Research of the influence of zinc on soil mycoflora with special attention to Zn-resistant fungi

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A study of fungal propagules was conducted in commune garden soil amended with $ZnCl_2$ (Zn^{2+} at 402 mg·kg^{-1}) and incubated at 18 °C and with moisture content approx. 60%. Fungal resistance to zinc ions was estimated by dilution plating on malt agar (MEA) containing Zn^{2+} at 2.6, 3.9, 5.2, 6.5 or 13.0 mg ml^{-1} (as $ZnCl_2$). The fungi *Emmonsiella capsulata, Eurotium herbariorum, Gliocladium penicilioides, Metarrhizium anisopliae, Paecilomyces lilacinus* and *Talaromyces flavus* were the most Zn-resistant species recovered from test soils.

Key words: fungi, soil pollution, zinc ions, indicator cultures

INTRODUCTION

There is a number of general points that must be considered before discussing the interaction of fungal cells with individual metal ions. These include the role of metals in normal fungal growth, deficiency and toxicity effects, and the relation of chemical properties of metals to their toxicity [1–4]. A metal may be regarded as toxic if it impairs growth or metabolism of an organism above a certain concentration. Zinc is essential for fungal growth [1], becoming toxic only at high concentrations [5]. The toxicity of zinc at a high concentration has been reported to be due to the creation of magnesium deficiency [6] and to the competition between Zn^{2+} and Mg^{2+} for transport into cell [1, 7]. High zinc concentrations inhibit organic acid and protein production [8] and the cellulolytic activity of soil fungi [5].

Mineral fertilization increases the content of mobile forms of heavy metals in soil in Lithuania [9]. Their amounts did not reach dangerous concentrations, however, the amounts of mobile zinc detected in soil were quite high (up to 102 mg Zn^{2+} kg^{-1} soil). The average Zn^{2+} concentration in Lithuanian soils varies from 28.4 to 58 mg kg^{-1} soil [10].

The aim of the present work has been to estimate how zinc ions in polluted soil can change the fungal community structure and which fungi remain resistant in the medium with high zinc concentrations.

MATERIALS AND METHODS

The soil was sampled in communal garden plots. It was a sandy loam (pH_{KCl} = 7.3). The sample was cleaned of vegetative detritus, slowly air-dried and sieved (2 mm). To one portion (2 kg) of the soil Zn^{2+} was added at 402 mg kg^{-1} soil as $ZnCl_2$ (545 mg kg^{-1}). After mixing the soil with zinc and moisturizing it up to 60% total water capacity, samples (control – without zinc addition) were incubated at 18 °C for 3 months, mixing the soil from time to time.

Fungi were isolated from the soil by dilution plating on 9 cm diam. Petri dishes, using a hydrous dilution medium with 8.5 g NaCl and 1 g peptone l^{-1}. Malt extract agar (MEA) was used as nutrient media. A medium was amended with ampicillin (100 µg/ml) to inhibit bacterial growth. Mycelial growth of the genus *Trichoderma* was reduced by addition of 0.1% Triton-X-100. The Petri dishes were incubated at 25 °C in the dark. The total number of fungi (cfu g^{-1} soil D. W.) was enumerated after 4 and 7 days. Six samples of soil were processed. The most common species present in the soil samples were studied on zinc-containing MEA. $ZnCl_2$ was added to MEA, the media were modified by adding 2.6, 3.9, 5.2, 6.5 or 13.0 Zn^{2+} mg ml^{-1}. Colonies on each Petri dish were counted, and representative fungal colonies were transferred on Sigma malt-extract agar (MEA), Sigma Czapek agar (CA), Sigma potato-dextrose agar (PDA), Sigma cornmeal agar (CMA) and incubated at 25 °C for seven days. The following systematic works were used: Domsch et al., (1980) [11], Raper et al., (1968) [12] and Кириленко (1977) [13].

RESULTS AND DISCUSSION

The total number of fungi in test soils (Fig. 1-A) after 1, 2 and 3 months of incubation under model

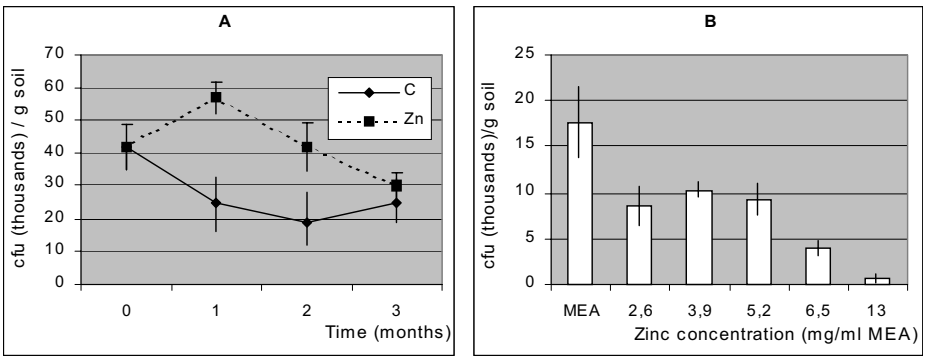

Fig. 1. Fungal concentration (10^3 cfu/g soil d.w.): A – in the control (C) and zinc-containing soils at the beginning of study and after 1, 2 and 3 months of incubation; B – on malt agar (MEA) and MEA-containing different Zn^{2+}concentrations

