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Assessing the long-term adverse effects of aluminium nanoparticles on freshwater phytoplankton using isolated-species and microalgal communities

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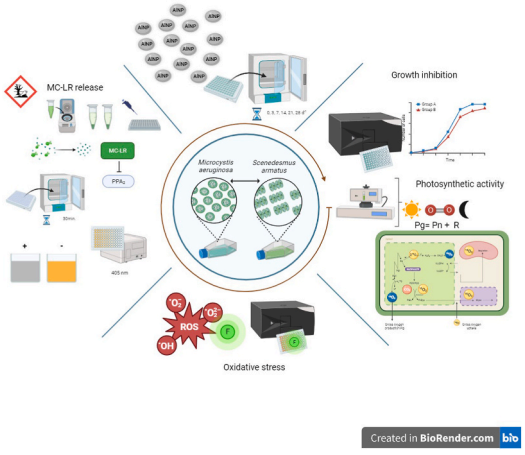
HIGHLIGHTS

- Long-term AlNPs exposure oppositely modified μ and photosynthetic balance in both strains.
- Pg, Pn and R were similarly oppositely modified by long-term AlNPs exposure in both strains.
- Long-term AlNPs exposure on bicultures gives competitive advantage to *M. aeruginosa* in μ and photosynthetic activity.
- ROS production increased in both strains exposed to AlNPs for 28 days but decreased in bicultures.
- Exposure to AlNPs for 28 days did not change either MC-LR production or release, but increased in bicultures.

GRAPHICAL ABSTRACT



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ABSTRACT

The physicochemical properties of aluminum oxide nanoparticles (Al_2O_3-NPs or AlNPs) allow them to remain suspended in water for extended periods. Despite this, AlNPs are one of the least studied types of metal nanoparticles and pose a significant risk to aquatic ecosystems. Therefore, it is essential to understand the toxic mechanisms of AlNPs on microalgae and cyanobacteria, as they can have adverse effects on the entire aquatic

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Growth rate
Photosynthetic activity
ROS
Microcystin (MC-LR)

food web. Our research aimed to assess the toxicity of continuous exposure to low environmentally relevant concentrations of AlNPs on the growth rate, photosynthetic activity, oxidative stress (ROS), and microcystin production (MC-LR) in a phytoplanktonic community (PCC) consisting of *Scenedesmus armatus* and *Microcystis aeruginosa*. Both single and community cultures were exposed to 1.0 μg mL-1 AlNPs for 28 days. The results showed a significant 20–40% inhibition of *S. armatus* population growth in both individual and community cultures after 28 days of exposure. In contrast, *M. aeruginosa* exhibited increased survival and cell division rates when exposed to nanoparticles, both individually and within the community. Additionally, *S. armatus* showed a substantial reduction in gross photosynthesis (Pg) and net photosynthesis (Pn), with less inhibition in respiration (R) after 28 days of exposure. Conversely, *M. aeruginosa* demonstrated higher rates of photosynthetic productivity in all three parameters (Pg, Pn, and R). In the PCC, respiration was inhibited from 14 to 28 days, and both Pg and Pn were also inhibited. Both *S. armatus* and *M. aeruginosa* showed 28–31% levels of ROS generation, while the phytoplanktonic community exhibited no significant ROS production. Moreover, the production and release of MC-LR decreased by 8–38% in *M. aeruginosa* compared to the control strain. These findings underscore the importance of monitoring the use and application of nanomaterials to mitigate their potential toxic effects on aquatic ecosystems.

1. Introduction

Nanotechnology uses nanomaterials like metal nanoparticles (MNPs), which have unique properties such as a large surface area and high stability. These particles, measuring 1–100 nm, are used in various fields, including biomedical science, pharmaceuticals, agriculture, and wastewater treatment (Aravantinou et al., 2015; Zhang et al., 2019). Aluminum oxide nanoparticles (AlNPs) are notable for their ability to absorb UV light, making them useful in water treatment systems (Javed et al., 2013; Zhou et al., 2016). They also have antibacterial properties and are employed in drug delivery and cancer therapy. (Doskocz et al., 2017; Hassanpour et al., 2018; Prabhakar and Samadder, 2018).

