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Using single-species and algal communities to determine long-term adverse effects of silver nanoparticles on freshwater phytoplankton

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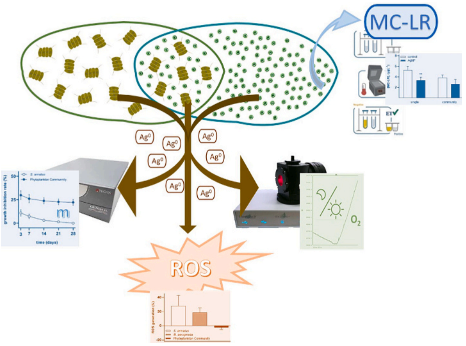
HIGHLIGHTS

- AgNP-IC_{10} affected the m-value of *S. armatus* during the 28 days of exposure.
- *S. armatus* and *M. aeruginosa* grown in single or community cultures showed different responses.
- Exposures in phytoplankton community significantly modified photosynthetic activity concerning single cultures.
- ROS generation was inhibited when both strains were exposed to community conditions.
- *M. aeruginosa* produced and released lower MC-LRs in both single and community cultures.

GRAPHICAL ABSTRACT



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ABSTRACT

The physical and chemical properties of silver nanoparticles (AgNPs) have led to their increasing use in various fields such as medicine, food, and industry. Evidence has proven that AgNPs cause adverse effects in aquatic ecosystems, especially when the release of Ag is prolonged in time. Several studies have shown short-term adverse effects of AgNPs on freshwater phytoplankton, but few studies have analysed the impact of long-term exposures on these populations. Our studies were carried out to assess the effects of AgNPs on growth rate, photosynthesis activity, and reactive oxygen species (ROS) generation on the freshwater green algae *Scenedesmus armatus* and the cyanobacteria *Microcystis aeruginosa*, and additionally on microcystin (MC-LR) generation from these cyanobacteria. The tests were conducted both in single-species cultures and in phytoplanktonic communities exposed to 1 ngL^{-1} AgNPs for 28 days. The results showed that cell growth rate of both single-species cultures decreased significantly at the beginning and progressively reached control-like values at 28 days

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post-exposure. This effect was similar for the community-cultured cyanobacteria, but not for the green algae, which maintained a sustained decrease in growth rate. While gross photosynthesis (Pg) increased in both strains exposed in single cultures, dark respiration (R) and net photosynthesis (Pn) decreased in *S. armatus* and *M. aeruginosa*, respectively. These effects were mitigated when both strains were exposed under community culture conditions. Similarly, the ROS generation shown by both strains exposed in single-species cultures was mitigated when exposure occurred in community cultures. MC-LR production and release were significantly decreased in both single-species and community exposures. These results can supply helpful information to further investigate the potential risks of AgNPs and ultimately help policymakers make better-informed decisions about their utilization for environmental restoration.

1. Introduction

The wide applications of nanotechnology through the synthesis of metallic nanoparticles such as silver nanoparticles (AgNPs), require a high demand in various products, in commercial and for medical, personal, industrial, and research uses. In this way, the AgNPs due to their plasmonic properties, electrical conductivity, excellent optical characteristics, and antimicrobial activity under specific circumstances, are the most commonly synthesized and used in textiles (e.g., socks, sportswear), food and pharmaceutical packaging, their use in the treatment of inactivation of bacterial resistance to antibiotics, cosmetic and personal hygiene products (e.g., toothbrushes), medical therapy and health care devices (e.g. prostheses, vascular catheters or wound dressings) to control the growth the pathogenic microorganisms, are also used in batteries, electronic and electric devices (e.g., mobile phones, refrigerators), photography, also used in drinking water treatment disinfection (e.g., ceramic and silver-spiked active carbon filters), etc. (Hadrup and Lam, 2014; Azimzada et al., 2017; Díaz Acosta, 2019).

In 2018, the annual global consumption of AgNPs was reported to be approximately 450 t per year (U.S. EPA, 2018). Besides, according to the inventory Nanodatabase, there are currently >800 products on the market that contain AgNPs (Nanodatabase, 2022). Therefore, the high global consumption, synthesis, and commercial use of AgNPs release considerable concentrations of silver in the order of 0.1 to 50 μg L^{-1} in water treatment systems (WHO, 2020). According to the U.S. EPA, and research by other authors, levels ranging from 0.1 to 100 ng L^{-1} or even more to 1.0 μg L^{-1}, have been found in AgNPs in freshwater ecosystems. That although these nanoparticles are very low concentrations, AgNPs present bioaccumulation and biomagnification responses in the aquatic trophic web (Hartemann et al., 2015; U.S. EPA, 2018; Zhang et al., 2019; Gagnon et al., 2021).

