PHYSIOLOGICAL RESPONSES OF MAIZE (*Zea mays* L.) TO COPPER (Cu) INDUCED STRESS

ABSTRACT

Z. mays L. is among the most essential staple crops globally. It has multifunctional uses both as food; feed as well as for other industrial purposes. Pollution of soil by heavy metals is however a huge factor limiting the optimal growth of this crop. The purpose of this study was to investigate the physiological responses of maize when induced with copper (Cu) at five different concentrations (0%, 39%, 77%, 115% and 154%) respectively. The plant material was acquired from Agricultural Development Program, Rumuodumaya, Port Harcourt while soil was sterilized and analyzed using standard protocols. Planting was done under controlled environment. After 1 week of planting, growth parameters such as Plant height, number of leaves, Leaf length and leaf area were recorded at weekly interval for 9 weeks. The study revealed that growth responses of the maize studied decreased as the concentration increased for copper (Cu). Higher treatments at 115% and 154% resulted in mortality of the maize at week three. There were conditions where growth performances fluctuated with variation in the treatments and there was also reduction in chlorophyll content as the concentration increases. It is therefore suggested that high concentration of heavy metal is detrimental to the growth of maize plant and the level of negative effect relies on the span of exposure.

KEYWORDS: Physiological, Maize, Copper, Stress

INTRODUCTION

Zea *mays* L. with the common name as maize is one of the species of the Poaceae family an annual crop with deep root, grows in warm weather and abundant moisture for optimum development. It grows from a seed with a stalk of about 2-20 feet depending on growth situation and type. The stalk ends in the staminate flowers. The nodes and smooth leaves are connected at the stem. Suckers or shoots arises from the stem's bottom where many seed are generated. The flower organs, which produce to grain kernels, are bounded by layers of papery tissue known as husks. Most varieties of corn require 80 to 140 days from seeding to full ripeness of the kernel. There are assorted shapes and sizes of Corn kernels or seeds. *Z. mays* is moderately cheap compared to most cereal food crops like wheat and rice hence occupies a prominent place in the Agricultural Development Agencies in most African Countries. All component of the plant are edible. Moreover, it is a basic ingredient in some indigenous drinks and food products. Maize is a key source of protein, iron, carbohydrate, minerals and vitamin B. The crop can also be prepared together with other foodstuff to give a variety of food. The grains also have high content in carbohydrate, vitamins A, C and E, and Minerals, and has protein (9%).

Zea mays is amongst the world's most important cereal crops [1,2,3,4]. Maize is an extremely versatile cereal which is valuable to man in different ways. It is essential in relation to its nutritional significance for both humans, livestock and also has various industrial applications. Maize plant is capable of adapting to various climates; moreover it is most widely dispersed across the globe compared to other local cereal crops [5,6]. The crop can possibly grow in diverse kinds of soil in Nigeria [7], which has an increased rate of weathering; mostly loamy which is regularly low in transferable bases and ability of exchanging cation.

Heavy metals accumulation due to pollution from industries causes serious soil degradation and rigorously inhibit plant growth and yield leading to Agricultural loss and is also harmful to human when it goes into the food chain [8,9,10]. [11] noted that abuse of pesticides and chemical fertilizer contributes largely to high level of heavy metal found in the soil. The harmful property of heavy metals depends largely on the extent of exposure of the plant to the heavy metal and the physical as well as ion's chemical characteristics to bypass the physiological obstruction [12]. Studies have shown that the injurious effect of heavy metal is noticeable as oxidative stress, as a consequence of the generation of free oxygen radical [13]. Copper is amongst the major nutrients needed by maize plant for adequate growth however excess of these nutrients may be detrimental to this plant species. A higher concentration leads to undesirable effects which include growth reduction in the plants resulting to death of the plant [14]. Pollution of Copper is common around the globe. Accumulation of this heavy metal in different parts of plant could hamper photosynthetic mechanism. Extreme quantity of copper (Cu) in soil influences the absorption of vital nutrients [15,16]. The elevated concentration of Copper (Cu) in addition generates the functional oxygen group which activates the oxidative effects and disrupts the permeability of cell membrane [17].

