Resistance and Activity of Phytopathogenic and Potential Antagonistic Soil Filamentous Fungi under the Action of Heavy Metals

E. V. Fedoseeva[a], *****, **Y. D. Sergeeva**[b], **V. D. Volkova**[b], **D. I. Stom**[c, d], **and V. A. Terekhova**[b]

[a] *Severtsov Istitute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 119071 Russia*
[b] *Faculty of Soil Science, Moscow State University, Moscow, 119991 Russia*
[c] *Irkutsk State University, Irkutsk, 664003 Russia*
[d] *Irkutsk National Research Technical University, Irkutsk, 664074 Russia*
*e-mail: elenfedoseeva@gmail.com

Abstract—The increase in the proportion of phytopathogenic micromycetes in soils contaminated with heavy metals (HMs) make it relevant to study the mechanisms of their resistance. It is important for choosing effective phytopathogen control methods. The issues of interaction between metal-resistant phytopathogens and nonpathogenic species against the background of pollution remain no less relevant and need to be studied. In laboratory experiments with pure cultures of four fungal species (phytopathogenic strains *Alternaria alternata* and *Fusarium oxysporum* and nonpathogenic strains *Trichoderma harzianum* and *Clonostachys rosea*), the resistance to Cu, Zn, and Pb cations, separately added to a nutrient medium with different contents of available carbon (sucrose), was studied. Colony growth, sporulation activity, and biomass accumulation were measured. The effective concentrations, resulting in the 50% inhibition of growth parameters (EC_{50}), were calculated. The fungi under study showed different resistances to HMs. T. *harzianum* and *C. rosea* are more resistant to Zn and Pb at any available carbon content. The pairs *A. alternata−C. rosea* and *T. harzianum−C. rosea* were the most resistant to Cu on media with lower and higher carbon contents, respectively. At the same time, Zn and Pb turn out to be less toxic than Cu for all fungi. The antagonistic activity, assessed by the dual culture method on media supplemented with HM cations, depends both on the growth characteristics and on the resistance to HMs that is revealed. The activity of fast-growing *T. harzianum* as a territorial antagonist is stimulated by the addition of Zn and Pb. The slow-growing *C. rosea* shows competitiveness due to its high resistance to HMs. It is concluded that it is necessary to take into account species resistance to HMs in order to predict the development of relationships between pathogenic and nonpathogenic species in fungal communities against the background of soil pollution.

Keywords: soil contamination, pathogen, antagonism, dual culture method, effective concentration, hormesis
DOI: 10.1134/S1995425523060069

INTRODUCTION

Heavy metals (HMs) can affect the development of fungi at different levels of biological organization: from the cellular to the community level. HM toxicity is due to several mechanisms of their effect on a living cell (Bagaeva et al., 2013; Singh et al., 2015). HM cations interact with various electron donating groups in many organic compounds. By combining with molecules involved in cell metabolism—proteins, nucleotides, coenzymes, phospholipids, and porphyrins—HMs affect the activity of these molecules. Increased HM accumulation causes oxidative stress and subsequent processes of increased lipid peroxidation, the depletion of glutathione and ergosterol, and changes in the activity of enzymes (dehydrogenase, catalase,

and peroxidase) (Pérez-Torres et al., 2020; Rola et al., 2022). HMs thus exert their toxic effects on most parts of the metabolic pathways of microorganisms.

The first external barrier protecting the cell from HM action is the cell wall surface, which can act as a ligand for binding metal ions and participate in their extracellular sequestration (Palanivel et al., 2023). HMs that have reached the plasma membrane can change its structure (Rola et al., 2022). The penetration of HMs into the cell can occur through specific transporters (Palanivel et al., 2023). After penetration, HMs can damage intracellular structures and macromolecules inside cells.

At the same time, fungi are characterized by high resistance and adaptability to HMs. The resistance of

fungi is due to several protective mechanisms (Bagaeva et al., 2013; Siddiquee et al., 2015; Skugoreva et al., 2019; Gajewska et al., 2022), in particular, the elimination of metals by a permeability barrier, the active transport of metals out of the cell, extracellular sequestration, enzymatic detoxification, the intracellular sequestration of metals, and a decrease in the sensitivity of cellular targets to metals.

Fungi have unique biochemical features that contribute to an increase in resistance to the toxic effects of HMs (Skugoreva et al., 2019). The cell wall contains chitin, a polysaccharide with a high sorption capacity due to HM chelation. Melanins, phenolic molecules associated with the cell wall, are another group of compounds that are produced by fungi in response to the action of HMs. Some micromycete melanins are effective copper biosorbents (Gadd and De Rome, 1988; Caesar-Tonthat et al., 1995). The functional groups of cell wall polymers are capable of ionic interaction and the formation of complex compounds with metal ions (Awofolu et al., 2006; Alluri et al., 2007). Features of fungi affect not only the structure of the cell wall, but the structure of the cell membrane and the features of intracellular metabolites. In this regard, possible universal strategies for the adaptation of fungi to adverse factors are distinguished: the accumulation of protective substances (osmolytes) in the cytoplasm and changes in the lipid composition of membranes (Danilova et al., 2020; Fedoseeva et al., 2021).

Representatives of the genera *Trichoderma, Fusarium, Aspergillus, Penicillium,* and *Alternaria* from the division Ascomycota, found throughout the soil, are some of the most resistant to HMs (Iskandar et al., 2011; Olapido et al., 2018; Torres Cruz et al., 2018; Njoku et al., 2020). Many metal-resistant fungi from the genera *Aspergillus, Fusarium, Penicillium,* and *Alternaria* are capable of exhibiting phytopathogenic properties. Among HM-resistant fungi, representatives of the genus *Trichoderma* are widespread. Trichoderma fungi are resistant to a number of agrochemicals, HMs, and organic pollutants; they are widely used in agriculture as plant growth stimulators and for the biological control of phytopathogens (Gorai et al., 2020). Under the action of HMs in the structure of fungal communities, the elimination of fungal species capable of suppressive activity occurs against the background of a shift towards the dominance of opportunistic resistant pathogenic groups, and the aggressiveness of pathogens increases (Pariaud et al., 2009; Korneikova et al., 2012). The danger posed by fungal pathogens is enhanced by the accelerated evolution of pathogens, mainly due to the use of fungicides and other human activities that contribute to the influx of toxic compounds, often containing HMs, into the habitat of microorganisms (Pandaranayaka et al., 2019).

The purpose of this work is to study the resistance to HMs of soil micromycetes differing in phytopathogenic and antagonistic properties and characterize the change in antagonistic activity in the growth medium of fungi containing HM cations. The effects of HMs (Cu, Zn, and Pb) were studied in model laboratory experiments using the example of two types of phytopathogenic micromycetes, *Alternaria alternata* and *Fusarium oxysporum,* and two nonpathogenic ones (but with potential antagonistic activity), *Trichoderma harzianum* and *Clonostachys rosea*.

MATERIALS AND METHODS

Fungal Cultures and Experiment Design

The objects of study were strains of the following species isolated from the soil: *Alternaria alternata* (Fr.) Keissl, *Fusarium oxysporum* Schltdl. *Trichoderma harzianum* Rifai 1969, and *Clonostachys rosea* (Preuss) Mussat 1901. Fungus *T. harzianum* does not synthesize melanin. Fungus *A. alternata* synthesizes 1,8-dihydroxynaphthalene (DHN) melanin-like compounds, which are mainly associated with ascomycetes (Toledo et al., 2017). Representatives of the genus *Fusarium* are capable of synthesizing less canonical melanins for ascomycetes, 5-deoxybostricoidein melanin (Toledo et al., 2017). We have previously obtained experimental confirmation of the synthesis of melanin-like pigments in *F. solani* (Fedoseeva et al., 2022).

