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Heavy Metal Tolerance of some Filamentous Fungi from Waste Water of Oxidation Ponds in Sadat City

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Abstract

Nowadays natural water resources are being polluted with heavy metals that became very common issue in the country as well as all over the world as a result of increase in anthropogenic activities that leads to excess heavy metals' release like Copper, Mercury, Lead, Nickel and Zinc to alarming level which may significantly affects human and other living organisms as well so, there have to be treatment of waste water. Reports revealed that biological treatment, especially by using filamentous fungi that have a significant part in removal of heavy metals from the contaminated water. Hence in the following study we have isolated filamentous fungi such as *Trichoderma* sp., *Aspergillus niger*, *Mucor* sp., *Penicillium* sp. and *Fusarium* sp. from Oxidation Ponds of Sadat City that have been exposed to heavy metal contamination with aim to find their tolerance properties towards selective heavy metal metals like, Copper and Mercury. Findings of our study highlighted that *Trichoderma* sp. have greater ability to grow under varying Copper and Mercury concentration and shows greater tolerance limit of 37.67mm (Diameter of fungal species) at 200 mg/L of Copper concentration and tolerance limits of 29.67 mm (Diameter of fungal species) at 200 mg/L of Mercury concentration. Thus, it is concluded that the filamentous fungi namely *Trichoderma* sp., *Aspergillus niger*, *Mucor* sp., *Penicillium* sp. and *Fusarium* sp. are efficient in removal of toxic heavy metals from the poluted water. Hence, we can employ these fungi individually or in consortium as effective agents for bioremediation of the contaminated water in order to enhance the amount of clean water and increase the yield of useful crop plants for supplying the demand of growing population.

Keywords: Heavy metals, Copper, Mercury, Bioremediation, Filamentous fungi.

Introduction

One form of waste whose presence frequently causes harmful effects on people's lives is liquid waste **(Satria *et al*., 2019)**. Industry-related liquid waste typically contains high concentrations of different organic compounds, heavy metals, chemicals, and hazardous and toxic materials **(Riani et al., 2018).** One serious issue that frequently has an adverse impact on both the environment and human health is heavy metal pollution. Therefore, creating efficient remediation plans is crucial. Biological approaches provide an alternate way to solve the issue in a more economical and environmentally friendly manner, whereas physical and chemical methods of remediation are usually unworkable and expensive. **(Iram *et al.*, 2015).** Many different types of microbes are naturally able to break down waste products and endure harsh environmental conditions. Numerous techniques, including bio-accumulation, bio-precipitation, biosorption, and uptake by pure biopolymers derived from microbial cells, could result in the bioremediation of heavy metals using microorganisms **(Qayyum *et al*., 2016 and Alves *et al*., 2022).** Microorganisms that are useful as adsorbents to eliminate various heavy metals include bacteria, fungi, and algae (**Edris *et al*., 2014 and Borah and Yadav, 2014).** The tested fugi in this research were, *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp., *Mucor* sp. and *Trichoderma* sp. *Aspergillus niger* is a member of a group of species named Aspergillus section Nigri, formerly known as A. niger group (**Varga *et al.*, 2011)**. This fungus causes the "black mold" disease and it is the most common contaminant of stored food, being responsible for postharvest decay of fresh fruits, grains, and crops worldwide **(Ajav *et al*., 2011).** The genus *Mucor* belongs to the zygomycotic order *Mucorales* Taxonomic classification of mucor fungi: Kingdom: Mushrooms; Phylum: *Zygomycota*; Order: *Mucorales*; Family: *Mucoraceae*; Genus: *Mucor*. *Mucor* has a filamentous form, it is an inhabitant of the soil, plants, decaying plant residues, it is widely distributed in nature. *Muco*r spp. can cause infections in humans, amphibians, cattle and pigs **(Alvarez, 2010 and Alvarez, 2009)**. Trichoderma belonging to Eumycota, Deuteromycotina, Hyphomycetes, Hyphomycetales, and Moniliaceae **(Kubicek *et al*., 2019).** Its sexual stage includes the *Ascomycota*, *Sordariomycetes*, *Hypocreales*, *Hypocreaceae*, and *Trichoderma* ssp. **(Sun *et al*., 2012)**. There are more than 370 *Trichoderma* spp. including *T. harzianum, T. viride, T. asperellum, T. hamatum, T. atroviride, T. koningii, T. longibrachiatum,* and *T. aureoviride* **(Sánchez-Montesinos *et al*., 2021; Sun *et al*., 2022).** Trichoderma has been used in biological control research, including *T. harzianum, T. hamatum, T. longibrachiatum, T. koningii, T. viride, T. polysporum,* and *T. asperellum* **(Di Marco *et al*., 2022).** The genus *Penicillium* was established in 1809, and *P. expansum* Link was designated as the type species. It is the most speciose in the order Eurotiales. In a monography published in 2014, 354 species were accepted in this genus **(Visagie *et al*., 2014)** and 483 species were recognized in one that was published in 2020 **(Houbraken *et al*., 2020)**. By the end of 2022, 64 species had been further added to this group **(Visagie *et al*., 2024)**. In the last year, 54 new species were described, and 43 of them were discovered in Southwestern China

