Improving Capacity For Phytoremediation Of Vetiver Grass And Indian Mustard In Heavy Metal (Al And Mn) Contaminated Water Through The Application Of Clay Minerals

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Abstract

Phytoremediation of areas contaminated by heavy metals using Vetiver grass and Indian mustard is cost-effective and environmentally friendly. However, strategies to optimize the remediation capacity of these plants to be more sustainable in combatting environmental pollution is lacking. This study aimed at enhancing remediation of heavy metal contaminated water through the simultaneous hybrid application of clay minerals (attapulgite and bentonite) and Vetiver grass or Indian mustard. A 21-day greenhouse experiment was carried out to investigate the effectiveness of the clay minerals to improve heavy metal phytoremediation. Attapulgite successfully improved the growth and tolerance index of Vetiver grass in heavy metal contaminated water. The addition of clay minerals did not, however, increase the tolerance of Indian mustard for Al and Mn. The best efficiency was from a hybrid system of 2.5% (w/v) bentonite treatment with Vetiver grass. However, Indian mustard showed no significant uptake of heavy metals, but suffered heavy metal toxicity despite the addition of clay minerals. The current laboratory-scale findings provided a basis for field trials earmarked for remediation in a post-mining coal environment in South Africa.

Highlights

- Attapulgite and bentonite assisted phytoremediation of Vetiver grass for Al and Mn.
- Bentonite (2.5% w/v) improved phytoremediation capacity of Vetiver grass for Al.
- Attapulgite (2.5% w/v) improved Vetiver grass tolerance index for Al and Mn.
- Clay minerals did not increase Indian mustard's tolerance for Al and Mn.

1. Introduction

Water is a scarce resource in most parts of the world and when affected by pollution, the proper ecosystem functioning and suitability for use diminishes. The rising human population drives industrialization, mining, agriculture, and poor sewage management, which then become major water resource pollutants (Danh et al., 2009). Of the various contaminants, heavy metals are constantly released into the environment from a multitude of anthropogenic sources, posing a risk to human and environmental health (Danh et al., 2009; Beniah Obinna and Ebere, 2019). The main sources of heavy metals pollutants are mining, manufacturing and processing industries, sewage, solid wastes, urban runoff, fuel leakages (WHO, 2017). Humans can readily ingest heavy metals by consuming contaminated water and aquatic biota (Beniah Obinna and Ebere, 2019).

Increased levels of trace metals in rivers are associated with mining and other industrial activities (Ali et al., 2018). For example, in South Africa, industrial effluents that end up in the Vaal River contained 14 mg/l Al, 56.7 mg/l Zn and 4.6 mg/l Pb, which are toxic at concentrations above 0.01 mg/l for Pb and 0.1 mg/l for Zn and Al (Iloms et al., 2020). Another example is the Leeuspruit River in proximity to a former coal mine which records concentrations of 2.72 mg/L and 5.4 mg/L for Mn and Al respectively (Wessels, 2013). Al and Mn are among the most abundant elements in the earth's crust. Al in nature is from weathered aluminosilicate rocks and minerals (Wang *et at.*, 2013). It is useful in various sectors and essential in production of many domestic products. There is evidence that solubilized Al in toxic amounts negatively affects plants, animals and human beings. E.g., root growth inhibition in plants, nervous disorders and Alzheimer's disease in humans (Wang et al., 2013). Likewise, Mn is applied in production of various materials such batteries, glass, fireworks, fertilisers, cleaning products and cosmetics (WQA, 2021). Excess Mn levels in human beings can result to several health issues such as neurological disorders, low IQ in children and low coordination and movement control (Wang et al., 2013). In Ecuador, elevated levels of Mn (970 µg/L) was detected in the Puyango River and children in proximity to this river had over 2 µg/g in their hair. This was found to be responsible for neurobehavioral disorders and low IQ among these children (Betancourt et al., 2015).

Over the years, the ecological and human health concerns of heavy metals have given rise to innovative solutions to rid them from contaminated sites and water bodies. One of the most accepted methods is phytoremediation, because it is affordable, and easily applied green technology whose by products can be used for other purposes such as bioenergy, essential oils and animal feed (Sricoth et al., 2018; Yang et al., 2019; Edgar et al., 2021). However, wider application of phytoremediation is inhibited by various challenges, including low biomass yield, extreme climatic influence, slow plant growth, long time required for remediation, pollutant-specific requirements, and adverse effects of contaminants on plant functions (Danh et al., 2009; Mioska, 2012; Shahid et al., 2020; Leng et al., 2021; Sharma, 2021).

Vetiver grass (*Chrysopogon zizanioides)* is notable in water remediation, because of its excellent physiological and morphological properties, which enable growth in contaminated substrates and under harsh climatic conditions (Truong and Hartm, 2001; Danh et al., 2009; Koupai et al., 2020). Kiiskila et al. (2019) observed that Vetiver removed Ni, Zn, sulphate, Mn, Cr, Al and Cu by 38%, 35%, 28%, 27%, 21%, 11 and 8% respectively from acid mine drainage. The authors noted up to 81% removal of Fe and Pb within a year. Indian mustard (*Brassica juncea*) is also recognized as a good plant for phytoremediation (Qadir et al., 2004; Rehana *et al.*, 2012; Raj et al., 2020), although studies on

water remediation in the field are limited. Some studies on the phytoremediation capacity of Indian mustard have confirmed its ability to survive and absorb heavy metals with concentrations as high as 50 ppm in substrates (Meyers et al., 2008; Singh and Fulekar, 2012; Napoli et al., 2019). Singh and Fulekar (2012) observed that Indian mustard absorbed 25,000 ppm, 32,750 ppm and 30,550 ppm of Cd, Pb and Zn from water and soil, respectively, after 21 days of exposure. Raj et al. (2020), remediated Hg contaminated fly ash in pot experiments using Indian mustard for up to 90 days with a heavy metal accumulation of up to 2.62 mg/kg, mostly in the roots followed by leaves and stems. Based on the bioconcentration factor (range 0.1–1), Indian mustard is classified as a moderate Hg accumulator (Raj et al., 2020).

