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GROWTH RESPONSE OF MAIZE (*ZEA MAYS* L.) TO METAL TOXICITY

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ABSTRACT

This study evaluated the phytotoxic effects of lead (Pb) and zinc (Zn) as $Pb(NO_3)^2$ and $Zn(NO_3)^2$ on maize (*Zea mays* L) considering the plants' growth indices, biomass as well as soil parameters and soil microorganisms after treatment. The results showed that plant growth significantly reduced with increasing concentrations (100,150 and 200 $mgkg^{-1}$) of Pb and Zn contamination. The results indicated significant inhibitory effect on the growth parameters (stem height, root length, leaf area, fresh and dry weight) measured, compared to control experiment. This study therefore revealed that the heavy metals have harmful effects on maize's growth. Consequently, the consumption of such contaminated cereal by man and animals can lead to their death after the metals have biomagnified in their systems. Lead and Zn application in general, affected the vegetative growth; both fresh and dry weights were reduced with increasing concentrations of the metals. These results show that these metals are toxic to human and animal health. As a result, farmers should be encouraged to use water from good source for irrigation and also cultivate on metal-free soils if possible to save lives.

Keywords: Biomass, Growth Indices, Heavy metal, Toxicity, Zea mays.

INTRODUCTION

Lead and Zinc are heavy metal pollutants among chromium, cobalt, copper, nickel and cadmium discharged through various chemical industries. These effluents are inadvertently used by farmers for irrigation which according to Haas and Brusca (1961) are stimulatory and inhibitory (Bracelo and Poschenrieder ,1985) to the crop plants. These industrial effluents contain a wide range of inorganic pollutants with heavy metals causing physiological disorders in living organisms. According to Arun *et al*., (2005), plant growth and development are essential processes of life and propagation of species. These life processes are continuous and mainly depend on external resources present in soil and in the air. In a study by Oladele *et al*., (2017), it was revealed that lead and zinc inhibited both seed germination and seedling growth especially lead. These effects were most significant on the roots compared to the shoots.

Maise (*Zea mays* L.), a typical cereal crop of temperate and subtropical zones is displayed as a variable stress tolerant plant under environmental extremities. Maize is known as an important crop plant for the grain and fodder. With all these in view, the present study will investigate the effect of different metal concentrations on the germination and growth of this important cereal; maize (*Zea mays* L), soil parameters and soil microorganisms.

MATERIALS AND METHODS

Dry seeds of cultivars of maize (*Zea mays*), (Accession ACR.91SUWANI-SRC1) were collected from the International Institute of Tropical Agriculture (I.I.T.A.), Ibadan. The maize seeds were surface-sterilized in 10^{-3} M $HgCl_2$ for 2 min (Azmat and Hasan, 2008), washed in distilled water and sown in different pots. The seedlings were treated with various concentrations of Pb and Zn (100,150 and 200mgkg^{-1}) as Pb $(NO_3)^2$ and $Zn(NO_3)^2$ 1 - 2 times per day for 8 weeks. These doses were decided on the basis of LD-50 and the regulatory limits. The parameters observed and measured were: Germination pattern, fresh weight (g), total dry weight (g) (Biomass), leaf area (cm^2), stem height (cm) and root length (cm) following methods used by Oladele *et al*., (2017).

Soil Analysis before and after Planting

The soil type, pH and Total Organic Matter were determined following the method by White (2006). The Total Nitrogen, Available Phosphorus and Moisture Content were estimated according to the procedure by AOAC (1990). The amount of Pb and Zn present in the soil was determined according to the procedure by Lone *et al*., (2008). The readings were taken from the equipment and the results were converted to actual concentration of metals in the samples.

Experimental Description

The soil was mixed thoroughly and then filled into 50 black cellophane bags. Four thousand grams of soil were placed in each bag. The bags were arranged in four (4) rows designated as control (untreated soil) and soil with metals. The experiment was carried out under a period of 60 days (Wu *et al*., 2003).

Soil Sample Digestion and Heavy Metal Determination in the Soil Samples using Atomic Absorption Spectrophotometer (AAS)

The soil samples were digested following method described by Lone *et al.,* (2008). The plant and soil samples were analyzed for lead (Pb) and zinc (Zn) accumulation (mgkg^{-1} DW) using Atomic Absorption Spectrometer (AAS) (Lone *et al.,* 2008).

