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ARTICLE

Effect of some metal salts on the cultivable part of soil microbial assemblage in a calcareous loam cropland 6 years after contamination

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ABSTRACT Heavy metal contamination is of great interest because of the accumulation in soil and a potential risk to get into the food chain. Effect of three heavy metal salts added to field plots on the soil microbial assemblage and their total biomass was investigated 6 years after the artificial contamination. The metal addition was 90 and 810 kg per hectare for cadmium, copper and nickel on a calcareous loamy chernozem soil. Cadmium at 90 and 810 kg ha^{-1} rates resulted in 80 and 670-fold increase; copper 2 and 9-fold increase; nickel 2 and 6-fold increase to the background level. Number of *Actinobacteria* and *Azotobacter* spp decreased while oligotrophic bacteria and microscopic fungi increased due to the metal additions especially in the case of cadmium. This indicated structural changes in soil microbial assemblage. Soil microbial biomass C was significantly lower in all metal treated soils compared to the control, but the higher doses did not result in further depletion. **Acta Biol Szeged 52(1):201-204 (2008)**

KEY WORDS

heavy metal
microbial counts
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The effect of heavy metal accumulation in soils is widely studied because of the risk of human health and environmental quality (Máthéné Gáspár and Anton 2004). Heavy metal uptake by plants depends on soil properties (Anton and Máthé-Gáspár 2005) and can be regulated by soil microorganisms in the rhizosphere especially those are in symbiotic relations (Biró and Takács 2007). Metal contaminants removal by plants is a new perspective to restore degraded soils (Máthé-Gáspár and Anton 2005). The other aspect of metal pollution is the effect on the transformation dynamics of soil organic matter and plant nutrients (Filip 1998; Máthé-Gáspár et al. 2006) which are mainly depended on the activity of soil microorganisms (bacteria and fungi). The activity, biomass and diversity of microbial community are the main characteristics that can be changed due to the soil pollution (Brookes 1995). The traditional method in soil microbiology is the plate dilution technique (Veres Lukács and Oláh Zsúpos 2006) to characterize the quantity of soil microbial assemblage. Colony development on selective media shows different sensitivity for different metals. Doelman (1985) suggested a sensitivity order among major groups of soil microbes as follows procaryotes (bacteria and actinomycetes) are more sensitive than eucaryotes (fungi) to heavy metal pollution; bacteria are more sensitive than actinomycetes and the Gram positive bacteria are more sensitive than Gram negatives. Arbuscular mycorrhizal (AM) fungi have metal sensitive and tolerant strains even within species (Takács et al. 2000). The metal tolerant AM fungi can be used for remediation purposes in polluted sites (Takács et al. 2005). Mikanová et al. (1998) have found that most sensitive group of soil bacteria are the free-living N_2-fixing *Azotobacter* while the number of oligotrophic bacteria increased with increasing heavy metal load. They established that filamentous fungi were less affected by soil pollution and their number was generally increased in highly polluted soils. Microbial biomass C decreased with metal-enriched (Cu, Ni, Zn and Cd) sewage sludge by chloroform fumigation incubation and also microscopic investigations (Brookes et al. 1986). Fliessbach et al. (1994) found that not only microbial biomass but the microbial biomass C to soil organic C ratio is also decreased after addition of metal enriched (Cr, Cu, Cd, Pb, Hg, Ni, Zn) sewage sludge to the soil. Biomass specific respiration i.e. soil respiration per unit microbial biomass increased especially the fungal respiration increased in higher extent due to the treatments. Knight et al. (1997) studied soils at the current UK limit values with Cu, Cd and Zn and found that Cd and Cu treatments decreased the microbial biomass C while Cu and Zn decreased the metabolic potential of the soil microbial community. Szili-Kovács et al. (2006) found a significant decrease in soil microbial biomass C and microbial biomass N in a floodplain resulted from heavy metal accumulations. Effect of a single heavy metal can not be distinguished in a real pollution situation that is why Kádár's experiment of peculiar interests (Kádár 1995). The aim of this study was to compare cultivable part of soil microbial assemblage and the total soil microbial biomass if they affected by metal loads

