Resistance and accumulation of heavy-metals by soil fungi of Basrah, Iraq

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Abstract

Bioremediation has emerged as an effective strategy for treating soil pollution, leveraging the capacity of microorganisms to degrade and eliminate pollutants. The soil fungal diversity in agricultural and oil areas in Basrah Province, Iraq, was studied to identify the fungal species and to examine their ability to accumulate heavy metals. From different lubricant and farming areas, eighteen soil samples were collected. The genus *Aspergillus* represented the highest occurrence percentage (100%) of all the fungal genera isolated during this study, the other genera showed 11–89% of occurrence. Sterile mycelia of seventeen fungal species were also isolated in this study. The ability of fungal species to accumulate three heavy metals namely copper, cadmium and cobalt were studied. Copper had the most potent effect on fungal growth, whereas cobalt revealed the least influence and cadmium had moderate effect on them. *Aspergillus niger* revealed the highest percentage of accumulation, whereas the other fungal species revealed different capabilities to accumulate heavy metals.
Keywords: *Aspergillus niger*, Fungal isolates, Heavy metals, Iraq, Mycoremediation.

Introduction

Heavy-metals contamination in the soil is an environmental and public health concern intensified by anthropogenic activities such as industrial processes, mining operations and inadequate waste management practices (Prakash and Chandran, 2023). These activities release heavy metals such as lead, cadmium, mercury and arsenic into the environment where they persist due to their chemical properties and pose significant risks to ecosystems and human well-being. Heavy metals are nonbiodegradable and tend to accumulate in the soil, posing long-term threats to plants, soil fertility, water quality and biodiversity (Shoaib and Javaid, 2021; Reddy *et al.*, 2024). Through plant uptake, these can enter the food chain, so human health and crop safety become in series danger. Different health problems including certain types of cancer, kidney damage and neurological disorders can be aroused due to exposure to heavy metals (Akram *et al*., 2023). Complete interdisciplinary background including biology, environmental science, chemistry, microbiology and engineering is needed for improving heavy-metals pollution requires. Remediation strategies to be successful should cover the nature of contaminants, evaluation of soil properties and the ecological communications within the adjacent environment (Deng *et al*., 2024). A reflective understanding of the effects of heavy metals in the soil, their origins and transport mechanisms are imperative for creating precise strategies aimed at mitigating environmental degradation and protection human (Chen *et al*., 2023).

According to the metal type and its presence in the soil, toxicity level of heavy metals differs widely. Higher levels of some metals such as manganese, nickel and zinc become harmful but at low concentrations they act as micronutrients for the organisms. On the other hand, some metals such as cadmium, lead and mercury not needed for the growth of organisms and they can be lethal even at low concentrations (Al-hejuje *et al*., 2017). Remediation methods including ion exchange, filtration, carbon adsorption, membrane technology and electrochemical methods have been used to eliminate heavy metals from polluted environments (Ali *et al*., 2018). However, many problems appear during the use of such approaches like high costs and complication, also these procedures lack consideration for metal-binding features (Wang *et al*., 2021). In recent years, many approaches have encouraged the usage of microorganisms like fungi and bacteria for heavy-metals remediation. There are several benefits by using these methods including cost-effectiveness, environmental sustainability, flexibility and possibility to use in large-scale applications (Javaid, 2011; Reddy *et al*., 2024). Mycoremediation is accessible and appropriate for treating widespread areas of polluted soil. Current progressions in fungal biotechnology improve its applied application (Prakash and Chandran, 2023). By using their extensive mycelial networks, fungi can effectively immobilise and accumulate heavy metals so they can reduce metal toxicity in soil (Qader and Shekha, 2023). One of the most important features that make fungi flexible to various soil types and contaminants is their ability to diverse metal tolerance and remediation abilities (Javaid *et al*., 2010; Reddy *et al*., 2024). In recent years, our environment has become heavily polluted with heavy metals, therefore, this work was designed to explore

the use of soil fungi in the remediation of heavy metals.

