

# Ecotoxicological assessment of arable field soils fertilized with sewage sludge

Irena Eitminavičiūtė<sup>1</sup>,

Audronė Matusėvičiūtė<sup>1\*</sup>,

Valerijus Gasiūnas<sup>2</sup>,

Milda Radžiūtė<sup>1</sup>,

Neda Grendienė<sup>1</sup>

<sup>1</sup> Institute of Ecology  
of Vilnius University,  
Akademijos 2,  
LT-08412 Vilnius,  
Lithuania

<sup>2</sup> Water Management Institute,  
Lithuanian University  
of Agriculture,  
Parko 6, Vilainiai,  
LT-58102 Kėdainiai distr.,  
Lithuania

The majority of cities have sewage treatment equipment where sludge is accumulated. The use of sludge is problematic even now, under a rapid development of different technologies, and this issue is the object of numerous discussions. Sewage sludge can be used for fertilizing soil, as it is an organic substance containing many biotic substances. However, the use of sewage sludge for fertilizing fields is limited by several factors such as high levels of heavy metals, pathogenic microorganisms and an unpleasant smell. Therefore, the use of this organic fertilizer is regulated by different normative acts.

The study was carried out in 1993–2008 in Vilnius District in *arenosols* (Pakenė, Skurbutėnai, Merešlėnai). The soils of intensively arable fields, especially in poor sandy soils, require special attention while analysing their biological processes related to mineralization and humification. Taking into account the complexity of the mechanism of the processes taking place in the soil, ecotoxicological methods based on biotesting are used for establishing the ecological state of soil. Pedobionts – organisms constantly living in the soil – are used for this purpose.

A single fertilization of arable field loam sandy soils with 8 to 20 t · ha<sup>-1</sup> of sewage sludge significantly improves the agrochemical qualities of the soil, does not contaminate it with heavy metals, and the crop grown on it is clean.

Improvement of agrochemical and biological qualities of the *arenosol* of arable fields is observed only after repeated fertilization of these soils with sewage sludge (20 t · ha<sup>-1</sup> in the fifth year). Then the amount of humus in them increases by 1.8 to 2.6%. The reaction of the soil becomes neutral. Biological processes become more active. The results of the research show that the crops grown on *arenosols* fertilized with sewage sludge is little contaminated with heavy metals and do not exceed the levels allowed for forage crops.

**Key words:** soil, arable fields, sewage sludge, heavy metals, microarthropods

## INTRODUCTION

Soil is the main component of land ecosystems. It is related to the whole biosphere, thus preservation of soil as a live natural body is one of the main, not only ecological, but also economic problems. Due to an intensive anthropogenic pressure and lately also climate change, the negative effect on the soil has become more intense. Data show that relatively natural land ecosystems on the Earth are declining catastrophically. Biological processes perform the main functions in the soils of these ecosystems. The processes of mineralization and humification maintain the balance. The greatest problem is disbalance of the biological processes of intensively arable lands. This happens due to the constant

overcrop in order to produce more agricultural products and the lack of care for the restoration of humus.

Intensively arable fields, especially poor sandy soils, require special attention to preserve the biological processes taking place in them and related to the mineralization and humification of organic substances. Sewage sludge is a fertilizer containing a lot of organics which enriches the soil with humus, nutritive substances (Al-Assiuty et al., 2000). Sewage sludge is widely used for recultivation and fertilization of soil, the possibilities of its use is an object of lively interest (McLaren, Smith, 1996; Kapots et al., 2000). The use of sludge is problematic even now, under a rapid development of different technologies, and this issue is the object of numerous discussions. Ecological methods should be used for evaluating the state of the soil recultivated with sewage sludge (Haimi, 2000). Ecological methods are directly related to soil biota.

\* Corresponding author. E-mail: audrone@ekoi.lt

Soil animals can be used in soil bioremediation processes as potential utilizers of organics and stimulators of activity of microorganisms (Haimi, Huhta, 1987; Edwards, 1998). Soil invertebrate animals can also be used as indicators of soil pollution (Haimi, 2000). The majority of *arenosols* are dominated by microarthropods, and earthworms are found as single organisms. Microarthropods that live in the soil are among the most suitable organisms to reflect changes of the habitat. Not only separate species, but also complexes of microarthropods as well as changes in their structure can be used in bioindication. They often reflect changes in the content of organics, pH and humidity of soil (Pankhurst, 1997).

It is often difficult to evaluate the negative effect of heavy metals on the total number of microarthropods (Bengtsson, Tranvik, 1989). However, even  $3 \text{ mg} \cdot \text{kg}^{-1}$  of cadmium can suppress the development of individual species (Spiel, 1995).

Using different concentrations of cadmium, it has been established that the concentration of  $5 \text{ mg} \cdot \text{kg}^{-1}$  of cadmium allowed in the sludge reduces the total number of microarthropods by half in 30 days. If the amount of cadmium increases 10 times ( $50 \text{ mg} \cdot \text{kg}^{-1}$ ), the number of microarthropods is reduced 1.5 times in 30 days and 2.5 times in 45 days (Eitminavičiūtė ir kt., 2002).

Upon analysing the distribution of microarthropods in soils contaminated with heavy metals, the sensitivity of Mesostigmata, especially of the family Parasitidae mites, which are predatory, to copper concentrations was established (Parmelee et al., 1993). Data on the tolerance of Mesostigmata mites to lead are also available (Hägvar, Abrahamsen, 1990). The distribution of the genus *Uropodina Trachytes* characterizes soils contaminated with lead whereas the genus *Veigaia* mites are very sensitive to lead.

Experiments using two different norms of sewage sludge exploited for recultivation of quarries were carried out (Andres, 1999). It was established that a larger norm of sewage sludge (15%) reduces the total number of microarthropods and their variety: oribatid mites *Punctoribates* sp. totally disappear, while the response of *Hemileius robustus* is opposite. Cryptostigmata and Collembola often show different tendencies. It is related to the content of organics and the humidity of the substratum.

Van Straalen et al. (1989) recommends distinguishing three levels of toxicity of biota pollutants: I – concentration of pollutants is equal to  $LD_{50}$ ; II – concentration is equal to NOEC (“no observed effect”), i. e. a certain value of the concentration in the soil, which produces no observed effect at the moment, should be reduced in the future; III – the concentration that does not produce any actual change in the association. The number of members of soil association at this level of pollution may decrease by no more than 5%. It is important to evaluate the concentrations allowed for soil fertilization as comprehensively as possible.

All these reasons indicate the necessity of immediate measures and new methods-technologies for the preservation of especially poor soils and their ecological evaluation.

The objective of our work was to assess the ecological state of soils of intensively arable fields fertilized with sewage sludge, using biotests, as well as to establish the direction of the biological processes in such soils.