Consequently, the increase in the use of AgNPs in different presentations, inevitably raises the emissions of MNPs in the different receiving ecosystems, being the aquatic environment of greatest global concern, due to its synthesis phases, properties, AgNPs-containing products, uses, and removal (Gottschalk et al., 2010). Besides, the role of AgNPs solubility has an important role, for example, in oxidized conditions, the Ag chemisorbed are formed by AgNPs, leading to a constant release of Ag in aquatic solution, and other studies mentioned that the AgNP solubility increased by bio-contact with microalgae cells (Navarro et al., 2008; Fabreg et al., 2011).

Likewise, despite innumerable research carried out and its non-target antimicrobial properties, there is not enough updated scientific information on its effects on phytoplanktonic communities of receiving aquatic bodies in chronic exposures at very low concentrations (ngL^{-1}) (Fabreg et al., 2011; Gagnon et al., 2021). Therefore, investigations on the actual environmental concentrations and the nanoparticle's toxicity are needed to provide the basis for evaluating the potential risks that these compounds represent on the freshwater ecosystem, as well as responses in the first aquatic trophic, such as microalgae. Besides, previous ecotoxicological research aimed at the nanoparticle toxicity effects on single-species strains of microalgae, however, the studies of effects in freshwater phytoplankton biocenosis cover almost whole water ecosystems (Zhang et al., 2019). The freshwater phytoplanktonic communities are the basis of the aquatic trophic web, which is why participate in energy transfer and represent >50 % of the planet's oxygen, and any toxicological effects of AgNPs produce a harmful impact on the aquatic food web by bioaccumulation and biomagnification properties, and its requirements to the elucidation of probable toxicity mechanisms in photosynthetic activity, allelochemical modifications in phytoplanktonic communities of the receiving aquatic environments, oxidative stress through ROS formation and lipid peroxidation, stimulation of toxin-producing cyanobacterial blooms, growth population inhibition, etc. (Książyk et al., 2015; Xia et al., 2015).

Hence, the aim of this work is to evaluate the long-term toxicity of AgNPs on the green alga *Scenedesmus armatus* and the cyanobacterium *Microcystis aeruginosa*, studying its effects on the cell growth rate, the impact on the oxygen balance (respiration and photosynthesis) and the generation of reactive oxygen species (ROS), as well as its possible influence on the production of microcystins (MC-LR) in the case of cyanobacteria. The results obtained are intended to provide a reliable knowledge base of potential adverse effects on freshwater phytoplankton following long-term AgNPs exposures.

2. Material and methods

2.1. Characterization of silver nanoparticles

Silver nanoparticles (AgNP) were purchased from Sigma Aldrich (Saint Luis, MO) as 0.02 mg mL^{-1} dispersion in an aqueous buffer. Characterization of AgNP nanoparticles was performed using Nanoparticle Tracking Analysis (NTA; NanoSight, Amesbury, UK) software package, and the results showed a monomodal size distribution for AgNPs with a primary strong peak at 46 ± 13 nm.

2.2. Organisms and culture conditions

Scenedesmus armatus (BEA 1402B) and *Microcystis aeruginosa* (BEA 1835B) strains were selected as representative green microalgae and cyanobacteria from freshwater ecosystems. Both strains were obtained from the Spanish Bank of Algae (BEA, Gran Canarias, Spain).

Both species, as single-species and both in community, were maintained in the laboratory growing under axenic conditions in culture flasks (Thermo Fisher Scientific Inc., MA, USA) containing 20 mL of BG-11 culture medium (Sigma Aldrich Chemie, Taufkirchen, Germany). The strains were maintained at 21 °C and exposed to an individual 12-h shift of the light-dark cycle (60 μmol m^{-2} s^{-1} over the 400 to 700 nm waveband). Serial transfers of single cell inoculums to fresh medium once a fortnight allowed maintaining mid-log exponential growth.

Single cultures of *S. armatus* and *M. aeruginosa*, as well as co-cultures of both under phytoplanktonic community conditions, with cell densities adjusted to 10^4 cells mL^{-1} each, were exposed to 1 ngL^{-1} AgNP dispersed in 20 mL of BG-11 culture medium. Control and AgNP-exposed cultures were incubated for 28 days at 21 °C in a thermostatically controlled chamber (Gilson Inc., Middleton, WI USA) at 60 μmol m^{-2} s^{-1}. The cultures were shaken by hand twice daily, once in the morning and once in the evening, for the 28 days of exposure.

