Microbial biomass dynamics after addition of EDTA into heavy metal contaminated soils

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ABSTRACT

An incubation experiment with addition of EDTA and alfalfa into soils contaminated with heavy metal over 200 years was carried out in order to evaluate the EDTA effects on microbial properties. Alfalfa was added to soils together with EDTA to examine its abilities to improve microbial activities affected by EDTA. The obtained results showed that the addition of EDTA led to a significant decrease of microbial biomass C during the first 24 days of incubation. At the end of the experiment the microbial biomass C significantly increased quite close to the original level. The EDTA amendment caused, probably due to the toxic effects, a significant increase in respiratory activities and of the metabolic quotient qCO_2. An addition of alfalfa significantly improved the microbial biomass C contents in arable soils treated together with EDTA. Both, respiratory activities and qCO_2 significantly increased after the soil treatment with EDTA together with alfalfa. EDTA alone decreased the microbial biomass, alfalfa alone as organic substrate was mineralised and utilised by soil microorganisms for their metabolism.

Keywords: heavy metal contamination; soils; EDTA; alfalfa; microbial biomass C; soil respiration; metabolic quotient (qCO_2)

Heavy metal contamination of soils represents a serious problem in industrial areas. Toxic elements accumulate in soils for a long time and are known to enter into the food chain. Remediation techniques that were developed in the past are one of the possibilities to decrease heavy metal concentrations in soils. One of them is based on phytoextraction of undesirable elements from soils by plant uptake. However, concentrations of certain metals (e.g. Pb) in the soil solution and subsequently their bioavailability in relation to the total concentration in soil is very low due to the complexation of metal ions on organic and inorganic colloids, sorption on clay minerals, oxyhydroxides Fe and Mn, precipitation of metals as carbonates, hydroxides or phosphates (Ruby et al. 1996). One way to increase the bioavailability of metals could be an addition of synthetic chelating agents to the soil, which have the potential to remobilise metals and to form soluble complexes (Nowack 2002). EDTA was used to increase the availability of metals for phytoextraction by accumulating plants (McGrath et al. 2006, Turan and Esringü 2007, Safari Sinegani and Khalilikhah 2008). In this context EDTA was also found to be effective in increasing Pb desorption from soils (Komárek et al. 2007).

The contents and activities of the soil microorganisms depend directly or indirectly on the concentrations of compounds present in a soil solution. Higher concentrations of toxic elements could negatively affect the soil microorganisms and their activities. The toxicity of metals depends on their availability for microorganisms. Free metal ions are considered the most toxic, whereas the metals complexed with organic compounds may be less available for soil microbes (Giller et al. 1998). Soil microorganisms can be adapted to heavy metal effects if these toxic elements are present in soils for longer periods of time (Chander and Joergensen 2008). Moreover, the tolerance to the toxic effects is also strongly correlated to the concentration of the metal as found by Ogilvie and Grant (2008) in their study with Cu.

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EDTA effects on the soil microorganisms are usually neglected (Römkens et al. 2002). The reports of the EDTA effects on microbial activities are various. The addition of EDTA can decrease microbial activities and utilization of substrates (Ultra et al. 2005). On the other hand, Chander and Joergensen (2008) showed that the microbial biomass C in long-term contaminated soils tolerated better EDTA amendments than in a low metal soil with zinc. Similarly Sapoundjieva et al. (2003) reported that EDTA application (0.5 and 1.5 mg/kg soil) dramatically increased the solubility of Pb, Cd, Zn and Cu in the soil without any negative effects on soil microorganisms.

In the past, more studies were concentrated on the geochemical situation and the origin of heavy metals in the area of the lead smelter of Příbram (Šichorová et al. 2004). Studies of EDTA addition on phytoextration efficiency were also realized in the same area (Neugschwandtner et al. 2008). Till now, the effects of EDTA on microbial activities have not been studied in this area.

The aim of this research was to evaluate the response of soil microbial activities on EDTA amendment in the area of the lead smelter near Příbram.

