Evaluation of the lead removal capacity of *Cladosporium* sp.

Evaluación de la capacidad de remoción de plomo por *Cladosporium* sp.

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ABSTRACT. Lead (Pb) is a highly toxic metal that can contaminate aquatic and terrestrial ecosystems. The objective of this study was to isolate and identify a filamentous fungus capable of removing Pb. Removal experiments were conducted at Pb concentrations of 50, 100, 250, 350, and 500 mg L^{-1}. The parameters evaluated were hyphal morphology, Pb removal efficiency, elemental composition and fungal biomass production. Fungi strain was identified as *Cladosporium* sp. Scanning electron microscopy (SEM) revealed morphological changes at 350 and 500 mg L^{-1}, including shorter, more compact, and deformed hyphae with granular deposits on their surface. Biomass production was not significantly affected, although reduced growth was observed at the highest Pb levels. Energy dispersive spectroscopy (EDS) confirmed Pb accumulation on the fungal surface, reaching 11.3% of the elemental composition in the 500 mg L^{-1} treatment. Pb removal efficiency increased with concentration and reached a maximum value of 97.3%.
Keywords: Filamentous fungus, toxic metals, biosorption, *Cladosporium* sp.

RESUMEN. El plomo (Pb) es un metal altamente tóxico que puede contaminar ecosistemas acuáticos y terrestres. El objetivo del estudio fue aislar e identificar un hongo filamentoso capaz de remover Pb. Los experimentos de remoción se realizaron con concentraciones de Pb de 50, 100, 250, 350 y 500 mg L^{-1}. Se evaluó la producción de biomasa fúngica, la eficiencia de remoción de Pb, la morfología de las hifas y la composición elemental. La cepa aislada fue identificada como *Cladosporium* sp. El análisis SEM reveló alteraciones morfológicas a 350 y 500 mg L^{-1}, incluyendo hifas más cortas, compactas y deformadas, con depósitos granulares en su superficie. La producción de biomasa no mostró una afectación significativa, aunque se observó menor crecimiento en la concentración más alta. El análisis EDS confirmó la acumulación de Pb en las hifas, alcanzando 11.3% de la composición elemental. La eficiencia máxima de remoción fue de 97.3%.
Palabras clave: Hongos filamentosos, metales tóxicos, biosorción, *Cladosporium* sp.

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INTRODUCTION

Industrial activities such as mining, electroplating, and battery manufacturing have significantly increased environmental contamination by toxic metals, particularly Pb, in aquatic systems and soils (Saedi *et al.* 2015). Pb exhibits several characteristics, such as high persistence, toxicity, and bioaccumulation potential in living organisms, making it one of the most dangerous pollutants (ATSDR 2025, WHO 2025). Some effects associated with lead exposure in humans include neurological, renal, cardiovascular, and reproductive disorders, even at low concentrations (El-Sherif *et al.* 2013, Zou *et al*. 2016, Taseidifar *et al.* 2017). Therefore, eliminating or reducing lead in the environment has become a significant environmental challenge, necessitating the development of sustainable and efficient strategies to achieve this (Fu and Wang 2011, Saha *et al.* 2021). Conventional methods based on physicochemical techniques for the removal of toxic metals often present certain disadvantages, such as high costs, generation of secondary pollutants, inefficiency at low concentrations, etc. (Fu and Wang 2011). In this context, bioremediation using microorganisms has emerged as an ecological and cost-effective alternative (Gadd 2010). In recent years, filamentous fungi have attracted considerable attention as potential candidates for removing toxic metals, mainly due to their high tolerance to growing in the presence of these contaminants, rapid growth, and large surface area containing functional groups capable of binding metal ions (Garcia-Rubio *et al.* 2020). These properties allow fungi to remove metals through various mechanisms such as biosorption, intracellular accumulation, and extracellular biomineralization (Gadd 2010, Dhankhar and Hooda 2011). Although several fungal species have been reported with the capacity to remove toxic metals, studies focused on analyzing the physiological and morphological responses of microorganisms to Pb exposure remain limited (Titilawo *et al.* 2023). Currently, there is still insufficient understanding of the structural changes associated with Pb exposure and the potential role of extracellular mineral precipitation during fungal-metal interactions (Lee *et al.* 2022). Furthermore, few studies have focused on investigating how Pb is associated with fungal biomass, as well as the localization of Pb within the hyphae (Wang *et al.* 2019). Recent studies on *Cladosporium* sp. have demonstrated its Pb biosorption capacity and ability to immobilize Pb on fungal biomass; however, the mechanisms involved in these interactions remain insufficiently understood (Lee *et al*. 2022, El-Gendy *et al.* 2023). Therefore, the aim of this study was to isolate and characterize a filamentous fungal strain capable of removing Pb from contaminated environments. In addition to evaluating Pb removal efficiency, this study investigated the morphological alterations and elemental composition of the fungal biomass under Pb exposure using SEM and EDS analyses to better understand the mechanisms involved in Pb biosorption.

