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Biosorption and Mitigation of Cd and Pb Polluted Soil Using *Abelmoschus esculentus* Grown with Rhizosphere Fungi Isolated from Sewage Treatment Plant

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The study was conceived and designed by ANA, EEO, CIC, AEN, CNO, FAA. The study was performed by ANA, EEO, CIC, AEN, CNO. The laboratory analyses by ANA, EEO, CIC, AEN, CNO. Statistical design and data analyses by ANA, FAA, CNO. Writing, editing and approval of manuscript for publication ANA, EEO, CIC, AEN, CNO, FAA.

Key words
Heavy metals, Sewage soil, bioremediation, *Abelmoschus esculentus*, *Aspergillus niger*, *Aspergillus flavus*

ABSTRACT

Microorganisms are metal cleanup technique to restore polluted soil. Hence, this study investigated the bioremediation role of fungi isolated from sewage treatment plant on the growth of *Abelmoschus esculentus* cultivated with sewage rhizosphere soil (As per using the total shoot length of *Abelmoschus esculentus*). *Aspergillus niger, Aspergillus flavus, Talaromyces purpureogenus* and *Curvularia coatesiae* were isolated and screened for heavy metals biosorption by inoculating 5 ml of fungi culture in 45 ml of potato dextrose broth having metal stock solutions. Fungi DNA were extracted and PCR amplification of all the isolates were performed. Reconstructed phylogenetic tree based on rRNA-ITS sequence showed that *T. purpureogenus* and *C. coatesiae* were of the same ancestral line. *A. niger* and *A. flavus* showed higher metals biosorption rate and *T. purpureogenus* was the least. *C. coatesiae* had the highest germination rate, fungal isolates significantly influenced the *Abelmoschus esculentus* vegetation growth rate in cadmium (Cd) and lead (Pb) contaminated soils and Pb had more effect on okra growth. *T. purpureogenus,* followed by *A. flavus* exhibited highest metal uptake, indicating their role in heavy metal accumulation. Interestingly, vegetated soil had metal uptake compared to non-vegetated soil which shows the significance of phytoremediation in synergy with fungal bioremediation.

INTRODUCTION

Most of agricultural practices are chiefly based on the need to increase food production or achieve food security in the field. Moreover, most farmers are often unconcerned with food safety, environmental advantages or risks, preferring to maximize yields and profits. On the other hand, some of the agricultural practices incorporate sewage irrigation in soil amendment to boost *Abelmoschus esculentus* vegetative growth rate and increase yield. However, long-term irrigation is a major source of farmland contamination and can significantly lead in substantial elevation of environmental metal contaminants absorbed by crops in the farms (Sankhla *et al*., 2016; Bedoya-Perales *et al*., 2023).

Then, pollution of agricultural soil with heavy metals has become a major environmental problem worldwide due to the current uncontrolled man-made sources of pollution in the farming sectors observed in the intensive applications of organic fertilizers, agrochemicals and sewage irrigations (Sankhla *et al*., 2016; Liu *et al*., 2020; Kalyabina *et al*., 2021). Other noted sources of the metal contaminants come from increased eruptions, emissions and discharges resulting from development processes, industrialization and urbanization (Raju *et al*., 2013; Prajapati and Meravi, 2014). Most of the heavy metals freely exists in nature and are required at safe levels for plant developmental activities

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(Beyersmann and Hartwig, 2018). However, when the safe levels are exceeded, the toxins accumulate in the food chain, therein impairing proper development or normal functioning of living systems and at long run endanger nature with adverse health consequences like diseases and disabilities (Wuana and Okieimen, 2014; Masindi and Muedi, 2018; Rahman and Singh, 2019). Therefore, heavy metals are non-biodegradable, toxic, persistent in nature and based on these, removal of these heavy metals from contaminated soil is of paramount importance. Moreover, exposure of crops and vegetables to continuous irrigation especially where there is poor sound sewage system may be a threat to food safety. In such a context, it is essential to understand the remediation role of sewage rhizosphere soil.

Microorganisms operate as biofertilizers which play crucial role in improving and maintaining soil fertility and plant growth (El-Shahir *et al*., 2021). Also, microorganisms (fungi and bacteria) even in combination, are eco-beneficial methods to remediate heavy metal polluted soils. They use their properties to dissolve contaminants in two mechanisms: active as in bioaccumulation, and passive as in adsorption (Kumar *et al*., 2018). Then, they can transform hazardous pollutants into less toxic inorganic salts, microbial biomass, carbon dioxide and water as well as other products by speeding up natural metabolic processes that result in these effects (Mohammadi-Sichani et al., 2019). Bioremediation of heavy metals can be achieved through biosorption, bioaccumulation, biotransformation, biomineralization and mycoremediation (fungi-mediated bioremediation). In this view, the investigation was to analyze heavy metals biosorption potentials of fungi isolates from sewage treatment plant. The focused of our investigation is on okra (*Abelmoschus esculentus*) because it is indispensable in the local soup diet and a popular annual garden crop, cultivated mostly within and outside season with different sources of irrigation water in Nsukka.

Okra is a multipurpose crop, used as vegetable and commonly consumed for its green delicate fruits, which contain appreciable amounts of oil, protein, calcium, potassium and other minerals (Kumar et al., 2018). It has been shown to have an outstanding nutritional quality. Okra seed oil is rich in unsaturated fatty acids like linoleic acid, which is necessary for human nutrition. The developed fruit and stems of this plant yield crude fiber, which is utilized in the paper industry (Kumar et al., 2018). Interestingly, *A. esculentus* species is known widely cultivated crop for its palatability.

The aims of this study are (i) to assess the biosorption potentials of fungi isolates from sewage treatment plant; (ii) to determine the influence of fungal inoculation on the germination rate of *A. esculentus* (i.e. okra seeds); to see whether fungal isolates significantly influenced *A. esculentus* vegetative growth in Cd and Pb contaminated soils; (iii) to evaluate heavy metal remediation efficacies of the fungi isolates and, and (iv) to investigate the synergy of vegetated and non-vegetated soil metal uptake in metal contaminated sewage soil.

