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Influence of Clay Mineral Amendments Characteristics on Heavy Metals Uptake in Vetiver Grass (*Chrysopogon zizanioides* L. Roberty) and Indian Mustard (*Brassica juncea* L. Czern)

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Citation: Otunola, B.O.; Aghoghovwia, M.P.; Thwala, M.; Gómez-Arias, A.; Jordaan, R.; Hernandez, J.C.; Ololade, O.O. Influence of Clay Mineral Amendments Characteristics on Heavy Metals Uptake in Vetiver Grass (*Chrysopogon zizanioides* L. Roberty) and Indian Mustard (*Brassica juncea* L. Czern). *Sustainability* **2022**, *14*, 5856. https://doi.org/10.3390/su14105856

Academic Editors: Kamal H. Shaltout, Ebrahem M. Eid and Tarek M. Galal

Received: 15 February 2022
Accepted: 7 May 2022
Published: 12 May 2022

Abstract: Phytoremediation is limited when heavy metals reduce soil quality and, subsequently, inhibit plant growth. In this study, we evaluated the use of attapulgite and bentonite as amendments in soil contaminated with multiple metals, to improve the phytoremediation capacity of Vetiver grass and Indian mustard. A 21-day greenhouse study was undertaken, to investigate plant tolerance in heavy-metal-contaminated soil, as well as heavy-metal absorption in plant roots and shoots. The results showed a generally higher root-uptake rate for Cr, Cu, Co, Ni, and Zn in Vetiver grass. Overall, the highest absorption for Ni, Cr, Co, Cu, and Zn was 1.37, 2.79, 1.39, 2.48 and 3.51 mg/kg, respectively, in the roots of Vetiver grass. Clay minerals inhibited the translocation of some heavy metals. The addition of attapulgite improved the phytoremediation capacity of Vetiver for Ni, Cr, and Co, while bentonite improved Vetiver's absorption of Cu and Zn. The translocation factor for Ni in one of the attapulgite treatments was 2, indicating that attapulgite improved the phytoextraction of Ni by Vetiver grass. Our results confirm that attapulgite at 2.5% (*w*/*w*) can successfully improve the phytostabilization of heavy metals by Vetiver grass. Indian mustard showed no significant metal uptake that could be detected by inductively coupled plasma optical emission spectrometry (ICP-OES), despite the addition of attapulgite and bentonite. This research contributes to the knowledge repository of suitable amendments that improve the phytoremediation properties of Vetiver grass.

Keywords: heavy metals; Indian mustard; phytoremediation; contaminated soil; Vetiver grass

1. Introduction

Extreme levels of chemical pollution in the soil are a common problem in any land where mining is active or in former mining landscapes. Additionally, post-mining land use, such as agricultural, waste management, and industrial activities, may contribute to the amount of pollution in old mining sites [1,2]. Soil pollution reduces soil fertility, productivity, and ecosystem functions, while increasing surface and groundwater pollution through the leaching of contaminants [3,4]. Heavy metals are one of the most challenging pollutants globally because they are highly toxic to living organisms even in trace amounts [5] and non-biodegradable, therefore, they are persistent in the environment over long periods [6,7]. Common heavy metal pollutants associated with mining include aluminum (Al), arsenic

(As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), and zinc (Zn) [6–9].

The associated heavy-metal-pollutant ecological and health risks include cancer, respiratory diseases, miscarriages, organ failure, skin disorders, and mental disorders [10,11]. Hence, there is the need to find more effective, economical, and environmentally friendly techniques to remediate contaminated media [12]. Phytoremediation has emerged as a promising remediation alternative that meets these conditions. Phytoremediation uses different mechanisms to either extract, stabilize, volatilize, or degrade contaminants in the environment [2]. Among these mechanisms, phytoextraction and phytostabilization are the most effective for the remediation of heavy metals [13,14]. Vetiver grass (*Chrysopogon zizanioides* L. Roberty) and Indian mustard (*Brassica juncea* L. Czern) have been demonstrated to be suitable for the removal of several heavy metals in contaminated media [5,15]. These species grow well under extreme climatic conditions, are easily cultivated and maintained, possess adequate root system mass, and meet the requirements of plants suitable for phytoremediation [16,17]. Previous studies have shown that Vetiver grass is a metal excluder and phytoextractor; therefore, it can be applied in phytostabilization and phytoextraction processes [18–20]. Vetiver is preferred among the grass crops for soil and water remediation and is recognized as the best species for tailings revegetation [21,22]. Vetiver can successfully remove 37 ppm As from water in 14 days, without suffering any signs of toxicity [23]. Similarly, Indian mustard can absorb high levels of heavy metals such as Pb, Ni, Cd, Hg, and Se in roots and shoots, without showing signs of toxicity [24–26], for instance, absorbing up to 1000 mg/kg Pb over 34 days [27].

The application of phytoremediation technologies to contaminated soils can be limited by several factors, such as low plant growth rate, low bioavailability of metals in soil, translocation rate of the metals, and extended periods required for remediation [2,28]. In highly polluted sites that have been left untreated over a long period, soil quality decreases and phytotoxicity can increase [29]. Moreover, plant growth may be naturally slow, requiring a longer time for sufficient remediation levels [30]. These concerns can be overcome through assisted phytoremediation, a process that involves the application of soil amendments to improve soil function, plant growth, and phytoextraction of contaminants [31–33].

