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An assessment of heavy metal remediation potential of *Lawsonia inermis* L. from iron ore tailings

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The present study aimed at studying the growth and physiological response of *L. inermis* on iron ore tailings. Pot experiments were conducted to investigate the effect of iron ore tailings both individually as well as in combination with soil (at different proportions) on the growth, pigment production as well as accumulation and translocation of various heavy metals from the tailings. The results suggested a decrease in the growth and chlorophyll content but increase in the carotenoids as well as metal accumulation capacity of *L.inermis* with increasing proportion of tailings in the soil. Furthermore, an increase in antioxidant (Carotenoids, Catalase, Peroxidase and Super Oxide Dismutase) activities in plants grown on tailings as compared to control was observed which suggests this plant's efficiency to overcome any stress generated due to excess of heavy metals. The order of accumulation of various heavy metals in the plant parts was observed to be Cr>Cu>Fe> Zn> Pb>Ni. However, the hundred percent survival rates of all the plants in all the treatments suggest its efficiency to survive metal stress. Thus the plant can be used for the reclamation of moderately contaminated areas.

Key words: Iron ore tailings (IOT), Catalase (CAT), Peroxidase (POD), Superoxide Dismutase (SOD), *L. inermis*

Both metal and non-metal mining activities generate huge quantity of waste rocks, which damages the aesthetics of the area. Particularly, in case of metal mining, activities such as crushing, grinding, washing, smelting and all the other process used to extract, concentrate metals, generate a large amount of waste rocks and tailings which scars the landscape, disrupts ecosystems and destroys microbial communities (Das and Maiti, 2008). Mine tailings remain a major concern to the mining industry and the environment. Mine refuse containing sulphur or sulphide minerals, such as pyrite, can be oxidized when exposed to the atmosphere and rainfall, forming large amounts of sulphuric acid. This results in pronounced pH decrease and acid mine drainage that can leach metals from the tailings, constituting a threat to the surrounding ecosystems (Spitz and Trudinger, 2008). Furthermore, the dry-stacked overburden and other mining wastes remain unstable and subject to eolian dispersion and water erosion with the potential to contaminate nearby communities and environmentally sensitive areas (Schwegler, 2006).

Most of the conventional remedial technologies like leaching of pollutant, vitrification, electrokinetical treatment, excavation and off-site treatment are expensive, technically limited to relatively small areas (Barceló and Poschenrieder, 2003) and deteriorate the soil fertility, which subsequently causes negative impacts on the ecosystem. Establishment of vegetation cover can fulfill the objectives of stabilization, pollution control, visual improvement and removal of threats to human beings (Freitas et al., 2004).This technique of using plants to remove contaminants is popularly regarded as 'Phytoremediation'. The generic term 'phytoremediation' consists of the Greek prefix "phyto" (plant), attached to the Latin root "remedium" (to correct or remove evil) (Cunningham and Ow, 1996). Phyto-remediation is a cost effective, environmental friendly, aesthetically pleasing approach with long term applicability. Phytoremediation is well suited for use at very large field sites where other methods of remediation are not cost effective or practicable (Williamson and Johnson, 1981). *Lawsonia inermis* is a much-branched glabrous shrub or small tree 2-6 m in height, which may be spiny. Bark greyish-brown, unarmed when young, older plants with spine-tipped branchlets. Young branches quadrangular green but turn red with age. Owing to it's adaptability to wide range of climatic conditions and tolerance towards high temperature, salt and drought it can be potential tool for revegetation of abandoned mines and other waste lands contaminated with metalliferous wastes. Thus, the objectives of this study were to examine the (1) growth response of *L. inermis* on iron ore tailings and (2) accumulation and translocation of various heavy metals within the plant body under different treatments.

Materials and Methods

Experimental design

Iron ore tailings (IOT) generated during the beneficiation of low grade iron ore from Orissa, India was taken and mixed at different proportions with the garden soil. The treatments were named as T_0-(Garden soil) as control, T_1-IOT (1:3), T_2-IOT (2:1), T_3IOT (3:1) and T_4- IOT. The samples were equilibrated completely for a month followed by the plantation of *L. inermis* seedlings collected from the local nursery at the rate of one seedling per earthen pot.