Copper plays major role in many fundamental physiological activities of plants and also act as catalyst in redox reaction in the chloroplast, cytoplasm and mitochondria of cells [18] or as an electron transporter throughout plant respiration [19]. Copper however becomes deadly once its concentration in plant tissue increases above best levels [20]. It is present in various forms in soils it is however readily used up by plants as $Cu2^{+}$ (21). The amount of Cu in the soil is usually between 2 -250 $\mu g \cdot g^{-1}$ and strong plants can take up 20–30 $\mu g \cdot g^{-1}$ DW [22].

Although presence of Cu depends largely on the soils pH and its phytoavailability rises as the pH decreases [23]. In accumulation, absorption of Cu and its detrimental effect relies on nutritional state of plant, concentration of the soil Cu^{2+}, duration of exposure, and genetic makeup of a species [24]. Various scientific studies like [25] in *Chloris gayana* Knuth [26,27,28] in some species of the Genus *Eucalyptus* show that Copper has a tendency of being built up in the root tissues with modest movement upward in the bearing of the shoots. As a consequence, the primary categorization of destructive effect of Cu is the obstruction of elongation of root and growth. The resultant symptoms or signs are leaf discoloration, necrosis and chlorosis [20]. The excess Cu has the capacity of becoming attached to the sulfhydryl group of the membrane of cells or trigger peroxidation of lipids, which results in membrane injury along with free radicals production in different parts of plant organelles [14]. This study therefore aims at evaluating the physiological responses of *Zea mays* to copper (Cu) induced stress.

MATERIALS AND METHODS

Description of study area

The research was carried out in the Department of Plant Science and Biotechnology Screen House, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.

Sources and collection of materials

Maize seeds (Oba 98) were obtained from Agricultural Development Program Rumuodumaya, Rivers State, and Port Harcourt, Nigeria. Copper (Cu) was obtained from Copper (II) Sulfate Penta hydrate which was of analytical grade.

Soil sample collection and sterilization

13 kg of soil sample (loamy soil) used for the research was collected and sterilized at the Department of Crop and Soil Experimental farm, Faculty of Agriculture, University of Port Harcourt, Rivers State before it was transferred into the planting buckets and were taken to the Screen House.

Determination of the physicochemical properties of the experimental soil

The physicochemical properties of the experimental soil were determined using atomic absorption spectrophotometer.

Determination of heavy metals

10ml of well mixed Perchloric, nitric and Sulphuric was added to 1g of dried soil sample which was heated using heating mantle for 10-20mins. It was allowed to cool and 20ml of distilled water was added and solution was subjected to boiling to bring metal into solution. After cooling the solution was filtered using whatmann filter paper into 100ml standard flask. And it was relocated to 100ml plastic container. The samples were then measured using AAS (Atomic Absorbance Spectrophotometer).

Soil pollution, planting of seeds and measurement of growth parameters

Soil was polluted with Copper (Cu) at 0g, 5g, 10g, 15g and 20g which gave the concentration 0%, 39%, 77% 115% and 154% w/w. The soil was homogenized with the pollutants and then was allowed for three weeks (21 days) before planting was done. Five seeds were planted per bucket with five replicates using Randomized Complete Block Design (RCBD) at the depth of 4cm and were watered every 2 to 4 days throughout the experimental periods. Data collection took effect from second week and various growth parameters measured included plant height, leaf length; number of leaves, and leaf width.

Chlorophyll Content determination

0.1g of sample was weighed and added into a vial containing 10ml acetone. The young plant were harvested from the screen house about three weeks old and some amount of acetone was introduced into the curvet to clean off every other chemical that would have altered the result and also to prepare the cuvette and the spectrophotometer for better result. 0.1g sample was weighed and added into a vial containing 10ml of acetone and kept under room temperature for 48hours (2days) to facilitate visibility of the green pigment thereafter the extract or the chlorophyll content was introduced or poured into the cuvette and was determined or read using the Spectrophotometer with different wavelength of 660nm and 643nm respectively. The acetone helps to increase the appearance of the chlorophyll determination. Spectrophotometer is the instrument utilized to determine the chlorophyll content.