## MATERIALS AND METHODS

The research was carried out in 1993–2008 in Vilnius District in *arenosols*: in Pakenė, Skurbutėnai, Merešlėnai. Arable fields were fertilized with dehumidified sewage sludge from the Vilnius city in two phases: a single fertilization with sludge  $8 \text{ t} \cdot \text{ha}^{-1}$  (Merešlėnai),  $10, 20 \text{ t} \cdot \text{ha}^{-1}$  (Skurbutėnai, Pakenė), and repeated fertilization of the same fields in the third year with  $10 \text{ t} \cdot \text{ha}^{-1}$  sludge (Merešlėnai) and in the fifth year with  $20 \text{ t} \cdot \text{ha}^{-1}$  (Skurbutėnai). The study was carried out on stationary sites – arable fields fertilized with sewage sludge and non-fertilized fields (control).

We analysed the following agrochemical indicators of soil: pH – according to LST ISO 10390 : 2005; humus – according to SVP-4 (GOST 2613-84);  $\text{N-NO}_3$  and  $\text{N-NH}_4 - 2\text{N}$  KCl extract FIA Star was analysed according to SVP-3, mobile  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$  – according to SVP-2 (GOST 26208-91), total nitrogen – according to ISO 11261 : 1995.

The content of heavy metals in the soil was analysed at the Hydrochemical Research Laboratory of the Institute of Geology and Geography, using the atomic absorption spectrometric method and the Varianz device with a flame. The total content of heavy metals was established by extracting  $2\text{M HNO}_3$  according to LST ISO 11047 : 2004 *Soil quality. Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc in aqua regia extracts of soil. Flame and electrothermal atomic absorption spectrometric methods.*

The content of heavy metals in plants was analysed by the atomic absorption spectrometric method at the Agrochemical Research Laboratory of the Lithuanian Institute of Agriculture according to LST EN 14082:2003 *Foodstuffs. Determination of trace elements. Determination of lead, cadmium, zinc, copper, iron and chromium by atomic absorption spectrometry (AAS) after dry ashing.* The concentrations of heavy metals in soil and plants were established in dry material.

The agrochemical indices of soil, concentrations of heavy metals in soil and plants were analysed in autumn, and the biological indices (number of microarthropods, structure of complexes) were analysed in spring and autumn.

The microarthropod complexes were studied by the conventional methods (Гиляров, Стриганова, 1987). Soil samples were taken with a cenometer ( $5 \times 5 \times 5 \text{ cm}$ ) from the top 0–5 cm soil layer with five repetitions in each territory under study. Microarthropods were extracted from the soil using the modified Tullgren–Berlese extractor. Three groups of microarthropods (Oribatida, Gamasina, Collembola) and partially Acaridae were identified before establishing the species.

To analyse species abundance,  $n$  (thousand organisms  $\text{m}^{-2}$ ) and the number of species ( $S$ ) were used. The structure

of complexes is expressed in percentage. The domination of species was established according to P. Cassagnau and O. Rouquet (1962): constant core species (>10%), constant satellite species (5.0–9.9%), constant sparse species (<5.0%).

## RESULTS

### Chemical-biological evaluation of Vilnius sewage sludge

The study has shown that the agrochemical qualities of the sludge used for fertilization may differ depending on the year, on the dehumidification technologies, the use of lime, etc. Therefore, its humidity can fluctuate from 66 to 84% (Table 1). The calcification of sludge is directly reflected in its pH reaction. In 1993–2002 the sludge was alkaline, its pH fluctuated between 11.5–13.2. In later years it was usually close to neutral.

The content of pathogenic microorganisms in the sludge greatly depends on its alkalinity. According to norms, only sludge of A and B categories is suitable for fertilizing fields. Data of our study show that the sludge used for fertilization during the research was mostly of category B (Table 1).

Table 1. Agrochemical data on sewage sludge used for soil fertilization

Index	Year					
	1993	1998	2002	2006	2007	2008
Humidity, %	66.4	70.13	76.8	84.0	67.2	81.0
Dry material, %	33.6	29.87	23.2	16.8	32.8	15.6
Organics matter, %	46.3	49.9	49.9	79.3	71.7	66.0
Common N, % d.m.	1.63	3.2	3.0	3.8	3.8	5.9
Common P, % d.m.	0.75	0.9	0.76	0.59	0.69	4.3
pH	11.5	10.35	9.97	5.5–6.6	6.2	5.1–12.4
Category of pathogenic microorganisms	B	B	B	B (C)	B (C)	B

Table 2. Concentrations of heavy metals (mg · kg<sup>-1</sup>) in sewage sludge used for fertilization

Year	mg · kg <sup>-1</sup>					
	Cu	Pb	Zn	Ni	Cr	Cd
1993	252	20.0	590	105	158	1.14
1998	246	32.0	1540	110	72.0	3.8
2002	200	18.0	650	70.0	60.0	5.0
2005	256	35.0	597	77.0	84.0	8.7
2006	253	38.8	592	81.0	60.3	3.54
2007	247	25.5	517	55.0	47.3	3.55
2008	201	41.0	832	29.0	56.0	3.4
Category II (LAND 20–2005)	75–600	140–500	300–2000	50–300	140–400	1.5–6.0

The concentrations of heavy metals in Vilnius sewage sludge used for fertilizing the fields did not exceed the requirements for category II sludge (Table 2).

### One time fertilization of arable fields with sewage sludge

The study was carried out on two stationary sites of arable fields: in Pakenė (fertilized with 20 t · ha<sup>-1</sup>) and Skurbutėnai (fertilized with 10 t · ha<sup>-1</sup>). The agrochemical analysis showed that the soil was average acid. The pH in non-fertilized soil fluctuated from 5.7 to 6.7. After fertilizing the soil with sewage sludge, its pH came closer to neutral (6.0–6.7). Humus accounts for the average of 1.8–2.0% of the analysed soil (Table 3). After fertilization with sewage sludge it remained in the same brackets as in non-fertilized soil. However, in different years (fourth, fifth and seventh) its increase was established. An increase of the content of biogenic elements, especially of phosphorus, was found in the seventh year. The content of biogenic elements in the soil of arable fields often depends on fertilization with mineral fertilizers and on the crop grown there. This was obvious in the seventh year of the research when the content of phosphorus increased both in the soil fertilized and not fertilized with sludge.

