Assessing the Heavy Metal Transfer and Translocation by Sida Acuta and Pennisetum Purpureum for Phytoremediation Purposes

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Assessing the heavy metal transfer and translocation by *Sida acuta* and *Pennisetum purpureum* for phytoremediation purposes

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Abstract

Field sampling of two prevalent weeds (*Sida acuta* and *Pennisetum purpureum*) growing on cement-polluted soil was carried out seasonally to assess the root and leaf contents of Pb, Cu, Cr, Cd and Zn. Concentrations of heavy metal in corresponding soils (total and bioavailable) were also determined and data generated were used to calculate the respective transfer factors (TFs) and translocation indices (Tis) of metals in the weed species. The results of the TFs and Tis were used to evaluate the potential ability of these weed species to accumulate heavy metals in their tissues and suitability or phytoremediation. Findings indicated that *S. acuta* and *P. purpureum* are suitable for phytostabilization of Cr, Cd and Zn in cement-polluted soil while Cr and Cd can be phytoextracted by these two weed species from cement-polluted soil.

Keywords: ***Accumulator plant, phytoremediation, weeds species, phytoextractor, transfer factor, translocation index.***

1. Introduction

Plants have developed the process for the acquisition of relatively less abundant micronutrients such as Cu, Zn, Ni and Mn from the soil. These essential micronutrients are also highly reactive and potentially toxic to plants [27]; hence the uptake, transport and accumulation of these micronutrients are thereby highly coordinated and regulated by necessity in plants. Many of these micronutrients of plants are also heavy metals which makes the contamination of the soil environment a problem through either the accumulation of essential micronutrients (Zn, Mn, Cu and Ni) in high amount or metals that can acts as analogues or replacements (such as Cd, Pb or Hg) [27]. Therefore, plants have evolved potential mechanisms like detoxification of metals in the roots and true tolerance to heavy metal stress which may involve primarily the avoidance of build-up of toxic concentrations at sensitive sites [26, 27]. Also, escaping from heavy metal toxicity by reducing or excluding the uptake of heavy metals may be another strategy for plants to resist metal toxicity [8].

Some plants are also tolerant to specific metals and are able to accumulate such metals in substantial quantities in their biomass due to their effective uptakes and translocations. Such plants with effective transfer and translocation concentrate metals in above-ground parts from low to great soil concentrations and are regarded as accumulators [8]. According to Nan et al. [31], the uptake and transfer of heavy metals from soil to plants is a process of significant importance and determining of transfer factor (TF) and translocation index (Ti) have been considered as a key parameter to assess the availability of elements in soil and hyper-accumulation capacity of the plants. So a detailed study on the metal transfer from soils to plants and translocation to aerial parts could possibly shed more light on the metal accumulation potentials of plants species and their phytoremediation potentials. D'Souza et al. [13] stated that all plants with phytoremedial potentials are not alike; some may be metal-specific while others may perform well for metals in combination. But the phytoremedial potential of plants is reported to be influenced by the mobility and availability of heavy metals in soil and plants, and thus the transfer factor (TF) (ratio of

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bioavailable metal content in root to that in soil) and translocation index (Ti) (ratio of metal content in leaves to that in root) can be used to assess the accumulation potential and hence the phytoremedial potential of plants [8, 17, 43]. And a critical limitation to the study of phytoremediation is that most studies are carried out *ex situ* in pots and hydroponic [14, 45,] without assessing the potentials on-field and even in-between seasons. Native or wild plants should be preferred for phytoremediation according to Antonsiewicz et al. [6] and Yoon et al. [44] because they are often better in terms of survival, growth and reproduction under environmental stress and require no agronomic inputs [14]. Therefore, this study aimed at assessing the transfer and translocation of heavy metals in native *S. acuta* and *P. purpureum* growing on soil in the environment of a mega cement factory. The seasonal assessment of TFs and Tis in the two weed species were used to evaluate possible phytoremedial potentials of heavy metals.

2. Material and Methods

The study was carried out around the factory plant of the Lafarge-Cement WAPCO in Sagamu, southwestern Nigeria (6°50' and 7°00' N; 3°45' and 4°00' E) (Fig. 1). This cement facility has been in full operation for the past 3 decades and Ogunkunle and Fatoba [33] have reported significant pollution of this soil with heavy metals. The area stands on a low-lying gently undulating terrain with altitude ranging between 30 and 61 m above sea level. The area is characterized by high annual temperature, high rainfall, high evapo-transpiration and high relative humidity which make it to be classified as humid tropical region [4].

