



Check for updates

**AUTHORS:**

Beatrice O. Otunola<sup>1</sup>   
Makhosazana P. Aghoghovwia<sup>2</sup>   
Melusi Thwala<sup>1,3</sup>   
Olusola O. Ololade<sup>1</sup>

**AFFILIATIONS:**

<sup>1</sup>Centre for Environmental Management, University of the Free State, Bloemfontein, South Africa  
<sup>2</sup>Department of Soil, Crop and Climate Sciences, University of the Free State, Bloemfontein, South Africa  
<sup>3</sup>Science Advisory and Strategic Partnerships, Academy of Science of South Africa, Pretoria, South Africa

**CORRESPONDENCE TO:**

Beatrice Otunola

**EMAIL:**

omobeat15@gmail.com

**DATES:**

**Received:** 05 Apr. 2023

**Revised:** 23 July 2023

**Accepted:** 07 Aug. 2023

**Published:** 29 Nov. 2023

**HOW TO CITE:**

Otunola BO, Aghoghovwia MP, Thwala M, Ololade OO. A mesocosm study on the use of clay minerals to improve heavy metal phytoremediation capacity of vetiver grass (*Chrysopogon zizanioides* L. Roberty). S Afr J Sci. 2023;119(11/12), Art. #15882. <https://doi.org/10.17159/sajs.2023/15882>

**ARTICLE INCLUDES:**

- Peer review
- Supplementary material

**DATA AVAILABILITY:**

- Open data set
- All data included
- On request from author(s)
- Not available
- Not applicable

**EDITOR:**

Teresa Coutinho

**KEYWORDS:**

heavy metals, contaminated soil, contaminated water, assisted phytoremediation, phytoremediation, vetiver grass

**FUNDING:**

University of the Free State



# A mesocosm study on the use of clay minerals to improve heavy metal phytoremediation capacity of vetiver grass (*Chrysopogon zizanioides* L. Roberty)

Fast-paced global industrialisation due to population growth poses negative environmental implications, such as pollution by heavy metals. We assessed the application of vetiver grass assisted by clay minerals for the remediation of soil and water contaminated by multiple metals in a mesocosm study. The technique was tested previously in a greenhouse study that confirmed the effectiveness of 2.5% (w/w) attapulgite and 2.5% (w/v) bentonite to improve vetiver grass remediation of soil and water contaminated by multiple metals. At the end of the experiment, the total accumulation of Co, Cr, Cu, Ni and Zn by vetiver grass from the soil was 1.8, 38.1, 19.0, 7.2 and 55.4 mg/kg, respectively, while in water, the total metal accumulation of Al and Mn by vetiver grass was 4534.5 and 104.5 mg/kg, respectively. The results confirm the effectiveness of attapulgite and bentonite as amendments to improve the remediation potential of vetiver in soil and water under natural conditions. Metal accumulation was generally higher in the roots than in shoots. We found the removal efficiency in the soil to be in the order Zn > Cr > Cu > Ni > Co and Al > Mn in water. Results also demonstrated that heavy metal accumulation was even better under natural conditions than in the greenhouse study. For example, Zn accumulation increased from 0.4 mg/kg in the greenhouse study to 55.4 mg/kg in the outdoor study. This study validates the application of bentonite and attapulgite-assisted phytoremediation for heavy metal contaminated soil and water.

**Significance:**

- Heavy metal pollution of soil and water is very common in industrialised and mining areas.
- It is important to find cost-effective, eco-friendly and easy-to-apply methods of removing these heavy metals from soil and water, so as to provide a clean and safe environment for living organisms.
- Phytoremediation is the use of plants to remove pollutants from the environment and is a cost-effective, aesthetically pleasing and eco-friendly method.
- Attapulgite and bentonite (clay minerals) are effective in improving the phytoremediation capacity of a phytoremediation plant known as vetiver grass.

## Introduction

Rising global industrialisation and urbanisation consequentially increase the release of pollutants into the environment. While some pollutants are natural elements, anthropogenic activities can increase their environmental release, for instance, heavy metals. Naturally, heavy metals are commonly associated with bedrock, but due to anthropogenic activities, they have become a major class of environmental pollutants that adversely affect soil ecology and productivity and surface water and groundwater quality, thereby threatening biodiversity.<sup>1</sup> Most heavy metals are highly toxic to biota even at low concentrations.<sup>2</sup> Although some heavy metals are essential nutrients, they can be toxic when present at excessive concentrations.<sup>3</sup> For instance, Al and Mn can be more easily accumulated by living organisms from water than from other sources<sup>4</sup>, and negatively affect many cell functions such as detoxification, brain function and metabolism, and can cause deoxyribonucleic acid (DNA) and tissue damage<sup>4,5</sup>.

South Africa is rich in natural resources and has some of the world's largest reserves of gold, coal and platinum.<sup>6</sup> As a result of the exploitation of these resources, the legacy of mining has caused considerable heavy metal pollution, posing a risk to human and animal life. For example, health effects such as chest pain, wheezing, tuberculosis, diarrhoea, cough and itchy skin due to people's proximity to and contact with mine tailings have been reported in Gauteng, Mpumalanga, North West and Limpopo Provinces of South Africa.<sup>7-9</sup> Furthermore, animals from mining communities in the North West and Gauteng Provinces have high levels of heavy metals in their faeces and serum due to the mining in these areas.<sup>10,11</sup>

