The Influence of Heavy Metals on Soil Biological and Chemical Properties

Michaela FRIEDLOVÁ

Crop Research Institute, Prague-Ruzyně, Czech Republic

Abstract: Soil samples were collected at alluvial sites of the Litavka River, which flows through the Beroun and Příbram cities in Central Bohemia Region of the Czech Republic in 2005 and 2006. Higher heavy metal content in soils (Cd, Pb, Zn, Cu) is due to composition of the parent rock, emissions from lead processing industry and the leak of toxic material from the steel works sludge ponds in the 1970s and 1980s. The samples were collected from six sites located at different distances from the contamination source (the former sludge ponds) and chemical and biological properties were determined. The ratio of the microbial biomass carbon to oxidisable carbon content dropped down significantly on more heavily contaminated sites. Basal respiration activity did not correlate with the content of heavy metals in soil, but there was certain declining tendency with increasing intensity of soil contamination. Respiration activities significantly correlated with the total carbon, oxidisable carbon and the total nitrogen content. The metabolic quotient showed higher values with increasing contamination. Dehydrogenases and arylsulphatase activities decreased with increasing contamination. Urease activity has also a declining tendency but its relation to different intensity of contamination was not unambiguous. Urease activity has shown a relationship with the content of total nitrogen in soil. No relationship was found between the total sulphur content and arylsulphatase activity. Dehydrogenases, arylsulfatase and urease activities significantly correlated with the microbial biomass carbon.

Keywords: biological activities; carbon; enzymatic activities; heavy metals; metabolic quotient; nitrogen; respiration activity; soil; sulphur

The contamination of soils by heavy metals is significant problem, which leads to negative influence on soil characteristics and limitation of productive and environmental functions. The soil microbial community has a fundamental role in the process of organic matter degradation and mineralization, which allows the recycling of nutrients (Castaldi *et al.* 2004). Heavy metals affect the number, diversity and microbial activity of soil microorganisms. They can cause slow down speed of growth and reproduction of microorganisms, in the soil then prevail slower growing microorganisms with lower diversity and higher resistance to heavy metals, but decreased biological activity (Šimon 1999). Concern about heavy metals in soil derives not only for their toxicity to living organisms inhabiting soil but also for their immobilization within different organic and inorganic colloids, in the immobilized form they can persist for long time before being again available to living organisms including plants (Nannipieri *et al.* 1997).

Monitoring methods characterizing microbiological and biochemical soil properties are successfully used to evaluate the intensity of the soil contamination. They are more sensitive and their reaction to soil contamination is faster in comparison with the monitoring of the chemical and physical properties of soil which are manifested after a long time (months to years) (Nannipieri *et al.* 1997). They reflect the real impact of stress

Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE 0002700604.

conditions caused by contamination on growth and activity of soil microflora.

The toxicity of heavy metals for the soil microflora depends on the pH, temperature, inorganic anions and cations, clay minerals, hydrous metal oxides, organic matter form and amount, chemical forms in which metals occurs, etc. (Bååth 1989; Giller *et al.* 1998). Hence, there are differences between many studies. Some of them confirm the negative influence of heavy metals on the soil microbiological activities, the others show that there is no evidence of correlation between microbiological soil properties and increasing heavy metal pollution (Castaldi *et al.* 2004).

The differences between studies are also caused by the fact that some of them work with the artificial contamination of soil prepared in laboratory, the others use the soil from the real contaminated areas. Each method has its pros and cons. However the microbiological characteristics bring the important information about the effect of pollutants on the soil ecosystem and it is necessary to take into account that single microbial characteristic can not be used universally for monitoring of the soil pollution (Brookes 1995). Therefore it is necessary to use wider spectrum of methods to assess the soil microbiological activities. The most frequently used methods are based on the monitoring of decomposition processes (respiration, C and N mineralization), synthetic processes (microbial biomass) and enzymatic activities.

The goal of this work was to evaluate the effect of heavy metal soil contamination on soil microorganisms using different methods for evaluation of the biological activities. For this purpose, the area along the Litavka River which flows through the Beroun and Příbram districts of the Central Bohemian Region of the Czech Republic was selected.

