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EFFECT OF SELECTED ANTIBIOTICS ON THE GROWTH AND MORPHOLOGY OF CYANOBACTERIA

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ABSTRACT

Antibiotics in the environment represent a significant pollutant with a great impact on the biota. With the increasing use of these substances, the resistance against them notably grows. The phototrophs, as the key part of microbial communities, often have different responses to antibiotics. Some species could be inhibited; on the other hand, some species show the ability to use antibiotics as a source of necessary nutrients. In our study, we investigated the impact of streptomycin, gentamicin, and sulfacetamide on six strains of cyanobacteria commonly present in water sources. The growth inhibitory effect of the studied antibiotics was measured in sterile 96-well microtiter plates, which contained different concentrations of antibiotics (1024 – 0.5 µg/mL) during 7 days at 23 °C and with 24h illumination (light intensity 48,6 µmol. foton m^{-2} s^{-1}). Then the results were statistically evaluated, and growth curves were designed for each studied strain. The minimum inhibitory concentration (MIC) of the investigated antibiotics was evaluated using probit analysis. The potential effect of antibiotics on tested strains morphology was also studied. The results show that the antibiotics have an inhibitory effect at higher concentrations (891.76 µg/mL to 1495.10 µg/mL). The highest values of MIC were estimated for *Synechocystis* sp. (PCC 6803) and *Cephalotrix* sp. (KL 18). The stimulation of growth was observed in the strains *Synechocystis* (PCC 6803) and *Cephalothrix* (KL 18). The most sensitive strains to selected antibiotics were *Chlorogleopsis fritschii* (CCALA 1005), *Lyngbya martensiana* (CCALA 930), and *Geitlerinema acuminatum* (CCALA 141). Strain *Cyanobium* sp. (LH14) was sensitive to streptomycin sulphate and gentamicin sulphate, but sulfacetamide stimulated its growth. The visible morphological changes were caused by streptomycin in *Chlorogloeopsis fritschii* (CCALA 1005). After five days of cultivation the bleached cells were present in the cultures of this strain.

Keywords: streptomycin, gentamicin, sulfacetamide, cyanobacteria

INTRODUCTION

Different antibiotics represent the most common substances present in aquatic ecosystems. These compounds can have a significant impact on water microbiota. Nowadays, for human or animal health treatment, around 250 different antibiotics are registered **(Kümmerer and Henninger, 2003)**. The use of antibiotics and their increasing presence in aquatic ecosystems cause concern regarding their potentially detrimental effects on microbial communities and the whole ecosystem **(Bengtsson-Palme *et al.*, 2018)**. Antibiotics and their residues represent a relatively low environmental risk. The exceptions are the cephalosporin cefalexin, the fluoroquinolone ciprofloxacin, and the macrolide azithromycin. These should be considered possible moderate environmental risks in water bodies, especially in Portugal, Spain, Cyprus, and Germany **(Rodriguez-Mozaz *et al.*, 2020)**. The concentration of antibiotics in most aquatic environments reached ng/L to µg/L **(Homem and Santos, 2011)**. These concentrations have a significant impact on the biomes in different environments. Cyanobacteria represent an essential part of most environments in the presence of light. These simple phototrophic microorganisms are primary producers of oxygen **(González-Pleiter *et al.*, 2013)**. They can fix carbon dioxide from water and terrestrial environments, and some can fix atmospheric nitrogen **(Berman-Frank *et al.*, 2003)**. Cyanobacteria are extremely sensitive to environmental contaminants and are often used as indicators of the contamination of the environment **(López-Rodas *et al.*, 2006)**. The most studied species in ecotoxicological studies where antibiotic toxicity was tested are *Microcystis aeruginosa*, *Anabaena flos-aquae*, and *Anabaena* sp. **(Välitalo *et al.*, 2017)**. Some antibiotics include oxytetracycline, chlortetracycline, tetracycline, tiamulin **(Halling-Sørensen, 2000)**, ampicillin, amoxicillin, ciprofloxacin, and clarithromycin **(Välitalo *et al.*, 2017)**, can have a highly toxic impact on cyanobacteria. Only trimethoprim is non-toxic to cyanobacteria **(Välitalo et al., 2017).** Antibiotics usually inhibit photosystem II (PSII) in cyanobacteria **(Berden-Zrimec *et al.*, 2007)**, can inhibit their growth **(Kvíderová and Henley, 2005),** inhibit protein syntheses **(Halling-Sørensen, 2000)**, and have an impact on toxin production **(Du *et al.* 2018)**. Ceftazidime and amoxicillin in *Microcystis aeruginosa* affect the content of soluble proteins and the reaction to oxidative stress. At the same time, norfloxacin affects cell size and growth capacity **(Du *et al.*, 2018)**, and ofloxacin **(Deng *et al.*, 2022)** and streptomycin **(Kang *et al.*, 2022)** have an inhibitory effect on the growth of this species. Antibiotics impact not only free-living cyanobacteria but also biofilms containing cyanobacteria. Low levels of kanamycin could affect biofilms with the dominance of *Synechoccoccus elongatus*, where the capacity of photosynthesis-mediated calcification and biofilm formation is impacted. This directly influences biofilms' function and formation and ecological functions (increased concentrations of atmospheric carbon dioxide due to the promoted precipitation of carbonate) **(Välitalo *et al.* 2017)**. Some species, such as *Phormidium valerian*, can use antibiotics (ampicillin) as a nitrogen source. *P. valerian* seems resistant to ampicillin up to a concentration of 2 mg/mL **(Dias *et al.*, 2015)**. *Gloeocapsa* sp. and *Chroococcidipsis* sp. are also resistant to ampicillin, carbenicillin, and penicillin at 10 mg/L concentrations **(Urbach *et al.*, 2008)**. Cyanobacteria represent an essential part of microbial communities. The studies of the impact of these contaminants on cyanobacteria can be used in the future prediction of biomass and biofilm production, as well as help suggest better treatments for water environments. Cyanobacteria can also serve as bioindicators, for which an understanding of their morphology and changes in their growth is essential.

Thus, this study aimed to evaluate the effect of selected antibiotics on the growth and potential morphology changes of different species of cyanobacteria. Firstly, the minimum inhibitory concentration of tested antibiotics was determined. The percentage growth of cyanobacterial strains treated by antibiotics was studied, and finally the effect of tested antibiotics on cyanobacterial morphology was evaluated.