Fungi were cultivated in the Czapek's medium with the following mineral composition (g/L): $NaNO_3$, 3.0; K_2HPO_4, 1.0; $MgSO_4$, 0.5; KCl, 0.5; $FeSO_4$, 0.001 (pH 5.5–6.0). Sucrose at a concentration of 3 and 20 g/L was added as a carbon source. Micromycetes were incubated at 22°C in flasks with Czapek's liquid medium or in Petri dishes containing Czapek's agar medium. In experiments on liquid nutrient media, the spore suspension was added to flasks with 100 mL of the medium up to a spore density of 10^5-10^6 U/mL; incubation was carried out on a shaker. In such cases, the fungal biomass was represented by mycelial pellets. Fungal inoculum was transferred to solid media with a bacteriological loop from test tubes with stock culture.

Heavy Metals

HM cations were used in the form of salts (copper, lead, and zinc nitrates). The use of the same anionic form made it possible to exclude the effect of different anions on the responses of micromycetes. In experiments on the accumulation of biomass in liquid nutrient media, HM salts were added to the media simultaneously with the introduction of fungal inoculum. In experiments on agar media, HM salts were added to the media after autoclaving and lowering the temperature. The multiple dilution method was used to create a range of concentrations of Cu, Pb, and Zn cations of 0.001–1 g/L. A wide range of concentrations made it possible to obtain both the effect of stimulation and the effect of inhibition of the growth rates of micromycetes. When choosing metal cations and test salt con-

centrations, we were guided by publications on the resistance of fungi to HMs (Iskandar et al., 2011; Mohammadian et al., 2017; Torres-Cruz et al., 2018). A nutrient medium without HMs served as control.

Estimation of Growth Rates of Fungal Cultures

Fungal endpoints were measured: mycelial biomass, fungal colony diameter, and conidial productivity. The kinetic indicators of the growth rate of colonies were evaluated in Petri dishes by the change in the diameter (radius) of the colonies, which was fixed with a ruler. When analyzing the structural and functional organization of mycobiota in natural environments, the express method for assessing the diversity of microbial communities by the radial growth rate is widely used (Netrusov et al., 2012). Based on this indicator, it is proposed to group the microorganisms isolated during the inoculation of samples on nutrient media into different classes with a certain range of values of the radial growth rate coefficient (Kr) and kinetic types (Polyanskaya et al., 1988). In this work, the studied species of fungi were ranked according to the growth rate in the control (without the introduction of HMs) and with the introduction of HM options. The growth rate was determined during the period of linear growth of colonies in Petri dishes with agar medium, measuring the diameter of the colony every 24–48 h. The growth rate (GR) was calculated using the formula

$$GR = R/\Delta t,$$

where R is the colony radius, mm; Δt is the duration of cultivation, h.

Mycelial biomass after cultivation on a liquid medium was collected by filtration on ashless paper filters. The mycelium was dried at 103°C to constant weight and weighed.

The intensity of sporulation was calculated from the conidial productivity per mm^2. To do this, three equal sections of the agar medium with spore-bearing mycelium were cut out from the center to the edge of the colony with a microbiological drill. The sections were placed in 5 mL of distilled water with a small addition of detergent (Tvin-80), shaken, and then the spore suspension was filtered through a nylon sieve. The intensity of sporulation was calculated using a Goryaev camera according to published recommendations (Sanin et al., 2008).

The effective concentrations causing 50% (EC_{50}) inhibition of colony growth on agar medium (on the 2nd, 4th, 6th, and 7th days of growth for *T. harzianum*, *F. oxysporum*, *A. alternata* and *C. rosea*, respectively), as well as the inhibition of biomass accumulation during growth in a liquid medium (3–4 days of growth for all cultures), were calculated.

Estimation of Antagonistic Properties by the Dual Culture Method

The antagonistic activity of fungi potentially possessing suppressive activity against phytopathogens was assessed by the dual culture method (Matarese et al., 2012). Disks cut from the edge of an actively growing antagonist and pathogen colony were placed in a Petri dish with an agar nutrient medium. The radius of the pathogen colony was taken into account, both facing towards the antagonist colony (Ra) and in the perpendicular (control) direction (Rc). To assess the antagonistic activity of fungi, the growth of colonies was evaluated on media with a reduced concentration of sucrose.

Statistical Processing

The experiments were carried out in 3–5 repetitions with the calculation of the arithmetic mean and standard deviations; the error bars were indicated on the graphs. The significance of differences between variants was determined using the one-way analysis of variance (ANOVA) and pairwise multiple comparison (Tukey's test). To calculate the EC, a bit analysis was used. All calculations were carried out in the statistical programs R, Excel, and ExcelStat.

RESULTS

Kinetic Types of Fungi

Based on the results of kinetic changes in the diameter of colonies on nutrient media without the addition of HMs, the fungal species were ranked by growth rate (Table 1). The growth rate of *T. harzianum* was in the range of 0.78 ± 0.03–0.89 ± 0.07 mm/h, which made it possible to attribute this strain to the kinetic type—fast growing with $Kr \geq 12$. The growth rate of *C. rosea* was in the range of 0.18 ± 0.03–0.22 ± 0.02 mm/h, which characterized the fungus as slow growing with $Kr = 4$. The growth rate of *A. alternata* was 0.38 ± 0.02–0.39 ± 0.03 mm/h, so this species was assigned to the kinetic type: fast growing with $Kr = 8$. Finally, *F. oxysporum*, whose growth rate 0.58 ± 0.01–0.61 ± 0.02 mm/h, was qualified as fast growing with $Kr = 10$–11. According to the decrease in the growth rate, the studied micromycetes can be arranged in the following order: *T. harzianum* > *F. oxysporum* > *A. alternata* > *C. rosea*. Different concentrations of sucrose (3 and 20 g/L) did not affect the described kinetic types of the studied micromycetes.

Effect of HMs on Colony Growth

HMs, when introduced into the nutrient medium, mainly inhibited the colony growth, reducing the growth rate of fungi (Table 1). The stimulating effect (hormesis) was observed at low concentrations of Zn and Pb, which provoked an increase in the growth growth rate of *T. harzianum*. Zinc at the lowest concentration increased the growth rate of *C. rosea*. An

Table 1. Growth rate of micromycetes in the control and samples with the introduction of HMs, mm/h*

