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Bioaccumulation of Copper, Cadmium and Cobalt by Heavy-metal Resistant Soil Fungi: *in Vitro* Investigation

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Abstract

Heavy-metal contaminants represent significant pollution in soil. Their high toxicity and propensity to accumulate in soil and crops constitute a serious risk to food security. Addressing this issue necessitates urgent restoration of disturbed lands. 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, Southern Iraq, was studied to identify the fungal species and examine their ability to accumulate heavy metals. Twenty-one soil samples were obtained from several oil and agricultural areas in Basrah Province. Nine fungal genera beside the sterile mycelia were isolated, with *Aspergillus* representing the highest percentage of occurrence (100%). The percentages of the appearance of other genera were between 11% and 89%. Seventeen fungal species were isolated followed by sterile mycelia. This study aimed to investigate the ability of fungi to bioaccumelate of heavy metals *in vitro*. The effects of copper, cadmium and cobalt were studied to assess the ability of fungal species to accumulate heavy metals. Results indicated that the most influential metal on fungal growth was copper, followed by cadmium, whereas cobalt exhibited the least influence. The fungal species showed different abilities to accumulate heavy metals, and *Aspergillus niger* exhibited the highest percentage of accumulation. This study aimed to investigate the ability of fungi to bioaccumelate of heavy metals in *vitro*.

Keywords: Fungal isolates; Heavy metals; In vitro; Mycoremediation

1. Introduction

Heavy-metals contamination in soil is a pressing environmental and public health concern, exacerbated 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 (Pb), cadmium (Cd), mercury (Hg), and arsenic (As), into the environment, where they persist due to their chemical properties and pose significant risks to ecosystems and human well-being (Reddy *et al.,* 2024)

Heavy metals are non-biodegradable, and they tend to accumulate in soil, posing long term threats to soil fertility, water quality and biodiversity. They can enter the food chain through plant uptake, affecting crop safety and human health. Chronic exposure to heavy metals has been linked to a range of health problems, including neurological disorders, kidney damage and certain types of cancer (Akram *et al.,* 2023)

Addressing heavy-metal pollution necessitates a comprehensive interdisciplinary framework that incorporates fields such as environmental science, chemistry, biology and engineering. Successful remediation tactics should encompass an assessment of soil properties, the nature of contaminants, and the ecological interactions within the surrounding environment (Deng *et al.,* 2024). A profound comprehension of the origins, transport mechanisms and ramifications of heavy metals in soil is imperative for crafting precise mitigation strategies aimed at mitigating environmental degradation and safeguarding human well-being (Chen *et al.,* 2023).

The toxicity level of heavy metals varies depending on the specific mineral metals and their availability in the soil. While some metals, such as zinc (Zn), copper (Cu), nickel (Ni) and manganese (Mn), act as micronutrients at low concentrations, they can become toxic at high levels. Conversely, metals, such as mercury (Hg), cadmium (Cd), and lead (Pb), are not essential for the development of organisms, and they can be toxic even at minimal concentrations (Al-hejuje *et al.,* 2017).

Conventional remediation techniques, including filtration, ion exchange, reverse osmosis, evaporation, membrane technology, carbon adsorption, electrowinning, pre-concentration, chelation, redox and electrochemical methods, were used to remove heavy metals from polluted areas (Ali *et al.,* 2018). However, such conventional methods come with numerous drawbacks, including complexity and rising expenses for large-scale deployment. In addition, these methods are difficult and lack consideration for metal-binding features (Wang *et al.,* 2021).

Recent years have moved towards the use of microorganisms like fungi for heavy-metals remediation, and this technique offers several advantages, emphasising its efficiency, environmental sustainability, cost-effectiveness, versatility and potential for large-scale applications (Reddy *et al.,* 2024). Mycoremediation is scalable and suitable for treating extensive areas of contaminated soil. Ongoing advancements in fungal biotechnology enhance its practical implementation (Prakash and Chandran, 2023). Fungi efficiently accumulate and immobilise heavy metals by using their extensive mycelial networks, reducing metal toxicity in soil (Qader and Shekha, 2023). Mycoremediation is often more cost-effective than traditional methods like excavation by minimising equipment and logistical expenses. Fungi possess diverse metal tolerance and remediation capabilities, making them adaptable to various soil types and contaminants (Reddy *et al.,* 2024).