Additional light absorption tests (OD627 and OD720) were performed to rule out a possible shading effect. The results showed that the light absorption obtained by emitting over 1 ngL^{-1} AgNPs in 20 mL of BG-11 culture medium did not exceed 0.007, so no shading effects are expected.

2.3. Cell growth rate

The cell growth rates of single-species or community cultures have been evaluated considering the fluorescence emitted by the photosynthetic pigments using a Tecan Genios plate reader (Tecan Group Ltd., Switzerland), with excitation-emission filters of 485-670 and 590-670 nm for the green algae *S. armatus* and the cyanobacteria *M. aeruginosa*, respectively. The relationship between fluorescence and cell density was estimated by comparing with parallel Neubauer chamber counts.

Measurements at 3, 7, 14, 21, and 28-days exposure of control and treated cultures were obtained, and the maximum cell growth rate was estimated with the Malthusian parameter (m), which was calculated as (Crow and Kimura, 1970):

$$m = log_e\ (N_t/N_0)/t$$

where N_t and N_0 are cell concentrations at time t and $t = 0$ (both estimated by fluorescence). The m-values are expressed as doublings d^{-1}.

2.4. Photosynthesis activity assessment

The O_2 production/consumption balance under light-dark conditions has been selected as model to evaluate photosynthetic activity. The light-dark O_2 balance was analysed using a Clark-type O_2 electrode.

Chlorolab 2 system (Hansatech, Norfolk, UK) has been selected to measure dissolved O_2 under automated illumination from red (660 nm) LED light and in darkness. In these photosynthesis activity tests, measurements were taken at 21 °C and 375 μmol m^{-2} s^{-1} irradiance. The light-dark oxygen balance, or gross photosynthesis rate (Pg), was estimated from the formula:

$$Pg = Pn + R$$

where Pg corresponds to the oxygen production rate under illuminated conditions, R (respiration) corresponds to the process by which phytoplankton consume oxygen and release carbon dioxide in darkness, and Pn (net photosynthesis rate) is defined as the difference between Pg and R.

The light-dark O_2 balance from control and treated cell cultures of both strains was measured at 3, 7, 14, 21 and 28 days of exposure on a cell concentration of 5 × 10^5 cells diluted in a final volume of 1 mL. Records of each sample were obtained after exposure to 5 min of darkness followed by 5 min of illumination. Four replicates of each experiment were performed ($n = 4$).

2.5. Generation of reactive oxygen species

Intracellular reactive oxygen species (ROS) produced by *S. armatus* and *M.aerugionsa* strains, both single-species and communities, were tested using the fluorescent probe 2′,7′-dichlorofluorescein diacetate (H_2DCFDA) (Sigma-Aldrich, St. Louis, MO, USA). Intracellular oxidation of H_2DCFDA induces the generation of 2,7-dichlorofluorescein (DCF), a fluorescent compound that behaves as an indicator of ROS.

Cell densities of *S. armatus* (2 × 10^6 cells mL^{-1}) and *M. aeruginosa* (5 × 10^6 cell mL^{-1}), exposed and unexposed to the selective concentration of Ag-NP nanoparticles, were adjusted to a final volume of 1.5 mL and incubated for 60 min at room temperature (23 °C) with a final concentration of 10 mM of H_2DCFDA.

Samples were monitored at 0, 1, 2, and 4 h, and measured on a Tecan Genios microplate reader (Tecan Group Ltd., Switzerland) at room temperature, with excitation-emission filters of 485-520 nm to determine relative ROS production. The BG-11 medium was also tested for ROS to measure "background" ROS formation, and the results were corrected by subtracting the fluorescence of the culture medium. As a positive control for ROS formation, 3 % H_2O_2 (v/v) was used.

All samples were measured at 0, 3, 7, 14, 21 and 28 days under dark conditions and after exposure to a light source of intensity 60 μmol m^{-2} s^{-1} (*S. armatus*) and 22.5 μmol m^{-2} s^{-1} (*M. aeruginosa*) for 2 h.

2.6. Microcystins (MC-LR) measurement

Densities of 2 × 10^6 cells from non-exposed and exposed *M. aeruginsa* cultures to 1 ngL^{-1} AgNP for 28 days were used to determine intra- and extracellular MC-LR. samples were centrifuged at 10000*g* and 4 °C for 5 min, and the supernatant was used for extracellular MCs analysis. The residue was resuspended in ultrapure water to the same original volume (10 mL), frozen at 80 °C, and rapidly thawed at room temperature thrice. The resulting solutions were centrifuged again under the same conditions and the supernatants were filtered through 0.22 μm cellulose acetate membranes for analysis of intracellular MCs.

