# Increased Soil Heavy Metal Concentrations Affect the Structure of Soil Fungus Community

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## Summary

Effects of heavy metals on soil fungi populations and soil fertility incidental to it were studied under laboratory conditions. Metal-amended antroposol type soil samples were incubated for a month at 17°C under natural light regime. Copper, zinc and lead were chosen as the most common industrial pollutants. Each metal was applied either of sulfate, chloride or acetate salt (at concentration varying from 0.4 to 16.14 g $kg^{-1}$ soil); control – soil without metal amendment. Fungal populations (dilution plate method) were investigated and soil phytotoxicity test was performed.

Elevated Cu, Zn and Pb concentrations in the soil influenced fungus community structure. Some species (*Absidia glauca*, *Acremonium kiliense*, *Aspergillus fumigatus*, and *Alternaria alternata*) detected in the control soil community were eliminated, while the abundance of the other species increased. *Paecillomyces* genus dominated in the soil amended with either of Cu or Zn. *P. farinosus*, *P. fumosoroseum* and fungal species from the *Clonostachys, Penicillium* and *Lecanicillium* genera were Zn-resistant. *P. lilacinus* and plant pathogenic fungi, *A. alternata, Fusarium oxysporum*, *F. solani* and *Phoma lingam* were very abundant in soil amended with Cu salts, followed by some saprotrophic fungi such as *Cunninghamella echinulata* and *Mucor hiemalis* f. *hiemalis*.

An overall change in the plant (cress, *Lepidum sativum*; wheat, *Ticicum aestivum*; lupine, *Lupinus polyphyllus*, and sunflower, *Heliannthus annus*) seed viability was observed in comparison with control. Most deleterious effects on the seed germination were observed in case of zinc, medium – in case of copper, and the least – in case of lead. Zinc salts at used concentrations were unfavorable to both fungus populations and consequently to the seed viability.

## Key words

heavy metals, zinc, copper, lead, soil, fungi, community, phytotoxicity

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## Introduction

Physical, chemical and biological factors in combination determine soil quality (fertility), which has a great importance to plant growth and development. Probably biological factor is the most variable and hard to determine. Wide variety of bacteria, actinobacteria and fungi comprises the greatest part of soil organisms (Ashraf et al., 2007). Many factors may influence a ratio of different groups of microorganisms (Rachel et al., 2006) as well as their intra-group diversity (Satish et al., 2007). Fungi are more resistant to changing environmental conditions, however their diversity and activity may be influenced by many factors as well (Garbeva at al., 2007).

Human activity negatively influences soil quality by different ways, the most common of them soil fertilization and industrial pollution should be noted. Unsustainable agriculture enhances soil pollution even more broadly than industrial pollution (Wolna-Maruvka et al., 2007; Digrak and Özçelik, 1998). Heavy metals are the most common pollutants that make the major problem of the environmental pollution all over the world (Ezzouhri et al., 2009). Results of investigations suggest that bacteria are reduced by heavy metals in higher degree than fungi (Fostergård et al., 1996; Gadd., 1990; Pečiulytė and Dirginčiutė-Volodkienė, 2009 a; Pečiulytė and Dirginčiutė-Volodkienė, 2009 b; Rajapaksha et al., 2004). However, high heavy metal concentrations affect soil fungus communities as well: reduce species diversity by shifting it to the heavy metal-resistant species and isolates (Gadd, 1993; Nordgren et al., 1983). Development of plants is closely related with both the soil quality and the community of soil microorganisms (Carney and Matson, 2005), therefore plants are influenced by heavy metals directly transferring them from the soil and indirectly via heavy metal impact on the microorganisms.

The aim of present research was to investigate possible effect of some heavy metals (zinc, copper and lead) to different plants via impact of heavy metals to the structure of soil microorganisms' community with special attention to fungal populations.

## Materials and methods

Soil preparation. Arable soil of antroposol type was sampled in late spring time of 2007. Three replicate bulk samples, consisting of 18-20 randomly collected sub-samples from the surface soil (0-10 cm horizon after litter removal) were taken with the aim to determine soil fungus community and soil quality via phytotoxicity test. The soil sample was transported to the laboratory, air-dried at the room temperature and sieved (2-mm mesh size) prior to further use in the experiment.

Zinc and copper were applied in the form of either sulfate, chloride or acetate salt, lead was applied as chloride and acetate salt. Stock solutions (zinc salts at 1 M concentration; copper and lead salts at 0.1M concentrations) were prepared in the sterile distilled water and required amounts were used to reach necessary metal salt concentrations (g $kg^{-1}$ fresh-weight soil): $ZnSO_4$ – 16.14; $ZnCl_2$ – 6.82; $Zn(CH_3COO)_2$ – 4.38; $CuSO_4$ – 0.47; $CuCl_2$ – 0.40; $Cu(CH_3COO)_2$ – 1.27; $PbCl_2$ –6.95; $Pb(CH_3COO)_2$ – 8.13. The concentrations of zinc, copper and lead salts corresponded to those determined in our earlier investigations (Dirginčiutė and Pečiulytė, 2007) as median lethal concentrations ($CL_{50}$), which stop development of soil fungi by 50%, when added to nutrient medium. Control treatment was soil moistened with the distilled water without heavy metal addition.

