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Variation of carbon and metal concentration in soil amended with sewage sludge

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Studies of metal impact on carbon cycle became more intense when the role of soil in climate change had emerged. This study aims at comparing changes in concentrations of soil carbon and Pb, Mn and Cd in soil amended with sewage sludge. The results indicate that mobile Pb, Mn and Cd concentrations have a higher correlation with soil carbon ($r > 0.766$; $r > 0.768$; $r > 0.653$ respectively, $p > 0.01$) than with the total soil metal contents (no significant correlation), which might be explained by the fact that mobile metals have more direct inhibitory effect on microorganism activity than total content. Significant correlation between mobile Mn, Pb, Cd and soil carbon shows their role in soil carbon cycle. Sewage sludge application on soil with an increased mobile metal fraction may lead to slower degradation of soil carbon and reduction of CO_2 evolution.

Key words: mobile Pb, mobile Cd, mobile Mn, soil carbon, sewage sludge, forest soil

INTRODUCTION

Metal pollution became an issue more than 50 years ago and has been a common object of analysis in a broad scope of research. A variety of available metal forms gives metals ability to act in different ways: from being more bio-available in soluble or ion form to more stable bound to carbonates and Fe–Mn with hydroxides. Though metals are inherent components of soil, a higher concern is related to their load resulting from anthropogenic activities (Liao, Xiao, 2006; Jankauskaitė et al., 2008), produced in larger amounts and of higher mobility.

Metals in organic and inorganic forms affect inhabitants of forest ecosystems ranging from trees to soil micro-organisms by causing abiotic stresses. (Ozolinčius, Sujetovienė, 2002).

Studies into metal impact on carbon cycle gained momentum when soil role in climate change had emerged. Soil carbon and metals were found to interact in different ways. Metals influence soil biota diversity (Mhatre, Paukhurst, 1997; Barajas-Aceves et al., 1997) by increasing the proportion of fungal population as compared to bacteria because fungi tend to be more resistant to metals than bacteria (Hiroki, 1992; Kelly et al., 1999; Akmal et al., 2005). They can also influence soil biota abundance (Mhatre, Paukhurst, 1997; Barajas-Aceves et al., 1997) and its activity (Mhatre, Paukhurst, 1997; Barajas-Aceves et al., 1997) as well as soil organic matter turnover (Dumat et al., 2006). Metal stress can induce changes in the microbial C : N ratio (McGrath et al., 1995; Huang, Khan, 1998; Khan et al., 1998). Metals cause lesser incorporation of organic carbon and organic nitrogen into microbial cells (Speir et al., 1995; Knight et al., 1997; Chander et al., 2004; Ghosh et al., 2004) since soil micro-organisms, being under metal stress, divert energy from growth to cell maintenance (respiration) (Killham, 1985). In other words, in metal-contaminated soils more energy is spent in order to survive, with faster respiration and less energy for incorporation of fresh substrate into new microbial biomass (Badgett, Saggar, 1994). An increase in C : N ratio in the microbial biomass was observed in Pb and Cd treated soils, which is caused by decline in the size of soil microbial community and reduction of C mineralization in these soils (Akmal et al., 2005). Cd has more adverse effect than Pb on reduction of C_{mic} and N_{mic} due to higher solubility and more direct toxicity to soil micro-organisms (Akmal et al., 2005).

Abiotic stress of metals in organic and inorganic forms affects the growth, morphology and metabolism of soil micro-organisms as well as size, composition and activity of microbial community (Doelman, 1985; Duxbury, 1985; Giller et al., 1998; Frostegård et al., 1996; Bardgett, Saggar, 1994; Chander, Brookes, 1991).

Over a threshold concentration, metals reduce microbial biomass and its activity depending on metal type, amount and combinatory effects of individual metals (Giller et al.,

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1998; Bååth, 1989; Frostegård et al., 1996; Bardgett, Saggar, 1994; Chander, Brookes, 1991).