However, AlNPs can remain suspended in water for long periods, posing risks to aquatic life. The U.S. Environmental Protection Agency (U.S. EPA, 2018) indicates that they are highly toxic to aquatic ecosystems. More ecotoxicological studies are needed to assess the risks of MNPs, as current research often overlooks long-term effects of low-level exposure in freshwater systems and focuses mainly on single microalgae strains instead of entire phytoplankton communities (Zhang et al., 2019).

Freshwater microalgae and cyanobacteria are the basis of the aquatic trophic web because they participate in energy transfer and represent more than 50% of the planet's oxygen (Prihanto et al., 2022). The harmful effects of MNPs on microalgae and cyanobacteria produce negative impacts on the entire aquatic food web (Nguyen et al., 2020) and it is necessary to identify the toxic mechanisms of Al_2O_3-NPs in microalgal and cyanobacterial cells. Likewise, the toxic effects of MNPs comprise entering microalgal cells in alterations on photosynthetic activity and pigment concentration, allelopathic responses in the phytoplankton community, ultrastructural alterations in microalgal cells, inhibition of population growth and induce oxidative stress through the reactive oxygen species (ROS) formation (Mahana et al., 2021). Besides, the MNPs induce nutrient sequestration, alterations in light uptake, as well as light shading effects (Liang et al., 2020).

Therefore, this study aims to assess the toxicity of continuous exposure to AlNPs at low environmentally predicted concentrations (Pakrashi et al., 2012; Gottschalk et al., 2013). Specifically, we will evaluate the impact on a strain of green freshwater microalgae *Scenedesmus armatus*, the *Cyanophyceae Microcystis aeruginosa*, and both under biculture conditions. To achieve this, we will evaluate the percentage of population growth inhibition, analyze oxidative stress by measuring the formation of reactive oxygen species (ROS), and estimate the levels of photosynthetic activity. Additionally, we aim to determine both the intra- and extracellular levels of microcystin (MC-LR) in isolated cultures of *M. aeruginosa* cells. This assessment will also be conducted under biculture conditions while exposing the cells continuously to AlNPs.

2. Material and methods

2.1. Characterization of aluminium nanoparticles

Aluminium oxide nanoparticles (Al_2O_3-NPs) were purchased from Sigma Aldrich (Saint Luis, MO). The supplier's data can be summarized as follows: molar weight of 101.96 g mol^{-1}, particle size <50 nm, specific surface area of >40 m^2g^{-1}, and 20 wt% in H_2O. Using Nanoparticle Tracking Analysis (NTA; NanoSight, Amesbury, UK) software package, a size-distribution for Al_2O_3-NPs with three different size peaks (51 ± 8 nm, 87 ± 11 nm, and 120 ± 13 nm) has been obtained.

The Al_2O_3-NPs were suspended in distilled water followed by sonication. A single nominal concentration of 1 mg L^{-1} was selected for testing, according to the predicted levels of Al_2O_3-NPs in freshwater environments estimated by Pakrashi et al. (2012) and Gottschalk et al. (2013).

2.2. Organisms and culture conditions

Two species of algae naturally present in freshwater ecosystems were used during the study. Strains of *Scenedesmus armatus* (BEA 1402B) and *Microcystis aeruginosa* (BEA, 1835B) were acquired from the Spanish algae bank (BEA. Gran Canarias, Spain) as representative samples of freshwater green algae and cyanobacteria. They were grown in flasks (Thermo Fisher Scientific Inc., MA, USA) with 20 mL BG11 culture medium (Sigma Aldrich Chemie, Taufkirchen, Germany) in mono- and bicultures. These were maintained in mid-log exponential growth by serial transfer of cell groups to fresh medium once every 15 days.

2.3. Cell growth rate

For the study, the contemplated concentration was 1 mg L^{-1} Al_2O_3-NPs. Both control and exposed cultures were maintained at 21 °C for 72 h in a thermostatically controlled chamber (Gilson Inc., Middleton, WI USA) at 375 μmol photons $m^{-2}s^{-1}$.

Cultures were maintained in 20 mL of BG-11 at a density of 10^4 cells mL^{-1} and every day were manually shaken twice a day. The cell growth rates of mono- or bicultures 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 microalgae *S. armatus* and the cyanobacteria *M. aeruginosa*, respectively. The relationship between fluorescence and cell density was estimated by comparing it with parallel Neubauer chamber counts.

