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Mercury resistance and bioremediation mediated by endophytic fungi

William Pietro-Souza[a], Felipe de Campos Pereira[b], Ivani Souza Mello[c], Fernando Ferrari Frutuoso Stachack[d], Ailton Jose Terezo[d], Cátia Nunes da Cunha[b], James Francis White[e], Haiyan Li[f], and Marcos Antônio Soares[b*]

[a] Federal Institute of Education, Science and Technology of Mato Grosso, Lucas do Rio Verde, Mato Grosso, Brazil

[b] Department of Botany and Ecology, Laboratory of Biotechnology and Microbial Ecology, Institute of Biosciences, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil.

[c] Department of Forest Engineering, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil

[d] Fuel Analysis Centre (CEANC), Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil.

[e] Department of Plant Biology, Rutgers University, New Brunswick, New Jersey, United States of America

[f] Medical School, Kunming University of Science and Technology, Kunming, Yunnan China

*** Corresponding author**: drmasoares@gmail.com

Highlights

- *Aspergillus* sp*, C. geniculata,* Lindgomycetaceae and *Westerdykella* sp were selected
- *Curvularia geniculata* and Lindgomycetaceae are dark septate endophytes
- The four strains bioremediate Hg^{2+} with high efficiency
- The four strains promote plant growth in the presence or absence of Hg^{2+}
- The four strains improve *Aeschynomene fluminensis* and *Zea mays* tolerance to Hg^{2+}

Abstract

The present study proposes the use of endophytic fungi for mercury bioremediation in *in vitro* and host-associated systems. We examined mercury resistance in 32 strains of endophytic fungi grown in culture medium supplemented with toxic metal concentrations. The residual mercury concentrations were quantified after mycelial growth. *Aspergillus* sp. A31, *Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp. P71 were selected and further tested for mercury bioremediation and bioaccumulation *in vitro*, as well as for growth promotion of *Aeschynomene fluminensis* and *Zea mays* in the presence or absence of the metal. *Aspergillus* sp. A31, *C. geniculata* P1, Lindgomycetaceae P87 and *Westerdykella* sp. P71 removed up to 100% of mercury from the culture medium in a species-dependent manner and they promoted *A. fluminensis* and *Z. mays* growth in substrates containing mercury or not (Dunnett's test, $p<0.05$). Lindgomycetaceae P87 and *C. geniculata* P1 are dark septate endophytic fungi that endophytically colonize root cells of their host plants. The increase of host biomass correlated with the reduction of soil mercury concentration due to the metal bioaccumulation in host tissues and its possible volatilization. The soil mercury concentration was decreased by 7.69% and 57.14% in *A. fluminensis* plants inoculated with Lindgomycetaceae P87+*Aspergillus* sp. A31 and Lindgomycetaceae P87, respectively (Dunnet's test, $p <0.05$). The resistance mechanisms of mercury volatilization and bioaccumulation in plant tissues mediated by these endophytic fungi can contribute to bioremediation programs. The biochemical and genetic mechanisms involved in bioaccumulation and volatilization need to be elucidated in the future.

Keywords: Mercury; Phytoextraction; *Aspergillus*; *Westerdykella*; Lindgomycetaceae; *Curvularia geniculata*

1. Introduction

Mercury is a toxic metal with unknown biological function that has high mobility and persistence in soil, bioaccumulates and biomagnifies through the food chain, and poses direct risk to human and animal health (Cozzolino et al., 2016; Dash and Das, 2012; Lopez et al., 2014). Human activities, such as illegal gold mining that indiscriminately uses mercury in the process of gold extraction, have contributed to the emission and accumulation of this metal in different ecosystems. Some wetlands in the Brazilian Pantanal have high soil mercury concentration due to the history of gold mining at these sites (Ceccatto et al., 2016; Dash and Das, 2012; Lázaro et al., 2015; Leady and Gottgens, 2001; Tiimpling et al., 1995). Mercury transformation in natural environments strongly depends on the environmental conditions and influences its toxicity and bioaccumulation potential (Clarkson, 1972). The low oxygen tension in wetland soils favors anaerobic bacterial processes of mercury methylation to form methylmercury (CH_3Hg^+), which is a highly toxic and bioaccumulative form of mercury (Ceccatto et al., 2016).

Bioremediation is a process that primarily uses microorganisms, plants or enzymes to detoxify contaminants in soils or other environments (Kurniati et al., 2014b, 2014a; Li et al., 2018; Suja et al., 2014). Endophytic microorganisms are important tools to remediate metals or assist their hosts in phytoremediation processes. Endophytic fungi resistant to different metals, including cadmium, lead, zinc, chrome, manganese and cobalt, are associated with plant species present in contaminated sites, indicating that these microorganism have metal bioremediation potential (An et al., 2015; Deng et al., 2014; Sim et al., 2016; Sun et al., 2017; Zahoor et al., 2017). Although phytoremediation is an economic and sustainable remediation method, the phytotoxicity of metals can make the sustainable use of this method unfeasible.

Plants that grow in contaminated soils often withstand unfavorable growing conditions, in part due to colonization by endophytic fungi that mitigate the toxic effects of contaminants on the host plants and consequently stimulate their growth (An et al., 2015; Deng and Cao, 2017; Khan et al., 2017a; Pietro-Souza et al., 2017; Shen et al., 2013; Zahoor et al., 2017).

Endophytes modulate morphological and physiological functions of the host plant, and improve its resistance to metals by providing different detoxification routes (Khan et al., 2017a) such as extracellular scavenging and precipitation, binding to fungi cell wall, intracellular scavenging and complexation, compartmentalization and volatilization (Fomina et al., 2005). In addition, fungi produce phytohormones, such as auxins and gibberellins, and solubilize nutrients that favor plant growth in metal-contaminated soils (Khan et al., 2017a).

High mercury levels affect all plant development stages, impair seed germination and water absorption, decrease biomass, denature proteins and inhibit photosynthesis (Patra and Sharma, 2000). In this sense, inoculation of plants with mercury-resistant endophytic fungi represents an interesting strategy to optimize the efficiency of phytoremediation of this metal. Fungi are resistant to mercury and play known roles in metal remediation *in vitro* (Chang et al., 2019; Kurniati et al., 2014b, 2014a; Urík et al., 2014). Some studies have determined that mercury biovolatilization and bioaccumulation are the main resistance mechanisms for filamentous soil fungi (Kurniati et al., 2014b, 2014a; Urík et al., 2014), yeast (Chang et al., 2019) and mycorrhizal fungi (Kodre et al., 2017).

There are few reports on mercury-resistant endophytic fungi and their functional roles in bioremediation, as well as on the phytoremediation promoted by the endophyte-host association. Inoculation of *A. fluminensis* with endophytic fungi effectively promotes host plant growth under contamination with mercury (Pietro-Souza et al., 2017). However, it is not known how endophytic fungi bioremediate mercury and how the host-endophyte association promotes mercury translocation and bioaccumulation in plant tissues. We hypothesize that

mercury-resistant endophytic fungi are capable of bioremediating mercury *in vitro*, promoting plant growth and assisting the host in the metal phytoextraction under greenhouse conditions. This study aims to: 1 - determine the degree of mercury resistance of endophytic fungi, 2 - evaluate functional traits related to plant growth promotion, 3 - quantify mercury bioremediation by fungi *in vitro*, 4 - determine the influence of the endophyte-plant association on metal bioremediation, translocation and bioaccumulation.

2. Materials and methods

2.1 Mercury resistance of fungal strains

We used mercury-resistant endophytic fungi isolated from the *Aeschynomene fluminensis* and *Polygonum acuminatum* root system (Pietro-Souza et al., 2017). The metal tolerance index determined in a previous study (Pietro-Souza et al., 2017) was used to screen for the most promising endophytic fungal strains. Thirty-two strains with tolerance index ≥ 0.9 were selected to determine the minimum concentration of mercury capable of inhibiting mycelial growth (Table S1).

The strains were activated in Sabouraud culture medium for seven days at 28 ºC. Mycelial plugs of 0.5 cm diameter were cut from fungal leading edges and inoculated in Petri dishes containing Sabouraud culture medium supplemented with 0, 300, 450, and 600 μg mL^{-1} Hg^{2+} ($HgCl_2$). The mycelial growth rate (μ) was determined daily by measuring the mycelial diameter and expressed as $mm.day^{-1}$. The values of μ and Hg^{2+} concentration were used to build a linear regression plot for each strain and the linear variation rate (*a*) was used to measure the strain resistance.

