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Cefixime and Aspirin alters antioxidant response, biochemical composition, and primary productivity of phytoplankton: A Mesocosm approach

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ABSTRACT

Pharmaceutical compounds have been described as compounds of emerging concern and interest due to their ability to exert changes in aquatic ecosystems. A mesocosm approach was used to evaluate the effects of aspirin, cefixime, a combination of cefixime and aspirin on phytoplankton community, alongside a control experiment for a period of 21 days. Cefixime and aspirin increased electrical conductivity and total dissolved solids but decreased nitrate by the 21st day of the experiment. pH was not significantly affected by the treatments. Total chlorophyll, chlorophyll a and chlorophyll b were all decreased by the combination of cefixime and aspirin by the 21st day of the experiment. Aspirin and a combination of aspirin and cefixime decreased total carotenoids by the 21st day of the experiment. Cefixime increased protein component by the last day of the experiment. Aspirin increased lipid content significantly by the 7th day of the experiment. Carbohydrate composition was not significantly affected by the treatments. Cefixime and aspirin decreased malondialdehyde by the 21st day of the study. The results suggest that cefixime and aspirin have the capacity to alter the dynamics of aquatic ecosystems. Further research should be conducted to determine effects of drug combination on the phytoplankton community.

Keywords Phytoplankton, Pharmaceuticals, Mesocosm, Ecotoxicology, Aquatic ecosystems

INTRODUCTION

The use of pharmaceuticals has increased in the recent decades, with over 20, 000 prescription drugs currently approved for marketing by the Unites States Food and Drug Administration (FDA, 2021). Pharmaceutical products are discharged into the environment through private household wastes, hospitals, pharmaceutical industries and wastewater treatment plants (Patel *et al.*, 2019; Jiang *et al.*, 2014). Pharmaceuticals are released as metabolites or unchanged compounds and their constant addition to the environment makes them prevalent (Kovalakova *et al.*, 2020). They have been detected in drinking water at extremely low concentrations (ng/L) (Odendaal *et al.*, 2015) and they have been found in ng/L – mg/L concentrations in the environment (aus der Beek *et al.*, 2016; Rzymski *et al.*, 2017). They have been detected in aquatic ecosystems globally due to their continuous release into the environment and inadequacy of water treatment plants (Roberts *et al.*, 2016; Gomaa *et al.*, 2021). Pharmaceuticals such as fluoroquinolones, sulphonamides and cephalosporins have been reported to be non-biodegradable and therefore continuously detected in aquatic ecosystems (Li, 2014).

The aquatic ecosystem acts as a sink for pharmaceuticals amidst many other chemical compounds and have high likelihood of affecting non-target biota such as phytoplankton (Pinckney *et al.*, 2017). Moreso,

pharmaceuticals are designed to target specific cellular pathways in humans and have a probability of affecting non-target organisms with similar cellular signals (Fabbri and Franzellitti, 2015). Pharmaceuticals have been reported to have deleterious effects on living organisms and the environment (Ding *et al.*, 2020) and have been tagged as emerging pollutants of concern (Busfield, 2015; Mojiri *et al.*, 2022). Recently, they have been included on the European Union Water Framework Directive "watch-list" as chemical compounds with potential of causing significant changes in aquatic ecosystems (Miller *et al.*, 2018). Aspirin, also known as acetylsalicylic acid (ASA) is a non-steroidal anti-inflammatory drug (NSAID) that is used to reduce fever, pain and inflammation and it also serves as an antithrombotic (Sachs, 2005). Cefixime is a cephalosporin antibiotic that is sold under the brand name, Suprax (WHO, 2009). It is used in the treatment of bacterial infections such as otitis media, pneumonia, urinary tract infections, strep throat, gonorrhoea and Lyme disease (Grayson, 2017). . Research studies have demonstrated that pharmaceutical compounds can exert a negative influence on phytoplankton, and higher trophic organisms (Gomaa *et al.*, 2021).

