

Pot Experiment: To Study the Uptake of Zinc by Different Plant Species in Artificially Contaminated Soil

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Abstract A novel, cost-effective and eco-friendly technologies are needed to remove Zinc from the contaminated soil environment. The present research study was designed to assess the naturally enhanced phyto extraction and phyto stabilization potential of different plant species from the Zinc (II) contaminated soil. Uptake of Zinc by plant species in a metal contaminated soil was studied in pot culture experiment. The pots were filed with 5 kg of garden soil. Weed plants were grown in pots and were irrigated with known heavy metal solutions in the concentration of 5ppm heavy metal Zinc solution was added to the pots alternate days up to 60 days. In controls normal water was used. The plants were grown for a period of 60 days. The initial soil heavy metal concentration was analyzed. After 20, 40 and 60 days soil heavy metal concentrations were also analyzed. Analysis of heavy metal was done in HNO₃/HClO₄ digested samples by Atomic Absorption spectrophotometer. The plant species had accumulated quantities of heavy metals per Kg of biomass. The plant species showed relatively good response to the higher level of heavy metal concentration in the roots, stem and leafs suggested that these plant species were good metal excluder with the possibility of extracting (Zn) from artificially contaminated soils.

Keywords: *pot culture experiment, contaminated soil, phyto remediation, zinc, plant species*

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1. Introduction

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand specificity, etc.) and atomic number >20. The most common heavy metal contaminants are: Cd, Cr, Cu, Hg, Pb, and Zn. Metals are natural components in soil. Contamination, however, has resulted from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition [1,2,3]. Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). Soils are the major sink for heavy metals released into the environment by aforementioned anthropogenic activities and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation and their total concentration in soils persists for a long time after their introduction [4]. Changes in their chemical forms (speciation) and bioavailability are, however, possible. The presence of toxic metals in soil

can severely inhibit the biodegradation of organic contaminants. Heavy metal contamination of soil may pose risks and hazards to humans and the ecosystem through: direct ingestion or contact with contaminated soil, the food chain (soil-plant-human or soil-plant-animal-human), drinking of contaminated ground water, reduction in food quality (safety and marketability) via phyto toxicity, reduction in land usability for agricultural production causing food insecurity, and land tenure problems [5]. The adequate protection and restoration of soil ecosystems contaminated by heavy metals require their characterization and remediation. Contemporary legislation respecting environmental protection and public health, at both national and international levels, are based on data that characterize chemical properties of environmental phenomena, especially those that reside in our food chain. While soil characterization would provide an insight into heavy metal speciation and bioavailability, attempt at remediation of heavy metal contaminated soils would entail knowledge of the source of contamination, basic chemistry, and environmental and associated health effects (risks) of these heavy metals. Risk assessment is an effective scientific tool which enables decision makers to manage sites so contaminated in a cost-effective manner while preserving public and ecosystem health [4]. Immobilization, soil washing, and Phyto remediation techniques are frequently listed among the best demonstrated available technologies (BDATs) for

remediation of heavy metal-contaminated sites [6]. In spite of their cost effectiveness and environment friendliness, field applications of these technologies have only been reported in developed countries. In most developing countries, these are yet to become commercially available technologies possibly due to the inadequate awareness of their inherent advantages and principles of operation. With greater awareness by the governments and the public of the implications of contaminated soils on human and animal health, there has been increasing interest amongst the scientific community in the development of technologies to remediate contaminated sites [7]. Phyto remediation of soil has attracted much attention in recent years due to its multiple advantages such as maintaining the biological activity and physical structure of soils, being potentially inexpensive and visually unobtrusive, and providing the possibility of bio recovery of metals. Identification of native species for Phyto remediation is a key to the success of the method. Keeping in view the heavy metals exposure to the soil environments, this study was planned under green house conditions with the objectives to evaluate the efficacy of

different plant species in the uptake and translocation of Zinc (Zn) from the artificially contaminated soils.

2. Materials and Methods

2.1. Study Aea and Selection of Pant Secies

In this study ten plant varieties were selected for their ability to absorb heavy metal (Zn) from the contaminated soil. The experimental plants were four weed species, three flowering plants, three grass varieties. The used plant species were 1. Weed species were; *Acalyphaindica*, *Abutilonindicum*, *Physalisminima* and *Cleome viscose*. 2. Flowering plants were; *Catharanthus roseus*, *Ruallia tuberosa*, and *Canna indica*. 3. Grass species were; *Perotis indica*, *Echinochloa colona* and *Cyperus rotundus*. The plant species are selected for the present study because of the qualities, such as large biomass production, perennial, robust rhizomes these are suitable for accumulation of heavy metals.



