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THE EFFECTS OF ETHYLENE DIAMINE TETRAACETIC ACID (EDTA) ON UPTAKE OF ZINC (Zn^{2+}) AND COPPER (Cu^{2+}) BY HYDROPONICALLY GROWN MINT LEAF (*Mentha piperita* L.)

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ABSTRACT
This study was carried out to assess the effects of EDTA on zinc and copper uptake in the hydroponically grown mint leaf (Mentha piperita L) as well as their translocation factors in both chelated and unchelated treatments. In the present work, effects of EDTA on Zn^{2+} and Cu^{2+} uptake by mint leaf (Mentha piperita L.) seedlings replanted in hydroponic solutions in a greenhouse were investigated. Two months old seedlings, were exposed to various doses of Zn^{2+} and Cu^{2+} (0, 0.0025, 0.005, 0.0075, and 0.025 mg/L) and constant concentration of EDTA (0.0025M). For unchelated treatments, the effects of increasing concentrations of Zn^{2+} and Cu^{2+} in the solution increases the uptake by the plants relative to control. Zn^{2+} contents in the shoots showed more accumulation than in the roots ($p<0.05$) and also Cu^{2+} content in the shoots was higher than in roots. Hence, for the chelated treatments i.e. addition of 0.0025M EDTA to the solutions of Zn^{2+} and Cu^{2+} inhibits their uptake in both shoot and root of the plant at (0.0025, 0.005, 0.0075 mg/L) when compared to the unchelated treatment of same concentration. But at 0.025 mg/L, chelation does not have much effect on the Zn^{2+} and Cu^{2+} uptakes in both shoot and root of the plant ($p>0.05$) as the uptake in both chelated and unchelated treatments was almost constant. Hence, chelation inhibits translocation of Zn^{2+} and Cu^{2+} to the shoots of the plant at highest concentration of zinc and copper in the solution.
Keywords: Ethylene diamine tetraacetic acid, chelation, mint leaf, hydroponic, greenhouse.

INTRODUCTION

Hydroponics is the growing of plants in a liquid nutrient solution with or without the use of artificial media. Commonly used mediums include expanded clay, coir, perlite, vermiculite, brick shards, polystyrene packing peanuts and wood fiber. (Arjina and Bruce, 2015). Hydroponic culture has been recognized as a viable method of producing vegetables (tomatoes, lettuce, cucumbers and peppers) as well as ornamental crops such as herbs, roses, freesia and foliage plants. Due to the ban on methyl bromide in soil culture, the demand for hydroponically grown produce has rapidly increased in the last few years (Jones, 2005). The aim of this study is to assess the effects of EDTA on the zinc and copper uptake in the hydroponically grown mint leaf (*Mentha piperita* L).

Zinc (Zn) is one of the important elements of plant growth and development (Bonnet *et al.*, 2000; Misra *et al.*, 2005). It plays essential metabolic roles in the plant, of which the most significant is its activity as a component of a variety of enzymes, such as dehydrogenases, proteinases, peptidases, and phosphohydrolases (Clarkson and Hanson, 1980; Bowen, 1979). However, high zinc concentrations, like other heavy metals, are toxic for plants (Zhao *et al.*, 2003). Zinc toxicity in crops is far less widespread than zinc deficiency. Zinc toxicity also occurs in soils contaminated by mining and smelting activities, in agricultural soils treated with sewage sludge, and in urban and peri-urban soils enriched by anthropogenic inputs of zinc (Chaney, 1993).

Zinc plays very important role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions and synthesis of cytochrome. Plant enzymes activated by zinc are involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, regulation of auxin synthesis and pollen formation. The regulation and maintenance of the gene expression required for the tolerance of environmental stresses in plants are zinc dependent (Cakmak, 2000).

Zinc seems to affect the capacity for water uptake and transport in plants and also reduce the adverse effects of short periods of heat and salt stress (Kasim, 2007; Disante *et al.,* 2010; Peck and McDonald, 2010; Tavallali *et al.,* 2010).