The KwaZulu-Natal, Mpumalanga, Limpopo and Free State Provinces hold the highest coal reserves in South Africa. Yearly, close to 65 Mt of waste is produced from coal processing; this waste contains high levels of sulfur and heavy metals which, upon release, pose an environmental risk.<sup>12</sup> Such risks include the disruption of soil and water ecosystems, the release of toxic metals into the food chain, and absorption through the skin.<sup>3,13</sup> Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn as environmental pollutants are commonly associated with coal.<sup>14,15</sup> In particular, heavy metals including Co, Cr, Cu, Ni, and Zn have been detected in the soil, while Al and Mn have been detected in water bodies surrounding a former coal mining environment located in Sasolburg in the Free State Province of South Africa.<sup>14,16,17</sup>

Considering the negative effects of heavy metals on the environment, several technologies have been identified for managing heavy metal polluted sites. Of these technologies, phytoremediation is a cost-effective and environmentally friendly option<sup>18</sup>, and there is growing interest in the application and optimisation of phytoremediation<sup>19,20</sup>. Vetiver grass (*Chrysopogon zizanioides*) is a terrestrial plant that has been adapted for soil and water remediation purposes because it is easily propagated, with rapid growth and can survive in extreme climatic conditions. It has been

applied to wetlands, industrial wastewaters, mine tailings and agricultural soils.<sup>21-23</sup> A combination of two or three remediation options can result in more efficient outcomes<sup>24</sup>, with combinations of amendments such as compost, red mud, clays, and biochar being applied for this purpose<sup>19,25</sup>. In addition, for a practical, real-life application of phytoremediation, mesocosm studies are essential to examine efficiency because mesocosms hold heightened environmental realism whilst allowing control of some environmental parameters.<sup>26</sup> Simply put, a mesocosm is an experimental setup in which some variables are controlled under natural conditions. Clay minerals are hydrous aluminosilicates that are naturally occurring, and possess high surface areas and cation exchange capacity, thus encouraging their application in many areas including adsorption and absorption of pollutants. Major examples of clay minerals include attapulgite, bentonite, montmorillonite, zeolite and kaolinite.<sup>13</sup> Previous studies by Otunola et al.<sup>16,17</sup> showed that attapulgite administered at 2.5% (w/w) was most effective to improve the phytoremediation capacity of vetiver grass in metals polluted soil, while bentonite administered at 2.5% (w/v) was best for water remediation.

In this study, we aimed to examine the efficiency of an optimised hybrid application of vetiver grass and clay minerals for remediation of soil and water contaminated with heavy metals (Al and Mn in water; and Co, Cr, Cu, Ni and Zn in soil) in a mesocosm setting as informed by success in previous greenhouse studies.<sup>16,17</sup> There is little documentation of mesocosm studies concerning assisted phytoremediation of soil and water<sup>27-29</sup>; therefore, this study contributes to the repository of available studies of phytoremediation in mesocosms, further encouraging its application.

## Materials and methods

### Sample collection

Soil and water samples were collected from a former coal mining area (26°50'50.4"S; 27°49'49.7"E) in Sasolburg, Free State Province, South Africa. The mining area is at a rehabilitation stage and several post-mining land uses have been implemented.<sup>16,17</sup> The general geology of Sasolburg comprises sandstone and shale, which have been intruded by dolerites in some localities. With a grazed grassland vegetation type, the area experiences summer rainfall and average temperatures of 21 °C during the summer season and 9 °C in winter.<sup>14</sup> A composite sampling method was employed to collect the soil samples from a depth of 20 cm using a shovel. The samples were stored in tightly sealed polypropylene bags and transported to the experimental site in Bloemfontein, Free State, South Africa (29°12'40.3"S; 26°20'42.4"E). The water samples were specifically collected from the Leeuspruit River, which flows through the mine boundaries. Water samples were collected in 25 L jerry cans following pre-rinsing with site water. Soil and water pH were measured before and after the experiment using a calibrated standard multi-parameter probe (YSI Incorporated, Model 85D, I.N058500, SN 09K 100684, Yellow Springs, Ohio, USA).<sup>30,31</sup> The pH in the soil and

water before the experiment was 6.2 and 6.6, respectively. Sampling was done in triplicate and samples were sent to the Central Analytical Facility, Stellenbosch University, South Africa for the determination of total heavy metal concentration using a Flexible Single Quadrupole Agilent 7900 Q ICP-MS.

### Outdoor experiments

Dead plant material and gravel were removed from the soil at the experimental site, then packed into plastic pots of 12 L capacity. Previous greenhouse experiments established that treatment AT2.5VT (attapulgite mixed with soil at 2.5% (w/w) + vetiver grass) and treatment BT2.5VT (bentonite mixed with water at 2.5% (w/w) + vetiver grass) were the most efficient of the tested hybrid treatments for metals contaminated soil and water<sup>16,17</sup>; therefore, only these two treatments were considered in this study as the aim was to test their performance under natural conditions.

Vetiver grass obtained from Hydromulch (Pty) Ltd, Johannesburg, South Africa, was used for both treatments. The vetiver grass was thoroughly washed using municipal tap water and then distilled water; after that, the grasses were trimmed to a shoot length of 30 cm and root length of 10 cm before being transplanted into the soil pots and watered with 500 mL of municipal tap water every two days in order to maintain the soil moisture content. For the water treatment, 8L plastic pots were filled with the water samples from the study area, and bentonite was added at 2.5% (w/v), after which vetiver grass of the same shoot and root lengths was transplanted into the water pots. The vetiver grass plants were placed over the water and pots which were maintained at the same level throughout the experiment i.e. refilled to the initial volume (8L) with water samples whenever the water levels became low due to transpiration, evaporation and/or plant uptake. Negative controls (no treatment) for soil and water were also set up. All the experimental pots were arranged in a randomised complete block design and maintained under natural sunlight, air, humidity and temperature (average 28 °C day and 10 °C night). Each treatment was done in triplicate (Figure 1).

