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Response of Cowpea (*Vigna unguiculata*) to Added Lead Nitrate in Soil

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Abstract: *Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification and thus tolerance to heavy metal stress. In this study, the response of cowpea (Vigna unguiculata) to added lead nitrate in soil was investigated. Four replicates each of 0, 1000 to 20,000mg of lead nitrate were added separately to 3kg soil and mixed thoroughly in plastic pots perforated at the base. The soil samples were watered with tap water for two days. Two cowpea seeds per pot were planted. Watering was continued until the plants were harvested. Available lead was determined from the concentrations of Pb^{+2} in water soluble and exchangeable fractions, which were extracted with deionized water and 1M ammonium oxalate respectively. Harvested plant materials were ashed at 450^{O}C to constant weight in porcelain crucibles in a muffle furnace. The ash was dissolved in 0.100moldm^{-3} nitric acid and filtered. Concentrations of Pb^{+2} in soil and plant extracts were determined by Atomic Absorption Spectrometry. On the basis of available lead in the soil before planting, three types of behaviours were exhibited by cowpea. The plant exhibited lead tolerance for available lead in the range 10.30 to 84.14mg/kg. Under this condition, plants grew to maturity and were harvested. For available lead greater than 84.14 to 99.27mg/kg, cowpea exhibited vegetative lead inhibition, where the plants grew and died after sometime. Total lead inhibition manifested when the available lead was greater than 99.27mg/kg. Under this condition, there was no seed germination. As a result of considerable lead uptake by cowpea, there was significant ($Pr < 0.05$) increase in the values of root, stem, leaf and seed Pb, with a corresponding decrease in weights of plants.*

Keywords: *Cowpea (Vigna unguiculata); Watering; Available lead; Lead tolerance; Vegetative lead inhibition, Total lead inhibition.*

1. Introduction

The general population is exposed to lead from air and food in roughly equal proportions (Jarüp, 2003). Lead is available to plants from soil and aerosol sources. Lead uptake studies in plants have demonstrated that roots have the ability to take up significant quantities of Pb whilst simultaneously greatly restricting its translocation to above ground parts (Lane and Martin, 1977). Soil particle size and cation exchange capacity as well as plant factors such as root surface area, root exudates, mycorrhization and rate of transpiration affect the availability and uptake of Pb (Davies, 1995). After being taken up by roots, the localization of Pb is greater in roots than in other parts of the plant. In general dicots accumulate significantly higher amounts of Pb in the roots than monocots (Huang and Cunningham, 1996). The normal Pb level in human blood is 10 – 20µgdm^{-3} (Lenntech, 2004). Up to 50% of inhaled inorganic lead may be absorbed in the lungs. Adults take up 10 – 15% of lead in food, whereas children may absorb up to 50% via the gastrointestinal tract. Lead in blood is bound to erythrocytes, and elimination is slow and principally via urine. Lead is accumulated in the skeleton and is slowly released from this body compartment. The half-life of lead in blood is about one month and in the skeleton 20 – 30 years (WHO, 1995).

2. Materials and Methods

2.1 The Study Area

The soil sample and cowpea seeds (*Vigna unguiculata*) used for this study were collected from International Institute of Tropical Agriculture (IITA) farm in Wase village, Minjibir Local Government Area of Kano State. Figures 1 to 3 show the locations of sampling and planting sites.