Copper (Cu) is considered as a micronutrient for plants (Thomas *et al.,* 1998) and plays important role in CO_2 assimilation and ATP synthesis. Cu is also an essential component of various proteins like plastocyanin of photosynthetic system and cytochrome oxidase of respiratory electron transport chain (Demirevska-Kepova *et al.,* 2004). Physiologically, Copper exists as Cu^{2+} and Cu^{+}. It acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signalling (Marschner, 1995). Copper is an essential heavy metal for higher plants and algae, particularly for photosynthesis (Chatterjee *et al.,* 2006). Excess copper in plant cell interferes with enzymes associated with chlorophyll and biosynthesis and protein composition of photosynthetic membranes (Lidon and Henriques, 1991; Quartacci *et al.,* 2000). Various studies have been conducted comparing the bioaccumulation ability of heavy metals by plants using pot and hydroponic solution. Chemically, hydroponic solutions form more homogenous media than soil (Degryse *et al.,* 2006).

Dagari and Umar (2016) investigated the effects of EDTA on Cu^{2+} uptake by spinach (*Spinacea oleracea* L.) seedlings replanted in hydroponic solutions in a greenhouse. Four week old seedlings, were exposed to various doses of Cu^{2+} (0, 5, 10, 15, and 20 mg/L) and constant concentration of EDTA (10mM). From 15 to 20 mg/L, there was a substantial increase in copper uptake in Chelated treatments ($p < 0.05$) compared to Unchelated treatments of same concentrations of Cu^{2+}. So, chelation enhanced Cu^{2+} uptake by spinach. In a similar research, Kralova and Masarovičova (2008) conducted a research on the effect of EDTA on Cu, Zn and Cd bioaccumulation in *Matricaria recutita* L. plants. The studied plants accumulated more Cd, Zn and Cu in the roots than in the shoots. Metal content in plant organs increased in hydroponic solution. On the other hand, application of EDTA caused sharp increase of Cu concentration in the shoots, whereas Cd shoot concentration increased only slightly and Zn concentration showed a moderate decrease. The values related to the accumulated metal amount in the shoots ranged were 69-168 for Zn, 3.8-11.5 for Cu and 56-100 for Cd (application without EDTA) and 62-162 for Zn, 34-39 for Cu and 78-129 for Cd (with EDTA application).

MATERIALS AND METHODS

Two months old Mint leaf *(Mentha piperita* L.) seedlings were carefully harvested from Department of Agronomy farm, Bayero University, Kano in October, 2017. They were washed with tap water to remove excess soil, and rinsed three times with deionized water before replanting in hydroponic solution and kept in a greenhouse (Dagari and Umar, 2016). The reagents for the preparation of hydroponic solutions are shown in Table 1.

The stock of each nutrient solution was prepared separately from which appropriate volume were carefully transferred into a 1000 cm^3 volumetric flask and diluted to mark. The treatment consists of three phases for each metal in a completely randomized design which composed of the following;

Phase I: contain only the nutrient solution (hydroponic solution), i.e. the control.

Phase II: contain the nutrient solution and four levels (0.0025, 0.005, 0.0075 and 0.025mg/L) of added Zn^{2+} as $Zn(NO_3)_2$ /Cu^{2+} as $Cu(NO_3)_2$.

Phase III: contain the nutrient solution and four levels (0.0025, 0.005, 0.0075 and 0.025mg/L) of added Zn^{2+} as $Zn(NO_3)_2$ / Cu^{2+} as $Cu(NO_3)_2$ and constant concentration (0.0025M) of the ligand (EDTA).

Each treatment in triplicate was allowed to stand for four days, after which the plants where harvested and subjected to chemical analysis (Dagari and Umar, 2016).

Table 1: List of reagents for the preparation of hydroponic solutions

Reagent	Volume(cm^3)
0.0075M KI	0.17
0.10M H_3BO_3	11.35
0.10M $FeCl_3.6H_2O$	5.15
0.05M $MnSO_4.H_2O$	23.10
0.05M $Ca(NO_3)_2.4H_2O$	3.57
0.05M $Na_2H_2P_2O_7$	5.00
0.10M KNO_3	1.28
0.05M $MgSO_4.H_2O$	5.00

