

## Long term effects of heavy metal enriched sewage sludge disposal in agriculture on soil biota

Kirsten Stöven\* and Ewald Schnug\*

### Abstract

Long term effects of heavy metal pollution caused by decennial sewage sludge disposal ( $12 \text{ t ha}^{-1} \text{ yr}^{-1}$ ) from 1980 to 1990 on soil biota of an experimental field in Northern Germany were investigated 16 years after the last sludge application. The plots still exist to date as permanent pasture. The consequences of the soil treatment were shown by means of heavy metal concentrations of soil and earthworms. Additional effects on soil micro-organisms by enzyme activities were investigated.

The disposal of heavy metal enriched sewage sludge caused increased soil heavy metal concentrations compared to mineral fertilised control plots. The increased soil heavy metal concentration was transferred to soil organisms. The earthworm biomass from the sewage sludge treated plots bore increased heavy metal concentrations. Cadmium showed the highest transfer factor from soil to earthworms. The amount of transfer into biomass is specific for each element and depends on heavy metal soil concentration. Despite the higher heavy metal concentration of earthworm biomass, the number of earthworms was increased in soil of the sewage sludge treated plots which is attributed to the enhanced soil organic matter (SOM) concentration. The number of earthworms did not correlate significantly with SOM but the weight of earthworms did.

The microbial activities of dehydrogenase and alkaline phosphatase enzymes were reduced in soil of the sewage sludge treated plots compared to the control plots. The enzyme activities of cellulase and protease however were enhanced by the application of heavy metal enriched sewage sludge compared to the control plots. On the one hand sewage sludge addition enhanced soil organic matter concentration; on the other hand its microbial decomposition was retarded by toxic effects of heavy metals due to reduced enzyme activities.

**Keywords:** *Sewage sludge, heavy metals, earthworms, microbial enzyme activities*

### Zusammenfassung

#### **Biotische Nachwirkungen der Befruchtung landwirtschaftlicher Böden mit schwermetallreichem Klärschlamm**

Die biotischen Auswirkungen einer durch zehnjährige Klärschlammentsorgung ( $12 \text{ t ha}^{-1} \text{ a}^{-1}$ ) verursachten Schwermetall-Belastung eines Bodens in Norddeutschland wurden 16 Jahre nach der letzten Schlammbehandlung untersucht. Bewertet wurden die Gehalte an Schwermetallen in Boden und Regenwürmern sowie die Enzymaktivitäten von Mikroorganismen im Boden. Die Ausbringung von mit Schwermetallen angereichertem Klärschlamm verursachte im Vergleich zur mineralischen Düngung erhöhte Schwermetallgehalte im Boden, welche auf Bodenorganismen übertragen wurden. Regenwürmer von Böden mit Klärschlammapplikation zeigten ebenfalls erhöhte Schwermetallkonzentrationen. Cadmium wies den höchsten Transferfaktor vom Boden zum Regenwurm auf. Die Höhe des Transferfaktors war elementspezifisch und abhängig von der Schwermetallkonzentration im Boden. Trotz der höheren Schwermetallkonzentration in der Biomasse der Regenwürmer war deren Anzahl im Boden der mit Klärschlamm behandelten Fläche erhöht, was auf den gleichfalls gesteigerten Gehalt an organischer Substanz (SOM) zurückgeführt wird.

Die Aktivitäten von Dehydrogenase und alkalischer Phosphatase waren in Klärschlamm-behandelten Böden vermindert. Cellulase und Protease-Aktivitäten wurden hingegen durch die Applikation von mit Schwermetallen angereichertem Klärschlamm erhöht. Klärschlamm-Befruchtung erhöhte somit zwar der Gehalt an organischer Substanz im Boden, die toxische Wirkung der Schwermetalle verzögerte jedoch den biologischen Abbau des Klärschlammes.

**Schlüsselworte:** *Klärschlamm, Schwermetalle, Regenwürmer, mikrobielle Enzymaktivitäten*

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\* Julius Kühn-Institut (JKI), Institute for Crop and Soil Science, Bundesallee 50, 38116 Braunschweig, Germany

## 1. Introduction

Sewage sludge is a by-product of wastewater treatment and described by Förstner (2004) as a “metal-containing waste material”. Sewage sludge however contains also reusable plant nutrients, essential trace elements and organic matter (Parat et al., 2005; Hamell and Holzwarth, 1999; Stevenson and Cole, 1999; Benickser, 1997). Thus the disposal of sewage sludge as fertiliser on agricultural land has become a common practice (Pescod, 1992) which was introduced in European countries during the 1970's (Giller et al., 1998). However sewage sludge can also be a hazardous waste as for heavy metals (Creamer et al, 2008). The heavy metals are associated with the organic matter (Hamell and Holzwarth, 1999; Stevenson and Cole, 1999). McBride (1995) used the term “time bomb” for sewage sludge to underline the danger of sewage sludge disposal on soil and explain that adsorbed heavy metals could be released in the course of organic matter decomposition, and affect herbal and faunal organisms. Long term effects of arable soil pollution caused by heavy metal enriched sewage sludge were assessed 16 years after the last application. Heavy metal concentrations in soil and earthworm biomass as well as microbial enzyme activities were used as indicators.

