Ecological Engineering xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Vetiver grass is a potential candidate for phytoremediation of iron ore mine spoil dumps

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ARTICLE INFO

Keywords:
Iron ore mine soil
Phytoremediation
Vetiver
Antioxidant enzymes
Oxidative damage

ABSTRACT

Vetiver grass, *Chrysopogon zizaniodes* (L.) Roberty, is a perennial C4 grass, valued for its aromatic essential oil in the roots. Vetiver attracted global attention as a natural means for diverse environmental applications including detoxification of degraded soil and water. A pilot study was conducted to investigate its potential to rehabilitate iron ore spoil dumpsites generated from the Joda East Iron mine located in Odisha, India. Four diverse genotypes of vetiver: S2 (diploid variety), S4 (tetraploid derivative of S2), TH (originated from Thailand), BL (a broad leaf) were grown over a period of 12 months to observe their growth performance, metal tolerance and metal uptake. The shoot/root length, photosynthetic pigments – chlorophyll, carotenoid and biomass production of plants grown on iron mine soil decreased initially as compared to the control plants grown on garden soil. At the end of 12 month the plants showed evidence of normal growth and appeared healthy. Scanning electron microscopy (SEM) and Perl's Prussian blue stain confirmed the uptake and localization of Fe in the roots and shoots of the plants grown on mine soil. In addition Zn, Mn, Cr and Cu, was detected in the plant tissues. Such accumulation of metals in plant tissues led to oxidative damage induced by reactive oxygen species (ROS). As a consequence the activities of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOD), glutathione reductase (GR) and glutathione peroxidase (GPX) were increased. Based on the results it can be inferred that vetiver grass *per se* can tolerate high concentrations of Fe along with other heavy metals in its tissues. Such potential vary across the four genotypes, and the genotype BL followed by S4 can be claimed of paramount importance in terms of phytoremediation. Thus vetiver grass can be effectively used for rehabilitation and soil stabilization of sites contaminated with high levels of heavy metals, particularly Fe, Mn, Zn and Cr.

1. Introduction

India is one of the fourth largest producers of iron ores in the world (Tuck et al., 2017). Mine over burden soil (OB) or spoil dumps are generated as waste products of large scale mine activity. They are inhospitable for plant growth due to lack of nutrients, low pH, metal toxicity, and are also erosion-prone owing to low water holding capacity (Juwarkar et al., 2009; Mendez and Maier, 2008; Pasayat and Patel, 2015; Verma et al., 2012). The mine wastes often release toxic heavy metals into the surrounding soil and contaminates the surface and ground water (Adhikary, 2015). Re-vegetation of such contaminated sites by suitable plants could remediate soil toxicity and facilitate the stabilization of soil surface in a long-term and prevent soil erosion. The required bioremediation strategies are eco-friendly (Aksorn and Chitsomboon, 2013; Prasad and Prasad, 2012), and could be quite effective for reclamation of mine spoil dumpsites.

Vetiver [*Vetiveria zizanioides* (L.) Nash. Syn. *Chrysopogon zizaniodes* (L.) Roberty; family Poaceae] is a perennial densely tufted C4 grass native to India. The grass has been traditionally used in India for centuries for extraction of aromatic essential oil from roots, and for the dense stand of stiff, erect stems forming a living barrier have been used for soil conservation (Lavania, 2008). Genetic analysis confirmed diversity in vetiver ecotypes for root and shoot morphotypes – (i) suitable for extraction of essential oil (Chakrabarty et al., 2015) and (ii) for diverse environmental applications (Chauhan et al., 2017; Lavania et al., 2016). This has led to the identification of appropriate genotypes for industrial and environmental applications. Lately, this grass is being grown successfully worldwide from tropical to Mediterranean regions for its environmental applications (Lavania and Lavania, 2009). The plant is extremely tolerant to a wide variety of heavy metals along with

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https://doi.org/10.1016/j.ecoleng.2018.10.012
Received 2 April 2018; Received in revised form 20 September 2018; Accepted 15 October 2018

variations in temperature, soil salinity, and pH (Danh et al., 2009). A study revealed that the vetiver roots and shoots can sequester approximately five times higher amount of Zn and Cr present in the soil compared to other such rehabilitating species (Truong, 1999). The ability of metal accumulation by this plant in addition to its tolerance to high metal concentrations and high biomass of roots and shoots makes it a choice plant for phytoremediation. Various reports have underpinned the importance of vetiver grass in restoration of overburden sites containing ores of Cu and Au (Knoll, 1997; Radloff et al., 1995; Truong, 1999), Pb/Zn (Shu et al., 2002; Pang et al., 2003; Chiu et al., 2006), Fe (Roongtanakiat et al., 2008), Cu (Das and Maiti, 2009), Pb (Meeinkuirt et al., 2013) and Cr/asbestos (Kumar and Maiti, 2015). But no systematic study on its phytoremedial potential including the site of metal uptake and scavenging mechanisms by vetiver grown over iron ore spoil dumps has been conducted.

It is well known that the toxic metals cause damage to plants causing alterations in their physiological and metabolic status (Hossain et al., 2009; Monni et al., 2001) by inhibiting photosynthesis, respiration and several enzyme activities (Villiers et al., 2011; Volland et al., 2014). Excessive reactive oxygen species (ROS) generation leads to the inhibition of photosynthetic machinery that subsequently results in extreme cellular damage and leaf chlorosis (Van Breusegem et al., 2001). ROS consists of free radicals and non-radical molecules which includes hydroxyl radical ($HO^{\cdot}$), superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2). Cellular ROS are produced endogenously as a consequence of aerobic metabolism or can be generated excessively due to disruption of cellular homeostasis by environmental or heavy metal stress. In order to protect the cellular components, the combined action of enzymatic and non-enzymatic detoxifying agents control the cellular ROS levels, before any visible symptoms of toxicity appear (Luna et al., 1994). On the other hand, plants develop various strategies to defend the external high concentration of metal which include sequestration of metals, restriction of its uptake and transport to control the accumulation and translocation of toxic metals (Anjum et al., 2015; Clemens, 2006). The antioxidant defense mechanism includes superoxide dismutase (SOD), catalase (CAT), amylase, peroxidase (POD), glutathione S transferases (GST), production of metal binding proteins and low molecular weight thiols (LMWT) and the synthesis of secondary metabolites such as phenolics compounds. In addition, phytochelatins-oligomers of glutathione act as chelators and are considered important for heavy metal detoxification (Ha et al., 1999).

In the present study, we have selected four genotypes of vetiver and grown them for a period of 12 months on iron mine OB soil with the objective to study their growth at an interval of 6 months. The parameters included physiological and biochemical assays, uptake and accumulation of iron and other metals in the plant tissues. In addition, oxidative damage induced by ROS, the involvement of defense mechanisms (antioxidant and intracellular enzymes), and the role of phytochelatins was evaluated. The results showed that vetiver grass in general can be used for the rehabilitation of iron mine OB soil and the genotypes BL and S4 were better adaptable to the iron mine soil conditions.

2. Materials and methods

2.1. Plant material

Four genotypes of Vetiver, *Chrysopogon zizanioides* (L.) Roberty available at the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India were selected for the study. The characteristics of Vetiver genotypes are presented in table 1. For the sake of easy usage they are named as: (i) S2 (ii) S4 (iii) TH and (iv) BL. The plants were grown and vegetatively multiplied in the Experimental Botanical Garden of the Department of Botany, University of Calcutta, Kolkata, India.

2.2. Collection of soil sample

The soil sample was collected in plastic containers from the iron mine OB dumpsite at Joda East Iron mine (22°00′19″N, 85°26′34″E), Odisha, India. The soil sample was sun-dried and stored at room temperature (28 ± 1 °C).

2.3. Experimental setup

An open pot experiment was designed to assess the development of vetiver plants grown on iron ore mine OB soil. Vetiver plants were planted in earthen pots (20 cm diameter × 17 cm height) containing OB and garden soil (control), 6 kg/pot for a period of 12 months. Three sets of four genotypes for each sampling period (0, 6 and 12 months) with three plants per pot were planted. Plants were kept under natural environmental conditions (annual mean temperature 24–35 °C, annual relative humidity 70.8%). The response of the vetiver genotypes was assessed at an interval of six months and continued till 12 months.

2.4. Physico-chemical characterization and analysis of metal content in mine and garden soil

For physicochemical analysis, the mine soil sample was sun-dried, ground and passed through a 2 mm sieve. 10 g of the sample was suspended in 100 ml of distilled water (1:10 w/v). The pH, conductivity, total dissolved solids (TDS), salinity and resistivity was estimated from the supernatant by Cyber Scan CD 650 (Eutech Instruments Pvt Ltd., Singapore) according to the method of Jackson (1967).

For analysis of metal content in mine soil, 1 g of soil sample was digested in a mixture of concentrated HNO_3 and $HClO_4$ (4:1 ratio, v/v) and analyzed for Fe, Zn, Mn, Cu, Cr, and Pb by Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES, ARCOS – Simultaneous ICP Spectrometer, SPECTRO Analytical Instruments GmbH, Germany). The metal concentrations were expressed as mg kg^{-1}. Characterization of size of the mine soil was done by means of scanning electron microscope (SEM) imaging (ZEISS EVO-MA 10; Carl Zeiss Pvt. Ltd., Oberkochen, Germany). Energy-dispersive X-ray spectroscopy (EDX) was performed simultaneously (Model No. 51-ADD0011, OXFORD Instruments, Germany) for semi-quantitative elemental analysis to identify the major and minor elements (as weight percentage) present.

2.5. Assessment of plant growth

The length and fresh weight of the shoot and root were measured immediately after washing. The root and shoot samples were dried for 48 h at 80 °C and the weights were recorded.

The tolerance index (TI) was determined to assess the ability of the vetiver varieties to grow in presence of iron mine OB soil according to the following equation (Wilkins, 1978):

TI (%) = Dry weight of the treated plants/Dry weight of the control plants × 100

(Dry weight of the roots or above-ground tissues of the vetiver were considered)

Relative water content percentage (RWC %) was measured following the method of Chen et al. (2009) using the following formula:

$$RWC\ \% = [(FW - DW)/FW] \times 100$$

where FW = fresh weight of the sample; DW = dry weight of the sample.

2.6. Estimation of heavy metals in vetiver plants and its phytoextraction ability

Oven dried plant samples (1 g) were digested and analyzed by ICP-AES (ARCOS, Spectro, Germany) following the method mentioned

Table 1
Morphometric characteristics of Vetiver varieties.

