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Phytoremediation Potential of *Chromolaena odorata* (L.) King and Robinson (Asteraceae) and *Sida acuta* Burm. f. (Malvaceae) Grown in lead-Polluted Soils

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Abstract

Heavy metals are non-biodegradable substances that can become deleterious and toxic if accumulated in higher concentrations in the environment. Translocation of nutrients from the plant to the soil is one of the key processes of human exposure to heavy metals through the food chain. The abilities of *Chromolaena odorata* and *Sida acuta* to bioaccumulate and translocate lead (Pb) are studied here under organic fertilizer amendment in order to determine their phytoremediation potentials. The experiment was a factorial combination of Lead at five levels of concentrations in a completely randomized design, replicated three times with two plants and two levels of an organic fertilizer. Seedlings of uniform height were transplanted from the nursery to experimental pots and were grown for ten weeks. The plants were then harvested and dried for the analysis of Pb accumulation both in the soil and the plant tissues using AAS. The results showed that the organic fertilizer enhanced the bioavailability of Pb because all the tested plants displayed a higher absorption of Pb. Significant concentrations of lead were easily taken up by the plants from the soil and were accumulated in the root, while only a small fraction was translocated upwards to the shoots. The two plants exhibited characteristics of a phytostabilizer because their transfer factors were less than one. The uptake of Pb observed in these plants were in the order of: *Sida acuta* > *Chromolaena odorata*, so *Sida acuta* remediates the soil better than *Chromolaena odorata*.

Keywords: AAS, Bioaccumulation, Bioavailability, Plant, Remediate, Soil.

1. Introduction

Heavy metals occur naturally in the ecosystem at varying concentrations due to anthropogenic activities such as metal-smelting, refuse dumping, car exhausts, and the sewage from industries which can all become accumulated to toxic levels (Adekola *et al.,* 2008). The presence of heavy metals in the environment is of great ecological significance due to their toxicity and non-biodegradable nature (Adekola *et al.,* 2008). The heavy metals that are commonly found in ecosystem include: Cadmium, Chromium, Copper, Lead, Mercury, Nickel and Zinc, and exposure to high levels of these metals is known to be hazardous and deleterious to the living organisms in the environment (USEPA, 1997; Hermen, 2011). Lead is the most prominent heavy metal in the soil due to its wide range of applications in human activities (Henry, 2000).

Globally, significant awareness and efforts have been geared towards using specific plants to mop up heavy metals in the ecosystem (Alushllari, 2015). One of the most touted and promising ways of reclaiming soils contaminated with lead is the phytoremediation which involves the use of some plants with significant hyperaccumulating potentials (Baker *et al.,* 2000; Ghosh and Singh, 2005). However, the plants must also be able to produce a large biomass. According to Baker and Brooks (1989), more than 400 plant species belonging to forty-five plant families have been identified and reported from temperate to tropical regions with the ability to tolerate and hyperaccumulate trace elements. One of these plants is *Tithonia diversifolia* whose effectiveness in the remediation of Pb has been reported by Adesodun *et al.* (2010).

Due to the invasive nature and ability of *Chromolaena odorata and Sida acuta* to produce a large biomass, and in addition to the paucity of information on their tolerance to lead toxicity, this study was conducted to unravel the bioaccumulation potentials of these two plants on lead-contaminated soils with organic fertilizer augmentation. This was done with the view to compare the abilities of *Chromolaena odorata* and *Sida acuta* to bioaccumulate and translocate lead. An organic fertilizer was added to the soil to improve its structure and the bioavailability of

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the Pb to the plant for translocation to their aerial parts (Xiao-lang *et al.*, 2011).

