Copper uptake by *Chrysopogon zizanioides* (L.) Roberty from aqueous solution: Effects of different factors and kinetic study

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Abstract

The high biomass, extensive root network, and fast growth of vetiver grass [*Chrysopogon zizanioides* (L.) Roberty] make it a good candidate for the treatment of heavy metal-polluted soil and water. For this reason, the influence of copper (Cu) concentration, iron (Fe) concentration, and presence of citric acid (CA) as chelating agent on the Cu accumulation in the roots and shoots of vetiver grass were investigated using a 2^3 factorial design. Moreover, Michaelis-Menten equation was utilised to describe the uptake of Cu after 24 hours. Vetiver grass was grown hydroponically using half-strength Hoagland solution adjusted to pH 4 and different conditions for the factorial experiment or varying Cu concentration for the kinetic experiment. Results indicate that the effects of Cu concentration on the Cu uptake of both roots and shoots were highly significant as determined by ANOVA. Meanwhile, there were significant interactions of Cu and Fe concentrations, Cu and presence of CA, and Fe concentrations and presence of CA on root Cu uptake only. For the kinetic study, the Michaelis-Menten equation was found adequate implying a saturable transport system. The calculated Michaelis-Menten constant, K_m, and maximum solute uptake influx, were V_{max}, 24.39 mg L^- and 272.21 mg kg FW^- day^-, respectively.

Keywords: copper, vetiver grass, uptake kinetics, wastewater

1. Introduction

Cu is one of the most valuable metals because of its various industrial applications such as electrical wires, pipes, component of brass and bronze, and constituent of pesticides and fertilizers. Its production involves mining Cu-containing ores, followed by either pyrometallurgical or hydrometallurgical processing. It is one of the Philippine's top mineral exports, amounting to PhP 18.98B in 2015 (Mines and Geosciences Bureau, 2016), as Philippines is ranked fourth globally in Cu production. Regrettably, Cu pollution of water bodies caused by leakage from tailings dam still persists today in some mining areas of the country. Although Cu exists naturally in the environment and is a micronutrient for both plants and animals including humans, excess levels is considered harmful as intake of high amounts can be toxic to living organisms. The damaging effects of elevated Cu in humans include mucosal and gastrointestinal irritation, capillary, kidney, liver and brain damages, and central nervous problems followed by depression. In plants, photosynthesis, pigment synthesis, membrane integrity, and root growth are disturbed (Fernandes and Henriques, 1991). Hence, the abatement of copper pollution is essential. Various physical and chemical methods have been investigated and employed in the remediation of heavy metal contaminated soil and water. High cost and labor requirements are some of the concerns related to the utlization of these approaches. Phytoremediation, considered a relatively recent technology and an active area of research (Ali *et al*., 2013), is seen to be a better alternative due to its cost-effectivity and efficiency. However, like its physical and chemical counterparts, there are issues concerning the application of phytoremediation. For instance, the selection of the plant to be used, the time required to decontaminate a site, the fate of heavy metal-laden biomass and the effects of the use of chemical for induced phytoremediation are some of the important setbacks attributed to phytoremediation. The tolerance of vetiver grass [*Chrysopogon zizanioides* (L.) Roberty] to increased amounts of heavy metals, including Cu, has been proven and documented. This physiological characteristic, along with high biomass, fast growth, extensive root network, and ability to survive in a wide range of conditions (Danh *et al*., 2009), makes vetiver grass one of the top choices in phytoremediation of contaminated soil and water. However, several studies have established that heavy metals mostly accumulate in the roots (Yang *et al*., 2003;Chen *et al*., 2004; Roongtanakiat *et al*., 2007; Aibibu *et al*., 2010). Thus, some researchers investigated the influence of chelating agents on heavy metal translocation to aboveground parts. EDTA, citric acid, and EDDS were some of the chelating agents considered (Chen *et al*., 2012). Chen *et al*. (2012) found that citric acid can increase the total uptake of Cu in vetiver grass in a hydroponic set-up. Citric acid as a chelating agent was deemed promising due to its natural origin, biodegradability and non-toxicity to plants (Ali *et al*., 2013). Aside from use of chelating agents, other factors affect the total metal uptake of plants. Liu *et al*. (2009) reported that increasing Cu levels in soil increased the Cu concentration in both the shoots and roots of elephant grass, vetiver grass and the upland reed. The presence of other metals may also influence Cu uptake by plants. Zinc, Fe and cadmium appear to be antagonistic to Cu uptake, while manganese acts synergistically(Fernandes and Henriques, 1991). The objectives of this paper are: 1) to examine the main and interaction effects of Cu concentration, Fe concentration and addition of citric acid on the Cu uptake by the different parts of vetiver grass, and 2) to study the uptake kinetics using Michaelis-Menten equation. The study aimed towards understanding the Cu accumulation in vetiver grass in an aqueous solution for subsequent recovery of Cu metal.