View	Sucrose (g/L)	T. harzianum		C. rosea		A. alternata		F. oxysporum	
		3	20	3	20	3	20	3	20
Control	–	0.78 ± 0.03ab**	0.89 ± 0.07c	0.22 ± 0.02a	0.18 ± 0.03bc	0.39 ± 0.03a	0.38 ± 0.02a	0.58 ± 0.01a	0.61 ± 0.02a
Type		Fast growing, $Kr \geq 12$		Slow growing, $Kr = 4$		Fast growing, $Kr = 8$		Fast growing, $Kr = 10$–11	
Pb, g/L	0.5	0.59 ± 0.02bc	0.88 ± 0.06c	0.16 ± 0.01b	0.15 ± 0.02bc	0.10 ± 0.005d	0.13 ± 0.02bc	0.15 ± 0.01cd	0.25 ± 0.02c
	0.05	0.80 ± 0.007a	1.21 ± 0.02abc	0.21 ± 0.01a	0.19 ± 0.01bc	0.36 ± 0.03a	0.34 ± 0.01a	0.57 ± 0.008a	0.54 ± 0.03a
	0.01	0.84 ± 0.008a	1.37 ± 0.16a	0.22 ± 0.01a	0.18 ± 0.02bc	0.39 ± 0.003a	0.34 ± 0.03a	0.56 ± 0.02a	0.56 ± 0.03a
Zn, g/L	0.5	0.16 ± 0.03d	0.31 ± 0.04de	0.10 ± 0.005c	0.19 ± 0.02bc	0.06 ± 0.004d	0.03 ± 0.03d	0.20 ± 0.01c	0.34 ± 0.02b
	0.05	0.71 ± 0.05ab	1.23 ± 0.03ab	0.21 ± 0.01a	0.21 ± 0.02b	0.28 ± 0.02b	0.21 ± 0.07b	0.53 ± 0.01a	0.52 ± 0.05a
	0.01	0.86 ± 0.02a	1.32 ± 0.13a	0.21 ± 0.03ab	0.28 ± 0.04a	0.30 ± 0.07b	0.31 ± 0.05a	0.54 ± 0.008a	0.50 ± 0.04a
Cu, g/L	0.05	0.15 ± 0.02d	0.08 ± 0.02e	0.05 ± 0.01d	0.014 ± 0.003d	0.11 ± 0.01cd	0.04 ± 0.02d	0e	0c
	0.01	0.23 ± 0.05d	0.48 ± 0.28d	0.16 ± 0.003b	0.14 ± 0.003c	0.09 ± 0.002d	0.11 ± 0.02cd	0.14 ± 0.02d	0.15 ± 0.13c
	0.005	0.44 ± 0.12c	0.91 ± 0.08bc	0.16 ± 0.04b	0.17 ± 0.01bc	0.17 ± 0.01c	0.17 ± 0.01bc	0.32 ± 0.06b	0.32 ± 0.01b

* Highest speed in exponential growth phase.
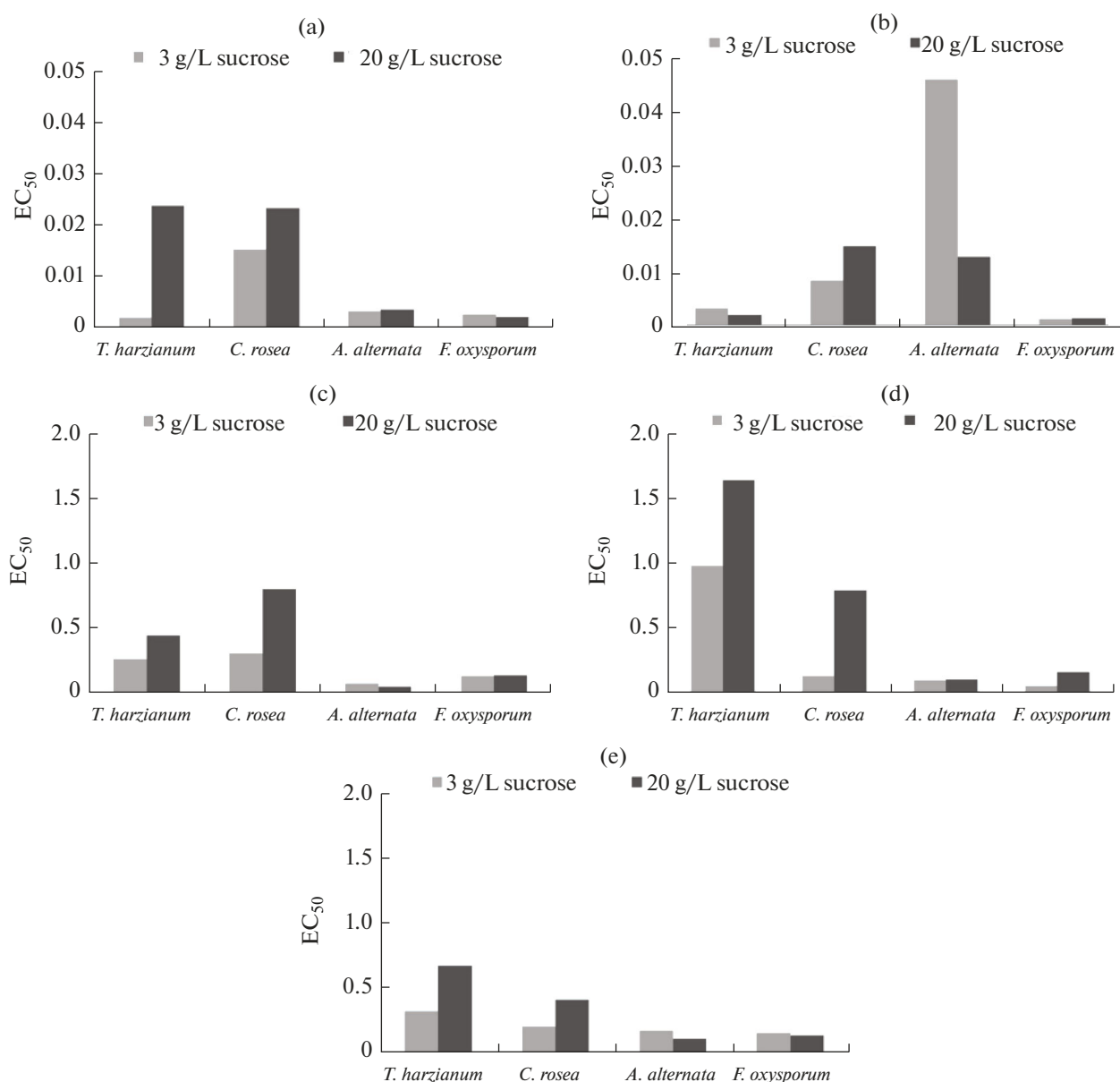** Values assigned to different letters differ significantly ($p \leq 0.05$, Tukey's test).

Fig. 1. Effective metal concentrations (EC_{50}, g/L of medium), calculated from the inhibition of colony growth ((a) copper, (c) zinc, and (e) lead) and inhibition of biomass accumulation ((b) copper and (d) zinc). The determination of lead EC was complicated by the fact that lead nitrate precipitated at the high concentrations required to create an inhibitory effect.

analysis of the calculated growth rates of fungi and effective concentrations of HMs that inhibit colony growth led to the conclusion that copper was the most toxic HM for the studied representatives of micromycetes. Within the studied group, micromycetes showed different resistance to HM exposure. The most resistant to the action of Zn and Pb were *T. harzianum* and *C. rosea*.

Different concentrations of sucrose influenced the degree of HM resistance in the studied strains. With an increase in the content of a readily available carbon source (an increase in the concentration of sucrose from 3 to 20 g/L), the resistance of *T. harzianum* and *C. rosea* to Zn and Pb practically doubled (Figs. 1c, 1e).

Copper resistance, characterized by EC_{50}, also depended on the content of sucrose in the growth medium. At 20 g/L sucrose, for *T. harzianum* and *C. rosea*, EC_{50} Cu were 0.024 and 0.023 g/L, respectively (Fig. 1a). With a decrease in the content of a readily available carbon source, the most resistant species were *C. rosea* and *A. alternata*: EC_{50} Cu 0.015 and 0.003 g/L, respectively (Fig. 1a).

Effect of HMs on Biomass Accumulation

The greatest resistance to Zn was found for *T. harzianum*: EC_{50} values of 1.16 and 1.64 g/L in media with

sucrose 3 and 20 g/L, respectively (Fig. 1d). The resistance of *C. rosea* to Zn was lower than that for *T. harzianum*: $EC_{50} = 0.79$ g/L at 20 g/L sucrose and $EC_{50} = 0.24$ g/L at 3 g/L sucrose (Fig. 1d). The resistance of *F. oxysporum* and *A. alternata* was significantly lower: EC_{50} from 0.09 to 0.15 g/L.

