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INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 7, Issue, 11, pp.22086-22092, November, 2015

ISSN: 0975-833X

RESEARCH ARTICLE

PHYTOREMEDIATION OF METALS CONTAMINATED SOILS BY *CATHARANTHUS ROSEUS* AND *RUELLIA TUBEROSA*

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ARTICLE INFO

Article History:
Received 19th August, 2015
Received in revised form
20th September, 2015
Accepted 02nd October, 2015
Published online 30th November, 2015

Key words:
Heavymetals,
Phytoremediation,
Catharanthus Roseus,
Ruellia Tuberose.

ABSTRACT

Heavy metal pollution causes potential ecological risk. Phytoremediation technology is cost effective and ecologically friendly in which plant utilizes its natural abilities to restore environment. Phytoremediation is a diverse collection of plant-based technologies that use either naturally occurring, or genetically engineered plants to clean contaminated environments. Plant selection is most important for determining the success or failure of the phytoremediation project. In the present study Catharanthus roseus and Ruellia tuberosa plant species was used for phytoremediation of Lead, Nickel, Zinc, Cadmium and Chromium contaminated soils. C. roseus was a shrub species belongs to Apocynaceae family and R. tuberosa was an herb species belongs to Acanthaceae family. The experiments were conducted using known metal solutions. The aqueous solutions were administered for 60 days to the experimental plants. The metal total accumulation in the plant (leaf, stem and root) was calculated. Bioconcentration factor (BCF) and Translocation factor (TF) was also calculated. Based on the results C. roseus highly accumulated all the five metals. R. tuberosa highly accumulated Pb, Zn and Cr. C. roseus species was recommended for phytoremediation of Pb, Ni, Zn, Cd and Cr contaminated soils. R. tuberosa was recommended for phytoremediation of Pb, Zn and Cr contaminated soils. Finally it was concluded that these two species was good accumulators of metals.

Citation: Subhashini, V. and Swamy, A.V. V. S. "Phytoremediation of metals contaminated soils by catharanthus roseus and ruellia tuberosa", *International Journal of Current Research,* 7, (11), 22086-22092.

INTRODUCTION

All components of the biosphere are facing threats of pollution by a variety of organic / inorganic pollution because of manmade activities that alter the normal biogeochemical cycle (Prasad and Freitas 2003). Heavy metal pollution has become one of the most serious environmental problems today (Annenkov 1982). Heavy metals make a significant contribution to environment as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and melting operations (Welch 1995; Samarghandi *et al*. 2007). Soil and water contaminated with metals pose a major environmental and human health problem that is still in need of an effective and affordable technological solution. Phytoremediation takes advantage of the fact that a living plant can be considered a solar-driven pump, which can extract and concentrate particular elements from the environment. Phytoremediation is becoming possible because of the productive interdisciplinary cooperation of plant biochemists, molecular biologists, soil chemists, agronomists, environmental engineers, and federal and state regulators.

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The metals targeted for phytoremediation include Pb, Cd, Cr, As and various radionuclides. The harvested plant tissue, rich in accumulated contaminant, is easily and safely processed by drying, ashing or composting. Some metals can be reclaimed from the ash, which further reduces the generation of hazardous waste and generates recycling revenues (Raskin *et al*. 1997).

Review of Literature

Current cleaning technology like physical removal of polluted soil from a site and dumping at somewhere else is too costly and destructive to environment (Meagher 2000). Nowadays, an emerging promising approach is Phytoremediation Technology. It is an in innovative field of science and technology for cleaning up contaminated soil, water and air (Salt *et al*. 1998; Blaylock and Huang 2000 and Blaylock *et al*. 1997). Maize and Indian mustard have been successfully used to remove Pb from solution culture and contaminated soil, respectively (Brooks *et al*. 1998). In order to make this technology feasible, the plants must extract large concentrations of heavy metals into their roots, translocation the heavy metals to surface biomass, and produce a large quantity of plant biomass. The removed heavy metal can be recycled from the contaminated plant biomass (Chunilall *et al*. 2005).