MATERIAL AND METHODS

Cyanobacterial strains origin and growth condition

This study used selected species of cyanobacteria: *Geitlerinema acuminatum* (CCALA 141), *Lyngbya martensiana* (CCALA 930), *Chlorogloeopsis fritschii* (CCALA 1005), *Cyanobium* sp. (LH 14), *Cephalothrix* sp. (KL18) and *Synechocystis* sp. (PCC 6803). The strains marked as CCALA were obtained from Culture Collections of Autotrophic Organisms (CCALA) (Třeboň, Czech Republic). Strain PCC 6803 and *Cyanobium* sp. (LH 14) were obtained from The

Pasteur Culture Collection of Cyanobacteria and strain KL 18 was isolated from water samples from Alaska. Strains were cultivated in BG 11 medium **(Stanier *et al.*, 1971)** with pH 7.5. Triplicate cultures of each strain were started by adding 10 mL of sterile BG11 medium to 1 mL of cyanobacteria inoculum to sterile tubes. Then they were incubated for 1-2 weeks at 23 °C and with 24h illumination (light intensity 48,6 µmol. foton m^{-2} s^{-1}).

Inoculum preparation

Before the inoculum preparation, the studied strains were cultivated as described above. Due to the optimal setting of the experiment, it was necessary to find out when the tested cyanobacteria strains reached their maximum biomas production. Cell numbers of the cultured strains were counted daily by Bürker chamber under a microscope Olympus CX23, and growth curves (cell density over time) were plotted to find the logarithmic phase (Figure 1).

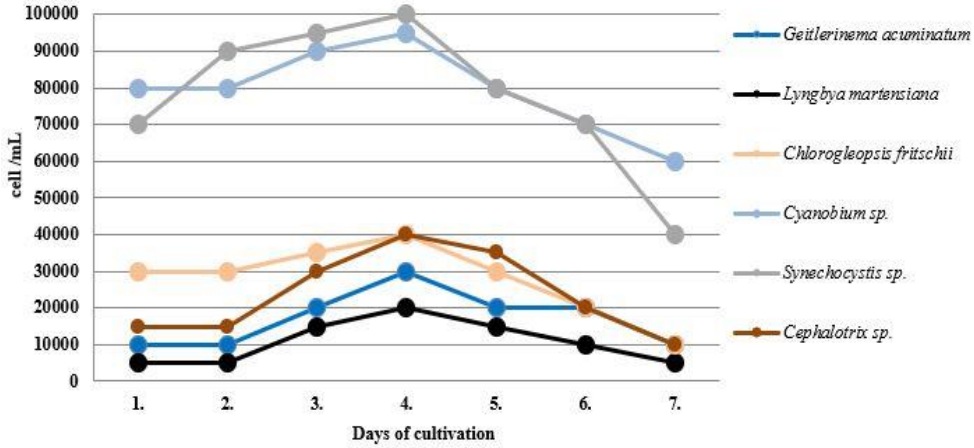

Figure 1 The growth curves of tested cyanobacterial strains

According to our results, all tested strains reached maximum biomass production on the 4th day of cultivation, and therefore the inoculum was prepared on the 4th day of cultivation. The inoculum was prepared by adding1 mL of medium BG11 containing the strain to 4 mL of fresh medium BG11 to a final concentration $2x10^6$ cell/mL according to **Diaz *et al*. (2015)**.

Growth inhibitory test and determination of the minimum inhibitory concentration

The growth inhibitory effect of antibiotics was performed in sterile 96-well microtiter plates. For the test, the following antibiotics were used: streptomycin sulphate (SS) (CAS-no.3810-74-0), gentamicin sulphate (GS) (CAS-no. 1405-41-0), sulfacetamide (S) (CAS-no. 144-80-9) (Sigma-Aldrich, Germany). Into each well, a 100 µL of BG11 medium (lines from A to F and line H) was added except line G with medium purity control, which contains 200 µL of BG11 medium. All used antibiotics (n=3) were tested on each plate (in lines A-B tested SC, in lines C-D tested GS, and in lines E-F tested S). The used antibiotics were diluted at an initial concentration of 2048 µg/mL in the BG 11 medium and were added to the first column as follows. Next, the two-fold dilution of each antibiotic at the concentration range from 1024 – 0.5 µg/mL was prepared. Then the 100 µL of inoculum with one selected strain (n=7) was added to each well, except the line G (medium purity control). The final volume of each well was 200 µL. Following the inoculation, plates were closed and pact in parafilm. All operations were undertaken in a sterilized chamber. After then, the plates were incubated for seven days under the same conditions as the cultures. Before and after the incubation period, the microplates were measured at 630 nm in the Opsys MRTM Microplate Reader.

Morphological study of tested strains treated by antibiotics

To monitor potential morphological changes in cyanobacteria, the tested strains cultivated with the same concentration range of selected antibiotics (1024 – 0.5 µg/mL) were cultivated simultaneously with the previous experiment. The test was performed in sterile 2 mL microtubes. The microtubes contain 250 µL BG11 medium and 500 µL of antibiotic diluted in BG11 medium to the required concentration. Then, the prepared inoculum of each tested cyanobacteria was added to the microtubes. The prepared microtubes were cultivated in the same way as described in the previous method. During the cultivation period (7 days), the density of cells was manually counted in the Bürker chamber in an optical microscope Olympus CX23. Also, the potential morphology changes were studied on the 7th day of cultivation.

Statistical analyses

In this study, all experiments were performed in independent triplicate. The results obtained from counting in the Bürker chamber (morphology study) and the growth inhibition of cyanobacteria were evaluated in Microsoft Office Excel computer software. The data were displayed as the growth curves for each tested strain. The results of MIC_{50} (MIC at which 50% of microorganisms are inhibited) and MIC_{90} (MIC at which 90% of microorganisms are inhibited) were evaluated using probit analysis ($p<0.0001$) in Statgraphics Centurion XVI program (version 16.1.11).