MATERIALS AND METHODS

Study area

The fungal isolation, purification, and biosorption screening test were performed at the National Biotechnology Development Agency (NABDA), South East Zonal Biotechnology Centre, Central Laboratory, University of Nigeria, Nsukka. Molecular identification of the fungal isolates was performed at the Bioinformatics Services Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, while field studies were carried out at the New Botanic Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka (UNN), Enugu State, located between longitude 7.39 ° E and latitude 6.86 ° N with an elevation of 456 m above sea level. The climate of Enugu is tropical with two major seasons, rainy/ wet (April–October) and dry (November-March) seasons (Asadu *et al*., 2018). The annual rainfall of Nsukka ranges from 1845 mm to 2000 mm, whereas during the dry season period (December-March), the surrounding temperature is largely influenced by harmattan (the cool dry wind that blows through the Sahara region) (Oparaku *et al*., 2021).

Procurement of materials

Seeds of early maturing Clemenson Spineless *A. esculentus* (Okra) *var.* 'Maha F1' used for this study were sourced from Adani-Ojo Ogurugu Agro Centre, Uzouwani, Enugu State. Ten kilograms of sewage soil, weighed into perforated experimental bags and displayed in a complete randomised design served as the medium for sowing seeds. Each of the soil was added-up with 10 ppm concentration of Cd and Pb respectively and were mixed thoroughly except in the control. It was allowed for 48 h before sowing of okra seeds for stabilization of the heavy metals. Test for seed viability was done via the flotation method prior to sowing. Meanwhile, rhizosphere soil samples were collected around different parts of the sewage treatment plant site (imhoff tank, oxidation tank and barricade chamber) located at the northeast corner of the UNN into a sterile airtight polyethylene bags following Oyeyiola (2009) method. Soil physicochemical properties analyses were performed before planting and after planting, at okra maturity at the Department of Soil Science, UNN through visible, near-infrared diffuse reflectance spectroscopy

and PLSR modeling procedures described by Vibhute *et al*. (2018). Soil texture, water holding capacity, amount of organic matter content, moisture content and pH of soil samples were the components of the soil attributes determined in this study.

Estimation of rhizosphere fungal populations

Isolation of fungi used dilution plate method from the rhizosphere soil (Eze and Amadi, 2014). Ten grams of the rhizosphere soil was weighed into a sterile test tube containing 1000 ml of distilled water and shaken vigorously. Five serial dilutions (1:100, 1:1000, 1:10000, 1:100000 and 1:1000000) were produced from the stock solution according to Onaebi *et al*. (2023). Then, 0.1 ml aliquots of 1:10000 dilution of the inoculum were spread and plated on potato dextrose agar (PDA) medium (Difco, Sparks, MD, USA). The inoculated media was incubated at 30 °C for 5-7 days. Colonies obtained after incubation were sub-cultured repeatedly to obtain pure cultures. The isolated pure cultures were stored in agar slants at 4 °C for further characterization and identification (Dilli *et al*., 2018).

DNA extraction and PCR procedure

DNA extraction, sequencing and basic local alignment search tool (BLAST) were performed at IITA, Ibadan using the method described by Srideepthi *et al*. (2017). Genomic DNA extraction was performed using fungal/bacterial DNA miniprep (Manufactured by Zymo Research). Then, for polymerase chain reaction (PCR) amplification, internal transcribed spacer (ITS) region of the universal *18S rRNA* gene was used for characterization of fungi. Also, the ITS universal primer set which is made up of ITS 1 (forward primer) and ITS 4 (reverse primer) regions were used. The nucleotide sequence for the ITS 1 primer is 5' TCC GTA GGT GAA CCT GCG G 3' and that of the ITS 4 is 5' TCC TCC GCT TAT TGA CAT GS 3'. PCR for the amplified fragments was performed using an Applied Biosystems Genetic Analyzer 3130xl sequencer and the BigDye terminator v3.1 cycle sequencing kit, according to the manufacturer's instructions. All genomic analysis was performed using Bio-Edit and MEGA X software.

Screening for phylogeny

Trimming was performed on the sequences obtained from the isolates to remove any unnecessary parts, and the trimmed sequences were then compared with existing sequences in the GenBank database through a BLAST search on the NCBI website. The best matches obtained from the BLAST search were aligned, and a neighbour-joining phylogenetic tree was constructed using the maximum composite likelihood method. The evolutionary analysis was carried out using the MEGA X software (Kumar *et al*., 2018).

Preparation of metal stock solutions

The stock solution of cadmium chloride ($CdCl_2$) and lead chloride ($PbCl_2$) were prepared by dissolving 1.34 g and 1.79 g, respectively in 1000 ml of distilled water. The stock solutions were agitated for 15 min and then allowed to stand for a period of 24 h in other to obtain a complete dissolution of salt (Oyewole *et al*., 2019).

Screening of fungi isolates for biosorption of heavy metals

Four fungi isolates represented with F1, F2, F3 and F4 were randomly screened for heavy metals biosorption by inoculating 5 ml of fungi culture in 45 ml of potato dextrose broth (PDB) having 10 ppm of Cd and Pb separately. The pH solution was adjusted to the pH value of 5.5 before the different isolates were added to the solution. The conical flasks containing PDB were incubated at 28 °C for 7 days and were centrifuged at 1000 rpm for 25 min. The supernatant was digested using nitric acid of 4 ml for every metal solution sample. The concentration of metal was determined by absorption spectrophotometry (Oyewole *et al*., 2019). The percentage of biosorption was determined by Beer Lambart's law:

$$\%\ \text{biosorption} = \frac{\text{initial metal concentration} - \text{final metal concentration}}{\text{initial metal concentration}} \times 100$$

Fungal inoculation on the germination rate was measured at six different time points on days 3, 4, 5, 6, 7, and 8.

For field experiment, seeds of *Abelmoschus esculentus* were inoculated by soaking them in a 20 ml suspension of each of the fungal isolates for 24 h. The inoculated seeds were sown six seeds per planting bag and thinned down to two seedlings per bag after germination. The un-inoculated seeds served as control. Bags were initially irrigated with 200 ml of fungi suspension for two weeks then sterile distilled water for the rest of the weeks. The study lasted for ten (10) weeks after planting (WAP). Plant morphological characteristics were determined following the methods of Nwadinigwe and Olawole (2010). Fresh and dry weight of pods, shoot and root were obtained with the aid of Golden-Mettler U.S.A electronic compact scale and recorded in gram, dried in an oven for 24 h at 60 degrees and weighed again.

