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Copper Uptake and Translocation of *Terminalia catappa* Linnaeus (Talisay) in EDTA Amended Soil

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Abstract **– This study evaluated the phytoextraction capability of *T. catappa* in soils that are artificially contaminated with 250, 1250, and 2500 mg·kg^{-1} of copper. Also, this study assessedthe accumulation and the translocation of copper by*T. catappa*if chelating agent EDTA is added. Results of the studyshowed a significant increase of copper accumulation with the addition of EDTA at 250 and 1250 mg·kg^{-1} of copper contaminated soils (*p<0.05*). Whilst translocation of copper was observed in all treatments, translocation of copper is not significantly enhanced by the addition of EDTA (*p*>0.05). This mechanism is not positively affected with the presence of EDTA. Furthermore,this study suggests that the *T. catappa* is not a hyperaccumulator of copper, and there is no relationship observed between the length of the plant and the copper uptake in all treatments.**

Keywords **– Hyperaccumulator, Phytoextraction, Phytoremediation, *Terminalia catappa,* Chelating Agent EDTA.**

I. Introduction

Philippines is one of the richest countries in terms of natural resources and precious metals particularly in Palawan and Mindanao. Increasing demand for advances in lifestyle and technology propelled the extraction of metals from the ground such as copper, gold, silver and zinc[15]. As a result, adverse side effects of mining are the formation and accumulation of some metal ions that are deposited in the soil and in the water system within the mining vicinity. These metals are considered to be essential to some animals and plants as they are used in some of its metabolic activity, yet elevated concentration of these metals is hazardous to both plants and animals [26]-[27]. Average concentration of copper in the soil is 100 -1500 ppm,concentrations higher than 2500 ppm is detrimental to the plants and other biotic organisms [4]. Scientists have turned their attention in using plants as a means of removing or stabilizing metal-contaminated soil, through phytoremediation, since removal of the metal ions deposited in soil and water systems is very expensive and often times less efficient [21]. A number of researches have already been made for *T. catappa*, however most of the studies were associated to medical research, which include antitumor properties [20], antioxidant properties [20] and the use of the plant in curing of wounds [8]. A good number of studies in *T. catappa*are related to biofuels [16], and as an indicator for acid-base reactions [12]. Some studies used the leaves to the plant in the biosorption of metal ions in polluted areas [24], [14], [22], [13] and some studies used the fruit in the biosorption of metal ions [15]. Phytoextraction of metal ions such as lead and mercury in polluted soils were also published [21], [26], however, this study is important since there is no apparent study on the phytoextraction of copper in soils heavily contaminated with copper and also studies using chelating agents as an enhancer in phytoextraction.

II. Materials and Methods

A. Seed and Plant Preparation

More than 350 seeds of *T. catappa* were gathered from the gazebo area of Ateneo de Davao University Jacinto campus. The seeds were planted in individual plastic containers and were kept inside the improvised greenhouse. The planted samples were then watered with distilled water of about 50 milliliters twice daily. More than 40 seedlings of good stature were produced after 2months. The seedlings were allowed to grow for another 7 days before the metal ion treatment commenced. The seedlings were properly distributed to the different treatments to ensure that all ages of the plant samples were properly represented.

B. Soil Preparation and Seedling transplantation

A total of 7 treatments including the control were used in this study. There were four individuals of *T. catappa* used for each treatment [25]. The 7 treatments were (a) the untreated soil-C, (b) soil which contains 250 mg·kg^{-1} copper– 250N, (c) soil which contains 1250 mg·kg^{-1}– 1250N, (d) soil which contains 2500 mg·kg^{-1} copper– 2500N, (e) soil which contains of 250 mg·kg^{-1} copper with 250 mL of 2.50 mmol·L^{-1} EDTA -250E, (f) soil which contains 1250 mg·kg^{-1} copper with 250 ml of 2.5 mmol·L^{-1} – 1250E, and (g) soil which contains 2500 mg·kg^{-1} copper with 250 ml of 2.5 mmol·L^{-1} EDTA - 2500E [23].

