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Assessment of Heavy Metal Levels in the Soils of Ohyia Kaolin Mining Site and Screening of Three Local Herbaceous Species for Use in Phytoremediation

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Abstract:
The study assessed three local plant species Chromolaena odorata, Ipomoea involucrata and Mariscus alternifolius commonly found at abandoned kaolin mining site at Ohiya, Umuahia Abia state for their efficacy in phyto-remediation of heavy metals contaminated soil using a pot experiment. Soils from Ohiya kaolin mining site were used as medium for growing the species. Pre-experiment assessments of the concentrations of six heavy metals (Cr, Pb, Ni, Cd, Co and Se) were carried out in the soil and plant tissues to be used for the experiment. The experiment was laid out in a Randomized Complete Block Design (RCBD) with 6 replicate pots each. The physico-chemical properties of the experimental soil showed high content of lead (Pb) and cobalt (Co) contamination which were (2.40mg/kg) and (9.84mg/kg) respectively. Post-trial plant analysis revealed that the three plant species used for the study accumulated appreciable quantities of the heavy metals. The ranges of Pb in the species were as follows C. odorata (0.80-1.00mg/kg), I. involucrata (1.00-2.00mg/kg), M. alternifolius (0.40-0.70mg/kg). The ranges of Cr were: C. odorata from (0.00-0.07mg/kg), I. involucrata (0.30-0.42mg/kg), M. alternifolius (0.01-0.10mg/kg). The ranges of Cd in the species were: C. odorata (0.02-0.30mg/kg), I. involucrata (0.10-0.70mg/kg), M. alternifolius (0.10-0.60mg/kg) and Co concentrations were: C. odorata (2.00-3.07mg/kg), I. involucrata (2.01-4.01mg/kg), M. alternifolius (2.01-2.10mg/k). Ipomoea involucrata had significantly higher accumulation of Pb and Co than the two other plant species used and should be preferred in phytoremediation activities in the kaolin mine site at Ohiya or soils with high concentrations of heavy metals.

Keywords: *Phytoremediation, Heavy Metals, Soil, Kaolin, Mining, Chromoneana odorata, Ipomea involucrata and Mariscus alternifolius*

1. Introduction

Kaolin mining is a type of mining that deals with a range of clay substances made up of Kaonite and several other minerals produced by the alteration of felspathic rock, often times, Kaolin appears in different colors like white, pink, grey, yellow or red with a soft plastic nature. Kaolin is used as filler and raw materials in the manufacture and production of several goods such as ceramics, bricks, tiles, cement, paint, paper etc. depending on its individual chemical component and the extent to which it is processed (Dme, 2005). Kaolin minerals possess negative charges that easily attract positively charged ion such as Pb, Cu, Zn, Fe, Cr, Co, Ni etc. Consequently, soils rich in kaolin easily retain heavy metals and accumulate them above acceptable levels hence affecting the ecosystem. (Gamiz *et al.*, 1988).

Most heavy metals are poisonous and can damage nervous connections especially in young children and causing blood and brain disorders. Heavy metal contamination are caused by anthropogenic and geological activities such as mining, smelting, industrial pollutants, military activities, fuel production and agricultural chemicals (Jadia and Fulekar, 2009)

Implication of soil contamination by heavy metals is that they are easily absorbed in the soil, and can accumulate along with food chain causing toxicity on plants and animals as well as adverse health effects (Nelson and Sommers, 1982; Gasparatos and Haidouti, 2001).

Phytoremediation is a low cost, long term and environmental friendly mechanism used to remove contaminant from the contaminated areas. Over the years, it has become a highly accepted means of cleaning up pollutants from the environment (U.S. EPA, 2001).
Plants differ in their ability to tolerate high heavy metal concentrations and potential use as phytoremediator. This study aimed to screen the efficacy of three selected plant species for phytoremediation of heavy metals soil concentration at Ohiya kaolin mining site as a means of reclamation.

2. Materials and Methods

2.1. Study Area

The study site is located at kaolin mining site at Ohiya community in Umuahia south L.G.A in Abia State. Ohiya is located near the Abia Tower junction on the Umuahia side, along the Enugu- Port Harcourt Express way. It has an area of 140km^2 and a population of 138,570 (NPC, 2006). The area lies at latitudes $5^0$26' and $5^0$34'N and longitudes $7^0$22 and 7^0 33'E. It has high relative humidity values over 70% and is characterized by high temperature of about 29^0 -31^0C. The area is part of the equatorial belt with average annual rainfall of about 2,400mm per annum.

