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Influence of citric acid on the uptake, bioconcentration and translocation factors of copper in *Chrysopogon zizanioides* (L.) Roberty

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Abstract: Phytoremediation is a cost-effective technology in remediating and stabilizing soil, as well as reducing and removing contaminants in surface waters and wastewaters. *Chrysopogon zizanioides* (L.) Roberty (vetiver grass) can tolerate high amount of heavy metals especially in its roots. Hence, the effect of citric acid (CA) as chelating agent on the uptake of copper by vetiver grass was investigated. Vetiver slips were grown in soil for four weeks, harvested and acclimatized for two weeks. The plants were then grown in half-strength Hoagland's nutrient solution with 50 mg L^{-1} copper at pH 4.0 for 3, 7 and 14 days. One treatment contained 5 mmol CA. Uptake, bioconcentration and translocation factors were determined. Bioaccumulation factors for the treatment with and without CA were 10.85 and 28.87, respectively. Translocation factor was not significantly enhanced by the addition of CA. A maximum concentration of 1,443.33 mg Cu kg^{-1} DW was detected in vetiver grown in solution without CA but an insignificant increase in Cu uptake after day 14 was observed. Addition of 5 mmol CA showed no significant difference in Cu uptake. The added CA concentration was not enough to have a significant change in the uptake, bioconcentration and translocation of copper in vetiver.

Keywords: phytoremediation, vetiver grass, copper uptake, bioconcentration, translocation

1. Introduction

Phytoremediation, besides being an economical, energy-efficient and environmental friendly method, has many applications and is very useful in treating a wide variety of contaminants, i.e., metals, radionuclide and organic substances; as well as growth media, i.e., soil, sludge, sediment and water (Cunningham *et al*., 1995). This technology uses plants to extract, immobilize, contain and/or degrade contaminants (Gerhardt *et al*., 2017). Vetiver grass, just like many other high-biomass plants, has been used in phytoremediation studies (Chen *et al*., 2004). The use of this plant became one of the leading biological systems of soil and water conservation, land rehabilitation, and stabilization in the past decades (Grimshaw, 1997; Truong, 2000; Chen et. al, 2004) because of its unique morphological, physiological and ecological characteristics such as its massive and deep root system, and its tolerance to a wide range of adverse climatic and edaphic conditions, including elevated levels of heavy metals (Pinthong et. al, 1998; Truong, 2000; Chen et. al, 2000; Chen et. al, 2004).

The success of phytoremediation lies on the ability to cultivate high biomass plants with a high content of toxic metals in a contaminated growth media (Evangelou *et al*., 2007). To enhance the metal accumulation capacities, uptake speed, and translocation from root to shoot of vetiver grass, the addition of chelating agents (Evangelou *et al*., 2007; Saifullah *et al*., 2009) has been widely used. Ethylene diaminetetraacetic acid (EDTA), ethylene diaminedisuceinate (EDDS), nitrontriaacetic acid (NTA) and other persistent and biological amino polycarboxylic acids (APCAS) have been used in the last decade (Evangelou *et al*., 2007). Although these synthetic chelators have shown positive effects on the improvement of phytoextraction, their use has disadvantages (Evangelou *et al*., 2006). EDTA, a non-selective metal extractor, has poor biodegradability and decreases plant growth (Barona et. al, 2005; Wasay et. al, 1998; Chen and Cutright, 2001; Evangelou *et al*., 2006). An alternative to synthetic chelators is natural low molecular weight organic acids (NLMWOA).

NLMWOA, such as citric acid, oxalic acid, or malic acid, may influence the solubility of essential and toxic ions both indirectly and directly; indirectly, through their effects on microbial activity, rhizosphere physical properties and root growth dynamics and, directly through acidification, chelation, precipitation and oxidificationreduction reactions in the rhizosphere (Uren &Reisenaeur, 1988; Marschner *et al*., 1995; Evangelou *et al*., 2007). These compounds play a significant role, because of their complexing properties, in heavy metal solubility (Mench and Martin, 1991; Krishnamurti *et al*., 1998; Nigam *et al*., 2001; Evangelou *et al*., 2007) and mobilization of mineral nutrients (Zhang *et al*., 1989; Jones *et al*., 1996; Evangelou *et al*., 2007). In addition, NLMWOA have also the capability to detoxify intracellular heavy metals via binding, as suggested by early studies (Lee *et al*., 1977;Evangelou *et al*., 2007).

In this study, the influence of citric acid to enhance the hydroponic phytoremediation process was investigated by assessing vetiver grass (*Chrysopogon zizanioides* (L.) Roberty). Bioconcentration and the efficiency of root-to-shoot translocation were also evaluated.

