Potential use of edible mushrooms *Pleurotus ostreatoroseus* Singer (*Pleurotaceae*) and *Lentinus sajor-caju* (Fr.) Fr. (*Polyporaceae*) in iron remediation processes

Rodrigo Paidano Alves
Núcleo de Estudos da Vegetação Antártica, Universidade Federal do Pampa, São Gabriel, Rio Grande do Sul, Brasil. E-mail: alvez_rdg@hotmail.com

Sibele Marques Bolson
Núcleo de Estudos da Vegetação Antártica, Universidade Federal do Pampa, São Gabriel, Rio Grande do Sul, Brasil. E-mail: sibelebolson@hotmail.com

Margéli Pereira de Albuquerque
Núcleo de Estudos da Vegetação Antártica, Universidade Federal do Pampa, São Gabriel, Rio Grande do Sul, Brasil. E-mail: margeli_albuquerque@hotmail.com

Filipe de Carvalho Victoria
Núcleo de Estudos da Vegetação Antártica, Universidade Federal do Pampa, São Gabriel, Rio Grande do Sul, Brasil. E-mail: filipevictoria@unipampa.edu.br

Antônio Batista Pereira
Núcleo de Estudos da Vegetação Antártica, Universidade Federal do Pampa, São Gabriel, Rio Grande do Sul, Brasil. E-mail: antoniopereira@unipampa.edu.br

Abstract: Increased heavy metal pollution generated through anthropogenic activities into the environment required the need for environmental recovery strategies, such as mycoremediation. The aim of the present study was to assess the effect of iron (Fe) in the mycelial growth of Pleurotus ostreatoroseus Singer and Lentinus sajor-caju (Fr.) Fr. The growth response was evaluated on Potato Dextrose Agar medium with varying concentrations (0 ppm, 10 ppm, 20 ppm, 40 ppm and 80 ppm) of the iron metal. Ferrous sulfate heptahydrate (FeSO4.7H2O) were evaluated in this study. The daily mycelia growths were measured and compared. The biomass production was determined using the same plates of the evaluation of the growth of mycelia. Results revealed that the growth of mycelia of P. ostreatoroseus occurs slowly in medium containing different iron concentrations, but in relationship to L. sajor-caju was able to produce the largest mycelial dry mass. Overall this study suggests that P. ostreatosroseus can be used as promising option for removal of iron metal in bioremediation strategies.

Keywords: Bioremediation, biosorption, filamentous fungi, heavy metals, mycoremediation.

O potencial uso dos cogumelos comestíveis *Pleurotus ostreatoroseus* Singer (*Pleurotaceae*) e *Lentinus sajor-caju* (Fr.) Fr. (*Polyporaceae*) em processos de remediação de ferro

Resumo: O aumento da poluição causada por metais pesados, gerados através de atividades antropogênicas no meio ambiente, tem exigido a necessidade de estratégias de remediação como a micorre-

mediação. O objetivo deste estudo foi avaliar o efeito do metal pesado (Fe) no crescimento micelial de *Pleurotus ostreatoroseus* Singer e *Lentinus sajor-caju* (Fr.) Fr. A resposta ao crescimento foi avaliada no meio de cultura Agar Dextrose De Batata com diferentes concentrações (0 ppm, 10 ppm, 20 ppm, 40 ppm e 80 ppm) do metal ferro. O sulfato ferroso heptahidratado (FeSO4.7H2O) foi avaliado neste estudo. O crescimento do micélio foi medido diariamente e comparado. A produção de biomassa foi determinada utilizando as mesmas placas da avaliação do crescimento micelial. Os resultados revelaram que o crescimento micelial radial de P. *ostreatoroseus* ocorre lentamente em meio contendo diferentes concentrações de ferro, mas em relação a L. *sajor-caju* foi capaz de produzir maior quantidade de massa seca micelial. Desse modo, estes resultados sugerem que P. *ostreatoroseus* pode ser usado como opção promissora para a remoção do metal ferro em estratégias de biorremediação.

Palavras-chave: Biorremediação, biossorção, fungos filamentosos, metais pesados, micorremediação.

