ISSN 2232-10603

PHYTOREMEDIATION OF CHROMIUM, COPPER AND LEAD FROM A FIREARM FACTORY IN MARIKINA CITY, PHILIPPINES USING *Ipomoea aquatica*

Jovita A. Anit, Eunice B. Billojan, Janella Kay P. Llaguno and Aizeen P. Malilay

College of Arts and Sciences, Rizal Technological University
Boni Avenue, Mandaluyong City, Philippines

jovita_anit@yahoo.com

ABSTRACT

The study was conducted to determine the capability of *Ipomoea aquatica* for phytoremediation. Plants were grown in a container with different concentrations of wastewater obtained from a firearm factory in Marikina City. The wastewater concentrations were 5%, 10%, 15% and 20%. A control using pure distilled water was also used. The experiment was carried out under hydroponic set-up. The absorption of heavy metals such as chromium, copper and lead were evaluated and tested after 21 days of submerging the plants in wastewater solution. The chromium, copper and lead that were accumulated by the plants were measured using Atomic Absorption Spectroscopy (AAS). The plant height, length of roots and number of nodes were observed for 21 days. The concentration of copper observed increased with increasing concentrations of wastewater. The plants were not able to absorb chromium and lead but survived and thus tolerated the concentration range of 0.001 to 0.002mg/L of chromium and 0.0015mg/L to 0.3mg/L of lead. The results showed that *Ipomoea aquatica* were not capable for the absorption of chromium and lead but tolerates the amount present in the wastewater and accumulate copper in different treatments. AAS showed that there were no amount of chromium and lead detected on the plant tissue samples. The initial copper concentration detected via AAS in pure distilled water was 2mg/L, 8mg/L in 5% wastewater, 9mg/L in 10%, 8mg/L in 15% and 12mg/L in 20% wastewater.

Keywords: phytoremediation, wastewater, chromium, copper and lead

1 INTRODUCTION

Marikina River is Marikina City's main waterway that is connected to Pasig River. The deterioration of the river over the last four decades is attributed to the uncontrolled encroachment and indiscriminate disposal of both domestic and industrial waste and garbage in the easement of the river. Untreated domestic and industrial wastes are discharged directly into canals that ultimately flow into the river. The worsening water quality is related to the incidence of water-borne diseases, and is likely to increase the negative impact of flood to human health.

Contamination of water due to the dispersal of industrial and urban wastes generated by human activities is a major problem. The controlled and uncontrolled disposal of waste in water is responsible for the migration of contaminants into non-contaminated sites and contributes towards contamination of our fresh water and marine ecosystem.

Heavy metal pollution is a global problem, although the severity and levels of contamination differ from place to place. Excessive level of heavy metals are exposed into the environment, for example industrial waste and fertilizers cause serious concern in nature as they are non- biodegradable and accumulate at high levels (Tiwari et al. 2012).They are one of the major means of environmental pollution, which heavily affect human health via ingestion through plants, foods, contamination of water bodies and other nutrient supplements (Mohotti et al. 2011). Common heavy metals such as chromium (Cr), copper (Cu) and lead (Pb) are phytotoxic at both low and very high concentrations. They are detected in wastewater from tanneries, electronics, electroplating, batteries and petrochemical industries as well as textile mill products (Singh et al. 2011). Ions of chromium, copper and lead which are frequently present in the wastewaters can cause renal dysfunction as well as chronic alterations in nervous system and gastrointestinal tract (Singh et al. 1996; Miretzkyet al. 2004).

One way to solve heavy metal pollution is phytoremediation. It is also called green remediation, botano-remediation, agro remediation and vegetative remediation (Erakhrumenet al. 2007). This is an ecological, cost effective, and an aesthetically environmental pollutants removal approach most suitable for developing countries (Pivertzet al. 2001). The plant used in the phytoremediation technique must have a considerable capacity for metal absorption, accumulation and the strength to decrease the treatment time (Mudgalet al. 2010). This plant based-green technology, has received increasing attention after the discovery of hyper accumulating plants which are able to accumulate, translocate, and concentrate high amount of certain toxic elements in their above-ground/harvestable parts. One of the most significant advantages of this technology is the ability to study the sub-lethal level of bio-accumulated contaminants within the tissues/components of organisms, an indicator of the net amount of pollutants integrated over a period of time (Lovett-Doustet al. 1994). Phytoremediation includes several technology processes namely, phytoextraction, phytodegradation, rhizofiltration, phytostabilization and phytovolatilization (Hasegawa et al. 2011).

