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Phytoremediation of heavy metals in *Golden doranda, Balsam* and *Eerwa* plants

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ABSTRACT

Phytoremediation is a technology that involves plants and trees to clean up the contaminated soils and ground water. The technology is based on the natural abilities of certain plants to bioaccumulate, degrade or convert the toxic contaminant into less harmful chemicals. Plants absorb water and nutrient through roots, and act as a transformation system to metabolize organic compounds or absorb and bioaccumulate toxic heavy metals such as copper, lead and zinc up to a (10^{-4}) concentration. Ability to tolerate the kind and quantity of contamination is another important factor in this regard. We investigated the physiological capacity of Golden Doranda, Balsam and Erwa plants in tolerating heavy metals in order to use them as an alternative approach for rehabilitating contaminated soils. The efficiency of metal removal by those plants were determined by pot culturing, and the analysis of heavy metals in the whole plant was done using inductively coupled plasma optical emission spectroscopy. These plants were also examined for their efficiency in removing chromium from the industrial effluents. In addition, the effect of heavy metals on seed germination especially in Balsam was studied.

Key words: Phytoremediation, Bioremediation, Heavy metals, Copper, Lead, Zinc, Hyperaccumulators, Potculturing.

INTRODUCTION

Phytoremediation, an emerging cleanup technology for contaminated soils, groundwater, and wastewater, is both low-tech and low-cost and is the engineered use of green plants, including grasses, forbs, and woody species, to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds and radioactive compounds in soil or water. It takes advantage of the unique and selective uptake capabilities of plant root systems together with the translocation, bioaccumulation, and contaminant storage/degradation abilities of the entire plant body. Plant-based soil remediation systems can be viewed as biological, solar-driven, pump and treat systems with an extensive, self extending uptake network (the root system) that enhances the below-ground ecosystem for subsequent productive use [1]. The phytoremediation of a site contaminated with heavy metals and/or radionuclides involves "farming" the soil with selected plants to biomine the inorganic contaminants, which are concentrated in the plant biomass [2, 3].

Heavy metals are important environmental pollutants and many of them are toxic even at very low concentrations. Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution [4,5]. The primary sources of this pollution are the burning of fossil fuels, the mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, and sewage [6,7]. The value of metal accumulating terrestrial plants for environmental remediation has been fully studied with an emerging technology and four subsets of this technology such as Phyto extraction, Rhizofilteration, Phyto stabilization, plant assisted bioremediation are being developed [8,9,10,11,12,13]. Plants are capable of developing three strategies such as metal excluders, metal indicators, hyperaccumulators for growing on contaminated and metalliferous soils [14].

Any toxic metal may be called heavy metal, irrespective of their atomic mass or density [15,16]. Heavy metals are a member of an ill-defined subset of elements that exhibit metallic properties. These include the transition metals, some metalloids, lanthanides, and actinides. One source defines heavy metal as one of the common transition metals, such as copper, lead, and zinc. These metals are a cause of environmental pollution from sources such as leaded petrol, industrial effluents, and leaching of metal ions from the soil into lakes and rivers by acid rain [3].

With these aspects the present study was aimed to determine the amount of Copper, Lead and Zinc accumulation and the percentage of seed germination based on the effect of copper, zinc and lead among *Golden Doranda*, *Balsam* and *Erwa* plants and as well as to determine the accumulation of chromium by those plants from the effluent.

EXPERIMENTAL SECTION

Collection of plant samples

Samples from *Golden Doranda* (*Plumeri species*), *Balsam* (*Impatiens species*) and *Erwa* (*Alternanthera species*) were collected from the greenhouse, SRM University, India. The plants were selected based on their ability to accumulate heavy metals at all concentrations of Copper, Lead and Zinc. The effluent sample (Rechroming water) was collected from a tannery at Pallavaram, Chennai, India.

Pot culturing

The explants were grown by sowing the seeds over the bed and then transferred into pots. Further the plants were grown in pots for 30 days. The heavy metals of concentrations (10^{-4}) of Zn, Cu and Pb were fed into the pots in triplicates for about 14 days. The plant samples were then collected on the 7 and 14 day for the analysis of heavy metals. The effluent sample containing chromium was induced into the pots containing the *Golden Doranda*, *Balsam* and *Erwa* plant for about 14 days. The samples were taken out from the pots at the intervals of 7^{th} and the 14^{th} day, and the concentration of chromium present in the sample was analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES).

