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Comparative Assessment of Cadmium, Chromium Resistance Capacity of *Amaranthus viridis*, *Trianthema portulacastrum*, *Alternanthera philoxeroides* Collected from Contaminated Site and Assessment of Phytoremedial Potential

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Abstract Among heavy metals cadmium and chromium pollution of soil is an alarming environmental issue as these metals are released in the atmosphere by anthropogenic activity. Their various use in industry, agriculture and domestic applications have led to their accumulation in the environment. Being biologically non essential both these metals are phytotoxic and injurious to animal also. Plants with greater capacity of metal accumulation are potential candidate of phytoremediation for cleanup of heavy metal contaminated soil. As, identification of metal tolerant native plant is prerequisite for phytoremediation, field studies were conducted to different Cd, Cr contaminated sites to assess metal tolerance of various plants. Comparative study was done with three different species (*Amaranthus viridis*, *Trianthema portulacastrum*, *Alternanthera philoxeroides*), collected from metal contaminated sites of Kolkata. Metal concentration of these plants and various biochemical parameters (lipid peroxidation, proline, non protein thiol content, SOD, glutathione reductase activity) were studied to assess metal detoxification capacity. Soil properties were studied to determine metal bioavailability. Alligator weed (*Alternanthera philoxeroides*), was found to be most abundant with considerable metal accumulation capacity and biochemical defences in comparison to others plants. To assess phytoremediation potential, this plant was potted in laboratory condition with artificial treatment of different concentration of Cd, Cr and metal uptake was estimated. Phytoextraction capacity was estimated also.

Keywords Cadmium, Chromium, Phytoremediation, Glutathione reductase, Non protein thiol content.

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Introduction

In recent years heavy metal pollution of soil is a global concern for its negative impact on our ecosystem. Cadmium and chromium contamination of soil and groundwater is a general health hazard throughout India. According to Agency of Toxic Substances and Disease Registry [1] Cd and Cr occupies 7^{th} and 77^{th} position respectively in the list of most hazardous substances of the world. Anthropogenic activity is responsible for excess production and accumulation of these metals in agricultural land also. These metals are not only phytotoxic after crossing a threshold level but also completely biologically non essential. Industrial sources of Cd and Cr include electroplating, leather tanning, phosphate fertilizer and Cd battery production unit, ceramic and soft drinks factories. Though Cr exists naturally in trivalent (Cr III) and hexavalent (Cr VI) forms, but Cr VI is more phytotoxic for its higher mobility and water solubility. Cd is a non redox metal still it produces considerable amount of reactive oxygen species which is detrimental for both animal and plant cells. Both of them are mutagenic as well as carcinogenic. Cd is involved in kidney and prostate cancer ; also in lung damage especially for chain smokers as Cd is used in cigarette paper for slow burning. Cr is potentially harmful to liver and kidney. Soil contamination of Cd and Cr almost affect all the metabolisms of a plant starting from chlorosis, abnormal nitrogen metabolism, mineral uptake, impaired photosynthesis and respiration even into cell death. Weed plants are more tolerant to these metals than crop plants ; due to their acute endurance and better capacity to uptake and scavenge heavy metals. Plants have various antioxidative mechanisms that ameliorate the excess toxic load and oxidative stress induced by Cd and Cr pollution. Our work provides a comparative estimation of metal uptake and tolerance capacity of different species growing in Cd and Cr polluted sites in and around Kolkata and elaborate *ex situ* study of metal accumulation and phytoextraction capacity of the most potential candidate, *A. philoxeroides*.

Materials and Methods

Specimen

Trianthema portulaccastrum : Common name Giant pigweed is an invassive annual herb of Aizoaceae family and forms a prostrate mat or clump. It grows in a wide variety of habitat types and it can easily take hold in disturbed, polluted lands.

Amaranthus viridis : Common name Green amaranth/ note shak is an edible annual herb, cosmopolitan species of Amaranthaceae family, eaten in various parts of India and preferably grows on marshy land.

Alternanthera philoxeroides (Mart.) Griseb : (Family-Amaranthaceae), common name. Alligator weed is a stoloniferous herb, widely grows in aquatic and terrestrial habits also. This fast growing weed has been found to grow profusely in soil of town and suburbans contaminated with heavy metals and in certain areas no vegetation other than this weed were found. It propagates by vegetative means as rarely produced seeds are non–viable.