Phytoplankton are primary producers for most food chains and factors that affect their functioning should be studied (Akinyemi, *et al.*, 2024). Most ecotoxicology studies have focused on single pharmaceuticals, but there is need to study how the mixture of pharmaceuticals affect living organisms as expected in natural aquatic ecosystems (Nallani *et al.*, 2011). Therefore, this study aims to evaluate the effects of aspirin and cefixime on the primary productivity, biochemical composition and oxidative stress response of phytoplankton.

MATERIALS AND METHODS

Study Area

The experiment was conducted in the screen house of the Department of Plant Biology, Faculty of Basic and Applied sciences, Osun state university, Osogbo.

Materials

The materials used are twelve (12) 20 L plastic containers, pond water containing phytoplankton, paper tape, permanent marker, laboratory coat, compound microscope, high-precision analytical balance, sample bottles (75cl) and(15cl), nitrate, aspirin, cefixime, sediment and pebbles.

Mesocosm Setup

The Pond water containing phytoplankton was collected from Owode, Ilesha garage, off riverside hotel bus stop, Osogbo, Osun State, Nigeria. The pond water was collected using eight (8) 25 L plastic containers and taken to the Osun State University's screen house. Twelve mesocosms were constructed by filling twelve (12) plastic containers of twenty (20) L capacity with fifteen (15) L of pond water containing phytoplankton.

Experimental Design

The study was a 4 x 3 factorial trial laid out in a randomized complete block design. Four treatments (control, aspirin, cefixime, aspirin and cefixime) and three replicates of each treatment were used in this study. The treatments used in the study are control (no aspirin and cefixime), 10 ug/L of aspirin, 10 ug/L of cefixime and the combination of aspirin and cefixime. Each tablet of aspirin and cefixime; 300 mg and 400 mg respectively, were used for the preparation of the treatments. One tablet of aspirin and cefixime were respectively dissolved in 1 L of distilled water. A stock solution of cefixime and aspirin was prepared at a concentration of 10 ug/L.

Samples Collection

Water samples were collected on day 0. The samples were collected from the twelve mesocosm set-up using twelve plastic containers (a plastic container for each mesocosm). The plastic container was dipped into each mesocosm and the water collected was transferred into a 75 cl bottle. Storage containers were labelled with date, time and treatment. After the collection of samples on day 0, treatment was added to each mesocosm and samples were collected on days 7, 14 and 21 to check for physical and chemical parameters. After checking for the water parameters each day, the samples were kept in the refrigerator. On day 21, all the samples collected from day 0 until day 21 were taken to the Department of Botany, Ahmadu Bello University for analysis.

Data Analysis

In this project, we conducted a comprehensive data analysis to explore the ecological dynamics of a biological system under different treatments. The analysis was performed in R, utilizing various packages for data manipulation, visualization, and statistical modelling. The data were loaded from two Excel files, 'Osun students' batch A.xlsx" and "phyto.xlsx," and pre-processed using the `openxlsx` package. The `tidyr` package was used to manage the phytoplankton data and separate multiple species names within a single cell. The processed data was organized and summarized using the `dplyr` package, allowing us to calculate means for multiple biological variables across different treatment groups and days. The `ggplot2` and `ggpubr` packages were employed to generate a series of visually appealing bar plots with error bars, allowing for comparisons of treatment effects on various physiological parameters.

Determination of Physicochemical Parameters

Prior to each weekly sampling (Days 0,7,14,21), the water in the mesocosm was mixed, and physicochemical parameters were measured using a multiparameter water quality portable Hanna instrument (model no. H1991300). Nitrate, phosphate, total dissolved solids, pH and electrical conductivity were determined from five hundred millilitres samples using the methods described by APHA (1992).

Determination of Antioxidant enzyme

The antioxidants that were analysed include peroxidase, malondialdehyde and glutathione S-transferase. The peroxidase activity of phytoplankton samples was assessed using the method of Reddy *et al.* (1995), whereas lipid peroxidation was determined by measuring the concentration of malondialdehyde (MDA) as described by Health & Parker (1968). The assay of glutathione S-transferase activity was determined using the method of Habig *et al.* (1974).

