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Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi

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Abstract

This work investigates the ability of ericoid mycorrhizal (ErM) and ectomycorrhizal (EcM) fungi to solubilize different toxic metal (Cd, Cu, Pb, Zn)-containing minerals. Minerals were incorporated into solidified agar media and solubilization assessed by measuring clearing of the agar after fungal growth. Measurement of radial growth and biomass dry weight provided indications of metal tolerance: accumulated metal in the biomass was measured by atomic absorption spectrophotometry. Metal tolerance and solubilizing ability varied widely between different mineral and fungal species, and strains derived from sites of differing degrees of metal pollution. Zinc phosphate exhibited the least toxicity and was the easiest to solubilize by the majority of tested fungal isolates. Solubilization of toxic metal minerals was connected with both the pH of the medium and growth and tolerance of fungi and it seems that acidification of the medium was the main mechanism of mineral dissolution for most of the mycorrhizal fungi studied. A very strong lethal effect was observed for ectomycorrhizal isolates ($>60\%$ of strains) in the presence of Pb phosphate, carbonate, sulphide and tetraoxide. In contrast, ericoid mycorrhizal isolates were able to grow on Pb-mineral-amended media. A significant proportion of ericoid mycorrhizal cultures (70–90%) solubilized Cd and Cu phosphates and cuprite. None of the ericoid mycorrhizal and ectomycorrhizal fungi were able to produce a clear zone in Pb mineral-containing agar. However, many fungi were able to accumulate mobilized Pb in their mycelia. Differences in toxic metal mineral tolerance, mineral solubilization and metal uptake between populations isolated from metal-polluted and uncontaminated sites were related to the toxic metal which was the main pollutant in the original contaminated environment. In general, metal-tolerant fungi grew and solubilized toxic metal minerals better than non-tolerant isolates.

Keywords: Mycorrhizal fungi; Toxic metal minerals; Solubilization; Tolerance; Metal accumulation; Interspecific variations

1. Introduction

The extent of metal and radionuclide contamination in the world is immense. In the soil environment, metals and radionuclides can be dissolved in solution, held on inorganic soil constituents through various sorption or ion exchange reactions, complexed with soil organic materials, or precipitated as pure or mixed solids (Knox et al., 2000). Unlike degradable organic contaminants and even short-lived radionuclides that can become less toxic over time, metals can be considered conservative because they are not decomposed in the environment. The influence of microbiological processes on contamination of the environment by toxic metals and radionuclides is of economic and environmental significance (Gadd, 1993, 2001). However, the potential of microbial processes for bioremediation may be dependent on the physical and chemical nature of the site which influences the form in which metals occur. Furthermore, mineral components contain considerable quantities of metals which are biologically unavailable. Certain microbial processes dissolve metal minerals thereby increasing metal bioavailability and potential toxicity, whereas others immobilize them and reduce bioavailability. As well as being an integral component of biogeochemical cycles for metals and associated elements, these processes

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may be exploited for the treatment of contaminated solid and liquid wastes. Bioremediation, especially if combined with phytoremediation, has been considered as a feasible approach for the remediation of metal-polluted soils (Van der Lelie et al., 2001).

Many fungi can survive and grow in high concentrations of toxic metals (Gadd, 1993). The mechanisms by which they are able to deal with these metals are numerous and varied in their action, e.g. extracellular metal sequestration and precipitation, metal binding to the fungal cell walls, intracellular sequestration and complexation, compartmentation or volatilization (Gadd, 1993). Ecto- and endomycorrhizal symbioses can play a crucial role in protecting plants from toxic metals. The ability of mycorrhizal associations to ameliorate metal toxicity to higher plants has been shown for ericoid mycorrhizas (Bradley et al., 1981), ectomycorrhizas (Brown and Wilkins, 1985a,b; Denny and Wilkins, 1987a,b; Van Tichelen et al., 2001) and arbuscular mycorrhizas (Gildon and Tinker, 1983; Heggo and Angle, 1990). The efficiency of protection, however, differs between distinct isolates of mycorrhizal fungi and different toxic metals and protective effects cannot be demonstrated for all associations in all circumstances (Meharg and Cairney, 2000). What is clear is that plant roots and their associated free-living and symbiotic microbial populations significantly alter the physico-chemical characteristics of the rhizosphere by metabolic activities, resulting in a geochemical environment that can be very different from the bulk soil (Olsson and Wallander, 1998; Whitelaw et al., 1999). This will have significant consequences for the biogeochemical mobility of metals and associated elements in such an environment.

Phosphorus is an essential element for plant and microbial nutrition and can only be assimilated as soluble phosphate species. However, in the soil, a large part of the P pool is poorly soluble. Numerous studies have concluded that an important role of mycorrhizal fungi is to improve plant P nutrition, emphasizing the phosphate-solubilizing ability of mycorrhizal fungi (Lapeyrie et al., 1991; Wallander et al. 1997). Of special interest are the mechanisms by which fungi and plants obtain phosphate since solubilization of inorganic phosphates can result in release of the associated metals (Gadd, 1986). Conversely, formation of insoluble metal phosphates will reduce both metal and phosphate bioavailability. For other insoluble metal compounds and minerals (e.g. various sulphates, oxides, carbonates), solubilization can also result in release of anionic species. The ability of ericoid- and ectomycorrhizal fungi to dissolve and transform Ca-containing insoluble compounds and minerals (phosphates, carbonate and sulphate) in pure culture and in mycorrhizal association has been reported (Callot et al., 1985; Lapeyrie et al., 1991; Gharieb and Gadd, 1999). However, the dissolution of toxic metal-containing minerals increases toxic metal mobility and therefore, possible toxicity to fungi, plants, and other organisms. Processes of toxic metal mineral solubilization by fungi should therefore be considered in connection with the metal tolerance of these organisms. However, this aspect has been neglected and only a few studies on toxic metal mineral dissolution by ericoid mycorrhizal fungi have been carried out (Martino et al., 2003). Lead, Zn, Ca and Cu are the most frequently identified inorganic contaminants in soil and groundwater (in the same order of their relative occurrence), with Pb being considered particularly dangerous because of its wide distribution and the existence of a variety of Pb-containing minerals (Knox et al., 2000). Therefore, our aims were to study the ability of a range of ericoid- and ectomycorrhizal fungi to solubilize Pb-, Cu-, Cd- and Zn-containing insoluble minerals; to elucidate the mechanisms involved and possible connections with tolerance to solubilized metals, and to compare these processes between ericoid and ectomycorrhizal species from differently polluted habitats.

2. Materials and methods

2.1. Organisms and their origins

We used 12 cultures of the ericoid mycorrhizal fungi *Hymenoscyphus ericae* (provided by Professor A. Meharg) and *Oidiodendron maius* (provided by Dr E. Martino), and 19 cultures of ectomycorrhizal fungi belonging to the genera *Cenococcum, Hebeloma, Laccaria, Lactarius, Paxillus, Rhizopogon, Suillus, Thelephora and Tylospora* (provided by Drs D. Genney (CEH Merlewood collection), J. Colpaert and D. Mitchell) (Table 1). Fungal cultures were isolated from metal-contaminated habitats (Table 1). These metal polluted sites were (1) the Devon Great Consols copper mine, UK; (2) Niepolomice Forest experimental plots, Poland; (3) Lommel field site, Belgium; and (4) Avoca mine spoil, Ireland. (1) The Devon Great Consols copper mine spoil low-organic soil (southwest England) is highly contaminated with As and Cu. Total (acid-extractable) concentrations of toxic metals in this soil were approximately: 17.1–39.2 mg As g^{-1} soil, 4.2–13.7 mg Cu g^{-1} soil and 1.0–1.4 mg Zn g^{-1} soil (Porter and Peterson 1997; Sharples et al., 2000). (2) The Niepolomice Forest experimental plots were contaminated (in 1980) with up to 5000 tonnes km^{-2} y^{-1} of industrial dusts containing different proportions of Zn, Cd, Al and other metal ions (Greszta et al., 1987). *O. maius* Zn was isolated in 1995 from a plot where Zn was the most abundant metal in the contaminating dust (22.06% ZnO; 0.63% CdO; 8.13% Al_2O_3); whereas Cd was the most abundant toxic metal in the dust (3.02% CdO; 1.75% ZnO; 21.83% Al_2O_3) in the plot where *O. maius* Cd was isolated from (Martino et al., 2000). (3) The EcM isolates from the Lommel Maatheide site were collected in the immediate vicinity of the 'zinc desert', an industrial site polluted with non-ferrous metals emitted by a zinc smelter dismantled in 1973. Toxic metal concentrations in Lommel Maatheide soils were for Zn 1.8 mg g^{-1} soil; for Cd

Table 1
Characteristics of the isolation site and hosts of the studied strains of mycorrhizal fungi

Strains	Origin site	Host
Ericoid mycorrhizal (ErM)		
Hymenoscyphus ericae C2(1)	Uncontaminated heathland site, Aylesbeare Common, Exeter, Devon, UK	*Calluna vulgaris*
H. ericae C3(1)	As above	*C. vulgaris*
H. ericae C4(8)	As above	*C. vulgaris*
H. ericae C5(10)	As above	*C. vulgaris*
H. ericae DGC1(H)	Devon Great Consols copper mine, UK, Cu ≫ Zn-polluted	*C. vulgaris*
H. ericae DGC3(UZ)	As above	*C. vulgaris*
H. ericae DGC5(E)	As above	*C. vulgaris*
Oidiodendron maius Zn	Niepolomice Forest, Poland, Zn > Cd-polluted	*Vaccinium myrtillus*
O. maius Cd	As above	*V. myrtillus*
O. maius A	Unpolluted site, Poland	*V. myrtillus*
O. maius E	Unpolluted site, Italy	*C. vulgaris*
O. maius 091	Unpolluted site, Canada	*V. angustifolium*
Ectomycorrhizal (EcM)		
Cenococcum geophyllum 45	Unpolluted site, Holland	Not known
Hebeloma crustuliniforme 46	Unpolluted site, France	Picea
Laccaria laccata 8	Unpolluted site, UK	*Picea*
Lactarius turpis 11	Unpolluted site, UK	*Betula sp.*
Paxillus involutus 15	Unpolluted site, UK	*Larix sp.*
P. involutus 23	Lommel, Belgium, Zn ≫ Cd/Cu-polluted	*Pinus sp.*
P. involutus 36	Unpolluted site, France	*Eucalyptus dalrympleana*
P. involutus 52	Unpolluted site, Teut, Belgium	*Pinus sp.*
P. involutus W	Avoca Mines, Ireland, Cu > Pb-polluted	*Betula sp.*
Rhizopogon luteolus 38	Unpolluted site, France	*Pinus pinaster*
Suillus bovinus 34	Unpolluted site, Sweden	*Picea*
S. bovinus LSt8	Lommel, Belgium, Zn ≫ Cd/Cu-polluted	*Pinus* sp.
S. bovinus MG1	Unpolluted site, Meeuwen, Belgium	*Pinus* sp.
S. luteus 21	Lommel, Belgium, Zn ≫ Cd/Cu-polluted	*Pinus nigra*
S. luteus 22	As above	*P. nigra*
S. luteus 33	Unpolluted site, Paal, Belgium	*P. nigra*
S. luteus 34	As above	*P. nigra*
Thelephora terrestris	Lommel, Belgium, Zn ≫ Cd/Cu-polluted	*Pinus* sp.
Tylospora fibrillosa 27	Unpolluted site, Sweden	*Picea*

