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Pb Removal from Contaminated Water Using EDTA with *Colocasiaesculenta* (L.) Schott at Klity Creek, Kanchanaburi, Thailand

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Abstract: This study investigated lead (Pb) removal using a plant called'Elephant Ear' (*Colocasiaesculenta* (L.) Schott) from contaminated waterat Klity Creek, located in Kanchanaburi Province, Thailand. Plants weregrown in contaminated water with lead carbonate ($Pb(CO_3)_2$) in a nursery. The experiment was divided into 4 sets; (1) With Pb but without EDTA, (2) With Pb and EDTA 0.01 millimole (mM) per liter (mM L^{-1}), (3) With Pband EDTA 0.02 mM L^{-1} and (4) With Pb and EDTA 0.03 mM L^{-1}. These plants were grown, maintained and harvested every 15, 30, 45, 60, 75 and 90 days. Plant samples were separated into three parts; leaf, petiole and root. They were analyzed in terms of total lead (TPb) content, including the water solution. The results showed that Pb accumulation in Elephant ear was relatively significant in all of the experiment sets ($p<0.05$) at 15 days. The results showed that Pb accumulation in the whole plant was highest at 90 days. This study showed that Pb absorption in plant was enhanced when the concentration of EDTA increased. Plants showed that Pb accumulation in roots > petioles > leaves were significant with 502.84, 126.19 and 91.06 mg kg^{-1} ($p<0.05$) at EDTA of 0.02 mM set, respectively. Plants exhibited signs of phytotoxicity, such as wilting and curling of their leaves, yellow color appearing in the leaf margins and the plants eventually dying. These effects could be used as an indicator for determining the presence of Pb in contaminated water and soil.

Keywords: Phytoremediation, Phytotoxicity, Lead, EDTA, Wastewater

Introduction

Lead (Pb) is a heavy metal that is used in many industries, such as in the production of batteries and telephone cables; as a result, is widely mined around the world. Sludge and waste from industrial usage and mining are major causes of Pb contamination in soil and water. This inevitably has an impact on the ecosystem, vegetation, animals and health and sanitation of humans, as well as disrupting the food chain (Chen *et al*., 2006). Since 2006, mining activities in Thailand have created mine tailings, causing Pb contamination in sediment, which, specific to this study, has been naturally brought to Klity Creek in Kanchanaburi. Official authorities have tried to solve and alleviate this problem, but to date, there has been no success in remediation (Phenrat *etal*., 2016). At present, there are numerous remediation technologies being used to clean up heavy metal contamination in water, soil and sediment. These techniques include in situ physical and chemical processes (soil flushing, solidification and stabilization), thermal processes, ex situ physical and chemical processes (soil washing, chemical reduction and oxidation)and other processes including excavation and off-site disposal (Sampanpanish *et al*., 2006; Sampanpanish, 2015). Specifically, chemical and physical treatment techniques are considered to be potentially more cost effective than biological treatments

(Tananonchai and Sampanpanish, 2014). It is worth noting that this treatment has been shown to be cost effective and environmentally friendly when using green plants or living organisms to clean up the contaminated sites called phytoremediation (USEPA 1998a; 1998b; 1999). Plants can be used for the uptake of heavy metals and translocate into the whole parts of the plant. Plants can accumulate heavy metals in their tissue, which might be a suitable treatment technique since this has a low cost and high performance in terms of successfully removing heavy metals (Aisien *et al*., 2012; Aransiola *et al*., 2013). A study by Tanhan (2008) showed a mechanism of phytoextraction using Pb as a remover from soil contaminated with Chromolaenaodorata. Whereas, Pb, Zn and Cd treatment with Arabispaniculata was shown in a study by Tang *et al*. (2008). As recently as 2016, further research has shown how using a chelating agent also helps to increase the uptake of heavy metal in plants (Dumbrava *et al*., 2015). It is worth noting that Tambamroong (2002)studied the effects of EDTA to remove As (Arsenic) from contaminated soil with Taro and Elephant ear plants. Cho *et al*. (2008) studied Pb removal from contaminated soil using EDTA with green onions used for the uptake process. This research used *Colocasiaesculenta* to remediate Pb contaminated water from Klity Creek, Kanchanaburi province. Pb mining has been closed and not in operation for more than 20 years. Previously when in operation, impacts to the environment including the contamination of water, sediment and soil as well as impacts to the health of humans and animals. Therefore, this study investigated the ability of *C. esculenta* to be used for Pb uptake and translocation into plants from contaminated water and additive chelating agents.