Table 1. Extent of contamination by heavy metals in sites

Site	Extent of contamination	Downstream distance from the contamination source (km)
1	no contamination	–
2	low	17.8
3	moderate	10.7
4	high	6.7
5	medium	5.0
6	high	0 (contamination source)

The Litavka River is characterised by significant instability and frequent heavy rain falls and floods. The predominant soil types are carbonate-free fluvisols with high humus content and lightly acidic to acidic pH.

The site has higher heavy metal content for several reasons. One of the reasons is the composition of the parent rock itself. The significance of this contamination type was confirmed by analysis of the soil layer 50–60 cm, where was assessed high lead content however the lead mobility in the soil is very low (Borůvka *et al.* 1996).

The second reason is anthropogenic contamination of a dual character. The first ones are the emissions from lead processing industry which have been present in the region since 1786. The second ones are the steel works sludge ponds whose dams broke in the 1970s and 1980s spilling their content into the Litavka River. The leak of toxic material and the subsequent frequent and very intense flooding caused an extremely high content of cadmium, lead and zinc in the alluvial soils.

MATERIAL AND METHODS

Soil samples were collected at alluvial sites of the Litavka River in the autumn of 2005 and in the spring and autumn of 2006 from six sites located at different distances from the contamination source (the former sludge ponds) (Table 1), all in direct proximity to the Litavka River.

Samples from soils with grass cover were taken from a layer ranging from 0–20 cm. After homogenisation and removal of unwanted content (stones, plant material, etc.) soil was sifted through a 2 mm sieve. For analysis requiring dry soil, samples were dried at laboratory temperature and ground using a Retsch RM 100 mortar grinder mill.

Heavy metals were determined in 2M HNO_3 (1:10) extract using a Varian SpectrAA 200 atomic absorption spectral photometer (Varian, Mulgrave, Australia). The content of total carbon (C_{tot}), nitrogen (N_{tot}) and sulphur (S_{tot}) in the soil was measured using a Vario Max analyser. Soil pH (H_2O) was measured in water suspension (w/v 1:2) and soil pH (KCl) in suspension with 1M KCl (w/v 1:2.5). Microbial biomass carbon ($C_{biomass}$) was determined by the fumigation-extraction method (Vance *et al.* 1987) and oxidisable carbon (C_{ox}) by incinerating

in potassium dichromate and subsequent iodometric titration (Alten *et al.* 1935). Basal respiration activity was measured by soil incubation in closed containers with the 1M NaOH pattern (testing periods 1–3 days and 4–7 days, incubation temperature 28°C). The content of CO_2 was analysed by titration with 0.25M HCl. The activities of dehydrogenases (Thalmann 1968), arylsulphatase (Tabatabai & Bremner 1970), and urease (Kandeler & Gerber 1988) were determined.

Statistic evaluation was performed by Statistica 7.1 software from Statsoft and MS Excel 2002. The analysis of variance was deeper evaluated using Tukey's test.

RESULTS AND DISCUSSION

The current state of contamination at the site is described in Table 2.

Basic chemical characteristics of the soils in the alluvial flood plane sites of the Litavka River are shown in Table 3. The soils are lightly acidic to acidic with high content of oxidisable carbon. This corresponds to the characteristic of sample areas with grass cover. No correlation between C_{ox} and content of heavy metals in soil was confirmed. High content of C_{ox} probably does not reflect the heavy metal soil contamination, which is characterised by the accumulation of soil organic matter and declining intensity of organic material transformation (Leita *et al.* 1999). Similar results were found for total carbon and nitrogen.

It was determined that the $C_{biomass}$ to C_{ox} ratio on more heavily contaminated sites drops significantly (Figure 1). Similar results were described by Nannipieri *et al.* (1997) and Šmejkalová *et al.* (2003), who identically described the significant declination of the $C_{biomass}$ to C_{ox} ratio with increasing level of contamination.

Table 2. Average content of heavy metals in soils (mg/kg)

Site	Extent of contamination	Cd	Pb	Zn	Cu
1	no contamination	1.41^{a}	81^{a}	40^{a}	10.37^{a}
2	low	4.82^{a}	341^{a}	362^{a}	15.21^{a}
3	moderate	9.36^{ab}	821^{a}	663^{a}	17.44^{a}
4	high	63.61^{d}	5 286^{b}	9 288^{c}	62.85^{b}
5	medium	28.46^{bc}	2 905^{ab}	3 383^{b}	89.20^{b}
6	high	34.76^{c}	4 490^{b}	3 558^{b}	101.44^{b}
Maximum admissible limit		0.4/1.0	50/70	50/100	30/50

