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Morphological Responses and the Evaluation for Remediation of *Aspergillus* under Cadmium and Lead Stress

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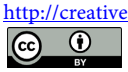

Abstract

Fungi have been a source of great concern because they are perfect candidates for heavy metal bioremediation. The fungus with tolerance to the heavy metal was screened from the mining soil samples. The fungus was characterized as *Aspergillus* sp. Y9 based on the morphological and ITS sequencing analysis. Y9 exhibited high levels of resistance to cadmium and lead. Y9 was investigated for its miro-morphology and application in heavy removal with different concentration Cd(II) (0, 50,100 and 200 mg/L), and with different concentration Pb(II) (0, 200, 500 and 1000 mg/L), respectively. Micro-morphological studies showed the reproduction of the fungus was affected under the higher concentrations of cadmium and lead, but the survival strategies of Y9 were different with cadmium and lead. Under higher concentrations of cadmium, the mycelium of Y9 became shorter, and the top of the hyphae swelled to prevent development. However, under higher concentration lead stress, the morphological changes of mycelium are not obvious, but the number of fruiting bodies decreases. The removal potential of Y9 was quantified by atomic absorption spectrometry. The highest Cd(II) biosorption capacity was 1.91 ± 0.02 mg/g in 200 mg/L initial concentration Cd(II), while that of Pb(II) was 5.87 ± 1.02 mg/g in 500 mg/L initial concentration Pb(II). The highest Cd(II) sorption removed 53.71% ± 0.31% in 50 mg/L initial concentration Cd(II), while that of Pb(II) is 66.91% ± 1.88% in 100 mg/L initial concentration Pb(II). Y9 showed the great potential as bioremediators for highly heavy metal-contaminated environments. Our present results provide a better understanding of the heavy metal resistance of *Aspergillus.*

Subject Areas

Biochemistry, Bioengineering

Keywords

Aspergillus, Cadmium Removal, Lead Removal, Morphological Response, Bioremediation

1. Introduction

Contamination of the natural ecosystem by heavy metals severely impacts the health and survival of all life forms because of their toxicity and persistence in the environment [1]-[3]. It is estimated that globally, there are >5 million sites covering 20 million ha of land, and the soils are contaminated by different heavy metals (1). The heavy metal ions present in the soil environment are rapidly absorbed into the food chain through the plant system [4]. It has been reported that popular agricultural products such as grains, vegetables, fruits and seafood may contain HM [5]. HMs enter the human body can lead to kidney dysfunction, carcinogenic effects, immune system imbalance, and in some cases even risk of death to human health [5] [6]. The Agency for Toxic Substances and Disease Registry (ATSDR) states that the four HMs, Hg, Pb, Cd, and As, they are extremely harmful to plants and humans [7]. In view of this, contamination assessment and remediation methods for contaminated soils have received considerable attention both locally and internationally.

The methods for remediation of heavy metal contaminated soil include physical remediation, chemical remediation, and bioremediation [8] [9]. Due to rapid multiplication and growth rates, microorganism having metal resistance are advocated for metal removal applications [10]-[13]. Most studies reported that the microorganisms including bacteria, actinomycetes, fungi and algae were used for restoration of heavy metal contaminated soil [14]-[16].

Fungal cells have high surface area with excellent HM-binding properties due to negative charges of functional groups present in cell wall components [17]. Additionally, fungi possess multiple antioxidant systems, metal transporters, metal-buffering molecules, metal-transformation enzymes, and secrete metal-precipitating compounds [18]. Filamentous fungi exhibited essential characteristics that recommend them as effective HM bioremediation agents [19]. Dell'Anno et al. reported the fungi could be more effective than acidophilic autotrophic and heterotrophic bacteria in HM bioleaching of marine sediments [20]. Fungal restoration had received increasing attention from researchers.

This study aims to 1) To isolate and screen target isolates with high resistance from heavily heavy metal-contaminated soils 2) To explore the growth and reproduction of fungi under heavy metals stress. 3) To determine the adsorption capability of fungi on heavy metal ions.

2. Materials and Methods

2.1. Sampling Sites

The soil samples were taken from 0 - 20 cm soil layer of farmland or forest land

within 1 km of the mine area of Qianwan Coal Mine (111.06˚E, 36.09˚N) in Xiangning Country, Shanxi Province. The soil samples were brought back to the laboratory in polyethylene plastic bags and be stored in a refrigerator at 4˚C.

2.2. Screening and Isolation of Heavy Metal-Resistant Fungi

The heavy metal compound was dissolved in distilled water to obtain the 10 g/L of ion concentration, and filtered through 0.22 μm filter, and then stored up for 1 week at 4˚C. 10 g of soil samples was dissolved in 90 mL of sterile water and incubated in a constant temperature shaker at 28˚C, 150 r/min for 24 h. The soil suspension was serially diluted and spread on sterile potato dextrose agar (PDA) medium (potato extract 200 $g \cdot L^{-1}$, dextrose 20 $g \cdot L^{-1}$, and agar 15 $g \cdot L^{-1}$) with the Cd(II) concentration of 200 mg/L, Pb(II) concentration of 1000 mg/L. Then placed in a biochemical incubator at 28˚C for 14 days, separated and purified twice. The isolated mold colonies were re-purified, and were stored at −80˚C for further use (17).

2.3. Minimum Inhibitory Concentrations of Isolate

The metal tolerance of the fungal isolate was evaluated against different concentrations of heavy metals following the standard protocol [21]. Heavy metals salts were used: Pb $(NO_3)_2$ for Pb, $CdCl_2$ for Cd, $HgCl_2$ for Hg and $K_2Cr_2O_7$ for Cr. PDA medium with different type of heavy metals and with different concentrations of metal salts were used to assess the resistance. The lowest concentration of each metal that inhibited noticeable growth of the fungal species was reported as the minimum inhibitory concentration (MIC).