Microbial analysis:

Population of microorganisms followed the method described by Anna *et al*., (2008).

Statistical Analysis

Results were analysed by using standard deviation as well as analysis of variance and tested for significance at 5% probability level using the Microcal origin 5.0 and Microsoft Excel

RESULTS

Percentage Germination

For maize treated with lead and zinc nitrate, germination decreased with increased heavy metal concentration. By the last day, the percentage germination was 75.0% and 62.5% in maize plants treated with 200 mg/kg of zinc nitrate and 200 mg/kg of lead nitrate respectively.

Growth Indices:

Leaf Area/Size (cm^2)

Table 1 shows the effect of different concentrations of lead and zinc nitrate on leaf area of maize seedlings. Leaf area increased with time (in days) in each treatment concentration whereas it decreased as the concentration of the heavy metals increased. It was observed that zinc had more inhibitory effect on the leaf area compared to lead. By the last day, the highest leaf area was observed in 100 mgkg^{-1} of lead with 44.60±2.32 cm^2 compared to control of 43.00±1.11 cm^2

while zinc at 100 $mgkg^{-1}$ was 32.30±0.72 cm^2. Statistical analysis revealed that the treatment effects on the leaf area were significantly different ($P<0.05$) from control for all treated maize plants.

Stem Height (cm)

Table 2 illustrates the effects of different concentration of Pb and Zn on stem heights of experimental seedlings. The stem height increased with time (in days) in each treatment concentration while it decreases as the treatment concentration increased. Statistical analysis revealed that the treatment effects were highly significant ($P<0.05$) on stem heights for the test plants. The untreated seedlings (control) of maize were observed to have the highest mean stem height (cm) of 14.0 ±0.08 while seeds treated with 200mg/kg of Pb had the least stem height (cm) as 4.0±0.05 at the end of thirty days.

Root length (cm)

Table 3, shows the effect of different concentrations of Pb and Zn on root lengths of experimental seedlings. The control seedlings of maize were observed to have the highest mean root length (cm) of 17.10±0.45 while seedlings treated with 200mg/kg of Pb had the least root length (cm) as 7.0 ±0.48 at the end of the treatment. Statistical analysis revealed that the treatment effects were highly significant ($P<0.05$) on root lengths for maize plants when compared to the control plants. Generally, the growth rate of roots and shoots were found to be retarded with increasing concentrations of lead and zinc nitrate for the maize plants.

Table 1: Effect of Different concentrations of Lead and Zinc Nitrates on Leaf Area (cm^2) of Maize (*Zea mays* Accession, ACR.91SUWAN I-SRC1)

Days	Treatment Concentrations						
	Control	100mg/kg Pb	150mg/kg Pb	200mg/kg Pb	100mg/kg Zn	150mg/kg Zn	200mg/kg Zn
10	24.00 ±1.28	22.30±2.29*	20.20±2.03*	18.30±1.28*	20.40±1.22*	18.20±1.20*	12.10±0.21*
15	30.40±2.77	28.20±3.06*	24.00±3.10*	22.50±1.29*	22.10±1.07*	20.30±0.86*	14.20± 0.80*
20	34.50±2.09	32.50±2.17*	30.20±1.45*	26.45±2.17*	26.15±1.15*	22.40±1.10*	16.10±1.23*
25	38.20±1.03	38.30±1.19*	36.50±2.01*	32.35±1.24*	28.20±0.61*	24.35±0.07*	18.00±1.02*
30	43.00±1.11	44.60±2.32*	40.65±0.89*	36.00±1.19*	32.30±0.72*	26.50±0.04*	22.40±0.04*

data were expressed as mean ± sem

when * p<0.05 = significantly different from control and when p>0.05 = not significantly different from control

Table 2: Effect of Different concentrations of Lead and Zinc Nitrates on Stem Length (cm) of Maize (*Zea mays,* Accession ACR.91SUWAN I-SRC1)

