ECOTOXICOLOGICAL ASSESSMENT OF THE SOIL FERTILIZED WITH SEWAGE SLUDGE

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Abstract: Sewage sludge and its management constitute one of the most important environmental problems. The introduced the Sewage Sludge Directive 86/278/EEC advocate utilization of sewage sludge in agriculture. However, this method is limited mainly by the availability of heavy metals and pathogens. An ecotoxicological assessment of sewage sludge doses applied to soil, recommended in the Polish Regulation of the Minister of Environment of 6th February 2015 on municipal sewage sludge, was carried out. The sewage sludge was added to soil in the amounts of 3, 6, 9, and 15 Mg/ha. The produced mixtures were subjected to physicochemical and ecotoxicological tests, involving the biomass and mortality tests of *Eisenia fetida* earthworms. The sewage sludge had a statistically significant impact on the increase in: electrical conductivity, total exchangeable cations, organic matter content, organic carbon, nitrogen and heavy metals of the considered soil. The impact on the biomass of earthworms was different; after 7 days in the mixtures with 3 Mg/ha and 6 Mg/ha sewage sludge addition, the biomass increased approximately by 1.3-fold, in comparison to non-modified soil. At 9 Mg/ha, it reduced by 1.3-fold, whereas at 15 Mg/ha – by 16-fold, in relation to the initial value. The studies indicated that the ecotoxicological assessment of wastes may be employed as environmental safety control measure of the sewage sludge application in agriculture.

1. Introduction

Sewage sludge and its management constitute one of the most important environmental problems. The European Union directives promote the sewage sludge management in agriculture. However, this method of utilization is limited mainly by the presence of heavy metals and pathogens.

In the presented research, an ecotoxicological assessment of the sewage

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sludge doses applied to soils recommended in the polish Regulation of the Minister of the Environment of February 6, 2015 on municipal sewage sludge, was carried out.

The sewage sludge was added to the soil at a dose of 3, 6, 9, 15 Mg/ha. The created mixtures were tested to physicochemical and ecotoxicological tests, consisting in conducting a biomass and mortality test for the *Eisenia fetida* earthworms. Sewage sludge increased statistically significantly: the values of electrical conductivity, the sum of exchangeable cations, the content of organic matter, organic carbon, nitrogen and heavy metals of the studied soil.

The ecotoxicological assessment of the doses of sewage sludge showed that the addition of 3, 6, 9 Mg/ha sewage sludge did not affect the mortality of earthworms, while the dose of 15 Mg/ha caused mortality of 38% of the earthworm population.

The doses of sediment had an effect on the earthworm biomass. Earthworm biomass after 7 days in the mixtures with 3 Mg/ha and 6 Mg/ha with the addition of sewage sludge increased about 1.3-fold compared to the soil without the addition of sludge. At the dose of 9 Mg/ha, the earthworm biomass decreased 1.3 times, while at the dose of 15 Mg/ha it was already 16 times lower, compared to the initial biomass of earthworms.

Studies have shown that the ecotoxicological assessment of waste can be used to control the safety of the environmental use of sludge in agriculture.

About half a million tonnes of dry matter of sewage sludge is generated in Poland annually. They primarily consist of the dead mass of microorganisms and are a by-product of the biological as well as mechanical-biological wastewater treatment process. According to the definition in the Act on waste, municipal sewage sludge is waste - sludge from digestion plants and other installations for the treatment of municipal wastewater and other types of wastewater with a composition similar to that of municipal wastewater (Act on the waste of 2019, item 701, 730, 1403, 1579).

Sewage sludge and its utilization is one of the most important environmental problems. The European Union directives introduced (Council Directive 86/278 / EEC of 12 June 1986) promote the use of sewage sludge in agriculture. However, introduction of sewage sludge to soils has some limitations due to its composition.

