HYDROPONIC SCREENING OF FIVE SUBMERGED PLANT SPECIES FOR LEAD PHYTOREMEDIATION POTENTIAL

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ABSTRACT

Heavy metals contaminated water is the one of the important water pollution problems in Thailand. Improper wastewater treatment and management are resulting in contamination of groundwater, surfacewater, and drinking water. Humans are exposed to the metals by ingestion or inhalation and they can be accumulated in living organisms causing various diseases and disorders. Aquatic plants were discovered to be a heavy metals phytoaccumulator for metals contaminated water environment, especially lead (Pb). This study aims to identify the potential candidates among 5 submerged plant species by comparing the plant's ability to remove and accumulate Pb. Hydrilla verticillata (L.f) Royle), Ceratophyllum demersum L., Limnophila heterophylla, Hygrophila difformis (L.f.) Bi, and Echinodorus amazonicus were cultured in 10% modified Hoagland's nutrients solutions supplemented with 10 mg/L as lead nitrate [$Pb(NO_3)_2$] for 11 days. Lead concentrations were determined by Inductively Couple Plasma-Optical Emission Spectrometer (ICP-OES) after microwave digestion. C. demersum, and L. heterophylla, showed Pb removal of 86.97 and 83.43%, respectively, after day 11^{th}. The highest concentration of lead was found in C. demersum (6269.6 mg/kg DW) and L. heterophylla (5171.7 mg/kg DW). These results indicated the potential application of C. demersum and L. heterophylla for phytofiltration of lead by constructed treatment wetland or introducing these plants into the contaminated water bodies.

Keywords: BCF, lead, phytoremediation, submerged plants, uptake.

INTRODUCTION

Heavy metals from agricultural sector, household activities, and improper waste management are resulting in water contamination. But the most concerned sources are industries such as mining, smelting, and manufacturing. Improper wastewater treatment and management are resulting in contamination of ground and surface water or drinking water. Generally, humans are exposed to these metals by ingestion or inhalation. Moreover, human can be exposed to Pb when working in an industrial site which utilizes Pb or living near a site where these metals have been improperly disposed [1]. These heavy metals tend to accumulate in living organisms and can cause various diseases and disorders [2].

Lead can be toxic to humans and animals causing health effects. They can move into and throughout ecosystems. Atmospheric lead is deposited in vegetation, ground and water surfaces [3]. Many researchers reported regarding effects of lead contamination in water, soil, food, and air. But this issue has received just little attention in most developing countries. Thailand, like many other developing countries, is now facing serious problems of Pb in the environment and concern about humans and animals health effects from lead [4].

Toxic heavy metals and organic pollutants are the major targets for phytoremediation [5]. There are several options for treating or cleaning up water contaminated with heavy metals such as, physical, chemical, biological remediation [6]. Phytoremediation is the use of metal accumulating plants for environmental clean-up [7]. The use of metal accumulating plants to clean soil and water contaminated with toxic metals is environmentally friendly and cost-effective technology [8]. Techniques of phytoremediation include phytoextraction (or phytoaccumulation), phytofiltration (or rhizofiltration), phytostabilization, phytovolatilization, and phytodegradation [9].

Submerged aquatic plants can be used for the removal of heavy metals [10]. Many species of aquatic plants has been reported to be heavy metals accumulator. Several aquatic species have the ability to remove heavy metals from water. *H. verticillata* is a potential As phytofiltrator for bioremediation [11]. It can be used for copper removal from moderately contaminated waters and can find application in as remediation in field conditions [12]. Rai et al. [13] reported that *C. demersum* plants responded positively to moderate Pb concentrations and accumulated high amount of metal. Chen et al. [14] reported that Pb accumulation increased with increasing concentrations and 40 μM is suggested to be *C. demersum*'s tolerance threshold for Pb in water.

The objective of the study is to investigate lead phytoremediation potential of five submerged aquatic plants (*H. verticillata, C.demersum, L. heterophylla, H. difformis, and E. amazonicus*) at 10 mg/L. Relative growth was observed to understand Pb tolerance. Total uptake and BCF were also investigated to identify potential accumulator.