Days	Treatment Concentrations						
	Control	100mg/kg Pb	150mg/kg Pb	200mg/kg Pb	100mg/kg Zn	150mg/kg Zn	200mg/kg Zn
10	15.00±1.75	6.00±0.21*	5.00±0.54*	4.00±0.52*	14.00±1.20*	12.00±0.83*	6.00±0.48*
15	20.00±3.07	8.00±0.72*	7.00±0.87*	6.00±0.44*	18.00±1.16*	14.00±0.87*	8.00±0.50*
20	28.00±2.11	11.00±1.10*	9.00±0.56*	8.00±0.45*	24.00±0.89*	18.00±0.92*	9.00±0.41*
25	32.00±1.04	13.00±0.89*	12.00±0.71*	9.00±0.52*	26.00±1.56*	20.00±0.65*	10.00±0.39*
30	35.00±2.48	16.00±1.04*	13.00±0.67*	10.00±0.48*	28.00±1.02*	22.00±0.88*	11.00±0.14*

data were expressed as mean ± sem

when * p<0.05 = significantly different from control and when p>0.05 = not significantly different from control

Table 3: Effect of Different concentrations of Lead and Zinc Nitrates on Root Length (cm) of Maize (*Zea mays,* Accession ACR.91SUWANI-SRC1)

Days	Treatment Concentrations						
	Control	100mg/kg Pb	150mg/kg Pb	200mg/kg Pb	100mg/kg Zn	150mg/kg Zn	200mg/kg Zn
10	14.00±0.30	3.50±0.97*	6.00±0.07*	5.00±0.09*	7.00± 0.18*	6.00±0.02*	5.00±0.30*
15	15.20±0.11	8.00±0.79*	7.00±0.32*	6.80±0.06*	7.50±0.27*	6.50±0.06*	6.00±0.02*
20	16.00±0.23	9.00±0.34*	8.20±0.45*	8.00±0.07*	8.00±0.03*	7.00±0.13*	7.00±0.12*
25	16.80±0.12	10.00±0.42*	8.50±0.16*	8.50±0.65*	9.00±0.04*	7.20±0.11*	7.50±0.17*
30	17.20±0.45	9.50±0.10*	8.00±0.03*	7.00±0.48*	9.80±0.62*	7.50±0.22*	8.00±0.59*

data were expressed as mean ± sem

when * p<0.05 = significantly different from control and when p>0.05 = not significantly different from control

Fresh and Dry Weights of Plants

Tables 4 shows the effects of different lead and zinc nitrate concentrations and augmentation on the fresh and dry weights of whole maize plants . The fresh weight of plants increased significantly with increase in lead treatment. The fresh weights of treated plants were significantly lower (P< 0.05) than the untreated plants (control). Maize control plants had 4.24±0.09 mean fresh weight (g), maize treated with 100mg/kg and 200mg/kg of lead concentration had a mean fresh weight (g) of 5.93±0.02 and 28.54±0.04 respectively. The same trend was observed for the different zinc concentrations but the effect was at a lower magnitude.

The dry weight of plants decreased significantly with increased lead and zinc supply for all treated plants (P< 0.05) when compared with the untreated plants (control). The dry weights of all treated plants were affected significantly at all concentrations. Statistical analysis showed that higher concentrations of the metals decreased dry weights of treated plants significantly (P< 0.05). Untreated maize plants (control) had 3.08±0.03 mean dry weight (g), maize treated with 100mg/kg and 200mg/kg of lead concentration had a mean dry weight (g) of 2.02±0.04 and 1.81±0.02 respectively. Statistical analysis revealed that higher concentrations of the metals decreased dry weights of treated plants significantly (P< 0.05). For Zn treatment, untreated maize plants (control) had 3.08±0.03 mean dry weight (g), maize treated with 100mg/kg and 200mg/kg of Zn concentration had a mean dry weight (g) of 3.10±0.03 and 2.22±0.03 respectively.

Table 4: Effect of Lead and Zinc concentrations on Fresh and Dry Weights (grams) of maize plant

Plants / Treatment concentration	Mean Fresh Weight (Unit)	Mean Dry Weight (Unit)
Control	4.24±0.09	3.08±0.03
100mg/kg Pb	5.93±0.02*	2.02±0.04*
150mg/kg Pb	10.21±0.03*	1.92±0.05*
200mg/kg Pb	28.54±0.04*	1.81±0.02*
100mg/kg Zn	6.13 ± 0.08*	3.10±0.03*
150mg/kg Zn	7.50 ± 0.03*	2.44±0.05*
200mg/kg Zn	11.13±0.04*	2.22±0.03*