2.2. Assessment of Heavy Metals Concentrations in the Kaolin Site

Core soil samples were randomly collected from the overburden excavated by the miners at 0-15cm depth each on the Kaolin mine site. Also core soil samples were randomly collected from an undisturbed site opposite to the kaolin mine site where a 20-year fallow is existing. Soil from the un-mined site located at about 2kilometers away from kaolin mine site was used as the control. These core soil samples were differently homogenized in a clean plastic bucket and a composite sample was drawn from each. The soil samples were air dried, sieved with a 2mm mesh and labeled according to treatment before being taken to the laboratory for heavy metals analysis.

2.3. Screening of Plant Species for Soil Remediation

One month old young seedlings of *Chromolaena odorata, Ipomoea involucrata and Mariscus alternifolius* were picked from germination boxes and transplanted to nursery bed to stabilize the seedlings for one month before being transplanted into experimental pots. Eighteen (18) pots were filled each with 5kg the overburden soil from the Ohiya kaolin mine site and another set of Eighteen (18) pots was filled with 5kg soil sourced from the un-mined site (control). Each of species seedlings were planted into six (6) pots containing the overburden soil and another six pots containing soils from the Un-mined site (Control). Pre- trial plant analysis were conducted on each of 3 species using leaf and stem samples to determine the heavy metal background levels using Atomic Absorption Spectrometer. The experiment was laid out as a Randomized Complete Design and was maintained until flowering at the Nursery of the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike. The plants were watered every other day.

2.4. Plant and Soil Samples Preparations and Heavy Metals Analysis

Stands of each plant species were harvested after four (4) months and separately washed with a clean water to remove pollen, dust and soil particles. The plant samples were oven dried at 60^0C to a constant weight, and milled for analysis.
Two grams of the milled samples were then mixed with 2mls of concentrated H_2S0_4 inside a fume cupboard for digestion at 150^oC for three and half hours (Odu et al. 1986). Drops of hydrogen peroxide as the oxidizing agent were added while heating. The samples were allowed to cool to a temperature of 25^oC, and ultra-pure water was added to boost up to a volume of 50m^3. The samples were then swirled and transferred to a centrifuge machine for 10 minutes at 3000RPM. The extract was then poured into a set of vials for the determination of heavy metals using Atomic Absorption Spectrophotometer (AAS). Also soil samples were collected according to their soil profiles from each six (6) replicate pots. The soil samples were air dried and labeled accordingly, three grams of air dried soil were weighed into a tube (50m^3 in volume) and 1ml of $HN0_3$ and 10ml of HCl (Aqua Regia of ratio 1:2) were added to the samples. The content was heated on a digested block in fume cupboard to dryness at 120^oC (Ademoroti, 1996). The residue was allowed to cool and leached with 5ml of 2ml HCl, placed inside the centrifuge machine for 10 minutes at 4500RPM (Revolution per minute) at 120^oC for 1 hour, then increased to 250^oC for 1 hour, allowed to cool before making it to volume with ultra-pure water. The extract was then poured into set vials for the determination of the metals (Pb, Cd, Ni, Co, and Se) using Atomic Absorption Spectrophotometer (AAS).Data generated from this study were subjected to analysis of variance (ANOVA). Mean separations were done using Duncan Multiple Range Test and fisher LSD at 0.05% probability.

3. Results and Discussion

Table 1: showed results of comparative evaluation of heavy metals concentration in soil of the kaolin mining site (overburden) and the control site (fallow field). The concentration of lead (Pb) was (2.4mg/kg) which was almost 60% higher than Pb concentration in the un-mined site (control) (1.53mg/kg). Ni, Cd, Co and Se contents of mined and un-mined soil samples were 0.77 and 0.49mg/kg,0.16 and 0.10mg/kg, 9.84 and 8.14mg/kg, 1.06 and 0.99mg/kg respectively. There was significant difference ($P<0.05$) between the heavy metal content contained in the mined soil and that of un-mined soil samples except in that of Cr (0.22 and 0.25mg/kg).
The high heavy metals concentration of lead (Pb) (2.40mg/kg) and cobalt (Co) (9.82mg/kg) indicated that the area is contaminated especially when compared with EPA'S natural level of lead (Pb) occurrence (50ppm) which is equal to (0.05mg/kg), (Chaney *et al*., 1984). These could be as a result of kaolin mining activities at the area. Moreover, kaolin develops negative charge ions between its

