Journal of Geochemical Exploration 182 (2017) 218–227

Contents lists available at ScienceDirect

Journal of Geochemical Exploration

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

Phytoremediation of metals using vetiver (*Chrysopogon zizanioides* (L.) Roberty) grown under different levels of red mud in sludge amended soil

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A R T I C L E   I N F O

Article history:
Received 10 July 2016
Revised 22 January 2017
Accepted 7 March 2017
Available online 8 March 2017

Keywords:
Biomass
Chrysopogon zizanioides
Metal tolerance index
Phytoremediation
Red mud
Sewage sludge

A B S T R A C T

Globally, 120 million tonnes of red mud is generated whose disposal and storage occupy large areas of potentially important arable lands. Red mud dumps are physically, nutritionally and biologically poor in nature. Vetiver (*Chrysopogon zizanioides* (L.) Roberty), a medicinally important perennial plant, known to control soil erosion, tolerates a wide range of pH and elevated levels of toxic metals. This information prompted to make use of red mud with sewage sludge, a nutrient rich bio-waste for growing vetiver. An experimental study was conducted using red mud at four rates (0, 5, 10 and 15% w/w) in soil amended with sewage sludge (soil: sludge: 2:1 w/w) to evaluate the effects on physico-chemical properties, plant growth performance, biomass and metal contents in vetiver under control (without sewage sludge and red mud) and different soil treatments. Application of red mud with sludge enhanced the levels of organic matter and nutrient status of the soil which offered suitable substrata to support plant growth. Heavy metal contents (Fe, Mn, Mg, Zn, Cu, Ni, Pb, Cd and Cr) increased with increase in red mud levels, however, their phytoavailable contents decreased. Addition of red mud resulted in significant improvement in root-shoot lengths, number of tillers culm^{-1}, root-shoot ratio and biomass compared to the control. However, maximum improvement occurred in root (125.27%), shoot (79.91%) and total plant biomass (88.07%) under 10% red mud treatment compared to control. Vetiver is found to be a potential metal tolerant plant as tolerance index was >100%. Based on translocation and bioconcentration factors, the plant was found efficient in translocation of Mn and Cu from roots to shoot, whereas it acted as a potential phytostabilizer for Fe, Zn, Mg, Cd, Pb, Ni and Cr. The study suggests utilization of 10% red mud in sludge amended soil to sustain maximum plant growth coupled with enhanced phytoremediation potential of vetiver.

1. Introduction

Bauxite residue commonly referred as red mud, is a by-product derived from the treatment of bauxite with concentrated sodium hydroxide under elevated temperature and pressure during the Bayer's process (Gräfe and Klauber, 2011). Worldwide production of red mud is about 120 million tonnes per annum (Power et al., 2011), while it's annual production in India is approximately 13.73 million tonnes (Samal et al., 2013). As per the jurisdictions (the European Union, U.S.A and Canada), red mud is a non-hazardous solid residue with no transport or regulatory controls (Klauber et al., 2011), whereas in China red mud is categorised as class II, general industrial solid waste (Liu et al., 2009). Red mud without any proper pre-treatment before disposal may lead to several harmful effects on surrounding environment and living beings, mainly due to its high alkalinity and elevated levels of toxic metals (Xue et al., 2016). It has very fine particle size distribution ranging from 2 to 2000 μm (Nguyen and Boger, 1998) with texture showing sand (0–30%), silt (9–66%) and clay (26–80%) (Wehr et al., 2006). Due to fine particle size of red mud, it has high bulk density, low hydraulic conductivity and porosity (Jones and Haynes, 2011). Typically, it is an alkaline and saline residue with high electrical conductivity, exchangeable sodium percentage and cation exchange capacity (Xue et al., 2016). Metals in red mud are dominated by iron (Fe), aluminium (Al), titanium (Ti), silica (Si), sodium (Na), calcium (Ca), phosphorous (P), vanadium (V), cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr) and nickel (Ni). Whereas, it contained low levels of essential nutrients such as zinc (Zn), manganese (Mn), magnesium (Mg), potassium (K) and copper (Cu) (Lacatusu et al., 2014; Mayes et al., 2011).

Several technological innovations are available to manage industrial solid wastes, but such applications are neither environmental friendly nor cost effective (Xue et al., 2016). However, in order manage such contaminated derelict sites, different phytotechnologies have been developed in last two decades (Marques et al., 2009), which are sustainable, eco-friendly, cost effective and have attained a special interest worldwide. Phytoremediation of red mud is a challenging task due to its physico-chemical properties that limit the establishment of plants

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http://dx.doi.org/10.1016/j.gexplo.2017.03.003
0375-6742/

on red mud dumps. Therefore, prior to establishment of vegetation on red mud dumps, properties of red mud need to be improved to support plant growth. Several studies have been conducted to develop the strategies for revegetation including field neutralization, soil capping and utilization of organic/inorganic amendments (Wehr et al., 2006). Utilization of organic amendments such as animal manures, sewage sludge, mushroom compost, vermicompost and saw dust are relatively inexpensive and effective in counterbalancing the negative impacts of red mud on plants (Courtney et al., 2013; Banning et al., 2011; Jones and Haynes, 2011; Chauhan and Ganguly, 2011; Fuller et al., 1982). Even though utilization of organic/inorganic amendments are found to improve the properties of red mud, thus offering a suitable substrata for revegetation. But, all the types of plant species may not necessarily grow on such soil due to high levels of toxic metal and salinity. Therefore, it is essential to select those plant species which are halophytes, drought resistant and metal tolerant (Gräfe and Klauber, 2011; Wu et al., 2013). Medicinally important perennial plants like *Cymbopogon citratus* and *Chrysopogon zizanioides* are useful for phytoremediation of metal contaminated land because they can withstand the harsh environmental condition and are not grazed by animals (Das and Maiti, 2009). Also, such plants are used as remunerative and viable option in preventing food chain contamination (Lal et al., 2008).

Chrysopogon zizanioides (L.) Roberty formerly known as *Vetiveria zizanioides* (L.) Nash and commonly referred as vetiver grass, belongs to family Poaceae. It is a fast growing, perennial plant with dense, fibrous and deep root system. Vetiver is a drought resistant plant which can thrive successfully under extreme alkaline and saline conditions and has an ability to tolerate wide range of toxic metals in the soil (Melato et al., 2016; Truong, 1999). This plant is widely grown for stabilization of slopes, erosion control, phytoremediation and in prevention of leaching of contaminants into ground water (Truong, 2002; Xia, 2004). Such plants attain different strategies to combat high metal contents in soil and plants through chelation, restricted uptake and translocation of metals as well as internal tolerance mechanisms to negate their toxic effects on growth and metabolism of plants (Emamverdian et al., 2015). Despite of having high tolerance of vetiver, accumulation of heavy metals in plant tissues beyond threshold levels may elicit other changes in plant that may lead to phytotoxic effects such as reduction in growth, photosynthetic activity, oil yield and biomass (Singh et al., 2011).

The objectives of the present study were (1) to evaluate the phytoremediation potential of vetiver grown under different levels of red mud in soil amended with sewage sludge, and (2) to gauge the influence of metals on growth performance of vetiver under different soil treatments. The study would suggest some future prospects for utilization of red mud in combination with sewage sludge for cultivation of this commercially important plant for phytomanagement of metal polluted soil.

2. Materials and methods

2.1. Study area

The experiment was conducted in Botanical Garden, Department of Botany of Banaras Hindu University (B.H.U.), Varanasi (25° 18′ N 82° 01′ E; and 76.19 m above sea level) from March 07 to September 07, 2014. Mean monthly maximum and minimum temperatures were 40.40 and 15.52° C, respectively, while mean monthly maximum and minimum relative humidity were 83.42 and 25.13%, respectively. Total rainfall during the experiment was 774.4 mm.

