Screening of heavy metal tolerant plants and assessment of its Phytoremediation capacity

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Abstract- **Phytoremediation is an emerging technology which is a cost-effective and eco-friendly way to solve the environmental stress of heavy metals by employing use of higher plants. The metal stress in the environment is caused due to the continuous application of chemical fertilizers and pesticides, industrial practices etc. The present study aimed to find the suitable plant species for the clean-up of heavy metal from polluted soil. In this work plant species like *Abelmoschus esculentus, Brassica juncea,Brassica rapa* were analyzed for their phytoremediation capacity for heavy metals-Copper, Lead and Mercury. *Abelmoschus esculentus* tolerated metal stress to high concentrations and also showed heavy metal accumulation property. Assessment of accumulation of metals in the plant body was determined by studying its different parameters such as root and shoot height, photosynthetic pigment content, bioaccumulation coefficient, antioxidant activity, protein and proline content. Atomic Absorption Spectrophotometry was used for the analysis of metal accumulated by plants. Heavy metal stress has a negative effect on the plant physical properties and the chemical properties were also considerably affected. The anti-oxidant activity of the enzymes Peroxidase and Catalase was more when compared with the normal plant. The protein content, proline content and antioxidant activity increased with increase in metal concentration. The Bio-Accumulation Co-efficient (BAC) study showed *Abelmoschus esculentus* is high bio-accumulator for Lead and moderate bio-accumulator for Copper. Thus, current investigation suggests this plant species can be used as a good Phyto-accumulator.**

Keywords: **Phytoremediation, Copper, Lead, Mercury, Bioaccumulation Coefficient, *Abelmoschus esculentus, Brassica juncea, Brassica rapa*.**

I. INTRODUCTION

The repeated application of large amounts of fertilizers and pesticides to agricultural land has raised up concern regarding the possible accumulation of high levels of their trace element constituents that cause potential harm to the environment. Soil serves as both a medium for contaminant accumulation, mobility and transfer medium of metals uptake by plants in different concentrations. From soil, heavy metals can enter plants and through them into the food chain. They can also migrate to surface, ground and underground waters and spread at longer distances, re-enter food chains and poison organisms. Heavy metals can disturb soil processes and sometimes cause soil degradation. Metals are non-degradable in nature and therefore remain for long periods in aquatic and terrestrial environments. They may be transportedthrough soils to reach groundwater or may be taken up by plants. Toxic and hazardous compounds including heavy metals cause various diseases and mutations (Garg and Kataria, 2009). The trace metal contaminations are usually introduced into soil environment through a variety of human activities, such as waste disposal, mining and smelting. Furthermore, increasing amounts of urban and industrial wastes which may contain significant quantities of heavy metals, are being disposed on the agricultural lands. Lead, Mercury and Cadmium belongs to the most toxic elements. Most of the heavy metals – Mercury, Lead, Cadmium, Copper, Nickel, Zinc,Beryllium and Uranium etc. are highly hazardous to human health. Severe heavy metal contamination in soil may cause a variety of problems, including the reduction of yield and metal toxicity of plant, animals and humans. The decontamination of these soils by engineering methods are high costing project.

Phyto extraction is the use of plants to remove heavy metals from contaminated soils. Phyto extraction may provide an attractive alternative for the clean-up of heavy metal-contaminated soils. The main aim of heavy metal Phyto-extraction is to reduce metal levels in the soil up to the acceptable levels within a reasonable time. A few plant species such as wheat, mustard, radish (Garg and Kataria, 2009), *Trianthema portulacastrum* (Tsafe *Et.al,2013*) etc. are able to survive and reproduce on soils heavily contaminated with Zn, Cu, Pb, Cd, Hg, Cr, Zn etc. The extreme level of metal tolerance in vascular plants is called hyper-accumulation. Heavy metals such as Copper is an essential mineral nutrient for higher plants, whereas Lead and Mercury do not play any important role in the plants. Generally, these heavy metals can cause oxidative stress, causing enzymatic and non-enzymatic antioxidative reaction responses and lipid per-oxidation in plants.

