Rhizobacteria effect on bioaccumulation and biotransformation of arsenic and heavy metal compounds in the technogenous soils

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Abstract. This study aimed to disclose migration and transformation of heavy metal and arsenic compounds in the soil-microorganisms-plant system. Plants have been grown on soil inoculated with experimental rhizobacteria Azotobacter and Bacillus. In our experiment, soil samples were collected close to the industrial site of the former Angarsk Metallurgical Plant (AMP) used to produce arsenic (Svirsk town, Eastern Siberia) and from background site. High As, Pb and Cd abundances, dozens and hundreds of times exceeding the Maximum Permissible Concentrations (MPC), were found in the soil around the former plant. Each soil sample was divided into control soil and soil inoculated with rhizobacteria used for plant growing. Concentrations of chemical elements were analyzed in easily exchangeable, carbonate, organic, and Fe hydroxide-associated fractions and chelate forms. Rhizobacteria initiate arsenic and heavy metals immobilization in organic fraction. The highest element abundances were found in the chelate form in soil inoculated by rhizobacteria. The intensity of As and heavy metals accumulation in the plants grown on the inoculated soil is markedly low, owing to biological adsorption of elements by the cells of rhizobacteria capable of creating a barrier for their supply into plants.

The study of As and heavy metals behavior under the influence of rhizobacteria is of great importance in developing new biotechnologies related to soil remediation and crop production.

1. Introduction
Recently, new biotechnologies based on rhizosphere bacteria of PGPR group (Plant Growth Promoting Rhizobacteria) are being widely used in plant cultivation [1]. These rhizobacteria allow reducing the use of mineral fertilizers, as they are capable of stimulating the accumulation of nutrients (phosphorus and silica) for their uptake by plants. This group of bacteria can promote the protection of plants against pathogens and diminish the use of toxic pesticides.

Many symbiotrophic microorganisms are highly resistant to heavy metals. There is an opinion that they can convert heavy metal compounds in the soil, thus, affecting their accumulation in plants [2]. However, the mechanisms of migration and transformation of As and heavy metal compounds in the soil-microorganisms-plant system are still poorly understood, as they are stipulated by complex biogeochemical processes. Biological preparations based on microorganisms are of particular
relevance in the field of soil remediation since their application opens up new avenues towards the development of new biotechnologies [3, 4]. Bioremediation is considered more effective and less expensive compared to physical remediation strategies [5]. Phytoremediation approaches can be used alone or in combination with microorganisms for stabilization of chemical elements in contaminated soils [6, 7].

The primary purpose was to identify the impact of rhizobacteria, the constituents of biopreparations used in plant cultivation, on migration and transformation of As and heavy metals compounds in the soil-plant system on the example of the technogenous soil. It is now widely recognized that speciation of heavy metals both in natural and technogenous migration cycles is the most important for the understanding of the particular environmental problems. New heavy metal compounds can be even more toxic to the living organisms since human beings and other living creatures lack the mechanisms of adaptation to them at the genetic level. As and many heavy metals, in particular, Cd and Pb, can induce DNA damage [8]. For people, consumption of foods of plant etiology, such as cereal, grains and root tubers is considered a potential source of exposure to cadmium intake. The study of this problem can have both theoretical and practical significance for the understanding of the biogeochemical cycles of heavy metals in the soil-plant system of natural and man-made landscapes as well as for disclosing the role of soil bacteria, which can be used to influence metal element speciation in a complex natural matrix.

2. Methods and materials

A simulation experiment was conducted to grow plants on soils inoculated with rhizobacteria: Azotobacter chroococcum (strain Az d 10, ACM B-2272 D) able to supply nitrogen as ammonium to plants; Bacillus megaterium var. phosphaticum (strain PI-04, ACM B-2357 D), a preparation based on live acid-forming soil bacteria able to convert phosphorus from an insoluble fraction into an available form; and Bacillus mucilaginosus (strain ACM B-1574) a preparation based on silicate bacteria. The bacteria in this preparation are capable of leaching silicon and other macro- and microelements from natural silicates and deliver them to the rhizosphere of plants. Biological preparations were developed at Tomsk State University [9]. The plants like oats (Avena sativa L.), peas (Pisum sativum L.), salad (Lactuca sativa), and radish (Raphanus sativus L.) were grown in a collective-use Phytotron chamber, operating at the Siberian Institute of Plant Physiology and Biochemistry, SB RAS in Irkutsk city (SIFIBR SB RAS). For the experiment, we selected the soil sites contaminated differently by the activities of the former Angarsk Metallurgical Plant (AMP) placed on the Angara River bank in the Svirsk town (Southern Baikal region). The plant used to produce white and gray arsenic from arsenopyrite concentrate until 1949. The remains that were left in the waste dumps of roasted products are a dangerous source of arsenic, lead and other metal contamination. As a result, soil contamination with arsenic penetrated not only the area of the former plant but also the entire territory of the town [10]. The samples of soil for the experiment were collected from the sites located at different distances from the AMP industrial site: 10 m away from the polluted site and 15 km away from it on conditionally background soils. The experiment was carried out in the humus horizon.