2.2. Experimental design and raising of plant

Dried cakes of red mud and sewage sludge were collected from the dumping yard of HINDALCO Industries Ltd., Renukoot and Dinapur municipal sewage treatment plant, Varanasi, respectively. Garden soil was dug out up to the depth of 30 cm from a natural vegetation site in Botanical Garden, B.H.U. Physico-chemical properties of red mud and sewage sludge are illustrated in Supplementary Table 1. The test plant *C. zizanioides* was incurred from Faculty of Ayurveda, Institute of Medical Sciences, B.H.U.

After removing stone and plant materials from red mud, sewage sludge and garden soil, these were air dried and grinded uniformly to get a homogenous mass. Dried samples were sieved through a 2 mm mesh and mixed in a definite proportion to obtain different soil combinations. Garden soil was mixed uniformly with sludge in the ratio of 2:1 (w/w). To the sludge amended soil thus obtained, red mud was added to achieve $S_{RM0,}$ 0%, S_{RM5} 5%, $S_{RM10,}$ 10% and S_{RM15}, 15% (w/w) concentrations, while the soil without sludge amendment and red mud treatment served as a control (C). Different soil combinations (10 kg) were filled into cylindrical plastic pots (height, 50 cm and diameter, 25 cm) and in total; there were 50 pots (5 treatments × 10 replicates). Pots were left at the experimental site for two weeks for stabilization. On fifteenth day, one plant slip of *C. zizanioides* (15 cm shoot length and 5 cm root length) was transplanted into each pot and equal amount of tap water was used to water the pots every alternate day, avoiding its leakage from the pots.

2.3. Soil sampling and analyses

For physico-chemical analyses of different soil treatments, soil samples from three pots per treatment were taken out using a soil corer (5 cm diameter and 10 cm depth) just before transplantation. Each sample was air dried and ground to pass through a sieve of 2 mm mesh size. The pH and electrical conductivity (EC) of soil samples were measured in an aqueous suspension of 1:5 (w/v) using pH meter (Model EA940, Orion, U.S.A) and conductivity meter (Model 303, Systronics, India), respectively. Total organic carbon (TOC), total nitrogen (TN) and available phosphorous (P) were assessed using Walkley and Black's rapid titration method (Allison, 1973), Gerhardt automatic nitrogen analyser (Model KB8S, Germany) and Olsen's method (Olsen et al., 1954), respectively. Exchangeable Na^+, K^+, Mg^{2+} and Ca^{2+} were determined by standard procedures given by Hesse (1971) and their contents were determined using Atomic Absorption Spectrophotometer (Analyst-800, Perkin Elmer Inc., Norwalk, CT, USA). The cation exchange capacity (CEC) and exchangeable sodium percentage (ESP) were calculated using formulae:

$$\text{CEC}\ (\text{meq}\ \ 100\ \text{g}^{-1}) = \sum \text{Ex.}\left(\text{Na}^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}\right) \tag{1}$$

$$\text{ESP}(\%) = \left(\text{Ex.Na}^+\right)/\text{CEC} \tag{2}$$

2.4. Plant sampling and growth parameters

Plants were harvested in triplicate by taking out entire plant along with roots from the pots at 180 days after transplantation (DAT). Plants were gently jerked and washed under running water to remove adhering soil particles. Numbers of tillers culm^{-1} and root-shoot lengths of the plants were measured. Thereafter, roots and shoot were separated and oven dried at 80 °C until their constant weights were attained. Dry weights of roots and shoot were measured for biomass determination and root shoot ratio (R/S ratio).

2.5. Metal contents in soil and plant

Oven dried samples of roots, shoot and soil (before transplantation) in triplicate were grinded to a fine powder using mortar and pestle and were digested using di-acid (HNO_3 and $HClO_4$ in 9:4 ratio) following Gaidajis (2003) method. Different soil treatments were extracted using 0.05 M EDTA solution (Quevauviller et al., 1997) for phytoavailable metals. Total and phytoavailable metal contents were determined using Atomic Absorption Spectrophotometer (Analyst-800, Perkin Elmer Inc., Norwalk, CT, USA).

Precision and accuracy of analysis was assured through repeated analysis of samples against National Institute of Standard and Technology, Standard Reference Material (SRM 1570) for all the metals. Blank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were run after every five sample to calibrate the instrument. The results were found within ±2% of the certified value. The coefficients of variation of replicate analysis were determined for different determinations and precision of analysis. Variations were found to be <10%.

Metal tolerance index (MTI) and metal extraction amount (MEA) for vetiver were determined following the method of Wang et al. (2014) and Zhang et al. (2014), respectively. Two important indices, translocation factor (TF) and bioconcentration factor (BCF), used for evaluating the phytoextraction potential of vetiver were calculated using the formulae given by Qihang et al. (2011).

$$\mathrm{MTI}(\%) = \{(\text{Plant biomass under treatment})/(\text{Plant biomass under control})\} \times 100 \tag{3}$$

$$\mathrm{MEA}\left(\text{mg plant}^{-1}\right) = \text{Metal content in plant tissue} \times \text{biomass} \tag{4}$$

$$\mathrm{TF} = (\text{Metal content in shoot})/(\text{Metal content in root}) \tag{5}$$

$$\mathrm{BCF} = (\text{Metal content in plant tissue})/(\text{Metal content in soil}) \tag{6}$$

2.6. Statistical analysis

Statistical significance of difference between the physico-chemical properties of different soil treatments, total and phytoavailable metal contents in soil, plant growth parameters, metal contents in roots and shoot, MEA, TF and BCF values were tested by one way analysis of variance (ANOVA) followed by Duncan's multiple range test as post hoc. Principal component analysis (PCA) on plant growth parameters, metal contents in roots and shoot were also performed based on the correlation matrix with the rotation method of Varimax with Kaiser normalization. All the statistical tests were performed using SPSS software IBM SPSS Statistics 20.0 (IBM, Armonk, NY, USA).

3. Results and discussion

3.1. Physico-chemical characterization, total and phytoavailable metal contents

Following the applications of red mud in different concentrations, there were significant increases in pH and EC values from 7.52 to 8.73 and 0.33 to 0.99 mS cm^{-1}, respectively across $\mathrm{S_{RM0}}$ to $\mathrm{S_{RM15}}$ treatments. Maximum ESP value was found under $\mathrm{S_{RM15}}$ followed by $\mathrm{S_{RM10}}$, $\mathrm{S_{RM5}}$, $\mathrm{S_{RM0}}$ and C (Table 1). Increases in pH, EC and ESP values of different soil treatments due to red mud addition are mainly due to utilization of high caustic soda during the process of alumina extraction. Total sodium content in red mud is in free, soluble and bound caustic soda forms (Kurdowski and Sorrentino, 1997). In red mud, free and soluble caustic soda contributes approximately 20–25% of total, whereas rest is in the form of sodalite complex (Kurdowski and Sorrentino, 1997). The CEC values showed insignificant changes across the treatments which could be attributed to relatively low CEC value of red mud (4.50 meq 100 g^{-1}) compared to sludge amended soil (5.12 meq 100 g^{-1}). In red mud, exchangeable cations are dominated by $\mathrm{Na^{+}}$ ion (53.58%), whereas in sewage sludge, the same is dominated by exchangeable $\mathrm{Mg^{2+}}$, $\mathrm{Na^{+}}$ and $\mathrm{Ca^{2+}}$ ions contributing 34.9, 30.7 and 22.0% of total exchangeable ions, respectively. The TOC values were maximum under $\mathrm{S_{RM10}}$ and $\mathrm{S_{RM15}}$ treatments, which insignificantly varied between them. Similarly, TOC values between $\mathrm{S_{RM0}}$ and $\mathrm{S_{RM5}}$ did not vary significantly (Table 1). Available P and TN contents were found maximum under $\mathrm{S_{RM0}}$ (712.18 mg $\mathrm{kg}^{-1}$and 0.69%, respectively) followed by a decline with increase in red mud levels in sludge amended soil.

Table 1

Selected physico-chemical properties, total and phytoavailable metal contents in control and red mud treatments in soil amended with sewage sludge (Mean ± SE).