14 μg g^{-1} soil, and for Cu 76 μg g^{-1} soil (Colpaert et al., 2000). (4) Copper mining began in Avoca around 1720 and there is more than 1,700,000 m^3 of acidic, metalliferous mine spoil at Avoca, Co. Wicklow (Gallagher and O'Connor 1997; Fay and Mitchell, 1999). Total metal concentrations in mine spoil and limed mine spoil were: Cu 527–1016 μg g^{-1} and Pb 517–974 μg g^{-1} (D.A. Fay, PhD Thesis, University college, Dublin, 2001). Note that we have mentioned only those metal species of interest for the present study (Zn, Cd, Cu, Pb) (Table 1). The comparative degree of metal contamination in the soils was considered regarding metal concentrations in unpolluted soils (e.g. Tables 1 and 4). According to various sources, including our own unpublished data, different European and American non-polluted soils can contain around 21–80 μg Zn g^{-1}, 0.2–10 μg Cd g^{-1}, 8–40 μg Cu g^{-1}, and 25–80 μg Pb g^{-1} (Porter and Peterson, 1977; Kabata-Pendias et al., 1992; Hopkin, 1993; Frink, 1996).

2.2. Media and culture conditions

Fungal strains were maintained at 25 °C on modified Melin-Norkrans (MMN) agar medium comprising: $(NH_4)_2HPO_4$ (0.5 g l^{-1}), KH_2PO_4 (0.3 g l^{-1}), $MgSO_4 \cdot 7H_2O$ (0.14 g l^{-1}), $CaCl_2 \cdot 6H_2O$ (50 mg l^{-1}), NaCl (25 mg l^{-1}), D-glucose (10 g l^{-1}), glutamic acid (1 mg l^{-1}), thiamine (100 μg l^{-1}) and agar No. 1 (Lab M, Bury UK) (14 g l^{-1}). Before adding the agar and prior to autoclaving, the liquid medium was adjusted to pH 5.5 using HCl. All screening experiments were carried out using MMN with the addition of appropriate metal compounds to the desired final concentration. Metal compounds were oven-sterilized for 36–48 h at 95 °C.

2.3. Metal compounds

Commercial preparations of $Cd_3(PO_4)_2$ (Alfa), $Cu_3(PO_4)_2 \cdot 2H_2O$ (Fluka), Cu_2O (BDH) (naturally occurring as cuprite), $Zn_3(PO_4)_2 \cdot 2H_2O$ (Alfa) (naturally occurring as hopeite), $Pb_3(PO_4)_2$ (Alfa), $PbCO_3$ (BDH) (naturally occurring as cerussite), Pb_3O_4 (Aldrich) (red lead) and PbS (Aldrich) (naturally occurring as galena) were used. Lead chlorophosphate or pyromorphite ($Pb_5(PO_4)_3Cl$) was synthesized by mixing solutions of Pb, P and Cl in stoichiometric proportions (5:3:1). 200 ml of 0.5 M $Pb(NO_3)_2$ was mixed with 200 ml of a solution containing

0.3 M Na_2HPO_4 and 0.1 M NaCl at approximately 90 °C. When cool, the precipitate was separated by filtration, washed with deionized water and dried at 60°C (Sayer et al., 1999). Samples of obtained pyromorphite were examined using XRPD to confirm their homogeneity.

2.4. Screening methods

2.4.1. Preparation of metal-amended plates and inoculation

Fungi were grown on 20 ml MMN agar, in 90 mm-dia. Petri dishes with metal compounds added to a final metal concentration of 15 mM (Sayer et al., 1995; Sayer and Gadd, 1997). Metal phosphates and Pb_3O_4 were added to a 5 mM final concentration (equivalent to 0.26% w/v $Cd_3(PO_4)_2$, 0.2% w/v $Cu_3(PO_4)_2$, 0.22% w/v $Zn_3(PO_4)_2$, 0.41% w/v $Pb_3(PO_4)_2$, 0.34% w/v Pb_3O_4). Cu_2O was added to 7.5 mM (or 0.11% w/v), PbS and $PbCO_3$ were added to 15 mM (or 0.36% and 0.4% w/v, respectively), and $Pb_5(PO_4)_3Cl$ was added to 3 mM (or 0.4% w/v) in order to equalize metal concentrations in molar terms. Prior to inoculation, 84 mm dia. discs of sterile cellophane membrane were placed aseptically on the surface of the agar in each Petri dish. The membrane allowed the passage of nutrients or metabolites between the agar and the fungus, and provided a convenient means of removing the mycelium from the agar (Sayer and Gadd, 1997). Inoculations were carried out using 7 mm dia. discs of mycelium cut from the leading edge of colonies which had been maintained on MMN at 25 °C for at least 14 d. All species were inoculated onto each metal compound (at least three replicates) and incubated at 25 °C for 2 months. Measurements were made of the size of the colonies every 3–4 days and of any clear zones present.

2.4.2. Estimation of solubilizing ability

The main criterion of mineral solubilizing ability was the diameter of the solubilization area (clear halo) in agar that is used in studies of fungal phosphate-solubilizing ability and mineral transformations (Paris et al., 1995; Sayer et al., 1995).

2.4.3. pH measurements

To obtain a pH profile of the agar surface under growing fungal colonies, pH measurements were made, in triplicate, across the agar surface, using a surface combination pH electrode (Orion, Model 720A, BDH, Poole, UK).

2.4.4. Biomass and metal analysis

Colonies were removed from replicate agar plates by peeling the biomass from the dialysis membrane. The mycelia were oven-dried at 80 °C until reaching constant weight, and after dry weight measurement, were digested (50 mg) in 3.0 ml conc HNO_3 at 159 °C overnight in a digestion block (Grant Instruments, UK). After appropriate dilution with ddH_2O, solutions were analyzed for metal ion content using a Pye Unicam SP9 atomic absorption spectrophotometer (AAS) with respect to appropriate standard solutions in acidified ddH_2O.

2.4.5. Metal tolerance

Growth of fungi was evaluated by extension of the colony and by the biomass yield of dry weight since extension of the colony alone does not take into account the density of fungal mycelium (Gadd, 1986; Vodnik et al., 1998). Tolerance results were expressed in terms of a tolerance index (TI) based on the dry weights of fungal biomass (DW): TI_{DW} = (DW of treated mycelium/DW of control mycelium) × 100 (%) (Colpaert et al., 2000; Vodnik et al., 1998).

2.4.6. Statistical analysis

Minitab for Windows 12 (Release 12.1) was used for statistical analysis. At least three replicate determinations were used in experiments.

3. Results

3.1. Toxic metal mineral solubilization

Growth and the ability to solubilize the insoluble toxic metal compounds depended on both the fungal strain and the nature of the insoluble compound. Some fungal cultures produced solubilization zones in the agar of larger diameter than the fungal colonies (Fig. 1a–c) but for others, solubilization zones were found only beneath fungal colonies (Fig. 1d–f). The highest values of Cd phosphate solubilization (halo dia > 17 mm) were observed for *H. ericae* DGC5(E), *O. maius* E, and *S. bovinus* LSt8 (Table 2). Most ectomycorrhizal fungi were unable to produce clear haloes in Cd phosphate-containing agar. Comparatively high values of solubilization (halo ≥ 20 mm) were found for Cu phosphate by *H. ericae* DGC1(H), *P. involutus* 23, *S. luteus* 21, and for cuprite by *H. ericae* DGC1(H). Among all the tested minerals, the largest clear halo diameters were found on Zn phosphate-containing medium (Table 2). Comparatively high values (halo ≥ 50 mm) were observed for Zn phosphate in several strains: *H. ericae* C4, DGC1(H), DGC3(UZ); DGC5(E), *O. maius* Cd, *P. involutus* 23, *S. bovinus* LSt8, *S. luteus* 21, 22, most of which were isolated from metal-contaminated soils (see Table 1). For all Pb-containing minerals, clear haloes in agar media were not produced for the tested isolates. It was also observed that solubilization was not necessarily connected with active fungal growth. For example, all isolates of *O. maius* were unable to grow on agar containing cuprite but most of them showed obvious clear haloes around the inoculum discs (Table 2, Fig. 2A). A similar phenomenon was observed for *H. ericae* C4(8) that did not grow on Cu phosphate but produced a small clear zone around the inoculum disc.

