



Growth of the fungus *Chaetomium aureum* in the presence of lead: implications in bioremediation

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Abstract

Filamentous fungi were isolated from semiarid soil in which the lead (Pb) concentrations were above regulatory limits as determined by the Brazilian standards. Among these fungi, four isolates were tested for their abilities to grow in a culture medium containing lead nitrate. *Chaetomium aureum* was the species that presented a comparatively better performance including mycelial growth in a lead-containing medium, ability to use pectin as a carbon source, as well as basophilic and thermotolerant properties. The *C. aureum* inoculum with either activated or inactivated native microbiota was able to reduce the free Pb in soil (61 and 54%, respectively) after 60 days of inoculation. Although the mechanism involved in decreasing water-soluble and exchangeable lead concentrations in soil has not been studied, either the processes of biosorption by organic molecules (called oosporein, produced by this species) or fungal mineral transformation is among its possible explanations. These findings support the use of filamentous fungi as potential tools for the bioremediation of contaminated sites and highlight *C. aureum* as a promising tool for environmental biotechnology. In addition, the ability of this fungus (collected in a lead-contaminated area) to grow efficiently in the presence of this metal indicates that the bioprospecting strategy of the indigenous microbiota should be encouraged, as they appear to be more likely to succeed.

Keywords Microcosm · Brazilian semiarid · Soil contamination · Trace metal · Filamentous fungi

Introduction

Lead (Pb) has been recognized as an extremely toxic metal of special interest among environmental pollutants (Casas and Sordo 2006). Pollution caused by anthropogenic activities mostly originates from mining, smelting, industrial uses, wastes incineration, and burning of coal and leaded gasoline (Cheng and Hu 2010). From toxicological perspective, lead

causes extensive damage in different organisms and it is considered a neurotoxic agent (Skerfving and Bergdahl 2014).

Microorganisms in soil are particularly susceptible to the effects of Pb. Unlike many other metals, Pb has no biological role and is potentially toxic to microorganisms (Sobolev and Begonia 2008). Its excessive accumulation in living organisms is always detrimental (Adelekan and Abegunde 2008). Nevertheless, certain microorganisms can tolerate the presence of high concentrations of heavy metals owing to different physiological mechanisms (Da Silva-Júnior and Pereira 2007, 2014; Li et al. 2015; Kang et al. 2016).

The strategies used by microorganisms to survive adverse environments with high concentrations of heavy metals are particularly interesting to the study of remediation in contaminated sites. In many cases, these organisms can decrease the bioavailability of toxic elements in the environment through mechanisms, including the absorption of lead by secreting extracellular polymers, like the secretion of polysaccharides by *Pseudomonas* sp. (Shiomi 2015). There is still, mechanisms such as adsorption at the cell wall (for example, in *Aspergillus flavus*) (Ozer and Ozer 2003),

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intracellular binding by substances like phytochelatins and siderophores (in *Schizosaccharomyces* sp.) (Shiomi 2015) or intracellular precipitation of lead (marine bacteria *Vibrio harveyi* and gram negative bacteria *Providencia alcalifaciens*) (Mire et al. 2004; Naik et al. 2012).

The efficacy of metal removal from the environment may be enhanced by selecting optimum conditions for parameters such as temperature and pH (Biswas et al. 2015). Determination of these optimal conditions may encourage faster microbial growth (Da Silva-Júnior and Pereira 2007) as well as the production and release of metabolites (Battilani et al. 2010). Furthermore, the specific relationship between the contaminant and the microorganism used for the bioremediation process should be considered.

Currently, the search for mechanisms and organisms for bioremediation programs has gained prominence, due to the increasing levels of contaminated sites. In order to look for these competent organisms we have to take into account their potential to grow in adverse conditions, adaptability, and a deeper investigation of the relationship between microorganisms and the environment

The study aimed to isolate filamentous fungi in a contaminated area of lead, as well as to evaluate the behavior of this isolated fungus tolerant to this metal in culture medium and to use a microcosm scale in the soil.