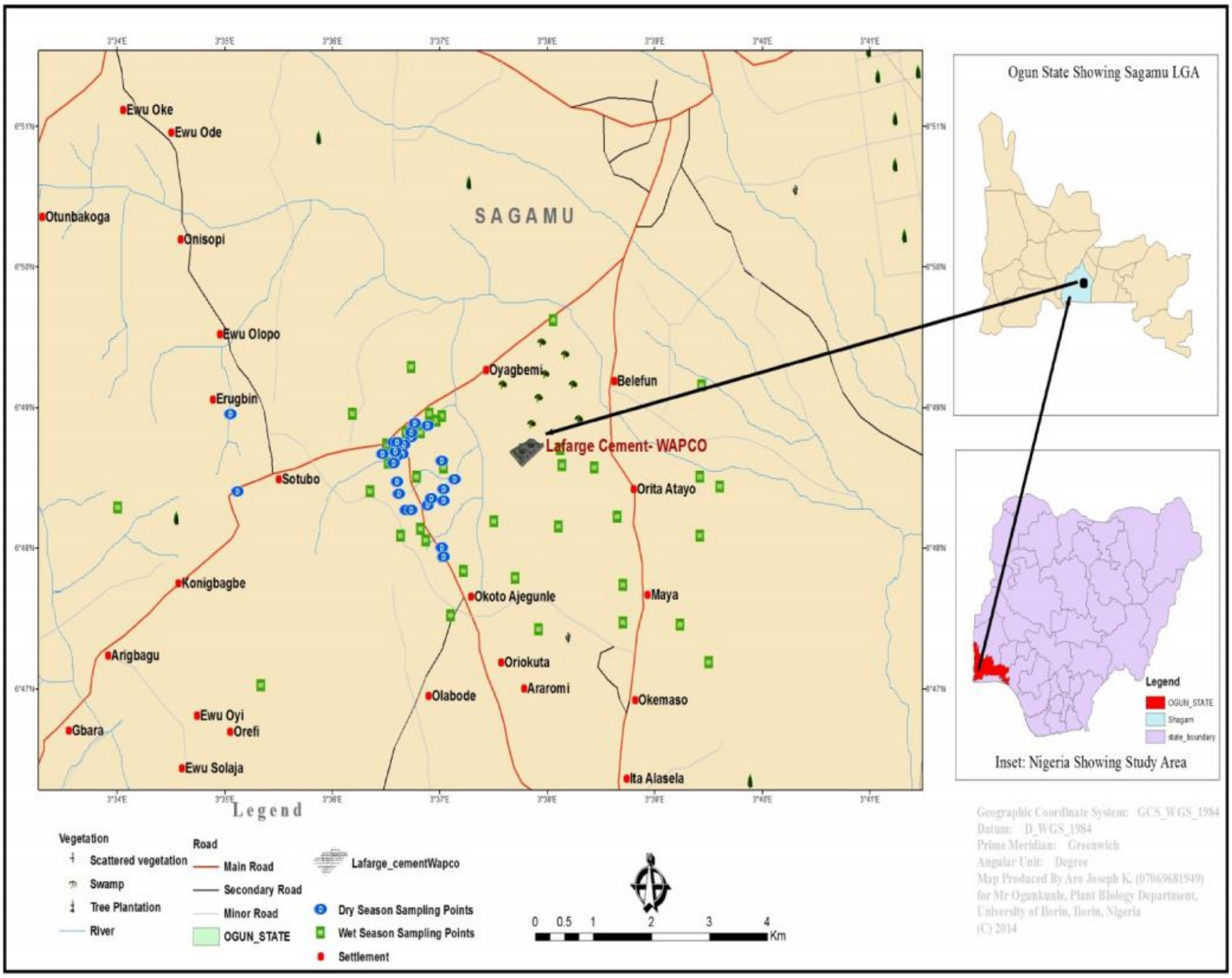

Figure 1. Map of Sagamu showing sampling points of soil and plant materials for the two seasons.

