Journal of Ecological Engineering
Volume 17, Issue 2, Apr. 2016, pages 33–37
DOI: 10.12911/22998993/62283

Research Article

THE TOXICITY OF TWO TYPES OF SEWAGE SLUDGE FROM WASTEWATER TREATMENT PLANT FOR PLANTS

Dana Adamcová[1], Magdalena Daria Vaverková[1,*], Eliška Broušková[2]

[1] Department of Applied and Landscape Ecology, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic, e-mail: dana.adamcova@mendelu.cz

[2] Department of Morphology, Physiology and Animal Genetics, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic, e-mail: eliska.brouskova@mendelu.cz

* Corresponding Author: magda.vaverkova@uake.cz

Received: 2015.02.12
Accepted: 2016.03.04
Published: 2016.04.01

ABSTRACT
The aim of the present study was the estimation of the phytotoxicity of soils amended with sewage sludge with relation to *Sinapis alba* L. The study was realized in the system of a plot experiment. Two kinds of sewage sludge: dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewatered sludge "Palikal" with dry matter content of about 92%. The results indicate that the tested samples are toxic. Growth inhibition at the studied samples ranged from 70.45% to 100%.

Keywords: sewage sludge, sewage treatment plant, phytotoxicity, *Sinapis alba* L., land application.

INTRODUCTION

The accumulation of sewage sludges from urban wastewater treatment plants is a growing environmental problem [Fuentes et al. 2004]. Sewage sludges constitute a very important element in sewage management. Sludge is formed during wastewater treatment. Wastewater is a combination of liquid- or water-carried wastes removed from residential, institutional, commercial and industrial establishments, together with ground water, surface water and storm water [Werthera and Ogada 1999]. A noted increase in sewage sludge production requires measures to ensure its utilization [Oleszczuk et al. 2011]. Studies carried out so far [Smith et al. 2001, Oleszczuk 2006a] showed that sewage sludge in the conditions of its land application can be a significant source of a lot of undesirable substances in soil and plants. Heavy metals and organic compounds are among the "most popular" pollutants present in sewage sludge [Stevens et al. 2003, Oleszczuk 2006b]. The influence of pollutants contained in sewage sludges on their toxicity has not, so far, been demonstrated unequivocally [Oleszczuk et al. 2011]. On the other hand, however, high contents of organic matter and nutrients make sewage sludge a perfect material for fertilization and recultivation of degraded soils [Albiach et al. 2001, Selivanovskaya et al. 2003]. Apart from soil enrichment in nutrients [Fytili and Zabaniotou 2008], an addition of sewage sludge causes an increase in the content of organic matter in soil [Epstein 2003]. Organic matter resources in soils are relatively low and frequently require replenishment. Therefore, the use of sewage sludge in agriculture is a desirable method of their utilization. The addition of sewage sludge to soils may thus be an inexpensive and effective alternative to the methods applied currently (mineral fertilisation, manure etc.) [Oleszczuk et al. 2012].

Plants are essential primary producers in the terrestrial ecosystem. In addition, the crop yield and quality are important success criteria in agriculture. Therefore, it is important to identify potential phytotoxins and understand the magnitude of their impact on different terrestrial ecosystems [Schowanek et al. 2004, Oleszczuk 2008]. Recent reports have considered phytotoxicity test to be useful in assessing environmental (soils, sediments) and anthropogenic (compost, sewage sludge) matrix toxicity [Czerniawska-Kusza et al. 2006, Oleszczuk 2008].

For the above-mentioned reasons, the aim of the present work was: (1) to characterize the sewage sludges, (2) to assess the phytotoxicity of two types of sewage sludges.

In addition, the effects on seed germination and primary root growth were determined in white mustard (*Sinapis alba* L.) to investigate the effect of stabilization strategy used on sludge phytotoxicity. Such bioassays are simple and rapid methods to indicate phytotoxicity [Wong et al. 2001, Zucconi et al. 1985].

MATERIAL AND METHODS

Sludges

Sewage sludge samples were collected from the sewage treatment plant (mechanical–biological treatment system) in Czech Republic. The wastewater treatment plant serves around 374 000 inhabitants with an influent flow rate of about 4.22 m^3/s. The treatment plant consists of a conventional extended aeration activated sludge process.

