Assessment of pesticides-contaminated soil: the case study from remediation viewpoint

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Abstract:
The remediation of highly contaminated sites around former chemical plants or storehouses is an important task for biotechnologists. In some cases initial information about contamination is not available, what makes remediation rather difficult. The aim of this study was to assess a possibility of soil bioremediation in the case of soil with unidentified contaminants of pesticides origin. Determination of pesticides in contaminated soil by gas chromatography has revealed three substances in concentration \( \leq 1 \text{ mg/kg soil} \). However, the concentration of the total N, C, S, as well organics in this sample was considerably higher as compared to the non-contaminated soil sample. A further search for appropriate methods for contaminant(s) identification is necessary. Soil assessment for its bioremediation showed that in this case it is possible. Activation of respiration, enzymatic activity, as well as microbial proliferation under certain conditions indicated to this possibility. Although an efficiency of this process, at this moment, is unpredictable until contaminant(s) and its degradation products are identified. The promoting role of additional nutrients, i.e. cabbage leave extract and buffered salt composition for activation of metabolic processes in contaminated soil was shown. Bioaugmentation in these experiments did not show a promoting effect for soil activation.

Keywords: bioremediation, soil, respiration, enzymatic activity, germination, root elongation

Abbreviations: FDA - Fluorescein Diacetate (3',6'-diacetylfluorescein); SIR – Substrate-Induced Respiration; TGA – Tryptone Glucose Yeast Extract Agar; CFU – Colony Forming Units.

1 Introduction

Since before 2500 BC, humans have utilized pesticides to protect their crops. In the 1940s manufacturers began to produce large amounts of synthetic pesticides and their use became widespread [1]. Over 98 % of sprayed insecticides and 95 % of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments, and food [2]. Pesticide use raises a number of environmental concerns. Development of effective remediation technological approaches for this purpose is of a great importance.

Soil activation by microorganisms with contaminant-degrading ability is widely used for bioremediation. However, their efficiency depends on many factors, including the chemical nature and the concentration of pollutants, their availability to degrade pollutants, and the physicochemical characteristics of the environment [3]. Various types of soil amendments used for soil remediation, revitalization and reuse are summarized in [4]. Beta-cyclodextrins solutions are employed to enhance the aqueous solubility of a hydrophobic organic compound. These additions increased hydrosolubility of the contaminants, which means the first step before microorganism uptake [5]. Addition of nutrients, such as carbohydrates, microelements, vitamins, can noticeably stimulate the process of biodegradation. Various amendments of natural origin are under investigation. The use of organomineral complex prepared from humic acids (organic part) bound on zeolite (inorganic part) was shown to be appropriate for bioremediation. Both components have good sorption properties and are of natural origin [6]. Organic wastes, i.e. compost, poultry manure, sewage sludge and organic municipal solid waste, were compared for their efficiency in the bioremediation process [7]. Application of minced shepherd’s purse root containing high peroxidase activity as a catalytic agent was studied in degradation of phenolic pollutants [8]. Plant extract as a source of additional nutrients for soil microorganisms was shown to be efficient in degradation of nitroaromatic compounds [9].

Thus, a lot of information regarding bioremediation of pesticides-contaminated soils can serve as a basis for technological approach. However, the model
experiments with known components and a real field-scale contaminated soil of unidentified origin are completely different in terms of their handling and experimental performance. The remediation of the highly contaminated sites around former chemical plants or storehouses is an important task for biotechnologists. In some cases initial information about contamination is not available, what makes remediation rather difficult. The aim of this study was to assess a possibility of soil bioremediation in the case of soil with unidentified contaminants of pesticides origin.

2 Materials and methods

Microbiological and biochemical study. For microbiological testing and fermentative activity 1g of average soil sample for each test was taken in duplicate. The number of CFU was determined using TGA medium (Sifin). FDA activity was determined by hydrolysis of fluorescein diacetate [10]. Substrate-induced respiration was determined according to [11-12] with some modification. The CO2 released from 30 g of glucose-amended soils after 24 h of incubation at +25°C was trapped in 5 mL of 0.15 mol L⁻¹ NaOH and determined by titration with 0.05 mol L⁻¹ HCl.

Eco-toxicological study. Germination and root elongation tests were performed according to EPA 712-C-96-154 [13].