Analysis of the Plant Samples

The Mint leaf *(Mentha piperita* L.) seedlings were harvested after four days exposure to Hydroponic solutions kept in a greenhouse at 65% relative humidity, 13hr/day, 11hrs/night under specified light intensity, and day/night temperatures 39/23°C. The harvested plant samples were washed first with tap water, followed by 1% HNO_3 and finally rinsed with deionised water (Dagari and Umar, 2016). The plant samples were separated into roots and shoots, and air dried at laboratory condition for 72 hr. They were ground with wooden mortar and pestle to a fine powder. A clean porcelain crucible was ignited on a hot electric plate for 5 min. 1g of each sample (root and shoot) was accurately weighed into the crucible and gently heated on hot electric plate until the smoking ceased. It was then transferred and ashed to constant weight in a muffle furnace at 550°C for 4 hr. The ash was cooled in a desiccator, dissolved in 0.10M HNO_3 acid, filtered into 50 cm^3 volumetric flask and made to mark. The Zn^{2+} and Cu^{2+}content in the roots and shoots were analyzed using Atomic Absorption Spectrophotometer (Buck Scientific, Model GP9900) at 213.9 nm and 324.8 nm respectively. The concentration of Zn^{2+} and Cu^{2+} was reported as $mgkg^{-1}$ dry weight (Dagari and Umar, 2017).

Statistical Analysis

All data were treated using Excel 2010 program for windows and significance test was performed using one-way ANOVA at 95% confidence level.

RESULTS AND DISCUSSION

Figure 1 shows the effect of increasing concentrations of added Zn^{2+} from 0.000 – 0.024 mg/L in the uptake and accumulation of Zn^{2+} in the shoots and roots of the plant. At 0.0025 mg/L, the shoot uptake was significant ($p < 0.05$), and it was highest (9.258±0.0219 mg/kg) when compared to the root (4.125±1.163 mg/kg) of same concentration. From 0.005 – 0.025 mg/L of Zn^{2+}, the uptake by the plant was constant in shoots (≈8.700 mg/kg) while it decreases in the roots. This finding is similar to the results of Tandy *et al.* (2006).

Figure 2 shows the effects of addition of 0.0025M EDTA (chelator) to the different concentrations of Zn^{2+} (0.000-0.025 mg/L). Uptake of Zn^{2+} by the plant increases linearly with increase in co ncentrations of Zn^{2+}.The highest value (9.086±0.123 mg/kg) was obtained at 0.025mg/L of Zn^{2+}, thus, the uptake was significant ($P<0.05$) relative to control, so, chelation enhances uptake by the plant to the shoot. The root also shows similar trend with that of the shoot, the highest root uptake was (3.865±0.400 mg/kg) at 0.025 mg/L.

Figure 3 shows the effects of translocation factor (TF) against the concentration of added Zn^{2+} in chelated and unchelated treatments. Translocation factor (TF): is defined as the ratio of the concentration of a metal in the aerial part of a plant to its concentration in the root, Chen *et al.* (2012).

In this study, the TF varied significantly ($P<0.05$). The unchelated shows better translocation (2.358, 1.630, 2.552, and 2.081) than the chelated treatments (1.603, 1.556, 2.512 and 2.336) of same concentration, this shows that a good fraction of Zn^{2+} is accumulated and translocated to the shoots in the unchelated treatment.

