

Research Articles

Effect of Clay Minerals on Immobilization of Heavy Metals and Microbial Activity in a Sewage Sludge-Contaminated Soil

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Abstract

Background, Aims, and Scope. Reducing heavy metal solubility and bioavailability in contaminated area without removing them from the soil is one of the common practices in decreasing the negative impacts on the environment and improving the soil quality. Therefore, our aim was to study the effect of clay minerals: Na-bentonite, Ca-bentonite, and zeolite applied to a contaminated soil on immobilization of heavy metals, as well as on some soil parameters related with microbial activity.

Methods. A soil derived from sewage sludge was incubated with clay minerals of either Na-bentonite, Ca-bentonite, or zeolite for 111 days (d). During the incubation experiment, concentrations of water soluble Zn, Cd, Cu, and Ni were measured after extraction of 2 g air-dry soil with 50 ml of H₂O for 2 h. After the water extraction, the soil sediment was extracted with 50 ml of 1 M NH₄NO₃ for 2 h to estimate the exchangeable amounts of heavy metals. Furthermore, soil microbial respiration, microbial biomass C, C_{org} mineralization, metabolic quotient (qCO₂), and inorganic N were also investigated.

Results and Discussion. Water extractable and exchangeable forms of heavy metals were changed by incubation and addition of clay minerals. Incubation of soil without addition of clay minerals (control) increased water extractable Cu by 12, 24 and 3.8% of initial content after 21, 62, and 111 d of incubation, respectively. The water extractable Zn decreased by 9% during 62 d of incubation and it tended to increase by 14% at the end of the incubation, as compared with the initial soil. Water extractable Cd decreased by 71, 66 and 33% of initial content, and Ni decreased by 54, 70, and 58%, after 21, 62, and 111 d of incubation, respectively. During the incubation experiment, the exchangeable form of all tested metals was decreased by incubation. The addition of clay minerals led to a significant decrease in water soluble and exchangeable forms of heavy metals during the incubation experiment, resulting in low metal extractability. The reduction in metal extractability was greater due to the addition of Na-bentonite or Ca-bentonite than that due to the addition of zeolite. During the first 3 weeks after addition of clay minerals, the studied biological parameters were not affected. However, as incubation progressed, the addition of Na- or Ca- bentonite led to a significant increase in soil respiration, microbial biomass C, C_{org} mineralization, and inorganic N; and a significant decrease in qCO₂. This result is explained by sorption of heavy metals on Na-bentonite and Ca-bentonite and strong reduction of their toxicity.

Conclusions. Our results clearly show that the addition of clay minerals, especially of Na-bentonite and Ca-bentonite, decreased the extractability of four metals during incubation. The decreased metal extractability was accompanied by an increase of soil respiration, C_{org} mineralization, microbial biomass C, and inorganic N and a decrease of metabolic quotient (qCO₂), showing positive effect of clay mineral addition on soil biological parameters.

Recommendations and Outlook. The use of Na-bentonite and Ca-bentonite is promising tool for reduction the extractability and possible toxicity of heavy metals in sewage sludge-contaminated soil. Therefore, the soils polluted with heavy metals may be ameliorated by addition of clay minerals, especially Na-bentonite and Ca-bentonite.

Keywords: Bentonite; clay minerals; heavy metals; inorganic N; metabolic quotient; microbial biomass C; organic matter mineralization; soil respiration; zeolite

Introduction

Disposal of sewage sludge to land can be beneficial because it contains plant nutrients (especially N and P) and organic matter. However, sewage sludge-applied heavy metals adversely affect biological and biochemical functions in soil (Fließbach 1994, Moreno et al. 1999, Chander and Brookes 1993). It is evident that heavy metals introduced with compost or sewage sludge caused the accumulation of soil organic matter (SOM) and decreased the turnover rate of organic matter, presumably because of inhibitory effects on microbial biomass (Leita et al. 1999, 1995, Chander et al. 1995, Chander and Brookes 1991). Moreno et al. (1999) found that the addition of cadmium-contaminated sewage sludge compost to the soil decreased microbial biomass C and stimulated the metabolic activity of the microbial biomass. The toxicity of heavy metals depends mainly on their bioavailability, and therefore on their chemical forms in both organic and inorganic matrices (Leita et al. 1999). The soluble and exchangeable fractions of heavy metals are the most important pools regarding toxicity and bioavailability of heavy metals. Thus, it is very important to reduce metal solubility and bioavailability in contaminated soils. In situ immobilization of HM in contaminated soil is one of the common practices reducing the negative impacts on the environment and improving the quality of contaminated soils (Oste et al. 2002). Welp (1999) reported that the sorption and immobilization of toxic metals in soil is an effective detoxification process and thus an essential part of the buffer

capacity of soil. Immobilization of heavy metals caused an increase in basal respiration, litter decomposition, and microbial activity (Kiikilä et al. 2001). There are several ways for immobilization of heavy metals in soil. Many additives have been screened for their potential to immobilize heavy metals in soil (Oste et al. 2002, Mench et al. 1998). The additives must obviously possess a high binding capacity, but they should not impair soil structure, fertility, and nutrient availability. Immobilization could be carried out by adding natural and synthetic chemical additives such as alkaline materials, phosphate minerals, iron and manganese oxides, aluminosilicates or coal fly ashes (Mench et al. 1998). Due to their very large specific surface area, clay minerals are the most promising materials interacting with a big variety of contaminants (Prost and Yaron 2001).