Materials and Methods

Water Preparation

Pb as lead carbonate ($Pb(CO_3)_2$) was dissolved in distilled water to prepare stock solutions at concentrations of 5 mg L^{-1}.

Pot Preparation

Plastics black pots without holes were used, 45 centimeters in diameter and containing 20 L of tap water. The experiment was divided into 4 sets: (1) Pb solution without EDTA, (2) Pb solution with EDTA 0.01 mM L^{-1}, (3) Pb solution with EDTA 0.02 mM L^{-1} and(4) Pbsolution with EDTA 0.03 mM L^{-1}. Each concentration was applied to 3 replications.

Plant Preparation

The plant selection used *C. esculenta* with lengths of between 15-20 centimeters and no traces of Pb were detectable prior to the study. Plant samples were maintained in a nursery for 30 days.

EDTA Preparation

EDTA concentrations were prepared by disodium salt of ethylenediaminetetraacetic acid at 0.01, 0.02 and 0.03 mM L^{-1} and was added into a water solution of 5 mg L^{-1} $Pb(CO_3)_2$.

Experimental Design

Plant samples (*C. esculenta*) were excavated from uncontaminated soil and transferred to the hydroponic system (this included 6 plants per pot). Each pot had Pb carbonate solution added at a dose of 5 mg L^{-1} with 20 L of tap water. After 30 days, the EDTA concentrations were added. Each concentration of EDTA was applied to 3 replications. No chemical fertilizer or any other functional chemicals were used during the experiment.

Water Analysis

Water samples were collected at 15, 30, 45, 60, 75 and 90 day intervals during the cultivation. The total Pb (TPb) in the water was analyzed with USEPA 3051 (USEPA, 1998) and analyzed by using Atomic Absorption Spectrometer (AAS).

Plant Analysis

Plant samples were harvested at 15, 30, 45, 60, 75 and 90 day intervals during the process of cultivation. Each time, samples were washed with tap water twice and rinsed with deionized water and air-dried at room temperature for 2-3 hours. These plants were separated into 3 parts: leaves, petiole and root and these were all measured in their wet-state. The plant was then dried in a hot-air oven at 105°C for 24-48 h until reaching a constant weight and then the leaves, petiole and root were all measured in their dry-state. Plant samples were digested by nitric acid, sulfuric acid and hydrogen peroxide with USEPA 3052 (USEPA, 1996). Pb was analyzed by Atomic Absorption Spectrometer (AAS).

Phytotoxicity

This study investigated the tolerance of *C. esculenta*growing in Pb solution and determined the phytotoxicity due to the addition of EDTA. Plant's toxicity was observed in terms of the symptoms exhibited by the plants as a whole.

Results and Discussion

pH in Water

The water pH in all sets of the experiment ranged of 7.1-8.1 at 15-90 days, (Fig. 1a) which is the base level (Chiyapreuk, 1993). The water pH showed a trend of increasing as the experimental period increased.