Amendments such as biochar, red mud, organic matter, clay minerals, microorganisms, compost, and organic chelates can improve soil function and plant growth, thereby promoting the phytoremediation potential of plants [7,31,34,35]. Clay minerals are suitable materials for the immobilization of heavy metals, thus reducing the bioavailability of toxic metals, improving soil pH, and retaining nutrients and moisture, thereby improving the soil quality [36]. Additionally, clay is affordable, and, generally, easily accessible [37]. Attapulgite and bentonite particularly have large pore spaces, high specific surface areas (SSA), and high cation exchange capacity (CEC), giving them the ability to adsorb and reduce the mobility of heavy metals and other contaminants [37]. The reflection of clay minerals may vary depending on the angle, impurities, or mineral compositions. According to Zotiadis and Argyraki [36], attapulgite is characterized by (001) reflection, and the highest reflection peak is at 26°, as a result of quartz within the mineral. Guo et al. [38], found that the characteristic reflections of attapulgite were at 8.5°, 19.8°, and 20.8° (2θ degrees). Montmorillonite is the major component of bentonite, characterized by (001) and (006) reflections between 54° and 68° (2θ degrees) [39]. The purer the clay, the higher its reflection.

Considering the quest to find sustainable solutions to environmental problems and the available knowledge on the advantages and disadvantages of using clay minerals and plants for heavy metals remediation, it could be worthwhile to combine these techniques in a bid to achieve better remediation results. The addition of clay minerals to soil during phytoremediation can be a feasible approach to improve the phytoremediation of heavy metal contaminated soil and reduce the leaching of heavy metals from soil to water bodies. This study, through a 21-day greenhouse experiment, investigated if better remediation results can be achieved through a hybrid application of clay minerals (attapulgite and bentonite) and phytoremediation (using Vetiver grass and Indian mustard). This application was undertaken to develop an effective remediation for heavy metals in a post-mining environment in Sasolburg, South Africa. This research is expected to contribute to the quest for knowledge on finding cost-effective, practical, and environmentally friendly remediation techniques for heavy-metal-contaminated soil. The problem of pollution from heavy metals in soil is common in several industries worldwide. Therefore, the findings of this study will be relevant and applicable in different industries, as well as various disciplines of environmental sciences, for the sustainable, cost-effective, and environmentally friendly management of soils polluted by metals.

2. Materials and Methods

2.1. Study Area

The soil was sourced from a former coal mining area (26°50′50.4″ S 27°49′49.7″ E) in Sasolburg, Free State Province, South Africa (Figure 1). Mining operations ceased in 2006, while rehabilitation and reclamation are ongoing [40]. The post-mining land uses that were established in the area includes animal grazing, livestock, and crop farming. Other land uses in the study area include a wastewater treatment plant, a municipal solid waste dumpsite, and industrial activities. Surface runoff and effluent discharge from these land-use activities potentially contribute to heavy metal pollution of surrounding soils and water bodies [8,40]. The geology of Sasolburg consists of sedimentary rocks from the Ecca Group, comprising sandstone, shale, and dolerite intrusions in some areas, which host some of the coal deposits in South Africa [8]. The topography is relatively flat, with gentle slopes, and is intersected by the Leeuspruit and Rietspruit rivers [8]. Average summer and winter temperatures are 21 °C and 9 °C, respectively. The area experiences summer rainfalls of about 776 mm per year. The major vegetation in Sasolburg is grazed grassland [8].

