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The Efficiency of some Locally Isolated Fungi on Removing Pb, Cd, Cr and Ni and their Mixture from Wastewater

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ABSTRACT

Resources reuse has become an important feature of wastewater management. Wastewater mainly from paint, leather, metal, and tanning industries contain huge amounts of heavy metals. As conventional methods to remove metals from aqueous solutions are not effective enough mainly at low metal concentration and too expensive, alternative methods are required. Microorganisms including fungi have been reported to remove heavy metals from wastewater through bioaccumulation and biosorption at low cost and in eco-friendly way. *Aspergillus terreus, A. niger, A. flavus* and *Talaromyces purpurgenus* were isolated from different wastewater samples in Egypt. These isolates exhibited high efficiency in removal of Pb, Cd, Cr and Ni from wastewater, respectively and they can tolerate up to 400 ppm concentration of Pb, Cd, Cr, and Ni. *Aspergillus niger* showed high efficiency in removing mixture of these heavy metals, this was confirmed by scanning electron microscope coupled with energy dispersive spectroscopy that showed a high amount of metals inside fungal mycelium.

1. Introduction

Water pollution with heavy metals is one of the greatest consequences of industrialization in the area of mining, petroleum refining, automobiles, paints etc. [1]. Heavy metals have been defined and described as "naturally occurring metals having atomic number greater than 20 and an elemental density greater than 5 g cm^{-3} [2]. Heavy metals are prominent contaminants because they are toxic, non-biodegradable in the environment, and easily accumulated in living organisms [3].

Various studies have been conducted to minimize or eliminate the heavy metals existing in the environment using conventional processes include precipitation, reverse osmosis, adsorption onto activated carbon or alumina, and redox processes [4]. Though, these conventional techniques have drawbacks such as slow and inefficient removal, generation of contaminated sludge requiring careful disposal, high cost and energy involved in the processes, and blockage of membranes [5, 6]. Consequently, there is a need for a cheap and effective technology to remove heavy metals with an eco-friendly method, this has been increasing the interest in the use of biological agents for heavy metals removal as an alternative to these methods [7].

Bioremediation is a technique for transforming harmful contaminants like heavy metals into less harmful materials; or removing toxic elements from the contaminated environment; or degrading organic substances and ultimate mineralization of organic

substances into carbon dioxide, water, nitrogen gas, etc., using dead or alive biomass [7]. Microorganisms including fungi have been reported to eliminate heavy metals through bioaccumulation and biosorption at low cost and in eco-friendly way.

Fungi can be developed easily, produce high yield of biomass, and can easily be genetically and morphologically manipulated. Fungi appear high resistance to the large amount of heavy metals and simultaneously can accumulate micronutrients (Cu, Zn, Ni, Co and Mn) and non-nutrient metals (Cd, Pb, Hg and Ag). Cell wall of fungi is composed of chitin, lipids, mineral ions, polysaccharides, polyphosphates, and proteins. Several mechanisms were included in heavy metal bioremediation by fungi as they could degrade heavy metal ions by extracellular and intracellular precipitation, energetic uptake or by converting the valency of the metal ions, many fungi also can accumulate metals into their spores and mycelium [8]. The present study was intended to explore local fungal isolates that have potential capability to bioremediate heavy metals Chromium, Lead, Nickle, Cadmium, from highly polluted wastewater, evaluate the performance of each isolated fungi using mixture of heavy metals and to evaluate its performance under laboratory conditions at different physical and chemical parameters.

2.Materials and Methods

2.1 Collection of wastewater samples

A total of six wastewater samples were collected from industrial effluents, tannery, drainage, and sewage wastewater during the period from October 2018 to December 2018 from different six areas in Egypt (El. Khosos, Shbin al kantar, Kalyobya, Sharkya, Alexandria, Soor magra el eyoon). All samples were collected in sterile containers and kept in refrigerator at 4ºC for further processing.

2.2 Isolation of fungi from wastewater

Fungal species were isolated from wastewater on potato dextrose agar (PDA) containing 25 ppm of Pb, Ni, Cd and Cr individually. Stock solutions (1000 ppm) of Pb, Ni, Cd and Cr were made in double distilled water using $Pb(NO_3)_2$, $NiCl_2.6H_2O$, $CdCl_2$ and $K_2Cr_2O_7$. The stock solutions of heavy metals were sterilized separately through bacteriological filters and added to sterilized PDA medium to reach the concentration 25 ppm [9].

A serial dilution of each sample was made up to 10^6 and one ml of dilution was added in sterile Petri plates in duplicate manner.

Twenty ml of prepared PDA medium containing 25 ppm of one of these heavy metals were poured in these sterilized Petri plates and incubated at 28ºC for 4-6 days. The colonies of predominant fungal genera were collected and purified by pour plate method. The purified fungal isolates were identified on the basis of their macroscopic characteristics on different culture media (PDA, SDA) [10, 11], and microscopic characteristics of preparations stained with lacto phenol cotton blue (LCB) [12, 13].

2.3 Screening of fungal isolates for tolerance to heavy metals

Recovered fungal isolates were further screened by fine methodology for tolerance to Pb, Ni, Cr and Cd at 50, 100, 400, and 800 ppm of heavy metals individually on PDA. Fungal isolates were streaked on PDA medium containing 50, 100, 400 and 800 ppm of each heavy metal. Normal PDA medium served as control. Observations on growth of fungal isolate were made after 72 hours of incubation at 28ºC. The growth of fungal isolates was recorded as normal growth or no growth in comparison to control respectively [9].

2.4 Removal of heavy metals by fungal isolates from liquid media

The most tolerant fungal isolates at different heavy metals were evaluated for uptake of heavy metals on potato dextrose broth medium containing 100 ppm concentration of different heavy metals (Pb, Ni, Cr and Cd) individually in duplicate. The Potato dextrose broth (PDB) containing 100 ppm of each of the prepared heavy metals was dispensed in 100 ml lots to 250 ml conical flasks and sterilized at standard conditions for 15 min and inoculated with 1 ml of freshly fungal spore suspension (10^6 spores/ml) and kept in shaking incubator at 150 rpm at 27±2ºC for 4 days. Control flasks having only PDB broth of 100 ppm concentration of Pb, Ni, Cr, Cd served as control [9].

Fungal growth was collected after 4 days through filtration using Whatman filter paper. The collected fungal mass was washed with double distilled water 2–3 times and dried in hot air oven at 70±5ºC for 1 day. The volume of the filtrate was made to 50 ml in volumetric flask. The concentration of heavy metals in filtrate was estimated by Atomic Spectrophotometer [14].

Tolerance and uptake of mixed heavy metals (Pb, Cd, Cr and Ni) by most tolerant fungal isolate in100 ml PDB broth containing 100 ppm each of Pb, Cd, Cr and Ni was studied by the same method.

The uptake of heavy metal by fungal biomass was calculated using the following equation:

qe (mg/g) = C *V *1000/W

Where, qe: is the concentration of heavy metal uptake by fungal biomass (mg/g); C is the concentration of heavy metal (ppm); V (ml): is the volume of the medium and W (g): is the dry weight of the fungal biomass [15].

