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Synergistic effect of compost and moringa leaf extract biostimulants on the remediation of gold mine tailings using chrysopogon zizanioides

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ABSTRACT

Phytoremediation is a cost-effective and environmentally friendly method for restoring degraded land. However, optimizing phytoremediation conditions and using locally sourced materials can further improve the sustainability of this approach. A 3×2×2 factorial design was used in determining the optimal parameters in the remediation of gold mine tailings using vetiver grass. The treatments consisted of three compost concentrations (0 %, 30 % and 60 %), two biostimulant types (laboratory-extracted and commercial biostimulants) and two application regimens (once and twice a week) in a randomized complete block design. The biomass and metal concentrations in the vetiver grass were measured after 16 weeks. All the vetiver that was planted in 0 % compost amendment died within four weeks regardless the MLE treatment. There was no significant difference in vetiver biomass between vetiver grown in 30 % and that grown in 60 % compost amendment. Biostimulant application frequency led to no significant differences. A difference on vetiver biomass due to the type of moringa leaf extract was eminent, with laboratory moringa leaf extract resulting in significantly higher biomass in vetiver grown on 60 % compost yet the commercial moringa leaf extract producing significantly higher vetiver biomass on vetiver grown on 30 % compost amendment. These findings suggest that the optimal combination for field study would be 30 % compost amendment with commercial biostimulant for a more cost-effective option.

Introduction

Mining and quarrying operations have led to the disruption of 0.4 million square kilometers of global land area [1]. This problem is compounded by the annual deposition of approximately five billion tons of mining waste, or tailings, into the environment [2]. In South Africa, for example, a total of 200,000 hectares of land were transformed for mining in 1997, with an additional 47,000 hectares used to store more than 471 million metric tons of mine waste [3]. The main concerns are the impact of mining on soils, groundwater and water channels [4,5] and the bioaccumulation of hazardous contaminants in plants, animals and humans [6]. The large-scale disruption of land for mining and quarrying operations has been linked to chronic health risks and hazards [7] especially to the surrounding [8].

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In South Africa, the reference for the obligation to remediate mine sites is to a greater extent contained in the Mineral and Petrolem Resources Development Act 28 of 2002 (MPRDA). The National Environmental Management Act 107 of 1998 (NEMA), National Environmental Management: Biodiversity Act 10 of 2004 (NEMBA), National Environmetal Management: Waste Act 59 of 2008 (NEMWA), and the National Water Act (NWA) are invariably triggered by the mining related activities. Section 43(1) ad Section 38(1)(d) of MPRDA, 2002; Section 2(4)(p) and Section 28(1) of NEMA, 1998 states that whoever pollutes remains responsible for the damage done until they remediate the damages to acceptable level as per the environmental management program (EMPr).

Remediation is a physical, chemical or biological action which is used to remove or reduce contaminants to minimize the risks to humans [9]. The conventional (physical and chemical) methods for remediation have been criticized due to their high cost, intensive labour requirements, irreversible changes in soil properties, disruption of native soil microflora, high energy demands, potential secondary pollution problems and difficulty to implement at large scale [10]. Phytoremediation, a low-cost, eco-friendly approach that harnesses the power of plants to remove contaminants from the environment, has been widely recognized as a viable alternative to conventional methods [11].

Phytostabilization and phytoextraction are the two most common phytoremediation technologies [12]. Phytoextraction is a phytoremediation technique in which plants absorb contaminants from the soil or water and accumulate them in their aboveground biomass [13,14]. Phytostabilization is a phytoremediation technique that involves establishing vegetation to concentrate metals in the roots or change their chemical state in the rhizosphere [15].

In recent years, there has been a shift in phytoremediation research toward more sustainable approaches [16,17]. The concept of 'sustainable remediation' has been a turning point for the remediation field, establishing new norms and standards for practitioners [16]. Sustainable remediation not only addresses pollution concerns but also provides value-added benefits and economic opportunities for communities in the areas being remediated. This includes its ability to improve food safety, promote the development of renewable energy sources, and sequester carbon [18]. The use of vetiver grass and locally extracted MLE biostimulants is a promising approach for sustainable phytoremediation. du Jardin, [19] defines biostimulants as "a group of substances from natural origin that contribute to boosting plant yield and nutrient uptake, while reducing the dependency on chemical fertilizers". The discovery of moringa leaf extract (MLE) as a biostimulant has gained importance in recent years [20]. *Moringa oleifera* leaves is rich in growth enhancing compounds such as ascorbate, phenolics and minerals (Ca, K, Zn and Fe), antioxidants [21], phytohormones i.e., zeatin (cytokinins) and auxins [22]. Several scholars have indicated that extracts of Moringa oleifera (Lam.) is capable of increasing plant growth in a vast number of crops due to high content of minerals, sugars, protein, proline, amino acids, cytokinins, auxins, gibberellins, and antioxidants (e.g. [23,24]). MLE has been extensively studied as a biostimulant for horticultural crops, but its potential for enhancing growth in mine-impacted soils has not been explored. This untapped potential presents an opportunity for further research into the use of MLE for sustainable phytoremediation purposes. The goal of this study was to compare the efficacy of a laboratory extracted MLE as a biostimulant to that of a commercially available biostimulant on vetiver grass planted on gold mine tailings amended with compost.

Vetiver grass is known for its ability to tolerate a range of environmental conditions, including those found in mine-impacted soils, and its potential for biomass valorization, a process of extracting value from plant biomass in the form of products or services. This includes carbon sequestration, the production of goods such as paper and textiles, and the generation of biofuels. The concept of biomass valorization is supported by several studies, including Danh et al. [25], who showed that vetiver grass can produce high amounts of biomass (>100 t/ha/year) and can potentially be utilized as a raw material for handicrafts, essential oils, raw material for pulp, paper, and fiberboard [26].

While Vetiver grass is not native to South Africa, it has been found to be non-invasive and does not pose a threat to native species [27]. In a greenhouse study conducted by [28], Vetiver grass was found to thrive on gold tailings from AngloGold Ashanti West Wits site in Gauteng, South Africa and accumulate Zn, Cu and Ni in its roots and limit their movement to the leaves.

Despite vetiver's resilience, the use of remedies, such as compost, is important in improving growth. Without amelioration, plants may not achieve the desired results. Organic amendments such as compost are preferred over inorganic chelating agents because they reduce metal bioavailability immediately and continues to decrease it gradually, add slow-release fertilizer, provide a microbial inoculum, and improve soil structure and cation exchange capacity. In addition, organic matter reduces erosion and increases infiltration of mine tailings [29–31].

Th synergy between vetiver, compost and MLE biostimulant can lead to a cost-effective, environmentally friendly solution and value-added benefits can be harnessed from the process. For example, communities may be able to produce MLE and propagate vetiver and this can create new employment opportunities and revitalizing communities especially in areas where mining is curtailing.

