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Variability of The Phytoremediation Potential of Different Tree Species in BCL Mine Tailings

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Abstract

Five different tree species were evaluated for their phytoremediation potential under Bamangwato Concessions Limited (BCL) mine tailings. The survival rate, dry matter yield/week, and uptake/removal capacity of heavy metal(loid)s by these species were used to evaluate their phytoremediation potential. The tree species selected for the study were *Eucalyptus globulus, Colophospermum mopane, Vachellia tortilis, Vachellia luederitzii, and Fairherbia albida.* Three amendments that were applied are: fly ash, compost, and arbuscular mycorrhiza. The best amendment that improved the survival rate and dry matter yield per week was the addition of fly ash (FA) and arbuscular mycorrhiza (FA+AM) followed by compost and arbuscular mycorrhiza (CM+AM). These amendments improved the survival rate of all species by 100%. The addition of compost alone was found to be the least effective amendment as some tree species recorded a survival rate of 50-70%. *V. luederitzii* recorded the highest overall dry matter yield/week. Overall, the results indicated that the addition of the correct amendments to all treatments allows tree species to survive in BCL mine tailings. Heavy metal uptake capacity or accumulation was affected by the amendments, and each amendment affected each heavy metal differently, allowing each tree species to have phytoremediation potential depending on the heavy metal(loid)s uptake.

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1. Introduction

Mining activities tend to affect the environment through land degradation, and the presence of heavy metal(loid)s. Generally, mining activities cause disturbances for many soil properties, including pH, electrical conductivity, and cation exchange capacity (Saleem et al., 2020). Subsequently, heavy metals such as Copper (Cu), Lead (Pb), Zinc (Zn), Nickel (Ni), Cadmium (Cd), Chromium (Cr), Iron (Fe), Manganese (Mn) and other organic pollutants such as hydrocarbons, have been found at elevated concentrations in the soil and sediments in mining areas (Gutiérrez-Ginés et al., 2014; Liu et al., 2017b; Song et al., 2017). Therefore, tree species could offer a solution to remediate these heavy metals from the environment through a process known as phytoremediation (Gomez et al., 2019). Phytoremediation is a method that utilizes plants to remediate contaminated environments. Phytoremediation is an inexpensive and environmentally favorable technique as it utilizes photosynthesizing plants to sequester or cleanse contaminants from contaminated soil and water (Ashraf et al., 2019). Different plants use one or more mechanisms to remediate different contaminants at different rates. Using a variety of methods, including genetic engineering, natural and chemical modifications, and natural microbial stimulation, the limitations of phytoremediation can be assessed (Kafle et al., 2022). Different plants utilize various strategies or combinations of strategies to remediate soil and water. The strategies should be contingent upon the pollutant types, structures, and media. Polluted groundwater can be remediated through phytodegradation, phytovolatilization, rhizo-filtration, rhizo-degradation, and phytodegradation. Surface and wastewater pollutants can be treated by rhizofiltration, phytodegradation, or rhizodegradation. Soil, dregs, or slime defilements are remediated through phytoextraction, phytodegradations, phytostabilization, rhizodegradation, or phytovolatilization (Kafle et al., 2022). Rapid growth, a high biomass, and a high tolerance to heavy metals are desirable qualities for the plants utilized in this process of phytoremediation (Marques, 2009). Immobilizing or limiting the mobility of heavy metals is the goal of phytostabilization. Metals can precipitate in the rhizosphere, be accumulated by the roots, or be absorbed on their surface (Podar and Maathuis, 2022). Plants with a strong root system and a high level of heavy metal tolerance are suitable candidates for phytostabilization. Additionally, these plants must have low metal translocation from roots to shoots and the ability to tolerate high metal buildup in the roots (Karczewska et al., 2013, Korzeniowska et al., 2011, Stanisławska-Glubiak et al., 2012). Therefore, the advantage of using multi-species outweighs the use of a single species for phytoremediation. Tree species are potential plants for phytoremediation due to their rapid growth and extensive root system (Shi et al., 2016). This study will evaluate the potential of five different tree species for the phytoremediation of BCL tailings. These are *Colophospermum mopane, Vachellia tortilis, Vachellia luederitzii, and Fairherbia albida.*

2. Materials and Methods

The data on survival rate, dry matter yield, and heavy metal accumulation by five different tree species was computed and analyzed through meta-analysis. Relative dry matter yield was calculated by dividing the dry matter yield of each species as affected by different amendments with the growing period (weeks) (Eq 1.).

$$\text{Relative Dry matter yield} = \frac{\text{Dry matter yield}}{\text{Number of weeks since planting trees}} \quad \text{[Eq.1]}$$