The sludge samples (about 1 kg) were collected in triples (during the autumn – sample A, B and C) at the end point of the sewage sludge digestion process. Sewage sludges were typical aerobically digested. The two types of sludges had been stabilized in different ways as follows: II – dewatered and anaerobically stabilized sludge with dry matter content of about 24%, I – dewatered sludge "Palikal" with dry matter content of about 92%. Chemical characteristic of the sewage sludges is presented in Table 1. The collected samples were stored in glass bottles and immediately transported to the laboratory. All sewage sludge samples were air-dried and crushed to obtain representative samples. Sewage sludges were crushed in a mortar and then sieved through a 2 mm sieve for chemical and ecotoxicological analysis.

Phytotoxicity test

The toxicity of sewage sludge was assessed with a commercial toxicity bioassay – Phytotoxkit^{TM} Test (Microbiotests, Nazareth, Belgium)[Phytotoxkit, 2004]. The Phytotoxkit (Figure 1) makes use of flat and shallow transparent test plates composed of two compartments, the lower one which contains soil saturated to the water holding capacity. In the experiment white mustard (*Sinapis alba* L.) was chosen because of its high sensitivity to sewage sludges. The phytotoxkit measures the decrease (or the absence) of seed germination and of the growth of young roots after 3 days of the exposure of selected seeds of higher plants to a contaminated matrix, in comparison to the controls in a reference soil. Water saturation is calculated according to the user's manual. The distilled water was spread over the entire surface of the soil in the test plate. Ten seeds of *Sinapis*

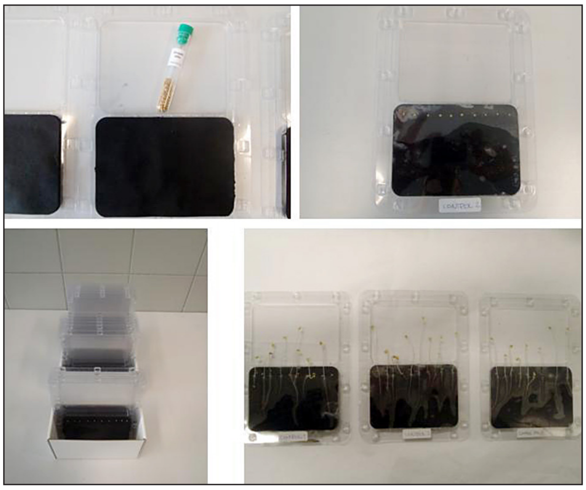

Figure 1. Phytotoxicity test

Table 1. Chemical characteristic of the sewage sludges

I – Dewater sludge "Palikal" (92% DM)	Hg (mg/kg)	Cd (mg/kg)	Ni (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
Sample A	1.60	0.640	31.6	68.6	200	964	30.4
Sample B	2.31	0.450	29.9	61.7	204	872	30.2
Sample C	1.86	0.570	28.4	65.5	213	907	28.4
II - Stabilized sludge (24% DM)	Hg (mg/kg)	Cd (mg/kg)	Ni (mg/kg)	Cr (mg/kg)	Cu (mg(kg)	Zn (mg/kg)	Pb (mg/kg)
Sample A	1.92	0.840	35.5	79.6	184	765	27.7
Sample B	1.69	0.880	32.8	71.2	199	895	24.8
Sample C	2.10	0.590	29.4	71.2	210	906	26.6

alba L. were positioned at equal distances near the middle ridge of the test plate on a filter paper placed on the top of the hydrated soil/sewage sludge mixture. After closing, the test plates were placed vertically in a holder and incubated at 25°C for 3 days. At the end of the incubation period a digital picture was taken of the test plates with the germinated plants. The analyses and the length measurements were performed using the Image Too l3.0 for Windows (UTHSCSA, San Antonio, USA). The bioassays were performed in three replicates. The percent inhibition of seed germination (*SG*) and root growth inhibition (*RI*) were calculated with the formula:

$$SG/RI = A–B/A \times 100$$

where: A – means seed germination and root length in the control,
B – means seed germination and root length in the test.

