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Heavy metal accumulation and changes in metabolic parameters in *Cajanas cajan* grown in mine spoil

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Abstract: *The extent of accumulation of some heavy metals in root and aerial plant parts, total chlorophyll, protein and yield of C. cajan exposed to mine spoil were investigated. Chlorophyll and protein level on the control site increased from the basal level to 1.49 fold and 1.92 fold respectively on 150 d and attained a plateau within 210 d. The maximum decline in leaf protein and yield in selected mine spoil has been observed 37% (18.46 mg g^{-1} fresh wt) and 76% at 150 d and maintained a slight decline when duration was extended up to 210 d as compared to control. Whereas in case of photo pigment content (Chlorophyll a and b) the maximum reduction was almost 42% (0.786 mg g^{-1} fresh wt.) during 210 d from its basal level. Plant tissues have accumulated maximum level of selected cations in control and mine spoil in the order (Fe > Mn > Zn > Cu > Pb > Ni > Cr > Cd). Metal accumulation in different plant parts was observed in the decreasing order roots > shoots > leaves > seeds. Invariably high accumulation of such cations in roots over shoots indicated accumulation, retention or restricted translocation from root to shoot. The metal share of seed varied from 1.3-39.5 fold as compared to their respective controls but their amount was quite below the toxic range. Thus the present work explores the metal accumulation in the plant tissues.*

Key words: *Cajanas cajan, Mine spoil, Metal accumulation, Protein, Chlorophyll*
PDF of full length paper is available online

Introduction

During opencast mining, the overburden is dumped containing soil, subsoil, small and big boulders of rock. The mine overburden is very much inhospitable for plant growth, but after stabilization, these dumps are derived by some temporary occupation of vegetation growth (Li *et al.*, 2007). Soil is the ultimate and most important sink of chemical components in the terrestrial environment (Rath *et al.,* 1994). In general mining areas, from a significant burden on the natural environment, where endemic plant species to be adapted to particular soil condition. Under controlled greenhouse or field conditions, some success has been achieved in the analysis of plant chemistry in relation to the soil chemistry. Investigations on functional relations between element concentration in plants and supporting soil over large geographic area have not received much attention. The relation of soil element concentrations and their accumulation in plants has shown differences among plant varieties, sub-species and cultivars (Xue *et al.,* 2004). In this regard, it has been suggested that elements in soil and their uptake in plants are influenced by a complex system in which factors such as soil mineralogy, soil chemistry (role of microbes and mycorrhizal association) and a plant genetic constitution collectively govern their accumulation in different organs of plants. There are numerous studies where changes in rhizobial population due to high concentration of metal as well as effect of heavy metal on legume plants are reported (Broos *et al.,* 2005). The presence of rhizospheric bacteria has been reported to increase the concentration of Zn, Cu, Pb or Cu in plants (Abou-Shanab *et al.,* 2008). The interaction between plants and rhizosphere micro-organisms can enhance biomass production and tolerance of plants to heavy metals and is considered to be an important component of phytostabilization or phytoremediation (Glick, 2003). Hence, a firm conclusion on the toxicity of heavy metals on legumes and their symbiotic partners can not be drawn (Wani *et al.,* 2008). A comparison of chemistry of plants in relation to the soils from natural and mine sites have been elucidated depending on the bioavailability of elements to plants (Xue *et al.*, 2003). Earlier, from polymetallic mine spoils, role on metal speciation and their interactions affecting uptake in plants has been suggested and total element levels in plants have been thought as representing the bioavailable fraction, whereas soil and biomass samples measure the biogeochemical load (Li *et al.*, 2007; Shu *et al.,* 2005). Very few studies have examined the chemistry of mine spoil and distribution of plant species with ground level element composition in comparison to undisturbed sites (Shen *et al.,* 2004; Ye *et al.,* 2002). In metal and coal mining wastes, the elements of most concern (Cd, Cr, Cu, Pb, Ni and Zn) are recognized and these are often found in large amounts in different sources and potentially toxic to plants. The amount of any metal taken up by plants from metal contaminated soil has been suggested as of central importance in assessing the risk of toxicity. However, the possibility of metals interaction with the root surface, uptake and accumulation in the plants and translocation to different organs and their toxicity levels have been considered as major factors by Kebata-Pendias and Pendias (1984). According to the available literature about the varying combinations of unmeasured factors, role of pH, organic

