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SCREENING OF CHROMIUM TOLERANCE POTENTIAL OF FEW WEEDS OF KOLKATA AND ASSESSMENT OF PHYTOEXTRACTION EFFICIENCY

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ABSTRACT

Soil polluted with heavy metals due to anthropogenic activities remains a great problem all over the globe especially in the developing countries. Agricultural processes, industrialization and domestic activities cause the increase of heavy metals in soils. Risk from the hexavalent chromium has become a worldwide concern. Chromium causes adverse impact on plant and animal health. The present study deals with the comparative estimation of Cr tolerance capacity of five common weed species (*Amaranthus viridis*, *Alternanthera philoxeroides*, *Euphorbia hirta, Solanum nigrum*, *Portulaca oleracea*) of Kolkata collected from Cr contaminated leather tanning industrial area of Bantala and car painting factory of Budgebudge. Among these Amaranthaceae family was found to be the most Cr tolerant species attributed to their enzymatic and non enzymatic antioxidative defence. Calculative analysis of phytoextraction potential of *A. philoxeroides* was established.

KEY WORDS : Accumulator, Translocation factor, Bioconcentration factor, Antioxidants, chromium

INTRODUCTION

In recent years, contamination of soil by chromium has become a major global concern. The soil environment is a major sink for multitude of chemicals and heavy metals, which inevitably lead to environmental contamination problems. Though Cr exists predominantly in the +III and +VI oxidation states, but due to higher mobility and water solubility Cr VI is more phytotoxic. Contrarily to Cr(VI), Cr(III) is considered as micronutrient in humans, being necessary for sugar and lipid metabolism (ATSDR) and is generally not harmful. Generally, Cr in soil represents a combination of both Cr(III) and (VI). The oxidants present in the soil (e.g., dissolved oxygen and MnO2) can oxidize Cr(III) to Cr(VI) (Oliveira, 2012). Hexavalent chromium are introduced into the environment due to various industrial processes including metallurgy, electroplating, production of paints and pigments, tanning, wood preservation, chemical production, pulp and paper production (Zayed and Terry, 2003). Being non biodegradable Cr is persistent environmental contaminants which may be deposited on the surfaces and then absorbed into tissues of vegetables. Cr into the environment creates unfavourable outcomes by altering the normal physiochemical properties of soil and water. Weed species appears to be a good choice for metal accumulation study since these hardy, tolerant species can grow in most harsh conditions and give a good amount of biomass as a secondary product. Cr is the principle threat of tannery industries. These industries of India alone contributes about 2000-3000 tons of Cr contamination to the environment in which Cr concentration ranges from 2000-5000 mg/L in the aqueous effluent (Dhal *et al*., 2013). Indiscriminate disposal of tannery waste is resulting in widespread Cr contamination of both soil and groundwater in the area of Bantala Leather complex, situated 15 km away on the southern periphery of Kolkata, and is a living hell. Cr is extremely venomous and carcinogenic (Alka *et al*., 2017). Cr produces considerable amount of reactive oxygen species which is detrimental for both animal and plant cells. Some of the direct toxic effects caused by high Cr concentration in soil include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress. Soil contamination by 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. Cr disturbs redox homeostasis by stimulating the formation of excessive ROS, such as hydroxyl radical ($OH^{.}$), singlet oxygen ($O^{.}$), superoxide (O_2) and hydrogen peroxide (H_2O_2). These toxic effects (both direct and indirect) cause lipid peroxidation, protein oxidation, damage to nucleic acids, and decline in plant growth which even results in the plant death. Plants have various antioxidative mechanisms that ameliorate the excess toxic load and oxidative stress induced Cr pollution.

