The availability of DTPA extracted heavy metals during laboratory incubation of contaminated soils with glucose amendments

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ABSTRACT

The laboratory incubation with glucose treatment was carried out in order to estimate the possible effects of increased microbial activity on heavy metal availability. The soils from vicinity of a lead smelter operating for more than 200 years were used for the experiment. The DTPA-extractable heavy metal contents increased after glucose addition and mostly reached the highest values the second day of the incubation. The comparative study, where the chloroform fumigation was used prior to the incubation in order to decrease the microbial activities, showed especially from second day of incubation significantly lower DTPA-extractable metal contents compared to non-fumigated treatments. The interactions among the maximum possible availability of DTPA-extractable heavy metal fractions and native soil microbial characteristics were studied in differently contaminated arable and grassland soils. Irrespective of different heavy metal contents in soils, significant correlations were found among the maximum percentage increase of DTPA-extractable Pb and Cd and the ratio B_c/TOC and metabolic quotient (qCO_2) which may be a result of the important role of organic matter and microbial characteristics in soils on the heavy metal availability.

Keywords: heavy metals; DTPA extractability; soil microbial biomass; metabolic quotient (qCO_2)

Heavy metals belong to the most dangerous contaminants of the environment. Once these elements enter the soil, they persist for thousands years there and it is very difficult to eliminate their effects in the soil-plant system. Their chemical forms depend on the origin of heavy metals and main factors, which affect the mobility of elements (i.e. pH, free oxides of Fe and Al, clay content, cation exchange capacity, organic matter content and hydrothermal conditions of soils) (Alloway 1990, Podlešáková et al. 1999). However, little is known about the effects of microorganisms on metal distribution *in situ* or in simulated soil ecosystems. Microbial activities can play an important role in transfer and mobility of toxic elements in soils (Welsch et al. 1997) but the properties of microorganisms and their ability to participate in mobilization-immobilization equilibrium of heavy metals in soil–plant system are not often considered (Naidu et al. 1997). The heavy metal bioavailability can be only assessed and is rarely quantified, particularly in microbial investigation (Giller et al. 1998), but the role of soil microorganisms in heavy metal mobilization and immobilization processes in soils has been recently discussed in some papers (Leita et al. 1995, 1999, Ledin et al. 1999). For example, the mobilization of strongly bound or fixed Cd was a microbially mediated process and this was affected by soil environmental factors such as soil type, aeration and moisture content (Chanmugathas and Bollag 1987). Frankenberger et al. (1995) described the release of elements i.e. by: bio-oxidation, bio-reduction, biosorption or methylation. The microorganisms may accumulate a considerable part of metals presented in a soil, microbial sorbents can respond to changes in the soil solution chemistry by other ways than other solid soil components do (Ledin et al. 1999).

The long-term heavy metal contaminated soils from the vicinity of a lead smelter were studied in the present experiment. The aim of this research was to evaluate the changes in heavy metal availability in function of altered microbial activity during the laboratory incubation and to assess the effects of soil microbial biomass on the distribution of metals in soil systems.

MATERIAL AND METHODS

The sampling site is situated in vicinity of a lead smelter in Pribram (Czech Republic) operating for over two centuries. Metal mining has been ceased in 1972, but a secondary lead smelter is in operation. Since 1982, a 98% efficient dust separator and a 160 m stack have been in use (Kalac et al. 1991, Riuwerts and Farago 1996).

The studied soils were Cambisols according to FAO classification. Arable (A, B, C) and grassland (D, E, F) soils were sampled in several distances from the smelter in order to obtain samples of different heavy metal content. The regular agricultural cultivation was performed on arable soils A, B and C. The soil D is the native grassland soil. The soils E and F were arable soils until 1995, thereafter their crop management changed and they became grassland because of their high heavy metal contamination. The soil cores of the depth 0–20 cm were sampled from each sampling area. The soils were after the manual removal of animals and plant debris sieved at ≤ 2 mm and stored at 4°C until the beginning of exper-

Table 1. Total heavy metal content (mean ± *SD*), total organic carbon (TOC), ratio B_c/TOC, metabolic quotient (qCO_2) and pH_{H2O} in arable (A, B, C) and grassland (D, E, F) soils; results within a column not followed by the same letters indicate significant differences determined by Duncan's multiple range test ($P < 0.05$)