Tailings substrata analysis

Organic carbon content of soil and tailing samples was determined by rapid dichromate oxidation technique (Walkey and Black, 1934); CEC by 1(N) ammonium acetate extraction method (Jackson 1973); exchangeable Ca, Na, K and Mg extracted by 1(N) ammonium acetate solution (Gupta 2000); diethylene triamine pentaacetic acid (DTPA) extractable (plant available) metals were determined using 0.005 M DTPA solution (LopezSanchez et al. 2000). Available N and P were determined by alkaline permanganate (Subbiah and Asija, 1956) and ammonium fluoride extraction (Bray and Kurtz, 1945) methods, respectively. Water holding capacity (WHC, in percent) of the samples was determined following the method of Chaturvedi et al. (2012). Five grams of soil and tailing samples were collected from the rhizosphere zone of the plants, dissolved in 50 ml of double- distilled water and stirred using a magnetic stirrer for 30 min. The filtrate obtained after filtering the supernatant in the previous step was used for measuring pH and electrical conductivity (EC) using the pH and EC meter (The HANNA instruments).

Biochemical parameters

Photosynthetic pigments like chlorophyll (Chl a, Chl b and total Chl) and carotenoids were quantified spectrophotometrically following the method of Porra et al. (1989) and Lichtenthaler (1987). The activities of SOD, CAT and POD were measured following the method of Misra and Fridovich

(1972), (Chance and Maehly, 1955) and Singh et al. (2006), respectively. The activity of these enzymes was expressed as specific activity (U^{-1} mg protein).

Heavy metal analysis from soil and plant samples

After harvesting plant samples were divided into root, stem and leaves and then carefully rinsed with tap water followed by double-distilled water. The samples were oven-dried at 60 °C to constant weight. The dried tissues were ground to powder. Rhizosphere soil samples were obtained by following the method of Yanai et al. 2003. The soil and tailing samples were then air-dried and ground using a mortar and pestle. Finally, the plant and soil samples were digested in aquaregia (HNO_3/ HCl, 1:3), and concentration of heavy metals were determined using the AA-6300 SHIMADZU Atomic Absorption Spectrophotometer after adjustment of required dilution factor.

Results and Discussion

Physico-chemical parameters

The general properties of soil and tailings samples are presented in Table 1. The soil and tailing samples showed large differences between their nitrogen content, while differences were marginal between the potassium and phosphorus content of the two. The tailings were comparatively acidic than the soil. The pseudo-total and plant available metal concentrations in soil and tailing samples are presented in Table 2. Both the total/ pseudo metal content of the tailings was higher than the soil.

Growth parameters

Hundred percent survival rates were observed in all the treatments but the plants grown on tailings and soil tailings mixture showed rather inhibited growth than those grown on soil (Fig.1). This inhibition in growth rate of these plants may be attributed to the excess accumulation of toxic metals like Pb, Cr, Ni and Zn (Mishra and Chaudhary, 1998; Espen et al., 1997; Farooqi et al., 2009). Furthermore, Overall Growth reduction may be generally linked to a loss of cellular turgor resulting in either a decrease of mitotic activity and/or an inhibition of cell elongation.

