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Influence of *Aspergillus Niger, Penicillium* and *Trichoderma* Fungi on the Growth of *Amaranthus Caudatus* in Heavy Metals Amended Soil From Tsamawa, Kano State

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ABSTRACT
Soil sample from Tsamawa irrigation site was amended with 10 mg/L of Cadmium (Cd), Chromium (Cr), Manganese (Mn), Nickel (Ni), Zinc (Zn) and Lead (Pb) separately in polythene bags. 4.0 x 10^{6} CFU/L *Aspergillus niger (An)*, *Trichoderma (Tr)* and *Penicillium (Pn)* fungal species were injected into the soil after planting *Amaranthus caudatus* seeds. Water was being added to the set-up after every 3 days interval for a period of 6 weeks during which measurements were taken from the 3rd to 6th week. The plants were uprooted and separated into shoot and root then prepared for AAS analysis to determine the effect of the fungi on metal uptake by the plants and also to determine Statistically the variations between the growth of the plants. The highest plant height (18.49 cm) was recorded in Ni amended soil with *Tr*, while *An* and *Pn* had slight influence on the plant growth. *Tr* generally promoted the best plant growth in Cd, and Ni amended soils, while *An* and *Pn* showed mixed effects, sometimes reducing plant growth. For the metal uptake, by the fungi in roots, *An* promoted high Cr (5.05 mg/kg), Pb (1.75 mg/kg) and Ni(4.15 mg/kg) accumulation, *Pn* accumulated Cd (0.78 mg//kg) and Mn (1.42 mg/kg while, *Tr* accumulated Zn (4.4 mg//kg).These findings proved that the selected fungi can be used in remediating contaminated soil as they are found to be good accumulators of the heavy metals hence, reducing their availability to the plants as well as promoting the plant growth.

Keywords:
Heavy metals,
Fungi, Soil,
Amaranthus caudatus

INTRODUCTION

The contamination of foods by heavy metals poses significant challenges for both producers and consumers. Vegetables primarily absorb heavy metals from their growing environments, such as soil, air or nutrient solutions through their roots or foliage (Doabi *et al.*, 2018). These metals become toxic and harmful when the contaminated vegetables are consumed over long periods. To safeguard public health, it is crucial to regularly monitor heavy metals levels in vegetables and other foods to prevent excessive accumulation in the food chain (Amin *et al.*, 2013).
Vegetables tend to absorb and accumulate heavy metals in quantities high enough to pose health risk to humans (Mosa *et al.*, 2016).
Leafy vegetables grown on heavy metal contaminated soils store higher amounts of metals than those grown in clean soils due to their root-based absorption (Ahmad & Ashraf, 2011).
Heavy metals are persistent in the environment (Samaila *et al.*, 2025), and are readily accumulated in the edible parts of leafy vegetables more than in grain or fruits (Parasakthi, 2023). Their presence not only disrupts plant physiology and biochemistry reducing crop yield, but also causes serious health hazards through their entry into the food chain (Akande & Ajayi, 2017; Zakari et al., 2022). Pollution of plants is of great concern for two major reasons; first, it can have direct toxic effects such as internal cell damage or biochemical disruptions due to the pollutant exposure. or indirect phytotoxic effects on the plants themselves, leading to a decline in crop yields and threatening food supplies (Igiri *et al.*, 2018).

Second, the plants may act as a vehicle for transferring pollutants into the food chain. For example, Cd can accumulate to levels which are dangerous to plants consequently posing a significant threat to animals and humans that consume them (Jaishankar *et al.*, 2014; Samaila *et al.,* (2025). Moreover, the presence of heavy metals such as, Iron, Lead and Mercury in soil can reduce its fertility and agricultural productivity (Tchounwou *et al*., 2012; Doabi *et al*., 2018).

Healthy soil is reservoir to diverse range of organisms, including fungi, bacteria, earthworms, algae, protozoa and nematodes. These beneficial microorganisms play a vital role in breaking down heavy metals through the process of bioremediation. Some microbes due to their metal tolerance can survive in contaminated areas and support plant growth while cleaning up pollutants (Dell'Anno et al. 2022). Given their ability to degrade persistent heavy metals which can reduce soil fertility, this study focuses on how fungal species such as *Aspergillus niger, Penicillium* and *Trichoderma* can aid in the uptake of heavy metals and promote plant growth in soils contaminated with Cadmium (Cd), Chromium (Cr), Manganese (Mn), Nickel (Ni), Lead (Pb) and Zinc (Zn)).