Determination of Biochemical Composition

Total lipid

Lipid extraction was conducted according to Parrish (1999) with chloroform : methanol (2:1) as the extraction solvent. 50 ml of water sample were centrifuged at 3000 x g for 10 min, and the resultant pellet was extracted in 6 ml of chloroform: methanol (2:1). Total lipids concentration was determined gravimetrically (weighed using a high-precision analytical balance).

Total carbohydrates

The total carbohydrate content of the phytoplankton community was determined using the phenol–sulfuric acid method (Liu *et al.*, 1973) with glucose as the standard. Five-millimetre (ml) of the water sample was centrifuged at 3000 rpm for 10 min to pellet the cells. The pellets obtained were re-suspended in 1 ml of distilled water in a test tube. 1ml of 5% (w/v) aqueous phenol solution was added to the test tube containing the sample and was vortexed for 5 minutes to ensure thorough mixing. Thereafter, 5ml of concentrated H_2SO_4 was quickly added to all test tubes, directing the flow at the liquid 49 surface to obtain a good mixing. The mixture was then left to stand at room temperature for 10 min and later centrifuged at 3000rpm for 10 min. The supernatant was decanted and read at 490nm against the reagent blank. (Liu *et al.*, 1973).

Total proteins

To determine the total intracellular protein content of the microalgal cells, the procedure of Bradford (1976) with bovine serum albumin (BSA) as protein standard was used. To every 500µl of the supernatant, 2.5mL of Bradford reagent (0.01% Coomassie blue, 47% ethanol, and 85% phosphoric acid) prepared just prior to the assay, was added and allowed to stand for 5 min at room temperature. The absorbance of the solution was then read at 595 nm. The BSA used as standard was prepared in phosphate buffer (pH 7.2) solution with concentrations ranging from 10 to 400 µg mL-1 . This value was used to plot the total protein standard calibration curve.

Determination of Chlorophyll content

Chlorophyll a content was determined according to Shoaf & Lium (1976), following extraction in 3 mL of 80% acetone. The absorbance of the extract was measured at 653 and 666 nm. Chlorophyll a concentration was determined using the equation provided by Ritchie (2006).

RESULTS

Physicochemical Parameters

Figure 1 provides the results of an experiment conducted to investigate the effect of different treatments (control, aspirin, cefixime, and cefixime + aspirin) on various water quality parameters and physiological characteristics of pond water over 21 days. The results suggest that the treatments significantly impacted the measured parameters ($p < 0.05$). TDS (total dissolved solids) and EC (electrical conductivity) were the highest in the cefixime + aspirin treatment on Day 21. At the same time, pH was relatively stable across all treatments and days (Figures 1a, b, and c). Also, the changes in total dissolved solids were significant ($p<0.05$) at the end of the study, with the control having the lowest

value on day 21. Phosphate phosphorus and nitrate nitrogen concentrations varied considerably among the treatments and days, indicating potential differences in nutrient uptake and assimilation by the phytoplankton. The phosphate-phosphorus and nitrate itrogen concentrations were highest in the aspirin treatment on days 14 and 21 respectively.

The nitrate concentration was lowest in the cefixime + aspirin treatment on day 14 (Figure 1 d and e). The changes in phosphate were significant ($p<0.05$) on days 14 and 21, while that of
nitrate was only significant ($p<0.05$) on day 21.