layers and easily attract positive charges ions like heavy metals such as (Pb, Co, Cd, Cu, Cr etc.) resulting to contamination of the soil (Gamiz *et al*., 1988). From the evaluation, it was also observed that lead (Pb) and cadmium (Cd) from the mined site was significantly higher than that of the un-mined site by almost (60%) while cobalt (Co) was highly significant by (70%) when compared with the un-mined site. However, the problem of lead (Pb) and cadmium (Cd) poisoning in animals has widely been recognized which needs a special attention for the environmentalist and health personnel's. It accumulates in different parts of the body especially in kidney, liver and brain (Mclaughlin *et al*., 1999; Swarup *et al*., 2005).

Sample	**Pb**	**Cr**	**Ni**	**Cd**	**Co**	**Se**
Mined Area (A) soil	2.40	0.22	0.77	0.16	9.84	1.06
Un-mined Area (B) soil	1.53	0.25	0.49	0.10	8.14	0.99
Probability	0.0014	0.1963	0.0000	0.001	0.0000	0.000
T- test (0.05%)	**	NS	***	**	***	**

Table 1: Concentrations of some heavy metals in soil samples from kaolin mine site (Overburden) and un-mined site used as control
*KEY: NS= Not Significant, ** = Very Significant, *** = Highly Significant*

Table 2: showed results of plant analysis conducted on samples of *C. odorata, I. involucrata and M. alternifolius* to determine their levels of heavy metals before the pot experiment. The levels of Pb, Cr, and Se in *I. involucrata* were 1.00mg/kg, 0.30mg/kg and1.00mg/kg respectively and these concentrations were significantly higher than that found in *M. alternifolius* by 30%, 15% and 20% respectively. The levels of Ni, Cd and Co were 0.00mg/kg, 0.10mg/kg, and 2.01mg/kg in both *I. involucrata* and *M.alternifolius*. The concentrations of all the heavy metals were lesser in *C. odorata* than in *I. involucrata* but the difference was only significant for Cr, Cd, and Se.

Sample	**Pb**	**Cr**	**Ni**	**Cd**	**Co**	**Se**
C. odorata	0.80^{a}	0.00^{b}0.01^{a}	0.02^{b}	2.00^{a}	0.20^{c}	
I. involucrate	1.00^{a}	0.30^{a} 0.00^{a}	0.10^{a}	2.01^{a}	1.00^{a}	
M. alternifolus	0.40^{b}	0.01^{b}0.00^{a}	0.10^{a}	2.01^{a}	0.60^{b}	
LSD (0.05%)	0.26	0.12	0.01	0.01	1.15	0.37

Table 2: Concentrations of heavy metals in plant samples of chromolaena odorata, Ipomoea involucrata and Mariscus alternifolus before the commencement of the pot experiment