1. *Acalypha indica*



2. *Abutilon indicum*



3. *Physalis minima*



4. *Ceome viscose*



5. *Catharanthus roseus*



6. *Ruellia tuberosa*



7. *Canna indica*



8. *Perotis indica*



9. *Echinochloa colona*



10. *Cyperus rotundus*

2.2. Plant Sampling

Fresh plant samples were collected in the morning by pulling carefully from the soil to avoid damage to the roots. samples were collected from the surface to subsurface portion of the soil around the plant roots at a range interval of 20 to 30 square meters apart.

2.3. Washing of Plant Samples

The collected samples were washed with distilled water remove dust particles. The samples were then cut to separate the roots, stems and leaves. The different parts (roots, stems and leaves) were air dried and then placed in a dehydrator for 2-3 days and then dried in an oven at 100 °C. Dried samples of different parts of the plants were ground into a fine powder using pestle and mortar and stored in polyethylene bags, until used for acid digestion. Before the experiment soil parameters were done and for every 20 days the soil samples were also collected and analysed for heavy metals by Atomic Absorption Spectrophotometer (make: Schimarze 6800).

2.4. Preparation of Plant Samples by Wet Digestion

Samples (0.5g) of each part (leaves, stems and roots) of the plant were weighed in digestion flasks and treated with 5 ml of concentrated HNO₃. A blank sample was prepared applying 5ml of HNO₃ into empty digestion flask. The flasks were heated for 2 hours on an electric hot plate at 80-90°C at which the samples were made to boil and digestion continued until a clean solution was obtained. After cooling, the solution was filtered with what man No.42 filter paper. It was then transferred quantitatively to a-25ml volumetric flask by adding distilled water. The filtrate was analyzed for metal content using Atomic Absorption spectrophotometer (make: Schimarze 6800).

2.5. Preparation of Standards and Analysis of Samples

Standard solution Zinc (Zn) was prepared from the stock standard solution containing 1000 ppm of element

in 2N nitric acid. Calibration and measurements of elements were done on atomic absorption spectrophotometer (make: Schimarze 6800). The calibration curves were prepared for this element individually. A blank reading was also taken and necessary correction was made during the calculation of concentration of various elements.

2.6. Pot Culture Experiment

Uptake of heavy metals by plants in a metal contaminated and normal soil was studied in pot culture experiment. Weed plants, flowering plants and grass species were grown in pots and were irrigated with known heavy metal solutions in the concentration of 5ppm heavy metal solution Zinc (Zn) was added to the pots alternate days up to 60 days. In controls normal water was used. The plants were grown for a period of two months (60days). The contaminated soil received the metals Zn as Zn (NO₃)₂ 3H₂O. The initial soil heavy metal concentration was analyzed. After 20, 40 and 60 days soil heavy metal concentrations was analyzed. Every 20 days the plant samples from each pot were collected and washed thoroughly under running tap water and distilled water so that no soil particles remained. Analysis of heavy metals was done in HNO₃/HClO₄ digested samples [8] and analyzed by Atomic Absorption spectrophotometer (make: Schimarze 6800). The experiment was carried out in the garden by using heavy metal solutions. The plant sample was uniform in size and which were free of disease symptoms. The pots were filed with 5 kg of garden soil. The plants were watered once every day and care was taken to avoid leaching of water from the pots. After 20 days from each pot plant samples were collected. The plants were harvested after 20 days, 40 days and 60 days for heavy metal analysis.

2.7. Statistical Analysis

Average data uptake, transfer coefficient were submitted for statistical analysis. The uptake and mobilization of potentially toxic metals (PTM) in plant tissues were assessed by bioaccumulation coefficient (BC). Differences in heavy metal concentrations among different parts of the weeds were detected using One-way ANOVA, followed by multiple comparisons using Turkey tests. A significance level of ($p < 0.05$) was used throughout the study. All statistical analyses were performed using STATISTICA (Version 7), MSEXCEL (Microsoft office 2012) and GrphPad Prism (Version 5).

