Stabilisation of metals in mine spoil with amendments and growth of red fescue in symbiosis with mycorrhizal fungi

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ABSTRACT

Metal stabilisation effects of amendments (1% m/m $CaCO_3$-L, 5% m/m municipal sewage sludge compost-MSSC, and 7.5% m/m natural zeolite-Z) were studied on acidic mine spoil (pH_{KCl} 3.73) from Gyöngyösoroszi (Hungary) containing Cd-15.0, Cu-336, Mn-568, Pb-1919, and Zn-3306 mg/kg. Red fescue (*Festuca rubra*, cv. Keszthelyi 2) was grown in a pot experiment in amended mine spoil, and was infected with spores of a Zn-tolerant arbuscular mycorrhizal fungi (AMF) *Glomus intraradices*. During 12 weeks of plant growth L + MSSC + Z amendments were more effective in stabilizing the pH, reducing the leaching and phytoavailability of metals in mine spoil than the lime application itself. Liming (L) had a negative effect on Cd, Mn and Zn concentrations in mine spoil water (leachate) as compared to the untreated control. In mine spoil stabilized with a combination of amendments red fescue growth can be established in symbiosis with AMF. Rate of AMF infection (F 4%) was low in limed mine spoil but was especially high (F 93%) in the case of L + MSSC + Z application. In shoots of these cultures – as a trend – AMF reduced the concentration of Cd by 35–55%, Cu by 9–34%, Mn by 14–55%, and Zn by 22–44%.

Keywords: stabilisation; heavy metals; mine spoil; amendments; red fescue; arbuscular mycorrhizal fungi

During the last decades as a consequence of mining, metal processing, industrialization, traffic, burning of fossil fuels, disposal of wastes, etc., soil and water resources were contaminated with heavy metals (i.e. Pb, Cd, Zn, Cu, Cr, Ni and Hg) all over the world. Most of the physical or physicochemical remediation methods (i.e. soil flushing, washing, vitrification) to clean the soils degraded with metals not only destroy the physical structure of the soils and stop their biological activity, but also generate secondary pollutants, which needs further handling (Adriano 2001). Phytostabilisation is a new promising strategy to handle polluted soils or mine spoils. During phytostabilisation the pollutants in soils, mine spoils, or industrial by-products are first stabilized with amendments and additives (e.g. liming agents, organic materials, aluminosilicates, phosphates, iron and manganese oxides, coal fly ashes) to reduce their solubility and mobility. Polluted areas are then covered with plants (usually first with grasses, later with bushes and trees) to prevent a contamination of groundwater, air or neighbouring uncontaminated areas (Berti and Cunningham 2000, Mench et al. 2000). Plant roots physically stabilize the soil, prevent erosion and deflation, may help to reduce water

Supported by the OTKA T043479, T046610 and EU-Kp5 Mycorem Projects, István Széchenyi Scholarship and EU COST 631 Action.

percolation through the soil, and can minimize the leaching of contaminants to groundwater. Metal-tolerant genotypes of red fescue (*Festuca rubra* L.) can be utilized to establish the vegetative cover in mine spoil heaps or contaminated soils previously treated with stabilisation amendments (Vangronsveld and Cunningham 1998, Li et al. 2000).

Fine roots of red fescue are usually colonized by arbuscular mycorrhizal fungi (Glomeromycota, Glomerales order; Colpaert 1998). Extraradical hyphae of mycorrhizal fungi can enhance the metal tolerance of host plants by sequestering of toxic metals in soil (Leyval et al. 1997, Colpaert 1998).

North from the village Gyöngyösoroszi (located in Mátra Mountains, North Hungary) a sphalerite and galenite mine had been operating for 40 years (till 1985). Careless handling of mine spoil caused serious heavy metal pollution of the local environment (Horvath and Gruiz 1996, Kovács and Tamás 2002, Lakatos et al. 2002). Nitric acid extractions of this mine spoil revealed that the highest concentrations of Cd, Cu, Pb and Zn are 55, 9000, 16 500 and 42 000 mg/kg (Kovács and Tamás 2002). During our previous pot experiment with red fescue we found that raising the pH of this strongly acidic mine spoil with lime ($CaCO_3$) and application of ammonium nitrate made this phytotoxic material suitable for plant growth (Simon 2005). Among tested amendments lime, municipal sewage sludge compost and natural zeolite had the best metal stabilisation effects in mine spoil, and reduced best the phytoavailability of metals (Simon 2005).