RESULTS AND DISCUSSION

To evaluate the toxicity tests with the test plants *Sinapis alba* L. the parameters shown in Table 2 (the basic characteristic of the growth inhibition and the degree of toxicity) were used.

Figure 2–4 presents the effect of the sewage sludge (concentration 100%, 10%, 25% and 50%) on the inhibition of seed germination and root growth as related to the test plants *Sinapis alba* L. (SIA), Samples A, B and C.

The growth inhibition (%) of *Sinapis alba* L. for dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewatered sludge “Palikal” with dry matter content of about 92%, Sample A was in the range of 79.3 – 100%. These samples are strongly toxic, the degree of toxicity 3, 50 <I.

The growth inhibition (%) of *Sinapis alba* L. for dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewater sludge “Palikal” with dry matter content of about 92%, Sample B was in the range of 70.45 – 100%. These samples are strongly toxic, the degree of toxicity 3, 50 <I.

The growth inhibition (%) of *Sinapis alba* L. for dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewatered sludge “Palikal” with dry matter content of about 92%, Sample C was in the range of 81.6 – 100%. These samples are strongly toxic, the degree of toxicity 3, 50 <I.

Table 2. The degree of toxicity

Inhibition (%)	The degree of toxicity	Evaluation
I* < 10	1	Non-toxic or slightly toxic
10 < I < 50	2	Toxic
50 < I	3	Strongly toxic

* I – growth inhibition (%).

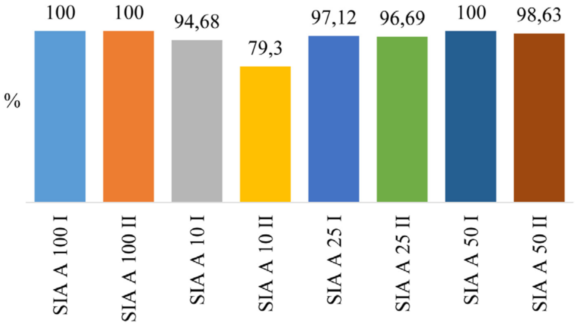

Figure 2. SIA Sample A

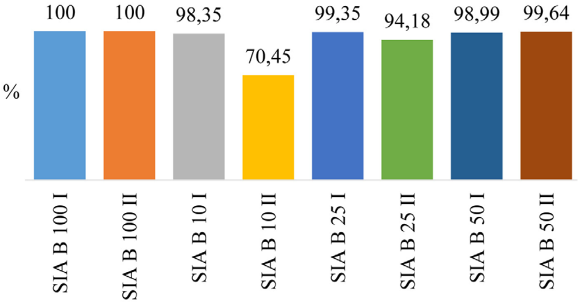

Figure 3. SIA Sample B

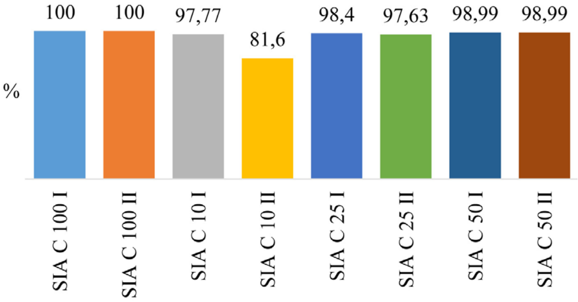

Figure 4. SIA Sample C

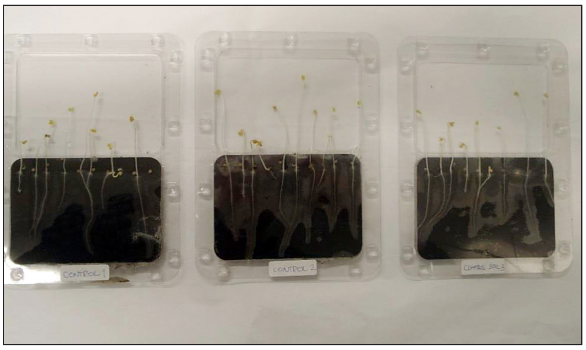

Figure 5. Illustration of the germinating capacity of *Sinapis alba* L. seeds control sample after three days

Figure 6. Illustration of the germinating capacity of *Sinapis alba* L. seeds sample A after three days

The image of the *Sinapis alba* L. control Sample and Sample A are shown on Figures 5 and 6.