The soil type of Sagamu is Oxic Haplustults according to USDA Soil Taxonomy [42] and Ferric Acrisols according to FAO System of Classification [15]. The climate is classified as humid tropical climatic zone [1] and controlled by the Tropical Maritime and Tropical Continental air masses [7]. The geology of Sagamu comprises of sedimentary rocks which consists of Abeokuta formation [2]. It is reported to be highly fossiliferous and consists of deposits of limestone [18, 22], sand with sandstone, siltstone, clay, mudstone and shale interbed. According to Agbaje [3], the basal conglomerate of Abeokuta formation has poorly rounded quartz pebbles with a silicified and ferruginous sandstone matrix.

2.1 Sampling method

Topsoil (0-15cm) at different locations within a radius of 3km of factory was randomly collected between August and October and between November to December of 2011 and 2012 respectively for rainy and dry seasons. Thirty-eight (38) and twenty-six (26) representative soil samples were collected randomly around the factory in wet seasons and dry seasons respectively, each sample representing a composite sample of 3 subsamples. The soil samples were air-dried in the laboratory to constant weight; after which the samples were grounded and passed through 2mm sieve to get fine fractions for chemical analyses. Two native species (*Pennisetum purpureum* and *Sida acuta*) were used for the study due to their relative abundance around the factory. At every point where soil sample was collected, matured plants (*P. purpureum* and *S. acuta*) were harvested, cleaned with tap water, sorted into roots and leaves while the reproductive parts and the stems were discarded. The plant samples were properly air-dried, powdered and well-tagged for chemical analysis.

2.2 Chemical and statistical analyses

The soil pH was measured in 1:2.5 (soil: water) suspension [38] with glass electrode pH meter (PHS-3C model) while the soil organic matter content was determined by Loss on Ignition method of Reddy et al., [37]. In order to assess the mobility and potential phyto-availability of the metals in the soil, the exchangeable/bioavailable fractions of the metals was estimated. The methods of Tessier et al. [41] and Chojnacka et al. [12] were adapted to determine the exchangeable/bioavailable fraction by extraction of bioavailable metal from soil with 8ml of 0.5M Magnesium chloride (Scharlau, Spain) at pH 7.0 for 20mins in a test tube with continuous agitation using a motorized shaker JK VXR S17 at 1000 rpm. Digestion of plant samples (roots and leaves) was performed using concentrated HNO_3 (70%, Sigma-Aldrich Corp, Germany) [21]. The solutions from the extraction and digestion processes were filtered using Whatman No 42 filter paper, diluted with deionized water to make up 25 ml and analyzed for Pb, Cu, Cr, Cd and Zn by Atomic Absorption Spectrophotometry method using AAS Peckin Elmer A Analyst 200.

Quality assurance and control were assessed using reference materials (CRM 193 BG 310-Meadow soil and IAEA 359-cabbage), duplicates and blanks method [9]. Recovery percentage of the reference materials was within the range of 96% to 127-9% and 80% to105% respectively for the soil and plants. The AAS was calibrated for each element determination using series of working standard solutions and the obtained calibration graphs of absorbance against standard concentrations were excellent with correlation coefficients r^2>0-980.

Analysis of Variance (ANOVA) was used to identify the significance of differences of the concentration of each element in the soil and plant samples. A multiple separation of means was determined by the Duncan Multiple Range Test (DMRT) and significance level of $P<0.05$ was used throughout the study. Statistical analysis was carried out using the SPSS 16 version at $P<0.05$ while Origin 7 software was employed to present the figures.

Transfer factor (TF) for the heavy metals was calculated using the method of Chojnacka et al. [12] and Prabu [35].

$$TF = Cp\ (\text{mg kg}^{-1}) / Cts\ (\text{mg kg}^{-1}) \qquad \text{Eq. 1}$$

Cp is the root/leaves metal content and *Cts* is the exchangeable metal content in the soil. According to Mendez and Maier [29] and Garba et al. [14], TF>1 refers to plant as phytostabilizer.

Translocation index (Ti) for the heavy metals was determined according to Ghosh and Singh [19] and Kidd et al. [25].

$$Ti = Cpl\ (\text{mg kg}^{-1}) / Cpr\ (\text{mg kg}^{-1}) \qquad \text{Eq. 2}$$

Cpl is the leaves metal content and *Cpr* is the root metal content and according to Garba et al. [14] and Baker [8], Ti>1 denotes accumulator plant while Ti<1 denotes excluder plant.