For the measurement of intra- and extracellular MCs, a commercial MC-LR detection kit based on the inhibition of phosphatase activity (MicroCistest, ZEU-IMMUNOTEC S.L., Zaragoza, Spain) was used. Samples containing MC-LR inhibit the enzyme activity proportionally to the amount of toxin contained in the sample that can be detected at 405 nm.

2.7. Data analysis

Statistical analysis was performed using the computer software package GraphPad Prism v6.0 (Graph-Pad Software Inc., USA). Data are expressed as the mean ± sd of four experiments ($n = 4$). Student's t-test and one-way analysis of variance (ANOVA) were used to check if there were significant differences ($p < 0.05$) among treatments, and a post-hoc analysis (Tukey test) to check differences among groups.

3. Results

3.1. Growth inhibition rate

Cell growth rate inhibitions exhibited by both phytoplankton strains, isolated or in the community, after 28 days of Ag-NP exposure are shown in Fig. 1.

Isolated cultures from both strains displayed a maximum inhibition of cell growth rate at 72 h of exposure, showing inhibition percentages of 10.91 ± 3.49 % and 26.32 ± 4.14 % for *S. armatus* and *M. aeruginosa*, respectively. Subsequently, the inhibition of m value decreases progressively throughout 28 days of exposure to similar values to the control assays.

Freshwater green algae *S. armatus* cultured in phytoplanktonic communities with *M. aeruginosa* showed 29.87 ± 4.54 % inhibition of m value, which is almost 300 % higher than those isolating cultured. This inhibition is maintained during the 28 days of exposure, showing an inhibition percentage of 22.22 ± 3.14 % of m-value. However, freshwater cyanobacterial *M. aeruginosa* exhibited a different behaviour by showing less inhibition of cell growth in community than in isolated cultures. Inhibition percentages on the order of 11.11 ± 1.40 % on community cultures were less than half of that obtained for isolated cultures. Moreover, this percentage of inhibition decreased progressively until reaching equal m values to the control at 28-days exposure.

3.2. Photosynthetic activity

The photosynthetic activity displayed by both freshwater strains grown alone or in phytoplanktonic community is plotted in Fig. 2.

Single cultures of the freshwater green alga *S. armatus* exposed to AgNp showed an initial increase in both Pn and R activity, showing increases of 35.17 ± 5.96 and 60.35 ± 6.16 % respectively compared to

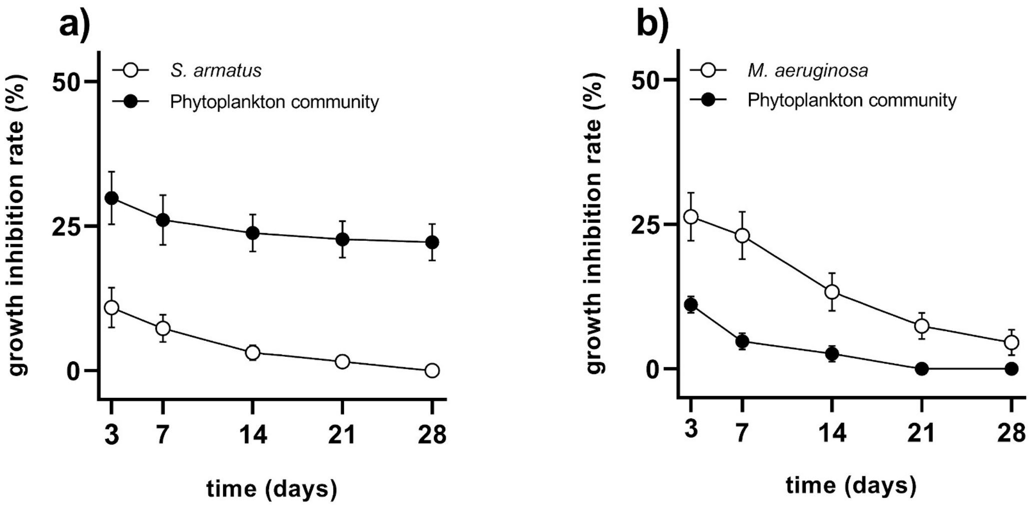

Fig. 1. Cell growth rate inhibition exhibited by *S. armatus* (a) and *M. aeruginosa* (b) cultures exposed in both single-species (empty dots) and community (filled dots) cultures to continuous 1 ngL^{-1} AgNPs to 28 days of exposure. Each point represents the mean ± sd of 4 independent experiments (n = 4) expressed as percentage inhibition (%) with respect to control values.