Letters in one of the columns show a significant difference between individual sites in the level of significance 0.05
Maximum admissible limits of heavy metal content in soil determined in 2M HNO_3, the Czech legislation (Ministry of Environment Decree No. 13/1994 Coll.), the first numerical information is valid for light soils, the second one for other types of soil

Table 3. Basic soil chemical characteristics

Site	pH (H_2O)	pH (KCl)	C_{ox} (%)	C_{tot} (%)	N_{tot} (%)	S_{tot} (ppm)
1	5.52^{ab}	4.91^{a}	6.67^{b}	7.187^{b}	0.635^{b}	912^{ab}
2	5.96^{c}	5.89^{bc}	2.97^{a}	3.384^{ab}	0.302^{ab}	464^{a}
3	5.42^{a}	4.68^{a}	2.78^{a}	3.192^{ab}	0.266^{a}	431^{a}
4	5.91^{c}	6.30^{b}	2.97^{a}	3.680^{ab}	0.254^{a}	957^{ab}
5	5.87^{bc}	5.96^{bc}	1.97^{a}	2.162^{a}	0.136^{a}	972^{ab}
6	5.84^{bc}	5.65^{c}	4.69^{ab}	4.765^{ab}	0.255^{a}	1 395^{b}

Letters in one of the columns show a significant difference between individual sites in the level of significance 0.05

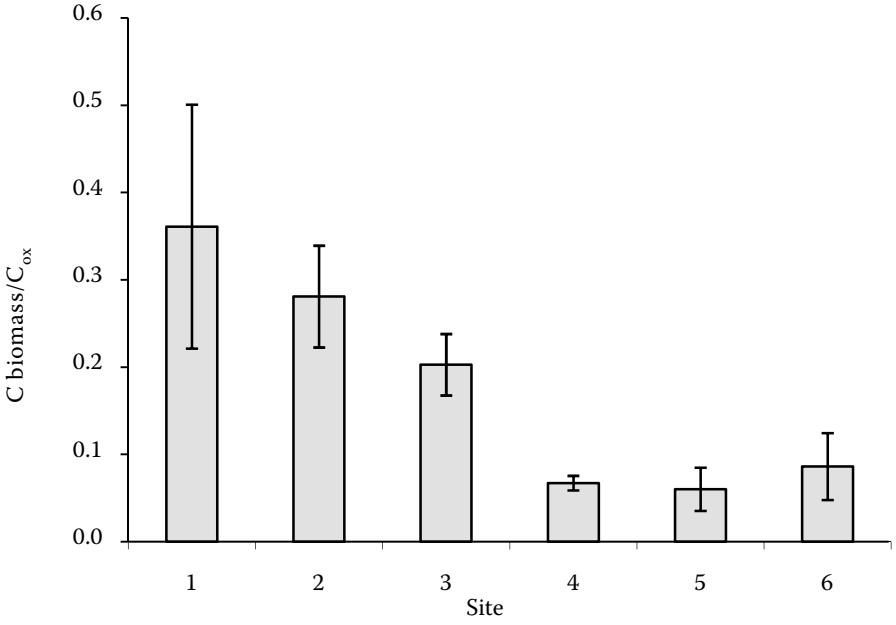

Figure 1. The ratio of microbial biomass carbon to oxidisable carbon

Respiration activity is a characteristic of the process of mineralization of organic matter in soil and of other metabolic processes in which CO_2 is released (Šantrůčková 1993). In our case the basal respiration activity does not correlate with the content of heavy metals in soil. However, it is possible to see a certain declining tendency with increasing intensity of soil contamination (Figure 2). Many studies differ in conclusions how soil contamination by heavy metals influences respiration activity. Tobor-Kapłon *et al.* (2005) stated that under stress, more resistant organisms respond by an increased respiration activity as oxygen consumption increases with ongoing decontamination processes, while more sensitive organisms are characterised by reduced respiration. Giller *et al.* (1998) attributed the different results of individual studies to different properties of available substrate in soil which is mineralised at the time when respiration activity is measured. The effect of heavy metals on respiration activity also strongly affects the content of clay minerals, organic matter and other factors influencing cation exchange capacity of soil. Respiration activity is a non-specific microbial process which in case of decrease does not indicate selective suppression