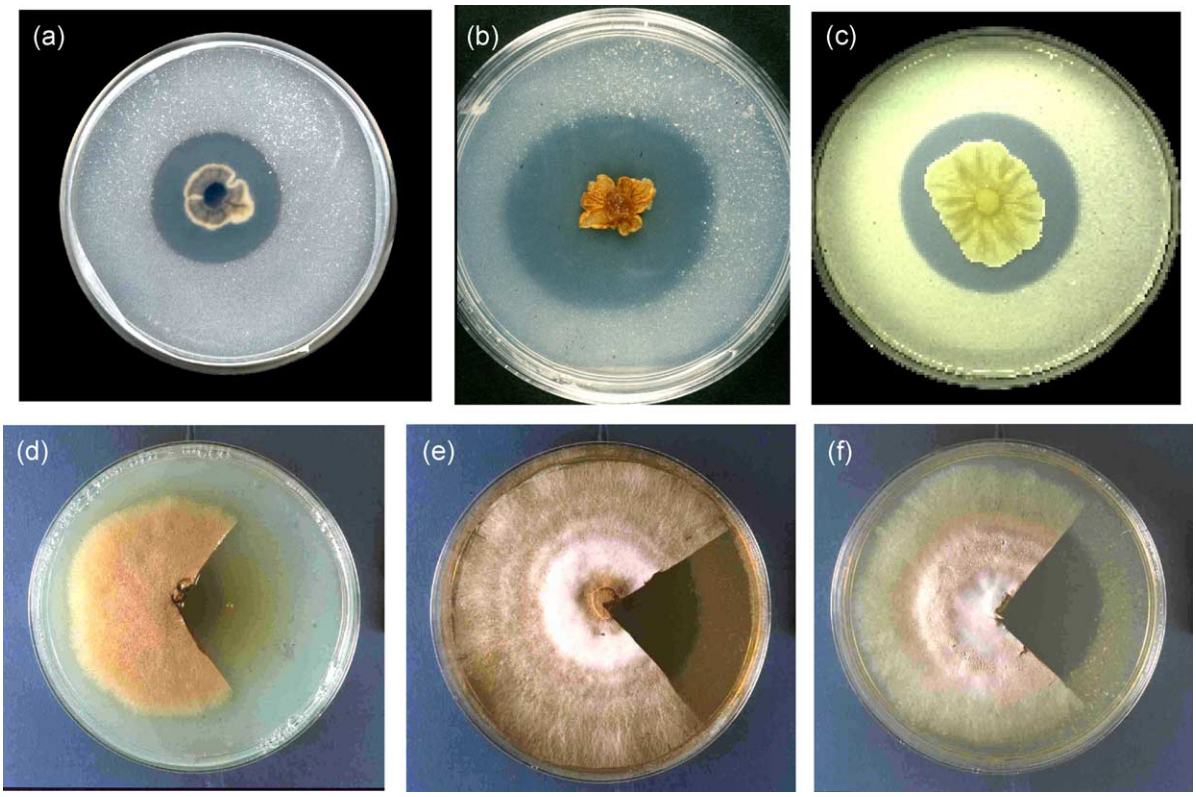

Fig. 1. Solubilization of toxic metal minerals in agar medium by ericoid- and ectomycorrhizal fungi: Zn phosphate (a–c, e,f) solubilization by *H. ericae* DGC3(UZ) (a), *O. maius* Cd (b), *L. laccata* 8 (c), *S. bovinus* LSt8 (e), *S. luteus* 21 (f), and Cu phosphate solubilization by *P. involutus* 52 (d). Segments of membrane with fungal mycelium were cut out to show the solubilization halo if it was less than the colony diameter (d–f).

3.2. Fungal tolerance to toxic metal minerals

The tolerance index varied widely with different minerals and between fungal species and strains originating from different polluted sites (Fig. 2). Some cultures were sensitive to most of the minerals and did not grow or their growth was strongly inhibited on mineral-amended media, e.g. *H. ericae* C2–C5 (Cd, Zn, Pb and Cu phosphates, cuprite, Pb tetraoxide); *C. geophilum* 45 (all minerals); *L. turpis* 11 (Cd, Zn and Cu phosphates, cuprite, pyromorphite); *R. luteolus* 38 (all minerals); *S. bovinus* 34 and MG1 (Cd and Cu phosphates, cuprite, and all Pb-minerals except pyromorphite); *T. terrestris* (copper phosphate, cuprite, and all Pb-minerals); *T. fibrillosa* 27 (all minerals except pyromorphite). Growth stimulation was also observed in the presence of some tested minerals, especially for Pb-containing minerals, probably due to impurities used as micronutrients by the fungi. Considerable growth stimulation was found for Zn phosphate on cultures of *O. maius* A, and *S. luteus* 21 and 22; for Pb phosphate in *O. maius* A and *L. turpis* 11; for pyromorphite on *O. maius* A, and *H. crustuliniforme* 46; for Pb carbonate on *O. maius* A, *O. maius* E, and *L. turpis* 11; for Pb sulphide on *O. maius* A and *O. maius* Zn; for Pb tetraoxide on *L. turpis* 11.

3.3. Relationship between solubilization, fungal growth and medium acidification

Regression analysis of the relationship between solubilization activity and the final medium pH, biomass yield, and tolerance index are presented in Fig. 3. Analysis of the relationship between mineral solubilization and pH demonstrated a linear regression with a strong negative correlation (Fig. 3A, C and E). ANOVA correlation coefficients (Pearson) were -0.894 for Zn phosphate, -0.702 for Cu phosphate and -0.832 for Cd phosphate with $P=0.000$ in all cases. The data for mineral solubilization also positively correlated with biomass yield and the tolerance index (Fig. 3B, D and F). Solubilization-biomass correlation coefficients were 0.876, 0.907 and 0.872 ($P=0.000$ in all cases) for Zn, Cu and Cd phosphates, respectively. Tolerance to Zn, Cu and Cd phosphates correlated with solubilization with R being 0.722, 0.833, and 0.757 ($P=0.000$ in all cases), respectively. Solubilization of toxic metal minerals was therefore connected with both the pH of the medium and growth and tolerance of the fungi suggesting that acidification, related to active fungal growth, was the main mechanism of mineral dissolution.

Table 2
Solubilization halo diameters (mm) for insoluble toxic metal compounds (Cd, Cu, Zn) produced by ericoid mycorrhizal (ErM) and ectomycorrhizal (EcM) fungi in agar plates

Fungi	$Cd_3(PO_4)_2$	$Cu_3(PO_4)_2$	Cu_2O	$Zn_3(PO_4)_2$
Ericoid mycorrhizal (ErM)				
H. ericae C2(1)	0±0	0±0	0±0	37.3±9.2
H. ericae C3(1)	5.3±3.2	0±0	0±0	38.8±2.8
H. ericae C4(8)	8.5±0.3	2.0±2.0	0±0	51.8±4.8
H. ericae C5(10)	5.3±3.1	0±0	5.0±5.0	21.0±1.5
H. ericae DGC1(H)	10.2±0.2	24.0±0.0	20.0±3.2	50.0±0.6
H. ericae DGC3(UZ)	9.2±0.4	13.2±0.2	9.3±1.7	61.2±1.9
H. ericae DGC5(E)	17.2±0.8	18.3±2.8	13.0±1.2	54.3±0.2
O. maius A	6.2±0.2	14.7±1.5	13.3±0.2	48.3±0.7
O. maius E	20.2±1.0	14.0±0	17.7±2.3	37.2±0.4
O. maius 091	12.5±0.3	13.0±2.6	8.4±0.8	33.3±5.6
O. maius Cd	10.5±0.5	0±0	0±0	56.7±4.3
O. maius Zn	12.2±0.2	6.0±1.0	1.0±1.0	40.2±2.8
Ectomycorrhizal (EcM)				
C. geophyllum 45	0	0	0	15.3±2.0
H. crustuliniforme 46	0	0	0	0
L. laccata 8	0	16.5±1.5	0	47.7±1.5
L. turpis 11	4.3±2.2	0	11.7±0.2	20.2±0.4
P. involutus 15	0	4.7±2.3	0	40.0±2.0
P. involutus 23	0	28.0±1.5	0	53.7±1.2
P. involutus 36	0	15.3±2.3	14.9±0.4	27.7±0.3
P. involutus 52	0	15.7±0.9	0	9.3±1.8
P. involutus W	0	15.5±2.5	0	6.6±1.5
R. luteolus 38	0	4.0±4.0	0	11.2±0.2
S. bovinus 34	0	0	0	30.3±4.3
S. bovinus MG1	0	0	0	43.7±3.5
S. bovinus LSt8	18.3±1.3	0	0	57.3±2.6
S. luteus 21	0	19.7±9.8	15.7±2.6	52.0±2.7
S. luteus 22	0	10.0±5.8	0	55.0±2.4
S. luteus 33	0	16.7±0.7	19.3±1.8	18.0±2.8
S. luteus 34	0	0	0	27.5±0.8
T. terrestris	6.7±1.7	0	0	42.3±2.4
T. fibrillosa 27	0	0	0	8.7±0.3

Values are the means of at least three replication determinations ±SEM.

3.4. Distribution of toxic metal mineral solubilization ability and tolerance among mycorrhizal fungi

Analysis of the overall distribution of the sensitivity to toxic metal minerals (TI = 0% or TI < 50%) and the mineral-solubilizing ability of tested fungal strains, expressed as a % of the total number of isolates, is shown in Fig. 4. All strains were able to grow in the presence of Zn phosphate (Fig. 4A). A similar proportion of ectomycorrhizal strains (up to 20%) could not grow on medium containing Cu and Cd phosphates and cuprite (Fig. 4A). One third of the tested ericoid mycorrhizal strains did not grow on Cu-containing minerals. A very strong toxic effect was observed for ectomycorrhizal isolates (>60% of strains) growing in the presence of Pb phosphate, carbonate, sulphide and tetra-oxide. In contrast, all ericoid mycorrhizal isolates were able to grow on Pb-mineral-containing media (Fig. 4A). Growth of most of the ericoid mycorrhizal and ectomycorrhizal isolates (≥60% of strains) was strongly inhibited (TI < 50%) by Cd and Cu phosphate (Fig. 4B). More than one third of the strains considerably reduced their biomass yield (TI < 50%) in the presence of Cu and Zn phosphate (for both ericoid- and ectomycorrhizal isolates), and Pb phosphate (for ericoid mycorrhizal isolates only). However, none of pyromorphite, Pb carbonate, Pb sulphide and Pb tetraoxide inhibited growth of ericoid mycorrhizal fungi (Fig. 4B).

Distribution of the ability to dissolve toxic metal minerals (in terms of solubilization zone diameter) showed that nearly all of the tested isolates (>97%) could solubilize Zn phosphate (Fig. 4C). A significant proportion of ericoid mycorrhizal cultures (70–90%) solubilized Cd and Cu phosphates and cuprite. Copper phosphate was dissolved by half of the tested ectomycorrhizal strains, but cuprite and Cd phosphate were solubilized by a lower proportion of ectomycorrhizal isolates (20–30%). None of the ericoid- and ectomycorrhizal fungi showed the ability to produce a clear zone in Pb-containing media as mentioned above.

Those mycorrhizal cultures able to effect mineral solubilization demonstrated very different activities for the different minerals (Table 2). For example, for most fungal cultures, Zn phosphate solubilization zone diameters were

30–60 mm, whereas for Cd phosphate they were only 10–15 mm and a large proportion of strains did not produce a clear halo on this mineral.

