of lead ions absorbed by *V. natans*.

2.4 Chlorophyll content determination of *V. natans*

The method outlined in the Scientific Engineering Response & Analytical Services (SERAS, 1994) was followed with minor modifications for the chlorophyll content determination of *V. natans*. The procedure in section 2.2 was used in the preparation of the setup.

After 15 days of exposure, the plant samples were removed from the setup, weighed, and cut to small pieces using scissors. The tissues were then placed in a mortar, added with 2 mL 1:9 v/v NH_4OH: acetone extraction solution, and ground for approximately 30 seconds or until the tissue was a fine slurry. Another 3 mL of the extraction solution was poured over the pestle to wash any sample material adhering to the pestle. The slurry was then poured into a test tube and refrigerated in the dark for two hours and then reground again using the mortar and pestle to extract any remaining chlorophyll. The extract was then poured carefully into a centrifuge tube and 5 mL of 80% aqueous acetone solution was pipetted over the mortar and pestle into the centrifuge tube. The sample extract in the centrifuge tube was then centrifuged for twenty minutes at high speeds. The supernatant solution was decanted into a 10 mL graduated cylinder and the volume was brought to 10 mL with 80% aqueous acetone.

The supernatant was then read by a UV-Vis Spectrophotometer at wavelengths of 663 nm and 645 nm in the Instrument Room of the Chemistry Department of Mindanao State University - Iligan Institute of Technology. The absorbance of each solution was recorded at these wavelengths and chlorophyll a and b concentrations were calculated.

The following calculations were made to ascertain sample chlorophyll concentrations. Concentrations were expressed on a wet weight basis.

$$Chl\ a\left(\frac{mg}{mL}\right) = 12.7A_{663} - 2.69A_{645}$$

$$Chl\ b\left(\frac{mg}{mL}\right) = 22.9A_{645} - 4.68A_{663}$$

where

$A_{645} = absorbance\ at\ a\ wavelength\ of\ 645nm$

$A_{663} = absorbance\ at\ a\ wavelength\ of\ 663nm$

$$Total\ Chl\left(\frac{mg}{mL}\right) = Chl\ a + Chl\ b$$

$$Total\ Chl(mg)\ in\ original\ tissue\ sample:$$
$$Total\ Chl(mg) = Total\ Chl\left(\frac{mg}{mL}\right) x\ final\ vol(mL)$$
$$Total\ Chl\ a(mg)\ in\ original\ tissue\ sample:$$
$$Total\ Chl\ a(mg) = Chl\ a\left(\frac{mg}{mL}\right) x\ final\ vol(mL)$$
$$Total\ Chl\ b(mg)\ in\ original\ tissue\ sample:$$
$$Total\ Chl\ b(mg) = Chl\ b\left(\frac{mg}{mL}\right) x\ final\ vol(mL)$$

The amount of chlorophyll was divided by the wet weight of the tissue analyzed to express the concentrations on the basis of wet weight.

3. RESULTS AND DISCUSSIONS

3.1 Lead biosorption on *V. natans*

V. natans was exposed to different lead concentrations for 15 days. The amount of lead in supernatant and residue were measured by atomic absorption sprectroscopy analysis.

Table 1 shows the concentration of lead bioaccumulated by *V. natans*. For the residue, mean lead uptake was lowest in 300 ppm lead with 4.44 ppm, then in 100 ppm lead with 6.05 ppm, and highest in 500 ppm with 6.58 ppm. For the supernatant, mean lead uptake was increased from 61.07 ppm in 100 ppm lead, then 185.06 ppm at 300 ppm lead, and 264.04 ppm at 500 ppm lead. Also, the concentration found in the supernatant was remarkably higher than that of the residue. It shows that even without acclimatizing *V. natans*, it is still capable of bio-accumulating lead in high levels.

Table 1. Mean lead uptake of *V. natans*

Samples	Mean lead uptake (ppm)		
	100 ppm	300 ppm	500 ppm
Actual solution	97.54	281.09	478.26
Residue	6.05[a]	4.44[b]	6.58[a]
Supernatant	61.07[a]	185.06[b]	264.04[c]

Means having the same letter are not significantly different at α=0.05 DMRT

The supernatant samples did not show significant difference in the total amount of lead accumulated at 100 ppm and 500 ppm but there is a significant difference in mean lead uptake at 300 ppm when compared to 100 ppm and 500 ppm based on the data obtained from One-way ANOVA. Mean lead uptake in supernatant samples increased as the concentration of lead exposure was increased. The data from one-way ANOVA shows that there is significant difference in mean lead uptake of *V. natans* between 100 ppm and 300 ppm, 100 ppm and 500 ppm, and 300 ppm and 500 ppm lead.

