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Use of Natural and Silver Nitrate Induced green Plant Technology for Phytoextraction Studies

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Abstract: - Phytoremediation is a green plant technology used recently to decontaminate heavy metal polluted soils. Natural and silver nitrate inducedZea mays, Abelmoschusesculentus and Amaranthusveriditis were used for phytoextraction studies of iron, copper, zinc, chromium, cadmium, lead, and nickel in soil using standard method. Induced Zea mays was found to be a good hyper accumulator of cadmium and lead only with plant concentration factors of 1.40 and 1.14 respectively. Natural Abelmoschusesculentus had the potential to extract heavy metals from the contaminated soil. Induced Amaranthusveriditis was found to be a good remediator and hyper accumulator of all the metals studied when compared with all other transgenic plants used with plant concentration factors of 25.8 (iron), 1.53 (copper), 2.16 (zinc), 12.3 (chromium), 1.39 (cadmium), 2.02 (lead) and 3.76 (nickel). Nickel had the highest factor of accumulation (47) followed by lead (21), an indication of soil nickel and lead contamination. Pearson correlation using CronbachsAlph at 0.0079 revealed positive correlations between pair of heavy metal in plants. A two sample test using Mann Whitney U test at p<0.05 showed statistical significant difference between means of heavy metals within the premises of the steel rolling mill and control site. Induced Zea mays and induced Amaranthusveriditis had better impact and potential to remediate heavy metal polluted soils and are thus good phytoremediators and hyper accumulators.

Keywords: **Abelmoschusesculentus, Amaranthusveriditis, heavy metals, phytoremediation, soil, Zea mays.**

I. INTRODUCTION

A rapid industrialization had led to regional and global redistribution of metals with consequent environmental pollution in the world today. The role of environmental pollution to produce various types of deleterious effects on diverse living system has been well-established. Heavy metals are the most hazardous pollutants as they are non-degradable & get accumulated and become toxic both to plants & animals. Heavy metal enters the food chain & adversely affects the flora & fauna (Fried land, 1990). In plants, enhanced level of heavy metals in soil caused significant reduction in plant height, root-shoot ratio, dry weight, nodule per plant, chlorophyll content in *Vignaradiata*(Tomar*et al.*, 2000) & in *Menthaspicata*(Bekiaroglou and Karatagli, 2002).Phytoremediation is a green technology for the sustainable remediation of surface soils contaminated with heavy metals. It is an innovative, novel & potentially inexpensive technology using metal polluted soils.(Baker et al., 1994; Salt et al., 1998). Phytoremediation is characterized by the use of vegetative species for in situ treatment of land areas that is believed to have been polluted by a variety of hazardous substances as a result of human activities (Skyeset al., 1999). Plants are especially useful in the process of bioremediation because they prevent erosion and leaching which can spread the toxic substances to surrounding areas. Various types of phytoremediation have been described that can be used to identify transgenic plants because of their ability to hyper accumulate heavy metals. These include phytoextraction, which relies upon a plant’s natural ability to take up certain substances (such as heavy metals) from the environment and sequester than in their cells until the plant can be harvested, phytodegradation, is a means by which plants convert organic pollutants into a non-toxic form, phytostabilization, where a plant releases certain chemicals that bind to the contaminant to make it less bioavailable and less mobile in the surrounding environment, and phytovolatilization, a process through which plants extract pollutants from soil and then convert them into a gas that can be safely released into the atmosphere (Bentjen, 2002). Of these techniques, phytoextraction & phytostabilization are used to remediate in organ iccontaminated soil especially by heavy metals. According to Freidland (1990) an ideal plant species for phytoremediation should have either one of the following characteristics: (a) a low biomass plant with a very high metal accumulation capacity or (b) a high biomass plant with enhanced metal uptake potential. Metal tolerant plants with lower metal accumulation are preferred for phytostabilization& heavy metal hyper accumulators are the best choice for phytoextraction as they tolerate high metal ions through various detoxification mechanisms which may include selective metal uptake, excretion, and complexing by specific ligands &compartmentation of metal ligand complexes (Cobbet 2000; Clemens 2001). Phytoextraction uses plants or large algae to remove contaminants from soils, sediments into harvestable plant biomass. Phytoextraction has been growing rapidly in popularity worldwide for the last twenty years or so. In general, this process has been tried more often for extracting heavy metals than for organics. At the time of disposal, contaminants are typically concentrated in the much smaller volume of plant matter than in the initially contaminated soil or sediment. The process that occurs in phytoextraction is such that the plants absorb contaminants through the root system and store them in the root biomass and/or transport them up into the stems and/or leaves. A living plant may continue to absorb contaminants until it is harvested. After harvest, a lower level of the contaminant will remain in the soil, so the growth/harvest cycle must