3. Results and Discussion

3.1 Distribution of total and bioavailable metal contents of the soil around the cement factory.

The distributions of total metal contents of the soil for the wet (mean of two wet seasons) and dry (mean of two dry seasons) seasons are presented in Table 1. In the wet season, heavy metal contents of the soil were significantly high; Pb has mean value of 469-2 mg/kg, Cu=404-4 mg/kg, Cr=185-2 mg/kg, Cd=298-9 mg.kg and Zn had a mean value of 168.1 mg/kg (Table 1). There was great variability in the metal contents of the soil in the wet season especially for Cu and Cd, thereby indicating the presence of outliner values. The high values in the wet season indicated that the soil was highly polluted as reported earlier by Ogunkunle and Fatoba [32] and Ogunkunle and Fatoba [33]. This pollution level was attributed to the activities of cement production in the study area by these authors. Significant reductions at P<0-05 were observed in the metal contents of the soil in the dry season especially in soil contents Pb, Cu and Cd. The main reason for the reduction was the adoption of filter bay dust collection as air pollution control in 2011 which reduced the dust emission level to less than 10mg/m^3 [28]. Another adducible reason could be the leaching of metals into the sub-soil layers by rainfall as reduction in metal concentrations in soils over time may occur due to breakdown and leaching [23]. And this is explained the significant reduction in total soil Cd in this study due to its low potential to adsorb to soil and organic matter [30], thereby readily leached into sub-soil.

Bioavailable metal contents extracted with $MgCl_2$ from the soil were presented Table 2. The soil around the cement factory was slightly acidic (pH=6-5) in the wet season whereas in the dry season, the soil tended towards alkalinity with a mean pH value of 7-6. The soil was rich in organic matter content with mean value of organic matter content of 5-6% in the wet season and significant increase was recorded in the dry season to 11-7% at P<0-05. Organic matter forms complexes with metals in the soil, so that it can either reduce mobility or increase bioavailability of metals when the complexes are soluble in soil solution [20]. Extractable Pb in the wet season was 4-% of the total metal content of the soil, 1-5% of Cu was extracted, 5-7% of Cr, 0-8% of Cd while 4-5% of Zn was extracted with $MgCl_2$. Significant increase (P<0-05) was observed in the amount of all the extractable bioavailable metals of the soil in relation to total metal concentrations in the dry season except for Zn that dropped significantly (P<0.05) (Table 2). Extractable Pb content was 26-0% of the soil total metal, Cu recorded 8-4%, Cr recorded 6-3%, and Cd recorded 8-2% whereas extractable Zn content dropped to 0-6% of the soil total metal content. Generally, the recovered bioavailable metal contents of the soil in the two seasons were low compared to the total metals and this could be linked to the pH of the soil and maybe the mineralogy of the soil [10]. Several reports have indicated that higher pH of soil reduces metal mobility [11, 40] but the considerable increase in the dry season could be linked to the large amount of organic matter content of the soil. Halim et al. [20] stated that bioavailability of metals could be increased when these metals form soluble complexes with organic matter content of the soil.

3.2 Uptake and metal partitioning in plants' parts.

Large amounts of extractable Cu, Cd and Zn (3.15 mg/kg, 3.15 mg/kg and 9.90 mg/kg respectively) were taken up by the roots of *S. acuta* from the phyto-available metals in the soil in the wet season (Table 3). And this is not a peculiar case for these plant species because Nan et al. [31] and Kalavrouziotis et al. [24] had reported that most plants ordinarily accumulate heavy metals mainly in plant roots system and to a more limited extent in leaves and/or in edible parts. Little variation was observed in the tissue partitioning of *S. acuta* in the wet season. There was no significant difference in the pattern of partitioning of Pb, Cu, Cr and Cd in roots and leaves of *S. acuta* whereas the amount of Zn in the roots was significantly greater than the accumulated Zn in the leaves at P<0.05 (Table 3). This lower amount of Zn in the leaves could be adduced to utilization of Zn for growth since wet season is a growing period for most plants and reports has it that Zn is mostly accumulated in green tissues like leaves [36]. A different pattern of partitioning was observed in *S. acuta* in the dry season. Leaf contents of Pb (2.39 mg/kg) and Cu (2.87 mg/kg) in the leaves were significantly higher (P<0.05) than roots contents (0.97 mg/kg and 0.84 mg/kg respectively) of *S. acuta*. This change of pattern could be due to the intense evapotranspiration experience in the dry season and also increase foliar uptake of metals by plants since report by Oluyemi et al. [34] stated that there is increase foliar deposition of metals the dry season. Oluyemi et al. [34]) also reported that dehydration of plant leaves by transpiration increases metal concentrations in the leaves. Cr and Cd partitioning between the roots and leaves in *S. acuta* in the two seasons did not differ statistically (P<0.05) which indicates that *S. acuta* has affinity for bioaccumulation of these metals.