T. catappa samples was then transferred by removing it from the plastic container (sec.A) and placed to another plastic garden pot with 1.25 kg of the treated soil. It was then watered with 50 mL deionized water. For the EDTA treated test groups, the EDTA was added 4 weeks after transplant. A total of 1 L of 2.5mmol·L^{-1} EDTA solution was added in the last 4 weeks to which 250 mL of the solution was added per week. The addition of EDTA solution was 50 mL per day for 5 consecutive days. On the 6th and 7th day of the week, the EDTA amended soils were watered with distilled water similar to the test groups without EDTA. The whole experiment lasted for 8 weeks [2], [23]

C. Initial Soil Analysis

The initial condition of the soil was established prior to the experimental phase. The macronutrients (Ca, Mg, Na and K) were determined using the procedure of the Department of Agriculture Bureau of Soils division [7] and the micronutrients (Cu, Zn, and Fe) were determined using the modified procedure from the [2]

D. Plant Analysis

The plants were uprooted, cleaned and analyzed in the Chemistry Laboratory of Ateneo de Davao University. Morphometrics of plant samples was also done. To obtain the dry weight of every part of the plants samples, the root, stem and leaves were separated and were dried in an oven for 24 hours at 80 °C. The analysis for copper uptake of the plant sample followed the method of AOAC with a slight modification [2]. To determine the efficacy of each treatments two tailed t-Test was used at 95% level of confidence [28].

III. Results and Discussion

Copper Uptake in Roots

The total copper uptake of the roots of *T. catappa* at different treatments showed variations as indicated by the high standard deviation (table 1 and Figure 1). Phytoextraction is a complex process, which is not affected by only a single factor like concentration of the metal in the soil. The presence of other biotic and abiotic substances in the soil may also vary the phytoextraction process.This variability of results is similar to the phytoextraction study of [19]. There is a significant increase ($p<0.05$) in the uptake of copper in the roots of *T. catappa*at 250 and 1250 mg·kg^{-1} of copper concentration (table 2). This increase might be due to the nonselective property of roots in accumulating metal ions from the soil [15], [5]. The high concentration of metals in the roots is associated with the diffusion of metals from the soil to the roots of the plant [9]. In addition, there is no significant increase of copper uptake in the roots of *T. catappa* when the concentration of copper in the soil is 2500 mg·kg^{-1} ($p>0.05$).Reference [10] stated that when the concentration of metals in the soil is very high, some plants tend to cease some of their metabolic functions to prevent further uptake of metals from the soil which can lead to the necrosis of plants.

Table 1: Average copper uptake in the roots of *T. Catappa* inmilligrams (mg).

Test Group	mg Cu	SD	Test Group	mg Cu	SD
250N	270	180	250E	780	170
1250N	290	120	1250E	1100	740
2500N	750	440	2500E	800	410
C	68	22			

Legend: 250, 1250 and 2500 – copper concentration in mg·kg^{-1} present in the soil; N – EDTA untreated samples; E – EDTA treated samples; C- Control

Table 2: Summary of t-test statistical analysis, comparing the total copper uptake (Roots) of the EDTA amended group with the group without EDTA at different contaminant levels.

Test Group concentration	T_{calc}	T_{crit}	*P*
250	2.2988	2.1448	0.0374
1250	3.1118	2.3646	0.0170
2500	0.2475	2.1448	0.8081

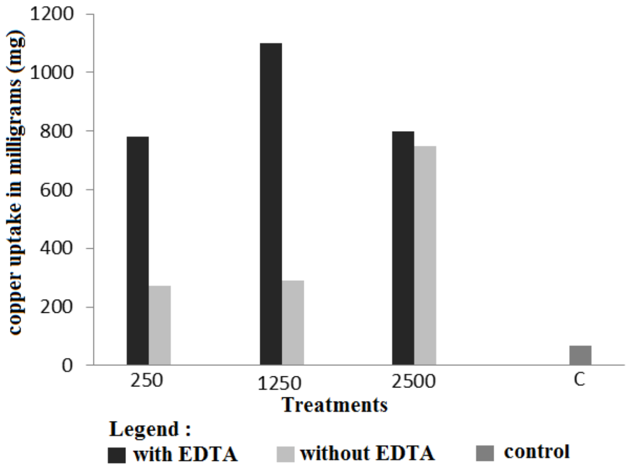

Fig.1. Average copper uptake of the roots of *T. catappa*.

