JOURNAL OF AGRICULTURE

RESEARCH ARTICLE ISSN: 2636-8757

Myosotis alpestris potential for extracting metal ions during wastewater pre-treatment at ore-mining facilities

Marija MENSHAKOVA[1*] | Valeria SUROVETS[1] | Anastasiya NİZİKOVA[1] | Nadezhda ORLOVA[1]

[1] Department of Biology and Bioresources, Institute of Natural Sciences and Technology, Murmansk Arctic University, Murmansk, Russia

Correspondence

Department of Biology and Bioresources, Institute of Natural Sciences and Technology, Murmansk Arctic University, Murmansk, Russia

Email: dendrobium@yandex.ru

This study was produced from the thesis of Marija MENSHAKOVA

Abstract: Phytoremediation and phytoextraction are seen as promising technologies which facilitate the development research on obtaining transgenic plants capable of metals hyperaccumulation. The article is devoted to the assessment of the possibilities of using Alpine forget-me-not for industrial wastewater treatment.The experiment was conducted on the industrial site of the Kovdorski GOK (Kovdor Ore-Mining Integrated Works), owned by the EuroChem Group. In 2020-2021 we assessed the content of heavy metals in plants on phyto-rafts made in the form of rectangular sheets of polyethylene foam with slots for plants. The rafts were fixed in running water at the beginning of the outlet channel, through which wastewater is discharged after primary purification. The most intensive accumulation in the organs of Alpine forget-me-nots appeared to be for physiological or essential metals, which is explained by their physiological role and the presence of membrane mechanisms for their intensive absorption. In terms of hyperaccumulation criteria, we can refer it in relation to iron, magnesium and manganese, especially when calculated for dry mass.

Keywords: Phytoextraction, Phytoremediation, Myosotis alpestris, Hyperaccumulation

INTRODUCTION

Currently, such topics as phytoremediation of contaminated areas, phytoextraction of pollutants from soil and water, as well as phytoextraction of useful substances draw a lot of attention since these technologies can simultaneously deal with the issues of industrial and household wastewater treatment as well as for deriving useful elements (Watanabe, 2007; Zhuang et al., 2007; Glass, 2009; Chaudhry et al., 2012; Miretzky et al., 2014).

The majority of works in this research area focus on industrial wastewater after-treatment (Dushenkov et al., 2005; Ghosh and Singh, 2015) and phytoextraction of metals from ore-mining and -processing waste. Also, there are some works devoted to phytoextraction of particular elements from household wastewater (Bennicelli et al., 2014). The use of inferior plants is generally seen as less promising due to its weaker strength of binding along with complex lifecycle making the control of phytoexctraction processes far more demanding (Lin et al., 2012). Particular difficulties are also associated with the use of unicellular algae for the reason of cell mass separation from water. Even though, some types of algae absorb metals so intensively that it makes it is impossible to ignore their potential for the practical use.

Phytoremediation technologies are also used to deal with the complex ecosystem pollution problems. For example, it allows to pursue several objectives of technogenic safety in cases of petrochemicals and heavy metals simultaneous presence (Groudeva et al., 2017). Planting the tailings dams allows not only to extract useful elements, but also to reduce the level of dust and thereby lower the negative impact on the environment. Most research works were tested in tropical, subtropical and equatorial areas (Reeves et al., 1999; Reeves, 2013). Meanwhile the temperate and cold climate

Received: 25-04-2024 | Accepted: 14-10-2024

territories also have many ore-mining and -processing enterprises where phytoextraction technologies could potentially be applied.

Speaking about the species capable of hyperaccumulating certain metals, the leading positions among them are occupied by Brassicaceae plant family (Vardanyan and Ingole, 2006; Begonia et al., 2008; Bani et al., 2009). Some of its species are adapted to temperate and cold climates and could be used in northern regions along with hydato- and hygrophytes of various systematic groups (Zhao and Duncan, 2008; Suseela et al., 2012; Zheng et al., 2013), growing in high latitudes. For phytoremediation of contaminated soils and phytoextraction of metals in arid climates, it is also suggested to use succulents (Wu et al., 2007), known for their high environmental tolerance.

Rhizofiltration and phytoremediation technologies are commonly applied for extraction of copper, nickel, zinc, cadmium, manganese, and lead. Meanwhile there are some research works devoted to searching for the plants which might be used for extracting radioactive elements (Eapen et al., 2013), as well as precious metals (Mkandawire et al., 2005). These research works cover the extraction of both, essential and nonessential metals and metalloids.

