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Original Article

Response of Cyanobacteria (Non-toxin producing=*Microcystis aeruginosa* EAWAG 198 and toxin producing=*Microcystis flos-aquae* UTEX-LB 2677) to Tetracycline

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ABSTRACT

The widespread use of antibiotics has led to their increasing presence in inland water systems, raising concerns about their ecological impact. Tetracycline (TC), a commonly used antibiotic, can negatively affect non-target aquatic organisms, potentially disrupting ecosystem structure and function and posing risks to human health, biodiversity, and environmental sustainability. This study investigated the effects of TC at concentrations ranging from 0.05 to 1.00 mg L^{-1} on two cyanobacterial species: *Microcystis flos-aquae* (toxin-producing, TP) and *Microcystis aeruginosa* (non-toxin-producing, NTP). The effects on pigment content, cell density, and antioxidant responses were evaluated. Results showed that the non-toxin producing strain demonstrated a recovery in cell density at lower TC concentrations (0.10–0.20 mg L^{-1}) after seven days. In contrast, the toxin-producing strain exhibited significantly slower growth at TC concentrations ranging from 0.10 to 1.00 mg L^{-1}, indicating greater sensitivity to TC. Pigment content in the non-toxin-producing strain increased at low TC levels but declined at higher concentrations. However, pigment content in the toxin-producing strain decreased steadily across all TC concentrations tested. Both species exhibited increased oxidative stress under TC exposure, as indicated by elevated reactive oxygen species (ROS), lipid peroxidation (measured as MDA), and peroxidase (POD) activity, while glutathione S-transferase (GST) activity remained largely unchanged. Principal component analysis revealed that lower TC concentrations were associated with improved growth and pigment content, whereas higher concentrations correlated with increased oxidative stress markers. Overall, the findings demonstrate that TC affects Microcystis species differently depending on concentration, exposure duration, and strain type, highlighting the ecological risks posed by antibiotic contamination in aquatic environments.

Keywords: Antibiotics, pharmaceuticals, toxicity, strain comparison; bloom-forming cyanobacteria

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INTRODUCTION

Tetracycline (TC) is a first-generation, broad-spectrum antibiotic widely used to treat infectious diseases [1].This group includes naturally occurring compounds such as tetracycline, chlortetracycline, oxytetracycline, and demeclocycline [2]. TC inhibits protein synthesis by reversibly binding to the bacterial 30S ribosomal subunit, thereby preventing aminoacyl-tRNA attachment and disrupting peptide elongation [3]. Due to its bacteriostatic and broad-spectrum activity, TC is considered one of the more harmful antibiotics in aquatic ecosystems. Research shows that TC can exert unintended biological effects on non-target organisms, including invertebrates, crops, algae, and cyanobacteria [4].

Cyanobacteria play a vital role in aquatic ecosystems, contributing significantly to oxygen production and forming the base of aquatic food webs. They exist in both toxin-producing and non-toxin-producing strains, and changes in their abundance or diversity can have cascading effects throughout the food chain [5]. Among aquatic organisms, cyanobacteria are particularly sensitive to antibiotic toxicity ($EC50 < 0.1$ mg L^{-1}), compared to green algae (EC50 between 0.3 and >1200 mg L^{-1}) [6]. Species of the genus *Microcystis* are especially important as model organisms for toxicity studies due to their rapid growth, ease of cultivation, widespread distribution, and ecological relevance [7].

Previous studies have reported that TC negatively affects the growth and antioxidant systems of cyanobacteria, particularly non-toxin-producing strains such as *Microcystis aeruginosa* [8], [9], [10]. However, no study has directly compared the effects of identical TC concentrations on both toxin-producing and non-toxin-producing species, such as *Microcystis aeruginosa* and *Microcystis flos-aquae*. Since multiple strains often coexist in aquatic environments and may respond differently to stress, such comparisons are essential to avoid overgeneralization. Therefore, this study compared the effects of TC on non-toxin-producing *M. aeruginosa* and toxin-producing *M. flos-aquae* by assessing growth parameters and antioxidant enzyme activity after seven days of exposure.

MATERIALS AND METHOD

Two cyanobacterial strains, *Microcystis aeruginosa* and *Microcystis flos-aquae*, were used in this study. The strains were obtained from the Gobler Laboratory, State University of New York, USA, and maintained at Ahmadu Bello University, Zaria. *M. flos-aquae* is toxin-producing, whereas *M. aeruginosa* is non-toxin-producing [11].