2.4. Tolerance Index

The isolates with high resistance were inoculated in PDA medium containing Cd(II) at concentrations of 50 mg/L, 100 mg/L, 150 mg/L and 200 mg/L, and Pb(II) at concentrations of 200 mg/L, 400 mg/L, 800 mg/L and 1000 mg/L. and the relevant control groups (without heavy metal ions) were set. Measurements of the colony diameters were performed every day in 1 week at 28˚C. The values were used to calculate the tolerance index (TI) values [22] [23]

$$\text{Tolerance index} = \frac{\text{Radial growth}(\text{cm}) \text{ of fungus in medium with } Pb^{2+} \text{ or } Cd^{2+}}{\text{Radial growth}(\text{cm}) \text{ of fungus in medium without } Pb^{2+} \text{ or } Cd^{2+}}.$$

2.5. Micro-Morphological Observations under Cd(II) and Pb(II) Stress

The isolate was inoculated on solid PDA and yeast extract peptone glucose (YPD) liquid medium (2% Trypton and 2% glucose) at 28˚C for 8 days. All of the culture medium were added with Cd(II) concentrations of 0 mg/L, 50 mg/L, 100 mg/L, 200 mg/L, or Pb) concentrations of 0 mg/L, 200 mg/L, 500 mg/L, 1000 mg/L, respectively. At the same time, the colonies were inoculated in small pieces of solid PDA medium in the control treatment and test treatments. After cultivation at

28˚C for 4 days, the micro-structure of the fungal was observed under a light microscope (Olympus, MoticNet, Japan). The experiment was repeated three times. The five parallel samples were made at each heavy metal concentration in each experiment for observation.

2.6. Physiological and Biochemical Characterization of Isolate

The physiological and biochemical identification of the isolate was carried out including the ester hydrolysis, cellulose decomposition, starch hydrolysis, H_2S, methyl red, and gelatin hydrolysis, as a further taxonomic identification of the isolate [24].

2.7. Identification of Heavy Metal-Resistant Fungus

The total genomic DNAs of the fungus isolates were isolated and purified using the method described by Al-Samarrai and Schmid [25]. To identify the species, the fresh solid PDA plates with fungus was cultured at 28˚C for 2 days. Afterwards, the cultures were sent to the Beijing Tsingke Biotechnology Identification Service (Beijing Tsingke Biotechnology Co., Ltd., P. R. China https://tsingke.com.cn) for species identification. For the Internal Transcribed Spacer (ITS), the fragments from the isolate was amplified using ITS1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3' primers [26]. The amplification programs were used with the following parameters: 2 min at 98˚C, 38 cycles (10 sec 98˚C, 10 sec 53˚C, 2 min 72˚C), 5 min at 72˚C. The sequences obtained was aligned using BLAST analysis network service of the National Center for Biotechnology Information (NCBI) for comparison with currently available sequences. The higher homology sequences were used for constructing the phylogenetic trees using Clustal X 1.83 and MEGA 5.0 [27]. A distance matrix method (with distance options set according to the Kimura two-parameter model), including clustering by neighbour-joining [28], was used for phylogenetic analysis.

2.8. Heavy Metal Sorption by Isolates

The Inoculation rate is 15% (V/V) and the volume of reaction is 100 mL. The final concentration of heavy metal ions in the reaction solution reached 50 - 200 mg/L for Cd(II) or 200 - 1000 mg/L for Pb(II). After the cultivation for 48 h with 150 r/min at 28˚C, the supernatant was centrifuged for 10 min at 10,000 r/min, 4˚C. Three groups were set up in parallel, and a control group was set up. The concentration of Cd(II) and Pb(II) in the supernatant was determined using the atomic absorption spectrophotometer (AAS), The harvested fungal biomass was rinsed 3 - 4 times with double-distilled water, and dried in a hot air oven (70˚C for 10 h) to weigh. The uptake of heavy metal for the isolate was calculated using the following equation. The removal rate η (%) and adsorption amount q_e (mg/g) of Cd(II) and Pb(II) by the fungus was calculated by the Equations (1) and (2).

$$\eta = \frac{C_0\text{-}C_e}{C_0} * 100\% \quad (1)$$

$$q_e = \frac{(C_0 - C_e)*V}{m}. \quad (2)$$

In above equation, η (%) was the removal rate; C_0 (mg/L) was the initial metal concentration; C_e (mg/L) was the final metal concentration; q_e (mg/g) was the adsorption capacity of fungi for heavy metal; m (g) was the amount of dry biomass; and V (L) was the volume of the medium.

2.9. Determination of pH under Heavy Metal Stress

The isolate was cultured in YPD medium with different heavy metal ions with 150 r/min at 28˚C. At a specific time, samples were centrifuged. The pH from the supernatant was measured with a precision pH meter, and repeated three times for each group of samples.

2.10. Statistical Analysis

All experiments were carried out by triplicate samples. Values reported in this paper was the Means ± SD. The significant difference in TI, the uptake capacity and pH were studied by one-way ANOVA followed by Tukey's test ($P < 0.05$) using SPSS 22.

3. Results

3.1. Screening and Isolation of Heavy Metal-Resistant Fungus

The isolation obtained from the soils was named Y9. The heavy metal-resistance of fungus Y9 is much higher than the threshold value and the international standardized risk control values in different environment reported (Table 1).

Table 1. Determination of minimum inhibitory concentration for Y9.

Substance	Cd(II)	Pb(II)	Hg(II)	Cr(VI)	References
MIC for Y9 (mg/L)	200	1000	40	10	In the study
Threshold value in agricultural soils (mg/kg)	1	60	0.5	100	[29]
Standard limit in water (mg/L)	0.003	0.01	0.006	0.05	[30]
Threshold value in residential land (mg/kg)	20	400	8	250	[31]

Note: A risk control value basically connoted that contaminant levels in soil and water exceeding the limit value under a particular land and water use pattern generally present an unacceptable risk to human health and require remediation or risk control actions. MIC screening was performed in PDA medium. All the results showed that Y9 had higher resistance for both Cd(II) and Pb(II) stress.

Tests for resistance to heavy metals showed that Y9 tolerated heavy metals well above the heavy metal threshold range. Except for Cr(VI), the tolerance ranges for other heavy metals had exceeded the international standardized risk control values. Pb and Cd are identified as priority control pollutants (29 - 31). Therefore,

we focused on the research for the heavy metal Cd(II) resistant and Pb(II) resistant of the isolate.

