

The availability of Cd, Pb and Zn and their relationships with soil pH and microbial biomass in soils amended by natural clinoptilolite

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ABSTRACT

The relationships among soil microbial biomass, pH and available of heavy metal fractions were evaluated in long-term contaminated soils during an incubation experiment with the amendment of zeolite (natural clinoptilolite) and the subsequent addition of glucose. The values of pH after the addition of glucose decreased during the first day of incubation approximately at about one unit and corresponded with the maximum increase of microbial biomass. The available heavy metal contents extracted by H_2O , 1 mol/l NH_4NO_3 and 0.005 mol/l DTPA increased during the first two days of incubation. Only a few significant relationships were found between the available metal contents and pH or microbial biomass. This fact could be ascribed to the different dynamics of the microbial biomass, pH and metal availability after glucose addition, when the highest metal contents during the incubation were usually reached one day later in respect to the greatest changes of pH and microbial activity. In comparison to soils without zeolite addition, the variants with natural clinoptilolite showed lower heavy metal contents in all used extractants with the exception of Cd which in H_2O extracts tended to increase.

Keywords: incubation experiment; heavy metal contaminated soil; glucose and zeolite amendment; Cd, Pb, Zn availability; pH; microbial biomass

Heavy metal contamination of soils derived from agricultural (e.g., fertilizers and sewage sludge) or industrial activities (e.g., metal mining and smelting) is one of the major environmental problems in many parts of the world. The determination of risks dealing with the release or binding of toxic compounds in soils is a complex problem, because a range of chemical, physical or biological soil properties can directly or indirectly affect these processes (Giller et al. 1998).

Desorption and bioavailability is dependent on soil characteristics such as organic matter content, clay mineralogy, pH, iron oxide content and redox conditions (Babich and Stotzky 1980). Of these, pH is often found to have the largest influence, due to its strong effects on solubility and speciation of metals both in the soil as a whole and particularly in the soil solution. Metals are bound to soil particles by different chemical bonds. These bonds determine, to a large extent, the bioavailability of the metals, although biological uptake mechanisms may contribute to a varying degree. Chemical bonds may change over time due to abiotic processes such as changes in temperature and redox conditions, or microbial activity, resulting in varying availability to other organisms (Schultz et al. 2004).

The bioavailability of metals, in soils is governed by the nature of microorganisms and microbially mediated processes (Megharaj et al. 2003). In addition, the important role in heavy metal bioavailability could play a big part in biosorption and the binding of elements in microbial cells, particularly their complexation with polysaccharides of the cell wall (Ford and Mitchell 1992). Till now, there is not enough information about the relationships between heavy metal mobility and soil microorganisms *in situ*, however poor mobilization of some elements in biologically active soils was studied (Chanmugatas and Bollag 1987). It is not easy to evaluate the role of the accumulation of metals by microorganisms for their distribution in soils and only a few studies have been focused on the evaluation of soil microorganisms in soil systems for their mobilization of heavy metals. Nederlof and Van Riemsdijk (1995) attributed the sorption of metal ions by soil organisms to the competition for binding of that metal ion by all reactive soil components (including the organisms). In addition, the total amount of bioavailable metal in soil is influenced by complexity of dissolved organic and inorganic ligands. Ledin et al. (1999) reported that microorganisms were able to accumulate a substan-

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Table 1. Main physico-chemical characteristics of soils and total metal content

Soil	Soil texture	pH (H ₂ O)	C _{org} (%)	P available (mg/kg)	CEC (mmol/kg)	Cd	Pb	Zn
						(mg/kg)		
P1	sandy silt loam	6.9	1.66	90.3	137	0.65	74	90
P2	sandy silt loam	6.3	1.77	33.1	171	4.06	1138	255

tially important part of metals, up to 38% for Hg. Microorganisms may also alter metal availability in their vicinity due to localized acidification of the environment or production of compounds which contain complex metals. However, as the studies involving microbial transformations have been performed mainly *in vitro* with pure cultures, the effect of these processes on metal bioavailability and their dynamics in soils is not clear (Giller et al. 1998, Megharaj et al. 2003).

The aim of the study was to evaluate the effects of the increased activity of soil microbial biomass on pH and on heavy metal availability. The effects of zeolites (natural clinoptilolite) on the mobilization processes of toxic elements were also studied.

