The effects of citric acid and varying concentrations of copper and iron on the phytoextraction of copper from aqueous solution by vetiver grass

Sorıano M. M. U.[1], Cenia M. C. B.[1], Mendoza H. D.[2,*] And Puzon J. J. M.[3]

Portion of the M.S. thesis of the first author at the Environmental Engineering Program, College of Engineering, University of the Philippines Diliman, Quezon City 1101, Philippines

[1]Environmental Engineering Program, College of Engineering, University of the Philippines Diliman, Quezon City 1101, Philippines

[2]Department of Mining, Metallurgical and Materials Engineering, College of Engineering, University of the Philippines Diliman, Quezon City 1101, Philippines

[3]Institute of Biology, College of Science, University of the Philippines Diliman, Quezon City 1101, Philippines

*corresponding author:

e-mail: judge.mendoza@gmail.com

Abstract

The effects of the presence of citric acid as chelating agent and varying concentrations of copper and iron on the phytoextraction of copper from aqueous solution by vetiver grass were investigated in a full factorial design. Vetiver slips were allowed to acclimatize in a half-strength Hoagland's Nutrient Solution for 14 days prior to the 7-day hydroponic study where the plants were exposed to different treatments. Plants were harvested, separated into roots and shoots, and prepared for Atomic Absorption Spectrometry analysis. Three-way analysis of variance was used to determine which factors significantly affect biomass change, total copper uptake, and translocation factor. Vetiver grass grown in solutions with citric acid significantly alleviated copper phytotoxicity symptoms resulting to lower decrease in biomass than those plants grown without the chelating agent. Higher copper concentration in solution led to higher total copper uptake, while higher iron concentration in solution significantly decreased copper translocation factors in vetiver grass. Average copper translocation factors were less than 1, i.e., more copper was accumulated in roots than in shoots.

Keywords: Phytoextraction; Vetiver Grass; Copper; Iron; Citric Acid

1. Introduction

Contaminated wastewater resulting from unmanageable growth of industries usually contains metals that can possibly be bioaccumulated due to their non-biodegradable nature (Kacalkova *et al*., 2009). Water pollution can be treated solely by or combinations of physical, chemical, and biological means. One of the biological wastewater treatment methods that is considered a green technology is phytoremediation. Phytoremediation has many categorical applications including phytoextraction. Phytoextraction uses plants to remove metals and it can be applied in the mineral processing industry to commercially produce metals (Ahmadpour *et al*., 2015). It may also be utilized in the field of phytomining.

Among the metals being removed from contaminated sites is copper (Cu). Some plants are Cu excluders, resisting Cu uptake and translocation from roots to shoots, while others are hyperaccumulators which permit uptake of at least 1,000 mg Cu per kg of dry biomass in its harvestable parts and allows translocation from roots to shoots (Song *et al*., 2003; Brooks *et al*., 1998). *Chysopogon zizanioides* (L.) Roberty commonly known as vetiver grass has been found to exhibit a Cu phytoextraction potential (Roongtanakiat *et al*., 2007). Similar to other non-hyperaccumulator plants, the phytoextraction potential of vetiver grass may be induced by the addition of chelating agents (Chen *et al*., 2012).

Most of the previous studies are done in parametric design and is focused mainly on general phytoremediation application which is to clean the environment. In this study, full factorial experiments dealt with a problem that may arise in phytomining, i. e., complexity of the wastewater to be treated. Presence of interfering metals may have an effect in the phytoextraction of the desired metal. In particular, in most Cu-containing wastewaters, iron (Fe) could also be present. Furthermore, this research aims to investigate the effects of citric acid and varying concentrations of Cu and Fe on the phytoextraction of Cu by vetiver grass in hydroponic medium. Hydroponic medium is more favorable for phytomining experiments than soil medium since soil-bound metals still have to be mobilized into the soil solution in order for the plants to accumulate the metals in its tissues (Ahmadpour *et al*., 2015). Individual and interacting effects of the said factors are presented in this paper.