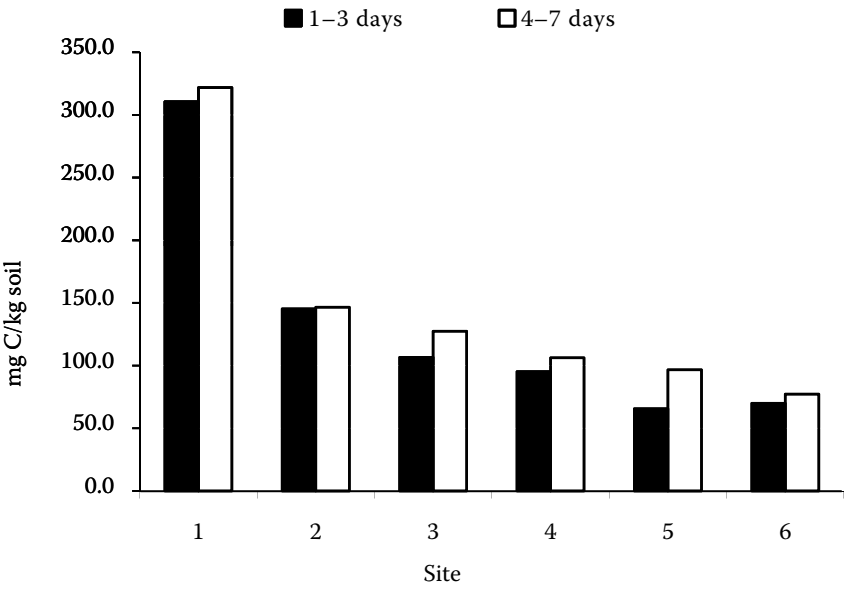

Figure 2. Basal respiration activity – autumn 2006

Table 4. Correlation coefficients for basal respiration activities

Respiration activity (days)	C_{tot}	C_{ox}	N_{tot}
Autumn 2005			
1–3	0.95*	0.87*	0.97*
4–7	0.96*	0.88*	0.97*
Spring 2006			
1–3	0.97*	0.99*	0.88*
4–7	0.97*	0.99*	0.89*
Autumn 2006			
1–3	0.86*	0.92*	0.97*
4–7	0.84*	0.90*	0.96*

*$P < 0.05$

of a certain soil process or a group of soil microorganisms (Nannipieri *et al.* 1997). In our case respiration activities significantly correlated with the total carbon, oxidizable carbon and the total nitrogen content (Table 4).

Respiration activity is better evaluated in relation to the quantity of microbial biomass. The relationship between respiration activity and microbial biomass is described by the so-called metabolic quotient (q CO_2).

Brookes (1995) identifies the metabolic quotient as the good indicator of negative impact of the heavy metal pollution on the soil microflora and this idea is documented by the case, where the basal respiration activity did not differ significantly between contaminated and non-contaminated soils, but the metabolic quotient was two times higher in contaminated soils. Also Giller *et al.* (1998) described a case when basal respiration activity was not affected by heavy metals but microbial biomass dropped. The results in Table 5 show that the higher the extent of contamination, the higher the metabolic quotient. Microbial population needs more energy for its own existence which is generated by increased respiration activity and lower incorporation of mineralised substrate into the organic matter of its own biomass (Nannipieri *et al.* 1997). High values of the metabolic quotient are characteristic of soils contaminated by heavy metals for a long time (Dahlin *et al.* 1997).

Although enzymatic activities are substrate specific and do not reflect the overall metabolic activity of soil microorganisms (Nannipieri *et al.* 1997), they are considered to be sensitive indicators of biological activity in soil (Mikanová 2006).

Our study has focused on monitoring the activity of dehydrogenases (intracellular enzymes involved in microbial metabolism of oxygen), arylsulphatase

Table 5. Metabolic quotient q CO_2 (mg CO_2 – C/mg $C_{biomass}$/h/10^4)

Site	Extent of contamination	Autumn 2005		Spring 2006		Autumn 2006	
		q CO_2	%	q CO_2	%	q CO_2	%
1	no contamination	15^{ab}	100	13^{a}	100	26^{ab}	100
2	low	21^{ab}	142	21^{ab}	162	29^{ab}	111
3	moderate	35^{abc}	235	25^{ab}	189	27^{ab}	104
4	high	63^{cd}	419	73^{d}	557	68^{d}	261
5	medium	89^{e}	594	67^{cd}	508	132^{f}	505
6	high	28^{ab}	188	47^{bcd}	356	62^{cd}	238

