Features of phytoextraction of rare earth elements by a complex of plants and microorganisms from technogenically polluted wastewater of mining enterprises

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Abstract. Rare earth elements are not always rare in their total mass, but their concentration in ores is usually so low that this limits the possibility of cost-effective extraction and enrichment for processing and use. At the same time, some rare earth elements accumulate as a by-product of mining ores containing, for example, copper, phosphates and iron. The article presents for the first time data on the accumulation of some rare earth elements in the tissues of phytoremediant plants Phragmites australis (Cav.) Trin. ex Steud., *Typha angustifolia* L., *Typha latifolia* L., used for the treatment of technogenically polluted wastewater from mining enterprises. The influence of the symbionts *Bacillus subtilis* strain DSM 32424, *Bacillus amyloliquefaciens* strain VKPM V-10642 (DSM 24614), *Bacillus amyloliquefaciens* strain VKPM V-10643 (DSM 24615), *Bacillus subtilis* 26D and *Azotobacter vinelandii* IB-4 on the efficiency of phytoextraction in different species of plants is considered. toremediants. The most effective symbiotic complexes of plants and microorganisms with a high phytoextractive ability to extract some rare earth elements have been identified.

1 Introduction

To date, “anthropogenic influence” has become a powerful, determining environmental factor causing “evolutionary changes” in the floras of various regions [1]. It consists in the extraction from the environment, concentration and rearrangement of a wide range of chemical elements and their mineral and organic compounds with secondary dispersion, and the results of anthropogenic impact on phytobiota are especially noticeable in the territories of old industrial regions [2].

In recent decades, due to the development of modern industry, the volume of production and consumption of rare earth elements (REE) has been constantly increasing. Rare earth

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elements are considered to be weakly to moderately abundant in the hydrosphere and are not traditionally considered as pollutants of aquatic ecosystems. At the same time, one of the most interesting areas of research is the study of the possibilities of associated phytoextraction of rare earth elements from technogenically polluted wastewater from mining enterprises (TPWME). In addition, increasing the efficiency of plant extraction of various substances can be achieved by creating symbiotic complexes in the plant-microorganism system.

Promising objects in the development of agrobiotechnological approaches to phytoextraction can be rhizosphere microorganisms that stimulate plant growth (PGPR from Plant Growth Promotion Rhizobacteria) and form stable symbiotic microbial-plant complexes (SMPC) [3-5]. The direct effect of symbionts on plant growth and development is associated with the fact that they are able to increase the availability of mineral nutrition elements for plants, enhance the formation of metabolites with hormonal and signaling functions (auxins, cytokinins and gibberellins), etc., as well as reduce the impact of pathogens on plants microorganisms [6-8]. According to the literature, microorganisms, in addition to the ability to increase the mobility of metals in the soil, are also capable of increasing the content of metals in plants by increasing plant biomass and activating the mobility of elements [9-10].

Purpose of the work: to study the degree of accumulation of some rare earth elements in the tissues of phytoremediant plants growing in combination with various microorganisms in conditions of technogenically polluted wastewater from mining enterprises.

2 Materials and methods

The study was carried out on the basis of the laboratory of experimental botany and the laboratory of physical and chemical methods of plant research of the Botanical Garden of the National Research University Belgorod State University using the equipment of a unique scientific installation (Botanical Garden of the Belgorod State National Research University).

The test plants that have proven themselves quite well for phytoremediation of wastewater were: *Phragmites australis* (Cav.) Trin. ex Steud., obtained by the in vitro method, *Typha angustifolia* L. and *Typha latifolia* L. [11-12], obtained from seeds. In laboratory conditions, plants were cultivated on phytoracks with LED lamps ECOLED-60-LX Fito IP 65 60W with a wavelength of 450-730 nm (LED).

