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Impact of copper and zinc on the growth of saprotrophic fungi and the production of extracellular enzymes

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To assess the impacts of Cu and Zn on fungi, we tested the growth of 18 taxonomically different saprotrophic (basidiomycete, ascomycete and zygomycete) fungi and their production of extracellular oxidative enzymes on Cu- (0, 100, 200, 400 mg kg^{-1}) or Zn- (0, 100, 200, 400 mg kg^{-1}) containing ABTS (2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) agar plates. *Coniothyrium* sp. was the most tolerant ascomycete and *Sordaria* sp. the most sensitive to both Zn and Cu. The growth of 8 basidiomyceteous fungi was reduced by 22%–100% on ABTS plates with 200 mg Cu kg^{-1}. Bioluminescent *Saccharomyces cerevisiae* yeast tests showed that Cu was 7-fold more toxic than Zn. Dose responses with respect to enzyme production varied among ascomycetous, zygomycetous and basidiomycetous fungi. Cu was more toxic than Zn for all tested fungi with all methods indicating that microbial functioning of soil is more vulnerable to Cu than Zn in the soil.

Introduction

Impacts of heavy metals (Cu and Zn) on biodiversity and function of fungal communities in contaminated soil are of concern. Cu and Zn are essential micronutrients, but above certain threshold concentrations they are toxic to both microbes and humans (Gadd 1993, Aelion *et al*. 2009). They are mined and used in industry worldwide, and are significant soil pollutants (4–6690 mg Cu kg^{-1} of soil and 16–9810 mg Zn kg^{-1} of soil) in mining, industrial and agricultural areas (Brock *et al*. 2006, Chopin and Alloway 2007, Martınez 2008, Coppolecchia *et al*. 2011, Fernandes-Calvino *et al*. 2010, Lei *et al*. 2010). In Finland, the mean values in the natural environment are 57 mg Zn kg^{-1} of soil and 20 mg Cu kg^{-1} of soil (Anon. 2007). Above-threshold concentrations of Cu and Zn can have deleterious effects on soil resources, and are a risk to the health of ecosystems and human populations in contaminated areas.

Bioavailability and microbial toxicity of Cu and Zn in soil are poorly characterized. Carbon and nutrient cycling is needed to maintain well balanced ecosystems. Basidiomycetous (Hatakka 1994) and ascomycetous (Durrens 1981, Kruus *et al*. 2003, Engh *et al*. 2007) fungi produce extracellular oxidoreductive enzymes that are involved in the cycling of carbon and other nutri-

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ents, and can depolymerize biopolymers and degrade recalcitrant xenobiotic compounds to carbon dioxide (Hatakka 1994). Heavy metals can decrease the ability of fungi to grow and produce extracellular enzymes (Gadd 1993, Baldrian 2003), and can consequently affect the cycling of carbon and nutrients. The response of a microbial population to toxic metals is characterized by a shift from bacteria- to fungi-dominated ecosystems (Chander *et al*. 2001, Khan and Scullion 2002, Turpeinen *et al*. 2004). Fungi are more resistant to metals than bacteria (Lechevalier 1977, Frostegård *et al*. 1993, Khan and Scullion 2002). Cu and Zn can hinder the carbon and nutrient cycles, causing deleterious impacts on the functioning and productivity of soil (van Beelen and Fleuren-Kemilä 1999). The growth of fungi and their production of extracellular enzymes associated with the carbon and nutrient cycles are good indicators of soil health. Little is known about the growth of fungi and their production of oxidative extracellular enzymes under heavy metal stress. Polluted soils often contain heavy metals, which can decrease the ability of fungi to degrade harmful xenobiotic compounds during bioremediation.

The aim of this study was to assess the effects of Cu and Zn on selected basidiomycetous, ascomycetous and zygomycetous fungi. We measured the growth of 18 saprotrophic fungi and changes in their production of extracellular oxidoreductive enzymes when grown in the presence of Cu and Zn on malt extract agar plates (MEA) containing 2,2´-azino-bis(3-ethylbentzthiazoline-6-sulfonic acid) (ABTS) as an indicator. To measure the bioavailability and toxicity of heavy metals, we used eukaryotic *Saccharomyces cerevisiae* yeast and procaryotic *Escherichia coli* bacteria, which have specific receptors for Cu and Zn.