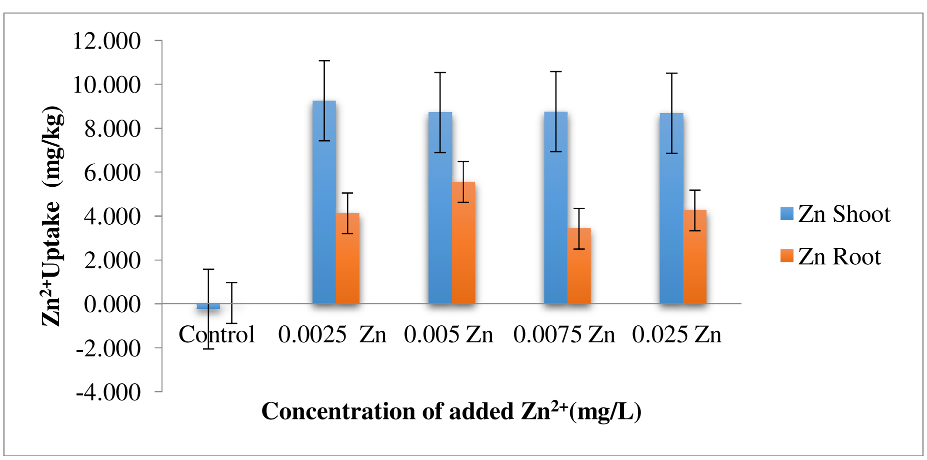

Figure 1: Zinc uptake by shoot and root of mint leaf *(Mentha piperita* L) seedlings in unchelated treatments of Zn^{2+} grown in different concentrations of zinc.

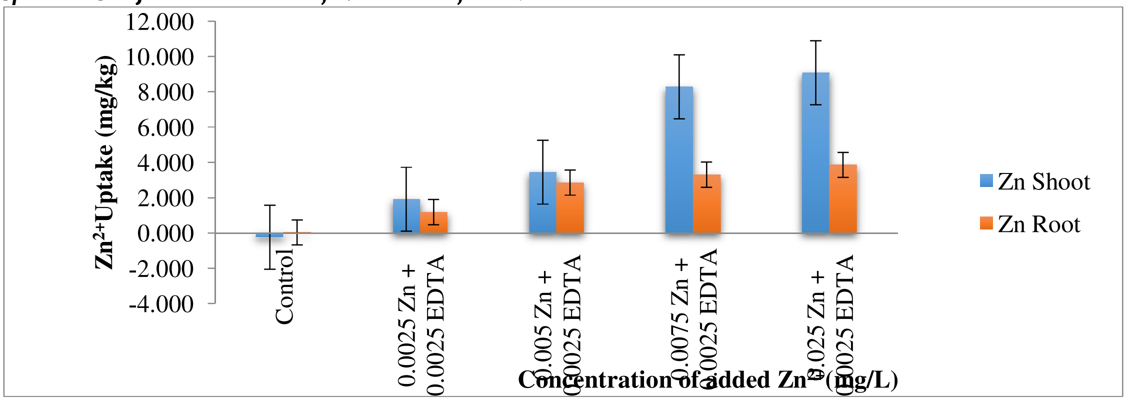

Figure 2: Zinc uptake by shoots and roots of mint leaf *(Mentha piperita* L.) at different concentrations of Zn^{2+} and same concentration of EDTA (chelator).

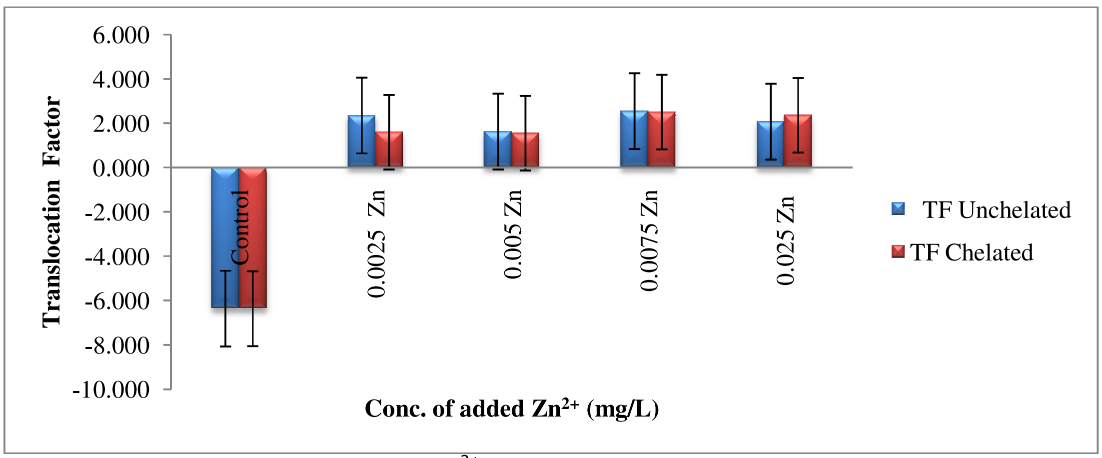

Figure 3: Translocation factor (TF) of Zn^{2+} in Mint leaf seedlings *(Mentha piperita* L.) grown in chelated and unchelated treatments of same Zn^{2+} concentrations grown in different concentrations of zinc.