Materials and methods

Sample collection and processing of soil

Soil sampling was performed at the perimeter of a metallurgical plant located in Belo Jardim, Pernambuco state, Brazil. The results of soil analysis are described in Table 1, and the lead concentration was considered to be above regulatory limits as defined by Brazilian standards (CONAMA 2009).

Soil samples were collected from random points at the depth of 0–20 cm, after removing plant residues from the

sampling sites. The samples were then stored in polyethylene bags and were transferred to the Soil Microbiology Laboratory of the Instituto Tecnológico de Pernambuco. At the laboratory, the samples were sieved (2 mm) and homogenized for further assays.

Isolation of filamentous fungi

For the isolation of filamentous fungi, 25 g of soil was suspended in 225 mL of sterile distilled water and the resulting suspension serially diluted. Triplicates were obtained from each suspension, placed in plates containing 0.2 mL potato dextrose agar (PDA) medium, and homogenized with a Drigalski spatula. The plates were incubated in the laboratory at room temperature (approximately 28 °C) for up to 6 days, and CFU g⁻¹ counts were then obtained (Warcup 1950).

Identification of the fungi

The identification was performed by macroscopic and microscopic observations of the isolated fungi. The morphological aspects of the cultures such as the aspects of their border, pigmentation, and color were visualized through macroscopic observation of the fungal colonies grown on either PDA or Czapek media. The microscopic characteristics of these isolates were observed through the description of the reproductive structures under an optical microscope using the slide culture technique. The fungal species were identified as described by Fennell et al. (1975), Domsch et al. (1980), and von Arx et al. (1986).

Growth in culture medium with lead nitrate

Tolerance analysis of the isolated species was performed by investigating the growth of the colonies in culture media containing different concentrations of lead nitrate (0, 93.75, 187.5, and 281.25 Pb mg kg⁻¹). A piece of the monosporic cultures of four isolates from the contaminated soil (*C. aureum*, *Thielavia* sp. *Aspergillus niger* and *Penicillium* sp.) was transferred to PDA media (in triplicate, individually). After 6 days of incubation, the colony diameters were measured with millimeter precision. Among the isolated colonies, the fungus *Chaetomium aureum* Chivers (1912) was selected considering the results from previous studies, which demonstrated the tolerance of the genus *Chaetomium* to high lead concentrations.

Growth using different substrates

Evaluation of the growth of colonies using different substrates was performed after transferring a fragment of monosporic cultures (*C. aureum*) to Petri dishes (also in triplicate) containing PDA medium with different substrates (dextrose,

Table 1 Soil analysis

Parameter	
pH (H ₂ O)	4.82
Organic matter (%)	2.48
V (%)	77
P (mg dm ⁻³)	6
Ca (cmol _c dm ⁻³)	3.25
Mg (cmol _c dm ⁻³)	4.05
K (cmol _c dm ⁻³)	0.41
Al (cmol _c dm ⁻³)	0.30
H (cmol _c dm ⁻³)	4.65
S (cmol _c dm ⁻³)	16.05
Pb mg kg ⁻¹	154.0

pectin, casein, and amide). The cultures were incubated at room temperature for 4 days, and the colony diameters were then measured with millimeter precision.

Growth at different temperatures

Fragments of *C. aureum* monosporic cultures were transferred to PDA media (six replicates) and incubated for 3 days at three temperatures (28, 35, and 42 °C). After this period, the colony diameters were measured with millimeter precision.

Growth in different pH values

Fragments of monosporic cultures of *C. aureum* were transferred to Petri dishes (six replicates) containing PDA media prepared at 4 different pH values (pH 6, 7, 8, and 9) and were incubated for 3 days. After this period, we measured the colony diameters with millimeter precision.

Experiment at the microcosm scale—*C. aureum* in contaminated soil

C. aureum inoculums preparation

Replicates of the fungal culture stored in the laboratory were used to prepare concentrated suspensions of spores which were placed in Petri dishes containing PDA. These replicates were incubated at a temperature of 28 °C for 10 days. After this period, the mycelial mass was transferred to tubes which containing a sterile saline solution (0.8%) and the final concentration of the inoculum ranged from 2.5 to 3 g L⁻¹.

