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RESIDUAL ACCUMULATION OF CHROMIUM (CR) AND COPPER (CU) AND THEIR TOXICITIES ON GERMINATION AND GROWTH OF THREE MAIZE (*Zea mays* L) VARIETIES

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ABSTRACT
High levels of heavy metals in polluted soils are common, and concerns about their possible hazards to humans, livestock, and crops cannot be overemphasized. This study was carried out to determine the effects of Chromium (Cr) and Copper (Cu) on germination and seedling growth of Sammaz, Oba super and Oba 98 maize (Zea mays) cultivars in order to determine their potentials as susceptibility (bioindicators) or tolerance (bioremediators). Treatments of the heavy metal ranged 2.88, 5.76, 8.63, 11.51, and 23.02 mg kg^{-1} for Cr, and 5000, 10000, and 15000 mg kg^{-1} for Cu, and no chemical treatment (control). Heavy metals from the plants were determined by Atomic Absorption Spectrophotometry (AAS) machine. All chemicals used had different levels of their effects on germination percentage (%) in the following pattern of increasing order of magnitude Cr >Cu. Initial seedling growth and development were strongly impeded by Cu, except Cr in all the three maize cultivars with a little bit development in the root and shoot. The concentration of Cr in the shoot of the three maize cultivars is in the order of Oba 98 >Oba Super> Sammaz while that of the root is in the order of Oba 98>Sammaz>Oba super. It can be concluded that the toxicity of Cr and Cu at the concentrations used in this experiment showed susceptibility of all the three maize cultivars and the toxic effects of these heavy metals. Thus, the maize cultivars could be bio-indicators of Cr and Cu toxicity.
Keywords; toxicity, chromium, copper, shoot, root, growth.

INTRODUCTION

Heavy metal contamination has received attention all over the world with comparatively little concern to address their risk assessment in developing countries particularly in the African continent (Nagajyoti *et al.,* 2010; Zeng *et al.,* 2013a; Awad *et al.,* 2014; Teng *et al.,* 2014; Tang *et al.,* 2015, Song *et al.,* 2017). Heavy metals are metallic components with a nuclear weight more than five and they are profoundly lethal even at low concentration (Järup, 2003; Yahaya *et al.,* 2020). Plants experience oxidative worry upon introduction to heavy metals that prompts cellular damage and unsettling influence of cell ionic homeostasis (Yadav, 2010).

Some plant species have capacity to grow in the metal contaminated soil and accumulate elevated amount of heavy metals (hyper-accumulation) as an eco-physiological adaptation in metalliferous (Singh, *et al., 2008*; Yugada *et al.,* 2018).

The presence of heavy metals in the soil as one of the major environmental stresses may cause retardation of plant growth and produce reactive oxygen species (Chehregani, and Malayeri, 2007; Deepesh*, et al.,* 2016).

Metal pollutants in soil may be absorbed by the plants through their roots and vascular system. Absorption of metals in soil could affect the ecosystem safety and cause a serious health risk to animals, plants, and human. High concentrations of metals in the plant could inhibit the ability of the plant to produce chlorophyll, increase the plant oxidative stress and weaken stomata resistance (Ashraf *et al.,* 2011; Sun *et al.,* 2009; Canivet *et al.,* 2015). Unnatural occurring heavy metals such as chromium (Cr) and cadmium (Cd) may suppress the growth of a plant, whether the pollution comes from soil or air (Street, 2012; Luo *et al.,* 2020). Heavy metals may enter human bodies through food chain, causing an increase rate of chronic diseases such as cancer (Ramadan and Al-Ashkar 2007) and affecting the central nervous system, especially in children (Zhao *et al*., 2009). Plants growing at the roadside may be exposed to high levels of metal pollution, especially vehicle emissions and trace content in the air. Heavy metal from traffic emission may accumulate in roadside plants from the soil (Feng *et al.* 2011).