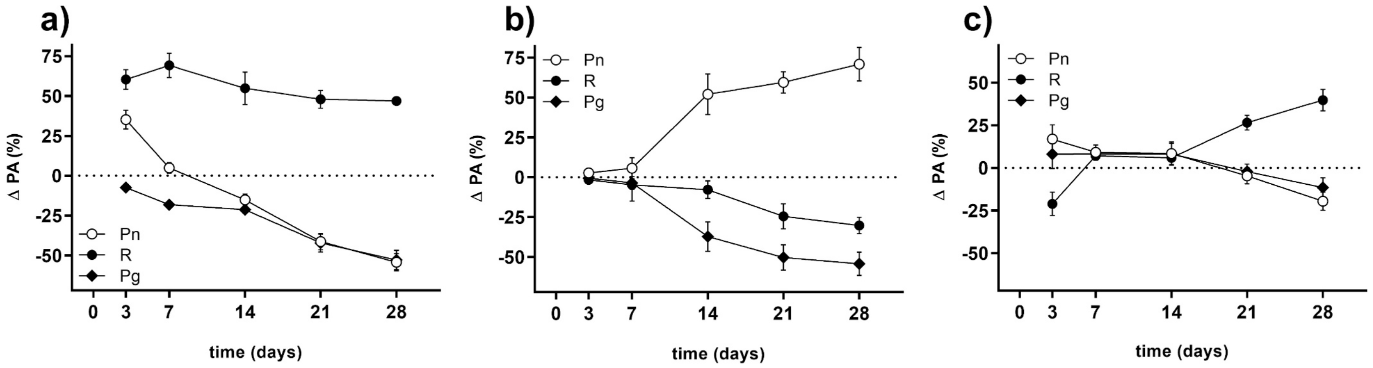

Fig. 2. Increased photosynthetic activity (ΔPA) displayed by *S. armatus* (a), *M. aeruginosa* (b), and both strains in community (c) under continuous exposure to 1 ngL^{-1} AgNPs for 28 days in gross photosynthesis (Pg; ◆), net photosynthesis (Pn; ○), and dark respiration (R; ●). Each point represents the mean ± sd of 4 independent experiments (n = 4) expressed as percentage inhibition (%) relative to control values.

control values. The initial Pn increase was progressively reduced until reaching photosynthetic activity percentages of -54.29 ± 5.46 %, significantly below control values. However, R activity remained increased during the 28 days of exposure, although with a slight decrease in activity until achieving a percentage increase of 46.94 ± 1.64 % with respect to control assays (Fig. 2a).

Single cultures of the freshwater cyanobacterial *M. aeruginosa* exposed to AgNP progressively increased Pn activity to show an increase of 70.86 ± 10.56 % at 28-day exposure. However, R activity showed an opposite effect by progressively decreasing during time exposure, achieving values of -30.22 ± 5.19 % with respect to the control assays (Fig. 2b).

In both cases, the isolated cultures showed a net negative balance of Pg activity, progressively decreasing to achieve a decrease at 28 days of exposure of 52.87 ± 6.03 % and 54.23 ± 7.30 % respect to control for *S. armatus* and *M. aeruginosa*, respectively.

However, when both strains were exposed under phytoplankton community conditions, the Pg balance showed non-significant differences compared to the control assays during the first 21 days post-exposure. The maximum increase in Pg activity was 8.19 ± 6.22 % and then decreased to reach an inhibition of 11.52 ± 5.75 % at 28 days post-exposure. In the initial exposure interval, Pn activity exhibited by the phytoplankton community showed an increase of 16.92 ± 8.29 %, while R activity decreased to 21.09 ± 6.86 %. From day 7 of exposure, both parameters stabilized in activity increments that in no case exceeded 10 % concerning the control trials. Finally, while Pn activity showed a reduction from day 21 post-exposure to a decrease of 19.46 ± 5.30 % at 28 days of exposure, R activity increased significantly to an overall increment of 39.82 ± 6.25 % in the same time interval (Fig. 2c).

3.3. ROS generation

The ROS generation exhibited by both strains exposed individually or in phytoplankton community to AgNP after 28 days of exposure is plotted in Fig. 3.

After 28 days of individual exposure, the green algae *S. armatus* and the cyanobacteria *M. aeruginosa* showed an increase in ROS generation of 27.19 ± 15.18 % and 18.60 ± 6.60 %, respectively. However, when both strains were exposed to AgNP under phytoplankton community conditions, no ROS generation occurred, and even the analyses showed a slight decrease of 2.51 ± 6.79 % with respect to the control assays.

