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Effects of Organic Matter on Translocation of Lead in Cowpea (*Vigna unguiculata*) Grown in Lead Contaminated Soil

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Abstract: *The influence of soil organic matter on the availability of trace metals is not well understood. While some workers reported that organic matter increases the fixation of these metals in soil, others claimed that it promotes their availability. Some authors found that organic matter has no influence on the availability of heavy metals. Yet, other authors found that organic matter has a negative effect on lead solubility. In this study, the effects of organic matter on translocation of lead in cowpea (Vigna unguiculata) 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, 5000, 10000 and 20000mg of cow dung 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. Lead accumulated in the roots and shoots decreased highly significantly ($Pr \leq 0.01$) in the control and leaded samples with increasing doses of cow dung. The largest concentration of Pb, was in the root. The control had the largest value of translocation factor (TF) which decreased with increasing dose of cow dung from 0 – 20,000mg in the soils. The effectiveness of cow dung in reducing Pb uptake and translocation of the element to the shoots could be related to their high organic matter content, and to a lesser extent to their phosphate content, pH and total nitrogen when compared with the untreated soils.*

.***Keywords:*** *Organic Matter; Cow dung; Cowpea; Translocation Factor; Ash; pH; Total nitrogen ; Phosphate Content.*

1. Introduction

Lead (Pb) as one of the major heavy metals in the environment has gained considerable importance as a potent pollutant. Apart from the natural weathering processes, Pb contamination of the environment has resulted from mining and smelting activities, Pb containing paints, gasoline and explosives as well as from the disposal of municipal sewage sludges enriched in Pb (Chaney and Ryan, 1994). Despite regulatory measures adopted in many countries to limit Pb input into the environment, it continues to be one of the most serious global environmental and human hazards. As many of the Pb pollutants are indispensable for modern human life, soil contamination with Pb is not likely to decrease in the near future (Yang *et al.*, 2000). Significant increases in the Pb content of cultivated soils has been observed near industrial areas. Pb tends to accumulate in the surface ground layer and its concentration decreases with depth (de Abreu *et al.*, 1998). It is easily taken up by plants from the soil and is accumulated in different organs. Pb 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).

The visual general symptoms of Pb toxicity are fast inhibitions of root growth, underdeveloped growth of the plant, blackening of root system and chlorosis. Lead inhibits photosynthesis, let downs mineral nutrition, water balance and enzyme activities. These disorders upset physiological activities of the plant. At high concentrations lead may finally result to cell death (Sharma and Dubey, 2005). Similarly, lead inhibits germination of seeds and retards growth of seedlings, decreases germination percent, germination index, root/shoot length, tolerance index and dry mass of roots and shoots (Mishra and Choudhary, 1998).

Earlier studies confirmed that the damage to plant root system and the decrease in transpiration strength was caused by excess of Pb, and brought about a reduction in water uptake, then inadequate supply of water to the above ground parts. Lead causes disorder in the composition of both the lipid membrane and protein fraction, enabling its permeation into cells (Sharma and Dubey, 2005).

The influence of soil organic matter on the availability of trace metals is not well understood. While some workers (Cheng and Quellette, 1971; Davies, 1956; Hassel, 1974; Hodgson, 1963; Rajagopal *et al*.1974; Shuman, 1975) reported that organic matter increases the fixation of these metals in soil, others on the contrary, claimed that it promotes their availability (Bloomfield *et al.*, 1976; Elliott and Blaylock, 1975; Ghanem *et al.,* 1971). Some authors found that organic matter has no influence on the availability of heavy metals (Lundblad *et al*., 1969).Mac Lean *et al.,* (1969), Karamanos *et al*., (1976) and Hassel (1974) found that organic matter has a negative effect on Pb solubility, while Bloomfield *et al.* (1976) demonstrated that this metal could be solubilized as an organic complexes in a true solution and/or colloidal solution.

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.