Therefore, the aim of this work was to study the effect of clay minerals: Na-bentonite, Ca-bentonite, and zeolite applied to sewage sludge-contaminated soil on water extractable and exchangeable forms of heavy metals (Zn, Cd, Cu, and Ni), as well as on soil respiration, soil organic matter mineralization, microbial biomass C, metabolic quotient (qCO_2) and the release of inorganic N.

1 Material and Methods

1.1 Soil characteristics

Soil samples were collected from a site located 15 km north-east of Stuttgart, in the southwest of Germany, where sewage sludge was deposited 50 years ago. According to FAO-UNESCO (1997), the soil type is an Urbic Anthrosol. The German name for this soil type is Reduktosol (Lehmann et al. 2000). The soil samples were taken from the 15- to 35-cm layer, corresponding to Ah2 horizon. X-ray diffraction analysis to determine the soil clay minerals was performed using a Siemens Instrument (D-500) with Cu-K α radiation (Zarei et al. 2001). In the clay fraction, poorly crystalline clay minerals of kaolinite, illite, and vermiculite/smectite were detected. This soil had a high content of C_{org} and $CaCO_3$ and a very high content of heavy metals (Table 1). The soil was air-dried and sieved to pass through a 2-mm sieve before the experiment and chemical analysis.

Table 1: Physico-chemical properties of soil derived from sewage sludge

Properties	Value
Particle size distribution [%]	
Sand	42
Silt	40
Clay	18
Chemical properties	
pH	7.0
CaCO ₃ , %	8.9
Organic C, %	13.3
Total N, %	1.7
Total heavy metal content [mg kg⁻¹]	
Zn	4500
Cd	77
Cu	2000
Ni	442
Pb	1650

1.2 Soil additives

Three clay minerals of Na-bentonite, Ca-bentonite, and zeolite (Fa. IKO Minerals GmbH, 45772 Marl) were used as additives to the soil as treatments. Na-bentonite and Ca-bentonite used in this study are composed of clay minerals of the smectite group, mostly montmorillonite (62–70%). Ca-bentonite with smectites that are predominantly occupied with Ca²⁺-ions originated from Bavarian mines. Na-bentonite with smectites whose initial composition of alkaline-earth-cations has been replaced with Na-ions in a technical process (alkali-activation). Clinoptilolite (zeolite) is the most abundant naturally occurring zeolite consisting of hydrated aluminosilicate with alkali and alkaline earth cations in three-dimensional crystal structure. Zeolite is composed of clay minerals of clinoptilolite (80%), montmorillonite (10%), and feldspar (5%).

1.3 Incubation experiment set-up

Each clay mineral (0.5 g) was added to 25 g soil. The soil-clay mineral mixtures were put in glass vessels (250 ml). Distilled water was added to each soil mixture to bring it to 70% of water retention capacity. A soil without addition of clay minerals was incubated as control. Small vials with 5 ml of 1 M NaOH solution were placed in vessels to trap CO₂. The NaOH solution in the vials was changed after 10, 15, 21, 30, 39, 45, 54, 62, 74, 83, 95, and 111 d. After the addition of NaOH, the vessels were closed air tight and incubated 111 d at 35°C. This temperature was chosen, because optimal temperature for microbial activity ranges from 20 to 35°C (Scheffer and Schachtschabel 2002).

1.4 Analyses

Soil pH was measured with a glass electrode using a soil-to-0.01 M CaCl₂ ratio of 1:2.5. Total soil C and N were measured by a LECO 2000 CN analyser. The inorganic carbon was determined using a Scheibler apparatus (carbonates were dissolved with 10% HCl and the volume of released CO₂ was measured). The carbonate concentrations were calculated using the universal Gas Law (Schlichting et al. 1995). Soil organic carbon was calculated as differences between total carbon and inorganic carbon. The soil texture was determined by a pipette method (Schlichting et al. 1995). The total content of Zn, Cd, Cu and Ni was determined by aqua regia microwave digestion method. Microwave digestion was performed using 10 ml of aqua regia with a nine-stage programme; microwave power ranged from 0 to 750 watt, temperature ranged from 70 to 105°C, and digestion time for 30 min. Heavy metal concentrations were determined by atomic-absorption spectrometry (AAS Perkin Elmer 3100). To validate the method for accuracy and precision, certified reference materials were analysed for each element.

1.4.1 Determination of water-soluble and exchangeable forms of heavy metals

After 21, 62, and 111 d of incubation, the soil was destructively sampled, concentrations of water soluble Zn, Cd, Cu, and Ni were measured after extraction of 2 g air-dry soil

with 50 ml of H₂O for 2 h. After the water extraction, the soil sediment was extracted with 50 ml of 1 M NH₄NO₃ for 2 h to estimate the exchangeable amounts of heavy metals (Schlichting et al. 1995). Heavy metal content in the filtered solution was analysed by AAS. The concentrations of sum of the water extractable and 1 M ammonium nitrate-extractable metal were calculated as percent of total metal content.

1.4.2 Estimation of soil microbial biomass C

Soil microbial biomass C was measured after 21, 62, and 111 d by a fumigation-extraction method (Vance et al. 1987). In this technique, three replicates of each treatment were fumigated with ethanol-free chloroform for 24 h at 25°C. Following this, the soil samples were extracted with 0.5 M K₂SO₄ for 30 min. Three replicates of non-fumigated soil samples were extracted similarly. The extracted C was determined by dichromate oxidation at 100°C (2 ml of extraction + 1.5 ml 15M H₂SO₄ + 1.5 ml saturated aqueous solution of K₂Cr₂O₇). The residual K₂Cr₂O₇ was determined photometrically at 565 nm (Kuzakov, 1997). The calibration of the extracted C measurements was carried using glucose. Microbial biomass C was calculated as Ec/k -factor, where Ec is organic C extracted from fumigated soils minus organic C extracted from non-fumigated soils and the k -factor = 0.45 (Jørgensen 1996, Wu et al. 1990).