2. Materials and Methods

2.1. Study Area

The study was carried out in a screen house in the Biological Gardens of Obafemi Awolowo University campus, Ile – Ife, Osun State, Nigeria. The town Ile – Ife lies within latitude 7^0 30' N – $7^0$35' N and longitude $4^0$30' – $4^0$35' E. The latitude of the study area is 7^0 31' N and the longitude is $4^0$31' E of Ile – Ife, Osun State, Nigeria. The average temperature of the screen house was 35.2^{O}C and the average light intensity was 11380 illuminance.

2.2. Collection of Materials

Samples of the soil were randomly collected from the top 0 – 20 cm depth at the back of Botany Department, Obafemi Awolowo University, Ile-Ife where anthropogenic activities were minimal. The soil samples were air-dried for a week, and thereafter air-dried and sieved using 2 mm mesh gauze to remove debris and stones. Seven – liter plastic pots of 23 cm in diameter were used for the experiment. The plastic pots were then perforated at the base using soldering iron and were filled with 5 kg of soil. Viable seeds of the selected plants *Sida acuta,* and *Chromolaena odorata* were obtained from the field.

2.3. Experimental Design

The experiment was a 3-factorial combination (5×2×2) of five concentrations of Pb, two plants species and two levels (0 and 9.4 g kg^{-1}) of amendment. The Pb concentrations were 0, 200, 400, 800 and 1000 ppm). The amendment was organic fertilizer OBD – Plus. *Sida acuta,* and *Chromolaena odorata* were the plant species used. The experiment was conducted in a completely randomized design with three replicates

2.4. Physical and Chemical Soil Analysis and Seeds Pre-germination

Soil samples were analyzed according to the methods described in Black *et al.* (1965) and Page *et al.* (1985) for some physical and chemical soil properties respectively.

A nursery bed was prepared for the two plant species with viable seeds sown at 2 cm depth. The nursery bed was watered to the field capacity.

2.5. Preparation of Lead Solution

Lead (II) nitrate {$Pb(NO_3)_2$} salt was used as the source of Pb at a concentration of 0, 200, 400, 800 and 1000 ppm. The organic fertilizer OBD-Plus with a Nitrogen (N) content of 0.95 % was used to augment the soil fertility at the concentration of 0 and 9.4 g/kg respectively.

2.6. Pollution of Soil

The bottoms of the plastic pots were perforated to allow aeration and drainage. Plastic trays were placed under each pot for the collection of excess water to prevent nutrient leakages by pouring it back to the experimental pot. After pollution, the pots were left for a week to allow equilibration to set in. Thereafter, an organic fertilizer was applied to augment the soil to improve the soil structure.

2.7. Transplanting the Seedlings to the Polluted Soils

Two weeks after the germination of the seeds in the nursery bed, seedlings with good growth and uniform height were selected and transplanted to each experimental pot at the rate of one seedling per pot.

2.8. Harvesting

At ten weeks after treatment (April, 2014), the plant samples were carefully rinsed under running tap water to remove clogged soil particles. Water droplets were removed from the plant roots using blotting papers. Then, the plant samples were separated into roots and shoots. The soil samples were collected from each experimental pot and prepared for laboratory analyses.

2.9. Analysis of Heavy Metal (Pb)

The soil samples were digested using the Jou (1982) method. The roots and shoots samples were digested using the Audu and Lawal (2005) procedure. Lead concentrations in each of the samples were analyzed spectrophotometrically using Spectronic 20 Absorption spectrophotometer.

2.10. Bioaccumulation and Translocation Factors

The bioaccumulation and transfer factors of the tested plant species were obtained using the equation below:

$$\text{Shoot bioaccumulation factor} = \frac{\text{Concentration of heavy metal in shoot}}{\text{Concentration of heavy metal in soil}}$$

$$\text{Root bioaccumulation factor} = \frac{\text{Concentration of heavy metal in root}}{\text{Concentration of heavy metal in soil}}$$

$$\text{Whole plant accumulation factor} = \frac{\text{Concentration of heavy metal in plant}}{\text{Concentration of heavy metal in soil}}$$

$$Transfer\ factor = \frac{\text{Concentration of heavy metal in shoot}}{\text{Concentration of heavy metal in root}}$$

(Cui *et al.,* 2007 and Yoon *et al.*, 2006).