Soil	Pb	Cd (µg.g^{-1} soil)	Zn	TOC (%)	B_c/TOC (%)	qCO_2 (µg CO_2-C.µg $C_{B_c}$$^{-1}$.h^{-1})	pH_{H_2O}
A	58.7 ± 7.4	0.20 ± 0.14	50.4 ± 4.4	1.41 f	1.24 cd	0.00299 a	5.28
B	595.7 ± 13.4	3.31 ± 0.47	295.9 ± 30.3	1.63 d	1.16 cd	0.00258 b	7.21
C	1 363.6 ± 53.0	5.45 ± 0.15	287.1 ± 14.0	1.51 e	1.12 d	0.00270 b	7.00
D	359.4 ± 19.7	2.24 ± 0.24	93.9 ± 3.5	3.50 a	2.91 a	0.00080 d	5.75
E	1 162.6 ± 44.0	4.99 ± 0.33	240.6 ± 32.8	2.10 c	1.49 b	0.00200 c	6.31
F	2 204.5 ± 21.5	8.34 ± 0.46	315.2 ± 27.8	2.23 b	1.34 bc	0.00201 c	6.98

iments. The basic organic and microbial characteristics (soil organic carbon, microbial biomass, respiratory rates) were determined prior the soil incubation. The soil characteristics and the total contents of heavy metals are reported in Table 1.

The soil incubation was performed as described by Mühlbachová (2001). All experiments were made in triplicate. After one week of preincubation the soils were treated with glucose in the dose 1000 µg C.g^{-1} soil and with $(NH_4)_2SO_4$ to give the ratio C:N 10:1. Soils were analysed at day 1, 2 and 10 of the incubation for the content DTPA-extractable heavy metals and microbial biomass content. In comparative study, approximately 1 kg of soil was placed into the desiccator and fumigated with ethanol-free chloroform for 24 h. Subsequently the $CHCl_3$ was removed by repeated evacuations of the desiccator, three