RESULTS AND DISCUSSION

Impact of the antibiotics on cyanobacterial growth

Antibiotics are commonly used to treat bacterial infections in humans and animals. Still, their overuse and improper disposal can lead to pollution of the environment, including water bodies where cyanobacteria live **(Bashir *et al.*, 2020)**. This pollution can negatively affect cyanobacteria by disrupting their natural balance with other microorganisms and causing changes in their growth and reproduction patterns. In addition, antibiotic exposure can lead to antibiotic resistance in cyanobacteria, threatening public health **(Gunathilaka *et al.*, 2023).** Cyanobacteria are crucial in the environment as primary producers, supplying food and oxygen for other organisms. They are also important in nutrient cycling and nitrogen fixation, which converts atmospheric nitrogen into a form that plants, and other organisms can use **(Hamilton *et al.*, 2016).** Overall, cyanobacteria play a significant role in the environment and have numerous potential benefits for human society, for example, in biotechnology, medicine or environmental remediation **(Zahra *et al.*, 2020).** Therefore, exposure to antibiotics can have adverse effects on cyanobacteria. One of the main negative impacts is the development of antibiotic resistance in cyanobacteria. For example, authors **Wang *et al.* (2020)** investigated the occurrence and spatiotemporal patterns of six ARG classes (Antibiotic Resistance Genes) in cyanobacteria isolated from Taihu Lake. Their results demonstrated that cyanobacteria could be a significant reservoir and source for acquiring and disseminating ARGs in aquatic environments. This is mainly due to the content of the same components related to gene transfer as in other bacteria (plasmids and transposable elements) **(Dias *et al.*, 2015)**. Also, **Yang *et al.* (2013)** studied the effect of tetracycline on *Microcystis aeruginosa* and found that after repeated exposure to this ATB, the cyanobacteria developed resistance to it after the first exposure. For this reason, it is very important to monitor the impact of different antibiotics on cyanobacteria. In our study, the effect of 3 antibiotics (streptomycin sulphate, gentamicin sulphate and sulfacetamide) on the growth and potential morphological changes of different species (*Geitlerinema acuminatum* (CCALA 141), *Lyngbya martensiana* (CCALA 930), *Chlorogloeopsis fritschii* (CCALA 1005), *Cyanobium* sp., *Cephalothrix* sp. (KL18), *Synechocystis* sp. (PCC 6803) and *Cyanobium* sp.) of cyanobacteria was investigated by microdilution method.

Minimum inhibitory concentration (MIC) determination

The lowest value of MIC_{50} was estimated by probit analysis for gentamicin sulphate (12.67 µg/mL in *G. acuminatum* (CCALA 141)), followed by streptomycin sulphate (25.32 µg/mL in *C. fritschii* (CCALA 1005)) > gentamicin sulphate (39.88 µg/mL in *L. martensiana* (CCALA 930)) > gentamicin sulphate (39.99 µL/mL in *Cyanobium* sp.) and finally also for gentamicin sulphate (62.73 µg/mL in *C. fritchii* (CCALA 1005)) (Table 1). Our results showed that gentamicin sulphate strongly inhibited the growth of tested cyanobacterial strains at relatively low concentrations.

Table 1 Minimum inhibitory concentration (MIC_{50} and MIC_{90}) for used antibiotics able to inhibit growth of tested cyanobacterial strains (n=3) estimated by probit analysis

Tested cyanobacteria	Used antibiotics					
	SS		GS		S	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Geitlerinema acuminatum (CCALA 141)	160.91	704.31	12.67	58.69	386.25	817.11
Lyngbya martensiana (CCALA 930)	245.58	358.34	39.88	127.89	551.39	808.47
Chlorogleopsis fritschii (CCALA 1005)	25.32	63.08	62.73	223.01	439.93	741.55
Cyanobium sp. (LH 14)	439.93	741.55	39.99	110.54	-*	-
Synechocystis sp. (PCC 6803)	-	-	1137.46	1294.69	-	-
Cephalotrix sp. (KL18)	-	-	891.76	1495.10	-	-

Note: SS – streptomycin sulphate, GS – gentamicin sulphate, S – sulfacetamide, -* - the growth of cyanobacteria was not inhibited

Our results agree with **Dias *et al.* (2015)**, who studied some antibiotics that included aminoglycosides, as in our study against different cyanobacterial strains and found that the MIC of aminoglycosides varied between 0.1 and 0.8 mg/L (strain LMECYA260), 0.2–0.4 mg/L (strains LMECYA 7 and LMECYA 40), and 0.4–1.6 mg/L (strain LMECYA 246). On the contrary, in the study of **Cameron and Pakrasi (2011)**, *Synechocystis* sp. was the most sensitive to gentamicin but in a higher tested concentration than in our study (1–10 mg/L). But for example, in an earlier study, *Synechoccocus* sp. was shown resistance when it was exposed to gentamicin in the concentration of 10 mg/L **(Reynaud and Franche, 1986).** The relatively higher inhibition of growth of the tested strains in this study found in gentamicin and streptomycin can be explained by the fact that streptomycin can inhibit protein synthesis due to its binding to the 30S ribosome subunit **(Harrass *et al.*, 1985)** and gentamicin increased the oxidative stress, as well as damage the photosystem I in cyanobacteria **(Cameron and Pakrasi, 2011)**.

Our results showed that tested ATB effectively inhibited cyanobacterial strains but at higher concentrations. The probity analysis showed that, the best MID_{90} values were found at a concentration of 58.69 (µg/mL), which inhibited the growth of *G. acuminatum* (CCALA 141) after seven days of cultivation. The highest value of MIC_{50} and MIC_{90} was estimated for *Synechocystis* sp. (PCC 6803) (MIC_{50} 1137.46 µg/mL and MIC_{90} 1294.69 µg/mL) and *Cephalotrix* sp. (KL 18) (MIC_{50} 891.76 µg/mL and MIC_{90} 1495.10 µg/mL). These ATBs were not effective in the inhibition of these strains.

After MIC evaluation, the percentage growth of cyanobacterial strains with antibiotics was studied. The percentage of cyanobacterial growth under treatment with tested ATB was determined based on the measured absorbance on a Microplate Reader Opsys MRTM, Dynex (Chantilly, USA). The obtained data were compared with the control samples (the control is marked with a red line in Figures 2, 3 and 4) and some differences between the MIC values and percentage growth of cyanobacterial strains were found. The most sensitive to streptomycin sulphate was strain *Chlorogleopsis fritchii* (CCALA 1005), similar to the results obtained from MIC evaluation, but it was also partially inhibited by concentrations of 32 µg/mL and 16 µg/mL compared to the control. This ATB inhibited its growth at 64 µg/mL (Figure 1). Our results agree with authors **Carvalho and Santos (2016)**, who also found that this thermophilic cyanobacterium could not resist the higher concentrations of antibiotics and is especially sensitive to β-lactam ATB. Streptomycin sulphate, tested in our study, belongs to aminoglycosides (sulphate salt form of streptomycin), but the effect of these ATB on the growth of *C. fritchii* was not available in studies yet.

The results showed that the tested antibiotics inhibited the growth of cyanobacteria differently depending on the concentration used.