MATERIALS AND METHODS

The materials used in this study include Soil sample, 10 mg/L of Cd, Cr, Zn, Mn, Ni and Pb salts solutions; 4.0 x 10^6 CFU/ml *Aspergillus niger, Trichoderma* and *Penicillium* fungal isolates solutions, *Amaranthus seeds*, polythene bags and sample containers.

The soil samples were collected at random from the irrigation site at Tsamawa, Kano State at uniform depth of 0 - 4 cm with the aid of hand trowel. The sample was collected in replicates which were mixed to form a homogenous sample. It was then air dried for Seven days at ambient temperature, stones and debris were removed before weighing (Yaro *et al*., 2017). Solutions of the fungal isolates were collected from the Department of Microbiology, UMYU, Katsina.

The experimental Set -up was the randomized block design (RBD), comprising the soil sample subjected to five different groups of treatments in triplicates. The treatments included unamended soil (control); soil amended with 10 mg/l Cd, Cr, Mn, Ni, Pb, and Zn) solutions obtained from stock solutions prepared by dissolving 4.398 g of Zinc Sulphate, 1.570 g of Lead ethanoate, 6.896 g of Cadmium Sulphate, 7.542 g of Chromium Sulphate, 2.894 g of Manganese Sulphate and 4.478 g of Nickel Sulphate and soil amended with heavy metals and the three selected fungal species (*Aspergillus, Penicillium* and *Trichoderma species*) separately. Seeds of *Amaranthus caudatus* (purchased from Agric. office in Ajiwa, Katsina) were identified and assigned a voucher No. UMYUH2121 by the head of Botanical Garden, Department of Biological Sciences, UMYU). The seeds were planted in each bag after the introduction of the metals to the soil (Bandurskar *et al.*, 2021). Size of the plants was reduced to 4 per bag on 3rd week of planting. Data were collected at 4th, 5th and 6th week on the plant stem height and leaves count from two representative samples in each polythene bag, after which the plants were uprooted from the soil. A total of 75 samples of the roots, shoots and soil were collected from each bag. Both soil and plant samples were collected at the same time after the whole plants were uprooted from the soils. Prior to analysis, the shoots and roots of the plants were placed under running tap water to washed off soil particles, placed in paper bags to air-dry at room temperature and later ground using a porcelain mortar and pestle. The ground plant samples were weighed and digested in the laboratory at UMYU before transferred to soil laboratory at ABU Zaria for elemental analyses to determine the concentrations of Cd, Cr, Mn, Ni, Pb and Zn using atomic absorption Spectrometry (AAS).

Digestion of soil samples

To determine the concentration of heavy metals in the soil after treatment 1g of the soil sample was measured in a beaker and 10 ml mixture of HNO_3 and HCl in the ratio 3:1 (aqua regia) was added. It was heated to 100°C on a hot plate until a clear solution was observed. A blank was also prepared for each digestion batch to check its homogeneity and processing efficiency. The digested soil samples were then cooled and filtered through Whatman No. 4 filter paper. They were then transferred into graduated flasks and deionized water added up to the 50 ml mark. The digests were then transferred to an acid-rinsed sample containers with a label for analysis (USDA, 2000) using AAS.

Digestion of vegetables

The heavy metals content in the mature vegetables were determined by removing the vegetables from the experimental site and then the root and shoots were properly separated and thoroughly washed with water. The shoots were then chopped into small pieces using a clean stainless table knife and afterward dried to a constant mass in an oven at 80 ^{0}C for 48hrs. Replicate samples of the dried vegetables were combined and pounded to fine powder using a porcelain mortar and pestle. Particle sizes of .2mm mesh were obtained using laboratory sieve.

The vegetables (0.5g each of shoots and roots separately) were treated with 10 mL of aqua regia (3:1 mixture of Conc. HCl to Conc. HNO_3). The mixture was heated on a hot plate in a fume cupboard at 80°C until a clear solution was obtained. The digested samples were filtered using Whatman No.4 filter paper into a 60 ml graduated plastic sample bottles and the final volume adjusted to 50 ml with deionized water (Akande & Ajayi, 2017).