2. Methodology

2.1 Plant preparation

Vetiver slips were obtained from the Vetiver Farms Philippines. The slips were planted in polyethylene bags filled with soil in a screenhouse located inside the Task Force on Solid Waste Management Facility, University of the Philippines Diliman, Quezon City. The plants were watered regularly and grown for a month. Plants with healthy 3 to 4 week-old leaves were carefully removed from soil, surface-sterilized with dilute sodium hypochlorite, and acclimatized in half-strength Hoagland nutrient solution (Hoagland and Arnon, 1950) for 2 weeks. Fresh weight of the plants were determined using an analytical balance (Shimadzu, ATX 224).

2.2 Factorial experiment

Acclimatized plants were grown in modified half-strength Hoagland nutrient solution (HNS) with pH 4. The modifications were based on a 3-factor, 2-level factorial design and the different experimental conditions are listed in Table 1. Low Cu and low Fe pertain to the metal's amount in a half-strength HNS, while high Cu and high Fe denote 50 mg L^{-1} and 7.5 mg L^{-1}. CA was supplemented for runs C,D,G, and H to achieve concentration of 5 mM. The solutions were continuously aerated and replaced every 2 days. Temperature and relative humidity inside the screenhouse ranged from 20.3-61.1 and 20->90%, respectively. Rectangular, 2.6 L plastic basins with an improvised Styrofoam cover were used to contain the solution and the plants.

2.3 Michaelis-Menten kinetic study

Acclimatized plants were grown in half-strength HNS spiked with 5 ppm, 10 ppm, 25 ppm and 50 ppm Cu for 24 hours. A nutrient solution without additional Cu served as the control and all solutions were adjusted to pH 4. The solutions were also continuously aerated. Temperatures of 24.1-50.0^{o}C and relative humidity of 34->90% were recorded inside the screenhouse. The same type of containment as with the factorial experiment was utilised.

2.4 Sampling and analysis

After the 7-day and 24-hour hydroponic experiments, the plants were harvested, rinsed with EDTA solution followed by deionized water to remove Cu that may have adhered to the surface of the roots, and dried gently with paper towels., The plants were separated into leaves, stems and roots. Fresh weights were measured immediately using an analytical balance. Then the different plant organs were oven-dried (Memmert) at 80^{o}C until constant dry weight was obtained. The dry biomass were then ashed in a muffle furnace (Thermolyne) at 600^{o}C with nitric acid as ash aid. Ash samples were brought to the Institute of Chemistry Analytical Services Laboratory, University of the Philippines Diliman for acid digestion with HCl and Cu analysis using air-acetylene flame AAS.

2.5 Data and statistical analysis

Each treatment is conducted in duplicate with each replicate having 3 samples. Data reported are the combined means ± SEM (n=6). ANOVA was applied to test for the significance of the main and interaction effects of the independent variables using R (R Core Team, 2013). Data in the short-term kinetic study were fitted to Michaelis-Menten equation using Solver in Microsoft Excel.

