

PAPER 26 – PHYTOREMEDIATION OF LEAD-POLLUTED DUMPSITE SOILS USING A NOVEL HYPER-ACCUMULANT IN COMBINATION WITH A BIODEGRADABLE CHELATING AGENT

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ABSTRACT

The effects of toxic substances including heavy metals (HMs) in the human environment have become a significant source of worry for the wellbeing of man and his dwellings. This work focused on the containment of HMs pollution in dumpsite soils through phytoremediation using Stinging Nettle (*Urtica dioica*) as macrophyte. The effects of macrophyte density (1 – 3 plants/m²) and bio-degradable chelating agent (Citric acid) concentration (3 – 9 mmol/kg) on the phyto-extraction efficiency of Pb was considered in this study. Soil trials were taken from a municipal dumpsite (depth 0 – 20 cm) in Makurdi, North-Central Nigeria which was already found to contain elevated Pb concentrations (106.20 mg/kg) for the phytoremediation experiments. Control pots that received same treatment without the addition of citric acid were also set up and monitored for comparisons. The bio-concentration factor for Pb was less than unity (BCF < 1), while the translocation factor was greater than one (TF > 1) at all macrophyte densities with the maximum occurring at macrophyte density of 3 plants/m². Overall, uptake of Pb by *Urtica dioica* increased with increment in the macrophyte density and at moderate lime concentration (6 mmol/kg).

Thus, *Urtica dioica* is an efficient natural hyper-accumulant for Pb pollution control in tropical soils.

KEYWORDS: Chelating agent, Dumpsites, Heavy metals, Soil, Phytoremediation, Stinging Nettle.

1. INTRODUCTION

The persistence and toxicity of heavy metals in the environment has been a global ecological and human wellbeing concern in recent times. Both natural and human-induced practices contribute to the accumulation of heavy metals in various environmental matrices including soils, water, food and air. The most common anthropogenic causes emerge through improper solid and liquid waste management practices. These result in the creation of huge quantities of non-useful substances that prompt the discharge of harmful features to the environment including heavy metals (Abdu, Yusuf, 2013; Nuhu *et al.*, 2014; Ma *et al.*, 2015). Additionally, the quantum of heavy metal occurrence in soils is influenced by the geological composition of the soils (Sivarajasekar *et al.*, 2018).

Food security, green revolution and agricultural endowment are greatly related to the quality and quantity of soil available. However, as a result of rapid urbanization and spring-up of industries, the quantity and quality of soils available for agricultural production have been greatly retarded (Sivarajasekar *et al.*, 2018). Heavy metals are among the most worrisome group of soil pollutants due to their toxicity to humans and the ecosystem. When present in soils, heavy metals solubilize in the soil-water-nutrient interaction and are taken up by plant/crop roots and translocated to the whole parts of the plants where they are persistently stored in stems and leaves of the plants. When such crops are consumed by man and his animals, they are exposed to the toxic and carcinogenic effects of heavy metals (Ayoub *et al.*, 2010; Ihedioha *et al.*, 2017).

Research efforts in the past have been focused on ascertaining the status of heavy metal (Pb, Zn, Cd, Ni, Cu etc.) contamination in and around dumpsites with little efforts given towards its removal for the safety of the ecosystem (Wang *et al.*, 2016; Gabarron *et al.*, 2017). Unfortunately, active or non-active municipal solid waste dumpsite soils which are associated with alarming heavy metal pollution status are frequently utilized for urban agriculture; a situation that subjects the end consumers of such food to the deleterious consequences of the toxic heavy metals due to bio-accumulation and food chain contamination problems (Wu *et al.*, 2018).

The most common methods for remediating heavy metal contamination in soils include; electro-chemistry, biochar addition, bioremediation and phytoremediation. Among these methods, phytoremediation which is the application of growing ornamental plants for the removal of toxic pollutants including heavy metals in soils is mostly considered. Phytoremediation is favoured for soil remediation because of its low cost, efficiency and low energy inputs when compared with the other methods. Several researchers have reported the deployment of phytoremediation as a treatment for reducing heavy metals in polluted soils. These researches cut across various continent of the world and have utilized different plants/macrophyte for phytoremediation trials. For instance, in

Asia, Massadeh and Massadeh (2019), used citrus lemon (Rutaceae), *Ceratonia siliqua* L., *Olea europaea* (Oleaceae), *Washingtonia filifera*, and *Myoporum* (Myoporaceae) to examine the removal of copper (Cu) and zinc (Zn) ions from aqueous solutions, while in Africa, Ameh *et al.*, (2020), used groundnut (*Arachis hypogaea*) and beans (*Phaseolus vulgaris*) plants to examine the removal of cadmium, chromium, copper and lead from soil.