3.2. The Heavy Metal Tolerance Index of Fungus

Fungus express tolerance to heavy metals through different growth at heavy metal concentrations, which can be quantified by the TI. The TI value is higher, the heavy metal-resistant is greater. The higher TI value indicated that the strain had good metal tolerance.

3.2.1. Cd(II) Tolerance Index of Fungus

In **Figure 1(a)**, the TI of Y9 decreased with the initial concentration rising. The TI < 1 indicated that the growth of Y9 under heavy metal stress was inhibited relative to the control. Statistical analysis was conducted on the TI value on the 7th day. The results showed that the TI value significantly decreased with the initial cadmium concentration increasing (**Figure 1(a)**).

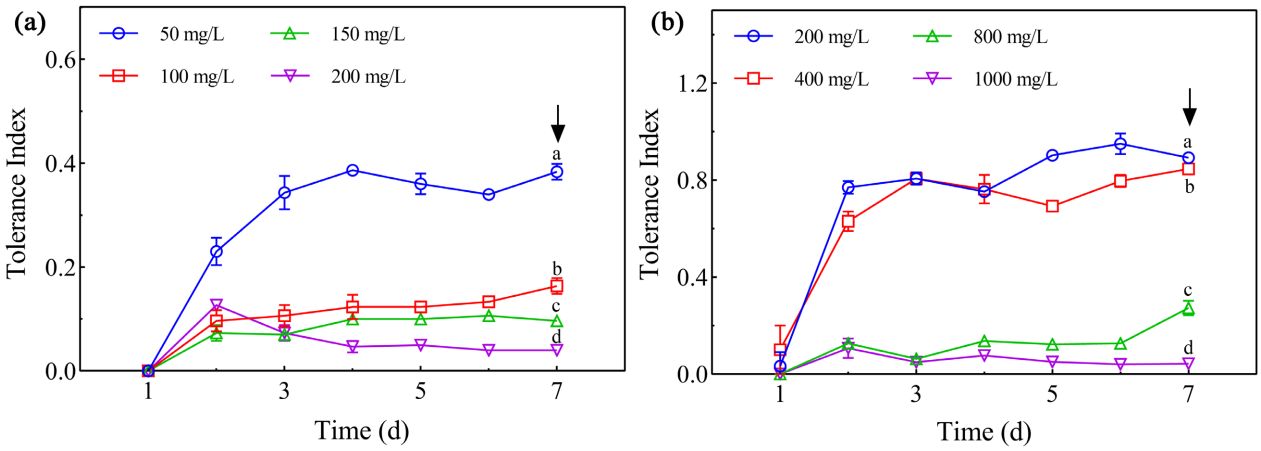

Figure 1. Tolerance index of Y9 under Cd^{2+} (a), and Pb^{2+} (b) stress measured data every 24 h. The same letters or part of the same meant that the difference was not significant, otherwise significant ($P < 0.05$) among the colony diameter by ANOVA followed by Tukey's test.

3.2.2. Pb(II) Tolerance Index of Fungus

In the **Figure 1(b)**, with the increase of concentration of Pb(II), the TI value decreased. TI of Y9 showed high tolerance (0.79 - 0.95) with the initial concentration 200 mg/L and 400 mg/L of Pb(II). Statistical analysis was conducted on the TI value on the 7th day. The results showed that the TI value significantly decreased with the initial lead concentration increasing (**Figure 1(b)**).

3.3. Morphological Observations under Cd(II) and Pb(II) Stress

3.3.1. Morphological Observations under Cd(II) Stress

When Y9 was inoculated on solid PDA with 50-200 mg/L Cd(II), the colony diameter decreased with the increasing of Cd(II) concentration. The fluffy morphology of the colony could not be observed and the color of colony showed greyish white from original green with 100 - 200 mg/L Cd(II) concentration (**Figures**

2(C)-(D)). The hyphae of the fungus became shorter and the top of the hyphae swelled with the increase of initial cadmium concentration (Figures 2(A')-(D')). With the increase of cadmium concentration, the diameter of fungal hyphae pellet decreased and biomass significantly declined in the liquid medium. When the initial concentration reached to 200 mg/L, the mycelial pellet couldn't be observed (Figures 2(a)-(d)). The results indicated that the high concentrations of cadmium inhibited growth and destroyed normal reproductive modes.

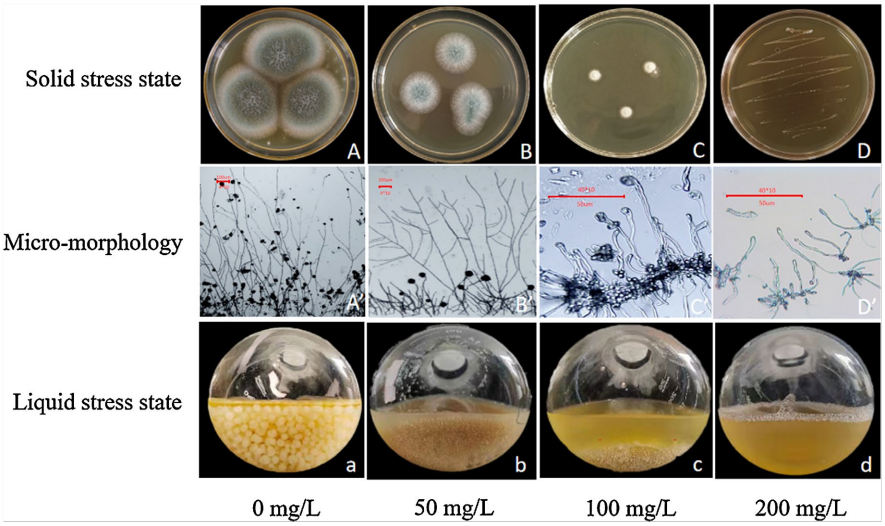

Figure 2. The morphology and growth of Y9 on solid PDA and in liquid YPD with Cd(II). Colony morphology of Y9 on solid PDA with different Cd(II) concentrations at 28˚C for 8 days (A)-(D); The micro-morphology observations of Y9 in the presence of different concentration Cd(II) for 4 days at 28˚C using Light microscopy (40×) (A')-(D'); The growth of Y9 in liquid medium with YPD at 28˚C for 8 days (a)-(d).

