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The effect of bioremediation methods involving different degrees of soil disturbance on the export of metals by leaching and by plant uptake

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Abstract

Lysimeter experiments were performed in order to investigate the effects of two bioremediation methods on the export of metals from a former uranium mine site located near Ronneburg, Thuringia, Germany. The first method consisted of the application of topsoil and compost to contaminated soil. Soil was sampled from two amended plots and from an unamended control, all located on a former uranium-leaching heap. The second method involved the mixing of the soil from the control plot with expanded clay, mycorrhizal fungi and *Streptomyces* sp. The lysimeters were sown with a mixture of two plant species. The export of metals by leaching and by plant uptake from the amended plots was higher than in the control, mainly because of the higher hydraulic conductivity and the higher plant biomass. The inoculation of control soil with mycorrhizal fungi decreased the leaching of metals, but the effect on metal uptake by plants was metal and species dependent. The extra inoculation with *Streptomyces* sp. increased the leaching of many metals, and also in many cases the metal uptake by plants. The cumulative export by plants and leaching was higher in the treatments with expanded clay in the case of Mg, Mn, Ca, Ni and Pb, smaller in the case of U and Cu, and did not differ in the case of Cr. Copper, Cr, Pb and, to some extent, U tended to accumulate in the top horizon. For these metals, plants played a more important role than the leaching in the total export. For the other metals, a significant decrease in concentrations in the top horizon was associated with a moderate to high export dominated by leaching.

Keywords: Metal leaching; Plant uptake; Lysimeters; Bioremediation; Succession

1. Introduction

The uranium mining area of Ronneburg and Seelingstädt, Eastern Thuringia (Germany) produced about 200 kt of uranium in the years 1946–1990 (Jakubick et al., 1997). These extensive mining operations resulted in, among other things, large open pit mines and waste rock piles. Rain infiltrated into the partly covered dumps and seepage water with a pH below 3 and a high metal load was observed. The seepage contaminated surface and ground water and the valley sediments.

During the active mining period (1971–1990), low grade ore substrate containing radionuclides was leached on the ancient leaching heap "Gessenhalde" with acid mine drainage (AMD) and 10 g/l sulphuric acid

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(Beleites, 1992). The leachate containing high concentrations of heavy metals infiltrated through the barrier soil and was retained in the glacial sediments underneath. Precipitating Fe-minerals contain large amounts of heavy metals due to co-precipitation (Carlsson and Büchel, 2005). Two types of stressors for plants were identified: contamination with metals and acidic conditions.

The risk of remobilization of heavy metals has to be minimized. Bioremediation could be a cost-effective means for decontaminating these slightly and heterogeneously contaminated sites.

There are two main pathways for the export of metals from contaminated areas: through bioaccumulation and further transfer of the organisms outside the area, and by surface or underground hydrological fluxes. These hydrological fluxes can be intercepted by surface water ecosystems, with consequent ecotoxicological and human health risks (e.g., Tian et al., 2006). One way of managing the metals fluxes is phytoremediation (McGrath et al., 2001; Robinson et al., 2006), that can be coupled with mycoremediation (Neagoe et al., 2004), and various soil treatments (Khan et al., 2000; Neagoe et al., 2005). Thus, it is an important issue to investigate the effects of the various phytoremediation methods on the leaching of metals to the groundwater.

Besides the field monitoring of the sites under remediation, the main instrument for studying the leaching of metals is provided by lysimeters. An area of research is the study of effects of sewage sludge and manure application on soil (Bojakowska and Kochany, 1985; Keller et al., 2002; McLaren et al., 2004; Pirani et al., 2006). It seems that phytoremediation methods involving a strong disturbance of the topsoil, such as mixing with different substrates, enhance the leaching of metals, at least in the first phases of plant development (Neagoe et al., 2006; Iordache et al., 2006). The numerous studies of the mechanisms controlling the transfer of metals to plants and groundwater have led to several attempts to model these processes (Seuntjens et al., 2004; Verma et al., 2006).

It is known that microbial activity and community structure not only determine the degradation of soil organic matter and dissolved organic matter as well as the transformation between these two carbon pools, but, as a consequence, also the speciation and solubility of metals with a high affinity for organic ligands (Hullebusch et al., 2005; Nowack et al., 2006). Moreover, in contaminated areas some bacterial groups such as streptomycetes can develop heavy metals resistance (Amoroso et al., 2000). *Streptomyces* sp. was used in the present study because it has previously been shown to be important in the microbial community of contaminated sites (Schmidt et al., 2005). The adsorption of heavy metal cations to the cells walls of Gram-positive bacteria is well known (e.g., Doyle et al., 1980), and the potential of *Streptomyces* strains to retain trace elements from AMD waters has recently been confirmed (Merten et al., 2004, 2005; Haferburg et al., 2007). Thus, coupling plants with fungi and bacteria might provide interesting opportunities for bioremediation. However, the effects of microorganisms (fungi or bacteria) on the metal leaching associated with phytoremediation have yet not received proper attention.

From an ecological perspective, it is documented that in secondary succession (the process of change and recovery of disturbed ecosystems) a high output rate of elements occurs in the first phases of successional development. The mechanisms supporting this pattern are governed both by the hydrological characteristics of the ecosystems (Gorham et al., 1979) and the plant diversity, which is low in the early succession stages and tends to increase towards later successional stages. However, this pattern has rarely been documented in the case of heavy metals. According to our knowledge there is only one study explicitly showing that in juvenile ecosystems developed on post-mining contaminated lands the deep percolation rate of water and associated fluxes of elements (including Ca and Mg) is much higher than in more mature ecosystems (Knoche et al., 2002). Other attempts have been undertaken in floodplains (Iordache, 2003), and in a restoration ecology context (mining areas, Hayes et al., 2003). Recently, it has become clear that the industrial barrens offer unique opportunities for conducting ecological research, in particular for testing some general theories in an evolutionary novel stressful environment (Kozlov and Zvereva, 2007).

In this context, the aim of this study is to investigate the export of metals from heavy metal contaminated soil by leachate and plants involving different degrees of soil disturbance, application of compost and topsoil and the addition of expanded clay, fungi and bacteria. One can refer to disturbance by type of disturbance and by the moment of the disturbance. We attempted to investigate soil disturbed at different moments and by different mechanisms. The working hypotheses were as follows:

(i) The inoculation of the recently disturbed contaminated soil with microorganisms will modify the export of metals.
(ii) The export of metals from the recently disturbed treatments will be higher than the export from the treatments disturbed 1 year before the experiment and will follow patterns interpretable by succession theory.

2. Material and methods

2.1. Experimental setting

The experimental site consisted of a contaminated brown-yellow loamy sand (10–20% loam) on the Gessenhalde uranium-leaching heap located in the Ronneburg mining area, Thuringia, Germany (Fig. 1; for a more detailed description of the area see Kothe et al., 2005; Carlsson and Büchel, 2005; Neagoe et al., 2005). Outdoor experiments using zero-tension, repacked and intact soil lysimeters were carried out to resemble natural field conditions.

The lysimeter experiment (50 cm height, 20 cm diameter, polyethylene material for repacked soil lysimeters and stainless steel for intact soil lysimeters, and gravitational drainage system) was designed using two sets of treatments. The first set consisted in the application of topsoil or municipal compost incorporated into the first 20 cm of experimental plots. One year after the application, intact soil monoliths were sampled from plots treated with compost (CK) and topsoil (CTS), down to a depth of 50 cm, as well as from a control contaminated area (C). The second set of treatments involved the mixing of the soil from the upper 50 cm of the control soil with expanded clay (commercial name Blähton; treatment code CB), with clay and mycorrhizal fungi (*Glomus intraradices*, VAM 510, 115 spores/g soil according to Alten, 2002; treatment code CBM), or with clay, mycorrhizal fungi and a mixture of *Streptomyces tendae* and *Streptomyces acidiscabies* (treatment code CBMS). For the second set of treatments, lysimeters containing homogenized and repacked soil samples were used.

Three types of disturbances can be compared: C with CK, vs. C with CTS, vs. C with CB. From the perspective of time, within the time scale of the experiment, the intact soil lysimeters group includes only one truly undisturbed soil, and two soils (CK and CTS) disturbed 1 year before sampling. In the longer term, even the control soil is actually a disturbed soil resulting from mining activity. It is not acceptable to compare the intact soil group with a mixed soil groups, but it makes sense to compare C soil with soils having different types – moments of disturbance, and modulated by other treatments such as inoculation.

The number of replicates for each treatment in both types of lysimeters (intact soil and repacked) was five. The characteristics of soils used in the experiments are presented in Table 1. For the intact soil values characterizing the upper (0–10 cm) and lower (40–50 cm) layer are presented (the layers in between generally showed intermediate values). The soil used for the repacked treatments (control – CB, with fungi – CBM and with fungi and bacteria – CBMS) had the same physico-chemical characteristics at the time of the experimental set up, and consequently only one set of values is presented in Table 1 (control – CB).

