BIOMASS PRODUCTION AND PIGMENT CONTENT IN *Arthrospira platensis* BY ADDING AuNP(PEG) AND AgNP(PEG) AT DIFFERENT GROWTH PHASES OF CULTIVATION CYCLE

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Abstract. The current study aimed to investigate the influence of growth phase on the response of cyanobacterium *Arthrospira platensis* to the presence of Au and Ag nanoparticles stabilized with polyethylene glycol in the culture medium. Nanoparticles of gold and silver in the applied concentrations altered the accumulation of spirulina biomass, regardless of the physiological growth phase of the culture to which they were added. Changes in the content of pigments in spirulina biomass indicated the presence of harmful effects on cyanobacterial strain. Gold nanoparticles, for instance, significantly reduced the amount of phycobiliproteins in spirulina biomass, and the inhibitory effect was more pronounced when adding nanoparticles in the *lag* phase of the cultivation cycle. Changes in the content of chlorophyll *a* and β-carotene occurred by approximately the same principle, while more pronounced effects were observed under the action of gold nanoparticles. Adding nanoparticles in the exponential growth phase showed a dose-dependent inhibitory effect in the case of chlorophyll. The amount of biomass accumulated during the cultivation cycle cannot be considered a sufficient parameter when assessing the possible toxic effects of nanoparticles, and biochemical tests are necessary to detect various deviations from the normal physiological state of spirulina culture.

Key words: *Arthrospira platensis*; gold and silver nanoparticles; growth phases; biomass production; pigment content.

INTRODUCTION

In recent decades, nanoparticles have become ubiquitous due to their chemical, physical, optical and biological properties. These specific properties give nanoparticles new physicochemical characteristics, which makes it possible to expand their field of application [2, 3, 21, 29].

Noble metal nanoparticles, in particular gold and silver nanoparticles, are constantly in the focus of researchers' attention. The antibacterial properties of silver nanoparticles (AgNPs) and the cost-effectiveness of their use have led to a wide range of applications, from food packaging, odour-resistant fabrics, sterile dressings, cosmetics, cleaning agents and food additives to various electronics, household appliances and medical devices [8]. Gold, being a colloidal element, is used as a vector in tumor therapy to enhance drug delivery. Gold nanoparticles are used in the production of conductors and catalysts as components of anti-aging cosmetics; in the production of dyes; manufacture of storage devices [9, 15, 30, 40]. Gold nanoparticles are known for various medical applications and biological activities [12].

The extended use of NPs in numerous products results in their release into the natural environment, especially in water. Aquatic organisms, including phytoplankton, are therefore subjected to their action and bear the consequences of contact with nanomaterials. Most studies of the action of nanoparticles on microalgae and cyanobacteria are aimed at determining their toxic effect. In aquatic systems, photosynthetic microorganisms are targets for most environmental pollutants. Silver is considered to be the most toxic metal for living organisms, after mercury [14, 22]. The mechanisms of AgNP toxicity include adhesion to cell membranes with changes in their properties, such as permeability and/or ion transport, inhibition of synthesis and DNA damage; generation of reactive oxygen species [13, 22, 24]. Two types of nanoparticle toxicity to microalgae were highlighted; first is direct toxic effects based on the chemical composition of nanoparticles and their reactivity with cell membranes, second is indirect toxic effects caused by the release of toxic ions [23]. Chemically stable metallic nanoparticles have no significant cellular toxicity, while nanoparticles able to be oxidized, reduced or dissolved are cytotoxic and even genotoxic for cellular organisms [1]. One of the reasons for the indirect toxic effect of small nanoparticles is their ability to form aggregates and/or be absorbed on membrane surface [11].

Gold is considered to be less toxic to organisms, with the exception of nanogold [25]. In the case of aquatic microorganisms, AuNP toxicity depends on the nature of metal core coatings and their spontaneous ability to form conglomerates [18].

It has been shown that non-functionalized gold and silver nanoparticles with small size are more toxic to some microalgae species such as *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum* [22, 34].

Coating nanoparticles with an organic and bioorganic shell alters the adhesion and aggregation properties of nanoparticles. The toxicity of Ag nanoparticles stabilized with poly(N-vinyl-2-pyrrolidone)/polyethyleneimine (PVP/PEI) for microalgae *Phaeodactylum tricornutum* has been demonstrated [33]. A reduction in the productivity of green microalgae *Chlorella vulgaris* was also confirmed in the case of using glucose-coated AgNPs.

In this case, direct toxicity of nanoparticles was caused by their dissociation with the formation of active ions that inhibited cell multiplication [20]. Gold nanoparticles stabilized with PVP are less toxic to microalgae *Raphidocelis subcapitata* compared to uncoated nanoparticles [7]. The gold nanoparticles stabilized with mannose in low concentrations induced cell aggregation of microalgae *Chlamydomonas reinhardtii*, and citrate-stabilized AuNPs inhibited cell growth of microalgae *Chlorella autotrophyca*, *Nannochloris atomus* and *Phaeodactylum tricornutum* [22, 25]. The toxic effect of stabilized nanoparticles also depends on the rate of dissociation of the coating compounds. The impact of coating (branched-polyethyleneimine: BPEI vs. poly-vinylpyrrolidone: PVP) silver nanoparticles in branched-polyethyleneimine and poly-vinylpyrrolidone on phytoplankton collected in the Eastern Mediterranean Sea were tested depending on the size and concentration of Ag nanoparticles. Toxicity of poly-vinylpyrroidone stabilized nanoparticles for which the dissolution rate was higher has been demonstrated [38].