2.2. Bioremediation screening and selection of promising endophytic fungal strains

Thirty endophytic fungal strains were assessed for their capacity to promote mercury bioremediation. The strains were activated in potato dextrose agar (PDA) medium, in Petri dishes, for seven days at room temperature. Ten mycelial plugs (0.5 cm diameter) from leading edges of each fungal strain were inoculated in 50 mL of PDA broth in 125-mL Erlenmeyer flasks and incubated for seven days at room temperature (28 ºC), under shaking (100 rpm). Sterile culture medium was used as non-inoculated control. Next, the cultures were centrifuged (10,000 rpm, 5 min) under aseptic conditions and the residual Hg^{2+} concentration in the supernatant was directly determined by inductively-coupled plasma atomic emission spectrometry. The percentage of bioremediation promoted by each strain was determined in relation to the Hg^{2+} concentration in the supernatant of the control group.

2.3. In vitro assay of mercury bioremediation and bioaccumulation

Aspergillus sp. A31, *Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp1 P71 were activated in PDA medium supplemented with 10 µg mL^{-1} Hg^{2+}, in Petri dishes, for seven days at room temperature (28 ºC). Ten mycelial plugs (0.5 cm diameter) from leading edges of each fungal strain were inoculated in 50 mL of PDA broth without Hg^{2+} in 125 mL Erlenmeyer flasks and incubated for seven days at 28 ºC. The cultures were centrifuged (1000 rpm, 5 min) under aseptic conditions. The resulting mycelia was suspended in 50 mL of PDA broth supplemented with two Hg^{2+} concentrations (30 and 90 µg mL^{-1}) and incubated for 7 and 14 days at room temperature (28 ºC), under shaking (100 rpm). The cultures were centrifuged again under aseptic conditions, and a 5 mL aliquot of the supernatant was withdrawn for the toxicity assay; the remaining supernatant was frozen to

Hg^{2+} quantification, and mycelia were dried until constant weight in an oven at 65 ºC to determine the mercury bioaccumulation capacity. The assays were repeated three times for each treatment: four endophytic fungi (*Aspergillus* sp. A31, *Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp1 P71), two Hg^{2+} concentrations (30 and 90 µg mL^{-1}) and two cultivation times (14 and 30 days). The metal tolerance index of the four strains to each Hg^{2+} concentration was determined by calculating the mycelium dry weight ratio between treated and untreated samples. Values of tolerance index equal to, lower than, and greater than 0 indicate inhibition, sensitivity, and resistance to mercury (Soares et al., 2016). Mycelial Hg^{2+} bioaccumulation was calculated as reported by Deng et al., 2014.

2.4. Toxicity to lettuce seeds

The toxicity assay was performed using lettuce seeds (*Lactuca sativa* L) (Bagur-gonzález et al., 2010; US.EPA, 1996). To examine the toxicity of compounds that were naturally produced by the endophytic fungi, the microorganisms were cultured in Hg^{2+}–free medium. It means that the samples whose toxicity were tested were: (i) supernatants of cultures of *Aspergillus* sp. A31, *C. geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp. P71 grown for 7 and 14 days in the absence or presence of 30 and 90 µg mL^{-1} Hg^{2+}; and (ii) non-inoculated culture medium without Hg^{2+} (-Endophytes-Hg) and culture medium supplemented with 30 or 90 µg mL^{-1} Hg^{2+} (-Endophytes+Hg) as controls.

The 5 mL of the supernatant collected in the *in vitro* bioremediation assay were added to 15 *L. sativa* seeds placed on filter paper in Petri dishes. The samples were assayed in triplicate. The dishes were incubated for 120 h in a seed germination chamber with 16/8 h light/dark photoperiod. The germination index (GE) and radicle length index (RL) were

determined using equations 1 and 2, respectively, as recommended by the OPPTS 850.4200 (US.EPA, 1996):

$$GE = (Germination_{sample\ (i)} - Germination_{control}) / Germination_{control} \quad (1)$$

$$RL = (Length_{sample\ (i)} - Length_{control}) / Length_{control} \quad (2)$$

The GE and RL values were used to determine the toxicity level: weak (0 to -0.25), moderate (-0.25 to -0.5), strong (-0.5 to -0.75), and very strong (-0.75 to -1). RL > 0 indicate that the sample stimulates radicle growth (Bagur-González et al., 2010).

2.5. Production of indoleacetic acid and siderophores and solubilization of phosphates

The ability of the endophytic fungal strains to produce indoleacetic acid (IAA) and siderophore and solubilize phosphate was examined *in vitro*. The isolates were incubated in PDA broth (50 mL) supplemented with tryptophan (2.5 mg mL^{-1}; pH 6.0) in 125-mL Erlenmeyer flasks for seven days, under shaking (100 rpm), at 28 ºC, in the dark. Sterile culture medium was used as control.

IAA production was determined using the Salkowski reagent (Nassar et al., 2005). To determine siderophore release, culture supernatants (1 mL) were mixed with equal volume of chrome azurol S solution (Cattelan, 1999). The siderophore units produced were calculated as reported by Giovanella et al. (2017).

2.6. Microscopy analysis of plant roots inoculated with *C. geniculata* P1 and Lindgomycetaceae P87

The presence of characteristic structures of dark septate endophytes (DSE) such as brown septate hyphae and microsclerotia in the root cortex (Jumpponen and Trappe, 1998) of

A. fluminensis and *Z. mays* inoculated with the dark endophytes *C. geniculata* P1 and Lindgomycetaceae P87 was analyzed using the method of Phillips and Hayman (1970), with modifications. Briefly, root fragments (1 cm) were clarified with 10% KOH for 15 min at 121 ºC in an autoclave, and with 1% HCl for 5 min at 60 °C. The cleared roots were stained with 0.1% trypan blue, fixed with polyvinyl alcohol in lactoglycerol in microscope slides, and visualized using a light microscope.

2.7. Mercury phytoremediation by *A. fluminensis* and *Z. mays* assisted by endophytic fungi

The fungal strains were activated in PDA medium and further inoculated in *A. fluminensis* and *Z. mays*. The *A. fluminensis* seeds were mechanically scarified and surface disinfested by soaking in 70% ethanol for 1 min and 2.5% NaOCl for 5 min, with further rinsing in sterile distilled water. The *Z. mays* seeds were disinfested using the same procedure. The *A. fluminensis* and *Z. mays* seeds were germinated for 15 and 5 days, respectively, in trays containing vermiculite and sand (1:1 v/v) that were irrigated daily with tap water.

Four seedlings were transferred to pots (3 dm^3) containing 1295 g of vermiculite and sand (1:1 v/v). One mL of *Aspergillus* sp. A31 spore suspension (10^6 conidia mL^{-1}) was inoculated near the root of each seedling. To inoculate plants using the strains that did not produce spores, the roots of seedlings were surrounded by two mycelial plugs (1 cm diameter) in PDA. Only one mycelial plug of each strain was used to coinoculate the seedlings.

The *A. fluminensis* and *Z. mays* plants were cultivated for 15 and 7 days, respectively, in the absence of Hg^{2+} (acclimation period). The field capacity was 80% and the plants were fertilized weekly with Hoagland solution (Hoagland and Arnon, 1950). Thereafter, the *A. fluminensis* and *Z. mays* substrate was contaminated with four subsequent applications of $HgCl_2$ solution in 48-h intervals until reaching the total dose of 180 and 80 mg kg^{-1} of Hg^{2+},

respectively. The influence of endophytic fungi on host plant growth was examined using the same protocol, without addition of Hg^{2+} to the substrate. The nine treatments performed were summarized in Table S2.

Lindgomycetaceae P87 promoted *A. fluminensis* growth, when compared with the other fungal strains selected in our previous study (Pietro-Souza et al., 2017). The *A. fluminensis* and *Z. mays* seedlings were grown in a greenhouse and collected 50 and 35 days after transplant, respectively. Their roots were rinsed and immersed in 10 mM EDTA solution for 30 min to remove excess of mercury bound to their surfaces. The chlorophyll levels were measured using a portable chlorophyll reader (SPAD-512, Minolta) at the time of collection.