conditions and fungal population density on media with different zinc concentrations (Fig. 1-B) were detected. The number of fungi in control soil at the beginning of study was estimated to 4213 cfu g^{-1} soil. Lower concentrations of fungi in control soil were detected after 1, 2 and 3 months of incubation (2521, 1986 and 2247 cfu g^{-1} soil, respectively). The fungal concentration recovered from zinc-containing soil was higher than from control soil. The viability of fungi in zinc-containing soil increased after one months, but after 3 months it was almost the same as in control soil (2989 cfu g^{-1} soil). The difference between the total cfu numbers in two soils after 1 and 2 months of incubation was statistically significant. In an earlier study A. Raguotis (1999) [5] examined the effect of $ZnSO_4 \cdot 5H_2O$ on the growth and activity of microorganisms in forest soil and reported a very weak stimulation effect of small zinc concentrations on fungi. The toxicity of zinc sulfate appeared only at a concentration greater than 700 mg kg^{-1} soil (or 243.32 mg kg-1 Zn^{2+}). Some fungal genera isolated from Zn-containing soil in our study lost their antagonistic activity on standard malt agar media. Their population density decreased or they grew very slowly.

Samples of test soil were analyzed for total numbers of different taxonomic groups of fungi. The major isolated fungal genera were *Aspergillus* Mich. Ex Fr., *Cylindrocarpon* Wollenw., *Gliocladium* Corda, *Fusarium* Link ex Fr., *Mortierella* Coemans, *Mucor* Mich. Ex St.-Am., *Paecilomyces* Bain., *Penicillium* Link ex Fr. and *Trichoderma* Pers. Ex Fr. (Fig. 2).

Stimulation of some fungi in Zn-containing soil was noticed according to a difference of genera in test soil fungal communities. Results presented in Fig. 2 show a high number of genera that were frequently recovered from Zn-containing soil. Detection of these genera and estimation of their concentrations was conducted by addition of $ZnCl_2$ at various concentrations to a malt agar medium. On the modified MEA, fungi of the genera *Cylindrocladium* Morgan, *Emmonsiella* Kwon-Chung, *Eurotium* Link ex Fr., *Metarrhizium* Sorok. and *Paecilomyces* Bain. were abundantly isolated from the Zn-containing soil (Fig. 3A). The concentration gradient of Zn^{2+} in MEA allowed us to detect Zn-resistant fungi. *Metarrhizium anisopliae* (Metschn.) Sorok.was most resistant to $ZnCl_2$ in test soil and on the dishes with Zn-containing MEA. *M. anisopliae* grew on the medium with Zn^{2+} at a concentration of 13 mg ml^{-1} and comprised 82.8% of the total number of Zn-resistant fungi (Fig. 3B). During investigation of the fungal communities in test soils, *M. anisopliae* was one of the most slowly growing fungi on standard MEA. In mixed cultures on MEA medium *M. anisopliae* was a weak antagonist, as it was a weak antagonist also in non-sterile soil [11]. The reduction in growth appeared to be related to a strongly aggressive action of mycoparasites dominating in the fungal associations.

Zn-resistant species of fungi were isolated on MEA medium. Zinc chloride was added to MEA at a concentration of 2.6, 3.9, 5.2, 6.5 or 13.0 Zn^{2+} mg ml^{-1}. A modified medium allowed us to isolate fungi of the genera *Metarrhizium* and *Cylindrocarpon* and to detect the concentration of viable fungi of the genera *Eurotium, Gliocladium* and *Paecilomyces* in Zn-containing soil (Fig. 3). On these media were

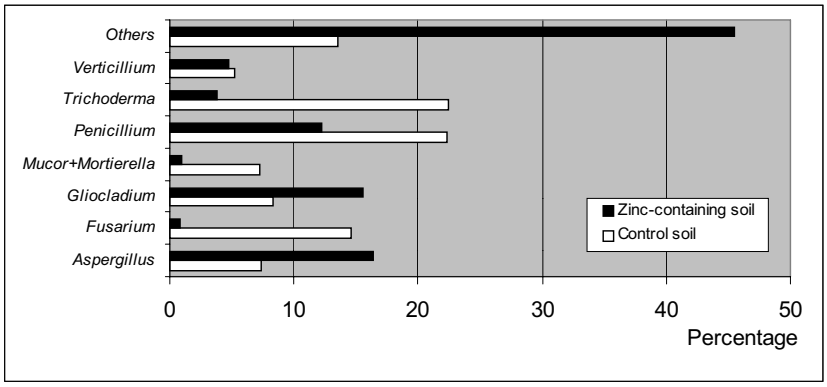

Fig. 2. Percentage of fungal genera isolated from control soil and soil with Zn^{2+} at 402 mg kg^{-1} during 3 months of incubation at 18 °C