2. Methodology

2.1 Experimentation

Vetiver slips were supplied by The Vetiver Farms Philippines. The full factorial experiment was done in 2 trials with 4 plants per 2-L solution. The study was conducted in a screenhouse inside the University of the Philippines Task Force on Solid Waste Management (UP TFSWM), also known as Materials Recovery Facility. The slips were acclimatized for 14 days in a half-strength Hoagland's Nutrient Solution (HNS). After weighing, the slips were surface-sterilized with dilute sodium hypochlorite solution and were exposed to varying hydroponic conditions for 7 days. Table 1 shows the 2^3 design followed in the study. Experiments were done at pH 4 where Cu was deemed mostly bioavailable (Peterson, 1982). Solutions were replaced every 2 days. Air temperature inside the screenhouse ranged from 20.3 to 61.1°C, while relative humidity measured from 20 to >90%. Upon harvesting, plants were washed with 5-mmol EDTA solution to remove surface-adhered substances. Plants were divided into root and shoot parts and were weighed after carefully drying with tissue. Plant samples were oven-dried at 80°C for at least 24 hours until constant dry weights were obtained and were dry-ashed in a muffle furnace at 600°C for at least 10 hours using concentrated nitric acid drops as ash aid. Ash samples were acid digested and subjected to Atomic Absorption Spectrometry analysis by the Institute of Chemistry, University of the Philippines Diliman, Quezon City. An MS Excel addin called Realstats were used in the statistical analyses. For each experimental trial, 3 replicates were considered in the three-way analysis of variance (ANOVA). Mean values for each condition were presented in graphs. Low metal concentrations were equivalent to its concentration in a half-strength HNS. High metal concentrations meant 50 ppm Cu and 7.5 ppm Fe. Five mmol citric acid was added to certain systems.

2.2 Theoretical Framework

The change in biomass and the ability of vetiver grass to accumulate Cu at different phytoextraction conditions were quantified using equations 1 to 3 (Chen *et al.*, 2012):

$$Percent\ change\ in\ biomass = \frac{Fresh\ weight\ after\ treatment - Fresh\ weight\ before\ treatment}{Fresh\ weight\ before\ treatment} x100 \qquad eq\ 1$$

$$Total\ copper\ uptake = \frac{Copper\ content\ in\ whole\ plant}{Total\ dry\ plant\ weight} \qquad eq\ 2$$

$$Translocaion\ factor = \frac{Copper\ concentration\ in\ shoots}{Copper\ concentration\ in\ roots} \qquad eq\ 3$$

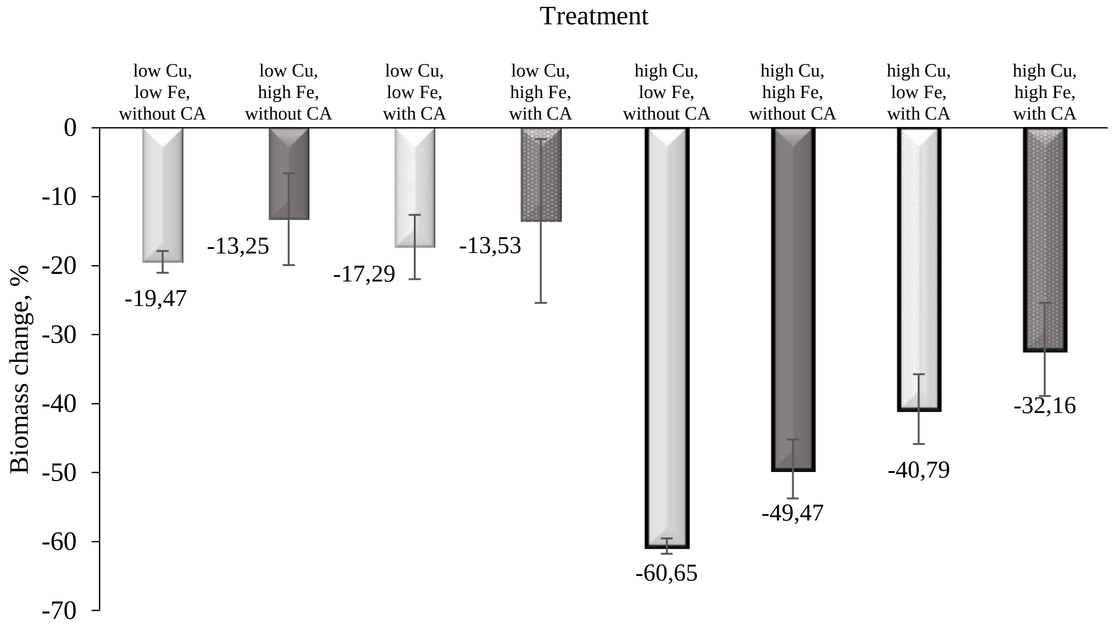

Figure 1. Change in the whole plant biomass of vetiver grass subjected to different phytoextraction conditions

Table 1. Experimental conditions

Treatment	Copper concentration	Iron concentration	Citric acid presence
A	low	low	without
B	low	high	without
C	low	low	with
D	low	high	with
E	high	low	without
F	high	high	without
G	high	low	with
H	high	high	with

