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The effects of Cefixime and Aspirin on phytoplankton community structure and dynamics: A Mesocosm approach

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Abstract:

Pharmaceuticals are emerging contaminants of interest due to their ability to induce changes in the environment. These chemical compounds are discharged into aquatic ecosystems with a high likelihood of affecting non-target organisms such as phytoplankton. However, there is a gap in knowledge regarding the effects of pharmaceuticals on phytoplankton. This study used the mesocosm approach to investigate the effects of cefixime, a cephalosporin antibiotic and aspirin, a nonsteroidal anti-inflammatory drug on the community structure (species diversity, richness, and abundance) of phytoplankton. The mesocosm approach studied the effects of three treatments of pharmaceuticals: cefixime, aspirin and a combination of cefixime and aspirin, alongside a control experiment for 21 days. A total of 31 phytoplankton species belonging to five groups were identified; with diatoms and green algae having higher diversity compared to the other algae groups. *Scenedesmus sp., Pediastrum sp. and Zygnema sp.* were the top three recurring species in all the samples taken throughout the experimental period. *Navicula sp.* were the most recurring diatoms. The exposure of phytoplankton to cefixime, aspirin, and a combination of cefixime and aspirin significantly reduced the diversity, richness, and abundance of all species by the 21st day of the experiment. The control had the highest cell density (8,910 cells mL) of phytoplankton species on the 21st day, while the cefixime treatment had the lowest cell density (48 cells mL) f phytoplankton species on the seventh day of the study. This study demonstrated that cefixime and aspirin had major impacts on the phytoplankton community.

Keywords: Pharmaceuticals, Phytoplankton, Ecosystem, Toxicology, Mesocosm

1. Introduction:

Pharmaceuticals are defined as chemical compounds used for preventive and therapeutic purposes (Xin *et al*., 2020). The use of pharmaceuticals has increased globally in recent decades and has been a concern for researchers (Ginebreda *et al*., 2010). Pharmaceuticals are often found in aquatic ecosystems worldwide (Osorio *et al*., 2016, Swiacka *et al*., 2022). This can be attributed to their continuous discharge from residential areas, industrial settlements, and wastewater treatment plants (Gomaa *et al*., 2021; Ngqwala and Muchesa, 2020). Due to the biological activity of pharmaceuticals, they have been identified as emerging contaminants, which could be detrimental to non-target aquatic biota (Pinckney *et al*., 2017). Recently, they have been included on the European Union Framework Directive watch list as chemical compounds that have detrimental effects on aquatic organisms (Miller *et al*., 2018).

Pharmaceutical products such as analgesics, antibiotics, antihypertensives and antidepressants used in the treatment of human diseases have been identified as the most prevalent pharmaceuticals in aquatic ecosystems (Swiacka *et al*., 2022). Advancements in science and technology have demonstrated the presence of pharmaceuticals in drinking water and aquatic ecosystems in the range of ng/L-ug/L concentrations (aus der Beek *et al*., 2016; Ding *et al*., 2020). The continuous use and discharge of pharmaceutical products coupled with their low degradation tendency are factors responsible for their pseudo-persistence and bioaccumulation in the environment (Ngqwala and Muchesa, 2020). Aspirin, also known as acetylsalicylic acid (ASA) is a non-steroidal anti-inflammatory drug (NSAID) that is used to reduce fever, pain and/or inflammation and serves as an antithrombotic (Sachs, 2005). Cefixime is a cephalosporin antibiotic sold under the brand name, Suprax (WHO, 2009). It is used in the treatment of bacterial infections such as pneumonia, strep throat, gonorrhoea, urinary tract infections, otitis media and Lyme disease (Grayson, 2017).

Phytoplankton are primary producers in freshwater ecosystems and are major contributors to the food chain. They play important roles in food and oxygen production (Falkowski and Raven, 2013). Research studies have implied that pharmaceutical substances can exert a negative influence on phytoplankton, and higher trophic organisms (Gomaa *et al*., 2020). Scientific studies have demonstrated that phytoplankton species have varying responses to different pharmaceuticals (Grzesiuk *et al*., 2016). It is therefore crucial to study the different pharmaceuticals and their influence on the community structure of phytoplankton.

