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Influence of Copper Chelation by EDTA on Growth, Survival and Development of Plumed Cockscomb (*Celosia argentea)* Cultivar (TVL 8) in Clay, Loamy, Sandy Garden Top and Red Earth Soils

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ABSTRACT: A greenhouse experiment was conducted to investigate the impact of chelation of Copper (Cu) toxicity on growth, survival and development by growing plumed cockscomb (*Celosia argentea)* cultivar (TLV 8) in 4 kg each of clay, loamy, sandy, garden topsoil and red earth contaminated with 500 mgL^{-1}. Cu and 5M Methylene diaminetetaacetic acid (EDTA) in the following combinations: 500 mg Cu L^{-1} as $CuSO_4 \cdot 5H_2O$ and 500 mgL^{-1} Cu + 5M EDTA with a control (absence of Cu and EDTA). This application was performed two weeks after planting (WAP) and was repeated at 6 WAP. Variations were observed in soil pH and organic matter content. Using the American soil texture triangle five soil types had the following soil textures: sandy-clay-loam (clay soil), sandy (garden-top soil), sandy (sandy soil), loamy-sandy (loamy soil) and sandy-loamy (red-earth). In the course of ten weeks of growth, growth parameters and morphometric characteristics were determined. Cu toxicity caused a significant reduction in growth parameters (plant height, dry root weight, dry stem weight, leaf area, leaf area ratio, and absolute growth rate) in all soil types but the maximum reduction was observed in sandy soil while the highest growth exhibited by plants grown in a loamy soil. Increasing soil copper concentration by a second applications (2 and 6 WAP) reduced the number of leaves (37% - 56%) and plant height (10% - 21%) in all soil types. Presence of EDTA in the medium effectively alleviates growth reduction induced by Cu toxicity in plants by increasing the physiological growth parameters of *Celosia argentea*. The performance of plant grown in the various toxic soil sample appeared to be inhibited by copper in the following order: Cu > Cu + EDTA > Control. Furthermore, loamy soil stimulated better growth of *Celosia* in a Cu-toxic environment.

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Keywords: Copper toxicity; Soil types; ethylenedianinetetraacetic acid; *Celosia argentea*.

Contamination of soil, water and food with toxic metals is a global environmental problem. Some of them are the most dangerous pollutants and have received much attention for their toxicity to humans (Moaref *et al.*, 2014; Malar *et al.*, 2014). Copper (Cu) is one of the most abundant heavy metals in agricultural soils. Copper-based pesticides are widely used in agriculture worldwide (Husak, 2015). Cu is an important redox-reactive transition metal involved in many physiological functions, such as photosynthesis, mitochondrial electron transport chain, cell wall metabolism or ethylene signaling. Plants can retain low level of Cu in their cells (about 10 µg g^{-1} dry mass); below these values, they show typical symptoms of Cu deficiency, tissue chlorosis and malformed leaves are displayed (Marschner, 1995). Cu is an essential nutrient for plant growth and is potentially phytotoxic at high concentrations. Cu phytotoxicity affects many morphological, physiological and biochemical processes in plants.

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Growth reduction is one of the most common responses and is first observed when plants are stressed by Cu toxicity (Adriano, 2001).

An Excess of copper can reduce growth by inhibiting enzymes involved in physiological processes necessary for growth and development (photosynthesis, respiration) or by inducing oxidative stress that can leads to cell death. Thus, copper toxicity results in the fixation of the metal on the thiol group of proteins, thereby inhibiting its activity and altering its structure (Ibrahim *et al.*, 2017). It has been reported that Cu concentrations in agricultural soil ranges from 20 to 30 mg kg^{-1} around the worldwide, but the value varies with conditions and locations. However, the addition of Ethylenediaminetetraacetic acid (EDTA) to soil induces the uptake and translocation of heavy metals from plant roots to shoots (Meers *et al*., 2008) and promote the growth and developments of plants when grown in metal- polluted soils (Zhang *et al*., 2013). *Celosia argentea. L*., a member of the Amaranthaceae family, is an important leafy vegetable crop grown in tropical and subtropical regions of the world especially in Lagos state (Usunobun and Ekpemupolo, 2016).

Research information on the influence of heavy metals on the growth of plumed cockscomb are limited or unavailable, therefore this work reports the investigation on the influence of copper chelation by EDTA on the growth, survival and development on plumed cockscomb (*Celosia argentea)* Cultivar (TVL 8) in clay, loamy, sandy, garden top and red earth soil.

MATERIALS AND METHODS

Plant materials and collections: The seeds of *Celosia argentea cultivar* T.L.V. 8 were collected from National Horticultural Research Institute (NIHORT) Ibadan. The five soil types used for planting in this experiment were collected from different sites within the University of Lagos environment. Seeds were surface-sterilized with sodium hypochlorite solution (1%) for 15 minutes and thoroughly washed with distilled water before planting to prevent fungal and bacterial surface contamination.