Materials and methods

Study site and design

Fleurhof is a settlement located in the Witwatersrand Basin, Gauteng Province, South Africa. This area is characterized by historic tailings dumps and are the main source of pollution, which they release into the surrounding environment through acid mine drainage and erosion of tailings deposits [32]. Tibane & Mamba [33] found that surrounding communities are prone to health risks and that due to their economic status, they cannot relocate; therefore, mitigation measures are ethical. The study site climate was described by Humphries et al. [34] as having winter and summer temperatures averaging 15°C and 20°C, respectively. The annual rainfall is between 600 mm and 700 mm [34]. Fig. 1 below shows the Google Earth image of the study site.

Preparation for trials

The gold mine tailings were collected using a soil auger at a depth of up to 30 cm on tailings adjacent to the Fleurhof Dam (26°11′53.4"S 27°54′33.5"E). The samples were stored in polypropylene bags and transported to the greenhouse, where they were amended with Culterra organic compost manufactured from dead decaying plant material. The compost was purchased from Schäffler's Garden Nursery at 28 Johannesburg Road, Lyndhurst, Johannesburg. The pH and EC of the tailings material and compost were measured prior to mixing. The average pH and EC of the unamended tailings were 3.10 and 2280 μS/cm, respectively. The compost had an average pH of 8.57 and an average EC of 2788 μS/cm. The baseline concentrations of elements in the unamended tailings material and compost were measured and are presented in Table 1 below. The metal concentrations in 30 % and 60 % of the compost amendments were obtained by using proportions. The formula Cmix= (C1xP1) +(C2xP2) +…., where C1, C2, etc., are the concentrations of each individual metal and P1, P2, etc., are the proportions of each metal in the mixture was used to determine the metal concentration in the amended tailings.

The levels of As, Cd and Cr, in the tailings were above the South African soil screening values (SSV) 1 and SSV 2 while Cu, and Pb exceeded SSV1 only. SSV1 are soil quality values that are protective of both human health and eco-toxicological risk for multi-exposure pathway including watercourse. SSV2 means soil quality values that are protective of risk to human health in the absence of a water resource. Only the SSV2 informal residence values have been considered in this study. As and Cd are classified as group 1 carcinogens, while Cr and Pb are classified as group 2 carcinogens by the International Agency for Research on Cancer. The health risks associated with these metals include various types of cancer, cardiovascular disease, respiratory problems, neurological disorders, and developmental delays in children. Therefore, corrective actions to remediate the tailings are inevitable. Table 2 provides the concentrations of some elements in the commercial and laboratory MLE.

Biostimulant preparation

The commercial biostimulant was purchased from a commercial supplier in Pretoria, South Africa. The solution was diluted at a ratio of 1:33 (v/v) with tap water to make a 3 % (v/v) solution that was used in the experiment. The dry moringa leaves for the laboratory MLE were obtained from Moringa Community Farm in Hammanskraal, South Africa. The MLE was extracted according to the methods of Rady & Mohamed [20]. The moringa leaves were ground to powder using a blender. MLE was obtained by blending 100 g of moringa powder with 675 ml of 80 % ethanol as suggested by Makkar and Becker [35]. The mixture was incubated for 4 hours on an orbital digital shaker, after which the volume was increased to 1000 ml. The extract was then centrifuged at 4000 rpm for 15 minutes, followed by filtering using No. 2 Whatman filter paper. The extract was subjected to a rotary evaporator to remove the ethanol. The extract was diluted 1:33 (v/v) with tap water to make the 3 % solution that was used in the experiment. The laboratory and commercial biostimulant (before dilution) were analyzed for the metal concentration using the method of Florian and Knapp [36].

Fig. 1. Google map image of the sampled area, a historic tailing storage facility near Fleurhof Dam in Johannesburg, South Africa.

Table 1
Average concentrations of elements in experimental tailings, compost and proportional representation on amended tailings, (mg/kg, mean, n=3).

Sample	100 % Tailings	Compost	30 % compost amendment	60 % compost amendment
As	90.73	62.69	82.34	73.91
Cd	19.70	61.69	32.30	44.89
Co	38.85	51.24	42.53	46.26
Cr	84.36	345.77	162.78	241.21
Cu	90.53	127.36	101.58	112.63
Ni	52.73	121.89	73.48	94.23
Pb	58.17	57.21	57.83	57.57
Zn	149.42	198.01	163.99	178.57
Al	10756.07	17684.58	12834.62	14913.18
Ca	10139.28	11583.08	10572.42	11005.56
Fe	55045.76	40850.75	50787.26	46528.75
K	795.46	6714.43	2571.15	4346.84
Mg	1769.63	2758.71	2066.33	2363.07
Mn	257.46	431.84	309.77	362.09
P	1092.32	14717.41	5179.85	9267.37
S	21997.61	3886.57	16564.35	11130.98

Table 2
Calculated concentrations of some elements from the measured stock solutions of laboratory and commercial MLE (mean ± std; n=3).

Element	Lab extracted MLE (µg/l)	Commercial biostimulant (µg/l)	P values
Al	7.66 ± 0.18^{a}	3.16 ± 0.35^{b}	<0.001
Ca	255.66 ± 1.2^{a}	469.14 ± 4.86^{b}	<0.0001
Co	0.32 ± 0.06^{a}	0.52 ± 0.03^{b}	<0.001
Cr	3.06 ± 0.42^{b}	2.28 ± 0.6^{a}	<0.01
Cu	2.58 ± 0.06^{a}	2.46 ± 0.16^{b}	0.047
Fe	5.22 ± 0.21^{a}	5.88 ± 0.11^{b}	<0.001
K	679.95 ± 25.14^{a}	681.9 ± 23.82^{a}	0.73
Mg	149.87 ± 4.13^{a}	149.79 ± 2.18^{a}	0.94
Mn	12.21 ± 0.24^{a}	12.19 ± 0.21^{a}	0.87
Na	149.2 ± 1.68^{b}	131.76 ± 1.52^{a}	<0.001
P	600.75 ± 3.96^{a}	642.2 ± 2.52^{b}	<0.001
S	50.5 ± 0.66^{a}	57.78 ± 1.2^{b}	<0.005
Zn	1.92 ± 0.54^{a}	2.61 ± 0.31^{b}	0.0003

Different letters in each row indicate significant difference ($P < 0.05$).

A 7 mL of each sample was put in a microwave digestion vessel, to it 1 mL of H_2O_2 and 50 uL of HNO_3 was added and the samples were digested at 1000W microwave power for 30 minutes. Using proportions, the obtained concentrations were multiplied by 3% and the results are presented in Table 2 above [36].

The description of different compost and moringa leaf extract treatments under which vetiver grass was exposed to for 16 weeks is provided in Table 3 below.

The above treatments were carried out in triplicate to validate the empirical data. One month old vetiver tillers donated by Hydromulch (Bapsfontein, Gauteng Province, South Africa) were removed from the soil that came with them and pruned to a height of 20 cm to establish the baseline height. The tillers were subsequently transplanted into the prepared potting pots. The 45 pots were arranged in accordance with a completely randomized block design in a glass greenhouse in Johannesburg, South Africa. The greenhouse humidity ranged from 55–65 %. The average daytime temperature was 30°C, and the nighttime temperature was 20°C. Fig. 2 shows the arrangement of the 45 pots in the greenhouse.