Sample preparation for heavy metal analysis

Microwave digestion

The plant samples were dried at 105°C for 6 to 8 hrs and 0.5g of dry material was decomposed in test tube using 1ml HCl and 4ml HNO_3. The mixture was heated 80°C for 1 hr to dissolve the residue. The digested samples were made upto 25ml using deionised water. The samples were then analysed for heavy metal accumulation in different concentrations using ICP-OES.

Effluent analysis (Chromium) in *Balsam*, *Golden Doranda* and *Erwa* plants

The effluent sample (Rechroming water) was collected from a tannery at Pallavaram, Chennai, India. The sample was poured to about 100 ml/day into the pots containing *Golden Doranda*, *Balsam* and *Erwa* plants in duplicates is shown in Fig 2a-c. The plant samples were taken out from the pots at the interval of 7^{th} and 14^{th} day. The samples were then prepared for the analysis of chromium using ICP-OES.

Seed germination

The *balsam* seeds were placed over the paper towel beneath the cotton in petriplates. The heavy metals of varied concentration of Zn, Cu and Pb respectively, were fed continuously for about a week to keep the seeds in moisture for germination. Proper cares were taken to keep the seeds under moisture at room temperature.

RESULTS AND DISCUSSION

Earlier, several cultivars of Indian mustard (*Brassica uncea, Brassica juncea* and *Brassica nigra*) were reported for their ability to remove lead, cadmium, copper, chromium, nickel, and zinc and *were reported to* have the highest metal-accumulating ability among the species tested [3]. The soil sample was collected and analyzed for the presence of heavy metals Cu, Pb and Zn (Figure 1).

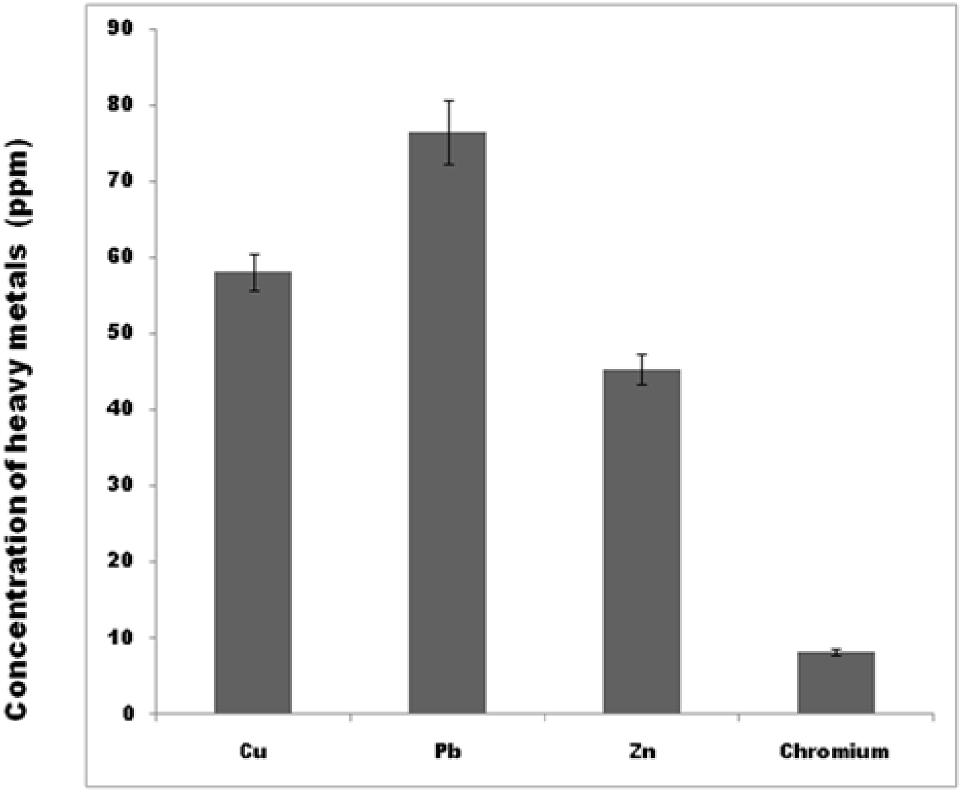


Figure 1: Analysis of heavy metals in soil sample

The *Golden Doranda* plants of 30-45 days grown (Figure 2a) upto a certain height were treated with heavy metals concentrations of Cu at 6.35 ppm, Pb at 20.72 ppm and Zn at 6.53 ppm of 10^{-4} M were used of about 100 ml/day to each plant in triplicates for 14 days. Then the samples were collected at the interval of 7^{th} and 14^{th} day and the amount of heavy metals present in the sample was analyzed using ICP-OES.