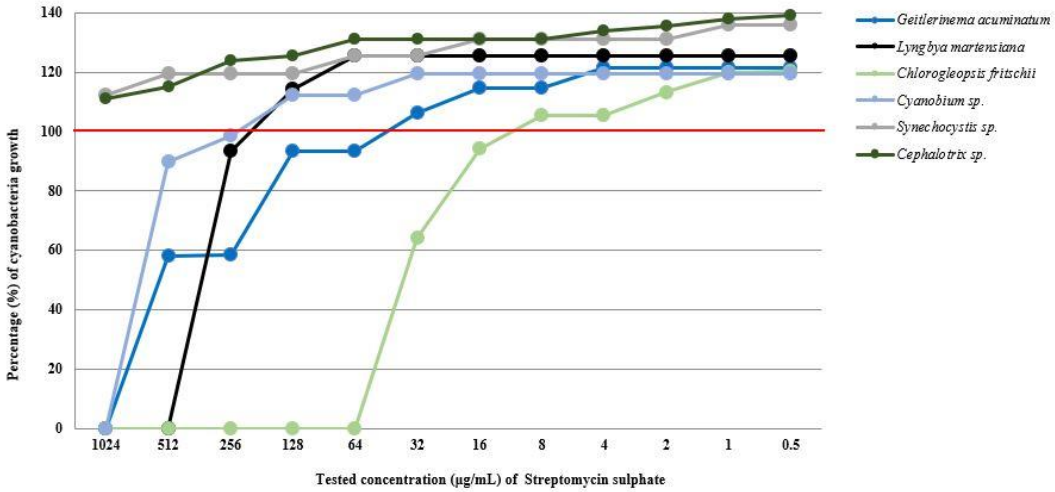

Figure 2 The growth curves mean (n=3) of tested cyanobacterial strains under treatment with streptomycin sulphate at different concentration (1024-0.5 µg/mL) after 7 days of cultivation at 23 °C and with 24h illumination (light intensity 48,6 µmol. foton m^{-2} s^{-1}), red line - control

The growth of *Lyngbya martensiana* (CCALA 930) was also inhibited by SS at 512 µg/mL. *Cyanobium* sp. (LH 14) and *Geilerinema acuminatum* (CCALA 141) were inhibited only at higher tested concentrations (1024 µg/mL). The growth of *Cephalotrix* sp. (KL18) and *Synechocystis* (PCC 6803) was not affected by this ATB at all, and in comparison, with control sets, their growth appeared to be poorly stimulated. Also, authors **Tan *et al.* (2018)** investigated the effects of two typical aminoglycoside antibiotics (tobramycin and kanamycin) on the aggregation of the model cyanobacterium *Synechococcus elongatus* and the *Microcystis aeruginosa*. They found that low-level of these aminoglycoside antibiotics (0.10 and 0.02 µg/mL) promoted the aggregation of *S. elongatus* and *M. aeruginosa* by 40 and 18%, respectively. Similarly, in the study of **González-Pleiter *et al.* (2019)**, they confirmed that some species, such as *Synechococcus* sp. and *Microcystis, aeruginous* could use some types of antibiotics for their growth (e.g., β-lactam ATBs). In our study, streptomycin sulphate inhibited the growth of some cyanobacteria at the lowest concentrations (*C. fritchii* (CCALA 1005) at 64 µg/mL). Similarly, in the study of **Harrass (1985)**, all of the cynobacteria tested were extremely sensitive to streptomycin. None grew at 0.9 mg/L, and only *Aphanizomenon flos-aquae* grew at 0.28 mg/L (with a Type I response); at 0.09 mg/L, only *Anabeana cylindrica* grew, as well as control cultures. On the contrary, **Han *et al.* (2014)** found that streptomycin was the less effective antibiotic (from seven tested) against *Nostoc flagelliform* and had the highest tolerance.

In our study, these strains, *Cephalotrix* sp. (KL18) and *Synechocystis* (PCC 6803) were also resistant to gentamicin sulphate (Figure 3). The most sensitive strains to this ATB were *Cyanobium* sp. and *Lyngbya martensiana* (CCALA 930). Their growth was inhibited at a 256 µg/mL concentration for both, respectively. **In their study, Le Page *et al.* (2019)** include *Cyanobium* sp. between cyanobacteria and wilt high sensitivity to antibiotics. For example, in the case of aminoglycosides, they found a MIC of 0.005 mg/mL. The different results obtained in our study were probably caused due to the slow growth of *Cyanobium* sp. (LH 14).

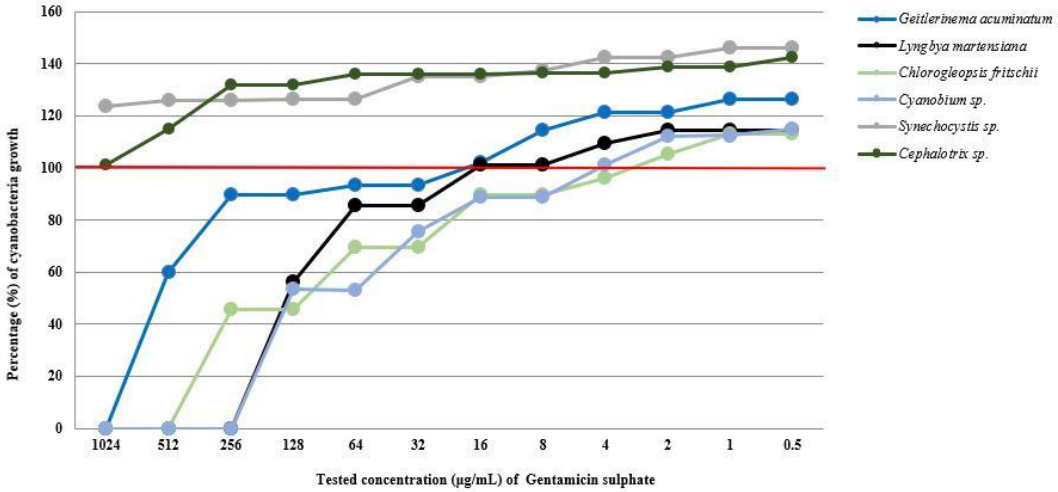

Figure 3 The growth curves mean (n=3) of tested cyanobacterial strains under treatment with gentamicin sulphate at different concentration (1024-0.5 µg/mL) after 7 days of cultivation at 23 °C and with 24h illumination (light intensity 48,6 µmol. foton m^{-2} s^{-1}), red line - control