MATERIALS AND METHODS

Fungi isolation, and morphological characterization

The fungal strain used in this study were obtained from soil samples from a mine located in San Felipe de Jesús, Sonora, México. The samples were transported in polyethylene bags and stored at 4 °C until use. The isolation of the fungal strain was carried out according to the method of Sivan *et al.* (2006) with modifications. The fungi isolation was carried out by selecting the colonies and

showing them in potato dextrose agar (PDA) culture medium until the purification of the strains was achieved. To document the main morphological characteristics of the fungi, a microculture was performed. Morphological analysis of the microscopic structures of the fungi was performed by optical microscopy and SEM. In addition, the identification of the strain was complemented by 18S rRNA gene amplification according to the method described by White *et al*. 1990. A partial sequence of the 18S rRNA gene was amplified using primers ITS1 and ITS4. The result of the 18S rRNA gene sequence was compared with those available in the GenBank public databases.

Microculture

The microculture consisted of placing a 1 cm square of PDA mounted on a slide in a Petri dish, and each isolated strain was inoculated in the corners of the agar with a culture loop. A coverslip was placed on the inoculum. A wet filter paper was placed under the slide. It was incubated for 7 days at 28 °C, to observe the microscopic structures then. A drop of the lactophenol blue stain was placed on a slide, the coverslip was removed from the microculture and mounted on the slide containing the stain. Characteristics such as spore shape and size, as well as hyphal type, were analyzed. Observations were made on a Leica inverted optical microscope, model 090-135.002, Wetzlar, Germany. The microculture of the isolated strain was performed in triplicate. Spore size was analyzed using Image J Fiji software, and 50 spores were analyzed.

Pb removal experiments

Sections of 2.5 cm x 2.5 cm were cut from PDA with the mycelium after 8 days in culture was placed in 250 mL Erlenmeyer flasks with 100 mL of Czapek-Dox broth at pH 6.5 (final pH value 5.9 ± 0.21). The culture broth was supplemented with different concentrations of Pb, 50 mg L^{-1}, 100 mg L^{-1}, 250 mg L^{-1}, 350 mg L^{-1} and 500 mg L^{-1}. The selected Pb concentrations were chosen to evaluate fungal tolerance and biosorption performance across a broad gradient of metal stress conditions, based on concentration ranges previously reported in fungal Pb biosorption studies (Wang and Chen 2009, Zhao *et al*. 2020, Senol *et al.* 2021). The flasks were kept under constant agitation for 8 days at 25 °C. The culture broth with fungi without Pb was used as a control. Culture media and glassware were sterilized at 121 °C for 15 min prior to use, and all experimental procedures were carried out under aseptic conditions. All treatments were performed in triplicate.

Biomass quantification

At the end of the 8 days of culture, the biomass of each flask was filtered on Whatman No. 4 paper and dried for 24 h at 60 °C. Once the biomass was dry, it was scraped from the surface of the filter paper to recover it and weigh it.

SEM and EDS analysis

For the analysis, characteristic fragments of the dry material (biomass) were selected and mounted on an aluminum sample holder with the aid of a graphite ribbon on the stage. A scanning electron microscope with an energy dispersive X-ray spectrometer (Hitachi TM3030plus with Bruker EDS and Quantax 70 software) was used. Analysis conditions: Primary energy source of 15 kV in vacuum using secondary (SE) and backscattered (BSE) electrons. The analysis was carried out by

selecting 3-5 characteristic particles through elemental mapping and compositional analysis with EDS of points of interest with the presence of Pb. Each treatment was analyzed in triplicate.

Pb quantification

The Pb present in the culture broth of each removal treatment was quantified, as well as in the fungal biomass. The solid samples were subjected to a digestion process while the culture media were analyzed directly. The biomass digestion procedure was carried out in clean 15 mL Savillex Teflon vessels containing 250 mg of the sample and 5 mL of concentrated HNO_3 (70%) and heating for 24 h. Subsequently, 1 mL of 30% H_2O_2 was added, and heating continued for another 24 h. Finally, the digest was allowed to cool and filled to the mark in 10 mL volumetric flasks. Pb determination was carried out in triplicate in a microwave plasma atomic emission spectrometer (MP-AES) (Agilent Technologies model 4210, USA) equipped with a cyclonic nebulization chamber and a Meinhard nebulizer using a main N_2 flow of 20 L min^{-1} and an air flow of 25 L min^{-1}. The detection limit of Pb of this equipment is 0.07 mg L^{-1} for aqueous solutions. The nebulization flows for each element were previously optimized in 2% HNO_3. The efficiency of fungal biomass to remove heavy metal at different concentrations was calculated by using the following equation:

$$RE\ (\%) = \frac{Ci - Cf}{Ci}\ x\ 100$$

Where RE (%) is removal efficiency; Ci and Cf are the initial and final concentrations of Pb (mg L^{-1}), respectively (Zhao *et al.* 2020).