Metals also have detrimental influence on biochemical parameters of soil (Bhattacharyya et al., 2008). Metals can inhibit production of soil enzymes by masking the active groups by protein denaturation, other effects on enzyme configuration or by competing with activating metal ions (Bååth, 1989; Ghosh et al., 2004).

Mobile form of metals is the available form of metals causing risk for groundwater quality, food safety if taken up, and other environmental threats because of fast transportability. Water-soluble forms of metals are more toxic to soil microorganism than insoluble forms (Ghosh et al., 2004). In this study mobile forms of three metals – Mn, Pb and Cd – were analysed, because two of them – Cd and Pb – are known to be harmful for environmental components, and Mn was chosen as a metal typical in high abundance in natural soil (Butkus, Baltrėnaitė, 2007; Baltrėnaitė, 2008). The aim of this study was to compare changes in concentration of soil carbon and metal in soil amended with sewage sludge.

METHODS

Site of investigation

In 1998, an experimental site was prepared in the woodcutting area of 2 ha in Gitėnai forest in Taruškos forestry located in Panevėžys region (Lithuania) (Fig. 1). It is located 12.5 km South from Panevėžys town (Lithuania), at E024°34'38.8"latitude and N55°43'31.6" longitude. Mean annual precipitation in the surroundings is 700 mm. 600 tons of the industrial sewage sludge from Panevėžys town was spread on the soil of experimental site. Sewage sludge amended on soil in 1998 presented a very heterogeneous content of metals (Table 1).

Fig. 1. Experimental site (white square) for industrial sewage sludge utilization in Gitėnai forest in Taruškos forestry located in Panevėžys region (Lithuania)

Table 1. **Selected properties of industrial sewage sludge prior to amendment on the soil (Katinas et al., 2002) (LOI – loss on ignition)**

	Heavy metals, mg/kg DW			pH	LOI, %
	Mn	Cd	Pb		
Range	224–572	3.1–18.7	597–1421	7.1–7.8	61.6–72.7
Median (n = 4)	393	9.5	962	7.7	66.4
EC limits 86/278/EEC	–	40	1 200	6–7	–

In 1999, aiming to regenerate the forest, pine (*Pinus sylvestris* L.) and birch seedlings (*Betula pendula* Roth) were planted in the site, and black alders (*Alnus glutinosa* (L.) Gaertn) had grown there naturally. Among the tree seedlings, the perennial grasses were dominated by *Calamagrostis epigeios*, *Artemisia vulgaris*, *Achillea millefolium*, *Solidago virgaurea*.

Loam and sandy loam soils, which make up the layer of 4–13 cm, prevail in the territory under investigation and therefore the forests in this area are turning into marshes. In the areas where marshes are widespread, the soil is alkaline (pH > 8.0). The soil in the territory under investigation consists of slightly decomposed forest floor of 20–25 cm in the higher parts of the forest and in the lower ones of 0.75–0.5 m in thickness low marshes type slightly decomposed peat consisting of 70 per cent of clayey fraction in the bottom part and of the forest floor and wood cuttings in the upper part (Katinas et al., 2002). Soil metal monitoring was carried out annually from 1998 to 2001 by researchers from the Institute of Geography and Geology. In 2006 and 2008 soil and plant samples from the field were taken by researchers of the Vilnius Gediminas Technical University.

Soil sampling

The sewage sludge amended territory was paired with the non-amended territory (control) with similar soil type and management history. The assumption was made that prior to the use of sewage sludge the background concentrations of metals were similar in both territories: Mn – 302 mg/kg; Zn – 5.2 mg/kg; Cu – 4.4 mg/kg; Ni – 6.2 mg/kg; Cr – 22.9 mg/kg; Pb – 14.9 mg/kg of dry weight (Katinas et al., 2002). Three soil sampling sites (5 × 10 m) were randomly selected from both the experimental and control sites. In each one a composite sample made up from 9 sub-samples was formed. Composite samples were taken from soil profile from the soil depths of 0–10 cm and 20–30 cm. Sampling was conducted two times: 8 and 10 years after sludge amendment. Soil samples were air-dried, ground and sieved through a 2 mm sieve and kept at 4 °C prior to analysis.

