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BIOREMEDIATION OF SOILS CONTAMINATED WITH HEAVY METALS

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ABSTRACT

Waste generated due to the growth of the modern industry is undergoing natural disposal in the environment for a long period of time. A special danger is caused by heavy metals that do not undergo biodegradation. Known purification methods of soils are not always effective and profitable. Correct selection of soil remediation methods contaminated with heavy metals ensures effective cleaning and restoration of soils. For this purpose, selecting representatives of various taxonomic groups of microorganisms binding heavy metals in the soil is carrying out. A complex method of purification of soils contaminated with have metals is being developed. Modified forms of humic acids were developed, geochemical barriers using local clays were created. Works to biostimulate local microorganisms required for bioremediation of soils contaminated with heavy metals are conducted. Remediation and increasing the fertility of soils contaminated with heavy metals are necessary for the prevention of further penetration of these metals into agricultural crops.

Introduction. Research of contamination sources of environment and their elimination is the most important problem. Waste generated due to the growth of the modern industry is undergoing natural disposal in the environment for a long period of time. In this regard, a special danger is caused by heavy metals that do not undergo biodegradation. Significant amounts of heavy metals accumulate in microorganisms and plant biomass, then by trophic chain enter organism of animals and humans, and negatively affect their activity. It should be mentioned, that the degree of contamination is

especially high in regions where the industry is highly developed. Heavy metals pollution causes changes in natural biocenosis. They cause inhibition of activity in some microorganisms.

There are several sources of heavy metal contamination in Georgia. Arsenic, copper, lead, cadmium, strontium, strontium, nickel, chrome belong to the main pollutants. The map of pollution with heavy metals of the territory of Georgia is drawn up, the level of contamination and polluted places are identified. The most contaminated places with radionuclides are identified (the southern part of Georgian Black Sea Coast of Adjara and Ozurgeti regions). It is necessary to determine the initial data of heavy metals concentration using modern technologies [1]. Thus, the development of effective methods for purification the environment from heavy metals contamination is relevant.

Different technologies to restrict the migration of heavy metals into the soil are widely used in the modern world. This can be achieved by immobilizing heavy metal ions on various solid surfaces, using ameliorants or stabilizers. For this purpose, it is effective to use natural and synthetic phosphates [2]. There are phosphorus-containing deposits in Georgia, but in this case, we did not consider their application appropriate due to unprofitability. Some basic oxides, hydroxides and sulfides can be used as ameliorators and stabilizers. Effective and accessible ameliorant has not been determined yet. It should be noted that it is difficult to find a universal ameliorator that can effectively immobilize all possible heavy metals in the soil. We are actively conducting research in this direction.

Heavy metals mainly accumulate in the upper layers of the soil. This depends on the mechanical and mineral composition of the soil. The degree of accumulation of heavy metals is especially high in soils with high humus content and heavy granulometric composition, as well as in dry soils rich with montmorillonite clays. The migration of heavy metals in such soils is restricted.

The rate of soil purification from heavy metals depends on the rate of decay of radioactive substances and the ability of these metal ions to migrate into the soil. Purification of soils contaminated with heavy metals is implemented using chemical and microbiological technologies [3, 4].

Heavy metals are divided into three groups according to the negative effect on the environment. The most hazardous: Hg, Pb, Zn, Cd, etc.; Medium hazardous: Ni, Cu, Cr, Sb, Co, and less harmful Mn, Sr. Heavy metals are also distinguished by their ability to migrate into soils. Heavy metals are found in soils in the form of free ions, as well as water-soluble inorganic and organic complexes.

Known methods of remediation of soils polluted with heavy metals are divided into three groups: restriction, *ex-situ* and *in situ*. The restriction method of heavy metals distribution in soils includes the creation of geo-barriers, mechanical insulation and sealing. This ensures the binding of contamination in a specific location and prevents the introduction of highly toxic heavy metal ions into the natural environment. *Ex-situ* method includes physical separation of soil contamination, leaching and pyrometallurgical treatment of soil. The application of this method is limited since its implementation requires an appropriately arranged area. The third group of existing cleaning methods includes the creation of isolated water-permeable barriers, electrokinetic treatment, soil washing, biological leaching, and phytoremediation.

Proper selection of these methods ensures effective soil purification and restoration. It should be noted that in the recent past, only phytoremediation has been used among biomethodes. To reduce soil contamination toxicity, mobile ions can be leached and other heavy metals converted to non-mobile forms. It should be noted, that this may result in the formation of more toxic compounds.

These technologies of restriction of heavy metals distribution in the soils are not able to completely remove hazardous xenobiotics but stabilize them in areas of contamination. The method of soil washing with organic acids and synthetic surfactants is based on chemical-technological processes. During this, such aggressive reagents are used that significantly worsen the existing ecological situation.

Research results. Described purification methods of soils are not always effective and profitable an effective and environmentally safe method of cleaning soils from heavy metals is the use of microorganisms. It is known that many microorganisms are not subjected to severe toxic effects and it is possible to increase their resistance. For this purpose, the selection of local bacteria and fungi able to bind heavy metals in the soils is conducted. Using representatives of different taxonomic groups of microorganisms is possible. Some microorganisms during life activity reduce water-soluble sulphates of certain heavy metals (Cd, Pb, Cu) into water-insoluble sulphides. This makes possible to bind pollutants in the soil that subsequently be washed away with sewage.