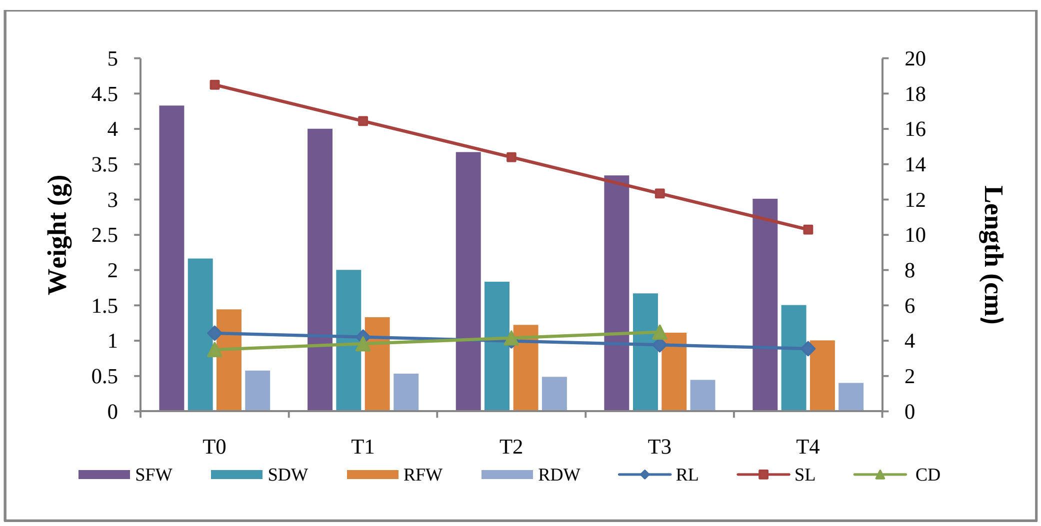

Fig 1. Effect of iron ore tailings on growth of *L.inermis*. Here, RL=Root length; SL=Shoot Length; RFW=Root fresh weight; SFW=Shoot fresh weight; RDW=Root dry weight; SDW=Shoot dry weight.

Table1. Showing the various Physico-chemical characteristics of soil and tailing samples (n = 4, Mean ± SE).

Treatment	pH	EC	OC (%)	CEC c mol (+)	WHC (%)	Available N	Available P	Exchangeable cations [c (+) mol/kg]			
		(µS/cm)		kg-1		(mg/kg)	(mg/kg)	Ca	Na	K	Mg
T0	6.2 ± 0.025	103 ± 0.017	3.3 ± 0.007	0.464 ± 0.033	38.7 ± 0.009	159 ± 0.003	7.6 ± 0.035	0.34 ± 0.015	1.36 ± 0.013	0.33 ± 0.009	2.8714 ± 0.013
T1	6.1 ± 0.26	108 ± 0.021	2.4 ± 0.024	0.533 ± 0.043	33.4 ± 0.012	116.99 ± 0. 019	6.1 ± 0.203	2.94 ± 0.002	1.97 ± 0.003	0.27 ± 0.012	2.5876 ± 0.005
T2	5. 9± 0.026	112 ± 0.015	1.4 ± 0.009	0.599 ± 0.025	29.8 ± 0.021	86.12 ± 0.120	4.2 ± 0.045	3.83 ± 0.009	1.22 ± 0.005	0.20 ± 0.035	1.8192 ± 0.017
T3	5.7 ± 0.017	115 ± 0.022	0.98 ± 0.013	0.654 ± 0.042	25.9 ± 0.020	44.67 ± 0.035	2.3 ± 0.111	5.18 ± 0.016	0.86 ± 0.029	0.14 ± 0.014	1.1856 ± 0.003
T4	5.5 ± 0.014	119 ± 0.029	0 ± 0.000	0.731 ± 0.030	22.7 ± 0.015	28.00 ± 0.009	1.2 ± 0.169	8.13 ± 0.005	0.58 ± 0.111	0.10 ± 0.005	0.3142 ± 0.009

Table2. Environmentally and plant available metal contents (mg kg^{-1}) in control and various treatments (n = 4, Mean ± SE).