Introduction

Heavy metals occur naturally in the environment from pedogenetic processes of weathering of parent materials and also through anthropogenic sources (Dixit et al., 2015). The contamination of heavy metals in various ecosystems has become a widespread environmental problem, as the results of agricultural, industrial, and urban activities (Niu et al., 2013). The impact of heavy metals pollution can cause severe environmental and human health problems because of their trophic transfer in organisms, biomagnification in food chains, and toxic effects on biota (Kapahi & Sachdeva, 2017; Oladipo et al., 2016a; Wu et al., 2016). Compared with traditional organic pollutants, heavy metal ions, such as Fe, among others metals, are difficult to degrade into cleaning products (Zou et al., 2016).

Iron (Fe) is an essential nutrient for all organisms(Slaninova et al., 2014; Zuo & Zhang, 2011). Fe content in soil is usually high, but a large proportion is fixed to soil particles and participates in many environmental processes (Bindraban et al., 2015; Li et al., 2014; Mimmo et al., 2014; Rui et al., 2016; Ye et al., 2015; Zargar et al., 2015). Iron is mainly used in galvanic industries, tanneries, foundries and mining. Increased Fe contamination is related to use or inappropriate handling, generated by industrial activities and accidents during manufacture, transport or storage (Mancilha, 2006). For this motive, many techniques for environmental remediation of heavy metals are being studied (El-Kassas et al., 2016; Oladipo et al., 2016b). For example, in USA, it was estimated that there are 350,000 contaminated sites requiring cleanup over the next 30 years. In Europe, there are 3,000,000 potential contaminated sites and need remediation (Gong et al., 2016).

Recently, diverse remediation strategies option has been explored for the restoration of contaminated environments, include the use of fungi (Oladipo et al., 2017). Mushrooms can act as effective biosorbent alternative to plants in removing toxic metals from soil and waste and the process is known as mycoremediation (Koutrotsios et al., 2016; Vaseem et al., 2017). Mushrooms interact with heavy metals physiological and morphologically, and mycelium reduces contaminants by different enzymatic mechanism to restore the natural environmental (Prakash, 2017). Fungi have chitin in their walls which can tolerate high concentrations of metals and are capable to survive in stressed or nonoptimal conditions, such as a low pH and a lack of nutrients and water (Kapahi & Sachdeva, 2017; Ong et al., 2017). Specifically, fungal species adopt metal tolerance strategies which include extracellular metal sequestration and precipitation, suppressed influx, enhanced metal efflux, production of intra-cellular/extracellular enzymes, metal binding to cell walls, intracellular sequestration and complexation (Oladipo et al., 2017; Ong et al., 2017).

Mushrooms belonging to the genera including *Lentinus* and *Pleurotus* have been investigated by some researchers for the uptake of heavy metals (de Albuquerque et al., 2011; Jia et al., 2017; Kapahi & Sachdeva, 2017; Wu et al., 2016). These genera are considered to be rich in proteins, fibres, carbohydrates, vitamins and minerals and own a very pleasant taste. It is rich in immense therapeutic properties (Kalač & Svoboda, 2000; Mleczek et al., 2016). Therefore, there has been a rise in research activities related to the genus because of its multiple uses including biosorption (Kapahi & Sachdeva, 2017). The aim of the present study was to assess the effect of heavy metal (Fe) in the mycelial growth of *Pleurotus ostreatoroseus* Singer and *Lentinus sajor-caju* (Fr.) Fr. and its potential use in bioremediation.

Materials and methods

Revival of fungal culture and preparation of mycelia discs

Agar blocks of approximately 9 mm from the pure stock culture of Pleurotus ostreatoroseus (POR) and Lentinus sajor-caju (PSC) (from the culture collection of the Institute of Biosciences, Universidade Estadual Paulista "Julio de Mesquita Filho" - Botucatu campus, São Paulo, Brazil), preserved in distilled water (Castella, 1967; Donini et al., 2005), were aseptically transferred into sterilized Potato Dextrose Agar (PDA) plates. Culture plates were incubated at 27ºC±1, in the absence of light, to allow growth of the secondary mycelia. After 7 days of incubation, the mycelial discs were prepared using the revived culture which served as source of inocula (de Albuquerque et al., 2011; Dulay, 2015).

