PLANT RESISTANCE AND PHYTOREMEDIATION IN SOIL CONTAMINANTED WITH HYDROCARBONS [1]

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Abstract
The phytotoxicity of leached chernozem, contaminated with kerosene (150-210ºC; 1, 2, 3, 5, 10, 15%) was estimated in laboratory scale for 13 fodder and wild plant species: 1) 2 kinds of alfalfa (Medicago x-varia), 2) amaranth (Amaranthus retroflexus), 3) sainfoin (Onobrychis viciifolia), 4) goat's-rue (Galega orientalis), 5) 2 kinds of clover (Trifolium pratense), 6) phacelia (Phacelia tanacetifolia), 7) fescue (Festuca pratensis), 8) timothy (Phleum pratense), 9) sedge (Carex vesicaria), 10) rastoropsha (Silybum marianum), 11) kostrez (Bromus inermis) in comparison with maize (Zea mays) and oats (Avena sativa) - the best in our previous study. The germination of seeds for most species at 1% kerosene was not significantly inhibited (80-100%). The depression of germination leguminous seeds was lesser then of cereal ones. Accumulation of above-ground biomass of 15-day seedlings was measured for species 3, 4, 5 (both kinds), 7, 8, 10, 11 in comparison with maize at 1, 2, 5% kerosene in soil, and also - the residual kerosene contents in leached chernozem by gas-liquid chromatography method. Sainfoin showed the least biomass depression in all variants. Effect of phytoremediation was established at 1 - 5% kerosene for sainfoin: residual hydrocarbon concentrations decreased in vegetated soils in comparison with unplanted one.

Introduction
Presently for remediation of soils contaminated by oil and petroleum products in Tatarstan agrotechnical and microbiological methods are mainly used **[4,5,8]**. However it is known that one of the promising cost and environment effective approach for remediation of soils contaminated with less-water-soluble materials, such as petroleum hydrocarbons (PHC) is the application of plants **[9,10]**. PHC could toxically affect plants and change soil conditions. Such negative phenomena may be the results of their effect on plant nutrition conditions (so, PHC can inhibit the activity of aborigine soil microorganisms and therefore decrease of nitrogen availability for plants) or the direct plants intoxication **[1,6,7,8]**.
But some plants successfully resist the soil contamination by PHC. Not only they adapt to unfavorable conditions but they also transform the soil around the roots in such a way that the soil becomes suitable for growing of less resistant crops. Plants may directly (by overground biomass and/or roots uptake) or indirectly (by stimulation of biodegradation processes of PHC in the rizosphere zone) influence the dissipation of contaminants in soils **[1,3,9,11,12]**. It was shown that the influence of residual PHC concentrations in contaminated soils on seed germination and plants growth essentially vary on plants sort and PHC "gravity" (API). The experimental investigations of the use of plants for soil remediation in Tatarstan are now still under development **[2,4,13]**. Therefore it is difficult to estimate the final criterions of soil clearing because of failure of available date on PHC toxicity for plants.

The objective of study was the comparison of resistance and remediation ability of different fodder and wild plant species growing in soil contaminated by petroleum hydrocarbons. The following basic tasks were solved:
1. The evaluation of phytotoxicity of soil, contaminated with petroleum hydrocarbons, for traditional and non- traditional plant species and sorts.
2. The study of the effect of different concentrations of petroleum hydrocarbons in soil onto the seed germination.
3. The study of the effect of different concentrations of petroleum hydrocarbons in soil onto the plant above-ground and root biomass accumulation.
4. The research of phytoremediation effect in soil contaminated with petroleum hydrocarbons.