2.11. Statistical Analysis

Data were subjected to analysis of variance (ANOVA), and means were separated using Fisher's LSD at 5 % probability level. All analyses were carried out using SAS version 9.1.

3. Results

3.1. Physical and Chemical Properties of the Soil Used in the Experiment

The physical and chemical characteristics of the soil used in the screen house are presented in Table 1. The texture of the soil was loamy sand. The pH in 1:1 soil to water ratio was 5.8 for the top soil indicating a slightly acidic soil condition. The organic carbon content of the soil was 40.10 g kg^{-1}, while the nitrogen, phosphorus and potassium values were 3.20 g kg^{-1}, 10.4 mg kg^{-1} and 2605 mg kg^{-1} respectively. Moreover, Calcium (Ca) 31.0 mg/kg, and magnesium (Mg) 2432.5 mg/kg, and Lead (Pb) 0.098ppm were found in the soil samples.

Table 1. Physical and Chemical Characteristics of the soil used in the study

Characteristics	Value
pH (H_2O)	5.8
Organic carbon (g kg^{-1})	4.10
Nitrogen (g kg^{-1})	3.30
Clay (%)	6.8
Silt (%)	4.0
Sand (%)	89
Phosphorus (mg kg^{-1})	10.4
Ca^{2+} (mg kg^{-1})	31.0
Mg^{2+} (mg kg^{-1})	2432.5
K^{+} (mg kg^{-1})	2605
Na^{+}(mg kg^{-1})	272.5
Lead (mg kg^{-1})	0.098

3.2. Bioaccumulation Concentration of Pb in the Tissue of the Plant Species

The lead (Pb) concentration in the plants tissue increased as the lead concentration in the soil increased, as shown in Table 2. The lead concentration in the soil also decreased. The concentration of extractable lead in the soil under all treatments decreased between 39.5 %-55.8 % and 53.6 %-65.9 % in *Sida acuta* without and with the fertilizer application respectively. Also 38.5 %-51.8 % and 46.6 %-62.2 % in *Chromolaena odorata* without and with the fertilizer application respectively. *Sida acuta* extracts more lead from the soil followed by *Chromolaena odorata.*

Table 2. Bioaccumulation of Pb in the tissue of the plant species and the soil after harvest.

Pb concentration (ppm)		Bioaccumulation concentration of Pb (ppm)					
		Sida acuta			*Chromolaena odorata*		
		Shoot	Root	Soil	Shoot	Root	Soil
NF	0	0.0^{e}	0.0^{e}	0.1^{e}	0.0^{e}	0.0^{e}	0.4^{e}
	200	28.8^{d}	54.3^{d}	121.0^{d}	16.7^{d}	41.6^{d}	123.1^{d}
	400	55.7^{c}	96.1^{c}	232.1^{c}	35.2^{c}	77.1^{c}	270.0^{c}
	800	192.3^{b}	275.0^{b}	438.4^{b}	152.8^{b}	237^{b}	451.8^{b}
	1000	255.7^{a}	290.4^{a}	442.1^{a}	181.1^{a}	256.8^{a}	482.3^{a}
	LSD	0.08	0.78	0.78	0.08	0.78	0.77
F1	0	0.0^{e}	0.0^{e}	0.2^{e}	0.0^{e}	0.0^{e}	0.2^{e}
	200	48.6^{d}	82.1^{d}	92.8^{d}	24.6^{d}	61.6^{d}	106.9^{d}
	400	64.3^{c}	106.8^{c}	207.0^{c}	43.2^{c}	83.4^{c}	220.8^{c}
	800	197.1^{b}	310.3^{b}	329.8^{b}	156.1^{b}	271.2^{b}	361.1^{b}
	1000	327.4^{a}	373.2^{a}	341.3^{a}	247.5^{a}	352.1^{a}	378.0^{a}
	LSD	0.08	0.50	0.95	0.08	0.09	0.78

