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Effect of Soil Heavy Metal Concentrations on Early Growth Performance of Cowpea (*Vigna unguiculata*) and Groundnut (*Arachis hypogea*) Seeds from Uyo, Nigeria

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Abstract: Pot experiment was conducted on seeds of cowpea and groundnut obtained from Uyo, Nigeria to evaluate their early growth performance. The seeds were grown in soil provoked with different concentrations of Zn, Fe, Cu, Cr and Pb. The heavy metals were added to the soil inform of solution of their salts: $Zn(NO_3)_2.6H_2O$, $FeSO_4.7H_2O$, $CuSO_4.5H_2O$, $K_2Cr_2O_7$ and $Pb(NO_3)_2$. Five (5) experiments, each corresponding to each metal were conducted. Each experiment had three (3) concentration treatments (10 ppm, 100 ppm and 1000 ppm) giving 15 experimental units for each of the two plants. Two other experiments, one for each plant in same soil but without heavy metal addition served as Control. The metal had different effects on seed germination rates. Growth performance was monitored for three weeks by measuring the shoot heights in centimetres using a metre rule. Growth Performance on soil enriched with Zn, Fe and Cu were higher than that of the Control. In Cr and Pb enriched soil, growth performances were lower than that of Control except for cowpea in 10 ppm and 100 ppm Cr-enriched soil. Pb was more toxic resulting to death of groundnut at second week at concentrations of 100 ppm. Fe caused mortality of groundnut at the same time but only at 1000 ppm enrichment. Generally, growth performance decreased as each of the heavy metal's concentration was increased in soil. It is concluded that cowpea showed more heavy metal tolerance in terms of mortality and growth performance compared to groundnut.

Keywords: *Growth performance, Concentrations, Heavy metal, Enrichment, Mortality*

1. Introduction

Heavy metals are a group of metallic chemical elements characterized by relatively high densities, and high relative atomic weight with an atomic number greater than 20 (Raskin *et al*., 1994; Yan *et al*., 2020). Järup (2003) and Khanna *et al.* (2018) referred to heavy metal as those metals, which possess a specific gravity greater than 5 g/cm^3 and adversely affect the environment and living organisms. Heavy metals can be classified into two groups: the essential and non-essential ones (Vanghele, 2020). Some of the essential heavy metals (HMs) like copper (Cu), cobalt (Co), molybdenum (Mo), nickel (Ni), selenium (Se), iron (Fe), manganese (Mn) and zinc (Zn) are important constituents for plants and humans, when present only in small and required amount (Arif *et al*., 2016; Oladebeye, 2017). Other trace heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg), Chromium (Cr) and lead (Pb) are nonessential as they do not contribute to plants growth and are toxic even at very small concentrations (Divrikli *et al*., 2006; chibuike, 2014; Tripathi *et al*., 2015; Sandeep *et al*., 2019). Heavy metals are non-biodegradable by any biological or physical process; hence, they persist in the soil environment with good potential to enter the food chain through edible crops, and may eventually accumulate in the body of human through bioaccumulation posing threat to health (Suman *et al*., 2018; Yan *et al*., 2020). Accumulation of heavy metals in the environment turns out to be a health hazard because of their persistency, bioaccumulation and toxicity to floras, faunas and human (Ogunwale *et al*., 2020; Karim *et al.* 2016).

HMs toxicity impacts plants growth and development, Kumar *et al*. (2019) reported that plants grown on soils contaminated with heavy metals from effluent showed several physiological changes in the biochemical process like nutrient accumulation, respiration and gaseous exchanges. Heavy metal toxicity due to high concentration reduces the number of beneficial soil microorganisms leading to decrease in organic matter decomposition making the soil to be less fertile (Asati *et al*., 2016; Kayode, 2021), this adverse effect of heavy metals on the growth and activities of soil microbes may also indirectly affect plant growth (Kumar *et al.,* 2016). Plants growing under heavy metal stress have to spend more energy on their survival; the energy that would have been used for other process, this leads to deficiency in the amount of energy required resulting to overall decrease in the growth of the plant (Kumar and Aery, 2016). Findings from Galal *et al*. (2021) showed a reduction in photosynthetic pigments, fruit production, and nutrients in *Pisum sativum* L grown in soil exposed to high levels of Cd, As, Cr, Cu, Ni, Fe, Mn, Zn, Ag, Co and V. Another finding by Al-muwayhi (2021) indicated negative effects of nickel on physiological and morphological traits of *Vigna sinensis* L.