More than 400 plant species belonging to 45 plant families have been identified and reported from temperate to tropical regions with the ability to tolerate and hyper accumulate heavy metals. These plants have been considered suitable for soil stabilization and extraction of heavy metals (Del Río-Celestino *et al*. 2006). Natural sensitivity or tolerance of plants to accumulate metals is substantially affected by plant species and genotypes. Based on these plants can be divided into three groups.

- Excluders - there are plants insensitive for uptake and accumulation of potentially toxic elements. Mainly monocotyledon grasses belong into this group (Sudan grass, fescue).
- Indicators - majority of agricultural plants whose content of elements more or less linearly responding to increasing available content of trace elements in soil (wheat, oats and maize).
- Accumulators - plants accumulating higher contents of elements in their tissues according to their increase in the soil. Accumulators include many species of Brassicaceae (mustard), and Compositae (lettuce, spinach) families.

Letachowicz *et al*. (2006) conducted a study on the phytoremediation capacity on heavy metals accumulation in different organs of *Typhia latifolia* L. The concentrations of Cd, Pb, Cu, Ni, Mn, Zn, and Fe were determined in different organs of *Typhia latifolia* from seven water bodies in the Nysa region in Poland. The *Typhia latifolia* species that can absorb heavy metals can be used as bio-indicator of pollutants is a macrohydrophyte and is widely present in the entire lowland and lower mountain sites.

Phytoremediation is an environmentally sound technology for pollution prevention, control and remediation. Depending on the applicability and type of contaminant, phytoremediation can be broadly categorized as:

- **Phytoextraction:** use plants to absorb, translocate and store toxic contaminants from a soil matrix into their root and shoot tissue.
- **Phytomining:** Phytomining is the planting of vegetation that will selectively concentrate specific metals
- from the soil into their tissues.
- **Rhizofiltration**: use of roots to uptake also store contaminants from an aqueous growth matrix.
- **Phytostabilisation**: plant-mediated immobilization or binding of contaminants into the soil matrix there
- by reducing their bioavailability.
- **Phytodegradation**: use of plants to uptake, store and degrade contaminants within its tissue.
- **Phytostimulation or rhizodegradation**: use of rhizospheric associations between plants and symbiotic soil microbes to degrade contaminants.
- **Phytovolatilisation**: use of a plant's ability to uptake contaminants from the growth matrix and subsequently transform and volatilize contaminants into the atmosphere.

Cheng *et al*., (2012) cited heavy metals can be absorbed by plants using their roots, or via stems and leaves, and stored the metals into different plant parts.

Shete *et al*., (2011) revealed in their study entitled, "Bio-accumulation of Zn and Pb in *Avicennia marina* (Forsk.) from urban areas of Mumbai (Bombay), India," that the mangrove species can bioaccumulate and survive despite heavy metal contamination. There is evidence that plants can accumulate heavy metals in their tissues such as *Sebera acuminate* and *Thlaspi caerulescens* (Cunningham and Ow, 1996), *Arabidopsis thaliana* (Delhaize, 1996), *Typha latifolia,* and *Phragmites australis* (Ye *et al*., 2001).

MATERIALS AND METHODS

A brief description of the plants selected for the present study

Catharanthus roseus **(L.) G. Don**

Catharanthus roseus (Periwinkle) is a species of *Catharanthus* native to Madagascar its belongs to Apocynaceae family. Synonyms include *Vinca rosea* (the basionym), *Ammocallis rosea*, and *Lochnera rosea*; other English names occasionally used include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle, and "Old-maid". It is also widely cultivated and is naturalized in subtropical and tropical areas of the world. It is an evergreen sub-shrub or herbaceous plant. As an ornamental plant, it is appreciated for its hardiness in dry and nutritionally deficient conditions, popular in subtropical gardens. It is noted for its long flowering period, throughout the year in tropical conditions. Numerous cultivars have been selected, for variation in flower colour (white, mauve, peach, scarlet and reddish-orange), and also for tolerance of cooler growing conditions in temperate regions. (Gamble, 2008a).