Table 1a shows the concentration of Cu, Pb and Zn accumulation by the *Doranda* plant. From the results, we observed accumulation of metals in the plant and increases as the days of treatment increases. The accumulation of Zn is higher in *Doranda* (14.49 ppm) relative to Cu (7.4 ppm) and Pb (10.84 ppm). We assumed that the uptake of heavy metals in *Doranda* is in the order as Zn<Pb<Cu.

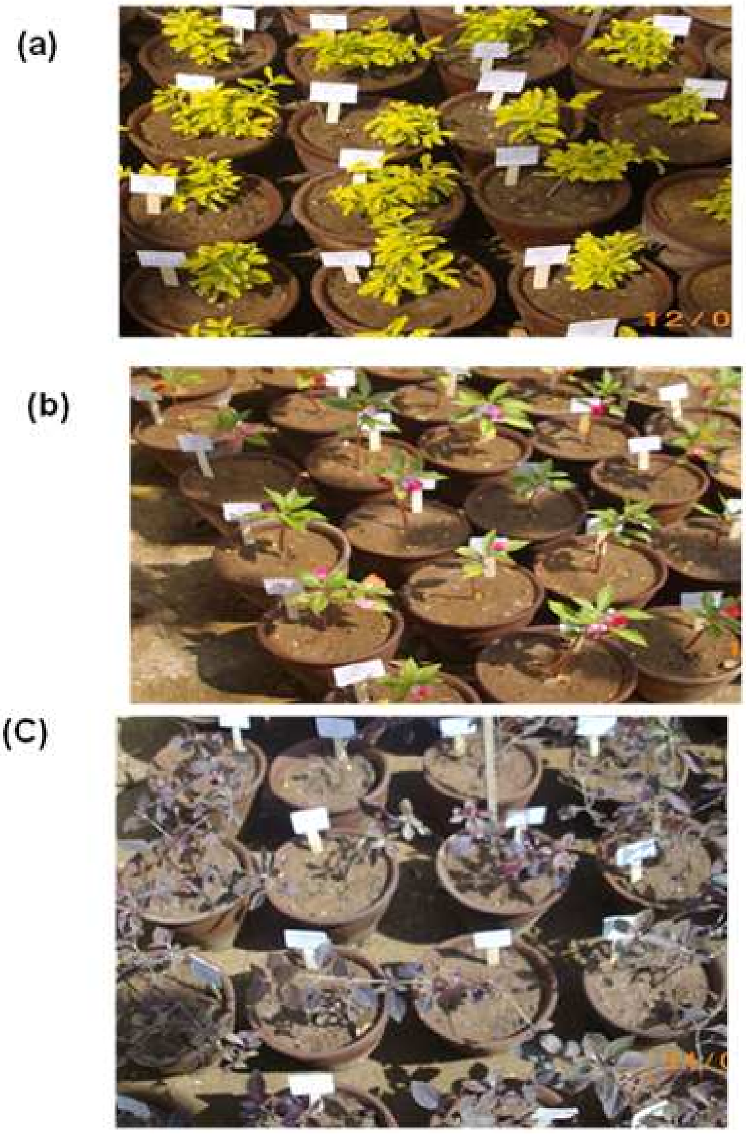


Figure 2. Pot culturing of (a) Golden Doranda Plant *(plumeri sp)*, (b) Balsam Plant *(Balsam impatiens)* (c) Erwa Plant *(Alternanthra sp)*

In Figure 2b shown the *Balsam* plants were grown in pots for about 30-45 days the heavy metals of Cu at 6.35 ppm, Pb at 20.72 ppm and Zn at 6.53 ppm of 10^{-4} M were used for about 100ml/day respectively, to each plant in triplicates for 14 days. The samples were collected at intervals of 7^{th} and the 14^{th} day and then the amount of heavy metals present in the sample was analyzed using ICP-OES. Finally, the concentration of Cu, Pb and Zn accumulation by the *Balsam* plant was determined and represented in Table 1b. These results indicate accumulation of metals in the plant increases as the days of treatment increases. The accumulation of Pb (7.45 ppm) is higher in *Balsam* rather than Cu (4.45 ppm) and Zn (3.16 ppm) (Table 1b). We assume that the uptake of heavy metals in *Balsam* is in the order as Pb<Cu<Zn. The *Erwa* plants were grown for about 30-45 days (Fig. 2c) and the heavy metals of concentrations Cu at 6.35 ppm, Pb at 20.72 ppm and Zn at 6.53 ppm of 10^{-4} M were used for about 100 ml/day to each plant in triplicates for about 14 days. The samples were collected at the intervals of 7^{th} and 14^{th} day and then the amount of heavy metals present in the sample was analyzed using ICP-OES. Finally, the concentration of Cu, Pb and Zn accumulation by the *Erwa* plant was showed in Table 1c. From the result, we observed that, the accumulation of metals in the plant increases as the days of treatment increases. The accumulation of Pb (45 ppm) is higher in *Erwa* rather than Cu (5.54 ppm) and Zn (6.20 ppm). Thus, the uptake of heavy metals in *Erwa* was in the order as Pb<Cu<Zn.

