UJMR, Conference Special Issue Vol. 9 No. 3. June, 2024, pp. 475 - 484

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.2493.055

Received: 13th March, 2024 **Accepted:** 19th June, 2024

Identification of Heavy Metals Tolerant Fungi from Mining Sites at Anka Local Government Area of Zamfara State, Nigeria

***[1]Ahmad, M., [1]Magaji. A. V., [1]Salisu F. S. and [2]Albarka, H. K.**

[1]Department of Microbiology, Kaduna State University (KASU), Kaduna, Nigeria

[2]Shehu Idris Institute of Science and Technology, Kaduna State University (KASU), Kaduna, Nigeria

*Correspondence author: muazukargi44@gmail.com,

Abstract

The indiscriminate release of heavy metals into the soil is a major health concern worldwide, as most of these heavy metals cannot be broken down into non-toxic forms. Soil samples were collected from mining and non-mining sites (as control) at Anka Local Government Area of Zamfara State, Nigeria. Soil samples analyzed had a pH ranging from 6.17 to 6.65, and the moisture content ranged from 1.8939 to 9.995, Carbon, Potassium, Phosphorus, Nitrogen, Vanadium Chromium, Manganese, Iron, Cobalt, Nickel, Copper Zinc, Stannum were detected in the soil samples contaminated with heavy metals. Iron (Fe), arsenic (Ar), and chromium (Cr) tolerance levels of the fungi isolated from mine site soil were investigated in this study. The highest fungal occurrence was Aspergillus niger with 38%; it was followed by Rhizophus sp and Penicillium sp with 24% and 11%, respectively; and lastly followed by Aspergillus fumigatus, Aspergillus flavus, and Fusarium sp all with 9% frequency of occurrence. The tolerance index (TI) of A. niger, Fusarium sp, and A. fumigatus were tested against Cr, Ar, and Fe at 50,100 and 200 parts per million (ppm). It was discovered that A. niger has TI at 50, 100, and 200 ppm, of 0.95 (high tolerance/HT), 0.87 (HT), and 0.82 (HT) respectively against Fe; against Ar was 0.85 (HT), 0.69 (medium tolerance/MT), and 0.54 (low tolerance/LT), respectively; and against Cr was 0.47 (LT), 0.39 (very low tolerance/VTL), and 0.34 (VLT). The findings demonstrated that A. fumigatus had TIs of 0.77 (MT), 0.71 (MT), and 0.66 (MT) against Fe at 50, 100, and 200 ppm, respectively; 0.93 (HT), 0.88 (HT), and 0.83 (HT) against Ar at 50, 100, and 200 ppm, respectively; and 0.95 (HT), 0.87 (HT), and 0.82 (HT) at 50, 100, and 200 ppm, respectively, against Cr. Specifically, the TI values for Fusarium sp against Fe, Ar, and Cr were determined to be 0.96 (HT), 0.85 (HT), and 0.48 (LT), respectively; likewise, the TI values for Fusarium sp against Ar and Cr were found to be 0.93 (HT), 0.91 (HT), and 0.84 (HT), at 50, 100, and 200 ppm, and 0.94 (HT), 0.90 (HT), and 0.86 (HT) at 50, 100, and 200 ppm, respectively. The findings of the study indicated that the isolates were found to be tolerant against Fe, Ar, and Cr (with A. fumigatus displaying the highest tolerance) and, therefore, could be potential candidates for the bioremediation of heavy metal-contaminated soil.

Keywords: Bioremediation, Fungi, Tolerance, Heavy metals, Contamination, Tolerance index (TI)

INTRODUCTION

The expansion of industries has resulted in a notable rise in the release of industrial waste into the environment, primarily into soil and water. This has caused a build-up of heavy metals, particularly in urban areas (Oladipo *et al.*, 2023). The widespread problem of heavy metal contamination in soil has significant effects on ecosystems and human health. Industrial activities, mining, improper waste disposal, and agricultural practices have led to the accumulation of heavy metals, such as lead (Pb), cadmium (Cd), arsenic (Ar), and chromium (Cr), thereby affecting the diversity of fungi in the soils worldwide (Kumar *et al.*, 2021). These contaminants persist in the environment, posing significant threats due to their toxic nature and potential for biomagnification in the food chain (Kumar *et al.*, 2021).