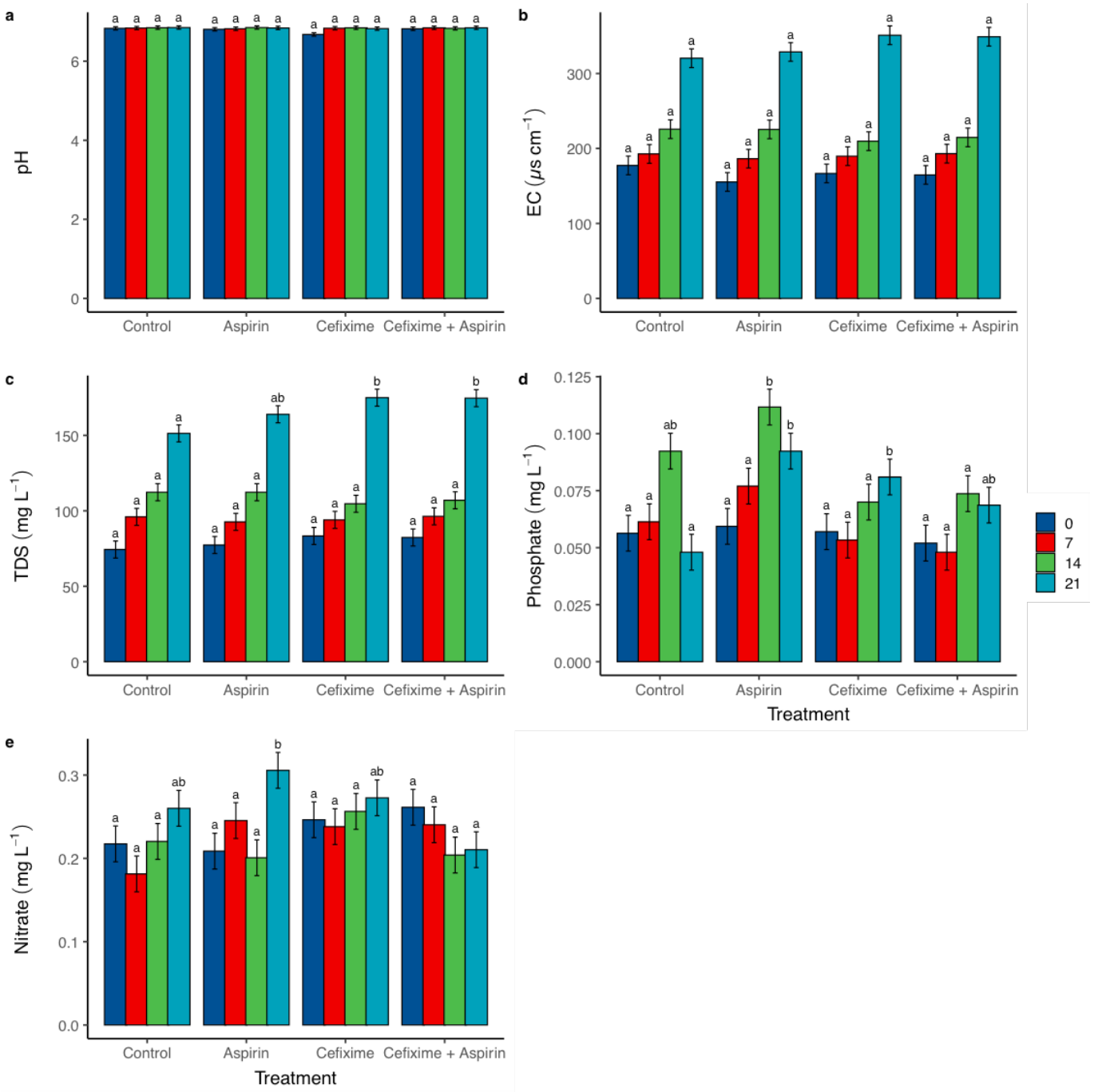

Figure 1: A graph showing the effects of different treatments on various physicochemical parameters.

Pigment Content

Figure 2 provides information concerning chlorophyll and carotenoid level alterations in phytoplankton communities in response to different treatments and time points. The values in the chlorophyll a, b, total chlorophyll, and total carotenoid columns indicate the mean levels of these pigments across different treatments (control, aspirin, cefixime, and cefixime + aspirin) and time points (0, 7, 14, and 21 days). Chlorophyll a, b, and total chlorophyll levels were lowest in the aspirin treatment on day 7. Also, the combination of cefixime + aspirin resulted in the lowest levels of these pigments on day 7. On the other hand, on day 14, the control had the lowest concentration of chlorophyll a, b, and total chlorophyll. Total carotenoid levels were highest in the cefixime and aspirin treatment on day 14, while the least concentration was found in the aspirin treatment on day 21. Throughout the study, the highest concentration of total carotenoids was recorded in the cefixime + aspirin treatment on day 14.