### Plant, soil and water sampling

The experiment lasted for 21 days, after which the plants were harvested and thoroughly cleaned in deionised water. Fresh biomass, root and shoot lengths were measured. The plant parts were oven dried at 75 °C for 72 h, after which dry biomass was recorded. The dried plant parts were then milled and microwave digested following the US EPA procedure (Method 3052). Translocation factor (TF) is a plant's ability to transfer heavy metals from its roots (below ground parts) to its shoots (above ground parts).<sup>17</sup> Bioconcentration factor (BCF) is a plant's ability to remove heavy metals from substrates (soil or water) and accumulate the heavy metals within its roots and shoots. TF and BCF were determined at the end of the experiment.



Figure 1: Outdoor experimental setup for soil and water remediation.

### Statistical analysis

All data were subjected to statistical analysis and expressed as the mean  $\pm$  standard deviation of three replicates and descriptive statistics were obtained. Using R software version 4.0.0 (2020)<sup>32</sup>, a one-way analysis of variance (ANOVA) was carried out separately for metals accumulated in the roots and shoots (at  $p < 0.05$ ) to compare the means of the accumulated metals and determine any statistically significant differences between the accumulated metals in each plant part. Tukey's post-hoc test was done to determine the treatments with significant differences.

## Results

### pH and heavy metal content in soil and water before treatment

The pH detected in the soil and water before the experiment was 6.2 and 6.6, respectively. The initial heavy metals in the soil before treatment were  $39.4 \pm 1.2$  mg/kg Cr,  $5.8 \pm 0.5$  mg/kg Co,  $13.5 \pm 0.1$  mg/kg Ni,  $9.1 \pm 0.4$  mg/kg Cu and  $28.4 \pm 4.3$  mg/kg Zn. The initial (before treatment) heavy metal concentration in water was  $0.05 \pm 0.2$  mg/L and  $0.18 \pm 0.3$  mg/L for Al and Mn, respectively.

### Biomass and heavy metal accumulation of plants

The vetiver grass under the influence of clay amended contaminated soil and water was assessed by observing its physical changes and metal accumulation. The morphological properties of the plants in soil and water treatments are presented in Table 1.

For the treated water, Al uptake was  $4177.7 \pm 0.47$  mg/L and  $356.8 \pm 0.22$  mg/L in roots and shoots, respectively, while uptake of Mn was

$68.2 \pm 38.8$  mg/L and  $36.3 \pm 3.2$  mg/L in the roots and shoots of vetiver grass, respectively.

For the treated soil, the concentrations of heavy metals in plant parts at the end of the outdoor experiment are shown in Figure 2.

### Translocation and bioconcentration factors

For vetiver grass in the BT2.5VT water treatment, the translocation factor (TF) for Al was very low (0.09), while the TF for Mn was 0.53. The bioconcentration factor (BCF) was very high for both Al and Mn (Table 2).

In the AT2.5VT treatment for soil, vetiver showed a TF of 0.06–0.36 for the various heavy metals (Table 3). The BCF obtained for Cr, Co and Ni was  $<1$ , whereas BCF values observed for Cu and Zn were  $>1$  (Table 3).

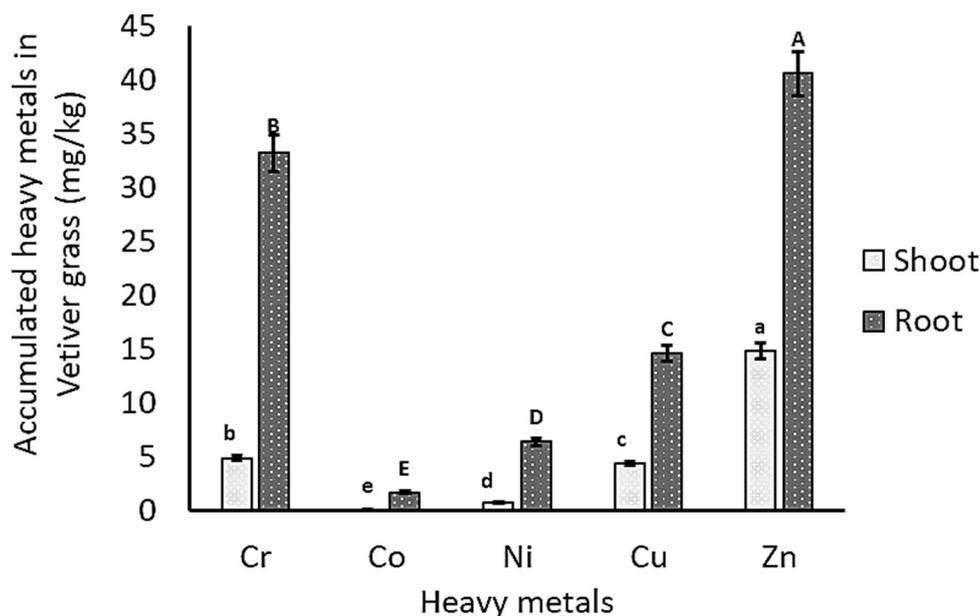
## Discussion

Outdoor mesocosm was used to mimic the real-life expected water and soil treatment conditions for vetiver grass in contaminated media. The pH observed in the soil and water were similar and close to neutral (6.2 and 6.6 for soil and water, respectively), thus encouraging moderate mobility of heavy metals. The final root and shoot lengths observed at the end of the experiment indicate that vetiver grass indeed is a fast-growing crop. An increase in shoot length of up to 4 cm was observed within 21 days, giving the plant more surface area for metal accumulation. This is because the rate of phytoremediation is proportional to the plant growth rate, where high biomass crops like vetiver are a very good option for phytoremediation. Vetiver grass uses a C4 photosynthetic pathway with higher rates of photosynthesis at high light intensities, supporting the better performance of vetiver in the outdoor experiment.<sup>21</sup> The presence of Cu in the soil may also have affected its biomass yield,