Two-sample T-test show that there is significant difference between the mean lead uptake of *V. natans* in the residue and supernatant in 100 ppm, 300 ppm, and 500 ppm lead. The statistical results and the results shown in Table 1 suggest that *V. natans* phytoremediate lead ions by adsorption when the plant is exposed to lead without acclimatizing it. This can be associated with the defense mechanism of plants to prevent heavy metal uptake inside the cells through the exudates secreted by the plant into the soil and water[13]. The cell wall can play a key role in the immobilization of toxic heavy metal ions by providing pectin sites and hystidyl groups, and extracellular carbohydrates such as callose and mucilage, and thus prevents heavy metal uptake into the cytosol[14]. Also, other components such as lignin, cellulose, hemicelluloses, lipids, proteins, simple sugars, starches, water, hydrocarbons, and many more that contain carboxyl, amino, alcohol, and ester functional groups have the ability to bind heavy metal ions by replacement of hydrogen ions for heavy metal ions in solution or by donation of an electron pair from these groups to form complexes with the metals in solution [15,16].

Table 2 shows the percentage uptake of *V. natans* in varying concentrations of lead. The percentage uptake of residue samples decreased as concentration of lead exposure is increased. The supernatant sample has its least percentage uptake at 500 ppm lead with 55.21%, and then followed by 100 ppm with 64.60%, and highest percentage uptake at 300 ppm lead with 65.84%.

Table 2. Percentage uptake of *V. natans* in varying concentrations of lead

Samples	Percentage uptake (%)		
	100 ppm Pb	300 ppm Pb	500 ppm Pb
Residue	6.20	1.58	1.38
Supernatant	64.60	65.84	55.21

The capability of *V. natans* to absorb lead is almost negligible when compared to its ability to adsorb as shown below in Figure 5.

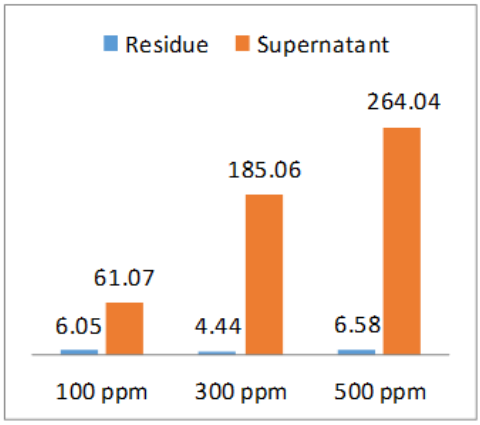

Figure 5. Comparison of lead levels (ppm) found in supernatant and residue

3.2 Effect of lead on the photosynthetic activity of *Vallisneria natans*

V. natans samples were exposed to different lead concentrations for 15 days and the chlorophyll content was analyzed in UV-Vis Spectrophotometer to determine the effect of lead contamination in its photosynthetic activity. The appearance of *V. natans* exposed in 0 ppm, 100 ppm, and 300 ppm lead does not show much difference. *V. natans* exposed in 500 ppm lead, however showed more yellow coloration in the leaves compared to the others.

3.2.1 Summary on the effect of lead to the concentrations of chlorophyll a, b and total chlorophyll content of *Vallisneria natans*

Table 3 presents the mean concentration of chlorophyll a, b and total chlorophyll at 0 ppm, 100 ppm, 300 ppm, and 500 ppm lead concentrations. One -way ANOVA showed that chlorophyll a content in 0 ppm lead showed significant difference compared to 100 ppm, 300 ppm, and 500 ppm lead. Chlorophyll b content in 0 ppm lead also showed significant difference to 100 ppm, 300 ppm, and 500 ppm. Chlorophyll b content in 300 ppm lead only showed significant difference to 0 ppm and 100 ppm lead but not to 500 ppm lead. Total chlorophyll content in 0 ppm and 100 ppm were not significantly different from each other but showed significant difference when compared to the total chlorophyll content at 300 ppm and 500 ppm lead.

Table 3. Mean chlorophyll a, b, and total chlorophyll concentration at varying concentrations of Pb

Chl type	Mean chlorophyll concentration (mg/kg)			
	0 ppm Pb	100 ppm Pb	300 ppm Pb	500 ppm Pb
Chl a	334.32^{a}	249.65^{b}	205.88^{b}	174.28^{b}
Chl b	193.71^{b}	215.55^{a}	168.71^{cd}	118.95^{d}
Total Chl	528.03^{a}	465.20^{a}	352.85^{b}	293.23^{b}

Means having the same letter are not significantly different at α=0.05 DMRT

Chlorophyll a is the pigment that participates directly in the light requiring reactions of photosynthesis. On the other hand, chlorophyll b is an accessory pigment that acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll a. Two-sample T-test for the effects of lead in the photosynthetic activity of *V. natans* showed that there is a significant difference in the concentration of chlorophyll a and b in 0 ppm lead. It coincides with the fact that plants contain more chlorophyll a than chlorophyll b. In samples exposed to 100 ppm, chlorophyll a and b content didn't show significant difference based on the two-sample T-test results. The same results are found on samples exposed to 300 ppm and 500 ppm lead. This means that lead affects chlorophyll a content of *V. natans* in the same way it affects chlorophyll b.