MATERIAL AND METHODS

The non-contaminated sample and a contaminated soil sample from the vicinity of a lead

smelter in Příbram (Czech Republic) were taken for the experiment. The soils from Příbram area (P1 and P2) were typical Cambisols according to FAO classification. The Příbram smelter (Czech Republic) has been in operation since 1786. In 1982 a 98% efficient dust separator and a 160 m stack had been in use (Kalac et al. 1991, Riuwerts and Farago 1996). The soil cores were sampled from a depth of 0–20 cm and after the removal of plant and animal debris sieved at < 2 mm. The soil characteristics are reported in Table 1.

Approximately 1 kg of each soil was mixed with zeolite (natural clinoptilolite) in order to give 3% zeolite amendment. Soils were adjusted to 50% of their water holding capacity (WHC) in plastic jars, and in three replicates, then placed into the three one litre plastic containers tightly covered with fitting lids to be conditioned at 28°C 14 days before treating with glucose. A jar with 25 ml of 1 mol/l NaOH was placed into the containers to take up the CO₂-evolved. The containers were daily

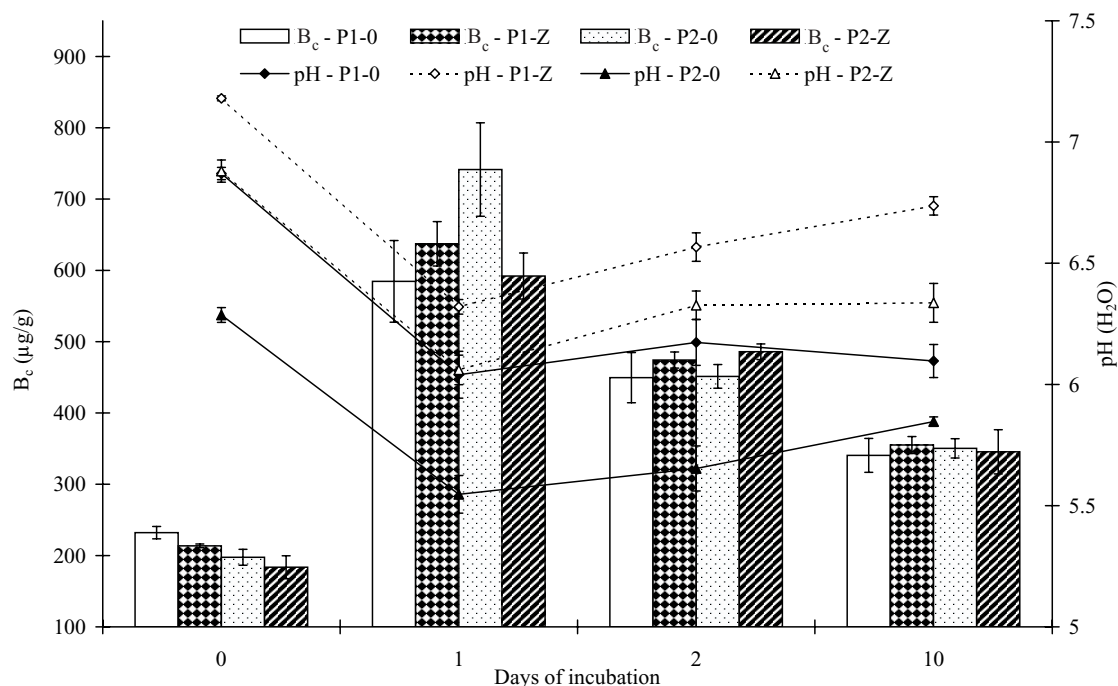


Figure 1. Microbial biomass (B_c , $\mu\text{g C/g}$ soil) and pH (H_2O) dynamics in soils from vicinity of Příbram smelter during incubation with glucose after previous zeolite amendment (P1-0: non-contaminated soil without zeolite amendment; P1-Z: non-contaminated soil with zeolite amendment; P2-0: contaminated soil without zeolite amendment; P2-Z: contaminated soil with zeolite amendment); standard deviation bars represent the variability of measurements

aerated to ensure a sufficient oxygen supply. The soils without zeolite amendment served as a control and they were preincubated in the same way as samples with zeolite. After 14 days of preincubation the mixture of glucose 1000 µg C/g and NH₄(SO₄)₂ given ratio C:N 10:1 was added. Soils were incubated for 10 days. The microbial biomass content, pH and available heavy metal fractions were determined at days 0, 1, 2, and 10 of the incubation. Detailed natural clinoptilolite characteristics were reported by Mühlbachová and Šimon (2003).