(a)

S.No	Heavy metals treatment (days)	Concentration of heavy metals	Control value for sample	Concentration of sample in (ppm or mg/l)
1	7	Cu-10^{-4} M Pb-10^{-4} M Zn-10^{-4} M	0.84±0.04	5.54±0.34 8.71±0.41 10.3±0.29
2	14	Cu-10^{-4} M Pb-10^{-4} M Zn-10^{-4} M	0.84±0.04	7.41±0.27 10.84±0.52 14.49±1.1

(b)

S.No	Heavy metal treatment (days)	Analyte name and concentration	Control value for sample	Concentration of sample in (ppm or mg/l)
1	7	Cu-10^{-4} M Pb-10^{-4} M Zn-10^{-4} M	1.00	1.99±0.04 0.46±0.002 0.53±0.003
2	14	Cu-10^{-4} M Pb-10^{-4} M Zn-10^{-4} M	1.00	4.45±0.12 7.45±0.32 3.16±0.09

(c)

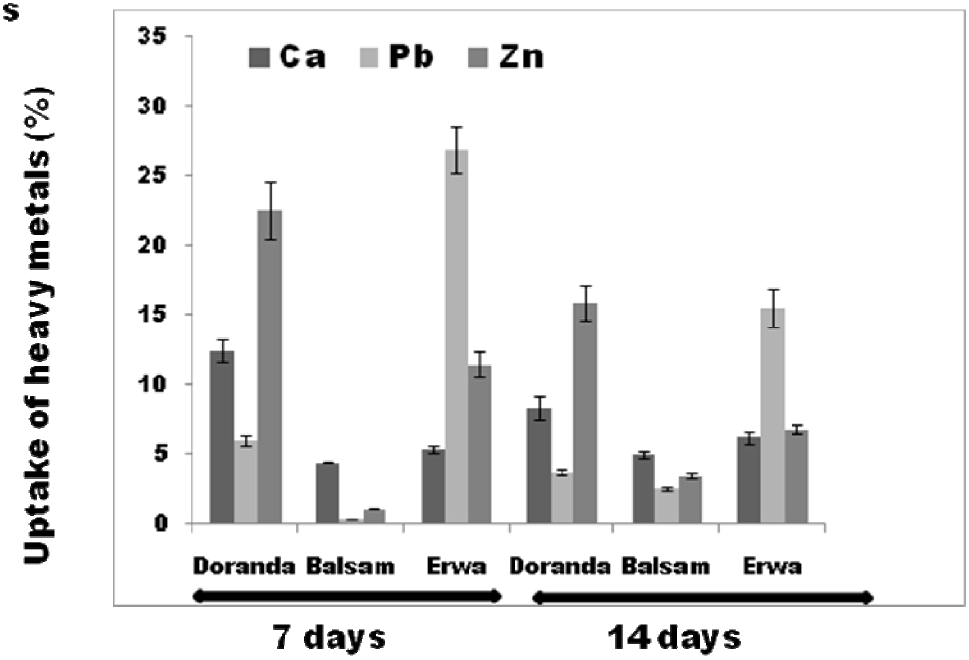
S.No	Heavy metal treatment (days)	Analyte name and concentration	Control value for sample	Concentration of sample in (ppm or mg/l)
1	7	Cu-10^{-4} M Pb-10^{-4} M Zn-10^{-4} M	0.90±0.002	2.41±0.03 39.00±2.11 5.24±0.2
2	14	Cu-10^{-4} M Pb-10^{-4} M Zn-10^{-4} M	0.90±0.002	5.54±0.025 45.00±1.1 6.20±0.34

Table 1. Accumulation of Copper, Lead and Zinc in (a) Golden *Doranda* (b) Balsam (c) *Erwa*