Recently, clay minerals and nanoparticles have received attention for application in the remediation of contaminated soil and water (Otunola and Ololade, 2020; Hussain et al., 2021). A review by Paz-Ferreiro et al. (2014) confirmed the use of several soil amendments such as biochar and compost in combination with hyperaccumulators for better remediation results. The approach of using immobilisers to improve phytoremediation has been tested in the laboratory showing increased phytoextraction of Pb and Sb, up to 533 times higher than phytoextraction alone without amendments (Katoh et al., 2016). Clay minerals play an important role is soil fertility because the soil structures depend on the proportion and types of clays present. These affect organic matter, nutrient and water retention capability of soil. Clays also buffer pH changes, and control soil microbial activities (Kome et al., 2019). In water, clays can control pH and provide adsorptive properties for heavy metals and other nutrients (Otunola and Ololade, 2020). These properties of clay minerals can improve phytoremediation (Salimizadeh et al., 2020).

In pursuit of sustainable solutions to environmental pollution and the associated challenges of phytoremediation, the present study's aim was to optimize the remediation of heavy metal contaminated water through a hybrid application of phytoremediation (using Vetiver grass and Indian mustard) and clay minerals (attapulgite and bentonite). In particular, the study investigated the impact of attapulgite and bentonite at two dosage levels (1% and 2.5%) on the growth and phytoremediation potential of Vetiver grass and Indian mustard in water. These dosage levels were chosen, because low clay dosages (between 0.5 and 8 g/kg) were effective in previous research (Zotiadis and Argyraki, 2013; Otunola and Ololade, 2020). Attapulgite and bentonite were selected for this study, because of their potential to adsorb and eliminate heavy metals from polluted water (Otunola and Ololade, 2020) and their capacity to serve as amendments that could improve soil properties and alleviate heavy metal toxicity in plants heavy metals (Salimizadeh et al., 2020; Otunola and Ololade, 2020). This experiment was carried out to evaluate the heavy metal uptake by Vetiver grass and Indian mustard under the influence of bentonite and attapulgite to decide the best treatment for small-scale field experiments. This application will be undertaken to develop a suitable solution for the remediation of heavy metals in a post-mining environment in Sasolburg, South Africa.

2. Materials And Methods

i. Water sampling

The study area is around a former coal mining area located in Sasolburg, Free State Province, South Africa. Mining operations were stopped in 2006 and the area is now at the rehabilitation and reclamation stage. Previous monitoring of this area established that the Leeuspruit River (26°50'16.1"S 27°48'42.3"E), one of the major water bodies in the area is polluted by nutrients and heavy metals including Al and Mn, emanating from mining as well as post-mining land-use activities (Wessels, 2013).

Physicochemical water parameters, including pH, temperature, electrical conductivity (EC), and total dissolved solids (TDS) were measured on-site using a calibrated standard multi-parameter probe (YSI Incorporated, Model 85D, I.N058500, SN 09K 100684, Yellow Springs, Ohio, USA). To determine the heavy metal concentrations, water samples were collected in triplicate from four sites along the course of the river, based on land use patterns and suspected pollution sources. Clean 500 mL polyethylene bottles were rinsed three times with the river water before samples were collected and stored in cooler boxes with ice. The samples were transported to the Institute for Groundwater Studies at the University of the Free State for heavy metal and nutrient analyses.

ii. Plant preparation

Vetiver grass (*C. zizanioides)* was supplied by Hydromulch (Pty) Ltd. Johannesburg, South Africa. The plants were thoroughly rinsed to remove soil particles and other possible contaminants and then trimmed to similar shoot and root lengths of 30 and 15 cm, respectively. Indian mustard (*B. juncea*) seeds were supplied by Seeds for Africa, South Africa. These were propagated in seedling trays using Hygrotech seedling starter composed of N (17.2%), P (7.1%), K (2.3%), Ca (0.8%), Mg (0.2%), Fe (785 mg/kg), Mn, Zn and Cu (398 mg/kg), B (204 mg/kg), and Mo (6.6 mg/kg). The seedlings were kept moist in a greenhouse at the Department of Soil, Crop and Climate Sciences, University of the Free State. Thirty-day-old seedlings of similar sizes were thoroughly rinsed and used for the experiment.

iii. Experiment set up

A 21-day randomized complete block design hydroponic experiment was set up in a greenhouse facility at the University of the Free State, South Africa. Pots were maintained under temperatures of 28°C (day) and 20°C (night) and exposed to natural light. The treatment codes and descriptions are presented in Table 1. This study seeks to determine the best treatment using a hybrid of clay minerals and plants, nutrient water was spiked with Al (5 mg/l) and Mn (1 mg/l), based on the concentrations found in the Leeuspruit River (Table 2). Plastic pots of 1 L were used to hold 800 mL contaminated water. These were covered with lids that had holes and wrapped with aluminium foil to minimize the effects of sunlight. The plants were placed over the water (Figure 1). The pots were refilled to the initial volume (800 mL) with prepared water each time the water levels were reduced through evaporation, transpiration and consumption by the plants.

Table 1
Water treatment codes and conditions

Treatment Code	Conditions
Control (Zero treatment)	Prepared water
AT1	Attapulgite Applied at 1% (w/v)
AT2.5	Attapulgite Applied at 2.5% (w/v)
BT1	Bentonite applied at 1% (w/v)
BT2.5	Bentonite Applied at 2.5% (w/v)
VT	Vetiver only (one plant per pot)
BJ	Indian mustard only (one plant per pot)
AT1VT	Attapulgite + Vetiver applied at 1% (w/v)
AT2.5VT	Attapulgite + Vetiver applied at 2.5% (w/v)
BT1VT	Bentonite + Vetiver applied at 1% (w/v)
BT2.5VT	Bentonite + Vetiver applied at 1% (w/v)
AT1BJ	Attapulgite + Vetiver applied at 1% (w/v)
AT2.5BJ	Attapulgite + Vetiver applied at 2.5% (w/v)
BT1BJ	Bentonite + Vetiver applied at 1% (w/v)
BT2.5BJ	Bentonite + Vetiver applied at 2.5% (w/v)
VTC	Vetiver only in nutrient water
BJC	Indian mustard only in nutrient water

Figure 1: Experimental setup of the various treatments at a greenhouse facility, University of the Free State, South Africa.

i. Plant harvesting and processing

At the end of 21 days, the plants were harvested and carefully rinsed with water. The length and weight of the roots and shoots of the plants' fresh biomass were recorded. The plant sections were then separately oven-dried at 75°C for 72 hours. The dry biomass of each plant was weighed, recorded and the tolerance index (TI) was calculated as follows (Equation 1):

$TI = DBcont/DBucont$ (1)

Where DBcont is the total dry biomass in the contaminated medium and DBucont is the total dry biomass in the uncontaminated medium (Beniah Obinna and Ebere, 2019).