Statistical Analysis

Results were expressed as mean ± SE (standard error) for the indicated number of measurements. The Shapiro-Wilk test was performed to verify normality and homoscedasticity of the data. Additionally, a one-way analysis of variance (ANOVA) was performed with a significance level of $P \leq 0.05$. Tukey's test was used to separate the means. The statistical package Origin Pro 8 for Windows was used.

RESULTS

Morphological characterization of the fungus

Morphological and genotypic analysis of the isolated strain identified it as *Cladosporium sp.* The morphological characteristics of *Cladosporium sp.* in the PDA culture medium were irregular, velvety, and dark olive-green colonies (Figure 1a). Spores were of variable size, 5–10 µm (length) × 4–5 µm (width), with a sub-globose shape (Figure 1b).

SEM observations

The scanning electron micrograph shows the mass of hyphae forming mycelia (biomass). The surface morphology of the biomass changed as Pb concentration increased in the exposure treatments. Without Pb exposure (control), the hyphae had smooth, intact, and uniform surfaces that maintained their original appearance (Figure 2a). This morphology remained unchanged at Pb concentrations of 50 to 250 mg L^{-1}, whereas at 350 and 500 mg L^{-1}, the hyphae showed

morphological changes in density and compactness (Figure 2b-c). These changes became more evident at 500 mg L^{-1} Pb, with shorter, deformed, and aggregated hyphae. Evidence of granular precipitation was also observed in the hyphae (Figure 2d-f). The presence of nanoparticles was associated with precipitation after Pb exposure because the surface of the hyphae was smooth before Pb exposure. Regarding the morphology the nanoparticles were almost spherical and varied in size from approximately 50 to 250 nm. Precipitation of larger crystal-like particles, up to approximately 20 μm, was also observed. The precipitated particles in the 50 mg L^{-1} exposure treatment were highly dispersed (Figure 2b), and as the Pb concentration increased, a greater presence of these particles was observed, mainly at 350 and 500 mg L^{-1} Pb (Figure 2e, f).

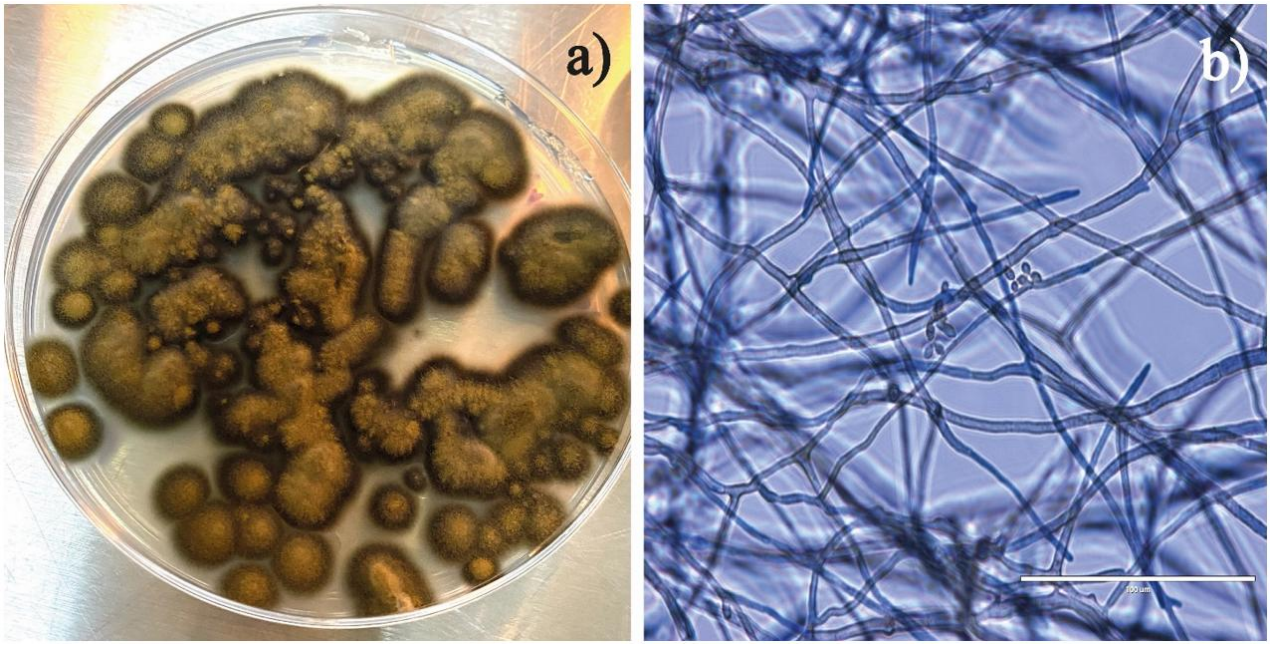

Figure 1. Filamentous fungal strain isolated in this study. a) Macroscopic morphological characteristics, colonies, and mycelium; b) Microscopic morphological characteristics, hyphae, and spores, observed by light microscopy. Scale bar: 100 μm.