Chlorophyll a is the primary pigment involved in the light-harvesting process of photosynthesis, while chlorophyll b is an accessory pigment that plays a complementary role. Total chlorophyll is the sum of chlorophyll a and chlorophyll b, indicating the overall amount of chlorophyll present in the phytoplankton cells. Carotenoids are another class of pigments that play essential roles in light absorption and photo protection.

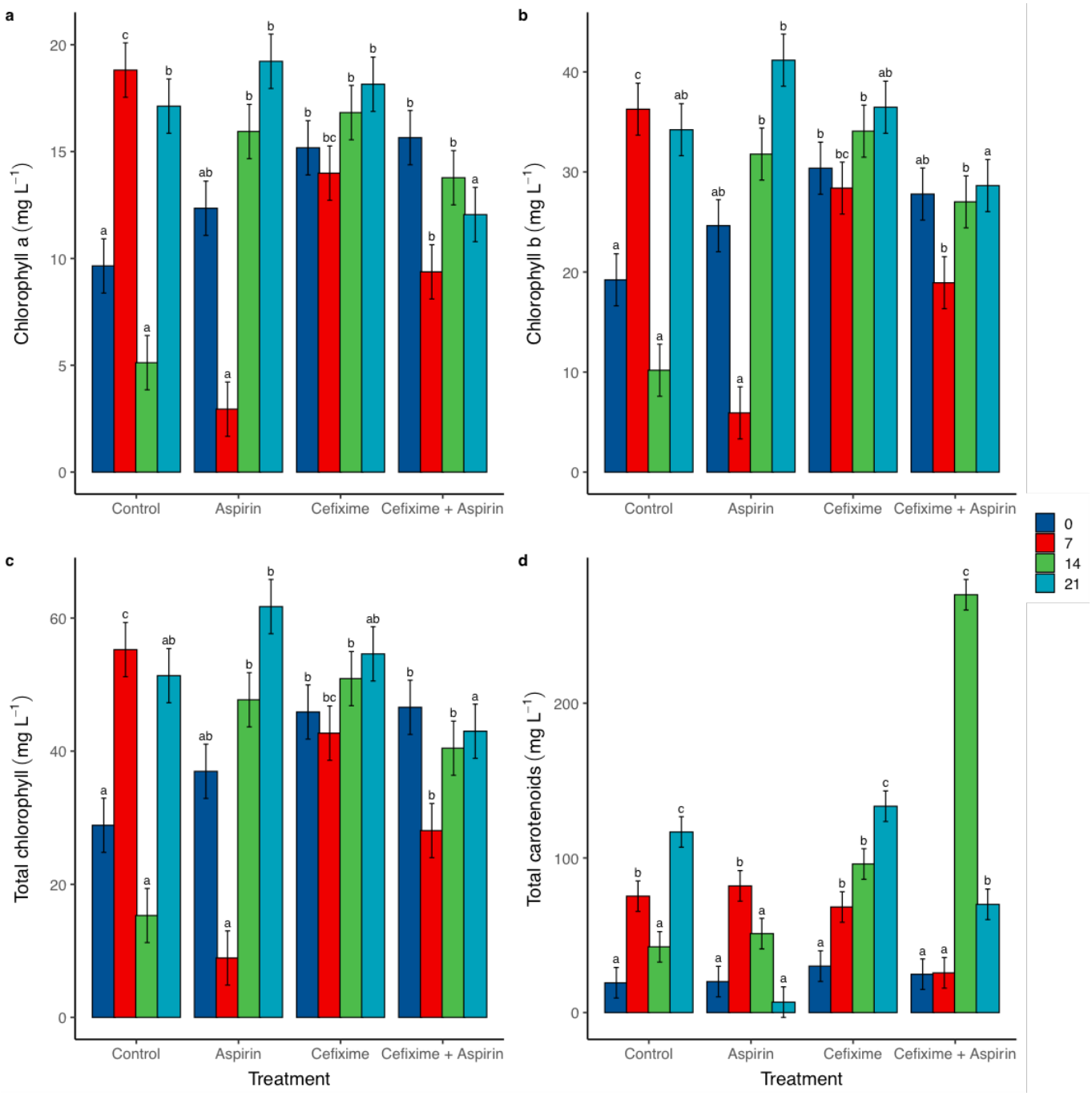

Figure 2: A graph showing pigment composition of phytoplankton community

Biomolecule Composition and Levels

The data in Figure 3 show changes in the concentration of molecules such as total proteins, carbohydrates, and lipids with the drug treatments and control group. On day 0, there was no significant change in total protein composition ($p>0.05$) for the control and treatments. In contrast, on days 7, 14, and 21, the alterations in the protein were significant ($p<0.05$, Figure 3a). On day 14, the lowest total protein was found in the control treatment, while the highest was in the cefixime treatment. The highest total protein concentration throughout the study was recorded in the cefixime treatment on day 21.

