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Comparative Study of Bioremediation Potential of Heavy Metal-Resistance Fungi Isolated from Mechanic Sites

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ABSTRACT

Soil polluted with heavy metals (HM) have become common across the globe due to increase in geologic and anthropogenic activities. Mycoremediation is a widely accepted and an effective method of treating heavy metal polluted soils. In this study, the effect of heavy metals on heavy metal resistance (HMR) fungi and bioremediation potential of HMR fungi isolated from two different mechanic sites, and at three different soil depth of 0cm, 15cm and 30cm were compared. The soil samples were analyzed to determine the level of heavy metal contamination and their resistance spectrum for the investigated elements was characterized by maximum tolerance concentrations (MTCs). The concentration of heavy metals used for MTC was determined based on the maximum concentration each fungal isolate could tolerate, are 10mg/L - 15mg/L for Cu^{2+} and 4.0mg/L - 4.5m/L for Zn^{2+} as quantified by atomic absorption spectrometry. On the basis of morphological characterization and biochemical analysis, the isolates belong to the genus *Aspergillus spp.*, *Penicillum spp.*, and *Saccharomyes spp*. respectively. *Penicillium spp.* has the least MTC for Zn^{2+} while *Saccharomyces spp.* showed the highest MTC for Cu^{2+}. Studies on bioremediation potential of the HMR fungi isolates in the soil samples revealed mixed fungal culture completely removed zinc and copper from MG soil samples, while in OPP soil samples, the zinc and copper concentration have a reduction effect of 82.59%. Based on the fungal isolate, *Aspergillus spp*. reduced copper in MG samples with a reduction effect of 88.98%, *Penicillium spp.* reduced zinc with a removal effect of 87.5% in MG samples, *Saccharomyces spp.* has a zinc remediation effect of 96.48% and *Penicillium spp.* has a copper removal effect of 88.26% in OPP samples respectively. These findings show that the fungi species have great bioremediation potential for highly contaminated Zn^{2+} and Cu^{2+} soils.

Keywords: bioremediation; heavy metal resistance fungi; contaminated soil

INTRODUCTION

Heavy metals are natural components of the earth crust. As trace element, some heavy metals (e.g. (Copper, Selenium and Zinc) are essential for maintaining the metabolism of the human body. The term heavy metals refer to any metallic chemical element that has a relatively high density but toxic or poisonous at low concentration (Fu and Wang, 2011). Heavy metals are a kind of natural resource used in several industrial, agricultural and domestic applications. However, conjointly they act as a pollutant, which can be very toxic to many life forms leading to various environmental issues. Unlike organic pollutant, heavy metals are non-biodegradable, exhibit toxicity even at low concentration and persistent in nature (Rose and Devi, 2015). Heavy metals at a higher concentration can be poisonous and dangerous because they bioaccumulate i.e. they increase in concentration in biological systems over time, compared to chemical concentrations in the environment. Compounds bioaccumulate in living things any time they are taken up and are stored faster than they are broken down (metabolized) or excreted (Narendra et al, 2015).

In addition, heavy metals can percolate into water supply system through industrial and consumer waste or through heavy metals released from the streams, lakes, rivers and ground waters as a result of soil decomposition by acidic rains. Heavy metal pollution occurs directly from industries (tannery, electroplating, dyeing, and mining), agricultural fields, sewage sludge and waste treatment plant.

Recent studies established that the long-term use of untreated wastewater from industrial sources can adversely affect water quality, making it unfit for human consumption (Wunna and Okieimen, 2011). Available untreated industrial wastewater is often colored, frothy and contains hazardous chemicals including heavy metals, toxic dyes, acid, alkalis and other toxic chemicals (Kaushik et. al, 2005). The resulting pollution leads to hazardous impacts on the health of occupants/ residents as well as constitutes occupational health hazards for workers (Jarup and Bull, 1998). Heavy metals such as chromium (Cr) when discharged in untreated effluents by electroplating plants have been reported to surpass permissible limits (Srisuwan and Thongai, 2002), while heavy metals like Copper (Cu), Iron (Fe), Manganese (Mn) and Zinc (Zn) are often present in tannery wastewater and groundwater supplies as heavy metals compared with stipulated standards for discharge of environmental pollutants (Devi, 2011).

However, there is a dearth of knowledge of the growth response and heavy metal tolerance of fungal species isolated from mechanical workshops sites. This study was therefore designed to isolate, identify and assess the growth response and tolerance/resistance of fungi species isolated from different mechanic workshop sites to varied concentrations of selected heavy metals associated with these sites.

MATERIALS AND METHODS

Sites Description and Soil Sampling

The geographical location investigated in this study were mechanic sites with Zinc and Copper contaminated soil located at Obafemi Owode Local Government, Abeokuta, Nigeria. These locations were selected based on their Zinc and Copper pollution densities. Soil samples were collected into a sterile polythene bag from two different mechanic sites and at different depths respectively. The sites are Oluwo Police Post (OPP) (at surface level, 15cm and 30cm) and Gbokoniyi MG mechanic site (at surface level, 15cm and 30cm) with each soil samples replicated in threes. The soil sample were taken to the laboratory for microbiological analysis. The control sample soil was obtained from Moshood Abiola Polytechnic (MAPOLY) botanical garden.

Collected soil samples were air dried, sieved with 2 mm sieve and weighed for wet digestion. The samples were oven-dried at 70^0C to constant weight, ground inside a hammer mill incorporated with 2mm sieve. Two grams of the ground samples were then placed in the crucible and ashed inside furnace at 580^0 C. The ash was washed into 100 ml volumetric flask and wet digested with a mixture of 1:1 per chloric and nitric acid respectively. The samples were placed in sterile glass bottles, kept and transported on ice (or 4^0C) for subsequent analysis. The soil samples were analyzed for the total content of Cu^{2+} and Zn^{2+} within 8 hours using the AAS (A.O.A.C, et al, 2005).

