Removal of Lead from Synthetic Wastewater using Aquatic Plants

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Abstract

Abilities of aquatic plant species, *Echinodosus cordifolius* (L.) Griseb., *Bacopa caroliniana* (Walt.) Rob., and *Hydrocotyle umbellata* L., for phytoremediation of lead (Pb) from synthetic wastewater were conducted in hydroponic system at 20-80 mg/L of Pb (II) and 7-28 days of exposure periods. Additional experimental set up for aquatic plant species under 60 and 80 mg/L of Pb (II) were added with 5 mM EDTA in order to enhance Pb accumulation in the plants. Results revealed that the capacity of Pb (II) accumulation at 80 mg/L of Pb (II), 28 days in roots of all aquatic plants was higher than that of shoots. *E. cordifolius* (L.) Griseb. had the highest capacity of Pb (II) accumulation about 12,566 (roots) and 8,686 (shoots) mg/kg dry wt, respectively giving 95% of Pb (II) removal, followed by *H. umbellata* L. and *B. caroliniana* (Walt.) Rob., respectively. Regarding EDTA addition, lead accumulation (80 mg/L of Pb (II), 7 days) was increased in the roots of *H. umbellata* L. about 19,883 mg/kg dry wt and it was increased in the shoots of *E. cordifolius* (L.) Griseb. about 14,516 mg/kg dry wt (80 mg/L of Pb (II), 21 days), respectively. The highest relative growth (13.85) and percentage of biomass productivity (98%) were in *H. umbellata* L. (60 mg/L of Pb (II), 7 days), whereas the highest bioconcentration factor (9,341.68) was in *E. cordifolius* (L.) Griseb.(80 mg/L of Pb (II), 21 days). According to the results, *E. cordifolius* (L.) Griseb. was recommended to remove the lead from synthetic wastewater with EDTA addition. Further studies in contaminated wastewater under various characteristics and flows should be investigated.

Keywords : *Echinodosus cordifolius* (L.) Griseb., *Bacopa caroliniana* (Walt.) Rob., *Hydrocotyle umbellata* L., Lead, EDTA, Wastewater

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1. Introduction

Nowadays, Thailand has been developed and expanded on economic rapidly. Lead (Pb) is the one of heavy metal using in the factories of battery, dry battery and vehicle materials, etc. It leads to the expansion of industries and occurrence of environmental problems [1]. Wastewater of forging lead, battery, and vehicle manufacture factories discharges without treatment into river sources and provides a large volume of heavy metal contaminated into the river, which has impacted on living things in river. It also has the ability to affect the entire food chain and to disrupt the health system of human being, animals and phytoplanktons [2]. Therefore, proper treatment of lead contaminated in water is very important. Given its potential hazard and widespread contamination, there is a high interest in the low cost method for removing Pb from the environment. Phytoremediation is a cost-effective and environmental friendly technology to clean up the aquatic system contaminated with lead using aquatic plants [3 - 4]. The principle involves growing plants that can hyperaccumulate heavy metals from contaminated sites, harvesting the plants, selling the harvested plants to buyers that can extract and process the metals for further use or disposal as hazardous waste [5]. Moreover, addition of synthetic chelator (ethylenediaminetriacetic acid; EDTA) has been shown to enhance the Pb accumulation in roots and to promote its translocation to shoots as well as to reduce Pb toxicity [6].

Hence, this study was designed to investigate the ability of aquatic plant species including *Echinodosus cordifolius* (L.) Griseb., *Bacopa caroliniana* (Walt.) Rob. and *Hydrocotyle umbellata* L. in Pb accumulation. The effects of synthetic chelator (EDTA) on relative growth, lead accumulation, biomass productivity, bioconcentration factor (BCF) and percentage of removal lead in aquatic plants, were also studied.

2. Materials and methods

2.1 Preparation of synthetic wastewater, hydroponic unit and aquatic plants

Synthetic wastewater was prepared by using an analytical grade lead nitrate ($Pb(NO_3)_2$). The stock solution contained 1,000 mg/L Pb (II). Then, the stock solution was diluted to the concentrations of 20, 40, 60 and 80 mg/L of Pb (II). pH of solution was maintained at 5.7 by using HNO_3 and NaOH for prevention of lead precipitation [7]. Synthetic chelator of ethylene diaminetetra-acetic acid (EDTA) was applied for experiment because EDTA enhanced the accumulation and transportation of Pb from root to shoot [6]. In the experiment, EDTA concentration of 5 mM was added to the lead synthetic solution in hydroponic system.

The hydroponic unit in experiment was a plastic bucket and contained 14 liters of synthetic solution. Pump was installed to continually circulate the synthetic solution with flow rate of 500 mL/min. The sheet of hydroponic gutter with 15 planting holes made from foam in the width of 23.50 cm and length of 34.50 cm was placed on hydroponic unit. The diameter of each hole was at 2.50 cm and the space of vertical hole was 4.00 cm and landscape hole was 3.50 cm.