Our results with gentamicin agree with other authors, although they worked with higher concentrations. **Dias *et al.* (2015)** evaluated the susceptibility of four cyanobacterial isolates (*Microcystis aeruginosa*, *Aphanizomenon gracile*, *Chrisosporum bergii* and *Planktothix agraphia*) and nine isolates from the same species (*M. aeruginosa*) to distinct antibiotics (amoxicillin, ceftazidime, ceftriaxone, kanamycin, gentamicin, tetracycline, trimethoprim, nalidixic acid, norfloxacin). They found the lowest concentration of gentamicin that inhibited *M. aeruginosa* at 0.2 mg/L and the highest (with 100% inhibition) at 0.4 and 1.6 mg/L, respectively. **Ahmaed and Buniya (2022)** found that all of the tested cyanobacterial strains (*Lyngbaya epiphytic*, *Wollea saccate*, *Chroococcus minutes*, *Chroococcus disperses*, and *Oscillatoria cerebriform*) were susceptible to gentamicin in all tested concentration. Gentamicin, similar to streptomycin, belongs to the aminoglycoside ATB, whose primary mechanism of influence on the cells is the inhibition of protein synthesis, and they also cause cytotoxicity through the induction of reactive oxygen species **(Cameron and Pakrasi 2011).**
The last antibiotic tested was sulfacetamide, which belongs to the sulphonamides group of ATB and appears to be the least effective in inhibiting the growth of the tested strains of cyanobacteria (Figure 4). The most sensitive strain to sulfacetamide was *G. acuminatum* (CCALA 141), which was inhibited at a concentration of 512 µg/mL, and the growth of *C. fritschii* (CCALA 1005) and *L. martensiana* (CCALA 930) were inhibited only at the highest tested concentration (1024 µg/mL). In our study, the growth of *Cephalotrix* sp. and *Synechocytis* sp. has poorly stimulated again like in treatment with gentamicin sulphate, but in addition, sulfacetamide inhibitory effect on *Cyanobium* sp. was also not detected. The growth of these strains seems to be poorly stimulated compared to control sets (the red line represents 100% of cyanobacterial growth - Figure 4). Also, **Pro *et al.* (2003)** observed no effect of ATB from this group (sulfachloropyridazine) on the growth of *Chlorella vulgaris*. **Le Page *et al.* (2017)** found that inhibition of cyanobacterial growth after exposure to sulphonamide was generally limited and, in some species (*Phormidium* sp.), the inhibitory effect was stabilized with increasing concentration of antibiotics, which may indicate the initiation of a possible mechanism of their resistance. Our results also agree with a meta-analysis analyzed in the study of **Crécy-Lagard *et al.* (2007),** who found that cyanobacteria are less sensitive to sulphonamides than microalgae and macrophytes. A possible explanation for their resistance to ATB could be that cyanobacteria contain protein (slr0642 identified in *Synechocystis* sp. (PCC 6803), which may act as a folate transporter and enables the absorption of folates from the environment **(Le Page *et al.*, 2019)**.

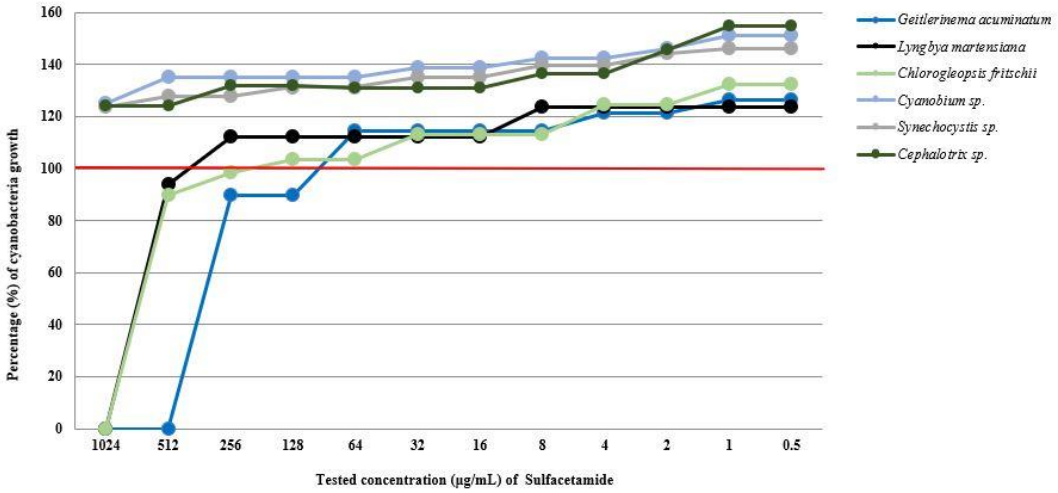

Figure 4 The growth curves mean (n=3) of tested cyanobacterial strains under treatment with sulfacetamide at different concentration (1024-0.5 µg/mL) after 7 days of cultivation at 23 °C and with 24h illumination (light intensity 48,6 µmol. foton m^{-2} s^{-1}), red line – control

The reason may be that some microalgae have already been used to remove some antibiotics, including sulfacetamide, from the aquatic environment **(Wang *et al.*, 2022)**. In addition, sulphonamides are the most widely used antibiotics, especially in veterinary medicine, and therefore their residues can also be found in the aquatic environment **(García-Galán *et al.*, 2009)**. And some species may be resistant to them. However, the effect of antibiotics on the growth, photosynthesis and transcriptome of cyanobacteria has not yet been fully understood, and it is clear from the available studies that the toxicity of various ATBs and their effect on the growth of cyanobacteria is different and primarily species-specific **(Zhao *et al.*, 2023)**. But in the study of **Ahn *et al.* (2022)** evaluated the ecotoxicological effects of aluminium oxide nanoparticles (Al_2O_3NP) and their influence on sulfacetamide (SA) biodegradation by a freshwater microalga, *Scenedesmus obliquus*. They found that the addition of 100 mg/L of Al_2O_3NP and 1 mg/L of SA reduced its total chlorophyll by 23.3% and carotenoids by 21.6%, and the genes responsible for ATP synthesis, and the photosynthetic system was significantly downregulated. So, the undesirable effect of ATB on cyanobacteria may not always be manifested only by inhibition of their growth but also in other sometimes observable ways. Therefore, our work also studied the effect of tested ATB on the morphology of cyanobacterial strains.