3. Results and Discussion

3.1. Physico-chemical Properties of Soil

The results obtained from the soil analysis were shown in Table 1. Soil particle size was determined using Bouyoucos hydrometer method. All reagents used were of analytical grade (BDH Laboratory supplies, Poole, England) The soil pH was determined in a mixture of soil and deionized water (1:2, w/v) with a glass electrode [9]. Total organic carbon content was determined using the Walkey-Black wet oxidation [10], Total Nitrogen was determined using the Kjeldhal method [11]. Cation

exchange capacity (CEC) and amounts of exchangeable Ca and Mg were determined using the ammonium acetate method [12]. Total phosphorus was determined calorimetrically. Total background cadmium concentration was determined using Atomic Absorption Spectrophotometer (make: Schimarze 6800). The electrical conductivity (EC) was measured by using digital meters (Elico, Model LI-120) with a combination of 1-cm platinum conductivity cell.

Table 1. Soil physico-chemical properties

S.No	Parameter	Result
1	pH	6.94
2	Organic matter	1.20
3	Total N (%)	0.85
4	Total (P) %	1.24
5	Sand (%)	18
6	Silt (%)	16
7	Clay (%)	42
8	CEC(mol/g soil)	10.99
9	EC(mS/cm)	455.00
10	Moisture content (%)	35.00
11	Zinc (Zn) mg/kg	13.085

CEC: Cation exchange capacity; EC: Electrical conductivity

The taxonomic classification of the experimental soil (Table 1) was sandy loam with pH of 6.94, EC of 455 mS/cm. The high pH level of the soil is generally within the range for soil in the region; soil pH plays an important role in the sorption of heavy metals, it controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates and also influences ion-pair formation, solubility of organic matter, as well as surface charge of Fe, Mn and Al-oxides, organic matter and clay edges [13].

3.2. Experimental Procedure

The experimental plants were intended to grow for 2 months (60 days) in the soil. The pots used for the experiment contained 5kg of soil. The seedlings of the experimental plants were collected from the uncontaminated soils. In each pot eight seedlings were planted. Grass species were also used for heavy metal accumulation. The plant species used for heavy metal accumulation are tabulated in Table-3. The total experimental period 60 days was divided into 3 stages. The first 20 days period was regarded as I stage. The next 40 days period regarded as II stage. The total 60 days period regarded as III stage. Zinc (Zn) total accumulation values in selected plants were shown in Table 2.

In the first stage: 5ppm /1000ml of Zn, solution was added alternate days to the all pots. The plants were growing. After 20 days two plants were collected from each pot and soil samples were also collected. The collected plant samples were separated into roots, stem and leaves. These are air dried first for two days, and then dried in hot air oven at 101°C for 12 hours. After the plant parts are powdered using mortar and pistle. Then 2g of the root leaves and stem powders are weighed and stored in polyethylene small bags for heavy metal analysis by Atomic Absorption Spectrometer. In the second stage: After 20 days 5ppm/1000ml of heavy metal Zinc (Zn) solutions was added alternate days to the all pots. The plants were growing. In these days the plants growth and physical changes were noted. After 40 days two plants from each pot were collected and soil sample was taken for heavy metal analysis. The collected plant samples plant parts were separated and dried in hot air oven at

101°C for 12 hours. After the plant parts are powdered using mortar and pestle, then 2g of the root stem and leaves powders are weighed and stored in polyethylene bags for heavy metal analysis. In the third stage After 40 days the heavy metal concentrations was increased. 5ppm/1000ml of heavy metal Zinc (Zn) solution was added alternate days to the all plots. The plants were growing. In all these days growth, development and physical characters were recorded. After 60 days two plants from each pot was collected for heavy metal analysis. The soil samples were also collected from each pot for heavy metal analysis. The collected plant samples the plant parts were separated into root, stem and leaves. These are air dried 24hours and then dried in the hot air

oven at 101°C for 12 hours. After the plant parts are powdered using mortar and pestle. 2g of the plant material was weighed and stored in small polyethylene bags for heavy metal analysis by AAS. In the third stage the collected soil samples were also measured for heavy metal analysis. All these stages all the plants were grown in identical environmental conditions. The experimental plants were four weed species, three flowering plants, three grass varieties. Generally grass species does not having stem and leaves except roots, hence we considered root analysis in grass varieties. Zinc (Zn) total accumulation values in selected plants were shown in (Figure 1).