Soil

Soil samples from the area, where the fungus was isolated, were used for the microcosm assay. Both natural and autoclaved (at 121 °C for 1 h for two consecutive days) soils were used.

Soil inoculation and analyzed variables

Recipients containing 300 g of soil were inoculated (in triplicate) with fungal suspensions for periods of 0, 30, and 60 days. The following parameters were then measured: pH, organic matter, lead content, and colony forming units per gram of soil (CFU g⁻¹).

The experimental design was entirely random in a scheme factorial 2 × 2 × 3 × 3. The treatments were: (1) non-autoclaved soil without *C. aureum* inoculum, (2) non-autoclaved soil inoculated with *C. aureum*, (3) autoclaved soil without *C. aureum* inoculum, and (4) autoclaved soil inoculated with *C. aureum*.

Water-soluble and exchangeable Pb determination

The soil samples were shaken with 0.1 M CaCl₂ for 16 h at room temperature and centrifuged at 3600 rpm. The supernatant then filtered with a Millipore filter (0.45 μm) (Orroño and Lavado 2009). The Pb was quantified using inductively coupled plasma-atomic emission spectrometry (ICP-OES).

Data analysis

The results were expressed as mean ± SD. The data were analyzed by one-way analysis of variance (ANOVA) following the Tukey's test. The Kruskal–Wallis nonparametric test was employed for the analysis of nonparametric data. Differences at *p* < 0.05 were considered as statistically significant.

Results and discussion

Microbial growth in the presence of lead nitrate

Among the colonies grown in soil from the residue storage patio at the metallurgical plant, four colonies were selected to evaluate growth in the presence of lead: one isolate of *C. aureum*, one of *Thielavia* sp., one of *Penicillium* sp., and one of *Aspergillus niger*. After 3 days, lead nitrate showed negative effects on the growth of *Thielavia* sp., *A. niger*, and *Penicillium* sp. isolates. However, it caused no significant difference in the growth of the *C. aureum* isolate (Fig. 1).

After 6 days, the *C. aureum* isolate showed increased mean growth efficiency in the medium containing lead nitrate, also displaying significantly greater mycelial growth in the two highest concentrations of lead compared to that in the control. In sequence, the *Thielavia* sp. isolate demonstrated mycelial growth that was superior to the control

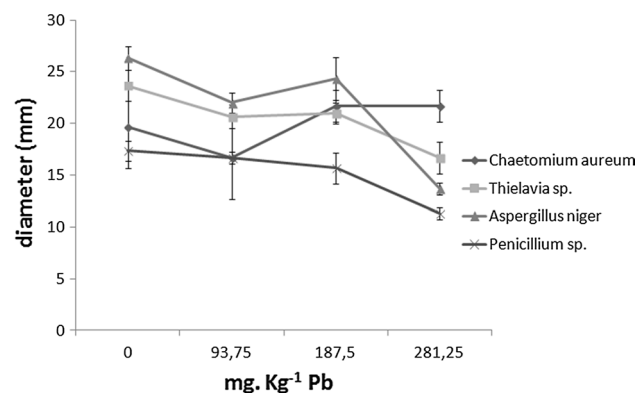


Fig. 1 Growth of colonies in culture mediums containing different concentrations of lead nitrate, after 3 days (0, 150, 300, and 450 mg kg⁻¹, which are equivalent to 0, 93.75, 187.5, and 281.25 Pb mg kg⁻¹, respectively)

