Using Different Tolerant Plant for Phytoremediation of Contaminated Soils with Total Petroleum Hydrocarbons

Smaranda Măsu¹, Florica Morariu², Neculai Dragomir²

¹National R & D Institute for Industrial Ecology, Branch of Timisoara, 300004-Timisoara, Regina Maria 1, Romania
²Banat’s University of Agricultural Sciences and Veterinary Medicine from Timisoara, Faculty of Animal Science and Biotechnologies, 300645-Timisoara, Calea Aradului 119, Romania

Abstract
The extraction activity, transportation and processing of crude oil caused soil contamination with total petroleum hydrocarbons (TPH). The develop phytoremediation process of contaminated soils with 2.8 % TPH, were studied in pots, with a tolerant grass species, *Lolium perenne*. Four treatments, each consisting of three replicates, were realized in randomized block design. The experimental variants are: control, uncontaminated soil, untreated and treated contaminated soil with anaerobic stabilized sewage sludge (50 t/ha) in absence/presence volcanic indigenous tuff amendment (5 t/ha). Removal efficiencies of TPH from the contaminated soils variants treated with organic fertilizer without volcanic indigenous tuff and mixed with tuff after eight months was 73.4 % and 78.9 % respectively. The results are supported by healthy plants with roots system well developed. The green biomass harvested from the variant fertilized with sludge mixed with volcanic tuff was similar to the harvested from control variant, 15.1-17.9 g / pot of vegetation The obtained results show that, the tolerant grass species, *Lolium perenne* must be applied safely to phytoremediation, on TPH contaminated soil.

Keywords: phytoremediation, stabilized sewage sludge, tolerant plant, total petroleum hydrocarbons, volcanic indigenous tuff.

1. Introduction
The extraction, transportation and processing of crude oil induce frequent pollution of soil, sediment, water, etc. Application of physico-chemical remediation processes in-situ or ex-situ of soils contaminated with TPH represents solutions with effective results but the methods generally greatly harm to the environment [1]. The phytoremediation of contaminated soils by using appropriate plants has the advantage that plants contribute to the biodegradation of TPH, pollutants [2-4], limits soil washing and prevents the migration of pollutants to groundwater [5]. Numerous plant species were tested to identify their phytoremediation potential.

Mostly, classifications were made with categories of plants being tested:
1. Plants with a demonstrated potential to phytoremediate soil with TPH compounds;
2. Plants with a demonstrated potential to tolerate TPH compounds in soil.

The first category includes a number of monocotyledonous (gramineae), i.e.: annual ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), sudangrasss (*Sorghum vulgare*), shorgum (*Sorghum bicolor*), tall fescue (*Festuca arundinecea*), red fescue (*Festuca rubra*), etc. [6-7]. It must be noted that soils contaminated with TPH are mixtures of soil particles with non-polar liquid (non-aqueous liquid phase - NAPL). These mixtures form some small amounts where microbial activity is strongly diminished. Water needed by microorganisms both as biotic component and as a carrier for nutrients, is not
available in these small amounts so that those areas remain in storage (as highly contaminated environments), biologically inert for long periods. [8-9]. It was reported that roots are more resistant to the presence of TPH compounds than the aerial part. Generally plants tolerate up to 3% TPH in the contaminated soil [9].

The biodegradation of 1% TPH in contaminated soils in the presence of perennial plants of the *Lolium perenne* species, get up to 24% [10]. The specificity of these plants is that they develop a fibrous root system containing large amounts of soil so that their contact surface with pollutants is much higher than the deep root systems [3].

In soils contaminated with TPH there is a shortage of bioassimilable nutrients and a decreased biocenosis settlement due to TPH toxicity. The addition of fertilizing agent is necessary both as nutritional agent and as supplement for maintaining the contaminated soil biocenosis activity and for the metabolic activities of the plant. For soils contaminated with TPH it is recommended to add fertilization agents that can facilitate the conversion of non bioassimilable compounds from crude oil to humic matter. [11].

Indigenous volcanic tuff comes from Mîrșid Romania careers and contains 70-72% clinoptilolites. The volcanic rock used has 0.2-2 mm and mixed with sewage sludge, fertilization agent.

