Effects of heavy metal concentrations on biological activity of soil micro-organisms

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ABSTRACT

The distribution of cadmium, lead and zinc in exchangeable, organic, and 2M HNO_3-extractable fractions as well as the effect of heavy metal concentrations on soil microflora was investigated. Six sampling transects were chosen in the Litavka River alluvium in 1999–2001. Concentrations of all metals increased with decreasing distance from the source of contamination. The concentrations of Cd and Zn in exchangeable fraction were higher than in organically bound fraction, a reverse trend was found in Pb speciation. All measured parameters of soil microbial activity were affected by heavy metal concentrations. The decrease in CFU was most significant in the case of oligotrophic bacteria and spore-forming bacteria. Significant inhibition of C-biomass occurred in soils highly contaminated by heavy metals. The $C_{biomass}$:C_{ox} ratio decreased with increasing soil pollution. Generally, the values of enzymatic activities were highest in the soil above the source of contamination and they were decreased as approaching the source of contamination. Our results demonstrate that several parameters of microbial activity could be used as good indicators of increasing concentrations of Cd, Pb, and Zn in soil.

Keywords: heavy metals; soil microflora; microbial and enzymatic activities; CFU

Heavy metals are a dangerous group of soil pollutants. The contamination by heavy metals causes a serious problem because they cannot be naturally degraded like organic pollutants and they accumulate in different parts of the food chain.

Under stress conditions caused by adverse anthropogenic effects such as dissemination of chemical pollutants, the development and biochemical activities of soil micro-organisms undergo several alterations. To prevent negative ecological consequences, microbiologically-related parameters should be involved in the indication of soil quality (Filip 2002). Chemical analyses measure the particular amounts of contaminants but they do not reflect the environmental consequences resulting from their mobility, food-chain input and mainly from their influence on key processes of soil metabolism. Biological methods can measure the actual impact of contaminants on soil organisms, they show the growth and activity inhibition under stress conditions. Therefore a set of effective, cheap and easily interpretable biological methods should be found.

There are several locations strongly polluted by heavy metals in the Czech Republic. The Litavka River alluvium was chosen on the basis of previous research by Borůvka (1997). He found heavy soil contamination mainly by cadmium, lead and zinc in this area. Repeated floods have been the main source of soil contamination; they have spread water from broken sedimentation basins of a lead-processing factory.

The objectives of this paper were to test various soil microbial parameters as indicators of Cd, Pb, and Zn contents as well as of exchangeable and organically bound fractions of these metals.

MATERIAL AND METHODS

Based on previous research by Borůvka in 1993 (Borůvka 1997), six sampling transects were chosen in the Litavka River alluvium in the years 1999–2001 (spring and autumn sampling): (i) control soil above the source of contamination; transect no. 20, (ii) soil taken just below the source; transect no. 16, (iii) transects no. 1, 4, 8, 12 downstream at the distance of 17.8, 10.7, 6.7, and 5.0 km, respectively, from the source of flood pollution. Soil was classified as Fluvisol. The basic chemical parameters of soils and vegetation cover are given in Table 1. For detailed transect and soil description see Borůvka (1997). Samples were taken from the depth 0–20 cm and passed through a 2mm-sieve (for chemical analyses after air drying). Analyses were performed in three replications and average values are presented. Statgraphics Plus for Windows 4.1 was used for statistical evaluation.

Heavy metals were extracted with 2M HNO_3 (soil to 2M HNO_3 ratio was 1:10). The amounts of heavy metals extracted with 2M HNO_3 represent maximum contents of potentially available metals for plants (potentially available fraction). Water-extractable + exchangeable fraction was

Table 1. Basic soil characteristics and type of vegetation on the sampling transects

Transect No.*	Contamination level	Vegetation	pH_{H_2O}	pH_{KCl}	$Q_{4/6}$	C_{ox} (%)
20	control background	grassland	5.71	4.96	5.99	3.07
1	very low	grassland	6.23	5.98	5.75	2.90
4	low	grassland	5.24	4.59	5.50	1.55
8	high	grassland	6.01	6.04	7.07	2.90
12	moderate	grassland	5.83	5.36	6.96	4.68
16	source of contamination	grassland	6.51	6.53	6.37	1.65