Letters in one of the columns show a significant difference between individual sites in the level of significance 0.05

Table 6. Soil enzymatic activities

Site	Dehydrogenases activity			Arylsulphatase activity		
	μg TPF/g/h			μg pNP/g/h		
	autumn 05	spring 06	autumn 06	autumn 05	spring 06	autumn 06
1	1 296^{h}	967^{g}	455^{d}	669^{h}	265^{f}	307^{g}
2	788^{f}	576^{e}	638^{e}	201^{e}	165de	147^{d}
3	314^{bc}	406^{cd}	423^{d}	85^{c}	75^{c}	73^{c}
4	68^{a}	103^{a}	106^{a}	50^{abc}	13^{a}	31^{ab}
5	26^{a}	67^{a}	47^{a}	23^{a}	25^{a}	22^{a}
6	95^{a}	286^{b}	94^{a}	69^{bc}	84^{c}	12^{a}

Letters in one of the columns show a significant difference between individual sites in the level of significance 0.05

(extracellular enzyme mineralising compounds containing sulphur and hydrolysing organic sulphates) and urease (extracellular enzyme hydrolysing C-N bonds in some amids and urea).

In our case, dehydrogenases and arylsulphatase activities decreased with increasing extent of contamination (Table 6). Heavy metals inhibit enzymatic reactions by bonding themselves to substrate, creating complexes with substrate, blocking reactive functional groups of enzymes or reacting with an enzyme-substrate complex (Mikanová 2006). Reduced enzymatic activities may also be caused by a lower content of enzymes in the soil. Heavy metals inhibit their synthesis (Bååth 1989). Urease activity has also a declining tendency but its reaction to different intensity of contamination is not completely unambiguous and there is rather an obvious relationship to the content of total nitrogen in soil (Figure 3). No relation to the content of total sulphur in soil was established in arylsulphatase activity.

Similar results, relating to the above-mentioned enzymatic activities in an environment contaminated by heavy metals, were obtained by Castaldi *et al.* (2004).

In our case all tested enzymatic activities statistically significantly correlated with the microbial biomass carbon (dehydrogenases $r = 0.84$, arylsulphatase $r = 0.99$, urease $r = 0.98$, $P < 0.05$)

The effect of heavy metals on soil microorganisms is not always identical since it depends on many physical and chemical characteristics of soils (quantity of pollutants, soil type, temperature, water content in soil, pH, effect of soil minerals and organic material) (Wood 1995; Nannipieri

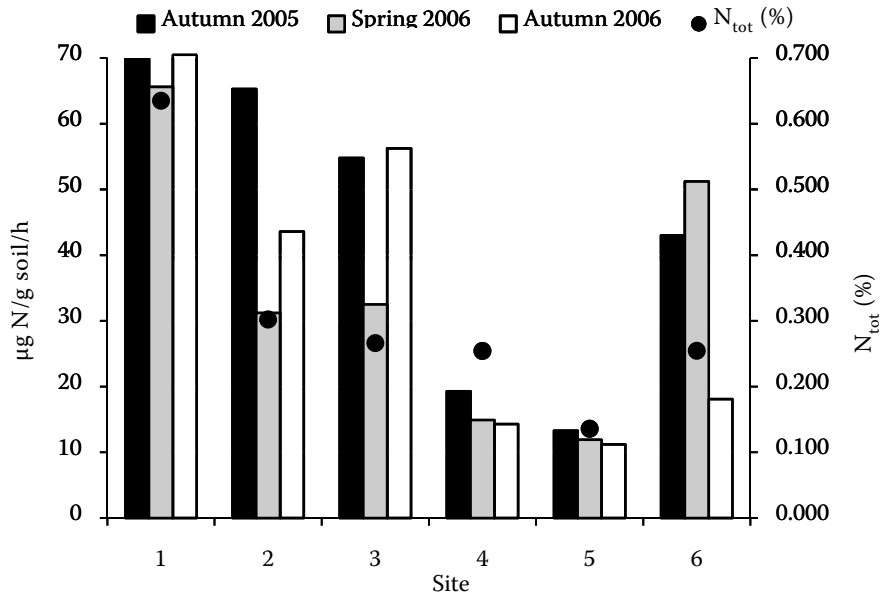

Figure 3. Urease activity and its relationship to the content of total nitrogen in soil

et al. 1997). In spite of that, based on the results of our study we can identify the following efficient indicators of soil degradation by heavy metals: the ratio of microbial biomass carbon to oxidisable carbon, the metabolic quotient and dehydrogenases and arylsulphatase activities.