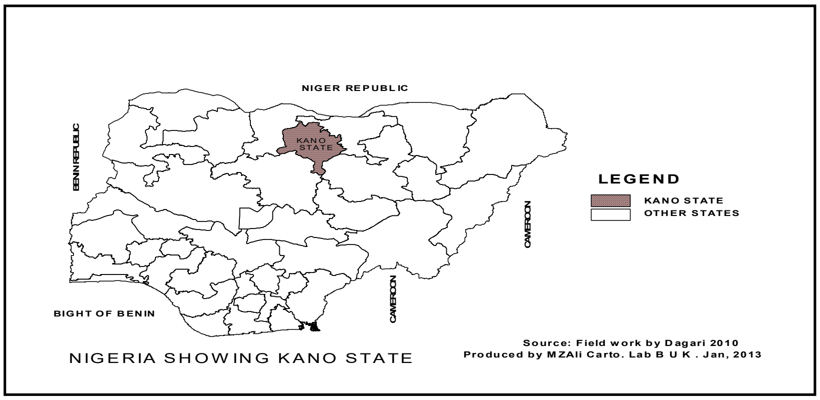


Figure 1: Map of Nigeria Showing Kano State

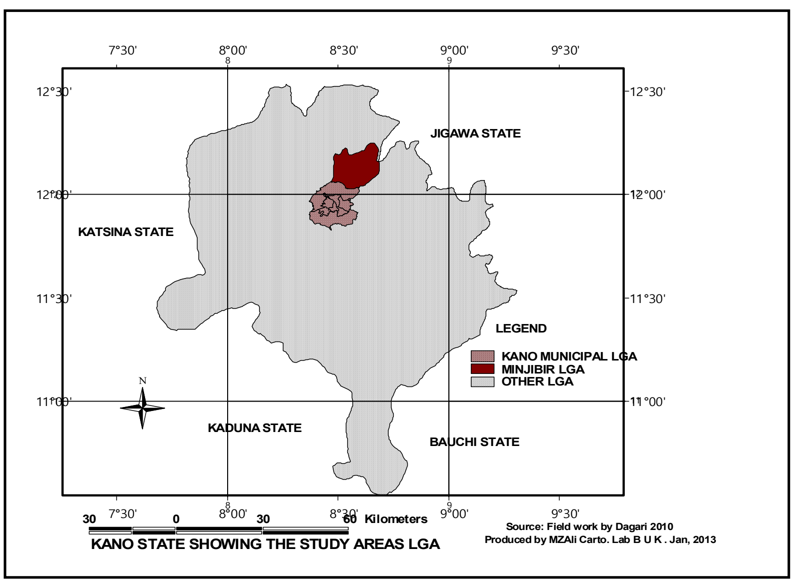


Figure 2: Map of Kano State Showing the Study Local Government Area

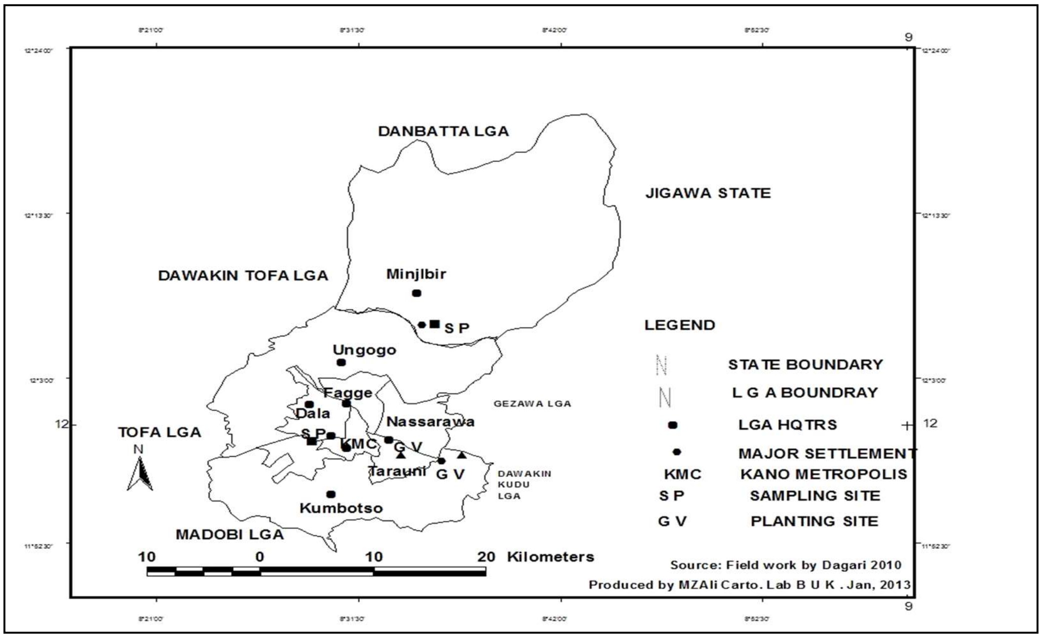


Figure 3: Kano State L.G.A. Map Showing Sampling and Planting Sites

2.2 Instruments, Apparatus and Reagents

All equipment and instruments used in this research were calibrated before conducting the experiments. All glassware used were thoroughly washed with detergents and tap water and then rinsed with deionized water. Suspected contaminants were cleaned with 10% concentrated nitric acid (HNO_3) and metal surfaces rinsed with deionized water. In preparation of reagents, chemicals of analytical grade purity and distilled water were used. All glassware and plastic containers were washed with detergents.

