Chemosphere 83 (2011) 502–509

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Comparison of sewage sludge toxicity to plants and invertebrates in three different soils

Patryk Oleszczuk [a,*], Henner Hollert [b]

[a] Institute of Soil Sciences and Environmental Management, University of Life Sciences in Lublin, ul. Leszczyńskiego 7, 20-069 Lublin, Poland
[b] Department for Ecosystem Analysis, Institute for Environmental Research (Biology V), RWTH Aachen University, 52074 Aachen, Germany

ARTICLE INFO

Article history:
Received 13 October 2010
Received in revised form 6 December 2010
Accepted 13 December 2010
Available online 13 January 2011

Keywords:
Toxicity
Sewage sludge
Soil
Heterocypris incongruens
Phytotoxicity

ABSTRACT

Understanding the effect of soil type on the overall toxicity of sewage sludge is one of the most important issues concerning environmental risks associated with the sewage sludge land application. The aim of the study was to determine the influence of different soils (sandy, loamy and OECD soil) on sewage sludges toxicity in relation to plants (*Lepidium sativum*, *Sorghum saccharatum*, *Sinapis alba*) and an invertebrate species (*Heterocypris incongruens*). The most evident negative influence of sewage sludges on root growth was observed in the case of OECD soil. The EC_{50} values determined on the basis of the root growth inhibition of all tested plants were in the range 0.1–6.4%, 0.03–9.4% and 6.6–22.1% (% of sewage sludge kg^{-1} soil) for OECD, sandy and loamy soil, respectively. Soil type also affects the sewage sludge toxicity in relation to *H. incongruens*. The LC_{50} (mortality) values ranged from 0.26% to 11.5% depending on the sludge tested. For EC_{50} (growth inhibition) values ranged from 10.7% to 36.2%.

1. Introduction

Because of unquestionable advantages in the agricultural utilisation of sewage sludges, this method of disposal is gaining wider interest. High contents of organic matter and nutrients make sewage sludge a perfect material for fertilization and restoration of degraded soils. Despite the obvious benefits, sewage sludges are still a waste, which when used without in-depth chemical and eco-toxicological evaluation, can lead to environmental contamination and degradation of the fertilized soil. Beside chemical analyses, biological tests are the best and most sensitive method of hazard evaluation relating to the agricultural usage of sewage sludges. Even though sewage sludge biological evaluation is not used in the routine assessment and is not legally regulated. The assessment of waste as hazardous or non-hazardous, according to the European Waste List, includes ecotoxicological characterization. Despite being made into national law by the Waste List Ordinance 2001, no recommendations on the methodology have been provided to cover the hazard criterion (Römbke et al., 2009). Biological assessment allows for a more practical evaluation of waste usability for agricultural purposes together with a better determination of possible hazards relating to their application than a chemical analysis alone. Studies conducted so far have shown that sewage sludges can significantly reduce plant growth and development (Oleszczuk, 2008b; Ramirez et al., 2008b) and increase mortality and inhibit the growth of invertebrates (Barrera et al., 2001; Domene et al., 2007; Oleszczuk, 2008a). Moreover the research have showed that some sewage sludges may have genotoxic properties (Klee et al., 2004) as well as affect on the behaviour of test organisms (Natal-da-luz et al., 2009). In most cases, the negative influence of sludges on these parameters was a result of the presence of organic and inorganic pollutants in the sewage sludges.

Developing bioassays to assess the safety of organic wastes including sewage sludges application to soil is a priority in Europe, given the increased production of these materials and concern about this practice (Domene et al., 2008). While the choice of suitable biotests should be based, primarily, on their ecological merit, also important are low experimental costs and practicality. In our previous research we have investigated the influence of sewage sludges on plant and crustaceans in OECD soils (Oleszczuk, 2008a,b). OECD artificial soil is a widely used substrate in soil toxicity tests. It has been recommended as a medium for ecotoxicological tests and it is a "reference soil" in the testing of complex solid samples (e.g. wastes or contaminated soils). OECD soil is a special type of the soil and can be significantly different then natural soil. The application of a standard OECD soil (or other reference soil) to ecotoxicological analyses is undoubtedly favourable as it allows the soil to be excluded as a toxic factor. Information obtained on this basis determines precisely the toxicity of the waste material being studied; moreover, it also makes comparisons possible between various laboratories and sewage sludges of various origins. However, it does not allow determining of the actual hazard relating to the application of sewage sludge to a particular soil. Together with the proper selection of bioassays to test toxicity of

* Corresponding author. Tel.: +48 82 534 35 48.
E-mail address: patryk.oleszczuk@ar.lublin.pl (P. Oleszczuk).

0045-6535/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2010.12.061

sewage sludge, the selection of the appropriate reference soil (to make control samples and to dilute the tested sample) is crucial. Due to varied soil properties, it is important to determine the influence of the soil type on the toxicity of sewage sludges. There are many papers (Kuperman et al., 2006; van Gestel et al., 2011) describing the effect of pollutants on different organisms depending on soil type. These studies, however, have focused on single chemicals whereas the present work analyzes environmental mixture (sewage sludge). Depending on the soil, different soil properties may have a multidirectional influence on the overall toxicity of a sewage sludge-amended soil. This issue is relatively new and as yet poorly recognized in the literature.

In present research we applied two type of soil (sandy and loamy) which were compared with OECD soil to check how type of soil influence sewage sludge toxicity. Two main aims of the present research were: (i) determine toxicity by two solid phase ecotoxicity tests of sewage sludge-amended different soils and (ii) evaluate of the degree to which soil type and sewage sludge type determines this toxicity.