Thus, the toxicity of nanoparticles is different for various species of microalgae and cyanobacteria and depends on the nature of nanoparticles (size, oxidation state, crystalline structure), their concentration in the nutrient media used and their composition, as well as the cultivation conditions.

However, nanoparticles can also be used as stimulators in microalgae and cyanobacteria cultivation technologies to promote their biosynthetic activity [39]. The addition of nanoparticles to microalga cultures can encourage CO_2 capture and light conversion efficiency. A growth-promoting effect of green microalgae *Chlamydomonas reinhardtii* was established when exposed to AgNPs in concentration range of 0.01-0.15 mg/L for 72 hours [34]. The application of small polyethylene glycol coated gold and silver nanoparticles stimulated the productivity of halophilic microalga *Dunaliella salina* by 15% under laboratory conditions and increased carotene content in biomass by 20-36% [19]. Poly(N-vinylpyrrolidone) protected gold nanoparticles ranging in size from 20 to 50 nm at a concentration of 0.014 mg/mL have also been reported to trigger the productivity of microalgae *Raphidocelis subcapitata* [7].

Cyanobacterium *Arthrospira platensis* is one of the most valuable microorganisms due to its unique biochemical composition. Most of the research is directed towards the selection of optimal growth parameters for spirulina; improvement of biomass harvesting and drying techniques; and the commercial use of spirulina as pharmaceutical and nutraceutical products [36]. The accumulation of silver and gold elements in the cellular structures of spirulina is closely related to the selection and optimization of cultivation conditions, which provide not only the fixation of these metals in cells, but also the intensification of biosynthetic activity, in order to obtain pharmaceutical and/or nutraceutical preparations.

The ability of spirulina to uptake silver and gold ions from nutrient media that were supplemented with silver nitrate ($AgNO_3$) in concentrations 0.01-1.0 mg/L and $Na[AuCl_4]$ in concentrations 18.5-370 mg/L was assessed. The presence of silver ions in the standard culture medium containing Cl^- ions reduced biomass productivity by 66% and protein content by 19%, while in the chlorine-free medium, the amount of biomass decreased by only 11.8%, instead the protein content was reduced by 30%. In the case of gold, the productivity and protein content in spirulina biomass decreased only when high concentrations of $Na[AuCl_4]$ were added to the culture medium. Scanning electron microscopy showed the presence of spherical metal nanoparticles that formed on the cell surface during the bioaccumulation of silver and gold ions [5]. Small nanoparticles in polyethylene glycol coating were non-toxic or showed low toxicity towards cyanobacterium *Arthrospira platensis* grown in a closed system. The amount of spirulina biomass increased by 24-31.6% in the presence of concentrations between 0.025 and 0.1 μM AgNP and by 29.4-35.8% in the presence of concentrations of 0.025-0.5 μM AuNPs [4].

One of the main parameters that may indicate toxic effect of nanoparticles is the productivity of photosynthetic organisms and the amount of biomass that accumulate during a growth cycle. Biomass accumulation strongly depends on the rate of photosynthesis ensured by light-harvesting pigments. These pigments absorb light and funnel the energy to the reaction center for conversion into chemical forms. Thus, monitoring the amount of biomass and changes in pigment contents is an important tool for highlighting the toxic effects of xenobiotics, including nanoparticles.

An important factor that determines the impact of nanoparticles on microalgae and cyanobacteria is the growth stage of their life cycle, to which it has been subjected to contact with nanoparticles. For example, it was found that citrate-stabilized Ag nanoparticles at a concentration of 46 μM determined the inhibition of microalgae *Chlamydomonas reinhardtii* growth in the lag phase, but not in the stationary phase [22].

Hence, the purpose of this study was to examine the influence of growth phase on the response of cyanobacterium *Arthrospira platensis* to the presence of Au and Ag nanoparticles stabilized with polyethylene glycol in the culture medium.

MATERIAL AND METHODS

Cyanobacterial strain, nutrient medium and cultivation conditions: The study was carried out on the strain of cyanobacterium *Arthrospira platensis* CNMN-CB-02 (spirulina), stored in the National Collection of Nonpathogenic Microorganisms (Institute of Microbiology and Biotechnology, Chisinau, Republic of Moldova).

Spirulina was grown in the mineral nutrient medium with the following chemical composition: 1)

macroelements (g/L): $NaNO_3$ – 2.5; $NaHCO_3$ – 8.0; NaCl – 1.0; K_2SO_4 – 0.6; Na_2HPO_4 – 0.2; $MgSO_4*7H_2O$ – 0.2; $CaCl_2$ -0.024 and 2) microelements (mg/L): H_3BO_3 – 2.86; $MnCl_2*4H_2O$ – 1.81; $ZnSO_4$ – 0.22; $CuSO_4*5H_2O$ – 0.08; MoO_3 – 0.015; Fe+EDTA - 1ml/L. The duration of the cultivation cycle was 6 days with the maintenance of the following parameters: pH 8 - 10, temperature of 28 - 30^0C, illumination of 37 - 55 µmol photons m^{-2} s^{-1} and periodic shaking. The amount of inoculum was 0.4 - 0.45 g/L in recalculation to absolutely dry biomass.