The root and shoot dry mass were determined after drying the plant material in an oven at 65 ºC until constant weight. The percentage of growth-promoting efficiency (GPE) (Equation 3) was estimated to examine to what extent inoculation with the endophytic fungal strains influenced the host plant growth, using the following equation proposed by Almoneafy et al. (2014):

$$GPE\ (\%) = [(GT - GC) / GC] \times 100 \qquad (3)$$

where GPE is the effectiveness of growth promotion, GT is the growth parameter in the group treated with endophytic fungi, and GC is the growth parameter in the control group.

2.8. Quantification of Hg^{2+} in the samples

The Hg^{2+} concentration in the culture supernatants, dry mycelia (*in vitro* bioremediation), soil samples and plant tissues (phytoremediation bioassay) were determined by inductively-coupled plasma atomic emission spectrometry. Culture supernatants were analyzed directly, but the other samples were previously digested with concentrated acidic solutions in a microwave oven (Berghof) according to the manufacturer's instructions. Soil

samples (1 g) were digested with $HCl:HNO_3$ (3:1 v/v), while mycelia, roots and shoots were digested with $HNO_3:H_2O_2$ (5:3 v/v).

The mercury translocation factor (TF) and mercury bioaccumulation factor (BF) were calculated using the Hg^{2+} concentrations detected in the soil and plant tissues according to equations 4 (Khan et al., 2017a) and 5 (Gonzalez-Mendoza et al., 2007):

$$TF = Hg^{2+} \text{ concentration in shoots } mg.Kg^{-1} / Hg^{2+} \text{ concentration in roots } mg.Kg^{-1} \quad (4)$$

$$BF = Hg^{2+} \text{ concentration in plant } mg.Kg^{-1} / Hg^{2+} \text{ concentration in soil } mg.Kg^{-1} \quad (5)$$

The amount of Hg^{2+} immobilized in the mycelium was determined considering the total amounts accumulated in the whole mycelium and present in the culture media (50 mL) contaminated with 30 and 90 $\mu g\ mL^{-1}$ Hg^{2+}, during the two culture periods (7 and 14 days).

2.9. Statistical analysis

The experiments were assayed in triplicate and the results were expressed as mean ± standard deviation. Data were analyzed using the R software v. 3.0.2 (Team R Development Core 2013). The Shapiro-Wilk and Levene tests were used to analyze data normality and homoscedasticity, to further select the type of analysis of variance (ANOVA or Kruskal-Wallis) and perform the subsequent tests, such as the Duncan, T, and Dunnett's test with confidence level of $p < 0.05$.

3. Results

3.1. Mercury tolerance and strain selection

The minimum concentration of mercury that completely inhibited mycelial growth was determined for each fungal strain selected (Table S1). Most strains (~84%) were highly tolerant to mercury and exhibited mycelial growth even when treated with the highest Hg^{2+} concentration tested (600 μg mL^{-1}) (Table S1). The strains *Aspergillus japonicus* A32, *Microsphaeropsis arundinis* A36, *Penicillium janthinellum* A56, and *Trichoderma brevicompactum* P35 did not grow when treated with 450 μg mL^{-1} Hg^{2+}.

The linear variation rate (*a*) scores estimated the mercury resistance when the resistance was directly proportional to *a*. The *a* value ranged from -0.09 (*Clonostachys rogersoniana* P62) to -8.97 (*Trichoderma brevicompactum* P35), indicating that these strains were the most tolerant and the most sensitive to mercury, respectively (Table S1).

Most endophytic fungal strains (60%) effectively bioremediated mercury *in vitro* and removed more than 80% of the metal added to culture media (Table S1). The strains *Aspergillus* sp. A31, *Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp. P71 remediated more than 97% of Hg^{2+} added to culture media. They were selected for the subsequent assays of mercury bioremediation and bioaccumulation *in vitro* and *A. fluminensis* and *Z. mays* growth promotion in the presence and absence of Hg^{2+}.

3.2. Mercury bioremediation by the endophytic fungal strains: influence of treatment period and Hg^{2+} concentration

The *Aspergillus* sp. A31 growth rate was reduced by increasing Hg^{2+} concentration and treatment period (Student's t-test, $p<0.05$) (Table 1). The *C. geniculata* P1 growth rate was reduced by increasing Hg^{2+} concentration (90 μg.mL^{-1}), in both cultivation periods (Student's t-test, $p<0.05$) (Table 1).

Table 1 Mycelial dry mass of endophytic fungal strains treated with different mercury concentrations for 7 and 14 days and the mercury bioremediation, bioaccumulation, recovery capacity, volatilization and tolerance index.

Fungal strain	Parameter	Hg^{2+} ($\mu g.mL^{-1}$)	Cultivation period (days)	
			7	14
Aspergillus sp. A31	Mycelial dry weight (g)	30	0.34 ± 0.01 aA	0.23 ± 0.02 bA
		90	0.17 ± 0.02 aB	0.13 ± 0.01 bB
	Bioremediation (%)	30	100 ± 0.00 aA	100 ± 0.00 aA
		90	95.13 ± 0.78 aB	94.64 ± 1.66 aB
	Bioaccumulation ($\mu g.g^{-1}$)	30	2026.79 ± 226.48 aB	2367.30 ± 204.57 aB
		90	14142.05± 751.50 aA	16050.09 ± 1301.24 aA
	Immobilization in mycelium (%)	30	45.79 ± 5.95 aA	35.86 ± 5.58 aA
		90	53.49 ± 6.24 aA	45.150 ± 0.74 aA
	Volatilization (%)	30	54.21 ± 5.95 aA	64.10 ± 5.6 aA
		90	41.60 ± 5.51 aB	49.50 ± 0.92 aB
	Tolerance index	30	1.07 ± 0.04 aA	0.51 ± 0.05 bA
		90	0.54 ± 0.05 aB	0.29 ± 0.02 bB
C. geniculata P1	Mycelial dry weight (g)	30	0.28 ± 0.02 aA	0.24 ± 0.03 aA
		90	0.12 ± 0.01 aB	0.16 ± 0.04 aB
	Bioremediation (%)	30	100 ± 0.00 aA	100 ± 0.00 aA
		90	87.38 ± 0.22 bB	92.48 ± 1.60 aB
	Bioaccumulation ($\mu g.g^{-1}$)	30	2653.91 ± 237.99aB	2213.20 ± 133.56 aB
		90	10583.49 ± 1914.79 aA	8607.69 ± 1169.16bA
	Immobilization in mycelium (%)	30	49.640 ± 7.02 aA	35.44 ± 3.11 aA
		90	28.17 ± 2,79 aB	30.74 ± 2.16 aA
	Volatilization (%)	30	50.260 ± 7,02 aA	64.60 ± 3.12 bA

		90	59.20 ± 2.91 aA	61.70 ± 2,82 aA
	Tolerance index	30	0.82 ± 0.06 aA	0.67 ± 0.07 bA
		90	0.35 ± 0.03 bB	0.46 ± 0.10 aB
Lindgomycetaceae P87	Mycelial dry weight (g)	30	0.14 ± 0.01 bA	0.16 ± 0.01 aA
		90	0.13 ± 0.01 aA	0.13 ± 0.02 aA
	Bioremediation (%)	30	93.71 ± 0.57 bA	98.60 ± 1.46 aA
		90	86.74 ± 0.37 aB	88.29 ± 3.27 aB
	Bioaccumulation ($\mu g.g^{-1}$)	30	2522.41 ± 322.94 aB	2779.84 ± 100.93aB
		90	8576.17 ± 567.48 aA	11977.09± 2448.33 aA
	Immobilization in mycelium (%)	30	23.340 ± 3.54 aA	29.270 ± 0.45 aA
		90	25.23 ±2.97bA	34..99 ± 3.45 aA
	Volatilization (%)	30	70.37± 5.92 aA	69.30 ± 1.59 aA
		90	61.520 ± 2.73 aB	54.31 ± 4.10 aB
	Tolerance index	30	0.38 ± 0.02 bA	0.42 ± 0.01 aA
		90	0.36 ± 0.03 aB	0.34 ± 0.05 bB
Westerdykella sp. P71	Mycelial dry weight (g)	30	0.13 ± 0.02 bB	0.30 ± 0.02 aA
		90	0.19 ± 0.01 bA	0.27 ± 0.02 aA
	Bioremediation (%)	30	85.70 ± 0.84 aB	86.10 ± 2.32 aB
		90	90.06 ± 0.70 aA	90.29 ± 0.74 aA
	Bioaccumulation ($\mu g.g^{-1}$)	30	3460.98 ± 297.88 aB	2209.14 ± 59.44 bB
		90	13467.93 ± 1113.72 aA	7472.69 ± 791.00 bA
	Immobilization in mycelium (%)	30	30.80± 5.4 bB	43.80 ± 4.2 aA
		90	57.60 ± 0.3 aA	44.40 ± 7.3aA
	Volatilization (%)	30	54.89 ± 5.97 aA	42.43 ± 6.20 aA
		90	32.47 ± 7.54 aB	45.87 ± 7.76 aA
	Tolerance index	30	0.39 ± 0.07 bB	0.92 ± 0.08 aA
		90	0.56 ± 0.04 bA	0.82 ± 0.06 aB