Phytoremediation as a sole strategy is conventionally limited for large scale uses targeting non-bioavailable heavy metals in soils. This is largely due to the long durations associated with the process (Wang *et al.*, 2019). Thus the use of improved techniques such as induced phytoremediation, chelate-assisted phytoremediation and genetic manipulation of the macrophyte prior to its use for phytoremediation has been recently advocated.

The choice of phytoremediation and its successful implementation for heavy metal control in soils is dependent on the availability of plant species that are hyper-accumulants of heavy metals. To this end, it is pertinent to study the suitability of local plants for their efficacy in phytoremediation with respect to heavy metals decontamination of soil of which several tropical ornamental and food crops are yet to be tested in this regard. The macrophyte of choice in the current work: Stinging nettle (*Urtica dioica*) was previously tested for its heavy metal uptake in soils without much success due to the non-bioavailability of heavy metals in the polluted soils (Shams *et al.*, 2009; Murtic *et al.*, 2021; Sharifi *et al.*, 2023). Recently, researchers have advocated the use of improved techniques for enhanced phytoremediation of heavy metals by *Urtica dioica*. For instance Viktorova *et al.* (2016) asserted that the phytoremediation ability of *Urtica dioica* can be greatly enhanced by genetic manipulation of the macrophyte using constitutive CaMV 35S promoter. Another improved technique is the use of biodegradable and non-biodegradable chelating agents to improve the bioavailability of heavy metals towards its effective phyto-extraction by the chosen macrophyte. These techniques have been explored by some researchers for enhanced phytoremediation of heavy metals using macrophyte such as *Amaranthus caudatus* L. and *Tagetes patula* L. (Aghelan *et al.*, 2021), *Sasa argenteostriata* (Yang *et al.*, 2022), and *Pfaffia glomerata* (Huang *et al.*, 2021) among others. The use of biodegradable chelating agents for improved phyto-extraction of heavy metals from contaminated soils is promoted ahead of the use of the non-biodegradable types due to its environmental friendliness and non-secondary pollution disposition. The most commonly tested biodegradable chelating agents and plant species have been chronicled by Shinta *et al.* (2021).

Despite the huge potentials of improved phytoremediation and environmental safety of biodegradable chelating agents towards heavy metal extraction from polluted sites, these techniques have not been tested using *Urtica dioica*; A macrophyte that has already shown significant prospects as a hyper-accumulator of heavy metals should the technique be improved. Therefore, this study was set out to fill this knowledge gap with the following specific objectives: (1) to comparatively study the phytoremediation of Pb by *Urtica dioica* under the application of citric acid (a biodegradable chelating agent) and non-application scenario in a controlled environment pot experiments using actual contaminated dumpsite soils, (2) to test the effects of macrophyte density and chelating agent concentration on the phytoremediation of Pb using *Urtica dioica*.

2. MATERIALS AND METHODS

The materials for this study were laboratory auger, aluminium foil, and polyethylene bags, lead contaminated soils, a screen house, standard analytical reagents and laboratory wares, stinging nettle plants, distilled water, concentrated citric acid and perforated cylindrical plastic pots. The experiments were carried out at the Research Farm of the Department of Agricultural and Environmental Engineering, Joseph Sarwuan Tarka University, Makurdi using standard pot cultures in a controlled environment devoid of external environmental influences. Soil samples from a municipal dumpsite that were already established to contain elevated levels of Pb in our previous study were used for the phytoremediation experiments. The Pb uptake ability of a locally available plant: Stinging nettle (*Urtica dioica*) was tested at varying plant/macrophyte densities and chelating agent (citric acid) concentrations using distilled water for irrigation. Control pots that did not receive chelating agent and/or not planted with *Urtica dioica* but received irrigation or planted in non-polluted soils were also set-up for comparisons.

Inferential indices considered in the evaluation of the system were the translocation factor, bio-concentration factor, and overall Pb uptake in the roots and aerial parts of the plants as well as the plant height over the growing period. These parameters were determined following the method of Das *et al.* (2014).

