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*Keywords: lead; vigna unguiculata; microbial activities; phytostabilization; bio-concentration factor, translocation factor; phytoremediation.*

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*Strictly as per the compliance and regulations of :*

# Phytoremediation Potential of *Vigna Unguiculata* on Lead Polluted Soil and Its Biotoxic Effects on Soil Microbial Activities

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*Abstract-* The aim of this research was to evaluate the impact of lead nitrate on soil microbial activities, growth performance, and the phytoremediation potential of *Vigna unguiculata* (cowpea) grown in agricultural soil. Pristine sandy loam soils were polluted with nitrate salt of lead at four different levels (50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg) in triplicates. A significant ($P < 0.05$) retarding effect of the metal salt on the studied parameters was observed. Consistently, total bacterial population was found decreasing with rise in lead dosage. Lead was also found to significantly ($P < 0.05$) affect the microbial metabolism as witnessed by the lowering of $CO_2$ evolved in the test samples. A generalized growth reduction in the performance of *Vigna unguiculata* observed when compared to the control showed that lead phytotoxicity was concentration dependent. Bioaccumulation capacity of the plant was determined using an Atomic Absorption Spectrophotometer (FS 240 AA Agilent Technology) after pulverization and digestion of the plants using $HCl/HNO_3$ (3:1 v/v). Favourably, roots appear to be a better site for metal residence than shoots. The Bio-concentration Factor (BCF) and Translocation Factor [BCF > 1 (3.99); TF < 1 (0.67)] value obtained conclusively suggest that cowpea is a suitable candidate for the phytostabilization of lead contaminated soil especially at highly polluted sites.

*Keywords: lead; vigna unguiculata; microbial activities; phytostabilization; bio-concentration factor, translocation factor; phytoremediation.*

## I. Introduction

The quest for recognized among committee of nations has made most countries especially developing and less developed nations to pay attention on economic growth and industrialization as their key developmental priorities, with little or no significant concern given to the ailing environment. Within the last century, the entire ecosystem has witnessed a sharp increase in the release of harmful substances due to industrialization and urbanization (Odoh, 2015). This with no doubt has caused global warming, climatic change, and food insecurity while lowering significantly life expectancy within the tropics. As soil and water bodies get continuously contaminated by industrial release, deposition and emission, they pose great threat to man and his agricultural activities (Ananda and Prasade, 2003; Alloway, 1995). In the developing world, most especially within the tropics (Nigeria), the deposition of wastes and substances loaded with heavy metals on agricultural soil has been a rising practice owing to lack of stern regulations, enforcement and monitoring of most industrial activities (Zhang *et al*., 2010).

As an emerging remediation strategy, phytoremediation has been highly explored in recent time for the clean-up of contaminated sites. Because of its aesthetic nature, ecofriedly, cost effective and long term applicability, unlike the conventional techniques (Knight *et al*., 1997), its use has gotten public acceptance. The classification of plants as either phytoextractor or phytostabilizer depends sole on their bioaccumulation and translocation strength. For a plant to be a suitable phytoremediation agent, it must have a rapid growth rate, high biomass, and potential to tolerate and accumulate high amount of heavy metal and translocate them to the aboveground part (Gisbert *et al.* 2003; Chaney *et al*., 1997). Efficiency of phytoremediation techniques depend largely on soil properties, type of contaminant, its mobility and bioavailability (Cunningham and Ow, 1996). As a promising ecofriendly remediation technique, it has been utilized in the decontamination of over 200 radiobiological sites in USA (Rajiv *et al*., 2009).

