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Research Article

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Impact of Cadmium, Chromium, Cobalt, Lithium and Manganese to the Growth of Fungi and Production of Enzymes

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Abstract

Impacts of metals to the growth and enzyme production of fungi were tested with Cd- (0-10 mg kg–1), Co- (0-100 mg kg–1), Cr- (0-100 mg kg–1), Li- (0-100 mg kg–1) or Mn- (0-400 mg kg–1) containing media with ABTS [2,2'-azino-bis (3-ethylbentzthiazoline-6-sulfonicacid)] as indicator of oxidative enzymes, laccase and/or peroxidases. All ascomycetes *Alternaria* sp., *Chaetomium* sp., *Epicoccum* sp., *Fusarium* sp. and *Trichoderma* harzianum, and basidiomycetes *Agrocybe praecox, Pleurotus pulmonarius, Phlebia radiata, Physisporinus rivulosus* and *Stropharia rugosoannulata*, fungi grew in the presence of metals. The growth of *A. praecox* was tolerant to Mn, Cr and Li. The color reaction with ABTS was present in all basidiomycetes with or without metals indicating production of oxidative enzymes. Ascomycetes did not show color zone formation with Mn, Cd, Li and without metals. *Alternaria* sp., *Chaetomium* sp., *Epicoccum* sp. and *Trichoderma harzianum* formed color zone with Co and Cr indicating enzyme production.

Keywords

Metals; Ascomycets; Basidiomycetes; Oxidative enzymes; Fungi

Highlights

I) The color reaction with ABTS was seen in all basidiomycetes without or with metals (Cd, Co, Cr, Li, Mn) indicating tested basidiomycetes produced oxidative enzymes.

II) All tested ascomycetes did not show color zone formation with Mn, Cd, Li and without metals indicating that ascomycetes did not produce oxidative enzymes.

III) The growth of *A. praecox* was tolerant to Mn, Cr and Li.

Introduction

Cadmium (Cd), chromium (Cr), cobolt (Co), lithium (Li) and manganese (Mn) are metals, which are in concern in the polluted environment. These metals have contaminated mining and industrial sites and their surroundings (0.5-4.2 mg Cd/kg soil^{-1}, 18-5750 mg Cr/kg soil^{-1}, 6^{-1}7 mg Co/kg soil^{-1}, 11-71 mg Li/kg soil^{-1} and 220^{-1}0400 mg Mn/kg) [1-6]. Cd, Cr and Li are used in electronic equipments and batteries [7,8]. Co is used in diverse products and materials including batteries, glass and polyester. Cr, Mn and Cd are released into the environment from fertilizers [9,10]. These five metals (Cd, Cr, Co, Li and Mn) are used in large scale globally.

Ligninolytic wood-rotting and litter-decomposing basidiomycetous fungi and also some ascomycetes produce different sets of extracellular oxidative enzymes consisting of peroxidases (lignin, manganese and versatile peroxidase) and laccase, which can depolymerize lignin, cellulose, hemicellulose and starch, and degrade recalcitrant xenobiotic compounds all the way to carbon dioxide [11-17]. White-rot fungi, such as *Phanerochaete chrysosporium, Phlebia radiata* and *Physporinus rivulosus* and several others, and litter decomposing basidiomycetous fungi *Stropharia* spp. and *Agrocybe praecox* produce lignin degrading enzymes [17-19]. Ascomycetes degrade mainly carbohydrates in soil and litter, but some ascomycetes can also degrade lignin [17,20-22]. Ascomycetes are the dominant fungi in agricultural soils and basidiomycetes are dominant in forest soils. The *Ascomycota* form the largest number of fungal clades with separate hyphae in the Kingdom Fungi. These clades have been differentiated from the *Basidiomycota* clade probably before Devon [23,24]. The ascomycetes represent the largest number of fungal species [25-27]. Heavy metals can decrease the ability of fungi to grow and produce extra- and intracellular enzymes and to bioremediate polycyclic aromatic hydrocarbons (PAHs) in the heavy metal contaminated soils [28-31]. Only a little is known about impacts of Cd, Cr, Co, Li and Mn on the growth and oxidative enzyme production of ascomycetous and basidiomycetous fungi. Particularly impact of metals to ascomycetous fungi is very little known. Fungi are crucial in the degradation of detritus and in the cycling of carbon in the soil in well-balanced ecosystems, and therefore their response to metal load in soil is important in soil health globally.

Five ascomycetous and basidiomycetous fungi were tested in the present study. Tested basidiomycetes *Phlebia radiata*, *Pleurotus pulmonarius* and *Physisporinus rivulosus* are wood decaying white-rot fungi found in boreal forests [32,33]. The litter-degrading basidiomycete *Agrocybe praecox* is a common inhabitant of bark mulch or wood chips [33]. The basidiomycete *Stropharia rugosoannulata* grows on wood chips, in gardens, and in other cultivated areas [34]. Tested ascomycetes *Alternaria* sp., *Chaetomium* sp., *Epicoccum* sp., *Fusarium* sp. and *Trichoderma harzianum* are common litter-degrading microfungi in boreal agricultural and forest soils [35].