***Ruellia tuberosa*, L.**

Ruellia is commonly known as ruellias or wild petunias. Ruellias are popular ornamental plants. *Ruellia tuberosa*, also known as Minnie Root, Fever Root, Snap dragon Root and Sheep Potato, is a species of flowering plant in the Acanthaceae family. It grows often as a weed even in ruderal habitats. Its names Popping Pod, Duppy Gun and Cracker Plant come from the fact that children like to play with the dry pods that pop when rubbed with spit or water. It is a small biennial plant with thick fusiform tuberous roots and striking funnel-shaped violet-colored flowers. It reaches an average height of about 25 cm in moist and shady environments. It has been used as a diuretic, anti-diabetic, antipyretic, analgesic, antihypertensive, gastroprotective, and to treat gonorrhea. It is also used as a natural dye for textiles. (Gamble, 2008b).

RESULTS AND DISCUSSION

ACCUMULATION OF HEAVY METALS IN *CATHARANTHUS ROSEUS*

Accumulation of Lead (mg/kg) in *Catharanthus roseus*

Catharanthu roseus absorbed Lead through root system in high quantities by 20th day itself and the lead was translocated in a slow manner throughout the remaining period of experimentation. As a result by the 60th day only 50% of the lead was absorbed and translocated to stem and leaves. There was lowest accumulation in leaves (0.92 mg/kg) and highest accumulation was recorded in roots (67.33 mg/kg) with a total accumulation of lead (77.05 mg/kg) in the whole plant. The results showed that *Catharanthus roseus* was good accumulator of lead.

Accumulation of Nickel (mg/kg) in *Catharanthus roseus*

Nickel accumulation in *Catharanthus roseus* was initially higher in stem followed by roots. The Nickel accumulation was consistent in leaves from the beginning of the experiment. However, the 60th day the accumulation in roots increased by manifold and reached highest followed by stem and leaves in that order (Table 2). Out of the 47.75mg/kg of Nickel accumulated in plant 20.63 mg/kg was retained in the roots, 16.63 mg/kg of Nickel was accumulated in stem and 10.49 mg/kg in leaves. Two thirds of the Nickel remained in roots and stem. The results revealed that *Catharanthus roseus* is a good accumulator of Nickel.

Accumulation of Zinc (mg/kg) in *Catharanthus roseus*

The initial concentration of Zinc was maximum in stem compared to roots and leaves. The increase of accumulation in roots and stem was consistent throughout the experimental period. However, from 40-60 day interval, the concentration of Zinc increased to 88.44mg/kg (from 46.99mg/kg) in leaves. The accumulation in stem also increased from 81.66 to 94.68mg/kg in stem. On the whole the leaves recorded highest accumulation of zinc followed by roots and stem (Table 3). The highest accumulation of zinc in leaves leaving low quantities of zinc in stem and roots reveal that maximum quantity of Zinc is translocated up to leaves. Out of the total 67.60 mg/Kg of Zinc absorbed 9.93 mg/kg was translocated to root and 9.85 mg/kg to stem and 47.82mg/kg to leaves. The results revealed that *Catharanthus roseus* is a good accumulator of Zinc.

Table 1. Accumulation of Lead (mg/kg biomass) in different plant parts of *Catharanthus roseus* during the Experimental period

Plant part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	24.03±0.41	24.53±0.15	24.5±0.15	24.95±0.08	0.92
Stem	60.69±0.16	67.31±0.18	68.09±0.08	69.49±0.17	8.79
Root	21.47±0.17	84.32±0.15	88.74±0.07	88.81±0.16	67.33
Total Accumulation	106.19	176.16	181.33	183.25	77.05

Table 2.Accumulation of Nickel (mg/kg biomass) in different plant parts of Catharanthus roseus during the experimental period

Plant part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	2.09±0.18	3.87±0.15	6.18±0.17	12.58±0.08	10.49
Stem	5.39±0.49	9±0.17	9.21±0.08	22.02±0.17	16.63
Root	4.65±0.16	5.94±0.14	7.41±0.07	25.28±0.18	20.63
Total Accumulation	12.12	18.82	22.8	59.87	47.75

Table 3. Accumulation of Zinc (mg/kg biomass) in different plant parts of Catharanthus roseus during the experimental period.

