Phytoremediation of Oil Polluted Soils and the Effect of Petroleum Product on the Growth of *Glycine max.*

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Selecting the tolerant plant species to grow on aged petroleum hydrocarbon-polluted soils is an important factor for a successful phytoremediation technology. Phytoremediation is a green technology that can become a promising solution for the aspect of decontaminating oil-polluted soils and for the ecological restoration of the landscape. Our comparative studies evaluate the effect of oil hydrocarbon pollution with a high initial concentration on growth characteristics of leguminous species: Glycine max. The experimental block contains control and non fertilized/fertilized polluted variants with 25 t.ha^{-1} sewage sludge anaerobically stabilized in absence/presence volcanic tuff variants. After six months of the experiment's soil samples in which plant species had grown well they were analyzed for total petroleum hydrocarbons) removal. Species showed promising efficiency in the phytoremediation of petroleum hydrocarbon polluted soil but there was noticed reduced growth of the surveyed plants Glycine max significantly for polluted and untreated variants. The efficiency of the total petroleum hydrocarbons diminution is increased in the case the addition of fertilizer as opposed to the initial quantity. In the case of the phytoremediation of polluted soils treated with fertilizer and volcanic tuff the soil total petroleum hydrocarbons quantity was decreased by two times vs. polluted and untreated soils.

Keywords: total petroleum hydrocarbons, phytoremediation, polluted soils, sewage waste, indigenous volcanic tuff

Polluted soils containing petroleum products have the risk potential for the agricultural circuit [1]. One of the techniques of rehabilitation of sites polluted with total petroleum hydrocarbons (TPH) is phytoremediation with appropriate plants which detoxify polluted soils. Phytoremediation of polluted soils is a soil remediation method using green plants in order to restore some physical, chemical and biological characteristics of the environment destroyed by anthropogenic activities [2-3] and is considered an alternative competitive method to remediate the environment [4, 5, 6]. There were identified numerous plants with potential of phytoremediation of TPH polluted areas [7-9]. Phytoremediation can be applied both in the case of soils polluted with metals, such as fly ash dumps, tailing areas etc. [5-10] and in the case of soils polluted with crude oil and its derivatives [11-13]. Phytoremediation depends on plant species [14-19]. From the used plants, those from the Graminee Family determine the formation in the rhizosphere area of a microbial culture based on a multitude of species, with a great number of individuals, but which do not have specificity for biodegrading petroleum products. On the other hand, leguminous plants determine the selection of bacterial groups, with less species but which have great selectivity for biodegradation of TPH compounds. Plant-bacteria consortium has marked capacity of biodegradation of petroleum products [13]. Some researchers suggest that the process of selection of plants for phytoremediation of soils polluted with TPH needs to be done monitoring several studies of plant development [8, 13, 20]. Gudin and Syratt, [21] shows examples of phytoremediation in approximately 15 area polluted with petroleum products from Europe, performed with leguminous plants, as dominant plants. The use of natural organic fertilizers which bring their intake of compounds that are easily bio-available to plants and with a rich and well developed biocenotic heritage positively act to biodegrade the petroleum products [16]. Moreover, the use of certain materials such as bentonite, natural zeolite, calcium oxide, etc, which absorb some petroleum products, reduces the initial pollution of soils. [22-23]. The goal of this study is to investigate phytoremediation of soils polluted with TPH compounds using the *Glycine max* species and the dependence of the biodegradation level of these products on the fertilizing matter in the absence/ presence of a natural and porous amendment, the indigenous volcanic tuff.

Experimental part

Materials and methods

The experimental study is done on soils polluted with TPH sampled from the area neighbouring the activity of petroleum collection, in a park of functional oil pumps, where crude oil is collected, then placed in great tanks and sent to the processing installations in the area. Polluted soil was taken from West Romania. Soil pollution has started with the oil extraction in the area, tens of years ago. The area got polluted with TPH because of accidental leaking, malfunctions etc. The polluted soil collected from the 0 to 30 cm profile was cleansed of organic and inorganic coarse materials and then well homogenised. The experimental soil variants are: 1). Non-polluted soil, control, M; 2). Soil polluted with petroleum products, P; 3). Soil polluted and fertilized with anaerobic stabilized sewage sludge PN; 4). Soil polluted, fertilized with sewage sludge

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and treated with indigenous volcanic tuff, PNT. The sewage sludge came from the Treatment Plant of municipal sewage waters and was used in a quantity of 25 $t.ha^{-1}$ D.M., which was well mixed with the polluted soil. Non polluted soil, control variant, was taken from farmland near the polluted area. Non polluted soil characteristics: cernosium cambic soil type, porosity =42-44%, density =2.45-2.65 $g{\cdot}cm^{-3}$, *p*H=6.18, total N=0.17%, P=13.08ppm.