(Wang *et al*., 2023). This leads to the species number of the genus over 600 at this moment. In China, more than 170 Penicillium species have been recorded, of which 91 were originally described from this country **(Wang et al., 2023).** Morphological characters are considered the most traditional criteria used to identify any fungal species. *Fusarium* produces a range of mycelia that are cottony with shades of pink, yellow, and purple. Some species produce either macroconidia or microconidia as asexual reproductive structures, while other species produce both macroconidia and microconidia **(Jay, 1987).** The morphology of microscopic characteristics, i.e., the general shape and dimensions of the macroconidia, the generation of microconidia, chlamydospores, sclerotia, sexual stages, and pigmentation, are the primary means used for the identification of *Fusarium* species. Members of the genus are variable in cultural characteristics because changes in the environment in which they grow can result in morphological changes both in culture and in conidia **(Moss *et al*., 2004).** Utilising living things like fungi, bacteria, and plants to naturally reduce the toxicity of various environmental pollutants and break down or detoxify dangerous compounds that endanger human health or the integrity of the environment is known as bioremediation (**Leong and Chang 2020 and Singh et al. 2020**). Numerous methods, including Land farming, biostimulation, bio-augmentation, bio-venting, bio-filters, bio-sorption, composting, and bioreactors, can be used to achieve the principles of bioremediation **(Huang *et al*., 1991; Kumar *et al*., 2015 and Lellis *et al*., 2019**). Metallic elements that have a high atomic weight and a density that is at least five times that of water are known as heavy metals. They are incapable of breaking down and are not biodegradable. Consequently, these are hazardous substances **(Paul, 2017 and Goher *et al*., 2019).** The greater risk to water bodies is as aresult of heavy metals that are thought to be among the most hazardous pollutants in the environment due to their potential for toxicity, bioaccumulation, and persistence. Heavy metals are especially concerning because of their toxic, carcinogenic qualities as well as other negative impacts on public health **(Joseph *et al*., 2019).** Heavy metals that are not biodegradable, like Copper (Cu), Mercury (Hg), Zinc (Zn), Cadmium (Cd), Lead (Pb), and Aluminium (Al), can be found in large amounts in agricultural nutrients or waste water **(Turan *et al*., 2018).** Heavy metal contamination of water is a recognised hazard that has been linked to human sources such as chemical spills, untreated home as well as agricultural leftovers, and discharges of industrial waste water **(Malyan *et al*., 2014)**. Heavy metals gradually find their way into the food chain through water, where they can have long-lasting effects that pose a threat to health, including mental illnesses, joint pain, stomach problems, and even cancer **(Caravanos *et al*., 2016).** The traditional techniques for getting rid of heavy metals are Ion exchange, chemical reduction followed by precipitation in an alkaline medium, and adsorption on activated coal, alum, kaolinite, and ash. The majority of these techniques have significant drawbacks, such as the requirement for high energy consumption, substantial chemical reagent requirements, insufficient metal removal, and the production of a significant amount of toxic waste sludge. Additionally, when the initial concentrations of heavy metals are between 10 and 100 mg/L, these procedures might be inefficient or very costly **(Zhao *et al*., 2022).** In waste water

biological treatment, microorganisms, namely bacteria and fungi, are the primary environmentally beneficial agents that aid in the breakdown and removal of heavy metal contaminants. By naturally using living things like fungi, bacteria, and plants to break down or detoxify dangerous substances that endanger human health or the environment, bioremediation can lower the toxicity of numerous environmental pollutants (**Leong and Chang 2020 and Singh *et al.*, 2020).** One of the best organisms for bioremediation is fungi. since they are able to grow and adapt to a variety of pH and temperature conditions, nutritional availability and at elevated heavy metal concentrations. Fungi are an eco-friendly, cost-effective, and efficient bioremediation tool. Fungal cell walls are made up of lipids, proteins, and polysaccharides that provide numerous active sites for metal binding **(Karcprzak and Malina, 2005)**. Fungi that are filamentous are regarded as biosorption agents Because of its capacity to extract concentrated heavy metal pollutants from liquid substrates, it is chosen over other organisms for the bioremediation process. Numerous fungal species, including *Aspergillus niger*, *T. virens*, *T. autroviride*, and *T. harzianum* are employed in the cleanup of contaminated areas. Numerous mechanisms, including metal transformation, extracellular and intracellular precipitation and active uptake contribute to the tolerance and capability of fungi. Extreme pH, different temperature, nutrient availability, and high metal concentrations are all circumstances in which filamentous fungi can do their function successfully **(López-Errasquín and Vázquez, 2003; Zafar *et al*., 2007 and Iskandar *et al*., 2011).** Numerous fungal species possess a rich network of filaments in large volumes that enable them to mineralize, release, or store multiple elements and accumulate numerous toxic substances while occupying the upper layer of the soil **(Irwin, 1996).** By using this filament network, the fungus is able to extract the necessary minerals from soil and water. Additionally, they play a significant role in decomposing organic compounds and recycling elements, a process that produces a wide range of essential substances including vitamins, mycotoxins, antibiotics, and different organic acids, among many other compounds that are secondary metabolites **(Mitchell, 1998).** More benefits than other types of microorganisms exist when using fungi for bioremediation of contaminated environments, especially waste water. These benefits include being more efficient, flexible, simple to use, yielding cost-benefit results and being environmentally friendly. **(Legorreta-Castañeda *et al*., 2020; Silva *et al*., 2019 and Ahuacizin-Pérez *et al*., 2014).** A significant method for the bioremediation of numerous pollutants has been investigated: biosorption. This process uses the viable or inactivated biomass of various fungal species. Because of their great selectivity, high binding capacity, and high degradation rate and level, a variety of filamentous fungi have been successfully employed to lower the levels of both organic and inorganic contaminants found in waste water produced from various industries **(Sharma *et al*., 2023; Danouche *et al*., 2021 and Igiri *et al*., 2018).** Owing to the effectiveness of fungus biomass, either by bioaccumulation or biosorption processes, in removing various pollutants, these organisms are regarded as highly valuable for bioremediation of various environments tainted by industrial and household pollutants **(Gunyar and Uztan, 2021; Patel *et al*., 2020 and Rudakiya *et al*., 2019).**

The *Trichoderma sp*. are genetically diverse with number of characteristics and having tolerance properties against wide range of pollutants including harmful heavy metals and also being tested for bioremediation of environmental toxic pollutants **(Tripathi *et al*., 2013).** *Aspergillus niger* is also very efficient for removing of the heavy metals and potent enough for bioremediation of heavy metals from polluted sites **(Juan *et al*., 2019).** Therefore, this study aims to isolation, characterization and identification of heavy metals tolerant indigenous fungi from sewage and industrial waste waters of Oxidation Ponds of Sadat City, and demonstrate their effectiveness in lowering the risk associated with heavy metal pollution.

Materials and methods.