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usually be reported through several crops to achieve a significant cleanup. After the process, the cleaned soil can support other vegetation. The process of phytoextraction used in this study induced the seedlings of plants with silver nitrate solution which is believed will help the plant in its hyper-accumulation capability. This is the first time that silver nitrate induced plants will be used for phyto-remediation in literature. The principle is based on the fact that heavy metals in soil will displace silver in these plants and can increase the rate at which the plants will hyper-accumulate these metals. Silver nitrate was also used because if present in soil might not pose a serious health effect as the studied heavy metals would since silver nitrate is present in some applications used in treating some human diseases and there has not been any report of health implications or death. Silver exhibits low toxicity in the human body, and minimal risk is expected due to clinical exposure by inhalation, ingestion, dermal application or through the urological or haematogenous route (Lansdown, 2006). Natural hyper-accumulation plants naturally take up the contaminants in soil unassisted. While induced or assisted hyper-accumulation is where a conditioning fluid containing a chelator or another agent is added to soil (or plant) to increase metal solubility or mobilization so that the plants can absorb them more easily. In many cases natural hyper-accumulators are metallophyte plants that can tolerate and incorporate high levels of toxic metals. The main advantage of phytoextraction is environmental friendliness. Traditional methods that are used for cleaning up heavy metal-contaminated soil disrupt structure and reduce soil productivity, whereas phytoextraction can clean up the soil without causing any kind of harm to soil quality. Another benefit of phytoextraction is that it is less expensive than any other clean-up process. However, the disadvantage is that the process is controlled by plants; it takes more time than anthropogenic soil clean-up methods. The use of different plant species as hyper-accumulators abounds in literature. Marchiolet al. (2007) reported the use of Sunflower (Helianthus annus) as hyper-accumulator for arsenic. Chinese Brake fern (Pterisvittata) was also reported by Wang et al. (2002) to store arsenic in its leaves. Greger and Landberg (1999) performed a research experiment and suggested that willow (Salix viminalis) has a significant potential as a phyto-extractor of cadmium, zinc, and copper. They reported that it can also be used for the production of bio energy in the biomass energy power plant. Sedum alfrediiHance was identified in China as hyper accumulator for Cd and Zn and has been intensively investigated by various researchers in their studies conducted in hydroponics and/or the uncontaminated and contaminated soils (Xionget al., 2004;Li et al., 2005; Li et al., 2005a; Liu et al., 2005; Yang et al., 2006). They reported that concentrations of cadmium and zinc in leaves and stems increased with increasing cadmium and zinc supply levels. The distributions of metals in different plant parts decreases in the order: stem>leaf>root for zinc and leaf>stem>root for cadmium. These results indicate that S. alfredii has an extraordinary ability to tolerate cadmium/zinc toxicities and to absorb and hyper accumulate cadmium and zinc under a range of cadmium/zinc combining levels.

The aim of this research was to use some natural and induced seedlings of Nigerian plants to extract heavy metals from polluted soil within the premises of a steel rolling mill. In achieving this, the scope of work was meant to achieve the following objectives:

- TO use natural seedlings of some plants for phyto-extraction studies.
- To induce seedlings of some plants with silver nitrate solution for phyto-extraction studies.
- To plant these seedlings in polluted soil collected from a steel rolling mill.
- To determine the concentrations of iron, copper, zinc, chromium, cadmium, lead and nickel in the harvested plant species.
- To compare the rate of accumulation of heavy metals in natural and induced plants for hyper-accumulation.

II. METHODOLOGY

Field Characteristics. Soil sample was collected from a steel rolling mill in Ikirun along Osogbo road. The soil sample was collected at 0-25 cm depth inside the within the premises of the environment where the raw materials are usually been dumped before processing. The soil sample contained both soil and some steel materials such as tin milk, nails, rods, bolts, nuts, etc. The steel materials were separated from the soil through sieving to get fine soil particles. The samples were then kept in a labeled polythene bag. Control samples were collected at Darus-Salam School along this expressway. The soil texture was dark sandy loamy soil with pH, EC.