Macrophytesare fast growing aquatic plants that absorb elements through roots and/or shoots (Hashimoto et al. 1985). They have been used during the last two decades for heavy metal removal (Denny & Wilkins 1987). They can either be floating on the water surface or submerged into the water.The floating aquatic hyper accumulating plants absorb or accumulate contaminants by its roots while the submerged plants accumulate metals by their whole body (Hasegawa et al. 2011). One such macrophyte is *Ipomoea aquatica* a semi-aquatic tropical plant grown as a leafy vegetable.Commonly proposed as a bioremediant, this species was found to be one of the most useful plant species in phytoremediation studies due to its ability to accumulate high concentration of heavy metals in the roots (Pip &Stepaniuk 1992). *Ipomoea aquatica* act as a good metal and toxin accumulators (De Jesus et al. 2011). It is because of this quality that this aquatic macrophyte has been suggested to be a potential heavy metal phytoremediant (Rahman et al. 2011). Plant assimilation of nutrients and its subsequent harvesting are another mechanism for pollutant removal. Low cost and easy maintenance make the aquatic plant system attractive to use (Kanabkaew&Puetpaiboon 2004).

The aim of this study was to determine the effectiveness of *Ipomoea aquatica* (kangkong) in absorbing heavy metals specifically chromium, copper and lead from a firearm factory in Marikina City. Also, to determine if *Ipomoea aquatica* is a hyperaccumulator or it has the capability to assimilate the heavy metals absorbed.

2 MATERIALS AND METHODS

2.1 Preparation of Materials

Chinese kangkong*(Ipomoea aquatica*) seeds were purchased from a seed company. The seeds were germinated in a soil-less planting medium and allowed to grow for 2 weeks. Germinated seedlings were submerged in distilled water for 2 days before being exposed to

the wastewater solution. Plant samples were subjected to tissue analysis.
Samples of wastewater were collected from a firearms factory at Armscor Avenue, Barangay Fortune Parang in Marikina City. Wastewater samples were subjected to chemical analysis before using to determine the chemical content and the concentrations of the chemicals present.

2.2 Experimental set-up

The experiment was done using the hydroponics technique. A preliminary set-up using the influent at 25%, 50% and 75% wastewater solution and distilled water was carried out. Plants placed in the different wastewater solutions were plasmolyzed just a few hours after exposure to the different concentrations. Only the plants in control condition (distilled water) were able to survive.

The result of the preliminary test was used as reference for the second set-up done. Instead of the influent, the effluent with the lower concentrations of chromium, copper and lead was used. The concentrations of wastewater used were 5%, 10%, 15% and 20%. A control set-up using distilled water was also done.

The height of the plant, length of the roots and number of nodes was measured with an interval of 3 days. Plant samples were gathered and subjected to tissue analysis after four weeks.

2.3 Statistical Analysis

The experiment was set-up using the Completely Randomized Designed (CRD) with four treatments (5%, 10%, 15% and 20% concentrations of wastewater) and a control set up (distilled water). Five samples per treatment were used. The whole experimental set-up was replicated three times. Analysis of data was carried out using one way analysis of variance (ANOVA).

3 RESULTS AND DISCUSSIONS

3.1 Preliminary Test

A preliminary test using the influent was carried out. The range of concentrations (0.05mg/li to 19mg/li chromium, 0.85mg/li to 3.39mg/li copper and 0.09mg/li to 36mg/li of lead) of the three heavy metals (table 1), chromium, copper and lead were found to be toxic to the plant as all plants treated with the wastewater exhibited plasmolysis 24 hours after exposing them to the wastewater (figure 1).

Table 1. Different percent concentration of heavy metals present in diluted Influent wastewater (mg/L) used in preliminary set-up

	Concentrations of the wastewater			
	25%	50%	75%	100%
Chromium (0.19 mg/L)	0.05 mg/L	0.10 mg/L	0.14 mg/L	0.19 mg/L
Copper (3.39 mg/L)	0.85 mg/L	1.70 mg/L	2.54 mg/L	3.39 mg/L
Lead (0.36mg/L)	0.09 mg/L	0.18 mg/L	0.27 mg/L	0.36mg/L

Data shows that the amount of heavy metals presents on the influent wastewater such as chromium, copper and lead in the diluted concentrations were not tolerated by the experimental plants. This is because the experimental plant has a limit on the concentration of substances that it can absorb*Ipomoea aquatica* can only uptake a concentration of 0.050

mg/li of copper (Estoque et al. 1997), 0.0010 mg/li of lead (Low et al. 1987) and chromium at 0.0016 mg/li according to the agency for toxic substances agency (MAL 1997). These values are way below the concentrations of the different wastewater sample.