Study Area and Sample Collection

Three sampling zones (nine total sampling sites) in Oxidation Ponds of Sadat City, Egypt (30º28.12 N, 30º35.86 E) and (30º28.09 N, 30º35.82 E) ((Figs .1 and 2), The water samples were taken from different regions of ponds that are contaminated with industrial wastes from the nearby industries and domestic sewege. The water samples were collected at April 2023 and the samples were kept in plastic tubes for further analyses. Every sample was 100 millilitres of sewage, collected in sterile bottles, and refrigerated before being brought to the lab **(Dick, 1994**).

Fig. 1. Geographical location of oxidation pond in Sadat City.

(A) (B)

Fig. 2. Collection of water samples from Oxidation Ponds in Sadat City**.**

The heavy metals and mineral analysis of tested water samples

The heavy metals and mineral analysis of tested water samples were operated in Quality control laboratory, Faculty of Agriculture, Mansoura university Accredited according to ISO 17025/2005.

The tested water samples were analyzed for heavy metals and minerals to determine the dominant heavy metals in Oxidation Ponds region of sadat city. Measurements and analysis were performed using double-induction plasma device (Thermo scientific iCAP 7000 plasma).

Isolation , identification and Purification of Fungal Species from tested water samples

Sterilization of apparatus

Syringes, flasks, Mccartney bottles, distilled water, petri plates, and media bottles were all autoclaved at 121°C for 40 minutes to ensure sterilisation. All sterilised materials were autoclaved before being dried at 95° in an oven.

Media preparation

Potato Dextrose Agar (PDA) media is used to revive fungal cultures. In order to create a broth, 200g of peeled, sliced, boiling, and sieved potatoes through a clean muslin cloth. Agar (7.5g) and dextrose sugar (7.5g) were then added. After that, the media was autoclaved at 121°C for 30 minutes. **(Razak *et al*., 1999).**

Prepration of plates

After pouring the media into Petri dishes, it is given a day to solidify. To inhibit bacterial growth, about 30 mg/l of streptomycin was added right before the plates were poured. The plates were left inverted at room temperature for a full day after the agar solidified. **(Murray, 2007)**.

Isolation and Identification of fungi

By using the serial dilution agar plate method, the fungi were separated from tested samples of sewage water. **(Aneja, 2009)**. The sewage water test samples were serially diluted in increments of 10^{-1} to 10^{-10}. Potato Dextrose Agar (PDA) plates were spread with 100µL of tested sewage water samples from each higher dilution (10^{-6} to 10^{-10}). In order to stop bacteria from growing, 30 mg/l of the antibiotic streptomycin was added to the PDA prior to pouring. For a period of three to five days, the inoculated petriplates were incubated at 28 °C to promote fungal growth. Following incubation, the morphologically distinct fungal colonies were separated and cultured further. For future use, The fungal isolates were stored at 4 ºC on PDA slants for preservation.The fungal colonies were identified using both macroscopic (colonial morphology, colour, texture, shape, diameter, and appearance of the colony) and microscopic (mycelium septation, presence of specific reproductive structures, conidia shape and structure, and presence of sterile mycelium) characteristics. Pure cultures of fungal isolates were identified with the help of published works. **(Domsch *et al*., 1980; Barnett & Hunter, 1999 and Cappuccino and Sherman, 2005).**

Purification of Fungi

Mccartney bottles were filled with prepared Potato Dextrose Agar (PDA) media, which was then autoclaved for 30 minutes at 121°C. The bottles were autoclaved and then left in a tilt position for a full day. After solidification, a sterile plug made entirely of fungal culture was inserted into the bottle's centre. For one week, the slants were incubated at 30°C. Slants were placed in the refrigerator for later use and preservation once the fungal colonies showed signs of full growth.

Metal tolerance test "Minimum Inhibitory Concentration (MIC)" for isolated fungal species

The ability of various fungal strains, such as *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp., and *Mucor* sp., to withstand varying concentrations of heavy metals [$CuSO_4$ and $HgCl_2$] was tested. The Minimum Inhibitory Concentration (MIC) test, which determines the lowest metallic concentration capable of inhibiting the growth of a fungal isolate, was employed to identify isolates that were resistant to metal. **(Zafar *et al.*, 2007)**. Initially, PDA medium was prepared with varying concentrations of [$CuSO_4$ and $HgCl_2$] (50, 100, 150, 200, and 250 mg/L). It was then transferred into sterilised plates, which were inoculated with the tested fungi by inserting a 5 mm diameter disc from an actively growing culture in the centre of plates containing PDA with ($CuSO_4$ and $HgCl_2$) after being sterilised at 121 °C for 15 minutes in the autoclave and allowed to cool to room temperature. For each treatment, three duplicate plates were used. For control, fungi were also grown on PDA that had not been treated. After that, the plates were incubated for five days at 28 °C in an incubator. There were three duplicates of every concentration used in the experiment. Following the incubation period, the minimum inhibitory concentration (MIC) of the heavy metal was identified as the lowest concentration of metal ions that could inhibit the visible growth of fungi. **(Price *et al.*, 2001).** The isolates which showed the better growth rate after incubation were considered concerning tolerance to metals.

Statistical analyses

It was decided to use the average of the three determinations. Using SPSS software version 16 **(SPSS Inc. 2007),** following the application of a one-way analysis of variance (ANOVA), the least significant difference ($p \leq 0.05$) was established.

Results

The heavy metals & mineral analysis of tested water

Data in Table 1 showed metals & mineral analysis of tested water samples, which collected from region of Oxidation pond in Sadate City, Egypt.The results revealed that high amounts of the heavy metals of Almunium (Al) 1,436.524 ppm, Zinc (Zn) 19.762 ppm, Mercury (Hg) 15.537 ppm, Ferrous (Fe) 19.073 ppm and Copper (Cu) 3.288 ppm respectively. So the present study select the heavy metals of Copper (Cu) and Mercury (Hg)

because they were found in high concentration in Oxidation pond region Sadat City, Egypt. The following table explains heavy metals & mineral analysis of tested water samples from three oxidation ponds :

Table 1. The heavy metals & mineral analysis of tested water sample from Oxidation Ponds in Sadat City (1), (2), (3).

