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Biological remediation of the petroleum and diesel contaminated soil with earthworms *Eisenia fetida*

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Abstract

The study was performed of the bioremediation efficiency of the soil contaminated with petroleum and diesel with the help of earthworms *E. fetida* in the presence of bacteria *Pseudomonas*, nitrogen fixing bacteria *Azotobacter* and *Clostridium*, yeasts *Saccharomyces*, fungi *Aspergillus* and *Penicillium*, as well as Actinomycetales, all being components of biopreparation Baykal-EM. It was demonstrated that in petroleum-contaminated soil with 20-60 g/kg of petroleum, the content of hydrocarbons decreases by 99% after 22 weeks in the presence of worms and biopreparation. In diesel-contaminated soil with diesel concentration of 40 g/kg in the presence of earthworms *E. fetida* and microbiological preparation, 30% of the earthworm species died after 14 days because of the toxic impact of the diesel fuel.

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1. Introduction

Currently, various methods for soil recultivation from oil and petrochemical spills have been developed and are widely used. The more perspective method for oil-contaminated soil recultivation is the method of bioremediation based on the application of microorganisms capable of hydrocarbons utilization in their vital activity [1]. This method is efficient at a concentration of petrochemicals in soil below 20 g/kg. At higher hydrocarbons concentrations, the bacteria population decreases and the soil bioremediation does not occur. A higher efficiency of soil bioremediation can be achieved by the introduction of earthworms into contaminated soil, i.e. by the

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vermiremediation method. Earthworms facilitate the removal of contaminants from soil and change the physical and chemical properties of soil by mixing it with organic substance and improving soil aeration, thus making contaminants accessible to microorganisms [2].

The earthworms are known to survive at high concentrations of contaminants. For instance, *E. fetida* survive in soil contaminated by 3500 mg/kg of oil [3], although the survivability depends on the duration of the exposition and metabolism [4, 5].

Investigations demonstrated that introduction of organic substance made a positive effect on earthworms survivability in contaminated soil, but the decrease in earthworms weight was also noted. Tejada and Masciandaro noticed the decrease of *E. fetida* weight by 38.46% after addition of 50 mg/kg benzopyrene to the soil, and after addition of dung the decrease of biomass was 20% [6]. Microorganisms can destroy polycyclic aromatic hydrocarbons in soils with earthworms [7-12]. Some scientists noted the decrease in polycyclic aromatic hydrocarbons concentration in the presence of earthworms *E. fetida* and *L. rubellus* [13]. It was demonstrated that polycyclic aromatic hydrocarbons bioremediation rates differ greatly and depend not only on polycyclic aromatic hydrocarbons composition, but mainly on the activity of soil microbial communities [5].

Fernández M.D. et al. reported on diesel-contaminated soil remediation with the help of plants such as reed fescue (*Festuca arundinacea*) and red clover (*Trifolium pratense*) and earthworms *E. fetida* for 90 days. They noticed that the plants had no effect on diesel fuel removal in spite of microbial biomass stimulation. On the other hand, in *E. fetida* cultivation 43% of diesel fuel were removed in surface soils and 52% were removed in deep soils whereas in the experiments without worms the decrease of diesel fuel concentrations was 30% and 34%, correspondingly. Nevertheless, it was noted that all worms died in diesel-contaminated soil after 90 days. It was explained by the absence of nutrients for the earthworms in the course of the experiment [14].

Jingchun Tang and Min Wang et al. studied the effect of various petroleum concentrations from 0.5 to 5 % on survival of *E. fetida* for 7 days. All earthworms died after 7 days at petroleum concentration higher than 3%. At petroleum concentration of 2%, the mortality was 90% after 7 days. At petroleum concentration of 0.5%, the survival of the earthworms did not change after a week [15]. The same results were obtained by Hanna and Weaver [16]. Geissen et al. noticed the increase in *E. fetida* mortality up to 70% - 90% in soils contaminated by 2% of petroleum [3]. Shin et al. mentioned earthworm *E. fetida* weight loss at diesel fuel concentrations higher than 1%, while at concentrations of 1.5% the decrease in the earthworms survival was 40% [17]. Kozlov experiments showed that when the concentration of gasoline in soil was 20 g/kg in the first day, the mortality of *E. fetida* was 90%, and on the seventh - 100%. When the concentration of diesel fuel was 20 g/kg in the first day, the mortality of *E. fetida* was 94 %, and on the seventh -100% [18].

Some investigations demonstrated that addition of organic substance as a nutrient had a positive effect on the earthworms survival in polycyclic aromatic hydrocarbons-contaminated soils. Conteras-Ramos et al. informed that after addition of sewage sludge to the soil contaminated by the mixture of 100 mg/kg phenanthrene, 500 mg/kg anthracene and 50 mg/kg benzopyrene, and during the cultivation of *E. fetida*, the earthworms survival was 93%, and their weight increased by 28% after 70 days. At *E. fetida* cultivation without organic substance addition, the earthworms weight decreased by 79% and the survival did to 60% [19].

Organic substance is of importance in the earthworms cultivation, providing nutrients and thus stimulating microorganisms activity in earthworms guts and increasing their survival. Sinha et al. discovered that in polycyclic aromatic hydrocarbons-contaminated soils with kitchen waste and cattle dung, 47% of contaminants were removed without earthworm addition and 80% were removed after earthworms *E. fetida* and *E. eugenaic* introduction [20].

Isabella Gandolfi and Matteo Sicolo registered great toxic effect of diesel light hydrocarbons with 2% concentration on earthworms, but compost introduction enhanced hydrocarbons remediation and earthworms survival. Compost introduction to diesel-contaminated soil resulted in diesel fuel remediation increase by four rings of PAHs and soil toxicity decrease, its effect on soil genotoxicity lowering [21].

Therefore, the undertaken literature analysis demonstrated that the usage of earthworms *E. fetida* for bioremediation of soils contaminated by petroleum and diesel fuel with hydrocarbons content of no more than 2 g/kg is an efficient technique. At higher concentrations of petroleum and diesel fuel, the increase in earthworms mortality is noticed. Taking the above mentioned into consideration, the aim of the present research was to study the efficiency of gasoline and diesel fuel-contaminated soil bioremediation with the help of earthworms *E. fetida* in the presence of bacteria *Pseudomonas*, nitrogen fixing bacteria *Azotobacter* and *Clostridium*, yeasts *Saccharomyces*,

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