In addition to nutrients such as nitrogen and phosphorus, the sludge contains heavy metals and pathogens, which is why regulations to specify the appropriate doses that can be introduced to the earth's surface have been introduced, imposing the heavy metal limit values and the ratio of nitrates to phosphorus (Artuso et al., 2011).

In Poland, the detailed conditions for the use of municipal sewage sludge, including the doses of these types of sludge that can be used on land, as well as the

scope, frequency and reference methods for testing municipal sewage sludge and land on which these sludges are to be applied, is provided by the Regulation of the Minister of the Environment of February 6th, 2015.

This regulation sets a maximum limit for the use of 3 Mg dm/ha (dry mass) of sewage sludge in agriculture and for land reclamation for the agricultural purposes, whereas, if the sludge is to be used for land reclamation for the non-agricultural purposes or for land development, for cultivating plants intended for the production of compost, for the cultivation of plants not intended for consumption and for the production of feed, the dose of sewage sludge may not exceed 15 Mg dm/ha/year. The regulations also allow cumulative doses, which may not exceed 6 Mg DS/ha/2 years and 9 Mg DS/ha/3 years, respectively.

The European and Polish legislation limits the use of sludge by taking into account only the chemical approach, imposing the limit values for heavy metals. Despite the positive influence on crops resulting from the use of sludge in agriculture, little is known about their actual impact on the entire soil system. It is important to determine what impact sewage sludge has on plant and soil organisms, their diversity and functions. It seems reasonable to conduct ecotoxicological tests to assess the risk of waste impact on soils.

The studies on the ecotoxicological assessment of waste in soil, which have shown that pollution causes harmful changes in soil, are known (Vikelsøe et al., 2002). Although the introduction of sediments into the soil provides nutrients and seems to favor invertebrates, harmful effects were found in laboratory studies (Andrés et al., 2011; Natal-da-Luz et al., 2009).

Notwithstanding these facts, in most countries the law does not require an ecotoxicological assessment. Striving to take into account all hazardous factors present in waste that cannot be measured by chemical analysis, Council Directive European Union 91/689 / EEC regulates the classification of wastes in terms of their potential harmful effects according to 14 properties (European Community 1991).

In recent years, the directive has been amended several times, the most significant change was the introduction of "ecotoxic" properties (European Community 2001). "Ecotoxic" (H14) is one properties assigned to the substances and preparations that pose or may pose a direct or delayed risk to the environment.

For this reason, the definition of a set of ecotoxicological tests for the quantitative assessment of the "ecotoxic" property of waste is urgent (Natal-da-Luz et al., 2009) Research is needed to develop, validate and standardize methods. (Artuso et al., 2011).

The purpose of the presented research was the ecotoxicological assessment of the doses of sewage sludge applied to soils recommended in the Regulation of the Minister of the Environment of February 6th, 2015 on municipal sewage sludge.

Sewage sludge was added to the soil at a dose of 3, 6, 9, 15 Mg/ha. The created mixtures were subjected to physicochemical and ecotoxicological tests, consisting in conducting the *Lepidium sativum* root test and *Eisenia fetida* earthworm mortality test.

2. Materials and methods

The mineral soil (G), was taken from the arable field in the vicinity of Lublin, from a depth of 10-20 cm. The hygienic sewage sludge (O) samples were obtained from Hajdow Wastewater Treatment Plant (in Lublin, Poland). The soil was mixed with sewage sludge in the doses specified in the Regulation of the Polish Minister of the Environment of February 6th, 2015 on municipal sewage sludge, namely: 3 Mg/ha (3O), 6 Mg/ha (6O), 9Mg/ha (9O) and 15 Mg/ha (15O).