2.1 Soil Sample Collection and Determination of Initial Pb Concentration

Soil samples were collected in the month of September, 2022 from an identified dumpsite with elevated concentration of lead in an urban settlement in Makurdi, North-central, Nigeria. The soils were randomly collected in sufficient quantities at a depth of 0 -20 cm using a laboratory auger in order to prevent the interference of wastes

and degrading materials in the samples. The soils were initially homogenized by thorough mixing before putting same in polyethylene bags, enclosed with aluminium foils and transported to the laboratory and experimental site for further analysis and use respectively (Ihedioha *et al.*, 2017).

In the laboratory, the soil were homogenized again and characterized for the physico-chemical properties (details not reported here) and then stored at room temperature before analysis for heavy metal content. For the determination of Pb concentration in the soil sample, the method of Hedge *et al.* (2009) was followed for sample digestion, while the concentration of Pb (mg/kg) in the sample was determined using a flame atomic adsorption spectrophotometer (Shimadzu model AA 700) equipped with a deuterium lamp and air/acetylene head burner as carrier gas system in accordance with the method of Wamalwa *et al.* (2018) at a wave length of 283.3 nm. Quality assurance was done by determining the concentration of Pb in spiked and unspiked control soil samples and the computing the percentage recovery following the procedure of Naz *et al.* (2018).

2.2 Experimental Set-up

The experiments were set-up in perforated cylindrical plastic pots of 250 mm² top areas and depth of 200 mm. the experiments were conducted for a total duration of 60 days with an additional initial seven day acclimatization period. The pots were filled with homogenized samples of contaminated soils (3 kg) with an inherent Pb concentration of 106.20 mg/kg. A drainage water collection vessel was placed underneath each experimental pot to collect and recycle water/nutrients in the system. The pots were initially treated with deionized water at 60 % of field capacity and left for one week. After that, the already established *Urtica dioica* plants grown in a nursery were transplanted into the experimental pots at the desired plant densities and following standard agronomic practices. Edge effects were minimized by routine randomization of the experiment pots in the screen house. The planting densities adopted were 1, 2 and 3 plants/m². Initially, the pots were planted with 6, 10 and 14 plants and later thinned to 4, 8 and 12 plants to give planting densities of 1, 2 and 3 plants/m² respectively as desired. The chelator treatments using citric acid were started on day 30 after transplanting at dosages of 3.0, 6.0 and 9.0 mmol/kg and planting density of 3 plants/m². Similar control pots that received only deionized water without the additions of citric acid (chelating agent) were also set up and monitored for comparison. Each experimental treatment was replicated thrice and arranged in a completely randomized design form.

In order to maintain the cleanliness of the experimental environment, weeding was done manually without the use of herbicides and insect infestation were prevented by the screen house, thus no insecticides were used. Photo and dark periods of 14 and 10 hours/day respectively were maintained throughout the experimental period in the screen house. Similarly, room temperatures of 30 ± 2 °C and 23 ± 2 °C were maintained during the day and night times respectively. Fig. S1 in appendix shows the schematic of the experimental set-up. The average height (ft) of the macrophyte was monitored using a flexible tape over the growing period at an interval of 5 days with emphasis on the experimental pots with three plants/m² and varying citric acid concentrations (0 -9 mmol/kg).

2.3 Determination of Pb Concentration in Harvested Plant (*Urtica dioica*) Parts and Data Analysis

At the end of the 60 days experimental period, the experiments were terminated and the *Urtica dioica* plants were separately harvested from the experimental pots and prepared for further analysis. The roots and shoots of each plant category were carefully separated using a disinfected knife. The separated parts of the plants were separately and carefully washed with deionized water; air dried and weighed using a digital weighing balance (Adam model PW 184). Each weighed sample was labelled and placed in dry plastic pots for further determination of the inherent Pb concentrations (mg/kg). Again the concentration of Pb in the component parts of the plants for each experimental treatments and control were determined after ashing in a muffle furnace set at 800 °C (Carbolite model GPC 12/81 + 103, Sheffield, England) and digestion using a flame atomic adsorption spectrophotometer in accordance with the methods of Rashid *et al.* (2016). Then the phytoremediation potentials of *Urtica dioica* for Pb under the various experimental treatments and control were determined as the Bio-concentration Factor (BCF), Translocation Factor (TF) and Enrichment Factor (EC_r) using equations 1, 2 and 3 respectively (Das *et al.*, 2014; Madanan *et al.*, 2021). The percentage reduction in Pb concentration in the soil was measured to indicate the chelating efficiency using equation 4, ANOVA was used to analyse the data sets (P = 0.05). The growth (height) profile of *Urtica dioica* was also measured at an interval of 5 days for the 60 day experimental period using a flexible meter rule.

