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Impact of *Arthrospira platensis* Morphology on Growth Performance and Zinc Bioaccumulation

Avisha Samimiazad[1], Saeed Mirdamadi[2]*, Abbas Akhavan Sepahi[3], Marjaneh Sedaghati[1], Maliheh Safavi[2]

[1] *Department of Food Science and Technology, NT.C., Islamic Azad University, Tehran, Iran.*
[2] *Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Iran.*
[3] *Department of Microbiology, NT.C., Islamic Azad University, North Tehran Branch, Tehran, Iran.*

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Abstract

The cyanobacterium *Arthrospira platensis* has garnered significant attention for its biotechnological applications; however, morphological and physiological variations among different species can considerably influence their industrial viability. This research provides a detailed comparative analysis of two variants of *A. platensis* (designated as L and S), which exhibit differing trichome morphologies—linear and spiral, respectively. The study investigates their molecular identity, growth characteristics, and resilience to zinc-induced stress. Notably, strain S, characterized by spiral trichomes, exhibited enhanced biomass production (OD590 = 2.6 ± 0.04). In contrast, strain L, with linear trichomes, demonstrated reduced growth (OD590 = 1.8 ± 0.03) and impaired metabolic efficiency at high zinc concentrations, suggesting reduced stress adaptability *($p < 0.05$).* Molecular identification through 16S rRNA sequencing confirmed both strains as *A. platensis*, showing 99% similarity to type strains in the NCBI database. Both morphologies exhibited concentration-dependent zinc enrichment, with enrichment factor (EF) values reaching 17.03 for strain S and 16.73 for strain L at a zinc concentration of 11 mg L^{-1}. The superior zinc accumulation capacity of the spiral morphotype is likely attributable to a combination of structural and physiological factors. These results underscore the significant influence of trichome morphology on stress tolerance, positioning morphology S as a promising candidate for bioaccumulation and large-scale cultivation. This study offers valuable insights into strain selection for industrial applications.

1. Introduction

Arthrospira platensis is a filamentous, photosynthetic cyanobacterium known for its high protein content and its capacity to succeed in extreme environmental conditions. The morphological plasticity of this organism, particularly in the arrangement of its trichomes (which can be either spiral or linear), is essential for its physiological performance under stress (Roy *et al.*, 2025; Samimiazad *et al.*, 2025). Among the various species, *A. platensis* is recognized as a model organism for large-scale cultivation due to its remarkable adaptability to extreme environments, including alkaline and saline waters, as well as its metabolic flexibility in response to stress conditions. However, the intraspecific variability, particularly concerning morphological characteristics such as trichome coiling, helix pitch, and gas vacuole distribution, remains poorly understood. This lack of understanding raises important questions regarding the implications of such variability for growth dynamics, stress resistance, and industrial productivity (Gómez *et al.*, 2021; Wang *et al.*, 2018). *A. platensis* undergoes cell elongation through the process of cell division, while its reproduction occurs through fragmentation (Chaiyasitdhi *et al.*, 2018; Roy *et al.*, 2025). Straight trichomes are observed to occur spontaneously in both laboratory and outdoor cultures. The ratio of spiral to straight trichomes in mixed populations is influenced by the specific conditions of the culture (Jeeji-Bai, 1985). Dimorphism in *A. platensis* is significantly influenced by various environmental factors, including light intensity, temperature, salinity, and mechanical stress (Bendezu Najarro, 2024; Zanolla *et al.*, 2022). Spiral trichomes revert to linear configurations under suboptimal conditions. This phenomenon is believed to result from genetic drift or environmental adaptation (Bendezu Najarro, 2024; Guenachi *et al.*, 2025; Zapata *et al.*, 2021). These morphological changes are not merely superficial; they have significant implications for buoyancy, light absorption, and resistance to photoinhibition, ultimately affecting biomass yield (Sili *et al.*, 2012). Furthermore, molecular investigations have identified distinct genotypic clusters within the genus *Arthrospira*, yet the relationship between genetic divergence and phenotypic plasticity remains unclear (Guenachi *et al.*, 2025).

