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Phytostabilization of mine waste : Growth and physiological responses of *Vigna unguiculata* (L.) Walp.

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Abstract: *Greenhouse experiments were carried out for phytoremediation of the Pb/Zn abandoned tailings (pH 3.2 and high metal content) of Rampura-Agucha Mines, Rajasthan. Vigna unguiculata (L.) Walp. (cowpea) was chosen as a test crop. On unamended tailings, the seeds of the test plant showed no germination. The tailing was amended with lime (3% on weight basis), 3% lime + NPK (diammonium phosphate @ 60kg/ha, muriate of potash @ 40kg/ha) and 3% lime + FYM @ 15 t/ha and used for experiments. Quantification of various parameters viz. shoot-root length, shoot-root dry weight, chlorophyll contents ('a', 'b' and total) and peroxidase activity of test crop revealed T + S + 3% lime + NPK to be the most suitable amelioration followed by FYM. The above treatments helped in improving the growth and productivity of the test plants by providing a favorable environment.*

Key words: *Mine tailings, Phytoremediation, Amendments, Zn/Pb/Cd, Growth, Physiology*
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Introduction

The state of Rajasthan, especially the south-eastern part, is rich in minerals. Metalliferous mining activities produce a large quantity of waste materials (such as tailings) which frequently contain excessive concentrations of heavy metals. The decontamination of soils and wastes polluted with heavy metals poses one of the most intractable challenges for soil clean-up. Both physical and chemical stabilization techniques, if used, require special equipment and operators, which are highly costly (Aery, 1991; Aery and Sharma, 2004). Further, these techniques remove all biological activity from the treated medium and adversely affect its physical structure.

Phytoremediation is an affordable technology that is useful when contaminants are within the root zone of the plants (Baek *et al.*, 2006). For sites, where contamination is spread over a large area, phytoremediation may be the only economically feasible technology (Aery and Panchal, 2007). Attempts were made to phytoremediate (phytostabilize) the tailings of Rampura-Agucha (Bhilwara, Rajasthan), lead-zinc mines having a pH of 3.2 and containing high amounts of Zn (893.23 $\mu g\ g^{-1}$), Pb (1330.35 $\mu g\ g^{-1}$), Cd (33.6 $\mu g\ g^{-1}$) and Fe (5972.7 $\mu g\ g^{-1}$). As the tailings are hostile to the growth of plants, their proper amelioration is a must before undertaking any large scale reclamation programme. The effect of tailings on the growth and physiology of crop plants was studied to know the extent of toxicosis, plants may experience during their life processes. The crop plant *Vigna unguiculata* (L.) Walp. (cowpea) var. C-152 was preferred due to its wide cultivation in the area and short and well-studied life cycle (3-4 months).

The effect of unamended and variously amended tailings was studied on the growth variables, chlorophyll contents and peroxidase activity of the cowpea plant during the fruiting stage.

Materials and Methods

The experiment was carried out in clay pots (30-cm height and 25-cm diameter) filled with four kilograms of tailings. One-cm thick layer of soil (approximately 15% of total weight) was put on top of every pot. The soil covering was carried out to ensure seed germination as our earlier experiments showed that seedlings were unable to develop directly on unamended tailings. Liming with calcium hydroxide was done to counter the extreme acidity (pH 3.2) of tailings. After a month of incubation, 3% liming (on weight basis) was found to be sufficient to increase the pH from an initial 3.2 to 6.28. In all the treatments, amendments were mixed with tailings in their respective treatments followed by covering with soil at the time of sowing. Inorganic fertilizers (NPK) in granular form (Diammonium phosphate @ 60kg/ha and muriate of potash @ 40kg/ha) were also added at the time of sowing as recommended for the above crop. Organic amendment with farmyard manure (FYM) @ 15t/ha (23.5 % organic carbon) was also carried out.