data were expressed as mean ± sem

when * $p<0.05$ = significantly different from control

when $p>0.05$ = not significantly different from control

Microbiological Study

Tables 5 and 6 give a summary of the total microbial population for some soil samples and treatment. In Table 5, the bacteria that were isolated were identified as *Bacillus spp, Pseudomonas aeruginosa, Micrococcus spp, Corynebacterim spp* and *Flavobacterium spp.* They were gram stained. The different bacteria specie appears to be motile, rod shaped, robust, cylindrical and coccus under the microscope. Lead nitrate had significant effect on the microbial population when compared to the control. As seen in Table 6, the Fungi that were isolated were identified as *Fusarium spp, Aspergillus wentii, Penicillum spp, Aspergillus flavus, Aspergillus niger, Rhizopus spp, Aspergillus fumigatus.* The total populations were also noted. Generally, Pb had detectable effects upon the community diversity and population even at the lowest concentration tested when compared with Zinc. Analysis of sample populations suggested a substantial change in microbial population. The least fungal population was observed in soil treated with 200 $mgkg^{-1}$lead maize.

Table 5: Microorganisms isolated from some of the treated soil samples

Soil sample / Parameters	Bacteria Types (Implicated Organisms) and their Population					
	Bacillus spp	*Pseudomonas aeruginosa*	*Micrococcus spp*	*Corynebacterim spp*	*Flavobacterium spp*	Total Bacteria population
Control Maize	–	–	–	–	(Gram +ve) +	9.2 x 10^6 cfu/gram
200 $mgkg^{-1}$ Pb Maize	(Gram +ve) +	(Gram-ve) +	–	–	–	7.2 x 10^6 cfu/gram

key:

+ = present

– = absent

Table 6: Microorganisms isolated from some of the treated soil samples

Soil sample/ Para-Meters	Fungi Types (Implicated Organisms) and their Population							
	Fusarium spp	*Aspergillus wentii*	*Penicillum spp*	*Aspergillus flavus*	*Aspergillus niger*	*Rhizopus spp*	*Aspergillus fumigatus*	Total Fungal population
Control Maize	+	–	–	+	+	–	+	(2.2 x 10^6cfu/ gram)
200 mgkg^{-1} Pb Maize	+	–	+	–	–	–	+	(9.0 x 10^5 cfu/gram)

KEY:
+ = Present
– = Absent

DISCUSSION

The results above reveal that at higher concentrations of the metals (lead and Zinc), especially lead seed germination was affected. The plant's growth was significantly affected negatively due to these metals treatment. Several researchers also reported similar observations. According to Malkowski *et al*. (2002), Pb at 10,100 or 1000µM was found to reduce the growth of maize. Also, Fodor *et al*. (1996) reported that 10µM of Pb was observed to be toxic to cucumber (*Cucumber sativus L*.). Peter *et al. (*2007), in their study observed significant toxic effect of lead at 0.2µM on cowpea's shoot and 0.06µM on the roots respectively; causing severe reductions in growth.

Oladele *et al*. (2017), also had similar observation of the effect of lead and zinc on bambara nut; indicating significant growth inhibition. These results also correspond with the study by Kibria *et al*. (2009), that Pb causes the shoot and root weight of *A. gangeticus* to decline by 28 and 53% and *A. oleracea* 46% and 37% respectively compared to control at 100mgkg^{-1}, which was the highest rate of lead application. *Kopittke et al*. (2007), reported a significant effect of lead on cowpea. Mohamad and Emad (2010) also discovered that the relative growth rate of carrot (*Daucus carota*) was reduced at the different concentrations of Cd and Zn combined but showed insignificant effect with Zn treatment alone. They discovered that Cd was more harmful than Zn. Also, generally, this present study shows that Pb effect was more significant than that of Zn. The observed effect of the metals, may be described as possible interference of Pb and Zn metals with growth processes, especially in the roots; thereby inhibiting the growth of the entire *maize* plant. This present experiment reveals that metals influence the population size of soil microorganisms due to reduced microbial population in some treated soils especially those treated with highest concentration of lead compared to control. Rajapaksha *et al*. (2004) reported a similar effect of lead on soil micro-organisms. They observed in their own study that lead-contaminated soils contained lower levels of microbial biomass than the uncontaminated soils (control). This present study agrees with the work reported by Kuperman and Carreiro (1997) that heavy metals contaminated soils reduced plant biomass and soil microbial population. Other researchers like Hutchinson and Symington (1997) also reported measurable decline in microbial population in metal-contaminated soils. Masakazu and Nagumo (1997), also observed no-table adverse effect of copper on microbial biomass. Anna *et al*., (2008), as well observed significant effect of metal toxicity on fungal and bacterial activities. It is however, important to note that lack of several plant communities and reduced soil organic matter content due to effect of these metal-contaminants could have resulted in low microbial population.

