Prospects of Using Leguminous Species in Phytoremediation of Total Petroleum Hydrocarbons Polluted Soils

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Abstract
Selecting the plant species to grow on aged petroleum hydrocarbons polluted soils is an important factor for a successful phytoremediation. Phytoremediation is a green technology that can become a promising solution for decontaminating polluted soils and ecological restoration of the landscape. Our comparative studies evaluate the effect of oil hydrocarbon pollution with high initial concentration on the growth leguminous plant species: *Vicia sativa and Glycine max*. The experimental block contains control variants, polluted soil unfertilized/fertilized with municipal sludge anaerobically stabilized in absence/presence of modified volcanic tuff amendment. After period of time the experiment's soil in which plant species had grown well was sampled and analyzed for petroleum hydrocarbons removal. Both species showed promising efficiency in the phytoremediation of petroleum hydrocarbon highly polluted soils but a reduced growth of the surveyed plants was noticed. The efficiency of the petroleum hydrocarbons diminution is increased in the case of the addition of fertilizer 16.6% for *Vicia sativa* and 30% for *Glycine max* vs. the initial quantity. In the case of the phytoremediation of polluted soils treated with fertilizer and volcanic tuff, the efficiency of the petroleum hydrocarbons reduction was 72.9% for *Vicia sativa* and 53.7% for *Glycine max.*

Keywords: indigenous volcanic tuff, leguminous species, phytoremediation, sewage waste, TPH polluted soils.

1. Introduction

The phytoremediation of contaminated soils with petroleum products, *i.e.* total petroleum hydrocarbons (TPH) is a biotechnology based on the use of plants as decontamination agents. Plants and their associated microorganisms can convert toxic compounds into less toxic or non-toxic compounds through biodegradation or biotransformation processes. Symbiotic systems plant-based are capable of transforming oil hydrocarbons into byproducts such as alcohols, acids, etc., less toxic and persistent compounds in the environment than the products the biotransformation started with. The final biotransformation of TPH is carbon dioxide and water. Plants can also influence the desorption of polycyclic aromatic hydrocarbons from the particles of soil or from humid conglomerates and even more to take these substances into their own tissue where they biodegrade by metabolic processes or are concentrated in the plant tissue [1].

The presence of large amounts of petroleum hydrocarbons, TPH in the soil is not harmful to the aerial parts of the crops, because the roots of the plants are more resistant to the presence of these types of pollutants than the leaves. The roots will limit the access of the hydrocarbons so that

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the leaves will be spared a fast and intense impact to affect them. In fact, some plants tolerate up to 100 g TPH•kg^{-1} of soil. At a concentration of 30 g TPH•kg^{-1} of soil, in contaminated soils, for the vast majority of plants there can be observed the phenomena of chlorosis and necrosis, leaf dehydration, a marginal increase to the aerial parts of the plants and ultimately death.

The hydrocarbons present in the soil may occur in the flow of the soil's aqueous solution or in the transfer of oxygen into the rhizosphere. Also the hydrocarbons may disrupt enzyme and hormone secretion. Plant seeds sown in soils contaminated with a large amount of hydrocarbons die before germination because the oil penetrates through the seed's wall and destroys the embryo. In other cases it was found that hydrocarbons adhere to the wall of the seed and dramatically limit the water and oxygen takeover thus producing the embryo's asphyxiation.

The literature shows many examples on the behavior of various plants to the presence of petroleum hydrocarbons in soils. For example, the hydrocarbon oil at low concentrations in the soil of 0.5% (5 g TPH•kg^{-1} D. M.), can dramatically reduce the harvest of barley. A small harvest of biomass is also due to the low bioavailability of nitrogen in soils polluted with oil. The amount of above ground crops decreases more significantly than the amount of root biomass in soils polluted with hydrocarbons [2, 3]. The typical response of the plant to the lack of water and nutrients is the overgrowth of the roots.