Experimental Procedures: This study was carried out in the greenhouse of the Botanic Gardens, University of Lagos. Experimental soil samples were randomly collected from five different soil types at depths of 0-15 cm within the university, bulked for analysis and mixed with 80 g of N.P.K. fertilizer per 100 kg of soil. Each type of soil was divided into 3 batches (A, B, C) of 18 pots (12 cm x 15 cm) each containing 4 kg of soil. The Seeds were sown in the nursery for 10 days before being transplanted into pots. Toxic concentrations of copper were applied when seedling were 3 weeks old as follows: Batch A was a control in which plants were not exposed to Cu stress (water only) in any soil type. Soils from Batch B were moistened with 2 g of $CuSO_4.5H_2O$ (500 mg Cu L^{-1}). In contrast, soils from Batch C were moistened with chelated Cu (500 mg Cu L^{-1} + 5 mM ethylenediaminetetraacetic acid (EDTA)). The experiment was performed in a 5 x 3 factorial arranged in a Randomized Complete Block Design (RCBD) and replicated three times. A total of 90 pots (12 cm x 15 cm) were used ln this experiment. Four plants were transplanted into each pot and thinned to two plants per pot after the first week. Heavy metal (Cu) and chelates were applied twice to each pot at the early and late vegetative stages (2 and 6 weeks). Samples of seedlings were harvested at the vegetative stages (6 weeks after planting (W.A.P.) while the final harvest was done at (10 W.A.P.).

Soil analysis: The soil samples were air-dried and subjected to physical and chemical analysis. Soil analysis was performed using a modified hydrometer method and pipette method with 5% Calgon (sodium hexametaphate) solution as dispersing agent by Bouyoucous (1962). Soil pH was determined in water at a 1:1 ratio of soil–water suspension and percentage organic matter was equally determined. The soil profile of soils from the 5 locations was conducted and the standard soil textures was extrapolated from the U.S Textural Triangle (Saxton, 1986).

Growth analysis: Three replicates of plants were harvested from each of the batches at 4, 6, 8 and 10 weeks after planting, Percentage germination was determined 14 days after planting, growth parameter measured include: plant height, number of leaves, leaf area, dry weight of shoot, root and whole plant. Leaf area ratio (LAR) and absolute growth rate (AGR).

The leaf area of plant was determined as outlined by Eze (1965). Each leaf was carefully traced on paper and leaf traced were weighed. $100cm^2$ of the same paper was weighed to give the standard weight (SW) using Mettler electronic balance. The leaf area of the leaf traces (LT) was determined using the formula:

$$LA = \frac{\text{W of LT xStandard area } (100\text{cm}^2)}{\text{SW (g)}}$$

Where LA = Leaf Area, LT = Leaf Traced, SW = Standard Weight.

Plants were dried in an oven at 800C for 3 days. Thereafter, dry weight of the whole plant, shoot and the root were taken. Leaf area ratio (LAR), Absolute growth rate (AGR) is determined using appropriate mathematical expression as outlined by Noggle and Fritz (1976).

Statistical Analysis: The values are expressed as means of three replicates ±SE. The data were subjected to ANOVA. The significance between treatment means at $p<0.05$ was determined by Turkey's Multiple Comparison Test.

RESULTS AND DISCUSSION

Soil physiochemical properties, including soil pH and organic matter (OM) are shown in Table 1. Loamy soils had the highest organic matter content (5.81 $mgkg^{-1}$) and were moderately acidic (pH: 5.4), while sandy soils had the lowest organic matter content and were slightly alkaline (pH: 7.9). The order of acidity is Sandy soil > Clay soil > Loamy soil > Garden topsoil > Red earth.

Table 1: The chemical properties of the soils before planting

Soil Samples	Organic Matter ($mgkg^{-1}$)	Soil pH	Description
Clay Soil	1.24	6.8	Neutral
Loamy Soil	5.81	5.4	Moderately acidic
Sandy soil	1.08	7.9	Moderately basic
G. Top Soil	4.32	7.0	Moderately basic
Red Earth Soil	2.98	6.9	Moderately basic

Percentage germination rates are presented in Table 2. Sandy soil exhibited the highest rate of plant emergence (78%), and clay soil showed the lowest germination rate (25%). The germination rate was in the order Sandy soil > Garden topsoil > Loamy soil > Red earth > Clay soil.

Table 2: Percentage germination rate of *Celosia argentea* in different soil samples

Treatment	Germination (%)
Clay Soil	25d
Loamy Soil	63b
Sandy soil	78a
G. Top Soil	69b
Red Earth Soil	48c

Similar letters are not significantly different (P> 0.5) according to Tukeys multiple comparison

Using the U.S Standard Soil Texture Triangle Figures 1-5 (Saxton et al 1986). The soil types had the following soil textures: sandy-clay-loam (clay soil), sandy soil (garden top soil), sandy soil (sandy soil), loam-sandy (loamy soil) and sandy-loam (red earth).

