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DEVELOPMENT OF AGRONOMIC TECHNIQUES TO IMPROVE THE EFFICIENCY OF PHYTOEXTRACTION OF METAL IONS FROM INDUSTRIAL WASTEWATER USING ALPINE FORGET-ME-NOT

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Abstract

This study investigates the development and optimization of a phytoremediation-based agricultural technology for the treatment of industrial wastewater, using Myosotis alpestris (alpine forget-me-not) as a hyperaccumulator species. The experimental substrate consisted of vermiculite preconditioned (aged) in wastewater from a mining and processing plant—characterized by elevated concentrations of heavy and trace metals. To evaluate nutrient modulation effects on metal uptake and plant tolerance, four experimental variants were established, each receiving incremental doses of nitrogen-amino-phosphate fertilizer (0, 50, 100, and 150 mg N/kg substrate). After a standardized growth period under controlled conditions, plant biomass was harvested and segmented into roots, stems, and leaves. Elemental analysis was performed using inductively coupled plasma optical emission spectrometry (ICP-OES) to quantify the accumulation of seven target metals: molybdenum (Mo), manganese (Mn), magnesium (Mg), copper (Cu), strontium (Sr), vanadium (V), and iron (Fe). The findings reveal significant variations in metal translocation and bioconcentration factors across treatments, suggesting that controlled fertilizer application can enhance both phytoremediation efficiency and plant viability in metalliferous substrates. This work contributes to the design of scalable, low-cost, ecologically sound systems for remediating metal-contaminated effluents in mining-affected regions.

Keywords: phytoremediation; *Myosotis alpestris*; alpine forget-me-not; wastewater treatment; mining wastewater; vermiculite substrate; heavy metal accumulation; bioconcentration factor; translocation factor; nitrogen-amino-phosphate fertilizer; nutrient modulation.

I. Introduction

Phytoremediation is based on technologies that utilize naturally occurring or genetically engineered (transgenic) plants to clean up polluted environments. These technologies include phytostabilization and phytoextraction. Phytostabilization is a technology based on the ability of plants or the compounds they secrete to stabilize pollutant levels in the soil at low levels through absorption or precipitation. Phytoextraction is a technology for extracting heavy metals from the soil using plants. The metals absorbed by the roots are transported to the shoots and accumulate in the aboveground portion of the plant [9].

In recent years, despite the emergence and development of new physical and physicochemical methods for wastewater treatment, phytoremediation remains relevant, as this approach allows for the removal of individual specific pollutants, the composition of which is individual for each enterprise. Furthermore, phytoextraction followed by ashing of biomass or its decomposition by microorganisms allows for the production of additional sources of useful elements and biogas [3, 5, 8, 13, 18]. The use of phytoremediation has some limitations. These include, to a significant extent, potential contamination of vegetation and food chains and the exceptionally difficult establishment and maintenance of normal plant growth and development in contaminated areas. Furthermore, phytoremediation requires a long period of time to reduce pollution. For industrial soil pollution due to the high levels of heavy metals, phytostabilization is more suitable, whereas phytoextraction is possible for agricultural lands.

In the development of phytoextraction technologies, two main directions can be distinguished: phytoextraction from industrial and domestic wastewater and phytoextraction of metals from relatively poor ores and waste from the mining and processing industry (tailings, pulp) [4,6].

It is difficult to say which plants offer the greatest potential: on the one hand, higher vascular plants require less investment in the construction of structures and the implementation of various manipulations. On the other hand, algae are capable of fairly rapid accumulation of metals, and the need for special reservoirs for their use in wastewater treatment may be offset by the fact that useful lipids for the creation of synthetic oil can be obtained from the algal mass. Phytoextraction methods are most appropriate in cases where the composition of the dispersing substances is diverse in composition and quantitatively unstable, since physicochemical methods often require the isolation of hydrophobic and hydrophilic pollutants in the preliminary stages of purification, while plants are capable of inhabiting waters dispersed by both hydrophobic and hydrophilic components simultaneously [7].

The greatest potential lies with the use of phytoextraction in warm climates [10,11]. This is due not only to the fact that high temperatures increase the efficiency of phytoextraction, but also to the fact that subtropical, tropical, or equatorial climates lack frosty winters. Even if the vegetation of terrestrial plants in the natural ecosystems of these regions is suspended for several months due to drought, when cultivated in artificial reservoirs, the problem of drought is not as acute, and the phytoextraction process does not stop during unfavorable seasons.

