Phytoremediation Potential of Kangkong (*Ipomoea Reptans* Poir*)* in Lead-Induced Hydroponic System

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ABSTRACT

This study examined the phytoremediation capacity of upland kangkong (Ipomoea reptans Poir) in a hydroponic system with elevated levels of lead (Pb). This study aimed to determine the concentration of Pb absorbed by I. reptans Poir and the time required for absorption, as well as to compare the absorbed concentration with the WHO tolerable daily intake for humans. To conduct the study, I. reptans Poir seeds were germinated in perforated cups containing charcoal and immersed in a hydroponic system filled with water and nutrients. The plants were exposed to the lead-induced hydroponic system for 10, 20, and 30 days. The concentration of absorbed Pb was analyzed using ICP-OES. The results revealed that Pb was not detected in the plants exposed for 10–20 days. However, detection began after 30 days, with the amount of Pb being <0.25 mg/kg, which is lower than the WHO's recommended tolerable daily intake for humans. This research suggests that further studies on phytoremediation using other Ipomoea species should be conducted to compare the bioaccumulation among different species. These findings contribute to the understanding of poir's potential for phytoremediation in lead-contaminated hydroponic systems and their safety for human consumption.

Keywords: Phytoremediation Potential, Lead-induced, Hydroponic system, Ipomoea reptans Poir

INTRODUCTION

Environmental pollution is a major global problem that affects biodiversity, ecosystems, and human health by contaminating the soil and water. Heavy metals in soil and water can contaminate plants and pose a risk to human health. One of the several ways these elements can enter the human body is through the ingestion of vegetables containing heavy metals. Once these heavy metals enter the human body, they are deposited in the bone and fat tissues, overlapping with noble minerals. Slowly released into the body, heavy metals can cause acute and chronic toxicities in humans. Lead (Pb), one of the extremely toxic heavy metals that does not play any biological functions. It is usually found in leaded gasoline, lead-acid batteries, and some household materials, such as old paints and/or lead-based paint in some toys, old water pipes, jewelry, and lead-glazed potterymade of lead. Once the lead (Pb) contaminates the soil, especially at high concentrations, disturbs various plant physiological processes, and may induce severe human complications such as abdominal colic pain, bloody diarrhea, and kidney failure when eaten. Furthermore, it adversely affects neurological, biological, and cognitive functions.

In conjunction with improvements brought about by Science and Technology, plants have been discovered to be effective accumulators of heavy metals, such as soil pollutants. Science is now referred to as phytoremediation, a bioremediation process that uses revegetation to remove, transfer, stabilize, and/or destroy contaminants in the soil and water. This is an effective, affordable, eco-friendly, and cost-effective technological approach for mitigating heavy metal pollution in soil and water.

Kangkong is a semi-aquatic herbaceous trailing vine that thrives naturally in water and moist soil. They are primarily found in Southeast Asian countries. Kangkong can also be cultivated in high elevation areas. It has high economic value and is used for various purposes, including, but not limited to, the use of medicines, vegetables, water purifiers, and soil conditioners. The plant grows well as a crop in regions with an average temperature above 25°C. Hence, the Philippines is a conducive environment for flourishing. The upland kangkong (*Ipomoea reptans* Poir) is a common type of water spinach. It is one of the cheapest vegetables and food staples in the normal Filipino diet. It is a low-maintenance crop with a high tolerance to heavy metals. Thus, the purpose of this study was to determine the potential role of upland kangkong (*Ipomoea reptans* Poir) in the absorption of Pb in a lead-contaminated hydroponic system and to compare the concentration to the tolerable daily intake for humans set by the World Health Organization (WHO).

METHODOLOGY

Construction of the Greenhouse

The greenhouse where the plants were grown was made up of materials such as a 6m green nylon net, thumbtacks, and thick plastic used as perimeter cover. A green nylon net was used to deter pests from infesting the experimental plants.

The makeshift greenhouse was roofed equally with a plastic cover. The area inside the greenhouse is enough to accommodate an old bamboo bed measuring 2.5m x 1.5m which was cleaned and installed inside the structure and served as a mount for the grow trays and for ease of observation. The experimental plants planted in styro cups were fitted in holes borne in 0.44-m by 0.20-m styro grow trays. In turn, growth trays consisting of rectangular styro foams were floated in reservoirs with a capacity of 16-L of water.

Preparation of hydroponic reservoirs and grow trays and perforated cups

The eight pieces of 0.44-meter by 0.20-meter rectangular flower pots, 8-oz styrocups and 4 pieces 1-in thick styroboards that were bought from the Cauayan City market and the National Bookstore respectively. Styroboards were cut to fit the tops of the rectangular reservoirs.

Soldering iron was used to make perforated cups, and a tin can with a diameter of 7.5 cm was used as a puncher to create 11 holes on the styroboard, which served as the growth tray. Rectangular flower pots were used as reservoirs and were filled with 16 liters of tap water to a depth of 9 cm, which was sufficient to partially immerse the bottom part of the perforated cups with seeds.

Planting of Experimental Plants

The experimental plants used in this study were upland kangkong (*Ipomoea reptans* Poir), which can be grown hydroponically and non-hydroponically (Syafitri and Fevria, 2021). Kangkong seeds from the East West Philippines company were submerged in distilled water for two hours to affect imbibition (Sen and Puthur, 2020; Hadas, 2005) before placing the seeds in perforated cups placed in the hydroponic system.

The 8-oz. The perforated cups were filled with ½ cup of charcoal as a hydroponic substrate and a piece of tissue on top, enough to cover the charcoal. A tablespoon of soil was used to cover the tissue before five pieces of seeds were placed in the soil. Additional soil was used to cover the exposed seeds. The styrocups were then sprayed with enough water to wet the soil. Perforated cups with kangkong (*Ipomoea reptans* Poir) seeds were partially immersed in water up to 3.6 cm from the bottom in the hydroponic reservoir. The setup was placed inside a greenhouse with a 12 h natural photoperiod. After seven days of germination, the recommended amount of prepared Yamasaki nutrients was added to the water of the hydroponic reservoir.