Parameters	C	$\mathrm{SS_{RM0}}$	$\mathrm{SS_{RM5}}$	$\mathrm{SS_{RM10}}$	$\mathrm{SS_{RM15}}$
pH	7.73 ± 0.01^{d}	7.52 ± 0.04^{e}	8.06 ± 0.02^{c}	8.18 ± 0.02^{b}	8.73 ± 0.04^{a}
Conductivity mS (cm^{-1})	0.16 ± 0.01^{e}	0.33 ± 0.01^{d}	0.45 ± 0.01^{c}	0.50 ± 0.02^{b}	0.99 ± 0.07^{a}
CEC (meq 100 g^{-1})	4.56 ± 0.29^{a}	5.12 ± 0.24^{a}	5.07 ± 0.50^{a}	5.04 ± 0.10^{a}	4.97 ± 0.40^{a}
ESP (%)	27.87 ± 0.59^{c}	27.69 ± 1.14^{c}	32.70 ± 1.86^{b}	35.57 ± 0.68^{b}	39.24 ± 0.45^{a}
TOC (%)	0.84 ± 0.04^{c}	5.68 ± 0.10^{b}	5.72 ± 0.05^{b}	5.90 ± 0.10^{ab}	6.03 ± 0.12^{a}
TN (%)	0.20 ± 0.01^{c}	0.69 ± 0.04^{a}	0.61 ± 0.02^{ab}	0.56 ± 0.03^{b}	0.54 ± 0.03^{b}
Available P (mg kg^{-1})	183.45 ± 13.52^{e}	712.18 ± 10.94^{a}	637.73 ± 8.51^{b}	510.0 ± 25.84^{c}	325.93 ± 7.56^{d}
Metal contents (mg kg^{-1})					
Fe	2321.67 ± 31.80^{e}	$21{,}540.4 \pm 230.47^{d}$	$35{,}150.51 \pm 116.29^{c}$	$51{,}143.28 \pm 1069.52^{b}$	$66{,}548.37 \pm 1351.75^{a}$
Mn	265.67 ± 1.76^{c}	310.39 ± 2.19^{b}	313.27 ± 4.52^{ab}	316.97 ± 1.49^{ab}	319.75 ± 1.40^{a}
Mg	217.41 ± 2.42^{c}	661.51 ± 15.99^{b}	723.29 ± 13.63^{a}	739.25 ± 5.88^{a}	757.72 ± 10.23^{a}
Zn	141.82 ± 9.31^{b}	578.94 ± 7.01^{a}	581.02 ± 3.28^{a}	585.94 ± 4.87^{a}	588.65 ± 11.76^{a}
Cu	81.90 ± 0.79^{b}	189.92 ± 2.27^{a}	192.40 ± 1.89^{a}	198.32 ± 1.91^{a}	199.71 ± 6.35^{a}
Ni	14.55 ± 0.49^{e}	28.75 ± 0.25^{d}	36.47 ± 1.24^{c}	41.18 ± 1.04^{b}	52.15 ± 0.27^{a}
Cr	17.40 ± 0.05^{d}	315.05 ± 5.03^{c}	360.06 ± 1.58^{c}	417.96 ± 1.57^{b}	486.42 ± 32.34^{a}
Pb	20.76 ± 0.48^{e}	42.07 ± 1.88^{d}	51.52 ± 0.08^{c}	63.30 ± 0.11^{b}	80.42 ± 3.40^{a}
Cd	2.29 ± 0.21^{e}	52.32 ± 1.38^{d}	74.79 ± 2.96^{c}	90.41 ± 0.55^{b}	110.06 ± 2.78^{a}
Phytoavailable metal contents (mg kg^{-1})					
Fe	93.04 ± 1.92^{b}	128.82 ± 5.95^{a}	96.38 ± 0.78^{b}	85.65 ± 1.58^{b}	73.45 ± 3.67^{c}
Mn	41.86 ± 0.18^{b}	55.19 ± 0.95^{a}	43.59 ± 0.82^{b}	35.57 ± 0.72^{c}	30.99 ± 0.96^{d}
Mg	22.71 ± 0.23^{e}	58.18 ± 0.52^{a}	41.53 ± 0.56^{b}	36.58 ± 0.91^{c}	32.58 ± 0.41^{d}
Zn	10.11 ± 0.46^{e}	24.44 ± 0.38^{a}	21.65 ± 0.13^{b}	16.59 ± 0.40^{c}	14.74 ± 0.10^{d}
Cu	7.61 ± 0.70^{c}	18.13 ± 0.41^{a}	15.54 ± 1.48^{a}	12.17 ± 0.88^{b}	10.39 ± 1.13^{bc}
Ni	2.69 ± 0.28^{b}	3.42 ± 0.04^{a}	2.82 ± 0.12^{b}	2.51 ± 0.13^{b}	2.42 ± 0.10^{b}
Cr	4.27 ± 0.13^{e}	2.83 ± 0.05^{a}	2.24 ± 0.05^{b}	1.83 ± 0.10^{c}	1.23 ± 0.03^{d}
Pb	0.82 ± 0.004^{b}	1.18 ± 0.09^{a}	0.9 ± 0.01^{b}	0.6 ± 0.01^{b}	0.4 ± 0.01^{b}
Cd	0.43 ± 0.01^{b}	0.57 ± 0.01^{a}	0.39 ± 0.01^{c}	0.35 ± 0.01^{d}	0.31 ± 0.02^{e}

CEC: cation exchange capacity, ESP: exchangeable sodium percentage, TN: total nitrogen, TOC: total organic carbon, P: phosphorous, C: control, $\mathrm{SS_{RM0}}$: soil with sewage sludge, $\mathrm{SS_{RM5}}$: 5% red mud $\mathrm{SS_{RM0}}$, $\mathrm{SS_{RM10}}$: 10% red mud in $\mathrm{SS_{RM0}}$, $\mathrm{SS_{RM15}}$: 15% red mud in $\mathrm{SS_{RM0}}$. Numbers with different letters in same row differ significantly at $p < 0.05$ as per the Duncan's test.

Insignificant change in TOC and decrease in TN and available P contents across red mud treatments may be attributed to their low levels in red mud (Gray et al., 2006). A decline in available P may also be ascribed to possible formation of insoluble metal phosphates at high pH and presence of sesquioxides in red mud which scavenge P from soil (Wang et al., 2008; Snars et al., 2004).

Total and phytoavailable metal contents in different soil treatments are illustrated in Table 1. Metal contents were significantly higher under all the treatments compared to the control and Fe was found most abundant followed by Mg, Zn, Cr, Mn, Cu, Cd, Pb and Ni. Due to low levels of essential nutrients (Mn, Mg, Zn and Cu) in red mud (Lacatusu et al., 2014); its increasing concentrations caused insignificant change in these metals from S_{RM5} to S_{RM15} treatments. However, Fe and Ni contents increased significantly with increase in red mud treatments. Similarly, contents of potentially toxic metals Cd, Pb and Cr showed significant increases due to red mud addition in soil amended with sludge. On the basis of ecological risk, the contents of Zn, Cr and Cd under different red mud treatments exceeded the guideline values for metals (250–400, 200–300 and 10–20 mg kg^{-1}, respectively) in soil, whereas Cu, Pb and Ni were well within their guideline values i.e. 150–200, 200–750 and 100–150 mg kg^{-1}, respectively (Ministry of Environment, Finland (MEF), 2007). However, no guidelines have been specified for Fe, Mn and Mg in soil (MEF, 2007). Metal contents (Cu, Zn, Ni, Cd and Cr) when compared to their threshold levels in soil required for the growth of vetiver, Cu and Cd contents exceeded their threshold levels 50–100 and 20–60 mg kg^{-1}, respectively, whereas other metals were within the specified threshold levels, Zn (>750), Ni (347) and Cr (200–600) (Truong, 2000).