As non-essential non degradable natural component of the earth crust, lead (Pb) has become one of the major and most frequently released environmental pollutants. Its affects all forms of life even at low concentration (Wierzbicka *et al*., 2007) by exerting negative pressure that alters membrane permeability, induce oxidative stress and reduce mineral/nutrient uptake in plants (Reddy *et al*., 2005). This pollutant often gets deposited via industrial activities, anthropogenic means, city effluent and sewage and excessive used of agrochemicals. With the emergence of an ecofriendly

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and cost effective sequestration technology of pollutants (phytoremediation), a good number of plants has been studied for their possible potential, notable among them include *Thlaspicaerulescence, Andropogangerardii* (Rajiv *et al*., 2009), *Populus* sp (Singh *et al.,* 2006) and *Euphorbia cheiradenia* (Chehregani and Malayeri, 2007).

Even though the call for the utilization of legumes in phytoremediation owing to their inherent potentials (*rhizobacteria*) has been stressed, it has not yet been fully harness due to rejection by some quarters who foresee food scarcity, especially in developing world where most of these crops serve as staple food. However, soil microorganisms, phosphorus solubilizing bacteria, plant growth promoting rhizobacteria (PGPR), mycorrhizal helping bacteria (MHB) and arbuscular mycorrhizal fungi (AMF) residence in legumes rhizosphere plays a vital role in environmental cleansing by lessening the harmful effects of heavy metals via secretion of proteins and biomolecules (Denton, 2007). Occasioned by the rising pollution of the environment by heavy metals through industrialization, there has been growing concern of the fate of plants and soil microorganisms, bearing in mind of their crucial role in the ecosystem. Hence the research was design to evaluate the impact of lead nitrate on soil microbial activities, growth performance and phytoremediation strength of *Vigna unguiculata* grown in contaminated soil.

## II. Methods and Materials

*a) Study Area and Sample Collection*

Soil samples were collected from Botanical Garden, University of Nigeria, Nsukka at the depth within 5cm to 15cm. The planting experiment was done in a greenhouse. Viable seeds of cowpea were purchased from Nsukka and stored at $20^0$C-$25^0$C for 24h. Healthy seeds were sorted and viability tested using floatation technique. Analytical grade nitrate salt of Lead [$Pb(NO_3)_2$] was used.

*b) Soil Analysis*

Soil particle size was determined using Boyoucos Hydrometer Method of Gee and Bauder (1987). Potential of hydrogen (pH), $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $K^{+}$,percentage organic matter, phosphorus and soil moisture were analyzed according to Black (1965). Soil nitrogen determination was done using Kjeldahl method of Keeney and Nelson (1982).

*c) Planting Experiments*

After the soil samples were air dried, stones sorted out and sieved, three kilogram (3kg) of the soil samples were dispensed in each twelve (12) plastics perforated pots. In triplicate, all the four different levels (50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg) of $Pb(NO_2)_3$ were used to treat the soils. The different concentrations of the salt were dissolved in 200ml distilled water and thoroughly mixed with a plastic miniature cement mixer. The treatments were kept in a greenhouse and allowed to stabilize for seven days to permit the disturbance caused by sampling and sieving to subside. Three soil samples with no metal amendment (controls) were prepared. After seven days of incubation, four viable seeds were sown on each pot. They were watered (200ml) every two days for eight weeks while being cautious of possible waterlog and leaching of the metal salt.

*d) Effect of Lead on Germination*

Viable seeds of cowpea were considered germinated when the radical reach a length of 1mm. The germination time (days) was recorded. The number of germinated seed and total number of planted seeds were used to determine the germination percentage.

$$G\% = \frac{nG}{nT} \times 100$$

Where: G% = Germination percentage
nG = Number of germinated seeds
nT = Total number of seeds

*e) Effects of Lead on Shoot and Root Length*

On weekly interval, the shoots length of *Vigna unguiculata* were monitored and measured with a meter rule and readings taken from both the test and control experiments. After 60 DAS (days after sowing), they were uprooted and washed with clean tap water to remove soil particles. The root length was then measured in relation with the control.

*f) Effect of Lead on Nodulation*

After Sixty days, the *Vigna unguiculata* was uprooted and washed to remove soil particles. The number of nodules formed on each plant and level of treatment were carefully counted and recorded.