The chlorophyll content of *V. natans* decreased as the concentration of lead exposure was increased. Chlorophyll a concentration has also decreased when lead concentration was increased. Chlorophyll b content of *V. natans* treated with 100 ppm lead is greater than that treated with 0 ppm lead. However, from 100 ppm to 500 ppm lead, chlorophyll b content has shown a decrease in concentration. Chlorophyll a/b ratio in varying lead concentrations is shown below in Table 4.

Table 4. Chlorophyll a/b ratio in varying lead concentrations

	Lead concentration			
	0 ppm	100 ppm	300 ppm	500 ppm
Chl a/b ratio	1.73	1.16	1.22	1.47

The a/b chlorophyll ratio, is 1.73 for 0 ppm, 1.16 for 100 ppm, 1.22 for 300 ppm, and 1.47 for 500 ppm. The ratio a/b at 0 ppm lead is higher compared to a/b ratio for 100, 300, and 500 ppm contamination levels.

However, when lead exposure is increased from 100, 300, and 500 ppm, a/b ratio has increased from 1.16 to 1.47. The expected 2:1 ratio between chlorophyll a and b was not achieved in *V. natans* in 0 ppm, 100 ppm, 300 ppm, and 500 ppm lead. One possible reason for the decreased chlorophyll ratio for *V. natans* in 0 ppm lead is that the treatment was done without acclimatization.

The disparity on the concentration of chlorophyll a, b and total chlorophyll of *V. natans* exposed to lead relative to the control is shown in Figure 6.

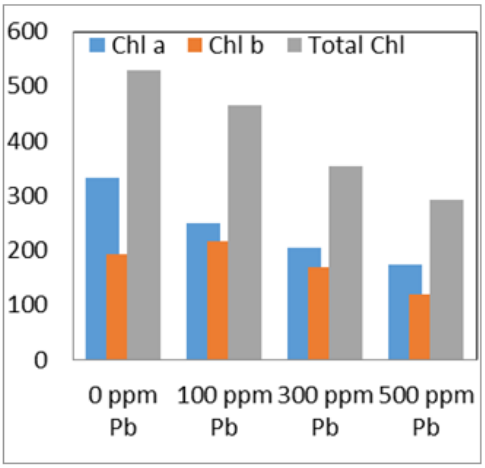

Figure 6. Chlorophyll a, b, and total chlorophyll (ppm) in varying Pb treatments

Table 5 shows the percentage loss of chlorophyll a, b, and total chlorophyll in 100 ppm, 300 ppm, and 500 ppm lead.

Chlorophyll	Percentage lost (%) in		
	100 ppm Pb	300 ppm Pb	500 ppm Pb
Chl a	25.33	38.42	47.87
Chl b	-11.27	12.91	38.59
Total chl	11.90	33.18	44.47

V. natans has lost 25.33% of chlorophyll a in 100 ppm lead, 38.42% in 300 ppm lead, and 47.87% in 500 ppm relative to chlorophyll content in 0. Chlorophyll b content has decreased in *V. natans* exposed to 300 ppm lead by 12.91% and 38.59% in 500 ppm lead but showed an increase in of 11.27% in 100 ppm lead. Total chlorophyll content has also decreased to 11.90% in 100 ppm lead, 33.18% in 300 ppm, and 44.47% in 500 ppm lead.

It is not clear as to how the chlorophyll b content of *V. natans* in 100 ppm lead is greater than the chlorophyll b content in 0 ppm lead since lead only affects chlorophyll synthesis by inhibiting the enzyme leavulinate dehydrogenase and not the chlorophyll itself [17]. Nevertheless, the total chlorophyll content has decreased and it is an implication that chlorophyll content decreases when the amount of lead exposure is also increased.

A study conducted by Zengin and Munzuroglu[18] showed that lead concentrations above 1.5 mM can cause significant reduction of total chlorophyll content of *Phaseolus vulgaris L.*, with about 8.04%-12.51% decrease.

Detailed studies have indicated that heavy metals have effects on chlorophyll content in plants. Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient [19].

4. CONCLUSION

The use of *V. natans* as a biosorbent in the field of bioremediation was proven to be a promising and efficient approach for the removal of lead ions up to 500 ppm as observed in this study. In immediate bioremediation, *V. natans* still proved to be effective as it is capable of adsorbing high levels of lead even in treatments without acclimatizing the plant. The percentage uptake of residue samples have decreased as the concentration of lead is increased which means that the ability of *V. natans* to absorb lead ions is weakened by the increasing presence of lead ions. Also, comparison of the mean lead uptake of *V. natans* in supernatant and residue samples at all lead treatments has shown that the macrophyte is more capable of sequestering lead ions via adsorption.

Results obtained have also shown that the photosynthetic activity of *V. natans* was appreciably affected by the presence of lead ions. Chlorophyll a content of the macrophyte has decreased as lead concentration was increased. Chlorophyll b content has increased as lead concentration was increased from 0 ppm to 100 ppm and then decreased as lead concentration was increased from 100 ppm to 300 ppm, and then to 500 ppm.

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