The translocation factor (TF), which is the ability of a plant to translocate metals from its roots to shoot was calculated as (Equation 2):

$TF = Hm\ conc\ in\ shoot/Hm\ conc\ in\ root$ (2)

Where Hm are the heavy metals (Beniah Obinna and Ebere, 2019).

ii. Plant sample digestion and analysis

The dried root and shoot samples were milled and digested using microwave-assisted digestion by nitric acid. Briefly, 0.5 g of homogenized powdered plant sample was weighed and transferred into a microwave vessel; 15 mL nitric acid was added based on a modification of US EPA method 3051 (US EPA, 2007; Sastre et al., 2002). The vessel was covered, de-pressured and placed in a microwave for 25 mins (Ramp for 10 mins). After cooling, the digestate was transferred into 250 mL volumetric flasks, diluted, and filtered through a 0.45 syringe filter. To determine the heavy metal uptake levels in the plant roots and shoots, the digested samples were analysed using a Prodigy7 ICP-OES Spectrometer (Teledyne Leeman Labs) at the Analytical Laboratory, Chemistry Department, University of the Free State.

iii. Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) to determine the effect of clay treatments on heavy metal adsorption. The calculations were performed using R software version 4.0.0 (R Development Core Team 2020) at a significance level of 0.05. Pearson Correlation Coefficient test was used to confirm the relationship between roots and shoots uptake.

3. Results And Discussion

i. Physicochemical properties and heavy metals

The physicochemical properties and heavy metal values of the water samples for each sampling site along the Leeuspruit River were compared with the In-stream Water Quality Guidelines for the Leeuspruit Catchment (In-stream WQG, 2021) (Table 2). The pH values of the water samples ranged from 6.02 ± 0.01 at RIV2 to 7.22 ± 0.1 at RIV3, indicating the pH of the Leeuspruit was close to neutral. The highest temperature was 29.8 ± 0.1°C at RIV3 while the average temperature of the river was 26.38°C. All the pH and temperature values were within the Leeuspruit Catchment Water Quality Limits. The mean values of EC and SO_4^{2-} were 104.18 mS/m and 91.26 mg/L, respectively. There was a high variation in the TDS values among the sample points, which ranged from 240 ± 0.70 mg/L to 965 ± 0 mg/L. The possible sources of the dissolved solids may have been from the dissolution of underlying sedimentary rocks or runoff from agricultural land (Fondriest Environmental, 2014). Based on the Leeuspruit Catchment Water Quality Guidelines, ideal EC values were exceeded in all the sites except for RIVC (Table 2). The lowest PO_4^{3-} value was 0.24 ± 0.35 mg/L from RIVC, which were within the acceptable limits of the Leeuspruit Catchment Water Quality Guidelines. The highest PO_4^{3-} values of 2.06 ± 0.61 mg/L was from RIV4, which exceeded this limit by 1.66 mg/L. High phosphate levels can promote eutrophication, lowering overall water quality (Mezgebe et al., 2015). The SO_4^{2-} concentrations ranged from 13.7 ± 0.41 mg/L to 238.7 ± 1.44 mg/L (RIV3), with a mean value of 91.26 mg/l, indicating mine water pollution. The ICP-OES results revealed that Mn ranged from 0.14 ± 0.003 to 0.54 ± 0.001 mg/L while Al ranged from 0.33 ± 0.01 to 4.58 ± 0.002 mg/L. The result for other heavy metals such as Cd, As, Co, Cr, Mo, and Cu were below detection limits. Al and Mn with concentrations of ~ 5 mg/L and 1 mg/L respectively were of importance in this research.

ii. Tolerance index (TI) and visual symptoms

Attapulgite and bentonite were investigated for their ability to improve the growth of Vetiver grass and Indian mustard in Al and Mn contaminated water. The rate of phytoremediation is affected by plant growth rate, which is why fast-growing and high biomass crops are the most appropriate (Danh et al., 2009; Beniah Obinna and Ebere 2019; Itam et al., 2019). Although TI varied within the different treatments, both plants showed a high tolerance with TI > 60 (Figures 2 and 3). The TI of Vetiver grass was significantly higher in the treatments assisted with attapulgite and bentonite. Attapulgite gave the best growth improvement with TI of 107.7% in the AT2.5VT treatment, while the lowest TI of 62.9% was obtained in the Vetiver grass +control (Figure 2a). This indicates that attapulgite and bentonite alleviated heavy metal stress in Vetiver grass. For the Indian mustard treatments, the highest TI was 116.8% with the BJ treatment (Indian mustard + control), while the lowest TI of 76.2% was in the AT2.5 BJ treatment (Figure 3a), indicating that the clay minerals did not improve the metal tolerance of Indian mustard. Despite the addition of more contaminated water leading to increased concentrations of Al and Mn, Vetiver grass showed no physical signs of heavy metal stress. The plant remained green and luscious throughout the experiment (Figure 2b), while Indian mustard became pale and yellowish with leaves drying out due to heavy metal stress (Figure 3b). This confirms that Vetiver has the ability to survive in highly contaminated environments (Danh et al., 2009; Suelee et al., 2017). This is similar to the observation of Gravand and Rahnavard (2021), who noted that there were no physical signs of toxicity in Vetiver grass in highly contaminated media.