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matter and colonization by mycorrhiza have also been on similar lines (Morghan, 1993). In mining areas, endemic plant species appear to be adapted to a particular soil conditions. Looking to the fact that a huge area of land is occupied by main dump or spoil, their rehabilitation is now getting importance. Metal, essential or not are toxic for plants, when they are present in high concentrations. Plants possess mechanisms to avoid the excess uptake and also to cope with metals, when the internal concentration in the cells is too high, interfering with normal cell metabolism (Turner, 1994). Very little information is available on mechanisms of adaptation and plants growing in metal enriched soil under field conditions (Ernst, 2008). The present investigation was aimed at the cultivation of an economically important leguminous cultivar, *Cajanas cajan* in selected mine spoils, located in Eastern part of India (Coal field in Dhanbad of Jharkhand state). We have investigated the heavy metal load in mine spoil and their accumulation in *C. cajan* growing in mine spoils and control soil. In addition, the present communication describes cultivation of *Cajanas cajan* on mine spoil and monitored the level of chlorophyll, protein and total yield.

Materials and Methods

Study site: Soil samples were collected randomly from 5 to 10 locations at 10 m intervals from 20 cm depth on control and spoil of Modidih mine sites located at Dhanbad, India (23°30'S -23°55'N). Soil study of coal mine spoil revealed the texture of rocky sandy loam and the ratio of coal: overburden being roughly 1:5 (Banerjee, 1989). Modidih dump (Western coal field area) was selected as study site with estimated coal production of about 2.1 million tons per annum. Control site (Topchanchi site) was occupied by luxuriant natural vegetation. Above dump sites were used for cultivation of *C. cajan* and samples of spoils were taken for laboratory analysis and pot experiments.

Plant material and growth conditions: Seeds of *Cajanas cajan* (leguminous plant) were collected from the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Samples of 2×20 seeds of *Cajanas cajan* were allowed to germinate at 24±1°C, after careful imbibition between moist filter papers in separate petri dishes. Normal germination was assessed according to ISTA (1999). Viable seeds were sown individually in 2 m^2 plots of control soils and mine spoils with row space of 40 cm and a plant density of 40±2 plants m^2. Plots were irrigated thrice a week untill fruiting (150 d).

Determination of metal uptake: To determine the metal uptake, plants were harvested at 30 and 150 d. Chlorophyll content, protein content in leaf and total yield were monitored at 30, 90, 150 and 210 d from each plot. Roots, shoots and leaves were separated and oven dried for 24 hr at 80°C. Equal biomass (1 g each) was 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 heavy metal analysis of soil, 1 g fine soil samples of each location 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, lead, chromium, nickel and cadmium were determined with the help of atomic absorption spectrophotometer (Perkin Elmer 2380, USA) (Jackson, 1958).

Determination of proteins and chlorophyll: About 500 mg fresh leaf of each plant, collected from different mine and control soils were homogenized in 5 ml of Tris-HCl buffer (pH-7.0) using mortar and pestle. Homogenates were centrifuged at 5000 rpm for 2 min. The supernatant was shifted to test tube and added with 5 ml of 10% TCA and solution boiled in a water bath for 3 min. Samples were again centrifuged at 5000 rpm for 20 min and the pellets were solubilized in 5 ml of 0.1 N NaOH as well as protein was estimated by using the method of Lowery *et al*. (1951). Protein content in leaf of control and mine spoil plants was estimated and expressed as mg g^{-1} fresh wt.

For chlorophyll extraction 250 mg (fresh wt) of each plant leaves from each site were washed with water and placed in 20 ml of acetone in stoppered conical flasks and left overnight at 4°C. On the subsequent day, samples were homogenized and centrifuged (4000 rpm for 10 min). Thereafter a volume of 25 ml was maintained with 80% acetone and optical density was recorded at 645 nm and 663 nm. The chlorophyll content was calculated according to the formula of Arnon (1949) and expressed in mg g^{-1} fresh wt.

Total yield: For yield, number of seeds/pod, number of seeds/plant, single seed weight, seed weight/pod, total seed wt/plant as well as weight of 100 seeds were recorded in plants growing in control soil and coal mine spoils.

Statistical analysis: Experiments were repeated four times and the results were expressed as arithmetic mean±SE of five replicates. The data was analyzed by using student's 't' test with statistical software (SPSS 12).