The removal percentage of heavy metal by fungal biomass was calculated using the following equation:

Metal removal % = (Ci-Cf)/Ci *100

Where Ci is the initial concentration of heavy metal and Cf is the final concentration of the heavy metal [16].

2.5 Optimization of parameters process

The most suitable parameters for the best uptake of Pb, Cd, Cr and Ni by the most tolerant, potent fungal isolates for each heavy metal, were chosen using potato dextrose medium supplemented with 100 ppm of each heavy metal $Pb(NO_3)_2$, $NiCl_2.6H_2O$, $CdCl_2$ and $K_2Cr_2O_7$.

These parameters include carbon source (glucose, pectin, sucrose, and potato extract) in 2 gm of each carbon source, pH adjusted to 5.5 with inoculum size 1×10^6 spores/ml of fungal isolate for 7 days at 28 °C in a rotary shaker at 150 rpm and samples were collected after 7days. For the optimization of pH, the potato dextrose medium supplemented with 100 ppm of Pb, Ni, Cd and Cr adjusted at different pH values; 5,7 and 9 with inoculum size 1×10^6 spores/ml of fungal isolate and incubated for 7 days at 28 °C with shaking in a rotary shaker at 150 rpm. For the optimization of inoculum size each flask containing potato dextrose medium supplemented with 100 ppm of each heavy metal, pH was adjusted to 5.5 then inoculated with 0.5 ml, 1 ml, 2 ml of each heavy metal with inoculum size 1×10^6 spores/ml of fungal isolate for 7 days at 28°C in a rotary shaker at 150 rpm. For the optimization of time interval flasks containing potato dextrose medium with 100 ppm of each heavy metal with inoculum size 1×10^6 spores/ml of fungal isolate and incubated for 3,7and 9 days at 28 °C with shaking in a rotary shaker at 150 rpm. While for the optimization of temperature each flask containing potato dextrose medium supplemented with 100 ppm of each heavy metal with inoculum size 1×10^6 spores/ml of fungal isolate and incubated for 7 days at different temperatures 20 °C, 30 °C, 40 °C with shaking in a rotary shaker at 150 rpm.

2.6 Molecular identification of the most potent fungal isolate to remediate Pb, Cd, Cr and Ni

Identification of the most potent fungal isolates capable of lead, cadmium, chromate, and nickel reduction were additionally confirmed by ITS1-5.8S-ITS2 rDNA region sequence analysis provided by Sigma Scientific Service Company. Sequencing was performed by using forward and reverse primers by combining the traditional Sanger technology with 454 technology (GATC Company, Germany). A phylogenetic analysis was performed by using MEGA 7.0 [17] , using the neighbor- joining method , clustering of the isolates and related species was performed . the tree was constructed by using the p- distance of nucleotide difference [18].

2.7 Morphological examination of Aspergillus niger mycelia growing on mixed heavy metals by SEM and EDX

Scanning electron microscopy coupled with EDX was applied in demand to observe the morphological change occurred in mycelium of *Aspergillus niger* upon the remediation of mixture of Pb, Cd, Cr and Ni in comparison with the organism incubated in absence of those heavy metals as a control sample .The fungal mycelium of two samples were separated from broth medium and cut into small pieces about 5x5 mm then treated with 2.5% glutaral solution at 4°C for two hours to perform an outer fixation of samples then washed with phosphate buffer (50mM,xPH 6.8) after that samples placed in osmium tetroxide (OsO_4) 0.1M which used as a secondary fixative for two hours then dehydration of samples using series of 30, 50, 70, 85, 90, 95 and 100% of ethanol each for 10 minutes [19].

After that, gold spraying of samples using S160A Sputter Coater (BOC Edwards, UK) was performed, finally the samples were examined and photographed using Quanta™ 250 FEG (FEI company, Netherland). Energy dispersive spectroscopy (EDX) analyses for both exposed and non-exposed samples were performed.

2.8 Statistical Analysis

All experiments were repeated 3 times, the data shown in tables and figures were the mean values of the experiments. Data were presented as mean and standard deviation (SD) values. One-way ANOVA test was used for the comparison between the levels of each factor. The significance level was set at α (p-value) $\leq$ 0.05.

3.Results

3.1 Isolation of fungal isolates from wastewater

A total of twelve fungal isolates belonging to seven genera (*Aspergillus*, *Alternaria*, *Penicillium, Cladosporium, Talaromyces, Trichoderma* and *Rhizopus*) were isolated from the six areas. These isolates were identified according to their macro and micro morphological characteristics as shown in Table 1.

Table 1 Fungal species isolated from studied sites

Fungal species	Isolate location					
	El.khosos	Shbin al kantar	Kalyobya	Sharkya	Alexandria	Soor magra el eyoon
1- *Aspergillus fumigatus*	√	√	√	√	√	√
2- *Aspergillus flavus*	√	√	√	√	√	√
3- *Aspergillus niger*	√	√	√	√	√	√
4- *Aspergillus terreus*	√	√	√			
5- *Alternaria* sp.		√			√	√
6- *Aspergillus parasiticus*				√		
7- *Penicillium chrysogenum*	√	√	√	√	√	√
8- *Penicillium brevicompactum*				√		
9- *Rhizopus* sp.		√	√			
10- *Trichoderma* sp.					√	√
11- *Cladosporuim* sp.						√
12-*Talaromyces purpuregenus*					√	

3.2 Tolerance and screening of fungal isolates from wastewater

The degree of removal of heavy metals varied from fungal isolates to another. *Aspergillus terrus* showed the highest removal of Pb (93%) after 7th day of incubation. *Talaromyces purpuregenus* showed the highest removal of Ni (65%). *Aspergillus niger* showed the highest percentage of Cd removal (84.4%), while *Aspergillus flavus* exhibited the highest Cr removal (49%) as shown in Fig. 1 - 4.

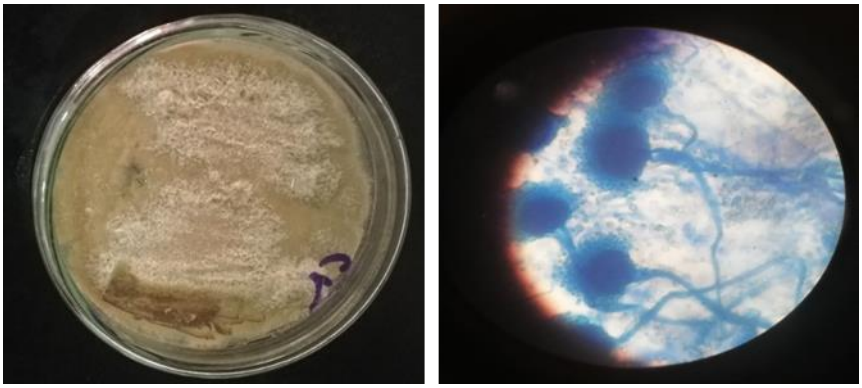

Fig. 1 Macroscopic and microscopic characteristics of *Aspergillus flavus* on PDA

