Philippine Journal of Science
149 (3): 479-489, September 2020
ISSN 0031 - 7683
Date Received: 07 Jan 2020

Fly Ash and Compost Amendments and Mycorrhizal Inoculation Enhanced the Survival and Growth of *Delonix regia* in Cu-Ni Mine Tailings

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***Delonix regia*, an ornamental tree that can tolerate an elevated level of heavy metals, was evaluated for phytoremediation potential in mine tailings with fly ash, compost, and mycorrhiza. A two-factorial experiment was conducted under screen house conditions using mine tailings from Bamangwato Concessions Limited (BCL) Cu-Ni mine. The treatments are mine tailing (MT) without mycorrhiza (MT – AM), MT with mycorrhiza (MT + AM), MT with 10% fly ash (FA) without mycorrhiza (MT + FA – AM), mine tailings with 10% FA with mycorrhiza (MT + FA + AM), MT with 10% compost (CP) without mycorrhiza (MT + CP – AM), and MT with 10% CP with mycorrhiza (MT + CP + AM). After 40 wk, results showed an interaction effect of amendments and mycorrhiza wherein compost and FA enhanced the survival, AM root colonization, plant height, and dry matter yield of *D. regia* seedlings. Mycorrhiza further enhanced these growth parameters, especially in pure mine tailings. The As, Cu, Mn, Ni, Pb, and Zn concentration in plant tissues were reduced substantially by FA and compost amendments. Mycorrhiza also reduced the As, Cu, Ni, Pb, and Zn in pure mine tailings but enhanced the concentration of these metals in the presence of FA and compost. These results could be attributed to the decrease of heavy metal availability and increase of nutrients due to FA and compost while mycorrhiza decreased the translocation of heavy metals regardless of the presence of FA and compost. Overall, results indicated that the survival and growth of *D. regia* in BCL Cu-Ni mine tailings could be enhanced by the combined application of fly ash, compost, and mycorrhiza.**

Keywords: *Delonix regia*, heavy metal accumulation, heavy metal availability, mine tailings

INTRODUCTION

The establishment of plants in heavy metal contaminated mine tailings are the key steps towards the successful implementation of the phytoremediation program. Mine tailings are difficult to revegetate because of its deleterious conditions unfavorable for plant growth characterized by very low pH, low nutrient contents, poor physical condition, and high heavy metal contents (Sun *et al.* 2018). Except for metalophytes, other plants have difficulty surviving and experience severe heavy metal toxicity and poor nutrition resulting in retarded growth and development (Han *et al.* 2012). To be successful with remediation of heavy metal contaminated sites through phytoremediation, studies have been conducted to identify strategies to enhance the efficiency and effectiveness of phytoremediation (Mahar *et al.* 2016; Ultra *et al.* 2016;

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Yenn *et al.* 2014). For example, in phytoextraction, strategies include the use of hyperaccumulators and plants that can accumulate high concentration of heavy metals with high above-ground dry matter yield by genetic engineering (Ibañez *et al.* 2014), use of chelating agents to improve heavy metal availability and plant uptake (Ultra *et al.* 2005), soil amendments that decrease metal availability and improve plant growth, inoculation of beneficial soil microorganisms (Kong and Glick 2017; Ultra *et al.* 2007), and its combinations.

Identification of potential plants that will successfully tribe in the BCL Cu-Ni mine tailings is a big challenge. Currently, BCL mine is under the liquidation stage after its operation of more than 40 years. The mine tailings produced during its operation are stored in dumpsite occupying more than 1.2 km^2 at a height of about 50 m. The tailings dump site at present is bare and a point source of contaminant through migration of heavy metal to adjacent areas and groundwater by acid mine drainage. With the semi-arid environment of the area, severe wind erosion transport particles loaded with heavy metals to nearby agricultural and residential lands, threatening human and ecosystem health as well as the danger of food contamination.

In this study, the potential of *Delonix regia* as candidate plants in the phytoremediation of BCL mine tailings dumpsite was investigated. It is very widely grown in Africa as an ornamental tree because of its vivid red/orange/yellow flowers and bright green foliage and a potential source of pulp because of its fast growth rate (Bhokare *et al.* 2018). Although *D. regia* is not a hyperaccumulator, studies have shown that it has a relatively high tolerance to elevated heavy metals and can accumulate a high amount of Pb (Ukpebor *et al.* 2010). Based on their findings, *D. regia* can accumulate Pb up to 70 g/kg in the bark. However – in a separate study – Pb concentration in dried leaves, stems, and roots of the *D. regia* grown in polluted soil were up to 216, 646, and 786 ug/g dry matter, respectively; these values are equal, approximately, to the value of 1000 µg/g Pb in plants (Kamel *et al.* 2012). Prasad *et al.* (2015) evaluated the bioaccumulation of heavy metals in plants near mining and non-mining areas and found that *D. regia* contains about 18.6 ppm Cr, 5.6 ppm Cd, 142 ppm Ni, 25.5 ppm Mn, and 4.9 ppm Cu in the leaves. Zhang *et al.* (2007) indicated the high potential of *D. regia* for the phytoextraction of Pb, Cd, Hg, and As, and recommended the planting of this tree to remedy the heavy metal pollution in soils.