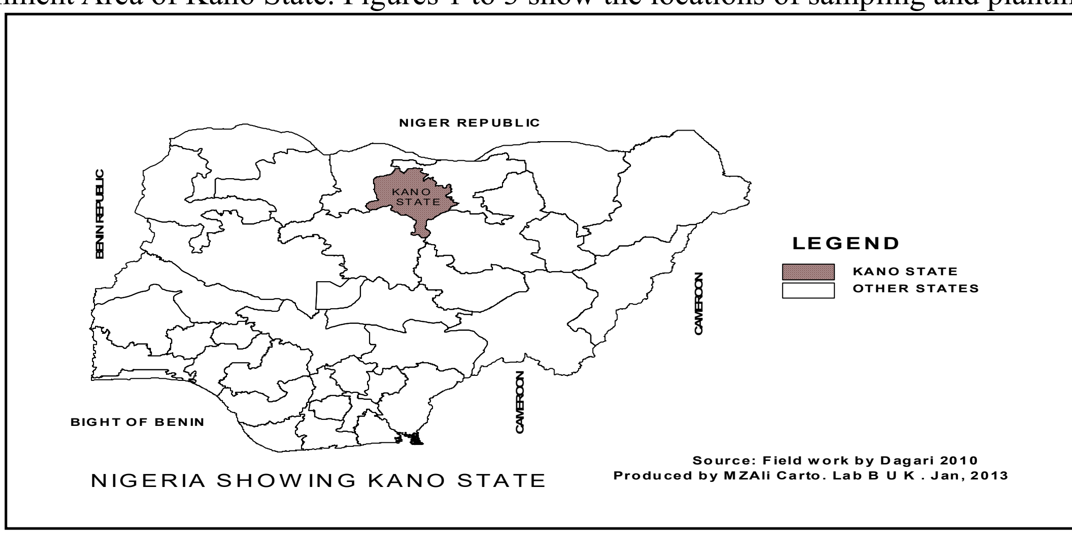


Figure 1: Map of Nigeria Showing Kano State

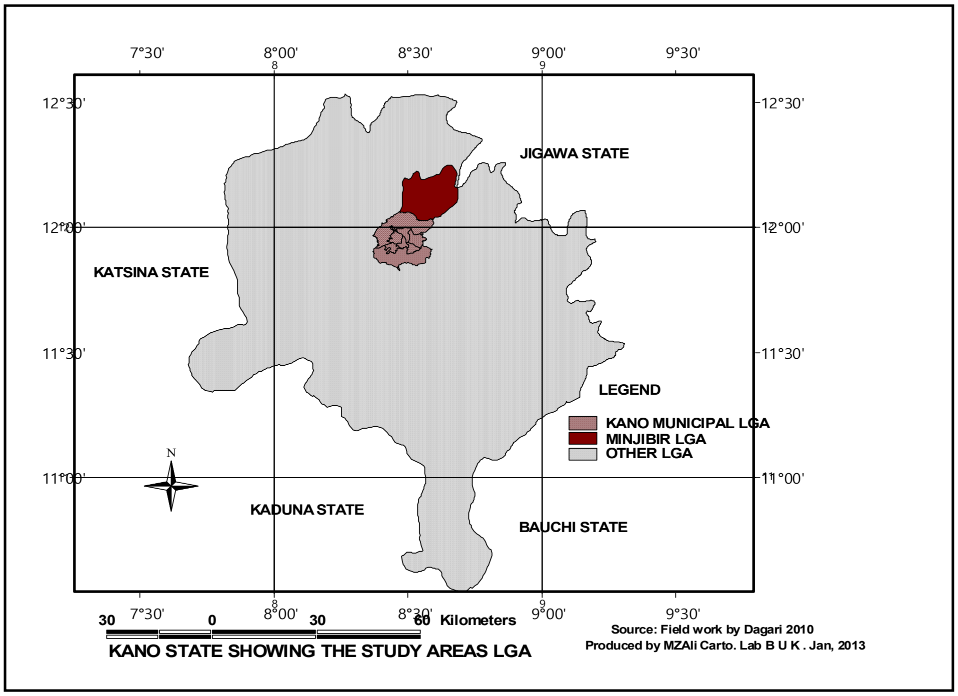


Figure 2: Map of Kano State Showing the Study Local Government Area

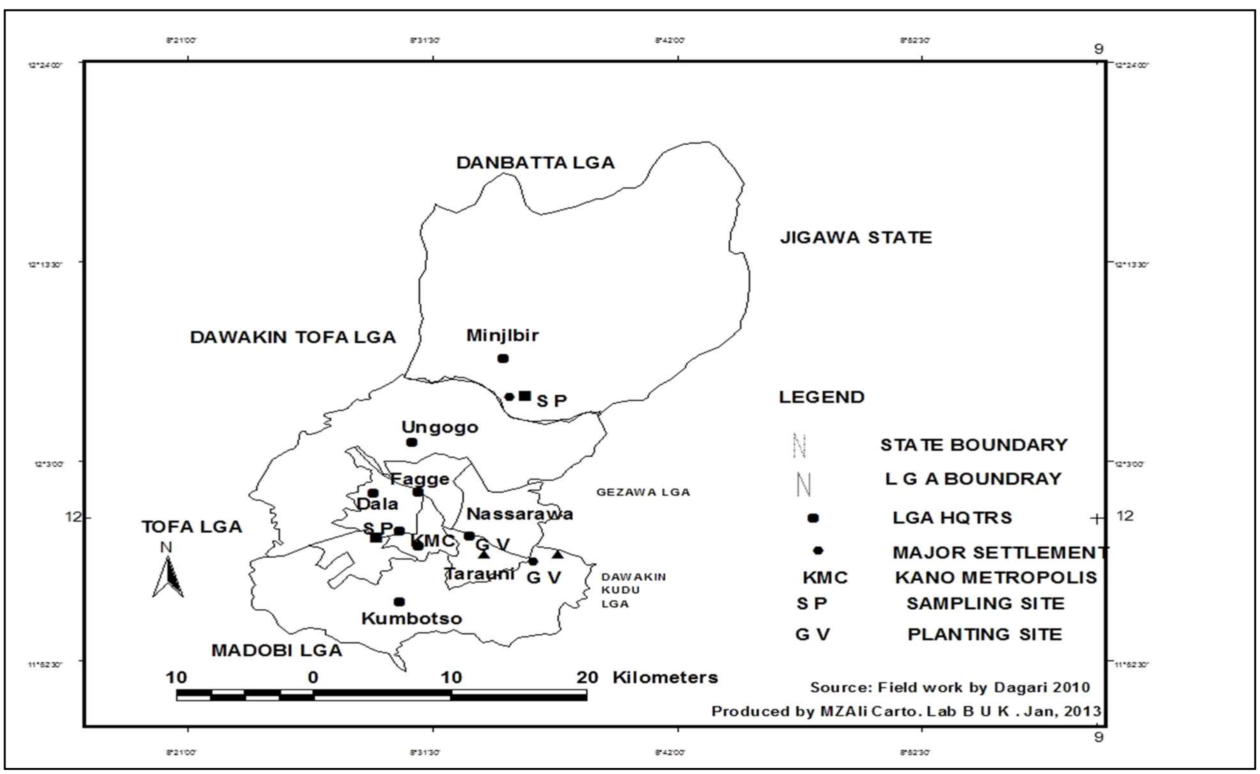


Figure 3: Kano State L.G.A. Map Showing Sampling and Planting Sites

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 from IITA farm, Wasai 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.

2.4 Preliminary 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 0 and 5,000mg of lead nitrate were applied alone or in combination with 0, 5000, 10000 and 20000mg of cow dung 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.