Indigenous volcanic tuff characteristics

The indigenous volcanic tuff comes from a quarry from Mârºid - Romania and contains up to 70-72% clinoptilolite. The volcanic rock used has a granulation of 0.2-2 mm and was mixed with the polluted soil. It was used in a quantity of 1% vs. soil quantity, distributed on the soil with the sewage sludge. The addition of the fertilizing agent was necessary as nutritional supplement recommended to maintain the activity of polluted soil biocenosis and the metabolic activities of plants. It is recommended that for soils polluted with petroleum products fertilizing agents be added to facilitate the conversion of compounds from petroleum in humic products. The water retention capacity dramatically decreases when petroleum products are present in soil. Water cannot infiltrate in soil when it contains high quantities of petroleum. Water retention capacity decreases also as a result of the capacity of degradation of the organic compounds via microorganisms which can biodegrade petroleum products. The addition of indigenous volcanic tuff was necessary to correct the property of the polluted soil to retain water, property that was deteriorated by the presence of petroleum products. Tuff retains water in the micro porous structure, and from here, water will be released in small quantities depending on the necessities of the plant. Also, the volcanic tuff absorbs the petroleum products modifying the level of pollution for plants.

Selected plants

Selected plants of the *Glycine max* species (soybean) are part of the Leguminous. From this family are beans, clover or alfalfa also. Soybeans play an important role in the food industry because they contain 39% proteins, 17% oils. Vegetation period is of 108 - 145 days, depending on the maturity group (very early - late). Seeding is done when soil temperature reaches 7-8°C at seeding level. Seeding density insures 40-50 plants/m^2 when seeding in rare rows and 50-55 plants/m^2 when seeding in dense rows. Harvest begins when seed humidity drops below 15%.

Experimental variants soil characteristics

The experimental study was done in experimental block in vegetation pots equipped with 6.5 kg of non-treated/treated soil. 12 seeds from the plant species are seeded, on 2 rows (6 on each row), at 2 cm depth. Each experimental variant is done with 3 replicas. Soils are watered with equal amounts. The experiment is undergone outside. The initial quantity of petroleum products from the used polluted soils to determine the tolerance level for soybean was 18.0 ±2.3 g THP /kg^{-1} D.M.

Soil physico-chemical characteristics determination

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: 0.5-1.0g of dry soil are weighed (M), then add 5g Na_2SO_4 anhydrous and 25mL solvent, petroleum ether, (Sigma Aldrich); 30 min stirring at 50 rotations/min and then filtered; the glass and filter paper (Whatman No. 4 paper) are washed with petroleum ether, which is added to the filtrate; the filtrate is evaporated on water bath; the residue is dissolved in petroleum ether, then passed through the chromatographic column filled with aluminium oxide. The elute was collected in a tarred capsule, m_1[g]; petroleum ether is evaporated at room temperature and weighed at constant mass, m_2 [g]; the same is done for the control from 28 ml petroleum ether (m_3 – mass of capsule without control residue [g], m_4 – mass of capsule with control residue [g]); Calculating TPH: $TPH\ [g \bullet kg^{-1}] = 1000 \bullet [(m_2 - m_1) - (m_4 - m_3)] \bullet M^{-1}$. Polluted soil characteristics: *p*H=7.2, total N=0.12%, P=53.0ppm. Polluted soil is composed of oil soaked aggregates Physical and chemical soil analyses were performed in ECOIND Laboratory.

Agricultural work

In table 1 are shown the agro-technical works done during March-August 2013 for soybean.

Statistical analysis

The statistical evaluation of the experimental data was made using PAST 2.14 free online software, which runs on standard Windows computers [24]. The chosen mathematical models to interpret experimental data were the Kernel density interpretation based on Gaussian kernel functions, with radius parameter *r,* and the General Linear correlation [25].

