

Research Article

Phytoremediation of Gold Mine Tailings Amended with Iron-Coated and Uncoated Rice Husk Ash by Vetiver Grass (*Vetiveria zizanioides* (Linn.) Nash)

F. S. Tariq,¹ A. W. Samsuri,¹ D. S. Karam,¹ and A. Z. Aris²

¹Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia (UPM),
43400 Serdang, Selangor Darul Ehsan, Malaysia

²Environmental Forensics Research Centre, Faculty of Environmental Studies, Universiti Putra Malaysia (UPM),
43400 Serdang, Selangor Darul Ehsan, Malaysia

Correspondence should be addressed to A. W. Samsuri; samsuriaw@upm.edu.my

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This study was undertaken to determine the effects of rice husk ash (RHA) and iron-coated rice husk ash (Fe-RHA) on phytoavailability of As, Cd, Cr, Cu, Mn, Pb, and Zn to vetiver grass grown in gold mine tailings amended with either RHA or Fe-RHA at 0%, 5%, 10%, and 20% (w/w). The results showed that the RHA amended tailings recorded higher concentration of As in the shoot and the root and higher concentration of Cr and Mn in the root compared to the untreated tailings which was used as a control. The biological accumulation coefficient (BAC) and bioconcentration factor (BCF) values of the vetiver grass for As and Zn increased with RHA application rate but the biological transfer coefficient (BTC) values of As and Zn were decreased. In Fe-RHA amended samples, As concentration in the shoot and root concentrations of Cd and Zn were significantly higher compared to the control. The Fe-RHA treated samples had lower BAC and BTC values for As and Zn than the control. However, the BCF values for those elements were higher than the control. The concentration of Pb was not detected in any of the samples.

1. Introduction

Tailings are the components of the primary mineral-bearing rock left after the extraction of minerals like gold, copper, and silver [1]. Mine tailings are regarded as a major source of environmental pollution due to the presence of high concentration of trace elements in the tailings, which may cause various health effects. The heavy metals in mine tailings are quickly discharged and distributed into the ground water causing serious environmental and health issues in the vicinity of the mine area [2]. Hence, gold mine tailings require proper management to reduce the release of heavy metals into the environment.

There are several methods of cleaning up the harmful effects of heavy metal on contaminated sites which include, among others, physical removal (excavation), *in situ* stabilization of these metals, and utilization of plants to extract the metals from the polluted site. Each of these approaches

nevertheless has its advantages and disadvantages and its success depends on the nature and size of the contaminated site. However, using green plants to contain, degrade, or eliminate metals, for the purpose of removing them is efficient and cost-effective and involves nonenvironmental disruptive compared to operations that require excavation of soil. Different plants have different phytoextraction potentials depending upon the environments and their genetic variability. The ability of plants to take up heavy metals and transfer them from the shoots to the roots is very important in phytoremediation [3]. Phytoremediation can be considerably advantageous when the metals are in the soil solution rather than held by soil constituents and this can be achieved by using different techniques such as adding soil acidifiers, organic and inorganic compounds, and chelates [4]. Organic amendments can be used to either mobilize or immobilize toxic metals in contaminated soils [5, 6]. In the mobilization technique, the metal(loid)s are released into the soil and are

subsequently removed through the native plant species. Plant growth on metal-contaminated soil may be enhanced as a result of organic amendments [7]. Chemical portioning and distribution are also affected by organic amendments [8], which may influence the phytoavailability of metals. A field study was conducted by Singh and Agrawal [9] to assess the suitability of sewage sludge amendment in soil for rice (*Oryza sativa* L.) by evaluating the heavy metal accumulation and growth of plants in soil amended with sewage sludge at either 0, 3, 4.5, 6, 9, or 12 kg m⁻². They found that sewage sludge amendment modified the physicochemical properties of soil, thus increasing the availability of heavy metals in soil and consequently resulting in higher accumulation in plant parts.

The use of waste-based materials for environmental conservation has been stressed under Malaysia's Green Strategies of the National Policy on the Environment [10]. Solid waste disposal is becoming a major issue in Malaysia; for example, milling processes generate approximately 300 000 mt (metric ton) of rice husk annually. Consequently, burning is estimated to produce more than 63 000 mt of ash a year [11]. Rice husk is regarded as agricultural waste, comprising around 20% of the paddy weight, and its annual gross production is 545 million metric tons worldwide [12]. Rice husk ash is a by-product produced from the burning of rice husk. The rice husk and its ash may be used as a natural, low cost adsorbent to remove toxic metals. The ash also contains compounds such as silica, cellulose, lignin, and hemicellulose with binding sites capable of taking up metals [13, 14]. Also, the ash derived from rice husk has good adsorptive ability and it has been used previously by researchers to remove metal ions [15], dye [16], and filtration of arsenic from water [17]. In addition, Nakbanpote et al. [18] tested the ability of RHA to adsorb gold-thiourea complex and the results have shown that the RHA absorb more gold than conventionally activated carbon. There are a number of studies that proved that RHA has a good ability to remove heavy metals such as Cd, Pb, Zn, Cu, Mn, and Hg from aquatic solution [12, 16, 19]. However, there is no information available in the literature for the simultaneous phytoremediation of heavy metals by RHA. Thus, the main objectives of the present study were to determine the effects of rice husk ash (RHA) and Fe-coated RHA on the ability of vetiver grass to phytoremediate mine tailings contaminated with As, Cd, Cr, Cu, Mn, Pb, and Zn.

2. Materials and Methods

2.1. Chemicals and Reagents. All the chemical reagents were of analytical grade, and the solutions were prepared using a Milli-Q system (Direct-Q® 3 UV) ultrapure water (electrical resistivity 18.2 MΩ cm⁻¹). Analytical grade sodium arsenate, cadmium nitrate tetrahydrate, chromium(III) nitrate nonahydrate, copper(II) chloride, manganese(II) sulfate monohydrate, lead(II) nitrate, zinc chloride, and iron(III) chloride were purchased from Sigma-Aldrich.

2.2. Tailings Preparation and Analyses. The tailings were collected from a gold mine located in the state of Pahang, Peninsular Malaysia. Composite samples were taken using

a stainless steel scoop from the upper 30 cm of the tailings. The samples were taken from this layer because they were fresh samples and not waterlogged. The samples were brought to the laboratory and immediately air-dried, homogenized, and grounded to pass through a 2 mm sieve. The samples were stored in plastic bags prior to analysis. The particle size distribution of the tailing was measured using a pipette method [20]. Samples of tailings/water ratio of 1:2.5 were equilibrated for 24 hours and then the pH and EC were measured by using Metrohm 827 pH meter and Eutech Instruments CON 700 EC meter, respectively. Exchangeable bases and CEC were determined using 1 M NH₄OAc buffered at pH 7 [21]. The extracted NH₄⁺ was measured using Lachat QuikChem 8000 Series FIA+ autoanalyzer. The carbons, nitrogen, and sulfur, components of tailings sample, were determined by using a CNS elemental analyzer (model: LECO TruMac CNS Analyzer). One gram of tailing sample was digested with a mixture of three concentrated acids (4 mL HCl, 2 mL HNO₃, and 2 mL HF and filtered through Whatman 44 ashless diameter 125 mm filter papers for total P, Ca, Mg, K, and Na determination) [22]. The concentrations of P, Ca, Mg, K, and Na elements in the digests were determined with Perkin Elmer AAnalyst 400 atomic absorption spectrophotometers and P was measured using an autoanalyzer (Lachat Instruments QuikChem 8000 Series FIA+ System). The acid neutralizing capacity (ANC) was determined using the method described by Shu et al. [23], while the amount of CaCO₃ was determined by acid neutralization method [24]. The total amount of heavy metals in the tailings was determined using a method modified from Güven and Akinci [25]. Details of the digestion programs are presented in Table 1. The digests were then filtered through a 0.45 μm membrane filter, diluted to 50 mL, and stored prior to analysis. The metal concentrations were analyzed by Perkin Elmer Optima 8300 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.3. Rice Husk Ash Characterization. Rice husk ash (RHA) was obtained from a factory located at Sungai Besar, in the state of Selangor, Peninsular Malaysia. The ash was passed through 1 mm sieve and analyzed for its chemical and physical properties. The pH was measured in a 0.5:100 (w/v) RHA:H₂O suspension and the electrical conductivity (EC) was measured by shaking 1 g sample in 20 mL water for two hours [26]. The pH and EC were measured using the instruments described previously. The ash content was determined by using a dry combustion method [27]. The cation exchange capacity (CEC) was measured using the method described by Song and Guo [28].