Characteristics	High Oil KH-20 (S2)	High Oil KH-40 (S4)	Medium Oil – Surathani (TH)	Low oil Broad Leaf Type (BL)
General morphology	Tall, green, profuse flowering, smooth and thick roots	Medium height, low flowering, no seed set, smooth and thick roots	Semi-spreading, profuse flowering, low seed set, smooth and thin roots	Medium height, broad leaf, high tillering, less green with profuse secondary roots
Growth Habit	Fast growing	Fast growing	Fast growing	Fast growing, profuse tillering
Plant height (cm.) (taken as leaf length)	157	142	135	158
Shoot yield (g) (culm/leaf dry matter) after 5 months	85	95	65	120
Number of leaves/tiller	6–8	7–9	5–7	8–9
Number of slip/ tillers after 5 months	20–23	18–20	25–28	25–28
Inflorescence stalk length (culm and inflorescence combined)	195	185	190	200
Culm length (cm.)	105	95	100	100
Leaf color	RHS 137 A Green group	RHS 138B Green group	RHS 137B Green group	RHS 143B Green group
Leaf Texture/No. of air chambers	Stiff/ 17	Stiff/20	Stiff/20	Smooth/22
Leaf Thickness at midrib (mm)	0.40	0.66	0.53	0.35
Leaf blade stomatal index	7.16	6.47	5.78	5.36
Average size of guard cell (μm^2)	99	119	88	108
Stomata/mm^2	115	77	133	113
Number of primary roots after 5 months	193	185	198	223
Root Length (cm.) after 5 months	150–155	155–185	130–140	135–160
Average root diameter/stele diameter (at the base of main root) (mm.)	2.0/1.2	2.3/1.4	1.9/1.4	2.19/0.91
Total root dry weight (g/plant) after 5 months	38	43	31	46
Oil Content (%) in fresh roots	1.0	1.5	0.8	0.3

Table 2
Physico-chemical characters of garden and iron mine overburden soil.

Parameters	Garden soil	Iron mine overburden soil
pH	7.47 ± 0.3	6.50 ± 0.17
Conductivity (μS/cm)	142.80 ± 2.5	86.03 ± 1.31
Total dissolved solids (TDS) (mg/kg)	129.05 ± 5.2	82.74 ± 3.09
Salinity (NaCl content) (mg/kg)	126.55 ± 4.1	80.01 ± 2.62
Resistivity (Ω)	3.94 ± 0.04	6.08 ± 0.07
Fe (mg/kg)	322.25 ± 11.02	19119.30 ± 59.36
Cr (mg/kg)	26.00 ± 2.2	239.45 ± 5.92
Zn (mg/kg)	45.60 ± 2.8	75.80 ± 3.28
Mn (mg/kg)	50.70 ± 3.6	68.15 ± 2.80
Cu (mg/kg)	14.50 ± 1.02	37.25 ± 4.11
Pb (mg/kg)	< 0.01	< 0.01

Values are mean of 3 samples ± SE.

above. The metal content was expressed as mg kg^{-1} DW.

The phytoextraction ability of the plants was evaluated by calculating translocation factor (TF) and bioaccumulation factor (BF) according to the method of Wu et al. (2011).

$$TF = [Metal]_{shoot}/[Metal]_{root}$$

$$BF_{shoot} = [Metal]_{shoot}/[Metal]_{soil}$$

$$BF_{root} = [Metal]_{root}/[Metal]_{soil}$$

2.7. Anatomical changes and localization of iron in leaves and roots

The morphology of the plant tissues, surface adsorption and internalization of Fe were studied by SEM followed by EDX for elemental analysis. Leaf and root samples were fixed in a solution of 0.4% paraformaldehyde and 2.5% glutaraldehyde in sodium phosphate buffer (0.1 M; pH 7.2). They were dehydrated in graded ethanol series, dried and coated with platinum in the sputtering device and observed by SEM (ZEISS EVO-MA 10; Carl Zeiss Pvt. Ltd., Oberkochen, Germany).

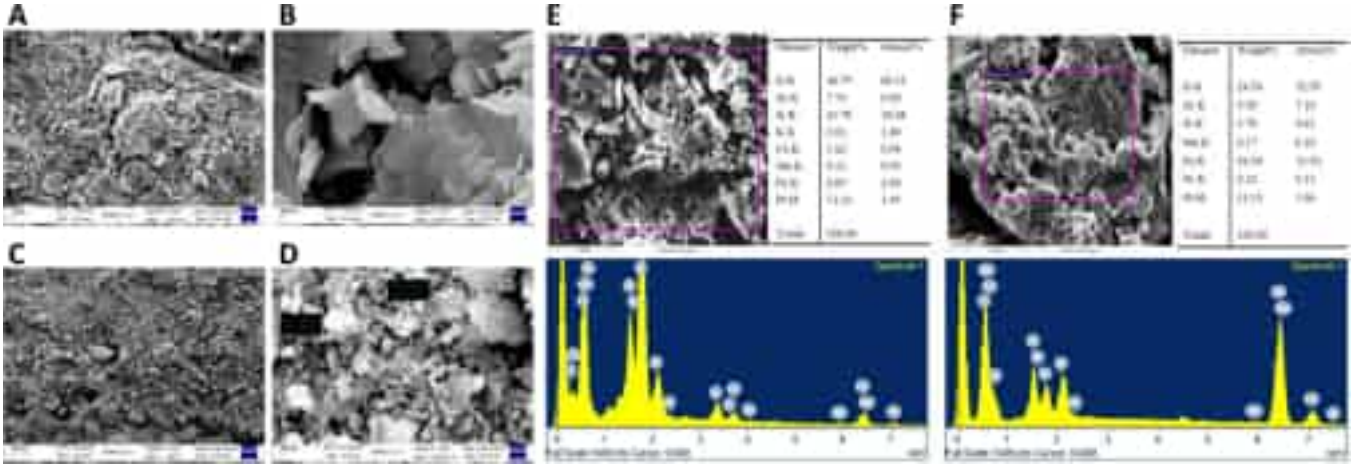

Fig. 1. Scanning electron microscopic images of (a, b) garden soil and (c, d) iron mine overburden soil; Energy-dispersive X-ray spectroscopic (EDX) analysis of (e) garden soil and (f) iron mine spoil dump soil.

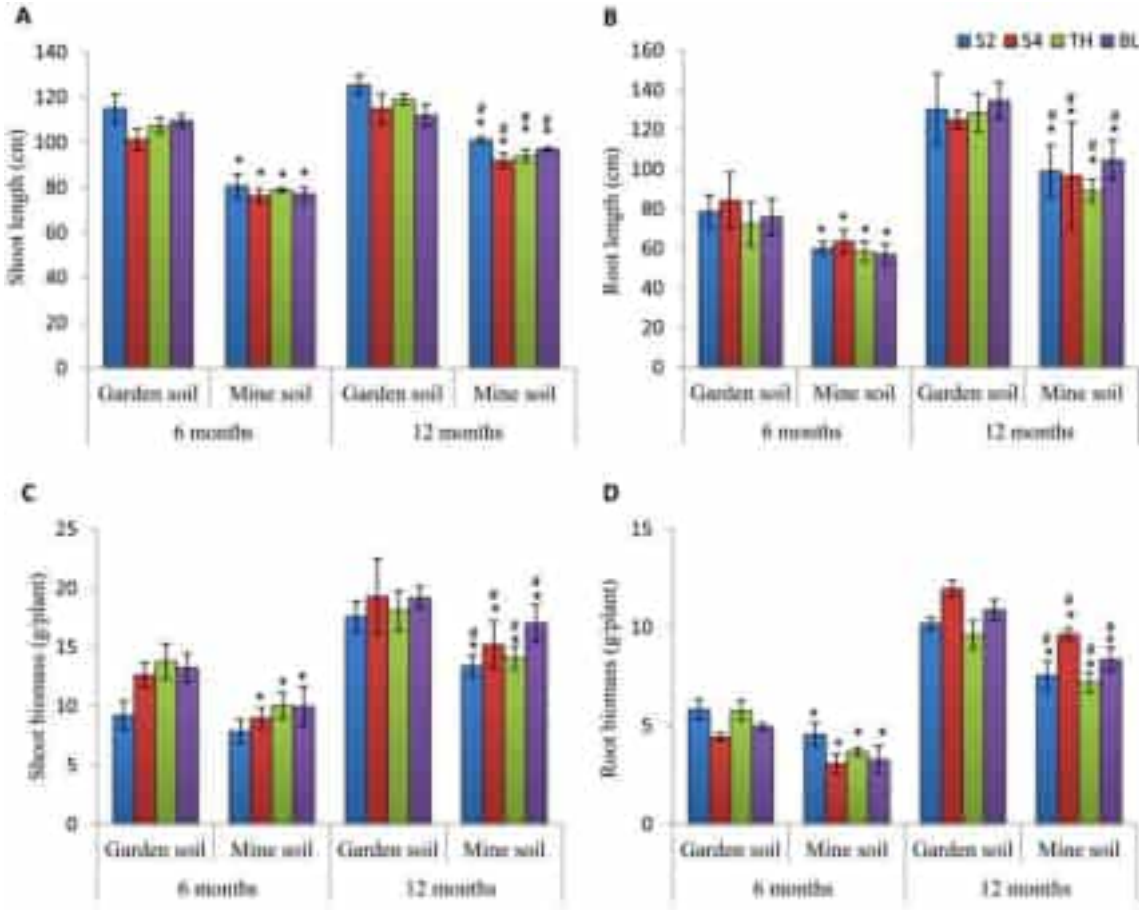

Fig. 2. Growth of vetiver varieties (S2, S4, TH and BL) grown on garden soil and iron mine overburden soil: (a) shoot length; (b) root length; (c) shoot dry weight; (d) root dry weight [*Statistically significant ($P \leq 0.05$) compared to respective control at 6 or 12 months, #Statistically significant ($P \leq 0.05$) between 6 and 12 months].

To observe the localization of Fe in vetiver plants, fresh leaf and root tissues were processed and stained by Perls Prussian blue (Stacey et al., 2008). The transverse sections of fresh leaf and root were infused with 4% (v/v) HCl and 4% (w/v) potassium ferrocyanide (Perls Prussian blue stain). Excess stain was washed with distilled water and the slides were observed under light microscope.

2.8. Estimation of chlorophyll and carotenoid content

Photosynthetic pigments including chlorophyll *a*, chlorophyll *b* and carotenoid were quantified from shoots (100 mg fresh weight) according to the method of Lichtenthaler (1987). Chlorophyll *a*, chlorophyll *b* and carotenoid were extracted in pure acetone and the absorbance was read at 470, 647 and 663 nm respectively, in a spectrophotometer (Beckman Coulter, DU 730, CA, USA). Chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content were calculated using the following equations and expressed as mg g^{-1} FW:

$$\text{Chlorophyll } a = 12.25A_{663} - 2.79A_{647}$$

$$\text{Chlorophyll } b = 21.50A_{647} - 5.10A_{663}$$

$$\text{Total chlorophyll} = \text{chlorophyll } a + \text{chlorophyll } b$$

$$\text{Carotenoid} = (1000A_{470} - 1.82\,\text{Chlorophyll a} - 85.02\,\text{Chlorophyll b})/198$$

A_{470}, A_{647} and A_{663} represent the absorbance at wavelengths of 470, 647 and 663 nm respectively.