*g) Effect of Lead on Weight*

After uprooting and washing with distilled water the plant was air-dry and the wet weight determined. This was followed by oven-drying at 105°C for 24h to check for the dry weight.

*h) Lead Uptake Potential of Vigna unguiculata*

At the end of the dry weight determination, the plants were separated into roots and shoots and pulverized using laboratory milling machine. The pulverized organic samples were digested using $HCl/HNO_3$ (3:1 v/v), followed by metal uptake determination using an Atomic Absorption Spectrophotometer (FS 240 AA Agilent Technology). The bioconcentration factor (BCF) and translocation factor (TF) were calculated. The BCF is the ratio of metal concentrations in the roots to those in the soil or water (Abdul and Thomas, 2009) while TF is the ratio of metal concentration in the shoots to that of the roots (Malik *et*

*al*., 2010). Plants are considered as phytoextractor when TF >1 and as phytostabilizer when BCF > 1 and TF < 1 respectively,

$$BCF = \frac{roC}{soC}, \quad TF = \frac{shC}{roC}$$

Where: roC = Concentration in root (mg/g)
soC = Concentration in soil (mg/g)
shC = Concentration in shoot (mg/g)

*i) Effects of Lead on Soil Bacterial Population*

Pristine sandy loam soil samples were air-dried sieved and dispensed in twelve 250ml conical flasks. The samples were contaminated with four different levels of Pb, with each level in triplicates. Controls of three unpolluted soil samples were prepared. The conical flasks were periodically watered for microbial sustenance. Bacterial population analysis was done by serially diluting the soil sample collected from each conical flask at a weekly interval over a period of four weeks and viable bacterial cells determined as described by Wistreich (1997).

*j) Effects of Lead on Soil Microbial Respiration*

Two hundred (200) grams of air-dried pollute soil samples were weighed into twelve kliner jar with three unpolluted soil samples serving as controls. Sterile water was sprinkled on the soil up to 60% water holding capacity to make it moist. In each jar, a vial containing 15ml of already prepared 0.05M NaOH was placed. Three empty kliner jars, each containing a vial with 15ml 0.05M NaOH, were used as blanks. The jar tops were greased and properly capped to avoid escape of $CO_2$ and incubated at room temperature. On weekly bases, the vials were removed and 3ml of 20% $BaCl_2$ were added followed by three drops of phenol red indicator, and subsequently titrated using 0.05M HCl until a colourless end point was observed. Values obtained were calculated as:

$$CO2\ (mg)/SW/T = \frac{(Vo\text{-}V) \times 1.1}{DWT}$$

*Where*
SW is the amount of soil dry weight in grams.
T is the incubation time in hours.
Vo is the volume of HCl used for titration.
V is the volume of HCl used for the soil sample.
DWT is the dry weight of 1g moist soil.
Is the conversion factor (1ml 0.05 NaOH equals 1.1mg CO2).

## III. Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using SPSS version 16 and reported as mean ± standard deviation (SD). Statistical values at $P < 0.05$ were considered to be significant.

## IV. Results

*a) Physicochemical Analysis*

Soil physicochemical characteristics were determined and their values summarized in Table 1. The soil was categorized as sandy loam soil with a pH of 6.3, organic carbon of 1.98 and cation exchange carbon (CEC) as 8.0. The pH is one of the most important parameters that directly affect the availability of trace metals in the soil (Sauveet *al*., 1997).