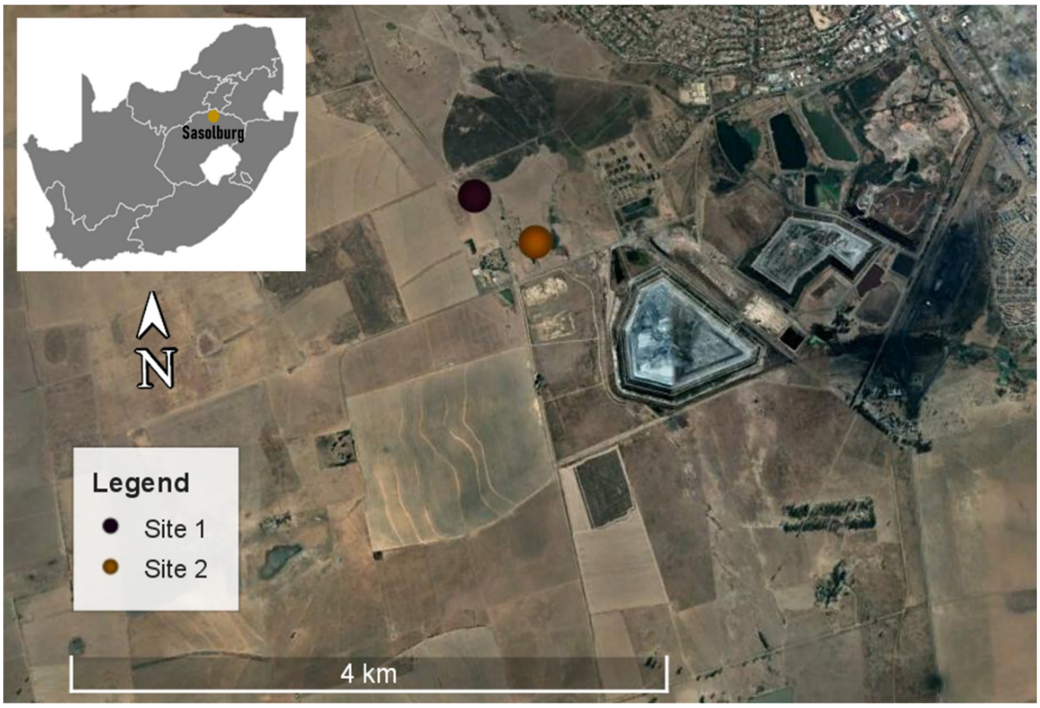

Figure 1. Map showing the study area and sampling sites located in Sasolburg, Free State Province, South Africa.

2.2. Sampling Procedure and Analyses

Soil samples were collected from the areas where the post-mining land use is agriculture, by scooping with a shovel to a depth of 20 cm. Samples were stored in tightly sealed polypropylene bags and transported to the laboratory. The soil samples were air-dried in the laboratory on a clean and flat surface for 48 h, after which they were analyzed for various parameters. The mineral composition was determined by X-ray diffraction (XRD), while the soil physico-chemical parameters and nutrient analyses were performed by the methods outlined by Madanan et al. [41]. Soil pH was determined by mixing 20 g soil with 50 mL distilled water in a 100 mL beaker. The mixture was stirred and left for 30 min, then the pH was measured using a calibrated pH meter. Organic carbon was determined by the Walkey and Black method, while the available nitrogen was determined using the Kjeldahl method. Particle size was determined using the hydrometer method [42]. To ensure complete metal recovery and minimize contamination risks from the surroundings, samples were digested by microwave-assisted acid digestion, using the US EPA guidelines 3052 [43], and, then, analyzed for heavy metals using ICP-OES at the Chemistry Department, University of the Free State.

2.3. Seed Germination and Transplanting

Indian mustard seeds were purchased from Seeds for Africa, Cape Town, South Africa. They were sterilized in 6% v/v hydrogen peroxide for 15 min and, then, washed with deionized water three times [44]. The sterilized seeds were germinated in seedling trays using Hygrotech seed starter, composed of 17.2% N, 7.1% P, 2.3% K, 0.8% Ca, 0.2% Mg, 785 mg/kg Fe, 398 mg/kg Mn, Zn, and Cu, 204 mg/kg B, and 6.6 mg/kg Mo. The seedlings were kept moist and a 95% germination rate was achieved by the third day. The 30-day-old seedlings, with at least three leaves and a height of ~6 cm, were transplanted to individual pots for the different selected treatments. Vetiver grass seedlings were obtained from Hydromulch (Pty) Ltd., Johannesburg, South Africa. They were washed thoroughly and trimmed to similar heights of 30 cm for shoots and 15 cm for roots, before they were transplanted to individual pots.

2.4. Characterization of Bentonite and Attapulgite

Natural bentonite and attapulgite were supplied in their raw forms by AttaClay (Pty) Ltd. (Germiston, South Africa). The Brunauer–Emmett–Teller (BET) surface area and micropore of the clays were analyzed, as described by Dogan et al. [45]. Specific surface area was determined for the clay minerals because it is an important parameter to quantify mineral dissolution, adsorption, and desorption in soils [46].

2.5. Experimental Design

A 21-day experiment was conducted in a temperature-controlled greenhouse at the Department of Soil, Crop and Climate Sciences, University of the Free State. The greenhouse was maintained under temperatures of 28 °C (day) and 20 °C (night) and provided a natural light cycle. Round plastic planting pots, having a holding capacity of 1.5 kg, were used as experiment vessels. One kg of soil was placed into each pot and weighed to ensure the same bulk density of 1 g/cm^3. The clays were applied at two dosage levels, 1% and 2.5% (w/w), since low clay dosages are required for adequate soil amendment [36,37]. A factorial experiment was set up with three replicates of each treatment, as described in Table 1. In this study, the clay minerals were introduced, homogeneously, within the soil in each pot. The soil pots were arranged in a randomized complete block design.

2.6. Harvesting and Analyses

At the end of the experiment (21 days), the plants were harvested and thoroughly rinsed with tap water to get rid of all soil particles. The plants' fresh weight was determined and recorded. Dry weight was determined after the plants were oven-dried at 75 °C for 72 h; then, they were milled and digested using microwave-assisted digestion with nitric acid. The powdered dry plant matter was weighted (0.5 $\pm$ 0.005 g) and digested using nitric acid (HNO_3) in a microwave and analyzed for heavy metals using ICP-OES [43]. The tolerance index (TI) of the plants was calculated as the total biomass in contaminated soil/total biomass in uncontaminated soil [47]. The TI was determined to evaluate the tolerance and growth rate of Vetiver grass and Indian mustard in heavy-metal-contaminated soil, as well as examine the influence of each clay treatment on plant growth. Translocation Factor (TF) is the ability of a plant to translocate metals from its roots to shoots and can be calculated as TF = HM concentration in shoot/HM concentration in the root (where HM = heavy metals) [47].