Total heavy metal(loid)s accumulated by different tree species as affected by amendments were estimated by multiplying the relative dry matter yield by the total HM concentration in the tissues to obtain heavy metal uptake capacity and removal rate through biomass extraction (Eq. 2).

$$\text{HM uptake capacity} = \text{Total HM x Relative Dry matter yield} \quad \text{[Eq.2]}$$

The transformed data were used to assess the heavy metal uptake and removal rate by these five different species as influenced by different amendments like fly ash, cow manure, and sewage sludge. 10% of these amendments were applied singularly or in combination. The tree species that were used for meta-analysis were grown in a pot experiment in the same tailings from BCL and the same amendments were applied. *C. mopane* was grown for 40 weeks, *V. tortilis*, *F. albida*, and *V. luederitzii* had a growth period of 24 weeks, and *E. globulus* was grown for 52 weeks.

2.1. Statistical analysis

The transformed data was analyzed using a single-factor ANOVA in CRD to determine the differences in heavy metal(loid)s concentration accumulation between tree species. When significant differences were observed, Tukey's test was performed for treatment mean (tree species) comparison at a 5% level of significance. Each parameter was analyzed separately per tree species.

3. Results and Discussion

3.1. Effects of amendments on the Survival Rate & Dry matter yield per Week of tree species

Table 1 indicates that all tree species grown in mine tailings without amendments (soil alone) had a survival rate below 100%. Specifically, *E. globulus* had a 0% survival rate. *V. tortilis* had a 30% survival rate whereas *C. mopane*, *V. luederitzii, and F. albida* each had a survival rate of 70%. Similarly, those tree species that have survived had low relative dry matter yield especially *C mopane*. This survival rate and relative dry matter yield were attributed to the acidic nature of mine tailings and the high concentration of heavy metal(loid)s in the tailings which hinders plant growth due to the deficiency of essential nutrients and toxicity of some metals. With the addition of mycorrhiza, the survival rate of all tree species improved with *V. tortilis* and *V. luederitzii* recording a 100% survival rate and *E. globulus* improving from 0% to 70%. In addiiton, *V. luederitzii* recorded a highly significant faster growth rate and significantly higher relative dry matter yield (3.18g/plant/week) compared to the corresponding plants in pure mine tailings (control) due to mycorrhiza inoculation.

Table 1: Survival rate and dry matter yield and relative dry matter yield/week of five different species.

Treatments	Species name	Survival Rate	Dry matter yield	Number of weeks planted	Relative Dry matter yield/week
		(%)	(g/plant)		(g/plant)
Soil Alone	*C. mopane*	70d	2.100	40	0.05i
	V. tortilis	30f	15.68j	24	0.65g
	E. globulus	0g	0.00m	52	0.00i
	F. albida	70d	60.09h	24	2.50e
	V. luederitzii	70d	76.40g	24	3.18e
Soil + AM	*C. mopane*	90b	3.80l	40	0.10i
	V. tortilis	100a	10.51k	24	0.44g
	E. globulus	75c	17.77j	52	0.34
	F. albida	90b	67.56h	24	2.82e
	V. luederitzii	100a	121.60e	24	5.07c
Soil+10% FA	*C. mopane*	100a	5.10l	40	0.13i
	V. tortilis	100a	16.18j	24	0.67g
	E. globulus	100a	82.87g	52	1.59f
	F. albida	100a	278.28c	24	11.60b
	V. luederitzii	100a	630.90b	24	26.29a
Soil+10% FA + AM	*C. mopane*	100a	3.80l	40	0.10i
	V. tortilis	100a	33.91i	24	1.41f
	E. globulus	100a	128.71e	52	2.48e
	F. albida	100a	263.08c	24	10.96b
	V. luederitzii	100a	727.00a	24	30.29a
Soil + 10% Compost	*C. mopane*	70d	7.90k	40	0.20h
	V. tortilis	100a	7.90k	24	0.33h
	E. globulus	50e	25.65i	52	0.49g
	F. albida	70d	110.25ef	24	4.59d
	V. luederitzii	70d	145.02e	24	6.04c
Soil + 10% Compost+AM	*C. mopane*	100a	13.40j	40	0.34h
	V. tortilis	100a	13.40j	24	0.56g

	E. globulus	100a	54.51h	52	1.05f
	F. albida	100a	161.33d	24	6.72c
	V. luederitzii	100a	188.54d	24	7.86c
P-value		0.000***	0.000***		0.000***

[1]Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at a 5% level of significance; *** very highly significant.

