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Mycoremediation of Heavy Metals Contaminated Soil by Using Indigenous Metallotolerant Fungi

Muhammad Bilal Akram[1], Ibrar Khan[1], Mujaddad Ur Rehman[1], Abid Sarwar[2], Najeeb Ullah[2], Shafiq ur Rahman[3], Tariq Aziz[4*], Metab Alharbi[5], Abdulrahman Alshammari[5], Abdullah F Alasmari[5]

[1]*Department of Microbiology, Abbottabad University of Science & Technology, 22010 Havelian, Pakistan*
[2]*Food and Biotechnology Research Center PCSIR, Labs Complex Lahore Pakistan*
[3]*Department of Environmental Sciences, Shaheed Benazir Bhutto University Sheringal Dir Upper*
[4]*Department of Agriculture, University of Ioannina, 47100 Arta, Greece*
[5]*Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*
[*]*Corresponding author: e-mail: iwockd@gmail.com*

The present study was aimed to identify the indigenous fungal strains which could possibly be applied to the bioremediation of heavy metal-contaminated soil. The contaminated soil samples of Korangi Industrial Estate Karachi were found to have total concentration of Cu 1.044 mgL^{1}, and Pb 0.631 mgL^{-1}. A total of eight indigenous strains of the fungus were isolated and screened for bioremediation capacity from heavy metals-contaminated soil. For the bioremediation of Lead (Pb) these same indigenous eight fungal strains were used for biological remediation. All the fungal isolated with enhanced bioremediation capability were through phenotypic and genotypical characterization. The topology of the phylograms established that the fungal isolates used in this study were allocated to: K1 (*Penicillium notatum*), K2 (*Aspergillus parasiticus*), K3 (*Aspergillus fumigatus*), K4 (*Aspergillus flavus*), K5 (*Aspergillus terries*), K6 (*Fusarium solani*), K7 (*Penicillium chrysogenum*), K8 (*Aspergillus niger*), K9 (*Penicillium piceum*) and K10 (*Penicillium restrictum*). Thus, K8 fungal isolate was found to be more efficient with maximum bioremediation capacity, for copper and lead removal efficiency, and selected for FTIR and SEM to find out the uptake of Cu and Pb which of the functional groups are involved, and further to detect the effects of bioleaching of both heavy metals on to the surface of K8 fungus biomass. The current study indicates that indigenous fungal isolates could be used with high potency to remediate or clean up the heavy metals-contaminated soil either by the technique of in situ or ex-situ bioremediation.

Keywords: Mycoremediation; Heavy Metals Contaminated Soil; Korangi Industrial Estate; FTIR; SEM.

INTRODUCTION

Environmental pollution is a major worry for society today, as a growing number of pollutants from human activities are being introduced into water sources, soil, and air both in developing and advanced countries[1, 2]. As per United Nations, "pollution is the presence of exogenous chemical compounds in an appropriate area, at the appropriate time, and in insufficient quantities", Pollution of the environment and the fatal impacts of polluting factors on the health of the public has been a growing global issue throughout the last three decades. Above 80% of dirty water has been used for agriculture in underdeveloped nations. With the establishment of industry in urban and semi-urban areas surrounded by more people and lower-income neighborhoods impurities including heavy metals invaded the environment[3]. Heavy metals are a class of elements that are known carcinogens, dangerous materials, and extremely water soluble[4]. Metal components are widely used in both our everyday lives and the development of the industrial sector Higher amounts of some metals, however, have the capability to be hazardous to people, animals, and even plants[5]. Due to their potential for long-term persistence in the environment, heavy metals are thought to be the primary cause of environmental pollution. Poisonous heavy metals may have negative effects on people, plants, and animals[6].

The toxicity of heavy metals can result in a variety of health issues, such as altered blood composition, impaired mental and effect function of the central nervous system, decreased energy production, damaged kidneys, liver, lungs, and other essential organs. Thus, Bioremediation like Mycoremediation is generally cheaper, cost-effective, and productive technique rather than used other traditional methods because it does not require the use of expensive equipment and complex procedure in disposing of those hazardous soils. These heavy metals may have a detrimental effect on groundwater quality, soil ecology, and agricultural output or product quality. These elements may potentially have a detrimental effect on the health of living organisms through the food chain[7]. These metal elements can have a direct impact on a variety of activities, such as growth, withering, and energy production, because of their high reactivity Heavy metals can either stop a plant's growth in its entirety or only in a specific area[8]. Heavy metals absorbed by plants could be harmful to humans if they build up in the food chain. High amounts of heavy metals, can be fatal or permanently harm the brain, kidneys, and nervous system[9].

In chemical-based remediation, the polluted soil is washed with clean water, chemicals, and other fluids or gases that might drain the pollutant from the soil[10]. Contaminated soil having heavy metals is transformed into a liquid phase employing techniques such as ion exchange, precipitation, adsorption, and chelation by using an inorganic eluent, chelation agents, and leachate[11]. Among remediation techniques, soil washing can lessen the amount of polluted soil while also removing any additional heavy metals that have been adsorbed into the soil[12]. While the components of biological remediation (eco-friendly and cost-effective approach)

include; phytoremediation, bioremediation, and combined remediation[13]. Environmental contaminants that are problematic in nature will be treated utilizing natural or genetically modified microbes or plants in the bioremediation process. By transforming harmful heavy metals into less toxic elements, these organisms will degrade xenobiotics and reduce their levels[14]. Korangi Industrial Estate Karachi is one of the largest industrial zones of Pakistan under Korani Association of Trade and Industry (KATI). About 4500 industries, commercial, and trade units are located inside the area. These include textile, steel mills, pharmaceutical, automotive plants, chemical and engineering plants, and flour mills. As this area is heavily populated with different industries therefore soil and water samples have high levels of heavy metals and other contaminants. Contamination of the soil by heavy metals occurs because of leakage and stacking practices during industrial production. Thus, the current study not only provides a brief knowledge about contaminants but also gives a detailed view of microbial flora found in the local environment that can be further used for different eco-friendly processes of bioremediation by using *in--situ* or *ex-situ* approach. The current study is aimed to conduct mycoremediation of heavy metals contaminated soil by using Indigenous Metallotolerant from Korangi Industrial Estate, Karachi.

MATERIAL AND METHODS

Collection of samples

The soil samples were collected with the aid of a sharp--edged plastic spatula to plough 0–20 cm of soil from various locations separated by 10–20 m from heavy metals contaminated effluent soil of Korangi Industrial Estate Karachi, Pakistan. Then the samples were labelled and sealed in a sterile polyethylene bag, the samples were carefully delivered to the laboratory of the Department of Microbiology at Abbottabad University of Science and Technology for Processing.

Sample preparation

The polluted soil samples with heavy metals were dried in a desiccator for two weeks, after being air dried in a lab, the samples were further dried at 75 °C to achieve a constant mass for further analysis. Then the samples were first converted into powder form with the help of mortar and pestle and stored in the polyethylene bags for examination.

Detection of the total heavy metal concentration

Atomic Adsorption Spectrophotometry was used to assess the concentration of total heavy metals at the laboratory of Karachi University. To access the total metal content from soil samples using atomic adsorption spectrophotometry, a standard solution was made using the acid digestion method described by Hseu, *et al.*[15]. Acids, such as acetic and hydrochloric, were used to decompose soil samples. For the acid digestion procedure, soil samples were weighed 5 grams. Weighted soil samples were placed inside the conical flask, and then the flask was filled with 5 ml of nitric acid and 2 ml of perchloric acid and then mixture in the flask was heated to a temperature of 250–350 °C in the fume hood. When fumes and a cloudy appearance in the sample were noticed, the flask was immediately removed from the heating source and placed in a designated sterilized location at room temperature then, Watsman filter papers were used to filter the solution. The serial dilution method was used to dilute the filtered sample described by[16]. By following this method for serial dilution, the sample was diluted to various concentrations ranging from 10^{-1} to 10^{-4} mL. By using atomic absorption spectrophotometry (AAS), the amount of heavy metals concentration in the sample was calculated from final dilution.