The present study was conducted for a comparative estimation of Cr accumulation and tolerance capacity of different weed species growing in Cr polluted leather tanning industrial site of Bantala and car painting industrial area of Budgebudge. Few weeds were found common to be grown these two sites. These test species are most dominant indigenous weed species. These were collected for metal assay and were subjected for study of antioxidative defence against Cr toxicity. An attempt was made to explore the phytoextraction potential of the most promising Cr tolerant Alligator weed (*A. philoxeroides*) as it was found to be the highest accumulator of Cr among all weeds of both experimental sites. This in situ study could be a stepping stone for identification of Cr tolerant native plant which can be further explored for phytoremediation purpose.

MATERIALS AND METHODS

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.

Solanum nigrum: Black nightshade of solanaceae family, native to Eurasia. It usually grows as weed in moist soil and has been reported as agricultural weed of 37 crops in 61 countries. Plant is glabrous to slightly hairy with non glandular hairs.

Euphorbia hirta: It is a pantropical weed of euphorbiaceae family, native to India. This is a erect annual, hairy herb, grows in open grasslands, roadsides and pathways. It is used in traditional herbal medicine.

Portulaca oleracea: It is an annual succulent of Portulacaceae, which may reach 40 cm in height. It is an exotic weed native to Europe. It has smooth, reddish, prostrate stem with alternate or oppositely arranged leaves. It is tolerant to poor compacted soil and drought.

Field work

Two 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 Cr in the environment contaminating soil and ground water. The study site (I) was situated at Kolkata Leather Tanning Complex, Bantala, East Kolkata, West Bengal, India. Random plots were selected in this area for collection of native weed plants grown in the close vicinity of dumped solid tannery waste. Site II was area of car painting factories of Budgebudge, Maheshtala which use Cr (VI) in paint manufacturing.

Though all these specimens were common in two experimental areas but members of amaranthaceae family (*A. philoxeroides and A. viridis*) were the most abundant species (Quadrat study was done) with luxuriant 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.

Metal assay

Total Cr analysis of the plant and soil samples were carried out by tri-acid digestion (HNO_3: $HClO_4$:H_2SO_4 = 10:40:1) and determined by AAS (Atomic Absorption Spectrophotometer) with of accurately 5 g and 10 g of plant tissue and soil sample respectively (APHA).

Metal concentration was measured by Translocation factor and Bioconcentration factor were calculated for all experimental plants 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, TF (Translocation factor): Metal concentration in shoot / metal concentration in root (Yoon *et al*., 2006).

Lipid peroxidation

Using thiobarbituric acid (TBA), the amount of malondialdehyde (MDA) content is determined to estimate lipid peroxidation according to the standard protocol of Heath and Packer (1968). 0.5g leaves were crushed in 5 mL of 0.1% TCA and centrifuged for 5 min at 1000 rpm. For every l 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 determined (µg/g tissue) from a previously prepared standard curve following the methods of Bates *et al.,* (1973). 500mg leaves were homogenized in 5 mL 0.1M sulfosalicylic acid and centrifuged at 5000 rpm for 30 minutes. To the supernatant (2 mL), 5 mL glacial acetic acid, 5 mL ninhydrin solution 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)

It was estimated by the standard protocol of Cakmak and Marchner **(**1992). 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 150 mM phosphate buffer (pH 7.4), 5 mM EDTA, 0.5 mL 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.

Estimation of endogenous hydrogen peroxide content

Leaf tissue (0.5 g) was homogenized with 3ml of 1% (w/v) TCA in an ice-bath. The homogenate was centrifuged at 10000 rpm for 10 minutes and the supernatant 0.5mL was added with 0.75 mL of 50mM potassium phosphate buffer (pH 7) and 1.5 mL of 1M potassium iodide (KI) and the absorbance was measured at 390nm. H_2O_2 content was expressed as nmol H_2O_2 g^{-1} of fresh weight tissue (Jessup *et al.*, 1994).