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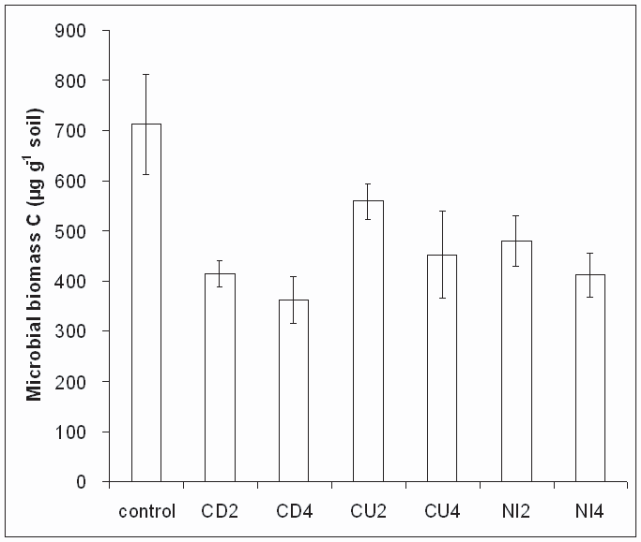

Figure 1. Mean (±standard error, 4 replicates) values of soil microbial biomass C six years after metal contamination.

six years after the artificial contamination with cadmium, copper and nickel salts.

Materials and Methods

Experimental site, experiment, treatments

Study site is located at Nagyhörcsök experimental station of the Research Institute for Soil Science and Agricultural Chemistry (RISSAC) of the Hungarian Academy of Sciences.

The soil type of the experimental plots is a calcareous loamy chernozem with medium to deep humus layer formed on loess. The upper 20 cm layer had 2.46% soil organic C, 0.23% total N, pH(KCl) 7.4, 3.5% lime and 21 meq/100 g soil cation exchange capacity. Particle distribution of the soil was 0.8% coarse sand, 15.7% fine sand, 60.4% silt, 23.1% clay. Kádár (1995) designed an artificial heavy metal contamination experiment to study the long-term changes in soil properties, plant uptake and vertical movement of metals. Experimental plots were polluted with a single addition of $CdSO_4$, $CuSO_4$, and $NiSO_4$ respectively that investigated in this study. The application rates were 90 (CD2, CU2 and NI2 plots) and 810 (CD4, CU4 and NI4 plots) kg ha^{-1}, which corresponded to 30 mg kg^{-1} and 270 mg kg^{-1} soil respectively, based on the soil bulk density value of 1.5 and the average plough layer of 0.2 m. The artificial contaminants were ploughed into the topsoil in April 1991. NPK fertilisers were given at 100 kg ha^{-1} y^{-1} N (as NH_4NO_3), 100 kg ha^{-1} y^{-1} P_2O_5 (as superphosphate), 100 kg ha^{-1} y^{-1} K_2O (as potassium salt). The fertiliser treatments are aimed to assess the effects of microelements on crops grown under conditions of regular cropping systems. No pesticides were applied, weed control was performed by manual cultivation. Experimental units were arranged in a split-plot design encompassing an area of 21 m^2. Soil samples were taken on 22 November, 6 years after metal pollution, sieved and stored in refrigerated (4°C) until the analyses have performed. A part of the samples were air dried for chemical analyses and another part was used for moisture determination after drying at 105°C until constant weight.

Table 1. Metal content of soil samples 6 years after pollution.

Treatments	Cu	Cd	Ni
		mg kg^{-1} soil	
Control	20.77	0.28	27.13
CD2	20.04	21.89	27.09
CD4	20.85	186.95	28.23
CU2	42.32	0.24	27.73
CU4	189.61	0.31	28.42
NI2	20.65	0.24	54.87
NI4	20.82	0.65	145.67

Soil chemical analyses

Soil organic C, total N, lime content, pH(H_2O), pH(KCl), cation exchange capacity (CEC) was determined with standard methods. The "total" (aqua regia) and "available" (NH_4-acetate-EDTA soluble) fraction of element was determined with ICP spectrometry.