Cultures were maintained in BG-11 medium (pH 7.4) under controlled laboratory conditions (16:8 h light–dark cycle, 23 $\pm$ 1°C). The medium was autoclaved prior to use, and cultures were acclimated through repeated transfers during the exponential growth phase to ensure stable physiological responses.

Tetracycline (TC) stock solutions were prepared according to OECD guideline 201 for chemical testing. Exponentially growing cells (1×10^6 cells mL^{-1}) were exposed to TC concentrations of 0 (control), 0.05, 0.1, 0.2, 0.5, and 1.0 mg L^{-1} for seven days, following a two-step range-finding and determination experiment [8]. All treatments were conducted in triplicate under sterile conditions.

Growth was assessed using microscopic cell counts with a Neubauer hemocytometer and optical density measurements at 750 nm. Specific growth rate was calculated according to [12]. Pigments (chlorophyll a and carotenoids) were extracted with acetone and quantified using Ritchie's equations [13].

Oxidative stress was evaluated by measuring intracellular hydrogen peroxide (H_2O_2) using Jana's method [14]. Lipid peroxidation was determined by quantifying malondialdehyde (MDA) following [15]. Antioxidant enzymes were extracted in phosphate buffer, and peroxidase (POD) activity was measured using the method of [16]. Glutathione S-transferase (GST) activity was determined using 1-chloro-2,4-dinitrobenzene as described by [17].

Data were analyzed using one-way ANOVA and Tukey's HSD test to determine significant differences among treatments. Principal component analysis (PCA) was applied to assess relationships among measured parameters. Statistical analyses were conducted using R software version 4.3 at a 5% significance level

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RESULTS

Cell density and photosynthetic activity of Microcystis

Tetracycline (TC) exposure affected the cell density, photosynthetic pigments, and antioxidant responses of two *Microcystis* strains differently. In the non-microcystin-producing strain, *Microcystis aeruginosa*, growth significantly increased after 4 days at 0.5 mg L^{-1} TC. However, after 7 days, cell density significantly ($p < 0.05$) decreased at 0.1 and 0.2 mg L^{-1} compared to the control, while no significant changes were observed at 0.5 and 1 mg L^{-1}. In contrast, the microcystin-producing strain Fig 1A. *Microcystis flos-aquae*, showed a significant ($p < 0.05$) reduction in cell density after 7 days at 0.5 mg L^{-1} and even at the lower concentration of 0.05 mg L^{-1} TC. At the highest concentration (1 mg L^{-1}), neither strain showed significant changes in cell density Fig 1B

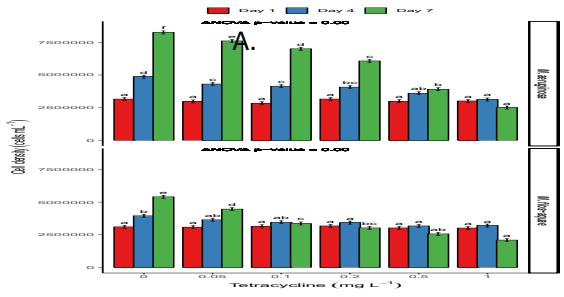

Fig.1 Changes in cell density (1x10^6 Cells mL^{-1}) of (*M. aeruginosa)* and (*M. flos-aquae*) after 7 days.

Bars with different letters ($p < 0.05$, and $p<0.01$) are significantly different compared to the control (0 mg L^{-1}). Bars with the same alphabets are not significantly different at $p < 0.05$ Error bars are standard deviation for $n = 3$

Photosynthetic pigment analysis revealed that *M. aeruginosa* experienced a significant ($p < 0.05$) decline in chlorophyll-a and total chlorophyll at 0.1 mg L^{-1}, and further reductions at 0.05, 0.2, and 1 mg L^{-1} after 7 days. No significant ($p > 0.05$) changes were

observed at 0.5 mg L^{-1} Fig 2A and B. Total carotenoid content also decreased significantly at 0.05, 0.1, and 0.2 mg L^{-1}, but remained unchanged at higher concentrations (0.5–1 mg L^{-1}) Fig 2C. Conversely, *M. flos-aquae* exhibited a significant ($p < 0.05$) increase in chlorophyll-a and total chlorophyll at 0.1 mg L^{-1}, with no significant ($p > 0.05$) changes at other concentrations Fig 2A and B. Its carotenoid levels remained largely unaffected by TC exposure Fig 2C