Given that the majority of heavy metals have long-lasting effects on ecosystems and human health and cannot be converted into non-toxic forms, their indiscriminate release into soil is a serious global health concern. These heavy metals are not only cytotoxic but also mutagenic and carcinogenic (Usman *et al.*, 2023). Heavy

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

metal pollution is a significant concern in many countries where industrial effluents comprise toxic metal ions (Oladipo *et al.*, 2023).

The first recyclers in nature, microorganisms transform harmful organic chemicals into innocuous by-products, most frequently carbon dioxide and water. According to Boregowda *et al.* (2022), research is leading to the development of a workable strategy to quicken the process of decay and removal of pollutants by promoting the microbial and associated biota (flora and fauna) in the ecosystem to break down, accumulate, or eliminate these pollutants from the environment by converting harmful substances into less toxic or non-toxic compounds.

Fungi are thought to be the best options for bioremediation out of all the bioleaching microbes because of their greater surface-to-volume ratio and high tolerance to heavy metals. Using an indigenous fungal strain has the primary benefit of allowing the fungal strain to adapt to both the site's environmental conditions and the presence of contaminants (Boregowda et al., 2022). As a result, new approaches to using local fungal strains to remove heavy metals from contaminated soil are required (Abdel-Wareth, 2023). The main objective of this research was to ascertain the heavy metal tolerance level of fungi isolated from mining sites.

MATERIALS AND METHODS

Study Area and Sample Collection

Soil samples were collected from mining sites and non-mining sites (as control) at Anka local government area of Zamfara State. Anka is a Local Government Area in Zamfara State, Nigeria. Its headquarters is in the town of Anka at 12°06′30″N 5°56′00″E. Four samples were collected as follows: three (3) as experimental samples from the mining site and one (1) sample as control from non-mining sites in the same community. Samples were placed in a clean polyethylene bag labeled appropriately and transported to the Microbiology Laboratory at Kaduna State University, Nigeria.

Determination of the physicochemical parameters of the soil sample

pH determination

A 250 mL beaker was filled with 20 g of soil sample, and 100 cm^3 of distilled water was added. To guarantee that all of the soluble components were effectively dispersed and dissolved, the mixture was agitated for an hour at regular intervals. The neutral range of a buffer solution was used to calibrate the pH meter. A pH meter was used to record the pH. Following each use, distilled water was used to rinse the electrodes (Ajai *et al.*, 2016).

Moisture determination

A porcelain dish was used to weigh 10 g of the material. For two hours, it was kept in a hot air oven set at 105°C to remove all of the moisture. The soil's moisture content is the weight difference. Using the formula, the amount of moisture in the soil was determined (Chukwuemeka *et al.*, 2017):

$$\text{Moisture content} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}}$$

Heavy metal determination

The AOAC (2005) method was employed to determine the presence of heavy metals. Ten millilitres (ml) of concentrated HNO_3 were added to a beaker containing 1 g of air-dried soil. After the mixture was heated, it evaporated until it was dry. After dissolving the residue in 25 milliliters of HCl, the residue was heated in open air for 15 minutes. After being moved into a 100 ml volumetric flask, the sample was diluted with distilled water to volume. Prior to examination, the sample underwent filtration to remove suspended particle matter. The atomic absorption spectrophotometer was used to precisely analyse the filtrates for heavy metals using the designated wavelengths for each metal.

Isolation and identification of fungi

Standard techniques for isolating fungi from the contaminated soil samples were used, involving serial dilution and plating on potato dextrose agar media (PDA). Dilutions were made by mixing 1g of the soil sample in 9mL of sterile distilled water for 10 min. 1mL suspension was

diluted up to 10^{-5}, after which 0.1 mL from 10^{-2} to 10^{-5} dilutions were inoculated onto the surface of the PDA medium and incubated aerobically at room temperature for five days. The isolates were sub-cultured on fresh PDA plates and incubated aerobically at room temperature for five days to obtain pure culture (Suleiman *et al.*, 2023). After incubation, distinct colonies were morphologically identified, as described by Hays *et al.* (2019).