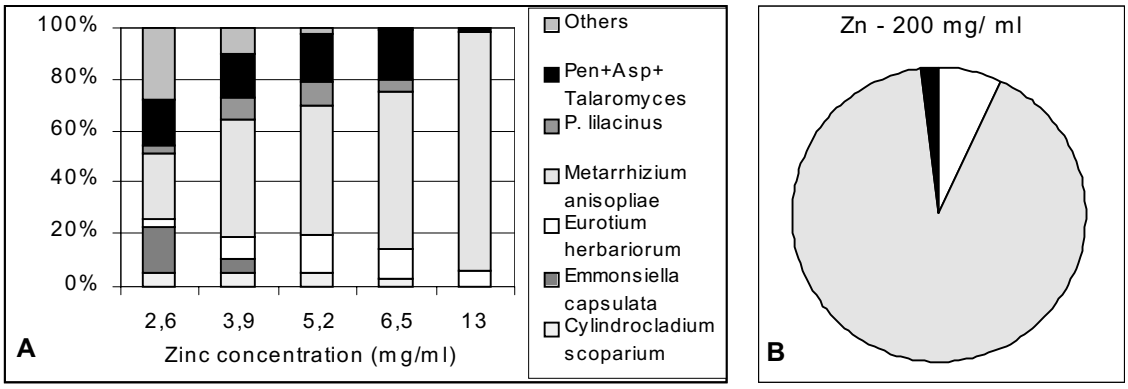

Fig. 3. Percentage of Zn-resistant fungal species on media with different (A) and with 6.5 mg/ml (B) Zn^{2+} concentrations

detected, isolated and studied the Zn-resistant species *Metarrhizium anisopliae, Zygorrhynchus moelleri* and *Cylindrocladium scoparium*.

Zinc at a concentration of 13 mg ml^{-1} was fungistatic to the most fungi of the test soil fungal community. *M. anisopliae* and *Eurotium herbariorum* (Wiggers) Link ex Gray were two fungi recovered on that medium. On a medium with Zn^{2+} at a concentration of 6.5 mg ml^{-1} fungi *Paecilomyces lilacinum* (Thom) Samson, *Cylindrocladium scoparium* Morgan, *Emmonsiella capsulata* Kwon.-Chung and some species of the genera *Aspergillus* and *Penicillium* were also abundant and were strong antagonists towards cellulolytic fungi (*Trichoderma, Fusarium* and *Chaetomium* Kunze ex Fr.). A negative influence on the growth of cellulolytic fungi was noticed. Population density of the genera *Trichoderma, Fusarium, Mucor* and *Mortierella* decreased in zinc-containing soil (Fig. 2A). However, after 3 months of study the *Trichoderma* population density in Zn-containing soil increased and almost reached the control level. A decrease of cellulolytic activity in soil samples was detected in forest soil [5]. According to the most research reffered to in Domsch et al., (1988) [11], zinc is essential for *Aspergillus niger, Emmonsiella capsulatum, Zygorrhynchus moelleri* and some species of the genera *Paecilomyces* and *Penicillium*. The influence of zinc on the production of antibiotics which contribute to the ecological competence of pseudomonads further indicates that this trace mineral is a key environmental signal in biocontrol [14].

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CINKO POVEIKIO DIRVO□EMIO MIKROFLORAI TYRIMAI AKCENTUOJANT CINKUI ATSPARIUS MIKROMICETUS

S a n t r a u k a

Mikromicetų pradų kiekio tyrimai atlikti kolektyvinio sodo dirvožemyje, praturtintame cinko chloridu (402 mg kg^{-1} Zn^{2+}), sudrėkintame iki 60% drėgnumo ir laikytame 18 °C temperatūroje. Mikromicetų atsparumas cinkui (cinko chlorido forma) buvo įvertintas suspensijos skiedimo ir auginimo ant skirtingas cinko koncentracijas (2,6, 3,9, 5,2, 6,5 ir 13,0 mg ml^{-1} Zn^{2+}) turinčios alaus misos terpės. Mikromicetai *Emmonsiella capsulatas, Eurotium herbariorum, Gliocladium penicilioides*, *Metarrhizium anisopliae, Paecilomyces lilacinus* ir *Talaromyces flavus* įvertinti kaip cinkui atsparūs tirtame dirvožemyje.