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Impacts of EDTA on Uptake and Accumulation of Cu^{2+} by Spinach (*spinacia oleracea L.*) Seedlings Replanted in Hydroponic Solutions

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ABSTRACT

The effects of EDTA on Cu^{2+} uptake by spinach (*spinacia oleracea L*) seedlings replanted in hydroponic solutions in a greenhouse were investigated. Four week old seedlings were exposed to various doses of Cu^{2+} (0, 5, 10, 15, and 20mg/L) and constant concentration of EDTA (10mM). During the exposure, the plant protected itself from the damage caused by Cu^{2+} uptake by using the antioxidant,(proline). The photosynthetic pigments (i.e. chlorophyll *a*, chlorophyll *b* and Carotenoid) gradually declined. In this study, dry weights and lengths of roots, shoots and contents of the photosynthetic pigments of both chelated and unchelated hydroponic treatments were investigated. Changes in photosynthetic pigments, root and shoot weights and lengths were significant ($p < 0.05$) with respect to addition of EDTA to different concentrations Cu^{2+} compared to unchelated treatments of same Cu^{2+} concentrations. Hence, chelation enhanced Cu^{2+} uptake with adverse effects on the plant.

Keywords: Photosynthetic, Chlorophyll, Chelation, Spinach, Hydroponic, Greenhouse

INTRODUCTION

Copper is an essential micronutrient in plants. It plays a significant role in a number of physiological processes such as the photosynthetic and respiratory electron transport chains, nitrogen fixation, protein metabolism, antioxidant activity, cell wall metabolism, hormone perception and as a structural and catalytic component of proteins and enzymes. However, when absorbed in excess quantities, Copper (Cu) is highly toxic to plant growth potentially leading to physiological disorders (Karimi *et al.*, 2012). It has been reported that excess copper at cellular level causes molecular damage to plants via the generation of reactive oxygen species (ROS) and free radicals (Erdei *et al.*, 2002; Shaw *et al.*, 2004). Oxidative stress by formation of ROS and oxidation of biomolecules such as lipids, proteins, nucleic acids, carbohydrates, and almost every other organic constituent of the living cell is an important aspect of copper toxicity. Excess Copper (Cu) 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). It also induces Fe deficiency (Patsikka *et al.* 2002) and displaces Mg required for chlorophyll biosynthesis (Kupper *et al.* 2003). 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 soils (Degryse *et al*, 2006). Furthermore solution medium is more ideal to study trace metals at very low concentrations in the range, (10^{-11} to 10^{-10} M) than soils.

The research was aimed at investigating the effects of EDTA on Cu^{2+}uptake, and oxidative stress in Spinach (*Spinaceae oleracea L.*) under copper toxicity replanted in hydroponic solutions.

MATERIALS AND METHODS

Growth Conditions of the Spinach Seedlings

Four week old Spinach *(Spinacea oleracea L.)* seedlings were carefully collected on Wednesday 10th December, 2014 by 4.00pm, from the Department of Agronomy farm, Bayero University, Kano with coordinates latitude 8^0 22', to 9^0 25', North and longitude 11^0 57' to 12^0 00' East,. They were washed with tap water to remove excess soil, and rinsed three times with deionise water before replanting in hydroponic solution and kept in a greenhouse. They were supplied with the Hoagland nutrient solution (pH 6.0-6.3) which contained the following nutrients: 1mM KH_2PO_4, 2mM $MgSO_4{\cdot}4H_2O$, 5mM KNO_3, and 5mM $Ca(NO_3)_2{\cdot}4H_2O$ and 9μM $MnCl_2{\cdot}4H_2O$, 4.6μM H_3BO_3, 0.8μM $ZnSO_4{\cdot}7H_2O$, 0.3μM $CuSO_4{\cdot}5H_2O$, and 0.1μM $H_2MoO_4{\cdot}H_2O$. Iron was supplied as Fe-EDTA (1.8 mM). Copper in five levels (0, 5, 10, 15 and 20 ppm) as $CuSO_4{\cdot}5H_2O$ were added to the nutrient solution. The concentration of EDTA used was 10mM. Each treatment in triplicate was allowed to stand for five days, after which the plants were harvested and subjected to physiological and biochemical analysis (Chen *et al.*, 2012).