Field work

Three experimental sites situated in urban areas of Kolkata metropolis were selected randomly. All these sites are localized in the close vicinity of industries that emit considerable amount of Cd and Cr in the environment contaminating soil and ground water. Such as factories of soft drink, leather tanning and Ni-Cd battery recycling unit. Some studied areas are co contaminated with both of these two metals. Soil of certain semi rural areas (Site III) which are about 30 km away from the main city are heavily Cd-contaminated where dry cell batteries are recycled and scrap material is dumped in the open areas adjacent to the agricultural field.

Though all these three specimens were common in those experimental areas but Alligator weed is the most abundant species (Quadrat study was done) among them with robust growth. In addition with soil sample, plant samples were collected and separated into root and shoots, washed, oven dried and metal assay was conducted. Soil samples were collected (3 replicas) from the sites at a depth of 15 cm. Plants collected from an uncontaminated site is considered as control plant and maintained in the laboratory garden.

Metal assay

Metal analysis of the plant and soil samples were carried out by acid digestion (3 : 1 conc HNO_3 and conc HCl v/v) of accurately 1 g of plant tissue and soil sample respectively. Metal concentration was measured by AAS (Atomic Absorption Spectrophotometer) [2].

Translocation factor and Bioconcentration factor were calculated from the result of metal analysis as these two parameters are immense important to ascertain the metals accumulation and uptake capacity of a plant from soil to the above ground part.

BCF (Bioconcentration factor): Metal concentration in root/metal concentration in soil [3], TF (Translocation factor): Metal concentration in shoot / metal concentration in root.

Table 1. Metal assay of different plants (*Alternanthera philoxeroides*, *Trianthema portulacastrum*, *Amaranthus viridis*) collected from contaminated sites (I, II, III). TF : Translocation factor, BCF : Bioconcentration factor.

Site	Soil metal mg/kg		Plants	Shoot metal mg/kg	
I	Cr	Cd		Cr	Cd
Adjoining area of Tannery industry	1833 ± 1.47	1.46 ± 0.31	*Alternanthera philoxeroides*	178.7 ± 0.92	0.95 ± 0.05
			Trianthema portulacastrum	52.2 ± 1.2	0.45 ± 0.87
			Amaranthus viridis	23.5 ± 0.76	0.26 ± 0.54
II					
Adjoining area of soft drinks factory	883.7 ± 1.22	BDL	*Alternanthera philoxeroides*	107.3 ± 0.66	–
			Trianthema portulacastrum	57.8 ± 1.1	–
			Amaranthus viridis	38.6 ± 1.04	–
III					
Adjoining area of battery re-cycling unit	64.4 ± 0.79	44.8 ± 0.8	*Alternanthera philoxeroides*	23.55 ± 0.5	18.6 ± 0.36
			Trianthema portulacastrum	17.6 ± 1.1	10.3 ± 0.58
			Amaranthus viridis	11.77 ± 0.68	BDL

Table 1. Continued.

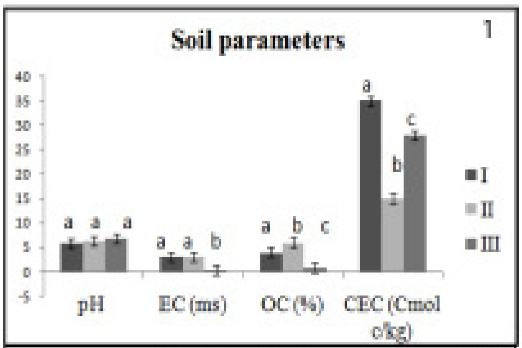
	Root metal mg/kg		TF		BCF	
I	Cr	Cd	Cr	Cd	Cr	Cd
Adjoining area of Tannery industry	164.3 ± 0.60	0.99 ± 0.2	1.08	0.96	0.09	0.67
	67 ± 1.3	0.63 ± 0.8	0.78	0.71	0.04	0.43
	31.7 ± 0.65	0.38 ± 0.6	0.74	0.68	0.017	0.26
II						
Adjoining area of soft drinks factory	135.7 ± 0.62	–	0.8	–	0.15	–
	83.6 ± 0.7	–	0.69	–	0.09	–
	66.3 ± 1.2	–	0.58	–	0.075	–
III						
Adjoining area of battery recycling unit	36.33 ± 0.75	28.9 ± 0.4	0.64	0.64	0.56	0.64
	26 ± 0.92	19.7 ± 0.5	0.55	0.6	0.4	0.44
	20.34 ± 0.8	BDL	0.57	–	0.32	–