* the numbers correspond to Borůvka (1997)

extracted with 0.1M $Ca(NO_3)_2$ (Brümmer et al. 1986) and the residue was used for the extraction of organically bound fraction with 0.05M $Na_4P_2O_7$ (Pospíšil 1981). Supernatants were used for measuring metal concentrations with atomic absorption spectrophotometer Spectr AA200 in acetylene-air flame; Varian software was used. pH_{H_2O} of soil was measured in water suspension (w/v 1:2), pH_{KCl} in soil suspension with 1M KCl (w/v 1:2.5). Humus content was estimated by modified Tjurin's method (Valla et al. 2000), where the end of titration is indicated potentiometrically. Ratios $Q_{4/6}$ were determined to estimate humus quality.

Colony forming units (CFU) of all bacteria, spore-forming bacteria, oligotrophic bacteria and micromycetes were determined by a plate dilution technique on Thornton agar, meat peptone agar, 100-fold diluted meat peptone agar and Martin agar, respectively (Angerer et al. 1998). Microbial biomass was measured using an extraction method of chloroform fumigation (Vance et al. 1987). Potential respiration was measured as CO_2 production after 20-hour incubation (27°C) after addition of glucose (concentration 100 mg C/ml) and ammonium sulphate (concentration 100 mg N/ml) to 50 g of soil.

The activity of various enzymes was estimated measuring the rate of product formation as follows: (i) dehydrogenase: formazane formation from triphenyltetrazolium-chloride after 24-hour incubation at 37°C (Thalmann 1968), (ii) amylase: maltose formation from water-soluble starch after 24-hour incubation at 37°C (Scherbakova 1968), (iii) invertase: glucose formation from saccharose after 4-hour incubation at 37°C (Scherbakova 1968), (iv) phosphatase: p-nitrophenol formation from Na-p-nitrophenyl-phosphate after 1 hour at 37°C (Tabatabai and Bremner 1969), (v) arylsulphatase: nitrophenol formation from K-p-nitrophenyl-sulphate after one-hour incubation at 37°C (Tabatabai and Bremner 1969). Phosphatase, amylase and invertase activity was measured only after the last sampling (autumn 2001), arylsulphatase in the spring and autumn 2000.

RESULTS

Generally, the concentrations of all metals increased with decreasing distance from the source of contamination. The amounts of contaminants exceeded the critical values for agricultural land in the Czech Republic in all cases except the control transect (Ministry of Environment 1994, Table 2).

The concentrations of exchangeable fractions of cadmium and zinc were higher than in organically bound fractions (Figure 1). A different trend was found in lead speciation. The concentration of organically bound fraction was higher than the concentration of exchangeable fraction. In less contaminated soils the exchangeable fraction concentrations increased almost to the same level as the potentially available fraction in some cases, in highly-polluted samples the ratio of exchangeable fraction decreased (Table 3).

As we supposed, there was a significant negative correlation between pH_{KCl} and exchangeable fraction of cad-

Table 2. Average values of the concentration of potentially available heavy metal fraction (mg/kg) on the transects and critical values (1, 70, 100 mg/kg, respectively) for agricultural land in the Czech Republic

Transect No.	Contamination level	$Cd-HNO_3$	$Pb-HNO_3$	$Zn-HNO_3$
		1	70	100
20	control background	0.98	101.2	51.2
1	very low	1.53	140.3	90.6
4	low	4.13	417.5	211.6
8	high	98.26	7 348.9	10 418.9
12	moderate	57.39	3 320.8	5 350.4
16	source of contamination	77.77	9 879.7	11 179.2