The highest resistance to Cu was found for *A. alternata,* EC_{50} 0.046 g/L (at 3 g/L sucrose) (Fig. 1b). Fungus *T. harzianum,* which can be attributed to the most resistant to Zn, did not show a high resistance to Cu.

Influence of HMs on the Intensity of Sporulation

For three types of micromycetes, *C. rosea, A. alternata,* and *F. oxysporum,* the introduction of HMs at concentrations that did not suppress the colony growth stimulated sporulation. The colonies of *C. rosea* produced conidia more actively when each of the three HMs was added (Figs. 2c, 2d, 3e−3h) and, those of *A. alternata,* when introducing Cu (Figs. 2e, 2f, 3i−3l); for *F. oxysporum,* it was when Zn was introduced (Figs. 2g, 2h, 3m−3p). The sporulation activity of *T. harzianum* against the background of adding HMs depended on the concentration of sucrose in the medium (Figs. 2a, 2b, 3a−3d). At 3 g/L of sucrose, Zn and Pb at high concentrations (1 and 0.5 g/L) inhibited conidial productivity, while Cu completely suppressed it. With an increase in sucrose to 20 g/L, Zn and Pb stimulated conidial productivity for all studied concentrations; the stimulation by Cu was noted at the lowest concentrations (0.005 g/L).

Influence of HMs on the Manifestation of the Antagonistic Activity of Fungi

On media with the addition of Zn and Pb, the activation of the growth of a fast-growing *T. harzianum* (Figs. 4b, 4c, 4f, 4g) was noted. Together with phytopathogenic *A. alternata* and *F. oxysporum,* characterized by a slower growth rate of colonies, *T. hazrianum* proved to be the most active territorial antagonist. However, on media with Cu, which inhibits the growth of *T. harzianum* to a greater extent than the growth of *A. alternata* and even *F. oxysporum*, the effect of enhancing the antagonistic properties of *T. harzianum* did not appear (Figs. 4d, 4h).

Fungus *C. rosea* manifested itself differently in relation to phytopathogenic fungi. It had a slower growth rate than *A. alternata* and *F. oxysporum*. On media with HMs, a noticeable activation of both growth and possible suppressor activity of *C. rosea* was not observed (Figs. 5a−5d). The introduction of Cu inhibited the growth of *F. oxysporum*, while *C. rosea* proved to be resistant to the action of this HM (Fig. 5h). Thus, in the presence of copper, *C. rosea* could potentially be more competitive than phytopathogenic *F. oxysporum*.

The ability to exhibit antagonistic properties (especially as territorial antagonists) depended on the growth rate of the fungus and the level of HM resistance. *T. harzianum* was the most active territorial antagonist on media with Zn and Pb against fungi with a lower growth rate (*A. alternata* and *F. oxysporum*). Slow-growing *C. rosea* could be potentially more competitive when compared to phytopathogenic fungi with a higher growth rate only due to its high resistance to HMs.

DISCUSSION

Based on the data on the effect of HMs on colony growth, sporulation activity, and biomass accumulation, it can be concluded that, for three strains, *T. harzianum, A. alternata,* and *F. oxysporum,* lead was the least toxic; for *C. rosea,* zinc was the least toxic. Copper was characterized by the highest toxicity for all studied micromycetes. An analysis of published data on the effect of HMs on different species of filamentous fungi showed that toxicity indicators vary (Table 2). Thus, the strain of *Curvularia lunata* proved to be more sensitive to the toxic effect of nickel than copper and zinc (Paraszkiewicz et al., 2009). Zinc made the lowest impact on *F. oxysporum* and *Pythium debaryanum,* while cadmium made the highest one (Golubović-Ćurguz et al., 2010). Hyphomycete strains *Heliscus lugdunensis* (*Neonectria lugdunensis*) and soil fungus *Verticillium* cf. *alboatrum* also showed higher resistance to Zn than to Cd (Jaeckel et al., 2005). The authors of (Sazanova et al., 2015) found an inhibitory effect of individual treatment with Zn (2 mM) and Cu (0.5 mM) on the growth of *Aspergillus niger* and *Penicillium citrine*. On the whole, it can be concluded that Cd and Ni are among the most toxic metals for filamentous fungi; Zn is the least toxic. Cu and Pb occupy an intermediate position.

The assessment of the toxicity of metals can be influenced by the characteristics of the development of fungi in various environments. For *A. alternata,* the EC values of zinc and copper, calculated from the inhibition of colony growth, were lower than when assessing the inhibition of biomass accumulation in a liquid medium (Figs. 1a−1d). This trend was manifested both on media with 3 and 20 g/L of sucrose. For *C. rosea* and *F. oxysporum,* EC values for colony growth inhibition were higher or equal to EC values for biomass accumulation inhibition (Figs. 1a−1d). For *T. harzianum,* no clear trend emerged. The effect in which the fungus showed greater resistance to HMs when growing on liquid media determined for *A. alternata* is consistent with other studies. Strain *C. lunata,* isolated from uncontaminated by HM soil, when grown on a solid medium (under conditions preventing the formation of a specific emulsifier), showed a significantly higher sensitivity to Cd^{2+}, Zn^{2+}, and Pb^{2+} ions than when grown on a liquid medium, when this fungus produces an extracellular emulsifier (Paraszkiewicz et al., 2009).

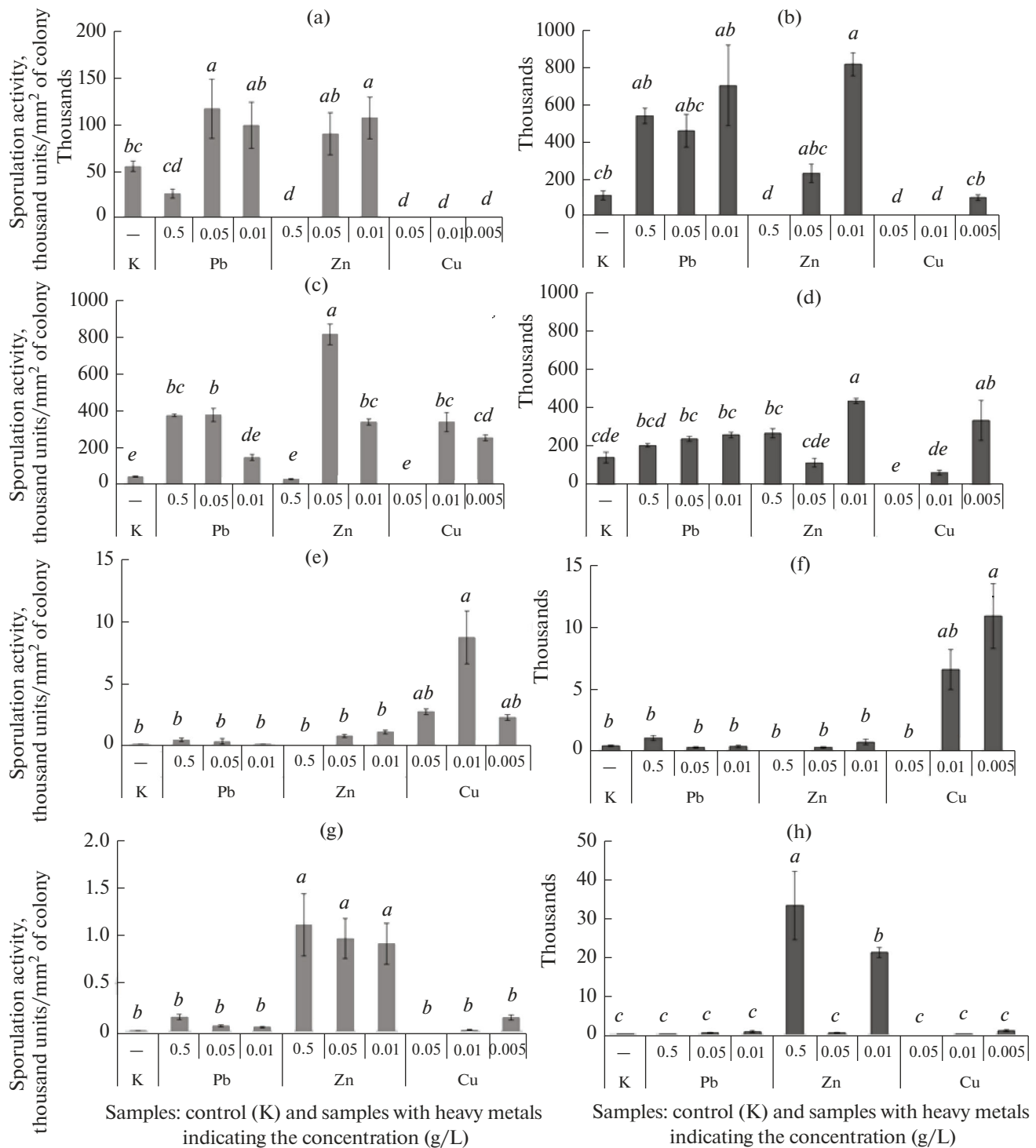The quantity of the readily available carbon source in the environment also affects the assessment of the

