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Bioaccumulation and translocation of heavy metals in pearl millet (*pennisetum glaucum*) depends on ectomycorrhiza *pisolithus arhizus* and soil type

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Abstract

This study was carried out to determine the combined effects of ectomycorrhizal fungus Pisolithus arhizus and different soil types on the accumulation of heavy metals by seedlings of pearl millet. The seedlings were grown on soils with a high and low concentration of heavy metals and a mixture of the two soil types. The soils were inoculated with the mycorrhiza. The plants were grown for six weeks and then harvested and analyzed for concentrations of heavy metals using an Atomic Absorption Spectrophotometer (AAS). The data obtained were used to determine the bioaccumulation factor (BCF) and translocation factor (TF) of the heavy metals. The result showed that millet accumulated manganese (Mn) up to 121.4% compare to its concentration in the soil, which means millet is a hyperaccumulator of Mn. It was also found to be hyperaccumulator of cadmium (Cd) (110%); moderate accumulator iron (Fe) (74.8%), copper (Cu) (20.9%), zinc (Zn) (22.2%) and lead (Pb) (18.8%). However, the inoculation of the experimental soils with the mycorrhiza increased the BCF of some of the heavy metals, while others were decreased. Also, the addition of waste dump soil to the experimental soil (soil amendment) decreased the BCF of some of the heavy metals, while others were increased. These results suggest that pearl millet can be used for phytoremediation of some heavy metals from contaminated soils. Also, the addition of waste dump soil and ectomycorrhiza Pisolithus arhizus to soil can be used to increase or decrease phytoremediation/amelioration potentials of pearl millet, which depend on the particular heavy metal involved.

Keywords: Bioconcentration, ectomycorrhiza, heavy metals, millet, soil, translocation

Introduction

Many people, particularly in developing countries depend on arable cultivation as a source of their livelihoods (Bekele *et al.*, 2014), but as a result of overpopulation and concomitant overuse of agricultural lands, there is currently a serious crisis of soil degradation, especially soil erosion and low availability of soil nutrient. These, in addition to the high cost of synthetic fertilizer, pose some of the major limitations of agricultural productivity in the developing countries (Baum, 2011; Sasson, 2012; Zakari, *et al.*, 2014). Therefore, farmers greatly patronize soil from refuge dumpsites as an alternative to fertilizer, which they use for the amendment of their farmlands in many places. The wastes dump soils are widely reported to contain a high amount of organic matter and nutrient elements, which improve the physicochemical properties of soil. This boosts productivity through increasing soil porosity and water holding capacity; reducing bulk density; enriching nutrient mineral content; improving cation exchange capacity and pH. Thus, wastes dump soils are view as a cheaper alternative source of fertilizer for many farmers (Pasquini and Harris, 2005; Azeez *et al.*, 2011; Mashi *et al.*, 2014).

However, the use of wastes dump soils for amendment of agricultural soil lead to the accumulation of heavy metals in the soil and subsequently in the crops, which is potentially harmful when consumed by humans or their animals (Mashi *et al.*, 2014). Some of the heavy metals can be toxic even at very low concentrations (Ghachtouli *et al.*, 2017; Fan *et al.*, 2017). Many different methods are used for remediation or amelioration of heavy metals from soils, but bioremediation, which employs the use of microorganisms generally considered to more environmentally friendly,

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cheaper, and effective (He et al., 2020; Khalid et al., 2021). The microorganism involved, particularly fungi, ameliorate heavy metals from soils by binding the heavy metal ions to the components of their cell walls, such as cellulose, chitin, and lignin. This mechanism limits the penetration of the heavy metals into soil matrix and plant systems (Chopra *et al.,* 2009). Ectomycorrhizas fungi are usually used for the amelioration of heavy metals in crops. Ectomycorrhizas form a mutualistic symbiosis with diverse host plants. They contribute to their host plants through efficient extraction and transport of water and mineral salts from the soil by the plants, while the host plants supply carbohydrates to the fungi (Khalid *et al.,* 2021). Also, the potential of any plant to uptake and accumulate any heavy metal is not determined only by the plant species but also by the soil conditions.

However, it should be noted that there is limited knowledge on the impact of different species of ectomycorrhizal on ameliorations of heavy metal by many species of crop plants. Specifically, no research has been performed to establish the impacts of the ectomycorrhizal fungus *Pisolithus arhizus* on the uptake and accumulation of different heavy metals by millet grown in different soil types. To fill this gap of knowledge, it is, therefore, necessary to conduct research on the impacts of *Pisolithus arhizus* and soil type on the dynamics of different heavy metals in millet. We hypothesized that the ectomycorrhiza *Pisolithus arhizus* and different soil types will affect the dynamics of different heavy metals in pearl millet seedlings in different ways.

