DOI: http://doi.org/10.21698/simi.2017.0004

IN-SITU PHYTOREMEDIATION VARIANTS FOR TPH POLLUTED SOILS

Smaranda Masu[1], Nicolai Dragomir[2], Stefanita Mirel Pana[3], Carol Blaziu Lehr[1]

[1]National Research and Development Institute for Industrial Ecology, Timisoara Subsidiary, 115 Bujorilor, 30043, Timisoara, Romania, *smarandamasu@yahoo.com*
[2]Banat´s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Department of Biotechnologies, code 300645, Timisoara, Romania
[3]S.C. DEMECO S.R.L. Arad, Romania

Abstract
A mixture of grassland species *Lolium perenne, Festuca arundinaceea, Medicago* sativa and Ttrifolium pratense were used in situ phytoremediation of oil polluted soil, with 70.45-120.52 g/kg d.m. Total Petroleum Hydrocarbons (TPH). They were studied variants of phytoremediation of oil polluted soils and fertilized with 50 t/ha stabilized sewage sludge in absence / presence of 50 t/ha fly ash, waste from coal burning power plants. Lolium sperenne herbs formed on the surfaces sown exclusive bouquets, in first weeks. After six weeks of vegetation appear and develop leguminous species Medicago sativa and Trifolium pratense. After five months, was a balanced distribution of species of legumes and grasses species on the surfaces sown. The efficiency reducing the TPH from oil polluted soil was 48.7% in variant fertilized with stabilized sewage sludge and fly ash. Efficiency was with 15.3% higher than the reduction efficiency of petroleum products in fertilized variants in fly ash absence. The process efficiency of oil polluted soil treated with stabilized sewage sludge and fly ash was similarly with phytoremediation variant with fertilizer and indigenous volcanic tuff realized.

Keywords: *fly ash, grassland species, in-situ phytoremediation, oil polluted soil, sewage sludge fertilizer*

Introduction
In addition to the phytoremediation in site, for soil polluted with oil products name total petroleum hydrocarbons (TPH) additional research is needed on specific method: selecting plant species tolerant and efficient evaluation of the merits of the polluted area, method and maintenance costs of implementing them, the prediction of risks occurring, likely results (Gerhardt et al. 2009). In polluted soil with compounds in oil are highly accumulations of derivatives of carbon. In relation to the high quantity of compounds with carbon content, the soil shows the amounts limiting nitrogen and phosphor. Bringing nutrients by plants based on C, N, P in relation to the optimal agronomic plant and provide the necessary micro-organisms in a complex process of development of the crop defaults. Biodegradation efficiency /reduction of petroleum products will grow in polluted soil (Basumatary et al. 2012). The addition of the adsorbent materials in polluted soil with TPH are able to correct soil characteristics polluted water loss in the petroleum polar compounds.
For example retain water in volcanic tuff and micro porous structure of this stock will release small amounts of water depending on the plants needs (Leggo et al. 2006). Fly

ash acts by adsorbing properties and therefore reduce the early stages of development of plant stress caused by the amount high petroleum products in the polluted soil (Kishor et al. 2010). In addition, fly ash provides the required microelements from the soil (Basu et al. 2009; Ram & Masto 2014). They performed several studies of phytoremediation soil polluted with TPH. Studies phytoremediation are conducted in many countries, both on plants of meadows and shrub. It investigated the behaviour of several species of plants in the presence of different amounts of TPH in soil. Plants of meadows eg. species rye grass used in tests phytoremediation determine the effectiveness of reducing petroleum hydrocarbons in soil polluted with 6 g/kg up to 59% time in which to monitor the effectiveness of reducing soil pollution was for 80 days (Alarcón et al. 2008). Other species of perennial grasses such as tall fescue (Festuca arundinacea Schreb., or Lolium perenne) were also selected for phytoremediation of soils polluted with sheer oil because these species form an extensive network of roots and a robust development after installing culture. Grasses presents roots can penetrate the soil to a depth of up to 3m, and also, these plants show genetic diversity inherent which could give a competitive advantage to establish under adverse soil. Plants characteristic of natural grasslands based on grasses Festuca spp., Lolium spp., Cynodon dactylon have been used for the phytoremediation of areas with a higher pollution of 9 g/kg d.m. (Alarcón et al. 2008; White et al. 2006). In conclusion, TPH contaminated soil phytoremediation process can re-integrate the land destroyed into the natural circuits of the Earth (Aprill & Sims 1990; White et al. 2006).