As a result of the environment being polluted with heavy metals and to obtain an environmental friendly method to treat and remove these pollutants from the environment, this study aimed to investigate the use of fungi in the remediation of heavy metals and assess their efficiency to enhance bioremediation techniques and contribute to the preservation of ecosystems and human health.

2. Methodology

2.1 Samples collection

Twenty-one soil samples from agricultural and oil areas (100 g for each one) were collected from the surface layer (5 – 10 cm) of six places in the south and north of Basrah Province between September and December 2023 (Figure 1). The samples were preserved in sterilised plastic bags and stored at 4 °C until further analysis.

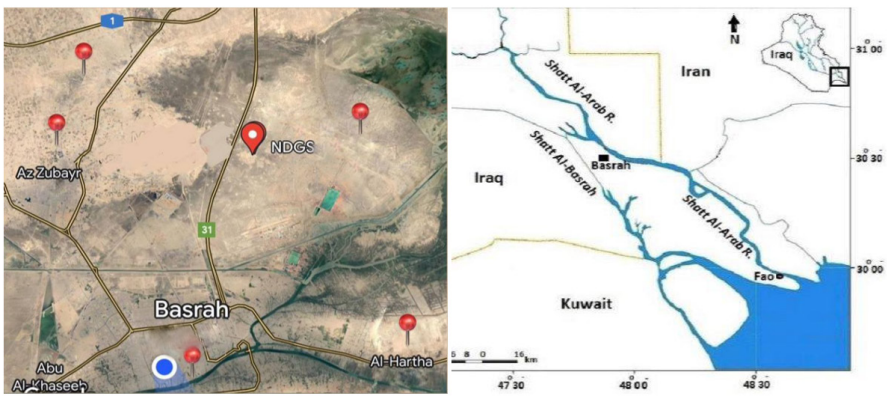

Figure 1. Samples collection area in Basrah Province, places and names.

2.2 Chemicals

All chemicals and media used in this study were obtained from Hi media company (India).

2.2.1 Heavy-metal stock solutions

Stock solutions of copper, cadmium, and cobalt ions were prepared at a concentration of 5000 mg/L by dissolving the pure salts of these metals in ion-free water, and 1.5 ml of concentrated nitric acid was added to prevent heavy-metal precipitation (Lobos *et al.,* 2020).

2.2.2 Culture media

Two types of culture media, malt extract agar (MEA) and potato dextrose agar (PDA), were used for the isolation of fungi from soil samples. PDA was also used for the preservation of fungal isolates. The two types of media were prepared in accordance with the manufacturing instructions of Hi media company (India). First, 250 mg/L of chloramphenicol was added for each media to inhibit bacterial growth. Second, the media were sterilised by autoclaving at 121 °C, under 15 pounds/inch² for 15 min.

2.3 Fungal isolation

Fungi were isolated from soil samples through dilution (Wicklow and Wittingham, 1974). Approximately 10 g of each soil sample was added to 90 mL of sterile physiological saline in a 250 mL conical flask to obtain 10^{-1} dilution, from which a serial dilution of up to 10^{-3} was prepared. Each dilution (1 mL) was transferred to sterile Petri dishes, and then MEA or PDA was added separately to each Petri dish containing the sample before solidification. The samples were incubated for 7 – 14 days at 25 °C ± 2 °C. The percentage of occurrence for the isolated fungi was recorded using the following equation:

$$\text{Occurrence \%} = \frac{\text{Number of samples in which the genus or species appeared}}{\text{Total number of samples}} \times 100$$

2.4 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 followed the established keys from seminal works by Watanabe (2002) and Guarro *et al.* (2012).