The experimental design included different combinations of plants and microorganisms:

- Control (plants only).
- Technogenically polluted wastewater from mining enterprises.
- Consortium of bacteria *Bacillus subtilis*.
- Technogenically polluted wastewater from mining enterprises + consortium of bacteria *Bacillus subtilis*.
- Bacterial strain *Bacillus subtilis* 26D.
- Technogenically contaminated wastewater from mining enterprises + bacterial strain *Bacillus subtilis* 26D.
- Bacterial strain *Azotobacter vinelandii* IB-4.
- Technogenically contaminated wastewater from mining enterprises + strain of the bacterium *Azotobacter vinelandii* IB-4.

Primary inoculation of plants with cultures of microorganisms was carried out on the 72nd day from the moment of emergence of seedlings and their introduction into the culture. The following were used in the experiment: a consortium of bacteria *Bacillus subtilis* strain DSM 32424, *Bacillus amyloliquefaciens* strain VKPM B-10642 (DSM

24614) and *Bacillus amyloliquefaciens* strain VKPM B-10643 (DSM 24615) at a concentration of at least 1×10^6 CFU/g; strain of the bacterium *Bacillus subtilis* 26D at a concentration of at least 2×10^9 CFU/g; strain of the bacterium *Azotobacter vinelandii* IB-4 at a concentration of at least $2\text{-}3\times10^9$ CFU/g. The plants were watered with tap water for six days, and on the 7th day they were treated according to the experimental design.

Determination of rare earth elements: cerium (Ce), lanthanum (La), niodymium (Nd), prosiodymium (Pr) was carried out on an ISP AVIO 220 Max optical emission spectrometer, the results are presented as % of the dry weight of the sample.

Statistical processing of data was carried out using Microsoft office Excel with calculation of the arithmetic mean (M) and confidence interval (±CI) at a significance level of $p = 0.05$.

3 Results

Figures 1-3 show the results of the accumulation of rare earth elements in phytoremediant plants under the influence of technogenically polluted wastewater from mining enterprises and their inoculation with symbionts.

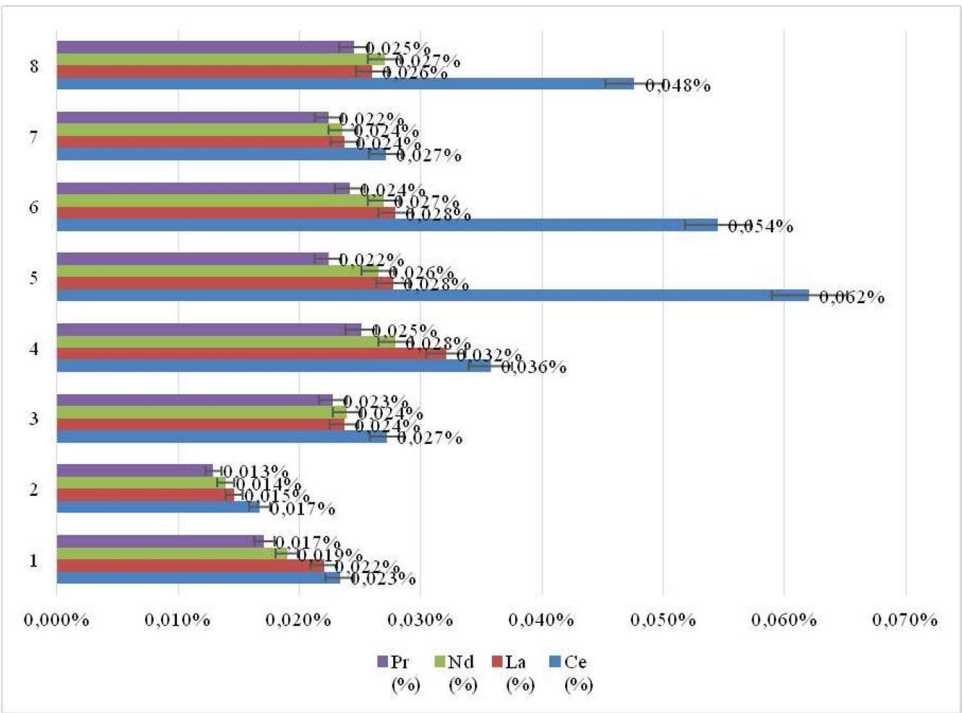

Fig. 1. Accumulation of rare earth elements in the leafy mass of *Phragmites australis* (Cav.) Trin. ex Steud. under the influence of technogenically polluted wastewater from mining enterprises and during inoculation with symbionts.