Results and Discussion

Metal content in soil and parts of plant: The data in Table 1 incorporate the evaluation of heavy metal contents in the collected soil samples 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 metal (Cr, Ni, Pb, Cu, Zn, Mn and Fe) level were 2 to 4 fold higher in mine spoil than the control soil. The Iron content was observed much higher (2.58 g kg^{-1}) over the elements in control soil and almost 3 fold in case of mine spoil. Manganese content (106 mg kg^{-1}) was found to be highest of all the metal tested followed by Zn (14.8 mg kg^{-1}) and Cr (12.2 mg kg^{-1}) in control and observed with 2.4 to 3 fold higher in mine spoil. While the Pb content was raised 4 fold (maximum) higher (25.6 mg kg^{-1}) in mine spoil as compared to control (6.3 mg kg^{-1}). Among Ni, Cd and Cu comparatively higher increase has been observed in Ni

Table - 1: Heavy metal concentration in control and mine spoil *g kg^{-1} (values are mean of 5 replicates)

Metals	Control soil mg kg^{-1} dry wt.	Mine spoil mg kg^{-1} dry wt.	Fold increase
Cr	12.20±0.071	34.00±0.71	2.79
Ni	6.00±0.09	23.10±0.86	3.85
Cd	1.20±0.031	1.93±0.05	1.61
Pb	6.30±0.025	25.60±0.11	4.06
Cu	10.40±0.16	27.80±0.017	2.67
Zn	14.80±0.13	35.70±0.049	2.41
Mn	106.00±1.32	315.60±0.29	2.98
Fe	2.58±0.61*	7.36±0.066*	2.85

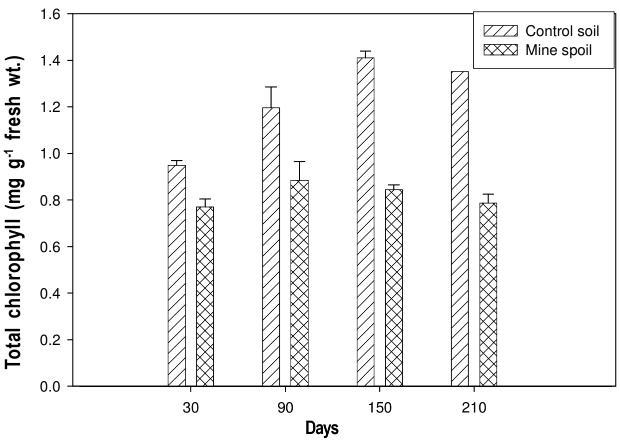

Fig. 1: Changes in total chlorophyll contents in leaf of *Cajanas cajan* L. grown in mine spoil at different growth periods

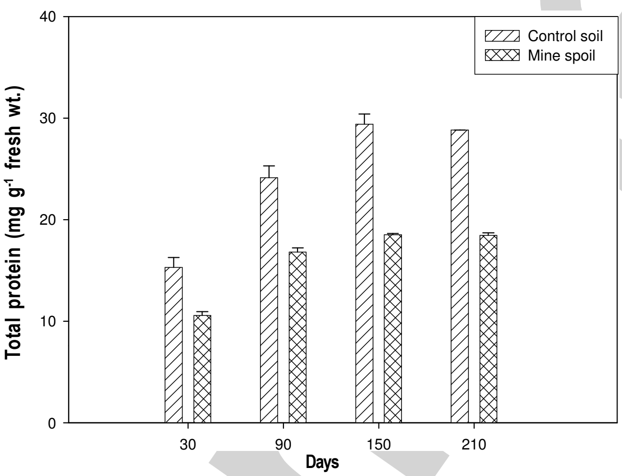

Fig. 2: Changes in Protein content in leaf of *Cajanas cajan* L. grown in mine spoil at different growth periods

(3.85 fold) followed by Cu (2.7 fold) and Cd (1.6 fold) in mine spoil as compared to control. Such findings support the earlier reports on the distribution pattern of metals in Indian coal mine spoil (Arya *et al.,* 2008; Rath *et al.,* 1994). On the basis of earlier observations, metals at own observed level in mine found eventually toxic to plants (Kabata-Pendias, 2001). Besides metal content, the decrease in soil water content in mine spoil has also been considered as limiting factor.

Metal uptake in different plant parts: Plant that frequently accumulate metals over the complete growth cycle belongs to phytoextraction strategies or the hyper accumulating plants inhabiting heavy metal enriched soils may contribute in accumulation of zinc, nickel, manganese or iron in roots more than 10% of shoot biomass and also helped in decontamination of heavy metal polluted soils (Arya *et al.,* 2008). Phytoremediation can be defined in angiosperms by their behavior of rapid colonization in environments having high metal concentration.

