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Effect of Water Soluble Phosphate onToxic Effect of Lead in Cowpea (*Vignaunguiculata*) Grown in Lead Contaminated Soil

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Abstract: *The interaction of lead (Pb) and phosphorus to form insoluble lead phosphates has been implicated as the limiting factor with regard to lead concentrations in soil solutions.Pb in soils can be transferred to plants, animals and even humans.The toxicity of Pb is worrisome and therefore environmental criteria, established by laws to support the management of contaminated sites, have been developed to prevent its deleterious effects in a wide range of soils, uses and applications. In this study, the effect of water soluble phosphate on toxic effect of lead in cowpea grown in lead contaminated soil was investigated. Four replicates each of 0 and 5,000mg of lead nitrate were applied alone or in combination with 0, 10, 50 and 250mg of disodium dihydrogen pyrophosphate 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. 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 plant extracts were determined by Atomic Absorption Spectrometry. The lead accumulated in all plant parts decreased highly significantly ($Pr \leq 0.01$) in the control and in presence of 5000mg of lead nitrate in the order root lead > seed lead > leaf lead > stem lead.According MacPherson and Martin (1994), addition of phosphate caused significant decreases in shoot–root Pb ratio due to precipitation of Pb^{+2}.*

Keywords: *Watering; Disodium dihydrogen pyrophosphate; Toxicity;Water Soluble Phosphate, Environmental Criteria.*

1. Introduction

Little is known about the inorganic buffer mechanisms for lead (Pb) in natural waters, in spite of the fact that this is a matter of considerable ecological, agricultural and biological importance. The interaction of Pb and phosphorus to form insoluble lead phosphates has been implicated as the limiting factor with regard to lead concentrations in soil solutions (Nriagu, 1974).The most important soil factors controlling lead availability to plants are pH (Cox and Rains, 1972; ArvickandZimdahl, 1974; Reddy and Patrick, 1977; Harter, 1983), redox potential (Swaineand Mitchell, 1960; Reddy and Patrick, 1977) and organic matter (GregsonandAlloway, 1984;

Stevenson, 1986). Romero - Freire*et al*. (2015) indicate that Pb availability in soils is controlled by characteristics such as pH, carbonate content, texture and surface specific area of soil particles. In general concentrations of Pb decreases with soil depth due to the important role of organic matter retaining metals (Zhao *et al.,* 2014).

Environmental pollution with Pb occurs through anthropogenic activities, such as mining, metallurgy, and electronics; among other applications, Pb is used in lead –acid batteries and in protection barriers against X- rays (Iball and Brettle, 2011). Human exposure to Pb may occur due to ingestion of contaminated water and food and due to contaminated air inhalation. Lead can accumulate in the human body, causing deleterious effects in all organs and systems; the nervous system is among the most affected (Grandjean and Herz, 2015). Lead can also damage many biological processes in plants, such as germination, growth, grain yield, nutrient absorption, ATP production and DNA replication by overproducing reactive oxygen species (Sengar*et al*., 2009; Pourrut*et al*., 2011).

2. Materials and Methods

2.1 The Study Area

The soil sample and cowpea seeds (*Vignaunguiculata*) 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.

