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Nontoxicity and oxidative stress caused by antibiotics in the green alga *Neochloris conjuncta*

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ABSTRACT

In this research, the biochemical and genotoxic impacts of three antibiotics, Ciprofloxacin, Levofloxacin and Amoxicillin were examined on a freshwater microalga, *Neochloris conjuncta*. Six concentrations (0, 5, 10, 15, 25, 50 and100 µg ml^{-1}) were used, and the pure algal cultures were exposed for nine days under laboratory conditions. MDA, SOD, CAT, ROS%, and Vitamin C were measured as reflective markers of oxidative stress responses; whereas the Comet Assay was conducted to detect DNA damage. The antibiotics resulted in different oxidative stress patterns, as shown regarding the results. Among drugs, amoxicillin produced the most intense biochemical disturbance, including high levels of CAT and SOD activities, which suggest considerable alterations in redox homeostasis. Moderate oxidative stress was provoked by Ciprofloxacin while Levofloxacin showed the less severe metabolic alterations. The genotoxicity study, however, revealed that DNA fragmentation was the highest in the case of Levofloxacin at its maximum concentration and subsequently Ciprofloxacin while Amoxicillin induced the lowest potent damages. Taken together, the results suggested that the antibiotics had different toxic effects on *Neochloris conjuncta*; among them Amoxicillin imposed the most oxidative stress, and Levofloxacin caused the most severe DNA injury. These findings also demonstrate the significance of including both biochemical and genetic endpoints into risk assessments concerning pollution since biochemistry was less sensitive than genotoxicity.

Keywords: *Neochloris conjuncta*, ciprofloxacin, emerging pollutants, levofloxacin, amoxicillin, DNA damage, oxidative stress.

INTRODUCTION

Pharmaceutical residues are continuously being released into freshwater ecosystems and have indeed become an actual environmental issue on a global scale. These include analgesics, anti-inflammatories, antibiotics and antidepressants as well as other effective drugs that reach the environment via household sewage, hospital effluents, agricultural run-off or when wastewater treatment is incomplete. Their persistence and chemical stability enable their retention in surface waters at trace nanograms to biologically active micrograms per liter concentrations, presenting a potential for chronic exposure and long-term ecological impacts. Recent studies have drawn attention to the fact that pharmaceuticals are now considered as emerging contaminants because they can interfere with biochemical and physiological processes of the primary producers in aquatic environments (Feijão *et al.*, 2020).

Microalgae are the primary trophic level of freshwater ecosystems and are major players in global oxygen production, primary productivity, carbon fixation and nutrient recycling. Their wide sensitivity range to environmental perturbations makes them powerful bioindicators for water quality monitoring. Previous examination showed that pharmaceutical compounds can interfere with basic physiological processes in the microalga such as photosynthetic efficiency, production of chlorophyll, cell morphology and membrane permeability. Pharmaceuticals in particular have been shown to allow to cause photosystem II inhibition breaking and decrease the growth rates, contributing an excessive generation of ROS which results

in oxidative stress causing metabolic unbalance and cell dysfunction (Hejna *et al.*, 2022).

In addition to physiological damage, it has been shown that chemical drugs disrupt molecular signaling circuits and antioxidant pathways. These disruptions trigger a series of intracellular stress responses that implicate antioxidant enzyme function, redox balance and energy metabolism. Protracted exposure has also been linked to genotoxic effects, i.e., DNA strand breaks and chromatin instability which reflect the greater cellular damage induced by persistent oxidative stress and progressive accumulation of damage (Chakraborty *et al.* 2023).

In addition, there is also evidence that the morphological and pigmentation status of freshwater microalgae are affected by class-wise different pharmaceuticals, such effects have been related to differences in chemical structure, persistence and biological reactivity of pharmaceutical molecules that in turn establish the level of toxicity as well as the ability of microalgae to induce protective biochemical responses. The presence of even very low levels of these pollutants is known to cause changes in cellular metabolism, suppression of photosynthesis, and disruption of enzymatic pathways that contribute to the maintenance of physiological equilibrium (Duarte *et al.*, 2023).

In this context, the model freshwater green alga *Neochloris conjuncta* stands out as being highly useful for testing the effect of pharmaceutical pollutants. This species is ecologically significant since it has a wide distribution range and adaptation ability to various environments as well as its ability to synthesize useful biochemicals, such as pigments and lipids, according to the source file. Due to its high sensitivity toward chemical stress and fast physiological reaction, this model has proved to be especially helpful in the indication of pollutant induced disturbances in aquatic environments. However, though important for ecology and application purpose, the physiological, biochemical and molecular mechanisms employed by *N. conjuncta* exposed to pharmaceutical pollutants are still insufficiently defined (Zheng *et al.*, 2020).

Closing this knowledge gap is crucial, as previous studies on different algal species have demonstrated that contamination by pharmaceutical compounds can trigger complex and highly variable oxidative, metabolic, and genetic responses. These responses are strongly influenced by the chemical nature of the compound, exposure duration, concentration, and the algal species involved. Pharmaceuticals and personal care products are widely recognized as emerging aquatic contaminants capable of inducing oxidative stress, disrupting cellular homeostasis, and causing genotoxic effects in algae, while antioxidant and metabolic responses often differ markedly among species and stress conditions. Consequently, understanding species-specific biochemical and genetic responses is essential for improving ecological risk assessments of pharmaceutical pollution in aquatic environments (Xin *et al.*, 2021; Hejna *et al.*, 2022; Koletti *et al.*, 2025). In light of these findings and the evident variability in algal responses to pharmaceutical contamination, targeted investigations focusing on species-specific biochemical and genetic endpoints are essential to improve ecological risk assessment frameworks, The present study aimed to evaluate the biochemical and genotoxic effects of three commonly used antibiotics (ciprofloxacin, levofloxacin, and amoxicillin) on the freshwater microalga *Neochloris conjuncta*. Specifically, the study investigated antibiotic-induced oxidative stress responses by assessing lipid peroxidation and antioxidant-related biomarkers (MDA, SOD, CAT, ROS%, and vitamin C), as well as DNA damage using the comet assay, in order to compare the relative toxic potential of the tested antibiotics and to assess the sensitivity of biochemical and genetic endpoints for ecological risk evaluation.

MATERIALS AND METHODS

Algal isolation and culture conditions

The isolate was purified and microscopically examined to confirm the absence of contaminants prior to its use in the experiments.

The microalgal cultures were maintained in BG-11 medium containing the essential macro- and micronutrients required for optimal growth. Cultures were grown at a constant temperature of 25 ± 2 °C under an illumination intensity of 40 µmol photons m^{-2} s^{-1} provided by cool-white fluorescent lamps, with a 12:12 h light–dark photoperiod to ensure stable photosynthetic activity and cellular metabolism throughout the culture period.