There have been scientific reports on the impacts of certain NSAIDs (such as Ibuprofen and diclofenac) and antibiotics on the phytoplankton community. However, there is a knowledge gap as regards the roles of aspirin and cefixime in aquatic ecosystems. Thus, this study aims to examine the impact of aspirin and cefixime on the community structure of phytoplankton.

2. Materials and Methods:

2.1. Study Area

The experiment was carried out in a screen house under standard conditions, at the Department of Plant Biology, Osun State University, Osogbo. The materials used include twelve (12) 20 L plastic containers, sample bottles, nitrate, compound microscope, aspirin, cefixime, pond water containing phytoplankton, sediments, and pebbles.

2.2. Mesocosm Set-up

The pond water containing phytoplankton was collected from Owode, Ilesha garage, off riverside hotel bus stop, Osogbo, Osun State with coordinates 7.74299 and 4.57233. 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) litres capacity with fifteen (15) L of pond water containing phytoplankton.

2.3. 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.

2.4. Sample Collection

Water samples were collected on day 0. The samples were collected from the twelve mesocosm set-up using twelve bowls (a bowl for each mesocosm). The bowl was dipped into each mesocosm, and the water

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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 physicochemical parameters. 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 at Ahmadu Bello University for analysis.

2.5. Identification and cell count of phytoplankton

Phytoplankton were identified using the standard keys of Prescott (1964) with the aid of a microscope. Phytoplankton cell count was carried out using the drop count technique (Chia *et al*., 2012).

2.6. Species richness and Species diversity

Species richness is a measure of the number of species in a community, while species diversity is the number of different species that are represented in each community. Species richness is simply a count of species, and it does not consider the abundances of the species or their relative abundance distributions. In this study, species richness was calculated as the number of individual species discovered in each mesocosm while Species diversity was determined using the Shannon diversity index (1948).

The Shannon diversity index is a popular metric used in ecology. The index demonstrates the number of species living in a habitat (richness) and their relative abundance (evenness).

$H = -\Sigma p_i * ln(p_i)$
Where:
H- Shannon diversity index
Pi- the proportion of individuals of species 'i' in the community. Where 'i'= 1
Where: n- Individual of a given species
N- Total number of individual species in a community
∑- sum symbol

3. Results & Discussion:

3.1. Species Diversity and Richness

A total of 31 species belonging to five groups; Bacillariophyta, Chlorophyta, Euglenophyta, Charophyta and Cyanophyta, were identified in the study. Fourteen (14) species were identified in Bacillariophyta including Navicula sp. Bory de Saint-Vincent, Coscinodiscus sp. Ehrenberg, Synedra sp. Ehrenberg, Actinocyclus sp. Ehrenberg, Caloneis bacillum (Grunow) Cleve, Fragilaria sp. Lyngbye, Nitzschia sp. Hassall , Chaetoceros sp. Ehrenberg, Melosira sp. C.Agardh, Achnanthes sp. de Saint-Vincent , Stauroneis sp. Ehrenberg, Cymbella sp. Krammer, Cyclotella sp. (Kütz.) Bréb. and Mastogloia sp. G.H.K.Thwaites. Ten (10) chlorophytes were identified including Scenedesmus sp. Meyen, Pediastrum sp. Meyen, Ankistrodesmus sp. Corda, Dictyosphaerium sp. Nägeli, Sphaerocystis sp. R.Chodat, Crucigenia sp. Morren, Chlorella sp., Beyerinck, Selenastrum sp. Reinsch, Oocystis sp. Naegeli ex A .Braun and Tetraedron trigonum (Nägeli) Hansgirg. Zygnema sp. C. Agardh, Closterium sp Nitzsch ex Ralfs and Micrasterias sp. C. Agardh ex Ralfs are the three desmids identified. Phacus sp. Dujardin and Euglena sp. Ehrenberg are the only euglenophytes. Microcystis sp. Lemmermann and Oscillatoria sp. Vaucher ex Gomont are the only blue-green algae species identified in this study.

The results of the study suggest that the aspirin and cefixime treatments influence the diversity indices and species richness of phytoplankton communities (figure 1). The control treatment has the highest diversity, followed by the cefixime treatment, then the aspirin treatment (figure 2). The cefixime and aspirin treatment have the lowest diversity.