*Table 1:* Soil physicochemical analysis

| Serial number | Properties | Sample result |
|---|---|---|
| 1 | Texture class | Loam Soil |
| 2 | Particle size% | |
| | a. Clay | 12 |
| | b. Silt | 3 |
| | c. Fine Sand | 28 |
| | d. Sand | 57 |
| 3 | pH value | |
| | i. $H_2O$ | 6.3 |
| | ii. KCl | 5.7 |
| 4 | Organic Matter (%) | |
| | i. Carbon | 1.15 |
| | ii. Organic matter | 1.98 |
| 5 | Nitrogen (%) | 0.126 |
| 6 | Exchange bases (Me/100g) | |
| | $Na^+$ | 0.11 |
| | $K^+$ | 0.05 |
| | $Ca^{2+}$ | 2.40 |
| | $Mg^{2+}$ | 1.20 |
| 7 | CEC (me/100g) | 8.00 |
| 8 | Base salt (%) | 47.00 |
| | - $Al^{3+}$ | - |
| | - $H^+$ | 1.60 |
| 9 | Phosphorus (ppm) | 11.19 |
| 10 | Moisture (%) | 6.2. |

*b) Effects of Lead on the Germination, Growth Performance and Phytoremediation Potentials of Vigna unguiculata.*

Table 2 shows the effect of lead on germination time and percentage of *Vigna unguiculata,* metal uptake with BCF and TF potentials. This demonstrates that cowpea exhibits a remarkable decrease in germination when found in Pb contaminated soil. At maximum level of treatment (400mg/kg), a prolonged germination time (7 days) was noticed unlike the control (4±1 days). At 50mg/kg treated soil and control, the cowpea seed sown in each pot germinated but there was a high reduction in other levels of treatment. The root showed to be the most preferential site for Pb accumulation than the shoot,as it uptake tend to be concentration dependent (Table 2).

*Table 2:* Effect of lead on germination, vegetative growth, metal uptake, BCF and TF potentials of Vigna unguiculata

| Parameters | 50mg | 100mg | 200mg | 400mg | Control |
|---|---|---|---|---|---|
| Germination time (days) | 5±1.6 | 5.5±1.3 | 6±1 | 7±0 | 4±1 |
| Germination percentage (%) | 100 | 75 | 58.30 | 33.30 | 100 |
| Shoot metal uptake (mg/kg) | 0.026±0.002 | 0.051±0.002 | 0.112±0.002 | 2.701±0.002 | 0.010±0.001 |
| Root metal uptake (mg/kg) | 0.118±0.002 | 0.225±0.000 | 0.295±0.006 | 4.006±0.191 | 0.102±0.003 |
| Bio-concentration factor (mg/kg) | 0.13 | 0 .22 | 0.29 | 3.99 | ND |
| Translocation factor (mg/kg) | 0.22 | 0.23 | 0.40 | 0.67 | ND |
| Nodulation | 28.33±1.53 | 21.33±1.53 | 16.66±1.53 | 9.0±1.73 | 41.05±0.42 |
| Wet weight (mg) | 3.06±0.15 | 3.36±0.15 | 1.76±0.15 | 1.26±0.305 | 2.9±0.10 |
| Dry weight (mg) | 1.76±0.1 | 1.6±0.1 | 1.1 ±0.1 | 0.9±0.05 | 2.05±0.26 |
| Root length (cm) | 15.4±0.5 | 12.5±0.15 | 12.2±0.47 | 11.2±0.36 | 13.0±0.75 |

*ND = Not Determined*

*c) Effects of Lead on the Shoot Growths and Microbial Activities*

Figure 1 shows the effects of lead on the shoot length of cowpea grown on soil polluted with varying levels of lead. Lead significantly ($P < 0.05$) retarded the length of cowpea and the degree of retardation was dose dependent. Although cowpea grew in all the treatment levels, there was a significant suppression of the shoot length at higher doses (100 mg/kg – 400 mg/kg).