Harnessing the role of beneficial microorganisms in the phytoremediation system is picking traction because of the wide benefits that can be derived from plant-microbe association especially during the seedling establishment of plants. Nie *et al.* (2011) elucidated how mycorrhiza improves the growth of plants in the deleterious environment by its capability to produce various metabolites that would improve the biogeochemical processes in rhizosphere, which will feedback to better plant growth and development. Ultra *et al.* (2007) also showed how mycorrhiza would induce transformation of As into less toxic form in the rhizosphere of sunflower. Others have shown that mycorrhiza can enhance the supply and absorption of nutrients – through cell extension and detoxication of metals – as well as the production of phytohormones and siderophore, thus decreasing the abiotic stress of plants in nutrient-deficient soil and improving plant growth (Agarwal *et al.* 2017; de Fátima Pedroso *et al.* 2018). Mycorrhiza could accelerate the mobilization or immobilization of metals through the release of chelating agents, acidification, phosphate solubilization, induced metalloid transformation, and redox changes that could be beneficial in the survival of seedlings in heavy metal contaminated environments (Gonzalez-Chavez *et al.* 2002; Krishnamoorthy *et al.* 2019).

Soil amendments such as coal fly ash and compost have shown to modify soil properties and improve the supply of essential nutrients in degraded soils (Agarwal *et al.* 2011; Lwin *et al.* 2018). Fly ash has fine-sized particles with low bulk density, higher water-holding capacity, and favorable pH (alkaline), thus making it a potential source of essential plant nutrients that favors plant growth (Pandey and Singh 2010). Depending on the chemical components of fly ash, this material could be used as a liming agent and improve the soil buffering capacity. Previous findings showed that fly ash amendments could modify the soil texture, enhance soil structure formation – which ultimately reduces bulk density and improves aeration, percolation, and water retention – and can decrease the mobility and availability of metals in the soil due to the alkaline nature of fly ash (Shaheen *et al.* 2014). However, caution should be considered on several harmful effects of fly ash as amendments, which include low availability of associated nutrients because the elements present are occluded in the mineral matter, excess salinity, low N contents, and sometimes the presence of heavy metals that may contaminate the soil.

Several studies have shown the benefits of compost amendments in a heavy metal contaminated environment (Venegas *et al.* 2015; Liang *et al.* 2017). Short term benefits include pH adjustments, provision of nutrients, and increase of humus in the soil. Increase in pH and humus decreases the exchangeable fraction of heavy metal in soils and changes the metal speciation because of complex processes that include adsorption, complexation, precipitation, and redox reactions (Venegas *et al.* 2015; Liang *et al.* 2017). Similarly, Venegas *et al.* (2016) have shown that an increase of dissolved organic carbon and pH changes had enhanced the metal sorption, thus reducing its availability. Kulikowska *et al.* (2015) indicated that humic substances from compost had good surfactant properties that can change the distribution and speciation of some heavy metals such as Zn, Ni, and Cu in soil.

In this study, fly ash from Morupule B Power Plant in Botswana and compost are evaluated as soil amendments in combination with mycorrhizal inoculation on *D. regia* in mine tailings of BCL Cu-Ni mine in Selebi-Phikwe Botswana. The rehabilitation of mine tailings dump in BCL mines needs an immediate solution because the mining company is currently under liquidation stage and at present, the dumpsite serves as a point source of the contaminants in the adjacent environment. Therefore, this study evaluated the combined application of fly ash and compost amendments and mycorrhiza on the potential of *D. regia* as a phytoremediation agent in BCL mine tailing. Specific objectives are the following: to evaluate the effect of fly ash and compost amendments, and mycorrhiza inoculation on survival, growth, and heavy metal uptake of seedlings of *D. regia* grown in mine tailings of BCL mines; and to evaluate the effect of fly ash and compost amendments, and mycorrhiza inoculation on the chemical properties and heavy metal availability in the mine tailings collected from the vicinity of BCL mines.

MATERIALS AND METHODS

Soil Sampling and Pot Preparation

The experiment was conducted using a mine tailing from BCL mine in Selebi-Phikwe, Botswana. MTs were collected, air-dried and sieved to pass through 5- and 2-mm mesh for the cultivation of plants and chemical analysis, respectively. The tailing's properties and the heavy metal contents are presented in Table 1. The tailings are highly acidic – characterized by sandy loam with very low organic matter and nutrient content – and have high amounts of Cu, Ni, Pb, and As. Fly ash was collected from the Morupule B Power Plant Station, Palapye, Botswana. The fly ash was air-dried and sieved using a 2-mm diameter mesh before application. The chemical properties of fly ash are also presented in Table 1. The compost used in the experiment was obtained from a local supplier that contains 7% N, 4% P, and 5% K; with 42% organic carbon, and having a pH of 8.2; the heavy metal contents were presented in Table 1. Plastic pots were filled with 5 L (approximately 8 kg) tailings, or a mixture of MT + fly ash, or MT + compost before mycorrhiza inoculation and plant establishment.