Energy dispersive spectroscopy (EDS) analysis

The results of EDS analysis of the hyphae without and with exposure to Pb showed differences in their elemental compositions. In control treatment, the hyphae were primarily composed of organic matter based on carbon (62.02%), oxygen (36.59%), and a small amount of phosphorus (1.39%) (Figure 3a). After treatment, in addition to carbon, oxygen, and phosphorus, Pb was detected in the mycelia, reaching 11.3% in the 500 mg L^{-1} Pb treatment (Figure 3f). The presence of elements such as Ca, Na, Cl, and K was also observed in the different Pb exposure treatments (Figure b-f). These elements showed variations in weight percentages during the treatment.

Furthermore, the X-ray mapping results confirmed the presence and exact location of Pb on the hyphal surface, as well as O, P, and the remaining elements mentioned previously, as shown for the 350 mg L^{-1} (Figure 4a, b) and 500 mg L^{-1} Pb (Figure 4c, d) treatments.

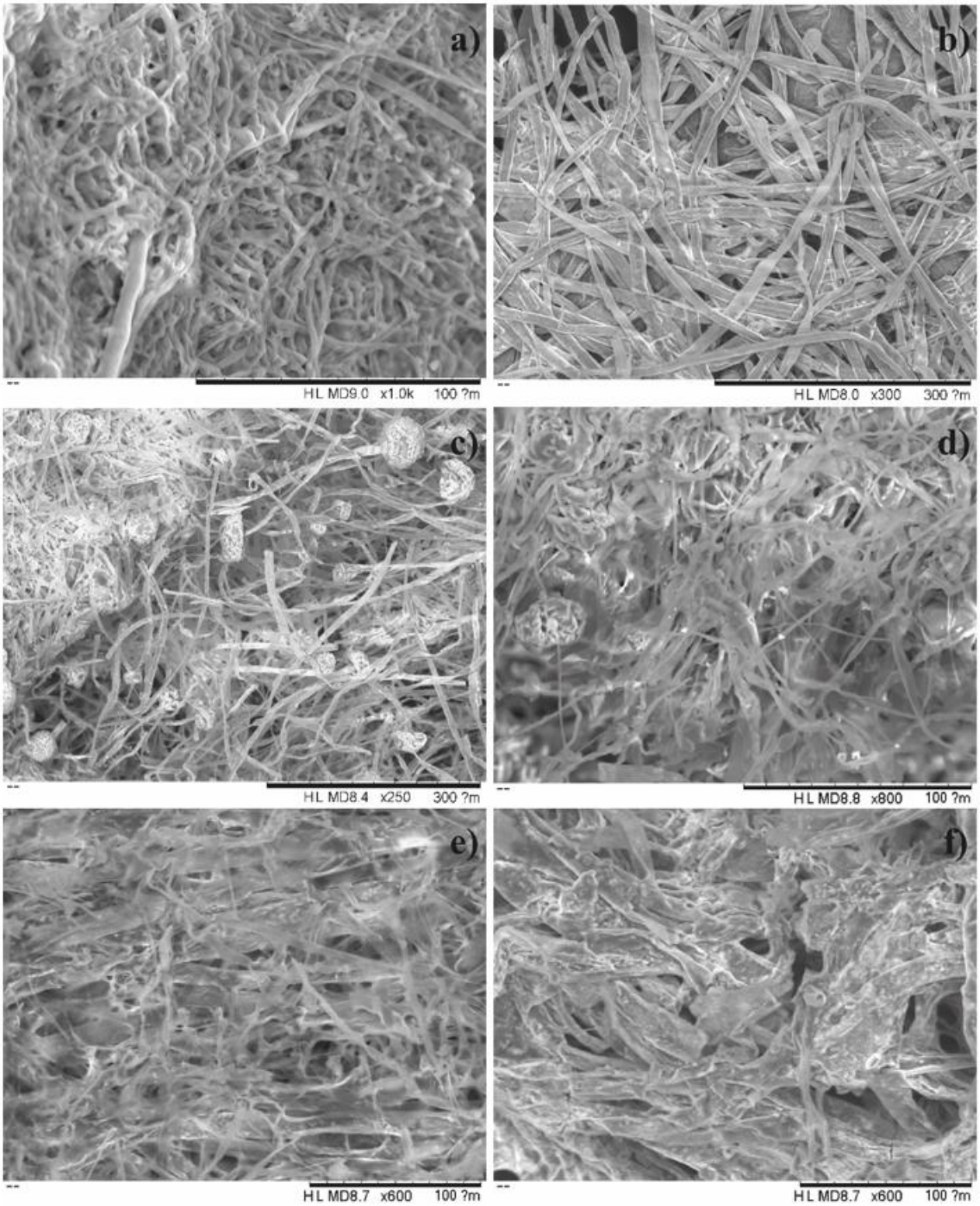

Figure 2. SEM observations of fungal biomass, control (a) and after (b) 50 mg L^{-1}, (c) 100 mg L^{-1}, (d) 250 mg L^{-1}, (e) 350 mg L^{-1}, (f) 500 mg L^{-1} biosorption of Pb. The arrows indicate particles precipitated on the surface of the hyphae in the Pb exposure treatments.

