JASEM ISSN 1119-8362

Full-text Available Online at
www.ajol.info and
www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *June* 2017
Vol. 21 (4) 761-768

The Influence of Pb and Zn Contaminated soil on the Germination and Growth of Bambara Nut *(Vigna subterranea)*

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ABSTRACT: This research work takes a look at the possible phytotoxic effects of Pb and Zn as $Pb(NO_3)^2$ and $Zn(NO_3)^2$ on Bambara nut (*Vigna subterranea*) planted in polluted. Lead and zinc are common environmental contaminant found in soils. Unlike Zn, Pb has no biological role, and is potentially toxic to microorganisms. The bambara nut seedlings were irrigated with various concentrations of Pb and Zn (100,150 and 200mgkg^{-1}) . Effect of these metals on the growth parameters (stem length/height, root length, leaf area, yellowness of leaves, fresh and dry weights) were measured. Influence of heavy metals on the micro-organisms in the soil used for planting as well as the organic matter, pH and moisture content of the soil were also evaluated. The results showed that plant performance significantly reduced with increasing concentrations (100,150 and 200mgkg^{-1}) of Pb and Zn contamination. The results show that a negative relationship existed between the different metal concentrations in the soil and the growth parameters (stem height, root length, leaf area, yellowness of leaves, fresh and dry weight) measured compared to control experiment. This study therefore reveals that these heavy metals have great implications on the sustainability of *V. subterranea's* growth. These metals could pass down to humans through the food chain if animals eat such plants during grazing. This will affect their health, which may eventually lead to their death due to bio concentration and bioaccumulation. There would therefore be a need to explore ways of improving bambara nut production in areas of metal pollution. © JASEM
https://dx.doi.org/10.4314/jasem.v21i4.17

Keywords: *V. subterranea*, Bioaccumulation, Pollution, Phytotoxicity, Contamination, bioaccumulation.

Bambara nut (*Vigna subterranean* (L.) Verdec) is a leguminous crop grown in semi-arid parts of Africa on a small scale. It can withstand drought, resist pests and diseases and is able to thrive well in soils that are not chemically rich. The Bambara nut is a member of the family *Fabaceae*. The plant originated in West Africa. Still a traditional food plant in Africa, this little-known vegetable has potential to improve nutrition, boost food security, foster rural development and support sustainable land care (National Research Council, 2006). It is cultivated principally by farmers as a "famine culture" crop because it has several natural agronomic advantages including high nutritional value, drought tolerance and the ability to produce in soils considered insufficiently fertile for cultivation of other more favoured species such as common beans and groundnuts (*Arachid hypogea)* (Anchirinah *et al*., 2001).

According to Horsfall and Spiff, (2004) a group of non-biodegradable elements with the tendency of bioaccumulation in living organisms are Heavy metals. Ademoroti (1996) referred to heavy metals as metals with higher atomic weights of groups III to V of the periodic table such as aluminium, cadmium, zinc, chromium, copper, manganese, nickel and lead. They are both biologically and industrially important. As a result, they are usually encountered in effluents and they have caused so much environmental distress (Guo *et al*., 2006). According to Menon *et al*., (2007) plants response to heavy metals are drawing attention greatly since metal pollution is still rampant and the ecosystem's stability is being threatened. Unlike Zn, Pb has no biological role, and is potentially toxic to microorganisms. Metals as we know occur naturally in the environment and are involved in the metabolism of plants, however beyond a tolerable level for various plant species, these metals cause phytotoxicity in them (Markovska *et al* ., 2009). This work therefore assessed the influence of Pb and Zn contaminated soil on the germination and growth of Bambara nut *(Vigna subterranea).*