To find out, if metals (Cd, Cr, Co, Li, Mn) have impact on the fungal growth and production of fungal extracellular enzymes, we measured the impact of selected metals (Cd, Cr, Co, Li, Mn) on the growth and enzyme production of five ascomycetous and five basidiomycetous fungi on the contaminated malt extract agar with ABTS [2,2'-azino-bis (3-ethylbentzthiazoline-6-sulfonic acid)] as an indicator.

The aim of the study is to assess impacts of metals (Cd, Cr, Co, Li, Mn) on the growth and the extracellular oxidoreductive enzyme production of five ascomycetous and five basidiomycetous fungi.

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Materials and Methods

Fungi

The selected ascomycetous fungi belonging to the genera *Alternaria* (HAMBI 3289), *Chaetomium* (HAMBI 3290), *Epicoccum* (HAMBI 3291) and *Fusarium* (HAMBI 3292) were identified morphologically on the level of genera by using an optical microscope. These fungi were cultivated from small fragments of hyphae isolated from straw residuals that were buried over winter in arable soil in southern Finland. Buried barley straw residuals were surface sterilized with 70% (v/v) EtOH and 2% (v/v) hypochlorite before placement on growth media containing 2% (w/v) malt extract. Isolated fungi were maintained on 2% (w/v) malt extract agar (MEA) as pure cultures. Pure cultures of identified ascomycetous fungi were stored on 2% (w/v) MEA in sterile glass tubes at +4° C. The four selected ascomycetous fungi are stored in HAMBI culture collection at the Department of Food and Environmental Sciences at University of Helsinki in Finland. Ascomycete *Trichoderma harzianum*, strain T22, is commercially available fungus, which is commonly used as biocontrol against several fungal soil borne plant pathogens.

The five selected basidiomycetous fungi were obtained from the Fungal Biotechnology Culture Collection (FBCC) at the Department of Food and Environmental Sciences at University of Helsinki in Finland. The basidiomycetes were *Agrocybe praecox* FBCC 476 (TM70.84), *Pleurotus pulmonarius* FBCC1465 (ESH1), *Phlebia radiata* FBCC43, *Physisporinus rivulosus* FBCC939 (T241i) and *Stropharia rugosoannulata* FBCC 475 (DSM 11372). They were maintained on 2% (w/v) MEA in sterile glass tubes at +4°C.

Indicator color plate tests

The ability of five ascomycetous and five basidiomycetous fungi to grow and produce extracellular oxidoreductive enzymes was tested with Cd- (0, 2, 5, 10 mg kg^{-1}), Co- (0, 20, 50, 100 mg kg^{-1}), Cr- (0, 20, 50, 100 mg kg^{-1}), Li- (0, 20, 50, 100 mg kg^{-1}) or Mn- (0, 10, 50, 100, 400 mg kg^{-1}) containing media. Used chemicals were $Cd(NO_3)_2$•4 H_2O, $CoCl_2$•6 H_2O, $KCr(SO_4)_2$•12 H_2O, Li_2SO_4•H_2O and $MnSO_4$•4 H_2O. The media contained malt extract agar (MEA, DIFCO) and 250 mg kg–1 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Sigma-Aldrich, USA) in the Petri dish plates. All were done as three replicates. Indicator color plate tests were incubated five days at 20 ºC. The diameters of the colored zones (due to the oxidation of ABTS to corresponding cation radical) and the growth of fungi in the plates were measured manually between 90° angels, which the average was calculated in each plate. The intensity of colour was not measured. The formation of the colored zones indicated the fungal extracellular enzymatic reactions.

Statistical tests

To find out statistically significant differences between the growth and enzyme production (color formation zone) of each fungus in the metal (Cd, Co, Cr, Li or Mn) -containing ABTS agar plates compared to the ABTS agar plates without added metal for a certain fungus species ($p<0.05$), a t-test was done [36]. All statistical tests were performed with Excel (Microsoft) program.

Results

All five basidiomycetous, *A. praecox, P. radiata, P. rivulosus, P. pulmonarius* and *S. rugosoannulata*, fungi grew in the presence of all tested metals (Cd, Cr, Co, Li, Mn) (Figure 1). The results show that the growth of *S. rugosoannulata* decreased most (45-53%) among five basidiomycetous fungi with 10 mg Mn/kg and with 20-100 mg Cr/kg compared to the non contaminated plates indicating that *S. rugosoannulata* was sensitive to Mn and Cr. The growth of *P. radiata* decreased most among five basidiomycetous fungi with 2 mg Co/kg (63%) and with 100 mg Li/kg (88%). The growth of *P. pulmonarius* decreased most (100%) among five fungi with 20 mg Co/kg. The results indicate that the growth of the basidiomycetes was most sensitive to Cd (2 mg kg^{-1}) and Li (100 mg kg^{-1}) in the case of *P. radiata* and to Co (20 mg kg^{-1}) with *P. pulmonarius*. The growth of *A. praecox* remained same or even increased with Cr (20-100 mg kg^{-1}), Li (20-100 mg kg^{-1}) and Mn (10-400 mg kg^{-1}) compared to the non contaminated plates indicating that *A. praecox* was tolerant to Cr, Li and Mn. The growth of *P. pulmonarius* remained same with Li (20-100 mg kg^{-1}) and Mn (20-400 mg kg^{-1}) indicating that *P. pulmonarius* was tolerant to Li and Mn.