1.4.3 Determination of soil respiration

CO₂ evolved during the incubation was trapped in 1 M NaOH and the excess of NaOH was titrated with 0.1 M HCl after addition of BaCl₂ (Black 1965). Soil microbial respiration was expressed as mg CO₂-C released per day and gram of soil (Moreno et al. 1999). Mineralized C_{org} was calculated as cumulative CO₂-evolution (% of total organic C) (Leifeld et al. 2002). The specific respiration activity (qCO₂) was expressed as the production of CO₂-C per unit biomass C and time (Anderson and Domsch 1978).

1.4.4 Determination of soil inorganic N

Soil inorganic N (NH₄-N and NO₃-N) in 0.02 M CaCl₂ extracts (1:4 soil-to-solution ratio) from all treatments was determined using an autoanalyser (Fa. Scalar).

1.5 Quality control

Quality control was achieved by analysing reference materials independently prepared from the standards, especially for determination of heavy metals, total N, total C, and pH. The accuracy of the methods was verified by subjecting standard reference material to the overall analytical procedures. The chemicals used were purchased from E. Merck (A.R., 99.9%). The used vessels were washed with 30% HNO₃, then rinsed three times with deionised water and dried in an oven. All stages of sample preparation, analysis, and measuring were carried out in a clean environment.

1.6 Statistical analysis

Differences among treatments were tested by separate two-way ANOVAs and subsequent post-hoc comparisons of means (LSD test, at $P=0.05$). Furthermore, simple correlation was used to establish the relationships between water extractable or exchangeable forms of heavy metals and biological parameters at the end of the incubation.

2 Results and Discussion

2.1 Effect of incubation time and clay minerals addition on extractability of heavy metals

The water extractable and exchangeable forms of heavy metals were changed by incubation and addition of clay minerals. For all the treatments, water extractable Cd and Ni concentrations decreased significantly during the first 3 weeks of incubation (Fig. 1). In the untreated soil (control), water extractable Cd decreased by 71, 66 and 33% of initial content, and Ni decreased by 54, 70, and 58% of initial content, after 21, 62, and 111 d of incubation, respectively. The reduction in water extractable Cd and Ni concentrations can be explained by their retention by soil organic matter and calcium carbonate (El-Bassam and Stühmeier 1978, Tyler and McBride 1982, McBride et al. 2000). Sewage sludge constituents also can lower Cd activity in soil solution via complexing by soluble ligands or colloidal surfaces (Bell et al. 1991). Gray et al. (1998) reported that soil organic matter was important in controlling Cd sorption and desorption. Our results showed that the rate of decrease of water extractable Cd tended to decline, as incubation time progressed; mainly due to formation of soluble Cd-organic associations over the time of incubation. Neal and Sposito (1986) found that soil sorption of Cd was reduced by the formation of soluble-organic associations in the aqueous solution.

In contrast, water extractable Cu in the untreated soil increased and it did not change much with time. The results showed that water extractable Cu increased by about 12, 24, 3.8% of initial content after 21, 62, and 111 d of incubation, respectively. While, water extractable Zn decreased by about 1.5% at 21 d and 9% at 62 d, but it increased by about 14% of initial content at 111 d. Thus, it did not also change much with time. These increases in water extractable Zn (at 111 d) and Cu (especially up to 62 d) may be explained by dissolved organic matter, which promoted the dissolution of Cu and Zn by building organic complexes. Almas et al. (2000) found that addition of organic matter increased the solubility of Zn by the formation of organo-metallic complexes. In this study, the increase in water extractable Cu tended to decrease at the end of the incubation. This result is attributed to the tendency of soluble Cu to bind with organic matter or solid phase materials. Organic matter in soluble and insoluble forms plays contrasting roles in controlling total soluble Cu, and other metals as well. Tyler and McBride (1982) reported that Cu has a great tendency to form more strongly covalent bonds to organic ligands.