* Values in a column or in a row not sharing the same letter are significantly different from each other ($p<0.05$; Student's *t* test). Upper case letters indicate the comparison between the concentrations in the same column for each parameter. Lower case letters indicate the comparison between cultivation times in the same row.

Lindgomycetaceae P87 and *Westerdykella* sp. P71 were more resistant to mercury than *Aspergillus* sp. A31 and *C. geniculata* P1, since their mycelial growth rates were not affected by increasing Hg^{2+} concentration (Student's t-test, $p<0.05$). *Westerdykella* sp. P71 mycelial growth was stimulated by increasing Hg^{2+} concentration after 7 days of cultivation; this strain provided the greatest dry mass after 14 days of cultivation with both Hg^{2+} concentrations (Student's t-test, $p<0.05$) (Table 1).

Although their growth rate decreased as a function of Hg^{2+} concentration and growing period, the four endophytic fungal strains removed 86-100% of Hg^{2+} added to the culture media, indicating that they had high mercury bioremediation capacity *in vitro* (Table 1). At both growing periods, (i) *Aspergillus* sp. A31 and *C. geniculata* P1 bioremediated all the mercury from culture media supplemented with 30 μg mL^{-1} Hg^{2+}; and (ii) the increase in Hg^{2+} concentration diminished the bioremediation efficiency of *Aspergillus* sp. A31, *C. geniculata* P1, and Lindgomycetaceae P87 by nearly 5.15, 8.95, and 10.55%, respectively, but enhanced the bioremediation efficiency of *Westerdykella* sp. P71 by 5% (Student's t-test, $p<0.05$) (Table 1).

Mycelial bioaccumulation of Hg^{2+} in all the fungal strains was proportional to the Hg^{2+} concentration added to culture media (Table 1). The growing period did not influence mycelial bioaccumulation of Hg^{2+} in *Aspergillus* sp. A31 and Lindgomycetaceae P87 (Student's t-test, $p<0.05$). In contrast, mycelial bioaccumulation of Hg^{2+} in *Westerdykella* sp. P71 treated with 30 and 90 μg mL^{-1} Hg^{2+} was respectively 56% and 80% greater at the seventh day of growth (Student's t-test, $p<0.05$) (Table 1).

The mercury amount immobilized in the mycelium was determined considering the total amount of the metal added to culture media. The mercury recovery rate of the strains grown for 7 and 14 days varied from 23.3-57.6% and 29.3-45.1%, respectively (Table 1). *Westerdykella* sp. P71 treated with 90 μg mL^{-1} Hg^{2+} significantly increased mercury immobilization; this strain also reduced the percentage of volatilized Hg^{2+} during 7 days of cultivation (Student's t-test, $p<0.05$). Treatment of *Aspergillu*s sp. A31 and Lindgomycetaceae P87 with increasing concentrations of Hg^{2+} decreased the percentage of volatilized Hg^{2+}, regardless the cultivation period (Student's t-test, $p<0.05$) (Table 1).

3.3. Toxicity of culture supernatants

Analysis of the toxicity index of culture supernatants towards *L. sativa* seeds (Table S3) revealed that radicle length index presented greater variability than germination index, indicating that the plant development stages differed with respect to their sensitivity to changes in the supernatant composition.

The culture supernatants of *Aspergillus* sp. A31 and *Westerdykella* sp. P71 grown in the absence of Hg^{2+} were strongly and weakly toxic to *L. sativa* radicle growth, respectively (Table S3), while the culture supernatants of *C. geniculata* P1 and Lindgomycetaceae P87 stimulated *L. sativa* radicle growth, affording positive radicle length index values (Duncan test, $p <0.05$) (Table S3).

Culture supernatants of the four endophytic fungal strains grown for 7 and 14 days in the presence of 30 μg mL^{-1} Hg^{2+} were not toxic to *L. sativa* seed germination but affected radicle growth in different ways (Duncan test, $p <0.05$) (Table S3). *Aspergillus* sp. A31 and *Westerdykella* sp. P71 supernatants suppressed radicle growth by exerting very strong and

strong toxicity, respectively; in contrast, Lindgomycetaceae P87 and *C. geniculata* P1 supernatants – especially those collected after 7 days of growth – stimulated radicle growth.

The non-inoculated culture medium samples supplemented with the metal (-Endophytes+Hg control), incubated for 7 and 14 days, were strongly toxic to *L. sativa* and impaired seed germination and radicle growth (Duncan test, $p < 0.05$). However, 7-day culture supernatants of *Aspergillus* sp. A31, *C. geniculata* P1 and Lindgomycetaceae P87, and 14-day culture supernatant of *Westerdykella* sp. P71 were weakly toxic towards *L. sativa* seed germination, indicating that the four fungal strains mitigated mercury toxicity.

On the other hand, radicle growth was very sensitive to toxicity of culture supernatants. Compared with the very strong toxicity of the -Endophytes+Hg control, the culture supernatants of *Aspergillus* sp. A31, Lindgomycetaceae P87 and *C. geniculata* P1 mitigated mercury toxicity towards *L. sativa* seeds (Duncan test, $p < 0.05$) (Table S3).

3.4. IAA and siderophore production and phosphate solubilization

Three out of the four endophytic fungal strains analyzed – *Aspergillus* sp. A31, Lindgomycetaceae P87, and *Westerdykella* sp. P71 – released siderophores, with values ranging from 12.9 to 82.2 (U). *Aspergillus* sp. A31 and *C. geniculata* P1 solubilized phosphate *in vitro*, while only *Westerdykella* sp. P71 synthesized IAA (Table S4 and Figure S1).

3.5. Microscopy analysis of plant roots inoculated with *C. geniculata* P1 and Lindgomycetaceae P87

The endophytic fungal strains *C. geniculata P1* and *Lindgomycetaceae P87* had brown mycelium and were able to colonize the roots of *A. fluminensis* and *Z. mays*, as

demonstrated by the presence of septate hyphae and microsclerotia typical of DSE in the host plant root cortex (Figure S2).

3.6. Effect of the endophytic fungi *Aspergillus* sp. A31, *Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp. P71 on *A. fluminensis* and *Zea mays* growth in the presence and absence of mercury

A. fluminensis and *Z. mays* seedlings were inoculated with *Aspergillus sp.* A31, *C. geniculata* P1, Lindgomycetaceae P87, and *Westerdykella sp.* P71 alone or in four combinations (Table S2): (1) Lindgomycetaceae P87+*Aspergillus sp.* A31; (2) Lindgomycetaceae P87+*C. geniculata* P1; (3) Lindgomycetaceae P87+*Westerdykella sp.* P71; (4) the four strains together-All.

The chlorophyll index and shoot and root dry mass were the parameters used to analyze the growth-promoting effects of endophytic fungi and compare them with non-inoculated plants (control). Data from cultivation substrates supplemented (Table 3 and Figure 2) or not (Table 2 and Figure 1) with mercury were analyzed separately.

Table 2 Growth-promoting efficiency (GPE) and chlorophyll index in *Aeschynomene fluminensis* and *Zea mays* seedlings inoculated with endophytic fungi in the absence of mercury.