The particle size distribution showed that all the soil samples contained a high percentage of sandy particles with garden topsoil (92%) and sandy soil (100%) exhibiting the highest.

The order of particle size distribution to percentage sand composition is as follows: Sandy soil > Garden topsoil > Red earth > Loamy soil > Clay soil.

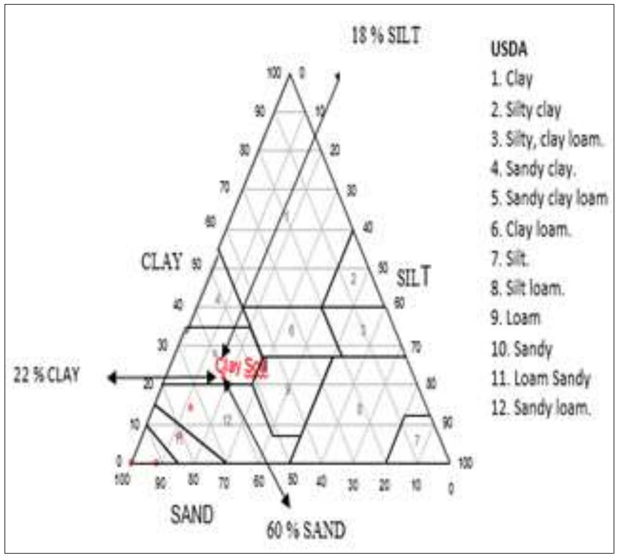

Fig 1: Soil Type: Clay Soil. USDA Classification: Sandy-clay-loam (60% Sand, 22% Clay and 18% Silt)

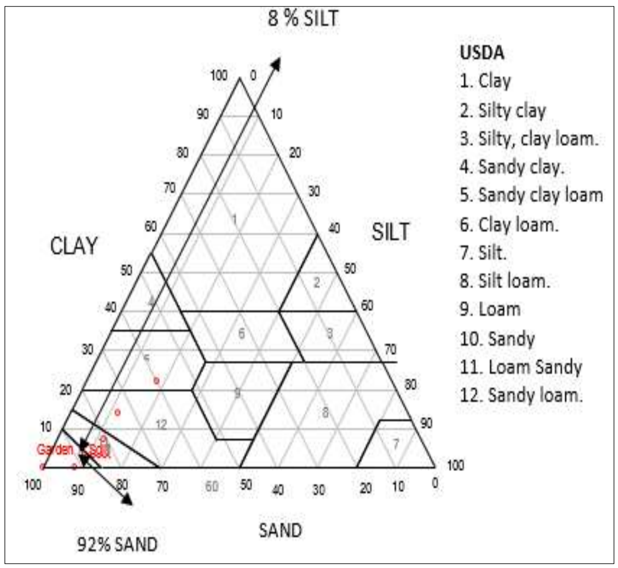

Fig 2: Soil Type: Garden Topsoil. USDA Classification: Sandy Soil (92% Sand and 8% Silt)

The impact of copper and EDTA on growth, survival and development of *Celosia argentea* grown in different soil types on plant height and leaf area are shown in Figures 6 and 7 respectively. Cu toxicity resulted in significant reductions in all growth parameters compared to the control. Increasing Cu concentration reduced the growth parameter of *Celosia argentea*, including plant height and leaf area. Furthermore, this reduction was pronounced at higher Cu concentrations (1000mg CuL^{-1}). Greater plant growth was recorded in the control treatment (0mg CuL^{-1}) compared with Cu trated plants grown in all soil types. Among the five soil types used, Cu-contaminated sandy soil exhibited minimum plant height (27.4 cm), while the maximum plant height (54.2 cm) was obtained from control loamy soil. Similarly, the maximum leaf area (51.9 cm^2) per plant was recorded in control loamy soil, while the minimum leaf area (29.9 cm^2) was observed in Cu-stressed sandy soil. The application of EDTA in the soil solution of Cu-contaminated mixture significantly (P<0.05) enhanced plant growth and biomass. The result revealed that Cu-stressed plants (1000mg CuL^{-1}) with the application of EDTA increased plant height by 24%, 17%, 28%, 27%, and 23% in clay, loamy, garden topsoil, and red earth, respectively over soil contaminated with only Cu.