The slide culture method was utilised for microscopic characterisation. Sterile cotton wool was placed in a sterile petri dish, and then 2 millilitres of sterile distilled water were poured into the dish. The sterile glass slide was placed on the cotton wool. A little (1 × 1 cm) piece of potato dextrose agar (PDA) agar block was cut using a sterile knife and put in the middle of the slide. A tiny pinch of the fungi was inoculated at the edge of the agar block using a sterile inoculation needle and the plate was then covered. After, the plate was incubated aerobically at room temperature for 3 days. The slides were observed under the microscope at x10 and x40 objectives (Agu *et al.*, 2021).

Heavy Metal Tolerance Test

The tolerance levels of some selected fungal isolates were determined using the method described by Valix *et al.* (2001) in 250 mL conical flasks containing 50ppm, 100ppm, and 200ppm concentrations of chromium, arsenic, and iron separately. About 50 mL of potato dextrose broth was added. After that, the inoculum of the fungal isolates was introduced to each flask. Each flask was incubated for seven (7) days at room temperature. The tolerance index was determined using the formula;

$$T.I. = \frac{\text{Radial growth rate of the test fungal in control medium}}{\text{Radial growth rate of test fungal in metal medium}}$$

Fungi heavy metal tolerance levels were determined by adopting the method described by Oladipo *et al.* (2018) as 0.00– 0.39 (very low tolerance), 0.40–0.59 (low tolerance), 0.60–0.79 (moderate tolerance), 0.80–0.99 (high tolerance) and 1.00–>1.00 (very high tolerance).

Statistical Analysis

Data on physiochemical and heavy metals analysis were subjected to a One-Sample t-test analysis on GraphPad Prism version 10.2.3 at 95% confidence interval. Physiochemical parameters and heavy metal content with two-tailed significance (p) value ≤0.05 were considered significantly different and did not occur by chance. pH, moisture, carbon, vanadium, chromium, and cobalt were significant, while the rest of the parameters were not significant.

RESULTS

Physicochemical Parameters of the Soil

Table 1 presents the physicochemical composition of the soil sample obtained from the mining site contaminated with heavy metal and the control sample from the non-mining site. The pH values, moisture content, and elemental composition for each sample, including control, were detailed in each column. The pH values range from 6.65/highest (control) - 6.17/lowest (AZ2); the moisture content ranges from9.995/highest (AZ3) to 1.8939/lowest (AZ2); the highest carbon, potassium, and nitrogen contents were 93.02, 10.89, 10.73 (mg/kg) and no phosphorus was detected.

Heavy metals concentration found in the contaminated soil (mg/kg)

Table 2 presents the various concentration of heavy metal found in the heavy metal-contaminated soil measured in milligram/kilogram(mg/kg). The control sample had the highest Cr (0.07) and Ni (0.006) concentrations; Az1 had the highest Cu (0.443) concentration; Az2 had the highest Mn (1.926), Fe (23.362) and Co (0.098) concentrations; and Az3 had the highest of V (0.1) and Sn (0.129).

Cultural, Morphological, and Microscopic Identification of Fungal Isolate

The cultural and microscopic attributes of fungi obtained from heavy metal-contaminated soil are illustrated in Table 3. A total of eight fungal isolates, originating from four distinct genera, were obtained. The identified fungal isolates include *Aspergillus niger* with a white surface at first and then black having non-septate hyphae with smooth-walled conidiophore, *A. flavus*with yellowish green velvety surface, and Septate hyphae with club shape conidia, *A. fumigatus* powdery white to tan colonies and small, smooth-walled conidia, *Rhizopus* spp with cottony white or gray colonies and non-septate

hyphae,, *Fusarium* spp with white cottony colony and short multiple branched conidiophores., *Penicillium* spp with flat cottony white to blue-green colony and brush-like septate hyphae. *Rhizoctoizia solani* with various shades of brown with cross wall within the hyphae (dolipore septum), and *Candida albican*with white wrinkled colonies and yeast, unicellular, budding form or pseudohyphae