The present research aimed to study the accumulation of copper, lead and mercury in roots, shoot and leaves of plants belonging to the family Brassicaceae and Malvaceae. This study also examined the growth performance and physiological response in respect to activity of important enzymes like peroxidase (POD) and catalase (CAT). The changes in the protein and proline content, photosynthetic pigment content and antioxidant activity was also examined. Bio-Accumulation Co-efficient (BAC) was

also calculated to determine the potential efficiency of these crops to remediate the metal from the contaminated soil in which they were cultivated.

II. MATERIALS AND METHODS

Collection of seeds:

The seeds of*Abelmoschus esculentus*, *Brassica juncea*,*Brassica rapa* plant were collected from PYRAMID SEEDS-Byculla, Mumbai.

Seed germination and soil treatment:

The seeds of *Abelmoschus esculentus*,*Brassica juncea*,*Brassica rapa* were soaked overnight and surface sterilized. Salts of Copper sulphate, lead acetate and Mercury chloride were used and solutions of different concentrations i.e. 25µM, 50µM, 100µM (Garg and Kataria,2009) ,200µM, 400µM, 800µM, 1600µM, 2400µM, 3200µM, 4000µM were prepared to determine the maximum tolerance limit of the plants for heavy metals. The germination of the seeds was carried out on respective concentrations of metals as mentioned in Garg and Kataria, 2009. Soil pre-treatment was given with the respective salts for 2 days. After germination, the seedlings were transferred into soil samples of respective concentrations of heavy metals and were irrigated with nutrient solution for 30 days (Garg and Kataria, 2009).

Plant harvest and analysis:

Plantlets were gently removed from the pots after 30 days of incubation and were analysed for various physical parameters and biochemical parameters.

Physical parameters: The full-grown plants after 30 days were analyzed for the germination rate, growth rate. Height of plant was also determined.

Chemical parameters: The estimation of chlorophyll content, carotenoids content, proline content, anti-oxidant scavenging activity, catalase and peroxidase enzyme activity, total protein content analysis was carried.

Chlorophyll and carotenoid content estimation:

The chlorophyll and carotenoid estimation was carried out by extracting photosynthetic pigments in 80 per cent acetone. The absorbance was read at 480 nm, 645 nm and 663 nm in spectrophotometer using 80 per cent acetone as blank. The chlorophyll and carotenoid content were calculated by the formula mentioned in Garg and Kataria, 2009.

Eq.1.Total chlorophyll (mg/ml) = (0.0202) x (4.645) + (0.00802) x (4.663)

Eq.2.Chlorophyll ‘a’ (mg/ml) = (0.0127) x (4.645) – (0.00438) x (4.663)

Eq.3.Carotenoid = ΔA 480 + 0.114 x ΔA 663 – 0.638 x Δ 4.645

Proline estimation:

The amino acid proline is known to play as an osmoprotecting in plants subjecting to stress conditions (Ajithkumar and Ibadapbiangshylla, 2017). The proline from the plant was extracted using 3% aqueous sulfosalicylic acid and was estimated colorimetrically at 520nm using toluene as a blank. The proline concentration was determined from a standard curve and calculated as follows: - [(µg proline /ml x ml toluene) /115.5 µg/ µmole] / [(g sample) /5] = µmoles proline / g of fresh weight material (Bates, 1973).

Antioxidant scavenging activity:

Antioxidants are substances that neutralize free radicals and their actions. There are natural antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin thiols, and disulphides bonding which form the buffering system in every cell and provide protection against the metal ion stress. The estimation of antioxidant activity was done by DPPH assay method. The absorbance was measured at 517nm using ethanol as blank and control of DPPH. Percentage scavenging activity was calculated based on control reading by following equation. (Ansari *et.al*, 2013)

Eq.4.DPPH scavenged (%) = $[(A_{control} - A_{test}) / A_{control}] \times 100$

$A_{control}$ = is absorbance of control reaction

A_{test} = is the absorbance in presence of sample of the extracts

Catalase and Peroxidase activity:

The catalase and peroxidase enzyme activity increase in plants due to the stress of the heavy metal. The Catalase and peroxidase enzymes were extracted by homogenizing the plant material with ice cold 50mM sodium phosphate buffer (pH 7.5).