Aquatic plant species with 2-3 weeks of plant age i.e., *Hydrocotyle umbellata* L. (4.0-5.0 g fresh weight), *Echinodosus cordifolius* (L.) Griseb. (4.0-5.0 g fresh weight), and *Bacopa caroliniana* (Walt.) Rob. (3.5-5.0 g fresh weight), were grown in the experiment plot. These aquatic plants were cleaned up with tab water and rinsed with distilled water to clean soil or dirty attached to plant's root and then measured the fresh weight prior to the experiment. They were acclimatized in a modified Hoagland's solution [8] that their roots were dipped into a nutrient solution about 2.0-3.0 cm depth and were naturally grown for 7 days before experimental setup (Fig. 1). The experiment was carried out in triplicate with control group.

2.2 Experimental procedures

2.2.1 Ability of aquatic plants in Pb accumulation

Three species of aquatic plants and liquid samples were collected after complete acclimatization as the starting sample of experiment (synthetic solution) for analysis. Then, lead concentration of synthetic solution at 20, 40, 60 and 80 mg/L of Pb (II) was added and pH of solution was maintained at 5.7 (using HNO_3 and NaOH) in the experiment. Exposure periods were 7, 14, 21, and 28 days in the experiment. Each Pb concentration in experiment was conducted in four hydroponic units for suitable plant sampling. Three species of aquatic plants and liquid were collected from a hydroponic unit in every 7 days, and then they were analyzed to find out the lead accumulation in aquatic plants and lead concentration of contaminated solution.

2.2.2 Effects of synthetic chelator (EDTA) on relative growth, Pb accumulation, biomass productivity, and bioconcentration factor in aquatic plants

Three species of aquatic plants were continually studied in the best accumulation of lead in aquatic plants (60 and 80 mg/L of Pb (II)). The synthetic solution of 60 and 80 mg/L of Pb (II) was added with 5 mM EDTA for enhancing lead accumulation and transporting Pb from root to shoot. At day 7, 14, 21, and 28 days, plants and liquid were collected from hydroponic unit in every 7 days for analysis.

2.3 Analytical methods

Each aquatic plant in hydroponic unit (60 and 80 mg/L of Pb (II) for 7, 14, 21, and 28 days) with EDTA addition was collected for observation, recorded on plant's growth, i.e., amount of leaves, a length of root, and a length of stem, as well as a plantation's image and plant's changing. Aquatic plant sampling's analysis (20, 40, 60 and 80 mg/L of Pb (II) without EDTA addition or 60 and 80 mg/L of Pb (II) with EDTA addition) was done. They were cleaned by water and rinsed with distilled water about 2-3 times, and done a wet weight after experiment and then heat them in a hot air oven at 60°C in 24 hours. Dry weights of aquatic plants were divided into 2 parts; roots and shoots. The dry weight (wt) of 0.5 g was put in a vessel to add the nitric acid (HNO_3) of 1 mL and hydrogen peroxide (H_2O_2) of 7 mL and it was brought to a microwave for digestion method [9]. The filtered Pb solution was analyzed by using an Atomic Absorption Spectrophotometer (AAS). The results were reported in unit of mg Pb (II)/kg Pb dry wt. The initial and final Pb concentrations of solution operated at 20, 40, 60 and 80 mg/L of Pb (II) (without EDTA addition), while the initial and final Pb concentrations of solution operated at 60 and 80 mg/L of Pb (II) (with EDTA addition) for exposure periods of 7, 14, 21, and 28 days in hydroponic unit were collected in every 7 days. Pb concentration in solution was analyzed by using the Atomic Absorption Spectrophotometer (AAS) [9].