Frequency of Occurrence of the Fungi Isolated from Contaminated and Control Soil Samples

Figure 1 and 2 depict the proportional prevalence of the identified fungal species across heavy metal contaminated soil samples and control soil sample(not contaminated with heavy metal) respectively; *A. niger* is the most frequently isolated fungus, occurring in all replications of AZ1, AZ2, and AZ3 samples, 29 occurrences, constituting 38.2% of the total isolates and *Rhizophus* spp is the second most frequently isolated fungus, also occurring in all replications of AZ1, AZ2, and AZ3 samples with 18 occurrences, representing 23.6 % of the total isolates, *Penicillium* spp had 8 occurrences, constituting 10.3% of the total isolates, while *Fusarium* spp and *A.fumigatus* both had 7 occurrences, constituting 9.2% of the total isolates, *R.solani* and *C.albicans* were isolated only from the control soil sample and both have a frequency of occurrence of 16.1%, and have five(5) occurrences for both isolates, *A.niger* also dominated the control soil sample with 15 occurrences and constituting 48.4% of the fungal isolates from the control sample.

Tolerance Level Assessment of Fungal isolates against some selected Heavy metals

Table 4 presents the tolerance index of fungal isolates to various concentrations (50 ppm, 100 ppm, and 200 ppm) of chromium, arsenic, and iron. *Aspergillus niger* demonstrates remarkable sensitivity across all chromium concentrations (50, 100, and 200 ppm). *A. fumigatus*: Showed high tolerance at all concentrations (50,100 and 200 ppm). *Fusarium* spp. Appears to have a high tolerance to Cr compared to all concentrations. Its growth diminishes progressively with increasing chromium concentrations, indicating lower tolerance. For arsenic tolerant, *A. niger* demonstrates high, moderate, and low tolerance at 50, 100, and 200 ppm concentrations. *A. fumigatus and Fusarium* exhibit high tolerance at all concentrations (50, 100, and 200 ppm). For iron tolerant, *A. niger* demonstrates high at 50, 100, and 200ppm concentrations. *Fusarium* spp. Demonstrated moderate tolerance at 50, 100, and 200 ppm concentrations. *A. fumigatus* showed high tolerance at 50 and 100 ppm and low tolerance at 200 ppm. Overall, *A. fumigatus* was found to be more tolerant against these metals compared to *A. niger* and *Fusarium* sp.

Table 1: Physicochemical parameters of the soil samples

Samples	pH	MC (%)	Atomic concentration(mg/kg)			
			Carbon	potassium	Phosphorus	Nitrogen
Control	6.65	9.332	89.11	10.89	0	0
AZ1	6.3	9.9502	88.07	1.2	0	10.73
AZ2	6.17	1.8939	71.48	0.91	0	4.93
AZ3	6.21	9.995	93.02	6.98	0	0
Mean±SEM	6.333±0.1093	7.793±1.972	85.42±4.767	4.995±2.411	*	3.915±2.552
t-value	57.95	3.952	17.92	2.071	*	1.534
d.f	3	3	3	3	*	3
p-values	<0.0001	0.0289	0.0004	0.1301	*	0.2225

Keys: MC=moisture content, AZ =Anka Zamfara, SEM = Standard error of mean, t = t test value; d.f =degree of freedom; p* = no value

Table 2: Various Concentration of Heavy metals found in the contaminated soil (mg/kg)

Sample code	Control	Az1	Az2	Az3	Mean±SEM	t-values	d.f	p-values
Vanadium	0.061	0.074	0.084	0.1	0.7975±0.008230	9.69	3	0.0023
Chromium	0.07	0.031	0.029	0.029	0.03975±0.01009	3.938	3	0.0292
Manganese	0.042	0.452	1.926	0.264	0.6710±0.4266	1.573	3	0.2138
Iron	2.134	20.488	23.362	13.587	14.89±4.722	3.154	3	0.0511
Cobalt	0.011	0.087	0.098	0.059	0.06375±0.01941	3.285	3	0.0462
Nickel	0.006	0.002	0	0.002	0.0025±0.001258	1.987	3	0.1411
Copper	0.053	0.443	0.054	0.118	0.1670±0.09325	1.791	3	0.1712
Zinc	0.001	0.012	0.046	0.031	0.0225±0.009987	2.253	3	0.1097
Stannum	0.034	0	0.009	0.129	0.043±0.02956	1.1455	3	0.2417