All five ascomycetes, *Alternaria* sp., *Chaetomium* sp., *Epicoccum* sp., *Fusarium* sp. and *Trichoderma harzianum*, grew in the presence of each of the five tested metals (Figure 2). The growth of all tested ascomycetous fungi was similar or even benefitted from Mn (10-100 mg kg^{-1}) compared to without Mn (Figure 2) indicating that all five ascomycetous fungi tolerated Mn. The growth of *Chaetomium* sp. among tested ascomycetes decreased most (12%) with 20 mg Cr kg^{-1}, (53%) 2 mg Co kg^{-1} and (25%) 100 mg Li kg^{-1}. The growth of *Alternaria* sp. among tested ascomycetes decreased most (60%) with 2 mg Cd kg^{-1}. The results indicate that the growth of tested ascomycetes was most sensitive to Cr, Co and Li in the case of *Chaetomium* sp. and to Cd with *Alternaria* sp. The growth of *Trichoderma harzianum* was similar with Cd (2-10 mg kg^{-1}), Co (20-100 mg kg^{-1}) and Li (20-100 mg kg^{-1}) compared without metal indicating that *Trichoderma harzianum* tolerated Cd, Co and Li.

The color zone formation showing the oxidation of ABTS to cation radical was seen with all five tested basidiomycetes *A. praecox*, *P. radiata*, *P. rivulosus*, *P. pulmonarius* and *S. rugosoannulata* fungi with metals (Cd, Cr, Co, Li, Mn) and without tested metals indicating that all five basidiomycetes produced oxidative enzymes (Figure 3). The color zone formation of *A. praecox* remained stable or even increased with Cd (2-10 mg kg^{-1}), Co (20-100 mg kg^{-1}) and Li (20-100 mg kg^{-1}) compared to the plates without added metals indicating that enzyme production of *A. praecox* was tolerant and even benefited from Cd, Co and Li. The color zone formation of all five tested basidiomycetes remained stable or even increased with Cr (20-100 mg kg^{-1}) and with Mn (10-50 mg kg^{-1}) compared to the plates without added metals. The color zone formation of *P. rivulosus* decreased most (40%) among five basidiomycetous fungi with 10 mg Cd kg^{-1} indicating that *P. rivilosus* was sensitive to Cd.

Ascomycetes did not show any color zone formation in the non contaminated ABTS indicator color plates. There was no color zone formation of any of the five studied ascomycetes, *Alternaria* sp., *Chaetomium* sp., *Epicoccum* sp., *Fusarium* sp. and *Trichoderma harzianum* with Mn (10-400 mg Mn kg^{-1}), Cd (2-10 mg Cd kg^{-1}) and Li (20-100 mg Li kg^{-1}). However, color zone formation was observed with added Cr and Co in some cases in ascomycetes plates. Color zone formation of tested ascomycetes was seen with *Alternaria* sp. with 50-100 mg Cr kg^{-1} and with *Chaetomium* sp. and *Trichoderma harzianum* with 50 mg Cr kg^{-1}. *Chaetomium* sp. induced color zone formation with 20-100 mg Co/kg. *Epicoccum* sp. showed color zone formation with 20 and 100 mg Co kg^{-1}. The color zone formation of