Impact of antibiotics on morphology

In our study, the possible effect of tested ATB on morphological changes was seen in the strain *Chlorogloeopsis fritschii* (CCALA 1005) treated with streptomycin. After five days of cultivation of this strain, the bleached cells were present in the cultures at all concentrations, compared with the control sample, where not detected. *C. fritschii* (CCALA 1005) was shown to decolourize their cells after five days of cultivation with streptomycin (Figure 5).

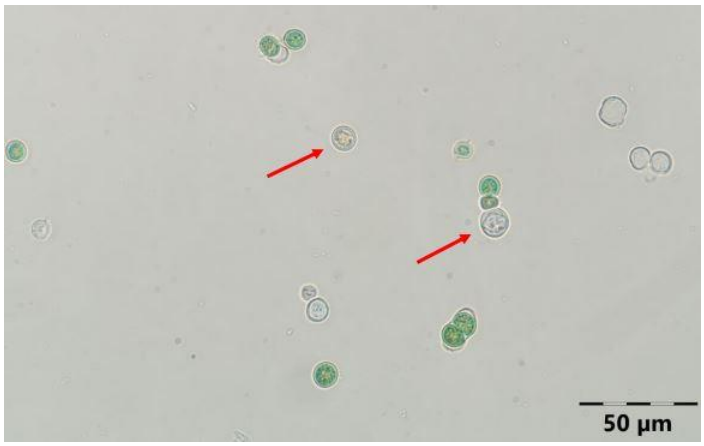

Figure 5 Discoloured cells of *Chlorogloeopsis fritschii* (CCALA 1005) after five days of cultivation at 23 °C and with 24h illumination (light intensity 48,6 µmol. foton m^{-2} s^{-1}) with streptomycin sulphate

Whitening/decolourization of *Microcystis* (PCC 7820) cells were mentioned in the study of **Bagchi *et al*. (1993)**, who studied the effect of antibiotics on some cyanobacterial strains. Other authors describe the degradation of phycobilisomes (whitening of cells) due to loss of nitrogen caused by ATB. Loss of pigmentation also occurred in *Synechocystis* sp. (PCC 6803) **(Ogawa and Sonoike, 2016)**. **Hunter and McVeigh (1961)** found that representative strains of Myxophyceae, Chlorophyceae, Bacillariophyceae, Xanthophyceae, and Euglenophyceae were inhibited after exposure to different antibiotics, but bacitracin caused a loss of pigmentation in Bacillariophyceae. Similar results were obtained **by Wan *et al*. (2014)**, who evaluated the effect of levofloxacin on *Microcystis flos-aquae*. They found that a concentration exceeding ten µg/L inhibited its growth significantly, and chlorophyll a continent was also considerably decreased. Authors **Chauhan *et al*. (1992)** found that the antibiotic inhibited PS II in isolated chloroplasts, primarily interacting at a site before P680 on the electron transport chain. In a study by **Yalcin *et al*. (2022)**, the impact of ampicillin, tetracycline, kanamycin, and cefotaxime on pigment fluorescence and photosynthetic capacity in *Fremyella diplosiphon* strains B481-WT and B481-SD was studied. It is clear from their conclusions that similar to our study, optimal concentrations of antibiotics can induce cell growth, while high concentrations can negatively affect cell functionality.

Therefore, the knowledge about the role of cyanobacteria in contaminated aquatic environments can clarify how aquatic ecosystems respond to pollution caused by antibiotics and define preventive measures regarding the spread of antibiotic resistance in the environment **(Prasanna and Coll, 2010).**

CONCLUSION

The current study demonstrated that following a 7-day exposure to the antibiotics streptomycin sulphate, gentamicin sulphate, and sulfacetamide, the investigated cyanobacteria exhibited varying levels of sensitivity towards them. The results showed that the antibiotics have an inhibition effect at higher concentrations (891.76 µg/mL to 1495.10 µg/mL). The gentamicine sulphate shows the ability to inhibit the growth of every studied strain. On the other hand, streptomycine sulphate and sulfacetamide inhibit the growth of strains *Chlorogleopsis fritschii* (CCALA 1005), *Lyngbya martensiana* (CCALA 930), *Cyanobium* sp. (LH 14) and *Geitlerinema acuminatum* (CCALA 141). They also slightly stimulated the growth of *Synechocystis* (PCC 6803) and *Cephalothrix* (KL 18). The studied strains were most sensitive to gentamicine sulphate, which influenced their growth at lower concentrations (256 µg/mL for *Cyanobium* sp. LH 14 and *Lyngbya martensiana* CCALA 930, 512 µg/mL for *Chlorogleopsis fritschii* CCALA 1005). The inhibitory impact of sulfacetamide on the growth of the studied strains was relatively low. This ATB inhibits only strains *Chlorogleopsis fritschii* CCALA 1005, *Geitlerinema acuminatum* CCALA 141 and *Lyngbya martensiana* CCALA 930, but the highest concentration was needed. From these, the most sensitive strains to antibiotics were *Chlorogleopsis fritschii* (CCALA 1005), *Lyngbya martensiana* (CCALA 930) and *Geitlerinema acuminatum* (CCALA 141). Strain *Cyanobium* sp. (LH 14) was sensitive to streptomycin sulphate and gentamicine sulphate, but sulfacetamide stimulated its growth. Changes in morphology due to the antibiotics were observed in strain *C. fritschii*, where the typical package colonies lost their homogeneity, and the strains produced solitary cells, which were often decolorized. These results showed that the cyanobacteria could survive in an environment contaminated by antibiotics and even use them for their growth. These key photosynthetic microorganisms, colonizing most of the water and soil environments, are potentially large reservoirs of genes for ATB resistance, and are able to horizontally transfer them to different bacterial strains. In order to gain a deeper understanding of antibacterial resistance in cyanobacteria, further investigation of the effects of a broader range of antibiotics on various cyanobacterial strains would be necessary. Additionally, exploring the genetic mechanisms underlying resistance in these strains would be valuable. Given the important role of cyanobacteria as primary producers, it is essential to conduct more comprehensive studies on cyanobacteria to better comprehend their antibacterial resistance and its implications.

REFERENCES

Ahmaed, M., Buniya, H. (2022). STUDY OF ANTIBIOTIC RESISTANCE FOR SOME LOCAL ISOLATES OF BLUE-GREEN ALGAE.