FIGURE 1: Area view of mechanic sites for sample collection.

Isolation of Fungal Strains

Fungal strains were isolated from the collected soil samples by serial dilution technique. Serial dilution was carried out separately for each of the samples using distilled water, at dilution factors 10^{-3}, 10^{-7} and 10^{-9} used, so as to reduce microbial load in samples. The procedure involved labeling series of sterile tubes with the appropriate dilution factor, arranged in a rack with each containing 9mls of distilled water. The contents were mixed thoroughly by pipetting up and down several times using a sterile syringe. 1ml of the mixture from the first tube (10^{-1}) was measured and added to the tube labeled 10^{-2}. The contents of the tube were thoroughly mixed and the entire procedure was repeated for factors 10^{-3} up till 10^{-9} until the desired dilution was obtained. Potatoes Dextrose Agar (PDA) and Sabouraud dextrose agar (SDA) were prepared according to the manufacturer's instruction. The media were heated in a water bath and autoclaved at 121^{o}C for 15 minutes. The media were cooled and 2.1g of ciprofloxacin was dispensed into the SDA while 2.1 g of Chloramphenicol was dispensed into PDA respectively.

Isolation of Heavy Metal Tolerance Fungal

The tolerance to selected bacterial strains to various heavy metals were determined by agar dilution method. Inoculum were picked from dilution $10^{-3,}$ 10^{-7}, and 10^{-9}, and were inoculated aseptically on PDA and SDA plates supplemented individually with vary amount of heavy metals. In other to select the tolerance strains, Zn^{2+} resistance strains, 4.0 mg/L and 4.5 mg/L were added into different separate agar, while 10.0 mg/L and 15.0 mg/L Cu^{2+} were added into different agar plates for tolerance fungi determination respectively. These plates were labelled according to the varying amount of metals and agar used. The plates were incubated at 27^{0}C for 10 days and were visually inspected for microbial growth daily. Several subculturing was conducted and the pure isolates were preserved in an agar slant (Oladapo et al, 2016) at 4^{o}C with subculturing every four weeks.

Identification and Characterization of Fungal Isolates

All resistant fungal isolates were initially identified by colony characteristics to the genus level on the basis of macroscopic characterization on SDA and PDA (morphology, colour, appearance and shape of the colony), microscopic characteristics (septation of mycelium, shape, diameter and texture of conidia). The method used in the identification is by staining the fungi isolates with Lactophenol cotton blue.

Maximum tolerance concentration (MTC)

The six fungi isolate were inoculated into SDA broth containing different concentrations of heavy metals. The concentration of heavy metals used was determine based on the maximum amount each of the fungi isolate can tolerate. The concentration of zinc used were 4.0 and 4.5mg/ml, for copper 10 and 15mg/ml was used. The growth of each fungi isolated was monitored with a spectrophotometer at a wavelength of 600nm using an SDA broth as a blank containing the same amount of heavy metals. (Pandit et al, 2013).

Determination of bioremediation potential of heavy metals fungi

Soil samples picked at different sites and specific depths were numerically labelled 1-39. Test tubes were sterilized in an autoclave at 121^{o}C for 15 mins.

Serial dilution was done and 1ml of each soil samples at different depth were picked from dilution factor 10^{-3}, dispensed into 9ml of SDA broth in 38 test tubes. Tube 1was not inoculated with the fungi isolate (Control), tube 2-37 was inoculated with each of *Aspergillus spp.* (20%), *Aspergillus spp.* (20%), *Aspergillus spp.* (10%), *Penicillium spp.* (20%), *Penicillium spp.* (20%), *Saccharomyces spp.* (10%), tubes 38-39 was inoculated with mixed fungal culture (MFC) containing mixed soil samples of the three depths (0cm, 15cm, 30cm) for each soil samples (MG and OPP). The tubes were kept at room temperature. The concentrations of copper and zinc were determined after 14 days of treatment with the isolates using atomic absorption spectrophotometer (Nwagu et al, 2016), the concentration was calculated using Beer Lambert equation while molar absorptivity was calculated using the standard graph.

Statistical Analysis

The data difference in the concentration of heavy metals in soil in relation to soil depth and metal site was compared using one –way ANOVA.

RESULTS

Marginal means of Cu^{2+} and Zn^{2+} concentration in the soil

Supplementary Table 1 shows that there is no direct correlation between soil depth and heavy metal concentration in the soil because at 0cm (surface level), Cu^{2+} has the lowest concentration compared to Zn^{2+} while at 15cm and 30cm depth, Zn^{2+} has the highest concentration compared to Cu^{2+}. The result therefore indicates that there is no significant effect of soil depth on metal concentration in the soil ($p > 0.05$). Supplementary Table 2 indicates that Cu^{2+} concentration in OPP soil samples is higher than that of MG soil samples, implying that OPP samples has a higher Cu^{2+} concentration than MG soil samples. However, the concentration of Zn^{2+} in MGA soil samples is higher than that of OPP soil samples, indicating a possible effect of different metal sites on Cu^{2+} and Zn^{2+} concentration in the soil ($p<0.05$). Moreover, the concentration of heavy metals detected in OPP and MG soil samples was in accordance with the permissible limits of heavy metals in soil recommended by WHO, 1996.