Prior to starting the experiment, soil moisture, redox potential (Eh), electrical conductivity (EC), (WTW 320 (WTW, Germany)), nitrate, nitrite, phosphate, and sulphate concentrations of the soil material used for packing the lysimeters were measured. Soil samples were stored at 4 °C and processed within 24 h of retrieval. 20 g of each sample were extracted with 100 ml 0.2 M KCl solution for nitrogen species and sulphate, and 5 g with 100 ml 0.5 M $NaHCO_3$ for phosphate analyses, filtered using glass filters (Whatman GF/C) and analysed by

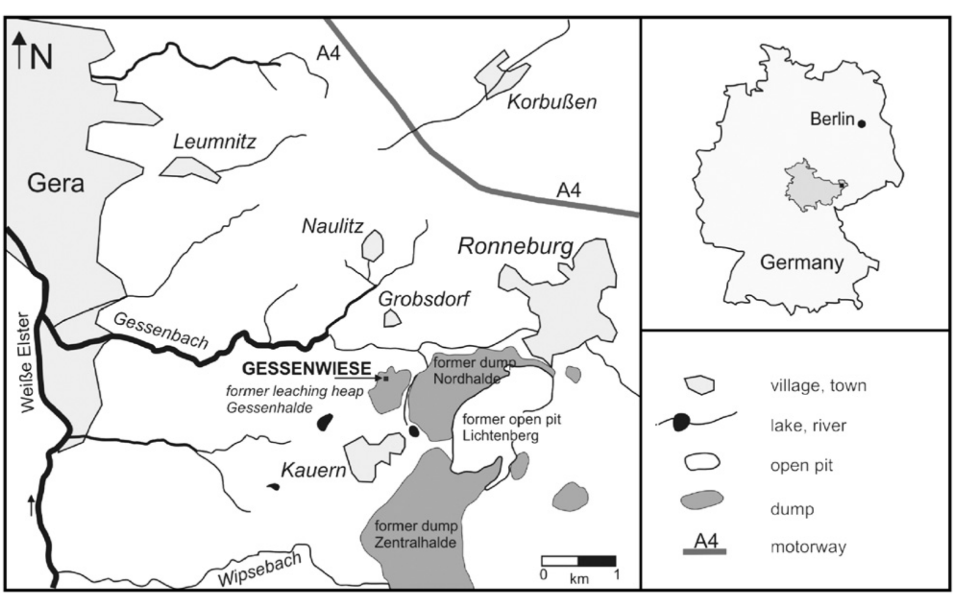

Fig. 1. Location of the former Gessenhalde sampling site (Grawunder 2006, modified).

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Table 1. General characterization of the soils used in the experiments

Parameter/soil code	C		CK		CTS		CB	
	10 cm	50 cm	10 cm	50 cm	10 cm	50 cm	Min.	Max.
Moisture (%)	9.7	8.0	23	10	13	8.9	9.7	9.9
pH (H_2O) (%)	4.8	5.1	8.1	4.1	6.6	3.8	3.4	5.1
pH ($CaCl_2$) (%)	4.2	4.5	7.5	4.0	6.1	3.8	3.0	4.4
Eh (mV)	470	390	230	500	360	510	440	460
EC (μS/cm)	670	269	412	940	146	1010	360	380
N–NH_4^+ (μg/g d.w.)	4.7	8.4	3.9	4.0	4.9	9.9	3.6	4.7
N–NO_3^- (μg/g d.w.)	0.4	1.0	1.6	1.4	0.1	0.9	0.2	0.3
N–NO_2^- (μg/g d.w.)	0.1	0.2	0.6	0.2	0.4	0.1	0.1	0.1
P–PO_4^{3-} (μg/g d.w.)	8.9	23	122	17	30	21	8.9	13
S–SO_4^{2-} (μg/g d.w.)	642	1699	461	2245	148	2431	642	1387

colorimetric methods as described by Neagoe et al. (2005).

The lysimeters were planted with a mixture of two invasive and acidophilic plant species. These were grass (*Festuca rubra* L.) and clover (*Melilotus albus* L.). During the study period of 3 months, the plants were watered if necessary with rainwater, in order to maintain the soil moisture. After harvest, the plants were cleaned with tap water and after that several times with deionised water, weighed (fresh weight) and partitioned for analysis into aboveground and underground part of plants. All plant material was frozen and lyophilised (Martin Christ, Germany) for determination of dry weight (d.w.) and then ground in a stainless steel mill that was carefully cleaned between samples (IKA, All basic, USA) and stored at −20 °C until processing.

Two heavy rains occurred during the experimental period (3 months), one in the first phase of the plant development, and the second when the plants were fully developed. In both cases, the leachates were collected, filtered by 0.45 μm filters (CA membrane and GF prefilter, Sartorius, Germany) and acidified using suprapur HNO_3 (Merck, Germany).

The different soils, harvested plants and the percolated water were analysed for Pb, U, Cr, Cu, Ni, Mn, Ca and Mg. For soil samples, a pseudo-total extraction with *aqua regia* was used (Hoffmann, 1991). For ground plant samples microwave assisted pressure digestion (Mars 5, CEM, Germany) was performed using suprapur nitric acid (0.2 g of plant material and 2 ml of HNO_3). The percolated water was analysed after 0.45 μm filtration. The analysis was performed by ICP-OES (Spectroflame, Spectro, Germany) or ICP-MS (PQ3S, Thermo Electron, UK).

The method for estimating the hydraulic conductivity is described in Grawunder (2006). The estimation of soil bulk density used for computing stocks of metal in the soil of intact treatments was provided by Grawunder (personal communication).

2.2. Statistical analysis

Averages were calculated from the data characterizing the five lysimeters of each experimental treatment. A Mann–Whitney *U*-test was used for comparison between experimental treatments at the level of significance $p<0.05$. The statistical processing was done with the program SPSS© (version 12) for Windows. Data processing was performed separately for each metal. However, as the number of metals is high, we also looked for synthetic representations of a dominant pattern of variation. For this purpose, standardized values were calculated in order to compensate for the differences between the absolute values of concentration of various metals, using the formula: $x_i^{st} = x_i - \bar{x}/\text{S.D.}$, where x_i is the concentration of a metal in a sample, x_i^{st} is the standardized value of x_i, $\bar{x}$ is the mean of the concentrations of the metal in all samples, and S.D. is the standard deviation of the mean. $\bar{x}$ was computed as mean of the concentrations in all experimental variants of a set of methods in order to allow visual comparison of the standardized concentrations in the different variants of the same set.

3. Results

3.1. The export of metals from repacked lysimeters

3.1.1. The export by water from repacked lysimeters

Table 2 presents data describing the export of metals by water from repacked lysimeters. The volume of leachate was significantly higher (Mann–Whitney test) after the second rain event than after the first rain event for any treatment. The leachate pH was not significantly different between the two events for any variant. The Eh was significantly lower in the water percolated during the second rain event in the control and in the treatment

Table 2. Presentation of the volume of exported leachate from the repacked lysimeters after the first and second rain, the values of physico-chemical parameters and DOC, and selected metal concentrations in the percolated water

Parameter	Treatments											
	Rain 1			Rain 2			Mann–Whitney					
	CB 1	CBM 2	CBMS 3	CB 4	CBM 5	CBMS 6	1 2	1 4	2 5	3 6	4 5	5 6
Volume (ml)												
Av.	260	316	308	1155	968	930	+*	+*	+*	+*	–*	NS
S.D.	12	15	10	62	49	187						
pH												
Av.	6.3	6.5	6.4	6.6	6.4	6.7	NS	NS	NS	NS	–*	+*
S.D.	0.3	0.4	0.3	0.2	0.1	0.1						
Eh (mV)												
Av.	510	506	522	406	416	548	NS	–*	–*	NS	NS	+*
S.D.	14	19	51	11	5	27						
DOC (mg/l)												
Av.	19	9.2	12	6.0	4.0	4.3	NS	–*	–*	–*	–*	NS
S.D.	17	1.2	4.2	1.0	0.8	0.8						
Pb (μg/l)												
Av.	0.7	0.6	2.3	0.7	2.2	1.4	NS	NS	+*	NS	NS	NS
S.D.	0.1	0.0	2.4	0.0	3.3	1.0						
U (μg/l)												
Av.	3.1	6.2	3.5	1.0	0.7	0.7	NS	–*	–*	–*	NS	NS
S.D.	1.8	2.7	1.5	0.7	0.0	0.0						
Cr (μg/l)												
Av.	0.8	0.8	1.9	4.7	5.8	4.1	NS	+*	+*	NS	NS	NS
S.D.	0.0	0.0	2.5	1.2	1.8	1.4						
Cu (μg/l)												
Av.	44	63	85	32	59	105	NS	NS	NS	NS	NS	NS
S.D.	17	52	52	21	75	120						
Ni (mg/l)												
Av.	9.5	13	8.8	4.6	2.4	6.0	NS	–*	–*	NS	NS	+*
S.D.	4.7	1.7	4.8	1.9	0.8	2.0						
Mn (mg/l)												
Av.	157	218	147	106	37	96	NS	NS	–*	NS	–*	+*
S.D.	88	28	93	40	16	31						
Ca (mg/l)												
Av.	417	549	454	392	280	442	+*	NS	–*	NS	–*	+*
S.D.	83	35	110	63	71	74						
Mg (mg/l)												
Av.	733	1053	750	519	235	469	NS	NS	–*	NS	–*	+*
S.D.	390	107	394	191	87	100						

Tests of significance have been performed between the average values (Av.) of the parameters coded with numbers as indicated in columns below the codes of the experimental variants (+*, significantly higher; –*, significantly lower; NS, no significant difference).

with mycorrhizal fungi (CBM), and the DOC concentration was significantly lower in all treatments. Lead and Cr concentrations were higher in the leachate during the second rain event from the mycorrhizal fungi treatment, as was the Cr concentration in water percolated from the control. Copper concentrations were not significantly different between the two rain events. The concentrations of the other metals were significantly lower in the leachate during the second event, as follows: U in all treatments, Ni in the control

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and mycorrhizal fungi treatment, and Mn, Ca, Mg in the mycorrhizal fungi treatment (with no significant decreases in any of the other treatments).

