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Glutathione and cysteine biosynthesis in two varieties of *Abelmoschus esculentus* in response to mine spoil

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Abstract: *The extent of accumulation of some heavy metals and glutathione and cysteine levels in the roots and aerial plant parts in two genotypically different varieties of A. esculentus (KS404 and BO2) exposed to mine spoil were investigated. Glutathione (GSH) level in both the varieties on control sites increased from basal level to 155.15 nmol g^{-1} dry weight (d.wt.), almost 1.5 fold on 30 day and attained a plateau within 60 day. Mine spoil exposure of both the varieties decreased glutathione 1.13 fold (89.2 nmol g^{-1} dry weight) during 60 day from its basal level. GSH concentration in shoots of these varieties increased accompanying growth contrary to roots where it finally declined 2 fold. Cysteine content in control plants increased 2 fold (31.6 nmol g^{-1} dry weight) on 30 day and finally declined 1.38 fold (22.35 nmol g^{-1} dry weight, at 60 day). Both the varieties, when exposed to mine spoil, showed enhanced cysteine content almost 2 fold during 30 day (50.95 nmol g^{-1} dry weight) but failed to increase further. For shoots in both the varieties challenged with mine spoil, cysteine maxima reached late (15.2 nmol g^{-1}dry weight, at 40 day) relative to control but the levels declined subsequently (11.85 nmol g^{-1} dry weight). Contrary to GSH, cysteine content in roots of both the varieties responded positively to mine spoil as apparent from the 2.23 fold increase during 30 d than basal level although it lowered to a level of 12.85 nmol g^{-1} dry weight finally at 60 day. Both the varieties accumulated almost maximum level of selected cations (Fe > Mn > Zn > Cu > Ni) during 30 day, but BO2 variety was significantly superior in this regard. Invariably high accumulation of such cations in roots over shoots indicated accumulation, retention or restricted translocation from root to shoot. The metal share of the edible part was just 6% of the plant load. Thus, present work reflects a genotypic differences in metal accumulation and that affected the major non-enzymatic traits or synthesis of sulfhydryl compounds as well. The present results also indicate that metal tolerance is in part associated with anti-oxidant system activity.*

Key words: *Abelmoschus esculentus, Cysteine, Glutathione, Mine spoil*
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Introduction

The ecology and physiology of plants colonizing on soils with elevated metal concentrations has been the subject of many investigations, and this aspect has been termed as phytoremediation (Schnoor *et al.,* 1995; Salt *et al.,* 1998; Baek *et al.,* 2006). Such an aspect also attracted the attention of investigators to look into the plant response to various heavy metals in order to revegetate the heavy metal contaminated sites (Salt *et al.,* 1998; Pandey *et al.,* 2007; Hooda *et al.,* 2007). Excess heavy metal ions as essential and nonessential micronutrients cause a series of alterations in biochemical processes related to photosynthesis (Lichtenthaler, 1996; Demirevska- Kepova *et al.,* 2004). Heavy metal stress increases the generation of active oxygen species (AOS) such as superoxide amino radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxy radicals (OH) (Weekx and Clijsters, 1996; Schutzendubel and Polle, 2002). To prevent oxidative damage, plants have evolved a complex antioxidant defense system, including both nonenzymatic and enzymatic constitutions (Demirevska Kepova *et al*., 2004). Of the nonenzymatic constituents GSH are most soluble antioxidants, which scavange O_2^- and H_2O, thus preventing the formation of highly toxic OH^- radical (Foyer *et al.,* 1997; John *et al*., 2007). During physiological investigations, induction of glutathione and cysteine synthesis have been documented in plants as a response to heavy metals or of metalbinding cysteine-rich compounds of which the majority, if not all belong to PC group *i.e*. (γ-glutamyl cysteinyl)-glycine. Glutathione (GSH) is the major non-protein thiol in plants and accumulates high amount in plants from orogenic soil (Grill *et al.,* 1995; Rennenberg, 1995). There are production of heavy metal binding complex ligands which are structurally related to glutahione (GSH), the substrate for PC biosynthesis (Zenk, 1996; Nagalakshmi and Prasad, 2001). There seems to be the need to monitor the extent of synthesis of such sulfhydryls in plants facing heavy metal stress, as these plays a pivotal role in cellular metal homeostasis and protection against oxidative stress to develop tolerance at organic soil (Foyer *et al.,* 1994). There are also reports on the metal-sensitive and -resistant plant ecotypes that could be used for reclamation of organic soils originating from mining, smelting and refining activities (Chardonnens *et al*., 1999; Ernst *et al.,* 2000). The utilization of metalliferous soils to raise vegetable crops also poses some questions as to whether such plants are suitable for revegetation purposes as: (a) permit metal accumulation in different plant parts and strongly impair the metabolism which could be expressed by enhanced synthesis of total GSH and cysteine as precursors of phytochelatin (b) heavy metals in cellular compartments of okra grown in orogenic soil is associated with antioxidant enzymes system, (c) sequestration of heavy metals and development of tolerance by the participation of antioxidant enzyme activity. In the latter case, attention has to be paid on the level of metal load in the edible parts.