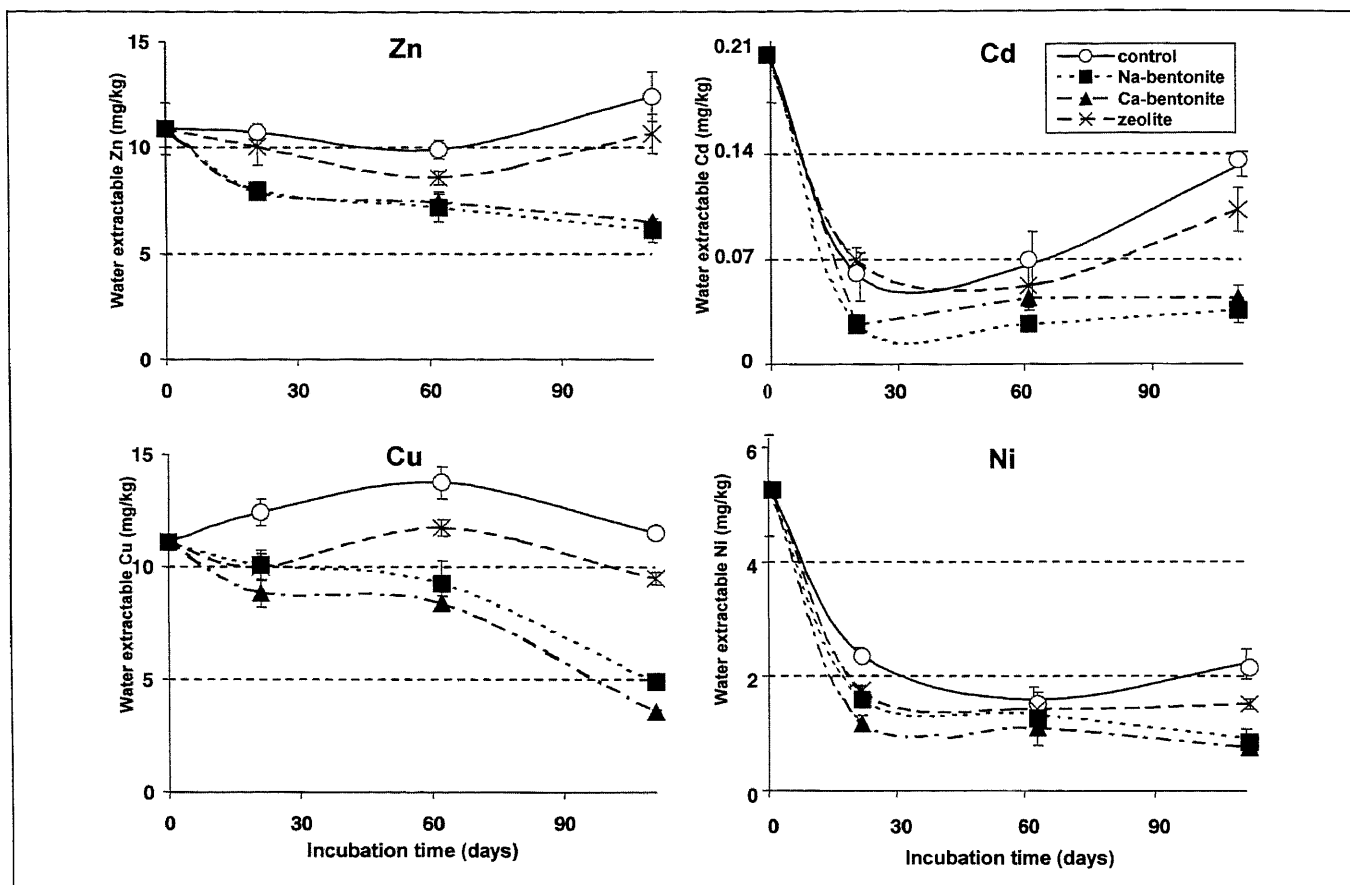


Fig. 1: Changes in water extractable heavy metals after clay mineral addition during the incubation experiment. Mean \pm SE; where absent, bars fall within symbols

The results indicated that the exchangeable form of all tested metals was reduced by incubation in all treatments (data not shown). During the first 3 weeks of incubation, in untreated soil, the exchangeable form of heavy metals decreased by 21% for Zn, by 38% for Cd, by 23% for Cu, and by 46% for Ni as compared with initial soil. After that, these decreases of exchangeable metals changed very little or almost remained constant up to the end of the incubation.

The water soluble and exchangeable heavy metals are a very important pool regarding their toxicity, lability, and bioavailability. Before incubation, the initial percent of total metal content extracted by H_2O and 1 M NH_4NO_3 was 1.13, 1.96, 2.88, 5.26% of total content, for Zn, Cd, Cu, and Ni, respectively (Table 2). In untreated soil (control), this initial percent of all tested metals was decreased by incubation. This interpreted that incubation of sewage sludge soil caused a reduction in extractability of heavy metals. This reduction in metal extractability may be attributed, in part, to the neutral pH, the presence of free calcium carbonate, and in part to the high organic matter content. This result concurs with that of McBride et al. (2000), who found that strong retention of heavy metals occurred in sewage sludge soil, which has a high organic matter and lime content. Ong and Bisque (1968) explained the phenomena of metal reduction by the fact that humic substances behave as negatively charged colloids, which may be coagulated by

the electrolytes. Another phenomenon is that after the release of heavy metals from the sewage sludge they react chemically with the soil so that metal toxicity declines with time (Lewin and Beckett 1980).

The decrease of water extractable and exchangeable forms of heavy metals in the soil treated with clay minerals was greater than without clay minerals. This result indicates that the water soluble and exchangeable forms of heavy metals were redistributed to other forms by clay mineral addition during the incubation experiment. As compared with untreated soil, the addition of clay minerals led to a significant decrease in water extractable (14% to 75%) and exchangeable (12% to 42%) forms of heavy metals. The highest reduction was due to the addition of Na- or Ca-bentonite, especially for water soluble forms of heavy metal (see Fig. 1). For example, addition of clay minerals decreased water extractable heavy metals at the end of the incubation as follows: Zn decreased by 51, 48, and 14%, Cd by 75, 68, and 25%, Cu by 57, 69 and 17, and Ni by 59, 63, and 29% for Na-bentonite, Ca-bentonite, and zeolite, as compared with untreated soil, respectively. While, addition of clay minerals decreased the exchangeable Zn by 31, 27, and 19%, Cd by 22, 23, and 16%, Cu by 40, 42, and 16%, and Ni by 27, 28, and 20% for Na-bentonite, Ca-bentonite, and zeolite, as compared with untreated soil, respectively (data not shown for exchangeable metals).

