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Simultaneous bioaccumulation and translocation of iron and aluminium from mining wastewater by *Scirpus grossus*

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ABSTRACT

Iron (Fe) and aluminium (Al) contamination due to mining activities has increased considerably and became a serious environmental problem. Phytoremediation is an emerging green technology that uses plants to treat heavy metal contaminated environment. In this study, *Scirpus grossus* was exposed to synthetic mining wastewater (binary mixture of Fe and Al with a mass ratio of 3:1) to assess its ability to phytoremediate Fe and Al with different treatment concentrations (90 mg/L Fe + 30 mg/L Al – 450 mg/L Fe + 150 mg/L Al). The plants were exposed for 102 d in a subsurface batch system. The results show that the *S. grossus* accumulated Fe and Al simultaneously in biomass throughout the study. The maximum accumulations of Fe and Al were found on Day 42 in the plant roots (50,277 mg/kg Fe in 450 mg/L Fe + 150 mg/L Al treatment and 7,744 mg/kg Al in 300 mg/L Fe + 100 mg/L Al treatment). The bioaccumulation factor and translocation factor of *S. grossus* were found to be greater than 1 and less than 1, respectively, for the two metals, indicating that this species is a hyperaccumulator that uses phytostabilization in the phytoremediation of Fe and Al.

Keywords: Phytoremediation; Bioaccumulation; Translocation; Heavy metals; Mining wastewater; *Scirpus grossus*

1. Introduction

Mining results in the extraction of metals, metalloids and other minerals. During the extraction, substantial quantities of mine waste are generated. It has been reported that approximately several thousand million tons of wastes are produced per annum at present and that this volume is increasing exponentially as demand and exploitation of lower-grade deposits increases [1]. Mine waste dissolution can lead to problematic drainage (one of the sources for mining wastewater), which strongly requires remediation and its quality depends on mine waste composition and time. Mine waste dissolution can occur through various pathways, for example, acid generation, acid neutralization, trace metals released by trace metal sulphide oxidation and key solid-phase factors controlling mineral oxidation and dissolution [2].

Dissolution of mine waste leads to acidic metal-rich mine drainage, often called as acid mine drainage (AMD), and is usually net acidic and sometimes extremely acidic [3]. The characteristics of AMD have been reported by researchers at different mining locations and it was found that the AMD are acidic and the pH can be as low as pH 0.5 [4–11]. Based on the data reported by them, AMD also contains

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high content of heavy metals especially Fe and Al. When this drainage reaches receiving water bodies, for example, lakes, streams or aquifers, the waters can cause undesirable turbidity and sedimentation, may alter temperatures or chemical compositions and may have toxic effects on plants and animals, including humans [12]. These risks can be minimized by utilizing suitable management or remediation strategies. One of the emergent and innovative remediation strategies to remediate AMD is by applying constructed wetlands through phytoremediation technology to treat the contaminated water [13].

Phytoremediation technology is seen to be one of the most potential and promising treatments for treating both organic and inorganic contaminants [14] due to the unique, selective and naturally occurring uptake capabilities of plant root systems and other processing abilities of the entire plant body, such as translocation, bioaccumulation, pollutant storage and/or degradation [15]. Phytoremediation can be defined as the use of plants to reduce, remove, degrade or immobilize environmental toxins with the aim of removing contaminants and heavy metals from contaminated soil or water, thereby restoring specific sites to a condition that is usable for private or public applications [16,17]. It is cheaper than other physical, chemical or thermal remediation methods because it can be performed in situ, is solar driven (and therefore does not require the consumption of fossil fuels), can function with minimal maintenance and requires less attention [15,18]. In phytoremediation process, the bioaccumulation factor (BF) and translocation factor (TF) values are important in evaluating whether a particular plant can be classified as a metal accumulator and also in determining the mechanism involved during the phytoremediation process, that is, phytostabilization, phytoextraction, phytovolatilization or rhizofiltration [19].

In spite of the fact that many studies on phytoremediation of a contaminated medium using different species of plants and contaminants have been reported [20–26], to the best of our knowledge, limited information is available regarding the phytoremediation potential of *Scirpus grossus* for binary mixture of metals, especially Fe and Al. Therefore, in this study, a native emergent tropical plant, that is, *S. grossus*, was used to determine the phytoremediation potential with respect to Fe–Al synthetic mining wastewater representing AMD. Therefore, this study measured the potential accumulation (mg/kg) of Fe and Al by *S. grossus* and calculated the BF and TF values to determine the potential type of accumulator for this species and the potential mechanism involved during phytoremediation of binary Fe and Al mixture using *S. grossus*.

2. Materials and methods

2.1. Selection of plant species and heavy metals

An earlier assessment was done near a mining area to select plant species and types of heavy metals for this study [27]. Based on the assessment, *S. grossus* was selected due to its ability to accumulate high concentrations of Fe and Al compared with other plants while synthetic mining wastewater containing binary mixture of Fe and Al (mass ratio was approximately 3:1) was used to simulate the mining wastewater.