Materials and Methods

The Study Area

This study was carried out in Bauchi L.G.A, North-East of Nigeria (at latitude 10º 17'N and longitude 9º 49'E). The climatic zone is typical of the northern guinea savanna. It has a seasonal climate with an average temperature that ranges from 10-12°C in December-January and 30-32°C in March-May (Mustapha *et al.,* 2007; Akande, 2010).

Soil sampling

In this experiment, the soil used was collected from domestic waste dumpsites in Yelwa area of Bauchi metropolis. These waste dump soils are usually patronized by the local Farmers in this area as a source of their farm manure. The background soil was also collected from cultivated fields at about 0-20 cm. All the soils were sieved through a 2 mm diameter mesh to remove gravel and other larger debris. The soil samples were then sterilized by autoclave (Azeez *et al.,* 2011; Mashi *et al.,* 2014).

Soil analysis

The physicochemical properties of the soils used in this experiment were analyzed using standard procedures. Samples of all the experimental soils were oven-dried at 80ºc for several hours. A portion (0.5 g) of each of the soil samples were digested in a binary mixture of HNO_3—HCl in 2:1 in a 250 ml beaker. The digested materials were subsequently analyzed for some heavy metals. The techniques used were as follows: 1) pH was determined in a 1:1 soil/solution ratio using a pH meter and a combination electrode; 2) the heavy metals were estimated using Atomic uptake spectrophotometer (AAS). Percentage carbon was determined using Walkey-Black chromic acid titration method (Pasquini and Harris, 2005; Azeez *et al.,* 2011; Mashi *et al.,* 2014; Fan *et al.,* 2017). Analyses for the pH, percentage of carbon content, and textural class of the soils were carried out in the soil laboratory of Abubakar Tafawa Balewa University, Bauchi, while analyses for the heavy metals were carried out in the Energy Center of the same institution (Table 1).

Experimental design and treatments

The experiment was conducted in a screen house of the Abubakar Tafawa Balewa University. Seeds of pearl millet were purchased from a local vendor. The seeds were then sterilized in 30% H_2O_2 (hydrogen peroxide) for 15min and then rinsed three times with plenty of distilled water (Miche´, L. and Balandreau, 2001). Three different soils were used for the experiment. These include 1) the background soil (S_1), which had the least of nutrients, organic matter content, and lesser levels of most of the heavy metals (Table 1, 2) the waste dump soil (S_2), which was the richest in terms of nutrient, organic matter and most

Table 1: physicochemical properties of soils used in this study (mg kg^{-1})

Soil type	Mn	Cd	Ni	Zn	Cu	Pd	Cr	Mg	Fe	pH	OM (%)	Soil Class
Background soil (S_1)	1.32	0.00	0.08	2.00	0.28	0.21	0.01	46.2	124	5.48	0.36	Loamy sand
Waste dump soil (S_2)	0.80	0.01	0.15	6.05	1.27	0.25	0.00	353	11.1	6.13	1.09	Sandy Loam
Amended soil (S_3) = 3:1 S_1/S_2	**1.52**	**0.00**	**0.11**	**5.35**	**0.58**	**0.22**	**0.00**	**193.33**	**120**	**5.74**	**0.54**	**Sandy Loam**

of the heavy metals; 3) the background soil that was amended with the waste dump soil in a mixture of 3:1 ratio (background soil/waste dump soil, S_3). One kilogram of each of these soil types was placed in one-liter plastic pots. These pots were perforated at the bottom. Dried fruiting bodies (sporocarps) of ectomycorrhiza *Pisolithus arhizus* were collected in a plantation of *Eucalyptus camaldulensis* in July, which is the onset of the rainy season. One kilogram of the dried sporocarps were gently crushed in four liters of sterile water and then sieved through a mesh. The mixture obtained was used to inoculate the experimental soils (Brundrett *et al.*, 1996). Each of the pots containing the different soils was inoculated with four tablespoonsful of the mycorrhizal inoculum at a depth of 2 cm. Some of the waste dump soil (S_2) were not inoculated with the mycorrhiza, which serves as a control. Four seeds of the millet were planted on top of the inoculated soils inside the pots. The pots were arranged and analyzed using a completely randomized design (CRD). Each soil type and the control had eight replicates. The plants were watered with ½ L of tap water twice a week. The amount of water used was just enough to saturate the soil with little or no leaking at the bottom of the perforated pots. The plants were harvested after six weeks.