$$BCF = \frac{s}{q} \quad (1)$$

Where s = the combined concentration (mg/kg) of Pb in the plant aerial parts and roots

q = the initial concentration of Pb (mg/kg) in the soil.

$$TF = \frac{z}{r} \quad (2)$$

Where, z = Pb concentration in aerial parts of the plant

r = Pb concentration in the plant roots

$$EC_r = \frac{z}{q} \quad (3a)$$

$$\text{Or } EC_r = \frac{r}{q} \quad (3b)$$

Where z, r and q are as defined above.

$$\% \text{ Reduction} = \frac{\text{initial concentration of Pb} - \text{final concentration of Pb in soil}}{\text{initial concentration of Pb in soil}} \times 100 \quad (4)$$

3. RESULTS AND DISCUSSION

The results of these experiments are presented in the following sections with discussion of findings and implications. These have been sub-divided into; The effects of plant density on the uptake of Pb in soils, The effect of planting density on the Pb phytoremediation potentials of *Urtica dioica*, effects of chelating agent concentration and plant density on percentage reduction of Pb in soils and the growth characteristics of *Urtica dioica* under Pb contaminated soils and control.

3.1 Effects of Plant Density on Phytoremediation of Pb by *Urtica dioica*

The influence of plant density (1 – 3 plants.m²) on the total uptake of Pb (mg/kg) from contaminated soil by *Urtica dioica* is shown in Table 1 for both the aerial parts and roots of the macrophyte. In the aerial parts, Pb uptake ranged from 0.00 ± 0.00 to 11.10 ± 0.23 mg/kg. The uptake of Pb from the dumpsite soils increased with increase in the plant density and was significant at P = 0.05 (Table 2). Similarly, the uptake of Pb in the roots of *Urtica dioica* increased with increase in planting density (0.00 ± 0.00 – 19.80 ± 0.14 mg/kg) and was significant at P ≤ 0.05 (Tables S1 – S2a-e in appendix). The control showed no uptake since the soils were not contaminated with Pb. The BCF was 0.00 ± 0.00 – 0.29 ± 0.20 (Table 1) and was significantly higher in experimental pots having 3 plants/m² (P ≤ 0.05).

The BCF is an indicator of the macrophyte ability to accumulate pollutants including heavy metals depending on its initial concentration in the polluted media (Madanan *et al.*, 2021). Increase in the BCF as the plant density was increased indicate that more Pb was accumulated when the extracting macrophyte were increased thus signifying a liner relationship. Macrophyte with BCF values between 1 and 2 are considered as suitable for phyto-extraction (Das *et al.*, 2014). The BCF values in phytoremediation are not enough to elucidate on the absolute suitability of a macrophyte as a hyper-accumulator, thus to unravel this, a more suitable index known as the TF is used. In this study, the TF ranged from 0.00 ± 0.00 in the control to 1.89 ± 0.06 in the 1 plant/m² experimental pots. It was further observed that an increase in the plant density lead to a decrease in the TF. This trend was ascribed to competition among the macrophyte to phyto-extraction of Pb in the polluted soil. Macrophyte that extract heavy metals and largely store it in the roots have TF < 1, while TF >1 indicate the transfer of the extracted metals to the aerial parts of the plant. In this vein, *Urtica dioica* was found to phytoextract Pb in the soil and efficiently transfer same to its shoots for all plant densities considered in this work (Table 1). This shows that *Urtica dioica* is a good accumulator of Pb ions from polluted soils.

Similar to the BCF and as shown in Table 1, the EF was also found to increase with an increase in the plant density (0.00 ± 0.00 – 0.17 ± 0.006). The EF shows the relationship between the concentrations of the extracted pollutant in the aerial parts of the macrophyte to the remaining concentration in the soil. Thus values > 1 show hyper-accumulation, while those lower than 1 indicate moderate to good accumulation of the pollutant (Madanan *et al.*, 2021).