A different pattern of tissue partitioning of heavy metals was observed in *P. purpureum* (Table 3). This supports the earlier assertion of Alloway [5] that plant species types dictate the patterns of accumulation of heavy metals. Accumulations of Pb (16.79 mg/kg), Cu (3.79 mg/kg) and Zn (7.25 mg/kg) in the roots were significantly greater than the leaves contents of 14.18 mg/kg, 3.05 mg/kg and 5.18 mg/kg respectively (Table 3).

Table 1. Variations in the total metal contents of the soil between the wet season and dry seasons.

Metal	Mean value† (wet season)	Mean value (dry season)	t-value	P-value
Pb	469.2±291.1	21.9±25.45	7.794*	0.000
Cu	404.4±313.7	22.6±38.44	6.159*	0.000
Cr	185.2±90.0	121.9±44.40	3.366*	0.001
Cd	298.9±326.0	9.8±6.39	4.510*	0.000
Zn	168.1±109.9	76.0±28.44	4.120*	0.000

*Values with symbols are significantly different at p<0.05,
† Values of soil metal concentrations already reported in Ogunkunle and Fatoba [32].

Table 2. Variations in some soil parameters and bioavailable metal contents of the soil between the wet season and dry seasons.

Metal	Mean value (wet season)	Mean value (dry season)	t-value	P-value
pH	6.5±1.10	7.6±0.39	5.231*	0-000
Organic matter (%)	5.6±3.10	11.7±7.26	4.539*	0-000
Pb	21.3±10.36	5.7±2.14	9.017*	0-000
Cu	6.2±6.59	1.9±0.26	4.184*	0-000
Cr	10.6±4.70	7.7±1.38	3.117*	0-003
Cd	2.5±1.19	0.8±0.17	7.418*	0-000
Zn	7.5±2.70	0.5±0.26	13.172*	0-000

*Values with symbols are significantly different at p<0.05.

Table 3. Seasonal variation in $MgCl_2$-extractable metal contents of the soil and metal contents of plant parts in *S. acuta* and *P. purpureum* around the cement factory.

	Wet season			Dry season		
	$MgCl_2$-extractable metal (mg/kg)	Root content (mg/kg)	Leaves content (mg/kg)	$MgCl_2$-extractable metal (mg/kg)	Root content (mg/kg)	Leaves content (mg/kg)
			S. acuta			
Pb	20.9±0.70	5.43^{a*}±2.27	4.85^a ±1.53	5.7±2.10	0.97^a±1.35	2.39^b ±2.87
Cu	5.9±7.90	3.15^a ±1.37	3.20^a ±1.83	1.9±0.30	0.84^a ±0.61	2.87^b ±1.95
Cr	10.8±4.50	4.95^a ±3.89	5.00^a ±4.89	7.7±1.40	10.3^a ±4.26	12.80^a ±5.69
Cd	2.5±1.50	3.15^a ±2.21	2.92^a ±2.14	0.8±0.20	0.72^a ±0.14	0.79^a ±0.13
Zn	7.4±2.40	9.90^a ±6.48	7.50^b±6.54	0.5±0.30	35.0^a ±5.13	67.50^a±55.9
			P. purpureum			
Pb	20.9±0.70	16.79^a±11.88	14.18^b±12.38	5.7±2.10	1.57^a ±2.0	1.20^a±1.49
Cu	5.9±7.90	3.79^a ±2.42	3.05^b ±2.07	1.9±0.30	1.30^a ±1.14	1.70^a±0.98
Cr	10.8±4.50	4.20^a ±3.50	3.86^a ±3.69	7.7±1.40	13.20^a ±6.23	10.2^a±4.23
Cd	2.5±1.50	2.45^a ±2.04	3.21^a ±2.26	0.8±0.20	0.75^a ±0.29	0.73^a ±0.16
Zn	7.4±2.40	7.25^a ±6.81	5.18^b±4.20	0.5±0.30	34.1^a ±6.37	44.4^a±16.9

*Values with different letters along the row for each plant species are significantly different at p<0.05.

