Lolium Perenne - A Phytoremediation Option in Case of Total Petroleum Hydrocarbons Polluted Soils

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Rehabilitation of polluted soils with petroleum products requires a re-vegetation strategy to obtain a green mass cover that can quickly and efficiently cover the polluted soil. For the gradual recovery of the destroyed soil, it was necessary: 1. adequate soil treatments with fertilizer i.e. sewage sludge and fly ash as amendment, 2. plant species selection, 3. agronomical works in accordance with geographical position and climatic conditions. Fertilizers and fly ash create conditions for plant installation, by nutrients insurance. Our experiment was conducted in pots with 91.73±11.12 [gKg^{-1} D.M (dry matter)] total petroleum hydrocarbons (TPH) polluted soil, fertilised with sewage sludge and fly ash 60 [tha^{-1}] derived from the burning of fossil fuels in thermal power plants. The selected plant species for bio-remediation is Lolium perenne. The selected plant species Lolium perenne is installed on 50-90% of the land surface giving 8 successive crops of grass in the warm season. The TPH reductions of polluted and treated soil were 38.4-56.3 [%]. The biomass did not bioaccumulate chromium at the detection limit. The amounts of cadmium, lead and zink bioaccumulated in the aerial parts were below acceptable limits. The obtained biomass can be used as animal feed or for bedding in shelters. The soil remediation efficiencies of 91.73 ± 11.12 [gKg^{-1} D.M] were directly proportional to the amount of fly ash used.

Keywords: Lolium perenne, sewage sludge, fly ash, phytoremediation fingerprint

Industrial pollutants such as hydrocarbons characterize a severe alarm due to their persistent and negative effects on the environment and the health of living organisms. Total petroleum hydrocarbons (TPH) consists of aliphatic and aromatic hydrocarbons that are composed of C5–35 chains with a variety of structural carbon atom configurations [22]. If the atomic structure is complex, the hydrocarbon compounds are more hydrophobic [28]. Because of these characteristics, TPH is persistent in soil and water, becoming barely degradable for microorganisms [7].

TPH contain compounds that have been related to environmental pollution but also regarding mutagenesis and carcinogenesis [17].

There are many technologies available for removing or stabilizing these types of pollutants from soil, but most of these approaches are costly, invasive and tricky to implement. Due to the fact that the classic removal technologies effect negatively on soil chemistry, physical structure and soil microorganisms [1], phytoremediation (the biological technology that uses the ability of plants to metabolize or concentrate chemical compounds) is recommended to restore polluted sites [26] and represents a cheaper, less environmentally invasive technology.

TPH reaching the surface of the soil will flow to its surface according to the geometry of the area and / or will gradually penetrate into the depth of the soil to an impermeable layer, eg clay. TPHs will migrate over a long distance and will pollute areas located far away from the pollution point [10].

Crude oil spills have always caused many shortcomings in agricultural productivity [10]. Often there have been oil spills, which occurred on the dry land due to releases of crude oil from tankers, offshore platforms, drilling rigs and wells, as well as spills of refined petroleum products (such as gasoline, diesel) and their by-products. These spills have affected crops of rice, corn, potatoes, Cassava, etc [3].

In this context, there was a need to reduce the negative effects of oil pollution and its derivatives. Specialists have been able to apply more efficient and comprehensive strategies for polluted land remediation due to the knowledge and understanding of the different biotechnological processes that occur on TPH-contaminated land.

Understanding the different biotechnological processes which occur on TPH-contaminated land, specialists have been able to apply more efficient and comprehensive strategies for polluted land remediation. Remediation biotechnologies are still the „green technologies” linked to the regulations governing polluted sites because they are related to rapid and effective rehabilitation efforts [2]. Of these, the phytoremediation method involving plants in the restoration of the contaminated ecosystem was predominantly used with positive results [10, 11, 19]

In the last decade, of the perennial herbs, the *Lolium perenne* species has been frequently selected for organic bioremediation or redevelopment of metallurgical, mining, chemical, etc. polluted land. Numerous studies have been

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conducted to assess the feasibility of *Lolium perenne* growth and development on soils contaminated with heavy metals [12-14, 16, 21].