Seeds produced by plants on such spoils showed the highest level of Fe (6.38 μg g^{-1} dwt.), while it was recorded to almost half for Mn (average 4.0 μg g^{-1}) or Zn in the decreasing order (2.1 μg g^{-1}). Cu was subsequently the next lower concentration in seeds (1.57 μg g^{-1}) followed by Ni (0.19 μg g^{-1}) or Pb (0.24 μg g^{-1}). Characteristically, Cr accumulation (0.126 μg g^{-1}) came next to Ni, while as before, Cd accumulation maintained the lowest level (0.044 μg g^{-1}) (Tables 2a,b). The increase in the content of Fe, Cd and Mn was noticed in the range of 1.3 to 3.3 fold. Whereas Cr and Zn were observed with almost 8 fold along with Pb (18 fold) and Ni (39.5 fold) as compared to control but all the metals in seed were under the toxic range.

The ratio of metal accumulation in root, stem and leaf fraction of *C. cajan* indicates preferences of plant for ion accumulation because its higher upper limit in shoot and immediate contact of roots with the mine spoil. Iron accumulation in stem and leaf was poor whereas root was observed with 2 fold increase at 30 d. Similar to iron, lead accumulation was also observed 2 fold at 30 d and 2.5 fold at 150 d. For Cr, Cu during 30 d as the root shared more than 3 fold accumulation but it was decreased to almost 2 fold at 150 d. Manganese accumulation followed almost same ratio between 30 and 150 d growth. Nickel accumulation was observed with 3.47 fold at 30 d and 8.3 fold at 150 d. For cadmium and zinc the accumulation was observed 1.78 fold and 1.94 fold respectively at 30 d but at 150 d the accumulation became 6.44 fold and 3 fold for cadmium and zinc respectively. To sum up, all the metal ions tested were accumulated and also retained by roots and here onwards theses get translocated to the aboveground plant parts.

Such a variance in the amount of metals present in the spoil and the extent of their accumulation by plant parts also taken into consideration the nature and magnitude of metal-metal interactions that ranges from synergism to antagonism as the observations pertain to a multi-element combination in the respective spoil. The variable accumulation in plant tissues at different sites may be interpreted in terms of decrease in spoil pH, increased solubility of metals in spoils and their mobility in the plant tissue (Kumar *et al.,* 2009). Not only the vegetative parts but also the ultimate seeds followed the same sequence of the extent of accumulation of metal in reference. The reason of high uptake of Fe may be due to presence of Fe in the form of iron pyrites lowering pH of the spoil and thus rendering soluble metals more available for plant uptake (Pandey *et al.,* 2008). Another reason for increased metal accumulation at low pH may be

Table - 2a: Heavy metal accumulation in root, stem and leaf of *Cajanas cajan L.* grown in mine spoil at 30 and 150 d of growth period as well as heavy metal accumulation in seed

Metal	Population	Plant part	30 day μg g^{-1} dry wt.	150 day μg g^{-1} dry wt.	Heavy metal accumulation in seed μg g^{-1} dry wt.
Cr	Control	Root	0.174±0.018	0.41±0.019	0.015±0.006
		Shoot	0.024±0.012	0.048±0.025	
		Leaf	0.010±0.006	0.022±0.01	
	Mine spoil	Root	0.274±0.018[b]	0.70±0.051[c]	0.126±0.018[c]
		Shoot	0.206±0.016[b]	0.40±0.028[b]	
		Leaf	0.10±0.012	0.20±0.027[b]	
Ni	Control	Root	0.111±0.011	0.44±0.025	0.020±0.009
		Shoot	0.048±0.010	0.072±0.026	
		Leaf	0.028±0.010	0.03±0.017	
	Mine spoil	Root	2.454±0.019	3.48±0.053	0.19±0.014[b]
		Shoot	1.102±0.014[c]	1.30±0.043	
		Leaf	0.708±0.018[c]	0.419±0.035	
Cd	Control	Root	0.038±0.008	0.18±0.027	0.034±0.005
		Shoot	0.012±0.007	0.024±0.012	
		Leaf	0.006±0.004	0.015±0.010	
	Mine spoil	Root	0.116±0.011[c]	0.25±0.035[a]	0.044±0.010[a]
		Shoot	0.055±0.012[b]	0.20±0.024[a]	
		Leaf	0.018±0.010[a]	0.14±0.017[a]	
Pb	Control	Root	1.106±0.29	2.74±0.081	0.030±0.025
		Shoot	0.076±0.061	0.11±0.107	
		Leaf	0.045±0.018	0.09±0.17	
	Mine spoil	Root	1.884±0.027	3.98±0.166[c]	0.24±0.013[a]
		Shoot	1.20±0.022[a]	2.05±0.271[a]	
		Leaf	0.90±0.170[c]	1.60±0.054[a]	