3.3.2. Morphological Observations under Pb(II) Stress

It was observed that the growth inhibition of Y9 was very obvious under Pb(II) stress in the different media (Figure 3). Compared to the control test, the difference in morphology and diameter of fungal colonies was not significant with Pb(II) concentration of 200 mg/L. However, the colony diameter decreased and the difference between the central and peripheral hyphae is not significant with Pb(II) concentration of 500 mg/L, which is typical for fungus colony morphology (Figures 3(A)-(D)). When the Pb(II) concentration was 1000 mg/L, the surface of the colony was dry and greyish white. From the micro-morphological images, with the increasing of the Pb(II) concentration, the number of conidia decreased. However, the changes of mycelium were not significant (Figures 3(A')-(D')).

With the increase of Pb(II) concentration, the diameter of fungal hyphae pellets decreased and biomass significantly declined in the liquid medium, indicating that the growth of Y9 was inhibited under 200 - 1000 mg/L concentration. When the Pb(II) concentration reached to 1000 mg/L, the culture medium became turbid, and no mycelial pellet was observed (Figures 3(a)-(d)).

All the results demonstrated that the higher concentrations of Cd(II) inhibited

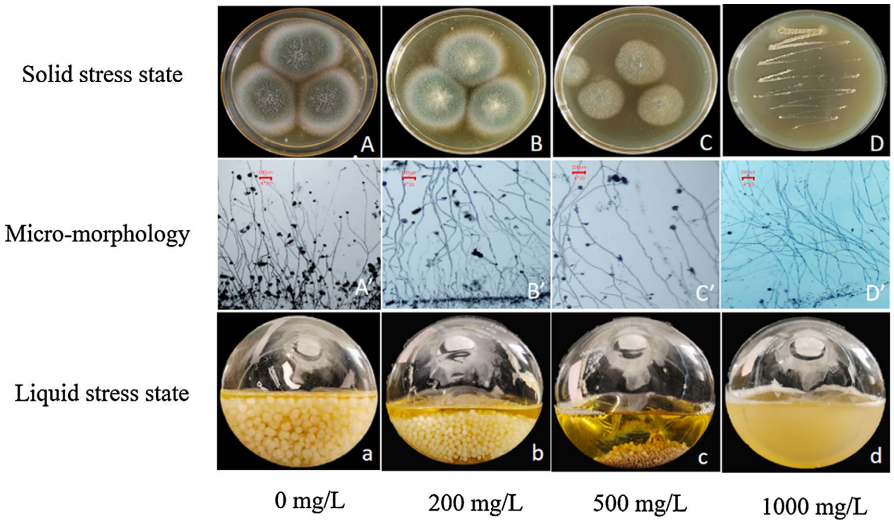

Figure 3. The morphology and growth of Y9 on solid PDA and in liquid YPD with Pb(II). Colony morphology of Y9 on solid PDA with different Pb(II) concentrations at 28˚C for 8 days (A)-(D); The micro-morphology observations of Y9 in the presence of different Pb(II) concentration for 4 days at 28˚C using light microscopy (40×) (A')-(D'); The growth of Y9 in liquid medium with YPD at 28˚C for 8 days (a)-(d).

the formation of fruiting bodies by destroying the normal growth of hyphae. However, the higher concentrations of Pb(II) had little effect on hyphal growth, but directly reduced the number of fruiting bodies. Some literatures indicate that microbial resistance to heavy metals can be categorized into two types: intracellular adsorption and extracellular adsorption. However, distinct microbial species exhibit divergent physiological mechanisms for heavy metal resistance. In extracellular adsorption, the functional groups involved in binding about Pb(II) and Cd(II) and the resulting precipitates differ significantly. Furthermore, some studies have reported that cadmium (Cd) may alter microbial reproductive strategies for some fungi, or (and) effected the nitrogen metabolism [32]. Nevertheless, few studies have systematically investigated the physiological basis of a single microbial strain's resistance to both heavy metals.

3.4. Physiological and Biochemical Characterization of Isolate

As for the physiological and biochemical identification results, it could be judged that Y9 had the ability to decompose esters, the ability to decompose starch. However, the Y9 does not have the ability to decompose cellulose. Y9 colonies were fluffy or flocculent, dark green in color, and their microscopic form had globular ascospores (**Table 2**).

3.5. ITS Sequencing and Phylogenetic Analysis

The sequence of Y9 was uploaded to NCBI (GenBank accession number PQ725423), and the homology was compared against known gene sequences present in GenBank, and the phylogenetic tree was constructed shown in **Figure 4**. The result showed that Y9 had a high homology with *Aspergillus fumigatus*

Table 2. Biochemical characteristics of Y9.

Characteristic	Isolate Y9
Ester hydrolysis	+
Cellulose decomposition	-
starch hydrolysis	+
Gelatin hydrolysis	+
Mycelial morphology	phragmatic, multicellular
Fruiting body character	Spherical, ellipsoidal
microbial colony color	Dark green
microbial colony character	Fluffy or flocculent

Note: "+" means positive; "-" means negative.

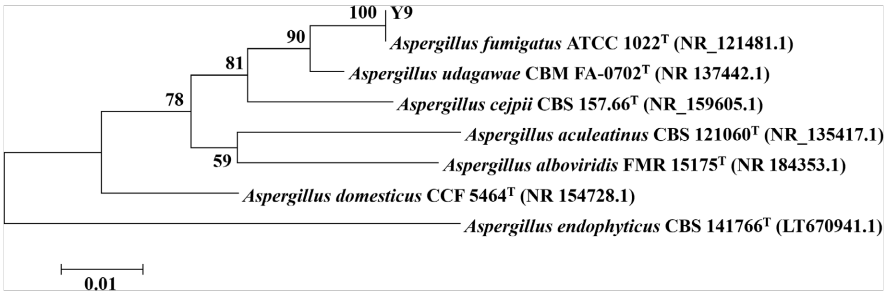

Figure 4. Phylogenetic tree constructed after multiple alignments using the ITS sequences available from the GenBank/EMBL/DDBJ database. The scale bar indicated the 0.01 evolutionary distance unit.