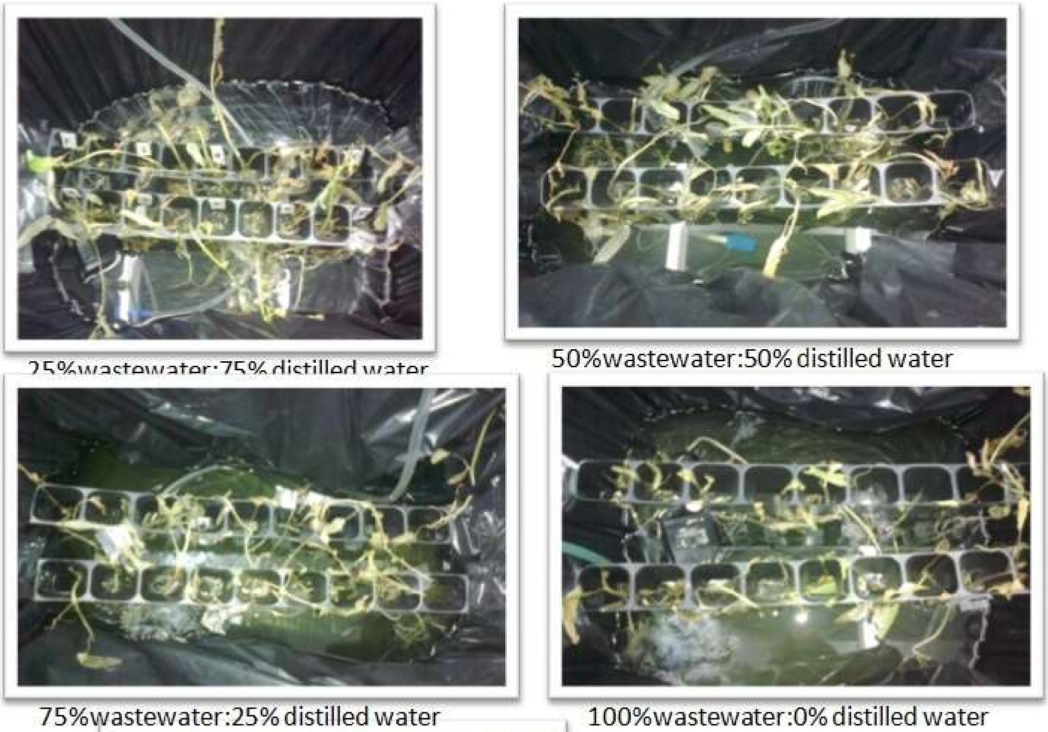
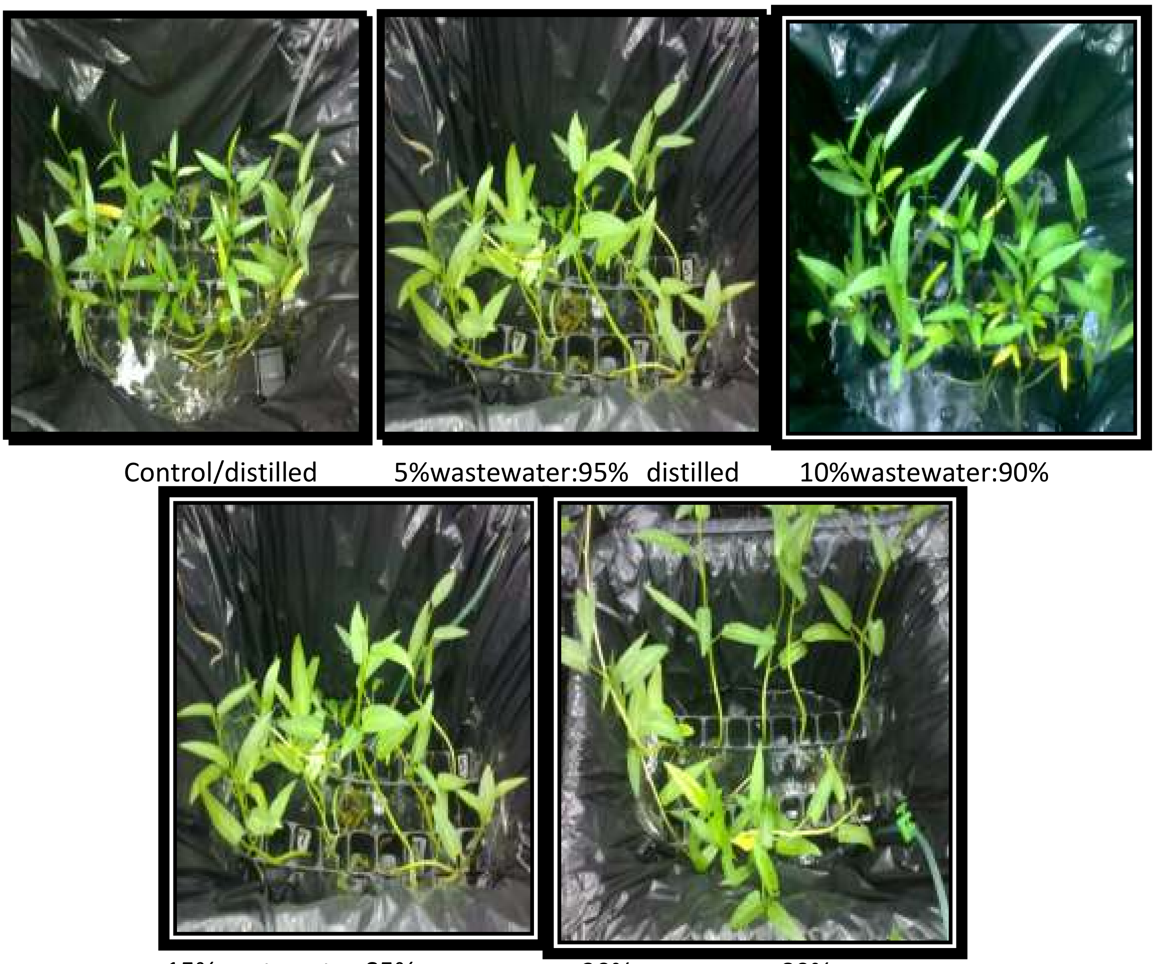