Description of the Treatments and Plant Culture

A two-factor experiment was established on the response on *D. regia* to soil amendments and mycorrhiza

Table 1. Chemical properties and heavy metal contents in mine tailings, fly ash, and compost.

Properties	Tailings	Fly ash	Compost
pH (1:2, soil: water)	3.14	8.8	8.2
Electrical conductivity (1:2, soil: water)	1033.54 mS/m	2290 mS/m	783 mS/m
% organic matter (Walky and Black 1957)	0.00	0.015	68.0
Total N (Kjeldahl distillation-acid titration)	0.00	0.78	7%
Total P		+	4%
Total K			5%
Particle size distribution (laser diffraction)			
% sand	7.60	21.05	
% silt	62.28	45.25	
% clay	30.12	33.7	

Heavy metals	Mine tailings (n = 3)			Fly ash (n = 3)			Compost (n = 3)
	Total	WS* mg kg^{-1}	Exch**	Total	Ws mg kg^{-1}	Exch	Total mg kg^{-1}
As	154.31	3.81	3.95	8.08	1.23	4.73	1.26
Cu	5311.62	12.05	15.71	45.20	0.07	0.13	12.42
Mn	1137.53	5.73	8.60	382.71	0.04	0.57	248.24
Ni	2025.22	15.24	25.40	82.25	0.01	0.10	56.28
Pb	552.81	3.69	6.11	129.33	0.88	2.10	22.46
Zn	220.27	0.18	0.25	26.21	0.04	0.21	138.42

*WS – water-soluble; **Exch – 1N ammonium acetate exchangeable inoculation in a heavy metal contaminated mine tailings. There are two levels of mycorrhiza and three types of soil amendments comprising of six treatment combinations (Table 2). The treatments are pure mine tailing (MT) without mycorrhiza (MT – AM), pure MT with mycorrhiza (MT + AM), MT with 10% (v/v) fly ash (FA) without mycorrhiza (MT + FA – AM), mine tailings with 10% (v/v) FA with mycorrhiza (MT + FA + AM), MT with 10% (v/v) compost (CP) without mycorrhiza (MT + CP – AM), and MT with 10%(v/v) CP with mycorrhiza (MT + CP + AM). For treatments with FA and compost, an equivalent to 10% of the total volume of mine tailings were mixed thoroughly before potting. Approximately 300 g of compost and 650 g of fly ash were added on 5 L of mine tailings for treatments involving compost (T5 and T6) and fly ash (T3 and T4), respectively. The seeds of the *D. regia* were collected from identified trees near the university. The seeds were scarified using concentrated sulfuric acid for 1 h, then washed 10 times with sterile distilled water and germinated on sterile Petri plates lined with moistened filter paper. The pre-germinated seed was then planted at the center of the pots at three seedlings per pot and was maintained at a moisture content of 40% of the field capacity under shade house conditions. For the mycorrhiza treatments, 5 g of sterile AM inoculants or non-sterile AM inoculants (Mykovam™) were mixed with the soil at the center of the pot just below the roots of the seedlings of the – AM and + AM treatments, respectively. The Mykovam™ was sterilized by autoclaving the samples at 121 °C, 15 psi for 30 min for three consecutive days. The Mykovam™ is a soil-based biofertilizer containing vesicular-arbuscular mycorrhiza produced by the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Banos, Philippines. Ten (10) replicates were used for each treatment laid out in a complete randomized design. The plants were irrigated regularly and monitored for survival and growth inside the shade house (80% exposure) located at the Department of Earth and Environmental Sciences, Botswana International University of Science and Technology (BIUST), Palapye, Botswana. The experiment was conducted from February 2018 to March 2019.

Plant Sampling and Chemical Analysis

During the 40-wk cultivation period, plants were periodically monitored for survival and growth. At harvest, the shoots were excised and the roots were carefully separated from the soil, after which their fresh weights were determined. A portion of the fresh roots, 1.0 g from each plant, was subjected to trypan blue staining for microscopic examination of mycorrhizal infection (Brundrett and Abbott 1994). The root length colonized by AMF was quantified using the gridline intersection method (Giovennetti and Mosse 1980). The remaining roots and all the shoots were oven-dried at 70 °C for three consecutive days and ground for chemical analysis after their dried weights were taken. The tissues were digested using a nitric acid and hydrogen peroxide mixture in an open vessel at 120 °C for a minimum of 4 h and analyzed for heavy metals using ICP-MS (Thermo Scientific™ iCAP Q ICP-MS, Illinois, USA). To test the variation in the analysis of heavy metals, a representative sample which was randomly drawn from the whole experimental samples were submitted for analysis at the Analytical Service Laboratory, University of Botswana. Variation in the values obtained between the two laboratories is less than 6%. The translocation factor (TF) for each metal was calculated as a ratio of the shoot divided by the root concentration.