Experimental design was set up as follows:

1. Soil (control)
2. Tailings (T)
3. T + 15% soil (S)
4. T + S + 3% lime
5. T + S + 3% lime + NPK
6. T + S + 3% lime + FYM

After ten days, seedlings were thinned to five per pot (three replications per treatment). Watering was done on alternate days. After 1½ months, chlorophyll contents ('a', 'b' and total) (Arnon, 1949) and peroxidase activity (Malik and Singh, 1980) were measured in leaf samples of each treatment. Plants were harvested after two months. Observations were taken for shoot-root length (expressed as length $plant^{-1}$) and Dry weight (dried at 80°C for 24 hr and expressed as dry weight $plant^{-1}$). All the treatments were

Table - 1: Effect of unamended and amended tailings on growth indices of *V. unguiculata*. Results are given as mean ± S.D. (% increase/decrease over the control is given in parenthesis)

Treatment	Shoot length/plant	Root length/plant	Shoot dry wt./plant	Root dry wt./plant
Soil (control)	34.91 ± 1.37	21.8 ± 0.49	1.2634 ± 0.02	0.1514 ± 0.01
Tailings	Not survived	Not survived	Not survived	Not survived
T1	26.89 ± 0.39(-22.9674)	14.72 ± 0.18(-32.4348)	0.5143 ± 0.07(-59.2923)	0.1013 ± 0.01(-33.0911)
T2	27.04 ± 0.30(-22.5323)	15.62 ± 2.00(-28.3321)	0.5489 ± 0.02(-56.5537)	0.1249 ± 0.01(-12.9616)
T3	36.80 ± 0.95(+5.4209)	16.45 ± 0.22(-24.5137)	1.0091 ± 0.09(-20.1282)	0.1414 ± 0.05(-1.4634)
T4	35.01 ± 1.98(+ 0.2855)	15.92 ± 0.98(-26.9472)	0.9461 ± 0.04(-25.1147)	0.1401 ± 0.04(-2.3693)

T1 = T+S; T2 = T + S + 3% lime; T3 = T + S + 3% lime+NPK; T4 = T + S + 3% lime + FYM
T = Tailings; S = 15% soil; FYM = Farmyard manure@15 t/ha; NPK = Diammonium phosphate @ 60 kg/ha and muriate of potash @ 40 kg/ha

compared with soil (control). The data were statistically analyzed for the computation of analysis of variance.

Results and Discussion

The tailings have high bulk density (1.28 g/cm^3), high specific gravity (2.62), low porosity (51.14%), low air content (2.35%), high water holding capacity (48.79%) and non detectable amounts of organic matter. The results (Table 1-2; Fig. 1-2) indicate that tailings are not a suitable environment for proper growth of plants. The seeds of experimental plant species (cowpea) even failed to germinate in the pure tailings. However, certain ameliorative treatments, given to the tailings, showed some positive results, although the growth of plants in these treatments was not as good as compared to control.

The toxic levels of heavy metals (Zn, Pb, Cd), high acidity (pH 3.2), deficiency of nutrients (N, P and K) and organic matter were the major constraints for plant establishment and colonization. Aery and Jagetiya (1997), have also observed significant growth inhibition in plants resulting from high concentrations of heavy metals such as Zn, Pb and Cd.

In the present study, the treatment T+S+3% lime + NPK resulted in better growth than the other treatmentss (Table 1). The results were found to be statistically significant (Table 2). Zn toxicity in acid soils can often be avoided by liming or applying phosphorus or organic fertilizers. Liming of an acid soil not only improves physical conditions and microbiological activities but also increases the availability of nutrients and efficient use of fertilizers by the crops.

In the present study, higher shoot length, over the control, was observed in the treatment T + S + 3% lime + NPK (Table 1). Since nitrogen, phosphorus and potassium are the key elements required for the growth of plants, fertilization of tailings resulted in better growth of roots although it was less than that of control. It is well known (Kshirsagar *et al.,* 2003; Sharma and Aery, 2001) that nitrogen stimulates root growth and uptake of nutrients whereas phosphorus is essential for metabolic processes and potassium balances the nutrient uptake by plants.

The amendment with 3% lime + FYM @15 t/ha also resulted in an increase in shoot length (0.285%), over the control (Table 1). The addition of organic matter has important effects both on the physical characteristics and the nutrient status of mine wastes. It increases the water and nutrient holding capacity, improves surface stability, aeration and water penetration by alteration of the soil structure, decreases surface run-off and improves germination and seedling emergence (Williamson *et al*., 1982). FYM has the ability to form stable metal chelates, in which metal ions are held between atoms of the complex organic molecules, thereby eliminating toxicity (Aery and Tiagi, 1985). Shuman and Zhenbin (1997), observed that for soils having mild Zn-toxicity, liming is the preferred remediation technique, but for soils having severe Zn-toxicity, organic amendments plus liming may be necessary. The toxic effect of tailings was found to be more pronounced in roots of test crop than in shoots. This may be due to the fact that heavy metal toxicity affects the roots more than shoots. Oncel *et al*. (2000) observed similar results, while studying the effect of heavy metals on growth performance of wheat seedlings.