Table 1: Effects of Plant Density on Phytoremediation of Pb

S/No	Phytoremediation Parameter	Plant Density (Plants/m ²)			
		1	2	3	Control
1.	Bio-concentration Factor	0.28 ± 0.006 ^b	0.28 ± 0.006 ^b	0.29 ± 0.20 ^c	0.00 ± 0.00 ^a
2.	Translocation Factor	1.89 ± 0.06 ^c	1.85 ± 0.01 ^c	1.74 ± 0.00 ^b	0.00 ± 0.00 ^a
3.	Enrichment Factor	0.15 ± 0.006 ^b	0.15 ± 0.02 ^b	0.17 ± 0.006 ^c	0.00 ± 0.00 ^a
4.	Aerial Pb Uptake (mg/kg)	10.00 ± 0.66 ^b	10.54 ± 0.96 ^b	11.10 ± 0.23 ^b	0.00 ± 0.00 ^a
5.	Root Pb Uptake (mg/kg)	19.20 ± 0.42 ^b	19.20 ± 0.11 ^b	19.80 ± 0.14 ^c	0.00 ± 0.00 ^a

Values are means and standard deviations of triplicate experiment. Values with same superscript across rows are not significantly different at P = 0.05.

3.2 Effects of Chelating Agent Concentration on Pb Removal by *Urtica dioica*

The effect of citric agent concentration as a chelating agent to aid bioavailability of Pb in polluted soils was tested in the range of 3.0 – 9.0 mmol/kg. A control that received 0.0 mmol/kg of citric acid was also monitored for comparison. One way ANOVA and Duncan Multiple Range Test (DMRT) indicated a significant difference in

the levels of citric acid considered ($P = 0.05$) as shown in Table S3 and S4 in the Appendix respectively. Pb removal efficiency ranged from $68.90 \pm 6.12 - 88.35 \pm 2.08$ % (Table S5 in appendix) with the lowest and highest values observed in the control and pots with citric acid concentration of 9.0 mmol/kg respectively (Fig. 1). ANOVA also showed that there was no significant difference in the Pb uptake efficiency of the 6.0 and the 9.0 mmol/kg set set-ups. Thus it was recommended that the moderate citric acid concentration of 6.0 mmol/kg is optimal for bioavailability and subsequent uptake of Pb from polluted soils by *Urtica dioica* macrophyte. Thus in this study it was found that a planting density of 3 plants/m² and citric acid concentration of 6 mmol/kg are ideal for efficient removal and translocation of Pb by ornamental *Urtica dioica* macrophyte.