Mine Tailings Sampling and Chemical Analysis

Representative mine tailings samples from each pot were collected after harvest of plants. The samples were passed through a 2-mm sieve, air-dried, and stored at 4 °C until chemical analysis. The soil pH and electrical conductivity (EC) were measured in water suspension (1:2, tailings: water) after shaking for 1 h. The total heavy metal contents were measured after wet acid digestion in an open vessel at 120 ºC for 6 h using HNO_3: HCl mixture (1:3) in a hot plate followed by quantification using ICP-MS (Ultra *et al.* 2016). The available fraction of heavy metal in the growing media was assessed by determining the water-soluble (WS) and ammonium acetate exchangeable fractions. Samples were shaken with distilled water (1:100) for 2 h at room temperature to obtain a WS fraction. The exchangeable fraction (Exch)

Table 2. Treatment combination of mycorrhizal inoculation and soil amendments on mine tailings.

Treatment	Factor A – soil amendments	Factor B – mycorrhizal inoculation
T1	Pure mine tailings (MT)	– AM (5 g sterile Mykovam™)
T2	Pure mine tailings	+ AM (5 g Mycovam™)
T3	MT + 10% fly ash (v/v)	– AM (5 g sterile Mykovam™)
T4	MT + 10% fly ash (v/v)	+ AM (5 g Mycovam™)
T5	MT + 10% compost (v/v)	– AM (5 g sterile Mykovam™)
T6	MT + 10% compost (v/v)	+AM (5 g Mycovam™)

was determined by using 1N ammonium acetate (pH 7) at a 1:100 ratio for 2 h at room temperature. The WS and the Exch fractions were analyzed for different heavy metals using ICP-MS. Samples were analyzed in duplicates. All the chemical analyses were conducted at the Soil Science Laboratory of the Department of Earth and Environment Sciences, BIUST, Palapye, Botswana.

Statistical Analysis

The data were analyzed using a two-factor ANOVA in a completely randomized design. When significant differences were observed, Tukey's test was performed for treatment mean comparison at a 5% level of significance.

RESULTS

Survival, Dry Matter Yield, and Mycorrhizal Infection in the Roots of *D. regia*

After 40 weeks of growth, significant interaction effects of amendments and mycorrhiza inoculation were observed on the survival, growth, and dry matter yield of *D. regia* in mine tailings (Table 3). Compost and fly ash amendments, regardless of mycorrhiza inoculation, improved the survival of *D. regia* in mine tailings from BCL-Cu-Ni mine by 100% survival rate. On the other hand, mycorrhiza inoculation also improved the survival of *D. regia* from 20–60% in the absence of amendments.

Plants from pure mine tailings and those grown in mine tailings with 10% fly ash amendments without mycorrhizal inoculation had zero roots colonized by mycorrhiza, while those inoculated with AM and tailings with compost had significantly higher colonization rate (Table 3). The tailings with compost had 38% mycorrhizal colonization in the roots of *D. regia* even in the absence of mycorrhizal inoculation. The colonization rate of inoculated mycorrhiza increased from pure tailings < tailings + FA < tailings + compost.

Plant height was significantly enhanced by FA and compost amendments, and mycorrhiza inoculation further improved the growth of *D. regia*. In pure tailings, mycorrhiza inoculation enhanced plant height by 30%, 11% in + FA treatments, and 12% in + compost treatments, respectively (Table 3). The tallest plants are recorded in treatment containing compost and mycorrhiza. Similarly, the shoot dry weight, root dry weight, and total dry matter yield were significantly enhanced by FA and compost amendments. Regardless of mycorrhiza inoculation, FA increased the shoot, root, and total dry matter yield by 1.97 x, 4.4 x, and 2.7 x, respectively based on the dry matter yield of the plants grown in pure tailings. Compost amendments also enhanced the shoot, root, and dry matter yield by 3.19 x, 7.45 x, and 4.5 x, respectively over the plants grown in tailings alone (Table 3). Mycorrhiza

Table 3. Survival, mycorrhizal colonization, and dry matter yield of *D. regia* seedlings in mine tailings from BCL mines as influenced by fly ash amendment and mycorrhiza inoculation at 40 weeks after planting[1].