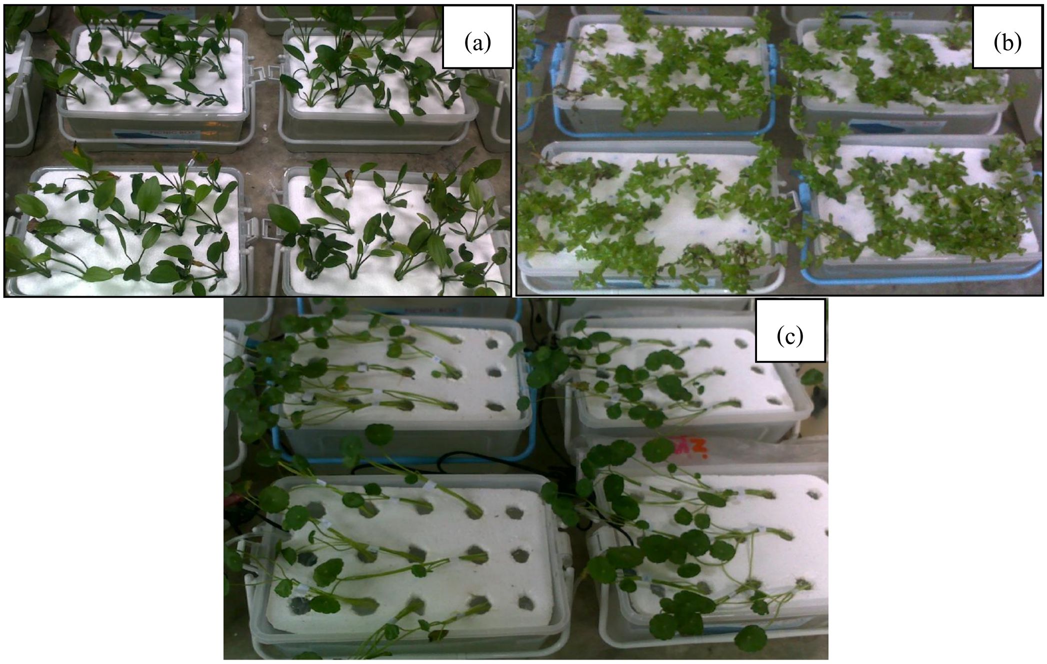

Fig. 1. Plant species were hydroponically grown in Hoagland's solution; (a) *E. cordifolius* (L.) Griseb. (b) *B. caroliniana* (Walt.) Rob., and (c) *H. umbellata* L.

The percentage removal of heavy metal from plant is calculated by an equation (1) [10].

$$\% \text{ Removal} = \left[(C_{inf} - C_{eff}) / C_{inf}\right] \times 100 \quad (1)$$

where C_{inf} is an initial contaminant concentration in and C_{eff} is a final contaminant concentration in effluent.

The biomass productivity was determined by dry weight of each plant sample which was contained in an oven at temperature 60°C for 24 hours. The dry weight of each plant at different Pb concentration and exposure time was expressed as percentage of biomass productivity. Relative growth was determined by the final and initial fresh weights [2]. The BCF provides an index of the ability of the plant to accumulate the trace element with respect to the trace element concentration in substrate [11].

3. Results and discussion

3.1 Efficiency of aquatic plants for Pb removal in hydroponic system

Efficiencies of *E. cordifolius* (L.) Griseb., *B. caroliniana* (Walt.) Rob., and *H. umbellata* L. for Pb accumulation in roots and shoots were detected at 20, 40, 60 and 80 mg/L of Pb (II) in exposure periods of 7, 14, 21 and 28 days. Regarding the Fig. 2 & 3, these aquatic plants could uptake Pb dissolved in hydroponic system, but they showed different abilities of accumulating Pb. In addition, Pb accumulation of aquatic plants had highly capacity with a significant at $P<0.05$ when Pb concentration and exposure period increased.

Among the tested plant species, tendency of Pb accumulation declined in the order of *E. cordifolius* (L.) Griseb. > *H. umbellata* L. > *B. caroliniana* (Walt.) Rob. There were quite high abilities of accumulating Pb in these aquatic plants at 60 and 80 mg/L of Pb (II). After 28 days, Pb accumulation in roots at 80 mg/L of Pb (II) was found that *E. cordifolius* (L.) Griseb. was in the highest Pb accumulation (12,566.67 mg/kg dry) (Fig. 2(a)) and followed by *H. umbellata* L. (10,950 mg/kg dry wt) (Fig. 2(c)) as well as *B. caroliniana* (Walt.) Rob. (10,640 mg/kg dry wt) (Fig. 2 (b)). For the Pb accumulation in shoots at 80 mg/L of Pb (II), they were found that *E. cordifolius* (L.) Griseb. was in the highest Pb accumulation (8,686.67 mg/kg dry wt) (Fig. 3(a)) and followed by *H. umbellata* L. (4,216.67 mg/kg dry wt) (Fig. 3(c)), whereas *B. caroliniana* (Walt.) Rob. was about 4,993.33 mg/kg dry wt at 60 mg/L of Pb (II) in 28 days. In the present study, it was indicated that tendency of Pb accumulation in roots was higher than in shoots. Similar results have been reported by several investigators in different species of plants. The differences in the ability of plants to accumulate Pb have been related to differences in their root morphology and metabolic pathway. The roots are indeed the primary site for absorption of solution and accumulate the heavy metal in the large quantities of culture solution as well as translocation to shoot may be in limitation due to binding of heavy metal on root surface and cell wall [12 - 13].

The study results were complied with the previous researches. Phetsombat et al. found that in *Salvinia cucullata*, exposure to 40 mg/L Pb (II) resulted in the shoot concentration of 3,982.60 μg/g dry wt and the root concentration of 14,305.60 μg/g dry wt [11]. Tayaparan et al. reported that the highest Pb accumulation in *Azolla pinnata* was 10, 590 mg/kg dry wt when exposed to 10 mg/L of Pb in 7 days [6]. Sunflower and mustard accumulated about 917.82 mg/kg dry wt and 835.54 mg/kg dry wt when exposed to 200 mg/L of Pb, respectively [14]. Hence, it can be seen that accumulation of complexes of heavy metals by aquatic plants depends on plant species, kinds or concentrations of metals [14].