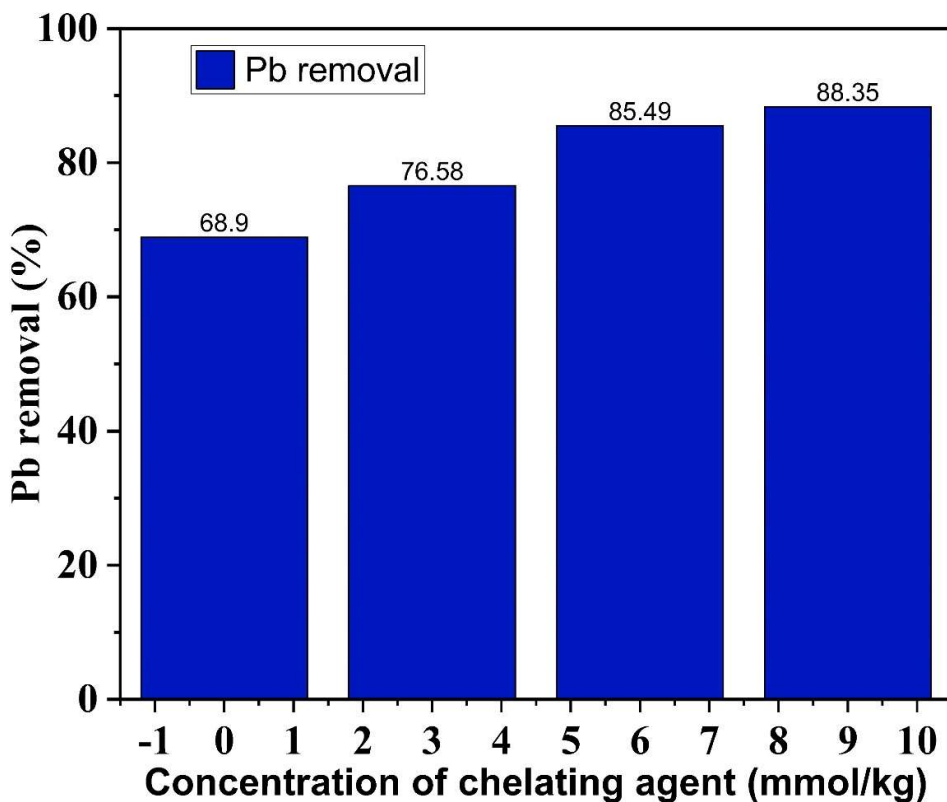


Figure 1: Pb Removal Efficiency as Influenced by Citric Acid Concentration

3.3 Growth of *Urtica dioica* at varying Chelating Agent Concentration in Polluted Soils.

The average height of *Urtica dioica* as a phytoremediation plant was monitored for 60 days in the pots containing 3 plants/m² and at the various chelating agent concentrations. The control pots which had 0.0 mmol/kg of citric acid were found to exhibit higher growth as compared with the pots that received various citric acid concentrations. Initially, before the introduction of the chelating agent to the experimental pots (0 -30 days), the plant height were not different, however, when the chelating agent was introduced (30 – 60 days), the growth of the macrophyte began to decline with increase in the concentration of the citric acid (Fig. 2). This was ascribed to the toxicity of Pb, which hindered its optimal growth. As stated earlier, when the concentration of citric acid was increased, the bioavailability of of Pb in the soils for phytoextraction by *Urtica dioica* was also enhanced. This situation encouraged Pb toxicity to the plants, leading to a diminished growth pattern as evidenced in Fig 1. Similar toxicities were documented by other researchers (Zair *et al.*, 2014).

4. CONCLUSION

In this work a novel macrophyte: *Urtica dioica* (Stinging nettle) was used for the first time towards the phytoremediation of Pb in polluted dumpsite soils. The influence of macrophyte density and biodegradable chelating agent concentration on the phytoremediation ability of the macrophyte was carefully studied in macrocosm pot experiments conducted under a controlled environment (screen house). The bio-concentration factor and enrichment factor were lower than unity at all planting densities and were significantly higher at higher plant density (3 plants/m²), while the translocation factor indicated the excellent phyto-extraction capability of

Urtica dioica as values were > 1 for all planting densities and significantly reduced with increase in the plant density. The Pb removal efficiency was found to depend on the chelating agent (citric acid concentration) and significantly increased with an increase in the chelating agent concentration up to a moderate value of 6.0 mmol/kg. The growth of *Urtica dioica* in the polluted soil media was inhibited by an increase in the chelating agent concentrations which promoted the bioavailability of Pb, its subsequent phyto-extraction and translocation which increased Pb toxicities in the plants morphological physiological functions.

It was therefore concluded that *Urtica dioica* is a good candidate for phytoremediation of Pb polluted soils at higher planting densities and moderate chelating agent concentrations.