Element	**Concentration (ppm) pond** (1)	**Concentration (ppm) Pond (2)**	**Concentration (ppm) Pond (3)**
Al 396.152 {85} (Radial)	1,436.524	103.762	5.502
V 290.882 {116} (Radial)	N.D	N.D	N.D
Hg 184.950 {482} (Radial)	15.537	2.469	1.365
Ag 328.068 {103} (Radial)	1.855	2.508	1.885
B 249.773 {135} (Radial)	7.631	4.742	3.172
Ba 455.403 {74} (Radial)	1.292	1.347	1.433
Ca 393.366 {86} (Radial)	1,217.614	997.993	944.397
Cd 226.502 {449} (Radial)	0.133	0.091	0.056
Co 228.616 {447} (Radial)	0.012	N.D	N.D
Cr 283.563 {119} (Radial)	0.530	0.611	0.465
Cu 324.754 {104} (Radial)	2.868	2.909	3.288
Fe 259.940 {130} (Radial)	9.601	19.073	7.759
Ga 294.364 {114} (Radial)	1.280	0.881	N.D
In 325.609 {103} (Radial)	2.291	N.D	N.D
Li 670.784 {50} (Radial)	N.D	N.D	N.D
Mg 279.553 {121} (Radial)	79.113	77.277	104.120
Mn 257.610 {131} (Radial)	0.569	0.965	0.501

Table 1. Cont.

Ni 216.556 {456} (Radial)	0.374	N.D	N.D
Pb 220.353 {453} (Radial)	N.D	0.596	0.305
K 766.490 {44} (Radial)	62.545	75.511	22.398
Sr 407.771 {83} (Radial)	2.214	2.222	2.166
Zn 213.856 {458} (Radial)	17.363	14.160	19.762
As 189.042 {478} (Radial)	N.D	N.D	0.184
Na 589.592 {57} (Radial)	503.009	452.058	296.411
Bi 223.061 {451} (Radial)	2.183	2.036	0.591
Se 206.279 {463} (Radial)	3.603	4.796	5.933

Isolation and identification of Fungal Species from tested water samples

Utilising Malt Extract Agar (MEA) colonies, micromorphological observations were conducted and potato dextrose Agar (PDA) colonies were used to describe the colony criteria. **(Hong *et al*., 2006).** All the isolated fungi were purified using single spore or the hyphal tip techniques suggested by **(Dhingra and Sinclair, 1985).** The purified fungi were identified according to their morphological features according to **(Booth, 1985)**. Every six to eight weeks, stock cultures were subcultured on fresh medium and kept in a refrigerator at 5 to 10°C on potato dextrose Agar (PDA) slants. The Department of Plant Pathology at Mansoura University in Egypt's Faculty of Agriculture verified the identification. Table (2) presented the results of the collection of nine water samples from the Oxidation Pond area in Sadat City, Egypt. Water samples were taken from various locations around the Oxidation Ponds region, and each water sample had its fungal species isolated and identified. Out of the grown dominant population, five isolates were chosen. According to the appearance features of the grown colonies, *Aspergillus niger, Penicillium* sp., *Fusarium* sp., *Mucor* sp. and *Trichoderma* sp. isolates formed the highest population (Table 2 and Figs.4, 5 ,6,7 and 8).

Table 2. list of fungi isolated from water samples of Oxidation Pond region of Sadat City.

Fungal spices	**water samples**								
	1	2	3	4	5	6	7	8	9
Trichoderma sp.	+	+	+	+	+	+	+	-	+
Penicillium sp.	+	-	-	+	+	-	+	+	-
Aspergillus niger	+	+	+	+	+	+	+	-	+
Mucor sp.	+	-	+	+	-	+	-	+	+
Fusarium sp.	+	-	+	+	+	-	-	+	+

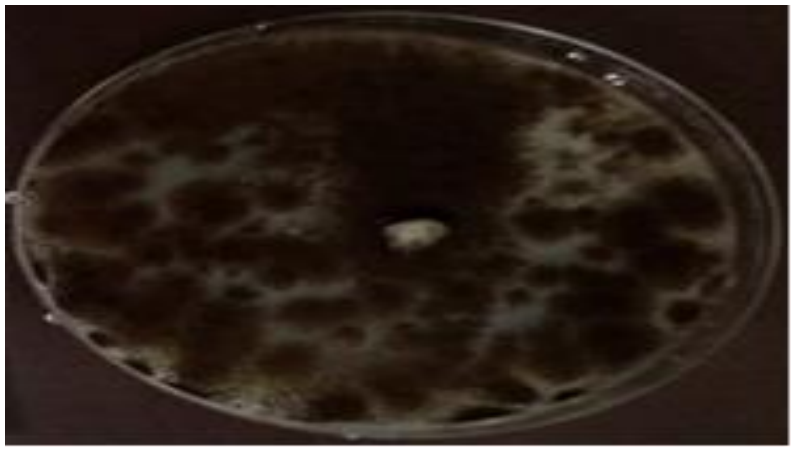


Fig. 4. *Aspergillus niger* fungal growth on PDA medium.

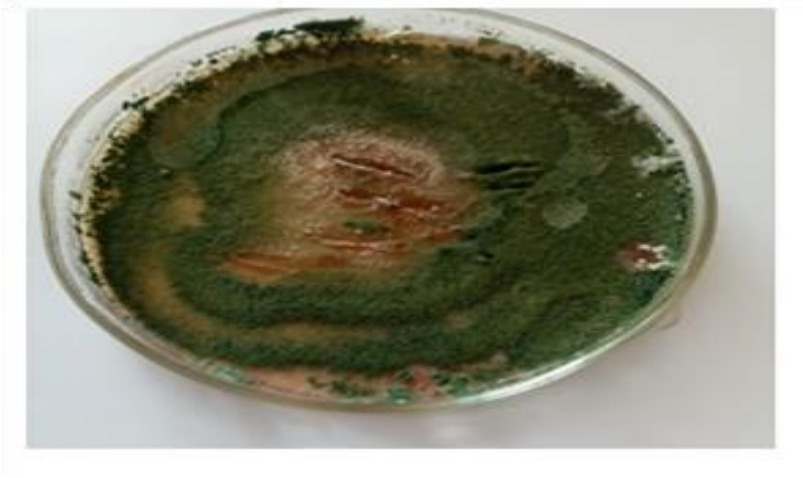


Fig. 5. *Trichoderma* sp. fungal growth on PDA medium.