Total carbohydrate concentration was generally stable throughout the study, but on day 21,the lowest and most notable change was recorded in the cefixime + aspirin treatment (Figure 3b). On day 7, the aspirin treatment resulted in the highest total lipids content, representing the highest level throughout the study (Figure 3c).

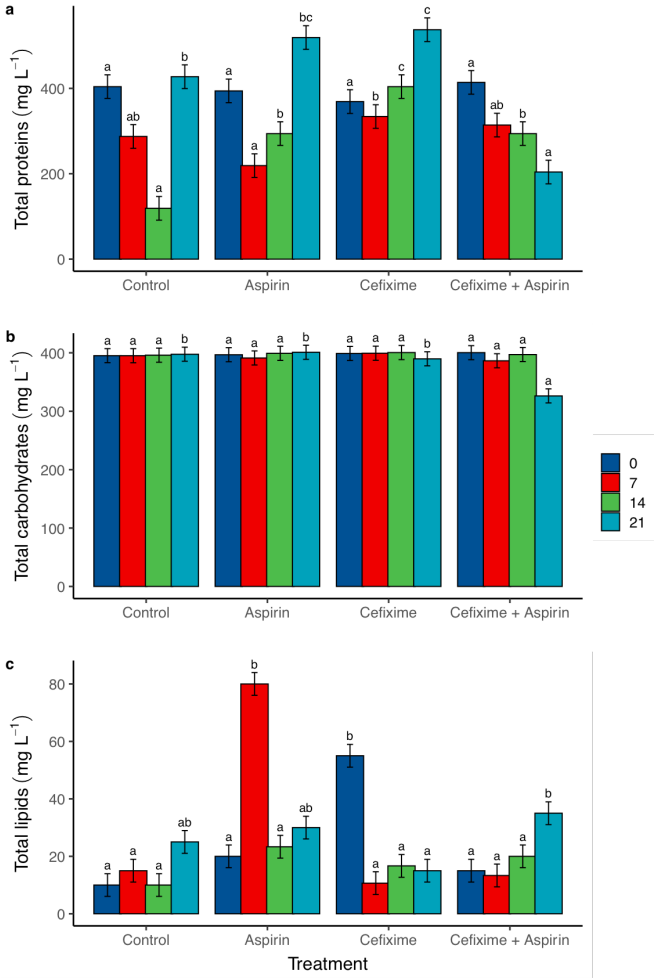

Figure 3: A graph showing changes in the biochemical composition of the phytoplankton community

Antioxidant Response

The antioxidant response of the phytoplankton communities revealed critical information concerning oxidative stress that the organisms underwent following exposure to aspirin, cefixime, and cefixime + aspirin treatments. At the end of the study, GST activity was not significantly different between the treatments, whereas, on days 0, 7, and 14, the changes in the enzyme's activity were significant (Figure 4a). On days 0 and 7, the lowest GST activity was recorded in the control, while those of the aspirin, cefixime, and cefixime + aspirin treatments were significantly increased. On day 14, the changes in GST activity in the control, cefixime, and cefixime + aspirin treatments were not significantly ($p>0.05$) different, while the reduction recorded in the aspirin treatment was significant ($p<0.05$).