The inverse Simpson, Simpson's Index, and Pielou's Evenness values decrease as the treatment becomes more toxic. This indicates that the aspirin and cefixime treatments have a negative impact on the diversity of phytoplankton communities. The species richness values decrease as the treatment becomes more toxic, but they do not decrease as much as the other diversity measures. This indicates that the aspirin and cefixime treatments have a negative influence on the abundance of the phytoplankton species but with a greater effect on the abundance of less abundant species.

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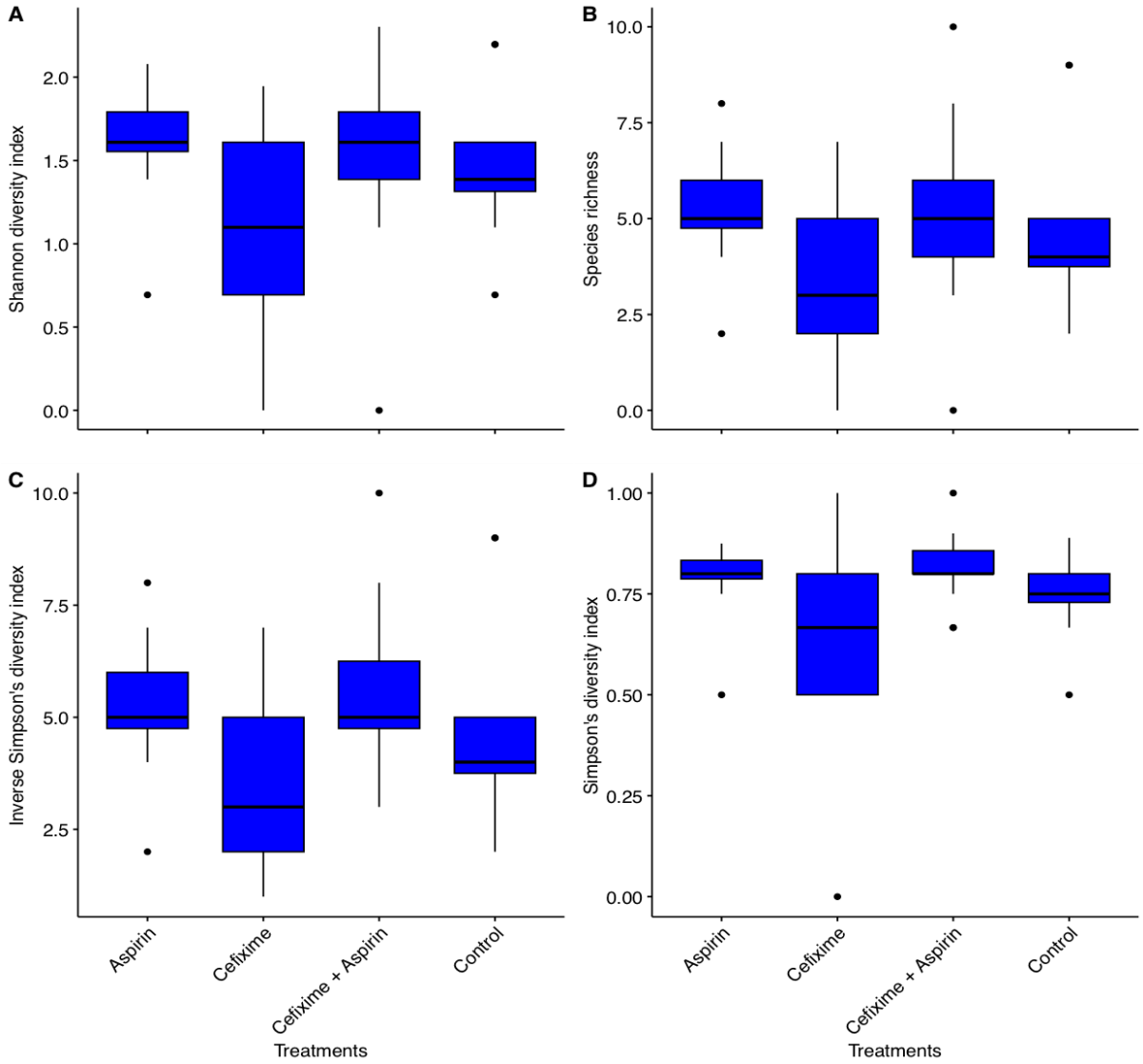