Colony forming units of some microbial groups

Microbial population (cultivables) was investigated by plate dilution method with different agar media. Soil suspension from 10-fold dilution series was spread on the surface of sterile agar plates. The visible colonies were counted and dilutions having 20 to 200 colonies were selected for calculations. Nutrient agar was used to count aerobic heterotrophic (copiotrophic) bacteria. The same media but the C-source in 100-times dilution was used for oligotrophic bacteria. Number of heat resistant spores were also counted on nutrient agar after heating of soil dilutions at 80°C for 10 min. Ashby agar was selected to count *Azotobacter* spp free-living nitrogen-fixing bacteria. Filamentous fungi were counted on Martin agar. Number of Actinomycetes was counted on glucose-casein agar (Szegi 1979).

Microbial biomass C

Soil microbial biomass C was measured by chloroform fumigation extraction (Vance et al. 1987). Briefly, 25 g of dry equivalent moist soil was fumigated with ethanol-free chloroform. The following day the chloroform was removed by repeated vacuum. After extraction of 1 to 4 soil:solute ratio with 0.5 M K_2SO_4 solution filtered through Schleicher & Schuell 589[3] paper and the organic C was determined by

Table 2. Mean logarithm CFU values of different groups of cultivable microbes plated from soil samples 6 year after metal pollution. An asterisk shows significant difference ($p<0.05$) from the control.

	Spores of aerobic bacteria	Actinomycetes	Copiotrophic bacteria	Oligotrophic bacteria	Microscopic fungi	*Azotobacter* spp.
Control	6.45	7.17	7.26	7.72	4.91	4.45
CD2	6.42	6.95*	7.32	7.68	5.06*	4.12*
CD4	6.51	6.94*	7.16	7.87	5.07*	3.39*
CU2	6.41	6.95*	6.98*	7.45*	4.94	4.48
CU4	6.32*	7.20	7.28	7.60	5.02	4.08*
NI2	6.37	6.95*	7.34	7.51*	5.07*	4.55
NI4	6.27*	7.15	7.11	7.55*	5.16*	4.38
$LSD_{0.05}$	0.055	0.059	0.096	0.079	0.060	0.119

redox titration with Mohr-salt solution after wet digestion with potassium dichromate and sulphuric acid and phosphoric acid (2:1 ratio). Soil microbial biomass was calculated as the difference of control and fumigated sample in organic C multiplied by 2.63 (Vance et al. 1987).

Results and Discussion

Six years after the metal amendments still significant accumulation can be observed in the plough layer (Table 1) however a slow decrease is shown. The identical application rates resulted in various enhancement compared to the background level of these metals. Cadmium at 90 and 810 kg ha^{-1} rates resulted in 80 and 670-fold increase; copper 2 and 9-fold increase; nickel 2 and 6-fold increase to the background level. Among the three investigated elements the cadmium is the most dangerous (Máthéné Gáspár et al. 2004) because of the easy uptake and accumulation in plants (Máthéné Gáspár et al. 2004) and toxicity for all livings.

The response of different groups of soil microbes altered to the heavy metal loads (Table 2). Copiotrophic bacterial number decreased only at low dose copper pollution while oligotrophs showed higher sensitivity to copper and nickel load and their number increased at high cadmium load. The strong selection pressure of cadmium load decreasing the interspecific competition and higher abundance of metal-tolerant population can explain it. Number of actinobacteria decreased by both level of cadmium loads and the low copper and nickel doses. The greatest metal load effect could be observed on the number of *Azotobacter* resulted in a significant decrease in both cadmium and high copper load. The only increase can be observed in case of microscopic fungi at both levels of cadmium and nickel while their number was unaffected by copper. Microbial biomass C was significantly reduced by all metal loads compared to control (Fig. 1). Microbial C to total soil organic was also decreased in both cadmium and the higher doses of copper and nickel treatments which is in agreement in other observations (Fliessbach et al. 1994). The studied soil microbial properties responded in different ways to the heavy metal loads. The cadmium contamination caused the highest changes while the effect of nickel was negligible on the soil microbes because of the weak solubility. Furthermore, other environmental factors might also influence these characteristics. Structural changes within the microbial assemblage can be occurred resulted from the metal driven selection.

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