Assay of antioxidative enzymes

Estimation of Catalase Activity

0.5 g of fresh leaf was homogenized in 0.067M phosphate buffer (pH 7) for estimation of catalase activity according to the method of Woodbury *et al*., (1971**)**. The homogenate was centrifuged at 12000 rpm for 30 minutes at 4 °C and the supernatant was used for the enzyme assay. In 1 mL of the reaction mixture containing potassium phosphate buffer (pH 7), 250 µL of enzyme extract and 60 Mm H_2O_2 was used to initiate the reaction. The readings were taken for 3 minutes at an interval of 10 seconds at 240nm in a spectrophotometer. The specific activity of the enzyme was calculated using molar extinction coefficient, $\varepsilon = 39.4\ mM^{-1}cm^{-1}$.

Estimation of Guiacol Peroxidase Activity

The guiacol peroxidase activity was measured according to the protocol of Fang and Kao, (2000). Reaction mixture (3 mL) containing 50Mm phosphate buffer (pH 5.8), 1.6 µL H_2O_2, 1.5 µL guiacol and 0.2 ml enzyme extract. The GPX activity was measured at 770 nm and then calculated using the extinction coefficient 26 $mM^{-1}cm^{-1}$ for tetraguiacol and was expressed in mol tetraguiacol/ min/mg tissue.

Superoxide dismutase

The activity of SOD was measured following the stardard method of Bauchamp and Fridovich (1971) and was expressed as unit per milligram protein. 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.

Glutathione reductase

GR activity was measured as described by Schaedle and Bassham (1977). 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 MgCl2 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.

Soil analysis: The bioavailability of metals to plant is dependent on different soil parameters. The pH and electric conductivity (EC) of soil samples were measured using digital pH and EC meters (Systronics). Other Soil parameters such as organic carbon (OC), cation exchange capacity (CEC) were measured by following standard protocols of Walkley and Black (1934)

Statistical analysis: All experiments were performed in random repetition of triplicates. Assigning different plot of tannery sludge as treatment factor, all datasets obtained from the experiments were analyzed with analysis of varience (ANOVA) followed by Tukey's Multiple Range Test (TMRT) using SPSS 11.0 statistical package for multi comparisons of means. Significance level was compared at $p < 0.05$. All results were expressed as means, with corresponding standard deviation.