After inoculation with fungi a significant increase of the volume of leachate during the first rain event, but a decrease of the volume during the second one was observed. The pH of the leachate and the DOC concentration also significantly decreased in the leachate during the second event as a result of the inoculation with fungi. The further inoculation with *Streptomyces* had no significant effect on the volume of leachate. However, it was associated with a significant increase of the pH and Eh of the leachate during the second rain event, as compared with the CBM treatment.

The effects of inoculation on the concentrations of metals in the leachate were almost absent during the first rain event. Only Ca had a significantly higher concentration in the mycorrhizal fungi treatment. But the effects of inoculation were visible at the moment of the second rain event. The concentration of Ni, Mn, Ca and Mg in the leachate decreased as a result of mycorrhization (significantly in most cases), but then significantly increased as a result of the inoculation with *Streptomyces*. However, the concentration of metals with low mobility such as Pb, U, Cr and Cu in leachate was not significantly different between the experimental treatments.

Thus, the patterns of variation between the experimental treatments differ between the first rain (low rainfall, small plants) and the second rain events (high rainfall, large plants). The total export of metals by water from the repacked treatments was controlled mainly by the second rain event, which happened to be more intensive. The differences between treatments in relation to the total metal export are controlled by differences specific to the stage of full plant development, when the second rain event occurred. It appears that the inoculation of the repacked contaminated soil with mycorrhizal fungi decreased the leaching of metals. The supplementary inoculation of the repacked contaminated soil with *Streptomyces*, partly reversed the effect of the mycorrhizal fungi by increasing the concentrations of metals in the leachate.

3.1.2. The export of metals by plants from the repacked lysimeters

The export of metals by plants in the repacked lysimeters is presented in Fig. 2. The biomass of *Festuca*

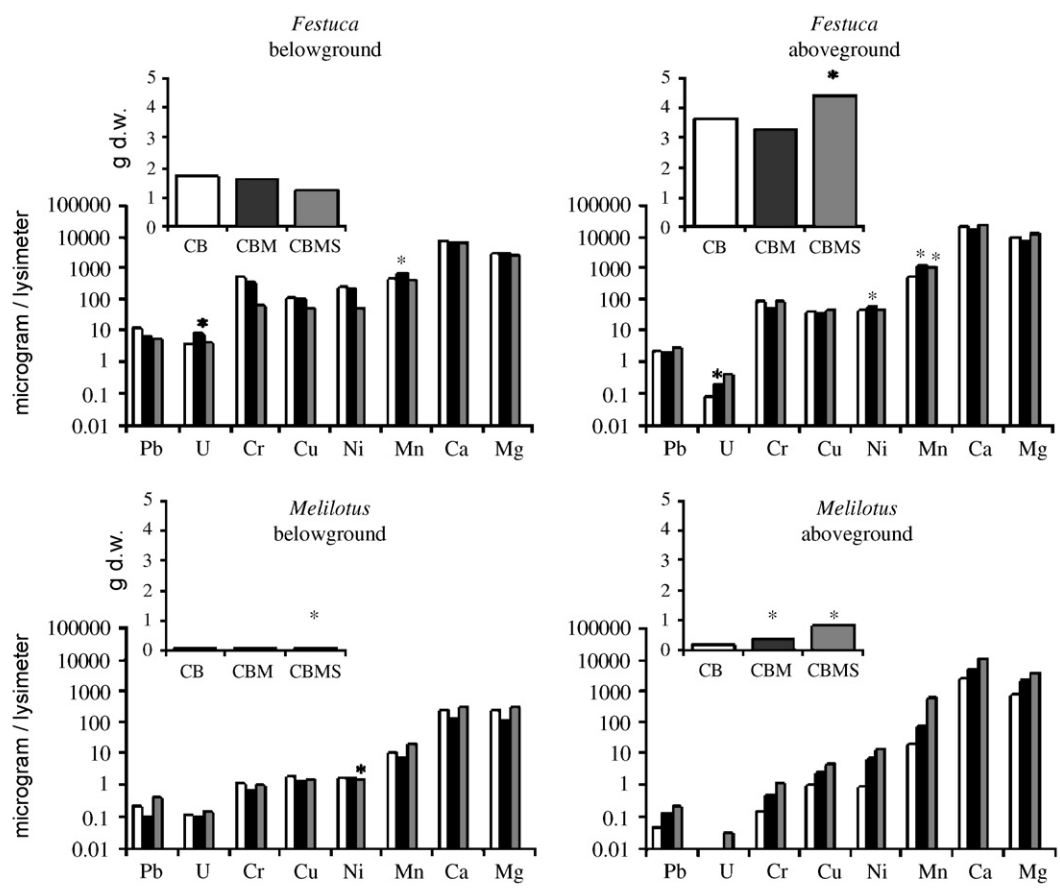

Fig. 2. Average stocks of metals exported from the repacked lysimeters; inserted: average dry weight biomass. The same scale was used in all graphs in order to allow visual comparison. Stars indicate significant variations of biomass compared to the nearby left variant.

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sp. was significantly larger ($p < 0.05$ Mann–Whitney test) than the biomass of *Melilotus* sp., in all treatments. Because of this situation the metal export pattern was not strongly influenced by *Melilotus* sp. in these experimental treatments. The mycorrhizal fungi significantly increased the belowground and aboveground biomass of *Melilotus* sp., and had no significant effects on the biomass of *Festuca* sp. The further inoculation with *Streptomyces* led to an even stronger increase of *Melilotus* sp. biomass, but also of the aboveground biomass of *Festuca* sp.

The mycorrhizal fungi decreased the concentration of metals in the belowground biomass of *Melilotus* sp. Its effect was metal dependent in the aboveground biomass of *Melilotus* and of *Festuca* sp. A significant increase of U, Ni and Mn in the aboveground biomass of *Festuca* sp. can be noticed in particular. The further inoculation with *Streptomyces* led to a decrease of the accumulation of Ni and Cu in the belowground parts of *Festuca* sp. and of *Melilotus* sp. and to a decrease of the accumulation of Mn in *Festuca* sp.

In most cases, the pattern of the stock of metals in plants followed the pattern of biomass variation. This occurred because the concentration of metals either had the same trend of variation as the biomass, or did not vary between experimental treatments. However, in some cases the changes in the concentration of metals were very strong and in opposite direction than the change in biomass (i.e., when the biomass increased the concentration of metal decreased, or vice versa). In this situation, the stock of metals followed the trend of concentration, not the trend of the biomass. The effect of the inoculation with mycorrhizal fungi on the total export of metals by plants was metal dependent. The effect of extra inoculation of the repacked contaminated soil with *Streptomyces* on the total export by plants was also metal dependent.

3.2. The export of metals from intact lysimeters

3.2.1. The export by water from intact lysimeters

In Table 3, data describing the export by water from intact lysimeters are presented. The volume of percolated water, pH, Eh, or DOC concentration were not significantly different between the two rain events for any of the experimental treatments. However, Pb concentration was significantly lower in the water percolated during the second rain event from the compost (CK) treatment. The U and Cu concentrations were significantly lower in the water percolated from the topsoil CTS treatment after the second rain. The concentrations of the other metals were not significantly different between the two rain events.

The volume of water from the control during the first rain event was too low to allow the determination of the parameters in all replicates. Consequently it is not possible to report significant differences between the treatments in this respect during the first rain event. The amendment with compost led to an increase of the volume of percolated water during both rain events. The amendment with topsoil led to an even higher significant increase of volume during the second rain. DOC concentrations significantly increased in the leachate during the second event as a result of the amendment with compost or topsoil. The pH, Eh and DOC concentrations did not significantly differ between the compost and topsoil treatments.

The effects of amendments on the concentrations of metals in the leachate could not be tested for the first rain event, but a visual inspection of Table 3 suggests large increases in the concentration of Pb, U and Cr in the leachate, and an increase in Cu concentration, as a result of amendments. This observation holds also for the data describing the second rain event and in this case is statistically confirmed. The treatment amended with topsoil did not differ from the treatment with compost during the first rain event. During the second event the concentration of Pb in the leachate from the CTS treatment was significantly higher than that in the leachate from the CK treatment and that of Ca was significantly lower.

Thus, contrary to the situation for the repacked lysimeters, for the intact lysimeters there are rather similar patterns of variation between the experimental treatments during the first and second rain events. The total export of metals by water from the intact treatments was controlled by the experimental treatment, and not by the time of the rain event. The amendment of the soil, either with compost or with topsoil strongly increased the quantity of leachate through the lysimeters, and also the concentration of some metals in the leachate.

The larger hydraulic conductivity of the lysimeters was confirmed by independent measurements in the field. Fig. 3 presents the distribution of the metals concentrations in the soil profile near the sampling places for the intact treatments, as well as the hydraulic conductivity of each soil horizon. One can notice increasing concentrations of metals in the control down to 50 cm, and decreasing concentrations in the amended treatments, i.e., most clear in CK experimental treatment. The apparently unexpected higher average of the standardized concentration in the upper 10 cm horizon of the CK treatment than the control is due to high concentrations of Ca and Mg in the 10 cm horizon of the soil amended with compost (while the concentration of most toxic elements are lower than in control). The peculiar pattern of the distribution of metals in control is associated with a comparatively much lower hydraulic conductivity of the soil than in the amended treatments.