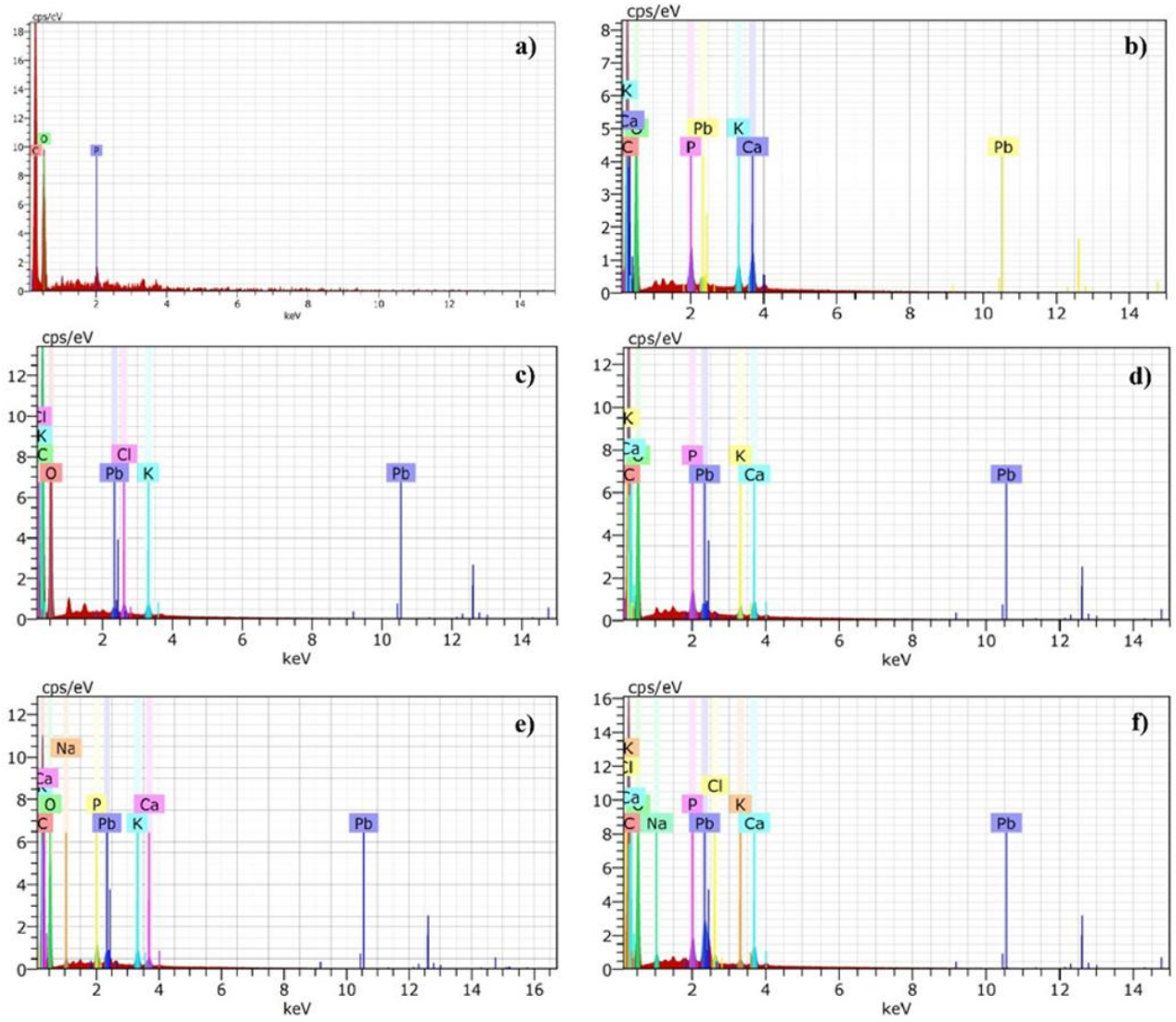

Figure 3. Elemental composition of fungal biomass, control (a) and after (b) 50 mg L^{-1}, (c) 100 mg L^{-1}, (d) 250 mg L^{-1}, (e) 350 mg L^{-1}, (f) 500 mg L^{-1} biosorption of Pb.

Fungal biomass

As shown in Table 1, fungal biomass growth at the lowest concentrations of Pb ions showed no inhibitory effect, as there were no significant differences compared to the control (without Pb exposure). However, a decrease in fungal growth was observed at the highest Pb concentration tested, i.e., 500 mg L^{-1}, which was statistically different from the control. According to the biomass morphology observed using SEM, the presence of Pb did not alter the microscopic structure of the fungus at any of the concentrations tested, except at 500 mg L^{-1}. At this final metal concentration, morphological alterations were observed in the hyphae, resulting in a collapsed appearance.