There were investigated 17 traditional and non- traditional for Russia plant species and sorts cultivated in Tatarstan for fodder green biomass purposes and also some wild plant species - in comparison with maize and oats - plant species most resistant in our previous studies to soil contamination by PHC **[2,13]**. Among them were 7 annuals plants: monocotyledones - maize *(Zea mays L.)*, sorts *"Katerina" and "Ross-151"*; oats *(Avena sativa L.)*, sort *"Los-3"* and panic *(Setaria Beauv.),* wild – and dicotyledones - amaranth *(Amaranthus retroflexus L.),* wild; rastoropsha *(Silybum marianum L.),* wild and phacelia *(Phacelia tanacetifolia Benth.)*, sort *"Ryazanskaya"* – and 10 perennials plants: monocotyledones - fescue *(Festuca pratensis Huds)*, sort *"Kazanskaya"*; kostrez *(Bromopsis inermis*

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Leys), sort *"Kazansky"*; timothy *(Phleum pratense L.)*, sort *"Kazansky"* and sedge *(Carex vesicaria L.),* wild – and dicotyledones (leguminous) - alfalfa *(Medicago x-varia Martyn.)*, sorts *"Muslima"* and *"Aislu"*; clover *(Trifolium pratense L.)*, sorts *"Ranny-2"* and *"Trio"*; goat's-rue *(Galega orientalis Lam.)*, sort *"Gale"* and sainfoin *(Onobrychis viciifolia Scop.)*, sort *"Petushok"*.

Methodology

Two series of experiments were conducted: (a) evaluation of seed germination in soil - in laboratory scale (*Exp.1*) and in 200 g planting pots (*Exp.2-4*) and (b) plant seedlings growing during 15-18 days in vegetation vessels – by artificial lighting – (*Exp.2,3*) and natural lighting – (*Exp.4*). Further the analysis of residual PHC contents in soil was made: extragent CCl_4; gas-liquid chromatograph Biochrom-1 with flame ionization detector; Inerton AW-HMDS + 5% SE-30, gas-carrier H_2, the flow rate 30 ml/min, temperature of column 70-170^oC and of sampler - 220^oC.

Soil characteristics.

In experiments leached chernozem was used (soil typical for regions with PHC contamination in Tatarstan) having pH (KCl) 5,9; humus contents 5,0%; texture: 22% sand, 40% silt and 38% clay; N (alkaly hydrolyzed) - 122 mg/kg; P_2O_5 and K_2O (available) – 189 and 251 mg/kg.

Type and doses of hydrocarbons investigated.

Preliminary soil contamination was conducted during 3 weeks by kerosene (KS) in *Exp.1,3,4* and diesel fuel (DF) in *Exp.2*. KS (fraction 150-200^oC) was applied in *Exp.1* as 10000, 20000, 30000, 50000, 100000 and 150000 mg KS /kg soil; in *Exp.3:* 10000 and 20000 mg KS /kg soil and in *Exp.4:* 10000, 20000, and 50000 mg KS /kg soil. The concentrations of DF in *Exp.2* were 10000 and 20000 mg DF /kg soil.

Results

1. The effect of different concentrations of PHC in soil onto the seeds germination.

1) The seed germination in soil contaminated by kerosene, laboratory scale (Exp.1) **(Fig. 1)**.

The following ranks of the decrease of relative seed germination capacity were noted for the plants: at 1-3% KS: maize *"Katerina"* and *"Ross"* > sedge > clover *"Trio"* = clover *"Ranny"* > oats, phacelia and sainfoin > alfalfa *"Aislu"* and *"Muslima"* > timothy and goat's-rue > rastoropsha > fescue > kostrez; at 5-10% KS: alfalfa *"Aislu"* > phacelia and clover *"Ranny"* = clover *"Trio"* > goat's-rue = sainfoin = alfalfa *"Muslima"* > maize *"Ross"* > fescue = timothy > sedge = oats = rastoropsha. The separate effect was found for amaranth: its germination capacity was sensitive to contamination, but was not so low and nearly constant at 1-15% KS. For the majority of studied plants the reliable reduction of germination rates with increasing of soil contamination level was observed.