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MATERIALS AND METHODS

Materials collection and Experimental Design: Dry seeds of cultivars of Bambara nut (*Vigna subterranea*), Tvsu 102 were collected from the International Institute of Tropical Agriculture (I.I.T.A.). The study was done in the Cell Biology and Genetics Department, also at the Chemistry department, University of Lagos and the garden along Lagos State University (LASU) Road, Akesan, Lagos. The seeds were subjected to viability test using floatation technique according to Agbogidi (2010). The Bambara nut 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 irrigated 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. Plant growth was determined by measurement of plant height using a meter rule at 1 week interval for 8 weeks when plants were harvested. The parameters observed and measured were: Germination pattern, fresh weight (g), total dry weight (g) (Biomass), colour of leaves, leaf size (cm^2), stem height/length (cm) and root length (cm). Measurement of the stem height began on the fifth day of planting. The leaf size, stem length and root length measurements began on the tenth day. After harvest, the soil samples were collected from the pots for Pb and Zn analysis using acid digestion method. The Percentage (%) germination was calculated using this formula:

$$\frac{\text{Number of seeds that sprouted}}{\text{Total number of seeds planted}} X\ 100\%$$

The fresh weights were obtained by uprooting the plant from each bag and weighing on a weighing balance (model PN 163) immediately after harvest to avoid water loss. The dry weights were obtained by oven-drying the plant at 60^{o}c for 48 hours to get rid of all moisture to ensure a constant weight and weighed using the same balance. The soil microbial population was considered to investigate the possible effects of these metals on them.

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 (4kg) 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*., 2000).

Soil Sample Digestion and Heavy Metal Determination in the Soil Samples using Atomic Absorption Spectrophotometer (AAS) The soil samples were digested with concentrated HNO_3 + $HClO_4$ following a modified 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: One gram of each soil sample was immediately used for microbial enumerations. The enumeration of bacteria and fungi was done according to a standard procedure (Kumar, 2004).The results were averaged for each soil samples. The fungal colonies were counted after 72-120 h. Samples were preserved at 4°C for further microbial analysis.

Statistical Analysis: All data collected were analyzed using standard deviation, t-test and analysis of variance (ANOVA) for statistical significance at 95% confidence interval. Descriptive statistics were calculated using the Microcal origin 5.0 and Microsoft Excel. Graphical illustrations were also carried out to get vivid representation of the data obtained.

RESULTS AND DISCUSSION

The result of the analysis of the heavy metal content and the physico-chemical parameters of the soil sample used for the experiment showed: Sandy loam (silt 28.58%, clay 30.93% and sand 49.81%), Soil pH 6.42, Organic matter 3.28%, Total Organic carbon 3.2%, Moisture content 8.18%, Total Nitrogen content 0.16mgkg^{-1}, Phosphorus content 0.026 mgkg^{-1}, Lead content 1.4220 mgkg^{-1}, Zinc content 0.7800 mgkg^{-1}.*

Viability of Seeds: The bambara nut seeds cultivar TVSU 102 showed 100% viability. It was also observed that the number of surviving seedlings treated with Lead nitrate reduced greatly compared with surviving seedlings in Zinc nitrate (Table 1). The percentage germination was 100% for the control test plant. However, no germination (0%) was observed in bambara nut seeds treated with 200 mgkg^{-1}of lead nitrate by day 15

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Table 1: Percentage of surviving seedlings 30 days after planting

plants/ treatment concentration	percentage of surviving seedlings
Control	100
100mgkg^{-1} Pb	75
150 mgkg^{-1} Pb	62.5
200 mgkg^{-1} Pb	50
100 mgkg^{-1} Zn	75
150 mgkg^{-1} Zn	62.5
200 mgkg^{-1} Zn	62.5

*Growth Experiments: Leaf Area/Size (cm^2), Stem Length/height (cm) and Root length (cm)***:** Figures 1, 2, 3 show the effect of different concentrations of Lead and Zinc nitrate on leaf-sizes, stem lengths and root lengths of experimental seedlings of cultivar TVSU 102 of *Vigna subterranean* respectively. Leaf-size increased with time (in days) in each treatment concentration whereas it decreased as the concentration of the heavy metals increased. The largest leaf size was observed on the 30th day in the control (non-treated) with a leaf size of 26.18cm^2 while the least leaf size of 12.24cm^2 was observed for seedlings treated with 200 mgkg^{-1}of Lead nitrate. The stem height increased with time (in days) in each treatment concentration while it decreases as the treatment concentration increased. The control seedlings of bambara nut were also observed to have the highest mean stem height of 14.6±3.98 while seeds treated with 200 mgkg^{-1}of lead had the least stem height(cm) as 8.0±3.16. The root length increased with time (in days) in each treatment concentration while it decreases as the treatment concentration increased. The control seedlings of bambara nut were also observed to have the longest mean root length of 7.14±0.74 while seeds treated with 200 mgkg^{-1}of Zinc had the least root length (cm) as 5.70±0.89*.

