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BIOTECHNOLOGICAL VALORIZATION OF *ARTHROSPIRA PLATENSIS* FOR HEAVY METAL AND RARE EARTH ELEMENT RECOVERY FROM MULTIMETALLIC EFFLUENTS

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Abstract

This study aimed to evaluate the bioaccumulation capacity of the cyanobacterium Arthrospira platensis for heavy metals and rare earth elements (REEs) from mono- and polymetallic effluents. Experimental systems were enriched with metals such as Ni, Cu, Zn, Cr, Fe, Sr, Mo, and REEs (Dy, La, Tb, Nd, Sm, Yb) at various concentrations. Metal accumulation in biomass was quantified using neutron activation analysis analysis. Biomass productivity was assessed spectrophotometrically based on a calibration curve, while oxidative stress was evaluated via malondialdehyde (MDA) content using the TBARS assay. Results demonstrated high accumulation capacities, with notable values for Cu (2870 mg/kg), Zn (up to 22500 mg/kg), Ni (1310 mg/kg), and La (>90% recovery). Biomass growth and MDA levels varied depending on the metal combination and concentration, indicating stress responses. A. platensis showed consistent resilience and was effective as a biosorbent, especially under moderate oxidative stress. The findings highlight its applicability in the bioremediation of wastewater containing diverse metal contaminants and its potential use as a matrix for biofortified supplements.

Keywords: ***Arthrospira platensis, bioaccumulation, heavy metals, oxidative stress, rare earth elements.***

INTRODUCTION

Environmental pollution by toxic metals and rare earth elements (REEs) is an escalating global challenge, largely driven by urbanization, industrial growth, and unsustainable resource use. As a result, hazardous substances, including heavy metals and REEs, are increasingly released into natural ecosystems, with water resources being especially vulnerable. Less than 3% of the world's water is

drinkable, and millions of tons of waste, including hundreds of tons of heavy metals, enter aquatic systems annually [1].

Research priorities have shifted from municipal and agricultural wastes to more complex contaminant mixtures, including microcontaminants, plastics, persistent organics, and REEs, all of which threaten environmental stability [2]. Effective management now requires innovative, sustainable solutions that move beyond traditional, energy-intensive physico-chemical treatments [3-6]. Biotechnological approaches, particularly bioremediation, offer eco-friendly and cost-effective alternatives by utilizing the metabolic potential of microorganisms to remove or transform pollutants.

Phototrophic cyanobacteria such as *Arthrospira platensis (Spirulina)* are promising bioremediators due to their minimal environmental requirements, mainly CO_2 and light [7-9], and high metabolic versatility. These microorganisms can efficiently accumulate and transform a broad spectrum of metal ions, including REEs, and their resulting biomass can be valorized for use as fertilizers or sources of bioactive compounds.

The application of *A. platensis* for metal and REE recovery aligns with recent advances in metabolic engineering and systems biology, enabling improved bioremediation efficiency and product valorization [10]. Its ability to thrive in diverse environments and its GRAS status further support its potential as a sustainable resource for wastewater treatment and resource recovery.

This study investigates the biotechnological valorization of *Arthrospira platensis* for recovering heavy metals and rare earth elements from multimetallic effluents, with the goal of developing integrated processes for both environmental remediation and biomass utilization.

MATERIALS AND METHODS

*Object of Study.*The object of study was the cyanobacterium *Arthrospira platensis*, a well-characterized strain (CNMN–CB-02) obtained from the National Collection of Nonpathogenic Microorganisms (Chisinau, Republic of Moldova).

Experimental Designs. To comprehensively evaluate the bioaccumulation capacity and physiological responses of *A. platensis*, four experimental models were established, each simulating different types of multimetallic contamination or supplementation scenarios relevant to environmental and biotechnological applications:

1. Nickel-Based Multimetallic Effluent Model: *A. platensis* was cultivated in mineral medium supplemented with nickel as a single metal or in combination with chromium, iron, copper, zinc, strontium, and molybdenum. Cultures underwent four consecutive cultivation cycles to simulate long-term exposure and adaptation to multimetallic synthetic effluents.