There is evidence that some plants also effectively accumulate non-metals, in particular, such a toxic element as arsenic (Vázquez et al., 2006). Efficient accumulation of selenium has also been shown in some works (Quin and Terry, 2003).

Along with applied investigation of possible metals phytoextraction from wastewater and contaminated soils of specific enterprises (Carpena and Bernal, 2007), there are fundamental works on the mechanisms of bioaccumulation and plant resistance to high doses of metals (Yang et al., 2004). Some authors study the influence of mineral growth factors on the intensity of metals phytoextraction (Bolan et al., 2003; De la Fuente et al., 2007; Fogarty et al., 2009.). Studies on the mechanisms of resistance through intracellular metal binding have shown that resistance to some metals is associated with glutathione (Freeman et al., 2014; Komives and Gullner, 2015). Much attention is also paid to investigating the transformations of transition metals in a living cell, in particular, to the processes of oxidation and reduction of metals, as well as to their inclusion in organic composition. (Limura et al., 2005)

Another topic of great concern is localization of absorbed metals in plant cells and tissues (Dominguez-Solis et al., 2001.). Such works not only provide better understanding of absorption and metal binding mechanisms, but also facilitate the development of phytoextraction technologies.

The influence of abiotic factors on phytoextraction intensity is also being studied, in particular, the influence of salinity on nickel accumulation (Lek, 2007)

Of great interest are the works aimed at exploring the biotic relationships that arise in plant communities in contaminated areas, tailings dams, and wastewater basins (Frérot et al., 2006; (McGrath et al., 2011). Understanding these processes allows to promote sustainability of artificial ecosystems created for phytoextraction.

Phytoremediation and phytoextraction are seen as promising technologies which facilitate the development research on obtaining transgenic plants capable of metals hyperaccumulation. For example, there are works on introducing genes involved in hyperaccumulation isolated from prokaryotes into the genome of higher hyperaccumulator plants (Vain, 2006; Doty et al., 2007; Eapen et al., 2017 (Hannink et al., 2011; Bennett et al., 2013; Cherian and Oliveira, 2015). The major prospects here are associated with the transfection of metallothionein genes into the plant genome (Raskin, 2006; Hesegawa et al., 2007), since metallothioneins are a universal and quite capacious mechanism for binding metals in both plant and animal cells. One of the factors enhancing the metals accumulation by plants is the activity of rhizosphere microorganisms (Kramer and Chardonnens, 2001). Apart from genetic engineering, traditional selection methods are also proposed to develop plants highly effective for phytoextraction. In particular, high accumulating capacity has been shown for cross-species poplar hybrids (Ma et al., 2004).

MATERIALS and METHODS

Research Subject

Myosotis alpestris F.W. Schmidt is a biennial plant. At the end of growing season we set the plants of the first year at the vegetative stage of development, and in 2021 – plants of the second year at the budding, flowering and bearing stages.

Research Design and Venue

The experiment was conducted on the industrial site of the Kovdorski GOK (Kovdor Ore-Mining Integrated Works), owned by the EuroChem Group. Wastewater there contains residues of calcium and iron hydroxides (used for the primary deposition of metal cations) as well as compounds of nitrogen, phosphorus and sulfur available for plants. In 2020-2021 we assessed the content of heavy metals in plants on phyto-rafts made in the form of rectangular sheets of polyethylene foam with slots for plants. The rafts were fixed in running water at the beginning of the outlet channel, through which wastewater is discharged after primary purification by means of deposition using calcium and iron hydroxides.