In the case of dry matter yield (top- root dry wt.), T + S + 3% lime + NPK showed better growth of plants as compared to other treatments (Table 1). Treatment with FYM also showed good growth. However, the dry weight of plants in these treatments was less as compared to control. Reduction of biomass production and nutritional quality was observed in crops grown in soils contaminated with moderate levels of heavy metal (Pierzynski and Schwab, 1993; Kramer *et al*., 2000).

In higher plants, net photosynthesis is reduced by heavy metal application. This causes yellowing of leaves which points towards a reduction in pigment status, therefore, the chlorophyll

Table - 2: Mean squares for different parameters in *V. unguiculata*

S. No.	Parameter	Treatment [4]	Error [10]
1	Shoot length	68.38**	1.36
2	Root length	24.05**	1.07
3	Shoot dry wt.	0.30**	0.003
4	Root dry wt.	0.001	0.001
5	Chlorophyll 'a'	0.17**	0.004
6	Chlorophyll 'b'	0.04*	0.01
7	Total chlorophyll	0.49*	0.13
8	Peroxidase	3.53**	0.37

** = significant at 5% level; * = significant at 1% level

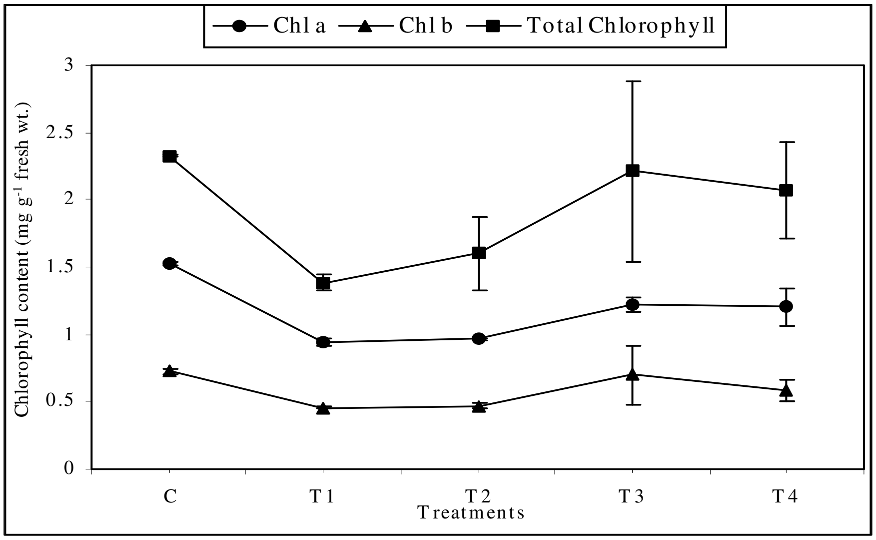

Fig. 1: Effect of unamended and amended tailings on chlorophyll content of *V. unguiculata*. Means of three replicates are presented, error bars represent standard deviation

C	=	Control (soil)	T	=	Tailings	S = Soil
T1	=	T + S	T2	=	T + S + 3% lime	
T3	=	T + S + 3% lime + NPK	T4	=	T + S + 3% lime + FYM	

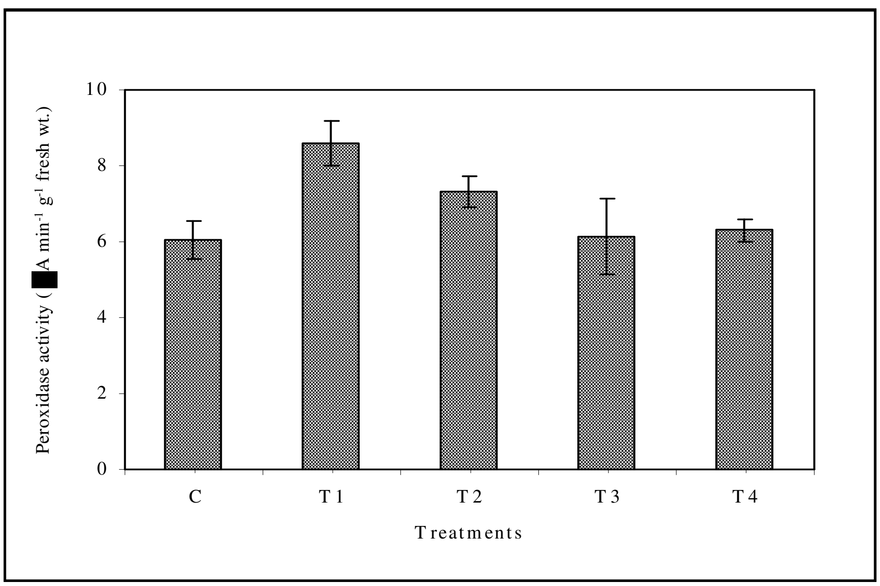

Fig. 2: Effect of unamended and amended tailings on peroxidase activity of *V. unguiculata*. Means of three replicates are presented, error bars represent standard deviation