NF = No fertilizer, F= Fertilizer. Means with the same letters within the column are not significantly different at $p < 0.05$

3.3. Bioaccumulation Factors of the Shoot

The shoot bioaccumulation factors (BCF) for the two plant species were presented in figure 1. The BCF for the shoots of the two plants increased with an increase in the contaminant concentrations as shown in figure 1a without the fertilizer application. Highest (0.6) bioaccumulation factor for the shoots was reported in *Sida acuta*, while shoot BCF of *Chromolaena odorata* was 0.4. Under organic fertilizer amendments (figure 1b), the BCF of *Sida acuta* increased by 67 %, while that of *Chromolaena odorata* increased by 17 % across the two plant species, the organic fertilizer amendment improved the shoot BCF.

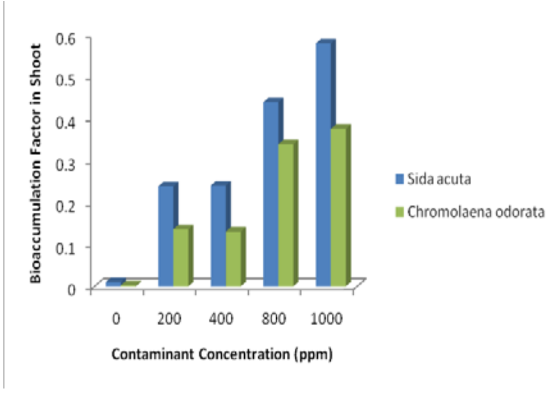

Figure 1a. Bioaccumulation factor for shoots without fertilizer application

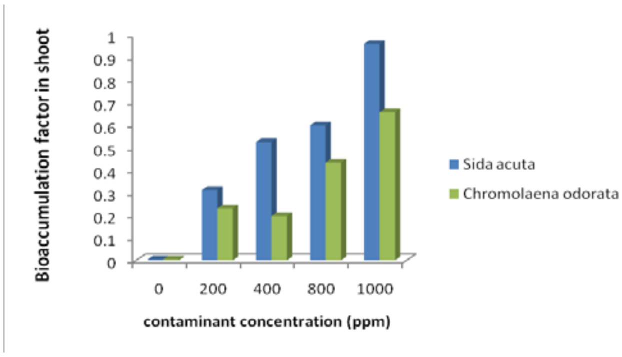

Figure 1b. Bioaccumulation factor for the shoots with the fertilizer application

3.4. Bioaccumulation Factors for the Roots

In figure 2, the BCFs for the roots of the two plant species with and without the organic fertilizer augmentations were presented. The highest was 0.7 in *Sida acuta* and 0.53 in *Chromolaena odorata* without fertilizer application as shown in figure 2a. With the organic fertilizer (figure 2b), the highest bioaccumulation factor for the roots was 1.1 in *Sida acuta* and 0.9 in *Chromolaena odorata.* Plants treated with the organic fertilizer had the highest bioaccumulation factor for the roots. For example, at a low-pollution strength of 200 mg kg^{-1}, the root BCF for *Sida acuta* was 0.52, while that of the *Chromolaena odorota* was almost 50 % less when the two plants were grown at the same pollution strength. Even without the fertilizer application, root BCF of *Sida acuta* was 41 % more than that of the *Chromolaena odorota*. Similarly, at the highest pollution strength of 1000 mg kg^{-1} ,*Sida acuta* accumulated more of the lead both in the roots and shoots than that of *Chromolaena odorota* with root BCF being less than about 42 % increase both with and without the fertilizer application.