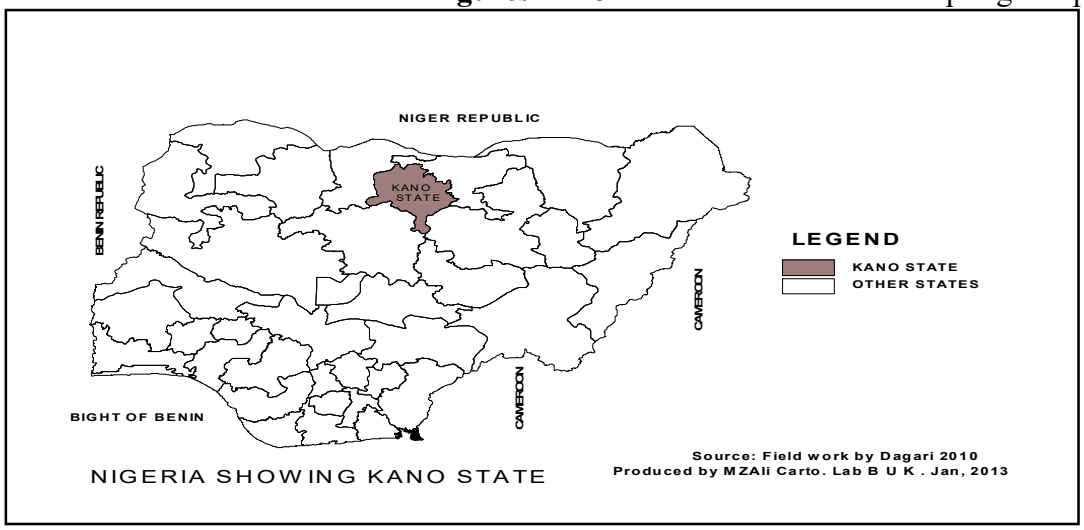


Figure 1: Map of Nigeria Showing Kano State

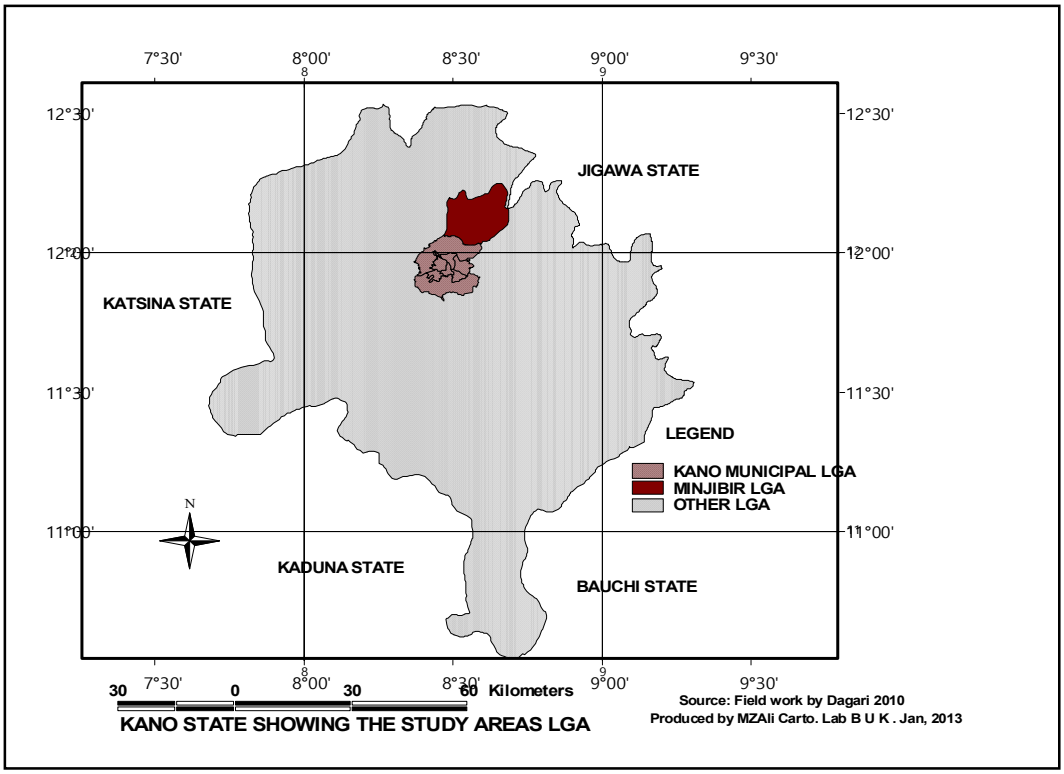


Figure 2: Map of Kano State Showing the Study Local Government Area

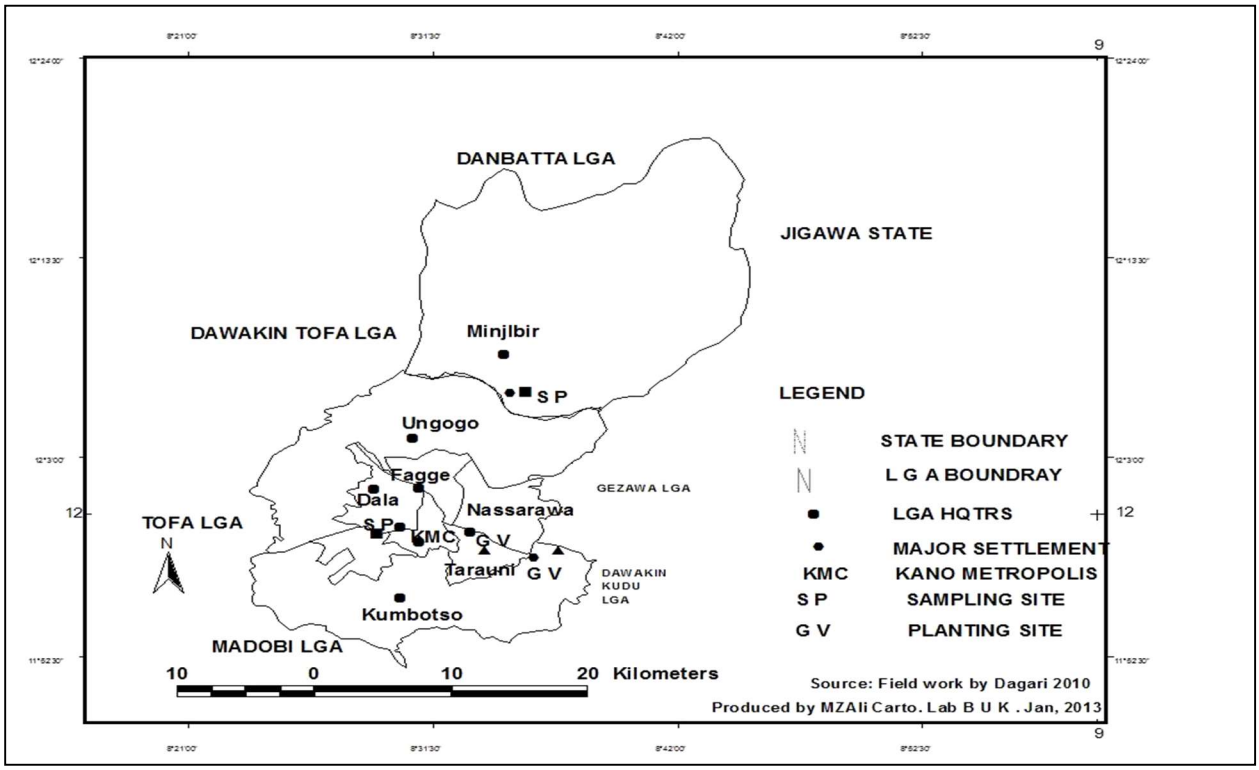


Figure 3: Kano State L.G.A. Map Showing Sampling and Planting Sites

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 collectedusing 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.

2.4Preliminary Soil Analysis

Preliminary soil analyses were carried out by standard methods; the pH and conductivity meters were used to measure the pH and electrical conductivity, respectively, in the soil suspension (1:2.5 w/v dilutions) (IITA, 1982). Organic carbon was determined by Walkley and Black method (Nelson and Sommers, 1996).The hydrometer method was used for the determination of particle size distribution (Gee and Or, 2002). Total nitrogen was determined using the Kjeldahl method (Bradstreet, 1954) and available phosphorus by Bray 1 extraction method (Bray and Kurtz, 1945), exchangeable cation by extraction with 1 N NH4OAc solution (Page *et al*., 1982), exchangeable acidity by leaching the soil sample with 1 N KCl solution (Agbenin, 1995), total metal concentration by tri-acid digestion (Stober*et al.*, 1976) and effective cation exchange capacity by summation method.

2.5 Soil Treatments and Planting of Cowpea Seeds

Four replicates each of 0and5,000mg of lead nitrate were applied alone or in combination with 0, 10, 50 and 250mg disodium dihydrogen pyrophosphate 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.