Total metal contents although showed increasing trends due to red mud addition, but their phytoavailable contents decreased with increasing red mud levels (Table 1). In soil, available metal contents are influenced by several factors such as pH, organic carbon content, oxides of Fe, Mn and Al, moisture content and soil texture (Sherene, 2010). Phytoavailable metal contents were found maximum under S_{RM0} which may be attributed to low soil pH. Increase in red mud levels in sludge amended soil raised the pH under different treatments which may elicit the reduction of mobile metal contents. Under neutral to alkaline soil condition, soluble and mobile metal contents decrease due to precipitation followed by adsorption onto charged colloids of red mud (Lombi et al., 2003). Low phytoavailabity of metals may also be ascribed to tectosilicate structures i.e. cancrinite and hematite, the two principal phases of red mud, which provide a high metal adsorption capacity (Santona et al., 2006). In cancrinite, metals are incorporated in cage and the channels of the negatively charged lattice, whereas in case of hematite, after hydrolysis, metals are adsorbed on both inner and outer sphere of the mineral surface resulting in reduced mobility of metals (Castaldi et al., 2009; Gray et al., 2006). Thus increase in red mud concentrations in sludge amended soil increased the absorption sites for metals thereby reducing their availabilities to plants. Unlike other metals, available Cu content showed insignificant change between S_{RM0} and S_{RM5} as well as between S_{RM10} and S_{RM15} which may be correlated with organic carbon contents under different treatments (Table 1). Guan et al. (2011) showed that available Cu content in soil is influenced by hydroxyl and carboxyl groups supplied by organic amendments, which lead to formation of insoluble and immobile Cu-complexes thereby lower the risk of Cu-phytotoxicity.

3.2. Effects of red mud treatments on plant growth parameters

Pictorial representation of plant growth parameters and biomass are given in Fig. 1. Root-shoot lengths and number of tillers culm^{-1} increased significantly with increase in red mud levels upto S_{RM10} treatment followed by a decrease under S_{RM15} treatment. Plant growth parameters under all treatments were significantly higher compared to the control, indicating that they are efficient enough to tolerate high metal contents in soil and in their tissues (Chen et al., 2004). A gradual increase in root biomass was found up to S_{RM10} treatment followed by a decline under maximum red mud treatment, whereas insignificant change in shoot biomass was observed under S_{RM0}, S_{RM5} and S_{RM10} treatments (Fig. 1). Maximum increase in total plant biomass was observed under S_{RM10} followed by S_{RM5}, S_{RM0}, S_{RM15} and C. Root, shoot and total plant biomass were higher under S_{RM10} treatment compared to the control with maximum percent increase by 125.27, 79.91 and 88.07%, respectively (Fig. 1). Reduction in plant growth parameters and biomass under S_{RM15} may be attributed to metal toxicity. A gradual change in colour of roots from creamy white to dark brown was observed under increasing levels of metals due to intense suberification (Fig. 2) (Piechalak et al., 2002). Apoplastic movement of metals through water and other solutes into stele is blocked by the suberized walls of the casparian strip, thus preventing metal movement through the symplast of the cell and arresting them in the cortex of the roots (Lane and Martin, 1977; Sharma and Dubey, 2005). Under such condition, root activity recovery is preceded by the emergence of primordial cells (Tataranni, 2009). Boonyapookana et al. (2005) found a decrease in

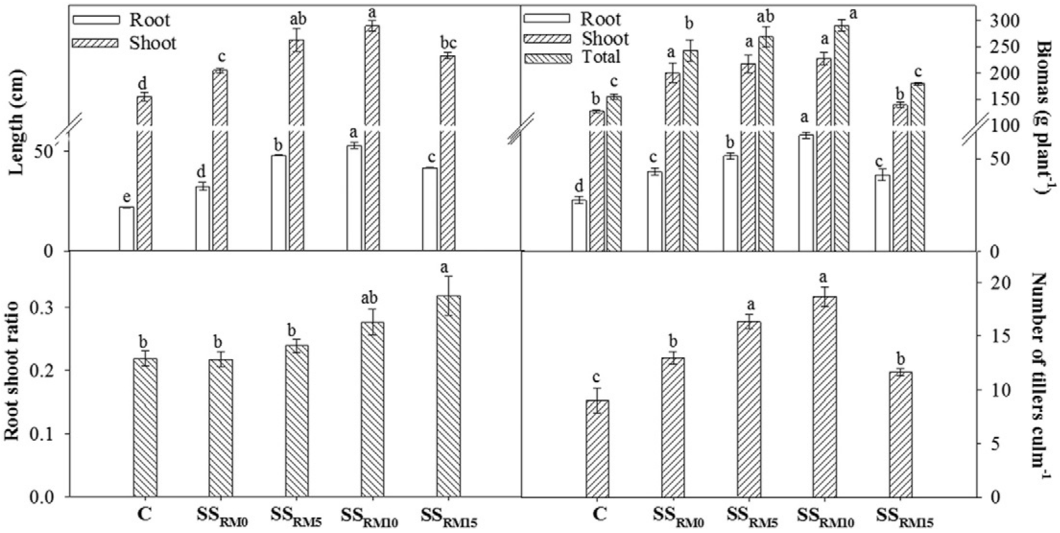

Fig. 1. Plant growth parameters and biomass of vetiver grown under control and different red mud treatments in sludge amended soil. Values are mean ± SE. Bars showing different letters indicate significant differences at $p < 0.05$ according to the Duncan's test. C: control; S_{RM0}: soil with sewage sludge; S_{RM5}: 5% red mud in S_{RM0}; S_{RM10}: 10% red mud in S_{RM0} and S_{RM15}: 15% red mud in S_{RM0}.

Fig. 2. Changes in colouration of vetiver roots under control and different red mud treatments in sludge amended soil. C: control; S_{RM0}: soil with sewage sludge; S_{RM5}: 5% red mud in S_{RM0}; S_{RM10}: 10% red mud in S_{RM0} and S_{RM15}: 15% red mud in S_{RM0}.

root elongation, growth and number of root hairs under heavy metal stress, which may have led to lower water uptake and nutrient transport to above ground plant parts, thereby affecting shoot as well as total plant biomass.

Biomass is the main indicator of energy accumulation in plants and changes in R/S ratio reflects the allocation of biomass that plant adopt to maximize their access to resources (Wilson and Tilman, 1995). Accumulators allocate equal proportion of energy towards both root and shoot parts of the plant, while excluders tend to allocate more energy towards root of the plant for absorption of metals and maintenance of their normal functioning by alleviating shortage of water under excessive metal effects (Zhang et al., 2014). In the present study, increase in R/S ratio in vetiver due to increase in red mud treatments reflects the metal exclusion behaviour of the plant (Fig. 1). The result is consistent with the study by Zhang et al. (2014) who found an increase in R/S ratio of *V. zizanioides* under increasing Cd contents (0 to 100 ppm) in soil.