Soil pH

The pH of the soils was determined using an Orion pH meter. All pH measurements were performed in duplicate using a ratio of 1 part of soil to 2 parts of 0.01M $CaCl_2$. The whole procedure followed the protocol described in ISO 10390.

Soil carbon, C %

Carbon measurements were made on 50 mg of ground sample by the dry combustion method (EC-12 LECO-carbon analyzer) (Schwartz, 1995) where the carbon of a sample was burned in the combustion tube to form carbon dioxide. The carrier gas containing the carbon dioxide and other combustion products flows from the combustion tube to a dehumidifier where it is cooled and dehydrated. Then it passes through a halogen scrubber before it reaches the cell of non-dispersive infra-red (NDIR) gas analyser where the carbon dioxide is detected. The analogue detection signal of the NDIR forms a peak and the area of this peak is measured by a data processor. The peak is proportional to carbon concentration of the sample. Therefore when a carbon standard has been analysed to create a calibration curve equation expressing the relationship between carbon concentration and peak area, the carbon concentration in the sample can be calculated. The standard used in preparing calibration curves was glucose containing 40% of carbon.

Mobile Cd, Mn, Pb

Mobile fraction was decided to measure because mobile metals pose higher risk to environment, thus to soil and its processes (Bhattacharyya et al., 2008). Mobile (and potentially bio-available) fraction of metals was determined in 0.01M $CaCl_2$ solution (Kabala, Singh, 2001). 5 g soil with 50 ml of 0.01M $CaCl_2$ solution were mechanically shaken for 2 hrs (Houba et al., 1997; Rauret, 1998) and then analysed with AAS graphite furnace (GFAAS). Detection limits of GFAAS are the following: 0.001 ppm for Mn, 0.0008 ppm for Pb and 0.0005 ppm for Cd.

Total Cd, Mn, Pb

0.2 g of each soil sample were digested with a mixture of HNO3 (65%) and HCl (37%) (Soon, Abboud, 1993) at the microwave digester *Milestone ETHOS*. The solution was poured in flasks of 50 ml and diluted with deionised water to the mark of 50 ml. Only 2008 soil samples were available for total Cd, Mn and Pb analysis.

Statistical analysis

Data were statistically analysed using SPSS 16.0 for Mac. Pearson correlation coefficient calculation was used to determine correlation between soil carbon and mobile metal concentration. Non-parametric test for two related samples was employed to determine changes in soil carbon and metal concentrations in soil samples in 2006 and 2008. All samples were prepared in duplicate.

RESULTS AND DISCUSSION

Variation in soil carbon

Fig. 2 shows variation of soil carbon within 2 years' period in two depths of soil profile: 0–10 cm and 20–30 cm. The mean value of soil carbon at soil depth of 0–10 cm varied from 1.31% in 2006 to 1.60% in 2008, whereas it ranged from

1.30% in 2006 to 1.16% in 2008 at soil depth of 20–30 cm. High standard deviation revealed high variation in soil carbon between samples. It is obvious in particular because of non-homogeneity of sewage sludge and difference in sludge layer thickness. However, statistical analysis applied to compare the difference between soil carbon concentrations after two years' period did not show significant difference ($p < 0.01$, $n = 12$).

