Simultaneous effect of nickel, cadmium and chromium(VI) on soil micromycetes

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Response of soil fungi *Alternaria alternata, Penicillium decumbens* and *Trichoderma viride* to cadmium, nickel and chromium(VI) as well as their mixture was studied. Fungal capacity to remove metals from their mixture added into the growth medium was investigated, too. *T. viride* was the fungus most tolerant towards all test metals and their mixture. This micromycete also removed the highest amounts of the metals.

Key words: micromycetes, heavy metals, metal response, metal mixture, metal sorption

INTRODUCTION

Pollution of soils by heavy metals affects the functioning of microorganisms and induces alterations in their population structure. Filamentous fungi were reported to exhibit considerable tolerance towards heavy metals and become dominant organisms in some polluted habitats [1–2]. Fungi are also known to accumulate high amounts of metals [3–4]. This property is of great importance to organisms growing in polluted habitats and for a possible binding of heavy metals in natural environments as well as for their removal from waste waters and other aqueous substrata [5–6]. Microorganisms in natural ecosystems often encounter not one but several heavy metals. Therefore, fungal tolerance towards a mixture of metals is of high importance both for fungal survival and their application for industrial purposes.

The aim of the present study was to assess the effect of a metal mixture *versus* single metals on the development of soil fungi and to evaluate fungal ability to remove metals from the mixture by growing fungal biomass.

MATERIALS AND METHODS

Micromycetes were isolated from rhizosphere zone soil under leaf-litter (Verkiai regional park). Three fungi were chosen for investigation: These were *Penicillium decumbens* Thom 102ML, a frequently encountered soil fungus; *Alternaria alternata* (Fries) Keissler 41L, able to cause plant diseases, and *Trichoderma viride* Persoon 11S, the fungus known as a bioagent against pathogens. Fungal sensitivity towards heavy metals and their mixture was tested on Czapek medium agar, pH 5.5 [7]. The medium was amended with Cd^{2+}, Ni^{2+} and Cr^{6+} at a rate of 0.1–3 mM. The metals were used as salts: $CdCl_2$, $NiCl_2{\cdot}6H_2O$ and $K_2Cr_2O_7$. All glassware used for metal studies was washed with 4% nitric acid and rinsed three times with distilled water.

Cultures were grown on a solid medium at 25 ± 2 °C and inspected after 7 days. The effect of heavy metals on the growth of fungi was evaluated as changes in the radial hyphal extension rate by measuring the diameter of fungal colonies. All experiments were conducted in triplicates.

For metal sorption experiments, fungi were grown in a liquid medium of the same composition with addition of a metal mixture at a concentration of 0.1 mM for each metal. The cultures were incubated on a rotary shaker for 5 days. Biomass amount was evaluated as dry weight obtained by drying at 105 °C for 8 hours. Measurements of metal residues in the growth medium after cultivation were conducted with a Perkin-Elmer Zeeman 3030 atomic absorption spectrophotometer in the Institute of Physics.

RESULTS AND DISCUSSION

First, the effect of a separate metal on fungi was studied. Fungal response to $NiCl_2$ revealed that the most sensitive fungus was *Alternaria alternata* 41L whose growth was reduced even by 0.15 mM nickel and absolutely inhibited at 1 mM nickel (Fig.). Most resistant to nickel was *Trichoderma viride* 11S. Development of *Penicillium decumbens* 102ML was in-