Figure 1. *Ipomoea aquatica* in different concentrations of wastewater during the first trial . All plants in wastewater were plasmolyzed while those in distilled water are normal

Noticeably, the amount of chromium, copper and lead in different concentrations of influent are higher than the maximum allowable limit and is too high for the *Ipomoea aquatica* plants to survive. According to Chojnackaet al.(2004), micronutrient elements such as chromium, copper and lead may be toxic to plants at high concentration. Actually, vegetation has its maximum level of heavy metal accumulation safe for human consumption. The amount of or concentration of heavy metals present in the effluent waste water are 0.02 mg/li for chromium, 0.24 mg/li for copper, 0.03 mg/li for lead as per the Atomic Absorption Spectroscopy (AAS) provided.

In the final set up, the concentrations of the heavy metals present in the effluent was more tolerable for *Ipomoea aquatic* (figure 2). Plants survived in different concentrations for twenty one days of observation. Table 2 shows the concentration of the different heavy metals present in the wastewater. Plants were able to tolerate the 0.02mg/li concentration of chromium but cannot tolerate the 0.05mg/li concentration.



Control/distilled 5%wastewater:95% distilled 10%wastewater:90%

15%wastewater:85% 20%wastewater:80%

Fig. 2. *Ipomoea aquatica* in diluted wastewater. Concentrations are at 5%, 10%, 15%

Wastewater when released into the bodies of water is diluted. Effluents on the canals, rivers and water course caused serious environmental pollution. They pollute natural water systems as well as ground water (Kashem&Singh 1998). The effluents contain different level of heavy metals such as chromium, copper and lead and Zn (Larsen et al. 1975; Arora et al. 1985). According to the AAS (Atomic Absorption Spectroscopy) results provided by Qualibet Testing Services Corporation the amount accumulated by experimental plants in different treatments are less than the maximum allowable limit of *Ipomoea aquatic* (table 2).

Table 2. **Different percent concentration of heavy metals present in diluted effluent wastewater (mg/L) used in the final set-up**

	Concentrations of wastewater			
	5%	**10%**	**15%**	**20%**
Chromium(0.02 mg/L)	0.001 mg/L	0.002 mg/L	0.003 mg/L	0.02 mg/L
Copper (0.24 mg/L)	0.012 mg/L	0.024 mg/L	0.036 mg/L	0.24 mg/L
Lead (0.03mg/L)	0.0015 mg/L	0.003 mg/L	0.006 mg/L	0.03mg/L

In treatment with 5% wastewater and 95% distilled water the total percentage of copper accumulated was only 0.0008 mg/L, treatment 2, 10% wastewater and 90% distilled water were only 0.0009 mg/L, in treatment 3, 15% wastewater and 85% distilled water were 0.0008 mg/L and in treatment 4, 20% wastewater and 80% distilled water were only 0.0012 mg/L. It is undoubtedly shown that there are no reasons for the experimental plants to be plasmolyzed compared to the *Ipomoea aquatica* plant samples used in the preliminary set-up using influent wastewater concentrations.

Table 3. presence of chromium, copper and lead on the plant tissues before and after the experimental set-up

PARAMETERS	PRE-TREATMENT (mg/Liter)	FINALTREATMENT (mg/Liter)				
	INITIAL	CONTROL	5:95%	10:90%	15:85%	20:80%
Chromium	NO DETECTION	NO DETECTION	NO DETECTION	NO DETECTION	NO DETECTION	NO DETECTION
Copper	0.0002 mg/L	0.0001 mg/L	0.0008 mg/L	0.0009 mg/L	0.0008 mg/L	0.0012 mg/L
Lead	NO DETECTION	NO DETECTION	NO DETECTION	NO DETECTION	NO DETECTION	NO DETECTION

Evidently, there are no substantial uptake of chromium and lead. Majeti and Helena (2003) reported that there are some plant species that can hyper accumulate a particular metal. While some others can hyperaccumulate more than one metal. Plants that hyper-accumulate metals have tremendous potential for application in remediation of metals in the environment. The total amount of copper was not accumulated by *Ipomoea aquatica*, it is in contrast to the studies and findings of Baldantonietal.(2005), where high accumulation of heavy metals registered in roots of*Ipomeaaquatica*was observed.Thus, experimental plants can tolerate the chromium and lead present in the effluent wastewater and can accumulate copper in different concentrations. Heavy metal tolerance is a relatively rare trait found only in a few highly adapted plant species. These plants are capable of growing especially at sites with normally or artificially elevated levels of heavy metals.