Table 2: Physicochemical parameters and heavy metals (± standard deviation) measured *in-situ* and laboratory chemical analysis of water samples from the Leeuspruit Assessment against the In-stream Water Quality Guidelines (WQG) for the Leeuspruit Catchment. EC: electrical conductivity; TDS: total dissolved solids

Sampling site and WQC values	pH	Temperature (°C)	EC (mS/m)	TDS (mg/L)	NO_3^-(mg/L)	PO_4^{3-} (mg/L)	SO_4^{2-} (mg/L)	Al (mg/L)	Mn (mg/L)
RIVC	6.02 ± 0.01	25.5 ± 0.40	39.3 ± 0.50	240 ± 0.70	0.06 ± 0.01	0.24 ± 0.35	13.7 ± 0.41	0.96 ± 0.01	0.54 ± 0.001
RIV1	6.00 ± 0.00	26.7 ± 0.10	61.9 ± 0.10	364 ± 0.58	0.41 ± 0.02	0.32 ± 0.02	62.6 ± 0.77	4.58 ± 0.002	0.14 ± 0.003
RIV2	7.22 ± 0.07	29.7±0.10	59.9±0.10	965 ± 0	0.56 ± 0.02	0.51 ± 0.02	238.7 ± 1.44	0.64 ± 0.001	0.26 ± 0.001
RIV3	6.63 ± 0.10	25.0 ± 0.20	255.5 ± 1.30	580 ± 2.6	2.09 ± 0.09	2.06 ± 0.61	49.9 ± 0.53	0.33 ± 0.01	0.14 ± 0.001
WQG for the Leeuspruit Catchment	6–8.5	-	<45	-	0.5	0.2	-	0.3	<8

Figure 2: Growth performance of Vetiver grass in the experiment (a) tolerance index (TI) of Vetiver grass with different treatments, (b) Vetiver grass in the AT2.5VT treatment appearing healthy at the end of the experiment. *Values are means ± standard deviations n=3 ($p \leq 0.05$). Error bars represent percent (%) errors. Key: VT: Vetiver grass only; AT1VT: Vetiver + attapulgite (1% w/v); AT2.5VT: Vetiver + attapulgite (2.5% w/v); BT1VT: Vetiver + bentonite (1% w/v); BT2.5VT: Vetiver + bentonite (2.5% w/v).*

Figure 3: Growth performance of Vetiver grass in the experiment (a) tolerance index (TI) of Indian mustard in the different treatments, (b) Indian mustard in the AT2.5BJ treatment, appearing pale and yellowish by the end of the experiment. **Values are Means ± standard deviations n=3 ($p \leq 0.05$). Error bars* represent percent (%) errors. *Key: BJ: Indian mustard only; AT1BJ: Indian mustard + attapulgite (1% w/v); AT2.5BJ: Indian mustard + attapulgite (2.5% w/v); BT1BJ: Indian mustard + bentonite (1% w/v); BT2.5BJ: Indian mustard + bentonite (2.5% w/v).*

The comparison of the TI obtained for Indian mustard and Vetiver within the different treatment groups showed that there were no statistically significant differences ($p > 0.05$). Vetiver grass with attapulgite applied at 2.5% (w/v) showed the highest TI, while Indian mustard with attapulgite applied at the same rate showed the lowest TI. This variation may be due to the plants' different morphological and cellular traits (Beniah Obinna and Ebere 2019). Attapulgite and bentonite did not necessarily favour an increase in heavy metal tolerance and growth of Indian mustard, but they successfully improved the growth and tolerance of Vetiver grass in Al and Mn contaminated water.

iii. Heavy-metal accumulation

At the end of the experiment, the concentration of Al and Mn was significantly reduced in the vegetated treatments compared to the unvegetated treatments (Table 3). The treatments comprising both clay minerals and plants showed higher heavy metal removal. In some of the treatments, the resulting concentration of Al and Mn was higher than the initial concentrations (Table 3). This was because more contaminated water was added to the initial 800 mL mark as the water evaporated or transpired, leading to an increasing concentration of heavy metals. The desired outcome was to determine the effects of attapulgite and bentonite on the growth rate and heavy metal accumulation of Vetiver grass. The experiment revealed the quantity of Al and Mn that the plants could take up in 21 days under different treatments. For the clay-only treatments, there may have been a regeneration of contaminants that were previously adsorbed. This explains the increased Al and Mn in some treatments that were expected to have been adsorbed by the clay minerals (Li et al., 2019; Said et al., 2020).

A statistically significant difference ($p = 0.014$) was recorded in the absorbed concentration of Al and Mn between the treatments, as well as in their roots and shoots (Figures 4 and 5). There was generally higher root uptake of both heavy metals in all the treatments. The significant variance in root and shoot uptake corresponds to the findings of previous studies and confirms that Vetiver accumulates most heavy metals in its roots, because of its high tolerance (Suelee et al., 2017; Hassan et al., 2020; Gravand and Rahnavard, 2021). This was confirmed in all the treatments with Vetiver, even those with attapulgite and bentonite.

Table 3
Residual Al and Mn contents in Vetiver grass under various treatments

Heavy metals	OT (mg/l)	BT1 (mg/l)	BT1VT (mg/l)	AT2.5VT (mg/l)	BT2.5VT (mg/l)	BT2.5 (mg/l)	VT (mg/l)	AT1VT (mg/l)	AT2.5 (mg/l)	AT1 (mg/l)
Mn	24,5 ± 8,21	13 ± 7,85	0,1 ± 0,11	1,6 ± 1,02	0,5 ± 0,26	2,9 ± 0,67	16,8 ± 3,82	3,8 ± 3,3	0,7 ± 0,08	15,1 ± 1,44
Al	39,3 ± 15,93	30,9 ± 23,53	0,7 ± 0,61	3,4 ± 6,01	2,9 ± 2,44	17,5 ± 2,46	15,9 ± 5,42	14,5 ± 19,22	1,2 ± 0,08	2,1 ± 0,42