MATERIAL AND METHODS

Sampling site. The sampling site is situated in vicinity of a lead smelter in Příbram (Czech Republic) operating since 1786. Metal mining was ceased in 1972, but a secondary lead smelter is still in operation. Since 1982, a 98% efficient dust separator and a 160 m stack have been in use (Riuwerts and Farago 1996). At present, the production of emissions is lower than 1 t per year. Soils were sampled from arable and grassland soil with known contamination making 6 samplings in the circle of 2 m from each sampling site. Each two single samplings were randomly mixed in order to give three replications for the arable or the grassland soil. The soil characteristics are shown in Table 1.

Incubation experiment. Seven days before the experiment, three replication of each treatment (1 kg of soil on an oven dry basis) in plastic jars were placed into the 3 litre plastic containers tightly covered with fitting lids and they were conditioned at 28°C. The soils were preincubated at the 40% water holding capacity (WHC) with the jar of 25 ml 1M NaOH to take up the CO_2-evolved and the distilled water at the bottom of container.

At the beginning of the experiment the following experimental design was used: control – no amendment; alfalfa – amendment with alfalfa corresponding to 1000 µg C/g soil; alfalfa + EDTA – amendment with alfalfa corresponding to 1000 µg C/g soil and with 2 g NaEDTA/kg soil; EDTA – amendment with 2 g NaEDTA/kg soil.

The alfalfa straw contained 43% C and 2.5% N and was ground to < 200 µm before the soil treatment. The formula of NaEDTA (Ethylendiaminetetraace tic acid disodium salt) is $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$.

The experimental soils were adjusted to the 50% of its WHC and incubated under above described conditions. The containers were daily aerated to ensure a sufficient oxygen supply. The soils were regularly adjusted to 50% WHC.

The respiratory activity was determined by separate incubation of 100 g of soil in three replicates for each treatment and incubated in 1 l tightly closed plastic containers containing 5 ml 1N NaOH to determine the CO_2-evolved. The microbial biomass content, respiratory activity and pH were determined at days 0, 3, 10, 24 and 38 of the incubation.

Analytical methods. The measurements of the soil microbial biomass C (B_c) were performed using the fumigation-extraction method (F.E.) according to the procedure described by Vance et al. (1987). The microbial biomass C was calculated from the relationship:

$$B_c = 2.64\ E_c$$

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.

Table 1. Basic characteristics of soils used for the incubation experiment

Soil	Soil texture	pH (H_2O)	C_{org} (%)	Mehlich III			Total concentration				
				P	K	Ca	As	Cd	Cu	Pb	Zn
				(mg/kg)							
Arable	loamy	6.24	1.77	33	92	2703	92.3	4.06	35.2	1138	255
Grassland	loamy	5.52	2.55	34	75	2833	80.1	3.56	22.9	1086	199

The CO_2-C evolved was 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 0.25M HCl on Mettler DL 28 automatic titrator. The metabolic quotient (qCO_2) was determined according to Anderson and Domsch (1990); the qCO_2 was calculated as a unit of respired CO_2-C per unit of microbial biomass C per day.

Soil pH was determined by combined glass electrode on pH meter in the solution soil/water (1/2.5 w/v) after 1 h shaking on overhead shaker.