Table 3 and 4 explains the bioremediation effect of HMR fungi on Cu^{2+} and Zn^{2+} reduction in MG and OPP soil samples at different depth. The results indicate that *Aspergillus spp.* has the highest remediation effect (88.98%) that remediate copper from MG soil samples at 30cm, Penicillium *spp.* has the highest remediation effect (88.26%) on reducing Cu^{2+} in OPP samples and Zn^{2+} in MG soil samples (87.5%), *Saccharomyces spp.* has the highest remediation effect (96.48%) on reducing Zn^{2+} in OPP soil samples. MFC has a complete removal effect (100%) on both Cu^{2+} and Zn^{2+} from MG soil samples as well as a remediation effect of 82.59% on Zn^{2+} and Cu^{2+} removal in OPP soil samples. At 0cm and 15cm depth however, MG soil samples has the highest concentration of Zn^{2+} while OPP soil samples has low values of Zn^{2+} concentration compared to MG soil samples. At 30cm depth, OPP soil samples has Cu^{2+} concentration compared to that of MG soil samples. Therefore, there is a significant interaction effect between the soil depth and metal site on metal concentration in the soil.

TABLE 1: Correlation of soil depth to metal sites on Cu $^{2+}$ and Zn^{2+} concentration in the soil.

Soil Depth	Metal site	Cu $^{2+}$ Conc.	Zn^{2+} Conc.
0 cm	Gbokoniyi (MG) Police post (OPP)	1.379± 0.211 2.559 ± 0.611	10.24± 1.486 6.267± 0.218
15cm	Gbokoniyi (MG) Police post (OPP)	1.267 ± 0.050 2.902±1.682	10.11± 1,055 6.797± 0.239
30 cm	Gbokoniyi (MG) Police post (OPP)	1.142 ± 0.450 3.050 ± 0.980	6.169 ±4.098 7.646 ± 1.386

TABLE 2: MTC for OPP and MG Fungi Isolates.

Fungal isolate	Composition (%)	Zn^{2+}		Cu $^{2+}$	
		4.0mg/L	4.5mg/L	10mg/L	15mg/L
		(mol/L)	(mol/L)	(mol/L)	(mol/L)
Aspergillus spp.	*10*	0.91	0.41	10.11	5.11
Aspergillus spp.	20	1.01	1.51	11.4	10.9
Aspergillus spp.	20	1.54	1.04	6.78	1.78
Penicillum spp.	20	2.09	3.05	25.9	21.6
Penicillum spp.	20	1.22	0.72	10.6	5.56
Saccharomyces spp.	10	1.22	0.72	10.1	5.09

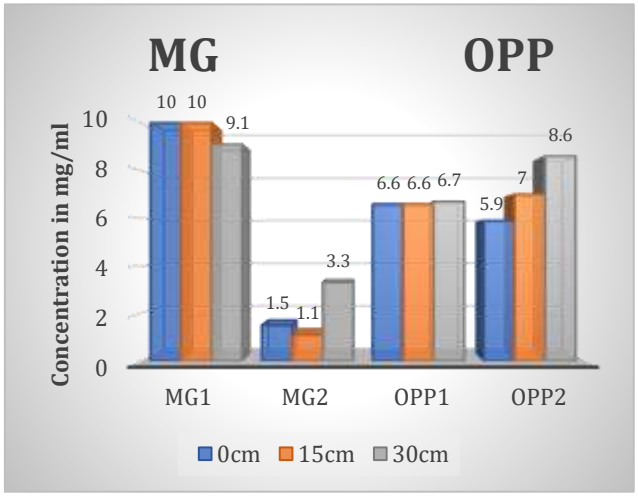

FIGURE 2A: Bar chart of correlation of soil depth and Zn^{2+} Concentration in the soil.

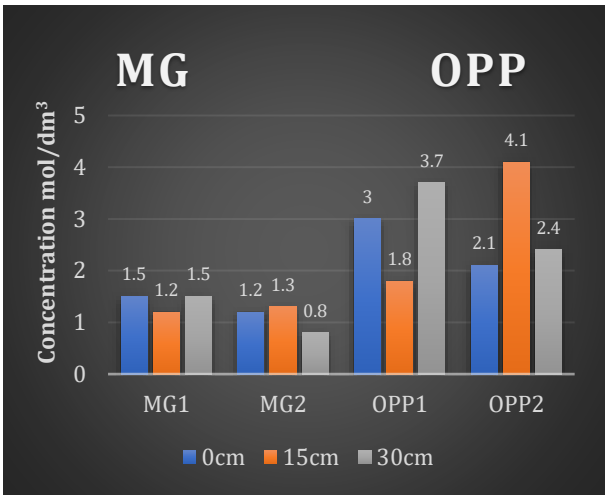

FIGURE 2B: Bar chart of correlation between soil depth and Cu^{2+} concentration.

FIGURE 2A AND B: Bar chart showing the effect of soil depths Zinc and Copper concentration in the soil (no significant difference in the concentration of heavy metals in correlation with depth, $p>0.05$).

Figure 2a explains the correlation of the depth and metal sites on Zn concentration. The figure shows that the OPP site has an almost even concentration of the metal at all depths unlike at the MGA site. Even though the concentration of Zinc is highest at the MG surface depth, it is consistently present at all depths at the OPP site. Interestingly, Figure 2b showed a higher concentration of Cu^{2+}in OPP site at 30 cm level while the concentration of the metal is generally evenly low at the MG site, and at other depths in OPP site.

Figure 3a and 3b indicated the effect of MTC on metals with respect to the fungi genus associated with each of these metals. Figure 3a is a graph that displayed the MTCs of all the fungi genus detected in this study. The output indicated similar MTCs for the detected fungi at 4.0mg/L of Zn metal concentration, while there are inconsistencies for these fungi genius at 4.5mg/L Zn^{2+} concentration. Meanwhile, Figure 3b shows outright undulation for all the detected fungi species, irrespective of the Cu^{2+} metal concentration observed in the study.