Figure 4 shows the effect of increasing concentration of Cu^{2+} from 0.00-0.025 mg/L. The Cu^{2+} uptake increase with increasing concentration of Cu^{2+} both in the shoot and root of the plant and it was observed to be significant ($P<0.05$). This finding is supported by free-ion activity hypothesis which states that "the uptake of trace metals by plants is commonly assumed to depend on the free metal-ion activity, rather than the total concentration of dissolved metal" (Degryse *et al.,* 2006). These results are in good agreement with the findings of Kumar *et al.*(1995), Ozounoudou (1994) and Karimi *et al.* (2012), who reported that higher levels of applied Cu^{2+} enhanced the uptake of the ion by different plants. Similarly Dagari and Umar (2016) obtained comparable result with spinach (*Spinaceaeoleracea* L.) seedlings exposed to various doses of Cu^{2+} (0, 5, 10, 15, and 20 mg/L) supplied as $CuSO_4.5H_2O$ in the nutrient solution.

Figure 5 shows the effects of addition of 0.0025M EDTA (Chelator) to different concentration of Cu^{2+} (0.000– 0.025 mg/L), There were significant changes ($P < 0.05$) in Cu^{2+} uptake by the plant. The uptake is proportional to the concentrations of Cu^{2+} in the solution. The highest shoot uptake was (10.882±0.505 mg/kg) at 0.025 mg/L and the lowest uptake occurred at (4.795±0.084 mg/kg) at 0.0025 mg/L. while the highest root uptake was (11.413±4.794 mg/kg) at 0.0075 mg/L while the lowest occured at (2.126±0.335 mg/kg) at 0.0025 mg/L of Cu^{2+}. This shows that chelation supressed Cu^{2+} uptake by mint leaf *(menthe Piperita* L*).* However, phyto-toxic effect of EDTA occured between 24 – 48 hr after replanting of mint leaf seedlings in hydroponic solution. Thus, the leaves appeared damaged and started to wilt, necrosis also manifested indicating phytotoxicity (Blaylock and Huang, 2000). In this study, TF was found to vary significantly with concentration of added Cu^{2+} ($P <0.05$). Thus, good fraction of Cu^{2+} was translocated to the shoot in the unchelated treatments more than the chelated treatments of same concentrations (Figure 6).

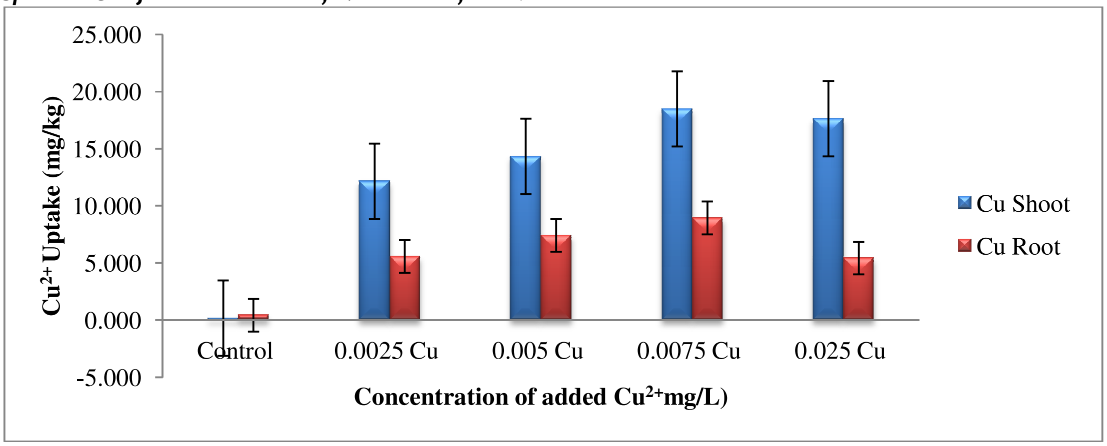

Figure 4: Copper uptake by shoot and root of mint leaf *(menthapiperita* L) seedlings in unchelated treatments of Cu^{2+} grown in different concentrations of copper.