2.9. Biochemical stress markers

2.9.1. Proline content

Extraction and biochemical quantification of proline content was performed according to the method of Bates et al. (1973). The absorbance was measured at 520 nm and the proline concentration was calculated from a standard curve and the values were expressed as μmol g^{-1} FW.

2.9.2. Lipid peroxidation

Lipid peroxidation was estimated by the amount of malondialdehyde (MDA) produced following the method of Dhindsa et al. (1981). The absorbance of the supernatant was read at 532 and 600 nm. The subtraction of non-specific absorbance at 600 nm from the absorbance at 532 nm was carried out. The content of MDA was determined from the extinction coefficient 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

2.9.3. Quantification of H_2O_2

H_2O_2 was extracted from plant tissues as described by Islam et al. (2008) and H_2O_2 content was read at 390 nm and expressed as nmol g^{-1} FW.

2.10. Estimation of antioxidants

For the extraction of enzymes fresh leaf samples (1 g) were homogenized in 50 mM Tris buffer (pH 7.8), 1 mM EDTA, 1 mM $MgCl_2$ and 1.5% polyvinylpyrrolidone (PVP) on an ice bath. The homogenate was

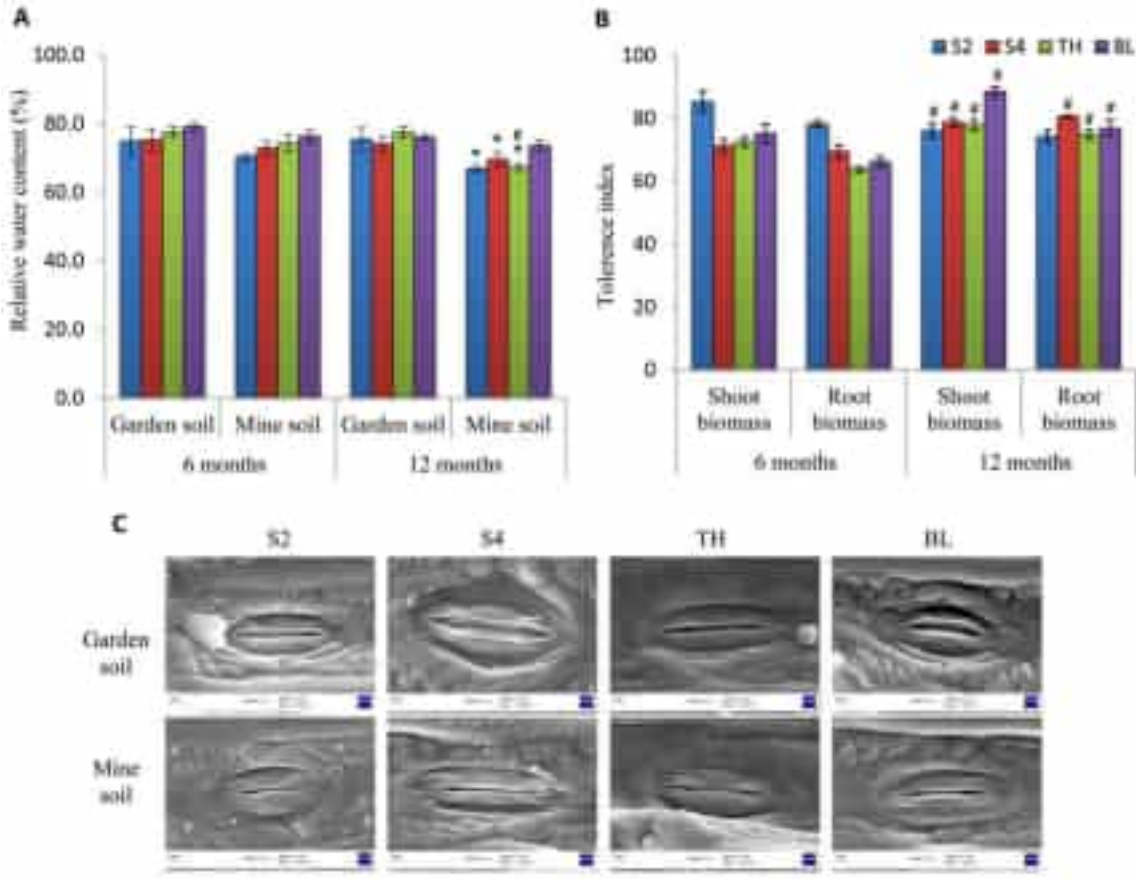

Fig. 3. (a) Relative water content (%) of vetiver varieties (S2, S4, TH, and BL) grown on garden and iron mine overburden soil; (b) Tolerance index of vetiver varieties (S2, S4, TH, BL) grown on garden soil and iron mine overburden soil, [*Statistically significant ($P \leq 0.05$) compared to respective control at 6 or 12 months, #Statistically significant ($P \leq 0.05$) between 6 and 12 months]; (c) Representative scanning electron microscopic images of stomata of the vetiver varieties.

centrifuged at 14,000 rpm for 30 min at 4 °C and the supernatant was used for enzyme analysis. An aliquot of the extract was used to determine the soluble protein content (Bradford, 1976). The absorbance was read at 595 nm using a microplate spectrophotometer (IMark, BioRad, USA). The protein content was calculated using bovine serum albumin (BSA) as standard.

The enzymatic antioxidants - superoxide dismutase (SOD, EC. 1.15.1.1), catalase (CAT, EC. 1.11.1.6), guaiacol peroxidase (GPOD, EC. 1.11.1.7), glutathione reductase (GR, EC. 1.6.4.2) and glutathione peroxidase (GPX, EC. 1.11.1.9) were analyzed from the enzyme extracts.

The activity of SOD was measured by the method of Beauchamp and Fridovich (1971); CAT activity was estimated by the method of Aebi (1984); GPOD activity was measured following the method of Chance and Maehly (1955); for GR activity the method of Smith et al. (1988) was followed and for GPX activity the method of Flohé and Günzler (1984) was adopted.

The changes in glutathione level were estimated by measuring the reduced (GSH) and oxidized (GSSG) glutathione content following the method of Anderson (1985). The absorbance was read at 412 nm and the total glutathione amount was determined. The amount of GSH was calculated using standard curve prepared from varying concentrations of GSH. The GSSG content was calculated by subtracting GSH from total glutathione content and was expressed as nmol μg^{-1} protein.

2.11. Synthesis of phytochelatins

The analysis of phytochelatins (PC) was carried out by pre-column derivatization of thiol compounds using monobromobimane (mBBr) as described by Sneller et al. (2000) with minor modifications. The sample was filtered through 0.45 µm nylon syringe filter and analyzed by Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) with C 18 column (5 µm, 4.6 × 150 mm) using a binary gradient of mobile phase A (0.1% TFA) and B (100% CH_3CN) with a flow rate 1 ml min^{-1} at room temperature. The derivatized samples (10 µl) were run using a linear gradient profile (0–10 min, 12–20% B; 10–40 min, 20–35% B; 40–50 min, 35–100% B; 50–55 min, 100% B; 55–65 min, 100–10% B) and equilibrated for 5 min with 12% B with the total run time of 70 min. The fluorescence intensity was recorded by a fluorescence detector with the excitation wavelength at 380 nm and emission at 470 nm. A sample blank with mBBr was run to identify the reagent peaks. Identification of individual PCs was based on the comparison of the retention time with standard PCs (PC3 and PC4).

2.12. Statistical analysis

Statistical analyses of the data were performed using the statistical program – SigmaStat 3.0 (SPSS Inc., Chicago, IL, USA). All data are expressed as mean ± standard error (SE) of three independent experiments with three replicates each. Student's *t*-test (at the significance level $P \leq 0.05$) was done for different parameters between control and exposed sets.

3. Results

3.1. Morphometric differentiation of vetiver varieties

There are a number of varieties of vetiver plants found across the

Table 3
Estimation of metal contents (mg kg^{-1} DW) in shoot and root of vetiver varieties (S2, S4, TH, and BL) grown on iron mine overburden soil.

