

Article

Evaluation of *Acacia karroo*'s Potential Aspect in the Phytoremediation of Soil Pollution

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Abstract: The rise in contaminated sites presents a significant issue for the environment and human health, necessitating the decontamination of the surroundings and the adoption of effective decontamination strategies. This investigation was initiated to assess the potential aspects of *Acacia karroo* in conjunction with enzyme activity, a method that shows promise for mitigating soil contamination. *Acacia karroo*, with its hyperaccumulator traits, demonstrates great capacity. Enzymes significantly efficiently convert and detoxify harmful substances to a non-toxic level. ICP-MS quantified the concentrations of trace elements in *Acacia karroo*, while colorimetric assays were used to determine the activity levels of the enzymes. Ten toxic elements were identified in leaf samples of *Acacia karroo* in the following sequence: Sr > Zn > Cr > V > Rb > Cu > Ni > Y > Sc > Co; concentrations ranged between 203.86 ± 4.48 ppm (Zn) and 10.12 ± 0.09 ppm (Sc). The concentration of these metals was very high, posing a potential risk of harming the environment. Meanwhile, the three identified enzymes, invertase (INV), phosphatase (PHO), and catalase (CAT), have high and average activity levels, respectively. PHO and CAT showed a positive correlation with Zn, Rb, Sr, and Y, while INV correlated positively with Sc, V, Cr, Co, Cu, and Ni content. The principal component analysis (PCA) findings in this study demonstrated an inconclusive correlation between soil enzyme activity and soil heavy metal content. Both positive and negative correlations between soil enzyme activity and heavy metals were observed. This investigation revealed *Acacia karroo* as an optimal botanical species for phytoremediation. Consequently, a correlation analysis demonstrated that incorporating the *Acacia karroo* species along with enzyme activity seems to be a highly promising environmentally friendly technique for remediating soil pollution. The *Acacia* species can also be used in phytoremediation efforts to help conserve biodiversity. Subsequent investigations should focus on the operational mechanisms of different plant parts used as herbal remedies, isolated compounds, their efficacy, adverse effects, and practical implications.



Citation: Maphuhla, N.G.; Oyedeji, O.O. Evaluation of *Acacia karroo*'s Potential Aspect in the Phytoremediation of Soil Pollution. *Sustainability* **2024**, *16*, 3315. <https://doi.org/10.3390/su16083315>

Academic Editors: Hongbiao Cui, Ru Wang, Yu Shi, Haiying Lu and Lin Chen

Received: 2 February 2024

Revised: 10 April 2024

Accepted: 11 April 2024

Published: 16 April 2024



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Keywords: soil health; remediate; trace elements; enzyme activity; soil contamination; principal component analysis

1. Introduction

Soil contamination in Alice has rapidly increased due to population expansion and growing commercial activity. The ecosystem is threatened by the emergence of harmful contaminants, such as heavy metals, and by inappropriate waste disposal practices [1].

In soil, harmful metallic elements merge with both organic and inorganic materials, becoming more resistant and corrosive. These metals accumulate in food systems, seep into human bodies, and contribute to the global accumulation of solid waste [2,3].

Municipal authorities face challenges in managing solid waste due to the lack of domestic waste treatment facilities. Consequently, solid waste is often disposed of in open spaces and around the city's outskirts, leading to trash burning to prevent buildup. Recent reports indicate that landfill garbage contains high levels of heavy metals exceeding WHO guidelines, which may accumulate due to soil erosion, percolation, and diffusion processes.

Some residents consume food scraps and leftovers from plastic garbage and use landfill soil for farming purposes as manure [2,4–6].

The increasing number of contaminated sites poses a serious threat to human health and the environment, necessitating effective decontamination techniques. This study aims to explore an innovative, low-tech, low-cost remedy that is environmentally friendly and capable of significantly reducing or eradicating pollution in soil ecosystems, the hydrosphere, and the atmosphere [7,8]. Phytoremediation, a promising approach, uses plants to absorb, eradicate, or diminish the bioavailability of elemental pollutants in the soil [9,10].

Soil enzymes, effective in altering pollutants at a measurable rate, contribute significantly to energy transfer, catalyze reactions essential to life, and serve as indicators of soil health. Their activity accurately reflects the overall state of the soil ecosystem [11,12]. This study examines *Acacia karroo* to explore its potential in enzyme activity for phytoremediation processes, aiming to reduce soil pollution and improve soil health [11,13]. *Acacia karroo*, capable of thriving under various environmental conditions, presents a viable option for phytoremediation in challenging environments [14].

Despite the limited literature on phytoremediation using *Acacia karroo* specifically, research on other native *Acacia* species demonstrates the potential of phytoremediation. Ettien et al., 2023, found *Acacia crassicarpa* to have the highest metal extraction capability from polluted soil among the species studied, making it suitable for phytoremediation [15]. The *Acacia* genus (*Fabaceae*) possesses beneficial properties for the phytoremediation of polluted soils [16], with *Acacia seiberiana* Tausch identified as highly effective in biodegradation [17].