2.6Ashing of Plant Parts

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^oC 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. The plant extracts and blank were stored at low temperature before analysis (IITA, 1979).

2.7Atomic Absorption Spectrometric Analysis

The 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 plant extracts were obtained in quadruplicates from calibration curves and expressed as mg/kg (IITA, 1979).

2.8 Statistical Analysis

The data were analyzed in triplets 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 Results of Routine Analyses

Table 1: Soil Fertility Parameters

Parameter	Result	RVAS
Texture: Sand (%)	73.8 ± 1.15	
Silt (%)	12.0 ± 0.00	
Clay (%)	14.2 ± 1.15	
Textural class	Sandy loam	*Sandy loam
pH	7.08 ± 0.05	6.00–7.80
Organic carbon (%)	0.671 ± 0.09	0.5–0.7
Total nitrogen (%)	0.02 ± 0.00	0.05–0.30
Carbon-nitrogen ratio	34.762 ± 0.04	2.3–10
Total organic matter (%)	1.16 ± 0.12	0.9 –1.2
Cation exchange capacity(cmol/kg soil)	3.44 ± 0.10	2.00–30.00
Exchangeable cations (c mol/kg soil)		
Na	0.15 ± 0.00	0.3 – 2.0
K	0.86 ± 0.01	0.2 – 1.2
Mg	0.11 ± 0.01	0.5 – 8.0
Ca	1.92 ± 0.01	2.0 – 15.0
Exchangeable sodium percentage (%)	4.37 ± 0.11	<15
Electrical conductivity (mS/cm)	0.02 ± 0.01	<4.00
Water soluble phosphate (mg/kg)	2.60 ± 1.00	<10

RVAS: Recommended values for agricultural soil (Landon, 1991)

*Best for cowpea **(IITA, 1970)**

The fertility parameters; pH, total organic matter, cation exchange capacity, exchangeable potassium and calcium, exchangeable sodium percentage, electrical conductivity and water soluble phosphate of the IITA farm soil were within the range of recommended values for agricultural soils (Landon, 1991). However, the total nitrogen, exchangeable sodium and magnesium were below the recommended values. So, the soil could be used for agricultural purposes.

Table 2: Heavy Metal Concentrations (mg/kg) in Soil

Metal	Total	Alloway (1990)	Landon (1991)	Wild (1996)
Chromium	375.00 ± 16.67	5 –1500	5 –1500	NA
Manganese	11.94 ± 1.08	NA	20 – 10,000	NA
Iron	60.00 ± 16.33	2 – 100	NA	NA
Cobalt	18.25 ± 0.92	NA	0.05 – 65	NA
Nickel	13.76 ± 1.87	2 – 1000	2 – 750	75
Copper	15.93 ± 6.17	2 – 250	2 – 250	140
Zinc	113.64 ± 26.24	10 – 300	1 – 900	300
Cadmium	1.09 ± 0.26	0.01 – 2.4	0.01 – 2.4	3
Lead	49.63 ± 3.68	2 – 300	2 – 300	300

NA means value not available

The total metal concentrations in the soil were compared with values reported byAlloway (1990), Landon (1991) and Wild (1996).There was no heavy metal contamination of the soil, since the concentrations of all metals determined were either less or within the range of these reported values.

3.2 Lead Uptake by Plants

Although considerable research has been done concerning contamination of vegetation by Pb, there are many unanswered questions. Research on Pb uptake by plants is incomplete but seems to indicate that plants take up Pb from the soil but generally only small quantities. Marten and Hammond (1966) studied Pb uptake by bromegrass (*Bromusenermis, Leyss*) from sandy loam soils with a range in Pb content between 12 and 680 ppm. Results indicated that only plants grown in the soil with the 680 ppm level accumulated a significant amount of Pb. This accumulation was enhanced by the addition of a chelate, but the maximum accumulation was only 34 ppm. Motto *et al.* (1970) grew several crop species in contaminated soil and in acid – washed sand to which soluble Pb was added in low concentrations. Their results established that Pb can be absorbed through the root system and that some translocation to other plant parts does occur.