RESULTS AND DISCUSSION

The soil samples from the contaminated site were collected ~15 m from a waste Cr(VI) heap of tanning plant in Bantala, Kolkata which use chromium salts, specially Cr alum and Cr sulphate for tanning of leather as Cr stabilizes the leather by cross linking of collagen fibre. So soil of adjoining areas (Table 2) of Bantala leather tanning industries (experimental site I) is highly contaminated with Cr (1141.8 mg/kg), that far exceeds the maximum permissible limits (300 mg/kg). 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 (Yoon *et al.*, 2006) 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 (Yoon *et al.* 2006). These parameters are usually used to evaluate plant ability to tolerate and accumulate heavy metals. Plants exhibiting BCF > 1 are suitable for phytoextraction, and plants with TF< 1 have the potential for phytostabilization (Rafati *et al.*, 2011). Among five considered specimens *Alternanthera philoxeroides* collected from site I showed maximum Cr uptake (289.8 mg/kg in root) evident from TF and BCF values (Table 2) which are higher than other plants of that same location. Site II was contaminated with effluents of car paint factory. The chemical plant produced potassium dichromate, which is a very common contributor of soil Cr (517.64 mg/kg) as Cr has been used as a coloring agent of commercial paint. Only *A. philoxeroides* showed highest TF closer to 1 (Table 2) proving that it is most capable of translocating Cr from root to shoot in considerable amount (Khankhane *et al.*, 2014). Second highest TF was reported in another member of amaranthaceae *A. viridis*. Our finding is in good accordance with Zou *et al.*, (2006) where *A. viridis* was found to be translocating higher amount of Cr in shoot from root. But *Portulaca oleracea* was not able to transport Cr to the shoot evident from its non detectable TF. *Solanum nigrum* and *E. hirta* showed insignificant Cr translocation as reflected by their compromised intermediate TF value. 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 so few soil parameters were studied such as ; soil pH, EC and CEC (cation exchange capacity). Among soil properties, soil pH had the greatest impact on the desorption and bioavailability of heavy metals, because of its strong effects on solubility and speciation of heavy metals both in the soil as a whole and particularly in the soil solution (Ibrahim *et al.*, 2017). The pH of soils collected from different locations varies from 5.87–6.8. CEC (cation exchange capacity) that measures the cation retention capacity as well as water holding capacity of soil particle is higher in soil of location 1 than location II (Fig. 1). Acidic pH and higher CEC enhance metal bioavailability (Takac *et al.*, 2009 and Stathi *et al.*, 2010). As lower CEC causes leaching out of ions, so higher CEC and acidic pH of soil of location I somehow supported higher Cr uptake in *A. philoxeroides* proven by higher BCF than site II (Table 2). *A. philoxeroides* collected from site I (area contaminated with tannery effluents) had highest TF values for Cr while *A. viridis* occupied the second highest value. It is very conspicuous from our study that all other plant populations had low TF and BCF values with regard to the absorption of Cr indicating its limited capacity for accumulation and translocation of Cr in comparison to plants of amaranthaceae family. Our study is a great contrary to other authors finding where *P. oleracea* was reported as Ni, Zn accumulator. Plants with TF>1 reveal better metal accumulation in roots without transporting to the above ground tissue. Our result is corroborated with Ramana *et al*., (2015) where considerable amount of Cr was scavenged in root cell vacuoles that resulted lesser toxic response of plant. Sundaramoorty *et al*., (2010) reported Cr accumulates mainly in roots and shoots; however roots accumulate the major part, being usually only a small part translocated to the shoots. Higher root metal concentration makes amaranthaceae plants suitable for phytoextraction (Ibrahim *et al*., 2017). Our result is in good accordance with the findings of different workers (Majid *et al.,* 2011), that during artificial heavy metal treatment, roots accumulate more Cd than shoots. Metal tolerant plants with considerably higher TF and BCF value are potential candidate for phytoaccumulation (Yoon *et al*., 2006).

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–1y–1) can be expressed as

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

bDW – 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] (Arsad *et al*., 2008).

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–1y–1) is 39.402 kg Cr ha–1y–1 considering the parameters from Site I because it has higher Cr concentration in soil.

MEASUREMENT OF OXIDATIVE DAMAGE

Lipid peroxidation

It is the indication of membrane damage, which can be initiated by Active Oxygen Species or by the

Table 1. Cr concentration of plants collected from experimental sites (BDL: below detectable limit)

Sites	*Amaranthus viridis*		*Alternanthera philoxeroides*		*Solanum nigrum*		*Euphorbia hirta* (mg/kg)		*Portulaca oleracea*	
	Cr concentration (mg/kg)		Cr concentration (mg/kg)		Cr concentration (mg/kg)		Cr concentration (mg/kg)		Cr concentration (mg/kg)	
Cr conc of Soil	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Site I (1141.8 mg/kg) Bantala Leather Tanning Industrial area	134.34	72.05	289.8	272.52	55.4	10.78	23.68	1.17	19.28	BDL
Site II (517.64 mg/kg) Budgebudge Car painting factory	93.54	41.62	194.61	180.06	21.98	9.21	12.312	BDL	9.31	BDL

Table 2. Translocation Factor (TF) and Bioconcentration factor (BCF)

Sites	Cr conc of Soil (mg/kg)	*Amaranthus viridis* TF	*Amaranthus viridis* BCF	*Alternanthera philoxeroides* TF	*Alternanthera philoxeroides* BCF	*Solanum nigrum* TF	*Solanum nigrum* BCF	*Euphorbia hirta* TF	*Euphorbia hirta* BCF	*Portulaca oleracea* TF	*Portulaca oleracea* BCF
I	1141.8	0.53	0.07	0.94	0.561	0.2	0.04	0.12	0.01	–	0.016
II	517.64	0.44	0.18	0.925	0.375	0.41	0.042	–	0.01	–	0.018