Although *Acacia nilotica* is noted for its medicinal and therapeutic qualities, Tiwari et al., 2017, discovered its ability to remediate soil and water environments [18]. The effectiveness of phytoremediation in removing organic pollutants from soil varies by contamination location, highlighting the need for a variety of plant species [19].

2. Materials and Methods

2.1. Study Area Description

The inquiry was conducted in Alice, Nkonkobe Municipality, located in the Eastern Cape Province. This spot is located along the southern slopes of the Winterberg Mountains range and cliffs. The sites selected for examination were the Alice dumpsite and the University of Fort Hare East campus. The landfill is situated at latitude 32°48'24.88" S and longitude 26°49'33.37" E, while the control site is positioned at latitude 32°47'07.35" S and longitude 26°57'26.10" E, respectively. The waste facility is approximately 2 km away from the Happy Rest residential area. The East campus, which is 3 km away from the disposal site, was used as a control site. The unpolluted site (Site D) descended towards the Somgxada hills, located opposite the university barrier. The soil is well protected by vegetation from the natural elements. Site 1 (landfill) has been divided into three sections: A, B, and C (see Figure 1). Section A is situated on the eastern side of the landfill, where the ground is strewn with corroded and charred tins, broken bottles or glass, and corroded wires from car tires. In contrast, Section B is located on the west side, where trucks and drivers transport waste. Finally, Section C is located outside the disposal facility fencing, with diverse wild vegetation covering the land.

2.2. Sample Collection and Preparation

Samples of soil and leaves were crushed into small pieces in a laboratory using a mortar and pestle, air-dried, and then ground into a fine powder. The smooth samples were then sealed in a Ziplock bag and refrigerated at -4°C for experimental use.



Figure 1. The study area map depicts the Alice region at a scale of 700 m, highlighting the location of the Alice landfill site along with three designated collection points labeled as A, B, and C. (www.googleearth.com, accessed on 18 January 2024).

2.3. Leaves Sample Digestion

A 1.0 g sample of *Acacia karroo* leaves was measured and mixed with 15.0 mL of nitric acid (HNO_3) and 5.0 mL of perchloric acid (HClO_4). The mixture was allowed to dissolve overnight in a fume cupboard and heated on a stove at 90 °C until it reached 170 °C, increasing by 10 °C every two minutes. Hydrogen peroxide (H_2O_2) drops were added to the solution at 170 °C until a white fume formed, indicating the completion of the reaction. After the supernatant cooled in a desiccator, 50 mL of deionized water was added. Subsequently, the slurry was sifted through the Whatman No. 42 filter paper. The resultant supernatant was analyzed for heavy metals using Inductively Coupled Plasma Quadrupole Mass Spectrometry (ICP-MS) (Agilent, Santa Clara, CA, USA). Mass Hunter v4.5 is equipped with an Agilent 7700 laser (Agilent, Santa Clara, CA, USA) [20].

2.4. Enzyme Activity Assay

Two duplicates of each subsample for three enzymes were prepared and assessed. To determine invertase (INV) activity, a phosphate buffer with a pH of 5.5 and a sucrose solution were mixed with 2.0 g of prepared soil, followed by the addition of a few drops of toluene. The solution was incubated at 37 °C for 24 h after being mixed. 3,5-dinitrosalicylic acid was added to determine the glucose produced using a 508 nm spectrophotometer [21].

The catalase (CAT) activity was measured by combining 5.0 g of soil with 0.5 mL of toluene in a conical flask. The mixture was placed in a refrigerator at 4 °C for 30 min. After refrigeration, 5 mL of 3% hydrogen peroxide (H_2O_2) was added, and the solution was cooled for one hour. Later, the mixture was treated with 2M sulfuric acid (H_2SO_4), and

the resulting liquid was titrated with 0.01 M potassium permanganate (KMnO_4) until a bright pink color was observed. The catalase enzyme activity was then quantified as mL of $\text{KMnO}_4 \cdot \text{g}^{-1} \text{ soil} \cdot \text{h}^{-1}$ [21].

Phosphatase activity (PHO) was discovered by combining 1.0 g of prepared soil with 5.0 mL of p-nitrophenyl phosphate substrate, 1.0 mL of toluene, and 5.0 mL of acetic acid buffer at a pH of 5.0. The solution was incubated for 12 h at 37 °C. Next, 1.0 mL of the filtrate was taken and mixed with a boric acid buffer at pH 9.0, 2.5% potassium ferricyanide, and 0.5% 4-amino-pyridine. The PHO activity was determined colorimetrically at 570 nm [21].

2.5. Statistical Analysis

All the output data and fundamental descriptive statistical analysis, including the mean, standard deviation, and coefficient of variation for various heavy metal variables and enzyme activity, were computed using XLSTAT Version 23. Pearson correlation analysis was performed to estimate the correlation between the concentration of trace elements and enzyme activity. Principal component analysis (PCA) was used to assess the correlation of multivariate data using XLSTAT 2023.