Analytical methods. The scope and analytical methods for determination of the physical and chemical parameters characterizing the mixtures and sewage sludge, soil were as follows:

Determination of physicochemical properties

- electrical conductivity– measured for 1:5 soil/water (weight/volume), (PN-EN 27888:1999 soil quality. Determination of the specific electrical conductivity) by means of a Orion Versa Star Pro benchtop meter,
- pH value by means of the potentiometric method in water solution and in 1 M solution of potassium chloride (PN-ISO 10390: 1997P soil quality. Determination of pH) by means of a Orion Versa Star Pro benchtop meter,
- dry matter content by means of loss on drying method at a temperature of 105°C (PN EN ISO 11465:1999P soil quality. Determination of the dry matter content of soil and water in soil per dry mass of soil. Gravimetric method),
- hydrolytic acidity (H) and total exchangeable bases (S) by means of the Kappen method,
- cation exchange capacity (CEC) was calculated based on the $CEC=H+S$ formula
- total organic carbon by means of a TOC-5050A Shimadzu analyzer (PN-ISO 4335 standard),
- total nitrogen content by Kjeldahl method using 1002 Kjeltex distillation unit,
- total heavy metals were determined by a ICP-OES Ultrace 238 (Jobin Yvon-Horriba France) using direct calibration method after microwave digestion (Multiwave 3000, Anton Paar). The samples of homogenized soil (1 g) were digested in an acid mixture of HNO₃: HCl (5:2 v:v). The digestion process lasted 45 min at 180°C and at the pressure of 18 bars.

Earthworm test

The assay was carried out in glass containers with the volume of 250 cm³, filled with soil containing an addition of sewage sludge.

Each sample contained 10 mature *Eisenia fetida* earthworms. Chernozem soil constituted the control sample. The water content amounted to 40÷60% (WHC). The assay was carried out over the period of 7 days. This method involves the determination of biomass and mortality of adult *Eisenia fetida* earthworms. The mortality coefficient of earthworms after 7 days was calculated with the following formula:

$$M_7 = \frac{(A - B_7)}{A} 100\%$$

where:

A – number of live earthworms in the control soil sample,

B7 – number of live earthworms in polluted soil after 7 days,

M7 – mortality coefficient after 7 days, %.

Test Phytotoxkit TM. The sewage sludge/compost toxicity was assessed with the commercial toxicity bioassay–Phytotoxkit TM. Test (Phytotoxkit, 2004). The phytotoxkit microbiotest measures the decrease (or the absence) of seed germination and the growth of the young roots after 3 days of exposure of seeds of selected higher plants to the contaminated matrix, in comparison to the control in the reference soil. Ten seeds of each plant were positioned at equal distance near the middle ridge of the test plate on a filter paper placed on top of the hydrated soil. After closing the test plates were placed vertically in a holder and incubated at 25°C by 3 days. At the end of the incubation period, a digital picture of the test plates with the germinated plants was taken. The analyses and the length measurements were performed using the Image Tool 3.0 for Windows (UTHSCSA, San Antonio, USA). The bioassays were performed in three replicates.

The percent inhibition root growth inhibition (RI) were calculated with the formula:

$$RI = \frac{(A - B)}{A} \cdot 100$$

where, A - Mean seed germination, root length and biomass in the control soil; B—mean seed germination, root length and biomass in the test soil.

According to Hoekstra et al. (2002), the germination index (GI) was calculated as well:

$$GI = \frac{RSD \cdot RPG}{100}$$

where, RSD—(number of seeds germinated in dung extract/number of seeds germinated in control) $\times$ 100; RPG—(mean root length in dung extract/mean root length in control) $\times$ 100.

Statistical analysis

Mean values with standard deviation were calculated for the results of this study. The data were also statistically analyzed through a parametrical test ANOVA (T Tukey's test), correlation analysis Pearson's (r) to investigate the significance of the treatments on the soil attributes. The letter indicators at the average content values indicate the statistically homogeneous groups (Tukey Homogeneous Groups). The presence of the indicator designates the lack of a statistically significant difference between them.

3. Results and discussions

The changes of the selected physicochemical properties due to the addition of sewage sludge to the soil are shown in Table 1.

