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ARTICLE

Phytoremediation of Pb Spiked Soils Amended with Iron Impregnated Rice Husk Ash Using *Ricinus communis* L. (Castor bean)

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ABSTRACT

Heavy metals pose a serious risk to the environment and living biota. Pot studies were carried out to determine the competence of Fe-coated rice husk in Pb spiked soils vegetated with *Ricinus communis*. Physicochemical properties of Fe- coated rice husk ash (Fe-RHA) were characterized on dry weight basis. Pot experiments were carried out with seedlings of *R.communis* for 60 days amended with Fe-RHA (0, 2.5% and 5% w/w) and Pb(NO3) [0, 400 and 800 mg kg^{-1}]. Addition of Fe-coated rice husk ash to Pb cntaminated soils improved soil pH and fertility. Treatment with 5% Fe-RHA decreased Pb accumulation in roots by 84%. Addition of Fe-RHA significantly ($p<0.05$) increased plant physiological parameters such as height, leaf diameter, nodes, and leaf number by 64%, 49%, 62% and 66% and chlorophyll contents (12–29%) compared to unamended plants. Our findings conclude that Fe-RHA is a low-cost, environmentally friendly and efficient adsorbent for stabilization of Pb spiked soils.

1. Introduction

Lead (Pb) is a toxic and global pollutant of great environmental concern. It is released into the environment through anthropogenic and geogenic processes. Pb is renowned as carcinogen and hazardous metal by United States Environmental Protection Agency [1] and International Agency for Research on Cancer [2]. Sources of Pb pollution to the environment include metallurgy (mining, smelting of ores), industrial (lead-based paint, battery industries, microelectronics, fertilizers, pesticides, automobile exhaust) and domestic waste disposal practices [3,4,5]. Lead is a toxic metal and its widespread usage has deleterious effects on biota through soil-plant-food interaction. Once Pb adheres to soil, it is bound strongly to soil granules on the surface layer of soil particles and water sediment for many years. Pb affects the physiological process of the plant which includes seed germination, chlorosis, stunted growth, chloroplast lamellar organization, substitution of divalent cations and inhibition of Calvin cycle enzymes [6]. Pb being redox active alters plant physiological process and generates reactive oxygen species (ROS) which often interfere with lipid bilayers, proteins and nucleic acids [7]. Pb induced phytotoxicity has been reported in several plant species such as *Sophora japonica & Platycladus orientalis* [8]; *Peganum harmala* [9]; *Jatropha curcas* [10]; *Zygophyllum fabago* [11] and *Spirodela polyrhiza* [12].

To date, several techniques emerged for soil remediation such as electrolyte extraction, ion exchange, reverse osmosis, vitrification, nanofiltration and dialysis/electrodialysis are generally cost effective and cause secondary pollution [13]. Recently, substantial research has focussed on environ-

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ment-friendly remediation such as aided phytostabilization because it is highly efficient and economically feasible process. It improves soil fertility, plant vegetation cover and at the same time reduces the mobility and phytoavailabilty of pollutants [14]. At this time, it becomes peremptory to select an appropriate metal tolerant plant, as it affects the efficiency of phytoremediation process. *Ricinus communis* (castor bean) being an agricultural crop, it is escaped and grown extensively in disturbed regimes and industrially polluted areas. It has high biomass, economic value, adequate adaptability, insipid nature and tolerant to nutrient poor soils, organic and inorganic pollutants [15,16].