Analysis for heavy metals content

The plants were grown for six weeks and then harvested by gently removing them from the soil so as not to lose the roots. The plants were then washed with running tap water and then cut into shoots and roots with a sharp blade. Each plant was tag according to the treatment they received and then dried in a hot air oven at 80ºC for 72 hours. After drying, the plant materials were grounded in a mortar and sieved through a 0.2 mm nylon sieve. The samples were digested following a standard procedure using concentrated nitric acid and hydrogen peroxide. After the digestion, the concentrations of different heavy metals in each digested plant material were analyzed in triplicate using atomic uptake spectroscopy (Krpata *et al.*, 2009; Mashi *et al.*, 2014; EL Ghachtouli *et al*., 2017; Fan *et al.*, 2017).

Data analyses

Bioconcentration factor (BCF) of Heavy Metals

The bioaccumulation factor of the metal, defined as a ratio of plant/soil metal concentration, was calculated as follows:

BCF = *C*plant/*C*soil X100

where *C*plant and *C*soil are the heavy metal concentrations in the plant part and the soil, respectively (Zhuang et al., 2013). The bioconcentration factor (BCF) shows the accumulation of heavy metals in a plant's root relative to the concentration of that heavy metal in the soil in which the plant was cultivated. The range of the BCF values >100% (hyperaccumulator); ≤100% (moderate accumulator); ≤10% (low accumulator) and ≤1% indicated non-accumulator (Syam et al., 2016, Sulaiman and Hamzah, 2018).

Translocation factor (Tf) is defined as a plant's ability to translocate a metal from its root to the shoot. The TF was determined by calculating the concentration of each heavy metals in the shoot relative to that of the root using the formula (Magaji et al., 2018):

Table 2: Analyses of Variance (ANOVA) of means of concentration (mg/kg) of different metals in root and shoot of millet seedlings planted in different soil types (α = 0.05)

	†Root S_1M_+	Root S_2M_0	Root S_2M_+	Root S_3M_+	Shoot S_1M_+	Shoot S_2M_0	Shoot S_2M_+	Shoot S_3M_+	Pooled StDev	P-Value
Fe	7.200c	8.300b	6.200d	9.606a	1.750f	1.910e	1.691g	1.522h	0.0021	0.001
Mg	16.74h	17.16g	17.73c	17.83b	17.24f	18.40a	17.44e	17.50d	0.0007	0.001
Ni	0.005g	0.012f	0.078b	0.084a	0.019d	0.023c	0.016e	0.001h	0.0004	0.001
Cu	0.237c	0.266a	0.148f	0.178e	0.148f	0.144g	0.242b	0.187d	0.0008	0.001
Zn	0.553d	1.344c	1.751a	1.387b	0.149f	0.084g	0.278e	0.148f	0.0005	0.001
Mn	0.870e	0.971d	1.460a	1.360b	0.670f	1.100c	0.271	0.380g	0.0005	0.001
Pb	0.039b	0.047a	0.033d	0.036c	0.019f	0.013g	0.024e	0.002g	0.0000	0.001
Cd	0.040b	0.011e	0.011e	0.035c	0.036c	0.013d	0.046a	0.011e	0.0005	0.001
Cr	**0.100b**	**0.004h**	**0.016g**	**0.033**	**0.093c**	**0.063d**	**0.118a**	**0.024f**	**0.0006**	**0.001**

Means that do not share a letter are significantly different.
†S_1M_+ Background soil inoculated with mycorrhiza
S_2M_+ Waste dump soil inoculated with mycorrhiza
S_2M_0 Waste dump soil without mycorrhiza
S_3M_+ Amended Background soil (Background soil +Waste dump soil (3:1) inoculated with mycorrhiza

$$Translocation\ factor = \frac{Metal\ concentration\ in\ shoot}{Metal\ concentration\ root}\ X\,100$$

The TF values obtained were expressed in percentages (Syam *et al*., 2016).

Statistical analysis

Mean values of concentration (mg/kg) of different heavy metals in root and shoot of the millet seedlings were obtained for plants cultivated in different soil types. The data were subjected to analysis of variance (ANOVA) followed by Tukey Multiple Comparison of means in other to find the significant differences of concentration of different the heavy metals in plant among all the treatments. The analysis was carried out in MINITAB® 18.1, 2017. The means were considered significantly different at $p \leq 0.05$ (Kaur, 2018).

Results

In this study, we determined the effects of ectomycorrhiza *Pisolithus arhizus* and soil type on uptake, accumulation, and translocation of heavy metals in millet seedlings.