Table 1. Chemical properties of the sewage sludge, soils and the mixtures ($\pm$ standard deviation)

Substrates	pH	EC	K_h	S	T
		[mS/cm]			
O	6.03 ± 0.1	1640	9.40 ± 1.90	28.1 ± 1.25	37.5
G	5.97a ± 0.08	3.5a	1.05a ± 0.32	12a ± 0.71	13.05a
3O	6.24ab ± 0.14	11.5b	0.82a ± 0.21	17.65a ± 5.16	18.47a
60	6.34ab ± 0.04	12.1b	0.97a ± 0.20	19.35a ± 1.9	20.32a
9O	6.24ab ± 0.19	19.2c	1.32ab ± 0.38	23.15b ± 1.34	24.47b
15O	6.23ab ± 0.21	33.6d	1.75b ± 0.42	24.6b ± 0.84	26.35b

a, b - the letter indicators at the average content values indicate the statistically homogeneous groups (Tukey Homogeneous Groups). The presence of the indicator designates the lack of a statistically significant difference between them.

Soils with sewage sludge had the soil pH range of 6.1-7.3 from slightly acid to neutral (Kabała et al., 2016; Stanowski et al., 2019). These values pH were slightly higher than in the control (5.97). The studies on the addition of sewage sludge to the soil have shown a different effect on soil pH, may cause an increase in soil pH

as in studies like (Hamd et al., 2019), but also a decrease as in studies (Wolejko et al., 2012; Martinez et al., 2002). The soil pH is an important parameter affecting the bioavailability of heavy metals.

The addition of sewage sludge statistically significantly increased the values of electrolytic conductivity (EC). In the mixtures with 3 Mg/ha and 6 Mg/ha of sewage sludge, the EC values were 4 times higher than in the soil without the addition of sludge, while the addition of sewage sludge already causes a 10-fold increase in EC in soil.

While comparing the EC values measured in the mixtures tested with the Jackson soil salinity scale, and taking into account the dilution of the samples, it was found that the soils with 3 Mg/ha, 6 Mg/ha and 9 Mg/ha with the addition of sewage sludge can be classified as "poorly saline" soils (1.6–3.2 mS/cm). The soil with the addition of sewage sludge is classified as "moderately saline" (3.2–6.4 mS/cm). While considering the requirements of plants in terms of the EC value of the substrates, it should be assumed that the substrates containing up to 9 Mg/ha of sewage sludge should not have a negative impact on the growth of plants that do not show strong sensitivity to salinity. The mixtures containing 15 Mg/ha of sediments can limit the plant growth to varying degrees, so it is necessary to select the species with high salinity tolerance, e.g. legumes (Parida et al., 2005).

The sorption properties are changed statistically significantly only at higher doses of 9 Mg/ha and 15 Mg/ha sewage sludge. The values of the hydrolytic acidity in the mixtures of 9O and 15O were slightly higher (1.32–1.75 cmol(+)/kg) than in soil (1.05 cmol(+)/kg), while the values of the sorption capacity in the said substrates were already twice as high as in the control soil. Many authors emphasize the positive effect of sewage sludge and brown coals on improving the sorption properties of soil (Ociepa, 2011; Farasat & Namli, 2017).

The organic substance is widely recognized as the main indicator of soil quality. Sewage sludge is characterized by a high content of organic matter (OM = 61.03%); therefore, it is recommended to introduce it into soils. The concentration of carbon, nitrogen and organic matter content increases along with the dose of sewage sludge. The highest values of the discussed parameters were obtained for the substrate with 15 Mg/ha of sewage sludge, these are statistically significantly higher values than other substrates. The addition of sewage sludge increased the C and OM content of soil to the values recommended for arable soils. When assessing the quality of arable soils, it is assumed that the content of organic matter (SO) should not be less than 3.4%, and organic carbon (Corg) – less than 2% (Loveland & Webb 2003).