Chlorophyll-mg/c (Ch) was calculated following the formula beneath;

12 x Abs at 660 + 16.8 x Abs at 643 x 100

Statistical analysis

The data were analyzed using One-way analysis of variance (ANOVA) to determine the significant difference within factors. Also, multiple comparisons were employed to determine significant disparity between paired factors. Descriptive statistics like mean and standard deviation were also used.

RESULTS

The result of the physicochemical properties of the experimental soil and heavy metals shows that pH was 4.97, electrical conductivity 51.2, Total Nitrogen 0.086%, Zinc 0.234mg/kg and Copper 0.105mg/kg (Table 1).

Table 1: Result of Soil sample analysis and heavy metals

Parameter/Unit	Method	Sample 1-UTS
Physiochemical		
pH	**ASTMD1293B**	**4.97**
Electrical conductivity	**ASTMD1125**	**51.2**
Total Nitrogen %	**ASTME258**	**0.086**
Phosphorus, mg/kg	**ASTMD5198**	**16.43**
Cation Exchange Capacity, meq/100g		**1.806**
Cations, mg/kg		
Potassium	**API-RP45**	**2.374**
Heavy metals, mg/kg		
Zinc	**API-RP45**	**0.234**
Copper	**API-RP45**	**0.105**

The results of the chlorophyll content determination shows that as the concentration of the Copper (Cu) increases there was reduction in the chlorophyll content and 77% Cu had the lowest chlorophyll content of 411.0mg/Kg (Table 2).

Table 2: Results of Chlorophyll content determination

S/N	Concentration	Total chlorophyll (Mg/kg)
1.	**0% of Cu**	**1758.0**
2.	**39% of Cu**	**802.0**
3.	**77% of Cu**	**411.0**
4.	**115% of Cu**	**483.0**
5.	**154% of Cu**	**974.0**

The results of the growth studies shows that as the concentration of the heavy metal (Cu) increases there was reduction in the growth performances of *Zea mays* and at 115% and 154% resulted in complete mortality of the crop from week 3 (Figures 1-4).

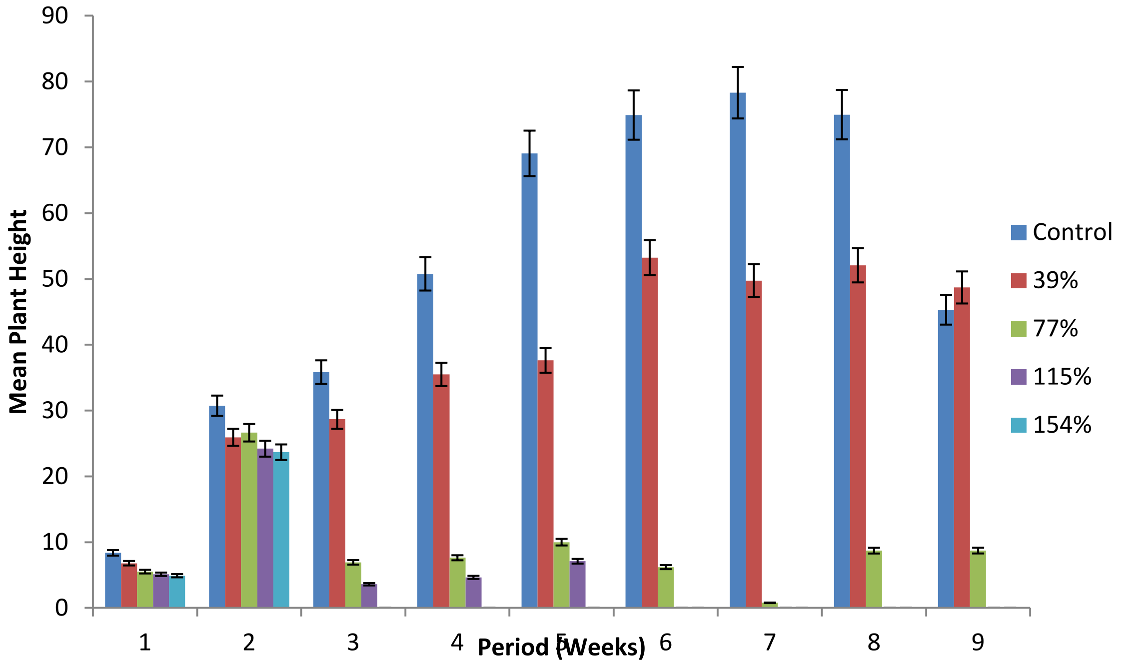

Figure 1: Effect of Copper (Cu) induced stress on mean plant height of *Zea mays* at 1-9 weeks after planting (WAP)