The objectives of our work were to study the stabilisation of metals (Cd, Cu, Mn, Pb and Zn) in mine spoil with combination of amendments (lime, municipal sewage sludge compost, natural zeolite), and the growth of red fescue in symbiosis with mycorrhizal fungi in this medium.

MATERIAL AND METHODS

Mine spoil samples (approximately 25 kg) were collected north of Gyöngyösoroszi from one terrace of tailing dumps of a former mine (WGS 84 coordinates: 47°50'34"N, 19°52'44"E, 352 m above the Baltic see level). Mine spoil sampling, mixing, drying, screening, elemental analysis, determination of pH and liming were detailed in our former publication (Simon 2005). Addition of 1.0% (m/m) $CaCO_3$ (*puriss.*, Reanal Ltd., Hungary) to this acidic mine spoil raised the pH above 6.5, which is suitable for plant growth. Municipal sewage sludge compost and natural zeolite origin and their mixing to mine spoil were described in Simon (2005). Pellets with spores of Zn-tolerant arbuscular mycorrhizal fungi (*Glomus intraradices* BrI isolate, Glomeromycota, Glomerales; Inox GmbH, Germany) were used as mycorrhizal inoculants (Hildebrandt et al. 1999).

Growth chamber pot experiment with red fescue

The non-sterile mine spoil (20 000 g) was divided into five 4000 g sub-samples. The treatments were as follows: 1. untreated mine spoil; 2. (1) + 1.0% $CaCO_3$; 3. (2) + *Glomus intraradices* infection; 4. (2) + 5% (m/m) municipal sewage sludge compost +7.5% (m/m) natural zeolite; 5. (4) + *Glomus intraradices* infection. These treated sub-samples were divided into 1000 grams, and were placed into plastic pots with 16 cm diameter and 13 cm height. Limed mine spoil was thereafter incubated for 9 weeks in room temperature receiving distilled water weekly to reach a constant weight (1250 grams). pH in mine spoil was determined before seeding and at the end of the pot experiment (Simon 2005).

Pot experiment (randomized experimental design with 4 replicates) was conducted with red fescue (*Festuca rubra* L. cv. Keszthelyi 2) in a growth chamber of the College of Nyíregyháza, Hungary. Light, temperature, humidity, water and nitrogen supply were controlled (Simon 2005). In every pot 0.6 g red fescue seeds were planted. In the case of treatments 3 and 5 pellets (0.03% m/m) with spores of *Glomus intraradices* were mixed with mine spoil under the roots of 2-week-old host plants. Untreated mine spoil without plants was watered during the whole experiment similarly as in the other treatments (to constant 1350 g weight).

Shoots of red fescue were harvested four weeks (1^{st} cut), eight weeks (2^{nd} cut) and 12 weeks (3^{rd} cut) after planting. At the 3^{rd} cut roots were also harvested. Sampling, washing, drying, digesting and element analysis of plant materials were described formerly (Simon 2005). From washed roots 0.2 g sub-samples were taken in triplicate. The frequency (F%) and intensity (M%) of the mycorrhizal infection, the absolute (a%) and relative (A%) arbusculum richness were estimated by rating the density of infection on 30 one-cm root segments, using a five-class system of Trouvelot et al. (1985).

Sampling of mine spoil and mine spoil water

Mine spoil samples were taken in 4 replicates at the end of the pot experiment (Simon 2005). Mine spoil water samples (leachates) were taken when plants were 4, 8 or 12 weeks old with soil water samplers (Prenart Super Quartz®, Prenart Equipment ApS, Frederiksberg, Denmark). After 24 hours of suction with 667 mbar vacuum the collected mine spoil water (appr. 80 cm^3) was thoroughly mixed and filtered on MN 619 G ¼ type filter paper (Machinery-Nagel, Düren, Germany). Three 10 cm^3 sub-samples were taken for element analysis.