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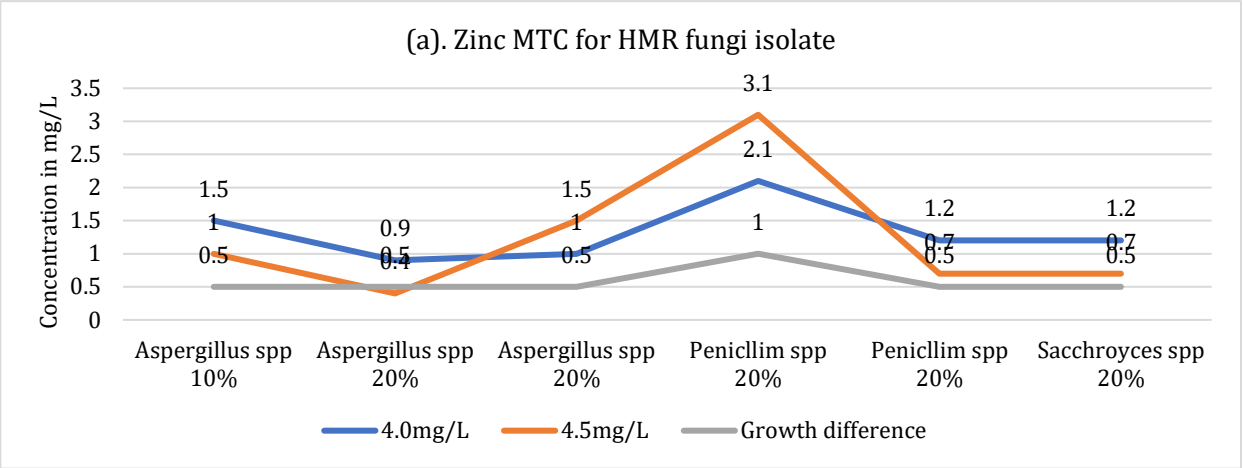


FIGURE 3A: Zinc MTC of HMR Fungi.