Preparation of Potato Dextrose Agar (PDA) with iron metal

Two hundred grams of potato was boiled in 1 L of distilled water until tender. After boiling, the decoction was obtained and 10 g of dextrose (Sigma-A1296, Merck KGaA company, Darmstadt, Germany) and 12 g of agar (Sigma-D9434, Merck KGaA company, Darmstadt, Germany) were added and subsequently boiled, stirred constantly until homogenized (Guzmán, 2008). One hundred ml of the medium was prepared for each concentration. Four concentrations (10 ppm, 20 ppm, 40 ppm and 80 ppm) (Sprynskyy et al., 2006) of the Iron metal namely; Ferrous sulfate heptahydrate (FeSO4.7H2O) were evaluated in this study. A heavy metal free medium was also prepared as the control, 0 ppm. Each prepared heavy metal medium was dispensed in a flask, cotton plugged and properly labelled. These were sterilized in an autoclave at 121°C for 20 min (Dulay, 2015).

Evaluation of the secondary mycelia growth of P. ostreatoroseus and L. sajor-caju

After sterilization, the different media were pour plated into sterilized Petri plates with ten replicates each concentration. Once solidified, the plated media were centrally inoculated with mycelia discs (9 mm), with mycelium facing upwards, from the revived culture of *P. ostreatoroseus* and *L. sajor-caju*. The inoculated plates were incubated at 27ºC±1, in the absence of light, to allow mycelia growth, during days (Dulay, 2015). The daily mycelia growths were measured and compared, following the method of de Albuquerque et al., (2011). A completely randomized design was conducted and factorial design consists: A x B x C (A = fungus specie; B = concentrations; C = days of incubation) for growth rate variable; and A x B (A = isolated species; B = concentrations) for mycelial biomass variable. The experimental units consisted of Petri plates, with ten repetitions for each test concentration, totaling 50 Petri plates (de Albuquerque et al., 2011; Donini et al., 2006).

Evaluation of fungal biomass

The evaluation of biomass production was determined using the same plates of the evaluation of the secondary mycelia after the last measured of mycelia growth. For this purpose, each inoculated plate was placed initially in an open beaker containing 500ml distilled water. The culture medium was dissolving in a boiling water for 5 minutes, picking up dispersed mycelium from the liquefied culture medium. The wet fungal biomass was determined using an analytical balance and incubated for 24 hours at 50 ° C for evaluation of dry fungal biomass (de Albuquerque et al., 2011; Donini et al., 2006).

Statistical data analysis

Data were analyzed using Tukey's test ($p \leq 0.05$). The Statistix 10.0 software program (Analytical Software, Tallahassee, FL, USA) for Windows computer was used for analysis (de Albuquerque et al., 2011). The graphics were built in R (Team, 2015) through the Rstudio (http://www.rstudio.com/) using the R packages ggplot2 (Wickham, 2009), reshape2 (Wickham, 2007) and RColorBrewer (Neuwirth, 2014).

Results and discussion

Mushrooms interact with heavy metals morphological. Some heavy metals are considered toxic at certain concentration in the fungal metabolism (Dulay, 2015). In the present study, the effect of iron metal on mycelia growths of *P. ostreatoroseus* (POR) and *L. sajor-caju* (PSC) was evaluated. The diameter of mycelial growth after 8 days of incubation is shown in Tab. 1.

Tab. 1. Mycelial growth diameter (mm) of *Pleurotus ostreatoroseus* (POR) and *Lentinus sajor-caju* (PSC) after 8 days of incubation in the different concentrations of iron metal.

Species	Concentration (ppm)	Number of days of incubation						
		2	3	4	5	6	7	8
PSC	0	11.93 ± 0.91	21.18 ± 1.03	30.16 ± 0.77	38.07 ± 1.13	39.57 ± 1.02	-	-
	10	11.41 ± 0.80	20.20 ± 0.95	28.89 ±1.25	37.24 ± 1.62	39.30 ± 0.34	-	-
	20	11.94 ± 0.46	21.31 ± 0.52	29.86 ± 0.61	37.13 ± 0.56	38.87 ± 1.16	-	-
	40	11.59 ±1.01	21.41 ± 0.78	28.02 ± 0.88	35.80 ± 1.02	39.37 ± 1.73	-	-
	80	11.65 ± 0.72	20.14 ± 0.61	27.87 ± 0.70	35.31 ± 1.05	39.23 ± 0.94	-	-
POR	0	10.81 ± 0.63	17.98 ± 0.74	25.56 ± 0.95	32.11 ± 0.96	36.23 ± 1.37	37.86 ± 1.99	39.07 ± 1.38
	10	10.11 ± 1.05	16.49 ± 0.96	23.33 ± 1.64	30.36 ± 1.99	34.51 ± 2.31	39.11 ± 3.10	39.07 ± 0.65
	20	10.37 ± 0.60	17.51 ± 0.81	25.20 ± 1.08	27.50 ± 6.40	36.28 ± 1.40	38.20 ± 0.90	39.38 ± 0.50
	40	9.52 ± 0.35	16.08 ± 0.55	23.01 ± 0.48	30.84 ± 1.66	35.23 ± 1.16	37.81 ± 0.99	39.03 ± 1.00
	80	10.62 ± 0.84	18.48 ± 1.25	27.19 ± 1.93	34.99 ± 2.26	38.93 ± 1.00	39.41 ± 0.53	39.41 ± 0.53

