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Soil pollution

VARIATION OF OIL PRODUCTS IN CONTAMINATED SOIL CULTIVATED WITH LEGUMINOUS SPECIES

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Abstract. The phytoremediation of destroyed soil by anthropogenic activities with appropriate species of plants has multiple applications in the treatment of contaminated areas and environmental management. The aim of the study was to determine the abilities of *Onobrychis viciifolia* species to form a vegetative cover on contaminated soil with 2.86% total petroleum hydrocarbons (TPH) and to assess the degradation efficiency from the rhizosphere. This study is performed on experimental variants of contaminated soil in the absence/presence of the fertiliser agent combined/non-combined with volcanic indigenous tuff. Each experimental variant was done in 3 replicates. The fertilisation of contaminated soil with 50 t ha^{-1} fertiliser agent, municipal sludge anaerobically bio-stabilised (biosolids), combined with indigenous volcanic tuff, 5 t ha^{-1}, determined the vegetation of *Onobrychis viciifolia,* resistant to prolonged drought. Furthermore, leguminous species, *Onobrychis viciifolia,* has demonstrated the ability to biodegrade oil products (TPH). The results obtained on the experimental variants fertilised with municipal sludge in the absence/presence of indigenous volcanic tuff showed oil products degradation by 55–70%, after the vegetative cycle (8 weeks). Furthermore, biomass quantity resulted from treated soil with fertiliser agent and volcanic tuff was 50% higher than the amount of biomass that resulted from volcanic tuff untreated experimental variants.

Keywords: *c*ontaminated soil*,* oil products, *Onobrychis viciifolia*, phytoremediation.

AIMS AND BACKGROUND

The aim of the study is to investigate oil products by total petroleum products (TPH) parameter, phytoremediation on contaminated soils using leguminous species *Onobrychis viciifolia* and the interdependence of biodegradation level of these products with the fertilising matter in the absence/presence of amendment porous volcanic indigenous tuff.

Contaminated soil containing oil products have the risk potential for the agricultural circuit[1]. One of the rehabilitation techniques of the contaminated sites with

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total petroleum hydrocarbons is the use of plants, i.e. phytoremediation[2,3]. Among the plants, the leguminous species show phytoremediation characteristics due to the fact that they have the capacity to release in the rhizosphere area enzymatic exudates that determine the selection of some specific bacterial groups. Plant-bacteria consortium shows increased capacity to biodegrade organic pollutants. Plants absorb pollutants as such or as products resulted from microbial, enzymatic degradation in rhizosphere[4]. It is suggested the examination of the vegetation on the fields and then deciding if the studied species shows abilities for phytoremediation[5]. An example of leguminous plants prevailing in approximately 15 areas contaminated with oil products in Europe are *Medicago lupulina*, and *Lotus corniculatus*[6]. For the intensification of the TPH degradation, organic fertilisers are used. They provide compounds easily bioavailable to the plants and a biocenosis succession which positively acts in the biodegradation of the TPH. The strategy for phytoremediation of organic products in contaminated soils is correlated with an appropriate treatment applied to polluted soils with high/medium crude oil concentration[7,8]. Moreover, the intensification of the biodegradation of TPH was reported when treating soils with inorganic material: i.e. bentonite, natural zeolite, etc., which reduce the soils pollution[9].

EXPERIMENTAL

The experimental study was carried out on non-contaminated/contaminated soils with 2.86% TPH in the following variants: (1) non-contaminated soil, control experimental variant, **M**; (2) Contaminated soil with 2.86% TPH, **P**; (3) Contaminated soil with 2.86% TPH and fertilised with anaerobically bio-stabilised municipal sludge (name biosolids), from municipal treatment plant, **PN**; (4) Contaminated soil with 2.86% TPH, fertilised with biosolids and indigenous volcanic tuff, **PNT**. The experiment was carried out in vegetation pots with 6.5 kg dry matter (DM) of soil. Each variant is done in 3 replicates.