The content of iron, molybdenum, manganese, vanadium, magnesium and strontium were determined by atomic emission spectrometry after preliminary ashing of the samples. Plants were dried at a temperature of 105 °C. Samples from each location were divided into 10 weighed portions. The metal content was determined using an inductively coupled plasma emission spectrometer ICPE 9000, manufactured by Shimadzu-Corporation (Japan) at the Environmental Control Laboratory Regionlab (St. Petersburg). Mineralization of the samples was carried out according to the following method: a sample of 1 g was heated to a temperature of 95 °C with 20 mL of concentrated nitric acid (EUROCHEM-11125.F01080) and 5 mL of 33% hydrogen peroxide (LNN-003.F01150K) to the state of wet salts, then another 5 mL of concentrated nitric acid and 20 mL of water were added and leached until the precipitate dissolved. After cooling, the sample was put into a volume of 100 mL of bidistilled water. The residual suspension was removed using a filter with a blue ribbon. measurements were carried out in parallel with a “blank” sample. The results were compared with each other and interpreted. During the analysis, we used state standard samples (SSS) of metal solutions of the following grades: Zn - SSS 7256-96 (Ural plant of chemical products, Russia); Cd—SSS 6690-93, Cu—SSS 7998-93, Pb—SSS 7012-93 (Samples and High-purity Substances, Russia).

The samples were analyzed for the content of metal ions such as V, Mn, Fe, Cu, Sr, Mg, Mo, Zn, since the concentration of these metals is subject to industrial environmental control.

Since setting up a reference sample for the experiment was impossible in this case, in order to evaluate the accumulation process, we used the plants grown in the botanical garden of Murmansk Arctic University before planting them on phytorafts. The content of elements from the garden (reference samples) is provided in the table with the study results.

The metal content in the underground part was assessed without any pre-treatment of the roots before ashing. Thus, it included the metals contained inside cells, metals sorbed on the surface of root hairs, as well as metals of insoluble particles retained between small roots.

RESULTS and DISCUSSION

The results of studying the accumulation of heavy metal ions in the organs of alpine forget-me-not grown on wastewater are presented in Tables 1 and 2.

Table 1. *Content of metal ions in above-ground plant organs (mg.kg^{-1} of dry mass)*

Above-ground organs	Harvesting year	V	Mn	Fe	Cu
mg.kg^{-1} of wet mass	2020	4.96±0.46*	4777±512	253.3±19.12	128.2±10.81
	2021	7.64±0.64	8740±791	391±40	186.3±1.63
mg.kg^{-1} of dry mass	2020	29.2±1.89	28100±1980	1490±132	753±54
	2021	33.2±2.71	38000±2900	1700±153	810±69
Reference sample, mg.kg^{-1} of dry mass		-	705±23.6	45.3±3.92	6.2±0.31
Above-ground organs	Harvesting year	Sr	Mg	Mo	Zn
mg.kg^{-1} of wet mass	2020	22.44±1.81	716±6.32	157±12.34	21.9±1.91
	2021	27.6±1.9	920±80.2	317.4±28.3	27.4±2.3
mg.kg^{-1} of dry mass	2020	132±11	4215±289	923±72	129±9.3
	2021	120±9	4000±312	1380±120	119±10.2
Reference sample, mg.kg^{-1} of dry mass		-	52±3.8	2.3±0.08	4.2±0.19

*: mean+ standard deviation

Table 2. *Content of metal ions in above-ground plant organs (mg.kg^{-1} of dry mass)*

Roots	Harvesting year	V	Mn	Fe	Cu
mg.kg^{-1} of wet mass	2020	6.98±0.51*	1151±112	2941±191	203±18
	2021	12.83±9.12	1365.3±109	2960.2±192	196.8±16.31
mg.kg^{-1} of dry mass	2020	17.9±1.3	2950±195	7540±612	520±39
	2021	31.3±2.7	3330±273	7220±615	480±32
Reference sample, mg.kg^{-1} of dry mass		-	23.3±1.92	41.3±3.81	8.3±0.72
Roots	Harvesting year	Sr	Mg	Mo	Zn
mg.kg^{-1} of wet mass	2020	206.3±17.2	3159±281	280.9±21.2	36.3±2.91
	2021	200.9±18.3	3157±194	241.9±18.1	41.8±3.2
mg.kg^{-1} of dry mass	2020	529±34	8100±681	720±54	93±6.2
	2021	490±38	7700±612	590±42	102±8.2
Reference sample, mg.kg^{-1} of dry mass		24.3±1.92	63.3±5.12	5.3±0.35	8.7±1.02

*: mean+ standard deviation

Noteworthy is the extremely high content of manganese in the above-ground parts of the forget-me-not, recorded in 2021. The accumulation of metal in the above-ground part indicates high binding strength and allows us to consider this plant to be one of the most promising for treating wastewater from this metal.

