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Potential of three plant species for phytoremediation of oil-contaminated soils in northern conditions

© 2023. D. V. Tarabukin ORCID: 0000-0001-8572-4902,
Institute of Biology of Komi Science Centre of the Ural Branch
of the Russian Academy of Sciences,
28, Kommunisticheskaya St., Syktyvkar, Russia, 167982,
e-mail: dim1822@yandex.ru

The aim of this work was to assess the potential of three plant species (*Brassica juncea* L., *Trifolium repens* L., *Agrostis stolonifera* L.) for phytoremediation of oil-contaminated soils. The germination time of seeds and their survival rate after sowing into the model oil-contaminated soil were found. The influence of the procedure of encapsulation of seeds in alginate gel was assessed. Green manure plants seeds are preferably added after 30 days of self-cleaning of the soil due to the absence of a continuous oil film and restoration of air exchange. *Brassica juncea* L. was found to have the shortest germination time, at the same time the encapsulation of seeds in a polymeric complex increased the time for the development of vegetative organs. The encapsulation of *T. repens* L. and *A. stolonifera* L. seeds in alginate gels, on the contrary, reduced the germination time. *A. stolonifera* was also found to be more drought-resistant. It was concluded that the use of an auxiliary gel coat is most effective for small plant seeds and increases the survival rate on oil-contaminated soil.

The contribution of the green manure plants root rhizosphere to the processes of biochemical transformation of oil was assessed by comparing such diagnostic indicators of the state of the soil as the activity of dehydrogenase and urease. The dehydrogenase activity in all versions of the experiment was higher than in the oil-free soil. Moreover, for *T. repens* and *A. stolonifera* the values were 20–25% higher than in the contaminated soil without plants. The urease activity also increased, however, in the experiments with plants it was less than in the control experiment with the oil. In general, the selected plants can act as green manures for the accelerated formation of phytomass and restoration of species diversity on the recultivation territories.

Keywords: green manure plants, petroleum pollution, alginate beads, encapsulation, enzymatic diagnostics.

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Потенциал трёх видов растений для фиторемедиации нефтезагрязнённых почв в условиях Севера

© 2023. Д. В. Тарабукин, к. б. н., н. с.,
Институт биологии Коми научного центра
Уральского отделения Российской академии наук,
167982, Россия, г. Сыктывкар, ул. Коммунистическая, д. 28,
e-mail: dim1822@yandex.ru

Целью работы была оценка потенциала трёх видов растений (*Brassica juncea* L., *Trifolium repens* L. и *Agrostis stolonifera* L.) для фиторемедиации нефтезагрязнённых почв. На модельной нефтезагрязнённой почве было определено время всхожести семян, выживаемость, оценено влияние процедуры включения семян в альгинатный гель. Выявлено, что вспомогательная гелевая оболочка наиболее эффективна для мелких семян растений, она повышает их выживаемость на нефтезагрязнённой почве. Вклад прикорневой ризосферы растений сидератов в процессы биохимической трансформации нефти был оценён через сравнение таких диагностических показателей состояния почвы, как активность дегидрогеназы и уреазы. В целом, выбранные растения могут выступать в качестве сидератов для ускоренного формирования фитомассы и восстановления видового разнообразия на участках рекультивации.

Ключевые слова: сидераты, нефтяное загрязнение, альгинатные шарики, инкапсуляция, ферментативная диагностика.

Phytoremediation as a method of restoration of oil-contaminated soils is widely used in different stages of recultivation [1–3]. The use of oil-resistant plants enables to create an environment in the soil for effective decomposition of oil products through the formation of near-root rhizosphere of hydrocarbon oxidizing microorganisms. For example, the work [4] shows the survival rate and, as a result, potential effectiveness of *Cynodon dactylon* L., *Eleusine indica* L., *Alternanthera sessilis* L. for phytoremediation. Moreover, *E. indica* and *A. sessilis*, besides rhizosphere biodegradation, were able to accumulate oil products in vegetative organs. *Bassia scoparia* L. is suggested for reclamation of arid lands in case of oil contamination within 2–3% [5]. It was found to have a good acclimation rate and also the ability to decrease the concentration of both hydrocarbons and sulphur in the soil. Another work [6] assessed the potential of *Crotalaria pallida* L. at different concentrations of crude oil. The plant turned out to be effective for phytoremediation up to 10 percent oil concentration, so it is recommended for preparation of the soil for further sowing of agricultural crops. The work [7] compared the efficiency of bioremediation of the crude oil-contaminated soil using *Testuca arundinacea* L. as compared to bioaugmentation and self-purification. It was found that bioaugmentation is more effective in the initial purification stages up to 30 days. Phytoremediation at the same time turned out to be more effective after 90 days of the experiment. Therefore, using *T. arundinacea* jointly with bioaugmentation turned out to be the most efficient approach to reclamation of oil-contaminated salinized soils. The role of rhizospheric fungi in oil decomposition is also worth noting. For example, the fungi of the genus *Fusarium* [8] turned out to be resistant to a ten percent (by mass) concentration of oil in the soil. The model experiments showed that the strains of the fungus *Fusarium* sp. played the main role in bioremediation of oil-contaminated soils, but the roots of the plants *Amaranthus retroflexus* L. reinforced this process.