Keys: AZ =Anka Zamfara, SEM = Standard error of mean,t = t-Test value; d.f =degree of freedom

Table 3: Cultural, morphological and microscopic identification of fungal isolate

Isolate Code	Cultural Characteristics	Microscopic Characteristics	Probable Organism
A1	White surface at first and then black	Non-septate hyphae with smooth walled conidiophore	*A. niger*
A2	Yellowish green velvety surface	Septate hyphae with club shape conidia	*A. flavus*
A3	Powdery white to tan colonies	Small, smooth-walled conidia	*A. fumigatus*
R1	Cottony white or gray colonies	Non-septate hyphae	*Rhizopus spp.*
R2	Various shade of brown	Cross wall within the hyphae (dolipore septum)	*R.solani*
C1	White wrinkled colonies	Yeast, unicellular, budding form or pseudohyphae	*C. albicans*
F1	White cottony colony	Short multiple branched conidiophores	*Fusarium spp*
P1	Flat cottony white to blue green colony	Brush-like septate hyphae	*Penicillium spp*

Keys: **A1**=*Aspergillus niger*, **A2**=*A. flavus*, **A3**=*A. fumigatus*, **R1**=*Rhizopus* spp.,R2= *Rhizoctonia solani* and **C1**=*Candida albicans*, F1= *Fusarium* spp, P1= *Penicillium* spp