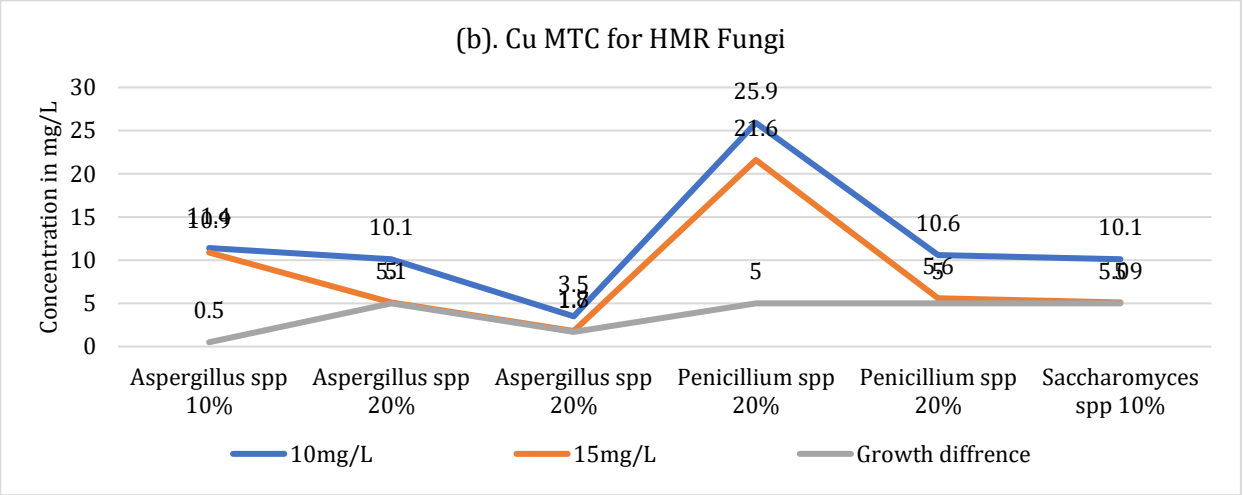


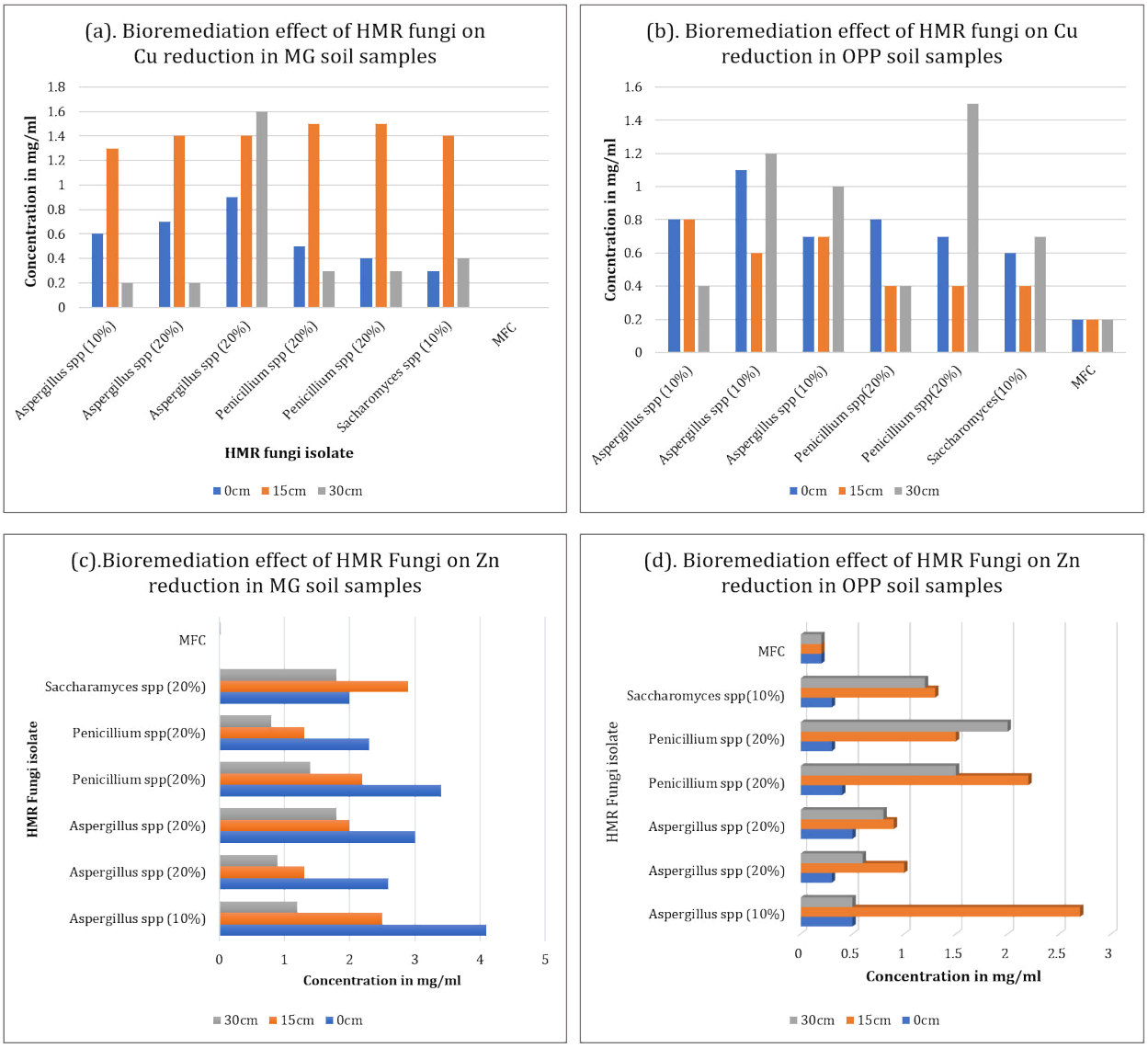
FIGURE 3B: Copper MTC of HMR Fungi.

TABLE 3: Bioremediation effect of fungal isolate on MG soil sample at different depth.

Fungal isolate	MG (0cm)		MG (15cm)		MG (30cm)	
	$[Cu^{2+}]$	Zn^{2+}] (mg/l)	$[Cu^{2+}]$	Zn^{2+}] (mg/l)	$[Cu^{2+}]$	Zn^{2+}] (mg/l)
Aspergillus spp. (10%)	0.552	4.097	1.318	2.527	0.228	1.233
Aspergillus spp. (20%)	0.689	2.561	1.417	1.348	0.152	0.949
Aspergillus spp. (20%)	0.919	2.927	1.362	2.022	0.163	1.763
Penicillium spp. (20%)	0.459	3.414	1.448	2.246	0.326	1.371
Penicillium spp. (20%)	0.394	2.276	1.492	1.263	0.283	0.771
Saccharomyces spp. (10%)	0.344	2.048	1.350	2.888	0.380	1.762
MFC: 0.00mg/ml						

TABLE 4: Bioremediation effect of fungi isolate on OPP soil sample at different depth.

Fungal isolate	OPP (0cm)		OPP (15cm)		OPP (30 cm)	
	$[Cu^{2+}]$	Zn^{2+}] (mg/l)	$[Cu^{2+}]$	Zn^{2+}] (mg/l)	$[Cu^{2+}]$	Zn^{2+}] (mg/l)
Aspergillus spp. (10%)	1.139	0.341	0.829	2.652	0.406	0.508
Aspergillus spp. (20%)	1.139	0.341	0.580	1.020	1.219	0.609
Aspergillus spp. (20%)	0.737	0.512	0.725	0.884	1.016	0.762
Penicillium spp. (20%)	0.783	0.426	0.414	2.210	0.358	1.524
Penicillium spp. (20%)	0.696	0.301	0.386	1.473	1.524	2.033
Saccharomyces spp. (10%)	0.626	0.626	0.362	1.326	0.677	1.219
MFC: 0.0234mg/ml						



FIGURES 4(A – D): Bar chart of bioremediation effect of HMR fungi on Cu and Zn reductions in MG and OPP soil samples at different soil depths respectively.

The results from this study indicated that on the overall, there are three species belonging to the *Aspergillus* genus, two species in the *Penicillium* genus while only one species belongs to the *Saccharomyces* genus, with each percentage concentrations: *Aspergillus spp.* (10%), *Aspergillus spp.* 20%, *Aspergillus spp.* (20%), *Penicillum spp. (*20%), *Penicillum spp. (*20%) and *Saccharomyces spp.* (10%) respectively (Supplementary Figure 3).

Further observation shows that after several isolations, most of the fungal strains grew well on SDA while a few of them did on PDA (Supplementary Table 3). In addition, Table 2 shows that higher concentration of metals is inversely proportional to fungi growth.

The resultant effect signifies that higher concentration of metals results in severe reduction of microbial activity which reflected as a reduction in the apparent growth rate and an increase in lag phase of the fungi.

At lower concentration of metals, however, there is an increase in fungi growth because lower concentration of metals stimulates microbial growth leading to an increase in the exponential phase of the fungi. Table 3 shows the microscopic characterization of the fungal isolates from the respective sites and depths in each of these locations. These characterizations led to the identification of six different fungal isolates, three in the *Aspergillus* genus, two in the *Penicillum* genus and one in the *saccharomyces* genus respectively (Supplementary figure 2).