S. grossus grows rapidly and can reach 2 m in length, with brown fibrous roots. The stems are triangular, with a spongy interior structure. The spikelet is very numerous, brown, ovoid and 5 to 8 mm long. It is a perennial emergent aquatic plant with the common names of giant bulrush, greater club-rush, rumput menderong (Malaysia), mensiang and walingi (Indonesia) [28]. These plant species efficiently treat domestic sewage [29,30], media contaminated with lead [28] and diesel [31] but has not yet been applied to a mixture of metals.

2.2. Selection of binary Fe–Al mixture concentrations

A preliminary test was performed to determine the range of concentrations of Fe and Al mixture that *S. grossus* has the ability to survive and tolerate [32]. These concentrations were then used during this study. After 21 d of exposure, the plants could grow and survive in concentrations up to 300 mg/L Fe + 100 mg/L Al. Therefore, in this study, the concentrations selected were 90 mg/L Fe + 30 mg/L Al, 150 mg/L Fe + 50 mg/L Al, 300 mg/L Fe + 100 mg/L Al and 450 mg/L Fe + 150 mg/L Al, together with a plant control (i.e., without the addition of Fe and Al). The plants exposed to the concentration of 450 mg/L Fe + 150 mg/L Al were selected to evaluate the phytotoxicity effects of *S. grossus* at higher concentrations of Fe and Al with the hypothesis; greater toxic effects on the plant would decrease the bioaccumulation potential of *S. grossus*.

2.3. Experimental setup of the subsurface batch system

This study was conducted under greenhouse conditions at an average temperature of 33°C ± 2.5°C at the Universiti Kebangsaan Malaysia (UKM) using a subsurface batch system for 102 d of exposure. For this study, 17 solid plastic (high-density polyethylene, HDPE) crates, each with dimension of 58 cm × 39 cm x 29.5 cm ($L \times W \times H$) were used as batch reactors. The treatment concentrations used were T1 = 90 mg/L Fe + 30 mg/L Al, T2 = 150 mg/L Fe + 50 mg/L Al, T3 = 300 mg/L Fe + 100 mg/L Al and T4 = 450 mg/L Fe + 150 mg/L Al.

The experiment was performed in triplicate with contaminant controls (CC1 was contaminant control for T1, CC2 was contaminant control for T2, CC3 was contaminant control for T3 and CC4 was contaminant control for T4) and a plant control (PC). The contaminants controls included the dedicated contaminant concentration but without plants, acting as an indicator for the performance of phytoremediation of Fe–Al binary mixture. In contrast, the plant control (PC) included plants but with no contaminants. This control acted as an indicator of plant growth in normal conditions for comparison with the plants in the crates with Fe–Al binary mixture. About 12 healthy plants of *S. grossus* (42 d old and second generation) were planted in T1–T4 and 8 L of synthetic mining wastewater containing Fe and Al mixture was poured into each dedicated crate.

Each crate was filled from the bottom layer to the upper layer with the following: (1) 3 cm of gravel with diameter size (ϕ) of 10–20 mm, (2) 2 cm of gravel with ϕ 1–5 mm and (3) 10 cm of fine sand with approximate ϕ 2 mm. Sand was used to minimize the nutrient content so that the direct

toxicity effect of the heavy metals on the plants can be directly observed. This was proven by the analysis done by Titah et al. [33] at which the sand contents for macronutrients were 29.2 mg/kg N (nitrate), 1.2 mg/kg K, 13.0 mg/kg SO_4^{2-}, 86.5 mg/kg Ca, 7.4 mg/kg Mg while the micro nutrient was 6.4 mg/kg, 5.5 mg/kg Fe, 0.04 mg/kg Zn and 1.62 mg/kg Mn. No additional nutrients were added during the exposure period.

The synthetic Fe–Al binary mixture of mining wastewater was prepared by mixing tap water with Fe and Al salts. The salts used were iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) (Friendemann Schmidt, UK) and aluminium sulphate ($Al_2(SO_4)_3{\cdot}16H_2O$) (R & M Marketing, UK). Prepared synthetic Fe–Al mining wastewater was added only at the beginning of the experiment (batch experiment). The watering process was performed on average once every 2 d with 0.5–1.0 L of tap water without Fe and Al was added to each treatment crates to maintain the subsurface system.

2.4. Sampling of effluent, sand and plants

Throughout the exposure period, effluent, sand and plants were collected on day 1, 7, 14, 42, 72 and 102. Effluent was collected from effluent sampling point. About 100 mL of effluent being sampled in a 100 mL clean plastic bottle from each crate. Samples of sand were collected as a composite sample as defined by Patil [34] for each crate, including PC, CC and treatments with plants. As for plant, one plant was taken out from each crate on each sampling day.