The addition of 10% FA on mine tailings significantly improved the survival rate and relative dry matter yield of all tree species as compared to those in the control. The relative dry matter yield of *V. luederitzii,* and *F. albida* is significantly higher than the increase of relative dry matter yield in the presence of Mycorrhiza alone. The combination of FA+AM resulted in 100% survival rate and significantly higher relative dry matter yield of all tree species as compared to control and FA alone, and in tailings amended with AM alone. The difference between FA alone and FA+AM on relative dry matter yield of *F. albida and V. luederitzii*, is not significantly different as denoted by the same letters in the Table 6.3.1. The addition of FA+AM significantly improved the dry matter yield of *E. globulus* compared to soil alone, AM alone and FA alone.

The addition of 10% compost significantly improved the survival rate of tree species especially *E. globulus and V. tortilis but not F. albida, V. luederitzii,* and *C. mopane.* In terms of the relative dry matter yield, a significant increase was also observed due to compost application as compared to the control, but such increase was insignificant compared to treatments involving mycorrhiza (+AM). Among the different tree species that were added with compost, the highest relative dry matter yield was observed in *V. luederitzii,* as compared to *E. globulus* and *C. mopane.* The combination of compost and AM resulted to a much higher survival rate (100% for all species). The relative dry matter also indicated a significant increase due to combined application of compost and AM as compared to the control for each species. The highest increase due to the addition of compost and AM was observed in *F. albida,* and *V. luederitzii*.

Overall performance of these different tree species indicated that there is a significant increase in survival rate with the application FA, compost, AM and its combination leading to 100% survival rate. In terms of the relative dry matter yield, *F. albida,* and *V. luederitzii* produced significantly high dry matter compared to other tree species especially in the presence of FA+AM and FA alone. These results indicate that in acidic soils with low nutrients and high heavy metal contents, a synergistic effect of compost and mycorrhiza or FA +AM on the growth of tree species will occur. The enhancement of survival growth, development, and dry matter yield/week of tree species can be attributed to the different roles these organisms and amendments played in the tree species and the properties of soils (Thoms and Gleixner, 2013). Studies by Aransiola et al. (2019) and Nie et al. (2017) indicated that mycorrhiza in roots can enhance the plant's ability to uptake water and essential nutrients and allow it to tolerate unfavorable environments through several mechanisms. These include the secretion of secondary plant metabolites such as siderophores, organic acids, biosurfactants, etc. that will affect numerous biogeochemical processes in the rhizosphere and improve plant growth, especially in a stressful environment such as those with high heavy metal contents. Through cell extension and the transformation of nutrient elements that occurs when they are either applied to seeds or incorporated into the soil, mycorrhiza can enhance the supply and absorption of nutrients of the host plants (Aransiola et al., 2019; Ultra et al., 2007). Second, amendments such as compost and FA can modify the soil's chemical, biological, and physical characteristics, making the soil less stressful to seedlings (Balasubramaniam, 2020; Sharma and Singh, 2019).

Compost enhances the soil buffering capacity and neutralize acidity aside from its ability to supply essential nutrients (Sánchez et al., 2017). The high microbial load of compost will also provide the necessary microbes that will help in the transformation of nutrients and hasten nutrient availability, and consequently absorption by plants (Akmal et al., 2019). The third amendment, fly ash from Morupule Power Plant was alkaline enabling it to neutralize the acidity of soil substantially as compared to compost. With the exception of nitrogen, it also contains all essential nutrients for plants, including P, S, K, Ca, Mg, Cu, Mn, and Zn (Yunusa et al., 2006). The increase in pH due to FA will decrease the acidity stress on tree species and thus enhance survival. Acidic soil is very detrimental to seedlings for non-acid-tolerant plants because it can cause dehydration, oxidative stress to roots, and reduced nutrient availability (Dos Reis et al., 2018). The enhanced survival, growth, and development of tree species seedlings due to the combined effects of mycorrhizal inoculation, FA, or compost amendments is due to these favorable effects on soil and the plant's ability to acquire water, nutrients and tolerate the environmental stress associated with acidity and high heavy metal(loid)s in soils (Ultra, 2020). However, results also showed that the addition of compost alone negatively impacted the survival rate, and dry matter yield/week for tree species like *E. Globulus, F. albida V. luederitzii*. This is largely because, without the addition of FA, compost alone could not alter the pH of the soil, leaving the soil acidic and toxic to plants (Latifah et al., 2018).