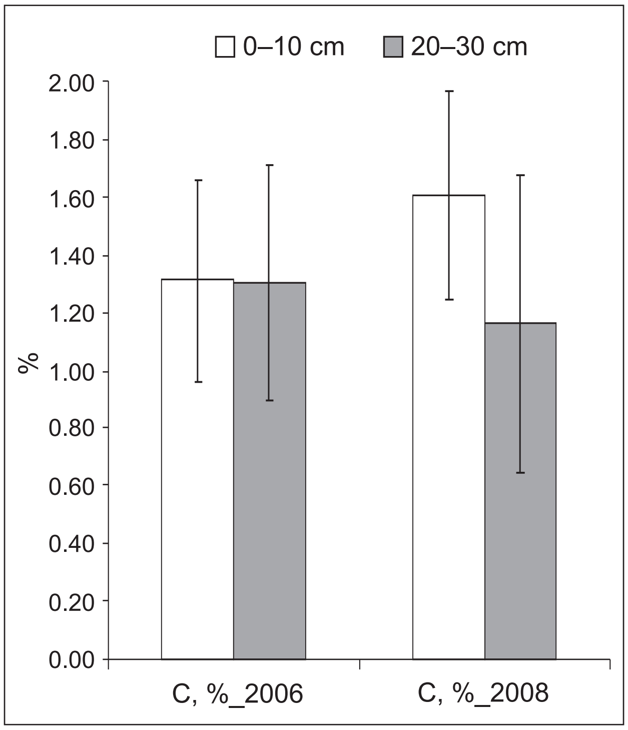

Fig. 2. Variation of soil carbon in two depths of forest soil in 2006 and 2008 (± SD, n = 6)

Statistical analysis revealed no significant difference between soil carbon variation in soil at a depth of 0–10 cm and 20–30 cm. The tendency of higher soil carbon concentration in upper soil layer than in deeper layers appeared in 2008. The differences were not significant, however, higher concentration in the deeper layers can be explained by new organic matter addition (e. g. annual increment of forest litter), mineralization of forest litter and root turnover (Armolaitis et al., 2008). Soil carbon concentrations in the investigated territory were similar to those determined in other Lithuanian forest soils, reaching 1.29% (Beniušis, Vaičys, 2008). Though a 2-year period is a short period for significant changes in carbon, however, some authors have found that addition of organic amendments showed noticeable increase in soil organic C after 1 year (Madejon et al., 2008).

Variation in mobile Cd, Mn and Pb within 2 years

After two years, mobile forms of Cd decreased from 0.24 mg/kg to 0.15 mg/kg DW, while those of Pb and Mn increased, from 0.02 mg/kg to 0.10 mg/kg DW and from 0.18 mg/kg to 0.30 mg/kg DW, respectively. Statistical analysis revealed the only significant difference in Mn concentration change. The role of Mn in the environment and contaminated soil is interesting. Comparison of Mn total concentration in control and sewage sludge amended soil showed higher concentration of Mn in control soil (168 mg/kg) than in sewage sludge amended soil (108 mg/kg) in 0–40 cm soil profile (Butkus, Baltrėnaitė, 2007). Mn is known to be important in photosynthesis, nitrogen metabolism and nitrogen assimilation (Mengel, Kirkby, 1987), therefore an increase of Mn mobile form in investigated soil can show that possibly more intensive microbe activity occurred because of nitrogen immobilization by micro-organisms, and nitrification processes. Since soil N and C amounts are closely related, increase of Mn may have also facilitated an increase in soil carbon.

Little difference was observed in change of Cd and Pb concentrations, showing no environmental risk towards groundwater. As previously reported by Katinas et al. (2002), no significant leaching of Cd and Pb was determined within 4 years after sewage sludge amendment. Our data also revealed that sewage sludge application on peaty woodcutting areas does not pose environmental risk from harmful metals, such as Pb and Cd.

Variation of mobile Cd, Mn and Pb within soil profile

Fig. 3 shows distribution of mobile metal form in soil horizon of 0–10 cm after two years. Mobile fraction of Mn and Pb increased from 0.02 mg/kg to 0.088 mg/kg DW and from 0.146 mg/kg to 0.313 mg/kg DW, respectively. For Cd, a slight decrease from 0.201 mg/kg to 0.189 mg/kg was determined.