Each soil from the two sites was divided into a control soil (without bacteria) and the one treated with biopreparations (experiment) where plants were grown. The working solution with three bacterial preparations (5 ml of concentrate per 10 l (0.5 ml/l)) of water was poured into the soil during planting, and then, in 5 days, – as the amount of 1 l/m². The bacteria titer in the working solution was 10⁶ cells/ml. After 37 days of growth on the technogenous polluted soils, the plants began to dry, thus, failing to reach the mature state. Plants dried to the air-dry state were used for chemical analysis. The method of sequential extracting was employed in the research of mobility and bioaccessibility of chemical elements in the soil [11]. This method with some modifications is currently extensively used for the study of microelement fraction composition in soils [12]. In the experiment, the following fractions were extracted from the control soil and the one inoculated with rhizosphere bacteria Azotobacter and Bacillus, as constituents of biopreparations: easily-exchangeable, carbonate, organic, and combined with iron hydroxides, in which arsenic and heavy metal contents were defined. To
isolate chelate forms, the distribution of these elements in ethylenediaminetetraacetic acid (EDTA) extract was studied. Chemical analyses of soils and plants were accomplished at the certified analytical center of the collective use of the Institute of Geochemistry SB RAS. To determine As and heavy metal mass fractions in soil samples the atomic adsorption method was applied. The measurements were carried out on 403 and 503 models of spectrometers manufactured by Perkin Elmer (the USA). The determination error did not exceed 10%. The chemical composition of plants was analyzed by inductively coupled plasma mass spectrometry (ICP-MS), whose determination error did not exceed 5-7%.

3. Results and discussion

Figure 1 shows the percentage distribution of As and heavy metal contents relative to the total amount of isolated fractions for the technogenous soil. Easily exchangeable (ion-exchangeable) and carbonate (sorption) fractions of As are the most mobile forms in soils, which represent the closest reserve of chemical elements that can be released when some physicochemical properties of the soil change (pH, Eh). As indicated by the experiment, the mobility of Cd and As was greater than that of Zn and Cu, and Pb was characterized by the lowest migration ability. It appears that 90% of all lead forms persistent and permanently bound residues (figure1). Figure 1 shows regularities in percentage distribution of chemical element contents: the concentrations of As, Cd, Cu, and Zn, occurring as carbonate fraction, significantly decrease in experiment, which can be explained by less intense sorption processes in the carbonate fraction of the soil inoculated by rhizobacteria. At the same time, there is a simultaneous increase in these elements in organic fraction, particularly cadmium and arsenic. The same regularity is found for concentrations of other elements, given in tables 1 and 2. This is characteristic of both weakly mobile elements, such as Pb, Cr, Ni, and Co, and biophile elements, phosphorus and silica (table 2). The obtained results suggest the ability of rhizobacteria Azotobacter and Bacillus to initiate the accumulation of chemical elements in organic compounds and partly convert them into easily-exchangeable (ion-exchangeable) fractions.

![Figure 1. Percentage contents of As and heavy metals in sequential extractions (percent of the total amount of fractions) in the technogenous soil after plants growing: 1 – control (without bacteria); 2 – experiments (with Azotobacter and Bacillus rhizobacteria applied). Fractions: F1 – easily-exchangeable; F2 – carbonate bound; F3 – organically bound; F4 – Fe oxide bound; F5 – residual.](image-url)
Table 1. Distribution of As and heavy metal contents in the technogenous soil after plants growing, mg/kg.