Materials and Methods

The experimental study was conducted on soils contaminated with petroleum products conglomerates with high oil content had a diameter of between 3-10 cm, and range of TPH concentrations between 70.45-120.52 g/kg d.m. The conglomerate are black colour, with thick consistency, glossy and sticky smell of oil. Soil high conglomerate represents about 80-90% of studied soil and could not be crumbled. Soil inter conglomerate containing amounts between 3.57-11.50 g/kg d.m. The amount of petroleum products from contaminated soils used for phytoremediation experimental study is presented in Table 1. The experimental block is located in the experimental 46°16'.96.66"N, 21°43'46.1"E, elevation 150 m. Experimental block includes four lots/variants:

- soil polluted with oil products, lot P;
- soil polluted with oil products, fertilized with sewage sludge originated from the city wastewater treatment station, 50 t/ha treated with tuff indigenous, the amount of 5 t/ha, lot PNT;
- soil polluted with oil products fertilized with sewage sludge originated from the city wastewater treatment station, 50 t/ha and fly ash from burning brown coal in power plant (50 t/ha), lot PNC ;
- soil polluted with oil products, fertilized with sewage sludge originated from the city wastewater treatment station 50 t/ha, lot PN.

Surface of each experimental variants was a lot of 20 sqm. The experimental variants are separated from each other by spaces 1 m.

Table.1. Characterization of soils polluted with oil products (TPH) of experimental variation mixture of herbaceous plants cultivated in order of phytoremediation

No.	Lot	Soil deepness [cm]	pH	TPH in high soil conglomerates (min - max) [g/kg d.m.*]	TPH in soil inter conglomerates (min – max.) [g/kg d.m.]
1	PN	5-10	6,75	108,5-120,52	6,47-11,2
2	PNC	5-10	7,04	80,81-89,7	6,88-11,50
3	PNT	5-10	7,07	102,8-119,3g	5,88-6.11
4	P	5-10	7,18	70,45-71,3	3,57-5.32

*dry matter

Method for determining soil TPH

In an Erlenmeyer flask were mixed 3-5 g polluted soil with 5g anhydrous Na2SO4 and 25 ml tetrachloroethylene (Fluka). The content of Erlenmeyer flask was stirred 30 min at 100 rpm and then filtered through Whatman filter paper no. 4. Then flask and the filter paper were 3 times washed with 3 ml of solvent added to the filtrate in a porcelain capsule. The filtrate was evaporated at 75 °C until complete removal of the solvent. The residue of porcelain capsule was dissolved in 3 portions of solvent (15 ml, 10 ml and 5 ml) and passed (together with the undissolved parts) on the chromatographic column filled with aluminium oxide. The eluate was collected in a porcelain capsule. The solvent was evaporated from the capsule and weighed to constant mass. The identical residue was obtained from 28 mL of tetrachloroethylene (solvent), representing blank sample (without soil sample).

$$TPH_{[mg/kg\ d.m.]} = [(m_2-m_1) – (m_4-m_3)]/m_1 \times 1000] \quad (1)$$

where m_1 - mass capsule 1 without residue, mg; m_2 - capsule 1 weight containing residue from polluted soil, mg; m_3 - mass capsule 2 without residue, mg; m_4 - capsule 2 weight containing residue from solvent, mg.

The volcanic rock comes from Marsid quarries and contains about 70% clinoptilolite 0,2-2 mm grained volcanic rock is used and strewn on the ground before spreading sewage sludge. Characteristics of sewage sludge ashes: humidity 99%, 35.4% organic matter, total nitrogen, 1.7%, 0.65% phosphorus, pH = 6.1. Fly ash and sewage sludge analysis were determined in ECOIND laboratory, as per national standardized. Metals content of fertilising materials / amendments is presented in Table 2.

Table 2. Metals content of fertilising materials / amendments

No.	Parameter	M.U.	Characteristics	
			sewage slugde	fly ash
1	Cd	mg/kg d.m.	3.43	2.43
2	Cr	mg/kg d.m.	134.7	92.4
3	Cu	mg/kg d.m.	333.9	74.3
4	Ni	mg/kg d.m.	27.4	51.7
5	Pb	mg/kg d.m.	157.8	67.4
6	Zn	mg/kg d.m.	304.6	169.0

The selection of plant species was made in view of resistance culture to extreme soil conditions with toxic elements, TPH and harsh climatic conditions, hot summers with

prolonged drought. Plants tolerant which were selected was legumes and grasses meadows. The mixture of plant species selected included: *Lolium perenne, Festuca arundineceea, Trifolium pretense, Medicago sativa*. The seeding was done with an amount of 18-20 kg/ha**.**
Vegetation cover degree was determinated after Braun-Blanquet and Ellenberg and is presented in Table 3 (Wikum & Shanholtzer 1974).