Lipid peroxidation

Using thiobarbituric acid (TBA), the amount of malondialdehyde (MDA) content is determined to estimate lipid peroxidation. 0.5g leaves were crushed in 5 ml of 0.1% TCA and centrifuged for 5 min at 1000 rpm. For every 1 ml of aliquot, 4 ml of 20% TCA containing 0.5% thiobarbituric acid was added and heated at 95°C for 30 min and quickly cooled on an ice bath. The resulting mixture was centrifuged at 10000 g for 15 min and the absorbance was taken at 532 nm and 600 nm. The non specific absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of MDA was calculated by using the extinction coefficient of 155/mM/cm.

Estimation of free proline

Free proline amount was fetermined (µg/g tissue) from a previously prepared standard curve. 500mg leaves were homogenized in 5ml 0.1M sulfosalicylic acid and centrifuged at 5000 rpm for 30 minutes. To the super-



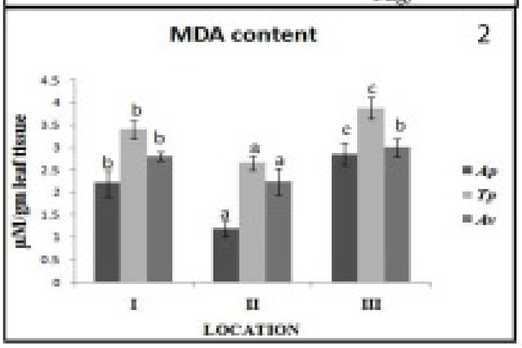


Fig. 1. Comparative analysis of soil parameters (pH, EC, OC, CEC) of experimental sites. **Fig. 2.** Comparative analysis of MDA content of *Alternanthera philoxeroides* (*Ap*), *Trianthema portulacastrum* (*Tp*) and *Amaranthus viridis* (*Av*) of contaminated sites.

natant (2ml), 5ml glacial acetic acid, 5ml ninhydrinsoln were added and heated at boiling water bath for 1 hour. The mixture was extracted with toluene and absorbance was taken at 520 nm.

Estimation of non protein thiol content (NP-SH)

0.5g fresh samples were homogenized in 5 ml of 5% meta-phosphoric acid and centrifuged at 12000 rpm. Reaction mixture was prepared containing 0.5 ml plant extract, 2.5 ml of 150mM phosphate buffer (pH 7.4), 5 mM EDTA, 0.5ml 6mM 2-nitro benzoic acid. Following incubation at RT, the OD was measured at 412 nm. Calculation was done from the standard curve of reduced GSH.

Assay of antioxidative isozymes

Glutathione reductase (*GR*)

50 mM Tris HCl buffer (pH 7.6) was used to homogenize 200 mg leaf and centrifuged at 14000 rpm for 20 min. The reaction mixture in total volume of 1 ml contained 50 mM Tris–HCl buffer pH 7.6, 0.15 mM NADPH, 1mM GSSG (oxidized glutathione), 3mM $MgCl_2$ and 200 µl enzyme extract. Decrease in absorbance of NADPH at 340 nm was monitored carefully by taking OD of reaction mixture. The specific activity of enzyme was expressed as µmol NADPH oxidized / min / mg protein.

Superoxide dismutase (*SOD*)

One unit of activity of SOD is the amount of protein required to inhibit 50% initial reduction of nitroblue tetrazolium under light. 3 ml reaction mixture was prepared with 50 mM potassium phosphate buffer (pH 7.8). 13 mM methionine, 75 µM nitroblue tetrazolium, 2 µM riboflavin, 0.1 mM EDTA and a suitable aliquot of enzyme extract. The test tubes were kept 30 cm below light source consisting of 15W fluorescent lamp for 20 min then the OD at 560 nm was recorded. The activity of SOD was expressed as unit per milligram protein.

Soil analysis

The bioavailability of metals to plant is dependent on different soil parameters. Soil parameters such as organic carbon (OC), cation exchange capacity (CEC) and electric conductivity (EC) and pH were measured following standard protocols.