Plant Growth Conditions. The seeds of *Zea mays, Abelmoschusesculentus and Amaranthusveriditis* (corn, okro and vegetable respectively) were used for the study and bought from Ikirun, Ada and Iree markets respectively. 0.00014 M of silver nitrate solution was prepared. The seeds to be used were divided into two. One portion was planted naturally in the polluted soil and the second portion was soaked for 48 hours in 50 mL silver nitrate solution. The seeds were later filtered and sundried. The silver nitrate changed the color of the *Zea mays* to black. *Abelmoschusesculentus* and *Amaranthusveriditis* retained their color. The two sets of seeds for all the plants were planted each in 2kg of the polluted soil. The induced seeds of the plants were named tagged (T) and those used without inducing was named as untagged (U).

Plant Harvesting. The plants were left to germinate for a period of six weeks. Some of the plants germinated and some did not grow especially those whose seeds were induced with silver nitrate solution. Plants samples

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collected were then harvested, sundried, crushed, sieved and kept for analysis.

Soil Samples: The soil samples for each plant was also sundried, crushed, sieved, labeled and kept for analysis.

Soil and Plant Analysis

Digestion: 1.0 g of the sample was weighed into digestion flask. 10mL of nitric perchloric acid was added. It was digested at 150°C for 1 hr 30 mins. The temperature was increased to 230°C and 2mL of hydrochloric acid and distilled water (1:1) was added and digested for another 30 mins. This was allowed to cool and washed into a 50mL standard volumetric flask and made up to the mark with distilled water and kept for instrumental determination.

Instrumental Analysis: The digest for plant and soil was analyzed using an Atomic Absorption Spectrophotometer (AAS) model 210 VAP Buck Scientific. The instrument was calibrated using metal standard preparations and was used to prepare calibration graph. The concentration of each metal was determined from the calibration graph and recorded as mg/kg metal. The detection limit of the instrument is as shown in Table .1 below.

Table .1: Detection limit of Heavy Metals

Metal	Wavelength (nm)	Detection Limit
Iron	283.5	0.001
Copper	324.7	0.003
Zinc	213.9	0.003
Chromium	357.5	0.003
Cadmium	228.8	0.002
Lead	283.7	0.050
Nickel	232.0	0.003

Statistical Determination

Statistical analyses such as mean, standard deviation, Pearson correlation, t-tests, were carried out using NCSS. Plant concentration factor, and accumulation factor was determined. The soil accumulation factor was calculated as the concentration of the metal in sampling site divided by the concentration of that metal in control site. Plant concentration factor (PCF) = C plant/C soil, according to Cui *et al.* (2005),Where plant is the concentration of the metal in plant, Csoil is the concentration of the metal in soil.

III. RESULTS AND DISCUSSION

Phytoextraction with Zea mays

The results obtained in the phyto-extraction of metals in the polluted soil of the sampling site are as shown in Figures 1-27. The Figures depict the concentrations of these metals in soil, tagged and untagged plants species used. Figures 1,2,3,4 and 7 showed that *Zea mays* that was not induced with silver nitrate was able to extract more of iron, copper, zinc, chromium and nickel metals from the polluted soil. However, it could be noticed from Figures 5 and 6 that induced *Zea mays* are able to extract cadmium and lead from the polluted soil compared to the untagged *Zea mays*.Amin (2011) reported the use of *Zea mays* (corn) to be an effective accumulator plant for phytoremediation of cadmium and lead polluted soils.Khaled*et al.* (2010) investigated the use of *Zea mays* in the decontamination of chromium polluted site.