Treatments	Survival (%)	Mycorrhizal roots (% colonized)	Plant height (cm)	Dry matter yield (g/plant)		
				Shoots	Roots	Total
MT						
– AM	20 c	nd	8.6 e	5.71 d	2.10 e	7.81 f
+ AM	60 b	55 b	11.22 d	14.25 c	6.78 d	21.03 e
MT + FA						
– AM	100a	nd	16.84 c	27.67 b	19.96 c	47.63 d
+ AM	100 a	70 a	18.76 b	31.69 b	27.89 b	59.58 c
MT + CP						
– AM	100 a	38 b	19.2 b	38.24 a	32.26 a	70.50 b
+ AM	100 a	82 a	21.5 a	45.38 a	42.82 a	88.20 a
p-value						
Amend	0.123ns	0.130ns	0.000**	0.000**	0.000**	0.000**
AM	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Amend x AM	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

[1]Means within the same column followed by the same letter(s) are not significantly different from each other at a 5% level of significance based on the LSD test.
** – highly significant; * – significant; ns – non-significant

inoculation further improved the dry matter yield of *D. regia*. In soil alone, mycorrhiza inoculation enhanced the shoot dry weight by 1.49 x, 2.23 x in roots, and 1.69 x in the total dry weight, respectively. In the + FA and + compost treatments, around 25% increased due to mycorrhiza inoculation was recorded in the total dry yield of *D. regia*. The highest dry weights are recorded in treatment containing compost and mycorrhiza.

Concentration of Heavy Metals in Plants

The heavy metal concentration in the tissues of *D. regia* is presented in Table 4. Depending on the type of metal, mycorrhizal inoculation and amendments could either increase or decrease the metal concentration in the tissues. Arsenic concentration in shoots was significantly reduced by FA and compost amendments wherein the reduction was more pronounced due to FA. Mycorrhiza inoculation in unamended mine tailings significantly reduced the As concentration in the shoot but such effect was not observed in the presence of FA and compost. In roots, the As concentration was significantly reduced by FA and compost amendments whereas mycorrhiza inoculation reduced the As in roots of plants grown in tailings without amendments, but the opposite occurred in FA and compost-amended tailings. In FA-amended tailings, mycorrhiza inoculation leads to a significant increase of As in the roots of *D. regia*.

Copper and lead concentration in shoots and roots of *D. regia* were reduced by FA and compost amendments. Fly ash had reduced the Cu and Pb concentration at a higher magnitude compared to compost. However, the mycorrhiza inoculation resulted in a slight but significant increase in Cu and Pb concentrations in shoots and roots when combined with FA and compost amendments. Without amendments, mycorrhiza significantly reduces the Cu and Pb concentrations in shoots and roots of *D. regia* but reduced the concentration in plants grown in pure tailings.

Manganese concentration in shoots was reduced primarily by FA but not with compost amendments, while mycorrhiza enhanced Mn in shoots in unamended tailings and the presence of compost. The Mn concentration in the roots was enhanced by mycorrhiza inoculation regardless of the amendments, while FA alone reduced the root's Mn concentrations. The nickel concentrations in shoots and roots were reduced significantly by FA and compost. However, mycorrhizal inoculation increased the Ni concentration in roots regardless of the amendments. The Zn concentration in shoots and roots was likewise reduced by FA and compost amendments. In pure tailings, mycorrhiza inoculation also reduced Zn concentration in shoots and roots. In contrast, a slight increase in Zn concentration in shoots and roots was observed due to mycorrhiza in FA and compost amended tailings.

Table 4. Heavy metal concentrations in shoot and roots of *D. regia* grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 40 weeks after planting[1].

Treatments	Heavy metals concentration (mg/kg)											
	As		Cu		Mn		Ni		Pb		Zn	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
MT												
– AM	9.27 a	33.65 a	665.50 a	1201.92 a	73.94 b	48.08 d	127.72 a	144.23 a	28.42 a	32.21 a	127.72 a	240.38 a
+ AM	1.41 b	5.27 c	77.30 b	531.13 b	92.76 a	77.46 c	88.63 b	151.22 a	14.22 b	26.16 a	26.80 b	73.77 b
MT + FA												
– AM	0.69 d	4.24 c	15.09 e	33.28 d	26.06 c	22.76 e	5.94 d	9.10 e	6.48 c	10.13 c	9.60 c	7.96 d
+ AM	0.93 c	11.56 b	27.21 d	91.29 c	27.88 c	50.80 d	6.64d	16.93 d	3.98 c	8.83 c	19.25 b	14.72 d
MT + CP												
– AM	1.64 b	10.17 b	31.84 c	94.86 c	67.22 b	178.03 b	25.95 c	52.24 c	18.26 b	20.58 b	20.05 b	26.81 c
+ AM	1.65 b	12.98 b	41.16 c	114.46 c	101.23 a	295.54 a	33.37 c	78.58 b	14.82 b	28.44	25.59 b	27.33 c
p-values												
Amend	0.042*	0.032*	0.038*	0.018*	0.022*	0.012*	0.036*	0.083ns	0.014ns	0.022*	0.012*	0.036*
AM	0.048*	0.142ns	0.202ns	0.024*	0.036*	0.022*	0.028*	0.046*	0.026*	0.036*	0.022*	0.028*
Amend x AM	0.032*	0.132ns	0.186ns	0.012*	0.002**	0.024*	0.016*	0.048*	0.032*	0.002**	0.024*	0.016*