Purpose of this work is to assess the potential of three plant species (*Brassica juncea* L., *Trifolium repens* L., *Agrostis stolonifera* L.) for phytoremediation of oil-contaminated soils.

Materials and Methods

The objects of study were soils selected in the vicinity of the Syktyvkar city and plants. The seeds of the following types of plants were used in the work: *Brassica juncea* L., *Trifolium repens* L. and *Agrostis stolonifera* L. The model soil for recultivation was prepared from the upper layers of meadow and forest soils of 10 cm in depth. The meadow soil was sampled from the former agrocenosis and was characterized by a low content of humus. The forest soil was selected in spruce forests with green moss. Soils are classified as Haplic Albeluvisols [9]. All the soil samples were selected in mid-April, the vegetative organs of the local flora were not yet developed. The soil acidity (pH) was 5.8 for the meadow soil and 6.3 for the forest soil.

The first experiment consisted of the assessment of the potential of self-cleaning of the meadow and forest soils after introduction of oil. Before the beginning of the experiment the soil samples were ground and sifted through a sieve with 5 mm mesh size. Then the soil samples with the mass of 100 g and 10% humidity were placed into vegetation vessels with 8 g of oil and 0.1 g of mineral fertilizer (ammonium nitrogen – 10%; total phosphates – 25%; potassium in terms of K_2O – 25%). Soil samples in the vessels were placed in conditions close to natural for a period of 90 days. The level of humidity maintained in the vessels was 20–30%. Dynamic changes of total petroleum hydrocarbons (TPH) in the soil were assessed after 30, 60 and 90 days.

Before the beginning of the experiment with plants, the meadow and forest soils were mixed with the ratio 50/50. Then the soil samples with the mass of 100 g and 10% humidity were placed into vegetation vessels, adding 8 g of oil. The vessels with the oil were held outdoors for 30 days before the introduction of the seeds. Then the soil in the vessels was slightly mellowed, the plant seeds were introduced together with 0.1 g of mineral fertilizer. The soil samples without seeds but with fertilizer were used as a control. Within a month the germination and survival rates were assessed, and after 30 days, the changes in dehydrogenase and urease activity. During the observation period significant temperature variations were noted, within the range from 0 °C at night to 28 °C by day. The level of humidity maintained in the vessels was 20–30%. However, twice in the course of the experiment water was not added for 2 days, modelling drought. All variants of the experiment were laid in three repetitions.

The polymeric environment to improve the safety of seeds was formed from a biocompatible polymer. To form gel beads with seeds 2% sodium alginate solution was prepared, which contained 0.1% of polyacrylic acid. The seeds of

every type of plant were suspended in polymeric solutions, then measured out drop by drop into 1% $CaCl_2$. Then the gel beads with seeds were taken out, washed with tap water and, before being planted into the soil, were kept for 3 days in 0.1 M potassium phosphate buffer (pH 6.0). Before sowing, untreated seeds were held for 12 hours in the same buffer solution.

The primary biochemical assessment of the state of soils in the process of phytoremediation was carried out by measuring the activities of dehydrogenase and urease. Dehydrogenase activity was determined using the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) method [10]. A sample of 1 g soil was transferred into test tube with stopper. 10 cm^3 of 0.5% TTC was added. The mixture was mixed on a vortex and incubated at 30 °C. After 24 h, the triphenylformazan, a product from the reduction of TTC, was extracted by adding 10 cm^3 acetone and shaken for 1 min. The sample was collected in a volumetric flask. The tube was washed with acetone until the red color disappeared. The filtrate was then diluted with additional acetone to a final volume of 50 cm^3. The color intensity was measured at 485 nm with acetone as a blank. One unit of dehydrogenase activity represented the number of enzymes in 1 g of the air-dried soil that form 1 μg of triphenylformazan per 24 hours in the presence of 0.5% triphenyltetrazolium chloride solution.