The physiological resilience of *A. platensis* is a significant area of research. This alkaliphilic cyanobacterium employs unique osmoregulatory

*Corresponding author E-mail: mirdamadi@irost.ir
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mechanisms, including the accumulation of trehalose and glucosyl-glycerol, to withstand salinity stress (Hagemann, 2011). Its photosynthetic system demonstrates adaptations to high light and oxidative stress through processes such as non-photochemical quenching and the turnover of the D1 protein (Gorbunov *et al.*, 2011; Wu *et al.*, 2011). However, the presence of strain-specific variations in stress responses highlights the need for comparative studies aimed at optimizing cultivation conditions. Although studies have been conducted on the characteristics of *A. platensis* dimorphism, its effect on biosorption is a topic that has received less attention.

This study aims to bio accumulation selection by synthesizing morphological, physiological, and molecular data, thereby contributing to a comprehensive understanding of cyanobacterial adaptation.

Therefore, elucidating the morphotype-specific stress responses undertaken in this study is a necessary step toward informing strategies designed to stabilize productivity in outdoor mass cultures against environmental fluctuations.

2. Materials and Methods

2.1. Strain cultivation and preparation

Two morphotypes of *Arthrospira platensis* were used: Morphotype L (dominant linear trichomes) and morphotype S (spiral trichomes), sourced from the Biotechnology Research Institute of the Scientific and Industrial Research Organization of Iran. Cultures were maintained in Zarrouk's medium (pH 9.0, 1.5% agar for solid subculturing) under a 16/8-h light-dark cycle at 25 ± 2°C, with 80 μmol m^{-2} s^{-1} illumination (cool white fluorescent lamps). Subculturing involved serial dilution (10^{-1} to 10^{-4}) in triplicate, followed by isolation of single colonies on solid medium. Axenic status was confirmed via microscopy and the absence of heterotrophic growth in nutrient-rich LB medium. Isolated colonies were transferred to liquid Zarrouk's medium for expansion (El Baky *et al.*, 2020).

2.2. Microorganism identification

Genomic DNA was extracted using the Pouya Gene Azma DNA Extraction Kit (Iran). The 16S rRNA gene was amplified by PCR with universal primers:

- 4F: 5′-TATCGGAGAGTTTGATCCTGG-3′
- 1505R: 5′-GATACGGCTACCTTGTTACGA-3′

Purified PCR products were sequenced by Stab Vida (Portugal) using the following primers:

- 27F: 5′-GAGTTTGATCCTGGCTCAG-3′
- 16F358: 5′-CTCCTACGGGAGGCAGCAG-3′
- 704F: 5′-GTAGCGGTGAAATGCGTAGA-3

Taxonomic identification followed ≥97% 16S rRNA similarity to reference strains. Negative controls were included.

2.3. Morphological assessment

Microscopy: Bright-field imaging (100× magnification) to document trichome structures.

2.4. pH Monitoring

The pH of the culture medium was measured daily using a calibrated pH meter (Metrohm, Switzerland) to monitor changes induced by microbial metabolism. The procedure included: Calibration of the pH meter with standard buffers (pH 4.0, 7.0, and 10.0) before each measurement session. Aseptically collected 5 mL samples were temperature-equilibrated (25°C). Triplicate measurements were recorded to ±0.01 pH units (Monod, 2012).

2.5. Growth optimization under zinc concentrate

Microbial cultures were grown in Zarrouk's liquid medium supplemented with different concentrations of zinc (Zn^{2+}) (2-4-7 and 11 mg L^{-1}) to assess the impact of zinc stress on growth dynamics. Zinc was introduced as zinc sulfate ($ZnSO_4 \cdot 7H_2O$) at predetermined concentrations. Cultures were maintained under controlled conditions: Temperature: 25 ± 2°C. Light intensity: 80 μmol m^{-2} s^{-1} (continuous illumination). Agitation: 150 rpm on a shaker. Biomass accumulation was quantified by measuring optical density at 590 nm (OD590) using a UV-Vis spectrophotometer (Jenway, UK). Measurements were taken every 48 hours over a 14-day experimental period (Gadd, 2010; Stanier *et al.*, 1971).