Statistical testing revealed that the treatment effects were highly significant (P<0.05) on leaf areas, stem lengths and root lengths of the test plants. Generally, the results indicated that the growth rate of root and shoots were found to be retarded with increasing concentrations of Lead and Zinc nitrate

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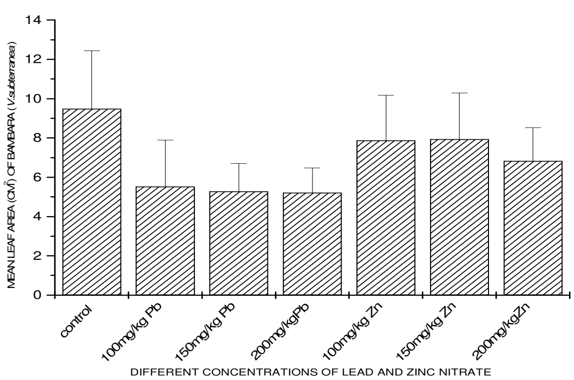

Fig 1: Effect of Lead and Zinc Concentrations on Mean Leaf Area **(cm^2)**

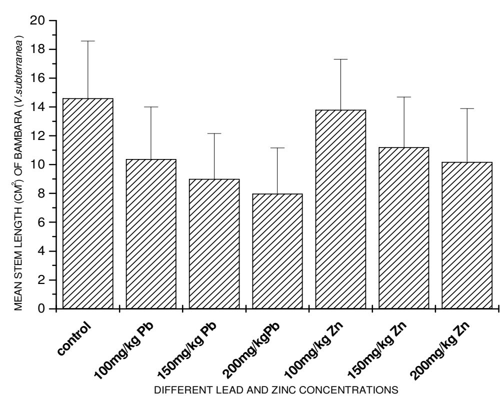

Fig 2: Effect of Lead and Zinc Concentrations on Mean Stem Length (cm)

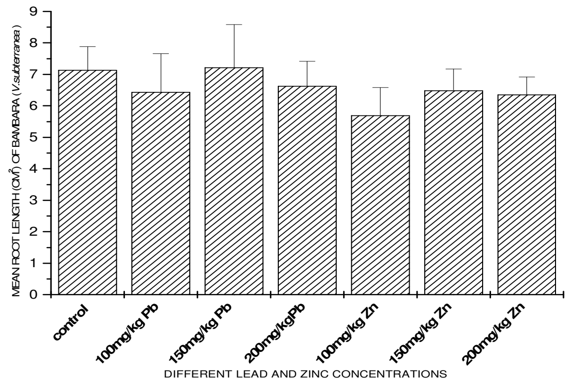

Fig 3: Effect of Lead and Zinc Concentration on Mean Root Length (cm) of Bambara Nut (*V.subterranea*)

Fresh and dry weight Characteristics: Table 2 show the effects of different Lead and Zinc concentrations on the Fresh and Dry weights of bambara nut. The fresh weight of plants decreased significantly with increased lead supply. A similar pattern of effect though with a lower magnitude was observed for plants treated with Zinc nitrate. For instance, control bambara nut plant had a mean fresh weight (g) of 4.44±1.0, 7.56±1.0 at 100 $mgkg^{-1}$of lead concentration and 4.47±1.0 at 200 $mgkg^{-1}$of lead concentration, indicating decrease in fresh weight with increased concentration. The same trend was observed for the different Zinc concentrations but at a lower magnitude. The dry weight of plants also decreased significantly with increased lead and zinc supply for all treated plants. The dry weights of all treated plants were affected significantly at all concentrations. The control bambara nut plant had a mean dry weight (g) of 2.16± 0.05, 1.14±0.03 at 100 $mgkg^{-1}$of lead concentration and 0.41± 0.01 at 200 $mgkg^{-1}$of lead concentration, indicating a decrease in dry weight with increased concentration. Statistical analysis showed that higher concentration of metals decreased both the fresh and dry weights of plants significantly