Table 3. Presentation of the volume of exported leachate from the intact lysimeters after the first and second rain, the values of physico-chemical parameters and DOC and selected metal concentrations in the percolated water

Parameter	Treatments											
	Rain 1			Rain 2			Mann–Whitney					
	C 1	CK 2	CTS 3	C 4	CK 5	CTS 6	1 2	1 4	2 5	3 6	4 5	5 6
Volume (ml)												
Av.	24	327	204	46	312	379	NA	NA	NS	NS	$+^{*}$	$+^{*}$
S.D.	54	170	11	92	297	390						
pH												
Av.	4.9	4.3	4.5	4.3	4.4	4.6	NA	NA	NS	NS	NS	NS
S.D.		0.3	0.3	1.0	0.2	0.3						
Eh (mV)												
Av.	520	572	602	487	564	506	NA	NA	NS	NS	NS	NS
S.D.		42	37	12	42	5.5						
DOC (mg/l)												
Av.	8.5	17	10	5.5	19	18	NA	NA	NS	NS	$+^{*}$	NS
S.D.		0.6	12		3.1	23						
Pb (μg/l)												
Av.	1.1	9.1	4.7	1.3	5.6	7.4	NA	NA	$-^{*}$	NS	$+^{*}$	$+^{*}$
S.D.		2.2	1.7	1.0	1.7	5.4						
U (μg/l)												
Av.	24	770	674	13	801	79	NA	NA	NS	$-^{*}$	$+^{*}$	NS
S.D.		452	475	12	289	115						
Cr (μg/l)												
Av.	0.8	16	53	10	30	15	NA	NA	NS	NS	$+^{*}$	NS
S.D.		12	88	1.6	17	5.2						
Cu (μg/l)												
Av.	868	1098	1562	70	1007	327	NA	NA	NS	$-^{*}$	$+^{*}$	NS
S.D.		557	855	58	260	423						
Ni (mg/l)												
Av.	4.9	11	9.8	5.3	9.6	10	NA	NA	NS	NS	NS	NS
S.D.		2.1	4.6	3.7	2.3	5.4						
Mn (mg/l)												
Av.	93	188	112	82	156	111	NA	NA	NS	NS	NS	NS
S.D.		29	66	66	40	56						
Ca (mg/l)												
Av.	630	583	535	617	587	500	NA	NA	NS	NS	NS	$-^{*}$
S.D.		35	50	34	18	48						
Mg (mg/l)												
Av.	634	831	682	905	682	948	NA	NA	NS	NS	NS	NS
S.D.		134	181	290	191	483						

Tests of significance have been performed between the average values (Av.) of the parameters coded with numbers as indicated in columns below the codes of the experimental variants ($+^{*}$, significantly higher; $-^{*}$, significantly lower, NS, no significant difference; NA, test not applicable due to single sample in C for rain one).

3.2.2. The export of metals by plants from intact lysimeters

The export of metals by plants in intact lysimeters is presented in Fig. 4. The biomass of *Festuca* sp. was significantly higher ($p<0.05$ Mann–Whitney test) than the biomass of *Melilotus* sp. in the control and topsoil amended treatment, and lower in the compost amended treatment. The differences between the biomasses of

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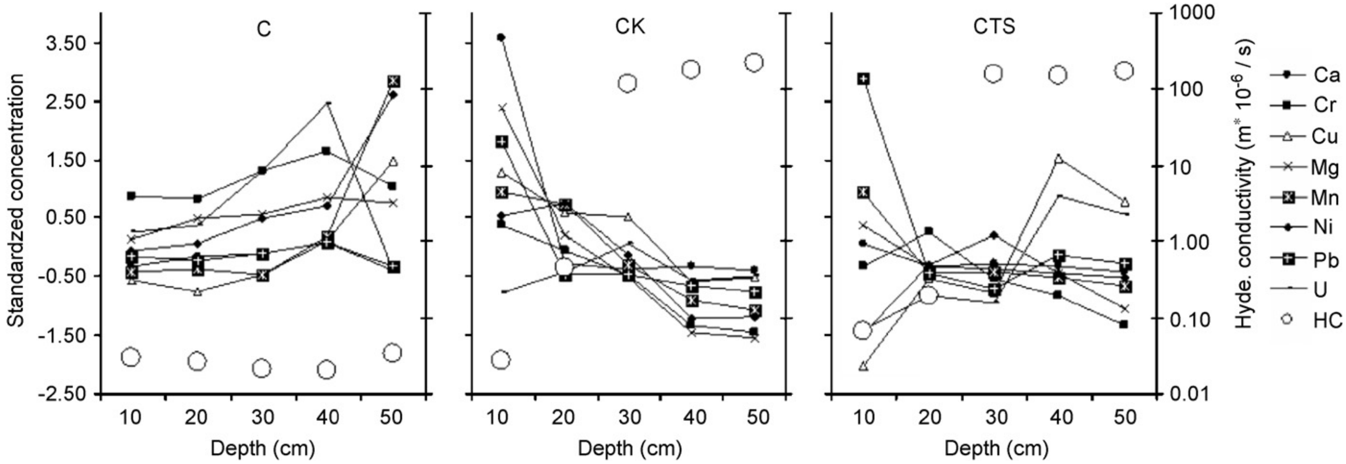

Fig. 3. The distribution of hydraulic conductivity (HC, round markers, right *y*-axes) and of concentrations of the analysed elements in the soil of the undisturbed lysimeters (left: control, middle: amended with compost, right: amended with topsoil). Hydraulic conductivity data are from Grawunder (2006), with permission.

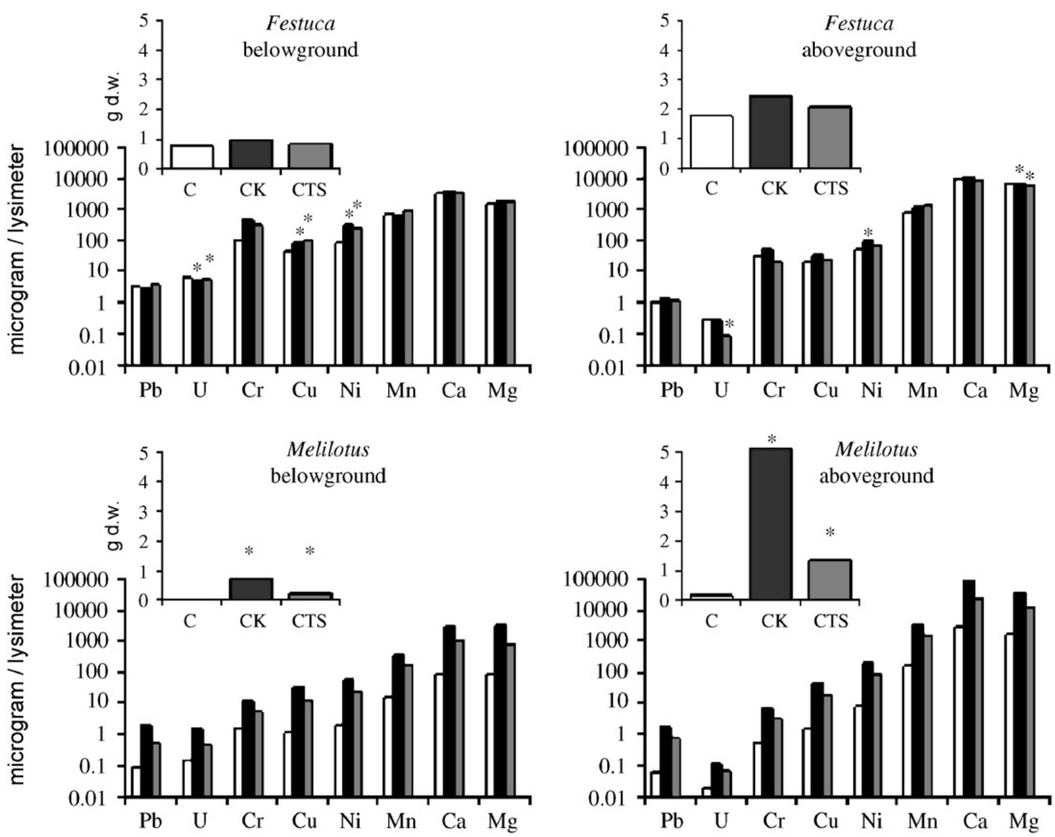

Fig. 4. Average stocks of metals exported from the intact lysimeters; inserted: average dry weight biomass (g d.w.). The same scale was used in all graphs in order to allow visual comparison. Stars indicate significant variations compared to the nearby left variant.

Festuca and *Melilotus* sp. were not as large as in the repacked treatments. As a result of this situation, *Melilotus* sp. played an important role in the export of metals by plants, in the treatments using intact lysimeters. The amendment with compost significantly increased the biomass of *Melilotus* sp., and possibly also the aboveground biomass of *Festuca* sp. The amendment with topsoil also had a positive effect on the biomass, but to less extent than the amendment with topsoil.