The surface area of the RHA was measured by N₂ adsorption at 77.3 K, using a Quantachrome version 2.01 (Quantachrome ASiWin™) surface area analyzer. The RHA sample was degassed at 100°C for 9 h prior to N₂ adsorption. The multipoint Brunauer-Emmett-Teller (BET) method was employed to calculate the total surface area. The Scanning Electron Microscope-Electron Dispersive Spectroscopy (SEM-EDS) was used to study the RHA morphology, and Fourier Transformed Infrared Spectroscopy (Perkin Elmer

TABLE 1: The operating conditions for the microwave digestion system.

Samples	Reagents (mL)	Steps program	Power (watt)	Time (min)	Temperature (°C)
Tailings					
0.5 g	65% HNO ₃ (6 mL) 37% HCl (2 mL) 40% HF (2 mL)	1	200	8	150
		2	400	7	180
		3	600	1	200
		4	0	10	200
Vetiver grass					
0.25 g	65% HNO ₃ (6 mL) 30% H ₂ O ₂ (2 mL)	1	250	5	100
		2	500	10	150
		3	750	5	200
		4	0	15	200

FTIR-2000 spectrometer) was used to determine the RHA surface functional groups.

2.4. Preparation of Iron-Coated Rice Husk Ash (Fe-RHA). Surface modification of the RHA was done by coating it with Fe(III) as described by Samsuri et al. [29]. The RHA was weighed and washed several times with deionized water and then dried for 48 h in an oven at 50°C. Then, the sample was soaked in a solution containing 2000 mg L⁻¹ of Fe(III) prepared using FeCl₃ salt. The solution pH was adjusted to 6 using either 0.1 M HCl or 0.1 M NaOH. The mixture of RHA and Fe(III) solution was stirred several times per day for four days manually and then filtered on Whatman no. 42 filter paper and the rice husk was washed with deionized water several times to remove the free Fe until no more Fe was detected in the filtrate.

2.5. Greenhouse Study. Untreated tailings (control) and tailings treated with three rates (5, 10, or 20% w/w) of either RHA or Fe-RHA were used for this experiment. Amended tailings were thoroughly homogenized in large plastic containers, and water was added to field capacity and then transferred to plastic bags and kept in a climate-controlled dark room for four weeks. From each bag, approximately 20 g sample was taken to determine the pH and EC at the end of incubation. Pot experiments were conducted in a glass house using square plastic tube pots measuring 5.4 × 5.4 cm at the top and 1.5 × 1.5 cm at the bottom and 29 cm in height with 50 g of acid washed sand placed at the base of the pots. Then, 500 g of tailing mixture was poured into each pot and a one-month age vetiver grass seedling was transplanted into the pot. The experimental units were arranged using a randomized complete block design. The seedlings were watered daily and were allowed to grow for 75 days. At the end of the experiment, the plants were harvested, and the shoots and roots were separated. The plant parts were washed with deionized water several times to remove the tailings and then dried at 60°C for 72 h and weighed for dry biomass determination. The heavy metals (As, Cd, Cr, Cu, Mn, Pb, and Zn) contents in the plant parts were analyzed by ICP-OES after grinding and approximately 0.25 g of sample digested with 6 mL of HNO₃ 65% and 2 mL of H₂O₂ 30% in microwave digestion system; according to the digestion program presented in Table 1, the resulting solutions were cooled and diluted to 50 mL

with ultrapure water. The biological accumulation coefficient (BAC) is defined as the concentration of metals in plant shoots divided by the metal concentration in soil [30] and is given in

$$\text{BAC} = \frac{[\text{Metal}] \text{ shoot}}{[\text{Metal}] \text{ soil}}. \quad (1)$$

The biological transfer coefficient (BTC) is defined as the ratio of metal concentration in shoot to the root of the plant [30] and is given in

$$\text{BTC} = \frac{[\text{Metal}] \text{ shoot}}{[\text{Metal}] \text{ root}}. \quad (2)$$

The bioconcentration factor (BCF) was calculated as the ratio of the concentration of metal in plant root to the soil [31] as given in

$$\text{BCF} = \frac{[\text{Metal}] \text{ root}}{[\text{Metal}] \text{ soil}}. \quad (3)$$

2.6. Statistical Analysis. All data were checked for normality and homogeneity of variances prior to statistical analysis. The precision of the data was calculated and expressed as a standard deviation (SD). Later the data were subjected to statistical analysis of variance (ANOVA) using MINITAB version 16 at the 5% significance level and Tukey's test was employed for mean separation.

3. Results and Discussion

3.1. Physicochemical Properties of the Gold Mine Tailings and RHA and Fe-RHA. The selected physicochemical properties of the gold mine tailings are shown in Table 2. The tailings texture was silty loam (14.23% clay; 36.02% sand; 49.68% silt) and the pH was slightly alkaline at 7.90 due to the use of lime to treat the waste water which was pumped back to the retaining pond. The tailings had high acid neutralization capacity and calcium carbonate content which were 63.9 H₂SO₄ t⁻¹ and 56 g kg⁻¹, respectively. The high concentrations of total heavy metals As (1625.251 mg kg⁻¹), Cd (57 mg kg⁻¹), Cr (31.44 mg kg⁻¹), Cu (75.6 mg kg⁻¹), Mn (790.03 mg kg⁻¹), Pb (81.8 mg kg⁻¹), and Zn (174.8 mg kg⁻¹)

TABLE 2: The selected physicochemical properties of the gold mine tailings.