The final results of plant tissue analysis revealed that there is no presence of heavy metals such as chromium and lead in the plants cultured in distilled water. Therefore, *Ipomoea aquatica* has a substantial uptake of copper while did not accumulate chromium and lead in different concentrations after 21 days. The results of the analysis provided by Qualibet Testing Services Corporation shows that *Ipomoea aquatica* does not accumulate chromium and lead but tolerates the amount of heavy metals present in the wastewater of Arms Corporation in Marikina City, It is contrary to the studies ofGoswami et al.(2005) and Cheng(2002) that *Ipomoea aquatica*has an ability to remove Cr (VI) from the contaminated water by transforming Cr (VI) to Cr (III) and can take up lead by their roots, or even by stems and leaves, and accumulate them in organs.

The result of AAS shows that in 5%wastewater *Ipomoea aquatica* uptakes 0.0008 mg/L of copper, it is less than in treatment with 10% wastewater which absorbed 0.0009 mg/L while *Ipomoea aquatica* in treatment with 15% wastewater showed that only 0.0008 mg/L was obtained. This is may be due to the ability of these plants to detoxify the heavy metal ions by degradation or sequestration before they cause damage (Gregory & Bradshaw 1995). Experimental plants in 20% wastewater have the highest percentage of copper accumulated which was 0.0012 mg/L. It shows that experimental plants absorbed copper and did not uptake chromium and lead but tolerates the amount present from the wastewater. The uptake of copper may be due by the predominant accumulation in the roots, and then some portions are transported to other parts of the plant. Generally, the contents of heavy metals in underground parts are higher than that found in those parts above the ground (Liao 1993) and follows a pattern, root>leaf>shoot (stem). *Ipomoea aquatica* shows that as the concentration is increased the amount of copper uptake is also increased and translocated copper from roots to shoot at high rates (Lombiet al.2000) and in such instances the metal ions get accumulated preferentially in the shoots.

Tolerance for the presence of heavy metals was indicated in the growth rate of the roots, growth rate of the shoot (height) and in the number of nodes. In figure 3, plants in 10% wastewater exhibited the highest growth rate, however, there are no significant difference on the rate of root growth for the different treatments and the control during the 21 experimental days. This only revealed that heavy metals specifically chromium, copper and lead do not affect the growth of the roots and can tolerate the heavy metals present in the wastewater being used. The growth rate of *Ipomoea aquatic* increases with the increasing concentration of the wastewater as reflected in figure 4. It can be observed that the control have the lowest growth rate while the 20% wastewater treatment exhibited the highest growth rate. However, just like in the growth rate of roots, there is no significant difference among the growth rate of shoots for all the treatments and control.