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

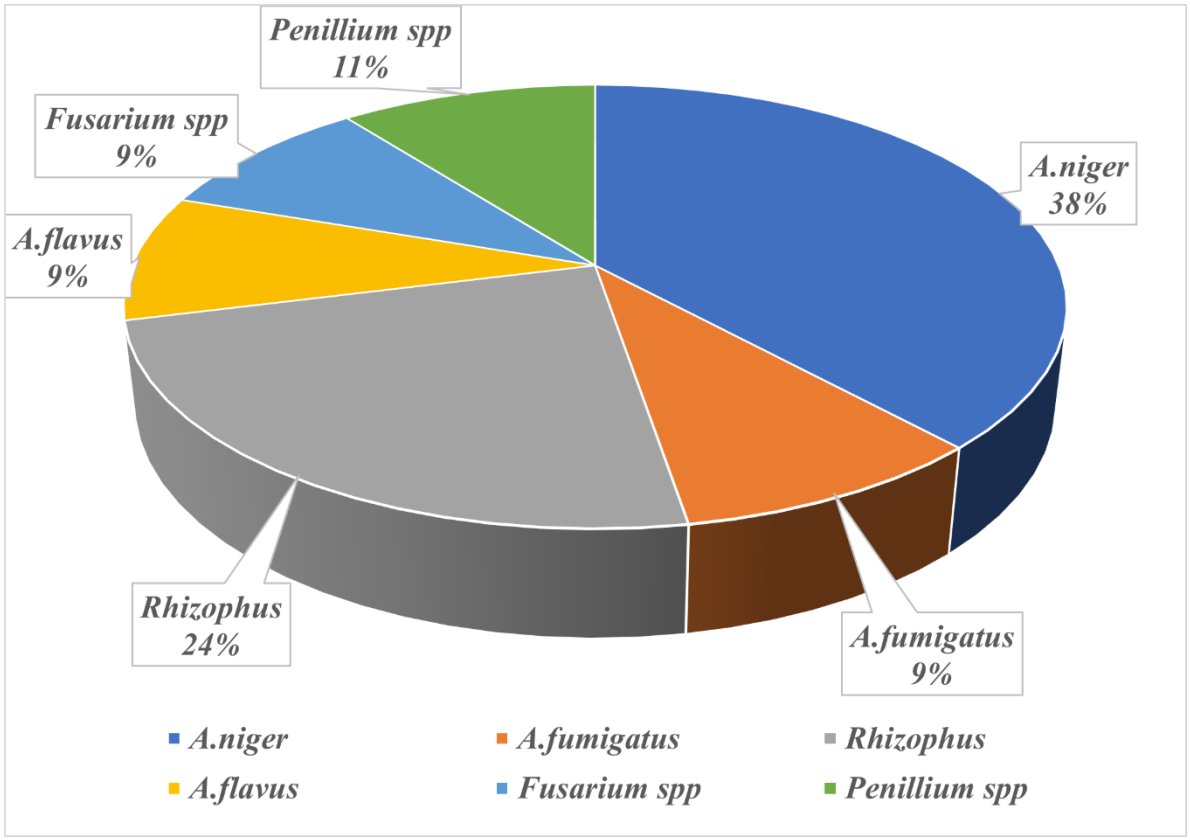

Figure 1: Frequency of occurrence of fungal isolates in contaminated soil samples

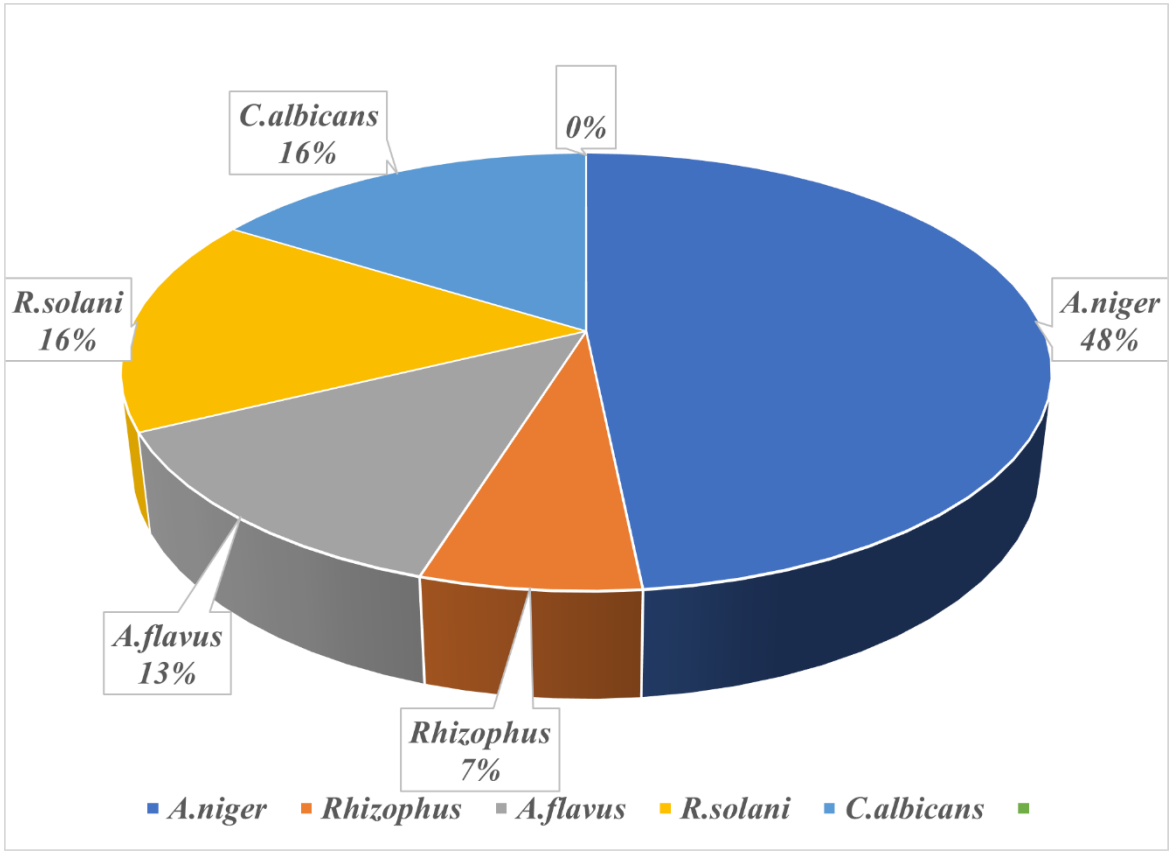

Figure 2: Frequency of occurrence of fungal isolates in control soil samples

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

Table 4: Tolerance Level Assessment of Fungal isolates against some selected Heavy metals