Figure 1 shows that in the control (1) and under the influence of technogenically polluted wastewater from mining enterprises (2), the distribution of rare earth elements in plant tissues correlates at the level of 0.93, however, plants exposed to technogenically polluted wastewater from mining enterprises are much less degrees accumulate elements. The use of a consortium of *Bacillus subtilis* bacteria (3 and 4) significantly increases the content of Pr, Nd and Ce. It can be noted that the use of this group of microorganisms

significantly increases the extractive ability of rare earth elements Ce, La and Nd by plants when combined with technogenically polluted wastewater from mining enterprises. The dynamics of accumulation of elements is also observed when using *Bacillus subtilis* 26D (5 and 6). The ability for phytoextraction of Ce increases 2.5 times; at the same time, the combined use of technogenically polluted wastewater from mining enterprises and microorganisms in the experiment did not lead to a significant increase in rare earth elements compared to the use of tap water. The third group of symbionts used significantly increased the degree of Ce accumulation when combined with technogenically polluted wastewater from mining enterprises and *Azotobacter vinelandii* IB-4.

In all variants, when inoculating plants with symbionts *Phragmites australis* (Cav.) Trin. ex Steud. increased phytoextractive properties compared to the use of only technogenically polluted wastewater from mining enterprises (2) and control (1).