Table 3
Description of different treatments under which vetiver grass was exposed to for 16 weeks.

Biostimulant	0 % compost	30 % Compost	60 % Compost
No Biostimulant	0 %O	30 % O	60 % O
Lab Extracted Biostimulant once a week	0 %L1	30 % L1	60 % L1
Lab Extracted Biostimulant twice a week	0 %L2	30 %L2	60 % L2
Commercial Biostimulant once a week	0 %C1	30 % C1	60 % L1
Commercial Biostimulant twice a week	0 %C2	30 % C2	60 % L2

Replicate 1	Replicate 2	Replicate 3
0%0	0%L1	30% 0
0%C1	0%C1	0%0
30% C2	30%L2	0%C2
60% C1	0%L2	30% C2
60% C2	30% L1	0%L1
30% 0	60% L1	0%C1
30%L2	60% C1	60% C2
60% L1	60% L2	30% C1
0%L1	0%C2	60% L1
60% 0	30% 0	60% C1
60% L2	30% C1	30% L1
0%C2	60% 0	60% 0
30% C1	30% C2	60% L2
30% L1	60% C2	30%L2
0%L2	0%0	0%L2

Fig. 2. Experimental design for the greenhouse study showing three replicates of each experimental pots randomly arranged in the greenhouse.

Chemicals and reagents

Analytical grade chemicals, solvents and reagents were used. Chemicals such as hydrochloric acid (HCl), nitric acid (HNO3), hydrofluoric acid (HF) and hydrogen peroxide (H2O2) were purchased from Fisher Scientific (London, UK). A direct-Q 3 UV Millipore system (Massachusetts, USA) was used to prepare ultrapure water that was used to dilute stock solutions and a range of working standard solutions.

Instrumentation

An analytical balance (Precisa 180A, Switzerland) was used to measure the mass of the sample. The digestion of plant and soil samples was performed using a Multiwave 3000 microwave digester (Anton Paar, Switzerland). Metals were analyzed using inductively coupled plasma optical emission spectroscopy (ICP−OES) (Spectro, Kleve, German). A Bante 900P multiparameter water quality meter (Bante Instruments) was used to measure electrical conductivity (EC) and pH.

pH and conductivity of the tailings

A mass of 2.000 g of the material was weighed into a 50 ml plastic centrifuge tube. A volume of 40 ml of deionized water was added, and the mixture was subsequently placed on an end- to-end rotator for 10 minutes prior to measuring the pH and EC.

Vetiver grass preparation for analysis

At harvest, the entire plant was removed from the pot. The number of tillers and leaves and the length of the leaves were measured. The plants were then washed under running tap water, rinsed in 0.10 % HNO3 to remove any metal on the surface and finally rinsed with distilled water. The roots and shoots were separated and air-dried in the laboratory at room temperature until they reached a constant weight. The dry masses of the roots and leaves were recorded.

Element content: tailings

The tailings material was dried in the laboratory at room temperature for 10 days, homogenized and sieved. To evaluate the total metal concentration, a mass of approximately 0.250 g of $<$ 25 μm particles was weighed and placed into acid-washed digestion tubes (PTFE-TFM liners), into which 9 ml of HCl, 3 ml of HNO3, 1 ml of HF and 6 drops of H2O2 were added before being placed into the microwave digester. Extraction was performed in 60 minutes. The solution was transferred to 50 ml centrifuge tubes, which were filled with deionized water. All the samples were filtered through 22 μm Whatman Puradisc syringe filters prior to analysis.

Element content: vetiver grass

The dried leaves and roots were ground to powder using a blender. A mass of approximately 0,1000 g of ground material was placed in a microwave digestion vessel. To this mixture, 8 mL of HNO3 and 2 mL of H2O2 were added. Digestion was carried out for 30 minutes in a microwave digester. The digested samples were then transferred to 50 ml centrifuge tubes, which were filled with deionized water. All the samples were filtered through 22 μm pore filters prior to analysis.

Chemical analysis

ICP−OES was used to determine the concentrations of the elements in the tailings, compost, vetiver roots, and leaves. A total of 16 elements consisted of trace metals such as cadmium (Cd), cobalt (Co), chromium (Cr), arsenic (As), nickel (Ni), lead (Pb), and copper (Cu); essential macro- and micronutrients, including phosphorus (P), magnesium (Mg), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), sulphur (S), zinc (Zn) and aluminum (Al) were determined. The method for determining the concentrations of the metals in the plant parts and soils was validated using standard methods. The concentrations of the calibration standards were 0,100, 0,200, 0,500, 1,00 and 2,00, and the average coefficient correlation (r) was 0,8997.

Data processing and statistical analysis

The data were processed and statistically analyzed using the Data Analysis Tool Pack in Microsoft Excel. The presented results are an average of triplicate samples. The values for the plant height are in cm, the mass was measured in grams (g), and the concentrations are expressed in mg/kg of plant or tailings dry weight. Two-way ANOVA with replication was used to determine statistically significant differences between the treatments.

Results and discussion

Conductivity and pH

The percentage change in pH and EC between the initial measurements of amended tailings and at 16-weeks (at the end of the experiment) is shown in Fig. 3 below.

The pH increased by 15 % - 20 % for the 30 % compost-amended tailings and between 25 % - 32 % for the 60 % compost-amended tailings. The increase in pH coincided with studies by Cheng et al. [37]; Tang et al. [38]. The pH of the compost was significantly higher than that of the unamended tailings. The interaction of the functional group in compost reacts with protons (H^{+}) in tailings leading to an increase in pH. The increase is greater in 60 % amendment than 30 % because of more functional group in 60 % compared to 30 % amendment. The percentage decrease in EC was between 80 and 110 % in the 30 % compost amendments. In the 60 % compost amendments, a decrease of between 100 to 160 % was observed across the five treatments. A similar trend was also noted in studies by Gil-Loaiza et al. [39] and Melato et al. [28]. Gil-Loaiza et al. [39] reported a significant positive relationship in response to compost