Table 2: Effect of clay minerals on the extractability of heavy metals (sum of water soluble and exchangeable metal concentration during the incubation experiment)

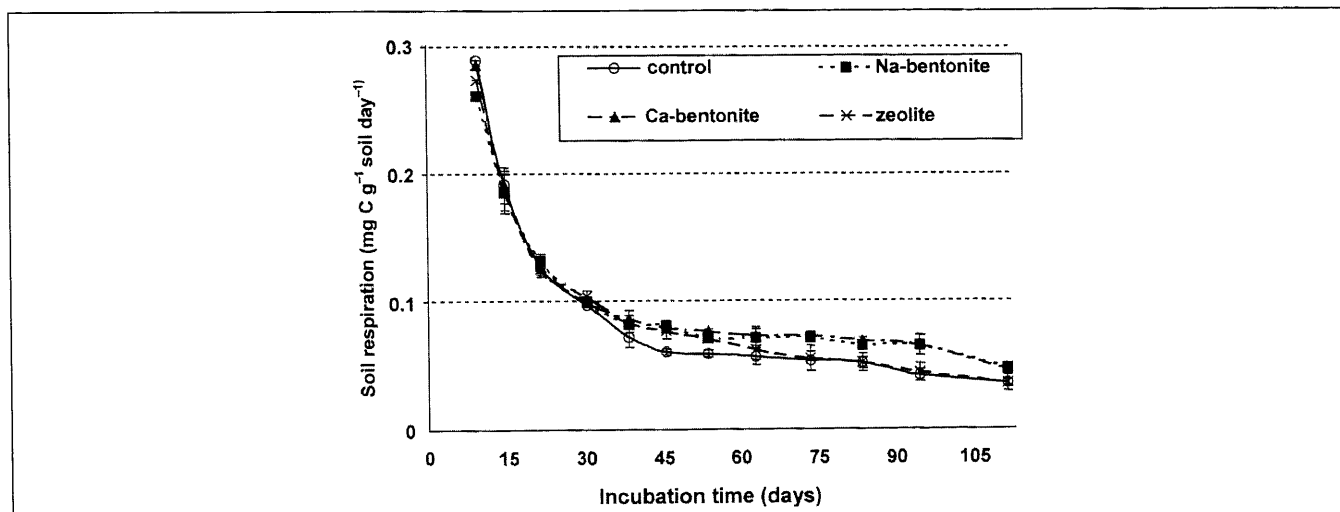
Treatments	Incubation time (days)			
	Initial (day 0)	21 d	62 d	111 d
Zn				
Control	1.13	0.94	0.87	0.92
Na-bentonite	–	0.73	0.70	0.58
Ca-bentonite	–	0.72	0.70	0.62
Zeolite	–	0.90	0.74	0.76
LSD ($P=0.05$)		0.13	0.13	0.10
Cd				
Control	1.96	1.12	1.17	1.33
Na-bentonite	–	1.04	0.97	0.94
Ca-bentonite	–	1.05	1.01	0.94
Zeolite	–	1.04	1.07	1.10
LSD ($P=0.05$)		NS	0.15	0.31
Cu				
Control	2.88	2.40	2.49	2.22
Na-bentonite	–	2.21	2.0	1.24
Ca-bentonite	–	2.05	1.91	1.13
Zeolite	–	2.26	2.36	1.86
LSD ($P=0.05$)		0.24	0.45	0.22
Ni				
Control	5.26	2.74	2.38	2.31
Na-bentonite	–	2.43	2.08	1.53
Ca-bentonite	–	2.25	2.04	1.49
Zeolite	–	2.62	2.09	1.81
LSD ($P=0.05$)		0.28	0.20	0.32

NS: Not significant

In general, the lowest percent of total metal content extracted by H_2O and NH_4NO_3 was found in the soil treated with either Na-bentonite or Ca-bentonite during the incubation experiment (see Table 1). This result suggests that Na-bentonite and Ca-bentonite have a strong effect on decrease of metal extractability more than zeolite. This could be explained by high contents of the clay minerals in montmorillonite. Ca-bentonite and Na-bentonite have a higher proportion of montmorillonite (62–70%) than for zeolite (10%). Bentonites are known as good adsorbents for heavy metals (Tiller et al. 1984; Stockmeyer and Kruse, 1991).

2.2 Effect of incubation time and clay minerals addition on soil microbial respiration, organic C mineralization, microbial biomass C, metabolic quotient, and N mineralization

Soil microbial respiration is expressed as $mg\ CO_2-C$ released per day and gram of soil and is a useful index for measuring soil microbial activity (Moreno et al. 1999). For all treatments, soil microbial respiration decreased with incubation time (Fig. 2). This result suggests that the easily biodegradable pool of organic C was gradually exhausted with time. During the first month of incubation, there were no significant differences in the effects of all treatments on soil mi-

**Fig. 2:** Effect of clay mineral addition on soil microbial respiration ($mg\ CO_2-C\ g^{-1}\ soil\ day^{-1}$) during the incubation experiment. Mean \pm SE; where absent, bars fall within symbols

crobal respiration. As incubation time increased, however, the addition of Na- or Ca-bentonite led to a significant increase in the soil microbial respiration compared with untreated soil. At the end of the incubation, the values of soil microbial respiration amounted to 0.035 (control), 0.047 (with addition of Na- or Ca-bentonite), and 0.036 mg CO₂-C g⁻¹ soil day⁻¹ (with addition of zeolite). Soil basal respiration is a widely used parameter to monitor microbial activity and soil organic matter break down (Pascual et al. 1999). Our results suggest that the increased soil microbial respiration, in soil treated with Na- or Ca-bentonite, was reflected as increased C_{org} mineralization. During the incubation, the cumulative C_{org} mineralized amounted to 7.24 (control), 7.99 (with addition of Na-bentonite), 8.25 (with addition of Ca-bentonite), and 7.43% of total organic C (with addition of zeolite) (data not shown). Addition of bentonites increased microbial respiration and then organic matter mineralization, because it reduced the availability of heavy metals to soil microorganisms. This result concurs with Kiiikilä et al. (2001), who reported that immobilization of heavy metals caused an increase in basal respiration, litter decomposition, and microbial activity.