METHODOLOGY

Experimental set-up

Transparent polyethylene plastic holding tanks with volume 5 L were used as reactors to study effect of Pb concentration on submerged plant. *H. verticillata, C.demersum, L. heterophylla, H. difformis, and E. amazonicus* were obtained from the Chatuchak's fish market, Bangkok, Thailand. Before cultivation, plants were washed by deionized water and soak in hydroponic culture in the laboratories and then acclimatized in 10% Hoagland's nutrients solution at least 7 days before use in the experiment [15]. A moderately acid regime (from pH 5.0 to 6.5) has been found to be suitable for most plants [16]. The pH values were adjusted between 5 and 6 during the batch experiments to prevent precipitation of Pb. Only healthy plants with uniform size and weight (approximately 20 ± 0.2 g) were chosen for the experiments. The experiments were performed at the same conditions such as ambient temperature, light, and nutrient solution under greenhouse. Five submerged plants were investigated for removal of Pb with 10 mg/L of Pb standard solution prepared from $Pb(NO_3)_2$ in deionized water and maintain in 10% Hoagland's solution for 11 days. Plants in 10% Hoagland's solution without Pb served as a control. The total volume of the solution were kept constant by adding deionized water every 3 days to compensate for water loss through plant evapotranspiration.

Heavy metal preparation

Instead of using natural wastewater, $Pb(NO_3)_2$ was used as the source of lead stock solution. As a precise of lead with varying concentrations value are essential. The required solutions were prepared with analytical reagents and deionized water for preparing lead stock solution of 1,000 mg/L. 1.599 g of $Pb(NO_3)_2$ was dissolved in 200 ml deionized water in 1 L volumetric flask and add 10 mL of 65% HNO_3. Add deionized water up to the mark to obtain 1000 mg/L of lead stock solution.

Heavy metal analysis in wastewater

Water samples were analyzed for Pb removal on day 0, 1, 3, 5, 7, 9, and 11 of experiment. The water samples were collected in clean plastic bottles. The water samples were filtered through Syringe Filter 0.22 µm Nylon and cooled to 4 °C immediately after collection. For Pb determination, the water samples were acidified to pH<2 with 1M HNO_3 and analysed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Model Optima 8000, PerkinElmer, USA.

Heavy metal analysis in plants

All parts of plants were harvested at the day 11 after starting the experiment. Plants were cleaned thoroughly with tap water, followed by several rinses with deionized water and weighed for fresh weight. Then they were dried at 70^{o}C for 48 h and weighed again for dry weight. After being dried, the dried plants were digested with 65%HNO_3 using a microwave digestion Model CEM MARS 6 (CEM Corporation, Matthews, NC) system. The lead content in both the water samples and the digested plant samples were estimated using ICP-OES. All experiments were set up in three replications.

Data analysis

1. Relative Growth Rate (RGR) = Final fresh weight (g)/ Initial fresh weight (g)
2. %Removal= [(Initial concentrations of metal in the medium - Remaining concentrations of metal in the medium) / Initial concentrations of metal in the medium] x 100%
3. BCF= concentration of Pb in dried plant (mg/kg dw)/ initial concentration of metals in solution (mg/L)

RESULTS AND DISCUSSION

Plant's physical observation

The visual changes were observed in, *H. verticillata, C.demersum, L. heterophylla, H. difformis, and E. amazonicus* over the period of experiments are summarized in Table 1. All plants species looked healthy before day 7th. At day 7th *H. verticillata* and *H. difformis* looked quite healthy with leaves margin dried. Whereas, *L. heterophylla* and *E. amazonicus* looked unhealthy, leaves turning yellow. Day 11th of the experiment, *H. verticillata* still looked quite healthy with leaves margin dried. On the other hand, *L. heterophylla* and *H. difformis* looked unhealthy, turning brown and going to die. *E. amazonicus* looked unhealthy, leaves turning yellow. Lastly, *C. demersum* looked healthy with green leaves through the last day of the experiment. *C. demersum* and *H. verticillata* could grow well through the experiment.