Table 3. Agrochemical data fluctuation in non-fertilized and once fertilized arable land fertilized with sewage sludge (10–20 t · ha<sup>-1</sup>)

Agrochemical analysis	Year							$\bar{x}$	Year				$\bar{x}$
	1	2	3	4	5	6	7		1	2	3	4	
	Fertilized 20 t · ha <sup>-1</sup>								Fertilized 10 t · ha <sup>-1</sup>				
pH	n	5.8	5.6	7	5.5	5.3	7.2	6.0	6.0	6.3	n	n	6.1
Humus, %	n	1.6	1.4	2.4	2.9	1.2	2.9	2.0	n	2.0	n	1.59	1.0
Common N, %	n	0.07	0.12	0.19	0.09	0.06	0.16	0.12	0.1	0.087	n	n	0.09
m. N, mg/100g	n	n	41.5	n	14.5	21.6	74.1	37.9	6.16	7.51	n	n	6.83
m. P <sub>2</sub> O <sub>5</sub> , mg/100g	n	135	153	550	176	215	570	299	18.1	30.2	n	19.4	22.5
m. K <sub>2</sub> O, mg/100g	n	62.6	54	70	69	25.5	137	69.6	15.6	17.6	n	12.4	15.2
	Not fertilized (control)								Not fertilized (control)				
pH	5.9	n	n	5.0	n	n	6.8	5.9	6.17	5.4	n	n	5.7
Humus, %	1.5	n	n	2.06	n	n	2.3	1.8	2.08	2.4	n	1.7	2.06
Common N, %	0.07	n	n	0.17	n	n	0.14	0.11	0.115	0.086	n	n	0.10
m. N, mg/100g	n	n	n	n	n	n	n	n	7.84	7.4	n	n	7.6
m. P <sub>2</sub> O <sub>5</sub> , mg/100g	115.6	n	n	175.8	n	n	428	197	15.5	26.3	n	17.3	19.7
m. K <sub>2</sub> O, mg/100g	66.6	n	n	82.5	n	n	133	85	15.6	24.6	n	15.2	18.4

n – not analysed.

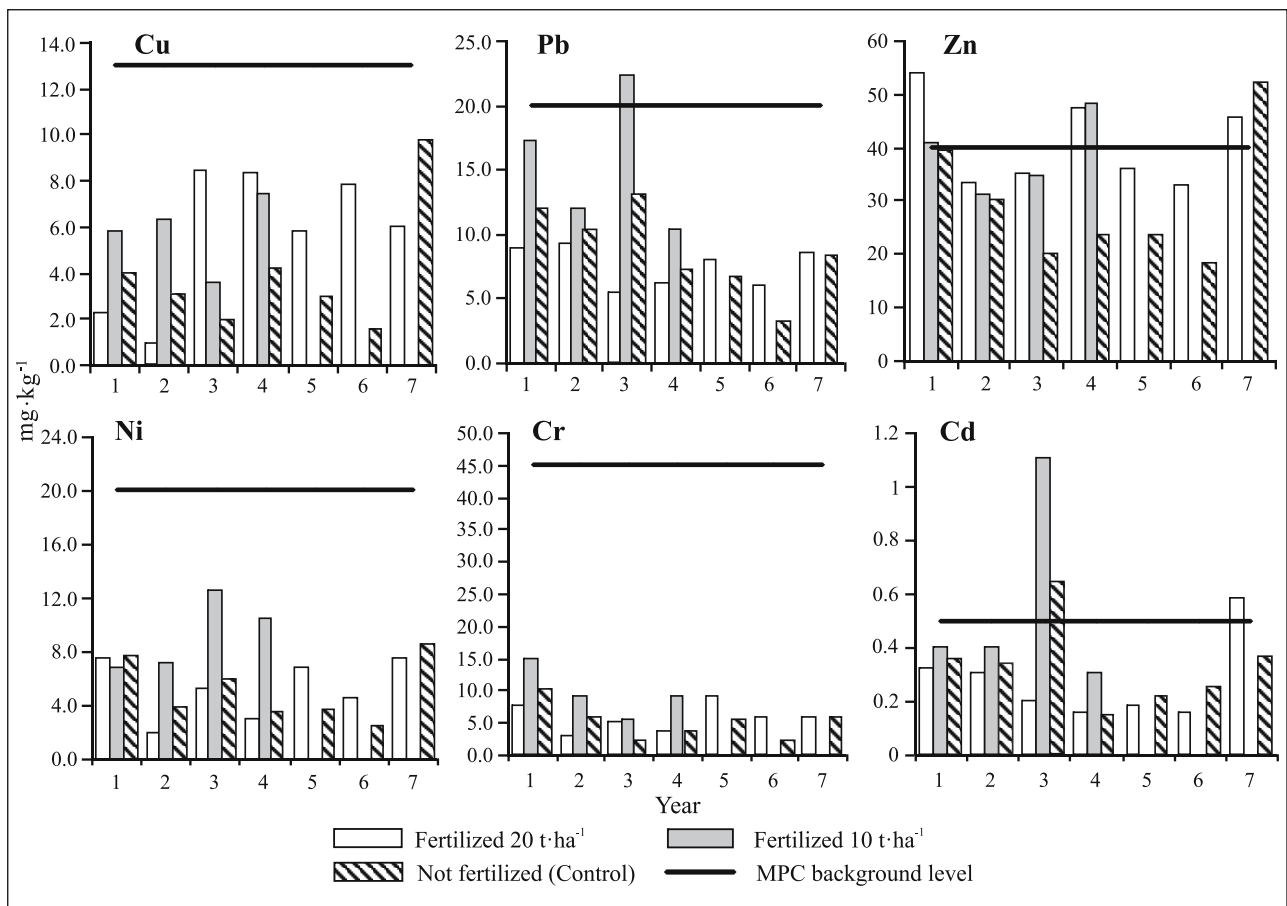


Fig. 1. Fluctuations of heavy metal concentrations in soils fertilized with sewage sludge one time  $10 \text{ t} \cdot \text{ha}^{-1}$  (over 4 years) and  $20 \text{ t} \cdot \text{ha}^{-1}$  (over 7 years), and in non-fertilized soil (control)

The concentrations of heavy metals were not high in the soils and did not reach the background levels allowed for such soils (LAND 20–2005). More Zn was found in separate years (Fig. 1).

After fertilizing the soil of arable fields with  $20 \text{ t} \cdot \text{ha}^{-1}$  sewage sludge, the concentrations of heavy metals were higher in the first year in comparison with non-fertilized soil: Cd – 1.3, Zn – 1.2 times, while the others were similar both in fertilized and non-fertilized fields (Fig. 1).

Fluctuations of the levels of heavy metals in the soil fertilized with  $10 \text{ t} \cdot \text{ha}^{-1}$  sewage sludge were slight. In four years, the concentrations of the majority of heavy metals were larger in the field fertilized with sewage sludge: Cr – 2.2, Cu and Ni – 1.3, Pb – 1.0, Zn 1.4 times; only the concentration of Cd remained similar to that in the soil not fertilized with sewage sludge (Fig. 1). In our opinion, the increase of the concentrations of heavy metals in soils fertilized with a single norm of sewage sludge (3, 4 and 7 years) is related to fertilization of the crops with mineral fertilizers.

The concentrations of heavy metals were analysed not only in the soil but also in the crops. Upon fertilizing the soil with  $10 \text{ t} \cdot \text{ha}^{-1}$  sewage sludge, the concentrations of Cu, Ni, Zn were slightly larger in barley and oats in the first and second year, but they did not exceed the levels allowed for forage

crops (Table 4). After fertilizing the soil with  $20 \text{ t} \cdot \text{ha}^{-1}$  sewage sludge, oats were grown there for the first year. The concentrations of all heavy metals were greater in the fertilized field than in the crop, but they were very small and did not exceed the norms allowed for forage crops (Table 4). The concentrations of heavy metals decreased 1.2 times in the crop grown in the fertilized field in the second year as compared to the first. The content of Cr, Cu and Ni decreased most significantly. The concentrations of Cr and Cu in barley became levelled with those in barley grown in a non-fertilized field in the third year. These fields were additionally fertilized with mineral fertilizers in the third and fourth years. In the fifth and sixth years, the content of heavy metals in perennial grass in both fields decreased greatly.