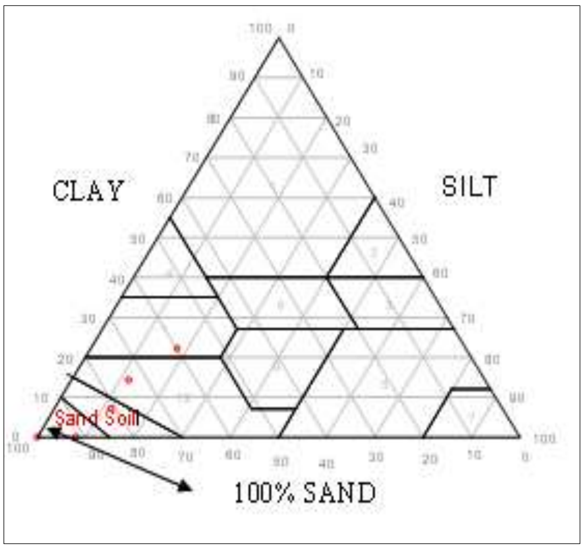

Fig 3: Soil Type: Sandy soil USDA Classification Sandy (100% sand)

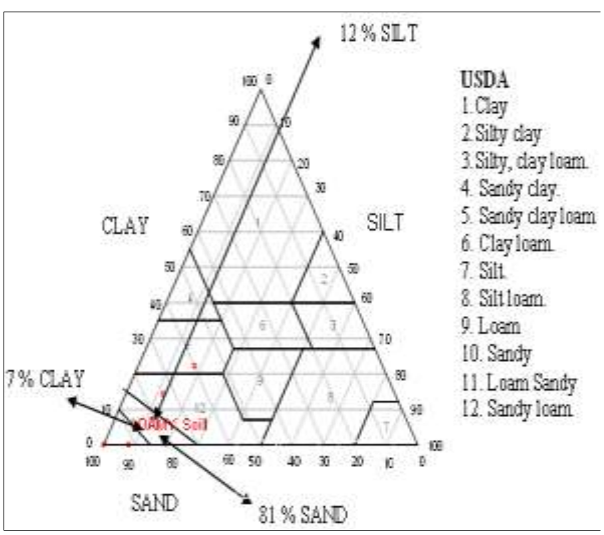

Fig4: Soil Type: Loamy soil USDA Classification Loamy-Sandy (81% sand, 12% silt, 7% clay)

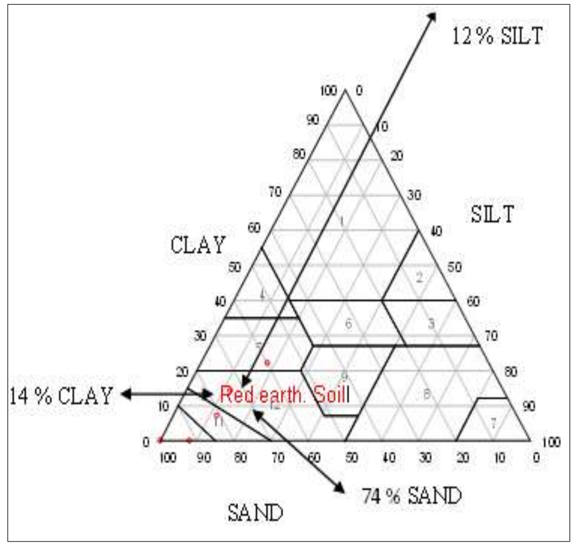

Fig 5: Soil Type: Red earth soil USDA Classification Sandy-Loamy (74 sand, 14%bclay, 12% silt)

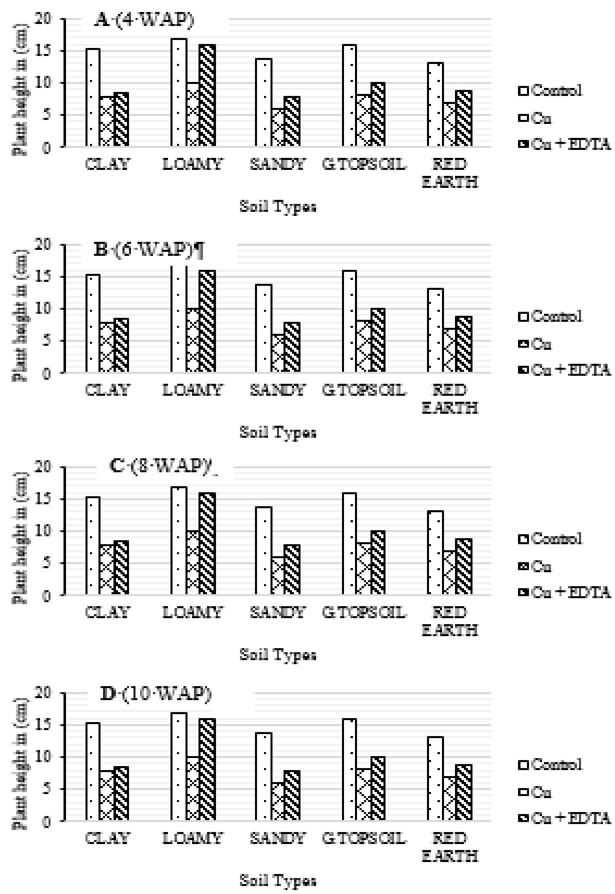

Fig 6: Plant height (cm) of *Celosia* treated with Cu and chelate in 5 types of soil at 4(A), 6(B), 8(C) and 10(D) weeks after planting. Each treatments interval represented by similar letters are not significantly different (P=0.05) according