Mean values down the columns with the same superscript are not significantly different (P>0.05)
Table 3: showed results of post planting plant analysis conducted on plant samples of *C. odorata* (P1), *I. involucrata* (P2), and *M. alternifolius* (P3) to determine their level of heavy metals concentrations after growing them on kaolin mined and un-mined soil samples. The levels of Pb, Cr, Ni, Cd, Co, and Se in P2 from mined soil sample were 2.00mg/kg, 0.42mg/kg, 0.10mg/kg, 0.70mg/kg, 4.01mg/kg, and 1.70mg/kg respectively and these concentrations were significantly higher than that found in P3 by 65%, 16%, 4%, 5% 95% and 32%. The concentrations of all the heavy metals were significantly lower in P1 than P2 but the difference was only in Ni in P1 which was significantly higher than P2 and P3 while Pb and Co in P1 was higher than P3. The levels of Cd and Se in P3 were significantly higher than that found in P1. The levels of Pb, Cr, Ni, Cd, Co, and Se in P2 from un-mined soil sample were 1.50mg/kg, 0.35mg/kg, 0.40mg/kg, 0.19mg/kg, 3.13mg/kg and 1.17mg/kg respectively and these concentrations were significantly higher than that found in P3 by 49%, 17%, 13%, 4%, 55%, and 10%. The concentrations of all the metals were lower in P1 than in P2 but was found to be significantly higher than P3 except Cd and Se in P3 which was invariably higher than P1 from un-mined soil samples.
To evaluate these three plant species for suitability use in the phytoremediation of established contaminated kaolin mining site, several features must be considered. Based on the fact deduced by Alkorta *et al.,*2004, none of the plant surveyed at the present study possess root system deep enough to penetrate to an adequate depth to remove contaminant from entire soil profile. However, species such as *C. odarata, I. involucrata and M. alternifolius* possess fibrous root system which may be effective in preventing surface erosion of contaminated soil and reduce the rate of leaching contaminants in ground water. Most notably in this study, *Chromleana odorata* showed high tolerance of Cr, Ni, and Cd concentrations while *Ipomoea involucrata* tolerated significantly higher Pb, Cr, Ni, Co and Cd concentrations than the other plant species used. *Ipomoea involucrata* showed the most promising characteristic for phytoremediation of the contaminated area under study. These species demonstrated adaptability to a wide range of Pb, Cd, Ni, and Cr soil concentrations. *Ipomoea involucrata* was able to absorb significantly higher Pb and Co concentrations by more than 50% than other species used, from (1.00mg/kg to 2.00mg/kg of Pb) and (2.01mg/kg to 4.01mg/kg of Co) respectively.

Sample	Pb		Cr		Ni		Cd		Co		Se	
	MS	ums	MS	ums	MS	ums	MS	ums	MS	ums	MS	ums
C. odorata	1.00^{b}	1.19^{b}	0.07^{b}	0.03^{b}	0.18^{a}	0.25^{b}	0.30^{b}	0.09^{c}	3.07^{b}	3.05^{b}	0.60^{c}	0.47^{c}
I. involucrate	2.00^{a}	1.50^{a}	0.42^{a}	0.35^{a}	0.10^{b}	0.40^{a}	0.70^{a}	0.19^{a}	4.01^{a}	3.13^{a}	1.70^{a}	1.17^{a}
M. alternifolus	0.70^{c}	0.52^{c}	0.10^{b}	0.02^{b}	0.01^{c}	0.14^{c}	0.60^{ab}	0.12^{b}	2.10^{c}	2.03^{c}	1.05^{b}	0.98^{b}
LSD(0.05%	0.03	0.48	0.06	0.23	0.02	0.02	0.04	0.16	1.18	0.40	0.33	0.40

Table 3: Post planting plant analysis of heavy metals concentrations on plant samples from mined site soil sample (MS) and un-mined site soil sample (UMS)
Mean values down the column with the same superscript are not significantly different (P>0.05)
Key: P1=Chromolaena odorata, on mined/un-mined soil, P2=Ipomoea involucrata on mined/un-mined soil, P3=Mariscus alternifolius, on mined/un-mined soil

Table 4: showed results of post planting soil analysis conducted on all the 3 plant species from mined site soil sample and un-mined site soil sample to determine their level of heavy metals after pot experiment. The background values obtained from pre-trial analysis (A) soil were used as controlalongside with results from the plant species of the un-mined site soil. The levels of Pb, Cr, Ni, Cd, Co, and Se in (P2 on MS) were 1.35mg/kg, 0.10mg/kg, 0.05mg/kg, 0.04mg/kg, 7.60mg/kg and 0.92mg/kg respectively and these concentrations were significantly lower than that found in the respective values from pre-trial analysis (A) soil by 53%, 6%, 36%, 6%, 85% and 7%. The levels of Pb, Cr, Ni, Cd, Co, and Se in (P1 on MS) were 2.00mg/kg, 0.15mg/kg, 0.10mg/kg, 0.06mg/kg, 8.30mg/kg and 1.00mg/kg respectively and was lower when compared with values from pre-trial analysis (A) soil by 20%, 4%, 15%, 5%, 77%, and 3%. The concentrations of all the heavy metals in (P3 on MS) were little lesser than the values from pre-trial analysis (A) soil but the difference was only significant for Ni, Cd, Co, and Se. In un-mined site soil sample (UMS), The levels of Pb, Cr, Ni, Cd, Co, and Se in (P2 on UMS) were 1.00mg/kg, 0.18mg/kg, 0.07mg/kg, 0.01mg/kg, 7.00mg/kg and 0.81mg/kg respectively and the concentrations were significantly lower than that found in values from pretrial analysis (B) soil by 27%, 4%, 21%, 5%, 57% and 9%. The levels of Pb, Cr, Ni, Cd, Co, and Se in (P1 on UMS) were 0.21mg/kg, 1.20mg/kg, 0.24mg/kg, 0.03mg/kg, 7.08mg/kg and 0.70mg/kg respectively and were lower when compared with the values from pre-trial analysis (B) soilby 17%, 2%, 13%, 4%, 53% and 15%. The concentrations of all the heavy metals in (P3 on UMS) were little lesser than the pretrial analysis (B) soil values, but the difference was only significant for Se.