2.6. Zinc enrichment analysis by Atomic Absorption Spectroscopy (AAS)

To assess zinc enrichment in variants S (spiral) and L (linear), cultures were grown in Zarrouk's medium supplemented with varying Zn^{2+} concentrations (2, 4, 7, and 11 mg L^{-1}). After 14 days, biomass was harvested by filtration, washed with deionized water, and zinc content was quantified via atomic absorption spectroscopy, with calibration curves prepared from $ZnSO_4$ standards. Atomic absorption spectroscopy with the Atomic Absorption Spectrometer (AA240FS, Varian). The enrichment factor (EF) was calculated as:

$$EF = CE/CC \tag{1}$$

Where CE represents the concentration of the microelement in dry algae within media containing elevated levels of metals, and CC denotes the concentration in control media. According to the EF equation, values below 1.5 suggest the absence of enrichment, values between 5 and 20 indicate substantial enrichment, and values between 20 and 40 reflect extremely high levels of enrichment (Molnár *et al.*, 2013).

2.7. Statistical analysis

Data were analyzed using one-way ANOVA with post-hoc Tukey's test to determine significant differences ($p < 0.05$) in biomass and pH across zinc concentrations.

3. Results and Discussion

3.1. Molecular Confirmation

Gel electrophoresis was conducted to assess the efficacy of the polymerase chain reaction (PCR). Distinct bands were observed at approximately 1500 base pairs, aligning with the anticipated size of the amplified 16S rRNA gene fragment. These findings substantiate the successful amplification of the target gene, with no notable non-specific products identified, thereby demonstrating effective PCR optimization and specificity. The amplifying 16S rRNA gene yielded fragments approximately 1,400 base pairs in length; the obtained nucleotide sequences were then analyzed using BLAST for identification. Pairwise alignment between the query and reference sequences revealed a high degree of similarity, with 99.30% identity and 85% query coverage. This strong similarity, combined with an E-value of 0.0, indicates a very close phylogenetic relationship to *Arthrospira platensis* sequences in genomic databases. These molecular findings conclusively confirm the species-level identification of the isolates as *A. platensis*.

3.2. Morphological Differences

Microscopic analysis of the two variants of *A. platensis* revealed significant morphological variations. Variant S mainly displayed a spiral morphology when observed at 100× magnification, consistent with the characteristic coiled structure typically found in *A platensis* species (Figure 1). In contrast, Variant L predominantly exhibited linear trichomes, with a diminished presence of coiled structures, suggesting a potential divergence in morphological adaptation in response to specific growth conditions.

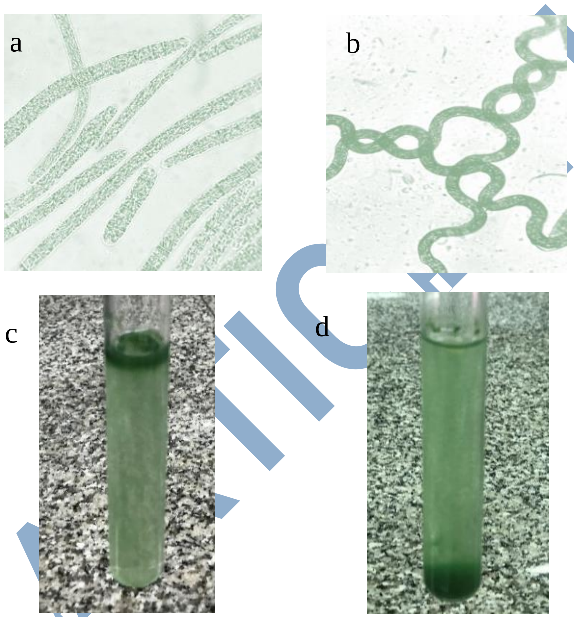

Figure 1. Microscopy of (a) Linear Trichomes and (b) Spiral Trichomes of A. Platensis Cultures each with an Absorbance of 0.5 at 590 nm. Visual Comparison of Trichome Morphology and Flotation Behavior after 24 Hours. c) Spiral Trichomes and d) Linear Trichomes.

