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DETOXIFICATION OF TOXIC HEAVY METALS IN CONTAMINATED SOIL USING SELECTED HYPERACCUMULATOR PLANTS

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ABSTRACT

Phytoremediation is a method of bioremediation process which involves the application of biological processes for the removal of inorganic and organic pollutants from the contaminated environment. This technology has been getting attention due to of its low cost, efficient and eco-friendly nature. Phytoremediation is a promising approach for removal of contaminants from the environment by the use of hyperaccumulator plants. The basis of this technology is the natural ability of a green plant to accumulate chemical elements and transport them from the substrate to above the ground parts. Heavy metals like cadmium (Cd), copper (Cu) and zinc (Zn) when present in high concentrations in soil exert potential toxic effects on growth and metabolism of plants. Bioaccumulation of toxic metals in the plant poses a risk to human and animal health. In the present study a model field experiment was conducted using *Talinum triangulare, Sansevieria roxburghiana and Plectranthus amboinicus* plant species for their ability to absorb the heavy metals such as cadmium, copper and zinc from the contaminated soil. The metal uptake of Cd (7.61 mg/kg) and Cu (6.96 mg/kg) is observed in experimental field (F – I) and Cd (5.06 mg/kg) and Cu (3.95 mg/kg) in experimental field (F – II) by Water leaf (*Talinum triangulare)*. Similarly, the metal uptake of Cu (7.3 mg/kg), Cd (6.54 mg/kg) and Cu (3.60 mg/kg), Zn (3.05 mg/kg) by *Sansevieria roxburghiana* and Cd (5.44 mg/kg) and Zn (3.86 mg/kg), Cd (3.35 mg/kg was noticed by Plectranthus *amboinicus* species in heavy metal contaminated soils. The results clearly suggested that, these plants can be used as potential bioindiators and metal scavengers for the removal of toxic heavy metals in contaminated soil.

Key words: Green plants, hyperaccumulation, phytoremediation, detoxification, heavy metals.

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INTRODUCTION

Heavy metal contamination of soil is one of the most important environmental problems throughout the world (Doumett *et al.*, 2008; Nouri *et al.*, 2006). Heavy metals are the primary inorganic contaminants introduced into the environment by urbanization, industrialization, various agricultural practices, anthropogenic sources and other man-made activities. It has caused extensive environmental pollution (Arti Hansda *et al*., 2014). Heavy metals are readily accumulated in ecosystem and transferred into food web (Rajkumar et al, 2010). Heavy metals cannot be degraded to harmless products and persists indefinitely in the environment complicating their remediation (Lasat, 2002). The ability of heavy metals to accumulate and cause toxicity in biological systems - humans, animals, microorganisms and plants has been reported (D'amore *et al*., 2005).

Phytoremediation is a method of bioremediation process which involves the application of biological

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processes for removal of inorganic and organic pollutants from the contaminated environment. It is a cost effective environmental friendly, aesthetically pleasing approach most suitable for developing countries (Alkorta and Garbisu, 2001; Ghosh and Singh, 2005; Subhashini *et al.*, 2017). It is convenient solution for remediation of heavy metal contaminated soil in comparison with physico-chemical remediation technologies which are too costly and harmful for soil characteristics (Quartacci *et al.*, 2006). Most of the conventional remedial technologies are expensive and inhibit the soil fertility; this subsequently causes negative impacts on the ecosystem (Pulford and Watson, 2003; Stephen J. Coupe *et al.*, 2013).

Phytoremediation is an emerging technology that involves the use of hyperaccumulator plants to remove pollutants from the environment or to reduce their toxicity (Panwar *et al.*, 2002; Clemente, 2005). Ideally, the crop specie producing high biomass can be used to remediate a heavy metal-contaminated soil. Such species can both tolerate and accumulate the contaminants of interest in soil (Ebbs and Kochian, 1997). Research has demonstrated that plants are effective in cleaning up contaminated soil (Chehregani *et al.*, 2009).