Biosolids were used in amounts of 50 t ha^{-1} DM for the experimental fertilised variants. The indigenous volcanic tuff from Romania (with 70/72% clinoptilolite), Marsid quarry, was used in quantities of 5 t ha^{-1} wt./wt. as amendment. The resulted biosolids from the Timisoara municipal water treatment plant exhibited the characteristics presented in Table 1, and were used to prepare the fertile layer of vegetation. Other characteristics of biosolids were 80±0.5% humidity, 64.4±0.6% organic matter, 2.85±0.2% N_{total}, 0.55±0.2% P and a pH about 7.3±0.3 units. The fertilising agent used contains nutrients for an optimum plant growth. Moreover, bring a bacterial consortium that completes the soil needs.

Table 1. Heavy metal content of used biosolids

Material	Heavy metal content of used biosolids* (mg kg^{-1}DM)							
	Cr_{tot}	Cu	Cd	Fe	Mn	Ni	Pb	Zn
Biosolids	112.3 ± 3.2	402.3 ± 3.5	3.3 ± 0.1	2040.7 ± 12.2	69.7 ± 1.8	13.5 ± 0.3	63.8 ± 12.0	205.6 ± 13.6

Values are means of 12 samples ±SE; *mean values.

In the vegetation pots 50 seeds from the *Onobrychis viciifolia* plant species were planted. To determine the TPH from the soils an analysis of the concentration was performed periodically[10], in the upper level: (1) 3–5 g of dry soil are weighed (**M**), then 5 g Na_2SO_4 anhydrous and 25 ml petroleum ether p.a. are added; (2) 30 min stirring at 50 rpm and then filtered; (3) the glass and filter paper are washed with petroleum ether, which is added to the filtrate; (4) the filtrate is evaporated on water bath; (5) the residue is dissolved in petroleum ether, then passed through the chromatographic column filled with aluminium oxide. The elute collected in a tarred capsule; $\mathbf{m_1}$ (g); (6) petroleum ether is evaporated at room temperature and weighed at constant mass $\mathbf{m_2}$ (g); (7) the same is done for the control with 28 ml petroleum ether ($\mathbf{m_3}$ – mass of capsule without control residue (g), $\mathbf{m_4}$ – mass of capsule with control residue (g)); (8) calculating TPH:

$$\text{TPH [g kg}^{-1}] = 1000 \times [(\mathbf{m_2} - \mathbf{m_1}) - (\mathbf{m_4} - \mathbf{m_3})]\ \text{M}^{-1}$$

RESULTS AND DISCUSSION

Table 2 shows the TPH quantities from the experimental variants of initial studied soils and varying the quantities of pollutants from soils cultivated with *Onobrychis viciifolia* leguminous species during vegetative cycle. In Table 2 we noticed that in the case of the contaminated soils, the quantity of TPH decreases from 28.60±0.30 to 25.50±0.20 g kg^{-1} DM during the 8 weeks vegetative cycle. In the case of the experimental variants fertilised with biosolids, the biodegradation induced by the plants from the *Onobrychis viciifolia* was greater. TPH quantity decreases during the 8 weeks from 28.60±0.30 to 21.42±0.10 g kg^{-1} DM. Soil treatment with fertiliser mixed with volcanic tuff determined a TPH biodegradation from 28.60±0.30 to 8.70±0.10 g kg^{-1}DM.

Table 2. Quantities of TPH from soils cultivated with leguminous species *Onobrychis viciifolia* during vegetative cycle

No	Variants	TPH quantity from the soil* (g kg^{-1}DM)			
		10.04.2012	04.05.2012 (4 weeks)	21.05.2012 (6 weeks)	11.06.2012 (8 weeks)
1	**M**	0.025±0.01	0.025±0.008	0.024±0.008	0.022±0.001
2	**P**	28.60±0.30	27.36±0.35	26.54±0.28	25.50±0.20
3	**PN**	28.60±0.30	25.93±0.39	25.11±0.24	21.42±0.10
4	**PTN**	28.60±0.30	25.42±0.32	12.87±0.23	8.70±0.10

*Mean values.

Table 3 shows the TPH removal efficiencies from the experimental variants of studied soils cultivated with leguminous species *Onobrychis viciifolia* during vegetative cycle. In Table 3 it can be seen that after a period of 4 weeks in the unfertilised/fertilised polluted soil, seeded with *Onobrychis* spp. plants, TPH reduction was less than 5%. When the fertilising agents have been used to treat the polluted soil, biosolids mixed with indigenous volcanic tuff, the TPH removal efficiency by bean plants was 5–10%.