Materials and Methods

Soil sampling

For isolation of fungi eighteen soil samples were collected (100 g each) from the top soil layer (5–10 cm). Study area including six oil and agricultural places south and north of Basrah province between September and December 2023 (Fig. 1). After collection sterilised plastic bags were used to preserve them at 4 °C waiting for further analysis

Chemicals

All chemicals and media used in this study were obtained from Hi Media Company, India.

Preparation of heavy-metal stock solutions

Stock solutions of copper, cadmium, and cobalt ions were prepared at a concentration of 5 g L^{-1} by dissolving pure salts of these metals in ion-free water, and concentrated nitric acid (1.5 mL) was added to prevent heavy-metal precipitation (Lobos *et al*., 2020).

Culture media

Two types of culture media, malt extract agar (MEA) and potato dextrose agar (PDA), were used to isolate of fungi from the soil samples. PDA was also used to preserve of fungal isolates. Two types of media were prepared according to the manufacturer's instructions of Hi Media Company. First, 250 mg L^{-1} of chloramphenicol was added to each medium to inhibit bacterial growth. Second, the medium was sterilised by autoclaving at 121 °C under 15 pounds/$inch^2$ for 15 min.

Fungal isolation

Isolation of fungi from soil samples was done by dilution method (Wicklow and Wittingham, 1974). Ten grams of the soil from each sample were transfererd to 90 mL of sterile saline solution in a 250 mL flask to prepare a 10^{-1} dilution. This mixture was serially diluted up to 10^{-3}. One milliliter of each dilution was loaded on MEA or PDA media in Petri dishes separately before solidification. The samples were incubated at 25±2 °C for one to two weeks. The percentage of occurrence for the isolated fungi was recorded using the following equation:

$$\text{Occurrence (\%)} = \frac{\text{No. of samples in which fungus appeared}}{\text{Total number of samples}} \times 100$$

Fungal identification

The isolated fungi were initially examined under a dissecting light microscope to document colony characteristics, sporulation rates and colours. Glass slides were then prepared from each fungal colony and morphological features were further studied using a compound light microscope (Samson *et al*., 2010). The taxonomic identification of fungi was done using the established keys (Watanabe, 2002; Guarro *et al*., 2012).

Screening of fungi to accumulate heavy metals

The potential of fungal isolates to tolerate and accumulate heavy metals was tested in accordance with the work of Mohamadhasani and Rahimi (2022). First, the fungi were activated by culturing on MEA for 7 days. Then Petri dishes with MEA medium were then supplemented with 100, 200, 400 and 800 ppm of Cu, Co and Cd heavy metals individually and each concentration was inoculated by a piece obtained using a 5 mm cork borer from the edge of each 7-day-old fungal isolate. Petri dishes containing MEA medium and fungal isolates without any heavy metals served as controls. The experiment was done in triplicates for each fungus and concentration. Petri dishes were incubated for six days at 25±2 °C and the colonies were examined for differentiation in size and colours every 48 h up to six days.

Heavy-metals tolerance index (TI) of fungi

The tolerance index (TI) of heavy metals was determined in accordance with Calvillo-Medina (2021) by dividing the growth of the fungus exposed to different concentrations of heavy metals (HMFG) by the growth of the fungus in the control medium (CFG).

$$\text{TI} = \frac{\text{HMFG}}{\text{CFG}}$$

Value of tolerance refer to the following:
0.00–0.39 - very low metal tolerance.
0.40–0.59 - low metal tolerance.
0.60–0.79 - moderate metal tolerance.
0.80–0.99 - high metal tolerance.
1.00 or more - very high metal tolerance.

Statistical analysis

One-way analysis of variance ANOVA was performed using Minitab version 16. Relative least significant values were calculated to identify important differences in fungal growth rate under $P \leq 0.01$. A completely randomised design was used.