The effectiveness of phytoremediation depends on many factors, particularly on the bioavailability of pollutants to the roots of the plants and the processes of absorption, translocation and accumulation of contaminants by plants (Anna Grobelak and Anna Napora, 2015). The transformation of pollutants in the soil is a dynamic process and that the bioavailability of metals varies with time and their solubility in soil (Calace *et al.*, 2006).

Phytoremedation can also become an income generating technology by extracting some useful metals from the plants which are used to remove the metals from the soil particularly known as the phytomining (Brooks *et al.*, 1998; Angle *et al.*, 2001). Particularly, phytoremediation process have been studied for cleaning up heavy metals such as cadmium, chromium, copper, mercury, Ni, lead and zinc from contaminated soil (Ndimele, 2010).

The main objective of this study is the investigation of detoxification process of metal pollutants such as Cd, Pb and Zn conducted soil supported with the application of effluent from the metal plating industry and with the use of hyperaccumulator plant species such as *Talinum triangulare, Sansevieria roxburghiana and Plectranthus amboinicus*.

Materials and Methods

Selection of plants

The following plant species are selected for the detoxification of heavy metal contaminated soil i.e., *Talinum triangulare, Sansevieria roxburghiana and Plectranthus amboinicus*.

Talinum triangulare

Water leaf (*Talinum triangulare)* is an herbaceous annual and perennial plant with a broad, worldwide distribution. Water leaf (*Talinum triangulare*) is a significant staple leafy vegetable grown in Africa in general and Cameroon in particular (Ndaeyo *et al.*, 2013). Water leaf cultivation improves nutritional requirement for the family and also provides additional complementary source of income for the farmers and contributes to the urban greening and environmental protection (Satterthwaite *et al.*, 2010; Predotova *et al.*, 2010). Tata *et al.*, (2016) reported that, it is playing a major role in efforts to eradicate malnutrition in Africa. The role of *Talinum* farming is often responded by declining soil fertility and its use is likely to enhance the yield of leafy vegetables (Billa Samuel Fru *et al.*, 2017).

Fig. 1. *Talinum triangulare*

Sansevieria roxburghiana

Sansevieria roxburghiana in the family of Agavaceae (Asparagaceae) is a stem less evergreen perennial plant. It is commonly known as “Indian bowstring-hemp”. It is producing succulent, erect, rigid leaves with 45-75cm or more long and 25mm wide from a rhizomatous rootstock (Obydulla, 2016). Traditionally it is used as a cardiotonic, expectorant, febrifuge, purgative, tonic in glandular enlargement and rheumatism (Pulliah, 2006). It has many medicinal and pharmacological properties. The juice of tender shoots is used for clearing viscid phlegm from throats in affected children. The roots are used as a febrifuge in snake bite and hemorrhoids (Roy *et al.*, 2013).

Fig. 2. *Sansevieria roxburghiana*

Plectranthus amboinicus

Plectranthus amboinicus is a perennial herb belonging to the family Lamiaceae. It has fleshy and highly aromatic, much branched, possessing short soft erect hairs, with distinctive smelling leaves. Leaves are undivided (simple), broad, egg/oval-shaped with a tapering tip (ovate) and very thick (*Nirmala Devi and Periyanayagam,* 2008). It is a medicinal plant used to treat malarial fever, hepatopathy, renal and vesical calculi, chronic asthma, hiccough, bronchitis, colic convulsions and epilepsy (Preeja G. Pillai *et al.,* 2011). It is identified as a good traditional medicine due to their wider pharmacological activities. The herb has therapeutic and nutritional properties. The plant leaves are often eaten raw and also incorporated as ingredients in the preparation of traditional food (Greetha Arumugam *et al.,* 2016).