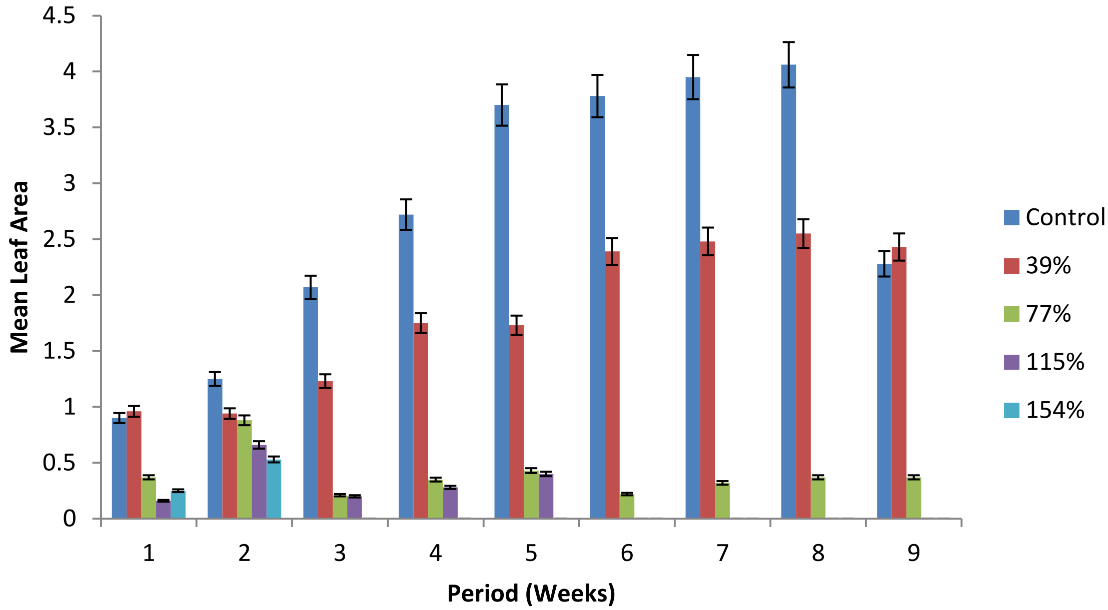

Figure 2: Effect of Copper (Cu) induced stress on mean leaf area of *Zea mays* at 1-9 weeks after planting (WAP)