3.4. MC-LR synthesis and release

The results obtained from the measurement of intra- and extracellular MC-LR from *M. aeruginosa* cultures exposed to 1 ngL^{-1} AgNP for 28 days are plotted in Fig. 4.

The cyanobacterial strain exposed to AgNP nanoparticles both single and in community with *S. armatus* released MC-LR concentrations of 3.4 ± 0.6 μgL^{-1} and 2.6 ± 0.9 μgL^{-1} to the culture medium, respectively

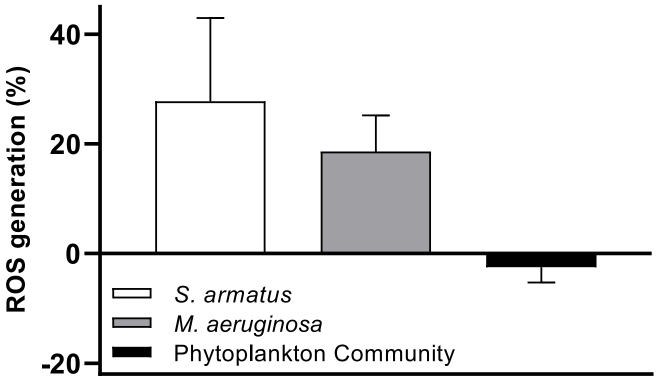

Fig. 3. Reactive oxygen species (ROS) generation from *S. armatus*, *M. aeruginosa,* and both strains in the community, continuously exposed to 1 ngL^{-1} AgNPs at 28 days post-exposure. Each bar represents the mean ± sd of 4 independent experiments ($n = 4$) expressed as percentage generation (%) with respect to control.

(Fig. 4a). These concentrations represent about 36 % and 31 % decrease, respectively, with respect to the control values.

Intracellular MC-LR analysis showed that *M. aeruginosa* cells, cultured both single and in community with *S. armatus*, contained concentrations of 76.9 ± 7.6 fg cell^{-1} and 63.9 ± 7.8 fg cell^{-1}, respectively (Fig. 4b). These values represent a decrease of about 21 % and 34 % with respect to the controls.

4. Discussion

It is widely recognized that AgNPs represent an emerging threat to aquatic systems, so it is necessary to study the fate and toxic effects associated with these nanoparticles in order to assess the risk to these ecosystems. Phytoplanktonic species in particular appear to have varied responses when exposed to AgNP. Decreased photosynthetic activity, reduced ATP production, or increased lipid peroxidation primarily through the generation of reactive oxygen species, are effects observed on phytoplankton over short-time scales (Oukarroum et al., 2012; Das et al., 2014; Ivask et al., 2014). However, these effects on phytoplankton communities are poorly referenced in the literature. Likewise, while there is abundant information about the short-term toxic effects of AgNPs on phytoplankton (Griffitt et al., 2008; Ribeiro et al., 2014; Pham, 2019), long-term studies in the literature are limited.

In our study, exposures of 1 ngL^{-1} AgNP on green algae *S. armatus* and cyanobacteria *M. aeruginosa*, grown in single or community cultures, led to different behaviors in terms of cell growth rate, photosynthetic activity, and ROS generation. The selected concentration is within the range of expected Ag concentrations and in agreement with the observations and predicted ambient concentrations described by Blaser et al. (2008). In European surface waters, 0.56-2.16 ngL^{-1} AgNP concentrations have been predicted (Gottschalk et al., 2009; Nowack et al., 2011), and more recently 0.9-2.3 ngL^{-1} AgNPs concentrations have been measured in sediments of the Isar River (Germany) (Li et al., 2016).

Cell growth rate assays showed an initial inhibition in both phytoplankton strains, followed by a sustained decrease over 28-day exposures, until growth rates similar to control values were achieved. These results are in agreement with those obtained by Sørensen and Baun (2015), who point to the timing issue as a fundamental aspect when performing ecotoxicity tests with AgNPs on freshwater phytoplankton. This time-dependent impact may in part be related to the kinetics of AgNP dissolution. Different authors have suggested that the release of silver ions from AgNPs follows a kinetic pattern of a very fast initial release of ions, followed by a stagnation or equilibrium phase, often occurring within few days (Liu and Hurt, 2010; Lee et al., 2012).