Ahn, H. J., Ahn, Y., Kurade, M. B., Patil, S. M., Ha, G. S., Bankole, P. O., ... Jeon, B. H. (2022). The comprehensive effects of aluminum oxide nanoparticles on the physiology of freshwater microalga Scenedesmus obliquus and it's phycoremediation performance for the removal of sulfacetamide. Environmental Research, 215, 114314. https://doi.org/10.1016/j.envres.2022.114314

Bagchi, S. N., Chauhan, V. S., Marwah, J. B. (1993). Effect of an antibiotic from Oscillatoria late-virens on growth, photosynthesis, and toxicity of Microcystis aeruginosa. Current Microbiology, 26, 223-228. https://doi.org/10.1007/bf01577380

Bashir, I., Lone, F. A., Bhat, R. A., Mir, S. A., Dar, Z. A., Dar, S. A. (2020). Concerns and threats of contamination on aquatic ecosystems. Bioremediation and biotechnology: sustainable approaches to pollution degradation, 1-26. https://doi.org/10.1007/978-3-030-35691-0_1

Bengtsson-Palme, J., Kristiansson, E., Larsson, D. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. FEMS microbiology reviews, 42(1), fux053. https://doi.org/10.1093/femsre/fux053

Berden-Zrimec, M., Drinovec, L., Zrimec, A., & Tišler, T. (2007). Delayed fluorescence in algal growth inhibition tests. Open Life Sciences, 2(2), 169-181. https://doi.org/10.2478/s11535-007-0014-1

Berman-Frank, I., Lundgren, P., Falkowski, P. (2003). Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. Research in microbiology, 154(3), 157-164. https://doi.org/10.1016/s0923-2508(03)00029-9

Cameron, J. C., Pakrasi, H. B. (2011). Glutathione facilitates antibiotic resistance and photosystem I stability during exposure to gentamicin in cyanobacteria. Applied and environmental microbiology, 77(10), 3547-3550. https://doi.org/10.1128/aem.02542-10

Carvalho, I. T., Santos, L. (2016). Antibiotics in the aquatic environments: a review of the European scenario. Environment international, 94, 736-757. https://doi.org/10.1016/j.envint.2016.06.025

de Crécy-Lagard, V., El Yacoubi, B., de la Garza, R. D., Noiriel, A., Hanson, A. D. (2007). Comparative genomics of bacterial and plant folate synthesis and salvage: predictions and validations. BMC genomics, 8(1), 1-15. https://doi.org/10.1186/1471-2164-8-245

Deng, Y., Debognies, A., Zhang, Q., Zhang, Z., Zhou, Z., Zhang, J., Qian, H. (2022). Effects of ofloxacin on the structure and function of freshwater microbial communities. Aquatic Toxicology, 244, 106084. https://doi.org/10.1016/j.aquatox.2022.106084

Dias, E., Oliveira, M., Jones-Dias, D., Vasconcelos, V., Ferreira, E., Manageiro, V., Caniça, M. (2015). Assessing the antibiotic susceptibility of freshwater Cyanobacteria spp. Frontiers in microbiology, 6, 799. https://doi.org/10.3389/fmicb.2015.00799

Du, Y., Wang, J., Zhu, F., Mai, D., Xiang, Z., Chen, J., Guo, R. (2018). Comprehensive assessment of three typical antibiotics on cyanobacteria (Microcystis aeruginosa): The impact and recovery capability. Ecotoxicology and environmental safety, 160, 84-93. https://doi.org/10.1016/j.ecoenv.2018.05.035

García-Galán, M. J., Díaz-Cruz, M. S., Barceló, D. (2009). Combining chemical analysis and ecotoxicity to determine environmental exposure and to assess risk from sulfonamides. Trac Trends in Analytical Chemistry, 28(6), 804- 819. https://doi.org/10.1016/j.trac.2009.04.006

González-Pleiter, M., Cirés, S., Hurtado-Gallego, J., Leganés, F., Fernández-Piñas, F., Velázquez, D. (2019). Ecotoxicological assessment of antibiotics in freshwater using cyanobacteria. In Cyanobacteria (pp. 399-417). Academic Press. https://doi.org/10.1016/b978-0-12-814667-5.00020-9

González-Pleiter, M., Gonzalo, S., Rodea-Palomares, I., Leganés, F., Rosal, R., Boltes, K., ... Fernandez-Pinas, F. (2013). Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: implications for environmental risk assessment. Water research, 47(6), 2050-2064. https://doi.org/10.1016/j.watres.2013.01.020

Gunathilaka, M. L., Bao, S., Liu, X., Li, Y., Pan, Y. (2023). Antibiotic Pollution of Planktonic Ecosystems: A Review Focused on Community Analysis and the Causal Chain Linking Individual-and Community-Level Responses. Environmental Science & Technology. https://doi.org/10.1021/acs.est.2c06787