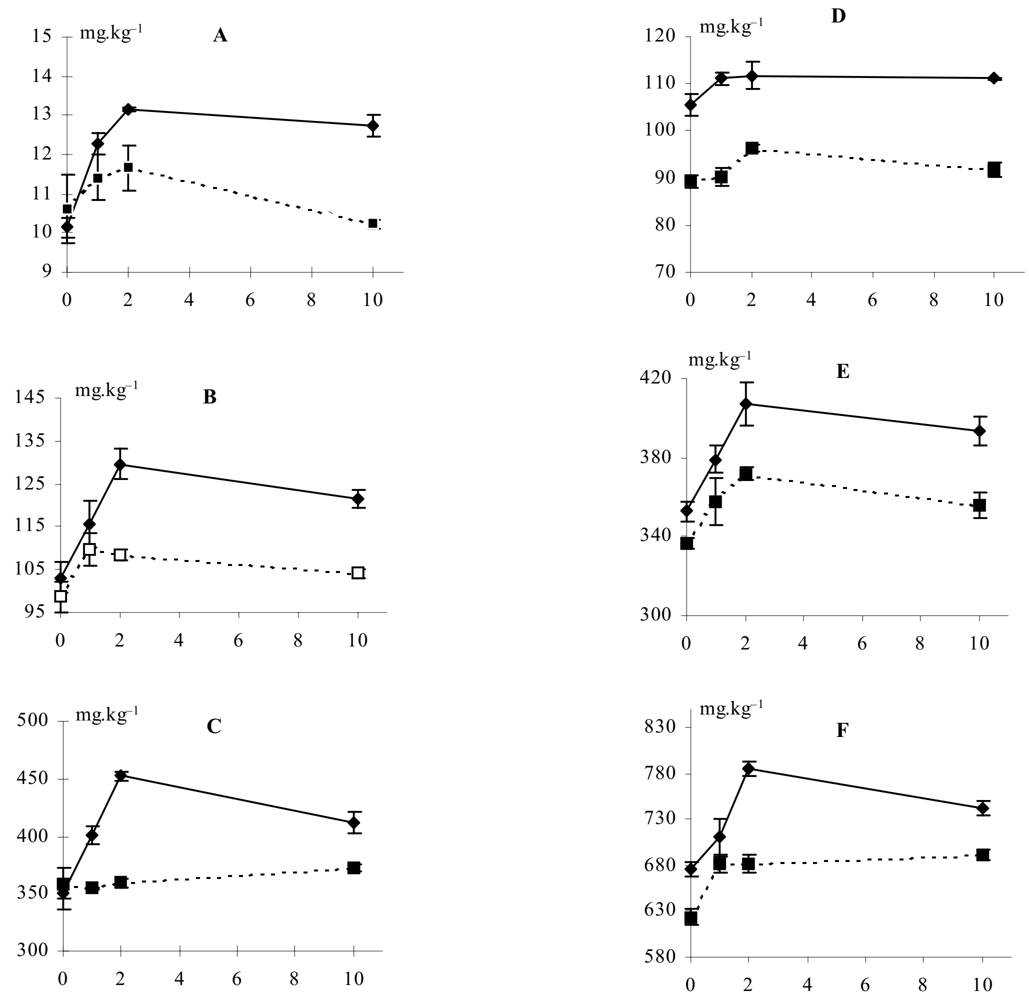

Figure 1. Changes of 0.005M DTPA-extractable lead in arable (A, B, C) and grassland (D, E, F) non-fumigated (nf – full line) and fumigated (f – interrupted line) soils during incubation experiment with glucose; x axis – days, y axis – content of Pb in mg.kg^{-1} of soil

replicates of every treatment were incubated with glucose and $(NH_4)_2SO_4$ and analysed equally as in the first part of the experiment.

The measurements of the soil microbial biomass (B_c) were performed using the fumigation-extraction method (F.E.) (Vance et al. 1987).

Total organic carbon (TOC) was determined by digestion of soil (1g of an oven dry basis) with 10 ml 1N $K_2Cr_2O_7$ and concentrated H_2SO_4 at 125°C and subsequent titration by 0.1N $Na_2S_2O_3$.

The respiratory rates were determined as amount of organic C released as CO_2 after absorption in NaOH and precipitation with $BaCl_2$ and was analysed by titration with standard HCl. The metabolic quotient (qCO_2) was calculated according to Anderson and Domsch (1990) equation: $qCO_2 = \mu g\ CO_2\text{-}C.\mu g\ C_B^{-1}.h^{-1}$.

DTPA-extractable fractions of heavy metals were extracted from 10 g weighing samples of soil with 20 ml of extracting solution (0.005M DTPA, 0.01M $CaCl_2$ and 0.1M TEA adjusted to pH 7.3) according to Lindsay and Norvell (1978) procedure.

DTPA extracts were filtered on Schleicher and Schuell filters No. 310645 and the solutions were then analysed for metal content using the ICP-OES axial sequential spectrometer Trace Scan f. Thermo Jarrell Ash corp. (USA).

RESULTS AND DISCUSSION

DTPA-extractable heavy metal contents increased during the incubation and mostly reached maximum values the second day of the incubation. The exception was the Zn availability, which continued to increase in soils B, D and F until the end of incubation (Figures 1–3). The chloroform fumigation of soils did not decrease the DTPA-extractable metal contents immediately and in correspondence with dead microbial biomass (Figure 4) suggesting that a simple decline of soil microorganisms cannot affect the availability of metals. On the other hand, the DTPA-extractable heavy metal contents decreased during the experiment compared to non-fumigated variants. The results obtained confirmed also the ANOVA statistics analysis, which showed that DTPA-extractable Pb and Cd contents in fumigated soils were from the second day mostly significantly lower compared to non-fu-