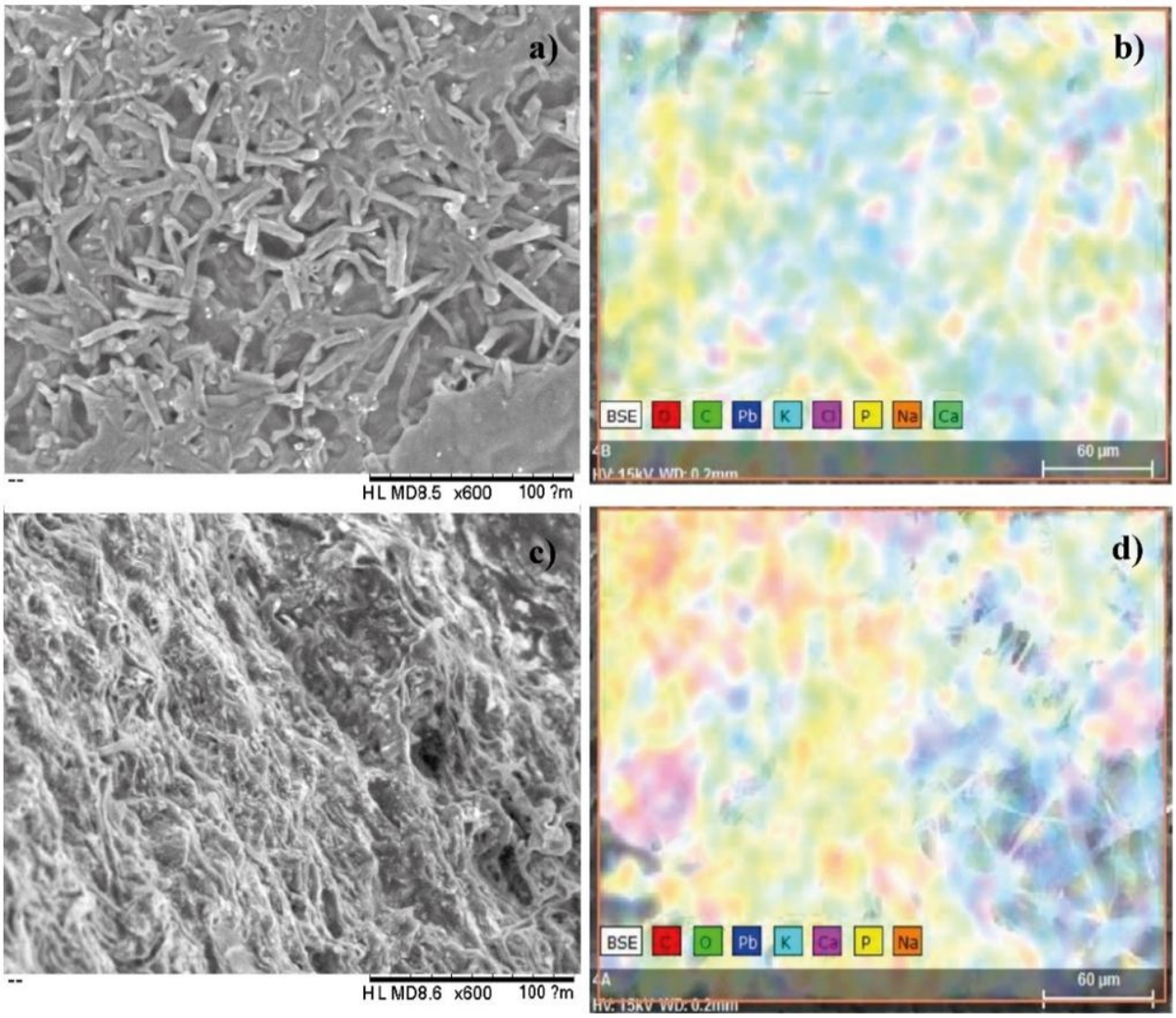

Figure 4. X-ray mapping also confirmed the presence and exact location of Pb and other metals on the hyphal surface. (a, b) 350 mg L^{-1} Pb, (c, d) 500 mg L^{-1} Pb.

Pb removal efficiency

The removal efficiency of Pb by *Cladosporium* sp. was determined under liquid culture conditions (pH 6.5) over 8 days of culture (Table 1). The results showed that the removal rate gradually increased from the lowest to the highest concentrations of Pb in the culture medium. The lowest values of 79.2% and 82.5% (at 50 and 100 mg L^{-1}, respectively) were not significantly different. However, these values were significantly lower than those in the other treatments. Although the maximum value was 97.3% at 500 mg L^{-1} Pb, no significant differences in the removal efficiency were observed among the 250, 350, and 500 mg L^{-1} Pb treatments, which remained between 95 and 97.3%.

Table 1. Fungal biomass, residual Pb concentration, and removal efficiency by *Cladosporium* sp. after Pb treatment (50–500 mg L^{-1}).