Atomic Adsorption Spectrophotometry (AAS)

Atomic Adsorption Spectrophotometry is used for the wavelength of light that is specifically absorbed by an element to figure out the amount of trace metals in complex matrices. When the power switch gas source and airflow were turned on, Atomic Adsorption Spectrophotometry became operational. After that, the fuel switch was turned on to set the burner on fire, a lighter was used. The burner head produced a 5–10 cm long, a few millimeters deep flame. The machine needed to be zeroed at that point. for this reason, Blank solution (distilled water) was provided by the nebulizer. The machine›s Auto button was then pressed to confirm that it was prepared for the next step in the process.

Using standard solutions of these metals in various concentrations the analysis of the contents was primarily started with two metals, copper, and lead. After that, readings were quickly taken while the nebulizer was continuously dipped into a series of standard solutions ranging in concentration from low to high. The final step involved placing the sample solution on an instrument tray after the standard had been dipped with the intention of having automatically sucked up by a nebulizer. Once there, the flame ingested the sample and produced an atom from the sample solution, which atomized the sample. A sample's anode and flame (from a hollow cathode lamp) were the focal points of the light. Then a high voltage was passed between the anode and the cathode. The radiation from the source ended up there and was focused by the mirror on the sample. Then, radiation was routed through an analyzer designed to choose the frequency that would reach the detector at a given time. The signal from the detector was then sent to a recorder that was connected to the analyzer to create a trace of the absorbance of various frequencies. After that, the system provided a standard curve to analyze the heavy metal content[17].

Isolation of indigenous fungal strains

By using the method of serial dilution on the Potato Dextrose Agar (PDA) medium and supplemented with chloramphenicol 0.05% the fungal indigenous strains from the heavy metal polluted soil samples were isolated. The standard dilution method was used to dilute each sample up to a 10^{-4} dilution. By using a pipette and a spreader, 1 ml of the microbial suspension from each of the diluted concentrations was added to the pre-sterilized PDA Petri plates. Then at 30 °C these plates were incubated for 96 h. Isolated fungi colonies were collected and purified using the streak plate method. The mixed or

mother culture's purified colonies were again subculture on new PDA plates and kept at 4 °C for preservation.

Microbial bioleaching

Five different types of media, including yeast peptone glucose (YPG), sabouraud dextrose broth (SDB), potato dextrose broth (PDB), yeast peptone dextrose (YPD), cezapek dox broth (CDB), were used to bioleach polluted soil samples. Then the bioleaching of the heavy metals was done in five types of media in the following way.

Optimization with YPG medium

The 1mL of spore suspension of each fungal strain was inoculated into 100 mL of YPG medium (1% Yeast Extract, 2% Peptone and 2% Glucose), in the 250 mL of sterilized conical flasks, then the flasks were incubated at 32 °C for 72 h. After 3 days of incubation, into these conical flasks, 1g of sterilized contaminated soil sample was added. These flasks were then placed in a shaking incubator for an additional 72 h while being shaken continuously at 150 rpm and 32 °C.

Optimization with SDB medium

The 1ml spore suspension of each fungal strain was inoculated into the 100 mL of autoclaved SDB medium (Peptone 1% and Dextrose 2%) in sterile 250 mL flasks and kept the flasks in a shaking incubator and incubated at 32 °C for 72 h at 150 rpm. After 72 h of incubation, then the autoclaved 1g of contaminated soil was added into each flask and again placed the flasks in a shaking incubator at 32 °C for a further 72 h at 150 rpm.

Optimization with PDB medium

The 1 ml spore suspension of each fungal strain was inoculated into the 100 ml of autoclaved PDB medium (Potato Infusion 0.4% and Dextrose 2%) in sterile 250 ml flasks and kept the flasks in a shaking incubator and incubated at 32 °C for 72 h at 150 rpm. After 72 h of incubation, the autoclaved 1g of contaminated soil was added into each flask and again placed the flasks in a shaking incubator at 32 °C for a further 72 h at 150 rpm.

Optimization with YPD medium

The 1 mL of spore suspension of each fungal strain was inoculated into the 100 mL of autoclaved YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) in a sterile 250 mL flasks and kept the flasks in a shaking incubator and incubated at 32 °C for 72 h at 150 rpm. After 72 h of incubation, the autoclaved 1g of contaminated soil was added into each flask and again placed the flasks in a shaking incubator at 32 °C for a further 72 h at 150 rpm.

Optimization with CDB medium

The 1 mL of spore suspension of each fungal strain was inoculated into the 100 mL of 250 flasks of autoclaved CDB medium (sucrose 3%, sodiumnitrate 0.3%, potassium chloride 0.05%, magnesium sulphate 0.05%, ferrous sulphate 0.001%, potassium phosphate dibasic 0.1%) in a sterile 250 ml flasks and kept the flasks in a shaking incubator and incubated at 32 °C for 72 h at 150 rpm. After 72 h of incubation, the autoclaved 1g of contaminated soil was added into each flask and again placed the flasks in a shaking incubator at 32 °C for a further 72 h at 150 rpm. While five uninoculated flasks containing 100 mL of each of the following media: YPG, SDB, CDB, YPD, and PDB served as the control in each case, and all experiments were conducted in triplicate. At regular intervals, supernatant from the growing culture was collected and the growing culture or biomass was weighed. Further, the amount of metal released in the media was measured using an atomic adsorption spectrophotometer.

Analysis of the uptake efficiency of heavy metals

The indigenous fungal isolates were grown in the presence of both heavy metal Cu and Pb, in the five different media (PDB, SDB, YPD, CDB and YPD). Each flask's broth was transferred into individual 50 ml centrifuge tubes and centrifuged at 14,000 rpm for 15 minutes. The supernatant was kept determining the metal contents using an atomic adsorption spectrophotometer. After being obtained through filtration, the fungus's biomass was rinsed with double-distilled water and weighed. After the centrifugation, the biomass of the fungus was dried in a hot air oven overnight at 80 degrees Celsius and weighed. The following equation was used to analyze or calculate the amount of the heavy metals taken up by biomass of fungus in each supernatant[18, 19].

$$Q_e = \frac{(C_i - C_f)V}{M}$$

Where V showed the volume of the aqueous medium, M signify the dry weight of the fungus, where Qe is the amount of heavy metal uptake by fungus in ($mg \cdot g^{-1}$), C_i is the amount of heavy metal at initial concentrations (mgL^{-1}), and C_f represents the final concentration of heavy metal (mgL^{-1}) at the end.

Fourier Infrared spectroscopy (FTIR)

The study of the infrared light wavelengths that are absorbed by a specimen preferentially is known as infrared spectroscopy[20]. Each molecule will absorb a particular set of infrared energies, according to the forms of chemical bonding, the types of molecular vibrational motion stimulated, and the masses of the atoms involved in FTIR spectroscopy, in contrast to visible light microscopy, reports absorbance peak energies in wavenumbers (cm^{-1}, inverse wavelength). Mid-infrared wavelengths between 2.5 and 12.5 m correspond to 4000–800 cm^{-1}, where typical vibrational energies are found. The information, which represents the infrared spectrum as a function of infrared energies (wavenumbers, x-axis) is shown as absorbance peaks[21]. The functional groups and bonds found in fungus that were responsible for the metal buildup in the cytosol were found using FTIR. The mycelium of fungal isolate with maximum bioremediation capability was isolated, cleaned with distilled water, and placed in oven, and dried at 80 °C for 4 hours to achieve constant weight. The dried biomass was then prepared using a potassium bromide pellet technique before being analyzed using and Thermo Nicolet 6700 FTIR spectrometer with a scan wave range of 400 to 4000 cm^{-1}.

Scanning Electron Microscopy (SEM)

Before and after the bioleaching morphology of the selected fungal biomass was observed under the microscope. First, the biomass of the selected fungal strain was placed in an oven and dried at the 60 °C. Later Glutaraldehyde was applied, and then it was incubated at 4 °C for 12 h. Then The biomass's water content was removed by using alcohol. Further, using a sputter coater under vacuum, gold particles were sputtered onto the specimens, which were then examined under the SEM at an accelerating voltage of 15 kV to record the images.