ATCC1022^T (100%).

In summary, combined with the morphological characteristics, physiological and biochemical identification of the isolate, and based on the results of ITS gene sequence analysis, the isolation was identified as *Aspergillus* sp. Y9.

3.6. Removal Capacity of Cd(II) and Pb(II) by Fungus

3.6.1. Removal Capacity of Cd(II) by Y9

When the initial concentration ranged from 20 mg/L to 50 mg/L, the removal rate increased. However, when the initial concentration ranged from 50 mg/L to 200 mg/L, the removal rate gradually decreased. When the concentration of Cd(II) was 50 mg/L, the highest removal rate of the Y9 was 53.71% ± 0.31%. As for the adsorption capacity, the adsorption capacity increased significantly with the increase of Cd(II) concentration. When Cd(II) concentration was 200 mg/L, the highest adsorption capacity reached to 1.91 ± 0.02 mg/g (**Figure 5(a)**).

3.6.2. Removal Capacity of Pb(II) by Y9

With the initial Pb(II) concentration rising, the removal rate decreased. The highest removal rate of Y9 was 66.91 ± 1.88%. As for the adsorption capacity, when the initial concentration ranged from 100 mg/L to 500 mg/L, the adsorption

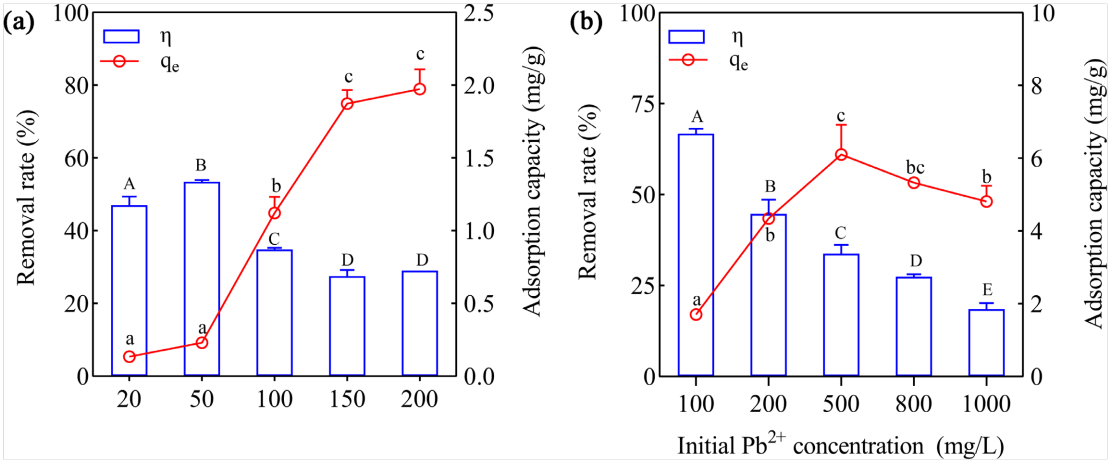

Figure 5. Effect of different initial concentrations of Cd(II) (a) and Pb(II) (b) on the adsorption capacity of Y9. (n = three experiments per condition. Data are mean ± SD. η (%) was removal rate and the q_e (mg/g) was adsorption amount of heavy metal ions. (The same letters or part of the same meant that the difference was not significant, otherwise significant by ANOVA followed by Tukey's test).

capacity increased significantly. However, when the initial concentration ranged from 500 mg/L to 1000 mg/L, the adsorption capacity gradually decreased. The maximum amount was 5.87 ± 1.02 mg/g (**Figure 5(b)**).

Under relatively confined liquid culture conditions, microbial growth phases undergo rapid sequential transitions within a short timeframe: lag phase, exponential phase, stationary phase, and decline phase. Thus, experimental constraints continuous monitoring of Y9 strain performance over extended periods.

3.7. pH Test after Culture at Various Heavy Metal Concentrations

As an indirect indicator, pH helped us understand the overall metabolic changes for fungi under different heavy metal stress. The pH levels on the 6th and 10th day of cultivation were used to detect changes.

In the control group (without Cd(II)), Y9 showed a significant increase in pH on the tenth day compared to the sixth day. When the initial Cd(II) concentration in the environment was 50 mg/L, the changes at the two time points were similar to those in the control group. When the initial Cd(II) concentration reached to 100 - 200 mg/L, the values at the two time points were close and there was no significant difference (**Figure 6(a)**).

In the control group (without Pb(II)), Y9 showed a significant increase in pH on tenth day compared to sixth day. When the initial Pb(II) concentration was between 200 - 1000 mg/L, the changes pattern at the two time points were similar to the control group. However, the pH level decreased with increasing of initial Pb(II) concentration (**Figure 6(b)**). All results indicated that higher concentrations of Cd(II) and (or) Pb(II) had a greater impact on the metabolism substance of Y9.

The result was consistent with the research findings by Feng *et al.* [33]. Lower concentrations of heavy metals could increase pH, while higher concentrations of heavy metals could decrease pH.