For the 28-day study, measurements were obtained at 3, 7, 14, 21, and 28 days for both control and exposed cells, and with these measurements, the maximum cell growth rate (μ) could be assessed by the Malthusian growth model, expressed in d^{-1} and calculated by the following equation (Crow and Kimura, 1970)

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

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}.

To rule out possible shading effects, additional light absorption tests were performed (OD627 and OD720). Results of 0.007 or less were obtained when emitting more than 1 mg L^{-1} of AlNPs in 20 mL of BG-11 culture medium so that we could discard possible shading effects.

2.4. Photosynthesis activity assessment

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

The 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 photons 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 ml^{-1}. 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 (ROS)

The intracellular oxidation process of 2′,7′-dichlorofluorescein diacetate (H2DCFDA) (Sigma-Aldrich, St. Louis, MO, USA) induces the generation of a fluorescent compound of 2,7-dichlorofluorescein (DCF). We used this compound as a ROS indicator to test intracellular reactive oxygen species produced by *S. armatus* and *M. aeruginosa* strains in mono- and biculture conditions.

We used an adjusted concentration of AlNP nanoparticles for cell cultures of *S. armatus* (2×10^6 cells mL^{-1}) and *M. aeruginosa* (5×10^6 cell mL^{-1}). All of them were adjusted to a volume of 1.5 mL with a final concentration of 10 mM of H_2DCFDA. Incubation was 60 min at 23 °C.

Sample data were collected at 0, 1, 2, and 4 h using a Tecan Genios microplate reader (Tecan Group Ltd., Switzerland) with excitation-emission filters of 485–520 nm and at room temperature. For the positive control, 3% H_2O_2 (v/v) was used. BG-11 culture medium was also tested to check for the presence of ROS that could interfere with the results. This fluorescence was subtracted from that produced by the culture. Each bioassay was repeated six times (n = 6).

All samples were tested on days 0, 3, 7, 14, 21, and 28 days in dark conditions at an intensity of 375 μmol photons m^{-2} s^{-1} (*S. armatus*) and 222.5 μmol photons m^{-2} s^{-1} (*M. aeruginosa*).

2.6. Microcystins (MC-LR) measurement

Intracellular and extracellular determination of MC-LR was done by using a density of 2×10^6 *M. aeruginosa* cells exposed to 1 mg L^{-1} AlNPs for 28 days. These were centrifuged at 10000 *g* and 4 °C for 5 min, and the supernatant was collected for the study. It was then frozen at −80 °C and thawed immediately. The process was repeated three times (Zambrozi and Martínez, 2012).

To perform the MC-LR study, phosphatase activity was evaluated using a commercial kit (MicroCistest, ZEU-IMMUNOTEC S.L., Zaragoza, Spain). The amount of toxins present in the sample is directly proportional to the amount of enzyme inhibited by the MC-LR, and quantification at 405 nm wavelength.

2.7. Experimental data analysis

The estimation of each percentage population growth inhibition value of *Scenedesmus armatus*, *Microcystis aeruginosa* and bicultures was established through multiple comparisons performed via multiple *t*-test. The oxidative stress and the microcystin MC-LR levels were measured by the comparisons estimated through One-way ANOVA. All data were expressed as the mean and standard deviation of each value (mean ± SD) and their respective 95% confidence limits. D'Agostino-Pearson test assessed data normality and the differences are considered significant at $p < 0.05$, the analysis of all data is established through the statistical software GraphPad Prism v 9.0 (Graph-Pad Software Inc., USA).

3. Results

3.1. Growth inhibition rate

According to the results obtained, growth rate (μ) inhibition is shown in Fig. 1A and B, with a reduction of 18.8 ± 7.3% at 3 days, while at 7 days only 6.7 ± 1.7% reduction of *S. armatus*. However, in bicultures, the inhibition of the population growth of *S. armatus* was increased between 20% and 40% with statistically significant differences ($p < 0.0001$) at 3, 7, 14, 21, and 28 days of exposure (Fig. 1a).