Manifestation of lethal and growth-inhibiting effects of toxic metal minerals and mineral dissolution in fungi were considered to be species-dependent if all the tested isolates of the same species uniformly demonstrated the same growth response to the minerals or the same mineral solubilizing ability. Such analysis of species-dependent toxic metal mineral tolerance and solubilization is summarized in Table 3. For example, the biomass yield of all tested isolates of *P. involutus* was strongly reduced (TI $\leq$ 20%) in the presence of Cd and Pb phosphates, and cuprite. Cuprite was also very toxic (TI $\leq$ 20%) to all isolates of *H. ericae, S. bovinus* and *S. luteus*. Lead phosphate significantly inhibited growth of *S. bovinus* (Table 3). All isolates of *O. maius* did not grow (TI = 0%) on cuprite-medium, and *S. luteus* did not grow on medium containing Pb phosphate or

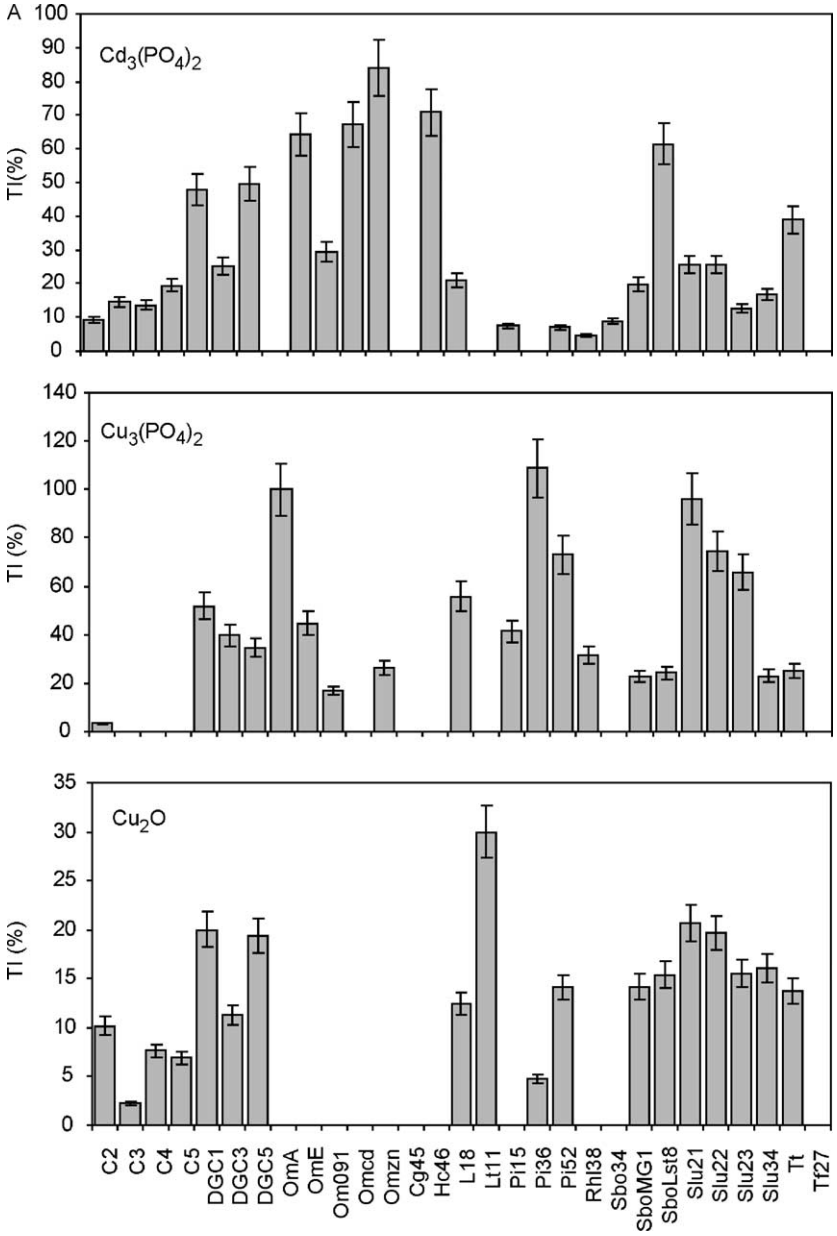

Fig. 2. Tolerance index for fungal biomass yield in the presence of different toxic metal minerals, as indicated in plot area: (A) Cd phosphate, Cu phosphate and cuprite; (B) Zn phosphate, Pb phosphate and pyromorphite; (C) Pb carbonate, Pb sulphide and Pb tetraoxide. Values are means and derived from at least three replicate determinations, bars indicate $\pm$SEM. Fungal cultures on the *x*-axis are (see Table 1): C2—*H. ericae* C2(1); C3–*H. ericae* C3(1); C4—*H. ericae* C4(8); C5—*H. ericae* C5(10); DGC1—*H. ericae* DGC1(H); DGC3—*H. ericae* DGC3(UZ); DGC5—*H. ericae* DGC5(E); OmA—*O. maius* Zn; OmE—*O. maius* E; Om091—*O. maius* 091; OmCd—*O. maius* Cd; OmZn—*O. maius* Zn; Cg45—*C. geophilum* 45; Hc46—*H. crustuliniforme* 46; L18—*L. laccata* 8; Lt11—*L. turpis* 11; Pi15—*P. involutus* 15; Pi36—*P. involutus* 36; Pi52—*P. involutus* 52; Rhl38—*R. luteolus* 38; Sbo34—*S. bovines* 34; SboMG1—*S. bovines* MG1; SboLSt8—*S. bovines* LSt8; Slu21—*S. luteus* 21; Slu 22—*S. luteus* 22; Slu 33—*S. luteus* 33; Slu 34—*S. luteus* 34; Tt—*T. terrestris;* Tf27—*T. fibrillose* 27.

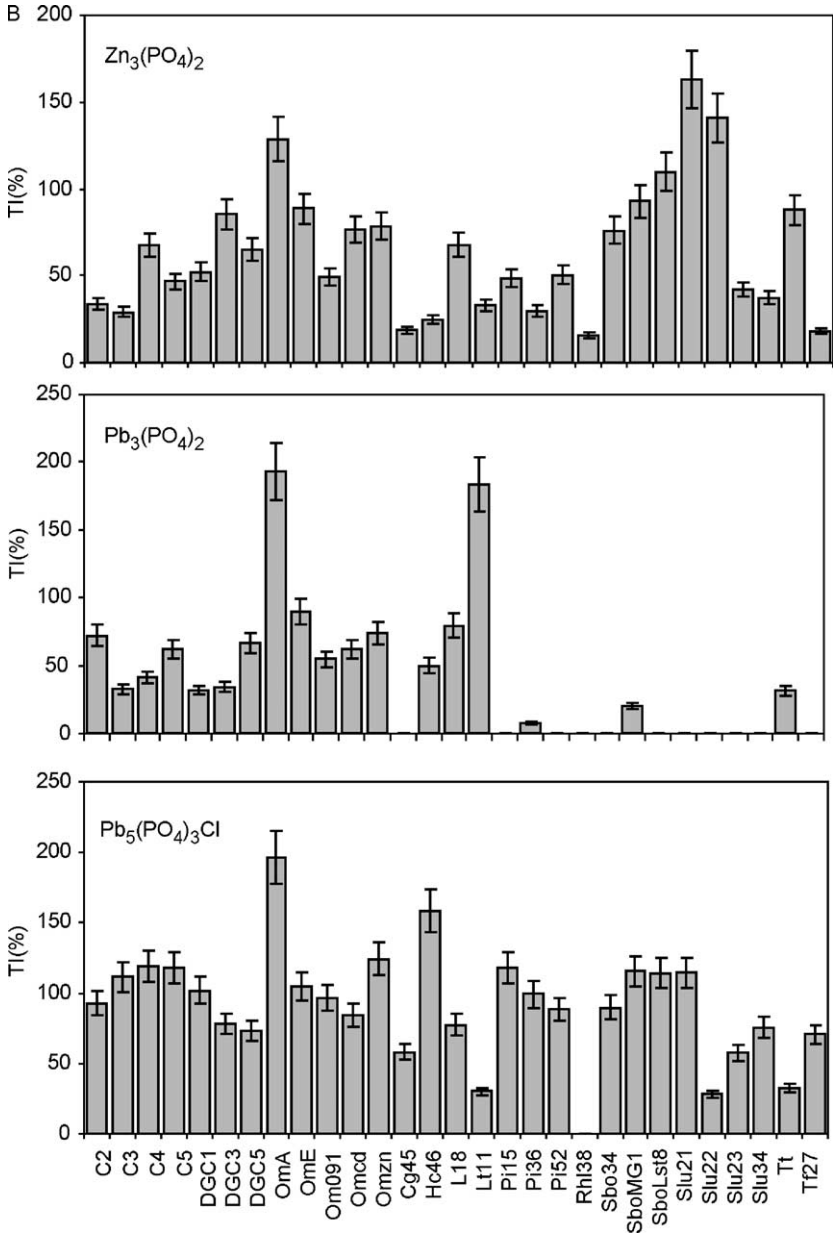

Fig. 2 (*continued*)

tetraoxide. Lead tetraoxide was also lethal for all isolates of *P. involutus* and *S. bovinus*. None of the *S. bovinus* isolates produced a solubilization zone in cuprite-agar, while *P. involutus* and *S. luteus* isolates were not able to produce clear haloes for Cd phosphates and all Pb-containing minerals. As mentioned above, dissolution of Pb-containing minerals in agar was not observed visually in all of the tested fungal cultures.

3.5. Metal accumulation by fungal biomass during toxic metal mineral solubilization

Metal accumulation by fungi can also be regarded as an indirect index of fungal solubilization potential. Metal accumulation by fungal biomass was expressed in terms of specific metal uptake (g dry wt)$^{-1}$ biomass (Fig. 5) and total metal uptake by each individual fungal colony per Petri dish (not shown). Specific metal uptake widely varied among fungal isolates. High Zn uptake values were found for *H. ericae* C3(1), *P. involutus* 52, *S. bovinus* LSt8 and *T. terrestris* (Fig. 5A and B). *S. luteus* isolates 21 and 22 (Lommel) accumulated more Zn than isolates 33 and 34 (from uncontaminated soil). Similarly Lommel-isolate *S. bovinus* LSt8 accumulated more Zn than *S. bovinus* MG1, the latter isolated from a non-polluted environment (Fig. 5B). It was also found that total Zn uptake by fungal colonies correlated ($R=0.7$) with solubilization zone data from Zn phosphate-containing media by those colonies (not shown).

