

Effects of zeolite amendment on microbial biomass and respiratory activity in heavy metal contaminated soils

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ABSTRACT

A laboratory incubation experiment with zeolite and glucose was performed to evaluate the effects of zeolite amendment in heavy metal contaminated soils from two smelter areas on some microbial characteristics [Kremikovtzi (K1, K2) in Bulgaria and Příbram (P1, P2) in the Czech Republic]. The content of microbial biomass showed a tendency to decrease in Kremikovtzi soils whereas in Příbram soils no significant effects were found after zeolite amendment. Respiratory activity and metabolic quotient (qCO_2) decreased on the second and third day in Kremikovtzi soils amended with zeolite, no effects were observed in Příbram soils. Heavy metals decreased the content of microbial biomass in Kremikovtzi soils whereas the contaminated soil from Příbram area had the highest microbial biomass compared to non-contaminated soil during incubation, probably due to lower mineralization of carbon. The respiratory activity did not show any significant effects of zeolites on the evolution of CO_2 and qCO_2 in heavy metal contaminated Příbram soil. The respiratory activity in non-contaminated Příbram soil remained during the experiment lower in comparison to contaminated one, however the addition of zeolite increased qCO_2 .

Keywords: incubation experiment; heavy metal contaminated soils; zeolite amendment; microbial characteristics

Smelters and mines are among the largest and often the oldest industrial sources of soil contamination by heavy metals. Once heavy metals enter the soil, they persist there for thousands years and it is very difficult to eliminate their effects on the soil-plant system and on their transfer into the food chain. Toxic effects of heavy metals could negatively affect soil microbial activities if they were present in excessive concentrations. A considerable body of information has been acquired on the effects of heavy metals on soil microorganisms and microbially mediated processes (Giller et al. 1998). A review of such studies (Bååth 1989) shows enormous disparity between the studies as to what metal concentrations are toxic. However, a decrease in microbial biomass and its activities in the presence of heavy metals is often reported (i.e. Brookes 1995, Kandeler et al. 1996, Nannipieri et al. 1997, Giller et al. 1998, Bajaras-Aceves et al. 1999, Šimon 2000). The possibilities to use microbial and biological methods in artificially intoxicated soils in the Czech Republic were studied by Němeček et al. (1998), who reported that counts of microorganisms and their activities were specific to particular soils and their response to liming was also specific according to the stability of soil aggregates. The response of microorganisms and biochemical processes to natural pollution of soils by metals is significant only in extreme cases whereas the studies with artificially contaminated soils show higher effects (Podlešáková et al. 2000). The rate of mineralization of native soil organic carbon has been extensively used as an assay for metal toxicity in laboratory ecotoxicological studies of forest soils around metal smelters, but far less in monitoring of metal-contaminated agricultural soils.

The ability to mineralise organic matters is a key function of soil microorganisms, its sensitivity to metal contamination has been a subject of many studies (Giller et al. 1998). The amount of C mineralised over a short period such as 24 h after the addition of glucose was found to be extremely sensitive to an addition of even small amounts of metal salts in the laboratory (Stadelmann et al. 1984).

The removal of heavy metals in polluted areas is very difficult because they persist in soils for very long periods. However, the fixation of heavy metals in a non-available form could be a useful method for soils that are already contaminated by heavy metals. Natural and artificial zeolites increase ion exchange sites in soils in addition to offering absorption sites for small molecules, due to their porous structure. Consequently, zeolites are able to retain heavy metals in soil. However, the consequences of zeolite amendments for soil microorganisms in contaminated soils are completely unknown (Chander and Joergensen 2002).

The aim of the study was to evaluate microbial biomass dynamics, mineralization of added carbon substrate and its respiratory activities in heavy metal contaminated soils from two different smelter areas. In addition, the effects of soil amendment by natural zeolites on microbial properties were evaluated in laboratory incubation experiments after zeolite and glucose amendments.