3. Results

3.1. Trace Elements in *Acacia Karroo*

The results for the determined trace elements are presented in Table 1 and graphically in Figure 2. Ten trace metals were measured in *Acacia karroo* samples collected in polluted and unpolluted areas. The concentrations measured in parts per million (ppm) of trace elements varied from one site to another. Their concentrations (overall mean and standard deviation) were ranked in the following sequence: Sr (134.24 ± 19.22 ppm) > Zn (104.70 ± 47.74 ppm) > Cr (70.00 ± 17.48 ppm) > V (69.90 ± 14.87 ppm) > Rb (62.31 ± 9.09 ppm) > Cu (57.93 ± 32.16 ppm) > Ni (22.73 ± 7.96 ppm) > Y (22.20 ± 2.75 ppm) > Sc (11.52 ± 1.12 ppm) > Co (11.30 ± 2.58 ppm). The highest concentration levels of these metallic elements were recorded at site B (a polluted site) as follows: Sr = 154.82 ppm, Zn = 145.58 ppm, Cu = 126.97 ppm, Rb = 73.60 ppm, and Y = 23.64 ppm. Meanwhile, unpolluted site D was found to contain high concentration levels of these elements: Cr = 103.71 ppm, V = 103.24 ppm, Ni = 40.62 ppm, Sc = 13.46 ppm, and Co = 15.08 ppm.

These findings are corroborated by reports from Dulama et al., 2012, and Maphuhla et al., 2019, where the accumulation of trace elements in *Acacia karroo* leaves was very high [2,22]. A contaminated study area (dumpsite) may have zinc (Zn) contamination from transportation-related sources due to human activities such as fossil fuels, brake pads, tire degradation, engine oil dispersion, and the burning of residential and commercial waste [23].

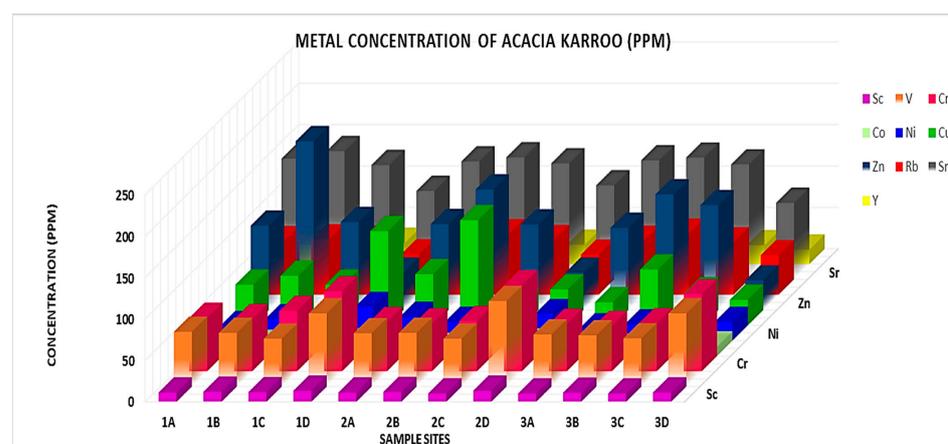


Figure 2. The concentration of trace elements in *Acacia karroo* leaves. [Note: A, B, C, and D represent sample sites, and numerical values from 1 to 3 represent the number of weeks; (Sc–Y): the metallic elements symbols].

Table 1. The trace elements concentration in *Acacia karoo* leaves (ppm).

Sample Sites	Scandium (Sc)	Vanadium (V)	Chromium (Cr)	Cobalt (Co)	Nickel (Ni)	Copper (Cu)	Zinc (Zn)	Rubidium (Rb)	Strontium (Sr)	Yttrium (Y)
1 A	11.25 ± 0.19	66.23 ± 0.65	64.45 ± 0.66	13.10 ± 0.30	17.58 ± 0.13	49.04 ± 3.95	101.93 ± 1.38	64.56 ± 0.28	145.83 ± 0.03	23.64 ± 0.01
1 B	12.21 ± 0.37	64.82 ± 0.27	62.95 ± 1.25	13.27 ± 0.49	25.87 ± 1.02	59.80 ± 1.92	203.86 ± 4.48	66.77 ± 0.53	154.82 ± 0.64	23.05 ± 0.54
1 C	11.75 ± 0.46	57.83 ± 2.01	72.67 ± 3.28	8.99 ± 0.54	17.84 ± 0.49	48.99 ± 0.15	106.28 ± 0.11	65.25 ± 0.59	138.11 ± 1.40	25.57 ± 0.35
1 D	13.46 ± 0.28	88.39 ± 1.19	96.42 ± 0.53	15.08 ± 0.11	40.62 ± 3.93	113.78 ± 4.36	45.29 ± 0.48	48.72 ± 0.03	106.75 ± 0.15	17.94 ± 0.04
2 A	11.25 ± 0.08	64.33 ± 1.81	59.18 ± 1.12	12.57 ± 0.40	28.74 ± 2.15	61.73 ± 2.98	103.70 ± 1.26	66.04 ± 0.61	142.40 ± 2.32	23.79 ± 0.09
2 B	11.93 ± 0.07	64.74 ± 0.12	57.57 ± 1.63	9.72 ± 0.31	22.56 ± 0.26	126.97 ± 0.50	145.58 ± 4.13	72.96 ± 0.22	147.36 ± 0.94	23.57 ± 0.46
2 C	10.21 ± 0.56	57.87 ± 2.81	60.53 ± 1.53	7.86 ± 0.37	12.68 ± 0.50	25.55 ± 0.57	103.33 ± 3.54	65.94 ± 1.50	140.09 ± 2.57	23.20 ± 0.47
2 D	13.43 ± 0.17	103.24 ± 0.21	103.71 ± 1.36	15.03 ± 0.46	31.97 ± 0.39	43.34 ± 1.10	45.20 ± 0.42	48.28 ± 0.29	113.59 ± 0.10	18.14 ± 0.25
3 A	10.20 ± 0.27	62.89 ± 0.04	57.19 ± 0.34	11.68 ± 0.50	14.87 ± 0.04	27.85 ± 0.77	99.18 ± 2.46	64.95 ± 0.31	143.77 ± 1.18	23.93 ± 0.47
3 B	11.12 ± 0.12	61.79 ± 1.28	54.56 ± 2.37	8.70 ± 0.23	19.55 ± 0.92	67.43 ± 0.27	139.64 ± 3.20	73.60 ± 2.14	147.08 ± 1.03	23.64 ± 0.12
3 C	10.12 ± 0.09	58.27 ± 1.10	57.78 ± 4.29	8.23 ± 0.22	18.01 ± 0.83	39.88 ± 2.54	126.67 ± 0.03	63.13 ± 0.34	138.80 ± 1.82	22.66 ± 0.39
3 D	11.33 ± 0.00	88.38 ± 1.01	92.93 ± 1.31	11.34 ± 0.01	22.47 ± 0.09	30.85 ± 0.32	35.79 ± 0.76	47.55 ± 1.81	92.32 ± 0.92	17.32 ± 0.16
FAO/WHO limits (mg/kg)	0.02	n/a	1–2.3	50	10	10–40	50	0.5	100	n/a [24–27]