2.3 Soil Sampling and Pre-Treatment

The soil sample was collected using the method recommended by (Petersen, 1994). 100m^2 of the land was divided into ten equal sized grid cells of 10m^2. A steel augur was used to dig the soil to a depth of 25cm. Samples collected from all cells were thoroughly air dried, mixed and stored in large plastic bags.

For the purpose of preliminary studies, 1kg of the air-dried sol was taken. After removing the debris, the soil was ground in a wooden mortar and sieved through a 2mm mesh. It was then stored in a labeled plastic container.

Soil Treatments and Planting of Cowpea Seeds

Four replicates each of 0, 1000 to 25,000mg of lead nitrate were added separately to 3kg soil and mixed thoroughly in plastic pots perforated at the base (Wong and Lau, 1985). The soil samples were watered with tap water for two days. Two cowpea seeds per pot were planted. Watering was continued until the plants were harvested.

All seeds germinated except in the pots containing 20,000 and 25,000mg lead nitrate. Plants grew and died after sometime in soils treated with 14000, 15000, 16000mg lead nitrate.

2.5 Ashing of Plant Materials

The various plant parts harvested were ground to fine powder. Based on availability, 0.125 or 0.25g (root), 1.00g (stem), 0.75g (leaf) and 0.50g (seed) were used for analysis. They were weighed into porcelain crucibles and ashed at 450°C in a muffle furnace to constant weight. The ash was dissolved in 0.100moldm^{-3} nitric acid, filtered and made to mark in a 25cm^3 volumetric flask (IITA, 1979).

2.6 Extraction of Water Soluble and Exchangeable Lead

The water soluble lead was extracted from a mixture of 10g of pre-treated soil and 100cm^3 of deionized water in a 120cm^3 plastic bottle. The exchangeable fraction was extracted by adding 100cm^3 of 1M ammonium oxalate to the residue of the water soluble fraction (Stober *et al,* 1976).

2.7Atomic Absorption Spectrometric Analysis

The soil and plant extracts were analyzed for lead at 283.5nm using flame atomic absorption spectrophotometry. Blank determinations were made prior to sample analysis. Concentrations of Pb^{+2} in soil and plant extracts were obtained in quadruplicates from calibration curves an expressed as mg/kg. (IITA, 1979).

2.8 Statistical Analysis

The data were analyzed in quadruplicates and expressed as mean and standard deviation. The mean of all treatments was subjected to a One-way analysis of variance (ANOVA) using IBM SPSS Statistics 23 software and mean differences were performed using the Tukey test. All graphs were plotted using Microsoft Excel 2013.

3. Results and Discussion

3.1 Available Lead in Soil

The different forms of heavy metals which exist in equilibrium in soil can be broadly classified as readily available, potentially available and non – available. Non – available $\leftrightharpoons$ potentially available $\leftrightharpoons$ readily available. The readily available and potentially available forms are found in the water soluble and exchangeable fractions respectively (Landon, 1991).

According to Stober *et al.* (1976), the significance of sequential extraction of metals in soil is to determine and compare the concentrations of metals in soil with threshold values for plant and animal growth. In this study, soil sample was treated with deionized water and 1M ammonium oxalate in succession to extract water soluble and exchangeable Pb^{+2} (Stober *et al.*,1976), the sum of which gave the available Pb^{+2} (Landon, 1991) as shown in table 1.