The present communication describes attempts on cultivation of two okra varieties (*Abelmoschus esculentus*) on control

and mine spoil and the monitoring of the extent of accumulation of selected heavy metals and the level of cysteine and glutathione in the below and above ground plant parts in response to the mine spoil environment.

Materials and Methods

Soil samples were collected randomly from 5 to 10 locations at 10 meter intervals from 20 cm depth on control site and spoil of Kusunda mine site, located at Dhanbad, India ($23^0$30'S - $23^0$55'N). Soil study of coal mine spoil revealed the texture of rocky sandy loam. Moisture content varied from 7.4% to 8.85% . Total organic carbon was 0.22%. The pH of mine spoil was acidic and varied from 5.0 to 5.6 (USDA soil taxonomy). For heavy metal analyses, 1g fine soil samples of each locations were added in ternary acid (HNO_3 : H_2SO_4 : $HClO_4$; 5:1:7) and incubated for 10-12 hr. After complete digestion, contents of iron, manganese, zinc, copper and nickel were determined with the help of atomic absorption spectrophotometer (Perkin Elmer 2380, USA) (Jackson, 1958).

Plant material and growth conditions: Seeds of two cultivars BO2 and KS404 of *Abelmoschus esculentus* L. Moench were collected from the Institute of Agriculture Sciences, Banaras Hindu University, Varanasi, India. Viable seeds of both the cultivars were sown individually in 2 m^2 plots of control soils and mine spoil with row space of 40 cm and a plant density of 40$\pm$2 plants m^2. Plots were irrigated thrice a week until fruiting (60 day). The study period was undertaken from mid-July to September.

Determination of metal uptake and biomass production: To determine the metal uptake, biomass production, cysteine and glutathione enzyme activities, plants were harvested at 20, 40 and 60 day from each plot. Plants were separated in to roots and shoot-leaves and were oven dried for 24 hr at 80^0C. Equal biomass (1 g each) were wet digested in concentrated HNO_3 : $HClO_4$ acid mixture (10 : 1, v/v). After cooling, contents of metals were determined by atomic absorption spectrophotometer (Perkin Elmer 2380, USA) (Ouzounidou, 1994). For determination of biomass production in intact plants, shoots and roots were washed and dried separately and then incubated at 80^oC until reaching to constant weight.

Determination of total glutathione content: Plant samples were homogenized in mixture of 6% metaphosphoric acid pH 2.8, 1 mM EDTA, and 10% PVPP. After centrifugation, total GSH content was determined in acid soluble extracts according to the method of Griffith (1980) and Anderson *et al.* (1992) with slight modification. After neutralizing with 500 mM potassium phosphate buffer pH 8.0 and incubating with 10 mM 5-5'dithio-bis (2-nitrobenzoic acid), GSH and GSSG were assayed where GSSG was used as standard. Each extract was assayed twice and only 3% variation was found between replicates.