Nanoparticles were custom-made and purchased from the company ”Nanomaterials & Technologies”, Togliatti, Russia. Their characterization was based on X-ray diffraction (XRD) patterns. According to calculated data, the average size of AuNP is ~ 4.7 ± 0.2 nm, while AgNP is ~ 12 ± 0.6 nm.

Exposure of spirulina culture to nanoparticle action: Two experimental variants were designed: (1) spirulina culture was subjected to the action of gold and silver nanoparticles at the beginning of *lag* phase. To this end, the selected concentrations of gold and silver nanoparticles were supplemented to the nutrient medium prior to inoculation of spirulina culture; 2) spirulina culture was subjected to the action of gold and silver nanoparticles at the beginning of the exponential growth phase. In this case, the selected concentrations of nanoparticles were supplemented to culture medium on the 3rd day of cultivation cycle. The concentrations of nanoparticles were: 1.0; 1.25; 2.0; 3.75; 5.0; 10.0 µM; 3) Spirulina culture grown under the same conditions without the addition of nanoparticles was taken as a control.

Growth estimation by optical density measurement: The amount of cyanobacterial biomass in the samples was determined spectrophotometrically with the recording of cellular suspension absorption at 680 nm and the recalculation in g/L, performed on the basis of the calibration curve.

Preparation of samples for biochemical analysis: Spirulina biomass was separated from the cultural liquid by filtration and standardized with distilled water at the final concentration of 10 mg/mL. The samples have been frozen and thawed repeatedly.

Determination of phycobiliprotein content: The content of phycobiliproteins was determined in water extracts obtained from spirulina biomass. The quantity of 1.0 mL of standardized biomass**,** after repeated freezing-thawing procedure, shall be centrifuged at 11000 rpm for 3 min. The absorption of samples shall be recorded at wavelengths 620 and 650 nm. The calculation of the quantitative content of phycobiliproteins was carried out on the basis of the equations [35].

Determination of chlorophyll and β-carotene content: The chlorophyll and β-carotene content was determined in ethanolic extracts obtained from spirulina biomass. The reactant ratio was 10 mg biomass per 1.0 mL of 96% ethyl alcohol. The extraction of pigments takes place at room temperature, by shaking for 120 min. Extracts are obtained by centrifugation at 11000 rpm for 3 min. The absorption of samples was recorded at wavelengths of 450 nm and 665 nm. Quantitative calculation of pigments was carried out on the basis of formulas [31, 37].

All the experimental results were subjected to formal statistical analysis with the application of descriptive statistics tools (calculation of arithmetic means, standard deviation, coefficient of variation), inferential statistics (tests of statistical *significance* and *validity*). Calculation of statistical indicators has been conducted using the possibilities of MS Excel.

RESULTS

Change in biomass amount

The introduction of Ag nanoparticles into spirulina culture medium on the first day of the cultivation cycle in the concentrations of 1.25-5.0 µM induced biomass production, which increased by 12-25% compared to control sample (Fig. 1). The maximum biomass value was determined in the variant with concentration of 3.75 µM AgNPs. At a concentration of 10 µM, the amount of biomass decreased by 10% compared to control.

Silver nanoparticles supplemented to spirulina culture on the 3rd day of the cultivation cycle corresponding to the beginning of the exponential growth phase showed a similar effect of stimulating biomass production (Fig. 1). Concentrations of 1.25-5.0 µM AgNPs increased the biomass content by 14-24%. It was shown that concentration of 10 µM stimulated biomass accumulation by 10%. The dose of 1.0 µM AgNPs has been shown to be inert to spirulina culture.

Gold nanoparticles added to the nutrient medium prior to inoculation of spirulina culture in the concentrations of 1.0-5.0 µM stimulated biomass production by 12-21%, while the maximum amount of 1.16 g/L was obtained in the presence of 2.5 µM AuNPs (Fig. 2). The introduction of gold nanoparticles to spirulina culture at the exponential growth phase shifted the stimulating effect of nanoparticles towards

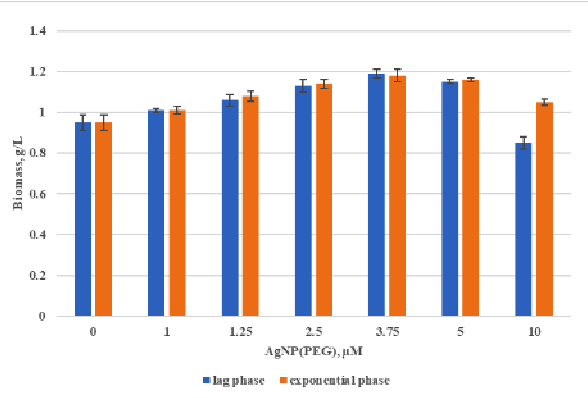

Figure 1. Biomass production of *Arthrospira platensis* grown in the presence of AgNPs applied in two experimental variants: 1) addition of NPs in the *lag* phase and 2) addition of NPs in the exponential growth phase

higher concentrations. Thus, concentrations of 1.25-5.0 µM applied to spirulina culture on the 3rd day of the cultivation cycle stimulated biomass production by 16-23%.