However, result from soil analysis shows that *Ipomoea involucrata* was able to reduce Pb and Co soil concentrations by almost 60% than other species used, from (2.40mg/kg to 1.35mg/kg) and (9.84mg/kg to 7.60mg/kg), indicating a significant decrease in Pb and Co soil concentration. It is possible that the apparent hyper accumulation of lead (Pb) by *Ipomoea involucrata* in this study may be due to its fibrous root and vigorous growth habit in a normal climatic condition, which could be the very feature that makes it suitable for phyto-extraction (Chaney, 1983; Brown *et al.,* 1995). It was observed from the result that *M. alternifolius* did not exhibit any significant accumulation on Pb, Cr, and Ni concentrations although there were few exceptions, this species was primarily found to be significantly high in accumulation of Cd, Co, and Se soil concentration. From the foregoing, *M. alternifolius* could be therefore less suitable for use in a phytoremediation of soil polluted with/high in lead (Pb). The high Cd accumulation observed in this study by *Mariscus alternifolius* corroborates with the findings of Baker *et al*., (2000) who opined that most plant species growing over lead (Pb)/Zinc (Zn) mineralization soils show elevated cadmium (Cd) concentration in range (10-100mg/kg).

	Pb		Cr		Ni		Cd		Co		Se	
Sample	**MS**	**UMS**	**MS**	**UMS**	**MS**	**UMS**	**MS**	**UMS**	**MS**	**UMS**	**MS**	**UMS**
C. odorata	2.00^{ab}	1.20^{b}	0.15^{b}	0.21^{bc}	010^{c}	0.24^{c}	0.06^{c}	0.03	$8.30b^{c}$	7.08	1.00^{b}	0.70^{bc}
I. involucrata	1.35^{b}	1.00^{b}	0.10^{c}	0.18^{c}	0.05^{d}	0.07^{d}	0.04^{c}	0.01^{b}	7.60^{c}	7.00^{b}	0.92^{c}	0.18^{ab}
M. alternifolius	2.10^{a}	1.40^{a}	0.21^{a}	0.24^{ab}	062^{b}	0.34^{a}	0.10^{b}	0.08^{a}	9.01^{ab}	8.12^{a}	0.98^{b}	0.60^{c}
PRE TRIAL VAL.	2.40^{a}	1.53^{a}	0.22^{a}	0.25^{a}	0.77^{a}	0.49^{a}	0.16^{a}	0.10^{a}	9.84^{a}	8.14^{a}	1.06^{a}	0.99^{a}
LSD(0.05%) 0.48 0.27 0.04 0.04					0.03	0.05	0.03	0.03	0.98	0.94	0.03	0.19

Table 4: Post planting soil analysis of heavy metals concentration in mined site soil sample (MS) and un-mined soil sample (UMS)
Mean values down the column with the same superscript are not significantly different (P>0.05).
KEY: P1=Chromolaena odorata, on mined/un-mined soil, P2=Ipomoea involucrata on mined/un-mined soil, P3=Mariscus alternifolius, on mined/un-mined soil, Pre-trial val.= preliminary soil analysis conducted from mined site soil sample (overburden) and un-mined site soil sample