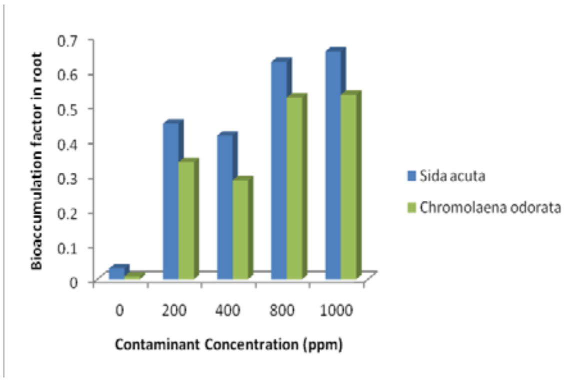

Figure 2a. Bioaccumulation factors for roots without fertilizer application

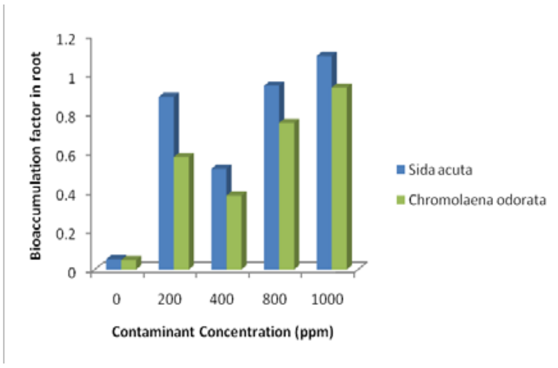

Figure 2b. Bioaccumulation factors for roots with fertilizer application

3.5. Bioaccumulation Factors for the Whole Plants

The bioaccumulation factors for the whole plants increase as the concentration of the contaminant increases, as shown in figure 3. The highest bioaccumulation factors for the whole plants were 1.2 in *Sida acuta* and 0.9 in *Chromolaena odorata* without the fertilizer application (figure 3a). With the organic fertilizer, the highest bioaccumulation factors were 2.1 in *Sida acuta* and 1.6 in *Chromolaena odorata*. Plants grown with the organic fertilizer (figure 3b) have the highest bioaccumulation factor for the whole plants. For *Chromolaena odorata*, the highest BCF value (0.9) was less than one while under fertilizer application. BCF values above one were reported with 31 % less than that obtained for *Sida acuta* exposed to the same pollution stress. BCF for the whole plant showed that the highest BCF was reported in *Sida acuta* grown in 1000 mg kg^{-1} of Pb polluted soils with and without fertilizer application.

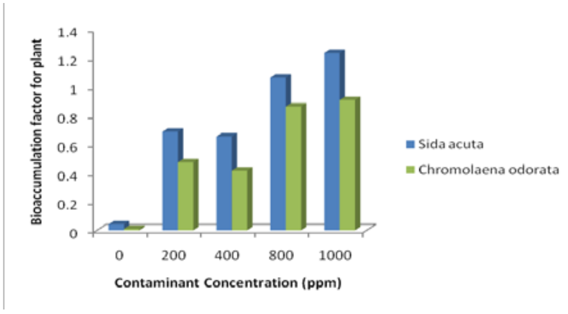

Figure 3a: Bioaccumulation factors for plants without fertilizer application

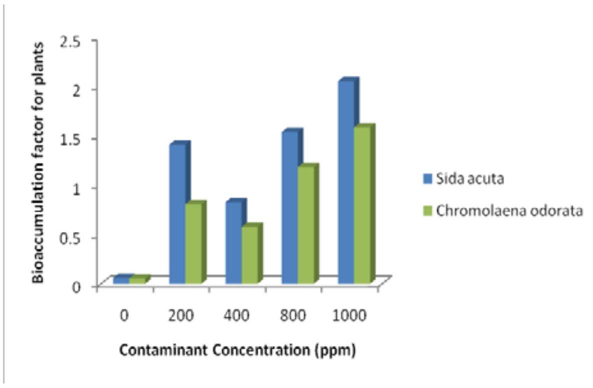

Figure 3b, Bioaccumulation factors for plants with fertilizer application

3.6. Transfer Factor (TF)

The TF of the plants increased with an increase in the contaminant concentration as shown in figure 4. The highest was 0.9 in *Sida acuta* and 0.7 in *Chromolaena odorata* under without fertilizer application (figure 4a). With the organic fertilizer application (figure 4b), the highest was 0.9 in *Sida acuta* and 0.7 in *Chromolaena odorata.* The TF were the same under the fertilizer and without fertilizer application for each plant.