For catalase activity estimation, the decomposition of H2O2 was determined by taking absorption at 240 nm whereas for the determination of peroxidase activity the amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract at zero time. The time activity was expressed in unit mg^{-1} protein which is the number of enzyme units per ml divided by the concentration of protein in mg per ml (Ajithkumar and Ibadapbiangshylla, 2017). Protein estimation by Folin Lowry method (Ansari *Et.al,2013)* was carried out for the calculation of enzyme activity.

Bioaccumulation coefficient (BAC) analysis:

Bioaccumulation coefficient is the content value of metal per plant that seems to be a better estimate for heavy metal extraction efficiency in a given species, and reflects the extent of metal which could be remediate by an individual plant. BAC was determined using Atomic Absorption Spectroscopy (Garg and Kataria, 2009). The instrument used was Atomic Absorption Spectrophotometer of Agilent Technologies 200 Series AA. The lamps used were of Copper and Lead.

III. RESULTS:

Seed germination and Physical parameter analysis:

Thirty seeds of each of all the plants i.e. *Abelmoschus esculentus (Malvaceae), Brassica juncea andBrassica rapa* (Brassicaceae) were exposed to the different metal stress of varying concentrations from 25µM to 4000µM of the metals copper, lead and mercury along with the control plant. The germination rate for okra was 100% for all the metals (Figure 1), for mustard it

was 50% and for turnip it was 0%. The work with mustard and turnip was not carried out further since when mustard was transferred to the soil it was not able to grow for 30 days for any of the metal and turnip did not show germination for any of the metals. Okra was selected as suitable plant which can tolerate the heavy metal stress. It was subjected to varying concentrations from 25µM to 4000µM for copper, lead and mercury to find out the highest concentration to which it can survive and can grow optimally. Okra was able to grow in presence of all the heavy metals (Graph 1) (Figure 2). It tolerated a concentration of 4000µM for lead, 1600 µM for mercury and 3200µM for copper (Table 1) (Graph 2). The physical parameter analysis of okra plants is mentioned in Table 1. The Okra plants was observed to be of wrinkled stem and edgy leaves with tap root system whereas the mustard plants were short in height with a few leaves and tap root system which is in a slender form.