Soil samples with each of heavy metal salts, mentioned above and without heavy metal addition (three replicates each variant) were used for phytotoxicity test and determination of the soil fungus community structure.

Soil characteristics. Some characteristics of the soil used for investigation are shown in Table 1. The $pH_{KCl}$ was measured with a glass electrode using a mixture of soil with a 1.0 M KCl solution. The concentration of nitrogen and phosphorous was determined with the photometer "SPECOL11", that of potassium by applying flame photometer "FLAPHO41", and content of humus calorimetrically (Mineev, 1989). Heavy metal content was determined when soil was digested by concentrated $HNO_3$ and HCl (1:3, v/v) (aqua regia). Quantity of the metals (Pb, Zn, Mn, Cr, Ni, Cu, and Cd) in soil was studied by electrothermal atomic absorption spectrophotometry (EAAS) using a Perkin-Elmer-Zeeman 3030 spectrophotometer.

Phytotoxicity test. Plants from different taxonomic groups suitable for investigating soil phytotoxicity under laboratory conditions (ISO 11269-2) were the following: cress (*Lepidum sativum* L.), wheat (*Triticum aestivum* L.), large-leaved lupine (*Lupinus polyphyllus* Lindl.), and sunflower (*Helianthus annus* L.). Seeds were reared into young seedlings in a controlled environment: in pots, containing 500 g of metal-contaminated or control soil in case of *Triticum aestivum* and *Helianthus annus,* and Petri dishes, containing 100 g metal-amended or control soil in case of *Lepidum sativum* and *Lupinus polyphyllus.* Every treatment was done in triplicate, in 16:8 (L:D) photoperiod, at 20 ± 2°C. Each treatment contained 30 (wheat, lupine and sunflower) or 50 (cress) seeds. After appropriate growth time (3, 7, 10 and 20 days cress, wheat, lupine and sunflower, respectively) seed germination percent and seedlings length (mm) were determined.

Fungus community structure. After phytotoxicity experiment soil samples from the each pot were removed and fungal community structure was analyzed by the serial dilution method, the level of the dilution being selected to give 30–50 colonies

**Table 1.** Soil physical and chemical properties, measured in field soil samples used for investigation

| Sand (%) | Clay (%) | Humus (%) | $pH_{KCl}$ | Total N (%) | $P_2O_5$ (mg $kg^{-1}$) | $K_2O$ (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|
| 36.4 | 24.8 | 14.50 | 6.89* | 0.663 | 3717.8 | 502.03 |
| Pb | Zn | Mn | Cr | Ni | Cu | Cd |
| | | | mg $kg^{-1}$ (dry weight soil) | | | |
| 5 | 8 | 125 | 5 | 2 | 3 | 0.1 |

*pH of soil samples after metal salts addition changed from 6.89 in control to 6.6 in samples with sulphates, to 6.74 – with acetate and left unchanged when chlorides were added.

on the medium plate. Fungi were isolated on Czapek medium (CA) comprising (g $l^{-1}$): $NaNO_3$ – 2.0, $KH_2PO_4$ – 1.0, $MgSO_4 \cdot 7H_2O$ – 0.5, KCl – 0.5, $FeSO_4 \cdot 7H_2O$ – 0.01, glucose – 20.0, agar – 20.0 (pH after sterilization – 6.8) with 30 mg $l^{-1}$ streptomycin to avoid bacterial contamination. Abundance of fungi in soil was evaluated as number of colony forming units (CFU) per gram of dry weight soil. Number of different morphotypes was evaluated and after that each morphotype was transferred on the potato dextrose agar (PDA, Liofilchem, ITALY), Czapek Dox agar (CDA, Liofilchem, ITALY) and malt extract agar (MEA, Liofilchem, ITALY) for identification purpose. Standard procedures based on colony, spore and structural morphology were followed for identification (Ellis, 1971; Watanabe, 1994; Domsch et al., 2007; Kiffer and Morelet, 1999). Fungus community structure of the initial soil, collected in the field, and soil of control and metal-amended treatments after their phytotoxicity tests was investigated.

Statistical analysis. Standard deviation was estimated for every trial. It is marked in Tables and in Figures 1 and 2 as error bars. Hierarchic clustering analysis was performed using the nearest neighbor joining method via single linkage. Euclidean distances were derived from the observations of fungal species and their abundance in community. *Statistica* 6.0 software was used for statistical analysis.