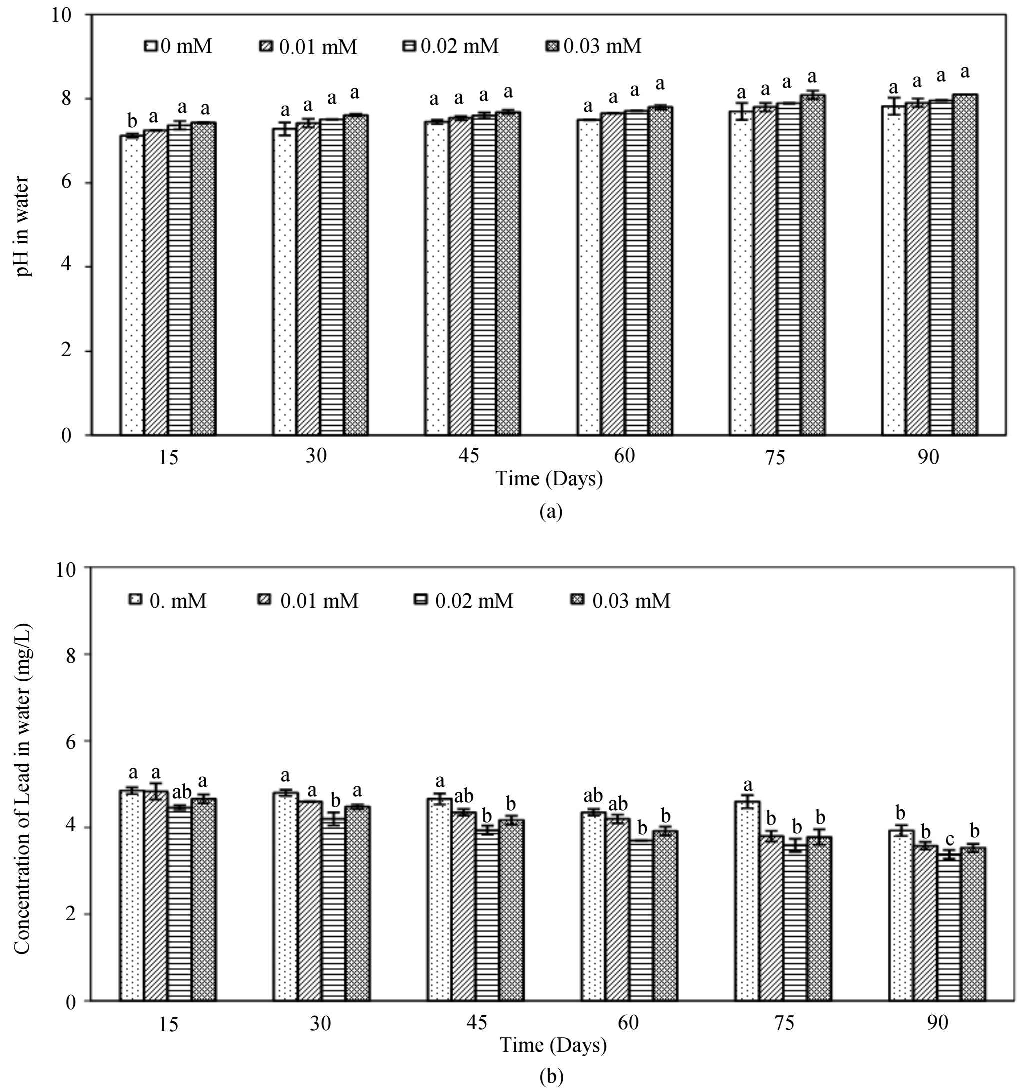

Fig. 1: (a) pH in contaminated water and (b) Concentration of Pb in contaminated water

Pb Dose in Water

Water samples showed the lowest Pb concentration of 4.47 mg L^{-1} at 15 days, (set of Pb solution with EDTA 0.02 mM L^{-1}), which can be seen as a statistically significant difference between the sets ($p<0.05$) (Fig. 1b). For the set of Pb solution without EDTA, Pb solution with EDTA 0.01 mM L^{-1} and Pb solution with EDTA 0.03 mM L^{-1} were 4.87, 4.84 and 4.67 mg L^{-1}, respectively. These water solutions showed the highest Pb concentration and a trend that decreased when the experiment time increased and EDTA dose increased. The Pb concentration showed the lowest decrease of 3.41 mg L^{-1} at 90 days. Li *et al*. (2008) studied Pb uptake using EDTA concentrations of 0.1 and 0.5 mM L^{-1} with Typhaorientalis. Their results showed that the Typhaorientalis absorbed Pb with the EDTA concentration at 0.1 mM L^{-1} higher than at 0.5 mM L^{-1}, while EDTA concentration of 0.5 mM L^{-1} in a water solution was higher than the additive EDTA concentration 0.1 mM L^{-1}. However, Poopa *et al*. (2015) studied the sequential extraction method, which was employed to investigate the distribution and chemical fractions of Pb in Klity Creek sediments, Kanchanaburi, Thailand. The objective was to define the Pb mobility in sediment and potential bioavailability in relation to sediment contamination levels. The results showed that the background value of TPb concentration in the sediments from this area was higher than those reported from other locations in Thailand. Sequential extraction results revealed that Pb was mainly associated with the reducible fraction, especially in the polluted zone in the vicinity of the ore dressing plant (factory).