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Table 2: Effect of Lead and Zinc concentrations on Fresh and Dry Weight (grams) of Bambaranut

Plants/ Treatment concentration	Mean Fresh Weight± SEM	Mean Dry Weight± SEM
Control	4.44±0.06	2.16±0.05
100 $mgkg^{-1}$ Pb	7.56±0.04*	1.14±0.03*
150 $mgkg^{-1}$ Pb	5.92±0.03*	0.98±0.02*
200 $mgkg^{-1}$ Pb	4.47±0.02*	0.41±0.01*
100 $mgkg^{-1}$ Zn	4.44 ± 0.01	0.84 ±0.03*
150 $mgkg^{-1}$ Zn	5.98 ±0.02*	0.63 ±0.02*
200 $mgkg^{-1}$ Zn	9.56± 0.03*	0.37±0.41*

- Data were expressed as Mean ± SEM
- When * $P<0.05$ = Significantly different from control
- When $P>0.05$ = Not Significantly different from control

Soil Sample Analysis after Planting: After planting, the soil samples were taken to investigate the impact of the heavy metals on the organic matter, pH and moisture content of the soil. The results are shown below:

Table 3: Effects of Heavy Metal On Some Soil Parameters After Treatment**.**

Different soil samples	Soil pH	Moisture Content	Organic Matter (%)
Soil sample before use	6.42	7.86	3.280
Control Bambara nut	6.18	12.24	3.986
100 $mgkg^{-1}$ Pb Bambara nut	6.83	19.71	4.046
150 $mgkg^{-1}$ Pb Bambara nut	6.63	15.29	6.111
200 $mgkg^{-1}$ Pb Bambara nut	6.02	11.33	2.606
100 $mgkg^{-1}$ Zn Bambara nut	6.45	9.55	4.946
150 $mgkg^{-1}$ Zn Bambara nut	6.23	13.7	3.648
200 $mgkg^{-1}$ Zn Bambara nut	6.08	12.41	5.698

From the result above, it was observed that there was an increase in soil pH only for the following treated soil samples: 100 $mgkg^{-1}$ Pb Bambara nut had soil pH of 6.83, 150 $mgkg^{-1}$ Pb Bambara nut had 6.63, and 100 $mgkg^{-1}$ Zn Bambara nut had 6.45. Soils with Pb treatments experienced decrease in soil pH compared to the initial values before planting. Generally, for all treated and control plants, the moisture contents increased. Also, for the organic matter contents all treated and control plants experienced an increase except for 200 $mgkg^{-1}$ Pb Bambara nut.

Microbiological Study: 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 appear to be motile, rod shaped, robust, cylindrical and coccus under the microscope. Tables 4 and 5 show summary of the total microbial population for the different soil samples and treatment. Microorganisms in soil sample containing 200$mgkg^{-1}$ lead nitrate Bambara nut appeared to be the most resistant to this metal. Lead nitrate had no significant effect on the microbial population when compared to the control. As seen in Table 5, the Fungi that were isolated were identified as *Fusarium spp, Aspergillus wentii, Penicillum spp, Aspergillus flavus, Aspergillus niger, Rhizopus spp, Aspergillus fumigatus.* Their total populations were also noted. Generally, Lead 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 control Bambara nut and soil treated with 200 $mgkg^{-1}$lead Bambara nut.

Table 4: 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 Bambara	–	(Gram -ve) +	–	–	–	1.2×10^6 cfu/gram
200 $mgkg^{-1}$ Pb Bambara	(Gram +ve) +	–	–	–	(Gram +ve) +	1.2×10^6 cfu/gram
200 $mgkg^{-1}$ Pb Bambara + Manure	–	(Gram +ve) +	–	–	–	8.0×10^5 cfu/gram

KEY:
+ = Present
– = Absent

Table 5: 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 Bambara	–	–	–	–	–	+	–	(2.0×10^5 cfu/gram)
200 $mgkg^{-1}$ Pb Bambara	+	–	+	–	–	–	–	(2.0×10^5 cfu/gram)
200 $mgkg^{-1}$ Pb Bambara + Manure	+	–	–	+	–	–	–	(9.0×10^5 cfu/gram)

KEY:
+ = Present
– = Absent

In this study, higher concentrations of the metals (lead and Zinc), especially lead inhibited both seed germination and seedling growth. However, roots showed higher degree of growth inhibition compared to shoots. Growth changes are the first most obvious reactions of plants under stress. Different effects of lead on plant growth have been observed by various researchers. This study also noted this from the morphology and physiology of plants exposed to the treatment. Seregin *et al*., (2004); Vojtechova and Leblova, (1991) reported that heavy metals such as lead inhibited seed germination and seedling growth.