Interestingly, it can be seen that 20 ppm of concentration stimulated the mycelial growth of *L. sajor-caju*, which were found higher than the mycelial growth of iron metal free (control) culture plates. But, *P. ostreatoroseus* showed a different result, mycelial growth was stimulated in control compared with other concentrations. However, lower growth diameters were significantly recorded at 10 ppm and 40 ppm, which strongly suggests its less toxicity in *L. sajor-caju* and *P. ostreatoroseus*, respectively.

After completion of remediation at 8 days, the overall remediation of iron metal by mushroom was also calculated in terms of percent at 8th day by using the formula- [(average of mycelial growth diameter X 100) / average of final mycelial growth diameter] (Vaseem et al., 2017). *L. sajor-caju* had a significant decrease in mycelia growth in the high levels of iron metal concentration, control had a mycelia growth of 97.02% in 5 days (Fig. 1). While the concentration of 80 ppm of iron had a mycelia growth of 89.66% in the same time.

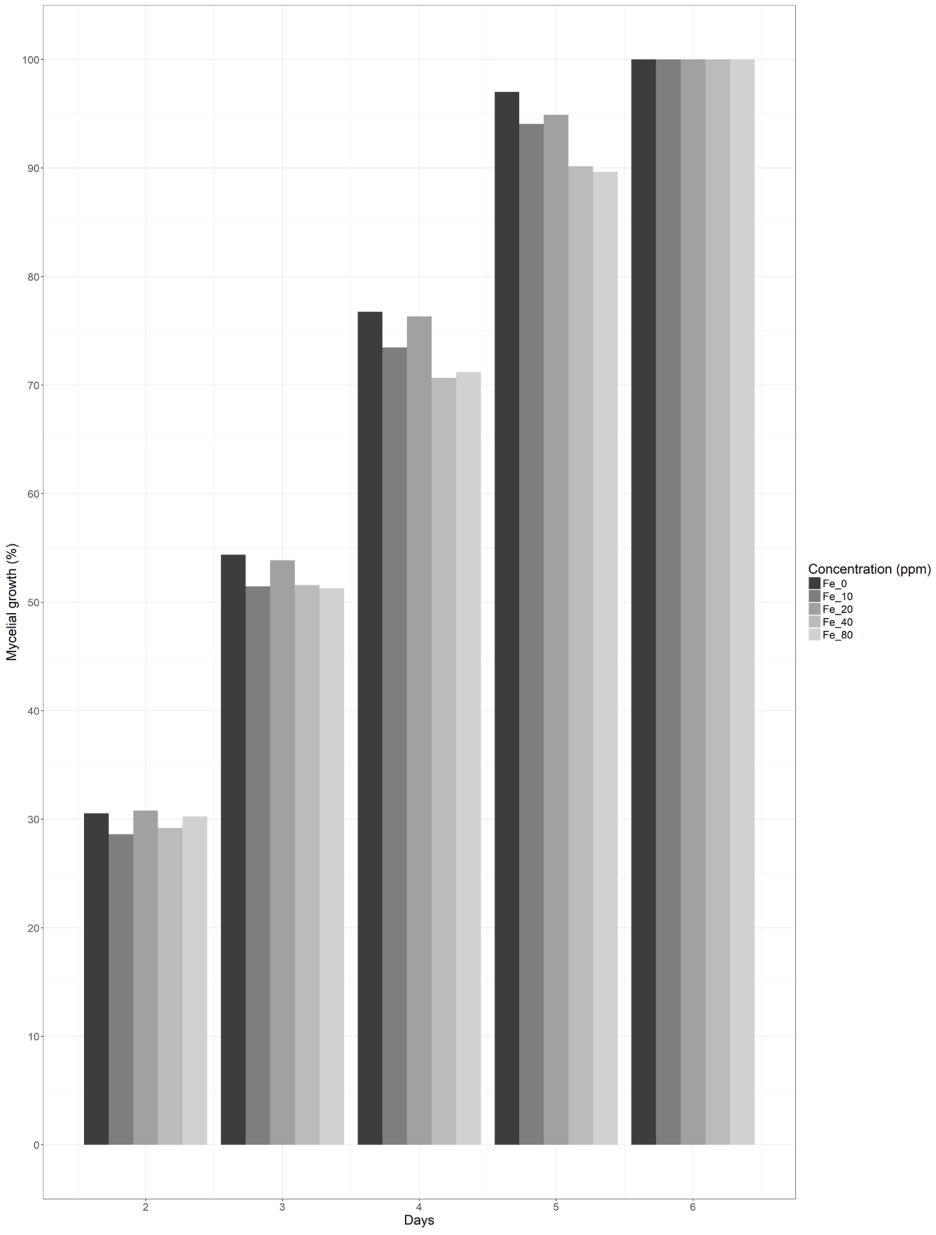

Fig. 1. Mycelial growth diameter (%) of *Lentinus sajor-caju* (PSC) after 8 days of incubation in the different concentrations of iron metal.

In contrast, *P. ostreatoroseus* had a significant increase in mycelia growth in the high levels of iron metal concentration, control had a mycelia growth of 92.28% in 6 days. While the concentration of 80 ppm of iron had a mycelia growth of 99.12% in the same time (Fig. 2).