MATERIAL AND METHODS

Soils from the vicinity of two smelters were used in experiments. Soils K1 and K2 were sampled from Kremi-

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kovtzi area near Sofia in Bulgaria with smelter history starting after 1945. Soils from Kremikovtzi were chromic Luvisols according to FAO classification. Soils P1 and P2 were typical Cambisols and were sampled in the area of Příbram smelter (Czech Republic) operating since 1786. Metal mining was 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). Non-contaminated soils (K1 and P1) were added as a control. Soils were sampled (layer 0–200 mm) from each sampling area in 2001, soil characteristics are shown in Table 1.

Approximately 1 kg of each soil was mixed with zeolite (natural clinoptilolite, Table 2) in order to give 3% zeolite amendment and adjusted to the 50% of its water holding capacity (WHC) in plastic jars, and in three replications placed into 3 litre plastic containers tightly covered with fitting lids to be conditioned at 28°C 14 days before treatment with glucose. A jar with 25ml of 1M NaOH was placed to the containers to take up the evolved CO₂. The containers were aerated daily to ensure a sufficient oxygen supply. The soils without zeolite amendment served as controls and they were pre-incubated in the same way as samples with zeolite. After 14 days of pre-incubation the mixture of glucose 1000 µg C/g and NH₄(SO₄)₂ giving the ratio C:N 10:1 was added. Thereafter 3 × 50 g of soil of each treatment were weighed and separately incubated in 750ml tightly closed glass jars containing 5ml 1N NaOH to determine the CO₂-evolved. Soils were incubated for 10 days. The content of microbial biomass, respiratory activity, pH and available heavy metal fractions were determined on days 0, 1, 2, 10 of the incubation.

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

K₂SO₄-extractable carbon was determined by a shaking of 20g soil samples (on oven dry basis) for 30 min on an overhead shaker with 80 ml of 0.5M K₂SO₄ solution. The extraction was performed according to determination of non-fumigated C described by Vance et al. (1987). Similarly like microbial biomass measurements, carbon con-

tent was determined in filtered extracts by digestion with a mixture of H₂SO₄ and H₃PO₄ and with K₂Cr₂O₇ and titration of excess dichromate with (NH₄)₂Fe(SO₄)₂·6H₂O.

Total organic carbon (TOC) was determined by digestion of soil (1g on oven dry basis) with K₂Cr₂O₇ and conc. H₂SO₄ at 125°C. The content of organic carbon was determined in digested solutions with 20% KJ by titration with Na₂S₂O₃. 2% starch solution was added closely before the point of equivalence (Alten et al. 1935).

The evolved CO₂-C was determined as an amount of organic C released as CO₂ after absorption in NaOH and precipitation with BaCl₂ and was analysed by titration with standard HCl. The metabolic quotient (qCO₂) was calculated according to Anderson and Domsch (1990) equation: qCO₂ = µg CO₂-C/µg C_B/h.

Values of pH were determined in a solution of soil/distilled water (1/2.5 w/v) after 1h shaking on an overhead shaker.

The soils were digested to determine total contents of heavy metals using the microwave digestion system Milestone MLS 1200. The soil samples (1 g on oven dry basis) were digested with 7 ml HNO₃, 2 ml HCl and 6 ml H₂O₂. Digested solutions were filtered on Schleicher and Schuell filters No. 310645 and clear solutions were then analysed for metal content using the OES-ICP axial sequential spectrometer Trace Scan manufactured by Thermo Jarrell Ash corporation (USA).

RESULTS AND DISCUSSION

The original content of microbial biomass in contaminated Kremikovtzi soil K2 was significantly lower before the start of the experiment in comparison with soil K1 whereas only a small decrease was obtained for Příbram soil P2 if compared to soil P1 (Table 1). Thereafter, the dynamics of microbial biomass in contaminated Kremikovtzi soil K2 remained significantly lower compared to soil K1 during the time of soil incubation with glucose. The zeolite amendment tended to slightly decrease the content of microbial biomass in soils, however these differences were not significant compared to soils without zeolite amendment (Figure 1). Kočí (1997), who studied possible toxic effects of water extracts of some cation zeolite forms on some water organisms, reported that

Table 1. The basic characteristics of soil samples from Kremikovtzi (K1, K2) and from Příbram (P1, P2) included in the experiment (mean ± deviation standard)