Fungal isolates identification

Phenotypic based identification

The selected indigenous fungal isolates from the soil sample that was polluted with heavy metals, with maximum bioremediation capacity were identified morphologically by observing morphology on PDA, SDA, and MEA media, supplemented with 0.05% chloramphenicol.

Molecular based identification

The gDNA of all the fungal strains were extracted by using the methodology mentioned by Khan, *et al.*[22], and PCR amplification was done by using thermocycler by using ITS primes conserve primers, with a reaction mixture system consisted of 10 buffer 2.5 ul, Taq DNA polymerase 1.25 U, dNTP 0.8 mM, ITS-F or ITS-R 0.5 mM, DNA template 1.0 l, and H_2O 16 ul (total 25 ul). Initial denaturation at 94 °C for 10 minutes, earlier denaturation at 94 °C for 1 minute, annealing for 1 minute, extension at 72 °C for 2 minutes, and final extension at 72 °C for 10 minutes were used in the PCR amplification. A total of 32 cycles of PCR will be performed. Finally, the PCR products will be separated using a 1% agarose gel electrophoresis.

The sequences acquired as mentioned above will combine for the construction of the phylogenetic tree using BLAST analysis (http://blast. ncbi.nlm.nih.gov/Blast.cgi). The sequences which showed over 98% resemblance with presently existing sequences will be considered the same species. Moreover, using Clustal X 1.83, multiple alignments will be performed and for the building of the phylogenetic tree, MEGA 10.02.X was used[23].

Preservation of fungal isolates

For the preservation of indigenous fungal isolates, 30% glycerol solution was used, the 30 ml of glycerin was dissolved in 70 ml of distal water and autoclaved for the time of 15 minutes at a specific temperature of 121 °C. Following the addition of fungal isolates for preservation, this solution was put in the refrigerator.

Statistical analysis

Using the LSD (least significant difference) test in the SPSS 20 program one way ANOVA was used to compare the differences between the methods at the P0.05 level of significance (SPSS, Inc.). Three duplicates of each procedure were carried out.

RESULTS

Total Metal Content

The heavy metals contaminated soil was collected and analyzed for heavy metals contents (Cu, Pb, Cr, and Zn), by using AAS procedure mentioned above (Table 1).

Table 1. Total heavy metals content of contaminated soil of Korangi Industrial Estate

Code	Cu (mgL^{-1})	Pb (mgL^{-1})	Cr (mgL^{-1})	Zn (mgL^{-1})
1	0.759	0.418	8.295	1.940
2	0.562	0.499	0.433	2.091
3	0.631	0.247	0.259	1.255

Isolation of indigenous fungal strains

A total of eight fungal strains (K1, K2, K3, K4, K5, K7, K8, and K10), were isolated from the heavy metals contaminated soil sample (S1) of Korangi Industrial Area Karachi and purified in accordance with the methods mentioned in the material and methods section.

Bioleaching analysis

Fungal heavy metal[1] removal efficiency

Heavy metals pollution leads to decrease in the number of microbial communities because they are susceptible to the heavy metals, and they cannot survive into the heavy metals contaminated environment. Fungi could survive into the heavy metals contaminated environment. All the fungal isolates were analyzed for resistance to different concentrations of heavy metals especially Cu and Pb (Table 2).

The K1 fungal strain showed high removal efficacy 96.6% for Cu removal in the PDB medium. While the least removal efficiency 90.2% of K1 strain was recorded in SDB medium. In the YPD medium, K1 showed 90.3% removal efficiency, whereas 91.4% removal efficiency was recorded in CDB medium. The K2 fungal strain showed the highest removal efficiency in the PDB medium which was about 96.2%. While 93.2% removal efficiency of K2 was monitored in the SDB medium. The least removal efficiency 91.0% of K2 was recorded in the YPD medium. Whereas 92.3% removal efficiency of K2 was monitored in the CDB medium.

The highest removal efficiency of K3 strain of fungus was showed in SDB medium 95.1%, while the lowest removal efficiency 91.9% of K3 was recorded in the YPD medium. Whereas removal efficiency 94.5% of K3 was monitored in the PDB medium. whereas 93.0% removal efficiency of K3 was recorded in CDB medium. While, K4 showed the highest removal efficiency 95.3% in PDB medium, while the lowest removal efficiency 91.1% of K4 was monitored in the YPD medium. The removal efficiency of 93.3% of the K4 was recorded in SDB medium. Whereas in CDB medium 92.7% removal efficiency of K4 was recorded.

The K5 showed the highest removal efficiency of copper 94.3% in the PDB medium, whereas, in the SDB medium 92.8% removal efficiency was recorded. The removal efficiency of 9 1.7% of K5 was monitored in YPD medium. While 91.8% removal efficiency of K5 was recorded in the CDB medium. The highest removal efficiency K7 strain was shown in PDB medium which

Table 2. Removal efficiency of Copper[1] by all the indigenous metallotolerant fungal isolates

Code of sample	Heavy metal	Initial concentration of heavy metal	Fungal isolate	Media	Final concentration of metal	Removal efficiency
S1	CU	1.044 mgL^{-1}	K1	PDB	0.0352 mgL^{-1}	96.6%
				SDB	0.1019 mgL^{-1}	90.2%
				YPD	0.1011 mgL^{-1}	90.3%
				CDB	0.0894 mgL^{-1}	91.4%
			K2	PDB	0.0392 mgL^{-1}	96.2%
				SDB	0.0701 mgL^{-1}	93.2%
				YPD	0.0937 mgL^{-1}	91.0%
				CDB	0.0798 mgL^{-1}	92.3%
			K3	PDB	0.0569 mgL^{-1}	94.5%
				SDB	0.0507 mgL^{-1}	95.1%
				YPD	0.0838 mgL^{-1}	91.9%
				CDB	0.073 mgL^{-1}	93.0%
			K4	PDB	0.0485 mgL^{-1}	95.3%
				SDB	0.0695 mgL^{-1}	93.3%
				YPD	0.0924 mgL^{-1}	91.1%
				CDB	0.0754 mgL^{-1}	92.7%
			K5	PDB	0.0595 mgL^{-1}	94.3%
				SDB	0.0742 mgL^{-1}	92.8%
				YPD	0.0859 mgL^{-1}	91.7%
				CDB	0.0847 mgL^{-1}	91.8%
			K7	PDB	0.0423 mgL^{-1}	95.9%
				SDB	0.0772 mgL^{-1}	92.6%
				YPD	0.0803 mgL^{-1}	92.3%
				CDB	0.0865 mgL^{-1}	91.7%
			K8	PDB	0.0822 mgL^{-1}	92.1%
				SDB	0.0819 mgL^{-1}	92.1%
				YPD	0.077 mgL^{-1}	92.6%
				CDB	0.0806 mgL^{-1}	92.2%
			K10	PDB	0.0712 mgL^{-1}	93.1%
				SDB	0.1295 mgL^{-1}	87.5%
				YPD	0.0851 mgL^{-1}	91.8%
				CDB		

was about 95.9%. In the SDB medium, 92.6% removal efficiency was monitored. In the YPD medium, K7 showed 92.3% removal efficiency of cooper. Whereas 91.7% removal efficiency of K7 was recorded in CDB medium.

In YPD medium K8 showed the highest removal efficiency 92.6%. In the CDB medium 92.2% removal efficiency of K8 was monitored. While the least removal efficiency 92.1% of K8 was monitored in SDB medium. Whereas 92.1% removal efficiency of K8 was recorded in the PDB medium. In the PDB medium, 93.1% removal efficiency was noted. Whereas in the SDB medium, 87.5% removal efficiency was monitored. while in the YPD medium, 91.8% removal efficiency was recorded.