Prior to antibiotic exposure, cultures were standardized based on chlorophyll content, which was used as a proxy for initial algal biomass to ensure comparable starting conditions among all treatments. Chlorophyll was extracted using 80%

acetone, and absorbance was measured spectrophotometrically at 663 and 646 nm. Chlorophyll a, chlorophyll b, and total chlorophyll concentrations were calculated according to the equations described by Lichtenthaler (1987) as follows:

$$\text{Chlorophyll a } (\mu g\ ml^{-1}) = 12.25 \times A663 - 2.79 \times A646 \quad (1)$$

$$\text{Chlorophyll b } (\mu g\ ml^{-1}) = 21.50 \times A646 - 5.10 \times A663 \quad (2)$$

$$\text{Total chlorophyll (a + b, } \mu g\ ml^{-1}) = 7.15 \times A663 + 18.71 \times A646 \quad (3)$$

Total chlorophyll content was subsequently used to normalize the initial algal biomass prior to the exposure experiments.

Antibiotic sources and preparation

Ciprofloxacin, levofloxacin, and amoxicillin used in this study were purchased from Samarra Drugs Industry (SDI), Iraq, with pharmaceutical-grade purity. For each antibiotic, 0.1 g of the compound was accurately weighed and dissolved in sterile deionized water to prepare the stock solutions. From these stock solutions, a series of working concentrations (0, 5, 10, 15, 25, 50, and 100 μg ml^{-1}) was prepared by serial dilution and used for the exposure experiments.

Experimental design

The purpose was to compare the biological and genotoxic effects of the three antibiotics on *Neochloris conjuncta*. seven concentrations were tested for each antibiotic: 0 (control), 5, 10, 15, 25, 50 and 100 μg ml^{-1}. 0 μg ml^{-1} was used as non-treated control group. All experiments are repeated three times for reliability and reproducibility.

The algal cultures were kept under similar controlled environmental conditions as stated above. The exposure was of 9 days and the algae were in contact with the antibiotic at different concentrations. Biochemical and genotoxic parameters were estimated at the end of ninth day after exposure.

Although pharmaceutical concentrations in surface waters are often reported in the nanogram per liter (ng/L) range, several studies have documented substantially higher concentrations in contaminated aquatic environments, particularly near point sources before dilution occurs.

A comprehensive global review by Maghsodian *et al.* (2022) reported that antibiotics have been detected in aquatic systems at concentrations ranging from <1 ng/L up to 100 μg ml^{-1}, depending on location, compound type, and proximity to contamination sources. In a field study conducted in surface waters impacted by runoff from informal settlements, Maraj *et al.* (2025) detected active pharmaceutical ingredients at concentrations reaching approximately 10–100 μg ml^{-1} prior to downstream dilution. Similarly, Abdel-Shafy and Mohamed-Mansour (2013) reported that pharmaceutical compounds in wastewater and receiving waters close to discharge points commonly occur in the 1–50 μg ml^{-1} range, with some compounds approaching or exceeding ~100 μg ml^{-1} before treatment and dilution processes reduce their concentrations.

These findings clearly demonstrate that pharmaceutical pollution can result in locally elevated concentrations reaching tens to hundreds of micrograms per liter, supporting the use of higher exposure concentrations in laboratory toxicity studies to simulate worst-case contamination scenarios.

Measurement of oxidative stress markers

Superoxide dismutase (SOD) activity

SOD activity was assayed by the pyrogallol auto-oxidation inhibition method of Marklund and Marklund (1974). Absorbance was taken at 420 nm and the enzyme activity was expressed as U/ml extract.

Catalase (CAT) activity

CAT was determined according to Johansson and Borg (1988) at 240 nm by monitoring the breakdown of hydrogen peroxide. Results were expressed as U/L.

Lipid peroxidation (MDA)

MDA concentration was determined using TBA method according to Buege and Aust (1978). The absorbance of the MDA–TBA complex was measured at 532 nm, and values were noted as mmol MDA/mg protein.

Reactive oxygen species (ROS%)

The ROS content was measured according to Venkidasamy et al. (2019) spectrophotometric method. and absorbance determined at 560 nm. ROS percentage values were given as %.

Vitamin C (ascorbic acid)

The content of vitamin C was measured by the colorimetric reduction method with DCPIP (2,6-dichlorophenol-indophenol). The results were presented in μg ml^{-1}.

DNA damage assessment (comet assay)

DNA damage was assessed by the Comet Assay (Comet tail length) according to similar conditions as Singh *et al.* (1988). Nuclei of algae were imbedded in low–melting point agarose, lysed and subjected to electrophoresis at 70 V for 60 min. After neutralizing the staining with ethidium bromide, cells were observed under a fluorescence microscope. Histological damage was divided into Low, Medium, and High according to tail length and DNA migration.

Statistical analysis

All data of experiments were analyzed by SPSS (version 25.0). The measured parameters were analyzed in response to the type and concentration of antibiotic by two-way ANOVA; significant differences between treatments were determined using a LSD post-hoc test at $p \leq 0.05$. The data is expressed as mean ± SE.

RESULTS

Impacts of antibiotics on oxidative stress marker of alga *Neochloris conjuncta*

The data of Table 1 indicate that the alga *N. conjuncta* had differentiated responses in oxidative stress to three different antibiotics Ciprofloxacin, Levofloxacin and Amoxicillin. These differential oxidative stress responses were evidenced by specific changes in such biochemical parameters as MDA, SOD, CAT, ROS % and Vitamin C, which are known to be reliable indicators of oxidative homeostasis in cells and ability of the organism to cope with stress caused by pharmaceuticals currently studied.

Effects of ciprofloxacin

Exposure of *Neochloris conjuncta* to ciprofloxacin resulted in a clear concentration-dependent alteration in oxidative stress parameters (Table 1). Lipid peroxidation, expressed as MDA content, showed a gradual increase with rising antibiotic concentration. MDA increased from 0.10 nmol ml^{-1} in the control to 0.12 nmol ml^{-1} at 5 μg ml^{-1}, followed by a further increase to 0.13 nmol ml^{-1} at 10 and 15 μg ml^{-1}. At higher concentrations, MDA reached 0.14 nmol ml^{-1} at 25 μg ml^{-1}, 0.15 nmol ml^{-1} at 50 μg ml^{-1}, and peaked at 0.16 nmol ml^{-1} at 100 μg ml^{-1}, indicating progressive enhancement of lipid peroxidation.

SOD activity increased markedly in response to ciprofloxacin exposure. Compared with the control value of 6.65 U ml^{-1}, SOD rose sharply to 20.25 U ml^{-1} at 5 μg ml^{-1} and further increased to 26.96 U ml^{-1} at 10 μg ml^{-1}. Similar elevated levels were observed at 15 μg ml^{-1} (26.68 U ml^{-1}), followed by a continued increase at higher concentrations, reaching 32.79 U ml^{-1} at 25 μg ml^{-1}, 31.87 U ml^{-1} at 50 μg ml^{-1}, and 33.59 U ml^{-1} at 100 μg ml^{-1}.