Synthetic surfactants are environmentally hazardous, highly toxic substances with a low level of biodegradation. They also actively accumulate in the soil

biocenosis. Therefore, treatment of soils contaminated with heavy metals using ecologically safe biogenic surfactants of bacterial origin is perspective. Biosurfactants have the ability to form complex compounds with metal ions. The active surface of surfactants promotes and facilitate the desorption of contaminants by microbial cells. Therefore, they are effective agents in the bioremediation of soils contaminated with heavy metals. Selection of biosurfactants with environmentally safe and metallo-chelating abilities is necessary. Their similarity with the type of contamination, high mobility in the soil and long-existing ability should be considered. Biosurfactants synthesized by microorganisms fully meet these requirements. They have almost completely replaced the synthesized analogues. Natural surfactants are biodegradable and characterized by low toxicity and high activity in extreme conditions [5, 6].

Bacteria of the genus Rhodococcus have high oxidizing activity, the ability to accumulate ions of heavy metals and synthesize non-toxic biosurfactants. This promotes to the restoration of soils contaminated with heavy metals. The leaching of heavy metals in the soils by means of *Rhodococcus*-biosurfactants is explained by the formation of a stable chelating complex between the metal and the biosurfactant molecule. Using immobilized *Rhodococcus* promotes the isolation of Ni 2+ from the soil. Despite various immobilization methods, induced *Rhodococcus* retain high functional activity during bioreduction. Their introduction into contaminated soils activates soil respiration processes, which is explained by the biodegradation of hydrocarbons by these species of bacteria. It is established that the number of *Rhodococcus* in the purified soil decreases and reaches the limit of the norm. This proves their ecological safety.

At the first stage of the experiment conducted by us, certain strains of microorganisms were selected for bioremediation, artificial contamination of soils with aqueous solutions of heavy metal salts have been done and soil acidity was determined. Correction of soils acidity with a slaked lime is performed, which helps to prolong soil fertility as well [7].

For absorption of heavy metals modified natural sorbents are used. As components of geochemical barriers, local clay minerals kaolinite and montmorillonite are used. That promotes the deposition of xenobiotics and prevent the transfer of contamination into groundwater [8]. Natural sorbents have been modified with derivates of humic acids extracted from peat, while inorganic acids and salts have been used to increase the sorption capacity. Humic acids were modified with iron ions Fe^{2+}, which decreased the solubility of heavy metals in humic acid. This prevents the formation of soluble and consequently mobile complexes of metals. It should be noted that modified humic acids are effective sorbents for Pb^{2+} and Cu^{2+} ions, whereas in the case of Zn^{2+} the degree of sorption is low.

Optimal conditions for biostimulation of local microorganisms selected for the accumulation of xenobiotics have been selected.

The humidity of the model contaminated samples was 40%, temperature 22-25°C. After 14 days from artificial contamination, local dry sorbents (kaolinite and montmorillonite) were introduced into the soils. During the studies, the watering and loosening of soil samples were carried out regularly once a week. Contaminated soil samples without sorbents are taken as a control. The quantity and quality of microflora have been studied using traditional microbiological methods [9].

We conducted quantitative and qualitative microbiological analyses of non-contaminated and artificially contaminated soil samples. 10 groups of microorganisms were isolated from the soil sample. The number of microorganisms was determined by sowing the soil suspension in the nutrient area. The results were evaluated on a petri dish, the calculation was made on 1 g of soil. At this stage of the study, remediation was performed by means of *Azotobacter* and *Nitrobacter*, representatives of the *Nitrogen-fixing bacteria* genus since their percentage distribution in the studied soils compare to other groups of microorganisms was high. Quantitative and qualitative analysis has shown that in the initial stage, a quantitative reduction of microorganisms in artificially contaminated soils is observed, and during the remediation process, their gradual recovery is noticed. It should be noticed that the low content of oligocarbophiles in contaminated soils is a sign of their low fertility and during the experiment, their number has increased (Table 1).

Table 1. Quantitative content of microorganisms (%) per 1 g of soil

Microorganism	Quantitative content of microorganisms (%) per 1 g of soil	
	Artificially contaminated soil before remediation	Soil in the early stage of remediation
Nitrate-reducing	16.1	21.25
Denitrifying bacteria	12.2	18.5
Cellulose-destroying	9	8
Nitrogen-fixing bacteria	20.1	27.05
Ammonifiers	4.2	2.5
Actinomycetes	18.6	9.75
Fungi / yeast	8.4	5.25
Nitrifiers I	7.4	3.70
Nitrifiers II	2	1
Oligo Carbophils	2	3

As can be seen from the table, there are some changes between soil artificially contaminated with heavy metals before remediation and soil at an early stage of remediation. The number of actinomycetes and fungi have been increased in soils contaminated with heavy metals, although the quantity of nitrogen-fixing bacteria has not changed much [10].

Currently, works for biostimulation of local microorganisms needed for bioremediation of soils contaminated with heavy metals is carrying out. Recommendations for soil remediation based on the microbiological analysis of the studied soil samples will be developed. Works to create geochemical barriers based on other local clays are planned. Remediation and increasing the fertility of soils contaminated with heavy metals are necessary for the prevention of further penetration of these metals into agricultural crops.

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