MICROORGANISMS OF FUEL CONTAMINATED SOIL UNDER PHYTOREHABILITATION

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Abstract
Microbiological parameters of soil polluted with kerosene under grasses milk vetch and kostrec have been investigated. It was shown, that at all tested pollution levels (1 %, 2 % and 5 %) number of heterotrophic microorganisms was higher under these plants at 2-30 times in comparison with unplanted soil, depending on type plant. Simultaneously with growth of heterotrophs number the increase of hydrocarbon utilizers on order was also revealed in comparison with quantity of the that group of microorganisms in unplanted soil.

Introduction
Environment pollution with oil and oil products has a global character. It determines constant interest to development of various methods of cleaning of soils from oil pollution, including technologies with use of plants – phytoremediation (2). It is considered, that plants influence to microclimate of soil (1, 3), make active it biochemicaly, raising its ability to transformation of pollution. In this connection studying of interaction of plants and microorganisms of soil in conditions of anthropogenous pollution is of interest.

Materials and methods
Object of the research was leached chernozem of Tatarstan Republic. Characteristics: salt pH - 5,28; nitrogen - 80 mg / 100g soil, phosphorus P_2O_5-99 mg / 100g soil, exchange potassium K_2O - 99 mg / 100g soil. Soil was artificially polluted with cleared kerosene (temperature of boiling 150-220 ^{0}C) - 1 %, 2 % and 5 % of air-dry soil weight.
Vegetation of plants – kostrec and milk vetch - was carried out in natural climatic conditions in greenhouse. During experiment humidity of soil in vessels was supported at a level of 24 %, average daily temperature - 25-27^{o}C. No polluted unplanted and planted soil was taken as the control (Tab. 1).

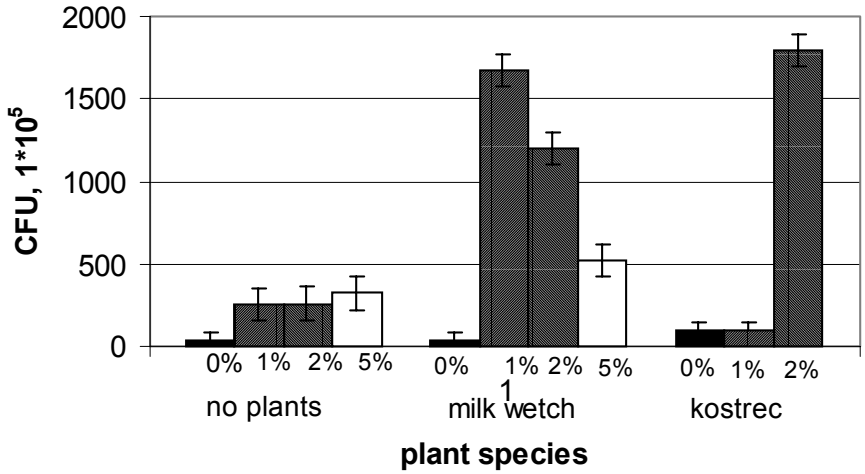
Table 1. Variants of experience

Plant species / Level of pollution, %	0	1	2	5
Unplanted soil				
kostrec				
milk vetch				

The soil for biochemical and microbiological analyses was selected after 18 days of grasses cultivation. The total heterotrophic bacterial number (THBN) and hydrocarbon utilizers number (HCN) was determined by method of decinal dilutions of soil suspension with the subsequent spreading on solid nutrient mediums – (Meat-Pepton Agar) and the synthetic medium for HCN.
Dehydrogenase activity of the soil was defined by Lenhard method (1962), protease activity – by method Mersi and Schiner (1991) (2).

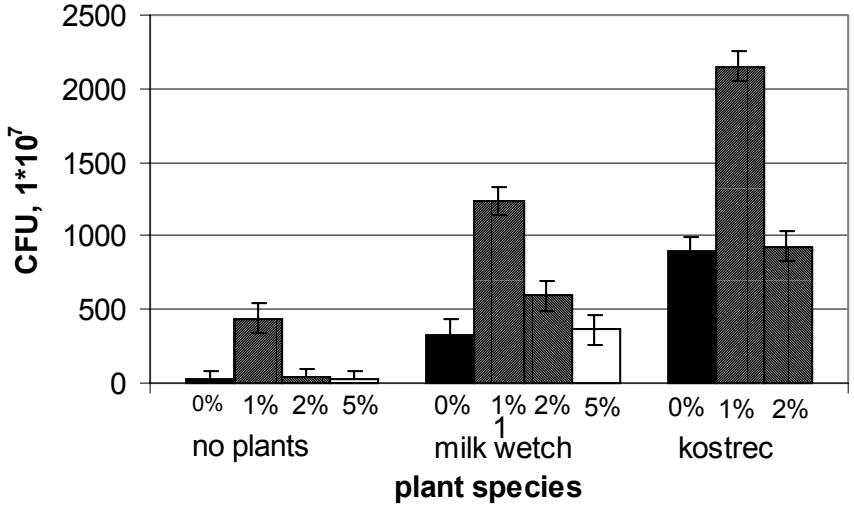
Results
Pollution of soil with kerosene has resulted in increase of number of HCN at one order in comparison with no polluted soil. At grasses cultivation this parameter was considerable higher - in 18,2-41,8 times in comparison with not polluted soil (Fig. 1). Development of microorganisms in not polluted planted soil did not exceed corresponding parameters of unplanted soil.
The variant with 5% of pollution with kostrec cultivation was not examined because the grass has not sprouted at this conditions.
As to heterotrophs in variants with planted soil their number exceeded the quantity of these microorganisms in unplanted polluted soil on the order. The maximum quantity of heterotrophic

Fig.1. The amount of HCU in different contamination variants.



microorganisms is revealed in all variants at 1 % kerosene pollution of soil (Fig. 2).