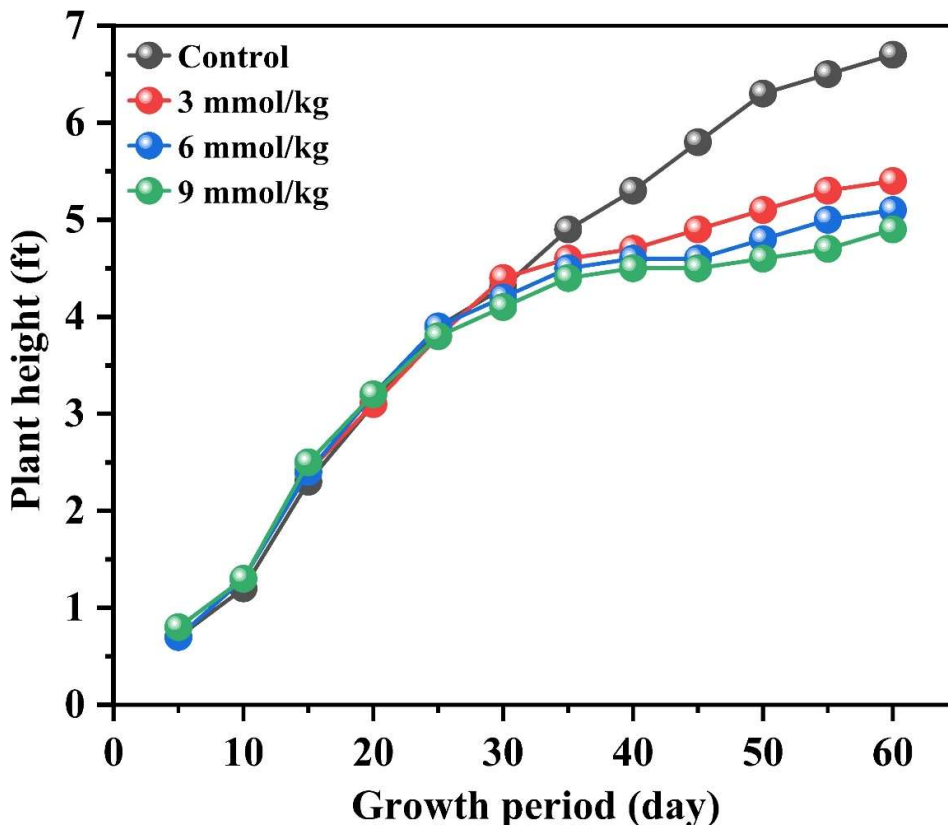


Figure2: Effects of Citric Acid concentration on the Growth of *Urtica dioica* in Pb Contaminated soils

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REFERENCES

- Abdu, N., Yusuf, A. (2013). Human health risk characterization of lead pollution in contaminated farmlands of Abare village, Zamfara State, Nigeria. *Afr. J. Environ Sci. Technol.* 7(9):911–916.
- Aghelan, N., Sobhanardakani S., Cheraghi M., Lorestani B., Mernkhpour H. (2021). Evaluation of some chelating agents on phytoremediation efficiency of *Amaranthus caudatus* L. and *Tagetes patula* L. in soils contaminated with lead. *J. Environ. Health Sci. Eng.* 19(1): 503 – 514.
- Ameh, G. I., Nwamba, O. H., Njam, V. S., Ofordile, E. C. (2020). Phytoremediation of heavy metals using some selected leguminous crops. *Afr. J. Agric. Res.* ISSN: 1991-637X. <https://doi.org/10.5897/AJAR>.