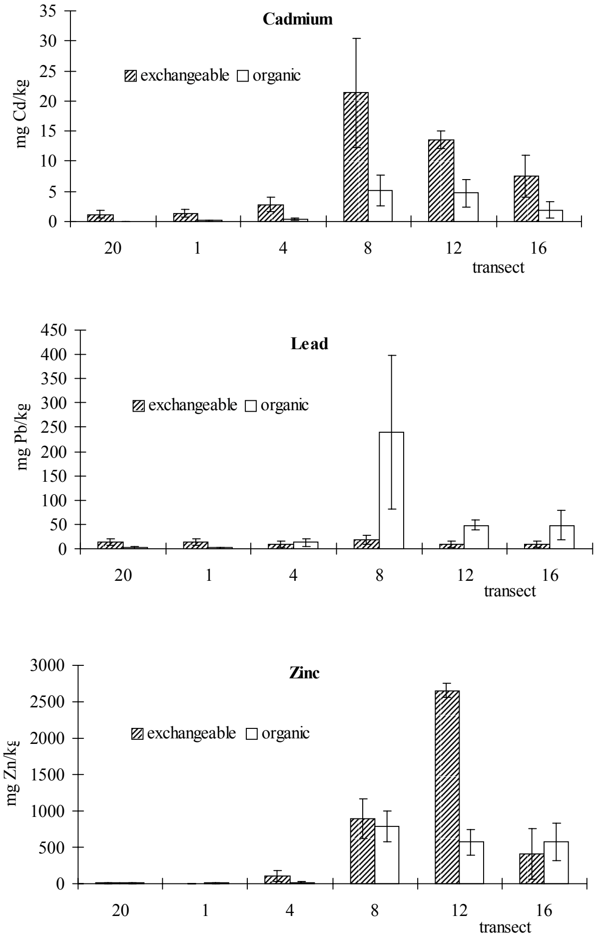

Figure 1. Average values of exchangeable fraction and organically bound fraction on different transects

mium ($P < 0.05$, correlation coefficient = –0.4461) and zinc ($P < 0.001$, correlation coefficient = –0.8811) because of the high mobility of these heavy metals in acid soils. A significant correlation ($P < 0.05$, correlation coefficient = –0.5580) between organic fraction of Cd and organic carbon content (C_{ox}) was found.

All measured parameters of soil microbial activity were affected by heavy metal concentrations. CFU of total bacteria, spore-forming and oligotrophic bacteria and micromycetes decreased with increasing heavy metal concentrations, but the decrease in CFU was most significant in the case of oligotrophic bacteria and spore-forming bacteria (Table 4).

Significant inhibition of C-biomass occurred in soils highly contaminated by heavy metals (Figure 2). Potential respiration was higher in less contaminated soils than in soils near the source of contamination (Figure 2), but the difference was not significant (Table 5). The $C_{biomass}$:C_{ox} ratio decreased with increasing soil pollution (Figure 2). Both dehydrogenase and arylsulphatase activity showed a similar trend like the other microbial parameters. The activities decreased with an increase in heavy metal concentrations (Figure 3, Table 5). Similar results were obtained for the other enzymatic activities (Figure 3). Except for the phosphatase activity, the highest values were found in soil above the source of contamination and in samples from transects far from this source; the values decreased as approaching the source.

DISCUSSION

Extractions of potentially available fraction were done to compare the results with limits for heavy metals in soils of the Czech Republic (Ministry of Environment 1994). The amounts of heavy metals in potentially available fraction represent maximum contents of potentially available metals for plants. The real plant uptake, however, is usually markedly lower (Valla et al. 2000). A decreasing distance from the source of contamination caused an increase in potentially available concentrations of all metals, except for transect no. 8. The concentration of heavy metals in this transect can be explained by higher pH (thus lower mobility) compared to the other transects and also by the long-term influence of flooding water on this transect due to the wide alluvium and very small slope on this transect.