Strain	**GPE % (dry mass)**				**Chlorophyll index**	
	Shoot	**Root**	**Shoot**	**Root**		
	A. fluminensis		*Zea mays*		*A. fluminensis*	*Z. mays*
Lindgomycetaceae P87+ *Westerdykella* sp. P71	60	55	7	-23	20.50 ± 1.69 *	32.93.63 ± 0.95
C. geniculata P1	46	75	59	4	17.80 ± 1.30	30.27 ± 1.20

Lindgomycetaceae P87	40	46	10	23	22.18 ± 1.40 *	33.10 ± 0.31
Westerdykella sp. P71	31	39	25	10	19.85 ± 0.82	32.60 ± 1.47
Lindgomycetaceae P87+ *Aspergillus sp.* A31	24	40	96	12	18.02 ± 1.60	33.33 ± 0.60*
Lindgomycetaceae P87+ *C. geniculata* P1	11	39	24	29	17. 68 ± 1.40	32.90 ± 0.10
All	1	21	44	22	16.10 ± 2.28	31.12 ± 1.15
Aspergillus sp. A31	-7	7	21	-7	20.80 ± 1.60 *	32.25 ± 0.76
-Endophytes-Hg (control)	-	-	-	-	16.80 ± 1.04	32.50 ± 0.21

GPE% estimates the growth promotion indicated in Fig. 1.* Statistical difference compared to control treatment -Endophytes-Hg (Dunnett's test, $p<0.05$).

C. geniculata P1, Lindgomycetaceae P87, *Westerdykella sp.* P71 and Lindgomycetaceae P87+*Westerdykella sp.* P71 promoted *A. fluminensis* growth in the absence of Hg^{2+}, with the greatest accumulation of root and shoot dry mass (Dunnett's test; $p<0.05$) (Figure 1A): these treatments respectively augmented the root dry mass by 75, 46, 39 and 55%, and the shoot dry mass by 46, 40, 31 and 60% (Figure 1A and Table 2).

The host growth promotion correlated with the increased chlorophyll content in plants inoculated with Lindgomycetaceae P87 and Lindgomycetaceae P87+*Westerdykella sp.* P71 (Dunnett's test; $p<0.05$) (Table 2).

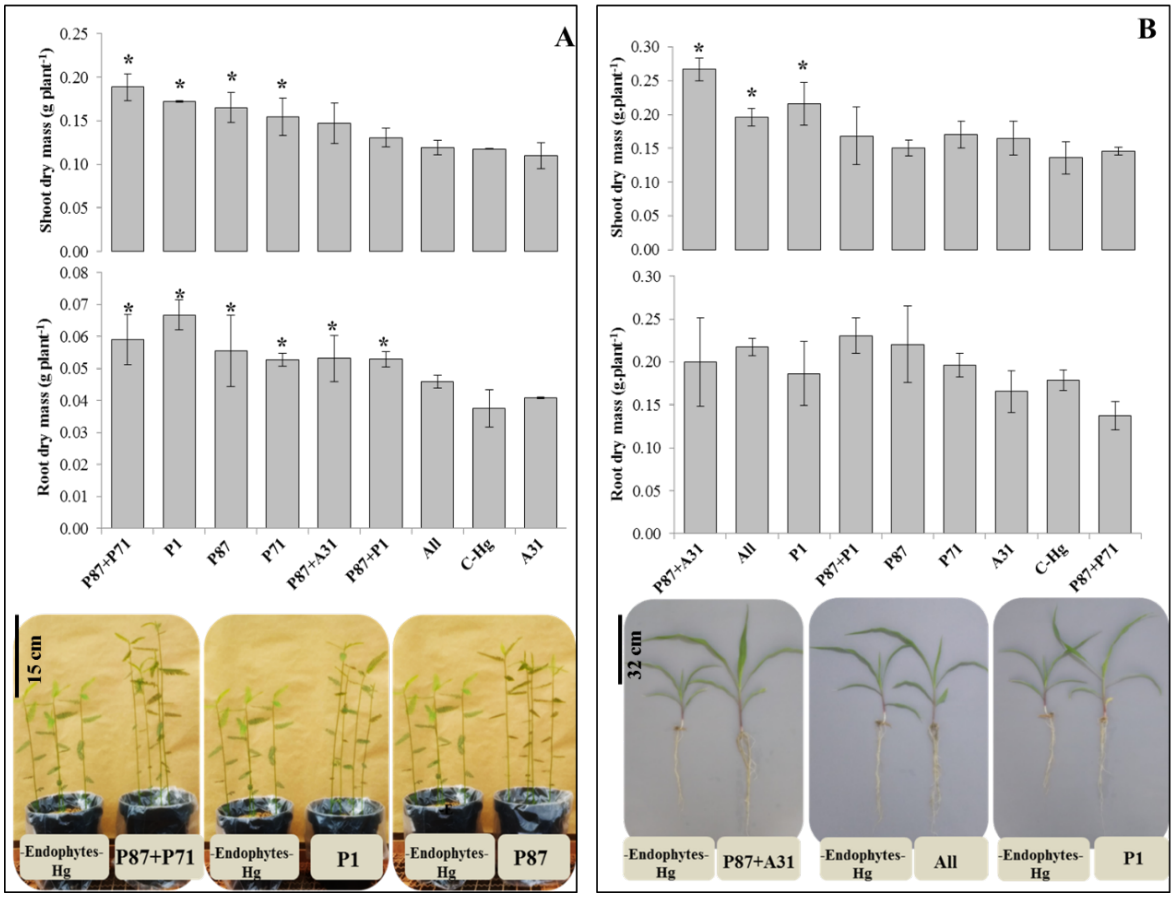

Fig. 1. Effect of endophytic fungi on *Aeschynomene fluminensis* (A) and *Zea mays* (B) growth in the absence of mercury. *A. fluminensis* and *Z. mays* seedlings were inoculated with *Aspergillus sp.* A31, *C Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella sp.* P71 alone or in four combinations: (1) Lindgomycetaceae P87+*Aspergillus sp.* A31; (2) Lindgomycetaceae P87+*C. geniculata* P1; (3) Lindgomycetaceae P87+*Westerdykella sp.* P71; (4) the four strains together (all); C-Hg = -Endophytes-Hg. * statistical difference compared to control treatment C-Hg (Dunnett's test, $p<0.05$).

Z. mays seedlings responded in a different manner to inoculation with endophytic fungi in the absence of Hg^{2+}. Inoculation with Lindgomycetaceae P87+*Aspergillus sp.* A31, *C. geniculata* P1, and the consortium of the four endophytic fungal strains significantly increased only the shoot dry mass (Dunnett's test, $p<0.05$) (Figure 1B); this parameter was augmented by 96, 59, and 44%, respectively (Figure 1B and Table 2). Inoculation with Lindgomycetaceae P87+*Aspergillus sp.* A31 improved the chlorophyll index (Dunnett's test, $p<0.05$) (Table 2).

Mercury negatively affected *A. fluminensis* and *Z. mays* growth, as evidenced by the diminished chlorophyll index and biomass accumulation (Table 3). The shoot dry mass decreased by 55% and 206% and the root dry mass decreased by 25% and 83% in *A. fluminensis* and *Z. mays* grown in the presence of mercury (Dunnett's test, $p<0.05$), respectively.

Table 3 Growth-promoting efficiency (GPE) and chlorophyll index in *Aeschynomene fluminensis* and *Zea mays* seedlings inoculated with endophytic fungi in the presence of mercury.

Strain	GPE % (dry mass)				Chlorophyll index	
	Shoot	Root	Shoot	Root		
	A. fluminensis		*Z. mays*		*A. fluminensis*	*Z. mays*
Lindgomycetaceae P87	40	43	28	52	11.99 ± 0.89*	27.28 ± 2.68
Lindgomycetaceae P87+ *Westerdykella sp.* P71	32	7	34	144	11.33 ± 0.33	28.67 ± 1.40*
Lindgomycetaceae P87+ *C. geniculata* P1	22	5	30	196	18.25 ± 0.55*	26.87 ± 2.07
Aspergillus sp. A31	10	-6	-3	67	11.63 ± 1.34	28.00 ± 1.26
Lindgomycetaceae P87+ *Aspergillus* sp. A31	3	1	2	69	11.18 ± 1.41	28.83 ± 2.97*
Westerdykella sp. P71	3	-9	52	143	12.35 ± 1.45*	29.52 ± 3.83*
C. geniculata P1	-11	-13	59	172	7.85 ± 0.55	31.33 ± 2.75*
All	-18	-17	20	133	10.73± 2.29	25.82 ± 4.45
-Endophytes -Hg	55	25	206	83	16.80 ± 1.04*	32.50 ± 0.21*
-Endophytes +Hg	-	-			8.77 ± 0.96	20.82 ± 5.70

GPE% estimates the growth promotion indicated in Fig. 2. * Statistical difference compared to control treatment -Endophytes+Hg (Dunnett's test, $p<0.05$).