Germination and early seedling growths have been regarded as critical phases, which are greatly influenced by stressful conditions (Shah, and Dubey, 1995). Growth changes are the first most obvious reactions of plants under stress. Heavy metals uptake and accumulation in plants have been shown to result in negative effects on plant growth (Breckle and Kahle, 1992; Nagajyoti *et al.* 2010, Krzesłowska, 2011; Deepesh *et al.,* 2016). Several plants such as *Amaranthus viridis L., Oryza sativa L., Vigna radiata L., Abelmoschus esculentus, Brassica juncea L.* (Vinod and Chopra, 2015), have been studied for their phytotoxic responses to chromium as well as for their phyto-remediation potential.

Heavy metals including Cu can affect the way land is used in the future because of their non-biodegradable nature. They can cause varying toxicities to plants and as such could affect vegetation growth (Chigbo and Batty, 2013). High concentrations of Cu in the environment pose a risk to plant species by reducing plant growth and photosynthesis as well as by inducing oxidative stress (Gunawardana *et al.,* 2011; Choudhury and Panda 2005; Sun *et al.,* 2009). In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair (Wang, and Shi, 2001). Plants such as maize species provide an alternative biomarker or bioremediation that can use for feasible investigation.

The main aim of this research is to determine the toxicities of copper, chromium zinc and lambda cyhalothrin on the germination and growth of three maize varieties viz SAMMAZ, OBA 98 and OBA Super with the following research question; Are SAMMAZ, OBA 98 and OBA Super maize cultivars susceptible to or tolerant to (bio indicators) of copper, chromium zinc and lambda cyhalothrin toxicity?

MATERIALS AND METHODS

Treatments

Five treatments were used in this experiment along with three cultivars of *Zea mays*, each treatment has three concentrations and three replicates for each concentration, and each replicates contains five seeds of *Zea mays* cultivar. The treatments includes;

Copper concentrations of 5gl^{-1} (5000mg/kg), 10 gl^{-1}(10000mg/kg), 15 gl^{-1} (15000mg/kg) and chromium concentration were, 25um (2.88mg/kg), 50um (5.76mg/kg), 75um (8.63mg/kg), 100um (11.51mg/kg), 200um (23.02mg/kg), (Yuguda *et al.,* 2018; Unitlab.com), water was used as control.

Collection and sowing of seeds

Plant material

Seeds of Oba 98, Oba Super and Sammaz were obtained from Bauchi State Agricultural Development program (BSADP)

Methods

Seeds of three maize (*Zea mays)* cultivars of Oba 98, Oba Super and Sammaz, were sterilized with 10% sodium-hypochlorite (NaOCl) solution for 20 min and washed with distilled water five times. The seeds were then soaked in distilled water for approximately 12 hour to accelerate seeds germination according to Kummerova, et al., 2012*,* and Benzinz, et al., (2013).

Seed germination, root and shoot elongation test were carried out on Whatman no 1 filter paper in plastic Petri dishes (110×20 mm).

5 seeds from each cultivar were placed on filter paper in the Petri dishes. Copper and chromium solutions were prepared in the required concentrations.

10ml of the solutions involved were added uniformly to each Petri dish, and 10ml of distilled water was used as a control. The petri dishes were sealed with parafilm and kept in the dark for 5 days (Di Salvatore, *et al.,* 2008, Kummerova, *et al.,* 2012). All the set up were run in triplicate. Final seed germination was determined by counting the number of seeds germinated in each treatment on the 5th day of planting, whereas seedling length of plumule and radicle were recorded. The pot experiment 1kg of sandy-loam soil were used from 0-20cm depth of the screen house vicinity, seeds of the three cultivars of *Zea mays* (Oba 98, Oba Super and Sammaz) were also sterilized as mentioned above before sowing with four replications, 65ml of prepared heavy metal (Copper and Chromium solutions) were added of the three different concentrations of heavy metal mentioned above in kilogram of soil respectively to each and control treatments were supplied with distilled water. Cu was in form of $CuSO_4.H_2O$ and chromium was in form of $CrKOSO_2.12H_2O$ respectively.