Table 3. Abundence-Dominance Index after Braun-Blanquet and Ellenberg

No	Level	Cover degree [%]	Abundence-Dominance mean, (*ADm*) [%]
1	+ (scattered plants)	0.1-1	0.5
2	1 (diminshed)	1-10	5
3	2 (1/20 cover from area)	10-25	17.5
4	3 (¼-½ cover from area)	25-50	37.5
5	4 (½-3/4 cover from area)	50-75	62.5
6	5 (>3/4 cover from area)	75-100	87.5

Results and Discussions
Characterization presence grasses species in the experimental study of phytoremediation of soils polluted with petroleum products is presented in Table 4. Plants of the species *Lolium spp*. presented in Week 2 of vegetation a coverage area that lot of 40-50% for experimental versions of polluted soil, fertilized and treated with fly ash from coal combustion or fossil indigenous tuff. For these variants experimental area covered by plants of *Lolium spp*. was extended in the 6th week vegetation at 60-80%.

Table 4. The presence of the *Lolium spp* in the experimental lot

Lot	Week 2 Total vegetation degree [%]	Week 6 Total vegetation degree [%]	Week 10-		Week 20 after harverst	
			Total vegetation degree [%]	*Lolium spp.* cover of Total vegetation degree [%]	Total vegetation degree [%]	*Lolium spp.* cover of Total vegetation degree [%]
PN	20	40-50	70	80	75	40
PNC	40	60	80	90	90	50
PNT	50	80	95	100	100	50
P	-	5	60	40	70	40

The 6th week of the lots were invaded by weeds, which required their removal repeated. At week 10, demonstrated an overall areas planted vegetation cover 80-95% for experimental versions of polluted soil, fertilized and treated with native tuff or fly ash. Of the total area covered by plant species of grasses *Lolium spp.* has occupied 90% where it was used as an additive to fertilizer fly ash and 100% if it was used as an additive to fertilizer tuff indigenous. A satisfactory result was obtained in soil fertilized with sewage sludge when the area occupied plants was 70%. In this case the presence of *Lolium spp.* was 80% of the area covered by plants. In Table 4 are presented the results obtained after harvest. The 20th week showed a degree of vegetation cover 90-100% for experimental versions of polluted soil, fertilized and treated with fly ash or tuff indigenous. Of the total area covered with grass plant,

species *Lolium spp*. occupies only 50%. A satisfactory result was obtained in soil fertilized when the plants area occupied was 75%. In this case the presence of *Lolium spp.* was 40% of the area covered by plants. Reducing the presence of *Lolium spp*., the total degree of vegetation cover resulting from expansion of other species of plants mixture was seeded lawns that lot. In the experimental variant unfertilized plants will recover delays due to late emergence. Of the total degree of vegetation cover 70% of this batch presence *Lolium spp.* up to 40%. In Table 5, is presented abundance–dominance index Braun-Blanquet for plant species to experimental variant PN after harverst.

Table 5. Abundance–dominance index Braun-Blanquet for plant species to PN after harvest (20th week)

No	Species of plants	The presence of plant species in total vegetation degree [%] / abundance –dominance index	Observations
		Grasses	
1	*Lolium spp.*	40% / Level 3	large areas of the plant
2	*Festuca spp.*	5% / Level 1	individuals scattered
		Legumes	
3	*Trifolium spp.*	5% / Level 1	some plants
4	*Medicago spp.*	20% / Level 2	plants as a group

It is noted from Table 5 that the experimental polluted variant and fertilized with sewage sludge and has developed best species of plants grasses *Lolium spp*. This plant has occupied 120 week after mowing, 40% of the area covered with plants and level 3 for Abundance–dominance index Braun-Blanquet. Ranked second in the standings is leguminous plant development (*Medicago sativa*) which occupied 20% of the area covered with plants and Level 2 for Abundance–dominance index Braun-Blanquet. *Festuca spp.* and *Trifolium spp.* occupied 5% of the area covered with plants and Level 1 for Abundance–dominance index Braun-Blanquet. In Table 6 is presented abundance–dominance index Braun-Blanquet for plant species to experimental variant PNC after harverst. Shown in Table 6 that the experimental variant polluted and fertilized with sewage sludge in fly ash presence was developed *Lolium spp*. and *Medicago sativa*. This plant has occupied after mowing, 50% of the area covered with plants and Level 4 for Abundance–dominance index Braun-Blanquet. Ranked second in the standings is leguminous plant development (*Medicago sativa*) which occupied 30% of the area covered with plants and Level 3 for Abundance–dominance index Braun-Blanquet. Addition of fly ash with sewage sludge fertilizer favoured the species *Medicago sativa and Lolium spp.*