Figure 3a shows the accumulation percentage of Cu, Pb and Zn in Golden *Doranda*, *Balsam* and *Erwa* plants were calculated. It was noted that, the overall percentage of accumulation of Cu, Pb and Zn in each plants is reduced on 14^{th} day compared to 7^{th} day. This is due to the toxicity of heavy metals that suppress the process of remediation in plants. Thus the pot experiment showed that plants such as *Golden Doranda, Balsam* and *Erwa* plants the accumulation of heavy metals increases day by day on induction. On comparison, it was noted that the *Doranda* plant has the ability to accumulate Cu, Pb and Zn to a larger range than the other two plants. Since, *Doranda* is very dense and it has the capability to absorb the metals from the soil, whereas the *Balsam* and *Erwa* do not have resistance to the metals, it is very sensitive. And the percentage of accumulation of Cu, Pb and Zn in those plants is reduced in the 14^{th} day compared to 7^{th} day, due to increase in metal toxicity. Compare to Cu and Zn the accumulation of Pb is higher in both *Balsam* and *Erwa* plant (Table.2b,c), as consistent with the previous report by Sharma and Dubey, [17] stated that Pb is easily absorbed and accumulated in different plant parts . Similarly, Deram and Petit [18] also found lead hyper accumulation in the grass *Arrhenatherum elatius* growing on calamine soils at France. Thus, in this results, these two species are suited for phytoremediation should have wide distribution, high above-ground biomass, high bioaccumulation, and sites, had high above-ground biomass and high propagation rates. It is generally preferable to use a perennial since phytoremediation will never take just a single year and the use of a perennial will prevent the need to annual planting.

Figure 3.

(a). Overall accumulation of heavy metals by the selected plants



(b). Accumulation of chromium in *Doranda*, *Balsam* and *Erwa* plants

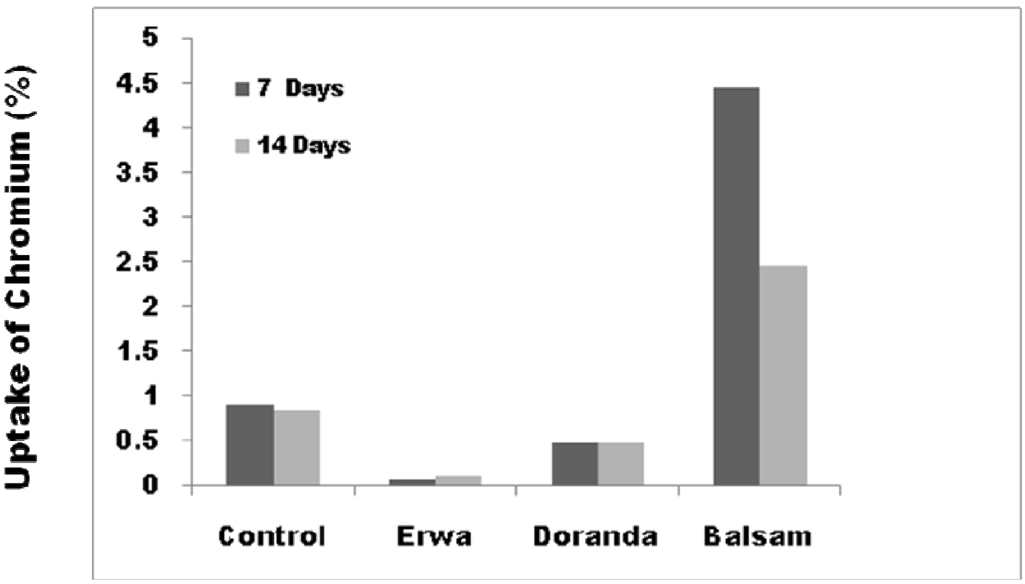


Figure 3. (a) Overall accumulation of heavy metals by the selected plants (b) Accumulation of chromium in *Doranda, Balsam* and *Erwa* plants