Fig. 2. Intensity of sporulation of micromycetes in the control (without the introduction of metals) and samples of the medium with the adding metals: at 3 g/L sucrose ((a) *T. harzianum,* (c) *C. rosea,* (e) *A. alternata,* and (g) *F. oxysporum*) and 20 g/L sucrose ((b) *T. harzianum,* (e) *C. rosea,* (f) *A. alternata,* and (h) *F. oxysporum*). Note: * Values assigned to different letters differ significantly ($p \leq 0.05$, Tukey's test).

toxicity of metals in the laboratory. During the experiments, there was a tendency for *T. harzianum* and *C. rosea* to increase resistance to the action of HMs with an increase in the content of a readily available carbon source (an increase in the concentration of sucrose up to 20 g/L) (Figs. 1a−1e). When additional sucrose was added, the resistance of the phytopathogenic fungus increased, *F. oxysporum* manifested
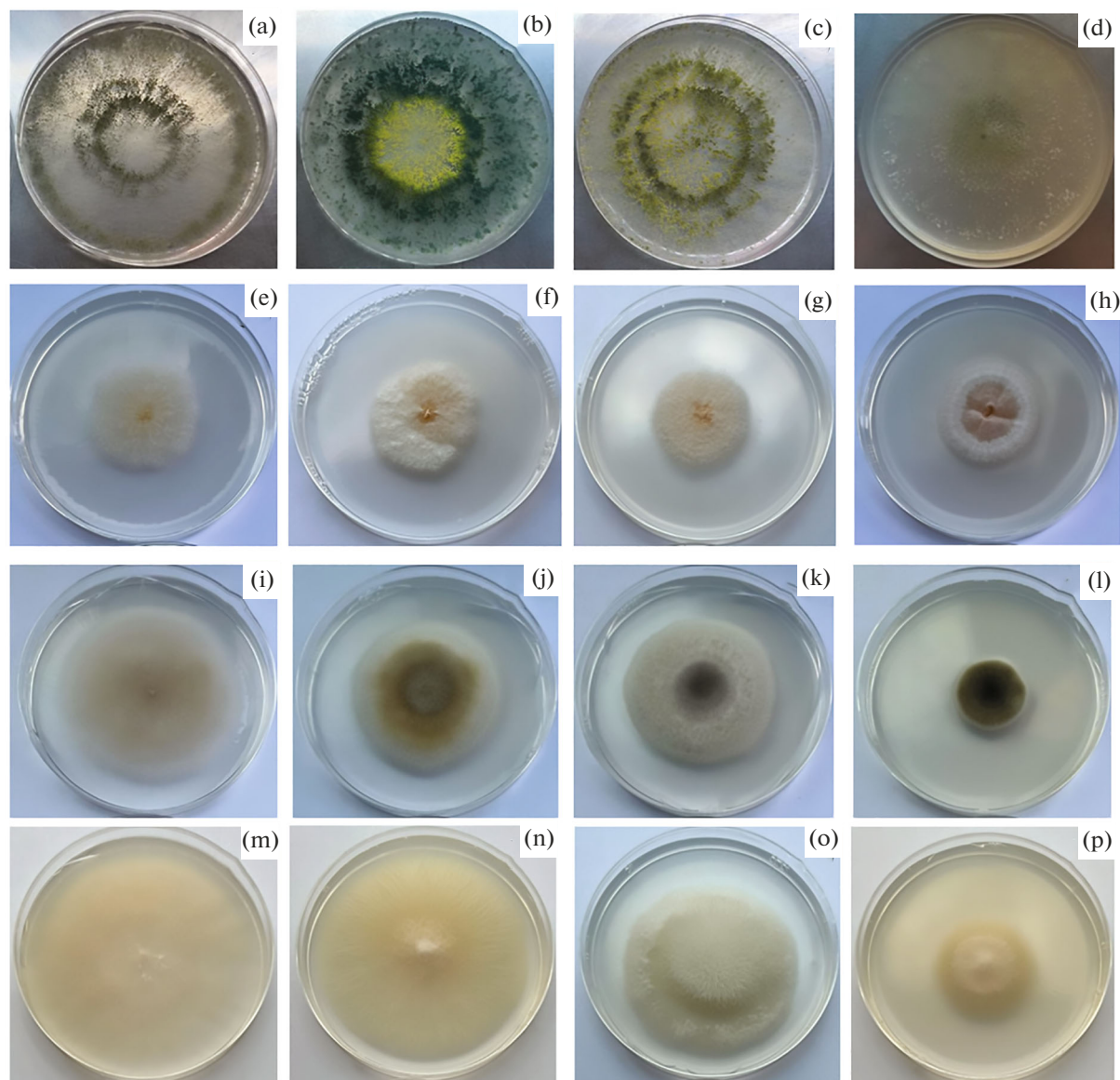
Fig. 3. Appearance of micromycete colonies in the control (without the metals) and on media with the adding of metals: *T. harzianum* ((a) control, (b) 0.01 g/L Pb, (c) 0.01 g/L Zn, and (d) 0.005 g/L Cu); *C. rosea* ((e) control, (f) 0.01 g/L Pb, (g) 0.01 g/L Zn, and (h) 0.005 g/L Cu); *A. alternata* ((i) control, (j) 0.01 g/L Pb, (k) 0.01 g/L Zn, and (l) 0.005 g/L Cu); and *F. oxysporum* ((m) control, (n) 0.01 g/L Pb, (o) 0.01 g/L Zn, and (p) 0.005 g/L Cu).

itself to a lesser extent, and the phytopathogenic fungus *A. alternata* generally showed less resistance (Figs. 1a–1e). The sporulation activity of *T. harzianum* against the background of the addition of HMs depended on the concentration of sucrose in the medium and was more intense on media with a high content of sucrose (Figs. 2 a, 2b, 3a–3c).