Bioconcentration factor and Transfer factor

The ability of plants to absorb, accumulate and translocate heavy metals was measured by means of bioconcentration factor (BCF) and transfer factor (TF) as described by Safana and Tasi.u (2018). The bioconcentration factor quantifies the relative differences in bioavailability of metals to plants and is a function of both soil and plant properties. This was calculated by dividing the concentration of the metal in the vegetable by the total metal concentration in the soil according to Equation 1.

$$BCF = \frac{Heavy\ metal\ concentration\ in\ shoot\ (mg/kg)}{Heavy\ metal\ concentration\ in\ soil\ (mg/kg)} \quad (1)$$

BCF signifies the number of metals that ended up in the vegetable (ref). Higher BCF reflect relatively poor retention in soils or greater efficiency of plants to absorb metals. Low BCF reflect the strong sorption of metals to the soil colloids.

Transfer factor (TF) signifies the fraction of total deposition on plant surfaces that is incorporated into edible parts of the plant. It was calculated by dividing concentration of the metals in shoot by the concentration of metals in root as indicated in equation (2).

$$TF = \frac{Heavy\ metal\ concentration\ in\ shoot\ (mg/kg)}{Heavy\ metal\ concentration\ in\ soil\ (mg/kg)} \quad (2)$$

Where as, TF>1 indicates that the plant translocated metals effectively from root to shoot (Safana and Tasiu, 2018) This one also gives indicators about the capacity of the plant to accumulate or to transfer the toxic elements from one organ to another.

RESULTS AND DISCUSSION

Method of data analysis

Descriptive statistics was used to calculate Mean concentration. The results were subjected to Analysis of Variance (ANOVA) to determine the level of significance between the groups ($P \leq 0.05$)., Correlation was used to determine association between heavy metals levels in soil and in the vegetables.

Table 1: Influence of *Aspergillus niger (*An), *Penicillium* (Pn*) and Trichoderma (*Tr) fungal species on the growth of *Amaranthus caudatus* in heavy metals polluted soil from Tsamawa, Kano State.

Plant Stem Height (cm) and Average number of Leaves												
Soil amendment	**Cd**		**Cr**		**Mn**		**Ni**		**Pb**		**Zn**	
M Only	12.56	12.22	16.99	9.99	13.22	7.55	12.88	6.66	11.44	6.88	12.11	7.99
M + An	9.66	4.44	14.78	8.55	13.59	9.22	12.56	8.55	13.67	9.22	9.22	5.66
M + Pn	11.22	17.00	11.44	8.00	15.11	7.10	8.44	6.21	10.56	7.11	13.56	7.44
M + Tr	5.99	11.99	8.00	5.88	15.44	7.55	18.48	11.22	10.99	7.66	13.33	7.66
Control	18.56 / 5.57											

Table (1) above showed average stem height and number of leaves in *Amaranthus caudatus* which has been grown in Tsamawa soil. The highest stem height of 18.48 cm was obtained n Ni amended soil with the addition of *Trichoderma* fungi, Mn and Cd amendments also showed *Trichoderma* having the highest growth influence when compared with metal only amendments, having heights of 15.44 cm and 17.00 cm respectively. This finding agrees with Bandurska *et al.*, (2021) who reported *Trichoderma* as having plant growth-attributes, producing phytohormones and improving nutrient uptake. *Aspergillus niger* fungi added to Pb amended soil also showed high growth influence when compared with metal only amendments, having stem height of 13.67 cm. *Penicillium* fungi showed highest growth influence in Zn amended soil with growth height of 13.56 cm when compared with the metal only treated soil. Both *Aspergillus niger* and *Penicillium spp.* have also been reported to influence root architecture and stimulate plant

growth indirectly by reducing heavy metal toxicity (Ezzouhri *et al*, 2009).
Addition of *Trichoderma* fungi indicated highest average number of leaves in Cd, Ni and Zn amended soils with values of 11.9, 11.22 and 7.66, while *Aspergillus* fungi showed its influence in Cr, Mn and Pb amended soils with values of 8.55, 9.22 and 9.22 respectively

Table 2: Metal concentrations (mg/kg) in shoots of *Amaranthus caudatus* grown in Tsamawa irrigation soil amended with *Aspergillus niger* (An), *Penicillium* (Pn), *Trichoderma* (Tr) fungi and 10 mg/L Cr, Cd, Mn, Pb, Zn and Ni.