Biological assays have been used for several decades in risk assessment and detection of water/sludge contamination have mainly involved aquatic invertebrates (chironomid larvae, mosquitoes, dragon flies, prawns, shells and hydras), aquatic vertebrates such as fish and algae, and aquatic plants such as *Lemna minor* L. Based on these facts, it is obvious that the use of plants as indicators of contamination has been generally underestimated and rarely used in toxicological studies, compared to animal organisms [Moor and Kroege, 2010]. However, the significance of research that involves phytoindicators should not be neglected because such data show the bioavailability of contaminants and enable risk assessment and creation of protocols for remediation of contaminated sites [Gvozdenac et al., 2013].

The problem of the effect of sewage sludge on seed germination and plant growth has been addressed by numerous researchers [Fjällborg and Dave 2004; Fuentes et al. 2006; Hu and Yuan 2012; Oleszczuk 2008; Ramirez et al. 2008a, Oleszczuk et. al., 2012]. Among different toxicity indices based on germination and seedling growth of various higher plants, the growth inhibition seemed to be a good method for the evaluation of the toxicity of sewage sludge [Czerniawska-Kusza, 2006]. In the present study, the plant species of the Phytotoxkit microbiotest responded differently to the degree of contamination of the sewage sludge samples. In general, growth inhibition values clearly revealed the inhibitory effects of sewage sludge contaminants on seed germination and root elongation of *Sinapis alba* L.

CONCLUSIONS

Sewage sludge to be utilized in agriculture must be subjected to comprehensive evaluation comprising not only the determination of the basic physicochemical properties, content of pollutants or pathogenic bacteria, but also of the ecotoxicological properties. Germination capacity known as Phytotoxicity test [Adamcová and Vaverková 2016] was determined to evaluate the suitability of sewage sludge for field application. The results of this study contribute to a novel approach to contamination detection using phytoindicators. The test species, white mustard (*Sinapis alba* L.) expressed different sensitivity levels to contamination. This is consistent with the findings of Gvozdenac et al. [2013] where tolerance levels of crops are species dependent and vary under different stress intensities (concentrations and types of pollutants) and growth stages (germination, emergence, vegetative growth, etc.). In the present study, two types of sewage sludge: dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewatered sludge "Palikal" with dry matter content of about 92% caused phytotoxic effects on the tested plant species, manifesting as root and shoot growth reduction or total inhibition. The results indicate that the tested samples are toxic. Growth inhibition (%) at the studied samples ranged from 70.45% to 100%. In the control, the average root length of *Sinapis alba* L. reached 46.37 mm. In conclusion, the data of this study revealed that the Phytotoxkit microbiotest was effective in identifying toxic sample. In this context, further studies should be performed on sewage sludge characteristics for a better understanding of the biological/ecotoxicological response to the contaminants present.

Acknowledgements

This study was supported by the IGA – Internal Grant Agency Faculty of AgriScience MENDELU No. IP 5/2016 "The relationship between the bedding and the occurrence of mastitis in dairy cows".