TABLE 5: Microscopic Characterization of Fungal Isolates.

Isolate Code	Characterization	Probable Identity
OPP1	They are typically powdery black. Conidiophores arising from long, broad thick-walled, sometimes branched for cells, it has tall conidiophores. Conidia are large with radiating heads, mostly globose and irregularly roughed.	*Aspergillus spp.*
OPP1	These colonies are powdery masses of yellowish-green spores on the upper surface and the reddish-gold on the lower surface.	*Aspergillus spp.*
OPP2	These colonies are powdery masses of yellowish-green spores on the upper surface and the reddish-gold on the lower surface	*Aspergillus spp.*
OPP2	Spreading yellow colonies, rough walled stipes, mature vesicles bearing phalides over the entire surface with conspicuously enchinulate conidia.	*Aspergillus spp.*
MG1	Septate hyaline, the scelerotia have all defined, complex, internal structure. Conidiophores are unbranched, the cell wall of the fungus has a distinct roughening.	*Penicillium spp.*
MG2	Septate hyaline hyphae with some branched conidiophores, phalides and conidia.	*Penicillium spp.*
MG3	Spherical to ovoid shaped	*Saccharomyces spp.*
MG1	Septate hyaline, the scelerotia have all defined, Complex, internal structure. Conidiophores are unbranched, the cell wall of the fungus has a distinct roughening.	*Penicillium spp.*
MG1	Septate hyaline hyphae with a branched conidiophores, phalides and conidia.	*Penicillium spp.*
MG2	Spreading yellow colonies, rough walled stipes, mature vesicles bearing phalides over the entire surface with conspicuously enchinulate conidia.	*Aspergillus spp.*

Key: MG1= 10cm, MG2 = 15cm, MG33 = 30cm, OPP1 = 10cm, OPP2 = 15cm and OPP3 = 30cm.

DISCUSSION

Heavy metals percolate the surroundings by natural means and through human activities. Various sources of heavy metals include soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops, and many others (Morais *et al.*, 2012). Microorganisms have been used as such a low-cost method to remove metals from effluents (Volesky, 2007), with fungi known to be more tolerant to metals and to have a higher microorganism surface to volume ratio than bacteria or actinomycetes (Turnauk *et a*l 2006; Mala *et a*l 2006). Fungi are not only a major component of the biota in soils and mineral substrates (Frossard et al, 2017; Chang et al, 2019; Zeng *et al,* 2020), but also under certain environmental conditions (low pH), they can serve as efficient biogeochemical agents and bio-accumulators of soluble and particulate forms of metals (Gonen *et al,* 2009). Among them, *Penicillium* spp. are described as prominent ones (Balakumaran et al 2016; Massacesi et al, 2022). A widely used approach is mycoremediation that involves the use of fungi-based technology to decontaminate polluted soils (Vimala *et al* 2009; Ayangbenro *et a*l 2017).

In this study, high concentrations of heavy metals (Zn and Cu) were detected at various depths (0cm, 15cm, and 30 cm) of the respective metal sites. Even though there are differences in the concentration of metals at different depths in the two sites investigated, the availability of these metals is in accordance with various reports from other various sites, as reported globally. Heavy metals have been isolated from polluted soils (Anam and Shazia, 2014; Mohammed et al, 2015), textile effluent (Milene et al, 2022), rhizosphere of plants (V˘acar et al, 2021) and from gold and gemstone mining sites (Oladipo et al, 2018).

It is informative and of cognizance notice to this study that metals in different concentrations are located in various sites globally, irrespective of the activities operational on these identified sites.

In addition, heavy metal analysis from this study indicated that at 15cm depth, MG site has a higher Zn^{2+} concentration compared to OPP site, but with seemingly low levels of Cu^{2+} across all depths examined. OPP soil samples have a higher concentration of Cu^{2+} at 15cm depth but with a seemingly even distribution of Zn^{2+} metal concentration across the varying depths investigated. This observation suggests that there is no correlation between soil depth and heavy metal concentration in this site, even though highest metal concentration was detected at 15cm depth. It must be taken into account that the contamination at polluted sites is usually caused by a combination of metals and that the selection is probably driven either by the most toxic element or by more different metals acting synergistically (Baldrian *et a*l, 2002). The types of organism present in these sites may be responsible for the distribution of the heavy metals examined in our study (Livia et al, 2007). More so, the history of soil use, topography of the area, and the specific activity being carried out on the site may be responsible for the heavy metal distribution at various depths investigated in this study.

The fungi species isolated in this study belongs to the genera *Aspergillus, Penicillium and Saccharomyces*. Out of the10 strains identified, 6 multiresistant strains including 3 *Aspergillus spp.*, 2 *Penicillium spp.*, and 1 *Saccharomyces spp.* were selected for further investigation and removal from heavy metals in the collected soil samples.

Species of the genus *Aspergillus* were mostly dominant in all the sites at different soil depth. The occurrence of these genera in soils polluted with heavy metals has been reported in different part of the world. For instance, molecular detection of fungal isolates of *Penicillium* sp. ITF 2, *Penicillium rubens* ITF 4, *Penicillium* sp. ITF 12 and ITF 20 were detected in Brazil (Milene et al, 2022) while various species of the *Aspergillus* genera has been isolated from Pakistan (Anam and Shazia, 2014), Iran (Mohammadian et al, 2015), and Brazil (Milene et al, 2022) respectively. The selected fungal strains showed varying resistance at different concentrations of metals. The differences in the sample sites regarding the richness in microbial isolates appear to be closely linked to the degree of heavy metal pollution present in each metal sites. In conjunction with the aim of this study, we found that at lower metal ion concentrations, the tested fungal isolates were very resistant and exhibited stronger growth. This observation might be as a result of expression of certain fungal genes which has evolved as a response to certain environmental stress and strain over time. Higher metal ion concentrations caused a reduction in growth and increased the length of the lag phase compared to the control. A reduction in the growth rate is a typical response of fungi to toxicants (Turnau, 2006), whereas the lengthening of the lag phase is not always present.