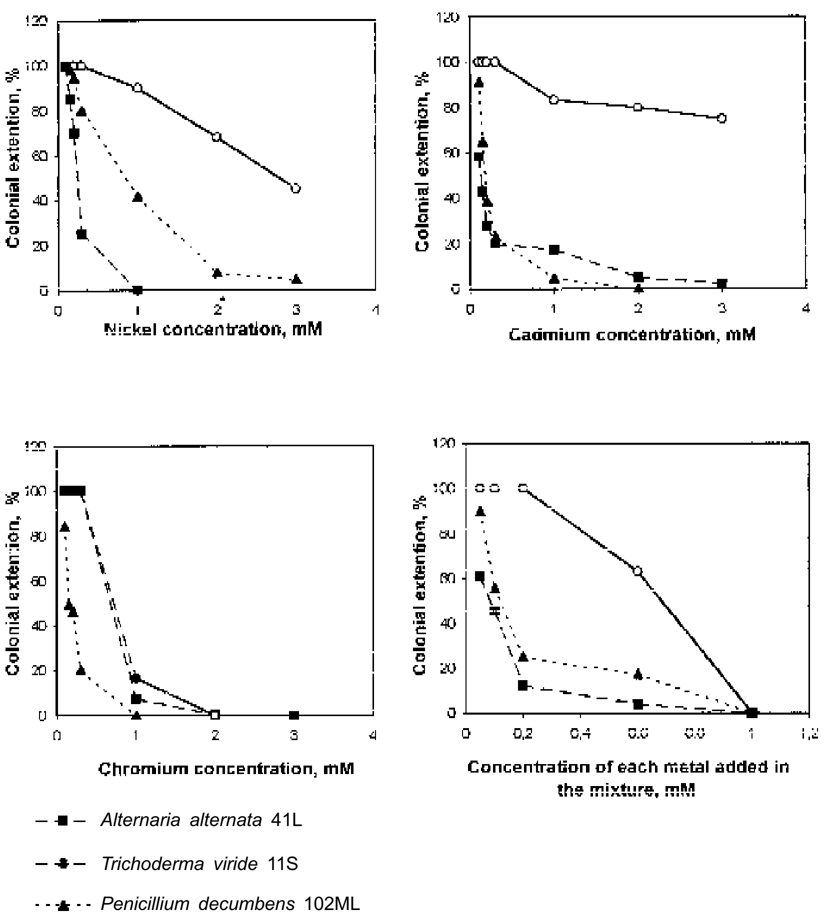

Fig. Growth response of fungi after 7 days towards nickel, cadmium and chromium (VI) and their mixture. The metals were added as salts: $CdCl_2$, NiCl2·6H2O and $K_2Cr_2O_7$

fluenced more negatively than that of *T. viride* 11S, but less than of *A. alternata* 41L.

In response to $CdCl_2$ *P. decumbens* 102ML was more sensitive as compared to its reaction towards nickel, and 2 mM cadmium for this fungus was lethal. *A. alternata* 41L growth was also fairly weak, but it was able to grow slightly when exposed to 3 mM cadmium. Meanwhile, *T. viride* 11S exhibited a considerably higher resistance to cadmium than did the other fungi. Its radial growth was reduced by a 3 mM concentration insignificantly and was equal to 75% of its growth on a metal-free medium.

The effect of $K_2Cr_2O_7$ on the radial growth of *A. alternata* 41L and *T. viride* 11S almost did not differ. A sharp decrease was noticed at a metal concentration of 1–2 mM and no growth was observed at 3 mM. *P. decumbens* 102ML was more sensitive than the other two fungi to $K_2Cr_2O_7$.

When fungi were cultivated on a medium amended with a metal mixture, they responded much more sensitively to a mixture of metals than to separate ones. *P. decumbens* 102ML was more tolerant than *A. alternata* 41L and more sensitive than *T. viride* 11S. The metal mixture even at a concentration of 1 mM inhibited the growth of *T. viride* 11S, whereas the fungus developed on a medium with separate metals at this and higher concentrations. A more negative influence of the mixture was noted on *A. alternata* 41L.

As the results show, *T. viride* 11S exhibited the highest resistance to all metals and their mixture, *P. decumbens* 102ML was quite resistant to nickel and sensitive to chromate, and *A. alternata* 41L was sensitive to nickel and more resistant to chromate. The variance of fungal response could be determined by their individual biology (all micromycetes were from different taxonomic groups), their different metabolites which can bind metals, and their ability to prevent cellular entry of a toxic metal or to compartmentize and detoxify it within the cell [5, 8–9].