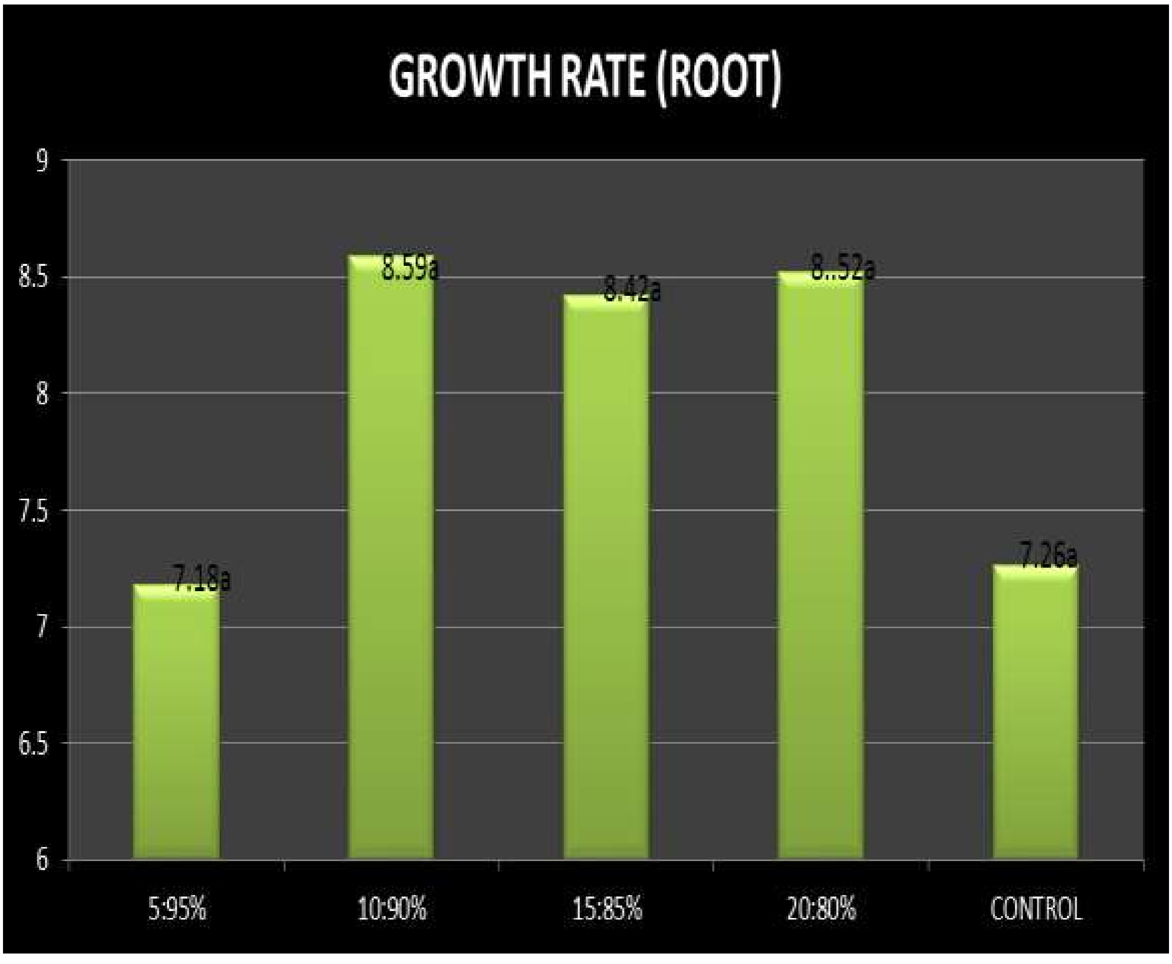


Figure 3: Growth rate of roots in different concentrations of wastewater 21 days after the experiment