2. Zinc-Based Multimetallic Systems: Cultivation was performed in four systems: (i) zinc alone, (ii) zinc with copper and strontium, (iii) zinc with copper and nickel, and (iv) zinc with copper, strontium, and barium. In each case, three increasing concentrations of metal ions were added on the fifth day of growth, at the onset of the stationary phase. The exposure lasted for 24 hours before biomass harvesting.

3. Rare Earth Element (REE) Model: *A. platensis* was exposed separately to dysprosium (Dy), samarium (Sm), terbium (Tb), lanthanum (La), neodymium (Nd), and ytterbium (Yb), each at three concentrations (10, 20, and 30 mg/L). Cultures were maintained for five days, after which the biomass was harvested for analysis.

4. Lithium Bioaccumulation Model: Lithium carbonate (Li_2CO_3) was introduced to the growth medium at concentrations ranging from 5 to 500 mg/L, either at inoculation or during the exponential growth phase, to investigate lithium enrichment and its physiological impact.

Analytical Methods

Following cultivation, the biomass was collected, washed, and dried for subsequent analyses. Metal accumulation in the biomass was quantified using neutron activation analysis (NAA) for transition metals and rare earth elements, and Proton Induced Gamma Emission (PIGE) analysis for lithium. Total protein content was measured spectrophotometrically by the Lowry method. The malondialdehyde (MDA) content was measured using the thiobarbituric acid reactive substances (TBARS) assay. All experiments were conducted in at least three replicates, and results were evaluated for statistical significance.

RESULTS AND DISCUSSION

This study comprehensively evaluated the bioaccumulation capacity and biochemical responses of Arthrospira platensis under exposure to a wide spectrum of metals, including transition metals, rare earth elements, and lithium, using four complementary experimental models.

Heavy Metal and Multimetallic Effluent Exposure. The first two experimental models focused on the ability of A. platensis to accumulate nickel, zinc, and other metals from mono- and multimetallic synthetic effluents. The results, summarized in Table 1, show that both nickel and zinc were efficiently accumulated from all tested systems, with the highest nickel content (1310 mg/kg) observed in the Ni/Cr/Fe variant and maximum zinc accumulation (up to 23-fold over control) in certain zinc-based mixtures. Alongside nickel and zinc, substantial uptake of additional metals - chromium, iron, copper, strontium, barium, and molybdenum, was observed depending on system composition. Notably, in most systems, accumulation peaked in the first or second cultivation cycle, then

stabilized or declined, reflecting both high initial uptake and subsequent cellular adaptation or efflux.

Exposure to metal mixtures led to a transient reduction in biomass productivity (up to 12% after the first cycle), but yields typically recovered in subsequent cycles, indicating physiological adaptation. Total protein content showed a similar dynamic: initial declines (up to 35% in some multi-metallic systems) were followed by partial or full recovery with prolonged cultivation. MDA levels, measured by the TBARS assay, increased during the initial exposure cycles (up to 2.5-fold), especially under multi-metallic stress, but subsequently decreased, indicating an enhanced antioxidative response in adapted cells.

Table 1. Metal bioaccumulation, protein content, and MDA Levels in *Arthrospira platensis* exposed to mono- and multimetallic systems