3.3. Metal contents in roots and shoot of vetiver

Studied metal (Fe, Mn, Mg, Cu, Zn, Ni, Cr, Cd and Pb) contents in roots and shoot of vetiver grown under different soil treatments are given in Fig. 3. Among all metals, content of Fe was found maximum, whereas Cd content was found minimum in roots and shoot of the plant. Overall metal contents were in the order of Fe > Mg > Mn > Zn > Cu > Cr > Pb > Ni > Cd, which varied in different plant parts. Similar result was obtained by Banerjee et al. (2016) who found high accumulation of Fe, Mn, Zn and Cu in roots and shoot of vetiver, whereas Pb, Ni and Cd bioaccumulation was relatively less. Minimum Cd content in vetiver may be because of inhibitory effect of metals such as Fe, Zn, Cu and Ni in extra cellular bindings and intracellular uptake of Cd thereby lowering its content in plant parts (Noraho and Gaur, 1995). The study showed maximum Fe, Mg, Zn, Cr, Pb, Ni and Cd contents in roots in comparison to shoot (Fig. 3). More metal accumulation in roots than in shoot is mainly because of positive charge on metals, enabling their absorption to negative charged sites of root cell walls (Yang et al., 2005). Potentially toxic metals such as Pb, Cd and Cr even in trace amounts can cause phytotoxic effects and their bioaccumulation in aboveground plant parts may directly lead to food chain contamination. Therefore, it is essential to cultivate such plants which restrict the translocation of toxic metals to above ground plant parts and vetiver is a suitable plant for cultivation in metal polluted sites. In the present study, more Pb content in roots than in shoot is because of formation of immobile phosphates or carbonates which restrict its movement to shoot system (Berti and Cunningham, 2000). Zeng et al. (2011) found higher Cd accumulation in roots of *Zea mays* than in shoot, due to connection of Cd in cation exchange sites of roots and formation of immobile metal complexes with ligands containing sulfhydryl group (Topcuoglu, 2012). Low mobility of Cr from roots to shoot within the plant can be because of saturation and accumulation of Cr in vacuoles and apoplast of cells (Topcuoglu, 2012; Park et al., 2011). On the contrary, more Cu and Mn contents in shoot than in roots could be attributed to their strong antagonistic behaviour with Pb and Cd, respectively (John and Leventhal, 1995). In shoot, Mn and Mg contents, although showed insignificant variations under different treatments, but were significantly higher compared to the control. Contents of Zn, Cr, Pb and Cu in shoot showed maximum increase under S_{RM0} followed by S_{RM15}, S_{RM10}, S_{RM5} and C (Fig. 3). A significant reduction in metal contents under red mud treatments in comparison to S_{RM0} may be ascribed to reductions in the levels of phytoavailable metal contents (Castaldi et al., 2009; Lombi et al., 2003).

Based on literature survey, threshold levels of metals for most vascular plants are low indicating their sensitivity towards metals, while vetiver has very high threshold levels indicating its metal tolerant behaviour (Truong and Director, 2006). The threshold levels for metals in shoot of vetiver are Cu, 13–15; Zn, 880; Ni, 347; Cd, 45–48; Pb, >78 and Cr, 5–18 mg kg^{-1} (Truong, 2000). However, no thresholds for Fe, Mn and Mg have been defined for vetiver. The results showed that metals such as Zn, Ni, Cd, Cr and Pb in shoot were found within the threshold levels for vetiver, whereas Cu content exceeded the threshold value. Although, Cu content was higher than the phytotoxic threshold for vetiver, but it did not cause any severe phytotoxic effects on growth performance and yield of the plant (Fig. 1). This is because of synthesis of Cu induced phytochelatin complexes that reduce the toxic effects of Cu (Athar and Ahmad, 2002). Boojar and Tavakoli (2010) also found high bioaccumulation of Cu in leaves of metal tolerant, *Alhagi camelorum* plant with no visual and conspicuous symptoms of Cu toxicity. This behaviour of vetiver represents its usefulness in phytoremediation of soil containing high levels of metals such as industrial wastes which do not support the growth of other plants due to their hostile conditions (Chen et al., 2004). Fe, Cu, Cd, Ni, Zn, Pb and Cr contents in vetiver exceeded the WHO permissible limits for medicinal plants. However, Mn content under all the treatments was within the permissible limit (200 mg kg^{-1}) (WHO, 2005).

Principal component analysis (PCA) was used to assess the relationship between plant growth parameters and metal contents in plant parts (Fig. 4). It is a multivariate technique to simplify a complex dataset by creating one or more new variables or factors, each representing a cluster of several interrelated dependent variables (Abdi and Williams, 2010). Results of PCA showed three principal components contributing 87.99% of variance (Fig. 4). Component 1, contributing 55.95% of total variance (Eigenvalue = 9.95) had strong positive loadings (≥ 0.70) for Zn, Cu, Ni, Pb, Fe and Cr contents in shoot, and Fe, Ni and Mg contents in roots (Supplementary Table 2). Contents of Zn, Fe and Cr in shoot showed significant correlation ($p < 0.001$) with Cu having high translocation from roots to shoot. Synergistic effects of Cu on Zn, Fe and Cr accumulations in *Hibiscus sabdariffa* have been found (Ondo et al., 2012). On the other hand, foliar content of Zn was significantly correlated ($p < 0.001$) with Pb and Ni contents in shoot of the plant. Second component (24.50% of total variance; Eigenvalues = 9.09) depicts strong positive loadings for Cu, Cr, Pb and Zn contents in roots, Mn content in entire plant and root-shoot lengths (Supplementary Table 2), thus showing close associations of these metals with root-shoot lengths.

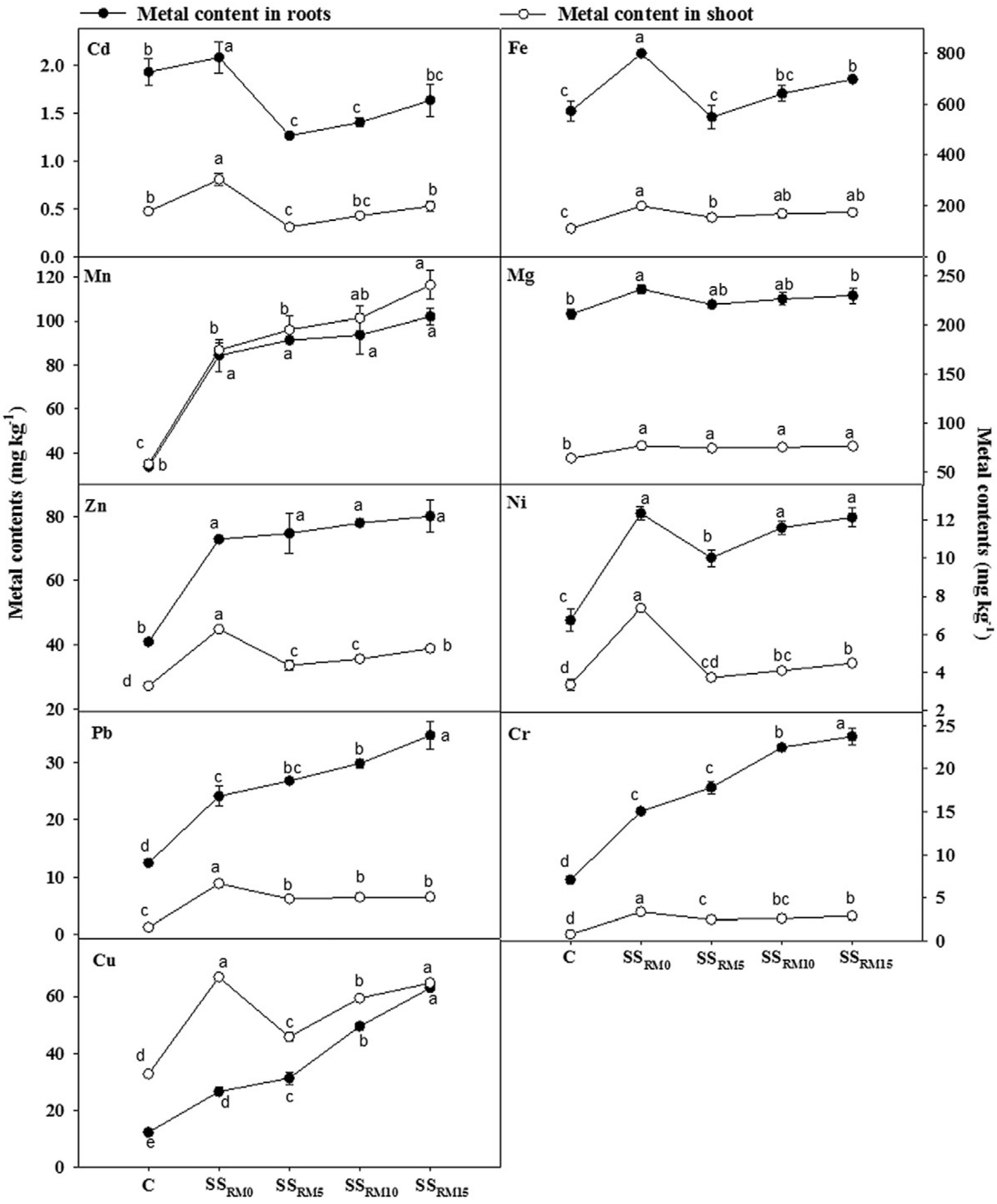

Fig. 3. Metal contents in roots and shoot of vetiver grown under control and different red mud treatments in sludge amended soil. Values are mean ± SE. Line graph showing different letters indicate significant differences at $p < 0.05$ according to the Duncan's test. C: control; S_{RM0}: soil with sewage sludge; S_{RM5}: 5% red mud in S_{RM0}; S_{RM10}: 10% red mud in S_{RM0} and S_{RM15}: 15% red mud in S_{RM0}.