Statistical analysis

Analysis of variance (ANOVA) was carried out using Statistical Product and Service Solution (SPSS) version 23.0 (SPSS Inc., Chicago, IL, USA) and tests of least significant difference (LSD) at mean values $P < 0.05$ were used to separate means.

RESULTS AND DISCUSSION

Physicochemical properties of the experimental soil

The water holding capacity, organic matter content, moisture content and pH of soil samples before planting okra seeds were 33.24 ml/g, 1.00%, 12.79% and 5.96 while at okra maturity the values were 24.00 ml/g, 1.73%, 10.18% and 6.70, respectively. Soil components before planting showed variation compared to the values after planting, at okra maturity except the soil texture class which was loamy soil (Table I). Chemical properties of soils before and after planting were optimal for growth which encouraged growth of okra. Moreover, this study found out that soil pH was slightly acidic which confirmed the earlier reports that most plants thrive best on soil that is mildly to slightly acidic (Onaebi *et al*., 2023). Previous report on variation in the soil properties showed that it can affect vegetation growth, plant form and development (Amujiri *et al*., 2018). However, this finding implies that okra can grow well in high acidic soil as was earlier reported on *T. africana* plants (Amujiri *et al*., 2018).

Molecular identification and characterization of the fungal isolates

Molecular characterization of the rhizosphere soil from UNN sewage treatment plant yielded four viable fungi colonies. The isolated fungi were identified as *Aspergillus niger* (F1), *Aspergillus flavus* (F2), *Talaromyces purpureogenus* (F3) and *Curvularia coatesiae* (F1). The pure cultures of the fungi isolates are presented in the Figure 1A. Sewage treatment plant reflects fungi potential adaptation in environments to aid in decaying fecal matter or waste, as highlighted by Iyanyi *et al*. (2021). This aligns with previous studies that emphasized on the association between fungi and the decomposition of organic matter in sewage systems and contaminated soils (Iram *et al*., 2013; Ogbuji *et al*., 2021; Lin *et al*., 2020).

PCR amplification (~600 bp) from the four isolates were subjected to automated sequencing followed by BLAST analysis to confirm the genomic sequence level (Fig. 2B). Results of the DNA Sequencing of the fungi isolates are presented in Table II.

Table I. The soil sample properties before planting and after planting.

Sample description	Texture class	OM (%)	WHC (ml/g)	MC (%)	pH value
Before planting	Loamy	1.00	33.24	12.79	5.96
After planting	Loamy	1.73	24.00	10.18	6.70

OM, Organic matter; WHC, Water holding capacity; MC, Moisture content.

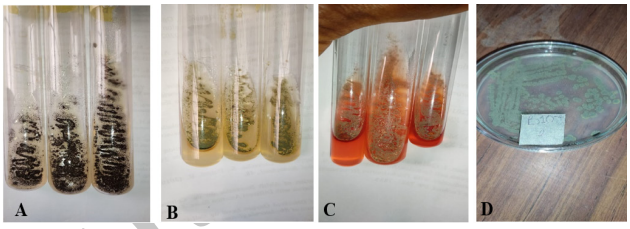

Fig. 1. Pure cultures of the fungi isolates. A, *Aspergillus niger;* B, *Aspergillus flavus;* C, *Talaromyces purpureogenus;* D, *Curvularia coatesiae*.

The reconstructed phylogenetic tree of the fungi isolates showed that the isolates were closely related as they descended from the same evolutionary lineage axis, though the generation root from which they tend to have evolved was not shown (Fig. 2B). Based on the rRNA-ITS sequence, *T. purpureogenus* and *C. coatesiae* were of the same ancestral line and were found to be more closely related than others. This phylogenetic tree implies that similar fungi unfold some development to enable species co-exist and survive. Earlier comparable terrestrial isolates species passed insignificant evolution to exist and ensure their survival (Onaebi *et al*., 2023). The strain identification of the isolates in the GeneBank showing the percentage sequence relatedness and accession number of the species are also presented in Table II.

Table II. Strain identification of the isolates using sequence homology, e value and accession number.

Isolates	% sequence homology	Strain no	E value	NCBI accession number
Aspergillus niger	90.01	ND89	6.00E-27	MG659683
Aspergillus flavus	99.03	RCBBR_AEAPL2	0	MZ798382
Talaromyces purpureogenus	96.07	113N3	7.00E-169	OP237340
Curvularia coatesiae	95.77	CPO 10.824	0	MG664783

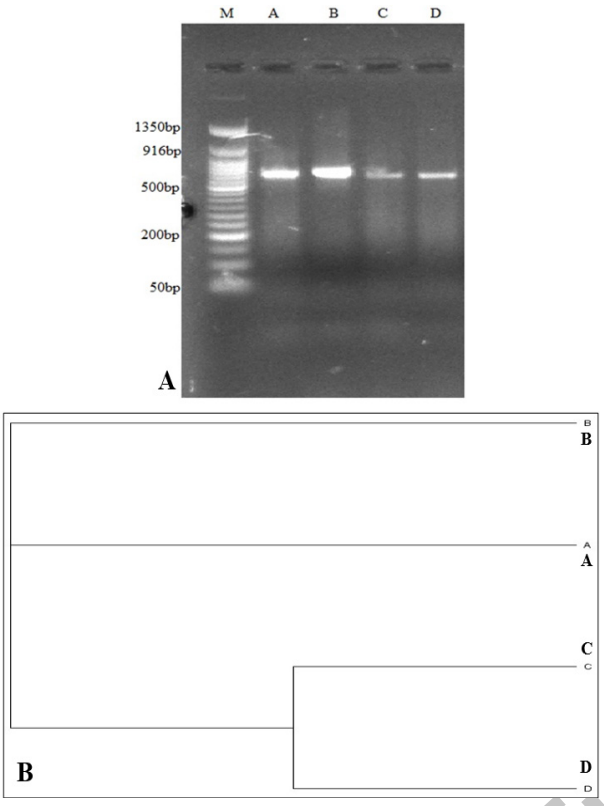

Fig. 2. A, gel image showing amplification of internal transcribed spacer gene (ITS) in the isolates. Lane M is a 50bp DNA ladder. B, Phylogenetic tree of the fungi isolates. A, *A. niger*; B, *A. flavus;* C, *T. purpureogenus*; D, *C. coatesiae.*