Table 1. Soil treatment codes and conditions.

Treatment Code	Conditions
Control (zero treatment)	Soil with no treatment
VT	Vetiver only (one plant per pot)
BJ	Indian mustard only (one plant per pot)
AT1VT	Attapulgite + Vetiver applied at 1% (*w*/*w*)
AT2.5VT	Attapulgite + Vetiver applied at 2.5% (*w*/*w*)
BT1VT	Bentonite + Vetiver applied at 1% (*w*/*w*)
BT2.5VT	Bentonite + Vetiver applied at 1% (*w*/*w*)
AT1BJ	Attapulgite + Vetiver applied at 1% (*w*/*w*)
AT2.5BJ	Attapulgite + Vetiver applied at 2.5% (*w*/*w*)
BT1BJ	Bentonite + Vetiver applied at 1% (*w*/*w*)
BT2.5BJ	Bentonite + Vetiver applied at 2.5% (*w*/*w*)
VTC	Vetiver only in uncontaminated soil
BJC	Indian mustard only in uncontaminated soil

2.7. Statistical Analyses

All data were subjected to statistical analysis and expressed as means ± standard deviation of three replicates using two-way analysis of variance (ANOVA). Means were considered significant at $\rho < 0.05$. The calculations were performed using R software version 4.0.0 [48]. The mean values were compared using the ANOVA test for normal data, after which a Tukey's post hoc test was performed to know the specific treatments with significant differences.

3. Results

3.1. Soil and Clay Characteristics

The soil properties are shown in Table 2. Soil texture analysis revealed that the soil is a loam soil, with a higher sandy fraction and close to neutral pH. According to the BET surface area plots shown in Figure 2, attapulgite had an SSA of 129.42 m^2/g, and bentonite had an SSA of 111.16 m^2/g. The micropore spaces of these clay minerals were 0.014 and 0.022 cm^3/g for attapulgite and bentonite, respectively. Attapulgite used for the study contained 91% clay, with pyroxene, quartz, and calcite being present at 5%, 3% and 1%, respectively. Bentonite on the other hand, had 89% clay content, with 4%, 4%, and 3% calcite, quartz, and plagioclase, respectively.

Table 2. Analyzed soil properties and content of heavy metals.

Parameter (Unit)	Value
pH	6.80 ± 0.20
N (%)	0.01 ± 0.00
C (%)	1.31 ± 0.09
Soil texture	Loam
Sand (%)	42.80 ± 8.87
Silt (%)	34.70 ± 0.61
Clay (%)	22.50 ± 0.09

Table 2. *Cont.*

Parameter (Unit)	Value
Mineralogy (%)	
Quartz	78.00 ± 0.14
Plagioclase	7.00 ± 0.17
Potassium feldspar	5.00 ± 0.17
Mica	6.00 ± 0.15
Serpentine	4.00 ± 0.06
Total trace elements (mg/kg)	
Cr	151.00 ± 8.39
Ni	50.50 ± 2.75
Co	26.00 ± 1.40
Cu	25.00 ± 0.92
Zn	57.50 ± 1.16