The soil microbial biomass was used as a sensitive indicator of changing soil conditions (Leita et al. 1999). The control soil initially contained about 16.2 mg biomass C g⁻¹ C_{org}. For all treatments, microbial biomass C increased during the first 3 weeks of incubation, as compared to the initial soil. After day 21, microbial biomass C decreased with incubation time (Fig 3). This result indicates greater availability of the mineralizable material at the beginning and stabilization of sludge organic matter with increase of incubation time (Pascual et al. 1997). During the first 3 weeks of incubation, there were no significant differences in the ef-

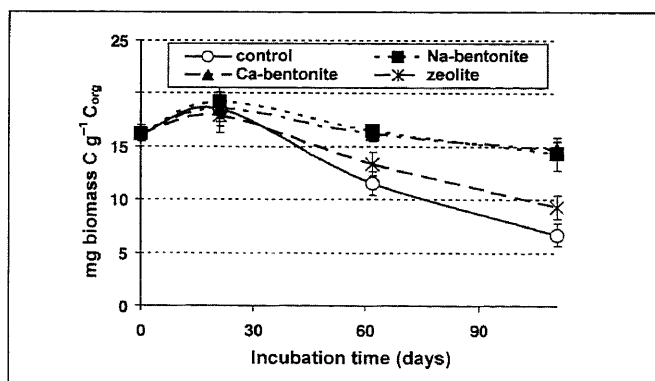


Fig. 3: Changes in microbial biomass C after clay minerals addition during the incubation experiment. Mean \pm SE; where absent, bars fall within symbols

fects of all treatments on the microbial biomass C levels. However, after 62 and 111 d of incubation, the addition of Ca-bentonite or Na-bentonite increased microbial biomass C significantly compared to untreated soil (control). Addition of zeolite increased microbial biomass C significantly only at the end of the incubation compared with untreated soil (control). It was observed that there were no significant effects on biomass C between Ca-bentonite and Na-bentonite, but the significant differences were found between bentonites and zeolite at 62 and 111 d of incubation. At day 21, microbial biomass C values were 18.5 (control), 19.2 (with addition of Na-bentonite), 18.6 (with addition of Ca-bentonite) and 17.9 mg g⁻¹ C_{org} (with addition of zeolite). At the end of the incubation (111 d), they were 6.7, 14, 15 and 9 mg g⁻¹ C_{org}, respectively. At the end of the incubation, clay mineral application increased the microbial biomass C content in soil about 53% for Na-bentonite, 55% for Ca-bentonite, and 28% for zeolite, as compared to untreated soil. The higher values of biomass C in soil treated with Na- or Ca-bentonite are due to decreased microbial toxicity of metals.

The metabolic quotient (qCO₂) is a relationship between soil respiration and microbial biomass C and is expressed as mg CO₂-C h⁻¹μg⁻¹ biomass C (Anderson and Domsch 1978). The qCO₂ can be used as a marker of the environmental stress of the microbial population (Anderson and Domsch 1993, Moreno et al. 1999). Our results showed that qCO₂ was higher in untreated soil (control) than in soil treated with clay minerals as incubation progressed (Table 3). The higher values of qCO₂ at the end of the experiment in the control were due to the lower values of microbial biomass C than in soil treated with clay minerals. The lower values of microbial biomass C and thus the higher values of the qCO₂ found in control at the end of the incubation are also related to the high availability of heavy metals (Leita et al. 1999).

The control soil initially contained 1.33 g inorganic N kg⁻¹ soil. After 21 d of incubation, this initial value of inorganic N decreased, but not significantly in all treatments (Table 4). After that, the inorganic N content of all treatments began to steadily increase until the end of the experiment; mainly due to mineralization of soil organic matter (SOM) and subsequent release of inorganic N. At 21 and 62 d of incubation, no significant differences were found between all treatments in inorganic N. However, after 111 d of incubation, addition of Na-bentonite or Ca-bentonite increased significantly amounts of inorganic N compared to untreated soil (control) and addition of zeolite. At the end of the incubation, amounts of inorganic N increased by about 1.8 and 2.2 and 0.35 g N kg⁻¹ soil for Na-bentonite, Ca-bentonite,

Table 3: Changes in the metabolic quotient (qCO₂) after clay mineral addition during the incubation experiment

Treatments	Incubation time (days)			LSD (P=0.05)
	21 d	62 d	111 d	
Control	2.16	1.54	1.67	0.42
Na-bentonite	2.15	1.35	1.03	0.13
Ca-bentonite	2.15	1.40	0.99	0.14
Zeolite	2.19	1.45	1.24	0.19
LSD (P=0.05)	NS	NS	0.32	