Parameters	Value
Clay (%)	14.23 ± 0.61
Silt (%)	49.68 ± 0.89
Sand (%)	36.02 ± 1.43
Texture (USDA)	Silty loam
Moisture content % at 33 kpa	25.90 ± 0.36
Moisture content % at 1500 kpa	4.04 ± 0.81
pH	7.90 ± 0.02
EC (dS m ⁻¹)	1.48 ± 0.01
CEC (cmol ₍₊₎ kg ⁻¹)	10.75 ± 0.83
Total carbon (%)	1.56 ± 0.01
ANC (kg H ₂ SO ₄ t ⁻¹)	63.90 ± 2.01
Total N (%)	0.036 ± 0.004
Total P (%)	0.076 ± 0.002
Total K (%)	0.196 ± 0.005
Total S (%)	0.140 ± 0.011
Total Ca (mg kg ⁻¹)	8600 ± 30.51
Total Mg (mg kg ⁻¹)	10750 ± 152
Total Na (mg kg ⁻¹)	1480 ± 21
Total As (mg kg ⁻¹)	1625.25 ± 12
Total Cd (mg kg ⁻¹)	57.00 ± 1.41
Total Cr (mg kg ⁻¹)	31.44 ± 0.61
Total Cu (mg kg ⁻¹)	75.60 ± 0.65
Total Mn (mg kg ⁻¹)	790.03 ± 9.98
Total Pb (mg kg ⁻¹)	81.80 ± 1.75
Total Zn (mg kg ⁻¹)	174.80 ± 1.18
Total Fe (mg kg ⁻¹)	16420 ± 402

Mean of three replicates ± SD.

indicated that the tailings were highly contaminated with these elements. On the other hand the tailings had low CEC (10.75 cmol₍₊₎ kg⁻¹), N (0.036%), P (0.076%), and K (0.196%) contents. In addition to that, the selected physicochemical properties of the RHA and Fe-RHA are shown in Table 3. The pH, EC, CEC, total C, DOC, total P, total K, total Ca²⁺, and ash content values of the RHA were higher than the Fe-RHA. On the other hand, total N and total Na were higher in the Fe-RHA than RHA. The Fe-RHA also had a larger total surface area, pore surface area, pore volume, and pore radius. However, there was no significant difference in the surface functional groups between RHA and Fe-RHA as indicated by the FTIR spectra (Figure 1). The bands at 615.73 and 619.50 cm⁻¹ are associated with the C=C-H (aromatic) functional group. The bands at 784.61 and 781.83 cm⁻¹ are associated with the =C-H out of plane bending and finally the bands at 1057.41 and 1055.05 cm⁻¹ are associated with the C-O stretch. The surface morphology, shape, and pores of RHA and Fe-RHA are shown in Figure 2. It can be seen that the meso- and macropores were more abundant than the micropores. Both intact and deformed cellulose and lignin strands are visible in the scanning electron micrograph images (Figure 2).

TABLE 3: The selected physicochemical properties of the RHA and Fe-RHA.

Parameters	RHA	Fe-RHA
pH	10.33 ± 0.33	7.24 ± 0.06
EC (dS m ⁻¹)	0.56 ± 0.04	208.33 ± 0.01
CEC (cmol ₍₊₎ kg ⁻¹)	13.36 ± 0.37	11.32 ± 0.05
Total C (%)	6.44 ± 0.09	5.45 ± 0.07
DOC mg kg ⁻¹	818.77 ± 2.43	800.8 ± 1.41
Total N (%)	0.047 ± 0.001	0.11 ± 0.01
Total S (%)	ND	ND
Total P (%)	0.23 ± 0.02	0.15 ± 0.01
Total K (%)	0.098 ± 0.003	0.03 ± 0.001
Ash content (%)	8.4 ± 0.23	6.8 ± 0.03
Total Mg (mg kg ⁻¹)	7800 ± 17.61	2600 ± 17.62
Total Ca (mg kg ⁻¹)	585 ± 6.03	ND
Total Na (mg kg ⁻¹)	740 ± 4.58	8100 ± 25.63
Total surface area (m ² g ⁻¹)	24.252	44.686
Pore surface area (m ² g ⁻¹)	4.417	5.648
Pore volume (cm ³ g ⁻¹)	0.016	0.017
Pore radius (Å)	17.165	19.201

Mean of three replicates ± SD; ND: not detected.

3.2. Chemical Properties of the Mine Tailings and Dissolved Organic Carbon (DOC) Content after Burning Rice Husk Application. The application of RHA significantly increased the pH of the tailings in all treatments while Fe-RHA amendment lowered the pH of the tailings compared with the control (Table 4). The highest pH value for RHA amended tailings was 9.05 at the highest application rate (20%). It is also observed that there was no effect of RHA application on the EC of the mine tailings except at the highest rate (20%). The results also revealed that both RHA and Fe-RHA application had no impact on the CEC of the mine tailings. The application of high rates of RHA and Fe-RHA significantly increased DOC of the mine tailings. The DOC of the tailings amended with 10% and 20% RHA and 20% Fe-RHA were higher compared with the control (Table 4). The highest DOC value for RHA amended tailings was 919.80 mg kg⁻¹ at the 20% (w/w) application rate.

The increases in the pH, EC, and DOC of the tailings with RHA application were possibly due to the high ash content, DOC, elemental concentrations, and the presence and dissolution of hydroxides and carbonates in the RHA (Table 3). Even though the CEC of the RHA was higher than the CEC of the tailings, the addition of RHA did not increase the CEC of the tailings significantly. This might be attributed to the short time span between samples incubation and the time of CEC measurement. Karmakar et al. [32] and Masulili et al. [33] found that the application of RHA to soil increased soil pH, organic carbon, and nutrient availability. Therefore, it can be concluded that the effects of RHA application on the pH, EC, and DOC of the tailings depend on both the properties of the RHA and the tailings.

3.3. Concentrations of Heavy Metals in Shoot, Root, and Total Heavy Metal Uptake of the Vetiver Grass. Figures 3(a) and

TABLE 4: The pH ($1:2.5 w_{\text{soil}}/v_{\text{water}}$), EC ($\mu\text{S cm}^{-1}$), and CEC ($\text{cmol}_{(+)} \text{kg}^{-1}$) of the mine tailings amended with the ashes (mean $n = 3$, $\pm\text{SD}$). Different letters correspond to significant differences between rates of the same ash ($P < 0.05$) in the same column.

Type	Rates (%)	pH	SD	EC (dS m^{-1})	SD	CEC ($\text{cmol} + \text{kg}^{-1}$)	SD	DOC (mg/kg)	SD
Control	0	7.90 ^d	0.02	1.48 ^b	0.012	11.75 ^a	0.83	823.03 ^b	5.28
RHA	5	8.45 ^c	0.01	1.48 ^b	0.015	10.05 ^a	1.47	844.86 ^b	7.84
RHA	10	8.55 ^b	0.00	1.49 ^b	0.015	11.75 ^a	0.63	904.43 ^a	6.59
RHA	20	9.05 ^a	0.02	1.58 ^a	0.045	12.46 ^a	1.46	919.80 ^a	10.30
Control	0	7.90 ^a	0.02	1.48 ^b	0.012	11.75 ^a	0.83	823.03 ^a	5.28
Fe-RHA	5	7.73 ^b	0.03	1.48 ^b	0.034	14.12 ^a	0.12	772.43 ^b	12.41
Fe-RHA	10	7.70 ^b	0.04	1.51 ^b	0.012	12.48 ^a	2.56	815.17 ^a	8.57
Fe-RHA	20	7.66 ^b	0.06	1.64 ^a	0.008	12.25 ^a	0.23	835.63 ^a	5.54

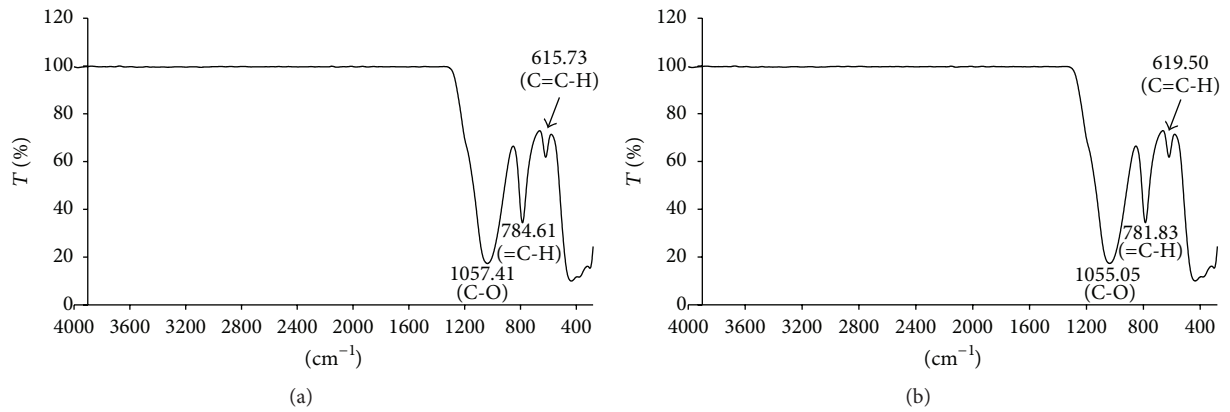


FIGURE 1: The FTIR spectra of RHA (a) and Fe-RHA (b).