** Values are means ± SD, n=3, p< 0.05. Key: OT: zero treatment; VT: Vetiver grass only; AT1VT: Vetiver + attapulgite (1% w/v); AT2.5VT: Vetiver + attapulgite (2.5% w/v); BT1VT: Vetiver + bentonite (1% w/v); BT2.5VT: Vetiver + bentonite (2.5% w/v); AT1: attapulgite (1% w/v); AT2.5: attapulgite (2.5% w/v); BT1: bentonite (1% w/v); BT2.5: bentonite (2.5% w/v).*

Figure 4: Manganese (Mn) accumulation in (a) roots and (b) shoots of Vetiver grass. **Values are Means ± standard deviations n=3 (p ≤ 0.05). Error bars represent percent (%) errors. Key: VT: Vetiver grass only; AT1VT: Vetiver + attapulgite (1% w/v); AT2.5VT: Vetiver + attapulgite (2.5% w/v); BT1VT: Vetiver + bentonite (1% w/v); BT2.5VT: Vetiver + bentonite (2.5% w/v).*

Figure 5: Aluminium (Al) accumulation in (a) roots and (b) shoots of Vetiver grass. **Values are Means ± standard deviations n=3 (p ≤ 0.05). Error bars represent percent (%) errors. Key: VT: Vetiver grass only; AT1VT: Vetiver + attapulgite (1% w/v); AT2.5VT: Vetiver + attapulgite (2.5% w/v); BT1VT: Vetiver + bentonite (1% w/v); BT2.5VT: Vetiver + bentonite (2.5% w/v).*

The absorption of Al (Figure 5) was generally higher than that of Mn (Figure 4) in both roots and shoots, probably because the initial concentration of Al in the water was five times greater than that of Mn, and in most cases, heavy metal accumulation in plants increases with increasing concentration in the substrates (He *et al*., 2021; Hussain *et al.,* 2021; Leng *et al*., 2021). In addition, Al could have reduced the availability of Mn because it exhibits an antagonistic behaviour towards Mn uptake (Yang *et al*., 2009). Al has more affinity to FeOH than Mn, therefore the iron plaques might also play an important role in this process. In previous studies, Vetiver showed more preference for Mn than other heavy metals, without a significant change in biomass yield even at high concentrations (Hassan *et al*., 2020; Thakur *et al*., 2021). For Mn, AT1VT showed the highest root uptake (28.6 ppm) while the VT (Vetiver only treatment) showed the least root uptake (7.9 ppm). The highest shoot uptake of 6.1 ppm was, however, observed in the VT treatment. According to Thakur *et al.* (2021), when heavy metals are taken up into plant cells, they can be excluded, immobilized, chelated, or compartmentalized. Therefore, cell growth determines biomass yield, which in turn promotes metals absorption (Ali *et al.,* 2013). For Al, treatment BT2.5VT showed the highest root and shoot uptake of 330.7 and 41.1 ppm, respectively, while the lowest root and shoot uptake was observed in treatment VT. There was a strong positive correlation between root and shoot Al absorption by Vetiver grass (r = 0.90, $p < 0.05$), while a weak positive coefficient was observed between Mn root and shoot uptake (r = 0.01, $p < 0.05$).

Generally, there was no significant uptake of Al and Mn by Indian mustard in all treatments, as none of the heavy metals were detected by ICP-OES. This was attributed to the increasing concentrations of contaminated water in the experimental pots. The final concentration in the untreated water was 25.4 and 39.3 ppm for Mn and Al, respectively (Table 3). These final concentrations resulted from the continual addition of contaminated water each time the initial volume was reduced by evaporation, transpiration and plant uptake. Phytoremediation studies indicate that this method is suitable for minimally contaminated sites (Beniah Obinna and Ebere, 2019). Although none of the plants died during the experiment, the resulting toxicity from increasing heavy metal concentration was likely to be responsible for the inability of Indian mustard to absorb Al and Mn significantly.

Previous studies indicated that Indian mustard can absorb high concentrations of metals (50–30,000 ppm) in water (Meyers et al., 2008; Singh and Fulekar, 2012; Napoli et al., 2019). However, studies have also indicated that Indian mustard performs better as a phytoremediation plant when only one metal type is present compared to when two or more metals or when heavy metal contaminants are present. For example, Yang et al. (2021) reported that Indian mustard performed better as a hyperaccumulator when only As or Pb was present compared to when both heavy metals were present. The authors noted up to 90% decrease in As absorption when Pb was present as a co-contaminant in solution, whereas, in As only solution, Indian mustard absorbed up to 1,786 ppm. Kim et al. (2010) observed a reduced uptake of Cd, Cu, Pb, and Zn due to the presence of multiple metals and competitive uptake of these metals. Chigbo et al. (2013) reported up to 85% decrease in Cu accumulation by *B. juncea* and a decrease of biomass in the presence of pyrene. The decrease in Cu accumulation was attributed to reactions of complexes with root exudates and pyrene (Jeelani et al., 2020), resulting in the formation of insoluble Cu complexes, thus limiting uptake.

The insignificant metal uptake by Indian mustard could also result from Mn-induced toxicity, which have been reported previously (Parashar et al., 2014; Fariduddin et al., 2015). From these studies, it was evident that excess Mn triggers reactive oxidative stress such as H_2O_2 and O_2 radicals in Indian mustard, threatening proper plant growth after damage to membrane lipids, stomatal functions, proteins, and enzymes

(Parashar et al., 2014). Crop productivity is dependent on several factors such as aeration, irrigation and abiotic stresses (Phusantisampan et al., 2016). According to Gayatri et al. (2019), higher contents of trace elements including Zn, Ni, Mn, Cu and Fe can inhibit plant growth and lead to toxicity in plants. This is likely to be the case with Indian mustard in this study, but the growth of Vetiver grass was not inhibited by Al and Mn toxicity. Increasing concentrations of Al and Mn may have lowered the ability of Indian mustard cells to function properly, thereby limiting its metabolic, morphological and absorptive properties (Srivastava et al., 2015; Phusantisampan et al., 2016). Mn and Al induce oxidative stress in Indian mustard, restrict plant growth, cell elongation and photosynthesis, leading to stunting (Fariduddin et al., 2015; Ahmad et al., 2018). In this study, attapulgite and bentonite could not increase heavy metal absorption by Indian mustard, neither could these clay minerals alleviate heavy metal stress in the plant.