| Fractions     | As  | Cd  | Pb  | Cu  | Zn  | Cr  | Ni  | Co  |
|---------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Easily-exchangeable | 41.3<sup>a</sup> | 0.91 | 2.84 | 9.8 | 80.5 | 0.385 | 4.20 | 0.016 |
|                | 31.5<sup>b</sup> | 1.23 | 2.70 | 7.0 | 91.0 | 0.455 | 5.25 | 0.033 |
| Carbonate     | 609  | 0.91 | 11.2 | 23.0 | 84.0 | 1.05 | 3.36 | 0.022 |
|               | 273  | 0.42 | 7.0  | 6.65 | 34.3 | 3.22 | 1.75 | 0.007 |
| Organic       | 32.6 | 0.49 | 2.91 | 46.9 | 37.5 | 2.66 | 2.17 | 0.037 |
|               | 47.6 | 1.44 | 5.60 | 52.9 | 45.8 | 3.47 | 3.85 | 0.244 |
| Fe-hydroxide  | 107 | 0.55 | 8.70 | 22.2 | 43.1 | 0.592 | 1.85 | 0.070 |
| Residual      | 310 | 1.88 | 3185 | 240 | 410 | 106 | 44.0 | 23.0 |
|               | 264 | 1.68 | 2400 | 250 | 390 | 85.0 | 41.0 | 25.0 |
| Total of fractions | 1100 | 4.7 | 3210 | 342 | 655 | 110 | 55.6 | 23.1 |
|               | 727 | 5.2 | 2423 | 334 | 594 | 93.0 | 53.4 | 25.3 |
| MPC of soils<sup>[13]</sup> | 10 | 0.5 | 32 | 3.0 | 23.0 | 6.0 | 4.0 | 5.0 |

Note: (<sup>a</sup>) control soil; (<sup>b</sup>) soil inoculated with rhizobacteria (experiment). Higher element concentrations in control and experiment soils are marked in bold.

Many chemical elements are bound to soil fulvic or humic acids. Different-metal-fulvic acid complexes demonstrate lower stability constants, implying that these complexes are more soluble and, thus, more available for plant uptake. Humic acids, on the other hand, form less soluble complexes with heavy metals<sup>[14]</sup>, which can be regarded as the organic reserve of heavy metals in soils. Such complexes are not readily available for plant uptake.

Table 2. Distribution of chemical elements contents in technogenous soil after plant growing, mg/kg.

| Fractions     | Fe  | Mn  | Ca  | Mg  | K   | Na  | Al  | Si  | P   |
|---------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Easily-exchangeable | 122.5<sup>c</sup> | 84.0 | 5600 | 245 | 87.5 | 101 | 382 | 104 | 26.6 |
|                | 126<sup>d</sup> | 98.0 | 5250 | 285 | 105 | 101 | 458 | 92.7 | 25.1 |
| Carbonate     | 714 | 140 | 3780 | 168 | 84.0 | 231 | 735 | 11.0 | 40.4 |
|               | 294 | 59.5 | 1190 | 66.5 | 35.0 | 84.0 | 311 | 9.4 | 23.9 |
| Organic       | 875 | 73.5 | 308 | 133 | 21.4 | 252 | 1155 | 23.9 | 192 |
|               | 1225 | 136 | 381 | 238 | 39.2 | 374 | 1645 | 51.6 | 324 |

Note: (<sup>c</sup>) control soil; (<sup>d</sup>) soil with the rhizobacteria applied (experiment). Higher element concentrations in control and experiment soils are marked in bold.

Some simple organic acids are more likely to form low-molecular-weight chelate complexes, which may increase chemical element mobility for plant uptake. The EDTA is currently widely employed for analyzing the bioavailability of microelements and some toxic elements due to its high chelating ability. In this experiment, the maximum As and heavy metal concentrations were found in EDTA represented by chelate forms, which were extracted from the technogenous rhizosphere soil inoculated with rhizobacteria. Figure 2 A exemplifies this regularity for the distribution of As, Cd and Pb contents and is characteristic of other heavy metals involved in this experiment.

The higher levels of elements in EDTA fraction suggest the formation of organic chelate compounds, whose major portion can be absorbed on the surface of bacterial cells. The uptake of heavy metals can occur via biosorption on the cell surface as chelate forms<sup>[15]</sup>. The absorption of chemical elements by microorganisms likely promotes the formation of weakly-mobile organic compounds in soils<sup>[14]</sup>. As a result, the plants grown on soils inoculated with rhizobacteria show an
opposite trend. In this experiment, the accumulation of heavy metals and arsenic significantly decreased, owing to biosorption of those elements by rhizosphere bacteria capable of creating a biogeochemical barrier for the supply of As, Pb and Cd into plants (figure 2 B), thus, implying a high tolerance ability of rhizobacteria to high levels of heavy metals and arsenic. This regularity is characteristic of other heavy metals, such as Cu, Zn, Cr, Ni, Co, Cu, whose concentrations markedly decrease in plants grown on the soils with the bacteria applied (table 3).

**Figure 2.** (A) – As, Pb and Cd concentrations (mg/kg) in EDTA fraction extracted from technogenous soil, (B) – average As, Pb and Cd concentrations (mg/kg) in plants (dry substance) grown on technogenic soils (10 m away from arsenic dumps). 1 – control, 2 – experiment.