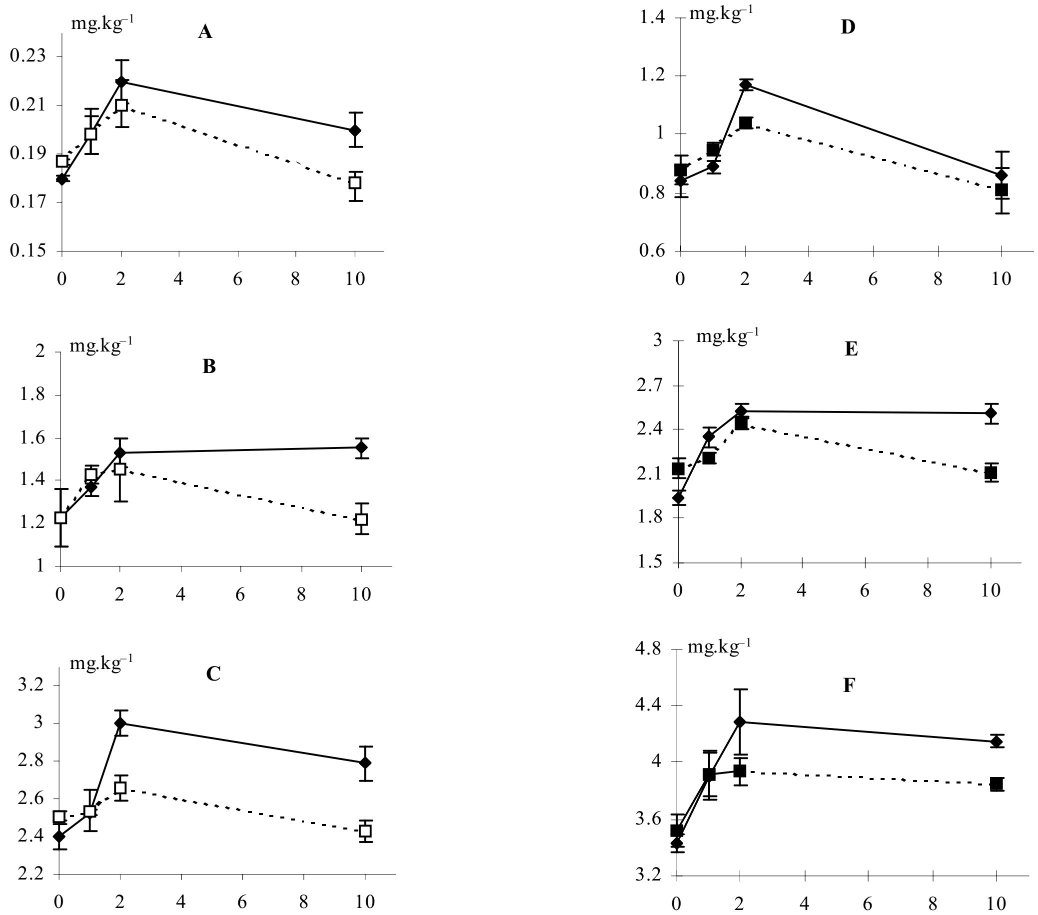

Figure 2. Changes of 0.005M DTPA-extractable cadmium in arable (A, B, C) and grassland (D, E, F) non-fumigated (nf – full line) and fumigated (f – interrupted line) soils during incubation experiment with glucose; x axis – days, y axis – content of Cd in mg.kg^{-1} of soil

migated soils. Significant differences of zinc contents between fumigated and non-fumigated soils were obtained especially in soils with greater total Zn contents. Only few significant correlations were found among the available heavy metal contents and microbial biomass in non-fumigated treatments (soil C: Pb and microbial biomass, $r = 0.6267$, $P < 0.05$; soil D: Cd and microbial biomass, $r = 0.6645$, $P < 0.05$). In fact, the maximum microbial biomass contents were observed usually the first day of incubation (Figure 4) whereas the maximum contents of DTPA-extractable metals were mostly reached the second day, which could be one of reasons of the results obtained. A possible explanation may be that heavy metals could be partly bound into microbial cell walls during first day of incubation in correspondence with increased microbial bio-mass content as describe Giller et al. (1998), Ledin et al. (1999), Mullen et al. (1989). Thereafter, the elements could be released from cells a day after into a soil and this might more increase the available metal contents. This hypothesis also could partly explain the lack of correlations between microbial characteristics and the DTPA heavy metal extractability.