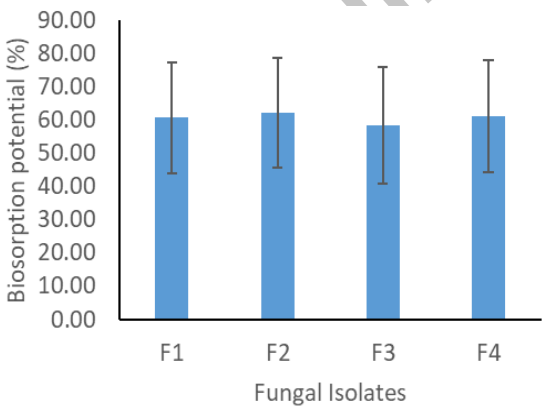

Fig. 3. Biosorption potential of the four fungi isolates.

Fungi isolates screened for biosorption potential

The biosorption potential of the isolated fungal strains reveals their capability in absorbing heavy metals. *A. niger* and *A. flavus* showed higher sorption rate followed by *C. coatesiae* while *T. purpureogenus* demonstrated the least biosorption rate, indicating its lower efficiency in removing heavy metals from the contaminated soil Figure 3. Majolagbe *et al*. (2023) reported similar trends in biosorption capacities of fungal isolates with *A. niger* and *A. flavus* exhibiting strong affinity and efficient biosorption of heavy metals, particularly cadmium and lead, as obtained in this study. Moreover, diverse studies confirmed that *Aspergillus* genus are capable of heavy metal biosorption (Akar and Tunali, 2009; Parameswari *et al*., 2010; Jena *et al*., 2012; Abioye *et al*., 2018; Liaquat *et al*., 2020). *Talaromyces* species are recognized for their resilience in challenging environments, indicating their potential usefulness in extreme conditions such as acidic and metal-contaminated soils like arsenic, copper, e.g. *Talaromyces helices* detoxified heavy metals and biacrylic compounds in biphenyl contaminated with copper (Cu) (Visagie *et al*., 2014; Hassan *et al*., 2019; Nam *et al*., 2019). Again, *Curvularia* genus exhibited significant tolerance to Pb, particularly under liquid culture conditions (Zhang *et al*., 2019) while *Curvularia coatesiae* was capable to adsorb heavy metals, specifically Cd and Pb (Di *et al*., 2022). The previous findings aligned with this present study on the adsorption potential of *C. coatesiae* for heavy metals. In terms of the heavy metals tested, the fungal isolates demonstrated a higher capacity for Pb sorption compared to Cd (Fig. 4). The higher sorption of lead by the fungal isolates could be attributed to their unique surface properties and functional groups that favour the binding and sequestration of Pb ions. This observation is consistent with the research of Majolagbe *et al*. (2023) and Iram *et al*. (2013), who also found that fungi, including *Aspergillus* species, exhibit greater affinity and uptake for lead ions.

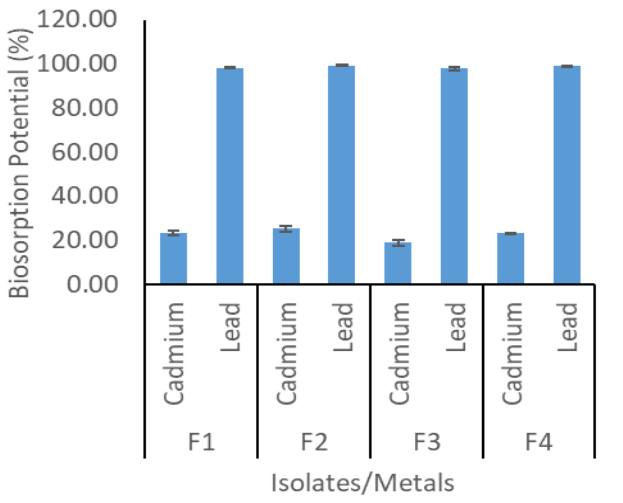

Fig. 4. Heavy metal uptake by different.

Table III. Effect of fungal inoculation on the germination rate using the total shoot of *A. esculentus* plant.

Fungal isolates	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
A. niger	1.33 ± 0.33	1.50 ± 0.22	2.17 ± 0.48	2.17 ± 0.48	2.17 ± 0.48	2.17 ± 0.48
A.flavus	2.17 ± 0.70	2.33 ± 0.61	2.50 ± 0.62	2.50 ± 0.62	2.33 ± 0.61	2.33 ± 0.61
T. purpureogenus	1.83 ± 0.48	2.00 ± 0.45	2.00 ± 0.45	2.00 ± 0.45	2.17 ± 0.40	2.17 ± 0.40
C. coatesiae	2.00 ± 0.68	2.67 ± 0.49	2.83 ± 0.48	2.83 ± 0.48	2.83 ± 0.48	2.83 ± 0.48
Control	2.84 ± 0.64	2.94 ± 0.60	2.80 ± 0.52	2.91 ± 0.55	2.80 ± 0.62	2.85 ± 0.48
LSD	NS	NS	NS	NS	NS	NS

Data are presented with mean ± standard error

Fungal inoculation on the germination rate of okra seeds

Germination rate of *A. esculentus* increased with time on all the fungi isolates, but the rate of increase varied and was not statistically significant Table III. *C. coatesiae* had the highest germination rate except in day 3 with mean values ranging from 2.67 ± 0.49 on day 4 to 2.83 ± 0.48 on day 5-8. *A. niger* and *T. purpureogenus* had lower germination rates respectively. The presence of fungal inoculation could have had an impact on the pace at which the seeds germinated, although in prior research, Pb has been demonstrated to have negative effects on seedling growth (Versieren *et al*., 2016). Therefore, it is important to take into account the possibility that the presence of heavy metals in the soil may have contributed to these findings. The effect of heavy metal exposure on the germination rate of *A. esculentus* seeds showed that lead had more effect on the growth generally when compared to cadmium (Fig. 5). This study aligns properly with the work of Li *et al.* (2005) which stated that the negative effects of heavy metals on plants are typically more pronounced during the early stages of seedling development rather than during the initial germination process.