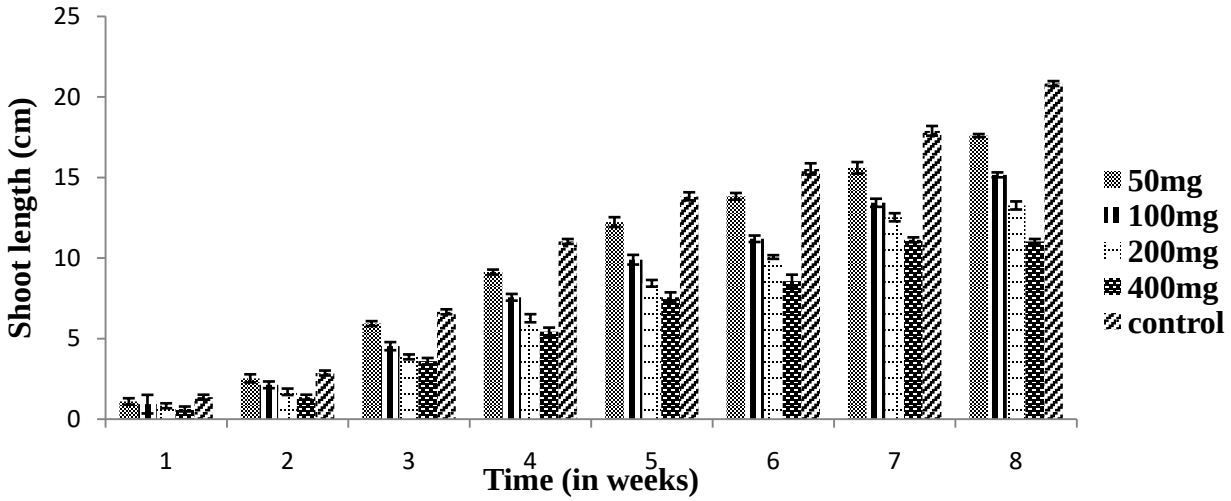


*Figure 1:* Effect of different levels of lead on the shoot length of cowpea grown in sandy loam soil.

Figure 2 shows the decrease in bacterial count with rise in heavy metals contamination. The figure illustrate that time is a major determining factor in heavy metal toxicity. The longer the persistence of the metal the more damage it has on soil bacteria population,

unlike the control group where progressive increased was observed with time.

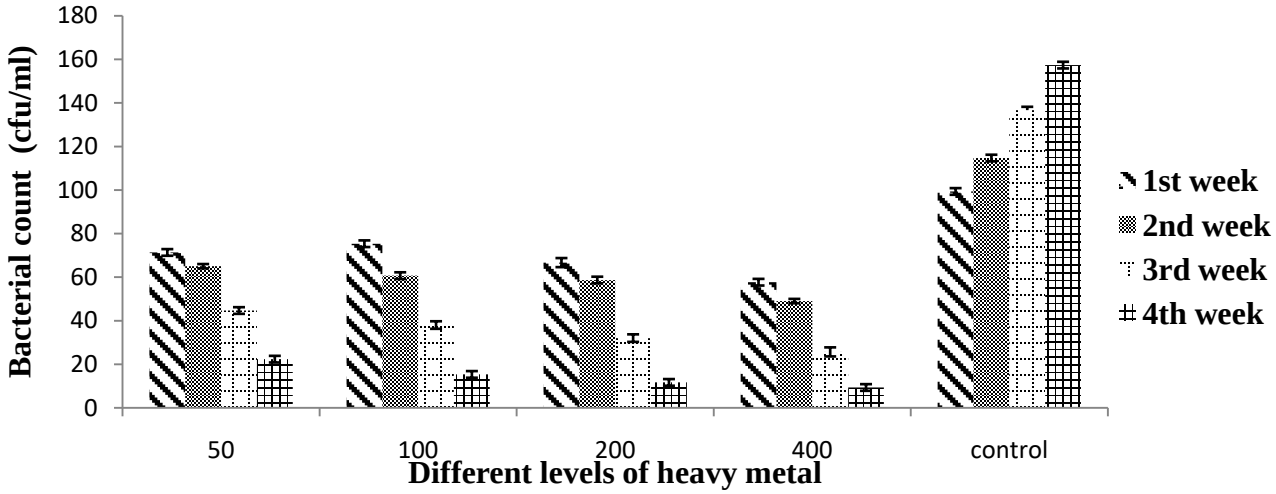


*Figure 2:* Effect of different levels of lead on bacterial population.

