

Role of EM technologies in solving bioremediation problems with regard to polluted soils

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Abstract. To clean and restore areas with man-made pollution, bioremediation methods are increasingly used. These methods are based on the use of the biochemical potential of microorganisms and are safe for the environment. The article is devoted to the investigation of the effectiveness of the use of a microbial preparation for the remediation of soils contaminated with benzo(a)pyrene. We experimented to test the ability of «Baikal EM1», a microbiological compound, to destroy benzo(a)pyrene in contaminated soils. The compound was found to be efficient at 96.7% in «dangerously» contaminated soils (up to 5 MAC), while less efficient at higher contaminations (38.7 to 52.6% at 15 to 19 MAC). When diluted 1 to 100, the compound was more efficient than in case of dilution 1 to 50. Studies have shown «Baikal EM1» a promising biological compound for further research into the remediation and restoration of polycyclic aromatic hydrocarbon-polluted soils.

1. Introduction

A high degree of the superficial contamination of soils with various harmful substances in industry-affected areas territories makes it necessary to find ways to remediate the soils near industrial centers. This is especially important in Russia, where modern urbanization processes, as a rule, cause a high concentration of contaminants near industrial cities, rural settlements, household plots and suburban areas, where urban citizens join the locals in farming. Our research has shown that eating vegetables grown in such areas may represent a high carcinogenic hazard to the public health [1].

The link between superficial soil quality and the quality of food products grown in technologically contaminated areas is obvious today and proven by many researchers. This is why methods of bioremediation exploiting the biochemical potential of various microorganisms find increasingly wider use, which determines their safety for the environment [2-6].

Currently, various microbial destructors specific to certain types of organic contaminants (oil residue, creosote, bitumen), as well as polycyclic aromatic hydrocarbons (PAH) contamination, are in use [7].

PAHs, with benzo(a)pyrene (B(a)P) as an indicator, are one of the most important classes of hazardous contaminants and belong to the primary environmental toxicants. Aside from hygienic aspects, PAH contamination has a negative environmental effect aspect as well, as it suppresses the soil biota, whereby, for example, dehydrogenase and catalase activity become observable [7]. Being persistent organic pollutants, these substances resist microbial degradation and accumulate in soils, getting adsorbed on soil particles. The danger of B(a)P accumulation in the soil is that it enters the



plants through the soil and enters the human body through the trophic chains. Using bioremediation technologies to remove such highly toxic contaminants from soils, will create a more favorable sanitary and hygienic environment and reduce the environmental risk for public health, especially in rural areas affected by the emissions from petrochemical, metallurgical, chemical and other enterprises

A large number of biocompounds for bioremediation has appeared in the global market recently. As a rule, they are intended to augment petroleum products, and therefore are not applicable to multiple PAHs. Currently, new compounds are being developed that can clean heavily contaminated soils from polynuclear PAHs. Studies have shown [7] the effectiveness of using the innovative compound BAK-PAH. It consists of a consortium of bacterial cultures *Shinella granuli*, *Pseudomonas ginsegsoli*, *Rhodococcus gingshengii*, and is able to clean the soil of polynuclear PAH, to completely destruct B(a)P.

However, these compounds are expensive and are not available to an ordinary buyer in the retail network. Therefore, EM compounds are a promising solutions for removal of various contaminants from soils. The EM technology is based on using unique biological compounds containing a balanced community of microorganisms of various groups.

The Russian microbiological compound of this series, "Baikal EM1", is developed on the basis of microorganisms of Lake Baikal's ecosystem. The main reason for the exceptional versatility of this compound is the very wide range of effect of its constituent microorganisms.

"Baikal EM1" is supplied in large containers as a result cultivating more than 80 species of microorganisms. The collected microorganisms belong to 10 orders, which in turn represent 5 families and include both aerobic and anaerobic varieties. They are created by mixing various groups of natural, non-pathogenic, aerobic, and facultative anaerobic microorganisms naturally occurring in nature, consisting mainly of phototrophic and lactic acid bacteria and yeast. EM cultures live in the natural environment and do not contain genetically modified strains. [8].

Analysis of research and practical results [9-11] shows that using biotechnologies based on Russian EM compounds allows solving various environmental problems. Those include: waste water and sewage treatment; processing of organic waste in a short time (30 to 60 days); elimination of bad odors; disinfection of water in recreational and decorative water reservoirs; reclamation of landfills; bioremediation of oil-contaminated soils.

The purpose of this work was to study the effectiveness of using «Baikal-EM1» for the remediation of B(a)P-contaminated soils contaminated.