The vanadium content in forget-me-not organs certainly seems to be less significant compared to other metals (manganese, magnesium, copper, etc.). However, given the minor presence of this metal in wastewater as well as its rarity and increasing value, it can be assumed that phyroextraction of vanadium from wastewater can be considered for both, treatment and obtaining the metal from ore-mining wastes.

We noted stable accumulation of iron ions, especially in the roots. Forget-me-not also absorbs quite actively vanadium. Meanwhile, molybdenum accumulation is very unstable

Analysis of heavy metal ions content in wastewater showed that concentrations can vary significantly throughout the year (table 3).

***Table 3**. Content of heavy metals in Kovdorski GOK wastewater (mg.l^{-1})*

Sampling period	Cu	Mg	Mn	Mo
March 2021	0.0087±0,0009*	23.1±1.9	0.0172±0.001	0.0108±0.002
June 2021	0.0145±0,001	24.7±1.6	0.0575-0.004	<0.005
September 2021	0.0115±0,001	15.7±1.3	<0.005	<0.005
December 2021	0.0091±0,001	12.7±1.3	<0.005	<0.005

*: mean+ standard deviation

Summer represents the most important period for plants it terms of metals content in wastewater. At the same time, in winter, when plants are dormant, metal ions can be accumulated in the bottom sediments of the secondary settling tank, from where they can subsequently be extracted by plant roots. Meanwhile it is worth mentioning that in different seasons, the concentration of metals in wastewater appears to be low and is often below the method' resolution capability.

The most intensive accumulation in the organs of Alpine forget-me-nots appeared to be for physiological or essential metals, which is explained by their physiological role and the presence of membrane mechanisms for their intensive absorption. In terms of hyperaccumulation criteria (Baker and Brooks, 1989; Brooks, 1994), we can refer it in relation to iron, magnesium and manganese, especially when calculated for dry mass. Thus, this species may well be considered for the purpose of additional obtaining of metals from wastewater (Carpena and Bernal, 2007), and not just for water treatment.

At the same time, it is in the above-ground parts where manganese accumulates more intensively which can be explained by its important role in the photolysis of water during the process of photosynthesis. Meanwhile, iron and magnesium accumulate mainly in the roots. The role of these elements is more diverse and their high concentrations are attributable to strong binding of cations by pectins in the cell walls of root cells, as well as to relatively low mobility. Some authors, who discovered in the course of their research a high metal content in the roots, note that such results do not yet indicate the absorption of metals into the root cells, but rather indicate the strong retention of soil particles on the root surface (Reeves and Kruckeberg, 2017). This surface binding can be very strong and the separation of metals from the root is possible only with the use of specific methods. In our opinion, to assess the possibilities of using plants for wastewater treatment, it is not the path, depth and mechanism of metal penetration that is more important, but the very possibility of transferring metals from a pool of dissolved water ions to a pool of ions bound in one way or another.

In our opinion, variations in metals accumulation in different years are explained by a number of factors: fluctuations in the concentration of wastewater metals, differences in weather conditions, and the exchange of metal ions between wastewater and bottom sediments. Changes in the effect of some biotic factors on plants in different years are also possible. For example, Durand et al., 2024 point out that it is the organisms of the rhizosphere and the presence of other microorganisms that have the greatest influence on the processes of accumulation of metals by plants, and parameters such as the concentration of metals and essential elements (nitrogen, phosphorus, potassium) are of secondary importance.

For metals such as zinc, copper, vanadium, strontium and molybdenum, it was not possible to detect the ability to hyperaccumulate in the studied species. The reasons for this may also be diverse: perhaps this is due to the low permeability of the membranes of the forget-me-not root cells to these metals. In addition, one of the reasons may be the presence of many other pollutants in the wastewater. So, Sherri et al., 2024 point to this reason as one of the most important for individual groups of plants.

CONCLUSION

In general, Alpine forget-me-not or Myosotis alpestris seems to be a fairly promising plant for additional wastewater treatment from metal cations in high latitudes. Its ample capability to accumulate metals goes well with unfastidiousness in terms of environmental conditions as well as with the ability to grow in wastewater and pronounced alkaline reaction. The shown accumulation of various metals in above-ground organs indicates the reliability of binding and the low probability of desorption when vegetating on phyto-rafts.

ACKNOWLEDGEMENTS

We thank the anonymous referees for their contributions to improve the manuscript.

AUTHOR CONTRIBUTIONS

The authors contributed equally to this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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