Effect of fungal inoculation on the germination rate of *A. esculentus* seeds contaminated with two heavy metals (Cd and Pb) is presented on Table IV. For cadmium contamination, *A. flavus* had highest germination rate of 3.33 ± 0.88 on days 3 to 6, which decreased to 3.00 ± 1.00 on days 7 to 8. On the other hand, for lead contamination, germination rate of *C. coatesiae* was highest ranging from 3.00 ± 1.00 on day 3 to 3.67 ± 0.67 on day 8.

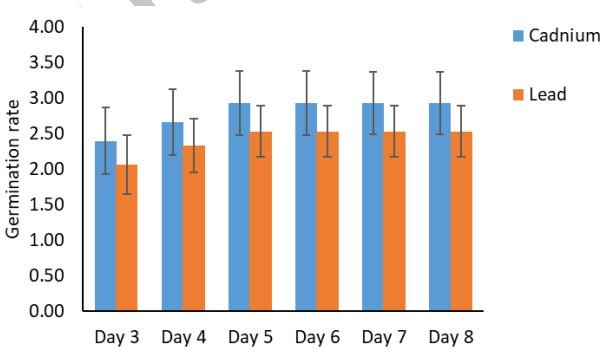

Fig. 5. Effect of heavy metal exposure on the germination rate of *A. esculentus* seeds.

Table IV. Effect of fungal inoculation on the germination rate of *A. esculentus* contaminated with heavy metal.

Fungal isolates	Heavy metals	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
A. niger	Cd	1.33 ± 0.33	1.33 ± 0.33	2.67 ± 0.88	2.67 ± 0.88	2.67 ± 0.88	2.67 ± 0.88
	Pb	1.33 ± 0.67	1.67 ± 0.33	1.67 ± 0.33	1.67 ± 0.33	1.67 ± 0.33	1.67 ± 0.33
A. flavus	Cd	3.33 ± 0.88	3.33 ± 0.88	3.33 ± 0.88	3.33 ± 0.88	3.00 ± 1.00	3.00 ± 1.00
	Pb	1.00 ± 0.58	1.33 ± 0.33	1.67 ± 0.67	1.67 ± 0.67	1.67 ± 0.67	1.67 ± 0.67
T. purpureogenus	Cd	2.00 ± 1.00	2.00 ± 1.00	2.00 ± 1.00	2.00 ± 1.00	2.33 ± 0.88	2.33 ± 0.88
	Pb	1.67 ± 0.33	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
C. coatesiae	Cd	1.00 ± 0.58	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
	Pb	3.00 ± 1.00	3.33 ± 0.88	3.67 ± 0.67	3.67 ± 0.67	3.67 ± 0.67	3.67 ± 0.67
Control	Cd	4.33 ± 1.20	4.67 ± 1.45	4.67 ± 1.45	4.67 ± 1.45	4.67 ± 1.45	4.67 ± 1.45
	Pb	3.33 ± 1.45	3.33 ± 1.45	3.67 ± 1.20	3.67 ± 1.20	3.67 ± 1.20	3.67 ± 1.20
LSD		NS	NS	NS	NS	NS	NS

Data are presented with mean ± standard error. Least significant differences of germination rate of *A. esculentus* measured in centimeters was significantly compared at $P < 0.05$.

Table V. Effect of fungal inoculation on the morphological features of *A. esculentus.*

Parameter	Treatment	Weeks				
		2	4	6	8	10
SL (m)	Control	12.78 ± 0.13	19.98 ± 0.26	24.33 ± 0.32	27.11 ± 0.44	27.22 ± 0.47
	F1	12.00 ± 0.15	18.26 ± 0.55	24.67 ± 0.43	27.50 ± 0.51	27.33 ± 0.52
	F2	11.61 ± 0.17	18.92 ± 0.33	22.78 ± 0.69	30.50 ± 0.76	30.11 ± 0.89
	F3	11.44 ± 0.16	19.59 ± 0.25	25.33 ± 0.31	28.72 ± 0.45	28.56 ± 0.44
	F4	11.50 ± 0.12	20.23 ± 0.35	24.83 ± 0.31	28.50 ± 0.46	28.33 ± 0.52
NL	Control	4.67 ± 0.06	5.78 ± 0.11	5.44 ± 0.11	4.11 ± 0.09	3.33 ± 0.11
	F1	4.89 ± 0.04	5.89 ± 0.10	4.78 ± 0.23	5.00 ± 0.17	4.44 ± 0.17
	F2	4.78 ± 0.05	6.11 ± 0.13	5.44 ± 0.14	4.67 ± 0.11	3.78 ± 0.09
	F3	5.00 ± 0.00	6.22 ± 0.09	5.44 ± 0.06	4.67 ± 0.14	3.44 ± 0.14
	F4	4.89 ± 0.04	5.89 ± 0.07	5.44 ± 0.08	4.44 ± 0.11	4.00 ± 0.12
LL (m)	Control	5.59 ± 0.04	9.33 ± 0.21	10.22 ± 0.14	10.33 ± 0.19	10.89 ± 0.23
	F1	5.63 ± 0.07	9.86 ± 0.27	10.64 ± 0.29	10.33 ± 0.31	9.72 ± 0.39
	F2	4.88 ± 0.09	8.89 ± 0.22	10.70 ± 0.24	12.61 ± 0.42	11.61 ± 0.32
	F3	5.69 ± 0.09	9.17 ± 0.13	10.37 ± 0.17	11.06 ± 0.28	10.50 ± 0.27
	F4	5.70 ± 0.08	10.00 ± 0.20	11.01 ± 0.17	11.00 ± 0.25	10.56 ± 0.22
LW (m)	Control	4.99 ± 0.06	10.17 ± 0.20	12.56 ± 0.21	14.06 ± 0.27	12.72 ± 0.36
	F1	4.88 ± 0.12	11.00 ± 0.36	13.39 ± 0.39	14.28 ± 0.44	12.94 ± 0.56
	F2	4.66 ± 0.07	10.06 ± 0.30	13.50 ± 0.32	17.11 ± 0.58	14.33 ± 0.46
	F3	5.22 ± 0.08	10.76 ± 0.17	13.69 ± 0.26	15.06 ± 0.38	13.61 ± 0.39
	F4	5.47 ± 0.06	11.44 ± 0.22	15.00 ± 0.37	15.56 ± 0.40	13.50 ± 0.36

Data are presented with mean ± standard error. Using least significant differences at $P < 0.05$; SL, Shoot length; NL, Number of leaves; LL, Leaf length; LW, Leaf width.
F1-F4, fungal isolates F1, F2, F3, F4.