Fig. 6. *Fusarium* sp. (a) fungal growth on PDA medium.

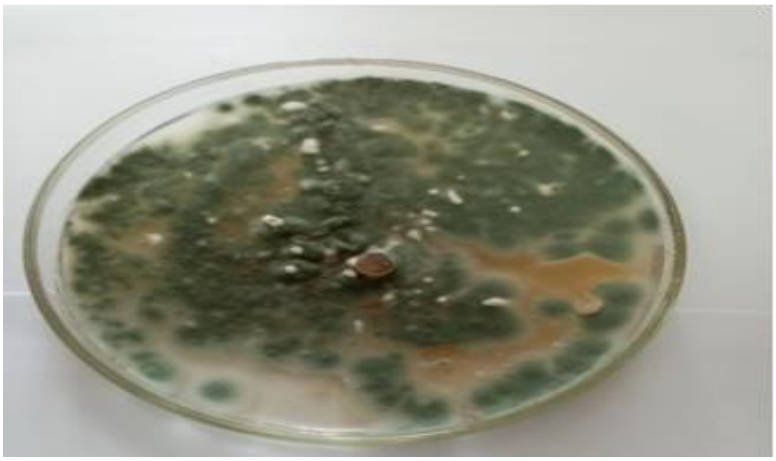


Fig.7. *Penicillium* sp. fungal growth on PDA medium.

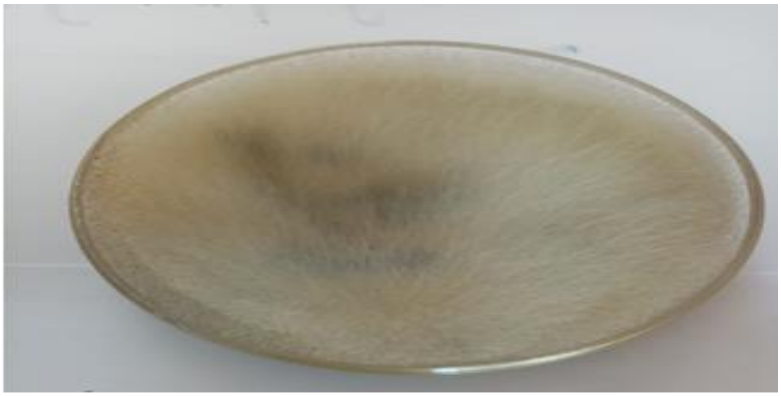


Fig. 8. *Mucor* sp. fungal growth on PDA medium.

Metal tolerance test "Minimum Inhibitory Concentration (MIC)

To select the metal – resistant isolates, the minimum inhibitory concentration (MIC) was utilized. Results of measuring the radial growth diameters of fungal colonies on the

medium contaminated with Cupper ions were showed in the following Tables 3, 4, 5, 6 and 7 and Figs. 9, 10, 11, 12 and 13. Five fungal isolates which had the growth potential were selected. Results in the following tables indicated that selected fungal isolates (*Mucor* sp., *Penicillium* sp., *Trichoderma* sp., *Fusarium* sp. and *Aspergillus niger*) had a good tolerance to Cupper.The most fungal isolates which showed high tolrant to Cupper were *Trichoderma* sp. and *Aspergillus niger* .The measured growth diameters were 37.67 and 20.33 (mm), respectively, at the metal concentration (200 mg/L). The Minimum Inhibitory Concentration (MIC) of Cupper (Cu) against *Mucor* sp., *Penicillium* sp., *Trichoderma* sp., *Fusarium* sp. and *Aspergillus niger* was 250 (mg/L). Metal tolerance range is between concentrations (50 mg/L) and (200 mg/L) in all tested fungi isolates.

Table 3. Minimum Inhibitory Concentration (MIC) of $CuSO_4$against *Aspergillus niger*.

Metal concentration (mg/L)	***Diameter of Fungal species (mm)**
	Aspergillus niger
0	90 ± 0
50	70 ± 1
100	6 2.33 ± 0.58
150	32 ± 2
200	20.33 ± 0.58
250	0.0

* A value is the average of three replicates of that value.

Using the Duncan's multiple range test procedure at (P≤0.05) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.

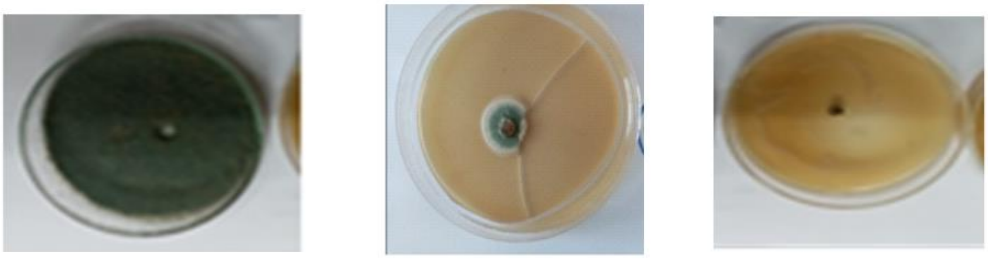
(A) (B) (C)

Fig. 9. Minimum Inhibitory Concentration (MIC) of (Cu) against *Aspergillus niger* (A) control, (B) concentration at (100 mg/L) and (C) concentration at (150 mg/L).

Table 4. Minimum Inhibitory Concentration (MIC) of $CuSO_4$ against *Penicillium* sp.

(Metal concentration mg/L)	***Diameter of Fungal species (mm)**
	***Penicillium* sp.**
0	86 ± 2
50	31.33 ± 1.15
100	24.67 ± 1.15
150	18.33 ± 1.15
200	11.33 ± 1.15
250	0.0

A value is the average of three replicates of that value.Using the Duncan's multiple range test procedure at ($P \leq 0.05$) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.