**Table 3.** Concentrations of chemical elements in plants (mg/kg, dry substance) grown on soils, collected in 10 m from AMP dumps

| Plants             | As  | Cd  | Pb  | Cr  | Ni  | Co  | Cu  | Zn  |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|
|                    |     |     |     |     |     |     |     |     |
| Control            |     |     |     |     |     |     |     |     |
| Salad              | 101 | 9.3 | 51.1| 0.958| 4.76| 2.30| 32.9| 325 |
| Redish             | 79.7| 3.01| 25.3| 0.531| 2.16| 1.06| 34.5| 345 |
| Peas (stem, leaves)| 18.7| 1.11| 5.04| 0.201| 2.97| 0.45| 10.8| 99.3|
| Peas (root)        | 110 | 6.18| 27.7| 0.696| 5.06| 2.41| 20.8| 308 |
| Oats (stem, leaves)| 19.2| 0.85| 0.82| 0.175| 2.54| 0.067| 3.73| 41.1|
| Oats (root)        | 120 | 1.44| 40.6| 0.810| 2.05| 0.97| 16.7| 103 |
| Experiment         |     |     |     |     |     |     |     |     |
| Salad              | 109 | 2.98| 62.1| 1.27 | 2.06| 1.04| 52.2| 121 |
| Redish             | 7.71| 0.25| 1.89| 1.17 | 2.05| 0.45| 6.46| 33.3|
| Peas (stem, leaves)| 11.8| 0.86| 2.90| 0.225| 2.30| 0.34| 8.92| 73.5|
| Peas (root)        | 2.28| 0.15| 0.96| 0.286| 0.72| 0.11| 2.33| 23.6|
| Oats (stem, leaves)| 14.8| 0.88| 1.71| 0.155| 1.68| 0.083| 3.21| 36.2|
| Oats (root)        | 30.7| 0.85| 8.26| 0.325| 0.78| 0.33| 7.77| 63.8|
| Average content in grass [14] | 0.2| 0.05| 0.5| 0.1-0.9| 0.1-1.7| 0.03-0.27| 1.8-10.5| 12-47 |

Note: Higher element concentrations in control and experiment soils are marked in bold

It appears that the plant toxicity of heavy metals and arsenic differs according to plant species: the salad turned out to be especially good at absorbing heavy metals and arsenic. The accumulation of heavy metals for this plant follows the barrier-free scenario, thus, the element concentrations in the salad were not taken into account for estimating the average concentrations (figure 2 B). The plants grown on the control soil show the maximum concentrations of heavy metals and arsenic, particularly in roots. In the experiment, the concentrations of all elements markedly decrease in all organs of plants.
The background soils characterized by low As and heavy metal concentrations show an opposite pattern. Contents of heavy metals and As are higher in the experiment, however, the levels of principal toxic elements do not exceed average concentrations in grass species. With relatively low and moderate pollution, the protective mechanisms of plants and microorganisms have not yet been induced, and the mobilization of heavy metals and arsenic in the soil-plant system may prevail. It leads to a slight increase in microelement levels in plants grown on soils with rhizobacteria. At high soil heavy metal concentrations, the plants excrete organic acids and other substances to the rhizosphere, which then convert microelements into the chelate forms [14]. The reduced bioavailability of chemical elements by plant uptake can be due to the accumulation of their organic compounds by rhizobacteria. At very high levels of heavy metals in the soil solution, protective reactions of microorganisms likely become more intense, and the microbiological immobilization processes begin to dominate in the rhizosphere. It can explain the reduced heavy metal concentrations in plants grown on the contaminated soils with rhizobacteria applied.

4. Conclusions

Therefore, the migration of heavy metals and As in the soil-plant system significantly relies on the impact of rhizobacteria *Azotobacter* and *Bacillus*. The accumulation of those elements in soils markedly depends on the soil contamination by heavy metals. Rhizobacteria initiate a slight accumulation of chemical elements in plants grown on the background soil.

On polluted technogenous soils, the rhizobacteria are capable of converting heavy metals and arsenic to organic compounds, including the chelate forms, which are not easily available for plant uptake, thereby, reducing the accumulation of element-toxicants in plants. The accumulation of heavy metals differs according to plant species and plant organs. Their maximum concentrations are found in barrier-free plant species and roots.

Rhizobacteria *Azotobacter* and *Bacillus* are persistent to toxic levels of heavy metals and could promote the inter-phase transfer of elements and their immobilization in the rhizosphere soil. The acquired results may be useful for developing new biotechnologies to be applied in soil remediation and plant cultivation.

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