Figure 1 (A) Shannon diversity index (B) Species richness (C) Inverse Simpson's diversity index (D) Simpson's diversity index; of phytoplankton species exposed to aspirin and cefixime treatments for 21 days

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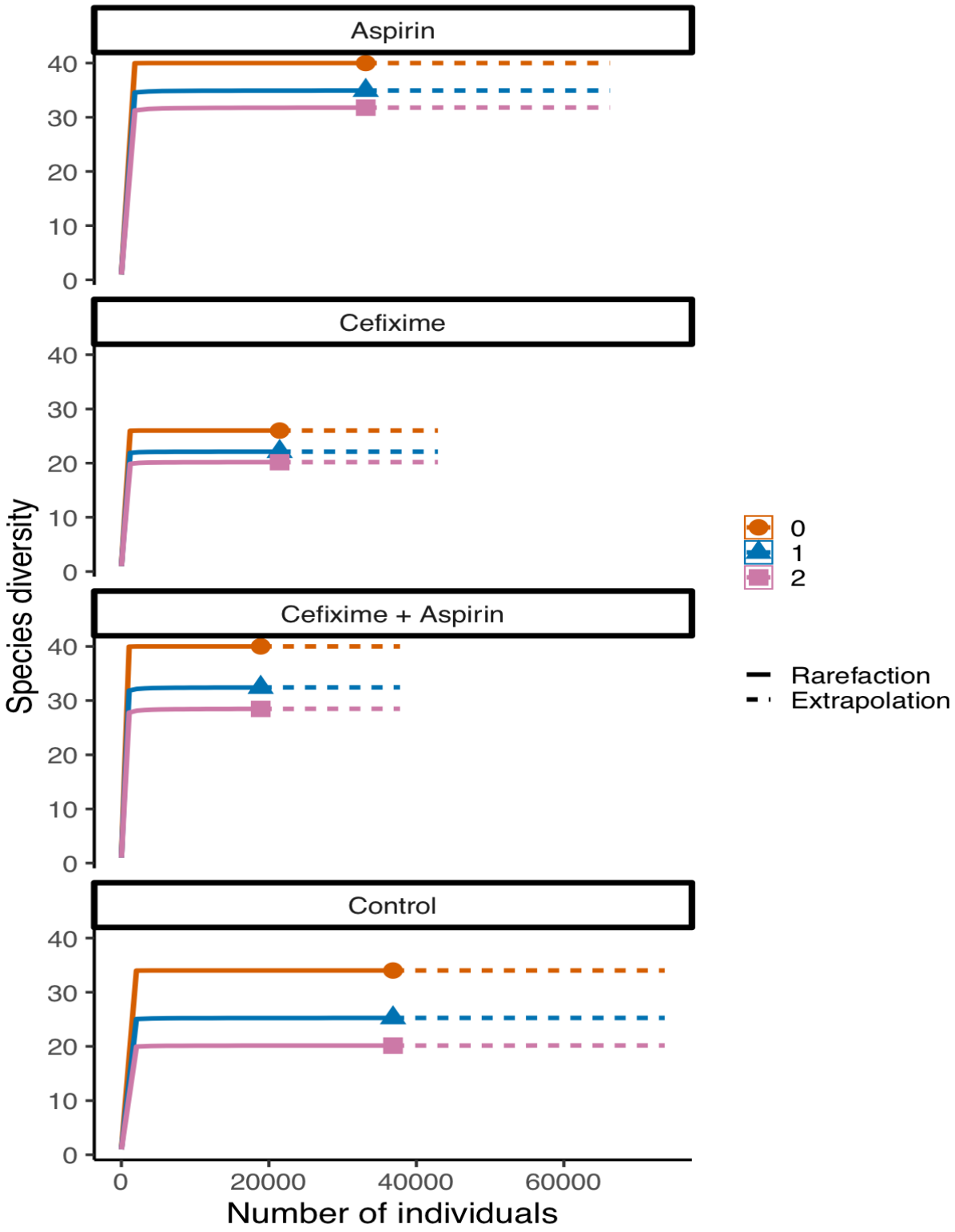