Fungal species	Conc(ppm)	Heavy metal Cr	Ar	Fe
A.niger	50	0.47(LT)	0.85(HT)	0.95(HT)
	100	0.39(VL)	0.69(MT)	0.87(HT)
	200	0.34(VL)	0.54(LT)	0.82(HT)
Fusarium sp	50	0.95(HT)	0.93(HT)	0.77(MT)
	100	0.87(HT)	0.88(HT)	0.71(MT)
	200	0.82(HT)	0.83(HT)	0.66(MT)
A.fumigatus	50	0.94(HT)	0.93(HT)	0.96(HT)
	100	0.90(HT)	0.91(HT)	0.85(HT)
	200	0.86(HT)	0.84(HT)	0.48(LT)

Keys= VL-very low tolerance, LT-low tolerance, ML-medium tolerance, HT- high tolerance.

DISCUSSION

The composition and abundance of fungal species in soil ecosystems are shaped by the physicochemical characteristics of the soil, which are significant factors that both directly and indirectly affect fungal diversity. In the present study, the soil samples analyzed had a pH value ranging from 6.17 to 6.65. This implies that the soils were slightly acidic. The pH range found in this investigation is in line with what Khalid et al. (2017) observed, which is between 6.2 and 7.7. However, compared to 5.8 to 5.83 and 5.82 to 6.20, published by Yuanan *et al.* (2013) and Radmila *et al.* (2013), it is marginally higher. The pH of the soil influences the solubility and mobility of metals in the soil. The soil samples had a mostly sandy texture with trace amounts of silt and clay particles. The soil's tendency to be sandy may make it less able to hold on to nutrients necessary for microbial existence. The variety of fungi in the soil is influenced by the size of the soil's particles (Song *et al.*, 2017).

A total of eight fungal isolates were identified in the course of this study belonging to four distinct genera: *Aspergillus* spp., *Rhizopus* spp., *Fusarium* spp., and *Penicillium* spp. Notably, *Candida albicans* and *Rhizoctozia solani* were present only in the control soil. *Aspergillus niger* dominated the fungal community in both control and contaminated soil, indicating its adaptability to diverse environmental conditions. Soraia *et al.* (2015) reported the occurrence of *Aspergillus* sp in heavy metal-polluted soil. This versatile fungus shines due to its remarkable adaptability and tolerance to a wide range of pH and moisture levels, coupled with its efficient organic matter utilization and potential metal resistance.

Additionally, *A.niger* is known for its fast growth and competitive edge, outperforming other fungi in resource acquisition. Due to its dominance and adaptability, *A.niger* could be employed in solid-state fermentation systems for treating contaminated soil directly as reported by Usman *et al.* (2020) and Li *et al.* (2020). Its tolerance to various metals suggests its potential for degrading organic pollutants and immobilizing heavy metals through biosorption onto its mycelial cell walls (Hassan *et al.*, 2019). *Rhizopus* spp also had outstanding prevalence and the genus is known for its strong cellulase and xylanase activity, making it potentially useful for degrading organic matter in contaminated soil. This breakdown process can enhance the bioavailability of heavy metals for other degrading microbes or facilitate their immobilization through humification (Jasu *et al.*, 2021). Some *Fusarium* species produce extracellular enzymes like laccases and peroxidases with the ability to oxidize and degrade certain organic pollutants and potentially detoxify some heavy metals, as reported in a study by Shanmugapriya *et al.* (2019) and certain *Penicillium* strains produce oxalate compounds that can chelate heavy metals, reducing their mobility and toxicity in the soil (Ding *et al.*, 2013). Some *Aspergillus* and *Penicillium* species can form symbiotic relationships with plants, such as mycorrhizal fungi. These fungi can enhance plant growth and uptake of nutrients while also aiding in metal sequestration and phytoremediation through their root interactions Adeyemi *et al.*, (2022). Interestingly, heavy metal contamination did not significantly alter the overall fungal diversity compared to the control, suggesting possible tolerance or adaptation mechanisms among the isolated fungi. However, in contrast, Van Der Heyde *et*