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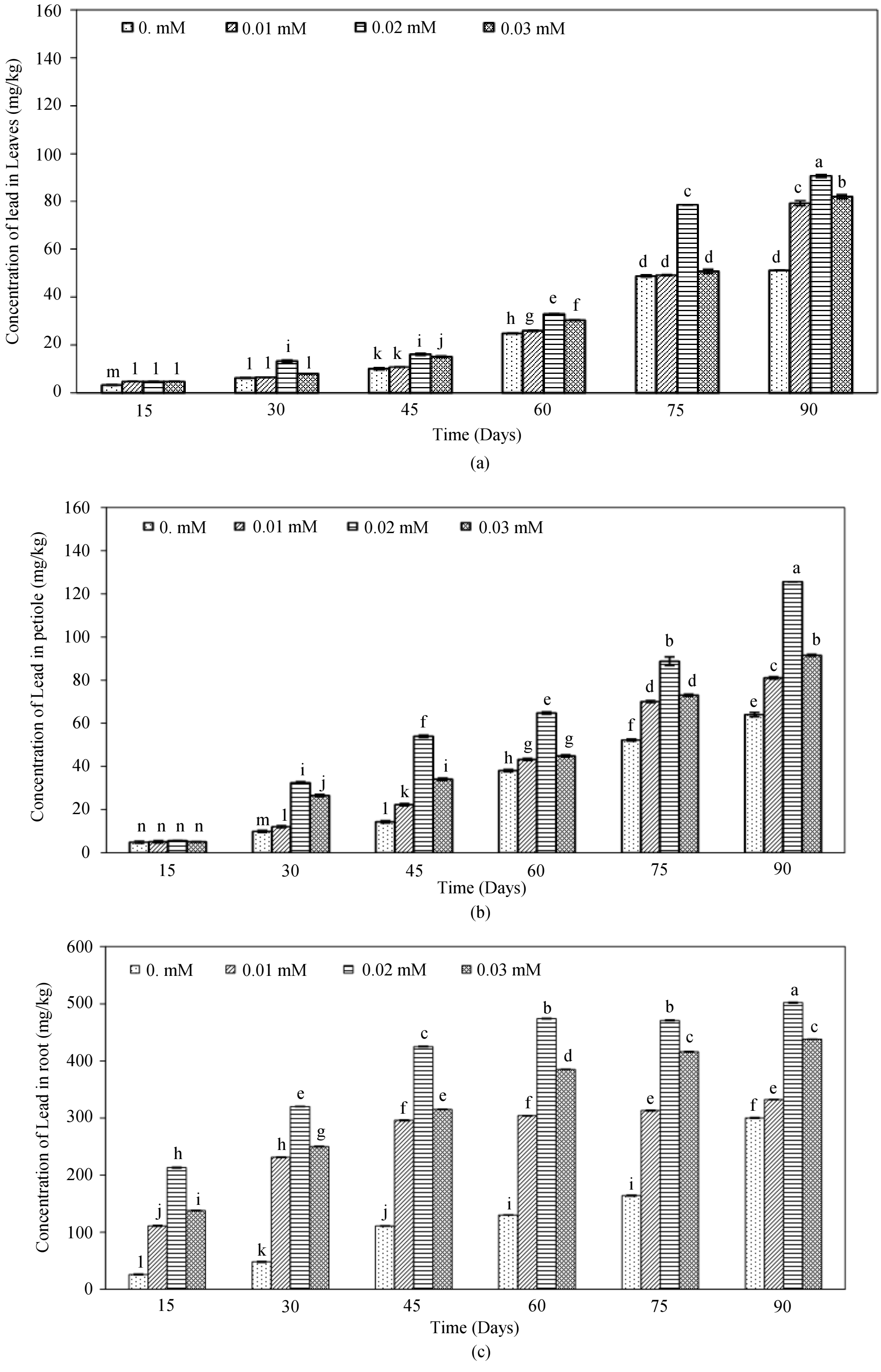

Fig. 2: Pb uptake in the various parts of *C. esculenta* (a) Leaves (b) Petioleand (c) Roots

This is different from the distribution of Pb fractions measured upstream and downstream within the polluted area, i.e. reducible fraction was the major component found upstream, whereas strongly dissociated fractions (oxidizable and residual) were the major components found in the downstream samples.