The measurements of the soil microbial biomass C (B_c) were performed using the fumigation-extraction method (F.E.) according to the Vance et al. (1987) procedure. The microbial biomass C was calculated from the relationship: $B_c = 2.64E_c$ where E_c is the difference between organic C extracted from the fumigated and non-fumigated treatments, both expressed as µg C/g oven dry soil.

The values of pH were determined after 1 h shaking of 20 g of soil and 50 ml of distilled water. The distilled water was boiled before the addition into the soil in order to eliminate possible effects on pH by CO₂ sorption.

H₂O-extractable contents of heavy metals were determined after 24 h overhead shaking of 20 g of soil sample weight with 50 ml deionised water at 150 rpm. The exchangeable heavy metal contents were extracted from the 20 g weighing soil samples with 1 mol/l NH₄NO₃ according to Zeien and Brümmer (1989). 0.005 mol/l DTPA-extractable fractions of heavy metals were extracted from a 10 g sample weight of soil with 20 ml of extracting solution (0.005 mol/l DTPA, 0.01 mol/l CaCl₂·2 H₂O and 0.1 mol/l TEA giving the ratio [w:w:w] 1:0.74:7.55 and adjusted to pH 7.3) according to the Lindsay and Norvell (1978) procedure.

Water, 1 mol/l NH₄NO₃ and 0.005 mol/l DTPA mixtures with soils were filtered with Schleicher and Schuell filters No. 310645 and clear solutions were then analysed for metal content using the OES-ICP axial sequential spectrometer Trace Scan f. Thermo Jarrell Ash corporation (USA).

RESULTS AND DISCUSSION

The initial pH values of the Příbram soil samples varied at the beginning of the experiment between 6.3 and 6.9 in variants non-amended by zeolite (P1-0, P2-0) and 6.9–7.2 in zeolite amended variants (P1-Z, P2-Z). During the first day of incubation pH decreased at about one unit in all experimental variants (Figure 1). The pH in the zeolite amended soils was significantly higher during the overall time of the experiment compared to the variants without zeolite, however pH tended to increase slightly from the second day of incubation. A de-

crease of pH during the first incubation day was accompanied by the increase of microbial biomass in incubated soils (Figure 1) giving significant relationships between these soil characteristics (Table 2). Microbial biomass contents did not demonstrate significant differences between contaminated and non-contaminated soil before the incubation. The

Table 2. Correlation coefficients between microbial biomass and pH in Příbram soils during soil incubation with glucose after previous amendment with zeolites (P1-0: non-contaminated soil without zeolite amendment; P1-Z: non-contaminated soil with zeolite amendment; P2-0: contaminated soil without zeolite amendment; P2-Z: contaminated soil with zeolite amendment)

Soil sample	Correlation coefficient (r)	Formula
P1-0	-0.74549**	$y = 7.0919 - 0.0020x$
P1-Z	-0.87545***	$y = 7.5753 - 0.0019x$
P2-0	-0.84299***	$y = 6.3792 - 0.0013x$
P2-Z	-0.74972**	$y = 7.0797 - 0.0019x$

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 3. Selected significant correlations between pH, microbial biomass and available heavy metal contents in Příbram soils (P1-0: non-contaminated soil without zeolite amendment; P1-Z: non-contaminated soil with zeolite amendment; P2-0: contaminated soil without zeolite amendment; P2-Z: contaminated soil with zeolite amendment)

Soil sample	Relationship	Correlation coefficient
P1-0	pH and Cd (H ₂ O)	0.902***
	pH and Zn (H ₂ O)	-0.763**
	pH and Zn (NH ₄ NO ₃)	-0.702*
	pH and Zn (DTPA)	-0.584*
P2-0	pH and Zn (H ₂ O)	-0.792*
P2-Z	pH and Cd (NH ₄ NO ₃)	-0.656*
P1-0	microbial biomass and Cd (H ₂ O)	-0.766**
	microbial biomass and Zn (H ₂ O)	0.663*
	microbial biomass and Zn (HN ₄ NO ₃)	0.846***
P1-Z	microbial biomass and Pb (DTPA)	0.663*
	microbial biomass and Zn (H ₂ O)	0.663*
	microbial biomass and Zn (HN ₄ NO ₃)	0.599*
P2-0	microbial biomass and Cd (NH ₄ NO ₃)	0.893***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, non-significant relationships are not shown