Results and Discussion

Identification of fungal genera

Nine fungal genera in addition to sterile mycelia were isolated from soils of different localities (Table 1). The number of fungi was less than that in other studies such as Wu *et al*. (2022), which may be due to the high air temperature during the time of samples collection which may have reached 45 °C which adversely affected growth of fungi in the soil. Moreover, some isolation areas were heavily contaminated with crude oil containing

different toxic compounds that had a negative effect on fungal growth. The current study agreed with other studies, such as Shatyeh *et al.* (2003) and Muhsen and Al-Dossary (2023) about fungal isolates and their appearance. The overall variations in the occurrence percentages of fungal genera likely arises from their resilience and adaptation to challenging environments wide temperature ranges and the secretion of various enzymes that facilitate the decomposition of diverse materials for energy and growth. Furthermore, prolific production of reproductive units enables widespread dispersion in the environment (Taylor and Sinsabaugh, 2015).

Anamorphic fungi representing asexual state dominated in this study. This group of fungi has good resistance, and their wide spread was due to the ability of most of them to produce reproductive units in large numbers and the composition of a well-developed reproductive structure. These features enabled them to resist harsh environmental conditions such as low humidity and high temperature and their enzymatic activity enabled them to use different organic materials including crude oil for growth and reproduction (Altaee and Al-Dossary, 2021). Fungi belong to Zygomycota were identified in the second classification category. This low rate can be attributed to their saprophytic nature that hinders their ability to compete with other species coupled with their limited resistance to harsh environmental conditions including low humidity and high temperatures (Singh *et al.*, 2014; Teigiserova *et al*., 2019).

The genus *Aspergillus* appeared in all samples with a frequency of 100%. It is a common genus in the world and it has a small reproductive unit with many numbers spread easily in the environment. Its ability to adapt to a wide range of temperatures, pH and high concentrations of salinity which enables it to grow in different environmental conditions. This finding is in agreement with those of other studies, such as Ahmad *et al*. (2023) and Muhsen and Al-Dossary (2023) on the high occurrence of *Aspergillus* compared with that of other genera. The percentage of occurrence for the other genera ranged from 11% in *Cladosporium* to 89% in *Penicillium.* The two genera *Fusarium* (72%) and *Penicillium* (89%) were similar to *Aspergillus* in having a high ability to tolerate different environmental conditions pose a small reproductive unit with high numbers that could spread easily in the environment and adapt to a wide range of temperatures, pH and high salinity, which enables them to grow in different environmental conditions (Slorach *et al*., 2020; Abass *et al*., 2021).

Genera such as *Cladosporium*, appeared at a low frequency of 11% because of their low ability to tolerate unfavourable environmental conditions or produce inactive reproductive units which affect their occurrence in culture media (Zhang *et al*., 2020; Ahmad *et al*., 2023; Al-hamdani and Al-Dossary, 2023). The occurrence of sterile mycelia was 22% which can be attributed to their potential loss of reproductive capabilities under the stressful environmental conditions from which they were isolated (Silva *et al*., 2015; El-Hanafy *et al*., 2017).

Identification of fungal species

Seventeen fungal species were isolated from soil samples (Table 2). The percentage of occurrence of the species ranged from 6–100%. The high percentages of occurrence belonged to species *Aspergillus niger* with 100% followed by *Aspergillus wentii* and *Fusarium* sp. 1 with 44%. These fungal species adapt well to their environment likely due to their ability to grow and reproduce in large quantities in contaminated soil, having a complex enzymatic system that enabled them to degrade many types of compounds that are difficult to degrade and the production of resistant reproductive units that enabled them to grow under different environmental conditions (Ahmad and Ahmad, 2024). The occurrence of other species ranged from 6% in *A. fumigatus* to 39% in *A. terreus*. The variation in occurrence rate may be attributed to their ability to tolerate contaminants in the environment and factors such as soil type, dryness and high temperaturewhich all influence species occurrence (Efremenko *et al*., 2024).

Screening of fungi for tolerance to heavy metals

All fungal isolates were examined for their resistance to the heavy metals Cu, Co and Cd at 100, 200, 400 and 800 ppm concentrations. The results revealed significant differences in the impact of heavy metals on fungal growth and activity. Cu was the most toxic metal exhibiting distinct inhibitory effects on the physiological processes of the tested fungi (Table 3). In contrast Co displayed the lowest toxicity resulting in minimal adverse effects on fungal performance (Table 4). Cd demonstrated intermediate toxicity with effects that were more pronounced than those of Cu but less severe than those observed with Cd (Table 5). The results showed that all concentrations tested for heavy metals affected the fungal growth and they could not grow well at almost all the concentrations, except in the case of *A. niger*, and could grow well at almost all concentrations of Cd, Co and Cu. Similar results were reported by Iram *et al*. (2013) who found that various strains of fungi originating from metal-contaminated sites did not have the same level of tolerance. The most probable reason for the difference in resistance levels could be the variation in the mechanisms of resistance (Ezzouhri *et al*., 2009).