Positive loading for Mn and negative loading for Cd in plant may be due to high translocation of Mn from roots to shoot. This could also be explained on the basis of competitive effect of Mn on Cd for transport binding sites in plant (Singh and Agrawal, 2007). Strong positive and negative loadings for Pb and Cd respectively, explained more bioaccumulation of Pb as compared to Cd in roots (Supplementary Table 2). Gupta and Sinha (2007) also found an antagonistic relationship between Pb and Cd in *Brassica juncea*, a metal tolerant plant grown in tannery waste contaminated soil. Thus, root-shoot lengths of the plant were strongly influenced by Cu, Cr, Pb, Mn and Zn accumulation in roots and Mn accumulation in shoot. Third component (7.53% of total variance; Eigenvalues = 4.72) had high loadings for number of tillers culm^{-1}, and root, shoot and total plant biomass; the most determinant plant parameters suggesting that these parameters were relatively less affected by metal accumulation in plant parts.

3.4. Metal tolerance index of vetiver grass

The metal tolerance index (MTI) measures the ability of plant to grow in the presence of a given concentrations of metals with respect to the control (Zacchini et al., 2009). Plants exhibiting higher MTI are more tolerant towards metals and there is a more possibility of growing such plants in the area contaminated with heavy metals (Pant and Tripathi, 2012). The MTI of vetiver based on total plant biomass under

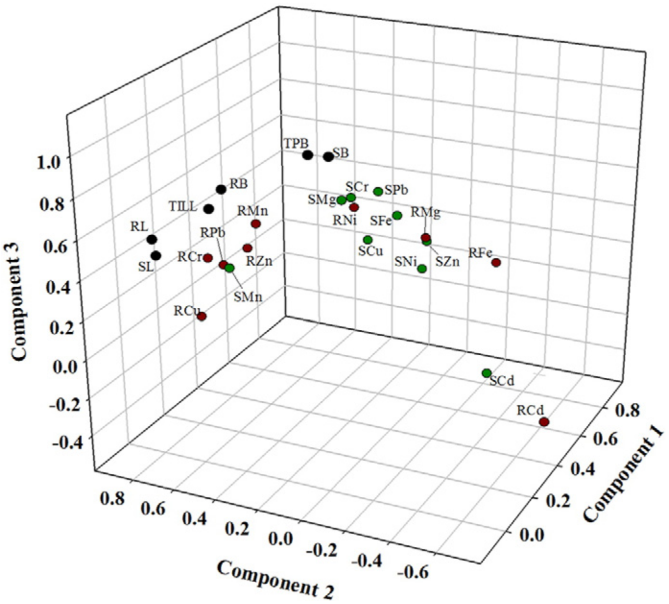

Fig. 4. Principal component analysis (PCA) for the relationship between plant growth parameters (black dots), metal contents in roots (brown dots) and shoot (green dots) of the plant. SL-shoot length, RL-root length, SB-shoot biomass, RB-root biomass, TPB-total plant biomass, TILL - Number of tillers culm^{-1}, RFe-iron content in roots, SFe-iron content in shoot, RMn-manganese content in roots, SMn-manganese content in shoot, RMg-magnesium content in roots, SMg-magnesium content in shoot, RCu-copper content in roots, SCu-copper content in shoot, RZn-zinc content in roots, SZn-zinc content in shoot, RNi-nickel content in roots, SNi-nickel content in shoot, RPb-lead content in roots, SPb-lead content in shoot, RCd-cadmium content in roots, SCd-cadmium content in shoot, RCr-chromium content in roots and SCr-chromium content in shoot.

different treatments were assessed, which showed a remarkable difference in tolerance to elevated levels of metals compared to the control. The MTI of vetiver were 157.08, 174.11, 188.07 and 116.30% under S_{RM0}, S_{RM5}, S_{RM10} and S_{RM15} treatments, respectively compared to control. Thus based of MTI values which was found >100% under all the treatments, vetiver grass is identified as a high metal tolerant plant, capable to withstand in soil containing elevated levels of Fe, Mn, Mg, Zn, Cu, Ni, Pb, Cd and Cr (Table 2). There are several studies supporting our result that vetiver is a metal tolerant plant, emerging as a primary choice for phytomanagement (Pidatala et al., 2016; Vargas et al., 2016; Chomchalow, 2011).

3.5. Metal extraction by roots and shoot

In phytomanagement technology, metal extraction amount (MEA) is an important indicator for assessing the phytoextraction efficiency of the plant (Zhang et al., 2010). Amount of metal extracted by both roots (MEA_{root}) and shoot (MEA_{shoot}) of the vetiver are illustrated in Table 3. The MEA_{root} values for Fe, Ni and Cd were maximum under S_{RM10} treatment, however, insignificant change was observed under S_{RM0}, S_{RM5} and S_{RM15} treatments. For Mn, Mg, Zn, Cu, Pb and Cr, MEA_{root} values increased from C to S_{RM10} followed by a decline under S_{RM15} treatment. Insignificant change was observed in MEA_{shoot} values for Fe, Mg and Cr from S_{RM0} to S_{RM10} treatments, while their values decreased under S_{RM15} treatment (Table 3). Cd and Ni showed significantly higher MEA_{shoot} values under S_{RM0} followed by S_{RM10} treatments and minimum under the control. In the present study, amounts of Mn and Zn extracted by vetiver were higher than the values recorded by Mudhiriza et al. (2015). Researches have shown that all the vetiver ecotypes have potential to extract Zn from the soil (Aksorn and Chitsomboon, 2013; Chen et al., 2012). In vetiver, amounts of potentially toxic metals such as Ni, Cd, Pb and Cr extracted by its roots were 2.23, 5.95, 5.99 and 6.60 mg plant^{-1}, whereas by the shoot were 0.26, 0.06, 0.44 and 0.39 mg plant^{-1}, respectively (Prasad et al., 2014). MEA_{root} values for Ni, Cd and Pb were low, while their MEA_{shoot} values were relatively high, whereas Cr extraction amount by both roots and shoot were comparatively higher when compared with the values reported by Prasad et al. (2014) (Table 3). Low extraction of metals by vetiver is possibly because of decrease in phytoavailable metal contents due to red mud addition in sludge amended soil (Castaldi et al., 2009).

Table 2
Classification of vetiver based on metal tolerance index (MTI).