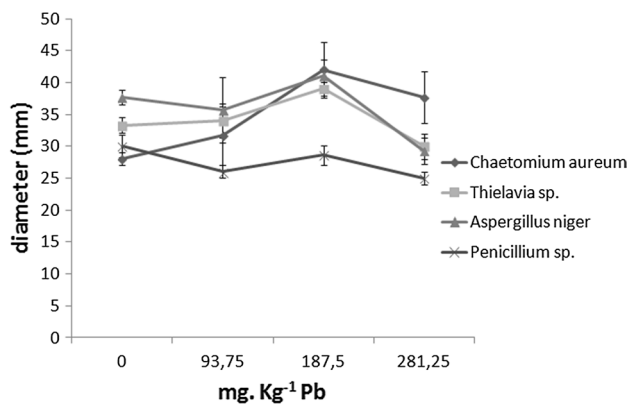


Fig. 2 Growth of colonies in culture mediums containing different concentrations of lead nitrate, after 6 days (0, 150, 300, and 450 mg kg⁻¹, which are equivalent to 0, 93.75, 187.5, and 281.25 Pb mg kg⁻¹, respectively)

at a Pb concentration of 187.5 mg kg⁻¹. On the other hand, isolates of the genera *Aspergillus* and *Penicillium* showed decreased mycelial growth with increasing lead concentration (Fig. 2).

These findings indicate that the filamentous fungus *C. aureum* showed tolerance to lead nitrate after 3 days of incubation, and presented a higher mycelial growth depending on the mean lead concentration in the mean after 6 days.

Interactions between fungi and toxic metals are not limited to studies with iron, nor are they restricted to the toxic effects of metals in different species of fungi, these studies also investigated their toxicity, resistance, tolerance, absorption, and excretion (Gola et al. 2016; Kaewdoug et al. 2016; Ruta et al. 2017). Elucidating these processes is an important step in decision-making for microbe bioremediation studies. The relationship between toxic metals and the fungi of the genus *Chaetomium* was addressed by only a few studies, which have shown both species-specific and metal-specific responses.

The fungi of the *Chaetomium* genus were isolated from the soils of peri-urban agricultural area irrigated by industrial and mine waste areas, presenting high concentrations of metals such as Fe, Mn, Cu, As, Sr, Mo, Cd, Sb, Ti, Zn, and Pb (Iram et al. 2009). Smith et al. (1978) studied the effects of trace-metals on the growth of urban tree-leaf pathogen fungi and observed that high concentrations of some metals such as Al, Fe, Mg, Pb, and Zn are extremely toxic to *Chaetomium* sp. However, the growth of this fungus was favored at some intermediate concentrations of Pb and Fe. An example of this high resistance in harsh environments can be seen in a review of the surveys conducted during 1986–2012 in natural ecosystems of the Russian Federation and Ukraine, which were contaminated due to the Chernobyl disaster (Shcheglov et al. 2013), showing a fungal activity of 3.7×10^5 – 3.7×10^7 Bq/Kg for *C. aureum* and *Paecilomyces*

lilacinus in medium and high levels of contamination (Zhdanova et al. 1995). Aguilera et al. (2016) also demonstrated a lower prevalence of parasitic fungi at the radiation levels near Chernobyl, but with similar fitness.

In the present study, four investigated isolates of *Chaetomium* sp. were tolerant to lead, since they were indigenous components of the contaminated soil adjacent to the metallurgical plant. In fact, fungi of this genus have already been used in isolates of contaminated soil from the same study area (Da Silva-Júnior and Pereira 2007) and have proven to be tolerant to the lead-containing mixture of natural soil and scum (Da Silva-Júnior and Pereira 2014). In the current study, *C. aureum* isolates displayed a better outcome in mycelial growth in relation to other isolates incubated in medium containing lead nitrate. The association between two factors must have contributed to this growth: the tolerance of the fungus to lead and the use of nitrate as a nitrogen source (Feeny and Curran 1992). This explains the necessity of evaluating some culture conditions of this fungus and inoculating it in a lead-contaminated soil in order to monitor some soil parameters at the microcosmic scale.

Analysis of mycelial growth in different substrates and conditions

The *C. aureum* isolate displayed a stronger ability to grow in the culture medium containing lead nitrate; therefore, it was used for physiological characterization as well as in the microcosm scale experiments. The ability of *C. aureum* to grow in different substrates was investigated (Fig. 3). This fungus displayed higher mycelial growth in media containing pectin compared to those containing starch and glucose. On the other hand, it was not capable of growing in media containing casein as the substrate.