Table 1: Mean Concentrations (mg/kg) of Available Pb^{+2} before Planting

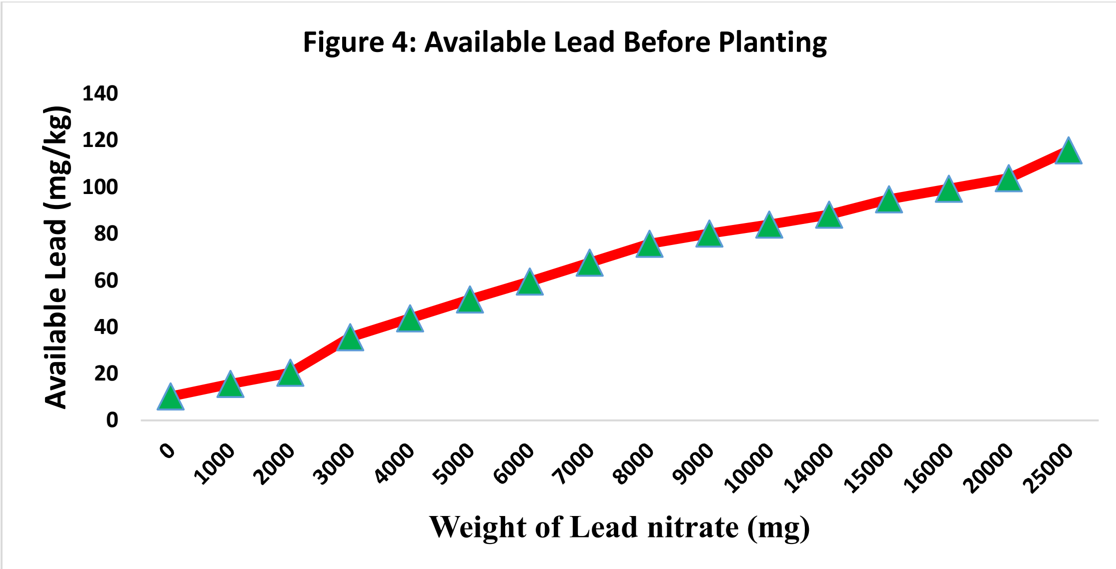
LNT	WSLBP	ELBP	ALBP
0	3.31±0.74	6.99±1.41	10.30±1.70
1000	3.68±0.85	11.95±5.51	15.63±5.00
2000	4.77±0.74	15.63±4.63	20.40±4.59
3000	6.25±1.30	29.41±12.01	35.66±12.01
4000	6.99±0.74	36.76±8.49	43.75±8.94
5000	7.72±0.74	44.12±12.01	51.84±12.03
6000	8.09±0.85	51.47±14.71	59.56±14.23
7000	8.82±1.70	58.82±0.00	67.64±1.70
8000	9.56±0.85	66.18±25.47	75.74±24.91
9000	10.29±0.00	69.85±7.35	80.14±7.35
10000	10.48±5.32	73.53±0.00	84.01±5.32
14000	11.03±7.35	77.21±7.35	88.24±8.49
15000	10.29±0.00	84.56±7.35	94.85±7.35
16000	11.03±7.94	88.24±0.00	99.27±7.94
20000	11.95±5.51	91.91±14.08	103.86±12.15
25000	12.87±10.61	102.94±16.98	115.81±27.59

KEY

LNT: Lead nitrate, **WSLBP**: Water soluble lead before planting
ELBP: Exchangeable lead before planting
ALBP: Available lead before planting

3.2 Response of Cowpea to Lead nitrate Treatment of Soil

The mean available Pb^{+2} in various soil treatments are shown in **Figure 4**



On the basis of available lead in the soil before planting, three types of behaviours were exhibited by cowpea (Barber, 1995), which include lead tolerance, vegetative lead inhibition and total lead inhibition.

3.2.1 Type1: Lead Tolerance

In the range 0 to 10,000 mg lead nitrate treatment, corresponding to 10.30 to 84.14mg/kg available lead, the plant exhibited lead tolerance. Under this condition, plants grew to maturity and were harvested. The concentrations of Pb^{+2} in various plant parts and dry weights of plants are given in table 2.

Table 2: Mean Concentrations (mg/kg) of Pb^{+2} in Plant Parts and Dry Weights of Plants

Concentration (mg/kg)