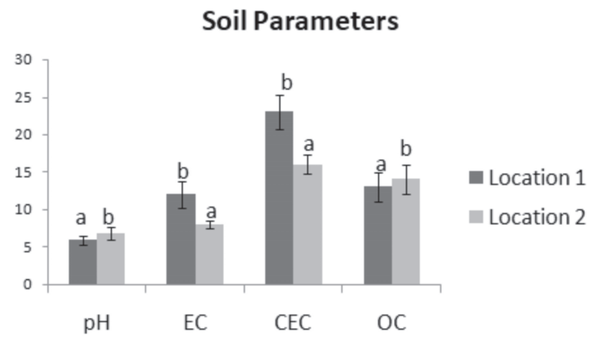

Fig. 1. Comparative analysis of soil parameters (pH, Electric conductivity EC; Cation exchange capacity CEC, Organic carbon OC) of experimental sites

action of lipoxygenase (Feng *et al*., 2013) so increase of malondialdehyde (MDA) concentration (as an indicator of non-enzymatic lipid peroxidation) is a common observation in stressed plants. 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 contaminated sites *A. viridis* showed highest membrane damage (Fig. 2) might be due to considerable amount of metal accumulation. But inspite of highest metal uptake MDA production was quite insignificant in *A. philoxeroides* probably due to other metal detoxification capacity (Simmons *et al*., 2007; Ansari and Sharma 2018). Our result is in good accordance with the finding of Xu *et al.,* (2012) which revealed that despite of considerable production of hydrogen peroxide *S. nigrum* showed minimum MDA due to its higher ROS scavenging capacity that ameliorate the Cr induced membrane damage. Least membrane damage of *P. oleracea* is pretty good explained by its least metal uptake and accumulation; though our result is a complete contrary with the record of Tiwari *et al*., (2008) where *P. oleracea* was found to be significant accumulator of Cr from industrial effluent irrigated water. Mihailovic *et al*., (2015), also reported Pb and Ni accumulation in *P. grandiflora*. This disparity regarding Cr uptake may be attributed to multimetal interactions and soil metal speciation (Mihailovic *et al*., 2015).

Endogenous H_2O_2

Production of reactive oxygen species is a biomarker of heavy metal poisoning. Formation of endogenous H_2O_2 is directly related to oxidative degradation of biological membranes. Maximum Cr sensitivity was recorded in *Euphorbia hirta* and *P. oleracea* evidenced by higher H_2O_2 formation (Fig. 3) which is directly proportional of membrane damage despite of their negligible Cr uptake and low BCF. Both amaranthaceae members were recorded with minimum endogenous H_2O_2 reflecting Cr tolerance ability due to enzymatic and non enzymatic antioxidative defences.

Antioxidants analysis

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

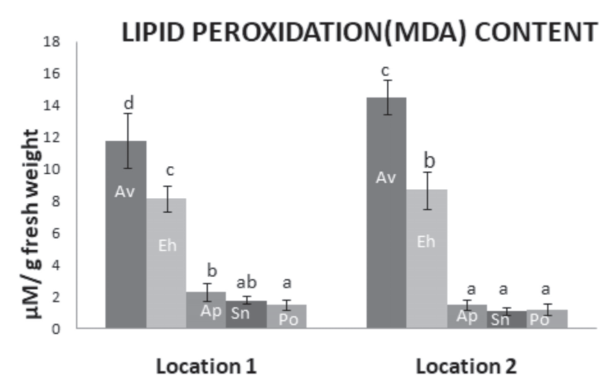

Fig. 2. Comparative estimation of MDA content of *Amaranthus viridis* (Av), *Euphorbia hirta* (Eh), *Alternanthera philoxeroides* (Ap), *Solanum nigrum* (Sn), *Portulaca oleracea* (Po) collected from contaminated sites.