Shivhare and Sharma (2012) assessed the effect of toxic heavy metal (Pb and Cd) contaminated soil on Dahlia Plant, their results indicated that increased concentration decreases the root and shoot elongation and consequently, leads to inhibition of the plant growth and development. Copper toxicity affected the growth, dry matter, and yield of *Vignaradiata*; growth and oxidative mechanism of tea plant (*Camellia sinensis)* (Manivasagaperumal *et al*., 2011; Dey *et al*., 2015). Sanjosé *et al*. (2021) assessed the effect of accumulation of heavy metals on the germination and growth of *Salsola vermiculata* L. seedlings and observed that Zn induced growth only at lower concentration and that there was linear relationship between the concentration in the tissue and soil medium. Work carried out on the impact of Fe on the growth of Cowpea (*Vigna unguiculata*) by Ifie *et al*. (2019) established that growth was reduced at increased concentration of Fe despite being one of the essential elements. This is in accordance to the report by Arif *et al.* (2016) where some essential heavy metals such as such as Co, Cu, Fe, Mn, Mo, Ni, and Zn enhanced plant growth but at the same time inhibited the growth at increased concentrations.

The effects of different concentrations of lead on seedling growth performance of cowpea (*Vigna unguiculata*) were studied by Mehboob *et al*. (2018), the result indicated that at concentration of 40 ppm lead treatment, seedling growth and germination were highly affected when compared to control (P = .05). Anitha *et al*. (2012) observed that germination, morphological and parameter s were retarded in Cowpea (*Vigna unguiculata*) subjected to different treatments in concentrations of chromium. Vineeth *et al*. (2014) in a pot experiment to study the effect of nickel, cadmium, and chromium on the seedling of *Arachis hypogea,* observed a suppressed growth of the plant resulting from increased concentration of the metals.

The objective of this work was to observe and to compare the growth performance and tolerance of cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogea*) under heavy metal stress at concentration up to 1000 ppm as most work including that of Mehboob *et al.* (2018), Anitha *et al.* (2012) and Kaya (2020) observed these at concentrations lower than 1000 ppm. This work used cowpea and groundnut seeds cultivated in Uyo, southern part of Nigeria since the obtainable work carried out were on the seeds from normal cultivation area of the savanna belt of Northern part of Nigeria.

2. Materials and Methods

2.1 Materials

Healthy and viable seeds of cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogea*) were obtained from Crop Science Department, University of Uyo, Akwa Ibom State, Nigeria. The heavy metal salts: zinc trioxonitrate (V) hexahydrate [$Zn(NO_3)_2.6H_2O$], Iron (II) tetraoxosulphate (VI) heptahydrate ($FeSO_4.7H_2O$) (green vitriol), copper (II) tetraoxosulphate (VI) pentahydrate ($CuSO_4.5H_2O$) (blue vitriol), Potassium heptaoxodichromate (VI) ($K_2Cr_2O_7$) and Lead (II) trioxonitrate (V) [$Pb(NO_3)_2$] were of analytical grade from Sigma-Aldrich Chemicals. Deionized water was used throughout the experiment.