Morphological differences correspond with the variability observed in this species and can be linked to mechanisms of environmental adaptation (de Morais *et al.*, 2008; Wang *et al.*, 2024). Variants S exhibited a distinctive helical or spiral shape, a characteristic typical of *A. platensis* that enhances its buoyancy and ability to endure environmental stresses (Rosic, 2022; Yadav *et al.*, 2022). This spiral form, observed at 100× magnification (Figure 1), increases the surface area available for nutrient uptake and photosynthesis, thereby facilitating its survival and growth under stressful conditions (Mühling *et al.*, 2006). In contrast, Variants L predominantly exhibited linear trichomes, with a reduced presence of spiral or coiled shapes. This linear morphology may signify an adaptive response to specific environmental pressures, potentially influencing cellular and filamentous structures (Chaiyasitdhi *et al.*, 2018). The diminished number of coiled structures could decrease susceptibility to mechanical stress or alter buoyancy, representing a morphological strategy to cope with adverse conditions (Sili *et al.*, 2012). These differences highlight the phenotypic plasticity of *A. platensis*, enabling it to modify its morphology in response to environmental cues, thereby enhancing its survival and metabolic flexibility.

Recent research has indicated that morphological plasticity in cyanobacteria, particularly in *A. platensis*, is often associated with abiotic stress factors (Kim *et al.*, 2025). Based on visual estimates, approximately 80-90% of the spiral trichomes are observed to be floating in the upper layer, indicating a high flotation efficiency in this morphology. Conversely, the linear trichomes appear to be only about 10-30% in the upper, buoyant layer, reflecting their limited flotation capacity, Figure 1c, d. Previous studies have indicated that the limited auto-flotation of linear trichomes is presumably due to their thin, densely packed structure (Walsby, 1994). When linear and coiled trichomes are mixed and subjected to agitation, the linear trichomes tend to primarily accumulate in the middle layer, while the coiled trichomes ascend to the upper layer. Consequently, *A. platensis* linear trichomes are likely excluded from the upper buoyant zone shortly after formation, owing to their weak flotation capacity (Kim *et al.*, 2025). This disparity highlights the critical influence of filament morphology on flotation behavior, with spiral structures providing enhanced buoyancy and separation performance compared to linear forms. The morphological differences thus play a pivotal role in determining the competitive dynamics and separation outcomes in mixed-culture flotation processes.

3.3. pH monitoring of *A. platensis* cultures under zinc stress response

The pH of the culture medium was monitored over a 14-day period to assess the impact on the growth rates of *A. plantensis* under two different morphologies (Figure 2). The initial pH values on day 0 were approximately 9.68 for both samples. The pH values gradually increased over the first two days, reaching approximately 9.83 to 9.94. From days 2 to 12, the pH continued to rise steadily, peaking at approximately 10.40 to 10.60 by day 12. Subsequently, the pH remained relatively stable with minor fluctuations, reaching values between 10.44 and 10.63 by day 14. Although statistical analysis using independent t-tests at each time point revealed no significant differences in pH values between the two variants (L and S) under the same conditions ($p > 0.05$), variant S exhibited a higher pH in Zarrouk medium culture, indicating better growth and biomass production.

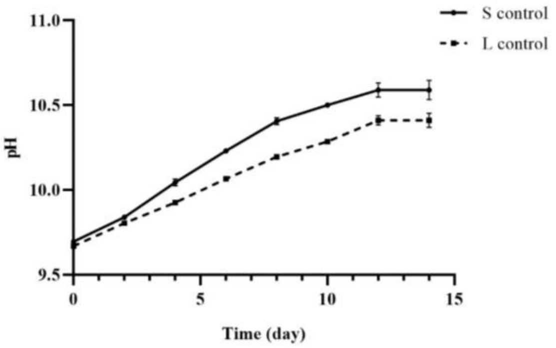

Figure 2. Changes in pH over 14 Days for Variants S and L under Control Conditions (**Note.** Data represent the mean ± standard error of 3 replicates. The pH values increase progressively over time in all treatments, with no significant differences observed between the variants or conditions.)