Atomic Absorption Spectrophotometric Determination of Cu^{2+} in Roots and Shoots of Harvested Spinach Seedlings

After five day exposure, the spinach seedlings were harvested and washed with tap water, followed by 1% HNO_3 and finally rinsed with deionised water. The roots and shoots were separated, and oven dried at 60°C for 48 hours. They were ground with wooden mortar and pestle to a fine powder. A washed dried porcelain crucible was ignited on a hot electric plate for 5minutes. 2g of each sample was accurately weighed into the crucible and gently heated on the hot electric plate until the smoking ceased. It was then transferred and ashed to constant weight in a muffle furnace at 550^0C for 4hours. The ash was cooled in a desiccator, dissolved in 0.10M HNO_3, filtered into a $50cm^3$ volumetric flask and made to the mark. The Cu^{2+} content in the roots and shoots was analyzed using Atomic Absorption Spectrophotometer at 324.7nm. The concentration of Cu^{2+} was reported as mg g^{-1} dry weight (IITA, 1979).

Determination of Photosynthetic Pigments

The photosynthetic pigments (chlorophylls *a, b* and carotenoid) were extracted by homogenizing 0.2g of a shoot in 80% acetone and then centrifuging at 1000rpm for 15minutes. Absorbance was determined spectrophotometrically in the supernatant at 645nm (chlorophyll *b*), 663nm (chlorophyll *a*), and 473 nm (carotenoid) according to the method of Lichtenthaler and Wellburn (1998).

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

There were significant changes in Cu^{2+} uptake at different concentrations of added Cu^{2+}.This finding is supported by free-ion 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). The unchelated treatments showed significant variation ($p < 0.05$) in the uptake of the Cu^{2+} ion. The uptake was 4.79 times higher between 5 to 10mg/L. However, no much difference was observed between 15 to 20mg/L. The results are in good agreement with the findings of Kumar *et al.*, (1990), Ozounoudou (1994) and Karimi *et al.*, (2012). They generally reported that higher levels of applied Cu^{2+} enhanced the uptake of the ion by different plants.

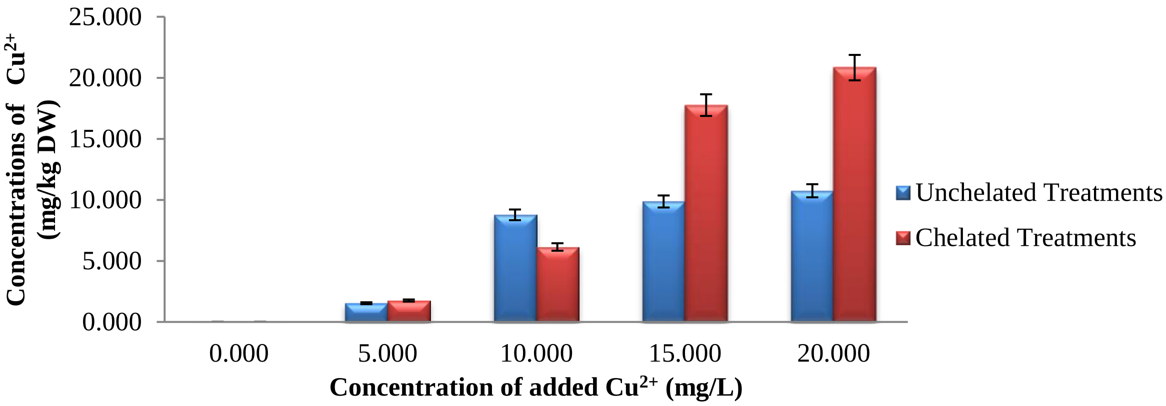

Figure 1: Copper uptake by Spinach Shoots (*Spinacia oleracea L)*s in Chelated and Unchelated Treatments of same Cu^{2+} Concentrations Grown in Different Concentrations of Copper.