2.1 Preparation of Heavy metal solutions

Heavy metal solutions were prepared by dissolving a known weight in 1000 dm^3 of deionized water separately for each metal. From this stock solution, different concentrations (10 ppm, 100 ppm and 1000 ppm) were obtained for each of the metals (Mumthas 2010). For Zn, 4.549 g of $Zn(NO_3)_2.6H_2O$ containing 1 g of Zn was placed in an empty volumetric flask and was diluted to 1 dm^3 with deionized water. This solution contained 1000 ppm of Zn. Other sub-standards were prepared by diluting 100 ml and 10 ml of the stock to 1000 ml of solution to obtain 100 ppm and 10 ppm respectively. The same procedure was used to prepare 1000 ppm, 100 ppm and 10 ppm of Fe, Cu, Cr and Pb solutions using 4.976 g of $FeSO_4.7H_2O$, 3.928 g of $CuSO_4.5H_2O$, 2.829 g of $K_2Cr_2O_7$ and 1.599 g of $Pb(NO_3)_2$ which contains 1 g of Fe, 1 g of Cu, 1 g of Cr and 1 g of Pb respectively.

2.2 Planting of seeds of cowpea (Vigna unguiculata) and groundnut (Arachis hypogea)

The seeds of the two plants were planted in pots on soil provoked with various concentrations of copper (Cu), Iron (Fe), Zinc (Zn), lead (Pb) and chromium (Cr). Twelve (12) seeds of each plant were sown in pots filled with I kg of soil respectively; the seeds were sterilized with 5% NaOCl (sodium hypochlorite) solution for five (5) minutes and then rinsed with deionized water before planting (Kaya, 2020). The heavy metals were added to the soil inform of solution of their salts. A 50 ml of the prepared concentrations for each of the heavy metals was added to each of the pots before planting the seeds. Based on the salts used, the metals existed in oxidation state of Zn^{+2}, Fe^{+2}, Cu^{+2}, Cr^{+6} and Pb^{+2}. The control was cultivated on the same soil but with no addition of any of the heavy metals.

2.3 Experiments and number of treatment treatments

Five (5) experiments (each corresponding to each metal) were carried out. Each experiment had three (3) treatments in metal concentrations (10 ppm, 100 ppm and 1000 ppm) giving 15 experimental units for each of the two plant which totaled 30 experiments. Two other experiments, one for each plant in same soil but without heavy metal addition served as control. Each plating pot was irrigated with same amount of deionized water during weeks of study. Growth performance of the plants was observed for three weeks by measuring the shoot heights in centimetres using a metre rule.

2.4 Percentage of seed germination

The percentage of seed germination was obtained by **Eqn. 1** as was stated by Kaya (2020).

$$\text{Percentage Germination} = \frac{\text{Nunber of Seeds Germinated}}{\text{Number of Seeds Planted}} \text{ X } 100 \ldots\ldots\ldots \textbf{ Eqn. 1}$$

3. Results and Discussion

3.1 Growth performance (shoot height)

Average growth performance in terms of shoot heights of cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogea*) in different chemical environment (heavy metal enriched soil and Control soil) in terms of heights in centimetres was monitored on weekly basis for three weeks as presented in **Figures 1 – 12**. Growth performance of cowpea in soil enriched with Zn (**Figures 1**), show that in 10 ppm enriched-soil, average growth in terms of shoot height varied from 11 cm in the first week to 30 cm in the third week, whereas in 100 ppm of Zn enriched-soil, the growth improved ranging from 12 cm to 31 cm. This improvement implies that cowpea needed some Zn up to 100 ppm in the soil; but the situation changed and was seen to reverse in 1000 ppm when the performance was poor ranging from 10 cm to 24 cm within the same period, this result attest that excess Zn ions at high concentration is detrimental to cowpea growth. This could be caused by the fact that higher concentration of Zn is toxic and can affect the morphology and physiology of cowpea leading to general growth inhibition including shoot height (Basha and Selvaraju, 2015).