## 2. Material and methods

### 2.1 Site and soil treatment

Investigations were carried out on soil from a long-term field trial which is located at the former Federal Research Centre for Agriculture (FAL) in Braunschweig, Germany (10° 27' E, 52° 18' N, 81 m height above sea level). The soil is composed of 46 % sand, 47 % silt and 7 % clay hence it is characterized as a sandy loam / loam or Cambisol, respectively; after FAO (2006) or USDA classification. Field plots were treated with sewage sludge which was enriched with heavy metals up to the permitted threshold levels of the German Sewage Sludge Ordinance which was in force at beginning of the experiment (German Federal Ministry of the Interior (1982)). Heavy metals were added as water soluble chlorides.

The sewage sludge was stored anaerobic for six weeks to enable transformation of heavy metal chlorides to less available organic or sulphidic species (Fließbach, 1991; Stöven et al., 2005). Total heavy metal concentrations in the sludge applied from 1980–1990 were (mg kg<sup>-1</sup>): Cd 65, Cr 2610, Cu 2011, Ni 387, Pb 2166, Zn 6652. Elements not enriched as As, Fe, Mn, Mo and U are also considered in this study. Results of chemical and biological investigations are compared to samples of plots treated with 90 kg ha<sup>-1</sup> yr<sup>-1</sup> N (= control plots).

### 2.2 Analytical methods

Soil was dried and sieved to 2 mm particle size. The total heavy metal concentrations of soil and earthworms were determined by acidic microwave digestion.

Microwave digestion of dry sample material followed the VDLUFA (2000) method for total phosphate concentration. 0.50 g sample material was digested in 6 ml HNO<sub>3</sub> (65 %) and 1.5 ml H<sub>2</sub>O<sub>2</sub> (30 %) in a PFA vessel at 120 °C for 5 min. and approximate 200 °C for 15 min. After cooling and filtering the extract was transferred to a 50 ml graduated flask and made up to 50 ml with aqua bidist.. The quantitative determination of heavy metals was performed with ICP-MS.

Earthworms were collected from soil and fixed in formalin according to DIN 23611-1:2006, rinsed with tap water and subsequently dried at 70 °C in an oven. Dried earthworms were ground by means of a ball mill (Retsch RS 1, Retsch, Haan, Germany) with heavy metal free zirconium tools.

The soil organic matter (SOM) concentration was determined by loss of ignition at 550 °C in a muffle furnace (DIN EN 12879).

Microbial parameters

Microbial enzyme activities of alkaline phosphatase (APA), cellulase (CLA) and protease (PTA) were determined according to Schinner et al. (1996). The method of Schinner et al. (1996) for microbial enzyme activity of dehydrogenase (DHA) was modified for only 40 % of the recommended soil weight.

### 2.3 Data analysis

The significance of heavy metal effects on soil and soil biota was evaluated using the least significance difference (LSD) test provided by the SPSS 12.0 software (SPSS, Chicago IL).

## 3. Results and discussion

### 3.1 Effect of sewage sludge application on soil parameters

The total heavy metal concentration of disposed sewage sludge from 1980-1990 is composed of the native sewage sludge heavy metal concentration and added heavy metals (Stöven et al 2005). The total amounts of heavy metals applied with sewage sludge were presented in materials and methods, site and soil treatment. Beside sludge disposal soil heavy metal pollution is constantly global caused by atmospheric depositions due to traffic emissions (Hildebrand et al., 2005, Legret and Pagotto, 2006, Li et al. 2007) and industrial processes. Also applied agricultural pesticides and fertilisers can pollute agricultural soil by heavy metals. The decennial application of heavy metal enriched sewage

Table 1:

Soil heavy metal concentrations in a Northern German Cambisol 16 years after the last sewage sludge application

Plot	Cd	Cr	Cu	Ni	Pb	Zn
Control plots	0.1	1.2	0.5	0.8	0.3	1.5
Sewage sludge treated plots	0.2	1.5	0.6	1.0	0.4	1.8
LSD5%	0.1	0.2	0.1	0.2	0.1	0.3

sludge on agricultural soil increased the soil concentrations of Cd, Cr, Cu, Ni, Pb and Zn significantly (Table 1).