Months	Vetiver varieties	Treatment condition	Fe		Cu		Zn		Cr		Mn	
			Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	S2	Garden soil	7.21 ± 1.06	10.33 ± 2.17	3.64 ± 0.68	2.17 ± 0.45	2. 46 ± 0.14	7.94 ± 0.52	2.57 ± 0.72	4.98 ± 0.49	1.44 ± 0.03	8.32 ± 1.04
		Mine soil	6.31 ± 1.39	9.12 ± 3.93	2.10 ± 0.23	1.83 ± 0.08	2.79 ± 0.63	7.82 ± 3.15	2.78 ± 0.32	4.85 ± 2.03	1.56 ± 0.79	8.50 ± 4.23
	S4	Garden soil	6.79 ± 0.94	9.02 ± 1.59	1.86 ± 0.58	1.63 ± 0.2	1.8 ± 0.05	6.71 ± 1.04	2.39 ± 0.08	3.16 ± 0.27	1.73 ± 0.2	8.19 ± 1.17
		Mine soil	5.13 ± 1.82	8.45 ± 4.08	1.92 ± 0.11	1.97 ± 0.14	1.73 ± 0.03	6.99 ± 2.46	2.52 ± 0.29	3.28 ± 1.71	1.70 ± 0.33	8.22 ± 4.16
	TH	Garden soil	5.67 ± 0.61	8.21 ± 1.09	2.09 ± 0.58	2.2 ± 0.29	1.28 ± 0.09	6.12 ± 0.83	2.67 ± 0.02	3.31 ± 0.78	1.19 ± 0.12	7.9 ± 1.54
		Mine soil	6.12 ± 1.71	8.13 ± 3.85	2.37 ± 0.35	2.54 ± 0.25	1.35 ± 0.12	5.99 ± 1.73	2.85 ± 0.46	3.17 ± 1.96	1.23 ± 0.16	7.96 ± 4.06
	BL	Garden soil	6. 11 ± 1.3	9.46 ± 2.02	2.72 ± 0.34	2.81 ± 0.08	2.19 ± 0.22	6.08 ± 1.1	3 ± 0.21	4.24 ± 0.32	1.26 ± 0.09	8.02 ± 2.31
		Mine soil	5.97 ± 1.88	8.82 ± 4.11	2.68 ± 0.58	1.89 ± 0.39	2.47 ± 0.59	6.27 ± 3.01	3.07 ± 0.25	4.36 ± 1.89	1.34 ± 0.31	8.27 ± 4.49
6	S2	Garden soil	76.19 ± 4.92	175.65 ± 17.3	13.56 ± 1.66	10.52 ± 1.21	3.95 ± 0.68	10.15 ± 1.71	4.11 ± 0.28	9.73 ± 2.07	9.36 ± 2.5	13.4 ± 3.3
		Mine soil	478.40 ± 50.46*	1498.81 ± 109.44*	47.42 ± 7.82*	36.83 ± 6.50*	5.91 ± 0.99*	14.12 ± 6.05*	8.37 ± 3.15*	15.94 ± 6.49*	16.37 ± 7.42*	21.40 ± 9.86*
	S4	Garden soil	85.69 ± 6.73	239.36 ± 21.8	25.32 ± 2.81	18.5 ± 2.47	3.69 ± 0.18	7.64 ± 1.98	5.51 ± 1.3	7.33 ± 1.42	6.52 ± 1.59	13.72 ± 2.97
		Mine soil	486.30 ± 49.28*	1614.12 ± 99.52*	61.27 ± 9.25*	45.57 ± 5.25*	6.47 ± 1.27*	10.70 ± 4.16*	8.40 ± 2.96*	12.38 ± 5.90*	10.61 ± 4.24*	18.23 ± 7.30*
	TH	Garden soil	79.77 ± 7.01	213.43 ± 15.31	16.92 ± 3.21	12.49 ± 0.98	3.65 ± 0.31	8.37 ± 2.6	5.37 ± 1.81	11.39 ± 3.01	9.13 ± 2.36	14.56 ± 3.82
		Mine soil	544.55 ± 47.03*	1642.01 ± 100.37*	37.72 ± 6.18*	44.85 ± 4.91*	7.31 ± 1.92*	13.50 ± 6.49*	9.36 ± 3.98*	14.36 ± 7.00*	16.96 ± 7.93*	21.25 ± 9.46*
	BL	Garden soil	101.52 ± 9.52	373.92 ± 28.9	20.28 ± 3.13	23.95 ± 3.90	3.99 ± 0.55	8.27 ± 1.79	4.21 ± 0.93	6.26 ± 1.35	6.68 ± 1.71	19.23 ± 4.07
		Mine soil	556.70 ± 52.39*	1661.37 ± 125.46*	46.67 ± 7.47*	56.35 ± 6.14*	6.93 ± 1.21*	11.90 ± 5.00*	7.39 ± 2.45*	9.95 ± 4.08*	12.57 ± 5.67*	27.49 ± 13.35*
12	S2	Garden soil	112.81 ± 8.39	414.46 ± 19.29	26.32 ± 5.61	16.41 ± 2.11	6.91 ± 2.39	14.39 ± 3.1	11.72 ± 2.07	19.3 ± 4.73	15.83 ± 2.5	27.14 ± 4.10
		Mine soil	1276.8 ± 98.43*	2992.86 ± 213.65*	128.03 ± 15.36*	84.71 ± 7.43*	10.64 ± 3.13*	36.71 ± 11.56*	16.74 ± 4.93*	39.85 ± 19.02*	29.47 ± 13.42*	51.36 ± 24.00*
	S4	Garden soil	120.35 ± 11.61	556.74 ± 26.14	32.55 ± 7.21	34.82 ± 3.17	6.28 ± 1.38	16.65 ± 3.09	10.49 ± 2.74	19.94 ± 3.52	13.64 ± 3.38	19.74 ± 4.87
		Mine soil	1041.6 ± 87.35*	3684.72 ± 285.59*	165.43 ± 17.60*	104.81 ± 14.38*	11.65 ± 1.54*	27.82 ± 10.28*	16.80 ± 3.99*	30.95 ± 13.76*	19.10 ± 9.88*	43.75 ± 19.79*
	TH	Garden soil	131.87 ± 14.86	434.01 ± 21.39	27.7 ± 4.57	22.52 ± 4.02	6.71 ± 1.53	15.84 ± 2.9	11.98 ± 2.3	16.8 ± 3.29	13.16 ± 4.05	20.89 ± 5.11
		Mine soil	916.1 ± 75.05*	3852.06 ± 269.72*	101.84 ± 15.01*	103.16 ± 13.71*	13.16 ± 1.49*	35.10 ± 15.42*	18.72 ± 7.02*	35.90 ± 12.48*	30.53 ± 12.48*	51.00 ± 22.59*
	BL	Garden soil	143.73 ± 16.62	674.05 ± 38.91	39.11 ± 6.34	42.01 ± 3.69	6.1 ± 2.02	18.33 ± 3.62	10.73 ± 3.09	17.36 ± 3.82	12.79 ± 3.53	24.41 ± 6.17
		Mine soil	1189.3 ± 86.09*	3968.22 ± 240.13*	126.01 ± 12.22*	129.61 ± 16.40*	12.47 ± 1.66*	30.94 ± 11.71*	14.78 ± 5.46*	24.88 ± 9.33*	22.63 ± 10.65*	65.98 ± 29.42*

Values are mean of 3 samples ± SE, *Statistically significant ($P \leq 0.05$) compared to respective control.

Table 4
Relative translocation (TF) and bioaccumulation (BF) of heavy metals in vetiver varieties (S2, S4, TH, and BL) grown on iron mine overburden soil.

	Time (Months)	Vetiver varieties	Treatment condition	Fe	Cu	Zn	Cr	Mn
TF	6	S2	Garden soil	0.434	1.289	0.389	0.422	0.699
			Mine soil	0.319	1.288	0.419	0.525	0.765
		S4	Garden soil	0.358	1.369	0.483	0.752	0.475
			Mine soil	0.301	1.345	0.605	0.679	0.457
		TH	Garden soil	0.374	1.355	0.436	0.471	0.627
			Mine soil	0.332	0.841	0.541	0.652	0.798
		BL	Garden soil	0.272	0.847	0.482	0.673	0.347
			Mine soil	0.335	0.828	0.582	0.494	0.457
	12	S2	Garden soil	0.272	1.604	0.480	0.607	0.583
			Mine soil	0.427	1.511	0.290	0.420	0.574
		S4	Garden soil	0.216	0.935	0.377	0.526	0.691
			Mine soil	0.283	1.578	0.419	0.543	0.437
		TH	Garden soil	0.304	1.230	0.424	0.713	0.630
			Mine soil	0.265	0.987	0.375	0.521	0.599
		BL	Garden soil	0.213	0.931	0.333	0.618	0.524
			Mine soil	0.300	0.972	0.403	0.329	0.343
BF_{shoot}	6	S2	Garden soil	0.236	0.935	0.087	0.158	0.185
			Mine soil	0.025	1.273	0.078	0.035	0.240
		S4	Garden soil	0.266	1.746	0.081	0.212	0.129
			Mine soil	0.025	1.645	0.085	0.035	0.156
		TH	Garden soil	0.248	1.167	0.080	0.207	0.180
			Mine soil	0.028	1.013	0.096	0.039	0.249
		BL	Garden soil	0.315	1.399	0.088	0.162	0.132
			Mine soil	0.029	1.253	0.091	0.031	0.184
	12	S2	Garden soil	0.350	1.815	0.152	0.451	0.312
			Mine soil	0.067	3.437	0.140	0.070	0.432
		S4	Garden soil	0.373	2.245	0.138	0.403	0.269
			Mine soil	0.054	4.441	0.154	0.070	0.280
		TH	Garden soil	0.409	1.910	0.147	0.461	0.260
			Mine soil	0.048	2.734	0.174	0.078	0.448
		BL	Garden soil	0.446	2.697	0.134	0.413	0.252
			Mine soil	0.062	3.383	0.165	0.062	0.332
BF_{root}	6	S2	Garden soil	0.545	0.726	0.223	0.374	0.264
			Mine soil	0.078	0.989	0.186	0.067	0.314
		S4	Garden soil	0.743	1.276	0.168	0.282	0.271
			Mine soil	0.084	1.223	0.141	0.052	0.341
		TH	Garden soil	0.662	0.861	0.184	0.438	0.287
			Mine soil	0.086	1.204	0.178	0.060	0.312
		BL	Garden soil	1.160	1.652	0.181	0.241	0.379
			Mine soil	0.087	1.513	0.157	0.062	0.403
	12	S2	Garden soil	1.286	1.132	0.316	0.742	0.535
			Mine soil	0.157	2.274	0.484	0.166	0.754
		S4	Garden soil	1.728	2.401	0.365	0.767	0.389
			Mine soil	0.193	2.814	0.367	0.129	0.642
		TH	Garden soil	1.347	1.553	0.347	0.646	0.412
			Mine soil	0.181	2.769	0.463	0.150	0.748
		BL	Garden soil	2.092	2.897	0.402	0.668	0.481
			Mine soil	0.208	3.479	0.408	0.187	0.968

globe that are cultivated mainly for their essential oil. Thus a dozens of divergent varieties have been identified varying in their physiological, morphological and ecological characteristics. A brief account of the morphotypic differentiation of the four genotypes / varieties used in this study is provided in Table 1.

3.2. Physico-chemical characterization and analysis of metal content of soil

The physicochemical properties and heavy metal content in iron mine OB soil are presented in Table 2. The pH of the mine soil (pH 6.5) was slightly acidic compared to the control garden soil which was slightly alkaline (pH 7.47). The electrical conductivity (EC) of the OB soil (86.03 µS/cm) was lower than the control (garden) soil (142 µS/cm). The content of Fe was evidently highest in OB soil followed by the metals Cr, Zn, Mn and Cu.

SEM images revealed a difference in the soil texture of iron mine OB soil and garden soil. Iron mine OB soil consists of fine irregularly shaped particles with diameters ranging from 98.4 nm to 800 µm (Fig. 1a–d). EDX images showed the presence of a significant amount of Fe along with Si, Mn and Ni in OB soil (Fig. 1e, f).

3.3. Assessment of growth of vetiver plants

The shoot and root lengths of vetiver genotypes (S2, S4, TH, BL) grown on garden soil and iron mine OB soil are shown in Fig. 2a, b. The plants appeared healthy without any signs of stress like change in colour, wilting or necrosis. The shoot and root lengths of plants grown on iron mine OB soil and on the control garden soil increased with exposure time from 6 and 12 months. The shoot and root length of the plants grown on mine OB soil were lower than garden soil and thus the biomass production in terms of dry weight was significantly lower in all the vetiver genotypes grown on OB soil than garden soil (Fig. 2c, d). Plants grown for 12 months showed a significant difference in biomass production than plants grown for 6 months. In terms of the growth parameters assessed, BL and S4 showed better results.

The changes in RWC % in vetiver varieties are presented in Fig. 3a. After 6 months the RWC values did not differ in plants grown on mine OB soil or garden soil. When compared to plants growing on control garden soil, after 12 months, RWC was significantly low in all vetiver genotypes except the BL growing on iron mine OB soil.