Inoculation with endophytic fungi mitigated mercury toxicity and promoted growth of both host plants under such environmental stress conditions (Figure 2 and Table 3). Compared with non-inoculated *A. fluminensis* grown on mercury-containing substrate (-Endophytes+Hg): (i) inoculation of *A. fluminensis* with Lindgomycetaceae P87 significantly increased shoot and root dry mass by 40% and 43%, respectively; (ii) coinoculation with Lindgomycetaceae P87+*Westerdykella sp.* P71 and Lindgomycetaceae *P87+C. geniculata P1* stimulated *A. fluminensis* shoot growth by 32% and 22%, respectively; (iii) inoculation of *A. fluminensis* seedlings with Lindgomycetaceae P87 and *Westerdykella sp.* P71, either alone or in combination, increased chlorophyll index (Dunnett's test, $p<0.05$) (Figure 2A and Table 3).

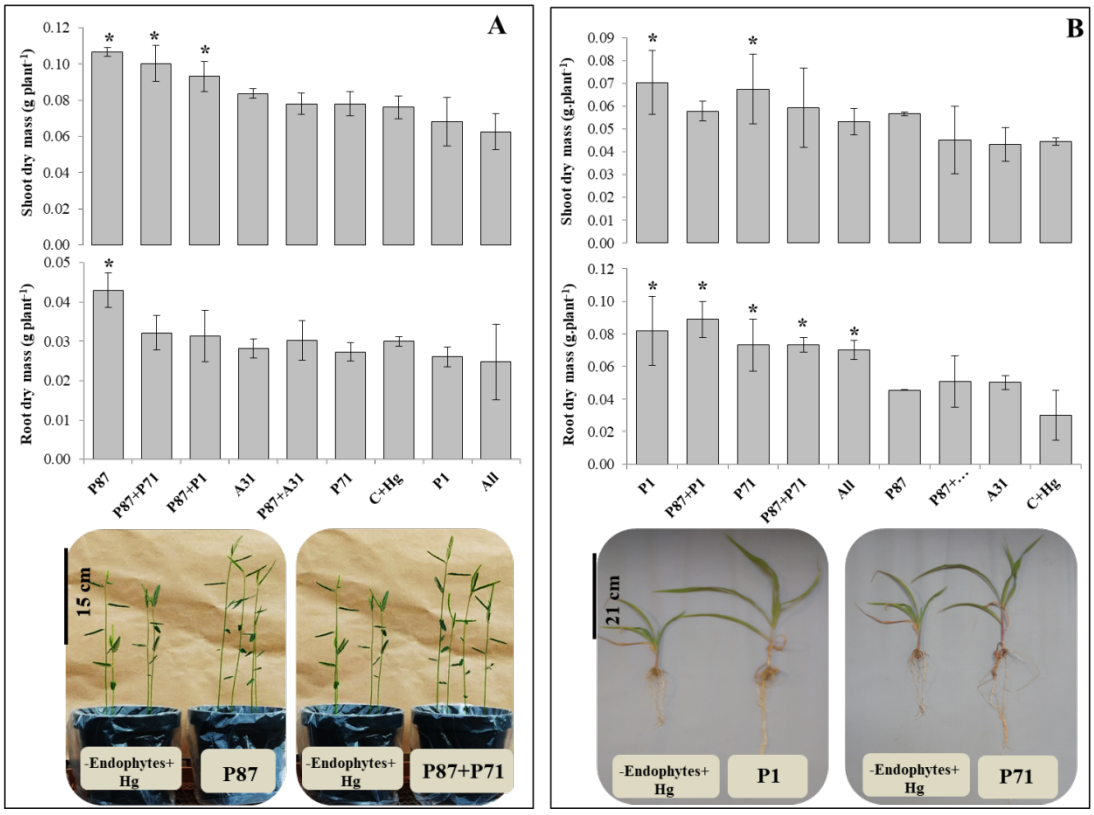

Fig. 2. Effect of endophytic fungi on *Aeschynomene fluminensis* (A) and *Zea mays* (B) growth in the presence of mercury. *A. fluminensis* and *Z. mays* seedlings were inoculated with *Aspergillus sp.* A31, *Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella sp.* P71 alone or in four combinations: (1) Lindgomycetaceae P87+*Aspergillus sp.* A31; (2) Lindgomycetaceae P87+*C. geniculata* P1; (3) Lindgomycetaceae P87+*Westerdykella sp.* P71; (4) the four strains together (all); C+Hg = -Endophytes+Hg. * statistical difference compared to control treatment C+Hg (Dunnett's test, $p<0.05$).

Inoculation of *Z. mays* with Lindgomycetaceae P87+*C. geniculata* P1, Lindgomycetaceae P87+*Westerdykella sp*. P71 and the four strains together mainly mitigated mercury toxicity to plant roots. They increased root dry mass by 196, 144, and 133%, respectively. *C. geniculata* P1 and *Westerdykella sp.* P71 augmented both the root (172 and 143%, respectively) and shoot (59 and 52%, respectively) dry mass and the chlorophyll index (Dunnett's test, $p<0.05$) (Figure 2B and Table 3). Lindgomycetaceae P87+*Westerdykella sp.* P71 also increased the chlorophyll index in *Z. mays* (Dunnett's test, $p<0.05$) (Table 3).

3.7. Effect of inoculation on mercury phytoextraction assisted by endophytic fungi

The four endophytic fungal strains selected, either alone or in combination, promoted mercury bioremediation. Compared with the control treatment (-Endophytes+Hg), all the treatments with endophytic fungi lowered the soil Hg^{2+} concentration, indicating that these microorganisms played important roles in the bioremediation process. Inoculation with Lindgomycetaceae P87+*Aspergillus* sp. A31 and Lindgomycetaceae P87 lowered soil mercury concentration the least and the most strongly – 7.69% and 57.14%, respectively –, representing the range of mercury bioremediation (Dunnet's test, $p < 0.05$) (Table 4). Such reduction in soil Hg^{2+} concentration was accompanied by the increased metal bioaccumulation factor in plant tissues (Dunnett's test, $p < 0.05$) (Table 4). Similar results were achieved in plants inoculated with *C. geniculata* P1, Lindgomycetaceae P87+*Aspergillus* sp. A31, *Aspergillus* sp. A31, *Westerdykella* sp. P71 and the consortium of the four species (Dunnet's test, $p < 0.05$) (Table 4). Plants inoculated with *C. geniculata* P1, *Westerdykella* sp. P71 and Lindgomycetaceae P87+*Aspergillus* sp. A31 exhibited greater mercury translocation factor than non-inoculated plants (-Endophytes+Hg), which favored mercury accumulation in the shoot (Dunnett's test, $p < 0.05$) (Table 4).

Table 4. Quantification, translocation factor (TF) and bioaccumulation factor (BF) of mercury in *Aeschynomene fluminensis* plants.

