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The optimal way of biological cleaning of oil-contaminated soils

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Abstract: The results of development of biological ways for cleaning soils from oil pollution in Boryslav are shown in this study. The prospects of tree species for the remediation of oil-contaminated soils are studied. The best results of cleaning oil contaminated soils were obtained with the application of *Hippophae rhamnoides* L. plants. We have shown that the *Hippophae rhamnoides* L. plants successfully adapt to the extreme conditions of oil contaminated soils, improve soil physical and chemical properties and reduce the amount of the contained oil. Purification degree of soils from oil pollution reaches 92.7%. The plants of these species decrease the toxicity of oil-contaminated soils and improve their microbiological properties. *Hippophae rhamnoides* L. plants are promising and attractive agents for remediation of oil-contaminated soils from both environmental and economic point of view.

Key words: oil-contaminated soils, phytoremediation, *Hippophae rhamnoides* L., a phytotoxicity.

Introduction:

Oil-extracting industry has extremely dangerous effects on soil ecosystem. It contaminates large areas by oil. Soils have the ability to accumulate pollution, and in turn, become a source of secondary pollution of air, surface and groundwater. Therefore, the problem of cleaning soil from oil pollution is important task at present.

Different methods have been used in the remediation of oil polluted soils and can be grouped as physical-chemical processes (shallow soil mixing, oxidation-reduction, hydrolysis-neutralization, stabilization-solidification, mobilization-immobilization, soil flushing-washing), thermal processes (heating to evaporate, burn, decompose, destroy or melt the contaminants) and biological processes (biopurging, soil bioinjection, phytoremediation). Physical methods, such as stripping or sorption, are not as effective as biological methods for treating hazardous organic compounds. Chemical methods may be used to remove heavy metals. The chemical structure (and then the behavior) of pollutants is changed by means of chemical reactions to produce less toxic or better separable compounds from the matrix. Thermal processes use heat to evaporate, to burn, decompose, destroy or melt the contaminants. Chemical constituents are burned and chemically oxidized by applying high temperature. The most important problem in incinerating hazardous wastes and soil is the generation of by-products such as polychlorinated dibenzofurans, chlorinated benzenes, chlorinated phenols and nitrogen oxides [1, 2]. In the remediation of oil polluted soils, physical and chemical methods (aeration, excavation, transportation and incineration) have been used for years. These methods however have been often expensive and laborious [3, 4].

It was shown that one of the most effective ways to rehabilitate contaminated areas are technologies of remediation [5-11]. Phytoremediation is an innovative technology that uses plants to remove environmental contaminants. Phytoremediation is the most promising environmentally friendly way of remediation of oil-contaminated soils in terms of simplicity and efficiency, characterized by long-term exposure and stable improvement of the environmental situation.

The analysis of scientific literature shows good results of use such plants as: *Glicine hispida* Maxim, *Helianthus annuus* L., *Vicia faba* L., *Carex hirta* L. mixed grasses etc.. for recultivation of contaminated soils [12-16]. Several studies have suggested the efficacy of various plants in eliminating different soil contaminants, particularly oil derivatives. In the study of decontamination of crude oil-polluted desert soils, Diab [13] reported oil degradation rate as 62.4%, 19.9%, and 17.6% using *Vicia faba*, *Zea mays*, and *Triticum aestivum*, respectively. A plot-culture experiments were conducted to examin the feasibility of Pharbitis nil L. and its microbial community to remedy petroleum contaminated soils [17]. The results showed that there was significantly greater degradation rate of oil derivatives in vegetated treatments, up to 27.63–67.42%, compared

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with the unvegetated controls (only 10.20–35.61%), after 127 days of incubation. In another research, after sowing Bidens maximowicziana for 50 days, the mean reduction in pyrene concentration was 28% more in treated soil compared to the control soil [18].

These known phytoremediation methods despite their positive aspects have certain weaknesses: they are multi-stage, long duration, need a thorough pre-treatment of soil, additional irrigation and the survival of remediating agents on highly contaminated soils is not guaranteed. These methods are based on the use of mainly one-year grassy plants, therefore, repetition of the process of planting seeds in next years is necessary. It is economically unprofitable and technically impossible on the dumps, inclined reliefs, soils which are exposed to erosion. Also thin root system is not able to fix such soil. Thus, these methods are limited to the possibility of use only on horizontal sections since the above-mentioned plants cannot be fixed on different soil surfaces.

Therefore we set for ourselves the task to improve a way of phytoremediation of oil-contaminated soils by means of selection and using resistant to oil pollution perennial vegetating plants, which can reduce the toxicity of soil, grow quickly, filling the "dead area" not only on horizontal sections, but also in bulk dump soils. This makes them economically promising.