Rice husk an agro-waste gaining stern considerations and emerged to be an invaluable source predominantly in the third-world economies [17]. On an average about 20% of rice husk is produced from total paddy milled. Rice husk is woody, lignocellulosic, insoluble in water and characterized by its silico-cellulosic structural arrangement and abrasive intrinsic behaviour. Its major constituents include 50% cellulose, 25-30% lignin, 15-20% hydrated silica and the rest of the mineral components are alkalis and trace elements [18]. RHA is renowned as low-cost adsorbent to immobilize toxic metals (Cd, Hg, Pb, As, Cu, Mn, Cr and Zn) from contaminated soils because of its high adsorptive ability and social wide acceptance [19]. The application of modified rice husk as adsorbent for removal of toxic contaminants is gaining considerable recognition in recent years. The impregnated rice husk has been reported to exhibit higher sorption than unmodified rice husk [20,21,22]. Biochar modified Ni/Fe bimetallic nanoparticles in soil showed high efficiency for removal of polybrominated diphenyl ethers (BDE-209) [23] and a Fe_3O_4 biochar composite to degrade Methylene Blue acted as a Fenton catalyst [24]. Lin et al. [25] reported that 2% Fe-Mn oxide modified biochar (FMBC) reduced the total As concentration in the rice grain by 78.3%. Due to the presence of functional groups like carboxyl, sulfate, imidazole, phenol, hydroxyl, thioether, sulphydryl, phosphate, amino, and amide the bio-adsorbents form stable metal complexes with toxic metal ions [26,27]. Previous studies have utilized RHA as adsorbent for changes in soil chemistry, interaction of RHA with the contaminant and pollutant accumulation in the targeted orgasm. Beyond accumulation in plant tissue, precise knowledge is known regarding Fe-RHA along with plant growth in immobilizing heavy metals. Thus the main aim of our study is to elucidate plant growth responses in Pb spiked soils impregnated with Fe-RHA.

2. Materials and Methods

Rice husk is procured from a local rice huller located in Hyderabad (17.514028º N, 78.300886º E), India. The obtained rice husk is burnt to greyish black coloured ash, grounded and finally sieved through a fine mesh of 100 mm.

2.1 Iron-coated Rice Husk Ash (Fe-RHA) Preparation

Surface impregnation of the RHA was done by following the method of Samsuri et al. [28]. The powdered RHA was weighed and washed with double distilled water to remove adherent dust particles. It is then oven dried for 72 h at 60 °C. The air dried RHA was soaked in a freshly prepared $FeCl_3$ solution containing 2000 mg L^{-1} of Fe(III) at pH 6. The RHA and Fe(III) solution mixture was stirred at regular intervals for three days and filtered through clean and transparent white cloth. The supernatant was discarded and final sample was washed with deionised water several times to remove the free Fe. It is then air-dried for 72 h at 50 °C. The amendments derived iron-coated rice husk ash hereafter referred as Fe-RHA and used for further analysis.

2.2 Physicochemical Characterization of Iron-coated Rice Husk Ash (Fe-RHA)

For pH and EC, triplicate samples of Fe-RHA and deionised water were added to achieve 1:2.5 (w/v) ratio and samples are left to stand overnight. Further samples were measured by using pH and electrical conductivity meters, respectively [29]. Bulk density was determined by calculating the ratio ratio of the weight of the biomass sample to the volume of the vessel following Richards [30] protocol. A muffle furnance (500 °C and 750 °C) was used to determine the total mineral content of Fe-RHA (ASTM #D-1762-84). Energy-dispersive X-ray spectroscopy (Oxford instruments) is an analytical technique used for elemental analysis. The air-dried Fe-RHA samples were made into fine powder, mounted on aluminum stubs and coated with gold-palladium. X-ray based emission spectral peaks were analysed after irradiation samples with 15-20 kV and woring distance of 8.5mm. The Fe-RHA samples were deposited onto a carbon coated grid and morphology of Fe-RHA was recorded using field emission scanning electron microscope (FESEM, Ultra 55-carl Zeiss). Thermogravimetric analysis (TGA) was carried out using Q50-TA Instruments under helium gas flow up to 1000 °C at a heating rate at 10 °C min^{-1}.

2.3 Experimental Design

A Pot study was conducted in the glasshouse at University of Hyderabad (latitude 17º 27′22.8″N and longitude 78º 18′49.8″E) under ambient conditions with temperature of 18-25 ºC (day/night) and humidity of 60±5%. DCS-108 varieties (castor seeds) were procured from Directorate

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of Oil Research, Hyderabad. Prior to the pot experiment, the soil was aged with 0, 400 and 800 mg kg^{-1} of $Pb(NO_3)_2$ and amended with Fe-RHA at concentrations of 0, 2.5% and 5% (w/w) for 10 days. Based on preliminary pot experiments, the concentration of amendments, Pb treatments, and time-period was selected. Experiments were performed in 3L plastic pots containing one plant each and nine treatments in triplicates. Treatments without amendment and metal concentration were considered as control. Plants were irrigated with 60% of water holding capacity throughout the growth period for 60 days.