In order to determine the variation of TPH in soils, their concentration is periodically determined [13], in the top layer of the vegetation pot (2 cm depth). The soil is dried and ground through a 5 mesh sieve. To determine the TPH from the soils an analysis is performed periodically of the concentration [8], in the upper level:

1. 0.5-1.0 g of dry soil are weighed (\(M\)), then add 5 g Na\(_2\)SO\(_4\) anhydrous and 25 ml solvent, CCl\(_4\) p.a.;
2. 30 minutes stirring at 50 rotations/min. and then filtered;
3. The glass and filter paper are washed with solvent CCl\(_4\), which is added to the filtrate;
4. The filtrate is evaporated on water bath;
5. The residue is dissolved in CCl\(_4\), then passed through the chromatographic column filled with aluminium oxide. The eluate collected in a tarred capsule; \(m_1\) (g);
6. CCl\(_4\) is evaporated at room temperature and weighed at constant mass \(m_2\) (g);
7. The same is done for the control from 28 ml CCl\(_4\) \((m_3 - m_4)\) – mass of capsule without control residue (g), \(m_4\) – mass of capsule with control residue (g);
8. Calculating TPH: TPH (g • kg\(^{-1}\)) = 
\[
1000 \times \frac{[(m_2 - m_1) - (m_4 - m_3)]}{M-1}
\]

2. Materials and methods

Vegetation pots were equipped with 6.5 kg of soil each, which is seeded with *Lolium perenne* species. The experimental study was done on non contaminated/contaminated soils with TPH in the following variants:
1. Normal soil, blank, M;
2. 2.8% TPH contaminated soil, P;
3. 2.8% TPH contaminated soil fertilized with anaerobic stabilized sewage sludge derived from municipal wastewater Station Timisoara, the amount of 50 t/ha D.M., PN;
4. 2.8% TPH contaminated soil, fertilized with sewage sludge in the amount of 50 t/ha D.M. mixed with indigenous volcanic tuff in the amount of 1 % vs. the amount of soil, PNT.

Each experimental variant is done in three replicates.

3. Results and discussion

The initial level of pollution on soil was 28±0.6 g/kg D.M and used in the experimental study. The variation of oil content in contaminated soils was studied in seeded experimental variant with *Lolium perenne* species, from 29.03-26.10.2012,
and is shown in table 1 (in the topsoil of the rhizosphere).

<table>
<thead>
<tr>
<th>No</th>
<th>Experimental variants</th>
<th>Depth (cm)</th>
<th>pH</th>
<th>Initial TPH (g/kg D.M.)</th>
<th>After 8 vegetation months TPH (g/kg D.M.)</th>
<th>Removal efficiency TPH(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>0-2</td>
<td>7.11</td>
<td>0.025±0.003</td>
<td>0.023±0.002</td>
<td>8.0</td>
</tr>
<tr>
<td>2</td>
<td>P</td>
<td>0-2</td>
<td>7.58</td>
<td>28.0±0.6</td>
<td>21.2±0.2</td>
<td>24.3</td>
</tr>
<tr>
<td>4</td>
<td>PN</td>
<td>0-2</td>
<td>7.12</td>
<td>28.0±0.6</td>
<td>7.44±0.1</td>
<td>73.4</td>
</tr>
<tr>
<td>6</td>
<td>PTN</td>
<td>0-2</td>
<td>7.46</td>
<td>28.0±0.6</td>
<td>5.63±0.1</td>
<td>79.8</td>
</tr>
</tbody>
</table>

From table 1 we can notice that the grown green cover of *Lolium perenne* species on contaminated soils with an initial pollution level of 28.0±0.6 g TPH/kg D.M., TPH biodegradation determine at the topsoil (the rhizosphere areas), an average of 6.8 g TPH/kg D.M. in the period of 8 vegetation months. As shown in table 1, this consumption represents TPH removal efficiency with 24.3% in a growth period of eight vegetation months. This result is similar to the one reported by literature [10].

The addition of anaerobic stabilized sewage sludge on TPH contaminated soil determined the achievement of more vigorous crops that form a green cover with grasses plants. Plants were harvested periodically. The TPH removal efficiency in fertilized soil gets up to 73.4% during the 8 months of vegetation. In the experimental variant fertilized with anaerobic stabilized sewage sludge mixed with the indigenous volcanic tuff amendment, TPH removal efficiency in the rhizosphere is up to 79.8%, and the residual TPH after eight vegetation months was 5.63±0.1 g/kg D.M.

The roots of plants grown on experimental variants of contaminated soil fertilized with anaerobic stabilized sewage sludge in presence of indigenous volcanic tuff are well developed and occupy the entire volume in the vegetation pot, after 8 months of vegetation. Roots networks are shown in figure 1.