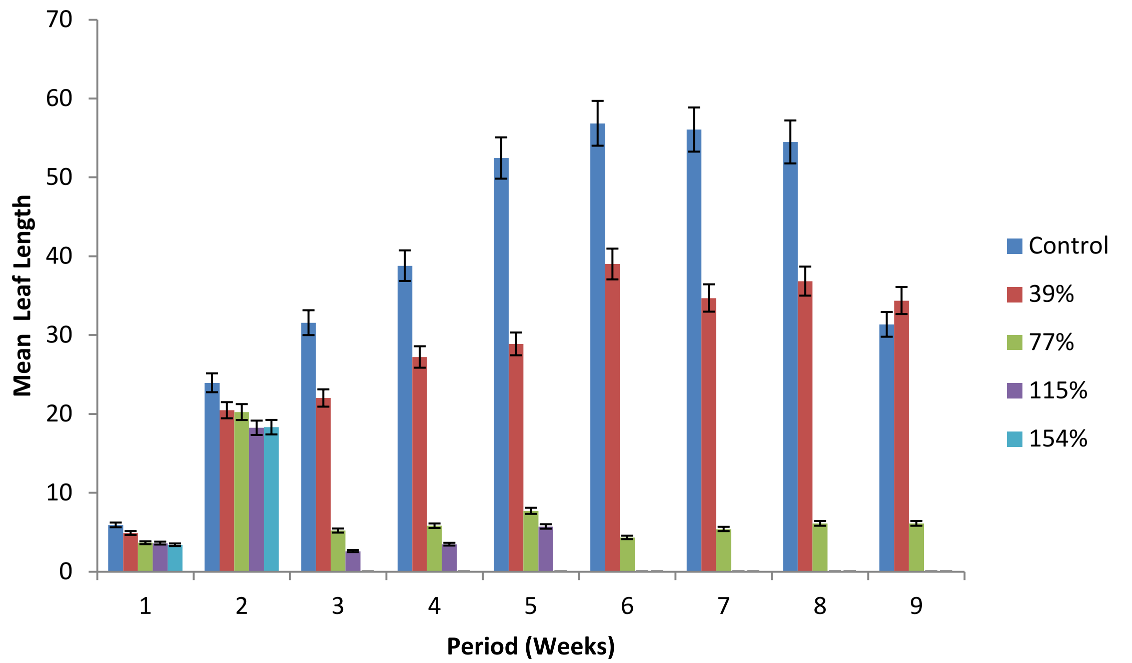

Figure 3: Effect of Copper (Cu) induced stress on mean leaf length of *Zea mays* at 1-9 weeks after planting (WAP)

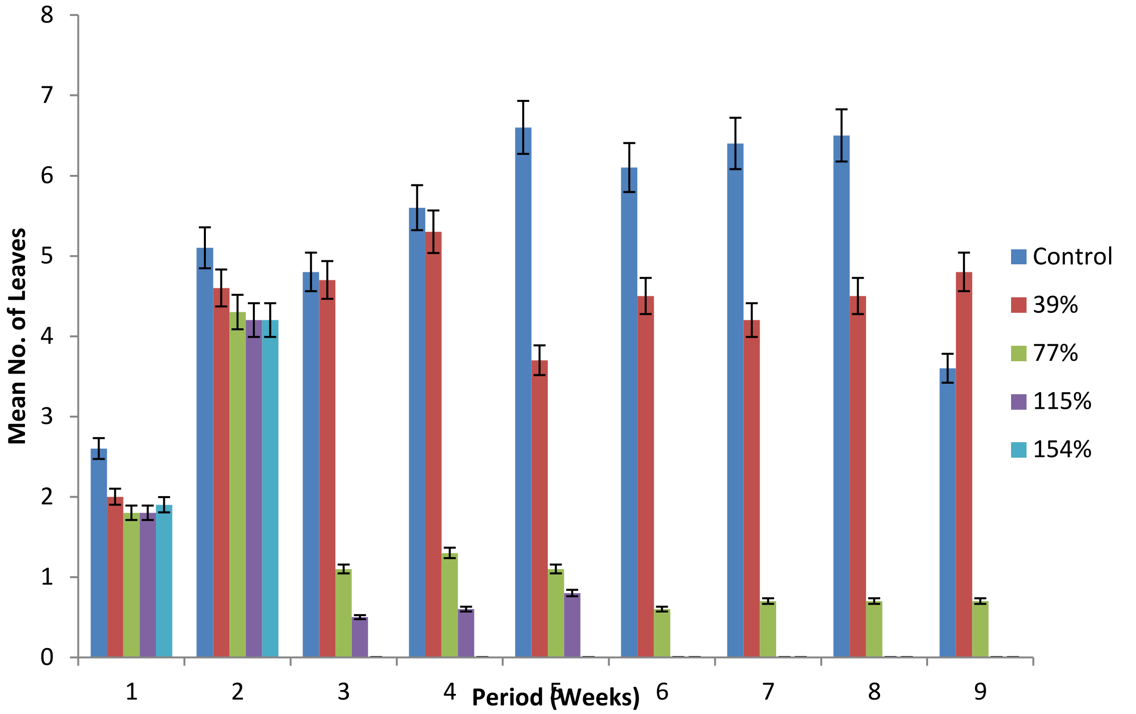

Figure 4: Effect of Copper (Cu) induced stress on mean number of leaf of *Zea mays* at 1-9 weeks after planting (WAP)