Figure 2 Species diversity of phytoplankton exposed to aspirin and cefixime treatments for 21 days

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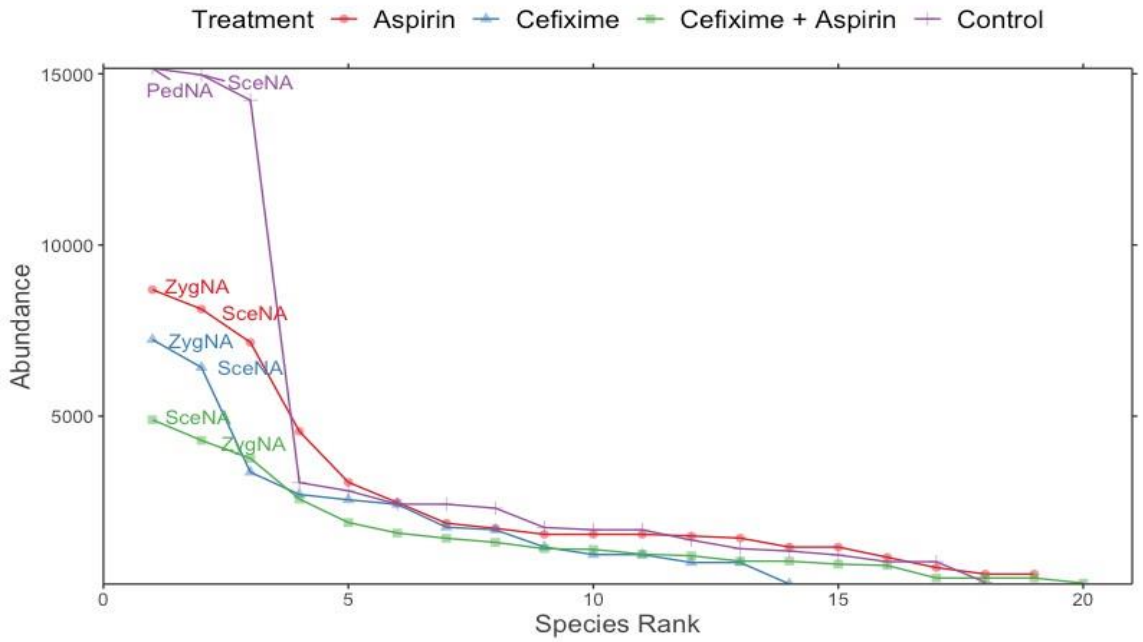

Figure 3 Rank abundance of phytoplankton species exposed to cefixime and aspirin for 21 days

3.2. Rank Abundance

The rank abundance curves are represented by figure 3. The rank abundance curves for the three treatments are similar in shape, with a few notable exceptions. The rank abundance curve for the control treatment is slightly higher than the curves for the cefixime and aspirin treatment at the beginning of the curve. This suggests that there are more abundant species in the control treatment than in the other two treatments.

The rank abundance curves for the aspirin and cefixime treatments are similar, but they are lower than the rank abundance curve for the control treatment. This suggests that the aspirin and cefixime treatments have negative effect on the abundance of phytoplankton species. The rank abundance curves for the aspirin and cefixime treatments cross at rank 10. This suggests that the two treatments have a similar effect on the abundance of the top 10 most abundant phytoplankton species. However, the aspirin treatment has a greater effect on the abundance of less abundant phytoplankton species. Overall, the results suggest that the aspirin and cefixime treatments have negative effects on the abundance of phytoplankton species.

3.3. Whitaker Beta Diversity

Table 1 represents the Whitaker beta diversity of the phytoplankton community exposed to different treatments. Whitaker beta diversity is a measure of the similarity between two communities. A high value indicates that the communities are similar, while a low value indicates that the communities are different. In this case, the table shows that the phytoplankton community exposed to aspirin is the most different from the control. The phytoplankton community exposed to cefixime is different from the control, but not as different as the phytoplankton community exposed to aspirin. The phytoplankton community exposed to cefixime, and aspirin is intermediate between the other two communities.

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Table 1: Whitaker beta diversity of phytoplankton community exposed to different treatments.