2.5. Physicochemical analysis of effluent

The physicochemical properties of the effluent, the temperature (T, °C), pH, dissolved oxygen (DO, mg/L) and oxidation–reduction potential (ORP, mV) were measured. The pH, ORP and T were measured using an IQ 150 multiprobe (IQ Scientific Instruments, Spectrum Technologies, Plainfield, USA), while the DO was measured using a dissolved oxygen sensor (GLI International, Model 63, USA). The effluent were filtered using a 0.45 μm cellulose nitrate membrane filter (Whatman, Germany) before it was sent for Fe and Al analysis using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 7300DV, PerkinElmer, USA). The filtered water samples were kept at 4°C before further analysis using an ICP-OES was done.

2.6. Plant dry weight and relative growth rate

All the plant samples were dried in a drying oven (MMM Venticell 404 Comfort Laboratory Oven, Germany) at 70°C to a constant weight to determine the dry weight [35]. The RGR of the plants was calculated to describe the plant growth responses to contaminants using Eq. (1) [36,37]:

$$\mathrm{RGR} = \frac{\ln W_2 - \ln W_1}{\mathrm{days}} \left(\mathrm{g\,g^{-1}d^{-1}}\right) \quad (1)$$

with W_2 final dry biomass (g) of *S. grossus* and W_1 represent initial dry biomass (g) of *S. grossus.*

2.7. Extraction of bioavailable Fe and Al from sand and total Fe and Al from plant

The bioavailability of Fe and Al in sand was determined using the EDTA extraction method, described by Holleman and Willberg [38] and Quevauviller [39]. Approximately 0.05 mol/L EDTA extraction solution was prepared by dissolving 18.61 ± 0.05 g $EDTANa_2$ (Merck, Germany) with 800 ± 20 mL distilled water, and the solution pH was adjusted to pH 8 using a NaOH solution (Merck, Germany). The volume of the solution was brought up to 1 L with distilled water. To determine the bioavailability of Fe and Al, 5 g sand sample was transferred to a centrifuge tube of 50 mL. Approximately 50 mL of 0.05 mol/L EDTA solution was added. The mixture was shaken using a programmable rotator–mixer Multi RS-60 (Biosan, Latvia) at 30 rpm for 1 h at 20°C ± 2°C. Then, it was centrifuged for 10 min at approximately 3,000 rpm. Filtration through filter paper (porosity of 0.2–1.1 μm) (Whatman, Germany) was performed to obtain a clean supernatant sample. This sample was stored in a polyethylene container at 4°C for further analysis using an ICP-OES.

Total Fe and Al were extracted from the plants (roots and shoots-stems and leaves) using procedures modified from Kalra [40] and Tangahu et al. [28]. Approximately 0.1–1 g of dried and grinded sample were added to a digestion tube. Then, 10 mL of 69% HNO_3 (R&M Chemicals, India) was added to the sample and covered with a watch glass. This mixture was left overnight. Then, the sample was heated in a block digester (AIM 600 Digestion System, Australia) to 95°C for 1.5 h. After 1.5 h, it was allowed to cool to 80°C before the addition of 8 mL of 30% H_2O_2 (R&M Chemicals, India). It was reheated to 95°C for 2 h. Then, 2.5 mL of aqua regia (HNO_3:HCl = 1:3) and then, deionized water were further added to achieve a volume of 50 mL. Filtration through filter paper (porosity of 0.45 μm) (Whatman, Germany) was performed to obtain a clean extracted sample for further analysis using an ICP-OES.

2.8. Analysis of Fe and Al contents in effluent, sand and plant

An Optima 7300 DV (PerkinElmer) ICP-OES was used with an auto-sampler. The instrument was operated with the computer software WinLab32 for ICP (Version 4.0.0.0305). The accumulation of both Fe and Al metals for *S. grossus* (plant roots and shoots) was calculated using Eq. (2).

$$\text{Metal accumulation}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right) = \frac{C_M\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) \times V_E(\mathrm{L})}{M_P(\mathrm{kg})} \quad (2)$$

with C_M = the total concentration of Fe or Al analysed by ICP-OES in plant, M_P = the mass of plant (either plant roots or shoots) and V_E = the extraction volume (0.05 L).