The results of the research show that the harvest of crops grown after fertilization of *arenosols* with sewage sludge is little contaminated with heavy metals and does not exceed the levels allowed for forage crops. Most of heavy metals are accumulated by crop in the first year of fertilization. The increase of the concentrations of heavy metals in the soil in later years may be related to fertilization of these fields with mineral fertilizers.

Microarthropod complexes in the soil were analysed in the first, second and seventh years after a single fertilization with

Table 4. Concentrations of heavy metals (mg · kg<sup>-1</sup>) in crops grown on soil one-time fertilized with sewage sludge (10–20 t · ha<sup>-1</sup>) in not fertilized soil

Study object	Year	Grown harvest	Fertilized						Not fertilized (control)					
			Cd	Cr	Cu	Ni	Pb	Zn	Cd	Cr	Cu	Ni	Pb	Zn
			mg · kg <sup>-1</sup>						mg · kg <sup>-1</sup>					
Pakenė 20 t · ha <sup>-1</sup>	1	oat corns	0.011	1.04	3.1	1.1	0.10	24.7	0.009	0.91	2.90	0.73	0.093	21.5
	2	oat corns	0.028	0.27	2.34	0.79	0.11	20.6	0.027	0.27	1.21	0.67	0.10	19.1
	3	barley corns	0.089	0.12	3.32	0.19	0.08	31.6	0.039	0.12	2.28	0.22	0.06	15.6
	4	oat corns	0.060	0.18	2.57	1.30	0.03	31.2	0.079	0.24	2.35	1.61	0.03	30.5
	5	perennial grasses	0.035	0.56	3.63	0.52	0.13	14.1	0.021	0.78	3.58	0.37	0.12	13.0
	6	perennial grasses	0.026	0.49	3.71	0.65	0.11	11.5	0.024	0.48	3.23	0.35	0.11	11.3
	7	fallow	n	n	n	n	n	n	n	n	n	n	n	n
Skurbutėnai 10 t · ha <sup>-1</sup>	1	barley corns	0.009	0.25	2.02	0.25	0.15	18.3	0.015	0.2	1.4	0.19	0.15	16.9
	2	oat corns	0.081	0.55	2.42	1.25	0.28	30.0	0.07	0.45	1.95	0.88	0.36	27.8
	3	mixture	0.16	3.21	5.0	3.46	2.75	22.0	0.17	2.62	5.5	3.2	2.45	22.8
	4	triticale	0.043	0.16	5.75	0.32	0.23	42.0	0.04	0.13	5.62	0.3	0.23	46.0
MPC for forage crops			0.3	0.5	30.0	3.0	5.0	20.0						
Direktyve 2002/32 EC for food crops									1.0			5.0		

n – not analysed.

sewage sludge. The number of microarthropods in this soil (control sample) fluctuated from 16.5 to 43.1 thou ind. m<sup>-2</sup>. Oribatida dominated among microarthropods, accounting for 31–50 % (Table 5). The nucleus of the association of microarthropods was composed of small euribiotic Oribatida species. It was dominated by *Micropopia minus*, *Oppiella nova*. Up to 30 types of microarthropods were found; their number could be up to 10.5 thou ind. m<sup>-2</sup>. A single fertilization of the soil with 10–20 t · ha<sup>-1</sup> sewage sludge in the first year reduced the number of microarthropods and the variety of their species (Table 6). Regrouping took place in the complex of microarthropods. Soil species were displaced by compost and raptorial Gamasina species and later by rapidly reproducing Acaridae. This was especially obvious in the second year when the number of microarthropods increased up to three times, and Acaridae mites *Caloglyphus radionovi* dominated the nucleus of the association. In the seventh year, this soil was dominated by the complex of microarthropods characteristic to agroecosystems (Table 6). The domination of Oribatida *Tectocephus velatus* in the nucleus of the association shows impoverishment of the soil.

Table 5. Microarthropod number (thou ind. m<sup>-2</sup>) and species variety in Vilnius district *arenosols*

Group	Skurbutėnai		Merešlėnai		Pakenė	
	Thou. ind. m <sup>-2</sup>	%	Thou. ind. m <sup>-2</sup>	%	Thou. ind. m <sup>-2</sup>	%
Oribatida	10.4	49.0	31.2	31.2	8.3	49.6
Gamasina	1.1	5.1	3.3	19.0	1.6	9.6
Acaridae	1.1	5.1	0.3	2.0	0.3	3.5
Tarsonemina	4.0	18.8	2.1	12.1	0.3	1.3
Other mites	3.1	15.0	3.2	18.4	1.2	7.1
Collembola	1.5	7.0	3.0	17.3	4.8	28.6
Total	21.2		43.1		16.5	
Sum of species	21		18		15	

### Repeated fertilization of arable fields with sewage sludge

Repeated fertilization of arable fields with sewage sludge was also analysed in two fields: in Merešlėnai (repeated fertilization in the third year, 10 t · ha<sup>-1</sup>) and Skurbutėnai (repeated fertilization in the fifth year, 20 t · ha<sup>-1</sup>).

After repeated fertilization with 10 t · ha<sup>-1</sup> sewage sludge in the third year, the reaction of the soil changed from close to neutral to neutral (pH 6.8–7.2) (Table 7) within the five years of research, and after 20 t · ha<sup>-1</sup> fertilization in the fifth year the reaction of the soil became neutral. Meanwhile the soil in the non-fertilized field gradually became acid. The content of humus after repeated fertilization of the fields with sewage sludge 10 t · ha<sup>-1</sup> increased from 1.8 to 2.2%, and after 20 t · ha<sup>-1</sup> fertilization it increased from 2.2 to 2.3 on average during the study period. However, the content of humus reached 2.9% in individual years. In the field fertilized with 20 t · ha<sup>-1</sup>, the content of humus was rather stable throughout the seven years of research and fluctuated between 2.0 and 2.9%. Thus, repeated fertilization of soil with sewage sludge 10 and 20 t · ha<sup>-1</sup> enriches it with humus as compared with non-fertilized soil. The content of biogenic elements was also increased (Table 7).

The concentrations of heavy metals after repeated fertilization of soil with 10 and 20 t · ha<sup>-1</sup> of sewage sludge were similar. The difference between fertilized and non-fertilized soils was very small, on average 1.2–1.3 times (Fig. 2), and fluctuated from 1.1 to 1.6 and 1.7 during the whole period of research in individual years. The difference was most pronounced in the fifth and seventh years of research. We suppose that the increase of these heavy metals is related to the additional use of mineral fertilizers for growing agricultural crops, as the same increase was also observed in the non-fertilized fields.

The concentrations of heavy metals in the crop after repeated fertilization of the soil in three and five years did not exceed the concentrations allowed by the norms.