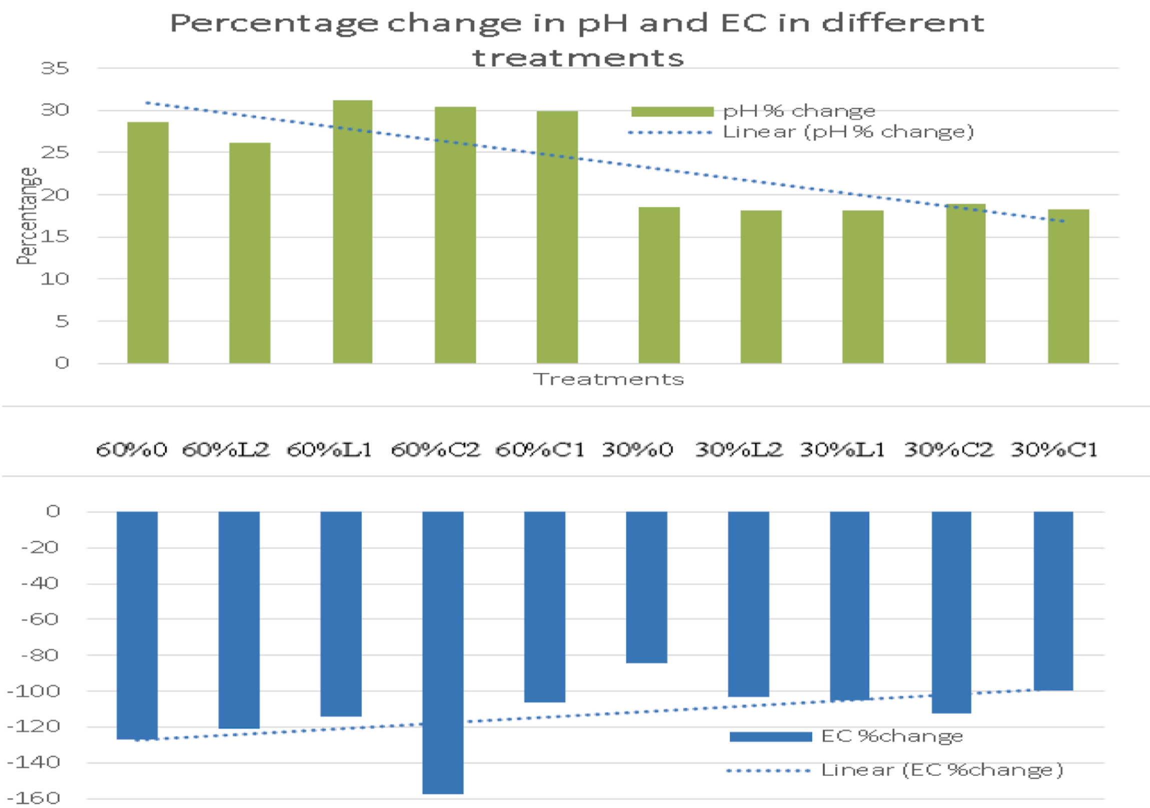

Fig. 3. Percentage change in pH and electrical conductivity (EC) across treatments during the study.

amendment ($R2 = 0{,}959$ $P < 0{,}205$). The decrease in EC could be due to the uptake of cations from the tailings by vetiver grass, leading to a decrease in the number of metal ions [28]. Biostimulant application had no significant influence on pH and EC on various treatments.

Growth parameters

The growth of the grass was monitored for 16 weeks. All the grass that was planted in the pots containing unamended tailings died within four weeks, regardless of whether they were sprayed with MLE or not. The grass that was planted in 30 % and 60 % amendment survived and the images after uprooting is shown on Fig. 4 below.

The results indicate that the unamended tailings were not suitable for vetiver growth, and that MLE alone was not sufficient to promote plant survival. Arthur et al. [40] also reported that B. juncea seedlings could not survive in unamended Krugersdorp Goldmine soils, regardless of biostimulant treatments used. Several reasons are attributed to these findings, and they include lack of essential nutrients in tailings; high levels of toxic heavy metals on tailings; high acidity; poor soil structure hence inadequate water holding capacity; high salinity or sodicity. These characteristics of tailings may have overwhelmed the beneficial effects of biostimulant and resilience of vetiver grass as they interfere with important cellular processes, damage membrane integrity, and ultimately cause plant death [41].

Although previous research has shown that biostimulants can improve plant growth and tolerance to abiotic stresses in agricultural soils for example, Yasmeen et al. [42]; Elzaawely et al. [23]; Zulfiqar et al. [24]; Abdalla [43]; Aluko et al. [44]; Merwad [45]; Mvumi et al. [46]; Rady & Mohamed, [20], the results of this study shows that the efficacy of MLE biostimulant on gold mine tailings is contingent upon compost amendment. Application of MLE alone on vetiver planted on gold mine tailings failed to yield any results. This finding underscores the critical role of compost amendment, indicating that MLE biostimulant's effectiveness on vetiver planted on gold mine tailings is dependent on compost amendment. The compost amendment increased the soil pH and organic carbon, which likely reduced the activity of the death causing chemoautotrophic sulphur oxidizers. Compost amendment can influence the diversification of the microbial community, which are beneficial to plant growth [47–49]. This explains the survival of vetiver grass on 30 % and 60 % compost amendment. However, Melato et al. [28] found that vetiver grass did not exhibit any signs of stress or death 16 weeks after planting in unamended acidic tailings. The difference in these study findings could be due to differences in the composition of the tailings and or the growth conditions under which vetiver was exposed to.

The growth parameters of all the grass that were planted on the 30 % and 60 % compost amendments was recorded in Table 4 below. Same superscripts indicate no significant difference while different superscript indicate a significant difference between measured parameters.

There was no significant difference between the 30 % and 60 % compost applications for all the measured growth parameters (Fig. 5).

While the positive effect of compost application has been noted in this study, the lack of difference between 30 % and 60 % compost application could be due to that, other than nutrients that are contained in the compost, it has high metals that probably interfered with vetiver growth. Excessive amounts of compost can cause problems such as nutrient imbalances, waterlogging, and reduced oxygen levels in the soil. These conditions can stunt plant growth and can even lead to plant death. This could explain why there was no significant difference in growth parameters between the 30 % and 60 % compost amendments.

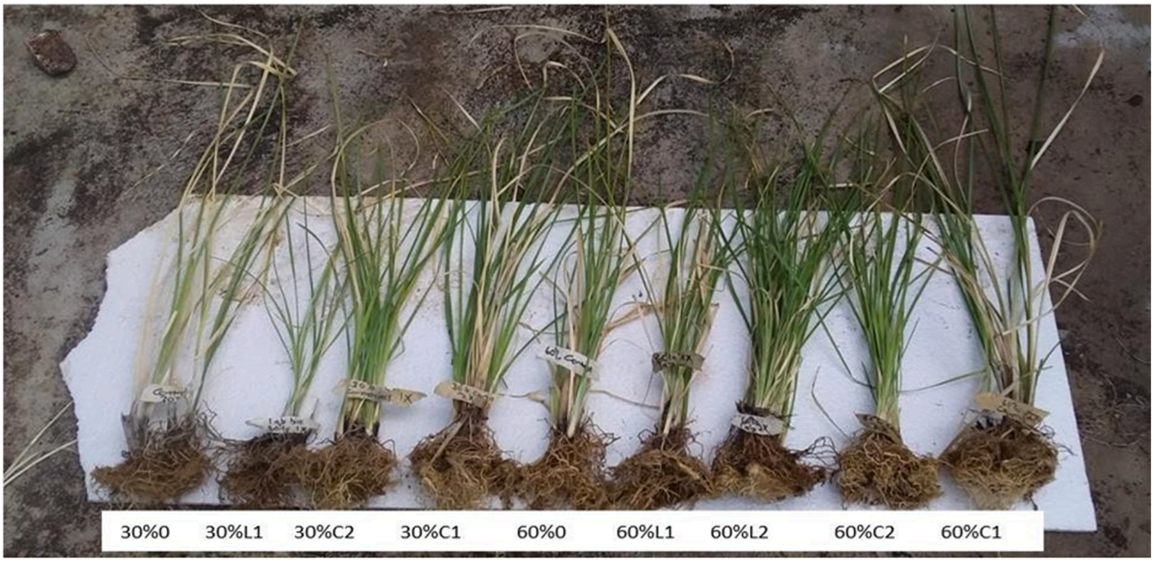

Fig. 4. Images of the vetiver grass plants from different compost and MLE treatments.