Fig. 4 shows distribution of mobile metal form in soil horizon of 20–30 cm after two years. Mobile fraction of Mn and Pb increased from 0.018 mg/kg to 0.111 mg/kg DW and from 0.226 mg/kg to 0.257 mg/kg DW, respectively. For Cd, a decrease from 0.354 mg/kg to 0.111 mg/kg was determined. The most significant differences were found in increase of mobile Mn and Pb at 0–10 cm after 2 years ($p < 0.01$, $p < 0.05$ respectively); decrease of Cd and increase of Mn at the soil depth of 20–30 cm after 2 years ($p < 0.05$). Increase of mobile Mn in both soil profiles supports an idea of intensified activity of soil micro-organisms. Decrease of mobile Cd in 20–30 cm soil layer may be explained by immobilization processes, in particular sorbtion by carbonates and Fe–Mn hydroxides (Katinas et al., 2002) with less mobile fraction of Cd available. Increase of Pb after 2 years in topsoil might have been affected by atmospheric deposition.

Soil pH changes and correlation with mobile metals

Soil acidity varied from 6.15 to 6.91 in 0–10 cm soil layer and from 5.82 to 6.75 cm in 20–30 cm soil layer. There was no significant change in soil pH values between soil layers ($p < 0.001$, $n = 24$). Significant ($p < 0.05$) negative correlation was found between soil pH and mobile metals concentrations. Correlation coefficient ranges from –0.453 for mobile

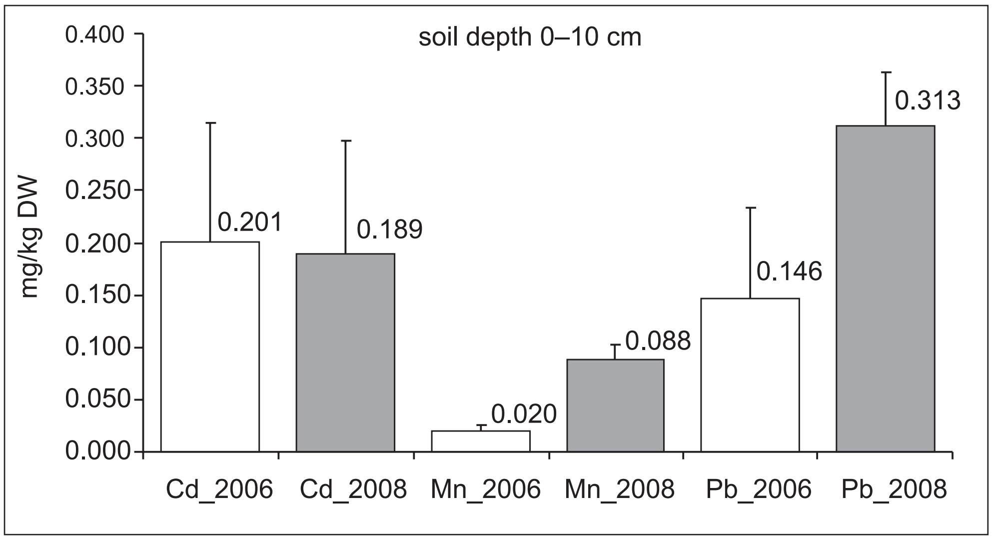

Fig. 3. Variation of mobile metal concentration (mg/kg DW) in soil depth of 0–10 cm measured in 2006 and 2008 (± SD, n = 6)