Material and methods

Fungi and inocula

Nine isolates of the litter-decomposing saprotrophic ascomycetes representing the genera *Chaetomium*, *Gliocladium*, *Sordaria*, *Cylindrocarpon*, *Pyrenophora*, *Coniothyrium*, *Alternaria*, *Fusarium,* and *Epicoccum* and one isolate of a saprotrophic zygomycete belonging to the genus *Mortierella* were isolated from straw that had been buried over winter in arable soil in southern Finland. The straw was surface-sterilized with 70% EtOH and 2% hypochlorite and placed on agar plates containing 2% malt extract (MEA). Pure cultures of isolated fungi were maintained on 2% MEA and identified morphologically under an optical microscope. Pure cultures were stored on 2% MEA in sterile glass tubes at 4 °C.

Eight litter-decomposing basidiomycetous fungi were obtained from the Fungal Biotechnology Culture Collection (FBCC) at the Department of Food and Environmental Sciences, University of Helsinki, Finland, and the culture collection of the International Graduate Institute of Zittau, Germany. All the basidiomycetous fungi except *Agaricus bisporus* were selected for their ability to grow in heavy metal (Pb) contaminated soil, as described in Kähkönen *et al*. (2008). The selected basidiomycetous fungi included *Agaricus bisporus* ATCC 62459, *Agrocybe praecox* FBCC 476, *Gymnopus peronatus* FBCC 635 (syn. *Collybia peronata*), *Gymnopilus sapineus* FBCC 100, *Mycena galericulata* FBCC 598, *Gymnopilus luteofolius* X9, *Stropharia aeruginosa* FBCC 521 and *Stropharia rugosoannulata* FBCC 475 (Table 1). All the fungi except *S. rugosoannulata*, which colonizes straw, occur in boreal forests (Salo *et al*. 2006). The selected strains were maintained on 2% w/w malt extract agar. The inocula for the soil cultures were made from two week-old malt agar (2% w/w, Biokar Diagnostics) plate cultures: 5 plugs (10 mm diameter) of the fungus growing on agar were harvested and added to 200 ml sterilized (pH 5) basal liquid medium (Steffen *et al*. 2002) containing 2 g rye bran. The two week-old inoculum was homogenized by shaking with sterilized glass beads for 15 s.

Color plate tests

The production of extracellular oxidoreductive enzymes by 18 litter-decomposing saprophytic fungi was tested in the presence of Cu or Zn (0, 100, 200, 400 mg kg^{-1}) containing media. The basal medium plates for the basidiomyc-

etous fungi and the malt extract agar plates for the ascomycetous fungi contained 250 mg kg^{-1} ABTS (Sigma-Aldrich, USA). The diameters of the growth and the colored zones, which indicated the fungal extracellular enzymatic reactions, were measured and compared to those grown on control ABTS agar plates without added Cu or Zn.

Soil plate tests

Soil was sampled with a shovel from the humus layer (0–6 cm) in the podzolized *Pinus sylvestris* forest soil in southern Finland (60°13´N, 25°2´E) with no known point source of metal contamination. Cu and Zn contents were 15 and 50 mg kg^{-1} soil (w/w), respectively. Contents of Cu and Zn in the soil were measured using microwave nitric acid digestion and ICP mass spectroscopy as described in Kähkönen *et al*. (1998). Solutions of $CuCl_2$ or $ZnCl_2$ (3 ml) were added to 9 g w/w of the soil in a Petri dish (10 cm diameter) to achieve a final concentration of 100 mg kg^{-1} soil. Control soil cultures did not contain added Cu or Zn. Eight selected basidiomycetous fungi were grown in liquid culture for two weeks. Two milliliters of the liquid culture was then added to the soil. Three plates per treatment were incubated at 25 °C for 3 weeks. The growth of fungi was visually observed using a stereomicroscope, and growth in a plate was measured on an area basis. The carbon and nitrogen content in the soil were analyzed using an LECO CHN analyzer, as described by Kähkönen *et al*. (1998). Carbon and nitrogen contents (mean ± SD) in the humus layer soil were 40.6% ± 1.3% dw and 1.7% ± 0.1% dw, respectively. The soil pH (4.3 ± 0.2) was determined as described by Kähkönen *et al*. (2002).