Out of six fungal isolates clearly tolerant to Cu at 100 ppm, only one isolate *A. niger* could tolerate Cu at 200 and 400 ppm, with growth rates of 8.5 and 5.3 cm respectively. However, its growth similar to that of other fungi was inhibited at 800

ppm. As previously mentioned this fungus has good tolerance to different heavy metals and possesses sophisticated biological systems that enable it to thrive in harsh environmental conditions and withstand toxicity from heavy metals such as Cu including the production of specialised enzymes that aid in the accumulation of Cu in their bodies (Akram *et al*., 2023). Except *A. niger,* the growth of all other fungi was clearly inhibited at 200, 400 and 800 ppm that might be due to several key properties of Cu. First Cu acts as a potent oxidizing agent within fungal cells reacting with biological compounds such as proteins and nucleic acids, thereby disrupting their essential functions. This oxidative stress leads to the impairment of cellular processes crucial for fungal viability, including respiration and cellular division. Secondly Cu ions interfere with fungal cell membrane potentially disrupting their integrity and causing an imbalance in vital cellular functions. Consequently, Cu effectively inhibits fungal growth by impeding normal cellular division and vegetative growth (Qader and Shekha, 2023).

The results of the statistical analysis ($P \leq 0.01$) showed significant differences in the growth rate between the control and 100, 200, 400 and 800 ppm of Cu except for *A. niger* in which the differences at 100 and 200 ppm were lowers from those of the control. Except for this fungus none of the other fungal species showed well tolerance to Cu at these two concentrations. The most fungi could grow well at 100 ppm concentration of Co and six species had a growth rate of more than 5 cm. Three species, *A. niger, A. flavus* and *A. wentii*, filled the plates at a growth rate of 9 cm. Similar results have been reported by Turnau *et al*. (2006). At high concentrations the number of isolates tolerant to Co decreased. At 200 ppm six species could grow well and this number decreased to four at 400 ppm. Meanwhile, no species could grow at all at 800 ppm (Table 4). The results of the statistical analysis showed significant differences ($P \leq 0.01$) in the growth rate of fungal species between the control and most species at different concentrations. Joshi *et al*. (2011) showed that the growth of fungi stopped at a concentration of 100 ppm for Co whereas the results of the current study showed that some fungi were tolerant possibly due to the development of membrane barriers preventing entry into cells, the chemical transformation of the metal into less toxic forms by fungi and the adaptation to toxic compounds in the environment (Dusengemungu *et al*., 2022).

Nine fungal species tolerated 100 ppm, showing moderate to good tolerance. Six species could tolerate 200 ppm. However, at higher concentrations, all the species either showed low tolerance or no tolerance at all (Table 4). The decrease in fungal tolerance at higher concentrations suggests that higher concentrations of Co have a poisonous effect on most fungal isolates, inhibiting their growth and survival in high concentrations. This phenomenon is consistent with previous findings showing that heavy metals can exert inhibitory effects on microbial growth, including fungi. The mechanism of toxicity typically involves interference with essential cellular processes such as enzyme function and DNA replication leading to cell damage and death (Akram *et al*., 2023). Out of seven fungal species tolerant to Cd at 100 ppm and showing moderate to good growth, only four species could tolerate Cd at 200 ppm namely *A. niger*, *A. flavus*, *Fusarium* sp.1 and *Fusarium* sp.2 with growth rates of 7, 2.4, 2.5 and 6.6 cm respectively. At 400 ppm *A. niger* and *Fusarium* sp.2 showed moderate growth rates of 4.5 and 3.6 cm respectively. However, at 800 ppm, all the fungal species did not show any growth at all. The results of the statistical analysis showed significant differences ($P \leq 0.01$) in the growth rate of fungal species between the control and different concentrations of Cd except for *A. niger*, *Fusarium* sp. 2 and *Penicillium* sp. 3 in which no differences were observed in their growth rate between the control and 100 ppm concentration. As for TI, most of the fungal species clearly could not tolerate the concentrations of Cd with a tolerance range of 0.1–0.4 for almost all the species except for *A. niger*, *Fusarium* sp. 2 and *Penicillium* sp. 3, which showed good to low tolerance at up to 400 ppm.