The reactions of micromycetes to the presence of HMs reflect the strategies of adaptation to unfavorable environmental factors (fungal life style). Fungus *T. harzianum*, characterized by a high growth rate, the ability to form extensive colonies, and active sporulation—both under optimal conditions and in the presence of HMs—was more resistant to the action of Zn and Pb at a higher content of available carbon in the medium. These characteristics make it possible to classify the fungus *T. harzianum* as an r-strategist. Phytopathogenic melanized fungus *A. alternata*, on the contrary, was more resistant on media with a reduced content of available carbon and showed a pronounced resistance to the action of Cu, which activated the sporulation of the fungus. The high stability of *A. alternata* to the action of Cu can also be associated with the presence of melanin, which is an effective copper biosorbent (Gadd and De Rome, 1988; Rizzo et al., 1992; Caesar-Tonthat et al., 1995). Slow
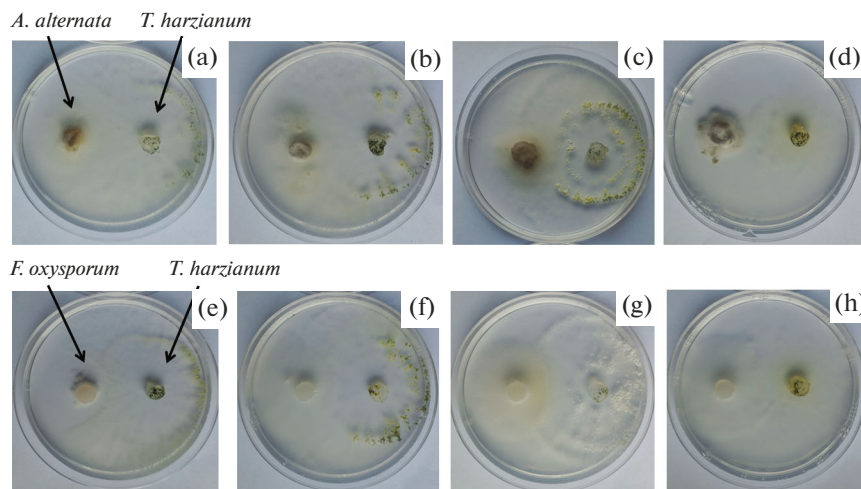
Fig. 4. Fixation of antagonistic properties of *T. harzianum* paired with *A. alternata*: (a) on Czapek's medium (control), (b) Pb 0.01 g/L, (c) Zn 0.01 g/L, and (d) Cu 0.01 g/L. Paired with *F. oxysporum* (e) on Czapek's medium (control), (f) Pb 0.01 g/L, (g) Zn 0.01 g/L, and (h) Cu 0.005 g/L.
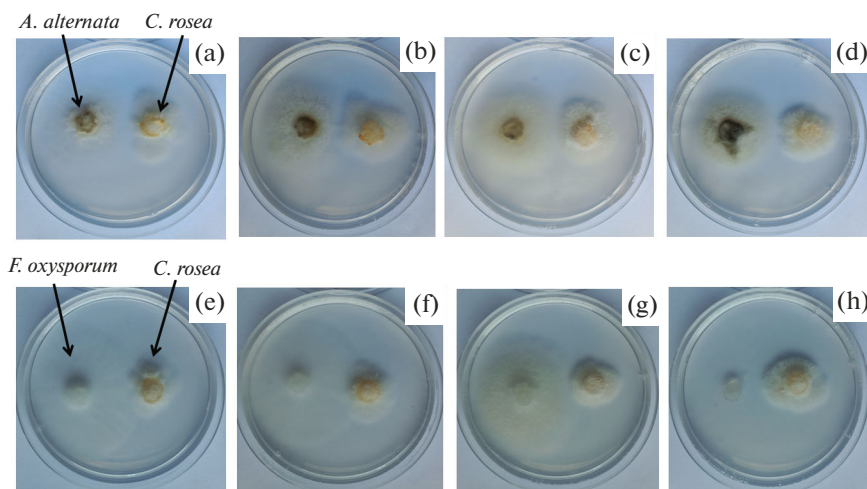

Fig. 5. Fixation of antagonistic properties of *C. rosea* paired with *A. alternata* (a) on Czapek's medium (control), (b) Pb 0.01 g/L, (c) Zn 0.05 g/L, and (d) Cu 0.005 g/L. Paired with *F. oxysporum* (e) on Czapek's medium (control), (f) Pb 0.01 g/L, (g) Zn 0.05 g/L, and (h) Cu 0.01 g/L.

growing fungus *C. rosea,* which did not form extensive colonies, was characterized by relatively high rates of resistance to the action of all HMs that activated sporulation of the fungus. Fungi *A. alternata* and *C. rosea* are most likely K-strategists, responding to stress by forming compact colonies and activating sporulation.

The intensity of spore formation is not always an indicator of a negative impact. The percentage of suppression of spore germination in swabs from colonies of *F. oxysporum* can serve as an indicator of the degree of HM toxicity (Terekhova, 2007). Under natural conditions, in the presence of nutrient components, HMs can provoke an increase in the proportion of spore biomass (Terekhova, 2007). In addition, environmen-

tal conditions optimal for active spore formation may differ from those favorable for colony growth (Marfenina et al., 2010).

The published data discusses the specific mechanisms of the toxic effect of HMs on fungal pathogens and the reasons for the high resistance of this group of fungi to the action of HMs (pathogen viability) (Gajewska et al., 2022). Indeed, among the most metal-resistant fungi, pathogenic species are most often recorded (Table 2). Some exceptions, as noted above, are representatives of the genus *Trichoderma*. In our study, a nonpathogenic fungus *C. rosea* also showed relatively high resistance to the action of Zn, Pb, and Cu. Thus, the EC values of the studied HMs

Table 2. Effective concentrations of HMs causing the 50% inhibition of biomass and diameter of filamentous fungus colonies (EC_{50})

Fungus type	Place of isolation	Patho-genicity	Incubation period, days	Growth inhibition	EC_{50}	Reference
		Cadmium (Cd)				
Paecilomyces marquandii	Heavily contaminated soil	Yes	5	Biomass inhibition	2.5 mM 281 mg/L	Slaba et al., 2013
Verticillium cf. *alboatrum*	Heavily polluted pond	Yes	4	Biomass inhibition	0.9 mM 101 mg/L	Jaeckel et al., 2005
		Nickel (Ni)				
Curvularia lunata	Metal-free soil	Yes	2	Biomass inhibition	3 mM 176 mg/L	Paraszkiewicz et al., 2009
Paecilomyces marquandii	Heavily contaminated soil	Yes	5	Biomass inhibition	3 mM 176 mg/L	Słaba et al., 2013
		Copper (Cu)				
Curvularia lunata	Metal-free soil	Yes	2	Biomass inhibition	10 mM 636 mg/L	Paraszkiewicz et al., 2009
Paecilomyces marquandii	Heavily contaminated soil	Yes	5	Biomass inhibition	4.5 mM 286 mg/L	Slaba et al., 2013
Aspergillus niger	Laboratory collection	Yes	10	Biomass inhibition	0.5 mM 32 mg/L	Sazanova et al., 2015
Alternaria alternata	Infected plants	Yes	6	Colony diameter	0.2 mM 33 mg/L	Ouda et al., 2014
Alternaria alternata	Metal-free soil	Yes	7	Colony inhibition	0.08 mM 5 mg/L	Fedoseeva et al., 2022
Trichoderma viride	Metal-free soil	No	4	Colony diameter	1.93 mM 123 mg/L	Fedoseeva et al., 2022
		Lead (Pb)				
Paecilomyces marquandii	Heavily contaminated soil	Yes	5	Biomass inhibition	3.7 mM 767 mg/L	Słaba et al., 2013
Alternaria alternata	Metal-free soil	Yes	7	Colony diameter	1.16 mM 240 mg/L	Fedoseeva et al., 2022
Trichoderma viride	Metal-free soil	No	4	Colony diameter	1.25 mM 259 mg/L	Fedoseeva et al., 2022
		Zinc (Zn)				
Curvularia lunata	Metal-free soil	Yes	2	Biomass inhibition	17.5 mM 1150 mg/L	Paraszkiewicz et al., 2009
Paecilomyces marquandii	Heavily contaminated soil	Yes	5	Biomass inhibition	12 mM 788 mg/L	Słaba et al., 2013
Aspergillus niger	Laboratory collection	Yes	10	Biomass inhibition	2 mM 131 mg/L	Sazanova et al., 2015

for *C. rosea* were higher than the EC values for the phytopathogenic fungus *F. oxysporum*.