For lead removal by three species of aquatic plants, their capacities of Pb removal from contaminated water were increased when Pb concentration and exposure period increased. Due to their root system and root surface could trap and race heavy metal or absorb contaminants into the roots when contaminants were in solution surrounding the root zone [15]. Another reason that the aquatic plants are able to remove of the heavy metals from contaminated water because metals in an aqueous solution are ready in soluble form, so accumulation by the plants can be achieved much easier [3]. According to the Pb removal by aquatic plants, *E. cordifolius* (L.) Griseb. had the highest capacity of Pb removal (95%) and followed by *H. umbellata* L. (93.90%) as well as *B. caroliniana* (Walt.) Rob. (93.70%) at 80 mg/L of Pb (II) in 28 days. Otherwise, *B. caroliniana* (Walt.) Rob. had the lowest capacity of Pb removal about 36.70% (40 mg/L of Pb (II),7 days) and followed by *H. umbellata* L. about 42.50% as well as *E. cordifolius* (L.) Griseb. about 54.50% (20 mg/L of Pb (II), 7 days).

The study results were similar to other researches. Singh et al. reported that common aquatic plant of duckweed could remove up to 90% of soluble Pb from water [3]. Chakraborty and Mukherjee showed that percentage of Pb removal by water lettuce was about 82% [16] and *Ca. caroliniana* showed the best removal efficiency (90-95%) at lead concentration 10 ppm after 12-15 days of exposure [17].

3.2 Effect of EDTA on growth and Pb accumulation of aquatic plants and Pb removal in hydroponic system

3.2.1 Effect of EDTA on growth of aquatic plants

According to the Table 1, it shows the values of relative growth, biomass productivity and BCF in aquatic plants that were tested at Pb concentration (60, 80 mg/L Pb (II) with and without EDTA additions. The results revealed that tendencies of these values in case of Pb (II) plus EDTA were higher than that of Pb(II).