	1st		2nd		3rd		4th		5th		6th	
	Fe		Pb		Zn		Cr		Ni		Cu	
	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
T1	41.09±0.014	7.55±0.019	8.26±0.020	0.105±0.003	22.16±0.002	0.424±0.001	12.98±0.009	1.23±0.002	22.24±0.002	0.346±0.003	7.31±0.001	0.46±0.003
T2	1592.35±0.026	144.45±0.002	11.49±0.012	0.284±0.002	22.87±0.001	0.457±0.004	16.23±0.002	1.31±0.001	23.77±0.001	0.376±0.001	7.71±0.003	2.08±0.003
T3	3143.62±0.002	281.35±0.023	14.75±0.001	0.463±0.001	23.59±0.003	0.495±0.003	19.47±0.002	1.38±0.002	25.29±0.002	0.405±0.001	8.10±0.002	3.70±0.002
T4	4694.89±0.003	418.26±0.018	18.00±0.003	0.643±0.002	24.31±0.004	0.528±0.002	22.70±0.003	1.46±0.002	26.81±0.001	0.436±0.002	8.50±0.003	5.32±0.002
T5	6246.15±0.003	555.18±0.014	21.25±0.004	0.824±0.003	25.03±0.001	0.565±0.002	25.94±0.001	1.53±0.003	28.34±0.002	0.464±0.001	8.89±0.002	6.94±0.003

Biochemical Parameters

Observations suggest a marked increase in Chl a, Chl b and total content with increase in time and treatment but the trend was completely reverse for Carotenoids. Similarly, Photosynthetic pigments were found to be reduced under the excessive concentrations of Hg (Rai *et al.*, 1981) and Cr (Corradi *et al.*, 1995) etc. The breakdown of photosynthetic pigment may be due to substitution of Mg ion in chlorophyll molecules by metal ions such as Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+} (Kupper et al, 1998). Carotenoids are generally regarded as the non enzymatic antioxidants and an increase in the concentration of carotenoids with increase in metal uptake proves its role as an antioxidant molecule (Prakash et al, 2007). Heavy metals are known to generate toxic reactive oxygen species (ROS) such as H_2O_2, O^{2-}, OH, OH_2 etc. which degrade important cellular components by inducing oxidative stress. The tolerance to damaging environmental stresses is correlated with an increased capacity to scavenge or detoxify reactive oxygen species (Smirnoff, 1993). Significant increase in the activities of SOD, POD and CAT was observed in the treatments than control (Fig.2).

Metal accumulation pattern

The comparative accumulation of different heavy metals by *L. inermis* subjected to various treatments is shown in Table 3. The metal concentrations in the parts (root, stem and leaves) of the plants grown on IOT was found to be significantly ($p < 0.01$) higher than the control and showed heterogeneous accumulation pattern. The mean metal concentration in the plants increased with increasing IOT (and hence metal) conc. in the soil. Also, the maximum and minimum values of each metal were found to be comparatively higher in treated plants than control. Regarding the accumulation of individual metals Pb, Zn, Cr and Ni accumulation was maximum in the root while Fe and Cu in the leaves. Restriction of these metals to the roots is one of the most popular metal tolerance mechanisms among the tolerant plant species. The order of accumulation of various heavy metals in plant body was observed to be Cr> Cu>Fe> Zn> Pb>Ni.