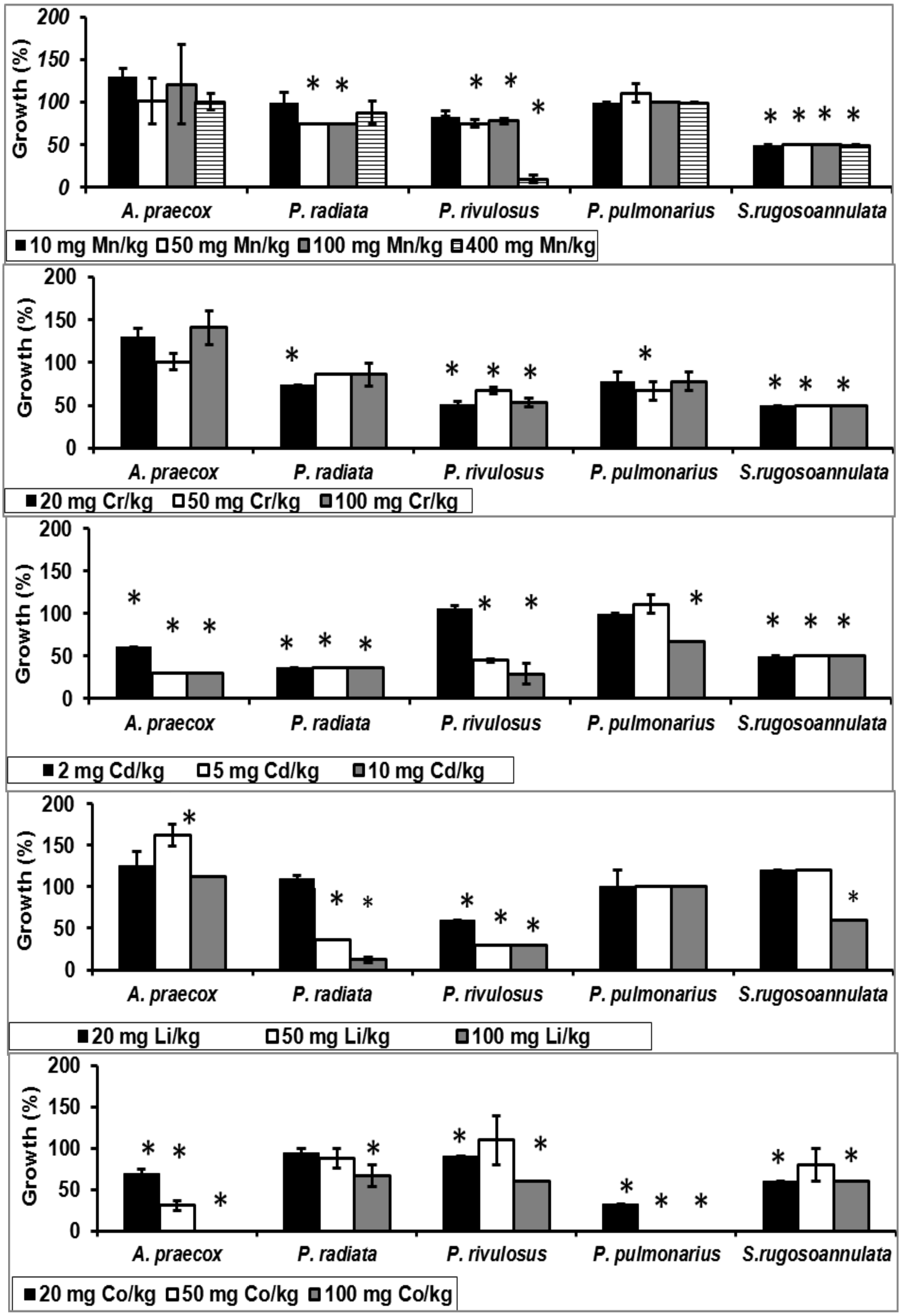

Figure 1: Growth of five basidiomycetous fungi in the presence of Mn, Cr, Cd, Co or Li compared to without (100 %) any added metal on ABTS malt extract agar plates (n=3). The error bar indicates standard error of mean. Asterisks (*)=Statistical differences (p<0.05) between with and without added metal.

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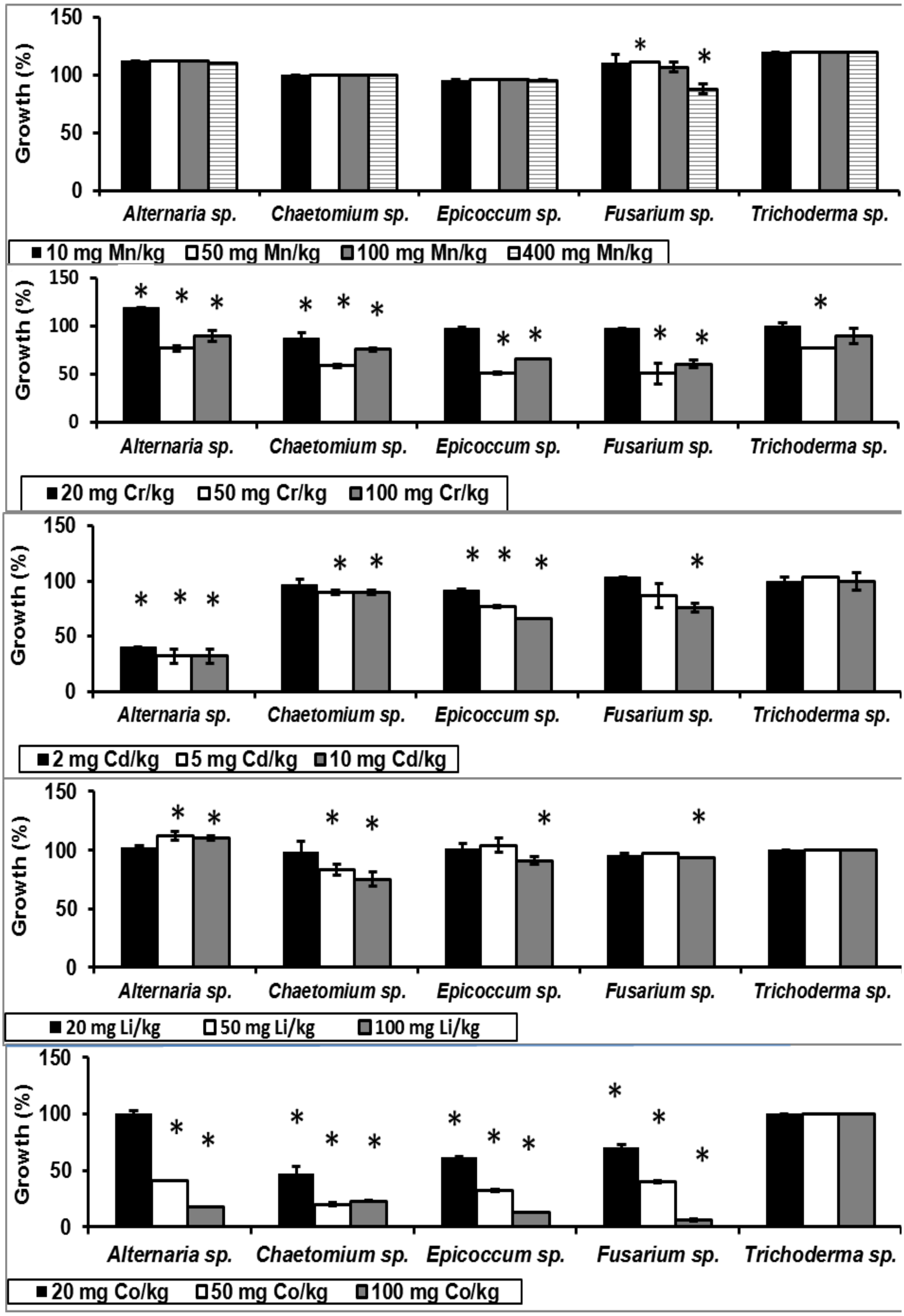