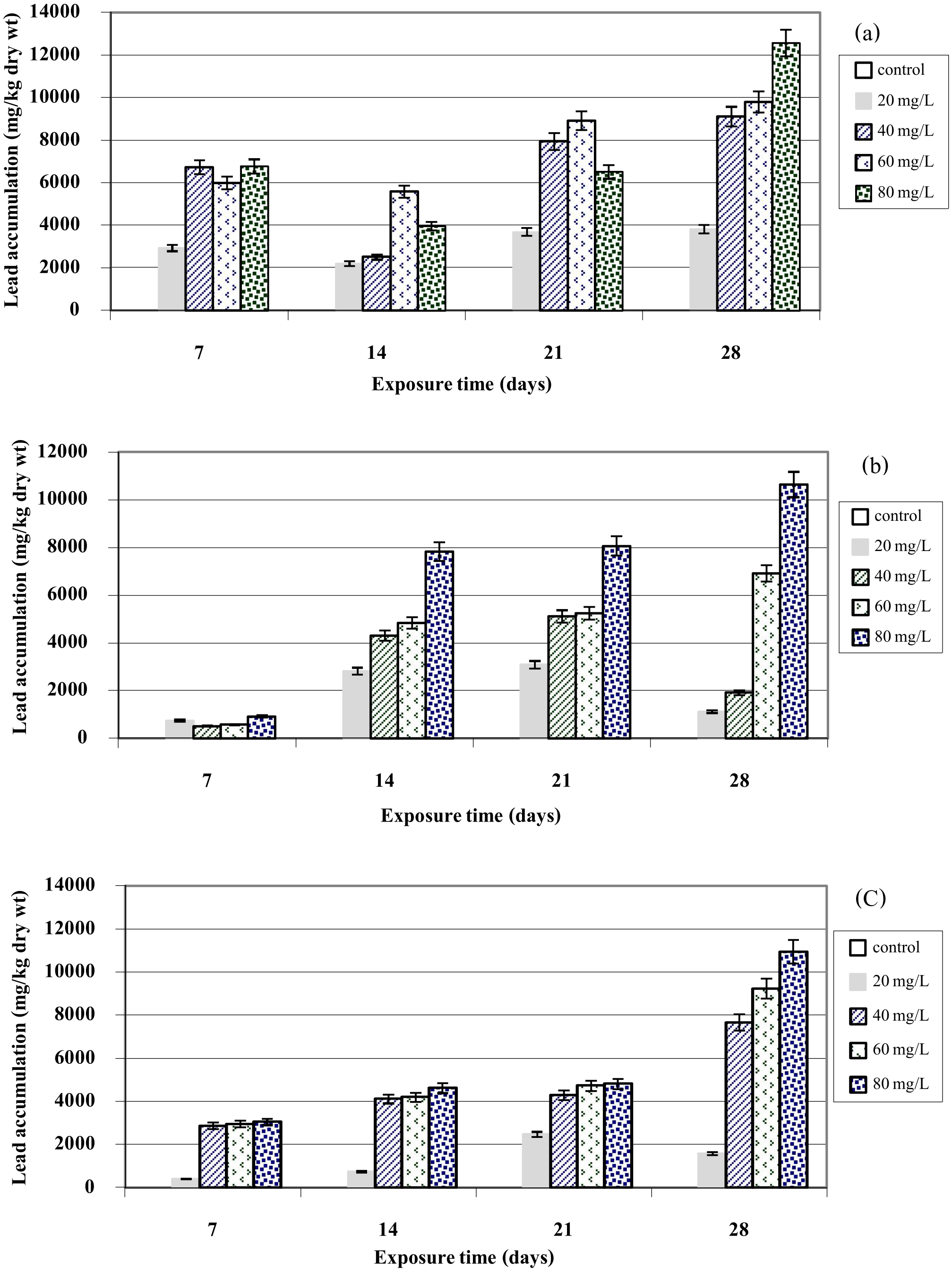

Fig. 2. Accumulation of lead in plant roots; (a) *E. cordifolius* (L.) Griseb. (b) *B. caroliniana* (Walt.) Rob., and (c) *H. umbellata* L.

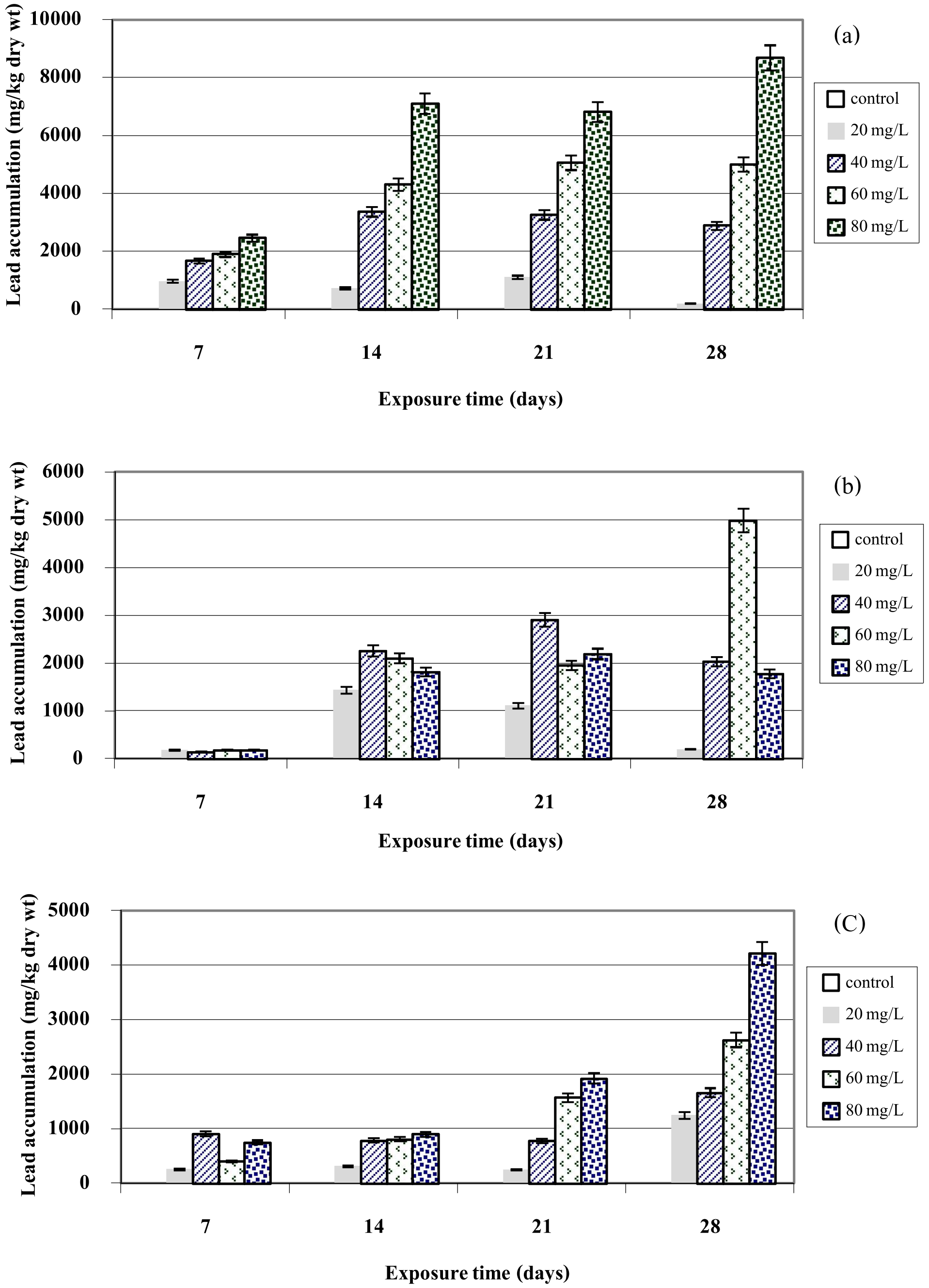

Fig. 3. Accumulation of lead in plant shoots (a) *E. cordifolius* (L.) Griseb. (b) *B. caroliniana* (Walt.) Rob., and (c) *H. umbellata* L.