Except for *A. niger,* which is known for its exceptional adaptability to environments contaminated with heavy metals like Cd it demonstrates the highest growth rates under such conditions due to several adaptive mechanisms (Iram *et al*., 2013). The decline in the growth rate of fungal species and their tolerance to Cd can be attributed to its toxic effects on fungal development. Factors contributing to this inhibition include cellular toxicity which disrupts vital cellular processes and oxidative stress caused by reactive oxygen species the interference with nutrient absorption leading to deficiencies in essential elements for growth (Jin *et al*., 2018). Inhibition of certain fungal isolates at increased concentrations was observed aligning with findings from Mohamadhasani and Rahimi (2022) regarding the detrimental impact of high concentrations of heavy metals on fungal growth.

Conclusion

Seventeen fungal species were isolated in the present study. Most of them represent anamorphic fungi, which are one of the largest groups of fungi in the environment. Many of these species can tolerate low concentrations of Cu, Cd and Co at 100 ppm, exhibiting moderate to good growth. Meanwhile, at high concentrations of 200, 400 and 800 ppm, very few species could grow, and 800 ppm showed no growth at all. *A. niger* showed the best growth rate amongst fungal species with all types of heavy metals.

Novelty Statement

This work directly addresses the issue of environmental safety by providing a sustainable solution throw using fungi towards the bioremediation of soils. We believe that the data we presented and discussed in this study is a novel and a timely contribution.

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Contribution of authors

MAA wrote the main text of the manuscript, ZSL analyzed the data and formatted tables while MAA carried out analysis of heavy-metals.

Conflict of interests

Authors declare that there is no conflict of interest.

Table 1: The isolated fungal genera with their percentage of occurrence.

No.	Fungal genera	No. of samples in which the genus appeared	Occurrence (%)
1	*Aspergillus*	18	100
2	*Cladosporium*	2	11
3	*Emericella*	2	11
4	*Fusarium*	13	72
5	*Mucor*	4	22
6	*Penicillium*	16	89
7	*Phoma*	3	17
8	*Rhizopus*	5	28
9	*Ulocladium*	2	11
10	Sterile mycelia	4	22

Table 2: The isolated fungal species with their percentage of occurrence.

No.	Species	No. of samples in which the species appeared	Occurrence (%)
1	*Aspergillus flavus*	4	22
2	*A. fumigatus*	1	6
3	*A. niger*	18	100
4	*A. terreus*	7	39
5	*A. versicolor*	5	28
6	*A. wentii*	8	44
7	*Cladosporium* sp.	6	33
8	*Emericella* sp.	4	22
9	*Fusarium* sp.1	8	44
10	*Fusarium* sp.2	5	28
11	*Mucor* sp.	4	22
12	*Penicillium* sp.1	6	33
13	*Penicillium* sp.2	4	22
14	*Penicillium* sp.3	6	33
15	*Phoma* sp.	4	22
16	*Rhizopus* sp.	5	28
17	*Ulocladium* sp.	2	11

Table 3. Fungal growth rates and tolerance index on solid media containing copper.