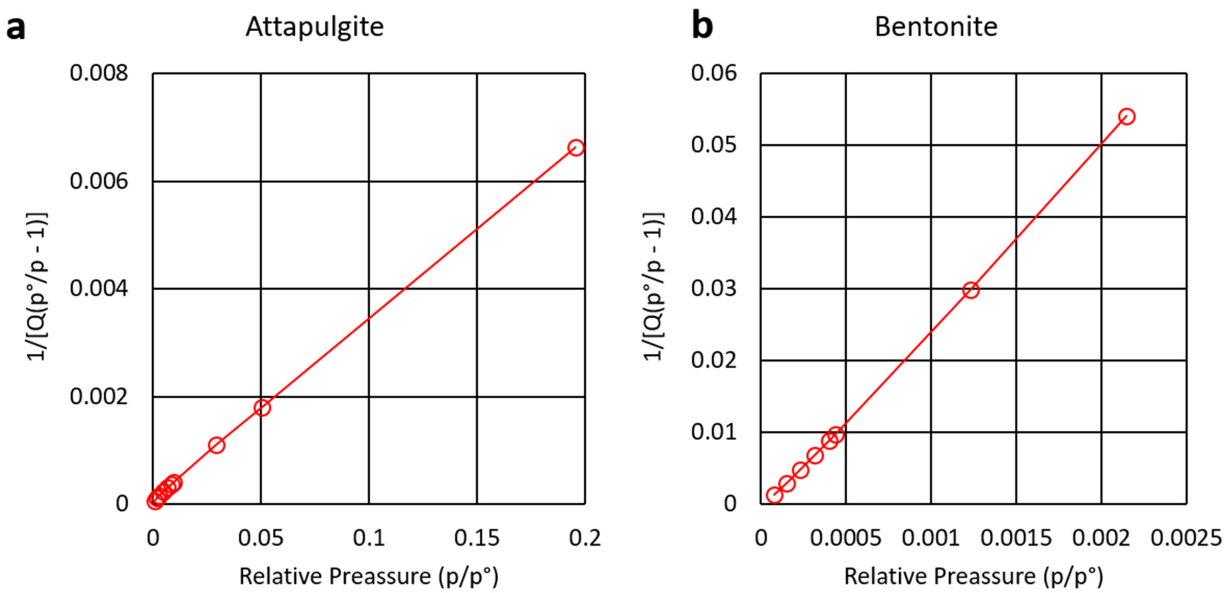

Figure 2. Brunauer-Emmett-Teller (BET) surface area plots for nitrogen gas adsorption on (**a**) attapulgite and (**b**) bentonite clay minerals.

3.2. Responses of Plant Growth Tolerance Index (TI)

The plant's dry weight biomass usually reflects the tolerance of plants to an adverse environment. The TI values for the various treatments of Vetiver grass and Indian mustard are presented and discussed in the current section. The TI of Vetiver grass varied among the different treatment types (Figure 3b). The highest TI was observed in the 1% bentonite treatment (BT1VT), while the lowest TI was observed in the 1% attapulgite treatment (AT1VT). Such findings indicated that bentonite improved the growth of Vetiver grass, while this was not the case with attapulgite because the attapulgite treatments had lower TI compared to the Vetiver-only treatment (without any amendments).

Indian mustard showed better growth in all the treatments, as its TI values were >100% (Figure 3a), indicating high tolerance of the plant in soil containing multiple heavy metals. Similar to the Vetiver grass treatments, the biomass yield for the Indian-mustard-only treatment was greater than the biomass yield observed in the clay-amended treatments, except for the BT2.5VT treatment, which showed the highest TI of 160.1%.

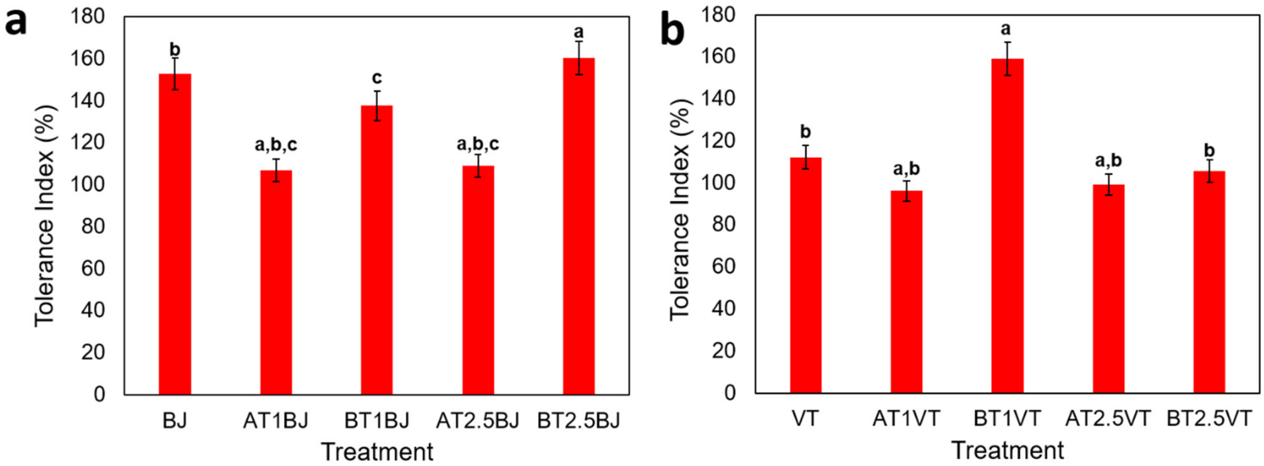

Figure 3. Tolerance index (TI) of (**a**) Indian mustard and (**b**) Vetiver grass in the various treatments at the end of the experiment among the different treatments. Data shown as mean ± SD of triplicates (error bars represent standard deviation). F value = 1.1679; total degrees of freedom = 14; lower case letters on top of error bars indicate statistically significant variance between means. Key: VT-Vetiver grass only; AT1VT-Vetiver + attapulgite applied at 1% (*w*/*w*); AT2.5VT-Vetiver + attapulgite applied at 2.5% (*w*/*w*); BT1VT-Vetiver + bentonite applied at 1% (*w*/*w*); BT2.5VT-Vetiver + bentonite applied at 2.5% (*w*/*w*); BJ-Indian mustard only; AT1BJ-Indian mustard + attapulgite applied at 1% (*w*/*w*); AT2.5BJ-Indian mustard + attapulgite applied at 2.5% (*w*/*w*); BT1BJ-Indian mustard + bentonite applied at 1% (*w*/*w*); BT2.5BJ-Indian mustard + bentonite applied at 2.5% (*w*/*w*).

3.3. Accumulation of Heavy Metals in Vetiver Grass

The concentrations of Co, Cr, Cu, Ni, and Zn absorption in the roots and shoots of Vetiver for each treatment are shown in Figure 4. There was a wide variation in the absorption of these metals within each treatment (Figure 4). However, the data from this study indicated that the heavy metals (Co, Cr, Cu, Ni, and Zn) absorbed by the plants were substantially retained in the roots, except Ni, whose shoot absorption was more than its root absorption in the AT1VT treatment (Figure 4b).