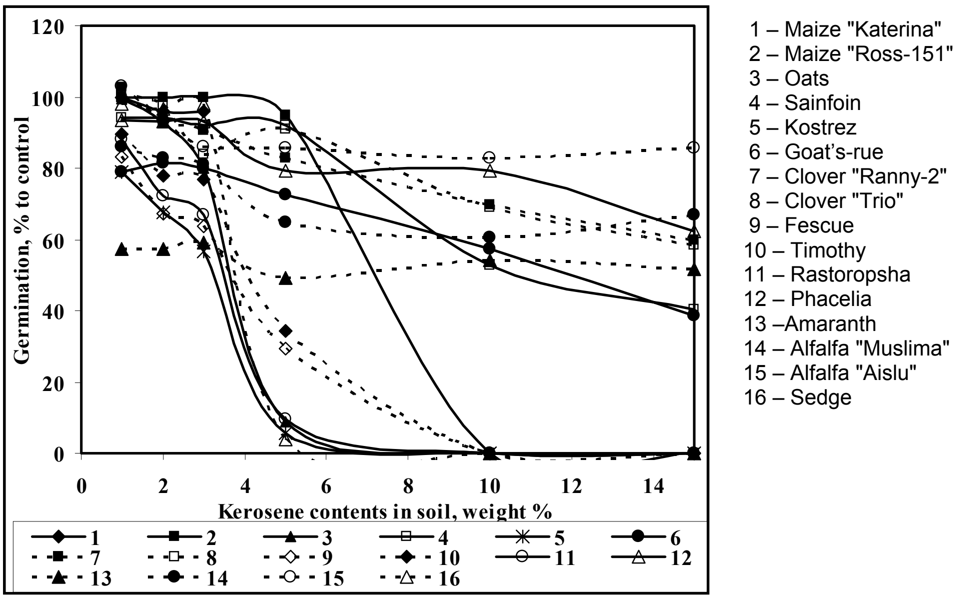
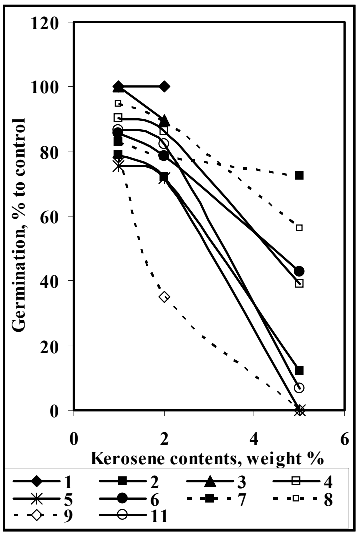


Fig.1. The seed germination in soil contaminated by kerosene (exp.1).



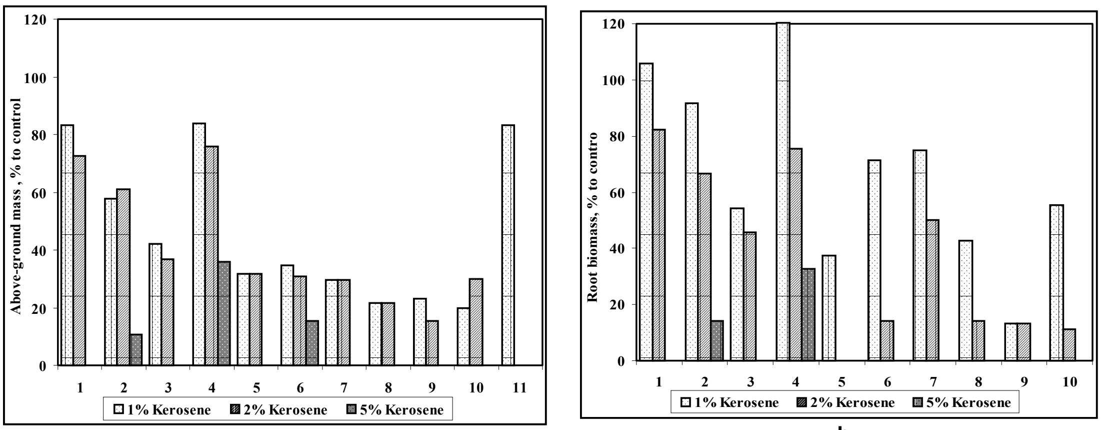
1 – Maize "Katerina"
2 – Maize "Ross-151"
3 – Oats
4 – Sainfoin
5 – Kostrez
6 – Goat's-rue
7 – Clover "Ranny-2"
8 – Clover "Trio"
9 – Fescue
11 – Rastoropsha

Fig. 2. The seed germination In vegetation pots with soil contaminated by kerosene (Exp. 4).