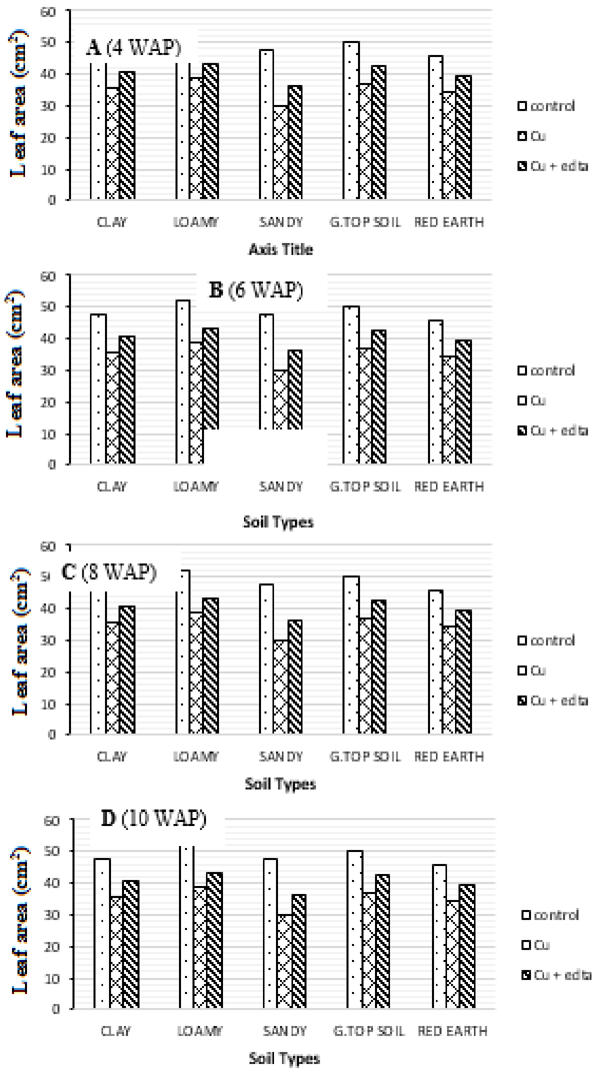

Fig 7: Leaf area (cm^2) of *Celosia* treated with Cu and chelate in 5 types of soil at 4(A), 6 (B), 8 (C) and 10 (D) weeks after planting. At each harvest treatments represented by similar letters are not significantly different (P=0.05) according to Tukeys multiple comparison test.

The impact of copper and EDTA on growth, survival and development of *Celosia argentea* grown in different soil types on number of leaves is shown in **Figures 8.** Plants grown on different soil types followed the trends in plant height and leaf area. Plants grown on the loamy and the garden topsoil appeared to be the most tolerant to the toxic concentration Cu in number of leaves at all application levels. The highest mean value for the number of leaves was recorded in the control loamy soil plants (28 ± 0.25), and the lowest value was recorded in the plants grown on sandy soil chelated with Cu at four weeks (3.36±0.21) WAP. The number of leaves on the plant was significantly inhibited ($P < 0.05$) by copper toxicity during growth. Copper toxicity drastically reduced the number of leaves in the treated plant over control by 31%, 47%, 75%, 43%, and 52% in clay, loamy, sandy, garden topsoil, and red earth at 8(C) WAP. Also, the addition of chelate did have any inhibitory effect on the number of leaves at 4(A) and 8(C) WAP. Generally, plants subjected to Cu + EDTA exhibited greater size, followed by those grown by copper only in all soil types. The performance of the plants grown on the various toxic soil samples appears to be inhibited by copper in the following order: Control > Cu+ EDTA > Cu only.