Figure 1. Germinated Okra seeds

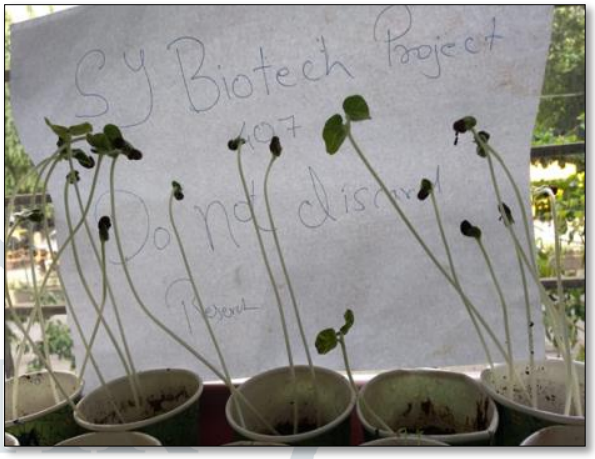
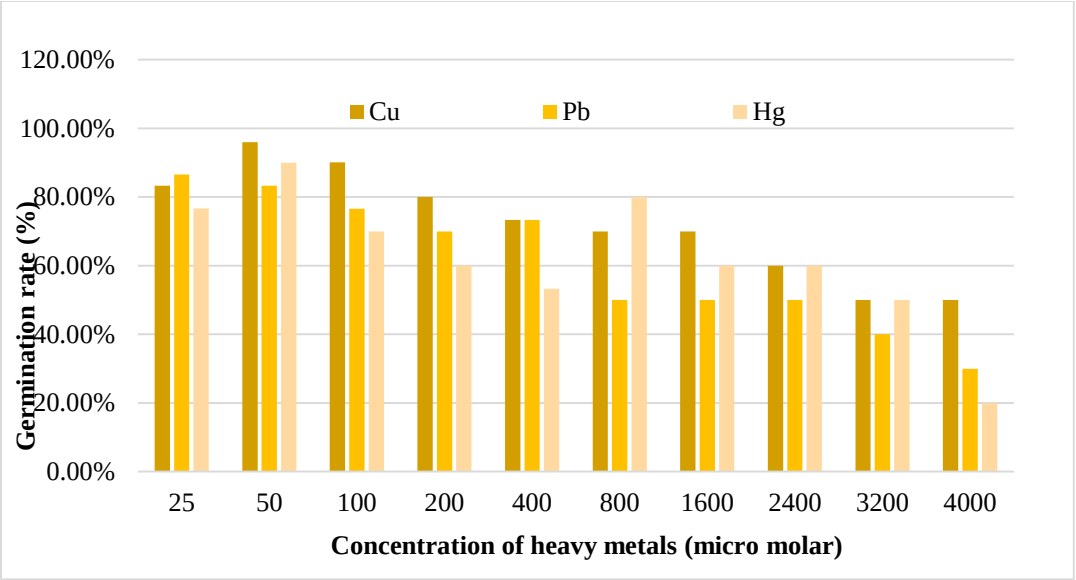


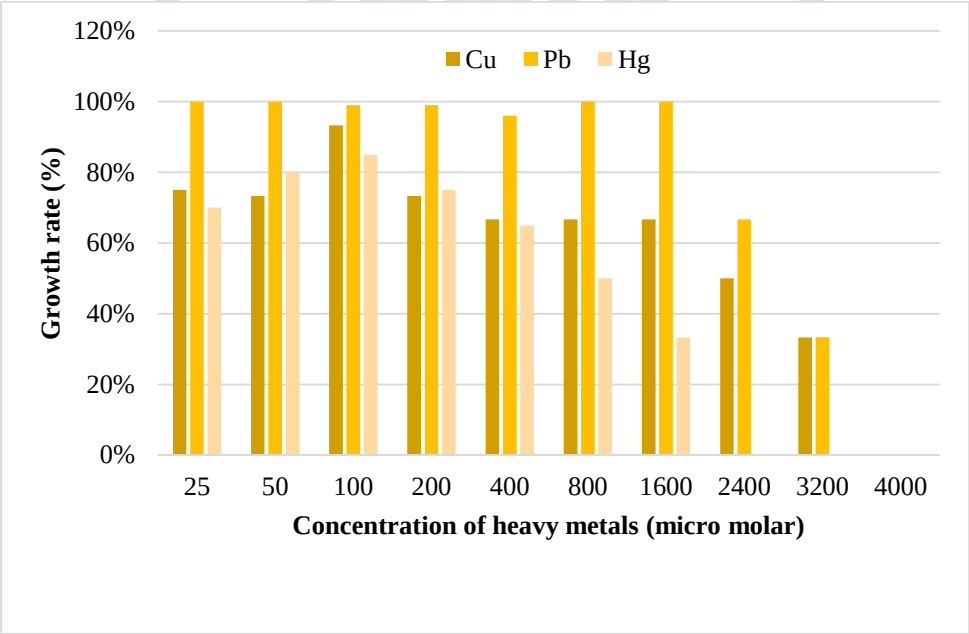
Figure 2. Plantlets grown after 30 days of incubation

Table 1. Physical parameter analysis of Okra plants grown in various concentrations of heavy metals