Figure 2: Growth of five ascomycetous fungi in the presence of Mn, Cr, Cd, Co or Li compared to without (100 %) any added metal on ABTS malt extract agar plates (n=3). The error bar indicates standard error of mean. Asterisks (*)=Statistical differences ($p<0.05$) between with and without added metal.

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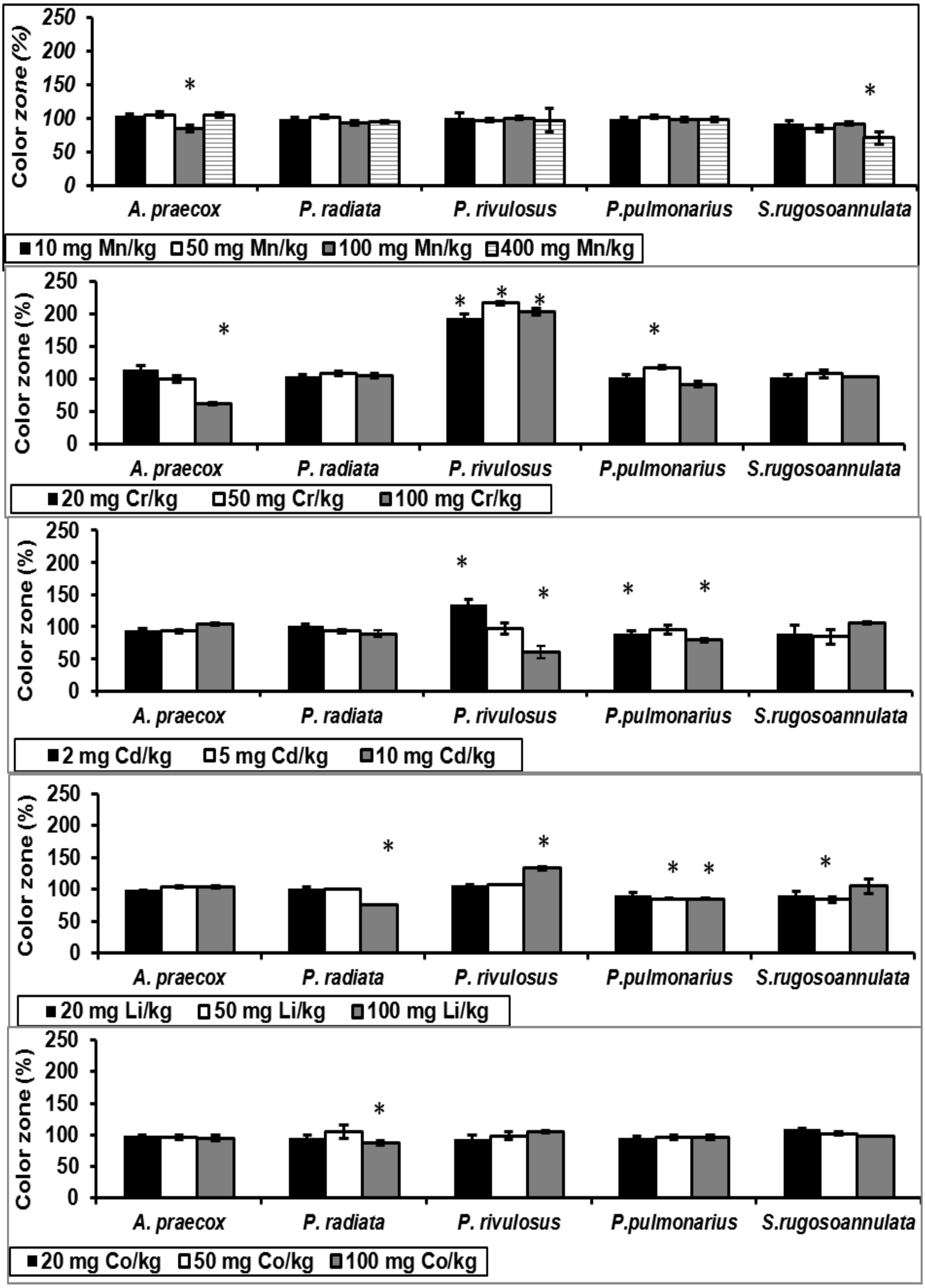