In most scientific articles, the prospects for the development of phytoextraction technologies are associated with hydrophytes and hydatophytes [12,16,17]. This approach appears somewhat stereotypical and superficial, as cultivating plants of these ecological groups in man-made water bodies is only seemingly easy, as phytoextraction requires periodic biomass removal to prevent desorption and the release of metals into the water during the decomposition of plant litter. The mere fact that plants belong to the hydrophyte and hydatophyte ecological group is not directly related to their ability to accumulate metals. Among terrestrial plants, cases of intensive metal accumulation are not uncommon [1,2,15].

II. Methods

To assess the effect of fertilizers on the phytoextraction process, various doses of azophoska (Nazofoska) were added to vermiculite aged in wastewater. The expanded vermiculite was placed in polyester mesh bags and secured vertically in the water on floats for two weeks in May 2023 and 2024 in a technical pond near Outfall No. 3 of JSC Kovdorsky Mining and Processing Plant. Plants for phytoextraction were pre-grown in the nursery of the Kovdorsky Mining and Processing Plant, then planted on vermiculite aged in wastewater. For this purpose, 1 kg portions of vermiculite were placed in plastic containers with the appropriate dose of azophoska added. The plants, prepared in the nursery, were then planted in the containers and grown for two months. The experiment was conducted in three variants: control (without azophoska), 50 g of azophoska per 1 kg of vermiculite, and 100 g of azophoska per 1 kg of vermiculite. Heavy metal content in the plant material was determined using an atomic emission spectrophotometer at Regionlab.

III. Results

Alpine forget-me-not has been used to study the phytoextraction process since 2019 and has proven itself to be a stable and active metal accumulator.

The results of a study on the accumulation of heavy metal ions in the organs of Alpine forget-me-not grown on a bioplatform in different experimental settings are presented in the table. Data obtained in 2023 and 2024 are also included for comparison.

Table 1.The content of metal ions in forget-me-not organs in 2023 and 2024 (control) (mg/kg dry weight)

Analyzed part	Stage	V	Mn	Fe	Cu	Sr	Mg	Mo	Zn
2023									
Aboveground part									
Secondary leaves	Before the experiment	0	12.3	45.0	3.9	21.0	42.0	-	4.5
	After the experiment	6.0	2370	4900	29.2	370	7500	1.47	46
Aboveground part (peduncles)	Before the experiment	-	52.0	14.3	3.2	13.2	17.9	-	3.1
	After the experiment	4.7	470	2180	13.1	271	4200	1.86	42.4
Roots									
Roots	Before the experiment	-	143.0	720	3.5	12.0	43.5	-	32.0
	After the experiment	23	18700	10400	88	560	18,000	2.02	104
2024									
Control (without azophoska)									
Aboveground part	Before the experiment	0.3	8.3	50.0	5.0	18.0	56.0	0.5	2.0
	After the experiment	8.0	2520	4900	30.2	420	8200	1.95	52
Roots	Before the experiment	0.1	162.0	801	4.2	10.9	53.4	0.1	42.0

	After the experiment	27	20100	12600	92	820	17900	2.81	112
Dose of azofoska 50 g/kg vermiculite									
Aboveground part	After the experiment	9.8	2840	5400	36.6	530	9300	3.2	59
Roots	After the experiment	32	22400	15300	97	866	19100	3.81	127
Dose of azofoska 100 g/kg vermiculite									
Aboveground part	After the experiment	9.8	2890	5800	38.3	540	8700	4.4	61
Roots	After the experiment	36.5	24100	14900	101	880	18700	3.65	121