Hippophae rhamnoides L is a deciduous shrub that belongs to the family of Elaeagnaceae. This flowering plant is native to fixed dunes and sea cliffs in Europe and Asia. It can grow 2–4 m (7–13 ft) high. The leaves are alternate, narrow and lanceolate, with silvery-green upper faces. The oval or lightly roundish fruits grow in compact grapes varying from pale yellow to dark orange and weighing from 0.2 g to 1 g. The plant has a very developed root system that can maintain the soil on high slopes. The roots live in symbiosis with actinomycetes. This relationship permits fixation of nitrogen from the air. They also transform insoluble organic and mineral matters from the soil to more soluble states.

Hippophae rhamnoides L i salso considered according to several studies, an important ally of our health. It is by far the most widespread and contains a number of compounds including carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, tannins. Such compounds are of interest not only from chemical point of view, but also because many of them have biological and therapeutic effects including antioxidant properties[19].

Materials and Methods

The study was performed in three replicates, on the experimental plots with different extent of pollution by oil: 97 g/kg, 123 g/kg, 150 g/kg. Each site was an area of 12 x 6 m^2 and contained 10 plants. Hippophae rhamnoides L. seedlings were transplanted in oil-contaminated soils at 15-20 cm depth. Four plants were introduced in each $10m^2$. Planting was carried out early in spring when the soil had still enough moisture, without additional fertilization, irrigation or micro-organisms [20].

Oil-contaminated soils samples were taken from experimental plots before the planting (Control) , from the place of planting and at a distance of 4-6 meters from the planting place and this annually over 4 years. The soil samples were collected by the envelop method at a depth of 0-10 cm [21]. A combined sample was prepared by mixing five point samples taken from the same plot. Oil content, phytotoxicity of contaminated soils and number of soil microorganisms were measured during the studies.

Determination of oil in soil

Oil content in the soil was determined by a modified method extracting oil from samples of soil by carbon tetrachloride. The resulting mixture is passed through a chromatography column filled with Al_2O_3 adsorbent to separate impurities from oil petroleum origin, followed by spectrophotometric determination in the infrared region of the spectrum (in wave numbers 2860, 2930 and 2960 cm-1), that correspond to the structural groups $–CH_3$, $–CH_2–$, C–H in petroleum products.The calculation of concentration was performed on the basis of the absorption peak intensity using the calibration graph [22]. The study was performed in three replicates.

Determination of phytotoxicity of oil-contaminated soils

Phytotoxicity of soil was determined using biotest-*Linum usitatissimum* L. The contaminated soil in an amount of 20 g was placed in a petri dish, adding 10 ml of water (moisture 33.3%), laid the seeds of test objects, covered with cup and placed in an incubator at temperature + 24ºC. The seed germination was determined in 3 days, in 5 days the lengths of roots and heights of shoots were measured. Following values were calculated using the above formula:

Relative seed germination % = (number of germinated seeds in contaminated soil / number of germinated seeds in control) × 100%;

Relative root length % = (average root length in contaminated soil / average root length in control) × 100%;

Relative shoot length % = (average shoot length in contaminated soil / average shoot length in control) × 100%. The study was performed in three replicates [23].

Determining the number of soil microorganisms

The method of serial dilutions was used for the analysis of soil microorganisms. 1 mm of each soil extract suspension was introduced to a Petri dish, incubated at 30ºC for five days, and the number of colonies was counted taking the dilution into account. The study was performed in five replicates [24]. The oil-degrading microorganisms ("oil-destructors") were determined on Shishkina-Trotsenko medium with oil and liquid paraffin as sources of carbon [25].

Statistical analyses

The experimental data were statistically processed for the determination of the deviation and the mean using Statist and Excel. For all indicators the arithmetic mean value of the sample and the standard error of the mean were calculated. To identify the significance of differences in the results of the study of experimental and control groups of plants, Student's coefficient was determined. Critical significance level was equal to 0, 05.

Results and discussion

Our research was conducted at the site of the old oil field, located in the Boryslav town. Boryslav town is located on the Tysmenytsia River (a tributary of the Dniester) in Lviv region in the western part of Ukraine. It is a center of petroleum industry. We have developed technologies for soil purification from oil pollution. The ways of soil cleaning from oil pollution are being developed and tested on the Boryslav oil deposit. Targeted search of tree species (*Salix babylonica* L., *Betula pendula* L., *Hippophae rhamnoides* L., *Prunus cerasifera* L.) was conducted for remediation of oil-contaminated soils and as a secondary fuel source.

The best results of cleaning oil contaminated soils were obtained with the application of *Hippophae rhamnoides* L. plants. *Hippophae rhamnoides* L. roots can tolerate long periods of flooding and waterlogging. The thick leaf of these plants and leaf thick cuticle are protective devices that provide economical consumption of water in the condition of high insolation and temperature. The elevated part of these plants can tolerate deficiency of water inside the plant, and actively adjust the rapid changes in osmotic pressure of the cell sap [26]. The combination of resistance to flooding and drought conditions makes these plants optimal for use in the phytoremediation of oil contaminated soil. We have shown that the *Hippophae rhamnoides* L. plants successfully adapt to the extreme conditions of oil contaminated soil, improve its physical and chemical properties, enrich it with nitrogen compounds and reduce the amount of oil.