The amendment with compost or topsoil decreased the concentration of U and Mg in the biomass of

Table 4. Concentrations of metals in the upper soil horizon before and after experiment

Parameter	Variants												
	Repacked lysimeteres				Intact lysimeters						Mann–Whitney		
	Before	After			Before			After					
	CB 1	CB 2	CBM 3	CBMS 4	C 5	CK 6	CTS 7	C 8	CK 9	CTS 10	1 2	1 3	1 4
Pb (μg/g)													
Av.	11	12	13	12	13	22	26	13	28	21	+*	+*	+*
S.D.	0	0.3	0.3	0.3				0.3	8.8	2.2			
U (μg/g)													
Av.	5.4	5.2	6.2	5.4	6.6	3.7	2.0	6.3	4.2	3.4	NS	NS	NS
S.D.	0	0.5	1.8	0.1				0.2	0.3	0.2			
Cr (μg/g)													
Av.	22	31	31	28	25	23	21	38	29	27	+*	NS	+*
S.D.	0	1.9	4.6	0.4				4.5	2.0	0.8			
Cu (μg/g)													
Av.	26	39	38	37	24	33	17	32	42	24	+*	+*	+*
S.D.	0	0.9	1.1	0.4				0.5	1.4	1.4			
Ni (μg/g)													
Av.	56	52	50	50	39	45	25	46	44	36	–*	NS	–*
S.D.	0	0.7	2.7	1.2				2.1	3.5	2.2			
Mn (μg/g)													
Av.	674	645	585	655	333	609	608	363	592	518	NS	NS	NS
S.D.	0	31	83	20				12	44	59			
Ca (μg/g)													
Av.	2155	1191	1366	1334	1109	14108	2398	1170	12745	1891	–*	–*	–*
S.D.	0	131	126	53				176	3462	219			
Mg (μg/g)													
Av.	3279	2859	2874	2817	3140	4465	3282	2994	4212	2998	–*	–*	–*
S.D.	0	80	60	47				80	426	91			

The concentrations of metals in the repacked soil were identical in all replicates before the experiments, so S.D. of the concentrations is 0.

Festuca sp. The addition of compost increased the concentration of Cr in both plant species. Effects of the amendments on the accumulation of metals in *Melilotus* were less noticeable, because they were masked by the huge effects on the biomass production.

In all *Melilotus* sp. cases, the pattern of the export of metals by plants followed the pattern of biomass variation. In case of *Festuca* sp., the changes in the concentration of metals mentioned in the previous paragraph were strong enough to reverse the export trends caused by the variation in biomass. However, the overall effect of amendments on the total export of metals by plants was less metal dependent than in the case of the repacked treatments.

3.3. Comparison of the export of metals from repacked and intact lysimeters

In the previous sections, differences between the repacked and intact lysimeters in the patterns of metal percolation during rain events, and in the pattern of metal export by plant species have been scrutinised. The focus will be now on the dynamics of metal concentrations in the upper soil horizon and on the relative importance of the export of metals by plants compared with the export by leachate.

Table 4 shows the concentrations of metals in the upper soil horizon before and after the experiment. In the repacked treatments, the concentrations of Pb and Cu increased with time (Mann–Whitney test) in all experimental treatments. In same treatments, the con-

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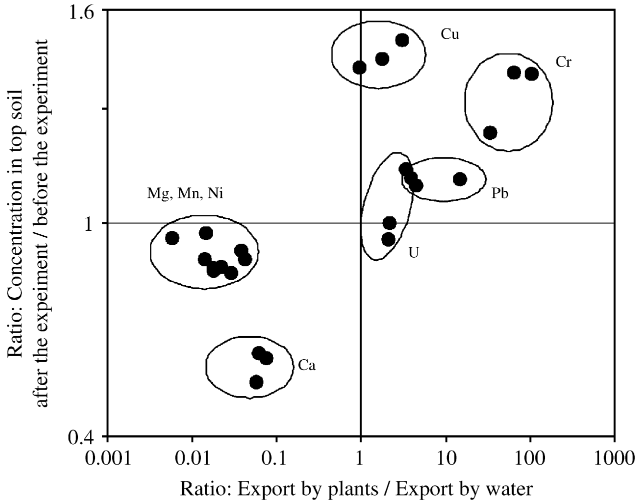

Fig. 5. Relationship between the ratio of the export by plants/ export by percolated water and ratio of the concentration in topsoil after the experiment/before the experiment, for the repacked variants.

centration of Cr increased significantly in the control and the CBMS treatment but not significantly in the CBM treatment. In contrast to this pattern, the concentrations of Ca, Mg and Ni decreased in all experimental treatments (significantly in all cases with exception of Ni in the CBM treatment). The differences characterizing the treatments using intact lysimeters, although following roughly the same pattern as for the repacked treatments, were significant in fewer cases. However, Cu concentrations significantly increased with time in all the intact treatments, Cr concentration significantly increased in the CB and CBMS treatment, and Mg significantly decreased in any treatment over time.

Fig. 5 shows the relationship for treatments using disturbed lysimeters between the ratio of the metal export by plants/export by leachate and the ratio of the metal concentration in topsoil after the experiment/ before the experiment. It can be observed that when plants played a more important role than the leachate in the export of metals, those metals also tended to accumulate in the topsoil. In contrast, when the water was more important in facilitating the export of metals, the concentration of those metals in the topsoil tended to decrease. This pattern, although present, was not as clear in the undisturbed treatments.

Fig. 6 compares the average total export of metals from the repacked and intact lysimeters. The point here is not to look at the absolute values (which depend on the experimental design), but to compare the experimental treatments and pathways of exports. The export of Pb, Cr, Ni, Mn, Ca, and Mg was larger in the repacked lysimeters compared with the intact lysimeters (computed as average of CK and CTS), while the total

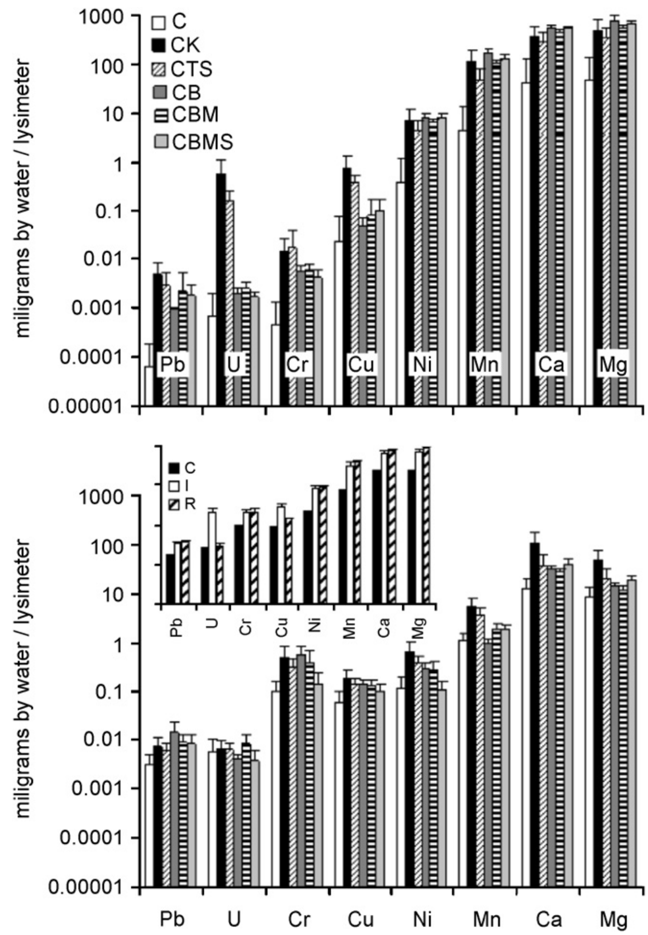

Fig. 6. The average export of metals (absolute amounts) from repacked and intact experimental treatments (up: by water, down: by plants, inserted: total). The total export is presented for control (C), intact treatments (I, computed as average of CK and CTS), and repacked treatments (R). The scale of the inserted graphs is the similar with that of larger graphs (units: milligrams per lysimeter).

export of U and Cu was smaller. The relative role of plants in the total export of Pb, U and Cu (estimated by the ratio of the export by plants/the export by water) was higher in the repacked lysimeters. The relative role of the leachate in the export of Ni, Ca and Mg was higher in the repacked lysimeters compared to the intact ones. The role of plants vs. water in the export of Cr and Mn was more or less the same in the two sets of experimental treatments.

The experimental treatments are characterized by different degrees of soil disturbance. The control is the least disturbed and the treatments mixed with expanded clay are the most disturbed. In this context, one could predict that the C variant would have the lowest relative export of metals compared to the stock of metals in the soil. At the other extreme, the CB, CBM and CBMS treatments would have the highest export of metals compared with the stock of metal in the soil. This

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Table 5. Total metals exported as a percent (%) of the stock of metals in the 0–10 cm soil horizon at the start of the experiment

Elements	Experimental treatments											
	C		CK		CTS		CB		CBM		CBMS	
	Av.	S.D.	Av.	S.D.	Av.	S.D.	Av.	S.D.	Av.	S.D.	Av.	S.D.
Pb	0.01	*0.00*	0.02	*0.01*	0.01	*0.00*	0.06	*0.03*	0.04	*0.02*	0.04	*0.02*
U	0.02	*0.01*	2.59	*2.27*	0.74	*0.36*	0.05	*0.01*	0.09	*0.04*	0.05	*0.02*
Cr	0.08	*0.06*	0.54	*0.38*	0.35	*0.12*	1.21	*0.49*	0.79	*0.66*	0.31	*0.18*
Cu	0.06	*0.07*	0.71	*0.54*	0.44	*0.10*	0.33	*0.05*	0.38	*0.19*	0.36	*0.09*
Ni	0.23	*0.38*	4.31	*2.93*	2.90	*1.48*	6.47	*1.82*	5.36	*0.82*	6.45	*1.25*
Mn	0.24	*0.40*	6.35	*4.31*	2.92	*1.71*	10.87	*3.18*	7.10	*1.13*	8.71	*1.73*
Ca	0.72	*1.20*	2.70	*1.61*	4.95	*2.80*	12.29	*1.87*	9.78	*1.49*	12.03	*1.38*
Mg	0.35	*0.61*	3.84	*2.58*	2.77	*1.43*	10.94	*3.23*	7.80	*1.13*	9.13	*1.65*