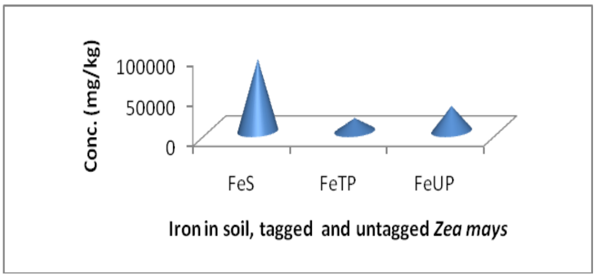

Fig 1: Level of iron in soil, tagged and untagged *Zea mays*

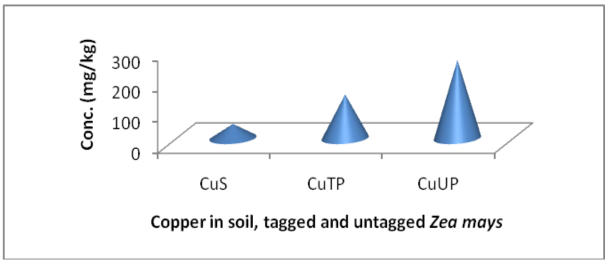

Fig 2: Level of copper in soil, tagged and untagged *Zea mays*

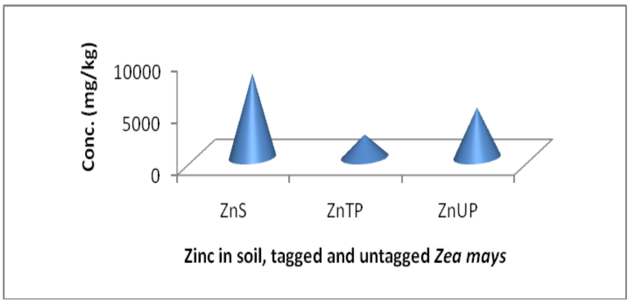

Fig 3: Level of zinc in soil, tagged and untagged *Zea mays*

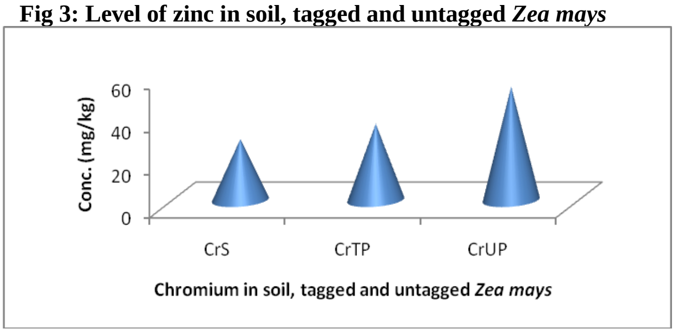

Figure 4: Level of chromium in soil, tagged and untagged *Zea mays*

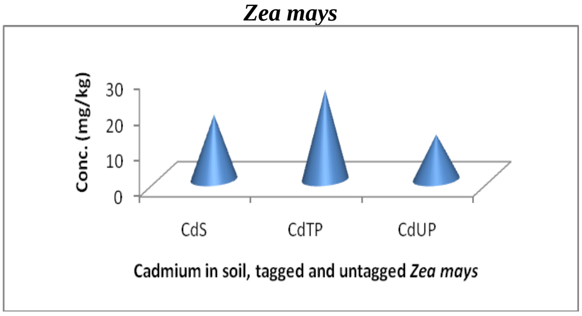

Fig 5: Level of cadmium in soil, tagged and untagged *Zea mays*

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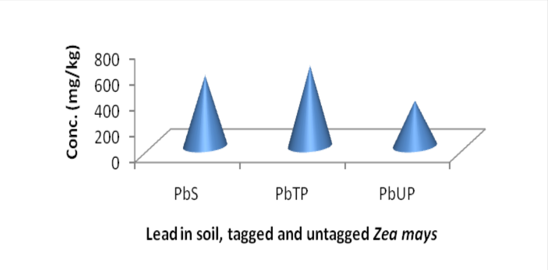

Fig 6: Level of lead in soil, tagged and untagged *Zea mays*

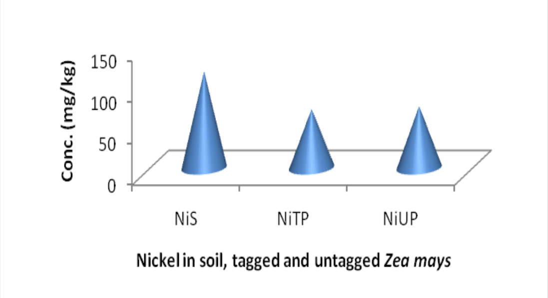

Fig 7: Level of nickel in soil, tagged and untagged *Zea mays*

Phytoextraction with Abelmoschusesculentus

The use of *Abelmoschusesculentus* to extract metals from polluted soil is as shown in Figures 8 to 14. The research revealed that after the immobilization of silver nitrate into the seeds of *Abelmoschusesculentus*, the seeds did not germinate. The reason might be due to change in the genetic modification of the seeds as a result of the concentration of the silver nitrate used for inducing the seed. Only the natural form of the seedling could then be used to discuss the phytoextraction ability of this Nigerian plant. All the Figures (8, 9, 10, 12, 13 and 14) showed that *Abelmoschusesculentus*hyperaccumulate iron, copper, zinc, cadmium, lead and nickel, chromium to some extent. The decontamination of chromium in this polluted soil was however high compared to other metals as shown in Table 1 in the plant concentration factor (1.93). Weeraingheeet *al*. (2009) reported that *Abelmoschusesculentus*, a dicotyledonous plant absorbed more cadmium compared to a monocotyledonous plant (*Bucholedsctyloides*). Use of *Abelmoschusesculentus* and *Zea mays* in the remediation of polluted soil as biotechnology approaches was reported by Anita and Sheo (2011).