The increase in pH can be attributed to the metabolic functions of *A. platensis*, particularly its photosynthesis, which utilizes CO_2 and generates hydroxide ions (OH^-), leading to the alkalization of the culture medium (Ismaiel *et al.*, 2016; Markou *et al.*, 2023). The spiral morphology of variant S likely plays a significant role in maintaining a stable, elevated pH throughout the 14-day culture period. This coiled structure enhances light scattering and minimizes self-shading, thereby facilitating sustained photosynthetic activity and effective bicarbonate utilization. In contrast, variant L, characterized by straight filaments, initially demonstrated a rapid increase in pH, enabling accelerated growth and CO_2 absorption in the early stages (Kim *et al.*, 2025). The resilience of the spiral morphology to environmental fluctuations may help maintain intracellular homeostasis (Rai *et al.*, 2016; Tiwari *et al.*, 2019).

3.4. Growth response under zinc stress

The data presented in the graph illustrate a gradual increase in optical density (OD) under zinc conditions in the culture media, starting from day one (Figure 3). Over time, both variants, S and L, show changes in OD values. Initially, the OD values for both variants are nearly equivalent; however, as the experiment progresses, variant S exhibits a significantly accelerated growth rate. By the conclusion of the 14-day observation period, variant S demonstrates better acclimation and growth performance under zinc-induced stress. The different growth behaviors observed between the variants under stress conditions suggest variability in their tolerance or adaptation mechanisms during exposure to zinc.

The enhanced growth performance of the spiral variant S of *A. platensis* under zinc stress can be attributed to the unique biomechanical and morphological advantages conferred by its spiral filament structure. Recent biomechanical studies suggest that alterations in the overall shape of cyanobacterial trichomes are crucial for their mechanical strength and adaptability to environmental conditions (Kim *et al.*, 2025). The spiral or spiral configuration increases the filament's resistance to external mechanical pressure by augmenting its flexural stiffness. This structural characteristic enables *A. platensis* to better withstand environmental turbulence and chemical stresses, such as zinc, compared to linear trichomes (Ogato & Kifle, 2014). Furthermore, the spiral shape offers increased flexibility and the capacity to deform without breaking, facilitating movement and nutrient absorption in challenging conditions (Kim *et al.*, 2025). Moreover, the spiral morphology increases the surface area-to-volume ratio, potentially enhancing detoxification and metal sequestration processes, thereby mitigating the cytotoxic effects of zinc (Sili *et al.*, 2012). This structural advantage facilitates faster acclimatization and sustained growth under zinc stress, which explains the superior growth performance observed in variant S.

3.5. Zinc bioaccumulation in morphologically distinct *A.platensis* variants

Zinc (Zn) accumulation profiles between the spiral and straight morphotypes of *A. platensis* are shown in Table 1. Both variants demonstrated concentration-dependent Zn enrichment, with EF values increasing from 12.86 (S) and 11.02 (L) at 2 mg L^{-1} Zn^{2+} to 17.03 (S) and 16.73 (L) at 11 mg L^{-1} Zn^{2+}. Notably, Variant S consistently maintained higher EF values across all tested concentrations, with the greatest difference observed at the lowest Zn concentration (16.7% higher than Variant L). The enhanced capacity for zinc accumulation observed in the spiral morphotype (Variant S) can be attributed to various structural and physiological characteristics. The spiral structure increases the surface area, providing approximately 30-40% more area for metal binding compared to linear trichome, as demonstrated by previous research (Al-Amin *et al.*, 2021). Furthermore, spiral trichomes often produce more extracellular polysaccharides (EPS) (Cepoi *et al.*, 2020), which chelate metals and improve accumulation.