## Results and discussion

Phytotoxicity test. Plant seeds germination in the control soil determined by phytotoxicity test was 99.5% (Fig.1). Effect of metal salts additions to the soil depended on the metal salt used and plant species. The greatest toxic effect of $ZnCl_2$ on plants was determined. This salt completely suppressed seed viability of wheat, lupine and sunflower, while the viability of cress seeds was suppressed by 20% and their sprouts were tenfold shorter as compared with control (Fig. 2). Cress was strongly affected by other zinc salts as well. $ZnSO_4$ and $Zn(CH_3COO)_2$ suppressed seeds viability by 17 and 22%, respectively; the length of their sprouts was smaller (2.5-fold and 1.5-fold, respectively) as compared with control (Fig.2). Zinc sulphate affected neither germination nor sprouts growth of the wheat, but the length of the lupine and sunflower sprouts decreased 2 and 2.5 times, respectively. Zinc acetate did not influence wheat viability and slightly reduced the length of the sprouts. Seeds viability of the lupine was not affected by this salt as well, but the sprouts were five-fold shorter.

Both copper salts (sulphate and acetate) significantly reduced viability of sunflower seeds. Interestingly, sprouts of the survived seeds developed better in those treatments as compared with control. The seeds viability and growth of the other plants changed negligible under effect of copper salts.

Surprisingly, lead salts just slightly decreased plant seeds viability; sunflower was not affected at all. It's sprouts were higher in $Pb(CH_3COO)_2$ treatment than in the control. Possibly it is due to known ability of sunflower to accumulate heavy metals from the soil (Azhar et al., 2006; Padmavathiamma and Li, 2006). Lupine was the most sensitive to lead addition to the soil. Its seed viability vas affected slightly, but the sprouts were two-fold smaller than those in the control.

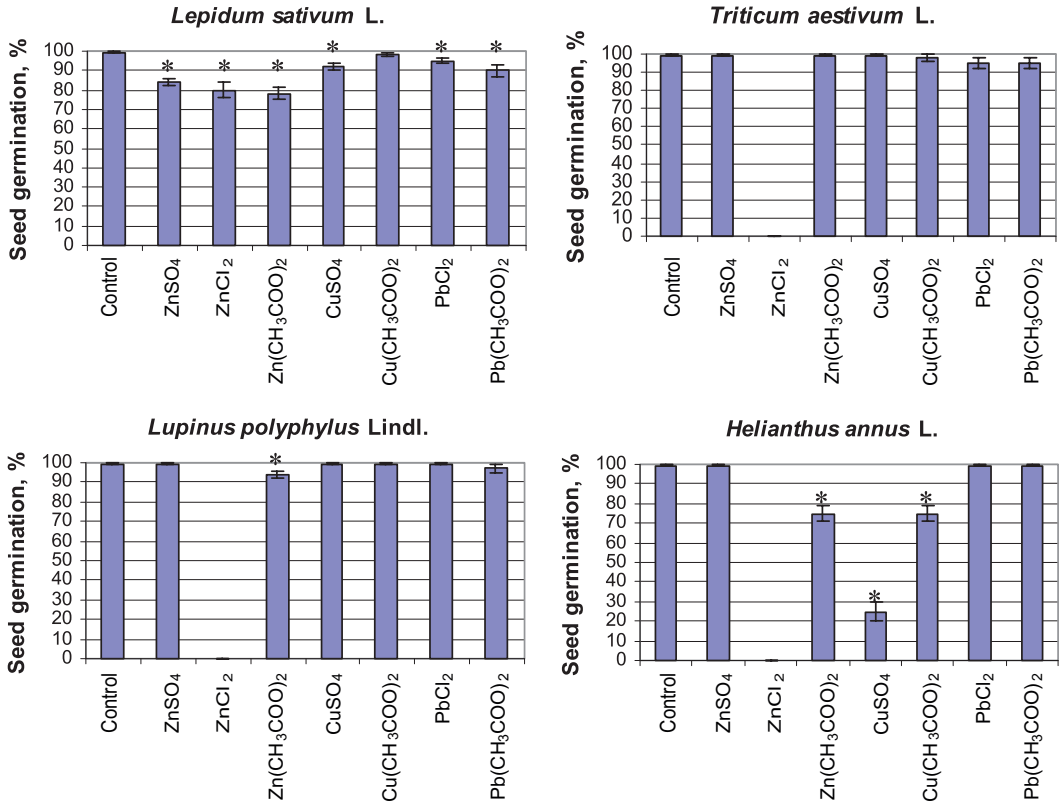


**Figure 1.**
Seeds viability (measured as percent of germination over five days) of crest (*Lepidum sativum*), wheat (*Triticum aestivum*), large-leaved lupine (*Lupinus polyphyllus*) and sunflower (*Helianthus annus*) (%) in soil amended with zinc [$ZnSO_4$ – 16.14, $ZnCl_2$ – 6.82, $Zn(CH_3COO)_2$ – 4.38 g $kg^{-1}$ of fresh weight soil], copper [$CuSO_4$ – 0.47, $Cu(CH_3COO)_2$ – 0.40 g $kg^{-1}$ of fresh weight soil] and lead [$PbCl_2$ – 6.95, $Pb(CH_3COO)_2$ – 8.13 g $kg^{-1}$ of fresh weight soil] salts and in soil without heavy metal-amendments (Control). Columns with an asterix indicate significant difference from the control treatments ($P \leq 0.05$).