Treatment	Hg^{2+} (mg.kg^{-1} dry weight)				TF	BF
	Total	Root	Shoot	Soil		
C. geniculata P1	7063.01 ± 473.68*	5247.32 ± 82.59	1816.49 ± 421.84*	78.29 ± 2.25*	0.34 ± 0.08*	90 ± 8*
All	6943.28 ± 1133.66*	6414.25 ± 1075.54*	529.02 ± 64.35	50.28 ± 0.69*	0.08 ± 0.01	138 ± 21*
Lindgomycetaceae P87+*Aspergillus* sp. A31	6726.40 ± 237.12*	5541.26 ± 157.19	1185.15 ± 115.93*	83.97 ± 0.94*	0.21 ± 0.02*	80 ± 3*

Aspergillus sp. A31	6398.22 ± 1200.62	5455.62 ± 1228.78	942.60 ± 30.72*	54.03 ± 0.70*	0.18 ± 0.04	119 ± 24*
Lindgomycetaceae P87+*Westerdykella sp.* P71	6352.40 ± 279.84	5759.10 ± 211.17	593.30 ± 61.95	50.96 ± 0.90*	0.10 ± 0.01	125 ± 4*
Lindgomycetaceae P87+*C. geniculata* P1	5101.19 ± 250.34	4394.81 ± 278.98	706.34 ± 33.96	60.13 ± 0.73*	0.16 ± 0.02	85 ± 4*
Lindgomycetaceae P87	4393.23 ± 559.28	3999.13 ± 564.62	394.10 ± 5.68	39.38± 0.55*	0.10 ± 0.02	111 ± 13*
Westerdykella sp. P71	3916.29 ± 158.02	2974.37 ± 136.89*	941.92 ± 27.19*	63.02 ± 0.76.57*	0.32 ± 0.01*	62 ± 2
-Endophytes+Hg	5050 ± 204.31	4496.36 ± 205.57	554.21 ± 16.13	91.54 ± 0.83	0.12 ± 0.01	55 ± 3

* Statistical difference compared to control treatment -Endophytes+Hg (Dunnett's test, $p<0.05$).

All the treatments with endophytic fungi, either alone or in combination – except with *C. geniculata* P1 alone – in *Z. mays* plants lowered soil Hg^{2+} concentration when compared with the control treatment (-Endophytes+Hg) (Dunnett's test, $p <0.05$) (Table 5). Inoculation of *Z. mays* plants with Lindgomycetaceae P87+*C. geniculata* P1 and Lindgomycetaceae P87+*Aspergillus* sp. A31 lowered the soil Hg^{2+} concentration the least and the most strongly (14.28% and 57.14%, respectively), and increased the bioaccumulation factor. The reduced soil Hg^{2+} concentration was not associated with increased metal bioaccumulation in plants inoculated with Lindgomycetaceae P87+*C. geniculata* P1 and *C. geniculata* P1 (Dunnett's test, $p <0.05$) (Table 5).

Mercury translocation factor decreased in plants associated with endophytic fungi, indicating a preferential accumulation of the metal in their roots, except for plants inoculated with *C. geniculata* P1 (Dunnett's test, $p <0.05$) (Table 5). Compared with non-inoculated plants, *Z. mays* plants inoculated with *Aspergillus* sp. A31, *Westerdykella* sp. P71, Lindgomycetaceae P87+Westerdykella sp. P71, Lindgomycetaceae P87+*C. geniculata* P1 and the consortium of the four strains had lower accumulation of mercury in the corn shoot (Dunnett's test, $p <0.05$) (Table 5). Inoculation with *Aspergillus* sp. A31, Lindgomycetaceae

P87+*Aspergillus* sp. A31 and the consortium of the four strains increased root mercury concentration and total dry mass of *Z. mays* plants (Dunnett's test, $p < 0.05$) (Table 5).

Table 5 Quantification, translocation factor (TF) and bioaccumulation factor (BF) of mercury in *Zea mays* plants.

Treatment	Hg^{2+} (mg.kg^{-1} dry weight)				TF	BF
	Total	Root	Shoot	Soil		
Aspergillus sp. A31	3535.43 ± 228.92 *	3181.58 ± 184.01 *	353.83 ± 47.13*	10.00 ± 0.16 *	0.12 ± 0.01 *	353 ± 18 *
All	3372.25 ± 87.82 *	2982.90 ± 75.45 *	389.34 ± 33.10 *	11.70 ± 0.74	0.13 ± 0.01 *	289 ± 19 *
Lindgomycetaceae P87+*Aspergillus* sp. A31	3239.93 ± 379.80 *	2769.31 ± 438.01 *	470.62 ± 74.74	5.57 ± 0.08 *	0.17 ± 0.05 *	581 ± 62 *
Lindgomycetaceae P87	2981.06 ± 30.70	2558.32 ± 26.17	422.74 ± 26.31	10.63 ± 0.33 *	0.16 ± 0.01 *	281 ± 7 *
Westerdykella sp. P71	2446.61 ± 247.73	2149.41 ± 271.22	297.20 ± 28.17 *	7.94 ± 0.54 *	0.14 ± 0.03 *	311 ± 53 *
Lindgomycetaceae P87+*Westerdykella* sp. P71	2273.52 ± 66.96	2017.60 ± 76.78	255.92 ± 40.59 *	9.02± 0.11 *	0.13 ± 0.02 *	252 ± 5 *
C. geniculata P1	2207.01 ± 224.67	1676.56 ± 141.58	530.45 ± 85.49	14.46 ± 2.39	0.31 ± 0.03	157 ± 35
Lindgomycetaceae P87+*C. geniculata* P1	1994.03 ± 206.50 *	1688.51 ± 249.61	305.52 ± 43.36 *	11.64 ± 1.48 *	0.19 ± 0.06 *	172 ± 6
-Endophytes+Hg	2633.71± 275.22	1998.39 ± 88.06	635.31 ± 200.50	14.31 ± 0.13	0.31 ± 0.09	184 ± 21

* Statistical difference compared to control treatment -Endophytes+Hg (Dunnett's test, $p<0.05$).

4. Discussion

Metal-resistant microorganisms have been isolated from different contaminated sites (Chang et al., 2019; Oyewole et al., 2019; Pietro-Souza et al., 2017). Their ability to grow in the presence of high toxic metal concentrations indicates that they have developed mechanisms of resistance or adaptation to toxic metals. *In vitro* studies on metal resistance have described fungal species that grow in the presence of zinc, lead, chromium, arsenic and mercury concentrations as high as 2000 mg L^{-1} added to culture media (Acosta-Rodríguez et al., 2018; Gaur and Adholeya, 2004). In the present study, the highest mercury concentration

tested – 600 mg mL^{-1} – did not suppress the growth of approximately 84% of the strains selected, distributed in 24 genera of endophytic fungi.

Aspergillus sp. A31, *Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp. P71 stood out for their ability to grow in mercury-containing media (> 600 mg mL^{-1}) and to remediate the metal under *in vitro* conditions. These fungi were originally isolated from hosts collected in mercury-contaminated wetlands (Pietro-Souza et al., 2017). Some *Aspergillus* species (Acosta-Rodríguez et al., 2018; Urík et al., 2014), *Curvularia* (Soares et al., 2016) and *Lecythophora* sp., DC-F1 (Chang et al., 2019) are recognized for their resistance to mercury. There are no reports on mercury resistance of *Westerdykella* sp (FNBR_3), but it is capable of biosorbing arsenic in aqueous solution (Srivastava et al., 2012).

Our findings suggest that *Aspergillus* sp. A31, *Curvularia geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp. P71 have potential application for mercury bioremediation due to their ability to remove > 97% of mercury added to culture media. *Aspergillus niger* and *Cladosporium* isolates remediate approximately 80% of mercury by volatilization (Kurniati et al., 2014b, 2014a; Urík et al., 2014), while *Mucor hiemalis* removes more than 99% of this metal from water through adsorption and bioaccumulation (Hoque and Fritscher, 2016).

The mechanisms of fungal resistance to mercury are not completely elucidated, but some studies suggest that volatization (Urik et al. 2014; Chang et al., 2019), biosorption (Martínez-Juárez et al., 2012) and intracellular bioaccumulation mediated by P-type ATPases (Hoque and Fritscher, 2016; Solioz and Vulpe, 1996) are important mechanisms. Our findings indicate that the four fungal species remediated mercury *in vitro* via mycelial volatilization and biosorption/bioaccumulation, according to the mass balance between metal concentrations in the beginning and at the end of the growth process, the amount of mercury retained in the mycelium mass, and the percentage of volatilized mercury.