- Ayoub K., van Hullebusch E. D., Cassir M., Bermond A. (2010). Application of advanced oxidation processes for TNT removal: a review. *J. Hazard. Mater.*, 178:10–28. <https://doi.org/10.1016/j.jhazmat.2010.02.042>.
- Das S., Goswami S., Talukdar A. D. (2014). A Study on Cadmium Phytoremediation Potential of Water Lettuce, *Pistia stratiotes* L.. *Bull Environ. Contam. Toxicol.* 92, 169–174.
- Gabarrón M., Faz A., Martínez-Martínez S., Zornoza R., Acosta J. A. (2017). Assessment of metals behaviour in industrial soil using sequential extraction, multivariable analysis and a geostatistical approach. *J. Geochem. Explor.* 172, 174–183.
- Hegde A. G., Verma P. C., and Rajan M. P. (2009). "Standard protocol for conducting pre-operational environmental surveillance around nuclear facilities". A Publication of the Government of India, Atomic Energy Group, Bhabha Atomic Research Centre, Mumbai, India, BARC/2009/E/004, 144 pp.
- Huang R., Cui X., Luo X., Mao P., Zhuang P., Li Y. *et al.* (2021). Effects of plant growth regulator and chelating agent on the phytoextraction of heavy metals by *Pfaffia glomerata* and on the soil microbial community. *Environmental Pollution*, 283: 117159.
- Ihedioha J., Ukoha P., Ekere N. (2017). Ecological and human health risk assessment of heavy metal contamination in soil of a municipal solid waste dump in Uyo, Nigeria. *Environ. Geochem. Health* 39(3):497–515.
- Ma L., Sun J., Yang Z., Wang L. (2015). Heavy metal contamination of agricultural soils affected by mining activities around the Ganxi River in Chenzhou, Southern China. *Environ. Monit. Assess*, 187(12):731.
- Madanan T. M., Shah G. K., Varghese G. K. (2021). Application of Aztec Marigold (*Tagete erecta* L.) for phytoremediation of heavy metal polluted lateritic soil. *Environ. Chem. Ecotoxicol.*, 3: 17 - 22.
- Massadeh M. A., Massadeh S. A. (2019). Removal of Cu and Zn from aqueous solutions by selected tree leaves with phytoremediation potential. *Water Air Soil pollution*, 230: 264.
- Murtic S., Zahirovic C., Civic H., Sijahovic E., Jurkovic J., Avdic J. *et al.* (2021). Phytoaccumulation of heavy metals in native plants growing on soils in the Spreca river valley, Bosnia and Herzegovina. *Plant, Soil and Environment*, 67(9): 533 – 540.
- Naz A., Chowdhury A., Mishra B. K. (2018). Distribution of heavy metals and associated human health risk in mine, agricultural and roadside soils at the largest chromite mine of India. *Environ Geochem Health*, 40: 2155–2175.
- Nuhu A. A., Sallau M., Majiya M. (2014). Heavy metal pollution: the environmental impact of artisanal gold mining on Bagega village of Zamfara State, Nigeria. *Res. J. Pharm. Biol. Chem. Sci.* 5(6):306–313.
- Rashid M. H., Fardous Z., Chowdhury M. A. Z. (2016). Determination of heavy metals in the soils of tea plantations and in fresh and processed tea leaves: an evaluation of six digestion methods. *Chemistry Central Journal* 10(7): <https://doi.org/10.1186/s13065-016-0154-3>.
- Shams K. M., Tichy G., Fischer A., Sagar, M., Peer T., Bashar A. Fllip K. (2009). Aspects of phytoremediation for chromium contaminated sites using common plants *Urtica dioica*, *Brassica napus* and *Zea mays*. *Plant and Soil*, 328(1): 175 – 189.
- Sharifi K., Rahnavard A., Saeb K., Fahimi F. D., Tavana A. (2023). Ability of *Urtica dioica* L. to adsorb heavy metals from (Pb, Cd, As and Ni) from contaminated soils. *Soil and Sediment Contamination: An International Journal*, 32(1): 51 – 84.
- Shinta Y. C., Zaman B., Sumiyati I. (2021). Citric acid and EDTA as chelating agents in phytoremediation of heavy metals in polluted soils: A review. *IOP conf. ser.: Earth Environ Sci.*, 896: 012023.
- Sivarajasekar N., Nainamalai M., Sivamani S., Ganesh M. I., Ram Kothandan, M. S. (2018). Comparative modeling of fluoride biosorption onto waste *Gossypium hirsutum* seed microwave-bichar using response surface methodology and artificial neural networks, *IEEE Xplore*. 1631–1635.

- Viktorova, J., Jandova Z., Madlenakova M., Prouzova P., Bartunek V., Vichotova B. *et al.* (2016). Native phytoremediation potentials of *Urtica dioica* for removal of PCBs and heavy metals can be improved by genetic manipulations using constitutive CaMV 35S promoter. *PLOS ONE*, 11(12): e0167927.
- Wang J.Z., Peng S.C., Chen T.H., Zhang L. (2016). Occurrence, source identification and ecological risk evaluation of metal elements in surface sediment: Toward a comprehensive understanding of heavy metal pollution in Chaohu Lake, Eastern China. *Environ. Sci. Pollut. Res.* 23: 307–314.
- Wamalwa K., Lusweti J., Lutta S., Anditi B. C., Imbila O. M., Muthoka T. M. (2018). Variation of Pollutant Levels in Vegetables: A Case Study of Kitale Municipality, Trans-Nzoia County, Kenya. *Afri. J. Edu. Sci. Technol.*, 2(2): 146-160.
- Wu W., Wu P., Yang F., Sun D.L., Zhang D. X., Zhou Y.K. (2018). Assessment of heavy metal pollution and human health risks in urban soils around an electronics manufacturing facility. *Sci. Total Environ.* 630: 53–61.
- Yang Y., Liao J., Chen Y., Tian Y., Chen Q., Gao S. *et al.* (2022). Efficiency of heterogeneous chelating agents on the phytoremediation potential and growth of *Sasa argenteostriata* (Regel) E.G. Camus on Pb-contaminated soil. *Ecotoxicol. Environ. Safety*, 238: 113603.
- Zaier H., Ghnaya T., Ghabriche R. (2014). EDTA - enhanced phytoremediation of lead-contaminated soil by the halophyte *Sesuvium portulacastrum*. *Environ. Sci. Pollut.* 21: 7607 - 7615.