Elemental composition of mine spoil, mine spoil water and plant samples was determined by inductively coupled argon plasma emission spectrometry (ICAP, model Perkin-Elmer Optima 3300 DV) technique in triplicate at Debrecen University, Centre for Agricultural Sciences, Hungary.

Statistical analysis of data was made with SPSS 12.0.1. software, using analysis of variance followed by Tukey's *b*-test.

RESULTS AND DISCUSSION

Decrease of pH in mine spoil

Figure 1 shows the pH decrease in untreated and limed mine spoil after 12 weeks of red fescue growth. Application of lime raised the pH by 3 units. Application of municipal sewage sludge compost and natural zeolite further raised the initial pH of the limed mine spoil by 0.5–0.8 units. After 12 weeks of plant growth the rate of pH decrease was definitely lower in treatments 4 or 5 than in treatments 2 or 3. Glomus inoculation of plants has not influenced the pH of limed and amended mine spoil. Application of sewage sludge compost and zeolite was found to be more advantageous in respect to pH stabilisation than the liming itself, since these materials slowed down the pH decrease during the plant growth in mine spoil. For stabilisation of metals in mine spoil the least decrease in pH is the most advantageous, since the lower is the pH the higher is the mobility of most heavy metals (including Cd, Cu, Mn, Pb and Zn) in soil-plant system (Kabata-Pendias and Pendias 2001). Decrease of pH can be explained by oxidation of pyrite that is present in high quantities in mine spoils (Vangronsveld and Cunningham 1998).

Elemental composition of mine spoil and mine spoil water

Table 1 demonstrates the effects of treatments on the metal composition of mine spoil after 12 weeks of red fescue growth. In treatments 2–5 significantly lower Cd, Cu, Mn and Zn concentrations were measured at the end of the pot experiment

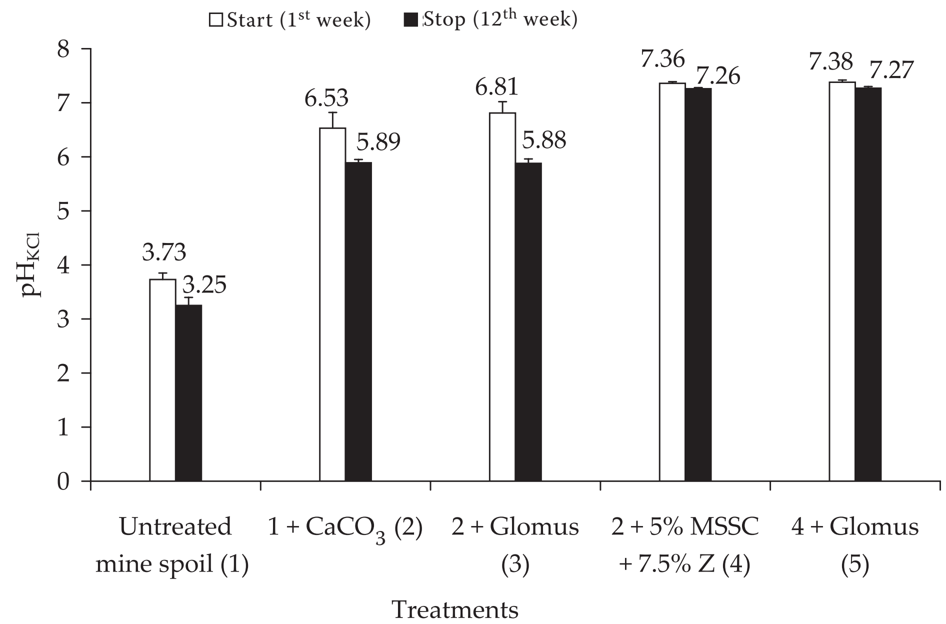

Figure 1. Effect of amendments and Glomus inoculation on pH decrease in untreated and limed mine spoil from Gyöngyösoroszi (Hungary) after 12 weeks of red fescue growth (pot experiment, Nyíregyháza, Hungary); MSSC – municipal sewage sludge compost; Z – zeolite; data are means of 4 replications

Table 1. Metal composition of mine spoil from Gyöngyösoroszi (Hungary) treated with various amendments and inoculated with *Glomus intraradices* (determined in cc. HNO_3/H_2O_2 extracts after 12 weeks of red fescue growth at the end of the pot experiment; Nyíregyháza, Hungary)