For the strain of *Microcystis aeruginosa* exposed to AlNPs, there is a slight increase in growth at 3, 7, and 14 days of exposure compared to the control strain. This translates into Fig. 1b, where indeed the cyanobacteria increase their survival, and their rate of cell division is not affected by individual exposure to AlNPs. *Microcystis aeruginosa* cells exposed under phytoplankton community conditions showed an increased cell growth ratio of 37.2 ± 2.3% at 3 days post-exposure concerning the control (Fig. 1b). Subsequently and up to 28 days post-exposure, the cell growth rate exhibited by the *M. aeruginosa* strain did not show significant differences concerning the control values.

3.2. Photosynthetic oxygen evolution

The *S. armatus* strain exhibited significant inhibition of Pn, amounting to 71.7 ± 9.3% within the first 72 h of exposure, followed by a gradual decrease to 11.0 ± 6.4% inhibition at 28 days post-exposure compared to control values (Fig. 2a). This pattern of inhibition is also evident in the changes observed in R, with inhibition of 51.2 ± 5.6% after 72 h, gradually decreasing to 16.2 ± 10.5% at 28 days of exposure (Fig. 2b). Similarly, both parameters led to initial Pg inhibitions of 40.56 ± 8.05%, which reduced to 9.9 ± 6.8% at 28 days of exposure (Fig. 2c).

The *M. aeruginosa* strain exhibited contrasting behavior compared to *S. armatus* when exposed individually. It showed a 1.5-fold increase in Pn activity (154.6 ± 13.8%) at 14 days of exposure, followed by a subsequent decrease to a 24.8 ± 11.5% increase at 28 days (Fig. 2a). Similarly, there was a moderate increase in R activity with values of 80.3 ± 13.0% and 14.6 ± 4.9% at 14 and 28 days of exposure, respectively (Fig. 2b). Moreover, Pn activity showed an increase at 14 days (117.9 ± 21.6%) and then decreased to 19.7 ± 9.1% at 28 days post-exposure (Fig. 2c).

Under biculture conditions, both strains exhibited changes in their activities. The Pn activity showed a mild inhibition of 20.7 ± 6.7% in the first 72 h, which continued throughout the exposure period, resulting in a reduction of 15.6 ± 1.8% compared to the control after 28 days (Fig. 2a). In contrast, the R activity significantly increased by 50.9 ± 5.8% in the first 72 h, but then gradually decreased, reaching an inhibition percentage of 30.7 ± 11.8% after 28 days (Fig. 2b). As for Pg

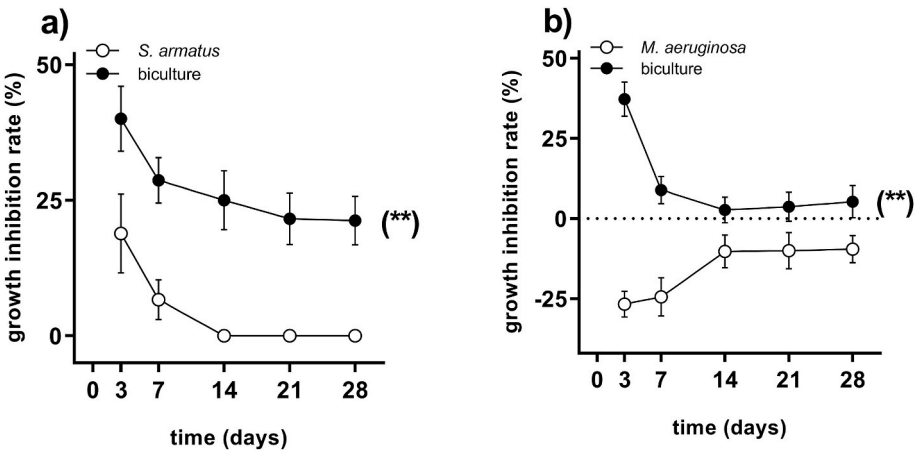

Fig. 1. Cell growth rate inhibition displayed by *S. armatus* (a) and *M. aeruginosa* (b) continuously exposed singly (empty dots) or in biculture conditions (filled dots) to 1 mg L^{-1} Al_2O_3 NPs to 28 days. Each point represents the mean ± SD of 4 independent experiments (n = 4) expressed as percentage inhibition (%) with respect to control values. (**): Statistically significant differences ($p < 0.01$) concerning to the representative values of the single cultures.