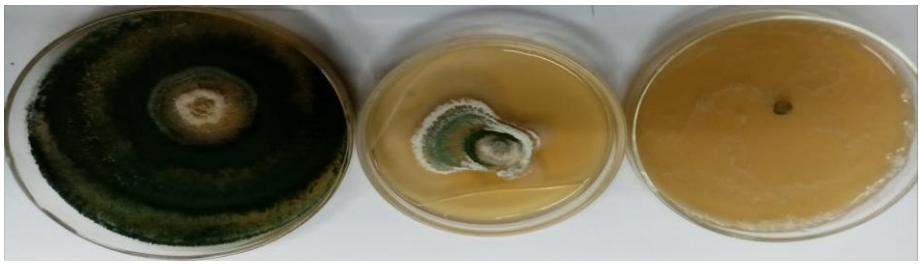
(A) **(B)** **(C)**

Fig. 10. Minimum Inhibitory Concentration (MIC) of (Cu) against *Penicillium* sp. (A) control (B) concentration at (150 mg/L) , (C) concentration at (200 mg/L).

Table 5. Minimum Inhibitory Concentration (MIC) of $CuSO_4$ against *Trichoderma* sp.

Metal concentration (mg/L)	***Diameter of Fungal species (mm)**
	***Trichoderma* sp.**
0	90 ± 0
50	75.33 ± 1.15
100	67.33 ± 1.15
150	46.33 ± 2.52
200	37.67 ± 1.15
250	0.0

*A value is the average of three replicates of that value.
Using the Duncan's multiple range test procedure at ($p \leq 0.05$) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.



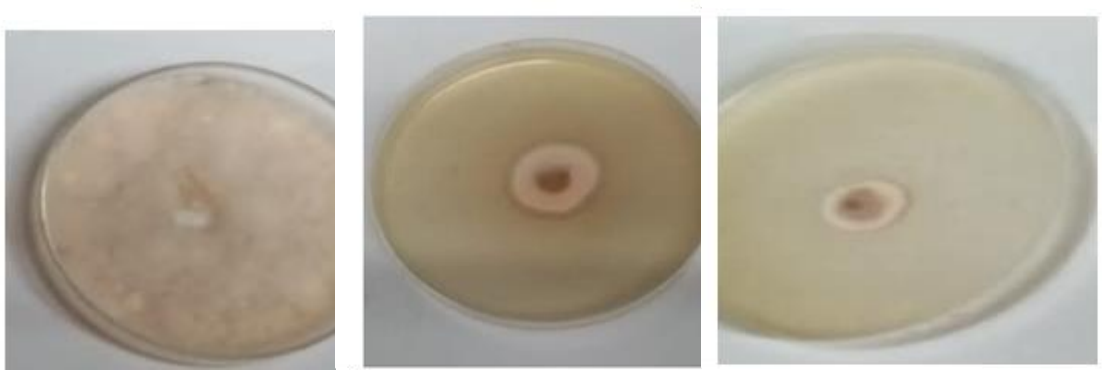
(A) **(B)** **(C)**

Fig. 11. Minimum Inhibitory Concentration (MIC) of (Cu) against *Trichoderma* sp. (A) control (B) concentration at (150 mg/L)(C) concentration at (250 mg/L).

Table 6. Minimum Inhibitory Concentration (MIC) of $CuSO_4$ against *Fusarium* sp.

Metal concentration (mg/L)	***Diameter of Fungal species (mm)**
	***Fusarium* sp.**
0	88 ± 2
50	51.33 ± 1.53
100	41.33 ± 1.53
150	21 ± 1
200	0.0
250	0.0

*Avalue is the average of three replicates of that value.
Using the Duncan's multiple range test procedure at ($p \leq 0.05$) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.



(A) **(B)** **(C)**

Fig. 12. Minimum Inhibitory Concentration (MIC) of (Cu) against *Fusarium* sp. (A) control (B) concentration at (100mg/L) (C) concentration at (150 mg/L).

Table 7. Minimum Inhibitory Concentration (MIC) of $CuSO_4$ against *Mucor* sp.

Metal concentration (mg/L)	***Diameter of Fungal species (mm)**
	***Mucor* sp.**
0	90 ± 0
50	64.33 ± 1.53
100	56.67 ± 1.53
150	30.33 ± 2.31
200	18.33 ± 1.15
250	0.0

*Avalue is the average of three replicates of that value.
Using the Duncan's multiple range test procedure at (P≤0.05) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.

(A) **(B)** **(C)**

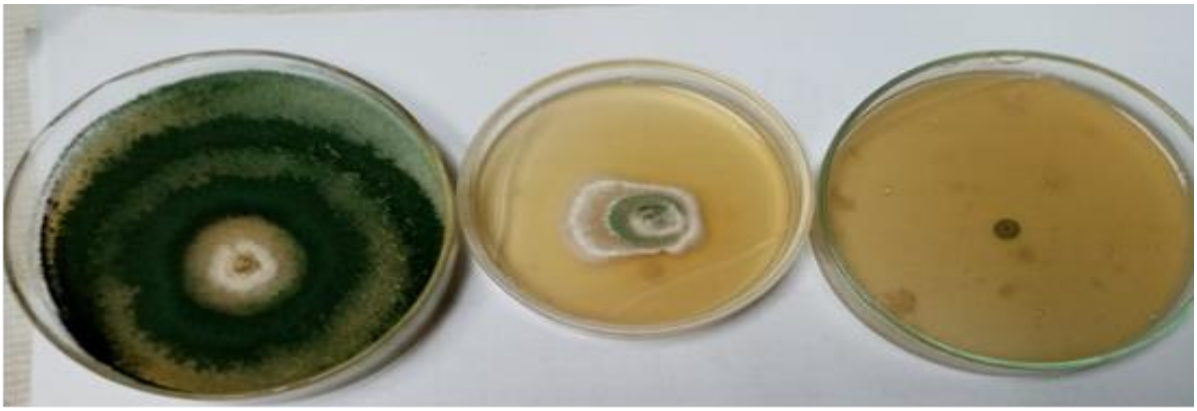
Fig. 13. Minimum Inhibitory Concentration (MIC) of (Cu) against *Mucor* sp. (A) control (B) concentration at (100 mg/L) (C) concentration at (200 mg/L).