The water-extractable + exchangeable and organically bound fractions are considered as the most dangerous fractions of Cd, Pb, and Zn in soils in terms of the food-chain input (Borůvka and Drábek 2001). In the case of Cd and Zn, in less contaminated soils the exchangeable frac-

Table 3. Comparison of exchangeable:potentially available ratio (e:p) and organic:potentially available ratio (o:p) between transects and between elements

Transect No.	Cadmium		Lead		Zinc	
	e:p	o:p	e:p	o:p	e:p	o:p
20	1.18	< 0.06	0.14	0.02	0.17	0.18
1	0.88	0.07	0.09	0.01	0.03	0.11
4	0.68	0.08	0.02	0.03	0.52	0.11
8	0.22	0.05	0.003	0.02	0.09	0.08
12	0.24	0.08	0.003	0.005	0.50	0.11
16	0.10	0.02	0.001	0.02	0.04	0.05

Table 4. Average CFU values (per g dry soil) of counted groups of micro-organisms (n = 30)

Transect No.	Contamination level	Total counts of bacteria		Spore-forming bacteria		Oligotrophic bacteria		Micro-mycetes	
		CFU/g dry soil							
20	control background	3.3×10^6	ab	12.0×10^5	c	19.0×10^6	b	1.2×10^5	ab
1	very low	3.9×10^6	b	7.1×10^5	bc	24.0×10^6	b	1.5×10^5	b
4	low	2.5×10^6	ab	1.9×10^5	ab	8.4×10^6	a	1.3×10^5	ab
8	high	1.3×10^6	a	0.3×10^5	a	3.4×10^6	a	0.5×10^5	ab
12	moderate	2.0×10^6	ab	2.1×10^5	ab	4.8×10^6	a	0.7×10^5	ab
16	source of contamination	1.4×10^6	a	0.2×10^5	a	3.1×10^6	a	0.2×10^5	a

a, b, c – different letters in one column show statistically significant differences between transects (P < 0.05)

tion contents increased markedly indicating high Cd and Zn mobility in soils. Filipek et al. (2001) also reported the high Cd mobility, more than 50% of Cd was extracted in mobile fraction. High concentration of zinc together with low pH can explain the high amount of zinc in exchangeable fraction at transect no. 12. Impellitteri and Allen (2001) suggested that pH is the most important parameter controlling distribution of heavy metals in soils. The

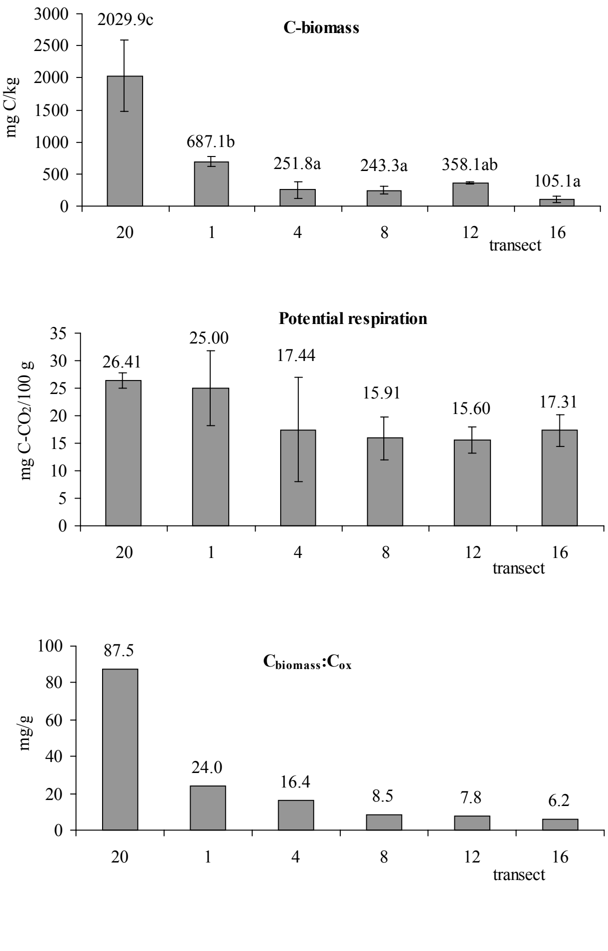

Figure 2. Average values of C-biomass, potential respiration and $C_{biomass}$:C_{ox} ratio
a, b, c – different letters show statistically significant differences between transects (P < 0.05)