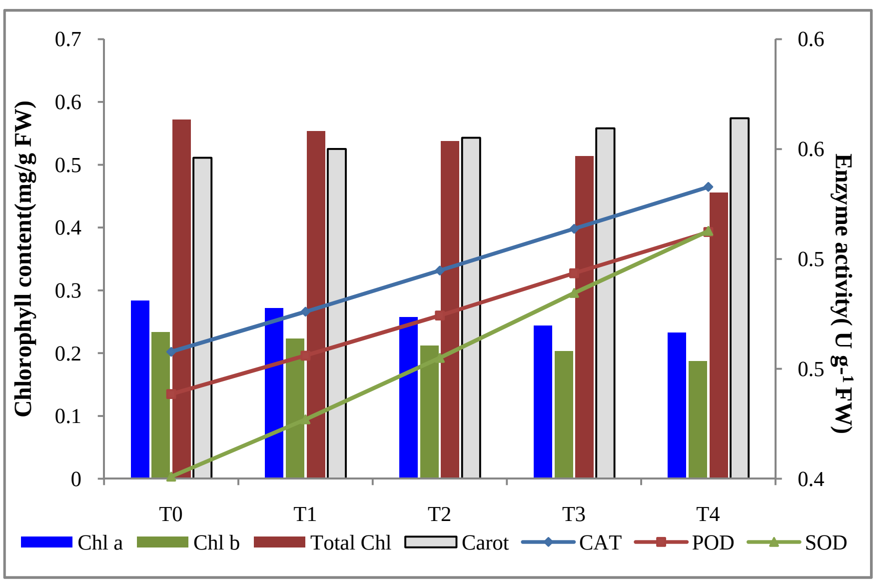

Figure 2. Effect of iron ore tailings on photosynthetic pigments and antioxidant enzyme activities of *L.inermis*. Here, Chl a,band Total=Chlorophyll a, b,& Total: Carot=Carotenoid; CAT=Catalase; POD=Peroxidase; SOD=Superoxide Dismutase.

Table 3. Metal concentration (µg g^{-1} DW) in different parts of *L. inermis* subjected to various metal treatments

		Fe			Pb			Zn	
Treatments	**Total**	**Shoot**	**Root**	**Total**	**Root**	**Shoot**	**Total**	**Root**	**Shoot**
T0	76.48±0.002	48.18±0.001	28.30±0.002	16.32±0.001	10.28±0.002	6.04±0.002	2.90±0.004	1.83±0.002	1.07±0.001
T1	84.48±0.002	53.22±0.002	31.26±0.002	24.13±0.002	15.20±0.001	8.93±0.003	20.33±0.002	12.81±0.001	7.52±0.002
T2	92.48±0.002	58.27±0.001	34.22±0.002	31.94±0.002	20.13±0.001	11.82±0.002	37.76±0.001	23.79±0.001	13.97±0.005
T3	100.49±0.003	63.31±0.003	37.18±0.002	39.76±0.003	25.05±0.002	14.71±0.002	55.19±0.003	34.77±0.002	20.42±0.002
T4	108.49±0.003	68.35±0.003	40.14±0.002	47.58±0.001	29.97±0.002	17.60±0.001	72.61±0.003	45.75±0.001	26.87±0.001
		S>R			**R>S**			**R>S**	
		Cr			**Ni**			**Cu**	
	Total	**Root**	**Shoot**	**Total**	**Root**	**Shoot**	**Total**	**Shoot**	**Root**
T0	127.15±0.004	80.10±0.001	47.05±0.001	3.00±0.002	1.89±0.002	1.11±0.003	16.28±0.002	10.26±0.004	6.02±0.001
T1	123.82±0.002	78.01±0.001	45.81±0.003	6.07±0.002	3.82±0.002	2.25±0.002	33.96±0.001	21.40±0.001	12.57±0.001
T2	126.45±0.000	79.67±0.002	46.79±0.001	4.80±0.001	3.02±0.004	1.78±0.001	61.76±0.003	38.91±0.004	22.85±0.003
T3	124.30±0.002	78.31±0.003	45.99±0.004	2.40±0.001	1.52±0.001	0.89±0.002	67.22±0.002	42.35±0.003	24.87±0.003
T4	228.21±0.002	143.77±0.001	84.44±0.001	10.93±0.001	6.89±0.001	4.04±0.002	141.16±0.001	88.93±0.003	52.23±0.002
		R>S			**R>S**			**S>R**	

n =4, Mean±SE; R=Root; S=Shoot.

Conclusion

The present study clearly revealed that *L. inermis* has the potential to survive in the tailings in spite of its extreme physio-chemical characteristics and minimal nutrient levels. Inhibition of plant growth with excess metal accumulation at later stages of growth indicates its moderate level of tolerance towards heavy metals. However, considering it's adaptability to a wide range of conditions like low air humidity and drought, this plant must be given a serious trial as a phytoremediation tool. Furthermore, as it requires high temperatures for germination, growth and development which are generally a limiting factor for the growth of other plants in the mining areas, this plant can be potential tool for reclamation of mining zones. However, further research related to the use of various amendments particularly organic ones, in order to boost its growth on tailings and tolerance towards toxic metals is urgently suggested.

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