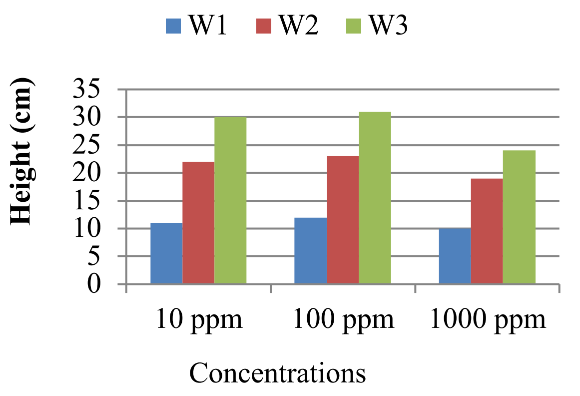
Average growth performance of groundnut in the same environment (Zn-enriched soil) presented in **Figure 2** shows growth increase from 8 cm to 23 cm in 10 ppm enriched soil, 6 cm to 21 cm in 100 ppm enriched soil and 6 cm to 20 cm in 1000 ppm enriched soil. This also shows that growth range reduced with increased concentration with more impact from 1000 ppm Zn-enriched soil. The reduced growth observed for groundnut at higher concentration of Zn was similar to what was observed by Sanjosé *et al*. (2021) when the effect of heavy metals on the germination and growth of *Salsola vermiculata* seedlings was studied. In comparism, cowpea showed better performance as maximum height of 31 cm in 100 ppm Zn-enriched soil was higher than that of groundnut at 21 cm across period of study. Hence, cowpea showed 46.62% increment in growth performance compared to groundnut under same condition. However, both plants decreased in growth performance as the concentration of Zn was increased to 1000 ppm.

Under iron (Fe) enriched soil, cowpea again showed higher growth performance compared to groundnut as contained in **Figure 3**. In 10 ppm Fe-enriched soil; the maximum shoot height of cowpea at end of experiment was 40 cm, while that of groundnut was 17 cm, implying that cowpea outperformed groundnut by 135.23% in term of shoot height. In 100 ppm of Fe-enriched soil; maximum height for cowpea was 32 cm and 19 cm for groundnut, this implies 68.42% difference in growth height. The maximum shoot height for groundnut (19 cm) observed at 100 ppm Fe-enriched soil, shows that groundnut needed up to 100 ppm Fe-enrichment as against 10 ppm by cowpea for maximum growth performance. In 1000 ppm Fe-enriched soil; maximum height for cowpea was 30 cm at end of study (third week). For groundnut, the maximum height (8 cm) was observed at first week as it did not survive to second week. Hence, in 1000 ppm Fe-enriched soil, the toxicity was lethal to groundnut and it died after first week as indicated in **Figure 4**, but cowpea survived, though with drastic reduction in growth (40 cm in 100 ppm to 30 cm in 1000 ppm). Hence, cowpea showed more Fe tolerance at higher concentrations compared to groundnut.

Ifie *et al.* (2019) reported a general growth suppression of cowpea planted in Fe-elevated soils in a study where growth and yield of cowpea was assessed. In 1000 ppm Fe-enriched soil, the toxicity was lethal to groundnut and it died at second week as indicated in **Figure 4**, but cowpea survived, though with drastic reduction in growth (40 cm in 100 ppm to 30 cm in 1000 ppm). Hence, cowpea showed more Fe tolerance at higher concentrations compared to groundnut.

In Cu-enriched soil as illustrated in **Figures 5 and 6**, cowpea exhibited same growth pattern with that in soil enriched with zinc. The average heights ranged from 12 cm to 32 cm, 12 cm to 35 cm and 13 cm to 30 cm in 10 ppm, 100 ppm, and 1000 ppm respectively (**Figure 5**), showing that cowpea thrived better in 100 ppm Cu-enriched soil and little improved than in Zn-enriched soil of same concentration (100 ppm). For groundnut, average shoot height ranged from 10 cm to 30 cm, 9 cm to 27 cm and 8 cm to 24 cm in 10 ppm, 100 ppm, and 1000 ppm respectively (**Figure 6**). This shows that groundnut performed better in 10 ppm Cu-enriched soil compared to other two concentrations (100 ppm and 1000 ppm). A closer look at the growth performance of groundnut as adjudged in **Figure 6** reveals that groundnut growth increased arithmetically from week 1 to week 3 for each concentration of the metal (Cu) and that the range of growth was reduced from 10 – 30 cm to 8 – 24 cm as the concentration increased from 10 to 1000 ppm. There was decrease in growth (shoot height) as soil metal enrichment was increased for the two plants. Decrease in growth could come from more absorption and tissue accumulation of Cu as the concentration increased from 10 ppm to 1000 ppm, which in turn could create stress and inhibit growth (Nguyen, 2015). In 10 ppm Cu-enriched soil; maximum shoot height for cowpea was 32 cm, that of groundnut was 30 cm, denoting 6.67% in difference in growth between cowpea and groundnut. In 100 ppm of Cu-enriched soil; maximum shoot height for cowpea was 35 cm and 27 cm for groundnut; this implies 29.63% difference in growth. In 1000 ppm Cu-enriched soil; maximum shoot height for cowpea was 30 cm and that of groundnut was 24 cm, showing 25% difference.