Analytical study. Soil pH was measured according to ISO 10390. Carbon and sulphur were measured using the C, S analyzator (ELTRA). Total ammonium was determined according to ISO 5983-2:2005.

Remediation study

Two average soil samples were used, i.e. the soil X with unidentified contaminant and the soil A without contamination taken from the same sampling site. Two soil samples were mixed in different ratios thus decreasing a toxicity level of the soil X. Liquid phase, according to the scheme of experiment, consisted of sterile distilled water, or M8* solution (Table 1). Cabbage leaf extract, as well as inoculum of bacteria were added when indicated. M8* solution contained, g/l: Na₂HPO₄ – 60, KH₂PO₄ – 30, NaCl – 5 (pH 6.9). Cabbage leaf extract was prepared according to [9]. Experiments were performed in duplicate.

3 Results and discussion

3.1 Preliminary soil testing

Soil samples X and A were tested from physical, chemical, microbiological, and eco-toxicological viewpoints with the aim to characterise soil properties for its bioremediation.

3.1.1 Physico-chemical properties of the soil samples X and A

The pH level in soil samples X and A was 7.7 and 8.3, respectively. Redox potential was -51.7 and -96.6, respectively.

The soil physico-chemical testing has revealed the presence of the total N, C, and S in the sample X in much greater concentrations as compared to the sample A (Table 2). Obviously, contamination in the soil X is of organic origin, because the content of organics in this sample was found to be of 16% (Table 2).

Table 1. Scheme of the experiment (+ 28 °C, 21 days)

<table>
<thead>
<tr>
<th>Variant</th>
<th>Sample A, g</th>
<th>Sample X, g</th>
<th>H₂O, ml</th>
<th>M8*, ml</th>
<th>CLE, ml</th>
<th>Inoculum, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>24</td>
<td>20</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>0</td>
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<td>0</td>
<td>-</td>
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</tbody>
</table>

Table 2. Physico-chemical characteristics of soil samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dry weight, %</th>
<th>N, %</th>
<th>C, %</th>
<th>Ash, %</th>
<th>S, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>89,71</td>
<td>0,32</td>
<td>3,18</td>
<td>83,82</td>
<td>12400,00</td>
</tr>
<tr>
<td>A</td>
<td>94,48</td>
<td>0,01</td>
<td>2,15</td>
<td>94,73</td>
<td>854,20</td>
</tr>
</tbody>
</table>
3.1.2 Toxicity assessment of soil sample X

Soil toxicity was assessed using germination, root elongation test, as well as the plating method for microorganism cultivation in the presence of contaminated soil. Thus, the number of colony forming units in the soil samples X and A was $2.0 \times 10^4$ and $1.0 \times 10^6$, respectively. It is important to note, that no colony formation was observed if cultivation was performed in the presence of soil X (5% and 50 % soil X in TGA medium).

Seed germination in the presence of soil X varied in the range of 90-95%, except cress and rape, which were more sensitive to this contamination. Thus, germination of cress and rape seeds was of about 60 %. Soil A did not affect seed germination, which was 100 % for all tested plants.

Root elongation test demonstrated a toxic effect of soil X for all tested plants (Fig.1).

![Fig.1. Toxicity assessment of soil X and soil A using the root elongation test.](image)

3.1.3 Determination of pesticides in the soil sample X

Determination of pesticides in the soil sample X by gas chromatography has revealed three substances in concentration $\leq 1 \text{ mg/kg soil}$ (results are not shown). These substances belong to the herbicides and known as slightly hazardous (class III) [14]. Analysis of these results and data shown in Table 2 comes to the conclusion, that the herbicides identified in this study cannot be the main source of contamination. Soil toxicity, content of the total nitrogen and carbon in the sample X did not correspond to the level of identified herbicides in this soil sample.

A search for appropriate analytical method to identify the main contamination of soil X is supposed to be continued in future. The identification of transformation products of pesticides is a crucial task difficult to tackle, due to the lack of standards and scarce information available [15].

3.2 Evaluation of soil sample X for its biodegradability

The scheme of the remediation experiment is shown in Table 1.

3.2.2 Microbiological, biochemical and physico-chemical changes in soil occurred upon remediation study

Considerable changes in microbiological, biochemical and physico-chemical properties of the tested soil samples were found after 21 days incubation.