CAT activity exhibited a pronounced concentration-dependent increase. CAT rose from 3.93 U ml^{-1} in the control to 8.13 U ml^{-1} at 5 μg ml^{-1}, then increased further to 13.96 U ml^{-1} and 14.12 U ml^{-1} at 10 and 15 μg ml^{-1}, respectively. At higher concentrations, CAT activity increased to 13.86 U ml^{-1} at 25 μg ml^{-1} and 27.78 U ml^{-1} at 50 μg ml^{-1}, before reaching a maximum value of 89.56 U ml^{-1} at 100 μg ml^{-1}, reflecting severe oxidative stress at high exposure levels.

ROS levels also increased with concentration, rising from 0.09% in the control to 0.12–0.13% at concentrations between 5 and 50 μg ml^{-1}, and reaching 0.16% at 100 μg ml^{-1}. Vitamin C content initially decreased from 0.45 μg ml^{-1} in the control to 0.10 μg ml^{-1} at 5 μg ml^{-1}, followed by a gradual increase to 0.25–0.30 μg ml^{-1} at 10–25 μg ml^{-1} and a maximum value of 0.51 μg ml^{-1} at 100 μg ml^{-1}.

Effects of levofloxacin

Levofloxacin exposure induced comparatively milder oxidative alterations. MDA values remained close to control levels, ranging from 0.09 to 0.13 nmol ml^{-1} across all tested concentrations, suggesting limited lipid peroxidation.

SOD activity initially decreased from 6.65 U ml^{-1} in the control to 5.16 U ml^{-1} at 5 μg ml^{-1}, then increased sharply to 19.93 U ml^{-1} at 10 μg ml^{-1} and 35.48 U ml^{-1} at 15 μg ml^{-1}. At higher concentrations, SOD reached 40.49 U ml^{-1} at 25 μg ml^{-1} and remained relatively stable at 39.85–40.04 U ml^{-1} at 50 and 100 μg ml^{-1}.

CAT activity increased steadily with concentration, rising from 3.93 U ml^{-1} in the control to

Table 1. Effect of different concentrations of antibiotics (Ciprofloxacin, Levofloxacin, Amoxicillin) on oxidative stress indices (MDA, SOD, CAT, ROS%, Vit C) in *Neochloris conjuncta* alga after 9 days of exposure (Mean ± SE)

Antibiotics concentrations		Antioxidant parameters				
		MDA (nmol ml^{-1})	SOD (U ml^{-1})	CAT (U ml^{-1})	ROS %	Vit C µg ml^{-1}
Ciprofloxacin	Control	0.10±0.01 F a	6.65±0.44 F a	3.93±0.17 E a	0.09±0.01 D a	0.45±0.03 C a
	5	0.12±0.01 E b	20.25±0.73 E b	8.13±0.34 D a	0.12±0.01 C b	0.10±0.01 E c
	10	0.13±0.01 D c	26.96±1.42 D b	13.96±0.78 C c	0.13±0.01 B c	0.25±0.02 D b
	15	0.13±0.01 D a	26.68±0.78 D b	14.12±0.61 C c	0.12±0.01 C c	0.30±0.02 D b
	25	0.14±0.01 C a	32.79±1.44 B b	13.86±0.70 C b	0.12±0.01 C c	0.30±0.02 D c
	50	0.15±0.01 B a	31.87±1.08 C b	27.78±0.69 B c	0.13±0.01 B a	0.35±0.01 B b
	100	0.16±0.01 A a	33.59±0.84 A b	89.56±3.90 A b	0.16±0.01 A a	0.51±0.01 A a
Levofloxacin	Control	0.10±0.01 F c	6.65±0.44 D a	3.93±0.17 G a	0.09±0.01 D a	0.45±0.03 A a
	5	0.09±0.01 E b	5.16±0.30 E c	7.28±0.27 E b	0.13±0.01 B b	0.4±0.01 B
	10	0.11±0.01 D b	19.93±1.25 C c	24.25±0.84 E	0.12±0.01 C a	0.20±0.01 D c
	15	0.11±0.01 C c	35.48±1.17 B a	35.89±2.05 D a	0.13±0.01 A a	0.20±0.01 C c
	25	0.12±0.01 B b	40.49±1.64 A a	39.94±1.81 B a	0.12±0.01 C b	0.30±0.02 B b
	50	0.12±0.01 B a	39.85±1.76 A a	36.03±0.79 C a	0.13±0.01 D c	0.31±0.02 B
	100	0.13±0.01 A a	40.04±1.59 A a	43.88±1.48 A c	0.13±0.01 B a	0.36±0.02 B b
Amoxicillin	Control	0.10±0.01 F a	6.65±0.44 D a	3.93±0.17 F a	0.09±0.01 D a	0.45±0.03 D a
	5	0.08±0.01 G c	20.11±0.71 C b	13.79±0.54 C c	0.12±0.01 C b	0.31±0.01 E b
	10	0.12±0.01 E c	39.59±2.03 B a	13.93±0.86 C b	0.12±0.01 C b	0.35±0.01 C a
	15	0.12±0.01 A c	40.50±1.77 A a	15.75±0.75 E c	0.13±0.01 B b	0.40±0.018 D a
	25	0.13±0.01 B b	40.09±1.80 A a	18.10±0.70 D b	0.13±0.01 A a	0.40±0.02 B
	50	0.13±0.01 C b	39.91±0.61 A a	32.16±1.22 B b	0.13±0.01 B b	0.45±0.03 A a
	100	0.14±0.01 A a	40.49±2.03 A a	161.17±8.61 A a	0.13±0.01 B b	0.45±0.01 A a
LSD		0.00203				

Uppercase letters indicate statistically significant differences between the three antibiotics at a given initial concentration, whereas lowercase letters reflect significant differences among concentration levels within each antibiotic treatment.

7.28 U ml^{-1} at 5 µg ml^{-1}, followed by marked increases to 24.25 U ml^{-1} at 10 µg ml^{-1} and 35.89 U ml^{-1} at 15 µg ml^{-1}. CAT reached 39.94 U ml^{-1} at 25 µg ml^{-1} and remained elevated at 36.03–43.88 U ml^{-1} at 50 and 100 µg ml^{-1}. ROS levels showed only slight changes, increasing from 0.09% in the control to approximately 0.12–0.13% across all concentrations. Vitamin C content varied within a narrow range, from 0.20 to 0.36 µg ml^{-1}, indicating a limited antioxidant response.