Mean values of five replicates ± SE, Data marked significantly differed from control according to student's 't' test (a, not significant; b, p<0.05; c, p<0.01; d, p<0.001)

the metal binding properties of the organic matter (Pandey *et al*., 2006b). In the present observations, the second group of elements comprised of Pb or Cr having increased in the same range of concentration. The root uptake of Fe, Ni or Mn also followed a proportionate increase with the increase in the ambient concentration was not the case as the values showed little fluctuation in this regard, if one ignores the minor fluctuation in uptake values.

Generally, metal accumulations in plant tissues increased with increasing metal concentration as well as the plant growth period. Following growth, toxicity symptoms were observed in terms of chlorosis and curling of leaves in plants, attributable higher accumulation of Mn and Fe by the aboveground parts. The statistical analysis showed that the levels of all the eight metals in plant tissues, could be arranged in order: Fe> Mn> Zn> Cu> Pb> Ni> Cr> Cd. Metal accumulation and its translocation in plant parts was in the order: roots> shoots> leaves > seeds.

Metal vs. total chlorophyll: Fig. 1 takes into account the relative amount of the major photo pigment (Chl a and b) during successive growth of control plants (30-210 d) and a comparison with the plants challenged to mine spoil. In a month time (30 d), the control plants showed an average of 1 mg of chlorophyll(s) on the g fresh weight basis that increased to an almost 1.5 fold in mature plants (at 150 d) followed by a slight decline if the duration was extended to 210 d. Mine spoil in close proximity as reflected by an average of 42% inhibition in chlorophyll content at 210 d. At 150, 90 and 30 d the decline in chlorophyll level was also observed 40, 26 and 19% respectively. Gradual inhibition of chlorophyll (a and b) synthesis after flowering is due to increased metal accumulation in leaf of mine plants, which result to loss of chlorophyll and decrease in photosynthetic activity (Sinha *et al.,* 1993). The inhibition in the chlorophyll biosynthesis can also be taken as the reflection of inhibitory effects of spoil throughout the period in comparison (Fig. 1). In this case also, the ultimate reductions (at 210 d) were very close to those as observed for leaf protein (Fig. 2). However disturbances of metabolism by excess heavy metals appear to happen in multiple ways like inhibit respiration because a reduction of chlorophyll content and inhibit some photosynthetic function in leaves (Asrar *et al.,* 2005). Toxic effect of heavy metals on photosynthesis is also reported by (Morita *et al.,* 2006; Wang *et al.,* 2004). In this study the level of heavy metals seems to be phytotoxic to *C. cajan* causing growth inhibition and effect on pigment content,

Table - 2b: Heavy metal accumulation in root, stem and leaf of *Cajanas cajan L.* grown in mine spoil at 30 and 150 d of growth period as well as heavy metal accumulation in seed.

Metal	Population	Plant part	30 day µg g^{-1} dry wt.	150 day µg g^{-1} dry wt.	Heavy metal accumulation in seed µg g^{-1} dry wt.
Cu	Control	Root	1.760±0.58	4.40±0.102	1.00±0.159
		Shoot	0.281±0.044	0.36±0.143	
		Leaf	0.112±0.033	0.22±1.38	
	Mine spoil	Root	2.798±0.059[d]	6.7±0.129[d]	1.57±0.170[a]
		Shoot	2.094±0.058[c]	3.54±0.123[b]	
		Leaf	1.20±0.025[c]	1.80±0.189[b]	
Zn	Control	Root	5.420±0.74	7.25±0.14	0.26±0.097
		Shoot	0.518±0.033	0.64±0.109	
		Leaf	0.312±0.032	0.46±0.119	
	Mine spoil	Root	9.090±0.053[c]	15.48±0.454	2.10±0.253[d]
		Shoot	5.508±0.049	10.26±0.209	
		Leaf	3.00±0.032	8.00±0.141	
Mn	Control	Root	10.95±0.16	21.00±0.50	1.20±0.292
		Shoot	2.696±0.083	4.86±0.387	
		Leaf	1.08±0.108	2.60±0.256	
	Mine spoil	Root	18.17±0.36	38.2±0.720[c]	3.92±0.289[b]
		Shoot	10.49±0.34	22.98±0.577[d]	
		Leaf	8.06±0.345[d]	14.54±0.368	
Fe	Control	Root	30.32±0.691	40.04±0.50	3.86±0.206
		Shoot	11.61±0.838	17.01±0.58	
		Leaf	6.80±0.601	12.13±0.43	
	Mine spoil	Root	60.81±0.65	85.38±0.689	6.38±0.692[c]
		Shoot	42.02±0.76	75.38±0.622	
		Leaf	30.28±0.62	58.44±0.798	