Copper and zinc toxicity and copper activity measurements using yeast assays

Cu and Zn toxicity was measured using the bioluminescent yeast *Saccharomyces cerevisiae* strain BMA64/luc (Leskinen *et al*. 2005) by measuring the decrease in light emission. The synthetic minimal (SM) medium contained 6.7 g Difco yeast nitrogen base without amino acids (BD, USA) per 910 ml. The synthetic complex (SC) medium contained 6.7 g Difco yeast nitrogen base without amino acids and 1.4 g yeast synthetic drop-out mix (Sigma-Aldrich Co., St. Louis, USA) per 910 ml. The pH of each medium was adjusted to 5.6.

Cultivation of *S. cerevisiae* for toxicity assays was performed as described by Leskinen *et al*. (2005). An incubation time of 30 min was sufficient to detect toxicity among eight concentrations of Cu or Zn (50–5000 mg kg^{-1} of soil) with duplicate samples. Toxicity was tested by measuring the decrease in light emission relative to a control containing only water as the solvent. Growth inhibition of BMA64/luc yeast was tested in duplicate on SC agar plates at eight concentrations of Cu or Zn ranging from 50–5000 mg kg^{-1}. SC agar (1 ml) was added to

Table 1. The growth of eight litter-degrading basidiomycetous fungi (n = 3) in soil contaminated with Cu or Zn (100 mg kg^{-1} of soil) or in non-contaminated soil.

Fungus	Cu-contaminated soil	Zn-contaminated soil	Non-contaminated soil
Agaricus bisporus ATCC62459	+	+	+
Agrocybe praecox FBCC 476	++	++	+
Gymnopus peronatus FBCC 635	–	+	–
Gymnopilus sapineus FBCC 100	–	+	+
Mycena galericulata FBCC 598	+	+	+
Gymnopilus luteofolius X9	+	+	+
Stropharia aeruginosa FBCC 521	+	+	–
Stropharia rugosoannulata FBCC 475	+	+	+
Control soil without inoculum	–	–	–

– = No growth, + = weak growth, ++ = moderate growth.

a well of a 24-well microtiter plate, and 20 μl of diluted overnight yeast culture was spread on the agar surface. The plate was incubated at 30 °C, and growth inhibition was visually assessed after 24 and 48 h. The EC_{50} value was calculated by fitting the curve to the results from the inhibition of light emission of *S. cerevisiae* at the various metal concentrations. EC_{50} value expresses concentration, where effect is 50 % of that in the control without added Cu or Zn.

Copper and zinc toxicity and activity measurements in the bacterial assay

Metal toxicity was measured using *Escherichia coli* strain pDNlux (J. Rajasärkkä unpubl. data), which constitutively expresses bacterial luciferase. The toxicity of soil containing various concentrations (8) of Cu or Zn ranging from 50–5000 mg kg^{-1} of soil was tested in duplicate by measuring the decrease in light emission relative to a control containing only water as the solvent.

The biological activity and toxicity assays were performed using a heavy metal morpholinepropanesulfonic acid (MOPS/HMM) medium supplemented with 0.5% (wt/vol) casein hydrolyzate. Freeze-dried *E. coli* cells were used (Tauriainen *et al*. 1998). An ampoule of freeze-dried cells was rehydrated in 6 ml HMM medium and incubated at room temperature for 2 h. For each test (carried out in triplicate) the rehydrated cells (50 μl) were mixed with 50 μl of sample in a well of a 96-well plate, and the plate was incubated at 30 °C for 2 h. The luminescence in the wells was measured using a Victor3 1420 multilabel counter (Perkin-Elmer Wallac, Turku, Finland) in a luminescence mode using 1-s counting time. The EC_{50} value was calculated by fitting the curve to the results from the inhibition of light emission of *E. coli* at the various metal concentrations. EC_{50} value expresses concentration, where effect is 50% of that in the control without added Cu or Zn.