[1]Means within the same column followed by the same letter(s) are not significantly different from each other at a 5% level of significance based on the LSD test.
** – highly significant; * – significant; ns – non-significant

Translocation of Heavy Metals in Plants

The TF of heavy metals in *D. regia* was significantly affected by amendments and mycorrhiza (Table 5). The TF for As was reduced by FA and compost amendments compared to the control, while mycorrhiza further reduced the TF of in FA amended tailings. In pure tailings, mycorrhiza reduced the TF of Cu, Mn, Ni, Pb, and Zn. When mycorrhiza was combined with FA, the translocation of Cu, Mn, Ni, Pb but not Zn was reduced, whereas the combination of mycorrhiza and compost did not significantly change the TF in Cu and Mn but reduced the TF of Ni, Pb, and Zn. The lowest TF for As and Pb was observed in the + FA + AM treatment, pure tailings + AM for Cu and Zn, and + compost + AM for Mn and Ni.

Chemical properties of tailings and heavy metal availability. Analysis of the tailings after harvest of *D. regia* showed a significant increase of pH and EC due to FA and compost amendments (Table 6). The magnitude of increase was higher due to FA compared to compost amendments. On the average, the pH in FA amended tailings was about 8.38 while in compost amended tailings was about 6.26 (Table 6). The EC was increased to about 2700 dS/m due to fly ash amendments while compost increased the EC up to 687 dS/m.

The WS fractions of heavy metal in tailings were significantly reduced by amendments and not by mycorrhiza inoculation (Table 6). There was no significant interaction effect between amendments and mycorrhiza on the concentrations of WS-fractions in tailings after plant cultivation. Specifically, the WS-As were significantly reduced by compost amendments to about 68% based on the control while FA did not result in significant difference when compared to the unamended tailings. The WS-Cu, WS-Mn, WS-Ni, WS-Pb, and WS-Zn were significantly reduced up to 97%, 96%, 99%, 41%, and 59%, respectively due to FA amendments. On the other hand, compost amendments resulted in 71%, 37%, 49%, 36%, and 50% reduction of WS-Cu, WS-Mn, WS-Ni, WS-Pb, and WS-Zn, respectively.

The concentrations of heavy metals associated with ammonium acetate exchangeable fraction were significantly affected by amendments but not with mycorrhiza (Table 6). The exchangeable As and Mn was significantly enhanced by FA and compost to about 20% and 43%, respectively, over the unamended mine tailings. On the other hand, exchangeable Cu, Ni, Pb, and Zn were reduced significantly by FA and compost amendments. Fly ash amendments reduced the exchangeable fraction of Cu by 66%, 77% of Ni, 28% of Pb, and 43% of Zn while compost reduced the exchangeable Cu by 60%, 43% of Ni, 18% of Pb, and 53% of Zn, respectively.

DISCUSSION

Arbuscular mycorrhiza had colonized the roots of *D. regia* in tailings from Cu-Ni mine. The percentage of colonized roots were significantly higher in compost and fly ash

Table 5. Translocation factor (shoot to roots ratio) of heavy metal concentration *D. regia* grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 40 wk after planting[1].

Treatments	Shoot/root ratio of heavy metal concentrations					
	As	Cu	Mn	Ni	Pb	Zn
MT						
– AM	0.28 a	0.55 a	1.54 a	0.89 a	0.88 a	0.53 d
+ AM	0.27 a	0.15 c	1.20 b	0.59 b	0.54 b	0.36 e
MT + FA						
– AM	0.16 b	0.45 a	1.15 a	0.65 b	0.64 b	1.21 a
+ AM	0.08 c	0.30 b	0.55 c	0.39 c	0.45 c	1.31 a
MT + CP						
– AM	0.16 b	0.34 b	0.38 d	0.50 b	0.89 a	0.75 c
+ AM	0.13 b	0.36 b	0.34 d	0.42 c	0.52 b	0.94 b
p-value						
Amend	0.031*	0.083ns	0.032*	0.000**	0.000**	0.000**
AM	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Amend x AM	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

[1]Means within the same column followed by the same letter(s) are not significantly different from each other at a 5% level of significance based on the LSD test. ** – highly significant; * – significant; ns – non-significant

amended tailings compared to the colonization rates in the roots of *D. regia* grown in pure mine tailings (Table 3). This indicates that FA and compost improved the mycorrhizal infection in roots. There were no mycorrhizal roots detected in – AM treatments in pure tailings and tailings with fly ash indicating the absence of native mycorrhiza in soil used in the experiment. However, in + compost – AM, 38% colonization of mycorrhiza was observed, which could be traced to the presence of mycorrhiza in compost that colonized the roots of *D. regia*. It should be noted that unsterilized composts were applied in this experiment.