The urease activity, content of microscopic fungi and residual TPH content were determined according to the methods described in the work [11]. For all methods, the Microsoft Excel statistical analysis package was used. In particular, "one-way ANOVA" was used.

Results and Discussion

This experiment revealed that during the first 30 days in natural conditions the most intensive TPH decomposition occurs in meadow soil, with its content decreasing by 35±5% (Fig. 1). In forest soil, the content decreases by 25±5%. On days 60 and 90, the process of TPH decomposition in meadow and forest soils stops. Therefore, after 30 days the potential for self-purification was exhausted, this is why additional measures for acceleration of the processes of biodegradation of oil products in the soil are necessary.

After 30 days outdoors, thanks to abiotic factors as well as self-regeneration processes, the oil film disappeared, and oil remains in the soil were gathered into local accumulations. The restoration of air change helped the survival of the seeds in contaminated soil (Fig. 2). As part of this work, the effectiveness of the procedure of encapsulation of seeds in alginate gel for better adaptation in the oil-contaminated soil was also assessed.

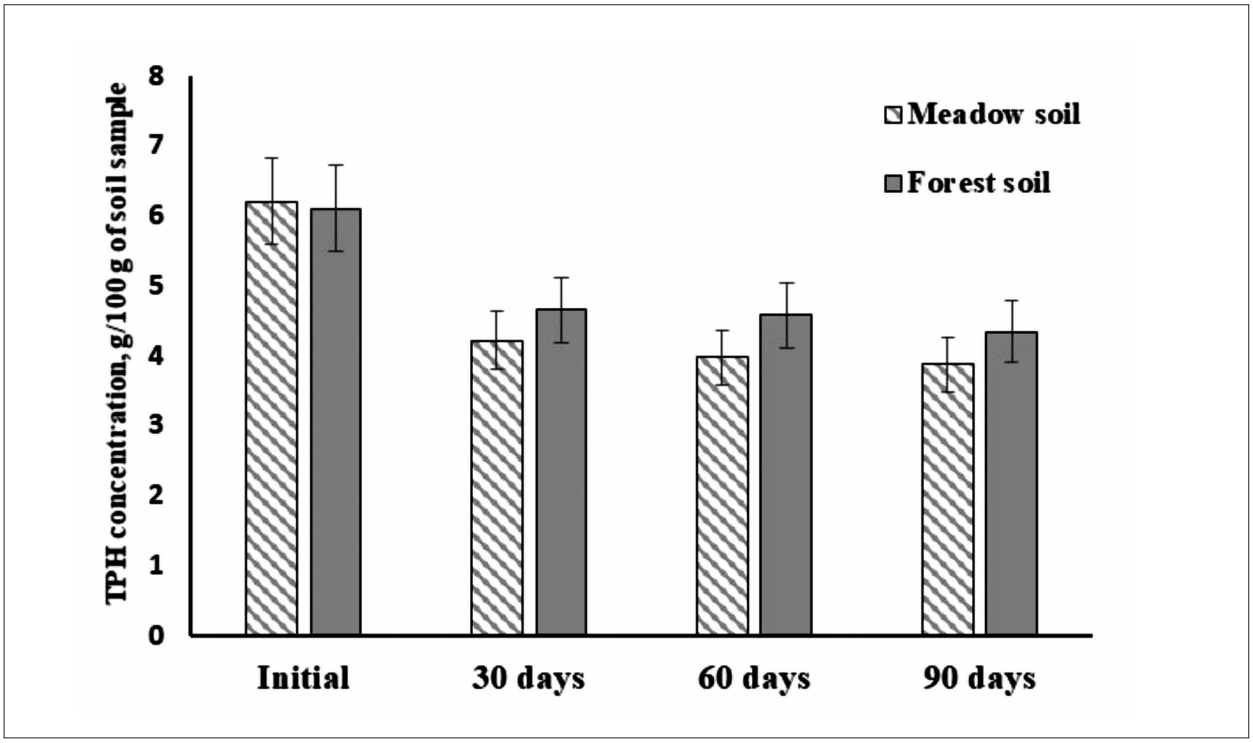

Fig. 1. Dynamics of TPH concentration changes in soil samples during *ex situ* remediation. The "Initial point" represents the data of TPH concentration after introduction petroleum to soil samples