Because in many cases pollution is caused by high amounts of TPH (> 100 gKg^{-1} D.M.), this causes high stress on the living matter in the polluted soil. For stress reduction, materials characterized by adsorbent properties can be used which temporarily reduce the aggressive nature of TPH components. Ashes with adsorbing properties [24] result from the burning of fossil coal in thermoelectric plants.

According to Singh RP et al, 2014 the effect of amending soils with fly ashes (FA) has been extensively investigated, many researchers reported FA as a depository of nutrients that improve soil properties. Ash is currently a byproduct, known as waste, characterised by high quantities of mineral substances, almost all in ionic form, essential to plant growth and having a positive impact on the physical-chemical properties of the damaged soil [18,20,25].

In the case of the destroyed soils, phytoremediation, the ash is considered a useful adjuvant for the growth of vegetal production [18,20]. In the soil containing large quantities of petroleum products the ratio C: N: P is poorly affected by the excess of carbon products. The correction of this report to the values needed to cultivate agricultural crop is achieved by administrating of fertilizers rich in nitrogen and phosphorus. For this the polluted soil can be treated with manure, sewage sludge or other recommended waste [4, 15, 23, 27].

The purpose of this study was rehabilitation by phytoremediation with grassland plants (*Lolium perenne*) of soils strongly polluted with petroleum products in the presence of fly ash and sewage sludge.

Experimental part

Material and methods

All reagents were of analytical grade and were used as received without further purification. The soil used in the study was a polluted soil with TPH, sampled from the excaveted land near the railways. The soil initially contains soil aggregates soaked in petroleum products ranging in size from 2 to 8 cm and impurities (pieces of wood, textiles, stones of different sizes etc). The soil used for the experiments was cleaned of dirt, and the soil aggregates embedded with petroleum products were ground and then mixed with agricultural soil in a ratio of 1: 1. The quantity of petroleum products in the soil mixture (TPH polluted soil: agricultural soil) was 91.73 ± 11.12 [gKg^{-1} D.M.] The phytoremediation study was carried out in pots having a useful surface of 0.08 mp. The mixture of polluted soil: unpolluted soil was fertilized with sewage sludge in an equivalent quantity of 60 [tha^{-1}]. The amount of fly ash used for different experimental phytoremediation variants was variable, ranging from 15 - 60 [tha^{-1}] (equivalent amount).

Figure 1 presents the characteristics of sewage slugde and fly ash used for fertilization of soil polluted with petroleum products. The analysis of sewage slugde and fly ash were determined in the ECOIND laboratory according to national standards.

Description of pot experimental variants

P_1-TPH polluted soil and 60 [tha^{-1}] fly ash treated; P_2-TPH polluted soil and 60 [tha^{-1}] sewage sludge treated; P_3-TPH polluted soil, 60 [tha^{-1}] sewage sludge and 15 [tha^{-1}] fly ash treated; P_4-TPH polluted soil, 60 [tha^{-1}] sewage sludge and 45 [tha^{-1}] fly ash treated; P_5-TPH polluted soil, 60 [tha^{-1}] sewage sludge and 60 [tha^{-1}] fly ash treated. The soil was sown with gramineous plants of the *Lolium perenne* species. The amount used for seeding was 20 g/pot. Each experimental variant was made in three replicas. The crop and soil characteristics were followed for a period of 6.5 months. The plants were watered once a week in the absence of summer rains.

Plant characteristics

The *Lolium perenne* species is spontaneously encountered in all permanent meadows, railroad slopes, and road edges and, of course, grown as forage crop. As this species has reduced soil requirements, the roots are well developed, exploit's a large mass of soil, shows a rapid increase and develops a rich vegetative mass [6], permitting repeating mowing [5], is a suitable option for the bioremediation of soils polluted with hydrocarbons and / or heavy metals [8, 9].