Mean values of five replicates ± SE, Data marked significantly differed from control according to student's 't' test (a, not significant; b, $p<0.05$; c, $p<0.01$; d, $p<0.001$)

protein and metabolism (Assach and Clijester, 1990). The way heavy metals affected the chlorophyll synthesis, it can be suggested that they are linked to specific change to some chloroplast protein and also results differences in metabolic level and give insight into the toxicity mechanism. Probably all these mechanisms were related with steady-state level on protein synthesis and protein breakdown. The finding of reduction in chlorophyll content may be due to alteration of chloroplast structure and thylakoid membrane composition under such growth conditions (Quartacci *et al.,* 2000).

Metal vs. leaf protein: Data in Fig. 2 evaluate the relative concentrations of leaf protein in control as well as plants exposed to mine spoil. Leaf protein for control plants showed a progressive increment between 30 to 150 d attaining a maximum of 28.52 µg g^{-1} (fresh weight) and further up to 210 d, did not show any increment as the values were very close to those as observed for 150 d old plants. Whereas, in mine spoil maximum decline in leaf protein 37% has been observed at 150 d. Whereas, at 30 to 90 d 31% decrease has been noticed as compared to control. Plant protein synthesis in general has been known to be sensitive to heavy metals and the inhibition in the amount of protein can also be due to the inhibitory effects of spoil during the period of comparison (Davis, 1992). It seems that during transport heavy metals act at different sites to inhibit a large number of enzymes having functional sulphydryl group. It results deleterious effects in the normal protein form (Nagoor, 1999; Yurela, 2005). The basic mechanism starts with formation of complexes between metals and biomolecules leading to conformational changes in nucleotides and polypeptides (Sharma and Talukder, 1989). Excess concentrations of heavy metals also adversely affect water status resulting water deficit in plants leading to subsequent changes in plant metabolism. Earlier metal toxicity to proteins and other biomolecules has been illustrated with subject to its binding with sulphydryl groups in proteins thereby inhibiting enzyme activity (Meharg, 1994). However it is well established that heavy metals disrupt the metabolic pathways and protein synthesis with increasing concentration but ultimately heavy metals stress produces more amount of sulphur rich peptides than amino acids.

Metal vs. total yield: The data incorporated in Table 3 displays a summary of productivity of *C. cajan* in terms of seed yield, number and weight. The average number of seeds in *C. cajan* control plants was in the average of 200 seeds/plant but this number declined

Table - 3: Changes in productivity of *Cajanas cajan* L. grown in control and mine spoil

Parameters	Control soil	Mine spoil
No. of seed / plant	200±2.90	80±1.70
Seed wt. / pod (g)	0.345±0.001	0.17±0.004
Per seed wt. (g)	0.038±0.00	0.023±0.001
Yield / plant (%)	14.3±0.780	3.46±0.100
Wt. of 100 seeds (g)	7.26±0.660	4.22±0.580

to 80 for plants exposed to mine spoil. Seed weight/pod and per seed weight has been observed 51 and 39% decrease respectively. There was 76% decrease in case of total yield/plant, whereas 42% decrease has been noticed in case of weight of 100 seed observed, as compared to control. It is reflected from the results that the test plant could grow in mine spoil only at the cost of the seeds and their total weight apart from the general depression of plant growth relative to control. Reduction in yield has been suggested to result from phytotoxicity in relation to uptake of metals. (Kabata-Pendias, 2001). The finding of our experiment concluded that metal ions taken up by plants from the spoil are retained primarily in roots and only small proportion translocated to aboveground mass as observed by others (Umebese and Motajo, 2008). Such observation indicates that moderate biomass production, yield and metabolic depression at internal metal concentration and detoxification in plant tissues and cell possibly ensure even up to reproduction phase or seed production. To sum up, legume plant like *Cajanas cajan* seemed to show acclimatization towards polymetallic and metal rich habitats and hence this crop could be exploited for re-vegetation of metal rich mining wastes.

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