The uptake pattern of heavy metals in the roots of millet seedlings

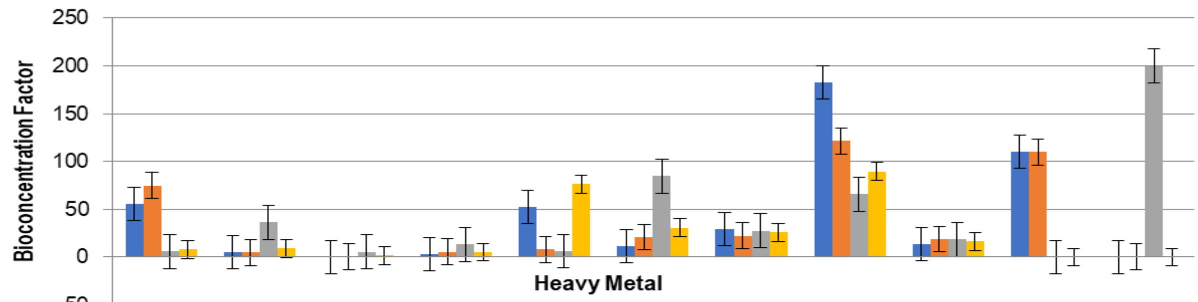
The result revealed that the ectomycorrhiza and soil type have affected the pattern of uptake and concentration of the heavy metals and other nutrient elements in the seedlings of millet. The magnitude of these effects depends on the soil type, the particular heavy metal involved, and the plant's part (Table 2). By comparing the result of the inoculated and non-inoculated waste-dump soil, the result showed that the mycorrhiza significantly decreased the concentration (mg/kg) of iron (Fe) in the root. The values were 6.20 and 8.30 for the inoculated and non-inoculated waste-dump soils, respectively. The concentration Fe was also found to be significantly lower (7.20) in the root of the inoculated background soil than the inoculated amended background soil (9.61), although the background soil contained a higher concentration of the Fe than the amended background soil. The mycorrhiza slightly but also significantly increased the concentration of magnesium (Mg) in the root of the millet seedlings in waste dump soil. The concentration values were 17.73 and 17.16 for the inoculated and non-inoculated waste-dump soils, respectively. The highest concentration value (17.83) was found in the root of the plant cultivated in the inoculated amended background soil, and the lowest (16.74) was in the inoculated background soil. However, the concentration of Mg in the waste-dump soil sample was four times more than that in the background soil sample.

The mycorrhiza increased the uptake of nickel (Ni) by the roots of millet seedlings. The concentration values were 0.012 and 0.078 for the inoculated and non-inoculated waste-dump soils, respectively. There was a higher concentration of Ni in the inoculated amended background soil (0.084) than in the inoculated background soil (0.005). Uptake copper (Cu) by the root was significantly suppressed by the presence of the mycorrhiza. The concentration values of the Cu in the root of millet seedlings were higher in the roots of plants cultivated in the non-inoculated waste-dump soil (0.266) than in the inoculated waste-dump soil. Also, the plant's roots accumulate more Cu in the inoculated background soil (0.237) than the inoculated amended background soil (0.178).

The uptake of zinc (Zn) by the plant's root was increased significantly by the mycorrhiza. The roots of plants in the inoculated waste dump soil accumulated 1.751 of Zn, and that of the non-inoculated waste dump soil was 1.344. The values of Zn concentration in root plants cultivated in the inoculated waste dump and the inoculated background soils were 1.387 and 0.553 respectively. The concentration of Zn in the waste-dump soil sample was three times more than its concentration in the background soil sample.

Uptake of manganese (Mn) by the roots of the millet seedlings was also increased by the mycorrhiza. The concentration values in the inoculated and the non-inoculated waste dump soils were 1.460 and 0.971, respectively. The values for the inoculated background soil and the inoculated amended background soil were 0.870 and 1.360, respectively. However, the concentration of Mn in the waste-dump soil sample was lower (0.80) than its concentration in the background soil sample (1.32). The concentration of lead (Pb) in the plant's roots was slightly but significantly decreased by the mycorrhiza. The concentration values were 0.047 and 0.033 for the roots of plants in the inoculated and the non-inoculated waste dump soils, respectively; and for the root plants cultivated in the inoculated background soil and the inoculated amended background soil, the concentration values were 0.039 and 0.036, respectively. However, the concentrations of Pd in the samples of the waste-dump soil and the background soil were nearly the same, which were 0.21 and 0.25, respectively.