No.	Species	Concentration (ppm)								
		Colony diameter (mm)					Tolerance index			
		0	100	200	400	800	100	200	400	800
1	*Aspergillus niger*	9	8.5	8.5	5.3	0	0.9	0.9	0.1	0
2	*Aspergillus flavus*	9	5.5	1	1	0	0.6	0.1	0.1	0
3	*Cladosporium* sp.	5	4	1	1	0	0.2	0.2	0.2	0
4	*Fusarium* sp.1	8.5	4	1	1	0	0.4	0.5	0.1	0
5	*Fusarium* sp. 2	8.5	4	1	1	0	0.4	0.1	0.1	0
6	*Penicillium* sp. 2	9	3.1	1	1	0	0.3	0.1	0.1	0
7	*Penicillium* sp. 1	9	1	1	1	0	0.1	0.1	0.1	0
8	*Penicillium* sp. 3	9	1	1	1	0	0.1	0.1	0.1	0
9	*Aspergillus versicolor*	9	1	1	1	0	0.1	0.1	0.1	0
10	*Rhizopus* sp.	9	1	1	1	0	0.1	0.1	0.1	0
11	*Mucor* sp.	9	1	1	1	0	0.1	0.1	0.1	0
12	*Ulocladium* sp.	9	1	1	1	0	0.1	0.1	0.1	0
13	*Phoma* sp.	7	1	1	1	0	0.1	0.1	0.1	0
14	*Aspergillus terreus*	6	1	1	1	0	0.1	0.1	0.1	0
15	*Aspergillus wentii*	5.5	1	1	1	0	0.1	0.1	0.1	0
16	*A. fumigatus*	5	1	1	1	0	0.2	0.2	0.2	0
17	*Emericella* sp.	5	1	1	1	0	0.2	0.2	0.2	0

RLSD=1.108

Table 4: Fungal growth rates and tolerance index in solid media containing cobalt.

No.	Species	Concentrations (ppm)								
		Colony diameter (mm)					Tolerance index			
		Control	100	200	400	800	100	200	400	800
1	*Aspergillus flavus*	9	9	9	4.7	0	1	1	0.5	0
2	*Aspergillus wentii*	9	9	5.3	3.6	0	1	0.6	0.4	0
3	*Aspergillus niger*	9	9	7.8	3.6	0	1	0.9	0.4	0
4	*Fusarium* sp.2	9	8.2	6.6	3.6	0	0.9	0.7	0.4	0
5	*Fusarium* sp.1	9	6.4	4.5	1	0	0.7	0.5	0.1	0
6	*Aspergillus terreus*	4.5	2.5	1	1	0	0.5	0.2	0.2	0
7	*Aspergillus fumigatus*	5.8	1	1	1	0	0.1	0.1	0.1	0
8	*Mucor* sp.	9	6	1	1	0	0.7	0.1	0.1	0
9	*Penicillium* sp.3	9	4.2	4.2	1	0	0.5	0.5	0.1	0
10	*Rhizopus* sp.	9	4	1	1	0	0.6	0.1	0.1	0
11	*Cladosporium* sp.	9	2.4	1	1	0	0.3	0.1	0.1	0
12	*Emericella* sp.	9	2.1	1	1	0	0.2	0.1	0.1	0
13	*Aspergillus versicolor*	9	1	1	1	0	0.1	0.1	0.1	0
14	*Penicillium* sp.1	9	1	1	1	0	0.1	0.1	0.1	0
15	*Penicillium* sp.2	9	1	1	1	0	0.1	0.1	0.1	0
16	*Phoma* sp.	9	1	1	1	0	0.1	0.1	0.1	0
17	*Ulocladium* sp	9	1	1	1	0	0.1	0.1	0.1	0

RLSD = 0.857

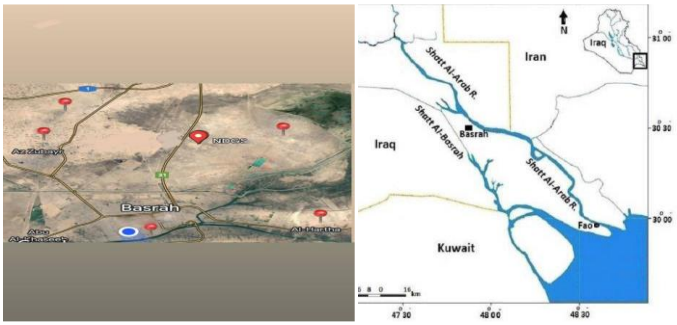

Fig. 1: Study areas in Basrah province, Iraq.

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