Soil concentrations of As and Fe which were not enriched in the applied sludge were also increased, however not significantly. The soil concentration of Mn was not affected by sewage sludge addition. The soil concentration of Mo was also increased significantly (Table 1). The soil concentration of U was not increased by sewage sludge addition (Table 1).

### 3.2 Soil and soil organic matter (SOM)

The decennial sewage sludge application caused a significant increase in soil organic matter concentration (Stöven et al. 2005; Table 2). The maintained soil organic matter concentrations support the "time bomb hypothesis" of McBride (1995).

Table 2:

Soil organic matter concentration, number and fresh weight of earthworms 16 years after the last sewage sludge disposal in a Cambisol in Northern Germany

Plot	SOM [%]	Number of earthworms [no m <sup>-2</sup> ]	Weight of earthworms [g m <sup>-2</sup> ]
Control plots	3.6	66	28.4
Sewage sludge treated plots	5.0	76	47.8
LSD5%	0.3	26.4	14.5

SOM = soil organic matter  
LSD = last significant difference

Heavy metals in soil occur in soil in different chemical species (Stevenson and Cole, 1999) and also in different association with soil organic matter. In sewage sludge heavy metals are mostly organically bound (Benickser, 1997). This relation is verified in the investigations reported here through correlations for Cd, Mo, Ni, Zn and Cr (Table 3).

### 3.3 Effects of long term sewage sludge application on earthworms

The presented results refer to the native occurring earthworms of the investigated site that were extractable by formalin. The number of earthworms was increased not significantly in soil of sewage sludge treated plots due to the enhanced soil organic matter (SOM) concentration. At the control plots with 3.6 % SOM, 66 earthworms m<sup>-2</sup> were found compared to 76 earthworm m<sup>-2</sup> at 5 % SOM in sewage sludge treated plots (Table 2). However, their biomass (EWB) was increased significantly in sewage sludge treated plots as has also been reported by (Lee, 1985). But also, the correlation between the number of earthworms and SOM was not significant (Table 2).

Earthworms are exposed to the soil properties of their surrounding habitat. They also feed on the soil, respectively the soil organic matter (Kizilkaya, 2005) and therefore it was expected that the element composition of earthworms is also affected by long term sewage sludge application. The heavy metal concentrations of earthworms clearly reflected the increase of soil heavy metal concentrations caused by sewage sludge application (Table 4).

Earthworms collected from sewage sludge treated plots showed generally increased heavy metal concentrations compared to those collected from control plots (Table 4). Significantly increased were the concentrations of Cd, Cr, Cu, Ni, Pb and Zn.

Ireland (1983) reported heavy metal accumulation in earthworms both in contaminated and not-contaminated soils. Earthworms feed on soil and can indicate soil pollution by heavy metals due to heavy metal accumulation in their biomass (Morgan and Morgan, 1993; Kizilkaya, 2004). In this study the accumulation factor of As, Cd, Cu, Fe, Mo and Zn in earthworms from control plots exceeded the accumulation factors of these elements in sewage sludge treated plots (Table 5). The accumulation of heavy metals in tissue makes earthworms useable as bio-indicator to monitor hazardous soil pollutions for human and environment (Edwards, 2004; Hinton and Veiga, 2002). However, heavy metal accumulation in earthworms is not only determined by soil heavy metal concentrations (Nahmani et al., 2005).

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The observed moderately high Cd concentration in earthworms which is not significantly correlated to the soil Cd concentration (Table 3) and taking into account the high transfer factor suggests an immobilisation of Cd in the tissue of earthworms.

### Molybdenum

Mo is an essential component of several animal and microbial enzymes (Anke, 2004).

In this investigation Mo was only transferred from soil to earthworms in sewage sludge treated plots but not in the control plots. Furthermore the Mo concentration of earthworms is significantly correlated to the soil Mo concentration (Table 3). Also Yoshida et al. (2005) found Mo transfer factors > 1. Asawalam and Johnson (2007) found increased Mo concentrations in soil in the direct vicinity of earthworms which might be derived from earthworm casts. Tomati et al. (1996) assume that casts are settled by micro-organisms rich in Mo-containing enzymes for N-fixation which might be ingested with soil.

### Zinc

Zinc is an essential trace element of all living systems (Peganova and Eder, 2004) and is dynamically regulated after uptake by earthworms (Eijsackers, 2004). The results of the research presented here also show a Zn concentration with significant correlation between concentrations in soil and earthworms (Table 3). The high transfer factor for Zn in control plots suggests also an active absorption of Zn by earthworms.