Effects of amoxicillin

Amoxicillin elicited the strongest oxidative response among the tested antibiotics. MDA values increased from 0.10 nmol ml^{-1} in the control to 0.08 nmol ml^{-1} at 5 µg ml^{-1}, followed by a steady rise to 0.12 nmol ml^{-1} at 10 and 15 µg ml^{-1}, 0.13 nmol ml^{-1} at 25 and 50 µg ml^{-1}, and 0.14 nmol ml^{-1} at 100 µg ml^{-1}. SOD activity increased sharply from 6.65 U ml^{-1} in the control to

20.11 U ml^{-1} at 5 µg ml^{-1} and further to 39.59 U ml^{-1} at 10 µg ml^{-1}. High SOD activity persisted at 40.50 U ml^{-1} at 15 µg ml^{-1} and remained elevated at 39.91–40.49 U ml^{-1} at higher concentrations.

CAT activity showed the most dramatic increase. CAT rose from 3.93 U ml^{-1} in the control to 13.79–15.75 U ml^{-1} at concentrations between 5 and 15 µg ml^{-1}, increased further to 18.10 U ml^{-1} at 25 µg ml^{-1} and 32.16 U ml^{-1} at 50 µg ml^{-1}, and reached an exceptionally high value of 161.17 U ml^{-1} at 100 µg ml^{-1}, indicating severe disruption of redox homeostasis.

ROS levels showed a modest increase from 0.09–0.12% to 0.13% across all concentrations. Vitamin C values ranged between 0.31 and 0.45 µg ml^{-1}, with no pronounced concentration-dependent increase.

Collectively, the results demonstrate that the magnitude of antibiotic-induced oxidative stress in *Neochloris conjuncta* followed the order:

Amoxicillin > Ciprofloxacin > Levofloxacin, With amoxicillin producing the most severe oxidative imbalance, ciprofloxacin inducing a moderate but clear response, and levofloxacin exhibiting the weakest oxidative effects.

Assessment of antibiotic-induced DNA damage in *Neochloris conjuncta* using the comet assay

The results of Comet assay (Table 2) suggest that DNA integrity was altered (increased levels) in *N. conjuncta* on exposure to the tested antibiotics with increasing concentration, Ciprofloxacin, Levofloxacin and Amoxicillin. DNA strand breakage was classified in 3 categories: Low damage, Medium damage and High damage which reflected the increasing degree of DNA fragmentation and genomic instability.

Effect of ciprofloxacin

Exposure of *Neochloris conjuncta* to ciprofloxacin resulted in a clear concentration-dependent alteration in DNA integrity, as assessed by the comet assay (Table 2). At control conditions and low concentrations (5, 10, and 15 µg ml^{-1}), cells were entirely classified within the low-damage category, with values remaining at 100%, indicating preserved DNA integrity.

At higher concentrations, a progressive decline in the proportion of low-damage cells was observed. Low-damage values decreased to 95.87% at 25 µg ml^{-1}, 92.31% at 50 µg ml^{-1}, and further to 91.61% at 100 µg ml^{-1}, reflecting a gradual shift toward DNA damage.

Moderate DNA damage was absent at concentrations up to 15 µg ml^{-1}, but became evident at higher doses. Moderate-damage values reached 4.13% at 25 µg ml^{-1}, increased to 4.81% at 50 µg ml^{-1}, and slightly declined to 3.93% at 100 µg ml^{-1}.

High DNA damage was detected only at the two highest concentrations, appearing at 2.88% at 50 µg ml^{-1} and increasing to 4.46% at 100 µg ml^{-1}. These findings indicate that ciprofloxacin induces measurable DNA strand damage in *N. conjuncta* at elevated concentrations, with damage severity increasing as exposure levels rise.

Effect of levofloxacin

Levofloxacin exposure produced a more pronounced genotoxic response compared with ciprofloxacin. At control conditions and concentrations up to 10 µg ml^{-1}, all cells remained within the low-damage category, with values maintained at 100%.

Beginning at 15 µg ml^{-1}, a marked decline in low-damage cells was observed, decreasing to 97.00%, followed by further reductions to 95.85% at 25 µg ml^{-1} and 95.00% at 50 µg ml^{-1}. The most substantial decline occurred at the highest concentration, where low-damage cells dropped sharply to 82.41% at 100 µg ml^{-1}. Moderate DNA damage emerged at 15 µg ml^{-1}, with a value of 3.00%, and increased progressively with concentration, reaching 4.15% at 25 µg ml^{-1}, 5.00% at 50 µg ml^{-1}, and peaking at 12.49% at 100 µg ml^{-1}.

High DNA damage was absent at concentrations up to 50 µg ml^{-1} but appeared prominently at the highest exposure level, reaching 5.10% at 100 µg ml^{-1}. These results demonstrate that levofloxacin exerts a strong concentration-dependent genotoxic effect, particularly at high doses.

Effect of amoxicillin

Amoxicillin induced the weakest genotoxic response among the three antibiotics tested. Low-damage values remained consistently high across all concentrations. Control and 5 µg ml^{-1} treatments showed 100% low-damage cells, while slight reductions were observed at higher concentrations, with values of 98.00% at 10 µg ml^{-1}, 96.15% at 15 µg ml^{-1}, 96.00% at 25 µg ml^{-1}, and 95.65% at 50 µg ml^{-1}. At 100 µg ml^{-1}, the low-damage proportion increased again to 98.00%.

Table 2. Evaluation of DNA damage in *Neochloris conjuncta* exposed to antibiotics using the comet assay

Antibiotics concentrations		Comet assay (Comet tail length)		
		Low %	Medium %	High %
Ciprofloxacin	con	100±4.60 A a	0±0 B a	0±0 Ba
	5	100±2.68 A a	0±0 B a	0±0 B a
	10	100±1.92 A a	0±0 B a	0±0 B a
	15	100±4.71 A a	0±0 B c	0±0 B a
	25	95.87±2.99 B b	4.13±0.13 A a	0±0 B a
	50	92.31±3.47 C b	4.81±0.13 A b	2.88±0.049 A a
	100	91.61±4.30 C b	3.93±0.18 A c	4.46±0.12 A b
Levofloxacin	con	100±4.60 AB a	0±0 D a	0±0 B a
	5	100 ±2.83 A a	0±0 D a	0±0 B a
	10	100±4.23 A a	0±0 D a	0±0 B a
	15	97.00±4.50 B a	3.00±0.13 C b	0±0 B a
	25	95.85±4.12 C b	4.15±0.13 C a	0±0 B a
	50	95.00±3.07 C a	5.00±0.32 B a	0±0 B a
	100	82.41±4.62 D c	12.49±0.59 A a	5.10±0.18 A a
Amoxicillin	con	100 ±2.28 A a	0±0 E a	0±0 B a
	5	100.±2.39 A a	0±0 E a	0±0 B a
	10	98.00±4.11 AB a	2.00±0.10 D b	0±0 B a
	15	96.15±1.53 B b	3.85±0.09 C b	0±0 B a
	25	96.00±5.35 C a	4.00±0.16 B b	0±0 B c
	50	95.65±2.94 C a	4.35±0.1616 C c	0±0 B b
	100	98.00±0.01 A a	1.00±0.44 A a	1.00±0.17 A a
LSD		2.223		

Uppercase letters indicate statistically significant differences between the three antibiotics at a given initial concentration, whereas lowercase letters reflect significant differences among concentration levels within each antibiotic treatment.