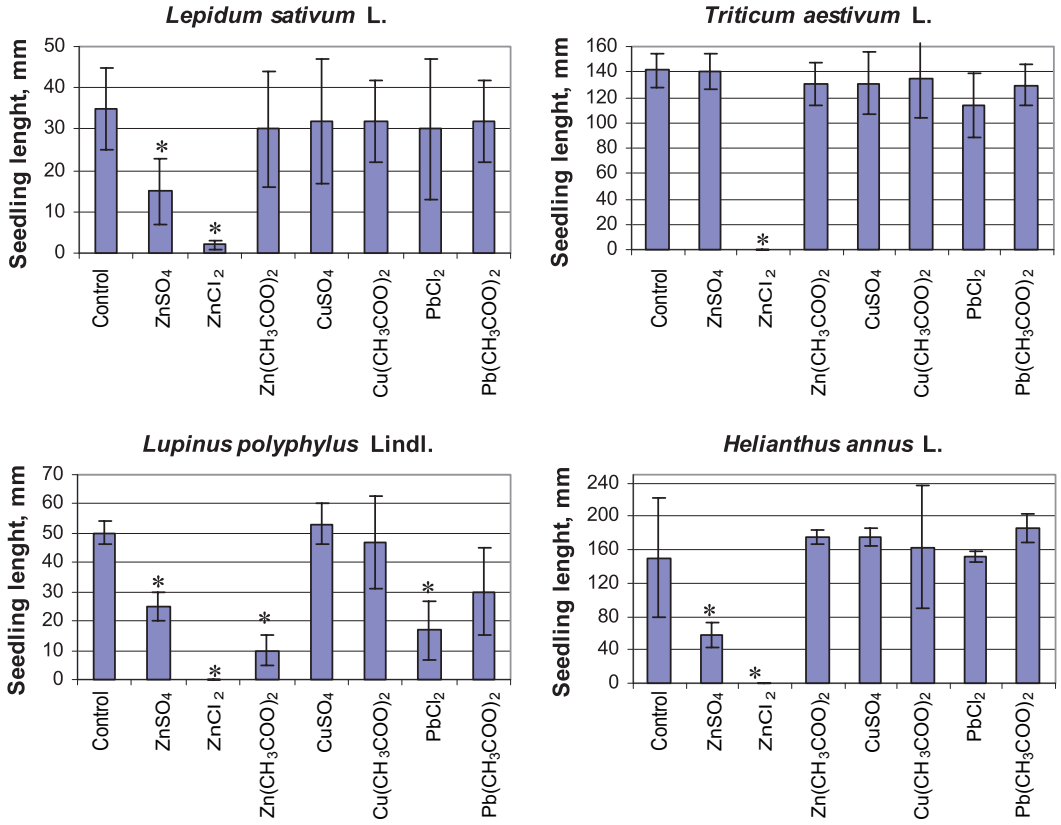


**Figure 2.**
Seedlings length (mm) of crest (*Lepidum sativum*), wheat (*Triticum aestivum*), large-leaved lupine (*Lupinus polyphyllus*) and sunflower (*Helianthus annus*) (%) in soil amended with zinc [$ZnSO_4$ – 16.14, $ZnCl_2$ – 6.82, $Zn(CH_3COO)_2$ – 4.38 g $kg^{-1}$ of fresh weight soil], copper [$CuSO_4$ – 0.47, $Cu(CH_3COO)_2$ – 0.40 g $kg^{-1}$ of fresh weight soil] and lead [$PbCl_2$ – 6.95, $Pb(CH_3COO)_2$ – 8.13 g $kg^{-1}$ of fresh weight soil] salts and in soil without heavy metal-amendments (Control). Columns with an asterix indicate significant difference from the control treatments ($P \leq 0.05$).