Treatments	Cd	Cu	Mn	Pb	Zn
	mg/kg				
1. Untreated mine spoil*	20.0^{c}	484^{b}	723^{c}	1749^{a}	5352^{c}
2. (1) + 1% $CaCO_3$	14.9^{a}	322^{a}	497^{a}	1990^{b}	3229^{a}
3. (2) + *Glomus intraradices*	15.3^{ab}	324^{a}	506^{a}	1988^{a}	3153^{a}
4. (2) + 5% MSSC + 7.5% Z	16.2^{b}	332^{a}	601^{b}	1652^{a}	3670^{b}
5. (4) + *Glomus intraradices*	16.1^{ab}	338^{a}	610^{b}	1694^{a}	3758^{b}

*in untreated mine spoil was no plant growth; MSSC – municipal sewage sludge compost; Z – zeolite; data are means of 4 replications; Tukey's *b*-test was conducted for each column; means marked with the same letter are not significantly different ($P < 0.05$)

than in untreated mine spoil. This reduction could be attributed to the metal concentration dilution effect of the mass of applied amendments, and/or to physico-chemical immobilization of metals by applied amendments (from treated mine spoil samples less metals were extractable after wet HNO_3/H_2O_2 destruction). If we compare the metal concentrations in treatments 2 or 3 and treatments 4 or 5, it is obvious that municipal sewage sludge compost and zeolite application significantly enhanced the manganese and zinc concentration in mine spoil. A slight increase in Cd concentration was also observed. Element analysis revealed that sewage sludge compost is only moderately contaminated with heavy metals (Cd-3.96, Cu-158, Mn-468, Pb-135 and Zn-1278 mg/kg). Only traces of heavy metals (Cd-0.77, Cu-2.99, Mn-156, Pb-25.1 and Zn-35.1 mg/kg) were found in zeolite.

Table 2 shows metal concentrations in mine spoil water (leachate) during the pot experiment. As a general trend, metal concentrations were the lowest in leachate of limed mine spoil amended with sewage sludge compost and zeolite (treatment 4). Very low metal concentrations present in leachate of treatment 4 can be attributed to combined effects of metal immobilization and

Table 2. Metal concentrations in mine spoil water (leachate) during the growth of red fescue in amended mine spoil (pot experiment, Nyíregyháza, Hungary)

Treatments	Sampling time (plant growth)	Cd	Cu	Mn	Pb	Zn
		μg/cm^3				
1. Untreated mine spoil*	4 weeks	0.777^{c}	0.044^{a}	23.9^{b}	u.d.l.	64.7^{b}
2. (1) + 1% $CaCO_3$	4 weeks	0.627^{b}	0.029^{a}	50.0^{c}	u.d.l.	82.6^{c}
4. (2) + 5% MSSC + 7.5% Z	4 weeks	0.067^{a}	0.038^{a}	0.57^{a}	u.d.l.	5.54^{a}
1. Untreated mine spoil*	8 weeks	0.249^{c}	0.034^{a}	10.0^{b}	u.d.l.	25.1^{b}
2. (1) + 1% $CaCO_3$	8 weeks	0.583^{a}	0.041^{a}	32.6^{c}	u.d.l.	83.2^{c}
4. (2) + 5% MSSC + 7.5% Z	8 weeks	0.057^{b}	0.024^{a}	0.88^{a}	u.d.l.	2.41^{a}
1. Untreated mine spoil*	12 weeks	0.303^{b}	0.023^{a}	5.60^{a}	u.d.l.	30.2^{b}
2. (1) + 1% $CaCO_3$	12 weeks	0.698^{c}	0.075^{b}	31.2^{b}	u.d.l.	100^{c}
4. (2) + 5% MSSC + 7.5% Z	12 weeks	0.039^{a}	0.031^{a}	1.68^{a}	u.d.l.	1.57^{a}

*in untreated mine spoil was no plant growth; MSSC – municipal sewage sludge compost; Z – zeolite; u.d.l. – under the detection limit; data are means of 3 replications; Tukey's *b*-test was conducted for each column; means within the mine spoil water collection times marked with the same letter are not significantly different ($P < 0.05$)

water retention by organic compounds present in sewage sludge compost (Kabata-Pendias and Pendias 2001), and to sorption or incorporation of metals into zeolite, and to highest mine spoil pH (Figure 1) among treatments. Cd, Mn and Zn concentrations were higher in limed mine spoil (treatment 2 with plant growth) than in untreated mine spoil (treatment 1 without plants and lower pH) leachate. This could be explained as a competition of Ca – dissolved from the lime in the acidic substrate – for sorption sites, thus releasing cations into the mine spoil leachate (Adriano 2001).