Pb Uptake in Plant Leaves

The results in Fig. 2a show that amount of Pb absorbed in *C. esculenta* leaves was very small for all sets of treatments at 15 days to the time of harvest, which were 3.35, 4.91, 4.94 and 4.93 mg kg^{-1}, respectively for Pb dose at 0, 0.01, 0.02 and 0.03 mM. This study shows the Pb accumulation in *C. esculenta*plant increased as the harvested time increased over the overall time period.The Pb set of 0.03 mM had the highest uptake rate of 91.06 mg kg^{-1} at 90 days. All sets showed a significant difference with the other treatments ($p<0.05$). The results indicated that treatment of Pb with EDTA absorbed higher amounts than treatment of Pb without EDTA at all times of harvesting. From the statistical analysis, it was shown that there was a significant difference between treatments ($p<0.05$). This was because of the addition of EDTA in the water, which affected the Pb translocation and accumulation in plants. Consistent with this, Liphadzi *et al*. (2003) studied EDTA assisted heavy metal uptake by using sunflowers when EDTA concentrations were 0, 0.5, 1 and 2 grams per kilogram (g kg^{-1}) in soil. They found that the sunflower was able to absorb and accumulate heavy metal in the leaves, with accumulation rates increasing when more EDTA was added. The concentration of EDTA affected the heavy metal uptake of the plant study. These results found that the concentration of EDTA at 0, 0.5 and 1 gram per kilogram (g kg^{-1}) had the absorbed a higher rate of heavy metals in the leaves as the concentration of EDTA increased. However, the leaves of the sunflowers absorbed less heavy metal when the concentration of EDTA increases at 2 g kg^{-1}. Moreover, Li *et al*. (2008) found that Typhaorientalis absorbed Pb at concentrations of EDTA at 0.1 mM L^{-1} which was higher than the concentration of EDTA at 0.5 mM L^{-1}.

Pb Uptake in Plant Petioles

The results show that the petiole sections of *C. esculenta* absorbed Pb only very slightly at the harvesting time of 15 days. For the experimental sets: (1) with Pb but without EDTA, (2) with Pb and EDTA 0.01 mM L^{-1}(3) with Pb and EDTA 0.02 mM L^{-1} and (4) with Pb and EDTA 0.03 mM L^{-1} (Fig. 2b) Pb uptake was at 4.78, 4.95, 5.51 and 4.98 mg kg^{-1}, respectively. The *C. esculenta* absorption had the highest trend of increase as the harvest time increased. Thus, the uptake by *C. esculenta* was highest with the set of Pb and EDTA 0.03 mM L^{-1} at day 90, 126.19 mg kg^{-1}. From the statistical analysis, it is shown that there were significant differences between treatments ($p<0.05$). The comparison of Pb treatment with and without EDTA sets; the result indicated that the treatment of Pb and EDTA absorbed at higher rates than treatment of Pb without EDTA during the harvesting times. These were significant differences between treatments ($p<0.05$). Moreover, treatment of Pb and EDTA 0.02 mM L^{-1} accumulated Pb at a higher rate than treatment of Pb without EDTA and such rate was 1.9 fold. This might be due to the ability of EDTA to combine Pb in the form useful to plants. As a result, the plant exhibited an ability to absorb Pb even more. This is consistent with Epstein *et al*. (1999)'s study of Brassica juncea grown in Pb contaminated soilwith the addition of EDTA concentrations of 0, 1, 5 and 10 mM kg^{-1} in soil. The results showed that Brassica juncea was able to accumulate Pb in the petiole section, whereby Pb increased in line with increasing EDTA concentrations of 0, 1 and 5 mM kg^{-1} and the Pb decreased at a concentration of EDTA 10 mM kg^{-1}. This result can also be related to Ebrahimi(2014), which found that the addition of EDTA can have positive effects on Pb and Cr uptake when using *Echinochloa crus-galli* (L) Beauv.