Pot culture experiments

After determination of Cd, Cr content in three specimens, *A. philoxeroides* was chosen for further *ex situ* study due to its maximum metal uptake capacity in comparison to other two plants. Seedlings of these plants were collected from Cd and Cr contaminated areas and potted in departmental garden. These seedlings were allowed to grow for one month then they were treated with various concentrations of Cd (cadmium chloride) and Cr (potassium dichromate) [0.5

Table 2. Metal concentration of soil and different parts of (Roots and Shoots) *Alternanthera philoxeroides* treated under laboratory condition. The data represents means ± SD of three independent replications.

Conc (mM)	Soil mg/kg	Root mg/kg	Cr Shoot mg/kg	TF	BCF
Conc	0.033 ± 0.015	BDL	–	–	–
0.5	25.6 ± 3.2	28.38 ± 2.54	26.38 ± 1.27	0.93	1.11
0.8	41.6 ± 2.8	39.53 ± 1.33	35.53 ± 0.63	0.898	0.95
1.0	52.0 ± 1.1	46.13 ± 1.2	47.31 ± 0.91	1.025	0.89
1.2	62.0 ± 2.2	51.81 ± 0.8	53.13 ± 1.0	1.02	0.84
1.5	80.0 ± 1.98	69.85 ± 1.73	46.48 ± 1.3	0.665	0.88
1.8	93.7 ± 1.2	73.77 ± 0.82	32.31 ± 1.0	0.505	0.79

Table 2. Continued.

Conc (mM)	Soil mg/kg	Root mg/kg	Cd Shoot mg/kg	TF	BCF
Conc	–	–	–	–	–
0.5	56.3 ± 1.8	29.98 ± 1.31	22.63 ± 2.51	0.75	0.54
0.8	90.0 ± 2.3	86.71 ± 0.55	107.8 ± 0.789	1.24	0.97
1.0	112.5 ± 1.67	98.11 ± 1.2	117.3 ± 0.916	1.2	0.88
1.2	135 ± 3.3	73 ± 2.17	67.91 ± 2.15	0.93	0.54
1.5	168.7 ± 2.0	87.162± 1.56	59.51 ± 1.46	0.68	0.52
1.8	202.34± 1.5	93.58 ± 0.97	36.38 ± 1.04	0.38	0.47

mM, 0.8 mM, 1 mM, 1.2 mM, 1.5 mM, 1.8 mM] individually and synergistically. Plants collected from uncontaminated sites were considered as control.

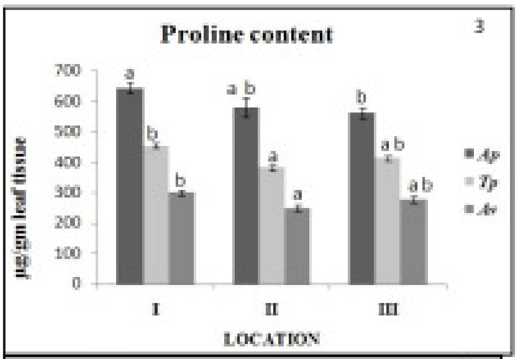
Statistical analysis

All experiments were performed in random repetition of triplicates. All datasets obtained from the experiments were analyzed with ANOVA followed by Tukey's Multiple Range Test (TMRT) for multi comparisons of means. Significance level were compared at $p < 0.05$. All results were expressed as means, with corresponding standard deviation.

Results and Discussion

Metal uptake capacity

Soil of adjoining areas (Table 1) of tannery industries is co-contaminated with Cd and Cr, but the Cr concentration of soil (1833 mg/kg) far exceeds the maximum permissible limits (300 mg/kg) as huge Cr is used for tanning of leather. Among three considered specimens *Alternanthera philoxeroides* showed maximum Cr uptake (178.7 mg/kg in shoot) evident from TF and BCF values which are higher than other two plants of that same sites. Site II was contaminated with effluents of soft drinks factory with below detectable amount of Cd, but quite moderate amount of Cr in soil (883.7 mg/kg) as Cr has been used as a coloring agent of soft drinks. Only *A. philoxeroides* showed TF closer to 1 proving that Cr has been translocated from root to shoot in considerable amount. But *Trianthema portulacastrum* showed TF value intermediate of other specimen while *A. viridis* had lowest TF. *A. philoxeroides* of Site III translocated both metals from root to shoot with same efficiency evident from same TF value (0.64). As metal bioavailability depends on the edaphic factors ; soil pH, EC, OC and CEC (cation exchange capacity) so these soil parameters were studied (Fig. 1). The pH of soils collected from different locations varies from 5.87–6.75. CEC (cation exchange capacity) that measures the cation retention capacity as well as water holding capacity of soil particle is highest in soil of location 1 and lowest in soil of location II. Acidic pH and higher CEC enhance metal bioavailability [4, 5]. As lowest CEC causes leaching out of ions, so high-