3. Results and Discussion

3.1 Factorial experiment

The copper uptake by the roots and shoots of vetiver grass exposed to different conditions is shown in Figure 1. It can be seen that higher Cu concentration in the solution elevated the Cu uptake in both the below- and above-ground parts although more remarkably in the roots. ANOVA results in Tables 2 and 3 lend support to this finding with P-values for both below 0.10 indicating the significant effect of Cu concentration. This dependency of Cu uptake in plants on the amount of Cu in nutrient solution or soil is exhibited by other plants, although the pattern of relationship differs among plant species and parts (Kabata-Pendias, 2010). The following interaction effects were identified for the roots: Cu×Fe, Cu×CA and Fe×CA. As for the shoots, no interaction effects were recognized. The interaction effects were examined in this study because real wastewater normally contains different substances that may have an influence on the uptake of Cu. The effect of Fe was determined as it is the most common substance in the earth's crust, hence it is most likely present in acid mine drainages. A significant interaction can conceal the significance of main effects, as a result, when interaction is present, the main effects of the factors involved may not have much meaning (Montgomery, 2011). This can be the reason why the main effects of Fe and CA are not significant

3.2 Michaelis-Menten kinetic study

Fitting of Cu uptake in relation to the Cu concentration in the solution to Michaelis-Menten kinetics can give insight to carrier-mediated transport. The Michaelis-Menten equation is given by:

$$V = \frac{V_{\max} \times C_{solution}}{K_m + C_{solution}} \qquad \text{Eq. 1}$$

where V=solute uptake flux (mg kg $FW^{-1}day^{-1}$), V_{max}=maximum solute uptake influx (mg kg $FW^{-1}day^{-1}$), $C_{solution}$= trace element concentration in soil solution (mg L^{-1}), K_m=root permeability coefficient or the Michaelis-Menten constant (mg L^{-1}), which is the $C_{solution}$ when V=50% of V_{max}. Irrespective of the concentration of the

Table 1. Experimental conditions for the 2^3 full factorial design

Run	Cu concentration	Fe concentration	Presence of CA
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

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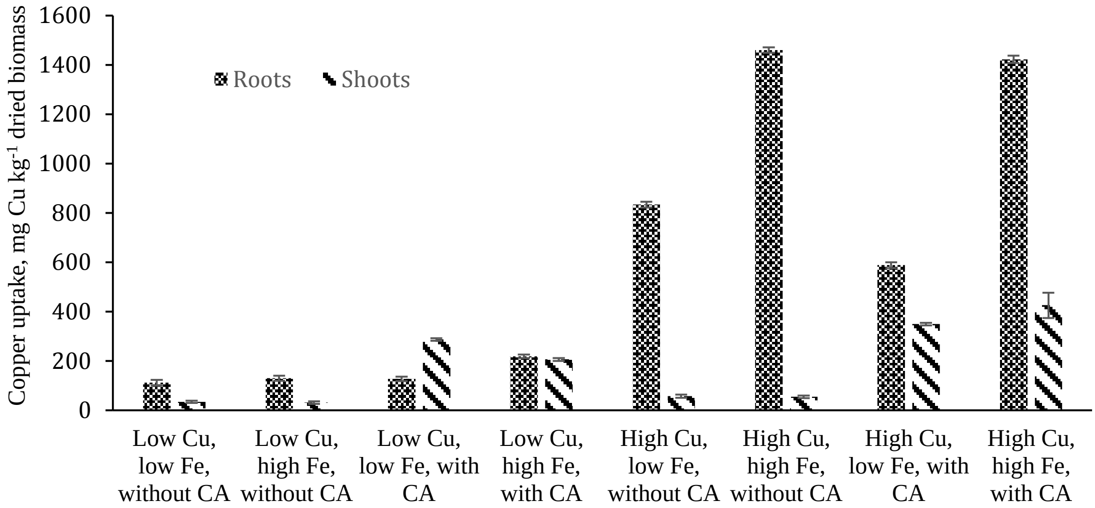

Figure 1. Cu uptake of roots and shoots of vetiver grass [*Chrysopogon zizanioides* (L.) Roberty] exposed to different conditions

solute, V_{max} cannot be exceeded. It is approached when the solute-binding site on the carrier is always occupied. Concentration of the carrier, not of the solute, becomes rate-limiting. K_m describes the affinity of the transport site to the transported substance with lower values of K_m indicating higher affinity for the substance (Taiz and Zeiger, 2010). The computed values for K_m, and maximum reaction rate, V_{max}, values are 24.39 mg L^{-} and 272.21 mg kg FW^{-} day^{-}, respectively. A high value of R^2=0.973 (Fig. 2) indicates a good fit and implies that the uptake of Cu by vetiver grass is facilitated by saturable transport system.