Fungal community structure. In total, 39 species of the fungi belonging to 24 genera were identified. A vide variety (36 species and two morphotypes) of fungi, isolated and identified from the initial soil samples (collected in field) and almost unchanged during the experiment in the control treatment is presented in Table 2. *Scytalidium aurantiacum*, *Mortierella alpina*, *Cylindrocarpon destructans*, *Penicillium expansum* and *Absidia glauca* folowed by *Penicillium thomi*, *Cladosporium cladosporioides* and *Fusarium oxysporum* were the most abundant species in the control. Changes of the fungus diversity were observed in the soil under a month impact of zinc, copper and lead salts: some species (*A. glauca*, *Acremonium kiliense*, *Aspergillus* spp., and *Alternaria alternata*) were eliminated from the fungus communities in soil amended with heavy metal salts, while the other species dominated. Zinc- and copper-resistant fungus species, survived in the polluted soil are listed in Table 3. Although total number of the fungal CFUs was higher in soil with heavy metal salts than in control soil, their diversity in the polluted soil was lower. Comparable results were obtained during our previous investigation of the deciduous forest soil under industrial pollution impact (Pečiulytė and Dirginčiutė-Volodkienė, 2009b) as well as in other investigations in case of arsenic-, chromium- and copper- contaminated soils (Turpeinen et al., 2004) and in soil affected by cadmium (Shentu et al., 2008). Lead salts (chloride and acetate) at used concentrations did not show significant impact on the soil fungi as well as on the plants' development. Fungal community structure was not affected by lead; therefore those results are not presented. Zinc salts additions to the soil (at 16.14, 6.82 and 4.38 g $kg^{-1}$ concentration of $ZnSO_4$; $ZnCl_2$ and $Zn(CH_3COO)_2$, respectively) led to fungus species diversity differences as compared with the control. The most abundant fungal isolates of the zinc-amended soil belonged to the *Paecilomyces farinosus* (syn: *Isaria farinosa* Holmsk.) species (Table 3). This species comprised 75.2, 37.8 and 71.4% of all fungi isolated from the soil with $ZnSO_4$, $ZnCl_2$ and $Zn(CH_3COO)_2$, respectively. Effect of $ZnSO_4$ and $Zn(CH_3COO)_2$ on the soil fungus structural characteristics was similar. Percentage of *P. farinosus*, *P. fumosoroseus*, and *Penicillium expansum* in the total number of CFUs in the soil with zinc salts was alike. *Lecanicillium lecanii* (syn. *Verticillium lecanii* (Zimm.) Viégas) dominated in soil with $ZnSO_4$ and *Clonostachys* sp. – in soil with $Zn(CH_3COO)_2$. Greater species diversity of the fungi was observed under impact of $ZnCl_2$. Complexes of the fungi, isolated from the soil samples incubated with copper salts (sulphate 0.47, chloride 0.40 and acetate 1.27 g $kg^{-1}$) differed from those isolated from the samples amended with zinc salts. It should be noted that some fungi isolated from the copper-amended soil did not sporulate in culture, remained unidentified and were assigned to the sterile mycelium morphotypes. They comprised 8.0; 6.1 and 12.3% of the total number of CFUs for $CuSO_4$, $CuCl_2$ and $Cu(CH_3COO)_2$ treatments, respectively. Effect of those salts on the soil fungus community structure differed marginally. *Fusarium oxysporum* dominated in the soil with $CuSO_4$; $CuCl_2$ or $Cu(CH_3COO)_2$ and comprised 11.8; 14.9 and 11.1% CFUs, respectively. *A. alternata*, the second copper-resistant species comprised 9.2; 10.1 and 10.5% in the $CuSO_4$; $CuCl_2$ and $Cu(CH_3COO)_2$ treatments, respectively and *Fusarium solani* – 6.7; 4.3 and 11.6% in the $CuSO_4$; $CuCl_2$ and $Cu(CH_3COO)_2$ treatments, respectively. *Cladosporium her-*

**Table2.** The list of fungal species isolated from arable soil on Czapek medium (CA), potato dextrose agar (PDA), Czapek Dox agar (CDA) and malt extract agar (MEA) and their abundance in the total number of isolated CFU (Colony forming units), %.