Plants physico-chemical characteristics determination

The surface was covered for each variant with *Lolium perenne*. Plants and the health of plants development as well as determinations were performed every 20-30 days; the amount of bio-accumulated metals were analyzed half-yearly. Plant sampling was done in agreement with the standardized methodology. Plant tissues were thoroughly washed with deionized water to remove any soil particles attached to plant surfaces. The tissues were dried at 105°C to constant weight. Plant samples with precise weight are then calcinated at 550°C; to the residual materials were added 5mL of concentrated hydrochloric acid and the samples were maintained 30 min on the dry sand bath followed by filtering operation on a paper filter with small porosity and taken to a calibrated flask with hydrochloric acid 1:1 solution.

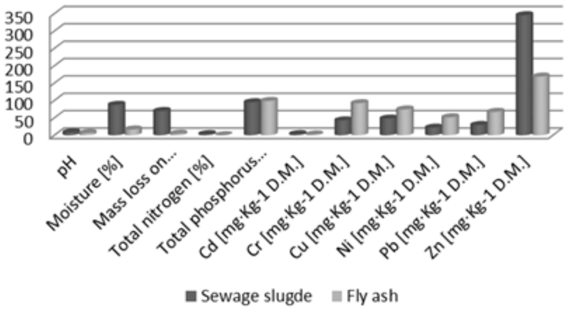

Fig.1. Sewage slude and fly ash characteristics used for the fertilization of soil polluted with petroleum products

Soil physico-chemical characteristics determination

The TPH concentration in soil was determined after 6.5 months following the protocol (analysis steps): 1). 3-5 g of dry soil (D.S. = m [mg]) are weighed into a 50 mL Erlenmeyer with a polished cover, over which 5g of anhydrous Na_2SO_4 and 25 mL of tetrachloroethylene solution (Fluka Analytical) were added; 2). the content was shaken for 30 minutes at 50 rpm (rotations/min) so that all soil particles come in contact with the extraction agent, then filtered through Whatman No. 4 filter paper into a 50 mL Berzelius beaker; 3). the glass and filter paper were washed 3 times with 3 mL of solvent added to the filtrate; 4). the filtrate was evaporated on a water bath in a niche at a temperature of maximum 75°C until complete removal of the solvent; 5). the residue were dissolved in 3 portions of 15, 10 and 5 mL solvent which pass (along with any non dissolved parts) on the chromatographic column filled with aluminum oxide and the eluate was collected in a porous porcelain capsule (m_1 [mg]); 6). the solvent was evaporated in a niche at room temperature and weighed to the constant mass (m_2 [mg]); 7). The procedure is followed identically for a control sample obtained from 28 mL solvent which was evaporated and resuspended as described above (m_3 - capsule mass without residue of the blank [mg]), (m_4 - mass of the control residue residue [mg]); 8). calculation of the TPH content based on equation 1:

$$TPH = 1000 \bullet [(m_2 - m_1) - (m_4 - m_3)] \bullet m^{-1} \quad |mg \cdot Kg^{-1}| \tag{1}$$

Plant and soil extracts analysis was done using a spectrophotometer, Varian Spectra AAS with a detection limit of the device (0.001 mg).

Statistical analysis was performed using the statistical software program PAST, version 2.17c., MVSP, version 3.22 and Microsoft Office Exel 2007.

Results and discussions

The plants rise two weeks after sowing (sowing date: 09.04.2016). The chart of agricultural works of the experimental variants is presented in table 1.

Harvesting of the plants were carried out when the plant height reached 25-30 [cm]. The soil cover of the plants was determined after each harvest and is presented in figure 2.

It is noted that for each variant the plant-covered surface increases due to the twinning of plants. After 6 months *Lolium perenne* culture will handle P4, P4, P5 sewage sludge and fly ash treated occupied 50-90 [%] of the harvested area. Figure 2 shows that the most effective phytoremediation variant was P5 (TPH polluted soil, 60 [tha^{-1}] sewage sludge and 60 [tha^{-1}]] fly ash treated). Figure 3 shows the amount of green mass obtained at harvests 1-8 and the total amount of each variant.