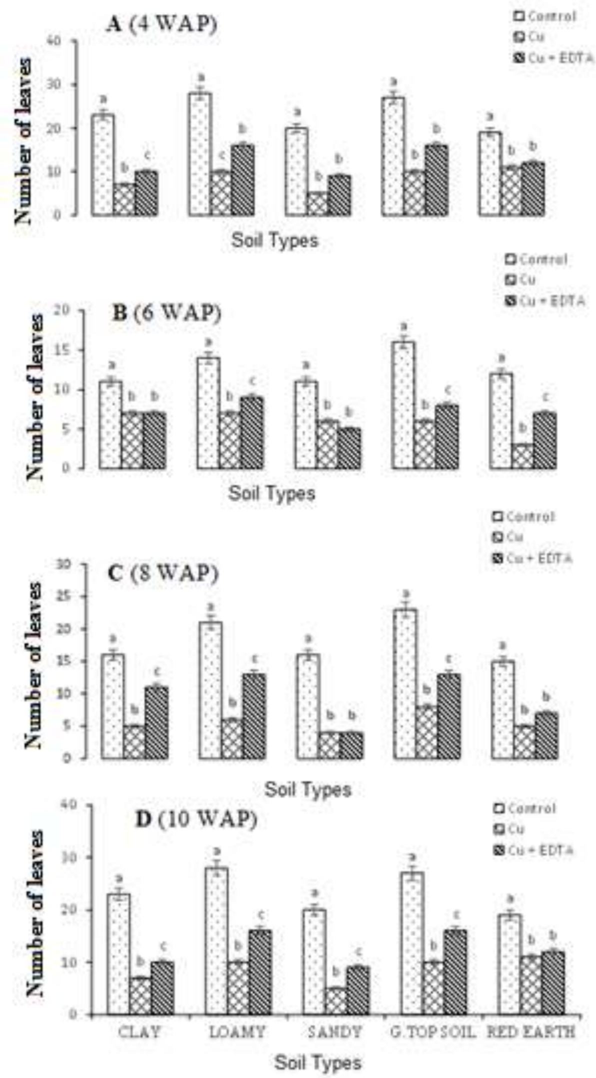

Fig 8: Number of leaves of *Celosia* treated with Cu and chelate in 5 types of soil at 4(A),6 (B), 8 (C) and 10 (D) weeks after planting. At each treatment represented by similar letters are not significantly different (P=0.05) according to Tukeys multiple comparison test

The impact of copper and EDTA on growth, survival and development of *Celosia argentea* grown in different soil types on the dry matter content, root and shoot dry weight are shown in **Figure 9** Root dry weight was significantly influenced by different levels of Cu and EDTA. Higher dry root weight was recorded in plants under control conditions than in plants grown in Cu + EDTA and Cu alone in all soil types. Cu significantly decreased root dry weight compared with all other treatments. Similarly, shoot dry weigh exhibited significant decrease when exposed to different applications of Cu and a combination of Cu and EDTA.

The higher shoot dry weight (3.9g) was recorded by control plants in garden topsoil at 6 WAP while minimum shoot dry weight (0.4 g) was recorded for 1000 mgkg^{-1} of Cu treatment in sandy soil. Similarly, maximum root dry weight (0.8 g) was observed in control loamy soil compared with other soil types. Plants exposed to Cu toxicity produced reduced root dry weight of 0.1 g, 0.2 g, 0.1 g, 0.1 g and 0.1 g, in clay loamy, sandy, garden top soil and red earth. Application of EDTA maximized the shoot dry weight with the highest increase (1.9 g) observed in a loamy soil.

Weight changes in shoot and root plant parts followed the increased pattern Control > Cu + EDTA > Cu. Plant subjected to the combination of EDTA and Cu has improved weight in all weeks. The impact of copper and EDTA on growth, survival and development of *Celosia argentea* grown in different soil types on absolute growth rate is shown in Figure 9. The result show obvious difference between the stressed and unstressed plant. EDTA maximized the absolute growth rate of treated plants.

Celosia argentea is sensitive to copper toxicity in different soil types. The physico-chemical properties of soil affect the plant growth, survival and development. Soil physical properties are strongly influenced by mineral particle size distribution which is an indicator of soil quality (Dexter, 2004; Schoenholz *et al*., 2000). Sand content was high in all soil types, but clay soil which was classified sandy-clay-loam had highest silt content (18 % silt, 22 % clay and 60 % silt). The grain size composition also plays an important role from a physiological point of view, as soil with unequal grain size composition but similar metal composition may have different bioavailability of heavy (CsathÒ, 1994).

There are also significant differences among the soil particle size distributions. This current study is in consistent with GyÒrgy and Krisztina, (2009) who revealed sandy soils with coarse grains and low absorption capacity have increased metal concentrations.
Significant differences were observed in the organic matter (humus) content of the five soil types. Organic matter is highest in loamy soil and lowest in sandy soil. In general, red earth soils and clay soils have low organic matter content.