Our experimental data suggest that sewage sludge compost and zeolite in combination with lime are more effective to prevent metal leaching, than the liming alone. This is in agreement with findings of other authors (Li et al. 2000, Berti and Cunningham 2000).

Metal accumulation and arbuscular mycorrhizal fungi colonisation of red fescue

Table 3 presents metal accumulation in red fescue grown in amended and *Glomus intraradices* inoculated mine spoil. In shoots and roots of plants grown in limed mine spoil significantly higher Mn and Zn concentrations were measured in all sampling times than in cultures grown in sewage sludge compost and zeolite amended limed mine spoil. At the 3rd cut a similar tendency was observed in Cd accumulation of shoots. This is in correlation with the highest Mn, Zn and Cd concentrations measured in the leachates of limed mine spoil (Table 2).

It was found in our previous pot experiment (Simon 2005) with the same mine spoil that in limed mine spoil the relative percentages of Cd or Zn concentrations in "plant available" (H_4EDTA in

Table 3. Metal accumulation in red fescue grown in amended and *Glomus intraradices* inoculated mine spoil (pot experiment, Nyíregyháza, Hungary)

Treatments	Root (µg/g)					Shoot (µg/g)				
	Cd	Cu	Mn	Pb	Zn	Cd	Cu	Mn	Pb	Zn
4 weeks old plants (1st cut)										
1. Untreated mine spoil*	–	–	–	–	–	–	–	–	–	–
2. (1) + 1% $CaCO_3$	–	–	–	–	–	2.64^{a}	24.2^{a}	1224^{b}	3.51^{a}	685^{c}
3. (2) + *Glomus intraradices*	–		–	–	–	2.69^{a}	26.4^{a}	1295^{b}	3.68^{a}	560^{bc}
4. (2) + 5% MSSC + 7.5% Z	–	–	–	–	–	2.69^{a}	42.4^{a}	158^{a}	2.48^{a}	423^{ab}
5. (4) + *Glomus intraradices*	–	–	–	–	–	1.76^{a}	28.0^{a}	71^{a}	3.19^{a}	238^{a}
8 weeks old plants (2nd cut)										
1. Untreated mine spoil*	–	–	–	–	–	–	–	–	–	–
2. (1) + 1% $CaCO_3$	–	–	–	–	–	4.53^{c}	34.5^{b}	2392^{c}	8.47^{a}	1090^{c}
3. (2) + *Glomus intraradices*	–	–	–	–	–	2.88^{b}	25.0^{a}	1501^{b}	3.01^{a}	607^{b}
4. (2) + 5% MSSC + 7.5% Z	–	–	–	–	–	3.13^{b}	21.4^{a}	80^{a}	1.36^{a}	365^{a}
5. (4) + *Glomus intraradices*	–	–	–	–	–	1.65^{a}	19.3^{a}	64^{a}	2.04^{a}	263^{a}
12 weeks old plants (3rd cut and harvest)										
1. Untreated mine spoil*	–	–	–	–	–	–	–	–	–	–
2. (1) + 1% $CaCO_3$	35.1^{a}	440^{b}	3665^{b}	571^{b}	4789^{b}	9.33^{b}	21.6^{a}	3778^{b}	19.7^{a}	1584^{b}
3. (2) + *Glomus intraradices*	38.6^{a}	346^{a}	2364^{b}	409^{ab}	4464^{b}	9.11^{b}	24.7^{a}	3367^{b}	4.6^{a}	1441^{b}
4. (2) + 5% MSSC + 7.5% Z	52.5^{b}	525^{bc}	150^{a}	334^{a}	2499^{a}	3.92^{a}	19.1^{a}	94^{a}	1.5^{a}	523^{a}
5. (4) + *Glomus intraradices*	41.4^{a}	547^{c}	116^{a}	222^{a}	2269^{a}	1.78^{a}	16.8^{a}	81^{a}	1.3^{a}	404^{a}