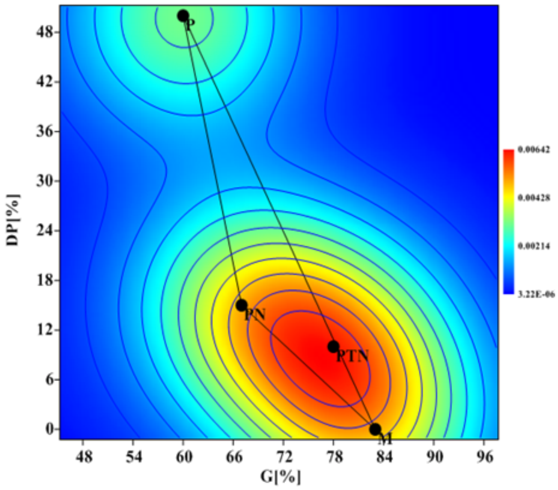
Table 1

AGRO-TECHNICAL WORKS DONE DURING MARCH-AUGUST 2013 FOR SOYBEAN CULTURES

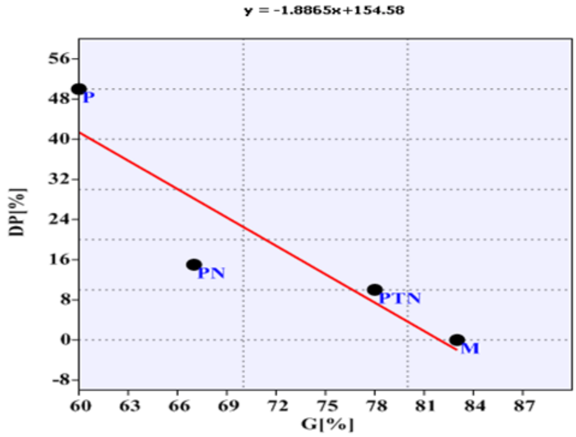
Agro-technical work performed	Date	Observations
1. Preparation of the vegetation pots: three vegetation pots are filled with established experimental variants of soils; total of 12 vegetation pots.	March 1st, 2013	Vegetation pots filled with soil variants selected for study are wetted with same water quantity. Soils are allowed to stabilise bio-geo-chemically.
2. Sowing with soybeans	March 14th, 2013	Sown soil is compressed and wetted with the same water quantity.
3. Periodically wetted with 100 ml of water	April 25th – August 14th, 2013	Germination level is established. Biometrical measurements. Establishing the level of plant covering of the sown surface. Plant aspect is monitored. Periodical analysis of TPH concentration in soils.
4. Plants are harvested	September 14th, 2013	Plant aspect is monitored. Analysis of TPH content from experimental soil variants.

Table 2
GERMINATION OF SOYBEANS AND PERCENTAGE OF DRY PLANTS FROM GERMINATED TOTAL

No.	Experimental variants	Germination period	Germination (G) %	Dry plants from germinated total (DP) %
1	M	01-07.04.2013	83.0	-
2	P	05-09.04.2013	60.0	50
3	PN	02-07.04.2013	67.0	15
4	PTN	01-08.04.2013	78.0	10



Legend: G = Germination percent [%]; DP = Percent of dried plants [%]; P, PN, PTN- experimental variants
Fig..1. Kernel density representation of DP[%] based on G[%]



Legend: G = Germination percent [%]; DP = Percent of dry plants [%] M, P, PN, PTN experimental variants
Fig.2. Graphical representation of generalised liniar model applied to DP[%] based on G[%]

Results and discussions

In monitored period moisture of experimental variant soil was assured by watering (table 1). Indicators monitored in our study are: germination level of seeds and percentage of dry plants of the germinated total; Fruition level/quantity of seeds resulted; Efficiency of reduction of TPH from soils cultivated with soybean, in the soil profile of 0-5.0 cm, during the vegetative cycle.

Germination level of seeds and percentage of dry plants of the germinated total;

Germination level of seeds represents an important parameter to verify the tolerance of plant seeds to diverse levels of soil pollution [26]. On polluted soils plants germinated 5-6 days later from sowing. Germination level was of 60%. The addition of fertilizer stimulates germination and plant development. Seeds germinate on the variant fertilized with municipal sludge at a level of 67%. The addition of indigenous volcanic tuff determined a germination level of 78%. Table 2 shows the germination levels of soybeans.

From figure 1 can be seen that on polluted soil -P we find the largest number of soybean dry plants whereas on treated soils (PTN and PN) behaviour more similar to the control variant M is present.

Appliying the liniar generalised model we obtain an equation which help us to appreciate the percent of dry plants based on the percent of germination. For the liniar generalised model it was used the normal distribution with identity function, $DP = -1.8865 \cdot G + 154.58$, (slope a: -1.8865; interc. b: 154.58; stderr a: 0.62972 and stderr b: 45.695 (fig. 2).