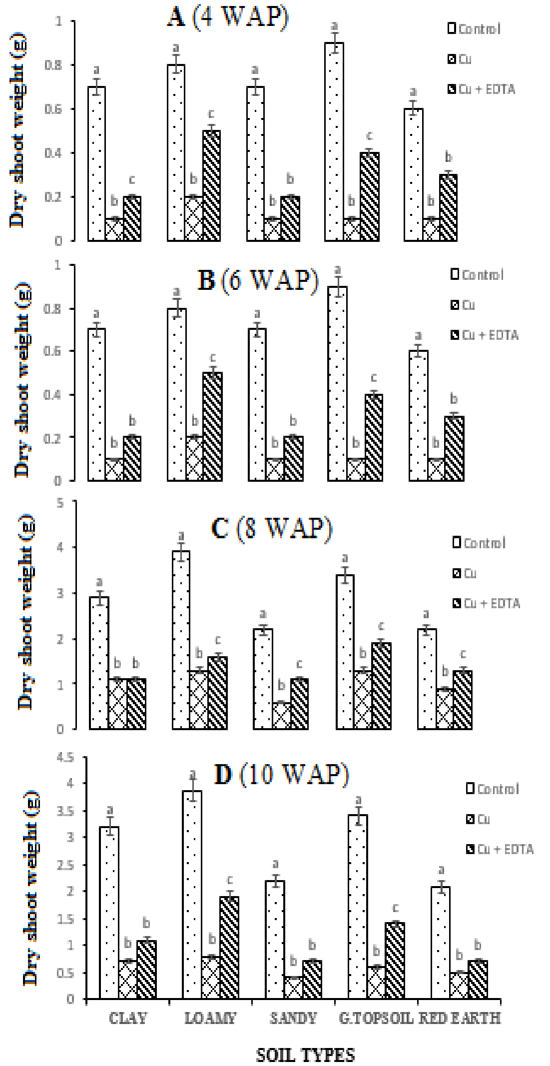

Fig. 9: Dry shoot weight (g) at 6 (A), 10 (B) and Dry root weight at 6 (C) 10, (D) weeks after planting of *Celosia* treated with Cu and chelate in 5 types of soil. Each interval represented by similar letters are not significantly different (P=0.05) according to Tukeys multiple comparison test.

They can contain 2.36-3.08% of humus under natural conditions. However, the organic matter contents of the studied soil samples is less than 2%, which may be due to intensive human activities in this area. Organic complexing agents promote the biological availability of heavy metals in the soil Stefanovits *et al.* (1999). Organic matter is important for the retention of metals by soil solids, but in this study, it decreases metal mobility and bioavailability. Thus, an increase in organic matter reduces metal availability. Clay soils and loamy soil had high copper availability, but their high organic matter content reduced the mobility and availability of this metal, while sandy soil had low organic content and high metal mobility.

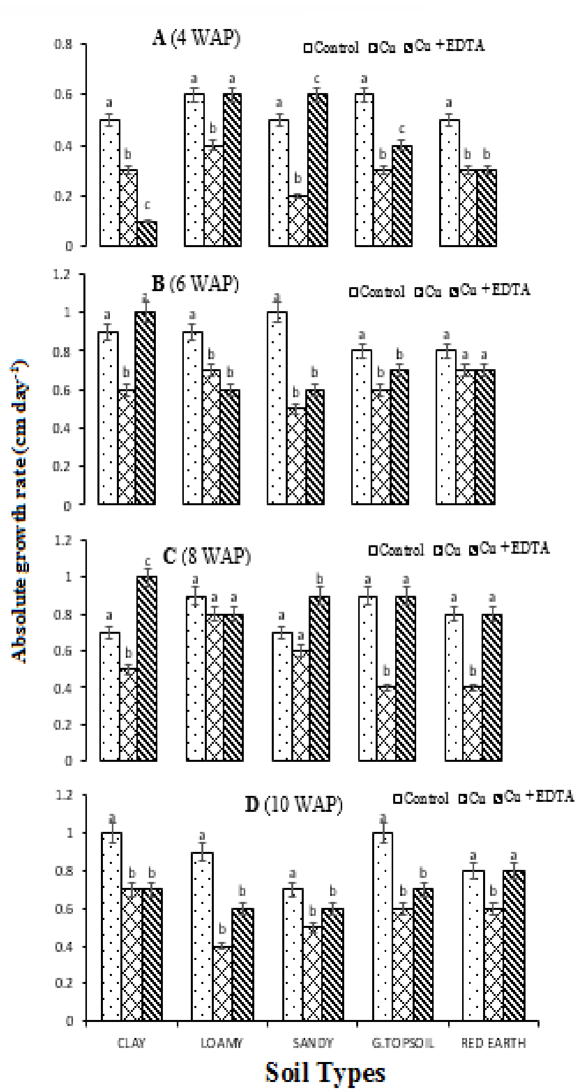

Fig 10: Absolute growth rate (cm day^{-1}) of *Celosia* treated with Cu and chelate in 5 types of soil at 4(A), 6 (B), 8 (C) and 10 (D) weeks after planting. At each harvest treatments represented by similar letters are not significantly different (P=0.05) according to Tukeys multiple comparison test.