Plant Part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	40.62±0.18	46.03±0.15	46.99±0.18	88.44±0.09	47.82
Stem	84.83±0.16	79.53±0.17	81.66±0.08	94.68±0.17	9.85
Root	78.05±0.15	87.79±0.14	87.86±0.07	87.98±0.16	9.93
Total Accumulation	203.5	213.35	216.51	271.1	67.60

Table 4. Accumulation of Cadmium (mg/kg) in different plant parts of *Catharanthus roseus* during the experimental period

Plant Part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	0.13±0.18	0.97±0.14	1.85±0.16	11.16±0.08	11.03
Stem	0.56±0.16	0.79±0.18	2.46±0.08	13.73±0.17	13.17
Root	0.84±0.15	3.37±0.19	3.81±0.06	4.09±0.18	3.25
Total Accumulation	1.53	5.13	8.12	28.98	27.45

Table 5. Accumulation of Chromium (mg/kg) in Catharanthus roseus during the experimental period

Plant part	Control	20th day	40th day	60th day	Total accumulation
Leaf	5.4±0.18	6.52±0.15	12.15±0.16	37.13±0.09	31.72
Stem	13.57±0.16	19.49±0.18	19.81±0.08	20.27±0.17	6.7
Root	6.07±0.15	36.73±0.14	36.57±0.07	36.96±0.16	30.89
Total accumulation	25.04	62.74	68.53	94.36	69.31

Table 6.Accumulation of Lead (mg/kg biomass) in different plant parts of Ruellia tuberosa during the experimental period

Plant part	Control	20th day	40th day	60th day	Total accumulation
Leaf	19.71±0.48	36.45±0.19	40.73±0.21	44.02±0.08	24.3
Stem	24.7±0.21	69.07±0.08	69.19±0.07	70.19±0.15	45.48
Root	34.56±0.11	59.94±0.16	60.21±0.13	60.31±0.16	25.75
Total Accumulation	78.98	165.46	170.13	174.52	96.54

Table 7. Accumulation of Nickel (mg/kg biomass) in different plant parts of Ruellia tuberosa during the experimental period

Plant Part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	1.86±0.08	2.27±0.18	19.09±0.22	20.67±0.08	18.82
Stem	3.17±0.21	3.3±0.08	7.53±0.08	13.67±0.16	10.49
Root	7.96±0.11	11.11±0.16	16.13±0.13	18.39±0.17	10.43
Total Accumulation	12.98	16.68	42.75	52.73	39.74

Accumulation of Cadmium (mg/kg) in *Catharanthus roseus*

The accumulation of Cadmium was highest in the stem (13.17 mg/kg) followed by leaves (11.03mg/kg) and lowest in roots (3.25 mg/kg). The overall rate of accumulation in leaves, stem and roots increased consistently up to 40th day and there was a sudden increase from 40-60th day (Table 4). The results revealed that as the plant continued to grow the absorption of Cadmium also increased but the Cadmium absorbed by roots was completely translocated to stem and leaves. Out of the total accumulated 27.45mg/kg of Cadmium roots retained only 3.25mg/kg and the remaining was translocated to stem (13.17 mg/kg) and leaves (11.03mg/kg).

ACCUMULATION OF HEAVY METALS IN *RUELLIA TUBEROSA*

Accumulation of Lead (mg/kg) in *Ruellia tuberose*

A total of 96.54 mg/kg of lead had accumulated in *Ruellia tuberose* by 60th day. The absorption was highest in all the plant parts during the first 20 days. The stem and roots accumulated marginal qualities from 20-60 days where as leaves have accumulated consistently higher quantities of lead during 20-40-60 days. The trend of accumulation of lead revealed that the lead concentrations reached saturation by 20th day itself and hence the increase of concentrations from 20-40 and 40-60 was less than 1 mg/kg of lead. After 20th day whatever the lead the plant has absorbed is tanslocated to leaves. The total accumulation was highest in stem followed by roots and leaves the difference of total accumulation between the roots and stem in meagre. Based on the results the species was good accumulator of lead.