These species occupied areas by 10% higher than in the case of variant fertilized in the absence of fly ash. *Festuca spp* and *Trifolium spp* occupied 5% of the area covered with plants and Level 1 for Abundance–dominance index Braun-Blanquet. In Table 7 is presented abundance–dominance index Braun-Blanquet for plant species to experimental variant PNT after harvest.

Table 6. Abundance–dominance index Braun-Blanquet for plant species to experimental variant PNC after harverst (20th week)

No	Species of plants	The presence of plant species in total vegetation degree [%] / abundance –dominance index	Observations
		Grasses	
1	*Lolium spp.*	50% / Level 4	large areas of the plant
2	*Festuca spp.*	5% / Level 1	individuals scattered
		Legumes	
3	*Trifolium spp.*	5 % / Level 1	some plants
4	*Medicago spp.*	30% / Level 3	bouquets branched

Table 7. Abundance–dominance index Braun-Blanquet for plant species to experimental variant PNT after harvest (20th week)

No	Species of plants	The presence of plant species in total vegetation degree [%] / abundance –dominance index	Observations
		Grasses	
1	*Lolium spp.*	50% / Level 4	large areas of the plant
2	*Festuca spp.*	Level + - 1-2 individuals	individuals scattered
		Legumes	
3	*Trifolium spp.*	10% / Level 2	some plant
4	*Medicago spp.*	30% / Level 3	bouquets branched

Shown in Table 7 that the experimental variant fertilized with sewage sludge polluted and treated with tuff native was developed best species of plants grasses *Lolium spp*. The presence of plant species in total vegetation cover grade and abundance – dominance index Braun-Blanquet it was similar to that obtained in the experimental variant of phytoremediation of polluted soils fertilized with sewage sludge and treated with fly ash. *Festuca spp.* only occurs sporadically, a few wires and legumes species of *Trifolium spp.* occupied 10% of the area covered with plants. The area covered with plants clover was 5% higher than in the experimental variant of phytoremediation of polluted soils fertilized with sewage sludge and fly ash treated with the surface covered with plants. 10% of the area covered by weed plants develop from the seeds made of sewage sludge (Level + respectively Level 2 for abundance –dominance index Braun-Blanquet). In Table 8 is presented abundance – dominance index Braun-Blanquet for plant species to experimental variant P after harvest. It is noted from Table 8 that the experimental variant polluted was developed best species of plants grasses *Lolium spp*. This plant has occupied 40% of the area covered with plants and Level 3 for abundance – dominance index Braun-Blanquet. The second place ranking is leguminous plant development which occupied 20% of the area covered with plants and Level 2 for index Braun-Blanquet. *Festuca spp.* appears only sporadically, *Trifolium spp.* increase as a few scattered groups.

In Table 9 is presented Total petroleum products (TPH) variation content in soils unfertilized / fertilized in the absence / presence amendments.

Table 8. Abundance–dominance index Braun-Blanquet for plant species to experimental variant P after harvest (20 th week)

No	Species of plants	The presence of plant species in total vegetation degree [%] / abundance –dominance index	Observations
		Grasses	
1	*Lolium spp.*	40% / Level 3	large areas of the plant
2	*Festuca spp.*	-	-
		Legumes	
3	*Trifolium spp.*	-1-2 plants Level +	few scattered groups.
4	*Medicago spp*	20 % / Level 2	bouquets branched

Table 9. Total petroleum products (TPH) content unfertilized / fertilized soil in the absence / presence amendments

No.	Lot	Total petroleum products **TPH [g/kg d.m.]**				
		Initial	**Week 6**	**Week 12**	**Veek 14**	**Veek 20**
1	PN	114,5	102,45	86,11	80.09	78,19
2	PNC	85,7	88,9	79,63	64,2	44,0
3	PNT	120,32	116,5	108,38	89,5	54,1

It notes that TPH products, reduce the variance of soil studied, planted with meadows. Noted that the polluted soil fertilized without amendments remain significant amounts of petroleum products: 78.19 g/kg d.m. The fertilized in the presence of polluted soil amendments remain after five months of growing quantity of oil in the range 44.0-54.1 g/kg d.m. TPH reduction efficiency was 48.7% in the experimental variant PNC and 54.8% in the experimental variant PNT. TPH reduction efficiency was 33.4% in the experimental variant polluted fertilized, but the non-amended.