Sewage sludge increased the nitrogen concentrations in the tested substrates statistically significantly, compared to the soil without sludge. Already 3 Mg/ha the

addition of sewage sludge increased the nitrogen concentration 1.5-fold compared to soil, while the 15 Mg/ha addition of sewage sludge – by 3-fold.

Table 2. Physical properties of the sewage sludge, soils and the mixtures ($\pm$ standard deviation)

Substrates	OM	C	N	C/N
	[%]			
O	61.03 $\pm$ 1.12	35.48 $\pm$ 0.65	4.34 $\pm$ 0.09	8.15
G	4.95a $\pm$ 0.39	2.88a $\pm$ 0.23	0.17a $\pm$ 0.01	16.9a
3O	5.44a $\pm$ 0.28	3.16a $\pm$ 0.16	0.26b $\pm$ 0.01	12.15b
60	6.86b $\pm$ 0.4	3.98b $\pm$ 0.23	0.31c $\pm$ 0.02	12.8b
9O	7.49b $\pm$ 0.23	4.36b $\pm$ 0.13	0.43d $\pm$ 0.01	12.8b
15O	9.45c $\pm$ 0.45	5.49c $\pm$ 0.26	0.53e $\pm$ 0.01	10.35c

a, b - the letter indicators at the average content values indicate the statistically homogeneous groups (Tukey Homogeneous Groups). The presence of the indicator designates the lack of a statistically significant difference between them.

The C: N ratio in the tested substrates ranged from 10.35 to 12.5, was lower compared to the values typical for arable soils (C: N = 17-19). If this ratio is below 15: 1, mineralization dominates, which consequently allows plants to absorb the ammonia formed in this way and its oxidation product, i.e. nitrate (Leite et al., 2017).

One of the factors limiting the natural management of sewage sludge is the excessive content of heavy metals (Zhang et al., 2017; Tytła, 2019; Spanos et al., 2016; Karwowska & Dąbrowska, 2017).

The metal concentrations in the sewage sludge depend on several factors such as: sewage origin, sewage treatment processes, and sludge treatment processes (Milik et al., 2017). Heavy metals increased due to the sewage sludge application, as compared to the control (Table 3).

Studies have shown that the addition of sewage sludge increased the content of heavy metals in soil, in proportion to the increase of sludge in soils. The mixture containing 15 Mg/ha of sewage sludge was characterized by the highest metal content. However, none of the analyzed heavy metals in the mixtures tested exceeded the concentration limits specified in the Regulation of the Minister of the Environment of 1 September 2016 on the method of assessing the surface pollution of land for group II, i.e. arable land (Journal of Laws 2016 item 1395).

The post hoc tests showed that the concentration of chromium and lead in the substrate with 15 Mg/ha sewage sludge addition differed statistically significantly from the other tested substrates. The concentrations of cadmium, copper, nickel and zinc in the control soil were statistically significantly lower than the substrates with the addition of sewage sludge.

Table 3. The concentrations of metals in soils, sewage sludge and drill cuttings ($\pm$ standard deviation)

Substrates	Cd	Cr	Cu	Ni	Pb	Zn
	[mg/kg]					
O	6.13 ± 0.02	51.4 ± 0.82	395.23 ± 0.71	68.18 ± 0.75	12.12 ± 0.42	1116.815 ± 0.80
G	0.29a ± 0.009	16.2a ± 0.73	9.81a ± 0.30	9.375a ± 0.18	5.27a ± 0.22	47.085a ± 0.29
3O	0.41b ± 0.01	17.8a ± 0.55	21.63b ± 1.02	10.95b ± 0.24	5.27a ± 0.39	81.40b ± 0.59
6O	0.47b ± 0.03	18.23a ± 0.22	20.14b ± 0.52	10.91b ± 0.01	5.82a ± 0.52	79.01b ± 0.41
9O	0.66c ± 0.005	19.38ab ± 0.39	32.19c ± 0.01	12.59c ± 0.23	5.78a ± 0.09	112.46c ± 0.22
15O	0.81d ± 0.04	20.77b ± 0.19	38.72d ± 1.94	13.69d ± 0.42	6.77b ± 0.09	153.10d ± 0.51
Limit*	2-5	150-200	100-300	100-300	100-500	300-1000