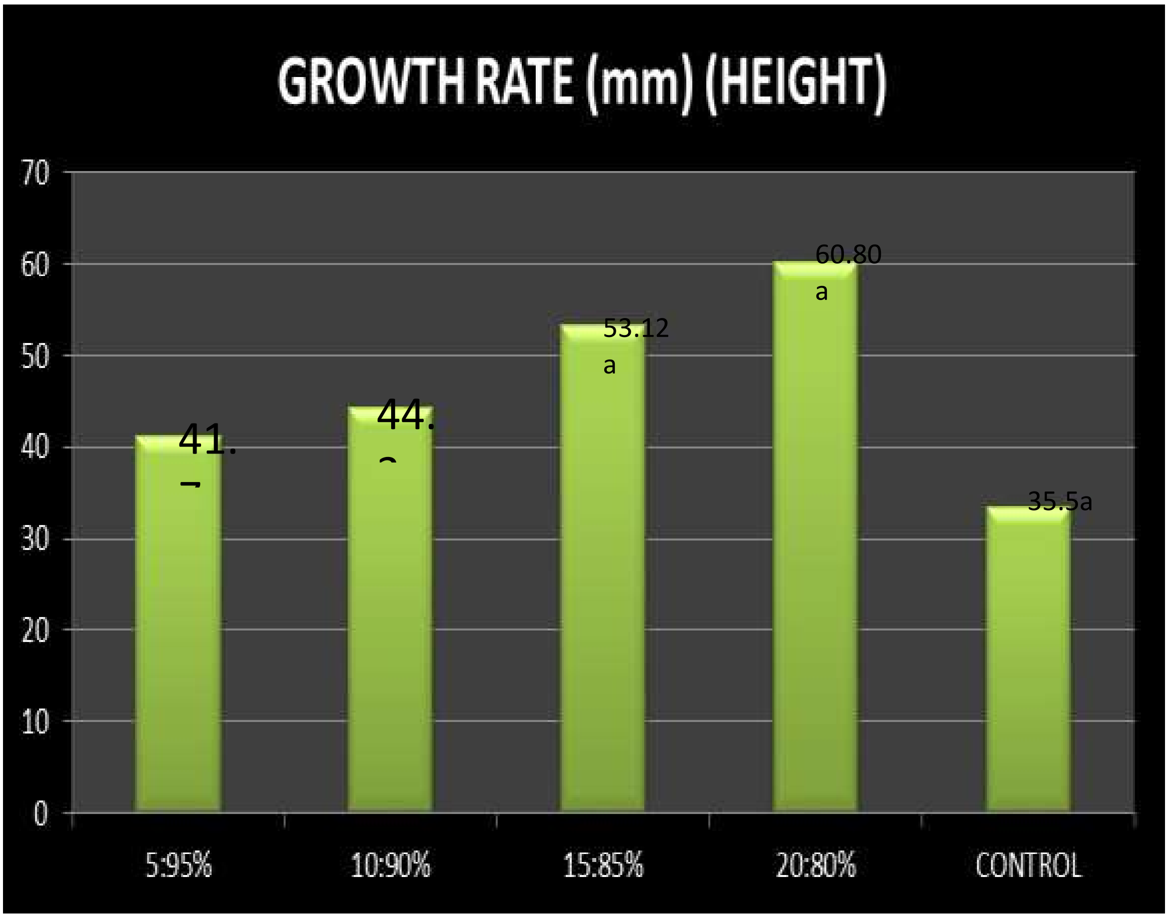


Figure 4: Growth rate (height) in different concentrations of wastewater 21 days after the experiment

The change in the number of nodes contrary to the growth rate of shoot is decreasing with increasing wastewater concentration as shown in figure 5. No significant differences in the number of nodes observed among treatments. This means that the change in number of nodes is not affected by the concentration of heavy metals in the wastewater.

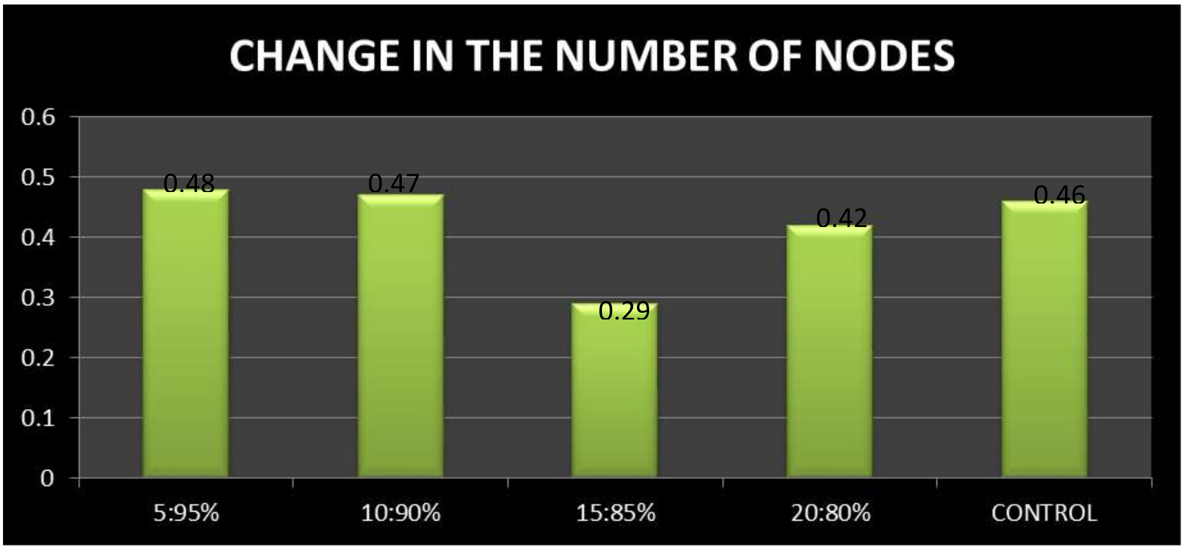


Figure 5: Change in the number of nodes in different concentrations of wastewater 21 days after the experiment.

4 CONCLUSION

The study showed that the *Ipomoea aquatica* has the ability to absorb and accumulate chromium and lead. Growth rate, root growth and number of nodes indicate that *Ipomoea aquatica* can tolerate the amount of heavy metals present in the different concentrations of wastewater. The Atomic Absorption Spectroscopy (AAS) showed that the total amount of copper accumulated within 21 days is lower than the total concentration present in the wastewater. The AAS analysis also showed that the concentration of copper present *in Ipomoea aquatica* is 0.0002mg/li. The concentration of copper in the plant tissue increased with increasing concentration of wastewater. The treatment with 5% wastewater has a concentration of 0.0008 mg/L copper while in 10% wastewater was 0.0009mg/L, treatment with 15% wastewater was 0.0008mg/L and 0.0012mg/L in treatment with 20% wastewater. Furthermore, there were no amount of chromium and lead detected from the plant tissues.

5 RECOMMENDATIONS

*Ipomoea aquatica*was used for the absorption of heavy metals such as chromium, copper and lead present in the wastewater of Marikina River. It is recommended to test for other aquatic macrophytes which is fibrous, hardy, thicker and more tolerable to different heavy metals present in wastewater specifically chromium, copper and lead. On the other hand, an improved set-up for the analysis of plant was also advisable in order to accumulate considerable amount of heavy metal. The plant tissue analysis in each concentration is recommended to be separated in testing to determine the amount of accumulation of heavy metals present in *Ipomoea aquatica.*

It is also suggested to use other wastewaters that are mainly connected to the main-gateway of rivers in the country. Considerably, there is a need to conduct a chemical analysis for the plant prior to the experiment and a wastewater analysis before and after the study to determine if there is really an uptake of the heavy metals present in the wastewater that will be used in the study.