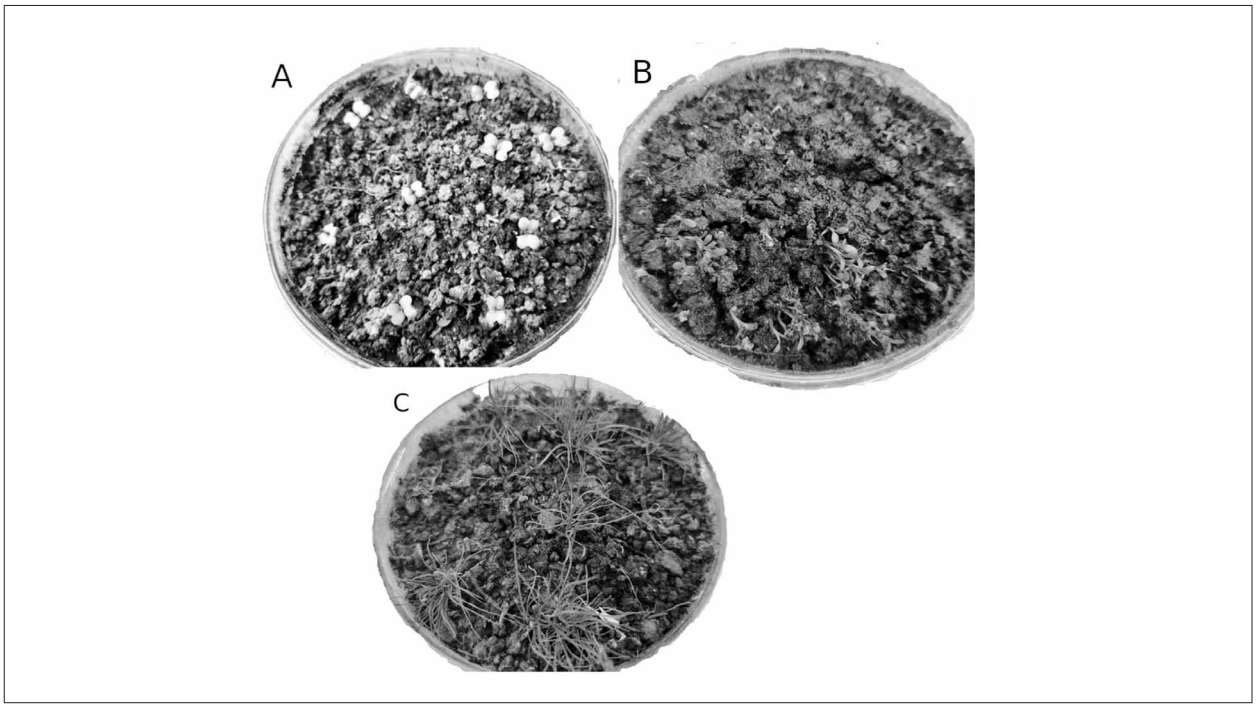

Fig. 2. Fresh sprouts of model plants on oil-contaminated soils in 20 days after sowing (A – *Brassica juncea*; B – *Trifolium repens*; C – *Agrostis stolonifera*; experiment versions with the insertion of seeds into alginate gel)

Previous studies show that the use of alginate gels for plant growing is intended to ensure the safety of sprouts and spores as well as better survival rate during reproduction [12, 13]. For example, the tips of the *Phyllanthus amarus* L. sprouts encapsulated in an alginate cover are able to develop a complete plant [14]. Immobilization of the *Hordeum vulgare* L. seeds paired with phosphataze helped to increase the bioavailability of phosphorus from the soil [15]. Encapsulating the fern seeds *Pteridium aquilinum* var. *latiusculum* (Desv.) Underw. ex A. Heller in alginate gel is viewed as an effective technology for commercial use [16].