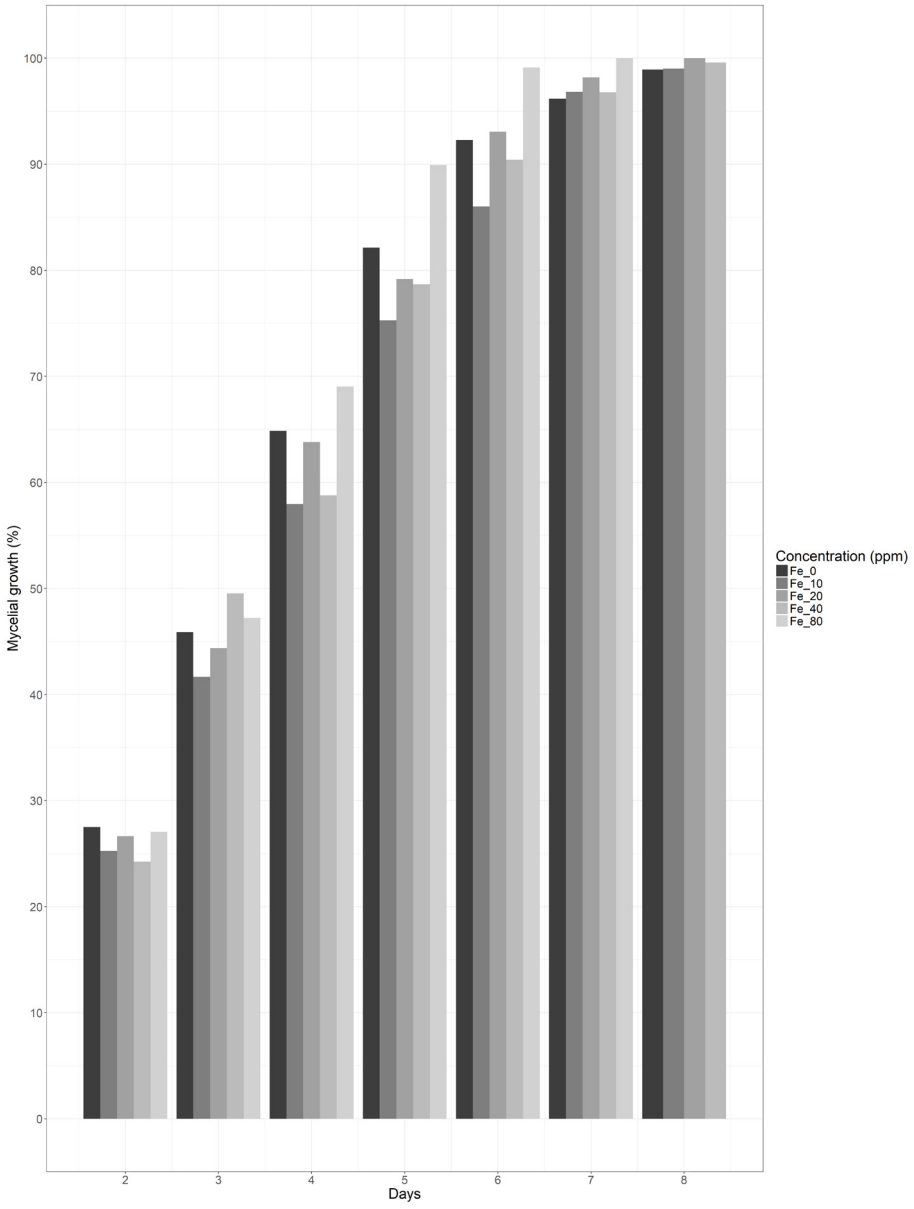

Fig. 2. Mycelial growth diameter (%) of *Pleurotus ostreatoroseus* (POR) after 8 days of incubation in the different concentrations of iron metal.

These different rates of mycelial growth on iron is in congruence to the previous findings of Zafar et al. (2007) and Vaseem et al. (2017) who reported fungus tolerance to iron depends on metal concentration and its capacity to adapt.

Fungi particularly mushrooms have powerful degrading capabilities. They have extracellular degrading system which enables them to degrade compounds that are not easily taken up by their cells such as lignin and many hazardous environmental pollutants like heavy metals (Dulay, 2015). The Tukey's test results indicate significant interactions ($p < 0.05$) between the lineages and iron concentrations for the dry mycelial mass variable (Tab. 2).

Tab. 2. Mean dry biomass of fungi (g) of *Pleurotus ostreatoroseus* (POR) and *Lentinus sajor-caju* (PSC) after 8 days of incubation in the different concentrations of iron metal.

Species	Concentration (ppm)				
	0	10	20	40	80
PSC	0.0880aA	0.0714abA	0.0772aA	0.0446bB	0.0464bB
POR	0.0760abA	0.0516cA	0.0812abA	0.0610bcA	0.0882aA

* Means followed by the same letters, uppercase in rows and lowercase in columns, do not differ amongst themselves ($p \leq 0.05$) by Tukey's test.

The best results were obtained with a *P. ostreatoroseus* in the medium with 80 ppm of iron concentration. Whereas, *L. sajor-caju* mycelial mass was significantly higher in heavy metal free medium. Among the two mushrooms, *L. sajor-caju* showed significantly lower in the medium with 40 ppm and 80 ppm of iron concentration. The slow growth of fungal mycelium increases the risk of contamination by other fast growing organisms, such as bacteria and fungi (de Albuquerque et al., 2011). The *P. ostreatoroseus* specie, despite show slow growth in medium containing different iron concentrations, but in relationship to *L. sajor-caju* was able to produce the largest mycelial dry mass, suggesting that biomass produced by this mushroom is not directly related to colony diameter.