The AT2.5VT treatment also had the highest absorption for Co, Cr, and Ni. For Cr, AT2.5VT was the only treatment that improved Cr root uptake, but Cr translocation to the shoots was inhibited. For Cu, the BT1VT, AT2.5VT, and BT2.5VT treatments showed increased Cu root uptake by 183%, 92%, and 41%, respectively, compared to the VT (control) treatment, and no shoot absorption was observed in the AT1VT and VT treatments. There was no shoot uptake of Co in all of the treatments, and only the AT2.5VT treatment showed a 70% increase in Co root uptake.

Clay minerals significantly reduced bioavailable fractions of heavy metals. Looking at Ni in the current study, the Vetiver treatment (with no added clays) showed the highest Ni root uptake, indicating that attapulgite and bentonite inhibited the absorption of Ni (Figure 4).

3.4. Translocation Factor (TF)

The translocation factor (TF) in the current study ranged from 0 to 2.0 (Table 3). The addition of attapulgite improved the phytoremediation capacity of Vetiver for Ni, Cr, and Co, while bentonite improved Vetiver's absorption of Cu and Zn. Attapulgite added at 1% (AT1VT treatment) was the most effective in enhancing Ni translocation to the shoots (51% more Ni in Vetiver shoots). The TF was very low for Cu, and uptake was mainly in the bentonite treatments, suggesting that bentonite favors the translocation of Cu.

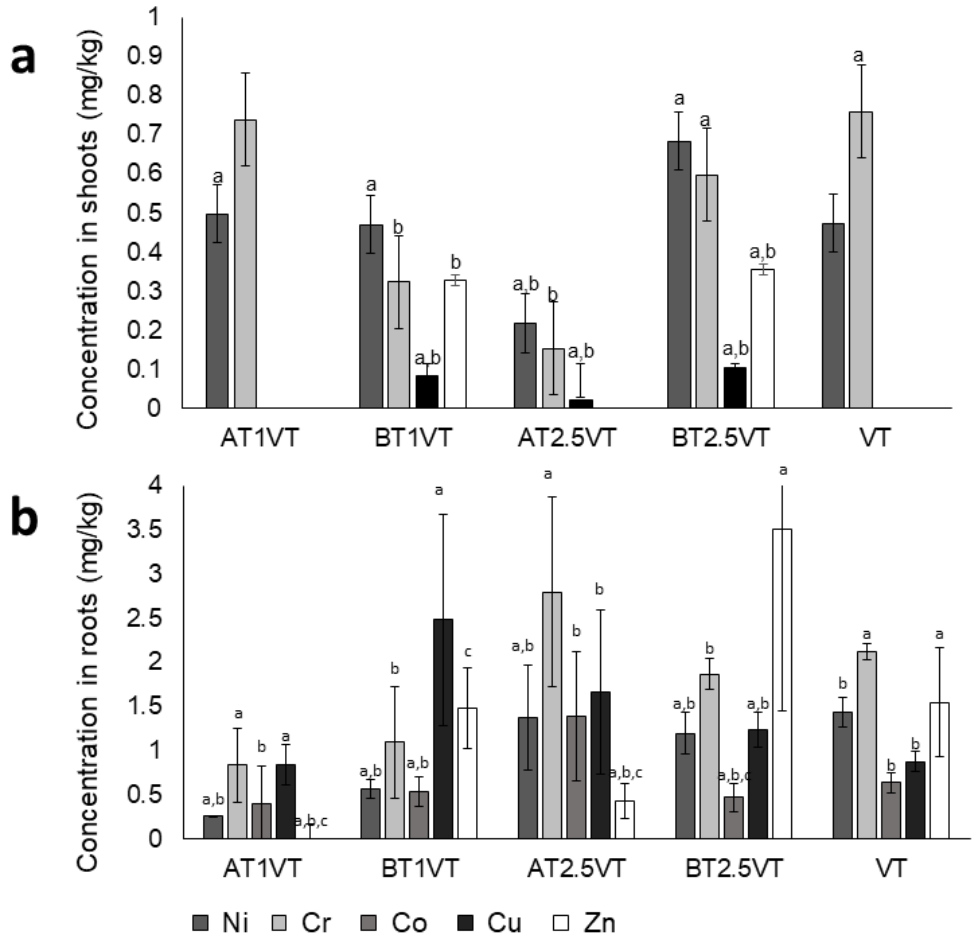

Figure 4. Concentrations of heavy metals in (**a**) shoots and (**b**) roots of Vetiver grass in the different treatments at the end of the experiment. Data shown as mean ± SD of triplicates (error bars represent standard deviation). F value = 3.012 for shoots and 3.8723 for roots; total degrees of freedom = 24; lower case letters on top of error bars indicate statistically significant variance between means ($\rho < 0.001$), based on Tukey's Honest Significant Difference (HSD) test. Data with non-significant variance have the same letter. Key: VT-Vetiver grass only; AT1VT-Vetiver + attapulgite applied at 1% (*w*/*w*); AT2.5VT-Vetiver + attapulgite applied at 2.5% (*w*/*w*); BT1VT-Vetiver + bentonite applied at 1% (*w*/*w*); BT2.5VT-Vetiver + bentonite applied at 2.5% (*w*/*w*).