Note: Metallic concentration results are presented as mean values (M) ± standard deviation (SD). (Column 1): A, B, C, and D represents sample sites, numerical values from 1 to 3 represent the number of weeks. (Row 1): The metallic elements symbols. n/a stands for Not Available.

3.2. Enzyme Activity Findings

Three (3) soil enzyme activities were discovered in soil samples, namely phosphatase (PHO), invertase (INV), and catalase (CAT), and their activity levels are tabulated in Table 2.

Table 2. Enzyme activity concentration levels in soil samples.

Sampling Sites	Invertase ($\mu\text{g glucose}\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$)	Catalase ($\text{mL KMnO}_4\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$)	Phosphatase ($\mu\text{g phenol}\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$)
1 A	$3.66 \pm 2.11^{\text{d}}$	$0.65 \pm 0.37^{\text{b}}$	$1.59 \pm 0.92^{\text{d}}$
1 B	$3.65 \pm 2.10^{\text{d}}$	$0.80 \pm 0.46^{\text{cd}}$	$3.49 \pm 2.01^{\text{ab}}$
1 C	$3.66 \pm 2.11^{\text{d}}$	$1.36 \pm 0.78^{\text{a}}$	$3.66 \pm 2.11^{\text{e}}$
1 D	$3.60 \pm 2.08^{\text{d}}$	$0.87 \pm 0.50^{\text{cd}}$	$1.42 \pm 0.82^{\text{d}}$
2 A	$3.35 \pm 1.93^{\text{d}}$	$0.97 \pm 0.56^{\text{cd}}$	$2.37 \pm 1.37^{\text{c}}$
2 B	$3.48 \pm 2.01^{\text{d}}$	$0.46 \pm 0.27^{\text{ab}}$	$3.49 \pm 2.01^{\text{ab}}$
2 C	$3.18 \pm 1.83^{\text{d}}$	$1.14 \pm 0.66^{\text{a}}$	$2.79 \pm 1.61^{\text{a}}$
2 D	$3.47 \pm 2.01^{\text{d}}$	$0.65 \pm 0.37^{\text{b}}$	$2.33 \pm 1.34^{\text{c}}$
3 A	$2.88 \pm 1.66^{\text{c}}$	$2.27 \pm 1.31^{\text{e}}$	$3.97 \pm 2.29^{\text{e}}$
3 B	$3.26 \pm 1.88^{\text{d}}$	$2.58 \pm 1.49^{\text{d}}$	$3.67 \pm 2.12^{\text{e}}$
3 C	$2.43 \pm 1.52^{\text{c}}$	$2.63 \pm 1.52^{\text{d}}$	$3.98 \pm 2.30^{\text{e}}$
3 D	$3.36 \pm 1.94^{\text{d}}$	$1.57 \pm 0.91^{\text{a}}$	$3.65 \pm 2.10^{\text{e}}$

Note: mean \pm SD with similar superscript letters indicates no statistically significant difference ($p > 0.05$).

All detected enzyme activities were observed to have low levels of activity, recorded below 5.0. The activity levels of PHO ranged from 3.98 ± 2.30 ppm to 1.42 ± 0.82 ppm, and INV ranged from 3.66 ± 2.11 ppm to 2.43 ± 1.52 ppm, which were higher in all sample sites compared to CAT activity levels (ranging from 2.58 ± 1.49 ppm to 0.46 ± 0.27 ppm), which varied across all sites. Low levels of catalase (CAT) activity indicate the adverse effects of the high concentration of trace elements found in *Acacia karroo*. The high levels of PHO and INV activity suggest that they are reliable indicators of soil pollution and have the potential to be utilized in remediation processes alongside other phytoremediation methods.