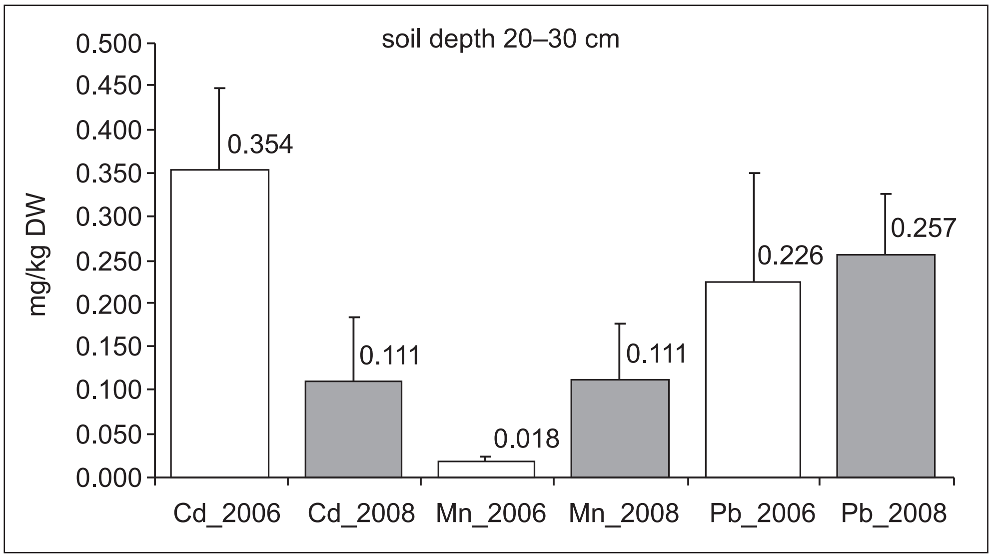

Fig. 4. Variation of mobile metal concentration (mg/kg DW) in soil depth of 20–30 cm measured in 2006 and 2008 (± SD, n = 6)

Pb and Mn to –0.549 for Cd under $p < 0.05$. This supports the fact that more acidic soil facilitates mobility of metals (e. g. Baltakis, 1993, Iyengar et al., 1981; Narwal, Singh, 1998; Ma, Rao, 1997). Results suggest that soil became less acidic, reaching values of 5.5–6.5, which means more suitable conditions for soil micro-organisms and favours their activity. It also supports the presence of less mobile metals, since in general, less acidic soil decreases metal mobility.

Correlation between soil carbon, total and mobile metal concentration

We have calculated correlation between soil carbon, total and mobile metals from all data available (Table 2). Most results showed strong positive correlation between soil carbon and mobile metals, in most cases reaching value of 0.8. No significant correlation was found between soil carbon and total concentration of investigated metals.

Table 2. **Correlation between total, mobile metals and soil organic carbon**

Year	Control soil			Soil amended with sewage sludge		
	Mobile Pb	**Mobile Cd**	**Mobile Mn**	**Mobile Pb**	**Mobile Cd**	**Mobile Mn**
1998–2001[a]	N / A	N / A	N / A	0.766**	0.768**	N / A
2006[b]	N / A	N / A	N / A	0.805**	0.807**	0.653**
2008	–0.305	0.652*	0.845*	0.884**	0.796**	0.845**
	Total Pb	**Total Cd**	**Total Mn**	**Total Pb**	**Total Cd**	**Total Mn**
2008	0.078	0.483	–0.404	–0.511	0.147	0.135

** – $p < 0.01$, n = 9–20; [a] data reported in Katinas et al., 2002; [b] data on total metal concentration in 2006 was not available.

The strong correlations between mobile metals and soil carbon may be related to a decrease of mineralization of organic carbon to CO_2 due to inhibition effect of Pb and Cd on soil micro flora involved in organic matter decomposition (Anderson, 1982; Liao, Xie, 2007; Bhattacharyya et al., 2008; Baltrėnaitė, 2008). Strong correlation between Mn and soil carbon can explain an important role of Mn in micro-organism activity. Another explanation could be that investigated soils had a higher content of stabilized fraction of soil organic matter (high in clay-humus complexes) which is known to be less degraded by micro-organisms and attract higher concentration of metals (Dumat et al., 2006). There is also an opinion that mineralization of organic materials may also be inhibited directly where metals are bound to them (Hattori 1996; Post, Beaby, 1996), a process of particular relevance to sludge application.