Digestion of samples

Plants (maize) were uprooted at six weeks after sowing, washed with running water to remove dirt, dust, and other contaminating agents. Samples were separated into root and shoot, and then dried at 70°C for 3 days (Ma *et al.,* 2001) and latter pulverised prior to further analysis and metal determination. One gram of plant sample (root and shoot) and soil were placed in 100ml conical flask separately, 1ml H_2SO_4, 1.2ml concentrated HNO_3 and 3.6ml concentrated HCl were added to the samples. The mixtures were digested at 95°C for 1hour till the solution became colorless (Adekiya *et al.,* 2018). The resulting solution were filtered to 20ml using deionized water and were analysed for Cr and Cu using Atomic Absorption Spectrophotometer (Perkin Elmer AAS-700) (APHA, 1990)were determined respectively.

Data collection

Numbers of growth were recorded after five days, the length of plumule and radicles were measured using a metre rule. Data collected were subjected to ANOVA using Minitab version 16 to determine the differences between and within the treatments and cultivars.

RESULTS AND DISCUSSION

The results of this research are presented in figure 1-2 and table 1-4. There was a remarkable decrease in percentage (%) germination as concentration of heavy metals in all the three maize cultivars with similar trend in effect, that shows the extent of inhibition of the developing embryo in these susceptible maize cultivars, this could be the effect of physiological process (Ranieri and Gikas, 2014), which may affect germination in general (Aly *et al.,* 2012). The highest percentage germination was recorded in the control of all the three maize cultivars and lowest germination percentage were observed on oba super and oba 98 treatment at 23.02mg/kg of chromium followed by oba 98 treated with 10000mg/kg and 15000mg/kg of copper. The percentage germination of all the three cultivars treated with chromium (Cr) ranged, 26.67-6.67% (Sammaz), 46.67-6.67% (Oba super), and 13.33-6.67% (Oba 98). Cu recorded, 26.67-6.67% (Sammaz), 13.33-6.67 (Oba super), only 20% for Oba 98.The work of Abdullah 2011, was similar to what was obtain in this research where seed germination and seedling growth were affected even at a lower heavy metal content than the one used in this study. The effect of Cr was reported by Hayyat *et al.,* (2015) and Nath *et al.,* (2008), which show inhibition on germination at high Cr levels. Previous works have also shown the effect of copper, and its effects on germination at comparatively lower concentration (0.03%, 0.003% and 0.0003%) than the one used in this study (Tomulescu *et al.,* 2004). Percentage germination of seeds of the three maize cultivars was significant ($p < 0.05$) in all the treatment as compare to control.