Removal efficiency of heavy metal (Lead) by fungal isolates

The heavy metal (Lead) removal efficiency of all the indigenously isolated fungal isolates was analyzed, as shown below which indicates the potency of fungal isolates that may be used in bioremediation of heavy metals contaminated soil (Table 3). The highest removal efficiency K1 fungal strain was recorded in the PDB medium which was about 93.6%. Whereas removal efficiency of 87.8% of K1 strain was recorded in YPD medium. In the SDB medium K1 strain showed 87.2% removal efficiency and 81.2% removal efficiency K1 strain was recorded in YPG medium for lead removal. while the lowest removal efficiency K1 strain was recorded at 70.8% in the CDB medium. While the highest removal efficiency K2 fungal strain to remove lead was recorded in the PDB medium which was 96.3%. whereas the removal efficiency of K2 strain recorded in CDB medium was 91.6%. In the SDB medium K2 strain showed 90.8% removal efficiency, whereas 81.3% removal efficiency of K2 strain was recorded in YPG medium. While the lowest removal efficiency 77.1% of K2 strain for lead removal was recorded in the YPD medium.

The highest removal efficiency 94.5% of K3 fungal strain to remove lead from the soil sample S1 was recorded in the PDB medium. Whereas 90.6% removal efficiency of K3 strain was monitored in the CDB medium. In the YPG medium K3 strain showed 90.3% removal efficiency, whereas 85.1% removal efficiency of K3 strain was recorded in CDB medium. While the lowest removal efficiency of K3 strain to remove lead was recorded at 73.4% in the YPD medium. The highest removal efficiency of K4 fungal strain to remove lead from the soil sample S1 was recorded in the PDB medium which was 92.9%. The removal efficiency of 88.3% of K4 strain was recorded in the SDB medium. In the YPG medium K4 strain showed 87.6% removal efficiency, whereas 84.4% removal efficiency of K4 strain was recorded in CDB medium. while the lowest removal efficiency 75.3% of K4 strain was monitored in the YPD medium.

The highest removal efficiency of K5 fungal strain to remove lead from the soil sample S1 was recorded in the PDB medium which was about 96.7%. The removal efficiency of 86.9% of the K5 strain was recorded in the CDB medium. In the SDB medium K5 strain showed 83.7% removal efficiency, whereas 78.9% removal efficiency of K5 strain was recorded in YPG medium. while the lowest removal efficiency 76.0% of K5 strain was recorded in the YPD medium. While the highest removal efficiency of K7 93.6% was recorded in the YPG medium. The 88.9% Removal efficiency of K7 strain was monitored in PDB medium. In the CDB medium K7

Table 3. Removal efficiency of Lead (Pb) by all the indigenous metallotolerant fungal isolates

Code of sample	Heavy metal	Initial concentration Pb	Fungal isolate	Media	Final concentration of Pb	Removal efficiency
S1	Pb	0.631 mgL^{-1}	K1	PDB	0.0399 mgL^{-1}	93.6%
				SDB	0.0801 mgL^{-1}	87.2%
				YPD	0.0765 mgL^{-1}	87.8%
				CDB	0.1841 mgL^{-1}	70.8%
				YPG	0.1182 mgL^{-1}	81.2%
			K2	PDB	0.0233 mgL^{-1}	96.3%
				SDB	0.0575 mgL^{-1}	90.8%
				YPD	0.1441 mgL^{-1}	77.1%
				CDB	0.0530 mgL^{-1}	91.6%
				YPG	0.1178 mgL^{-1}	81.3%
			K3	PDB	0.0343 mgL^{-1}	94.5%
				SDB	0.0589 mgL^{-1}	90.6%
				YPD	0.1676 mgL^{-1}	73.4%
				CDB	0.0936 mgL^{-1}	85.1%
				YPG	0.0611 mgL^{-1}	90.3%
			K4	PDB	0.0446 mgL^{-1}	92.9%
				SDB	0.0734 mgL^{-1}	88.3%
				YPD	0.1557 mgL^{-1}	75.3%
				CDB	0.0982 mgL^{-1}	84.4%
				YPG	0.0781 mgL^{-1}	87.6%
			K5	PDB	0.0208 mgL^{-1}	96.7%
				SDB	0.1026 mgL^{-1}	83.7%
				YPD	0.1508 mgL^{-1}	76.0%
				CDB	0.0823 mgL^{-1}	86.9%
				YPG	0.1329 mgL^{-1}	78.9%
			K7	PDB	0.0698 mgL^{-1}	88.9%
				SDB	0.0854 mgL^{-1}	86.4%
				YPD	0.1327 mgL^{-1}	78.9%
				CDB	0.0797 mgL^{-1}	87.3%
				YPG	0.0398 mgL^{-1}	93.6%
			K8	PDB	0.0476 mgL^{-1}	92.4%
				SDB	0.1093 mgL^{-1}	82.6%
				YPD	0.0450 mgL^{-1}	92.8%
				CDB	0.1105 mgL^{-1}	82.4%
				YPG	0.0473 mgL^{-1}	92.4%
			K10	PDB	0.0659 mgL^{-1}	89.5%
				SDB	0.0955 mgL^{-1}	84.8%
				YPD	0.1129 mgL^{-1}	82.1%
				CDB	0.1547 mgL^{-1}	75.4%
				YPG	0.0609 mgL^{-1}	90.3%

strain showed 87.3% removal efficiency, whereas 86.4% removal efficiency of K7 was observed in the SDB medium. while the lowest removal efficiency of K7 78.9% was recorded in the YPD medium.

The highest removal efficiency of K8 fungal strain to remove lead from the soil sample S1 was recorded in the YPD medium which was recorded at 92.8%. The removal efficiency of 92.4% of K8 strain was recorded in the PDB medium. In the YPG medium K8 strain showed 92.4% removal efficiency. whereas 82.6% removal efficiency of K8 strain was recorded in SDB medium. while the lowest removal efficiency of K8 strain was recorded 82.4% in the CDB medium. On the other hand, the highest removal efficiency of K10 fungal strain to remove lead from the soil sample S1 was recorded in the YPG medium which was 90.3%. The removal efficiency 89.5% of K1 strain was recorded in the PDB medium. In the SDB medium K1 strain showed 84.8% removal efficiency, whereas 82.1% removal efficiency of K10 was recorded in YPD medium. while the lowest removal efficiency 75.4% of K10 was recorded in the CDB medium.

Uptake efficiency of indigenous fungal strains for Pb ($mg \cdot g^{-1}$)

The uptake efficiency for Lead (Pb) of all the indigenous fungal isolates was calculated as mentioned below and shown in Figure 1. From the soil sample S1 the highest uptake value efficiency for lead removal was shown by K1 strain in the PDB medium which was 0.1037 $mg \cdot g^{-1}$. The lowest uptake value of efficiency was noticed by K1 strain in the CDB medium was recorded at 0.0784 $mg \cdot g^{-1}$. In the YPD medium K1 showed 0.0972 $mg \cdot g^{-1}$, Whereas 0.0899 $mg \cdot g^{-1}$ uptake value was recorded by K1 strain in the YPG medium. In the SDB medium K1 showed 0.0966 $mg \cdot g^{-1}$ uptake value efficiency for copper removal from the soil sample S1. The K2 fungal isolate showed the highest uptake efficiency at 0.0187 $mg \cdot g^{-11}$ in PDB medium, K2 showed 0.01783 $mg \cdot g^{-1}$ in CDB medium and the least uptake value of efficiency of K2 was recorded at 0.0150 $mg \cdot g^{-1}$ in the YPD medium. Whereas 0.01783 $mg \cdot g^{-1}$ uptake value was recorded in CDB medium. while 0.01583 $mg \cdot g^{-1}$ uptake value was recorded in the YPG medium. The K3 fungal isolate showed the highest uptake efficiency in PDB medium 0.044 $mg \cdot g^{-1}$. While the lowest uptake efficiency was recorded by K3 strain in YPD medium 0.034 $mg \cdot g^{-1}$. K3 showed 0.040 $mg \cdot g^{-1}$ uptake value of efficiency in the CDB medium, while 0.04269 $mg \cdot g^{-1}$ and 0.04252 $mg \cdot g^{-1}$ uptake efficiency of K3 was recorded in the SDB and YPG medium.

