THE EFFECT OF ZnO NANOPARTICLE MORPHOLOGY ON BIOMASS AND PHYCOCYANIN CONTENT IN THE CYANOBACTERIUM *ARTHROSPIRA PLATENSIS*

Tudor BRANIȘTE[1], Ludmila RUDI[2], Tatiana CHIRIAC[2], Liliana CEPOI[2]

[1]*National Center for Materials Study and Testing, Technical University of Moldova, Chisinau, Republic of Moldova, tudor.braniste@cnstm.utm.md*

[2]*Institute of Microbiology and Biotechnology, Technical University of Moldova Chisinau, Republic of Moldova, ludmila.rudi@imb.utm.md*

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Abstract

Metallic nanoparticles, particularly zinc oxide nanoparticles (ZnONPs), are innovative tools for modulating the metabolic activity of photosynthetic microorganisms and are increasingly used in bionanotechnology, as controlled stress inducers that can redirect biosynthetic pathways toward the accumulation of high-value compounds. The morphology of nanoparticles significantly influences their interaction with biological systems and the resulting effects, making the study of their properties essential for the development of biotechnological applications. In this study, the effects of lab-synthesized ZnONPs with multipod morphology, were compared with those of commercially available spherical ZnONPs (Sigma-Aldrich) on the cyanobacterium *Arthrospira platensis* (spirulina). Nanoparticles were added to the spirulina culture at the beginning of the exponential growth phase (day 3), in two concentration ranges: 0.01–1.0 mg/L and 10–30 mg/L. To evaluate the cyanobacterial response to nanoparticle interaction, two parameters were assessed at the end of the cultivation cycle (day 7): accumulated biomass and C-phycocyanin content, a photosynthetic pigment with high biotechnological value. The results demonstrated that both ZnO multipods and spherical ZnONPs stimulated biomass production by 12–14% at higher concentrations. However, a marked difference in pigment response was observed. Spherical nanoparticles caused a substantial reduction in C-phycocyanin content by 57.5–87.9%, whereas multipod ZnONPs stimulated pigment synthesis at low concentrations and exerted a weaker inhibitory effect at high concentrations (50.3–59.7%). ZnONP morphology significantly modulates pigment biosynthesis and cytotoxicity in *Arthrospira platensis*, with multipod structures showing a more favorable interaction profile. These data support the morphological optimization of metal oxide nanoparticles for use in controlled cyanobacterial cultivation systems.

Keywords: *Arthrospira platensis,* ZnO multipods, Spherical ZnONPs, biomass, C-phycocyanin

1. INTRODUCTION

Zinc oxide nanoparticles (ZnONPs) are valued for their multifunctional properties, such as antimicrobial activity, UV radiation absorption ability, photocatalytic activity, electronic conductivity, and biocompatibility. These characteristics have enabled their broad application in medicine, biotechnology, advanced materials, sensors, cosmetics, environmental protection, and the food industry [1,2,3]. Morphology is a critical determinant for these applications, since the shape, size, and specific surface area of ZnONPs influence their efficiency of interaction with microorganisms, human cells, or various food matrices. For example, nanorods and nanoflowers exhibit enhanced antibacterial activity and are often used in medical coatings and contact materials [4], whereas uniformly dispersed spherical particles ensure effective UV protection and high stability without compromising the transparency of cosmetic products. In the food sector, morphology governs the controlled release of Zn^{2+} ions, which is essential for the development of active packaging systems [2,3]. Alongside their beneficial applications, ZnONPs can enter the environment through anthropogenic sources, particularly wastewater and industrial discharges, and thus reach aquatic ecosystems. Once released, these nanoparticles can affect microalgae and cyanobacteria by generating reactive oxygen species, releasing Zn^{2+} ions, and directly damaging cell membranes. Such processes disrupt vital functions and inhibit growth in photosynthetic organisms [5]. The cyanobacterium *Arthrospira platensis* (spirulina) is recognized for its high biotechnological value, as its ability to produce a wide range of biologically active compounds, including essential amino acids, polysaccharides, polyunsaturated fatty acids, carotenoids, phycobiliproteins - particularly C-phycocyanin, and others [6]. Bionanotechnology, an interdisciplinary field in which nanomaterials are integrated into biological systems to modulate cellular functions and direct biosynthetic processes toward compounds of interest, has generated growing interest in metallic nanoparticles. In this context, these nanoparticles are frequently investigated as stimulators of lipid synthesis in microalgal cultures used for biofuel production [7]. *Arthrospira platensis* is widely used as an experimental model for evaluating both toxicological responses and beneficial metabolic effects induced by metallic nanoparticles, owing to its well-characterized cellular structure and high sensitivity to environmental factors. Exposure to ZnONPs can elicit dual effects: at high concentrations, they inhibit growth, reduce pigment levels, and induce oxidative stress, whereas at lower doses, they may stimulate biomass accumulation and activate specific metabolic pathways [8,9]. The present study was designed to evaluate how the multipod-shaped morphology of ZnONPs influences biomass accumulation and C-phycocyanin content in *Arthrospira platensis*, in comparison with spherical ZnONPs.