Soil	Total organic carbon (TOC) (%)	pH (H ₂ O)	Microbial biomass (µg C/g soil)	Respiratory activity (µg C/g soil/h)	Pb	Cd	Zn	As
					(mg/kg)			
K1	1.74 ± 0.04	8.46 ± 0.12	200.22 ± 12.12	1.1608 ± 0.0547	15.3 ± 2.5	0.23 ± 0.04	90.0 ± 4.5	14.1 ± 0.6
K2	1.99 ± 0.13	8.28 ± 0.04	132.83 ± 8.57	1.2153 ± 0.0803	996.8 ± 64.7	2.73 ± 0.33	1074.5 ± 68.4	85.8 ± 11.3
P1	1.66 ± 0.03	6.87 ± 0.05	232.17 ± 8.71	0.6838 ± 0.2207	43.5 ± 10.5	0.55 ± 0.19	72.08 ± 3.6	25.4 ± 10.3
P2	1.78 ± 0.12	6.28 ± 0.03	197.60 ± 11.14	0.3862 ± 0.1273	1363.6 ± 101.0	5.45 ± 0.08	287.1 ± 6.0	92.3 ± 12.3

zeolites were not usually toxic to organisms. However, when 4A-Na zeolite was transformed by ion exchange to 4A-Ca form, the toxic effects were very low, but water-flea mortality was 10% (Kočí 1997). Matulová and Klokočnicková (1994) also reported 50% inhibition of algae growth after zeolite addition to water. Chander and Joergensen (2002) stated that the effects of zeolites on microbial populations and their activities in soils were completely unknown, but they found out an increase in microbial biomass and incorporation of added ^{14}C into microbial biomass after zeolite amendment. The obtained results suggest that such effects of zeolite or of the combination of zeolite, pH and heavy metals could partly affect the dynamics of microbial biomass in Kremikovtzi soils whereas its effects in Příbram soils were more pronounced only in contaminated soil P2.

The content of microbial biomass in Příbram soils was slightly lower in contaminated soil P2 before the start of the experiment. However, microbial biomass increased more during the first day of incubation in contaminated soil P2 than in soil P1 (Figure 2). The addition of zeolite decreased the dynamics of microbial biomass in contaminated soil P2 whereas no effects were observed in soil P1. A possible explanation of these results is that high pH in Kremikovtzi soils in combination with zeolite addition could cause lower availability of nutrients that could be absorbed into zeolite structures more readily. A decrease in microbial biomass in Příbram soils was observed only for zeolite-amended contaminated soil P2 where the heavy metal content could play such a role.

The differences in microbial biomass between Kremikovtzi and Příbram soils could also result from different zinc contents in these soils. While lead and cadmium contents in soils K2 and P2 were comparable, zinc in soil K2 exceeded its content in soil P2 about three times. Zinc is considered as one of the most toxic elements to microbial biomass (Chander and Brookes 1993, 1995, Brookes 1995, Leita et al. 1995, Nannipieri et al. 1997, Ledin et al. 1999) whereas low toxicity is reported for lead, the main polluting element in Příbram soils (Bajaras-Aceves et al. 1999). The higher content of microbial biomass in contaminated soil P2 obtained during incubation with glucose could result from lower carbon consumption by the microbial pool in the more contaminated soil.

This hypothesis could be confirmed by determination of 0.5M K_2SO_4 -extractable carbon (Figures 3 and 4). The highest content of 0.5M K_2SO_4 -extractable carbon during the first day of incubation was observed in soil P2. The content of K_2SO_4 -extractable carbon in non-contaminated soil P1 during the first day of incubation was significantly lower compared to soil P2. However, the content of K_2SO_4 -extractable carbon in soil K2 was higher, the dynamics of K_2SO_4 -extractable carbon in Kremikovtzi soils was similar in both soils K1 and K2. The differences between these observations could reflect different energy requirements of soils from different areas. The ability to mineralise organic matters is a key function of soil microorganisms, its sensitivity to metal contamination has been a subject of many studies (Giller et al. 1998).