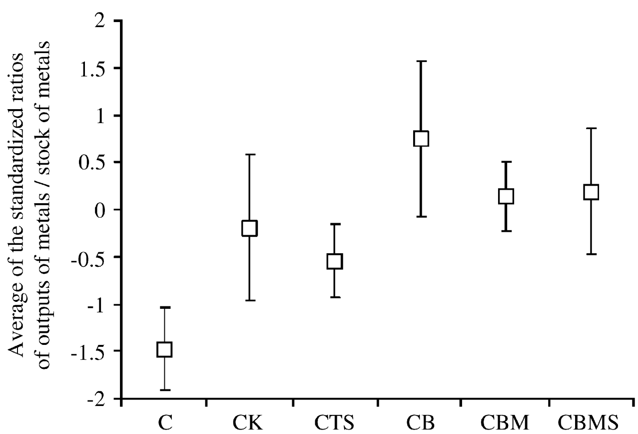

Fig. 7. The ratio between the total export of metals from the lysimeters and the stock of metals in soil (average for the studied metals and S.D.). In order to ensure an equal influence each metal on the final pattern the ratios of a metal in an experimental variant have been standardized using the mean of the ratios of that metal in all variants.

prediction is confirmed by our computations excepting for U and Cu, as presented in Table 5 (the total metals exported as a proportion of the concentrations of metals present in the upper soil horizon). The general picture described by these data is depicted in Fig. 7.

4. Discussion

The fact that there were almost no significant differences between the repacked treatments during the first rain event (small plants, low rainfall), but many significant differences during the second rainfall (large plants, high rainfall) is not surprising taking into consideration that the soils at the start of the experiment were identical, excepting for the different inoculations. The treatment with mycorrhizal fungi resulted in a significant decrease of the volume of leachate during the second rain event, probably due to the stronger root development of the plants. The concentrations of metals in the leachate during the second rain were in many cases lower than those in the water percolated during the first rain event. This might be due to some depletion of the mobile fraction after the first rain, but most probably to a dilution effect resulted from the large quantity of water percolated during the second rain event. This quantity was larger than that percolated from the intact lysimeters, where the decreasing pattern of metal concentrations between rain events was not as obvious.

Uranium bioaccumulation increased greatly as a result of inoculation with mycorrhizal fungi. One reason of the change in the phyto-availability of uranium could have been the soil pH. At a pH lower than 5.5, uranium is in the most available form for plants (Ebbs et al., 1998; Shahandeh and Hossner, 2002). However, the data provided in Table 2 shows that the pH of the repacked experimental treatments is always larger than 5.5, so we cannot follow this line of argumentation. Another possible reason could be the behaviour of the plant species under mycorrhization. The uptake and translocation of U is plant species dependent (e.g., Saric et al., 1995), *Festuca* sp. being a genus with high bioaccumulation factor for uranium (Bykova et al., 2006). Mycorrhization was reported to reduce the uptake of uranium in clover (Rufyikiri et al., 2004), which is confirmed by the results presented (Fig. 2). This is not the case for *Festuca* sp., in which bioaccumulation of U was strongly enhanced by mycorrhization in our experimental conditions (Fig. 2). This behaviour coupled with the higher biomass of *Festuca* lead to the more important role of plants than of leachate in the export of U from the repacked lysimeters.

The double inoculation with *G. intraradices* and *Streptomyces* sp. strongly stimulated the developments of plants. However, in an early study on the interaction of streptomycetes and mycorrhizal fungi the micro-organisms interacted antagonistically with respect to the growth and phosphorous nutrition of the studied plant species (Krishna et al., 1982). The authors suggested that the antagonistic effect of their *Streptomyces* isolate on *Glomus fasciculatum* was the result of antibiotic production. In later studies, this pattern was neither confirmed nor disconfirmed. However, Ames et al. (1987) documented that the rhizosphere microbial populations from VAM and non-VAM plants differ, and Wyss et al. (1992) reported that mycorrhiza formation with *Glomus mosseae* was significantly depressed in presence of biocontrol agents, in particular of *Streptomyces griseoviridis*. In contrast, Ames (1989) tested 12 actinomycetes and reported that seven of them significantly increased the percentage of mycorrhizal root colonization and the density of hyphae, predominantly those of VAM. Posta et al. (1994) found that the number of actinomycetes increased both in the rhizosphere and in the bulk soil as a result of mycorrhization. Abdel-Fattah and Mohamedin (2000) found that the inoculation with *Streptomyces coelicolor* strain 2389 significantly increased the intensity of mycorrhizal root colonization and arbuscular formation. It is today widely accepted that studies on mycorrhizal interactions with rhizosphere bacteria show variable results, depending on the species, on the abiotic environment and on the structure of the soil community (Hodge, 2000; Wamberg et al., 2003).

Lorenz et al. (2007) reported elevated Mn concentrations in leachate (up to 1060 μg/l) after passage through soil columns inoculated with *S. acidiscabies*. The repeated flushes performed by the authors (corresponding to different rainfall events in our experiment) were associated with a decrease in Mn and of DOC concentrations, as well as a decrease of the redox potential in the leachate. In the CBMS treatment, there was a significant decrease of DOC concentration in leachate during the second rain event compared with the first rain, but the changes in Mn concentration and of Eh were not significant. A long term *in situ* lysimeters experiment would be needed in order to fully confirm the patterns noticed by Lorenz et al. (2007).

Another aspect deserving attention is the significantly larger values of the pH of leachate in CBMS treatment compared with the CBM variant, associated with larger values of Eh and concentration of Ni, Mn, Ca and Mn, during the second rain event. This was also associated with a lower accumulation of Ni and Mn in *Festuca* sp. (the dominant plant species) in the CBMS compared with the CBM treatment. Thus, while the phytoavailability of Ni and Mn decreased as result of inoculation with *Streptomyces* sp., the potential of these elements to be transported in leachate increased. Crawford et al. (1993) found a transient increase in the soil solution pH as a result of inoculation with a *Streptomyces* strain. In another experiment, the higher pH of the leachate was clearly associated with higher concentrations of Ca and Mg and higher concentrations of DOC (Tatar et al., 2004). In the experiment described by Barcan (2002), large leaching rates of nickel and copper were observed in treatments at pH 5–6, and small ones at pH 2–3. This pattern (large percolation at higher pHs) was interpreted by Barcan as evidence for the formation and the further leaching of organic ligand–metal complexes. His point is that the organic ligand–metal complexes are the determinants for leaching of heavy metals from soils, and not the induced acidity. It is possible that dissolved organic carbon is a key parameter that might help to explain the results observed in our experiment, although the DOC concentrations were not significantly higher in the CBMS compared with the CBM treatment. However, it might be that low molecular weight molecules are involved in the mobilization of Ni and Mn in the lysimeters, which is reasonable taking into consideration the poorly developed organic layer in the original control soil. Such complexing molecules might have prevented the precipitation of trace elements in the present of Ca^{2+} and in soil solutions having a relatively high pH level (Kabata-Pendias and Pandias, 2001). As Q2 the main enzymatic activities in the soil of the experimental treatments and the detailed structure of the dissolved organic carbon in the leachate have not been investigated, one cannot speculate on the detailed causes leading to the noticed increase in the pH of the leachate and the mechanisms supporting it.

Another important clue concerning the metal leaching patterns comes from comparing the values characterizing the first rain event with those describing the second rain event (Table 2). One can see that the values of the discussed parameters (pH, Eh, most metals) in the water percolated from CBMS treatment during the second rain are actually not significantly different from those of the water percolated during the first rain. The larger values of the parameters in CBMS compared with CBM during the second rain are actually due to a significant decrease in Eh and in concentrations of Ni, Mn, Ca and Mn in the leachate from CBM during the second rain event compared to the first one. This decrease in the values of the parameters is probably due to a change in the functioning of the microbial community in the CBM treatments. This means that during the plants development the function of the microbial communities in the two variants, CBM and CBMS, had different dynamics, with different biogeochemical consequences.

Based on the above results and discussion, it can be concluded that the first working hypotheses is confirmed, i.e., the inoculation of the repacked contaminated soil with microorganisms modified the export of metals. One can recommend the use of the mixed inoculation method in the bioremediation of sites contaminated with metals because it can lead to an increase of plants biomass. Attention should be paid, however, to the eventual mobilization of metals

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(followed by leaching) as a result of changes in the quality of the DOC in soil.

Prior to discussing the differences between intact and repacked treatments, it can be noted that the atypical distribution of the metals in the soil profile in the control (Fig. 3) is supported also by the results of Carlsson and Büchel (2005) who found that a perched groundwater table was present on the top of control soil. Cemented Fe-rich layers in the unsaturated zone (hardpan) with a thickness of ~5 cm were found in the control plot at a depth of ~20–50 cm.