Findings of the present study indicate that fungal populations isolated from heavy metal contaminated sites have the ability to resist higher concentrations of metals. The resistance of the isolates depended much more on the fungus tested than on the sites of its isolation. This variation may be explained by the development of tolerance or adaptation of the fungi species to heavy metals. Iskander et al, (2011) found that *Aspergillus spp.* has the ability to tolerate higher concentrations of Cu^{2+} (Iskander *et al,* 2011). Furthermore, many researchers and studies have described *Penicillium spp.* as being isolated from various metal stress conditions, indicating the ability of this fungi to withstand and tolerate higher concentrations of examined heavy metals (Zafar *et al,* 2007; Iram *et a*l, 2012). Our study also indicated that *Aspergillus* isolates were the most resistant to Cu^{2+} and also has the highest remediation effect of Cu^{2+} in MG soil samples at 30cm depth, *Penicillium spp.* has the highest remediation effect of Zn reduction in MG soil samples, *Saccharomyces spp.* has a high bioremediation effect of Zn^{2+} reduction in OPP soil samples and *Penicillium spp*. has the highest bioremediation effect of Cu^{2+} in OPP soil samples. These observations may be an indication that each of these fungal isolates have different tolerant and resistant capacity, hence their different bioremediation effect on each of the metals considered in this study. Our study also showed that mixed fungi culture completely removed copper and zinc in MG soil samples, an observation which has been supported with a similar report from Oaikhena et al. (2016) who submitted that, mixed culture was more efficient in the removal of heavy metals than pure isolates.

CONCLUSION

This study reiterates the presence of heavy metals such as Zinc and Copper, and the presence of heavy metal-resistant fungi in contaminated environments with high metal content. Findings from the study showed that the fungal population isolated from heavy metal-contaminated sites has the ability to tolerate higher concentrations of metals. Among the isolated fungi species from soil, *Aspergillus* isolates were the most resistant to Cu^{2+} while *Penicillium spp.* and *Saccharomyces spp.* has a higher resistant to Zn^{2+}, an observation which may be explained by the development of tolerance and adaptation of the fungi to heavy metals.

The outcome of this study emphasizes the need to investigate and make use of microorganism consortia adapted to the particular conditions of each location. Future study will also investigate these fungi at the molecular level using molecular detection methods and gene characterization techniques to accurately identify and study the various species present in the fungal genus isolated.

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SUPPLEMENTARY TABLES

TABLE S1: Significant effect of soil depth on Cu $^{2+}$and Zn^{2+} concentration in the soil.

Soil Depth	Cu $^{2+}$ concentration in soil	Zn^{2+} concentration in soil
0cm	1.969 ± 0.834	8.254± 2.809
15cm	2.085± 1.555	8.453 ± 2.343
30cm	2.096 ± 1.349	6.908 ± 1.045

TABLE S2: Significant effect of metal site on Cu $^{2+}$ and Zn^{2+} concentration in the soil.

Metal site	Cu $^{2+}$ concentration in Soil	Zn^{2+} concentration in soil
Gbokoniyi (MG)	1.263 ± 0.117	8.840 ±2.313
Police post (OPP)	2.837 ± 0.251	6.903± 0.695

TABLE S3: Morphological characteristic of pure isolate of OPP and MG samples.

Isolate code	Media	Colony colour	Colony reverse colour	Size (s)
OPPAI	SDA	Black	Brown	30mm
OPPB1	SDA	Light-green	Grey	25mm
OPPA2	PDA	Light-green	Grey	20mm
OPPB2	SDA	Black	Brown	25mm
MGA1	SDA	Light orange	Yellow	5mm
MGA2	SDA	Blue green	Grey	3mm
MGA3	PDA	Cream	Light yellow	3mm
MGA4	SDA	Light orange	yellow	5mm
MGB1	SDA	Blue green	light green	5mm
MGB2	PDA	Black brown	brown	30mm