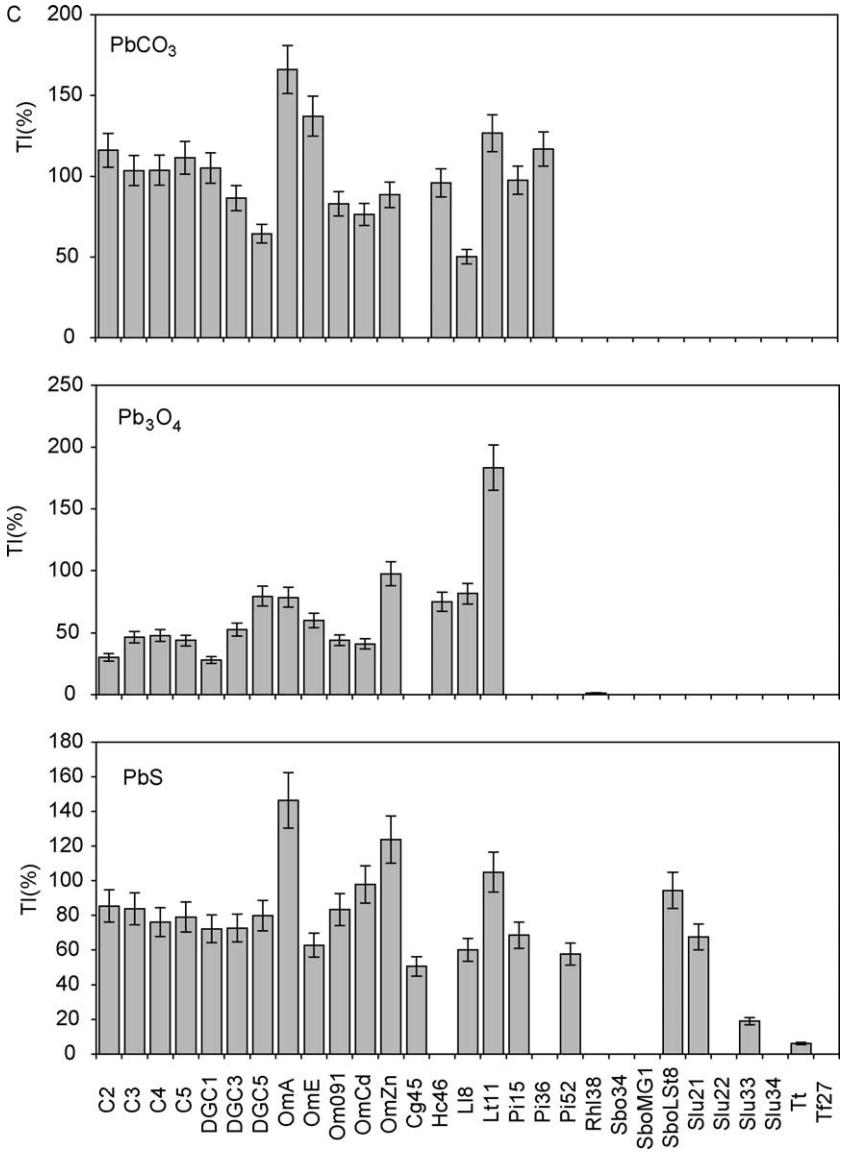

Fig. 2 (*continued*)

Despite the absence of clear haloes in the agar media, some minerals (e.g. pyromorphite) were dissolved with the mobilized metal (Pb) accumulated in the biomass of many strains (Fig. 5C, D). The absence of correlation between data for clear halo formation in pyromorphite-amended agar (as a visual indication of mineral solubilization) and total Pb accumulation by fungal colonies suggests an important limitation of the agar-plate screening method for certain perhaps more intractable minerals. Of all the cultures, *S. bovinus* isolates accumulated the highest (at least a 2-fold) quantity of Pb in their mycelia (Fig. 5D). There was no significant difference between specific Pb uptake data for groups of strains of *S. luteus*, *S. bovinus* and *O. maius*, isolated from sites of differing degrees of metal contamination (Fig. 5C and D). However, *H. ericae* C2–5 from non-polluted soil accumulated considerably more Pb (2–5 fold) than *H. ericae* DGC from the Devon Great Consols copper mine (Fig. 5C).

3.6. Differences in tolerance, solubilization and metal accumulation between populations from metal-polluted and uncontaminated soils

Comparative analysis of the data on the tolerance to toxic metal minerals, mineral solubilization and metal accumulation for populations of the same species originating from metal-contaminated and uncontaminated environments was carried out using one-way ANOVA (Table 4, Fig. 6). The difference between populations in tolerance and solubilizing ability depended on (i) the fungal species, (ii) the toxic metal mineral, and (iii) the characteristics of the metal-polluted site (metal species, and combination and proportion

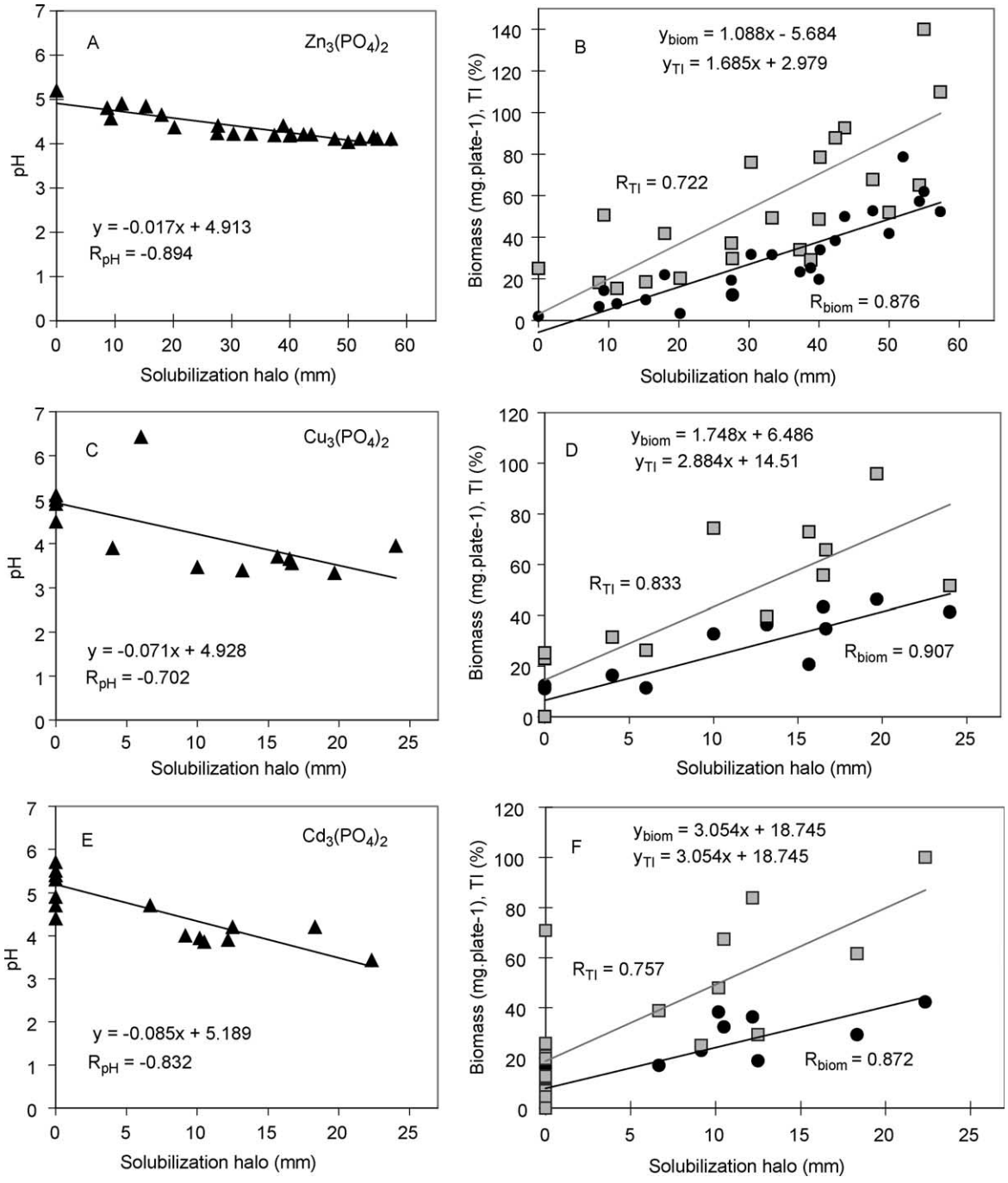

Fig. 3. Relationship between solubilization of (A, B) Zn phosphate, (C, D) Cu phosphate and (E, F) Cd phosphate and (A, C, E) pH of agar medium and (B, D, F) biomass yield (biom) [black circles] and tolerance index (TI) [grey squares]. Regression analysis equations and correlation coefficient (*R*) data are shown within the plot area.

of different toxic metals). It was found that a special role was played in such an analysis by the main contaminating metal pollutant. *H. ericae* isolates from the Devon Great Consols copper mine were considerably more tolerant to Cu and Cd phosphates, and cuprite, and solubilized Cu-minerals than isolates from non-polluted soil (Table 4, Fig. 6A and B). For the minerals containing metals that were absent or were minor pollutants in the metal-contaminated site where the strains originated, in most cases there were no statistically significant differences in tolerance, solubilization and metal uptake. Moreover, the opposite phenomenon was sometimes observed when solubilization or metal uptake were significantly greater in isolates from uncontaminated environments, e.g. Pb uptake by *H. ericae* grown on pyromorphite-medium (Figs. 5C and 6, Table 4). For *O. maius,* it was found that strains from Zn/Cd-polluted sites solubilized Cu minerals significantly less than control strains from uncontaminated sites (Table 4). No statistical