Figure 3: Enzyme production (as measured by color zone formation) with five basidiomycetous fungi in the presence of Mn, Cr, Cd, Co or Li compared to without (100 %) any added metal on ABTS malt extract agar plates (n=3). Asterisks (*)=Statistical differences ($p<0.05$) between with and without added metal.

Alternaria sp. was seen with 100 mg Co kg^{-1}. Color zone formation of tested ascomycetes was not seen with *Epicoccum* sp. and *Fusarium* sp. with Cr (20-100 mg kg^{-1}) and with *Fusarium* sp. and *Trichoderma harzianum* with Co (20-100 mg kg^{-1}).

Discussion

Our results showed that the growth of the five tested ascomycetous and basidiomycetous fungi had variations between sensitivity to the five tested metals (Mn, Cr, Cd, Li, Co). Impacts of tested metals (Cd, Cr, Co, Li, Mn) on the growth and the production of oxidative enzymes of five basidiomycetes and of five ascomycetes in the present study were not tested in any earlier studies. The growth of the basidiomycetes *P. radiata*, *P. rivulosus*, *P. pulmonarius* and *S. rugosoannulata* was inhibited in the Cr containing ABTS plates (20-100 mg Cr kg^{-1}) in the present study. These concentrations were 3-25 less than the minimum growth inhibition concentration for *Candida* sp. isolated from tannery wastes 0.3 g Cr l^{-1} and for the *Rhodosporium* sp. isolated from metallurgical wastes 0.5 g Cr l^{-1} [37]. *Phlebia gigantea* and *Pleurotus ostreatus* did not grow with Li (0.5 g l^{-1}) during two weeks in the liquid media [38], which bioavailability of metals is higher than in the solid media in the present study. The contaminant concentration limits set by the Finnish government are 100 mg Co kg^{-1} soil, 200 mg Cr kg^{-1} soil and 10 mg Cd kg^{-1} soil [39]. Finnish Government has not set limit values in the contaminated soil for Mn and Li, which were tested in the present study. The growth of *Agaricus bisporus, Agrocybe praecox, Gymnopus peronatus, Gymnopilus sapineus, Mycena galericulata, Gymnopilus luteofolius, Stropharia aeruginosa and Stropharia rugosoannulata* was inhibited with 20 mg Ni kg^{-1} [31] and 22-100% with 200 mg Cu kg^{-1} [30] on ABTS containing indicator color agar plates. Our results showed that the growth of the most of the tested basidiomycetous fungi was sensitive to Co, Cr and Cd even below the levels of contaminants limits set by the Finnish Authorities. The growth of basidiomycetes *P. rivulosus*, *P. radiata* and *S. rugosoannulata* fungi were vulnerable to Li (100 mg kg^{-1}) and Mn (100 mg kg^{-1}).

The growth of our tested ascomycetous fungi, *Alternaria* sp., *Chaetomium* sp., *Epicoccum* sp. and *Fusarium* sp., was sensitive to Co, Cr and Cd with concentrations below the soil contaminants limits set by the Finnish government (100 mg Co kg^{-1} soil, 200 mg Cr kg^{-1} soil and 10 mg Cd kg^{-1} soil; [39]. The growth of ascomycetes *Chaetomium* sp., *Epicoccum* sp. and *Fusarium* sp. was sensitive to Li, but limit values have not been set for Li in the contaminated soil. Li is used in batteries and electronic equipments [7,8]. They are mass products and therefore Li is emerging harmful metal in the environment. *Trichoderma* viride grew 25%, *Trichoderma* koningii, *Trichoderma* longibrachiatum, *Trichoderma* pseudokoningii grew 25-50%, *Trichoderma harzianum* grew 50-75% and *Alternaria* alternata, *Chaetomium* globosum, *Trichoderma* polysporum and *Trichoderma* reesei grew 75-100% with Li (0.5 g l^{-1}) compared to the control in the liquid media during two weeks [38]. The inhibitory concentrations of Li were 5-25 times higher for ascomycetous fungi than those in the present study for the growth of ascomycetes *Chaetomium* sp., *Epicoccum* sp. and *Fusarium* sp. fungi. Fertilizers contain Cd [10], and this increases the Cd levels of agricultural soils. Finnish agricultural soils are classified as clean if Cd level is less than 0.06 mg kg^{-1}, as normal if Cd level is between 0.06-0.3 mg kg^{-1} and as unusual if Cd level is more than 0.3 mg Cd kg^{-1} [10]. Our results indicate that increased level of Cd in the agricultural soil is a threat for the tested ascomycetous fungi, which were isolated from the Finnish agricultural soil. Novel finding in the present study is that the growth of tested ascomycetous fungi was sensitive to Co, Cr, Cd and Li indicating that these metals cause damage and change in the fungal communities in the contaminated soil. These changes can have changes in the cycling of carbon in the contaminated soil.