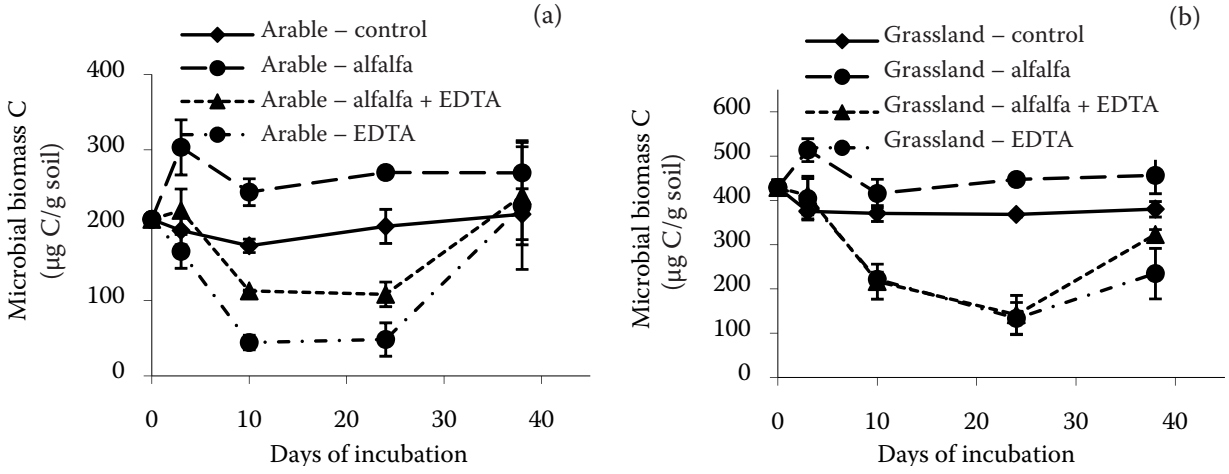
For the total heavy metal concentrations, the soil samples were digested in the microwave Ethos 1 (MLS GmBh, Germany) in a mixture of nitric, hydrochloric and hydrofluoric acid. The soil digests were determined by optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration, Varian, VistaPro, equipped with autosampler SPS-5 (Australia).

All experimental analyses were carried out in triplicate, the mean values and standard deviations were calculated. The statistical significance of differences among the experimental treatments was evaluated by ANOVA Duncan's multiple range test.

RESULTS AND DISCUSSION

Microbial biomass C. The microbial biomass C content in the control arable and grassland soils remained quite stable during the experiment. In comparison with control soils the addition of alfalfa increased significantly the microbial biomass in both soils (Figure 1). During the first 24 days of incubation the amendment of EDTA decreased significantly the microbial biomass in all soils including those with alfalfa. Compared to the grassland soil the microbial biomass C in the arable soil which received alfalfa + EDTA decreased less in the arable soil and alfalfa enhanced significantly the microbial biomass contents. In the period between day 24 and day 40 of incubation, a sharp significant increase of soil microbial biomass was found in all treatments receiving EDTA, however differences were found between arable and grassland soil. The microbial biomass C in the arable soil with EDTA treatments was restored to the level of the control soil. On the other hand, the microbial biomass in the grassland soil was not restored completely to the control level and this difference was significant.

Soil respiration. The respiratory activity differed in dependence on the treatment (Figure 2).



Statistical analysis. Data with the same letter represent statistically identical values according to the ANOVA Duncan's multiple range test ($P < 0.05$)

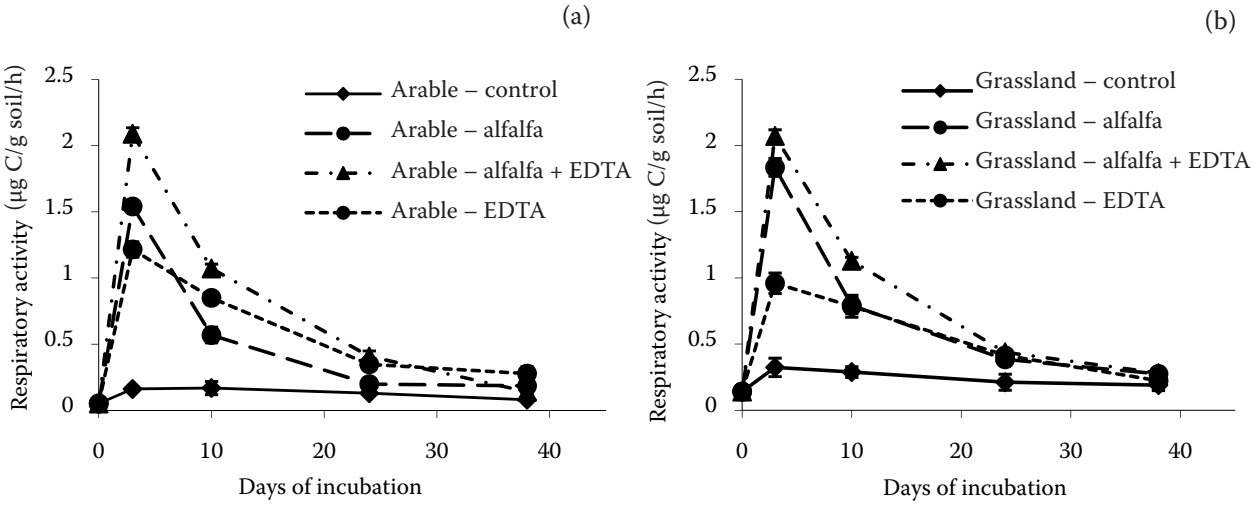
Treatment	Day					Treatment	Day				
	0	3	10	24	38		0	3	10	24	38
Arable – control	cde	cd	c	cd	cde	Grassland – control	ef	de	cd	cd	de
Arable – alfalfa	cde	f	de	ef	ef	Grassland – alfalfa	ef	g	def	f	f
Arable – alfalfa + EDTA	cde	cde	b	b	cde	Grassland – alfalfa + EDTA	ef	def	b	a	c
Arable – EDTA	cde	bc	a	a	cd	Grassland – EDTA	ef	def	b	a	b