The concentration of chromium, present in the effluent sample was found to be about 593 ppm. We focused on the accumulation of chromium by the plants from the rechroming water (effluent) of dying industry. We found, the *Balsam* plant has the capacity of accumulate chromium to a large amount rather than the other two plants. The concentration of chromium accumulated by the *Balsam* plant after 14 days was about 20.46 ppm (Fig.3b). We also found, the chromium uptake is also reduced in the plants on 14th day due to metal toxicity. The accumulation of chromium in the plants was in the range as *Balsam*>*Doranda*>*Erwa*. It was prominent that, the *Balsam* plant has the capacity of accumulating chromium to a large amount rather than the *Doranta* and *Erwa*. This reveals that the mechanism of Chromium tolerance involved in the *Balsam* plant is possibly different from that of the *Doranta* and *Erwa* plants. The distribution of elemental concentrations and the metal uptake in different organs of plant varies widely due to complex process of metabolism. Each plant species has its own requirements and tolerance to elemental uptake and retention. Published report from Dunn *et al*., [19], revealed that the composition of an individual plant varies substantially among its various tissues types, *i.e.* roots, wood, bark, twigs, needles-leaves and flowers. Generally the plants take up metals to varying degree from the substrates in which they are rooted and developed [20], and the level of tolerance developed can often be related to the amount of metal in the soil [21].

Generally, metal concentration in plants is depends on not only the total soil concentrations, however depends on the chemical speciation of metals in soil and soil solutions [22] and the involvement of metal in biological functions [23]. The plant uptake mechanism normally restricts the nonessential element concentration to a constant level in spite of the higher metal abundance of such metals in the soil [24]. In our study, the Chromium uptake was high in *Balsam* than that of *Golden Doranta and Erwa*. The evaluation of plant metal concentrations can be used to obtain information about specific plant behavior in the soil environment and reveals the metal distribution and their mobility.

(a)

Metal	Concentration of copper	No. of seeds sowed	No. of seeds germinated	Seed germination (%)
Cu	Control	15	15	100
	Cu-10^{-2}M	15	11	73.33
	Cu-10^{-3}M	15	13	86.66
	Cu-10^{-4}M	15	13	86.66
	Cu-10^{-6}M	15	14	93.33
	Cu-10^{-7}M	15	15	100
	Cu-10^{-8}M	15	15	100

(b)

Metal	Concentration of Lead	No. of seeds sowed	No. of seeds germinated	Seed germination (%)
Pb	Control	15	15	100
	Pb-10^{-2}M	15	13	86.66
	Pb-10^{-3}M	15	12	80
	Pb-10^{-4}M	15	13	86.66
	Pb-10^{-6}M	15	15	100
	Pb-10^{-7}M	15	15	100
	Pb-10^{-8}M	15	15	100

(c)

Metal	Concentration of Zinc	No. of seeds sowed	No. of seeds germinated	Seed germination (%)
Zn	Control	15	15	100
	Zn-10^{-2}M	15	10	66.66
	Zn-10^{-3}M	15	13	86.66
	Zn-10^{-4}M	15	12	80
	Zn-10^{-6}M	15	14	93.33
	Zn-10^{-7}M	15	15	100
	Zn-10^{-8}M	15	15	100

Table 2. Effect of (a) Copper (b) Lead (c) Zinc on seed germination (Balsam)

Among the three plants, the *Balsam* only grows from its seeds, whereas the *Doranda* and *Erwa* grown from their cut ends. Thus, the *Balsam* seeds were selected to determine the percentage of seed germination with the effect of heavy metals and shown in Supplementary 2. The heavy metals (Cu, Pb and Zn) of different concentrations 10^{-2}, 10^{-3}, 10^{-4}, 10^{-6}, 10^{-7}, 10^{-8} were prepared. They were fed into the seeds daily, placed in petriplates, for about a week. The number of seeds germinated on each day was also noted. Based on this, the percentage of seed germination was calculated for each metal with their varying concentrations as given in (Table 2a,b,c). Control plants showed 100% of seed germination. From the result, we observed that, as the concentration of heavy metals increases the percentage of seed germination decreases only in *Erwa* and *oranda*, but the germination increases in *Balsam* (supplementary 2).

If the concentration of heavy metals decreases the percentage of seed germination increases. It was renowned that, the percentage of germination was low in the Zn- 77.14% whereas the Cu at 91.42% and the Pb at 93.33% showed greater germination percentage. Zinc act as superior hyper accumulator of *Balsam* than to *Doranda* and *Erwa*. Earlier studies [24] have shown that, the seed germination and plant growth was significantly affected by heavy metals at higher concentrations (P<1%). As a result of induction of Cu, Pb and Zn at different concentrations onto seeds, it was noted that, the germination percentage increases as the concentration of heavy metals decreases. The doses used in the study were about Cu at 6.35 ppm, Pb at 20.72 ppm, Zn at 6.53 ppm. With respect to the control, the overall germination percentage of Cu, Pb and Zn at 91.42%, 93.33%, and 77.14% were observed respectively. It was confirmed that the effect of Cu and Pb on seed germination is greater whereas the effect of Zn on seed germination is poor (Fig.4).