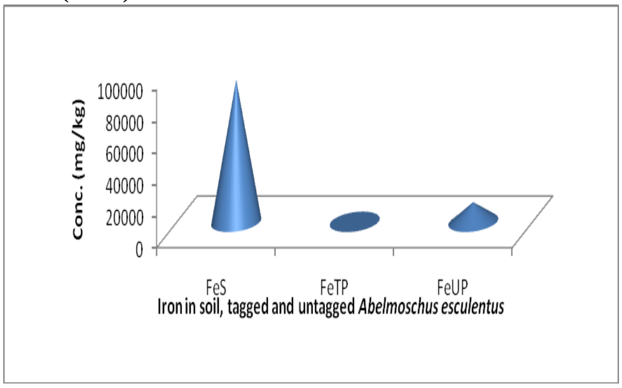

Fig 8 : Level of iron in soil, tagged and untagged soil of Abelmoschusesculentus

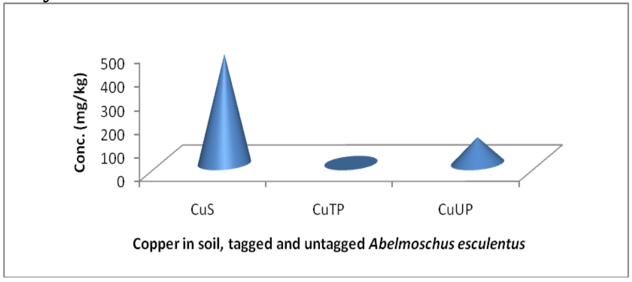

Fig 9: Level of copper in soil, tagged and untagged *Abelmoschusesculentus*

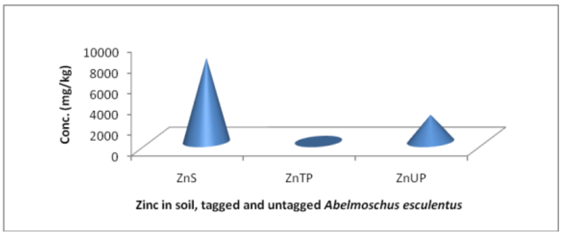

Fig 10: Level of zinc in copper, tagged and untagged *Abelmoschusesculentus*

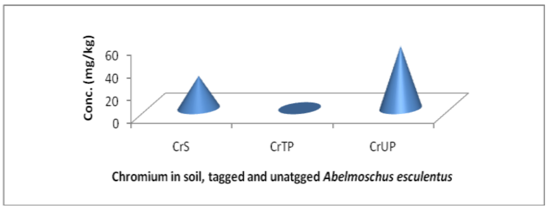

Fig 11: Level of chromium in soil, tagged and untagged *Abelmoschusesculentus*

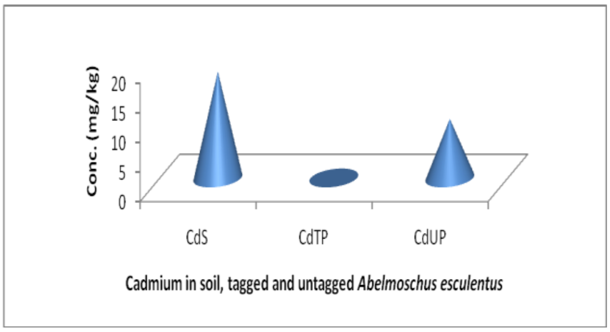

Fig 12: Level of cadmium in soil, tagged and untagged *Abelmoschusesculentus*

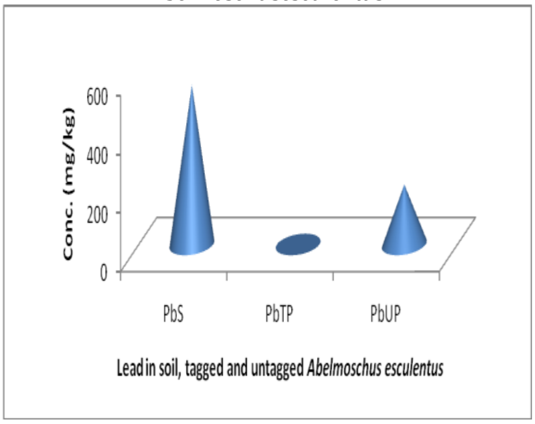

Fig 13: Level of lead in soil, tagged and untagged *Abelmoschusesculentus*

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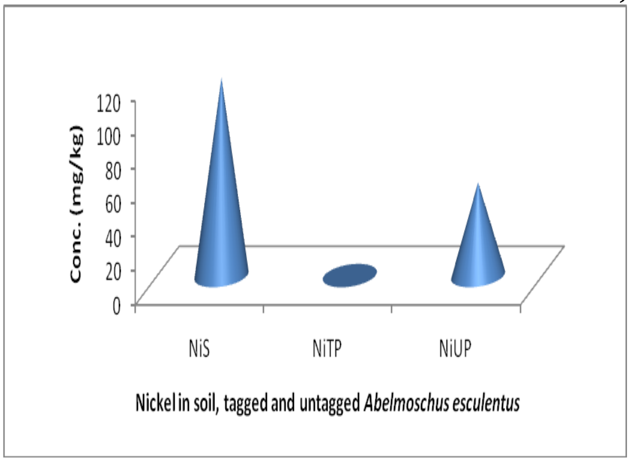

Fig 14: Level of nickel in soil, tagged and untagged *Abelmoschusesculentus*

Phytoextraction with Amaranthusveriditis

The ability of *Amaranthusveriditis* to extract metals from polluted site is as shown in Figures 15 to 21. The natural seedlings of *Amaranthusveriditis* did not germinate but the induced seed germinated and was used for the phytoextraction study. All the Figures showed that tagged *Amaranthusveriditis* are good hyper-accumulator for all the metals. These simply implies that inducing the seeds of *Amaranthusveriditis* with silver nitrate at the concentration used has helped to extract more of this metals from the polluted soil sample. It could also be suggested that perhaps, if the concentration of the silver nitrate is increased it could increase the rate at which it will hyper-accumulate heavy metals. The plant concentration factor was however very high for iron (25.8) followed by chromium (12.3) and low for copper (1.5) as show in Table 1. This implies that *Amaranthusveriditis* is a good hyper-accumulator of iron especially when the seed is induced with silver nitrate solution.