iv. Translocation factor (TF)

Translocation factor values <1 indicate a plant is suitable for phytostabilisation or root storage of heavy metals, and TF values >1 indicate suitability for phytoextraction (Beniah Obinna and Ebere, 2019). The TF for all treatments was less than 1 (Table 4), although Mn showed a higher translocation to the shoots of Vetiver compared to Al in all the treatments (Table 4). The highest TF of 0.78 was observed in the VT treatment for Mn, indicating that attapulgite and bentonite might have prevented translocation of Mn by promoting stronger adsorption of Mn within the root zone. According to Ramos-Arcos et al. (2019), the removal of Mn was the fastest among heavy metals including Al, B, Ba, Be, Co, Cr, Cu, Fe, Mg, Ni, Pb, S, Se, Tl, V and Zn, but TF was <1. This is similar to the present study as TF values below 1 (ranging between 0.22 to 0.77) were observed for Mn in Vetiver grass. Another study showed that within 30 days, 0.15 ppm of Mn can be removed from landfill leachate by Vetiver grass (Thakur et al., 2021), with TF >1. The high TF observed in the study may have been due to the low initial concentration of Mn, which encouraged faster translocation (Thakur et al., 2021). For Al, the highest TF of ~ 0.14 was observed in the AT2.5VT treatment (Table 4), but reasonable amounts of Al were stored within the roots of Vetiver. Generally, results indicated that the roots of Vetiver grass could both tolerate and accumulate high concentrations of Mn and Al.

Table 4
Translocation factor (TF) observed for Vetiver grass in each treatment

Heavy Metal	BT2.5VT	AT1VT	AT2.5VT	VT	BT1VT
Mn	0,242	0,21	0,337	0,776	0,165
Al	0,124	0,092	0,137	0,052	0,1

**(p<0.05), strong negative correlation was observed between the TF of Al and Mn. Coefficient (r) = -0,68. *Values are means ± SD, n=3, p< 0.05. Key: VT: Vetiver grass only; AT1VT: Vetiver + attapulgite (1% w/v); AT2.5VT: Vetiver + attapulgite (2.5% w/v); BT1VT: Vetiver + bentonite (1% w/v); BT2.5VT: Vetiver + bentonite (2.5% w/v)*

The low TF observed in this study was similar to the findings of Suelee et al. (2017) and Thakur et al. (2021). The cell membrane is negatively charged; therefore, Mn and Al ions enter plant cells easily. However, Mn is more easily translocated to the shoots because it is an essential element for plant growth (Ramos-Arcos et al., 2019; Shahid et al., 2020; Thakur et al., 2021). Although the TF indicates a plant's ability to translocate heavy metals to its shoots, it should not be solely considered when determining the suitability of plants as hyperaccumulators, because although TF < 1, the shoots may still have absorbed high levels of heavy metals. For instance, in a study on the absorption of Cd by Himalayan balsam, TF was < 1, but the plant's shoots contained about 70% of the total Cd root uptake (Coakley et al., 2019). The TF was < 1 for Al and Mn in this study, a situation that can be considered an advantage, because it prevents metals from reaching the plant shoots and damaging the photosynthetic machinery as well as limiting post-remediation use of Vetiver grass (Beniah Obinna and Ebere, 2019). This also prevents the heavy metals from getting into the food chain (if animals eat shoots and leaves).

4. Conclusion

The study investigated the TI and heavy metal accumulation ability of Vetiver grass and Indian mustard to simultaneously remove Al and Mn from contaminated water in a hydroponic system. Results from the experiment demonstrated that while Vetiver grass could bioaccumulate significant amounts of both heavy metals in its roots within 21 days, Indian mustard could not absorb significant amounts of Al and Mn that could be detected by ICP-OES analysis of the digested plants. In Vetiver grass, the uptake of Al and Mn in the roots was more than two-fold that of the shoot uptake in most of the treatments. In addition, there was low Al and Mn translocation, as the TI in all the treatments were < 1.

Attapulgite and bentonite successfully increased the tolerance index and heavy metal phytoremediation potential of Vetiver grass. These results suggest that Vetiver grass can be a suitable candidate for removal of Al and Mn in water under controlled greenhouse conditions and its performance can be improved by the addition of clay minerals such as bentonite added at 2.5% (w/v), which performed best for Al

remediation and significantly good for Mn. Therefore, it is recommended that the efficacy of this combination of Vetiver grass and clay minerals be tested under field (natural) conditions to ascertain full scale application for heavy-metal contaminated waters. Field studies would be recommendable to test the application of bentonite to improve the phytoremediation capacity of Vetiver grass in Al and Mn contaminated river water. If similar results are obtained in field trials, then bentonite could be confirmed as a beneficial material for improving the phytoremediation capacity of Vetiver grass. This is the first study to prove that the use of bentonite improves the performance of the Vetiver grass during phytoremediation of heavy metals such as Al and Mn.

Declarations

Availability of data and Materials

The authors confirm that the data supporting the findings of this study are available within this published article.

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Statements and Declarations

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors Contributions

Beatrice Omonike Otunola, Makhosazana Aghoghovwia, Melusi Thwala and Olusola Oluwayemisi Ololade contributed to the study conception and design. Beatrice Omonike Otunola, Alba Gómez-Arias, Rian Jordaan and Julio Castillo Hernandez contributed to chemical analysis and interpretation. Literature search and first draft of the manuscript was done by Beatrice Omonike Otunola. All authors contributed to the critical revision of manuscript and approved the final manuscript.

Consent to Participate

All the authors consented to participate in the drafting and submission of this manuscript.

Consent for Publication

All the authors consented to publish this manuscript.

Ethics approval

Not applicable.