Preparation of Yamasaki Nutrients Stock

Yamsak Nutrient A is composed of K_2O 20% and Nitrogen total of 14% total nitrogen, while Yamasaki

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Nutrient B is composed of P_2O_5 9%, K_2O 28% and total Nitrogen of 8% total nitrogen. Yamasaki Nutrient A (50 g) was dissolved in 500 ml tap water and stirred well. 50 grams of Yamasaki Nutrients B was also dissolved in another 500 ml of tap water and stirred. The prepared Yamasaki nutrient stock solutions A and B were added equally to 8 ml per liter of water in the hydroponic setup. Each hydroponic setup contained 16 liters of water; thus, 128 ml of the prepared nutrients was added per hydroponic setup.

Lead Induction of Plants

After ten days of germination, the hydroponic reservoir filled with 16 liters of tap water and prepared nutrients was contaminated by dissolving 10 g of Pb $(CH_3COO)_2$ salt (available in the ISU laboratory) in 16 liters of water in the hydroponic reservoir, resulting in 625 ppm lead acetate, which is less than the recommended 10 to 20 thousand ppm concentration in a study by Cañizares (2016). Pb acetate was used because of its potential toxicity, which is generally attributed to the presence of Pb (Alexandrino *et al.*, 2021). Lead acetate is an ionic compound with the formula Pb $(CH_3COO)_2$, where lead is present in the +2 oxidation state. It has a Pb^{+2} cation and a CH^3COO^- anion. (https://www.vedantu.com/chemistry/lead-acetate) It is water-soluble and is one of the most bioavailable forms of Pb (Ghaz and Millette, 2004). The pH of the hydroponic solutions was measured using a digital pH meter. It is has a pH of 5.19.

The design was pre-experimental, particularly a one-shot case study with no control. Each treatment (10, 20, and 30 days, induced with 625 ppm concentration of lead acetate).

Table 1. Treatments received by upland Kangkong in the polluted hydroponic setup and concentration of contaminants.

Treatment	Length of Exposure	Concentration
T_1	10 days treated	625 ppm $Pb(CH_3COO)_2$
T_2	20 days treated	625 ppm $Pb(CH_3COO)_2$
T_3	30 days treated	625 ppm $Pb(CH_3COO)_2$

Measurement of pH reservoir solution

The protective cap of the calibrated pH meter was removed and the electrode was rinsed with distilled water and dried using filter paper. The pH meter was turned on by pressing the “ON/OFF” button. The pH meter electrode was immersed in the solution used in the hydroponic setup containing lead acetate, not over the immersion line. The solution was gently stirred, and the pH meter was released for approximately 30 s until the reading of the instrument stabilized. The 5.19 pH level of the solution was recorded. After measuring the pH level of the solution, the pH meter was rinsed with distilled water and dried using filter paper, and the meter was turned off by pressing the “ON/OFF” button. Finally, the protective cap was recapped after use.

Monitoring of Plants

The experimental setup was visited every day to monitor the progress of the plants, and the water level was monitored using a simple depth measuring device (1 foot ruler) to maintain the base of the perforated cups above 2 cm.

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Harvesting of Samples for Analysis

Samples were taken for analysis at 10-day intervals for up to 30 days (10, 20, and 30 days). The experimental plants chosen for analysis (samples) were uprooted from the perforated cups. Each perforated cup was lifted from a styro board. The entire plant was then removed from the roots to the shoots. The roots were carefully rinsed using a spray bottle with tap water to remove any dirt. The same process was performed for the plants collected after 20 and 30 days.

The contaminated water reservoir was collected and placed in a heavy-duty container with a liters of five gallons capacity, to be sent to the Barangay 176 Treatment, Storage, and Disposal Facility in Phase 2 Package 3, Barangay 176, Caloocan City, NCR Thirds District, which is a registered treatment, storage, and disposal facility in the National Capital Region.

Drying and Shipping

The harvested experimental plants were air-dried on top of the table until brittle (Andress and Harrison, 2000). Dried samples were weighed using a digital weighing scale. One hundred grams (required sample weight of Sentrotek Laboratory) were placed in labelled (7x 10 cm) and 0.12 mm thinkness transparent zip lock plastic bags. The samples were sent through LBC Express to Sentrotek Laboratory in Mandaluyong City, where they were analyzed using Inductively Coupled Plasma optical emission spectrometry (ICP-OES).

Laboratory Analysis

The phytoaccumulation potential of the experimental plants was analyzed at the Sentrotek Laboratory in Mandaluyong City from August to December 2021. Acid digestion was performed to prepare the samples, and lead analysis was performed using Inductively Coupled Plasma optical emission spectrometry (ICP-OES). The procedures used in the preparation of the sample (acid digestion) and lead analysis are shown in Appendix A Procedure 4.

RESULTS AND DISCUSSION

This study aimed to determine the Pb absorption capacity of upland kangkong in a hydroponic system induced with 625 ppm $Pb(CH_3COO)_2$. The Pb concentration absorbed was determined on a dry weight basis, involving the whole plant: leaves, stems, and roots.

Analysis of lead-induced kangkong for 10 days

Kangkong plants were harvested for analysis after exposure to Pb for 10 days using 625 ppm $Pb(CH_3COO)_2$ in the hydroponic system. The collected and air-dried kangkong samples were analyzed using Inductively Coupled Plasma Emission Spectrometry (ICP-OES) to measure the lead concentration absorbed by the plant samples.