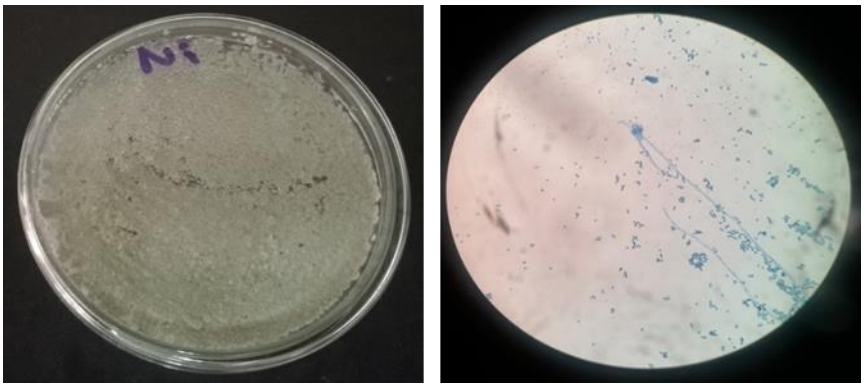

Fig. 2 Macroscopic and microscopic characteristics of *Talaromyces purpurgenus* on PDA

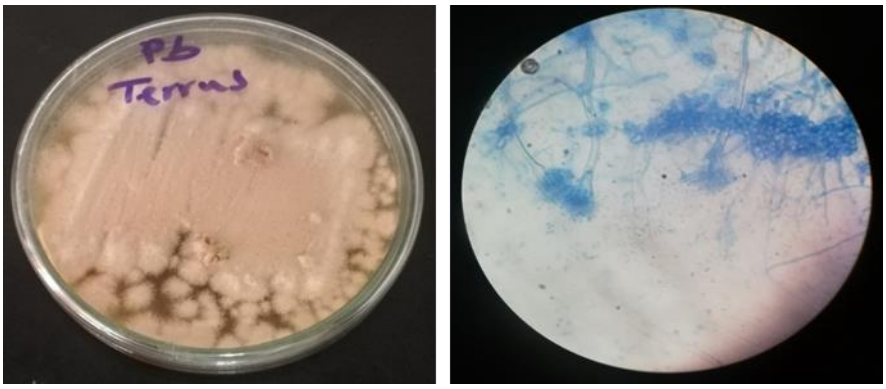

Fig. 3 Macroscopic and microscopic characteristics of *Aspergillus terreus* on PDA

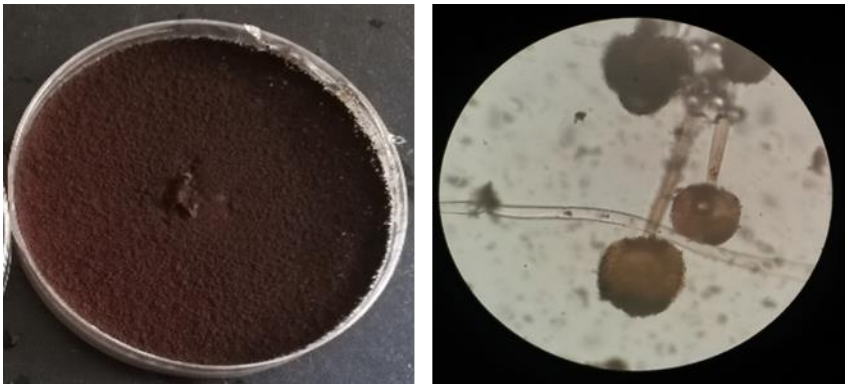

Fig. 4 Macroscopic and microscopic characteristics of *Aspergillus niger* on PDA

3.2.1 Growth and uptake of Pb by fungal isolates

A. terreus exhibited the value quantity of Pb uptake (281.995 mg/g) followed by *A. fumigatus* (11.093 mg/g), *A. flavus* (9.027 mg/g), *A. niger* (6.0985 mg/g), *P. chrysogenum* (2.206mg/g), *P. brevicompactum* (1.807mg/g) and *A. parasiticus* (1.135 mg/g), While *Alternaria* sp.showed the lowest value of Pb uptake (0.382 mg/g).

For absorbance of Pb by the fungal isolates, *A. terreus* showed the highest absorbance value (23.113 ppm), followed by *A. niger* (21.183 ppm), *P. brevicompactum* (15.577 ppm), *A. flavus* (14.65 ppm), *A. fumigatus* (11.603 ppm), *P. chrysogenum* (8.207 ppm) and *A. parasiticus* (5.517 ppm) while *Alternaria* sp. showed the least absorbance value of Pb (4.957 ppm) Fig. 5.

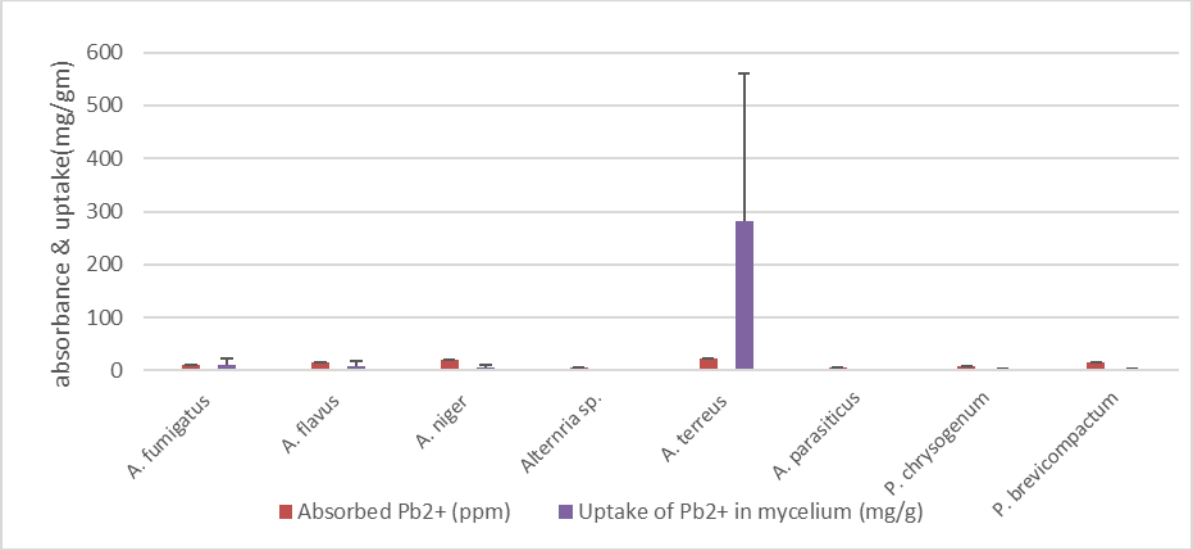

Fig. 5 Absorbance and uptake of Pb by different fungal isolates

3.2.2 Growth and uptake of Ni by isolated fungal species

Talaromyces purpuregenus exhibited the highest value of Ni uptake (0.625 mg/g) followed by *A. flavus* (0.222 mg/g) and *Rhizopus* (0.177 mg/g) while *P. chrysogenum* showed the least uptake quantity of Ni uptake (0.018 mg/g). For the absorbance of Ni by the fungal isolates *T. purpuregenus* showed the highest absorbance value (6.55 ppm) followed by *Rhizopus* (2.693 ppm) and *A. flavus* (2.474 ppm) while *P. chrysogenum* showed the lowest absorbance value (0.294 ppm) Fig. 6.