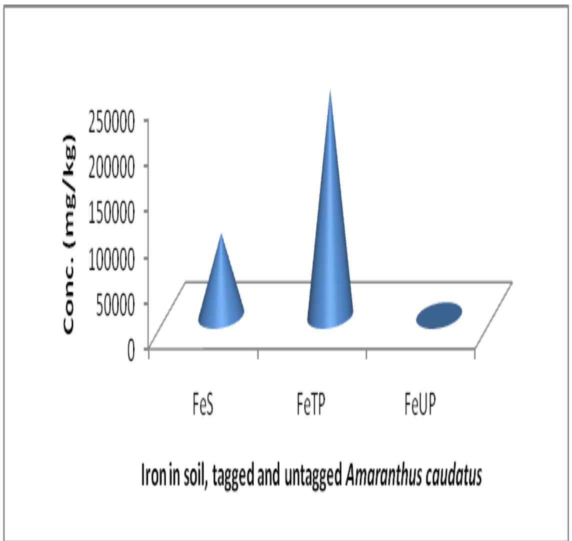

Fig 15: Level of iron in soil, tagged and untagged *Amaranthusveriditis*

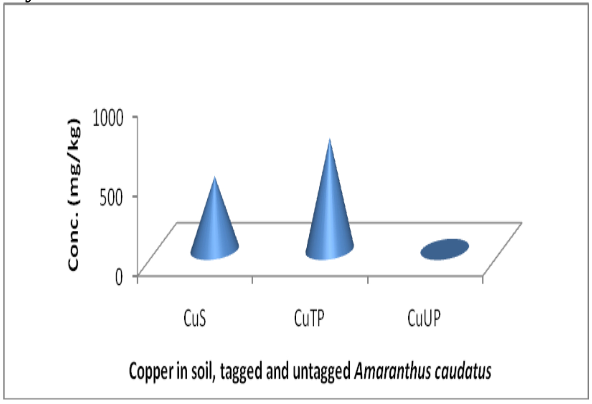

Fig 16: Level of copper in soil, tagged and untagged *Amaranthusveriditis*

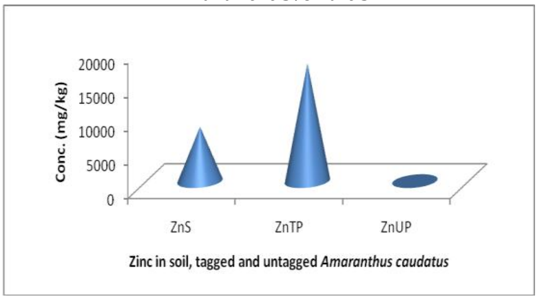

Fig 17: Level of zinc in soil, tagged and untagged *Amaranthusveriditis*

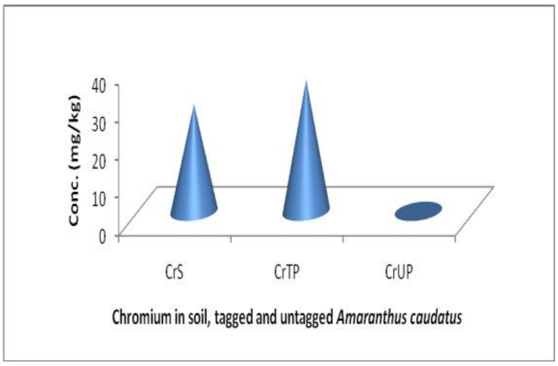

Fig 18: Level of chromium in soil, tagged and untagged *Amaranthusveriditis*

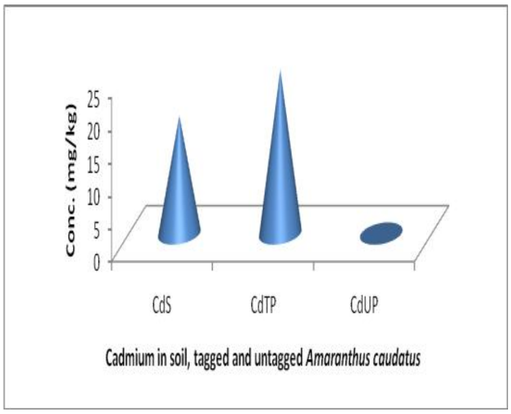

Fig 19: Level of cadmium in soil, tagged and untagged *Amaranthusveriditis*