Table 6. Microarthropods complex characteristic of arable poor sandy soil fertilized with sewage sludge (10 and 20 t · ha<sup>-1</sup>)

Dominance	Species	Thou	%	Species	Thou	%	Species	Thou	%
		ind. m <sup>-2</sup>			ind. m <sup>-2</sup>			ind. m <sup>-2</sup>	
One year after fertilization (10 t · ha <sup>-1</sup> )				Two years after fertilization (10 t · ha <sup>-1</sup> )			Seven years after fertilization (20 t · ha <sup>-1</sup> )		
Constant core species >10%	<i>Brachychthonius</i> sp.	0.25	13.5	<i>Caloglyphus rodionovi</i>	46.5	53.0	<i>Tectocephus velatus</i>	2.5	39.0
	<i>Artoseius cetratus</i>	0.25	13.5	<i>Microppia minus</i>	9.25	29.7	<i>Parisotoma notabilis</i>	1.5	23.0
	<i>Isotomiella minor</i>	0.20	10.8				<i>Dendrolaelaps</i> sp.	0.7	11.0
	<i>Rhodacarellus silesiacus</i>	0.20	10.8						
	<i>Caloglyphus rodionovi</i>	0.20	10.8						
Satellite species 5.0–9.9%	<i>Microppia minus</i>	0.15	8.1						
	<i>Tectocephus velatus</i>	0.10	5.4						
	<i>Folsomia quadrioculata</i>	0.10	5.4						
	<i>Hypogastrura assimilis</i>	0.10	5.4						
	<i>Hypoaspis praesternalis</i>	0.10	5.4						
Sparse species <5.0%	<i>Acotyledon</i> sp.	0.10	5.4						
	<i>Oppiella nova</i>	0.05	2.7	<i>Oppiella nova</i>	1.50	4.8	<i>Trichoribates trimaculatus</i>	0.3	4.7
	<i>Alliphis sículus</i>	0.05	2.7	<i>Brachychthonius</i> sp.	1.25	4.0	<i>Lepidocyrtus cyaneus</i>	0.3	4.7
				<i>Acotyledon</i> sp.	0.95	3.0	<i>Asca bicornis</i>	0.2	3.1
				<i>Mesaphorura krausbaueri</i>	0.80	2.6	<i>Hypogastrura assimilis</i>	0.2	3.1
				<i>Sminthurinus aureus</i>	0.30	1.0	<i>Latilamellobates incisellus</i>	0.1	1.6
				<i>Tectocephus velatus</i>	0.15	0.5	<i>Oppiella nova</i>	0.1	1.6
				<i>Brachystomella parvula</i>	0.10	0.3	<i>Pergamasus lapponicus</i>	0.1	1.6
				<i>Rhodacarellus silesiacus</i>	0.10	0.3	<i>Isotomiella minor</i>	0.1	1.6
				<i>Ceratophysella denticulata</i>	0.05	0.2	<i>Mesaphorura krausbaueri</i>	0.1	1.6
				<i>Folsomia quadrioculata</i>	0.05	0.2	<i>Isotoma</i> sp. juv.	0.1	1.6
				<i>Isotomiella minor</i>	0.05	0.2	<i>Podura aquatica</i>	0.1	1.6
				<i>Parisotoma notabilis</i>	0.05	0.2			
				<i>Alliphis sículus</i>	0.05	0.2			
	Total	13	1.85	100.0	15	31.15	100.0	14	6.4

Table 7. Agrochemical characteristic of repeated fertilization with sewage sludge in third (10 t · ha<sup>-1</sup>) and fifth (20 t · ha<sup>-1</sup>) years in arable soils

Agrochemical characteristics	Year					$\bar{X}$	Year							$\bar{X}$
	1	2	3	4	5		1	2	3	4	5	6	7	
Fertilized 10 t · ha <sup>-1</sup>							Fertilized 20 t · ha <sup>-1</sup>							
pH	6.8	n	n	7.5	7.5	7.2	6.49	6.49	7.2	6.7	7.0	6.8	7.6	6.8
Humus, %	2.72	n	1.9	2.06	2.49	2.2	2.01	2.27	2.72	2.3	2.9	2.32	2.15	2.3
Common N, %	0.142	n	n	0.11	0.115	0.12	0.1	0.124	1.157	0.125	0.15	0.13	0.14	0.27
m. N, mg/100 g	9.24	n	n	n	n	0.24	n	n	n	n	n	n	n	n
N-NO <sub>3</sub> , mg/kg	n	2.23	6	4.43	1.61	3.56	n	4.0	26.2	18.0	5.9	8.5	8.6	11.8
N-NH <sub>4</sub> , mg/kg	n	2.69	1.7	0	1.93	1.58	n	1.93	2.1	0.33	1.6	1.41	0.69	1.3
N <sub>min</sub> , mg/kg	n	4.91	7.7	4.43	3.54	5.14	n	5.93	28.3	18.33	7.4	9.9	9.29	13.2
m. P <sub>2</sub> O <sub>5</sub> , mg/100 g	42.15	n	20.3	38.8	21.8	30.76	43.5	64.5	81.0	81.0	17.2	52.8	57.6	56.8
m. K <sub>2</sub> O, mg/100 g	33.3	n	19.5	15.3	25.4	23.37	16.9	12.1	17.4	15.9	17.2	10.8	16.3	15.2
Control							Control							
pH	6.65	n	n	6.9	6.9	6.8	5.2	5.26	n	5.1	5.4	4.8	4.8	5.0
Humus, %	2.18	n	1.5	1.92	1.72	1.83	1.69	2.09	n	2.03	2.6	3.31	1.63	2.2
Common N, %	0.091	n	n	0.1	0.08	0.09	0.10	0.107	n	0.119	0.12	0.18	0.11	0.12
m. N, mg/100 g	7.84	n	n	n	1.48	4.66	n	2.68	n	n	n	n	n	2.68
N-NO <sub>3</sub> , mg/kg	n	1.19	5.6	0.21	0.94	1.98	n	2.4	n	15.44	1.53	6.6	6.0	6.3
N-NH <sub>4</sub> , mg/kg	n	2.16	1.8	0.09	0.54	1.14	n	0.28	n	0.33	1.9	1.31	0.57	0.8
N <sub>min</sub> , mg/kg	n	3.35	7.5	0.3	1.48	3.15	n	2.68	n	15.8	3.43	7.91	6.57	7.2
m. P <sub>2</sub> O <sub>5</sub> , mg/100 g	26.7	n	24.4	32.8	18.3	25.55	32.7	23.0	n	27.6	17.6	20.5	18.3	23.2
m. K <sub>2</sub> O, mg/100 g	28.1	n	12.2	14.3	15.0	17.4	9.9	24.1	n	26.9	34.0	38.0	32.4	27.5

n – not analysed.