The less clear correlation between the ratio of the metal export by plant/the export by water and the ratio concentrations in topsoil after/before the experiment in the intact treatments might be due, to some extent, to the larger heterogeneity of the samples resulting from field sampling, in comparison with the repacked treatment, which were packed with homogenized soil. However, we will further assume that at least part of this effect is due to other, more interesting, phenomena. The less clear pattern between the ratio of the export by plant/export by water and the ratio between the concentrations in topsoil after/before the experiment in the intact treatments might be due to the lower total export occurring for most metals as illustrated in Fig. 6, at least for the case of C and CTS treatments (the differences are large for these variants). As a result of this lower export, the variation of the concentration of all elements in control can be expected to be smaller than in the intact and repacked treatments. With the same argument, the variation of Pb, Cr, Ni, Mn, Ca, Mg in the upper soil layer can be expected to be lower in intact treatments than in repacked ones. This is in agreement with the smaller number of significant differences found in the intact treatments for these metals (Table 4). However, according with this argument, the variation of U and Cu concentrations in the upper soil layer should be larger in the intact treatments, because the total export of Cu and U is larger in these treatments than in the repacked ones. U confirms this prediction: the average increase in concentrations is 0.5 μg/g d.w. in the upper soil of the intact variants, and 0.2 μg/g in the upper soil of the repacked treatments. But the variations of Cu were larger in the repacked treatments (11–13 μg/g significant increase in the upper soil layer) than in the intact ones (7–8 μg/g significant increase). The pattern of Cu apparently contradicts what would be expected. The peculiar situation of Cu is due to the relative role of plants (hypothetically responsible for the enrichment of the upper soil horizon, as suggested by Fig. 5) compared with the role of leachate in the total export. The role of plants was much larger in the repacked than in the intact treatments. An average of 128 μg Cu was exported by plants and 77 μg were exported by water in the intact treatments, and an average of 134 μg Cu was exported by plants and 380 μg were exported by water in the not repacked treatments. It is possible that part of the Cu tending to accumulate in the upper soil horizon was washed out by the percolating water.

The patterns presented in Figs. 5 and 6 are the results of the interaction between two processes: export by leachate (associated with a decrease of metals concentrations in the upper soil layer), and export by plants (associated with an increase of the concentration of metals in this layer). The variation of the total metal export from intact treatments to repacked ones results from variations in export by water and by plants. An increase of the relative role of plants in the export of metals can be due simply to a decrease of the export by percolating water without an important change in the export by plants (this is the case for Cu and U). But it can also be due to an important increase in the export by plants coupled with a slight decrease in the export by leachate, as in case of Pb (an average of 6 μg was exported by plants and 2.8 μg was exported by leachate in intact treatments, and an average of 11 μg was exported by plants and 1.7 μg was exported by water in the repacked treatments). Consequently, Pb has a larger total export in the repacked treatments than in the intact treatments. Uranium and Cu show lower total export in repacked treatments than in intact ones, despite the increase of the relative role of plants in total metal export, and the tendency of increasing concentrations in the upper soil horizon at the end of the experiment.

After inspecting Table 5 and Fig. 7, one can notice that the amendment of the control soil with compost or topsoil had a disturbing role at the time scale of the experiment (the amendment involved a mechanical mixing of soil down to 20 cm depth – Neagoe et al., 2005). It induced an increase in the relative export of metals, in agreement with the secondary succession theory. On the other hand, the inoculation of CB treatment with microorganisms led in many cases to a significant decrease of the relative export of metal, corresponding to systems more mature from succession point of view. This is a reasonable result taking into consideration that the natural colonization of a CB treatment with microorganism would take a long time, and this step was shortcut by the inoculation. There are two other clues suggesting that the treatments with expanded clay correspond to more juvenile systems than the compact variants. One comes from their reactions to rains. More mature systems make a more effective buffering of the incoming fluxes than juvenile ones. Compact soil variants reacted similarly to the two rain events, while the mixed treatment reacted very differently, which represents a confirmation of the mentioned theoretical statement. The other one comes from the relative abundance of the plant species. A common hypothesis in the ecological theory is that the systems with higher plant diversity are closer from the point of

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view of the cycling of elements, and thus the relative exports are lower. As species richness could not vary in our experiment, being set to two species, we can look only at the other component of diversity, evenness (which can be estimated in our case as indirectly proportional with the abundance of the dominating species). The abundance of the dominating species is extremely high in the treatments with expanded clay mixture (86–97% relative biomass abundance of *Festuca* sp.), but lowest in the treatments with compost and topsoil (37% and 65%, respectively), being very high in the C treatment (92%). This can be interpreted as an indication that the amendment with compost and topsoil treatments actually would induce a fast succession advancement of the ecosystem in the long run, although at the moment of the experiment there are clear signs of disturbance in terms of exported fluxes from the system. The export patterns were controlled mainly by alterations in the abiotic environment (mechanical mixing leading to accessibility for oxygen and larger pore size/hydraulic conductivity), but there are indications that biological processes modulated strongly these physical processes.

A final note is that these patterns should be cautiously extrapolated to the field situation. On the one hand the fluxes by plants are usually locally recycled in the field, and thus only the transfer by percolating water counts as an export from the system. On the other hand, other mechanisms of metal export are possible in the field, especially by runoff, which might change the pattern depicted in Fig. 7. After all, the plant development is very low on the control soil and thus the erosion potential is high, the purpose of the sets of bioremediation methods tested in this study being mostly the phytostabilization of the area.

With these precautions, it can be concluded that the second working hypotheses was confirmed: the export of metals from the repacked treatments was in many cases higher than the export from the intact treatments and the results are interpretable in terms of succession theory (Fig. 8).

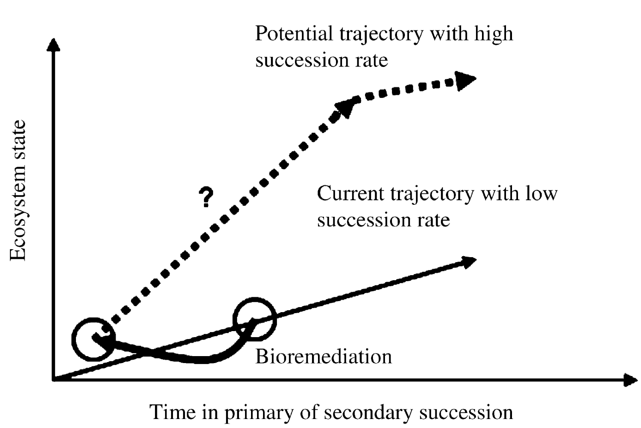

Fig. 8. The bioremediation could be interpreted as a switch between ecosystem trajectories with different successional speed.

5. Conclusions

The average total export of metals from the repacked variants was higher than the export from the intact treatments for most metals, and the relative role of the export by leaching compared to the export by plants' uptake increased as a result of strong soil disturbance. One can also notice that the plant uptake in the export of metals from the repacked treatments frequently played a more important role than the leaching. After the inoculation of the repacked contaminated soil with mycorrhizal fungi a decrease in the leaching of metals was observed, accompanied to a certain extent, by an increase in the export of metals by plants as a results of stimulating plants growth. The extra inoculation of the repacked contaminated soil with *Streptomyces* sp. reversed the effect of the mycorrhization by increasing metal concentrations in the leachate, and also increased the metals exported by plants to some extent, by stimulating plants growth. The results support the idea that the success of bioremediation approaches depends on the appropriate knowledge of the initial system state and of successional processes in contaminated areas. Bioremediation techniques can induce a disturbance of the system in the first phases of the process. Long-term studies on population of contaminated sites are needed in order to fully assess the effects of the manipulation on the successional processes and the real success of bioremediation.

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References

Abdel-Fattah, G.M., Mohamedin, A.H., 2000. Interactions between a vesicular–arbuscular mycorrhizal fungus (*Glomus intraradices*) and *Streptomyces coelicolor* and their effects on sorghum plants grown in soil amended with chitin of brawn scales. Biol. Fertil. Soils 32, 401–409.

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Alten, H., 2002. Quality control of arbuscular mycorrhizal fungi inoculum in Europe. In: Gianinazzi, S., Schuepp, H., Barea, J.M., Haselwandter, K. (Eds.), Mycorrhizal Technology in Agriculture, Birkhäuser Verlag, Switzerland, pp. 281–196.

Ames, R.N., 1989. Mycorrhiza development in onion in response to inoculation with chitin decomposing actinomycetes. New Phytol. 112, 423–427.

Ames, R.N., Mihara, K.L., Bethlenfalvay, G.J., 1987. The establishment of microorganisms in vesicular–arbuscular mycorrhizal and control treatments. Biol. Fertil. Soils 3, 217–223.

Amoroso, M.A., Schubert, D., Mitscherlich, P., Schumann, P., Kothe, E., 2000. Evidence for high affinity nickel transporter genes in heavy metal resistant *Streptomyces* spec. J. Basic Microbiol. 40, 295–301.

Barcan, V.S., 2002. Nature and origin of multicomponent aerial emissions of the copper–nickel smelter complex. Environ. Int. 28, 451–456.

Beleites, M., 1992. Altlast Wismut: Ausnahmezustand: Umweltkatastrophe und das Sanierungsproblem im deutschen Uranbergbau, first ed. Brandes und Apsel, Frankfurt (Main), Germany.

Bojakowska, I., Kochany, J., 1985. Studies on the removal of heavy metals from sludges by leaching and uptake by plants. Plant Soil 86, 299–302.

Bykova, E.I., Gorborukova, L.P., Kostenko, E.S., Namazbekova, S.Sh., 2006. Man-caused uranium contamination of biosphere objects in the territory of Kyrgyzstan. J. Environ. Sci. Health A 41, 2665–2682.

Carlsson, E., Büchel, G., 2005. Residual soil contamination at a former uranium heap leaching site, Thuringia, Germany. Chem. Erde 65 (S1), 75–95.