In soil enriched with Cr (**Figures 7 - 8**), the growth performance decreased more when compared to other metal enriched soil (Zn, Fe, and Cu) with worst growth rate exhibited in 1000 ppm Cr-enriched soil. Cowpea increased slowly from 10 cm to 32 cm in 10 ppm Cr-enriched soil, 9 cm to 30 cm in 100 ppm enriched soil. In 1000 ppm Cr-enriched soil, zero (0) shoot height was observed at first week as there was no germination of any seed, but at 2nd week the height was 4 cm and remained at this height to the end of the third week (**Figure 7**). This signals that Cr at very high concentration can cause delayed germination of cowpea seed. The stagnation of growth at 4 cm shows that Cr can retard the growth of cowpea at higher concentration in the soil. Prakash *et al.* (2018) observed that Cr delayed cowpea germination when the effect of Cr in cowpea was studied, but in contrast, Prakash *et al*. (2018) observed this in soil with Cr enrichment less than 100 ppm as against the enrichment above 100 ppm in this study. Suppression of cowpea germination rate under Cr (VI) stress may be due to disruption of seed coat permeability and increase in protease activity (Parmar *et al*., 2002; Hou *et al*., 2014). Groundnut in Cr-enriched soil also exhibited low growth, as highest shoot height was 12 cm at third week in 10 ppm with lowest height of 4 cm in 1000 ppm. The range of height for groundnut (4 – 12 cm) when compared to that of cowpea (4 – 32 cm) across all concentrations is an indication that groundnut was more impacted by Cr toxicity and that cowpea has more Cr tolerance compared to groundnut considering the 62.5% growth reduction in groundnut. At 1000 ppm of Cr-enrichment, groundnut displayed germination characteristics of that of cowpea as there was complete dormancy in the two plant seeds in 1000 ppm Cr-enriched soil at first week.