These partitioning pattern could be peculiar to grasses because Yoon et al., [44] also reported higher Pb, Cu and Zn contents in the roots of some grasses (bahia, bermuda and wire grasses) than the leaves contents. This occurrence of larger amount of heavy metals (Pb, Cu and Zn) in the roots of *P. purpureum* in the wet season could be due to the complexation and sequestration of metals in cellular structures like the vacuoles in the plant root and render them unavailable for translocation to the shoot [39]. There was no difference in the tissue partitioning of all the metals in the dry season (Table 3) and this could probably be due to the low concentration of bioavailable metals in the soil in the dry season since the pH was slightly alkaline thereby rendering the metals non-mobile. It is also important to note that root and leaf contents of *P. purpureum* showed no significant difference in the tissue partitioning of Cr and Cd in the two seasons and this also indicates the high bioaccumulation potential of this plant species for Cr and Cd.

3.3 Heavy metal transfer and translocation in *S. acuta* and *P. purpureum.*

The TF and Ti values (Figures 2 &3) help to identify the suitability of plants for phytoextraction and phytostabilization by depicting the characteristics of accumulation and the patterns of translocation of metals in plants. Figure 1 presents the seasonal variations in the transfer factor (TF) of *S. acuta* and *P. purpureum* growing around the cement factory. TFs of Cr, Cd and Zn in *S. acuta* were all greater than 1.0 in both the wet and dry season while TFs of Pb and Cu for both season were below 1.0 but still higher than 0.5 (Fig. 2a).