When Pb concentration with EDTA addition and exposure period increased, the relative growth of plant decreased ($P<0.05$). Regarding the relative growth of aquatic plants, the highest value (12.89-13.92) was found in *H. umbellata* L. and followed by *E. cordifolius* (L.) Griseb. (7.31-7.90) as well as *B. caroliniana* (Walt.) Rob. (5.78-7.11) (80 mg/L of Pb (II), 7-28 days) (Table 1). Moreover, the highest biomass productivity of *H. umbellata* L. was about 60-98% and followed by *E. cordifolius* (L.) Griseb. (61-93%) as well as *B. caroliniana* (Walt.) Rob. (53-92%) (80 mg/L of Pb (II), 7-28 days) (Table 1).

BCF values can be index to estimate a plant's ability of accumulating heavy metals according to the bioconcentration [14]. From the results of BCF values with EDTA addition, they showed that the highest BCF values of *E. cordifolius* (L.) Griseb. was about 4,904-9,341 and followed by *H. umbellata* L. (2,529-6,500) as well as *B. caroliniana* (Walt.) Rob. (1,643-3,991) (80 mg/L of Pb (II), 7-28 days) (Table 1). Moreover, BCF values over 1000 are generally considered as a good accumulator and evidence of a useful plant for phytoremediation [2, 4]. Based on these criteria, the results showed that *E. cordifolius* (L.) Griseb. is the best accumulator of Pb. BCF values were in different trends, they were caused from the Pb concentration, accumulation ability and physiological factors of plants, and environmental condition [14].

3.2.2 Effect of EDTA on Pb accumulation of aquatic plants

Among the tested plant species, tendency of Pb accumulation declined in the order of *E. cordifolius* (L.) Griseb. > *H. umbellata* L. > *B. caroliniana* (Walt.) Rob. Pb accumulations of those aquatic plants were quite increased in the presence of EDTA, except *B. caroliniana* (Walt.) Rob (Fig. 4. & 5.). Regarding Pb accumulation in roots at 80 mg/L of Pb (II) with EDTA addition, it found that *H. umbellata* L. had the highest Pb accumulation about 19,833.33 mg/kg dry wt in 7 days (Fig. 4(c)) and followed by *E. cordifolius* (L.) Griseb. (16,766.67 mg/kg dry wt) in 21 days (Fig. 4(a)), whereas *B. caroliniana* (Walt.) Rob. was about 15,600 mg/kg dry wt (60 mg/L of Pb (II), 21 days)(Fig. 4(b)). For Pb accumulation in shoots, it found that *E. cordifolius* (L.) Griseb. had the highest Pb accumulation about 14,516.67 mg/kg dry wt (80 mg/L of Pb (II), 21 days) (Fig. 5(a)) and followed by *H. umbellata* L. was about 9,416.67 mg/kg dry wt (60 mg/L of Pb (II), 14 days) (Fig. 5(c)) and *B. caroliniana* (Walt.) Rob. was about 2,083.33 mg/kg dry wt (60 mg/L of Pb (II), 7 days) (Fig. 5(b)). Based on those results, EDTA facilitated both the Pb uptake by roots and also helped it translocation from roots to shoots [18].

According to the Pb removal by aquatic plants in hydroponic system of EDTA addition, the highest capacity of Pb removal by *H. umbellata* L. was about

96.90% (80 mg/L of Pb (II), 7 days) and followed by *E. cordifolius* (L.) Griseb. (91.40%) as well as *B. caroliniana* (Walt.) Rob. (81.40%) (60 mg/L of Pb (II), 21 days).

The study results were similar to other researches of Pb with EDTA addition that Pb accumulation was enhanced in roots and promoted its translocation to shoots in *H. annuus* L. [18]. Also, results on increasing Pb accumulation of the aboveground plant parts were in *B.juncea*, corn, alfafa [19 - 20].

4. Conclusion

Three aquatic plants could phytoextract Pb in hydroponic system in absence of EDTA, and *E. cordifolius* (L.) Griseb. showed the better ability of accumulation than the others. The ability of accumulation differed with plant species and Pb concentrations. The greatest Pb uptake was observed in the roots. Minimal amounts of Pb were translocated to the shoots. In case of Pb with EDTA addition, it enhanced the Pb accumulation in roots and promoted its translocation from roots to shoots, especially the roots of *H. umbellata* L. and the shoots of *E. cordifolius* (L.) Griseb., respectively. In any case, the usage of EDTA as chelating agent has the limitation of low biodegradability, thus its practical usage requires careful site-specific evaluation to minimize the risk of living things exposure in environment. Consequently, *E. cordifolius* (L.) Griseb. was a good accumulator for Pb and could be applied for Pb removal in wastewater. However, since the results obtained were from lab-scale experiment, the Pb accumulation presented here may not be valid under natural field conditions with flow of water, the presence of dissolved oxygen and rhizosphere microorganism activity. Further studies under simulated field conditions should be done.