The uptake increased substantially in the chelated treatments ($p < 0.05$) compared to unchelated treatments of same concentrations of Cu^{2+}. So, chelation enhanced Cu^{2+} uptake by spinach. This result agrees with the findings of Bell *et al.* (1991).

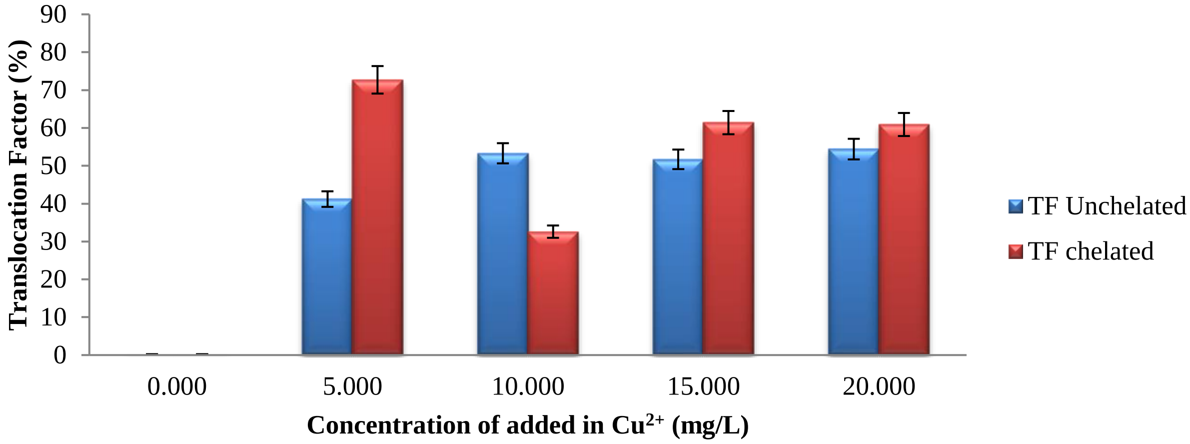

Figure 2: Translocation Factor (TF) of Cu^{2+} in Spinach Seedlings (*Spinacia oleracea L)* Grown in Chelated and Unchelated Treatments of Same Cu^{2+} Concentrations

According to Chen *et al*. (2012), translocation factor (TF) is the ratio of the concentration of a metal in the aerial part of a plant to its concentration in the root. In this study, TF was found to vary significantly with concentration of added Cu^{2+} ($p < 0.05$). Thus, a relatively good fraction of the Cu^{2+} was translocated to the shoots. The results which suggested that EDTA aided the translocation of Cu^{2+} to the shoots was supported with the findings of Kralova *et al*, (2008), in which EDTA was found to promote the translocation of Cu^{2+} to the shoots of Chamomile plant (*Matriacaria recutita L.)*. Copper toxicity has a significant effect on translocation factor and dry weight of plant. It increased the translocation factor and decreased the dry weight of plant significantly (Minnich *et al.*, 1987).

Changes in Dry Shoot and Root Lengths Due to Addition of Cu^{2+} and EDTA

The morphological parameters monitored in the two different treatments include changes in root and shoot lengths of the seedlings.

Change in shoot length (ΔShl) = (Shoot length for a given treatment) – (Shoot length for control)

Change in root length (ΔRtl) = (Root length for a given treatment) – (Root length for control)

Figures 3 and 4 show the changes in root and shoot lengths which were significant ($p < 0.05$) with respect to addition of Cu^{2+} and at constant EDTA concentration. The changes in root and shoot lengths were -1.030 ±8.917 and -1.217±1.826cm relative to control. In paper birch (*Betula papyrifera*) and honey suckle (*Lonicera tatarica)* seedlings, high Cu concentrations have been shown to inhibit the production of root hairs (Patterson and Olson, 1983). The effects of EDTA were visible between 24 - 48hours of replanting where the leaves appeared damaged and started to wilt,a manifestation of necrosis which indicated phytotoxicity, (Chen *et al*, 2011).