A (low Cu, low Fe, w/o CA)

B (low Cu, high Fe, w/o CA)

C (low Cu, low Fe, with CA)

D (low Cu, high Fe, with CA)

E (high Cu, low Fe, w/o CA)

F (high Cu, high Fe, w/o CA)

G (high Cu, low Fe, w/ CA)

H (high Cu, high Fe, with CA)

Figure 2. Representative shoots of vetiver grass after the 7-day hydroponic study on copper phytoextraction at different conditions

3. Results and Discussion

3.1 Plant biomass

Cu is an essential micronutrient for living microorganisms including plants. However, at certain levels, plants suffer from metal intoxication called phytotoxicity (Zaheer *et al*., 2015). Figure 1 shows that vetiver grass manifested phytotoxicity symptoms such as larger decrease in biomass in high Cu-containing solutions than in low Cu-containing solutions. Apparent at high Cu concentration, the addition of citric acid resulted to smaller decrease in plant biomass. Citric acid application might have enhanced antioxidant enzymes activities and reduced oxidative stress (Afshan *et al*., 2015; Shakoor *et al*., 2014). It may be possible that citric acid was able to alleviate Cu-stress in vetiver grass. Generally, as shown in Figure 2, plants grown in solutions with citric acid looked greener and healthier than those grown without the chelating agent. The latter dried up and exhibited necrosis characterized by browning of the leaves. Relatively low-Fe concentration in solution resulted to lower decrease in biomass although not found to be statistically significant. Three-way analysis of variance confirmed that the significant factors affecting change in biomass were Cu concentration, citric acid presence and the interaction of these factors. The physiological variations that occurred in vetiver grass were affected by these factors.

3.2 Total copper uptake

Figure 3 shows that higher Cu concentration in solution resulted to greater uptake of Cu in vetiver grass. This corresponded with the results of the study of Roongtanakiat *et al*. in 2007. ANOVA results indicate that the effect of Cu concentration was highly significant in terms of total Cu uptake. High Cu concentration in solution provided more bioavailable Cu for vetiver grass to accumulate in its tissues. After 7-day exposure to various conditions, the highest total Cu uptake of 438.68 was obtained in Treatment F (high Cu-high Fe-without citric.

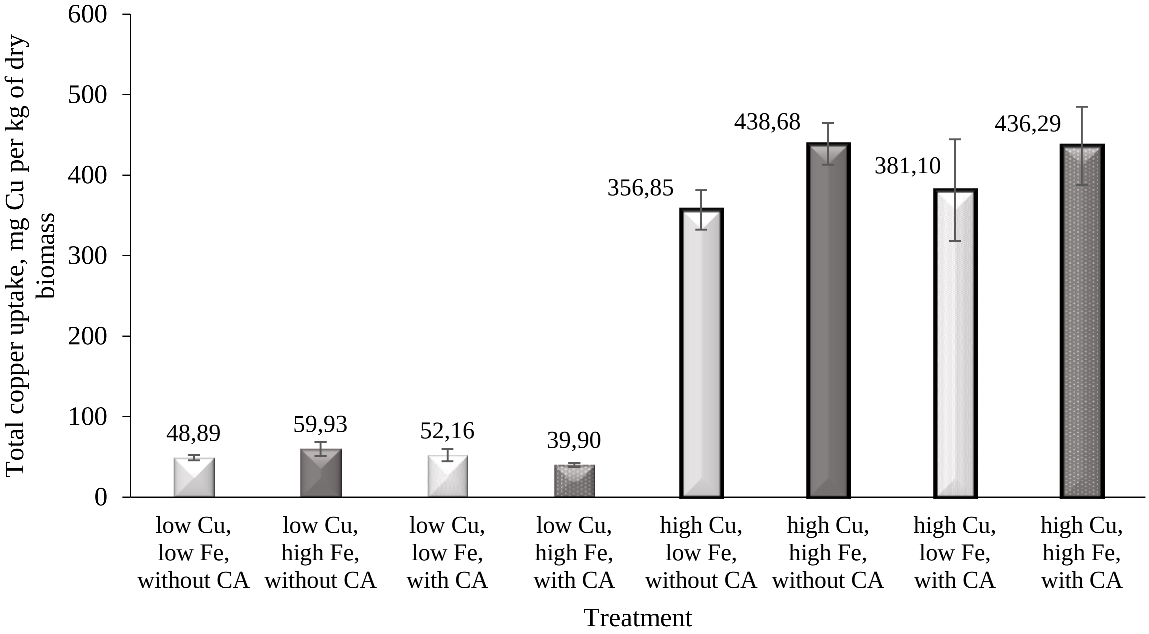

Figure 3. Total copper uptake of vetiver grass subjected to different phytoextraction conditions