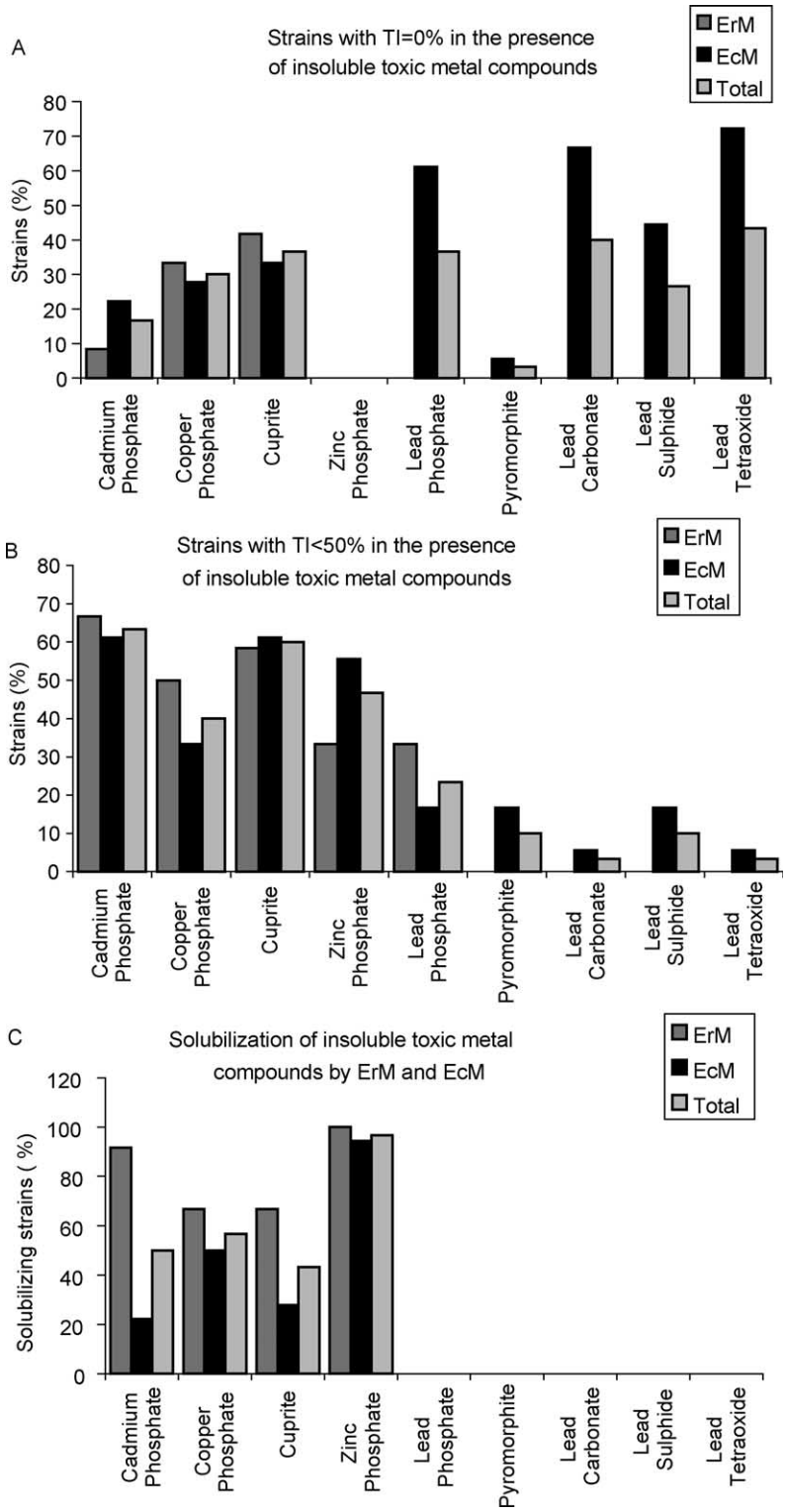

Fig. 4. Lethal effects (TI = 0%) (A) and inhibiting growth effects (TI < 50%) (B) of toxic metal minerals, and mineral solubilization (as presence of any solubilization halo) (C) among strains of ericoid- and ectomycorrhizal fungi (total $n = 32$ strains).

differences between the two groups of *O. maius* isolates were detected in all the other cases. *S. luteus* isolates from Lommel demonstrated a higher tolerance to Cd and Zn phosphates and cuprite, and higher solubilization of Zn phosphate and Zn accumulation in biomass (Fig. 6, Table 4). However, they accumulated less Cu than isolates from uncontaminated soil (Table 4). In general, the higher the concentration of the metal pollutant at the site of origin,

Table 3
Fungal species that showed no growth (TI = 0%) or very inhibited growth (TI ≤ 20%) and no solubilization zone on medium containing insoluble toxic metal compounds for all the strains tested

Compound	Species		
	TI = 0%	TI ≤ 20%	Solubilization zone = 0 mm
$Cd_3(PO_4)_2$	NA	*P. involutus*	*P. involutus, S. luteus*
Cu_2O	*O. maius*	*H. ericea, P. involutus, S. bovinus, S. luteus*	*S. bovinus*
$Pb_3(PO_4)_2$	*S. luteus*	*P. involutus, S. bovinus*	*H. ericea, O. maius, P. involutus, S. bovinus, S. luteus*
Pb_3O_4	*P. involutus, S. luteus, S. bovinus*	NA	*H. ericea, O. maius, P. involutus, S. bovinus, S. luteus*

NA—data not available due to lack of the number of strains.

the more statistically significant was the observed increase in solubilization and tolerance data compared to cultures from uncontaminated sites.

4. Discussion

Fungi may interact with toxic metals and metal-containing minerals in a variety of ways depending on their tolerance and ability to influence the mobility of toxic metals. Metals can be mobilized by fungi by protonolysis, complexation by microbial metabolites and siderophores, and methylation which can result in volatilization. Two main mechanisms of metal mineral dissolution by fungi are proton-promoted and ligand-promoted (Gadd, 2001). Organic acids provide both source of protons for solubilization and metal-chelating anion to complex the metal cation with complexation being dependent on such factors as relative concentrations of the anions and metals, pH, and the stability constants of the various complexes (Devevre et al., 1996). Conversely, immobilization can result from sorption to cell components or exopolymers, transport

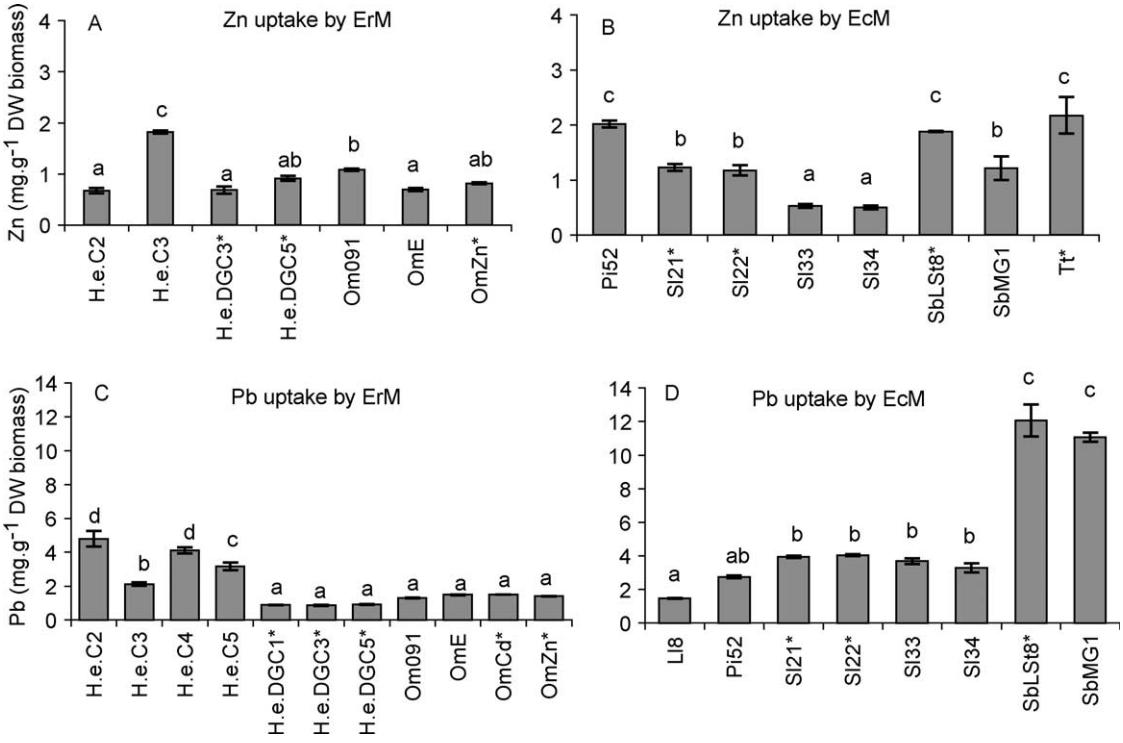

Fig. 5. Accumulation of zinc (A, B) and lead (C, D) in mycelia of ericoid mycorrhizal (A, C) and ectomycorrhizal (B, D) strains grown on media amended with Zn phosphate and pyromorphite, correspondingly. Ericoid mycorrhizal fungi are shown on the left, ectomycorrhizal fungi are shown on the right side of plots. Values are means and derived from at least three replicate determinations, bars indicate ±SEM. DW biomass on axis *y* means the dry weight of biomass. One-way ANOVA was carried out for each parameter and different letters (a–d) denote significant differences for Zn and Pb accumulation at the 5% level using Fisher's LSD test. Fungal cultures on the *x*-axis are (see Table 1): H.e.C2—*H. ericae* C2(1); H.e.C3—*H. ericae* C3(1); H.e.C4—*H. ericae* C4(8); H.e.C5—*H. ericae* C5(10); H.e.DGC1—*H. ericae* DGC1(H); H.e.DGC3—*H. ericae* DGC3(UZ); H.e.DGC5—*H. ericae* DGC5(E); OmA—*O. maius* Zn; OmE—*O. maius* E; Om091—*O. maius* 091; OmCd—*O. maius* Cd; OmZn—*O. maius* Zn; L18—*L. laccata* 8; Pi52—*P. involutus* 52; SbMG1—*S. bovinus* MG1; SbLSt8—*S. bovines* LSt8; Slu21—*S. luteus* 21; Slu 22—*S. luteus* 22; Slu 33—*S. luteus* 33; Slu34—*S. luteus* 34; Tt—*T. terrestris.*

Table 4
Differences in biomass yield, tolerance index (TI), solubilization ability and toxic metal uptake between ErM and EcM strains isolated from toxic metal-contaminated soils ('M'-metal) and from non-contaminated soils ('NM'-non-metal) when grown on medium containing insoluble toxic metal compounds

Compound	TI (%)	Solubilization (mm)	Metal uptake	
			Specific (mg g^{-1} dry weight biomass)	Total (mg $plate^{-1}$)
H. ericae ('M' strains isolated from Cu-contaminated environment)				
$Cd_3(PO_4)_2$	**M > NM** $P=0.013$	M = NM $P=0.055$	–	–
$Cu_3(PO_4)_2$	**M > NM** $P=0.000$	**M > NM** $P=0.000$	–	–
Cu_2O	**M > NM** $P=0.021$	**M > NM** $P=0.000$	–	–
$Zn_3(PO_4)_2$	M = NM $P=0.133$	M = NM $P=0.072$	M = NM $P=0.525$	M = NM $P=0.973$
$Pb_5(PO_4)_3Cl$	M = NM $P=0.053$	*No clear zone*	**M < NM** $P=0.000$	**M < NM** $P=0.000$
O. maius ('M' strains isolated from Cd/Zn-contaminated environment)				
$Cd_3(PO_4)_2$	M = NM $P=0.171$	M = NM $P=0.917$	–	–
$Cu_3(PO_4)_2$	M = NM $P=0.304$	**M < NM** $P=0.000$	–	–
Cu_2O	No growth	**M < NM** $P=0.000$	–	–
$Zn_3(PO_4)_2$	M = NM $P=0.727$	M = NM $P=0.095$	M = NM $P=0.867$	M = NM $P=0.991$
$Pb_5(PO_4)_3Cl$	M = NM $P=0.564$	*No clear zone*	M = NM $P=0.256$	M = NM $P=0.963$
S. luteus ('M' strains isolated from Zn ≫ Cd/Cu-contaminated environment)				
$Cd_3(PO_4)_2$	**M > NM** $P=0.032$	*No clear zone*	–	–
$Cu_3(PO_4)_2$	M = NM $P=0.232$	M = NM $P=0.354$	**M < NM** $P=0.025$	M = NM $P=0.578$
Cu_2O	**M > NM** $P=0.016$	M = NM $P=0.700$	–	–
$Zn_3(PO_4)_2$	**M > NM** $P=0.010$	**M > NM** $P=0.000$	**M > NM** $P=0.002$	**M > NM** $P=0.030$
$Pb_5(PO_4)_3Cl$	M = NM $P=0.926$	*No clear zone*	M = NM $P=0.136$	M = NM $P=0.830$

P—probability determined by one-way ANOVA. Statistically significant results ($P<0.05$) are shown in bold. '–' data not available.