Results of measuring the radial growth diameters of fungal colonies on the medium contaminated with Mercury ions were showed in the following Tables 8,9,10,11 and 12. Five fungal isolates which had the growth potential were selected. Results in the following Tables (8, 9, 10, 11 and 12) and Figs. 14, 15 ,16, 17 and 18 indicated that selected fungal isolates (*Mucor sp., Penicillium sp., Trichoderma sp., Fusarium sp. and Aspergillus niger*) had a good tolerance to Mercury (Hg). The most fungal isolates which showed high tolrant to Mercury were *Trichoderma sp* . and *Mucor sp*.The measured growth diameters were 29.67 and 21.67 (mm), respectively, at the metal concentration (200 mg/l). The Minimum Inhibitory Concentration (MIC) of Mercury (Hg) against *Mucor* sp., *Trichoderma* sp. and Aspergillus *niger* was 250 (mg/L) but, it was 200 (mg/L) against *Fusarium* sp. and *Penicillium* sp Metal tolerance range is between concentrations (50 mg/L) and (200 mg/L) in all tested fungi isolates, except in case of *Penicillium* sp. and *Fusarium* sp. that is between metal concentrations (50 mg/L) and (150 mg/L).

Table 8. Minimum Inhibitory Concentration (MIC) of ($HgCl_2$) against *Trichoderma* sp.

(Metal concentration mg/L)	***Diameter of Fungal species (mm)**
	***Trichoderma* sp.**
0	90 ± 0
50	75.33 ± 0.58
100	66.33 ± 1.15
150	44.33 ± 1.53
200	29.67 ± 0.58
250	0.0

* Avalue is the average of three replicates of that value.
Using the Duncan's multiple range test procedure at ($p \leq 0.05$) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.



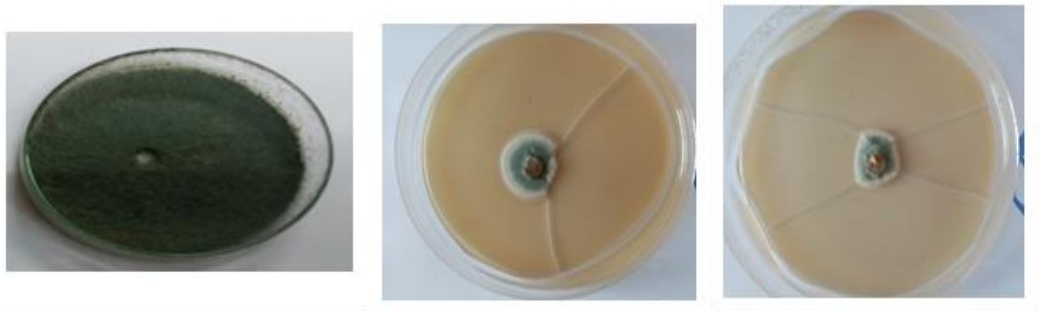
(A) **(B)** **(C)**

Fig. 14. Minimum Inhibitory Concentration (MIC) of (Hg) against *Trichoderma* sp. (A) control (B) concentration at (150 mg/L) (C) concentration at (250 mg/L)**.**

Table 9. Minimum Inhibitory Concentration (MIC) of ($HgCl_2$) against *Penicillium* sp.

Metal concentration (mg/L)	***Diameter of Fungal species (mm)**
	***Penicillium* sp.**
0	85.33 ± 2.31
50	41.67 ± 2.31
100	23.33 ± 1.15
150	15.67 ± 1.15
200	0.0
250	0.0

*Avalue is the average of three replicates of that value.
Using the Duncan's multiple range test procedure at ($p \leq 0.05$) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.



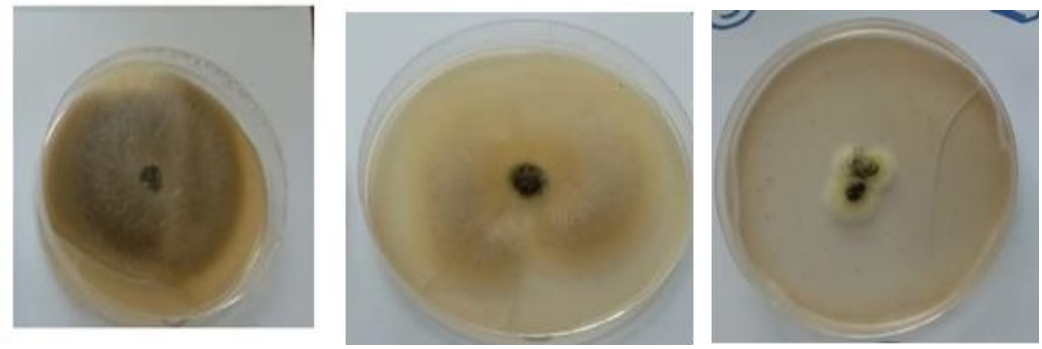
(A) **(B)** **(C)**

Fig. 15. Minimum Inhibitory Concentration (MIC) of (Hg) against Penicillium sp. (A) control (B) concentration at (100 mg/L), (C) concentration at (150 mg/L).

Table 10. Minimum Inhibitory Concentration (MIC) of ($HgCl_2$) against *Mucor* sp.

Metal concentration (mg/L)	***Diameter of Fungal species (mm)**
	***Mucor* sp.**
0	90 ± 0
50	71 ± 1.73
100	63.3 ± 1.53
150	34.67 ± 1.15
200	21.67 ± 0.58
250	0.0

* Avalue is the average of three replicates of that value.
Using the Duncan's multiple range test procedure at ($p \leq 0.05$) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.



(A) **(B)** **(C)**

Fig. 16. Minimum Inhibitory Concentration (MIC) of (Hg) against *Mucor* sp. (A) control (B) concentration at (50 mg/L) (C) concentration at (200 mg/L).

Table 11. Minimum Inhibitory Concentration (MIC) of ($HgCl_2$) against *Fusarium* sp.