Metal (µM)	Germination rate	Growth rate	Height of plant (cm)	Germination rate	Growth rate	Height of plant (cm)	Germination rate	Growth rate	Height of plant (cm)
	Copper (Cu)			Lead (Pb)			Mercury (Hg)		
Control	100%	90%	29						
25	83.3%	75%	20	86.6%	100%	19	76.67%	70%	22
50	96%	73.3%	19	83.3%	100%	22	90%	80%	22
100	90.07%	93.3%	25	76.6%	99%	22	70%	85%	24
200	80%	73.3%	26	70%	99%	23	60%	75%	25
400	73.3%	66.67%	27	73.3%	96%	26	53.3%	65%	26
800	70%	66.67%	28	50%	100%	27	80%	50%	27
1600	70%	66.67%	28	50%	100%	26	60%	33.3%	27
2400	60%	50%	28	50%	66.67%	28	60%	0%	-
3200	50%	33.3%	30	40%	33.33%	30	50%	0%	-
4000	50%	0%	-	30%	33.33%	30	20%	0%	-



Graph 1. Effect of heavy metal concentration on percentage germination rate of Okra



Graph 2. Effect of heavy metal concentration on percentage growth rate of Okra

Chemical parameter analysis:

Metal exposure influences several biochemical and physiological parameters of plants. Administration of excess amount of copper, lead and mercury was followed by an increase of metal ions and its associated symptoms of toxicity in leaves. Typical symptoms of metal toxicity developed 30 days after the beginning of treatments. Chlorophyll concentration was decreased in response to heavy metal toxicity. The chlorophyll content was determined in terms of total chlorophyll (a + b) and carotenoids. There was decrease in the chlorophyll content and increase in the carotenoids content in the plant was reported with the increase in metal stress (Table 2). CAT (catalase enzyme) activity increased as increase in metal ions concentration for all three heavy metals (Table 2). The similar effect was observed for POD (peroxidase enzyme). The antioxidant scavenging activity in the plant was too increase under the metal concentration stress (Table 2). The proline analysis was performed for the plants by the use of sulphosalicylic acid for easy extraction. The proline content was observed to increase with the increase in the metal ions exposure (Table 2). Protein content was determined by the Folin-Lowry method and it was found to be increased with the increase in the concentration of metal (Table 2).

Table 2. Chemical parameter analysis of Okra plants grown in various concentrations of heavy metals

Metal/Tests	Carotenoids content	Chlorophyll content	POD (U)	CAT (U)	Scavenging activity (%)	Proline content	Protein content

	(mg/ml)	(mg/ml)				(µmole/g wt. plant)	(mg/g dry wt. of plant)
Control	17.56	10.3	4.8	3.2	3.82	0.866	2.4
Cu 25	16.7	9.227	10.2	6.8	6.92	1.73	3.400
50	17.9	7.7	10.5	7.0	10	3.29	3.800
100	20.63	7.1	10.85	7.1	11.2	3.62	3.360
200	21.34	4.8	11.1	7.4	8.46	4.16	4.350
400	21.56	3.9	11.7	7.8	12.3	3.81	4.800
800	22.16	5.4	12.6	8.0	14.6	4.67	1.450
1600	22.82	7.3	12.8	8.5	15.4	6.79	3.200
2400	26.0	6.7	13.2	9	16.92	8.31	3.250
3200	30.927	5.1	15.4	9.5	17.3	8.45	4.350
4000	-	-	-	-	-	-	-
Pb 25	14.6	9.3	6.3	4.2	7.69	2.1	2.100
50	14.8	7.49	8.7	5.8	6.92	2.6	3.100
100	15.82	7.16	10.2	6.8	10	3.46	3.500
200	6.01	5.52	11.5	7.5	10.5	4.2	4.250
400	10.62	3.4	12.3	8.2	7.69	5.35	4.550
800	4.31	6.68	12.45	8.35	9.26	8.4	1.900
1600	19.68	5.1	12.6	8.4	11.54	14.02	2.350
2400	19.7	4.98	13.2	8.8	13.08	15.24	2.800
3200	36.14	4.8	13.5	9.1	13.6	15.6	4.600
4000	37.0	4.62	14.9	9.5	14.1	16.5	4.650
Hg 25	7.39	18.05	10.5	10.5	6.92	2.1	3.500
50	15.97	9.25	10.8	10.8	8.46	2.1	3.800
100	16.22	5.88	11.1	11.1	10.77	2.42	4.050
200	16.622	5.62	11.4	11.4	12.30	2.94	4.350
400	19.4	5.49	12.2	11.7	14.6	3.46	4.600
800	23.65	5.822	12.69	12.2	16.92	6.23	3.100
1600	26.74	5.7	13.5	12.8	17.3	7.4	3.950
2400	-	-	-	-	-	-	-
3200	-	-	-	-	-	-	-
4000	-	-	-	-	-	-	-