Statistical tests

For each fungus a *t*-test (Sokal and Rolf 1969) was used to test the significance of differences in fungal growth or enzyme production (color formation zone) on ABTS plates containing Cu or Zn compared with that on ABTS plates without added Cu or Zn. Statistical calculations were performed using the Excel (Microsoft) program. The suitability of the data was tested using the Shapiro-Wilk test (Sokal and Rolf 1969).

Results

Growth of the tested ascomycetous fungi decreased by 4%–98% in the presence of 100 mg Cu kg^{-1}, and by 4%–78% in the presence of 100 mg Zn kg^{-1} (Fig. 1). The only exception was growth of *Coniothyrium* sp., which increased by 40% in the presence of 200 mg Zn kg^{-1}.

The levels of enzyme production in the presence of Cu or Zn varied among the ascomycetous fungi (Fig. 2). As compared with the control, the zone of color formation increased by 15%–27% for *Chaetomium* sp., *Epicoccum* sp. and *Cylindrocarpon* sp. in the color agar plates containing 100–200 mg Cu kg^{-1}, but decreased by 20%–40% for *Coniothyrium* sp. and 63%–100% for *Sordaria* sp. and *Pyrenophora* sp. in this Cu concentration range. *Fusarium* sp., *Gliocladium* sp. and *Mortierella* sp. did not show any color reaction at any Cu concentration tested on ABTS agar plates. As compared with the control, the zone of color formation increased by 12%–62% for *Sordaria* sp., *Alternaria* sp., *Mortierella* sp., *Cylindrocarpon* sp. and *Chaetomium* sp. in the color ABTS agar plates containing 100 mg Zn kg^{-1}. Production of oxidative enzymes by *Epicoccum* sp. increased by 40% on the color agar plates containing 100–400 mg Zn kg^{-1}. For *Coniothyrium* sp. and *Pyrenophora* sp., the color zone formation decreased by 17%–31% on the ABTS color agar plates containing 100 mg Zn kg^{-1}. *Gliocladium* sp. did not show any color reaction in the presence of Zn.

The growth of all eight litter-decomposing basidiomycetous fungi was dramatically inhibited (22%–100%) in the presence of 200 mg Cu kg^{-1} (Fig. 3). In contrast, on the plates containing 100 mg Zn kg^{-1}, *A. praecox*, *G. peronatus*, *G. sapineus*, *M. galericulata*, *S. aeruginosa* and *S. rugosoannulata* increased their growth by 2%–272%.