| Fungal species | % in the total number of isolated CFU |
|---|---|
| *Absidia glauca* Hagem | 5.1±0.5 |
| *Acremonium kiliense* Grütz | 2.6±0.6 |
| *Alternaria alternata* (Fr.) Keissl. | 0.3±0.1 |
| *Aspergillus fumigatus* Fresen. | 0.8±0.3 |
| *Cladosporium cladosporioides* (Fresen.) G.A. de Vries | 4.7±0.2 |
| *Cladosporium herbarum* (Pers.) Link | 0.9±0.1 |
| *Chaetomium globosum* Kunze | 1.0±0.4 |
| *Clonostachys rosea* f. *rosea* (Link) Schroers, Samuels, Seifert & W. Gams | 2.3±0.6 |
| *Clonostachys* sp.Corda | 1.8±0.4 |
| *Cylindrocarpon destructans* (Zinssm.) Scholten | 5.3±0.8 |
| *Cunninghamella echinulata* (Thaxt.) Thaxt. ex Blakeslee | 1.4±0.2 |
| *Fusarium oxysporum* Schltdl. | 4.1±0.7 |
| *Fusarium solani* (Mart.) Sacc. | 2.8±0.4 |
| *Gliomastix murorum* (Corda) S. Hughes | 3.5±0.6 |
| *Lecanicillium lecanii* (Zimm.) Zare & W. Gams | 2.8±0.3 |
| *Metarhizium anisopliae* (Metschn.) Sorokīn | 0.4±0.1 |
| *Mortierella alpina* Peyronel | 6.7±1.5 |
| *Mortierella verrucosa* Linnem. | 3.4±1.2 |
| *Mucor hiemalis* f. *hiemalis* Wehmer | 1.2±0.3 |
| *Mucor racemosus* Fresen. | 0.7±0.2 |
| *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm. | 1.4±0.2 |
| *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm. | 2.5±0.3 |
| *Penicillium chrysogenum* Thom | 1.3±0.2 |
| *Penicillium expansum* Link | 0.8±0.1 |
| *Penicillium funiculosum* Thom | 5.3±1.2 |
| *Penicillium glabrum* (Wehmer) Westling | 2.2±0.6 |
| *Penicillium janczewskii* K. M. Zalessky | 1.2±0.3 |
| *Penicillium oxalicum* Currie & Thom | 0.8±0.5 |
| *Penicillium restrictum* J.C. Gilman & E.V. Abbott | 2.5±0.4 |
| *Penicillium thomii* Maire | 4.8±1.1 |
| *Penicillium* sp. Link | 2.7±0.6 |
| *Scytalidium aurantiacum* Klingström & L. Beyer | 9.5±3.0 |
| *Trichoderma polysporum* (Link) Rifai | 1.2±0.6 |
| *Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx | 0.4±0.02 |
| *Trichoderma viride* Pers. | 2.7±1.2 |
| *Verticillium tenerum* Nees | 1.9±0.2 |
| Sterile mycelium – dark | 2.4±0.3 |
| Sterile mycelium – white | 1.1±0.5 |
| Nonidentified | 3.5±1.5 |
| Total number of CFU $g^{-1}$ dry weight soil x $10^3$ | 150±7 |

*barum* and *Paecilomyces lilacinus* dominated in the soil with $CuSO_4$ and $CuCl_2$, whereas *Clonostachys* sp. – in the soil with $Cu(CH_3COO)_2$ additions. *P. fumosoroseus* was found only in the soil with $CuSO_4$ addition (Table 2). Other fungal species (*Mucor hiemalis*, *Scytalidium aurantiacum*, *Phoma lingam,* and *Trichoderma viride*) were found in the soil affected with copper sulphate as well as chloride and acetate.

Single linkage dendrogram derived from the fungal species abundance in the communities data confirms high similarity of the communities of soil with $CuSO_4$ and $CuCl_2$ (Fig. 3). These salts showed the most similar interdependent effect on fungus diversity which was comparable with $Cu(CH_3COO)_2$ effect. Conversely, all three zinc salts influenced fungus diversity unlike: similarity of those communities was low. Moreover,

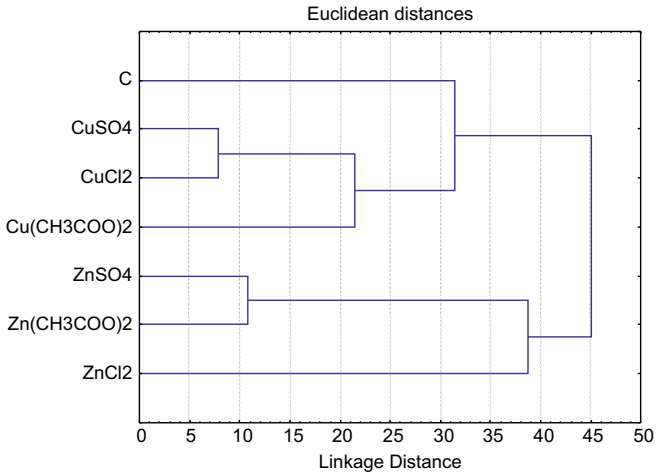


**Figure 3.**
Dendrogram of soil fungus communities under effect of zinc ($ZnSO_4$, $ZnCl_2$, $Zn(CH_3COO)_2$) and copper ($CuSO_4$, $CuCl_2$, $Cu(CH_3COO)_2$) salts. C – sample without effecting.

effect of zinc salts markedly differed from that of copper salts; they appear in different clusters of the dendrogram. The fungal communities of copper-amended soil were more similar with that of control, while the fungus communities of zinc-amended soil differed from the control in higher degree. Those results suggest that overall effect of copper to soil fungus community was less than effect of zinc in the concentrations used in the present experiment. Therefore effect of zinc was significantly dependent on the salt used. This is not so noticeable in case of copper.