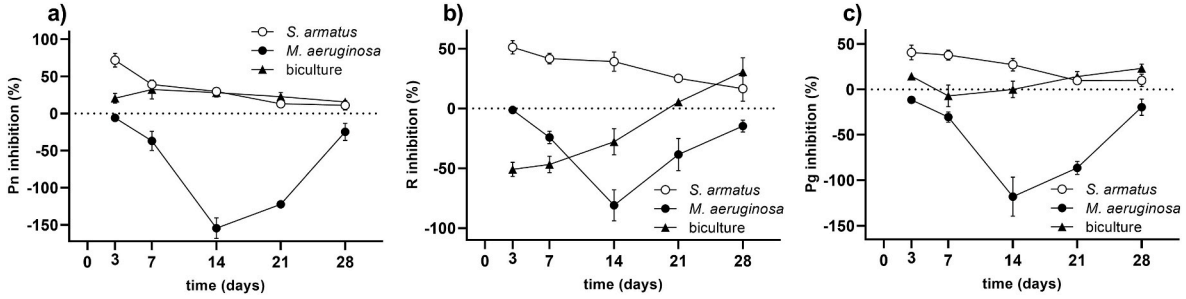

Fig. 2. Effects of 1 mg L^{-1} Al_2O_3 NPs exposures on the net photosynthesis (a), respiration (b), and gross photosynthesis (c) of *S. armatus* (◯) and *M. aeruginosa* (●) evaluated after 28 days of growth in both isolation and in biculture conditons (◆). The results, representing the mean ± SD of 4 independent experiments (n = 4), were expressed as percentage inhibition (%) relative to control values.

activity, there was an initial inhibition of 14.5 ± 3.7% which decreased until 14 days, after which the activity became comparable to the controls. Subsequently, it increased again to an inhibition value of 23.1 ± 4.7% after 28 days of exposure (Fig. 2c).

3.3. ROS assessment

Isolated *S. armatus* and *M. aeruginosa* cells experienced intracellular ROS increases of 31.4 ± 4.4% and 28.8 ± 6.2% (Fig. 3). Interestingly, when both strains were exposed under biculture conditions, the ROS values decreased by 21.7 ± 2.1% compared to the control values.

3.4. Microcystin (MC-LR) synthesis and release

When *M. aeruginosa* cells were grown in monoculture and exposed to 1 mg L^{-1} of AlNPs, they released a concentration of 6.1 ± 0.2 μg L^{-1} of MC-LR into the medium after 28 days, compared to 5.6 ± 0.4 μg L^{-1} released by control cells, marking an 8.2% increase in ROS. In bicultures, the exposed cells released 5.2 ± 0.3 μg L^{-1} compared to 3.6 ± 0.2 μg L^{-1} released by control cells, representing a 31.2% increase in ROS (Fig. 4a).

In monocultures, the intracellular concentration of MC-LR in exposed *M. aeruginosa* cells was 67.9 ± 11.1 fg $cell^{-1}$, a concentration very similar to that of control cells (71.3 ± 10.8 fg $cell^{-1}$). However, under biculture conditions, exposed cells exhibited a 32.8% increase in MC-LR concentration (100.2 ± 7.8 fg $cell^{-1}$) compared to controls (67.3 ± 7.4 fg $cell^{-1}$) (Fig. 4b).

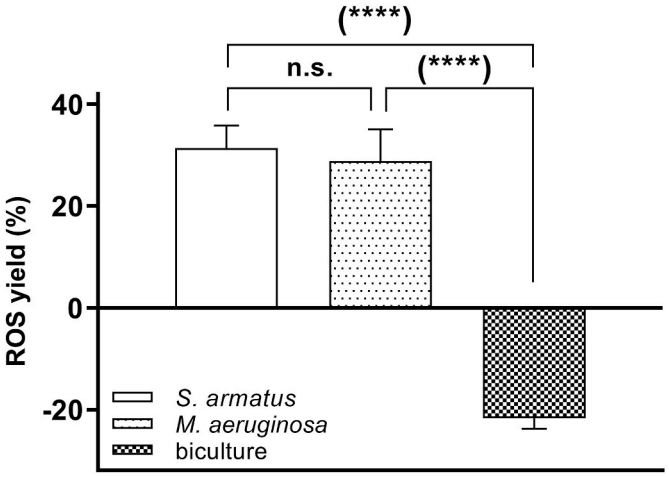

Fig. 3. ROS levels measured in isolated and bicultures of *S. armatus* and *M. aeruginosa* exposed to 1 mg L^{-1} Al2O3 NPs for 28 days. Each bar represents the mean ± SD of 4 experiments (n = 4), expressed as percentages of intracellular ROS yield compared to controls. (n.s.): no significant differences; (****): significant differences to control at $p < 0.0001$.