Pb Uptake in Plant Roots

The results show that the *C. esculenta*'s roots absorbed Pb very slightly in all of sets or treatments at 15 days of harvesting time, which were recorded as 26.33, 111.96, 215.52 and 138.77 mg kg^{-1}, respectively at treatment (1) with Pb but without EDTA, (2) with Pb and EDTA 0.01 mM L^{-1}(3) with Pb and EDTA 0.02 mM L^{-1} and (4) with Pb and EDTA 0.03 mM L^{-1} (Fig. 2c). The harvesting time at 90 days shows that the treatment with Pb solution added with EDTA 0.02 mM L^{-1} exhibited the highest uptake of Pb into *C. esculenta*'s roots at 502.84 mg kg^{-1} which was higher than all of treatments and were 1.67, 1.51 and 1.15 fold, respectively. These were significant differences between treatments ($p<0.05$). These results also related with Hegazy *et al*. (2011) found that the Pb accumulation was higher in roots than the other parts of plants. These findings are similar to those reported by Abrantes *et al*. (2007), who found the direct effect of a chelating agent on Cd accumulation in *Halimioneportulacoides*, especially in the roots (Sampanpanish and Tantitheerasak (2015). Consistent with the research of Duo *et al*. (2010) who studied how grass (Turf) absorbs heavy metals when adding EDTA at a dose of 0, 10, 15, 20, 25 and 30 mM kg^{-1}. The results showed that the Turf grass absorbed heavy metals, with the trend of absorption increasing when the concentration of EDTA increased. The highest level of heavy metals absorbed was at the EDTA dose of 20 mM kg^{-1}. Turf

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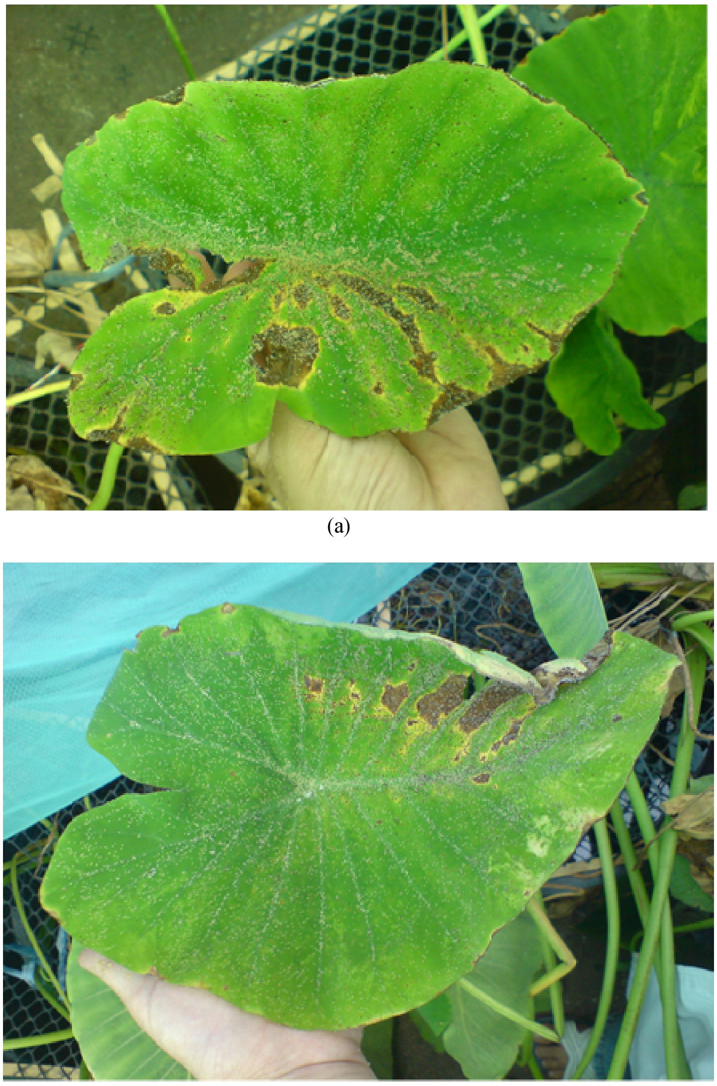
grass showed the heavy metal uptake trend to decrease at an EDTA dose of 25 and 30 mM kg^{-1}. Grcman *et al*. (2001) studied the absorption of Pb with Brassica rapa by adding EDTA at 3, 5 and 10 mM kg^{-1}in soil. They found that with the concentrations of EDTA at 3 and 5 mM kg^{-1}, Brassica rapa absorption and accumulation of Pb in the root increased when the concentration of EDTA increased. But Brassica rapa absorption of Pb also showed a trend of decrease at a concentration of EDTA 10 mM kg^{-1}. Moreover, Khamla and Sampanpanish (2015) showed that arsenic (As) accumulation in roots was significantly higher than in stems and leaves ($p<0.05$) and was maximal after 120 days of cultivation, at which point the As concentration reached 29.71 mg As/kg in the roots compared to 6.32 mg As/kg in the stem and leaves. The average Asaccumulation in all parts of the plant over four months was 2.71-36.03 mg As/kg per plant.