Fig. 3. *Plectranthus amboinicus*

Field experiments

The plant growth was conducted in model experimental fields measuring 12 x 12 Sq.ft areas for 120 days under ambient greenhouse experimental conditions. The temperature is of 28°C during the day and 20°C during the night at 75% relative humidity. During the plant growth, only ground water was applied for irrigation for every two days. In the model experimental plots, field (F – I) is served with spiked metal ion concentration at the rate of 100 mg/L and the second field (F – II) is contaminated only with the addition of metal plating industry wastewater periodically. Initially, in all the fields, compost fertilizer is added to maintain the soil fertility. After that period biomass such as roots, stems and leaves were collected rinsed under running tab water. The plant biomass samples were dried at 70°C for 48 h and then were ground and stored for further analysis.

Chemical analysis of soil samples and biomass

The soil samples in control site and experimental fields were collected from the top of the soil (30 cm deep) separately. The samples were dried at 70^{o}C for 3 hrs and then crushed to fine powder and stored in airtight polythene containers for further analysis. The soil pH and EC values are determined using pH meter and conductivity meters. The pH and EC values were determined by immersing the electrode in the soil paste with a soil: water ratio of 1:2.5 (Black *et al.,* 1982). Organic carbon content was measured by the rapid titration method (Nelson and Sommers, 1986). The cation exchange capacity (CEC) of the soil was determined by the method of Thomes (1982). Total nitrogen content in the soil samples was determined using Kjeldahl method (Nelson and Sommers, 1980; Jones *et al.,* 1991). The heavy metal concentrations in soil samples were determined by digesting 0.5 g soil in a mixture of concentrated HNO_3 / $HClO_4$ (10: 7, v: v) (McGrath and Cunliffe, 1985).

The biomass samples such as roots, stems and leaves parts in each plant species were collected separately and dried at 70 ^{o}C for 2hrs. The samples were powdered and 2g of plant samples was digested with 3 a mixture of HCl / HNO_3 (4:1, v/v) and the heavy metal contents were determined using AAS by the method outlined by McGrath and Cunliffe, (1985).

Results and Discussion

Characteristics of the soil

The characteristics of the soil collected at control and experimental fields were determined at time of the start experimental plantation. The soil analytical results were shown in the Table 1. The physical and chemical properties of the soil impact the form of the metal contaminant, its mobility and bioavailability. The pH of the soil is a very important parameter, which determines the solubility and mobility of many mineral compounds including heavy metals and nutrient elements (Karaca, 2004). The solubility of heavy metals is generally greater in the pH range of normal agriculture soils. Generally it is observed that, increasing pH value prevented the leaching of biogenic components. The experimental sites had a pH of 7.28 and 7.55 respectively. Bramryd (2013) and Kacprzak et al. (2014) reported that, application of sewage sludge is increases the pH vale of the soil.

The humic acids and the cation exchange capacity (sorption capacity) of soil have a protective function. In general, soils with high CEC can adsorb larger amounts of heavy metals than soils with low CEC (Singh *et al.,* 2001). These parameters are constituents of the soil and have direct relationship with heavy metals and influencing the solubility and migration of metallic elements in soil. It is noted that, in the experimental sited, humic acid level is 19.45 and 16.96 and CEC is 17.48 and 15.85respectively. The level of carbon content and nutrients (K and P) are also good in soil and contribute the growth of the plants.

Characteristics of metal plating industry wastewater

In the second experimental field metal plating industry wastewater was used to study the metal uptake capacity of the hyperaccumulator plants selected in this research study. The wastewater was collected from the factory combined treatment plant. Mainly, the analytical results of important wastewater quality parameters were presented in the Table 2.

After the periodical application of the metal plating industry wastewater in the experimental field (F – II), at the end of the irrigation period (120 days), pooled soil samples collected from the experimental field and tested for the available heavy metal content. It was observed that, Zn (3.56 mg/kg), Cd (5.25 mg/kg) and Cu (4.35 mg/kg) were present (Table 1). This result indicates that, the distribution or mobilization of heavy metals in the soil mainly influences the physical and chemical properties of the soil.