al. (2020) emphasise the importance of considering functional diversity (enzyme activity, metabolic pathways) rather than just species richness in assessing microbial communities for bioremediation applications.

This study assessed the tolerance of some of the isolates to chromium, arsenic, and iron sulfate for representative fungal isolates. While a detailed analysis requires further investigation, preliminary results suggest differential tolerance amongst species. Some were found to be sensitive and moderately tolerant, and others were tolerant. Thus, they behaved differently against different metals. This is in line with the work of Malik (2004), who reported different tolerant levels by fungi against different heavy metals. Fungal resistance to heavy metals typically varies depending on the strain and environmental factors. For instance, *Fusarium* and *Penicillium* species have tolerances for Cr(VI) of up to 300 mg/L, according to García-Hernández *et al*. (2017). Additionally, *Aspergillus fumigatus* biomass was shown by García-Hernández *et al*. (2017) to be capable of effectively removing Cr(VI) from aqueous solutions.

A. niger demonstrated remarkable resilience across all arsenic and iron concentrations (50-200 ppm), as evidenced by its consistent growth patterns under different metal concentrations, indicating a relatively high tolerance to heavy metals. However, the reduction in growth at 50-200ppm chromium with an index of less than 0.5 may indicate a threshold beyond which the fungus experiences inhibitory effects and susceptibility to Cr; this is substantiated by Oladipu *et al*. (2018), whose report recorded significant tolerance of *A. niger* and other *Aspergillus* species to varying concentrations of heavy metals. The ability of *A.niger* to thrive in the presence of arsenic and iron implies adaptability and potential participation in metal detoxification processes. This suggests exceptional arsenic and iron tolerance, making *A. niger* a prime candidate for potential bioremediation strategies in arsenic and iron-contaminated environments (Elhamouly *et al.*, 2022).

Aspergillus fumigatus showed tremendous tolerance at all concentrations (50-200ppm) for chromium and arsenic but exhibited a decline in growth at higher levels of 200ppm for iron. This suggests partial tolerance. Although *A. fumigatus* showed some tolerance at lower metal concentrations, its sensitivity at higher levels limits its potential to thrive in heavily iron-contaminated soils (Mohapatra *et al.*, 2022).

Fusarium spp appears less tolerant and more sensitive to iron compared to the others. Its growth diminishes progressively with increasing iron concentrations, indicating lower tolerance but not as low as *A.niger's* sensitivity to chromium. This could be because chromium can disrupt multiple metabolic pathways in fungal cells, affecting energy production, enzyme activity, and DNA replication. *Fusarium* species might be more susceptible to these disruptions at higher chromium concentrations compared to other fungi with more robust or adaptable metabolic processes (Tuo *et al.*, 2023).

CONCLUSION

In conclusion, six (6) fungi were isolated; *Aspergillus niger* (38%), *Aspergillus fumigatus* (9%), *Aspergillus flavus* (9%), *Penicillium sp* (11%), *Fusarium sp* (9%), *and Rhizophus* sp (24%). Even at 200ppm concentration, *A. niger* has a high tolerance to Fe, *Fasarium,* and *A. fumigatus* has a high tolerance to Cr and Ar. From the findings of the study, these isolates were found to be tolerant against Fe, Ar, and Cr and, therefore, could be potential candidates for the bioremediation of heavy metal-contaminated soil. Notably, *A. fumigatus* showed the highest level of tolerance across all metals tested compared to *A. niger* and *Fusarium* sp. This makes it a more suitable candidate for further investigation of its potential in removing these metals.

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