Information about the individual resistance of fungi to the action of HMs can help in understanding the interspecies relationships within fungal soil communities against the background of HM contamination. Thus, *T. harzianum* showed itself to be an active territorial antagonist on media with Zn and Pb, but not with Cu. Soil contamination with some HM cations can provoke a shift of fungal communities to the elimination of forms that have suppressor activity against phytopathogens.

CONCLUSIONS

Based on the data on the effect of HMs on colony growth, sporulation activity, and biomass accumulation, it can be concluded that the three species *T. harzianum, A. alternata,* and *F. oxysporum* proved to be the most resistant to Pb and *C. rosea* proved to be the most resistant to Zn. All studied micromycetes were characterized by low resistance to Cu when compared to the other two metals. Thus, the sample of micromycetes showed different sensitivity to HMs. The high resistance of *T. harzianum* and *C. rosea* to the action of Zn

and Pb increased with an increase in the content of available carbon in the medium. The pairs *A. alternata−C. rosea* and *T. harzianum−C. rosea* on media with lower and higher carbon content, respectively, exhibited the greatest resistance to the action of Cu. Various effects of HMs on the growth, development, and physiology of filamentous fungi emphasize that HM toxicity depends not only on the type and concentration of metals, but also on the life style of fungi. The ability to manifest antagonistic properties (especially as territorial antagonists) depends on at least two factors: the growth rate of the fungus and the level of HM resistance. Information about the species resistance of fungi to the action of HMs is necessary to predict the development of relationships between pathogenic and nonpathogenic species in fungal communities against the background of soil pollution.

COMPLIANCE WITH ETHICAL STANDARDS

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

Statement of the welfare of animals. The article does not contain any studies involving animals in experiments performed by any of the authors.

REFERENCES

Alluri, H.K., Srinivasa, R.S.R., Settalluri, V.S., Singh, J., Suryanarayana, V., and Venkateshwar, P., Biosorption: An eco-friendly alternative for heavy metal removal, *Afr. J. Biotechnol.*, 2007, vol. 6, no. 25, pp. 2924−2931.
https://doi.org/10.5897/AJB2007.000-2461

Awofolu, O.R., Okonkwo, J.O., Merwe, R.R.D., Badenhorst, J., and Jordaan, E., A new approach to chemical modification of *Aspergillus niger* and sorption of lead ion by fungal species, *Electron. J. Biotechnol.*, 2006, vol. 9, no. 4, pp. 340−348.
https://doi.org/10.2225/vol9-issue4-fulltext-1

Bagaeva, T.V., Ionova, N.E., and Nadeeva, G.V., *Mikrobiologicheskaya remediatsiya prirodnykh sistem ot tyazhelykh metallov* (Microbiological Remediation of Natural Systems from Heavy Metals), Kazan: Kazan. Gos. Univ., 2013.

Caesar-Tonthat, T.C., Kloeke, F.V., Geesey, G.G., and Henson, J.M., Melanin production by a filamentous soil fungus in response to copper and localization of copper sulfide by sulfide-silver staining, *Appl. Environ. Microbiol.*, 1995, vol. 61, pp. 1968−1975.
https://doi.org/10.1128/aem.61.5.1968-1975.1995

Danilova, O.A., Ianutsevich, E.A., Bondarenko, S.A., Georgieva, M.L., Vikchizhanina, D.A., Groza, N.V., Bilanenko, E.N., and Tereshina, M.V., Osmolytes and membrane lipids in the adaptation of micromycete *Emericellopsis alkalina* to ambient pH and sodium chloride, *Fungal Biol.*, 2020, vol. 124, pp. 884−891.
https://doi.org/10.1016/j.funbio.2020.07.004

Fedoseeva, E.V., Danilova, O.A., Ianutsevich, E.A., Tereshina, V.M., and Terekhova, V.A., Micromycete lipids and stress, *Microbiology*, 2021, vol. 90, no. 1, pp. 37−55.
https://doi.org/10.1134/S0026261721010045

Fedoseeva, E., Patsaeva, S., Stom, D., and Terekhova, V., Excitation-dependent fluorescence helps to indicate fungal contamination of aquatic environments and to differentiate filamentous fungi, *Photonics*, 2022, vol. 9, no. 10, p. 692.
https://doi.org/10.3390/photonics9100692

Fedoseeva, E.V., Kiryushina, A.P., Stom, D.I., and Terekhova, V.A., Resistance of soil micromycetes *Trichoderma viride* and *Alternaria alternata* to heavy metals Cu and Pb, *Theor. Appl. Ecol.*, 2022, no. 3, pp. 118−127.
https://doi.org/10.25750/1995-4301-2022-3-118-127

Gadd, G.M. and De Rome, L., Biosorption of copper by fungal melanins, *Appl. Microbiol. Biotechnol.*, 1988, vol. 29, pp. 610−617.
https://doi.org/10.1007/2FBF00260993

Gajewska, J., Floryszak-Wieczorek, J., Sobieszczuk-Nowicka, E., Mattoo, A., and Arasimowicz-Jelonek, M., Fungal and oomycete pathogens and heavy metals: An inglorious couple in the environment, *IMA Fungus*, 2022, vol. 13, p. 6.
https://doi.org/10.1186/s43008-022-00092-4

Golubović-Ćurguz, V., Tabaković-Tošić, M., Veselinović, M., and Rajković, S., The influence of heavy metals on the growth of pathogenic fungi, *For. Ideas*, 2010, no. 16, pp. 121−125.

Gorai, P.S., Barman, S., Gond, S.K., and Manda, N.C., *Trichoderma*, in *Beneficial Microbes in Agro-Ecology*, 2020, part 28, pp. 571−591.
https://doi.org/10.1016/B978-0-12-823414-3.00028

Iskandar, N.L, Izzati Mohd Zainudin, N.A., and Tan, S.G., Tolerance and biosorption of copper (Cu) and lead (Pb) by filamentous fungi isolated from a freshwater ecosystem, *J. Environ. Sci.*, 2011, vol. 23, no. 5, pp. 824−830.
https://doi.org/10.1016/S1001-0742(10)60475-5

Jaeckel, P., Krauss, G.J., and Krauss, G., Cadmium and zinc response of the fungi *Heliscus lugdunensis* and *Verticillium cf. alboatrum* isolated from highly polluted water, *Sci. Total Environ.*, 2005, no. 346, pp. 274−279.
https://doi.org/10.1016/j.scitotenv.2004.12.082

Korneykova, M.V., Evdokimova, G.A., and Lebedeva, E.V., The complexes of potentially pathogenic microscopic fungi in anthropogenic polluted soils of Kola North, *Mykol. Fitopatol.*, 2012, vol. 46, no. 5, pp. 322−328.

Marfenina, O.E., Fomicheva, G.M., Vasilenko, O.V., Naumova, E.M., and Kul'ko, A.B., Sporulation in saprotrophic and clinical strains of *Aspergillus sydowii* (Bain. & Sart.) Thom & Church under various environmental conditions, *Microbiology*, 2010, vol. 79, no. 6, pp. 753−758.