Fugure 1. Cowpea growth perfomance in Zn-enriched soil

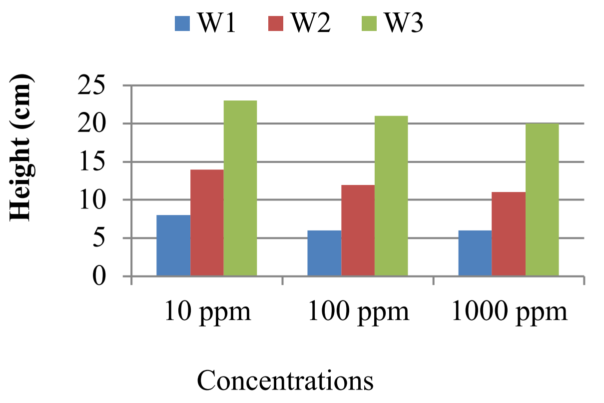


Figure 2. Groundnut growth perfomance in zn - enriched soil

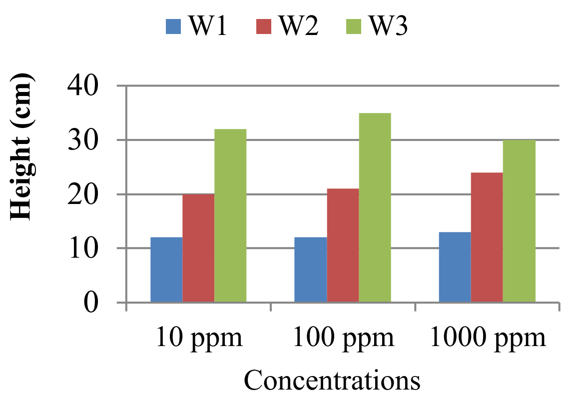
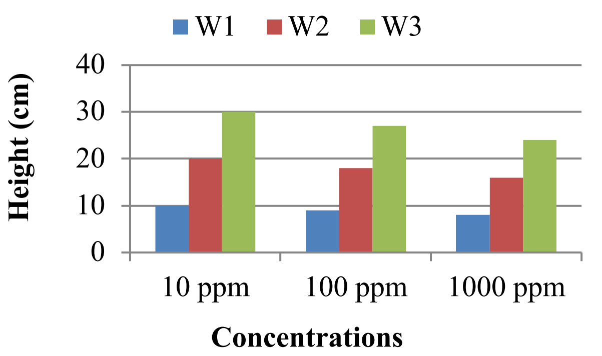


Figure 5. Cowpea growth performance in cu-enriched soil



Figue 6. Groundnut growth performance in cu-enriched soil

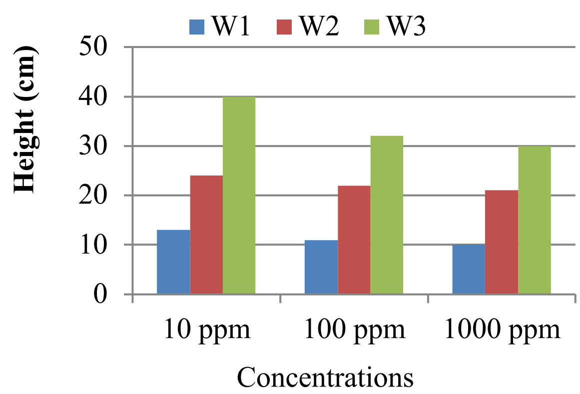


Figure 3. Cowpea growth perfomance in fe - enriched soil

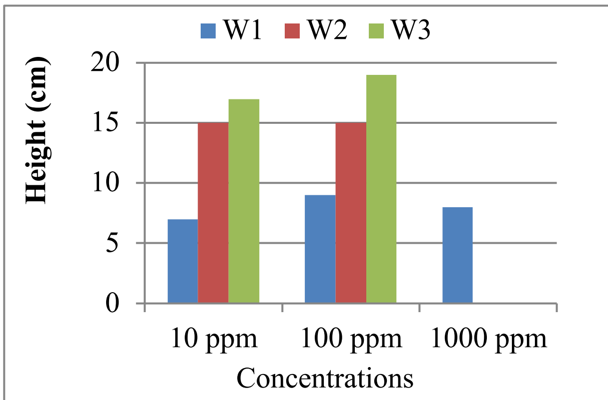


Figure 4:. Groundnut growth perfomance in fe - enriched soil

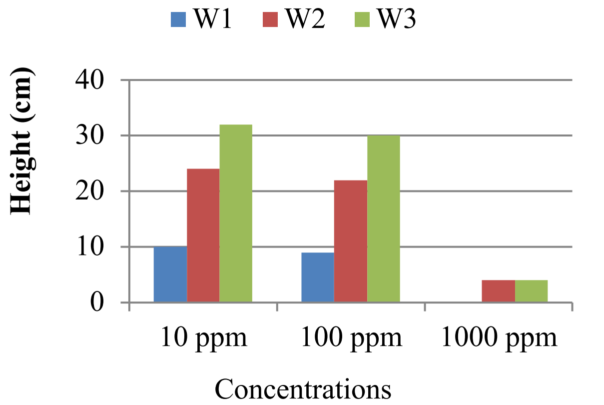
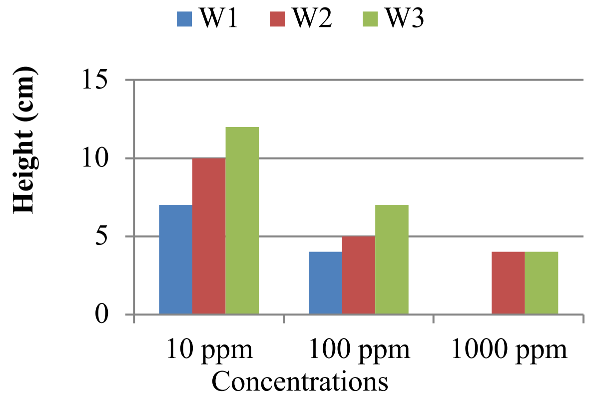