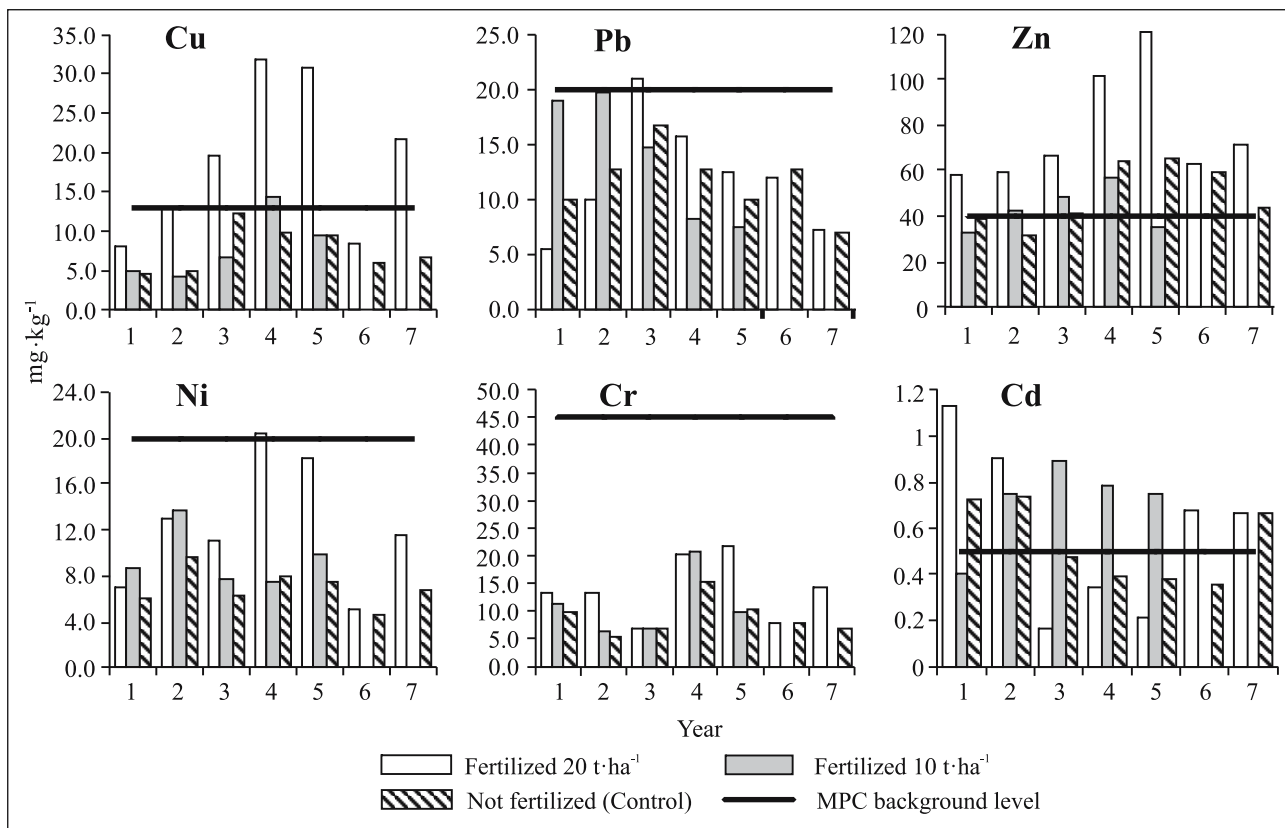


Fig. 2. Concentrations of heavy metals in soil after repeated fertilization with sewage sludge  $10 \text{ t} \cdot \text{ha}^{-1}$  and  $20 \text{ t} \cdot \text{ha}^{-1}$ , and in non-fertilized soil (control)

Only the content of Zn was insignificantly higher (Table 8). The concentrations of heavy metals in hay were also negligible.

Thus, we can state that repeated fertilization of *arenosols* with sewage sludge in the third and fifth years does not contaminate the soil with heavy metals. The grown crop is clean, and the soil retains the content of humus stable at the average level of 2.2% for the period of seven years. The reaction of the soil comes close to neutral from average acid.

Repeated fertilization of soil with sewage sludge ( $20 \text{ t} \cdot \text{ha}^{-1}$ ) in the fifth year after the first fertilization with sludge caused an immediate increase of microarthropods up to  $135.5 \text{ thou ind. m}^{-2}$  during the first year. A massive distribution of Acaridae was observed. The dominating *Schwiebea pachyderma* accounted for even 82.3% of all microarthropods. The satellite Gamasina species *Alliphis siculus* was also significant. A total of 20 species were found (Table 9).

Table 8. Heavy metals the concentrations ( $\text{mg} \cdot \text{kg}^{-1}$ ) in crops after repeated fertilization with sewage sludge

Study object	Year	Grown crops	Fertilized						Not fertilized (control)					
			Cd	Cr	Cu	Ni	Pb	Zn	Cd	Cr	Cu	Ni	Pb	Zn
			$\text{mg} \cdot \text{kg}^{-1}$						$\text{mg} \cdot \text{kg}^{-1}$					
Merešlėnai $10 \text{ t} \cdot \text{ha}^{-1}$	1		n	n	n	n	n	n	n	n	n	n	n	n
	2	rye	0.07	0.93	2.15	0.82	0.45	17.95	0.07	1.16	2.6	1.5	0.4	15.5
	3	perennial grasses	n	n	n	n	n	n	n	n	n	n	n	n
	4	perennial grasses	0.11	0.58	11.8	0.98	2.1	20.8	0.09	0.51	8.5	0.83	1.96	16.0
	5	perennial grasses	0.31	1.35	7.4	2.75	0.031	18.7	0.07	0.62	6.6	0.46	0.94	17.7
Skurbutėnai $20 \text{ t} \cdot \text{ha}^{-1}$	1	barley	0.13	0.35	15.0	0.65	0.71	64.0	0.073	0.24	9.3	0.37	0.7	30.3
	2	hay	0.057	0.74	7.1	0.45	0.057	20.9	0.047	0.79	5.9	0.34	0.44	13.4
	3	hay	0.047	0.95	9.33	1.36	0.43	30.6	0.045	0.92	5.23	0.82	0.43	12.5
	4	hay	0.087	0.67	2.75	0.6	1.13	13.3	0.093	0.699	2.85	0.52	1.12	12.2
	5	hay	0.015	1.15	2.6	0.65	0.25	11.9	0.02	1.05	1.76	0.58	0.27	13.1
	6	hay	0.036	0.33	1.79	0.39	0.08	11.8	0.03	0.31	1.88	0.39	0.06	10.4
	7	hay	0.04	0.43	1.57	0.56	0.21	15.1	0.05	0.4	2.24	0.55	0.08	15.9
		MPC Background level	0.3	0.5	3.0	2.0	5.0	50.0						

n – not analysed.