2. Materials and methods

2.1. Experimental design

Vetiver grass used in the experiment was supplied by The Vetiver Farms Philippines. Hydroponic studies were done in a screenhouse inside UP Task Force on Solid Waste Management. Fourteen (14) days of acclimatization in a half-strength Hoagland's Nutrient

Solution was conducted prior to the actual hydroponic studies in quadruplicates. After acclimatization, plants were weighed, surface-sterilized with dilute sodium hypochlorite solution and were exposed to different conditions. Six different experimental setups (3, 7, 14 days of exposure with or without citric acid) were treated. All the setups contain 50 ppm Cu and were done at pH 4. Plants were collected thereafter for analysis preparation which includes separation of roots and shoots, weighing, washing with dilute-EDTA solution, drying in an oven at 80°C until constant dry weights were obtained and dry-ashing in a muffle furnace at 600°C for at least 10 hours. Ash samples were sent to University of the Philippines, Diliman, Quezon City, Institute of Chemistry for Atomic Absorption Spectroscopy (AAS) analysis.

2.2. Evaluation of phytoremediation efficiency

Two important parameters, translocation factor (TF) and bioconcentration factor (BF), were used to evaluate the phytoremediation efficiency of vetiver. The quantitative transfer of available metals from the solution to plant parts is defined as bioconcentration factor (BF). The plants ability to translocate them form roots to shoot is determined through translocation factor (TF) (Gautam and Agrawal, 2017). The two parameters were calculated by the following equations:

$$\text{Translocation factor (TF)} = \frac{[Cu]_{shoot}}{[Cu]_{root}}$$

$$\text{Bioconcentration factor (BF)} = \frac{[Cu]_{plant}}{[Cu]_{hydroponic\_medium}}$$

Data were analyzed using one-way ANOVA to determine if there was significant difference between phytoremediation efficiency with and without the addition of chelators at 0.05 significance level.

3. Results and discussions

3.1 Effect of citric acid on Cu uptake

Copper accumulation in vetiver plants was not increased significantly even in the presence of citric acid. Figure 1 shows the Cu uptake of vetiver grass for days 3, 7 and 14 from solutions with and without CA.A maximum of 1,443.33 mg Cu kg^{-1} dry weight of the vetiver in the absence of CA compared to 542.49 mg Cu kg^{-1} dry weight with the addition of CA was observed. The Cu uptake by plants is strongly dependent upon the concentration of metal-chelate complex (Vigliotta *et al*., 2016). The chelators help in metal solubility and stabilization to prevent that metal from reacting to other ions present in the solution, thus making it possible to pass through the membrane of the roots (H. Ali *et al*., 2013). The citric acid (CA) helped the Cu metal to be more soluble by forming Cu-CA complex and stabilizing the metal, thus increasing the uptake by vetiver. However, due to a low concentration and the biodegradability of the organic acids, an insignificant change was observed in the mg Cu uptake per kg dry weight of biomass.

3.2 Effect of citric acid on Cu translocation to shoot tissues

The Cu concentration in the shoots per Cu concentration in the roots was determined and the effect of the translocation factor of the Cu added with citric acid and Cu alone was assessed. Figure 2 shows the Cu translocation from roots to shoot tissues after 3, 7 and 14 days of exposure with 5 mmol and 0 mmol CA. These results suggested that insignificant change was observed even after the addition of the CA. Based on Figure 3, at 14^{th} day of exposure to Cu stress only (0 mmol of CA added), Cu metal was translocated more in the shoots with a TF value of 1.15 which is higher than one and can be classified as hyperaccumulator. Accumulation of metal in roots is greater than in shoot because of positive charge on metals, enabling their adsorption to negative charged sites of root cell walls (Gautam and Agrawal, 2017). However, in the presence of CA, 0.32 TF value was measured, that is < 1. This indicates that vetiver prefers to accumulate heavy metal in the roots more than in the shoots. Plants that translocate low metal concentration to their aerial parts and restrict metals in their roots are called metal excluders (Ghosh and Singh, 2005; Aksorn and Chitsomboon, 2013). Shoot concentrations were not influenced by treatments, indicating that the translocation of the metal from roots to shoots is very limited.