Table 2. Results of analysis of kangkong after 10 days of exposure to Pb

Parameter	Results			Methodology
	Trial 1	Trial 2	Trial 3	
Lead (Pb), mg/kg	ND	ND	ND	ICP-OES

Legend. ND-none detection

Table 2 shows the results of the analysis of the kangkong induced with lead after 10 days. It revealed that there was no detection returned by the instrument at 0.25 ppm detection limit after three (3) trials of analysis were conducted. This could probably be due to the natural tendency of plants to resist Pb intrusion into their systems. Punz & Sieghardt (1993), described in their study of roots of herbaceous plant species that most heavy metals, such as Pb, have proven the existence of uptake exclusion, which is also known as the avoidance system. Another reason is the pH level of 7.0, which is the appropriate environment for the plant and is more suitable for accumulating the metal ions based on Saygideger *et al*. (2004), whereas Lee *et al*. (1988) stated that as the pH increases, the plant Pb absorption also increases. The 5.19 pH level of the hydroponic solution could possibly have affected the lower Pb absorption because the roots of the upland kangkong were immersed in the Pb-contaminated solution.

Analysis of lead-induced kangkong for 20 days

A similar result was obtained after a 20-day lead induction period in the experimental plants (Table 3).

Table 3. Results of analysis of kangkong after 20 days of exposure to Pb

Parameter	Results			Methodology
	Trial 1	Trial 2	Trial 3	ICP-OES
Lead (Pb), mg/kg	ND	ND	ND	

Legend. ND-none detection

Based on Tables 2 and 3, there was no apparent absorption of Pb at the detection level of 0.25 mg/kg (ppm) after exposing the kangkong with 625 ppm of $Pb(CH_3COO)_2$ concentration. However, this does not necessarily mean that no lead is absorbed. This may be because of three reasons. First, the sensitivity of the instrument (0.25 mg/kg) may have masked the level of Pb absorption, particularly after 20 days. Second, there is a natural tendency of plants to resist Pb intrusion into their systems. Punz and Sieghardt (1993), reported in their study of roots of herbaceous plant species that most heavy metals, such as Pb, have proven the existence of uptake exclusion, which is also known as the avoidance system. According to Saygideger *et al*. (2004) pH 7.0, is the more appropriate environment for the plant and more suitable for the plant to accumulate metal ions, and Pb absorption also increases as the pH increases. The 5.19 pH level of the hydroponic solution could possibly have affected the lower Pb absorption because the roots of the upland kangkong were immersed in the Pb-contaminated solution.

The root defense of some plants is to stop metal ions, such as lead, from entering the root tissues by rejecting them (Mishra *et al*.,2006). Yanai *et al.* (2006) and Chen *et al.* (2009) found that aside from plant species, the concentration of metals, soil or nutrient solution acidity, and organic matter composition are factors affecting the absorption and accumulation of heavy metals in plants. The formation of a mechanical barrier is the immediate response of roots to the presence of Pb (Krzesłowska, 2011). Callose forms in the root tip as an efficient barrier against Pb penetration (Samardakiewicz *et al.*, 1996). Lastly, the experimental plant has a slow absorption rate, as evidenced by the study of Muryani and Hernanda (2020), who concluded that *Ipomoea reptans* Poir is a food crop not suitable as a phytoremediation agent for the heavy metal lead because the efficiency of Pb uptake in the plants is very low.

Analysis of lead-induced kangkong for 30 days

Table 4. Results of analysis of kangkong after 30 days of exposure to Pb

Parameter	Results			Methodology
	Trial 1	Trial 2	Trial 3	ICP-OES
Lead (Pb), mg/kg	<0.25	<0.25	<0.25	

Legend. ND-none detection

Table 4 shows the Pb absorption after 30 d of exposure to Pb. After three (3) trials were conducted, the result revealed that there was a detection of <0.25 mg/kg Pb absorbed by upland kangkong (*Ipomoea reptans* Poir), but still the instrument cannot quantify the exact amount due to its detection limit. Less than 0.25 mg/kg is a very small amount compare to 1.0 mg /kg maximum limit of Pb in food according to SNI No. 7387: 2009 (Badan Standarisasi Nasional, 2009) and compared to the established a provisional tolerable weekly intake of 3 mg of Pb per person by the Joint FAO/WHO Expert Committee on Food Additives which, correspond to 0.05 mg/kg body weight for adults. The maximum tolerable daily intake of Pb for a 60 kg man set by the WHO (2001) is equivalent to 0.42 mg/kg. Therefore, even if there is <0.25 mg/kg detected Pb absorbed by upland kangkong (*Ipomoea reptans* Poir) after 30 days of exposure to lead-induced hydroponic system. The absorbed Pb was below the maximum tolerable daily intake of Pb for the 60 kg man set by the WHO, and it will be less once the roots are removed, due to the higher accumulation of Pb in roots than in shoots (Jones *et al.,* 1973; Verma & Dubey, 2003; Ng *et al.,* 2016). Thus, upland kangkong (*Ipomoea reptans* Pior) did not exceed the maximum limit set by the World Health Organization.

Summary of analysis of lead-induced kangkong for 10, 20 and 30 days

Table 5 shows that there was no absorption of Pb at the detection level of 0.25 mg/kg after inducing the upland kangkong (*Ipomoea reptans* Poir) in the first 20 days in the 625 ppm $Pb(CH_3COO)_2$ concentration. Hence, after 30 days of exposure to the upland Kangkong, the ICP-OES equipment detected a <0.25 mg/kg Pb in its plant tissue.