The present study examines the effect of water soluble phosphate on toxic effect of Pb in cowpea grown in contaminated soil. Table 3 shows the concentrations of Pb in different parts of cowpea.

Table 3: Concentrations of Pb^{+2} in Different Parts of Cowpea (*Vignaunguiculata*)

LNT	DSDHP	RtL	StL	LfL	SdL	ShL
0	0	36.76±14.71	9.19±3.68	12.25±4.90	14.71±12.01	36.15±7.31
0	10	30.57±8.41	7.35±0.00	8.85±2.24	9.78±8.05	25.98±229
0	50	27.43±6.03	4.21±0.73	6.36±0.54	7.26±6.71	17.83±4.61
0	250	22.96±4.22	2.10±1.23	3.17±0.00	4.16±1.15	9.43±1.18
5000	0	176.47±0.00	27.57±7.04	31.86±9.39	44.12±0.00	103.55±7.71
5000	10	148.35±32.47	22.06±0.00	24.34±3.27	27.78±2.84	74.18±2.52
5000	50	127.89±8.84	13.22±2.59	20.11±3.27	24.22±1.39	57.55±1.20
5000	250	112.83±8.54	8.24±1.09	10.33±0.00	14.15±2.36	32.72±0.43

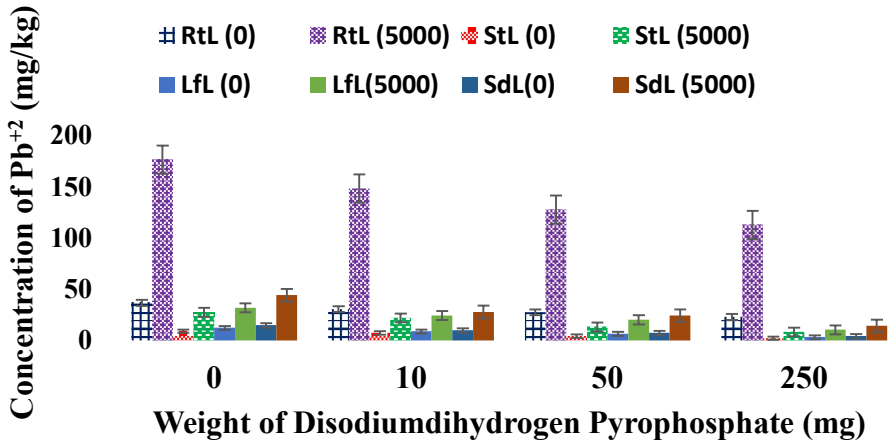
KEY

LNT: Lead nitrate (mg in 3kg Soil) **DSDHP:**Disodium dihydrogen pyrophosphate

RtL: Root Lead **StL:** Stem Lead
LfL: Leaf Lead **SdL:** Seed Lead **ShL:** Shoot Lead

Concentrations of Pb^{+2} in various plant parts are shown in **Figure 4**

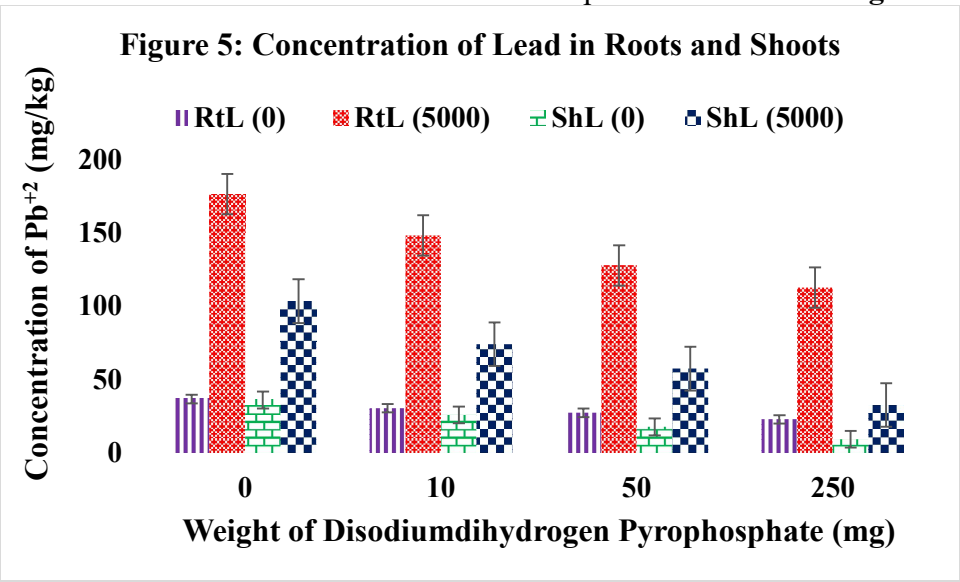
Figure 4: Concentration of Lead in Different Plant Parts