3.2. Effects of amendments on heavy metal accumulation capacity of different tree species

3.2.1. Effects of amendments on heavy metal accumulation capacity of different tree species

Table 2 show the heavy metal accumulation of different tree species as influenced by the addition of amendments (AM, FA, FA+CM, and CM). Within the control (soil alone), there is a significant variation in the concentration of metals and metalloid(s) accumulated in different tissues by different tree species. The addition of AM reduces the uptake of As in shoots and roots of all tree species. AM also significantly reduces the uptake of Cu, Mn, Ni, Pb and Zn in shoots and roots in all tree species. Compared to heavy metal(loid)s accumulation in the control, the addition of FA also reduces the accumulation of all the analysed metals and metalloid(s). The addition of FA+AM significantly decreased the concentration of these metals and metalloid(s) in shoots and in roots of all tree species. The application of 10% compost and 10% + AM significantly decreased the presence of these metals in shoots and roots when compared to the control. The addition of 10% compost proved to be the amendment that had the highest significant reduction of metals and metalloids especially As, Cu, Mn in most species. The lowest concentration of As and Cu was observed in *E. globulus* (shoots and roots) when grown in tailings with 10% FA and AM respectively. As for Mn, the lowest concentration was observed in the shoots of *V. luederitzii* and in the roots of *F. albida* under the +AM treatment which are significantly lower comapred to other treatments.

Table 2: Heavy metal accumulation by five tree species as influenced by amendments.

Treatments	Species	Heavy Metals Concentration (mg/kg)											
		As		Cu		Mn		Ni		Pb		Zn	
		Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Soil Alone	*C. mopane*	21.20c	66.18b	63.92f	705.88b	37.42h	29.41h	34.3f	112.75d	54.03j	68.15j	54.56d	98.04c
	V. tortilis	44.42b	63.28b	473.33 a	586.67c	103.49c	93.48c	119.08b	104.26d	176.48e	185.24f	132.85b	110.67b
	E. globulus	-	-	-	-	-	-	-	-	-	-	-	-
	F. albida	55.42a	53.28c	383.23 b	336.87d	83.59d	73.48d	219.08a	194.66b	276.48d	245.26e	102.8c	90.97c
	V. luederitzii	39.44b	96.1a	445.9a	1212.2a	19.4i	52.37f	93.11c	265.1a	322.8c	918.6b	24.7h	70.31e
Soil +AM	*C. mopane*	15.35d	25.55e	40.62g	250.11e	48.95g	25.01i	20.83g	66.1f	56.09j	66.32j	28.12g	39.3h
	V. tortilis	22.11c	49.96c	158.14 d	136.72f	72.76e	84.23c	68.25d	92.15e	168.44e	224.27e	59.38d	65.78e
	E. globulus	1.08e	2.11h	4.21k	5.01j	45.28g	66.33e	25.03g	22.02k	32.05l	38.02k	45.88e	66.02e
	F. albida	42.11b	69.96b	138.08 e	126.82f	61.71f	56.29f	78.55d	72.19f	168.44e	154.17f	55.31d	50.58g
	V. luederitzii	22.31c	65.19b	256.76 c	759.25b	15.39j	46.64g	54.88e	173.3c	167.5e	562.15c	14.31j	46.42g
Soil+10 % FA	*C. mopane*	8.94e	36.82d	22.36h	85.16g	48.94g	30.06h	3.02j	22.54k	25.33	41.08k	19.94i	30.06h
	V. tortilis	38.21b	77.01a	68.48f	243.23e	55.31f	77.02d	10.54i	40.54i	5.42n	8.26l	28.97g	44.59g
	E. globulus	2.50e	1.90h	2.81k	87.55g	100.57c	144b	4.67j	71f	8.20m	70.38i	41.08e	54g
	F. albida	34.13b	67.2b	11.79i	51.53h	32.91h	48.99f	6.49j	17.52l	590.87a	1061.84a	268.94a	267.69a
	V. luederitzii	32.25b	84.44a	157.27 d	226.4e	130.54b	162.06b	30.54f	123.6d	240d	312.83ld	29.43g	47.79g
Soil+10 % FA + AM	*C. mopane*	11.69d	39.79d	18.92i	109.57f	66.2f	54.79f	2.58	28.84j	3.07n	7.55	16.34j	60.55f
	V. tortilis	23.86c	72.57a	88.12e	293.74e	48.46g	71.92d	13.22h	60.27g	6.61m	7.22l	37.45e	89.63c
	E. globulus	5.02e	2.11h	5.45j	99.21fg	105.33c	154.07b	5.07j	23.22k	405.33b	305.21d	44.31e	65.02f
	F. albida	16.54d	75.35a	16.55i	66.19g	26.22i	36.65h	9.37i	17.99l	566.47a	1121.29a	32.75f	80.39d
	V. luederitzii	21.27c	48.85c	15.52i	61.69gh	47.74g	35.24h	2.82j	10.5m	290.00c	287.76e	15.52j	12.53j
Soil + 10% Compost	*C. mopane*	7.38e	11.63f	22.54h	147.47f	86.05d	36.87h	12.29h	49.98h	35.25l	28.02l	21.51h	32.77h
	V. tortilis	6.21e	36.13d	10.03j	42.08h	20.75i	28.21i	10.03i	11.54m	56.88j	52.08j	28.36g	15.53j
	E. globulus	4.20e	2.30h	16.48i	66.25g	13.35j	213.35a	22.05g	89.04e	0.05n	97.20h	10.08k	49.09g
	F. albida	10.52d	45.08c d	9.33j	40.01h	24.21i	30.85h	10.78i	10.25m	78.03h	66.21j	10.22k	25.02i
	V. luederitzii	15.22d	56.45c	15.09i	55.33h	31.12h	45.22g	15.31h	18.36l	118.02f	115.08g	18.21i	38.52h
Soil + 10% Compost +AM	*C. mopane*	4.15e	8.13f	25.96h	106.3f	92.03c	49.96f	17.02h	43.05i	45.21k	97.2h	33.75f	22.85i
	V. tortilis	10.55d	44.25c d	15.07i	52.24h	25.03i	35.44h	14.06i	13.74m	60.01i	58.22j	30.07f	18.25i
	E. globulus	5.22e	5.65g	17.25i	156.35f	222.54a	224.55a	22.06g	95.24e	4.67n	119.34g	19.47i	68.55e
	F. albida	5.32e	28.01e	5.21j	25.21i	21.08i	21.22i	9.25i	10.08m	65.02i	71.05i	11.08k	23.09i
	V. luederitzii	10.22d	44.22c d	23.07h	39.18h	20.09i	35.14h	19.22h	19.25k	98.22g	111.58g	17.21j	36.15h
P-value		0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