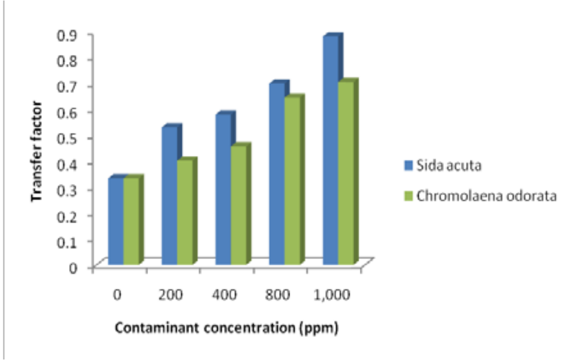

Figure 4a. Transfer factors of the plants without fertilizer application

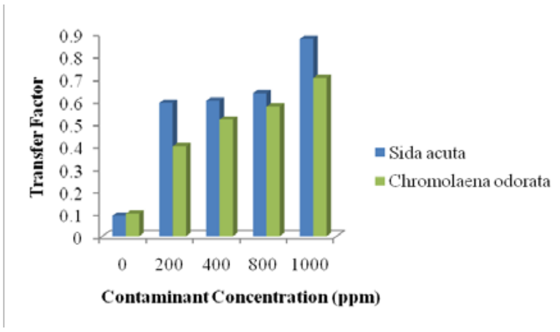

Figure 4b. Transfer factors of the plants with fertilizer application

4. Discussion

The success of the phytoremediation process through the techniques of phytoextraction of the contaminant depends largely on the ability of the candidate plant to transfer contaminants from the below-ground biomass to the aboveground, In contrast, the success of the phytoremediation process through the techniques of phytostabilization of the contaminant depends largely on the restrictive ability of the plant to stabilize the contaminants below-ground biomass (Baker, 2000; Dada and Awotoye, 2013).

In this study, *Sida acuta* and *Chromolaena odorata* showed varied bioaccumulation potentials after exposure to different concentrations of lead pollution. In addition, the level of the lead contamination determines the Pb-uptake in all the studied plants. This observation was similar to that reported by Wang *et al.* (2007) for *Bidens maximowicziana* and Oseni *et al.* (2015) using *Momordica charantia.* This may be because the roots of plant are the first to have direct contact with the contaminants through passive transport. The current study comes in agreement with previous studies such as Blaylock *et al.* (1997); Salido *et al.* (2003); Aiyesanmi *et al.*(2012) where a higher Pb absorption was observed in the plant species grown in soils treated with an organic fertilizer. This implies that *Chromolaena odorata* and *Sida acuta* were able to absorb and bioaccumulate significant concentrations of lead in this study. Also, it was found that the organic fertilizer enhanced the bioavailability of Pb in the soil-environment.