Contrary to these findings, a similar study by Nurzhan et al., 2022, reported that low levels of enzyme activity in soils were due to high arsenic (As) content (exceeding 1000 mg/kg) and the most severe pollution of the soils, which more severely suppressed enzyme activity [28]. Moreover, Attademo et al., 2021, reported enzymes with minimal activity throughout each crop cycle [29,30]. Meanwhile, Jaworska and Lemanowicz (2019) reported similar findings. They found that the levels of enzyme activity examined were higher in soil samples collected between layers of 0–20 cm compared to those collected between layers of 20–40 cm [31].

3.3. Pearson Correlations and PCA Analysis

The results in Table 3 indicate two component factors with eigenvalues greater than >1 , suggesting a two-factor solution ($F1 = 6.67$ and $F2 = 1.96$). Thus, these two factors were selected for further analysis. The other small eigenvalue of less than <1 was not utilized to estimate the probable number of contributing source factors. This reveals that the first two principal components (F1 and F2) are the most accurate options, as confirmed by the scree plot, which shows an elbow-shaped graph after the second component (Figure 3). Many recent researchers have endorsed and projected that the findings of the first two principal components (F1 and F2) are adequate to represent the entire dataset information, accounting for a total percentage of variance exceeding 70%, which signifies the overall inertia [32,33]. The first principal component (F1) contains the greatest variance values (66.70%), with maximum loadings of Sc, V, Zn, Rb, Cr, Co, Ni, Sr, and Y. This indicates a high concentration of heavy metals as pollutants in *Acacia karroo* leaves, suggesting that these elements may originate from the same sources. The second principal component (F2) accounts for 19.59% of the variance, with high loadings of Sc, Ni, Cu, and Zn, suggesting the dominance of essential elements supplied by the soil for plant development or growth. Despite the lack of

knowledge regarding Sc's biological function in plants, Table 1 shows that its concentration is minimal. The fact that the rule states that there is a substantial amount of Sc on plant roots suggests that the Sc value in the soil does not bioaccumulate in plant tissue [34]. The first two principal components account for a cumulative variance contribution of 84.28%, explaining a total variance of 84% with strong correlations to the identified elements.

Table 3. The total percentage of variance in *Acacia karroo* leaves.

Variables	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	6.670	1.958	0.631	0.273	0.238	0.133	0.068	0.020	0.008	0.000
Variability (%)	66.698	19.585	6.315	2.732	2.380	1.327	0.681	0.201	0.081	0.000
Cumulative %	66.698	86.283	92.597	95.329	97.709	99.037	99.717	99.919	100.000	100.000

Note: Bold values are deemed significant for analysis.