Our results suggest that while AgNPs adversely influenced on the phytoplankton community over the 28-day analysed period, they had an uneven effect on the growth rate exhibited by *S. armatus* and *M. aerugionsa* strains. Thus, while cyanobacteria showed a low impact, which progressively decreased to the level of the control tests, green algae showed a much higher impact maintained throughout the 28 days of exposure. These results could be influenced by the production of microcystins from *M. aeruginosa* strain present in the consortium. Sedmak and Eleršek (2005) observed increased cell aggregation in *Scenedesmus quadricauda* in the presence of *M. aerugionsa*, as well as changes in the cell volume of these green algae, which decreased the biodiversity in these consortia. Inhibitory effects on the proliferation of specific phytoplanktonic organisms have been observed previously (Sedmak and Kosi, 1998; Singh et al., 2001), which would demonstrate that microcystin-producing cyanobacteria gain a substantial ecological advantage. In any case, the proper mechanism of green algae-cyanobacteria interaction has not yet been sufficiently explored and further studies are needed to understand the mechanism and effect.

Analysis of the photosynthetic activity exhibited by both phytoplankton strains showed that exposure to 1 ngL^{-1} AgNP induced a significant decrease in Pg activity compared to controls. However, when the effect was disaggregated into their corresponding Pn and R activities, the impact on both strains was different. Thus, while the green algae *S. armatus* showed a sustained increase in R activity, gradually decreasing their Pn activity, the cyanobacterial *M. aeruginosa* showed opposite effects.

Most of the references we have found on the photosynthetic activity of phytoplankton affected by AgNP were based on short-term studies, and there are very few studies that extend the time exposure over 96 h. There is consensus in all of them that AgNP can be toxic to

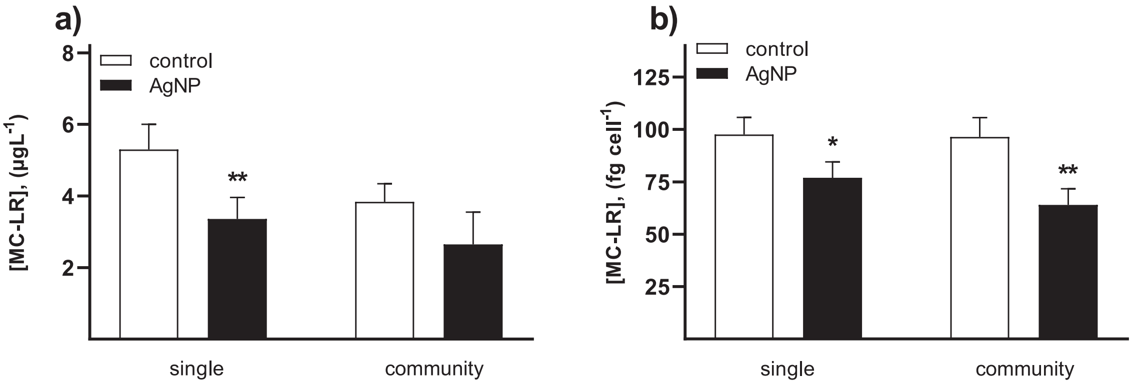

Fig. 4. Release into the culture medium (a) and cell production (b) of MC-LR generated by *M. aeruginosa* cells exposed both on single cultures and in phytoplankton community conditions to 1 ngL^{-1} AgNP. Each bar represents the mean ± sd of 4 independent experiments ($n = 4$) expressed as percentage generation (%) with respect to control. (*) and (**) represent significant differences respect to control at $p < 0.05$ and $p < 0.01$, respectively.

phytoplankton photosynthesis (Wijnhoven et al., 2009; Julia et al., 2011; Behra et al., 2013; Ribeiro et al., 2014), even under nanomolar or sub-nanomolar level exposures (Gonçalves et al., 2016). Additionally, some studies have documented adverse effects of AgNp on the physiology (photosynthesis, lipid peroxidation, enzymatic activity) of prokaryotic and eukaryotic algae (Navarro et al., 2008; Pugliara et al., 2016). Considering the AgNP concentration differences used in each of the referenced studies, our results agree with those obtained by these authors, showing a weak Pg inhibition at short-term exposure times. Additionally, our results show that this inhibition increased gradually until 28 days of exposure.

To our knowledge, no data on the effect of AgNP on Pn and R are available in the literature so far. However, studies by Qian et al. (2016) on expression of proteins involved in photosynthesis from *Chlorella vulgaris* and *M. aeruginosa* exposed to AgNP showed that several of the most dramatically regulated proteins identified were parts of the Calvin cycle and photosynthetic metabolism, both of which are directly associated with light-independent reaction. The progressive decrease in R activity exhibited by *S. armatus* and *M. aeruginosa* strains in our assays agrees with these findings.