At low concentrations of petroleum hydrocarbons in soils, plants can grow vigorously, resulting in higher yields. For example soy can give increased yields when grown on soils contaminated with 7.5 g TPH•kg^{-1} D.M (crude oil)/weight of soil in the case when this amount is added to sandy soils. The presence of 5 g TPH•kg^{-1} D.M oil in a polluted soil caused an increased harvest of legumes like white clover. Plant growth on soils polluted with crude oil determine plant conversion after rotting into stable humus respectively the increase in the soil's organic carbon content. Furthermore it can be noted that the application of fertilizers on oil polluted soils is required because it facilitates the conversion of hydrocarbons into humus [2, 3]. Telysheva et al., 2011 [4] reported the use of annual grain legumes (*Vicia faba,)* corn *(Zea mays)* and perennial red clover (*T. pratense)* and alfalfa (*Medicago sativa*). Nyoku et al. 2009 [5] reported that the plants selected for phytoremediation of polluted areas should be suited to the climatic conditions of the affected areas. Plants must be able to tolerate stress conditions.

The purpose of the study is to determine: 1 the tolerance degree of plants pursued in the phenonogical phases of development, 2 TPH reduction efficiency from the soil planted with leguminous species.

2. Materials and methods

Studies were carried out in experimental devices comprising of growing vessels. The vessels are each equipped with 6 kg of soil polluted unfertilized/soil fertilized with stabilized sewage sludge in the absence/presence of indigenous volcanic rock amendment with 72% clinoptilolite in which the legume species *Vicia sativa* and *Glycine max* are to be seeded. The experimental variants of soil for each species are shown in Table 1. The volcanic rock comes from the Mârşid quarries and contains about 70% clinoptilolite. The grained volcanic rock used is 0.2-2 mm and was scattered on the ground before spreading the sewage sludge.

In order to determine the variation of TPH in soils, their concentration is periodically determined, 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 in the upper level:1) 0.5-1.0 g of dry soil are weighed (M), then add 5 g Na_2SO_4 anhydrous and 25 ml solvent, CCl_4 (Fluka Analytical); 2) 30 minutes stirring at 50 rotations/min and then filtered; 3) the glass and filter paper (Whatman no. 4 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 aluminum oxide. The elute was 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–mass of capsule without control residue [g], m_4–mass of capsule with control residue [g]); 8). Calculating TPH: TPH [g•kg^{-1}]=1000•[(m_2-m_1)-(m_4-m_3)]•M^{-1}.

Table 1. Experimental variants of soil culture on which two species of leguminous were cultivated

No	Experimental variants	Experimental block	
		Vicia sativa species	*Glycine max* species
1	Normal soil, blank	Unpolluted soil, **M 1**	Unpolluted soil, **M 2**
2	Soil polluted with TPH	Soil polluted with 2.8% TPH symbol **P 1**	Soil polluted with 1.8% TPH symbol **P 2**
3	Soil polluted with TPH fertilized with stabilized sewage sludge from municipal wastewater Timisoara	Soil polluted with 2.8% TPH fertilized with 50 $t \cdot kg^{-1}$ sewage sludge, **PN 1**	Soil polluted with 1.8% TPH fertilized with 50 $t \cdot kg^{-1}$ sewage sludge, **PN 2**
4	Soil polluted with TPH fertilized with sewage sludge mixed with indigenous tuff.	Soil polluted with 2.8% TPH, fertilized with 50 $t \cdot kg^{-1}$ sewage sludge and mixed with 5 $t \cdot kg^{-1}$ indigenous tuff, **PNT** 1	Soil polluted with 1.8% TPH, fertilized with sewage sludge 50 $t \cdot kg^{-1}$ and mixed with 5 $t \cdot kg^{-1}$ indigenous tuff, **PNT 2**

3. Results and discussion

1. The tolerance degree of plants pursued in the phenonogical phases of development.

Vetch crops are analyzed periodically over 60 days from emergence focusing on certain biometric characteristics of the plant. The plants did not reach fruition. At the end of the 60 days monitoring period the plants were harvested. The biometric characteristics are set out in Table 2.