4. Conclusions and Recommendations

Real wastewater contains a variety of contaminants, hence it is important to understand how their interactions affect the uptake of a particular metal by a plant for a successful phytoextraction activity. This research showed that Cu concentration is the most important determinant of the amount of Cu that vetiver grass can uptake for the conditions studied. The 2-way interaction of Cu, Fe and CA significantly impacts the root Cu uptake but not shoot uptake. The V_{max} value obtained may assist in deciding the quantity of vetiver grass to be utilized in practice. A similar factorial study is recommended if the technology will be applied using other plant species, in wastewater with different characteristics, and in recovering other metals. The factors to be investigated may be varied depending on what the researcher may see fit.

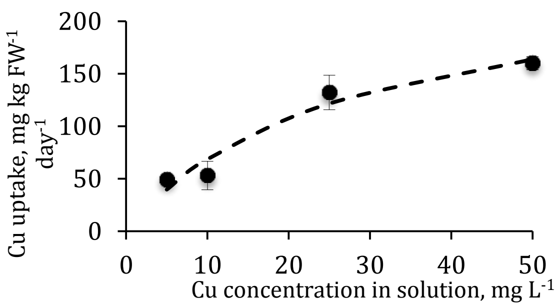

Figure 2. Curve-fitting to Michaelis-Menten of the Cu uptake of vetiver grass [*Chrysopogon zizanioides* (L.) Roberty] in 24 hours

Table 2. Significance test for the main and interaction effects of the different factors on the Cu uptake by roots of vetiver grass [*Chrysopogon zizanioides* (L.) Roberty]

Factors	Coefficients	Standard error	T value	P value
Constant	4.70993	0.09617	48.973	<2 e^{-16}***
Cu	2.01245	0.13601	14.796	9.38 e^{-11}***
Fe	0.14920	0.13601	1.097	0.2889
CA	0.12440	0.13601	0.915	0.3740
Cu×Fe	0.40264	0.19235	2.093	0.0526*
Cu×CA	-0.47941	0.19235	-2.492	0.0240**
Fe×CA	0.38828	0.19235	2.019	0.0606*
Cu×Fe×CA	-0.05725	0.27202	-0.210	0.8360

Note: Significance codes: ***p<0, **p<0.05, *p<0.10

Table 3. Significance test for the main and interactiion effects of the different factors on the Cu uptake by shoots of vetiver grass [*Chrysopogon zizanioides* (L.) Roberty]

Factors	Coefficients	Standard error	T value	P value
Constant	3.74110	0.08215	45.540	<2 e^{-16}***
Cu	1.92838	0.11618	16.599	1.66 e^{-11}***
Fe	0.15449	0.11618	1.330	0.202
CA	0.02973	0.11618	0.256	0.801
Cu×Fe	0.01131	0.16430	0.069	0.946
Cu×CA	0.06755	0.16430	0.411	0.686
Fe×CA	-0.17074	0.16430	-1.039	0.314
Cu×Fe×CA	-0.03161	0.2326	-0.136	0.893

Note: Significance codes: ***p<0, **p<0.05, *p<0.10

Acknowledgement

The authors acknowledge the Office of the University of the Philippines Diliman, Quezon City through the office of the Vice Chancellor for Research and Development (OVCRD), for funding support through the Outright Research Grant, the Engineering Research and Development for Technology (ERDT) of the Department of Science and Technology of the Philippines for additional funding support, The Vetiver Farms for supplying the plant materials and UP Task Force on Solid Waste Management (TFSWM) for logistical support.

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