Determination of cysteine: Cysteine was extracted and estimated according to the method as described by Maas *et al.* (1985). Root and shoot tissues were homogenized in a mixture of 80 mM sulfosalicylic acid, 1 mM EDTA and 0.15% sodium ascorbate (w/v). After incubating in boiling water bath followed by centrifugation, the determination of cysteine was carried out on the basis of reactivity of its sulfhydryl group with a mixture of 1 ml 0.05 M MES, pH 5.8 and 0.1 M methylgyoxal in the supernatant. After 10 min incubation at 30^oC in mixture of 10 mM DTNB and 0.2M Tris-HCL, absorbance was measured at 415 nm by UNICAM SP 8000 spectrophotometer. The cysteine content was calculated by substracting the sulfhydryl content of a sample incubated in a mixture with methylglyoxal from sulfhydryl content in which methylglyoxal was replaced by H_2O.

Statistical analysis: Experiments were repeated four times and then results were expressed as arithmetic mean ±SE and mean differences were performed using statistical software (state soft).

Results and Discussion

Metal content in soil: The data in Table 1 incorporate the evaluation of heavy metal content in the collected soil sample from different locations of coal mine and control site. A comparison of heavy metal content between control soil and mine spoil showed that most of the metals (nickel, zinc and copper) level were 4 to 7 fold higher in mine spoil than control soil. Nickel content was found to be highest of all the metal tested followed by zinc and copper. While manganese was raised to more than six fold higher in mine spoil. Comparatively the iron concentration was much higher (2.58 g kg^{-1}) over such elements in control soil and almost 3 fold in case of mine spoil. Such findings were almost similar with reports on the distribution pattern of metals in Indian coal mine spoil (Ratha *et al.,* 1994). According to earlier reports, metals at above observed levels in mine spoil are eventually toxic to plants (Bowen, 1966). Metal content in spoil was not only limiting factor but decrease in soil water content in mine spoil could be included in this.

Metal accumulation in different plant parts: Angiosperms often rapidly colonize environments having high metal concentrations (Bradshaw, 1984) and led to concept of phytoremediation (Schnoor *et al.,* 1995; Salt *et al.,* 1998). Among the various strategies, phytoextraction involves plants that accumulate metals over the complete growth cycle, whereas in the hyperaccumulating plants inhabiting heavy metal enriched soils may contribute to accumulation of zinc, nickel, manganese or iron in roots more than 10% of shoot biomass (Baker and Brooks, 1989) and also, the crops of metal accumulating plants have been

Table - 1: Metal content in soil

Metals	Control soilmg kg^{-1} dry wt.	Mine spoilmg kg^{-1} dry wt.
Ni	6.0	45.6
Zn	14.8	49.8
Cu	10.4	50.0
Mn	100.0	618.6
Fe	2.58*	7.36*

*g kg^{-1}
Values are mean of 4 replicates

Table - 2: Changes in metal concentration in whole plant, roots and shoots of *A. esculentus* varieties KS404 and BO2 during 30 day and 60 day of growth in garden soil (control) and on mine spoil (n= 4)