2. EXPERIMENTAL DESIGN

Zinc oxide nanomultipods (ZnONP-MP) were synthesized via the chemical bath deposition method, using zinc nitrate hexahydrate and potassium hydroxide as precursors. An aqueous solution of 0.5 mol/L zinc nitrate [$Zn(NO_3)_2$] was gently mixed with a 4 mol/L KOH solution at room temperature. Once the solution became transparent, the temperature was increased to 85°C and maintained under continuous stirring for 2 hours. The resulting ZnO nanomultipods were collected by centrifugation, washed with deionized water, and dried in a lyophilization system at –80°C. The average diameter of the ZnO nanomultipods arms is approximately 200 nm, while their length is <1µm, as determined by SEM. Spherical ZnO nanoparticles (ZnONP-S), with a size <100 nm TEM), used in this study, were purchased from Sigma-Aldrich, Merck KGaA (Darmstadt, Germany).

The cyanobacterium *Arthrospira platensis* (strain CNMN-CB-02, National Collection of Nonpathogenic Microorganisms, Institute of Microbiology and Biotechnology, Technical University of Moldova) was cultivated in mineral medium under autotrophic conditions, at constant temperature and continuous illumination [8]. Nanoparticles were added at the tested concentrations at the onset of the exponential growth phase (day 3). Biomass was harvested on day 7, quantified spectrophotometrically using a calibration curve for absolute dry weight, separated from the culture medium, and subsequently resuspended to a standardized concentration of 10 mg/L. The C-phycocyanin (C-PC) content was determined spectrophotometrically from aqueous extracts obtained after six successive freeze–thaw cycles, with quantitative calculations performed according to the formulas proposed by Siegelman (1978) [10].

3. RESULTS AND DISCUSSION

In this study, two types of ZnO nanoparticles were tested: nanomultipods and nanospheres. For each type, two concentration ranges were established: low concentrations (0.01, 0.1, 0.5, and 1.0 mg/L) and high concentrations (10, 20, and 30 mg/L). The selection of these ranges was based on data from the literature [7], previous experience with nanoparticle testing [9], and studies on microalgae exposed to ZnONPs with different morphologies at concentrations between 0.01 and 1 g/L [11,12]. To avoid acute toxic effects previously observed at high concentrations [8], ZnONPs were added to the *Arthrospira platensis* culture at the onset of the exponential phase (day 3). The exposure lasted for 96 hours, after which biomass and C-phycocyanin content were determined.

The application of ZnO nanomultipods at concentrations ranging from 0.01 to 1.0 mg/L stimulated *A. platensis* biomass by 6.22% ($p < 0.05$) to 8.76% ($p < 0.01$), with no direct correlation observed between concentration and accumulation ($r = 0.3798$), indicating a very weak relationship between the variables (**Figure 1**). At higher concentrations, 10 and 20 mg/L, biomass increases of 14.33% ($p < 0.01$) and 11.62% ($p < 0.01$), respectively, were recorded. At 30 mg/L biomass yield was not significantly altered; however, a decreasing trend was observed with increasing concentration, with a strong negative correlation ($r = –0.9614$).