The K4 fungal isolate showed the highest uptake efficiency 0.068 $mg \cdot g^{-1}$ for lead removal from the soil sample S1 in the PDB medium. While the least upta-

ke value of 0.0559 mg · g^{-1} of K4 was recorded in the YPD medium. K4 showed 0.066 mg · g^{-1} uptake value of efficiency in the CDB medium, while 0.0650 mg · g^{-1} and 0.065 mg · g^{-1} uptake efficiency of K4 was recorded in the YPG and SDB medium. The K5 fungal isolate showed the highest uptake efficiency in PDB medium 0.0701 mg · g^{-1}. While the least uptake efficiency was noticed at 0.05518 mg · g^{-1} in the YPD medium. In the YPG K5 showed 0.0572 mg · g^{-1} uptake efficiency. In the CDB and SDB medium, 0.063066 mg · g^{-1} and 0.0607 mg · g^{-1} uptake efficiency of K5 for Pb removal was recorded. The K7 fungal isolate showed 0.0398 mg · g^{-1} highest uptake efficiency for lead removal in the YPG medium. The uptake value of K7 0.0397 mg · g^{-1} was monitored in the PDB medium. The least uptake efficiency value 0.0353 mg · g^{-1} was observed in the YPD medium. whereas in the CDB and SDB medium 0.0390 mg · g^{-1} and 0.0386 mg · g^{-1} uptake value of K7 was recorded for the Pb removal. The K8 fungal strain showed the highest uptake efficiency for the removal of lead in the YPD medium which was 0.1542 mg · g^{-1}. K8 showed 0.1535 mg · g^{-1} uptake value in the PDB medium. whereas, in the YPG medium 0.1536 mg · g^{-1} and in SDB medium 0.1387 mg · g^{-11} uptake value was monitored. The least uptake value of K8 fungal strain was recorded at 0.1369 mg · g^{-1} in the CDB medium.

The highest uptake value of K10 for lead removal was recorded at 0.0231 mg · g^{-1} in the YPG media. whereas 0.0229 mg · g^{-1} uptake efficiency of K10 was recorded in PDB medium. In the CDB medium lowest uptake efficiency was recorded at 0.0193 mg · g^{-1}, while 0.02176 mg · g^{-1}, 0.02106 mg · g^{-1} uptake efficiency 0f K10 was monitored in the SDB and YPD medium.

Uptake efficiency of indigenous fungal isolates for Cu (mg · g^{-1})

However, the Uptake efficiency of indigenous fungal isolates for Copper[1] also showed promising results that are mentioned below and represented in Figure 2. From the soil sample S1 the highest uptake value of K1 for copper removal was recorded at 0.1769 mg · g^{-1}. The lowest uptake value noticed by K1 strain in the SDB medium was 0.1652 mg · g^{-1}. K1 showed 0.1654 mg · g^{-1} in the YPD medium. K1 showed 0.1674 mg · g^{-1} uptake value for Cu removal in the CDB medium. The K2 fungal isolate showed the highest uptake efficiency 0.0310 mg · g^{-1} in the PDB medium. The lowest uptake value 0.0293 mg · g^{-1} of efficiency of k2 was recorded in the YPD medium. K2 showed 0.0300 mg · g^{-1} in SDB medium and 0.0297 mg · g^{-1} in CDB medium. The K3 fungal isolate showed the highest uptake efficiency in SDB medium 0.0741 mg · g^{-1}. The least uptake value was 0.0716 mg · g^{-1} efficiency recorded of the K3 strain in the YPD medium. K3 displayed 0.072 mg · g^{-1} uptake value in the CDB media. While 0.0736 mg · g^{-1} value of K3 was recorded in the PDB medium.

The K4 fungal isolate showed the highest uptake value efficiency 0.1171 mg · g^{-1} for Cu removal from sample S1 in the PDB medium. The uptake value 0.1146 mg · g^{-1} K4 was displayed in the SDB medium. And the uptake efficiency value 0.1119 mg · g^{-1} was recorded in YPD medium. While 0.1139 mg · g^{-1} uptake value efficiency was observed in the CDB media of the K4 strain. The K5 fungal isolate showed the highest uptake efficiency 0.1131 mg · g^{-1} in the PDB medium. K5 lowest uptake efficiency was noticed at 0.1101 mg · g^{-1} in the YPD medium. In the SDB and CDB medium 0.1114 mg · g^{-1} and 0.1102 mg · g^{-1} uptake efficiency of K5 was recorded for Cu removal. The K7 fungal isolate showed the highest uptake efficiency for Cu removal was 0.0710 mg · g^{-1} in

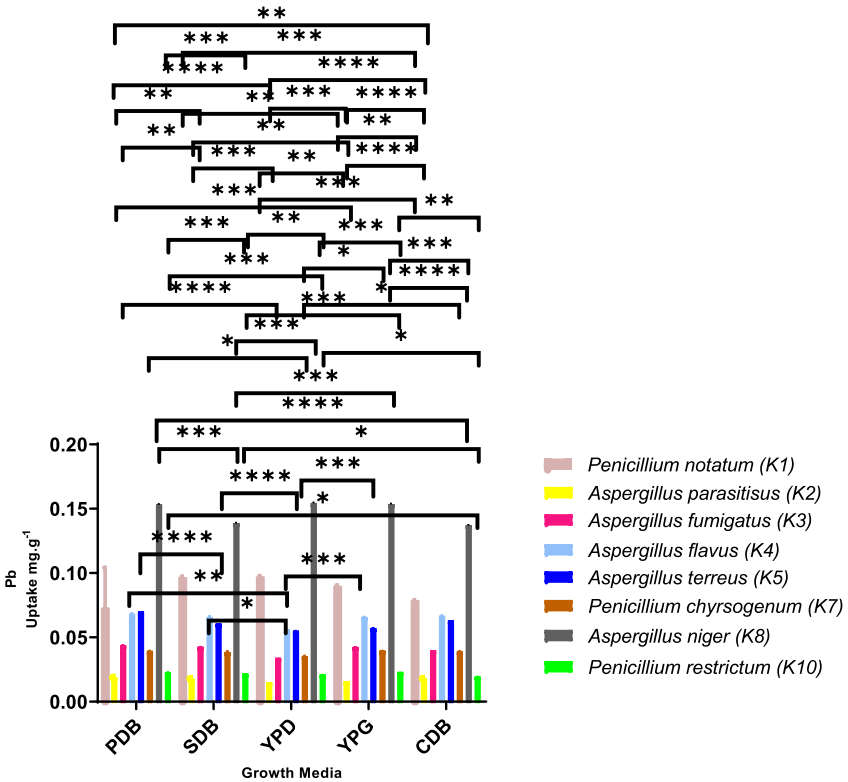

Figure 1. Uptake of Pb (mg · g^{-1}) by indigenous fungal isolates in PDB, SDB, YPD, YPG & CDB medium. Values are presented as mean ± SEM (n =3). Bar with asterisks differ significantly, *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