The TI of plants measured on the basis of shoot and root biomass are

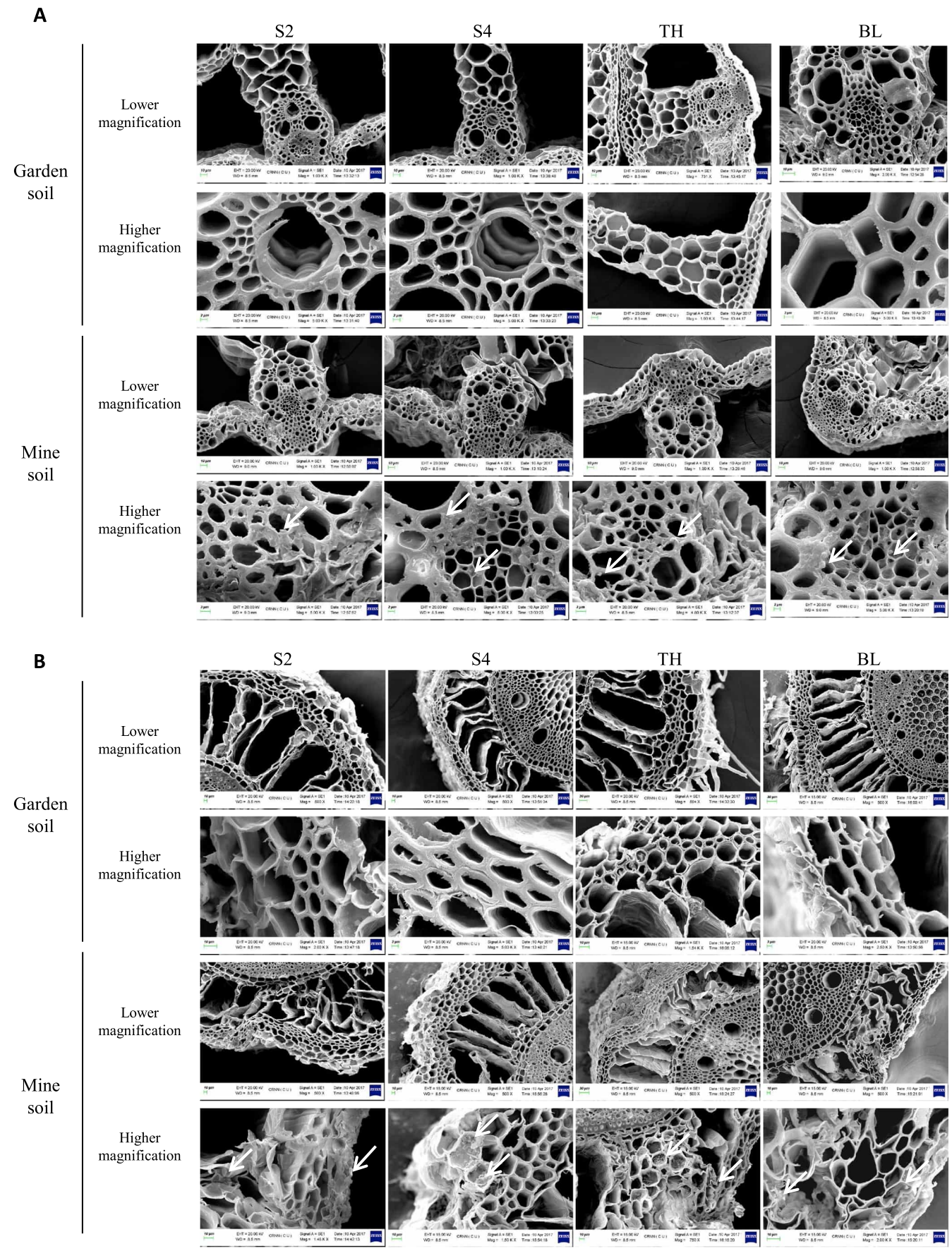

Fig. 4. Scanning electron microscopic images: (a) transverse section of leaves; (b) transverse section of roots of vetiver varieties (S2, S4, TH and BL) grown on garden soil and iron mine overburden soil; Arrows indicate localization of iron.

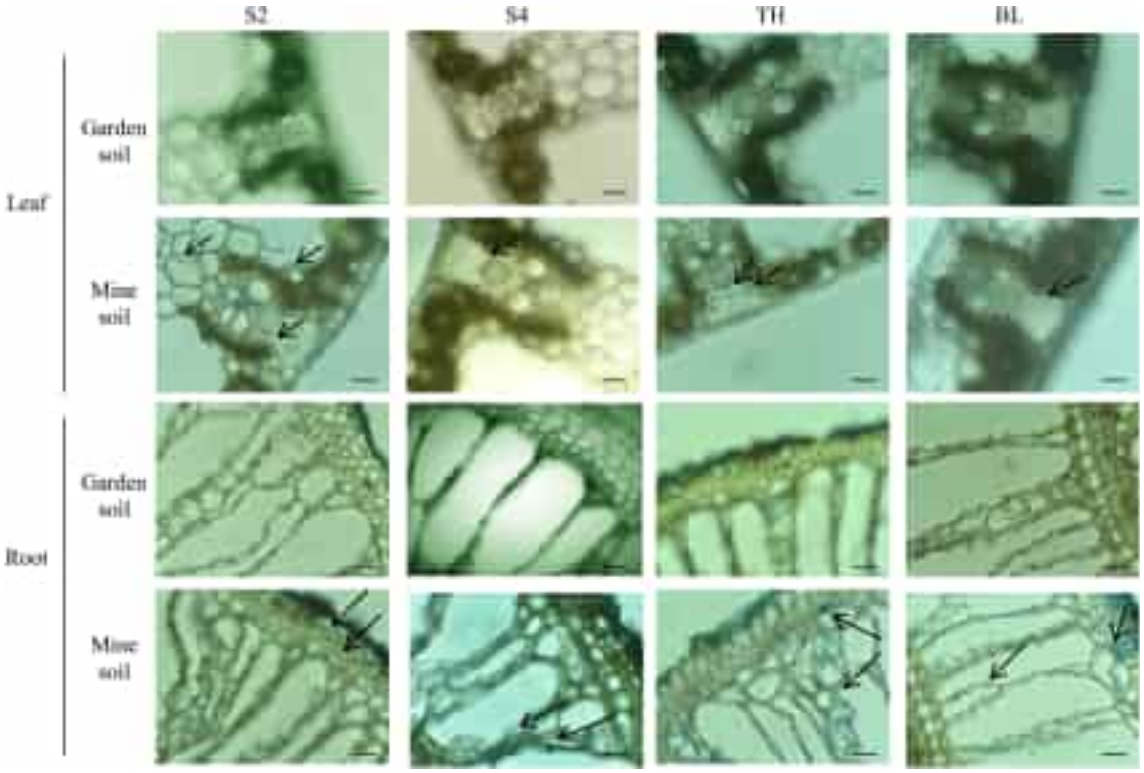

Fig. 5. Iron localization in vetiver leaves and roots: Perl's Prussian blue stained transverse sections of leaf and root of vetiver varieties (S2, S4, TH and BL) grown on garden soil and iron mine overburden soil. Arrows indicate localization of iron; scale bar = 50 μm.

presented in Fig. 3b. After 12 months of exposure, the TI of shoot biomass was highest in BL followed by S4, and the TI of root mass was highest in S4 followed by BL. Such differences can be related to the growth habit of the two genotypes (Table1).

SEM images of stomata of vetiver genotypes (S2, S4, TH, and BL) grown for 12 months on garden and iron mine OB soil showed marked differences in size of stomatal aperture (Fig. 3c).

3.4. Phytoextraction of heavy metals

The capability of vetiver genotypes to absorb metals (Fe, Cu, Zn, Cr and Mn) in shoots and roots was measured by ICP-AES (Table 3). The roots accumulated a higher amount of Fe, Mn, Cr and Zn than the shoots with the exception of Cu. The content of Fe in roots of the vetiver plants was highest in BL followed by TH, S4 and S2. Mn content was highest in BL followed by S2, TH and S4. The content of Cu was highest in BL followed by S4, TH and S2. The content of Cr was highest in S2 followed by TH, S4 and BL. Zn content was highest in S2 followed by TH, BL and S4.

Bioaccumulation factor (BF) and translocation factor (TF) are two useful parameters to study the metal accumulation and distribution within the plants. The ability of translocation of metals from root to shoot was assessed using TF expressed as the ratio of $[Metal]_{shoot}/[Metal]_{root}$ (Maiti and Nandhini, 2006). In most of the genotypes the TF values of the metals Fe, Zn, Cu, Mn and Cr were lower than 1 (Table 4).

In S2 and S4 grown on mine OB soil for 6 and 12 months, the TF value of Cu was greater than 1, that substantiate that a greater amount of Cu was translocated to the shoots.

The potential of the plant to accumulate metals in root and shoot with respect to the metal concentrations present in the soil was measured by BF. Accumulation of Fe, Zn, Cr and As was higher in the roots than that in shoots in all the vetiver genotypes after growing for 6 and 12 months on mine OB soil. Content of Cu was more in the shoots than that in the roots of S2 and S4 variety plants grown on mine OB soil collected after 12 months.

3.5. Localization of iron

SEM imaging demonstrates the accumulation and transport of Fe in root and leaf tissues of vetiver grown on iron OB soil after 12 months of exposure. The presence of Fe particles was observed in the vascular bundle region of leaves (Fig. 4a), and in hypodermal and cortical regions of roots (Fig. 4b).

This was further confirmed by staining root and leaf sections by Perls' Prussian blue stain. Fe deposition was less in the leaves, and was found on the hypodermal and vascular bundle region of leaves (Fig. 5). Most of the Fe deposition was located on the cell walls. The intensity of blue staining of Fe was higher in the hypodermal and cortical tissues of roots (Fig. 5).

3.6. Chlorophyll and carotenoid content

The photosynthetic pigments – chlorophyll *a*, chlorophyll *b*, total chlorophyll and total carotenoid content in leaves of vetiver genotypes S2, S4, TH and BL grown on garden soil and iron mine OB soil are presented in Fig. 6a–d. After 6 months of growing on mine soil the values of total chlorophyll and total carotenoid content decreased significantly, but there was an increase in their content after 12 months exhibiting recovering trend, yet less than the corresponding plants growing on control garden soil. In general, the vetiver genotypes grown on iron mine OB soil showed a decrease in photosynthestic pigments as compared to control plants. Chlorophyll and total chlorophyll content was highest in BL followed by S4, TH and S2.