Table 4
Average number of leaves, number of tillers, vetiver leaf length, root dry mass and leaf dry mass (n=3, mean± std) at week 16 measured on vetiver harvested from different treatments.

Parameters	Leaf length (cm)	No. of leaves	No. of Tillers	Root dry mass (g)	Leaf dry mass (g)
60 % 0	47.95 ± 5.81 [a]	9.88 ± 4.94 [a]	3.25 ± 0.96[a]	8.82 ± 3.34 [a]	5.22 ± 2.03 [c]
60 % L2	34.18 ± 3.16 [c]	16.63 ± 8.21 [b]	6.40 ± 2.22 [b]	29.28 ± 7.32 [b]	17.93 ± 5.24 [b]
60 % L 1	44.68 ± 6.76 [b]	12.25 ± 6.02 [c]	4.50 ± 1.73 [c]	19.62 ± 6.45 [c]	11.64 ± 4.54 [a]
60 % C 2	40,13 ± 4.09 [b]	8.63 ± 4.34 [a]	3.25 ± 1.50 [a]	15.23 ± 5.32 [c]	8.01 ± 3.07 [d]
60 % C 1	51.55 ± 10.07 [c]	8.63 ± 5.78 [a]	3.25 ± 1.26 [a]	15.29 ± 6.01 [c]	7.21 ± 2.75 [d]
30 % 0	48.65 ± 3.21 [a]	9.63 ± 3.89 [a]	3.50 ± 0.58 [a]	7.54 ± 3.24 [a]	3.44 ± 1.21 [c]
30 % L 2	33.99 ± 5.44 [c]	9.13 ± 2.17 [a]	3.25 ± 0.54 [a]	7.01 ± 3.96 [a]	2.34 ± 0.76 [c]
30 % L 1	33.63 ± 7.23 [c]	9.00 ± 2.27 [a]	3.25 ± 0.50 [a]	6.57 ± 2.31 [a]	2.02 ± 0.95 [c]
30 % C 2	44.05 ± 6.61 [a]	9.50 ± 3.16 [a]	4.25 ± 0.96[a]	16.03 ± 6.02 [c]	8.91 ± 2.77 [d]
30 % C 1	49.40 ± 2.54 [a]	9.38 ± 4.41 [a]	3.50 ± 1.29 [a]	15.44 ± 6.29 [c]	7.74 ± 2.99 [d]
P value	0.0012	0.013	0.001	<0.001	<0.001

Different letters in each column indicate significant difference ($P < 0.05$).

The effect of application frequence for both the biostimulants on 60 % compost amendment and 30 % compost amendments were investigated and the results are presented in Fig. 6 below.

The commercial and laboratory MLE application frequency on vetiver grown on 30 % compost amendment led to no significant differences. However, application frequency of laboratory MLE biostimulant led to significant differences in all growth parameters except for the number of tillers, with twice a week application leading to significantly higher number of leaves, root and leaf mass. The application frequency of commercial biostimulant to vetiver grown on 60 % compost amendment led to a significantly high length for once-a-week application compared to twice a week application. The reasons for these differences are not yet understood especially where once a week application led to improved growth parameter compared to twice a week application. This could be contributed to the interaction of the compounds in the biostimulant and those in the compost. Fig. 7 below shows the comparison between commercial and laboratory biostimulants on vetiver grown on 30 % and 60 % amendments.

Biostimulants are known to contain cytokinins and auxins [50], which are expected to stimulate shoot initiation and plant growth [51]. This has proven to be true, as shown by the high number of tillers and leaves in treatments such as 60 %L1, 60 %L2, 30 %C1 and 30 %C2. Application of laboratory biostimulant on vetiver grown on 60 % biostimulant led to a significant improvement in root and leaf mass in both once a week and twice a week application compared to commercial biostimulant yet in vetiver grown in 30 % compost amendment, commercial biostimulant led to significantly high leaf and root biomass.

The contrasting effect of the laboratory and commercial biostimulant is intriguing. This could be due to (i) biostimulant composition (partly provided in Table 2) or uptake mechanisms at different compost levels; (ii) the commercial biostimulant may have undergone processing, formulation, and quality control measures to enhance its stability, shelf life and efficacy; (iii) the laboratory

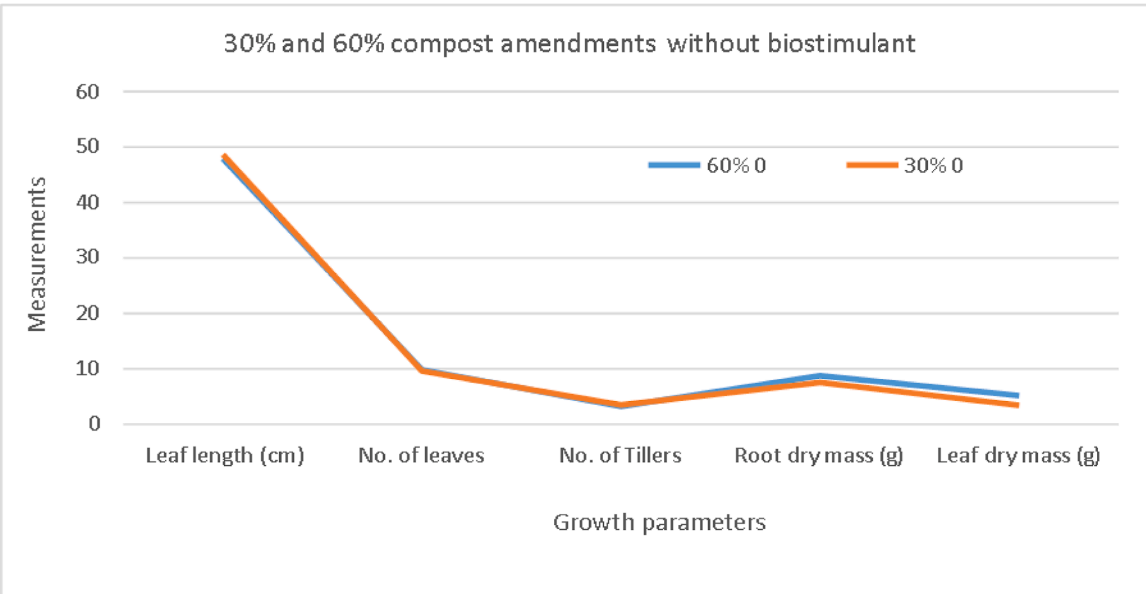

Fig. 5. Effect of 30 % and 60 % compost on the length, number of leaves, root and leaf dry mass of vetiver grass planted in the footprint and tailings material in the Witwatersrand Goldfields in South Africa.