Plant relative growth rate

A comparison of plant relative growth rate (RGR) of five submerged plants in Hoagland's nutrient solution and Pb contaminated water is shown in in Table 2. The relative growth of *H. verticillata*, which was 1.18 Hoagland's nutrient solution and 1.13 in Pb contaminated water, was the highest amongst all species. The relative growth of *L. heterophylla* decreased drastically (0.36). However, the relative growth of all plants slightly decreased. The relative growth was 0.9, 0.77, and 0.85 for *C. demersum, H. difformis,* and *E. amazonicus*, respectively. This shows inhibition of growth due to Pb toxicity.

Pb toxicity is reported to inhibit growth of plants [17], which is in agreement with decrease in biomass production observed in this study. The inhibited growth of submerged plant species due to Pb toxicity was reported in many researchers [13] reported that *C. demersum* exhibited weight loss due to Pb exposure. Mishra et al., [18] also reported the toxic effect and oxidative stress of *C. demersum* caused by Pb were evident by the reduction in biomass with increase in metal concentration and exposure duration. It can be concluded that *H. verticillata* is the most tolerant to the Pb and *L. heterophylla* depicts most toxic effect amongst five species.

Table 1 Plants physical observation

Species	Day 0				Day 3				Day 7				Day 11			
	Control	Reactor 1	Reactor 2	Reactor 3	Control	Reactor 1	Reactor 2	Reactor 3	Control	Reactor 1	Reactor 2	Reactor 3	Control	Reactor 1	Reactor 2	Reactor 3
H. verticillata	H	H	H	H	H	H	H	H	QH	QH	QH	QH	QH	QH	QH	QH
C. demersum	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
L. heterophylla	H	H	H	H	H	H	H	H	UH	UH	UH	UH	UH	UH	D	D
H. difformis	H	H	H	H	H	H	H	H	QH	QH	QH	QH	QH	UH	UH	D
E. amazonicus	H	H	H	H	QH	QH	QH	QH	UH	UH	UH	UH	D	UH	UH	UH

H: Plants looked healthy with green leaves
QH: Plant looked quite healthy with leaves margin dried
UH: Plants looked unhealthy, leaves turning yellow
D: Plant turning brown and going to die.

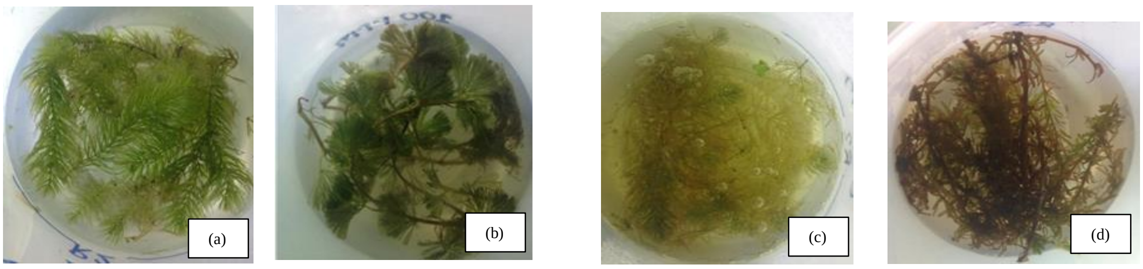

Figure 1 Physical observation of healthy plants (a) *C.demersum* (b) L. heterophylla and unhealthy plants (c) *C.demersum* (d) *H. verticillata*

Table 2 Relative Growth Rate

Species	Relative Growth Rate	
	Control	Pb contaminated water
Hydrilla verticillata (L.f) Royle)	1.18	1.13
Ceratophyllum demersum L	0.94	0.9
Limnophila heterophylla	0.93	0.36
Hygrophila difformis (L.f.) Bi	0.98	0.77
Echinodorus amazonicus	1.08	0.85