Matarese, F., Sarrocco, S., Gruber, S., Seidl-Seiboth,V., and Vannacci, G., Biocontrol of *Fusarium* head blight: Interactions between *Trichoderma* and mycotoxigenic

Fusarium, *Microbiology*, 2012, no. 158, pp. 98–106.
https://doi.org/10.1099/mic.0.052639-0

Mohammadian, E., Babai Ahari, A., Arzanlou, M., Oustan, S., and Hossein Khazaei, S., Tolerance to heavy metals in filamentous fungi isolated from contaminated mining soils in the Zanjan Province, Iran, *Chemosphere*, 2017, vol. 185, pp. 290–296.
https://doi.org/10.1016/j.chemosphere.2017.07.022

Netrusov, A.I., Bonch-Osmolovskaya, E., Gorlenko, V.M., Ivanov, M.V., Karavayko, G.I., Kozhevin, P.A., Kolotilova, N.N., Kotova, I.B., Maksimov, V.N., Nozhevnikova, A.N., Semenov, A.M., Turova, T.P., and Yudina, T.G., *Ekologiya mikroorganizmov* (Ecology of Microorganisms), Moscow: Yurayt-Moscow, 2012.

Njoku, K.L., Asunmo, M.O., Ude, E.O., Adesuyi, A.A., and Oyelami, A.O., The molecular study of microbial and functional diversity of resistant microbes in heavy metal contaminated soil, *Environ. Technol. Innovation*, 2020, no. 17, p. 100606.
https://doi.org/10.1016/j.eti.2020.100606

Oladipo, O.G., Awotoye, O.O., Olayinka, A., Bezuidenhout, C.C., and Maboeta, M.S., Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites, *Braz. J. Microbiol.*, 2018, vol. 49, pp. 29–37.
https://doi.org/10.1016/j.bjm.2017.06.003

Ouda, S.M., Antifungal activity of silver and copper nanoparticles on two plant pathogens, *Alternaria alternata* and *Botrytis cinerea*, *Res. J. Microbiol.*, 2014, vol. 9, pp. 34–42.
https://doi.org/10.3923/jm.2014.34.42

Palanivel, T.M., Pracejus, B., and Novo, L.A.B., Bioremediation of copper using indigenous fungi *Aspergillus species* isolated from an abandoned copper mine soil, *Chemosphere*, 2023, vol. 314, p. 137688.
https://doi.org/10.1016/j.chemosphere.2022.137688

Pandaranayaka, E.P., Frenkel, O., Elad, Y., Prusky, D., and Harel, A., Network analysis exposes core functions in major lifestyles of fungal and oomycete plant pathogens, *BMC Genomics*, 2019, vol. 20, p. 1020.
https://doi.org/10.1186/s12864-019-6409-3

Paraszkiewicz, K., Bernat, P., and Długoński, J., Effect of nickel, copper, and zinc on emulsifier production and saturation of cellular fatty acids in the filamentous fungus *Curvularia lunata*, *Int. Biodeterior. Biodegrad.*, 2009, vol. 63, pp. 100–105.
https://doi.org/10.1016/j.ibiod.2008.03.015

Pariaud, B., Ravigné, V., Halkett, F., Goyeau, H., Carier, J., and Lannou, C., Aggressiveness and its role in the adaptation of plant pathogens, *Plant Pathol.*, 2009, vol. 58, no. 3, pp. 409–424.
https://doi.org/10.1111/j.1365-3059.2009.02039.x

Pérez-Torres, E.J., Camacho-Luna, V., Pérez-Ocampo, S., Rodríguez-Monroy, M., and Sepúlveda-Jiménez, G., Tolerance to oxidative stress caused by copper (Cu) in *Trichoderma asperellum* To, *Biocatal. Agric. Biotechnol.*, 2016, vol. 29, p. 101783.
https://doi.org/10.1016/j.bcab.2020.101783

Polyanskaya, L.M., Kochkina, G.N., Kozhevin, P.A., and Zvyagintsev, D.G., Kinetic description of the structure of soil actinomycetes complexes, *Microbiology*, 1988, vol. 57, no. 5, pp. 854–859.

Rizzo, D.M., Blanchette, R.A., and Palmer, M.A., Biosorption of metal ions by *Armillaria rhizomorphs*, *Can. J. Bot.*, 1992, vol. 70, pp. 1515–1520.
https://doi.org/10.1139/b92-190

Rola, K., Latkowska, E., Ogar, W., and Osyczka, P., Towards understanding the effect of heavy metals on mycobiont physiological condition in a widespread metal-tolerant lichen *Cladonia rei*, *Chemosphere*, 2022, vol. 308, p. 136365.
https://doi.org/10.1016/j.chemosphere.2022.136365

Sanin, S.S., Neklesova, N.P., Sanina, A.A., and Pacholkova, E.V., *Metodicheskie rekomendatsii po sozdaniyu infektsionnykh fonov dlya immunogeneticheskikh issledovanii pshenitsy* (Guidelines for the Creation of Infectious Backgrounds for Immunogenetic Studies of Wheat), Moscow: Vseross. Nauchno-Issled. Inst. Fitopatol., 2008.

Sazanova, K., Osmolovskaya, N., Schiparev, S., Yakkonen, K., Kuchaeva, L., and Vlasov, D., Organic acids induce tolerance to zinc- and copper-exposed fungi under various growth conditions, *Curr. Microbiol.*, 2015, vol. 70, pp. 520–527.
https://doi.org/10.1007/s00284-014-0751-0

Siddiquee, S., Rovina, K., and Azad, S.A., Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: A review, *J. Microb. Biochem. Technol.*, 2015, vol. 7, pp. 384–395.
https://doi.org/10.4172/1948-5948.1000243

Singh, A., Maitreyi, M., Tripathi, P., and Shweta, S., Resistance of heavy metals on some pathogenic bacterial species, *Afr. J. Microbiol. Res.*, 2015, vol. 9, pp. 1162–1164.
https://doi.org/10.5897/AJMR2014.7344

Skugoreva, S.G., Kantor, G.Ya., and Domracheva, L.I., Biosorption of heavy metals by micromycetes: Specificity of the process, mechanisms, kinetics, *Theor. Appl. Ecol.*, 2019, vol. 2, pp. 14–31.
https://doi.org/10.25750/1995-4301-2019-2-014-031

Słaba, M., Szewczyk, R., Piątek, M.A., and Długoński, J., Alachlor oxidation by the filamentous fungus *Paecilomyces marquandii*, *J. Hazard. Mater.*, 2013, vol. 261, pp. 443–450.
https://doi.org/10.1016/j.jhazmat.2013.06.064

Terekhova, V.A., *Mikromitsety v ekologicheskoi otsenke vodnykh i nazemnykh ekosistem* (Micromycetes in the Environmental Assessment of Aquatic and Terrestrial Ecosystems), Moscow: Nauka, 2007.

Toledo, A.V., Ernesto Franco, M.E., Yanil Lopez, S.M., MaríIné, T., Nazareno Saparrat, M.C., and Balatti, P.A., Melanins in fungi: Types, localization and putative biological roles, *Physiol. Mol. Plant Pathol.*, 2017, vol. 99, pp. 2–6.
https://doi.org/10.1016/j.pmpp.2017.04.004

Torres-Cruz, T.J., Hesse, C., Kuske, C.R., and Porras-Alfaro, A., Presence and distribution of heavy metal tolerant fungi in surface soils of a temperate pine forest, *Appl. Soil Ecol.*, 2018, vol. 131, pp. 66–74.
https://doi.org/10.1016/j.apsoil.2018.08.001