Table 2. Zinc total accumulations in selected plant

Name of the plant	CONTROL (Mg/Kg)			20THDAY (Mg/Kg)			40THDAY (Mg/Kg)			60THDAY (Mg/Kg)		
	LEAF	STEM	ROOT	LEAF	STEM	ROOT	LEAF	ROOT	STEM	LEAF	STEM	ROOT
Acalypha indica.	29.7793	48.9722	38.4049	33.05	53.8019	45.6835	34.1653	53.9266	45.7921	49.92	53.9295	131.3603
Abutilon indicum.	22.8463	28.8163	25.6596	67.4217	37.1321	76.8127	74.6456	45.0032	83.6296	163.6737	102.5231	89.214
Physalis minima	48.3428	34.5489	29.9299	73.7037	46.513	52.3171	74.2565	62.0509	56.6891	79.3537	63.0091	104.7213
Cleome viscosa	31.5445	19.5026	32.616	49.221	46.8497	38.3337	49.7722	19.5051	36.1734	51.8111	40.461	65.2623
Catharanthus roseus	40.6246	84.8266	78.0478	46.0301	79.5308	87.7971	46.9897	81.6626	87.8919	88.4428	94.679	87.9852
Ruellia tuberosa	26.4505	37.6097	39.956	30.5539	44.0739	49.853	31.159	44.1071	49.9721	32.7741	44.8587	49.9933
Canna indica	36.4509	52.6121	11.9051	28.2461	62.7024	48.109	43.0045	72.1997	48.2021	49.014	77.0191	49.302
Perotis indica	-	-	48.0155	-	-	49.0703	-	-	62.4583	-	-	132.4386
Echinocloa colona	-	-	29.4016	-	-	69.1586	-	-	29.7868	-	-	161.4223
Cyperus rotundus	-	-	32.0948	-	-	32.2373	-	-	44.3653	-	-	44.712

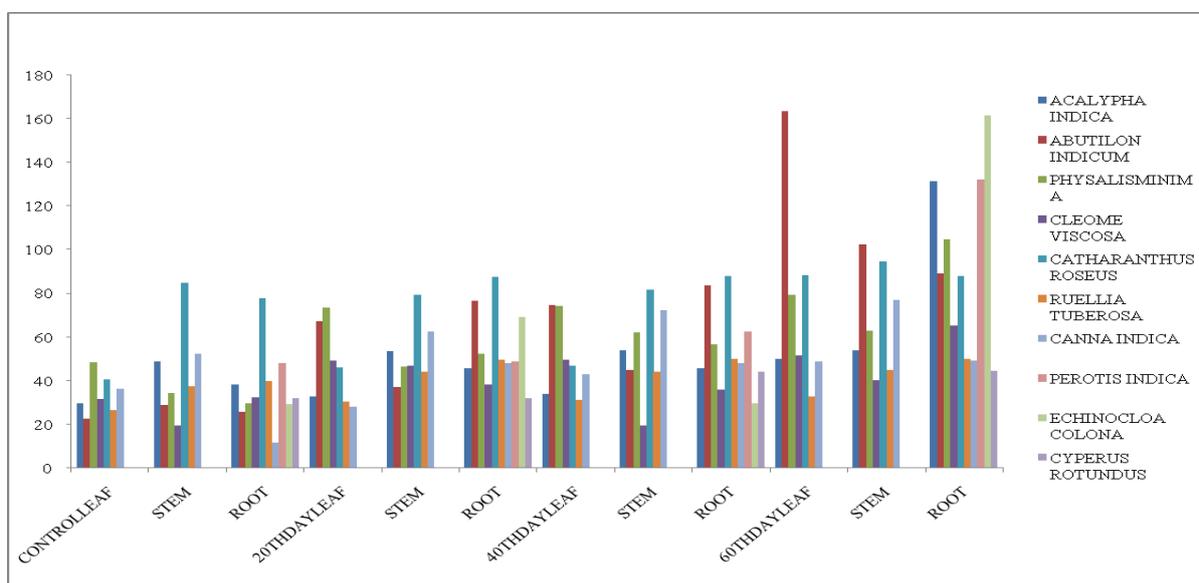


Figure 1. Zinc total accumulation in selected plant

3.3. Metal Transfer Co-efficients

3.3.1. Accumulation Factor (AF)

plants divided into three categories i.e. accumulator, excluder and indicator [14]. In accumulator plants the concentration ratio of the element in the plant to that in the soil is >1 . In excluder plant metal concentrations in aerial parts are maintained low ($\ll 1$) and constant over a wide range of soil concentrations. In indicator plants the uptake and transport of metals were regulated in such a way that the ratio of the concentration of element in the plant to

that in the soil is near 1. As total heavy metal concentration of soils is poor indicator of metal availability for plant uptake, accumulation factor was calculated based on metal availability and its uptake by a particular plant were shown in Table 3. Accumulation Factor for plants was calculated as:

$$\text{Accumulation Factor (AF)} = \frac{\text{Mean Plant Concentration } (\mu\text{g g}^{-1})}{\text{Mean Soil available } (\mu\text{g g}^{-1}) \text{ Concentration}}$$