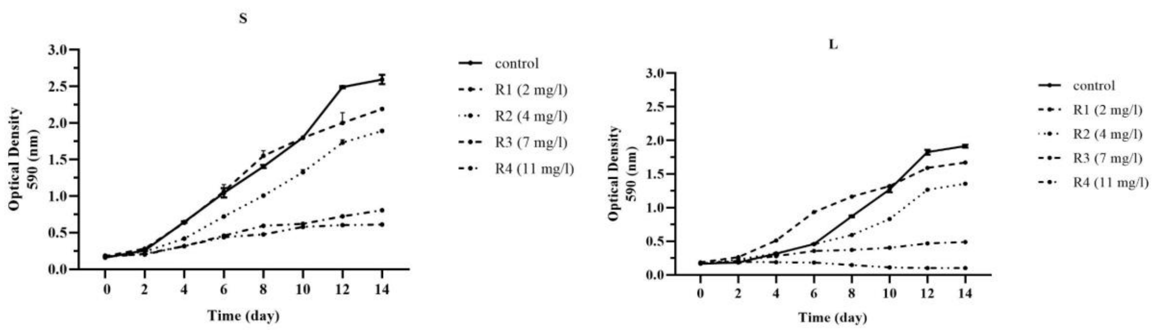

Figure 3. Growth Curves of A. platensis Variants S and L under Zinc Stress (2,4,7, and 11 mg L^{-1}) in Culture Media over 14 days, Measured as Optical Density at 590 nm (**Note**. Data represent the mean ± standard error of 3 replicates.)

Table 1. Zinc enrichment factors (EF) for *A. platensis*. (mean ± SD, n = 3) variant S (spiral), variant L (liner)

$ZnSO_4$ (mg L^{-1})	variant S (EF)	variant L (EF)
2	12.86 ± 0.45^{a}	11.02 ± 0.38^{b}
4	13.79 ± 0.51^{a}	13.48 ± 0.42^{a}
7	17.02 ± 0.62^{a}	16.43 ± 0.55^{a}
11	17.03 ± 0.59^{a}	16.73 ± 0.58^{a}

Values with different superscript letters (a, b) within the same row indicate significant differences ($p < 0.05$). Shared letters (a) denote no significant difference between variants at that concentration.

4. Conclusion

This research presents a comprehensive comparative study of two morphologically distinct variants of *A. platensis*, highlighting the complex interactions among filament structure, growth behavior, environmental adaptability, and stress resistance mechanisms. Molecular analysis via PCR and sequencing confirmed the identity of both variants, with the amplification of the 16S rRNA gene producing fragments of approximately 1400 base pairs, exhibiting 99% similarity to *A. platensis* reference sequences. Importantly, the two strains were found to be genetically identical, with primary differences limited to their morphological characteristics. The findings indicate that the spiral strain demonstrates superior growth performance under both normal and zinc-stressed conditions.

This advantage is largely attributed to its distinctive biomechanical and morphological features, such as enhanced mechanical stability and resilience, which improve tolerance to environmental fluctuations and facilitate more efficient nutrient absorption and zinc detoxification. The morphological plasticity and structural benefits of the spiral filament contribute significantly to the stress resistance capacity of variant S, enabling it to thrive in challenging conditions.

Overall, the interplay between filament morphology, growth efficiency, and stress resistance emphasizes the importance of morphological and physiological adaptability in cyanobacteria.

The spiral morphology of variant S offers a clear advantage for zinc enrichment in low-concentration environments, while variant L's competitive performance at high Zn^{2+} and rapid growth may suit high-throughput systems. Morphological optimization through variant selection or culture conditions could thus tailor *Arthrospira* to specific bioremediation or nutraceutical goals.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Authors' Contributions

Conceptualization, A.S., S.M., A.A.; methodology, A.S., S.M., A.A. and M.Se; software, A.S., M.Se. And M.Sa; validation, A.S. and S.M.; formal analysis, S.M., A.A. and M.Sa; investigation, A.S.; resources, A.S., M.Se. And M.Sa; data curation, A.S., S.M., and A.A.; writing—original draft preparation, A.S., S.M.; writing—review and editing, A.S., S.M., A.A., M.Sa and M.Se; visualization, A.S. and S.M.; supervision, S.M., A.A., M.Sa and M.Se; project administration, S.M. and A.A. All authors have read and approved the final version of the manuscript.

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