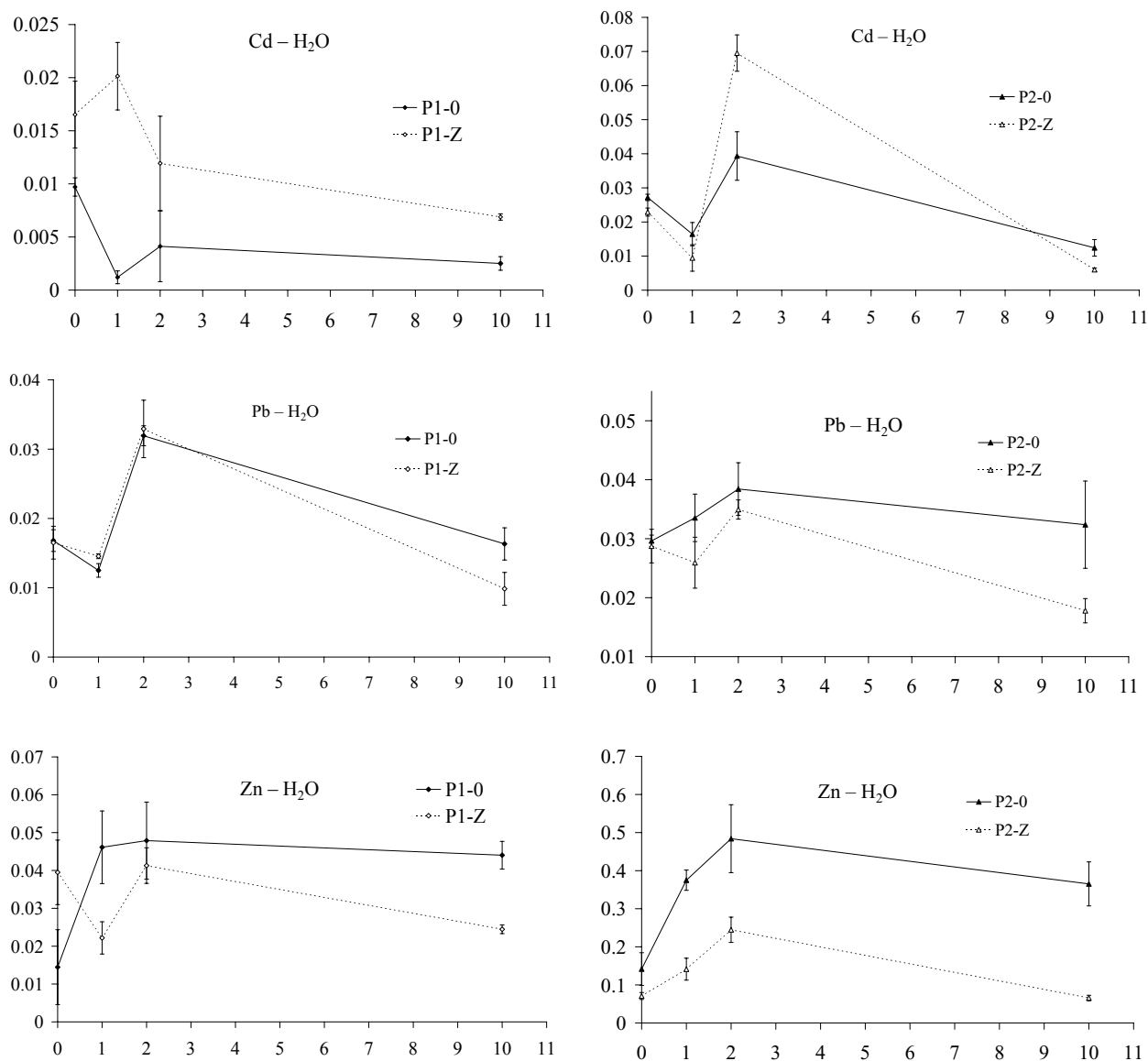


Figure 2. Water-extractable heavy metal contents in soils from vicinity of Příbram smelter during incubation with glucose after previous zeolite amendment (P1-0: non-contaminated soil without zeolite amendment; P1-Z: non-contaminated soil with zeolite amendment; P2-0: contaminated soil without zeolite amendment; P2-Z: contaminated soil with zeolite amendment); standard deviation bars represent the variability of measurements (x-axis: days of incubation, y-axis: mg/kg of soil)