The concentration of 10.0 µM AuNPs, supplemented to spirulina culture medium on the first day of the cultivation cycle, reduced the biomass content by 31%. For spirulina culture at the beginning of the exponential growth phase, the concentration of 10.0 µM AuNPs did not alter biomass production that was similar to control sample.

Change in phycobiliprotein content

The content of phycobiliproteins, major photosynthetic accessory pigments in cyanobacteria, has changed in the presence of Ag and Au nanoparticles. Thus, in the presence of 1.0-10.0 µM AgNPs supplemented to the nutrient medium of spirulina at the beginning of the cultivation cycle, phycobiliprotein content in spirulina biomass ranged from 9.54 to 13.85% biomass (Fig. 3). While the highest pigment content was in biomass obtained at adding concentrations of 2.5 and 10.0 µM AgNPs. In other experimental variants, the content of phycobiliproteins decreased by 10-14%. The smallest amount of phycobiliproteins was determined in spirulina biomass cultivated in the presence of 1.0 µM AgNPs, and at concentrations of 2.5 µM and 10.0 µM AgNPs, an increase of up to 14% in the content of phycobiliproteins in spirulina biomass was found.

The effect of gold nanoparticles on phycobiliprotein content in spirulina was more pronounced. In spirulina biomass grown on mineral medium, supplemented with gold nanoparticles on the first day of the cultivation cycle, the content of phycobiliproteins varied from 4.19 to 8.41% biomass, which was 30.5-65.0% lower than in control sample (Fig. 4).

The amount of phycobiliproteins of 7.17 and 8.41% biomass was obtained at concentrations of 1.25-3.75 µM AuNPs added to spirulina culture at the beginning of the exponential growth phase. The most severe reduction in phycobiliprotein content in spirulina biomass was observed at a concentration of 10.0 µM AuNPs.

Changes in chlorophyll and β-carotene content

The content of chlorophyll and β-carotene in spirulina biomass grown in the presence of Ag nanoparticles added to the mineral medium on the first day changed significantly (Fig. 5). It was found that in the concentration range of 1.25-5.0 µM AgNPs, the chlorophyll content in biomass decreased by 14-24%.

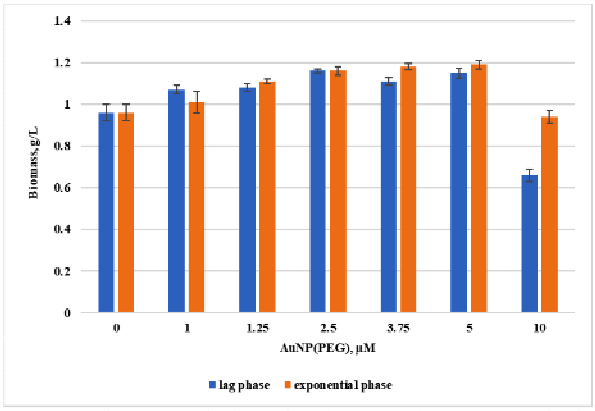

Figure 2. Biomass production of *Arthrospira platensis* grown in the presence of AuNPs applied in two experimental variants: 1) addition of NPs in the *lag* phase and 2) addition of NPs in the exponential growth phase

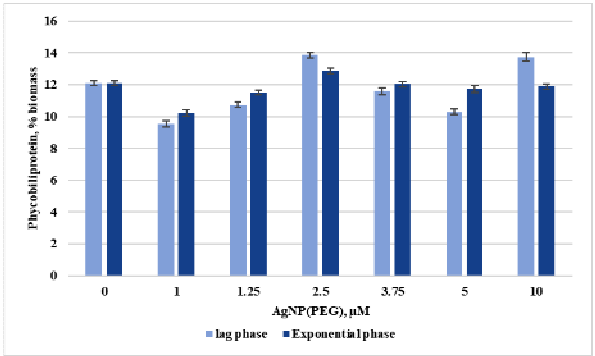

Figure 3. Changes in phycobiliprotein content in *Arthrospira platensis* biomass grown in the presence of concentrations of 1.0-10.0 µM AgNPs applied in two experimental variants: 1) addition of NPs in the *lag* phase and 2) addition of NPs in the exponential growth phase

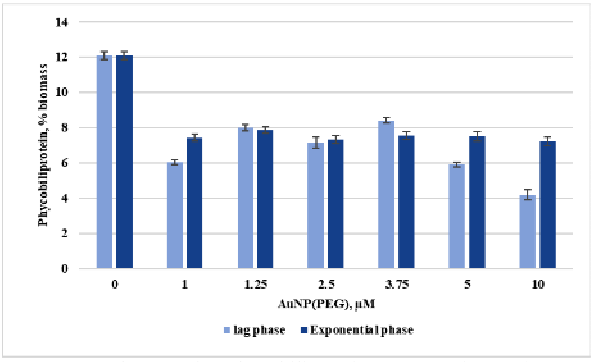

Figure 4. Changes in phycobiliprotein content in *Arthrospira platensis* biomass grown in the presence of concentrations of 1.0-10.0 µM AuNPs applied in two experimental variants: 1) addition of NPs in the *lag* phase and 2) addition of NPs in the exponential growth phase