Figure 1. Microbial biomass C dynamics in long-term heavy metal contaminated arable (a) and grassland (b) soils during incubation with EDTA and alfalfa. Vertical bars represent the standard deviation

In control arable and grassland soils a very small non-significant increase during the first days of incubation was observed, thereafter a slow decrease followed. The addition of alfalfa together with EDTA produced the greatest and significant increase of respiratory activities during the first three days of incubation in comparison with other experimental treatments; thereafter a significant decrease of these activities was registered. The comparison of arable and grassland soils showed that the effects of treatment with alfalfa + EDTA together on soil respiration were quite similar. The significant differences could be observed especially the third day of incubation between respiratory activities in single alfalfa or EDTA treatments, alfalfa treatments showed significantly higher respiratory activities in comparison with EDTA treated soils. However, the soil respiration decreased more slowly in EDTA treatments. In comparison with the grassland the EDTA amendment caused a higher respiratory activity in the arable soil. On the other hand, simple alfalfa treatment showed the opposite trend and arable soil with alfalfa respired less than the grassland one.

Metabolic quotient (qCO_2). The highest qCO_2 was observed at day 3 in treatments amended with alfalfa + EDTA (Figure 3). The qCO_2 remained significantly higher in the alfalfa + EDTA treatment in the grassland soil during the first 24 days of incubation in comparison with other treatments; thereafter practically identical values were obtained in simple treatment with EDTA and that with alfalfa + EDTA. A different course was observed in the arable soil where, from the 10th day of incubation, the qCO_2 was the highest in the treatment only with EDTA. Values of qCO_2 significantly higher than the control levels were found also in the treatment of the experimental soils with alfalfa during the first 10 days of incubation. In addition, at day 3 the treatment with alfalfa in the grassland soil was significantly higher than in the sample with single EDTA treatment.