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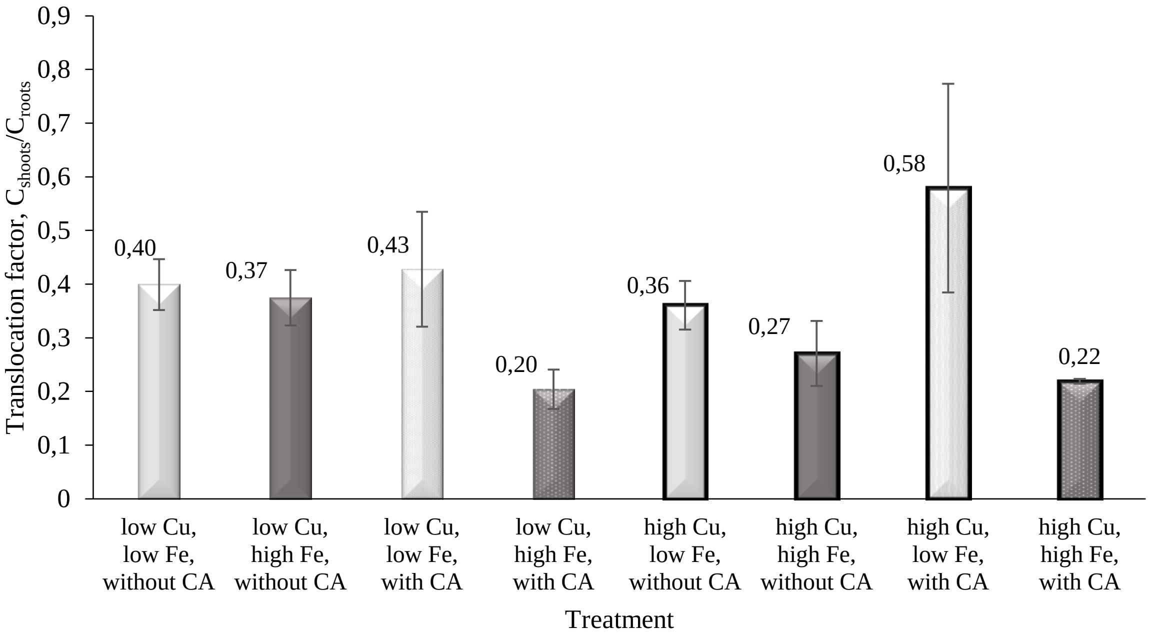

Figure 4. Translocation factor of copper by vetiver grass subjected to different phytoextraction conditions

acid) followed by Treatment G (high Cu-high Fe-with citric acid) with total Cu uptake of 436.29 ppm. Probably, the effect of citric acid on total Cu uptake maybe observed upon an extended duration of phytoextraction. Also, high Cu and high Fe concentrations consistently led to higher total Cu uptake than solutions with high Cu and low Fe concentrations

3.3 Translocation factor

Translocation factor provides information on the ability of vetiver grass to transport Cu from below-ground parts into its aerial parts. In Figure 4, average Cu translocation factors of vetiver grass grown in solutions with higher Fe concentration were slightly smaller than those grown in lower Fe concentration. Based on ANOVA, only Fe concentration was found to be significant. Translocation factors obtained in all of the conditions in this study were less than 1. More Cu was accumulated by plant roots than its shoots. At 7.5 ppm Fe, metal competition occurred and manifested in Cu translocation factor but not in total Cu uptake

4. Conclusions

The addition of citric acid significantly alleviated Cu phytotoxicity symptoms in vetiver grass wherein lower decrease in biomass was obtained. High Cu concentration in solution resulted to a high total Cu uptake. After 7-day phytoextraction from aqueous solution of 50 ppm Cu, vetiver grass already accumulated almost half of the threshold for the plant to be considered a hyperaccumulator. It should be noted that not all effluent discharges and contaminated water bodies may contain same amounts of Cu used in this study. Mean total Cu uptake values of vetiver grass grown in solutions with or without citric acid at 50 ppm Cu were almost equal. Nevertheless, differences in plant appearance and change in biomass were apparent. The authors recommend a longer duration of similar hydroponic study in order to determine whether citric acid can help increase total Cu uptake and translocation factor as well. Less metal competition occurred in conditions with relatively lower Fe concentration. Less interference by Fe led to higher Cu translocation factors. Since Cu accumulation was larger in roots than in shoots, better Cu recovery might be obtained by harvesting the whole plant during actual wastewater treatment application.

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