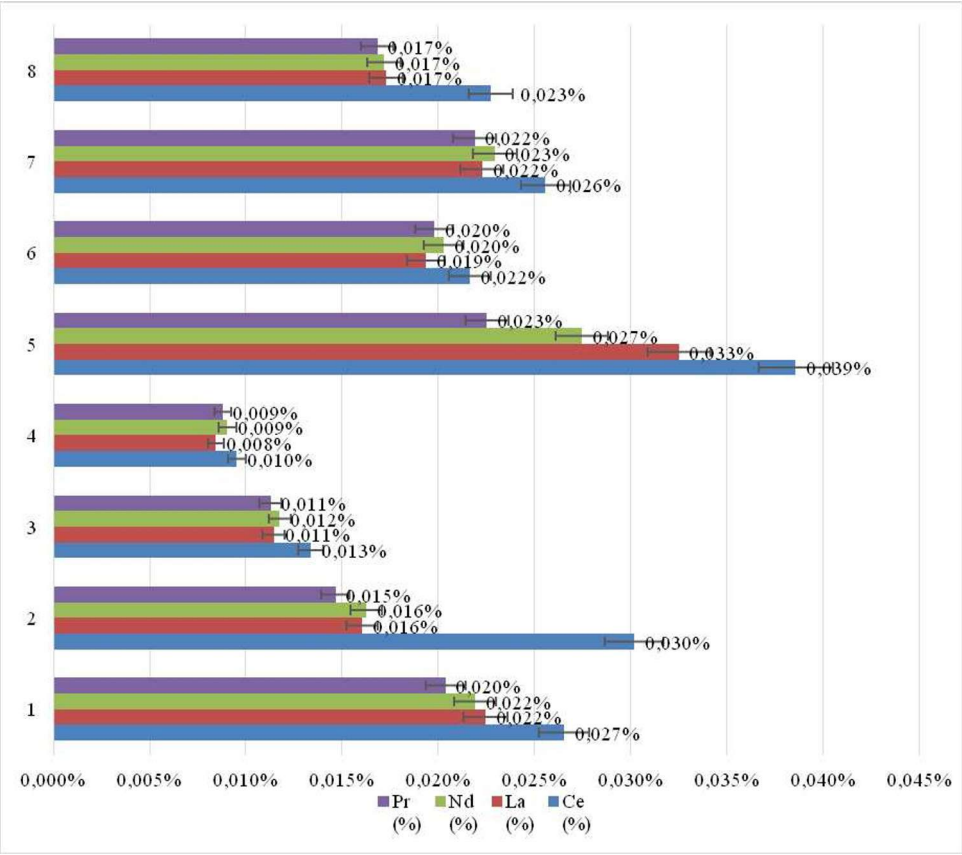

Fig. 2. Accumulation of rare earth elements in the leaves of *Typha angustifolia* L. under the influence of technogenically polluted wastewater from mining enterprises and during inoculation with symbionts.

The research results presented in Figure 2 indicate that the type of phytoremediant used significantly affects its phytoextractive properties. Thus, *Typha angustifolia* L. accumulates significantly more Ce in leaves when using technogenically polluted wastewater from mining enterprises (2) than in the control (1). Inoculation of plants with a consortium of bacteria *Bacillus subtilis* (3 and 4) inhibited the accumulation of rare earth elements. At the same time, the use of *Bacillus subtilis* 26D led to a significant increase in the accumulation of all four elements (5), and the combined use of the symbiont and technogenically polluted

wastewater from mining enterprises inhibited the accumulation processes (6). Symbiotic microbial-plant complexes based on *Azotobacter vinelandii* IB-4 turned out to be ineffective catalysts for phytoextraction in leaves compared to the complex using *Phragmites australis* (Cav.) Trin. ex Steud.