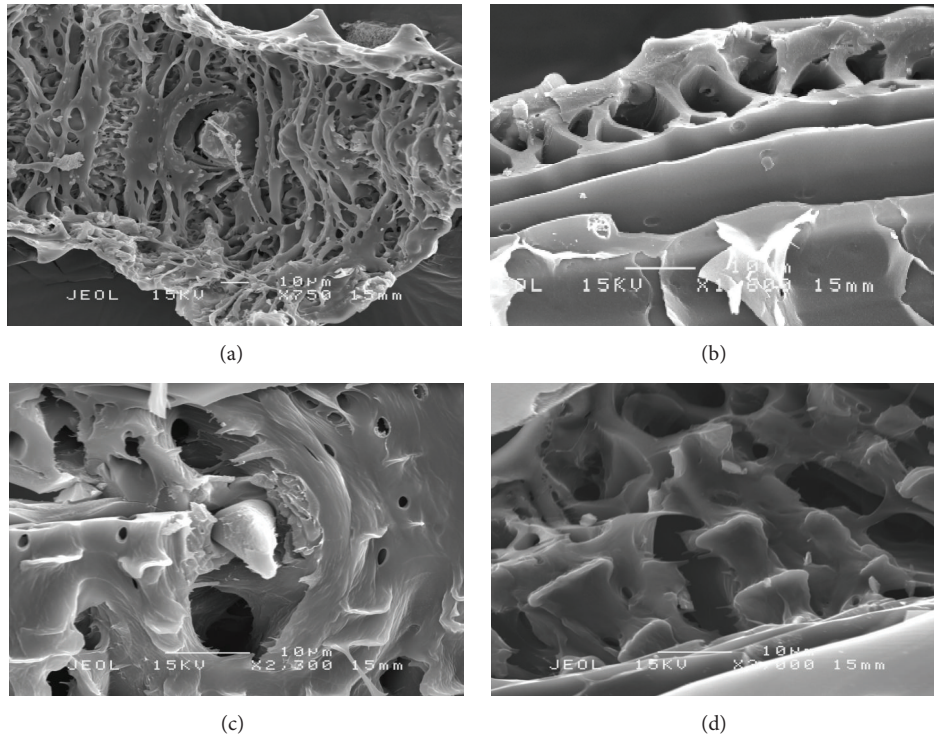


FIGURE 2: The scanning electron microscope images of the RHA magnified at 750 (a), 1600 (b), 2300 (c), and 3000 times (d). The accelerating voltage of the instrument was maintained at 15 kV.

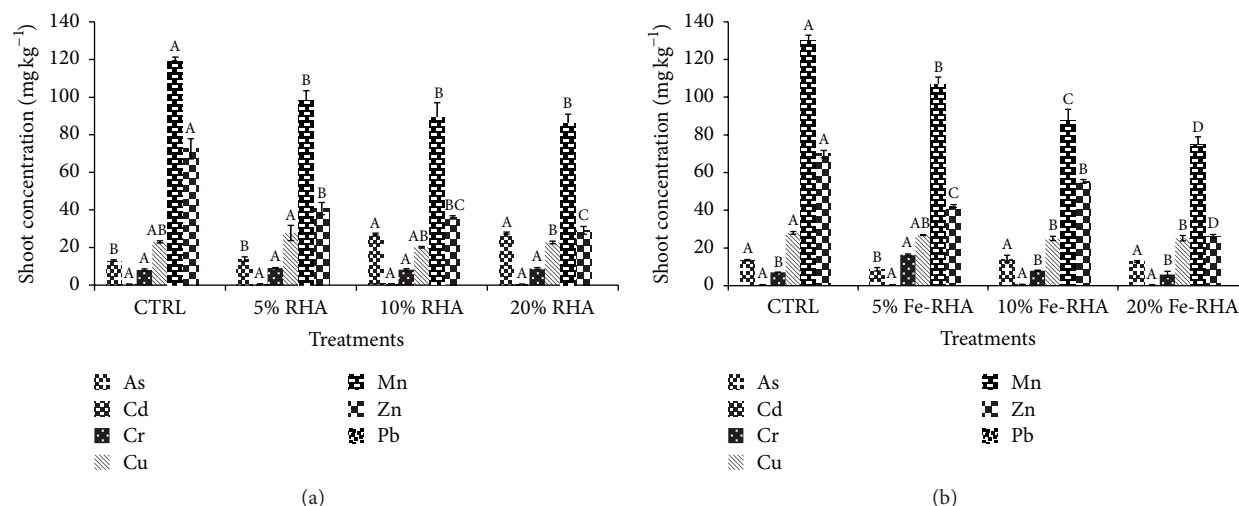


FIGURE 3: Concentration (mg kg^{-1}) of As, Cd, Cr, Cu, Mn, Zn, and Pb in shoot of vetiver grass grown in gold mine amended with (a) RHA and (b) Fe-RHA (mean $n = 3$, \pm SD). Different letters correspond to significant differences between rates of the same ash ($P < 0.05$). Pb was not detected.

3(b) show the concentration of metals in the shoot of the vetiver grass. The As concentration in all RHA amended samples, except at 5% application rate, was higher than the control (Figure 3(a)). The As concentration in all Fe-RHA treatments, except at 5% application rate, was not significantly different from the control, while it was significantly decreased in 5% Fe-RHA application rate (Figure 3(b)). The highest shoot concentration of As ($27.733 \text{ mg kg}^{-1}$) was recorded at 20% RHA. The concentrations of Mn in the shoot of vetiver grass grown in all RHA treated samples were significantly lower than the control. The lowest shoot concentration of Mn ($86.133 \text{ mg kg}^{-1}$) was recorded at 20% RHA (Figure 3(a)). The shoot concentrations of Cr and Cu in all RHA treated samples were not significantly different compared with the control (Figure 3(a)). However, the concentrations of Cr, Cu, and Mn in the shoot of vetiver grass grown in all Fe-RHA treated samples were significantly lower than the control except at 5% application rate for Cr and Cu in which the shoot Cr concentration was higher than control and the shoot concentration of Cu was not significant compared to the control. The lowest shoot concentrations of Cr (6.000 mg kg^{-1}), Cu ($25.000 \text{ mg kg}^{-1}$), and Mn ($74.867 \text{ mg kg}^{-1}$) were recorded in tailings amended with 20%, 10%, and 20% Fe-RHA, respectively (Figure 3(b)). The shoot concentrations of Cd in all RHA and Fe-RHA treated samples were not significantly different compared with the control. However, the shoot concentrations of Zn in all RHA and Fe-RHA treated samples were lower than the control. The lowest shoot Zn concentration ($26.267 \text{ mg kg}^{-1}$) was recorded at 20% Fe-RHA.