Figure 3 shows that the combined sludge and fly ash treatment of sewage resulted in the doubling of the total mass of green mass obtained from the experimental variants P_3 - P_5 compared to the quantity of green mass harvested in the experimental variants where the polluted soil was treated with sewage sludge or fly ash. Table 2 lists TPH after 6.5 months of grass cultivation on variants P_1-P_5.

Table 1
THE CHART OF AGRICULTURAL WORKS CARRIED OUT FOR THE EXPERIMENTAL VARIANTS

Sowing	Parameter	Harvest 1 (H1)	Harvest 2 (H2)	Harvest 3 (H3)	Harvest 4 (H4)
09.04	Data	10.05	27.05	14.06	03.07
	Parameter	Harvest 5 (H5)	Harvest 6 (H6)	Harvest 7 (H7)	Harvest 8 (H8)
	Data	28.07	29.08	14.09	23.09

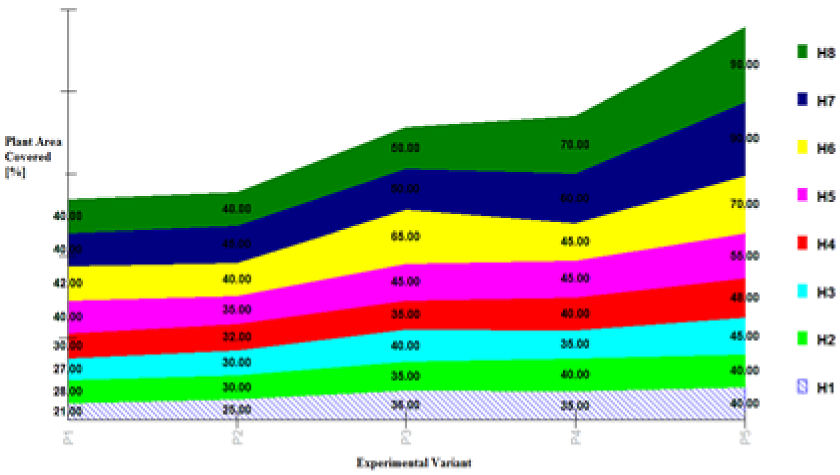

Fig. 2. Fingerprint of covered plant area (%) at different harvest data
Legend: H1-8 = harvest data , P_1, P_2, P_3, P_4, P_5 -TPH polluted variants

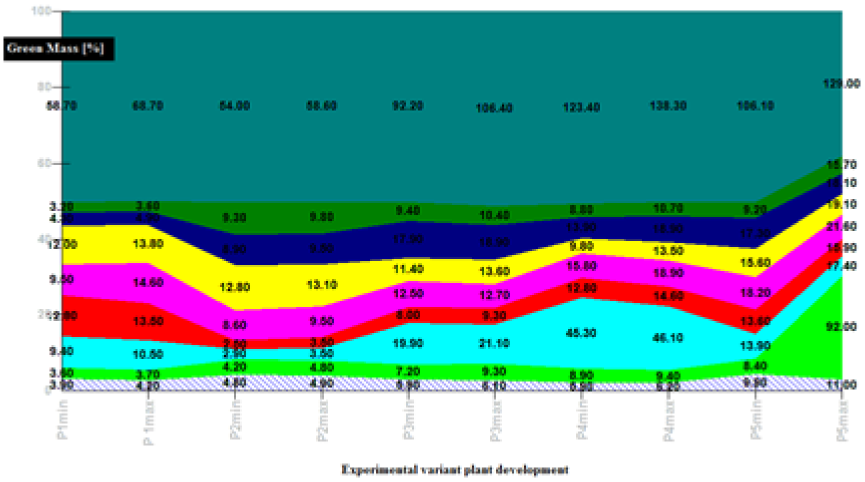

Fig. 3. Harvests and total quantity of each experimental variant green mass fingerprint
Legend: H1-8 = harvest data , P_1, P_2, P_3, P_4, P_5 - TPH polluted variants