Table 9. Microarthropod complex structure in arable fields in the fifth year of repeated fertilization with sewage sludge (20 t · ha<sup>-1</sup>)

Dominance	Species	Thou ind. m <sup>-2</sup>	%	Species	Thou ind. m <sup>-2</sup>	%
	One year after fertilization			Three years after fertilization		
Constant core species >10%	<i>Schwiebea pachyderma</i>	111.6	82.3	<i>Isotomiella minor</i>	17.0	48.0
				<i>Protaphorura armata</i>	4.4	12.4
Satellite species 5.0–9.9%	<i>Alliphis siculus</i>	10.15	7.49	<i>Parisotoma notabilis</i>	3.15	8.9
				<i>Ceratophysella denticulata</i>	2.25	6.3
				<i>Proisotoma minima</i>	2.05	5.8
Sparse species <5.0%	<i>Tectocephus velatus</i>	5.10	3.76	<i>Isotoma</i> sp.	1.10	3.1
	<i>Acotyledon</i> sp.	1.85	1.36	<i>Mesaphorura krausbaueri</i>	0.90	2.5
	<i>Protaphorura armata</i>	1.70	1.25	<i>Tectocephus velatus</i>	0.80	2.3
	<i>Artoseius cetratus</i>	0.90	0.66	<i>Brachychthonius</i> sp.	0.70	2.0
	<i>Brachychthonius</i> sp.	0.85	0.63	<i>Hypogastrura</i> sp.	0.70	2.0
	<i>Micropia minus</i>	0.65	0.48	<i>Oppiella nova</i>	0.50	1.4
	<i>Parisotoma notabilis</i>	0.60	0.44	<i>Alliphis siculus</i>	0.45	1.3
	<i>Oppiella nova</i>	0.45	0.33	<i>Micropia minus</i>	0.25	0.7
	<i>Caloglyphus rodionovi</i>	0.45	0.33	<i>Acotyledon</i> sp.	0.25	0.7
	<i>Schwiebea rossica</i>	0.30	0.22	<i>Isotoma viridis</i>	0.15	0.6
	<i>Ceratophysella denticulata</i>	0.25	0.18	<i>Lepidocyrtus</i> sp. 1	0.15	0.4
	<i>Hypoaspis aculeifer</i>	0.25	0.18	<i>Schwiebea pachyderma</i>	0.15	0.4
	<i>Brachystomella parvula</i>	0.10	0.07	<i>Suctobelbella</i> sp.	0.10	0.3
	<i>Sphaeridia pumilis</i>	0.10	0.07	<i>Caloglyphus rodionovi</i>	0.10	0.3
	<i>Rhodacarus</i> sp.	0.10	0.07	<i>Bourletiella arvalis</i>	0.05	0.1
	<i>Macrocheles muscaedomesticae</i>	0.05	0.04	<i>Bourletiella hortensis</i>	0.05	0.1
		<i>Acotyledon krameri</i>	0.05	0.04		
	<i>Schwiebea tshernyshevi</i>	0.05	0.04			
Total	20	135.5	100.0	21	35.7	100.0

Insertion of this sludge also greatly influenced the number of maggots of minor insects participating in the destruction of organic substances. Those were Cyclorharpha, Chironomidae, etc. Their number increased 4 to 17 times in comparison with control samples. Repeated fertilization with sludge favoured the distribution of earthworms. In comparison with control samples, the number of earthworms was 1.7–2.5 times bigger than in the fields fertilized with more sludge. On average, 7.2 to 37.3 ind. m<sup>-2</sup> were found, and *Aporrectodea caliginosa caliginosa* dominated (Strazdienė, 2001).

Significant changes took place in the structure of the complex of microarthropods in the third year, the nucleus of the association was dominated by Collembola. Collembola is also on the level of satellite species (Table 9). *Schwiebea pachyderma*, which had dominated in the first year, was a sparse species, their density being only 0.4%. The species of microarthropods dominant in the soil showed that a large part of organic substance had been decomposed. The number of microarthropods decreased four times as compared with the first year of research.

Thus, repeated insertion of sewage sludge into soil triggers the same biological processes as a single fertilization: an intensive succession of groups and species of microarthropods takes place, clearly reflecting the level of mineralization of sewage sludge. Such processes are most active during the first three years, while no indications are found in the seventh year.

## DISCUSSION

The concentration of heavy metals in Vilnius Region poor sandy soils is smaller than the established background concentration, but these soils are not rich in biogenic elements and humus. Our research has shown that humus accounts for the average of 1.8% in this soil. If the soil is not fertilized with organic fertilizers, the crop depends on fertilization with mineral fertilizers.

Directives restricting the use of sewage sludge have been prepared in the European Union (Directive 91 / 271 / EEC, Directive 86 278 EEC, Ordinance on the use and disposal of sewage sludge). In our country, the use of sewage sludge for soil fertilization is also regulated by normative documents (LAND 20–2005). All these documents allow reducing the risk of environment pollution with sewage sludge. However, there are opinions that sewage sludge is not very hazardous to the environment. It is specified that fertilization of forests with 18 t · ha<sup>-1</sup> sewage sludge and 112 t · ha<sup>-1</sup> for recultivation of soil, using it once, is not dangerous to the environment (Schafer, 2007).

The problem of the use of sewage sludge is twofold: there are the agronomic and the ecological sides. Intensively used arable fields, especially those in poor soils, are impoverished without the use of organics. Sewage sludge is a substratum that helps to maintain humus. Maintenance of humus and its formation in the soil of arable fields is a very complex process



comprising many factors. It also depends on the mechanical composition of the soil (Eidukevičienė, 2001).

The research carried out using manure without hay and manure with hay showed that in case of constant fertilization of soil with such fertilizers only manure with hay ( $20 \text{ t} \cdot \text{ha}^{-1}$ ) produced positive results with respect to formation of non-deficit humus (СафоНОВ, 1989).

Therefore, upon fertilizing arable fields with liquid or very humid sludge, stabilization of humus in the soil may be problematic. A single fertilization with sewage sludge ( $20$  and  $10 \text{ t} \cdot \text{ha}^{-1}$ ) with the average humidity of 75% showed that this amount of sludge only insignificantly (by 1.8 to 2.0%) enriched arable fields with humus over the period of seven years.

The improvement of the agrochemical and biological qualities of the soil of arable fields is observed only after repeated fertilization in the fifth year with  $20 \text{ t} \cdot \text{ha}^{-1}$  of sewage sludge. Then the content of humus increases on average by 1.8 to 2.6%. The reaction of the soil becomes neutral. The biological processes become more active.

The ecological part of this problem is related to contaminants entering the soil with the sludge: heavy metals, different organic compounds and pathogenic microorganisms. Complex processes forming technogenic associations of heavy metals take place in the soil contaminated with heavy metals. The distribution of heavy metals and the formation of new compounds depend on the qualities of soil (pH, humus %). It has been established that heavy metals in sandy soils according to stability can be listed in the following order:  $\text{Cu} > \text{Pb} > \text{Zn} > \text{Cd}$ . Cadmium is nine times more mobile than copper (Ладонин, 2000). Besides, the mobility of heavy metals may change depending on soil humidity and the activity of biota.