Pb removal eficiency

All submerged plants were able to remove Pb from contaminated water as shown in Figure 1. The initial concentrations of Pb (10 mg/L) in each reactor were decreased in high amount on the 1st day of experiment. All species removed Pb slowly after 1st day. Pb removal in *C. demersum, L. heterophylla, H. difformis,* and *E. amazonicus* slightly increased and reached highest removal percentage on day 11th whereas *H. verticillata* reached highest removal percentage on day 9th. Comparing Pb removal of each plant, the highest Pb removal was found in *C. demersum* (86.97%±4.80), and lowest by *H. verticillata* (47.36±3.06). Although the *L. heterophylla* and *H. difformis* did not look healthy as other species, their removal efficiency was better than some other. *L. heterophylla*, had the lowest relative growth, but could remove as well. The result conforms to the earlier report [13] that more than 70% Pb was removed *by C. demersum*. It is in agreement with a study of [14] which reported that the amount of lead accumulated by *C. demersum* was significantly influenced by exposure concentration and exposure time as well as the interplay between concentration and time. Mishra et al. [18] also reported that the maximum rate of metal accumulation in *C. demersum* was found after 1 day when about 70% of the total metal accumulated was taken up by the plant. With increase in duration, metal accumulation increased though rate was quite low. It seemed that Pb uptake is two-step process, a rapid first step up to 1 d and a slow second step till the end of experiment. Moreover, the results of Keskinkan et al. [19] showed that submerged aquatic plant *C. demersum* can be successfully used for heavy metals (Pb, Zn and Cu) removal under dilute metal concentration. It can be concluded that *C. demersum* had a highest Pb removal potential, additionally *L. heterophylla* can also be employed for phytofiltration of lead by constructed treatment wetland or introducing these plants into the contaminated water bodies.

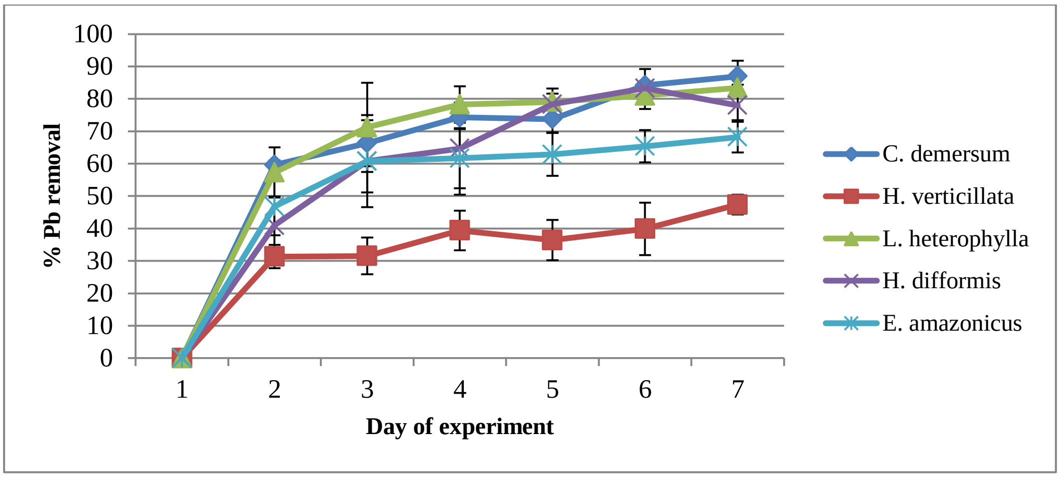

Figure 2 Pb removal with time by different species (Pb concentration=10mg/L)

Pb uptake efficiency

Lead concentration in plants vary with plant species. The results on Pb uptake by these five species are shown in Figure 3. The highest Pb accumulation in plants was in *C. demersum* (6269.63± 666.20 mg/kg dw), while the lowest one as found in *H. verticillata* (1479.7±447.06 mg/kg dw). Thus, *C. demersum* can be considered as Pb hyperaccumulator according to the threshold value of Pb in plants (1000 mg kg^{-1}) [20]. Chen et al., [14] revealed their studied about Bioaccumulation and tolerance characteristics of a submerged plant (*C. demersum L.*) exposed to toxic metal lead (5–80 µM) for 7, 14 or 21 days. The results found that *C. demersum* appears to possess a high potential for lead bioaccumulation. From their study, the maximum amount of lead accumulated in the plants reached 4016.4 mg kg^{-1}. Rai et al., [13] reported about *C. demersurn* which accumulated high amounts of Pb 12.06 µmol g^{-1}dw at Pb concentration 7.784 µM. Mishra et al., [18] also reported about Pb accumulation by *C. demersum* plants when exposed to Pb 1–100 µM for 1–7 days. Plants accumulated high amount of Pb in concentration and duration dependent manner. Their maximum rate of metal accumulation was found after 1 d when about 70% (1222 µg g^{-1} dw at 100 µM) of the total metal accumulated was taken up by the plant and showing a gradual increase throughout the experiment. The two highest accumulators were *C. demersum and L. heterophylla* need to be investigated further.