Table 2
THE RESIDUAL TPH FROM THE POLLUTED SOIL AFTER 6.5 MONTHS CULTIVATION OF PERENNIAL *LOLIUM PERENNE* ON VARIANTS P_1-P_5

Crt. No.	Total Petroleum Hydrocarbon	Experimental variant // TPH quantity				
		P 1	P 2	P 3	P 4	P 5
1	Initial [gKg^{-1} D.M.]	107.10	112.30	99.50	96.10	97.30
2	After 6.5 months of cultivation [gKg^{-1} D.M.]	92.70	85.90	63.24	47.10	42.50
3	Reduction efficiency in soil [%]	13.40	23.50	36.40	50.90	56.30

Table 3
AMOUNTS OF BIOACCUMULATED METALS OF PLANTS AT THE HARVEST H8

Metal	Experimental variants/ Metals content of plants aerial tissue [$mgKg^{-1}$ D.M.]					
	Maximum Romanian admited limit for organic matter with 12% humidity [29]	P_1	P_2	P_3	P_4	P_5
Cd	1.0	*	*	*	*	0.44
Cr	* *	*	*	*	*	*
Cu	* *	13.0	20.8	12 .9	19.4	16.9
Ni	* *	7.1	7.4	6. 5	6.7	5.4
Pb	100	20.8	23.5	12 .9	13,7	25,7
Zn	* *	11.5	63.9	63 .9	45.3	47.5

* under the detection limit, * * without analyzing

It can be noticed that in the polluted soil treated with sludge, the plants participate at the reduction of TPH quantity with 23.5 [%]. The addition to the quantity of 6 [tha^{-1}] fertilizer of an ash quantity of 15 [tha^{-1}] resulted in higher reduction efficiency of TPH of up to 36.4 [%] of the perennial *Lolium perenne* cultivated soil. The increase in the amount of ash to 45 - 60 [tha^{-1}] determined the increase of efficient TPH reduction between 50.9 and 56.3 [%]. Table 3 presents the quantities of bio-accumulated metals in the grass on variants P_1 - P_5 at the harvest H8.

The aerial parts of plants grown on variants P_1 - P_5 did not accumulate Cr. The aerial parts of the plant tissue harvested from the experimental variants P_1-P_4 did not bioaccumulate Cd, but in P_5 variant the harvested crop bioaccumulated 0.44 [$mgKg^{-1}$ D.M.] Cd. As it can be seen, the bioaccumulated amount of Cd was below the maximum admited limit. The amount of bioaccumulated Pb in the fertilized sludge and fly ash variants varied between 12.9-25.7 [$mgKg^{-1}$ DM], and it was below the maximum allowed limit in these conditions. Based on these observations we can affirm that *Lolium perenne* plants were not behaving like heavy metal bioaccumulators.

Conclusions

The method of phytoremediation with *Lolium perenne* species of the petroleum polluted areas using urban sludge and fly ash presents the advantages of rapid recovery of the surface of the polluted area by covering with a layer of grass that can be harvested periodically at 15-20 days during the warm season; using an optimal 6o tha^{-1} fertilizer, an ash quantity of 15-60 [tha^{-1}] has determined effective 50-90 [%] crop coverage with successive grass layers leading to high yields. Effective TPH reduction in soil fertilized with fly ash during intense vegetation season, the efficiency of TPH reduction in the polluted and treated soil was 38.4-56.3 [%]. The biomass did not bioaccumulate Cd and Cr at the detection limit. The amount of Pb and Zn bioaccumulated by the aerial plant parts was below maximum admitted limits. The obtained biomass can be used as a supplement to animal feed or for bedding in shelters. The soil remediation efficiencies of 91.73 ± 11.12 [gKg^{-1} D.M] were directly proportional to the amount of fly ash used.

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