Ascomycetes did not show color zone formation in the case of all five studied ascomycetes with Mn, Cd and Li and without added metals. Color zone formation was seen with Cr with *Alternaria* sp., *Chaetomium* sp. and *Trichoderma harzianum* and with Co with *Alternaria* sp., *Chaetomium* sp. and *Epicoccum* sp. indicating that Cr and Co induced oxidative enzyme production or at least cation radical formation from ABTS. Cr induced the production of oxidative enzymes with *P. rivulosus* and three tested ascomycetous fungi. 10-20 mg Cd l^{-1} has no effect to the activity of laccase with the basidiomycete *Pleurotus ostreatus*, but laccase activity increases with the increasing metal concentration at 60-600 mg Cd l^{-1} [40]. Thus taken together, our results show that basidiomycetous fungi were able to produce more oxidative enzymes than ascomycetous fungi with Cd, Cr, Co, Li and Mn and without added metals. Our results indicate that basidiomycetous fungi are more involved in the oxidation of detritus biopolymers with extracellular enzymes in depolymerization process than ascomycetous fungi. Depolymerization of biopolymers is needed in the cycling of nutrients in well balanced ecosystems to maintain soil health globally.

Conclusions

All tested ascomycetes *Alternaria* sp., *Chaetomium* sp., *Epicoccum* sp., *Fusarium* sp. and *Trichoderma harzianum*, and basidiomycetes *Agrocybe praecox*, *Pleurotus pulmonarius, Phlebia radiata, Physisporinus rivulosus* and *Stropharia rugosoannulata*, fungi grew with Cd- (0-10 mg kg–1), Co- (0-100 mg kg^{-1}), Cr- (0-100 mg kg^{-1}), Li- (0-100 mg kg^{-1}) or Mn- (0-400 mg kg^{-1}) and without tested metals. The growth of *A. praecox* was tolerant to Mn, Cr and Li with tested concentrations. The color reaction with ABTS was present in all basidiomycetes without or with metals (Cd, Co, Cr, Li, Mn) indicating tested basidiomycetes produced oxidative enzymes. All tested ascomycetes did not show color zone formation with Mn, Cd, Li and without metals indicating that these fungi did not produce oxidative enzymes. *Alternaria* sp., *Chaetomium* sp., *Epicoccum* sp. and *Trichoderma harzianum* formed color zone with Co and Cr indicating oxidative enzyme production. Basidiomycetous fungi are more potential to depolymerize detritus biopolymers with oxidative extracellular enzymes than ascomycetous fungi.

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References

1. Fernandez-Turiel JL, Acenolaza P, Medina ME, Llorens JF, Sardi F (2001) Assessment of a smelter impact area using soils and plants. Environ Geochem Health 23: 65-78.
2. Hu X, Ding Z (2009) Lead/cadmium contamination and lead isotopic ratios in vegetables grown in peri-urban and mining/smelting contaminated sites in Nanjing, China. Bull Environ Contam Toxicol 82: 80-84.
3. Johnson J, Schewel L, Graedel TE (2006) The contemporary anthropogenic chromium cycle. Environ Sci Technol 40: 7060-7069.
4. Kien CN, Nguyen VN, Son LT, Ngoc HM, Tanaka S, et al. (2010) Heavy metal contamination of agricultural soils around a chromite mine in Vietnam. Soil Sci Plant Nutr 56: 344-356.
5. Li MS, Yang SX (2008) Heavy Metal Contamination in Soils and Phytoaccumulation in a Manganese Mine Wasteland, South China. Air Soil Water Res 1: 31-41.

6. Palmer MA, Bernhardt ES, Schlesinger WH, Eshleman KN, Foufoula-Georgiou E, et al. (2010) Mountaintop mining consequences. Science 327: 148-149.

7. Ramesh Babu B, Parande AK, Ahmed Basha C (2007) Electrical and electronic waste: a global environmental problem. Waste Manag Res 25: 307-318.

8. Hua L, Chan YC, Wu YP, Wu BY (2009) The determination of hexavalent chromium (Cr6+) in electronic and electrical components and products to comply with RoHS regulations. J Hazard Mater 163: 1360-1368.

9. Fan C, Lu A, Li Y, Wang C (2008) Synthesis, characterization, and catalytic activity of cryptomelane nanomaterials produced with industrial manganese sulfate. J Colloid Interface Sci 327: 393-402.

10. Mäkelä-Kurtto R, Louekari K, Nummivuori S, Sippola J, Kaasalainen M, et al. (2003) Cadmium in Finnish agro-ecosystems: concentrations, balances and risks (English Summary, in Finnish). Maa-jaelintarviketalous 27: 1-51.