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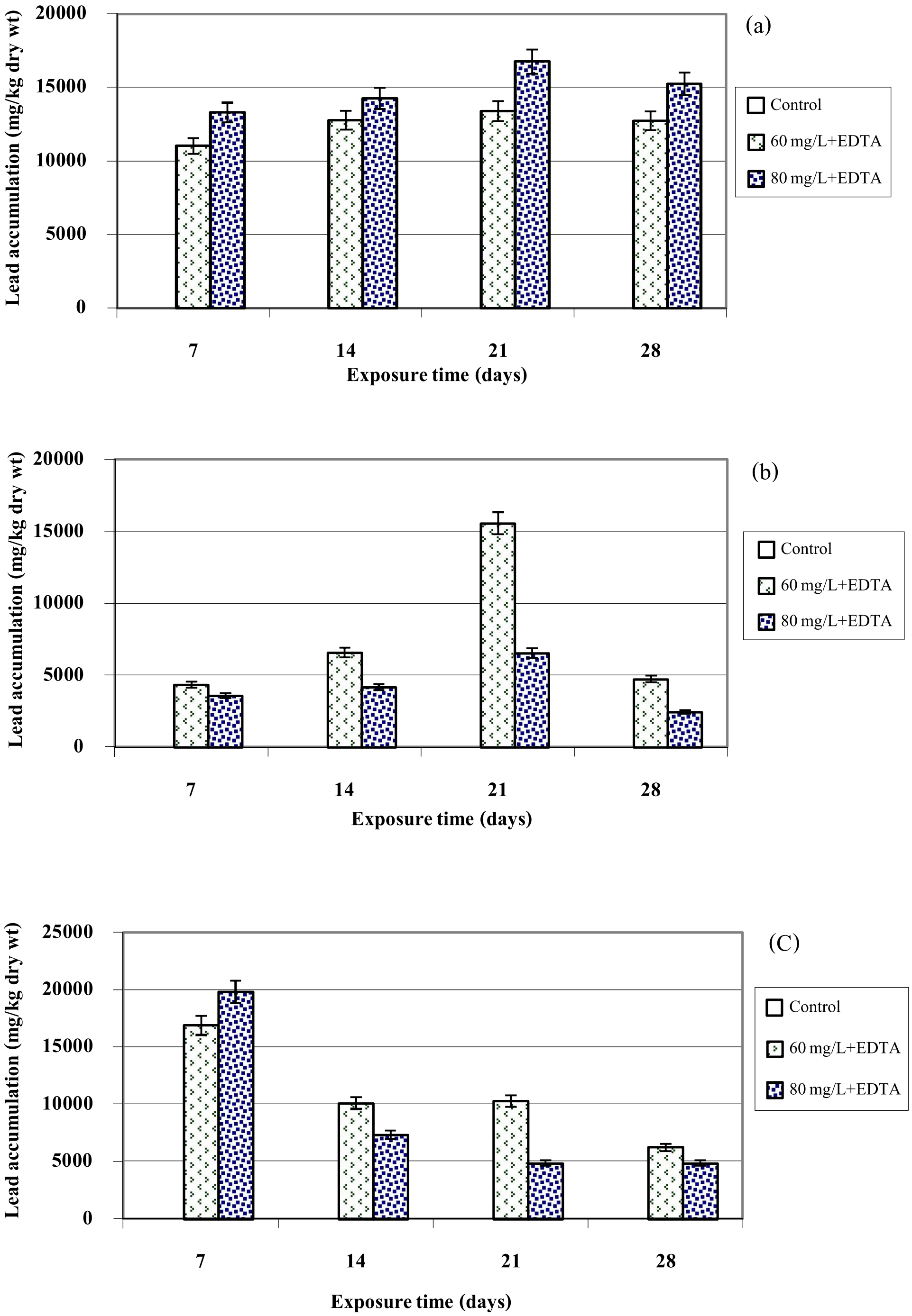

Fig. 4. Effect of EDTA on accumulation of lead in plant roots; (a) *E. cordifolius* (L.) Griseb. (b) *B. caroliniana* (Walt.) Rob., and (c) *H. umbellata* L.