Abiotic culture parameters, such as temperature, pH, and water activity, may influence in the physiological state

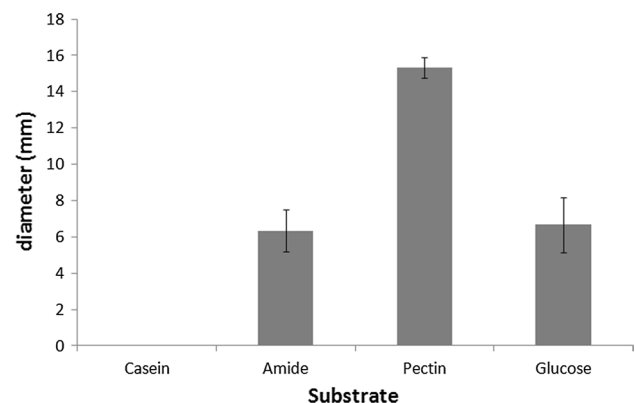


Fig. 3 Growth of colonies in culture mediums containing different substrates, after 6 days

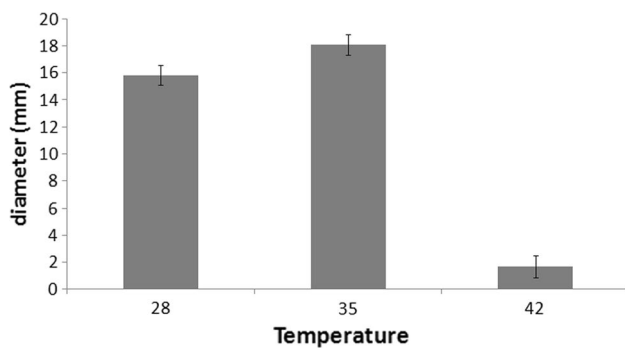


Fig. 4 Growth of colonies in culture medium in different temperatures, after 6 days

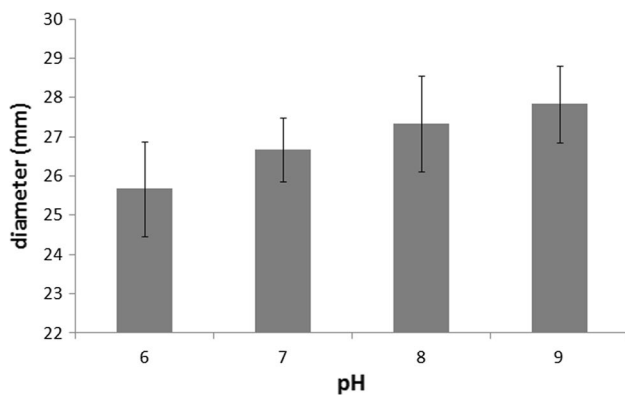


Fig. 5 Growth of colonies in culture mediums in different pH values, after 6 days

of microorganisms (van Long et al. 2017). The *C. aureum* isolate demonstrated the ability to use pectin, glucose, and starch as substrates, but not casein. Among the three substrates, pectin promoted the highest mycelial growth. The ability to degrade materials of vegetal origin, such as cellulose and pectin, has already been described for the fungi of the genus *Chaetomium* by Batista and Pontual (1948), besides the detection of the enzymatic activity of pectinase (Reddy and Sreeramulu 2012).

The genus *Chaetomium* is composed of fungi with an enzymatic surface machinery, which is capable of breaking down complex molecules such as cellulose (Cragg et al.

2015). This strategy has been investigated in studies on the biodegradability of organic polymers (Oprea and Doroftei 2011), nitrocellulose (Auer et al. 2005), hydrocarbons (Aranda 2016), pesticides (Tiedje and Hagedorn 1975; Hu et al. 2015), and natural products (Zheng et al. 2016).