Conclusions

Plants of the species *Lolium spp.* sprung up exclusively on the cultivated variants. The plants had during sprouting a degree of surface vegetation cover seeded 40-50% for experimental variants of polluted soil, fertilized and treated with amendments, fly ash resulting from burning coal on fossil power plant or native tuff. For these variants remains six week exclusively experimental area covered by plants of *Lolium spp*. After 10 week of vegetation, coverage sown variants reaches 80-95%. Plants of the species *Lolium perenne* have a twin. As a consequence the presence of the species reaches 90-100%. After harvesting, week 20, total vegetation coverage of the lot fertilized in the absence / presence amendments reaches 75-100%. After harvest the presence of *Lolium spp*. of the total coverage area was 40-50%. Leguminous plants have sprouted with delay of up to 6 weeks toward *Lolium spp.* The decrease in the area occupied by the *Lolium spp.* was due to the extension of the areas occupied by the leguminous plants. Effectiveness of reducing TPH products from studied soil variants, cultivated with grassland plants was 48.7% for the polluted, fertilized and fly ash treated, PNC, variant. The TPH reduction efficiency in the polluted, fertilized and volcanic tuff treated variant was 54.8%. The polluted soil fertilized in the presence amendments remain after 5 months of grass quantities of petroleum products in the range 44.0-54.1 g/kg d.m. TPH reduction efficiency was 33.4% in the polluted fertilized, but the non-amended variant. It should be noted, that in the polluted soil

and fertilized in the absence of amendments, significant quantities of oil products remain in the soil, *i.e*. 1.5-1.8 times more than in polluted soil, fertilized in amendment presence and grass.

References

Alarcón, A, Davies Jr, FT, Autenrieth, RL & Zuberer, DA 2008, 'Arbuscular mycorrhiza and petroleum-degrading microorganisms enhance phytoremediation of petroleum-contaminated soil'. *International journal of phytoremediation*, vol. 10, no. 4, pp. 251-263.

Aprill, W & Sims, RC 1990, 'Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil'. *Chemosphere*, vol. 20, no. 1-2, pp. 253-265.

Basu, M, Pande, M, Bhadoria, P & Mahapatra, S 2009, 'Potential fly-ash utilization in agriculture: a global review'. *Progress in Natural Science*, vol. 19, no. 10, pp. 1173-1186.

Basumatary, B, Bordoloi, S & Sarma, HP 2012, 'Crude oil-contaminated soil phytoremediation by using Cyperus brevifolius (Rottb.) Hassk'. *Water, Air, & Soil Pollution*, vol. 223, no. 6, pp. 3373-3383.

Gerhardt, KE, Huang, X-D, Glick, BR & Greenberg, BM 2009, 'Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges'. *Plant science*, vol. 176, no. 1, pp. 20-30.

Kishor, P, Ghosh, A & Kumar, D 2010, 'Use of flyash in agriculture: A way to improve soil fertility and its productivity'. *Asian Journal of Agricultural Research*, vol. 4, no. 1, pp. 1-14.

Leggo, PJ, Ledésert, B & Christie, G 2006, 'The role of clinoptilolite in organo-zeolitic-soil systems used for phytoremediation'. *Science of the Total Environment*, vol. 363, no. 1, pp. 1-10.

Ram, L & Masto, R 2014, 'Fly ash for soil amelioration: a review on the influence of ash blending with inorganic and organic amendments'. *Earth-Science Reviews*, vol. 128, no. pp. 52-74.

White, PM, Wolf, DC, Thoma, GJ & Reynolds, CM 2006, 'Phytoremediation of alkylated polycyclic aromatic hydrocarbons in a crude oil-contaminated soil'. *Water, Air, & Soil Pollution*, vol. 169, no. 1, pp. 207-220.

Wikum, D & Shanholtzer, G 1974, 'Cover Application of the Braun-Blanquet, Scale for Vegetation Analysis in Land Development Studies'. *Environment Management*, vol. 2, no. pp. 323-329.