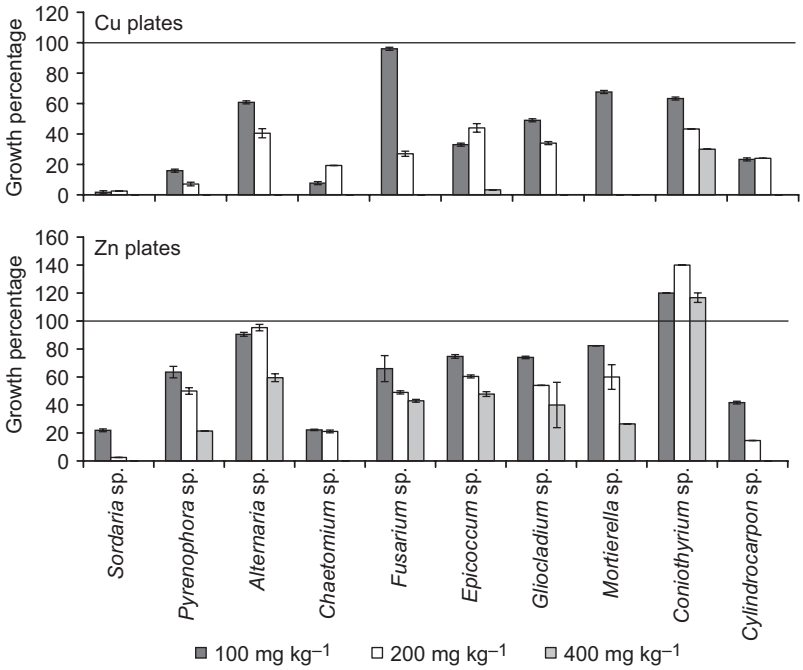

Fig. 1. Growth of nine ascomyceteous and one zygomycetous fungi in the presence of Cu or Zn (100, 200, 400 mg kg^{-1}) on ABTS malt extract agar plates (n = 3). The error bars indicate standard deviations; horizontal line = control (0 mg kg^{-1}). Growth of all fungi was significantly different (p < 0.05) from controls grown in the absence of added Cu or Zn.

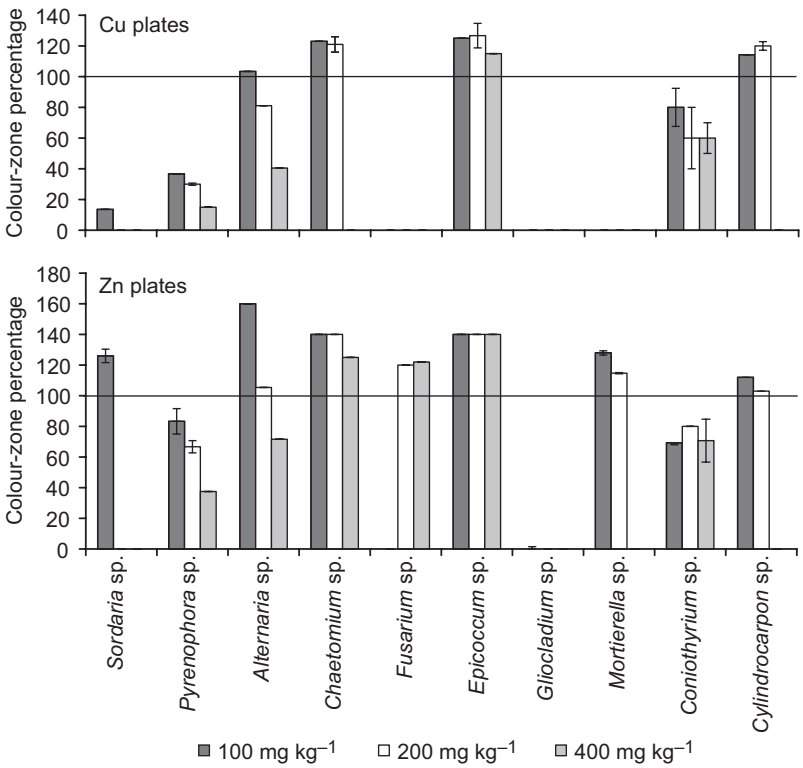

Fig. 2. Enzyme production (as measured by color zone formation) of nine ascomycetous and one zygomycetous fungi in the presence of Cu or Zn (100, 200, 400 mg kg^{-1}) on ABTS malt extract agar plates (n = 3). The error bars indicate standard deviations; horizontal line = control (0 mg kg^{-1}). Enzyme production by all fungi was significantly different (p < 0.05) from controls without added Cu or Zn.

Growth of basidiomycetous fungi was tested on the Cu and Zn containing soil plates. The best-growing fungus was *A. praecox*, both in the presence of Cu- and Zn-contaminated soil (Table 1). *Mycena galericulata* and *S. rugosoannulata* grew in the Cu- or Zn-contaminated soil. Neither