* The limit specified in the Regulation of the Minister of the Environment of September 1, 2016 on the method of conducting soil pollution assessment for land from group II, i.e. arable land (Journal of Laws 2016 item 1395).

a, b - the letter indicators at the average content values indicate the statistically homogeneous groups (Tukey Homogeneous Groups). The presence of the indicator designates the lack of a statistically significant difference between them.

Figure 1 shows the result of an ecotoxicological test conducted on earthworms. The doses of 3 Mg/ha, 6 Mg/ha, 9 Mg/ha of sewage sludge did not affect the mortality of earthworms. Only a dose of 15M g/ha caused mortality in

Fig. 1. The mortality coefficient of *Eisenia fetida* in soil, sewage sludge and mixtures after 7 days.

38% of the earthworm population. No statistical differences were found when comparing the number of surviving adults placed in control and in the soil amended with 3 Mg/ha, 6 Mg/ha and 9 Mg/ha of sludge. The exception occurred in the soil amended with 15 Mg/ha of sludge, where a significantly lower survival rate was observed.

In research Moreira et al. (2008) and Natal-da-Luz et al. (2008) found no effects on either mortality or growth of *Eisenia spp.* At 6 Mg/ha of sewage sludge, suggesting the absence of toxicity at lower rates of sewage sludge.

Table 4 shows the impact of drilling waste on the earthworm biomass after 7 days. The earthworm biomass increased after 7 days with the addition of 3 Mg/ha and 6 Mg / ha of sewage sludge. At the dose of 9 Mg/ha of sediment, a decrease of biomass by about 1.3 times was observed, while at the dose of 15 Mg/ha, it was already 16 times lower compared to the initial biomass of earthworms. The post hoc tests showed that the biomass of earthworms bred on 15 Mg/ha sewage sludge was statistically significantly different from the others. The correlation analysis showed strong negative correlations between the earthworm biomass and all the parameters studied, the only exception was the reaction of the substrates. Moreira et al. (2008) also observed a statistically significant reduction in the weight gain of adult earthworms at a dose of 12 Mg/ha of sludge to artificial soils (OECD) compared to controls.

The literature indicates that the reason for the reduction in the weight of earthworms is the effect of heavy metals (Lukkari et al., 2004). Fountain and Hopkin, showed that the concentration of zinc over 50 mg/kg reduces the earthworm biomass (Fountain & Hopkin 2005).

Table 4. Comparison of the earthworm biomass in soil, sewage sludge and mixtures

Substrates	Total biomass [g]	
	Start	After 7 days
G	13.64± 0.96	10.9a±0.26
3O	12.72± 0.98	13.75a± 0.63
6O	14.07±0.65	14.62a ± 0.65
9O	13.71 ± 0.51	10.27 a± 0.27
15O	13.2 ±0.67	0.82 b ± 0.16

Lepidium root test results. The root test aimed at assessing the effect of substrates on germination of cress seeds and the growth of its roots. The assessment was based on the value of germination index (GI) and the root growth inhibition index (RI).

Figure 2 presents the values of the germination index of the substrates with sewage sludge. Already with the addition of 3 Mg/ha inhibition of germination

index (GI = 23.5%) was observed, with 15 Mg/ha sewage sludge substrate there was complete inhibition of cress germination. The post hoc tests showed that the GI values for the tested substrates were statistically significantly different.

The GI values of all tested substrates reached values below 25%, which according to the scale developed by Wong, qualifies them as very phytotoxic (Wong et al., 2001). Phytotoxicity in the soil sewage extract typically results from the presence of partially degraded organic acids, fermentation intermediates, ammonia, salts and heavy metals (Jagadabhi et al., 2019).