3.2.3 Growth and uptake of Cd by isolated fungal species

A. niger exhibited the highest value of Cd uptake (34.336 mg/g) followed by *P. chrysogenum* (7.036 mg/g), *Alternaria* sp. (3.025 mg/g), *Trichoderma* sp. (1.954 mg/g) and *A. fumigatus* (1.282 mg/g) while *Rhizopus* sp. showed the lowest uptake of Cd with (0.115 mg/g). For the absorbance of Cd by the fungal isolate, *A. niger* showed the highest absorbance value (17.283 ppm) followed by *P. chrysogenum* (11.867 ppm), *Rhizopus* sp. (5.9 ppm), *Alternaria* sp. (2.88 ppm) and *Trichoderma sp*. (2.35 ppm) while *A. fumigatus* showed the lowest of Cd absorbance (0.227 ppm) Fig. 7**.**

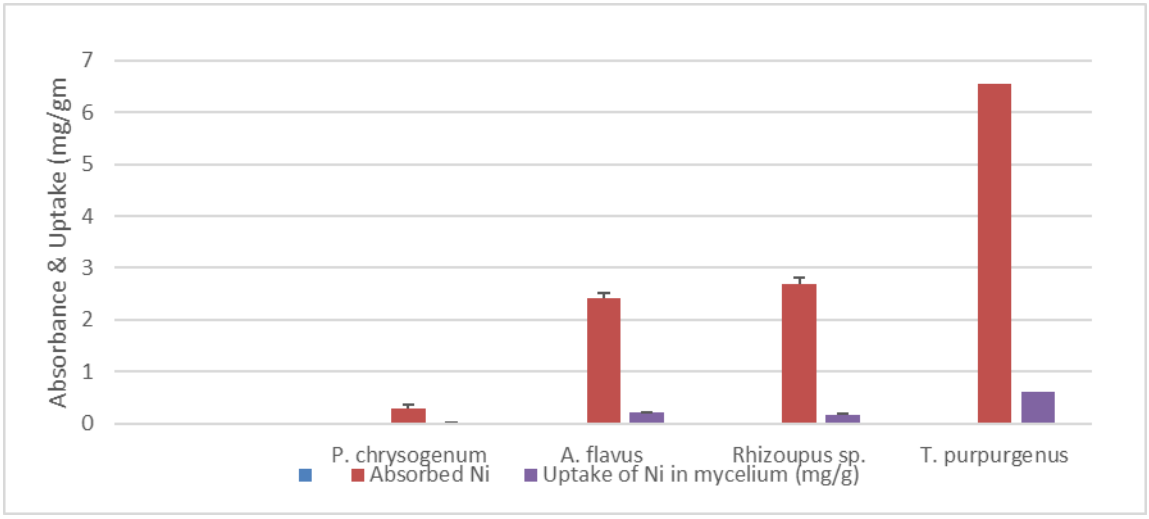

Fig. 6 Absorbance and uptake of Ni by different fungal isolates

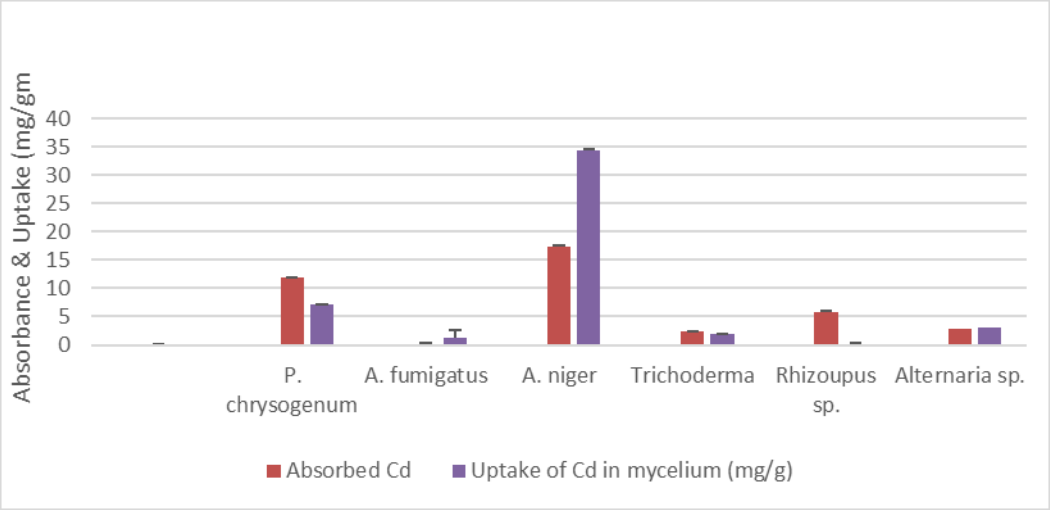

Fig. 7 Absorbance and uptake of Cd by different fungal isolates

3.2.4 Growth and uptake of Cr by isolated fungal species

A. flavus exhibited the highest value of Cr uptake (0.951mg/g) followed by *A. fumigatus* (0.628 mg/g), *A. niger* (0.397mg/g), *Cladosporium* sp. (0.385 mg/g) and *P.chrysogeum* (0.345 mg/g) ,while *A.terreus* Showed the lowest quantity of Cr uptake (0.235mg/g). For the absorbance of Cr by the fugal isolate *A. flavus* showed the highest absorbance value (7.377 ppm), *A. niger* (3.653 ppm), *P.chrysogenum* (2.067 ppm), *A. fumigatus* (1.0927 ppm) and *A.terrus* (1.303 ppm) while *Cladosporium sp*. Showed the lowest of absorbance value of Cr (0.367 ppm) Fig. 8.

3.3 Molecular identification of the most potent fungal isolate:

The identification of the most potent fungal species in removing each of heavy metals (Lead, Nickle, Cadmium and Chromium) was further confirmed by molecular technique, as *Aspergillus terreus* (**MW673533**), *Aspergillus niger* (**MW673534**), *Aspergillus flavus* (**MW673531**), and *Talaromyces purpuregenus* (**MW673532**) respectively Fig. 9.