Figure 4.

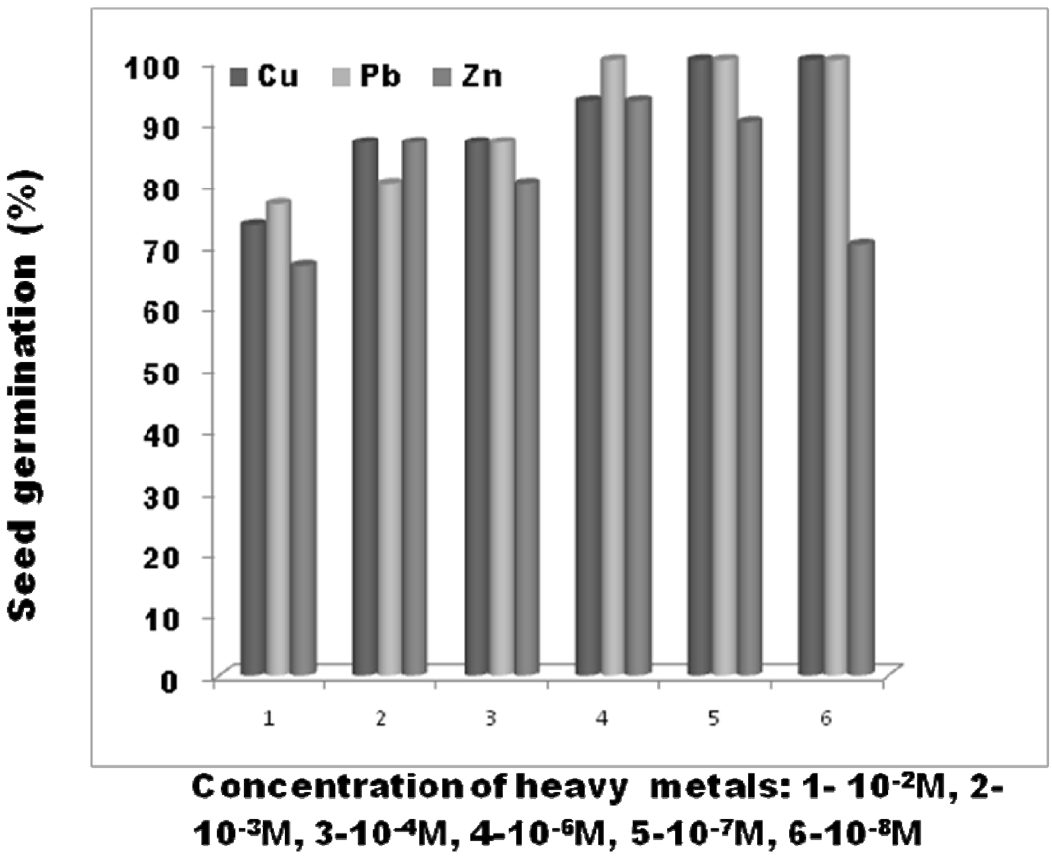


Figure 4. Overall percentage of seed germination on copper, Lead and Zinc

Supplementary 1.