Halling-Sørensen, B. (2000). Algal toxicity of antibacterial agents used in intensive farming. Chemosphere, 40(7), 731-739. https://doi.org/10.1016/s0045-6535(99)00445-2
Hamilton, T. L., Bryant, D. A., & Macalady, J. L. (2016). The role of biology in planetary evolution: cyanobacterial primary production in low-oxygen Proterozoic oceans. Environmental microbiology, 18(2), 325-340. https://doi.org/10.1111/1462-2920.13118
Han, P. P., Sun, Y., Wu, X. Y., Yuan, Y. J., Dai, Y. J., Jia, S. R. (2014). Emulsifying, flocculating, and physicochemical properties of exopolysaccharide produced by cyanobacterium Nostoc flagelliforme. Applied biochemistry and biotechnology, 172, 36-49. https://doi.org/10.1007/s12010-013-0505-7
Harrass, M. C., Kindig, A. C., Taub, F. B. (1985). Responses of blue-green and green algae to streptomycin in unialgal and paired culture. Aquatic Toxicology, 6(1), 1-11. https://doi.org/10.1016/0166-445x(85)90015-3
Homem, V., Santos, L. (2011). Degradation and removal methods of antibiotics from aqueous matrices–a review. Journal of environmental management, 92(10), 2304-2347. https://doi.org/10.1016/j.jenvman.2011.05.023
Hunter Jr, E. O., & McVeigh, I. (1961). The effects of selected antibiotics on pure cultures of algae. American Journal of Botany, 48(2), 179- 185. https://doi.org/10.1002/j.1537-2197.1961.tb11623.x
Chauhan, V. S., Marwah, J. B., Bagchi, S. N. (1992). Effect of an antibiotic from Oscillatoria sp. on phytoplankters, higher plants and mice. New phytologist, 120(2), 251-257. https://doi.org/10.1111/j.1469-8137.1992.tb05661.x
Kang, L., Mucci, M., Lürling, M. (2022). Compounds to mitigate cyanobacterial blooms affect growth and toxicity of Microcystis aeruginosa. Harmful Algae, 118, 102311. https://doi.org/10.1016/j.hal.2022.102311
Kümmerer, K., Henninger, A. (2003). Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clinical microbiology and infection, 9(12), 1203-1214. n https://doi.org/10.1111/j.1469-0691.2003.00739.x
Kvíderová, J., Henley, W. J. (2005). The effect of ampicillin plus streptomycin on growth and photosynthesis of two halotolerant chlorophyte algae. Journal of Applied Phycology, 17, 301-307. https://doi.org/10.1007/s10811-005-7293-6
Le Page, G., Gunnarsson, L., Snape, J., Tyler, C. R. (2017). Integrating human and environmental health in antibiotic risk assessment: a critical analysis of protection goals, species sensitivity and antimicrobial resistance. Environment international, 109, 155-169. https://doi.org/10.1016/j.envint.2017.09.013
Le Page, G., Gunnarsson, L., Trznadel, M., Wedgwood, K. C., Baudrot, V., Snape, J.,; Tyler, C. R. (2019). Variability in cyanobacteria sensitivity to antibiotics and implications for environmental risk assessment. Science of the Total Environment, 695, 133804. https://doi.org/10.1016/j.scitotenv.2019.133804
López Rodas, V., Maneiro, E., Costas, E. (2006). Adaptation of cyanobacteria and microalgae to extreme environmental changes derived from anthropogenic pollution. limnetica, 25(1-2), 403-410. https://doi.org/10.23818/limn.25.28
Ogawa, T., & Sonoike, K. (2016). Effects of bleaching by nitrogen deficiency on the quantum yield of photosystem II in Synechocystis sp. PCC 6803 revealed by Chl fluorescence measurements. Plant and Cell Physiology, 57(3), 558-567. https://doi.org/10.1093/pcp/pcw010
Ogawa, T., Sonoike, K. (2016). Effects of bleaching by nitrogen deficiency on the quantum yield of photosystem II in Synechocystis sp. PCC 6803 revealed by Chl fluorescence measurements. Plant and Cell Physiology, 57(3), 558-567. https://doi.org/10.1093/pcp/pcw010
Prasanna, R., Madhan, K., Singh, R. N., Chauhan, A. K., Nain, L. (2010). Developing biochemical and molecular markers for cyanobacterial inoculants. Folia microbiologica, 55, 474-480. https://doi.org/10.1007/s12223-010-0079-5
Pro, J., Ortiz, J. A., Boleas, S., Fernández, C., Carbonell, G., Tarazona, J. V. (2003). Effect assessment of antimicrobial pharmaceuticals on the aquatic plant Lemna minor. Bulletin of environmental contamination and toxicology, 70, 0290-0295. https://doi.org/10.1007/s00128-002-0208-1
Reynaud, P. A., Franche, C. (1986). Isolation and characterization of nonheterocystous tropical cyanobacteria growing on nitrogen-free medium. MIRCEN journal of applied microbiology and biotechnology, 2, 427-443. https://doi.org/10.1007/bf00933366
Rodriguez-Mozaz, S., Vaz-Moreira, I., Della Giustina, S. V., Llorca, M., Barceló, D., Schubert, S., Manaia, C. M. (2020). Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment. Environment international, 140, 105733. https://doi.org/10.1016/j.envint.2020.105733
Stanier, R. Y., Kunisawa, R., Mandel, M. C. B. G., Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriological reviews, 35(2), 171-205. https://doi.org/10.1128/br.35.2.171-205.1971
Tan, L. R., Xia, P. F., Zeng, R. J., Li, Q., Sun, X. F., Wang, S. G. (2018). Low-level concentrations of aminoglycoside antibiotics induce the aggregation of cyanobacteria. Environmental Science and Pollution Research, 25, 17128-17136. https://doi.org/10.1007/s11356-018-1894-5
Urbach, C., Fastrez, J., Soumillion, P. (2008). A new family of cyanobacterial penicillin-binding proteins: a missing link in the evolution of class A β-lactamases. Journal of Biological Chemistry, 283(47), 32516-32526. https://doi.org/10.1074/jbc.m805375200
Välitalo, P., Kruglova, A., Mikola, A., Vahala, R. (2017). Toxicological impacts of antibiotics on aquatic micro-organisms: a mini-review. International Journal of Hygiene and Environmental Health, 220(3), 558-569. https://doi.org/10.1016/j.ijheh.2017.02.003
Wan, J., Guo, P., Zhang, S. (2014). Response of the cyanobacterium Microcystis flos-aquae to levofloxacin. Environmental Science and Pollution Research, 21, 3858-3865. https://doi.org/10.1007/s11356-013-2340-3
Wang, J., Chen, X. (2022). Removal of antibiotic resistance genes (ARGs) in various wastewater treatment processes: An overview. Critical Reviews in Environmental Science and Technology, 52(4), 571-630. https://doi.org/10.1080/10643389.2020.1835124
Wang, J., Chu, L., Wojnárovits, L., Takács, E. (2020). Occurrence and fate of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in municipal wastewater treatment plant: An overview. Science of the Total Environment, 744, 140997. https://doi.org/10.1016/j.scitotenv.2020.140997
Yalcin, Y. S., Aydin, B. N., Sayadujjhara, M., Sitther, V. (2022). Antibiotic-Induced Changes in Pigment Accumulation, Photosystem II, and Membrane Permeability in a Model Cyanobacterium. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.930357
Yang, W., Tang, Z., Zhou, F., Zhang, W., Song, L. (2013). Toxicity studies of tetracycline on Microcystis aeruginosa and Selenastrum capricornutum. Environmental Toxicology and Pharmacology, 35(2), 320-324. https://doi.org/10.1016/j.etap.2013.01.006
Zahra, Z., Choo, D. H., Lee, H., Parveen, A. (2020). Cyanobacteria: Review of current potentials and applications. Environments, 7(2), 13. https://doi.org/10.3390/environments7020013
Zhao, Q., Zhu, L., Jiang, L., Zhao, Q. (2023). Photosynthetic and transcriptomic responses of Chlorella sp. to tigecycline. Algal Research, 71, 103033. https://doi.org/10.1016/j.algal.2023.103033
Zrimec, M. B., Drinovec, L., Zrimec, A., Tišler, T. (2007). Delayed fluorescence in algal inhibition tests. Cent Eur J Biol, 2(2), 169-181. https://doi.org/10.2478/s11535-007-0014-1