3.7. Changes in biochemical stress markers

The increase of proline can be considered as an indicator of tolerance to the iron mine OB soil. The vetiver grown on OB soil at all the

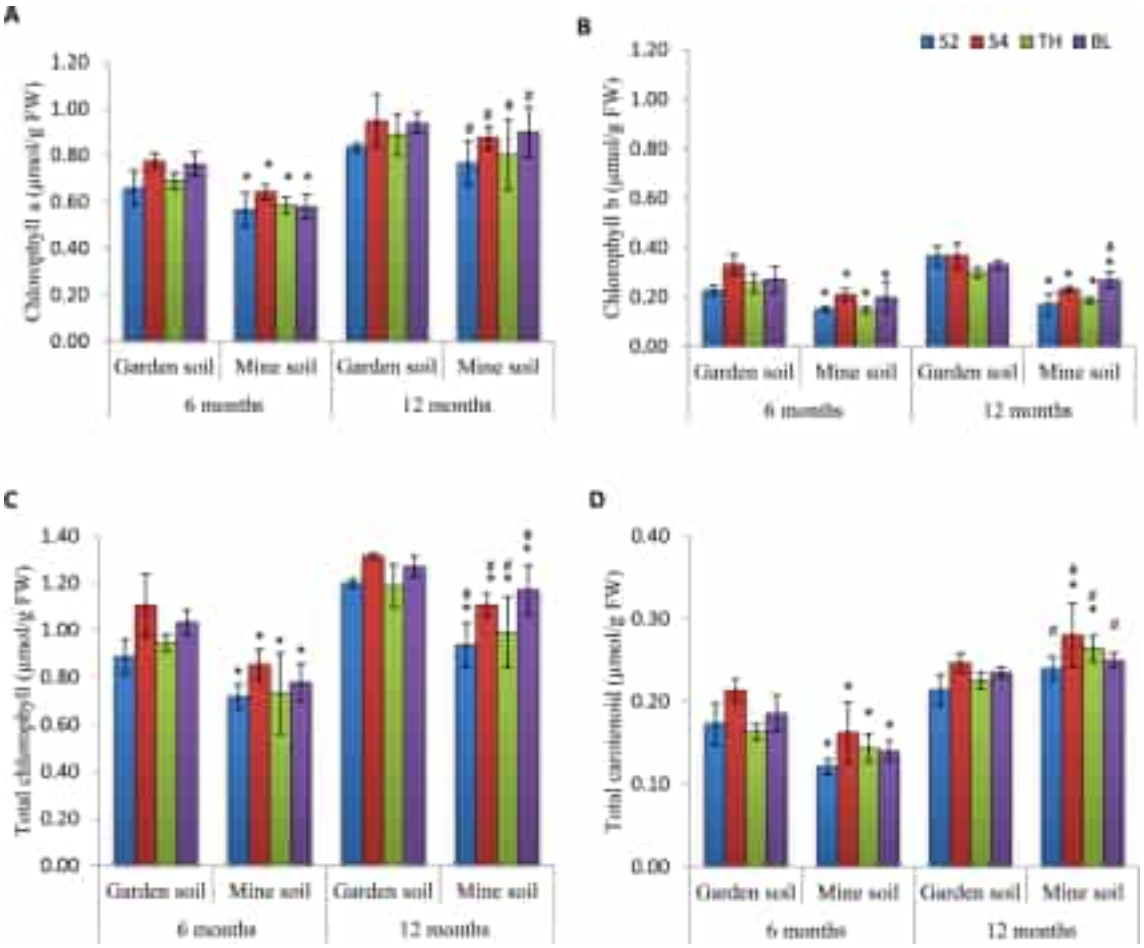

Fig. 6. Estimation of photosynthetic pigments: (a) chlorophyll *a*, (b) chlorophyll *b*, (c) total chlorophyll and (d) total carotenoid content in leaves of vetiver varieties (S2, S4, TH and BL) grown on garden soil and iron mine overburden soil [*Statistically significant ($P \leq 0.05$) compared to respective control at 6 or 12 months, #Statistically significant ($P \leq 0.05$) between 6 and 12 months].

durations showed a significant raise in proline content (Fig. 7a). Among them S4 and S2 showed highest proline content at 6 and 12 months respectively.

The peroxidation of cell membrane and cellular lipid in leaves of the 4 genotypes of vetiver was estimated by the content of MDA formed (Fig. 7b). The MDA content was significantly higher in the 4 genotypes grown on the OB soil than their corresponding plants grown on control soil. The highest level of MDA was found in S4 (52.90 ± 1.08 nmol/g FW) at 6 months, and in TH (51.03 ± 0.648 nmol/g FW) after 12 months.

Vetiver genotypes grown on iron mine OB soil after 6 and 12 months showed a significant higher cellular H_2O_2 quantity than the control plants (Fig. 7c). BL shows the least H_2O_2 Content.

3.8. Antioxidant defense responses

The involvement of enzymatic and non-enzymatic antioxidants associated with metal stress is presented in Fig. 8. The SOD, CAT, GR, GPOD and GPx activity in the leaves of vetiver plants was significantly higher in the plants exposed to mine OB soil than in the control plants. The highest SOD activity was shown by BL after 6 months exposure, whereas after 12 months the S4 showed highest activity. Similarly increase in CAT activity was observed with increasing exposure time from 6 to 12 months and highest activity was found in the BL. When compared to the control plants, GR activity in the leaves of vetiver plants growing on mine OB soil increased progressively with increasing exposure time from 6 to 12 months.

The GPOD and GPX activity in vetiver plants was found to be increased after 6 months exposure and a slight inhibition in month 12, when compared to the control.

The non-enzyme antioxidant, contents of GSH was higher in the leaves of the vetiver grown on OB soil than control garden soil during all periods (Fig. 9a). An altered level of GSSG was observed in S4, TH and BL variety after month 12 months (Fig. 9b).

3.9. Synthesis of phytochelatins

Vetiver genotypes exposed to iron mine OB soil for 12 months produced considerable amount of phytochelatins (Fig. 10). Two types of phytochelatins PC3 and PC4 were identified in the leaves of vetiver growing on iron mine OB soil (Fig. 10d, f, h, j) .Synthesis of phytochelatins was absent in the plants growing on control garden soil (Fig. 10c, e, g, i). PC3 accumulation was highest (25.25 ± 3.32 μg/g FW) in BL,and highest accumulation of PC4 (70.23 ± 6.27 μg/g FW) was observed in S4.

4. Discussion

Extensive mining operations and improper disposal of mine wastes are the major source of heavy metals that lead to significant soil contamination (Tordoff et al., 2000). The adverse impact of mine waste on environment includes water and air pollution, soil erosion, heavy metal contamination, loss of biodiversity, geo-environmental disasters and ultimately loss of economic wealth. To ensure continued beneficial use

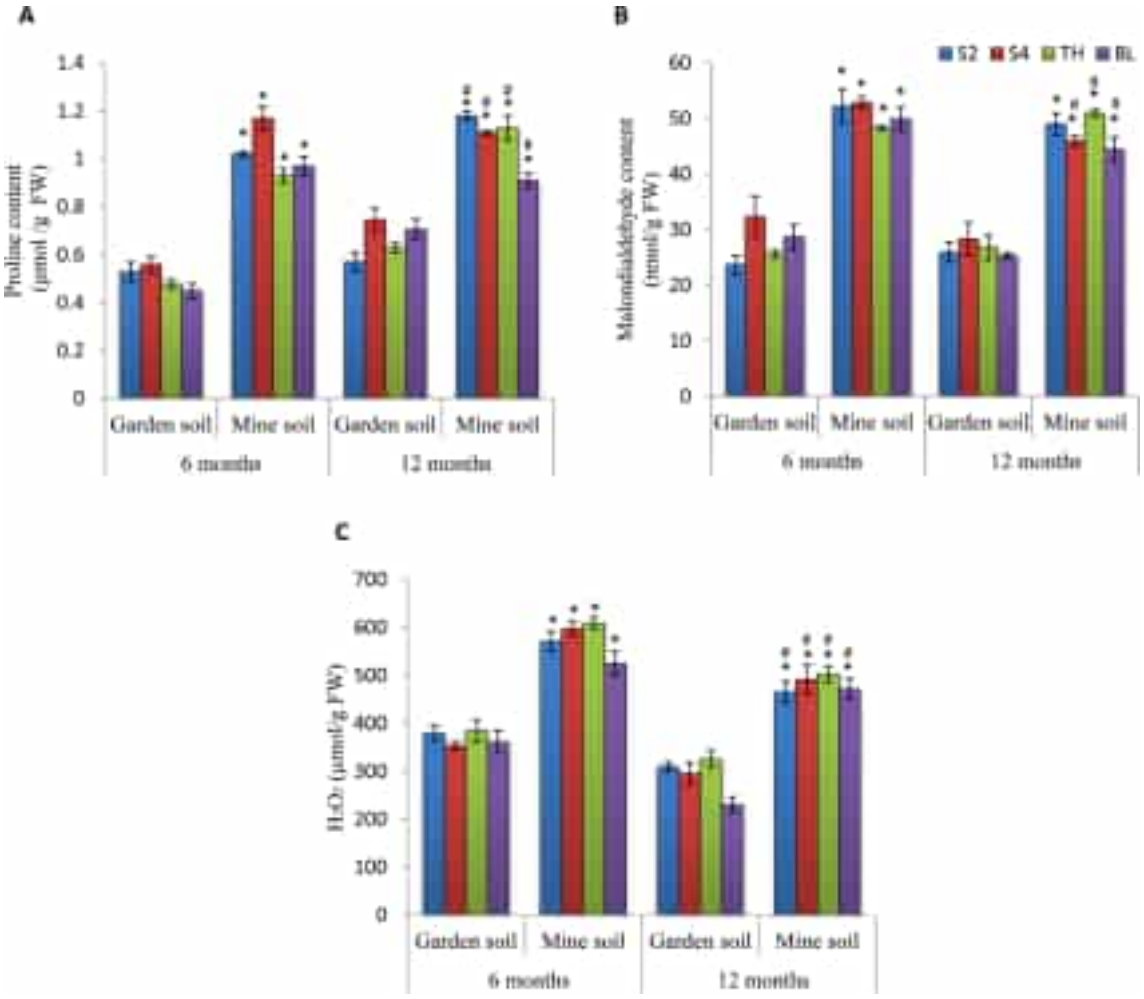

Fig. 7. Changes in biochemical stress markers: (a) proline, (b) lipid peroxidation, (c) H_2O_2 in leaves of vetiver varieties (S2, S4, TH and BL) grown on garden soil and iron mine overburden soil [*Statistically significant ($P \leq 0.05$) compared to respective control at 6 or 12 months, #Statistically significant ($P \leq 0.05$) between 6 and 12 months].

of land resources conservation and reclamation efforts are essential. These highly degraded lands could be returned to productivity by reclamation process which involves plant growth and microbial process to restore biotic function and productivity of the waste lands (Kavamura and Esposito, 2010; Sheoran et al., 2010; Singh et al., 2002).

In the present study analysis of the physico-chemical properties of the mine soil revealed slightly acidic nature of the soil in comparison to the garden soil (Table 2). Most OB soils of iron ore mines in India are near neutral (NEERI, 2004). The metal analysis data showed very high amount of Fe in OB soil along with Cr, Zn, Mn, Cu and Pb. This is quite in agreement with previous reports from related sites (Chen et al., 2004; Verma et al., 2012).

Plants employ combating strategies to alleviate rigours of external high metal concentration, and activate internal tolerance mechanisms which control toxic metal accumulation and translocation within different parts of the plant (Anjum et al., 2015; Clemens, 2006). Selection of plant species with high tolerance to heavy metals is very important criteria for remediation of mine spoil-dump sites. Vetiver plants are found efficient in remediation of arsenic (As) from the hydroponic system (Singh et al., 2017), lead-contaminated soils with firing ranges (Wilde et al., 2005), and could survive in gold mine tailings (Melato et al., 2016). Vetiver can also help in remediation of radionuclides 90Sr, 137Cs, and 239Pu from spiked solutions as well as low level nuclear waste (Singh et al., 2008) from hydroponic system as well as soil (Singh et al., 2017).