2.5 Screening of the ability of fungal isolates to accumulate heavy metals

The ability of fungal isolates to tolerate different concentrations of heavy metals and accumulate them were tested in accordance with the work of Mohamadhasani and Rahimi (2022). First, the fungi were activated by culturing them on MEA media for 7 days. Then, Petri dishes with MEA medium were 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 media and fugal isolate without any heavy metals served as the control. The experiment was performed in triplicate for each fungus and concentration. All Petri dishes were incubated for 6 days at 25 °C ± 2 °C, and the colonies were examined for differentiation in size and colours every 48 h for 6 days.

2.6 Heavy-metals tolerance index (TI) of fungi

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

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

Tolerance index rating values indicate: 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 to > 1.00 very high metal tolerance.

2.7 Statistical analysis

One-way ANOVA was applied using Minitab (version 16). Relative least significant difference (RLSD) values were calculated to identify important differences in fungal growth rate. A complete randomised design was employed.

3. Results and Discussion

3.1 Identification of fungal genera

The overall variations in the occurrence percentages of fungal genera likely appear 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, their prolific production of reproductive units enables widespread dispersion in the environment (Taylor and Sinsabaugh, 2015).

Nine fungal genera in addition to sterile mycelia were isolated from the soil samples (Table 1). The number of isolated fungi was less than that in other studies, such as Wu *et al.,* (2022), may be due to high air temperature during the time of samples collection, which may have reached 45 °C, which negatively affected fungal growth 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 Shtayeh *et al.,* (2003) and Muhsen and Al-Dossary (2023), about fungal isolates and their appearance.

Anamorphic fungi representing the 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 factors and 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).

Zygomycota was 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 ratio 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 and its ability to adapt to a wide range of temperatures, pH and high concentrations of salinity, which enabled it to grow in different environmental conditions. This finding is in agreement with those of other studies, such as Ahmad *et al.* (2024) 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 6%, such as *Cladosporium*, to 89%, such

Table 1. Isolated fungal genera with their percentage of occurrence

No.	Fungal genera	No. of soil samples in which the genus appeared	% of fungal 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

as *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 concentration of salinity, which enabled 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 % due to their low ability to tolerate unfavourable environmental conditions or produce inactive reproductive units, which affect their occurrence on the culture media (Zhang *et al.,* 2020; Ahmad *et al.,* 2023; Al-hamdani and Al-Dossary, 2023). The occurrence of sterile mycelia was at 22%, and this finding 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).

3.2 Identification of fungal species

Seventeen fungal species were isolated from soil samples (Table 2). The percentage of occurrence of the species ranged from 6% to 100%. The high percentages of occurrence belonged to species *Aspergillus niger* with 100% followed by *A. 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 in 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 temperature, which all influence species occurrence (Efremenko *et al.,* 2024).

Table 2. Isolated fungal species with their percentage of occurrence

No.	Species	No. of soil samples in which the species appeared	% of fungal species 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

3.3 Screening of fungal isolates for tolerance to heavy metals

All fungal isolates were examined for their resistance to heavy metals Cu, Co and Cd at 100, 200, 400 and 800 ppm. The results revealed significant differences in the impact of heavy metals on fungal growth and activity. Cu was revealed as the most toxic metal, exhibiting distinct inhibitory effects on the physiological processes of the tested fungi (Table 3). By contrast, Co displayed the least 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 presented 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,* which 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 mechanism 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, such as in other fungi, was inhibited at 800 ppm. As previously mentioned, this fungus has good tolerance ability for 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; this adaptation includes the production of specialized enzymes that aid in the accumulation of Cu in their bodies (Akram *et al.,* 2023).

Table 3. Fungal growth rates and tolerance index in MEA solid medium containing copper in various concentrations

No.	Species	Colony diameter at different concentrations of copper (mm)					Tolerance index at different concentrations of copper			
		Control	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	*A. 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	*A. 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	*A.terreus*	6	1	1	1	0	0.1	0.1	0.1	0
15	*A. 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

Note: RLSD = 1.108

Except for this fungus, the growth for all other fungi were clearly inhibited at 200, 400 and 800 ppm possibly because of the several key properties of Cu. Firstly, Cu acts as a potent oxidising 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 membranes, 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 processes (Qader and Shekha, 2023).