Despite the different heavy metal contents extracted by DTPA from six experimental soils, the maximum DTPA availability of Pb and Cd expressed as the percentage increase between the initial and maximum metal content for each single soil during incubation is well correlated to the native ratio B_c/TOC (Figures 5a and 6a) and the native metabolic quotient (qCO_2) (Figures 5b and 6b). In fact, soil organic matter seemed to be able, despite the increased microbial activity during glucose incubation, not to release Pb so readily from organic complexes nevertheless the original qCO_2 values increased in correspondence to the maximum DTPA availability of Pb. On the other hand, Cd demonstrated increasing metal mobility in soils with greater organic matter content, but the qCO_2 showed its decreasing tendency. Lead, which is known to create stable complexes with organic matter, was less extractable in soils with greater organic carbon content. It accumulates in soils and relatively may not be affected by microbial degradation (Davies 1990), therefore, its lower extractability may be explained also by its greater ability to create strong organic complexes and chelates which were not releasable so readily by in-

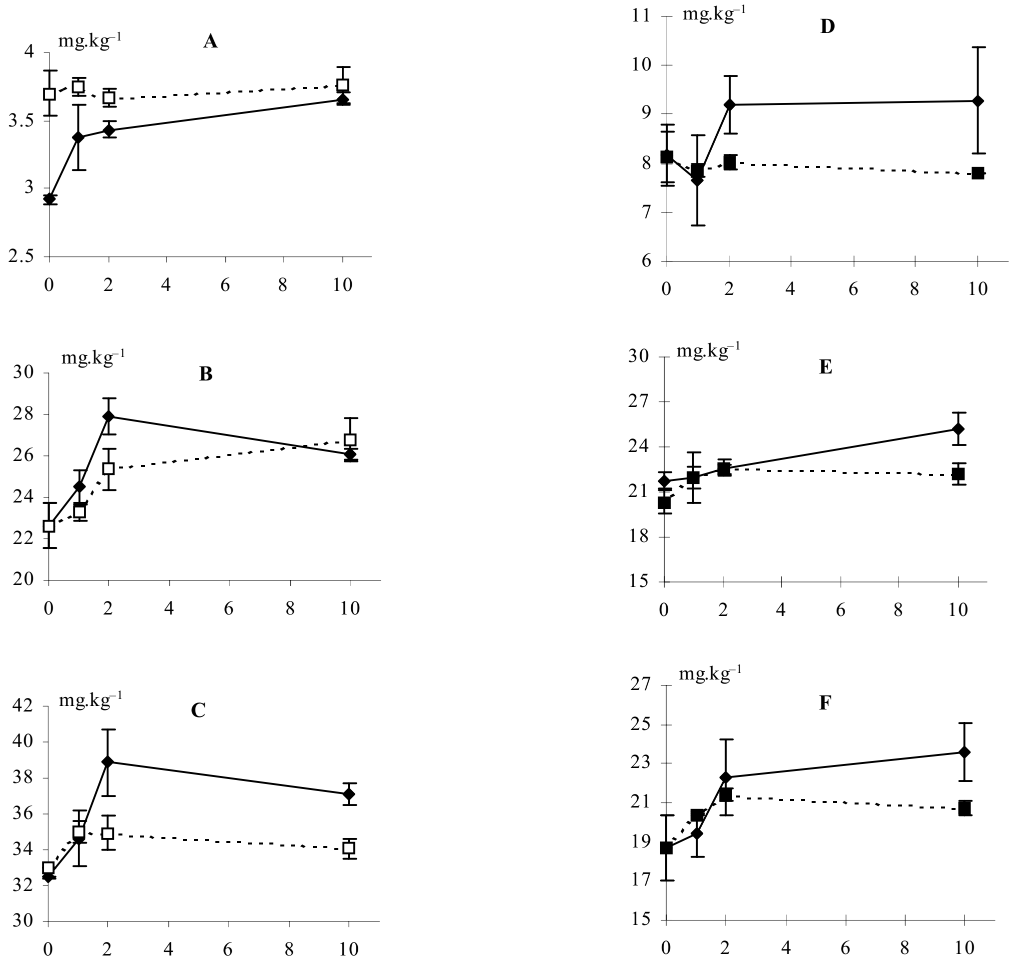

Figure 3. Changes of 0.005M DTPA-extractable zinc in arable (A, B, C) and grassland (D, E, F) non-fumigated (nf – full line) and fumigated (f – interrupted line) soils during incubation experiment with glucose; x axis – days, y axis – content of Zn in $mg.kg^{-1}$ of soil