Although some changes in the trends of POD activity were recorded, these variations were not significantly different between the treatments and control (Figure 4b). The indicator of lipid peroxidation, MDA, had significant changes in its levels throughout the study (Figure 4c). However, the changes found on day 0 showed the values of the controls were not significant ($p>0.05$) from those of the aspirin, cefixime, and cefixime + aspirin treatments. On day 7, the lowest and most meaningful change in MDA content was recorded in the cefixime treatment, while on day 14, it was in the aspirin treatment. By day 21, all the treatments significantly reduced MDA content, with the cefixime + aspirin treatment having the lowest compound level. The indicator of lipid peroxidation, MDA, had significant changes in its levels throughout the study (Figure 4c).

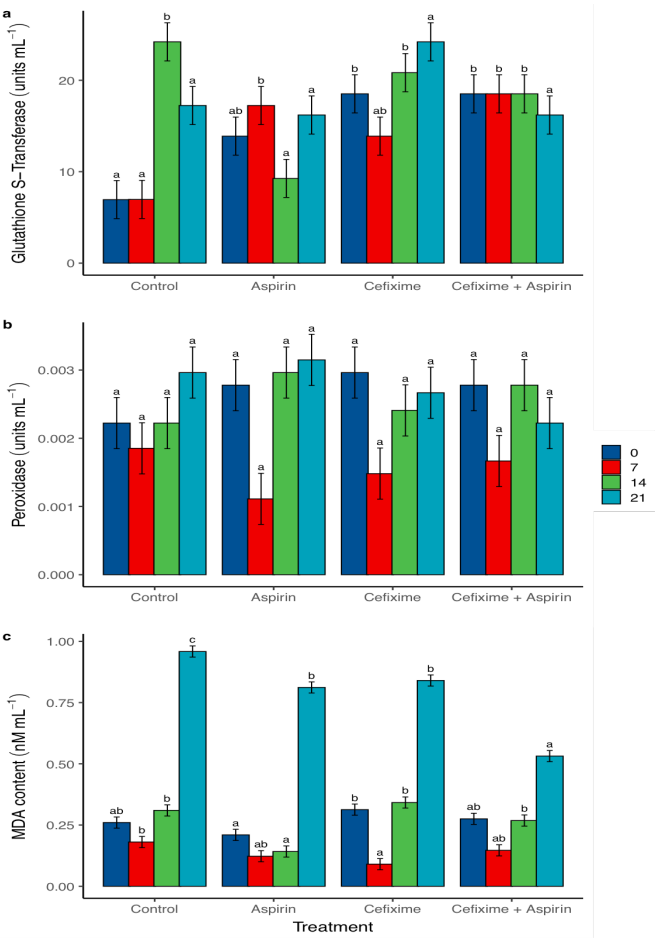

Figure 4: The graph showing antioxidant response of the phytoplankton community

DISCUSSION

Diatoms contribute to 25% of world oxygen and 40% of primary marine production. Cyanobacteria are majorly responsible for CO_2, Nitrogen fixation and free O_2 production. Phytoplankton serve as major primary producers in aquatic ecosystems and the evaluation of factors that affect their functions is important. Although the activities of antibiotics are targeted at bacteria; they pose severe risks to non-target organisms such as phytoplankton due to their structural and evolutionary similarities to bacteria (Guo *et al.*,2016). Potential factors that can influence responses of algal species to APIs include differences in effluent pumps used in elimination of toxic substances, bioavailability of APIs based on pH of medium and binding sites of algal species (Halling-Sorensen, 2000).