Characteristics of ground water

The physico-chemical analysis of ground water used for the growth of plant species both in control and experimental sites were analysed and presented in the Table 3.

The quality of the ground water used for the irrigation of the plants in the control and experimental fields are good and does not harm the soil fertility and pH.

Analysis of metal contents in plant biomass

The bioaccumulation of heavy metals in root zone, stem and leaf parts of the *Talinum triangulare* plant grown in control soil and in experimental fields (F – I) and (F – II) were presented in the Figure 4 and 5 respectively.

In Figure 6 and 7, the heavy metal bioaccumulation behaviour of *Sansevieria roxburghiana* plant grown in control and experimental fields were presented.

Similarly, the bioaccumulation of heavy metals in root zone, stem and leaf parts of the *Plectranthus amboinicus* plant grown in control and experimental fields were presented in the Figure 8 and 9.

In the experimental observation of detoxification of heavy metals present in contaminated soils by hyperaccumulator plants reveals that, each plant species has its own capacity in the bioaccumulation process of heavy metals into their metabolism. In this study it is noticed that, higher amount of heavy metals are accumulated in mainly root zone and in stem. The leaves part comparatively has fewer amounts. In the case of Water leaf (*Talinum triangulare)*, the metal uptake of Cd (7.61 mg/kg) and Cu (6.96 mg/kg) is observed in experimental field (F – I) and Cd (5.06 mg/kg) and Cu (3.95 mg/kg) in experimental field (F – II).

By observing the bioaccumulation capacity of *Sansevieria roxburghiana*, the results indicates that, the metal uptake of Cu (7.3 mg/kg), Cd (6.54 mg/kg) and Cu (3.60 mg/kg), Zn (3.05 mg/kg) has noticed in experimental field (F – I) and experimental field (F – II) respectively. Similarly, the results of metal uptake capacity of Plectranthus *amboinicus* is measured and it is observed that, Zn (6.4 mg/kg), Cd (5.44 mg/kg) and Zn (3.86 mg/kg), Cd (3.35 mg/kg) were removed from the experimental field (F – I) and experimental field (F – II) respectively.

Table 1. Physical and chemical parameters of soil of the control and experimental fields.

Parameters	Units	Results (Average value ± SD)		
		Control Field	Exp. Field (F – I)	Exp. Field (F – II)
pH in H_2O		6.52 ± 0.50	7.28 ± 0.50	7.55 ± 0.50
Electrical Conductivity	µS/cm	1356 ± 2.50	1448 ± 2.50	1386 ± 2.25
Sodium	mg/kg	135 ± 1.60	138 ± 2.10	142 ± 2.15
Potassium	mg/kg	46 ±	53 ± 0.5	50 ± 0.6
Humidity	%	16.46 ± 1.30	19.45 ± 1.39	16.96 ± 1.22
CEC	Cmol / kg	12.45 ± 1.05	17.48 ± 1.00	15.85 ± 1.05
Humic acids	%	0.28 ± 0.02	0.42 ± 0.02	0.55 ± 0.02
C total	g / kg	19.80 ± 1.25	23.50 ± 1.10	26.30 ± 1.20
N Kjeldhal	mg / kg	485.00 ± 8.35	525.00 ± 2.55	538.00 ± 1.85
P available	mg / kg	32.35 ± 1.08	48.32 ± 1.02	47.98 ± 1.08
P total	mg / kg	86.44 ± 2.85	96.45 ± 1.82	99.34 ± 1.26
Zn	mg / kg	1.95 ± 0.02	6.65 ± 0.02*	3.56 ± 0.56**
Cd	mg / kg	1.20 ± 0.01	8.38 ± 0.50*	5.25 ± 0.65**
Cu	mg / kg	0.95 ± 0.01	7.26 ± 0.35*	4.35 ± 0.85**

Note: *Available spiked metal concentration, **Available metal concentration after the addition of plating industry wastewater in the test field.