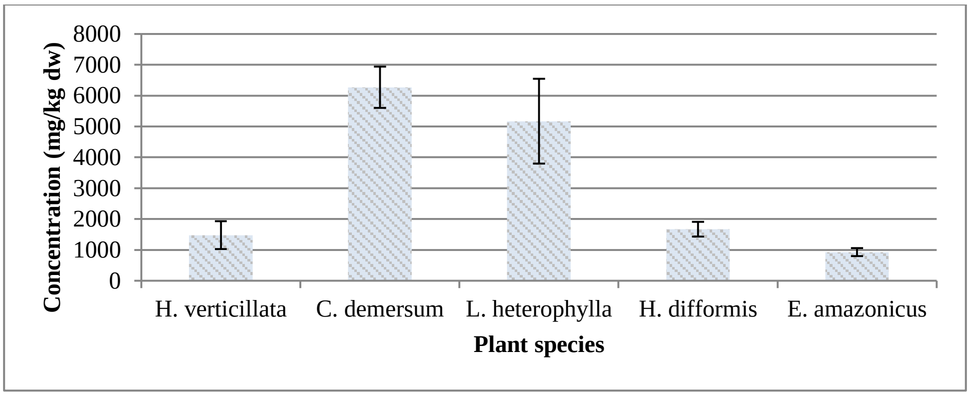

Figure 3 Pb uptake by plants

Bioconcentration factor

Plant bioconcentration factor (BCFs) is more significant than the amount accumulated in plants as it shown an index of the ability of the plants to accumulate metal element with respect to the element concentration in water [21]. The BCFs for different plant species are shown in Table 3. The highest BCF was found in *C.demersum* (626.96), indicating that Pb uptake was better than other species, followed by *L. heterophylla*, *H.difformis H. verticillata*, and *E. amazonicus*, respectively. Zayed et al. [22] reported that plant which is considered as a good heavy metals accumulator must have a BCF over 1000. Thus, *C. demersum* and *L. heterophylla* considered as moderate accumulator for Pb at 10 mg/L.

Table 3 Bioconcentration factor

Species	Bioconcentration factor
Hydrilla verticillata (L.f) Royle)	92.72
Ceratophyllum demersum .	626.96
Limnophila heterophylla	517.17
Hygrophila difformis (L.f.) Bi	167.46
Echinodorus amazonicus	147.97

CONCLUSION

The five aquatic plants investigated **(***Hydrilla verticillata (L.f) Royle), Ceratophyllum demersum L., Limnophila heterophylla*, *Hygrophila difformis (L.f.) Bi*) have the ability to remove lead from contaminated water. In view of their tolerance, relative growth, Pb removal and accumulation efficiency, and bioconcentration factor *C. demersum* had the highest relative growth rate, bioconcentration factor, Pb removal, and accumulation efficiency amongst all species. *H. verticillata* had the lowest BCF value, Pb removal and uptake efficiency species, but they were highest in RGR value. From this study, it was found that *C. demersum*, and *L. heterophylla* were the three highest potential Pb accumulators among five species. *C. demersum and L. heterophylla* were found as the Pb moderate hyperaccumulator. These plants can be found easily in Thailand, and can be easily harvested. They have potential for use in the phytoremediation of aquatic environments that are contaminated with Pb. However, harvesting techniques, metal recovery technology, and safe disposal of used plants will have to be worked out before large scale application is adopted.

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