Fruition level/quantity of seeds resulted

Table 3 shows the quantities of soybeans and dry pot harvested from the non-treated/treated experimental variants polluted with TPH vs. quantities harvested from the control.

The table 3 shows that the soybean production resulted on the variant of soil polluted and non-fertilized represented only 21.3% of the quantity harvested from the control. Dry pod quantity was of only 19.6% of the quantity harvested from the control. The addition of fertilizer has a positive effect both on plant health and the obtained production [27]. For the undergone experiment, the addition of fertilizer, stabilized sewage sludge, determined the development of more rigorous plants which bloomed and fruited thus that the soybeans production reached 47.5% from the quantity harvested from the control and the pods quantity reached 30.4% of the quantity harvested from the control. The addition of porous amendments mixed with fertilizing agent determined the adsorption of some parts of TPH, thus reducing the aggressiveness of pollutants in the rhizosphere area. As a result, plant cultures were superior quantitatively and qualitatively [17,21]. Table 3 shows that the soybean harvested from the variant fertilized with sewage sludge and treated with modified volcanic tuff was

Table 3
QUANTITIES OF HARVESTED SOYBEANS FROM NON-TREATED/TREATED EXPERIMENTAL VARIANTS POLLUTED WITH TPH VS. QUANTITIES HARVESTED FROM THE CONTROL

No.	Experimental variants	Harvest vs. control, M [%]	
		Soybeans,	Dry empty pods
1	P	21.3	19.6
2	PN	47.5	30.4
3	PTN	55.7	57.5

**Values are means of three replicates*

Table 4
QUANTITIES OF TPH FROM SOILS CULTIVATED WITH SOYBEAN IN THE EXPERIMENTAL VARIANTS

No.	Experimental variants	Quantities TPH in soils [g/kg^{-1} D.M.]			
		Initial 01.03.2013	30.05.2013	14.06. 2013	14.08. 2013
1	M	0.025±0.008	0.025±0.008	0.024±0.008	0.022±0.001
2	P	18.46±2.3	16.17±0.35	13.65±0.28	12.50±0.20
3	PN	18.46±2.3	15.85±0.39	12.92±0.24	11.32±0.10
4	PTN	18.46±2.3	12.74±0.32	8.55±0.23	6.6±0.30

over 55.7% of the quantity harvested from the control and pods quantity reached 57.5% of the harvested from the control, Ml.

Efficiency of reduction of the TPH from soils cultivated with soybean in the soil profile during the soybean culture.

Table 4 shows the efficiencies of reduction of TPH quantities from soils cultivated with soybean in the four experimental variants during March 1st – August 14th, 2013.

Table 4 shows that after cca.12 weeks, in the studied soils the TPH quantity decreases. The decrease of TPH quantity was determined by the plant development, tolerance to soil pollution level, type of treatment applied to improve the characteristics of the deteriorated soil. When the polluted soil was not fertilized, the TPH quantity dropped after 12 weeks with 2g/kg D.M. and at the end of the vegetative cycle it reached 6g/kg D.M. The treatment of soil with fertilizer determined a slight increase of the biodegraded quantity of TPH after 12 weeks to 5g/kg D.M. and at the end of the vegetative cycle up to 7g/kg D.M. In this case the development of plants on phenophases was better than the non-fertilized polluted variant, but there were plants that dried during the monitoring period which determined the reduction of the TPH biodegrading activity from soil. The treatment of soil with fertilizer mixed with indigenous volcanic tuff determined an increase of the biodegraded quantity of TPH after 12 weeks to 6 g/kg D.M. and at the end of the vegetative cycle the quantity was doubled. .The modifications brought about by the presence of the amendment in the polluted soil determined the formation of a culture with more vigorous and resistant plants which through their metabolic activity determined the biodegradation of a quantity of TPH up to 12 g/kg D.M. from the polluted soils.

Conclusions

Plants from the *Glycine max* species showed tolerance to petroleum products from soils polluted with 18g/kg D.M. when a quantity of soybeans up to 21.3% of the quantity harvested from the control was obtained. The TPH quantity biodegraded during the vegetative cycle was 1/3 of the initial quantity present in the polluted soil. The addition of the fertilizing agent determined the doubling up of the plant fruition level and the increase of the quantity of the biodegraded compounds. The use of a treatment of the polluted soil with aerobic stabilized sewage sludge and indigenous volcanic tuff determined the increase with over 50% of the soybean production vs. the production from the polluted and non-treated variant. Soybean culture determined a biodegradation of 2/3 of the quantity of petroleum products found initially in the soil during the vegetative cycle.

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