Metal	Population (KS404)		30 day µg g^{-1} d.wt.	60 day µg g^{-1} d.wt.	Population (BO2)		30 day µg g^{-1} d.wt.	60 day µg g^{-1} d.wt.
Fe	Control	Root	15±0.375	9±0.27	Control	Root	15±0.34	11.2±0.34
		Shoot	7±0.175	6±0.18		Shoot	8±0.24	7±0.175
		Whole plant	22±0.66	15±0.52 (13.6)*		Whole plant	23±0.69	18.2±0.45 (6.13)*
	Mine spoil	Root	80±2	80±2.2	Mine spoil	Root	80±2	89±0.65
		Shoot	46±1.28	50±1.25		Shoot	45±1.25	48.2±1.15
		Whole plant	126±3.15	130±3.77 (6.82)*		Whole plant	125±3.12	62±1.55 (9.03)*
Mn	Control	Root	2±0.05	1.8±0.65	Control	Root	2.2±0.05	1.77±0.44
		Shoot	0.8±0.02	0.6±0.01		Shoot	0.7±0.01	0.58±0.01
		Whole plant	2.8±0.07	2.4±0.07 (11.6)*		Whole plant	2.90±0.05	2.34±0.05 3.23)*
	Mine spoil	Root	20±0.56	19±0.055	Mine spoil	Root	17.6±0.52	17.6±0.61
		Shoot	7.9±0.22	7.75±0.05		Shoot	6.8±0.9	7±0.21
		Whole plant	27.9±0.8	20.8±0.62 (5.76)*		Whole plant	24.4±0.88	24.6±0.73 (3.73)*
Zn	Control	Root	1.4±0.04	0.8±0.02	Control	Root	1.2±0.03	0.7±0.021
		Shoot	0.56±0.01	0.40±0.01		Shoot	0.3±0.01	0.25±0.008
		Whole plant	1.96±0.05	1.20±0.03 (5.83)*		Whole plant	1.50±0.04	0.95±0.027 (11.9)*
	Mine spoil	Root	10±0.3	10.2 ±0.24	Mine spoil	Root	9.4±0.32	9±0.26
		Shoot	2.8±0.07	3±0.08		Shoot	2.5±0.07	2.6±0.07
		Whole plant	12.8±0.38	13.2±0.36 (6.96)*		Whole plant	11.9±0.33	11.6±0.34 (5.86)*
Cu	Control	Root	0.46±0.01	0.4±0.01	Control	Root	0.48±0.01	0.4±0.01
		Shoot	0.18±0.005	0.15±0.005		Shoot	0.12±0.004	0.12±0.004
		Whole plant	0.64±0.01	0.55±0.01 (5.95)*		Whole plant	1.50±0.04	0.95±0.027 (15.3)*
	Mine spoil	Root	5±0.12	4.6±0.13	Mine spoil	Root	4.5±0.12	4.3±0.12
		Shoot	1.6±0.04	1.5±0.04		Shoot	1.6±0.04	1.4±0.04
		Whole plant	6.6±0.16	6.1±0.18 (6.5)*		Whole plant	6.1±0.18	5.7±0.16 (9.12)*
Ni	Control	Root	0.6±0.01	0.25±0.007	Control	Root	0.3±0.01	0.3±0.1
		Shoot	0.26±0.007	0.15±0.005		Shoot	0.12±0.003	0.12±0.003
		Whole plant	0.86±0.02	0.40±0.01 (20.0)*		Whole plant	0.42±0.01	0.42±0.01 (7.14)*
	Mine spoil	Root	4.5±0.13	4.1±0.12	Mine spoil	Root	3.8±0.11	3.8±0.11
		Shoot	1.3±0.04	1.2±0.03		Shoot	0.1±0.003	0.1±0.003
		Whole plant	5.8±0.17	5.3±0.15 (3)*		Whole plant	4.8±0.14	4.8±0.13 (1.25)*

* = Represents percentage of metal accumulation in fruits. All values are mean ±SE

successfully deployed for *in situ* decontamination of heavy metal polluted soils (Baker *et al.,* 1991).

Investigations on plant exposed to organic soils have resulted in sufficient knowledge about the physiological responses of plant and the extent of regulation of metal intake during initial stages of development (Ernst *et al.,* 2000). A genetic approach in this regard has been used in zinc and cadmium resistant ecotypes of *Silene vulgaris* (Chardonnens *et al.,* 1999). Investigations on similar lines also revealed delayed transport of metal from roots to shoots (Lou *et al.,* 2004; Song *et al.,* 2004) and leaves accumulated high metal levels compared to seeds (Ernst *et al.,* 1990) thus suggesting, that metal accumulation by plant parts regulates metal tolerance at the whole plant level. The data in Table 2 also offer similar comparisons adopting two varieties of *A. esculentus* grown on control site (garden