The concentration of cadmium (Cd) in the roots of the plant seedlings was not affected by the mycorrhiza. The concentration value was 0.011 in both the inoculated and the non-inoculated waste dump soil. The concentration value for the Cd was significantly higher in the roots of plants in the inoculated background soil (0.040) than the inoculated amended background soil (0.035). There was a higher



	Fe	Mg	N	K	Ni	Cu	Zn	Mn	Pb	Cd	Cr
S2M+	55.856	5.023	0.106	3.214	52	11.654	28.942	182.5	13.2	110	0
S2M0	74.775	4.861	0.129	5.357	8	20.945	22.215	121.375	18.8	110	0
S1M+	5.806	36.234	5.367	13.333	6.25	84.643	27.65	65.91	18.571	0	200
S3M+	8.005	9.224	1.836	5.206	76.364	30.69	25.925	89.474	16.364	0	0

Figuren1: Bioconcentration Factor (BCF) of heavy metals in the root of millet seedlings

S_1M_+ Background soil inoculated with mycorrhiza
S_2M_+ Waste dump soil inoculated with mycorrhiza
S_2M_0 Waste dump soil without mycorrhiza
S_3M_+ Amended Background soil (Background soil +Waste dump soil (3:1) inoculated

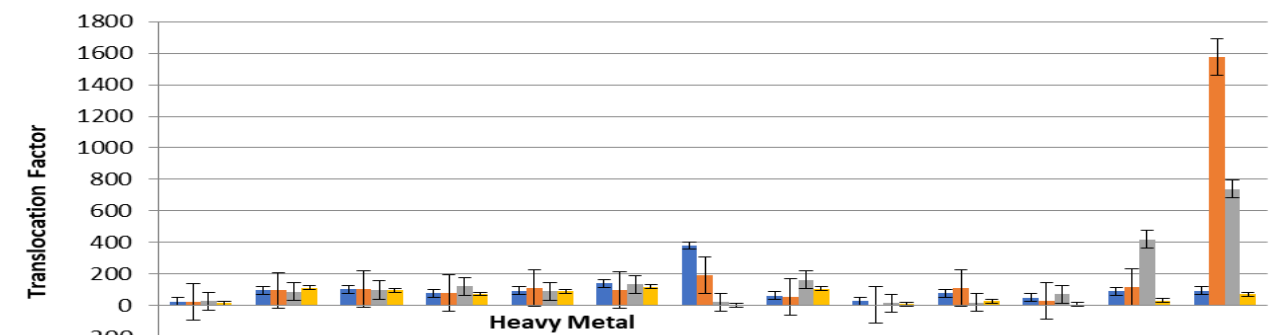
concentration of Cd in the sample of the waste-dump soil than in the sample of the background soil. The mycorrhiza had positively affected the uptake of chromium (Cr) by the roots of the millet seedlings. The concentration values were 0.016 and 0.004 for the inoculated non-inoculated waste-dump soil, respectively. There was also a significantly higher concentration of the Cr in the roots of plants cultivated in the inoculated background soil (0.100) than the root of plants in the inoculated amended background soil (0.033). The background soil sample had a higher concentration of Cr than the sample of the waste-dump soil.

Bioconcentration factor (BCF) of heavy metals in the root of millet seedlings

The bioconcentration factor (BCF) shows the accumulation of heavy metals in a plant's root relative to the concentration of that heavy metal in the soil in which the plant was cultivated. The range of the BCF values >100% (hyperaccumulator); ≤100% (moderate accumulator); ≤10% (low accumulator) and ≤1% indicated non-accumulator. The result of this study revealed that the BCF of the root of millet seedlings was affected by both the mycorrhiza and the soil type. In the non-inoculated waste dump soil, the BCF values showed that millet is a hyperaccumulator of Mn (121.4%) and Cd (110%); and moderate accumulator Fe (74.8%), Cu (20.9%), Zn (22.2%), and Pb (18.8%) (Figure 1). It is a low accumulator of Mg (4.9%) and Ni (8.0%). The BCF values for some of the heavy metals changed in the plants that were cultivated in the inoculated waste dump soil, which indicated the effect of the mycorrhiza on the BCF of millet. For Mn and Cd, the plant remained hyperaccumulators but the BCF of Mn increased from 121.4 to 182.5%. The plant remained a moderate accumulator of Zn but its BCF value increased from 22.2% to 28.9%; while concerning Fe, Cu, and Pd, the millet seedlings remained moderate accumulators but their BCF decreased from 74.8 to 55.8, 20.9 to 11.7, and 18.8 to 13.2, respectively. However, the plant changed from low accumulator (8%) to moderate accumulator (52%) for Ni but remained low accumulator Mg with a slight increase of the BCF from 4.9 to 5.0.

For the macro-nutrients N and K, the mycorrhiza decreased their BCF in the root. The BCF of N for the plants cultivated on the inoculated and non-inoculated waste dump soils, was 0.106% and 0.129%, respectively. While that of K for the same treatment, the BCF were 3.214% and 5.357%, respectively.