Table 3. Zinc Total Accumulation Standard Deviation of selected plants

Name of the plant	Zinc (Mg/Kg)		
	20TH DAY	40TH DAY	60TH DAY
<i>Acalypha indica</i>	15.379±0.1949	16.7276±0.2557	118.0615±0.0422
<i>Abutilon indicum</i>	104.0443±0.0229	125.9562±0.6557	278.0886±0.0764
<i>Physalisminima</i>	59.7122±0.2830	80.1749±0.1826	134.2625±0.0597
<i>Cleome viscosa</i>	21.7876±0.3572	50.7413±0.3855	73.8713±0.0983
<i>Catharanthus roseus</i>	9.859±0.3063	13.0152±0.2337	67.608±0.05722
<i>Ruellia tuberosa</i>	20.4646±0.1628	21.222±0.1756	23.6099±0.03711
<i>Canna indica</i>	38.0894±0.0515	62.4382±0.3270	74.367±0.2291
<i>Perotis indica</i>	1.0548±0.0323	62.4583±0.1684	84.4231±0.2114
<i>Echinocloa colona</i>	39.757±0.6758	79.7868±0.2849	132.0207±0.01237
<i>Cyperus rotundus</i>	1.1425±0.0480	12.2705±0.03861	12.6172±0.08806

Based on the zinc total accumulation standard deviation values , the selected plants were good accumulators because the concentration ratio of the element in the plant to that in the soil is >1.

3.3.2. Translocation Factor (TF)

Translocation factor is a measure of the ability of plants to transfer accumulated metals from the roots to the shoots. It is given by the ratio of concentration of metal in the shoot to that in the roots [15].

TFs were also varied under different zinc concentrations in the growth media and a significant difference ($p \leq 0.05$) observed among treatments in TFs. This index was in the range of 0.27 to 0.9885 shown in Table.4. It was observed that TFs increased with increase in the applied zinc concentration in the growth media. However, the TFs under different zinc levels were <1 in *Acalypha indica*, and *Physalisminima*, indicating that all plant species were less able to translocate Zinc from the

roots to the shoots efficiently thus labeling these plants as trace metal excluders. The uptake and transport of metals were regulated in such a way that the ratio of the concentration of element in the plant to that in the soil is near 1 in *Canna indica* so labeling of this plant as metal indicator ,where as *Abutilonindicum*, *Cleomeviscosa*, *Catharanthus roseus*, and *Ruelliatuberosa* were only translocate zinc from the roots to the shoots efficiently hence it is considered as accumulator plant (Zn levels was >1).The translocation of Zn is often restricted due to the ability of this element to create Zn-phyto chelatin complex by sequestration in the vacuole [16]. Zn movement from root to shoots probably occurs within the xylem. The levels of free Zn in the symplast can be influenced highly by cellular sequestration of Zn and therefore it can affect the movement of Zn throughout the plants [17].

Table 4. Zinc Translocation Factor of selected plants

Name of the plant	Shoot(Mg/Kg)	Root(Mg/Kg)	Translocation factor Table
<i>Acalypha indica</i>	25.1061	92.9554	0.27
<i>Abutilon indicum</i>	214.5342	63.5544	3.3755
<i>Physalisminima</i>	59.4711	74.7914	0.7951
<i>Cleome viscosa</i>	41.225	32.6463	1.262
<i>Catharanthus roseus</i>	57.6706	9.9374	5.8033
<i>Ruellia tuberosa</i>	13.5726	10.0373	1.3522
<i>Canna indica</i>	36.9701	37.3969	0.9885
<i>Perotis indica</i>	84.4231		-
<i>Echinocloa colona</i>	132.0207		-
<i>Cyperus rotundus</i>	12.6172		-

3.3.3 Bio Concentration Factor(BCF)

Bioconcentration factor (BCF) is defined as the ratio of heavy metal concentration in plant roots to that in soil [18]. Bioconcentration factor (BCF) was used to evaluate the efficiency of Zn phyto extraction and was calculated using the following Equation:

$$BCF = C_{Zn - Roots} / C_{Zn - soil}$$

where, $C_{Zn - roots}$ (mg/kg) is the Zn concentration in roots part of the plant. $C_{Zn - soil}$ (mg/kg) is the Zn concentration in soil [19] and [20].