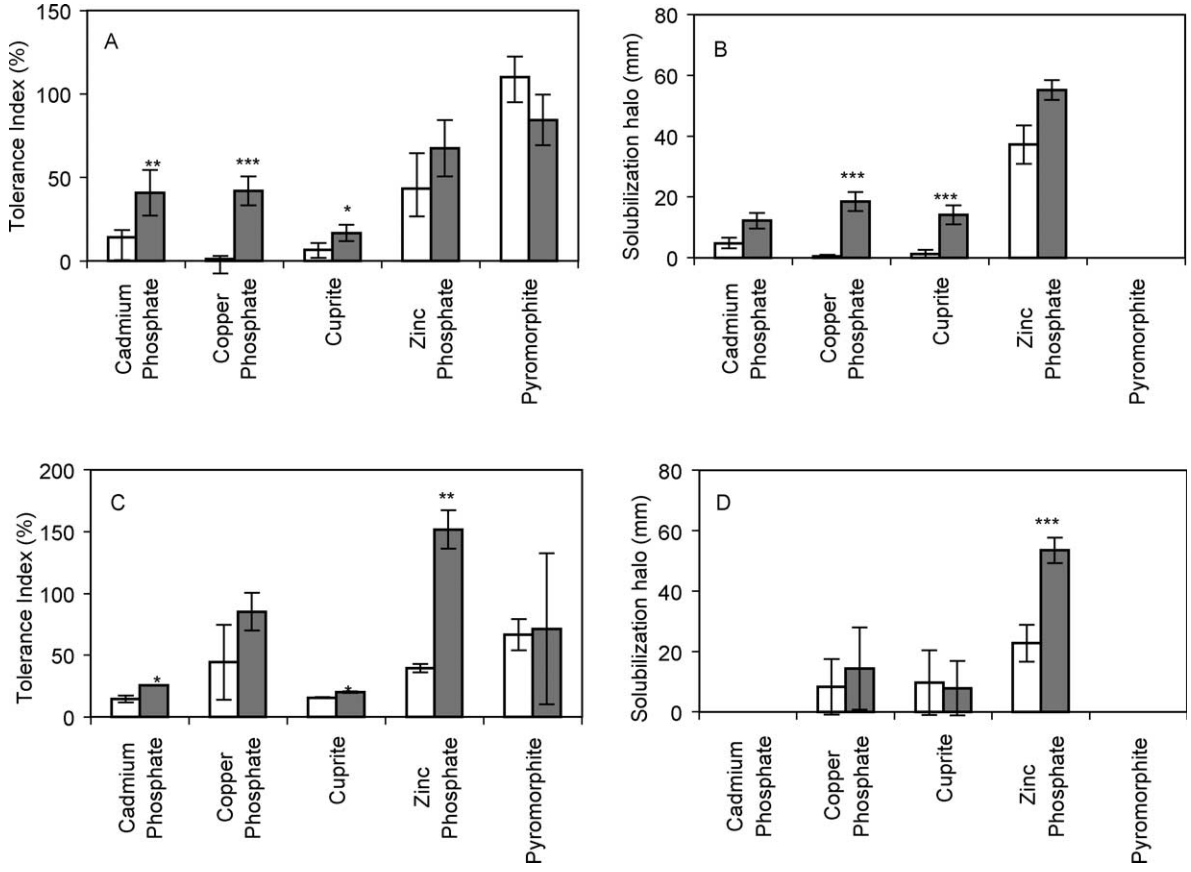

Fig. 6. Tolerance index (A, C) and solubilzation haloes (B, D) data for the populations of ericoid mycorrhizal species *H. ericae* (A, B) and ectomycorrhizal species *S. luteus* (C, D) isolated from metal polluted sites (grey columns) and uncontaminated environment (white columns). Bar represent ±SEM. ($n=7$ for A, B, and $n=4$ for C, D). One-way ANOVA was carried out for each parameter. *, ** and *** denote levels of significant difference at 5, 1, and 0.1%, respectively.

and intracellular sequestration or precipitation as insoluble compounds, e.g. oxalates (Gadd, 1993, 2001; Sayer and Gadd, 1997).

In terms of mineral weathering and dissolution, mycorrhizal fungi form one of the most prominent groups of soil microorganisms (Devevre et al., 1996; Jongmans et al., 1997; Lundstrom et al., 2000). For example, a number of experimental studies have shown the ability of ectomycorrhizal fungi (e.g. *Paxillus involutus*, *Pisolithus tinctorius, Laccaria laccata, L. bicolor, Hebeloma cylindrosporum, H. crustuliniforme, Cenococcum geophilum*) to dissolve Ca-bearing minerals (Callot et al., 1985; Lapeyrie et al., 1991; Gharieb and Gadd, 1999). The ability of some ericoid mycorrhizal fungi (mycorrhizal endophytes of *Woollsia pungens* (Epacridaceae), *Hymenoscyphus ericae* and *Oidiodendron maius*) to dissolve hydroxyapatite, and Zn oxide and phosphate have also been reported (Van Leerdam et al., 2001; Martino et al., 2003). For dissolution of Ca sulphate (gypsum) and different forms of Ca phosphate, it was found that solubilization was significantly affected by the nature of the supplied N source and greater mineral dissolution has been demonstrated in the presence of NH_4^+ than in the presence of NO_3^- (Lapeyrie et al., 1991; Gharieb and Gadd, 1999; Whitelaw et al., 1999; Van Leerdam et al., 2001). This is presumed to reflect acidification of the medium as a result of H^+ excretion from hyphae during NH_4^+ uptake. Our results clearly indicate that, for the majority of tested mycorrhizal fungi grown on solid medium with NH_4^+ as the N source, acidification was the main mechanism of mineral dissolution, which could be enhanced by metal chelation by organic acid anions. It can be also concluded that many ericoid- and ectomycorrhizal fungi are able to solubilize different toxic metal (Cd, Cu, Pb, Zn)-containing minerals. Such dissolution releases toxic metal constituents. In a study of metal toxicity towards basidiomycetes using a micro-well technique, it was shown that fungitoxicity generally increased with increasing electronegativity of the element: Zn was found to be moderately toxic, Cu and Pb toxic and Cd very toxic (Hoiland, 1995). Our results showed that Zn phosphate was the least toxic to the majority of tested fungal isolates despite this mineral being the easiest to dissolve. Lead-bearing minerals were very toxic to ectomycorrhizas but not for ericoid mycorrhizal isolates.

The ability of fungi to withstand stress induced by toxic metals in their environment may be connected with their ability to immobilize and bind toxic metals (e.g. cell wall adsorption, precipitation in extracellular matrix, intracellular chelation by metallothioneins or phytochelatins, sequestration within vacuoles) (Gadd, 1993; Howe et al., 1997; Perotto and Martino, 2001). For mycorrhizal fungi, binding properties appear to be a highly important factor in ameliorating the effects of metal toxicity on the symbiosis (Galli et al., 1994; Vodnik et al., 1998). Most studies have indicated that the extramatrical mycelium of mycorrhizal fungi provides the major binding sites for toxic metals to such components as chitin, melanin, or in the interhyphal spaces to extracellular polysaccharide slime (Denny and Wilkins, 1987b; Colpaert and Van Assche, 1992, 1993; Turnau et al., 1996; Van Tichelen et al., 2001). It has been reported that Pb uptake (determined using ^{210}Pb tracer) was much higher and lead binding to mycelium was much stronger for *S. bovinus* than for other tested ectomycorrhizal species (*Laccaria laccata*, *Lactarius piperatus, Pisolithus tinctorius* and *Amanita muscaria*) (Vodnik et al., 1998). Our results also showed the highest Pb accumulation by *S. bovinus* isolates compared to the other cultures of ericoid- and ectomycorrhizal fungi.

Investigations on metal toxicity in mycorrhizal, especially ectomycorrhizal, fungi at a species and community level have revealed wide inter- and intraspecific variation in sensitivity to metals (Hartley et al., 1997; Vodnik et al., 1998; Blaudez et al., 2000; Meharg and Cairney, 2000). For fungi growing on wood at metal-contaminated sites, natural selection for metal resistant wood-rotting fungal strains was not observed (Baldrian, 2003; Baldrian and Gabriel, 2002). However, the situation may be different in soil, where the concentrations of toxic metals could be higher, and the process of adaptation to metal stress is probably accompanied by the exclusion of metal-sensitive fungal strains (Baldrian, 2003). Ectomycorrhizal fungi show considerable interspecific responses to toxic metals, yet the extent to which intraspecific (adaptive) resistance exists remains unclear (Meharg and Cairney, 2000). The studies of Denny and Wilkins (1987a, b) and Brown and Wilkins (1985a, b) are often cited as showing that there may be no relationship between the soil of origin and metal resistance of EcM fungi isolated from such habitats. The same conclusion has also been made in some other studies of ericoid- and ectomycorrhizal fungi (Blaudez et al., 2000; Martino et al., 2003). However, other studies suggest that, where the selection pressure (i.e. the degree of toxic metal contamination) is high enough, selection for resistant ecotypes can indeed occur (Sharples et al., 2000, 2001; Colpaert et al., 2000). The absence of a uniform and strong enough selection pressure for a whole group of fungal isolates from metal-polluted environments seems to be the main reason for the absence of any clear relation between metal tolerance and metal pollution of the sites of their origin. This is probably why the grouping of fungal cultures that have been isolated from different metal-polluted sites often show no relationship between metal contamination of the original soil and metal tolerance (Blaudez et al., 2000). The degree of selection pressure will depend not only on such characteristics of the metal-polluted site as metal species and speciation, and the combination and proportion of different toxic metals, soil type and pH, but also on the species of fungi present. Our study has demonstrated that differences in toxic metal mineral tolerance, mineral solubilization and metal uptake between populations isolated from metal-polluted and uncontaminated sites were clearly related to the toxic metal which was the main pollutant in the original contaminated environment.