Effects of Pb and EDTA on Phytotoxicity of C. esculenta

The phytotoxicity during the time period over 90 days of the experiment shows that *C. esculenta* did not have exhibit toxicity symptoms in the treatment process with Pb but without EDTA, with Pb and EDTA 0.01 mM L^{-1} and treatment of Pb and EDTA 0.02 mM L^{-1}. Note that, *C. esculenta* exhibited toxicity in the treatment of Pb and EDTA 0.03 mM L^{-1}. Phytotoxicity (Fig. 3) symptoms included, for example, wilting leaves, curling leaves, yellow color appearing in leaf margins and plants eventually dying (Akkajit, 2015). Moreover, Pb might be able to interfere with and resist the process of photosynthesis by absorbing nutrients into plants.



(a)

(b)

Fig. 3: (a) Yellow leaf margins, and (b) wilting and curling leaf

The significantly lower phytotoxicity between EDTA 0.03 mM L^{-1} compared with EDTA 0.02 mM L^{-1} in Pb solution also relates with Wu *et al*. (2004), who used Chinese mustard (*Brassica juncea*) with EDTA to remediate the heavy metal. The results showed that EDTA potentially reduced the phytotoxicity of heavy metal to Chinese mustard and also enhanced the cadmium accumulation. This finding also agreed well with the effects of Cd Zn Pb and Cr uptake with Arum (*Colocasiaesculenta* L.); Grass (*Kochiascoparia*) phytotoxicity and growth reported by Islam *et al*. (2017) and Zhao *et al*. (2015).

Conclusion

This study reproduced the effects of Pb removal in contaminated water using EDTA by *C. esculenta*. The results showed that plants exhibited an increase in Pb uptake in trend with an increase of time. *C. esculenta* was grown well in contamination water with different sets of Pb and EDTA. The set with Pb and EDTA showed the highest potential uptake of Pb compared to the set with Pb and without EDTA. Moreover, *C. esculenta* showed a trend of absorbing more Pb when the dose of EDTA increased. The treatment with Pb and EDTA at 0.03 mM L^{-1} showed a trend of decreasing Pb absorption and showed toxicity on the leaves of *C. esculenta*. The phytotoxicity symptoms were also exhibited, such as wilting leaves, curling leaves, yellow color appearing in the leaf margins and plants dying. Thus, it can be concluded that *C. esculenta* can be useful for the monitoring and cleanup of Pb from Pb contaminated water and soil. It is worth considering that any following research should focus the types of plants that have a higher Pb uptake potential than this the *C. esculenta* plant used in this research. Such plant species should also be hyperaccumulators, non-edible and able to be used as alternative energy such as *Jatrophacurcas*, *Meliosmapinnata*, *Leucaenaleucocephala* and*Eucalyptus camaldulensis*. Especially, *PennisetumpurpureumSchum* cv. Mott (Napier grass) can be utilized as a biomass for producing heat and electricity.

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Author's Contributions

This article is original research paper. Authors participated in all experiments, coordinated the data analysis and contributed to the written and read of this manuscript. Authors give final approval of the version to be submitted this journal.

Ethics

The authors declare no conflicts of interest and confirm that the manuscript has been submitted solely to this journal and is not published, in press, or submitted elsewhere.

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