2.9. Bioaccumulation factor and translocation factor

The BF is defined as the ratio of the metal concentration in the plant roots to that in the soil/water medium, while the TF is the ratio of the metal concentration in the plant shoots to that in the roots [41]. The BF and TF values can be

calculated using Eqs. (3) and (4), respectively. Four categories of heavy metal accumulation are proposed by Sekabira et al. [42] based on the BF values: <0.01 as non-accumulator plants, 0.01–0.1 as low accumulator plants, 0.1–1 as moderate accumulator plants, and 1–10 as high accumulator/hyperaccumulator plants. High TF ratios (TF > 1) represent considerable translocation of metals from the bottom tissues (roots) to the upper tissues (shoots) of a plant [43].

$$\mathrm{BF} = \frac{\text{Metal concentration in plant roots } \left(\frac{\mathrm{mg}}{\mathrm{kg}}\right)}{\text{Metal bioavailable concentration in sand } \left(\frac{\mathrm{mg}}{\mathrm{kg}}\right)} \quad (3)$$

$$\mathrm{TF} = \frac{\text{Metal concentration in plant shoots } \left(\frac{\mathrm{mg}}{\mathrm{kg}}\right)}{\text{Metal concentration in plant roots } \left(\frac{\mathrm{mg}}{\mathrm{kg}}\right)} \quad (4)$$

2.10. Statistical analysis

The experimental results were statistically evaluated using IBM SPSS Statistics Version 21 (IBM, USA). Paired *t*-tests were used to determine the significance of the difference in Fe and Al accumulations between the plant roots and shoots. Statistical significance was defined as $p < 0.05$. The total fresh and dry weight of *S. grossus* (dependent variables) and the Fe and Al concentrations and time (independent variables) were analysed using the general linear model test with Tukey's multiple range tests to separate means.

3. Results and discussion

3.1. Variation in physicochemical parameters during exposure

Physical and chemical parameters for effluent such as temperature, pH, ORP and DO are recorded for 102 d of exposure for plant control (PC) and treatment with plants (T1, T2, T3 and T4). The temperature and pH during the study did not show a significant difference between PC and treatment with plants. Temperature range for PC and treatment with plants is 23.96°C ± 1.29°C. According to Yang and Simbeye [44], the optimum temperature for plant growth is between 20°C and 36°C. Whereas, the average value of pH is pH 7.07 ± 0.20 for PC and treatment with plants. The highest ORP values are on the first day with 62.00 ± 0.47 mV while the lowest is on day 102 with values of –72.10 ± 3.39 mV. According to Dabkowski [45], ORP value is in the anoxic zone when the value is in the range of –100 and 100 mV, while less than –150 mV is classified as an anaerobic zone. The anoxic condition indicated by the ORP reading was in line with the DO reading recorded for PC and treatment with plants that showed a decrease from 6.17 ± 0.05 mg/L on the first day of exposure to 0.86 ± 0.03 mg/L on the last day of exposure. According to Muda et al. [46] and USEPA [47], DO concentration in anaerobic condition is between 0.2 and 2.0 mg/L while in the anoxic condition occurs when the DO reading is less than 0.2 mg/L [48]. Therefore, it can be concluded based on the ORP value that the process involved in the study is heading towards the anoxic condition.

3.2. Plant dry weight and relative growth rate with respect to Fe and Al contamination

Fig. 1 depicts the dry weight of *S. grossus* during the 102-d study. The dry weight of the plants in all the treatments increased up to Day 72 and continued to increase until 102 d for PC and T1, with dry weights of 12.7 and 10.1 g, respectively. However, the dry weight of plants decreased after Day 72 for treatments T2, T3 and T4 due to the effects of Fe and Al toxicity. It is known that photosynthetic activity of plants can decrease due to metal toxicity [49,50]; thus reducing the growth and biomass of the plants. Although the dry weight of the plants in T1 continued to increase until the last day of exposure, it also experienced the effects of Fe and Al toxicity, as the dry weight of these plants was significantly different ($p < 0.05$) than that of the PC plants on Day 42 onwards. Therefore, it can be concluded that the inhibition of growth occurred in plants grown under TI, T2, T3 and T4. Based on the ANOVA results, the dry weight of *S. grossus* between PC and other treatments generally differed significantly ($p < 0.05$) on Day 102 of the study, indicating that the growth of *S. grossus* in the PC treatment was greater than that in all the other treatments.

Additionally, the growth effects of plants in response to Fe and Al can be seen in the plot of RGR in Fig. 1 at which the value of RGR indicates the health of plants during a treatment period. The RGR values for PC, T1, T2, T3 and T4 were 0.0209, 0.0165, 0.0154, 0.0134 and 0.0136 g g^{-1} d^{-1}, respectively. Therefore, the RGR decreased as the concentration of contaminants increased indicating that the growth of *S. grossus* was affected by the contaminants in all the treatment (T1, T2, T3 and T4).