	Fungal biomass (g L^{-1})	Concentration of Pb after treatment (mg L^{-1})	Removal efficiency (%)
Control	1.95 ± 0.1a	<DL	-
50 mg L^{-1}	1.8 ± 0.13a	10.4 ± 0.43ab	79.2 ± 2.24a
100 mg L^{-1}	1.99 ± 0.23a	17.5 ± 0.37c	82.5 ± 3.16a
250 mg L^{-1}	1.8 ± 0.24a	12.4 ± 0.28b	95.0 ± 2.82b
350 mg L^{-1}	1.8 ± 0.24a	9.9 ± 0.44a	97.2 ± 1.95b
500 mg L^{-1}	1.3 ± 0.18b	13.5 ± 0.31b	97.3 ± 1.75b

DL:detection limit of Pb. Values are expressed as mean ± standard error (SE). Different letters within the same column indicate statistically significant differences among treatments according to Tukey's post hoc test ($p \leq 0.05$). One-way ANOVA detected significant treatment effects on fungal biomass production (F = 6.31, p = 0.022), residual Pb concentration (F = 66.84, $p < 0.001$), and Pb removal efficiency (F = 12.69, $p = 0.0006$).

DISCUSSION

Morphological characterization of the fungus

The morphological identification of the isolated fungus *Cladosporium* sp. was consistent with the classic descriptions of the genus (Bensch *et al*. 2012), further supported by the results of the nucleotide sequence analysis. The isolation of this fungal strain from soils contaminated with toxic metals suggests its adaptation to stress conditions, indicating its potential for use in bioremediation strategies. Morphologically similar species have been reported to exhibit different biosorption capacities (Gadd 2010), thus necessitating individual- or species-specific characterization.

SEM observations

Based on the morphological analysis, the fungal biomass exhibited alterations in the normal hyphal structure at the highest Pb concentrations, suggesting cellular stress. SEM images revealed an accumulation of nanoparticles and crystal precipitates on the hyphal surface, which increased over time. Furthermore, the culture medium became slightly acidified during the 8 days incubation period. Under this pH condition, Pb is expected to remain predominantly in soluble form. Note that this condition is unfavorable for the chemical precipitation of the metal, as this process generally occurs under alkaline conditions, typically at pH values above 7–8 (Sparks 2003, Gadd 2010, Zhao *et al.* 2020). Therefore, the Pb removal observed was primarily attributed to biologically mediated mechanisms involving *Cladosporium* sp. The hyphal cell wall contains various functional groups, such as carboxyl, hydroxyl, phosphate, amino, and sulfhydryl groups, that can bind metal cations through ion exchange, complexation, and electrostatic interactions (Gadd 2007, Gadd 2009, Dhankhar and Hooda 2011). At near neutral pH values, these negatively charged groups provide efficient binding sites for Pb^{2+}, facilitating its immobilization on the hyphal surface. Therefore, Pb removal by *Cladosporium* sp. was likely dominated by biosorption processes occurring at the fungal cell wall via direct interactions between the metal and fungal biomass; however, bioaccumulation cannot be entirely excluded.

EDS analysis showed that Pb is associated with the fungal biomass, supporting this mechanism and the aforementioned findings. The simultaneous detection of Pb and P in the fungal biomass, along with the presence of precipitates observed by SEM, suggests a possible association between these elements. Meanwhile, the granular and crystalline structures observed in the hyphae by SEM indicate potential Pb immobilization on the fungal surface. Fungi have been reported to promote metallic precipitate formation through interactions involving extracellular metabolites and cell wall-associated compounds, including phosphates, oxalates, and carbonates (Gadd 2007, Fomina and Gadd 2014, Zhao *et al.* 2020). However, as this study did not characterize the precipitates, their mineralogical nature has not been confirmed. These results suggest that Pb removal by *Cladosporium* sp. predominantly occurred through biosorption mediated by cell wall associated functional groups, followed by partial biomineralization on the hyphal surface. Because the experiments in this study were conducted under controlled laboratory conditions, the similarity of fungal behavior in more complex environmental matrices remains uncertain.

EDS analysis

EDS analysis confirmed the presence of P on the surface of fungal hyphae, the origin of which is associated with the phosphoryl groups of the fungal cell wall. The formation of precipitates and crystalline nanoparticles on the cell surface indicates Pb immobilization. However, the available SEM-EDS data cannot sufficiently confirm the formation of specific inorganic compounds resulting from the Pb–P interactions. Similar processes have been reported for other fungi, such as *Phanerochaete chrysosporium* (Zhao *et al*. 2020) and *Pleurotus ostreatus* (Wang *et al.* 2019). Zhao *et al.* (2020) observed the extracellular precipitation of an inorganic Pb–P compound identified as hydroxypyromorphite $Pb_5(PO_4)_3OH$ in *P. chrysosporium*, which was mediated by a phosphatase enzyme induced by Pb exposure. The results in this study indicate that *Cladosporium* sp. has considerable potential to transform soluble Pb into insoluble inorganic forms, thereby reducing its toxicity and bioavailability, particularly in contaminated water. However, EDS analysis alone cannot differentiate between surface adsorption and intracellular accumulation. Therefore, complementary spectroscopic techniques such as X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR) are recommended to better elucidate the molecular mechanisms involved (Wang and Chen 2009).