NS: Not significant

Table 4: Changes in the amounts of inorganic N in soil after clay mineral addition during the incubation experiment

Treatments	Incubation time (days)			LSD ($P=0.05$)
	21 d	62 d	111 d	
Control	0.90	1.84	3.06	0.73
Na-bentonite	1.09	2.19	4.89	0.84
Ca-bentonite	0.99	2.34	5.25	0.97
Zeolite	1.15	2.06	3.28	0.91
LSD ($P=0.05$)	NS	NS	0.97	

NS: Not significant

Table 5: Simple correlation coefficient (r) between water extractable or exchangeable forms of heavy metals and biological parameters at the end of the incubation

Biological Parameters	Water extractable heavy metals				Exchangeable heavy metals			
	Zn	Cd	Cu	Ni	Zn	Cd	Cu	Ni
Soil respiration	-0.74	-0.84	-0.82	-0.72	-0.74	-0.67	-0.72	-0.66
Mineralized C_{org}	-0.92	-0.92	-0.93	-0.88	-0.81	NS	-0.87	-0.77
Biomass C	-0.80	-0.78	-0.90	-0.80	-0.76	-0.68	-0.80	-0.60
qCO_2	0.80	0.77	0.82	0.78	0.80	NS	0.86	0.77
Inorganic N	-0.86	-0.81	-0.89	-0.72	-0.77	NS	-0.89	NS

NS: Not significant

and zeolite, respectively. The extractability of heavy metals in soil treated with Na-bentonite or Ca-bentonite was less than that in untreated soil and in soil treated with zeolite. This result is related to higher amounts of inorganic N in soil treated with Na-bentonite and Ca-bentonite. N mineralization is very sensitive to heavy metal pollution (Liang and Tabatabai 1977, 1978).

2.3 Relationships between water extractable or exchangeable forms of heavy metals and biological parameters at the end of the incubation

Simple correlation coefficient (r) between water extractable or exchangeable forms of heavy metals and biological parameters are reported in Table 5. The results suggest that the water extractable or exchangeable forms of metals were negatively related with soil respiration, C_{org} mineralized, biomass C, and inorganic N. However, they were positively related with metabolic quotient (qCO_2). This result indicated that the immobilization of water soluble and exchangeable forms of toxic metals in soil, due to the addition of bentonites, is an effective detoxification process. Any of the studied biological parameters may be used as a biomarker to measure metal toxicity. Among the studied metals, the exchangeable Cd was not significantly related with C_{org} mineralization, qCO_2 , and inorganic N. Moreover, the exchangeable Ni was not significantly related with inorganic N.

3 Conclusions

An addition of Na-bentonite or Ca-bentonite decreased the extractability of heavy metals during incubation. The decreased metal extractability over the time of incubation was reflected as increased soil respiration, C_{org} mineralization,

microbial biomass C, and inorganic N and as decreased metabolic quotient (qCO_2), as compared to untreated soil and addition of zeolite. The use of Na-bentonite and Ca-bentonite is a promising tool for a reduction of the extractability and possibly toxicity of heavy metals in sewage sludge-contaminated soil. Therefore, the soils polluted with heavy metals may be ameliorated by addition of Na-bentonite and Ca-bentonite.

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References

- Almas AR, McBride MB, Singh BR (2000): Solubility and lability of cadmium and zinc in two soils treated with organic matter. *Soil Sci* 165, 250–259
- Anderson JPE, Domsch KH (1978): A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biol Biochem* 10, 215–221
- Anderson T, Domsch KH (1993): The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of the soil. *Soil Biol Biochem* 25, 393–395
- Bell PF, James BR, Chaney RL (1991): Heavy metal extractability in long-term sewage sludge and metal salt-amended soils. *J Environ Qual* 20, 481–486
- Black CA (1965): *Methods of soil analysis*. American Society of Agronomy, Inc., Madison, Wisconsin, USA