The data in Table 2 shows that plants cultivated on polluted soil grow at a slower pace compared with plants grown in the experimental variants fertilized with sludge in the absence/presence of volcanic tuff or plants grown on control variant M 1.

Table 2. Biometric measurements of the species *Vicia sativa* plant grown in the four experimental variants. Emergence date 11.04.2012

No	Experimental variants	Plant height (Minim-maxim values) * mean value (cm)				
		16.04.2012	23.04.2012	04.05.2012*	14.05.2012*	14.06.2012*
1	M1	*8/2 leaves	10-14 *12	16-17 *15	30-40 37*	45-50 46.5
2	P1	*4/2 leaves	6-8 *7.2	14-15 *14.4	25-30 *28.5	25-30 *29.2
3	PN1	*8/2 leaves	10-14 *13.2	14-15 *14.8	30-35 *33.5	40-45 *43.2
4	PTN1	*12/2 leaves	14-16 *15.0	16-18 *16.8	30-40 *36.8	50-55 *53.5

Plants grown on the experimental variant P1 had an average height of 29.2 cm at harvest, presenting yellow leaves with drying tendencies from the first week of development. The use of fertilizer allowed the culture to achieve an average height of 43.2 cm at harvest. The use of fertilizer mixed with volcanic tuff resulted in a culture with an average height of 53.5 cm at harvest, height exceeding by a few cm the average height of plants harvested normally from unpolluted land.

Table 3 shows the results of biometric measurements performed on soybean plants grown on the four experimental variants pursued during a five months period: 14.03-24.08.2013. The data in Table 3 shows that plants cultivated on polluted soil grow at a slower rate compared with plants grown in the experimental variants fertilized with sludge in the absence/presence of volcanic tuff and control variant M2.

Plants grown in the experimental variant P2 presents yellow leaves and drying tendencies from the first week of development. Plants grown in the experimental variant P2 reached an average height of 26.6 cm at grain harvest. The plants showed yellow leaves and drying tendencies starting from the first week of development.

Using fertilizer resulted in a culture with an average height of 31.9 cm at harvest. Using fertilizer mixed with volcanic tuff allowed the culture to achieve an average height of 37.5 cm harvest, height exceeding by few cm the medium height of plants harvested from a normal unpolluted field.

Table 3. Biometric measurements of plants of the genus *Glycine max* grown in four experimental variations during the followed period

No	Experimental variants	Plant height (Minim-maxim values) * mean value (cm)				
		05.04.2013	25.04.2013	04.05.2013	04.06.2013	24.08.2013
1	M2	3-5 *4	10-14 *12.5	16-21 *18.6	21-30 *25.6	26-37 *33.4
2	P2	3-4 *3.5	8-10 *8.9	12-14 *13.0	21-30 *25.5	22-32 *26.6
3	PN2	3-5 *3.8	10-14 *11.8	12-15 *13.0	18-30 *18.7	25-33 *31.9
4	PTN2	3-5 *3.8	10-16 *14	12-17 *15.5	20-36 *30.5	25-39 *37.5

2. TPH reduction efficiency from the soil planted with leguminous species.

In the second part of the paper are presented the changes of the content of petroleum products from the contaminated soil used in the experimental variants treated/fertilized in the absence/presence of indigenous volcanic tuff while being cultivated with the two types of legumes. In Figure 1 are shown the TPH reduction efficiencies on the surface of the soil cultivated by *Vicia sativa* species. The culture on the variant fertilized with the sewage sludge resulted in a reduction of the TPH content on the soil's surface by 16.6%.