Copper Translocation in the Stem and leaves

There is no significant difference of the translocation of copper in the stem and leaves of *T. catappa* in all test groups ($p>0.05$) (table 3). Stem transport minerals taken up by the roots and transfers them to the leaves, shoots and fruits, however, the amount of copper translocated in the stem of *T. catappa*is relatively lower than the amount of copper present in the roots (Figures 1 and 2). This result is coherent to the result of [27] that the necessary minerals are translocated by the stem and those that are irrelevant for the growing plant are left in the roots. High levels of copper in the roots may not be all translated in the stem as indicated in the results. Copper is needed in some photosynthetic processes of the plant, but the needed amount is minimal, therefore, the plant does not need to translocate a lot of the copper from the roots [6]. As shown in figure 2 translocation of copper is not significantly ($p>0.05$) enhanced by the addition of EDTA. This study may recommend that the presence of EDTA would not positively affect the mechanism of copper translocation in *T. catappa* (table 3).

Table 3: Summary of test statistical analysis, comparing the total copper Translocation (Stem and Leaves) of the EDTA amended group with the group without EDTA at different contaminant level.

Test Group concentration	T_{calc}	T_{crit}	*P*
250	1.3121	2.1448	0.2106
1250	1.8741	2.306	0.0978
2500	1.1992	2.1448	0.2503

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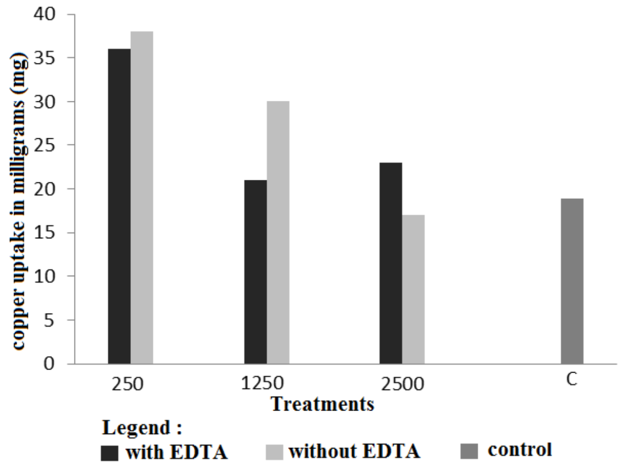

Fig.2. Average copper (in mg) translocated in 1000g basis of the stem of *T. catappa*.

The distribution of copper was observed at below-ground (roots) and the above-ground part (stem and leaves) of *T. catappa* (Figure 3). Whilst, the translocation of copper of *T. catappa* is increasing in the presence of EDTA, however, the extent of the increase is minimal in the 2500 groups. Large amount of copper that is localized in the root zone of the *T. catappa*showed its greatest accumulation at the 1250E group (Figure 4). A significant increase $p<0.05$ in the accumulation of copper when the concentration of copper in the soil is less than 2500 $mg \cdot kg^{-1}$ (table 4), thus, to use EDTA as an enhancer for copper accumulation is ineffective when the concentration of copper in the soil is relatively high. These results were consistent to other phytoextraction studies and are also supported by plant physiology where the roots serve as a depository of minerals present in the soil [1], [3], [23], [17]. To be considered a hyperaccumulator, the mass of the metal accumulated divided by the total dry mass of the plant must exceed 1% to be considered a hyperaccumulator [4], [15] therefore *T. catappa* plant is not a hyperaccumulator of copper since the percent of copper accumulated in all test group was less than 1% (table 5).

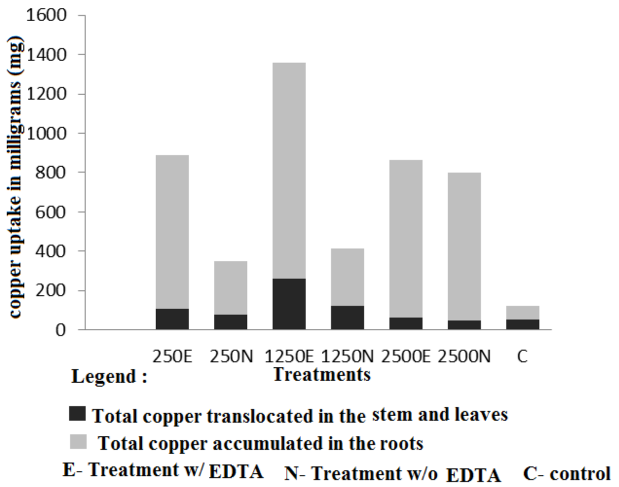

Fig.3. Total amount of copper accumulated and the total amount of copper translocatedof *T. catappa* plant.