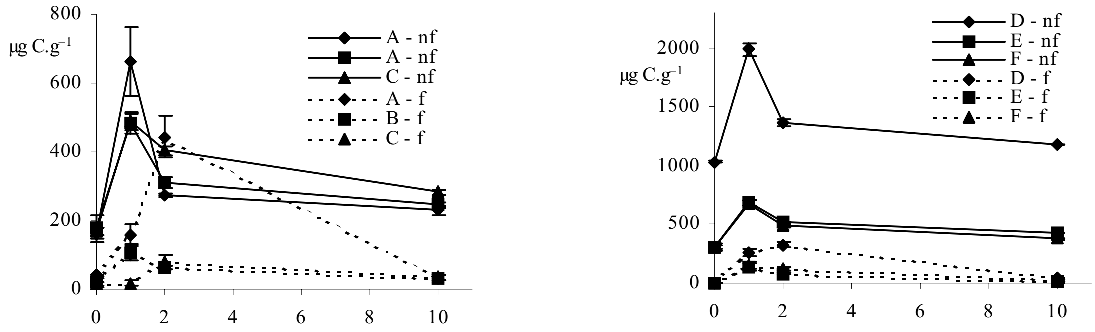

Figure 4. Dynamics of microbial biomass in arable (A, B, C) and grassland (D, E, F) soils non-fumigated (nf) and fumigated (f) by chloroform during incubation experiment with glucose; x axis – days, y axis – microbial biomass in µg $C.g^{-1}$ of soil

creased microbial activity. Cadmium is the highly labile metal and is more mobile in soils than lead. Cadmium from inorganic sources also tended to be more readily available for the soil environment (Alloway 1990), which could be one of possible explanations of the increase of maximum percentage release of cadmium in studied soils with greater organic matter content. No correlations were obtained between DTPA-extractable percentage increase of Zn and the ratio B_c/TOC and the qCO_2 and it released from the soil complexes unpredictably and therefore it is not possible to estimate more precisely any mechanisms involved in zinc availability from soils in this experiment.

Different ways may be involved when metals are released from soil. Leita et al. (1995, 1999) suggest that soil microorganisms can be also significantly involved in mobilization-immobilization processes of toxic elements in soils, but their mechanisms are not completely known yet. The different DTPA-extractable heavy metal contents released from soils when the percentage of maximum availability of heavy metals (Pb and Cd) was related to B_c/TOC and qCO_2 suggest, that the microorganisms and their activities could increase the availability of heavy metals in different ways special for every element, but the effects of organic matter on metal availability can

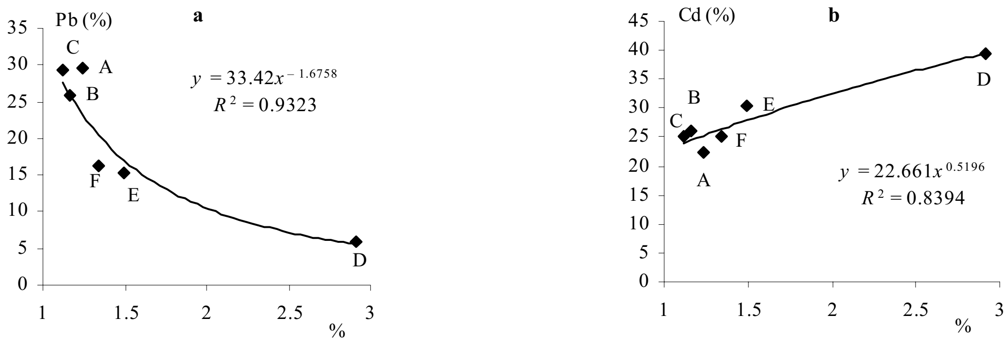

Figure 5. Relationships between the index B_c/TOC (x axis in %) and DTPA-extractable lead (a) and cadmium (b); the letters A–F indicate the single soils used in the experiment; y axis – maximum release of Pb and Cd, respectively