These results are in agreement with the reports of the present study. Vetiver plants were able to grow and survive in iron mine OB soil, tolerating the metals accumulated in different tissues of the plant. Vetiver could endure the high concentrations of Fe along with other heavy metals in the soil and in their tissues as well (Truong, 2000).

The plants take up toxic metals from the OB soil (Table 3). The four genotypes of vetiver, particularly BL and S4, planted on iron mine soil accumulated much higher concentration of Fe in the roots than in the shoots. The uptake of other heavy metals – Cr, Zn, Mn and Cu were also higher in roots. Roongtanakiat et al. (2007) reported a higher accumulation of metals Fe, Mn, Zn and Cu in roots of several ecotypes of vetiver exposed to industrial waste water. The vetiver varieties grown in mine soils under the study period of 12 months had apparently no severe phyto-toxicity symptoms, indicating that they could tolerate the high concentrations of Fe along with other heavy metals in the soil and in their tissues as well. The ability of plants to remediate heavy metals from the soils can be moderately reflected by the translocation factor (TF) and bioaccumulation factor (BF) (Baker et al., 1994; Dahmani-Muller et al., 2000). BF measures the potential of the plants to accumulate the heavy metals in their different parts with respect to the metal concentrations present in the soil (Branquinho et al., 2007). TF measures the plant's potential to translocate heavy metals from roots to the aerial shoots (Gupta et al., 2008; Kisku et al., 2000; Maiti and Nandhini, 2006). Our data reveal that the roots accumulated more heavy metals as the TF values are lower than 1, therefore validating

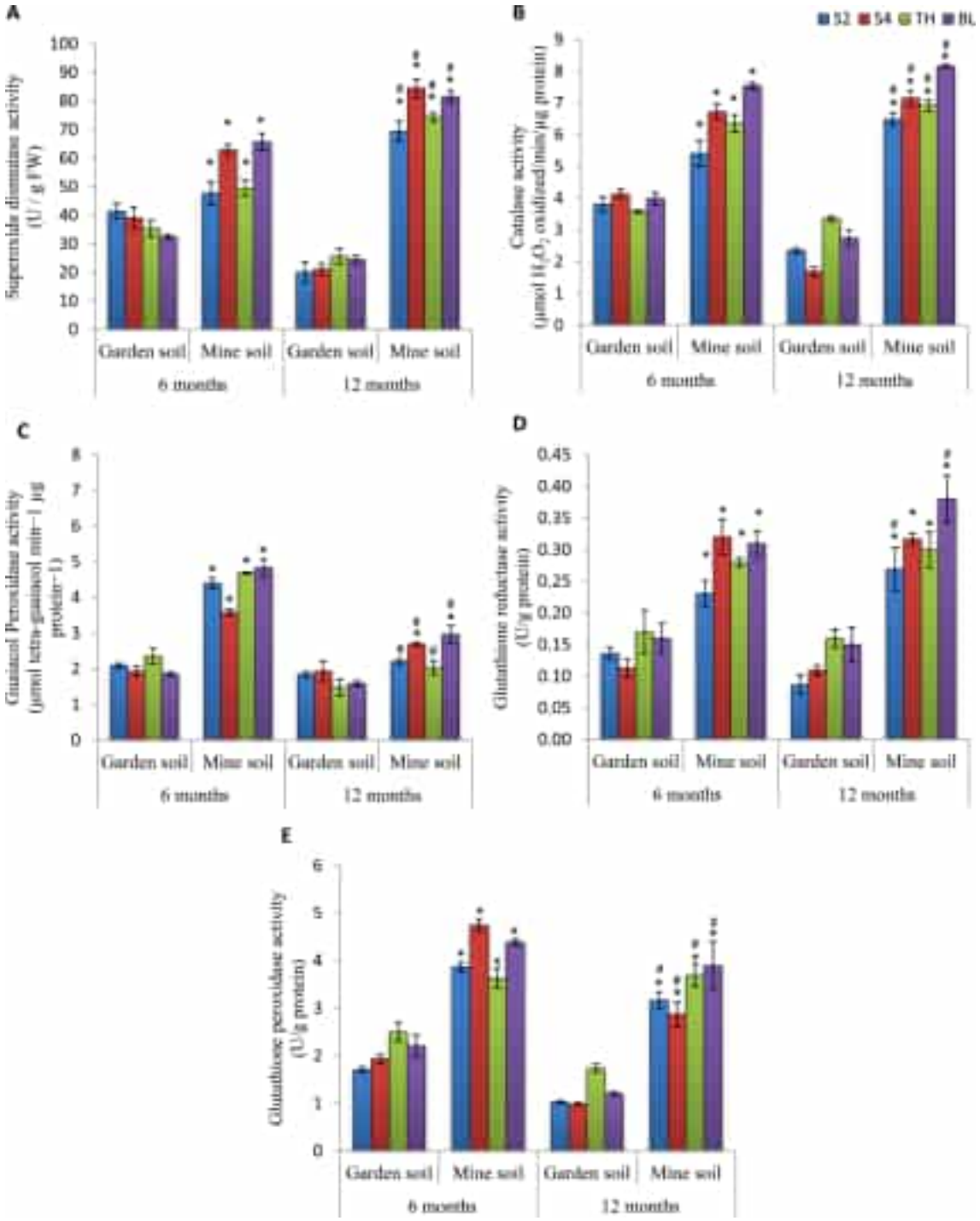

Fig. 8. Changes in antioxidant enzyme responses: (a) superoxide dismutase activity, (b) catalase activity, (c) guaiacol peroxidase activity, (d) glutathione reductase activity, (e) glutathione peroxidase activity in leaves of vetiver varieties (S2, S4, TH and BL) grown on garden soil and iron mine overburden soil [*Statistically significant ($P \leq 0.05$) compared to respective control at 6 or 12 months, #Statistically significant ($P \leq 0.05$) between 6 and 12 months].

their suitability for phytostabilization. This is in accordance to an earlier report by Roongtanakiat et al. (2009).

The presence of Fe in plant tissue, in particular, was confirmed by SEM and simple microscopy by Perls blue staining (Stacey et al., 2008). This is in agreement with our initial report (Banerjee et al., 2016) where Fe deposition was observed in lower epidermal and vascular bundle regions of leaf and in sub-epidermal and cortical regions of the root. In addition SEM images of the transverse sections of leaves and roots of vetiver growing on mine soil revealed no noticeable structural abnormalities. Similar observations were made by Melato et al. (2016) on vetiver plants growing on gold mine tailings, and Sridhar et al. (2011) on brake fern exposed to As. In our study, the SEM images confirmed Fe depositions in the vascular bundle region of leaves and in case of roots it was observed in hypodermal and cortical regions. Previous studies have demonstrated the deposition of Fe, Mn and Cu in roots of *Phragmites australis* growing in coal mine drainage (Batty et al., 2000).

The parameters like root length, shoot length, root biomass and shoot biomass have been used as indicators for the overall health performance of plants growing in the presence of heavy metals (Fayiga

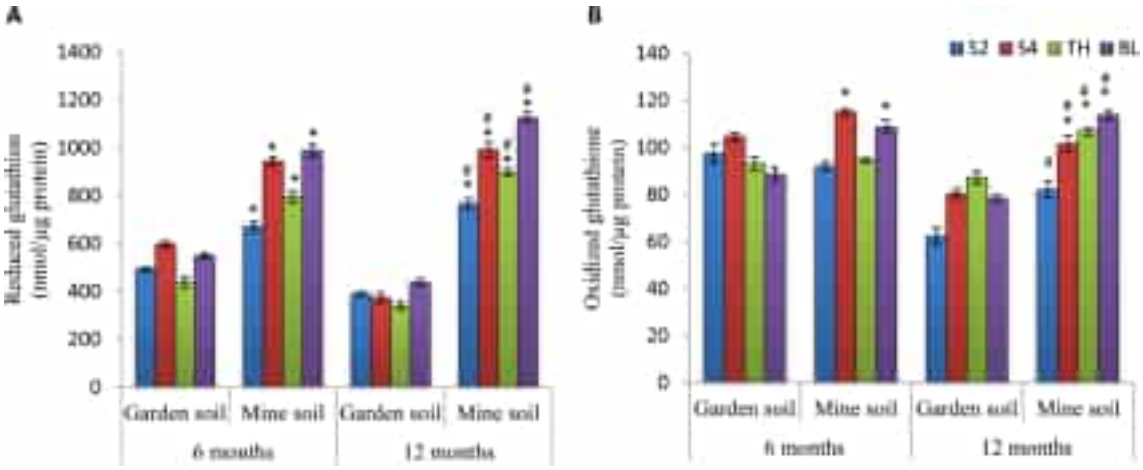

Fig. 9. Changes in glutathione level: (a) reduced glutathione, (b) oxidized glutathione in leaves of vetiver varieties (S2, S4, TH and BL) grown on garden soil and iron mine overburden soil [*Statistically significant ($P \leq 0.05$) compared to respective control at 6 or 12 months, #Statistically significant ($P \leq 0.05$) between 6 and 12 months].

et al., 2004). Vetiver grown on OB soil over a period of 6 months till 12 months showed nearly similar values for the growth parameters with an overall decrase in shoot and root length, shoot dry weight and root biomass of vetiver plants growing in OB soil in relation to control. Tolerance index (TI) is one of the most known parameters for selection of plants for phytoremediation (Zacchini et al., 2009). In the present study this could be expressed in terms of shoot and root biomass of vetiver plants growing in OB soil in relation to control. The results of TI obtained in the present study highlight the notable ability of this plant species to tolerate Fe. RWC decreased significantly in vetiver plants grown in OB soil at 12 months expressing reduction in size of the stomatal aperture. However, there were variations in TI across the genotypes. It is inferred that BL and S4 show better adaptability to the iron mine OB induced stress conditions. Other studies also revealed high tolerance of vetiver grass against heavy metals, acidity, salinity and agrochemicals (Truong, 1999, 2000).

Abiotic stresses can potentially decrease the chlorophyll content in plants (Ahmad et al., 2007). In our study a reduction in chlorophyll *a* and *b* content was observed whereas carotenoid content was found to increase in all the vetiver varieties exposed to mine OB soil. Carotenoid being a non-enzymatic antioxidant pigment can protect chlorophyll against heavy metal stress induced ROS generation (Hou et al., 2007). It can be further suggested that increase in carotenoid content can protect the plants against heavy metal stress (Rastgoo and Alemzadeh, 2011).

Plants are generally equipped with their enzymatic and non-enzymatic antioxidant defense system in order to minimize the detrimental effects of heavy metal induced oxidative stress. The first line of tolerance mechanism includes heavy metal detoxification by absorption of these metals into plant tissues in their reduced form. The second line of defense involves up-regulation of antioxidant enzymes and sequestration of heavy metals by glutathione and phytochelatins. These strategies work together to lessen the amount of oxidative stress for the protection of plants against the harmful effects of ROS.