Relationship of heavy metals, soil fungus community and plant growth. Copper is one of the microelements essential to plat growth at low concentration (Yruela, 2005). Concentration of copper used in the present investigation was unfavorable to tested plants with exception of lupine, which grew better in the soil with $CuSO_4$. It was determined that plant-associated fungi (potential plant pathogens such as *F. oxysporum, F. solani* and *P. lingam* as well as plant symbiotic fungi such as *T. viride*) survived and dominated in the soil with different copper salts. Possibly seed viability and plant development were affected by these fungi abundant in the pot soil. Special attention should be paid to the facultative plant pathogen *F. oxysporum*, which overgrew on the surface of not germinated seeds and poorly growing sprouts. This was observed only in the soil with copper additions independently on the salt used, thus this fungus could be the reason of development suppression in plants.

Plants and soil fungi were differently affected by zinc due to the salt form (acetate, sulfate or chloride) added to the soil. The greatest number of fungal species survived in the soil with $ZnCl_2$, though the affect to plants was huge: the seeds of wheat, lupine, and sunflower did not germinate, seeds of cress showed low viability and their sprouts grew very poorly. Controversially, other two zinc salts showed less affect on the plant development, however, less fungus diversity was determined. Such a great phytotoxic effect of $ZnCl_2$ could be explained by the high salt impact due to additional affect of $Cl^-$ ions. According to Marchner (1995), minimal requirement of chlorine for crop

**Table 3.** Abundance (percent in the total number of CFU) of the species of fungi, isolated from arable soil amended with zinc, and copper salts after long-term experiment. Fungi were isolated on Czapek medium (CA), potato dextrose agar (PDA), Czapek Dox agar (CDA) and malt extract agar (MEA). * Numbers in brackets – concentrations of salts, g $kg^{-1}$ of fresh-weight soil.

| Fungus species | $ZnSO_4$ (16.14*) | $ZnCl_2$ (6.82) | Zn $(CH_3COO)_2$ (4.38) | $CuSO_4$ (0.47) | $CuCl_2$ (0.40) | Cu $(CH_3COO)_2$ (1.27) |
|---|---|---|---|---|---|---|
| *Absidia glauca* Hagem | | | | 1.5±0.2 | 1.3±0.2 | 0.9±0.2 |
| *Alternaria alternata* (Fr.) Keissl. | | | | 9.2±0.8 | 10.1±1.0 | 10.5±1.2 |
| *Arthrinium sp.* Kunze | | 10.1±1.2 | 4.6±0.6 | | | |
| *Cladosporium herbarum* (Pers.) Link | | | | 10.3±2.2 | 8.5±1.8 | |
| *Clonostachys rosea* f. *rosea* (Link) Schroers, Samuels, Seifert & W. Gams | 4.6±0.4 | | | | | |
| *Clonostachys sp.* Corda | | 12.0±2.3 | 5.2±1.1 | | | 14.6±2.2 |
| *Cunninghamella echinulata* (Thaxt.) Thaxt. ex Blakeslee | | 5.2±1.2 | | 9.0±3.2 | 8.1±2.5 | 5.5±1.2 |
| *Fusarium oxysporum* Schltdl. | | | | 11.8±3.9 | 14.9±4.2 | 11.1±6.5 |
| *Fusarium solani* (Mart.) Sacc. | | | | 6.7±1.2 | 4.3±0.8 | 11.6±2.4 |
| *Gliomastix murorum* (Corda) S. Hughes | 4.1±0.9 | 7.4±1.2 | 6.8±1.0 | 2.0±0.7 | 1.7±0.5 | 1.1±0.4 |
| *Lecanicillium lecani* (Zimm.) Zare & W. Gams | 5.1±1.4 | 12.3±1.3 | | | | |
| *Mortierella alpina* Peyronel | | 3.7±0.7 | | | | |
| *Mucor hiemalis* f. *hiemalis* Wehmer | | 2.4±1.5 | | 9.1±3.5 | 7.1±2.0 | 6.2±1.5 |
| *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm. | 71.1±5.4 | 30.4±4.0 | 64.6±4.3 | | | |
| *Paecilomyces fumosoroseus* (Wize)A.H.S. Br. & G.Sm. | 12.2±1.4 | 4.5±0.9 | 12.2±1.0 | 3.3±0.1 | | |
| *Paecilomyces lilacinus* (Thom) Samson | | | | 9.2±0.2 | 10.1±0.1 | |
| *Penicillium expansum* Link | 2.0±0.3 | 4.2±0.3 | 3.9±0.3 | 1.0±0.1 | 0.8±0.1 | 1.1±0.1 |
| *Penicillium janczewskii* K. M. Zalessky | 0.9±0.2 | 2.0±0.1 | 2.7±0.2 | 0.9±0.1 | 1.0±0.2 | 1.3±0.1 |
| *Phoma lingam* (Tode) Desm. | | | | 7.3±2.5 | 7.5±2.9 | 5.1±2.1 |
| *Scytalidium aurantiacum* Klingström & L. Beyer | | | | 8.1±1.1 | 11.0±0.3 | 12.0±1.2 |
| *Trichoderma viride* Pers. | | | | 2.6±0.5 | 7.5±1.0 | 6.7±1.6 |
| *Umbelopsis ramanniana* (Möller) W. Gams | | 5.8±0.7 | | | | |
| Nonidentified | | | | 8.0±0.6 | 6.1±0.9 | 12.3±3.6 |
| Total number of CFU $g^{-1}$ dry weight soil x $10^3$ | 163±2 | 151±3 | 173±6 | 173±7 | 180±8 | 207±2 |