Metal concentration (mg/L)	***Diameter of Fungal species (mm)**
	***Fusarium* sp.**
0	90 ± 0
50	37.67 ± 1.15
100	20.33 ± 0.58
150	11.33 ± 0.58
200	0.0
250	0.0

*Avalue is the average of three replicates of that value.
Using the Duncan's multiple range test procedure at ($p \leq 0.05$) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.

(A) **(B)** **(C)**

Fig. 17. Minimum Inhibitory Concentration (MIC) of (Hg) against *Fusarium* sp. (A) control (B) concentration at (100 mg/L) (C) concentration at (150 mg/L).

Table 12. Minimum Inhibitory Concentration (MIC) of ($HgCl_2$) against *Aspergillus niger*.

Metal concentration (mg/L)	***Diameter of Fungal species (mm)**
	Aspergillus niger
0	90 ± 0
50	67.67 ± 1.53
100	56.33 ± 1.15
150	32.67 ± 0.58
200	19.33 ± 0.58
250	0.0

*Avalue is the average of three replicates of that value.
Using the Duncan's multiple range test procedure at ($p \leq 0.05$) level of significance, means with the same alphabetical letter in the column within a comparable group of means do not differ notably. All values are indicative of the average values.

(A) **(B)** **(C)**

Fig. 18. Minimum Inhibitory Concentration (MIC) of (Hg) against *Aspergillus niger* (A) control (B) concentration at (50 mg/L) (C) concentration at (200 mg/L).

Discussion

In the present study, five isolates forming the grown dominant population were isolated and identified from Oxidation Pond region of Sadat City, Egypt. Then identification was confirmed in fungal plant pathology Lab, Faculty of Agriculture, Mansoura Univerisity. According to the appearance features of the grown colonies the isolates of *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp., *Mucor* sp. and *Trichoderma* sp. were formed the highest population as in Table (2)

and Figure (4, 5, 6, 7 and 8). Excess levels of nutrients and other chemicals lead to changes in aquatic life **(Wesbster and Descals, 1979)**. The Oxidation Pond area of Sadat City, Egypt has recorded high fungal population because of the presence of dumping municipal solid waste, industrial chemicals, and domestic sewage. In the aquatic environment, these nutrients function as catalysts and promote the growth of microorganisms. **(Umesh *et al.*, 2012)**. The fungal population increases with the increase of pollution **(**Vaidhya and Paradeshi**, 2015; Somashekhar *et al.*, 1982 and Bhupendra singh *et al.*, 2014).** Heterotrophic fungi are typically found in aquatic environments. **(Goh *et al.*, 2003)**. Through their active participation in the biodegradation and utilisation of organic materials, aquatic fungi contribute to the energy flow and productivity of ecosystems **(Khuble, 2001)**.

Aspergillus, *Mucor*, and *Trichoderma* species have all been reported to exhibit remarkable resistance to Copper and Mercury heavy metals at varying concentrations; our findings are consistent with their findings (**Zafar *et al.*, 2007; López and zquez, 2003 and Harman et *al.*, 2004)**. Certain *Trichoderma* strains demonstrated a high level of resistance to several heavy metals, including Cu and Hg. Furthermore, *Penicillium* strains were able to withstand concentrations of Cu and Hg up to 150 mg/L. Additionally, it was discovered that a strain of *Trichoderma* could withstand 200 mg/L concentrations of Cu and Hg in medium. **(Iskandar *et al.*, 2011; Volesky, 1994; Babu and Shea, 2014 and Vala and Sutariya, 2012).** Certain Trichoderma strains demonstrated a high level of resistance to several heavy metals, including Cu and Hg. Furthermore, *Penicillium* strains were able to withstand concentrations of Cu and Hg up to 150 mg/L. Additionally, it was discovered that a strain of Trichoderma could withstand 200 mg/L concentrations of Cu and Hg in medium **(Kredics *et al.*, 2001).** According to the findings, strains of *Trichoderma* that can withstand Copper so, they could be the best option for bioremediation agents. Fungal species tolerate heavy metals at elevated metal concentrations **(Baldrian, 2003 and Deng *et al.*, 2004).** Specifically, indigenous filamentous fungi that were isolated from contaminated areas have demonstrated a tolerance to heavy metals **(Iram *et al.*, 2013).** The isolates' methods for adapting to increased levels of heavy metal contamination may be responsible for this remarkable feature. **Vala and Sutariya, 2012** outlined the various mechanisms of tolerance, including complexation, the production of intracellular and extracellular enzymes, increased metal efflux, decreased influx, extracellular metal sequestration and precipitation, and metal binding to cell walls. Most people are aware that fungi can be found in a wide range of contaminated or polluted sites with high levels of heavy metals. Specifically, **Zafar *et al.*, 2007 and Fazli *et al.*, 2015** documented the presence of fungal strains in soils that had high concentrations of Cd, Cu, and Zn.

Composed of nitrogen-rich polysaccharides, proteins, lipids, chitin, inorganic ions, and polyphosphates, the fungal cell wall is inflexible **(Gupta *et al.*, 2015)**. Fungi generally use both intracellular and extracellular sequestration to detoxify metal ions. In order to facilitate the precipitation of metals and, ultimately, their immobilisation, fungal metabolites such as siderophores and organic acids (such as citric, malic, acetic, succinic, and gluconic acids) are secreted during the extracellular sequestration process. **(Gajewska *et al.*, 2022)**. Metal ions are bound by the surface of the cell wall, which ensures that metals are removed from the environment. Many biomolecules (such as lipids, polysaccharides, and peptidoglycans) with metal ligands such as metal binding to the fungal cell wall that involves hydroxyl, carboxylic, sulfhydryl, phosphoryl, amine, and thiol groups. **(Igiri *et al.*, 2018)**.

Conclusion

Based on the study's findings, five indigenous fungal isolates that could survive in environments with Copper and Mercury were discovered. The five isolates were believed to possess the capacity to function as bioremediation agents for waste water that contains Copper and Mercury. The majority of the isolates, including *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Mucor* sp., and *Trichoderma* sp., were able to grow in the range of 50 to 200 mg/L when Copper and Mercury were present.

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