2. Materials and methods

Samples of soil for analysis were taken in the area of the Southern Baikal region exposed to aluminum emissions: the village of Olkha 3 km away from Shelekhov, a large industrial center of Irkutsk Oblast.. The city's industry revolves around alloyed metals. The presence of a large number of industrial enterprises causes a high level of air pollution, which inevitably leads to a high level of soil contamination in Shelekhov and nearby rural settlements (villages of Olkha and Baklashi). The research identified an extremely dangerous level of hygienic contamination of agricultural soils with fluorine and B(a)P in a 0.5 to 8 km area around this aluminum-production industrial center [12].

According to the complex anthropogenic impact study for 2009-2011, the sanitary and hygienic situation in the city and its surroundings is "critical" [13]. In 2013, more than 54 thousand people lived under the conditions of increased soil contamination in residential areas.

Soil samples from Olkha were taken in Fall 2015 from a superficial layer (0-10 cm) differently purposed lands (arable land, pasture land) by an envelope method with a one-meter square. Sampling was carried out according to [14,15].

Prior to the experiment, the soil was prepared in accordance with ISO 11268-1, 1993 [16]. The prepared soil was placed in 2-liter polypropylene containers with a 15 cm layer (1 kg of soil). In total, 3 series of contaminated soil samples were prepared: one sample of arable soil, and two samples of meadow (pasture) soil. Baikal EM1 was applied to the first and second soil samples as recommended

by the manufacturer, i.e. with a 1 to 100 dilution (by 1 ml); for the third sample, it was diluted 1 to 1 to 50 (5 ml). The compound was applied once a week.

Two soil samples (arable and pasture) were also prepared as control ones, with adding distilled water without Baikal-EM1 compound.

Sampling from soil samples for B(a)P analysis was carried out in 14, 30 and 60 days according to [14,15,17].

The content of B(a)P mass concentration in soils was determined in accordance with MUK 4.1.1274-03 [18] by HPLC method with fluorimetric detection using liquid chromatograph Agilent 1200 G1322A (USA). The error of detecting B(a)P in the soil for fractions from 0.005 to 0.040 and from 0.040 to 2.0 would be 35 to 25%, respectively, with a confidence of $P = 0.95$.

3. Results and discussions

The results of B(a)P analysis showed that the studied soils of different purpose, sampled at a distance of 3 to 4 km from the contamination source (Irkutsk Aluminum Plant) according to the wind diagram, had an increased content of the carcinogenic substance. It was noted that the content of B(a)P was dependent not only on the proximity of the sampling site to the contamination source, the industrial zone, but also on the soil type, as pastures were more contaminated than arable land (Table 1). The observed discrepancy in the average concentrations of B(a)P in arable land and pasture soils was statistically significant ($P = 95\%$). This was due to the fact that arable soils would undergo seasonal treatment whereas pastures would not. The results obtained were consistent with previous studies in the Southern Baikal area [12], which showed that the vertical profile distribution of B(a)P in soil would depend less on its type than on its purpose and watering. In untreated soils, at least 90% of the total amount of B(a)P accumulates in the surface layer up to 10 cm. In arable soils, it is evenly distributed in the layer up to 30 cm deep.

Table 1. The results of the Baikal-EM 1 biocompound use on soils contaminated with B(a)P.

Soil designation	Biocompound dilution	B(a)P concentration, mg/kg			
		Before treatment	After the application of the compound, in		
			14 days	30 days	60 days
Arable land	control	0.085±0.02	0.082±0.02	-	0.078±0.02
	1:100	0.091±0.02	0.047±0.01	0.014±0.004	0.003±0.001
Pasture	control	0.39±0.10	0.42±0.10	-	0.35±0.09
	1:100	0.38±0.10	0.23±0.06	0.31±0.08	0.18±0.05
	1:50	0.31±0.08	0.24±0.06	0.28±0.07	0.19±0.05

In all soil samples, the B(a)P concentration exceeded the MAC [19]: 4.6 times in arable-land soil, 15.5 to 19 times in pasture soil (Tab. 1). Evaluation of the soil contamination degree showed that such soils in accordance with SanPiN 2.1.7.1287-03 were classified as "dangerous" and "extremely dangerous", respectively. According to MU 2.1.7.730-99 arable soils could be classified as "moderately dangerous", as the translocation and water-migration indicators were below the permissible level and such soils could be used for any crops provided adequate quality control of agricultural products; pasture soil had a "highly dangerous" contamination degree. Such soils would be permitted for use for technical crops only and, therefore, vegetable and green cultures grown on these soils might pose a danger to human health.

Thus, decontaminating the soils intensively used for growing agricultural products in private farms in the rural area of Olkhavill necessitate a set of measures. One of those consists in using bioremediation technologies, the most important advantage of which is their safety for the environment, as they are based on wildlife self-cleaning.

As a result of the model experiment, it was determined that the Baikal-EM consisting of a consortium of microorganisms is capable of cleaning soils of different contamination degrees and decomposing B(a)P and possibly other toxic polynuclear PAHs (Table 1).