2.6 Ashing 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^{o}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. 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: Properties of Cow Dung

Parameter	Results	Cheung and Wong, (1981)
pH	7.45±0.01	6.76–7.44
Organic carbon (%)	179.35±4.12	0.76–1.71
Total nitrogen (%)	2.136±0.06	0.02–0.23
Carbon-nitrogen ratio	83.97±2.13	NA
Total organic matter (%)	309.20±3.45	1.31–2.95
Cation exchange capacity(cmol/kg soil)	12.58±0.05	7.64–10.27
Na	4.07±0.05	0.08–0.41
K	4.57±0.04	0.17–1.42
Mg	0.19±0.01	2.70–4.65
Ca	3.24±0.01	1.47–2.97
Exchangeable sodium percentage (%)	32.35±0.05	NA
Electrical conductivity (mS/cm)	8.81±0.01	38–127.47
Water soluble phosphate (mg/kg)	165.77±1.00	NA

Differences in values obtained in this work compared to reported values for cow dung by Cheung and Wong, (1981) could be attributed to environmental factors, age, eating habit and health status.

Table 3: 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 Alloway (1990), Landon (1991) and Wild (1996). There was no heavy metal contamination of the soil, since the concentrations of all metals were either less or within the range of these standard values.

Table 4: Heavy Metal Concentrations (mg/kg) in Cow Dung

Metal	Total	heung & 1981	Won¦PA, 1993
Chromium	615.00±57.45	NA	NA
Manganese	8.36±0.97	5.75 –390.0	NA
Iron	82.23±5.81	NA	NA
Cobalt	2.14±0.82	NA	3–100
Nickel	27.87±1.18	NA	17.5–250
Copper	19.85±5.70	14.5–105.0	NA
Zinc	46.18±1.13	7.5–372.5	100–450
Cadmium	5.08±1.61	NA	4–83
Lead	61.21±3.31	8.3 –33.2	2.5–340

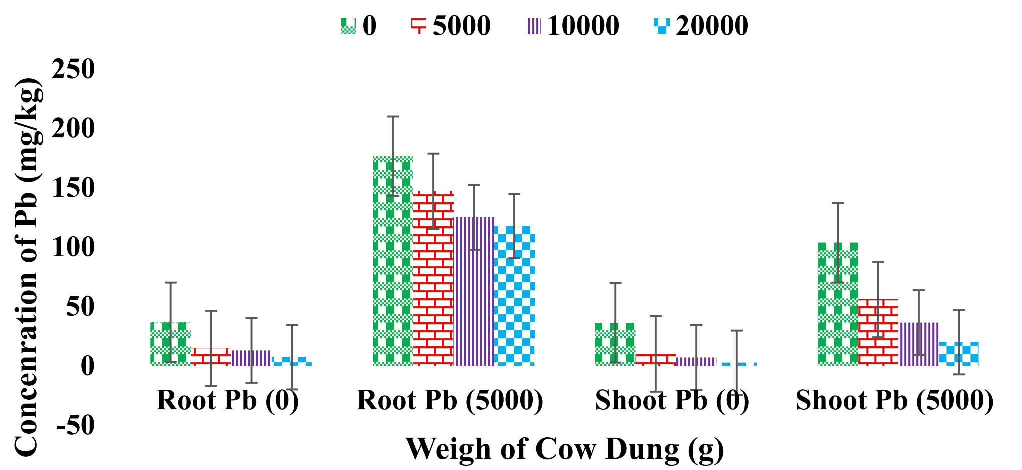
The properties of cow dung and soil used in this study are shown in **Tables 1 – 4**. Cow dung had higher pH, total nitrogen, cation exchange capacity, exchangeable bases and electrical conductivity. The total organic matter, exchangeable sodium percentage and water soluble phosphate of cow dung were extremely high when compared with that of the soil.