- Chander K, Brookes PC (1991): Microbial biomass dynamics during the decomposition of glucose and maize in metal-contaminated and non-contaminated soils. *Soil Biol Biochem* 23, 917–925
- Chander K, Brookes PC (1993): Residual effects of zinc, copper and nickel in sewage sludge on microbial biomass in a sandy loam. *Soil Biol Biochem* 25, 1231–1239
- Chander K, Brookes PC, Harding SA (1995): Microbial biomass dynamic following addition of metal-enriched sewage sludge to a sandy loam. *Soil Biol Biochem* 27, 1409–1421
- El-Bassam N, Stühmeier K (1978): Immobilisierung einiger Spuren- und Schadelemente durch Zufuhr organischer Siedlungsabfälle. *Mittelgen Dtsch Bodenkdl Ges* 27, 221–230
- FAO-UNESCO (1997): Soil map of the world, revised legend, with corrections. *World resources report 60*, FAO Rome. Reprinted as Technical Paper 20, ISRIC (Int. Soil Reference and Information Center) Wageningen, The Netherlands
- Fließbach A, Martens R, Reber HH (1994): Soil microbial biomass and microbial activity in soil treated with heavy metal contaminated sewage sludge. *Soil Biol Biochem* 26, 1201–1205
- Gray CW, McLaren RG, Roberts AHC, Condorn LM (1998): Sorption and desorption of cadmium from some New Zealand soils: Effect of pH and contact time. *Aust J Soil Res* 36, 199–216
- Jørgensen RG (1996): The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{EC} value. *Soil Biol Biochem* 28, 25–31
- Kiikilä O, Perkiömäki J, Barnette M, Derome J, Pennanen T, Tulisalo E, Fritze H (2001): In situ bioremediation through mulching of soil polluted by a copper-nickel smelter. *J Environ Qual* 30, 1134–1143
- Kuzyakov YV (1997): The role of amino acids and nucleic bases in turnover of nitrogen and carbon in soil humic fractions. *Eur J Soil Sci* 48, 121–130
- Lehmann AEH, Holland K, Stahr K (2000): Flows and chemical fractions in a soil from sewage sludge. *Zeitschrift für Kulturtechnik und Landentwicklung* 41, 206–210
- Leifeld J, Siebert S, Kögel-Knabner I (2002): Biological activity and organic matter mineralization of soil amended with bio-waste composts. *J Plant Nutr Soil Sci* 165, 151–159
- Leita L, De Nobili M, Muhlbachova G, Mondini C, Marchiol L, Zerbi G (1995): Bioavailability and effects of heavy metals on soil microbial biomass survival during laboratory incubation. *Biol Fertil Soils* 19, 103–108
- Leita L, Nobili MD, Mondini C (1999): Influence of inorganic and organic fertilization on soil microbial biomass, metabolic quotient and heavy metal bioavailability. *Biol Fertil Soils* 28, 371–376
- Lewin VH, Beckett PHT (1980): Monitoring heavy metal accumulation in agricultural soils treated with sewage sludge. *Effluent and Water Treatment Journal* 20, 217–221
- Liang CN, Tabatabai MA (1977): Effects of trace elements on nitrogen mineralization in soils. *Environ Pollut* 12, 141–147
- Liang CN, Tabatabai MA (1978): Effects of trace elements on nitrification in soils. *J Environ Qual* 7, 291–293
- McBride M, Martinetz CE, Topp E, Evans L (2000): Trace metal solubility and speciation in a calcareous soil 18 years after no-till sludge application. *Soil Sci* 165, 646–656
- Mench M, Vangroensveld J, Lepp NM, Edwards R (1998): Physico-chemical aspects and efficiency of trace element immobilization by soil amendments. pp 151–182. In: Vangroensveld J, Cummingham SD (ed), *Metal-Contaminated Soils: In Situ Inactivation and Phytoremediation*. Springer-Verlag and R.G. Landes Company, Berlin, Germany
- Moreno JL, Hernandez T, Garcia C (1999): Effects of a cadmium-contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil. *Biol Fertil Soils* 28, 230–237
- Neal RH, Sposito G (1986): Effects of soluble organic matter and sewage sludge amendments on Cd sorption by soils at low Cadmium concentrations. *Soil Sci* 142, 164–172
- Ong H, Bisque RE (1968): Coagulation of humic colloids by metal ions. *Soil Sci* 106, 220–224
- Oste LA, Lexmond TM, Van Riemsdijk WH (2002): Metal immobilization in soils using synthetic zeolites. *J Environ Qual* 31, 813–821
- Pascual JA, Garcia C, Hernandez T (1999): Lasting microbiological and biochemical effects of the addition of municipal solid waste to an arid soil. *Biol Fertil Soils* 30, 1–6
- Pascual JA, Garcia C, Hernandez T, Ayuso M (1997): Changes in the microbial activity of an arid soil amended with urban organic wastes. *Biol Fertil Soils* 24, 429–434
- Prost R, Yaron B (2001): Use of modified clays for controlling soil environmental quality. *Soil Sci* 166, 880–894
- Saggar S, Parshotam A, Sparling GP, Feltham CW, Hart PBS (1996): ^{14}C -Labelled ryegrass turnover and residence time in soils varying in clay content and mineralogy. *Soil Biol Biochem* 28, 1677–1686
- Scheffer F, Schachtschabel P (2002): *Lehrbuch der Bodenkunde*. 15. Auflage. Spektrum Akademischer Verlag Heidelberg, Berlin, Germany
- Schlichting E, Blume HP, Stahr K (1995): *Bodenkundliches Praktikum*, 2nd ed. Blackwell, Berlin, Germany
- Schulthess CP, Huang CP (1990): Adsorption of heavy metals by silicon and aluminum oxide surfaces on clay minerals. *Soil Sci Soc Am J* 54, 679–688
- Stockmeyer M, Kruse K (1991): Adsorption of Zn and Ni ions and phenol and diethylketones by bentonites of different organophilicities. *Clay Min* 26, 431–434
- Tiller KG, Gerth J, Brümmer G (1984): The relative affinities of Cd, Ni, and Zn for different soil clay fractions and goethite. *Geoderma* 34, 17–35
- Tyler LD, McBride MB (1982): Mobility and extractability of cadmium, copper, nickel, and zinc in organic and mineral soil columns. *Soil Sci* 134, 198–205
- Vance ED, Brookes PC, Jenkinson DS (1987): An extraction method for measuring microbial biomass C. *Soil Biol Biochem*, 19, 703–707
- Welp G (1999): Inhibitory effects of the total and water-soluble concentrations of nine different metals on the dehydrogenase activity of a loess soil. *Biol Fertil Soils* 30, 132–139
- Wu J, Jørgensen RG, Pommerening B, Chaussod R, Brookes PC (1990): Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. *Soil Biol Biochem* 22, 1167–1169
- Zarei M, Sommer M, Stahr K (2001): Neoformed Halloysite in podzols developed on the Bärhalde granite, southern Black Forest, Germany. A Clay Odyssey, proceedings of the 12th International Clay Conference, Bahia Blanca, Argentina, pp 227–234

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