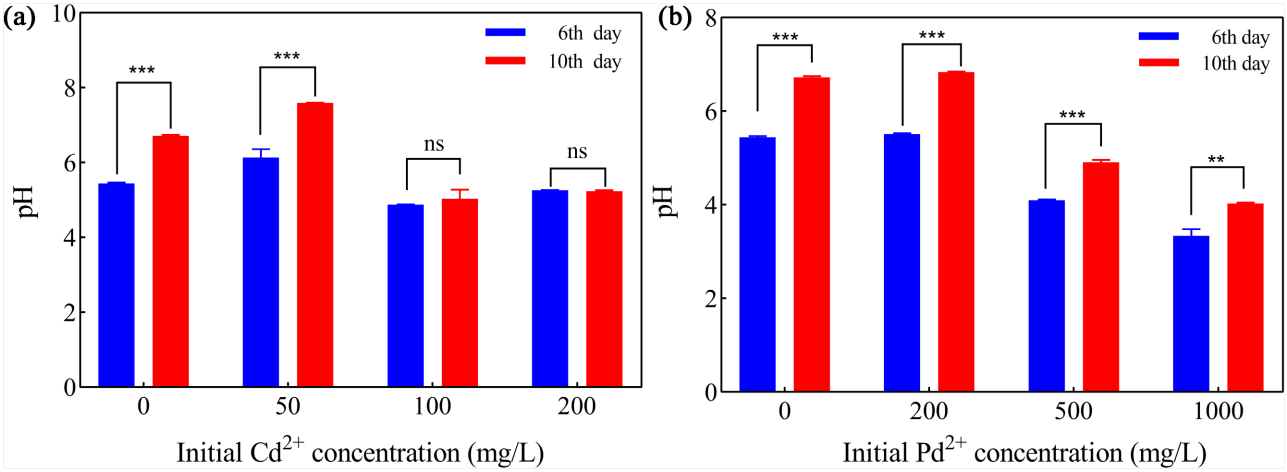

Figure 6. Changes of pH under Cd(II) (a) and Pb(II) of Y9 (b) at the 6th day and 10th day. (A paired t test was applied to test for differences between every pair (P < 0.05). ***P < 0.001; ****P < 0.0001; ns meant not significant n=three experiments per condition. Data were mean ± SD).

4. Discussion

Although lots of reports on cadmium and Pb(II) resistance from different fungi genera, few reports on the growth response of fungi under heavy metals [34] [35]. As we all known, there is different response for different Cd-resistant and Pb-resistant fungi under heavy metal stress. Generally, fungal remediation agents exhibit specificity towards heavy metal contaminants, and show effective remediation for particular types of pollutants. The target strain Y9 in the study demonstrates favorable adsorption capacities for both Pb and Cd. In some research, the heavy metal resistance was reported by the radial growth rates of fungi or determined by measuring of the minimum inhibitory concentration (MIC) under heavy metal stress [36]-[38]. At present, the morphological changes of heavy metal tolerant fungi were focused on the surface changes of mycelium from scanning electron microscopy under heavy metal stress. Tian et al reported that the surface of the *Aspergillus fumigatus* hyphae became rough with particulate substances, and a concave shape after Cd treatment [39]. Talukdar et al pointed the mycelium of *Aspergillus fumigatus* FS2 was completely disrupted and disintegration in the presence of 100 mg/L of Cd(II) after incubation of 120 h [40]. Feng et al reported that the *Verticillium insectorum* J3 hyphae warped and aggregated at higher Pb(II) concentrations [33]. However, they didn't refer to the impact of fruiting body and (or) the hyphae growth under heavy metal stress.

Light microscopy is a basic imaging technique that is useful for providing the visual identification. The morphological variant was the inherent adaptive immunity based strategies for metal toxicity induced environmental implications of challenge, and it was one of the mechanistic actions in combating heavy metals [10] [41] [42]. In a previous report from our lab, cadmium can induce the change of reproduction mode of the *Sarocladium*, from conidia to arthrospores, which made the colony morphological modifications, from the fungi colony morphology to the bacteria colony morphology [32]. In terms of experimental design, we

focused on the effects of heavy metals on the growth and reproduction of fungus. Our research results concluded that Cd disrupted the mycelium tip growth and made it swell, which inhibited the hyphae development with Cd concentration increasing, and the number of fruiting body significantly decreased with the Pb for Y9. The microscopic morphology was consistent with the colony morphology on solid and in liquid plates for Y9. All of the results demonstrated that the toxicity of Pb(II) is lower than that of cadmium for the isolate. The study focused on laboratory conditions, where both the cultivation and adsorption processes were conducted under artificially controlled environmental parameters, to obtain optimal remediation data and establish the best adsorption model for the strain. In subsequent experiments, the strain was introduced into soil environments to further refine the research.

Our present results may provide a better understanding of the heavy metal resistance of *Aspergillus* under Cd and Pb stress. However, the specific mechanism of how regulates the resistance of Y9 against Cd and Pb stress is not fully understood and thus awaits further elucidation in the future.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Lihong Zhang], [Zidi Yuan], [Min Yang], [Xueyong Zhou]. The first draft of the manuscript was written by [Lihong Zhang] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Sarker, A., Masud, M.A.A., Deepo, D.M., Das, K., Nandi, R., Ansary, M.W.R., *et al.* (2023) Biological and Green Remediation of Heavy Metal Contaminated Water and Soils: A State-Of-The-Art Review. *Chemosphere*, **332**, Article ID: 138861. https://doi.org/10.1016/j.chemosphere.2023.138861

[2] Lin, H., Wang, Z., Liu, C. and Dong, Y. (2022) Technologies for Removing Heavy Metal from Contaminated Soils on Farmland: A Review. *Chemosphere*, **305**, Article ID: 135457. https://doi.org/10.1016/j.chemosphere.2022.135457

[3] Pal, A., Bhattacharjee, S., Saha, J., Sarkar, M. and Mandal, P. (2021) Bacterial Survival Strategies and Responses under Heavy Metal Stress: A Comprehensive Overview. *Critical Reviews in Microbiology*, **48**, 327-355.

https://doi.org/10.1080/1040841x.2021.1970512

[4] Yang, D., Chu, Z., Zheng, R., Wei, W., Feng, X., Zhang, J., *et al.* (2021) Remediation of Cu-Polluted Soil with Analcime Synthesized from Engineering Abandoned Soils through Green Chemistry Approaches. *Journal of Hazardous Materials*, **406**, Article ID: 124673. https://doi.org/10.1016/j.jhazmat.2020.124673

[5] Ahmed, A.S.S., Sultana, S., Habib, A., Ullah, H., Musa, N., Hossain, M.B., *et al.* (2019) Bioaccumulation of Heavy Metals in Some Commercially Important Fishes from a Tropical River Estuary Suggests Higher Potential Health Risk in Children than Adults. *PLOS ONE*, **14**, e0219336. https://doi.org/10.1371/journal.pone.0219336