C	=	Control (soil)	T	=	Tailings	S = Soil
T1	=	T + S	T2	=	T + S + 3% lime	
T3	=	T + S + 3% lime + NPK	T4	=	T + S + 3% lime + FYM	

content of leaf tissue has been used as criterion for metal toxicity (Burton *et al*., 1986; Zengin, 2006). In our studies, tailings led to a reduction in the chlorophyll contents ('a', 'b' and total) during the plant's fruiting stage (Fig. 1). On amelioration, maximum enhancement was observed in the treatment T + S + 3% lime + NPK, though it was less than control.

Evidences show that increased nitrogen supply stimulates photosynthetic capacity of leaves (Evans and Terashima, 1988) and consequently plant growth and productivity (Lawlor, 1995; Joel *et al*., 1997). Pankovic *et al*. (2000) observed that the inhibitory effect of Cd on photosynthetic reactions depends on the nitrogen nutrition of plants.

It is well documented that in plants subjected to metal stress, the activity of peroxidase increases (Gasper *et al*., 1991). Peroxidase (POD) induction is a general response of higher plants to uptake toxic amounts of heavy metals (Shaw, 1995). Such isoenzymes are considered to protect cell membrane against the active oxidants and enable plants to be resistant to the stress factor. In the present investigation, high peroxidase activity (8.6) was observed in the treatment T + S (Fig. 2). This may be accounted for high metal content and nutrient deficiency of the substratum (rooting medium) that resulted in the development of stressful environment for plants. On amelioration, the POD activity was reduced. The activity of POD was found to increase with the decline in the level of total chlorophyll. Durmus and Kadioglu (1998), Mishra and Agrawal(2006), also observed high POD activity together with low chlorophyll content. Baycu *et al*. (1999), detected increased POD activity and decreased chlorophyll content in coniferous tree species in Istanbul due to cadmium and lead concentrations in soil.

In our studies, the amelioration of tailings with lime, NPK or FYM resulted in better growth of cowpea and helped the plants to survive up to the fruiting stage but in unamended tailings, the seeds could not even germinate. This indicates that the ameliorative treatments reduced the toxicity of metals present in the tailings and provided a better environment for the growth of plants. It is, therefore, concluded that these treatments can be successfully used for the restoration of mine waste under study as well as other such wastes.

References

Aery, N.C. and Y.D. Tiagi: Studies on the reclamation of tailings dam at Zawar Mines, Udaipur, India. Proc. Asian Mining, IMM, London. pp. 65-70 (1985).

Aery, N.C.: Stabilization of tailing dams. *In:* Botanical Researches in India (*Eds.:* N.C. Aery and B.L. Chaudhary). Himanshu Publications, Udaipur. pp. 345-358 (1991).

Aery, N.C. and B.L. Jagetiya: Relative toxicity of cadmium, lead and zinc on barley. *Commun. Soil Sci. Plant Anal.*, **28**, 949-960 (1997).

Aery, N.C. and J. Panchal: Phytoremediation of heavy metal polluted sites. *In*: Proceedings of DAE-BRNS National Symposium of Limnology, Udaipur, 19-21 Feb. pp. 74-81 (2007).

Aery, N.C. and A. Sharma: Phytoremediation: A novel stretegy for the clean-up of contaminated lands (*Ed.*: P.C. Trivedi). Aavishkar Publ., Jaipur. pp. 67-93 (2004).

Arnon, D.J.: Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, **24**, 1-15 (1949).

Baek, Kyung-Hwa, Joo-Yun Chang, Yoon-Young Chang, Bum-Han Bae, Jaisoo Kim and In-Sook Lee: Phytoremediation of soil contaminated with cadmium and/or 2,4,6-Trinitrotoluene. *J. Environ. Biol.,* **27**, 311-316 (2006).