CONCLUSION

This study has shown that at high concentration, Pb and Zn are highly toxic to living tissues (plants) especially Pb. Also, the study revealed that metal-induced stress affected soil microbes and plants 'growth indices negatively.

REFERENCES

Anna M.S, Maria,N., Ryszard, L .2008. Metals affect soil bacterial and fungal functional diversity differently. *Environmental Toxicology and Chemistry* 27(3):591-598.

AOAC.1990. Official Methods of Analysis. 15th Editionn., AOAC, Washington, DC., USA. pp: 200-210.

A**run, K.S, Carlos, C, Herminia, L, Avudainayagam,S.** 2005. Chromium toxicity in Plants. *Environmental International* : 31:739-753pp

Azmat, R. S. Hasan. 2008. Photochemistry of light harvesting pigments of Lens culinaris under Al stress. Pakistan. Journal of Botany, 40(2): 779-784.

Barcelo, I., Posxhenrieder, C. 1985. Effect of chromium (VI) on mineral element composition of bush beans. *Journal of Plant Nutrition.*8: 211-217pp

Fodor F., Sarvari E., Lang F., Szigeti Z., Cseh E., 1996. Effects of Pb and Cd on cucumber depending on the Fe-complex in the culture solution. *Journal of Plant Physiology*. 148(3/4): 434-
439.

Haas, A.R.C., Brusca, J.N .1961. Effects of chromium on Cirrus and Avocado grown in nutrient solution.*CIII. Agriculture .IS*:10-11

Hutchinson T.C., Symington, M.S. 1997. Persistence of metal stress in a forested ecosystem near Sudbury, 66 years after closure of the O'Donnell roast bed. *Journal of Geochemistry*.*Exploration*.58:323-330.

Kibria, M.G, Islam, M., Osman, K.T.2009. Effects of lead on growth and Mineral Nutrition of *Amaranthus gangeticus L*. and *Amaranthus oleracea L*. Soil and Environment 28(1): 1-6pp

Kopittke, P.M, Asher, C.J, Kopittke, R.A, Menzies N.W, 2007. Toxic effects of Pb^{2+} on Growth of cowpea (*Vigna unguiculata).Environmental Pollution* 150, 280-287pp

Kumar G., S. Kesarwani. 2004. Cytotoxic effect of mercuric chloride in root meristem of *Lens culinaris* . Medik. *International Journal of Mendel* 21(1-2): 41-42.

Kuperman, R.G, Carreiro, M.N.1997. Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. *Soil Biology*. *Biochemistry*.29:179-190

Lone .M.I, He, Z., Stoffella, P.J., Yang .X. 2008. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives, *Journal of Zhejiang University Science* **9**: 210-220.

Malkowski, E., A. Kita, W. Galas, W.

Karez., K. Michael .2000. Lead distribution in corn seedlings (*Zea mays* L.) and its effect on growth and the concentration of potassium and calcium. *Plant Growth Regulation*, 37: 69-76.

Masakazu, A., Nagumo, T.1997. Effect of heavy metal accumulation in apple orchard soils on microbial biomass and microbial activities. Soil Science. *Plant Nutrition*. 43:601-612.

Mohamad Abou Auda., Emad El Shakh Ali .2010. Cadmium and Zinc toxicity on growth and Mineral nutrients of Carrot (*Daucus carota*).*Pakistan Journal of Botany 42 (1): 341-351pp*

Peter, M.K, Colin, J.A, Rosemary, A.K, Neal, W.M .2007. Toxic effects of Pb^{2+}on Growth of Cowpea (*Vigna unguiculata*).*Environmental Pollution* .150, 280-287pp

Rajapaksha, R. M. C. P, Tobor-Kapłon, M. A., E. Bååth, 2004. Metal Toxicity affects Fungal and Bacterial Activities in Soil Differently *Applied and Environmental Microbiology*. 70 (5) 2966-2973pp

White R. 2006. *Principles and Practice of Soil Science: The Soil as a Natural Resource, 4th Edition* . Blackwell. **250-310pp**

Wu, F., Zhang, G., Dominy, P. 2003. Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. *Environmental and Experimental Botany,* 50: 67–78.

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