REFERENCES

1. Adamcová D., Vaverková M.D. 2016. Does composting of biodegradable municipal solid waste on the landfill body make sense? J. Ecol. Eng. 17(1), 30–37.
2. Albiach R., Canet R., Pomares F., Ingelmo F. 2001. Organic matter components, aggregate stability and biological activity in a horticultural soil fertilized with different rates of two sewage sludges during ten years. Bioresource Technol. 76, 109–114.
3. Czerniawska-Kusza I., Ciesielczuk T., Kusza G., Cichoń A. 2006. Comparison of the phytotoxkit microbiotest and chemical variables for toxicity evaluation of sediments. Environ. Toxicol. 21, 367–372.
4. Epstein E. 2003. Land application of sewage sludge and biosolids. Lewis: Boca Raton.
5. Fjällborg B., Dave G. 2004. Toxicity of Sb and Cu in sewage sludge to terrestrial plants (lettuce, oat, radish), and of sludge elutriate to aquatic organisms (*Daphnia* and *Lemna*) and its interaction. Water Air Soil Pollution. 155, 3–20.
6. Fuentes A., Llorens M., Sacz J., Aguilar M.I., Perez-Marin A.B., Ortuno J.F., Meseguer V.F. 2006. Ecotoxicity, phytotoxicity and extractability of heavy metals from different stabilized sewage sludges. Environmental Pollution. 143, 355–360.
7. Fytili D., Zabaniotou A. 2008. Utilization of sewage sludge in EU application of old and new methods – A review. Renewable and Sustainable Energy Reviews. 12, 116–140.
8. Gvozdenac S., Inđić D., Vuković S. 2013. Phytotoxicity of Chlorpyrifos to White Mustard (*Sinapis alba* L.) and Maize (*Zea mays* L.): Potential Indicators of Insecticide Presence in Water. Pestic. Phytomed. (Belgrade) 28(4), 265–271.
9. Hu M., Yuan J. 2012. Heavy metal speciation of sewage sludge and its phytotoxic effects on the germination of three plant species. Advan. Mathem. Research. 347–353, 1022–1030.
10. Mercedes A.F., Sáez L.J., Aguilar M.I., Ortuño J.F., Meseguer V.F. 2004. Phytotoxicity and heavy metals speciation of stabilised sewage sludges. J. Hazard. Mater. 108(3), 161–169.
11. Oleszczuk P. 2006b. Influence of different bulking agents on polycyclic aromatic hydrocarbons (PAHs) disappearance during sewage sludge composting. Water Air Soil Pollut. 175, 15–32.
12. Oleszczuk P. 2008. Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. Ecotox. Environ. Safe. 69(3), 496–505.
13. Oleszczuk P., Jośko I., Xing B. 2011. The toxicity to plants of the sewage sludges containing multiwalled carbon nanotubes. J. Hazard. Mater. 186, 436–442.
14. Oleszczuk P., Malara A., Jośko I., Lesiuk A. 2012. The Phytotoxicity Changes of Sewage Sludge-Amended Soils. Water Air Soil Pollut. 223(8), 4937–4948.
15. Oleszczuk P. 2006a. Characterization of Polish sewage sludges with respect to fertility and suitability for land application. J. Environ. Sci. Health A, 41, 1119–1217.
16. Phytotoxkit, Seed germination and early growth microbiotest with higher plants, in: Standard Operation Procedure, 2004. MicroBioTests Inc., Nazareth, Belgium, 1–24.
17. Ramirez W.A., Domene X., Andrés P., Alcañiz J.M. 2008. Phytotoxic effects of sewage sludge extracts on the germination of three plant species. Ecotoxicology 17, 834–844.
18. Schowanek D., Carr R., David H., Douben P., Hall J., Kirchmann H., Patria L., Sequi P., Smith S., Webb S. 2004. A risk-based methodology for deriving quality standards for organic contaminants in sewage sludge for use in agriculture – conceptual framework. Regul. Toxicol. Pharm. 40, 227–251.
19. Selivanovskaya S.Y., Latypova V.Z., Artamonova L.A. 2003. Use of sewage sludge compost as the restoration agent on the degraded soil of Tatarstan. J. Environ. Sci. Health A, 38, 1549–1556.
20. Smith K.E., Green M., Thomas G.O., Jones K.C. 2001. Behavior of sewage sludge-derived PAHs on pasture. Environ. Sci. Technol. 35, 2141–2150.
21. Stevens J.L., Stern G.A.,. Tomy G.T, Jones K.C. 2003. AHs, PCBs, PCNs, organochlorine pesticides, synthetic musks and polychlorinated n-alkanes in UK sewage sludge, survey results and implications. Environ. Sci. Technol. 37, 462–467.
22. Werthera J., Ogada T. 1999. Sewage sludge combustion. Prog. Energy Combust. Sci. 25 (1), 55–116.
23. Wong J.W.C., Li K., Fang M., Su D.C. 2001. Toxicity evaluation of sewage sludges in Hong Kong. Environ. Int. 27, 373–380.