[6] Shamsudduha, M., Joseph, G., Haque, S.S., Khan, M.R., Zahid, A. and Ahmed, K.M.U. (2019) Multi-hazard Groundwater Risks to Water Supply from Shallow Depths: Challenges to Achieving the Sustainable Development Goals in Bangladesh. *Exposure and Health*, **12**, 657-670. https://doi.org/10.1007/s12403-019-00325-9

[7] Mansoor, S., Kour, N., Manhas, S., Zahid, S., Wani, O.A., Sharma, V., *et al.* (2021) Biochar as a Tool for Effective Management of Drought and Heavy Metal Toxicity. *Chemosphere*, **271**, Article ID: 129458. https://doi.org/10.1016/j.chemosphere.2020.129458

[8] Jiang, Q., He, Y., Wu, Y., Dian, B., Zhang, J., Li, T., *et al.* (2022) Solidification/Stabilization of Soil Heavy Metals by Alkaline Industrial Wastes: A Critical Review. *Environmental Pollution*, **312**, Article ID: 120094. https://doi.org/10.1016/j.envpol.2022.120094

[9] Azhar, U., Ahmad, H., Shafqat, H., Babar, M., Shahzad Munir, H.M., Sagir, M., *et al.* (2022) Remediation Techniques for Elimination of Heavy Metal Pollutants from Soil: A Review. *Environmental Research*, **214**, Article ID: 113918. https://doi.org/10.1016/j.envres.2022.113918

[10] Mathivanan, K., Chandirika, J.U., Vinothkanna, A., Yin, H., Liu, X. and Meng, D. (2021) Bacterial Adaptive Strategies to Cope with Metal Toxicity in the Contaminated Environment—A Review. *Ecotoxicology and Environmental Safety*, **226**, Article ID: 112863. https://doi.org/10.1016/j.ecoenv.2021.112863

[11] Varshney, S., Bhattacharya, A. and Gupta, A. (2023) Halo-Alkaliphilic Microbes as an Effective Tool for Heavy Metal Pollution Abatement and Resource Recovery: Challenges and Future Prospects. 3 *Biotech*, **13**, Article No. 400. https://doi.org/10.1007/s13205-023-03807-5

[12] Syed, Z., Sogani, M., Rajvanshi, J. and Sonu, K. (2022) Microbial Biofilms for Environmental Bioremediation of Heavy Metals: A Review. *Applied Biochemistry and Biotechnology*, **195**, 5693-5711. https://doi.org/10.1007/s12010-022-04276-x

[13] Verma, N. and Sharma, R. (2017) Bioremediation of Toxic Heavy Metals: A Patent Review. *Recent Patents on Biotechnology*, **11**, 171-187. https://doi.org/10.2174/1872208311666170111111631

[14] Ashraf, M.A., Hussain, I., Rasheed, R., Iqbal, M., Riaz, M. and Arif, M.S. (2017) Advances in Microbe-Assisted Reclamation of Heavy Metal Contaminated Soils over the Last Decade: A Review. *Journal of Environmental Management*, **198**, 132-143. https://doi.org/10.1016/j.jenvman.2017.04.060

[15] Junpradit, C., Thooppeng, P., Duangmal, K. and Prapagdee, B. (2021) Influence of Cadmium-Resistant Streptomycetes on Plant Growth and Cadmium Uptake by *Chlorophytum comosum* (Thunb.) Jacques. *Environmental Science and Pollution Research*, **28**, 39398-39408. https://doi.org/10.1007/s11356-021-13527-z

[16] Qi, X., Fu, K., Yue, M., Shou, N., Yuan, X., Chen, X., *et al.* (2023) Kynurenic Acid Mediates Bacteria-Algae Consortium in Resisting Environmental Cadmium Toxicity.

Journal of Hazardous Materials, **444**, Article ID: 130397. https://doi.org/10.1016/j.jhazmat.2022.130397

[17] Khan, I., Aftab, M., Shakir, S., Ali, M., Qayyum, S., Rehman, M.U., *et al.* (2019) Mycoremediation of Heavy Metal (Cd and Cr)-Polluted Soil through Indigenous Metallotolerant Fungal Isolates. *Environmental Monitoring and Assessment*, **191**, Article No. 585. https://doi.org/10.1007/s10661-019-7769-5

[18] Kumar, V. and Dwivedi, S.K. (2021) Mycoremediation of Heavy Metals: Processes, Mechanisms, and Affecting Factors. *Environmental Science and Pollution Research*, **28**, 10375-10412. https://doi.org/10.1007/s11356-020-11491-8

[19] Văcar, C.L., Covaci, E., Chakraborty, S., Li, B., Weindorf, D.C., Frențiu, T., *et al.* (2021) Heavy Metal-Resistant Filamentous Fungi as Potential Mercury Bioremediators. *Journal of Fungi*, **7**, Article 386. https://doi.org/10.3390/jof7050386

[20] Dell'Anno, F., Rastelli, E., Buschi, E., Barone, G., Beolchini, F. and Dell'Anno, A. (2022) Fungi Can Be More Effective than Bacteria for the Bioremediation of Marine Sediments Highly Contaminated with Heavy Metals. *Microorganisms*, **10**, Article 993. https://doi.org/10.3390/microorganisms10050993

[21] Joo, J. and Hussein, K.A. (2012) Heavy Metal Tolerance of Fungi Isolated from Contaminated Soil. *Korean Journal of Soil Science and Fertilizer*, **45**, 565-571. https://doi.org/10.7745/kjssf.2012.45.4.565

[22] Ge, W., Zamri, D., Mineyama, H. and Valix, M. (2011) Bioaccumulation of Heavy Metals on Adapted *Aspergillus foetidus*. *Adsorption*, **17**, 901-910. https://doi.org/10.1007/s10450-011-9359-x

[23] Mohammadian Fazli, M., Soleimani, N., Mehrasbi, M., Darabian, S., Mohammadi, J. and Ramazani, A. (2015) Highly Cadmium Tolerant Fungi: Their Tolerance and Removal Potential. *Journal of Environmental Health Science and Engineering*, **13**, Article No. 19. https://doi.org/10.1186/s40201-015-0176-0

[24] Smibert, R. and Krieg, N. (1981) General Characterization. In: American Society for Microbiology, Eds., *Manual of Methods for General Bacteriology*, American Society for Microbiology, 409-433.