Figure 7. Cowpea growth performance in cr-enriched soil



Figue 8. Groundnut growth performance in cr-enriched soil

Pb-enriched soil was also observed to favour cowpea more compared to groundnut as in the case of Cr-enriched soil (**Figures 9 - 10**). The growth rate in terms of shoot height was quite low and similar to that observed for Cr-enriched soil. At second week of growth, Pb toxicity was too lethal to groundnut resulting to dead in 100 ppm and 1000 ppm of Pb-enriched soil; this was similar to that of Fe-enriched soil. However, for that of Fe; mortality was observed only at 1000 ppm enrichment. This is an indication that cowpea has more Pb tolerance compared to groundnut. The mortality caused by Pb at 100 ppm shows that Pb was more toxic among the heavy metals especially to groundnut. Cowpea exhibited higher growth rate as it range of height (9- 16 cm) in 10 ppm Pb-enriched soil was higher than the range (6 – 9 cm) for groundnut in the same 10 ppm of Pb-enriched soil, revealing 63.75% in height reduction for groundnut.

The growth performance for cowpea and groundnut on soil enriched with Zn, Fe and Cu were higher compared to the Control. In Cr and Pb enriched soil; the maximum heights for the two plants were lower than that of Control except in 10 ppm and 100 ppm Cr-enriched soil, where the maximum height of cowpea in 10 ppm (32 cm) and in 100 ppm (30 cm) were higher than the 24 cm observed for Control. However, at 1000 ppm of Cr, there was delayed germination at first week and a drastic reduction in height for the two plants compared to Control (**Figures 7 – 8**). Higher growth performance from Zn, Fe and Cu enriched soil compared to that of Control could emanate from the fact that Zn, Fe and Cu are essential heavy metal which in any way could contribute to the plants morphology leading to higher growth rate

3.2 Average growth performance (shoot height) in heavy metal-enriched soil and control

The average growth performance of the plants in terms of shoot height in the heavy metal enriched soil is presented in **Figures 1 – 10.** For the control, average growth performance is presented in **Figures 11 -12**.