addition of zeolite decreased during the first day of incubation microbial biomass dynamics in the contaminated soil P2, whereas no effects were observed in the soil P1. Particularly, microbial biomass increased more sharply after the addition of glucose in contaminated soil P2 than in the soil P1 indicating a lower capacity to utilize the substrate for its synthesis in the contaminated soil P2 (Mühlbachová and Šimon 2003). In fact, the amount of C mineralised over a short period such as 24 hours after the addition of glucose has been found to be extremely sensitive to addition of even low amounts of metal salts in laboratory experiments (Stadelmann et al. 1984). Significant

relationships between pH and microbial biomass content in soils during the incubation (Table 2) indicate the acidification of the soil environment caused by increased microbial activities after the addition of glucose. In fact, stimulated nitrification, CO₂ evolution or the enzymatic activities could decrease soil pH. Giller et al. (1998), Megharaj et al. (2003) reported that microbial communities are able to acidify their immediate microenvironment in soils by their activities. Natural clinoptilolite increased soil pH in comparison to non-zeolite variants. In fact, addition of zeolites in soils can increase a number of ion exchange places (Edwards et al. 1999); therefore they could play an important

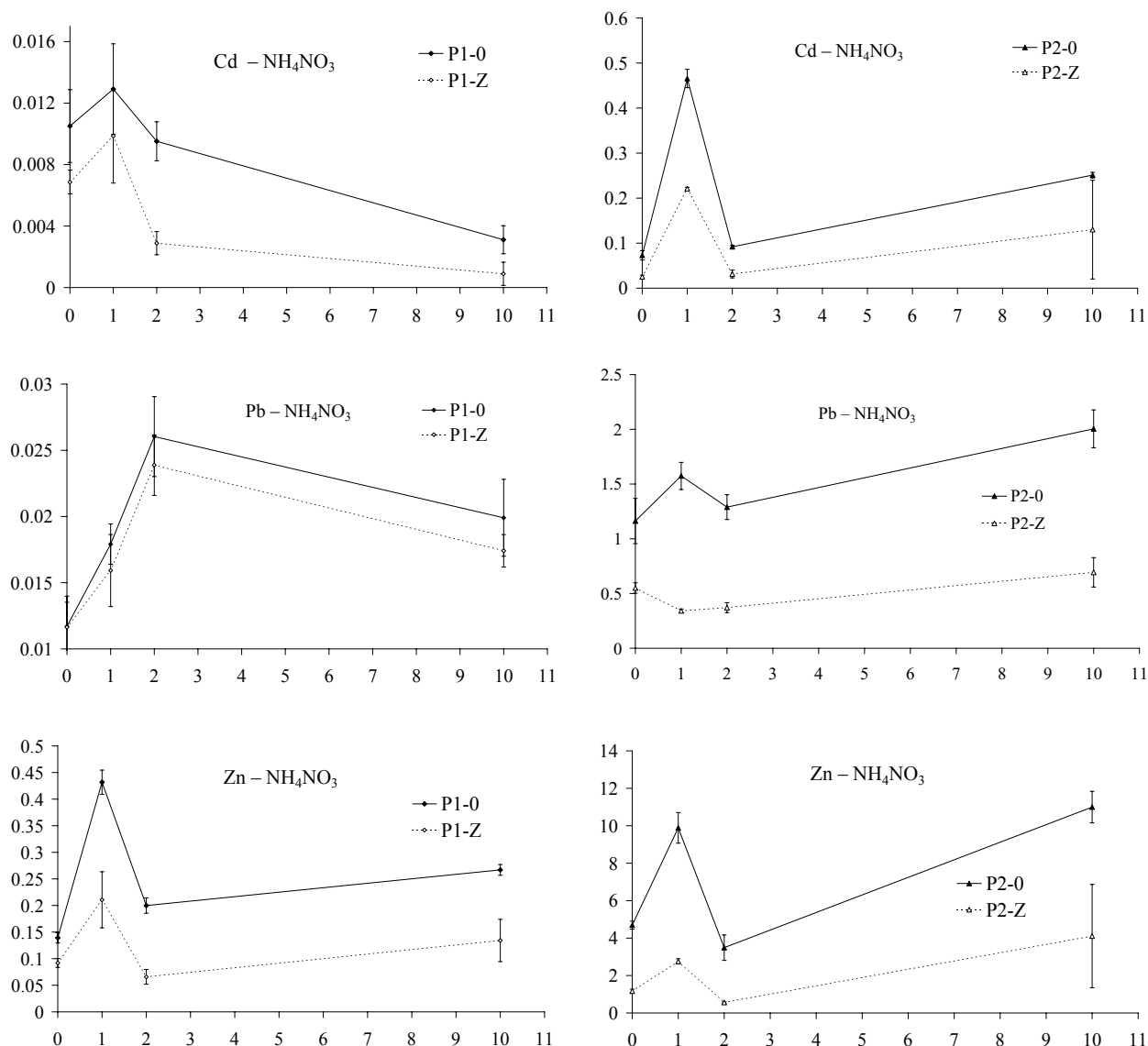