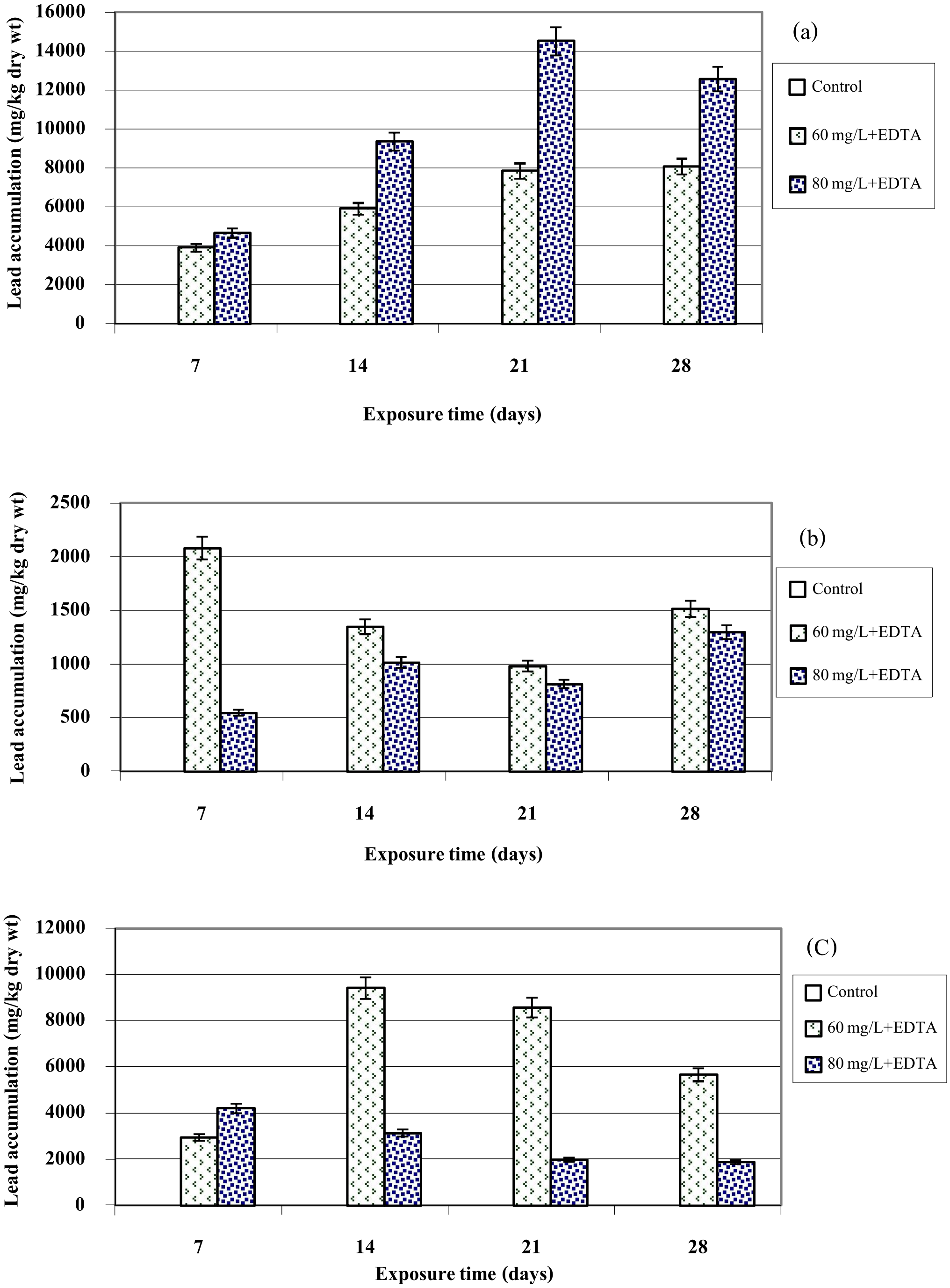

Fig. 5. Effect of EDTA on accumulation of lead in plant shoots; (A) *E. cordifolius* (L.) Griseb. (B) *B. caroliniana* (Walt.) Rob., and (C) *H. umbellata* L.

Table 1. Ralative growth, biomass productivity and BCF of three species of aquatic plants at 60 and 80mg/L Pb (II) with and without EDTA additions (7-28 days).

Aquatic plant species	**Relative growth**			
	Non EDTA addition		**EDTA addition**	
	60 mg/L Pb (II)	**80 mg/L Pb (II)**	**60 mg/L Pb (II)**	**80 mg/L Pb (II)**
E. cordifolius (L.) Griseb.	1.20-1.46	1.12-1.28	7.33-7.99	7.31-7.90
B. caroliniana (Walt.) Rob.	0.72-0.87	0.67-0.86	4.11-7.03	5.78-7.11
H. umbellata L.	1.14-1.25	1.04-1.21	13.32-13.85	12.89-13.92
Aquatic plant species	**Biomass productivity (%)**			
	Non EDTA addition		**EDTA addition**	
	60 mg/L Pb (II)	**80 mg/L Pb (II)**	**60 mg/L Pb (II)**	**80 mg/L Pb (II)**
E. cordifolius (L.) Griseb.	57-94	53-94	59-96	61-93
B. caroliniana (Walt.) Rob.	53-72	49-59	50-91	53-92
H. umbellata L.	56-92	49-91	54-98	60-98
Aquatic plant species	**BCF**			
	Non EDTA addition		**EDTA addition**	
	60 mg/L Pb (II)	**80 mg/L Pb (II)**	**60 mg/L Pb (II)**	**80 mg/L Pb (II)**
E. cordifolius (L.) Griseb.	2,764-4,853	2,547-6,648	4,163-6,635	4,904-9,341
B. caroliniana (Walt.) Rob.	491-4,163	618-3,469	757-2,415	1,643-3,991
H. umbellata L.	1,228-4,224	1,460-4,454	2,615-6,878	2,529-6,500

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