Table 5. Summary of results of analysis of kangkong after 10, 20, 30 days of exposure to Pb

Methodology	10 days	20 days	30 days	Parameter
ICP-OES	ND	ND	<0.25	Lead, mg/kg

Legend. ND-none detection

Same results were found in the study of Anit *et al.* (2015) where there is also no amount of Pb detected from the plant tissues of kangkong using the Atomic Absorption Spectroscopy (AAS) while Ng *et al.* (2016), found out, in their study of heavy metals phyto-assessment that water spinach or kangkong (*Ipomoea aquatic*) has a good potential as Pb phytoremediation and highest accumulation of Pb was found in its shoots.Water spinach and kangkong (*Ipomoea aquatic*) are mostly cultivated in moist soils (Fontanilla, 2014; Medenilla, 2021).

Huang and Cunningham (1996) found that upland kangkong, a dicot plant, accumulates significantly higher amounts of Pb in the roots than monocots, and that the absorption and bioaccumulation of heavy metals in plants depend on the plant species (Sharma and Dubey, 2005). This also agrees with the findings of Yanai *et al.* (2006) and Chen *et al.* (2009) that aside from plant species, the concentration of metal, soil or nutrient solution acidity, and organic matter composition are factors affecting the absorption and accumulation of heavy metals in the plant. Therefore, it is safe to say that different cultivars of kangkong are likely to have a different array of heavy metal accumulations, as shown by He *et al.* (2015) and Alia *et al.* (2015).

These findings are somehow supportive of the results of this study because the analysis showed that Pb was not detected from 10 to 30 days of exposure.

An important implication of this result is that the upland kangkong (*Ipomoea reptans* Poir) is a foodstuff among Asians, children, and adults. Shoots are good for many food preparations in the Filipino diet. People, especially those in poor places, are forced to obtain food at all costs. Upland kangkong (*Ipomoea reptans* Poir) grows easily, even in minimal soil or in lead-contaminated bodies of water, and with regular consumption of upland kangkong (*Ipomoea reptans* Poir) harvested from a contaminated area. Humans may bioaccumulate Pb at low concentrations, stimulating respiration and increasing the ATP content, whereas higher concentrations inhibit respiration and decrease ATP (Sharma & Dubey, 2005). Pb causes an imbalance in K, Ca, Mg, Mn, Zn, Cu, and Fe within the tissues by physically blocking the access of these ions to the absorption sites of the roots (Aslam, 2021).

However, children are more vulnerable to Pb or other metal poisoning. Children are more easily affected by behavioral patterns. Pb absorption is higher among children, despite the level of exposure (Kazi *et al.*, 2006), and children's poor nutritional status and low intake of essential metals also increase Pb absorption (Wright *et al.*, 2018). For this reason, the Food and Drug Administration has set a threshold level of 0.0005 mg/L for lead in any food product intended for infants and children (FDA, 1994), as cited by ATSDR (2021).

A study by Göthberg and Bengtsson (2002) suggested that the roots of kangkong should be avoided when the accumulation of heavy metals is higher. Lead concentrations in roots are higher than those in shoots (Jones *et al.,* 1973; Verma and Dubey, 2003; Ng *et al.,* 2016; Peláez *et al.,* 2016; Ashraf *et al.,* 2017; Castro-Bedriñana *et al.,* 2021). Outridge and Noller (1991) found that in aquatic plants, roots are frequently reported to contain higher concentrations of most metals than the aboveground parts. Since roots are not consumed as food, the real danger lies in the shoots that humans consume, although accumulation is lower compared to the roots, as reported by Rahman *et al.* (2002).

CONCLUSION

1. The study showed that upland kangkong (*Ipomoea reptans* Poir) can absorb Pb in its tissues, but less than the instrument's detection level of 0.25ppm.
2. It took 30 days of exposure to upland kangkong (*Ipomoea reptans* Poir) in the lead-induced hydroponic system to detect absorption of <0.25 mg/kg.
3. Upland kangkong *(Ipomoea reptans* Poir) absorbed less than 0.25 mg/kg of Pb, which is a very small amount and within the safe limits prescribed by the WHO in 2002 for a 60 kg man.

RECOMMENDATION

In accordance with the findings and conclusions, the following recommendations were made:

1. The amount of Pb per plant tissue, such as roots, stems, and leaves, was analyzed to identify the part with the highest accumulation.
2. Similar studies should be conducted using other treatments such as 30, 45, and 60 days.
3. Soil was used instead of hydroponics to compare where the absorption of Pb is more efficient.

4. Further studies on phytoremediation using other *Ipomoea* species should be conducted to compare the bioaccumulation among *Ipomoea*