The very high heavy metal content, acidic pH, and low nutrient content in the mine tailings is detrimental to the survival and growth of *D. regia* as observed in the poor performance of the control plants. However, compost and FA amendments – as well as the mycorrhizal inoculation – improved the survival and growth of *D. regia* grown in the tailings collected from Cu-Ni mines in Selebi-Phikwe, Botswana (Table 3). This improvement of survival and growth of *D. regia* is due to the synergistic interaction of soil amendments and mycorrhiza. Compost and fly ash modified the chemical properties of mine tailings affecting nutrient and heavy metal availability, thus improving growth and dry matter yield, and the presence of mycorrhiza further improved such benefits. Compost amendments modified the mine tailings to favor plant growth and development and supplied essential nutrients to plants. Fly ash contained essential nutrients for plant growth. These growth enhancements by compost and FA are complemented by the ability of mycorrhiza to mobilized and increased absorption of nutrients in the mine tailings and improved the nutrition of *D. regia* under this type of growing media, as evident in the heavier dry matter yield in plants (Table 3). Based on the study of He *et al.* (2018), FA increased the Exch-P, Exch-Mg, Exch-Cu, Exch-Zn, Exch-Mo, and Exch-Se; and, consequently, enhanced the dry matter yield and the nutrient uptake of alfalfa grown in loessial soil. In the present study, it was apparent that the essential nutrient in FA helped the nutrition of *D. regia* in mine tailings contaminated with heavy metals and enhanced their survival and growth (Mora-Romero *et al.* 2015; Ravnskov and Larsen 2016).

The alkaline pH of FA neutralized the acidity from the initial pH 3.14 of the tailings to about pH 8.8, which resulted in various chemical changes as reflected in the variation of the concentration of WS and Exch metals (Table 6). This increase in the pH of mine tailings decreased the solubility of heavy metals through the different mechanisms, which include precipitation of cationic heavy metals or increased adsorption of heavy metals on soil matrix (Sommers and Lindsay 1979). This is very evident in the low concentration of WS-Cu, WS-Mn, WS-Ni, WS-Pb, and WS-Zn and Exch-Cu, Exch-Ni, Exch-Pb, and Exch-Zn in fly ash amended soils (Table 6), thus effectively reducing the availability of these metal elements, which are similar to the findings of Sitarz-Palczak and Kalembkiewicz (2012). The reduction of available metals was attributed to increased sorption sites and metal precipitation in the form of hydroxides as the pH increases due to FA amendments (Lwin *et al.* 2018). On the other hand, FA amendments decreased the WS-As but increased the exchangeable, As which could be attributed to changes in speciation and solubility of As in response to pH change (Signes-Pastor *et al.* 2007). Consequently, Cu, Ni, Pb, Mn, and Zn uptake of *D. regia* was reduced due to decreased amounts of the available fraction of metals in tailings after FA and compost amendments.

The uptake of micronutrients including heavy metal contaminant is deduced to be a function of the concentration in soil within the rooting zone (Kutrowska *et al.* 2017). The different patterns and trends of heavy metal concentration in response to amendments and mycorrhiza inoculation in *D. regia* indicate that there are other factors that influence the uptake of heavy metals within the same plant. Some of these factors include the presence of competing ions, concentration ratio to another micro- and macro-nutrients, biochemical transformations in the rhizosphere induced by plant roots and microorganisms, and the presence or absence of transport facilities on plant membranes (Koptsik 2014). This study showed that the reduction of available metals in the mine tailings due to fly ash and compost amendments consequently reduced substantially the metal concentration in shoots of *D. regia*. The influence of mycorrhiza on metal uptake and plant survival had been documented in several studies, and most of these studies dealt with single to few metals in soils (de Fátima Pedroso *et al.* 2018; Krishnamoorthy *et al.* 2019). In this current study, mycorrhiza inoculation increased or decreased metal uptake depending on the properties of the growing media, and the presence or absence of other factors affecting heavy metal availability. Mycorrhizal inoculation in pure tailings reduced the As, Cu, Ni, Pb, and Zn in shoots of *D. regia* but enhanced the Mn concentration in the shoots. In the presence of FA and compost, this reduction of heavy metals in shoots was not observed. AM inoculation in tailings with FA resulted in significantly higher Cu and Zn in shoots and compost amended tailings, as + AM significantly enhanced Mn concentration in shoots. These differences could be attributed in part to the different uptake and transport mechanisms involved with different metals and nutrients (Becklin *et al.* 2016). Reduced concentration of different heavy metals due to mycorrhizal inoculation could be attributed to heavy metal localization at the external mycelium of the arbuscular mycorrhiza (Krishnamoorthy *et al.* 2019). The differences on the degree of reduction

Table 6. Chemical properties of soils after cultivation of plants.