-: no-survival; [1]Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at a 5% level of significance; *** very highly significant

Within the control, significantly higher uptake of as was found in *V. tortilis* and *F. albida;* while more Cu was significantly up taken by *V. luederitzii* and *V. tortilis* in shoots and roots. The uptake of Pb was significantly lower in the shoots of *E. globulus*'s *grown in* tailing with compost alone and the combination of compost and AM. Similarly, the shoots of *C. mopane* also had low concentration of Pb when grown in tailings with +FA+AM. The lowest Ni concentration was recorded in the shoots of *C. mopane* when grown in tailings with +FA+AM. The lowest uptake of Zn was observed in the shoots of *F. albida* in treatments involving compost+AM, and in the roots of *V. luederitzii* in +FA+AM treatments.

This data showed that the different tree species have different capacities to uptake these metals and the different amendments had generally reduced the amount accumulated in the plant tissues of the different tree species. It appeared that FA, and compost would reduce the availability of these heavy metal(loid)s in soils, thus reducing its uptake and accumulation in plant tissues. The reduction in the concentration in plant tissues due to the colonization of mycorrhiza on these tree species could be attributed to the ability of mycorrhiza to retain these metals on the fungal tissues in roots. The combination of FA or compost with mycorrhiza synergistically enhanced the growth and the dry matter yield which also resulted to reduction in the concentration of these metals in tissue by dilution effect. The high capacity of *V. luederitzii* to accumulate these metals is consistent with other studies (Bech, 2021; Scalbert, 1992).

Table 3 shows the heavy metal uptake capacity and removal rate through biomass extraction for different tree species from the BCL mine tailings. The uptake capacity was based on the dry matter yield of plants and the concentration of metals in the tissues. Among the five species growing in pure BCL mine tailings, *V. luederitzii* and *F. albida* have the highest capacity to remove metals from the soil, as these metals were accumulated mostly in their shoots. The application of AM led to a significant reduction of these metals and metalloids in the tissues all tree species compared to the control, but the mycorrhizal fungus enhances the biomass yield resulting to higher potential removal of these metals from the tailings. Similarly, the application of 10% FA on tailings led to a significant increase of the uptake of this metal(loid)s as compared to the control in most of the tree species. However, FA application did not significantly enhance the uptake of Cu, Ni and Mn by *C. mopane and V. tortilis and E. globulus* as compared to the control. Across the different tree species, all the metals except Mn, recorded a significantly high uptake in the roots compared to shoots. The addition of amendments did not significantly alter the pattern of accumulation of metals by roots compared to shoots. The only exception on this accumulation pattern were observed for As by *F. albida* (soil alone), V. *tortilis* and *E. globulus* (Compost+AM) wherein a higher concentration in shoots than in roots was recorded. The other exception on this accumulation pattern was observed in Cu accumulated in *V. tortilis* after the application of Compost+AM.

Table 3: Meta-analysis showing heavy metal uptake capacity and removal rate through biomass extraction for different tree species[1].