[25] Al-Samarrai, T.H. and Schmid, J. (2000) A Simple Method for Extraction of Fungal Genomic DNA. *Letters in Applied Microbiology*, **30**, 53-56. https://doi.org/10.1046/j.1472-765x.2000.00664.x

[26] Pedersen, L.H., Skouboe, P., Boysen, M., Soule, J. and Rossen, L. (1997) Detection of *Penicillium* Species in Complex Food Samples Using the Polymerase Chain Reaction. *International Journal of Food Microbiology*, **35**, 169-177. https://doi.org/10.1016/s0168-1605(96)01216-0

[27] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**, 2731-2739. https://doi.org/10.1093/molbev/msr121

[28] Saitou, N. and Nei, M. (1987) The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution*, **4**, 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

[29] Tóth, G., Hermann, T., Da Silva, M.R. and Montanarella, L. (2016) Heavy Metals in Agricultural Soils of the European Union with Implications for Food Safety. *Environment International*, **88**, 299-309. https://doi.org/10.1016/j.envint.2015.12.017

[30] Rahman, Z. and Singh, V.P. (2018) Assessment of Heavy Metal Contamination and Hg-Resistant Bacteria in Surface Water from Different Regions of Delhi, India. *Saudi*

Journal of Biological Sciences, **25**, 1687-1695.
https://doi.org/10.1016/j.sjbs.2016.09.018

[31] Peng, J., Zhang, S., Han, Y., Bate, B., Ke, H. and Chen, Y. (2022) Soil Heavy Metal Pollution of Industrial Legacies in China and Health Risk Assessment. *Science of the Total Environment*, **816**, Article ID: 151632.
https://doi.org/10.1016/j.scitotenv.2021.151632

[32] Zhang, L., Wang, C., Guo, B., Yuan, Z. and Zhou, X. (2024) Reproductive Strategy Response of the Fungi *Sarocladium* and the Evaluation for Remediation under Stress of Heavy Metal Cd(II). *Ecotoxicology and Environmental Safety*, **271**, Article ID: 115967. https://doi.org/10.1016/j.ecoenv.2024.115967

[33] Feng, C., Li, J., Li, X., Li, K., Luo, K., Liao, X., *et al.* (2018) Characterization and Mechanism of Lead and Zinc Biosorption by Growing *Verticillium insectorum* J3. *PLOS ONE*, **13**, e0203859. https://doi.org/10.1371/journal.pone.0203859

[34] Xie, Y., Li, X., Huang, X., Han, S., Amombo, E., Wassie, M., *et al.* (2019) Characterization of the Cd-Resistant Fungus *Aspergillus aculeatus* and Its Potential for Increasing the Antioxidant Activity and Photosynthetic Efficiency of Rice. *Ecotoxicology and Environmental Safety*, **171**, 373-381.
https://doi.org/10.1016/j.ecoenv.2018.11.123

[35] Deng, Z., Cao, L., Huang, H., Jiang, X., Wang, W., Shi, Y., *et al.* (2011) Characterization of Cd- and Pb-Resistant Fungal Endophyte *Mucor* sp. CBRF59 Isolated from Rapes (*Brassica chinensis*) in a Metal-Contaminated Soil. *Journal of Hazardous Materials*, **185**, 717-724. https://doi.org/10.1016/j.jhazmat.2010.09.078

[36] Pan, R., Cao, L. and Zhang, R. (2009) Combined Effects of Cu, Cd, Pb, and Zn on the Growth and Uptake of Consortium of Cu-Resistant *Penicillium* sp. A1 and Cd-Resistant *Fusarium* sp. A19. *Journal of Hazardous Materials*, **171**, 761-766.
https://doi.org/10.1016/j.jhazmat.2009.06.080

[37] Mohammadian, E., Babai Ahari, A., Arzanlou, M., Oustan, S. and Khazaei, S.H. (2017) Tolerance to Heavy Metals in Filamentous Fungi Isolated from Contaminated Mining Soils in the Zanjan Province, Iran. *Chemosphere*, **185**, 290-296.
https://doi.org/10.1016/j.chemosphere.2017.07.022

[38] Shalaby, M.A., Matter, I.A., Gharieb, M.M. and Darwesh, O.M. (2023) Biosorption Performance of the Multi-Metal Tolerant Fungus *Aspergillus* sp. for Removal of Some Metallic Nanoparticles from Aqueous Solutions. *Heliyon*, **9**, e16125.
https://doi.org/10.1016/j.heliyon.2023.e16125

[39] Tian, Q., Wang, J., Shao, S., Zhou, H., Kang, J., Yu, X., *et al.* (2024) Combining Metabolomics and Transcriptomics to Analyze Key Response Metabolites and Molecular Mechanisms of *Aspergillus fumigatus* under Cadmium Stress. *Environmental Pollution*, **356**, Article ID: 124344. https://doi.org/10.1016/j.envpol.2024.124344

[40] Talukdar, D., Sharma, R., Jaglan, S., Vats, R., Kumar, R., Mahnashi, M.H., *et al.* (2020) Identification and Characterization of Cadmium Resistant Fungus Isolated from Contaminated Site and Its Potential for Bioremediation. *Environmental Technology & Innovation*, **17**, Article ID: 100604. https://doi.org/10.1016/j.eti.2020.100604

[41] Mohite, B.V., Koli, S.H. and Patil, S.V. (2018) Heavy Metal Stress and Its Consequences on Exopolysaccharide (EPS)-Producing *Pantoea agglomerans*. *Applied Biochemistry and Biotechnology*, **186**, 199-216.
https://doi.org/10.1007/s12010-018-2727-1

[42] Chakravarty, R. and Banerjee, P.C. (2008) Morphological Changes in an Acidophilic Bacterium Induced by Heavy Metals. *Extremophiles*, **12**, 279-284.
https://doi.org/10.1007/s00792-007-0128-4