Considering the BCF of *Sida acuta Chromolaena odorata* under the organic fertilizer application tend to accumulate in the roots more than in the shoots. This was in line with the findings of Parsadoost *et al.* (2008), Cho-Ruk (2006) and also the work of Mojiri (2011) when studying the potentials of Corn *(Zea mays)* in the phytoremediation of soil contaminated with Cadmium and Lead using EDTA for the bioavalability of the heavy metal ions. Moreover, Adejumo *et al.* (2011) in their study of *in-situ* remediation of heavy-metal contaminated soils using the Mexican sunflower (*Tithonia diversifolia*) and cassava waste as composts reported a similar trend in their study. Likewise, the work of Wang *et al.*, (2007) revealed that *Bidens maximowicziana* is a new hyperaccumulator of lead (Nie *et al*., 2004). However, EDTA application had also been reported to enhance the phytoremediation of lead by *Bidens maximowicziana* when planted in lead-contaminated soils. Lead-uptake studies in plants have demonstrated that the roots have an ability to take up significant quantities of Pb while simultaneously restricting its translocation to the aboveground parts (Oseni *et al.,* 2015). The majority of the lead was easily taken up by plants from the soil and accumulated in the roots, while a lesser fraction was translocated upwards to the shoots (Patra *et al.,* 2004). This may be attributed to the fact that passive transport of ions occurred in the roots which were directly exposed to ionic environment. This may extend to the endodermis but not beyond because of the casparian strip. According to Mojiri (2011), the capacity of the soil to adsorb lead increases with increasing of pH, cation exchange capacity (CEC), organic carbon content, soil/water Eh (redox potential), and phosphate levels. Some ways to induce Pb solubility are to decrease the soil pH and lower its organic matter because Pb binds with organic material in the soil (McBride, 1994; Sharma and Dubey, 2005).

The results of the current study revealed BCF of 2.1 and 1.6 for *Sida acuta* and *Chromolaena odorata* respectively. The BCF were higher than one in the two plants, depicting their potentials to accumulate Pb from the soil. These results respond to the work of Pitchtel *et al.* (2000) when analyzing Pb concentrations in plants collected from a dump site. Similarly, Stoltz and Greger (2002) reported a range of 3.4 to 920 mg kg^{-1} of Pb concentrations in different wetland plant species collected from mine tailings. So, plants exhibiting bioaccumulation factors greater than one are suitable for phytostabilization of contaminants. According to Fitz and Wenzel (2002), they are tolerant plants which restrict root–shoot transfers.

Another important mobility index that should be taken into consideration when assessing the phytoremediation potentials of any plant is the ability of the plants to translocate metals from the roots to the shoots which can be measured using a mobility index referred to as the transfer factor (TF). Enrichment occurs when a contaminant taken up by a plant is not degraded rapidly, resulting in an accumulation in the plant (Baker, 2000; Dada and Awotoye, 2013). The highest transfer factors of the studied plants were were less than one, which shows that the plants restricted the Pb transfer from their roots to the shoots. This observation may be attributed to the effects of Pb toxicity. Tang *et al.* (2009) indicated that in *Arabis paniculata*, with the elemental Pb concentration of 9- 267 μM, the transfer factor of the plant was below one. Moreover, Aiyesanmi *et al.* (2012) also reported BCF and TFs of less than one in Lead accumulation in Siam weed (*Chromolaena odorata*), Nodeweed (*Synedrella nodiflora*), and Water leaf (*Talinum triangulare*).

The two plants used in this experiment absorbed a significant concentration of lead into their root tissues under the fertilizer application with TFs being less than one. This implies that the two plants exhibit characteristics of a phytostabilizer. However, the uptake of Pb in these plants were in the order *Sida acuta* > *Chromolaena* odorata. Thus, the treatment of the soil with the organic fertilizer enhanced the uptake of lead in the plants. This is because the spiking of the soil environment with an organic fertilizer increases the bioavailability of lead in the soil solution.

5. Conclusion

The two plants used in this experiment showed a significantly higher absorption of lead. It could also be observed that the treatment of the soil with the organic fertilizer enhanced the uptake of Pb in the plants by increasing the bioavailability of Pb in the soil solution. The two plants exhibited the characteristics of a phytostabilizer because their transfer factors were less than one. The uptake of Pb observed in these plants were in the order *Sida acuta*> *Chromolaena odorata* both in the roots and the shoots and this order holds both with organic fertilizer and without organic fertilizer soil.

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