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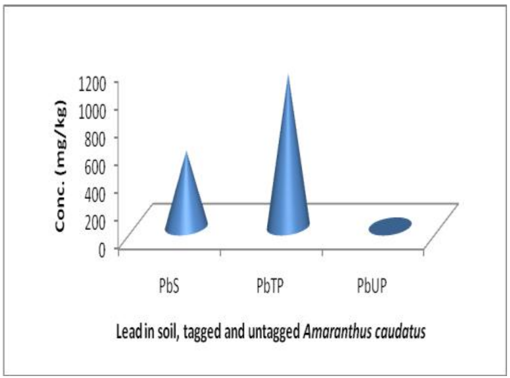

Fig 20: Level of lead in soil, tagged and untagged *Amaranthusveriditis*

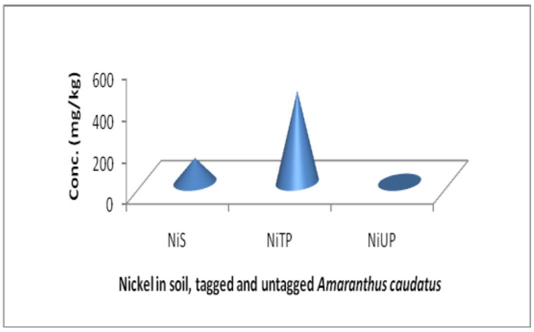

Fig 21: Level of nickel in soil, tagged and untagged *Amaranthusveriditis*

Table 2: Plant concentration factor of metals

Metal	*Zea mays*		*Abelmoschusesculentus*		*Amaranthusveriditis*	
	Tagged	Untagged	Tagged	Untagged	Tagged	Untagged
Fe	0.18	0.35	-	0.14	25.8	-
Cu	0.31	0.55	-	0.23	1.53	-
Zn	0.27	0.59	-	0.32	2.16	-
Cr	1.25	1.86	-	1.93	12.3	-
Cd	1.40	0.69	-	0.56	1.39	-
Pb	1.14	0.64	-	0.38	2.02	-
Ni	0.61	0.64	-	0.47	3.76	-