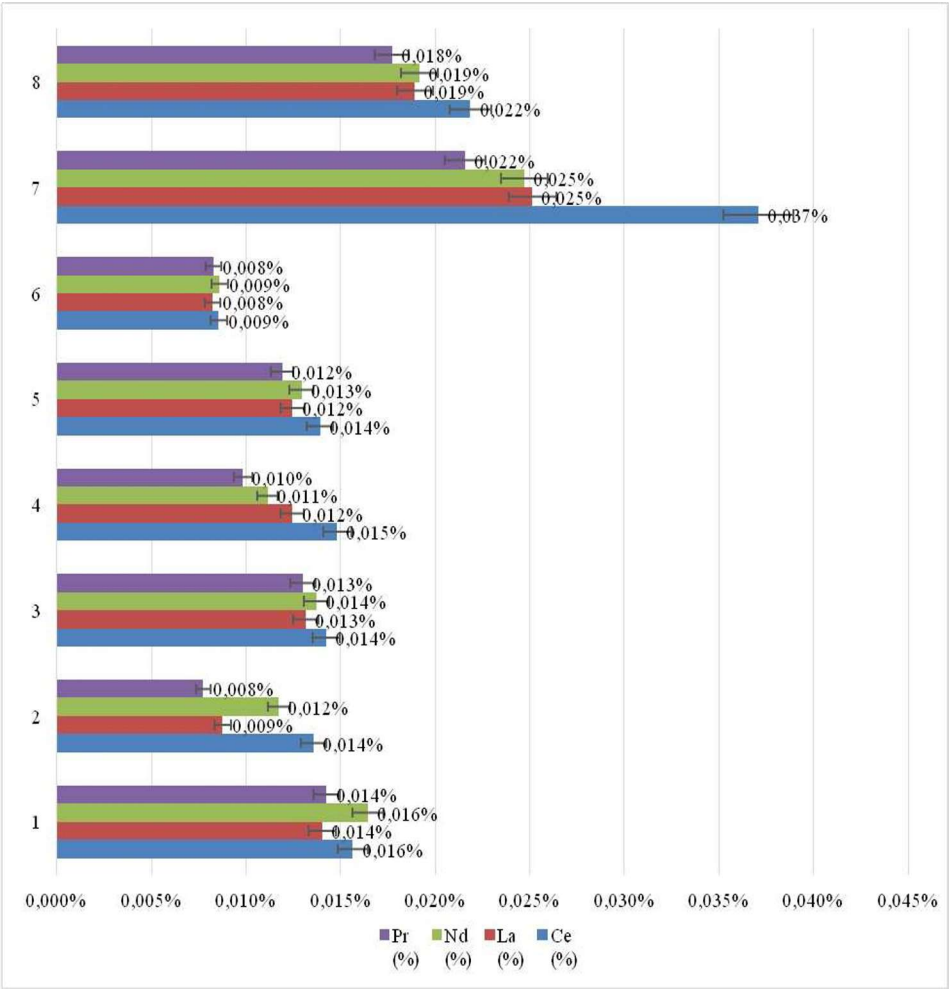

Fig. 3. Accumulation of rare earth elements in the leaves of *Typha latifolia* L. under the influence of technogenically polluted wastewater from mining enterprises and during inoculation with symbionts.

When *Typha latifolia* L. was used as a phytoremediant, only inoculation with the *Azotobacter vinelandii* strain IB-4 led to an increase in the content of rare earth elements when tap water was used in the experiment. The use of technogenically polluted wastewater from mining enterprises also led to an increase in the detected elements, but in smaller quantities. The use of technogenically polluted wastewater from mining enterprises for this species contributed to the inhibition of the accumulation of rare earth elements. A similar dependence was observed in the symbiotic microbial-plant complexes *Typha angustifolia* L. and the bacterial consortium *Bacillus subtilis*.

4 Discussion

The conducted research indicates that technogenically polluted wastewater from mining enterprises can be a source of rare earth elements that they contain. The use of plant phytoremediants for phytoextraction is most effective when inoculated:

- *Phragmites australis* (Cav.) Trin. ex Steud. consortium of bacteria: *Bacillus subtilis*, *Bacillus subtilis* 26D, *Azotobacter vinelandii* IB-4.
- *Typha angustifolia* L. – *Bacillus subtilis* 26D, *Azotobacter vinelandii* IB-4.
- *Typha latifolia* L. – *Azotobacter vinelandii IB-4.*

The different phytoextraction effects demonstrated when using different phytoremedinates are species specific and related to the genetic nature of the species used. This is apparently explained by the peculiarities of plant defense mechanisms, which differently ensure the export of unnecessary (excretory) substances from the cell through carrier proteins built into the plasma membrane. It is also necessary to take into account the influence of consortia of microorganisms on phytoextraction processes. They determine, on the one hand, the resistance of plant-microorganism complexes to anthropogenic impact, and on the other, the ability to increase the bioavailability of elements contained in technogenically contaminated wastewater from mining enterprises. The third factor influencing the degree of phytoextraction of rare earth elements by plants and the accumulation abilities of the studied complexes is the nature of the interaction between microorganisms and phytoremediant plants, which is determined by their compatibility.

5 Conclusion

The conducted research indicates that aquatic plants can be successfully used to solve problems of phytoextraction of various substances from technogenically polluted wastewater. The use of soil microorganisms (rhizosphere bacteria) makes it possible to increase the phytoextractive abilities of plants and expand the range of species used for phytoremediation. Inoculation of plants with rhizobacteria is an important and necessary procedure for increasing the phytoextractive ability of phytoremediants.

Further research in this direction will not only expand the range of phytoremediant plants and evaluate the effectiveness of their use in treating wastewater from pollutants, but also determine the prospects for the use of various plant species and their complexes with microorganisms in relation to phytoextraction of various substances. Solving these problems is undoubtedly extremely important for optimizing production processes, since they will not only contribute to compliance with environmental requirements and standards, but will also expand the economic opportunities of enterprises.

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