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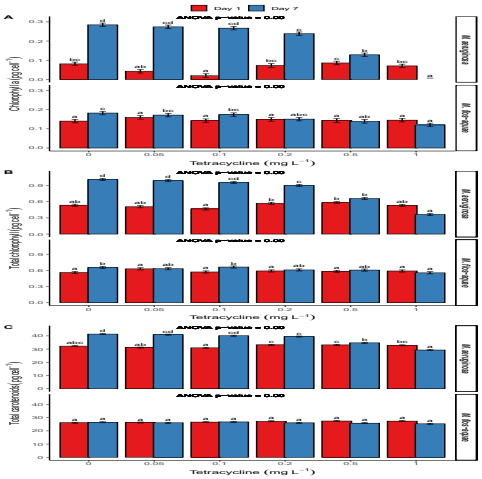

Fig. 2 Changes in (A) chlorophyll a (pg cell^{-1}), (B) Total chlorophyll (pg cell^{-1}) and, (C) total carotenoid (pg cell^{-1}) of *M. aeruginosa* and *M. flos-aquae* exposed to tetracycline for 7 days.

Antioxidant and enzyme responses indicated oxidative stress in both strains. In *M. aeruginosa*, increasing TC concentrations led to significant ($p < 0.05$) rises in reactive oxygen species (ROS), malondialdehyde (MDA) Fig 3A and B, and peroxidase (POD) activity Fig 4A, with the highest levels recorded at 1 mg L^{-1}. Similarly, *M. flos-aquae* showed elevated ROS and MDA levels as TC concentrations increased Fig 3A and B, with peak POD activity at 0.5 and 1 mg L^{-1} Fig 4A. Glutathione S-transferase (GST) activity also increased significantly ($p < 0.05$) after 7 days, indicating enhanced stress defense mechanisms Fig 4B. Overall, TC exposure induced concentration-dependent physiological and oxidative stress responses in both strains, though their sensitivity patterns differed.

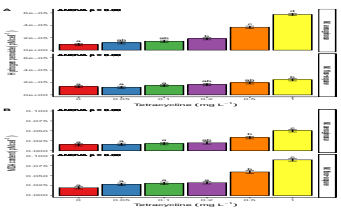

Fig. 3 Changes in (A.) H_2O_2 (µmol cell^{-1}) and (B.) MDA (pM cell^{-1}) of *M. aeruginosa* and *M. flos-aquae* exposed to tetracycline for seven days.

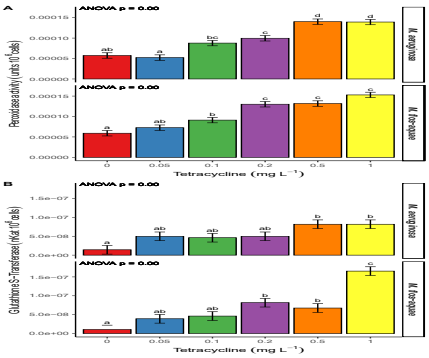

Fig. 4 The activities of (A.) Peroxidase (units mg^{-1}), and (B.) Glutathione S-transferase (µmol mL^{-1} min^{-1}) of *M. aeruginosa* and *M. flos-aquae* exposed to tetracycline for 7 days.

Correlations between response parameters

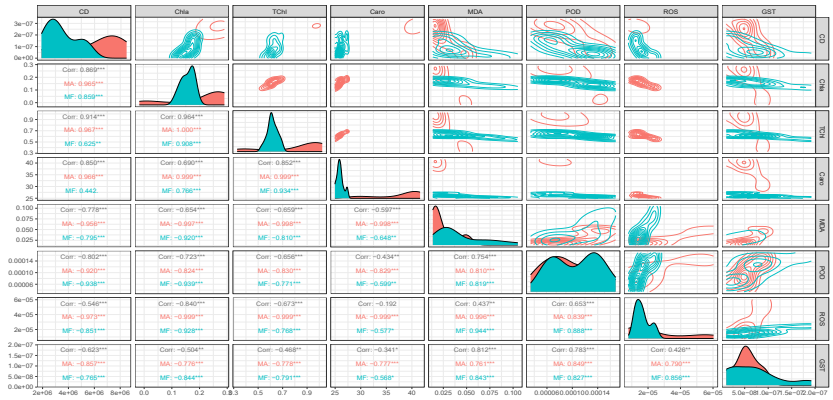

Fig. 5 Pearson's Correlation scatter plot showing the relationship between response parameters of *M. aeruginosa* and *M. flos-aquae* following exposure to different TC concentrations.

The parameters grouped on the same orthogonal axis are positively correlated, whereas those on the opposite axes have a negative correlation.

The first two PCA components data constituted 84.1% of the total variance, with PC1 representing 71.1% and PC2 representing 13%, respectively (Fig. 5 and 6). The antioxidant response (POD, ROS, MDA, and GST) was linked to high TC concentrations, whereas cell density, and pigment content, were linked to low TC concentrations and the control treatment.