Figure 3 shows the effect of different levels of lead on microbial respiration. In same dose group of Pb, the concentration of $CO_2$ decreased with an increase in time (week) while the concentration of $CO_2$ in control group increased with an increase in time.

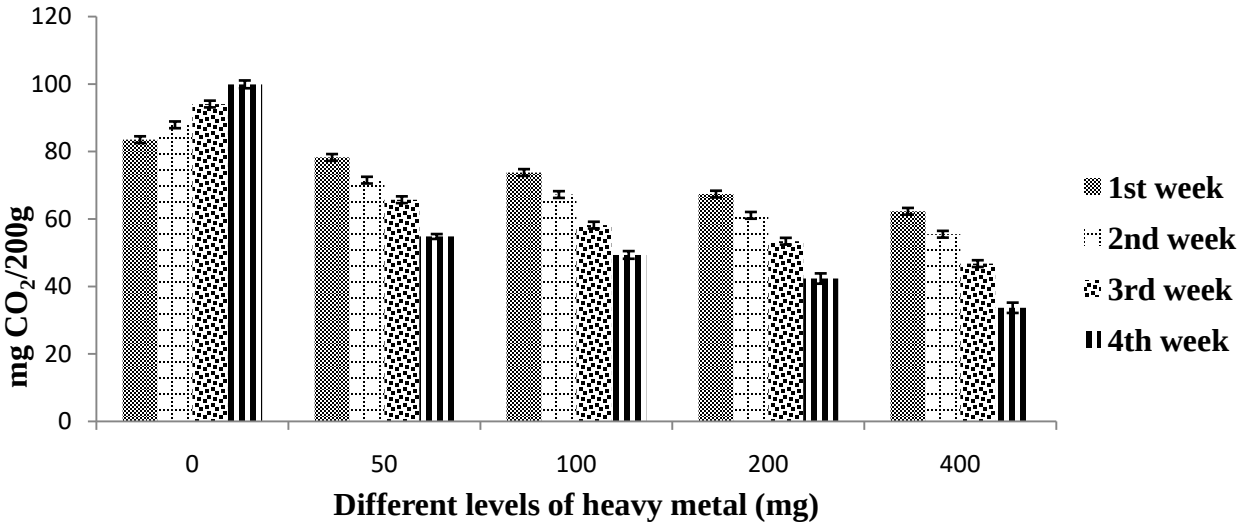


*Figure 3:* Effect of different levels of lead on microbial respiration

## V. Discussion

Seed germination, the first visible indicator of plant growth was monitored. Being a unique growth condition regulated by abiotic and biotic factors, it revealed an adverse effect in the germination study with rise in levels of Pb treatment. At lower concentration, Pb seems to pose no much harm to seed viability but shows evidence of retardation at high concentration, thus attributing it to soil metal toxicity (Luilo and Othman 2006). At low level of application, the legume phytate content which is an innate resistance property against some abiotic stress makes the plant more tolerant. Delayed germination has been linked to accelerated breakdown of stored nutrients in seeds, alteration of selection permeability properties of cell membrane (Shafiq *et al.* 2008) and enzymatic degradation of β-amylase and protease found in plants seeds (Verma and Dubey, 2003).

The observed reduction in the shoots growth of *Vigna unguiculata* witnessed in our study is similar to that reported by Imtiyaz *et al.* (2014). This may be due to the alteration of root cell leading to poor nutrient and water uptake. Interestingly, this retardation is said to be dose dependent. Even though cowpea grew in all the levels of treatment, there was still a significant

suppression of the shoot length at higher doses (100-400 mg/kg). In a related research, lead affected the growth of mash bean cultivars (Mumtaz *et al.,* 2006) by altering various enzymatic activities and stomatal actions. Elsewhere, it has been attributed to the accumulation of pollutant in the root region thus inhibiting shoot development (Diwan *et al.,* 2010) as in concomitant with our discovery. Bahri *et al.* (2015) identified reduced root growth as a major developmental challenge faced by plants in high Pb-contaminated soil.