2.4 Plant Growth Assessment

Plant physiological parameters such as number of leaves, nodes, height and leaf diameter were measured during harvesting period. Leaf measurements were performed on second and third leaves from the basal of the stem and the repetition values (n=3) were denoted in centimeters.

2.5 Metal Accumulation in Plant

At the end of the experiment (60 days) plants were harvested and excised into roots, stem and leaves. Metal treated plant parts were washed with deionised water to clean it from adherent dust particles and then oven-dried at 100 ˚C for 3 days. The Pb content in the plant samples was analysed by inductively coupled plasma mass spectrometry (GBC, USA) after grinding and digested with concentrated HNO_3 (v/v) using microwave digester (Perkin Elmer, Australia).

2.6 Photosynthetic Pigments

Chlorophyll a and b were carried out by the method of Arnon [31] and Duxburg and Yentsch [32]. The leaf sample (0.1g) was grounded in 5 ml chilled 80% acetone in dark. After centrifugation at 10,000 rpm for 10 min at 4 ˚C, absorbance of supernatant was taken at 480, 645 and 663 nm. The units of chlorophylls were calculated in mg g^{-1} fresh weight (fw).

2.7 Statistical Analysis

One-way analysis of variance (ANOVA) and Turkey multiple comparison tests was used to statistically analyse the data using Graph pad prism (6.0). Significant levels ($p \leq 0.05$) were used to show the differences between each treatment in a group.

3 Results and Discussion

Soil pH is an important parameter influencing soil-surface chemistry. With increasing pH, the number of negatively charged surface sites in soil increases and sorption capacity of soil to cationic metals correspondingly increases. The pH of Fe-RHA was alkaline (8.81±0.58) while the electrical conductivity was 2.35±0.17, respectively (Table 1). Fe-RHA showed increased pH because of its ash content and dissolution of carbonates, phosphates, silica, organic nitrogen, nutrients and sesquioxides. The data obtained are similar with Raven et al. [33] for the adsorption of As(III) by ferrihydrite. Singh et al. [27] reported that addition of RHA leads to increase in soil pH. The bulk density of Fe-RHA was 0.18±0.03 and exhibited ash content of 231±3.74 respectively. The high ash content was ascribed due to addition of Fe oxides [34]. The macro and mironutrients such as (C, O, Si, N, P, K, Mn, Fe, and Zn) were detected in the amendment by energy-dispersive X-ray spectroscopy. The carbon (41.68%) and oxygen (35.12%) were contributed as major elements followed by silica (16.45%). The high carbon content in Fe-RHA was due to carbonization, and low oxygen content was attributed to the removal of water and hydrocarbons during pyrolysis process [35]. Surface area is a vital indicator of adsorbent sorption ability. The scanning electron microscopy images of Fe-RHA had high porous structure and inhomogeneous surface dominated by various meso and macropores (~3 nm) with deformed cellulose and lignin strands (Figure 1). Previous studies also showed that iron impregnated amendments were used for sorption of metal ions [20, 36, 22]. TG analyses show the weight loss curves versus temperature of iron-coated rice husk ash and rice husk biomass (Figure 2). Majority of the weight loss (60%) occurred between 200 °C to 400 °C for rice husk biomass. It is due to the solid decomposition of cellulose fraction of rice husk. On the other hand, the weight loss in Fe-RHA was gradual up to 600 °C and around 62% of Fe-RHA remained even at 600 °C which confronts the thermal stability of Fe-RHA over rice husk biomass [37].

Table 1. Physical and chemical characteristics of iron-coated rice husk ash (Fe-RHA) on a dry weight basis

Characterization of iron-coated rice husk ash (Fe-RHA)	
Physicochemical properties	
pH	8.81±0.58
Electrical conductivity (dS m^{-1})	2.35±0.17
Bulk density (g cm^{-3})	0.18±0.03
Mineral (ash) content (g kg^{-1})	231±3.74
Elemental composition (% on dry wt. basis)	
Carbon (C)	41.68
Oxygen (O)	35.12
Silica (Si)	16.45
Nitrogen (N)	0.11
Phosphorous (P)	0.15
Potassium (K)	1.28
Manganese (Mn)	3.99
Iron (Fe)	1.07
Zinc (Zn)	0.15