Moderate DNA damage appeared at relatively low levels, beginning at 2.00% at 10 µg ml^{-1} and increasing modestly to 3.85% at 15 µg ml^{-1} and 4.00–4.35% at concentrations between 25 and 50 µg ml^{-1}. At the highest concentration, moderate damage declined to 1.00%.

High DNA damage remained absent across most concentrations and appeared only at 100 µg ml^{-1}, with a value of 1.00%, indicating minimal severe DNA fragmentation. Overall, amoxicillin caused only limited DNA damage in *N. conjuncta*, even at elevated concentrations. Collectively, the comet assay results indicate that all tested antibiotics affected DNA integrity in *Neochloris conjuncta*, but with markedly different severities. The extent of DNA damage followed the order:

Levofloxacin > Ciprofloxacin > Amoxicillin, with levofloxacin producing the strongest genotoxic effects, ciprofloxacin inducing moderate DNA damage at higher concentrations, and amoxicillin exerting the weakest impact on genomic stability.

DISCUSSION

Antioxidant responses to antibiotic treatment

Analysis of the antioxidant responses of *Neochloris conjuncta* to antibiotics is important for elucidating the effects of these pharmaceuticals on algal physiology at biochemical level. Pharmaceutically active contaminants (PACs), antibiotics in particular, are known to cause disruptions in cellular redox processes through inhibitions of photosynthetic and/or respiratory electron transfer chains leading to leakages involving excessive generation of reactive oxygen species (ROS) (Santos *et al.*, 2025; Cirulis *et al.*, 2013; Murali, 2024). This progressive increase of ROS% across most antibiotics in this study is obviously indicative of that mechanism and shows that the antibiotics exerted oxidative stress beyond the usual detoxification ability of the cells. This oxidative increment was followed by a significant increase in malondialdehyde (MDA) content, a major product of lipid peroxidation indicating structural damage to membrane lipids as a result of ROS attack which is supported by the studies describing MDA as one of the major biomarkers for pollutant-induced oxidative degradation in microalgae (Murali, 2024; Gu *et al.*, 2017). Thus, the high MDA levels attained under Amoxicillin and Ciprofloxacin reflect an obvious augmentation of peroxidative reactions, indicating that a profound membrane gross injury effect was caused by these molecules.

In reaction to these oxidative perturbations, *N. conjuncta* turned on its enzymatic antioxidant machinery starting with SOD. SOD over expression reflects increased detoxification of superoxide radicals that are generated during exposure and this was also noted previously to occur in *Neochloris conjuncta*, which is naturally highly antioxidant stress resistant and undergoes rapid SOD activation when exposed to oxidative challenge (Kumar *et al.*, 2014; Cirulis *et al.*, 2013). Increase in the activity of SOD led to the formation of hydrogen peroxide as a byproduct that still required detoxification by CAT. The extremely high CAT activity observed, especially in the presence of Prescription Medicines is consistent with other studies indicating that pharmaceutical contaminants could result in significant hydrogen peroxide accumulation leading to rapid increase in CAT activity as an adaptive response (Lee *et al.*, 2018; Kumar *et al.*, 2014). The degree of CAT activation observed in our study points to a marked oxidative stress caused by the antibiotics, particularly Amoxicillin seems to disturb redox balance more significantly than other drug substances.

Together with the activity of enzymes, the relatively weak enhanced ascorbate concentration indicates that another antioxidant has a role in this. Known ascorbate has also been found to assist in the maintaining of redox balance after enzymatic defenses are weakened or exceeded, serving as a backup ROS scavenger (Pereira *et al.*, 2024; Gu *et al.*, 2017). The elevation of Vitamin C however was also not as much as SOD and CAT; but reflects the triggering of non-enzymatic anti-oxidants in limiting oxidative abuse over prolonged exposure.

Oxidative stress induction capacities of the three antibiotics are thus different, what is also in correlation with previously published patterns. The well-marked oxidative effect of Amoxicillin observed, which resulted in the greatest elevation on ROS as well as MDA, and mainly CAT activity, indicates that this molecule is the most harmful by biochemical interference among all the other antibiotics tested. This result is consistent with reports from literature where some pharmaceutical pollutants cause more intense and prolonged oxidative damage since they interfere with electron transport processes in a greater extent (Santos *et al.*, 2025; Cirulis *et al.*, 2013; Murali, 2024). The significantly high MDA levels in the Amoxicillin treatment group agrees with a report indicating that powerful pollutants cause severe lipid peroxidation and membrane leakage damage (Murali, 2024; Gu *et al.*, 2017). Therefore, it's not surprising to see Amoxicillin as the most harmful antibiotic since previous biochemical mechanisms show that some pharmaceuticals disturb more algal redox systems.

Ciprofloxacin seems to show medium toxicity. Its power to enhance ROS and MDA less than that of Amoxicillin shows moderate disturbance in the redox status. These trends are in concordance with the fact that intensity of oxidative process is dependent upon the chemical structure as well as penetration ability of pollutants from literature (Kumar *et al.*, 2014; Cirulis *et al.*, 2013). Thus, the weak oxidative answer under Ciprofloxacin exposure corresponds to its intermediary reactivity and justo a middle interference in cellular seelectron-transport.

Levofloxacin brought with it the least oxidation damage, which was reflected in the small

ROA, MDA, SOD and CAT increases. This lower response fits in the context that not all pharmaceutical pollutants exhibited same biochemical responses and their toxicological implications depend on molecular structure or interaction with algal physiology (Santos *et al.*, 2025; Cirulis *et al.*, 2013). The mild oxidative stress detected by Levofloxacin indicates lower potential to interfere with the electron transport system and production of reactive intermediates, leading to less intense oxidative pressure as previously presented, showing differences in pollutant-dependent oxidative effects (Kumar *et al.*, 2014; Rezayian *et al.*, 2019).