When both strains were exposed to 1 ngL^{-1} AgNP under multispecies community conditions, the photosynthetic balance was significantly equilibrated with the control assays from day 7 post-exposure. Analysis of the Pn and R activities exhibited by both strains exposed in monoculture and in phytoplankton community seems to indicate that the final photosynthetic balance would be the sum of each independently, without being altered by other factors, such as irradiance availability or microcystin formation from *M. aeruginosa*. These results agree with Conine et al. (2018) who concluded that AgNP exposures at environmentally relevant concentrations for 2 years did not affect phytoplankton communities. The absence of microcystin-induced adverse effects on community dynamics agrees with Kaur et al. (2019), who observed that exposure to AgNP at environmentally relevant concentrations for 10 days did not affect phytoplankton communities in freshwater aquatic environments.

Our studies showed that, after 28 days of exposure to AgNP, a significant increase in ROS production was observed in both strains. It is widely accepted that AgNPs provoke oxidative damage through the generation of ROS (Oukarroum et al., 2012; Qian et al., 2016; Taylor et al., 2016), activating the antioxidant system and thus increasing the production of antioxidants. The fact is that, despite these increases in ROS production, neither the rate of cell growth nor photosynthetic activity is affected. This fact may be explained by the ability of the microalgae to form exopolysaccharides (EPS) as a protective system against stress. Yilancioglu et al. (2014) have reported EPS increases when microalgae are exposed to different stress conditions.

This protective effect was more pronounced when both strains were exposed under phytoplankton community conditions. Although some studies conducted under these conditions showed a decrease in the toxic response (Baker et al., 2014; Sharma et al., 2014; Lodeiro et al., 2017), further studies are needed to establish the protective mechanisms of toxicity capable of significantly decreasing the generation of ROS in mixed exposures to AgNP nanoparticles.

After 28 days to AgNPs exposure, *M. aeruginosa* cells produced and released lower amounts of MC in both single and community cultures.

The decrease in intracellular MC content obtained in our studies agrees with the results of Qian et al. (2016), which showed a reduction of close to 40 % after 96 h of exposure. However, the percentage decrease in MC release to the medium was significantly lower than that obtained by us. This discrepancy between short- and long-term studies, as well as the possible differences in cell membrane integrity reported by Singh et al. (2021), call for further studies in the future.

Studies by Mohamed (2008) demonstrated the relationship between MC and oxidative stress in the freshwater green algae *Chlorella vulgaris* and *Scenedesmus quadricauda*, as well as the existence of a protective response from polysaccharide production. This fact could justify that, after 28 days of exposure, the toxins did not affect the cell growth of the *S. armatus* strain growing in community with *M. aeruginosa*.

5. Conclusions

The present study has shown that exposure to 1 ngL^{-1} AgNP decreased the cell growth rate of the phytoplankton population exposed in the short term. This effect was higher in *M. aeruginosa* than in *S. armatus*, and in both strains was time-dependently mitigated until it disappeared at 28 days post-exposure. In both strains, although photosynthetic activity increased at 28 days of exposure, significant decreases in dark respiration and net photosynthesis were observed in *S. armatus* and *M. aeruginosa*, respectively. After 28 days of exposure, AgNPs induced ROS generation in both strains. Additionally, *M. aeruginosa* cells exposed to AgNPs produced and released lower amounts of MCs in the same time interval. However, when both strains in phytoplanktonic community long-term exposed to 1 ngL^{-1} AgNPs, green algae did not decrease the growth rate during the 28 days of exposure, in contrast to the induced effect on cyanobacteria. Photosynthetic activity and ROS generation were also different from those observed in single-species cultures, mitigating the effects of AgNPs. In addition, MC-LR generation was significantly lower than that produced in single-species cultures.

CRediT authorship contribution statement

A.A. Cortés-Téllez: Writing – original draft, Visualization, Investigation, Formal analysis. **A. D'ors:** Visualization, Investigation, Formal analysis. **C. Fajardo:** Visualization, Supervision, Investigation. **G. Mengs:** Software, Investigation. **M. Nande:** Software, Investigation. **C. Martín:** Visualization, Investigation. **G. Costa:** Visualization, Investigation. **M. Martín:** Visualization, Supervision, Investigation, Funding acquisition. **M.C. Bartolomé-Camacho:** Writing – original draft, Supervision, Investigation. **S. Sánchez-Fortún:** Writing – original draft, Investigation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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