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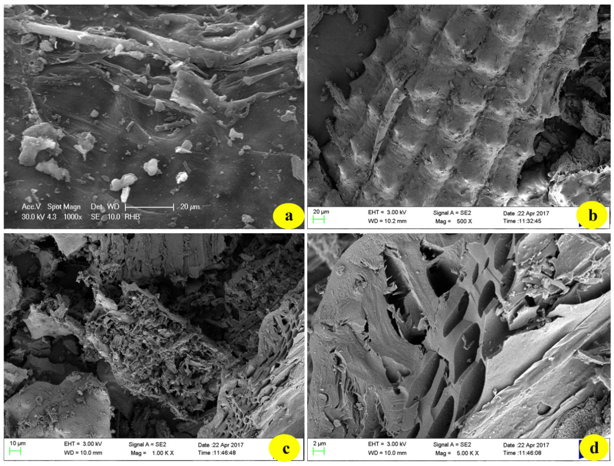

Figure 1. SEM micrographs of rice husk biomass (a) and iron coated rice husk ash (b-d) at 500X, 1.0 KX and 5.0 KX magnifications

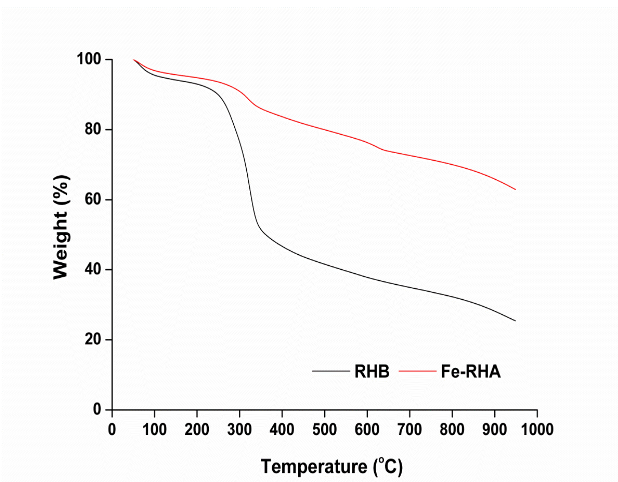

Figure 2. Change in temperature during the thermal decomposition of rice husk biomass (RHB) and iron coated rice husk ash (Fe-RHA) under a heating rate of 10 °C min^{-1}

Pb is regarded as a global environmental contaminant and increased accumulation in plants leads to membrane injury, chloroplast damage, necrosis, stunted growth, alters cell metabolism and reduces productivity [7]. Adsorption, complexation and/or precipitation are the possible mechanisms involved in immobilization of metals by amendments. Organic matter has reactive groups (hydroxyl and carboxyl) that react with soluble Pb to form stable complexes [38,19]. As the exogenous application of Pb in soil increases, accumulation of Pb in different plant parts increases rapidly (Table 2). Pb accumulation in different parts of *R.communis* followed the trend as root>shoot>leaf. The Pb concentration in all the iron-coated rice husk ash treatments were significantly ($p<0.05$) different compared to Pb treatments alone. Fe-RHA at 5% application rate and 400 and 800 mg kg^{-1} treatments decreased Pb content in leaves by 98% and 97%, shoots by 71% and 57% and roots by 84% and 78% with respect to the Pb treatments alone. Our results are in harmony with Lin et al. [25] where total As the concentration of the roots, stems, and leaves was decreased by 79.1%, 50.9%, and 64.7% with 2% FMBC (Ferromanganese oxide impregnated biochar) supplementation. In the present study, Pb concentration was higher in roots than aerial parts of *Ricinus*. A hypothesis drawn which block the translocation of Pb was due to the silicon content of RHA through depositing metal bound with silica in the endodermis, root pericycle or Si-induced apoplastic binding in roots [39]. Gu et al. [40] reported similar results which showed decreased uptake of Cd and Pb and their translocations in rice upon application of steel slag enriched in silicon. Hamid et al. [21] reported reduction of Pb content in rice grains was 82 mg kg^{-1} by 1% Fe impregnated biochar.