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Figures

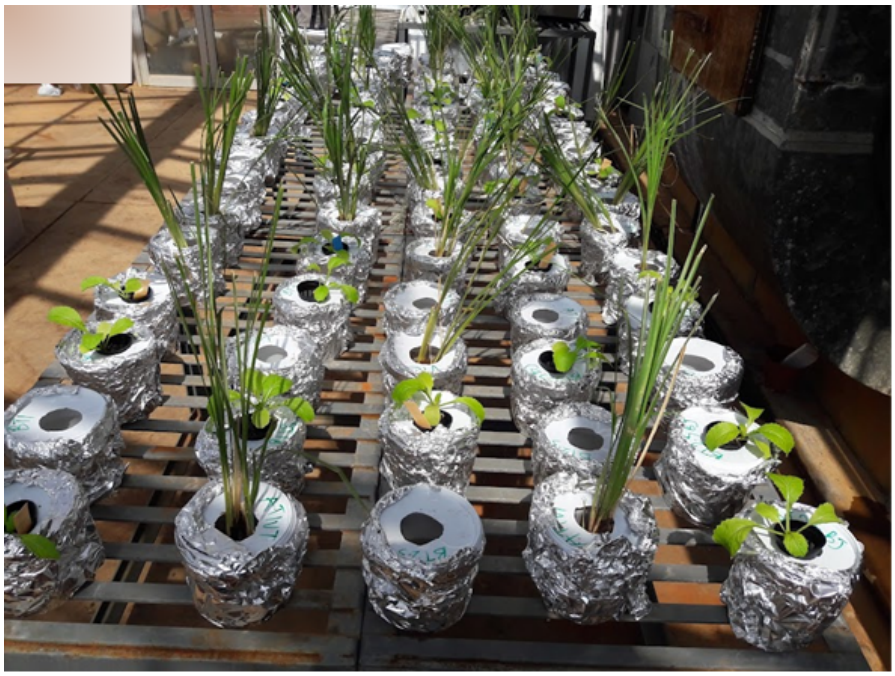

Figure 1

Experimental setup of the various treatments at a greenhouse facility, University of the Free State, South Africa.

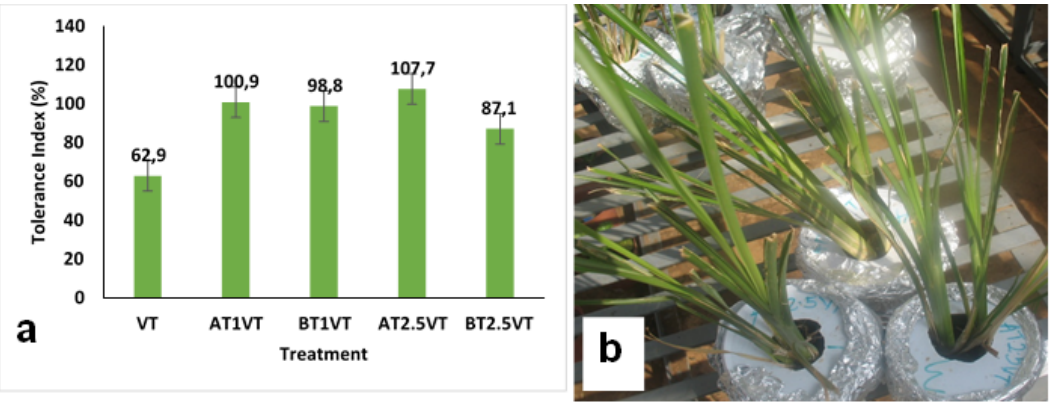

Figure 2

Growth performance of Vetiver grass in the experiment (a) tolerance index (TI) of Vetiver grass with different treatments, (b) Vetiver grass in the AT2.5VT treatment appearing healthy at the end of the experiment. *Values are means ± standard deviations n=3 ($p \leq 0.05$). Error bars represent percent (%) errors. Key: VT: Vetiver grass only; AT1VT: Vetiver + attapulgite (1% w/v); AT2.5VT: Vetiver + attapulgite (2.5% w/v); BT1VT: Vetiver + bentonite (1% w/v); BT2.5VT: Vetiver + bentonite (2.5% w/v).*

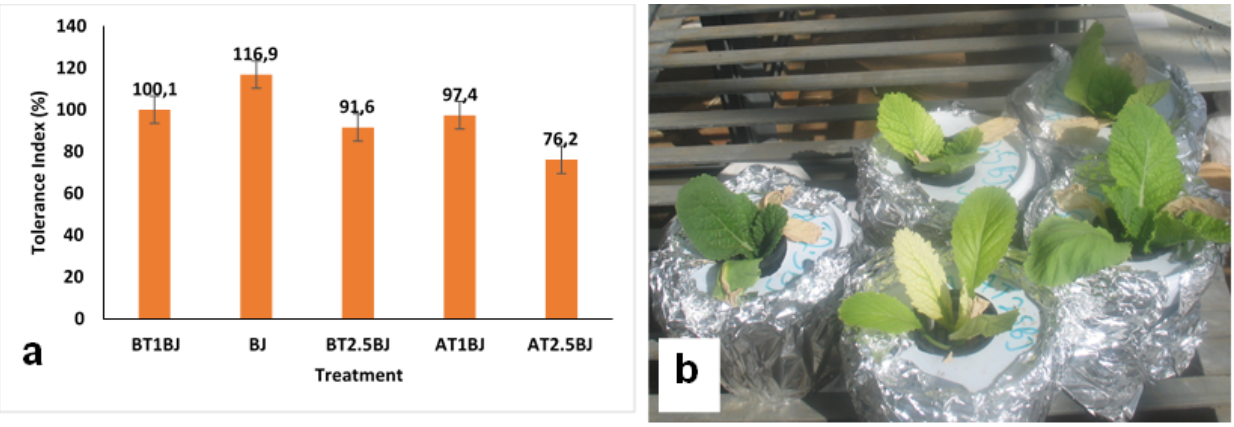

Figure 3

Growth performance of Vetiver grass in the experiment (a) tolerance index (TI) of Indian mustard in the different treatments, (b) Indian mustard in the AT2.5BJ treatment, appearing pale and yellowish by the end of the experiment. **Values are Means ± standard deviations n=3 ($p \leq 0.05$). Error bars* represent percent (%) errors. *Key: BJ: Indian mustard only; AT1BJ: Indian mustard + attapulgite (1% w/v); AT2.5BJ: Indian mustard + attapulgite (2.5% w/v); BT1BJ: Indian mustard + bentonite (1% w/v); BT2.5BJ: Indian mustard + bentonite (2.5% w/v).*