DISCUSSION

From the result it has been shown that Copper (Cu) is essential micronutrient for normal plant growth and development, but it could be poisonous either reversibly and irreversibly to changes in metabolism, known to respond to a good number of stressors such as natural and xenobiotic [29]. The outcome of this research proves that when maize was induced with Cu at various concentration of 0%, 39%, 77%, 115% and 154%, there were momentous differences in the growth performance. It was noticed that growth rate reduced as the concentration increases which is in accordance with an investigation by [30] and [16]. Copper (Cu) induce stress significantly (5%) reduced the growth performance of maize with increased concentration compared to the control.

The mortality at 154% concentration could be as a consequence of elevated concentration of the metals [15] which probably implies that excess of metals are detrimental to maize plant [31]. Hence consequences of the accumulated Copper (Cu) in various part of the maize plant disrupted the photosynthetic machinery due to direct role in photosynthetic pigment and protein [32]. In addition excess Copper (Cu) causes discoloration, necrosis, and leaf chlorosis. Crops cultivated on soil with an elevated content of Copper (Cu) are characterized by inhibited growth, reduce transpiration, chlorosis of leaves, limited seed germination [33] and the consequences are stronger during developmental stages. One key role of Copper (Cu) is that they are essential for proper functioning of plants [34].

This research further shows that as the concentration of the Copper (Cu) increases there was reduction in the chlorophyll content. This is in line with the works of [35] who reported that the chlorophylls contents (Chl *a* and Chl *b*) and carotenoids of *Brassica napus L*. noticeably reduced upon exposure of this plant to 6 μmol-dm^{-3} treatment of Cu. Several reports exist concerning reduction in chlorophyll content as a result to heavy metal toxic activities in plants [36,32, 33, 34].

Cu is an essential micronutrient that participates in many vital physiological functions of plants including acting as a catalyser of redox reaction in mitochondria, chloroplasts, and cytoplasm of cells [18] or as an electron carrier during plant respiration [20]. However, Cu becomes toxic when its concentration in the tissue of plants rises above optimal levels [[21]. In addition, uptake of Cu by plants and its toxicity are contingent on nutritional condition of plant, Cu^{2+} concentration in soil, length of exposure, and genotype of a species [24]. Therefore, the initial characterization of Cu toxicity is the hindrance of root elongation and growth [36]. The subsequent symptoms include chlorosis, necrosis, and leaf discoloration [20]). Decreased photosynthetic competence, low quantum efficiency of PSII, and reduced cell elongation are also associated with Cu toxicity [27]. These trends have been observed in various levels of Copper applied to different plants. Moreover, the results obtained with rapeseed (*Brassica napus* L.) indicated that content of chlorophylls (Chl *a* and Chl *b*) as well as carotenoids was markedly dropped when this plant was exposed to 6 μmol·dm^{-3} concentration of Cu (35].

CONCLUSION

Maize is a staple crop and one of the most imperative both for consumption and for industrial uses globally. Adequate nutrition of this crop is a requirement for its optimal growth whereas excess of these nutrients is disadvantageous to the plant. Findings from this research demonstrates that appropriate amount of nutrient such as Copper (Cu) potentially to enhance maize growth however, excess amount of this nutrient hinder the growth. The morphological examination of the plant indicated that growth rate reduced as the concentration of the heavy metal increased and the chlorophyll content also reduced with increased concentration of Copper (Cu).

REFERENCES

1. Tollenaar M, and Lee, E. (2002). Yield potential, yield stability and stress tolerance in maize. *Field Crops Res* 75, 161-169.

2. Shahab Q, Afzal M, Hussain. B. , Abbas N., Hussain. S. W, Zehra, Q, Hussain. A, Hussain Z, Ali. A, and Abbas Y. (2016). Effect of different methods of zinc application on maize (Zea mays L.). *International Journal of Agronomy and Agricultural Research (IJAAR)* 9,(3), 66-75.