The BCFs were varied under different Zinc concentrations in the soil and it was in the range of 1.0642 to 21.2518 has shown in Table.5. There was a significant difference ($p \leq 0.05$) among treatments in BCFs. The highest BCF (that is 21.2518 ± 0.050) of Zinc was found in *Abutilonindicum* as compared to other treatment levels. The BCFs were >1 under various treatment levels. The

BCFs decreased with increase in the Zinc concentration in the growth media, which may indicate the restriction in soil-root transfer at higher Zinc concentrations in the soil [21].

3.3.4 Relation between Translocation Factor and Bio Concentration Factor

phyto stabilization, metal-tolerant plants are used to reduce the mobility of metals, thus, the metals are stabilized in the substrate [22] Plants with both Bioconcentration factor and translocation factor greater than one (TF and BCF > 1) have the potential to be used in phyto extraction. Hence *Abutilon indicum*, *Cleome viscosa*, *Catharanthus roseus*,and *Ruellia tuberosa* have the potential to be used in phyto extraction. *Acalypha indica*, *Physalisminima* and *Canna indica* have the potential for phyto stabilization because plants with bioconcentration factor greater than one and translocation factor less than one (BCF > 1 and TF < 1) have the

potential for phyto stabilization. *Perotisindica*, *Echinocloacolona* and *Cyperusrotundus* were good metal accumulators because in accumulator plants the concentration ratio of the element in the plant to that in the soil is >1 . By comparing BCF and TF, the ability of different plants in taking up metals from soils and translocating them to the shoots can be compared. As

shown in Table 4 and Table 5, among the sampled plants, like *Abutilo indicum*, *Cleomeviscosa* *Catharanthus roseus*, and *Ruelliatuberosa* were suitable for phyto extraction of Zn-metal where as plants like *Acalyphaindica*, *Physalishminima* and *Cannaindica* were most suitable for phyto stabilization.

Table 5. Zinc - bio concentration factors of selected plants

Name of the plant	Total accumulation	Bioconcentration factor
<i>Acalypha indica</i>	118.0615	9.0223
<i>Abutilon indicum</i>	278.0886	21.2518
<i>Physalishminima</i>	134.2625	10.2605
<i>Cleome viscosa</i>	73.8713	5.6453
<i>Catharanthus roseus</i>	67.608	5.1666
<i>Ruellia tuberosa</i>	23.6099	1.8042
<i>Canna indica</i>	74.367	5.6832
<i>Perotis indica</i>	84.4231	6.4517
<i>Echinocloa colona</i>	132.0207	10.0892
<i>Cyperus rotundus</i>	12.6172	1.0642

4. Conclusion

Metal-contaminated soils are notoriously hard to remediate. Current technologies resort to soil excavation and either land filling or soil washing followed by physical or chemical separation of the contaminants. Because of the high cost, there is a need for less-expensive cleanup technologies. Phyto remediation is an effective and affordable technology used to remove inactive metals and metal pollutants from contaminated soil and water. It includes phyto extraction, phyto stabilization, phyto volatilization, and phyto degradation/ phyto transformation. In this research the accumulated toxic metal (Zn) concentration in roots, stems and leaves, growing conditions, and their economic values were three major factors for selecting the plants. According to pot experiments results the selected plants were good accumulators because the concentration ratio of the element in the plant to that in the soil is >1 . Among the 10 sampled plant species, *Abutilon indicum*, *Cleome viscosa*, *Catharanthus roseus* and *Ruellia tuberosa*, *Perotisindica*, *Echinocloacolona* and *Cyperusrotundus* have the potential to be used in phyto extraction (TF and BCF >1). *Acalypha indica*, *Physalishminima* and *Canna indica* have the potential for phyto stabilization (TF <1 and BCF >1). The results of this study are of considerable significance in demonstrating the practical application of selected plants for Phyto remediation of Zn-polluted soil although further research on their relevant mechanisms are very much required. Extensive research was in progress in Department of Environmental sciences, Acharya Nagarjuna University, India, regarding the results obtained from Pot experiments like Physico-chemical properties of Soil, Metal transfer co-efficients in the direction of pilot-scale reactor operation for removal of heavy metals from the aqueous solutions and contaminated soils.

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Competing Interests

The authors declare that they have no competing interests.

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