soil) and those on mine spoil. The ratio of metal accumulation in root and shoot fraction of KS404 indicates preference of the plant for iron accumulation because its highest upper limit in shoot and immediate contact of roots with the mine spoil. The subsequent plant growth (up to 60 day) retained the same ratio shared by root and shoot. Manganese accumulation share of shoots was poor whereas the amount increased more than 3 fold for roots at 30 day. Interestingly, manganese content in both the plant parts remained the same with subsequent growth up to 2 month. For zinc also, the overall accumulation trend remained the same as applicable to manganese during prolonged growth (60 day). Copper accumulation by the two plant parts in comparison followed almost the same ratio between 30 day or 60 day growth and it was almost 2 fold low compared to zinc. For nickel, during 30 day, as the shoot shared almost 4 fold less than root and the pattern of lowering remained the same as in case of copper during subsequent growth. To sum up, all the metal ions tested were accumulated and also retained by roots at least up to 30 day and it was only here onward that these get translocated to the aboveground plant parts of both the varieties. The sequence of metal levels in roots in the decreasing order could be arranged as Fe > Mn > Zn > Cu > Ni. Noticeably, similar varieties growing on control sites also accumulated metal in the same sequence but the average was almost 8 fold low. A comparison of metal load in fruits of the respective varieties indicated that nickel accumulation in fruits of control was highest for the cations estimated whereas for treated plants, it declined almost 7 fold followed by iron in KS404 even as control but it declined to almost 50% for plants exposed to mine spoil. Manganese was next to iron but during exposure to mine spoil, the cation level followed the same declining trend as applicable to iron. The interesting trend was shown for zinc as it increased during mine spoil exposure of plants and the same trend was applicable for copper. The other variety (BO2) presented a contrast in having low iron as control that increased to 9.03% following mine spoil exposure. However, the manganese level was almost 4 fold low than the earlier variety and it remained unchanged in the mine spoil exposed. This variety was also characterized by high accumulation of either zinc or copper but it appears that both the cations had restricted entry into fruits during mine spoil exposure. However, the average subsequent growth of both the varieties on mine spoil also indicated a marginal decline (1%) in metal content on the biomass dry weight basis. Such observations indicate that moderate biomass production and growth at internal metal concentrations, detoxification of metal in plant tissues and cells possibly ensure survival even up to reproduction phase or seed production.

Metal *vs.* glutathione biosynthesis: For the risk assessment of heavy metal load in soil, there are sufficient indicators available *e.g.* specific enzymes, metabolic biomarkers, parameters of plant growth and reproduction. A comparison of metal sensitive genotypes with regard to metabolic processes may enhance the understanding of metal sensitivity and tolerance on metal-enriched soils (Schat *et al.,* 1996). Here, field assay did not show a clear correlation between metal stress tolerance and activity of antioxidant enzyme. However, basis of differences in biomass production and degree of metal accumulation allowed us to select these two cultivars for studying defensive enzyme system under metal stress condition in spoil (Weckx and Clijsters, 1996). As the effect of metal on specific enzymes and their traits were very complex and difficult to analyze with respect to tolerance/susceptibility in both the cultivars. The data in Fig. 1a-c compare total glutathione in whole plant, roots and shoots of two varieties of *A. esculentus* KS404 and BO2 exposed to mine spoil along with the respective controls (0-60 day). In controls of both the varieties there was an apparent lag of 10 day although it followed a sharp rise during 30 day for KS404 and BO2, respectively, and remained unchanged during the subsequent time span tested. This indicate that constitutive GSH level in both varieties associated with low and high metals level in soils, GSH content increased during period of highest metal accumulation in root and shoot. At the exposure with mine spoil to KS404 showed no fluctuation in glutathione level up to 30 day and also, the subsequent incubations seemed unfavorable to glutathione synthesis. Likewise, BO2 also remained equally sensitive to mine spoil thus reflecting that both the varieties met with the same fate of glutathione inhibition. Such changes in levels of sulfhydryl compounds may be indicative of transient disturbance of metabolism as in *Silene cucubalus*, glutathione depletion was attributed to copper induced phytochelatin synthesis (Devos *et al.,* 1992). The latter investigators observed that copper in the sensitive plants, caused a 50% decline in total glutathione even during the initial 6 hr of exposure thus suggesting that the cation also induced the synthesis of some non-protein SH compounds other than glutathione; the copper resistant *S. cucubalus* was at variance in this regard. The observed decline in glutathione in both the cultivars of *A. esculentus* indicates that such precursors were possibly diverted towards the synthesis of stable metal complexing ligands or for the maintenance of optimal sulfhydryl rich intracellular environment to complex metals (Ernst *et al.,* 2000).