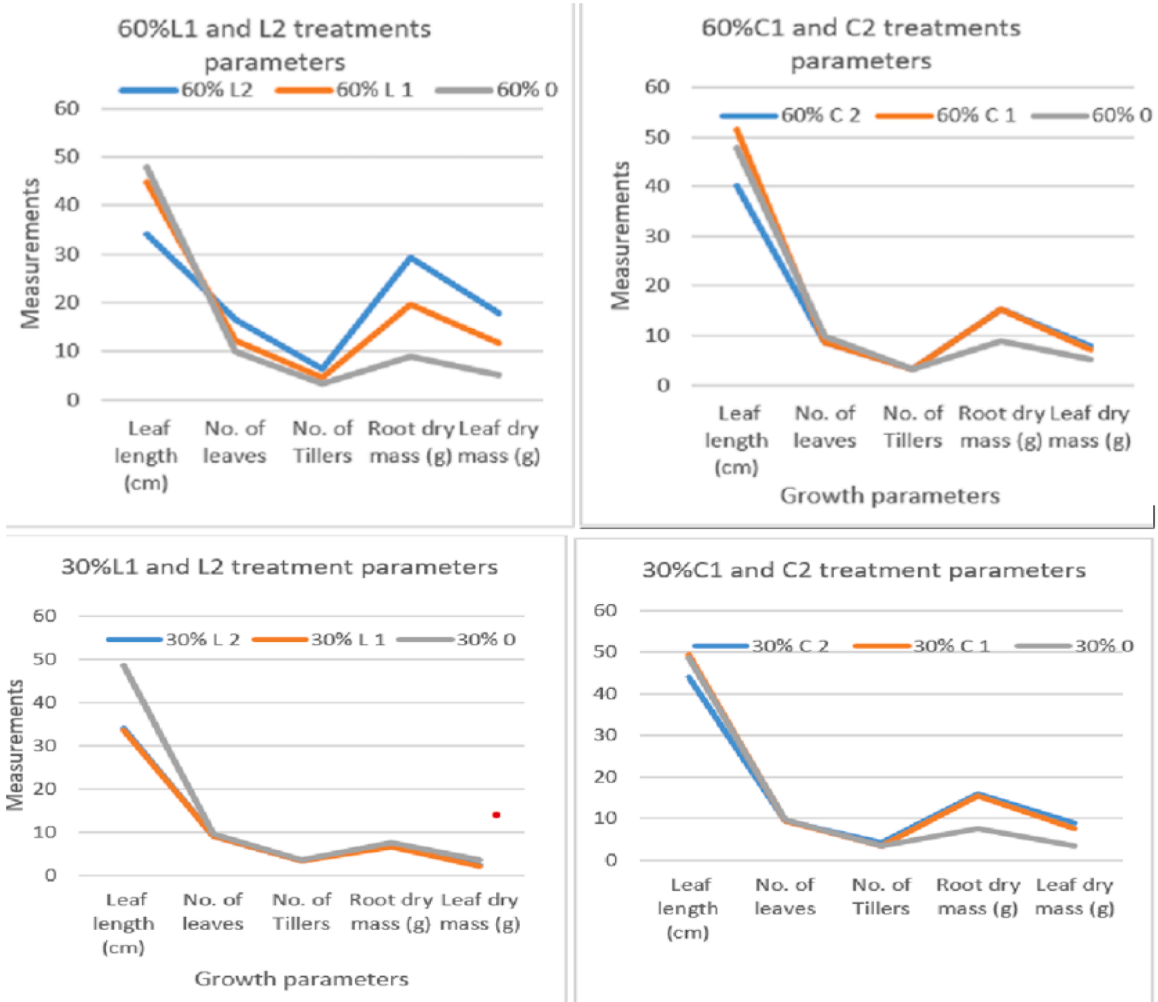

Fig. 6. Effect of commercial and laboratory MLE application frequences on vetiver laboratory planted on 30 % and 60 % compost amendments.

extracted biostimulant may have retained more of the plant's natural compounds and potentially offer a subtle effect on plant growth; (iv) the commercial biostimulant might be more effective in lower compost amendment level (30 %) because it contains nutrients or growth promoters that complement the limited nutrients available in the soil whereas the laboratory-extracted biostimulant might be more effective in higher compost amendment levels (60 %) because it contains compounds that enhance nutrient uptake or utilization, which becomes more relevant when nutrients are abundant; (v) the laboratory-extracted biostimulant might contain a lower concentration of phytochemicals such as phenolic compounds or terpenes, that are more effective in higher compost amendment levels, which can enhance plant growth or stress tolerance and (vi) there might be synergistic interactions between the biostimulants and the compost amendments, leading to enhanced effects at specific amendment levels.

It has been determined that the commercial and laboratory biostimulants varies in their composition. Yang et al. [52] alluded that there are various factors that influence chemical and nutrient variability. These factors include harvesting technique and post harvesting handling technique, genetic makeup and growth environment. Additionally, the mineral composition of M. oleifera varies greatly from one plant to the other [53]. It is therefore not surprising that the laboratory and commercial biostimulant led to different results.

Although the elemental analysis of the two biostimulants, showed that commercial biostimulant contains higher concentrations of essential elements such as Ca, Fe, P, S, and Zn, which are crucial for plant growth and development. Additionally, it has lower concentrations of potentially toxic elements like Al and Cr. In contrast, the laboratory MLE treatment had lower concentration of beneficial elements and has higher levels of toxic elements, which may hinder plant growth. However, it is important to note that this analysis only considers the elemental composition of the biostimulants and does not account for other potentially influential factors such as amino acid profiles and phytohormone content, which were beyond the scope of the present study. Secondly, biostimulants typically work by stimulating plant physiological processes, not by providing significant amounts of nutrients. Their mode of action often involves signaling pathways, hormone regulation, antioxidant activity, microbiome modulation [19,54,55]. These mechanisms can lead

Fig. 7. Effect of commercial MLE and laboratory MLE on vetiver laboratory planted on 30 % and 60 % compost amendments.

to improved plant growth, stress tolerance, and nutrient uptake, even if the biostimulant itself doesn't contain significant amounts of nutrients.

It is however puzzling how the laboratory extracted biostimulant had led to a reduction in the length of vetiver leaves across the treatments and the application of commercial MLE biostimulant leading to no significant differences on the number of leaves produced in most of the treatments. The reasons for reduction and no difference in length could because the biostimulant promoted the increase in leaf thickness and density instead. MLE contains plant hormones such as auxins, gibberellins or cytokinins, amino acids and phenolic compounds. Plant hormones regulate cell growth and differentiation. Amino acids can stimulate cell growth and protein synthesis and phenolic compounds can contribute to cell wall thickening and plant growth [23,24].

While the exact mechanisms of the biostimulant's action are not fully understood, it is likely that they involve complex interactions with plant physiological processes and soil microbiology. Further research is needed to elucidate these mechanisms and determine how they contribute to the biostimulants effects on plant growth and development.

Element concentrations at week 16

The concentrations of the elements measured at week 16 in the vetiver grass leaves, roots and amended tailings are presented in Figs. 8–10 below.