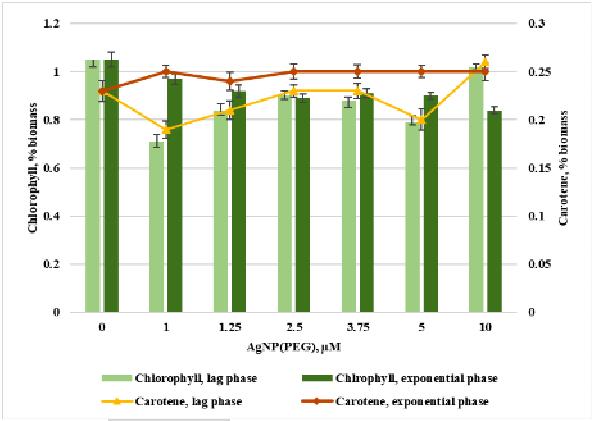

Figure 5. Changes in chlorophyll and β-carotene content in *Arthrospira platensis* biomass grown in the presence of AgNPs applied in two experimental variants: 1) addition of NPs in the *lag* phase and 2) addition of NPs in the exponential growth phase

Chlorophyll values ranged from 0.71 to 0.9% biomass. The reduction of chlorophyll content by 32% was observed at a nanoparticle concentration of 1.0 µM. At a concentration of 10 µM, the chlorophyll content in spirulina biomass did not change.

In the case of spirulina exposure to silver nanoparticles at the beginning of the exponential growth phase, a decrease in the chlorophyll content in biomass by 12-20% was found. The most significant reduction was observed in the presence of 10.0 µM AgNPs.

The carotene content also changed according to the pattern observed for chlorophyll. When nanoparticles were added at the beginning of the cultivation cycle, the least amount of β-carotene was observed at a nanoparticle concentration of 1.0 µM, while at a concentration of 10.0 µM, β-carotene content in biomass was even higher by 13% compared to control. In the case of the experimental variant with the addition of nanoparticles in the exponential growth phase, no changes in the carotene content were observed.

Under the action of gold nanoparticles, the content of β-carotene and chlorophyll *a* also changed differently depending on the time of adding nanoparticles (Fig. 6). The chlorophyll content in spirulina biomass grown by supplementing AuNPs to nutrient medium at the beginning of the cultivation cycle varied between 0.66% and 1.08% biomass. The inhibitory effect of chlorophyll content to a greater or lesser extent (up to 32% compared to control sample) was observed in the case of the concentration range from 1.0 to 5.0 µM, and at a concentration of 10.0 µM AuNPs, the chlorophyll content in biomass was at the level of control sample.

The lowest chlorophyll values of 27-38% in comparison with control sample were determined for concentrations of 3.75-10.0 µM AuNPs.

In spirulina biomass exposed to gold nanoparticles on the first day of cultivation, some changes in the carotene content were found. Concentrations of 2.5-5.0 µM AuNPs reduced the content of β-carotene by 17-26%. In the presence of 10.0 µM nanoparticles, the carotene content increased by 39% compared to control sample.

The content of β-carotene in spirulina biomass grown on nutrient medium supplemented with gold nanoparticles at the beginning of the exponential growth phase did not change, which led to a slight tendency to increase by 8-13% compared to control for concentrations of 1.25-5.0 µM AuNPs. In the presence of 10.0 µM AuNPs, β-carotene content in biomass increased by 21%.

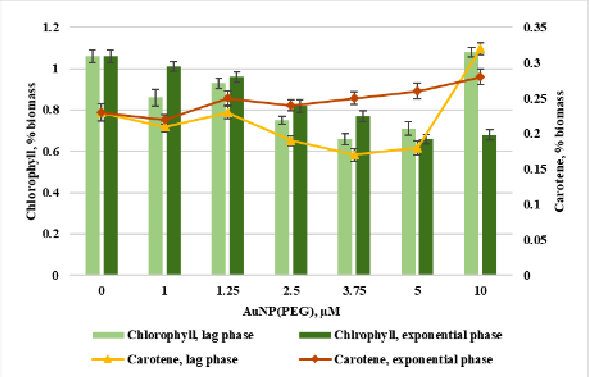

Figure 6. Changes in chlorophyll and β-carotene content in *Arthrospira platensis* biomass grown in the presence of AuNPs applied in two experimental variants: 1) addition of NPs in the *lag* phase and 2) addition of NPs in the exponential growth phase

DISCUSSION

Most studies of the action of nanoparticles on microalgae and cyanobacteria are aimed at determining the toxic effect on aquatic organisms. It has been shown that non-functionalized gold and silver nanoparticles with a small size are more toxic to some species of freshwater and marine microalgae compared to large ones, or those with different types of metallic core coatings [22]. Silver nanoparticles ranging in size from 2 nm to 15 nm, applied in concentrations of 10-300 µg/L, turned out to be toxic to microalgae *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*. At the same time, AgNPs of average size 30-50 nm in concentrations of 10-150 µg/L stimulated the growth of microalgae *Phaeodactylum tricornutum* [34].