The values BCF in the root of the millet seedlings differed among the three inoculated soils, i.e., waste dump soil, background soil, and amended background soil. Comparison for the BCF between the inoculated background soil and the inoculated amended background soil showed that soil amendment decreased the BCF values for Mg (from 36.2% to 9.2%), Cu (from 84.6 to 30.7), Zn (from 27.7 to 25.9), and Pd (from 18.6 to 16.4). However, soil amendment increased the BCF values for Ni (from 6.3 to 76.4), and Mn (from 65.9 to 89.5); while concentration values for Cd were not detected for plants grown in these soils. Also, BCF values for Cr in the plants that were



	Fe	Ca	Mg	N	P	K	Ni	Cu	Zn	Mn	Pb	Cd	Cr
S1M+	24.306	97.686	102.99	78.947	95.331	141.63	380	62.447	26.944	77.011	48.718	90	93
S2M0	23.012	95.517	107.23	81.818	110.33	97.8	191.67	54.135	6.25	113.29	27.66	118.18	1575
S2M+	27.274	89.001	98.364	122.22	91.188	133.33	20.513	163.51	15.877	18.562	72.727	418.18	737.5
S3M+	15.844	111.54	98.149	73.684	91.7	120.05	1.19	105.06	10.671	27.941	5.556	31.429	72.727

Figure 2: Translocation Factor of heavy metals in millet seedlings

S_1M_+ Background soil inoculated with mycorrhiza
S_2M_+ Waste dump soil inoculated with mycorrhiza
S_2M_0 Waste dump soil without mycorrhiza
S_3M_+ Amended Background soil (Background soil +Waste dump soil (3:1) inoculated

cultivated in the inoculated background soil showed that millet is a hyperaccumulator (1000%) of Cr, but concentration values for the Cr were not detected in other soil types.

Translocation factor (TF) of heavy metals in the root of millet seedlings

Translocation Factor (TF) measures the ability of a plant to translocate a particular heavy metal from the root to the shoot. TF is usually reported as a percentage or ratio of the concentration of that metal in a plant's shoot relative to its concentration in the plant root. The mycorrhiza increased the TFs of Cd, Cu, Fe, Mg, Pb, and Zn (Figure 2). The TF for these heavy metals in the millet seedlings cultivated in the inoculated and non-inoculated waste dump soil, respectively, were 118.2% and 418.2% for Cd, 54.1% and 163.5% for Cu, 23.0%, and 27.3% for Fe, 27.7% and 72.7% for Pb, and 6.6% and 15.9% for Zn. However, the TFs for Mg, Ni, Mn, and Cr was decreased by the mycorrhiza. Their TFs in the inoculated and non-inoculated waste dump soil, respectively, were 107.2% and 98.4% for Mg, 191.7% and 20.5% for Ni, 113.3% and 18.6% for Mn, and 1575% and 737.5% for Cr.

For the macro-nutrients N and K, mycorrhiza increased their rate of translocation from the root to the shoot. The TF of N for the plants cultivated on the inoculated and non-inoculated waste dump soils, were 122.22% and 81.82%, respectively. While that of K for the same treatment, the TF were 133.33% and 97.8%, respectively.

The result of the comparison between the TFs of heavy metals in plants cultivated in the background soil and the amended background soil revealed that soil amendment with the waste dump soil affected the TFs of all the heavy metals. The TFs of all the heavy metals were decreased except for Cu. The TF values of heavy metals that were decreased by less than half of their values as the result of the soil amendment include Cd (from 90% to 31.4%), Pb (from 48% to 5.6%), Mn (from 77% to 27.9%), Zn (from 26.9% to 10.7%), and Ni (from 380% to 1.2%). The heavy metals whose TF values were decreased by the soil amendment but are were still more than half of their original values include Fe (from 24.3% to 15.8%), Mg (from 103% to 98.1%), and Cr (from 93% to 72.7%). However, the TF of Cu was increased from 62% to 105%.

Discussion

In this study, we investigated the effects of ectomycorrhiza *Pisolithus arhizus* on the pattern of uptake and translocation of heavy metals in seedlings of millet cultivated on different soil types. The result showed that the mycorrhiza and soil type determined the concentration (mg/kg) of the heavy metals in the roots and their rate of translocation from the root to the shoot. It had been reported earlier that species of *Pisolithus* are frequently found to thrive soils that are polluted with different types of heavy metals; and that *Pisolithus arhizus,* in particular, can absorb and accumulate several heavy metals, which depend on the concentration of the heavy metals on the growth substrates (Colpaert *et al.*, 2011; Şen *et al.*, 2012). Most of the ectomycorrhizal fungi are known to tolerate and, therefore, can accumulate a high concentration of heavy metals. This is usually done through both extracellular and intracellular mechanisms. The extracellular mechanisms include chelation and cell-wall binding; while the intracellular mechanisms include binding the metal ions to organic acids, peptides, and other compounds (Aladesanmi *et al.,* 2019).