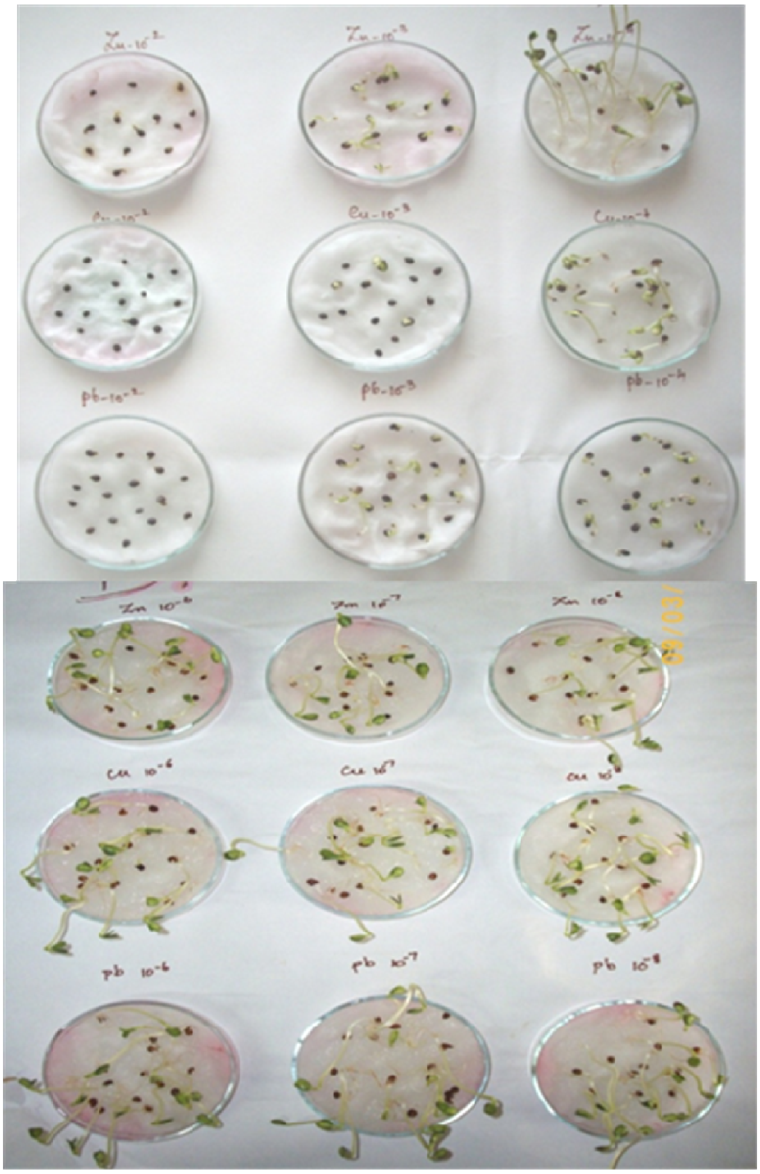
Supplementary 1. Effluent treatment (chromium) in Balsam, Golden Doranda and Erwa Plants
Supplementary 2. Effect of heavy metals on (Balsam) seed germination from different concentration 10^{-2}, 10^{-3}, 10^{-4}, 10^{-6}, 10^{-7}, 10^{-8} respectively

In Figure 4 shows the Zn, decreased the seed germination of *Balsam,* the highest germination percentage was estimated in the Pb and cu induced plant metals. Percentage of seed germination of *Balsam* was significantly ($p < 0.05$) decreased by Pb treatment at higher level (75, 100 mg/L) as compare to control (Fig. 4). Based on the report, from evaluated the hindering effect of Pb on *Brassica penkinensis Rupr* [25]. The reduction in germination percentage of *Balsam* was due to the effect of heavy metal Pb [26]. Copper treatment at 100 mg/L concentration affected ($p < 0.05$) germination percentage of *Balsam* when compared to control (Fig.4). It was consistant with report from kumar [27] dissected effect of zinc and copper on *Vigna mungi (L).* We concluded that the heavy metal pollution on seeds is very detrimental to seed germination. It was observed that the huge amount of different varieties of chemical substances using in agriculture field leads to soil pollution and turn into adverse effects on crop plants. Thus, there was consequents reduction in the seed germination of *Balsam* plant. The reason for low percentage in germination of *Balsam* might be due to physiological mechanism. Further work is necessary to reduce the enhancement of heavy metal pollution in all sources.

Supplementary 2.

(a)

(b)



CONCLUSION

We conclude that, among the three selected plants, the accumulation of Zn is higher in *Golden Doranda*, and the accumulation of Pb is higher in *Balsam* and *Erwa* plants, since accumulation of Cu is very low. The percentage of accumulation of heavy metals in the plants is reduced as treatment days prolong, and this may be due to the increase in toxicity of metals. Anyhow, the three selected plants have the ability to accumulate Cu, Pd and Zn. It was noted that, the accumulation of chromium from the effluent is higher in *Balsam* but lower in *Doranda* and *Erwa*. It was also noted that, the selected plants have the ability to accumulate heavy metals but of very low concentration. Thus, these plants are not best suited to be used for phytoremediation to clean up the contaminated soils. They can be used for phytoremediation but the accumulation percentage was not that much satisfied. But still because of their ability to accumulate heavy metals, they are planted to maintain the environmental pollutions. Our results indicate there were a total of 2 species of Pb hyper accumulators among this, Balsam contains highest Pb content. These accumulators were confirmed only under the laboratory condition but not at the field. Hence, field study of the above mentioned plants could be useful in practical phytoremediation approaches and reduction of the risk from metals to human health.

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