It was assumed that one of the reasons for the indirect toxic effect of small nanoparticles is their ability to form aggregates and/or be absorbed on the surface of membranes. A direct and more pronounced toxic effect of Ag nanoparticles can be caused by the formed Ag^+ ions. Thus, at the concentration of 50 µM AgNPs, about 20% of *Chlorella vulgaris* cells remained viable, and at the same concentration of Ag^+ ions only 10% of viable cells [11].

To date, for many types of nanoparticles, it has not been possible to demonstrate the role of organic coatings as a factor that could minimize their toxic effect. It was compared the toxicity of 5 nm Ag nanoparticles stabilized with poly(N-vinyl-2-pyrrolidone)/polyethyleneimine (PVP/PEI) for microalgae *Phaeodactylum tricornutum*, which exceeded toxic effects of uncoated nanoparticles with a size of 47 nm. It was assumed that the coating components are involved in the formation of small conglomerates with a high affinity for algal cell membranes [33].

Organic or bioorganic stabilizing compounds used to coat the metallic core of nanoparticles, being very diverse in structure, can influence microalgae and cyanobacteria cultures differently. Thus, gold nanoparticles stabilized with PVP at a concentration of 0.4823 mg/ml reduced the growth of green microalgae *Desmodesmus subspicatus* by 50% under laboratory conditions, while the result was similar to uncoated gold nanoparticles in a concentration of 0.028 mg/ml. The stimulating effect on the productivity of microalgae *Raphidocelis subcapitata* was determined

at a concentration of 0.014 mg/mL AuNP(PVP) with a size of 43.67± nm [7]. Studied gold and silver nanoparticles stabilized with polyethylene glycol in concentrations from 0.025 to 0.5 μM, stimulated biomass production of *Spirulina platensis* [4].

Mannose-stabilized gold nanoparticles in concentration of 6-12 ng/mL induced cell aggregation in the culture of microalgae *Chlamydomonas reinhardtii* [25]. A 50% decrease in the productivity of microalgae *Chlorella autotrophyca*, *Nannochloris atomus* and *Phaeodactylum tricornutum* was obtained in the presence of citrate-coated AuNPs at a concentration of 1.5 μM [22]. The use of glucose-coated AgNPs also reduced the productivity of microalgae *Chlorella vulgaris*. In this case, the direct toxicity of nanoparticles caused by their dissociation with the formation of active ions was thought to inhibit cell multiplication [20].

The study on the effect of 4.7 nm gold and silver nanoparticles in a polyethylene glycol coating on cyanobacterium *Spirulina platensis* showed an increase in biomass production by 24-36% at concentrations from 0.025 to 0.5 μM [4]. The small size and coating of polyethylene glycol resulted in low toxicity of gold and silver nanoparticles for cyanobacterial culture.

The relationship between the concentration of nanoparticles and the manifestation of toxic effects on microalgae does not always exhibit a direct positive correlation. For instance, Ag nanoparticles used for the cultivation of microalgae *Chlamydomonas reinhardtii* at concentrations of 10, 40, 75, 150 and 300 μg/L for 24 hours did not alter cell density [34]. Although high concentrations of nanoparticles are considered harmful, no toxic effects were reported in the example presented. On the contrary, low concentrations can cause positive effects, for example, at a concentration of 0.025 μM AuNP(PEG), an increase in the biomass of cyanobacterium *Arthrospira platensis* was achieved by 36% [5].

Cultivation of cyanobacterium *Arthrospira platensis* in the presence of gold and silver NPs with a diameter ≤5 nm, stabilized with polyethylene glycol in concentration range from 1.0 to 10 μM showed no inhibitory effect, except for the concentration of 10 μM, for which a decrease of 10% in the amount of biomass was recorded for AgNPs and 30 % for AuNPs (Fig. 1, Fig. 2).

In addition to variable factors such as concentration and size, the effect of nanoparticles on microalgae depends on the contact time of culture with nanoparticles. The 7-day duration of exposure to glucose-stabilized AgNPs was found to be lethal for microalgae *Chlorella vulgaris* [20]. The effect of Ag nanoparticles (PEG) on microalgae *Chlamydomonas reinhardtii* at a concentration of 0.209 μM for 60 min resulted in a 50% reduction in the amount of biomass [22]. The highest degree of inhibition of *C. vulgaris* growth was observed when microalga was exposed to AgNPs at a concentration of 90 μg/L for 96 hours. At concentrations of 720 and 1440 μg/L, suppression of microalgae growth occurred after 72 hours of contact with nanoparticles [32].

An important factor that can determine the effect of nanoparticles on microalgae is the physiological phase of development of microalgae culture, during which contact with nanoparticles occurs. For example, citrate-stabilized Ag nanoparticles at a concentration of 46 μM added during the *lag* phase caused inhibition of the growth of microalgae *Chlamydomonas reinhardtii*. However, no effect was observed when nanoparticles were added to the culture in the stationary growth phase [22].