pattern of lead speciation was opposite to Cd and Zn. It can be caused by high Pb affinity to complexation with insoluble humic substances; thus its fixation and immobilisation in humic horizons (Xian 1987, Angehrn-Bettinazzi et al. 1989). Low amounts of exchangeable lead in soil samples confirmed low Pb mobility in soils in general. A higher amount of exchangeable lead in control soil than in contaminated soils (except soil no. 8) was found (Figure 1), which can indicate the effect of low pH_{KCl} on this transect.

Soil pollution causes a pressure on sensitive micro-organisms and so changes the diversity of soil microflora, representation of trophic groups of micro-organisms (Zaguralskaya 1997). The decrease in microbial density caused by a high level of heavy metal contamination found at the sites we examined is in agreement with Kikovic (1997). The influence of heavy metals on total CFU of bacteria was not as strong as the influence on specific trophic groups (Table 3). Total CFU represents the whole community of bacteria and also involves those components that are more resistant to the heavy metal pollution. Likely, these resistant bacteria form a larger part of total CFU in contaminated soil compared to control transect. The oligotrophic bacteria show the highest sensitivity to heavy metal pollution indicating that the limitation of bacterial community is more pronounced in soils poor in organic matter and nutrient content.

Microbial biomass is a sensitive parameter and can be used as an indicator of changes in organic matter composition earlier than it could be registered in another way (Brookes 1995). The inhibition of C-biomass in soils highly contaminated by heavy metals supports the data of Brookes and McGrath (1984) that show only a half content of microbial biomass in soil contaminated by heavy metals compared to uncontaminated soils. Dias et al. (1998) observed an inhibition of C-biomass by heavy metals even higher than 80%. The synthesis of microbial biomass in soils polluted by heavy metals can be less effective than in non-polluted soils due to the stress caused by heavy metals. Micro-organisms in less polluted soils use a higher amount of consumed carbon for assimilation and a smaller part is released as CO_2 in dissimilation processes. In contaminated soils, micro-organ-

Table 5. Correlation coefficients between microbial characteristics and heavy metal content in soil (n = 30, arylsulphatase n = 12)

	C-biomass	Potential respiration	Total number of bacteria	Spore-forming bacteria	Oligotrophic bacteria	Micro-mycetes	Dehydrogenase activity	Arylsulphatase activity
Cd	–0.589***	–0.381	–0.514**	–0.530**	–0.625***	–0.462*	–0.727***	–0.634*
Pb	–0.735***	–0.353	–0.500**	–0.517**	–0.584***	–0.495**	–0.682***	–0.606*
Zn	–0.683***	–0.349	–0.511**	–0.528**	–0.606***	–0.494**	–0.712***	–0.625*

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

isms need more energy to survive in unfavourable conditions. Therefore, a higher portion of consumed carbon is released as CO_2 and a smaller part is built into organic components. This assumption is supported by the lower $C_{biomass}$:C_{ox} ratio in contaminated soils (Figure 2).

Enzymatic activities can sensitively reflect the biological situation in the soil (Šíša 1993). There are several reasons why enzymatic analyses could be a good indicator of soil quality (Dick et al. 1996): (i) they are strongly connected with important soil characteristics such as organic matter, physical properties, microbial activity or biomass; (ii) they change earlier than other characteristics; (iii) they involve relatively simple methods compared to other important parameters of soil quality. Our results show significant inhibition of enzymatic activities by a high level of soil contamination. It is considered that heavy metals mainly inhibit enzymatic reactions through either their complexing with substrate or blocking the functional groups of enzymes or reacting with complex enzyme-substrate (Speir et al. 1995).