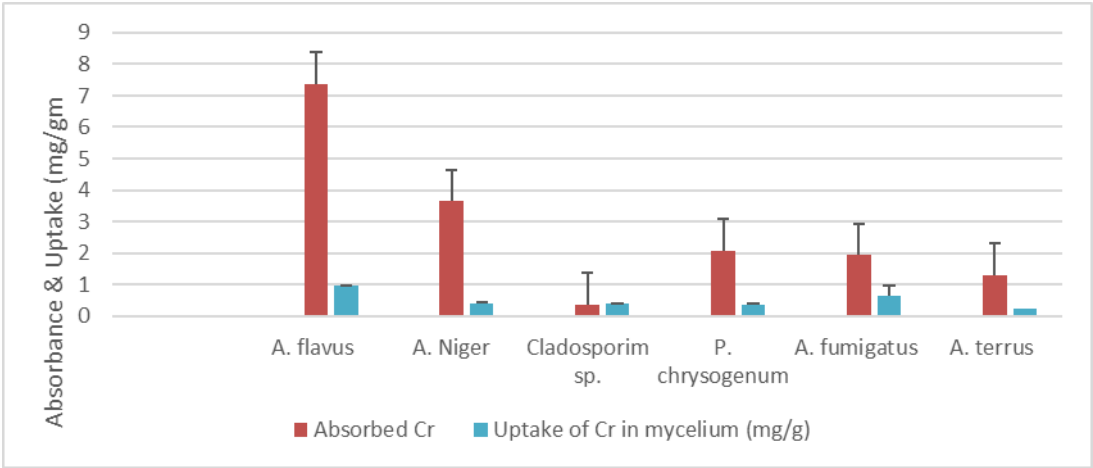

Fig. 8 Absorbance and uptake of Cr by different fungal isolates

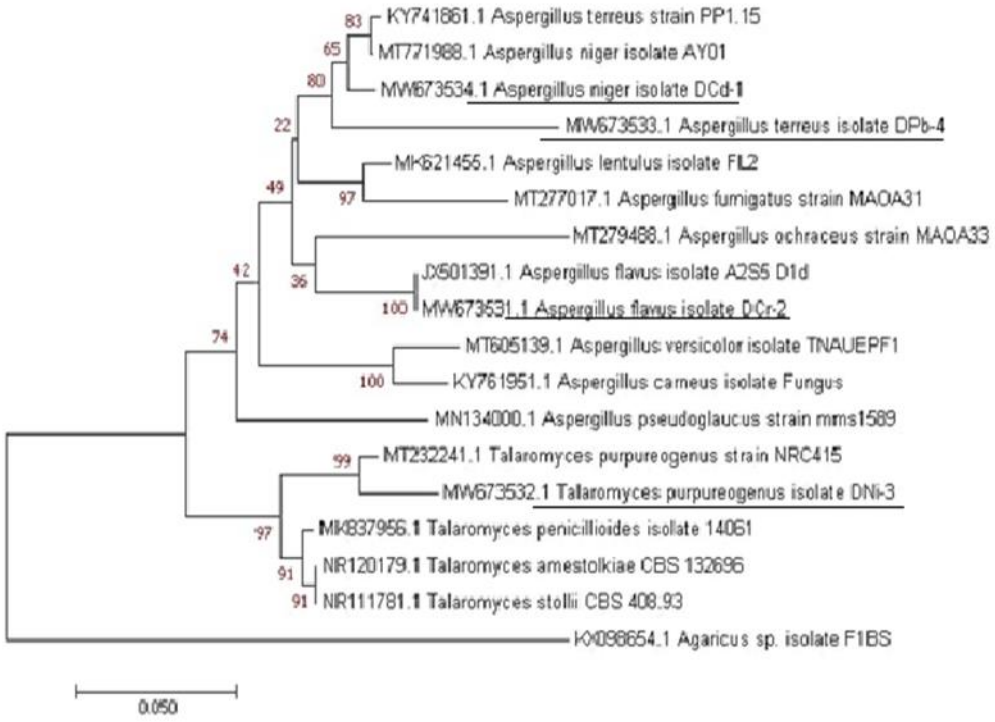

Fig. 9 Phylogenetic tree of the *Aspergillus niger*

3.4 Optimization of process parameters

A. niger exhibited the highest uptake with Potato dextrose broth containing 1.6220 mg/gm for glucose as a carbon source, while *A. flavus* and *A. terreus* showed the highest uptake (1.2323 and 1.4643 mg/gm) with sucrose as a carbon source respectively. As for *Talaromyces purpuregenus* pectin was the most suitable carbon source with uptake 1.6000 mg/gm as shown in Table 2**.**

In applying different pH intervals, *A. niger* showed at the highest uptake with 1.5797 mg/gm pH 7, while at pH 5 *A. flavus* and *A. terreus* showed uptake with 1.0863 mg/g, and 0.9747 mg/g respectively, while the highest uptake by *Talaromyces purpuregenus* occured at pH 9 with uptake 1.0200 mg/gm Table 2**.**

Different inoculum sizes of the heavy metals are applied as a parameter in the optimization process. *A. niger* and *A. terreus* showed the highest uptake of heavy metal at 0.5 ml inoculum size with uptake 1.3327 and 0.4197 mg/gm respectively, while *A. flavus* showed the highest uptake 1.17 mg/gm at 1 ml inoculum size, *Talaromyces purpuregenus* exhibited the highest uptake with 0.8030 mg/gm after applying 2ml inoculum size of the heavy metal Table 2**.**

Concerning the incubation period, the highest uptake of the heavy metals was obtained at 9 days' as shown in Table 2**.**

For the different temperatures, the highest uptake of heavy metals obtained at 30 ºC as shown in Table 2**.** with uptake 1.32 mg/gm for *Talaromyces purpurgenus*, 1.99 mg/gm for *A. niger*, 0,89 mg/gm for *Aspergillus flavus* and 0.59 mg/gm for *A. terreus.*

3.5 Scanning electron microscopy and energy-dispersive X-ray analysis (EDX)

The morphological changes in response to accumulation of mixture of Pb, Cd, Cr and Ni, in *Aspergillus niger* mycelium and the quantification of these heavy metals within fungal mycelium were analyzed by scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDX). SEM analysis of the fungus after 72 hrs of incubation without exposure to mixture of Pb, Cd, Cr and Ni (Fig. 10: a1) showed that fungal conidiophore were branched and smooth, with no peak of any heavy metals at 5.4 keV as shown in (Fig. 10: a2**)** Conversely, when cells treated with 400 ppm of mixture heavy metals, a significant peak of each heavy metal of the mixture at 5.4 keV was detected, indicating fungal adsorption to the mixture (Fig. 10: b2**)**, which was confirmed by the presence of particles of the heavy metals on the rough mycelium of *Aspergillus niger* as observed by SEM (Fig. 10: b1 **)**.

Table 3 Optimization factors for different heavy metals by selected fungal species

Optimization factors		Fungal isolates			
		T. purpuregenuse	*A. niger*	*A. flavus*	*A. terreus*
Carbon source	Potato	0.73±0.15	1.11±0.34	1.06±0.04	0.70±0.01
	Pectin	1.60±0.21	0.86±0.18	0.71±0.03	0.74±0.22
	Sucrose	0.71±0.04	0.94±0.18	1.23±0.04	1.46±0.15
	Glucose	0.60±0.11	1.62±0.08	0.86±0.04	0.60±0.11
pH	pH 5	0.88±0.02	1.17±0.11	1.09±0.06	0.97±0.05
	pH 7	0.98±0.03	1.58±0.08	0.83±0.09	0.75±0.03
	pH 9	1.02±0.11	0.41±0.09	0.88±0.05	0.49±0.10
Exposure time	3 days	1.02±0.09	0.35±0.05	0.39±0.06	0.63±0.09
	7 days	1.08±0.12	1.18±0.09	1.10±0.09	0.94±0.09
	9 days	1.42±0.14	2.99±0.02	2.20±0.21	1.12±0.06
Inoculum size	0.5 ml	0.42±0.02	1.33±0.06	0.12±0.02	0.42±0.03
	1 ml	0.49±0.04	1.17±0.13	0.91±0.06	0.14±0.04
	2 ml	0.80±0.08	1.21±0.10	0.15±0.04	0.41±0.03
Concentration of heavy metals	0.0025 ppm	1.35±0.05	1.41±0.01	1.05±0.05	0.44±0
	0.05 ppm	1.72±0.10	2.08±0.03	1.63±0.03	2.01±0.01
	0.1 ppm	1.01±0.01	1.46±0.04	0.96±0.06	0.92±0.00
Temperature	20 °C	0.62±0.10	1.25±0.08	0.77±0.05	0.35±0.06
	30 °C	1.32±0.05	1.99±0.09	0.89±0.04	0.59±0.07
	40 °C	0.65±0.04	0.67±0.06	0.45±0.01	0.29±0.03