The results of the statistical analysis ($P < 0.01$) 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 low compared with the control. Except for this fungus, no other fungal species showed good tolerance to Cu at these two concentrations.

The Co data indicated that most fungi could grow well at 100 ppm concentration, and six species had a growth rate of more than 5 cm. Three species, *A. niger, A. flavus*, and *A. wentii,* filled the plates with a growth rate of 9 cm. Similar results were reported by Turnau *et al.,* (2006). At high concentrations, the number of isolates tolerant to Co decreased. Some fungal species could not tolerate the different concentrations of Co, and their growth was negatively affected 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 ($P < 0.01$) in the growth rate of fungal species between the control and most species at the concentrations of 100 and 200 ppm and all species at 400 and 800 ppm of Co.

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 are tolerant, may be due to the development of membrane barriers preventing entry into cells, the chemical transformation of the metal into

Table 4. Fungal growth rates and tolerance index in MEA solid medium containing cobalt in various concentrations

No.	Species	Colony diameter at different concentrations of cobalt (mm)					Tolerance index at different concentrations of cobalt			
		Control	100	200	400	800	100	200	400	800
1	*A. flavus*	9	9	9	4.7	0	1	1	0.5	0
2	*A. wentii*	9	9	5.3	3.6	0	1	0.6	0.4	0
3	*A. 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	*A. terreus*	4.5	2.5	1	1	0	0.5	0.2	0.2	0
7	*A. 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	*A. 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

Note: RLSD = 0.857

less toxic forms by fungi and the adaptation to toxic compounds in the environment (Dusengemungu *et al.,* 2022).

Nine fungal species could tolerate 100 ppm, showing moderate to good tolerance. Six species could tolerate 200 ppm. However, at higher concentrations, all species either showed low tolerance or no tolerance at all (Table 4).

The decrease in fungal tolerance with 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 ($P < 0.01$) in the growth rate of fungal species between the control and 100, 200, 400 and 800 ppm 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 the 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

Table 5. Fungal growth rates and tolerance index in MEA solid medium containing containing cadmium in various concentrations

No.	Species	Colony diameter at different concentrations of cadmium (mm)					Tolerance index at different concentrations of cadmium			
		Control	100	200	400	800	100	200	400	800
1	*A. niger*	9	9	7	4.5	0	1	0.8	0.5	0
2	*Fusarium* sp.2	9	8.2	6.6	3.6	0	0.9	0.7	0.4	0
3	*Fusarium* sp.1	9	4.4	2.5	1	0	0.5	0.3	0.1	0
4	*Penicillium* sp.3	9	9	1	1	0	1	0.1	0.1	0
5	*Rhizopus* sp.	9	3.8	1	1	0	0.4	0.1	0.1	0
6	*A. flavus*	9	2.5	2.4	1	0	0.3	0.3	0.1	0
7	*Penicillium* sp.2	9	2.2	1	1	0	0.2	0.1	0.1	0
8	*A. wentii*	9	1	1	1	0	0.1	0.1	0.1	0
9	*Cladosporium* sp.	9	1	1	1	0	0.1	0.1	0.1	0
10	*Emericella* sp.	9	1	1	1	0	0.1	0.1	0.1	0
11	*Penicillium* sp.1	9	1	1	1	0	0.1	0.1	0.1	0
12	*A. fumigatus*	6.5	1	1	1	0	0.1	0.1	0.1	0
13	*A. terreus*	6.5	1	1	1	0	0.1	0.1	0.1	0
14	*A. versicolor*	9	1	1	1	0	0.1	0.1	0.1	0
15	*Mucor* sp.	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

Note: RLSD = 1.0741

such conditions due to several adaptive mechanisms (Iram *et al.*, 2013). The decline in the growth rate of fungal species and 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; oxidative stress caused by reactive oxygen species; and 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.

4. Conclusion

Heavy metals represent toxic compounds in the environment and different organisms, including microorganisms. Fungi are one of the important microorganisms that can accumulate heavy metals in their bodies, and they can be used in the bioremediation of heavy metals. Seventeen fungal species were isolated in this 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 and 400 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.

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