3.3. Bioavailable Fe and Al in the sand

Fig. 2 shows the Fe and Al bioavailable concentrations in the sand in the controls and the experimental treatments under normal greenhouse conditions. The differences in bioavailable concentrations of Fe and Al in sand for crates with and without plants was analysed with two-way analysis of variance (ANOVA). The analysis was done to determine the interaction between models (with plants and without plants) within the same treatment concentrations (using letters A and a) as illustrated in Fig. 2. The bioavailable concentrations of Fe and Al decreased significantly with time, model (with and without plants) and concentrations of synthetic mining wastewater. Furthermore, there was a statistically significant interaction between (model × time), (model × concentration), (time × concentration) and (model × time × concentration) both for bioavailable Fe and Al. For any given heavy metal, only a portion is generally bioavailable and can be taken up by plants [51]. Letter A-a represent statistically significant difference at $p < 0.05$ in Al concentrations (mg Al/kg) between sand from crates with plants (T1–T4) and crates without plants (CC1–CC4). The decreases in bioavailable Fe and Al concentrations in the sand are due to the uptake by the plants, resulting in the accumulation of both metals in *S. grossus* tissues. Other researchers have also experienced

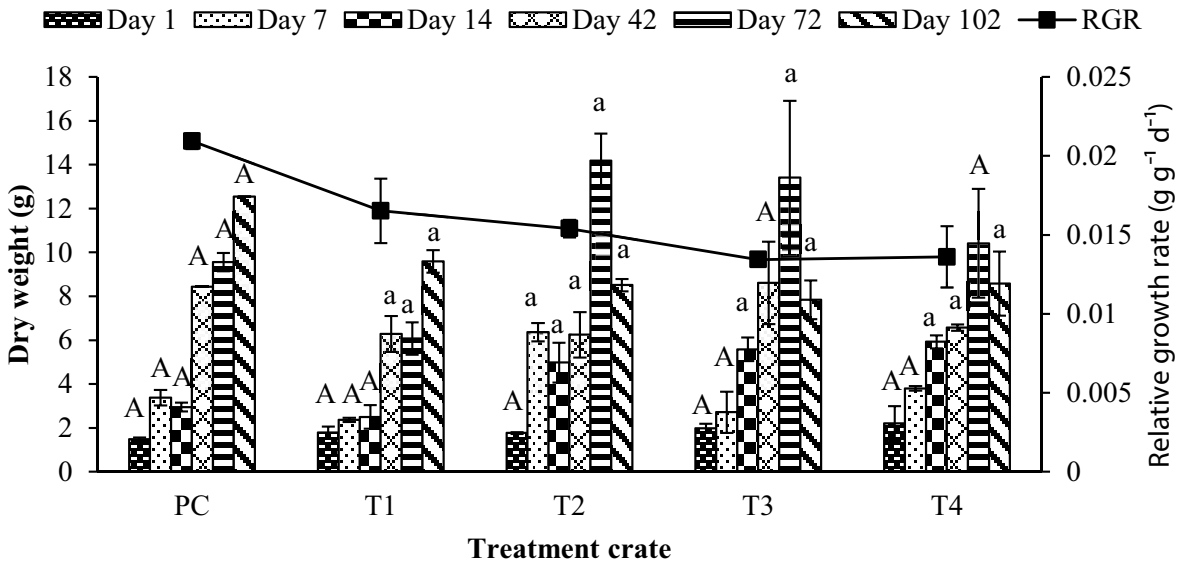

Fig. 1. Dry weights and the relative growth rate (RGR) of *S. grossus*. Vertical bars indicate ± S.D. of three replicates. Letter A-a represent statistically significant differences at $p < 0.05$ in dry weight (g) in control when compared with other treatments (i.e., T1, T2, T3 and T4) at the same sampling time; A-A represent no significant differences in dry weight (g) in control when compared with other treatments (i.e., T1, T2, T3 and T4) at the same sampling time.

similar decreasing metal bioavailability in the sand for arsenic phytoremediation with *Ludwigia octovalvis* [52].

3.4. Accumulation of Fe and Al in the plants

The accumulation of both Fe and Al in the plant roots and shoots of *S. grossus*, throughout the 102 d of the study was illustrated in Figs. 3a and b, respectively. The accumulation of Fe and Al in both parts of *S. grossus* was analysed with two-way analysis of variance (ANOVA). The analysis was done; (1) for interaction between metals (Fe and Al) within the same treatment concentrations (using letters A and a) and (2) for Fe accumulation (mg Fe/kg) and Al accumulation (mg Al/kg) between two consecutive days within the same treatment concentrations (using letters B and b). There was a statistically significant interaction between (metals × time), (metals × concentration), (time × concentration) and (metals × time × concentration) both in plant roots and shoots. Based on the ANOVA, the accumulation of Fe in plant roots of *S. grossus* showed significant difference ($p < 0.05$) than that of Al within all treatments (i.e., T1, T2, T3 and T4). This might be due to the mass ratio of Fe to Al (i.e., 3:1).