There are data showing that use of sewage sludge with higher concentrations of Cd, Cu, Ni, Pb and Zn (close to EC limits) may lead to short-term changes in soil microbial communities and their activities with increased loss of C to the atmosphere and N availability. It happened because of microbial responses to metal characterized by a shift within the microbial population from bacteria to fungi, increased mineralization of organic matter and reduced assimilation of mineralized N (Khan, Scullion, 2002).

In our study soil was amended with sewage sludge where Pb and Cd content did not exceed EC limits. However, these concentrations might have had impact on microbial activities. Based on the literature data (e. g. Witter et al., 1993; Dar 1996; Insam et al., 1996), concentrations higher than 9 mg/kg for Cd and 40 mg/kg for Pb were found to have an inhibitory effect on micro-organism biomass. Total concentration of 25 mg/kg for Cd (Dar, Mishra, 1994) and 200 mg/kg for Pb (Leita et al., 1995) was found to reduce soil microbial respiration and thus CO_2 evolution. Even if concentration of Cd was lower in our case (3.1–18.7 mg/kg), the decrease of respiration might have been caused by synergetic effect of Pb and Cd.

In our study, the results showed that mobile Pb, Mn and Cd concentrations have higher correlation with soil carbon than total soil metal contents. This was proved by all data on soil amended with sewage sludge available in 1998–2001, 2006 and 2008. High correlation might be explained by the fact that mobile metals have more direct inhibitory affect on micro-organism activity than total content.

CONCLUSIONS

1. No significant soil carbon change was determined in soil amended with sewage sludge after 8 and 10 years.

2. Furthermore, mobile fraction of typical anthropogenic metals, Pb and Cd, was not found to have changed significantly ($p < 0.01$) and thus did not pose environmental risk.

3. The results showed that mobile Pb, Mn and Cd concentrations have higher correlation with soil carbon ($r > 0.766$; $r > 0.768$; $r > 0.653$, respectively, $p > 0.01$) than that with total soil metal contents (no significant correlation), which might be explained by the fact that mobile metals have more direct inhibitory affect on micro-organism activity than total content.

4. Significant correlation between mobile Mn, Pb, Cd and soil carbon proves their role in soil carbon cycle. Sewage sludge application on soil with increased mobile metal fraction may lead to slower degradation of soil carbon and reduction of CO_2 evolution.

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ANGLIES IR METALŲ KONCENTRACIJŲ POKYČIAI NUOTEKŲ DUMBLU PATRĘŠTAME DIRVOŽEMYJE

Santrauka

Metalų poveikio dirvožemio anglies apykaitai tyrimai, siejant juos su dirvožemio vaidmeniu klimato kaitos kontekste, tapo labai svarbūs. Šio tyrimo tikslas buvo palyginti dirvožemio anglies ir metalų (Pb, Mn ir Cd) koncentracijų pokyčius dirvožemyje, ant kurio buvo paskleistas nuotekų dumblas. Iš rezultatų matyti, kad judriesiems metalams Pb, Mn ir Cd buvo būdinga stipresnė koreliacija su dirvožemio anglimi (atitinkamai $r > 0,766$; $r > 0,768$; $r > 0,653$, $p > 0,01$) negu šių metalų bendrosioms formoms (koreliacija nereikšminga). Tai gali būti paaiškinta faktu, kad judrioji metalų forma labiau nei bendroji daro didesnį neigiamą poveikį dirvožemio mikroorganizmų veiklai. Reikšminga judriųjų Mn, Pb ir Cd ir dirvožemio anglies koreliacija rodo šių metalų svarbą dirvožemio anglies apykaitoje. Nuotekų dumblo paskleidimas ant dirvožemio ir didėjanti judriųjų metalų dalis gali paskatinti lėtesnę dirvožemio anglies degradaciją ir mažiau intensyvias CO_2 emisijas.

Raktažodžiai: judrusis Pb, judrusis Cd, judrusis Mn, dirvožemio anglis, nuotekų dumblas, miško dirvožemis