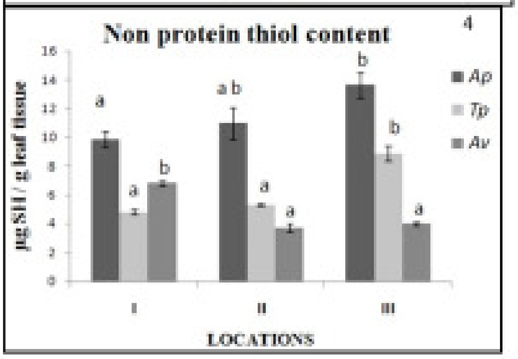


Fig. 3. Comparative analysis of proline content of *Alternanthera philoxeroides* (*Ap*), *Trianthema portulacastrum* (*Tp*) and *Amaranthus viridis* (*Av*) of contaminated sites. **Fig. 4.** Comparative analysis of non protein thiol content of *Alternanthera philoxeroides* (*Ap*), *Trianthema portulacastrum* (*Tp*) and *Amaranthus viridis* (Av) of contaminated sites.

est CEC and acidic pH of soil of location I somehow supported highest Cr uptake in *A. philoxeroides* [6].

From *ex situ* study of metal accumulation of Alligator weed (Table 2) it was observed that root Cr concentration had a linear increase with soil Cr concentration (28.38 mg/kg to 73.77 mg/kg root Cr corresponds to 25.6 mg/kg to 93.7 mg/kg soil Cr). But in contrast, Cr uptake by shoot was not linearly proportional to the soil Cr concentration. Upto 1.2 mM of soil Cr concentration (62 mg/kg soil) shoot Cr increased then declined sharply. At soil treatment of 1 mM and 1.2 mM Cr, this plant behaved as hyperaccumulator, which is evident from TF >1 [7, 8, 9] i.e. Cr uptake of shoot exceeds the Cr content of root.

For Cd, the accumulation of root and shoot didn't follow concentration dependent linear increase with soil Cd. Highest root accumulation was recorded at 1 mM concentration (98.11 mg/kg root Cd at 112.5 mg/kg soil Cd), then declined at 1.2 mM and insignificant increase was observed at 1.8mM concentration of Cd (93.58 mg/kg root Cd at 202.34 mg/kg soil Cd). Shoot accumulation of Cd showed significant variation with soil Cd amount. Highest shoot accumulation was found at 1mM treatment (117.3 mg/kg shoot Cd) ; then slowly decreased. Interestingly, in 0.8 mM and 1 mM treatment shoot accumulation far exceeds the root accumulation (TF > 1). So it can be concluded that in certain condition this plant has the potentiality to be have as hyperaccumulator. From this study it is quite obvious that *A. philoxeroides* can tolerate considerable amount of soil Cd (phytotoxic level–Cd: 5–20 mg/kg [10], Cr: 5–30 mg/kg ; [11] in artificial *ex situ* condition, but remarkable Cr tolerance was recorded from field study (1833 mg/kg soil Cr).

Heavy metal accumulation capacity of a higher plant is judged by its BCF (Bioconcentration factor) i.e. the ratio of metal concentration in the roots to that in the soil [3] and its ability to translocate metals from underground parts to the shoot is measured by TF (Translocation factor) i.e. ratio of metal concentration in the shoots to the roots. Plants collected from site I (area contaminated with tannery effluents) had TF values > 1 for Cr and nearer to 1 for Cd. All other plant populations had low TF and BCF values with regard to the absorption of Cr and Cd indicating its limited capacity for accumulation and translocation of Cr and Cd.