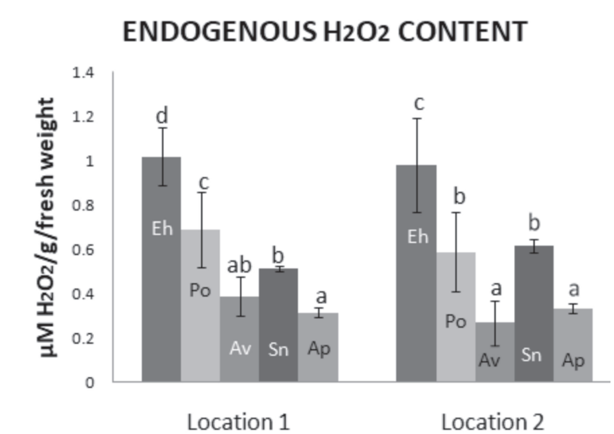

Fig. 3. Comparative estimation of endogenous H2O2 production of *Amaranthus viridis* (Av), *Euphorbia hirta* (Eh), *Alternanthera philoxeroides* (Ap), *Solanum nigrum* (Sn), *Portulaca oleracea* (Po) collected from contaminated sites

peroxidation (Khatamipur *et al*., 2011; Panda and Chowdhury, 2005). The metal detoxification capacity of *A. philoxeroides* was attributed to the highest production of free proline (Fig. 4) and NPSH (Fig.5) and significantly lower MDA content. Overproduction of proline might have provided the antioxidative defence against the generation of ROS under Cr stress. Our result is in good accordance with the findings of previous workers (Verney *et al*., 2008 and Handique *et al*., 2009). Better metal detoxification capacity was again recorded in two weeds of amaranthaceae family which is evidenced by their maximum proline and NPSH formation that was distinctly related to their significant Cr accumulation.

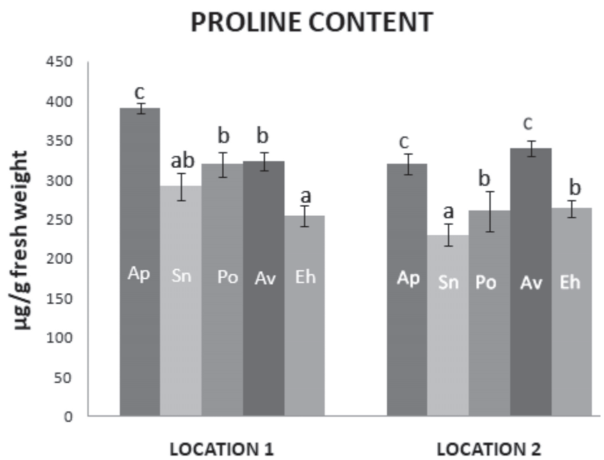

Fig. 4. Comparative estimation of Proline content of *Amaranthus viridis* (Av), *Euphorbia hirta* (Eh), *Alternanthera philoxeroides* (Ap), *Solanum nigrum* (Sn), *Portulaca oleracea* (Po) collected from contaminated sites.

The tripeptide glutathione is sulphur containing major non protein thiol (NPSH) and is involved in sequestration of heavy metals and detoxification of xenobiotics (Kao 2015). Excess formation of glutathione is the prerequisite for the synthesis of heavy metals scavenger peptide phytochelatins (Yu *et al*., 2018). Maximum NPSH content of *A. philoxeroides* collected from sites I with higher load of soil Cr was recorded. (Fig. 5). This is responsible for its better ability to resist cellular metal load, which might be due to the promotion of phytochelatin biosynthesis. This is in conformity with the documentations of other workers (Pandey and Singh, 2012; Pandey *et al*., 2009) that among heavy metals Cd and Cr are better potent inducer of NP-SH. The NPSH content of *P. oleracea* showed significant difference with *A. viridis and A. philoxeroides* but not with *E. hirta* of site I. Our finding is in good accordance with the study of Laeo *et al*., (2014) that revealed concentration dependent increase of non protein thiol in *Lemma* against as toxicity. There is strong evidence that phytochelatins, a class of nonprotein thiols, play a constitutive role in the detoxification of As and Cd (Noctor *et al*., 2012). But recently various workers have reported that under Cr stress in rice, phytochelatin synthesis increase for metal sequestration in plant vacuole resulting Cr tolerance (Yu *et al*., 2018).