*in untreated mine spoil was no plant growth; MSSC – municipal sewage sludge compost; Z – zeolite; data are means of 4 replications; Tukey's *b*-test was conducted for each column; means within the harvests marked with the same letter are not significantly different ($P < 0.05$)

Table 4. Colonisation of arbuscular mycorrhizal fungi on red fescue grown in amended and *Glomus intraradices* inoculated mine spoil (10 weeks of plant growth after Glomus inoculation; pot experiment, Nyíregyháza, Hungary)

Treatments	Mycorrhizal colonisation			
	F%	M%	a%	A%
1. Untreated mine spoil*	0	0	0	0
2. (1) + 1% $CaCO_3$	0	0	0	0
3. (2) + *Glomus intraradices*	4[a]	0	0	0
4. (2) + 5% MSSC + 7.5% Z	10[a]	0	0	0
5. (4) + *Glomus intraradices*	93[b]	48	35	17

*in untreated mine spoil was no plant growth; MSSC – municipal sewage sludge compost; Z – zeolite; F – frequency of AMF infection (%); M – intensity of AMF infection (%); a – arbusculum richness in the stained root segments (%); A – arbusculum richness converted to the whole root system (%); data are means of 3 replications; Tukey's *b*-test was conducted for first column; means marked with the same letter are not significantly different ($P < 0.05$)

ammonium acetate buffer soluble metals according to Lakanen and Erviö 1971) fractions compared to "total" (HNO_3/H_2O_2 extractable) concentrations were higher than in the sewage sludge compost treated limed mine spoil. In the case of zeolite this trend was not so clear, but metal immobilizing was also effective (Simon 2005).

Lower plant metal accumulation in plants can be attributed to metal immobilizing effects of organic compounds and phosphates present in sewage sludge compost, and to metal sorption or incorporation to applied zeolite. Our observations regarding to differences in metal stabilization efficiency of applied amendments confirm the findings of Li et al. (2000). In their study the limestone amendment added to Zn and Cd contaminated soil was somewhat effective, but limed biosolids-compost was highly effective in reducing Zn and Cd uptake of red fescue shoots.

The rate of mycorrhizal infection was absent (F% = 0) or very low (F% = 4–10) in treatments 2, 3 or 4, while it was especially high (F% = 93, M% = 48) in treatment 5 (Table 4), in connection with improved mine spoil properties. In these cultures the increased absolute (a% = 35) and relative (A% = 17) arbusculum richness indicated good functioning of the symbiosis (Table 4). The observed high rate of mycorrhizal infection of red fescue in our sewage sludge compost amended mine spoil is in contrast with results of Thorne et al. (1998), who found reduced arbuscular mycorrhizal fungi colonization of *Secar bluebunch* wheatgrass grown in sewage sludge compost amended mine spoil.

Although not statistically proven, there is a trend in reduction of metal accumulation of red fescue shoots (also roots) by Zn-tolerant Glomus infection in the case of treatment 5 (compare treatment 5 to 4 in Table 4). During all 3 sampling times 35–55% less Cd, 9–34% less Cu, 14–55% less Mn, and 22–44% less Zn was detected in shoots of Glomus infected red fescue grown in sewage sludge compost and zeolite amended limed mine spoil. When total metal transfer to shoots (µg metal/pot) was calculated by multiplying the metal concentrations in shoots (Table 3) with dry matter yield of shoots (data not shown), a similar tendency was found.

Arbuscular mycorrhizal fungi colonisation reduced the zinc toxicity to grass species (including red fescue) grown in zinc-polluted soil (Dueck et al. 1986). To verify the reduced metal accumulation of our host plant we plan to select metal-tolerant arbuscular mycorrhizal fungi from the sporadic vegetation in Gyöngyösoroszi mine spoil, which are adapted to extreme conditions (low pH, high metal content) of mine spoil. Presumably these fungi will be even more effective in metal stabilization than the Zn-tolerant *Glomus intraradices* tested in this experiment.

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Received on October 27, 2005

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