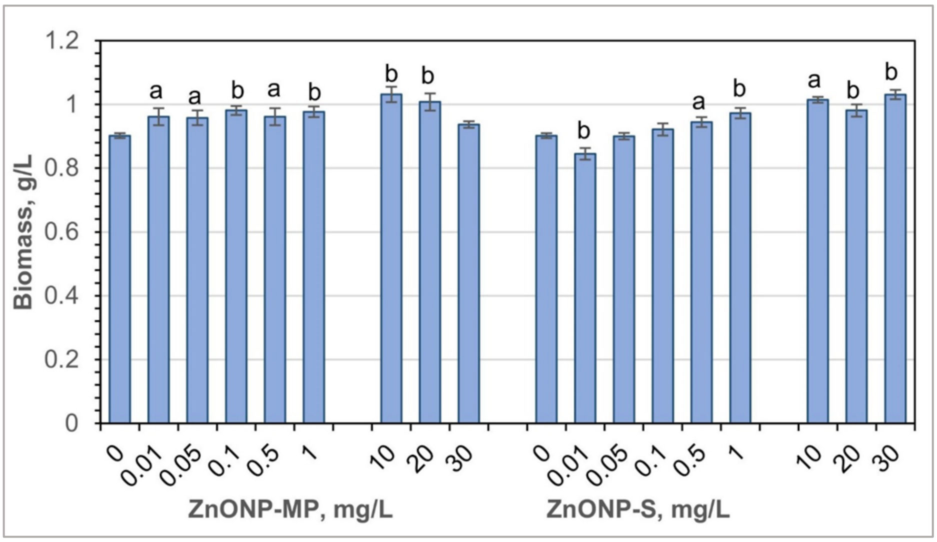

Figure 1 Biomass of *Arthrospira platensis* accumulated after exposure to ZnO nanomultipods (ZnONP-MP) and spherical ZnO nanoparticles (ZnONP-S) at low (0.01-1 mg/L) and high (10-30 mg/L) concentrations. 0 – control; a – $p < 0.05$; b – $p < 0.01$

For spherical ZnO nanoparticles, a slight inhibition of biomass accumulation (by 6.3%, $p < 0.01$) was observed at the lowest tested concentration of 0.01 mg/L. Starting from 0.05 mg/L, the biomass level approached that of the control, indicating the overcoming of the initial inhibitory threshold. In the 0.1–1.0 mg/L range, exposure resulted in moderate stimulation of growth, with biomass increases ranging from 2.2% ($p < 0.01$) to 7.8% ($p < 0.01$) compared to the control. Statistical analysis revealed a strong positive correlation ($r = 0.8419$) between the concentration of ZnO nanospheres and biomass accumulation, suggesting that subtoxic doses may act as stimulatory factors for cyanobacterial metabolism. At higher concentrations, the stimulatory effect was significant, with biomass increases of 12.46% ($p < 0.05$) at 10 mg/L and 14.30% ($p < 0.01$) at 30 mg/L.

For both types of nanoparticles, a stimulatory effect on biomass accumulation was recorded at higher concentrations (10–30 mg/L). The stimulatory effect of high concentrations of nanoparticles on microalgal biomass production has also been reported for other microalgae species. For example, in *Chlamydomonas monadina*, a concentration of 0.2 g/L stimulated biomass production by over 30%, whereas a lower concentration of 0.02 g/L ZnONPs inhibited growth. This effect was associated with ZnONPs of “flaky” morphology and sizes ranging from 400 to 1100 nm, with the proposed mechanism being related to the optimal availability of Zn^{2+} ions to the cell [12].