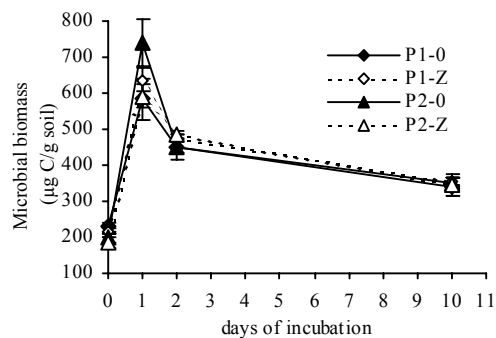
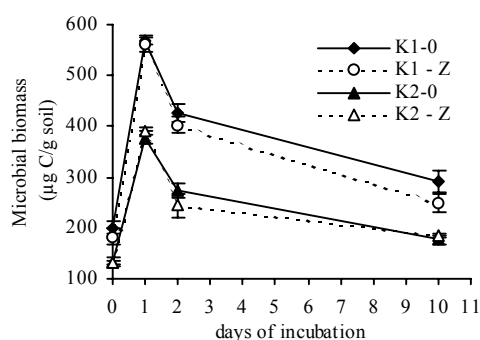
Table 2. Characteristics of zeolite (natural clinoptilolite) used for the incubation experiment

Compound	Concentration (%)
SiO_2	66.6
Al_2O_3	12.6
CaO	5.00
K_2O	3.84
Fe_2O_3	1.89
MgO	1.40
BaO	0.2
TiO_2	0.193
Na_2O	0.189
SrO	770 ppm
MnO	390 ppm
Rb_2O	350 ppm
ZrO_2	230 ppm
Co_3O_4	77 ppm
H_2O	6.67
CO_2	1.34

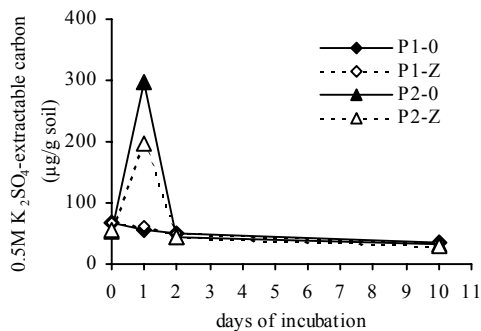
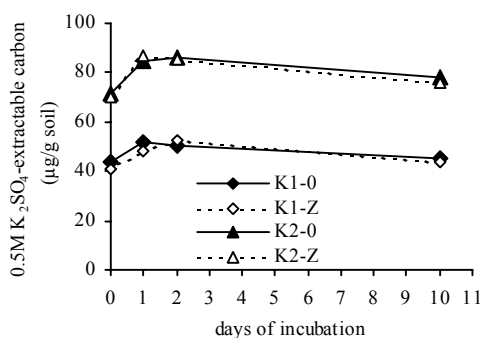
Brookes (1995) suggested that microbial populations under an environmental stress had higher energy requirements for their maintenance and they transformed less energy into new synthesis. In fact, contaminated soil K2 could use a major part of the added substrate for its maintenance and less glucose was used for new synthesis. Possibly due to the long-term contamination, Příbram soil P2 was not probably able to consume glucose as quickly as non-contaminated soil P1 and more 0.5M K_2SO_4 -extractable carbon was determined on day 1 of the experiment, which could lead to the higher content of microbial biomass during the first day of the incubation.

Microbial biomass content and soil respiratory activity correlated well during incubation (Table 3). The respiratory activity of contaminated and non-contaminated soils differed significantly in relation to the sampling area. The overall respiratory activity in Kremikovtzi soils without zeolite amendment did not differ significantly in soils K1 and K2 during incubation with glucose (Figure 5). The lower respiratory activity was found in variants with zeolite addition, which could be caused by sorption capacities of zeolite. The levels of evolved CO_2 -C were close to zero on the second day of incubation. In fact, the dynamics of respiratory activity in soils from Kremikovtzi area could be partly affected by high pH of the studied soils and so the sorption mechanisms of added zeolite.