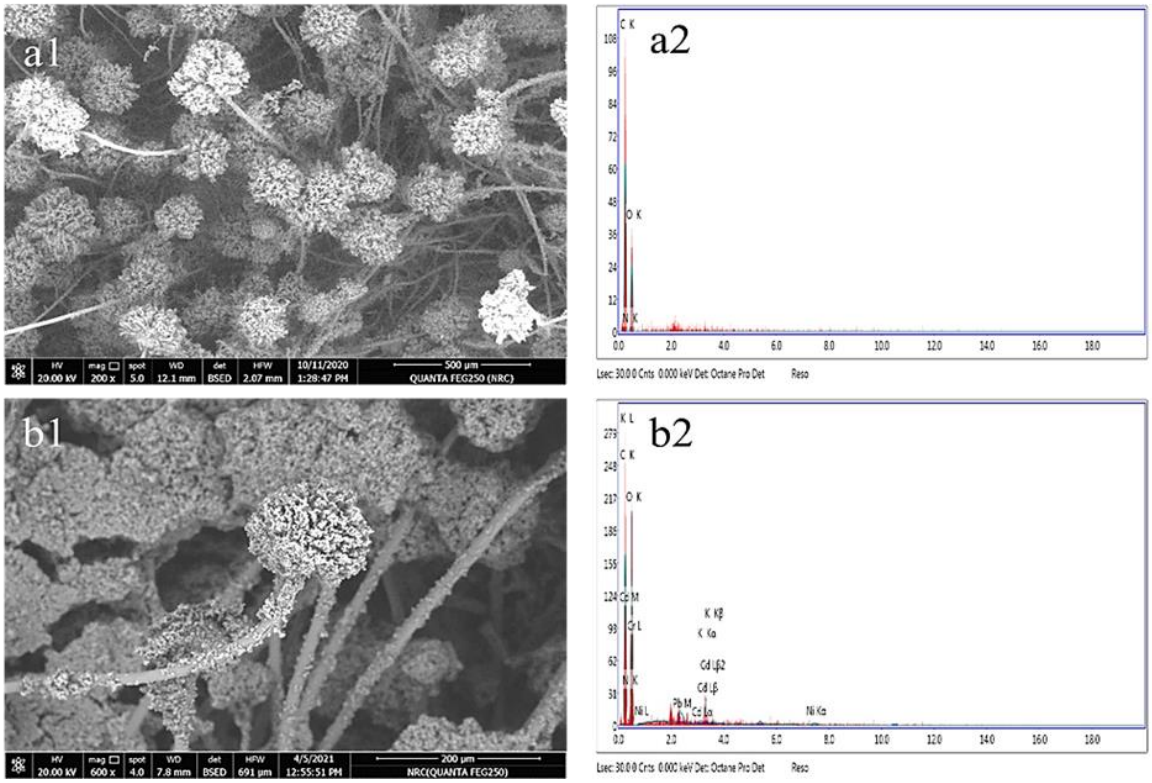

Fig.10:(a1) SEM image of *Aspergillus niger* incubated without mixture of heavy metals, (**a2**): EDX analysis of non-exposed sample, (**b1**): SEM image of *Aspergillus niger* after incubation in the presence of mixture of heavy metals, (**b2**): EDX analysis of exposed sample showing heavy metals peaks

4. Discussion

Twelve fungal isolates from different contaminated areas in Egypt were tolerant to heavy metals including Pb, Cd, Cr and Ni. *Aspergillus terreus, Aspergillus niger, Aspergillus flavus* and *Talaromyces purpurgenus* were the most potent isolates on removing Pb, Cd, Cr and Ni. Similarly, **Joshi *et al*.(2011)** [9] and **Sharma *et al*. (2020)** [20] found that *A. terreus* is an efficient bio-adsorbent it removed 80% of Pb from a liquid medium, the highest uptake of Pb by *A. terreus* indicated more binding sites on cell wall of this fungus and its potential as biosorbent to remove Pb from industrial wastewater containing higher concentration of Pb. Also **Kapoor *et al*.(1999)** [21] and **Tsekova *et al*.(2010)** [22] found that *A. niger* show an ability for binding and removing of Cd from waste water. For *A. flavus* according to **Kumar and Dwivedi (2019)** [23] it has the ability to remove, reduce and up take chromium. *A. flavus* CR500, isolated from electroplating wastewater, can form Cr-sulfhydryl compounds in its vacuole and accumulate in cells with non-protein sulfhydryl groups produced by fungi [23].

Talaromyces sp. are known to be tolerant for tough conditions, which made them possibly useful in extremely severe environments, which includes acidic and metalloid contaminated mine drainage and soils [24 , 25]. previous studies indicated that metal ion adsorption by *Talaromyces* sp. **Romero *et al*.(2006)** [26] used *T. helicus* to degrade biphenyl treated with high levels of copper (Cu), and it indicated the ability of detoxification of this species and its adjustment to heavy metals and biacrylic compounds.

In the current study the maximum growth in the presence of various mixture of heavy metals was observed by *A. niger.* It was tolerant to the four heavy metals and biosorbed considerable amount of the heavy metals from PD broth containing 100 ppm of each heavy metals (Pb, Cd, Cr and Ni) with capacity 2.0787 mg/g, other studies showed that the resistance and the removal capacity of *A. niger* and its resistance to some heavy metals, the fungus grew in 2000 ppm of zinc, lead, and mercury, 1200 and 1000 ppm of arsenic (III) and (VI), 800 ppm of fluor and cobalt, and least in cadmium (400 ppm)[27]. The best suitable conditions for the removal of 100 mg/L of the heavy metals were 28°C, pH between 4.0 and 5.5, 100 ppm of heavy metal, and 1 g of fungal biomass, these results agree with in in our study where *A. niger* was able to remove Cd, Pb, Ni and Cr (400 ppm).

Heavy metals toxicity not only depends on concentration but also rotates around bioavailability [28, 29]. *A. niger* showed a lower uptake of all the four heavy metals (Pb, Cd, Cr and Ni) in combination, compared to individual heavy metal uptake. This was mostly due to competition of heavy metals for same and limited adsorption sites on fungal cell wall of these fungi. These results showed the potential of these fungi to remove heavy metals from liquids media and industrial wastewater containing higher concentration of heavy metals. Similar observations regarding the uptake of heavy metals in comparison to individual heavy metal have been described by **Ahmed and Ansari(2006)** [30].