Chlorella vulgaris in the phase of exponential growth reacted in different ways to the action of silver nanoparticles, depending on the concentration and contact time. The growth inhibition rate for glucose-stabilized AgNPs in concentrations of 0.1, 1.0 and 10 μg/L on the first day of contact with nanoparticles was negative. Within a week, the inhibition rate increased by more than 20%, depending on the concentration of nanoparticles [20]. Concentrations of 0.054-0.108 mg/L AgNP(PEG) supplemented to culture medium of microalga *Dunaliella salina* on the first day of the cultivation cycle increased biomass production by 12-33%, and Au nanoparticles at a concentration of 0.081 mg/L induced an increase in the amount of algal biomass by 21% [19].

The purpose of this study was to establish the possibility of modelling the response of cyanobacterium *Arthrospira platensis* to the presence of PEG-stabilized gold and silver nanoparticles in culture medium, depending on the physiological phase of the culture at the time of contact and identifying the conditions under which cyanobacterium was sensitive to the action of nanoparticles, but at the same time managed to annihilate their possible toxic effect. In the experimental variant of the introduction of nanoparticles into the nutrient medium in the exponential growth phase of the cultivation cycle, an increase of 10 % above the control value of the amount of biomass in the sample with 10 μM AgNPs (PEG) was shown. Under the same conditions, AuNPs (PEG) did not alter the amount of spirulina biomass.

These nanoparticles also caused the remodeling of the content of photosynthetic pigments. Phycobiliproteins, which act as auxiliary photosynthetic pigments, are very sensitive to cultivation conditions. It is known that the synthesis of phycobiliproteins in the biomass of cyanobacteria and microalgae depends on the presence of metals in the nutrient medium. This has been shown to stimulate the synthesis of phycobiliproteins by red microalgae *Porphyridium cruentum* in the presence of iron and cobalt in concentrations of 3.15 and 6.30 ppm. A direct correlation has been demonstrated between the accumulation of metals into biomass and the increase in phycobiliprotein content [27]. A stimulating effect of 33.8-55% in phycobiliprotein synthesis was established in cyanobacterial biomass of *Synechocystis*

sp. for cultivation in the presence of titanium oxide nanoparticles [41].

Arthrospira platensis grown on polymetallic systems with increased concentrations of Cr, Fe, Ni, Zn, Cu lost 70-90% of phycobiliproteins, but retains the ability to uptake metals from nutrient medium during several cultivation cycles. A sharp decrease in the content of phycobiliproteins was the result of toxic effect of metals [4]. After 24 hours of exposure to selenium, the content of phycocyanins in spirulina biomass decreased up to 65%, and when exposed to Ag^+ ions for 72 hours, the content of phycobiliproteins in biomass decreased by 90% [6].

Cultivation of spirulina in the presence of concentrations of 0.025-0.5 μM AgNPs(PEG) did not alter the content of phycobiliproteins, which remained at the level characteristic to the strain in the study. In the case of spirulina cultivation in the presence of AuNPs(PEG), the content of phycobiliproteins in biomass was reduced by 21 % at 0.025 μM. An effect of stimulating the synthesis of phycobiliproteins, the content of which increased by 10%, was recorded for the concentration of 0.1 μM AuNPs(PEG) [4].

The phycobiliprotein content in spirulina biomass varied depending on the phase of life cycle to which the nanoparticles were added, as well as on the type of nanoparticles. Thus, both an increase and a decrease in the content of phycobiliproteins were observed upon the addition of silver nanoparticles in the *lag* phase. When nanoparticles were added in the exponential growth phase, regardless of concentration, the phycobiliprotein content remained stable at the level of control sample. Gold nanoparticles caused a decrease in the phycobiliprotein content regardless of the growth phase of life cycle, but if they were added in the exponential growth phase, the effect was more predictable and dose-dependent.

Some authors consider the parameter of changes in the chlorophyll content as an indicator of nanoparticle toxicity, since there is a strong direct correlation between the accumulation of algal biomass and the chlorophyll content as a result of contact with nanoparticles [16]. Thus, according to this point of view, the decrease in the chlorophyll content in biomass can be considered as a result of the toxic effect of nanoparticles on spirulina culture.

For small silver nanoparticles 2-15 nm in size, the effect of reducing the content of chlorophyll *a* in the biomass of microalgae *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum* was found. Silver nanoparticles with a size of 30-50 nm stimulated chlorophyll synthesis in the biomass of microalgae *Phaeodactylum tricornutum* and did not alter the pigment content in *Chlamydomonas reinhardtii* [34].

Exposure to AgNP concentrations of 5 and 20 μg/L did not alter the chlorophyll content in the biomass of microalgae *Scenedesmus sp*., while AgNP concentrations of 100 and 200 μg/L caused a reduction in chlorophyll *a* content by more than 21%. In the case of marine diatoms *Thalassiosira sp*. at concentrations of 20, 100, 200 μg/L AgNPs, the decrease in chlorophyll content was 14.5% [26]. It has been demonstrated the relationship between the concentration of Ag nanoparticles and contact time with microalga *Chlorella vulgaris*. Exposure of the culture for 24h to concentrations of 1, 10, 100 μg/L and 1.0 mg/L of glucose-stabilized silver nanopartivles reduced chlorophyll content in biomass by 80%. Cultivation of microalga for 7 days in the presence of AgNP-coated glucose in concentrations of 10, 100 μg/L and 1.0 mg/L resulted in 50% decrease in the content of chlorophyll *a* in algal biomass [20].