Treatments	Species	Heavy Metals Concentration (mg/kg)											
		As		Cu		Mn		Ni		Pb		Zn	
		Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Soil Alone	*C. mopane*	1.06l	3.31m	3.20m	35.29j	37.42j	1.47m	1.72l	5.64l	2.70l	3.41l	2.73k	4.90l
	V. tortilis	28.87j	41.13j	307.66e	381.34f	67.27h	60.76j	77.40g	67.77i	114.71h	120.41g	86.35f	71.94i
	E. globulus	-	-	-	-	-	-	-	-	-	-	-	-
	F. albida	138.55e	133.20h	958.08c	842.18d	208.98e	183.70h	547.70b	486.65c	691.20e	613.15e	257.00d	227.43f
	V. luederitzii	125.42e	305.60e	1417.96b	3854.80b	61.69h	166.54h	296.09c	843.02b	1026.50c	2921.15c	78.55f	223.59f
Soil +AM	*C. mopane*	1.54l	2.56m	4.06m	25.01j	4.90l	2.50m	2.08l	6.61l	5.61l	6.63l	2.81k	3.93l
	V. tortilis	9.73k	21.98k	69.58i	60.16i	32.01j	37.06k	30.03j	40.55j	74.11i	98.68h	26.13i	28.94j
	E. globulus	0.37l	0.72m	1.43m	1.70k	15.40	22.55k	8.51l	7.49l	10.90l	12.93k	15.60j	22.45j
	F. albida	118.75f	197.29g	389.39e	357.63f	174.02f	158.74h	221.51d	203.58e	475.00f	434.76f	155.97e	142.64g
	V. luederitzii	113.11f	330.51e	1301.77b	3849.40b	78.03h	236.46g	278.24c	878.63b	849.23d	2850.10c	72.55f	235.35f
Soil+10% FA	*C. mopane*	1.16l	4.79m	2.91m	11.07k	6.36l	3.91m	0.39l	2.93l	3.29l	5.34l	2.59k	3.91l
	V. tortilis	33.64i	51.60j	45.88j	162.96h	37.06j	51.60j	7.06l	27.16k	3.63l	5.53l	19.41i	29.88j
	E. globulus	3.98l	3.02m	4.47m	139.20h	159.91f	228.96g	7.43l	112.89g	13.04k	111.90g	65.32g	85.86h
	F. albida	395.91c	779.52d	136.76g	597.75e	381.76c	568.28c	75.28g	203.23e	6854.09b	12317.34a	3119.70a	3105.20a
	V. luederitzii	847.85a	2219.93a	4134.63a	5952.06a	3431.90a	4260.56a	802.90a	3249.44a	6309.60b	8224.30b	773.71b	1256.40b
Soil+10% FA + AM	*C. mopane*	1.17l	3.98m	1.89m	10.96k	6.62l	5.48m	0.26l	2.88l	0.31l	0.76l	1.63k	6.06l
	V. tortilis	33.64i	102.32i	124.25g	414.17e	68.33i	101.41i	18.64k	84.98h	9.32l	10.18k	52.80g	126.38g
	E. globulus	12.45k	5.23m	13.52l	246.04g	261.22d	382.09e	12.57k	57.59i	1005.22c	756.92d	109.89e	161.25g
	F. albida	181.28d	825.84c	181.39f	725.44d	287.37d	401.68d	102.70f	197.17e	6208.51b	12289.34a	358.94c	881.07c
	V. luederitzii	644.27b	1479.67b	470.10d	1868.59c	1446.04b	1067.42b	85.42g	318.05d	8784.10a	8716.25b	470.10c	379.53d
Soil + 10% Compost	*C. mopane*	1.48l	2.33m	4.51m	29.49j	17.21k	7.37m	2.46l	10.00l	7.05l	5.60l	4.30k	6.55l
	V. tortilis	2.05l	11.92l	3.31m	13.89k	6.85l	9.31m	3.31l	3.81l	18.77k	17.19k	9.36j	5.12l
	E. globulus	2.06l	1.13m	8.08m	32.46j	6.54l	104.54i	10.80k	43.63j	0.02l	47.63i	4.94k	24.05j
	F. albida	48.29h	206.92f	42.82j	183.65	111.12g	141.60	49.48i	47.05j	358.16g	303.90f	46.91h	114.84g

	V. luederitzii	91.93g	340.96e	91.14h	334.19f	187.96f	273.13f	92.47g	110.89g	712.84d	695.08e	109.99e	232.66f
Soil + 10% Compost +AM	*C. mopane*	1.41l	2.76m	8.83m	36.14j	31.29j	16.99l	5.79l	14.64l	15.37k	33.05j	11.48j	7.77l
	V. tortilis	24.78j	8.44l	29.25k	14.02k	19.85k	7.87m	7.69l	33.61k	32.60j	16.84k	10.22j	18.25k
	E. globulus	5.48k	5.93m	18.11l	164.17h	233.67de	235.78g	23.16j	100.00g	4.90l	125.31g	20.44i	71.98i
	F. albida	35.75i	188.23g	35.01k	169.41h	141.66f	142.60h	62.16h	67.74i	436.93f	477.46f	74.46f	155.16g
	V. luederitzii	80.33g	347.57e	181.33f	307.95f	157.91f	276.20f	151.07e	151.31f	772.01d	877.02d	135.27e	284.14e
P- value		0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