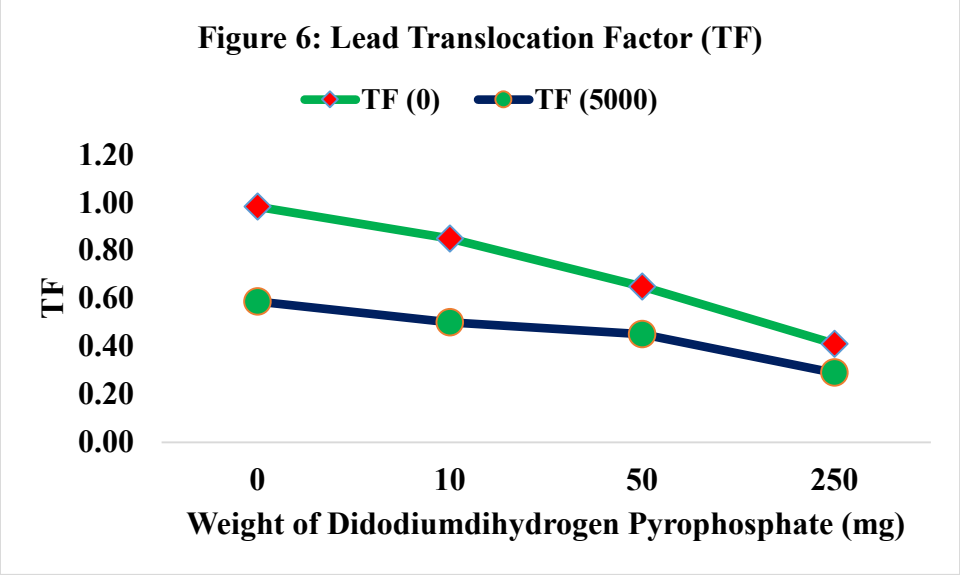
The numbers **0** and **5000** in bracket indicate the weights of lead nitrate added to soil samples.

Lead accumulated in all plant parts and decreased highly significantly ($Pr \leq 0.01$) in the control and leaded samples with increasing doses of disodium dihydrogen pyrophosphate (DSDHP).The largest concentration of Pb, was in the root. It decreased from176.47 to 112.83 mg/kg with increasing dose of DSDHP from 0 – 250 mg applied to the soils.This result is in agreement with other authors, who reported that Pb when absorbed by the plant tends to concentrate in roots, translocating very little to the shoot, which varies between different plant species (Zang et al.,