Of the 16 elements investigated, 14 were detected in the amended tailings. Cd and Co concentrations were below the detection limits in the tailings and Cd, Co, Cr, As, Ni and Pb were below the detection limits in leaves and roots. P, Mg, K, Ca, and Mn were greater in the leaves than in the roots of the grasses across all the treatments. The Fe and Al concentrations were greatest in the roots, while the Zn concentration in the roots and leaves varied from treatment to treatment. The 60 % L2 treatment had the highest concentrations of Zn and Mg in its leaves compared to those in the other treatments. The second highest Zn concentration in the leaves was recorded in the 30 % C2 treatment group. Zn and Mg are important for plant growth. High concentrations of these metals in 60 %L2 treatment correlates well with the highest growth parameters observed under this treatment. This suggest that the combination of 60 % biostimulant and laboratory MLE enhanced Zn and Mg uptake, leading to improved growth parameters. This is also true for the combination of 30 % compost and commercial biostimulant. Zn plays a role in enzyme activity, protein synthesis, and stress tolerance while Mg is responsible for photosynthesis, cell wall structure and nutrient uptake. The highest P concentration was recorded in vetiver grown in 60 %0 treatment, while the roots of plants in this treatment had the lowest P concentration. The 30 %C1 and 30 %C2 treatments presented higher P concentrations in the leaves than in the roots. The roots presented higher concentrations of Al and Fe than the leaves across all the treatments. This shows limited translocation of these elements to the leaves, possibly to prevent toxicity. Al and Fe can be beneficial in small amount but toxic in excess. The concentration of K in the leaves was highest in the 60 %0 treatment group.

The undetected levels of Cr, As, Ni, and Pb in the leaves and roots of vetiver grass grown in both 30 % and 60 % compost-amended tailings are likely the result of metal adsorption by the compost [56,57], which led to low bioavailability of metals in compost-amended tailings, as suggested by [58]. In addition to adsorption, the increase in pH caused by compost amendment can also lower the bioavailability of metals. For example, [59] reported that the available fraction of As is largely determined by soil pH, even in tailings containing high As concentrations.

Although the results of this study did not demonstrate the uptake of metals of concern by vetiver grass, the observed increase in root and leaf biomass in the compost-amended tailings in the 30 % C1, 30 % C2, 60 % L1 and 60 % L2 treatment groups indicates that this technique may be effective at stabilizing contaminants in the rhizosphere. Singh [60] argues that the objective of phytostabilization is to establish plants in contaminated soils and trap metals in the rhizosphere instead of extracting them from the soil [61]. The vegetative cap, plant– root exudates, organic matter, and established microbial community promote the stabilization of inorganic contaminants in the root zone [62,63]. While Wuana & Okieimen [64] suggested that vegetation cover can complex or reduce the valence of metals in the rhizosphere, they also noted that the accumulation of metals in the roots by vegetation cover also reduces metal mobility, thereby improving the phytostabilization process.

However, the results of this study differ from the findings of Andra et al. [65]; Danh et al. [25]; Datta et al. [66]; Truong et al. [67], who reported that vetiver can accumulate high levels of toxic metals in its roots and shoots. According to Melato et al. [28], vetiver grass can take up toxic metals from tailings and accumulate them in its roots, limiting translocation to the shoots, which is desirable for phytostabilization. Zhang et al. [68] observed high accumulation of Cd in vetiver grass exposed to Cd. Banerjee et al. [69,70] confirmed the findings that vetiver uptake and accumulate large amounts of Cd and can be utilized in the reclamation of Cd-contaminated soil. The findings of this study were unexpected and can be attributed to the physical-chemical properties of the tailings being strongly influenced by compost amendments.

Vetiver grass is highly likely to absorb nutrients such as P, K, Mg, Mn, Cu and Ca at elevated concentrations. This could also explain the ability of vetiver to grow under the experimental conditions, as these elements are essential for plant growth. Overall, the least nutrient accumulation in the roots and leaves of vetiver grass was recorded in the 30 %0, 30 %L1 and 30 %L2 treatments. This explains the poor growth of vetiver grass under these treatments and the signs of chlorosis noted in vetiver grass subjected to these treatments. This suggests a strong correlation between nutrient uptake and plant growth. We cold argue that 30 % compost treatment without biostimulant likely had limited nutrient availability, leading to reduced plant growth. But, because no significant difference was noted between the 60 %0 and 30 %0, therefore this implies that, other factors like nutrient uptake efficiency might be influencing the growth.

Although vetiver grass is not as effective as some other plants at accumulating heavy metals, its wide tolerance to adverse climate and soil conditions makes it a promising candidate for phytostabilization. Its high biomass has the potential for carbon offsetting and bioenergy production while also providing a valuable source of material for phytoproducts. These benefits can improve the economic activities and livelihoods of local communities.

Conclusions

The results of the study showed that there was no significant difference between the once a week and twice a week MLE application frequency except between the 60 %L1 and 60 %L2 treatments, where the vetiver biomass in the 60 %L2 treatment was significantly greater. Therefore, the application frequency is generally not a significant factor in determining plant biomass.

Among the 60 % compost amendment treatments, the laboratory-extracted MLE-treated plants produced the highest plant biomass. In the 30 % treatment group, the plants treated with commercial MLE produced the highest biomass compared to plants treated with laboratory extracted MLE. Generally, the order of increase in growth parameters was 60 % L treatments >30 % C treatments > 60 % C treatments > 60 % 0 treatments = 30 % 0 =30 % L treatments. The results suggest that the interaction between the biostimulant and compost has the most significant influence on plant biomass. This conclusion is supported by the fact that the effect of the biostimulants alone did not prevent plants from dying when they were grown in unamended tailings. These findings suggested that it is important to obtain right ratios of the compost and biostimulants to achieve the desired results.

The results showed that vetiver grass could not accumulate some of the metals of concern (As, Cd, Cr and Pb) in its biomass. The lack of significant accumulation of metals in vetiver grass does not negate the phytostabilization of mine tailings using compost, vetiver, and MLE. Although metal accumulation in plant tissue is one of the measures of phytostabilization, vegetation establishment is the most important factor in the phytostabilization of mine tailings because it improves soil stability, prevents erosion, provides