Early seedling growth was also reported to be inhibited in rice (Verma and Dubey, 2003), corn plants (Tung and Temple, 1996). Spruce (Vodnik *et al*., 1999). Opeolu *et al.,* 2009 observed that lead contamination had adverse effect on growth parameters of tomato. These adverse effects were noticeable on number of leaves, branching and plant height. Fargasova (2001) has reported that Pb significantly inhibited growth of *Sinapis alba L.*
This study also established that the metals (Zn and Pb) used affected the fresh and dry weights of test plants. Inhibition in fresh weight, dry weight and length of root and shoot of *Sesamum indicum* Cv. HT-I by lead was reported by Kumar *et al*., (1992). These inhibitory effects of lead on the biomass accumulation and growth are possibly a consequence of its effect on metabolic activities of the plants (Van Assche and Clijsters, 1990).

Stunted growth, chlorosis, necrosis, white lesions and wilting were all observed as direct implication of metal treatment. Heavy metals uptake and accumulation in plants have been shown to result in negative effects on plant growth (Breckle and Kahle, 1992). Stunted growth, chlorosis and necrosis, leaf epinasty and red-brown discoloration are visible symptoms of severe metal phytotoxicity (Vassilev, *et al.,* 1998). The observed Lead and Zinc induced decrease in growth of *Vigna subterranea* may be explained on the basis of these metals interference with cell division and cell enlargement, especially in roots; thereby reducing its growth rate which in turn affects the uptake of water and nutrients, and this influences growth of the entire plant.

This present study showed that elevated levels of heavy metals in soils have significant impacts on the population size, community structure, and overall activity of the soil microbial communities. There was increased microbial population in some treated soils especially those augmented with manure while others were reduced significantly. Experiments showed that the number of bacteria in the rhizosphere of *D. fusca* reached 1.0×107 cfu/g. Relatively low bacterial count can be attributed to the presence of heavy metals in high concentrations (39 mg Co/kg, 3 mg Cd/kg, 79 mg Ni/kg, 30 mg Cu/kg, 4834 mg Zn/kg, 123 mg Cr/kg and 114 mg Pb/kg dry soil) (Abou-Shanab *et al.,* 2005). Chaudri *et al.*, (1992) also found that rhizobium populations were reduced at concentrations greater than 7 $mgkg^{-1}$soil in their Cd treatments. Yao *et al.,* (2003) have also reported that heavy metals have adverse effects on soil microbial community, structure and activity. Rhizosphere soil adjacent to the roots of plants has been observed to contain greater microbial densities than those observed outside the soil rhizosphere (Paul and Clarke 1989). Short, gram-negative rods (especially *Pseudomonas spp* and *Flavobacterium spp*) are the most common microorganisms found in the rhizosphere (Barber, 1984). These microorganisms and some others were found in the soils used for this study. For this study, presence of plant exudates and organic matter are primarily possibly responsible for the increased microbial population densities found in the rhizosphere soil. These materials serve as sources of energy, carbon, nitrogen and growth factors for these populations. Prevalent fungi were *Aspergillus niger*, *Penicillium spp* and *Fusarium spp*.

*Conclusion***:** Pb and Zn contamination led to decrease in soil pH compared to the initial values before planting. The micro-organisms present within the soils treated with lead, also contributed to this increased soil pH. Generally, for all treated and control plants, the moisture contents increased. The organic matter contents of all treated and control plants experienced an increase. The micro-organisms present within the soils of the study plant also contributed to this increased moisture and organic matter contents. Similar research on cereals and other legumes need to be carried out.

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