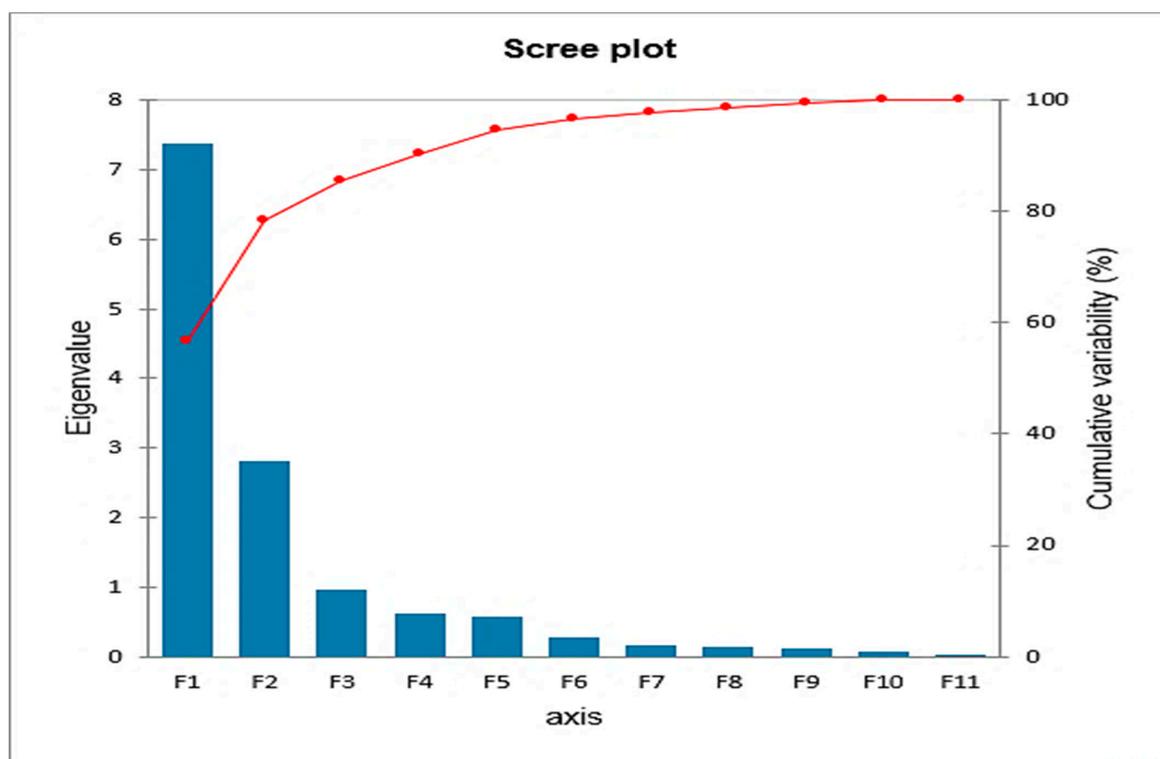


Figure 3. Scree plot for eigenvalues representing the two important components.

3.4. Correlation Matrix

The first principal component is strongly correlated with nine identified elements (Zn, Rb, Sc, Ni, V, Sr, Y, Co, and Cr), except for the Cu element, which has a correlation coefficient of less than <0.5 (Table 4). The F1 component increases with increasing concentration levels of Zn, Rb, Sc, Ni, V, Sr, Y, Co, and Cr. The strong negative correlation observed between F1 and Zn, Rb, Y, and Sr reveals an unhealthy effect of these elements on the physiology of *Acacia karroo* leaves. This suggests that these heavy metals vary together. If the concentration of one element increases, then the concentrations of the remaining elements also tend to increase. Based on the correlation levels of the F1 component, we can conclude that the nine elements detected in *Acacia karroo* leaves are the sources of pollution in the environment.

Table 4. Correlations between trace elements and enzyme activity.

Variable	Sc	V	Cr	Co	Ni	Cu	Zn	Rb	Sr	Y	INV	CAT	PHO
Sc	1												
V	0.733	1											
Cr	0.736	0.929	1										
Co	0.728	0.719	0.622	1									
Ni	0.860	0.707	0.661	0.765	1								
Cu	0.549	0.101	0.039	0.209	0.566	1							
Zn	−0.280	− 0.712	− 0.769	−0.358	−0.346	0.153	1						
Rb	−0.515	− 0.873	− 0.934	− 0.597	−0.575	0.141	0.822	1					
Sr	−0.427	− 0.819	− 0.884	−0.376	−0.496	0.044	0.870	0.925	1				
Y	−0.553	− 0.915	− 0.870	−0.552	− 0.642	−0.068	0.706	0.906	0.905	1			
INV	0.691	0.309	0.376	0.490	0.418	0.396	−0.044	−0.124	−0.102	−0.109	1		
CAT	− 0.629	−0.357	−0.356	−0.546	−0.452	−0.413	0.081	0.192	0.082	0.184	− 0.768	1	
PHO	−0.524	−0.438	−0.432	− 0.653	−0.569	−0.283	0.426	0.389	0.254	0.344	−0.510	0.640	1

Note: values in bold are different from 0 with a significance level alpha (α) = 0.05.

The F2 component revealed a strong projected correlation with concentrations of Sc, Cu, Ni, and Zn, which represent 19.59% of the total variance. According to the plotted correlation (Figure 4), there is a significant positive correlation between the Sc, Ni, and Co content (the R-value is close to 1). Zn, Rb, Sr, and Y are positively correlated with each other at F2. Lastly, a negative correlation was observed between V and Cr content. The arrangement of the dense metallic substances encountered revealed that the majority of the components were discovered in close proximity to one another, except for Cu, which clearly distinguished itself from other elements. This statistical information suggests that elements clustered together in each component share a common geochemical source of pollution [33].

