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Phytoaccumulation of trace elements by *Grevillea pteridifolia* Knight grown on iron ore tailings: Implications for phytoremediation

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A B S T R A C T

Pot experiments were conducted to investigate the effect of iron ore tailings (IOT) both individually as well as in combination with soil (at different proportions) on growth, photosynthetic pigments, antioxidant enzymes and accumulation heavy metals (Fe, Cu, Zn, Ni, Cr, and Pb) from Iron ore tailings by *Grevillea pteridifolia*. Results suggested that the plants grown on tailings showed an increased growth, chlorophyll content, as well as metal accumulation with increasing proportion of tailings in the soil. Further, an increase in antioxidant activities in plants grown on tailings as compared to control suggests plant efficiency to overcome stress generated due to excess accumulation of heavy metals. The order of accumulation of various heavy metals in the plant parts was observed to be Fe> Zn>Cr> Cu> Pb> Ni. Overall, *Grevillea pteridifolia* was found to be well adapted in iron ore tailings and it may be recommended for phytoremediation of most of the studied metals.

1. Introduction

Mining operations generate considerable amount of waste materials and tailings, which are either deposited on the surface as mine spoil dumps or stored in large size ponds called tailing ponds. Removal of fertile topsoil, formation of unstable slopes prone to sliding and erosion, and siltation of water bodies due to wash off of mineral overburden dumps are some other negative effects of mining. The metals released from mining, smelting, forging, and other sources would accumulate in the soil, altering its chemistry (Khan *et al*., 2009; Kumar, 2013). Mine contaminated soils represent a very harsh environment for crop production with low pH, nutrients and limited topsoil availability. Thus, reclamation of mine dumps and abandoned mine lands is a complex multi-step process involving improvement of physical and chemical nature of the site (ameliorative) and careful selection of species, cultivars, or ecotypes (adaptive), both to be used in juxtaposition with one another (Johnson *et al.,* 1994).

In recognition of the role of trees to improve soil fertility (Nair *et al.,* 2010), agro-forestry systems (growing trees and crops in an integrated manner) are believed to have a great potential to reclaim the mine contaminated sites. This conjecture is based on the notion that tree incorporation would result in greater export of pollutants, improve site fertility, and render the sites productive (Kumar, 2013). The present study aimed at studying the phytoremediation potential of *Grevillea pteridifolia* Knight, which is widely used in afforestration owing to its fast growing nature and pleasant appearance. Because of thick and fleshy leaves with petioles flexible and capacity to withstand vibration *Grevillea pteridifolia* is also used for noise control at the industry sites (Kumar *et al*, 2013). In spite of its wide spread use in agro-forestry programmes and multiple uses, phytoremediation potential of *Grevillea pteridifolia* was not explored yet. Thus, the objectives of the study were to examine the (1) growth of *Grevillea pteridifolia* on iron ore tailings and (2) accumulation and translocation of various heavy metals within the plant body under different treatments.

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2. Materials and methods

2.1 Tailings substrata analysis

pH, EC and WHC (%) of IOT as well as soil samples were determined according to Chaturvedi *et al*. (2013). Exchangeable Ca, Na, K and Mg were extracted by 1(N) ammonium acetate solution (Gupta, 2000). Organic carbon content was determined by rapid dichromate oxidation technique (Walkey and Black, 1934) and CEC by 1(N) ammonium acetate extraction method (Jackson, 1973). Diethylene triamine penta acetic acid (DTPA) extractable (plant available) metals were determined using 0.005 M DTPA solution (Lopez- Sanchez *et al.*, 2000) while Available N and P by alkaline permanganate (Subbiah and Asija, 1956) and ammonium fluoride extraction (Bray and Kurtz, 1945) methods, respectively.

2.2. Biochemical parameters

2.2.1. Photosynthetic pigments

A photosynthetic pigment like chlorophyll a, b and total was quantified spectrophotometrically following the method of Porra *et al*. (1989).

2.2.2. Antioxidant enzyme assay

Activities of CAT, POD and SOD were measured following the method of Chance and Maehly (1955), Singh *et al*. (2006) and Misra and Fridovich (1972) respectively. The activity of these enzymes was expressed as specific activity (U^{-1} mg protein).

2.2.3. Heavy metal analysis from soil and plant samples

Rhizospheric soil samples were obtained following Yanai *et al.* (2003). Oven dried (60° C) soil tailing and plant samples were ground using a mortar and pestle and digested in aquaregia (HNO3/ HCl, 1:3), and thereby, concentration of heavy metals were determined using the AA-6300 SHIMADZU Atomic Absorption Spectrophotometer after adjustment of required dilution factor. All the reagents and reference standards were of analytical grade from Merks (Darmstandt, Germany) and Suprapure hydrochloric and nitric acids (Merks, Darmstandt, Germany) were used for sample digestion and preparation of standards.