Table 2. Pb Concentration in leaf, stem and root of *Ricinus communis* L. grown with iron coated rice husk ash (Fe-RHA) (mg g^{-1})

Treatments	Iron coated rice husk ash (Fe-RHA)		
	Leaf	Shoot	Root
Control	nd	$1*10^{-3}$	$1*10^{-2}$
Pb 400	$0.34±0.002^{a}$	$1.59±0.03^{a}$	$3.63±0.13^{a}$
Pb 400 (2.5%)	$0.02±0.005^{b}$	$1.15±0.01^{b}$	$2.91±0.01^{b}$
Pb 400 (5%)	$0.01±0.001^{c}$	$0.75±0.02^{c}$	$1.57±0.01^{c}$
Pb 800	$0.49±0.004^{a}$	$2.56±0.05^{a}$	$9.60±0.91^{a}$
Pb 800 (2.5%)	$0.06±0.003^{b}$	$1.49±0.01^{b}$	$5.29±0.06^{b}$
Pb 800 (5%)	$0.01±0.002^{c}$	$1.11±0.01^{b}$	$2.13±0.02^{c}$

Note: Bars and rows represented by small-case letters indicate significant ($p<0.05$) differences within each group (n=3) according to Tukey comparison test.

Plant growth inhibition is one of the direct and reliable indicators of heavy metal stress. Pb accumulation at higher concentrations in plants causes stunted growth, wilting and inhibits reproducibility. [41]. Under Pb stress, significant plant growth was observed throughout the experimental period. Without Fe-RHA supply, 800 mg kg^{-1} Pb treatment reduced the plant height, leaf diameter, node number and leaf number (Figure 3a-d) by 21%, 16%, 9% and 41% with respect to control respectively. On contrary 800 mg Pb kg^{-1} + 5.0% Fe-RHA of soil, increased the plant height, leaf diameter, node number and leaf number by 64%, 49%, 62%, and 66% compared to Pb treatment alone. Pb immobilization in technosol as a result of binding of Pb to Fe-RHA was a significant factor for the improvement of plant growth parameters. RHA is also regarded as soil conditioner as its application increases soil fertility and plant growth by retaining nutrients and increasing pH, soil organic matter, CEC and soil physical properties [42, 27]. This activity decreases the contaminants

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in soil thereby reducing phytotoxicity and leading to a significant increase in yield. The obtained findings were consistent with Cao et al. [22] in *Eichhornia crassipes* and Lin et al. [25] in *Oryza sativa* who reported that Fe impregnated RHA could alleviate Pb toxicity and increase the *O.sativa* growth parameters. Fresno et al. [43] showed that iron combined with bio-char increases shoot biomass in white lupine on As and Cu contaminated technosol.

Chlorophyll reduction is one of the most obvious symptoms of heavy metal stress in higher plants. Increase in Chl content reflects plant health. Excess accumulation of Pb damages the chloroplast ultrastructure, pigment biosynthetic pathways, affects electron transport chain and replacement of central Mg^{2+} ions in Chl molecules with Pb^{2+}. Any above-mentioned reasons denature pigment-protein complexes and lead to Chl reduction [5, 44]. Significant reduction of chlorophyll in leaves of *R.communis* was observed under Pb stress (Figure 4a-c). In the absence of amendment, at 800 mg kg^{-1} of Pb soil, there was a decrease in Chl a, Chl b and Total Chl by 23%, 9% and 17% with respect to control respectively. Contrarily Fe-RHA at 5% application + 800 mg kg^{-1} Pb treatment increased the Chl a, Chl b and total Chl by 29%, 12%, and 21% compared to control respectively. Addition of Fe-RHA ($p<0.05$) significantly improved the chlorophyll content in leaves of *R.communis*. The impregnation of Fe to Pb spiked soils lead to immobilization of Pb and also altered the surface potential of RHA and nutrient enhancement in soil rhizosphere [42]. In Pb spiked soils of *R.communis* addition of 5% RHA increased the chlorophyll content by 22% compared to Pb treatments alone [18]. Field study conducted by Hamid et al. [21] in rice plants reported that addition of Fe-biochar considerably enhanced the net photosynthetic rate in Pb-contaminated soils. From our study we suggest that utilization of alkaline amendments (Fe-RHA) had the ability to reduce the phytoavailabilty of toxic cationic elements and sustainable plant development.