Our observations contrast with some of the data reported in the literature, where certain studies have highlighted inhibitory effects of ZnONPs on microalgae, with the intensity depending on nanoparticle morphology. For example, 72-hour exposure of the microalga *Pseudokirchneriella subcapitata* to ZnONPs of various shapes (spherical/aggregated, nanoplatelets, and nanoflowers) at concentrations ranging from 0.01 to 200 mg/L showed that concentrations below 10 mg/L did not induce toxic effects. On the contrary, higher concentrations inhibited growth in a morphology-dependent manner. Nanoflowers proved to be the most toxic, an effect attributed to more aggressive mechanical contact, possibly enhanced by a higher degree of aggregation; additional mechanisms such as shading or Zn^{2+} ion release was also suggested [13]. Another relevant example is the case of *Raphidocelis subcapitata* exposed for 96 hours to spherical ZnONPs of 20 and 40 nm, where a similar toxic effect was observed regardless of particle size, while 500 nm rods were less toxic. Toxic effects became apparent starting from a concentration of 0.1 mg/L, while lower concentrations were considered non-toxic [11].

In the case of marine diatoms, the morphology of ZnO nanoparticles strongly influences toxicity, especially at high concentrations and in species tolerant to Zn^{2+}. Studies on *Thalassiosira pseudonana*, *Chaetoceros gracilis*, and *Phaeodactylum tricornutum* have shown that all forms of ZnONPs (spherical, nanorods, nanoneedles) completely inhibited growth starting from 10 mg/L. This effect was correlated with the level of dissolved Zn^{2+}, which exceeded the EC50 for these species. Nanorods and nanoneedles proved to be more toxic than spheres, with possible mechanisms including the local accumulation of Zn^{2+} near cell membranes and the induction of mechanical or oxidative stress determined by nanoparticle morphology [14].

The results obtained in the present study indicate that the multipod morphology of ZnONPs is not toxic to *Arthrospira platensis* cultures and may even exert stimulatory effects on biomass growth. This characteristic could be explained by the extended surface area and complex structure of the multipods, which promote a gradual and controlled release of Zn^{2+} ions into the culture medium. These ions, essential for cyanobacterial metabolism and cell division processes, may be transferred more efficiently due to enhanced interactions between the multipods and the cell surface, thereby stimulating growth at subtoxic concentrations. Likewise, spherical ZnO nanoparticles did not show any toxic effects on *Arthrospira platensis* biomass within the tested concentration range, with a stimulatory effect observed at higher doses.

Despite the absence of an inhibitory effect on biomass, both multipod- and spherical-shaped ZnONPs were found to be involved in certain specific metabolic processes of the cyanobacterium, manifested particularly by a reduction in C-phycocyanin content (**Figure 2**).