Taken together, the sequential response: elevation of initial ROS levels, lipid peroxidation, strong activation of enzymes SOD and CAT activity; perturbation on Vitamin C, indicated that antibiotics induced different extents of oxidative stress (Amoxicillin > Ciprofloxacin > Levofloxacin), in *Neochloris conjuncta*. These patterns are very similar to oxidative mechanisms presented in the cited literature, and also indicate that the degree of oxidative imbalance is dependent on both: the specific chemistry of a given antibiotic in addition to an organism's inherent physiological resistance capability at *N. congjucta* cell (Santos *et al.*, 2025; Cirulis *et al.*, 2013; Kumar *et al.*, 2014).

Antibiotic impact on DNA damage (comet assay)

The genotoxic responses to antibiotics in *Neochloris conjuncta* are important for the interpretation of the cellular level where these pharmaceuticals compromise DNA stability especially considering that pharmaceuticals can induce oxidative and electrophilic stress capable of damaging nucleic acids and causing breaks in strands (Nadar & Nugroho, 2022; Emtyazjoo *et al.*, 2025). Comet assay findings of the present investigation showed that, depending on their concentrations, DNA damage shifted from low toward moderate and high damage categories by exposure to the three antibiotics, indicating a gradual compromise in genomic stability upon increasing antibiotic levels. The above results are in agreement with previous reports showing that pharmaceutical pollutants, such as ciprofloxacin, carbamazepine, diclofenac and sulfamethoxazole induced the ROS formation which leads to oxidative DNA lesions and alkali-labile (AL) sites in microalgal cells resulting in significant increase of tail length, tail %DNA and tail moment (Gupta *et al.*, 2024; Hejna *et al.*, 2022; Christudoss *et al.*, 2024). The strong genotoxic response when exposed to levofloxacin, particularly the drastic reduction on low damage cells and the high increase in medium and high levels at higher concentrations, is coincident with previous reports indicating that some drugs had a high redox-interfering capacity capable of generating dramatic DNA fragmentation through disruption of electron transport chains coupled to an overload and inhibition of DNA repair pathways (Harshkova *et al.*, 2021; Sánchez-Sandoval *et al.*, 2022; Jan-Roblero and Cruz-Maya, 2023). The moderate but pronounced rise of medium and high-level DNA damage induced by ciprofloxacin at high concentrations supports previous studies that have demonstrated differential or intermediary DNA-damaging potencies of fluoroquinolones due to their capability to develop reactive intermediates and induce oxidative imbalance without inducing the extensive chromatin destabilization seen for more potent pharmaceutical pollutants (Poddar *et al.*, 2025; Alsubih and Khan, 2024). On the contrary, amoxicillin generated the lowest genotoxic effect, since most of the cells were low damage at all concentrations and only marginal high damage was observed at the highest concentration, a scenario compatible with results published on other pharmaceuticals when their reactivity with DNA/ROS or capacity to generate ROS is diminished (Sharma *et al.*, 2021; Rios-Miguel *et al.*, 2021). These discrepancies between antibiotics were consistent with the compound-specific mechanisms of genotoxicity reported, by which substances with greater redox activity or a more potent alteration of cellular metabolism are more effective in inducing DNA fragmentation than those exerting weaker oxidative or electrophilic effects (Zhang *et al.*, 2019; Procopio *et al.*, 2021; Zheng *et al.*, 2024). The general order for the genotoxic potential of compounds in the present study Levofloxacin > Ciprofloxacin > Amoxicillin confirms previous reports that microalgae are differentially sensitive to chemically distinct drugs, as a function of their capacity to generate ROS, inhibit DNA repair systems or bind directly to genomic material (Baaloudj *et al.*, 2025). As a whole, the DNA comet assay results can conclude that antibiotic treatment brings substantial and different contents of DNA damage to *N. conjuncta*, suggesting the great potential value for the comet assay as sensitive biomarker in determination of genotoxic effect from emerging pharmaceutical pollutants.

Growth inhibition, photosynthesis, and biomass

Although growth rate, photosynthetic performance, and biomass accumulation were not directly measured in the present study, the pronounced biochemical and genetic disturbances observed provide strong evidence for potential adverse effects on overall algal health. In microalgae, oxidative stress and DNA damage are closely associated with growth inhibition and impaired photosynthetic efficiency, as cellular energy production and biomass accumulation depend on the integrity of metabolic and genetic systems (Hejna *et al.*, 2022; Xin *et al.*, 2021; Koletti *et al.*, 2025).

Link between oxidative stress and growth inhibition

The severe oxidative stress induced by amoxicillin, as evidenced by markedly elevated ROS levels, strong lipid peroxidation, and exceptionally high CAT activity, is likely to have detrimental consequences for algal growth. Excessive ROS production can disrupt membrane integrity, impair nutrient transport, and interfere with key metabolic pathways required for cell division and biomass formation. Lipid peroxidation, indicated by increased MDA levels, compromises membrane fluidity and functionality, which in turn limits cellular expansion and division. Such oxidative imbalance has been widely associated with growth inhibition and reduced biomass accumulation in microalgae exposed to pharmaceutical pollutants, particularly when antioxidant defense systems are overwhelmed (Gu *et al.*, 2017; Murali, 2024; Santos *et al.*, 2025). Therefore, the strong oxidative disturbance observed under amoxicillin exposure suggests a high potential for growth suppression and biomass reduction in *Neochloris conjuncta*.

Genotoxicity and its implications for photosynthesis

Levofloxacin induced the strongest genotoxic response among the tested antibiotics, characterized by a sharp decline in low-damage DNA classes and substantial increases in medium and high DNA damage categories at higher concentrations. DNA damage can impair photosynthetic efficiency by disrupting the transcription and translation of genes encoding photosystem components, electron transport proteins, and enzymes involved in carbon fixation. Damage to nuclear and chloroplast DNA has been shown to reduce the synthesis of essential photosynthetic proteins, leading to inefficient light harvesting, impaired electron transport, and decreased photosynthetic output (Zhang *et al.*, 2019; Procopio *et al.*, 2021; Zheng *et al.*, 2024). Consequently, the pronounced genotoxicity observed under levofloxacin exposure is likely to negatively affect photosynthetic performance and cellular energy availability, even when oxidative stress markers appear less severe.

Integrated effects on algal health and biomass

Taken together, the results indicate that different antibiotics compromise algal health through distinct but interconnected mechanisms. Amoxicillin primarily exerts its toxicity through severe oxidative stress, which is expected to inhibit growth and biomass accumulation, while levofloxacin predominantly affects genomic integrity, with potential downstream consequences for photosynthetic efficiency and cellular productivity. Ciprofloxacin showed intermediate effects, inducing moderate oxidative stress and DNA damage, which may result in partial growth limitation and reduced physiological performance. These findings are consistent with previous studies demonstrating that pharmaceutical pollutants can affect microalgal growth and productivity indirectly through oxidative and genotoxic pathways, even in the absence of direct growth measurements (Hejna *et al.*, 2022; Xin *et al.*, 2021; Baaloudj *et al.*, 2025). Overall, this integrative interpretation highlights the importance of combining biochemical and genetic endpoints when assessing the ecological risks of pharmaceutical contaminants, as molecular-level disturbances can ultimately translate into reduced algal growth, biomass, and ecosystem functioning.