Additionally, Fe is accumulated at greater rates than Al since it is required as an essential micronutrient [53] required by plants at low concentrations for growth [54]. Fe is necessary for chlorophyll synthesis and is a component of cytochromes and ferredoxin [55]. High Fe concentrations and strongly acidic conditions can result in Fe toxicity [56]. It can be physically observed that the toxicity effects occurred predominantly in *S. grossus* grown in T4 (450 mg/L Fe + 150 mg/L Al) with some withering effects on the plants. From Fig. 3a, as the treatment concentrations increased, the accumulation of Fe increased progressively until Day 42. There was significant difference ($p < 0.05$) in the plant shoots between Fe accumulation on Day 14 and Day 42 in T3 and T4, while Al accumulation showed significant difference only in T2 and T3.

The maximum Fe accumulation in this study was found during Day 42 in the plant roots of the *S. grossus* grown in T4 with 50,277 mg Fe /kg, and the maximum Al accumulation was found in the plant roots grown in T3 with 7,744 mg/kg Al. Although the biomass of *S. grossus* in T4 was lower than that of the other treatments (Fig. 1), the uptake of Fe was the greatest (Fig. 3a). This is due to Fe can be transported into the roots by diffusion from a region of high concentrations to a region of lower concentrations (Fe was taken up by the plant at the root surface) [57].

Based on the statistical analysis, the plant roots had significantly more metal than that in plant shoots, indicating that the translocation of Fe from roots to shoots was ineffective. According to Hochmuth [57], Fe is taken up by plant roots in greater amounts in the root zone between cell elongation and maturation, approximately 1 to 4 cm behind the root tip. It is absorbed into the rhizodermal (epidermal) cells and endodermal cells in the root. From the endodermis, Fe is transported into the pericycle cells and then to the xylem. Most of the Fe transported to the shoots ends up in the shoot apoplast. From there, it can be moved across the cell plasmalemma and into the cytoplasm and into organelles. However, Fe is relatively immobile once incorporated into compounds in the plant shoots [57]. Re-translocation of Fe from one shoot tissue or plant part to another is not easy.

3.5. BF and TF values

The BF and TF values for all the treatments are shown in Table 1. The values of BF in all the treatments are higher than 1 both for Fe and Al. In contrast, the TF values in all the treatments are less than 1 for both Fe and Al. Thus, *S. grossus* is a hyperaccumulator. For mechanism involved