Table 3. Translocation factor (TF) of Co, Cr, Cu, Ni, and Zn in each treatment (0 indicates no uptake in shoots or roots, therefore, TF could not be calculated for some heavy metals). Different superscript letters within a column indicate statistically different values in each treatment (ANOVA; Tukey's *t*-test, $\rho < 0.05$), indicating that there is a significant difference in the in TF observed for each heavy metal within each treatment. Total degrees of freedom = 24.

Heavy Metal/ Treatment	Co	Cr	Cu	Ni	Zn
VT	0	0.4 ± 0.08^{b}	0	0.4 ± 0.11	0
AT1VT	0	0.9 ± 0.44^{a}	0	2.0 ± 0.82	0
BT1VT	0	0.3 ± 0.82	0.1 ± 0.13	0.9 ± 0.24	0.3 ± 0.07
AT2.5VT	0	0.1 ± 0.02	0.1 ± 0.07	0.2 ± 0.06^{a}	0
BT2.5VT	0	0.4 ± 0.02^{ab}	0.1 ± 0.01	0.6 ± 0.34	0.2 ± 0.35

- Values are means (n = 3 ± SD). Key: VT-Vetiver grass only; AT1VT-Vetiver + attapulgite applied at 1% (*w*/*w*); AT2.5VT-Vetiver + attapulgite applied at 2.5% (*w*/*w*); BT1VT-Vetiver + bentonite applied at 1% (*w*/*w*); BT2.5VT-Vetiver + bentonite applied at 2.5% (*w*/*w*).

3.5. Absorption of Heavy Metals in Indian Mustard

In this study, there was no significant absorption of heavy metals by Indian mustard that could be detected by ICP-OES.

4. Discussion

Factors such as pH, organic carbon, and soil texture can affect the nutrient availability and retention in soil [34]. pH and clay fractions in the soil can lower or increase the mobility of heavy metals in soil, since clay particles increase pH and attract metals onto their charged surfaces. The low nitrogen (N) observed may be due to undecomposed organic materials, which use up the available nitrogen in the soil. Studies have revealed that properties such as surface area and pore volume can determine the environmental application of some materials such as clay minerals [37,45]. The pore spaces in attapulgite and bentonite make them suitable for the adsorption of heavy metals.

Bentonite improved the growth of Vetiver grass, and this was confirmed by other studies [49,50]. Similar to our observations, Kumararaja et al. [49] confirmed that bentonite added at 2.5% (w/w) significantly improved plant growth, by up to 80%. Bentonite improved soil properties, water-holding capacity, biomass, and transpiration rate [48]. Over the years, clay minerals have been used as additives in manufacturing composts and fertilizers, emphasizing their potential to improve soil properties [36,50]. Attapulgite and bentonite have adsorptive properties similar to red mud [34] and can improve Vetiver growth as well as reduce the bioavailability of heavy metals.

For Indian mustard, increased TI was only observed in the BT2.5VT treatment. This indicates that bentonite performed better in improving the yield and tolerance of Indian mustard. Similar to our observation, the addition of biochar to soil significantly stimulated the growth of the Physic nut in Cd-contaminated soil [51]. Biochar enhanced water and nutrient retention, cation exchange capacity, pH, and total organic carbon, due to its high surface area and adsorptive properties, which limited uptake of Cd, thus displaying similar characteristics to clay minerals. Rahman et al. [52] noted that despite the addition of N-fertilizer, Indian mustard produced lower biomass compared to sunflower in Cu- and Pb-contaminated soil.

The heavy metal concentrations observed in Vetiver grass show that it is tolerant to, and can survive in, multiple heavy-metal-polluted environments, corresponding to the findings of previous studies [20,23]. Comparing the absorption of all the heavy metals in Vetiver grass, the highest Ni shoot absorption was observed in the BT2.5VT treatment. It is evident that the amendments did not improve root Ni absorption, but enhanced shoot absorption in all the treatments except AT2.5VT. Attapulgite and bentonite, generally, limited the root absorption of Ni. De Bernadi et al. [53] confirmed that bentonite amendment sequesters Ni and, thus, reduces its bioavailability. Kumararaja et al. [49] found that pillared bentonite stabilized heavy metals in soil and reduced absorption by plants, particularly, at a dosage of 2.5% (w/w) (one of the dosages considered in this study). Kumararaja et al. [49] observed improved plant growth as well as reductions in the bioavailable metals in metal-spiked soils. They also observed that metal concentration in plant biomass reduced with increasing bentonite dosage; the same trend was observed for Cu in our study, but the opposite was the case for attapulgite. Cu and Ni in Vetiver increased with increasing dosage of attapulgite. According to Banerjee et al. [19], Vetiver absorbed heavy metals in the following order, Cu > Zn > Cr > Ni in the roots, but in this study, absorption in Vetiver with no clay treatment was in the order, Cr > Zn > Ni > Cu > Co, similar to the findings of Ng et al. [5]. The findings from this study suggest that attapulgite added at 2.5% could improve the phytostabilization properties of Vetiver for Cr, Co, Cu, and Zn, while Vetiver with attapulgite added at 1% has potential for phytoextraction of Ni. The results confirmed that attapulgite added at 2.5% is a good choice for improving contaminated soil quality and the phytostabilization ability of Vetiver grass.