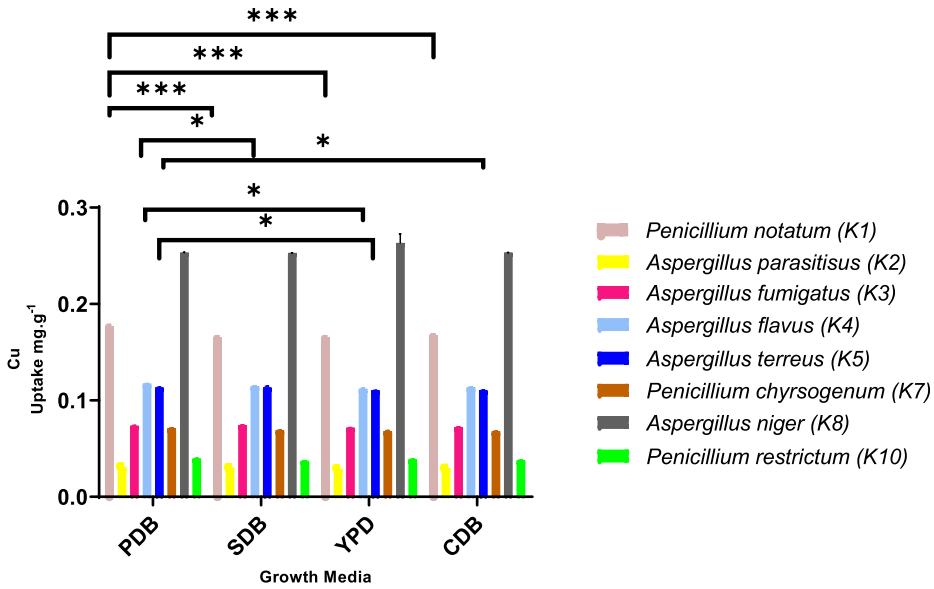

Figure 2. Uptake of Cu (mg · g^{-1}) by indigenous fungal isolates in PDB, SDB, YPD & CDB medium. Values are presented as mean ± SEM (n = 3). Bar with asterisks differ significantly, *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

the PDB medium. The least uptake value was recorded at 0.0679 mg · g^{-1} in the CDB medium of K7 fungal strain, whereas in the SDB and YPD medium 0.0685 mg · g^{-1} and 0.0683 mg · g^{-1} uptake efficiency of K7 was recorded for the removal of Cu from the soil sample S1. The K8 fungal strain showed the highest uptake efficiency for the removal of copper in the YPD medium which was about 0.2544 mg · g^{-1}. In the CDB medium, 0.2535 mg · g^{-1} was shown. Whereas, in the SDB and PDB medium 0.2531 mg · g^{-1} and 0.2531 uptake efficiency of K8 fungal strain was noted.

The maximum uptake value of efficiency of K10 fungal was recorded at 0.0395 mg · g^{-1} in the PDB medium. Whereas, 0.0389 mg · g^{-1} uptake efficiency for Cu removal of K10 was recorded in YPD medium. In the SDB medium, 0.0371 mg · g^{-1} uptake efficiency of K10 fungal strain for the removal of Cu was monitored.

Detection of a highly effective metal-resistant fungal isolate by using bioleaching analysis

The results of the current study showed that the K8 fungal isolate could biosorbate the maximum amounts of the heavy metals copper (0.2544 mg · g^{-1}) and lead (0.1542 mg · g^{-1}) in different growth mediums. Based on current results, the said strain was used for further investigation of identification and bioremediation mechanism.

Identification of fungal isolates

Phenotypical characterization

All the fungal isolates were cultured on the plate of PDA, SDA, and MEA media for 7 days at 25 °C for morphological identification and microscopy was done for investigation of hyphal morphology and spores' production (data not shown).

Genotypical characterization

Phylogenetic analysis of the amplified 18S rDNA sequences of all the fungal isolates was done for similarities index analysis between ITS and those in the NCBI database. The topology of the phylograms confirmed that the indigenous metallotolerant fungal isolate belongs to different fungal species. The topology of the phylograms established that the fungal isolates used in this study were allocated to; K1 (*Penicillium notatum*), K2 *(Aspergillus parasiticus),* K3 (*Aspergillus fumigatus*), K4 (*Aspergillus flavus*), K5 (*Aspergillus terries*), K6 (*Fusarium solani*), K7 (*Penicillium chrysogenum*), K8 (*Aspergillus niger*), K9 (*Penicillium piceum*) and K10 (*Penicillium restrictum*) (Figure 3).

Identification of functional groups using Fourier transform infrared spectrum.

The FTIR spectra of K8 fungal isolates which showed maximum uptake and removal efficiency of heavy metals, were analyzed to identify which of the functional groups are involved in the bioremediation mechanism. The protonated amido of protein and the N-acetyl glucosamine polymer of chitin are primarily involved when metals bind during bioleaching. The K8 strain's infrared peak absorption frequencies are listed along with the corresponding functional groups. The FTIR spectra showed peaks at 3347.26, 2145.24, 1639.25 and 723.68 cm^{-1}. The value at these peaks confirms the presence of hydroxyl group, alkynes, amide, and alkyl halide (Figure 4 & Table 4).

Scanning Electron Microscopy (SEM) of K8 fungal strain

The SEM was used to detect the results of the bioleaching of both Copper and Lead onto the surface of the fungal strain that showed maximum bioleaching capability. By conducting SEM studies, it was noticed that the

Table 4. The corresponding FTIR absorption frequencies for the functional groups of K8 biomass[24]

Peaks of FTIR	Frequency	Functional group	Assignment
1	3347.26	Hydroxyl(-OH)	-NH stretching
2	2145.24	C=C	Stretching bond of the alkyne's molecule
3	1639.45	C=O chelate stretching of O-C NH2	-NH wagging vibration of amide I
4	723.68	C-Cl	Scissoring vibrations

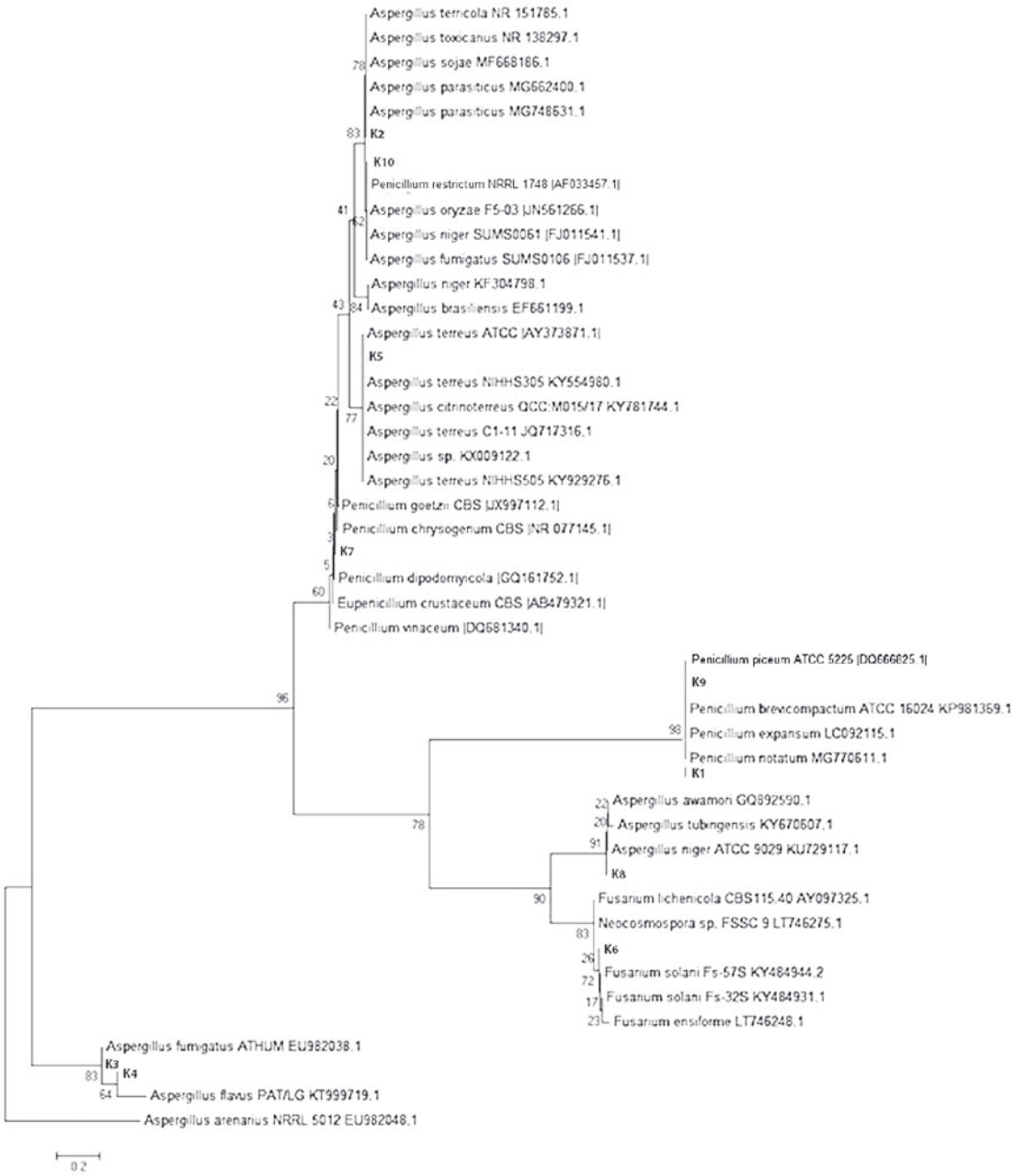

Figure 3. Phylogenetic tree of the fungal isolates with closest relatives based on a maximum parsimony analysis of ITS sequences by using Mega 4.0 software