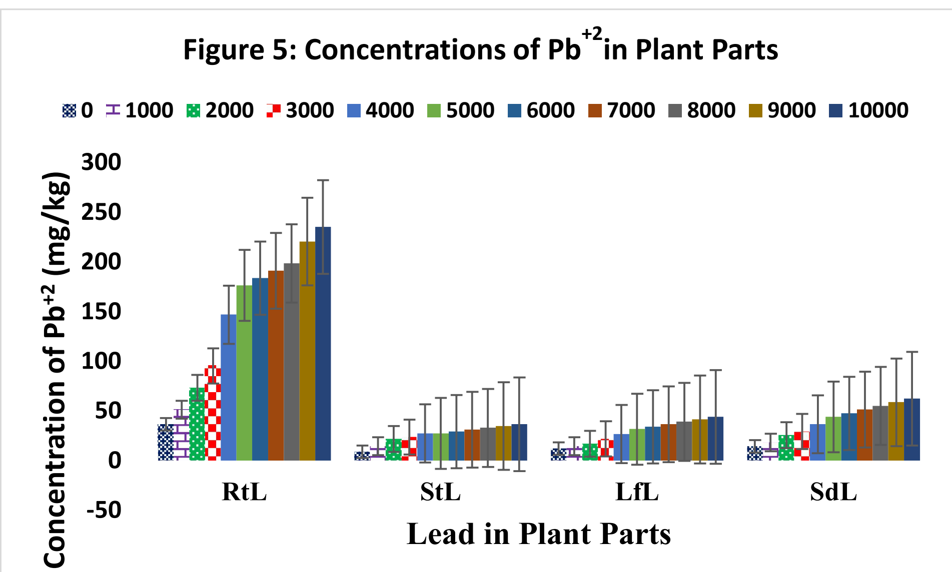
LNT	RtL	StL	LfL	SdL	DWP(g)
0	36.76±14.71	9.19±3.68	12.25±4.90	14.71±12.01	6.36±1.51
1000	51.47±14.71	14.71±0.00	14.71±5.66	18.38±14.08	5.80±0.94
2000	73.53±50.94	22.06±6.00	17.16±4.90	25.74±25.12	5.61±1.68
3000	95.59±37.01	23.90±7.04	22.06±14.71	29.41±0.00	5.44±1.70
4000	147.06±24.01	27.57±3.68	26.96±18.56	36.76±18.99	5.28±0.98
5000	176.47±0.00	27.57±7.04	31.86±9.39	44.12±0.00	4.86±1.07
6000	183.82±14.71	29.41±0.00	34.31±9.80	47.79±30.32	4.59±0.95
7000	191.18±16.98	31.25±3.68	36.76±9.39	51.47±30.61	4.30±0.48
8000	198.53±14.71	33.09±4.25	39.22±8.00	55.15±30.32	4.16±0.94
9000	220.59±29.41	34.93±3.68	41.67±9.39	58.82±20.80	3.83±0.62
10000	235.29±0.00	36.76±0.00	44.12±9.80	62.50±32.61	3.57±0.25

KEY

LNT: Lead nitrate **RtL :** Root lead **StL:** Stem lead

LfL: Leaf lead **SdL:** Seed lead **DWP:** Dry weight of plant

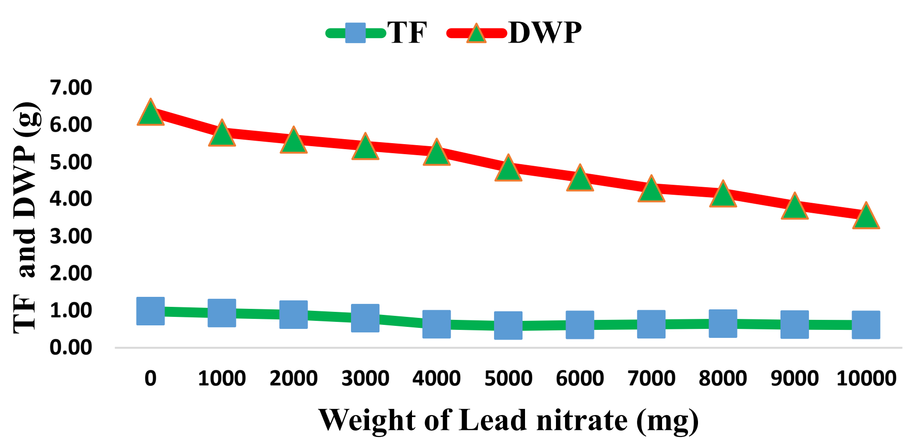
The Mean Concentrations (mg/kg) of Pb^{+2} in various Plant Parts are shown in **Figure 5**



Lead is available to plants from soil and aerosol sources. Lead uptake studies in plants have demonstrated that roots have an ability to take up significant quantities of Pb (Lane and Martin, 1977; Huang and Cunningham, 1996). Davies (1995) reported that soil particle size and cation exchange capacity as well as plant factors such as root surface area, root exudates, mycorrhization and rate of transpiration affect the availability and uptake of Pb. In general, the apparent concentration of Pb in aerial parts of the plant decreases as the distance from the root increases. This occurs due to greater localization of Pb in walls of the root than in other parts of plant. Furthermore, blinding of Pb occurs more in lignified rather than non-lignified tissues (Tung and Tample, 1996). The content of Pb in various plant organs tends to decrease in the following order: roots>leaves>stems> inflorescence>seeds. However this order can vary with plant species (Antosiewicz, 1992).