Antioxidative enzyme assay

The oxidative damage of plant cell imposed by heavy metal toxicity is ameliorated by enhanced activities of various antioxidative isozymes (SOD, GR, CAT). Superoxide dismutase (SOD EC 1.15.1.1) 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 oxygen. H_2O_2 is further converted to H_2O and O_2 by catalase (CAT, EC 1.11.1.6). Glutathione reductase (GR, EC 1.8.1.7) 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.

The highest SOD and CAT activity (Figs. 6 and 7) of *A. philoxeroides* collected from both sites supported its better detoxification capacity of Cr in comparison to *E. hirta* and *P. oleracea* which showed almost 60% less activity attributed to their lesser metal uptake capacity. Our result is in good

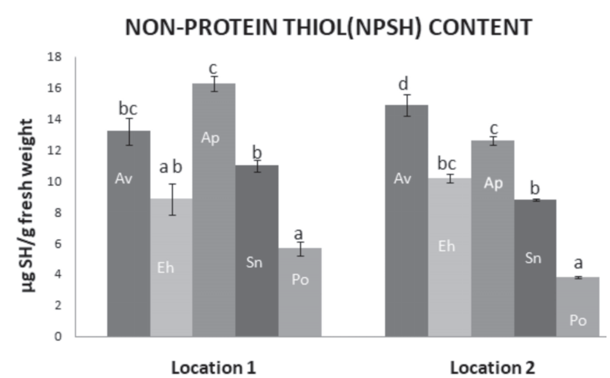

Fig. 5. Comparative estimation of non protein thiol content of *Amaranthus viridis* (Av), *Euphorbia hirta* (Eh), *Alternanthera philoxeroides* (Ap), *Solanum nigrum* (Sn), *Portulaca oleracea* (Po) collected from contaminated sites.

accordance with the Cd concentration dependent increase of SOD activity conferring ROS scavenging capacity reported in *Hibiscus* (Feng *et al*., 2013). Inspite of showing significant expression of non enzymetic antioxidants such as proline and NPSH under in situ Cr contamination, *A. viridis* revealed insignificant variation in CAT, SOD activity with *E. hirta* or *P. oleracea*. This observation can be supported by the findings that CAT activity was decreased in *A. viridis* exposed to Cr (VI) (Liu *et al*., 2008). *S. nigrum* showed more or less significant SOD and CAT activity that can be attributed to lesser amount of membrane damage (MDA). This finding can be supported with the study of Xu *et al*., (2012) where *S. nigrum* was proven to be Cd tolerant with higher concentrations of the ROS-scavenging antioxidative enzyme (SOD, CAT, and GR) activities.

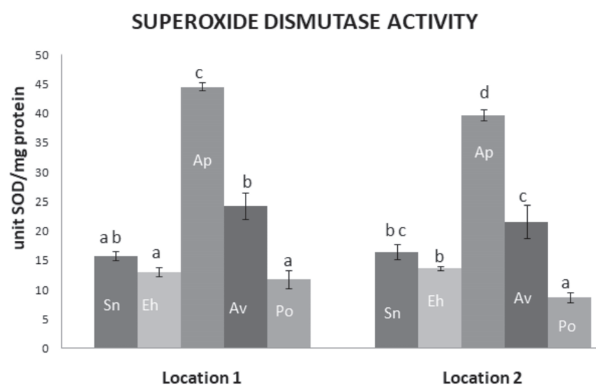

Fig. 6. Comparative estimation of SOD activity of *Amaranthus viridis* (Av), *Euphorbia hirta* (Eh), *Alternanthera philoxeroides* (Ap), *Solanum nigrum* (Sn), *Portulaca oleracea* (Po) collected from contaminated sites.