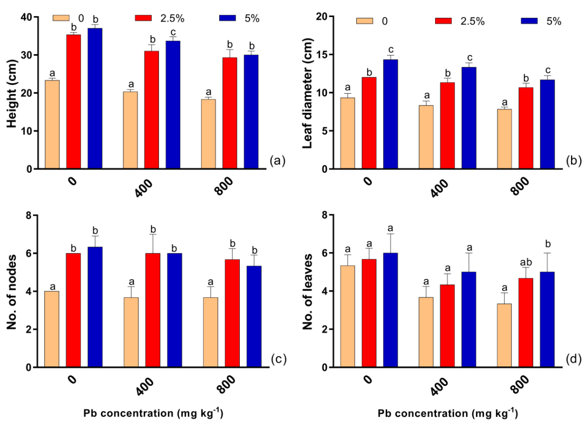

Figure 3. Effect of iron coated rice husk ash (Fe-RHA) on growth parameters of *R.communis* after 60 days in Pb-spiked soils

Note: (a) Height, (b) Leaf diameter, (c) No. of nodes and (d) No. of leaves. Bars and rows represented by small-case letters indicate significant ($p<0.05$) differences within each group (n=3) according to Tukey comparison test.

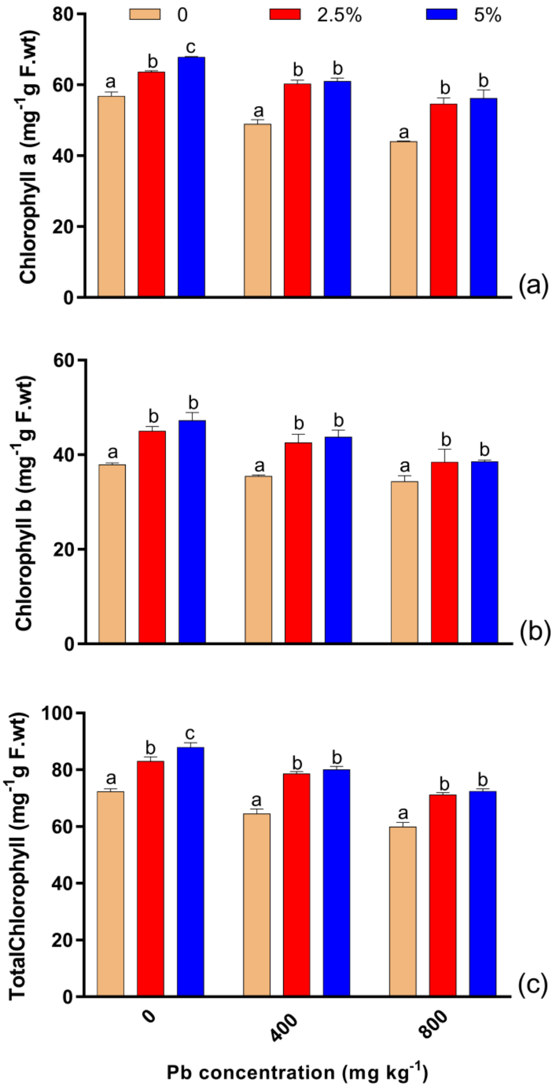

Figure 4. Photosynthetic pigment content of *R.communis* leaves treated with dierent doses of iron coated rice husk ash (Fe-RHA) in Pb contaminated soil

Note: Bars and rows represented by small-case letters indicate significant ($p<0.05$) differences within each group (n=3) according to Tukey comparison test.

4. Conclusions

Iron impregnated rice husk ash was successfully synthesized and utilized in the effective immobilization of Pb spiked soils. The Fe-RHA composite showed excellent physicochemical properties such as enhanced pH, large surface area, fine porous structures, enhanced nutrient content, thermal stability, and functional groups which lead to forming stable metal complexes. *R.communis*

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showed a positive influence by addition iron-coated rice husk ash to Pb contaminated soils. Control plants enhanced the Pb uptake whereas supplementation with Fe-RHA to Pb-contaminated soils reduced the translocation of Pb to different plant parts. Furthermore, Fe-RHA addition successfully reduced Pb phytoavailabilty, increased plant growth parameters, Pb uptake by plant and increased the chlorophyll content. However, further explorations are needed in molecular and field level for long term application of amendment and increased metal tolerance.

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