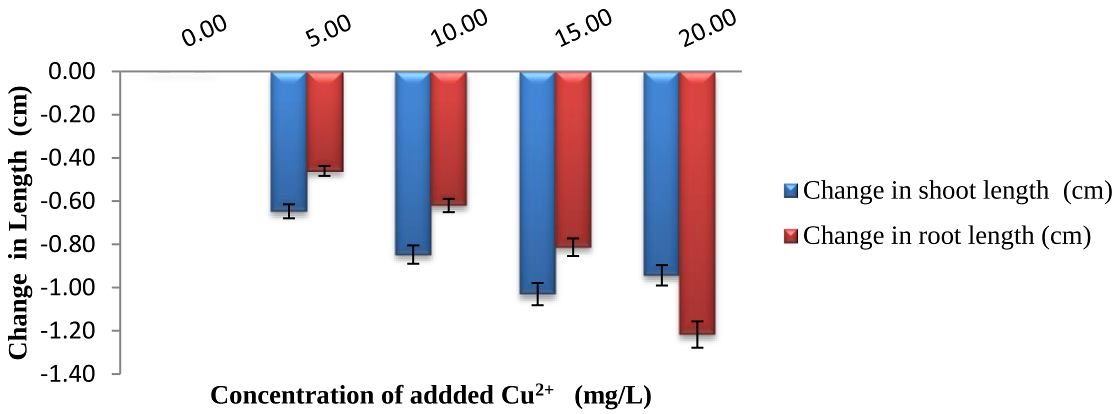

Figure 3: Changes in Root and Shoot Lengths Caused by Addition of $CuSO_4.5H_2O$ only to Hydroponic Treatments.

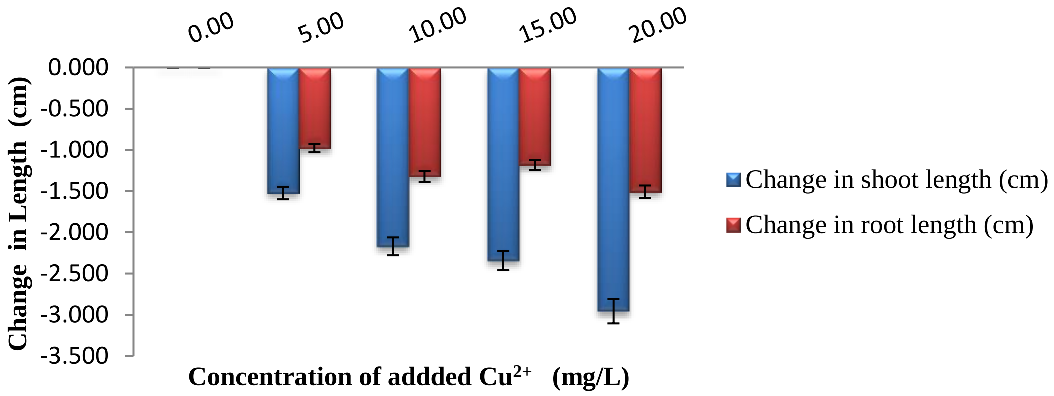

Figure 4: Changes in Root and Shoot Lengths Caused by Addition of 10mM EDTA to Different Hydroponic Treatments.

Changes in Dry Shoot and Root Weights Due to Addition of Cu^{2+} and EDTA

Figures 5 and 6 show that changes in dry weights of roots and shoots were significant (*$p < 0.05$*) with respect to addition of Cu^{2+} at different concentrations. The changes in shoot and root dry weights which were -1.637±1.815 g and -3.573±1.444 g relative to control were found to be significant (*$p < 0.05$*) with respect to addition of EDTA at different concentrations of Cu^{2+}. Plants grown in the presence of high levels of Cu^{2+} normally show reduced biomass and chlorotic symptoms (Baszynski *et al.*, 1988; Lidon and Henriques, 1993; Ciscato *et al.*, 1997; Quartacci *et al.*, 2000; Pätsikkä *et al.*, 2002). Servillia *et al,* (2005) found that heavy metals inhibit growth due to structure damage, decline in physiological and biochemical activity. This result was in good agreement with the above findings.