Bioaccumulation and bioavailability is regulated by several edaphic factors, such as chemical speciation of the metal, soil pH, organic chelators, cation exchange capacity, synergistic and antagonistic effects of other metals and anions [5]. So metal uptake pattern may vary between *in situ* and *ex situ* study of same plant sample and same metals (Cd, Cr) concerned which is quite conspicuous in our study. Plants treated with 1 mM and 1.2 mM Cr behaved as metal accumulator which is evident by TF value (TF > 1). Further increase in soil Cr concentration (1.5 mM, 1.8 mM), reduced metal uptake and accumulation capacity suggested that 1 mM and 1.2 mM soil Cr concen-

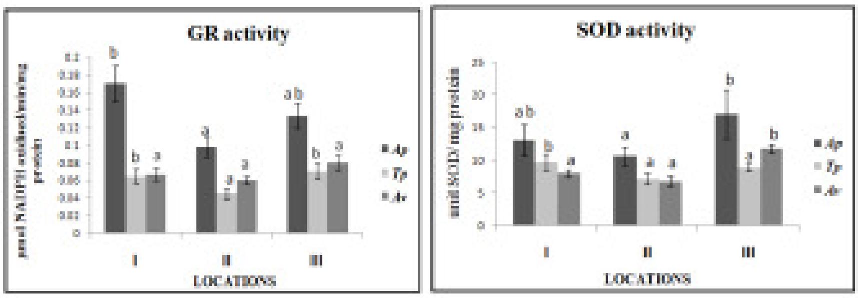


Figure 5a Figure 5b

Fig. 5. Comparative analysis of antioxidative isozymes: GR activity (5a) and SOD activity (5b) of *Alternanthera philoxeroides* (*Ab*), *Trianthema portulacastrum* (*Tp*) and *Amaranthus viridis* (*Av*) of contaminated sites.

tration was the optimum tolerance level. Other than 1 mM and 1.2 mM Cr treated plant, all other Cr treated plants showed better Cr accumulation in roots. This is because considerable amount of Cr could be scavenged in root cell vacuoles that results lesser toxic response of plant.

At 0.8 mM and 1 mM Cd treatment this plant satisfied the criteria of hyperaccumulator for Cd, regarding shoot metal accumulation (> 100 mg/kg) and TF > 1. But in all other concentrations root Cd content (Table 2) was higher than shoot making it suitable for phytoextraction rather than labeling this plant as Cd hyperaccumulator. Our result was also corroborated with the findings of different workers [12], that during artificial Cd treatment, roots accumulate more Cd than shoots. Metal tolerant plants with considerably higher TF and BCF value are potential candidate for phytoaccumulation [3]. Few worker [13] studied the response of *A. philoxeroides* leaves to cadmium stress and reported that bioaccumulation of Cd was concentration dependent, which is in accordance with our findings. As it is impossible to create the exact field condition in the laboratory, there was a disparity of the metal accumulation property of *A. philoxeroides* between *in situ* and *ex situ* condition. But the potential Cd, Cr tolerance of this weed is unquestionable.

Phytoextraction efficiency of *Alternanthera philoxeroides* for Cd

Assuming that Cd phytoextraction follows a linear pattern, the quantity of Cd extracted per hectare per year (Qcd : kg Cd $ha^{-1}y^{-1}$) can be expressed as

$$Q_{cd}=(10^{-3} \times b_{DW} \times D)\times(10^{-6} \times [Cd]_{DW}) \times C$$

b_{DW} – Dry weight of plant biomass per plant (g $plant^{-1}$ DW) ; D–Density of plant per hectare ; $[Cd]_{DW}$ – Total Cd concentration measured in plants (mg Cd / kg DW) ; C-Number of plants per year] [14].

By applying this equation from the present study it was calculated that the quantity of Cd extracted per hectare per year (Qcd : kg Cd $ha^{-1}y^{-1}$) is 3.424 kg Cd $ha^{-1}y^{-1}$ considering the parameters from Site III because it has highest Cd concentration in soil (44.82 mg/kg).