The two-way analysis of variance (ANOVA) procedure shows the significant ($P < 0.05$) effects of rice husk type and the interaction term of type \times rate on the concentration of As, Cr, Cu, and Zn in the shoot systems of the vetiver grass. However, the effects of rice husk type and the interaction term of rate and type were not significant ($P > 0.05$) on Cd and Mn shoot concentration of the vetiver grass. However,

the application rate of both RHA and Fe-RHA had a significant effect on the concentrations of all elements in the shoot of the vetiver grass.

The concentrations of As, Cd, Cr, Cu, Mn, and Zn in the roots of the vetiver grass are shown in Figures 4(a) and 4(b). The root concentrations of As in Fe-RHA and RHA amended samples were higher than the control (Figures 4(a) and 4(b)). The highest root As concentration ($267.400 \text{ mg kg}^{-1}$) was recorded at 20% RHA treated sample (Figure 4(a)). In contrast, the application of both Fe-RHA and RHA at all rates decreased the concentration of Cu in the root of vetiver grass compared with the control (Figures 4(a) and 4(b)). The lowest root Cu concentration (9.133 mg kg^{-1}) was recorded in 5% RHA treated sample.

In RHA treated samples, the Cd concentration in the roots decreased with the application rate. However, in Fe-RHA samples only the 5% Fe-RHA treated sample had a lower Cd concentration in the root compared with the control. In contrast, the roots Cr concentration in all RHA treated samples, except at 5% treated sample, increased compared with the control. The root Cr concentrations in Fe-RHA treated samples, except at 20%, were not significantly different from the control. The highest root Cr concentration (3.733 mg kg^{-1}) was recorded at 20% RHA treated sample. The application of Fe-RHA at all rates decreased the concentration of Mn in the root of vetiver grass compared with the control. However, RHA rates had different effects on Mn concentration in root. For instance, root Mn concentration at 5% RHA treated sample was lower than the control. However, the highest root Mn concentration ($69.067 \text{ mg kg}^{-1}$) was recorded in 10% RHA sample. The application of RHA at all rates increased the concentration of Zn in the roots of vetiver grass compared with the control. However, in Fe-RHA samples only the 5% Fe-RHA had a higher Zn root concentration than the control. The two-way ANOVA analysis for As, Cd, Cr, Cu, Mn, and Zn concentration in

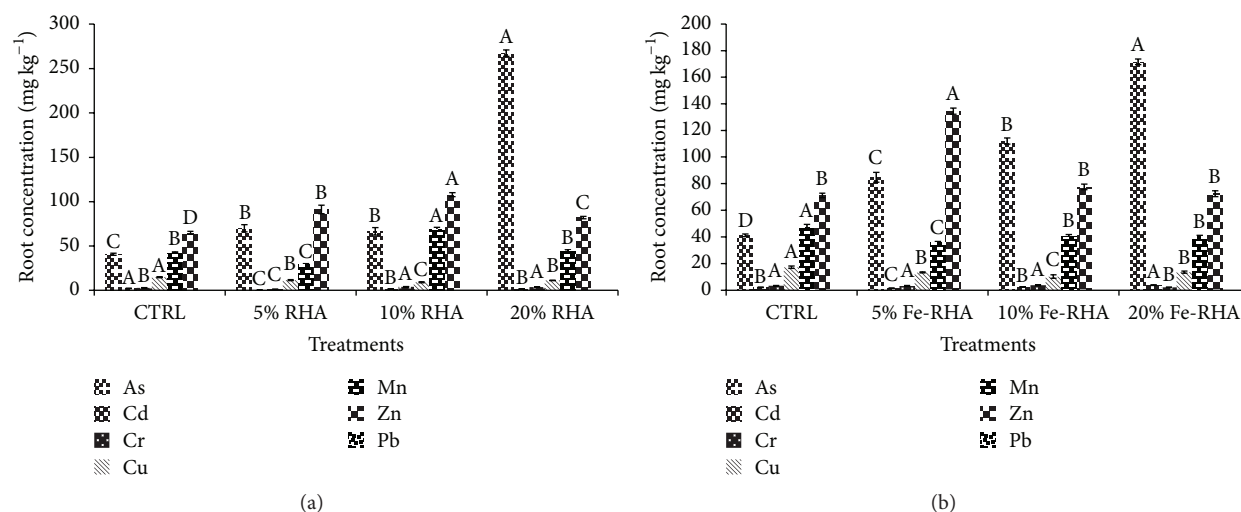


FIGURE 4: Concentration (mg kg⁻¹) of As, Cd, Cr, Cu, Mn, Zn, and Pb in root of vetiver grass grown in gold mine amended with Fe-RHA and RHA (mean $n = 3$, \pm SD). Different letters correspond to significant differences between rates of the same ash ($P < 0.05$). Pb was not detected.

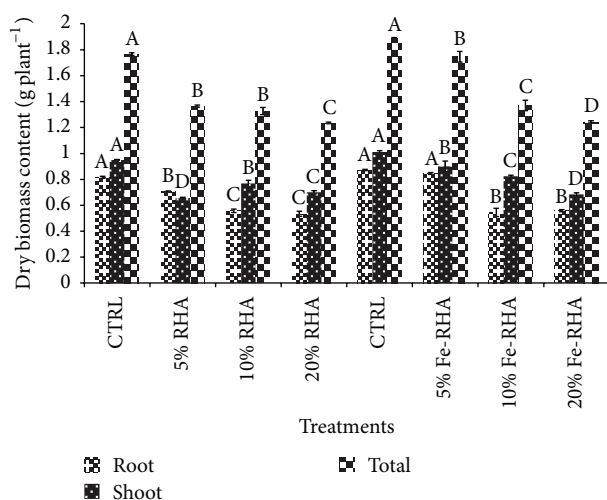


FIGURE 5: Shoot, root, and total dry biomass of vetiver grass grown in gold mine amended with Fe-RHA and RHA (mean $n = 3$, \pm SD). Different letters correspond to significant differences between rates of the same ash ($P < 0.05$).

the root system of the vetiver grass is shown. Rice husk ash type had significant ($P < 0.05$) effect on As, Cd, Cu, and Mn concentrations in the roots of the vetiver grass, but not for Cr and Zn. The two-way ANOVA results also revealed that both rates and the interaction term (type * rates) had a significant effect on the concentrations of all elements in the roots of the vetiver grass, except for Cu.

Figure 5 shows the root, shoot, and total plant dry biomass production of the vetiver grass. Vetiver grass grown in all Fe-RHA and RHA amended tailings had lower root, shoot, and total biomass production compared with the vetiver grass grown in the controls. The lowest dry root, shoot, and total biomass of the vetiver (0.535, 0.657, and 1.236 g plant⁻¹) were observed in 20%, 5%, and 20%

RHA amended samples, respectively (Figure 5). The ANOVA results show that type, rate, and interaction term (type * rate) of Fe-RHA and RHA had a significant effect ($P < 0.05$) on root, shoot, and total plant dry biomass production.