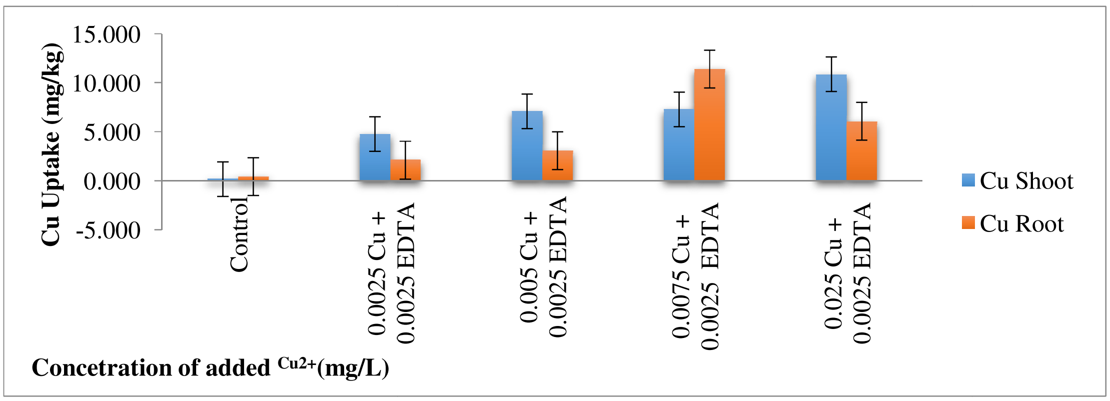

Figure 5: Copper uptake by shoots and roots of mint leaf (*menthapiperita L*) at different concentrations of Cu^{2+} and same concentrations of EDTA (chelator).

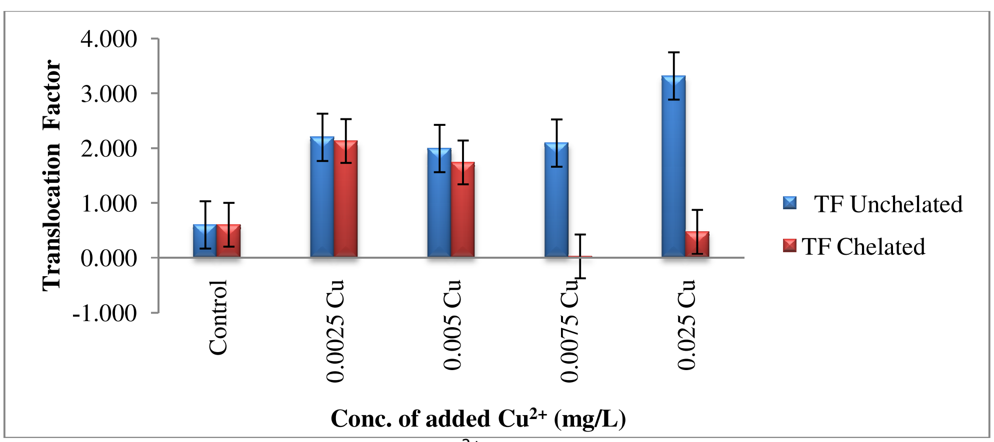

Figure 6: Translocation factor (TF) of Cu^{2+} in Mint leaf seedlings (*menthapiperita* L.) grown in chelated and unchelated treatments of same Cu^{2+} concentrations grown in different concentrations of copper.

CONCLUSION

Application of various doses of Zn^{2+} and Cu^{2+} (0.00, 0.0025, 0.005, 0.0075, 0.025 mg/L) to hydroponic solution enhance the phyto-extraction of Zn^{2+} and Cu^{2+} in mint leaf (*menthe piperita*L.) seedlings. Furthermore, addition of 0.0025M EDTA to the different treatments suppresses the uptake of both Zn^{2+} and Cu^{2+} by the seedlings. Varying degrees of Phytotoxic symptoms such as chlorosis, necrosis, leaf wilting, and stunted growth were observed due to Cu^{2+} and Zn^{2+} toxicity. This research could be used in phytoremediation of soils that are contaminated by heavy metals. The process is widely accepted as a cost effective environmental restoration technology.

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