Comparative analysis of TPH reduction efficiencies presented in Table 3 for experimental soil variants, after a period of 6 weeks of *Onobrychis viciifolia* vegetation led to the following conclusions:

1. For unfertilised/fertilised cultivated soil, the TPH reduction efficiency was between 9–12%;

2. For the TPH-polluted soil where the indigenous tuff has been used in addition to fertilisation agent, the plants grown better and caused a reduction of 25.1% TPH versus initial amount present in the polluted soil.

Table 3. TPH removal efficiencies from the experimental variants of studied soils cultivated with leguminous species *Onobrychis viciifolia* during vegetative cycle

No	Variants	TPH removal efficiencies from the soil versus initial quantity (%)		
		4 weeks	6 weeks	8 weeks
1	**P**	4.3	7.2	10.8
2	**PN**	9.3	12.0	25.1
3	**PTN**	11.1	55.0	69.9

Moreover, after 8 weeks of vegetation and at the end of *Onobrychis viciifolia* growing cycle, the removal efficiency of TPH from experimental variants of unfertilised polluted soil was 11.1%. The effective removal of THP was 5 times higher in fertilised soils than in unfertilised one, i.e. 55.0%. An extra-addition of indigenous tuff allowed achieving TPH removal efficiencies up to 7 times higher than for the unfertilised polluted variant, cultivated with *Onobrychis viciifolia*, i.e. 70.0%.

The comparative analysis revealed that the presence of 2.86% TPH in soil caused a dramatic reduction in plant growing. This aspect is confirmed by the fact that at the plant harvesting, a quantity of green biomass 12 times lower than the one obtained for the control variant was obtained. Moreover, the harvest is mostly dry before fructification. In this case, the dry matter was 31.2%, as it is shown in Table 4.

The addition of fertiliser resulted in:

1. The green biomass production of the fertilised variant was 4 times higher than the amount recorded for the unfertilised variant.

2. The content of dry matter was similar with the one determined for the biomass harvested on the control variant.

Table 4. Harvested *Onobrychis viciifolia* biomass on 11.06. 2012

Crt. No	Variants	Green biomass* (g/vegetation pot)	Dry biomass*	
			(g/vegetation pot)	(%)
1	**M**	32.97±0.35	6.15±0.06	18.6
2	**P**	2.74±0.03	0.86±0.05	31.2
3	**PN**	10.64±0.13	1.76±0.05	16.5
4	**PTN**	29.23±0.34	5.97±0.44	20.4

*Mean values.

For the soil treated with natural amendments (indigenous volcanic tuff) as addition to the fertilisation agent, the harvest increased over 10 times versus quantity resulted from polluted variant untreated. The harvest resulted from this variant shown similar characteristic to the one resulting from the control variant.

The tolerance of the leguminous *Onobrychis viciifolia* species has been tested in the presence of petroleum products on contaminated soils. The plants survive in soils as long as the grasses, in agreement with the results reported in literature[11,12] . The high amount of biomass in the testing period of 8 weeks of harvesting did not show stress polluting components, fact attributed both to nutrients coming from biowaste and biocenosis capable of TPH biodegradation and transformation for the phytoremediation stage. The used porous material as a soil amendment, indigenous volcanic tuff, reduces the TPH toxic level in the first stage of plant growing, which leads to a healthy harvest. Moreover, the retention water from tuff provides the water needs for drought periods.

CONCLUSIONS

The leguminous *Onobrychis viciifolia* species stands out as a plant with tolerance in soils contaminated with TPH for a pollution level of 2.86% and with phytoremediation potential. To obtain increased efficiencies for the reduction of TPH from soils it is absolutely necessary to fertilise with an organic fertilising agent. Moreover, the addition of a porous amendment as a indigenous volcanic tuff with retention/binding properties of some toxic TPH components in soils determined an increased level of germination, the installation of the crop on vast areas and a high capacity of biodegradation of TPH up to 70% through more intense processes than in the absence of the bio-waste anaerobically biostabilised municipal sludge and combined with indigenous volcanic tuff. The quantity of dry biomass produced under conditions of mixed fertilisation with addition of indigenous volcanic tuff was similar to that harvested on the experimental variants of unpolluted soil.

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