In this study, concentrations of Pb^{+2} in various plant parts varied significantly ($Pr < 0.05$). The root accumulated the highest concentrations of Pb^{+2} in the range 36.76±14.71 to 235.29±0.00 mg/kg. The stem had the lowest concentrations in the range 9.19±3.68 to 36.76±0.00 mg/kg. The concentrations Pb^{+2} of were found to decrease in the order root >> seed > leaf > stem. The translocation factor (TF), defined as the ratio of shoot-to-root metal concentrations (Adriano 2001; Liu *et al,*2009) provides an indicator of the ability of a plant to transport metal from roots to shoots. The translocation factor and dry weights of plants are shown in **Figure 6.**

Figure 6: Translocation Factor (TF) and Dry Weight of Plant (DWP)



TF and DWP generally decreased with increasing weight of lead nitrate added to soil. Requirement for a plant to hyperaccumulate Pb is that the concentration of Pb must be at least

1000mg/kg dry weight in its tissues (Blaylock *et al*, 1997). Clearly in the range 0 to 10,000mg lead nitrate, cowpea does not hyperaccumulate Pb. The dry weights decreased from 6.36±1.51 to 3.57±0.25g with increase in amount of lead nitrate. Krobrukhv *et al*, (2004) reported a considerable decrease in dry weights of plant parts under Pb treatement. According to Mishra and Choudhari (1998), very high Pb concentration may eventually lead to cell death.

3.2.2 Type 2: Vegetative Lead Inhibition

For available lead greater than 84.14 to 99.27mg/kg, cowpea exhibited vegetative lead inhibition, where the plants grew and died after sometime. The parameters for plants which germinated and died are presented in **Table 3.**

Table 3: Parameters for plants that germinated and died

LNT (mg)	ALBP (mg/kg)	LDP(mg/kg)	WDP (g)	PPS (days)
14000	88.24±8.49	47.79±7.35	0.82±0.05	20.00±0.82
15000	94.85±7.35	55.15±7.35	0.76±0.18	18.25±0.50
16000	99.27±7.94	62.50±7.35	0.63±0.14	17.00±1.41

LNT: Lead nitrate **ALBP**: Available lead before planting
LDP: Lead in dead plant **WDP:** Weight of dead plant **PPS:** Period plant survived
The lead contents in dead plants increased with increasing concentration of lead nitrate in soil. The weights of dead plants followed a reversed trend. Plants with higher lead content died earlier, which is consistent with the report of McLean et al, (1969**).**

3.2.3 Type 3: Total Lead Inhibition

Total lead inhibition manifested when the available lead was greater than 99.27mg/kg. Under this condition, there was no seed germination. The available Pb before planting in soil treatments with no seed germinated are given in Table 4.

Table 4: Available lead in treatments with no seed germination

LNT (mg)	ALBP (mg/kg)
14000	103.86±12.15
15000	115.81±27.59

Lead is considered a general protoplasmic poison, which is cumulative, slow acting and subtle. Soils contaminated with Pb cause sharp decreases in crop productivity thereby posing a serious problem for agriculture (Johnson and Eaton, 1980).Plant roots rapidly respond to absorbed Pb, through a reduction in growth rate and change in branching pattern. Several workers have reported the inhibition of root growth at 1μM to 1cM Pb or at a soil Pb content of 10μgg–1 (Breckie, 1991). A considerable decrease in dry weights of plant parts is observed under Pb treatment (Krobrukhv *et al,* 2004). Pb toxicity inhibits germination of seeds, retards growth of seedlings, root/shoot length, tolerance index and dry mass of roots and shoots. Very high Pb concentrations may eventually lead to cell death (Mishra and Choudhari, 1998).

Lead is a cumulative poison that can damage nervous connections (especially in young children) and cause blood and brain disorders. Lead poisoning typically results from ingestion of food or water contaminated with lead. It may also occur after accidental ingestion of contaminated soil, dust, or lead based paint. Long-term exposure to lead or its salts (especially soluble salts or the strong oxidant PbO_2) can cause nephropathy. The effects of lead are the same whether it enters the body through breathing or swallowing. Lead can affect almost every organ and system in the body. The main target for lead toxicity is the nervous system, both in adults and children. (Lenntech, 2004).

4. Conclusion

Cowpea exhibited three types of behaviours when planted in lead contaminated soil. These include lead tolerance, vegetative lead inhibition and total lead inhibition depending on the amount of available lead in the soil. The plant may accumulate considerable amount in the root and other parts, but it does not hyperaccumulate the heavy metal.

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Authors' Contributions

Dagari M.S.: Conceptualization, design, undertaking the research work, write-up and data analysis

Jimoh W.L.O.: Supervision of the research work; Editing of the write-up

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