Concentration of heavy metals in the root of millet seedlings

The result of this study revealed that the ectomycorrhiza *Pisolithus arhizus* significantly increased the concentrations of chromium (Cr). magnesium (Mg), manganese (Mn), nickel (Ni), and zinc (Zn) by the root of millet seedlings. While the mycorrhiza significantly decreased the concentrations of copper (Cu), iron (Fe), and lead (Pb) in the root plant. However, the concentration of cadmium (Cd) was not affected by the mycorrhiza.

The result also showed that amendment of the background soil with the waste dump soil greatly increased the uptake and concentration of Fe and Mn in the root. however, the concentrations of these heavy metals were higher in the millet seedlings cultivated on the amended background soil than the background soil without the amendment (both inoculated), although the sample of the background soil had higher concentrations of these heavy metals than the amended background soil. This suggested that some components that made up the waste dump soil must have affected the mycorrhizal efficiency in the uptake of Fe and Mn by the plant. For example, studies showed that waste-dump soils are rich in nutrients (Opaluwa *et al.,* 2012). Plants growing on such soils grow and add more biomass faster and subsequently leads to higher accumulation of the heavy metals in the plants' tissues (Cui *et al.,* 2004). Additionally, the process of faster growth can be further enhanced by the mycorrhizal facilitations through more efficient water and nutrients acquisition from the soil by the root of the plants. These will lead to greater extract of heavy metals from the soil by the roots (Singh *et al.,* 2019).

On the other hand, accumulations of Cu and Cd were decreased by the soil amendment. Although there was a higher concentration of Cu in the sample of the amended background soil than the non-amended background soil, concentrations of this metal were higher in the roots of millet seedlings cultivated on the background soil without the amendment than the amended background soil (both inoculated). Soil composition and characteristics, such as organic matter and pH, are known to affect the rate of uptake of heavy metals by plants. The waste-dump soil contains a high amount of organic matter, which is well known to played role in the suppression of heavy metals uptake by some plants (Adekiya *et al.,* 2018; Aladesanmi *et al.,* 2019), which might have been the case in this study.

Additionally, it was not clear whether the uptake and concentrations of Cr, Mg, Ni, and Zn by the plant's root were affected by the soil amendment or not. The level concentrations of these metals in the root millet seedlings were in respect to the level of their concentrations in the soil in which the plant was cultivated; i.e., the higher a metal concentration in the soil, the higher was its concentration in the plant's root. However, the concentrations Zn and Mg were more than two to three times, respectively, higher in the amended soil than the background soil, but their concentration in the plant's root cultivated on the amended soil was not that higher than in the root of plants cultivated on background soil. This suggested that soil amendment reduced the rate of the uptake and concentrations of these metals. Some previous studies reported that the rate of uptake and concentration of heavy metals by the root of plants increases with increasing concentration in the soil (Kumar and Chopra, 2014). Additionally, it was found that millet plants accumulate heavy metals mainly in the roots, but the straw and the grain also can accumulate significantly high amounts of heavy metals far above the WHO's recommended limits (Asdeo, 2014; Sab-Udeh and Okerulu 2017; Toroni *et al.,* 2019). Millet is a popular staple food and fodder especially, in the drier tropical regions of Africa, Asia, and Latin America (Kumar and Chopra, 2014). Therefore, toxic metals accumulation in this plant can be currently a serious threat to the health of people in this region due to its high consumption rate (Felagha and Ogbolosingha, 2018).

Bioconcentration factor (BCF) of heavy metals in the root of millet seedlings

The bioconcentration factor (BCF), which indicates the accumulation of heavy metals in the root of millet seedlings relative to its concentration in the soil (Syam et al., 2016, Sulaiman and Hamzah, 2018), was found to be affected by both the mycorrhiza and the soil type. Without the soil inoculation with the mycorrhiza, the millet cultivated on the waste dump soil was found to be hyperaccumulator of Mn and Cd; moderate accumulator of Fe, Cu, Zn, and Pb. It is a low accumulator of Mg and Ni. Also, BCF values for Cr in the plants that were cultivated in the inoculated background soil showed that millet is a hyperaccumulator Cr. However, the bioconcentration ability of millet changed when cultivated in the inoculated waste dump soil. This indicated that the mycorrhiza affected the BCF of this plant. The values of BCF for Mg, Mn, Ni, and Zn were increased by the mycorrhiza; while that of Fe, Cu, and Pd were decreased. The BCF of Cd remained unaffected by the mycorrhiza. The result of this study also revealed that the BCF of the millet seedlings was affected by soil type. Soil amendment decreased the BCF values for Mg, Cu, Zn, and Pd. However, the soil amendment increased the BCF values for Ni, and Mn; while the BCF value of Cd and Cr were not estimated because their concentrations were not detected in the background soil and the amended background soil. Previous studies revealed that the pattern accumulation of heavy metals by plants is extremely complex because it is affected by numerous environmental and edaphic factors, and the plants' mechanisms (Aladesanmi *et al.,* 2019; Shehu *et al.,* 2019). Bioaccumulation of heavy metals differs among different plant species or even the variety in question. Each plant species or variety employs a specific set of mechanisms to absorb and translocate the metals into different parts of the plant and in different ratios (Asopa, *et al.,* 2016). Therefore, the pattern of bioaccumulation of different heavy metals by millet cannot be generalized because it depends on a particular heavy metal, soil type, and the presence or absence of mycorrhiza (He *et al.,* 2020).