4. Discussion

Despite numerous ecotoxicity studies focusing on metal and oxide nanoparticles (Déniel et al., 2019; Chen et al., 2018; Sendra et al., 2018, 2023; Yang et al., 2023), AlNPs have received comparatively less

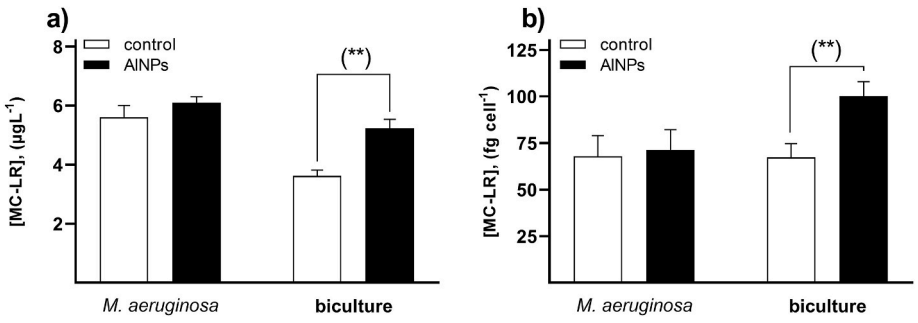

Fig. 4. Release into the culture medium (a) and cell production (b) of MC-LR generated by *M. aeruginosa* cells exposed both on single and biculture conditions to 1 mg L^{-1} Al_2O_3 NPs. Each bar represents the mean ± SD of 4 independent experiments (n = 4) expressed as μg L^{-1} and f gL^{-1} for extra and intracellular MC-LR, respectively, with respect to control values. (**) represent significant differences to control at $p < 0.01$.

attention. This is noteworthy given their potential for long-term accumulation in aquatic environments due to their high stability and low solubility (U.S. EPA, 2018). In this study, mono- and bicultures of *S. armatus* and *M. aeruginosa* were exposed to AlNPs concentrations of 1 mg L^{-1}. These levels align with environmental concentrations reported by other researchers (Pakrashi et al., 2012; Gottschalk et al., 2013) and highlight the need for careful regulation and control to mitigate their environmental impact. After reviewing the literature, we found limited information on the long-term risks of AlNPs in freshwater phytoplankton, with most studies focusing on short-term exposure (48–72 h). The 72-h median inhibitory concentration (72h-IC_{50}) for AlNPs was reported as 39.3 mg L^{-1} for *Scenedesmus* sp. and 45.4 mg L^{-1} for *Chlorella* sp. (Sadiq et al., 2011). Our results showed a slightly lower inhibition (18.9%) at 72 h, confirming the adverse effects on these algae. Notably, cell growth reduced to 6.6% at 7 days but returned to control levels by 28 days. This aligns with Karwowska et al. (2012), who observed a 5% decline in *S. quadricauda* after 7 days, but they noted a 55% inhibition after 21 days, likely due to higher exposure concentrations (20 mg L^{-1}) and the use of Al_2O_3 nanopowders in their study.

In our research, we observed an initial increase in the growth rate of the *M. aeruginosa* strain when exposed to AlNPs, followed by a gradual decrease until reaching levels similar to the control. Due to the insolubility of AlNPs, the bioavailability of Al^{+3} described by Pakrashi et al. (2013), which would explain the inhibitory effects of cell growth on cyanobacteria of the genus *Nostoc* sp. described by Kannaujiya et al. (2020), cannot be decisive in our case. The phenomenon of hormesis explains how low concentrations of MNPs can stimulate the growth rate of algae (Wu et al., 2019). Similar effects have been observed with iron-based (D'ors et al., 2023) and silver nanoparticles (Cortés-Tellez et al., 2024) on different cyanobacteria strains. This might be linked to variations in adsorption surface area and particle size, as higher surface area increases biological reactivity (Oberdörster et al., 2005). Notably, significant differences in surface area exist between *S. armatus* and *M. aeruginosa*. Previous research (Sadiq et al., 2011; Shirazi et al., 2015; Ameri et al., 2020) shows that aluminum affects various phytoplankton species differently, corroborating Wong et al. (2020) who noted that the impact of nanoparticles on aquatic organisms varies with the type.