3. Results and discussion

3.1. Physico-chemical parameters

The physico-chemical characteristics of soil and tailings samples are presented in Table 1. The chemical analysis of the iron ore tailings revealed about 59.70% Fe_2O_3, 18.1% Al_2O_3 and 1.77%, SiO_2 and 9.7% LOI. Tailings were comparatively acidic than the garden soil with pH 5.5 for tailings and 6.2 for garden soil. The percentage of WHC was found maximum for the garden soil (38.7%) and minimum for IOT (22.7%). Scanning Electron images revealing the morphology of soil and tailing samples has been presented in Fig 1.

The soil and tailing samples showed large differences between their nitrogen content, but little differences were observed between the potassium and phosphorus content of the two (Table 2). The concentration of environmentally and plant available metals increased or decreased proportionately with the increasing or decreasing proportion of tailings in the soil for most of the metals (Fig 2).

3.2. Growth and photosynthetic pigments

A significant increase in plant height was observed in all the treatments in *Grevillea pteridifolia* (Fig 3). Significant positive correlations ($p < 0.01$) between root length, shoot length, root fresh weight, shoot fresh weight, root dry weight

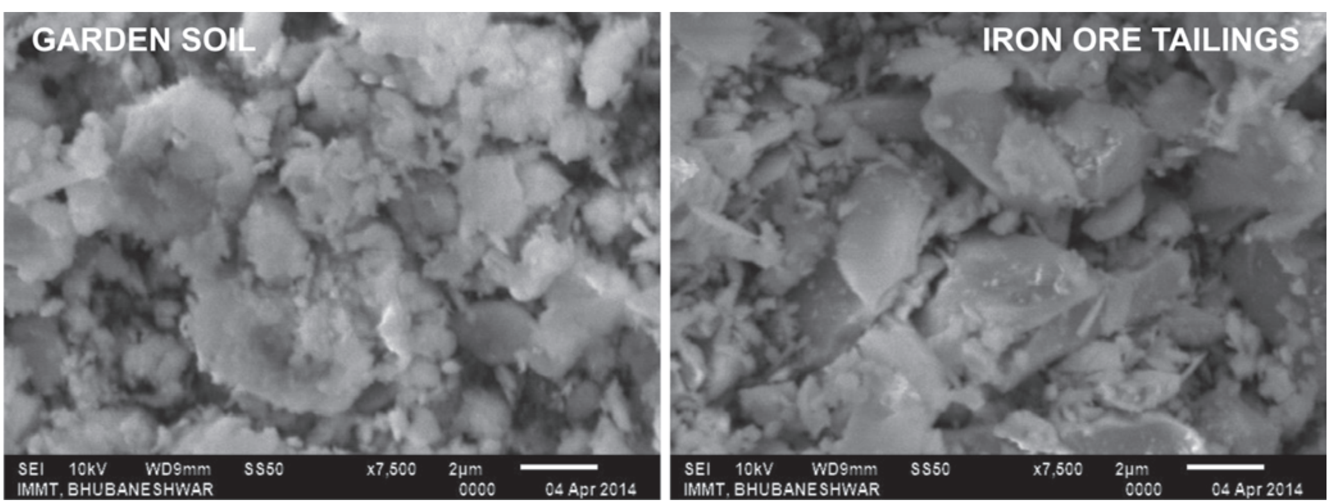

Fig. 1: Scanning electron image of garden soil and iron ore tailings (IOT)

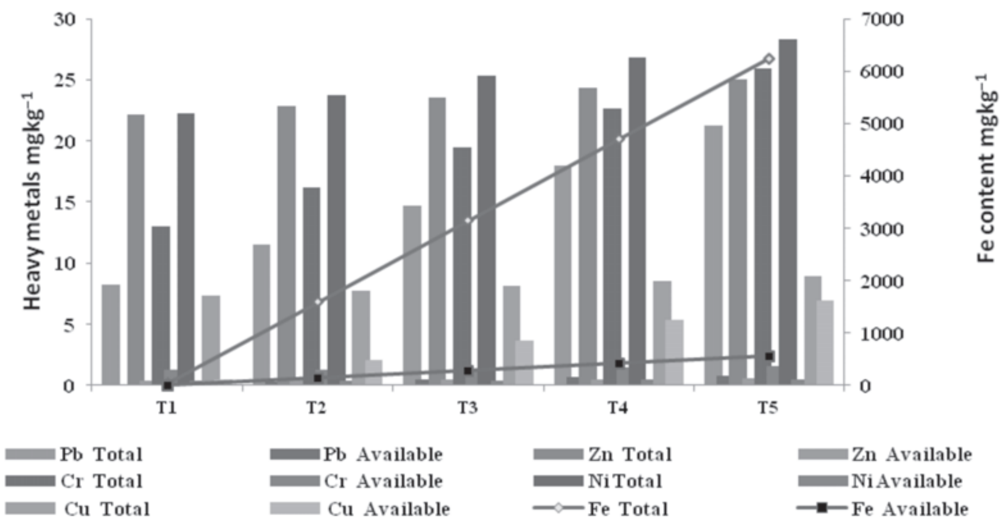

Fig. 2: Environmentally and plant available metal contents (mgkg^{-1}) in control and various treatments