2) The seed germination in vegetation pots in soil contaminated by kerosene (Exp. 3,4) **(Fig. 2)**.
The results of experiments in laboratory scale and in vegetation pots were close, but the seed germination in the second ones for the majority of plants was lower.

3) The seed germination in vegetation pots in soil contaminated by diesel fuel (Exp.2).
For oats the seed germination in soil contaminated by diesel fuel was higher in comparison with kerosene – probably because of the highness of less phytotoxic heavy PHC fractions in diesel fuel.

2. The effect of different concentrations of PHC in soil onto the plant above-ground and root biomass accumulation.
1) The above-ground plant biomass accumulation at artificial lighting in soil contaminated by kerosene (Exp. 3).
The least depression of dry biomass at 1% KS was found for sainfoin no more than 10% and maize "Ross" (43-62% at 1-2% KS). For other plant species it was 65-75% at 1% KS and 80-90% at 2% KS.
2) The above-ground plant biomass accumulation at artificial lighting in soil contaminated by diesel fuel (Exp.2).
The comparison of these data with results of experiments with kerosene pointed on a smaller toxicity of diesel fuel.
3) The above-ground plant biomass accumulation at natural lighting in soil contaminated by kerosene (Exp.4) **(Fig. 3 a)**.
Here relative findings were close to artificial lighting results, but at natural lighting the depression was lesser.



a b

Fig. 3. The effect of different concentrations of kerosene in soil onto the plant above-ground (a) and root (b) biomass accumulation.
1 – Maize "Katerina" 2 – Maize "Ross-151" 3 – Oats 4 – Sainfoin 5 – Kostrez 6 – Goat's-rue 7 – Clover "Ranny-2" 8 – Clover "Trio" 9 – Fescue 10 – Timothy 11 – Rastoropsha

4) The comparison of effects of above-ground and root plant biomass accumulation at natural lighting in soil contaminated by kerosene (Exp.4) **(Fig. 3 a, b)**.
For maize "Katerina" and sainfoin the depression of above-ground biomass and the stimulation of root biomass simultaneously were noted at 1% KS. For the other plants the depression both above-ground and root biomass was observed.

3. The phytoremediation effect in soil contaminated with kerosene.
At cultivation of plants in leached chernozem under the natural lighting in soil contaminated by kerosene *(Exp.4)* effect of phytoremediation was established at 1 - 5% kerosene for sainfoin: residual hydrocarbon concentrations decreased in vegetated soils in comparison with unplanted one.
Generally we have not found the overlap of tolerance of plants (seed germination and biomass accumulation) and their phytoremediation capacity.

Conclusions
1. The plants which had on soil contaminated with kerosene the depression less than 30% were: at 1% KS – everything except amaranth; at 2% KS - except kostrez, fescue, amaranth; at 3%KS - except kostrez, fescue, rastoropsha, amaranth; at 5% KS - both alfalfas, clovers, maize *"Ross"*, sainfoin, goat's-rue, phacelia; at 10% KS - both alfalfas, clovers, phacelia; at 15% KS - both alfalfas. Hereby at high KS contents (5-15% KS) the seed germination depression for leguminous seeds was lesser then for cereal ones. The oats seed germination on soil contaminated by diesel fuel was higher than by kerosene.
2. The kerosene contents more than 1% KS suppressed the plant development for the majority of species. The more resistant at 1% KS and 2% KS were sainfoin and both sorts of maize. The majority of plants were less oppressed by diesel fuel than by kerosene.
3. Effect of phytoremediation was established at 1 - 5% kerosene for sainfoin: residual hydrocarbon concentrations decreased in vegetated soils in comparison with unplanted one. We have not found the overlap of tolerance of plants (seed germination and biomass accumulation) and their phytoremediation capacity.

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