2. Materials and methods

2.1. Sewage sludges and samples preparation

Soil samples for the present studies were collected during the summer season of 2009. The soil was collected from the following sites: field – from the inter-row of winter wheat (loamy soil) and woodland – from places with limited reach of tree roots (sandy soil). Soil samples were collected from horizon of 0–20 cm. The samples were homogenized to provide a composite sample. The soil was dried at room temperature and sieved through a 1 mm sieve. OECD artificial soil was prepared as a mixture of 70% fine quartz sand (50% particles 0.05–0.2 mm), 20% kaolin clay (kaolinite content preferably above 30%), and finely ground *Sphagnum* peat. Physico-chemical properties of the soils are presented in Table 1.

Two sewage treatment plants localized in Zamość (ZM) and Biłgoraj (BJ) in southeast part of Poland were selected to collect sewage sludge samples. Sewage treatment plants treat about 12500 and 4500 $m^3\,d^{-1}$, respectively of domestic wastewater. The sludge samples (about 50 kg) were collected (during the summer) at the end point of the sewage sludge digestion process. Sewage sludges were typical aerobically digested. Physico-chemical characteristic of the sewage sludges is presented in Table 1.

Sandy (SS), loamy (LS) or OECD soil was mixed with sewage sludge at ratios at a level of 1%, 3%, 6% and 12% (v/v). The amount of sludge applied was established taking into account fertilizing (1%), melioration (3%, 6%) and extreme usually used for land remediation/reclamation (12%) doses. Dried soils and sewage sludges were mixed mechanically. The mixtures of soil and sewage sludge were used immediately after mixing. Both of the biological tests used allow for the direct determination of the toxicity of a solid sample. For physico-chemical analysis samples (sewage sludges, sewage sludge-amended soil) were air-dried and crushed to obtain a representative sample. Sewage sludges were crushed in a mortar and then sieved.

Table 1
Physico-chemical properties of sewage sludges and soils used in the experiment.

Properties	Soils			Sewage sludges	
	SS	LS	OECD	ZM	BJ
Clay	85	51	19	–	–
Silt	13	31	31	–	–
Sand	2	18	50	–	–
pH	3.6	7.1	6.0	6.1	7.6
CEC	0.2	19	0.7	44	97.6
TEB	5.5	20.5	–	144	118
TOC	5.3	10.7	4.0	188.2	157.2
N_t	0.8	1.5	0.01	40.6	22.1
TOC/N_t	6.6	7.1	400.0	4.6	7.1

SL, SH and OECD – light, heavy and OECD soil, respectively. ZM and BJ – sewage sludges. pH – reactivity in KCl, CEC – cation exchange capacity (mmol kg^{-1}), TEB – the total of the exchangeable bases (mmol kg^{-1}), TOC – total organic carbon content (g kg^{-1}), N_t – total nitrogen content (g kg^{-1}).

2.2. Determination of physico-chemical properties of soils and sewage sludges

The chemical properties of soils or sewage sludges studied were determined by standard methods. The pH was measured potentiometrically in 1 M KCl after 24 h in the liquid/soil ratio of 10 (Misztal et al., 1997), the total of the exchangeable bases (TEB) and cation exchange capacity (CEC) were determined in the 0.1 M HCl extraction (Misztal et al., 1997). The total nitrogen (N_t) was determined by the Kjeldahl's method (van Reeuwijk, 1995) without the application of Dewarda's alloy (Cu–Al–Zn alloy-reducer of nitrites and nitrates).

2.3. Ostracodtoxkit test

Toxicity determination of bulk samples was performed in a short-term contact test using an Ostracod test kit (Ostracodtoxkit F™, MicroBioTests, Nazareth, Belgium). Cysts (*Heterocypris incongruens*) were transferred into a Petri dish filled with 10 mL standard fresh water (US EPA medium hard reconstituted water) and were incubated at 25 °C and permanent illumination (approximately 3000–4000 lx). Prefeeding was performed with algae (spirulina powder) that were contained in the test kit. Ostracods started to hatch after approximately 38 h and were straightly used for testing. Algae (*Selenastrum capricornutum*) used as feed in the test plate were reconstituted according to the manufacturer's procedure. Each well of a test plate was filled in the following order: 1 mL algae suspension (sedimentation for 45 min), 10 Ostracods, 1 mL algae suspension, and 300 μL sewage sludge/compost–soil mixture. The test plate was sealed by a laboratory film (Parafilm), covered by a lid, and incubated at 25 °C in the dark. After 6 d, mortality of test organisms was determined. The length of the organisms was measured according to the user guide provided by manufacturer (Ostracodtoxkit F™, 2004). Growth inhibition (GI) of *H. incongruens* was calculated as:

$$GI = 100 - \left(\frac{A}{B} \cdot 100\right)$$

where *A* is the increment of the Ostracods in the reference control soil and *B* is the increment of the Ostracods in the investigated sewage sludge-amended soil.

2.4. Phytotoxkit test

The Phytotoxkit (MicroBioTests, Nazareth, Belgium) microbiotest measures the decrease (or the absence) of seed germination and of the growth of the young roots after a few days of exposure of seeds of selected higher plants to contaminated matrix in comparison to the controls in a reference soil. The Phytotoxkit makes use of flat and shallow transparent test plates composed of two compartments, the lower one of which contains soil saturated to the water holding capacity. Ten seeds (*Lepidium sativum*, *Sorghum saccharatum* or *Sinapis alba*) 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 with their transparent cover, the test plates were placed vertically in a holder and incubated at 25 °C for 3 d. 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