Treatments	pH	EC (mS/m)	Water soluble (mg/kg)						NH_4OAc extractable (mg/kg)					
			As	Cu	Mn	Ni	Pb	Zn	As	Cu	Mn	Ni	Pb	Zn
MT														
– AM	3.18 c	352.5 c	3.86 a	3.58 a	0.27 a	1.79 a	3.49 a	0.12 a	4.62 b	4.88 a	0.21 b	3.04 a	4.54 a	0.29 a
+ AM	3.66 c	463.4 c	3.70 a	1.35 b	0.21 a	0.65 b	3.45 a	0.10 a	4.72 b	4.66 a	0.25 b	3.03 a	4.38 a	0.24 a
MT + FA														
– AM	8.41 a	2700.0 a	3.94 a	0.06 c	0.01 c	0.01 c	1.99 b	0.04 b	5.79 a	1.61 b	1.26 a	0.56 c	3.35 b	0.14 b
+ AM	8.36 a	2682.5 a	3.58 a	0.14 c	0.01 c	0.01 c	2.10 b	0.05 b	5.29 a	1.65 b	1.01 a	0.84 c	3.02 b	0.16 b
MT + CP														
– AM	6.21 b	652.2 b	1.21 b	1.15 b	0.14 b	0.85 b	2.12 b	0.06b	5.38 a	1.92 b	1.32 a	1.65 b	3.65 b	0.12 b
+ AM	6.32 b	687.4 b	1.18 b	0.82 b	0.16 b	0.04 c	2.26 b	0.05 b	5.87 a	1.89 b	1.28 a	1.82 b	3.58 b	0.13 b
p-values														
Amend	0.022*	0.001**	0.021*	0.016*	0.012*	0.002**	0.042*	0.012*	0.038*	0.036*	0.042*	0.022*	0.032*	0.042*
AM	0.136ns	0.246ns	0.089ns	0.334ns	0.128ns	0.116ns	0.126ns	0.222ns	0.124ns	0.134ns	0.226ns	0.118ns	0.216ns	0.236ns
Amend x AM	0.148ns	0.238ns	0.126ns	0.048*	0.034*	0.020*	0.138ns	0.138ns	0.042*	0.098ns	0.019ns	0.144ns	0.124ns	0.138ns

[1]Means within the same column followed by the same letter(s) are not significantly different from each other at a 5% level of significance based on the LSD test.
** – highly significant; * – significant; ns – non-significant

between different metals are due to the selective exclusion of toxic and nontoxic metals by adsorption onto chitinous cell wall structure, or onto extracellular glycoprotein called glomalin, or intracellular crystallization that will vary in response to other factors such as the concentration of metals in the mycorrhizosphere (Shi *et al.* 2018). The addition of FA and compost also resulted to increase of other elements and changed the concentration ratios between different metals and nutrients in the soil (Table 6). Consequently, these changes may cause result in differential uptake and transport of other metals in plants. As proposed by Huang *et al.* (2016), the increase or decrease in the translocation of metals due to AM symbiosis is not entirely dependent on improvement or impedance of immobilization in roots or the uptake to shoots but rather the allocation of the metal concentration to different organs; this was regulated by the concentration present in the substrate. Similarly, different elements are transported from roots to shoots in plants at different patterns and extent (Vogel-Mikuš *et al.* 2006; Umar 2017). For example, Kutrowska *et al.* (2017) indicated that in contrast with other metals, copper is widely translocated in an acropetal direction. This would indicate that the heavy metals in the soil-plant system are dominated by competitive and inhibitory interactions with the major ions present, which control the concentrations of trace ions in soil solution and the cellular plasma membrane transport responsible for root uptake and translocations of these trace elements. Therefore, it is apparent that the modification on the chemical properties of tailings due to FA and compost amendments, together with the presence of mycorrhiza resulted in variable availability and translocation of metals from roots to shoots of *D. regia*. Nonetheless, the concentration of As (9–34 mg/kg), Mn (48–74 mg/kg), Zn (128–240 mg/kg) in *D. regia* grown in pure mine tailings are within the range of the normal concentration of plants (Reeves and Baker 2000), while the concentration of Cu (665–1202 mg/kg), Ni (128–144 mg/kg), and Pb (28–32 mg/kg) were above the minimum concentration of most heavy metal accumulator plant. The application of FA and compost with or without mycorrhiza reduced these heavy metal contents to normal concentration in plants (Reeves and Baker 2000).

CONCLUSIONS

Overall, this study demonstrated the potential of compost, FA, and mycorrhiza in improving the survival rate and growth of *D. regia* in heavy metal-contaminated mine tailing from Cu-Ni mines. Compost supplied the nutrients and reduced the heavy metals in shoots, which is possibly due to the reduction of the heavy metal availability by providing additional sorption sites for heavy metals, thus increasing survival and improved growth of *D. regia*. Similarly, FA improved the growth and lowered the heavy metal uptake of *D. regia* by neutralizing the acidic pH of the tailings and reducing the availability of heavy metals. Mycorrhiza successfully colonized the roots of *D. regia* and altered the heavy metal accumulation in plants which is metal-specific. For the BCL mine tailings, the establishment and growth of *D. regia* could be enhanced substantially by the application of soil amendments such as compost and FA combined with mycorrhiza inoculants.

ACKNOWLEDGMENT

This paper is supported by BIUST's initiation grant [Ref. DVC/RDI/2/1/161 (50)] to V. Ultra.

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