Table 2. Physical and chemical analysis of metal plating industry wastewater.

Parameters	Units	Results
pH	--	6.85
EC	µS/cm	1265
Copper	mg/L	5.75
Zinc	mg/L	6.45
Cadmium	mg/L	7.35

Table 3. Physico-chemical analysis of ground water.

S.No.	Parameter	Unit	Results
1	pH Value at 25 ^{o}C	--	7.68
2	Turbidity	NTU	2.3
3	Electrical Conductivity at 25 ^{o}C	µS/cm	1150
4	Total Dissolved Solids	mg/L	755
5	Total Hardness as $CaCO_3$	mg/L	485
6	Calcium as Ca	mg/L	125
7	Magnesium as Mg	mg/L	110
8	Sodium as Na	mg/L	78
9	Potassium as K	mg/L	32
10	Total Iron as Fe	mg/L	0.2
11	Total Alkalinity as $CaCO_3$	mg/L	325
12	Chloride as Cl	mg/L	165
13	Sulfate as SO_4	mg/L	238
14	Copper (Cu)	mg/L	0.20
15	Zinc (Zn)	mg/L	0.06
16	Cadmium (Cd)	mg/L	BDL

Fig. 4. Bioaccumulation of metals in the plant parts of *Talinum triangulare*.

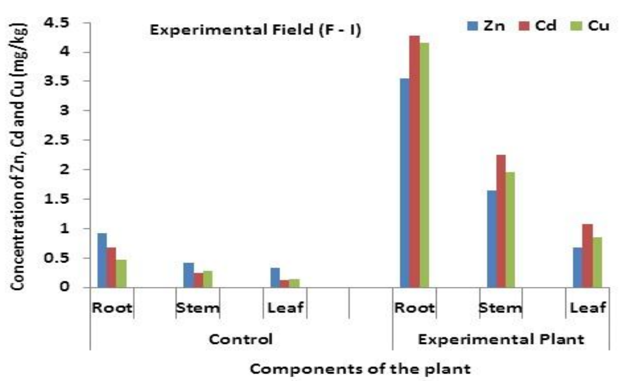


Fig. 5. Bioaccumulation of metals in the plant parts of *Talinum triangulare*.

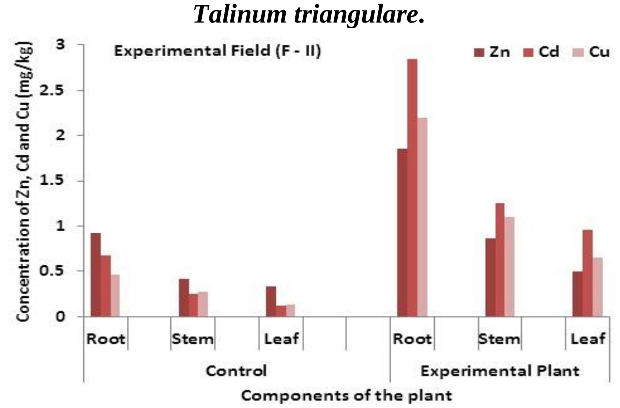


Fig. 6. Bioaccumulation of metals in the plant parts of *Sansevieria roxburghiana*

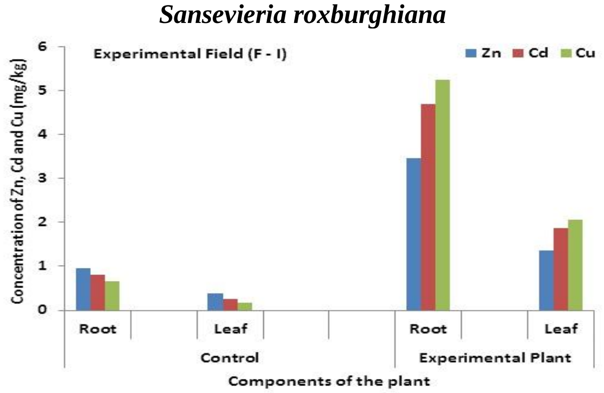


Fig. 7. Bioaccumulation of metals in the plant parts of *Sansevieria roxburghiana*