LITERATURE CITED

1. Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, *91*(7), 869-881.
2. Alia, N., Sardar, K., Said, M., Salma, K., Sadia, A., Sadaf, S., … & Miklas, S. (2015). Toxicity and bioaccumulation of heavy metals in spinach (Spinacia oleracea) grown in a controlled environment. *International Journal of Environmental Research and Public Health*, *12*(7), 7400-7416.
3. Andress, E. L., & Harrison, J. A. (2000). *Preserving food: Drying fruit and vegetables*. University of Georgia.
4. Anit, J., Billojan, E., Llaguno, J. K., & Malilay, A. (2015). Phytoremediation of Chromium, Copper and Lead from a Firearm Factory in Marikina City, Philippines Using *Ipomoea aquatica*. *BIMP-EAGA Journal for Sustainable Tourism Development*, *4*(2), 132-141.
5. Arthur, E. L., Rice, P. J., Rice, P. J., Anderson, T. A., Baladi, S. M., Henderson, K. L., & Coats, J. R. (2005). Phytoremediation—an overview. *Critical Reviews in Plant Sciences*, *24*(2), 109-122.
6. Ashraf *et al.* (2017) Ashraf U, Kanu AS, Deng Q, Mo Z, Pan S, Tian H, Tang X. Lead (Pb) toxicity; physio-biochemical mechanisms, grain yield, quality, and Pb distribution proportions in scented rice. *Frontiers in Plant Science.* 2017;8:259. doi: 10.3389/fpls.2017.00259.
7. Aslam, M., Aslam, A., Sheraz, M., Ali, B., Ulhassan, Z., Najeeb, U., … & Gill, R. A. (2021). Lead toxicity in cereals: Mechanistic insight into toxicity, mode of action, and management. *Frontiers in Plant Science*, 2248.
8. *ASTDR. 2021. Lead Toxicity. Environmental Health and Medicine. Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/csem/ leadtoxicity/safety_standards.html*
9. Auguy, F., Fahr, M., Moulin, P., Brugel, A., Laplaze, L., Mzibri, M. E., … & Smouni, A. (2013). Lead tolerance and accumulation in Hirschfeldia incana, a Mediterranean Brassicaceae from metalliferous mine spoils. *PLoS One*, *8*(5), e61932.
10. Austin, D. F. (2007). Water Spinach (Ipomoea aquatica, Convolvulaceae): A food gone wild. *Ethnobotany Research and Applications*, *5*, 123-146.
11. Australian Centre for International Agricultural Research (ACIAR). (2010). NUTRITIOUS LEAFY VEGETABLES FOR ATOLLS. *KANGKONG*, *6*(08), 1–2. https://www.adelaide.edu.au/directory/graham.lyons?dsn=directory.file;field=data;id=40381;m=view
12. Aydinalp, C. and S. Marinova, 2009. The effects of heavy metals on seed germination and plant growth on alfalfa plant (Medicago sativa). Bulg. J. Agric. Sci., 15: 347-350
13. Azevedo, R. A., & Lea, P. J. (2005). Preface: toxic metals in plants. *Brazilian Journal of Plant Physiology*, *17*, 1-1.
14. Baysa, M. C., Anuncio, R. R. S., Chiombon, M. L. G., Cruz, J. P. R. D., & Ramelb, J. R. O. (2006). Lead and cadmium contents in Ipomoea aquatica Forsk. grown in Laguna de Bay. *Philippine Journal of Science*, *135*(2), 139.
15. Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., … & Raskin, I. (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science & Technology*, *31*(3), 860-865.
16. Budianto, A. (2017). *Analisis kandungan timbal (Pb) pada tanaman kangkung air (Ipomoea aquatic forrsk) di Sungai Lesti Kabupaten Malang dengan variasi metode destruksi basah tertutup menggunakan spektroskopi serapan atom (SSA)* (Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim).
17. Canizares, Henry. (2016). Study of the Effects of Zinc, Cadmium, and Copper on Plant Survival and Growth of Water Spinach (Ipomoea aquatica Forsk-Ching Quat Variety), Grown in Commercial Soil. 10.13140/RG.2.2.10209.71529.
18. Castro-Bedriñana, J., Chirinos-Peinado, D., Garcia-Olarte, E., & Quispe-Ramos, R. (2021). Lead

transfer in the soil-root-plant system in a highly contaminated Andean area. *PeerJ*, *9*, e10624. https://doi.org/10.7717/peerj.10624

19. Chen, C., Huang, D., Liu, J., 2009. Functions and toxicity of nickel in plants: recent advances
20. Cheng S. Effects of heavy metals on plants and resistance mechanisms. A state-of-the-art report with special reference to literature published in Chinese journals. Environ Sci Pollut Res Int. 2003;10(4):256-64. doi: 10.1065/espr2002.11.141.2. PMID: 12943010.
21. Daintith, J. (Ed.). (2008). *A dictionary of chemistry*. OUP Oxford.
22. Dalvi, A. A., & Bhalerao, S. A. (2013). Response of plants towards heavy metal toxicity: an overview of avoidance, tolerance and uptake mechanism. *Ann Plant Sci*, *2*(9), 362-368.
23. Depkes. (1990). Peraturan Menteri Kesehatan RI No 416/Menkes/Per/IX/1990, Tentang Syarat-Syarat dan Pengawasan Kualitas Air. Departemen Kesehatan RI: Jakarta.
24. Dhanasekaran, S. and Muralidaran, P. (2010).CNS depressant and anti epileptic 55 activities of the methanol extract of the leaves of Ipomoea aquatica Forsk. E. J. Chem., 7: 15-61.
25. Dhanasekaran, S., Palaya, M. and Shantha Kumar, S. (2010). Evaluation of antimicrobial and anti-inflammatory activity of methanol leaf extract of Ipomoea aquatica Forsk. Res. J. Pharm. Biol. Chem. Sci., 1: 258-64.
26. Dubchak, Sergiy & Bondar, Olexander. (2019). Bioremediation and Phytoremediation: Best Approach for Rehabilitation of Soils for Future Use. 10.1007/978-3-319-73398-2_9.
27. *E.L. Andress and J.A. Harrison. 2000. So Easy to Preserve (5th Edition). University of Georgia Cooperative Extension Service.*
28. EPA (2000). A Citizen's Guide to Phytoremediation. EPA 542-F-98-011. United States Environmental Protection Agency, p. 6. Available at: http//www.bugsatwork.com/XYCLONYX/EPA_GUIDES /PHYTO.PDF
29. *EPA. 2022. Basic Information About Lead in Drinking Water. Ground Water and Drinking Water. https://www.epa.gov/ground-water-and-drinking-water/basic-information-about- lead-drinking -water*
30. Etim, E. E. (2012). Phytoremediation and its mechanisms: a review. *Int J Environ Bioenergy*, *2*(3), 120-136.
31. Fahr, M., Laplaze, L., Bendaou, N., Hocher, V., Mzibri, M. E., Bogusz, D., & Smouni, A. (2013). Effect of lead on root growth. *Frontiers in plant science*, *4*, 175.
32. Farhandimas, Ragil & Purbaningtias, Tri & Muhaimin, Muhaimin. (2017). Determination of Lead Metal (Pb) in Indonesian Kangkung (Ipomoea Aquatica) by Atomic Absorption Spectrophotometer (AAS).
33. *FDA. 1994. Guidance for Industry.*
34. FONTANILLA, C. (2014, February 24). *How to Grow Kangkong Plant in a Hydroponics Garden*. Hubpages. Retrieved June 28, 2022, from https://discover.hubpages.com/living/How-To-Grow-Kangkong-in-a-Hydroponics-Garden
35. Ghani, A. (2010). Toxic effects of heavy metals on plant growth and metal accumulation in maize (Zea mays L.).
36. Ghazi, A. M., & Millette, J. R. (2004). Environmental forensic application of lead isotope ratio determination: a case study using laser ablation sector ICP-MS. *Environmental Forensics*, *5*(2), 97-108.
37. Gjorgieva, D., Kadifkova Panovska, T., Ruskovska, T., Bačeva, K., & Stafilov, T. (2013). Mineral nutrient imbalance, total antioxidants level and DNA damage in common bean (Phaseolus vulgaris L.) exposed to heavy metals. *Physiology and molecular biology of plants : an international journal of functional plant biology*, *19*(4), 499–507. https://doi.org/10.1007/s12298-013-0196-0
38. Göthberg, A., Greger, M., & Bengtsson, B. E. (2002). Accumulation of heavy metals in water spinach (Ipomoea aquatica) cultivated in the Bangkok region, Thailand. *Environmental Toxicology and Chemistry: An International Journal*, *21*(9), 1934-1939.
39. Graham, A. (2004). DMOZ — Directory Mozilla The Open Directory Project, http://dmoz.org. *The Physics Teacher*, *42*(4), 255. https://doi.org/10.1119/1.1696607
40. Hadas, A. (2005) Encyclopedia of Soils in the Environment.