Figure 3. 1 mol/l NH₄NO₃-extractable heavy metal contents in soils from vicinity of Příbram smelter during incubation with glucose after previous zeolite amendment (P1-0: non-contaminated soil without zeolite amendment; P1-Z: non-contaminated soil with zeolite amendment; P2-0: contaminated soil without zeolite amendment; P2-Z: contaminated soil with zeolite amendment); standard deviation bars represent the variability of measurements (x-axis: days of incubation, y-axis: mg/kg of soil)

role in pH dynamics also if the microbial activities were enhanced by glucose addition.

Water-extractable contents of Cd, Pb and Zn representing easily soluble heavy metal fractions usually increased during the first two days of incubation (Figure 2). However, water-extractable metal contents were very low in all studied soils during the whole incubation period. Zeolite addition decreased water-extractable content of Pb and Zn, whereas Cd increased in the presence of zeolite. pH values in zeolite amended soils decreased less than in variants incubated only with glucose suggesting that metal ions could not be so readily released from soil complexes in the presence

of natural clinoptilolite. Significant correlations were found in the soil P1-0 between pH and Cd and Zn, the significant relationship between Zn and pH was found in the soil P2-0 (Table 3). Only significant correlations are shown in Table 3 due to the fact that little significant relationships between the measured parameters were found.

Exchangeable heavy metal contents in comparison to water-extractable fractions more often reached higher values during the first day of incubation (Figure 3). However, exchangeable metal fractions remained usually lower in zeolite-amended variants. An increase of exchangeable Cd, Pb and Zn fractions was observed at the end of soil incubation