It is noted that *B. juncea* fairly easily develops a complete plant even without insertion into a polymeric matrix (Table). The large seeds of *B. juncea* have a sufficient supply of nutrient substances, this is why they do not require a gel cover as an auxiliary instrument. At the same time the plant turned up to be more sensitive to drought. Most probably the conditions of the experiment did not allow *B. juncea* to form a full root system. For *T. repens* and *A. stolonifera* the procedure of encapsulation in alginate gel more clearly influences the germination time (Table). We also noted the significantly higher survival rate of the seeds of *A. stolonifera* as compared to sowing directly onto the surface of the oil-contaminated soil. Moreover, the plant achieved a much bigger biomass as compared to the control after encapsulation in polymer. *T. repens* grew in a similar way in all experiments and formed sufficiently pronounced vegetative parts. The development of primary turf layer was found in all versions. Therefore, large seeds of green manure plants are resistant to residual oil products and are able to grow on their own in case of sufficient humidity and absence of the oil film. For small seeds, the alginate cover is an additional factor of the survival rate. At the same time, additional experiments are needed to discover the mechanisms of biodegradation of oil products both through the near-root rhizosphere and through assimilation by vegetative organs.

The determination of the activities of the soil ferments is a convenient instrument for the analysis of the state of contaminated soils in the process of bioremediation [17, 18]. The assessment of their functions in supporting the quality of the soil, the balance of carbon and nitrogen, the nutrient utilization combined with other methods allows making a general judgment on the effectiveness of regeneration of disturbed soils [19]. One of the most significant indicators of fermentative activity of the oil-contaminated soil is the activity of dehydrogenase. For example, [20] the activity of dehydrogenase significantly decreased after the introduction of the polyaromatic hydrocarbon pyrene into model soil and noticeably increased after the introduction of *Scirpus triqueter* L. On the other

Table

Some parameters of the soil in the process of phytoremediation

Conditions of recultivation	Emergence of seedlings (days)	Dehydrogenase activity after 30 days, units/g of soil	Urease activity after 30 days, units/g of soil	Fungal mycelium content after 30 days, $\times 10^3$ g of soil
Brassica juncea (control)	5–6	60±6	125±16	6±1
B. juncea + oil	6–7	85±7	271±25	7±1
B. juncea (alginate) + oil	8–9	88±8	241±27	7±1
Trifolium repens (control)	8–9	73±5	98±10	3±1
T. repens + oil	10–11	109±8	285±24	7±1
T. repens (alginate) + oil	6–7	93±9	256±30	8±1
Agrostis stolonifera (control)	9–10	62±5	105±12	6±1
A. stolonifera + oil	20 (small shoots)	86±7	262±22	4±1
A. stolonifera (alginate) + oil	6–7	102±8	275±31	3±1
Oil without plants	–	82±6	376±25	6±1
Control without oil	–	48±4	107±9	4±1

*Note: * – initial dehydrogenase activity of model soil 53±5, units/g of soil, urease 112±8 units/g of soil.*

hand, the presence of up to 3% of straight chain hydrocarbons in the soil increased the dehydrogenase and urease activities [21]. In our experiment the analysis showed increased activities for all types of oil-contaminated soils. And the presence of oil influenced the increase of dehydrogenase activity the most. For *T. repens* and *A. stolonifera* the values were 20–25% higher than in the contaminated soil without plants. At the same time urease activity was maximum for the control oil-contaminated soil. So, viable green manure plants in the oil-contaminated model soil form the rhizosphere, which can subsequently make an additional contribution to the soil self-cleaning processes.

The calculation of content of fungal mycelium did not show any notable difference for different versions of the experiment. It can be assumed that the experiment conditions were not conducive to the development of rhizospheric fungi, and a significant role in this was played by sudden temperature drops as well as drought modelling. Therefore, for a more nuanced picture additional long-term studies are necessary, which will enable to realize the full potential of phytoremediation through model plants as well as near-root microbiota.

Conclusion

Meadow and forest soils are capable of intensive self-purification from oil products within the first 30 days. It is mostly the low molecular weight oil components that are disposed of. This is due to both abiotic factors and the activity of soil microorganisms. Then the process almost stops, which requires creation of additional conditions for the intensification of the processes of oil biodegradation. It is suitable to sow seeds after 30 days of self-purification due to absence of the oil film and restoration of air change in the soil. It was found that *Brassica juncea* L., *Trifolium repens* L. and *Agrostis stolonifera* L. are able to produce vegetative organs on the model oil-contaminated soil after sowing the seeds. The technological process of encapsulation of plant seeds in gels is sufficiently effective and can be used to increase the survival and germination rates in the oil-contaminated soil, accelerated formation of phytomass and restoration of species diversity on the recultivation territories. Even without strict microbiological control in the soil, biochemical indicators show a strong reaction to oil and, as a result, activation of self.

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