41. He, B., Ling, L., Zhang, L., Li, M., Li, Q., Mei, X., … & Tan, L. (2015). Cultivar-specific differences in heavy metal (Cd, Cr, Cu, Pb, and Zn) concentrations in water spinach (Ipomoea aquatic 'Forsk') grown on metal-contaminated soil. *Plant and soil*, *386*(1), 251-262.
42. Herlina, L., Widianarko, B., & Sunoko, H. R. (2020). Phytoremediation potential of Cordyline fruticosa for lead contaminated soil. *Jurnal Pendidikan IPA Indonesia*, *9*(1), 42-49.
43. Huang, J. W., & Cunningham, S. D. (1996). Lead phytoextraction: species variation in lead uptake and translocation. *New phytologist*, *134*(1), 75-84.
44. Jiwan, S and Ajay, K. (2011). Effects of Heavy Metals on Soil, Plants, Human Health and Aquatic Life. International Journal of Research in Chemistry and Environment. Vol. 1 Issue 2 Oct. 2011(15-21). ISSN 2248-9649. (Abstract)
45. Joint FAO/WHO Expert Committee on Food Additives. Meeting, Joint FAO/WHO Expert Committee on Food Additives, & World Health Organization. (2002). *Evaluation of certain mycotoxins in food: Fifty-Sixth report of the Joint FAO/WHO Expert Committee on Food Additives* (Vol. 56). World Health Organization.
46. Joint, F. A. O., WHO Expert Committee on Food Additives, & World Health Organization. (2000). Evaluation of certain food additives and contaminants: fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization.
47. Joint, F. A. O., WHO Expert Committee on Food Additives, & World Health Organization. (1972). Evaluation of certain food additives and the contaminants mercury, lead, and cadmium: sixteenth report of the Joint FAO. https://inchem.org/documents/jecfa/jecmono/v21je16.htm
48. Jones LHP, Clement CR, Hopper MJ (1973) Lead uptake from solution by perennial ryegrass and its transport from roots to shoots. Plant Soil 38:403-414.
49. Kalaivanan D., Ganeshamurthy A.N. (2016) Mechanisms of Heavy Metal Toxicity in Plants. In: Rao N., Shivashankara K., Laxman R. (eds) Abiotic Stress Physiology of Horticultural Crops. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2725-0_5
50. Kaur, Rawat, Renu, Kumar & Narain (2017). Taxonomy, Phytochemistry, Traditional Uses and Cultivation of Ipomoea Aquatica Forsk. Imperial Journal of Interdisciplinary Research (IJIR). Vol-2, Issue-10, 2016 ISSN: 2454-1362, http://www.onlinejournal.in
51. Kazi TG, Afridi HI, Kazi GH, Jamali MK, Arain MB, Jalbani N (2006) Evaluation of essential and toxic metals by ultrasoundassisted acid leaching from scalp hair samples of children with macular degeneration patients. Clin Chim Acta 369(1):52–60
52. Khan, S. R., Sharma, B., Chawla, P. A., & Bhatia, R. (2022). Inductively coupled plasma optical emission spectrometry (ICP-OES): a powerful analytical technique for elemental analysis. *Food Analytical Methods*, *15*(3), 666-688.
53. Kratky, B. A. (2003, February). A suspended pot, non-circulating hydroponic method. In *South Pacific Soilless Culture Conference-SPSCC 648* (pp. 83-89).
54. Krzesłowska, M. (2011). The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. *Acta physiologiae plantarum*, *33*(1), 35-51.
55. Krzesłowska, M., Lenartowska, M., Mellerowicz, E. J., Samardakiewicz, S., & Woźny, A. (2009). Pectinous cell wall thickenings formation—a response of moss protonemata cells to lead. *Environmental and Experimental Botany*, *65*(1), 119-131.
56. Lee, A., Boswell, C. C., & Watkinson, J. H. (1988). Effect of particle size on the oxidation of elemental sulphur, thiobacilli numbers, soil sulphate, and its availability to pasture. *New Zealand journal of agricultural research*, *31*(2), 179-186.
57. Malakar, C., & Choudhury, P. P. N. (2015). Pharmacological potentiality and medicinal uses of Ipomoea aquatica Forsk: a review. Asian J Pharm Clin Res, 8(2), 60-63.
58. Medenilla, V. (2021, July 15). How to plant and grow kangkong. *Manila Bulletin*. Retrieved June 19, 2022, from https://mb.com.ph/2021/07/15/how-to-plant-and-grow-kangkong/
59. Miller, R., 1996. Phytoremediation: Technology Cpeo.org. 2020. Phytoremediation. [online] Available at: <http://www.cpeo.org/techtree/ttdescript/phytrem.htm [Accessed 29 December 2020]. Overview Report. [online] Clu-in.org. Available at: <https://clu-in.org/download/toolkit/phyto_o.pdf>

[Accessed 29 December 2020].