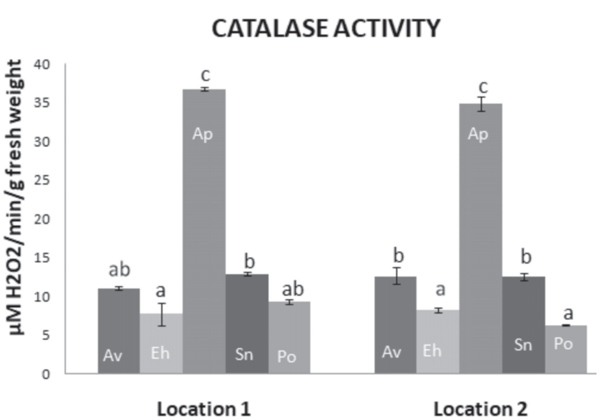

Fig. 7. Comparative estimation of CAT activity of *Amaranthus viridis* (Av), *Euphorbia hirta* (Eh), *Alternanthera philoxeroides* (Ap), *Solanum nigrum* (Sn), *Portulaca oleracea* (Po) collected from contaminated sites.

Glutathione Reductase is a member of flavoenzyme family that catalyzes the NADPH dependent reduction of glutathione disulfide (GSSG) of cell to glutathione (GSH), which in turn acts as precursor of metal chelator proteins (Ding *et al*., 2009). Over expression of GR or increase in GR activity (Melchiorre *et al*., 2009) has been related to metal stress tolerance. Again better Cr tolerance was proven in (Fig. 8). *A. philoxeroides* and *A. viridis* by their two folds higher GR activity with respect to other weeds. This biochemical defenses are responsible for their luxuriant, immense growth despite of considerable soil Cr contamination. During oxidative damage induced by heavy metals these antioxidative isozymes (SOD, GR) contribute to nullify the membrane damage evident by low MDA with higher proline, NPSH, SOD and GR activity of Alligator weed. An increase in GR activity alone is not sufficient to confer stress tolerance. More likely, a coordinated and finely regulated action of all enzymes of the ascorbate-glutathione cycle in conjunction with that of other ROS-processing enzymes in all cell compartments is required for plant stress tolerance (Yousuf *et al*., 2012 and Le *et al*., 2011).

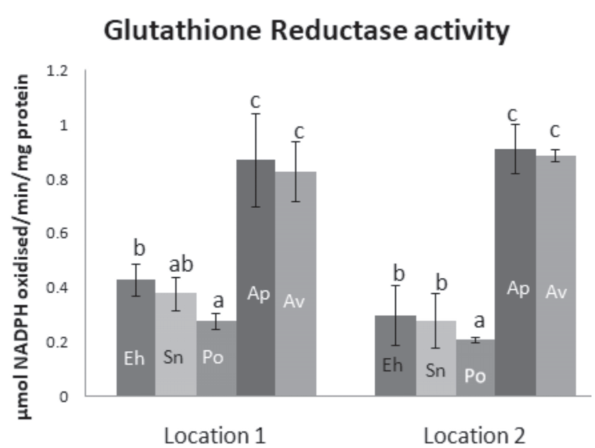

Fig. 8. Comparative estimation of Glutathione reductase activity of *Amaranthus viridis* (Av), *Euphorbia hirta* (Eh), *Alternanthera philoxeroides* (Ap), *Solanum nigrum* (Sn), *Portulaca oleracea* (Po) collected from contaminated sites.

CONCLUSION

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 attributed to Cr uptake capacity. *Amaranthus viridis* became the second most promising Cr tolerant candidate among all experimental weeds collected from Cr polluted sites of Kolkata. Since, Amaranthaceae family plants can be grown at sites contaminated with multiple metals, may produce high biomass due to high regeneration potential, good growth and also show potentiality to Cr accumulation and tolerance, it appears that these plants may be successfully employed in phytoremediation purposes.

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