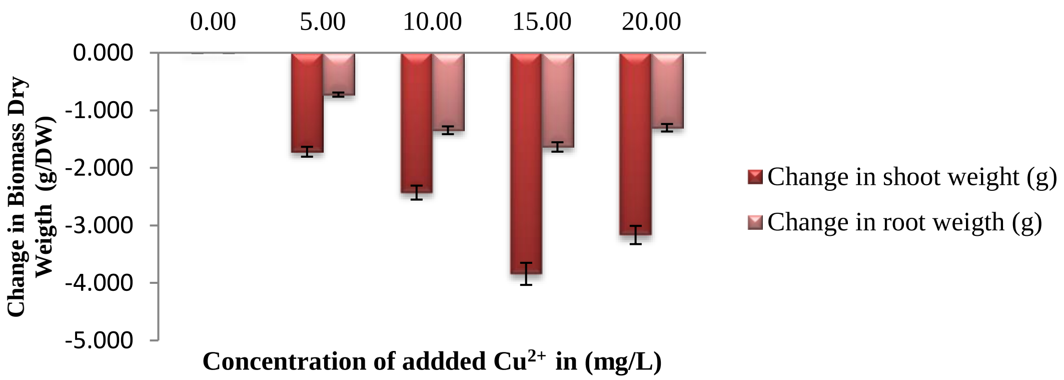

Figure 5: Changes in Shoot and Root Weights Caused by Addition of Cu^{2+} to Different Hydroponic Treatments.

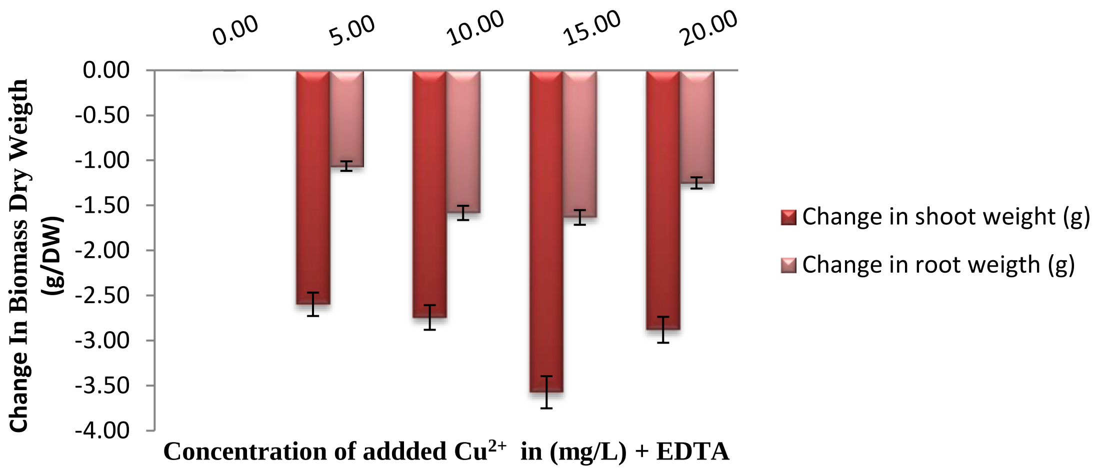

Figure 6: Changes in Shoot and Root Weights Caused by Addition of 10mM EDTA to Different Hydroponic Treatments.

Changes in Chlorophyll *a, b* and Carotenoid Contents

The plant pigments, chlorophylls *a*, *b* and carotenoid were determined to justify the Cu^{2+} uptake by the plants. The results revealed Cu^{2+} uptake decreased chlorophylls *a*, *b* and carotenoid contents in the plants (Karimi *et al*, 2012). However, significant reduction ($p < 0.05$) was achieved between 15 and 20mg/L concentrations levels (En-Jang *et al,* 1998). This indicated the levels of damage caused by Cu^{2+} uptake in chelated and unchelated treatments to the plants.