Thus, when arable soil with a concentration of B(a)P equal to 0.091 mg/kg was treated, the contaminant content decreased by 48.4% in 14 days of bioremediation, and by 96.7% in 2 months. The final B(a)P concentration in the treated soil was 0.003 mg/kg, i.e. the soil contamination level decreased to 0.15 MAC (Figure 1).

In pasture soils with an "extremely high" level of contamination (15.5 to 19 MACs), the study was carried out with different compound concentrations in the following ratios: 1 to 100 and 1 to 50. In general, higher soil contamination resulted in lower efficiency of the compound. Thus, treatment of soil with Baikal-EM in a 1 to 100 concentration (1ml per 1 kg of soil), the contents of B(a)P were comparable between 14 and 30 days of treatment, and statistically insignificant considering the measurement error. At the same time, the degree of bioremediation was 18 to 39.5%. After 60 days, there was a significant decrease in the concentration of B(a)P, which was statistically significant, the degree of its degradation was 52.6% (Figure 1, 2).

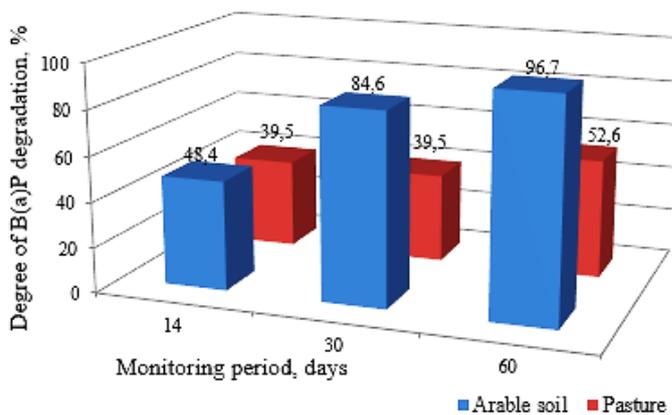


Figure 1. B(a)P degradation on the contaminated soils treated with "Baikal EM1".

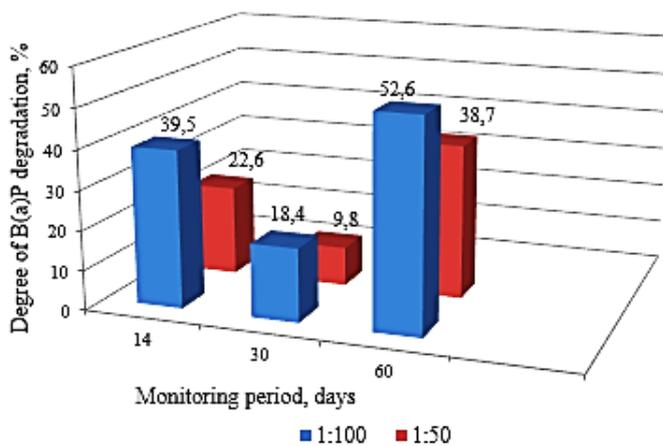


Figure 2. B(a)P degradation on the contaminated soils treated with "Baikal EM1" in different concentrations.

An increase in the biocompound concentration (1 to 50, or 5 ml per 1 kg of soil) did not give a positive result; on the contrary, there was a decrease in the degree of bioremediation of soils. After 1 and 30 days, the B(a)P content in the soil was also comparable and statistically insignificant, and the degree of its degradation was 9.8 to 22.6%. After 60 days, the degree of bioremediation reached 38.7%, with the final concentration of B(a)P being 0.19 mg / kg (9.5 MACs). It is possible that for remediation of soils with a very high and "old" contamination level, a longer period (2 or 3 summer seasons) of recovery is needed whereas other methods have to complement the remediating substance, example.g. phytoremediation (seeding of contaminant gathering plants). Also, for a more effective work of the biological compound, it is necessary to create a more favorable nutrient medium, i.e. contaminated soils should be sufficiently enriched with mineral elements, namely nitrogen,

phosphorus and potassium. Consequently, it is necessary to additionally apply mineral fertilizers in optimal proportions.

4. Conclusion

The conducted studies showed the effectiveness of using Baikal-EM 1 for bioremediation of soils contaminated with B(a)P. So, at a dangerous contamination levels (up to 5 MACs), its effectiveness after 2 months of bioremediation was 96.7%. At a higher soil contamination level of up to 15 to 19 MACs, the microbiological compound was less effective and the degree of bioremediation was 38.7-52.6%. It is possible that for remediation of soils with a very high and "old" level of contamination, a longer recovery period is required in combination with other methods. It was found that the greater degree of B(a)P degradation was achievable when using the biological product diluted in water 1 to 100 as compared to 1:50.

The conducted research showed the prospect of further study of the "Baikal EM1" biocompound for cleaning and remediating the soils contaminated with PAH. However, the results obtained by us are preliminary and need to be further tested on technologically stressed areas in the field.

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