The production and accumulation of higher level of proline in plants is a clear indicator of environmental stress (Hayat et al., 2012; Sharma and Dietz, 2006). It is an important parameter to determine the heavy metal toxicity (Sharma et al., 2016) which also plays various roles to combat stress in plants (Fidalgo et al., 2013). In the present study metal-induced stress caused an increase in the proline content in the leaves of the vetiver plants. Similar upsurge in proline content was found in vetiver plants growing on Pb and Zn mine tailings (Pang et al., 2003). Earlier studies have reported increase in lipid peroxidation level and H_2O_2 content during oxidative stress (Chen et al., 2000; Farmer and Mueller, 2013; Mittler, 2002; Parlak, 2016). A significant increase in MDA content was observed in *Artiplex hortensis* and *Artiplex rosea* exposed to soil polluted by Cu, Ni, Pb, Zn (Sai Kachout et al., 2010). Increased accumulation of H_2O_2 in the shoots of *Sedum alfredii* on exposure to Zn and Cd was observed by Chao et al. (2008) where an initial increase of H_2O_2 content was observed after up to 15 days of treatment, but decreased subsequently on further exposure. In our study similar increase in MDA and H_2O_2 quantity was observed as a result of the oxidative stress induced by the mine OB soil. An initial increase in the MDA and H_2O_2 content in the leaves of vetiver plants was noted after 6 months of exposure period with subsequent decrease after 12 months due to long term adaptation of plants to heavy metal stress conditions. These are in line with the earlier reports (Malar et al., 2016; Singh et al., 2008; Zhang et al., 2007). Interestingly, the BL and S4 genotypes manifest least oxidative stress in terms of proline, lipid peroxidation and generation of H_2O_2. This implies that BL and S4 are notably more tolerant for growing on OB soil incurring less oxidative stress.

To combat the enhanced level of oxidative stress caused by heavy metals, plants trigger their antioxidant enzymes to detoxify reactive oxygen species (ROS) (Manara, 2012; Shahid et al., 2014; Štolfa et al., 2015). In our study, the activities of antioxidant enzymes like SOD, CAT, GPOD, GR and GPX increased in the leaves of vetiver plants. Increase of CAT, SOD and POD was observed to reduce the metal induced ROS in vetiver plants growing on gold mine tailing (Melato et al., 2016) and Pb/Zn tailing (Pang et al., 2003). This is in agreement with our findings where increase in the antioxidant enzymes (SOD, CAT, GR, GPOD and GPX) was recorded in vetiver plants grown on mine OB soil. The BL and S4 contribute high SOD, CAT, GPOD, GR and GPX activities than S2 and TH varieties.

Glutathione plays an important role both as an antioxidant and reducing agent to balance the stress level in plant cells by maintaining the redox state of the cells (Hossain et al., 2012; Yadav, 2010). Under stress condition, intracellular GSSG concentration increases and for the maintenance of glutathione in its reduced form (GSH) is necessary for the production of phytochelatins which helps in the chelation of metal ions to defend plant cells from their toxic effects (Cobbett, 2000; Seth et al., 2012). In the present study, the enhanced level of reduced glutathione was observed as a result of stimulated GR activity which converts GSSG to GSH to maintain the glutathione pool. These results are similar to the observations of Mishra et al. (2006) where increased amount of GSH actively contribute in metal detoxification of *Bacopa monnieri*.

Phytochelatin (PC) synthesis is considered as one of the most important strategy for heavy metal detoxification (Sneller et al., 1999; Zenk, 1996). Significant induction of PCs (PC_2 and PC_3) in

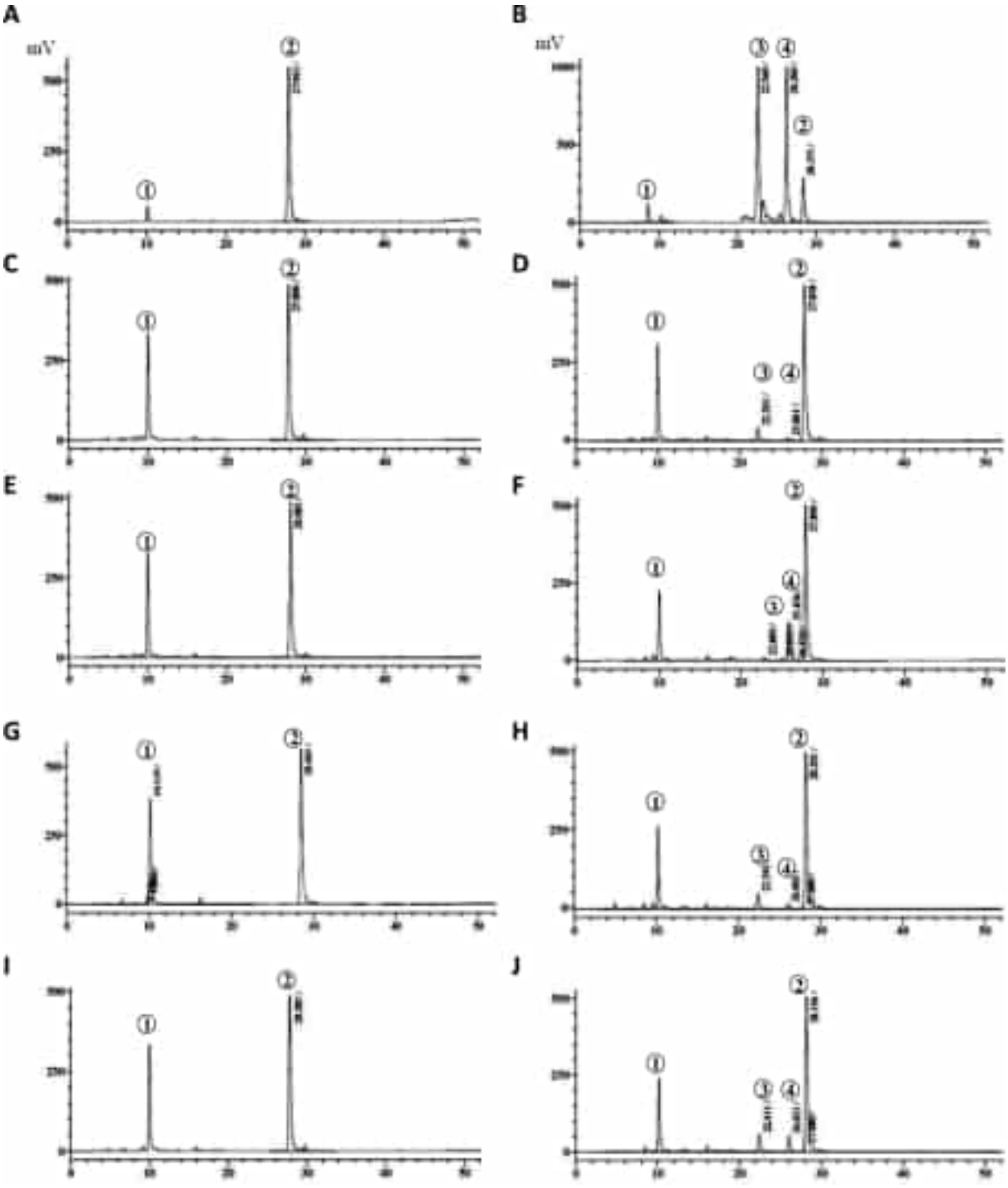

Fig. 10. Analysis of phytochelatins: HPLC analysis of phytochelatins in leaves of vetiver varieties (S2, S4, TH and BL) grown on garden soil (c, e, g, i) and iron mine overburden soil (d, f, h, j); (a) Blank (mBBr), (b) Phytochelatin (PC3, PC4) standards, (c, d) S2, (e, f) S4, (g, h) TH, (i, j) BL; where peak 1 – unidentified peak, peak 2 – mBBr, peak 3 – PC3, peak 4 – PC4.

Ceratophyllum demersum was observed due to Pb-induced oxidative stress (Mishra et al., 2016). Zhang et al. (2008) reported that *Sedum alfredii* collected from Pb/Zn mine tailing were found to produce enhanced level of PCs in their leaves, stems and roots. Up-regulation of the level of PCs showed a linear relationship with Pb content in vetiver tissues (Andra et al., 2010). Similarly in the present study, we found elevated levels of PCs (PC_3 and PC_4) in vetiver plants grown on mine OB soil for 12 months. This provides a comprehensive understanding on the metal tolerance and detoxification mechanism of the vetiver plants.

5. Conclusion

Vetiver grass could be suitably grown on iron mine OB soil to help rehabilitate soil as these plants could well tolerate metal contamination. Tolerance of vetiver plant to mine OB soil is related to its effective protective mechanisms to eliminate or reduce ROS induced damages. The present study demonstrated an increase in proline, MDA and H_2O_2 generation that were all indicative of oxidative stress. As a consequence antioxidative enzymes are increased to scavange ROS. Activity of intracellular GSSG concentration increases for the maintenance of

glutathione in its reduced form (GSH) and the production of phytochelatins. The Vetiver varieties exposed to iron mine OB soil for 12 months produced considerable amount of phytochelatins that help in the chelation of metal ions to defend plant cells from their toxic effects.

The accumulation of absorbed metals (Fe, Mn, Zn, Cr and Cu) in plant tissues does not inflict any major adverse affect on plant metabolism and productivity, since major amount is retained in the roots and only a fraction goes to shoots. Such a restricted translocation of metals into shoots and confinement of major amount in roots, as observed in the present study on vetiver, is considered ideal for phytoremediation. Therefore the vetiver grass could be recommended to remediate and stabilize degraded and toxic soils like mine dump contaminated with Fe, Mn, Zn and Cr. Of the four diverse plant types, BL followed by S4 are more efficient to remediate toxic metals from the OB soil.

Conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

AM and UCL developed the idea, designed the experiments and wrote the manuscript. RB and PG performed the experiments, analyzed the data and prepared the figures. SL and UCL identified the diverse morphotypes. All authors reviewed the manuscript.

Acknowledgments

The authors would like to acknowledge the Council of Scientific and Industrial Research, India for financial support (Sanction No. 38 (1367)/13/EMR-II dt. 01.10.2013 – “Investigation on iron ore mine site restoration and species performance in spoil dumps slope stabilization with vetiver system technology”), and Emeritus Scientist grant (Sanction No. 21(0949)/13/EMR-II dt. 28.04.2014) to UCL. The Center for Research in Nanoscience and Nanotechnology and the Department of Botany, Calcutta University is gratefully acknowledged for providing the core facilities. The authors would also like to thank Dr. Rajesh Mukherjee, Principal Geologist, Tata Steel limited, Natural Resources Division, Ore Mines & Quarries, Noamundi and Manager Mr. Rajesh Kumar at Joda mines for technical support in collection of iron ore spoil dump soil samples.

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