3.2 Effects of Organic Matter on Lead Uptake by Plants

It is evident that majority of Pb in plant tissues is absorbed via the root system and the availability of soil Pb to plants is governed by a number of soil factors. MacLean *et al.* (1969) reported that Pb uptake by oats (*Avena sativa*) and alfalfa (*Medicago sativa*) could be reduced by increases in pH and organic matter. Page *et al.* (1971) found that the toxicity of Pb to maize, beans, lettuce and radishes were obtained by Cox and Rains (1972) who reported that liming decreased uptake of Pb by plants.

Cow manure, which usually contains high percentages of organic matter, was found to effectively reduce Pb availability to plants (Zimdahl and Foster, 1976; Scialdone et al. (1980). Zimdahl and Skogerboe (1977) reported that soils with higher organic matter accumulated more metals near the surface. The ability of organic matter to retain Pb in soil can be a result of its great absorptive capacity, which lowers Pb mobility in soil.
Concentrations of Pb^{+2} in roots and shoots are shown in **Figure 4.**

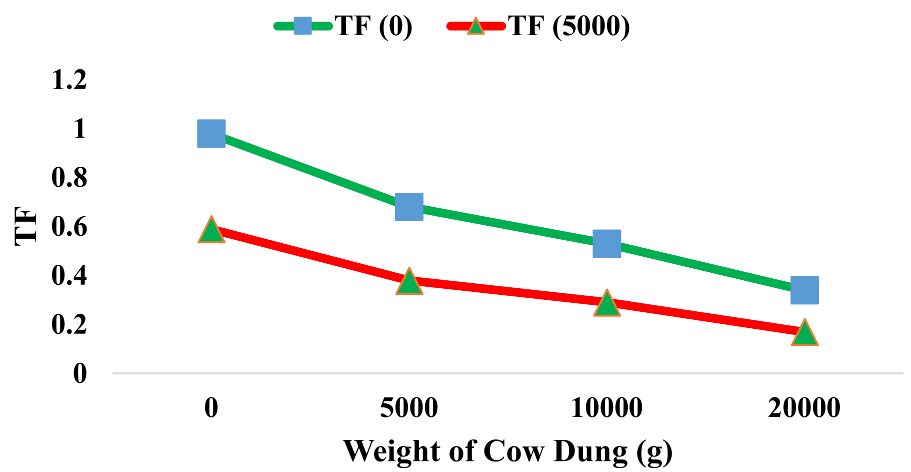
Figure 4: Concentrations of Pb in Roots and Shoots



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

Lead accumulated in the roots and shoots decreased highly significantly (Pr $\leq$ 0.01) in the control and leaded samples with increasing doses of cow dung. The largest concentration of Pb, was in the root. It decreased from176.47 to 117.65 mg/kg with increasing dose of cow dung from 0 – 20,000 mg applied to the soils. This result is in agreement with Wong and Lau (1985), who reported that Pb uptake by B. *chinensis* and R. *sativus* was significantly reduced in presence of pig manure and refuse compost. The lowest concentration of Pb in the shoot, decreased from 36.15 – 2.50mg/kg with increasing dose of cow dung.
Translocation factor of Pb^{+2} from the roots to shoots is shown in **Figure 5.**

Figure 5: Lead Translocation Factor (TF)



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 0.34 with increasing dose of cow dung from 0 – 20,000mg in the soils. At 5000 mg lead nitrate, TF was 0.59 which decreased to 0.14 with increasing dose of cow dung from 0 – 20,000mg in the soils.

The effectiveness of cow dung in reducing Pb uptake and translocation of the element to the shoots could be related to their high organic matter content, and to a lesser extent to their phosphate content, pH and total nitrogen when compared with the untreated soils (Tables 1 and 2). The importance of organic matter to immobilize Pb in soil has been commonly recognized (Griffeth, 1979; Zimbahl and Foster, 1976; Stevenson, 1976). Also, addition of cow dung might raise the pH (Tables 1 and 2).

4. Conclusion

Cow dung has been found to considerably decrease Pb^{+2} accumulated in plant parts which decreased in the order root lead > shoot 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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