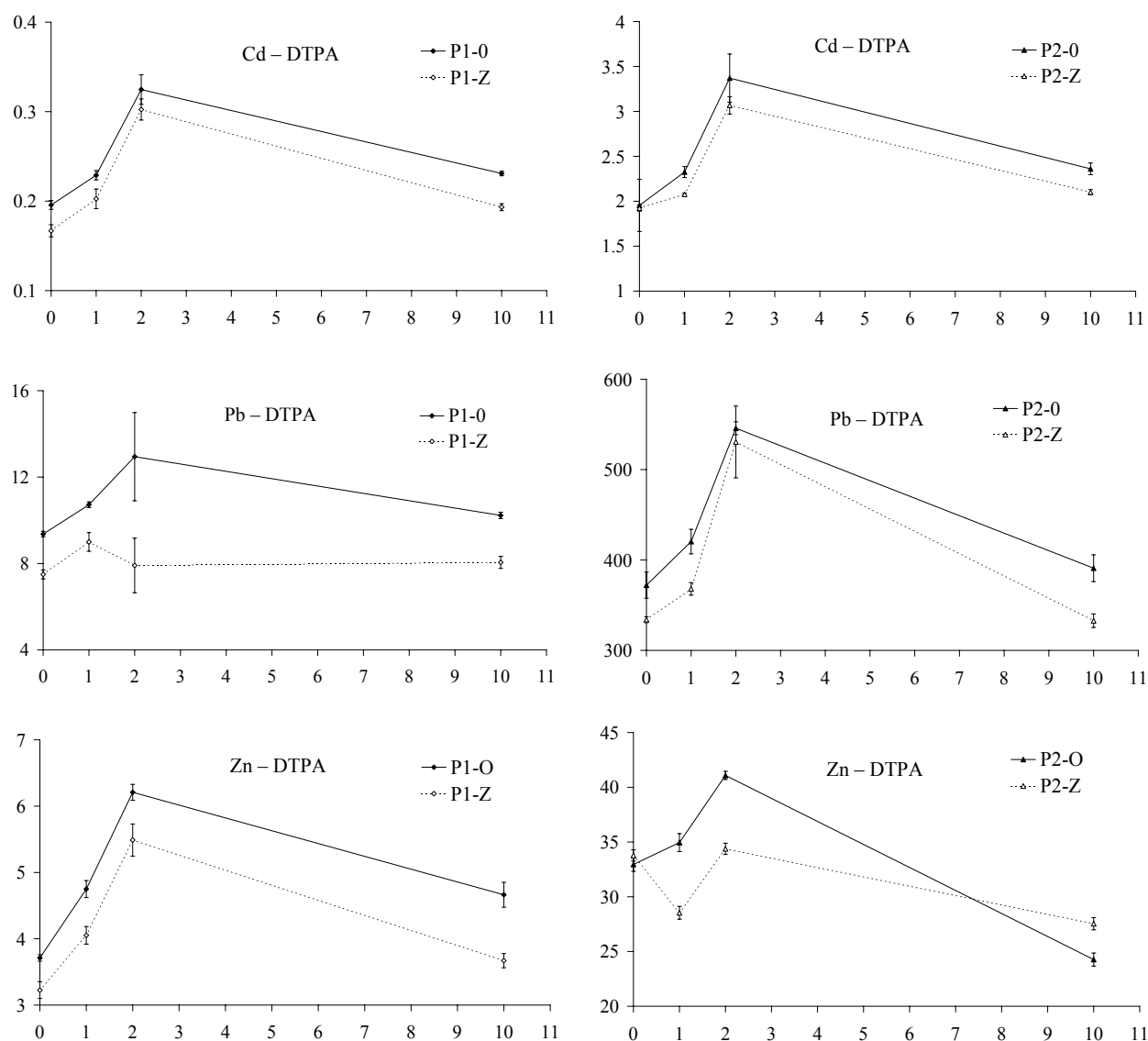


Figure 4. 0.005 mol/l DTPA-extractable heavy metal contents in soils from vicinity of Příbram smelter during incubation with glucose after previous zeolite amendment (P1-0: non-contaminated soil without zeolite amendment; P1-Z: non-contaminated soil with zeolite amendment; P2-0: contaminated soil without zeolite amendment; P2-Z: contaminated soil with zeolite amendment); standard deviation bars represent the variability of measurements (x-axis: days of incubation, y-axis: mg/kg of soil)

in the soil P2. Significant relationships between exchangeable heavy metal fractions and pH or microbial biomass were found only between Zn and microbial biomass in both experimental variants of the soil P1 and between zinc and pH in the soil P2 (Table 3). Significant relationships were found also for Cd and microbial biomass in the variant P1-0 and between Cd and pH in the variant P2-Z.

DTPA-extractable Cd, Pb and Zn in the Příbram soils usually reached its highest content in the second day of incubation (Figure 4) and the zeolite amendment decreased the DTPA-extractable metals in all studied soils if compared to non-zeolite variants. Significant correlations were found between DTPA-extractable Zn and pH in variant P1-0 and

between DTPA-extractable Pb and microbial biomass in the variant P1-Z (Table 3). No relationships were obtained for DTPA-extractable metals and pH or microbial biomass of the soil P2. DTPA-extractable metal contents in the Příbram soils reached often their highest level during the second day of incubation, therefore the direct effects of pH and microbial biomass on availability of elements was not possible to evaluate in terms of direct relationships with studied characteristics.