11. Tabatabai MA, Fu M (1992) Extraction of enzymes from soils: Soil Biochemistry. Marcel Dekker Inc, New York, USA.

12. Tomme P, Warren RA, Gilkes NR (1995) Cellulose hydrolysis by bacteria and fungi. Adv Microb Physiol 37: 1-81.

13. Warren RA (1996) Microbial hydrolysis of polysaccharides. Annu Rev Microbiol 50: 183-212.

14. Cai YJ, Chapman SJ, Buswell JA, Chang ST (1999) Production and distribution of endoglucanase, cellobiohydrolase, and beta-glucosidase components of the cellulolytic system of Volvariellavolvacea, the edible straw mushroom. Appl Environ Microbiol 65: 553-559.

15. Wittmann C, Kähkönen MA, Ilvesniemi H, Kurola J, Salkinoja-Salonen MS (2004) Areal activities and stratification of hydrolytic enzymes involved in the biochemical cycles of carbon, nitrogen, sulphur and phosphorus in podsolized boreal forest soils. Soil Biol Biochem 36: 425-433.

16. Hatakka A (1994) Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation. FEMS Microbiol Rev 13: 125-135.

17. Hatakka A, Hammel KE (2010) Fungal biodegradation of lignocelluloses: Industrial Applications. Springer-Verlag, Berlin, Germany.

18. Hatakka A (2001) Biodegradation of lignin: Biopolymers: Lignin, humic substances and coal. Wiley-VCH, Weinheim, Germany.

19. Hakala TK, Lundell TK, Galkin S, Maijala P, Kalkkinen N, et al. (2005) Manganese peroxidases, laccases and oxalic acid from the selective white-rot fungus *Physisporinus rivulosus* grown on spruce wood chips. Enzyme MicrobTechnol 36: 461-468.

20. Rodriguez A, Perestelo F, Carnicero A, Regalado V, Perez R, et al. (1996) Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti. FEMS Microbiol Ecol 21: 213-219.

21. Regalado V, Rodriguez A, Perestelo F, Carnicero A, De La Fuente G, et al. (1997) Lignin Degradation and Modification by the Soil-Inhabiting Fungus *Fusarium* proliferatum. Appl Environ Microbiol 63: 3716-3718.

22. Kluczek-Turpeinen B, Tuomela M, Hatakka A, Hofrichter M (2003) Lignin degradation in a compost environment by the deuteromycete Paecilomyces inflatus. Appl Microbiol Biotechnol 61: 374-379.

23. Berbee ML, Taylor JW (1993) Dating the evolutionary radiations of the true fungi. Can J Bot 71: 1114-1127.

24. Berbee ML, Taylor JW (2001) Fungal molecular evolution: gene trees and geologic time: The Mycota, Vol VIIb. Springer, Berlin, Germany.

25. Eriksson OE (2006) Outline of Ascomycota. Myconet 12: 1-82.

26. Eriksson OE (1995) DNA and ascomycete systematics. Can J Bot 73: 784-789.

27. Samuels GJ, Seifert KA (1995) The impact of molecular characters on systematics of filamentous ascomycetes. Annu Rev Phytopathol 33: 37-67.

28. Baldrian P (2003) Interactions of heavy metals with white-rot fungi. Enzyme Microbl Technol 32: 78-91.

29. Gadd GM (1993) Interactions of fungi with toxic metals. New Phytol 124: 25-60.

30. Hartikainen ES, Lankinen P, Rajasärkkä J, Koponen H, Virta M, et al. (2012) Impact of copper and zinc on the growth of saprotrophic fungi and the production of extracellular enzymes. Boreal Env Res 17: 210-218.

31. Lankinen P, Kähkönen MA, Rajasärkkä J, Virta M, Hatakka A (2011) The effect of nickel contamination on the growth of litter-decomposing fungi, extracellular enzyme activities and toxicity in soil. Boreal Env Res 16: 229-239.

32. Niemelä T (2005) Käävät, puiden sienet. Norrlinia 13: 1-320.

33. Salo P, Niemelä T, Salo U (2006) Suomen sieniopas. Botanical museum & WSOY Publishing house, Helsinki, Finland.

34. Korhonen M (1986) Uusi sienikirja Otava. Keuruu 1-318.

35. Crous PW, Verkley GJM, Groenewald JZ, Samson RA (2009) CBS Laboratory manual series. Fungal biodiversity CBS-KNAW Fungal Biodiversity Centre (Centraal bureau voor Schimmel cultures), Utrecht, The Netherlands.

36. Sokal RR, Rolf FJ (1969) Introduction to Biostatistics. WH Freeman and Company, San Francisco, USA.

37. Pepi M, Baldi F (1992) Modulation of chromium (VI) toxicity by organic and inorganic sulfur species in yeasts from industrial wastes. Biometals 5: 179-185.

38. Richter DL, Robinson SC, Beardslee MP, Habarth ML (2008) Differential sensitivity of fungi to lithium chloride in culture media. Mycol Res 112: 717-724.

39. Anon (2007) 39.Valtioneuvoston asetus 2007. Maaperän pilantuneisuuden ja puhdistustarpeen arvioinnista (214/2007).

40. Baldrian P, Gabriel J (2002) Copper and cadmium increase laccase activity in Pleurotus ostreatus. FEMS Microbiol Lett 206: 69-74.

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