Table VI. Effect of heavy metal contamination on the morphological features of *A. esculentus.*

Parameter	Heavy metal	Weeks				
		2	4	6	8	10
SL (m)	Ctrl	11.90 ± 0.10	20.27 ± 0.10	24.67 ± 0.03	29.50 ± 0.14	29.67 ± 0.14[a]
	Cd	11.90 ± 0.10	18.20 ± 0.25	23.87 ± 0.32	26.53 ± 0.42	25.33 ± 0.43[b]
	Pb	11.80 ± 0.08	19.72 ± 0.25	24.63 ± 0.32	29.37 ± 0.31	29.93 ± 0.33[a]
	LSD	NS	NS	NS	NS	0.98
NL	Ctrl	4.87 ± 0.02	5.67 ± 0.03	5.33 ± 0.03	4.33 ± 0.03	3.67 ± 0.03
	Cd	4.80 ± 0.03	6.20 ± 0.07	5.40 ± 0.07	4.33 ± 0.09	3.53 ± 0.10
	Pb	4.87 ± 0.02	6.07 ± 0.06	5.20 ± 0.12	5.07 ± 0.08	4.20 ± 0.08
	LSD	NS	NS	NS	NS	NS
LL (m)	Ctrl	5.51 ± 0.05	9.50 ± 0.11	10.43 ± 0.06	10.83 ± 0.10[b]	10.67 ± 0.10
	Cd	5.51 ± 0.05	9.05 ± 0.13	10.17 ± 0.15	9.97 ± 0.18[c]	9.73 ± 0.20
	Pb	5.47 ± 0.05	9.80 ± 0.13	11.17 ± 0.13	12.40 ± 0.21[a]	11.57 ± 0.19
	LSD	NS	NS	NS	0.49	NS
LW (m)	Ctrl	5.00 ± 0.05	10.50 ± 0.08	14.17 ± 0.02	15.83 ± 0.07[a]	12.83 ± 0.24
	Cd	5.00 ± 0.05	10.45 ± 0.19	12.77 ± 0.26	13.33 ± 0.28[b]	12.20 ± 0.27
	Pb	5.13 ± 0.05	11.10 ± 0.17	13.95 ± 0.20	16.47 ± 0.31[a]	15.23 ± 0.22
	LSD	NS	NS	NS	0.68	NS

Data are presented with mean ± standard error. Using least significant differences at $P < 0.05$. Ctrl, control; Cd, cadmium; Pb, lead; SL, Shoot length; NL, Number of leaves; LL, Leaf length; LW, Leaf width.

Table VII. Effect of fungal inoculation on the fresh and dry weight of *A. esculentus.*

Isolates	Fresh weight (g)			Dry weight (g)		
	Pod	Root	Shoot	Pod	Root	Shoot
F1	10.14 ± 0.44^{c}	1.60 ± 0.20^{b}	8.83 ± 0.43^{c}	1.65 ± 0.13^{c}	0.65 ± 0.06^{c}	1.57 ± 0.06^{d}
F2	11.65 ± 0.75^{b}	1.87 ± 0.13^{a}	13.07 ± 1.31^{a}	1.80 ± 0.09^{a}	0.59 ± 0.02^{d}	1.94 ± 0.13^{b}
F3	10.39 ± 0.97^{c}	1.62 ± 0.10^{b}	9.14 ± 0.68^{c}	1.71 ± 0.17^{b}	0.74 ± 0.03^{a}	1.68 ± 0.07^{c}
F4	12.28 ± 0.34^{a}	1.93 ± 0.12^{a}	11.41 ± 0.83^{b}	1.85 ± 0.15^{a}	0.67 ± 0.02^{c}	2.00 ± 0.07^{a}
Ctrl	8.65 ± 0.78^{d}	1.37 ± 0.17^{c}	8.12 ± 0.57^{d}	1.55 ± 0.13^{d}	0.70 ± 0.04^{b}	1.72 ± 0.06^{c}
LSD	0.38	0.12	0.55	0.07	0.02	0.05

Data are presented with mean ± standard error. Means with different alphabet superscripts along each column represents significant differences using least significant differences at $P < 0.05$

Effect of fungal inoculation on morphological characteristics of A. esculentus *in the field*

Throughout the study, shoot length consistently exhibited variation among the different fungal isolates and were generally low compared to the control. Fungal treatments F2, and F3 generally had higher leaf numbers compared to F1, F4 and the control, though not significant. Over the course of the experiment, a noticeable trend of consistent, gradual increase in leaf length was observed from week 2 to week 8 for all fungal isolates. Also, leaf width increased over time for all treatments, including the control group with F4 exhibiting non-significant lower mean. The varying increase in most of these parameters in the presence of fungal inoculation, with majority displaying no significant differences suggest that the fungal inoculation did not have a significant effect on the morphological characteristics of *A. esculentus* (Table V). Therefore, these findings suggest that the fungi isolates used may have a role in modulating the germination and growth of *A. esculentus*. A similar trend was reported that the inoculation of *Aspergillus* species used in their experiment increased number of leaves, plant height and stem girth as well as contributed to alleviating heavy metal stress encountered by *A. esculentus*.

Results observed that both Cd and Pb showed variable increase on the morphological features of *A. esculentus* across the weeks except in weeks 8 and 10 when leaf length (9.97 ± 0.18) and shoot length (25.33 ± 0.43) in Cd treatment were significantly lower compared to the lead treatment and the control. At all weeks, the mean for Cd, Pb and control treatments indicated no significant differences which implied that the observed variations are within the range of expected random variation. Also, the variable increase in the morphological features across the weeks without a notable disparity in significant of heavy metals suggest overall growth and development of the plants (Table VI). Therefore, fungal inoculations alleviated heavy metal stress in the studied plant.