No.	MTI (%)	Classification of plant
1	0 ≤ 25	Highly sensitive
2	25 ≤ 50	Sensitive
3	50 ≤ 75	Moderate
4	75 ≤ 100	Tolerant
5	≥100	Highly tolerant

3.6. Bioconcentration and translocation factors

Bioconcentration factor (BCF) represents a quantitative transfer of available metals from soil to varying plant parts (Branzini et al., 2012), while plants's ability to translocate them from roots to shoot is determined through translocation factor (TF) (Maiti and Nandhini, 2006). A plant with BCF value >1, indicates its efficiency to uptake higher metal content from the soil, while with BCF value <1 is metal excluder (Yanqun et al., 2005). In the present study, BCF values for metals in both roots and shoot decreased significantly under different soil treatments except for Mn and Cu in shoot which increased with increase in red mud levels in sludge amended soil (Table 4). For all the metals, BCF values were found higher in roots than in shoot except Mn and Cu which may be attributed to higher translocation of Mn and Cu from roots to shoot. In the present study, BCF values for Fe, Zn, Cu, Pb, Ni and Cr in the plant were less than those found in vetiver grass grown on gold mine tailings (Melato et al. 2016). Similarly, BCF value for Cd in both roots and shoot was found less than the value reported by Ghosh et al. (2015) in vetiver grown under different fly ash treatments. Low BCF values for studied metals in the plant under different red mud levels may be attributed to decrease in phytoavailable metal contents with increase in soil pH, and cancrinite and hematite levels due to red mud addition (Castaldi et al., 2009). Based on BCF values, vetiver was not found as a suitable candidate for phytoextraction of metals (Fe, Mn, Mg, Zn, Cu, Pb, Cd, Cr and Ni), rather it acted as a potential metal excluder. The finding of the present study that vetiver is a metal excluder and tolerant plant was supported by previous studies (Banerjee et al., 2016; Ghosh et al., 2015; Zhang et al., 2014).

The TF values for all the studied metals were less than unity except Mn and Cu (Table 4). Insignificant changes in TF values for Fe, Mn and Mg were found under S_{RM0} to S_{RM15} treatments, whereas Zn, Cu, Ni, Cd, Pb and Cr showed maximum TF values under S_{RM0} followed by significant reductions under increasing red mud levels. A significant reduction in TF values under red mud treatments could be due to low available metals absorbed by the roots and their further transport to shoot. The data of the present study indicate that considerable amounts of Fe, Zn, Cu, Ni, Cd, Pb and Cr were absorbed by the roots, but were not transferred to shoot system, as shown by TF values <1. The findings of this study is consistent with Banerjee et al. (2016), who found higher Fe, Al, Zn, Cr and Ni contents in roots than in shoot of vetiver grown on mine soil. Specialized features of vetiver grass such as fibrous root system and long, narrow and waxy leaves are also helpful towards its metal tolerant behaviour. Such specialized features of vetiver reduce evapotranspiration rate, resulting in restricted transport of metals to shoot through xylem (Boonyapookana et al., 2005). The TF values are widely affected by antagonistic and synergetic behaviour of different metals, which ultimately affect the metal uptake and their distribution

Table 3
Metal extraction amounts (mg plant^{-1}) in roots and shoot of vetiver grown under control and different red mud treatments in soil amended with sewage sludge (Mean ± SE).

Metals	Parameters	C	SS_{RM0}	SS_{RM5}	SS_{RM10}	SS_{RM15}
Fe	MEA_{root}	15.83 ± 0.57^{c}	34.42 ± 1.29^{b}	28.52 ± 3.27^{b}	40.16 ± 0.90^{a}	28.89 ± 1.61^{b}
	MEA_{shoot}	13.98 ± 0.26^{c}	39.54 ± 2.21^{a}	33.80 ± 5.29^{a}	38.36 ± 2.56^{a}	24.01 ± 0.72^{b}
Mn	MEA_{root}	0.94 ± 0.07^{d}	3.64 ± 0.41^{c}	4.72 ± 0.19^{b}	5.85 ± 0.43^{a}	4.22 ± 0.25^{bc}
	MEA_{shoot}	4.44 ± 0.15^{d}	17.25 ± 1.06^{bc}	20.93 ± 2.11^{ab}	23.02 ± 0.46^{a}	16.12 ± 1.15^{c}
Mg	MEA_{root}	5.90 ± 0.55^{d}	10.18 ± 0.39^{bc}	11.42 ± 0.47^{b}	14.19 ± 0.13^{a}	9.49 ± 0.39^{c}
	MEA_{shoot}	8.19 ± 0.42^{b}	15.47 ± 1.52^{a}	16.41 ± 2.13^{a}	17.26 ± 0.69^{a}	10.65 ± 0.44^{b}
Zn	MEA_{root}	1.14 ± 0.09^{d}	3.14 ± 0.19^{c}	3.83 ± 0.28^{b}	4.88 ± 0.10^{a}	3.31 ± 0.30^{bc}
	MEA_{shoot}	3.44 ± 0.04^{d}	8.93 ± 0.68^{a}	7.28 ± 0.45^{b}	8.12 ± 0.42^{ab}	5.38 ± 0.23^{c}
Cu	MEA_{root}	0.34 ± 0.04^{e}	1.15 ± 0.11^{d}	1.61 ± 0.08^{c}	3.10 ± 0.04^{a}	2.60 ± 0.17^{b}
	MEA_{shoot}	4.16 ± 0.17^{c}	13.33 ± 1.23^{a}	9.91 ± 0.43^{b}	13.54 ± 0.73^{a}	8.95 ± 0.33^{b}
Ni	MEA_{root}	0.19 ± 0.03^{c}	0.53 ± 0.03^{b}	0.52 ± 0.04^{b}	0.73 ± 0.03^{a}	0.50 ± 0.02^{b}
	MEA_{shoot}	0.42 ± 0.03^{d}	1.47 ± 0.13^{a}	0.81 ± 0.06^{bc}	0.93 ± 0.05^{b}	0.62 ± 0.04^{cd}
Cd	MEA_{root}	0.05 ± 0.003^{c}	0.10 ± 0.003^{a}	0.07 ± 0.002^{b}	0.09 ± 0.003^{a}	0.07 ± 0.004^{b}
	MEA_{shoot}	0.61 ± 0.06^{c}	1.64 ± 0.15^{a}	0.67 ± 0.01^{c}	0.98 ± 0.08^{b}	0.74 ± 0.04^{bc}
Pb	MEA_{root}	0.35 ± 0.02^{d}	1.05 ± 0.12^{c}	1.38 ± 0.03^{b}	1.87 ± 0.04^{a}	1.43 ± 0.11^{b}
	MEA_{shoot}	0.16 ± 0.001^{d}	1.77 ± 0.17^{a}	1.35 ± 0.12^{b}	1.48 ± 0.11^{ab}	0.91 ± 0.07^{c}
Cr	MEA_{root}	1.96 ± 0.10^{d}	6.45 ± 0.21^{c}	9.21 ± 0.66^{b}	14.07 ± 0.58^{a}	11.35 ± 1.60^{b}
	MEA_{shoot}	0.10 ± 0.01^{c}	0.68 ± 0.06^{a}	0.54 ± 0.04^{a}	0.59 ± 0.03^{a}	0.40 ± 0.01^{b}

MEA_{root}: metal extraction amount in root, MEA_{shoot}: metal extraction amount in shoot, C: control, SS_{RM0}: soil with sewage sludge, SS_{RM5}: 5% red mud SS_{RM0}, SS_{RM10}: 10% red mud in SS_{RM0}, SS_{RM15}: 15% red mud in SS_{RM0}. Numbers with different letters in same row differ significantly at $p < 0.05$ as per the Duncan's test.

in plants (Eid and Shaltout, 2014; Bonanno and Lo Giudice, 2010). More translocation of Mn and Cu to shoot could be due to their strong antagonistic relationship with Cd and Pb, respectively (John and Leventhal, 1995). Thus based on TF values for different metals, vetiver was found efficient in translocation of Mn and Cu to shoot and acted as a phytostabilizer for Fe, Zn, Cu, Ni, Cd, Pb and Cr in roots.