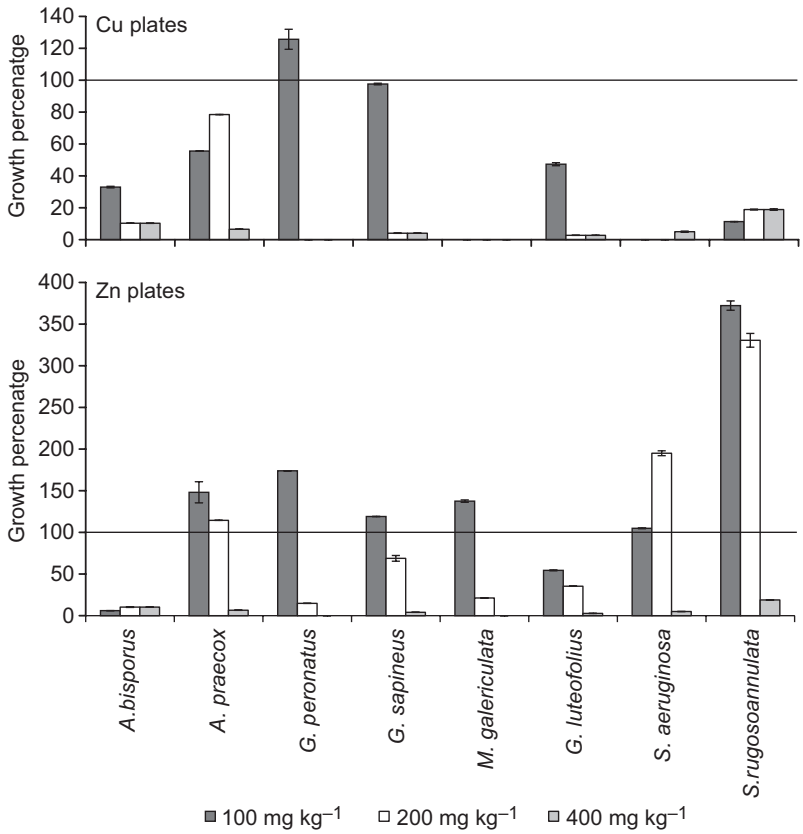

Fig. 3. Growth of eight basidiomycete fungi was tested in the presence of Cu or Zn (100, 200, 400 mg kg^{-1}) on ABTS malt extract agar plates (n = 2). The error bars indicate standard deviations; horizontal line = control (0 mg kg^{-1}). Growth of all fungi was significantly different ($p < 0.05$) from controls grown in the absence of Cu or Zn.

G. peronatus nor *G. sapineus* grew in the Cu-contaminated soil.

The toxicity of Cu, determined by the growth of *S. cerevisiae* (BMA64/luc) in the plate tests (EC_{50} = 75 mg kg^{-1} soil), was up to an order of magnitude higher than the toxicity determined by the production of bioluminescence by *S. cerevisiae* (EC_{50} = 550 mg kg^{-1} of soil) or *E. coli* (pDNlux) (EC_{50} = 260 mg kg^{-1} of soil) (Table 2). The toxicity of Zn, determined by measuring bioluminescence from *E. coli* (pDNlux) bacteria (EC_{50} = 60 mg kg^{-1} of soil), was 1–2 orders of magnitude higher than the toxicity determined by measuring bioluminescence from *S. cerevisiae* yeast (EC_{50} = 3500 mg kg^{-1} of soil) and the growth of *S. cerevisiae* in plate tests (EC_{50} = 1750 mg kg^{-1} of soil). The toxicity of Cu was 7–20 times greater than that of Zn, as determined by the corresponding *S. cerevisiae* yeast tests.

Table 2. EC_{50} values for the Cu receptor-containing bioluminescent bacterium *E. coli*, the Zn receptor-containing bioluminescent bacterium *E. coli*, the Cu or Zn receptor-containing bioluminescent yeast *S. cerevisiae*, and the growth of the bioluminescent yeast *S. cerevisiae*. All values are mg kg^{-1} of soil.

