

Phytoremediation Potentials of Common Nigerian weeds for the purpose of cleaning up a Lead-zinc Derelict Mine

ABSTRACT

Six common Nigerian weeds namely *Helianthus tuberosus* (Sunflower), *Imperata cylindrical* (Sword grass), *Sida acuta* (Broom weed), *Gossypium spp* (Cotton), *Eleusine indica* (Goose grass) and *Chromolaena odorata* (Siam weed) grown around Enyigba lead mines were investigated within a period of two years (2008-2010) for their abilities to remove heavy metals from the soils of the derelict mines. X-ray Fluorescence (XRF) spectrometric method was used to determine the concentrations of (As), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), Nickel (Ni), lead (Pb) and zinc (Zn) in the top and sub soils and in the roots, stems and leaves of the studied plants. Their corresponding Pollution Indices (PI), Bioaccumulation Factors (BAF) and Translocation Factors (TF) were evaluated and used to determine their phytoremediation potentials. The results obtained revealed the mean concentrations (mg/Kg) of metals in the studied plants were of the range: Pb (12.62 – 417.2); As (0.38 – 2.26); Cd (8.46 – 144.6); Cu (32.81 – 420.40); Cr (30.2 – 184.2); Zn (0.26 – 16.87); Mn (12.94 – 155.80) and Ni (3.86 – 47.42). Levels of heavy metals in *Chromolaena odorata* and *Imperata Cylindrical* were significantly higher than in the other weeds. Higher accumulation of metals was observed in roots than in the stems and the leaves. High BAF and TF (<1) observed in some of the studied plants suggest that they could be employed as efficient phytoremediation agents in cleaning up polluted soil. Statistical analysis of variance (ANOVA) at $P < 0.05$ showed variations in the heavy metal levels between and within groups while Fisher's Least Significant Difference (LSD) Correlation analysis identified a strong relationship between the investigated plant and soil samples.

Keywords: *Phytoremediation, heavy metals, weeds, pollution index, bioaccumulation factor, translocation factor*

INTRODUCTION

Increasing industrialization has lead to extraction and distribution of mineral substances from their natural deposits (Kaplan *et al.*, 2005). Unlike other pollutants, heavy metals are non-biodegradable and they have the tendency to bioaccumulate and biomagnify from one trophic level to another (Clark, 1992;Khan *et al.*2003a,2004a). Heavy metals, at different sites, have been mined, smelted, or used in other industrial processes. The tailings, smelter slag and other wastes left behind often pollute the agronomic soil and also surface and ground water. Heavy metal contamination can be carried with soil particles swept away from the initial areas of pollution by wind and rain. (Ademoroti, 1996;Khan *et al.*,2004a,2005b). In many Nigerian cities and rural areas, there is inefficient environmental control system which results to contamination of immediate surroundings and the ground water with heavy metals (Oti *et al.*, 2012;Khan *et al.*,2006a,2007a). This is the reason why this present study was carried at Enyigba lead-zinc derelict in Ebonyi State, Nigeria where farmers are still actively cultivating their crops around the mine waste.

Phytoremediation is a plant based bioremediation technologies which employs the engineered use of green plants and their associated micro biota for the in-situ treatment of contaminated

soil and ground water (Sadowsky, 1999;Khan et al.,2008a,2009a). The process is environmentally friendly and takes advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant storage/degradation abilities of the entire plant body. Phytoremediation is cost-effective, and aesthetically pleasing as the plants can be easily monitored and metals absorbed by the plants may be extracted from harvested plant biomass and then recycled. The major disadvantage of phytoremediation is that it is limited to the surface area and depth occupied by the roots. Moreover it relies on natural cycle of plants and therefore takes time (William, 2008;Khan et al.,2010a,2011a).