Fig.2. The amount of THBN in different contamination variants.



The total number of heterotrophs on 2-4 order exceeded number of HCU in soil under plants The share of CHU and THBN was higher in unplanted soil (Tab. 2)..

Table 2. Percentage of HCU in microbic community

Plant species	Percentage of HCU in microbic community			
Contaminant concentration	0 %	1 %	2 %	5 %
Milk vetch	0,123	1,351	2,022	1,445
Kostrec	0,11	0,043	1,929	-
Unplanted soil	1,03	0,588	5,73	10,0687

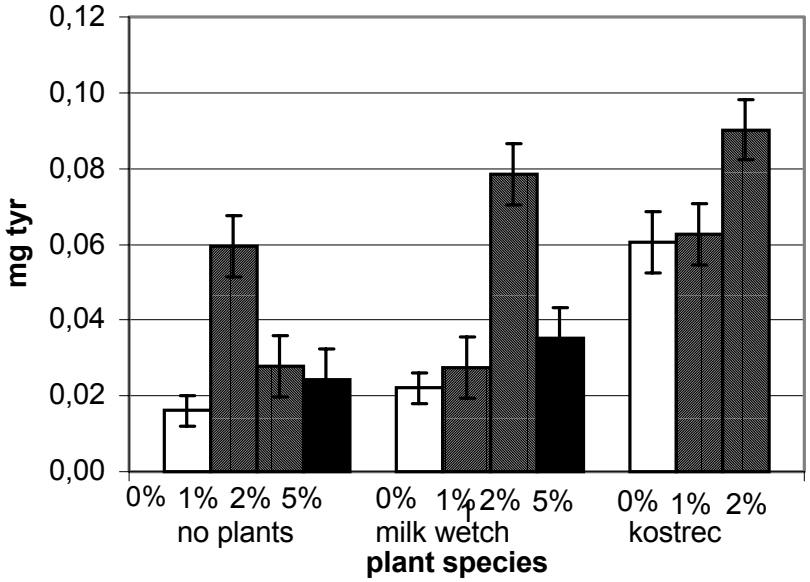
The dehydrogenase soil activity was maximal in a variant without plants at 1 % and 5 % pollution - 1,4*10-2 mg TPF/g dry soil and 1,1*10-2 mg TPF/g dry soil accordingly. Parameters of that activity under plants did not vary and they were lower of the corresponding parameters of unplanted soil (Tab. 3).

Table 3. Dehydrogenase activity of vegetated and unvegetated soil

	Dehydrogenase activity, mg TPF/g dry soil			
	0 %	1 %	2 %	5 %
No plants	0,00526	0,01407	0,00526	0,01169
Kostrec	0,00308	0,003461	0,003077	-
Milk vetch	0,0046	0,0056	0,0063	0,0046

Protease activity of unplanted soil was lower at 2.0-2.5 time that of a root zone soil at all levels of kerosene pollution. Plant cultivation rendered positive influence on protease activity of soil (Fig. 3). It achieved the maximal value at 1 % pollution level for kostrec (6,3*10-2 mg tyr/g soil) and 2 % for milk vetch (4,8*10-2 mg tyr/g soil). In soil without plants the higher enzyme activity was observed at kerosene 1 % (3,6*10-2 mg tyr/g soil).

Fig.3. Protease activity of vegetated and unvegetated soil



Discussion

The grasses used in this experiment were selected earlier by reserchers of Botany Garden of Kazan State University among the sainfoin (sort Cockerel), timothy (sort Kazan), fescue, clover Early, clover Trio, milk thistle, kostrec, milk vetch and maiz (sort Ross 151) upon to their resistance to toxic impact of kerosene and ability to speed the remediation of kerosene polluted soil.

The increase in number of heterotrophic microorganisms block and specialized hydrocarbonoxidizing bacteria in polluted planted soil in comparison with unplanted soil can be evidence of protective abilities of these grasses on soil microbiocenosis at anthropogenous disturbance. Probably, development of HCN under the plants at all investigated pollution levels can be considered as a stimulating action of these grasses on them at that kind of contamination (Fig. 1). These plants also support a variety of bacteria community of soil at it hydrocarbon pollution (Tab. 2).

As to the enzyme activity in leached chernozem, it was show that the tendency of protease activity has the same character as the tendency of bacterial amount. The highest protease activity was detected in planted soil. Dehydrogenase activity was higher in unplanted soil, whereas in planed soil it changed insignificantly.

So, the data presented above show that these plants could be a substantial factor for acceleration of remediation of kerosin polluted soil.

Conclusions

Kostrec and milk vetch vegetation has resulted to significant increase of investigated microorganisms

groups number and protease activity of leached chernozem of Tatarstan Republic at all levels of pollution.

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