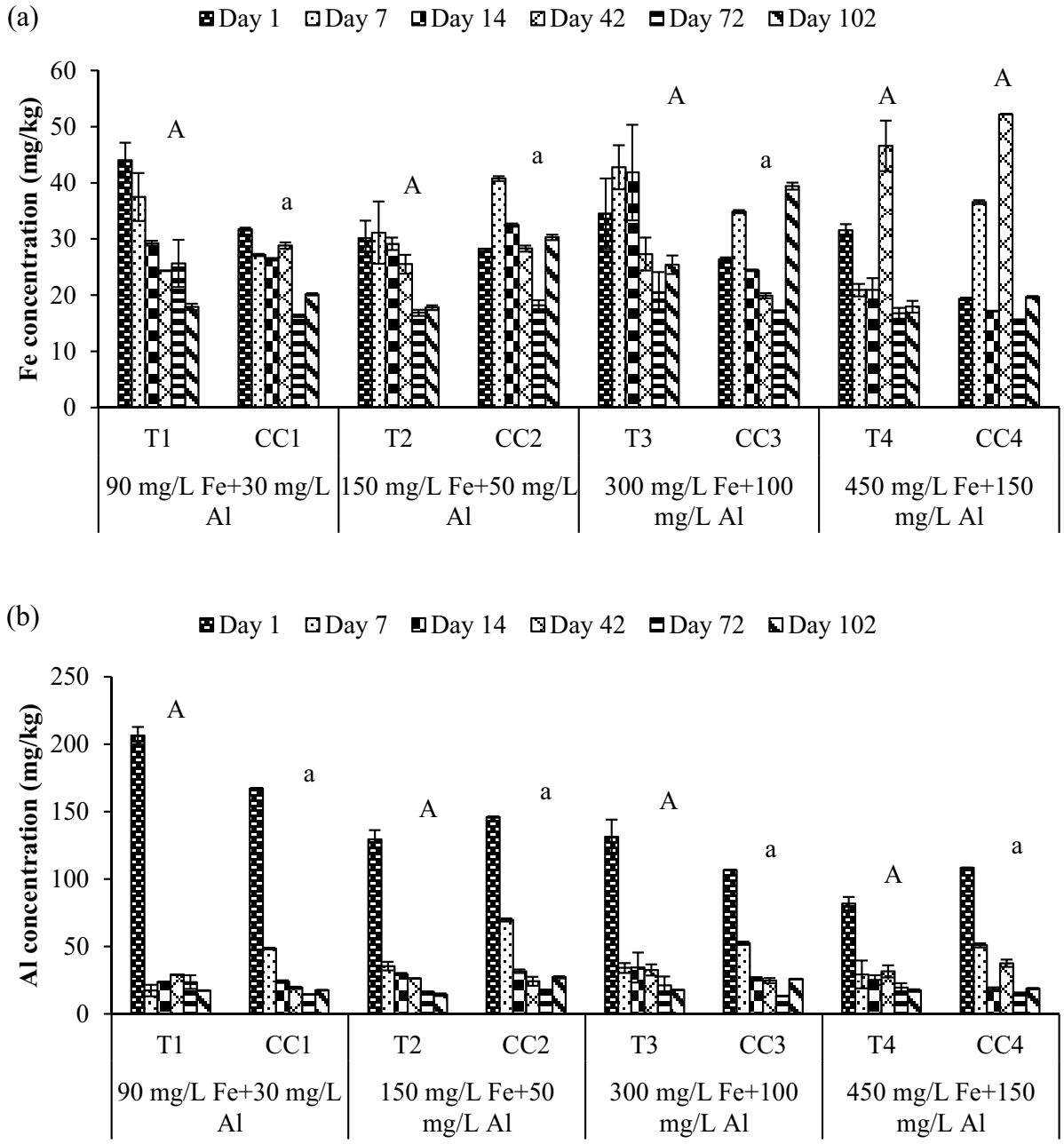

Fig. 2. (a) Fe bioavailable concentration and (b) Al bioavailable concentration in sand from crates with plants (T1–T4) and crates without plants (CC1-CC4). Vertical bars indicate ± S.D. of three replicates. Letter A-a represent statistically significant difference at $p < 0.05$ in Fe concentrations (mg Fe/kg) between sand from crates with plants (T1–T4) and crates without plants (CC1–CC4).

during phytoremediation, plants with both BF and TF values greater than unity have the potential to be used in phytoextraction [58] whereas plants that accumulate heavy metals in their roots, with little or no translocation to plant shoots, can be considered to perform a phytostabilization process [59] resulting in BF values greater than unity and TF values less than unity. Therefore, in this study, *S. grossus* performed the phytostabilization process during phytoremediation of Fe and Al.

Other researchers have identified different species of plants that have the ability to phytoremediate contaminated media. A list of these species is provided in Table 2. Some of the species were identified as hyperaccumulator plants (BF = 1–10) for Fe, including *Populus alba* L., *Populus nigra* L. and *Azolla caroliniana*, whereas others, including *S. grossus* and *Typha domingensis*, were identified as hyperaccumulator plants for both Fe and Al. From Table 2, it can be seen that the BF of Fe for *S. grossus* is much higher than that of *A. caroliniana*, that is, 337.0 to 972.0 vs. 1.7 to 18.6, respectively. *Populus alba* L. *and Populus nigra* L. have TF values that are greater than 1, indicating high degrees of translocation of Fe from stem to leaves, whereas *S. grossus* and *T. domingensis* have