Other growth parameters investigated were the pH of the culture medium and incubation temperature (Figs. 4, 5). The increase in pH values had a positive effect on the growth of *C. aureum*, and the best temperature for growth was observed at 35 °C. The *C. aureum* isolate appeared to be both thermo- and alkaline-tolerant. Both properties have already been described for many *Chaetomium* species (Milner 1977; Ravindran et al. 2011; Ravindran and Thangaiah Naveenan 2011; Srivastava et al. 2017). However, fungi of the genera *Chaetomium* have already been isolated in soils with pH higher than 10 in the same study area (unpublished data). This specific case, where the pH value of industrial waste is 10.4, demonstrates a basophilic condition that favors the use of this fungus in future bioremediation programs. Furthermore, since this region is located in the Brazilian semiarid region, fungi that can survive and grow in high temperatures may be considered in planning the recuperation of contaminated sites.

Microcosm scale experiment

In order to evaluate the effect of lead-contaminated soil on the fungus, we prepared an experiment at the microcosm scale by adding an inoculum of *C. aureum* (concentrations between 2.5 and 3 g L⁻¹) to soil samples. Tables 1 and 2 show that the four treatments, with or without *C. aureum* inoculum in autoclaved and non-autoclaved soil, reduced the concentration of water-soluble and exchangeable lead after 60 days.

Treatment 2 (non-autoclaved soil with the fungus inoculum) showed a reduction of approximately 40% in the levels of exchangeable lead in the first 30 days of inoculation. This reduction is twice as large as that observed in treatment 1 (non-autoclaved soil without the fungus inoculum). Between 30 and 60 days, treatment 4 (autoclaved soil with fungus inoculum) was the most efficient in reducing the concentration of free Pb. The reduction rate after 60 days revealed that treatments with the fungus inoculum were

Table 2 Reduction in water-soluble and exchangeable Pb in the microcosms experiment

	Day 0	Day 30		Day 60	
	Reduction (%)	Reduction (%)	Cumulative reduction (%)	Reduction (%)	Cumulative reduction (%)
Treatment 1	0	21.89	21.89	8.13	28.24
Treatment 2	0	40.81	40.81	34.25	61.08
Treatment 3	0	-5.14	-5.14	24.29	20.40
Treatment 4	0	6.89	6.89	50.65	54.05

at least in two different settings, non-autoclaved and autoclaved soil, more effective at decreasing the concentrations of exchangeable lead than in the treatments without the inoculum.

The effect on pH and the organic matter content in the soil is illustrated in Table 3. These two parameters only varied after 60 days of inoculation. The pH decreased significantly in treatments 1, 2, and 3, whereas organic matter content increased significantly in treatment 2 after the same period. Microbial density during the microcosm experiments is illustrated in Fig. 6. This parameter displayed similar behavior in treatments with both autoclaved and non-autoclaved soils. In the former experiment, microbial density grew after 30 and 60 days of inoculation, whereas in the latter, treatments 1 and 2 displayed an increase in density after 30 days, followed by a decrease after 60 days of inoculation.

C. aureum thus seemed to be a promising bioremediation element at the microcosm scale. Its presence in soil, associated with the native microbiota, reduced the levels of exchangeable lead by two times more than that in the absence of the fungal inoculum after 60 days of incubation. During the same period, inoculation of *C. aureum* played an important role in maintaining the pH values of the soil, while inoculation of the fungus in association with the native microbiota increase the organic matter concentration in the soil. On the other hand, the responses in microbial abundance in soil were related to the process of autoclaving.

Even though the number of studies encompassing microbial remediation focused on organic matter decomposition is highly varied, during the last few years, an increased interest in fungal species with the capacity for efficient metal removal has been observed (Sargin et al. 2016; Xin et al. 2016). The most recent studies show the importance of biosorption to reduce the bioavailability of metals in the environment (Gola et al. 2016) and as in the current study, the high tolerance strategy is accompanied by a decrease in the availability of metals in the environment (Dey et al. 2016). Manoliu et al. (1999) suggest that the cellulolytic fungus *C. globosum* is capable of internalizing iron, thus removing it from the surroundings through molecules called siderophores. In the current study, the