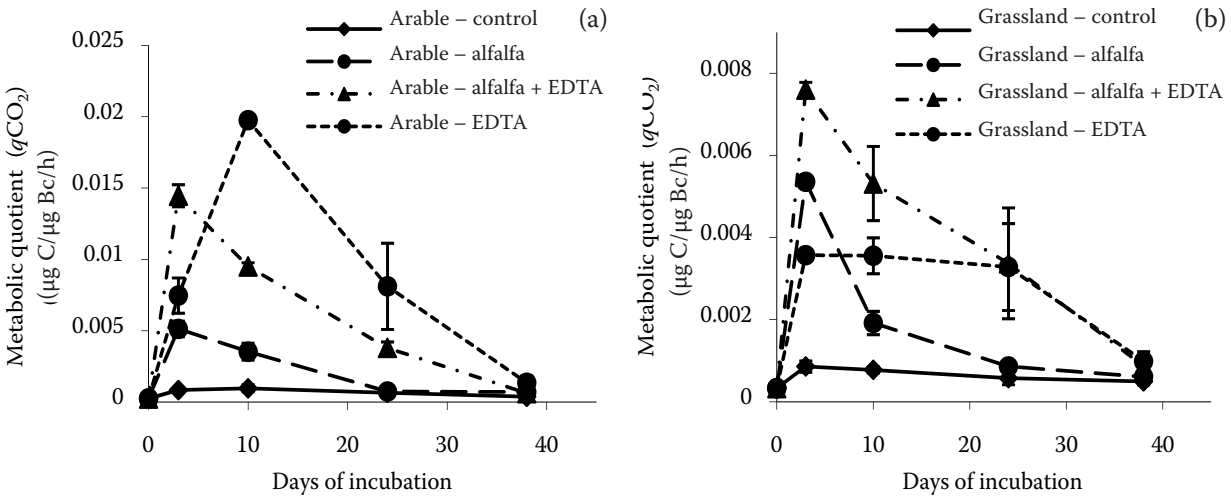
pH values. Together with other parameters, the pH-values were determined (Table 2). The values of pH ranged between 6.26–6.38 on the third day of incubation and 5.96–6.11 at day 38 in the arable soil. The pH values between 5.49–5.81 on the third day of incubation and 5.31–5.37 at day 38 were determined in the grassland soil. In comparison with control soils, significantly higher pH-values were observed in treatments amended



Statistical analysis: data with the same letter represent statistically identical values according to the ANOVA Duncan's multiple range test ($P < 0.05$)

Treatment	Day					Treatment	Day				
	0	3	10	24	38		0	3	10	24	38
Arable – control	ab	cde	cd	de	a	Grassland – control	ab	cd	bc	bc	ab
Arable – alfalfa	ab	l	h	cd	de	Grassland – alfalfa	ab	k	g	cd	bc
Arable – alfalfa + EDTA	ab	m	j	fg	bc	Grassland – alfalfa + EDTA	ab	l	i	g	bc
Arable – EDTA	ab	k	i	ef	ef	Grassland – EDTA	ab	j	h	f	ab

Figure 2. Respiratory activity in long-term heavy metal contaminated arable (a) and grassland (b) soils during incubation with EDTA and alfalfa. Vertical bars represent the standard deviation



Statistical analysis: data with the same letter represent statistically identical values according to the ANOVA Duncan's multiple range test ($P < 0.05$)

Treatment	Day					Treatment	Day				
	0	3	10	24	38		0	3	10	24	38
Arable – control	a	ab	ab	ab	a	Grassland – control	a	ab	ab	ab	ab
Arable – alfalfa	ab	de	cd	ab	ab	Grassland – alfalfa	a	g	cd	ab	ab
Arable – alfalfa + EDTA	ab	h	g	cd	ab	Grassland – alfalfa + EDTA	a	h	fg	efg	bc
Arable – EDTA	ab	f	i	efg	bc	Grassland – EDTA	a	e	e	def	bc

Figure 3a, b. Metabolic quotient (qCO_2) in long-term heavy metal contaminated arable (a) and grassland (b) soils during incubation with EDTA and alfalfa. Vertical bars represent the standard deviation

with alfalfa and with alfalfa + EDTA at day 3 and 10 of incubation in the grassland soil and at day 10 in the arable soil. The EDTA amendment significantly decreased the soil pH during the first three and ten days of incubation.

The incubation experiment with long-term heavy metal contaminated soils showed that EDTA amendment seriously affected the microbial biomass C. References on EDTA effects on soil microorganisms differ and some authors report no significant actions (Sapoundjieva et al. 2003), in other cases an increase in some microbial parameters was found (Chander and Joergensen 2008). On the other hand, negative effects of EDTA on microbial characteristics were observed (Ultra et al. 2005). The course of this experiment showed that EDTA strongly affected the microbial biomass C during the first 24 days of incubation; particularly in the arable soil the microbial biomass contents approached nearly zero values. The regeneration of the microbial biomass C was observed at day 38 in EDTA treatments in the arable soil when it was practically identical with the control sample. The microbial biomass C contents in EDTA treatments in the grassland soil came close to the control values. The toxic effects of EDTA on microbial biomass seem to be evident, however, in later phases after EDTA amendment the microbial biomass could restore possibly due to the utilization of organic substrates having their origin in dead cells or by synthesis of microorganisms more resistant to EDTA effects or by both processes.