The TF values < 1 indicate a plant is suitable for phytostabilization or root storage of heavy metals, while TF values > 1 indicate a plant's suitability for phytoextraction [47].

A low TF (TF < 1) was observed for most of the heavy metals considered in this study. This may be due to the metal-adsorbent capacity of the clay minerals' internal and external sites, which may also reduce metal bioavailability [37,49]. Ni translocation to the shoots may be due to metal sequestration in leaf vacuole and apoplast [34]. According to Sharma et al. [54], the AtITEG2 transporter encourages increased Ni sequestration in the vacuoles in the absence of Fe, which may explain the results of this experiment. It was observed that bentonite supported translocation of Cu compared to the other treatments. Banerjee et al. [19] observed that although heavy metals reduced chlorophyll contents, enzyme activities were improved, and the TF of Cu was greater than one in Vetiver. Low TF observed for Cu in this study may be due to its lower bioavailable fraction in the soil. Also, the immobilization of metals by root absorption may have prevented translocation because there is increased immobilization, due to the adsorptive effects of clay minerals [52].

Various factors could be responsible for the insignificant absorption of heavy metals by Indian mustard. A suspected reason for this may be due to the competitive behavior of heavy metals. For example, Yang et al. [55] observed a 90% decrease in uptake when Pb and As were both present in water, compared to when only one trace element was present. Another reason could be the short experimental period (21 d), as Indian mustard did not reach full maturity. According to Rahman et al. [52], Sunflower and Amarant were better than Indian mustard at absorbing Cu and Pb from polluted soil, although a significant concentration of Cu and Pb was still recorded for Indian mustard. In addition, other studies confirmed a reasonable uptake of heavy metals by Indian mustard in contaminated soils [56–58]. Goswami and Das [59] also conducted a greenhouse study that lasted 21 days. They observed reasonable uptake amounts (up to 400 ppm Cd) in the roots and shoots of Indian mustard, but this was in Cd-spiked soil. The Cd may have been easier to absorb in artificially contaminated soil.

5. Conclusions

The present findings confirm that Vetiver grass presented a higher root metal uptake in all the treatments. There was an indication that attapulgite added at 2.5% (*w*/*w*) could improve the phytostabilization properties of Vetiver for Cr, Co, Cu, and Zn, while Vetiver with attapulgite added at 1% had promising potential for the phytoextraction of Ni. Although root absorption increased in the AT2.5VT treatment for Cr, Ni, and Co, shoot uptake was the lowest, suggesting that attapulgite at 2.5% (*w*/*w*) was a better amendment to promote the phytostabilization of heavy metals by Vetiver grass. Based on the translocation factors observed, Vetiver grass behaved as a phytostabilizer for all the heavy metals (Co, Cr, Cu, and Zn) and efficiently translocated Ni (TF > 1), serving as a good phytoextractor and phytostabilizer. The results from this study signify that attapulgite can successfully improve the phytostabilization of heavy metals by Vetiver grass. This research contributes to the knowledge repository of suitable amendments that can improve the phytoremediation properties of Vetiver grass.

Author Contributions: Conceptualization, B.O.O., M.P.A., M.T. and O.O.O.; data curation, B.O.O., A.G.-A., R.J. and J.C.H.; formal analysis, B.O.O., A.G.-A., R.J. and J.C.H.; funding acquisition, O.O.O.; investigation, B.O.O., M.P.A., M.T. and O.O.O.; methodology, B.O.O., M.P.A., M.T. and O.O.O.; project administration, B.O.O., M.P.A., M.T. and O.O.O.; resources, B.O.O., M.P.A., M.T., A.G.-A., R.J., J.C.H. and O.O.O.; software, B.O.O.; supervision, M.P.A., M.T. and O.O.O.; validation, B.O.O.; visualization, B.O.O.; writing–original draft, B.O.O.; writing–review and editing, B.O.O., M.P.A., M.T., A.G.-A., R.J., J.C.H. and O.O.O. All authors contributed to the critical revision of the manuscript and approved the final manuscript.

Funding: This research was funded by the Faculty of Natural and Agricultural Sciences Central Research Fund, University of the Free State.

Institutional Review Board Statement: This study was approved by the Ethical Committee, University of the Free State, with clearance number: UFS-ESD2021/0070/21.

Informed Consent Statement: All the authors consented to participate in the drafting and submission of this manuscript.

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

Acknowledgments: The authors would like to thank Hydromulch (Pty) Ltd., Johannesburg, South Africa, for sponsoring the vetiver grass used in this research. The authors are grateful to AttaClay (Pty) Ltd., Germiston, South Africa, for sponsoring the clay minerals (bentonite and attapulgite) that were used in this study.

Conflicts of Interest: 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.

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