SUPPLEMENTARY FIGURES

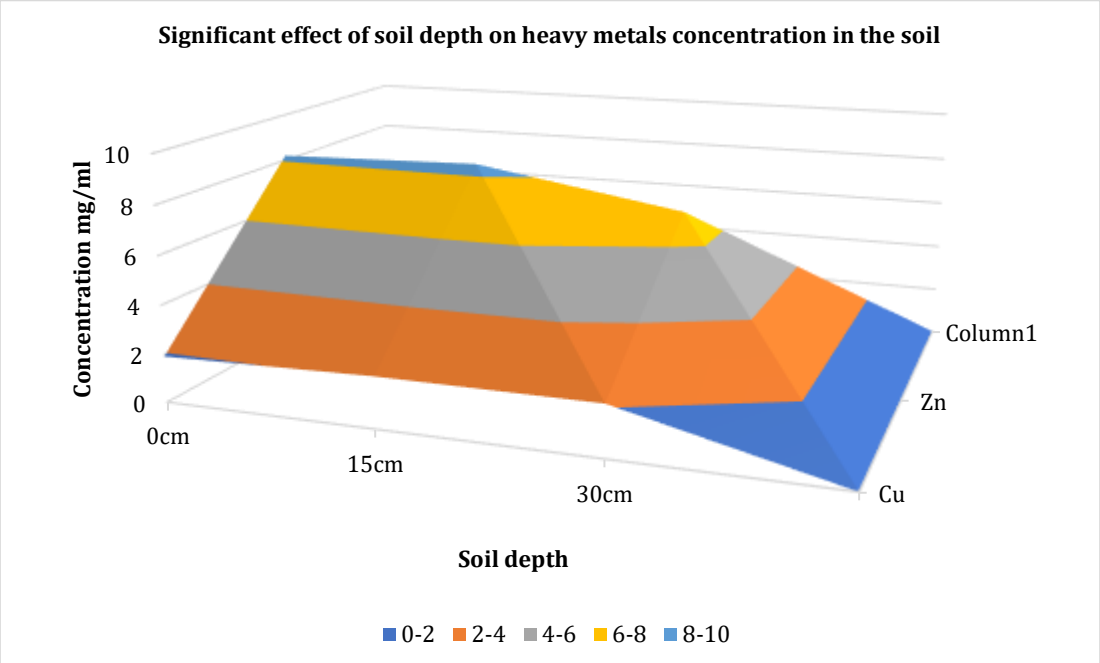

FIGURE S1: Chart of significant effect of soil depth on Cu $^{2+}$and Zn^{2+} concentration in the soil.

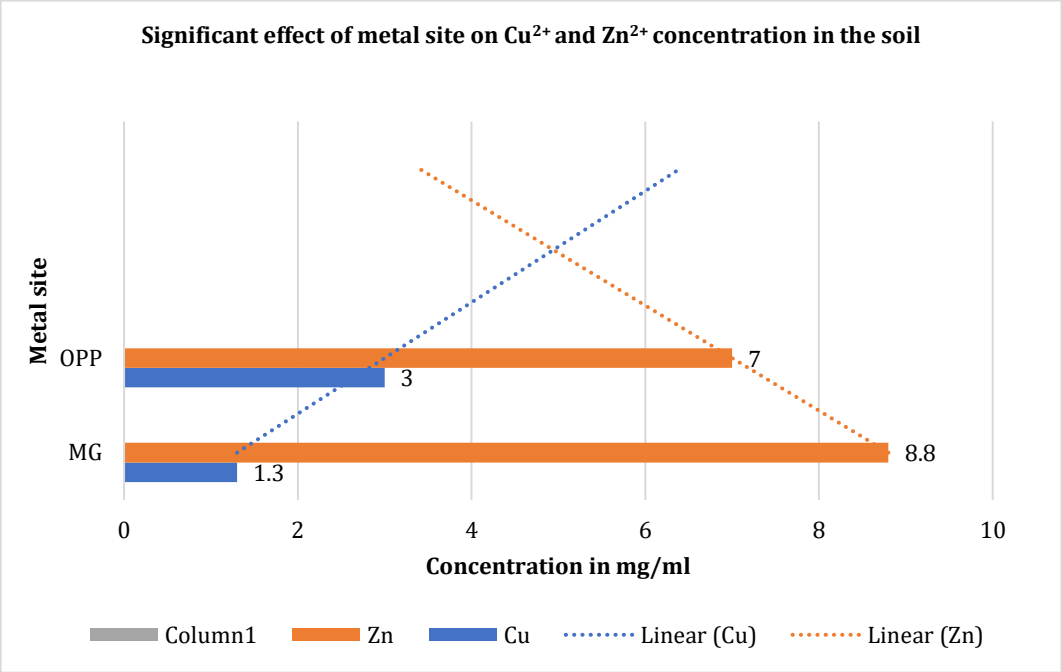

FIGURE S2: Chart of significant effect of metal sites n Cu^{2} and Zn^{2+} concentration in the soil.

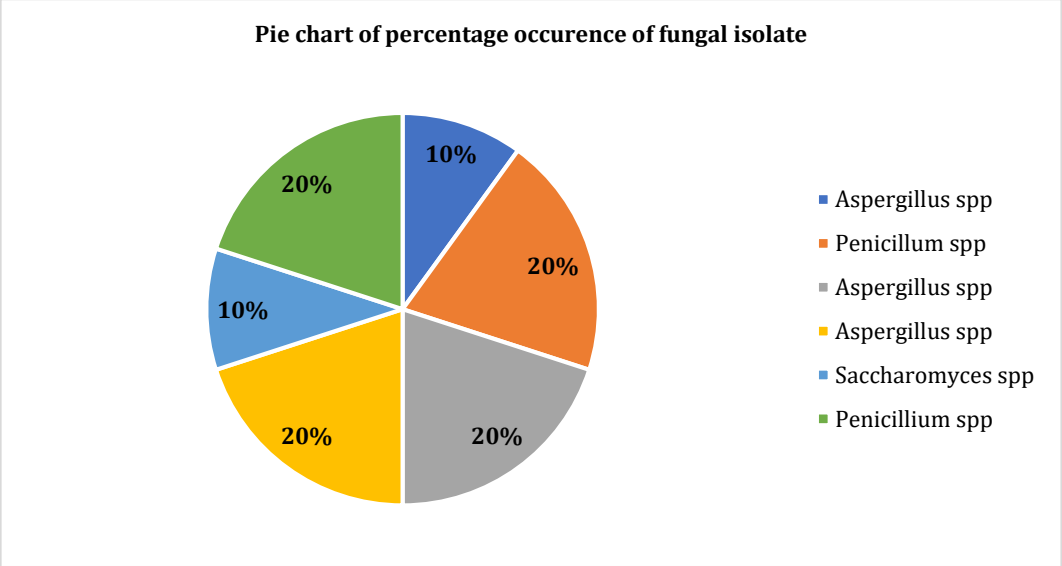

FIGURE S3: Pie chart of Growth occurrence of HMR fungi.