Figures 6(a) and 6(b) show the results of metal uptake in the shoot of vetiver grass grown in mine tailings amended with Fe-RHA and RHA. Amending the tailings with RHA at all rates, except at 5%, increased the shoot uptake of As in the vetiver grass compared with the controls. The highest As uptake (15.225 μ g plant⁻¹) was recorded at 10% RHA (Figure 6(a)). In contrast, application of Fe-RHA at all rates decreased As uptake in the shoot of vetiver grass (Figure 6(b)). In addition, the high rate (10% and 20%) of RHA and Fe-RHA Application reduced Cr, Cu, Mn, and Zn uptake in the shoot compared with the control. The lowest Cr (3.346), Cu (11.770), Mn (41.809), and Zn (14.697 μ g plant⁻¹) uptakes were observed in the samples amended with the 20% Fe-RHA, 10% RHA, 20% Fe-RHA, and 20% Fe-RHA, respectively. However, application of RHA and Fe-RHA at all rates had no significant effect on Cd uptake in the shoot compared with the control. The type of RHA had significant ($P < 0.05$) effect on shoot uptake of As, Cr, Cu, Mn, and Zn by the vetiver grass but not for Cd. However, both application rates and the interaction term (type * rate) had significant ($P < 0.05$) effect on the shoot uptake of all elements by the vetiver grass, except interaction for Cu.

Figures 7(a) and 7(b) also show metal uptake in the roots of vetiver grass grown in mine tailings amended with Fe-RHA and RHA. The root uptakes of As in RHA and Fe-RHA amended samples at all rates were higher than the control. The highest root As uptake (187.504 μ g plant⁻¹) was recorded in 20% RHA treated sample (Figure 7(a)) while the lowest root As uptake (38.723 μ g plant⁻¹) was recorded in the control. In contrast, the root uptakes of Cu in RHA and Fe-RHA amended samples at all rates were lower than the control. The lowest root Cu uptake (7.031 μ g plant⁻¹) was recorded at 10% RHA treated sample. The Cd and Cr uptake in the

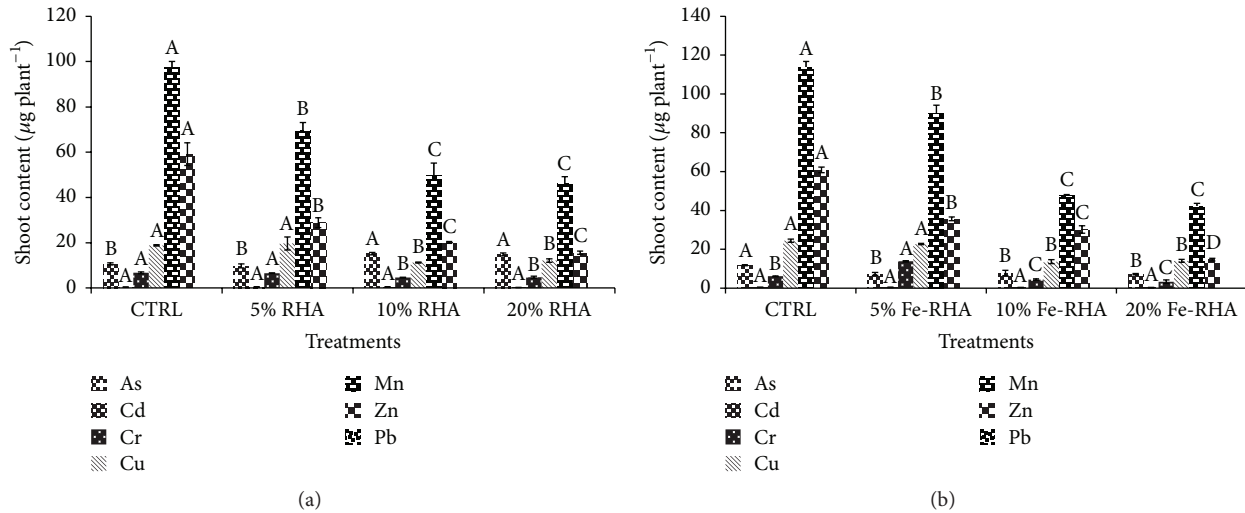


FIGURE 6: The uptake of As, Cd, Cr, Cu, Mn, Zn, and Pb ($\mu\text{g plant}^{-1}$) in shoot of vetiver grass grown in gold mine amended with Fe-RHA and RHA (mean $n = 3$, $\pm\text{SD}$). Different letters correspond to significant differences between rates of the same ash ($P < 0.05$). Pb was not detected.

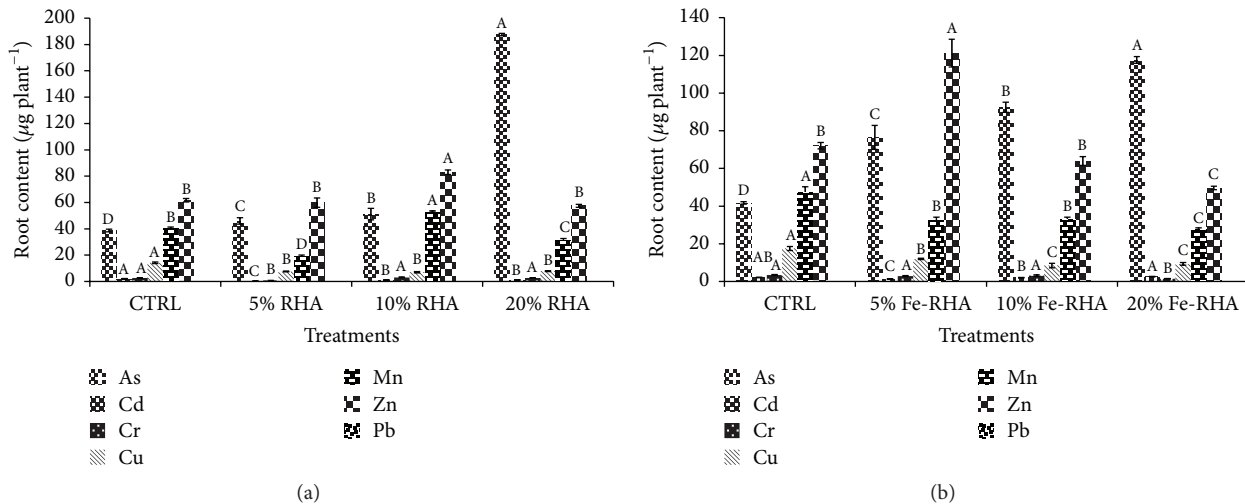


FIGURE 7: The uptake of As, Cd, Cr, Cu, Mn, Zn, and Pb ($\mu\text{g plant}^{-1}$) in root of vetiver grass grown in gold mine amended with Fe-RHA and RHA (mean $n = 3$, $\pm\text{SD}$). Different letters correspond to significant differences between rates of the same ash ($P < 0.05$). Pb was not detected.

root decreased with increasing RHA rates compared to the control. However, application of Fe-RHA at all rates had no significant effect on Cd and Cr uptake in the root, except 5% Fe-RHA for Cd and 20% Fe-RHA for Cr when they had a lower uptake in the root compared with the control (Figure 7(b)). Similarly, the application of either Fe-RHA or RHA at all rates, except the 10% RHA, decreased the root uptake of Mn of vetiver grass compared with the control. The two-way analysis of variance (ANOVA) procedure shows the significant ($P < 0.05$) effects of rice husk type on the uptake of Cd, Cr, Cu, and Zn in the root systems of the vetiver grass but not for As and Mn. However, both application rates and the interaction term (type * rate) had significant ($P < 0.05$) effect on the root uptake of all elements by the vetiver grass.