Single Pollution Index (PI) is often used to determine the level of the pollutant in the environment by comparing the concentration of the pollutant to the allowable maximum limit of regulatory bodies such as World health Organisation (WHO) (Jamali *et al.*, 2007;Khan et al.,2012a,2013a). Bioaccumulation factor (BAF) is the ability of the plant to accumulate the heavy metals with respect to the metal concentration in the ecosystem (Wong, 2000). For a plant to be an efficient phytoremediation tool in the contaminated soil, the BAF have to be higher than one (Brown *et al.*, 1994;Khan et al.,2014a,2015). Translocation factor is the plant's ability to translocate heavy metal from root to harvestable aerial part. When values of $BAF > 1$ and/or $TF > 1$ is obtained, it indicates a preferential partitioning of metals from soil to root and from root to shoot respectively. (Branquinho *et al.*, 2007;Khan.,2003b,2004b). TF and BAF values of the studied weeds are strong indices of their phytoremediation potential (Baker and Whiting, 2002;Khan et al.,2005b,2006b).

The aims of this study are in two fold; first is to determine the levels of heavy metals in the investigated soils and establish the Pollution Index (PI). Second is determine levels of heavy metals in the weeds and from the data, evaluate their Bioaccumulation Factor (BAF) and Translocation Factor (TF) which suggest their phytoremediation potential. (Ma *et al.*, 2001;Khan et al.,2007ab,2008b).

METHODOLOGY

Collection and Preparation of Soil and Plant Samples

Soil: From each sampling site, composite top and sub soil samples were collected and labeled at 0-30 cm (n=6) and 60-90 cm (n=6) depths respectively. The samples were air-dried, ground mechanically with stainless steel soil grinder and sieved to obtain < 2 mm fraction. 30 g sub-sample was drawn from the bulk (< 2 mm fraction) and reground with laboratory mortar and pestle to obtain $< 200 \mu\text{m}$ fraction. Further drying was done in an open inert vessel in a muffle furnace at 105°C for 2 hours so as to remove soil moisture, after which the samples were cooled in desiccators (Danish EPA, 2000).

Plant: Plant samples were collected within Enyigba lead-zinc mine derelict and were authenticated at Applied Biology Department of Ebonyi State University, Nigeria. The roots, stems and leaves of the studied plants were separated in each case and the components were cut into pieces. The plant tissues were cleaned to remove dust, soil and other particles by putting them through a three step washing sequence (Reuter *et al.*, 1983). First they were washed with water, then with P-free detergent and followed by de-ionized water. The samples were air dried, and placed in a dehydrator at approximately 80°C for 48 hours so as to stop enzymatic activity. The samples were mechanically grounded into fine powdery with the aid of an agate mortar and were further dried at 65°C in an oven to obtain a constant. The pre-treated soil and plant samples were stored in treated plastic bottles for XRF analysis

X-Ray Fluorescence (XRF) Analyses of Heavy Metal in Plant and Soil Samples

A 13mm pellet of the each sample was formed using CAVER model manual palletizing machine at a pressure of 6 - 8 torr. Procedure for XRF was followed to determine the concentrations of metals in the soil and plant samples in accordance with Shefsky (1995). A voltage of 25KV and current of 50 μ A produced from X-ray tube was used to bombard the sample in XRF system for 18 minutes at 1000 counts. Si-Li detector was used to detect the characteristic X-ray of the metals and their corresponding concentrations were computed in the read out device. In addition to metal concentration, the pH, percentage of Organic Matter and percentages of sand, silt, and clay of the soil were determined using Orion 920A pH meter; Walkley & Black method and Hydrometer method respectively (White, 2006).

Statistical Analysis

The samples were assayed and analyzed in triplicates and data generated from XRF were reported as Mean $\pm$ Standard Error. One way analysis of variance (ANOVA) and Fisher's Least Square Difference (LSD) were used to determine significant difference within and between groups, considering a level of significance of less than 5% ($P < 0.05$) and from the generated data, PI, BAF and TF were calculated.

FINDINGS/DISCUSSION

The following tables present the results of XRF analyses and the PI, BAF and TF calculated from the generated data

Table 1: XRF Mean Concentrations (mg/kg) of Heavy Metals in Soil of Enyigba Mine Derelict and their Pollution Indices, (Ave pH = 6.5 $\pm$ 0.29)