(b_{DW} = 6 g/plant), D=400×10^4 plants/hectare ;
$[Cd]_{DW}$ = 47.56 mg/kg ; C=3 crops/year

Phytoextraction efficiency of *Alternanthera philoxeroides* for Cr

Assuming that Cr phytoextraction follows a linear pattern, the quantity of Cr extracted per hectare per year (Qcr : kg Cr $ha^{-1}y^{-1}$) can be expressed as

$$Q_{Cr} = (10^{-3} \times b_{DW} \times D) \times (10^{-6} \times [Cr]_{DW}) \times C$$

b_{DW} – Dry weight of plant biomass per plant (g $plant^{-1}$ DW) ; D–Density of plant per hectare ; $[Cr]_{dw}$ – Total Cr concentration measured in plants (mg Cr / kg DW) ; C-Number of plants per year] [14].

By applying this equation from the present study it was calculated that the quantity of Cr extracted per hectare per year (Qcr : kg Cr $ha^{-1}y^{-1}$) is 49.402 kg Cr $ha^{-1}y^{-1}$ considering the parameters from Site I because it has highest Cr concentration in soil (1833 mg/kg).

(b_{DW} =8 g/plant), D=600×10^4 plants/hectare ; $[Cr]_{DW}$ = 343.07 mg/kg ; C=3 crops/year.

Biochemical parameters

Lipid peroxidation is the indication of membrane damage, which can be initiated by Active Oxygen Species or by the action of lipoxygenase [15]. Estimation of malondialdehyde (a cytotoxic product of lipid peroxidation) acts as vital parameter of oxidative stress due to heavy metal toxicity. Among plants collected from three experimental sites *T. portulacastrum* showed highest MDA content reflecting the maximum membrane damage, while *A. philoxeroides* showed the lowest and intermediate reading was found in *A. viridis* (Fig. 2).

Proline and non protein thiol (NPSH) are two important non enzymatic ROS scavenging mechanisms of plants. Free proline accumulation is the initial step of heavy metal toxicity. Though the production and accumulation of free proline which acts as an osmoprotectant, protein stabilizer, metal chelator ; OH^- scavenger and inhibitor of lipid peroxidation [16, 17]. The metal detoxification capacity of *A. philoxeroides* was attributed to the highest production of free proline (Fig. 3) and NPSH (Fig. 4) and minimum MDA content in field condition. Overproduction of proline might have provided the antioxidative defence against the generation of ROS under Cd and Cr stress. Our result is in good accordance with the findings of previous workers [18, 19].

Glutathione is a major non protein thiol (NPSH) and excess formation of glutathione is the prerequisite for the synthesis of Cd scavenger peptide phytochelatins. Maximum NPSH content of *A. philoxeroides* reflected greater amount of NPSH synthesis (Fig. 4) during co contamination of Cd Cr which is responsible for its better ability to resist cellular metal load, which might be due to the promotion of PC biosynthesis. Maximum NPSH was found from Alligator weed of site III, highly contaminated with Cd. This is in conformity with the documentations of other workers [20, 21] that Cd is more potent inducer of NP-SH. The NPSH content of *T. portulacastrum* and *A. viridis* collected from various sites show insignificant variations in readings.

Superoxide dismutase (SOD) and GR (Glutathione reductase) are antioxidative enzymatic defence of the plant. Superoxide dismutase plays a pivotal role in the first line of defense against ROS, reducing the oxidative stress by the dismutation of two superoxide radical to H_2O and O_2. H_2O_2 is further converted to H_2O and O_2 by peroxidases. GR is an important member of ascorbate–glutathione cycle that acts as antioxidative defence in any type of oxidative damage. For uninterrupted supply of phytochelatin, GSH pool must be maintained continuously which is done by GR [22].

The highest GR (Fig. 5a) and SOD activity (Fig. 5b) of *A. philoxeroides* supported its detoxification capacity of heavy metals in comparison to *T. portulacastrum* and *A. viridis.* This biochemical defences are responsible for its immense growth inspite of soil heavy metal load. Our result is in good accordance with the concentration dependent increase of SOD activity of Indian mustard [23], proving that metal accumulators are well adapted with better SOD activity conferring ROS scavenging capacity [15, 24]. During oxidative damage induced by Cd and Cr these antioxidative isozymes (SOD, GR) contribute to nullify the membrane damage evident by low MDA with higher proline, NPSH, SOD and GR activity in Alligator weed.

From, the study this can be interpreted that plants grown in contaminated sites are adapted to high heavy metal load by their various biochemical detoxification system. Alligator weed has considerable metal accumulation capacity, though not proven to be hyperaccumulator but a potential candidate for phytoextraction.

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