Lonergan et al. (1993), comparing *Phanerochaete crysosporium* Burdsall strains, not observed also the relationship between these parameters on fungal growth. These differences are related to the fact that measuring the colony diameter result only the area of surface growth of the mycelium in culture medium, while measurements of biomass involved aerial mycelium, ramification and density of hyphae.

By analyzing these results, it can be suggested that *P. ostreatosroseus* could be a good option for decontamination of iron metal because this macrofungi had a significant increase in mycelia growth in the high levels. In addition, fungal biomass production was observed visibly, being less vigorous in PDA culture medium containing higher concentrations of iron, and more vigorous and agglomerated in control.

In the present study, the varying quantities of iron metal taken up by the mycelium could be due to the role that metal plays in fungal metabolism. In every living organism, high amounts of any heavy metals may cause toxic effects. In human, these include damaged to vital organs and reduced mental and central nervous function. Thus, collection of mushrooms for food from the wild particularly in areas with heavy metal contamination is unsafe and highly not recommended for human consumption (Dulay, 2015).

Thus, the ability of P. *ostreatosroseus* to tolerate metal (Fe), compared to *L. sajor-caju*, strongly dictates its ecological importance and environmental impact. This basidiomycete can be used as mycoremediation agent to treat or rehabilitate areas contaminated. However, understanding the mechanisms of accumulation is highly considered in the future studies.

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