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COMPLEX ASSESSMENT OF BIOSOLID FOR AGRICULTURE USING LIVING ORGANISMS UNDER LABORATORY CONDITIONS

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ABSTRACT

The use of biosolids (treated municipal sewage sludge) as a fertilizer is the best way of their disposal. However, not all of them are suitable for use as a fertilizer. Biosolids should be subject to mandatory laboratory control to confirm their safety. Two directions of research on biosolids are being improved: chemical and biological. Chemical analysis methods allow us to determine the qualitative composition of complex waste. The biological approach (use of living organisms) allows us to estimate the total toxicity of all the components. Accordingly, a distinctive characteristic of biological methods is the integrated approach. We examined biosolid extract using a wide range of bioassay methods. As test organisms, we took *Daphnia magna* Straus, *Paramecium caudatum* Ehrenberg, *Tetrahymena pyriformis*, luminescent bacteria *Escherichia coli*. In addition, a phytotest was carried out on the culture of *Avena sativa* L. and *Raphanus sativus* L. None of the tests revealed a high toxicity of biosolid. Biosolid safety was confirmed by a low content of potentially toxic water-soluble elements – (µg /l): Al^{3+} – 980; Ba^{2+} – 19; B – 140; Mn – 360; Cu – 61; As – 57; Ni – 200; Pb – 1,4; Sr^{2+} – 302; Cr – 18; Zn^{2+} – 310; Co – 30; Mo – 56; (mg/l): Na^{+} – 16,8; Fe – 1,0. The bioassay methods make it possible to give an indicative safety assessment of this type of object by the effect of readily soluble (readily available) components from this object on living organisms and plants. The use of bioassay methods using soil extraction as a control tool allows to take into account the combined effect of the presence in the extraction of not only toxic elements that suppress the vital activity of organisms, but also of elements that attract and stimulate the activity of test-organisms.

Keywords: *bioassay, biosolid, sewage sludge, environmental safety, agriculture.*

INTRODUCTION

Continuously forming municipal sewage sludge (SS) is not only waste, it can also become a valuable resource for agriculture due to its similarity with traditional organic fertilizers on the composition of nutrient elements and organic substance.

However, the concomitant presence of heavy metals, organic pollutants (polychlorinated hydrocarbons, polycyclic hydrocarbons, pesticides, pharmaceuticals, including hormones and antibiotics) and pathogenic microorganisms becomes a significant limiting factor in their use as a fertilizer (Smith, 2009; Clarke, 2011; Delibacak, 2020). SS safety for agriculture (AC) is defined by the results of chemical analysis (Collivignarelli, 2019). However, the discrepancy in the regulation of chemicals, the inability to set an exact vector in the search for all toxic components makes it difficult to adequately assess the safety of the given object. Within this context, biological methods being integral methods of assessing toxicity are gaining great demand (Lyashenko, 2012; Zhmur, 2012). Biological methods (or bioassays) are a set of simple inexpensive and relatively fast in their implementation methods. With their help it is possible to identify the contribution of toxic components unaccounted by chemical and to give a primary integral assessment of the influence of all water-soluble components present in SS on living organisms (Gelashvili, 2016). The lack of a clear protocol on SS research using biological methods dictates the necessity in the development of the given direction. In this regard, we set a goal to estimate the approaches and methods of bioassay of waste water sludge processing products for their safe use in agriculture.

MATERIAL AND METHODS

The product of processing (compost) of aerobic-stabilized sewage sludge is studied in the work. The sewage sludge formed in Vladimir (Russia) (56.1428° N, 40.3896° E) treatment facilities was subjected to a biennium mesophilic composting with sawdust of coniferous trees in the ratio of 1:0.8 without the use of microbiological preparations. The compost represented a homogeneous mass of dark gray color (Figure 1). Determination of the total amount, potentially active forms (ammonium acetate buffer – AAB, pH=4.8), water-soluble forms of elements (water extract in the ratio of distilled water being 1:1) were performed in the water extract of compost using inductively coupled plasma mass spectrometry (ICP-MS). Bioassay (including phyto-testing) was performed using a wide range of test organisms of various trophic groups. The samples were examined using *Daphnia magna* Straus, *Paramecium claudatum* Ehrenberg, *Tetrahymena pyriformis*, an «Ecolume» biosensor based on a luminescent strain of *Escherichia coli, Avena sativa* L., *Raphanus sativus* L. Table 1 provides a brief characteristic of each method with of test organisms' toxicity indicators and criteria. An extraction (pH=7,2) of uncontaminated soil – Umbric ALBELUVISOLS (WRB) (the upper 20 cm of soil layer) formed on the binomial glacial deposits the plough layer of which is thicker loamy sediments overlying heavy moraine loam was used as a control for bioassay apart from the provided methods of distilled water or culture medium. SS and its processed products are local fertilizers and their use is confined to this type of soils. Extractions for bioassay are prepared in the following ratio compost (soil): distilled water – 1:1, 1: 10. In domestic guidelines, it is mainly recommended to use an extract of 1: 10 for waste and 1:5 for soil in a ratio with distilled water. In order to avoid breeding errors, we studied the extraction of compost and soil 1:1.