Metal	Topsoil	PI	Subsoil	PI	US-EPA
As	4.8 $\pm$ 1.8	0.06	2.12 $\pm$ 1.6	0.03	75*
Cd	126.0 $\pm$ 42	1.5	28.8 $\pm$ 6.2	0.34	85
Cu	812.2 $\pm$ 141.2	0.19	322.2 $\pm$ 12.2	0.07	4300
Cr	2.12 $\pm$ 0.2	—	1.02 $\pm$ 0.2	—	
Mn	424.0 $\pm$ 50.4	—	120.0 $\pm$ 44.0	—	
Ni	82.6 $\pm$ 22.0	1.1	34.8 $\pm$ 8.2	0.46	75
Pb	1116.8 $\pm$ 43.2	2.7	91.7 $\pm$ 16.7	0.22	420
Zn	995.2 $\pm$ 82.4	0.13	322.0 $\pm$ 62.4	—	7500

* Values refer to metal concentration in typical soils (Miroslav & Vladimir, 1999),

PI = Pollution index was calculated using $C_{\text{soil}} / C_{\text{USEPA-standard}}$ where C is concentration of metal

Table 2: Properties of Soil from Enyigba Mine Derelict

Properties	(n = 3)
Sand (%)	61.28 $\pm$ 5.2
Silt (%)	7.12 $\pm$ 0.8
Clay (%)	31.60 $\pm$ 2.6
Organic Matter (%)	1.34 $\pm$ 0.5

121 **Table 3: XRF Level of Heavy Metals of Non-edible Weeds from Enyigba Mine (n=3)**

Botanical name	Common name	Plant parts	Heavy metals concentration (mg/kg)							
			Pb	As	Cd	Cu	Cr	Zn	Mn	Ni
<i>Imperata cylindrical</i>	Sword grass	Leaves	53.22	0.38	66.42	340.1	46.3	11.24	53.26	56.41
		stem	68.40	0.35	66.48	312.2	56.2	14.25	62.82	61.30
		Root	128.6	0.62	66.40	366.4	113.2	14.26	61.08	61.34
<i>Sida acuta burn</i>	Broom weed	Leaves	12.62	ND	24.26	42.82	164.2	12.62	32.48	3.86
		stem	14.84	ND	22.82	46.26	112.4	12.04	36.26	4.88
		Root	22.65	ND	34.64	52.88	170.2	13.26	42.68	8.94
<i>Helianthus tuberosus</i>	Sunflower	Leaves	18.61	1.12	12.63	122.6	32.6	0.89	12.94	11.82
		stem	42.85	1.49	14.81	168.8	42.9	1.27	13.06	14.87
		root	84.68	2.26	22.67	222.6	71.3	2.32	13.12	15.20
<i>Gossupium spp</i>	Cotton	Leaves	24.85	0.88	8.46	32.81	33.4	6.46	40.18	25.92
		stem	32.23	1.12	18.41	44.43	32.9	8.08	42.06	27.14
		root	66.81	1.48	26.42	46.26	30.2	8.82	45.88	34.16
<i>Eleusine indica</i>	Goose grass	Leaves	24.83	ND	43.28	76.44	44.7	0.28	17.32	6.98
		stem	26.75	ND	52.24	86.48	49.2	0.26	21.44	8.23
		root	29.86	0.62	60.49	102.2	52.8	0.48	23.38	12.72
<i>Chromoleaeceae odorata</i>	Siam weed	Leaves	417.2	0.46	122.2	420.4	98.2	16.42	155.8	47.42
		stem	234.2	1.22	102.6	224.8	122.4	16.85	80.28	44.27
		root	164.5	1.88	144.6	264.1	184.2	16.87	80.18	40.19

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123 **Table 4: Bioaccumulation Factors (TF = C_{shoot}/C_{soil})**

Botanical name	Common name	Pb	As	Cd	Cu	Cr	Zn	Mn	Ni
<i>Imperata cylindrical</i>	Sword grass	0.06	0.07	0.53	0.38	2.45	0.01	0.13	0.72
<i>Sida acuta burn</i>	Broom weed	0.01	-	0.18	0.06	52.8	0.01	0.09	0.06
<i>Helianthus tuberosus</i>	Sunflower	0.04	0.31	0.12	0.21	20.2	0.00	0.03	0.18
<i>Gossupium spp</i>	Cotton	0.03	0.23	0.15	0.06	15.5	0.00	0.10	0.33
<i>Eleusine indica</i>	Goose grass	0.024	-	0.42	0.11	23.2	0.00	0.05	0.10
<i>Chromoleaeceae odorata</i>	Siam weed	0.21	0.25	0.82	0.28	57.7	0.02	0.65	0.54