Table 4: Summary of the t-test Statistical analysis, comparing the total copper Accumulation (Roots, Stem and Leaves) of the EDTA amended group with the group without EDTA at different contaminant levels.

Test Group	T_{calc}	T_{crit}	*P*
250	2.5537	2.1448	0.0230
1250	4.3871	2.3646	0.0032
2500	0.3046	2.1448	0.7651

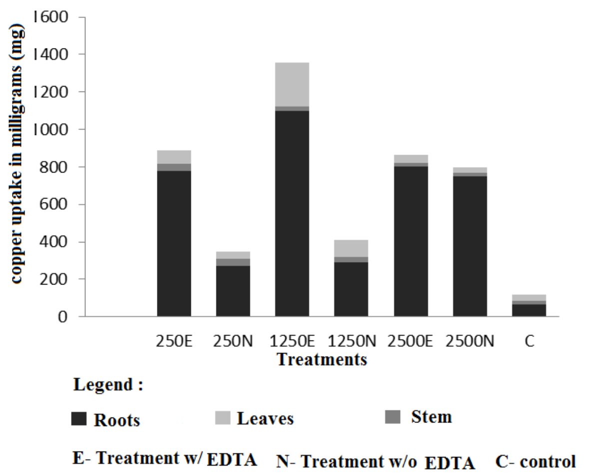

Fig.4. Average milligrams (mg) copper accumulated in the roots, stem, and leave of *T. catappa*

Table 5. Percentage of copper accumulated by *T. catappa*

Sample Group	% copper (g/g)	SD
250E	0.0056	0.0017
250N	0.0037	0.0020
1250E	0.018	0.010
1250N	0.0046	0.0016
2500E	0.0103	0.0048
2500N	0.0099	0.0055
C	0.00111	0.00023

Plant height and Mass

Reference [19] mentioned that plant height should be determined in order to give an estimate on the scope of the growth. The growth of the plant means survivability and sustainability of the phytoextraction. Essentially, the plant must be able to thrive normally at toxic conditions. If the plant accumulates more but dies eventually, the whole process is void. In this study, the length of the plant is categorized to below ground, above ground and total plant length. Results of the study showed that the length and weight of the plant decreases as the concentration of copper in the soil increases (Figures 5 and 6). Plants respond to abiotic stresses by decreasing their mineral uptake from the soil and in the process reduce their growth [10].

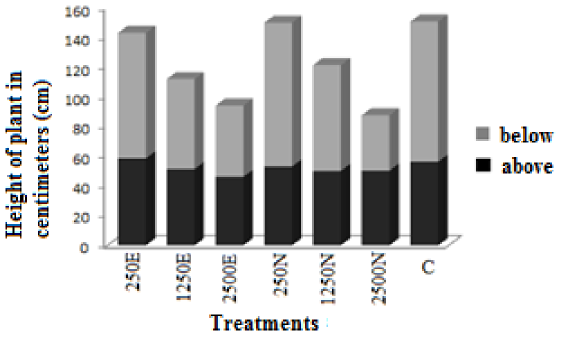

Fig.5. Mean height of plant in the different test group with above ground and below ground lengths in centimeters

Likewise, the length of the plant above ground is also decreasing but the difference is marginal, however, the length below ground is greatly affected (Figure 5). The root system did not develop well because of the presence of the metal. Root development is associated to soil condition. If the soil is highly contaminated, the roots will no longer spread out to look for minerals thus reducing its length and focuses its attention to survival thus limiting its growth [11], [12]. In the 250 treatments, *T. catappa*tends to fare well alongside with the control. This might be due to the high tolerance of *T. catappa*for metal ions in soil and copper is a micronutrient at low concentrations (Figure 5) [6].