Element /elements	**Metal concentration, mg/l**	**Total proteins % of biomass**	**MDA, nM/g of dry biomass**	**Bioaccumulation capacity, mg/kg of biomass**
Zn	2,5-10,0	64.1 – 71.4	12.0 – 14.8	90 - 16000
Zn/Cu/Sr	Zn 2,5-10,0 Cu 1,0-5,0 Sr 0.5-2,0	42.2 – 65.3	15.2 – 20.7	Zn 250.0-20000.0 Cu 125.0-500.0 Sr 5.0-50.0-
Zn/Cu/Ni	Zn 2,5-10,0 Cu 0,5-2,0 Ni 0.5-2,0	43.2 – 66.0	15.2 – 18.9	Zn 750.0-20500.0 Cu 60.0-250.0 Ni 40.0-240.0
Zn/Cu/Sr/Ba	Zn 2,5-10,0 Cu 0,5-2,0 Ni 0.5-2,0 Ba 0,5-1,0	43.1 – 66.0	15.0 – 15.9	Zn 120.0-22500.0 Cu 125.0-500.0 Sr 5.0-50.0 Ba 60.0-25.00
Ni	2,5-10	52.5 - 71.4	10.4 – 16.5	50-250
Ni/Cr/Fe	Ni 2,5-10 Cr 1,0-5,0 Fe 1,0-5,0	51.0 – 65.4	15.8 – 20.8	Ni 50.0-1310.0 Cr 1.05-110.0 Fe 2500.0-17000.0
Ni/Cu/Sr/Zn	Ni 2,5-10 Cu 0,5-1,0 Sr 1,0-5,0 Zn 0,5-2,0	51.8 – 72.1	17.2 – 28.4	Ni 60.0-51.00 Cu 70.0-820.0 Sr105.0-1370.0 Zn 20.0-1860.0
Ni/Cu/Zn/Mo	Ni 2,5-10 Cu 1,0-5,0 Zn 0,5-2,0 Mo 0,5	40.2 – 64.3	15.8 – 44.6	Ni 40.0-480.0 Cu 50.0-2870.0 Zn 20.0-1750.0 Mo 3,5-4,4

Rare Earth Element (REE) Bioaccumulation. In the third experimental model, A. platensis demonstrated selective and efficient bioaccumulation of rare earth elements, particularly La and Dy (up to 39.8 and 25.5 mg/g, respectively),

with removal efficiencies exceeding 85% even at elevated concentrations. Other elements (Sm, Nb) were accumulated at moderate levels, while Tb and Yb showed limited uptake. REE exposure had minimal impact on biomass productivity and total protein content, except for high ytterbium concentrations, which reduced growth and increased oxidative stress (MDA up to 60% above control). For most REEs, MDA and protein content remained near control values, suggesting low toxicity under tested conditions (Table 2).

Table 2. RREs bioaccumulation, protein content, and MDA levels

Element	Element concentration, mg/l	Total proteins % of biomass	MDA µM/L	Bioaccumulation capacity, %
La	10-30	64 - 66	1.10 – 1.30	88-95%
Dy	10-30	66 - 70	1.20 – 1.60	85-90%
Tb	10-30	62 - 65	0.70 – 0.95	<20%
Yb	10-30	62 - 66	2.00 – 2.10	<20%
Sm	10-30	59 - 62	1.10 – 1.35	42-97%
Nd	10-30	60 - 66	1.75 – 1.90	46-86%

Lithium Enrichment. The final model assessed lithium enrichment of *A. platensis* via bioaccumulation. The strain displayed high tolerance to lithium, with stable biomass production across the tested concentration range (5–500 mg/L). Lithium content in the biomass increased proportionally to medium concentration, reaching 2.1–2.5 mg/g dry weight. Importantly, total protein content remained stable (60–66% of biomass), and MDA increases (11–141% over control) were moderate and within safe dietary limits.

Collectively, these results highlight the remarkable adaptability of *A. platensis* to a range of metallic stressors. The organism combines efficient metal bioaccumulation with the ability to maintain, or quickly restore, key physiological parameters such as protein content and oxidative balance. These properties, together with the specific selectivity for different metal ions demonstrated in each experimental scenario, underline its potential for biotechnological applications in the remediation of multimetallic wastewaters and the production of functionalized biomass with added nutritional or technological value.

CONCLUSIONS

This study demonstrates that *Arthrospira platensis* is highly adaptable and effective in accumulating a broad range of metals, including heavy metals, rare earth elements, and lithium, from both mono- and multimetallic systems. Despite initial reductions in biomass productivity and protein content under metal stress, the strain exhibited strong physiological adaptation, with growth and protein levels recovering over time.

The results highlight the organism's high metal uptake capacity and resilience, enabling not only efficient bioremediation of complex, metal-rich effluents but also the production of protein-rich biomass with retained nutritional value. Notably, lithium enrichment did not compromise biomass yield or protein synthesis, further supporting its application in functional food development.

Overall, *A. platensis* stands out as a robust candidate for sustainable bioremediation and biotechnological valorization, combining metal recovery with the generation of valuable biomass for diverse applications.

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