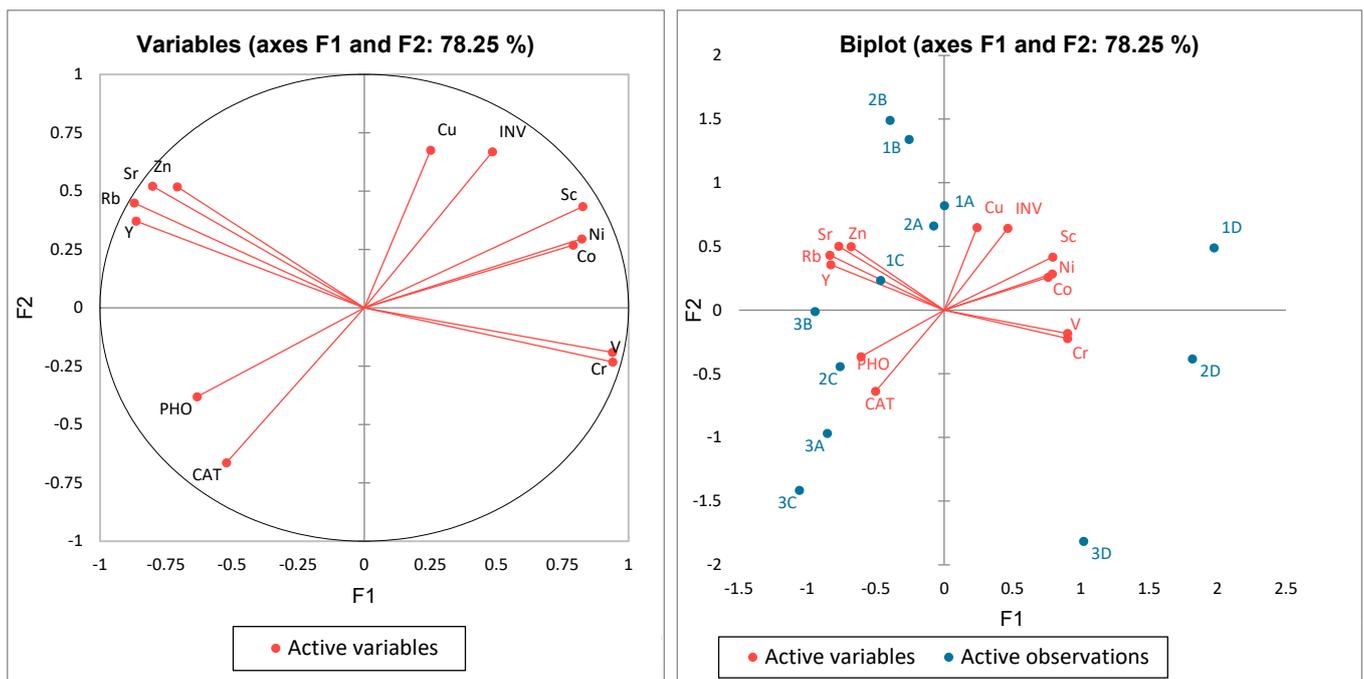


Figure 4. Diagram of the principal components F1 and F2 for trace elements and enzyme activity.

The Pearson correlation matrix between soil enzyme activity and detected heavy metals in *Acacia karroo* leaves reveals a significant positive correlation between invertase (INV) activity and Sc, V, Cr, Co, Cu, and Ni content but a negative correlation with Zn, Rb, Sr, and Y elements. A vice versa relationship was observed between catalase (CAT) and phosphatase (PHO) and the concentrations of Zn, Rb, Sr, and Y, which showed a positive association. Meanwhile, they were negatively correlated with Sc, V, Cr, Co, Cu, and Ni. The concentrations of Sc, V, Cr, Co, Cu, and Ni are inversely related to the levels of Zn, Rb, Sr, and Y.

4. Discussion

These findings suggest that the heavy metals studied in this investigation may be mobile and biologically active in soil. Nonetheless, since certain plants obtain most of their nutrients from the less accessible portions of the soil, some heavy metals serve as vital constituents. The presence of these metals in the above-ground parts of plants suggests a higher potential for extracting metals from contaminated sites [22]. The high concentration of these metals in the environment poses a risk to both humans and the ecosystem. Therefore, treating the soil using a phytoremediation procedure with indigenous plants is necessary.

Acacia karroo is the most prevalent species in the Alice region and can support phytovolatilization processes. This process involves converting pollutants within plant parts, specifically from roots to leaves, into a gaseous form that is emitted into the atmosphere through evapotranspiration procedures [35]. This plant can be found in various settings, including dry thornveld, river valley scrub, bushveld, forest, grassland, riverbanks, and coastal dunes. It exhibits a wide range of growth styles, and plants from different regions often have distinctive appearances. *Acacia karroo* can thrive under diverse environmental conditions, such as high salinity, poor soil quality, and drought, making it suitable for phytoremediation in challenging environments. It contains the nitrogen-fixing bacterium *Rhizobium*, which helps enhance soil fertility. This species can also aid in phytoremediation efforts to help conserve biodiversity [14,36].

Although *Acacia karroo* is not considered a hyperaccumulator tree, it exhibits traits such as survival in poor soil, easy cultivation, resistance to pathogens, rapid biomass production, high extraction ability, abundant shoots, and adaptability to various environments [35].

Enzymes have great potential to effectively transform and detoxify polluting substances, recognized for their ability to convert pollutants at a detectable rate and suitability for restoring polluted environments [8]. Fungi and other microorganisms in the soil release extracellular and oxidative enzymes [37]. White rot fungi are the main producers of oxidative enzymes essential for cleaning up contaminated areas. Due to their resilience and ability to withstand higher pollution concentrations than bacteria, these microbes are incredibly efficient [8]. The heightened enzyme activity can be attributed to the increased proliferation of microbes, facilitated by the greater availability of nutrients made accessible by the deceased segment of the microbial communities [38].

Therefore, combining enzyme activity and *Acacia karroo* could effectively clean up pollutants. Several studies have documented the use of *Acacia karroo* for phytoremediation, highlighting its significant potential in this field. Research has demonstrated the possibility of using other native *Acacia* species for soil phytoremediation. One study revealed that *Acacia karroo*'s ability to rejuvenate and demonstrate resilience in difficult conditions indicates its potential for phytoremediation and great promise in repairing soils poisoned by acid mine drainage [39].