Treatment	**Concentrations in mg/kg**					
	Cr	**Cd**	**Mn**	**Pb**	**Zn**	**Ni**
Control	0.07	0.01	0.19	0.03	0.01	0.05
Metal only	0.71	0.10	0.52	0.97	2.13	1.38
Metal + An	0.96	0.22	0.73	0.44	0.90	2.50
Metal + Pn	0.41	0.26	1.12	0.56	1.89	0.13
Metal + Tr	0.87	0.16	0.88	1.58	0.43	1.36

Table (2) above showed AAS analysis result for metal concentrations (mg/g) in shoots of Amaranthus caudatus grown in four different amended and control soils with graphical representation in Fig. (1) below
In Cr amended soil, the treatment with Penicillium (*Pn)* showed lowest metal concentration of 0.41 mg/kg < 0.71 mg/kg (control) indicating influence of the fungi in reducing the transfer of Cr to the shoot of the plant. In Cd and Mn amended soil, all the metal concentrations in fungal treatments exceed the control with metal only treatment showing no effect of the fungi in preventing metal transfer to the shoot. *An* showed the lowest metal concentration of 0.44 mg/kg < 0.97 mg/kg (control), indicating its ability to reduce metal transfer to the shoot. In Zn amended soil, all the fungal treatments succeed in decreasing the transfer of metals to the shoot when compared with metal- only amendment. Whereby, in Ni amendment, *Pn* showed its influence with a value of 0.13 mg/kg lower than the metal-only treatment (1.32 mg/kg). The role of fungi in reducing metal mobility aligns with findings by Anahid *et al.*, (2011) and Parasakthi *et al*, (2023), who observed that microbial activity can immobilize heavy metals, reducing their transfer to shoots.
Below is the graphical representation of table 2 where variations in the metal uptake between metal-only and fungal amendments (*Aspergillus, Trichoderma* and *Penicillium*) were shown clearly.

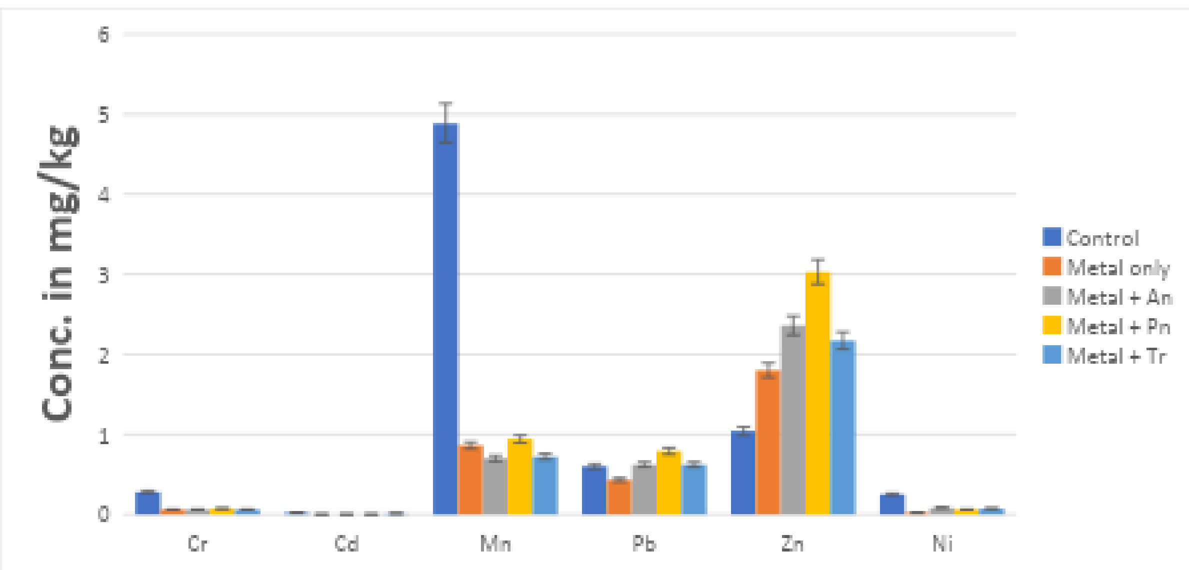

Fig.1. Metal concentrations in shoots of *Amaranthus caudatus grown* in Tsamawa irrigation soil.