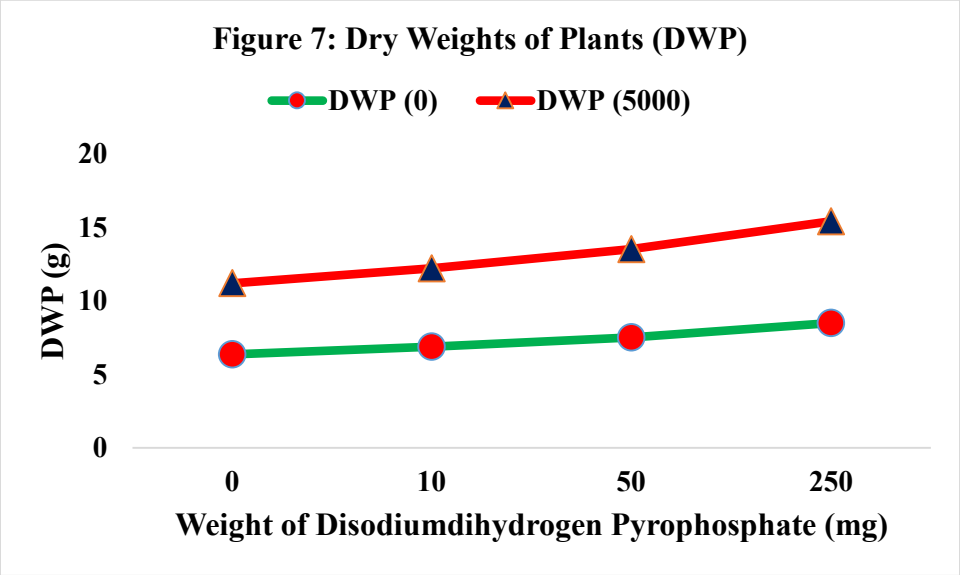
2013).The lowest concentration of Pb in the stem, decreased from 9.19 – 2.10mg/kg with increasing dose of DSDHP. Lead accumulated in other parts of the plant followed similar trend.Concentrations of lead in various plant parts which decreased in the order root lead > seed lead > leaf lead > stem lead agreed with the report of Antosiewicz, (1992).
Concentrations of Pb^{+2} in roots and shoots of plants are shown in **Figure 5**



The numbers **0** and **5000** in bracket indicate the weights of lead nitrate added to soil samples. The shoot consists of plant parts above the soil surface. Concentrations of Pb in the roots and shoots ranged from 22.96 to 176.47 and 9.43 to 103.55 mg/kg respectively. This is in consonance with the result of (Eugeniusz *et al*., 2002) who reported that root growth of corn seedlings (*Zea mays L*.) was more sensitive to lead than shoot growth and was inhibited by lead concentrations in the range 10^{-5} to 10^{-3} M.
The ratio of the concentrations of Pb in shoot to root called the Pb translocation (TF) is shown in**Figure 6.**



The numbers **0** and **5000** in bracket indicate the weights of lead nitrate added to soil samples. The largest TF was in the control, with a value of 0.98 which decreased to 0.42 with increasing dose of DSDHP from 0 – 250mg in the soils. At 5000 mg lead nitrate, TF was 0.59 which decreased to 0.29 with increasing dose of DSDHP from 0 – 250mg in the soils.
The results are in agreementwith other authors, who reported that Pb when absorbed by the plant tends to concentrate in roots, translocating very little to the shoot, which varies between different plant species (Berger, 1962; Berg, 1970; Rolfe, 1973 and Zang*et al*., 2013).
The dry weights of plants (DWP) are shown in **Figure 7.**



The numbers **0** and **5000** in bracket indicate the weights of lead nitrate added to soil samples. The DWP in the control was 6.36g which increased to 8.50g with increasing dose of DSDHP from 0 – 250mg in the soil. In presence of 5000mg of lead nitrate the DWP was 4.86g which increased to 6.93g with increasing dose of DSDHP from 0 – 250mg in the soil. So, the DWP decreased in presence of lead nitrate and increased with increasing dose of DSDHP. The DWP is often used as a measure of heavy metal toxicity, which decreased with concentration of heavy metal in the soil (Khan and Khan 1983; Burzynski 1987; Trivedi and Erdei 1992; Antosiewicz 1993; Begonia et al. 1998). In presence of phosphate, Pb, is insoluble and cannot move freely in the vascular system of the plant because the metal is immobilized in the apoplastic and symplastic compartments by the formation of precipitates (Raskin et al., 1997). In a similar research, MacPherson and Martin (1994) reported that root phosphate levels increased with addition of phosphate to soil. Excess phosphate in root then reacted with Pb to prevent its translocation to shoots. In the studies reported by MacLean (1969) and TerHaar (1970), limited translocation of Pb from root to the top in some edible crops was observed.

4. Conclusion

Disodium dihydrogen pyrophosphate has been found to considerably decrease Pb^{+2} accumulated in various plant parts which decreased in the order root lead > seed lead > leaf lead > stem lead.

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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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