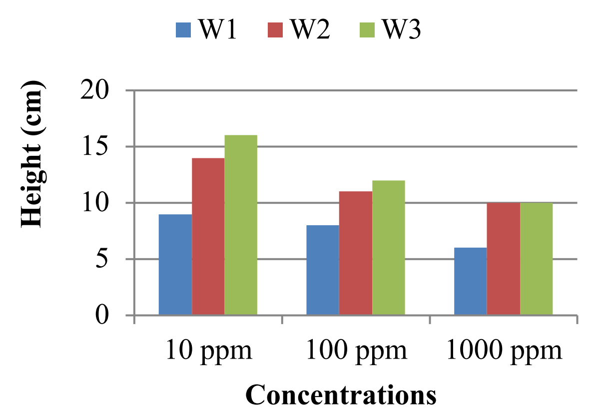


Figure 9. Cowpea growth performance in pb-enriched soil

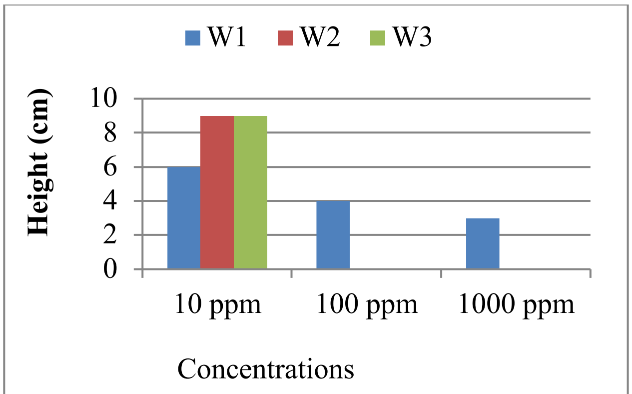


Figure 10. Groudnut growth performance in pb-enriched soil

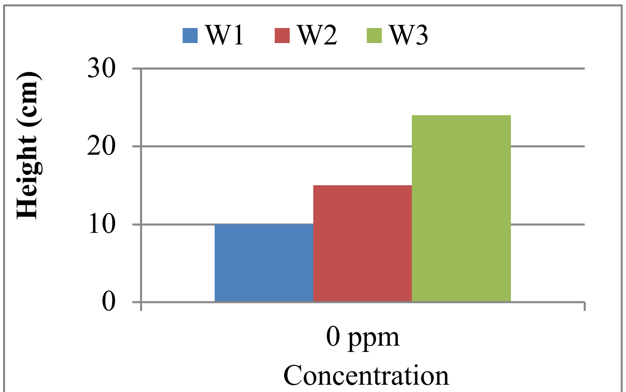


Figure 11. Cowpea growth performance in control soil

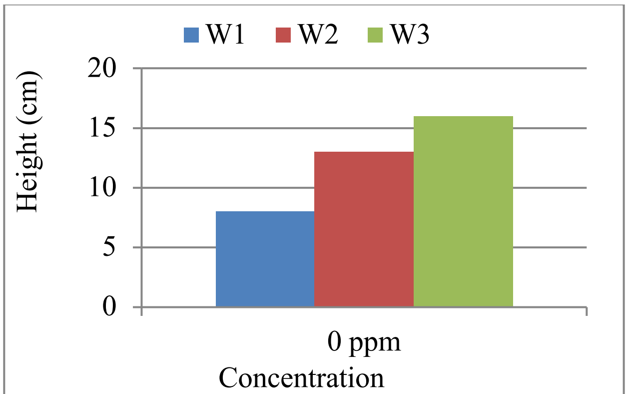


Figure 12. groundnut growth performance in control soil

3.3 Germination rate (%)

The metal had different effects on seed germination rates of cowpea and groundnut (**Table 1**). The lowest germination rate (58%) was obtained from groundnut in 1000 ppm Pb-enriched soil while the highest (100%) was observed for the two plants in 10 ppm and 100 ppm of Zn, Fe and Cu enriched soil (**Table 1**). For Cr-enriched soil, 100% germination rate was observed only for cowpea and at 10 ppm enrichment only. 100% germination rate was not observed from any of the plant from Pb-enriched soil (**Table 1**).

Table 1. Seed germination rate

Plants	Concentrations (ppm)	Zn-enriched soil	Fe-enriched soil	Cu-enriched soil	Cr-enriched soil	Pb-enriched soil
	10	100%	100%	100%	92%	92%
Groundnut	100	100%	92%	92%	83%	67%
	1000	92%	83%	83%	67%	58%
	Control	100%				
	10	100%	100%	100%	100%	92%
	100	100%	100%	100%	92%	67%
Cowpea	1000	92%	92%	83%	75%	67%
	Control	100%				

Conclusion

From the result obtained, growth performance of cowpea and groundnut were reduced as each of the heavy metal concentration was increased in soil. Cowpea showed more heavy metal tolerance with better growth performance at all concentrations than groundnut for each of the metal studied. Performance in shoot height for cowpea and groundnut on soil enriched with Zn, Fe and Cu were higher compared to the Control. In Cr and Pb enriched soil, growth performance in height for the two plants were lower than that of Control except in 10 ppm and 1000 ppm Cr-enriched soil where the height of cowpea were higher than the 24 cm observed for Control. Pb was more toxic resulting to dead of groundnut seedling at second week of study at concentrations of 100 ppm and 1000 ppm, Fe also caused mortality of groundnut seedling but only at 1000 ppm enrichment.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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