Table 3: ANOVA for Metal concentrations in shoots of *Amaranthus caudatus* grown in Tsamawa irrigation soil.

Sources	Df	Sum sq	Mean sq	F-value	Pr(>F)
TR	5	2.978	0.5955	1.837	0.1512
MC	4	3.480	0.8701	2.684	0.0611
RESIDAULS	20 6.483		0.3242		

From the result obtained in Table 3 it is observed that the P-value (0.1512) is greater than the typical significance level (0.05) indicating that the effect of metals concentration on *Amaranthus caudatus* growth may not be statistically significant. Moreover, the P-value for treatment is (0.0611) which is greater than the significance level, suggesting that the effect of treatments on plant growth may not be statistically significant. This weak statistical significance could be due to variability in fungal colonization or uneven distribution of metals as suggested by similar work from Garbisu and Alkorta, (2003) on bioremediation variability.

Table 4: Metal concentrations (mg/kg) in roots of *Amaranthus caudatus* grown in Tsamawa irrigation soil amended with *Aspergillus niger* (An), *Penicillium* (Pn), *Trichoderma* (Tr) fungi and 10mg/L Cr, Cd, Mn, Pb, Zn and Ni.

Treatment	Concentrations in mg/kg					
	Cr	Cd	Mn	Pb	Zn	Ni
Control	0.91	0.09	0.37	0.66	0.85	0.45
Metal only	2.81	0.17	0.86	0.99	3.02	2.73
Metal + An	5.05	0.55	0.82	1.75	0.95	4.45
Metal + Pn	1.05	0.78	1.42	0.72	3.43	0.52
Metal + Tr	2.56	0.75	0.94	1.94	4.42	3.16

Table 4 represents metal concentrations in roots of *Amaranthus caudatus.* The result shows that the fungal amendments with *Aspergillus niger* (An) increased Cr (5.05 mg/kg), Pb (1.75 mg/kg) and Ni (4.45 mg/kg) uptake, *Penicillium* increased Cd (0.78mg/kg) and Mn (1.42 mg/kg) uptake, while Trichoderma can be seen to reduce the transfer of Zn to the shoot as it showed highest concentration (4.42 mg/kg) when compared with meta-only and other treatments. This supports the findings of Anahid et al, (2011) who showed fungi have metal – specific biosorption preferences based on their cell wall chemistry and exudates. These findings also indicate that fungi accumulated heavy metals differently, meaning they vary in their metal uptake depending on the metal and the binding capacity of the fungi. But all of them can effectively aid in bioremediation of heavy metals contaminants.

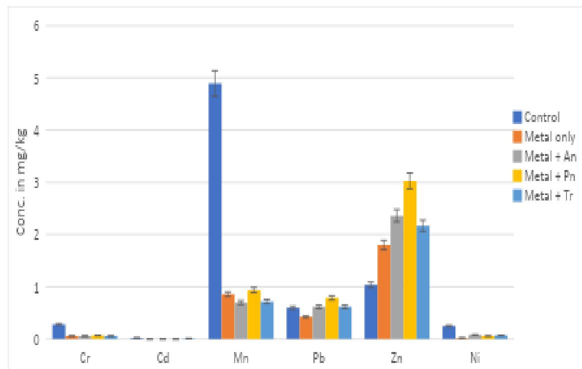

Fig. 2. Metal concentrations in roots of *Amaranthus caudatus* grown in Tsamawa irrigation soil.

Above is the graphical representation of Table 3 indicating metal accumulation variation in roots of *A. caudatus.*

Table 5: ANOVA for Metal concentrations in roots of *Amaranthus caudatus* grown in Tsamawa irrigation soil.

Sources	Df		Sum sq	Mean sq	F-value	Pr(>F)
TR	4		12.66	3.165	2.749	0.0569
MC	5		20.08	4.016	3.488	0.0199
RESIDUALS		20 23.03		1.151		

From the result obtained in Table 5 it is observed that the P-value (0.0560) is greater than the typical significance level (0.05) indicating that the effect of treatments on *Amaranthus caudatus* growth may not be statistically significant. Moreover, the P-value for treatment is (0.0199) which is less than the significance level, suggesting that the effect of metals concentrations on plant growth may be statistically significant. This finding is supported by Jaishankar *et al*, (2014) who reported that metal identity strongly governs root accumulation.