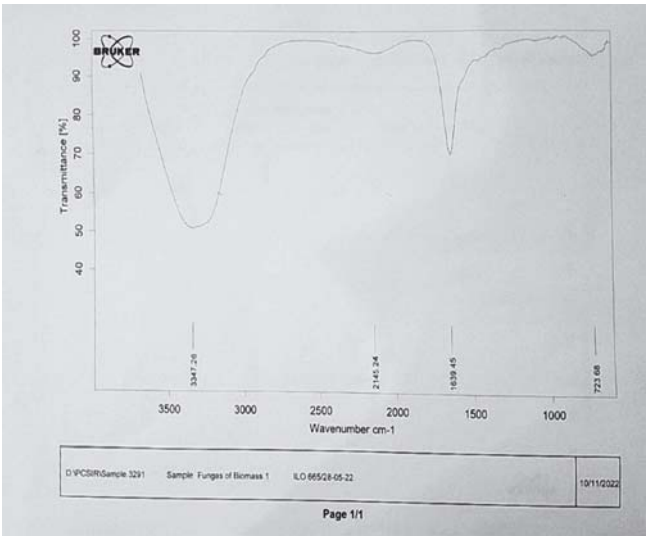

Figure 4. FTIR spectra of metal loaded biomass with Cu and Pb

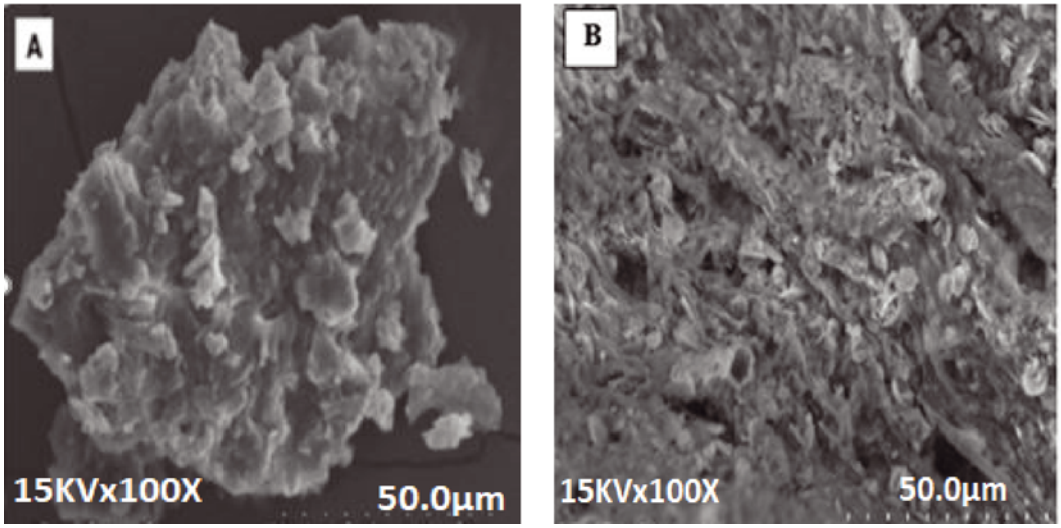

Figure 5. SEM of K8 biomass (A) before and (B) after the bioleaching

hyphae of the K8 strain were branched, conidial, and septate in Figure 5A, before contact with heavy metals. Whereas after the bioleaching due to the exposure of heavy metals, the typical change was monitored in the morphology of hyphae in Figure 5B, due to the metal stress K8 formed hyphal coils and curling of hyphae (Figure 5).

DISCUSSION

The term "environmental pollution" refers to the normal environmental processes, such as biological and physical components of the earth and atmosphere, which are extremely contaminated. Heavy metals are the major class of environmental pollutants[24]. In developing nations, the concentration of heavy metals has risen above normal levels to a high level due to anthropogenic and natural pollutants, which have a significant negative impact on the environment. The following list of sources contributes to the high rate of heavy metal pollution: natural weathering, mining, soil erosion, industrial effluent discharge, urban runoff, sewage effluents, air pollution fallout, and along with a few others[25]. Heavy metals are to blame for long-term negative health effects. Cu, Ni, Cd, Zn, Cr, and Pb are a few of the many metals that contribute significantly to soil pollution. Heavy metals' hazardous nature appears to have immediate effects on organisms and the environment and can spread up the food chain[26]. In terrestrial ecosystems, heavy metal pollution is transported by soil. The release of solid wastes, waste air, and wastewater by industrial activities pollutes the soil environment[27].

In the current study, soil samples were taken from the Korangi Industrial Estate, which is a major source of heavy metal pollution. Due to the lack of adequate waste treatment facilities, Korangi Industrial Estate contributes to pollution. The total metal content of Cu and Pb in the soil sample (S1) was recorded at 1.044 mg/l for copper and 0.631 mg/l for lead. The current study is in corresponds with another study in which, topsoil in the Shenyang Tiexi Industrial District had average copper and lead contents of 92.45 mg/kg and 116.76 mg/kg, respectively[28]. About seven heavy metals were isolated in the soil in the agricultural production area of the Shenyang suburb. As was found to be the least concentrated heavy metal at 11.96 mg/kg, followed by Cr at 96.2 mg/kg, Cu at 43.7 mg/kg, Pb at 102 mg/kg, and Zn at 52.7 mg/kg in the results[29]. A total of eight indigenous fungal strains were isolated from contaminated soil in the current study and process for their capability to remediate the heavy metals from the soil. The ability of fungi to remove heavy metals from contaminated soil is strong, and they have a high tolerance rate for heavy metals[30]. Fungi, among other microorganisms, are important because they can adapt to and grow in a variety of extreme conditions, including pH, temperature, the availability of nutrients, and high metal concentrations[31]. Indigenous fungi can grow in the presence of heavy metals due to their physiological adaptation[32].

In the current study, eight indigenous fungal strains (K1, K2, K3, K4, K5, K7, K8, and K10) were used and optimized for bioremediation of copper and lead from heavy metals contaminated industrial soil by using five different types of medium i.e., PDB, SDB, CDB, YPG, and YPD. Potato dextrose agar (PDA) is recommended as a growth medium for fungi isolated from various environmental sources. Fungi have a high potential for remediation due to their aggressive growth, concentrated biomass, and increased hyphal production in the soil. Fungi-based bioremediation used for industrially contaminated soil and water[30]. Different fungal strains exhibited different behaviors when exposed to heavy metals, with some being sensitive, moderately tolerant, and tolerant. According to an observational study, some Aspergillus flavus species were more resistant to the heavy metals (Cr, Pb), whereas other species could tolerate Pb but were sensitive to Cr The isolates of *Aspergillus niger* were identified as Cr-tolerant strains in a related study[33]. The K1 strain showed the highest uptake efficiency of 0.1769 mg/g and 0.1037 mg/g with 96.6% and 93.6% for Cu and Pb by using PDB medium, respectively, and lowest uptake efficiency of 0.1654 mg/g and 0.0784 mg/g with 90.3% and 70.8% was observed on YPG and CDB media. While K2 fungal strain showed the highest uptake efficiency of 0.0310 mg/g and 0.0187 mg/g with 96.2% and 96.3% for Cu and Pb in PDB medium and the least uptake efficiency of 0.0293 mg/g and 0.0150 mg/g with 90.0% and 77.1% was observed in YPG medium.