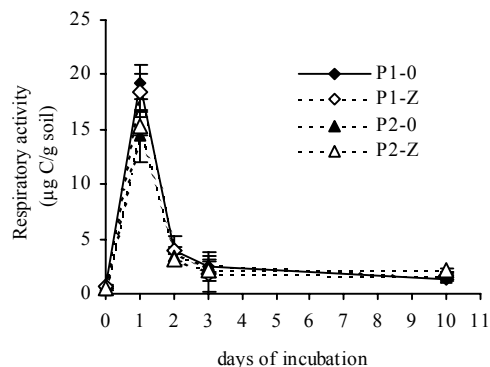
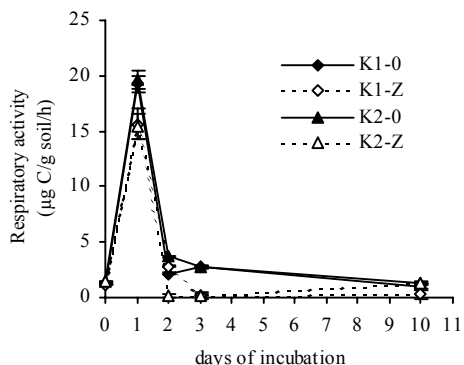
The respiratory activity of soils from Příbram area was higher in non-contaminated soil P1 during incubation with glucose compared to soil P2 (Figure 6). The addition of zeolites did not have any significant effects on respiratory activity in Příbram soils. Chander and Joergensen (2002) reported that the addition of synthetic zeolite led to mineralization of ^{14}C in favour of evolved $^{14}\text{CO}_2$. This is a unique result reported in literature and as Chander and Joergensen (2002) concluded the zeolite effects on soil microbial populations and their activities



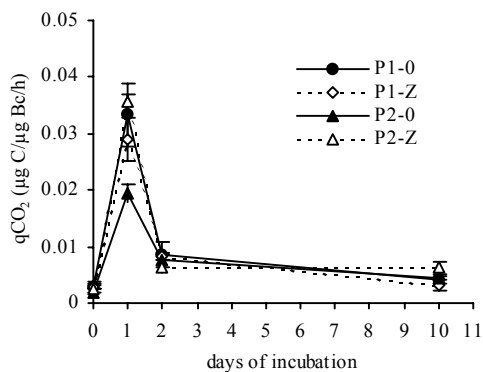
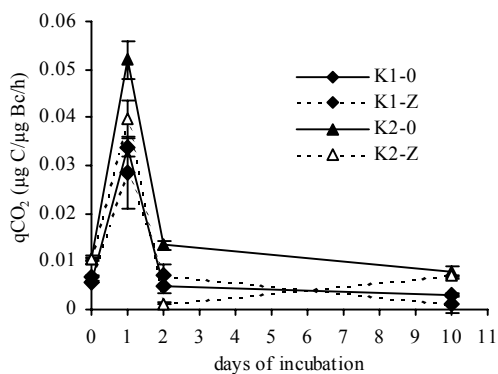
Figures 1 and 2. Microbial biomass dynamics in Kremikovtzi soils (K1, K2) and Příbram soils (P1, P2) during incubation with zeolite and glucose amendment (0 – without zeolite amendment, Z – with zeolite amendment)



Figures 3 and 4. Dynamics of 0.5M K₂SO₄-extractable carbon in Kremikovtzi soils (K1, K2) and Příbram soils (P1, P2) during incubation with zeolite and glucose amendment (0 – without zeolite amendment, Z – with zeolite amendment)



Figures 5 and 6. Respiratory activity in Kremikovtzi soils (K1, K2) and Příbram soils (P1, P2) during incubation with zeolite and glucose amendment (0 – without zeolite amendment, Z – with zeolite amendment)



Figures 7 and 8. Metabolic quotient (qCO_2) in Kremikovtzi soils (K1, K2) and Příbram soils (P1, P2) during incubation with zeolite and glucose amendment (0 – without zeolite amendment, Z – with zeolite amendment)

are unknown. However, the results obtained in other fields of research showed that zeolites decreased CO₂ concentrations in pig stables by 48% (Stankov and Veizovic 1993), a decrease in CO₂ amount was observed during storage of vegetables and fruits in zeolite presence (Oh-SeYoung et al. 1996, Di Matteo et al. 1998). The sorption abilities of zeolites could therefore explain the decreased respiratory activity in Kremikovtzi soils. On the contrary, no significant differences between the variants with and without zeolite were observed in Příbram soils. A possible explanation of different zeolite effects on Kremikovtzi and Příbram soils could be for instance the high pH value and possible higher sorption of CO₂ by zeolites in Kremikovtzi soils.