**Table 1:** Morphological properties of vetiver grass in water treated with vetiver + bentonite applied at 2.5% (w/v) (BT2.5VT) and soil treated with vetiver + attapulgite applied at 2.5% (w/w) (AT2.5VT)

Property / treatment	Shoot length (cm)	Root length (cm)	Fresh shoot biomass (g)	Fresh root biomass (g)	Dry shoot biomass (g)	Dry root biomass (g)
BT2.5VT	$36.5 \pm 2.1$	$16.5 \pm 2.1$	$33.8 \pm 17.3$	$23.7 \pm 2.6$	$10.9 \pm 5.1$	$8.2 \pm 1.1$
AT2.5VT	$44.0 \pm 4.2$	$15.5 \pm 2.1$	$30.3 \pm 5.8$	$17.8 \pm 1.6$	$11.9 \pm 2.6$	$6.7 \pm 0.4$

Values are means  $\pm$  SD,  $n = 3$



**Figure 2:** Concentrations of heavy metals in roots and shoots of vetiver grass in the soil treatment at the end of the experiment. Values are means ( $\pm$  SD;  $n = 3$ ). Error bars represent per cent errors. Uppercase letters on top of the bars show statistically significant differences in root accumulation, while the lowercase letters show statistically significant differences in shoot accumulation.

**Table 2:** Root and shoot metals concentration, translocation factor (TF) and bioconcentration factor (BCF) observed for vetiver grass in the water treatment

Heavy metal	Initial concentration <sup>a</sup> in water (mg/L)	BT2.5VTS (mg/kg)	BT2.5VTR (mg/kg)	TF	BCF
Al	0.05 ± 0.2	356.80 ± 0.2	4177.70 ± 0.5	0.09 ± 0.4	90 690.00 ± 3.5
Mn	0.18 ± 0.3	36.31 ± 3.2	68.21 ± 38.8	0.53 ± 0.1	580.60 ± 1.7

BT2.5VTS, metal concentration in shoots of vetiver; BT2.5VTR, metal concentration in roots of vetiver

<sup>a</sup>Before treatment

Values are mean ± SD, n = 3

**Table 3:** Root and shoot metals concentration, translocation factor (TF) and bioconcentration factor (BCF) observed for vetiver grass in the soil treatment

Heavy metal	Initial concentration <sup>a</sup> in soil (mg/kg)	AT2.5VTS (mg/kg)	AT2.5VTR (mg/kg)	TF	BCF
Cr	39.4 ± 1.2	4.9 ± 0.4	33.2 ± 12.9	0.15 ± 0.0	0.96 ± 10.8
Co	5.8 ± 0.5	0.1 ± 0.0	1.7 ± 0.2	0.06 ± 0.2	0.31 ± 0.4
Ni	13.5 ± 0.1	0.8 ± 0.0	6.4 ± 0.8	0.13 ± 0.4	0.53 ± 0.8
Cu	9.1 ± 0.4	4.4 ± 0.1	14.6 ± 1.3	0.30 ± 0.1	2.08 ± 3.5
Zn	28.4 ± 4.3	14.8 ± 0.2	40.6 ± 12.4	0.36 ± 0.0	1.95 ± 2.9

AT2.5VTS, metal concentration in shoots of vetiver; AT2.5VTR, metal concentration in roots of vetiver

<sup>a</sup>Before treatment

Values are mean ± SD, n = 3

as Liu et al.<sup>33</sup> observed that Cu at high concentrations can enhance the growth and dry weight of vetiver grass.

The vetiver grass that was grown in the water treated with bentonite applied at 2.5% (w/v) (BT2.5VT treatment) showed root Al and Mn accumulation that was greater than shoot accumulation, but the higher the root uptake, the higher the shoot uptake as well. Al accumulation was 4177.7 ± 0.5 mg/L and 356.8 ± 0.2 mg/L in roots and shoots, respectively, while Mn was 68.2 ± 38.8 mg/L and 36.3 ± 3.2 mg/L in roots and shoots, respectively (Table 2). The higher root accumulation of both Al and Mn corresponds to findings of previous studies on the application of vetiver grass for metal removal.<sup>19,34</sup> The total (combined root and shoot) Mn accumulation in this experiment was 104.5 mg/L, which is 88% higher than Mn accumulation in a previous greenhouse experiment.<sup>17</sup> Likewise, the total amount of Al accumulated by vetiver in the mesocosm experiment was 4534.1 mg/L, while the total Al accumulation in the greenhouse experiment was only 371.8 mg/L.<sup>17</sup> It is evident that vetiver absorbed much higher amounts of Al and Mn in the outdoor mesocosm experiment than in the greenhouse experiment. This could be due to the lower initial concentrations of Al and Mn in the outdoor experiment. In the outdoor experiments, the Al and Mn concentrations were 0.05 mg/L and 0.18 mg/L, respectively, which was much lower than the concentrations in the greenhouse study – 5 mg/L and 1 mg/L, respectively. The natural conditions (such as temperature, sunlight, humidity and air) of the present mesocosm experiment may also account for the higher metal accumulation observed.<sup>26</sup> Different periods of sampling may also be a reason for the higher accumulation rates, because wet seasons increase the solubility of nutrients, thus increasing accumulation by plants. The rate of water evaporation from the plant leaves is also higher in the summer and spring seasons than in colder periods, and evaporating water serves as a pump for nutrients and heavy metals.<sup>35</sup>