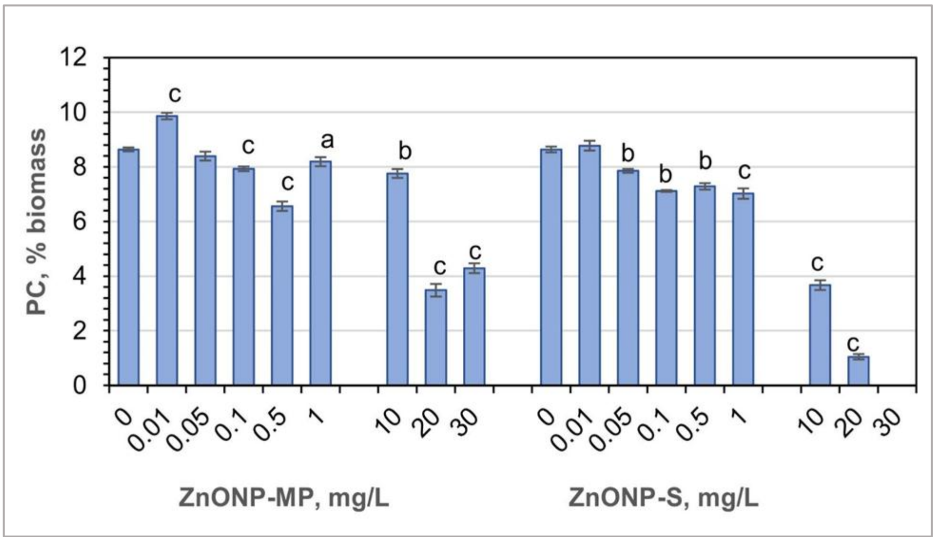

Figure 2 C-Phycocyanin content in *Arthrospira platensis* biomass after exposure to ZnO nanomultipods (ZnONP-MP) and spherical ZnO nanoparticles (ZnONP-S) at low (0.01-1 mg/L) and high (10-30 mg/L) concentrations. 0 – control; a – $p < 0.05$; b – $p < 0.01$; c – $p < 0.01$

For ZnO nanomultipods, within the low concentration range, only at 0.01 mg/L was an increase in C-phycocyanin content observed, by 14.17% ($p < 0.001$) compared to the control, indicating a slight stimulatory effect. At concentrations of 0.1 mg/L and 0.5 mg/L, pigment content decreased by 8.20% ($p < 0.001$) and 24.06% ($p < 0.001$), respectively, while at 1 mg/L the reduction was 5.20% ($p < 0.05$). At higher concentrations, 20 and 30 mg/L, a pronounced decrease in C-phycocyanin was observed by 59.70% ($p < 0.001$) and 50.30% ($p < 0.001$), respectively, confirming a strong inhibitory effect of nanoparticles at high doses.

For ZnO nanospheres, the lowest tested concentration (0.01 mg/L) did not affect the C-phycocyanin content in the cyanobacterial biomass. Conversely, at other low concentrations (0.05–1 mg/L), pigment content was progressively reduced, with values ranging from 9.07% ($p < 0.01$) to 18.74% ($p < 0.001$) below the control. At higher concentrations (10 and 20 mg/L), a drastic reduction in C-phycocyanin was observed, between 57.50% ($p < 0.001$) and 87.90% ($p < 0.001$), culminating in the complete loss of pigment at 30 mg/L. This response highlights a precise dose - effect relationship, consistent with observations reported for other microalgae, where spherical ZnONPs selectively affect photosynthetic pigments without directly influencing biomass growth [7,8]. For both ZnO nanoparticle morphologies, the C-phycocyanin content in the cyanobacterial biomass depended strictly on the nanoparticle concentration, with a strong negative correlation observed for both nanomultipods ($r = –0.8396$) and nanospheres ($r = –0.9768$).

The results indicate that the morphology and concentration of ZnO nanoparticles determine differentiated effects on accessory pigments. A combination of mechanisms, including nanoparticle - induced oxidative stress, physical interactions with photosynthetic structures, and possible shading effects or disruptions of pigment metabolism, may explain the significant reduction of C-phycocyanin at high doses. Notably, these changes were not mirrored by a reduction in biomass, suggesting a selective action of the nanoparticles on phycobiliproteins.

4. CONCLUSIONS

The results of this study demonstrate that both ZnO nanoparticle morphology (multipod vs. spherical) and concentration determine differentiated responses in *Arthrospira platensis*. At the biomass level, the effects were predominantly stimulatory, especially at higher concentrations (10–30 mg/L). ZnO nanomultipods were associated with a steady, non-toxic increase, whereas ZnO nanospheres induced a more pronounced and dose - dependent response. Analysis of accessory pigments revealed a marked inhibitory effect, particularly on C-phycocyanin, which was more pronounced in the case of ZnO nanospheres and closely dependent on concentration. These findings confirm that nanoparticles morphology is a key determinant of metabolic response in *Arthrospira platensis*. Multipods primarily promote biomass accumulation, while nanospheres exert a stronger impact on pigment metabolism. Overall, the observed dual effect, stimulation of biomass coupled with inhibition of photosynthetic pigments, highlights the necessity of evaluating multiple metabolic markers and underscores the critical importance of controlling nanoparticle morphology in microalga–nanomaterial interaction studies.

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