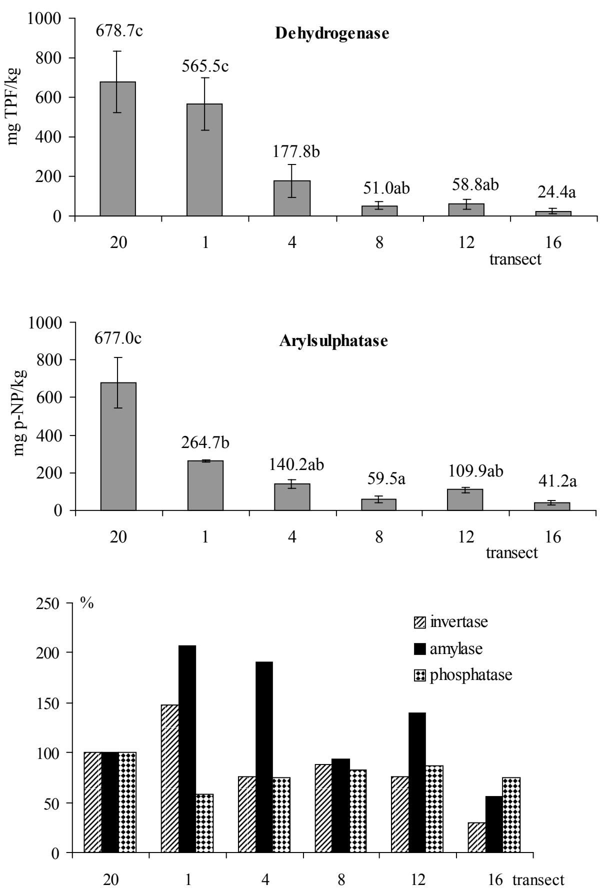

Figure 3. Average values of measured enzymatic activities
a, b, c – different letters show statistically significant differences between transects (P < 0.05)
To show all activities in one plot, their values were converted to percents; 100% are the values of background control soil

In conclusion, different microbial parameters were found as good indicators of the level of soil contamination by heavy metals. Specific groups of (spore-forming, oligotrophic) micro-organisms should be chosen instead of total counts of bacteria. Other characteristics that react to increasing concentrations of heavy metals are C-biomass and $C_{biomass}$:C_{ox} ratio. Increasing amounts of heavy metals inhibit enzymatic activities; especially dehydrogenase activity seems to be a sensitive indicator of soil pollution by heavy metals. Our results are in agreement with Tesařová (2000) and Kubát et al. (1999), who also considered the activity of dehydrogenase, microbial biomass, soil respiration, counts of N-fixing bacteria, etc. as sensitive bio-indicators of soil quality.

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ABSTRAKT

Vliv koncentrace rizikových prvků na biologickou aktivitu půdních mikroorganismů

Byla sledována speciace kadmia, olova a zinku a vliv koncentrace těchto prvků na půdní mikroflóru v půdách šesti odběrových míst v povodí řeky Litavky. Největší množství všech prvků byla nalezena na lokalitách nejblíže zdroji znečištění. Vyšší koncentrace Cd a Zn byly stanoveny ve výměnné frakci, naopak Pb bylo více vázáno na organické látky. Všechny zkoumané parametry mikrobiální aktivity byly ovlivněny koncentrací rizikových prvků v půdě, jejich hodnoty se snižovaly s rostoucími koncentracemi Cd, Pb a Zn. Počet KTJ byl nejvýznamněji snížen v případě sporulujících a oligotrofních bakterií. Byla zaznamenána významná inhibice mikrobiální biomasy zvyšujícími se koncentracemi rizikových prvků. Poměr $C_{biomasa}$: C_{ox} rovněž klesal s rostoucí kontaminací. Výsledky ukazují, že některé vybrané parametry struktury a aktivity mikrobiálních společenstev by mohly být použity jako vhodný ukazatel úrovně znečištění Cd, Pb a Zn.

Klíčová slova: rizikové prvky; půdní mikroflóra; mikrobiální a enzymatická aktivita; počet bakterií

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