Considering the complexity of the processes taking place in the soil, ecotoxicological methods based on biotesting are used for establishing the ecological state of soil. Pedobionts – organisms constantly living in the soil – are used for that purpose. Several tests have been standardized in Europe (earthworm *Eisenia fetida* and Collembola *Falsomia candida*) (Riepert, Kula, 1996). Also, other tests are available: groups, species, populations of individual organisms as well as genetic methods (Van Gestel, Van Diepen, 1997; Løkke et al., 1994; Van Gestel, Mol, 2003; Полянская, Звягинцев, 2005).

The analysed sandy soil is of microarthropod type (Eitminavičiūtė, 2001), therefore, we chose the density of microarthropods, the structure of their complexes and indices of the variety of species as test objects for evaluating the ecotoxicity of arable fields fertilized with sewage sludge. The research showed that after a single intensive fertilization of arable fields with sewage sludge ( $20 \text{ t} \cdot \text{ha}^{-1}$ ) after seven years the number of arthropods was lower than that in arable fields not fertilized with sludge. The variety of species in them was also poorer, but after analysing the data we can state that the concentrations of heavy metals which do not exceed the

background values is not the main reason for the density of microarthropods after a single fertilization with sewage sludge. Intensive tillage of land, the use of mineral fertilizers and pesticides should be considered the main reasons for the decreasing number of microarthropods (Eitminavičiūtė, 2001).

Data in the literature indicate that the influence of small concentrations of heavy metals in the soil on the biota is difficult to prove, especially in intensively arable fields. It can be more influenced by the intensive mechanical tillage of land.

An increase of Cu and Zn concentrations was observed in the third and fourth years. It is known that the use of mineral fertilizers increases the concentrations of these heavy metals in soil (Mažvila, 2001).

After repeated fertilization of soil with sewage sludge, we found that in the third year after fertilization with  $10 \text{ t} \cdot \text{ha}^{-1}$  the concentration of certain heavy metals in comparison with the control sample increased 1.1 times (Pb and Cd) and 1.6 times (Cu). After fertilization with sewage sludge ( $20 \text{ t} \cdot \text{ha}^{-1}$ ) in the fifth year, the concentration of Cu and Zn increased twice. A dynamic study of the concentrations of heavy metals shows that they increase in 3–5 years after fertilization of the soil with sludge ( $10$ – $20 \text{ t} \cdot \text{ha}^{-1}$ ), both in case of single and repeated fertilization.

Application of sludge to soil stimulates changes in the dominating microarthropods. The changes take place even in the nucleus of the association. After fertilization with sewage sludge, the total density of microarthropods decreased to 17% in the first year. In the second year, the number of microarthropods increased 3 ( $10 \text{ t} \cdot \text{ha}^{-1}$ ) to 13 times ( $20 \text{ t} \cdot \text{ha}^{-1}$  repeated fertilization) depending on the amount of inserted sludge. This density of microarthropods is caused by Acaridae, namely *Schwiebea pochyderma* species. The massive distribution and density of microarthropods persists in the soil for 3 years depending on the amount of sludge. Soil Collembola species start to dominate in the third year, indicating the end of sewage sludge decomposition. Only the repeated insertion of sludge ( $20 \text{ t} \cdot \text{ha}^{-1}$ ) in the fifth year enriched these soils with earthworms, their density reaching up to 37 organisms  $\text{m}^{-2}$ , i. e. 2.5 times more than in the control sample.

## CONCLUSIONS

1. A single fertilization of poor arable fields of *arenosols* with sewage sludge ( $10$ – $20 \text{ t} \cdot \text{ha}^{-1}$ ) insignificantly improves the organic qualities of soil.
2. The increase of humus and biotic elements in the soil takes place only after a repeated fertilization of the same fields with  $20 \text{ t} \cdot \text{ha}^{-1}$  sewage sludge in the fifth year.
3. Repeated fertilization of the soil ( $20 \text{ t} \cdot \text{ha}^{-1}$ ) stimulates the biological processes, increases the number of pedobionts.
4. The crop grown on the soil fertilized with sewage sludge is not contaminated.

5. Depending on the type of soil, the quality and norms of sewage sludge as well as the complexity of the mechanism of chemical-biological processes, it is necessary to undertake the ecotoxicological monitoring of soil fertilized with sludge.

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Irena Eitminavičiūtė, Audronė Matusevičiūtė,  
Valerijus Gasiūnas, Milda Radžiūtė, Neda Grendienė

### DIRBAMŲ LAUKŲ DIRVOŽEMIŲ, TRĘŠTŲ NUOTEKŲ DUMBLU, EKOTOKSIKOLOGINIS VERTINIMAS

#### *S a n t r a u k a*

Daugelis miestų turi nuotekų valymo įrenginius, kuriuose kaupiamas dumblas. Dumblo panaudojimas net ir dabar, kai sparčiai kuriamos įvairios technologijos, yra problemiškas ir daug diskusijų keliantis klausimas. Nuotekų dumblas, gausiai prisotintas biotinėmis medžiagomis, gali būti naudojamas dirvožemiams tręšti. Tačiau nuotekų dumblas laukams tręšti naudojamas ribotai dėl keleto veiksnių: sunkiųjų metalų koncentracijos, patogeninių mikroorganizmų ir nemalonaus kvapo. Todėl šios organinės trąšos naudojimą reglamentuoja įvairūs normatyviniai aktai.

Tyrimai atlikti 1993–2008 m. Vilniaus rajono skurdžiuose smėlžemio dirvožemiuose: Pakenėje, Skurbutėnuose, Merešlėnuose. Intensyviai dirbamų laukų dirvožemiams, ypač skurdiems smėlžemiams, reikia skirti daug dėmesio tiriant juose vykstančius

biologinius procesus, susijusius su organinių medžiagų mineralizacija ir humifikacija. Atsižvelgiant į dirvožemyje vykstančių procesų mechanizmo sudėtingumą, dirvožemio ekologinei būklei nustatyti taikomi ekotoksikologiniai metodai, kurių pagrindą sudaro biotestavimas. Tam yra naudojami nuolat dirvožemyje gyvenantys organizmai – pedobiontai.

Dirbamų laukų smėlžemio dirvožemius vieną kartą patręšus 8–20 t · ha<sup>-1</sup> nuotekų dumblo nežymiai pagerėja dirvožemio agrocheminės savybės ir jis neužteršiamas sunkiaisiais metalais.

Dirbamų laukų smėlžemio dirvožemių agrocheminės ir biologinės savybės pagerėja tik pakartotinai penktais metais tręšiant šiuos dirvožemius 20 t · ha<sup>-1</sup> nuotekų dumblo. Tuomet humuso kiekis juose padidėja vidutiniškai 1,8–2,6 %. Dirvožemio reakcija tampa neutrali. Suaktyvėja biologiniai procesai. Tyrimo rezultatai rodo, kad javų derlius, išaugintas patręšus skurdų smėlžemio priemolio dirvožemį nuotekų dumblo, yra mažai užterštas sunkiaisiais metalais ir koncentracijos yra ne didesnės nei leidžiamos pašariniuose javuose.

**Raktažodžiai:** dirbami laukai, nuotekų dumblas, sunkieji metalai, mikroartropodai