Acknowledgements. The author is grateful to Prof. L. Borůvka (Czech University of Life Sciences, Prague) for helpful discussions and informations about the experimental sites and Dr. O. Drábek (Czech University of Life Sciences, Prague) for heavy metals analyses of soil samples.

References

Alten F., Wandrowski B., Knippenberg E. (1935): Beitrag zur Humusbestimmung. Ergebniss der Agrikultuchemie **4**: 61–69.

Bååth E. (1989): Effects of heavy metals in soil on microbial processes and populations (a review). Water, Air and Soil Pollution, **47**: 335–379.

Borůvka L., Huan-Wei CH., Kozák J., Krištoufková S. (1996): Heavy contamination of soil with cadmium, lead and zinc in the alluvium of the Litavka river. Rostlinná výroba, **42**: 543–550.

Brookes P.C. (1995): The use of microbiological parameters in monitoring soil pollution by heavy metals. Biology and Fertility of Soils, **19**: 269–279.

Castaldi S., Rutigliano F.A., Virzo De Santo A. (2004): Suitability of soil microbial parameters as indicators of heavy metal pollution. Water, Air and Soil Pollution, **158**: 21–35.

Dahlin S., Witter E., Mĺrtensson A.M., Turner A., Bååth E. (1997): Where's the limit? Changes in the microbial properties of agricultural soils at low levels of metal contamination. Soil Biology and Biochemistry, **29**: 1405–1415.

Giller K.E., Witter E., McGrath S.P. (1998): Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils. Soil Biology and Biochemistry, **30**: 1389–1414.

Kandeler E., Gerber H. (1988): Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils, **6**: 68–72.

Leita L., De Nobili M., Mondini C., Műhlbachová G., Marchiol L., Bragato G., Contin M. (1999): Influence of inorganic and organic fertilization on soil microbial biomass, metabolic quotient and heavy metal bioavailability. Biology and Fertility of Soils, **28**: 371–376

Mikanová O. (2006): Effects of heavy metals on some soil biological parameters. Journal of Geochemical Exploration, **88**: 220–223.

Nannipieri P., Badalucco L., Landi L., Pietramellara G. (1997): Measurement in assessing the risk of chemicals to the soil ecosystem. In: Zelikoff J.T. (ed.): Ecotoxicology: Responses, Biomarkers and Risk Assessment. OECD Workshop. SOS Publ. Fair Haven, New York, 507–534.

Šantrůčková H. (1993): Soil respiration as a measure of soil biological activity. Rostlinná výroba, **39**: 769–778. (in Czech)

Šimon T. (1999): The effect of increasing rates of nickel and arsenic on the growth of radish and soil microflora. Rostlinná výroba, **45**: 421–430. (in Czech)

Šmejkalová M., Mikanová O., Borůvka L. (2003): Effects of heavy metal concentrations on biological activity of soils microorganisms. Plant, Soil and Environment, **49**: 321–326.

Tabatabai M.A., Bremner J.B. (1970): Arylsulphatase activity of soils. Soil Science Society of America Proceedings, **34**: 225–229.

Thalmann A. (1968): Zur Metodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC). Landwirtschliche Forschung, **21**: 249–258.

Tobor-Kapłon M.A., Bloem J., Rőmkens P. F. A.M., Ruiter P. C. (2005): Functional stability of microbial communities in contaminated soils. Oikos, **111**: 119–129.

Vance E.D., Brookes P.C., Jenkinson D.S. (1987): An extraction method for measuring soil microbial biomass. Soil Biology and Biochemistry, **19**: 703-707.

Wood M. (1995): Environmental Soil Biology. The University Press, Cambridge, 1–150.

Received for publication May 8, 2009
Accepted after corrections November 6, 2009

Corresponding author:

Ing. Michaela Friedlová, Výzkumný ústav rostlinné výroby, v.v.i., Drnovská 507, 161 06, Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 284, e-mail: friedlova@vurv.cz