60. Mishra, S., Srivastava, S., Tripathi, R. D., Kumar, R., Seth, C. S., & Gupta, D. K. (2006). Lead detoxification by coontail (Ceratophyllum demersum L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere*, *65*(6), 1027-1039.
61. Muryani, E., Mulyanto, D., & Hernanda, R. M. (2020, July). Phytoremediation of lead (Pb) polluted soil by Cordyline fruticosa and Ipomea reptans Poir (case study: Used battery smelting industry at Cinangka Village, Bogor). In *AIP Conference Proceedings* (Vol. 2245, No. 1, p. 090011). AIP Publishing LLC.
62. Najeeb, U., Ahmad, W., Zia, M. H., Zaffar, M., & Zhou, W. (2017). Enhancing the lead phytostabilization in wetland plant Juncus effusus L. through somaclonal manipulation and EDTA enrichment. *Arabian Journal of Chemistry*, *10*, S3310-S3317.
63. Nascimento, Clístenes Williams Araújo do, & Xing, Baoshan. (2006). Phytoextraction: a review on enhanced metal availability and plant accumulation. Scientia Agricola, 63(3), 299-311. https://doi.org/10.1590/S0103-90162006000300014
64. National Risk Management Research Laboratory (US). (2000). *Introduction to phytoremediation*. National Risk Management Research Laboratory, Office of Research and Development, US Environmental Protection Agency.
65. Ng, C. C., Rahman, M. M., Boyce, A. N., & Abas, M. R. (2016). Heavy metals phyto-assessment in commonly grown vegetables: water spinach (I. aquatica) and okra (A. esculentus). *SpringerPlus*, *5*(1), 1-9.
66. Novaes, C. G., Bezerra, M. A., da Silva, E. G. P., dos Santos, A. M. P., da Silva Romao, I. L., & Neto, J. H. S. (2016). A review of multivariate designs applied to the optimization of methods based on inductively coupled plasma optical emission spectrometry (ICP OES). *Microchemical journal*, *128*, 331-346.
67. Oancea, S., Foca, N., & Airinei, A. (2005). Effects of heavy metals on plant growth and photosynthetic activity. *Analele Univ."Al. I. Cuza*, *1*, 107-110.
68. Outridge, P.M. and B.N. Noller, 1991. Accumulation of toxic metals by freshwater vascular plants. *Rev. Environ. Contam. Toxicol*., 121: 1–63
69. Peer, W. A., Baxter, I. R., Richards, E. L., Freeman, J. L., & Murphy, A. S. (2005). Phytoremediation and hyperaccumulator plants. In *Molecular biology of metal homeostasis and detoxification* (pp. 299-340). Springer, Berlin, Heidelberg.
70. Peláez-Peláez, Bustamante & Gómez (2016) Peláez-Peláez MJ, Bustamante CJ, Gómez LE. Presencia de cadmio y plomo en suelos y su bioacumulación en tejidos vegetales en especies de *Brachiaria* en Magdalena medio colombiano. *Revista Luna Azul.* 2016;43:82–101. doi: 10.17151/luaz.2016.43.5
71. Piechalak, A., Tomaszewska, B., Baralkiewicz, D., & Malecka, A. (2002). Accumulation and detoxification of lead ions in legumes. *Phytochemistry*, *60*(2), 153-162.
72. Piršelová, B., Mistríková, V., Libantová, J., Moravčíková, J., & Matušíková, I. (2012). Study on metal-triggered callose deposition in roots of maize and soybean. *Biologia*, *67*(4), 698-705.
73. Punz, W. F., & Sieghardt, H. (1993). The response of roots of herbaceous plant species to heavy metals. *Environmental and experimental Botany*, *33*(1), 85-98.
74. Rahman, M. M., Shivakoti, G. P., Uddin, M. J., & Khandakar, S. M. A. T. (2002). Arsenic Accumulation in Kangkong (lpomoea reptans) and the Effects of Phosphate Fertilizer in its Availability in Soil. *Asia-Pacific Journal of Rural Development*, *12*(1), 65-75.
75. Raskin, I., and Ensley, B. D. (2000). Recent developments for in situ treatment of metal contaminated soils. In: Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. John Wiley & Sons Inc., New York. Available at: http//clu-n.org/techfocus
76. Robinson,D.(1991)."Rootsand resourcefluxesinplantsandcom- munities,"in *PlantRootGrowth.An EcologicalPerspective* (Special Publi- cation oftheBritishEcologicalSoci- ety,No10),ed.D.Atkinson(London: BlackwellScientific),103–130.
77. Rzymski, P., Tomczyk, K., Poniedzialek, B., Opala, T., & Wilczak, M. (2015). Impact of heavy metals on the female reproductive system. *Annals of agricultural and environmental medicine*, *22*(2).