Chlorella vulgaris in the exponential growth phase was exposed to Ag nanoparticles 50 and 100 nm in size, which were applied in the concentration range from 20 to 200 mg/L. The content of chlorophyll *a* in biomass decreased by 40-60% compared to control. The lowest chlorophyll value was recorded at a concentration of 200 mg/L AgNPs [10]. Exposure to concentrations from 0.01 to 10 mg/L AgNPs for 24 hours reduced the chlorophyll content in microalgae *Chlorella vulgaris* by 24-51% and in microalgae *Dunaliella tertiolecta* by 44-75% [24].

At the same time, for concentrations of 90-1440 μg/L AgNPs introduced into the culture of microalgae *C. vulgaris*, an increase in chlorophyll content by 80% was found. The carotene content in biomass increased by 77%, the maximum value was recorded for a concentration of 360 μg/L AgNPs [32].

The content of chlorophyll *a* in biomass of microalgae *Chlorella zofingiensis* exposed to concentrations of 40-80 ppm AuNPs with a size of 5, 15 and 30 nm for 5 days remained at the level of control sample, therefore it was not possible to highlight a dependence on the effect caused by the size of nanoparticles used [17]. The effect of increasing the synthesis and accumulation of carotenoids in microalgae *Chlorella zofingiensis* was of particular interest when nanoparticles were added during the exponential growth phase. In the presence of 5 nm gold nanoparticles, the carotene content in biomass increased by 30%, and in the presence of 15 and 30 nm nanoparticles, the carotene content remained at the control level [17]. AuNPs(PEG) and AgNPs(PEG) 5 nm in size, used in the concentration range from 0.05 to 0.5 mg/L during the cultivation of microalga *Dunaliella salina* on the first day of life cycle, stimulated the synthesis of β-carotene by 20-36% [19].

Concentrations from 0.025 to 0.5 μM AgNP(PEG) added to culture medium did not alter the content of chlorophyll and β-carotene in the biomass of cyanobacterium *Arthrospira platensis*. Gold nanoparticles introduced into spirulina nutrient medium on the first day of the cultivation cycle in concentrations of 0.025-0.05 μM reduced the β-carotene content by 19.8-22.5% and the chlorophyll content by 11-14.7%. Concentrations of 0.1-0.5 μM AuNPs(PEG) did not change the chlorophyll content, while β-carotene content decreased by 8.4%-16.3% [4].

The investigated gold and silver nanoparticles in the concentration range from 1.0 to 5.0 μM had no negative effects on the accumulation of spirulina biomass, regardless of the physiological growth phase of the culture to which they were added. Moreover, both types of nanoparticles in concentrations of 1.25-5.0 μM increased the amount of biomass by up to 24% compared to control. In contrast, the concentration of 10.0 μM of both nanoparticles proved to be an obvious inhibitor of spirulina, but only when the nanoparticles were added in the *lag* phase. In this physiological phase, cells are vulnerable, undergoing a process of adaptation to a new medium, and the presence of xenobiotics can adversely affect the subsequent multiplication of cells, which was observed in our experiments. *Spirulina* culture in the exponential growth phase was stable, that is, it manifested all physiological and biochemical properties, which led to better resistance to the action of studied nanoparticles. In our case, this was expressed in the fact that the amount of biomass obtained by adding nanoparticles to this growth phase was at the control level.

Although concentrations of 1-5 μM nanoparticles of both types did not reduce the amount of biomass, they even provided a slight increase in this parameter. The change in the pigment content of spirulina biomass indicated the presence of a harmful effect on cyanobacterium. In the case of silver nanoparticles added in the *lag* phase, the content of phycobiliproteins varied, showing a tendency to both increase and decrease. It is known that phycobiliproteins, in addition to their main function, also act as antioxidants, annihilating free radicals formed in response to the presence of xenobiotics. When nanoparticles were added to the exponential growth phase, the content of phycobiliproteins was at the level of control sample. Gold nanoparticles, on the other hand, significantly reduced the amount of phycobiliproteins in spirulina biomass, and the inhibitory effect was more pronounced when adding nanoparticles in the *lag* phase. Changes in the content of chlorophyll *a* and β-carotene occurred by approximately the same principle. In the case of these two pigments, more pronounced effects were also observed under the action of gold nanoparticles. The addition of AuNPs in the exponential growth phase showed a dose-dependent inhibitory effect in the case of chlorophyll. Increasing the content of chlorophyll and β-carotene at a concentration of 10 μM had a compensatory character oriented towards free radical scavenging.

Thus, the amount of biomass, although an indicator of major importance for biotechnology, cannot be considered sufficient in assessing the possible toxic effects of nanoparticles. Therefore, biochemical tests are necessary to detect various deviations from the normal physiological state of algal culture.

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