The total plant metals uptakes of vetiver grass grown in mine tailings amended with Fe-RHA and RHA are presented

in Figures 8(a) and 8(b). The amount of heavy metals uptake by vetiver grass was altered among the treatments. The total As uptake in vetiver grass grown in both Fe-RHA and RHA treated samples at all rates was higher compared with the control. The highest As uptake ($202.342 \mu\text{g plant}^{-1}$) was recorded in 20% RHA treated sample (Figure 8(a)). But, the application of RHA and Fe-RHA at all rates decreased the total plant uptake of Cu and Mn compared with the control and the lowest Cu (18.307) and Mn ($69.465 \mu\text{g plant}^{-1}$) uptakes were recorded at 10% RHA and 20% Fe-RHA, respectively. In addition, the application of RHA at all rates reduced the total vetiver grass uptake of Cd, Cr, and Zn compared with the control. The lowest Cd (0.643), Cr (7.304), and Zn ($73.103 \mu\text{g plant}^{-1}$) uptakes were recorded by plants grown in 5%, 5%, and 20% RHA, respectively. However, in Fe-RHA samples, only plants grown in the 5% Fe-RHA had lower Cd

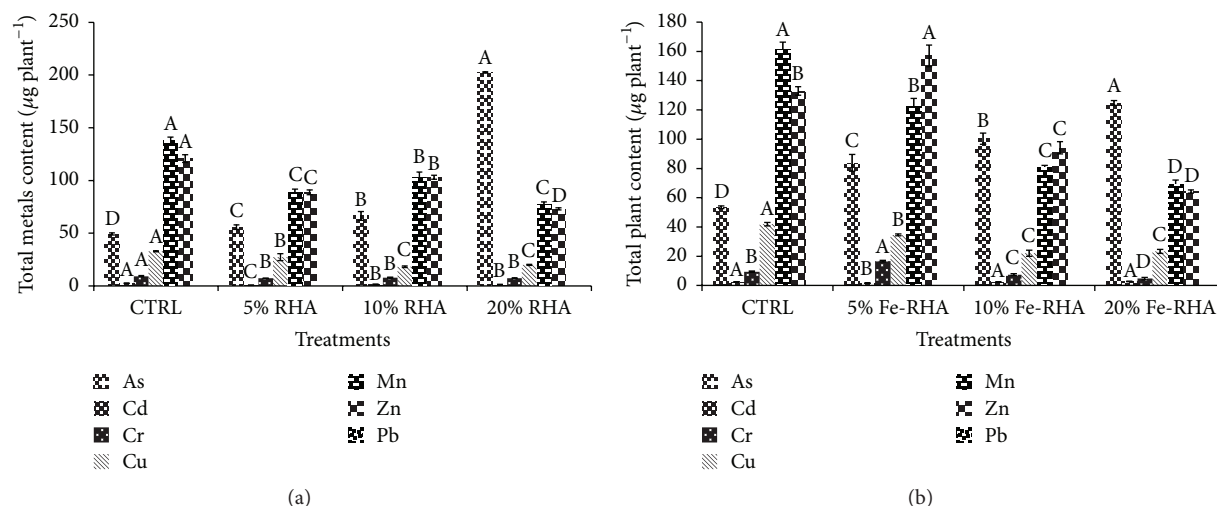


FIGURE 8: Total As, Cd, Cr, Cu, Mn, Zn, and Pb uptake ($\mu\text{g plant}^{-1}$) by vetiver grass grown in gold mine amended with Fe-RHA and RHA (mean $n = 3$, $\pm\text{SD}$). Different letters correspond to significant differences between rates of the same ash ($P < 0.05$). Pb was not detected.

uptake compared with the control. In contrast, the Cr and Zn uptakes by plants at 5% Fe-RHA treated samples were higher compared with control (Figure 8(b)). However, the total uptake of Cr and Zn by the vetiver grass decreased in 10% and 20% Fe-RHA treated samples. The application of type, rate, and the interaction term (type * rate) of RHA had significant ($P < 0.05$) effect on the total plant uptake of all elements by the vetiver grass.

Reduction in root, shoot, and total dry biomass of vetiver grass grown in samples treated with RHA and Fe-RHA might be due to the increase in As uptake and other metals since the elements are toxic and could inhibit plant growth when present at high concentrations. Arsenic has direct toxic effect on the biochemical and physiological processes in the plant. When it is translocated to the shoot, As resulted in a decrease of plant growth and biomass accumulation by severely inhibiting plant growth and slowing or arresting expansion [34, 35]. Eissa et al. [36] studied the phytoextraction of Ni, Pb, and Cd from contaminated soils using different field crops and EDTA treatment and they found that the root and shoot dry weights decreased by more than 25% due to the increase in metal plant uptake as a result of EDTA treatment.

Higher heavy metal uptake by the application of RHA on the gold mine tailings might be due to the presence of dissolved organic carbon (DOC). Previous studies found the positive correlations between mobility and availability of As and Cu with DOC [37]. The DOC affects the mobility of metals and metalloids through either the formation of soluble organic complexes or competition between the DOC and the metals for binding sites on the soil surface [38].

3.4. The Biological Accumulation Coefficient (BAC), Biological Transfer Coefficient (BTC), and Bioconcentration Factor (BCF) of the Vetiver Grass Grown in Mine Tailings Amended with RHA and Fe-RHA. The BACs values of As, Cd, Cr, Cu, Mn, and Zn for vetiver grass grown in RHA and Fe-RHA amended tailings are shown in Table 5. The RHA treated samples at all

rates showed a higher BAC for As compared with the control. The highest BAC for As (0.025) was recorded at 20% RHA amended sample. The application of RHA at all rates did not significantly affect BACs value for Cd, Cr, and Mn compared with the control but the BAC value for the Zn was reduced with the application RHA at all rates. The lowest BAC value for Zn (0.393) was recorded at 20% of the RHA amended sample. In Fe-RHA amended samples, only the 5% treatment had lower BAC value for As compared with the control while there is no significant effect at all rates on BACs value for Cd and Cu. On the other hand, the BAC values of Mn and Zn were reduced by the application of Fe-RHA at all rates compared with the control. The lowest BAC values for Mn (0.118) and Zn (0.356) were both recorded in 20% Fe-RHA samples (Table 5).

The RHA mended samples showed higher BTC values for Cu at all rates compared with the control. The highest BTC for Cu (2.389) was recorded at 5% RHA treated sample, respectively. In contrast, the BTC values for As, Mn, and Zn were lower than the control except for 10% RHA for As and 5% RHA for Mn amended samples. The lowest BTC values for As (0.104), Mn (1.283), and Zn (0.334) were observed at 20%, 10%, and 10% RHA, respectively. The BTC values for Cr were lower than the control except for 5% RHA amended samples. It is also observed that there was no significant effect of RHA application on the BTC values of vetiver grass for Cd.

Meanwhile, in Fe-RHA amended samples, the BTC values for As, Mn, and Zn were lower than the control except at 5% Fe-RHA for Mn. The lowest BTC values for As (0.075), Mn (1.857), and Zn (0.363) were all observed in 20% Fe-RHA amended samples. In addition, there is no effect of Fe-RHA application at all rates on BTC value of Cd, Cr, and Cu except at 5% Fe-RHA for Cd and Cr and 10% Fe-RHA for Cu.