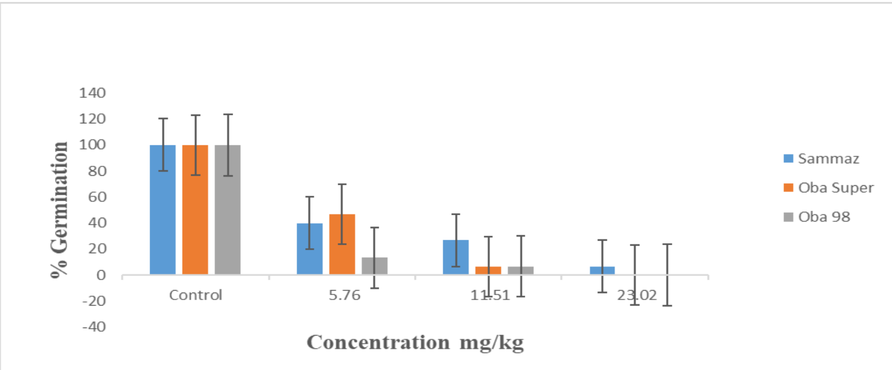

Fig 1. Effect of Chromium on % germination of three maize cultivars

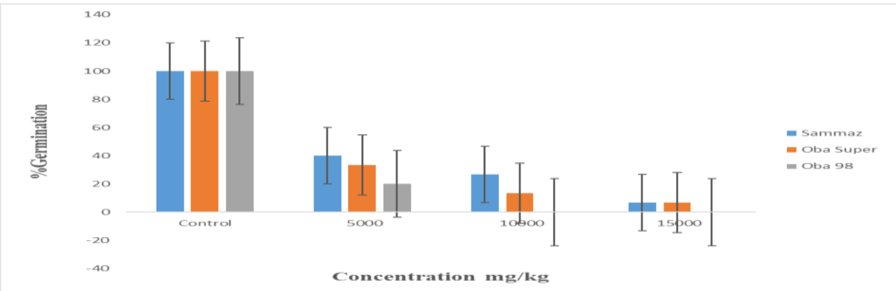

Fig 2. Effect of Copper on % germination of three maize cultivars

Effect of heavy metals on length of plumule and radicle of three maize cultivars

Tables 1-4 showed the % reduction on effect of copper and chromium at different concentration on radicle and plumule elongation of the three maize cultivars. The effect of the three level of Cr used on Sammaz, Oba super and Oba 98 showed a remarkable decrease in plumule and radicle length which significantly affect the development of plumule and radicle as compared with the control, ($p < 0.05$), this could be attributed to the effect of Cr on oxidative and enzymatic action and the morpho-physiological on the plant generally (Hayyat *et al.,* 2015; Anjum *et al.,* 2017). The results of this findings is similar with the findings of Nath *et al.,* (2008), where plumule and radicle were affected in all treatments with chromium increase concentration.

Even though, the three concentration (5000mg/kg, 10000mg/kg and 15000mg/kg) of Cu used were generally higher than that of Cr (5.76mg/kg, 11.51mg/kg and 23.02mg/kg), all the three levels were toxic and have virtually the same marked effect on the development of the plumule and radicle. Other factors that could be attributed to the effect of heavy metals on seedling growth may include inducement of chromosomal abbreviations and distorted mitosis (Liu *et al.,* 2003).

Table 1. Effect of Chromium on length of plumule of Sammaz, Oba super and Oba 98 maize cultivars

Length of plumule in cm				
Cultivars/$mgkg^{-1}/HM$	**CONTROL**	**5.76**	**11.51**	**23.02**
SAMMAZ	10. 54 ± 1.19 a	0.47 ± 0.13 (95.5) b	0.53 ± 0.05 (94.9) b	0.33 ± 0.12 (96.8) b
OBA SUPER	5.21 ± 0.57 a	0.90 ± 0.18 (82).b	0.33 ± 0.10 (93.6) b	0 (100) b
OBA 98	6.26 ± 1.12 a	0.33 ± 0.14 (94.7) b	0.30 ± 0.17 (95.2) b	0 (100) b

Means that do not share the same letter are significantly different.
Values in parentheses are percentage reduction in length of plumule.
($p < 0.05$)

Table 2. Effect of Copper (Cu^{2+}) on length of plumule of Sammaz, Oba super and Oba 98 maize cultivars

Length of plumule in cm				
Cultivars/$mgkg^{-1}/HM$	**CONTROL**	**5000**	**10000**	**15000**
SAMMAZ	10. 54 ± 1.19 a	1.13 ± 0.14 (89.2) b	0.87 ± 0.33 (91.7) b	0.17 ± 0.10 (98.3) b
OBA SUPER	5.21 ± 0.57 a	0.97 ± 0.16 (81.3) b	0.53 ± 0.31 (89.8) b	0.27 ± 0.15 (97.1) c
OBA 98	6.26 ± 1.12 a	0.67 ± 0.22 (89.3) b	0 (100) b	0 (100) b

Means that do not share the same letter are significantly different.
Values in parentheses are percentage reduction in length of plumule.
($p < 0.05$)

Table 3. Effect of Chromium (Cr^{3+}) on length of radicle of Sammaz, Oba super and Oba 98 maize cultivars

Length of radicle in cm				
Cultivars/$mgkg^{-1}/HM$	**CONTROL**	**5.76**	**11.51**	**23.02**
SAMMAZ	11.79 ± 1.65 a	0.60 ± 0.18 (94.9) b	0.23 ± 0.05 (97.9) b	0.37 ± 0.21 (96.9) b
OBA SUPER	6.33 ± 0.42 a	0.67 ± 0.11 (89.4) b	0.10 ± 0.06 (98.4) b	0 100 b
OBA 98	9.62 ± 2.12 a	0.87 ± 0.42 (91) b	0.03 ± 0.02 (99.7) b	0 100 b

Means that do not share the same letter are significantly different.
Values in parentheses are percentage reduction in length of radicle.
($p < 0.05$)