The metabolic quotient (qCO₂) that expresses respiratory activity per unit microbial biomass was lower in zeolite amended Kremikovtzi soils, which could be caused by higher CO₂ sorption into zeolite. Comparing non-contaminated soil K1 with contaminated K2, qCO₂ was significantly higher in soil K2 (Figure 7). A possible explanation of the results obtained in Kremikovtzi soils could be the higher energy requirement of microbial pool in soils stressed by heavy metals resulting in higher qCO₂ values as it was suggested by Brookes (1995) and Giller et al. (1998). Different qCO₂ dynamics was observed in Příbram soils (Figure 8). Similarly like the values of respiratory activity, the significantly lower qCO₂ was determined in contaminated soil P2 if compared to P1. Comparing these two soils, it was not possible to confirm the hypothesis of Brookes (1995), Giller et al. (1998) or Nannipieri et al. (1997) about the higher energy requirement of microbial populations necessary for their survival and new synthesis. On the contrary, Insam et al. (1996) did not found any increased qCO₂ levels as a consequence of microbial stress.

The incubation experiment with non-contaminated and contaminated soils from two smelter areas showed that the microbial characteristics could respond to the environmental stress differently. The heavy metal contents in Kremikovtzi soil K2 affected the microbial activities significantly, but the long-term contaminated Příbram soil P2 did not confirm the hypothesis of Brookes (1995), Nannipieri et al. (1997) or Giller et al. (1998) that less microbial biomass and higher respiratory activity would be found under a heavy metal stress. Furthermore, soils from both smelters did not respond to zeolite amendment in the same way. The microbial populations could respond to zeolite amendment in different ways, but further studies should be performed to understand better the zeolite effects on soil microbial properties.

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Table 3. Coefficients of correlation between the content of microbial biomass and respiratory activity in Kremikovtzi – K1, K2 and Příbram – P1, P2 soils (0 – without zeolite amendment, Z – with zeolite amendment)

Soil	<i>r</i>	Soil	<i>r</i>
K1-0	0.838***	P1-0	0.852***
K1-Z	0.837***	P1-Z	0.873***
K2-0	0.887***	P2-0	0.959***
K2-Z	0.805**	P2-Z	NS

NS – non-significant at $p < 0.05$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

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ABSTRAKT

Vliv přídavku zeolitu na mikrobiální biomasu a respirační aktivitu v půdách zatížených těžkými kovy

V laboratorním inkubačním pokusu byl na půdách kontaminovaných těžkými kovy sledován vliv přídavku zeolitu a glukózy na některé mikrobiální vlastnosti těchto půd. Půdy pocházely z oblasti okolo hutí – Kremikovtzi (K1, K2) v Bulharsku a Příbram (P1, P2) v ČR. V průběhu pokusu vykazovala mikrobiální biomasa půd z oblasti Kremikovtzi tendenci ke snížení svého obsahu, zatímco u půd z Příbrami se tento trend projevil pouze u kontaminované půdy P2. Respirační aktivita a metabolický kvocient (qCO_2) klesaly druhý a třetí den inkubačního pokusu u půd z oblasti Kremikovtzi, ke kterým byl přidán zeolit, tento efekt byl však u půd z Příbrami pozorován pouze v kontaminované půdě. Těžké kovy snížily obsah mikrobiální biomasy u půd z oblasti Kremikovtzi, naopak během pokusu vykazovala kontaminovaná půda P2 z Příbrami nejvyšší obsah mikrobiální biomasy ve srovnání s nekontaminovanou půdou P1, a to pravděpodobně díky nižší mineralizaci uhlíku. Respirační aktivita a qCO_2 u kontaminované příbramské půdy nebyly přídavkem zeolitu významně ovlivněny. Respirační aktivita u nekontaminované příbramské půdy zůstala v průběhu pokusu ve srovnání s půdou kontaminovanou nižší, přídavek zeolitu však zvyšoval qCO_2 .

Klíčová slova: inkubační pokus; půdy kontaminované těžkými kovy; přídavek zeolitu; mikrobiální charakteristiky

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