Exposure to AlNPs under biculture conditions initially had a strong impact on both *M. aeruginosa* and *S. armatus* strains, followed by a gradual recovery of the growth rate, with a more pronounced effect on *M. aeruginosa*. The competitive nature of both strains became evident under biculture conditions. It is generally agreed within the scientific community that *Microcystis* sp. tends to dominate phytoplankton communities due to its ecophysiological traits and environmental factors. The formation of colonies by *Microcystis* provides protection against external stressors, while the release of microcystins also contributes to its predominance. Our findings suggest that the adverse effects observed on *M. aeruginosa* cells are proportional to the exposure time, leading to larger colonies and increased MC-LR production. Some studies have suggested that low concentrations of microcystins do not provide a competitive advantage under biculture conditions, while others have indicated that only at higher concentrations does the growth of *Scenedesmus* sp. get affected. *Scenedesmus* sp., a green microalgae, coexists with *Microcystis* sp. in natural environments and acts as its competitor. It has been found that under certain circumstances, *Scenedesmus* sp. can partially resist the predominance of *Microcystis* sp. Allelopathic inhibition of *Scenedesmus* sp. growth is only possible in the early stages of competition, which aligns with the results obtained from our long-term exposures.

The photosynthetic activity of both strains was affected by AlNPs, but in different ways. Initially, AlNPs inhibited Pn and R in *S. armatus* cells, though these effects diminished over time to match control values. In contrast, *M. aeruginosa* showed no changes in Pn and R during the first 72 h, but these parameters more than doubled after 14 days before returning to control levels. The differences may be linked to the varying surface areas in contact with AlNPs, which can affect algal photosynthesis (Zhang et al., 2013; Sadiq et al., 2011). Our research found that AlNPs did not reduce the photosynthetic activity of *M. aeruginosa*, contrasting with previous studies (Cheng et al., 2021; Xu et al., 2021; Tseytlin et al., 2024) that indicated otherwise. However, some authors reported no effect of nanoparticles on cyanobacterial photosynthesis (Wu et al., 2021), highlighting the need for further studies to understand the toxic mechanisms of NPs on cyanobacterial photosynthesis. The Pg balance under biculture conditions showed only a slight difference, with 14–23% inhibition compared to the controls. These results can be attributed to the interplay between the inhibitory effect of *S. armatus* cells and the increase in Pg activity by *M. aeruginosa* cells. This phenomenon is also evident in Pn and R photosynthetic parameters. On the other hand, our findings of increased photosynthetic activity by *M. aeruginosa* cells are in agreement with the proposal of Wang et al. (2022), which indicated that under stress conditions, *M. aeruginosa* cells are able to increase photosynthetic potential by stimulating the maximum relative electron rate and thus maintain the population by enhancing photosynthetic activity.

Our research found that 28 days of exposure to AlNPs increased oxidative stress by 29–31% compared to controls in both isolated strains. This aligns with prior studies showing that ROS generation is common in microalgae and cyanobacteria exposed to metal NPs (Déniel et al., 2019). For instance, ROS levels rose by 13–19% in *Chlorella ellipsoidea* exposed to Al_2O_3-NPs (Pakrashi et al., 2013). Some researchers attribute the ROS increase to AlNPs' enzymatic effects on *Scenedesmus* sp. (Ameri et al., 2020), while others noted lipid peroxidation and protein oxidation in cyanobacteria exposed to Al^{+3} for 7 days (Hamed et al., 2019). These findings provide context for our long-term exposure results. When the AlNPs were exposed under biculture conditions, they had an unexpected impact. While the levels of reactive oxygen species (ROS) increased similarly in both strains when grown in monocultures, these levels decreased significantly under biculture conditions, dropping to around 20% below the control tests. This shift in ROS levels could be attributed to the activation of antioxidant

mechanisms and secondary metabolites induced by the phytoplanktonic community itself, with a particular emphasis on the role of *S. armatus* cells. Beneficial interactions between the two strains have been noted to help them cope with stressors (Harel et al., 2013). Antioxidant enzymes like catalase and superoxide dismutase are crucial for regulating ROS levels. Wu et al. (2019b) proposed that some MNPs with enzyme-like properties (nanozymes) could mimic these enzymes, reducing their harmful effects on phytoplankton. Given our specific experimental conditions, including the initial population size and growth rates, these factors likely explain our observed results.