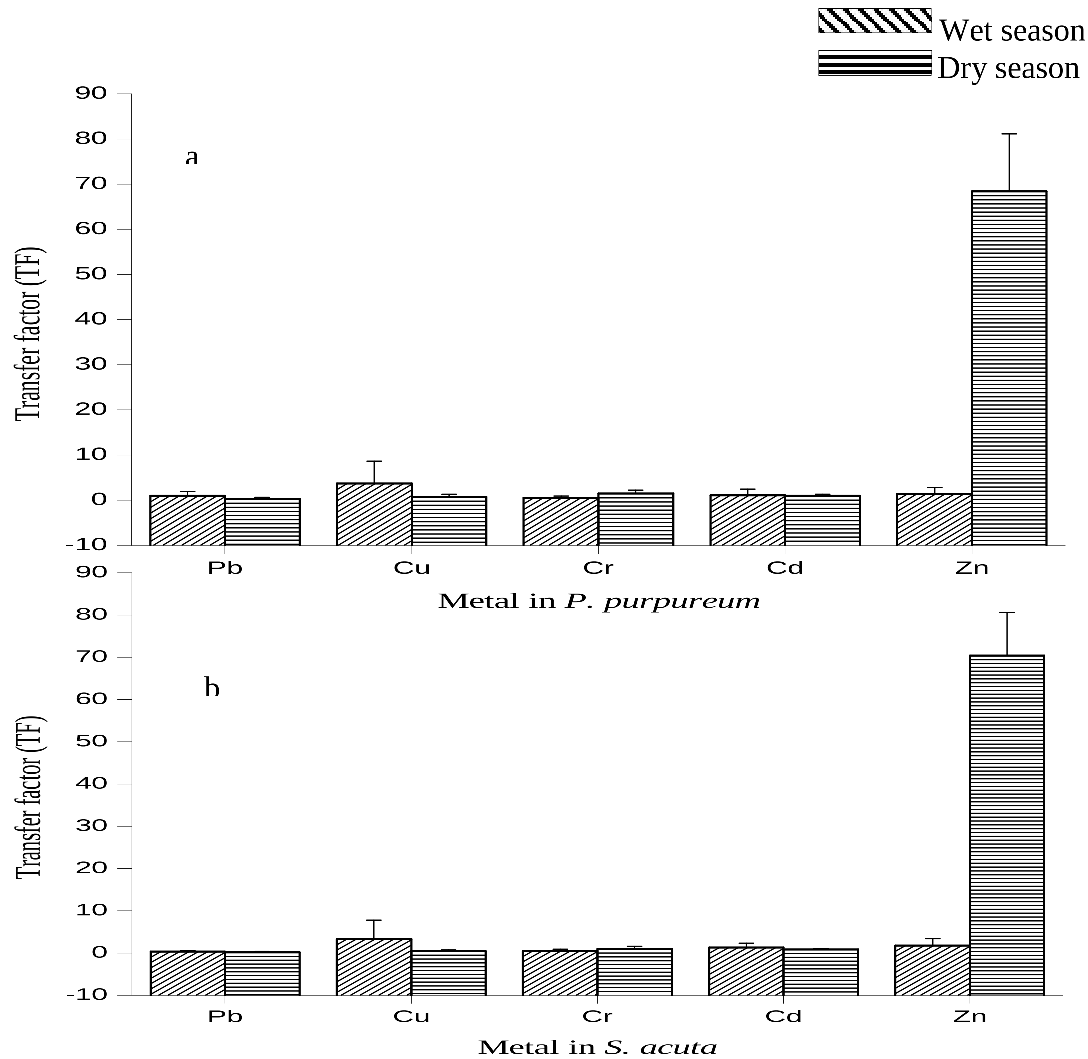

Figure 2. Seasonal variations in the transfer factor (TF) in (a) *S. acuta* and (b) *P. purpureum* growing on cement-polluted soil.