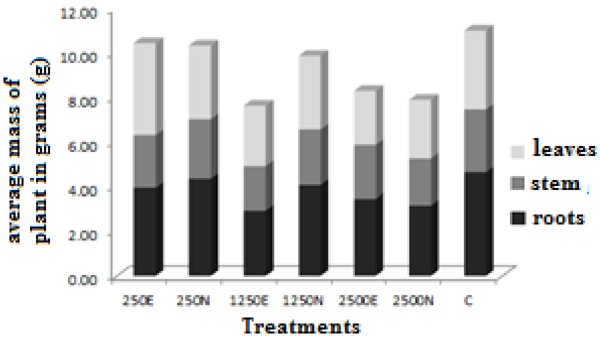

Fig.6. Distribution of plant mass in grams (g)

At 250 and 1250 treatments, the *T. catappa* without EDTA amendment tends to grow better than to others with EDTA except in the 2500 group (Figure 5). The presence of EDTA makes metal ions more soluble in the soil, making it easier to be accumulated thus making the plant absorb more and in the process. Too much copper concentration in the plant disrupts its photosynthetic ability eventually stunts its growth [23]. The high concentration of copper in the soil and the presence of EDTA made the soil phytotoxic [27], the nonselective property of roots when absorbing Cu from the soil lead to the chlorosis, stunted growth and necrosis of the *T. catappa*.

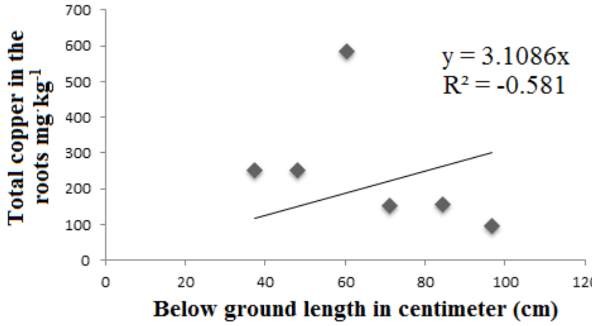

Fig.7. Relationship between the length of below ground part of *T. catappa*and the total copper accumulated

According to some reviews, there is no relationship between the length of the plant in both above and below ground length to the accumulation of minerals given [11], [17]. Mineral uptake of a plant is a very complex subject that involves soil composition [6] and condition, plant type, variety and metabolism and other biotic and abiotic factors [26]. As shown in figures 7, 8 and 9 there is no relationship between the length of the plant and the copper uptake in all treatments.The development of the root has no relative effect in the copper accumulation as well as the development of the stem and leaves to the copper accumulation.

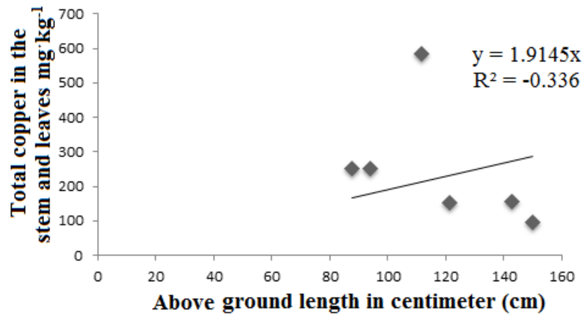

Fig.8. Relationship between the length of above ground part of *T. catappa* and the total copper accumulated in stem and leaves

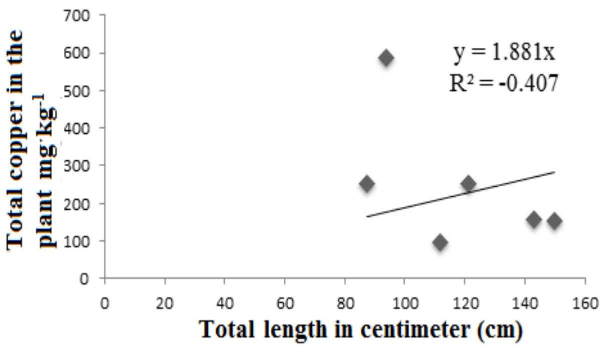

Fig.9. Relationship between the total length of *T. catappa* and the total copper accumulated

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