3.3. Effect of citric acid on bioconcentration The ability of a plant to accumulate heavy metals is evaluated by bioconcentration factor (BF) (Zayed etal., 1998; Liu et al., 2009). According to Agunbiade et al. (2009), the enrichment factor, also known as BF, > 1, is one of the features of an hyperaccumulator wherein the accumulation of heavy metals is greater in the shoot (Zhang et al., 2014).Figure 3 clearly shows that all the BFs are more than 1. However, the Cu concentration in the whole plant did not significantly increase when 5 mmol CA was added although the highest Cu concentration in the whole plant per Cu concentration in the hydroponic medium was recorded after 14 days, i.e., 28.87 (without CA) compared to 10.85 (with 5 mmol CA). The observed BFs were > 1which indicates that vetiver, even without addition of citric acid as chelators, is a good candidate for phytoremediation as supported in by Ali *et al*.(2013). Vetiver plant was proven to have high tolerance and adaptability to heavy metal stresses. Vetiver has a very high threshold levels compared to other vascular plants whose threshold levels of metals are low, thus showing their sensitivity towards various metals (Gautam and Agrawal, 2017).

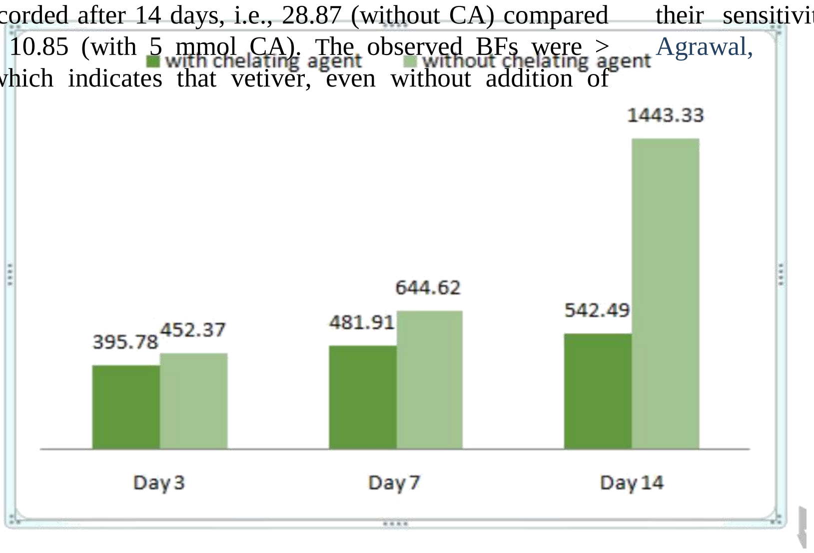

Figure 1. Cu uptake on *Chrysopogon zizanioides* (L.) Roberty after day 3, day 7, and day 14 of exposure in hydroponic solutions with or without citric acid.

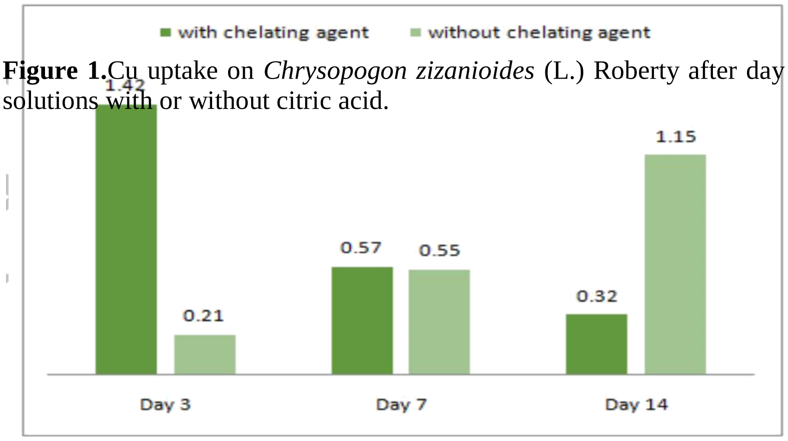

Figure 2. Cu translocation to shoot tissues in *Chrysopogon zizanioides* (L.) Roberty after day 3, day 7, and day 14 of exposure in hydroponic solutions with or without citric acid.

Figure 3. Bioconcentration of *Chrysopogon zizanioides* (L.) Roberty after day 3, day 7, and day 14 of exposure in hydroponic solutions with or without citric acid

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4. Conclusion

Citric acid was used as a chelating agent to enhance the phytoremediation efficiency of the vetiver plant. However, based on the measured results, insignificant effects were observed on biomass reduction, Cu uptake, bioconcentration and translocation from roots to shoots as compared to the control. There was no observed severe change in plant biomass, as well as no significant increase in Cu uptake after addition of citric acid. The highest Cu uptake (1,443.33 mg Cu kg^{-1} dry weight biomass) was recorded without the addition of CA.

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