The resulting low nodulation of cowpea grown in Pb polluted soil could be a response of lead phytotoxocity. There has been suggestion that toxic metals such as Pb, Cd and Cr inhibit nitrogenase activity, nitrogen fixation and photosynthesis. Hence, affecting severely the number of nodules, shoot cum root growth, leaf area, nodule biomass, and inducing nodule senescence (Balestrasse *et al.,* 2005). Emerging report on non-essential metals implicated induce oxidative stress, leghemoglobin depletion, decrease nodulation and inhibition of antioxidant activity (Carpena *et al*, 2003; Balestrasse *et al*, 2004) as a vital consequence on plants. More so, heavy metals penetrate the root and travel through the xylem by an apoplastic or symplastic pathway (Sanita di Toppi and Gabbrielli, 1999), thereby having severe effects on bacteriods multiplication and nodules formation.

Both wet and dry biomass of the studied plant was found to be an index indication of lead phytotoxicity. This is comparable with the work of Hosseini *et al.* (2007), where a decrease in plant biomass at high level of heavy metal was observed. This finding suggests that the decrease in biomass production under Pb stress could be due to the impairment caused by the pollutant. At 50-100mg/kg, lead encouraged more wet weight formation than the control just as reported by Bahri *et al.* (2015) who observed that Pb, Zn, and Cd at low level of application increases both fresh and dry weight matter of *Paulownia tomentosa.*

The deleterious effect of lead on bacterial growth is dose dependent. The sensitivity to metal toxicity at sufficient level of exposure results to immediate cell death, due to change in viability and competitiveness (Giller *et al*., 1998; Kelly and Tate, 2008). Our findings like that of Oliveira and Pampulha's (2006), shows that microbes were significantly reduced in heavy metal contaminated soil. The higher the metal dose, the more significant ($P < 0.05$) the retarding effects it has on the total bacterial count, due to change in the ecotoxicity. Microbial respiration study which involves the evolution of carbon (IV) oxide by soil microbes was found significantly ($P < 0.05$) decreased in the presence of lead. Sethi and Gupta (2014) in their work opined that heavy metals affects microbial metabolism with rise in the level of application.

In agreement to the work of Fatnassi *et al.* (2014), cowpea root was found to be the preferential site for Pb accumulation. This could be credited to the soil physicochemical properties and the metal (Pb) mobility in *Vigna unguculata*. Bearing in mind the inherent potential of legumes (root exudate), it is understandable to observe an increased microbial activity as a result of sufficient nutrient supply within the rhizosphere, thus lowering metal uptake to the above ground part as seen in our study. The translocation and bioconcentration factors (TF, BCF) shows the potency of a plant in remediating soil contaminated with heavy metals. This ability is always considered positive when $BCF_{\text{plant root/soil}}$ or $TF_{\text{plant shoot/plant root}} > 1$. The average BCF and TF of cowpea as observed in our studies is 1.15 and 0.38 respectively. Since the TF is less than 1, it indicates that the transfer of Pb to the aerial part was extremely low; this is in par with the work of Wei and Chei (2006). BCF illustrate the stabilization of heavy metals within the root system ($BCF > 1$, $TF < 1$) and also agrees with the report of Ma *et al.* (2001), and Cao and Ma (2004).

## VI. Conclusion

Lead contamination in soil via human activities affect soil fertility through their inhibitory effect on microbial population and metabolism. As non biodegradable pollutants, it reduces significantly the vegetative growth rate and nodulation of legumes which help in nutrient cycling and ecosystem restoration. Cowpea (*Vigna unguculata*) was found to preferably accumulate the pollutant within the root region. As a promising agent with phytostablization ability, *Vigna unguculata* could be utilized for the remediation of lead polluted soil.

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