The heavy metals accumulated by vetiver grass grown in the contaminated soil varied with metal type and initial concentration in the soil. After treatment, the order of abundance of heavy metals in the soil was Cr > Zn > Ni > Cu > Co. A similar order was observed for Zn and Cu by Kafil et al.<sup>36</sup> and there was a slight reduction of heavy metals in the untreated media. The order of accumulation in the roots of vetiver in this mesocosm experiment was Zn > Cr > Cu > Ni > Co, while for the greenhouse experiment, the order was Zn > Cr > Ni > Cu > Co, and

the total amount of Zn was ~ 4 mg/kg, while the total Zn in this outdoor study was 55.4 mg/kg. Also, there was no shoot accumulation of Co and Zn in the greenhouse experiment reported by Otunola et al.<sup>16</sup>, but these were found in concentrations of 0.1 mg/kg and 14.8 mg/kg, respectively, in the mesocosm study. A similar trend was observed in the root and shoot accumulation for all the heavy metals, whereby root accumulation was significantly greater than shoot accumulation (Table 4). It should also be noted that the same trend was observed for root and shoot metal accumulation in the previous greenhouse studies.<sup>16,17</sup>

In comparing the results of this outdoor experiment to previous greenhouse studies by Otunola et al.<sup>16,17</sup>, as shown in Table 4, we found that in both the greenhouse and outdoor experiments, vetiver showed no signs of growth inhibition in the soil and water treatments. Also, the outdoor experiment yielded better results for both water and soil remediation. A reason for the better performance of vetiver in the outdoor experiment could be due to its exposure to natural conditions, including sunlight, air, humidity and the right temperatures for vetiver.<sup>21</sup> For soil remediation, the order of metals accumulation in the roots of vetiver grass showed a very similar trend to the observation from the greenhouse experiment.<sup>16</sup>

According to Gravand et al.<sup>37</sup>, vetiver absorbed Ni (69.4 mg/kg), Mn (63.3 mg/kg) and Pb (282.5 mg/kg). After adding humic acid, Zn and Cu accumulation was promoted, although translocation was reduced due to low bioavailability.<sup>22</sup> Chelating agents and red mud have also been used to promote the growth and remediation capacity of vetiver grass.<sup>38,39</sup> These studies indicate that there was more root accumulation than shoot accumulation of heavy metals in vetiver grass. Vetiver mostly stores heavy metals in its roots or cell walls and heavy metals are likely to reduce water transport to shoots, thereby limiting the translocation of heavy metals. Coupled with the effects of clay minerals, metals sequestering in the roots and vacuoles may be responsible for reduced translocation. The positive side of metal sequestering and reduced translocation is that it limits translocation, so heavy metals will not damage photosynthetic organs.

Suelee et al.<sup>34</sup> observed that root length and density affected heavy metal accumulation by vetiver grass. Higher metal accumulation was achieved at higher root length and density and lower initial metal concentration.<sup>34</sup> In an experiment that tested the use of vetiver for industrial wastewater

**Table 4:** Comparison of heavy metals accumulated by the roots and shoots of vetiver grass in the present mesocosm study and previous greenhouse studies

Heavy metal	Present mesocosm study		Greenhouse studies <sup>16,17</sup>	
	Shoot (mg/kg)	Roots (mg/kg)	Shoot (mg/kg)	Roots (mg/kg)
Al	356.80 ± 0.2	4177.70 ± 0.5	41.10 ± 0.3	330.70 ± 0.6
Co	0.10 ± 0.0	1.70 ± 0.2	BDL	1.39 ± 0.7
Cr	4.90 ± 0.4	33.20 ± 12.98	0.15 ± 0.0	2.79 ± 1.1
Cu	4.40 ± 0.1	14.60 ± 1.38	0.02 ± 0.0	1.67 ± 0.9
Mn	36.31 ± 3.2	68.21 ± 38.8	3.49 ± 0.9	14.40 ± 0.7
Ni	0.80 ± 0.0	6.40 ± 0.8	0.22 ± 0.1	1.38 ± 0.6
Zn	14.80 ± 0.2	40.61 ± 12.4	BDL	0.43 ± 0.2

BDL, below detection limit

treatment, it was found that vetiver behaved differently depending on the industry and wastewater type.<sup>40</sup> The study also revealed that Cu toxicity resulted in stunted growth, but organic fertiliser increased vetiver yield.<sup>40</sup> The addition of bentonite also reduced the bioavailable Ni in lime and wastewater.<sup>20,41</sup> This is typical of clay minerals, which reduce the bioavailable properties of heavy metals as they adsorb these metals onto their surfaces.<sup>13,19</sup>

Vetiver is also tolerant to Zn, absorbing up to 10 000 mg/kg within 30 days with a high translocation factor.<sup>42</sup> Ni is an essential trace element that improves crop yield, but its behaviour in plants is not yet well understood.<sup>21</sup> The concentration of Ni in the soil before treatment was 13.5 mg/kg, and a total of 7.2 mg/kg was accumulated within 21 days in the AT2.5VT treatment, which is a promising amount.