The pH of the soil samples varied within a narrow range, the average value was neutral or slightly alkaline and only one soil sample had a slightly acidic pH value. Sandy soil had the highest alkaline pH. Soil pH is important factor in the mobilization of heavy metal, as acidity influences the solubility colloidal stability and the cation binding capacity, as well as microbial activity (Szabó *et al*. 2006; Szalai, 2008). Cavallaro and McBrode, (1984) reported that the proportion of copper in the soil solution increased as pH decreased. Copper toxicity is therefore more common in acidic soils. *Celosia* seedling emergence date was strongly influenced by soil type. *Celosia argentea* seed sown on clay soils generally had the lowest germination rates. Seedling emergence was faster in sandy soil. This may be due to the physical and chemical properties of clay. Dey (2001) highlighted that clay can pose a major challenge to germination and emergence due to its poor drainage, and it tends to clod easily. Seeds sown in sandy soil outperformed seeds sown in all other soil types in term of emergency. The results are similar to that of Okello and Young (2000) who reported *A. drepanolobium* seed germination was higher in red sand soils than in the clay loam. Sand contains loosely aggregated particles that allow the free exchange of gases between the germination medium and the embryo. Sandy soil is the best germination medium for vegetable seeds due to its nutrient availability, low water retention and suitability for vegetable seeds (Spicer *et al*., 2004). Copper was considered as trace element at low concentrations and maximum seed germination were observed at 500 $mgCuL^{-1}$ (first application) in all soil types.

Plants grown on garden top soils and loamy soil performed well with both Cu and EDTA, which may indicate that loamy and garden topsoils have better soil properties and microbial loads than others. The results confirmed the findings of Odiaka (2001) that *Telfairia occidentalis* grown on clay soils were less healthy and significantly lòwer height, shoot length and leaf area than those grown on humus and loamy soil respectively. The root length of *Telfairia occidentalis* growning in sandy soil was also observed to have fewer roots. This finding is consistent with the work of Abdulazeez (2017) who studied the effects of soil texture on vegetative and root growth of *Senna obtusifolia* seedlings from Bichi, Sudan Savannah of Northern Nigeria, under greenhouse conditions. He reported that humus and loamy soil supported the growth of the plants because humus and loamy soils had the highest organic matter and nitrogen content, also had the highest proportions of phosphorous and potassium. In contrast, the clayey soil exhibited the second-best performance, while sandy soil had the least. There was a drastic reduction in the growth parameters such as leaf area, number of leaves, plant height, root dry weight and shoot dry weight, over control. The present result has been supported by Shen *et al*. (1998) who confirmed that Cu and Zn toxicity can have multiple direct and indirect effects on all physiological and metabolic processes in plants. Although *Celosia argentea* is sensitive to high concentrations of Cu, the impact was not observed at the first application of non-chelated copper in some soil types. Conversely, no apparent symptom was detected in plants growing on the loamy soil and garden topsoil. Chlorosis initially appeared in the older leaves and moved progressively up to the youngest leaves at increased application of both Cu and chelated Cu in all soil types. Root tips exhibited a reddish-brown colour. Plants exposed to toxic concentrations of Cu in red earth soil showed more pronounced lateral root arrest, stunted growth and discolouration compared to plants grown in clay soil.

Plants grown in sandy soils treated with Cu and chelated Cu exhibited a significantly reductions in all growth stages, which may be a result of the high mobility of Cu in sandy soils. The application of EDTA has been showed to increase metal availability due to its chelating ability (Evangelou and Marsi 2001, Zhang *et al* 2008). Plants grown in garden topsoil, red earth and clay soil showed few foliar symptoms when exposed to Cu toxicity, but maximum effects were observed after the second application at six weeks. This confirms previous work reported by Karataglis and Babalonas (1985), who reported that plant height, shoot and root biomass, and flower and fruit production decreased with increasing Cu concentration. Although Cu toxicity significantly reduces plant growth and economic value, the presence of EDTA in the medium has been showed to effectively ameliorate growth reduction due Cu toxicity by increasing plant physiological growth parameters. This may be as a result of formation of chelate with Cu^{2+} that reduces the toxic effect of Cu (Habiba *et al.* 2015; Ebrahimi, 2014; Lambrechts, *et al.*, 2011). The negative impact of Cu toxicity in *Celosia argentea* can be overcome by the application of EDTA, which increases plant growth and biomass accumulation. Cu toxicity causes significant reduction in the plant growth and biomass. Concentration Copper concentrations in *Celosia* were generally above the FAO/WHO vegetable acceptable level which of 4.0 mg/100 g dry weight for copper. The study concluded that the use of copper contaminated soil for growing crop vegetables increased the concentration of copper in their tissues. Thus, it was shown that the presence of 5 mM EDTA in the medium effectively alleviates growth reduction induced by Cu toxicity in *Celosia argentea* plants by increasing the physiological growth parameters of the plant. In this experiment, loamy soil was considered a suitable soil for growing *Celosia argentea* in a copper-toxic environment. Therefore, farmers and the public need to be aware of the potential environmental consequences of using copper-contaminated soil to grow vegetables and other crops. *Celosia*, which grows in a variety of copper-contaminated soils is therefore dangerous to human health.

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