Repeatability for each test takes three times. Statistical processing of results was performed using MS Excel and STATISTICA 6.1 programs in accordance with the guidelines.

Figure 1. Compost of aerobic-stabilized sewage sludge

Table 1. Brief characteristic of bioassay methods used in the work.

Bioassay method	Test-organism	Toxicity indicator/criterion
Determination of the toxicity of water extractions by the survival rate of *Daphnia* (multicellular organism)	*Daphnia magna* Straus	Death of test organisms in 48 hours ≥50%
Determination of toxicity of water extractions by survival and chemotactic reaction of infusoria (single-celled organism)	*Paramecium caudatum* Ehrenberg	Decrease of chemotactic response (chemotaxis) *Paramecium caudatum* Ehrenberg/≥40%
Determination of the toxicity of water extractions by the growth function of infusoria (single-celled organism)	*Tetrahymena pyriformis*	Augmentation decrease of *Tetrahymena pyriformis* cells in 48 hours /≥50%
Determination of the toxicity of water extractions by the glow activity of lyophilized fluorescent bacteria *Escherichia coli*	"Ecolume" biosensor (luminescent bacteria *Escherichia coli*)	Suppression of bacterial bioluminescence intensity for a 5-30-minute exposure period /≥20%
Phyto-testing (eluated method)	*Avena sativa* L., *Raphanus sativus* L.	Suppression of root length of seedlings /≥20%

RESULTS AND DISCUSSION

The study of the chemical composition of complex multicomponent objects is mandatory, at that special attention should be paid to the form of finding the detected components. Knowing their form we can assume its behavior under various conditions. Table 2 represents the content of several components in the total amount and potentially active form of elements.

Table 2. The total content and the content of potentially active form.of elements in compost

Element	Total amount	Potentially active form – AAB (pH=4,8)
mg/kg of dry solid		
Li	3,8 ± 0,6	-
Be	1,2 ± 0,2	-
B	17,0 ± 2,6	-
Na	430,0 ± 65,0	-
Mg	5620,0 ± 840,0	267,0 ± 32,0
Al	14000,0 ±2100,0	31,0± 4,0
Ti	79,0 ± 12,0	-
V	22,0 ± 3,3	-
Cr	82,0 ± 12,0 (–/90-500)*	-
Mn	440,0 ± 66,0	34,0 ± 0,4
Fe	14000,0 ± 2100,0	39,0 ± 4,7
Co	5,2 ± 0,8	0,1 ±0,01
Ni	37,0 ± 5,6 (300-400/80-200)	1,0 ± 0,2
Cu	240,0 ± 36,0 (1000-1750/132-750)	0,6 ± 0,04
Zn	540,0 ± 81,0 (2500-4000/220-1750)	-
Ga	11,0 ± 1,7	-
As	5,0 ± 0,8 (–/2-10)	-
Se	0,7 ± 0,1	-
Br	640,0 ± 96,0	-
Rb	9,4 ± 1,4	-
Sr	100,0± 15,0	14,0 ±1,4
Cd	1,6± 0,2 (20-40/2-15)	0,1 ± 0,02
Sn	5,6 ± 0,8	-
W	1,3 ± 0,2	-
Pb	22,0 ± 3,3 (750-1200/130-250)	1,6±0,2
Ba	230,0 ± 35,0	19,0 ± 2,5
Zr	13,0± 2,0	-
Ag	3,5 ± 0,5	-
Th	1,0 ± 0,2	-
U	4,6 ± 0,7	-

La	5,5 ± 0,8	-
Ce	11,0 ± 1,7	-
Pr	1,2 ± 0,2	-
Nd	4,3 ± 0,7	-
ppb		
Hg	590 ± 89 (16000-25000/2100-7500)	-
Sb	350 ± 53	-
Mo	3,4 ± 0,5	-
Yb	270 ± 41	-
Hf	300 ± 45	-
Ta	14 ± 2	-
Tl	68 ± 10	-
Ge	50 ± 8	-
Nb	75 ± 11	-
Te	28 ± 4	-
Cs	560 ± 84	-
Sm	820 ± 120	-
Eu	240 ± 36	-
Gd	860 ± 130	-
Dy	580 ± 87	-
Ho	110 ± 17	-
Er	310 ± 47	-
Tm	43 ± 7	-

*() – concentration limits for heavy metals in sludge according to Directive 86/278/EEC / Russian State standard

The content of heavy metals in the test compost according to Directive 86/278/EEC does not exceed the limits. However, according to national standards that have stricter standards, compost is not recommended to be used for growing forage, technical, grain crops, green manures and for growing seedlings of vegetable and flower crops on a personal household nonetheless it is suitable for planting forestry crops along roads, in nurseries of forest and ornamental crops, floriculture, for cultivating depleted soils, recultivation of disturbed land and slopes of motor roads and recultivation of solid waste dumps (National Standard GOST R 54651-2011).