A study of metal accumulation showed that the sorption of metals from the medium by different fungi was not the same. The residual cadmium content in the cultural liquid was the lowest when calculated as a percentage – 5.6–17% (Table). The range of chromium amount found in the supernatant after fungal cultivation was from 18.1 to 32.2%, and the nickel content varied within 41.8–70.6%. There are reports that fungi can bind metals on the cell surface and uptake inside. High contents of chitin, chitosan and glycans in the cell wall can sorb considerable amounts of metals [9–10]. Metal uptake inside the cell is determined by various and inside and outside factors [9, 11].

T. viride 11S accumulated the highest amount of all metals. A particularly high sorption by this fungus was observed for cadmium (625 µg/l or 5.6% of the residual metal). *A. alternata* 41L accumulated high amounts of cadmium (remained 13.1%). *P. decumbens* 104ML also sorbed cadmium well (left 17.0%), nevertheless, chromium sorption was also high. An increase in the uptake of chromium by microorganisms can result from reduction of Cr^{6+} to Cr^{3+}, which is more amenable to complexation [12–13]. Metal sorption occurred in the medium rich in various components, including fungal metabolites, and with an altering medium pH during fungal growth. These reasons could also influence the fungal ability to sorb metals.

Table. **Removal of metals from growth medium by fungi**

Fungus	Biomass amount, (dry weight), g	Metals that remained in the cultural liquid					
		cadmium		chromium		nickel	
		μg/l	%*	μg/l	%	μg/l	%
Alternaria alternata 41L	0.98 ± 0.12	1473 ± 196	13.1	1673 ± 301	32.2	3320 ± 426	56.4
Penicillium decumbens 102ML	1.24 ± 0.26	1910± 384	17.0	1157 ± 253	22.2	4159 ± 358	70.6
Trichoderma koningii 11S	0.87 ± 0.15	625 ± 109	5.6	940 ± 124	18.1	2460 ± 395	41.8

* The percentage was calculated considering added metal concentration as 100%.

No clear correlation between metal accumulation and fungal sensitivity was observed. Some works showed that more sensitive microorganisms sorbed higher amounts of metals as they did not possess the mechanisms determining the efflux of toxic metals from the cell [9, 14]. On the other hand, the resistant organism can translocate heavy metals into intracellular structures up to particular amounts by binding and thereby detoxifying them [5]. In our experiment, 0.1 mM concentration of the metal mixture was considerably less toxic to *T. viride* 11S than to the other fungi, and *T. viride* 11S sorbed the highest amounts of all metals. *A. alternata* 41L and *P. decumbens* 104ML differed in significantly in their sensitivity to a metal mixture and more to separate metals. No correlation was observed between the accumulation of metals by the latter two fungi and their sensitivity.

The results have shown that fungi can withstand rather high concentrations of heavy metals and are able to sorb them from metal mixture in surroundings or aqueous systems rich in organic and inorganic components. *Trichoderma viride* 11S, which usually is a desirable fungus in many cultural soils as a bioagent against pathogens, manifested a high metal resistance and a high sorption capacity.

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BENDRAS NIKELIO, KADMIO IR CHROMO(VI) POVEIKIS DIRVOŽEMIO MIKROMICETAMS

S a n t r a u k a

Atlikti dirvožemio mikromicetų *Alternaria alternata, Penicillium decumbens* ir *Trichoderma viride* reakcijos į kadmio, nikelio ir chromo (VI) bei jų mišinio poveikį tyrimai. Taip pat tirtas grybų gebėjimas sorbuoti metalus iš jų mišinio, įdėto į grybų augimo terpę. Didžiausiu tolerantiškumu visų metalų atžvilgiu pasižymėjo *T. viride.* Šis mikromicetas taip pat akumuliavo didžiausius metalų kiekius.