3. FAO. 2011. FAO Statistical Year. http://faostat.fao.org. Accessed. 12/11/2018

4. Eteng, E.U., Asawalam, D.O. and Ano, A.O. (2014). Effect of Cu and Zn on Maize (*Zea mays L*.) Yield and Nutrient Uptake in Coastal Plain Sand Derived Soils of Southeastern Nigeria. *Open Journal of Soil Science*. 4, 235-245.

5. Ibeawuchi, I. I., Matthews-Njoku, E; Oerfor, M. O; Anyanwu, Chinyere P and Onyia; V. N. (2008). Variation in the harvest index of tropical maize: evaluation of recent evidence from Mexico to Malawi. *Annals of Applied Biology*. 138: 103–109. 13.

6. Ayisi, K.K and Poswall M. A.L. (1997). Effect of plant population on leaf area index, cob characteristics and grain yield of early maturing maize cultivars. *Europ. J. Agron*.16: 151-159.

7. Chude, V.O., Malgwi, W.B., Amapu, I. Y. and Ano, A.O. (2004). Manual on Soil Fertility Assessment. Federal Fertilizer Department/National Special Food Programme for Security, Abuja, 89.

8. Michael, W. H. A., Evangelou, K., Hockmann, R., Pokharel, A., Jako, A. and Schulin, R. (2012). Accumulation of Sb, Pb, Cu, Zn and Cd by various plants species on two different military shooting range soils. *Journal of Environmental Chemists*.108: 102-107

9. Metwali E.M. R., Gowayed S. M. H., Al-Maghrabi O. A and Mosleh Y.Y. (2013). Evaluation of Toxic Effect of Copper and Cadmium on Growth, Physiological Traits and Protein Profile of Wheat (*Titicum aestivium* L.), Maize (*Zea may* L.) and Sorghum (*Sorghum bicolor* L.) *World Applied Sciences Journal* 21 (3): 301-314

10. Youssef, M.M. and Azooz, M.M. (2013).Biochemical Studies on the Effects of Zinc and Lead on Oxidative Stress, Antioxidant Enzymes and Lipid Peroxidation in Okra (Hibiscus esculentus cv. Hassawi). *Sci. Int*. 1:29-38.

11. Bhatia, D. and Singh, K.(2012). Heavy metals remediation from maize (*Zia mays L*.) crop by the use of vermicomposts through vermicomposting by Eisenia fetida. *Am.Eurasian J. Agric. Environ. Sci.* 12:12151222.

12. Seregin, I.V. and Kozhevnikova, A.D. (2008). Roles of root and shoot tissues in transport and accumulation of cadmium, lead, nickel, and strontium. *Russ. J. Plant Physiol*. 55:1–22.

13. Gao, Y., Miao, C.Y., Xia, J., Mao, L., Wang, Y.F. and Zhou, P. (2011). Effects of coexisting plant species on soil enzyme activities and microbial community structure in multiple heavy metals contaminated soils: PCR-RAPD method. *Front Environmental Science and Engineering China.*

14. Chen, T., Zhang, Y.C. and Rossow, W. B. (2000). Sensitivity of atmospheric radiative heating rate profiles to variations of cloud layer overlap. *Journal of Clim.* 13:2941-2959.

15. Chen L.-M., Lin C. C., and Kao, C. H. (2000). Copper toxicity in rice seedlings: changes in antioxidative enzyme activities, H_2O_2 level, and cell wall peroxidase activity in roots. *Botanical Bulletin of Academia Sinica*. 41(2):99–103

16. Gupta, D. and Abdullah. (2011).Toxicity of copper and cadmium on germination and seedling growth of maize (*Zea mays* L.) seeds. *Indian Journal of Scientific Research*. 2: 67-70.

17. Patsikka, E., Kairavuo, M., Sersen, F., Aro, E.M. and Tyystjarvi, E. (2002). Excess copper predisposes photosystem II to photoinhibition in Vivo by out competing iron and causing decrease in leaf chlorophyll. *Plant Physiol*. 129:1359-1367.