	Aspirin	Cefixime	Cefixime + Aspirin	Control
Aspirin	0.00000000	0.39393939	0.28205128	0.35135135
Cefixime	0.39393939	0.00000000	0.47058824	0.37500000
Cefixime + Aspirin	0.28205128	0.47058824	0.00000000	0.36842105
Control	0.35135135	0.37500000	0.36842105	0.00000000

3.4 Discussion

Phytoplankton play important roles in nutrient cycling and oxygen production (Falkowski and Raven, 2013; Schiermeier, 2010). A shift in their population can disrupt the balance of the entire ecosystem. Research studies have shown that APIs (Active Pharmaceutical Ingredients) have detrimental effects on phytoplankton, which exert influence on higher trophic organisms (DeLorenzo and Fleming, 2008). Phytoplankton have receptors and metabolic pathways that are like bacteria which makes them susceptible to pharmaceuticals (Guo *et al.*, 2016). Several factors such as nutrient availability, physicochemical parameters and sensitivity to APIs are major contributors to phytoplankton community structure (Litchman and Klaumeier, 2008). According to Gomaa *et al.* (2021), pharmaceuticals affect the structure and diversity of phytoplankton with different responses from different taxa of organisms. Pharmaceuticals can exert synergistic, additive, or antagonistic effects on phytoplankton community (DeLorenzo and Fleming, 2008). Chia *et al.* (2021) stated that pharmaceuticals could lead to decrease in abundance and diversity of organisms.

Eukaryotic algal groups such as Chlorophyta and Bacillariophyta generally dominate other phytoplankton groups of ecosystems exposed to pharmaceuticals (Porsbring *et al.*, 2009). This is confirmed in this study, where diatoms and green algae contributed to more than 70% of the total species composition. The study by Duarte *et al*. (2023) also Observed a high composition of diatoms and green algae in the treatments with sulfamethoxazole and Diclofenac. Green algae are abundant in aquatic ecosystems with varying responses to nutrient concentrations, nutrient, light availability, and environmental factors (Kruk and Segura, 2012). In this study, *Scenedesmus* and *Pediastrum* contribute majorly to the abundance of the chlorophyta division. However, the richness, diversity and abundance of phytoplankton species were decreased with aspirin and cefixime treatments. Duarte *et al.* (2023) observed that the Genera *Closteripsis* and *Desmodesmus* had highest abundance at lower concentrations of sulfamethoxazole and diclofenac. Diatoms possess silica in their cell wall. Silica is the second most abundant element on earth and polymerization of monosilic acid into silica is a process that consumes less energy in diatoms. Therefore, diatoms can save energy in cell wall synthesis, while channeling it to other cellular activities such as growth (Martin-Jezequel *et al*., 2000). There was an increase in the diversity of diatoms over days of exposure to treatments, but an overall decrease in abundance by the end of the experiment. *Navicula sp* were the most recurring of the diatoms, which may be attributed to their higher survival ability compared to the other species.

Studies reveal that APIs, even at low concentrations, reduce the population of cyanobacteria (Azevedo *et al.*, 2019). Taskan *et al.* (2016) demonstrated that populations of blue-green algae were reduced with increasing concentrations of tetracycline. This study noted only two species of cyanobacteria. Duarte *et al*. (2023) reported that cyanobacteria were most

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abundant at high and intermediate concentrations of diclofenac. *Zygnema spp* were the most abundant of the desmids and were recurrent in almost all treatments of aspirin and cefixime on different days of exposure. This may suggest that they are tolerant species with the capacity to survive polluted environments. Duarte *et al*., (2023) noted that desmids had the highest abundance with low and intermediate concentrations of both diclofenac and sulfamethoxazole. Euglenophyta was represented by only two genera in this study, with *Phacus sp* being the most abundant. This may be attributed to the ability of *Phacus sp* to form mucilaginous walls for protection under unfavorable environmental conditions. The exposure of phytoplankton community to pharmaceuticals modifies their adaptability to the environment, therefore exerting a negative impact on the richness, diversity, and abundance of species in the community. This is subject to the interplay between the response of different phytoplankton species to stress and physicochemical parameters of the ecosystem.

4. Conclusion:

This study demonstrates a decrease in diversity, richness and abundance of phytoplankton species when exposed to aspirin and cefixime treatments. Further studies should be conducted to determine the individual and compound effects of pharmaceuticals belonging to different classes on phytoplankton communities.

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