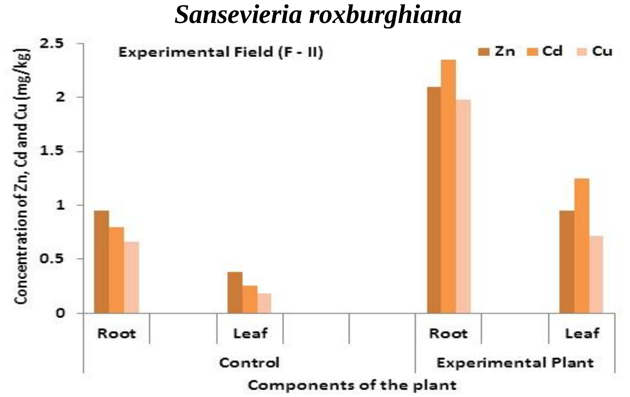


Fig. 8. Bioaccumulation of metals in the plant parts of *Plectranthus amboinicus*

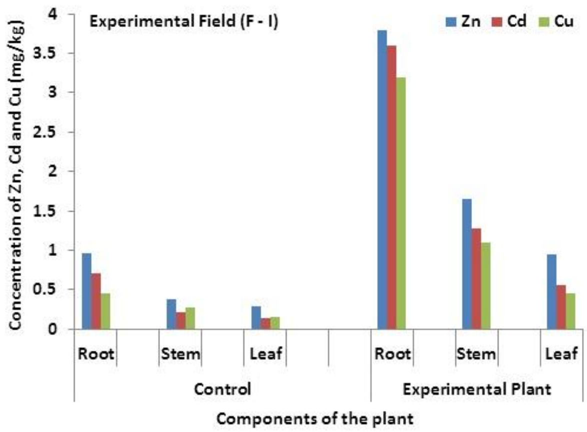
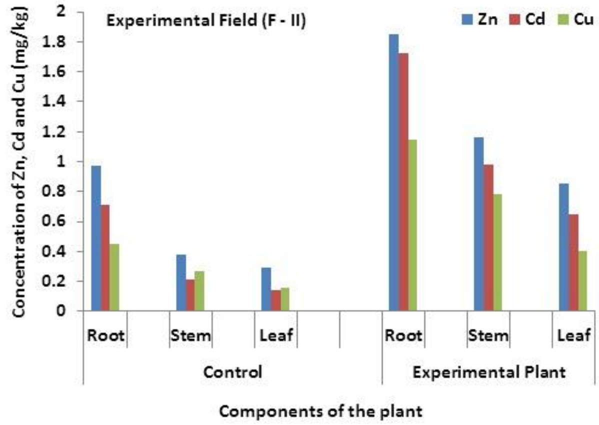


Fig. 9. Bioaccumulation of metals in the plant parts of *Plectranthus amboinicus*



CONCLUSION

Phytoremediation is a fast-emerging field in the removal of metal pollutants from the contaminated soil. The success of this technique is largely dependent on the continuous bioavailability of the metal of interest to the phytoextraction plants. Further research should focus on identifying remediating plants that are adapted to the local climate and soil conditions. Since, phytoremediation is a slow process; the knowledge of biotechnological advancement as well as classical hybridization techniques should be used to develop more efficient metal hyperaccumulator plant species for the remediation of contaminated soil environment. The results in this study, clearly suggested that, these plants can be used as potential bioindiators and metal scavengers for the removal of toxic heavy metals in contaminated soil.

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