Most published studies (Wong et al., 2001, Fuentes et al., 2006) indicate that the root length assessment is a more sensitive parameter than seed germination. Such results were also obtained in the presented studies. The values of root growth inhibition coefficients (Figure 3) for substrates with 3 Mg/ha sewage sludge and 6 Mg/ha sewage sludge reached the values below 50%. A 100% inhibition of root growth was obtained in the medium with 15 Mg/ha. The diversity of RI values was statistically significant.

Fig. 2. Germination index (GI) of *L. sativum*.

Fig. 3. The inhibition of root growth (RI) of *L. sativum*.

In the search for the causes of cress sprouting inhibition, a Pearson correlation matrix between the tested substrate parameters and the germination index was prepared (Table 5). Strong negative correlations were observed between all tested parameters, except for the pH, and germination index.

Lepidium root test showed the toxicity of the sewage sludge used. Similar results were obtained by Fuentes et al. (2006). These studies demonstrated an inhibitory effect of sediment on germination and root growth; in the case of watercress (*L. sativum*) and barley (*Hordeum vulgare*). Cress turned out to be a more sensitive plant compared to barley.

Table 5. The correlation coefficient and GI and earthworm Biomass for the mixture with sewage sludge

R	GI	Earthworm biomass
Cd	-0.960	-0.882
Cr	-0.806	-0.773
Cu	-0.991	-0.903
Ni	-0.976	-0.882
Pb	-0.719	-0.757
Zn	-0.958	-0.917
OM	-0.855	-0.848
C	-0.856	-0.849
N	-0.954	-0.863
H	-0.902	-0.802
S	-0.779	-0.715
pH	0.241	0.224
EC	-0.943	-0.936

In the case of cress, a reduction of the germination rate by more than 50% (Fuentes et al., 2006) was observed in sludge extracts. In Oleszczuk's research on the impact of the sewage sludge addition to soils (sandy soil, loamy soil, OECD soil) on the growth of plants: *Lepidium sativum*, *Sorghum saccharatum*, *Synapis alba* (Oleszczuk et al., 2010) the germination rate of *L. sativum* reached the values below 10% on the OECD soil with the addition of 90 Mg/ha sewage sludge. The high sensitivity of *L. sativum* to the compounds contained in sewage sludge was confirmed. Researchers attribute this inhibitory effect of sludge on germination to high EC and other toxic compounds present in sediments such as ammonia, heavy metals, and short-chain aliphatic acids in sediments (Asgharipour & Ali, 2012).

Conclusions

The addition of sewage sludge to soils caused an increase in organic matter, carbon, nitrogen, electrolytic conductivity (EC) and heavy metals, which affected the results of the ecotoxicological tests.

The use of sewage sludge in low doses of 3 Mg/ha to 6 Mg/ha did not adversely affect the number of earthworms in laboratory tests. However, the doses of 9 Mg/ha and 15 Mg/ha of sewage sludge showed toxicity to earthworms in the short duration of the laboratory experiment. The dose of 9 Mg/ha of sewage sludge did not affect the mortality of earthworms, but reduced the biomass. However, the dose of 15 Mg/ha of sewage sludge caused the earthworm mortality at the level of 38% and a significant decrease in the biomass of worms.

Soil *Lepidium* root test with the addition of sewage sludge will show significant toxicity, even at the lowest doses. Watercress exhibits a high sensitivity to the pollution present in the studied sediments.

The results of the ecotoxicological studies carried out cannot be used to predict the possible long-term effects of the continuous spread of sediments. The results of the ecotoxicological tests should be interpreted with caution as they may be associated with the artificial conditions of the experiment. However, it seems reasonable to use biological tests using various test species; they can constitute an additional tool for assessing the effects of changes in soil organic waste. It seems necessary to constantly monitor the soils on which sewage sludge is used.

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