The respiratory activity is closely connected to the decomposition of the organic substrate (alfalfa) which was added to the soil. The fact that the treatment of soils with EDTA together with alfalfa increases the respiratory activity over the simple alfalfa treatment indicates that probably two processes are engaged simultaneously. Alfalfa amendment enhances the mineralization of the organic substrate. EDTA might produce toxic effects on soil microorganisms and a part of respiratory activity is probably due to the effect of the decomposition processes of the dead microorganisms. This effect is pronounced in the treatment receiving EDTA only. On the other hand, simple alfalfa treatment showed an opposite trend and arable soil with alfalfa respired less than the grassland. One of

Table 2. pH values in long-term heavy metal contaminated arable (a) and grassland (b) soils during incubation with EDTA and alfalfa. Data with the same letter represent statistically identical values according to the ANOVA Duncan's multiple range test ($P < 0.05$)

Treatment	Day				
	0	3	10	24	38
Arable soils					
Arable – control	6.29^{efgh}	6.24^{bcdef}	6.22^{bcde}	6.20^{def}	6.11^{b}
Arable – alfalfa	6.29^{efgh}	6.38^{efgh}	6.41^{gh}	6.27^{fgh}	6.13^{bc}
Arable – alfalfa + EDTA	6.29^{efgh}	6.35^{efgh}	6.33^{defgh}	6.21^{def}	5.98^{a}
Arable – EDTA	6.29^{efgh}	6.24^{bcde}	6.26^{def}	6.11^{bcd}	5.96^{a}
Grassland soils					
Grassland – control	5.62^{efg}	5.50^{cd}	5.57^{def}	5.41^{abc}	5.33^{a}
Grassland – alfalfa	5.62^{efg}	5.81^{i}	5.73^{ghi}	5.41^{abc}	5.36^{ab}
Grassland – alfalfa + EDTA	5.62^{efg}	5.72^{ghi}	5.76^{hi}	5.51^{bcd}	5.37^{ab}
Grassland – EDTA	5.62^{efg}	5.50^{cd}	5.66^{fgh}	5.46^{cde}	5.31^{a}

possible explanations could be that grassland soil, having more organic matter, could better enhance the mineralization processes.

The measurements of respiratory activities and metabolic quotient showed that more metabolic processes could be involved: the microorganisms could spend more energy for their survival than for synthesis of new biomass as it was suggested by Giller et al. (1998). Their findings were confirmed e.g. by Jiang et al. (2003). The results of the respiratory activities and qCO_2 values in the present study on the long-term heavy metal contaminated soils do not exclude this contention. It is also possible to accept the idea that qCO_2 might not provide sufficient information about soil quality or degradation (Bastida et al. 2008) as the respiratory activities and qCO_2 values obtained during this incubation experiment could be partly explained by mineralization of the organic substrate in treatments containing alfalfa or by the death and subsequent autolysis of microbial cells after EDTA amendment. In fact, Wardle and Ghani (1995) showed that this index could behave unpredictably and some soil applications such as fertilization or liming can either decrease or increase qCO_2. Therefore, it is possible that different processes could be responsible for the dynamics of respiratory activity and qCO_2 in present experiment.

Higher pH values are usually favourable for the microbial growth. The microbial biomass was described to be larger after liming in comparison with unlimed control (Bezdicek et al. 2003). The growth of bacteria as the response to increased pH was reported also by Lorenz et al. (2001). The pH-values in the present study among the experimental treatments differed by 0.14–0.15 unit in the arable soil and by 0.32 unit in the grassland soil during the third day of incubation and 0.07 unit at day 38. It is therefore possible to suppose that the relatively small differences in pH among experimental treatments probably did not play a decisive role for the described changes.

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