The K3 fungal strain showed the highest uptake efficiency 0.0741 mg/g and 0.044 mg/g for Cu and Pb with

95.1% and 94.5% in SDB and PDB media, respectively. The least uptake efficiency of 0.0716 mg/g and 0.034 mg/g with 91.9% and 73.4% was observed in YPG medium. The K4 fungal strain on the other hand showed the highest uptake efficiency of 0.1171 mg/g and 0.068 mg/g for Cu and Pb with 95.3% and 92.9% in PDB medium, respectively, and the least uptake efficiency of 0.1119 mg/g and 0.0559 mg/g with 91.1% and 75.3% was recorded in the YPD medium. The K5 fungal strain showed the highest uptake efficiency of 0.1131 mg/g and 0.0701 mg/g with 94.3% and 96.7% for Cu and Pb in PDB medium, respectively, and the lowest uptake efficiency of 0.1101 mg/g and 0.05518 mg/g with 91.7% and 76.0% in the YPD medium. Furthermore, The K7 fungal strain showed the highest uptake efficiency 0.0710mg/g and 0.0398 mg/g for Cu and Pb with 95.9% and 93.6% in PDB and YPG media, respectively. The least uptake efficiency of K7 was recorded in the CDB and YPG media i.e., 0.0679 mg/g and 0.0353 mg/g with 91.7% and 78.9% removal efficiency of Cu and Pb, respectively. The K8 showed the highest uptake efficiency of 0.2544 mg/g and 0.1542 mg/g for the copper and lead removal with 92.6% and 92.8% in YPG, while low uptake efficiency value of K8 was recorded in the PDB and CDB media i.e., 0.2531 mg/g and 0.1369 mg/g with 92.1% and 82.4% removal efficiency. The K10 fungal strain optimized on PDB and YPG medium showed the highest uptake efficiency of 0.0395 mg/g and 0.0231 mg/g for Cu and Pb removal with 93.1% and 90.3% bioleaching efficiency. The least uptake efficiency of K10 i.e., 0.0371 mg/g and 0.0193 mg/g was recorded in the SDB and CDB media with 87.5% and 75.4% removal efficiency, respectively.

In the alike study, *Aspergillus niger* showed the highest tolerance to each metal salt, according to soil samples taken from Faisalabad, according to an observational study of different industrial areas. *Aspergillus niger* of the Rawalpindi district soil samples showed the highest tolerance, followed by *Aspergillus* sp. *Penicillium sp.* and *Fusarium sp.* of Jappaywala, Faisalabad, showed tolerance towards Pd > Zn, Ni > Cd. *Aspergillus niger, Aspergillus species, Penicillium species,* and *Fusarium species* have been used to remove the heavy metals Cu, Zn, Ni, Pd, and Cd[32]. Similarly, when compared to *Penicillium* sp.*, Fusarium* sp.*,* and *Aspergillus* sp.*, Aspergillus niger* and *Aspergillus* sp. demonstrated greater tolerance (Zn > Ni > Pd >Cd)[33]. Among all the eight indigenous fungal isolates, K8 showed the highest uptake efficiency and removal efficiency for both copper and lead, that why the K8 was selected and phenotypical characterizations and topology of phylogram confirm the K8 isolate as *Aspergillus niger*. In the alike study*, Aspergillus, Rhizopus, Penicillium, Fusarium, Chaetomium, Geomyces*, and *Paecilomyces species* have all been found in heavy metal-contaminated soil (Cu, Cd, Pb, As, and Zn)[34]. Numerous fungus genera have been discovered in the soil contaminated with metal[35]. *Aspergillus niger, Penicillium simplicissimum, Penicillium purpurogenum, Rhodotorula rubra,* and *Acidithiobacillus ferrooxidans* are among the significant fungi species for heavy metal removal[36]. Numerous isolates of *Aspergillus* spp.*, Cladosporium cladosporioides, Fusarium oxysporum, Gliocladium roseum, Penicillium* spp.*, Tala-romyceshelicus*, and *Trichoderma koningii* were found in the highly industrialized and metal-polluted area of La Plata, Argentina, according. Further research reveals that different 41 fungal species, including *Aspergillus flavus, Aspergillus niger, Fusarium* sp.*, Penicillium* sp.*,* and *Rhizopus* sp.*,* were isolated from Rawalpindi's industrially polluted soil and that were found to be metal resistant[37].

The FTIR spectra of *Aspergillus niger* (K8) strain were examined after the bioleaching experiment. The protonated amido of protein and the N-acetyl glucosamine polymer of chitin are primarily involved in metal binding during bioleaching. The K8 strain's peaks' infrared absorption frequencies are listed along with their corresponding functional groups. The broad adsorption peak of the FTIR spectra of the -OH groups of glucose and the -NH groups of proteins was found at a wavelength of approximately 3347 cm^{-1} [38, 39]. The functional groups in the N-acetyl glucosamine polymer or the protein peptide bond around the absorption peak at 1639, respectively, cause the amide I bands of the amide bond[38, 40]. Alkynes molecules' stretching bond is responsible for the absorption band at 2145. It is most likely that the C-Cl scissoring of alkyl halide is responsible for the weak absorption band between 600 and 800 cm^{-1} [24]. FTIR spectra of metal-loaded biomass with Cu and Pb. Alkynes, hydroxyl, and carboxyl groups can be found on the surface of K8 biomass. Because the shifting of peak characteristics to these groups was observed, it is evident that the mechanism of leaching for Cu and Pb involves a chemical interaction between Cu and Pb ions and hydroxyl, alkynes, and carboxyl group from the surface of fungal biomass.

On PDB medium, two-week-old cultures were grown, and the fungal biomass was then dried at 60 °C, treated with 10% glutaraldehyde, and incubated for roughly 10 to 12 hours at 4 °C. The pretreated specimen was then imaged under an SEM at an accelerating voltage of 12 or 15 kV after being sputtered with gold particles using a sputter coater under vacuum. Scanning electron microscopy (SEM) was used to detect the effects of the bioleaching of heavy metals (Cu and Pb) onto the surface of K8 fungal strain. By conducting SEM studies, it was noticed that the hyphae of the K8 strain were branched, conidial and septate before contact with heavy metals. Whereas after the bioleaching due to the exposure of heavy metals, the typical change was monitored in the morphology of hyphae due to the metal stress K8 formed hyphal coils and curling of hyphae.

Heavy metals toxicity to fungi involves many factors essential functional groups of enzymes are blocked, in the cells the conformational changes of polymers occur, essential metals are displaced, and many other transport processes are responsible[41]. In another similar study in response to other heavy metals exposure[42]. Paraszkiewicz and Courbot have also examined that the metal stresses presence had led to the formation of thiol compounds, according to them, intracellular vacuoles production is increased which results in the cell wall protrusions that perform the function of storage compartments for thiol--containing compounds which leads to binding of metal ions into the intracellular regions and store them in the vacuoles[43–50].

CONCLUSION

Mycoremediation is an effective productive and advanced technique for the removal of heavy metals from contaminated soil and water. In the metal contaminated environment microorganisms adopt different mechanisms for their survival in the metal polluted environment. In the current study eight indigenous fungal isolates showed the capability to remove the heavy metals but, K8 demonstrated the greatest capacity to remove Cu and Pb from the environment, making it a promising candidate for making the economical and environment eco-friendlier. The limitation, however, is that these fungi strains could be further analyzed for any type of toxin production that may affect human health as well as other living things. Therefore, they must be treated in a way that limits their capacity to spread disease and increases their ability to tolerate metals, which will help keep the environment free of metal pollution.

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