### 3.4 Effects of long term sewage sludge application on microbial enzyme activities

The metabolic activity of soil micro-organisms is essential for organic matter turnover. The microbial community in soil responds to changes in the organic matter composition and environmental conditions in a shift in metabolic rates (Schinner et al., 1996).

Giller et al (1998) assume that micro-organisms are far more sensitive to heavy metal soil pollution than soil fauna. The authors' differentiate between chronic and acute effects of heavy metals on micro-organisms.

By all means, 26 years after the first application of heavy metal enriched sewage sludge effects observed in enzyme activities are chronic ones.

The enzyme dehydrogenase is involved in intracellular metabolism as oxidoreductase (Schinner et al., 1996). The activity of dehydrogenase activity (DHA) is thought to reflect total oxidative activity of soil micro-organisms (Ladd, 1978). Alkaline phosphatase activity (APA) is mainly produced by micro-organisms in order to mineralise organically bound P-components of organic matter (Schinner et al., 1996). The cellulase activity (CLA) refers to the cleavage of cellulose the most abundant natural organic compound in

soil and belongs to the carbon metabolism. Proteins in soil derive from dead plants and animals which decomposition is performed by microbial protease (PTA) enzymes of the nitrogen metabolism (Schinner et al., 1996).

Table 6:

Microbial enzyme activities in soil 16 years after the last sewage sludge application

Treatment	DHA [µg TPFg <sup>-1</sup> dm 24 h <sup>-1</sup> ]	APA [µg p-NP g <sup>-1</sup> dm h <sup>-1</sup> ]	CLA [µg GE g <sup>-1</sup> dm 24 h <sup>-1</sup> ]	PTA [µg tyr g <sup>-1</sup> dm 2 h <sup>-1</sup> ]
Control plots	220	1084	22772	539
Sewage sludge treated plots	153	890	89586	628
LSD <sub>5%</sub>	11	69	9258	12
DHA	= dehydrogenase activity			
APA	= alkaline phosphatase activity			
CLA	= cellulolytic activity			
PTA	= proteolytic activity			
LSD	= last significant difference			

The microbial activities of dehydrogenase and alkaline phosphatase were significantly decreased by sewage sludge addition but the activities of cellulase (CLA) and protease (PTA) were increased (Tab. 6). The investigated enzymes are involved in the degradation of soil organic matter. Thus significant negative correlations of DHA and APA with soil organic matter were found (Table 3). The enzyme activities of CLA and PTA however were significantly positively correlated to soil organic matter (Table 3). This result is yielded by the sum of distinct heavy metal effects on micro-organisms. Heavy metals are associated to soil organic matter and become effective to micro-organisms during decomposition process (McBride, 1995). The microbial enzyme dehydrogenase (DHA) is involved in a wide range of oxidative decomposition processes (Ladd, 1978). DHA was negatively affected with all investigated metals except Cr (Table 3). APA showed a not significant but poor positive correlation to nearly all considered soil heavy metals except to Fe which was positive and significantly correlated. Positive significant correlations between CLA and distinct trace elements and heavy metals, respectively, were found for Mo, Cu, Zn, Ni, Cr, Pb, As and Cd. PTA was significantly positively correlated to Mo, Zn, Cu, Cr, Pb and As (Table 3).

Heavy metals originating from sewage sludge can enhance or reduce microbial enzyme activity depending on their specific concentration and availability and this decides its role between a beneficial trace element and a toxic hazard (Spain, 2003; Kuperman and Carreiro, 1997). The positive correlation of CLA and PTA to As and Cd could be caused by metal tolerant enzymatic active micro-organisms for example (Table 5).

Cd, Mo and Zn which showed high transfer factors to

and accumulation in earthworms were significantly negative correlated to microbial DHA enzyme activity. APA showed not significant smaller positive correlations to these elements while CLA and PTA were significant positive correlated to Cd, Mo and Zn soil concentrations (Table 3).

All correlations between earthworm biomasses and enzyme activities were not significant. Between earthworm biomass and DHA a negative relationship was found. A poor positive correlation to APA was found. The correlation of earthworm biomass to CLA was not significant as also PTA.

## Conclusions

Earthworms and micro-organisms are faced by same soil conditions. Still 16 years after the last sewage sludge application both were affected showing beneficial and adverse effects. Both groups are conducted by their ecological function to decompose organic matter in cooperation. On the one hand earthworms prepare the soil organic matter for successive microbial decomposition by mechanical destruction. Otherwise microbial settlement of organic matter surfaces improves its digestibility by earthworms. In result the degradation of soil organic matter is impeded by heavy metals. Heavy metals were transferred to earthworms presumably introduced to the food chain of earthworm predators. The effects of heavy metals added to soil sustain and are persistent perceivable at all stages of the degradation process of soil organic matter.

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