In evaluating a plant's ability for phytoremediation, after determining the amount of heavy metals taken up by the plant, the translocation factor (TF) and bioconcentration factor (BCF) should be calculated to examine the absorption and transfer of the metals. The TF is the ability of a plant to translocate metals from its roots to shoot and is calculated as the ratio of heavy metal concentration in the shoot to the concentration in its roots. TF values <1 indicate a plant is suitable for phytostabilisation or root storage of heavy metals, while TF values >1 indicate a plant's suitability for phytoextraction.<sup>31</sup> The BCF is the capability of plants to remove heavy metals in soil or water and accumulate them within their shoots and roots. This is expressed as the ratio of heavy metals in plants to that of the substrate.<sup>31</sup>

The observed TF values in this study correspond to the findings of Roongtanakiat<sup>40</sup> who observed TF values of 0.07 to 0.67 and indicated that the maturity of vetiver affects its ability to translocate heavy metals. The older vetiver gets, the less it can translocate heavy metals.<sup>31</sup> According to Roongtanakiat<sup>40</sup>, vetiver demonstrated higher TF values for heavy metals in soil compared to water. The type and nature of the amendment applied can also increase or reduce translocation.<sup>19</sup> In this study, clay minerals were noted to increase root sequestration of heavy metals and reduce translocation. In the work of Danh et al.<sup>21</sup>, a low TF was observed for As, Cd, Cr and Hg in vetiver, leading to only 16–30% translocation of heavy metals. Similar to the observation of Kafil et al.<sup>36</sup>, BCF was 1.30 for Cu and 1.98 for Zn. Siyar et al.<sup>43</sup> found that, within 21 days, the phytoremediation potential of vetiver can be improved by electrokinetic energy. They also observed a BCF >1 in multiple metal contaminated sites.

Although the concentrations of Al and Mn at the start of the experiment were low (0.05 ± 0.2 and 0.18 ± 0.3 mg/L, respectively), Bokhari et al.<sup>44</sup> also observed that metal removal percentage was high (up to 80%) in *Lemna minor* L. despite a very low initial metal concentration. Aisien et al.<sup>45</sup> observed metal concentrations as high as 4870 mg/kg, 4150 mg/kg and 710 mg/kg for Zn, Pb and Cd, respectively, while a BCF of up to 1674 was recorded for Zn. Likewise, Rai et al.<sup>46</sup> recorded a BCF as

high as 36 500 for Cd, confirming *Spirodella polyrrhiza* as an excellent hyperaccumulator for heavy metals.

As the observed BCFs are higher than 1 for most of the heavy metals in this study, while TF is <1, vetiver is recommended for phytostabilisation/rhizofiltration of Al, Co, Cu, Cr, Mn, Ni and Zn. Overall, the BCF and TF values differed by metal type and the treated media in this study.

## Conclusion

In the present study we have shown that vetiver grass has the potential to remediate heavy metal contaminated soil and water. There was a high BCF in the water experiment and for some metals in the soil experiment. BCF was <1 for Co, Cr and Ni but >1 for Cu, Zn, Al and Mn, while the translocation factor was <1 for all the heavy metals. The clay minerals restricted the translocation of some heavy metals from roots to shoots, which is considered an advantage because the adsorptive properties of these clays restricts the leaching of heavy metals from soil to water while controlling the amount of metals translocated to the shoots, thereby reducing metal toxicity in vetiver. Overall, the remediation levels achieved were higher than those obtained in greenhouse experiments, meaning that vetiver grass shows better performance in soil and water remediation in situ (outdoors under natural conditions). This demonstrates that attapulgite and bentonite are suitable for improving the phytoremediation capacity of vetiver for removing metal contaminants from soil and water. The results suggest that vetiver grass survives different concentrations of heavy metals in soil and water; and, in combination with clay minerals, it could be useful in a real-life scenario for the remediation of heavy metals contaminated soil and water.

## Acknowledgements

We thank Hydromulch (Pty) Ltd, Johannesburg, South Africa, for sponsoring the vetiver grass used in this research. We are grateful to AttaClay (Pty) Ltd, Germiston, South Africa, for sponsoring the clay minerals (bentonite and attapulgite) that were used in this study. Funding was provided by the Faculty of Natural and Agricultural Sciences Central Research Fund, University of the Free State.

## Competing interests

We have no competing interests to declare.

## Authors' contributions

B.O.O.: Conceptualisation; methodology; data collection; sample analysis; data analysis; validation; data curation; writing – the initial draft; writing – revisions; project management. M.P.A.: Conceptualisation; methodology; writing – revisions; student supervision; project leadership. M.T.: Conceptualisation; methodology; data collection; writing – revisions; student supervision; project leadership. O.O.O.: Conceptualisation; methodology; writing – revisions; student supervision; project leadership; funding acquisition.