4. Conclusion and Recommendation

The soils from the kaolin mined site had elevated concentrations of some heavy metals namely Pb, Cr, Ni, Cd, Co etc. when compared to the un-mined area.These native flora*C. odarata, I. involucrata and M. alternifolius* used in the study displayed varying abilitiesto withstand high concentrations of heavy metals in the soil.They showed different accumulation patterns for metals at different soil Concentrations (Wong, 2003).The levels of Pb and Co in *Ipomoea involucrata* were higher than that of *Chromoneana odorata* and *Mariscus alternifolius* showing the capacity to accumulate and tolerate the potential toxicity of these heavy metals. However, it is recommended that exposure to the mined kaolin site should be done with caution because it has high concentrations of heavy metals like Pb, Cr, Ni, Cd, Co, Se etc. It can be also deduced from the present study that, *Ipomoea involucrata* proved to be a good candidate for phytoremediation of Pb, and Co than *Chromolaena odorata* and *Mariscus alternofolius*. Though the three plant species showed a considerable level of accumulation of heavy metals, *Ipomoea involucrata* should be preferred in remediating the kaolin mine site at Ohiya because of its ability to grow well and accumulate the heavy metals than others considered. It is also recommended that some native plant species growing locally at kaolin mined site should be preferred for heavy metals accumulation. Rajakaruna *et.al*., (2006) also recommended that native plant species growing locally should be used in phytoremediation.

5. References

i. Ademoroti, C.M.A. (1996). Environmental Chemistry and Toxicology, Foludex Press Ltd Ibadan. Pp 17-24.
ii. Alkorta, I. Hernández-Allica, J., Becerril, J., Amezaga, I., Albizu, I., Garbisu, C. (2004). Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev. Environ. Sci. Biotechnol. 3(1):71-90.
iii. Brown, S.L., Chaney, R.L., Angle, J.S., and Baker, A.J.M. (1995a). Zinc and cadmium uptake by hyper accumulator Thlaspi caerulescens and metal tolerant Silene vulgaris grown on sludge amended soils. Environmental Science and Technology.29, 1581–1585.
iv. Chaney, R.L. (1983). Plant uptake of inorganic waste. In Land Treatment of Hazardous Waste, eds JE Parr, PB Marsh, JM Kla, pp 50-76, Noyes Data Corp, Park Ridge Il
v. Chaney, R.L. et al. (1984). The potential for heavy metal exposure from urban gardens and soils, pp. 37-84. In: J.R. Preer ed. Proceedings of the symposium on heavy metals in urban gardens. Agricultural Experiment Station, University of the District of Columbia. Washington.
vi. DME (2005). South Africa's mineral industry 2004/2005. Department of Minerals and Energy, Republic of South Africa, pp: 148-154, http://www.infomine.com/publications/docs/DMESouthAfrica/DME_Annual_Report_04 05.pdf. (05/07/2014)
vii. Gamiz, et al., Caballero, M.D. Rodriguez, and R.D.C. Flores, (1988). Characterization of Spanish kaolins for pharmaceutical use. I. Chemical and mineralogical composition, physico- chemical properties. Boll. Chim. Farm, 127: 114-121.
viii. Jadia, C.D. and Fulekar, M.H., (2009). Phytoremediation of heavy metals: Recent techniques. Afr. J. Biotechnol. 8(6): 921-928.
ix. Mclaughlin, M.J., Parker, D.R. and Clarke, J.M (1999). Field Crop Research 60:143-163.
x. Nelson, D.W. and Sommers, L.E. (1982). Total Carbon, organic carbon, and organic matter. Method of soil analysis. Part 2. Chemical and Microbiological properties. 2nd Edition, no.9 (part 2) in series. 539-579.
xi. Odu, C. T. I., Babalola, O., Udo, E. J., Ogunkule, A. O., Bakere, T. A. and Adeoye, G. O.(1986). Laboratory Manual for Agronomic Studies in Soil, plant and Microbiology University of Ibadan, Ibadan press Ltd p.83.
xii. Rajakaruna, N., Tompkins, K.M. and Pavicevic, P.G. (2006). Phytoremediation: An affordable green technology for the clean-up of metal-contaminated sites in Sri Lanka. Ceylon J. Sci. (Biological Sciences) 35:25-39.
xiii. Swarup, D., Patra, R. C., Naresh, R., Kumar, P. and Shekhar, P. (2005). Science of the Total Environment. 15(1-3):67-71.
xiv. United States Environmental Protection Agency. (2001). Citizen's guide to phytoremediation. Retrieved 4 January, 2011, from in.org/download/citizens/citphyto.pdf