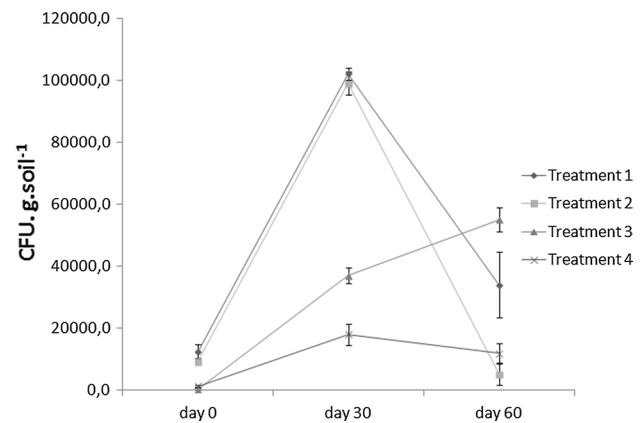


Fig. 6 Microbial density during the microcosms experiments

mechanism for decreasing exchangeable lead concentration is not clear, but two hypotheses can be proposed.

The first hypothesis is that exchangeable lead may be removed through biosorption by molecules released by the fungus, thus reducing its availability in soil. In fact, it is reported that *C. aureum* produces a metabolite called oosporein, which we believe, may bind to the metal, thus forming a complex. Oosporein is a red-colored pigment that presents the typical properties of a polyhydroxyquinone (Lloyd et al. 1955). Molecules of this nature are capable of binding to metals and actively participate in oxy-reduction reactions (Ragimov et al. 1974; Greenaway and Dabrowiak 1982).

The other hypothesis is the mineral transformation of lead by the fungus. This kind of biologically mediated mineralization has already been described for two fungal species, *Metarhizium anisopliae* and *Paecilomyces javanicus*, which transformed lead into pyromorphite (Rhee et al. 2012). Although it has not been investigated in *C. aureum*, mineralization of lead is a possible bioremediation mechanism, as it reduces metal solubility, and therefore, its bioavailability.

Irrespective of the strategy used by the fungus, our findings are relevant to the study of lead contamination in terrestrial environments. *C. aureum*, aided by the native microbial community in soil, was capable of influencing both the availability of this metal and the chemical parameters observed in the soil at the microcosm scale. This ability

Table 3 pH and organic matter behavior in the microcosms experiment

	pH			Organic matter		
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Treatment 1	6.8±0.02	6.8±0.02	6.6*±0.06	4.7±0.21	3.4±3.65	3.0±2.65
Treatment 2	6.8±0.02	6.7±0.02	6.6*±0.06	3.9±0.75	7.5±1.68	10.0*±0.61
Treatment 3	7.3±0.02	7.2±0.02	6.6*±0.07	8.0±1.29	7.9±1.31	6.5±1.04
Treatment 4	7.3±0.01	7.2±0.01	7.3±0.01	5.5±1.15	6.0±0.67	6.4±0.82

*Represents statistical difference ($p < 0.05$) in relation to day 0 (zero)

of interfering in edaphic processes is only possible due to the physiological mechanisms of lead tolerance, which have not been enlightened yet for *C. aureum*. In this context, we believe that the strategy of using indigenous organisms in attempts of bioremediation may be useful to increase their success rates. Recently, the authors also associated the increased efficiency in the removal of metals by fungi when used in consortia (Awasthi et al. 2017). Therefore, microbial processes must be considered in recuperation techniques at lead-contaminated sites.

Conclusions

Lead showed no evidence of toxicity in *C. aureum* at the concentrations tested in vitro. In terms of substrate use, the *C. aureum* isolate was considered versatile, temperature-tolerant, and basophilic. In addition, when inoculated in lead-contaminated soil, it was capable of decreasing the exchangeable lead concentration, irrespective of its association with the non-indigenous microbiota.

These findings support the use of filamentous fungi as potential tools for the bioremediation of contaminated sites by metals and highlight *C. aureum* as a promising tool for environmental biotechnology. The strategy of using indigenous species seems promising to seek candidates for bioremediation of contaminated sites.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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