It was very clear that sucrose is the optimum carbon source for *A. flavus* and *A. terreus,* the bioleaching efficiency of *A. flavus* with sucrose as a carbon source was better than that with glucose [31, 32] .The result may be due to the osmotic pressure of the bioleaching environment is maintained significantly better by sucrose than by glucose, which is favorable for the growth and metabolism of *A. flavus* but for *Talaromyces purpuregenus* pectin is the optimum carbon source, Pectinases are enzymes that are capable of degrading pectin materials. The action of these enzymes may be at the extremities or random cleaving [33]. while glucose is optimum for *A. niger.* This could be due to that simple carbon compounds are assimilated directly while complex ones (i.e., polysaccharides) must be transformed into simpler forms before their use. Glucose (a monosaccharide) and sucrose (a disaccharide) are well used by the waterborne fungi [34]. After applying different pH intervals, the optimum pH of *A. flavus* and *A. terreus* is 5, but for *Talaromyces purpuregenus* pH 9 is optimum pH which provids the best uptake for the heavy metal these results are agreed with **Della Monica *et al*. (2018)** [35], even though pH 7 is the optimum pH for *A. niger*. The pH regulating system make sure that secreted enzymes (e.g., alkaline and acid phosphatases, xylanases) are produced under pH conditions in which they are physiologically completely functional. At acidic pH, more protons (H^+) are available to saturate metal-binding sites; consequently metals are less likely to form insoluble precipitates with phosphates when the pH of the system is lowered because lots of the phosphate has been protonated [36, 37] .

Different inoculum sizes were used in this study, it was noticed and observed that 0.5 ml was the optimum size for both *A. niger* and *A. terreus* with uptake (1.3327 mg/gm) and (0.4197mg/gm) respectively, but for *A. flavus* the optimum inoculum size was 1 ml the gives the best uptake, while for *Talaromyces purpuregenus* 2 ml was the optimum inoculum size. Electrostatic interaction between the cells plays a significant role in metal uptake. Metal uptake depends on binding sites, increasing amount of metal adsorbed by the biomass will be increased with initial concentration of metals. Optimum and best percentage of metal removal can be taken at low initial metal concentration. Therefore, at a certain concentration of biomass, the metal uptake increase with increase in initial concentration [38].

A range of incubation periods are applied on each organism in order to get the best uptake of the heavy metal, and the best incubation period for all the organisms (*A. niger*, *A. flavus, A. terreus, Talaromyces purpuregenus*) is 9 days, as the incubation period increase the uptake of the heavy metal increase , our results are in agreement with **Iraq *et al*.(2020)** [39].

In the current study different temperatures were applied on each organism to obtain the best uptake of heavy metals, the optimum temperature for all the organisms (*A. niger*, *A. flavus*, *A. terreus*, *Talaromyces purpuregenus*) is 30°C, in live fungi, the optimum temperature for the growth of fungi usually varies between 25–35 °C, and the removal rate mainly depends on the growth of the fungi [23, 40]. At lower and higher temperatures, enzymatic mechanisms become affected that lead to reduction in the metabolic rate of the fungus; eventually, the growth and biomass productivity get hampered [23, 41].

5.Conclusion

In this study, it showed that *Talaromyces purpuregenus*, *A. niger*, *A. flavus* and *A. terreus* were the most potent fungal isolates on removing and up taking Ni, Cd, Cr and Pb from different contaminated areas in Egypt up to 800 ppm. While in the presence of different mixture of these heavy metals *A. niger* was the most tolerant fungal isolate to the four heavy metals.

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7. Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis, preparation of all figures and tables were performed by Doaa Ahmed Montaser. The design of the study was performed by Doaa Ahmed Montaser and Samar Samir Mohamed. Easa.S.M, Maisa M.A.Mansour and Samar Samir Mohamed helped in reviewing the manuscript. The first draft of the manuscript was written by Doaa Ahmed Montaser and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

8. Data availability statement

The data generated or analyzed during the current study are available in this published article. Sequence data that support the finding of this study have been diposted in "GenBank" with the accession codes [MW673533], [MW673534], [MW673531] , [MW673532].

9. Compliance with ethical standards

9.1 Conflict of interest

The authors declare that they have no conflict of interest.

9.2 Ethics statement

This article does not contain any studies with human participants or animals performed by any of the authors.

9.3 Consent for Publication

Not applicable.

10. Consent to Participate

Not applicable.

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12. References

1. **Iram, S., Arooj, A. and Parveen, K. (2012).** Tolerance potential of fungi isolated from polluted soil of Multan Pakistan. **2:** 27 – 34.
2. **Gomri, M. A., El Moulouk Khaldi, T. and Kharroub, K. (2018).** Analysis of the diversity of aerobic, thermophilic endospore-forming bacteria in two Algerian hot springs using cultural and non-cultural methods. Ann. Microbiol,m **68:** 915 – 929. https://doi.org/10.1007/s13213-018-1401-8

3. **Zhuang, W. and Gao, X. (2013).** Acid-volatile sulfide and simultaneously extracted metals in surface sediments of the southwestern coastal Laizhou Bay, Bohai Sea: Concentrations, spatial distributions and the indication of heavy metal pollution status. Mar. Pollut. Bull., **76:** 128 – 138.https://doi.org/10.1016/j.marpolbul.2013.09.016
4. **Jacques, R. A., Lima, E. C., Dias, S. L. P. and *et al* (2007).** Yellow passion-fruit shell as biosorbent to remove Cr(III) and Pb(II) from aqueous solution. Sep. Purif. Technol., **57:** 193 – 198. https://doi.org/10.1016/j.seppur.2007.01.018
5. **Ahalya, N., Ramachandra, T. and Kanamadi, R. (2003).** Biosorption of Heavy Metals. Mycoremediation, **7:** 484 – 532. https://doi.org/10.1002/0470050594.ch11
6. **Gunatilake, S. K. (2015).** Methods of Removing Heavy Metals from Industrial Wastewater. Multidiscip. Eng. Sci. Stud., **1**(1): 12 – 18.
7. **Kapahi, M. and Sachdeva, S. (2019).** Bioremediation options for heavy metal pollution. J. Heal. Pollut., **9**: 1 - 20. https://doi.org/10.5696/2156-9614-9.24.191203
8. **Xie, Y., Fan, J., Zhu, W. and *et al.* (2016).** Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. Front. Plant. Sci., **7:** 1 – 12. https://doi.org/10.3389/fpls.2016.00755
9. **Joshi, P. K., Swarup, A., Maheshwari, S. and *et al.* (2011**). Bioremediation of Heavy Metals in Liquid Media Through Fungi Isolated from Contaminated Sources. Indian J. Microbiol., **51:** 482 – 487. https://doi.org/10.1007/s12088-011-0110-9
10. **Samson, Reenen-Hoekstra, Ellen, S. and van, R. A. (1988).** Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures, Institute of the Royal Netherlands Academy of Arts and Sciences, Baarn.
11. **Pal, M. (2007).** Veterinary and Medical Mycology. 1 st edition. Indian Council of Agriculture Research, New Dalhi, India.
12. **Asan, A. (2016).** Mycotaxon Aspergillus , Penicillium and Related Species Reported from Turkey This database , available online , reviews 876 published accounts and Turkey , respectively . According to the published records , 428 species have Key Words : Aspergillus , Peni.
13. **Mushimiyimana, I., Kimonyo, A. and Nsabimana, P. (2016),** Colonial and Morphological Characteristics of various fungi Species Isolated from soil in Bangalore city. Bull. Env. Pharmacol. Life Sci., **6:** 17 – 21.
14. **Radulescu, C., Dulama, I., Stihi, C. and *et al.* (2014**). Determination of heavy metal levels in water and therapeutic mud by atomic absorption spectrometry. Rom. J. Phys., **59:** 1057 – 1066.
15. **Dwivedi, S., Mishra, A. and Saini, D. (2012).** Removal of Heavy Metals in Liquid Media through Fungi Isolated from Waste Water. Int. J. Sci. Res., **1**: 2319 – 7064.
16. **Taha, A. W., Dakroury, A. M., El-Sayed, G. O. and El-Salam, S. A. (2010).** Assessment Removal of Heavy Metals Ions from Wastewater by Cement Kiln Dust (CKD). J. Am. Sci., **6:** 1545 – 1003
17. **Kumar S, Stecher G, Tamura K (2016**) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054
18. **Saitou, N. and Nei. M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., **4:** 406 – 425.
19. **Ban, Y., Tang, M., Chen, H. and *et al.* (2012).** The Response of Dark Septate Endophytes (DSE) to Heavy Metals in Pure Culture. PLoS One **7:** 1 - 11. https://doi.org/10.1371/journal.pone.0047968