CONCLUSIONS

The three antibiotics induced different degrees of biochemical and genetic stress in *Neochloris conjuncta*. Amoxicillin caused the strongest oxidative imbalance, as reflected by marked increases in CAT and SOD activities, whereas ciprofloxacin induced moderate oxidative stress

and levofloxacin showed the weakest biochemical response. In contrast, comet assay results indicated that levofloxacin produced the highest DNA damage at elevated concentrations, followed by ciprofloxacin, while amoxicillin induced the lowest level of genotoxicity.

These findings suggest that antibiotic-induced oxidative and genetic disturbances may have broader consequences for algal health. Severe oxidative stress, such as that caused by amoxicillin, is likely to impair cellular metabolism and growth, whereas pronounced DNA damage, as observed under levofloxacin exposure, may negatively affect photosynthetic efficiency and cellular productivity. Overall, the results highlight the importance of considering both biochemical and genotoxic endpoints when evaluating the ecological risks of pharmaceutical pollutants.

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REFERENCES

1. Abdel-Shafy, H. I., Mohamed-Mansour, M. S. (2013). Issue of pharmaceutical compounds in water and wastewater: sources, impact and elimination. *Egyptian Journal of Chemistry, 56*(5), 449–471.
2. Alsubih, M., Khan, R. A. (2024). Pharmaceutical active compounds contamination and seasonal-based ecotoxicity assessment of the Yamuna River for the Delhi stretch. *Rocznik Ochrona Środowiska*, *26*, 587–602.
3. Awang, N. A., Jusoh, M., Said, N. F., Yusuf, N., Lani, M. N., Ahmad, F. T. (2025). Microalgae as potential antioxidants: Assessment of antioxidant capacities in microalgae from selected regions of Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural Science*, *48*(1).
4. Baaloudj, O., Scrano, L., Bufo, S. A., Modley, L. A. S., Lelario, F., Zizzamia, A. R., Brienza, M. (2025). Environmental fate, ecotoxicity, and remediation of heterocyclic pharmaceuticals as emerging contaminants: A review of long-term risks and impacts. *Organics*, *6*(1), 1.
5. Buege, J. A., Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in enzymology*, *52*, 302–310.
6. Chakraborty, A., Adhikary, S., Bhattacharya, S., Dutta, S., Chatterjee, S., Banerjee, D., Rajak, P. (2023). Pharmaceuticals and personal care products as emerging environmental contaminants: prevalence, toxicity, and remedial approaches. *ACS Chemical Health & Safety*, *30*(6), 362–388.
7. Chen, S., Wang, L., Feng, W., Yuan, M., Li, J., Xu, H., Zhang, W. (2020). Sulfonamides-induced oxidative stress in freshwater microalga Chlorella vulgaris: Evaluation of growth, photosynthesis, antioxidants, ultrastructure, and nucleic acids. *Scientific Reports*, *10*(1), 8243.
8. Christudoss, A. C., Chandrasekaran, N., Mukherjee, A. (2024). Polystyrene nanoplastics alter the ecotoxicological effects of diclofenac on freshwater microalgae Scenedesmus obliquus. *Environmental Science: Processes & Impacts*, *26*(1), 56–70.
9. Cirulis, J. T., Scott, J. A., Ross, G. M. (2013). Management of oxidative stress by microalgae. *Canadian journal of physiology and pharmacology*, *91*(1), 15–21.
10. Duarte, J. A. P., Ribeiro, A. K. N., De Carvalho, P., Bortolini, J. C., Ostroski, I. C. (2023). Emerging contaminants in the aquatic environment: phytoplankton structure in the presence of sulfamethoxazole and diclofenac. *Environmental science and pollution research*, *30*(16), 46604–46617.
11. Emtyazjoo, M., Sheibani nia, A., Payvar, S., Mozdarani, H. (2025). Comparison of genotoxic and cytotoxic effects of single-walled and multi-walled carbon nanotubes on Chlorella vulgaris. *Toxicological & Environmental Chemistry*, *107*(6), 1171–1185.
12. Estrada-Almeida, A. G., Castrejón-Godínez, M. L., Mussali-Galante, P., Tovar-Sánchez, E., Rodríguez, A. (2024). Pharmaceutical Pollutants: Ecotoxicological Impacts and the Use of Agro-Industrial Waste for Their Removal from Aquatic Environments. *Journal of Xenobiotics*, *14*(4), 1465–1518.
13. Feijão, E., Cruz de Carvalho, R., Duarte, I. A., Matos, A. R., Cabrita, M. T., Novais, S. C., Duarte, B. (2020). Fluoxetine arrests growth of the model diatom *Phaeodactylum tricornutum* by increasing oxidative stress and altering energetic and lipid metabolism. *Frontiers in microbiology*, *11*, 1803.
14. Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. *Aquatic Toxicology*, *191*, 122–130.
15. Gupta, A., Kumar, S., Bajpai, Y., Chaturvedi, K., Johri, P., Tiwari, R. K., Trivedi, M. (2024). Pharmaceutically active micropollutants: Origin,

hazards and removal. *Frontiers in Microbiology*, *15*, 1339469.