Fresh and dry weight of A. esculentus

The results observed increase in the fresh and dry weight of pods, shoots, and roots resulting from fungal inoculation compared to the control. The fresh weight of *A. esculentus* from fungi isolate F4 and F2 respectively produced the highest fresh and dry weights in almost all the plant parts. Also, fungi isolate F1 and F3 have intermediate weights while control consistently produced the lowest fresh and dry weights for all plant parts (Table VII). The increase in the fresh and dry weight resulting from fungal inoculation when compared to the control aligned with the findings of Twum-Ampofo (2008) that reported a significant increase in plant dry weight for *Gliricidia sepium* when subjected to *Arbuscular mycorrhizal* fungi treatment, in contrast to the non-mycorrhizal counterpart. Similarly, Shuab *et al*. (2014) reported that fresh weight of bulb and shoot of the onion plant increased in *Arbuscular-mycorrhizal* fungi inoculated plants compared to their respective controls.

Again, results indicate that fungal isolates had varying effects on the level of soil metal contamination. The fresh weight of pod and root, and dry weight of pod and shoot contaminated with cadmium significantly ($P < 0.05$) weighed higher than those contaminated with lead. Root contaminated with lead recorded highest dry weight than roots from and cadmium contamination and control (Table VIII). Specifically, *Talaromyces purpureogenus* demonstrated the highest uptake of heavy metals from the soil followed by *Aspergillus flavus*. This suggests that *Talaromyces* species might have potentials for efficient metal remediation in contaminated soils (Table IX). This finding showed that certain fungal species have natural affinity for heavy metal binding and can play significant role in soil remediation efforts. This agreed with studies of El-Shahir *et al*. (2021) which reported role of endophytic fungi in metal uptake and accumulation absorption, adsorption, complexation and ion exchange. The metal uptake capacity was attributed to the unique characteristics

Table VIII. Effect of heavy metal contamination on the fresh and dry weight of *A. esculentus.*

Fungi isolates	Heavy metals	Fresh weight (g)			Dry weight (g)		
		Pod	Root	Shoot	Pod	Root	Shoot
F1	Cd	10.13 ± 0.46^{cd}	1.60 ± 0.63	8.52 ± 0.78^{d}	1.49 ± 0.28^{bc}	0.45 ± 0.08^{f}	1.38 ± 0.08^{f}
	Pb	8.97 ± 0.85^{de}	1.36 ± 0.15	9.47 ± 0.48^{cd}	1.43 ± 0.11^{bc}	0.85 ± 0.02^{a}	1.60 ± 0.05^{de}
F2	Cd	9.45 ± 0.28^{d}	2.26 ± 0.22	15.19 ± 0.65^{a}	1.69 ± 0.24^{b}	0.62 ± 0.02^{d}	2.40 ± 0.22^{a}
	Pb	14.18 ± 0.99^{a}	1.49 ± 0.04	15.52 ± 1.83^{a}	1.67 ± 0.04^{b}	0.51 ± 0.01^{e}	1.66 ± 0.02^{de}
F3	Cd	12.72 ± 1.63^{ab}	1.71 ± 0.19	10.94 ± 1.03^{bc}	2.06 ± 0.04^{a}	0.83 ± 0.05^{a}	1.85 ± 0.07^{c}
	Pb	7.14 ± 0.54^{ef}	1.31 ± 0.02	7.96 ± 0.98^{de}	1.03 ± 0.03^{d}	0.77 ± 0.03^{ab}	1.45 ± 0.05^{ef}
F4	Cd	12.24 ± 0.37^{b}	2.28 ± 0.17	12.96 ± 0.54^{b}	2.24 ± 0.16^{a}	0.66 ± 0.04^{cd}	2.07 ± 0.04^{b}
	Pb	13.29 ± 0.52^{a}	1.68 ± 0.21	12.77 ± 0.72^{b}	1.28 ± 0.03^{cd}	0.72 ± 0.04^{bc}	2.18 ± 0.11^{b}
	Ctrl	11.31 ± 0.17^{bc}	1.85 ± 0.05	8.51 ± 1.02^{de}	2.03 ± 0.07^{a}	0.64 ± 0.04^{d}	1.75 ± 0.04^{cd}
LSD		1.45	NS	2.10	0.25	0.08	0.18

Data are presented with mean ± standard error. Means with different alphabet superscripts along each column represents significant differences using least significant differences at $P < 0.05$.

Table IX. Effect of fungal inoculation on the fresh and dry weight of *A. esculentus* contaminated with heavy metals.

Metals	Fresh weight (g)			Dry weight (g)		
	Pod	Root	Shoot	Pod	Root	Shoot
Cd	10.49 ± 0.58^{b}	1.76 ± 0.19^{b}	10.97 ± 0.85^{a}	1.79 ± 0.11^{b}	0.63 ± 0.04^{b}	1.91 ± 0.10^{a}
Pb	10.06 ± 0.88^{c}	1.43 ± 0.07^{c}	10.87 ± 0.86^{a}	1.31 ± 0.06^{c}	0.74 ± 0.03^{a}	1.69 ± 0.07^{c}
Control	11.31 ± 0.06^{a}	1.85 ± 0.02^{a}	8.51 ± 0.39^{b}	2.03 ± 0.03^{a}	0.64 ± 0.01^{b}	1.75 ± 0.01^{b}
LSD	0.23	0.07	0.33	0.04	0.01	0.03

Data are presented with mean ± standard error. Means with different alphabet superscripts along each column represents significant differences using least significant differences at $P < 0.05$

of fungi, including their extensive mycelial networks and secretion of metal-binding compounds (Vaishaly *et al*., 2015; Dusengemungu *et al*., 2022).