20. **Sharma, R., Talukdar, D., Bhardwaj, S. and *et al*. (2020)**. Bioremediation potential of novel fungal species isolated from wastewater for the removal of lead from liquid medium. Environ. Technol. Innov., **18:** 100757. https://doi.org/10.1016/j.eti.2020.100757
21. **Kapoor, A., Viraraghavan, T. and Cullimore, D. R. (1999**). 1999 Anoop kapoor bio 1. Bioresour. Technol. **70:** 95 – 104.
22. **Tsekova, K., Todorova, D. and Ganeva, S. (2010**). Removal of heavy metals from industrial wastewater by free and immobilized cells of Aspergillus niger. Int. Biodeterior. Biodegrad., **64:** 447 – 451. https://doi.org/10.1016/j.ibiod.2010.05.003
23. **Kumar, V. and Dwivedi, S. K. (2019).** Hexavalent chromium reduction ability and bioremediation potential of Aspergillus flavus CR500 isolated from electroplating wastewater. Chemosphere, **237:** 124567. https://doi.org/10.1016/j.chemosphere.2019.124567
24. **Visagie, C. M. and Jacobs, K. (2012).** Three new additions to the genus Talaromyces isolated from Atlantis sandveld fynbos soils. Persoonia. Mol. Phylogeny. Evol. Fungi, **28:** 14 – 24. https://doi.org/10.3767/003158512X632455
25. **Hassan, N., Rafiq, M., Rehman, M. and *et al*. (2019)** Fungi in acidic fire: A potential source of industrially important enzymes. Fungal Biol. Rev., **33:** 58 – 71.
26. **Romero, M. C. H., Reinoso, E., Urrutia, M. I. and Moreno Kiernan, A. (2006).** Biosorption of heavy metals by Talaromyces helicus: a trained fungus for copper and biphenyl detoxification. Electron. J. Biotechnol., **9:** 221 – 226. https://doi.org/10.2225/vol9-issue3-fulltext-11
27. **Acosta-Rodríguez, I., Cardenás-González, J. F., Pérez, A. S. R. and *et al.* (2018)**. Bioremoval of different heavy metals by the resistant fungal strain aspergillus Niger. Bioinorg. Chem. Appl., **2018:** 1 - 7. https://doi.org/10.1155/2018/3457196
28. **N, A. J., Udayashankara, T. H. and Lokesh, K. S. (2014).** Review on Bioremediation of Heavy Metals with Microbial Isolates and Amendments on Soil Residue. **3:** 118 – 123.
29. **Naveen Kumar, K. J. and Prakash, J. (2021**). Bioremoval of Different Heavy Metals in Industrial Effluent by the Resistant Fungal Strain Aspergillus niger. Nat. Environ. Pollut. Technol., **20:** 1437 – 1448. https://doi.org/10.46488/NEPT.2021.v20i04.006
30. **Ahmad, I., Ansari, M. I. and Aqil, F. (2006).** Biosorption of Ni, Cr and Cd by metal tolerant Aspergillus niger and Penicillium sp. using single and multi-metal solution. Indian J. Exp. Biol., **44:** 73 – 76.
31. **Qayyum, S., Meng, K., Pervez, S. and *et al*. (2019**). Optimization of pH, temperature and carbon source for bioleaching of heavy metals by Aspergillus flavus isolated from contaminated soil. Main Gr. Met. Chem., **42:** 1 – 7. https://doi.org/10.1515/mgmc-2018-0038
32. **Daza, A., Manjón, J. L., Camacho, M. and *et al*. (2006**). Effect of carbon and nitrogen sources, pH and temperature on in vitro culture of several isolates of Amanita caesarea (Scop.:Fr.) Pers. Mycorrhiza, **16:** 133 – 136. https://doi.org/10.1007/s00572-005-0025-6
33. **White, C. A. and J. F. K. (1998).** The Carbohydrate-Directed Enzyme. Carbohydrate Chemistry. Clarendon Press, Oxford, 343 - 377.
34. **Sati, S. C. and Bisht, S. (2006).** Utilization of various carbon sources for the growth of waterborne conidial fungi. Mycologia., **98:** 678 – 681. https://doi.org/10.3852/mycologia.98.5.67
35. **Della Mónica, I. F., Godoy, M. S., Godeas, A. M. and Scervino, J. M. (2018).** Fungal extracellular phosphatases: their role in P cycling under different pH and P sources availability. J. Appl. Microbiol., **124:** 155 –165. https://doi.org/10.1111/jam.13620

36. **Arjoon, A., Olaniran, A. O. and Pillay, B. (2013).** Co-contamination of water with chlorinated hydrocarbons and heavy metals: Challenges and current bioremediation strategies. Int. J. Environ. Sci. Technol., **10:** 395 – 412. https://doi.org/10.1007/s13762-012-0122-y
37. **Hughes, M. N. and Poole, R. K. (1991).** Metal speciation and microbial growth-the hard (and soft) facts. Microbiology, **137:** 725 – 734.
38. **Abbas, S. H., Ismail, I. M., Mostafa, T. M. and Sulaymon, A. H. (2014).** Biosorption of Heavy Metals : A Review.
39. **Iraq, Badr, S. Q. and Sahib, F. A. (2020).** EFFECT OF INCUBATION TIME ON COPPER UPTAKE AND ABSTRACT : Key words : biological removal accumulation , pollution , fungi , heavy metals .INTRODUCTION : MATERIAL AND METHODS : Sample collection and fungus isolation : Preparation of adsorbent : RESU., **23:** 1 – 5.
40. **Prasad, G., Kumar, V. and Dwivedi, S. K. (2018**). Antifungal activity of some selected medicinal plants against Fusarium solani causing wilt and rot in Pearl millet. Asian J. Bio. Sci., **13:** 21 – 27. https://doi.org/10.15740/has/ajbs/13.1/21-27
41. **Pundir, R., Chary, G. H. V. C and Dastidar, M. G. (2018**). Application of Taguchi method for optimizing the process parameters for the removal of copper and nickel by growing Aspergillus sp. Water Resour. Ind., **20:** 83–92.