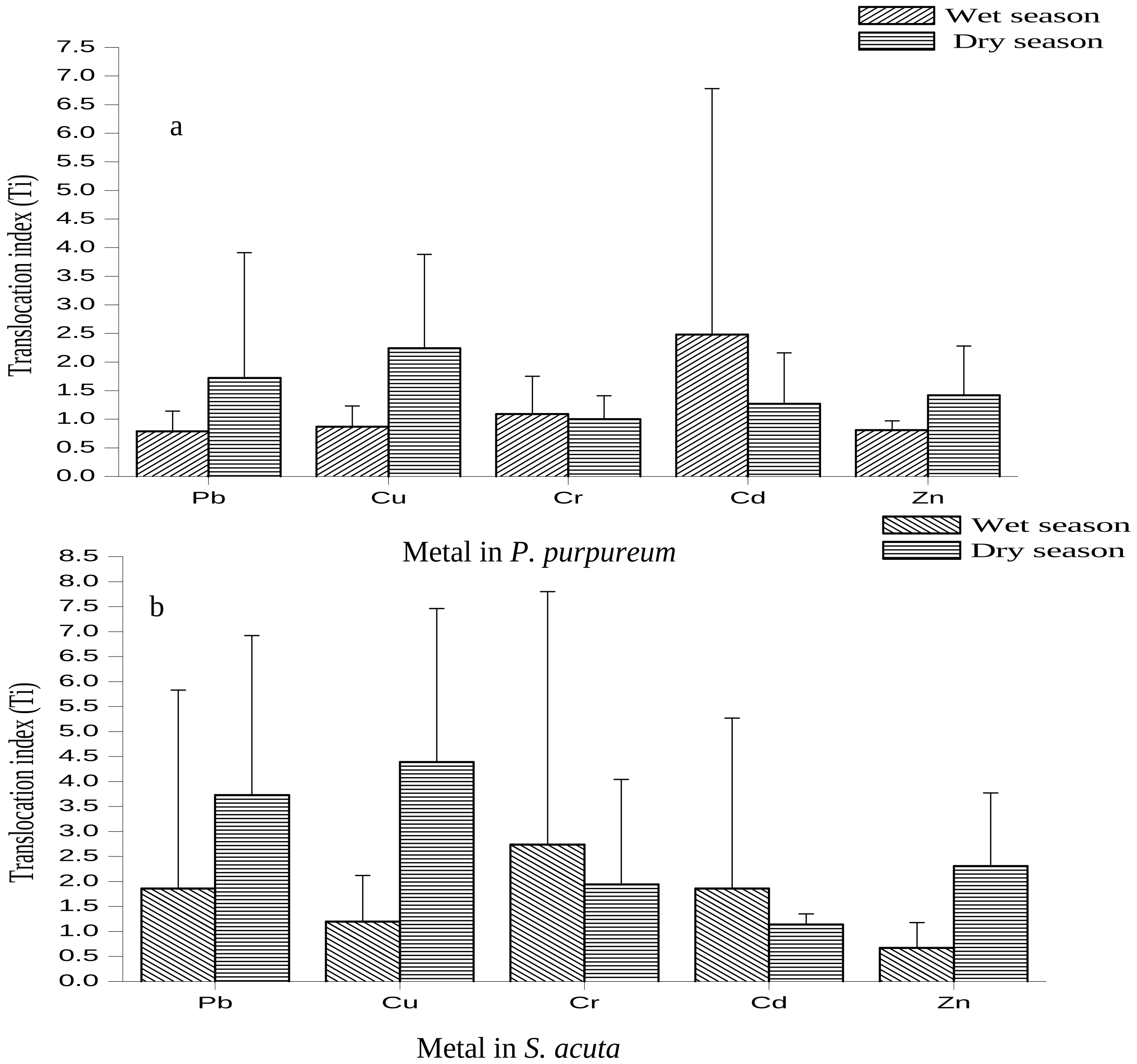

Figure 3. Seasonal variations in the translocation index (Ti) of (a) *S. acuta* and (b) *P. purpureum* growing on cement-polluted soil.

This is an indication that *S. acuta* has the potential of taking up Cr, Cd and Zn from phyto-available pool into the roots without much restriction and this could be a possible indication of *S. acuta* being a phytostabilizer of Cr, Cd and Zn. Garba et al. [17] have indicated that plants with TF>1.0 for a particular metal are good phytostabilizers. From Figure 2b, TFs of Cr, Cd and Zn in *P. purpureum* were also above 1.0 in both seasons while Pb had TF that was above 1.0 in the wet season and Cu with TF above 1.0 in the dry season. This is also an indication the suitability of *P. purpureum* as phytostabilizer of Cr, Cd and Zn in cement-polluted soil without restriction by seasons. Phytostabilization uses the ability of plants' roots to limit mobility of contaminants and bioavailability in the soil through the process of sorption, precipitation, complexation or metal valence reduction [19]. Therefore, *S. acuta* and *P. purpureum* possess the potentials to phytostabilizer Cr, Cd and Zn contaminated soil. The inconsistency in the TF of Pb and Cu for both weed species is an indication these plants species had limited capabilities of mobilizing these metals in the roots zone cross seasons and hence possess limited phytostabilization potential.

Translocation indices of metal for *S. acuta* and *P. purpureum* for the two seasons are presented in Figure 3. Pb had Tis >1.0 in *S. acuta* for both seasons, likewise Cr and Cd and according to Baker [8] and Garba et al. [17], a Ti that is above 1.0 denotes plant to be an accumulator. Tis of Cu and Zn were below 1.0 in the wet season and greater than 1.0 in the dry season (Fig. 3a). *S. acuta* cannot be regarded as accumulator of Cu and Zn because of its inconsistency in the Tis for Cu and Zn in the two seasons. Figure 3b showed that *P. purpureum* could also be regarded as accumulator of Cr and Cd because the Tis were also above 1.0. Due to the inconsistency in the Ti of *P. purpureum* in the two seasons (Tis of Pb, Cu and Zn <1.0 in the wet season and Tis>1.0 in the dry season), this plant species cannot be regarded as accumulator. Though, a study on similar grass (*Eleusine indica*) revealed the potential of *Eleusine indica* to exclude Pb, Cu and Zn and this supported the earlier assertion of Alloway [5] that plant species type determines metal absorption and accumulation patterns. The Ti showed the movement of metals from soil to root/shoot and indicate the efficiency of uptake by plants which is an important factor in determining accumulator or excluder plants.

4. Conclusions

The results from this study showed that *S. acuta* and *P. purpureum* displayed different metal partitioning in their tissue biomass. In spite of the different patterns of metal partitioning, these two weed species qualify as potential phytostabilizers of Cr, Cd and Zn in cement-polluted soil. This study has also been able to identify *S. acuta* and *P. purpureum* as accumulators (phytoextractors) of Cr and Cd which could be a sustainable option in remediating these metals from cement-polluted soil. This is indicated by the fact that these two weed species allocated greater proportion of Cr and Cd absorbed from the soil to aboveground biomass (leaves). And this is a convenient way of phytoremediation because these leaves are going to be senesced later thereby helping to discarding these toxic metals.

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