The highest content of heavy metal fractions extracted from soils by used extractants was found usually on the second day of incubation. The values of pH decreased during the first 24 hours of soil incubation with glucose simultaneously

as microbial biomass content increased. In fact, significant relationships were found between microbial biomass content and pH (Table 2) and the interaction of these soil characteristics could be a possible explanation of increased heavy metal contents during the incubation experiments. Soil microorganisms can participate in the mobilization and immobilization processes of heavy metals in soils by localized acidification of their microenvironment or production of compounds, which are complex metals (Giller et al. 1998, Megharaj et al. 2003). Significant relationships found between soil microbial biomass and pH values could confirm this assumption. In addition, available metal fractions increased in the performed experiment. Relationships between available metal contents and microbial biomass were usually studied after the amendment of soils by metal salts under laboratory conditions, significant correlations were obtained for instance, between available zinc contents and microbial biomass (Leita et al. 1995) where the contents of Zn decreased in correspondence to a lower amount of microbial biomass. However, this relationship is not able to explain the direct effect of microbial biomass on zinc availability, because zinc was added into the soils artificially, and therefore it is possible to suppose that when zinc entered a soil, it started to create complexes with different soil particles independently on the microbial biomass. This suggests that available metal contents might not be regulated only by the total quantity of soil microorganisms as reported Leita et al. (1995) or Valsecchi et al. (1995) who found significant relationships between heavy metal contents and microbial biomass contents. However, metabolic activities of soil microorganisms could play an important role already due to their participation in changes of physico-chemical and biological soil characteristics properties and the abilities of heavy metals to create more or less stable complexes in soils (Mühlbachová 2002).

Heavy metals can be also bound on microbial cell walls. In accordance with Ledin et al. (1999) and Chanmugatas and Bollag (1987) the microbial biomass could bind into its cell walls the part of heavy metals which could be released after the death of microbial communities and their subsequent autolysis. The heavy metal binding onto microbial cells was described also by Mullen et al. (1992). In our experiment, the highest heavy metal content was often obtained at the second day of incubation when microbial biomass already decreased after its maximum which was reached during the first day of the experiment. A possible explanation could be for example that after partial metal binding onto cell walls, available elements could be released again in the soil solution after the death of soil microorganisms and their subsequent autolysis.

Available heavy metal contents were lower in zeolite-amended soils in comparison to non-zeolite variants, however an increase of available metals was observed during glucose incubation in all studied soils. Only Cd contents in water extracts tended to have an increase in zeolite presence, however the determined water Cd contents were low and nearer to detection limits. This could suggest that the addition of zeolite favoured the metal sorption into their exchange places. The addition of natural or synthetic zeolites in the soils increased a number of ion exchange places in a soil due to its porous structure and they are able to absorb small molecules (Edwards et al. 1999). Therefore, they are the useful material for sorption of toxic elements present in soils (Chlopecka and Adriano 1997). On the other hand, influence of zeolites on microbial biomass and its activities is until now practically unknown (Chander and Joergensen 2002) and their addition was not completely able to eliminate the effects of microbial activities on metal availability.

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ABSTRAKT

Přístupnost Cd, Pb a Zn a jejich vzájemný vztah s pH a mikrobiální biomasou v půdách obohacených přírodním klinoptilolitem

V laboratorním inkubačním pokusu s dlouhodobě kontaminovanými půdami, ke kterým byl přidán zeolit (přírodní klinoptilolit) a následně glukóza, byla sledována aktivita půdní mikroflóry, pH a přístupnost těžkých kovů a jejich vzájemné vztahy. Hodnoty pH poklesly první den inkubace a korespondovaly s maximálními dosaženými obsahy mikrobiální biomasy. Obsahy přístupných frakcí těžkých kovů extrahovaných H_2O , 1 mol/l NH_4NO_3 a 0,005 mol/l DTPA se zpravidla zvyšovaly během prvních dvou dní inkubace. Mezi obsahy přístupných kovů a pH nebo mikrobiální biomasou bylo zjištěno jen několik statisticky významných korelací. Na tuto skutečnost měl pravděpodobně vliv rozdílný průběh obsahů mikrobiální biomasy a pH na jedné straně a dosahované maximální obsahy rizikových prvků na straně druhé, kdy po přidavku glukózy byly nejvyšší obsahy kovů dosaženy ve většině případů až druhý den inkubace. Přídavek přírodního klinoptilolitu v porovnání s variantami bez zeolitu snižoval obsahy těžkých kovů extrahované všemi extraktanty s výjimkou Cd, které mělo při extrakci H_2O tendenci se zvyšovat.

Klíčová slova: inkubační pokus; půda kontaminovaná těžkými kovy; přídavek glukózy a zeolitu; přístupnost Cd, Pb, Zn; pH; mikrobiální biomasa

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