	EC_{50}-[Cu]	EC_{50}-[Zn]
Bioluminescent test		
S. cerevisiae (BMA64/luc)	550	3500
E. coli (pDNlux)	260	60
Growth test in a plate		
Inhibition of growth of *S. cerevisiae*	75	1750

Discussion

Growth of the nine ascomycetous fungi and one zygomycetous fungus decreased by 4%–98% on Cu or Zn (100 mg kg^{-1}) containing ABTS plates. The only exception was *Coniothyrium* sp., which showed increased growth in the presence of 100–400 mg Zn kg^{-1}. Tolerance of *Coniothyrium* sp. to Zn is an advantage in the Zn-contaminated soil, where it may became the dominant

fungus. The toxic effects of heavy metals on fungal growth and development vary among species (Gadd 1993, Plaza *et al*. 1998). In an agar plate assay, Arnebrant *et al*. (1987) tested the effect of Cu (0–1600 mg kg^{-1}) on growth of various ascomycetous fungi, and reported EC_{50} values for *Beauveria bassiana* (EC_{50} = 250 mg kg^{-1}), *Verticillium* spp. (EC_{50} = 170–400 mg kg^{-1}), *Penicillium brevicompactum* (EC_{50} = 490 mg kg^{-1}), *Mortierella* sp. (EC_{50} = 41–65 mg kg^{-1}), *Oidiodendron* sp. (EC_{50} = 87 mg kg^{-1}) and *Penicillium* sp. (EC_{50} = 44–98 mg kg^{-1}). A novel finding of the present study was that growth of the ascomycetes *Chaetomium* sp., *Gliocladium* sp., *Sordaria* sp., *Cylindrocarpon* sp., *Pyrenophora* sp., *Coniothyrium* sp., *Alternaria* sp., *Fusarium* sp. and *Epicoccum* sp. was affected by Cu, as was observed for the other ascomycetous species tested by Arnebrant *et al*. (1987). The novel finding is that growth of the ascomycetes, *Chaetomium* sp., *Gliocladium* sp., *Sordaria* sp., *Cylindrocarpon* sp., *Pyrenophora* sp., *Coniothyrium* sp., *Alternaria* sp., *Fusarium* sp., and *Epicoccum* sp., in the present study was sensitive to Zn even at a slightly higher Zn concentration, which equals the mean value (57 mg Zn kg of $soil^{-1}$) in the natural Finnish environment (Anon. 2007). Our results indicate that ascomycetes *Chaetomium* sp., *Gliocladium* sp., *Sordaria* sp., *Cylindrocarpon* sp., *Pyrenophora* sp., *Coniothyrium* sp., *Alternaria* sp., *Fusarium* sp. and *Epicoccum* sp. are vulnerable even in slightly Cu- or Zn-contaminated soils.

Toxicity of Cu and Zn was determined by measuring the bioluminescence of *S. cerevisiae* and *E. coli* in the presence of these metals in soil. Cu was 7–20 times more toxic than Zn in the corresponding *S. cerevisiae* yeast tests, whereas Zn was 4 times more toxic than Cu in the *E. coli* bacterial test. Growth of *S. cerevisiae* yeast in plate tests was more sensitive to Cu and Zn than inhibition of bioluminescence in the *S. cerevisiae* yeast tests. Luciferases are suitable for measuring toxicity because signal production depends on the presence of viable cells containing cellular ATP (Fan and Wood 2007). Furthermore, the signal degenerates rapidly upon cell death. The genes involved in Cu resistance in yeasts include the metallothionein protein CUP2, which is a Cu-binding factor (Jin *et al*. 2008), and CUP1, which binds both Cu and Zn (Stroobants *et al*. 2009). Zn resistance may be conferred by several genes that function mainly in the processes of vacuole organization and biogenesis (Jin *et al*. 2008). Cu inhibited growth of the basidiomycetous and ascomycetous fungi in the ABTS plate tests (200–400 mg Cu kg^{-1}). Ni inhibited growth of *A. bisporus*, *A. praecox*, *G. peronatus*, *G. sapineus*, *M. galericulata*, *G. luteofolius*, *S. aeruginosa* and *S. rugosoannulata* at lower concentrations (20 mg kg^{-1}) (Lankinen *et al*. 2011) than those of Cu. This indicates that Cu is less toxic than Ni to the tested basidiomycetes. Soares *et al*. (2003) measured toxicity of Cu to starved yeast cells in solution, and reported an EC_{50} value of 200 mg Cu dm^{-3}. This value was lower than the EC_{50} value measured for the inhibition of bioluminescence for *S. cerevisiae* yeast in the soil tests reported here, because of a lower bioavailability of Cu in soil than in solution due to binding of Cu into the soil particles.