Our research shows that *Hippophae rhamnoides* L. plants are able to survive in heavily contaminated soils, 97-150 g of oil per 1 kg of soil. These plants significantly accelerate the process of biodegradation of oil even during the first year of growth. Total cleaning of soil from oil pollution reaches 76,7-84,6% after 1 year of vegetation (Table 1).

Table 1. Effect of *Hippophae rhamnoides* L. plants on degradation of oil in the soils during 1 year of vegetation.

Initial concentration of oil in the soils before remediation, g/kg	Remaining concentration of oil in the soil after remediation, g/kg	Total cleaning of soil from oil pollution, %
97	15,5	84,6
123	26,5	77,5
150	34,9	76,7

These three experimental plots represent different extent of pollution by oil: 97 g/kg, 123 g/kg, 150 g/kg and total cleaning of soil from oil polution soils during 1 year of vegetation. The following tables present data of the research in the area of oil pollution 123 g / kg over 1-4 years.

Purification degree of soils from oil pollution reaches 92.7% in four years of growth of *Hippophae rhamnoides* L. plants on the soil with initial oil contamination of 123 g / kg (Table 2). The soil loses its toxicity which also decreases in a radius of 4-6 m from the site of remediation, owing to the particular advantages of *Hippophae rhamnoides* L. plants: fast vegetative propagation and fast occupation of neighboring areas (Table 3).

Table 2. Effect of *Hippophae rhamnoides* L. plants on degradation of oil in the soil during 1-4 years of growth. The initial contamination of soil by oil 123 g / kg.

	The period of remediation			
	0 year	1st year	2nd year	4th year
The concentration of oil in the soil, g/kg	123	26,5	13,9	9,0
Total cleaning of soil from pollution, %	0	77,5	88,7	92,7

Table 3. Number of soil microorganisms and phytotoxicity of oil-contaminated soils before and after phytoremediation by *Hippophae rhamnoides* L. plants.

Sample	Toxicity of oil-contaminated soils Test organisms - *Linum usitatissimum* L.			Soil microorganisms, CFU/g	
	Relative seed germination, %	Relative root Lengh,%	Relative root Lengh,%	Hetertrophic microorganisms	
Oil-contaminated soils, 123 g/kg	0	0	0	$2x10^4$	$5x10^2$
The soil after remediation during four years	108,57	163,39	142,44	$2x10^8$	$3x10^5$
4-6 m from the site of remediation	88,57	181,25	52,37	$4x10^6$	$6x10^5$

The plants of these species decrease the toxicity of oil-contaminated soil and improve its microbiological properties too. Thus, the number of soil microorganisms after four years of growth of *Hippophae rhamnoides* L. plants on the oil-contaminated soil increased significantly: heterotrophs 10^4 times, oil destructive microorganisms $6×10^2$ times compared to non-reclaimed soil. The number of soil microorganisms also increases in a radius of 4-6 m from the site of remediation (Table 3).

A particular advantage of *Hippophae rhamnoides* L. plants is their fast vegetative propagation capability. The land reclamation area increased in 3 times during four years of phytoremediation. It is shown on Figures 1 and 2. This makes the method economically attractive.

Figure 1. The experimental reclamation area, first year of phytoremediation.

Figure 2. The experimental reclamation areaafterfour years of phytoremediation.

Hippophae rhamnoides L. plants are also able to grow both on the horizontal sections, and the backfill of oil-contaminated soils, due to the peculiarities of the root system, which is superficial and rapidly growing. This allows plants to be fixed on different soil surfaces. Moreover, wood of *Hippophae rhamnoides* L. plants has a high specific heat of combustion – 4,8 kcal/kg, which is significantly higher than most species of wood. 1 ton of wood of *Hippophae rhamnoides* L. plants corresponds to 0.68 tons of coal. It can be used as an independent fuel or for liquid fuel production.

Conclusion

Hippophae rhamnoides L. plants for phytoremediation of oil-contaminated soils was evaluated for the first time in this study. The presence of *Hippophae rhamnoides* L plants accelerates biodegradation of oil in the oil-contaminated soils. The plants of these species improve physical, chemical and biological properties of oil-contaminated soils, decrease their toxicity, reduce the amount of the contained oil, provide long-term phytoremediation and easily spread to unoccupied areas. *Hippophae rhamnoides* L. plants are also able to grow both on horizontal sections, and backfill of oil-contaminated soils. *Hippophae rhamnoides* L. plants can be recommended for phytorecultivation of the territories polluted with oil and oil products. These plants present an environmentally and economically profitable way of biological cleaning of oil-contaminated soils.

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