Baycu, G., E. Eruz, H. Caner and B. Gonencgil: Heavy metal stress and peroxidases: I. Peroxidase activity and chlorophyll content in response to cadmium and lead in *Pinus pinea*. *In*: Plant biochemistry and physiology (*Ed.:* M.C. Wuest). LABPV Newsletters University of Geneva, Switzerland. pp. 8-15 (1999).

Burton, K.W., J. B. King and E. Morgan: Chlorophyll as an indicator of the upper critical tissue concentration of Cd^{+2} in plants. *Water Air Soil Pollut.*, **27**, 147-154 (1986).

Durmus, N. and A. Kadioglu: Effect of benzyladenine on peroxidase activity during senescence of sunflower (*Helianthus annuus* L.) cotyledons. *Phyton.*, **37**, 253-261 (1998).

Evans, J. R. and I. Terashima: Photosynthetic characteristics of spinach leaves grown with different nitrogen treatments. *Plant Cell Physiol.*, **29**, 157-165 (1988).

Gasper, T., C. Penel, D. Hagege and H. Greppin: Peroxidases in plant growth differentiation and development processes. *In:* Biochemical, molecular and physiological aspects of plant peroxidases (*Eds.:* J. Lobarzewsky, H. Greppin, C. Penel and Th. Gasper). Univ. M. Curie-Sklodowska, Lublin. Poland and Univ. Geneva, Switzerland. pp. 249-280 (1991).

Joel, G., J. A. Gamon and C. B. Field: Production efficiency in sunflower: The role of water and nitrogen stress. *Remote Sens. Environ.*, **62**, 176-188 (1997).

Kramer, P.A., D. Zabowski, G. Scherer and R.L. Everett: Native plant restoration of copper mine tailings: II. Field survival, growth and nutrient uptake. *J. Environ. Qual.*, **29**, 1770-1777 (2000).

Kshirsagar, S., A. Sharma and N.C. Aery: Experimental studies on the phytostabilization of Pb/Zn mine wastes. Proce. of XII National Symp. on Environ., Tehri Garhwal, India, Sponsored by Board of Research in Nuclear Sciences, DAE, Mumbai, India, 5-7 June. pp. 507-512 (2003).

Lawlor, D.W.: Photosynthesis, productivity and environment. *J. Exp. Bot.*, **46**, 1449-1461 (1995).

Malik, C.P. and M.B. Singh: Plant Enzymology and Histo-enzymology. Kalyani Publishers, New Delhi (1980).

Mishra, S. and S.B. Agrawal: Interactive effects between supplemental ultra violet-B radiations and heavy metals on the growth and biochemical characteristics of *Spinacia oleracea* L. Braz. *J. Plant Physiol.*, **18**, 307-314 (2006).

Oncel, I., Y. Keles and A. S. Ustun: Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environ. Pollut.*, **107**, 315-320 (2000).

Pankovic, D., M. Plesnicar, I. Arsenijenic-Makimovic, N. Petrovic, Z. Sakac and R. Kastori: Effects of nitrogen nutrition on photosynthesis in Cd-treated sunflower plants. *Annl. Bot.*, **86**, 841-847 (2000).

Pierzynski, G.M. and A. P. Schwab: Bioavailability of zinc, cadmium and lead in a metal-contaminated alluvial soil. *J. Environ. Qual.*, **22**, 247-254 (1993).

Shaw, B.P.: Effects of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of *Phaseolus aureus*. *Biol. Plant*, **37**, 587-596 (1995).

Sharma, A. and N.C. Aery: Phytoremediation studies on the tailings of Rajpura- Dariba, Udaipur (Rajasthan) lead-zinc mines. Proceedings of X National Symposium on Environment, Mumbai, India, Sponsored by Board of Research in Nuclear Sciences, DAE, Mumbai, India 5-7 June. pp. 244-248 (2001).

Shuman, M.L. and L. Zhenbin: Amelioration of zinc toxicity in cotton using lime or Mushroom compost. *J. Soil Contam.*, **6**, 425-438 (1997).

Williamson, N.A., M.S. Johnson and A.D. Bradshaw: Mine waste reclamation: The establishment of vegetation on metal mine wastes. Mining Journal Books, London (1982).

Zengin, Fikriye Kirbag: The effects of CO^{2+} and Zn^{2+} on the contents of protein, abscisic acid, proline and chlorophyll in bean (*Phaseolus vulgaris* cv. Strike) seedlings. *J. Environ. Biol.,* **27**, 441-448 (2006).