![Figure 1](image1.png)

**Figure 1.** Roots of harvested plants from experimental variant fertilized with anaerobic stabilized sewage sludge mixed with volcanic tuff, after 8 months of vegetation

Note that plants from the experimental variant of untreated contaminated soil, dry slowly so that at the end of the study period on this version only biomass is harvested dry.

The average amount of green biomass resulting from phytoremediation experimental variants taken in the vegetation pots, with plants of the *Lolium perenne* species is shown in table 2.

Table 2 shows that the amount of biomass harvested from contaminated soil. In June, it was 10.32 g/pot of vegetation. At the second harvest, in July, the amount of grass decreases by more than 50% vs. the first harvested and on the third harvesting, in August, the amount of grass decreases by more than 75% of the quantity harvested in June. Addition of anaerobic stabilized sewage sludge determined that in June the amount of grass to grow by up to 60% from the quantity harvested from untreated contaminated variant. At the second harvest, in July, the amount of grass decreases in this case more than 50% of the quantity harvested on the first, in June. Note,
however, that on the third harvest, in August, the amount of grass was similar to the amount harvested in June. Addition of experimental variant of anaerobic stabilized sewage sludge mixed with indigenous volcanic tuff not induced increase in yield at the first mowing vs. quantity harvested from fertilized experimental variant without indigenous volcanic tuff. The positive effect of indigenous volcanic tuff was found on the second harvest when the amount of grass had increased by 50% vs. amount harvested from fertilized experimental variant without indigenous volcanic tuff. On the third harvesting, in August, was obtained from the experimental variants treated with anaerobic stabilized sewage sludge mixed with indigenous volcanic tuff, a quantity of 17.9 g/pot of vegetation, amount 2.8 g/vegetation pot larger than the blank experimental variant, third harvesting. Indigenous volcanic tuff submitted a stimulatory effect on plant metabolism or their development during the warm and drought summer months.

Table 3 presents the amount of dry biomass resulting from experimental variants with grass of the *Lolium perenne* species.

<table>
<thead>
<tr>
<th>No</th>
<th>Experimental variants</th>
<th>Dry matter vs. green biomass (%)</th>
<th>Harvest 1</th>
<th>Harvest 2</th>
<th>Harvest 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>18.2 - 20.0</td>
<td>23.8</td>
<td>19.1 – 19.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>P</td>
<td>16.6 - 17.3</td>
<td>25.1 - 27.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PN</td>
<td>15.0 - 17.2</td>
<td>28.9 - 30.1</td>
<td>28.0 - 33.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>PTN</td>
<td>18.0 - 21.7</td>
<td>22.9 - 23.8</td>
<td>24.4 - 25.8</td>
<td></td>
</tr>
</tbody>
</table>

From table 3 is observed that the addition of indigenous volcanic tuff determined the formation of a mass containing dry grass similar to the amount of dry mass resulting from grass harvested from the control variants for the three harvests in June, July and August. For the untreated contaminated experimental variants and of the variant contaminated and treated with sewage sludge, the amount of grass dry matter was up to 25% lower in June. In July and August, however, these experimental variants were found gradually drying plants for which at harvest time, there was a considerable share of dry weight. Addition of indigenous volcanic tuff has the net benefit of keeping crops in healthy green biomass during warm and drought summer months.

4. Conclusions

The *Lolium perenne* species stands out as a plant with tolerance in soils contaminated with TPH for a pollution level of 2.8% and with phytoremediation potential. To obtain increased efficiencies for the reduction of TPH from soils it is absolutely necessary to fertilize with an organic fertilizing agent. Experimental variant fertilized with anaerobic stabilized sewage sludge led in increased efficiency of TPH amount biodegraded from 24.3% to 73.4% in 8 months of vegetation. Furthermore addition of indigenous volcanic tuff has determined a more advanced decrease of TPH content in the topsoil of up to 79.8%, due to the acceleration of metabolic processes by soil biocenosis in connection with porous materials, indigenous volcanic tuff introduced. Increased removal efficiency of TPH from contaminated soils fertilized with anaerobic stabilized sewage sludge in the absence/presence of indigenous volcanic tuff, seeded with *Lolium perenne* species were due to the plant ability to form well developed and branched roots, that led to the development of aerial part of plants and give an amount of green biomass of 5-8 times higher vs. the harvest resulted on unfertilized experimental variants Addition of indigenous tuff determined...
the maintaining of a healthy cover *Lolium perenne* species in the warm and drought period during the summer months.

**References**

1. Meuser, H., Soil Decontamination, Soil Remediation and Rehabilitation, Environmental Pollution, 2013, 23, 201-278