The Table revealed that *Zea mays* was a good hyper accumulator of chromium (1.86) when untagged and a good hyper accumulator of cadmium and lead when the seed was induced with silver nitrate solution. This is biotechnology which should further be investigated. The normal routine in literature is to induce the soil to be used with a chelator or an agent that can increase the rate of accumulation of metals in plant. The use of this silver nitrate in seeds is a new technology that helped to reduce contamination of soil with heavy metals. This method is cheap, easy and safe. The accumulation factor is as shown in Table 2. The Table shows that nickel had the highest accumulation factor (47) and then lead (21). This result agrees with the findings of Bergmann, 1992; Madejon*et al*., 2002; Gune*et al*., 2004 who estimated the critical level of nickel in soil to be in the range of 2-50 µg/g, the present study shows mean Ni to be 117 mg/kg which is alarming suggesting that Ni pollution is critical within the premises of investigated area. Olayiwola (2013) investigated the level of heavy metals outside the premises of this steel rolling mill. The result showed that the outside premises were polluted with nickel. This study revealed that tagged *Amaranthusveriditis*seems to be the best plant species to reduce the contamination of nickel in this study. Tagged *Zea mays* and tagged *Amaranthusveriditis* could also be used to decontaminate lead within this factory premise as shown in Table 2. The control site is not as polluted as the sampling area. The accumulation factor for lead might be as a result of exhaust from big trucks loading within the factory premises and the fact that it is located beside a major highway.

Table 3: Accumulation factor

Metal	Concentration at Sampling site (mg/kg)	Concentration at control site (mg/kg)	Factor of Accumulation
Fe	90,600	19800	5
Cu	463	29.9	16
Zn	8100	6500	1
Cr	28.5	20	1
Cd	18.0	ND	0
Pb	545	25.5	21
Ni	117	2.5	47

Pearson correlation using Cronbachs Alph at 0.078867 reveals a positive correlation between pairs of metal for the plants used as shown in Table 4.The positive correlation among pairs shows that they are from the same source.FeTC/PbUO and FeTC/PbUO had the highest correlation among the pairs (+0.94).

Table 4: Pearson correlation matrix

Metal pair	r value	Metal pair	r value
FeTC/PbUO	+0.94	FeUC/NiUC	+0.91
CuUC//CuUO	+0.82	CrTC/NiTV	+0.91
FeUC/ZnTC	+0.87	CrTV/CdTC	+0.78
PbTV/CdUC	+0.92	FeTC/PbTC	+0.75
FeUC/FeUO	+0.91	FeTC/PbUO	+0.94
ZnTC/NiUO	+0.93	PbTC/ZnTV	+0.81
CdTV/CrTV	+0.77	FeTV/CrUO	+0.82

T= tagged, U= untagged, C = corn (*Zea mays*), O= okro (*Abelmanthusesculentus*), V= vegetable (*Amaranthusveriditis*)

A two-sample test using Mann Whitney U test at $p<0.05$ revealed statistical significant differences between means of heavy metals in the steel rolling mill and control site as shown in Table 5.

Table 5: Two-sample test between metals in steel rolling mill and control site ($p<0.05$).

Metal	p-value	Level of significance
Fe	0.030	Significant
Cu	0.016	Significant
Zn	0.029	Significant
Cr	0.012	Significant
Cd	0.027	Significant
Pb	0.030	Significant
Ni	0.033	Significant

IV. RESEARCH IMPLICATIONS

The data generated from this study can be used as a guideline for future studies on phyto-extraction of polluted soil. Lower concentrations of silver nitrate could also be used to see if there will be improvement of the level of heavy metals extracted.

V. CONCLUSION

The findings in this study show that the tagged and untagged transgenic plants are good extractor of heavy metals from contaminated soil. The tagged seedlings of *Zea mays* showed that the method will be good to extract cadmium and lead from polluted soil compared to the untagged seedlings of *Zea mays*. The tagged seedlings of *Abelmoschusesculentus* did not germinate meaning that the concentration of silver nitrate used to induce the seedlings might be too high. A lower concentration could be used in further research work. The tagged seedlings of *Amaranthusveriditis* revealed the method to be effective as shown from the plant concentration factor and in comparism with the plant concentration factors obtained for *Zea mays*. This is the first time that seedlings of transgenic plants will be induced with silver nitrate in literature. The research shows that inducing seedling of these varieties of transgenic plants with this silver nitrate solution can help decontaminate polluted soil from industrial site and it is eco-friendly because silver nitrate does not have a deleterious effect on humans as the studied heavy metals. The method is also fairly inexpensive compared to alternatives. The mechanisms behind this hyper accumulation& detoxification could be due to change in genetics of the tagged seedlings or it may be due to its larger biomass apart from the stronger metal uptake ability. Since higher amount of some of this metals are extracted into the plant species, this metals can also be obtained from this plant and recycled. The whole study shows that tagged *Zea mays* and tagged *Amaranthusveriditis* have better impact and potential to remediate heavy metal polluted soils and are thus good phytoremediator and hyper accumulator.

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