Another variable that influences the magnitude of the adverse effect induced by AlNPs on the consortium is the capacity of *M. aeruginosa* cells to produce MC-LR. In our study, after continuous exposure to AlNPs for 28 days, there were no significant differences in either the release or production of MC-LR by *M. aeruginosa* monocultures. However, under biculture conditions, a significant increase in MC-LR release and production compared to controls was observed at 28 days post-exposure (Fig. 4). The impact of AlNPs on the production and release of MC-LR by *M. aeruginosa* cells has not been extensively documented. However, some studies have explored this effect using other MNPs, such as CeO_2-NPs Zhao et al. (2020), or TiO_2-NPs (Wu et al., 2019), yielding varying results. Our findings, in conjunction with these studies, suggest that the influence of MNPs on MC-LR production and release is likely influenced by multiple factors. Furthermore, Kumar et al. (2022) proposed that the impact of MNPs on MC-LR production and release is dependent on the specific characteristics of each type of nanoparticle. Our research has demonstrated that exposure to 1 mg L^{-1} of AlNPs under biculture conditions significantly boosts the production and release of MC-LRThe competition between *Microcystis* sp. and *Scenedesmus* sp. is a well-documented phenomenon with various explanations proposed for it. Some researchers have suggested two models: one that focuses on competition for limited resources and another that considers interference, where the competitors themselves create secondary effects that impact one another (You et al., 2007). Kuwata and Miyazaki (2000) others opt for a single competition model between *Microcystis* sp. and *Scenedesmus* sp. (Kuwata and Miyazaki, 2000), and finally others noted how in eutrophic conditions, *Scenedesmus* sp. is often replaced by *Microcystis* sp. (Zhu et al., 2016). Several mechanisms have been proposed to explain the increased production of MC-LR. Wang et al. (2022) found that phosphorus limitation due to *S. obliquus* competition enhances MC-LR production in colonial *M. aeruginosa* and is positively correlated with its photosynthetic potential. MC-LR from *Microcystis* induces changes in *Scenedesmus* sp., such as increased cell volume and photosynthetic pigment production (Teneva et al., 2023). Likewise, the release of microcystins may be linked to the inhibition of membrane protein synthesis, leading to increased cell permeability (Wu et al. (2019). Additionally, higher MC-LR synthesis in bicultures is associated with the regulation of McyA, McyD, and McyH genes (Zhang et al., 2017). These findings support Omidi et al. (2021) hypothesis that MC-LR production provides an environmental advantage.

These findings clarify the competition between *S. armatus* and *M. aeruginosa* in freshwater environments contaminated with AlNPs, as noted by Zhang et al. (2013). The presence of AlNPs appears to impact both species differently, with *M. aeruginosa* potentially gaining a competitive advantage. However, further studies are necessary to understand the mechanisms behind AlNPs effects on phytoplankton coexistence in freshwater ecosystems.

5. Conclusions

In conclusion, exposure to AlNPs initially affected the growth rate of *S. armatus* and *M. aeruginosa*, but over time, both strains approached control values. Under biculture conditions, *M. aeruginosa* matched controls by 28 days, while *S. armatus* maintained a 25% inhibition in cell growth rate. *S. armatus* showed a similar trend in Pg, influenced by photosynthetic parameters Pn and R. *M. aeruginosa* experienced a rise in Pg until day 14, with a gradual return to control levels by day 28. With minimal Pg variations in bicultures, both strains increased ROS production after 28 days, though bicultures led to a significant decrease in ROS levels. Notably, *M. aeruginosa* under monoculture conditions demonstrated increased production and release of MC-LR, but levels rose significantly in biculture exposures to AlNPs. This suggests that *M. aeruginosa* could gain a competitive edge over *S. armatus* in their shared environment.

CRediT authorship contribution statement

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

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.

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Data availability

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

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