Regarding, individual plant parts, this has been reported that GSH content in roots of maize seedling exposed to cadmium and zinc declined 2 fold with the exception of a slight stimulation during first week of growth (Meuwly and Rauser, 1992). A similar approach was also adopted to compare the root/ shoot share of glutathione in both the cultivars of *A. esculentus*. The two varieties showed considerable difference in the root glutathione level with respect to time (0-60 day). In such comparisons, KS404 increased glutathione in roots was 1.55 fold from a basal level and the rising trend persisted up to 40 day. Onwards for the remaining 20 days GSH level remained unchanged. The same variety when challenged with mine spoil amendment, showed lowering in GSH during the initial 10 day and the decreasing trend continued until 60 day. The BO2 as control in such comparisons had high GSH content in the root fraction as compared to KS404 just described and followed by no change up to 60 day. Response of this variety to mine spoil also reflected retardation of GSH synthesis in line with that of KS404 with the exception of a slight resistance up to 20 day and also in the degree of final decline in GSH level. The shoot share of the ultimate GSH amount in KS404 was 1.1 fold higher over its root counterpart at 60 day, however the overall trend of increase followed the same course. Whereas, GSH

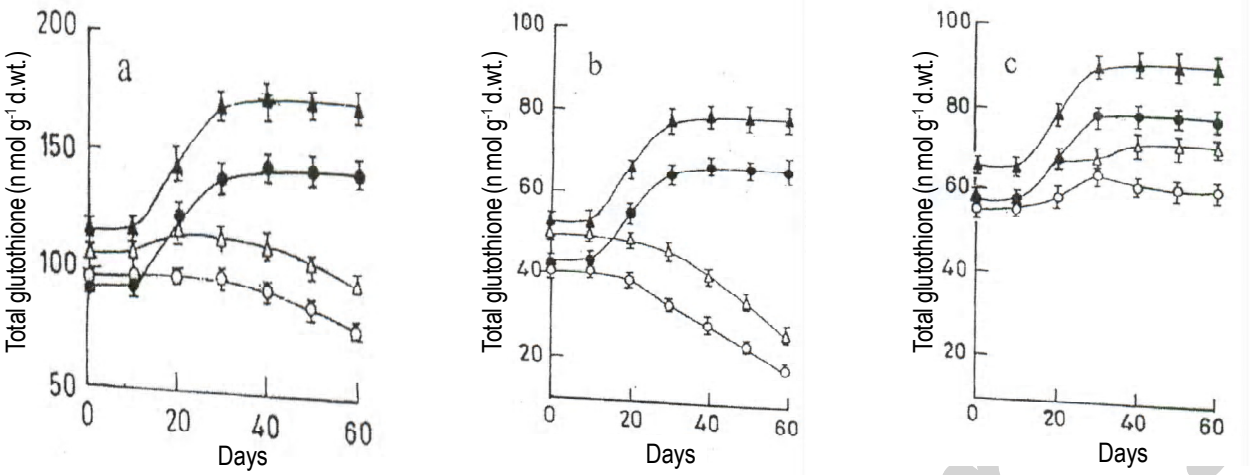

Fig. 1: (a) Total glutathione concentration in whole plant of KS404 on control soil (●) and mine spoil (❍) and BO2 on control soil (♦) and those on mine spoil (Δ)
(b) Total glutathione concentration in roots of KS404 on control soil (●) and mine spoil (❍) and BO2 on control soil (♦) and those on mine spoil (Δ)
(c) Total glutathione concentration in shoots of KS404 on control soil (●) and mine spoil (❍) and BO2 on control soil (♦) and those on mine spoil (Δ)