Fungal biomass

The fungal biomass was not significantly affected by Pb concentrations up to 350 mg L^{-1}, indicating a high tolerance of *Cladosporium* sp. to stress from exposure to this metal. This tolerance can be attributed to the mechanisms used by the fungus to remove Pb, which have been described in the previous sections and reported for other fungal species (Gadd 2007, Gadd 2010, Dhankhar and Hooda 2011). These mechanisms likely favored the ability of *Cladosporium* sp. to maintain biomass production, even under high Pb concentrations. However, a significant reduction in biomass was observed at 500 mg L^{-1} Pb, suggesting that detoxification mechanisms were partially overwhelmed at the highest metal concentration analyzed in this study. Exposure to high Pb concentrations has been documented to have adverse effects on fungal cells, including oxidative stress, membrane damage, and metabolic alterations that inhibit enzymatic activities, ultimately impairing cell growth and function (Gadd 2010, Wang *et al.* 2019). These effects are consistent with the SEM

observations, which revealed shorter, deformed, and aggregated hyphae as well as a collapsed mycelial structure at 500 mg L^{-1} Pb. An evident discrepancy was observed between the 350 and 500 mg L^{-1} treatments. Nevertheless, although the residual Pb concentration was significantly lower at 350 mg L^{-1} (9.9 ± 0.44 mg L^{-1}) than at 500 mg L^{-1} (13.5 ± 0.31 mg L^{-1}), both treatments showed statistically similar removal efficiencies (97.2 and 97.3%, respectively). This behavior is likely because the removal efficiency was calculated relative to the initial Pb concentration. Consequently, the 500 mg L^{-1} treatment removed a substantially greater absolute Pb amount despite maintaining a slightly higher residual concentration. These results show that *Cladosporium* sp. can maintain a high Pb removal capacity across a wide concentration range. However, the increase in residual Pb concentration and reduction in biomass observed at 500 mg L^{-1} suggest an approach to saturation of the binding sites.

Pb removal efficiency

Cladosporium sp. achieved 97% removal efficiency, demonstrating its remarkable capacity to remove Pb from the culture medium, even at high metal concentrations. This high removal efficiency was associated with fungus-mediated biological processes, as evidenced by the SEM and EDS results. The presence of P in the biomass and formation of granular and crystalline structures suggest the significant contribution of extracellular immobilization and biomineralization to Pb removal. These observations indicate that the fungal cell wall acts as the primary interface for Pb sequestration by interacting with functional groups capable of binding metal ions. Although *Cladosporium* sp. maintained growth at all evaluated Pb concentrations, a decrease in biomass production was observed at 500 mg L^{-1}. This result suggests that although the detoxification mechanisms remained effective, they were partially saturated at higher metal concentrations. The morphological alterations observed by SEM, including the presence of shorter and more aggregated hyphae and partial collapse of the mycelial structure, indicate physiological stress induced by Pb exposure. Wang *et al*. (2019) reported similar results for *Pleurotus ostreatus* ISS-1 and observed intracellular bioaccumulation, albeit to a lesser extent. In addition to living cells, dead biomass can adsorb metals via electrostatic adsorption onto the cell surface (Srivastava and Thakur 2006). If the cell surface is saturated with metals, they enter the intracellular space through cellular uptake, which depends on cellular metabolic processes and only occurs in living cells (Velásquez and Dussan 2009). However, in another study, Zhang *et al*. (2016) found that Pb was primarily eliminated through bioaccumulation by *P. ostreatus* HAU-2, exceeding the amount of Pb adsorbed onto the cell surface. This was likely due to acclimation of the HAU-2 strain to Pb stress. Furthermore, Zhang *et al*. (2016) demonstrated that extracellular Pb biosorption was faster than its intracellular accumulation. The high Pb tolerance exhibited by *Cladosporium* sp. is comparable to that reported for other filamentous fungi, including *Aspergillus flavus*, *Mucor hiemalis*, *Trichoderma viride*, and *Rhizopus arrhizus*, which can grow at high Pb concentrations via various detoxification mechanisms (Kurniati *et al*. 2014, Senol *et al*. 2021, El-Morsy *et al*. 2023).

CONCLUSIONS

Cladosporium sp. demonstrated high Pb tolerance and Pb removal efficiency under the experimental conditions evaluated in this study, highlighting its potential as a biological agent for Pb removal or immobilization. In terms of biological processes, the SEM and EDS analyses suggest that Pb removal was linked to the formation of inorganic metal precipitates. Further research is necessary to accurately determine the mechanisms involved and their relative contributions as well as evaluate the performance of the fungus under more complex environmental conditions.

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STATEMENT ON THE USE OF GENERATIVE ARTIFICIAL INTELLIGENCE TOOLS

In the preparation of this manuscript, the authors used ChatGPT for the purpose of reviewing formatting aspects such as detecting inconsistencies in cited references, typographical errors, and grammatical aspects. The review, verification, editing, and interpretation of the input provided by ChatGPT were carried out by the authors, who assume full responsibility for the published content.

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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