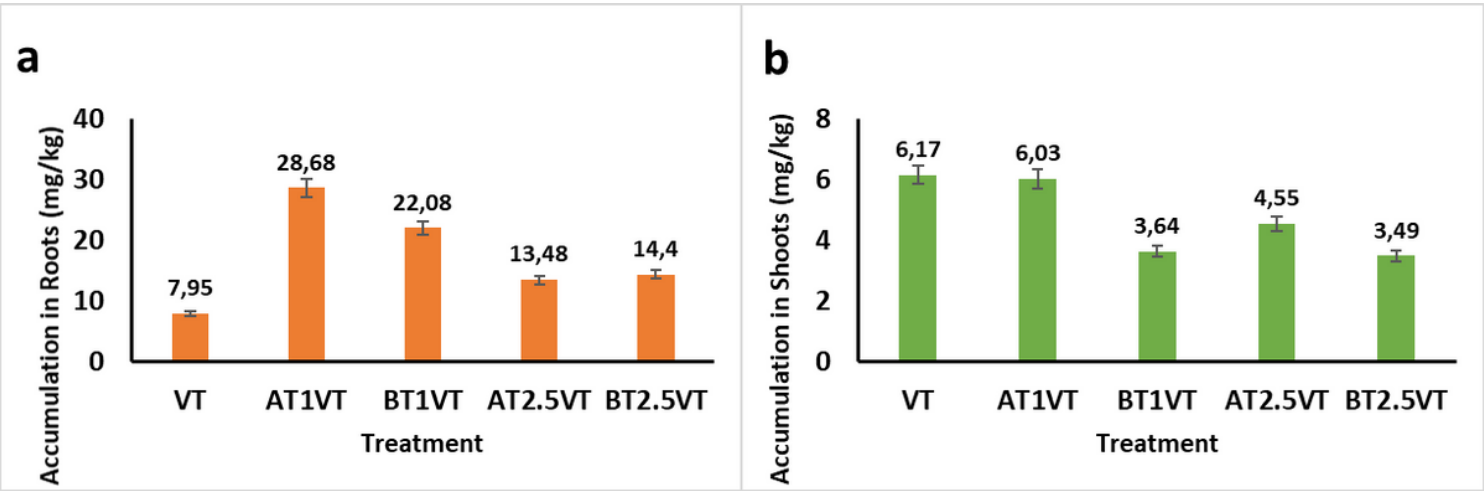

Figure 4

Manganese (Mn) accumulation in (a) roots and (b) shoots of Vetiver grass. **Values are Means ± standard deviations n=3 ($p \leq 0.05$). Error bars represent percent (%) errors. Key: VT: Vetiver grass only; AT1VT: Vetiver + attapulgite (1% w/v); AT2.5VT: Vetiver + attapulgite (2.5% w/v); BT1VT: Vetiver + bentonite (1% w/v); BT2.5VT: Vetiver + bentonite (2.5% w/v).*

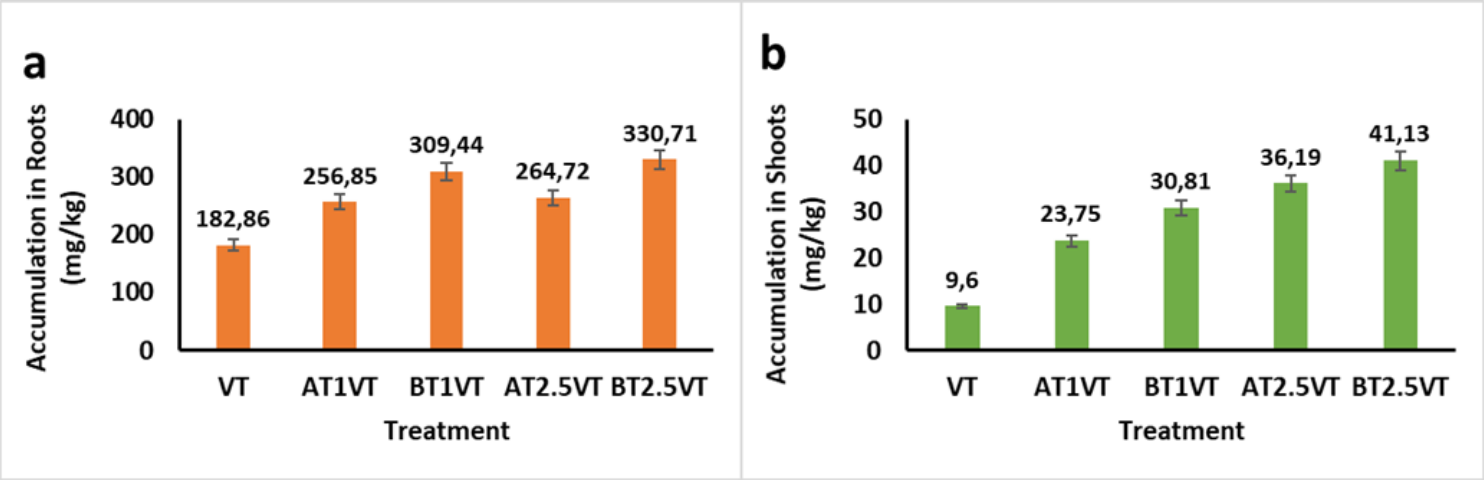

Figure 5

Aluminium (Al) accumulation in (a) roots and (b) shoots of Vetiver grass. **Values are Means ± standard deviations n=3 ($p \leq 0.05$). Error bars represent percent (%) errors. Key: VT: Vetiver grass only; AT1VT: Vetiver + attapulgite (1% w/v); AT2.5VT: Vetiver + attapulgite (2.5% w/v); BT1VT: Vetiver + bentonite (1% w/v); BT2.5VT: Vetiver + bentonite (2.5% w/v).*