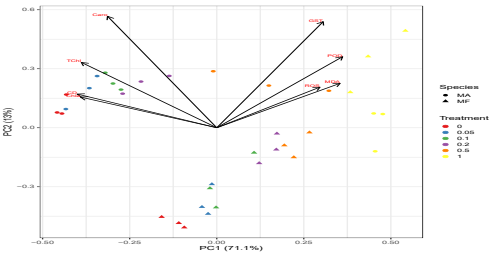

Fig. 6 PCA biplot showing the relationship of the cell density, pigment content, and enzyme and antioxidant responses of *M. aeruginosa* and *M. flos-aquae* under different TC concentrations of 0, 0.05, 0.1, 0.2, 0.5, and 1.0 mg/L.

The parameters grouped on the same orthogonal axis are positively correlated, whereas those on the opposite axes have a negative correlation.

DISCUSSION

Antibiotics in aquatic environments can severely affect phytoplankton by disrupting photosynthesis, inducing oxidative stress, and interfering with gene expression [18]. Tetracycline (TC), along with erythromycin, florfenicol, and thiamphenicol, has been widely reported to inhibit cyanobacterial growth and physiological functions, particularly photosynthesis [8], [19]. Early exposure to TC often reduces cell density, demonstrating toxicity during initial growth stages [20]. Similarly, [18] observed reduced biomass in *Coelastrella* sp. under TC treatment, especially in early growth phases.

In this study, both *Microcystis aeruginosa* (non-microcystin-producing) and *Microcystis flos-aquae* (microcystin-producing) showed concentration-dependent growth inhibition after seven days of TC exposure. Although very low concentrations occasionally stimulated slight growth responses, increasing TC levels generally reduced cell density. *M. flos-aquae* appeared more sensitive to TC than *M. aeruginosa*, particularly at lower concentrations. These findings contrast with [21], who reported growth stimulation of *M. aeruginosa* at low oxytetracycline and sulfamethoxazole concentrations, and with [22] and [18], who found that high TC doses inhibited algal growth while low doses had minimal effects. Limited differences between low TC treatments and controls suggest some tolerance, which diminishes as concentration increases.

Photosynthetic pigment analysis revealed species-specific effects. Chlorophyll-a and total chlorophyll levels decreased significantly in *M. aeruginosa* under TC stress, particularly at moderate concentrations. Antibiotics are known to damage thylakoid membranes and inhibit chlorophyll precursor synthesis and gene expression [23][22]. Structural alterations such as distorted thylakoid lamellae and enlarged vacuoles further impair photosynthesis and carbon fixation [20], [24].

Carotenoids, which function as antioxidants, initially increased in *M. aeruginosa* under oxidative stress, likely as a protective mechanism [25]. Similar findings were reported by [26], who observed increased carotenoid production alongside reduced chlorophyll under antibiotic stress. However, prolonged exposure led to carotenoid decline, indicating reduced protective capacity. In contrast, *M. flos-aquae* showed little carotenoid variation, highlighting species-specific adaptation [27], [28]

TC exposure significantly elevated reactive oxygen species (ROS) levels in both species, with the highest accumulation at 1 mg L^{-1} (Almeida et al., 2017). Excess ROS damages proteins, lipids, and membranes, causing oxidative stress and potentially cell death [4], [29]. Malondialdehyde (MDA), a lipid peroxidation marker, increased with TC concentration, especially in *M. flos-aquae*, indicating greater membrane damage [8], [9].

Antioxidant enzymes responded accordingly. Peroxidase (POD) activity increased at higher TC concentrations, helping detoxify hydrogen peroxide [18], [30]. Glutathione S-transferase (GST), important in xenobiotic detoxification, also rose with increasing TC exposure [31], [32]. Overall, although both strains activated defense systems, *M. aeruginosa* exhibited greater tolerance, while *M. flos-aquae* showed higher oxidative sensitivity under tetracycline stress

CONCLUSION

This study found that TC has a dose-response relationship with *M. aeruginosa* and *M. flos-aqua*. In both toxin producing and non-toxin producing strains, the inhibitory effect of TC on cell density was observed by altering the pigment content, which induces ROS generation and other enzymes and antioxidants. ROS, MDA, POD, and GST activities in the presence of the highest TC concentrations against cell density, pigment content, and biochemicals were all favorably linked with lower TC concentrations and the control treatment. These findings indicate that there was a strain- or species-specific response to the investigated strains, where non-toxin producing strain appeared to be more tolerant to TC exposure.

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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