78. Samardakiewicz, S., & Woźny, A. (2005). Cell division in Lemna minor roots treated with lead. *Aquatic Botany*, *83*(4), 289-295.
79. Samardakiewicz, S., Strawiński, P., & Woźny, A. (1996). The influence of lead on callose formation in roots ofLemna minor L. *Biologia plantarum*, *38*(3), 463-467.
80. Saygideger, S., Dogan, M., & Keser, G. (2004). Effect of lead and pH on lead uptake, chlorophyll and nitrogen content of Typha latifolia L. and Ceratophyllum demersum L. *International Journal of Agriculture and Biology*, *6*(1), 168-172.
81. Sengar RS, Gautam M, Sengar RS, Garg SK, Sengar K, Chaudhary R. Lead stress effects on physiobiochemical activities of higher plants. Rev Environ Contam Toxicol. 2008;196:73-93. doi: 10.1007/978-0-387-78444-1_3. PMID: 19025093.
82. Sharma, P., & Dubey, R. S. (2005). Lead toxicity in plants. *Brazilian journal of plant physiology*, *17*, 35-52.
83. Sijabat, N. Uji Aktivitas Antioksidan Ekstrak Etanol Daun Kangkung Air (Ipomoea aquatica Forsk.) dan Kangkung Darat (Ipomoea reptans Poir.) yang Ditanam di Daerah Berbeda Ketinggian.
84. Singh MR. Impurities-heavy metals: IR prespective. 2007. [Last cited on 2009 Aug 10].: http://www.usp.org/pdf/EN/meetings/asMeetingIndia/2008Session4track1.pdf
85. Srivastava, R. K., Kharisov, B. I., & Kharissova, O. V. (2021). Industrial useful bioproducts and devices development from microbial strains assisted nanosystems or nanoparticles. In *Handbook of Greener Synthesis of Nanomaterials and Compounds* (pp. 735-752). Elsevier.
86. Stephens, J.M. 2003. Kangkong — *Ipomoea aquatica* Forsk., also *Ipomoea reptans* Poir. Professor, Horticultural Sciences Department, Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville FL 32611. http://edis.ifas.ufl.edu/MV085)
87. Suman, J., Uhlik, O., Viktorova, J., & Macek, T. (2018). Phytoextraction of heavy metals: a promising tool for clean-up of polluted environment?. *Frontiers in plant science*, *9*, 1476.
88. Syafitri, S. D., & Fevria, R. (2021). Chlorophyll Ratio of Kale (Ipomea reptans Poir.) Which Are Cultivation With Hydroponick And Non Hydroponick. *Serambi Biologi*, *6*(1).
89. Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*, *2011*.
90. Tchounwou P.B., Yedjou C.G., Patlolla A.K., Sutton D.J. (2012) Heavy Metal Toxicity and the Environment. In: Luch A. (eds) Molecular, Clinical and Environmental Toxicology. Experientia Supplementum, vol 101. Springer, Basel. https://doi.org/10.1007/978-3-7643-8340-4_6
91. Thakur, S., Singh, L., Wahid, Z. A., Siddiqui, M. F., Atnaw, S. M., & Din, M. F. M. (2016). Plant-driven removal of heavy metals from soil: uptake, translocation, tolerance mechanism, challenges, and future perspectives. *Environmental monitoring and assessment*, *188*(4), 1-11.
92. Tong, Y. P., Kneer, R., & Zhu, Y. G. (2004). Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. *Trends in plant science*, *9*(1), 7-9.
93. Trap, S., Kohler, A., Larsen, L. C., Zambrano, K. C., and Karlson, U. (2005). Phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. J. Soil Sediments, 1: 71-76.
94. Trap, S., Kohler, A., Larsen, L. C., Zambrano, K. C., and Karlson, U. (2005). Phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. J. Soil Sediments, 1: 71-76
95. United States Environmental Protection Agency (USEPA). (2000). Introduction to Phytoremediation. EPA 600/R-99/107, U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.
96. Verma S, Dubey RS (2003) Lead Toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plants Sci. 164:645-655.
97. Wang, J., & Chen, C. (2006). Biosorption of heavy metals by Saccharomyces cerevisiae: a review. *Biotechnology advances*, *24*(5), 427-451.
98. WHO. (2021, October 11). *Lead Poisoning*. Retrieved June 25, 2022, from https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health#:~:text=Lead%20is%20a%20cumulative%20 toxicant,where%20it%20accumulates%20over%20time.

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99. Woolf, A. D., Goldman, R., & Bellinger, D. C. (2007). Update on the clinical management of childhood lead poisoning. *Pediatric Clinics of North America*, *54*(2), 271-294.
100. World Health Organization Food SND Agriculture Organization of the United Nations Rome. (2002). Human Vitamin and Minerals Requirements. Pages http://www.fao.org/3/y2809e/y2809e0m.htm#bm22
101. World Health Organization. 1993. Evaluation of certain food additives and contaminants. The 41st report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Report Series 837. Geneva, Switzerland.
102. World Health Organization. 1999. Summary and conclusions. Joint FAO/WHO Expert Committee on Food Additives, 53rd Meeting, Rome, Italy, June 1–10. Geneva, Switzerland.
103. Wright, R. O., Tsaih, S. W., Schwartz, J., Wright, R. J., & Hu, H. (2003). Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. *The Journal of pediatrics*, *142*(1), 9-14.
104. Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., & Chen, Z. (2020). Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. *Frontiers in Plant Science*, *11*, 359.
105. Yanai, J., Zhao, F.J.,McGrath, S.P., Kosaki, T., 2006. Effect of soil characteristics on Cd uptake
106. Zhuang, P., Ye, Z. H., Lan, C. Y., Xie, Z. W, and Hsu, W. S. (2005). Chemically assisted phytoextraction of heavy metal contaminated soils using three plant species. Plant Soil, 276: 153-162.
107. Zhuang, P., Ye, Z. H., Lan, C. Y., Xie, Z. W, and Hsu, W. S. (2005). Chemically assisted phytoextraction of heavy metal contaminated soils using three plant species. Plant Soil, 276: 153- 162.