16. Harshkova, D., Liakh, I., Bialevich, V., Ondrejmišková, K., Aksmann, A., Bišová, K. (2021). Diclofenac alters the cell cycle progression of the green alga *Chlamydomonas reinhardtii*. *Cells*, *10*(8), 1936.
17. Hejna, M., Kapuścińska, D., Aksmann, A. (2022). Pharmaceuticals in the aquatic environment: A review on eco-toxicology and the remediation potential of algae. *International journal of environmental research and public health*, *19*(13), 7717.
18. Hernández-García, C. I., Martínez-Jerónimo, F. (2023). Changes in the morphology and cell ultrastructure of a microalgal community exposed to a commercial glyphosate formulation and a toxigenic cyanobacterium. *Frontiers in Microbiology*, *14*, 1195776.
19. Husen, A. (Ed.). (2022). *Environmental pollution and medicinal plants*. CRC Press.
20. Jan-Roblero, J., Cruz-Maya, J. A. (2023). Ibuprofen: toxicology and biodegradation of an emerging contaminant. *Molecules*, *28*(5), 2097.
21. Johansson, L. H., Borg, L. H. (1988). A spectrophotometric method for determination of catalase activity in small tissue samples. *Analytical biochemistry*, *174*(1), 331–336.
22. Koletti, A., Skliros, D., Dervisi, I., Roussis, A., Flemetakis, E. (2025). Oxidative stress responses in microalgae: Modern insights into an old topic. *Applied Microbiology, 5*(2), 37.
23. Komazec, B., Vitko, S., Balen, B., Cindrić, M., Biba, R., Peharec Štefanić, P. (2025). Multi-parameter analysis of photosynthetic and molecular responses in chlorella vulgaris exposed to silver nanoparticles and ions. *Toxics*, *13*(8), 627.
24. Kumar, R. R., Rao, P. H., Subramanian, V. V., Sivasubramanian, V. (2014). Enzymatic and non-enzymatic antioxidant potentials of *Chlorella vulgaris* grown in effluent of a confectionery industry. *Journal of food science and technology*, *51*(2), 322–328.
25. Lee, H. W., Park, B. S., Joo, J. H., Patidar, S. K., Choi, H. J., Jin, E., Han, M. S. (2018). Cyanobacteria-specific algicidal mechanism of bioinspired naphthoquinone derivative, NQ 2-0. *Scientific Reports*, *8*(1), 11595.
26. Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology, 148*, 350–382.
27. Maghsodian, Z., Sanati, A. M., Mashifana, T., Sillanpää, M., Feng, S., Nhat, T., Ramavandi, B. (2022). Occurrence and distribution of antibiotics in the water, sediment, and biota of freshwater and marine environments: A review. *Antibiotics (Basel, Switzerland), 11*(11), 1461.
28. Maraj, K., Nicklin, E., Edmonds-Smith, C., Winter, K. (2025). Detection of active pharmaceutical ingredients in surface water polluted by an informal settlement. *Environmental Monitoring and Assessment*, 197(11), 1–16.
29. Marklund, S., Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European journal of biochemistry*, *47*(3), 469–474.
30. Moustafa, A. A., Mofeed, J., Abd-El Hakim, R. M., Soror, A. S. (2024). Response of *Chlorella vulgaris* to some selected pharmaceutical drugs. *Catrina: The International Journal of Environmental Sciences*, *29*(1), 79–92.
31. Murali, V. (2024). From prescription to pollution: The ecological consequences of NSAIDs in aquatic ecosystems. *Toxicology Reports*, *13*, 101775.
32. Nadar, Y. E., Nugroho, A. P. (2022). Combined effect of pesticides containing active ingredients of chlorpyrifos and mancozeb on the DNA damage of *Chlorella Sorokiniana* Shihira and Krauss. *Jurnal Pendidikan IPA Indonesia*, *11*(4), 640–648.
33. Pereira, L., Cotas, J., Valado, A. (2024). Antioxidants from microalgae and their potential impact on human well-being. *Exploration of Drug Science*, *2*(3), 292–321.
34. Poddar, B., Mandal, S., Das, S., Mukherjee, A. (2025). Interactive toxicity effects of metronidazole, diclofenac, ibuprofen, and differently functionalized nanoplastics on marine algae *Chlorella* sp. *Environmental Science: Processes & Impacts*, *27*(4), 901–916.
35. Procopio, Z. S., Roberts, J. B., Hunter, C., Pahl, O. (2021). Multifactorial evaluation of atenolol, caffeine, carbamazepine and ibuprofen on *Raphidocelis subcapitata* and *Chlorella vulgaris. Biology*, *10*(9), 926.
36. Rezayian, M., Niknam, V., Ebrahimzadeh, H. (2019). Oxidative damage and antioxidative system in algae. *Toxicology reports*, *6*, 1309–1313.
37. Rios-Miguel, A. B., Jetten, M. S., Welte, C. U. (2021). Effect of concentration and hydraulic reaction time on the removal of pharmaceutical compounds in a membrane bioreactor inoculated with activated sludge. *Microbial Biotechnology*, *14*(4), 1707–1721.
38. Sánchez-Sandoval, D. S., González-Ortega, O., Vazquez-Martínez, J., García de la Cruz, R. F., Soria-Guerra, R. E. (2022). Diclofenac removal by the microalgae species Chlorella vulgaris, *Nannochloropsis oculata*, *Scenedesmus acutus*, and Scenedesmus obliquus. *3 Biotech*, *12*(9), 210.
39. Santos, D., Cabecinha, E., Gago, J., Monteiro, S. M., Luzio, A. (2025). Oxidative stress, phytochemical screening, and antioxidant activity on microalgae (*Arthrospira platensis*) after exposure to glyphosate and microplastics. *Journal of Xenobiotics*, *15*(4), 106.
40. Sharma, L., Siedlewicz, G., Pazdro, K. (2021). The

toxic effects of antibiotics on freshwater and marine photosynthetic microorganisms: State of the art. *Plants*, *10*(3), 591.

41. Singh, N. P., McCoy, M. T., Tice, R. R., Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental cell research*, *175*(1), 184–191.
42. Tang, D., Li, X., Zhang, L., Xiao, P., Nie, Y., Qiu, F., Zhao, Y. (2025). Reactive oxygen species-mediated signal transduction and utilization strategies in microalgae. *Bioresource Technology*, *418*, 132004.
43. Tsai, T. Y., Khozin-Goldberg, I., Vonshak, A., Lee, T. M. (2024). Ascorbate-glutathione cycle alleviates low-temperature-induced oxidative stress for augmented growth of *Nannochloropsis oceanica* Rose Bengal mutants. *Physiologia Plantarum*, *176*(1), e14168.
44. Venkidasamy, B., Karthikeyan, M., Ramalingam, S. (2019). Methods/protocols for determination of oxidative stress in crop plants. *Reactive Oxygen, Nitrogen and Sulfur Species in Plants: Production, Metabolism, Signaling and Defense Mechanisms*, 421–435.
45. Wang, L., Yang, T., Pan, Y., Shi, L., Jin, Y., Huang, X. (2023). The metabolism of reactive oxygen species and their effects on lipid biosynthesis of microalgae. *International Journal of Molecular Sciences*, *24*(13), 11041.
46. Xin, X., Huang, G., Zhang, B. (2021). Review of aquatic toxicity of pharmaceuticals and personal care products to algae. *Journal of hazardous materials, 410*, 124619.
47. Zhang, Y., Guo, J., Yao, T., Zhang, Y., Zhou, X., Chu, H. (2019). The influence of four pharmaceuticals on Chlorellapyrenoidosa culture. *Scientific reports*, *9*(1), 1624.
48. Zheng, C., Aslam, M., Liu, X., Du, H., Xie, X., Jia, H., Li, P. (2020). Impact of Pb on Chlamydomonas reinhardtii at physiological and transcriptional levels. *Frontiers in Microbiology*, *11*, 1443.
49. Zheng, Y., Shao, Y., Zhang, Y., Liu, Z., Zhao, Z., Xu, R., Zhang, H. (2024). Metformin as an emerging pollutant in the aquatic environment: Occurrence, analysis, and toxicity. *Toxics*, *12*(7), 483.