content decreased in roots of KS404 against mine spoil challenge, shoots invariably had a rise at least up to 30 day. GSH in shoots of BO2 as control also increased with passage of time as applicable to KS404 but with an increased final amount. The noteworthy observation was that shoot response of both the varieties against mine spoil exposure was the same as in both the cases, the ultimate lowering in GSH was common. The present study shows that plants of these two varieties exposed to polymetallic soils had stimulation of glutathione, PC precursors but antagonistic behaviour of metal might be a cause of marked decrease in glutathione. As GSH is a substrate for PC synthase, so the depletion of glutathione could be ascribed to the metal induced phytochelatin synthesis and as acquiring of tolerance in plants of these two varieties (Schutzendubel *et al.,* 2001). In addition, GSH content in mine and control plants showed apparent differences at varietal level with regard to metals accumulation (Sharma *et al*., 1999).

In present finding, a lower capacity of synthesizing GSH and ultimate lowering of GSH in stem indicate a compensation for metal induced loss of GSH in the roots leading to tolerance development (Ruegsegger *et al.,* 1990). In control and mine plants, the production of GSH explains the constitutive difference on the level of GSH between plants of these two varieties and correlated with the different rate of induction of metal oxidative stress (Schutzendubel *et al.,* 2001). A general picture of an initiation of metal induced GSH activity as antioxidant fitted well with earlier field study reports on *Betula pendula* and *Brascica rapa* grown on organic soil (Ernst, 1999).

Metal *vs.* cysteine content: As PCs isolated from algae, fungi and higher plants are basically γ-glutamyl peptides comprised mainly of three amino acids cysteine, glutamic acid and glycine, the present experiments adopted estimation of the cysteine level in the two varieties of *A. esculentus* as control and those exposed to mine spoil. It has been suggested that γ-glutamyl-cysteinyl-glycine could be involved in donating reduced sulfur to O-acetyle serine in the synthesis of cysteine (Noji *et al.,* 2001; Youssefian *et al.,* 2001). The data in Fig. 2 a-c compare cysteine level in whole plant, roots and shoots of both the variety as applicable to glutathione just described including control and mine spoil sets (0-60 day). Controls in both the varieties increased cysteine level with passage of time, however, it was limited to 30 day followed by a significant decline during subsequent growth periods. The noteworthy observation was that contrary to glutathione, both the varieties responded positively to mine spoil exposure in increasing cysteine biosynthesis at least up to 30 day. However, this appears to be a threshold as cysteine content invariably decreased drastically for both the varieties during 30-50 day and the trend continued until end thus corresponding to an average 50% reduction. A comparison of cysteine content in roots of KS404 and BO2 indicates that as control, the latter was a marginally superior because it was almost doubled to the basal level within 30 day. KS404 as control had the low initial cysteine level relative to BO2 but almost the same sequence of increase was also apparent corresponding to the growth periods. To sum up, KS404 was invariably inferior to BO2 in this context. Roots of BO2 challenged with mine spoil seem to have triggered cysteine synthesis/ accumulation right from the 5th day as reflected from slight increase and the topmost curve once again reflects the superiority of BO2. However, the roots failed to retain this cysteine level with passage of time as also applicable to control plants during 60 day. Cysteine amount in the shoots of BO2 was 3.5 fold over the root counterpart and a follow-up of growth of such plants up to 60 day also showed increased cysteine biosynthesis with passage of time but restricted to 30 day as ascertained from the overall trend of increase as in roots just described. For similar plants exposed to mine spoil, the overall trend of cysteine levels revealed : (a) the maximum attained only after 40 day *i.e.* 10 day later than in case of roots and (b) the maximum was 2.38 fold over the control at 40 day. The KS404 plants as control, increased cysteine content in shoots analogous to the trend in BO2 but with lowered values either in terms of maximum at 30 day or the final value at 60 day. Such plants if exposed to mine spoil also responded positively in increasing