Table 6 - Metal concentrations in soil after uprooting *Amianthus caudatus*

Treatment	**Concentrations in mg/Kg**					
	Cr	**Cd**	**Pb**	**Mn**	**Zn**	**Ni**
Control	0.07	0.01	0.03	0.19	0.01	0.05
Metal only	0.32	0.60	0.17	0.54	0.05	0.11
Metal + An	0.29	0.50	0.10	0.63	0.06	0.08
Metal + Pn	0.08	0.13	0.06	0.42	0.04	0.11
Metal + Tr	0.21	0.08	0.09	0.55	0.05	0.16

Table 6 above represents heavy metals concentration in soil after uprooting the plants grown under various treatments. The results when compared with metal-only treatment shows that *Penicillium* is the most effective in reducing metal concentration especially for Cr (0.08 mg/kg) and Cd (0.13 mg/kg). *Aspergillus niger* was less effective than *Penicillium* but it also reduced some metal concentrations. *Trichoderma* showed moderate effectiveness with notable reduction in Cd (0.08 mg/kg) indicating potential ability of the fungi in bioremediation of contaminated soil. This confirms fungi can reduce bioavailable metal fractions, aiding in bioremediation as reported by Tchounwou et al., (2012) & Romani et al., (2006).

Below is the graphical representation of Table 4 above showing variations in metal reducing capability of the fungal species.

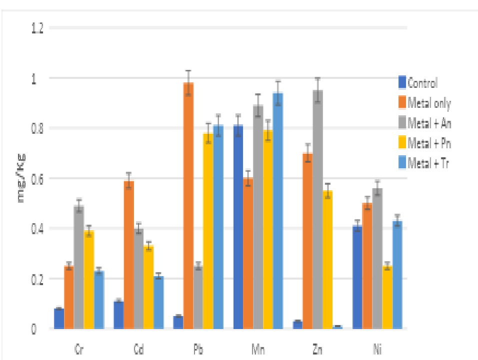

Fig. 3. Metal concentration in soil after uprooting *Amaranthus caudatus* subjected to various treatments.

Table 7. Bio-concentration factor of Amaranthus caudatus grown in Tsamawa (KISS) soil.

Treatment	**Cr**	**Cd**	**Pb**	**Mn**	**Zn**	**Ni**
Control	0.02	0.01	0.33	0.35	0.94	0.08
Metal only	0.02	0.01	0.32	0.37	0.97	0.07
Metal + An	0.02	0.01	0.35	0.36	0.99	0.09
Metal + Pn	0.18	0.01	0.36	2.90	1.28	0.14
Metal + Tr	0.03	0.01	0.39	0.67	0.00	0.10

Table (7) represents bioconcentration factor of the uprooted *Amaranthus caudatus.* In control and metal only treatments the values remain similar indicating that the presence of metal alone does not drastically change metal uptake. In metal + An treatment, the values are similar to control except for Pb (0.35) and Zn (0.99), which slightly increased. In metal + Pn: here is a significant increase in Mn uptake (2.90) and Zn uptake (1.28), suggesting an influence on the amendment on the metal's mobility or plant absorption. In metal + Tr, the uptake of Mn (0.67) and Pb (0.39) increased compared to the control, but Zn uptake dropped indicating possible inhibition of Zn absorption. Highest BCF indicates increased uptake efficiency, potentially beneficial in phytoremediation but risky for food crops (Parasakthi *et al.*, 2023). Lower BCF under *Trichoderma* aligns with safer accumulation profiles.

Below is the graphical representation of the above table showing the differences in metal translocation through the charts.

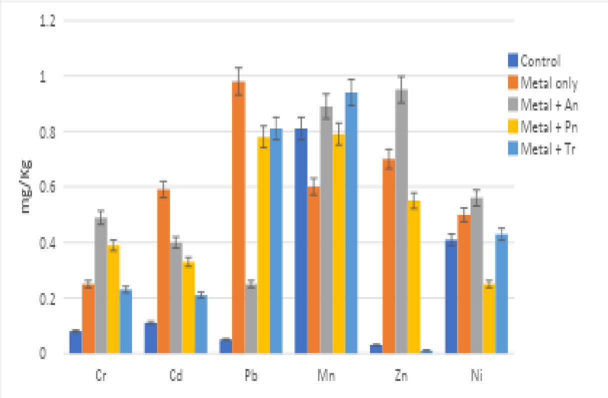

Fig 4. Bio-concentration factor in *Amaranthus caudatus* grown in Tsamawa soil.