REFERENCES

Denny, H & Wilkins, D (1987), 'Zinc tolerance in Betula spp. II. Micro analytical studies of zincuptake into root tissues', *New Phytol.* Vol. 106, pp. 525–534.

Dushenkov, V, Kumar, P, Motto, H &Raskin, I (1995), 'Rhizofiltration: the use of plants to remove heavy metals from aqueous streams', *Environ. Sci. Tech.*, vol. 29 (5), pp. 1239–1245.

European Commission. (2004) Stimulating technologies for sustainable development: An environmental technologies action plan for the European Union. Communication from the Commission to the Council and the European Parliament, Brussels, Belgium.

Erakhrumen, AA (2007), 'Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries', *Educational Research and Review*, vol. 2 (7), pp. 151-156.

Hasegawa, H (2011), *Aquatic arsenic: Phytoremediation using floating macrophytes.*

Bhatt, I, Nirmal Kumar, JI, Soni, H & Kumar, RN (2008), *Hyperaccumulation and mobility of heavy metals in vegetable crops in India*.

Kumar, S, Meshram, J, Fan, M, Singh, SK &Juwarkar, AA (2007), 'Effect of amendment on phytoextraction of arsenic by VetiveriaZizanioides from soil', *Int. J. Environ. Sci. Tech.*, vol. 4 (3), pp. 339-344.

Lee, CW, Jackson, MB, Duysen, ME, Freeman, TP & Self, JR (1996), 'Induced micro-nutrient toxicity in 'Towndown' Kentucky bluegrass', *Crop Science.,* vol. 36, pp. 705-712.

Luo, Y &Rimmer, DL (1995), 'Zinc-copper interaction affecting plant growth on metal contaminated soil', *Environ Pollution* vol. 88, pp. 79-83, Meagher, 2000.

Miretzky, P, Saralegui, A & Fernandez Cirelli, A (2004), 'Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina)', *Chemosphere*, vol. 57 (8), pp. 997-1005

Mudgal, V, Madaan, N &Mudgal, A (2010), 'Heavy metals in plants: phytoremediation: Plants used to remediate heavy metal pollution', *Agric. Biol. J. North Am.*, vol. 1 (1), pp. 40-46.

Negri, MC &Hinchman, RR (1996), 'Plants that remove contaminants from the environment Lab', *Medicine, vol.* 27, pp. 63-40.

Pip, E &Stepaniuk, J (1992), 'Cadmium, copper and lead in sediments and aquatic macrophytes in the Lower Nelson River System., Manitoba, Canada, Inter-specific differences and macrophyte – sediment relations', *Arch. fur Hydrobiol.*, vol. 124, pp. 337-355.

Pison, I &Menut, L (2004), 'Quantification of the impact of aircraft traffic emissions on tropospheric ozone over Paris area', *Atmospheric Environment*, vol. 38, pp. 971-983.

Pivertz, EB (2001). Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites. Environmental Research Services Corporation. EPA/540/S-01/500.

Prasad, MNV (2004), 'Phytoremediation of metals in the environment for sustainable development', *Proc. Indian natn. Sci. Acad.*, vol. B70 (1), pp. 71-98.

Rahman, MA, Kadohashi, K, Maki, T & Hasegawa, H (2011), *Transport of DMAA and MMAA into rice (Oryza sativa L.) roots.* [Corrected Manuscript] Environ. Exp. Bot., In Press.

Raskin, I, Kumar, PBAN, Dushenkov, V & Salt, DE (1994), 'Bioconcentration of heavy metals by plants', *Curr. Op. Biol.*, vol. 5, pp. 285-290.

Salt, DE, Blaylock, M, Kumar, PBAN, Dushenkov, V, Ensley, BD, Chet, I &Raskin I (1995), 'Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants', *Biotechnology,* vol. 13, pp. 468-474.

Salt, DE, Kumar, PBAN, Dushenkov, S &Raskin, I (1994), 'Phytoremediation: A New Technology for the Environmental Cleanup of Toxic Metals', International Symposium Research on Conservation and Environmental Technology for Metallic Industry. Toronto, Canada.

Singh, D, Gupta, R & Tiwari, A (2011), 'Phytoremediation of Lead from Wastewater Using Aquatic Plants', *Int. J. Biomed. Res.*, vol. 7, pp. 411-421.

Singh, DB, Prasad, G &Rupainwar, DC (1996), 'Adsorption technique for the treatment of As (V) rich effluents', *Colloids Surf*, vol. 111, pp. 49-56.