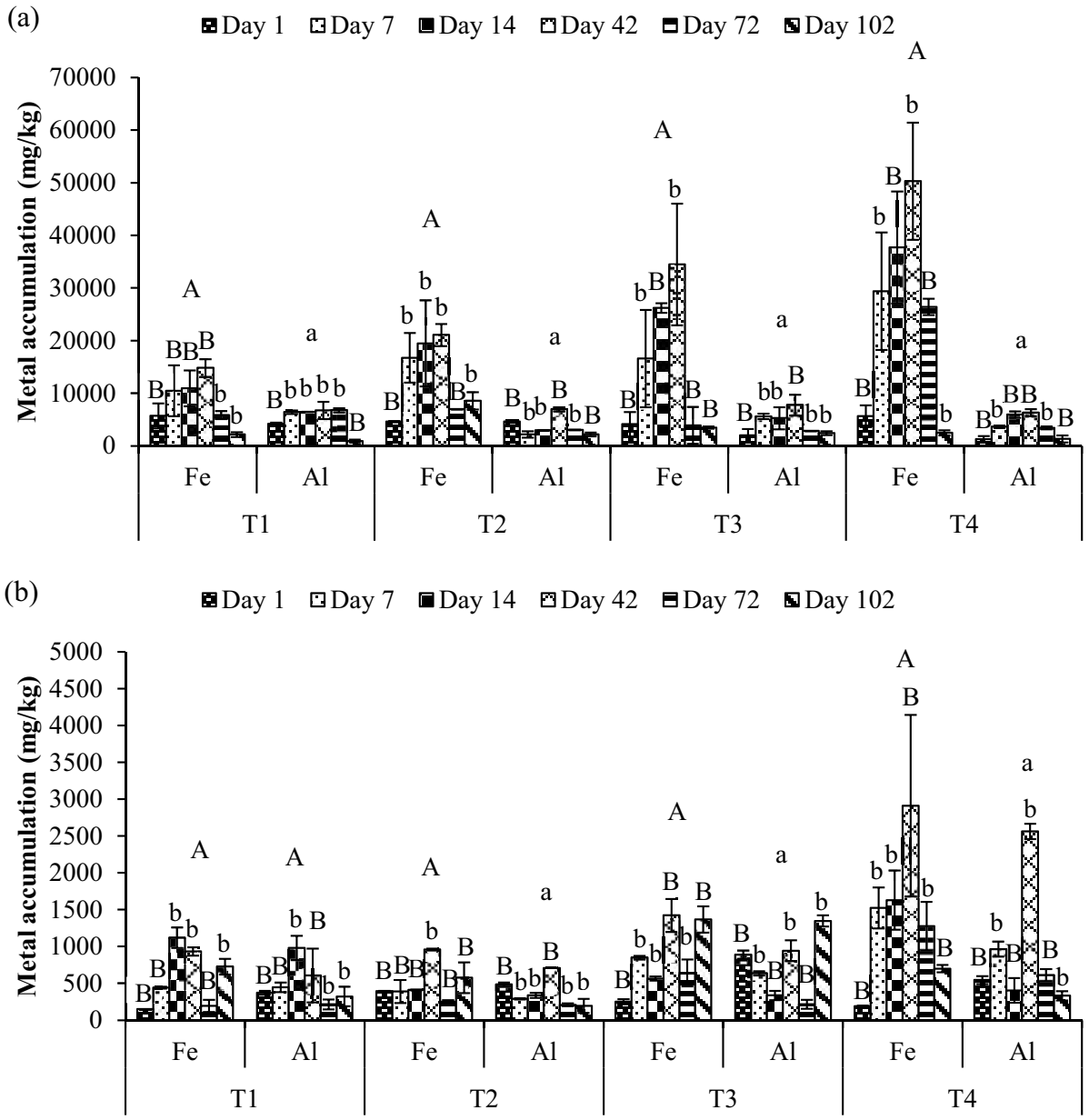

Fig. 3. Accumulation of Fe and Al: (a) accumulation of Fe and Al in the roots of *S. grossus* and (b) accumulation of Fe and Al in the shoots of *S. grossus*. Vertical bars indicate ± S.D. of three replicates. Letter A-a represent statistically significant differences at $p < 0.05$ in Fe accumulation (mg Fe/kg) when compared with Al accumulation (mg Al/kg) within the same treatment concentrations. Letter B-b and b-B represent statistically significant differences at $p < 0.05$ in the Fe accumulation (mg Fe/kg) and Al accumulation (mg Al/kg) between two consecutive days within the same treatment concentrations.

Table 1
Bioaccumulation factor (BF) and translocation factor (TF) values for both metals

Treatment	BF		TF	
	BF for Fe	BF for Al	TF for Fe	TF for Al
T1	338	153	0.11	0.20
T2	592	123	0.05	0.24
T3	518	532	0.07	0.07
T4	972	155	0.12	0.33

Table 2
Fe and/or Al hyperaccumulator plants

Plant species	Type of metal	Description on BF	Description on TF	Reference
Scirpus grossus	Iron and aluminium	BF values for Fe and Al in root ranged from 337.7 to 972.0 and 123.0 to 531.6 for Al, respectively.	TF values for Fe and Al ranged from 0.05 to 0.12 and 0.07 to 0.33, respectively.	This study
Chyrsopogon zizanioides (L.) Roberty	Iron and aluminium	BF values for both metals were less than 1 in roots and shoots.	TF values for both metals were less than 1 in roots and shoots.	[60]
Populus alba L. *and Populus nigra* L.	Iron	–	TF (i.e., stems to leaves) for *P. alba* and *P. nigra* were 5.670 and 53.206, respectively.	[61]
Azolla caroliniana	Iron	BF values in roots and fronds ranged from 1.7 to 18.6 and from 1.8 to 1.10, respectively.	TF ranged from 0.37 to 1.4 for various heavy metals.	[62]
Typha domingensis	Iron	–	Low translocation ratios make it suitable for metal ion phytostabilization	[63]
Typha domingensis	Iron and aluminium	BF values between roots and wastewater and shoots and wastewater were greater than 1.	–	[64]

low translocation values, indicating low degrees of translocation of metals from one part of the plants to another.

4. Conclusion

Exposing the *S. grossus* in synthetic mining wastewater containing a binary mixture of Fe and Al showed that Fe and Al were highly accumulated in roots rather than in shoots. It accumulated the greatest amount of Fe (50,277 mg/kg Fe) and Al (7,744 mg/kg/Al). BF values for Fe and Al meet the standard value for hyperaccumulators. The BF and TF values of *S. grossus* were found to be greater than 1 and less than 1, respectively, for both Fe and Al, indicating that the mechanism involved in phytoremediation of Fe and Al was phytostabilization rather than phytoextraction at which it prevents the distribution of both metals to the other area.

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