Our results showed that among the basidiomycetes tested, *A. praecox* grew best in Cu or Zn containing soil plates. Kähkönen *et al*. (2008) showed that *A. praecox* and *G. peronatus* were the best growing fungi in Pb-contaminated soil (40 mg Pb kg^{-1} of soil). The contamination limits set by the Finnish Government are 150–200 mg Cu kg $soil^{-1}$ and 250–400 mg Zn kg $soil^{-1}$ (Anon. 2007). The mean values in the natural Finnish environment are 57 mg Zn kg^{-1} soil and 20 mg Cu kg^{-1} soil (Anon. 2007). Soils polluted with organic compounds commonly also contain heavy metals, which decrease the ability of fungi to degrade xenobiotic compounds during bioremediation (Gadd 1993, Baldrian 2003, Park *et al*. 2009). The ascomycete *Fusarium oxysporum* GJ4 is able to use phenol as a sole carbon source, but is unable to degrade phenol at concentrations greater than 126 mg Cu_2O dm^{-3} in a liquid growth medium (Park *et al*. 2009). Mineralization of phenanthrene was reported to decrease in soil contaminated with 700 mg Cu kg^{-1} of soil as compared with that in non-contaminated soil (Sokhn *et al*. 2001). Cu and Zn can affect competition between indigenous soil fungi and fungal inocula during bioremediation. Resistant fungal species are commonly present at low rates of occurrence in non-contaminated soils, but can become dominant in soils contaminated with

toxic metals (Kunito *et al*. 1998). Amongst the fungi tested, the basidiomycetes were more tolerant to Zn than the ascomycetes and the zygomycete, and exhibited even increased growth in plates containing Zn at concentrations of 100–200 mg kg^{-1}. However, in plates containing 400 mg Zn kg^{-1}, growth of all ascomycetes and the zygomycete was greater than that of the basidiomycetes. The results indicate changes in the fungal communities exposed to varying concentrations of Zn, and differences in usefulness of ascomycetes, zygomycetes and basidiomycetes in bioremediation of Zn-contaminated soils. All of the ascomycetes and the zygomycete included in the study showed similar or better tolerance to Cu than the basidiomycetes tested, indicating that ascomycetes and the zygomycete are more suitable than basidiomycetes for the bioremediation of xenobiotics in the Cu-contaminated soil. Cu was more toxic than Zn to the ascomycetous and basidiomycetous fungi tested in the present study. Therefore, Cu may have a greater effect than Zn on the competition between fungal species, and on the structure of fungal communities in contaminated soil.

Conclusions

Eighteen taxonomically distinct fungi belonging to the ascomycetes, zygomycetes or basidiomycetes were tested for growth in the presence of Cu or Zn contamination. Growth of the ascomycetous fungi and the zygomycetous fungus was less affected by Zn than Cu indicating that the ascomycetous fungi and the zygomycetous fungus are more suitable for bioremediation of xenobiotics in the Zn than Cu contaminated soil. *Sordaria* sp. was the most sensitive and *Coniothyrium* sp. was the most tolerant ascomycetous fungus to both Zn and Cu in the ABTS plate cultures. Our results show that the basidiomycetous fungi tolerated lower Zn concentrations better than the ascomycete and zygomycete fungi, but the opposite occurred at higher Zn concentrations. This indicates that changes in fungal communities occur with changing Zn concentrations. The bioluminescent bacterium *E. coli* was up to 60-fold more sensitive to Zn than the bioluminescent yeast *S. cerevisiae*, indicating that fungi (yeasts) are more tolerant to Zn than bacteria. Cu was 7–20 fold more toxic than Zn, as evidenced by the *S. cerevisiae* yeast tests. All tested fungi groups (ascomycetes, zygomycetes and basidiomycetes) tolerated Zn better than Cu.

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