-: no-survival; 1Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at a 5% level of significance.; *** very highly significant

The addition of a combination of FA+AM and AM alone lead to a highly significant increase of heavy metal uptake capacity by *F. albida* and *V. ludereritzii*. This increase was observed in all the metals under study. *E. globulus* species had a significantly low metal uptake capacity compared to all the species under study. Overall, *V. luederitzii* accumulated the highest concentration of heavy metal(loid)s in roots and shoots, followed by *F. albida*, *V. tortilis*, *C. mopane* and *E. globulus*.

Generally, the reduction of heavy metal in shoots compared to roots could be attributed to the capacity of mycorrhizal roots to retain heavy metal by localization at the external mycelium of the arbuscular mycorrhiza (Thoms and Gleixner, 2013); or selective exclusion of toxic and nontoxic metals by adsorption onto the chitinous cell wall structure; or onto extracellular glycoprotein called glomalin; or intracellular crystallization which is a very important mechanism in reducing the plant's exposure to potentially toxic metals (Shi et al., 2019). Shabani and Sabzalian (2016) showed that for both mycorrhizal and non-mycorrhizal plants, Ni concentrations in shoots and roots increased according to the addition of the metal into the soil, but inoculation with *F. mosseae* led to significantly lower Ni translocation from roots to the aboveground parts compared to non-inoculated plants. This is attributed to the improved root system due to arbuscular mycorrhiza (Yang et al., 2015). Additionally, the reduction of As, Cu, Ni, and Zn in shoots of most tree species could be due to the plant dilution effect because the translocation factor for these metals did not decrease but rather increased due to mycorrhizal inoculation in pure soil (Baycu, 2006). Vogel-Mikuš et al. (2005) showed that mycorrhiza colonized plants showed significantly improved nutrients, higher biomass, and a decreased Cd and Zn uptake, especially at higher soil metal contents, as a strategy for metal tolerance. On the contrary, Mn concentration in shoots and roots was increased by mycorrhiza inoculation. This was also supported by the findings of Moon and Aggangan (2018) which showed that mycorrhiza could enhance Mn uptake in the tree species. This was attributed the increase of Mn uptake to the larger volume of roots that would facilitate uptake. Mardukhi et al. (2015) attributed the enhanced Mn uptake of mycorrhizal plants to the increase of exudates capable of chelating Mn and facilitating absorption by plants.

Fly ash and compost reduced the concentrations of As, Cu, Ni, and Zn in shoots of all five tree species. This reduction in concentrations in plant tissues could be attributed to the capacity of these amendments to neutralize the soil acidity thus decreasing the heavy metal availability (Yunusa et al., 2006). FA neutralized the acidity of soil from the initial pH 4.35 to about pH 8.2 which resulted in various chemical changes as reflected in the reduction of the concentration of water-soluble metals. The reduction of heavy metal availability due to pH increase is due precipitation of cationic heavy metal(loid) or increased adsorption of metal(loid)s on the soil matrix (Lwin et al., 2018). Compost also neutralized the acidic soil (Khater, 2015), but the neutralizing power is lower compared to FA. The low concentration of heavy metal(loid)s in shoots of most plants compared to roots could be due to humic substances, which can decrease the content of free metal ions due to their complexation with dissolved organic substances and adsorption by humic substances, which enter the solid soil phase, or through adsorption in the mineral phase (Barsova et al., 2019). The interaction effect of soil amendments and mycorrhiza inoculation on heavy metal concentration in shoots and roots of these tree species is metal-specific and could be due to complex processes affecting availability, root absorption, and translocation mechanisms (Bonanno et al., 2018).