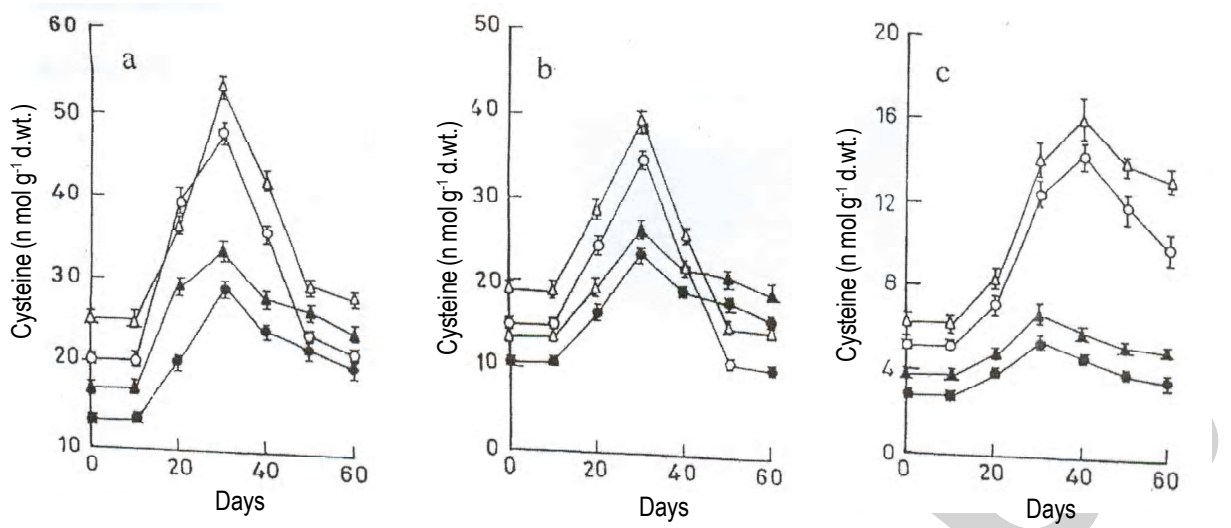

Fig. 2: (a) Cysteine concentration in whole plant of KS404 on control soil (●) and mine spoil (❍) and BO2 on control soil (⬧) and those on mine spoil (Δ)
(b) Cysteine concentration in roots of KS404 on control soil (●) and mine spoil (❍) and BO2 on control soil (⬧) and those on mine spoil (Δ)
(c) Cysteine concentration in shoots of KS404 on control soil (●) and mine spoil (❍) and BO2 on control soil (⬧) and those on mine spoil (Δ)

cysteine with passage of time in line with that of BO2 with the only difference that cysteine level were low relative to BO2 and supported the view of cysteine induced GSH synthesis (Noctor *et al.,* 1996; Bonner *et al.,* 2005). This enzyme is feed back inhibited by GSH and thought to have a major role in regulatory step of GSH synthesis. In this study, increasing level of cystein (as GSH derivative) indicate involvement of induction mechanisms in response to oxidative stress and this might approach to a delivery system to overcome the production of more protective molecule GSH in response to the challenge of heavy metals stress that permitted plant survival and production of good seed lot (Ernst, 1999; Noji *et al.,* 2001; Youssefian *et al*., 2001). Plants exposed to high environmental concentration of manganese, iron and copper gives a general view of measurable stimulation of GSH and cysteine as precursors of PC synthesis by SH- reactive heavy metals is quite in agreement with high stimulation of PC synthase by cadmium, copper and zinc in *Silene vulgaris* and *Pisum sativum* (Grill *et al.,* 1988; Klapheck *et al.,* 1995). The present result also shows that GSH accumulation accompanied by an increase in cysteine content corresponding to the metal accumulation in root and shoot. The basis of biomass production in both the varieties explained that GSH and its precursor are consumed in hardening of cells depending on the rate of oxidative stress in plants (de Kok and Osterhuis, 1983). In this case also the antagonistic metals influence on the content of cysteine and its participation in the assembly of glutathione synthesis could be suggested (Scheller *et al*., 1987).

The overall data infer the emergence of two genotypes of *A. esculentus* with a varietal response in terms of metal accumulation and the synthesis of sulfhydryl compound like glutathione and cysteine as sensitive indicator of anti-oxidant system under excess metal exposure. Their successful growth in mine spoil is indicative of the fact that such shallow rooted vegetable crops could be exploited for re-vegetation of nutrient poor and metal rich mining wastes.

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