Table 1

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

Treatments	pH	EC (µS/cm)	OC (%)	CEC c mol (+) kg^{-1}	WHC (%)
T0	6.2 ± 0.025*	103 ± 0.017*	3.3 ± 0.007*	0.464 ± 0.033*	38.7 ± 0.009*
T1	6.1 ± 0.26*	108 ± 0.021*	2.4 ± 0.024*	0.533 ± 0.043*	33.4 ± 0.012*
T2	5. 9± 0.026*	112 ± 0.015*	1.4 ± 0.009*	0.599 ± 0.025*	29.8 ± 0.021*
T3	5.7 ± 0.017*	115 ± 0.022*	0.98 ± 0.013*	0.654 ± 0.042*	25.9 ± 0.020*
T4	5.5 ± 0.014*	119 ± 0.029*	0 ± 0.002*	0.731 ± 0.030*	22.7 ± 0.015*

Table 2

Nitrogen, phosphorus and exchangeable cations of soil and tailing samples (n=4, Mean ± SE).

Treatments	Available N (mg/kg)	Available P(mg/kg)	Exchangeable Cations [c (+) mol/kg]			
			Ca	Na	K	Mg
T0	159 ± 0.003*	7.6 ± 0.035*	0.34 ± 0.015*	1.36 ± 0.013*	0.33 ± 0.009*	2.8714 ± 0.013*
T1	116.9 ± 0. 019*	6.1 ± 0.203*	2.94 ± 0.002*	1.97 ± 0.003*	0.27 ± 0.012*	2.5876 ± 0.005*
T2	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	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	28.00 ± 0.009*	1.2 ± 0.169*	8.13 ± 0.005*	0.58 ± 0.111*	0.10 ± 0.005*	0.3142 ± 0.009*

as well as shoot dry weight and the concentration of various heavy metals (Fe, Cu, Pb, Zn, Cr and Ni) in root and shoot parts of *Grevillea pteridifolia* further confirms synergistic effect of these metals/ IOT on growth of the plant Regarding the effect of heavy metals on photosynthetic pigments significant positive correlations between Zn, Ni and chlorophyll content was observed which suggests excellent tolerance mechanism of *G. pteridifolia* towards these toxic metals. Moreover, Chl b synthesis was much lower than Chl a in all the treatments as well as in control. This change in the ratio of Chl a/b suggests differential effect of metals on light-harvesting complexes like LHC2 of PS2, (Aravind and Prasad, 2004).

Furthermore Carotenoids, which are regarded as non-enzymatic antioxidants, serve as an accessory pigment for photosynthesis and protect the chlorophyll pigment under stress conditions by quenching the photodynamic reactions, replacing peroxidation and collapsing membrane in chloroplasts (Kenneth *et al*., 2000). An increase in the concentration of carotenoids with increase in metal uptake confirms the same (Prakash *et al*., 2007).

3.3. *Antioxidant activity*

Exposure of plants to tailings (both with and without additives) led to an increase in the activities of CAT, POD and SOD (Fig 4). The activity of enzymes increased with increase in doses and duration of exposure of tailings. Significant positive correlations ($p < 0.01$) were observed between the shoot heavy metals (Fe, Cu, Zn, Ni, Cr and Pb) and activity of CAT, POD and SOD in both control and the treatments. The higher oxidative enzymes activity is possibly a result of gradual shift of reductive metabolism to oxidative metabolism. These results suggest that heavy metals (Fe, Cu, Zn, Ni, Cd and Pb) present in the tailings induced oxidative stress in the plants and that elevated activity of antioxidant enzymes could play an important role in mitigating oxidative injury.

3.4. *Metal accumulation pattern*

The comparative accumulation of different heavy metals by *Grevillea pteridifolia* subjected to various treatments is shown in Fig 5. The metal concentrations in IOT was found to be significantly ($p <0.01$) higher than the control. 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. Accumulation of Pb, Cr, Ni and Zn was maximum in the root while Fe and Cu in the shoot. The overall order of accumulation of various heavy metals by *Grevillea pteridifolia* was Fe> Zn>Cr> Cu> Pb> Ni. Though there is severe dearth of literature on accumulation of heavy metals by *Grevillea pteridifolia,* a different species of *Grevillea* namely *Grevillea exul* has been reported to accumulate substantial amount of manganese content in the epidermal tissues. Similarly, Léon *et al*. (*2005*) and Rabier *et al*. (*2008*) reported accumulation of *Ni* in different parts of *Grevillea exul* like seed coat and phloem of basal stem and roots respectively (Rabier *et al*., *2008*). Rabier *et al*., (*2008*) reported accumulation of *Ni* in different parts of *Grevillea exul.* like seed coat and phloem of basal stem and roots respectively.

4. Conclusion

Thus, the present study which is to the best of our knowledge is the first detailed report on assessment of heavy metal accumulation potential of *Grevillea pteridifolia* on iron ore tailings clearly suggests that, the plant has not only the potential to survive on metallic wastes like Iron ore tailings but can also accumulate substantial amount of heavy metals. Hence, it can be used as a potential tool for remediation of industrial wastes. Considering its multipurpose uses and beautiful appearance *Grevillea pteridifolia* must be given a serious try in remediation and re-vegetation of industrial wastes and mining zones.

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