## References

- Mishra S, Bharagava RN, More N, Yadav A, Zainith S, Mani S, et al. Heavy metal contamination: An alarming threat to environment and human health. In: Sobti R, Arora N, Kothari R, editors. Environmental biotechnology: For sustainable future. Singapore: Springer; 2019. p.103–125. [https://doi.org/10.1007/978-981-10-7284-0\\_5](https://doi.org/10.1007/978-981-10-7284-0_5)
- Vardhan KH, Kumar PS, Panda RC. A review on heavy metal pollution, toxicity and remedial measures: Current trends and future perspectives. *J Mol Liq*. 2019;290:111197. <https://doi.org/10.1016/j.molliq.2019.111197>
- Masindi V, Muedi KL. Environmental contamination by heavy metals. *Heavy Metals*. 2018;10:115–132. <https://doi.org/10.5772/intechopen.76082>
- Bouchard M, Laforest F, Vandelay L, Bellinger D, Mergler D. Hair manganese and hyperactive behaviors: Pilot study of school-age children exposed through tap water. *Environ Health Perspect*. 2007;115(1):122–127. <https://doi.org/10.1289/ehp.9504>
- Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. In: Luch A, editor. Molecular, clinical and environmental toxicology. Basel: Springer; 2012. p. 133–164. [https://doi.org/10.1007/978-3-7643-8340-4\\_6](https://doi.org/10.1007/978-3-7643-8340-4_6)
- Simplified geological map of South Africa [webpage on the Internet]. c2023 [cited 2020 May 01]. Available from: <http://www.geoscience.org.za/index.php/publication/downloadable-material>
- Kamunda C, Mathuthu M, Madhuku M. Health risk assessment of heavy metals in soils from Witwatersrand Gold Mining Basin, South Africa. *Int J Environ Res Public Health*. 2016;13(7):663. <https://doi.org/10.3390/ijerph13070663>
- Overy N. Mining communities bear the burden of disease [webpage on the Internet]. c2020 [cited 2020 May 20]. Available from: <https://www.newframe.com/mining-communities-bear-the-burden-of-disease/#:~:text=Research%20has%20shown%20that%20living,coughs%2C%20pneumonia%20and%20chronic%20bronchitis>
- Rice B, Boccia D, Carter DJ, Weiner R, Letsela L, De Wit M, et al. Health and wellbeing needs and priorities in mining host communities in South Africa: A mixed-methods approach for identifying key SDG3 targets. *BMC Pub Health*. 2022;22(1):1–11. <https://doi.org/10.1186/s12889-021-12348-6>
- Dzoma BM, Moralo RA, Motsele LE, Ndou RV, Bakunzi FR. Preliminary findings on the levels of five heavy metals in water, sediments, grass and various specimens from cattle grazing and watering in potentially heavy metal polluted areas of the North West Province of South Africa. *J Anim Vet Adv*. 2010;9(24):3026–3033. <https://doi.org/10.3923/javaa.2010.3026.3033>
- Sedibe M, Achilonu MC, Tikilili P, Shale K, Ebenebe PC. South African mine effluents: Heavy metal pollution and impact on the ecosystem. *Int J Chem Sci*. 2017;15(4):198.
- South African Department of Mineral Resources and Energy.Coal resources [webpage on the Internet]. No date [cited 2020 May 01]. Available from: [http://www.energy.gov.za/files/coal\\_frame.html](http://www.energy.gov.za/files/coal_frame.html)
- Otunola BO, Ololade OO. A review on the application of clay minerals as heavy metal adsorbents for remediation purposes. *Environ Technol Innov*. 2020;100692. <https://doi.org/10.1016/j.eti.2020.100692>
- Wessels L. Decant of sigma colliery [dissertation]. Bloemfontein: University of the Free State; 2013.
- Digby Wells Environmental. Aquatic impact assessment [webpage on the Internet]. c2018 [cited 2020 May 03]. Available from: [https://sahris.sahra.org.za/sites/default/files/additionaldocs/Appendix7\\_Aquatics.pdf](https://sahris.sahra.org.za/sites/default/files/additionaldocs/Appendix7_Aquatics.pdf)
- Otunola BO, Aghoghovwia MP, Thwala M, Gómez-Arias A, Jordaan R, Hernandez JC, et al. Influence of clay mineral amendments characteristics on heavy metals uptake in vetiver grass (*Chrysopogon zizanioides* L. Roberly) and Indian mustard (*Brassica juncea* L. Czern). *Sustainability*. 2022;14(10), Art. #5856. <https://doi.org/10.3390/su14105856>
- Otunola BO, Aghoghovwia MP, Thwala M, Gómez-Arias A, Jordaan R, Hernandez JC, et al. Improving capacity for phytoremediation of vetiver grass and Indian mustard in heavy metal (Al and Mn) contaminated water through the application of clay minerals. *Environ Sci Pollut Res*. 2023;30:53577–53588. <https://doi.org/10.1007/s11356-023-26083-5>
- Ali H, Khan E, Sajad MA. Phytoremediation of heavy metals-concepts and applications. *Chemosphere*. 2013;91(7):869–881. <https://doi.org/10.1016/j.chemosphere.2013.01.075>
- Ng CC, Boyce AN, Rahman MM, Abas MR. Effects of different soil amendments on mixed heavy metals contamination in vetiver grass. *Bull Environ Contam Toxicol*. 2016;97(5):695–701. <https://doi.org/10.1007/s00128-016-1921-5>
- De Bernardi A, Casucci C, Businelli D, D'Amato R, Beone GM, Fontanella MC, et al. Phytoremediation potential of crop plants in countering nickel contamination in carbonation lime coming from the sugar industry. *Plants*. 2020;9(5):580. <https://doi.org/10.3390/plants9050580>
- Danh LT, Truong P, Mammucari R, Tran T, Foster N. Vetiver grass, *Vetiveria zizanioides*: A choice plant for phytoremediation of heavy metals and organic wastes. *Int J Phytoremediation*. 2019;11(8):664–691. <https://doi.org/10.1080/15226510902787302>
- Vargas C, Pérez-Esteban J, Escolástico C, Masaguer A, Moliner A. Phytoremediation of Cu and Zn by vetiver grass in mine soils amended with humic acids. *Environ Sci Pollut Res*. 2016;23(13):13521–13530. <https://doi.org/10.1007/s11356-016-6430-x>
- Singh S, Suvarna S, Kiran K, Fulzele P. Investigation of arsenic accumulation and biochemical response of in vitro developed *Vetiveria zizanioides* plants. *Ecotoxicol Environ Saf*. 2017;145:50–56. <https://doi.org/10.1016/j.ecoenv.2017.07.013>
- Sabeen M, Mahmood Q, Irshad M, Fareed I, Khan A, Ullah F, et al. Cadmium phytoremediation by *Arundo donax* L. from contaminated soil and water. *BioMed Res Int*. 2013;1231. <https://doi.org/10.1155/2013/324830>
- Diwan H, Ahmad A, Iqbal M. Genotypic variation in the phytoremediation potential of Indian mustard for chromium. *Environ Manage*. 2008;41(5):734–741. <https://doi.org/10.1007/s00267-007-9020-3>
- Berger SA, Nejtgaard JC. A Worldwide Web-portal for Aquatic Mesocosm Facilities: WWW.MESOCOSM.EU. Washington DC: American Geophysical Union; 2016.
- Nanekar S, Dhote M, Kashyap S, Singh SK, Juwarkar AA. Microbe assisted phytoremediation of oil sludge and role of amendments: A mesocosm study. *Int J Sci Technol*. 2015;12(1):193–202. <https://doi.org/10.1007/s13762-013-0400-3>
- Barbafieri M, Pedron F, Petruzzelli G, Rosellini I, Franchi E, Bagatin R, et al. Assisted phytoremediation of a multi-contaminated soil: Investigation on arsenic and lead combined mobilization and removal. *J Environ Manage*. 2017;203:316–329. <https://doi.org/10.1016/j.jenvman.2017.07.078>
- Sánchez V, López-Bellido FJ, Cañizares P, Villaseñor J, Rodríguez L. Scaling up the electrokinetic-assisted phytoremediation of atrazine-polluted soils using reversal of electrode polarity: A mesocosm study. *J Environ Manage*. 2020;255:109806. <https://doi.org/10.1016/j.jenvman.2019.109806>
- Iloms E, Ololade OO, Ogola HJ, Selvarajan R. Investigating industrial effluent impact on municipal wastewater treatment plant in Vaal, South Africa. *Int J Environ Res Public Health*. 2020;17(3), Art. #1096. <https://doi.org/10.3390/ijerph17031096>
- Madanan MT, Shah IK, Varghese GK, Kaushal RK. Application of Aztec Marigold (*Tagetes erecta* L.) for phytoremediation of heavy metal polluted lateritic soil. *J Environ Chem Ecotoxicol*. 2021;3:17–22. <https://doi.org/10.1016/j.enceco.2020.10.007>
- R Development Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. [cited 2021 November 21]. Available from: <https://www.R-project.org>
- Liu X, Shen Y, Lou L, Ding C, Cai Q. Copper tolerance of the biomass crops Elephant grass (*Pennisetum purpureum* Schumacher), vetiver grass (*Vetiveria zizanioides*) and the upland reed (*Phragmites australis*) in soil culture. *Biotech Adv*. 2009;27(5):633–640. <https://doi.org/10.1016/j.biotechadv.2009.04.017>
- Suelee AL, Hasan SNMS, Kusin FM, Yusuff FM, Ibrahim ZZ. Phytoremediation potential of vetiver grass (*Vetiveria zizanioides*) for treatment of metal-contaminated water. *Water Air Soil Pollut*. 2017;228(4):158. <https://doi.org/10.1007/s11270-017-3349-x>
- Tangahu BV, Sheikh Abdullah SR, Basri H, Idris M, Anuar N, Mukhlisin M. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *Int J Chem Eng*. 2011;10:11. <https://doi.org/10.1155/2011/939161>
- Kafil M, Boroomand Nasab S, Moazed H, Bhatnagar A. Phytoremediation potential of vetiver grass irrigated with wastewater for treatment of metal contaminated soil. *Int J Phytoremediation*. 2019;21(2):92–100. <https://doi.org/10.1080/15226514.2018.1474443>