Table 4. Effect of Copper (Cu^{2+}) on length of radicle of Sammaz, Oba super and Oba 98 maize cultivars

Length of radicle in cm				
Cultivars/$mgkg^{-1}/HM$	**CONTROL**	**5000**	**10000**	**15000**
SAMMAZ	11.79 ± 1.65 a	0.27 ± 0.04 (97.7) b	0.63 ± 0.25 (94.7) b	0.07 ± 0.04 (99.4) b
OBA SUPER	6.33 ± 0.42 a	0.70 ± 0.15 (88.9) b	0.40 ± 0.08 (93.7) b	0.30 ± 0.06 (95.3) b
OBA 98	9.62 ± 2.12 a	0 (100) b	0 (100) b	0 (100) b

Means that do not share the same letter are significantly different.
Values in parentheses are percentage reduction in length of radicle.
($p < 0.05$)

Residual concentration of heavy metals in the root and shoot.

The effect heavy metals have on plants largely depends on the amount taken by the plant and this could cause stress during early development, the toxicity of some heavy metals could be so enormous that the development of seedlings is impeded; this was observed in this study with respect to copper. The effect of heavy metal on seedling growth reported in this study conforms to the findings of (Houshmandfar and Moraghebi, 2011). Chromium was the only heavy metal that was tolerated by the three maize cultivars with a little bit development in the root and shoot at the concentration used this may be attributed to the fact that Chromium III is insoluble in most soil condition (Smith *et al.,* 1992). The highest residual concentrations of Cr were recorded in the maize roots as showed in table 5, this result is suggestive of the high maintenance of Cr in the root despite the relatively low concentration used in the experiment, and this is similar to the findings of Lu *et al.,* 2015 and Yuguda *et al.,* 2018, who reported higher Cr concentration in the root of maize than in the shoot and other parts of vegetables due its does not exceed the permissible limits by WHO/FAO and its significant at ($p < 0.05$). However, findings on other heavy metals such as Zn show contrasting result with higher accumulation in the shoot than in the root, the difference in the higher accumulation of heavy metals in different part of the plant is determined by the direction of accumulation either from down to up or up to down. Generally the concentration of Cr in the shoot of the three maize cultivars is in the order of Oba 98 > Oba super> Sammaz while that of the root is in the order of Oba 98>Sammaz>Oba super. Tolerance to heavy metals depends on whether the plant is an accumulator which can take heavy metals from the soil and translocate from root to shoot resulting in the accumulation in the shoot system. On the other hand, it can be an excluder plant that inhibit entrance into plant and thus, restrict root to shoot translocation (Rascio and Navari-Izzo, 2014). Even though maize plants are known to be potentials accumulators of heavy metals (Li *et al.,* 2007).

Table 5. Chromium concentration of Shoot and Root of three maize cultivars

$MgKg^{-1}/HM$(Cr^{3+})	**Sammaz**		**Oba super**		**Oba 98**	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
Control	0.12 a	0.01 a	0.09 a	0.03 a	0.05 a	0.02 a
2.88	0.17 b	0.07 b	0.43 b	0.01 b	0.13 c	0.08 b
5.76	0.06 c	0.09 bc	0.53 c	0.06 c	0.06 ab	0.10 bc
8.63	0.03 cd	0.12 cd	0.63 d	0.12 d	0.06 ab	0.11 abc
Mean(-control)	0.12	0.09	0.53	0.06	0.08	0.09

Means that do not share the same letter are significantly different.
($p<0.05$)

CONCLUSION

The results of this research has revealed the toxicity effect of Cr and Cu at the concentration used in the experiment and showed that at those concentration these maize cultivars are highly susceptible and could be bio-indicators of Cr and Cu due to the marked effects on seedling growth and germination of the three maize cultivars used. It is worthy of note to mention that the concentration used in this present study was much higher than, those reported by previous researchers (Tomulescu *et al.,* 2004; Mahmood *et al.,* 2005; Abdullah *et al.,* 2011; Shafiq *et al.,* 2008).

The maize cultivars used in this study could be considered more tolerant because higher percentage germination were recorded at comparatively higher concentration of heavy metals than those reported in several studies in different part of the world.

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