Table 5 also shows the BCF value for the elements in vetiver grass grown in tailings amended with RHA and Fe-RHA. The BCF values for As and Zn in RHA and Fe-RHA amended samples at all rates were higher than the control.

TABLE 5: Biological accumulation coefficient (BAC), biological transfer coefficient (BTC), and bioconcentration factor (BCF) of heavy metals by vetiver grass grown in the gold mine tailings amended with Fe-RHA and RHA (mean $n = 3$). Different letters correspond to significant differences between rates of the same ash ($P < 0.05$) in the same column.

	Type	Rate (%)	As	Cd	Cr	Cu	Mn	Zn
BAC	Control	0	0.009 ^c	0.011 ^a	0.315 ^a	0.493 ^b	0.174 ^a	0.858 ^a
	RHA	5	0.010 ^c	0.015 ^a	0.375 ^a	0.640 ^a	0.148 ^a	0.533 ^b
	RHA	10	0.020 ^b	0.017 ^a	0.343 ^a	0.422 ^b	0.129 ^a	0.436 ^c
	RHA	20	0.025 ^a	0.016 ^a	0.369 ^a	0.542 ^{ab}	0.137 ^a	0.393 ^c
	Control	0	0.009 ^a	0.009 ^a	0.257 ^b	0.619 ^a	0.200 ^a	0.816 ^a
	Fe-RHA	5	0.006 ^b	0.011 ^a	0.643 ^a	0.603 ^a	0.166 ^b	0.567 ^b
	Fe-RHA	10	0.011 ^a	0.015 ^a	0.314 ^b	0.564 ^a	0.134 ^c	0.739 ^c
	Fe-RHA	20	0.011 ^a	0.011 ^a	0.256 ^b	0.610 ^a	0.118 ^c	0.365 ^d
BTC	Control	0	0.321 ^b	0.211 ^b	3.089 ^b	1.554 ^b	2.802 ^a	1.119 ^a
	RHA	5	0.198 ^c	2.000 ^a	7.821 ^a	2.389 ^a	3.355 ^a	0.449 ^b
	RHA	10	0.411 ^a	0.532 ^b	2.054 ^b	2.215 ^a	1.283 ^c	0.334 ^b
	RHA	20	0.104 ^d	0.351 ^b	2.394 ^b	2.031 ^a	1.925 ^b	0.356 ^b
	Control	0	0.329 ^a	0.201 ^{ab}	2.153 ^b	1.631 ^b	2.782 ^a	0.981 ^a
	Fe-RHA	5	0.104 ^b	0.357 ^a	5.350 ^a	2.031 ^{ab}	2.957 ^a	0.314 ^d
	Fe-RHA	10	0.127 ^b	0.273 ^{ab}	2.183 ^b	2.497 ^a	2.193 ^b	0.715 ^b
	Fe-RHA	20	0.075 ^c	0.112 ^b	2.927 ^b	1.867 ^b	1.857 ^c	0.363 ^c
BCF	Control	0	0.027 ^c	0.051 ^a	0.102 ^b	0.318 ^a	0.062 ^c	0.770 ^c
	RHA	5	0.051 ^b	0.008 ^c	0.049 ^c	0.267 ^a	0.044 ^d	1.192 ^{ab}
	RHA	10	0.050 ^b	0.032 ^b	0.167 ^a	0.191 ^b	0.100 ^a	1.307 ^a
	RHA	20	0.243 ^a	0.044 ^{ab}	0.155 ^a	0.267 ^a	0.071 ^b	1.108 ^b
	Control	0	0.027 ^d	0.045 ^b	0.119 ^{ab}	0.380 ^a	0.072 ^a	0.832 ^c
	Fe-RHA	5	0.062 ^c	0.032 ^c	0.120 ^{ab}	0.296 ^{ab}	0.056 ^{ab}	1.806 ^a
	Fe-RHA	10	0.084 ^b	0.056 ^b	0.145 ^a	0.229 ^b	0.061 ^b	1.035 ^b
	Fe-RHA	20	0.145 ^a	0.101 ^a	0.088 ^b	0.327 ^a	0.064 ^{ab}	1.005 ^b

The highest BCF values of As (0.243) and Zn (1.806) were recorded by 20% RHA and 5% Fe-RHA samples, respectively. The application of RHA at all rates except at 20% reduced BCF value for Cd compared with the control and the lowest BCF value for Cd (0.008) was recorded at 5% RHA. The application of 10% and 20% RHA increased the BCF values for Cr, while at 5% RHA the values decreased compared to the control. However, for Cu only the 10% RHA amended sample had lower BCF value compared with the control. Samples treated with Fe-RHA at lower rates had lower BCF values for Cd than the control but, at 20% Fe-RHA, the BCF value was higher than the control. The application of Fe-RHA did not significantly affect the BCF values of Cr, Cu, and Mn at all rates, except at 10% Fe-RHA for Cu and Mn.

A plant is a hyperaccumulator when the heavy metal concentration in its stem and leaf is 10–500 times higher than in plants grown in nonpolluted area and enrichment coefficient is greater than 1 [39]. In general, it can be concluded that the success of a heavy metal-phytoextraction process depends on the ability of the plant to move the metals quickly from the root to the aerial parts of the plant [40]. Though the concentrations of metals were high in the shoot system of the vetiver grass, the BAC values for all heavy metals were less than 1 (Table 5), and this may be attributed

to the very high concentration of contamination in gold mine tailings because the BAC value is the ratio of concentration from the tailings to the shoots.

The mobility of heavy metals from the roots to the shoots is reflected by the BTC values. The results show that the BTC values for Cd, Cr, Cu, Mn, and Zn were greater than 1, while the BTC values for As were less than 1. The BTC values suggest higher concentrations of Cd, Cr, Cu, Mn, and Zn in the shoots than the roots system of the vetiver grass, while for As, the opposite was true. Also, BTC and BAC results indicated the ability of vetiver grass to absorb Cd, Cr, Cu, Mn, and Zn and they were rapidly transferred to shoot. In contrast, the results of As clearly showed the affinity of roots to accumulate a good amount of metals from the tailings but low transfer to the aerial parts. The BCF indicates root metal concentration divided by the tailings concentration. The value was used to explain the uptake of vetiver in roots. The BCF values for all metals except Zn were less than 1 (Table 5) which considered normal because the values demonstrated the ability of vetiver to accumulate a good amount of metals in the root. This might be due to variation in heavy metal concentration and plant species. The BCF values reflected the negative effect of adding RHA to uptake of heavy metals by the vetiver. The overall results indicate that the vetiver

grass can grow in the gold mine tailing containing high concentrations of As, Cd, Cr, Cu, Mn, Pb, and Zn and can accumulate the elements in its roots and shoots.

4. Conclusions

It can be concluded from this study that the vetiver grass was tolerant to high concentrations of As, Cd, Cr, Cu, Mn, and Zn in the tailings. Application of RHA and Fe-RHA reduced the phytoavailability of Cd, Cr, Cu, Mn, and Zn. This may be important during the early part of a phytoremediation project whereby establishment of plant species on tailings containing very high concentrations of heavy metals is difficult. On the other hand, both amendments especially the RHA can also be used to increase the phytoavailability of As. Therefore, the RHA and Fe-RHA can be used in a phytoremediation project to either reduce the phytoavailability of cationic elements like Cd, Cr, Cu, Mn, and Zn or increase the phytoavailability of anionic elements like As. We recommend that field experiments should be carried out to confirm the results obtained in this glass house study.

Competing Interests

The authors declare that they have no competing interests.

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