In general, metal tolerant fungal strains solubilized toxic metal minerals better than non-tolerant isolates. It may be predicted that metal-tolerant fungal cultures will find more use in bioremediation and reforestation of metal-contaminated areas simply because they will be able to grow and develop mycorrhizas better than non-tolerant fungi. However, toxic metal-bearing minerals dissolution and metal mobilization or immobilization by ectomycorrhizal fungi with different abilities to withstand metal toxicity might also be strongly affected by mycorrhizal and host plant status, and physico-chemical conditions of growth. Assessment of the potential application of metal-tolerant and non-tolerant mycorrhizal fungi to bioremediation requires further study of their biogeochemical activity in both axenic culture and mycorrhizal association under different nutritional conditions such as phosphorus limitation.

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References

Baldrian, P., 2003. Interaction of heavy metals with white-rot fungi. Enzyme and Microbial Technology 32, 78–91.

Baldrian, P., Gabriel, J., 2002. Intraspecific variability in growth response to cadmium of wood-rotting fungus *Piptoporus betulinus*. Mycologia 94, 428–436.

Blaudez, D., Jacob, C., Turnau, K., Colpaert, J.V., Ahonen-Jonnarth, U., Finlay, R., Botton, B., Chalot, M., 2000. Differential responses of ectomycorrizal fungi to heavy metals in vitro. Mycological Research 104, 1366–1371.

Bradley, R., Burt, A.J., Read, D.J., 1981. Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris*. Nature 292, 335–337.

Brown, M.T., Wilkins, D.A., 1985a. Zinc tolerance of *Amanita* and *Paxillus*. Transactions of the British Mycological Society 84, 367–369.

Brown, M.T., Wilkins, D.A., 1985b. Zinc tolerance of mycorrhizal *Betula*. New Phytologist 99, 101–106.

Callot, G., Mousain, D., Plassard, C., 1985. Concentration of calcium carbonate on the walls of fungal hyphae. Agronomie 5, 143–150.

Colpaert, J.V., Van Assche, J.A., 1992. Zinc toxicity in ectomycorrhizal *Pinus sylvestris*. Plant and Soil 143, 201–211.

Colpaert, J.V., Van Assche, J.A., 1993. The effect of cadmium on ectomycorrhizal *Pinus sylvestris* L. New Phytologist 123, 325–333.

Colpaert, J.V., Vandenkoornhuyse, P., Adriaensen, K., Vangronsveld, J., 2000. Genetic variation and heavy metal tolerance in the ectomycorrhizal basidiomycete *Suillus luteus*. New Phytologist 147, 367–379.

Denny, H.J., Wilkins, D.A., 1987a. Zinc tolerance in *Betula* spp. III. Variation in response to zinc among ectomycorrhizal associates. New Phytologist 106, 535–544.

Denny, H.J., Wilkins, D.A., 1987b. Zinc tolerance in *Betula* ssp. IV. The mechanism of ectomycorrhizal amelioration of zinc toxicity. New Phytologist 106, 545–553.

Devevre, O., Garbaye, J., Botton, B., 1996. Release of complexing organic acids by rhizosphere fungi as a factor in Norway Spruce yellowing in acidic soils. Mycological Research 100, 1367–1374.

Fay, D.A., Mitchell, D.T., 1999. A preliminary study on the mycorrhizal associations of tree seedlings growing on old mine spoil at Avoca, Co. Wicklow. Biology and Environment Proceedings of the Royal Irish Academy 99B, 19–26.

Frink, C.R., 1996. A perspective on metals in soils. Journal of Soil Contamination 5, 329–359.

Gadd, G.M., 1986. Fungal responses towards heavy metals, in: Herbert, R.A., Codd, G.A. (Eds.), Microbes in extreme environments. Academic Press, London, pp. 83–110.

Gadd, G.M., 1993. Interactions of fungi with toxic metals. New Phytologist 124, 25–60.

Gadd, G.M., 2001. Metal transformations, in: Gadd, G.M. (Ed.), Fungi in Bioremediation. Cambridge University Press, Cambridge, pp. 359–382.

Gallagher, V., O'Connor, P., 1997. Characterization of the Avoca Mine Site: Geology Mining Features, History and Soil Contamination Study. GSI Technical Report MS97/1. Geological Survey of Ireland, Beggars Bush, Haddington Road, Dublin 4.

Galli, U., Schuepp, H., Brunold, C., 1994. Heavy metal binding by mycorrhizal fungi. Physiologia Plantarum 92, 364–368.

Gharieb, M.M., Gadd, G.M., 1999. Influence of nitrogen source on the solubilization of natural gypsum. Mycological Research 103, 473–481.

Gildon, A., Tinker, P.B., 1983. Interactions of vesicular arbuscular mycorrhizal infection and heavy metals on the development of vesicular–arbuscular micorrhizas. New Phytologist 95, 247–261.

Greszta, J., Braniewski, S., Chrzanowska, E., Nosek, A., Chlodny, J., Zwolinski, J., 1987. The influence of dusts from chosen industrial lands on particular links of forest ecosystems of the Niepolomice Forest. Ekologia Polska 35, 291–326.

Hartley, J.W., Cairney, G., Meharg, A.A., 1997. Do ectomycorrhizal fungi exhibit adaptive tolerance to potentially toxic metals in the environment?. Plant and Soil 189, 303–319.

Heggo, A., Angle, J.S., 1990. Effects of vesicular–arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biology&Biochemistry 22, 856–869.

Hoiland, K., 1995. Reaction of some decomposer basidiomycetes to toxic elements. Nordic Journal of Botany 15, 305–318.

Hopkin, S.P., 1993. Ecological implication of 95% protection levels for metals in soil. Oikos 66, 137–141.

Howe, R., Evans, R.L., Ketteridge, S.W., 1997. Copper-binding proteins in ectomycorrhizal fungi. New Phytologist 135, 123–131.

Jongmans, A.G., Van Breemen, N., Lungstrom, U., Van Hees, P.A.W., Finlay, R.D., Srinivasan, M., Unestam, T., Giesler, R., Melkerud, P.A., Olsson, M., 1997. Rock-eating fungi. Nature 389, 682–683.

Kabata-Pendias, A., Dudka, S., Chlopechka, A., Gawinowska, T., 1992. Background levels and environment influences on trace metals in soils of temperate humid zone of Europe, in: Adriano, D.C. (Ed.), Biogeochemistry of Trace Metals. CRC Press, Boca Raton, FL, pp. 61–84.

Knox, A.S., Seaman, J.C., Mench, M.J., Vangronsveld, J., 2000. Remediation of metal- and radionuclide-contaminated soils by in situ stabilization techniques, in: Iskandar, I.K. (Ed.), Environmental Restoration of Metals-Contaminated Soil. Lewis Publishers, Boca Raton, FL, pp. 21–61.

Lapeyrie, F., Ranger, J., Vairelles, D., 1991. Phosphate-solubilizing activity of ectomycorrhizal fungi in vitro. Canadian Journal of Botany 69, 342–346.

Lundstrom, U.S., Van Breemen, N., Bain, D., 2000. The podzolization process. A review. Geoderma 94, 91–107.

Martino, E., Turnau, K., Girlanda, M., Bonfante, P., Perotto, S., 2000. Ericoid mycorrhizal fungi from heavy metal polluted soils: their identification and growth in the presence of zinc ions. Mycological Research 104, 338–344.

Martino, E., Perotto, S., Parsons, R., Gadd, G.M., 2003. Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. Soil Biology&Biochemistry 35, 133–141.

Meharg, A.A., Cairney, J.W.G., 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Advanced Ecological Research 30, 69–112.

Olsson, P.A., Wallander, H., 1998. Interactions between ectomycorrhizal fungi and the bacterial community in soils amended with various primary minerals. FEMS Microbiology Ecology 27, 195–205.

Paris, F., Bonnaud, P., Ranger, J., Lapeyrie, F., 1995. In vitro weathering of phlogopite by ectomycorrhizal fungi. Plant and Soil 177, 191–201.

Perotto, S., Martino, E., 2001. Molecular and cellular mechanisms of heavy metal tolerance in mycorrhizal fungi: what perspectives for bioremediation?. Minerva Biotechnologica 13, 55–63.

Porter, E.K., Peterson, P.J., 1997. Arsenic tolerance in grasses growing on mine waste. Environmental Pollution 14, 255–265.

Sayer, J.A., Gadd, G.M., 1997. Solubilisation and transformation of insoluble metal compounds to insoluble metal oxalates by *Aspergillus niger*. Mycological Research 101, 653–661.

Sayer, J.A., Raggett, S.L., Gadd, G.M., 1995. Solubilization of insoluble compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. Mycological Research 99, 987–993.

Sayer, J.A., Cotter-Howells, J.D., Watson, C., Hillier, S., Gadd, G.M., 1999. Lead mineral transformation by fungi. Current Biology 9, 691–694.

Sharples, J.M., Chambers, S.M., Meharg, A.A., Cairney, J.W.G., 2000. Genetic diversity of root-associated fungal endophytes from *Calluna vulgaris* at contrasting field sites. New Phytologist 148, 153–162.

Sharples, J.M., Meharg, A.A., Chambers, S.M., Cairney, J.W.G., 2001. Arsenate resistance in the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. New Phytologist 151, 265–270.

Turnau, K., Kottke, I., Dexheimer, J., 1996. Toxic element filtering in *Rhizopogon roseolus*/*Pinus sylvestris* mycorrhizas collected form calamine dumps. Mycological Research 100, 16–22.

Van der Lelie, D., Schwitzguebel, J.P., Glass, D.J., Vangronsveld, J., Baker, A., 2001. Assessing phytoremediation's progress in the United States and Europe. Environmental Science and Technology 35, 446A–452A.

Van Leerdam, D.M., Williams, P.A., Cairney, J.W.G., 2001. Phosphate-solubilizing abilities of ericoid mycorrhizal endophytes of *Woollsia pungens* (Epacridaceae). Australian Journal of Botany 49, 75–80.

Van Tichelen, K.K., Colpaert, J.V., Vangronsveld, J., 2001. Ectomycorrhizal protection of *Pinus sylvestris* against copper toxicity. New Phytologist 150, 203–213.

Vodnik, D., Byrne, A.R., Gogala, N., 1998. The uptake and transport of lead in some ectomycorrhizal fungi in culture. Mycological Research 102, 953–958.

Wallander, H., Wickman, T., Jacks, G., 1997. Apatite as a source in mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. Plant and Soil 196, 123–131.

Whitelaw, M.A., Harden, T.J., Helyar, K.R., 1999. Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum*. Soil Biology and Biochemistry 31, 655–665.