It should be noted that elements the presence of which isn't standardized and reaches tens of mg/kg are found in the test compost. Whereby the role of some of them hasn't yet been completely studied. Their presence can affect the total estimation of toxicity. Table 3 represents the values of content of water-soluble elements that pass into the extraction 1:1.

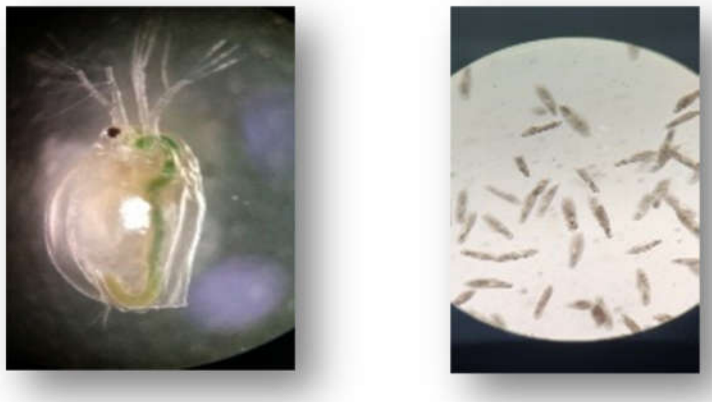
Table 3. The content of several elements of water extraction of compost (1:1).

Element	Mean value
mg/l	
Na^{+}	16,8
Fe	1,0
µg /l	
Al^{3+}	980
Ba^{2+}	19
B	140
Mn	360
Cu	61
As	57
Ni	200
Pb	1,4
Sr^{2+}	302
Cr	18
Zn^{2+}	310
Co	30
Mo	56

The content of elements in the compost extraction doesn't exceed the limits stipulated in the national standards for the content of adverse elements in drinking water (Sanitary Rules and Regulations SanPiN 2.1.4.1074-01).

According to the results of bioassay, *Daphnia magna* Straus test-organisms (Figure 2a, Table 4) showed 100% survival in all the variants at 48-hour exposure which can reveal the absence of acute toxicity of compost extractions on the basis of sewage sludge for the given test.

According to the influence of water extractions of compost on the change in the intensity of bioluminescence of bacteria (biosensor "Ecolume", represented by luminescent bacteria *Escherichia coli*), no acute toxic effects were detected as well (Table 4).



a b

Figure 2. Daphnia magna Straus (a), Paramecium caudatum Ehrenberg (b)

The components that are most sensitive to sewage sludge turned out to be organisms belonging to the same type: *Paramecium caudatum* (Figure 2b) and *Tetrahymena pyriformis* infusoria. As can be seen from Table 4 regarding the Lozina-Lozinsky medium provided by the method (*Paramecium caudatum*), all the extractions did not have a high acute toxicity (2% and 13%). As for soil extractions, compost extractions are less attractive and a significant increase in the percentage of toxicity (67% and 46%) indicates it.

Table 4. Compost toxicity in regard to various test-organisms, %.

Compost extraction	In regard to control	In regard to soil extraction
	Daphnia magna Straus	
Compost extraction 1:1	0	0
Compost extraction 1:10	0	0
luminescent bacteria *Escherichia coli*		
Compost extraction 1:1	0	0
Compost extraction 1:10	0	0
Paramecium caudatum Ehrenberg		
Compost extraction 1:1	2	67
Compost extraction 1:10	13	46
Tetrahymena pyriformis		
Compost extraction 1:1	13	7
Compost extraction 1:10	-19	-5
Avena sativa L.		

Compost extraction 1:1	17	26
Compost extraction 1:10	-17	-14
Raphanus sativus L.		
Compost extraction 1:1	13	20
Compost extraction 1:10	-14	-13

*symbol "-" stands for stimulation

Dolgov's researches have shown no toxicity of compost extractions based on sewage sludge with minimal dilution with distilled water (1:1) for augmentation of *Tetrahymena pyriformis* cells (Dolgov, 2014). According to the results of our studies presented in table 4, according to the influence of compost extractions based on sewage sludge on the augmentation of *Tetrahymena pyriformis* cells, the absence of their acute toxicity can be noted. Moreover, the extraction of compost 1:10 had a stimulating effect on the augmentation of infusoria cells. It is important to emphasize that by 48 hours, there was an inhibition of the augmentation of infusoria cells under the influence of extraction 1:1, that constituted 13%. In reference to soil extracts, the effect was somewhat lower.