The mercury bioremediation efficiency *in vitro* and tolerance index of *Aspergillus* sp. A31, *C. geniculata* P1, and Lindgomycetaceae P87 decreased with increasing Hg^{2+} concentrations in the culture media. The metal concentration probably limits the resistance to mercury, and its toxic effect becomes evident at higher concentrations (Xiao et al., 2010; Xu et al., 2015). On the other hand, the mercury bioremediation efficiency *in vitro* and tolerance index of *Westerdykella* sp. P71 increased as a function of the metal concentration, stressing the reports that this strain uses specific resistance mechanisms that are activated by the presence and amount of mercury (Sun et al., 2017). Mycelial mercury bioaccumulation was proportional to the increment of the metal concentration, corroborating literature reports (Joshi et al., 2011; Urík et al., 2014). The distinct bioaccumulation capacity of the fungal strains may be related to the intrinsic nature of the fungi biomass, including the cell wall composition, release of compounds that interact with the metals available (Gadd, 2007), and the number of biosorption sites on the cell surface (Vullo et al., 2008). Although the highest Hg^{2+} concentration decreased the biomass and tolerance index of *Aspergillus* sp. A31 and *C. geniculata* P1, it increased the metal bioaccumulation and recovery rate. This finding suggests the existence of a low correlation between metal capture and the fungi biomass and tolerance (An et al., 2015); for instance, the decreased growth rate of *A. terreus* does not influence Pb^{2+} accumulation (Joshi et al., 2011).

Biovolatilization is an important but poorly elucidated mercury detoxification mechanism in fungi (Urík et al., 2014). We hypothesize that volatilization associated with bioaccumulation were the mechanisms by which Lindgomycetaceae P87 remediated mercury because bioaccumulation decreased but the percentage of bioremediation remained constant during the first 7 days of cultivation with the highest Hg^{2+} concentration. Independently of the bioremediation mechanism, the reduced mercury concentration in culture media correlated

with the decreased toxicity of supernatant from *Aspergillus* sp. A31, *Curvularia geniculata* P1, Lindgomycetaceae P87 and *Westerdykella* sp. P71 culture media.

Our findings indicate that endophytic fungi influenced *A. fluminensis* and *Z. mays* growth either in the presence or absence of Hg^{2+}. These results are in line with a previous report on the protective effect of *Aspergillus* sp. A31, *C. geniculata* P1, Lindgomycetaceae P87 and *Westerdykella* sp. P71 towards *A. fluminensis* grown in a mercury-containing substrate (Pietro-Souza et al., 2017). Endophytic fungi promote host plant growth through different mechanisms, including production of phytohormones, increase of nutrient availability, and protection against biotic and abiotic stress (Deng et al., 2014; Deng and Cao, 2017; Pietro-Souza et al., 2017; Soares et al., 2016; Zahoor et al., 2017). The endophytic fungal strains studied herein exhibited at least one of growth-promoting trait (IAA production, siderophore production, or phosphate solubilization) that can improve host plant tolerance to metals and growth in the absence or presence of the contaminant (Babu et al., 2014; Khan et al., 2017b; Waqas et al., 2014). IAA release by rhizobacteria promotes root growth by stimulating plant cell elongation or cell division (Gontia-Mishra et al., 2016; Jha et al., 2012). *Westerdykella* sp. P71 released the phytohormone IAA and siderophores, which were functional traits that could be associated with increased root and shoot biomass in *A. fluminensis* grown in Hg^{2+}-free substrate.

The combinations of endophytic fungi Lindgomycetaceae P87+*Westerdykella sp.* P71 and Lindgomycetaceae P87+*Aspergillus sp.* A31 maximized *A. fluminensis* and *Z. mays* plant growth in Hg^{2+}-free substrate, respectively. The endophytic consortium can be used as bioinoculant for enhancing host growth in soil subjected to stress conditions (Silambarasan et al., 2019). The simultaneous inoculation with the mycorrhizal fungus *Rhizophagus irregulares* and the saprophytic fungi *Bjerkandera adusta* and *Mortierella* sp has favored *Solanum lycopersicum* growth (Fuentes et al., 2016).

Mercury contamination directly affects all plant development and growth stages by causing abnormal germination, reducing biomass production, inhibiting photosynthesis, and impairing water absorption (Ranieri et al., 2019). The ability of endophytic fungi to promote the growth of their hosts in the presence of mercury is specific to host species. Lindgomycetaceae P87, either alone or associated with *C. geniculata* P1 or *Westerdykella* sp. P71, mitigated mercury toxicity by promoting *A. fluminensis* growth. In contrast, corn plants grew faster when inoculated with *C. geniculata* P1 and *Westerdykella* sp. P71, either alone or in combination with Lindgomycetaceae P87. Plant-endophyte interactions may be species-specific to impart essential benefits to each other (Wani et al., 2015). Metal-resistant endophytic fungi promote host plant growth in metal-contaminated soils, as reported for *Solanum nigrum* (Khan et al., 2017b, 2017a), *Brassica napus* (Shi et al., 2017), and *Z. mays* (Wang et al., 2016; Yihui et al., 2017). The endophytic fungi-host plant symbiotic association can act as a barrier against the harmful effects of metals and increase plant tolerance to this environmental contaminant (Schultz and Boyle, 2006).

Most of the root-absorbed mercury (95-99%) is not translocated and tends to remain in the organ (Bishop et al., 1998; Schwesig and Krebs, 2003). In the present study, inoculation with endophytes altered the mercury translocation factor, favoring its accumulation in the host shoots or roots. We found the predominant mercury accumulation in the shoots of *A. fluminensis* inoculated with *C. geniculata* P1, *Westerdykella* sp. P71 and Lindgomycetaceae P87+*Aspergillus* sp. A31, and in the roots of *Z. mays* inoculated with *Aspergillus* sp. A31, *Lindgomycetaceae* P87+Aspergillus sp. A31.

Aspergillus sp. A31, *C. geniculata* P1, Lindgomycetaceae P87 and *Westerdykella* sp. P71 assisted the host plant in mercury bioremediation when associated with *A. fluminensis* or *Z. mays* roots (except *C. geniculata* P1 associated with *Z. mays*). Lindgomycetaceae P87 associated with *A. fluminensis* roots, and the consortium Lindgomycetaceae P87 + *Aspergillus*

sp. A31 associated with *Z. mays* roots, reduced approximately 57% of the soil mercury concentration. Fungal hyphae also work in symbiosis with the host plant root system by amplifying absorption of metals (Göhre and Paszkowski 2006; Ren et al., 2011).

Dark septate endophytes are often associated with the host root system in soils contaminated by different metals (Diene et al., 2014; Jin et al., 2017; Li et al., 2011; Likar and Regvar, 2013; Yamaji et al., 2016; Zhang et al., 2008; Zhou et al., 2013); they increase tolerance of maize plants to lead, zinc and cadmium (Li et al., 2011). Lindgomycetaceae P87 and *C. geniculata* P1, which bear typical DSE features (Jumpponen and Trappe, 1998), promoted host plant growth in the presence or absence of mercury. Brown septate hyphae and microsclerotia present in Lindgomycetaceae P87 and *C. geniculata* P1 were morphologically similar to those that exist in DSE fungi (Siqueira et al., 2017). Plant association with these fungi favors root nutrient absorption and thereby increases host plant growth (Likar and Regvar, 2013). In the present study, Lindgomycetacea P87 associated with *A. fluminensis* and *Z. mays* decreased soil mercury concentration without increasing the metal bioaccumulation in the host tissues. As this species volatilized from 54 to 70% of mercury under *in vitro* conditions, it is possible that the metal remediation occurred via fungi-induced volatilization processes. *Lecythophora* sp. DC-F1 volatilizes mercury in culture medium, reduces soil mercury content, and decreases mercury uptake by lettuce shoots (Chang et al., 2019).

These findings indicated that the four fungal strains selected enhanced the phytoremediation potential of plants and played relevant roles on mercury hyperaccumulation and translocation by increasing the metal phytoextraction and contributing to its phytostabilization in the host plant, besides influencing volatilization of the metal.

5. Conclusion

The endophytic fungi *Aspergillus* sp. A31, *C. geniculata* P1, Lindgomycetaceae P87, and *Westerdykella* sp. P71 were promising microorganisms for mercury bioremediation in *in vitro* and host-associated systems. They promoted host growth (*A. fluminensis* and *Z. mays*) in the presence or absence of mercury, as well as assisted them in metal bioremediation by favoring its bioaccumulation and/or volatilization. The reduction of soil mercury concentration promoted by endophytic fungi, especially Lindgomycetaceae P87, may be mediated by volatilization processes. Lindgomycetaceae P87 and *C. geniculata* P1 are dark septate endophytic fungi. Further studies are required to elucidate the precise mechanisms by which endophytic fungi mediate mercury resistance and growth promotion.

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