Bioaccumulation coefficient (BAC) analysis:

The bioaccumulation coefficient/ phytoextraction rate of metals, is defined as the ratiosbetween µg of metal/g dry weight of shoot, root or leaf and µg of metal/g dry weight of soil. The heavy metal was analyzed by acid digested samples of plant and soil for Copper and Lead at concentrations 3200µM and 4000µM respectively. The BAC value for Copper (3200µM) was 0.154ppm and for Lead (4000µM) it was 8.227ppm.

IV.CONCLUSION:

Heavy metals are conveniently defined as elements with metallic properties (ductility, conductivity, stability as cations etc.) and a high atomic number. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb and Zn. Plants grown in metal enriched media take up metal ions in varying degrees. Uptake of metal ions is largely influenced by the availability of metals, which is in turn determined by both soil associatedand plant associatedfactors. Only a limited number of plant species exhibit heritable tolerance or resistance, which enables these plants to grow on metal contaminated soils. Soil remediation is needed to eliminate risk to humans or the environment from toxic metals. Phyto extraction of metals was a feasible remediation technology for the decontamination of metal polluted soils.

In this study the plants used were Okra, Turnip, Mustard for the Phyto-remediation analysis against the metals mercury, lead and copper. The okra and mustard were found to be tolerant to metal stress, whereas mustard was resistant for lower concentrations of metal. The okra was found to be a good metal stress tolerant plant. The plants physical parameters were also affected by the metal stress. Copper, lead and mercury treatment caused a decrease in chlorophyll in leaves of metal accumulator plants. The decrease of chlorophyll after metal dose may be due to blocking of enzymes acting in chlorophyll synthesis or degradation of chlorophyll that can also be observed in the form of necrosis of the leaves. The carotenoids that act as a secondary anti-oxidants were seen to increase in the plants. Cell can also be protected from reactive oxygen species by the combined action of enzymatic antioxidant systems like catalase (CAT: EC1.11.1.6), peroxidase (POD: EC 1.11.1.7) and non-enzymatic antioxidant like ascorbate, glutathione and phenolic compounds. In present study, peroxidase and catalase enzyme activity was increased under metal stress condition. These enzymes remove superoxide radicals, which are harmful to cell membranes. Peroxidase activity and photosynthetic pigments are sensitive indicators of heavy metal stress on the plants. The amino acid proline content is an indicator of high metal accumulationand current study showed the increase in proline.

The bioaccumulation coefficient (BAC) analysis of the plants can be used to categories plants as Phyto accumulator (Garg and Kataria,2009). Different categories can be as follows:
1] High accumulator for value of BAC between 1-10.
2] Moderate accumulator for 0.1-1 and
3] Non-accumulator for value of 0.01.

Based on this study of BAC of Okra plant for the metal Copper and Lead, it can be concluded that the plant is a high accumulator of lead and a moderate accumulator of copper. Considering the metal accumulation capacity, current investigation suggests that Okra can be used effectively for phytoremediation process as plant has an ability to clean up the contaminated soil by the accumulation of metal element in its various parts.

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