USE OF PHYTOTOXKIT™ TEST IN ASSESSMENT OF TOXICITY OF TWO TYPES OF SEWAGE SLUDGE

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Abstract: The investigations aimed to determine a possible application of Phytotoxkit™ biotest for the assessment of soils amended with sewage sludge. The experiment was performed in a laboratory on samples of sewage sludge. The test bases on estimation of germination and early growth inhibition of sweet sorghum (Sorghum saccharatum L.). Two kinds of sewage sludge: dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewatered sludge with dry matter content of about 92%. The results indicate that the tested samples of sewage sludge are toxic. Growth inhibition (%) at the studied samples ranged from 94.97% to 100%. Phytotoxkit is a good method to evaluate the toxicity of sludge’s, and can be a valuable addition to the physico–chemical methods.

Key Words: sewage sludge, sewage treatment plant, phytotoxicity, sweet sorghum (Sorghum saccharatum L.), land application

INTRODUCTION

Sewage sludge generated from wastewater treatment process is a menace to environment. The cost for disposal of excess sludge accounts for 25–60% of the total operating cost of wastewater treatment plant (Zhang et al. 2009). Sewage sludge pose a threat to the environment and the problem of their utilization. Alongside the undeniable fertilizing properties, the sludge characteristics include the presence of pollutants, mainly heavy metals, pathogens and harmful organic compounds (Oleszczuk 2008), which are associated with a potential danger to the environment (Rosińska and Karwowska 2016).

Studies carried out (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 the soil and plants. Heavy metals and organic compounds are among the “most popular” pollutants present in the sewage sludge (Stevens et al. 2003, Oleszczuk 2006b). The sewage sludge’s application can pose an indirect risk to human health; due to the possibility of pollutants migration to groundwater, or their accumulation in plants (Adamcova et al. 2016). 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, Oleszczuk 2008).

There has been a series of research studies carried out in recent years (Stevens et al. 2003, Oleszczuk 2006b) that has been aimed at the evaluation of the organic and inorganic pollutant contents in sewage sludge and composts. Every year new pollutants that may have, or actually have a negative influence on the living organisms are recognized. Biological tests are a method which determines their negative influence and possible interactions among them (Oleszczuk 2008, Adamcova et al. 2016).

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, Adamcova et al. 2016). 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). Many of authors confirmed...
that the Phytotoxkit microbiotest is effective in identifying toxic samples contaminated with heavy metals, PAHs, pesticides (Czerniawska–Kusza et al. 2006, Wadhia and Thompson 2007, Mankiewicz–Boczek et al. 2008, Oleszczuk 2008, Sekutowski and Sadowski 2009).

The research aimed at assessing the toxicity of sewage sludge by means of Phytotoxkit™ test. The general aim of the present work was: (1) to characterize the sewage sludge’s, (2) to assess the phytotoxicity of two types of sewage sludge’s and (3) to investigate the effect of stabilization strategy used on sludge phytotoxicity. The effects on seed germination and root growth were determined in Sorghum saccharatum L. Such bioassays are simple and rapid methods to indicate phytotoxicity (Wong et al. 2001, Adamcova et al. 2016).

MATERIAL AND METHODS

Characteristic of sludge’s

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 m³/s. The treatment plant consists of a conventional extended aeration activated sludge process (Adamcova et al. 2016).

The experiment was carried out on two sludge’s. The samples (1 kg) were collected in triples (sample A, B and C) at the end point of the sewage sludge digestion process. Sewage sludge’s were typical aerobically digested. The two types of sludge’s had been stabilized in different ways as follows: II – dewatered and anaerobically stabilized sludge with dry matter content of about 24%, I – dewatered sludge with dry matter content of about 92%.

Chemical characteristic of the sewage sludge’s 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 sludge’s were crushed in a mortar and then sieved through a 2 mm sieve for chemical and ecotoxicological analysis (Adamcova et al. 2016).

Table 1 Chemical characteristic of the sewage sludge’s (Adamcova et al. 2016)

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

Phytotoxicity test

The toxicity of sewage sludge was assessed with a commercial toxicity bioassay–Phytoxkit™ Test (Microbiotests, Nazareth, Belgium) (Phytotoxkit™ 2004). The test was based on measurement of germination and growth of the plant roots after three days of exposure to the soil/sewage sludge in comparison with germination and growth of these plants in the soil. The test was conducted following the procedure recommended by the manufacturer (Phytotoxkit™ 2004). The phytoxkit 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 (Adamcova et al. 2016). In the experiment *Sorghum saccharatum* L., was chosen.

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 *Sorghum saccharatum* 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 Tool 3.0 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 (1):

\[
SG / RI = A - B / A \times 100 (1),
\]

where A means seed germination and root length in the control; B means seed germination and root length in the test (Adamcova et al. 2016).

**RESULTS AND DISCUSSION**

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

*Table 2 The degree of toxicity (www.um.prf.jcu.cz, adjust 2016)*

<table>
<thead>
<tr>
<th>Inhibition (%)</th>
<th>The degree of toxicity</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I* &lt; 10</td>
<td>1</td>
<td>Non – toxic or slightly toxic</td>
</tr>
<tr>
<td>10 &lt; I &lt; 50</td>
<td>2</td>
<td>Toxic</td>
</tr>
<tr>
<td>50 &lt; I</td>
<td>3</td>
<td>Strongly toxic</td>
</tr>
</tbody>
</table>

*I – growth inhibition (%)*

Figure 1–3 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 *Sorghum saccharatum* L. (SOS), Samples A, B and C.

*Figure 1 SOS Sample A*

The growth inhibition (%) of *Sorghum saccharatum* L. for dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewatered sludge with dry matter content of about 92%, Sample A was in the range of 95.33–100%. These samples are strongly toxic, the degree of toxicity 3,50 < I.
The growth inhibition (%) of *Sorghum saccharatum* L. for dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewatered sludge with dry matter content of about 92%, Sample B was in the range of 94.97–100%. These samples are strongly toxic, the degree of toxicity $I_{50} < 3$.

The growth inhibition (%) of *Sorghum saccharatum* L. for dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewatered sludge with dry matter content of about 92%, Sample C was in the range of 97.27–100%. These samples are strongly toxic, the degree of toxicity $I_{50} < 3$. The image of the *Sorghum saccharatum* L. control Sample and Sample A.

Sewage sludge is the by–product from the wastewater treatment plant. It is a complex mixture of organic and inorganic materials and contains a wide variety of substances and microorganisms in suspended or dissolved form (Werther and Ogada 1999, Niu et al. 2016).

Due to mixed contamination of sewage sludge with potentially hundreds of different substances, environmental quality assessment of sewage sludge or composted sewage sludge is challenging and cannot be achieved with chemical analysis alone. Ecotoxicity assessment provides valuable information on the environmental fate of these materials. Biotest could be used as an indicator for potential risk for example when sewage sludge–based products are targeted to agricultural or landscaping applications (Kapanen 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. 2008, Oleszczuk et. al. 2012 and Adamcova et al. 2016). 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, Adamcova et al. 2016). 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 *Sorghum saccharatum* L.

**CONCLUSIONS**

In the present study, two types of sewage sludge: dewatered and anaerobically stabilized sludge with dry matter content of about 24%, and dewatered sludge 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 94.97% to 100%. In the control, the average root length of *Sorghum saccharatum* L. reached 33.47 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.

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