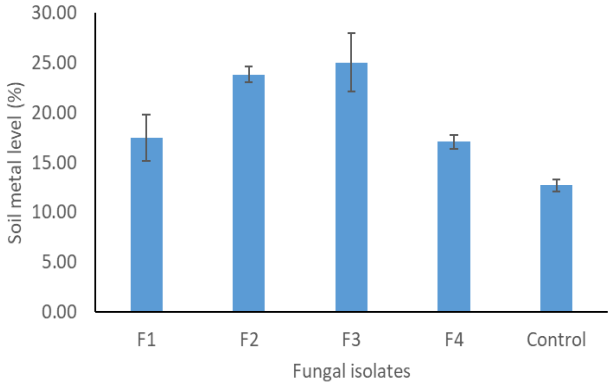

Fig. 6. The effect fungal isolates on soil metal level.

Soil metal content

Effect of fungal isolates on the soil metal level showed that isolate F3 had higher heavy metal uptake from the soil followed by isolate F2 (Fig. 6) and Cd was found to be higher than Pb in the soil (Fig. 7). The discrepancy in metal accumulation between Cd and Pb can be attributed to their varying physicochemical properties and availability in the soil. Cd is known to have higher mobility and bioavailability compared to lead, leading to its higher accumulation in the soil (Yang *et al*., 2019).

In general, the vegetated soil had significant metal uptake than the non-vegetated soil (Fig. 8). The significant differences in their metal uptake can be attributed to the presence of plant roots and associated mycorrhizal fungi. The roots provide a physical pathway for metal uptake, and mycorrhizal fungi facilitate the uptake process by extending the root system and increasing nutrient absorption (Putra *et al*., 2022). This finding aligns with the concept of bioremediation, where plants and their symbiotic fungi work together to enhance metal uptake and accumulation in the soil (Yan *et al*., 2020). Comparing the effects of fungal isolates between the vegetated and non-vegetated soil conditions, generally, the vegetated soil showed a high impact of bioremediation compared to the non-vegetated soil for both Cd and Pb contamination (Table X).

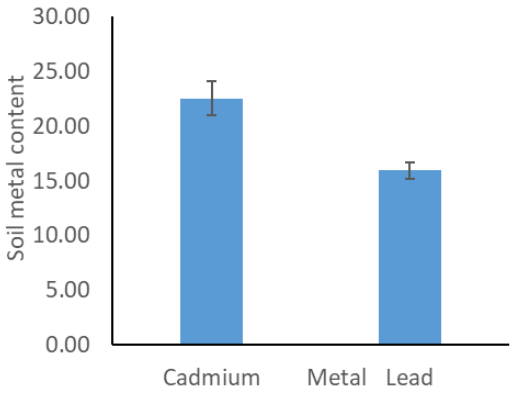

Fig. 7. The effect of heavy metals on soil metal.

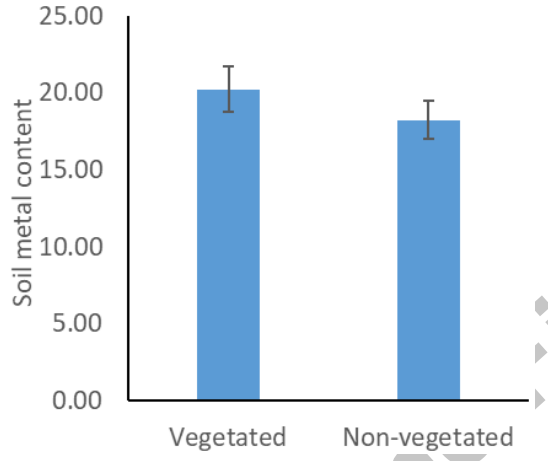

Fig. 8. The effect of soil types on content soil metal content.

Table X. Effect of fungal inoculation on the soil type contaminated with heavy metals.

Fungal isolates	Heavy metals	Vegetated	Non-vegetated
F1	Cadmium	$30.34 \pm 1.11^{b,1}$	$13.84 \pm 0.59^{de,2}$
	Lead	$14.58 \pm 1.16^{ef,1}$	$11.23 \pm 0.44^{e,2}$
F2	Cadmium	$25.15 \pm 2.96^{c,1}$	$24.77 \pm 1.14^{b,1}$
	Lead	$23.03 \pm 0.68^{c,1}$	$22.43 \pm 0.33^{b,1}$
F3	Cadmium	$34.37 \pm 5.03^{a,1}$	$33.50 \pm 2.25^{a,1}$
	Lead	$16.42 \pm 0.49^{de,1}$	$15.83 \pm 0.19^{cd,1}$
F4	Cadmium	$18.16 \pm 1.46^{d,1}$	$17.97 \pm 1.12^{c,1}$
	Lead	$17.67 \pm 0.36^{de,1}$	$14.62 \pm 1.63^{d,1}$
Control	Cadmium	$11.64 \pm 0.88^{fg,2}$	$15.55 \pm 0.88^{cd,1}$
	Lead	$10.90 \pm 0.36^{g,1}$	$12.82 \pm 0.42^{de,1}$

LSD = 3.10; Data are presented with mean ± standard error. Means with different alphabet superscripts along each column represents significant differences using least significant differences at $P < 0.05$.

CONCLUSION

The studied fungi isolates showed potential for heavy metal uptake and bioremediation in contaminated soils. *Talaromyces purpureogenus* and *Aspergillus flavus* exhibited higher levels of heavy metal uptake from the soil, with *Talaromyces purpureogenus* showing greater uptake for both Cd and Pb. Therefore, by incorporating metal-resistant fungal strains into bioremediation approaches, the detoxification and biosorption of pollutants in contaminated areas can be enhanced. This highlights the relevance of *Talaromyces* species in the context of the present study, where their biosorption potential and tolerance to heavy metals are essential considerations for the remediation of heavy metal polluted soil.

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IRB approval

This study was approved and conducted in accordance to the guidelines of the Faculty of Biological Science Institutional Review Board for research in the University of Nigeria (Ref: 2020/2024).

Ethical statement

All experimental proce-dures were performed following standard laboratory and envi-ronmental safety pro-tocols to minimize ecological risk and prevent contamination. No ethical approval was required for this study.

Credit author statement

This manuscript has not been published or presented elsewhere and is not under consideration by any other journal. All study participants provided informed consent, and the study design was approved by the appropriate ethics review boards. All authors approved the manuscript and agreed about its submission to your esteemed journal. We have no conflict of interest to declare.

Data availability

Data will be made available on request.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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