4. Conclusions

The present study indicated that red mud with sewage sludge improved the organic matter content and nutrient status of the soil which supported better plant growth in comparison to the control. Increasing red mud levels although raised total metal contents, but was found effective in reducing their phytoavailable contents. Applications of red mud in sludge amended soil showed significant improvements in plant growth performance and biomass compared to the control, with maximum increase under 10% red mud treatment. All the studied metals except Cu in the plant were found within the phytotoxic threshold levels for vetiver growth. Whereas, metal contents (except Mn) exceeded the WHO limit for medicinal plants. From the result of PCA analysis, it is clear that root-shoot lengths were strongly affected by accumulation of Cu, Cr, Pb, Mn and Zn in roots. However, number of tillers culm^{-1}, root, shoot and total plant biomass were less influenced by metal accumulation in plant parts. Based on metal tolerance index, translocation and bioconcentration factors, vetiver is found to act as a potential metal tolerant plant, efficient in transportation of Mn and Cu from roots to shoot and effective in phytostabilization of Fe, Zn, Mg, Ni, Pb, Cd and Cr in roots. Phytostabilization of metals using vetiver increased with increasing concentrations of red mud; of all treatments, 10% red mud in sludge amended soil sustain maximum plant growth

Table 4
Translocation and bioconcentration factors for different metals in vetiver grown under control and different red mud treatments in soil amended with sewage sludge (Mean ± SE).

Metals	Parameters	C	SS_{RM0}	SS_{RM5}	SS_{RM10}	SS_{RM15}
Fe	TF	0.19 ± 0.01^{c}	0.25 ± 0.01^{ab}	0.28 ± 0.02^{a}	0.27 ± 0.04^{a}	0.25 ± 0.01^{ab}
	BCF_{root}	0.25 ± 0.02^{a}	0.04 ± 0.001^{b}	0.02 ± 0.002^{b}	0.01 ± 0.001^{b}	0.01 ± 0.001^{b}
	BCF_{shoot}	0.05 ± 0.001^{a}	0.01 ± 0.001^{b}	0.004 ± 0.0001^{c}	0.003 ± 0.0001^{cd}	0.003 ± 0.0001^{d}
Mn	TF	1.04 ± 0.01^{a}	1.05 ± 0.10^{a}	1.05 ± 0.08^{a}	1.11 ± 0.08^{a}	1.14 ± 0.03^{a}
	BCF_{root}	0.13 ± 0.01^{b}	0.27 ± 0.03^{a}	0.29 ± 0.01^{a}	0.30 ± 0.03^{a}	0.32 ± 0.01^{a}
	BCF_{shoot}	0.13 ± 0.01^{c}	0.28 ± 0.01^{b}	0.31 ± 0.02^{b}	0.32 ± 0.02^{ab}	0.36 ± 0.02^{a}
Mg	TF	0.31 ± 0.004^{a}	0.33 ± 0.01^{a}	0.34 ± 0.01^{a}	0.33 ± 0.01^{a}	0.33 ± 0.01^{a}
	BCF_{root}	0.97 ± 0.01^{a}	0.36 ± 0.01^{b}	0.31 ± 0.01^{c}	0.31 ± 0.01^{c}	0.30 ± 0.01^{c}
	BCF_{shoot}	0.30 ± 0.005^{a}	0.12 ± 0.002^{b}	0.10 ± 0.01^{c}	0.10 ± 0.002^{c}	0.10 ± 0.003^{c}
Zn	TF	0.66 ± 0.02^{a}	0.62 ± 0.02^{a}	0.46 ± 0.04^{b}	0.46 ± 0.01^{b}	0.49 ± 0.03^{b}
	BCF_{root}	0.29 ± 0.02^{a}	0.13 ± 0.002^{b}	0.13 ± 0.01^{b}	0.13 ± 0.001^{b}	0.14 ± 0.01^{b}
	BCF_{shoot}	0.19 ± 0.01^{a}	0.08 ± 0.002^{b}	0.06 ± 0.002^{c}	0.06 ± 0.001^{c}	0.07 ± 0.001^{bc}
Cu	TF	2.67 ± 0.09^{a}	2.53 ± 0.15^{a}	1.47 ± 0.05^{b}	1.20 ± 0.03^{bc}	1.03 ± 0.01^{c}
	BCF_{root}	0.15 ± 0.004^{c}	0.14 ± 0.01^{c}	0.16 ± 0.01^{c}	0.25 ± 0.003^{b}	0.32 ± 0.01^{a}
	BCF_{shoot}	0.40 ± 0.01^{a}	0.35 ± 0.01^{b}	0.24 ± 0.01^{e}	0.30 ± 0.01^{d}	0.32 ± 0.01^{c}
Ni	TF	0.51 ± 0.09^{a}	0.60 ± 0.01^{a}	0.37 ± 0.02^{b}	0.35 ± 0.01^{b}	0.37 ± 0.02^{b}
	BCF_{root}	0.47 ± 0.05^{a}	0.43 ± 0.01^{a}	0.28 ± 0.01^{b}	0.28 ± 0.01^{b}	0.23 ± 0.01^{b}
	BCF_{shoot}	0.23 ± 0.02^{a}	0.26 ± 0.004^{a}	0.10 ± 0.003^{b}	0.10 ± 0.003^{b}	0.09 ± 0.002^{b}
Cd	TF	0.27 ± 0.02^{ab}	0.36 ± 0.03^{a}	0.25 ± 0.02^{b}	0.31 ± 0.02^{ab}	0.32 ± 0.01^{ab}
	BCF_{root}	0.78 ± 0.05^{a}	0.04 ± 0.002^{b}	0.02 ± 0.0001^{b}	0.02 ± 0.001^{b}	0.01 ± 0.001^{b}
	BCF_{shoot}	0.215 ± 0.04^{a}	0.015 ± 0.001^{b}	0.004 ± 0.0002^{b}	0.005 ± 0.0001^{b}	0.005 ± 0.0004^{b}
Pb	TF	0.10 ± 0.003^{c}	0.37 ± 0.03^{a}	0.23 ± 0.001^{b}	0.22 ± 0.01^{b}	0.19 ± 0.01^{b}
	BCF_{root}	0.60 ± 0.01^{a}	0.57 ± 0.04^{a}	0.52 ± 0.004^{ab}	0.47 ± 0.01^{b}	0.43 ± 0.04^{b}
	BCF_{shoot}	0.06 ± 0.001^{e}	0.21 ± 0.001^{a}	0.12 ± 0.003^{b}	0.10 ± 0.002^{c}	0.08 ± 0.006^{d}
Cr	TF	0.11 ± 0.01^{c}	0.22 ± 0.01^{a}	0.14 ± 0.004^{b}	0.12 ± 0.01^{bc}	0.11 ± 0.10^{c}
	BCF_{root}	0.41 ± 0.01^{a}	0.05 ± 0.001^{b}	0.05 ± 0.002^{b}	0.05 ± 0.001^{b}	0.06 ± 0.004^{b}
	BCF_{shoot}	0.45 ± 0.003^{a}	0.011 ± 0.0004^{b}	0.007 ± 0.001^{b}	0.006 ± 0.001^{b}	0.006 ± 0.0004^{b}

TF: translocation factor, BCF_{root}: bioconcentration factor for root, BCF_{shoot}: bioconcentration factor for shoot, C: control, SS_{RM0}: soil with sewage sludge, SS_{RM5}: 5% red mud SS_{RM0}, SS_{RM10}: 10% red mud in SS_{RM0}, SS_{RM15}: 15% red mud in SS_{RM0}. Numbers with different letters in same row differ significantly at $p < 0.05$ as per the Duncan's test.

coupled with enhanced phytoremediation potential of vetiver. The study, thus offers a potential avenue towards management of red mud using vetiver grass.

Acknowledgement

Authors are thankful to the Head, Department of Botany, Institute of Science, Banaras Hindu University (B.H.U.) and HINDALCO Industries Ltd., Renukoot, U.P. for providing all the necessary facilities during the research work. Meenu Gautam is thankful to the Council of Scientific and Industrial Research (09/013/(0469)/2012-EMR-I), New Delhi, for financial assistance. We are thankful to Coordinator, Centre of Advanced Study in Botany, Institute of Science, B.H.U., for AAS facility and Indian Meteorological Department, Geophysics for furnishing meteorological data of my experimental period. The authors also extend their thanks to the Head, Department of Ayurveda, Institute of Medical Sciences, B.H.U. for providing vetiver slips and to the In-charge, Dinapur sewage treatment plant for providing sewage sludge.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gexplo.2017.03.003.

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