Ettien et al., 2023, found that *Acacia crassicarpa* exhibited the highest metal extraction capability from polluted soil compared to two other *Acacia* species (*Acacia mangium* and *Acacia auriculiformis*), making it the most suitable species for phytoremediation of polluted soils [15]. Whereas Tiwari et al., 2017, discovered that *Acacia nilotica* has the ability to remediate soil and water, in addition to possessing significant medicinal and therapeutic qualities [18]. In a study conducted by Abdallah et al. in 2023, compelling evidence

was presented showing that *Acacia seiberiana* Tausch is highly effective in biodegradation, indicating the plant's unique ability to remediate soil contaminated with crude oil [17].

Furthermore, findings from chromated copper arsenate by Rathna Kumari and Nagaraja in 2023 discovered that the *Acacia* genus (*Fabaceae*) possesses beneficial properties and has the potential to be utilized in the phytoremediation process of polluted soils [16]. This study supports the findings reported by Ettien et al., 2023 [15].

Acacia species can store metallic elements in their roots and shoots due to their strong tolerance. Increased metal accumulation in *Acacia* species may be due to their ability to detoxify metals. Woody species, such as acacias, produce substantial biomass, aiding in the accumulation of heavy metals in their shoots [15,16]. Therefore, the combination of *Acacia karroo* with enzyme activity appears to be a promising approach for reducing soil contamination. Phytoremediation is the technique of using plants to clean up, purify, or stabilize pollutants in the soil. The activities of enzymes and *Acacia karroo* can collaborate to enhance the decomposition and removal of contaminants from the soil [40]. An essential component of phytoremediation is enzyme activity. A study investigating the effects of exogenous Cd on the soil enzyme activities of three herbs, *Solanum nigrum* L., *Phytolacca acinosa* Roxb, and *Bidens Pilosa* L., revealed that different types of plants recovered to different extents under different Cd solution stresses. The soil enzyme activities of PHO, CAT, and URE were influenced by Cd solution stress. The outcomes revealed that the phytoremediation process (enzyme activity in soil with grown herbs) improved, with the rate of recovery ranging from 70.19% to 84.57% [21].

PCA findings demonstrated an inconclusive correlation analysis between soil enzyme activity and soil heavy metal content.

The investigation observed both positive and negative correlations between soil enzyme activity and plant metallic elements. A statistically significant positive correlation was observed among the metallic components (Sc, Ni, Co, Rb, Sr, and Y), suggesting that these pollutants may originate from a common source. Conversely, a negative correlation was found between the content of vanadium (V) and chromium (Cr). The activities of CAT and PHO showed a statistically significant negative correlation with Sc, V, Cr, Co, Cu, and Ni content, whereas the INV activity exhibited a significant positive correlation. The concentration levels of Zn, Rb, Sr, and Y showed a significant positive correlation with the activity of CAT and PHO but a negative association with the INV activity. Haroun et al., 2023, reported a similar discovery, where the soil enzyme activities showed a significant negative correlation with heavy metals [41]. Another study by Yang et al., 2022, reported different results in correlation analysis. The study showed a positive correlation between forestland soil enzyme activity and heavy metals, but a negative correlation in wasteland soil. These findings indicate that the activities of these enzymes are highly sensitive and can rapidly decrease when the concentration of heavy metals is high [42].

Although the efficacy of *Acacia karroo* in conjunction with enzyme activity for soil phytoremediation has not been directly proven, previous studies indicate that the combination of plants and enzymes may be useful in eliminating pollutants from soil. These studies demonstrate that utilizing enzyme activity in combination with *Acacia karroo* can be an effective method to remediate pollutants. It is imperative to acknowledge that implementation and efficacy may differ depending on the nature and degree of contamination being addressed [40,43]. Therefore, further research and site-specific studies are necessary for optimal application.

5. Conclusions

Research has uncovered promising evidence that supports the use of native *Acacia karroo* for soil phytoremediation as the most suitable technique, showcasing its ability to extract metals from polluted soils. Utilizing plants and enzymes to restore soil health and fertility and remediate pollution presents a promising strategy. Moreover, the use of enzymatic proteins may provide a viable alternative to address the majority of drawbacks associated with employing other microorganisms in phytoremediation.

Acacia karroo possesses beneficial properties, making it a potential candidate for use in the phytoremediation process of polluted soils. This relates to the varied responses of different plants to contaminated soil and their respective outcomes. It is crucial to note that relying solely on plants for soil remediation can be time-consuming. Therefore, incorporating supplementary methods such as chelating agents, microbes, and foliar sprays is essential to enhance the effectiveness of phytoremediation.

Author Contributions: Conceptualization, N.G.M.; methodology, N.G.M.; software, N.G.M. and O.O.O.; writing and editing, N.G.M.; investigation, N.G.M.; data curation, N.G.M.; data analysis and interpretation, N.G.M.; data verification, O.O.O.; supervision, O.O.O.; funding acquisition, N.G.M. and O.O.O. All authors have read and agreed to the published version of the manuscript.

Funding: Govan Mbeki Research and Development Centre (GMRDC)-University of Fort Hare.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon request.

Acknowledgments: The authors are grateful to the Govan Mbeki Research and Development Centre (GMRDC) for funding this study, the University of Fort Hare Directorate of Research and Development, and the University of Fort Hare Chemistry Department.

Conflicts of Interest: The authors declare no conflicts of interest for this study.

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