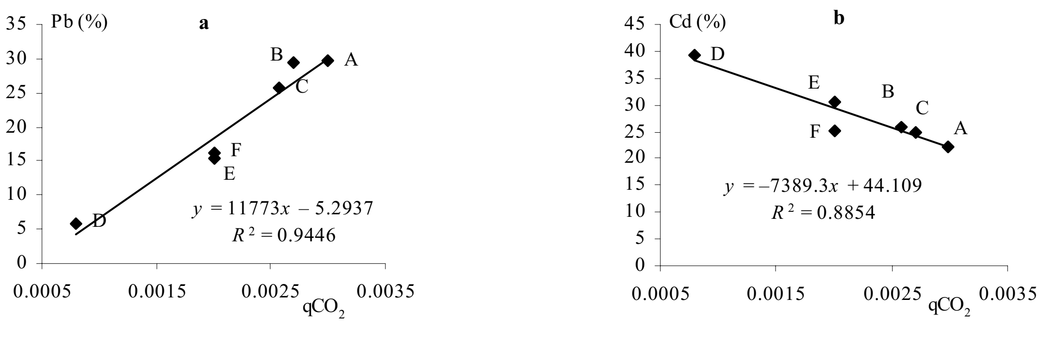

Figure 6. Relationships between the metabolic quotient qCO_2 (x axis in µg CO_2-C.µg $C_{B_c}^{-1}.h^{-1}$) and DTPA-extractable lead (a) and cadmium (b); the letters A–F indicate the single soils used in the experiment

not be omitted. The microorganisms for instance could partly alter some organic and inorganic complexes in the soil compartments and so affect the easier release of metal ions and compounds from different soil organic and inorganic complexes and ligands. Some of these mechanisms could be involved also in the experiment performed. In fact, microorganisms may also alter metal availability in their vicinity due to localised acidification of the environment, or production of compounds with complex metals (Giller et al. 1998). The lower microbial activity could be a possible explanation of the greater decrease of available metal fractions during incubation in fumigated soils. The significantly greater DTPA-extractable contents of heavy metals during glucose incubation in non-fumigated variants if compared to fumigated soils may support this hypothesis.

Thus, chemical properties of each element are different and their mobility can be affected by many soil parameters. The effects of soil microbial biomass, its metabolic activity and their interactions with organic matter and other soil constituents are one of possible factors affecting the heavy metal availability, but their common effects on heavy metal availability should be studied also in the further research.

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ABSTRAKT

Přístupnost těžkých kovů extrahovaných roztokem DTPA během laboratorní inkubace kontaminovaných půd s přídavkem glukózy

V laboratorním inkubačním pokuse byly po přídavku glukózy sledovány možné vlivy zvýšené mikrobiální aktivity na změny v přijatelnosti rizikových prvků. Pro pokus byly použity více než dvě stě let kontaminované půdy z oblasti příbramských kovohutí. Obsahy těžkých kovů extrahovaných DTPA vzrůstaly po přídavku glukózy a zpravidla dosáhly nejvyšších hodnot druhý den desetidenní inkubace. Ve srovnávacím pokuse, kde byly půdy nejprve fumigovány chloroformem, aby byly maximálně sníženy obsahy mikrobiální biomasy, a teprve poté inkubovány s přídavkem glukózy, byly nalezeny především od druhého dne inkubace statisticky významně nižší obsahy DTPA extrahovatelných rizikových prvků. Dále byly sledovány možné vzájemné vztahy mezi maximálními obsahy DTPA extrahovatelných podílů kovů a přirozenými půdními mikrobiálními charakteristikami. Přestože vybrané půdy měly různé obsahy těžkých kovů, byly nalezeny statisticky významné korelace mezi procentuálním nárůstem Pb a Cd extrahovatelných DTPA a koeficienty charakterizujícími půdní organickou hmotu (B_c/TOC a qCO_2), což může potvrzovat významnou úlohu půdní organické hmoty pro přijatelnost jednotlivých kontaminujících prvků.

Klíčová slova: těžké kovy; extrahovatelnost DTPA; půdní mikrobiální biomasa; metabolický kvocient (qCO_2)

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