Figure 7 depicts the extent of decrease in the levels of chlorophyll *a*, *b* and Carotenoid relative to control.

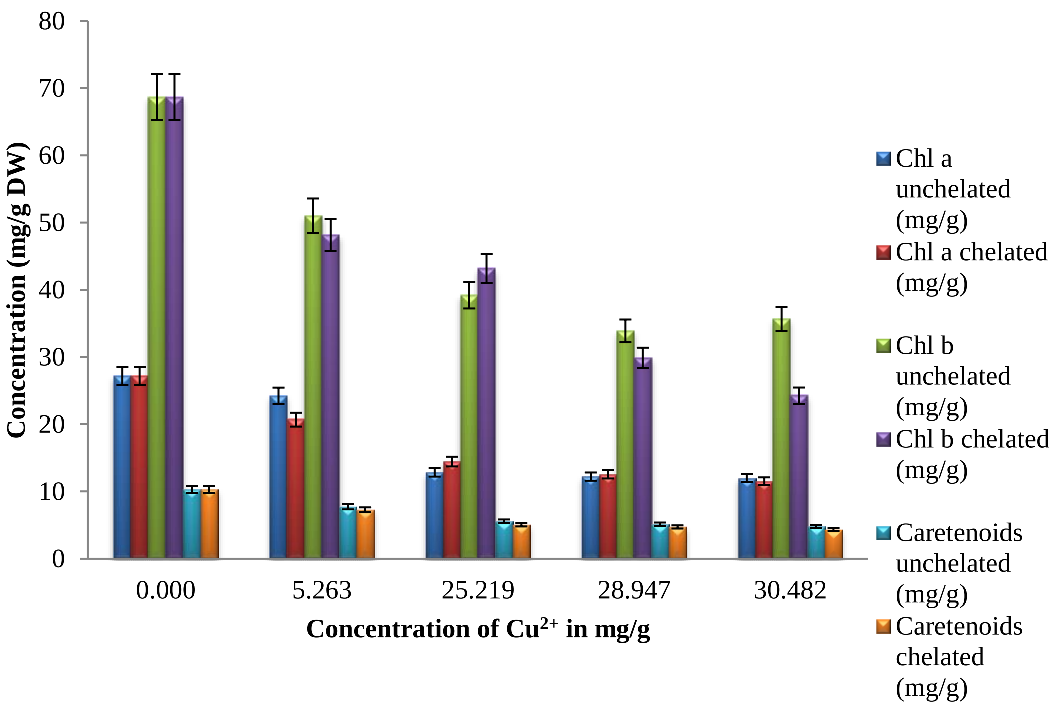

Figure 7: Changes in Chlorophylls a, b and Carotenoid contents in the shoot of spinach (*Spinacia oleracea L)* grown in different concentrations of copper.

The chlorophylls *a*, *b* and carotenoid contents of seedlings in chelated treatments were relatively lower than those in unchelated treatments of same concentrations of Cu^{2+} ($p < 0.05$). At 20mg/L, the chelated treatments showed the highest decrease (42%) of the pigments. Similar finding was reported by Karimi *et al.,* (2012).

CONCLUSION

Application of various doses of Cu^{2+} (0, 5, 10, 15 and 20mg/L) and 10mM EDTA to hydroponic solutions enhanced the phytoextraction of Cu^{2+} in spinach (*Spinacia oleracea L)* seedlings. Varying degrees of phytotoxic symptoms which include chlorosis, necrosis and reduction in root and shoot lengths were observed. Chlorophylls *a, b* and Carotenoid levels were found to decrease significantly ($p < 0.05$).

This research could be used in monitoring and phytoremediation of soils contaminated with heavy metals. The process is a cost effective way of cleaning and reclaiming contaminated sites.

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