3.3. Effects of amendments on tree species

Figure 1 summarizes the effect of amendments on the heavy metal uptake and other parameters that were analyzed. Overall, without the addition of amendments, tree species recorded survival rate below 100% and poor growth due to high levels of heavy metal(loid), which led to the reduced uptake of HM by plants, limiting their phytoremediation potential. A study by Sharma and Reynnells (2018) indicated that amendments are important to plant growth because they provide plants with soil nutrients and improve soil fertility which is very limited in heavy metal-contaminated soil. The addition of amendments like fly ash, compost, and arbuscular mycorrhiza increases the survival rate and growth of all tree species and lowered the availability of heavy metal(loid)s, and, in return, increases the uptake and stabilization of heavy metal(loid)s by all tree species, and increases their phytoremediation potential. This was also corroborated by Ultra (2020) in the study of lemon grass species as affected by amendments. The results indicated that these plants could accumulate high concentrations of several heavy metal(loid)s while others can either accumulate one particular metal or maintain a low concentration of other metals to survive in an environment with multi-metal contamination. Furthermore, this was supported in a study by Rascio and Navari-Izzo (2011) who indicated that plants could accumulate high amounts of heavy metal(loid)s in their shoots while some plants are excluders. Therefore, mine tailings in the BCL mine and the vicinity can be phytoremediated with these identified tree species and the phytoremediation potential could be improved by the addition of amendments.

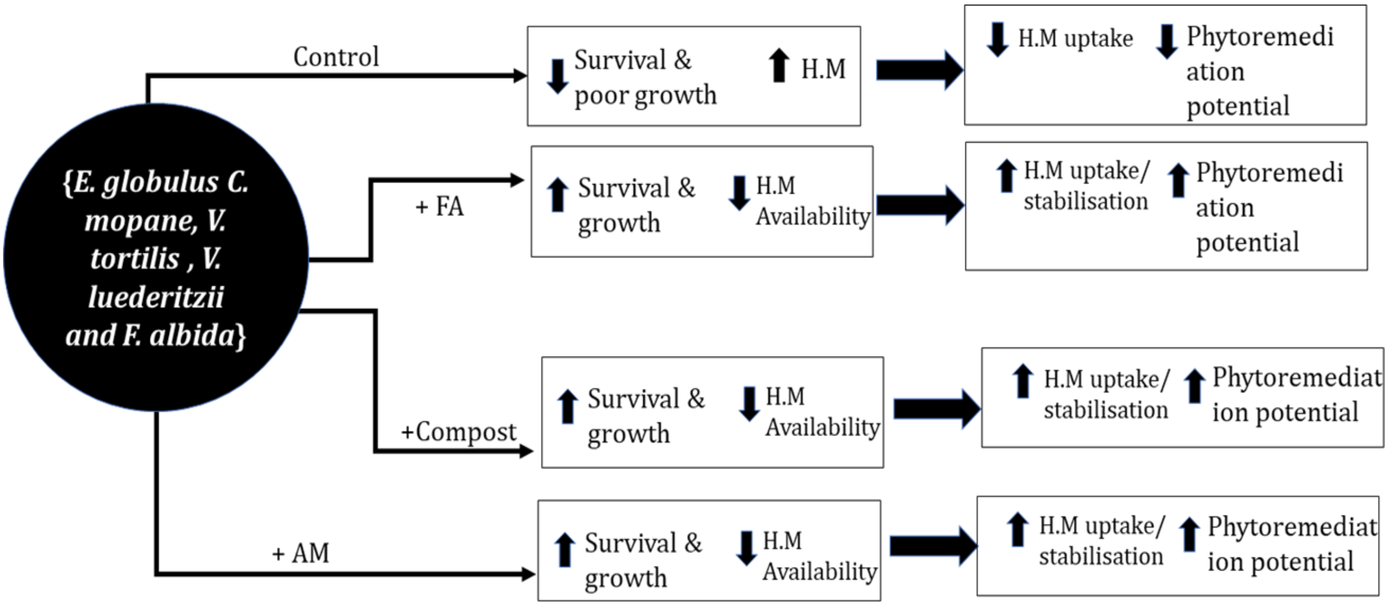

Figure 1: Summary of the effects of amendments on the phytoremediation potential of different tree species.

4. Conclusions

In conclusion, of the five tree species, *V. luederitzii* had a high survival rate and dry matter yield/week followed by *F. Albida & V. tortilis. Tree* species accumulated concentrations of heavy metal(loid)s in roots and shoots in this order *V. luederitzii >F. albida>V. tortilis>C. mopane>E. globulus.* This indicates that these trees can accumulate heavy metal(loid)s or tolerate high concentrations of heavy metal(loid)s without interference with their growth and development. *V. luederitzii* species also accumulated high concentrations of metal(loid)s compared to other species. Other tree species were ranked according to the decreasing preference based on survival rate, dry matter yield and heavy metal accumulation. The rank was as follows*: V. luederitzii>F. albida>V. tortilis>C. mopane>E. globulus.* Therefore, these trees should be used in combination to successfully remediate BCL mine tailings through the addition of amendments. Future studies should explore the use of other indigenous tree species on their potential for phytoremediation through the application of readily available amendments in Botswana.

Disclosure statement

The authors report there are no competing interests to declare.

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