According to Chia *et al.* (2021), pharmaceuticals are capable of altering the process of photosynthesis and can exert changes in antioxidant enzyme activities. Phytoplankton produce organic compounds and energy mainly through photosynthesis

(Chia *et al.*, 2021). Antibiotics have been confirmed to decrease pigment concentration and photosynthetic ability of algal species (Siedlewicz *et al.*, 2020). Alterations in photosynthetic pigments would affect the light absorption and photosynthesis of organisms (Grzeswik *et al.*, 2016; Grzeswik *et al.*, 2018). Reduction of proteins involved in absorption of light, electron transport and CO_2 fixation can lead to reduced photosynthesis, growth and biomass production of phytoplankton (Chia *et al.*, 2021). In this study; aspirin, cefixime and the combination of cefixime and aspirin significantly decreased concentrations of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations on different days of treatments. This corresponds with other studies that have reported negative effects of antibiotics on chlorophyll and carotenoid composition of phytoplankton (Guo *et al.*, 2016).

Aerobic organisms utilize O_2 for respiration, which is often reduced by free electrons to form toxic radicals. The radicals often react with O_2 to form reactive oxygen species (Mallick and Mohn, 2000). The ROS can affect activities of biochemical compounds such as carbohydrates, lipids, proteins and DNA (Pikula *et al.*, 2019).

Phytoplankton use enzymatic and non-enzymatic methods to detoxify accumulating ROS. Antioxidant enzymes such as GST, POD and MDA helps to detoxify ROS and increase the survival chances of phytoplankton (Pan *et al.*, 2018). Peroxidase (POD) eliminates super oxide anions (O^{2-}) while GST removes ROS and contributes to the biotransformation of chemical compounds (Patra *et al.*, 2008). Malondialdehyde (MDA) is an important marker of lipid peroxidation and free radical damage to lipids. The study of Wang *et al.*, (2017) examined the impact of thiamphenicol and florfenicol on *Microcystis flosaquae* and revealed increase in the levels of SOD (superoxide dismutase), CAT and MDA at high concentrations of treatments which is indicative of oxidative stress.

However, this study did not show any significant changes in POD. Activities of GST and MDA were decreased with treatments on different days of exposure. An increase in antioxidant enzyme was only demonstrated by GST with aspirin and cefixime treatments on days 0 and 7. Phytoplankton can adapt to increased ROS production by activating antioxidant enzymes. A failure of antioxidant defense can result in oxidative damage (such as enzyme inactivation, protein degradation, lipid peroxidation) which could lead to tissue damage and cell death (Rezayian *et al.*, 2019).

Biomolecules such as carbohydrates, proteins and lipids are produced by phytoplankton through photosynthetic assimilation of dissolved inorganic carbon into organic compounds (Fichez, 1991). Scientific reports suggested that the other biochemical components are of negligible weight, and that carbohydrates, proteins and lipids are easier to assimilate and digest (Kreeger *et al.*, 1997 ; Fichez, 1991). Phytoplankton are the primary producers in aquatic ecosystems, which makes their biochemical composition an important determinant of nutrient cycling (Bhavya *et al.*, 2019). According to Bhavya *et al.* (2019), biomolecular compounds produced by phytoplankton has close association to the environmental conditions (e.g., availability of nutrients and light), major phytoplankton groups, and the growth phase of phytoplankton. Therefore, environmental pollution with APIs would most likely exert effects on the biomolecules produced by phytoplankton. Exponential growth phase of phytoplankton plays a key role in protein synthesis (Danovaro *et al.*, 2000). High light intensity, low temperature and low nitrogen concentration have been found to support the synthesis of carbohydrates and lipids (Lee *et al.*, 2008; Morris, 1980). Proteins and lipids significantly increased with Cefixime and Aspirin respectively in the course of the experiment, which is an indicator of environmental pollution altering the bioaccumulation of compounds in phytoplankton.

CONCLUSION AND RECOMMENDATION

This study revealed alteration in physicochemical parameters, pigments, biomolecules and slight changes in antioxidant enzyme activities of phytoplankton, with exposure to cefixime and aspirin. Further studies should be conducted to evaluate the influence of different concentrations and mixtures of pharmaceuticals over extended period of time, to understand the pattern of changes in phytoplankton communities. The human community should be educated on the importance of proper sewage disposal to combat the effects of pharmaceuticals on non-target organisms through seminars, workshops, conferences and adult literacy programmes.

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