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125 **Table 5: Translocation Factors (TF = C_{shoot}/C_{root})**

Botanical name	Common name	Pb	As	Cd	Cu	Cr	Zn	Mn	Ni
<i>Imperata cylindrical</i>	Sword grass	0.53	0.56	1.00	0.85	0.49	1.00	1.03	1.00
<i>Sida acuta burn</i>	Broom weed	0.65	-	0.66	0.87	0.66	0.91	0.85	0.55

Helianthus tuberosus	Sunflower	0.51	0.66	0.65	0.76	0.60	0.55	1.00	0.98
Gossupium spp	Cotton	0.48	0.76	0.70	0.96	1.09	0.92	0.92	0.79
Eleusine indica	Goose grass	0.90	-	0.86	0.85	0.93	0.54	0.92	0.65
Chromoleaeceae odorata	Siam weed	1.42	0.65	0.71	0.85	0.66	1.00	1.00	1.10

DISCUSSION

Table 1 shows a comparison of the concentrations of the metals with the US-EPA Regulatory Limits (US-EPA, 1993). Only Pb, Ni and Cd from the Enyigba top soil exceeded the limits with their pollution index (PI) indicated in the order, Pb (2.7) > Cd (1.5) > Ni (1.1). The overall pollution status of soils of Enyigba mine derelict was calculated as 1.96 and this value indicated that Enyigba soils were polluted and it agrees with Chukwuma (1994) and Nweke *et al.*, (2008). Soils with PI > 1 are known to affect plants, animal and ultimately distort the food chain they support (Alloway and Jackson, 1991; Khan *et al.*, 2009b, 2010b; Khan *et al.*, 2013c, 2013d, 2014c). Table 2 shows the soil characteristics by their organic matter, sand, silt and clay contents. The percentage of organic matter content was 1.34 % and this is comparable to results obtained by Okoronkwo *et al.*, (2005). Organic matter content increases with decrease in pH and an increase in metal concentrations (Adhikari *et al.*, 2004; Khan *et al.*, 2011b, 2012b). Table 3 shows the levels of heavy metals in the plants and they did not follow a particular trend. However in most cases heavy metal concentrations were found to be higher in the root than in the stems and leaves. *Chromoleaeceae odorata* significantly accumulated high concentrations of Pb, Cd, Cu, Cr Mn and Ni (at $p < 0.05$) compared to other weeds. Low bioaccumulation factors (BAF < 1) were generally observed for all the investigated heavy metals except Cr (Table 4). Bioaccumulation factor is known to decrease with increasing metal concentration in the soil (Alam *et al.*, 2003). High BAF values of Cr were obtained for *Chromoleaeceae odorata* (57.7), *Sida acuta* (52.8), *Eleusine indica* (23.2) and *Helianthus tuberosus* (15.5). Based on classification of plants into excluders and accumulators, all the studied weeds can be considered as accumulators of Cr (Ma *et al.*, 2001 and Cluis, 2004; Khan *et al.*, 2013b, 2014b). Table 5 revealed low translocation factors (TF < 1) of As and Cu which was observed in all the weeds. TF = 1 was observed for Cd, Zn and Ni in *Imperata cylindrical*; Zn and Mn in *Chromoleaeceae odorata* and Mn in *Helianthus tuberosus*. However, high Translocation Factors (TF > 1) was observed in *Chromoleaeceae odorata* (1.42) for Pb and Ni (1.10); in *Imperata cylindrical* for Mn (1.03) and in *Helianthus tuberosus* (1.09) for Cr. A key trait of metal hyperaccumulators is the efficient metal transport from roots to shoots, characterized by the TF > 1 one (Zhao *et al.*, 2006; Ahmad *et al.*, 2015).

CONCLUSION

Based on high TF and BAF values, *Chromoleaeceae odorata* has a greater potential to clean up Pb and Ni contaminated soil than the rest of the studied weeds, while *Imperata cylindrical* and *Helianthus tuberosus* have the potential to clean up Mn and Cr contaminated soils respectively.

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