Table 8. Accumulation of Zinc (mg/kg biomass) in different plant parts of Ruellia tuberosa during the experimental period

Plant Part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	26.45±0.08	30.55±0.19	31.16±0.23	32.77±0.08	6.32
Stem	37.61±0.21	44.07±0.08	44.1±0.08	44.86±0.16	7.25
Root	39.96±0.11	49.85±0.16	49.97±0.13	49.99±0.15	10.04
Total Accumulation	104.02	124.47	125.23	127.62	23.61

Table 9. Accumulation of Cadmium (mg/kg) in different plant parts of Ruellia tuberosa during the experimental period

Plant Part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	0.35±0.08	1.31±0.11	1.37±0.21	1.37±0.08	1.03
Stem	0.08±0.21	0.55±0.18	1.29±0.08	1.3±0.15	1.22
Root	0.3±0.11	3.04±0.16	4.97±0.13	5.88±0.15	5.58
Total Accumulation	0.73	4.9	7.63	8.55	7.83

Total 10. Accumulation of Chromium (mg/kg biomass) in different plant parts of Ruellia tuberosa during the experimental period

Plant part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	1.63±0.08	2.2±0.18	8.86±0.21	8.87±0.08	7.23
Stem	1.94±0.21	4.75±0.08	4.81±0.08	14.87±0.15	12.93
Root	4.98±0.11	12.27±0.16	18.95±0.13	19.74±0.19	14.76
Total accumulation	8.55	19.22	32.62	43.48	34.92

Table 10. Bio concentration factor (BCF) and Translocation factor (TF) of metals in the experimental plants

Name of the plant	Lead		Nickel		Zinc		Cadmium		Chromium	
	BCF	TF	BCF	TF	BCF	TF	BCF	TF	BCF	TF
Catharanthus roseus	8.13	0.15	5.66	1.31	5.17	5.8	74.99	7.43	5.1	1.24
Ruellia tuberosa	10.19	2.71	4.71	2.81	1.8	1.35	21.39	0.4	2.57	1.36

Accumulation of Chromium (mg/kg) in *Catharanthus roseus*

From the experiments conducted it is recorded that the roots have accumulated highest chromium content (6.7 to 36.73 mg/kg) and then the absorption of chromium was consistent throughout. The accumulation in stem was very less compared to leaves and roots. Chromium accumulation in leaves was doubled from 20th to 40th day and threefold for 40th day to 60th day finally resulting in highest accumulation of chromium in leaves followed by roots and stem. *Catharanthus roseus* is a good accumulator of Chromium.

Accumulation of Nickel (mg/kg) in *Ruellia tuberose*

The accumulation of nickel was highest in leaves while stem and roots have accumulated equal quantities of nickel. The accumulation of nickel in leaves was maximum between 20-40 days and from 40-60 days it was only marginal. However, the accumulation was consistent in stem and roots for 20-40 and

40-60 days. A total of 39.74 mg/kg of nickel was accumulated in all plant parts in 60 days out of which approximately 50% of nickel accumulated in leaves.

Accumulation of Zinc (mg/kg) in *Ruellia tuberosa*

Ruellia tuberosa accumulated a total of 23.61 mg/kg of zinc during the experimental period. Out of which roots accumulated 10.04, stems 7.25 and leaves have accumulated 6.32 mg/kg. The absorption of zinc was maximum in the first 20 days and the increase of concentration was marginal from 20-40 and 40-60 days (Table 8). The absorption by roots and translocation to the above ground parts showed almost negligible increase.

Accumulation of Cadmium (mg/kg) in R*uellia tuberosa*

The total accumulation of cadmium in all plant parts was only 7.83 mg/kg, of which 5.58 mg/kg remained in the root system. Very less concentrations of cadmium was observed to be translocated (1.03 mg/kg in leaves and 1.22 mg/kg in stem) the increase of cadmium concentrations at different intervals of experimental period was higher during first 20 days. The leaves and stem together accumulated less than 30% of the cadmium that is accumulated in all the 60^{th} days.

Accumulation of Chromium (mg/kg) in *Ruellia tuberosa*

The total accumulation of chromium in all the plant parts was 34.92 mg/kg. Leaves have accumulated lowest chromium compared to stem and roots. The absorption of chromium by roots reached maximum by 40^{th} day and from 40-60 days the absorption was negligible. The similar trend of absorption was exhibited by leaves. Where as in the stem the accumulation suddenly increased by twofold from 40-60 days.