18. Madejonb, P., Ramirez-benitezc, J.E., Corralesa, I., Barceloa, J. and Poschenriedera, C. (2009). Copper-induced oxidative damage and enhanced antioxidant defenses in the root apex of maize cultivars differing in Cu tolerance. *Environmental Exp. Bot*. 67:415–420.

19. Fargašová A. (2004). Toxicity comparison of some possible toxic metals (Cd, Cu, Pb, Se, Zn) on young seedlings of *Sinapis alba* L. *Plant, Soil and Environment*. 50(1):33–38

20. Yruela I. (2009). Copper in plants: acquisition, transport and interactions. *Functional Plant Biology*. 36(5):409–430.

21. Lombardi L and Sebastiani L. (2005). Copper toxicity in *Prunus cerasifera*: growth and antioxidant enzymes responses of in vitro grown plants. *Plant Science*. 168(3):797–802

22. Maksymiec, W. (1977). Effect of copper on cellular processes in higher plants. *Photosynthetica*. 1997; 34(3):321–342.

23. Azooz M. M., Abou-Elhamd M. F., and Al-Fredan, M. A. (2012). Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat (*Triticum aestivum* cv. Hasaawi) at early growing stage. *Australian Journal of Crop Science*. 6(4):688–694.

24. Nicholls, A. M., and Mal T. K. (2003). Effects of lead and copper exposure on growth of an invasive weed, *Lythrum salicaria* L. (Purple Loosestrife). *Ohio Journal of Science*. 103(5):129–133.

25. Sheldon A. R. and Menzies N. W. (2005). The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Knuth.) in resin buffered solution culture. *Plant and Soil*. 278(1-2):341–349.

26. Yau P. Y., Loh C. F.,and Azmil I. A. R. (1991).Copper toxicity of clove (*Syzygium aromaticum* (L.) Merr. and Perryl) seedlings. *Mardi Research Journal*. 19(1):49–53.

27. Alaoui-Sossé B., Genet P., Vinit-Dunand F., Toussaint M.-L., Epron D. and Badot P.-M. (2004) Effect of copper on growth in cucumber plants (*Cucumis sativus*) and its relationships with carbohydrate accumulation and changes in ion contents. *Plant Science*. 166(5):1213–1218.

28. Assareh M. H., Shariat A., Ghamari-Zare A. Seedling response of three Eucalyptus species to copper and zinc toxic concentrations. *Caspian Journal of Environmental Sciences*. 2008;6(2):97–103.

29. Aly, A. A. and Amal A. Mohamed, A. A (2012). The impact of copper ion on growth, thiol compounds and lipid peroxidation in two maize cultivars (*Zea mays* L.) grown invitro *Australian Journal of Crop Science. 6(3):541-549.*

30. Liu J, Wang J, Lee S, and Wen, R. (2018). Copper-caused oxidative stress triggersthe activation of antioxidantenzymes via ZmMPK3 in maize leaves. *PLoS ONE*. 13(9).

31. Peško M., and Kráľová K. (2013). Physiological response of *Brassica napus* L. plants to Cu (II) treatment. *Proceedings of ECOpole*. 7(1):155–161.

32. Baldantoni, D., Ligrone, R., and Alfani, A. (2009). Macro- and trace elements concentrations in leaves and roots of Phragmites australis in a volcanic lake in Southern Italy. Journal of Geochemical Exploration, 101, 166-174.

33. Calheiros, C. S. C., Rangel, A. O. S and Castro, P. M. L. (2007). Constructed wetland systems vegetated with different plants applied to the treatment of tannery waste water. Water Research, 41(8),

34. Bonanno, G., and LoGiudice, R. (2010). Heavy metal bioaccumulation by the organs of Phragmites australis (common reed) and their potential use as contamination indicators. Ecological Indicators, 10, 639-645.

35. Bragato, C., Schiavon, M., Polese, R., Ertani, A., Pittarelo, M., and Malagoli, M. (2009). Seasonal variations of Cu, Zn, Ni, and Cr concentration in *Phragmites australis* in a constructed wetland of North Italy. Desalination, 246, 35-44.

36. Tsay C. C., Wang L. W., Chen Y. R. (1995). Plant response to Cu toxicity. *Taiwana*. 40(2):173–181.

.