For the macro-nutrients N and K, the mycorrhiza decreased their BCF in the root. Macro-nutrients are used by plants for their primary metabolism and are required in larger quantities (Koch *et al.,* 2020). For these reasons the macro-nutrients should be expected to be readily translocated from the root to the shoot, hence they will not accumulate in the root (see below).

Translocation factor (TF) of heavy metals in the root of millet seedlings

Translocation Factor (TF) estimates the degree of mobility of a particular heavy metal to be transported from the root to the shoot of a plant and is reported as the shoot/root ratio of the concentration of the metal (Syam et al., 2016; Magaji et al., 2018). In this study, the ectomycorrhiza increased the TFs of Cd, Cu, Fe, Mg, Pb, and Zn. However, the TFs of Mg, Ni, Mn, and Cr was decreased by the mycorrhiza. The soil amendment affected the TFs of all the heavy metals. The TFs of all the heavy metals were decreased by the soil amendment except for Cu. The TF values of heavy metals that were decreased by less than half of their values as the result of the soil amendment include Cd, Pb, Mn, Zn, and Ni. The heavy metals whose TF values were decreased by the soil amendment but are were still more than half of their original values include Fe, Mg, and Cr. However, the TF of Cu was increased. It was reported that endomycorrhiza enhanced heavy metal accumulation in roots tissue but restrict their translocation to the shoot portion (Singh *et al.,* 2019; Abdelmoneim *et al.,* 2014); although there is no consistency in the species of the ectomycorrhizas used in research related to bioaccumulation and translocation of heavy metals. In this study, it was found that ectomycorrhiza affects the translocation of heavy metals at a rate that depends on the heavy metals and soil type (Zielonka *et al.,* 2020).

For the macro-nutrients N and K, the mycorrhiza increased their rate of translocation from the root to the shoot. Since the macro-nutrients are used for the plants' primary metabolism and are required in larger quantities (Koch *et al.,* 2020), they should be expected to be readily translocated from the root to the shoot.

These findings suggest that the ectomycorrhiza *Pisolithus arhizus* and soil manipulations can be employed to increase or decrease the bioaccumulation and translocation of heavy metals by millet. This, of course, will depend on a particular heavy metal and the purpose of the millet cultivation. Millet is known to be cultivated for human and animal consumption, but it can also be used for the phytoremediation of some heavy metals. The result of this study suggested that pearl millet can be used potentially for the phytoremediation of Mn, Cd, Fe, Cu, Zn, and Pb from soils contaminated by these metals. The ectomycorrhiza *Pisolithus arhizus* can be introduced to the millet plant to increase its phytoremediation potentials for Mn, Zn, Mg, and Ni. Soil amendment with the waste dump soil can also be employed to increase the millet's phytoremediation potentials concerning Mn and Fe. However, the ectomycorrhiza *Pisolithus arhizus* can be used for alleviation of Cu, Fe, and Pb millet during cultivation to minimize human and animal health risks due to consumption of the millet. Additionally, soil amendment with waste dump soil can also be introduced for the amelioration of Mg, Cu, Zn, and Pb in the pearl millet plant during cultivation (Raklami *et al.,* 2021).

Conclusion

In this study, we determine the combined effects of ectomycorrhizal fungus *Pisolithus arhizus* and different soil types on the dynamics of different heavy metals in pearl millet seedlings. The result revealed that the ectomycorrhiza and soil type have affected the pattern of uptake and concentration of the heavy metals by the millet, which depends on the particular heavy metal. The BCF values showed that millet is a hyperaccumulator of Mn and Cd; and moderate accumulator Fe, Cu, Zn, and Pb. It is a low accumulator of Mg and Ni. These suggest that pearl millet can be used potentially for the phytoremediation of Mn, Cd, Fe, Cu, Zn, and Pb. The ectomycorrhiza *Pisolithus arhizus* increased the plant's phytoremediation potentials for Mn, Zn, Mg, and Ni. Soil amendment increased the millet's phytoremediation potentials concerning Mn and Fe.

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