***Catharanthus roseus*:** *Catharanthus roseus* showed a differential tendency of accumulation of different metals. The leaves have accumulated lowest quantities of lead and nickel while zinc accumulated in highest quantities (47.82mg/kg) in leaves. The leaves showed a vide variation in effinity of accumulation i.e. from 0.92 mg/kg of lead to 47.82 mg/kg of zinc. The total accumulation of metals showed a moderate range, the lowest being cadmium (27.44 mg/kg) followed by nickel (47.75 mg/kg), zinc(67.61 mg/kg), chromium (69.31 mg/kg) and lead(77.05 mg/kg) in that ascending order. Among the three plant parts roots showed highest accumulation of lead and nickel, while stem accumulated highest quantities of cadmium and no other metal accumulated in highest quantity in stem compared with leaves and roots. Lead showed a very slow increase in leaves and stem from 20-40 and 40-60 while the roots showed a faster accumulation of lead during the first 20 days and then on the accumulation slowed down. Also it was evident most of the lead absorbed remained in the roots itself and the translocation was very poor. The strong root system and considerable biomass of the root system are favouring the accumulation of lead.

***Ruellia tuberosa*:** *Ruellia tuberosa* exhibited a tendency of differential absorption of different metals in its parts. Chromium, cadmium and zinc recorded a highest accumulation in roots while accumulation of nickel in stem leaves in highest quantities. The difference between control and total accumulation was more than 10 times higher for cadmium. Though concentration wise it is 7.83mg/kg and also lowest total accumulations, maximum accumulation was that of cadmium. The cadmium was not effectively translocated to stem and leaves. The highest total accumulation was exhibited by lead (96.54 mg/kg) followed by nickel (39.75 mg/kg), chromium (34.92 mg/kg), zinc (23.65 mg/kg) and cadmium (7.83 mg/kg) in that order. A very well developed root system with considerable proportion of biomass might be the reason for maximum accumulation of three out of five metals in root system.

The plants that absorb or accumulate high quantities are categorized as good accumulators, moderate accumulators and poor accumulators based on the total accumulation in the plant body. Plants exhibit different affinity to different metals. A particular plant cannot act as good accumulator for all the metals hence the plant which acts as good accumulator for a particular metal is recommended for use as phytoextractor of phytostabilizer for that metal. The plants selected for the present study are categorized as good accumulator, moderate accumulator and poor accumulator based on the total accumulation of metals in the plant body.

The metal concentration, transfer and accumulation of metals from soil to roots and shoots was evaluated in terms of Biological Concentration factor (BCF) or Bioconcentration Factor (BCF) and Translocation Factor (TF). The Bioconcentration factor (BCF) of metals was used to determine the quantity of heavy metals absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil. Translocation factor (TF) was described as ratio of heavy metals in plant shoot to that in the plant root. TF was calculated to evaluate the potential of the species for phytoextraction or phytostabilzation.

TF is an indication of the ability of the plant to translocate metals from the roots to the aerial parts. The TF value will be higher for those plants which retain the metal in roots without translocating to aerial parts. The TF value increases with increasing ability of the plant to translocate metals to stem and leaves. Thus the plants showing high BCF and TF values (greater than one) are suitable for phytoextraction. While the plants showing TF value less than one can be used for phytostabilization. Phytoextraction ensure elimination of the metal from the soil, as the plant absorbs the metals and store them in the roots, stem and leaves. Phytostabilisation ensures adsorbtion metals on roots, concentration or agglomiration or precipitaion of metals in rhyzosphere. The plant which fails to translocate the metals can be used for phytostabilization. In the present study the plants have shown varied BCF and TF for each metal. *Catharanthus roseus* was good accumulator of lead, nickel, zinc and chromium and moderate accumulator of cadmium. *Ruellia tuberosa* was good accumulator of lead and moderate accumulator of nickel, zinc and chromium.

Conclusions

The total accumulation of metals in selected species revealed the potential of plants to accumulate heavy metals and accordingly, the selected species were categorized as good accumulators, moderate and poor accumulators. All good accumulator plants are recommended for phytoextraction of metals from contaminated soils. *Catharanthus roseus* was good accumulator of lead, nickel, zinc and chromium. The species can be recommended for the Phytoextraction of lead, nickel, zinc and chromium contaminated soils. *Ruellia tuberosa* was good accumulator of lead. The species can be recommended for the remediation of lead contaminated soils.

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