Table 8. Transfer factor (TF) in *Amaranthus caudatus* grown in Tsamawa soil.

Treatment	Cr	Cd	Pb	Mn	Zn	Ni
Control	0.08	0.11	0.05	0.81	0.03	0.41
Metal only	0.25	0.59	0.98	0.60	0.70	0.50
Metal + An	0.49	0.40	0.25	0.89	0.95	0.56
Metal + Pn	0.39	0.33	0.78	0.79	0.55	0.25
Metal + Tr	0.23	0.21	0.81	0.94	0.01	0.43

Table (8) represents transfer factor between the treatments where *An* amendment showed further increase in TF for Zn (0.95) and Mn (0.89), suggesting enhanced uptake. Pb (0.25) is significantly reduced compared to the metal-only treatment. *Pn* amendment showed high Pb uptake (0.78) when compared with metal only treatment. Mn (0.79) and Zn (0.55) remain high but lower than metal + An treatment. There is notable Ni uptake (0.25). *Tr* amendment showed highest Mn uptake (0.94) and Cr (0.23), among the remaining amendments Pb uptake remain higher (0.81), but Zn is almost eliminated (0.01) suggesting the treatments effectively reduced Zn uptake.
Pb showed the highest bio accumulation, while Mn and Zn uptake very significantly depending on the treatment, with metal + An maximizing Zn uptake, while metal + Tr nearly eliminates it. Ni uptake is significantly reduced by metal + Pn suggesting its effectiveness in limiting Ni absorption. Transfer factor values < 1 are encouraging (Safana & Tasi'u, 2018). The reduced Zn TF with *Trichoderma* suggests it is a safe bioremediation tool where food safety is a concern.

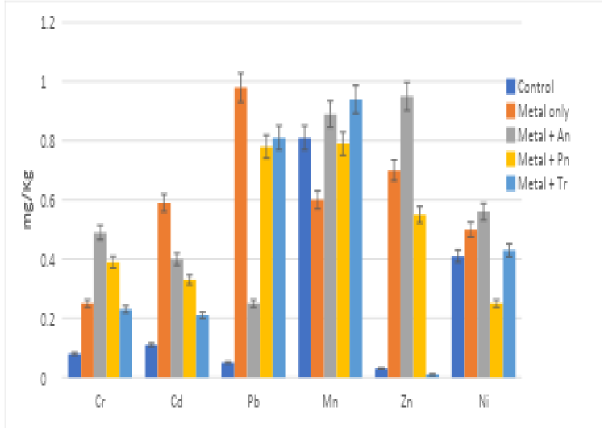

Fig. 5. Transfer factor of Amaranthus caudatus grown in Tsamawa soil.

CONCLUSION

The study provides experimental evidence supporting the beneficial role of fungi in promoting plant growth in heavy metal – polluted soil, enhancing or mitigating metal uptake depending on the fungus - metal combination and reducing residual metal concentrations in soil.
It was observed from the results that *Trichoderma* generally promoted the best plant growth, especially in Cd and Ni amended soils, while aspergillus and *penicillium* hard mixed effects the fungi also played a significant role in metal uptake *aspergillus niger* enhanced the accumulation of Cr, Pb and Ni in roots, while *penicillium* facilitated Cd and Mn uptake, and *Trichoderma* accumulated Zn. This result suggest that fungi can reduce the bioavailability of heavy metals to plant while also supporting plant growth. *Trichoderma* can be prioritized in bioremediation effort because it showed highest influence in plant and metal uptake *Aspergillus* and *penicillium* should be applied selectively as they exhibit varying metal uptake capacity. Additional research is also needed to focus on optimizing fungal concentration.
It is therefore recommended that regular soil and plant monitoring should be conducted in contaminated areas to assess heavy metal level; Fungal treatment could also be combined with hyper accumulator plants to further improve heavy metal removal from the soil.

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