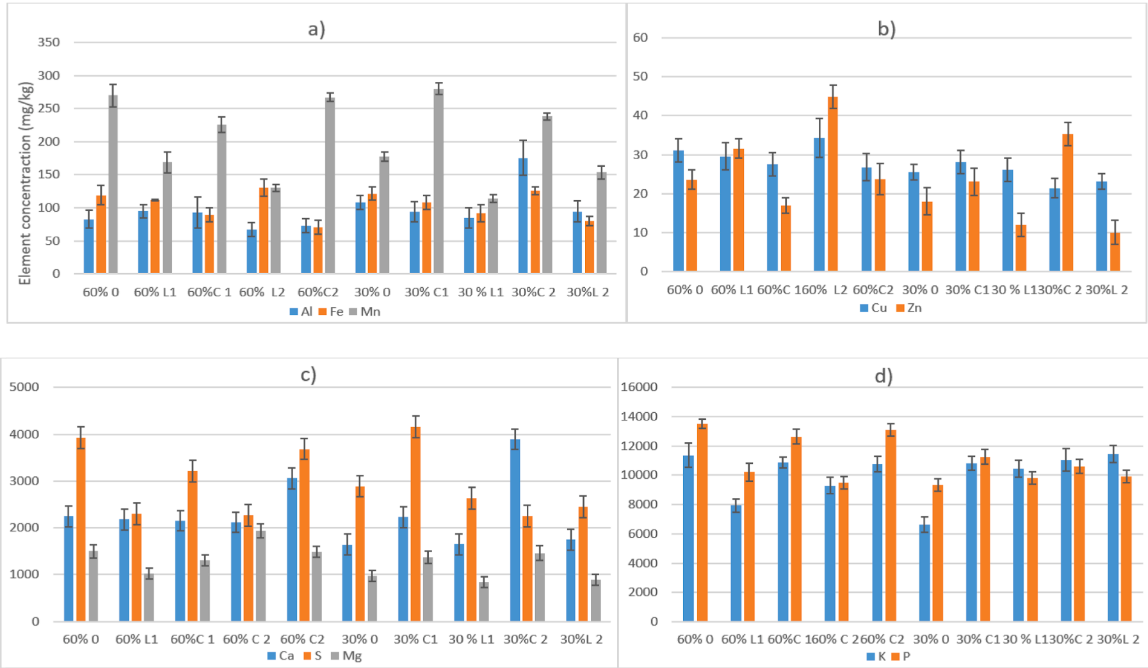

Fig. 8. a) Al, Fe and Mn; b) Cu and Zn; c) Ca, S and Mg; d) K and P concentrations in the vetiver grass leaves from different treatments.

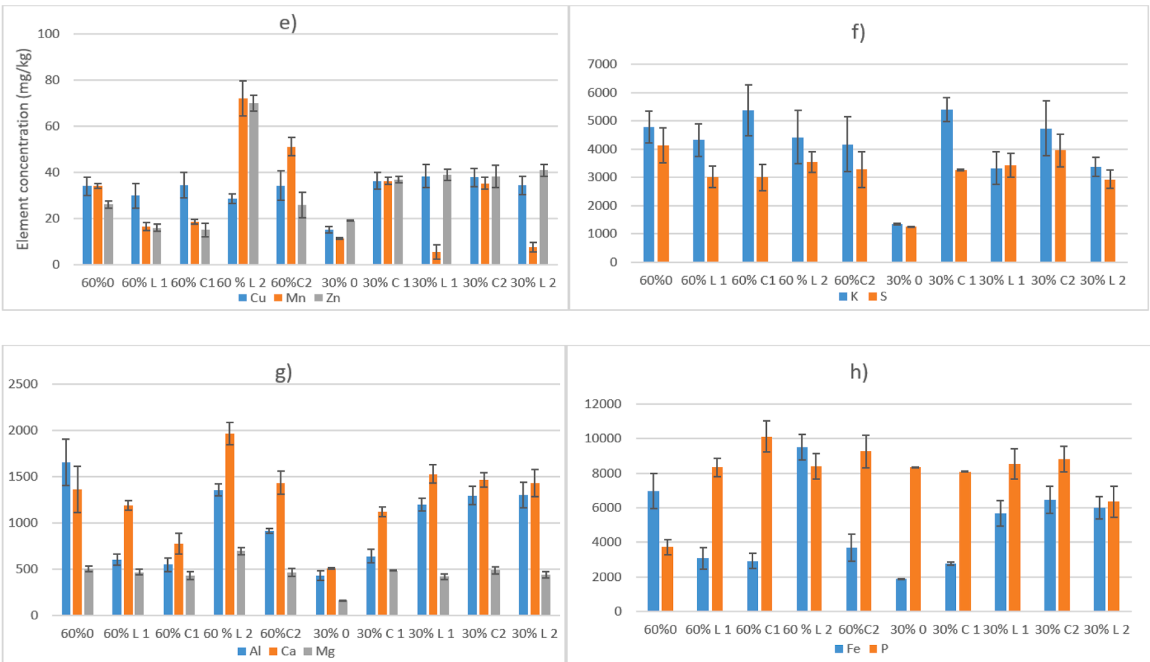

Fig. 9. e) Cu, Mn, and Zn; f) K and S; g) Al, Ca and Mg; h) Fe and P concentrations in the roots of vetiver grass from different treatments.

aesthetic benefits and provides other phytoproducts and services. Future studies should, however, focus on finding the lowest effective compost percentage when combined with laboratory extracted MLE, which can maximize biomass production and the phytoavailability of metals. Consequently, the laboratory moringa leaf extraction method must be optimized. The ultimate goal is to create a cost-effective, sustainable environmental cleanup strategy that reduces contaminants while providing social, economic, and other environmental benefits.

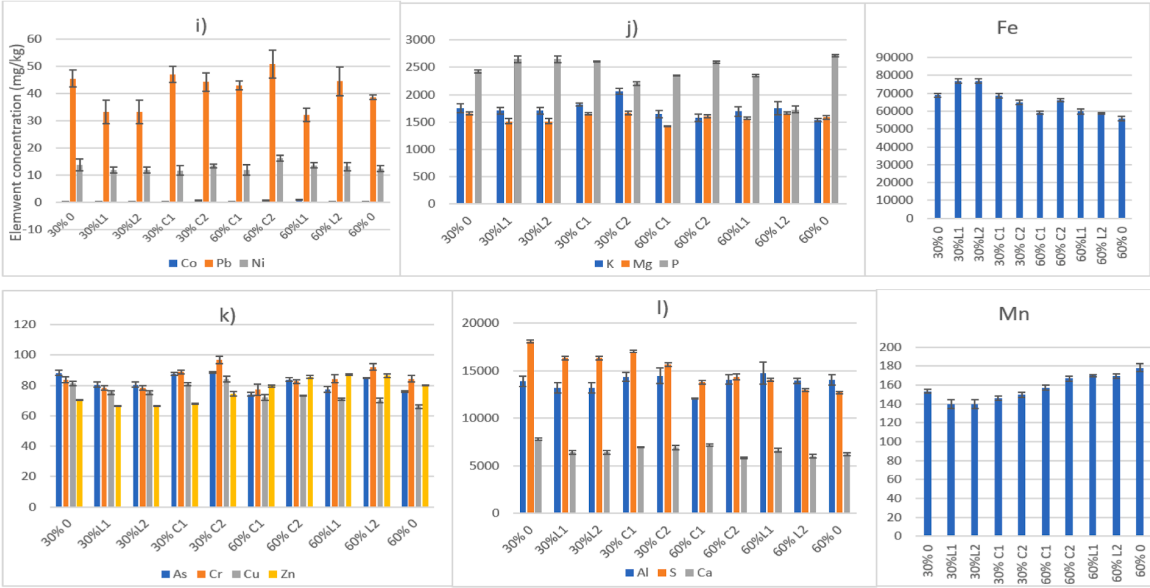

Fig. 10. Concentrations of Mn, Fe, i) Co, Pb and Ni; j) K, Mg and P; k) As, Cr, Cu and Zn; and l) Al, S and Ca in amended tailings measured at week 16.

Declaration of competing 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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