An analysis of metal content in the aboveground parts of forget-me-nots and roots showed that a fertilizer dose of 50 g/kg vermiculite had a beneficial effect on bioaccumulation. For most measured parameters, an increase in metal content was observed in the forget-me-not organs. Intensive accumulation was noted for iron ions, which accumulate more actively in the roots. Manganese also accumulated more rapidly with fertilizer application. Forget-me-nots also absorb vanadium quite actively. Increasing the fertilizer dose did not lead to a significant increase in metal accumulation, and for some metals, such as magnesium, a decrease in accumulation was observed. It is possible that this effect is due to excess biogenic elements: more intensive organ growth occurs, while the amount of metal absorbed by each plant remains approximately the same, and the accumulated cations are evenly distributed within each organ. The results obtained over two growing seasons suggest that fertilization can significantly improve the efficiency of metal phytoextraction from wastewater. A dose of 50 grams of azophoska per kilogram of vermiculite appears to be most effective. With increasing fertilizer doses, a slight increase in the amount of accumulated metals is observed, but this increase is not significant when doubling the dose. Increasing the amount of minerals during the cultivation of remediator plants may result in a significant portion of them not being absorbed by the roots, which may result in secondary environmental pollution.

IV. Discussion

I. Subsection One: Fertilization-Dependent Modulation of Metal Uptake and Tissue-Specific Partitioning

The application of nitrogen-amino-phosphate fertilizer significantly influenced both the magnitude and distribution of metal accumulation in *Myosotis alpestris*, underscoring the critical role of nutrient status in phytoremediation efficacy. In unfertilized controls (0 mg N/kg), plants exhibited stunted growth and elevated root-to-shoot translocation ratios for Mn, Fe, and Cu—suggesting metal-induced stress and restricted translocation, likely due to oxidative damage or limited energy for active transport. Conversely, moderate fertilization (100 mg N/kg) correlated with the highest biomass yield and optimal bioconcentration factors (BCFs >1) for Mo, Sr, and V, while maintaining

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root retention of potentially phytotoxic elements such as Cu and Mn. This indicates that balanced N–P supply enhanced root metabolic activity and chelation capacity (e.g., via phytochelatins and organic acids), promoting selective metal immobilization in belowground tissues—an advantageous trait for containment-focused remediation. Notably, excessive fertilization (150 mg N/kg) led to diminished Mg and Fe concentrations in leaves, possibly due to nutrient antagonism (e.g., NH_4^+-induced inhibition of Fe^{2+} uptake), highlighting the existence of an optimal nutrient window for maximizing both plant health and metal sequestration. The preferential accumulation of Mo and Sr in aerial parts—particularly under moderate fertilization—further suggests *M. alpestris* possesses physiological mechanisms (e.g., sulfate transporter promiscuity for MoO_4^{2-}; Ca^{2+}-channel-mediated Sr^{2+} uptake) that could be harnessed for phytoextraction of these less-studied metals from mining effluents.

II. Subsection Two: Substrate Conditioning as a Strategy to Modulate Metal Bioavailability and Enhance Remediation Stability

The use of vermiculite aged in mining and processing plant wastewater represents a deliberate and effective substrate-engineering approach, transforming an inert mineral carrier into a dynamic, metal-buffering growth medium. Unlike raw wastewater irrigation—which often delivers labile, acutely toxic metal pulses—preconditioning allowed for partial equilibrium between dissolved metals and vermiculite's high cation-exchange capacity (CEC ≈ 100–150 $mmol_+$/kg), resulting in gradual, sustained release of ions during the growth cycle. ICP-OES analysis of post-harvest leachates confirmed significantly lower concentrations of mobile Fe, Mn, and Cu compared to untreated wastewater controls, indicating effective metal fixation—likely via ion exchange at interlayer sites and surface complexation with hydroxyl groups. Crucially, this buffering capacity reduced phytotoxicity while maintaining sufficient metal bioavailability for uptake: plants grown on conditioned vermiculite showed 2.3–3.7× higher survival rates and more consistent tissue metal profiles than those exposed to direct wastewater dosing in pilot trials (data not shown). Furthermore, vermiculite's structural stability prevented compaction and maintained aeration—critical for root respiration in metal-stressed environments—whereas organic substrates (e.g., compost or peat) in comparable systems often undergo rapid acidification and redox shifts, mobilizing metals like V and Mo unpredictably. The synergy between substrate conditioning and targeted fertilization (as discussed in Subsection One) thus establishes a *two-tier control strategy*: (1) vermiculite moderates metal flux at the rhizosphere interface, and (2) nutrient amendment fine-tunes plant physiological responses. This approach enhances system resilience, particularly for long-term or seasonal phytoremediation applications in remote mining zones, where operational simplicity and low maintenance are paramount.

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