Crawford, D.L., Doyle, J.D., Wang, Z., Hendrick, C.W., Bentjen, S.A., Bolton, H., Fredrickson, J.K., Bleakley, B.H., 1993. Effects of a lignin peroxidase-expressing recombinant, Streptomyces lividans TK23.1, on biogeochemical cycling and the numbers and activities of microorganisms in soil. Appl. Environ. Microbiol. 59, 508–518.

Doyle, R.J., Matthews, T.H., Streips, U.N., 1980. Chemical basis for selectivity of metal ions by the Bacillus subtilis cell wall. J. Bacteriol. 143, 471–480.

Ebbs, S., Brady, D., Kochian, L., 1998. Role of uranium speciation in the uptake and translocation of uranium by plants. J. Exp. Bot. 49, 1183–1190.

Gorham, E., Vitousek, P.M., Reiners, W.A., 1979. The regulation of chemical budgets over the course of terrestrial ecosystems succession. Annu. Rev. Ecol. Syst. 10, 53–84.

Grawunder, A., 2006. Erstellen eines konzeptionellen geologisch-hydrogeologischen Modells des Testfeldes "Gessenwiese" im ehemaligen ostthüringischen Uranbergbaugebiet. Diploma Thesis, Friedrich-Schiller University, Jena (in German).

Haferburg, G., Reinicke, M., Merten, D., Büchel, G., Kothe, E., 2007. Microbes adapted to acid mine drainage as source for strains active in retention of aluminum or uranium. J. Geochem. Explor. 92, 196–204.

Hayes, W.J., Chaudhry, T.M., Buckney, R.T., Khan, A.G., 2003. Phytoaccumulation of trace metals at the Sunny Corner mine, New South Wales, with suggestions for a possible remediation strategy. Aust. J. Ecotox. 9, 69–82.

Hodge, A., 2000. Mycrobial ecology of arbuscular mycorrhiza. FEMS Microbiol. Ecol. 32, 91–96.

Hoffmann, G., 1991. Methodenbuch. Die Untersuchung von Böden, vol. 1. VDLUFA-Verlag, Darmstadt, Germany.

Hullebusch van, E.D., Lens, P.N.L., Tabak, H.H., 2005. Developments in bioremediation of soils and sediments polluted with metals and radionuclides. 3. Influence of chemical speciation and bioavailability on contaminants immobilization/mobilization bio-processes. Rev. Environ. Sci. Biol./Technol. 4, 185–212.

Iordache, V., 2003, Ecotoxicology of heavy metals in the landscape of the Small Island of Braila, Danube floodplain. Ph.D. Thesis, University of Bucharest.

Iordache, V., Neagoe, A., Bergmann, H., Kothe, E., Büchel, G., 2006. Factors influencing the export of metals by leaching in bioremediation experiments. 23. Arbeitstagung in Jena, Lebensnotwendigkeit und Toxizität der Mengen-, Spuren- und Ultraspurenelemente, pp. 288–295.

Jakubick, A.T., Gatzweiler, R., Mager, D., Robertson, A.M., 1997. The Wismut waste rock pile remediation program of the Ronneburg mining district, Germany. In: Proceedings of the Fourth International Conference on Acid Rock Drainage, vol. III, Vancouver, BC/Canada, May 31–June 6 1997, pp. 1285–1301.

Kabata-Pendias, A., Pandias, H., 2001. Trace Elements in Soils and Plants, third ed. CRC Press, Boca Raton, Ann Arbor, London, UK.

Keller, C., McGrath, S.P., Dunham, S.J., 2002. Trace metal leaching through a soil–grassland system after sewage sludge application. J. Environ. Qual. 31, 1550–1560.

Khan, A.G., Kuek, C., Chaudhry, T.M., Khoo, C.S., Hayes, W.J., 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41, 197–207.

Knoche, D., Embacher, A., Katzur, 2002. Water and element fluxes of red oak ecosystems during stand development on post-mining sites (Lusatian lignite district). Water Air Soil Pollut. 141, 219–231.

Kothe, E., Bergmann, H., Büchel, G., 2005. Molecular mechanisms in bio-geo-interactions: from a case study to general mechanisms. Chem. Erde 65 (S1), 7–27.

Kozlov, M.V., Zvereva, E.L., 2007. Industrial barrens: extreme habitats created by non-ferrous metallurgy. Rev. Environ. Sci. Biotechnol. 6, 231–259.

Krishna, K.R., Balakrishna, A.N., Bagyarajt, D.J., 1982. Interaction between a vesicular–arbuscular mycorrhizal fungus and *Streptomyces cinnamomeous* and their effects on finger millet. New Phytol. 92, 401–405.

Lorenz, C., Merten, D., Haferburg, G., Büchel, G., 2007. Laboratory investigations on the interactions of soil, water, and microorganisms with manganese. Adv. Mater. Res. 20–21, 311–314.

McGrath, S.P., Zhao, F.J., Lombi, E., 2001. Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232, 207–214.

McLaren, R.G., Clucas, LM., Taylor, M.D., Hendry, T., 2004. Leaching of macronutrients and metals from undisturbed soils treated with metal-spiked sewage sludge. 2. Leaching of metals. Aust. J. Soil Res. 42, 459–471.

Merten, D., Kothe, E., Büchel, G., 2004. Studies on microbial heavy metal retention from uranium mine drainage water with special emphasis on rare earth elements. Mine Water Environ. 23, 34–43.

Merten, D., Geletneky, J., Bergmann, H., Haferburg, G., Kothe, E., Büchel, G., 2005. Rare earth element patterns: a tool for understanding processes in remediation of acid mine drainage. Chem. Erde 65 (S1), 97–114.

Neagoe, A., Mascher, R., Iordache, V., Voigt, K., Knoch, B., Bergmann, H., 2004. The influence of vesicular arbuscular mycorrhiza *Glomus intraradiceae* on mustard (*Sinapis alba* L.) grown on a soil contaminated with heavy metals. Mengen und Spuren Elemente 22, 597–606.

Neagoe, A., Ebenå, G., Carlsson, E., 2005. The effects of soil amendments on plant performance in an area affected by acid mine drainage. Chem. Erde 65 (S1), 115–130.

Neagoe, A., Iordache, V., Mascher, R., Knoch, B., Kothe, E., Bergmann, H., 2006. Lysimeters experiment using soil from a heavy metals contaminated area. 23. Arbeitstagung in Jena, Lebensnotwendigkeit und Toxizität der Mengen-, Spuren- und Ultraspurenelemente, Friedrich-Schiller Universität, pp. 568–575.

Nowack, B., Schulin, R., Robinson, B.H., 2006. Critical assessment of chelant-enhanced metal phytoextraction. Environ. Sci. Technol. 40, 5225–5232.

Pirani, A.L., Brye, K.R., Daniel, T.C., Haggard, B.E., Gbur, E.E., Mattice, J.D., 2006. Soluble metal leaching from a poultry litter–amended Udult under pasture vegetation. Vadose Zone J. 5, 1017–1034.

Posta, K., Marschner, H., Romheld, V., 1994. Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize. Mycorrhiza 5, 119–124.

Robinson, B.H., Schulin, R., Nowack, B., Roulier, S., Menon, M., Clothier, B.E., Green, S.R., Mills, T.M., 2006. Phytoremediation for the management of metal fluxes in contaminated sites. Forest Snow Landscape Res. 80, 221–234.

Rufyikiri, G., Huysmans, L., Wannijn, J., Van Hees, M., Leyval, C., Jakobsen, I., 2004. Arbuscular mycorrhizal fungi can decrease the uptake of uranium by subterranean clover grown at high levels of uranium in soil. Environ. Pollut. 130, 427–436.

Saric, M.R., Stojanovic, M., Babic, M., 1995. Uranium in plant species grown on natural barren soil. J. Plant Nutr. 18, 1509–1518.

Schmidt, A., Haferburg, G., Sineriz, M., Merten, D., Büchel, G., Kothe, E., 2005. Heavy metal resistance mechanisms in actinobacteria for survival in AMD contaminated soils. Chem. Erde 65 (S1), 131–144.

Seuntjens, P., Nowack, B., Schulin, R., 2004. Root-zone modeling of heavy metal uptake and leaching in the presence of organic ligands. Plant Soil 265, 61–73.

Shahandeh, H., Hossner, L.R., 2002. Role of soil properties in phytoaccumulation of uranium. Water Air Soil Pollut. 141, 165–180.

Tatar, E., Mihucz, V.G., Tompa, K., Poppl, L., Zaray, G., Zambo, L., 2004. Study of soil leachates in doline above the Beke Cave, Hungary. Geoderma 120, 155–164.

Tian, G., Granato, T.C., Pietz, R.I., Carlson, C.R., Abedin, Z., 2006. Effect of long-term application of biosolids for land reclamation on surface water chemistry. J. Environ. Qual. 35, 101–113.

Verma, P., George, K.V., Singh, H.V., Singh, S.K., Juwarkar, A., Singh, R.N., 2006. Modeling rhizofiltration: heavy-metal uptake by plant roots. Environ. Mod. Assess. 11, 387–394.

Wamberg, C., Christensen, S., Jakobsen, I., Muller, A.K., Sørensen, S.J., 2003. The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). Soil Biol. Biochem. 35, 1349–1357.

Wyss, P., Boller, T., Wiemken, A., 1992. Testing the effect of biological control agents on the formation of vesicular arbuscular mycorrhiza. Plant Soil 147, 159–162.

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