growth is 1 g $kg^{-1}$ dry weight soil. High $Cl^-$ concentrations can be toxic to plants (White and Broadley, 2001). Consequently 3.55 g $kg^{-1}$ of chlorine added to the soil in the form of $ZnCl_2$ could be toxic to plants in our research. The abundance of phytopathogenic fungi in those treatments was decreased and thus they could not be a reason of plant death. On the other hand, plant relationship with other soil microorganisms is important as well. For example, number of bacteria in soil with zinc chloride was significantly reduced during the present investigation (data not presented). Proportional decrease of bacteria, noticed by several researchers (Khan et Scullion, 2000; Rajapaksha et al., 2004; Turpeinen at al., 2004), is most likely due to the fact that fungi tend to be more resistant to heavy metals than bacteria. We did not investigate mycorrhizal microorganisms, which a great importance on plant physiology under enhanced concentrations of zinc is known (Cavagnaro et al., 2009). However, it should be noted that *Trichoderma* genus, stimulating plant development, was suppressed by the increased zinc concentration and that could be the reason of the affected plant development as well. Phytotoxic effect of $ZnSO_4$ and $Zn(CH_3COO)_2$ was much lower, although fungal diversity under effect of these salts was very poor.

Lead salts (chloride and acetate) at used concentrations did not show strong toxic effect on either the fungi or plants, differently as we expected. This could be due to the strong binding of lead compounds to organic matter in the soil. Moreover, adsorption of lead compounds by plants is highly dependent on soil pH. Blaylock et al. (1997) reported that in soil with a pH between 5.5 and 7.5 lead is little available to plants. Initial soil pH in this study was 6.89 (Table 1) and this could be a reason of relatively low phytotoxic effect of lead salts. It should be noted that heavy metal salts just slightly reduced soil pH: up to 6.60 and 6.74 in the cases with sulphate and acetate addition, respectively, and left unchanged in the samples where chlorides were used (pH 6.8). One of the reasons of such soil pH stability could be a buffering capacity of the soil (James, 2004). Microorganisms are far more sensitive to heavy metal stress than plants growing on the same soils (Giller et al., 1998). Despite the possible plant-resistance, plant pathogens resistant to appropriate metal could damage seedlings affecting their development during our investigation. Humus content comprising 14.5 % of soil could facilitate both plant seedlings' development and fungus viability and activity, and bind metal ions in its mass. Nutrient conditions (P and N content in the soil) were also beneficial for plats and fungi. Heavy metal pollution cannot only result in adverse effects on various parameters relating to plant quality and yield but also causes changes in the size, composition, and activity of the microbial community (Giller et al., 1998). Abiotic stress caused by heavy metals, in inorganic and organic forms, affects the growth, morphology, and metabolism of the microorganisms in soils (Liao and Xie, 2007).

## Conclusions

Heavy metals Cu, Zn and Pb applied either of sulphate, chloride or acetate salt in the soil influenced fungus community structure. Metal additions eliminated a wide variety of species, namely *Absidia glauca*, *Acremonium kiliense*, *Aspergillus fumigatus*, and *Alternaria alternata* that were common in the control soil.

Other species conversely dominated under heavy metal impact. Abundance of the dominating species increased total number of CFUs, despite the low species diversity. *Paecillomyces* genus dominated in the soil amended with either of Cu or Zn. *P. farinosus* and *P. fumosoroseum* followed by *Arthrinium* sp., *Clonostachys* sp., *Lecanicillium lecani*, and *Penicillium janczewskii* were Zn-resistant. *P. lilacinus*, plant pathogenic (*Fusarium oxysporum, F. solani* and *Phoma lingam)* and other fungi such as *Alternaria alternata*, *Cladosporium herbarum*, *Cunninghamella echinulata*, *Mucor hiemalis*, *Scytalidium aurantiacum* and *Trichoderma viride* were abundant in soil amended with Cu salts. Lead effect to soil fungus diversity was slight.

Zinc effect on the soil fungus community depended on the zinc salt (sulphate, chloride or acetate) used. Conversely, effect of the copper and lead did not depend on the salt used.

The poorest plant development evaluated by phytotoxicity test was observed in the soil with increased zinc concentration, medium – in soil with increased copper concentration, whereas effect of Pb to the tested plants was the least.

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