A number of authors have identified the phytotoxicity of compost extractions based on sewage sludge on various crops (Mitelut, 2011; Adamcová, 2016). During phytotesting on barley (*Hordeum vulgare* L.) and garden-cress (*Lepidium sativum* L.) the author Fuentes, detected no toxicity, which may be due to a low content of easily soluble toxic components (Fuentes, 2004).

Table 4 also shows the results of testing compost extractions based on sewage sludge to detect an inhibitory effect on the germination of roots of monocotyledonous and dicotyledonous crops. Low inhibition of root growth of *Avena sativa* L. and *Raphanus sativus* L. was detected (13% and 20%). A stimulating effect was noted in the study of the extraction 1:10.

The use of bioassay methods using soil extraction as a control tool allows us to take into account the combined effect of the presence in the extraction of not only toxic elements that suppress the vital activity of organisms, but also of elements that attract and stimulate the activity of test-organisms. There are following quantities of important components for plants: N total – 2,6±0,2%, P_2O_5 – 8,45±0,3%, K_2O – 0,51±0,05%, Ca total – 24 g/kg. However, the choice of control in each particular case requires a clear scientific and practical justification.

CONCLUSIONS

Biotesting along with chemical analysis is an important component of the study of complex multicomponent objects. The use of chemical composition is not sufficient for determining the total toxicity due to the inability to set an exact vector in the determination of all the toxic components. Bioassay methods enable to detect or confirm the absence of toxicity of all active substances present. The use of water extractions can not only reveal the toxicity of sewage sludge and waste products, but also enable to estimate the possibility of migration of hazardous components at their disposal in the environment. The use of complex non-polluted natural objects, such as soil, as a control tool for bioassay of sewage sludge, will, in our opinion, give a more objective assessment compared to the use of unpolluted environment.

Certainly, when using sewage sludge as a fertilizer, their toxic properties can be neutralized in soil, but the initial assessment of such a complex object using biotesting methods is important in order to avoid unpredictable consequences.

REFERENCES

Adamcová D., Vaverková M. D., Břoušková E. The toxicity of two types of sewage sludge from wastewater treatment plant for plants //Journal of Ecological Engineering. – 2016. – Vol. 17. – №. 2.

Biotesting of products, feedings and environmental objects [Electronic resource] / Dolgov [et al.] /Bulletin of the Russian University of Peoples' friendship. Series: agronomy and cattle breeding. – 2014. – № 3. – pp. 63-72.

Clarke B. O., Smith S. R. Review of 'emerging'organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids //Environment international. – 2011. – Vol. 37. – №. 1. – pp. 226-247.

Collivignarelli M. C. et al. Legislation for the Reuse of Biosolids on Agricultural Land in Europe: Overview, Sustainability. – 2019. – Vol. 11. – №. 21. – p 6015.

Delibacak S. et al. Use of sewage sludge in agricultural soils: Useful or harmful //Eurasian Journal of Soil Science. – 2020. – Vol. 9. – №. 2. – pp. 126-139.

Fuentes A. et al. Phytotoxicity and heavy metals speciation of stabilised sewage sludges, Journal of hazardous materials. – 2004. – Vol. 108. – №. 3. – pp. 161-169.

Gelashvili D.B. et al. The principles and methods of environmental toxicology. – 2016.

Lyashenko O.A. Bio-indication and biotesting in environmental protection: a textbook. St-Petersburg, – 2012. – p. 67.

Mitelut A. C. et al. Seed germination bioassay for toxicity evaluation of different composting biodegradable materials //Romanian Biotechnological Letters. – 2011. – Vol. 16. – №. 1. – pp. 121-129.

National Standard GOST R 54651-2011. Organic fertilizers based on sewage sludge. Technical conditions.

Sanitary Rules and Regulations SanPiN 2.1.4.1074-01 Drinking water. Hygienic requirements to water quality of centralized drinking water supply systems. Quality control. Hygienic requirements to ensuring the safety of hot water systems.

Smith S. R. Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling //Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences. – 2009. – Vol. 367. – №. 1904. – pp. 4005-4041.

World Reference Base URL: http://www.fao.org/soils-portal/soil-survey/soil-classification/world-reference-base/en/ as of 20.06.2020

Zhmur N.S. Application of biotesting methods in Russia and the world //Methods of conformity assessment. - 2012. - №. 1. - pp. 10-14.