37. Gravand F, Rahnavard A, Pour GM. Investigation of vetiver grass capability in phytoremediation of contaminated soils with heavy metals (Pb, Cd, Mn, and Ni). *Soil Sediment Contam.* 2021;30(2):163–186. <https://doi.org/10.1080/15320383.2020.1819959>
38. Chiu KK, Ye ZH, Wong MH. Enhanced uptake of As, Zn, and Cu by *Vetiveria zizanioides* and *Zea mays* using chelating agents. *Chemosphere.* 2005;60(10):1365–1375. <https://doi.org/10.1016/j.chemosphere.2005.02.035>
39. Gautam M, Agrawal M. Phytoremediation of metals using vetiver (*Chrysopogon zizanioides* (L.) Roberty) grown under different levels of red mud in sludge amended soil. *J Geochem Explor.* 2017;182:218–227. <https://doi.org/10.1016/j.gexplo.2017.03.003>
40. Roongtanakiat N. Vetiver phytoremediation for heavy metal decontamination. *PRVN Tech Bull.* 2009;1.
41. Chang YS, Au PI, Mubarak NM, Khalid M, Jagadish P, Walvekar R, et al. Adsorption of Cu (II) and Ni (II) ions from wastewater onto bentonite and bentonite/GO composite. *Environ Sci Pollut Res.* 2020;27(26):33270–33296. <https://doi.org/10.1007/s11356-020-09423-7>
42. Antiochia R, Campanella L, Ghezzi P, Movassaghi K. The use of vetiver for remediation of heavy metal soil contamination. *Anal Biochem.* 2007;388:947–956. <https://doi.org/10.1007/s00216-007-1268-1>
43. Siyar R, Ardejani FD, Farahbakhsh M, Norouzi P, Yavarzadeh M, Maghsoudy S. Potential of vetiver grass for the phytoremediation of a real multi-contaminated soil, assisted by electrokinetic. *Chemosphere.* 2020;246:125802. <https://doi.org/10.1016/j.chemosphere.2019.125802>
44. Bokhari SH, Ahmad I, Mahmood-Ul-Hassan M, Mohammad A. Phytoremediation potential of *Lemna minor* L. for heavy metals. *Int J Phytoremediation.* 2016;18(1):25–32. <https://doi.org/10.1080/15226514.2015.1058331>
45. Aisien FA, Faleye O, Aisien ET. Phytoremediation of heavy metals in aqueous solutions. *Leonardo J Sci.* 2010;17(4), 37–46.
46. Rai UN, Sinha S, Tripathi RD, Chandra P. Wastewater treatability potential of some aquatic macrophytes: Removal of heavy metals. *Ecol Eng.* 1995;5(1):5–12. [https://doi.org/10.1016/0925-8574\(95\)00011-7](https://doi.org/10.1016/0925-8574(95)00011-7)