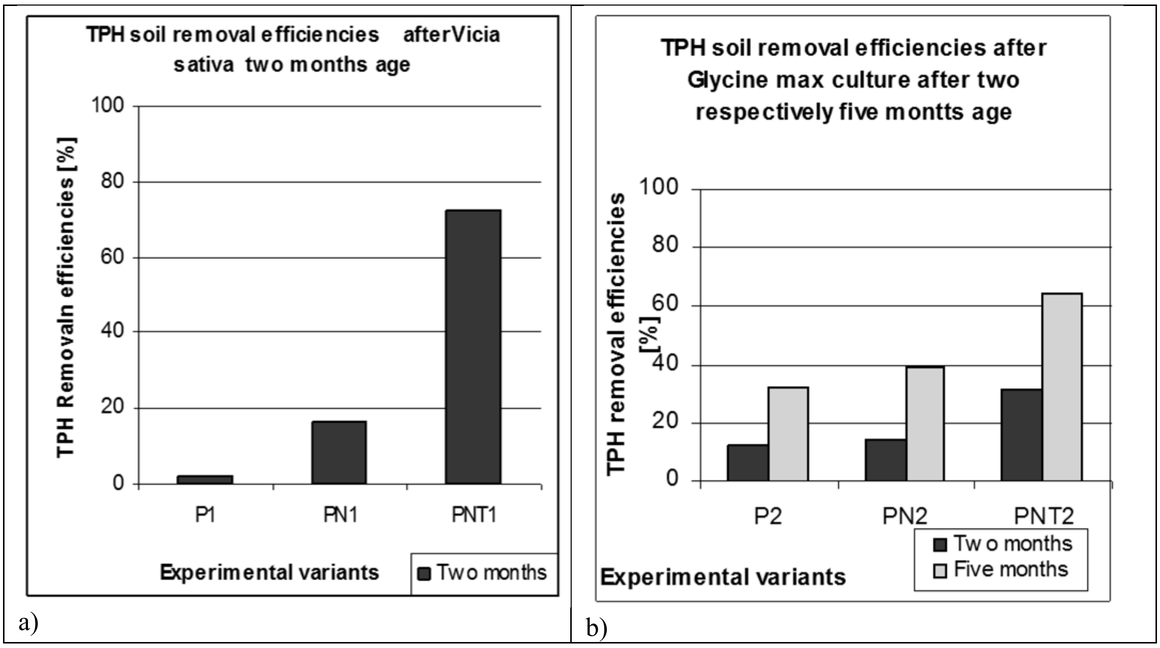

Figure 1. Reduction effectiveness of TPH from the surface of the cultivated soil
a) a two months culture of the plant species *Vicia sativa*
b) a two months culture respectively five months, of the plant species *Glycine max*

The vetch culture obtained from fertilized with sewage sludge mixed with volcanic tuff has reduced the soil's TPH content by over 72.9%.

In Figure 1 b are shown the efficiencies of TPH reduction from the soil cultivated with species *Glycine max,* a two months culture respectively five months. The culture grown on the soil fertilized with the sewage sludge resulted in a reduction of the content of TPH after two months of growth at over 14%. The reduction of the soybeans. The culture of soybeans fertilized with sewage sludge mixed with volcanic tuff reduced the content of TPH after two months of growth up to 30%. The efficiency of TPH content reduction on the soil's surface doubled at the harvest of the soybeans.

4. Conclusions

Vetch crops tolerated in greater measure the pollution of 2.8% TPH in soils fertilized in the absence/presence of indigenous volcanic tuff vs. untreated polluted soils. Vetch cultivated on soil fertilized with sludge caused the reduction of TPH in the soil's surface up to 16.6% over a two month growing period, and in the case of the variant fertilized with sewage sludge mixed with indigenous volcanic tuff the TPH content on the surface was reduced by over 72.9%.

Soybean cultures tolerated in greater measure the pollution of 1.8% TPH in soils fertilized in the absence/presence of indigenous volcanic tuff vs. cultures on untreated polluted soils. The soybean culture formed on soils fertilized with sewage sludge has caused the reduction of TPH at the surface of the soil to over 14% in two months of growth and up to 40% over a period of five months. The efficiency of the reduction of TPH content of the soil's surface in the variant fertilized with sewage sludge mixed with indigenous content of TPH at the surface of the soil has reached 40% at the stage of harvesting the

volcanic tuff reached 30% during the two months of vegetation and doubled during five months of growth. Vetch and soybean crops can be used for rapid phytoremediantion of polluted soils with TPH in terms of completion nutrient.

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