After incubation, dry weight of all tested samples varied in the range of 53-63 %. The pH level in the samples varied from 7.0 to 8.27. The highest pH level was found to be in the sample containing only non-contaminated soil A (Table 1, No.9). Redox potential varied from -14.2 to -83.7. The number of CFU in soil samples for all 10 variants was determined after 2, 12, and 21 days incubation. The number of CFU varied in the range from $3.0 \times 10^6$ to $5.0 \times 10^7$ after 2 days; from $5.0 \times 10^8$ to $9.0 \times 10^{11}$ after 12 days; and from $2.0 \times 10^8$ to $1.5 \times 10^{10}$ after 21 days incubation. Thus, microbial proliferation was detected in all tested samples and was found to be the highest in the middle of incubation period.

Microbial respiration of soil is known to be one of the more important criteria for evaluation of the metabolic processes occurred in soil. In this study, respiration and substrate-induced respiration was measured. Among tested samples, the highest respiration activity was detected in the variant No. 6 (Fig.2, Table 1). It is important to note, that addition of glucose into soil before determination of SIR, the noticeable increase of respiration activity was found in the samples containing the buffered liquid phase (M8*), as compared to the samples amended with water. Moreover, among these samples, the highest SIR activity was in the cases with the highest concentration of the soil X in the sample, i.e. No.4, 6, 8, and 10 (Fig.2, Table 1).

Soil enzymatic activities are among the measurements of soil quality that are the most
frequently and consistently able to distinguish between management systems.

![Fig.2](image1.png)

**Fig.2.** Microbial respiration of soil samples after 21 day incubation. (Description of the samples in Table 1). R – respiration, SIR – substrate-induced respiration.

Hydrolysis of FDA has been suggested as an appropriate method in integrated soil bioecosystem studies because the ubiquitous lipase, protease, and esterase enzymes are involved in the hydrolysis of FDA [16].

![Fig.3](image2.png)

**Fig.3.** FDA hydrolysis activity in soil samples after 21 days incubation. OD_{490} = 1 corresponded to the concentration of FDA=2.98 mg/l. (Description of the samples in Table 1).

In this study the higher FDA hydrolysis activity was detected in the samples containing plant extract (Fig.3, samples No. 5-10). The same effect, as was shown for microbial respiration (Fig.2), was found for FDA hydrolysis activity, i.e. soil samples with the higher content of soil X demonstrated the higher FDA hydrolysis activity (Fig.3, samples No.6, 8, and 10).

After 21 days incubation, the concentrations of the total N, C and S were measured in the soil samples. As shown in Fig.4, no noticeable decrease of these elements was detected. The longer period of incubation is necessary to achieve sufficient results of bioremediation.

![Fig.4](image3.png)

**Fig.4.** Concentration of the total N, C, and S in soil samples after 21 day incubation. (Description of the samples in Table 1).

### 3.3 Ecotoxicological evaluation of soil

The results of germination test, as well as root elongation test showed that phytotoxicity of soil X after 3 week incubation remained rather high (Fig.5).

![Fig.5](image4.png)

**Fig.5.** Germination test for soil samples after 21 day incubation. (Description of the samples in Table 1).

The most sensitive to soil X were rape and cress. It is interesting to note, that the seeds of wheat have germinated in the presence of soil samples amended with M8* solution and plant extract, much better as compared to soil amended with water or M8* solution alone (Fig.5).
4 Conclusions

The use of alternative methods of soil physico-chemical testing revealed that 3 herbicides identified by gas chromatography are not the main source of contamination. A further search for appropriate methods for contaminant(s) identification is necessary.

Soil assessment for its bioremediation showed that in this case it is possible. Activation of respiration, enzymatic activity, as well as microbial proliferation under certain conditions indicated to this possibility. Although an efficiency of this process, at this moment, is unpredictable until contaminant(s) and its degradation products are identified.

The promoting role of additional nutrients, i.e. cabbage leaf extract and M8* buffered salt composition for activation of metabolic processes in contaminated soil was shown. These results are in a good agreement with data obtained earlier for degradation of nitro aromatic compounds [17]. Bioaugmentation in these experiments did not show a promoting effect for soil activation.

In future it is supposed to continue experiments on possible bioremediation of soil X.

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References: