COMPARATIVE ASSESSMENT OF BREWERY EFFLUENT USING MARINE MICROALGAE

BY

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LSC1705888

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY
FACULTY OF LIFE SCIENCES
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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE UNIVERSITY OF BENIN, BENIN CITY, AWARD OF BACHELOR OF SCIENCE (B.Sc HONS) DEGREE

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CERTIFICATION

This is to certify that this project work was carried out by Happiness Chimakpa MBAGWU (Miss) of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City.

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External Examiner Date

DEDICATION

This project work is dedicated to Almighty God.

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I have taken efforts in this project. However, it would not have been possible without the kind support of many individuals including lecturers and students.

Firstly, my utmost appreciation goes to God almighty for his abundant grace in my life all through my years in school and also for seeing me through this project work.

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ABSTRACT

A study on the effect of brewery effluent on two microalgae *Thalassiosira sp.* and *Chaetoceros gracilis* was carried out for fourteen (14) days. The experiment was done in triplicate using concentrations 0% (control), 5%, 10%, 15%, 20%, 25% and 30% of brewery effluent. Growth responses were measured using a visible spectrophotometer at 750nm and some physicochemical parameters were analyzed before and after the experiment using standard methods. Descriptive statistics, inferential (one way analysis of variance) ANOVA repeated measures, paired t-test and Tukey test were done using Microsoft excel 2010 and statistical package for social sciences SPSS20. The results showed that there was a significant difference ($p < 0.05$) in the growth response of both microalgae. Different concentrations of brewery effluent affected the growth of *Thalassiosira sp.* and *Chaetoceros gracilis*. Higher concentrations (20%, 25% and 30%) had more stimulatory effect than lower concentrations (5%, 10% and 15%) when *Thalassiosira sp.* was used. *Chaetoceros gracilis* grew best in lower concentrations of 10%, 15% and 20%. A decrease in temperature was observed in all treatments while TOC and COD decreased at lower concentrations of brewery effluent then remained constant at higher concentrations.

CHAPTER ONE

INTRODUCTION

Brewery industries, is a vital part of the producing country's economy, it consume large volumes of water during the production processes, and later release about 70% of it as wastewater (Sun *et al.,* 2016). Wastewater byproducts such as yeast surplus, spent grains, produced from two main beer production stages (brewing and packaging) are the main contributors to environmental pollution when mixed with effluent. Furthermore, cleaning of floors, bottles, tanks and machines also contribute to the contamination of water bodies. Brewery wastewater may be discharged either directly into: municipal sewers, water bodies, or the brewery's wastewater treatment plant and water bodies/municipal sewer system after pretreatment. Discharge of untreated/partially treated brewery wastewater into water bodies raises environmental concerns. The major environmental concerns raised by the operation of breweries include water consumption, wastewater, solid waste and by-product generation, energy use and emissions to air (Capodaglio, 2020). This leads to environmental problems such as water scarcity, excessive growth of undesirable microbes that cause loss of aquatic life forms and health-related problems in communities around the discharge areas. There is therefore a need for brewery industries to adequately treat and manage their wastewaters before their final discharge into the environment.

Conventional treatment methods; though extensively used in brewery wastewater treatment, usually generate huge amounts of sludge (Shahid *et al.,* 2020). Conventional treatment methods are also characterised by high operation and maintenance costs, which further makes them economically unfeasible. Also, the excessive use of chemicals may cause ecological imbalances. The use of microalgae, is environmentally friendly and cost effective water treatment method, and has been identified as a way to address these problems (Mohd-Udaiyappan *et al.,* 2017), because, microalgae have the potential to efficiently remove organic loads from wastewater, and provide a useful biomass byproduct. Currently, algae wastewater treatment and its biomass use are attracting attention worldwide.

Brewery wastewater is usually voluminous with high moisture content. This wastewater usually has high chemical oxygen demand (COD) and BOD due to the presence of organic components (sugars, soluble starch, ethanol, volatile fatty acids). The temperature of brewery wastewater usually ranges from 25 ^{o}C to 38 ^{o}C. Its pH levels are variable and dependent on the amount and type of chemicals used in cleaning and sanitizing (e.g., caustic soda, phosphoric acid, nitric acid, etc.). Nitrogen (N) and phosphorus (P) levels present are also dependent on the handling of raw material and the amount of yeast present in the effluent (Nie *et al.,* 2020).

Wastewater could contain several compounds at elevated concentrations that inhibit biological (e.g., microalgal) growth (Muñoz and Guieysse, 2006). Similarly, the turbidity and pH of wastewater could inhibit microalgal growth. Physicochemical pretreatment of wastewater would improve the conditions for microalgal growth and reduce wastewater's strength. Muñoz and Guieysse (2006) adopted a three-step strategy for efficient treatment of textile industry water (TWW): adsorption of toxic compounds by granular activated carbon, followed by anaerobic digestion to produce electricity and reduce the load of the TWW before cultivating microalgae in the partially treated TWW. Although ammonium is the most preferred nitrogen source for microalgal growth, the ammonium tolerance limit for microalgal strains was reported as high as 1000 µmol NH4-N L−1. A pretreatment would be required to reduce the ammonium concentration in wastewater, or wastewater should be diluted to a tolerable limit where the selected strain could grow efficiently (Sutherland and Ralph, 2019). For several wastewater types (e.g., metal, mining, paper, oil, and grease), electrocoagulation was used as a pretreatment step, which could effectively remove various chemical additives, turbidity, pathogens. Microalgal biomass cultivation in such pretreated wastewater could also reduce residual nutrients and turbidity from the treated wastewater.

High concentrations of heavy metals in wastewater could inhibit microalgal photosynthesis. Nevertheless, microalgae efficiently concentrate the metal pollutants both internally and externally and could be adopted for metal removal from wastewaters (Ansari *et al.*, 2017). Microalgal cells need several metals (i.e., Fe, Mn, Cu, Co, Zn, and Mo) in trace amounts as their growth requirements. However, microalgae are also capable of concentrating on various heavy metals (e.g., Cd, Hg, Ni, Zn, Fe, Cu, Pb, Cr, etc.) through different mechanisms.

1.1 Current Brewery Wastewater Treatment Approaches

Brewery wastewater is characterized by dark brown color, TSS, TS, etc. that requires pretreatment to minimize suspended particles and other organic loads. Generally, the brewery wastewater pretreatment process is meant to change the physical, chemical and or the biological properties of the feed water (Wollmann *et al.*, 2019). The pretreatment process is carried out by physical, chemical or biological means or combinations of two or more methods. However, the selection of pretreatment method largely depends on the final discharge point of the effluent. For example, in a situation where the brewery does not discharge into the municipal drain, only primary and secondary treatments are required, but if the brewery is allowed to discharge into the municipal drain, pretreatment is required to reduce the organic loads of the municipal treatment plant and to also meet municipal wastewater treatment bylaws (Miazek *et al.*, 2015). However, municipalities at times impose higher sewer discharge fees on the effluent volumes as well as organic.

1.2 Physical Treatment

Physical wastewater treatment has been used generally to reduce suspended solids from wastewater through sedimentation by gravitational force. The process also separates materials such as grease and oil from the effluent. However, physical treatment methods only remove solid coarse materials but do not degrade pollutants. According to Ansari *et al.* (2017) screening, flow equalization mixing, flotation and sedimentation are the physical methods currently used in effluent treatment. Flow equalization is a technique used to consolidate wastewater effluent in holding tanks for equalization before the wastewater is introduced into downstream treatment processes. Usually, physical treatment serves as a pretreatment stage of brewery wastewater treatment.

1.3 Chemical Treatment

Chemical treatment processes involve pH adjustment or coagulation/flocculation by adding different chemicals to the effluent to alter its chemistry (Arbib and Garridope, 2013). Coagulation-flocculation is the first treatment step in the chemical wastewater treatment method. Flocculation involves stirring/agitation of chemically-treated effluent to induce coagulation that improves sedimentation performance by increasing particle size, thereby increasing settling efficiency. Inorganic coagulants such as aluminum sulfate and ferric chloride have been widely applied in wastewater treatment. During this treatment process wastewater, organic compounds are oxidized via the addition of chemical compounds like chlorine, ozone-oxygen, or permanganate to generate CO_2, H_2O and other inoffensive materials (Gupta *et al.*, 2016).

Chemical flocculants are highly efficient but are dangerous to human health and the environment. The wastewater pH needs to be maintained between 6 and 9 in order to protect the microorganisms (bacteria) present. Usually, neutralization of wastewater pH using H_2SO_4 and HCl is not recommended due to their corrosive nature and the discharge limitation of sulfate and chloride. However, the waste CO_2 could be utilized as an acidifying agent to decrease alkalinity (high pH) of wastewaters before the anaerobic digestion. The *Detarium microcarpum* is reported to be an effective bio-coagulant for removal of turbidity from brewery effluent. Ferreira *et al.* (2017), conducted a study on optimizing bio-coagulants for brewery wastewater treatment using response surface methodology. The method was used to evaluate the effects and the interactioin of three factors i.e., coagulants dosage, pH and the stirring time for solid particle removal on the treatment efficiency using *Detarium microcarpum* seed powder (DMSP) and oyster dried shell powder (ODSP) as coagulants. The results demonstrated the optimum conditions for coagulant dosage (100.53 mg/L), effluent pH (2.001) and stirring time (24.47 min) with 90.44% solid particle (SP) removal for DMSP and coagulant dosage (104.19 mg/L), pH (3.34) and stirring time (27.54) with 96.55% SP removal for ODSP (Farooq *et al.*, 2013).

1.4 Biological Treatment Approaches

Brewery effluent requires efficient treatment methods that can break down the organic loads in the wastewater. The effluent is passed through both anaerobic (using aerobic bacteria) and aerobic (using activated sludge) digestion processes aiming to reduce effluent's COD before its discharge into the municipal sewer (Dickinson *et al.*, 2013).

1.5 Anaerobic Treatment

Anaerobic digestion is a natural process in which various microbial species work together in the absence of oxygen to transform organic wastes through a variety of intermediates into biogas (Darpito *et al.*, 2014). This treatment method is widely used in brewery because the generated biogas may be used to maintain operational temperature or to generate revenue. Anaerobic digestion, however, is influenced by a number of factors, including nutrients, organic loads, carbon/nitrogen ratio, temperature and pH of the wastewater. An optimum temperature is required by anaerobic bacteria to effectively digest organic pollutants. Usually, most commercial anaerobic plants operate in the mesophilic range (Chaudhary *et al.,* 2017). However, the working bacteria are classified based on their optimum pH range. Acidogenic and methanogenic bacteria work perfectly at optimum pH values of less than 6.0 and 7–8, respectively.

1.6 Aerobic Treatment

The aerobic treatment method takes place in the presence of oxygen by aerobic microorganisms (bacteria) that metabolize organic matter in the wastewater. They produce more microorganisms and inorganic end-products such as carbon dioxide, ammonia and water. Aerobic processes are more efficient in the digestion of pollutants (Choi, 2016). In the aerobic biological treatment process, microorganisms convert non-settleable to settleable solids, followed by sedimentation which allows the settleable solids to settle and separate out. The widely used aerobic treatment method in wastewater treatment include: (1) activated sludge process, (2) attached growth (biofilm) process (3) trickling filter process. However, the choice of aerobic treatment methods strongly depends on the strength of pollutants in the effluent (Hu *et al.,* 2019). Usually activated sludge and trickling filter processes are used in brewery wastewater treatment due to the strong organic pollutants in the effluent. Activated sludge processes are mostly used in wastewater treatment. In this process, the wastewater flows into an aerated agitated tank that is primed with activated sludge. The suspension of aerobic bacteria in the aeration tank is mixed vigorously by aeration devices, which supply oxygen to the biological suspension (Kwon *et al.,* 2020). In the attached growth process, the aerobic biological process creates a favorable environment for microorganisms that desire to attach to the solid surface. During the trickling filter process, effluent from the brewery is sprayed on the surface of solid materials like gravel, stone/plastics, and these materials allow the effluent to trickle down via decomposed microorganism media. Application of both anaerobic and aerobic processes in wastewater treatment is associated with huge capital cost (Lee and Lee, 2001). However the operational cost of the anaerobic process is comparatively lower than that of the aerobic treatment. Aerobic processes are also hindered by the physical and chemical variation of the wastewater, high cost of treatment and the formation of excessive sludge by the microorganisms.

1.7 Microalgae

Microalgae are microscopic algae, typically found in freshwater and marine systems, living in both the water column and sediment. They are unicellular species which exist individually, or in chains or groups (Raposo *et al.,* 2010). Depending on the species, their sizes can range from a few micrometers (μm) to a few hundred micrometers. Unlike higher plants, microalgae do not have roots, stems, or leaves. They are specially adapted to an environment dominated by viscous forces. Microalgae, capable of performing photosynthesis, are important for life on earth; they produce approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow photoautotrophically (Rasoul-amini *et al.,* 2014). Microalgae, together with bacteria, form the base of the food web and provide energy for all the trophic levels above them. Microalgae biomass is often measured with chlorophyll a concentrations and can provide a useful index of potential production. The biodiversity of microalgae is enormous and they represent an almost untapped resource. It has been estimated that about 200,000-800,000 species in many different genera exist of which about 50,000 species are described (Marchão *et al.,* 2018). Over 15,000 novel compounds originating from algal biomass have been chemically determined. Most of these microalgae species produce unique products like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols.

1.8 *Thalassiosira* sp.

Thalassiosira is a genus of centric diatoms, comprising over 100 marine and freshwater species. It is a diverse group of photosynthetic eukaryotes that make up a vital part of marine and freshwater ecosystems, in which they are key primary producers and essential for carbon cycling (Martinez *et al.,* 2000). *Thalassiosira* is a diverse genus, however one species within the genus, *T. pseudonana,* has gained particular significance as the first marine phytoplankton to have its genome sequenced. *T. pseudonana* has since become a key model organism for studying diatom physiology. The *T. pseudonana* genome revealed novel genes for intracellular trafficking and metabolism in diatoms. This species was again used to develop methods for genetic manipulation of diatoms and for the study of silica biomineralization (Rawat *et al.,* 2011).

Thalassiosira sp. comes in a variety of shapes, from box-shaped to cylindrical, discoid or spherical. Some *Thalassiosira* cells are found alone while others form chains. *Thalassiosira* have a cell wall made of silica, known as a frustule. *Thalasiosira* harbor several discoid plastids and a circular valve, which contains pores arranged in rows or arcs, opening outwards. The valve's mantle edge is pattered with a series of bands (Tripathi *et al.,* 2019). Different species of *Thalassiosira* can be identified by the morphological characteristics of their areolae and the processes on the valve. During colony formation, *Thalassiosira* release chitin filaments through strutted processes known as fultoportulae. By extruding chitin fibers, and thereby increasing drag, *Thalassiosira* can slow the rate at which they sink.

1.9 Habitat and Ecology

Thalassiosira occupy diverse habitats, both marine and freshwater. Of note, they are a vital primary producers in temperate and polar regions. *Thalassiosira* can thrive in low temperature and light, as well as mixed waters, and are therefore a large part of diatom blooms during spring in temperate regions, such as Canadian and Alaskan waters (Whitton *et al.*, 2016). Species in this genus are also capable of assembling defensive threads against zooplankton, allowing them to survive the predation that normally keeps phytoplankton blooms in check. *Thalassiosira* species are diverse in both their ecology and physiologies, with variable mechanisms for nitrogen storage or requirements for iron. Iron concentrations, temperature and macronutrient availability have been identified as important factors for the composition of *Thalassiosira* species communities in marine waters (Sun *et al.*, 2016).

1.10 Life cycle of *Thalassiosira*

Thalassiosira can undergo both asexual and sexual reproduction in processes shared by other diatoms. During asexual reproduction, the parent cell divides into two daughter cells of unequal size one equal in size to the parent and one smaller. This constraint on size during mitotic division is due to the presence of the rigid silica cell wall (Li *et al.,* 2019). As a result, over multiple cell divisions, the cells size of each daughter cell will decrease. To cope with diminishing cell size, *Thalassiosira* can transition to sexual reproduction, which is triggered by an assortment of environmental factors, which are not well understood, once cells reach a critically small size (Capodaglio, 2020). In sexual reproduction, sperm and egg, which can arise from the same cell, fuse to form a diploid zygote, which is referred to as an auxospore. This progeny can then emerge from the parental frustule (silica wall) and reconstruct its own cell wall, thus becoming a cell of a larger size.

1.11 *Chaetoceros gracilis.*

Chaetoceros is probably the largest genus of marine planktonic diatoms with approximately 400 species described. It is often very difficult to distinguish between different *Chaetoceros* species. Several attempts have been made to restructure this large genus into subgenera and this work is still in progress (Shahid *et al.,* 2020). However, most of the effort to describe species has been focused in boreal areas, and the genus is cosmopolitan, so there are probably many tropical species still undescribed. Some species are known from the fossil record, from the Quaternary of Sweden. It is the type genus of its family.

Chaetoceros is primarily a marine genus, but there are also accounts of species within inland waters of the United States. It is a type of centric diatom that contains a frustrule or cell wall composed of silica that contain long, thin spines (setae). The spines connect the frustules together creating a colony of cells. Cells colonies can form chains that are coiled, straight, or curved. Cell size can range from <10 um to 50 um (Mohd-Udaiyappan *et al.*, 2017). Due to its high growth rates and high lipid concentrations, research has been conducted to potentially use *Chaetoceros* as a biofuel. Studies suggest that colonies of *Chaetoceros* serve as an important food source within the water column and major carbon contributor to the benthic environment. Within the North Water, located in northern Baffin Bay, *Chaetoceros* has been reported to contribute about 91% of total phytoplankton cells serving as an important primary producer within this area (Nie *et al.*, 2020). Therefore, contributing to oxygen production in the North Water. Overall, phytoplankton contributes to over half of Earth's oxygen production. Due to its high growth rates and high lipid concentrations, research has been conducted to potentially use *Chaetoceros* as a biofuel. Studies suggest that colonies of *Chaetoceros* serve as an important food source within the water column and major carbon contributor to the benthic environment. Within the North Water, located in northern Baffin Bay, *Chaetoceros* has been reported to contribute about 91% of total phytoplankton cells serving as an important primary producer within this area (Sutherland and Ralph, 2019).

1.12 Literature Review

Hu *et al.* (2019) investigated the removal of sulfamethoxazole (SMX) and ofloxacin (OFX) with *Chaetoceros muelleri*, a marine diatom and find out. The optimization process was conducted using response surface methodology (RSM) with two independent parameters, i.e., the initial concentration of antibiotics and contact time. The optimum removal of SMX and OFX were 39.8% (0.19 mg L-1) and 42.5% (0.21 mg L-1) at the initial concentration (0.5 mg L-1) and contact time (6.3 days). Apart from that, the toxicity effect of antibiotics on the diatom was monitored in different SMX and OFX concentrations (0 to 50 mg L-1). The protein (mg L-1) and carotenoid (µg L-1) content increased when the antibiotic concentration increased up to 20 mg L-1, while cell viability was not significantly affected up to 20 mg L-1 of antibiotic concentration. Protein content, carotenoid, and cell viability decreased during high antibiotic concentrations (more than 20 to 30 mg L-1).

Lee and Lee (2001) carried out a study to test the efficiency of additions of secondary sewage as a culture medium for *Chaetoceros gracilis* and *Thalassiosira* sp. (Chrysophyceae) under laboratory conditions. These algae were cultivated in sea water with concentrations of 10%, 20%, 30% and 40% of wastewater. The results were compared with those obtained by the nutritive medium f2 of Guillard (1975). The best results in terms of cellular densities were observed at 40% additions. There were significant differences (significance levels of 5%) between the nutritive medium f2 and the 40% additions for both the species. Maximum cellular densities observed for all additions tested were, 4,125.00 x 10^3 cells/ml for *Chaetoceros gracilis* on the ninth day and 834.00 x 10^3 cells/ml for *Thalassiosira* sp on the fifth day. Biomass was higher in the nutritive medium f2 than in the other treatments, reaching average values of 2,363µg/ml for *Chaetoceros gracilis*. At all experimental units, the best results were registered at 40% addition for *Chaetoceros gracilis*, where average values of 0.768µg/ml were observed on the fifth day, and at 30% additions for *Thalassiosira* sp where 0.883µg/ml were observed on the thirteenth day.

Whitton *et al.* (2016) stated that their study evaluated the biomass and lipid production of *Chlorella vulgaris* and its nutrient removal capability for treatment of brewery wastewater effluent. The results indicate that the maximum biochemical oxygen demand (BOD) (91.43%) and chemical oxygen demand (COD) (83.11%) were removed by *C. vulgaris* with aeration in the absence of light. A maximum of 0.917 g/L of dry biomass was obtained with aeration in the dark conditions, which also demonstrated the highest amount of unsaturated fatty acids at 83.22%. However, the removal of total nitrogen (TN) and total phosphorus (TP) with these aeration and light conditions was 9.7% and 11.86% greater than that of other conditions. The removal of BOD and COD and the production of biomass and lipids with aeration in the dark and the TN and TP removal with aeration and light were more effective than other conditions in the brewery wastewater effluent in the presence of *C. vulgaris*.

Marchão *et al.,* (2018) investigated the interaction between algae growth and pH, the effect of aeration on algae growth and pH, the effect of initial inoculation on algae growth, the relationship between pH and bubbling with air containing 5% CO_2, and algae growth in beer wastewater comparing with BG11 for different periods. It was concluded that: (1) *Scenedesmus obliquus* FACHB-276 can adapt to a wide pH range; (2) Aeration level affected the pH and algae growth; (3) Non-aseptic culture was possible with suitable initial algae inoculation to establish their dominance; (4) Beer wastewater slowed down the pH change when CO_2 was injected to the water; (5) CO_2 injection promoted algae growth in beer wastewater; (6) Algae could accumulate lipid in the algae cells grown in the beer wastewater but not in BG11. Based on this study, we believe that it is possible to build an algae based system coupling beer wastewater treatment and CO_2 sequestration with algae biomass production.

Arad and Spharim (2008) determined the effect of two heavy metals (Cu and Zn) on the photosynthetic pigments of chlorophyll *a, b* and the carotenoids found in the cells of algae *Chlorella vulgaris*. In the treatment with a concentration of 0.15 mg $CuSO_4/dm^3$, a decrease in the content of chlorophyll was observed, which was lower by 63% in comparison to the control sample, 7 days after incubation was observed. In the second study using zinc(II) sulphate at a concentration of 100 mg $ZnSO_4/dm^3$, the death of *Chlorella vulgaris* was observed after 5 days of incubation.

A report of Perez *et al.* (2010) stated the effect of mixed petroleum products (diesel, kerosene and petrol) and heavy metal, on two test microalgae: *Desmodesmus quadricauda* and *Eudorina elegans* was conducted in the laboratory for 14 days using various concentrations (0%, 10%, 25%, 50%, 75% and 100%). The growth response was measured spectrophotometrically using optical density at 680 nm. A general assessment indicates that both microalgae used were impaired by the high concentrations of WSF of these fuel oils, while lower concentrations stimulated growth. For *Eudorina elegans*, maximum growth was obtained at 10% in all fuel oils except kerosene, where maximum growth was obtained at 25%.

Neff *et al.* (2006) reported that lead, kerosene, petrol, diesel and furnace oil administered into the culture suspension of *Anabaena doliolum* as whole oil or aqueous extract exerted concentration dependent toxic effects. The hierarchy of toxicity of the test substances was diesel > furnace oil > petrol > kerosene > lead. The oils rich in aromatics were most toxic and therefore estimation of this fraction might enable prediction of toxicity of oil. In case of crude and kerosene, the whole oil application was more inhibitory than their respective aqueous extracts, whereas reverse trend was obtained in case of other oils.

Phatarpekar and Ansari (2000) report the effects of the water soluble fractions (WSF) of diesel and nickel (Ni) on the growth responses of a marine microalgae, *Chaetoceros calcitrans* studied in the laboratory for a short-term period of 96 hours revealed differential growth responses at comparatively lower concentrations (5, 10, 20 and 40%), though higher concentration (80%) was found to suppress the growth effectively.

1.13 Aims and Objectives

This study hopes to determine the bioremediation potentials of *Thalassiosira* sp. and *Chaetoceros* sp. on brewery wastewater.

The specific objectives of the study were to:

i. determine the effect of different concentrations of brewery wastewater on Thalassiosira sp. and *Chaetoceros gracilis*

ii. evaluate the bioremediation potential of *Thalassiosira* sp. and *Chaetoceros* gracilis.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Test Microalgae:

The test microalgae used in the experiment are *Thalassiosira* sp. and *Chaetoceros gracilis*.

2.1.1 Microalgae sample collection:

The marine microalgae sample was imported from Carolina Biological Supply Company, a science supply company located in North Carolina, United State of America.

2.1.2 Effluent sample collection:

The effluent was collected from International Breweries PLC, Head bridge industrial layout, SABmiller drive, Onitsha, Anambra State.

2.1.3 TAXONOMY

Taxonomy of Thalassiosira

Kingdom: Chromista

Division: Bacillariophyta

Class: Bacillariophyceae

Subclass: Coscinodiscophycidae

Order: Thalassiosirales

Family: Thalassiosiraceae

Genus: Thalassiosira

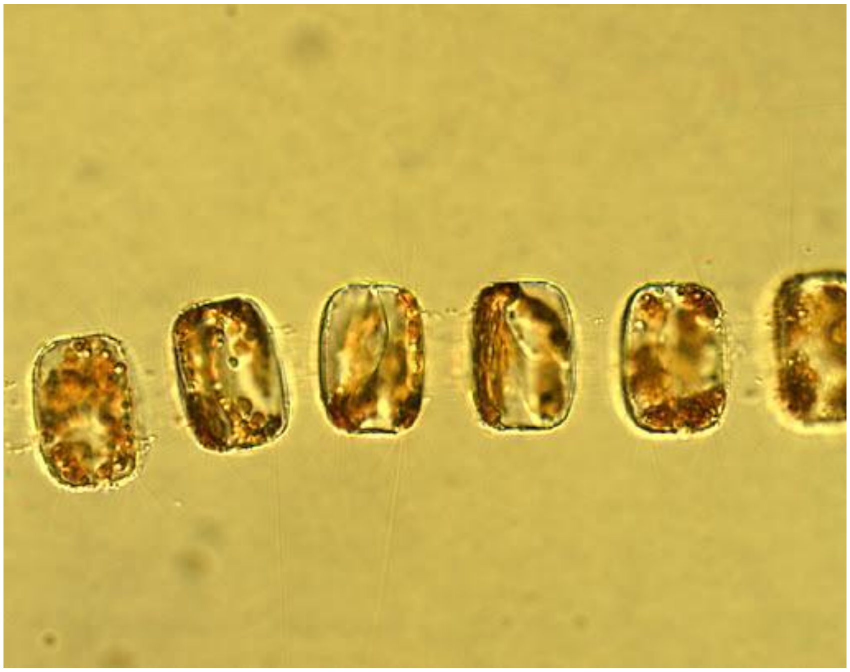

Plate 1: *Thalassiosira* sp.

Taxonomy of Chaetoceros

Kingdom: Chromista

Division: Bacillariophyta

Subdivision: Bacillariophytina

Class: Mediophyceae

Subclass: Chaetocerotophycidae

Order: Chaetocerotales

Family: Chaetocerotaceae

Genus: Chaetoceros

Species: gracilis

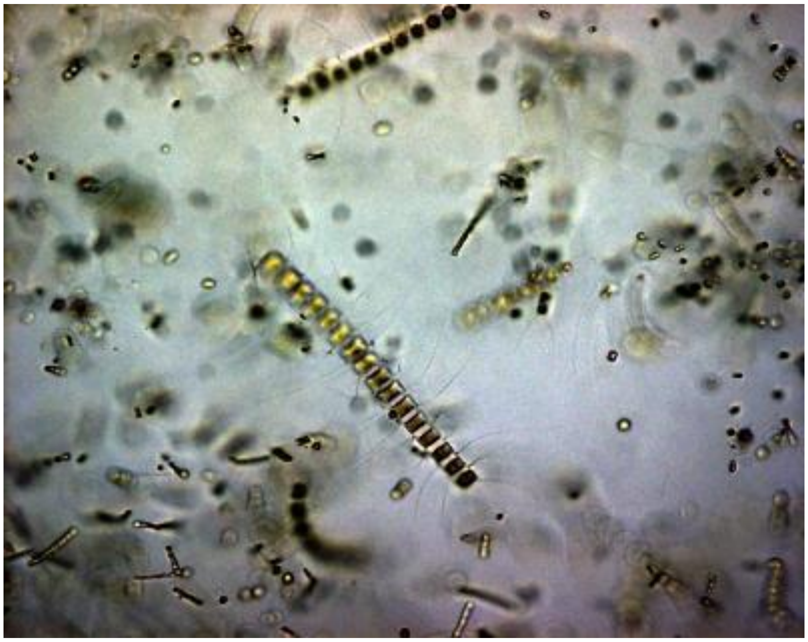

Plate 2: *Chaetoceros gracilis*

2.2 Culture Vessels:

In this research, 500ml flat bottomed glass bottles were used as the culture vessels for the experiment. They were washed properly with detergent at first, and further acid-washed with diluted Hydrochloric acid solution in order to remove any impurities and contaminants that were present. The laboratory work table was sterilized using cotton wool and acetone to wipe the surface before the vessels were placed on it. The culture vessels were covered with cotton wool to prevent contamination by microorganisms.

Plate 3: Culture vessels used for the experiment.

2.3 Culture Medium:

The culture medium used in this experiment was F/2 medium. The constituent of the F/2 medium was defined by Guillard and Ryther (1962); Guillard (1975). The medium is suitable for marine algae as it contains enriched seawater medium for growing marine algae. The preparation of F/2 growth medium was done using 950ml of filtered natural seawater which was added to the following components shown in table 1 below. The mixture was then autoclaved.

Table 1: Composition of the stock solution of F/2 culture medium.

Component	**Stock solution (g/LdH_2O)**	**Quantity (m/l)**
$NaNO_3$	75	1
$NaH_2PO_4H_2O$	5	1
$NaSiO9H_2O$	30	1

The F/2 trace element solution was prepared by dissolving the salts in table 2 below to the 950ml of distilled water and bringing the final volume to 1L using distilled water and then autoclaved.

Table 2: Trace metal composition of F/2 culture medium.

Component	Primary stock solution	Quantity
$FeCl_2$ $6H_2O$	-	3.15g
Na_2 EDTA $2H_2O$	-	4.36g
$CuSO_4$ $5H_2O$	9.8g/L dH_2O	1ml
Na_2MnO_4 $2H_2O$	6.3g/L dH_2O	1ml
$ZnSO_4$ $7H_2O$	22.0g/L dH_2O	1ml
$CoCl_2$ $6H_2O$	10.0g/L dH_2O	1ml
$MnCl_2$ $4H_2O$	180g/L dH_2O	1ml

The F/2 vitamin stock solution was prepared as seen in table 3 below by first preparing primary stock solutions. The final vitamin solution was prepared by dissolving all three vitamins in 950ml of distilled and 1ml of the primary stock added. The resulting solute was then made up to 1 litre with distilled water. Thereafter, the solution was filtered, sterilized and stored in a refrigerator.

Table 3: Composition of vitamin stock solution of F/2 culture medium.

Component	Primary stock solution	Quantity
Thiamine (Vitamin B1)	-	200mg
Biotin (Vitamin B7)	1.0g/L dH_2O	1ml
Cyanocobalamin	1.0g/L dH_2O	1ml

(Vitamin B12)

2.4 Preparation Of Experimental Mixture:

Table 4 shows the different constituents used in preparing the experimental mixture and their concentrations.

Table 4: Preparation of experimental mixture.

Brewery Effluent % Concentration	Brewery Effluent % in volume	Microalgal Culture (ml)	Seawater (ml)	Distilled Water (ml)	Total Volume (ml)
0	0	5	150	145	300
5	5	5	150	140	300
10	10	5	150	135	300
15	15	5	150	130	300
20	20	5	150	125	300
25	25	5	150	120	300
30	30	5	150	115	300

2.6 Experimental Setup:

The marine microalgae species was grown in F/2 medium (Guillard and Ryther 1962) and setup in triplicates for fourteen (14) days using seven (7) concentrations (0%, 5%, 10%, 15%, 20%, 25%, 30%). Each vessel was then inoculated with microalgae and their growth response were measured optically using absorbance at 750nm on a 721 visible spectrophotometer.

2.7 Inoculation:

Each of the vessels was later inoculated with 5ml of the micro algal culture using a 5ml capacity syringe. After inoculation, the experimental vessels were covered immediately with cotton wool in order to allow air passage, reduce evaporation and prevent contamination. After inoculation, the culture vessels were placed at the east-facing window of the final year laboratory, department of Plant Biology and Biotechnology, University of Benin. This position was chosen in order to prevent direct exposure of sunlight.

2.8 Growth Measurement And Monitoring:

After inoculation, the growth was measured using a 721 visible spectrophotometer at an absorbance on 750nm. The growth was monitored every two (2) days for two (2) weeks.

Plate 4: A 721 Visible Spectrophotometer.

2.10 Physicochemical Analysis f the Culture:

The culture sample was analyzed for physical and chemical parameters and this was done before and after the experiment.

2.10.1 Temperature (^oC):

The temperature was measured using a pH/TDS/salinity/temperature/conductivity meter model EZ-9909. The probe was submerged into the culture sample and allowed to stabilize for five (5) minutes before taking the reading in degree Celsius (^oC).

2.10.2 Total Dissolved Solids (ppm):

The total dissolved solid (TDS) was measured using a pH/TDS/Salinity/Conductivity/ Temperature meter model EZ-9909. The probe was immersed into the vessels containing the culture and allowed to stabilize for five(5) minutes before taking the reading in part per million (ppm).

2.10.3 Conductivity (mS/cm):

Conductivity was measured also using a pH/TDS/Salinity/Conductivity/Temperature meter model EZ-9909. The probe was immersed into the culture and allowed to stabilize for five (5) minutes before taking the reading in mS/cm.

2.10.4 pH:

The pH values for the culture were determined using a pH/TDS/Salinity/Conductivity/ Temperature meter model EZ-9909. The probe was submerged into the culture and allowed to stabilize for five minutes before taking the reading.

2.10.5 Salinity (%):

Salinity was measured by immersing the probe of the pH/TDS/Salinity/Conductivity/ Temperature meter model EZ-9909 in the culture and also allowed to stabilize for five minutes before taking the reading in percentage (%).

2.10.6 Phosphate:

Phosphate concentration was determined by pipetting 5ml of culture sample into a 25ml volumetric flask. Distilled water was added to bring the volume to appropriately 15ml. 8ml of ascorbic acid solution was added, it was made up to mark using distilled water. The mixture was then allowed to stand for 30 minutes for the color change before absorbance level was measured at 660nm on a Unicam on a 5625 visible spectrophotometer.

Calculation:

Phosphate (mg/l) = A × K

Where,

A =Absorbance, K =Calibration factor.

2.10.7 Nitrate (ppm):

Nitrate level of culture sample was estimated using Brucine method (ASTM, 1980).10mls of the sample was pipetted into a 10ml volumetric flask. 2ml of brucine reagent was added and immediately, 10ml of concentrated H_2SO_4 was added. The resultant mixture was stirred for 30 seconds and allowed to stand for 15 minutes. The sample was allowed to cool for 20 minutes and was made up to mark distilled water.

2.10.8 Dissolved Oxygen (mg/l):

The dissolved oxygen values were determined using a compact dissolved oxygen meter model DO210. The electrode was immersed into the culture sample and allowed to stabilize for 3 minutes before taking the reading in mg/l.

2.10.9 Chemical Oxygen Demand (mg/l):

The chemical oxygen demand (COD) was measured using a COD/TOC meter. The culture sample was diluted with distilled water in the ratio of 1:19 (19ml of distilled water was used to dilute 1ml of the culture sample) in a beaker before pouring it in the meter that has already been on. Afterwards, the readings were taken in mg/l.

2.10.10 Total Organic Carbon (mg/l):

The total organic carbon (TOC) was measured using the same COD/TOC meter with same procedure used to take the readings in mg/l.

2.11 Percentage Yield:

Percentage yield is the percentage ratio of the standard yield to theoretical yield. Percentage yield of each of the treatment was calculated using the formula below.

$$\text{Percentage yield (Y)} = \frac{G1 - G0 \times 100}{t}$$

Where:

G_0 = Growth at day 0 of the experiment

G_1 = Growth at the end of the experiment

t = Time (day) at the end of the experiment

2.12 Statistical Analysis

Descriptive statistics, inferential one-way analysis of variance (ANOVA) repeated measures, paired t-test and Tukey test were done using Microsoft excel 2010 and Statistical package for social sciences (SPSS) 20.

CHAPTER THREE

RESULTS

3.1 Effect of different concentrations of brewery effluent on the growth of *Thalassiosira* sp.

The results of a one-way repeated-measures ANOVA indicated a statistically significant change across the repeated measurements, $F(7, 98) = 85.774$, $p < 0.05$. Tukey test showed that 30 %, 25

% and 20 % concentrations had higher mean difference and were followed by 15% and 10% concentrations. There were no mean difference between the control and 5% concentrations. From the graph below, Thalassiosira sp grew more in higher concentrations of 30%, 25% and 20%. The highest growth recorded was seen in concentration 30%. In 30% concentration, there was a steady increase from day zero (0) to day four (4) with a little decline in day six (6) and day eight (8). Then the growth became stable from day ten (10) to day fourteen (14) of the experiment. Concentration 20% had the second highest growth which was observed in day four (4) followed by 25% concentration which was also observed in day four (4) of the experiment.

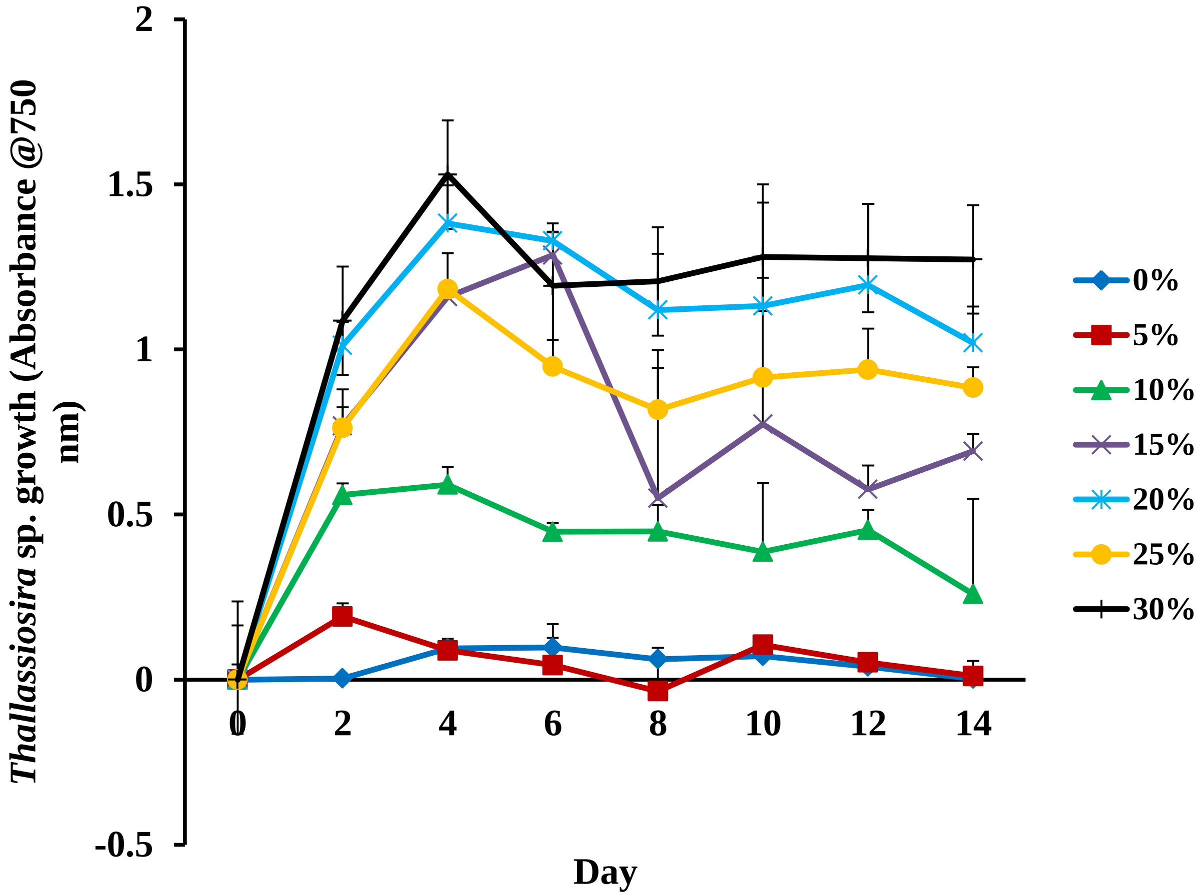

Figure 1: Effect of different concentrations of brewery effluent the growth *Thallassiosira* sp.

3.1.1 Effect of different concentrations of brewery effluent on the growth of *Chaetoceros gracilis.*

The results of a one-way repeated-measures ANOVA indicated a statistically significant change across the repeated measurements, $F(7, 98) = 25.435$, $p < 0.05$. Tukey test showed that 30 % and 25 % concentrations had higher mean difference; then followed by 20 % and was followed by 15% and 10% concentrations. There were no mean difference between the control and 5% concentrations. From the graph below *Chaetoceros gracilis* grew best in lower concentration of 10%, 20% and 15% with the highest growth recorded in 20% concentration on day four (4) and second highest growth recorded in 10% concentration on day two (2) followed by 15% concentration which had the highest value on day eight (8). After day eight (8), there was a decline in all concentration till day fourteen (14) with 30% concentration having the least decline.

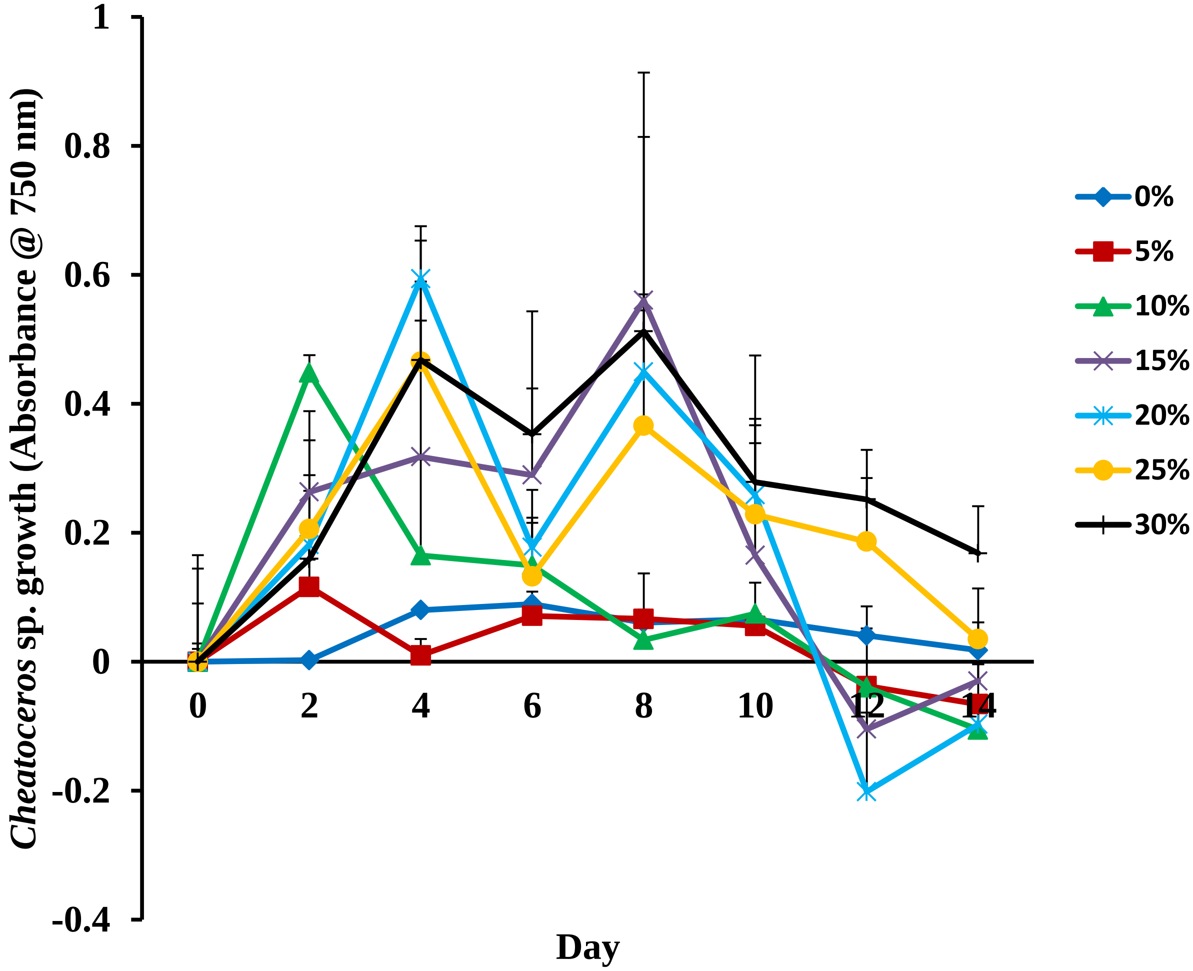

Figure 2: Effect of different concentrations of brewery effluent the growth *Chaetoceros gracilis*.

3.2 Percentage algal biomass at the end of the experiment

The results of a paired-samples *t*-test indicated a statistically significant difference between *Thallassiosira* sp. and *Chaetoceros gracilis*. $t(6) = 3.08$, $p < .05$. From figure 3 below, *Thalassiosira sp.* grew more than *Chaetoceros gracilis* in all the concentration with exception of 0% concentration (control). Concentration 30% had the highest yield followed by 25% and 20% (they had more yield in higher concentrations of the brewery effluent).

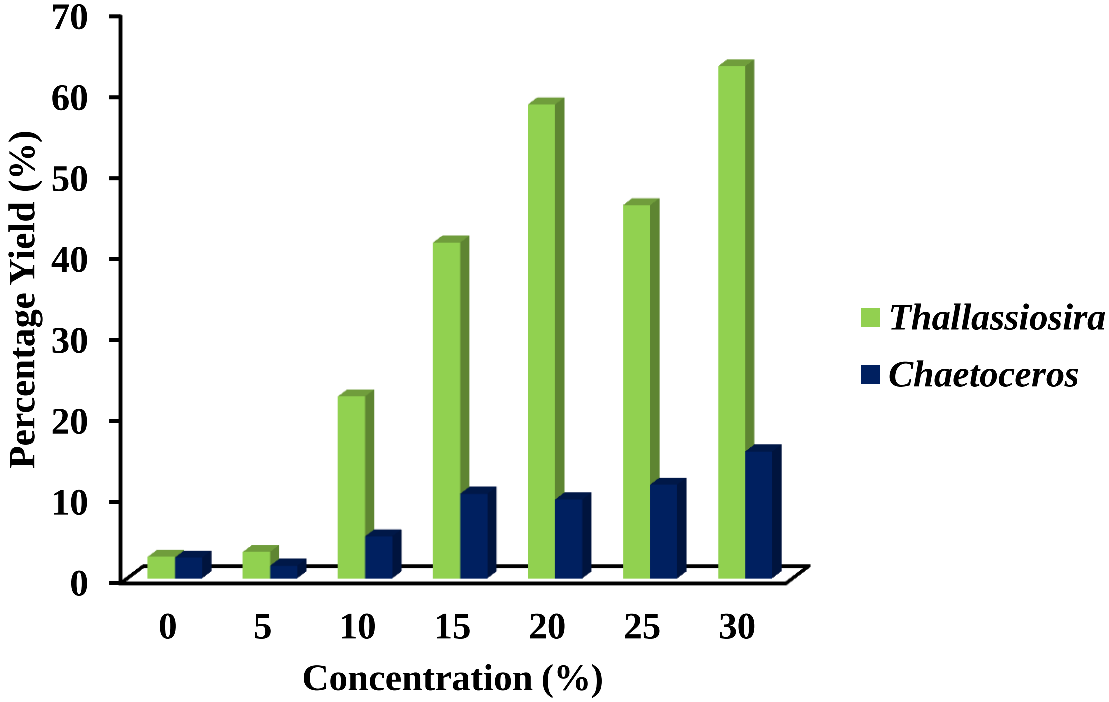

Figure 3. Percentage Yield of *Thallassiosira* sp. and *Chaetoceros gracilis.*

3.3 Temperature of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = 6.481$, $p < .05$, with a decrease in temperature in all the concentrations from pre-treatment to post-treatment. From the graph below, a decrease in temperature was observed in all the concentrations with least decrease recorded in concentration 30% followed by 25% and 0% (control).

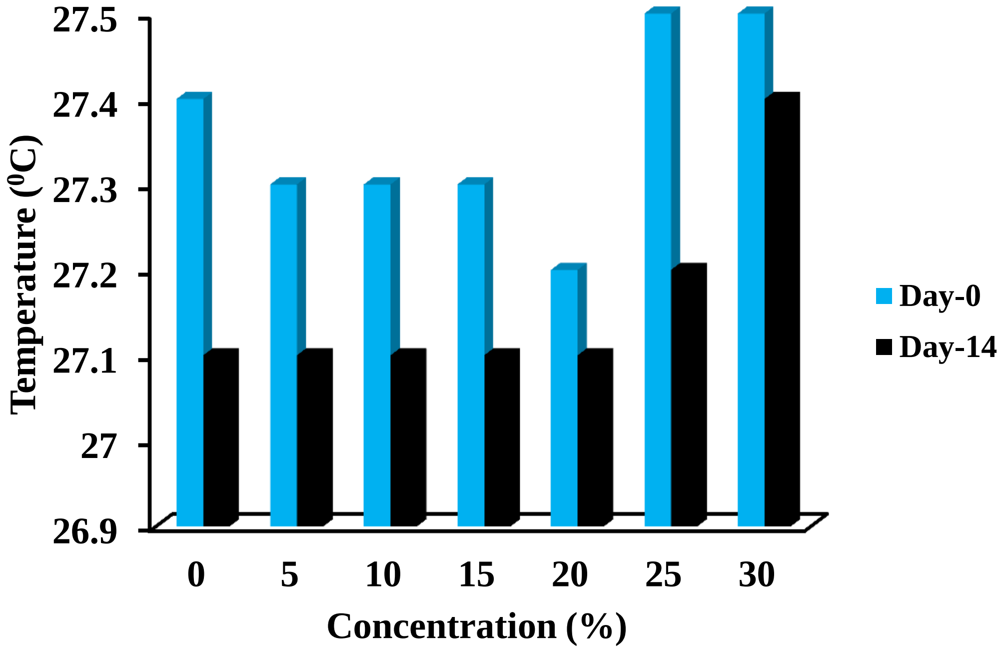

Figure 4a: Temperature of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.3.1 Temperature of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = 22.913$, $p < .05$, with a decrease in temperature in all the concentrations from pre-treatment to post-treatment. From the graph below, it was observe that there was a decrease in temperature in all the concentration with a highest decrease recorded in concentration 20% with concentration 30% coming next and 25%.

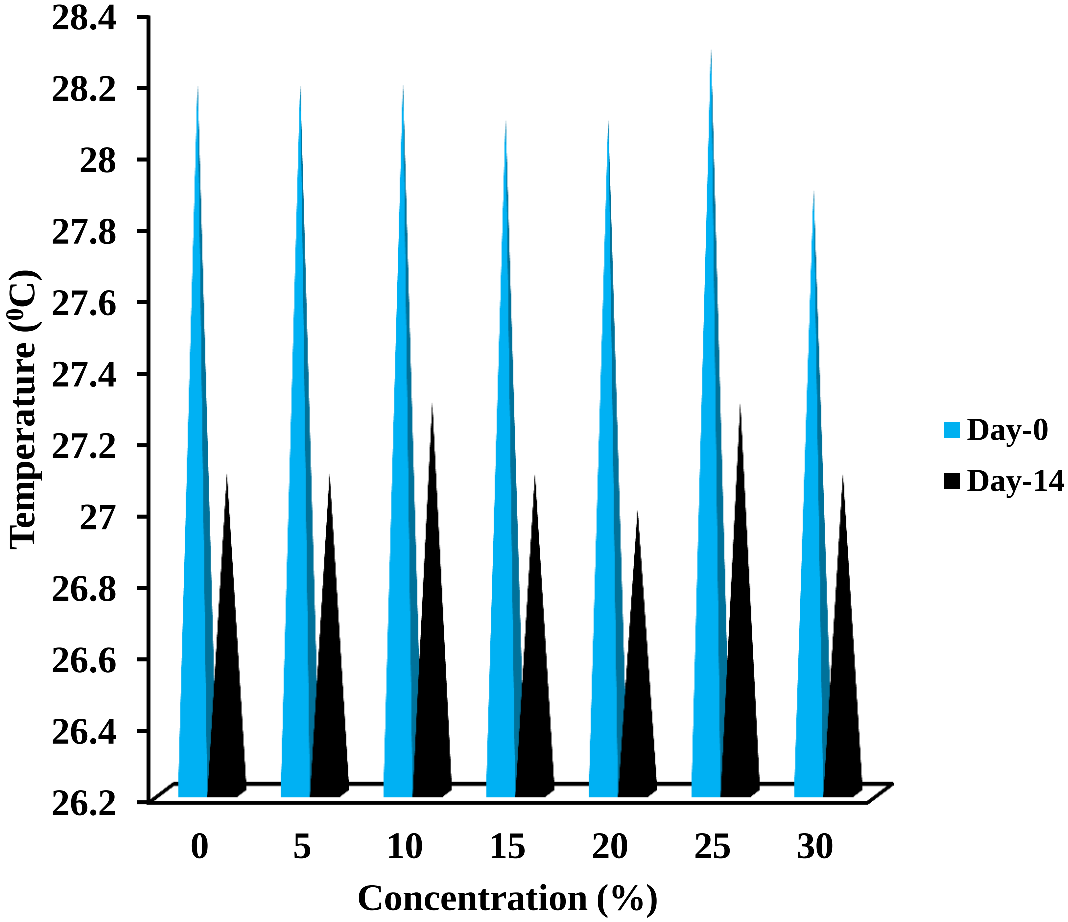

Figure 4b: Temperature of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

3.4 pH of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = -8.433$, $p < .05$, with an increase in pH in all the concentrations from pre-treatment to post-treatment. From the graph below, after the experiment, there was an increase in pH in all the concentrations with the highest at 20% and the least at 0% (control).

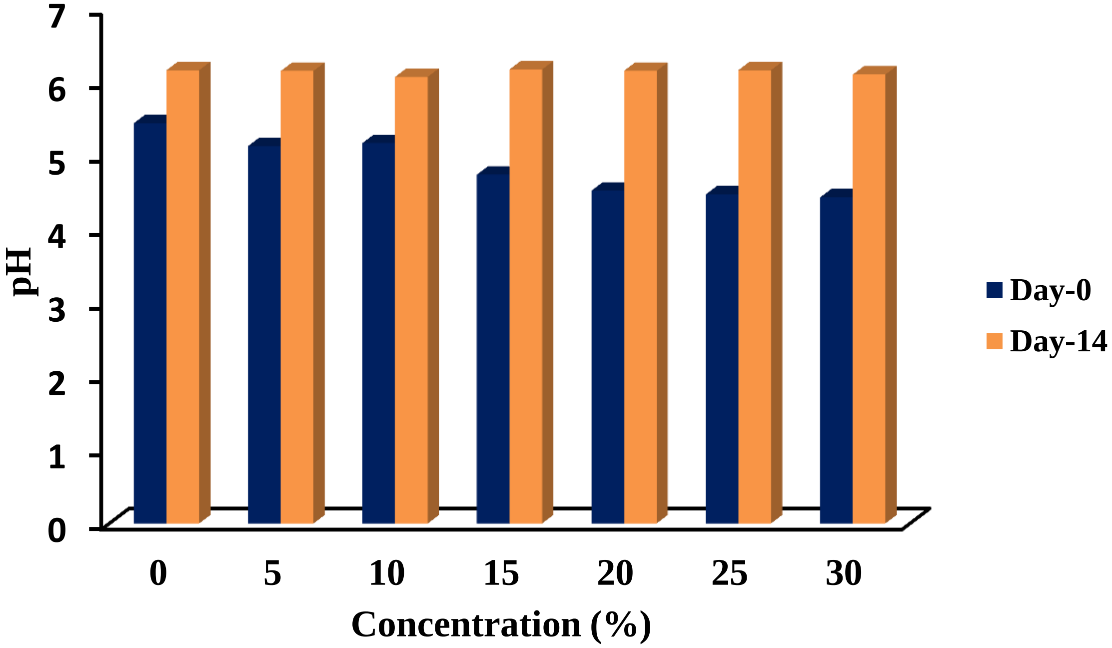

Figure 5a: pH of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.4.1 pH of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = -4.570$, $p < .05$, with an increase in pH in all the concentrations from pre-treatment to post-treatment. In figure 5b below, there was a slight decrease in the control (0%) and an increase in other concentrations with the highest increase recorded in concentration 30% followed by 25% before 20%.

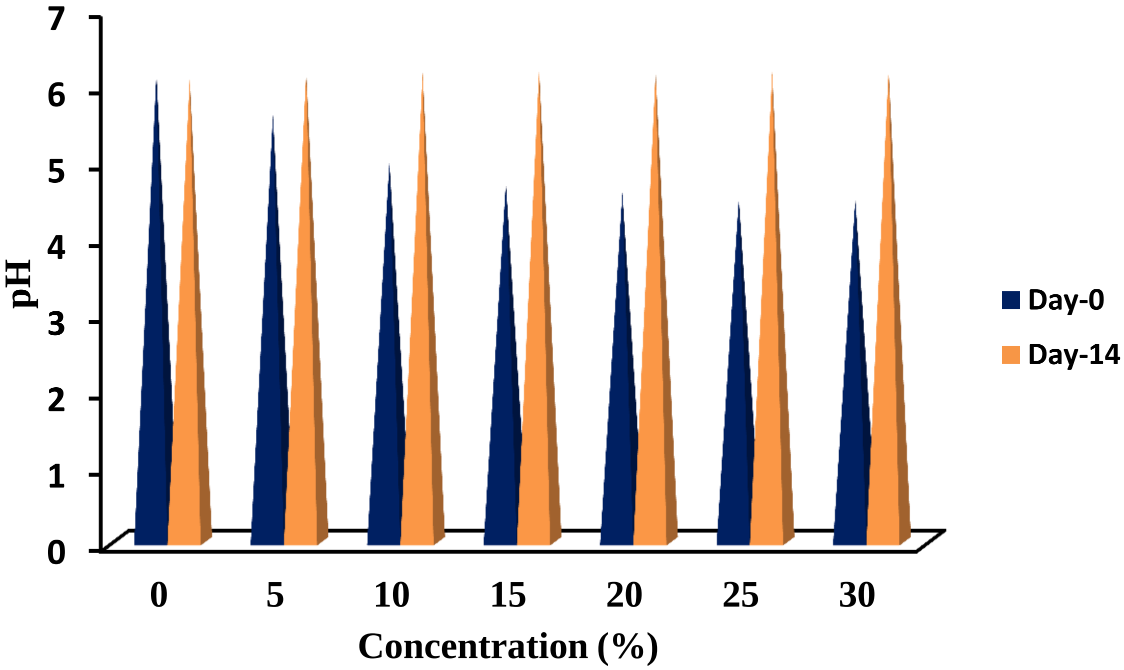

Figure 5b: pH of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

3.5 Total Dissolved Solids of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically insignificant treatment effect, $t(6) = -1.357$, $p < .05$, with an increase in total dissolved solids (TDS) 0%, 5%, 10%, 25% and 30% and a decrease in 15% and 20% pre-treatment to post-treatment. From the graph below, it was observed that the TDS values were fluctuating. In concentrations 0%, 5%, 10%, 25% and 30%, there was an increase in TDS and in concentrations 15% and 20%, TDS reduced.

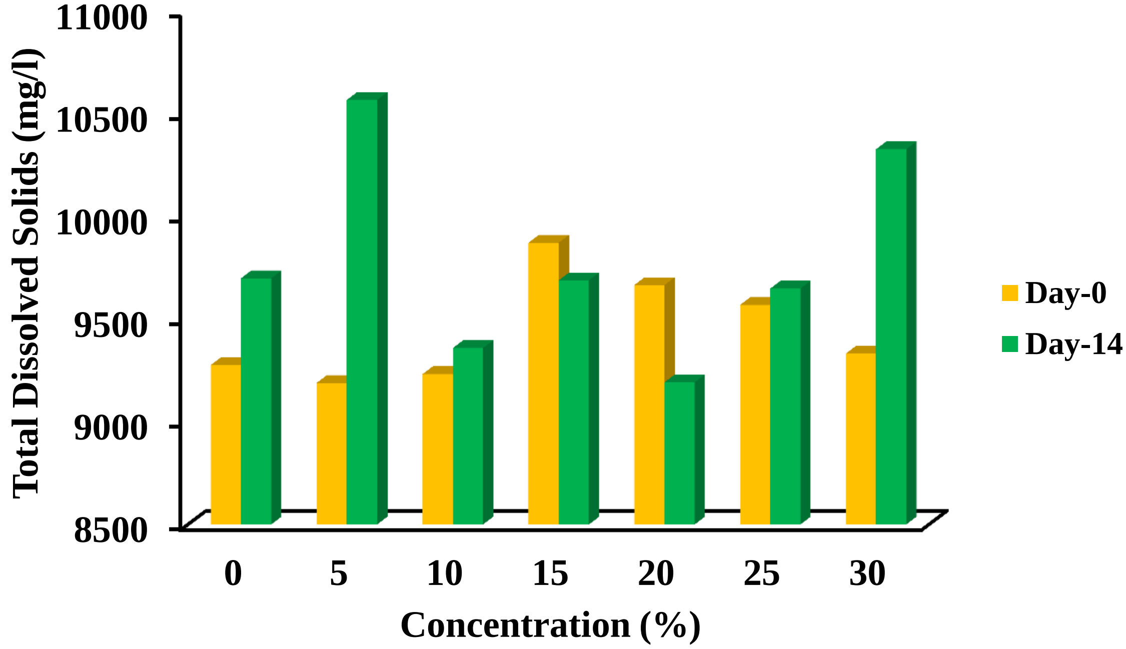

Figure 6a: TDS of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.5.1 TDS of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = -12.416$, $p < .05$, with an increase in total dissolved solids in all the concentrations from pre-treatment to post-treatment. From the graph below, there was an increase in TDS in all the concentration with the highest recorded in concentration 30% followed by 15% and 0% (control).

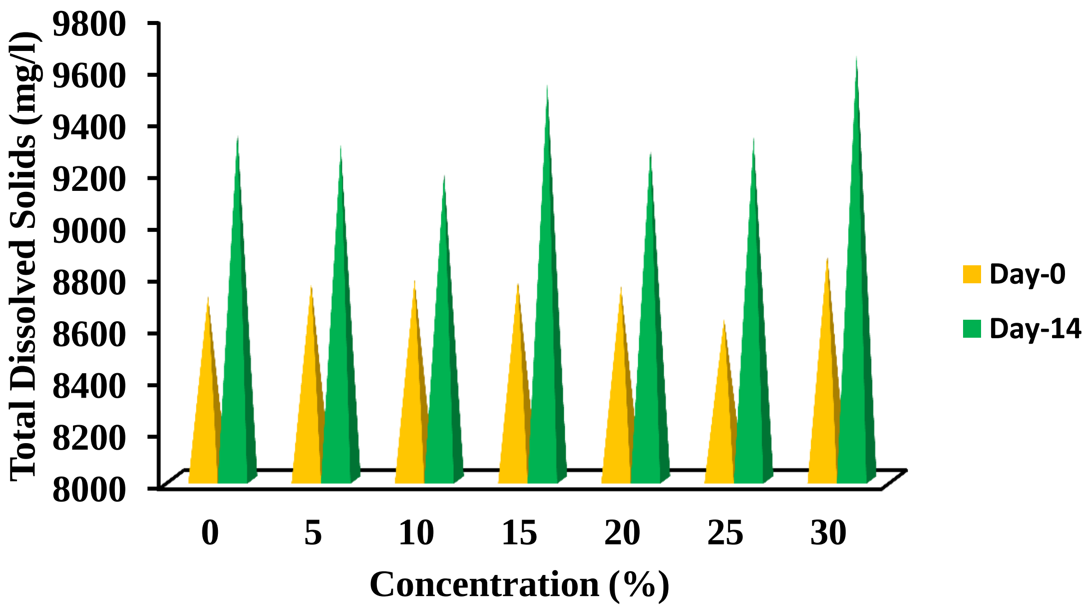

Figure 6b: TDS of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

3.6 Conductivity of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically insignificant treatment effect, $t(6) = -1.271$, $p < .05$, with an increase in conductivity 0%, 5%, 10%, 25% and 30% and a decrease in 15% and 20% pre-treatment to post-treatment. From figure 7a below, it was observed that concentration 5% had the highest increase and 15% had the highest decrease.

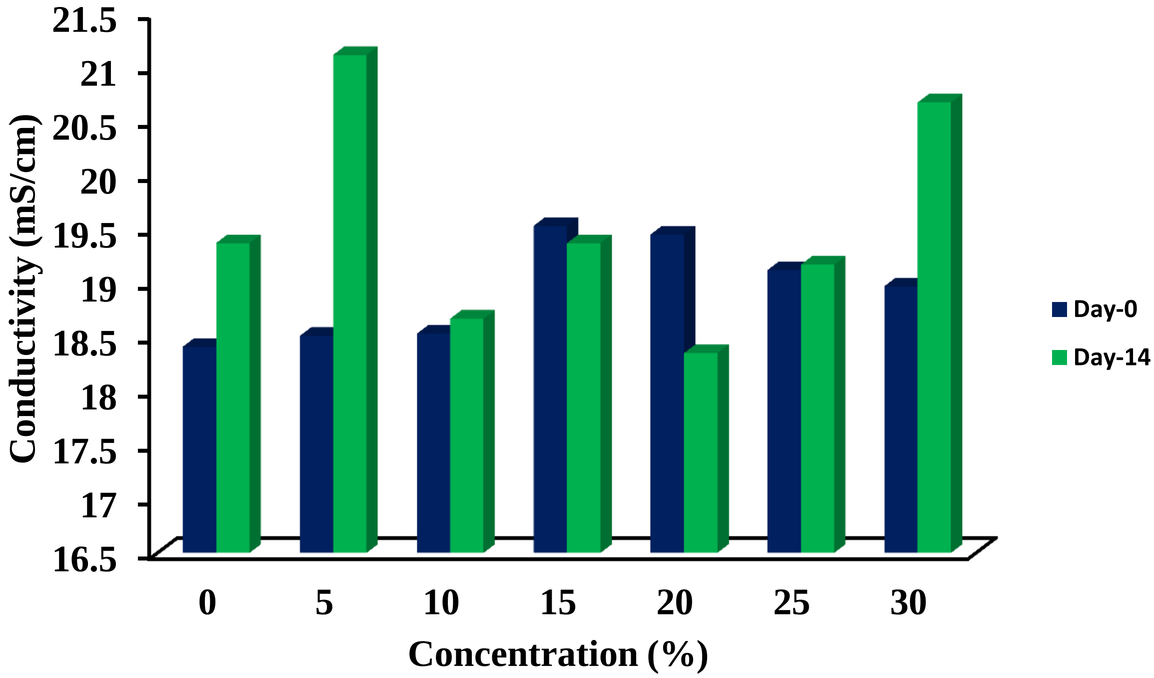

Figure 7a: Conductivity of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.6.1 Conductivity of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = -11.555$, $p < .05$, with an increase in conductivity in all the concentrations from pre-treatment to post-treatment. The highest increase was observed in concentration 30% followed by 15% and 5%.

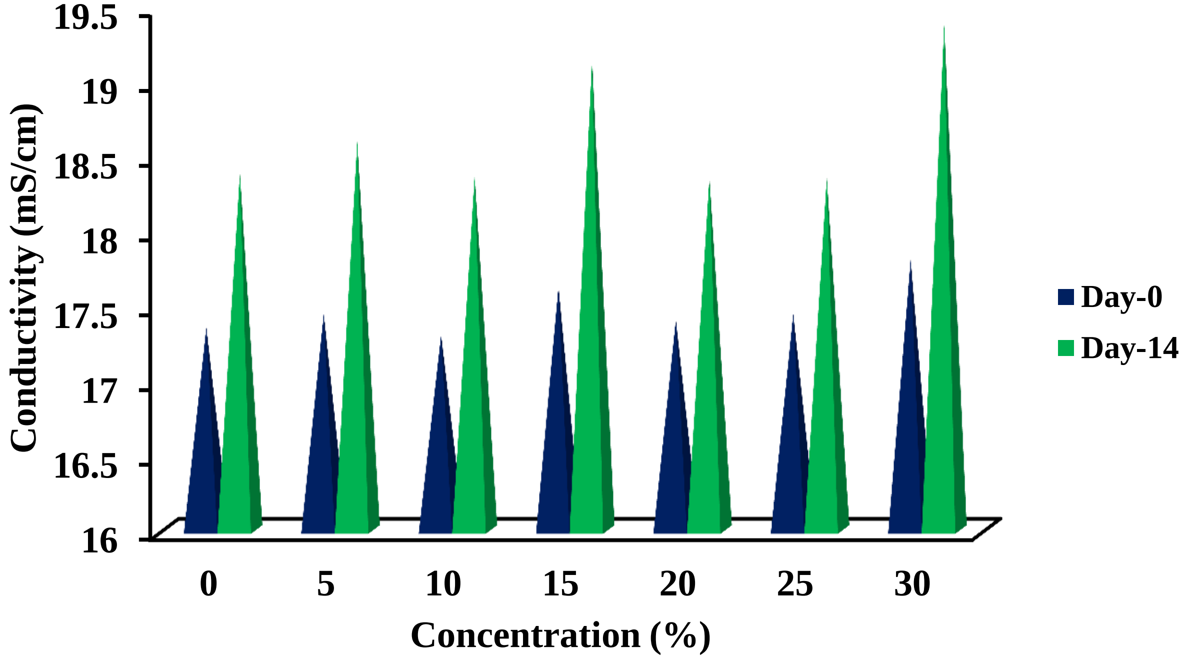

Figure 7b: Conductivity of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

3.7 Salinity of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically insignificant treatment effect, $t(6) = -1.317$, $p < .05$, with an increase in salinity 0%, 5%, 10%, 25% and 30% and a decrease in 15% and 20% pre-treatment to post-treatment. It was observed that 5% had the highest increase while 25% had the least increase and 15% had the highest decrease with 20% having the least decrease.

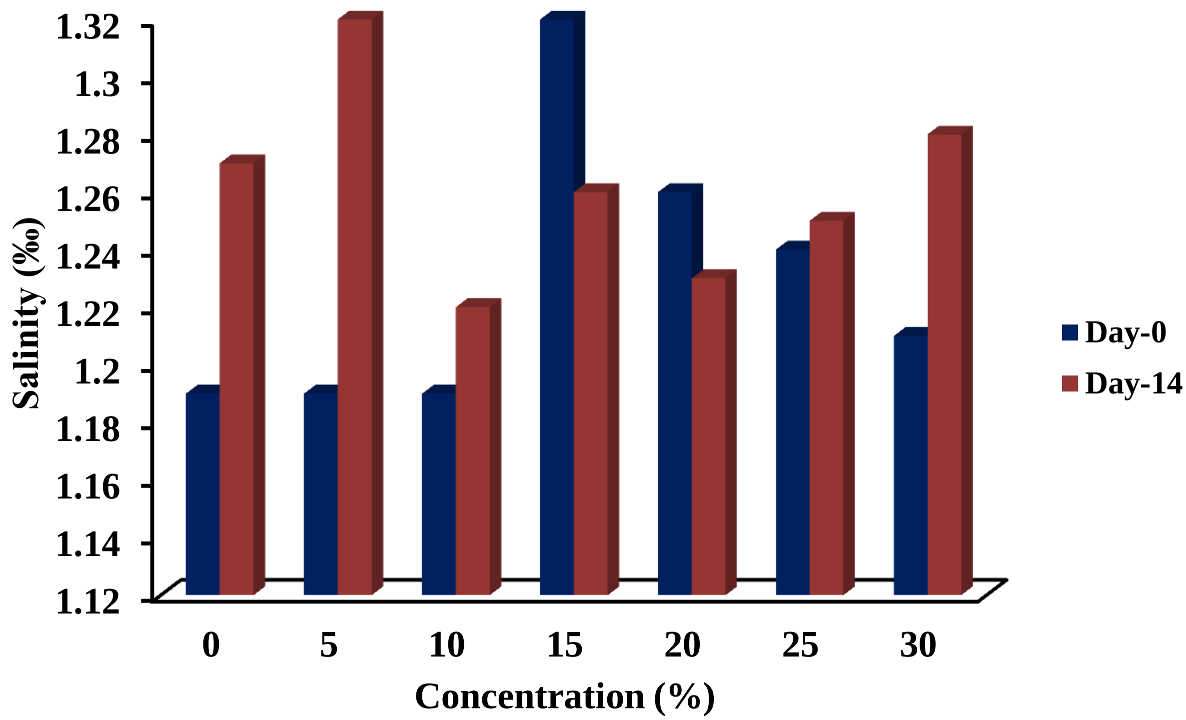

Figure 8a: Salinity of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.7.1 Salinity of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = -7.556$, $p < .05$, with an increase in salinity in all the concentrations from pre-treatment to post-treatment. 30% had the highest increase followed by 15% and 0% (control).

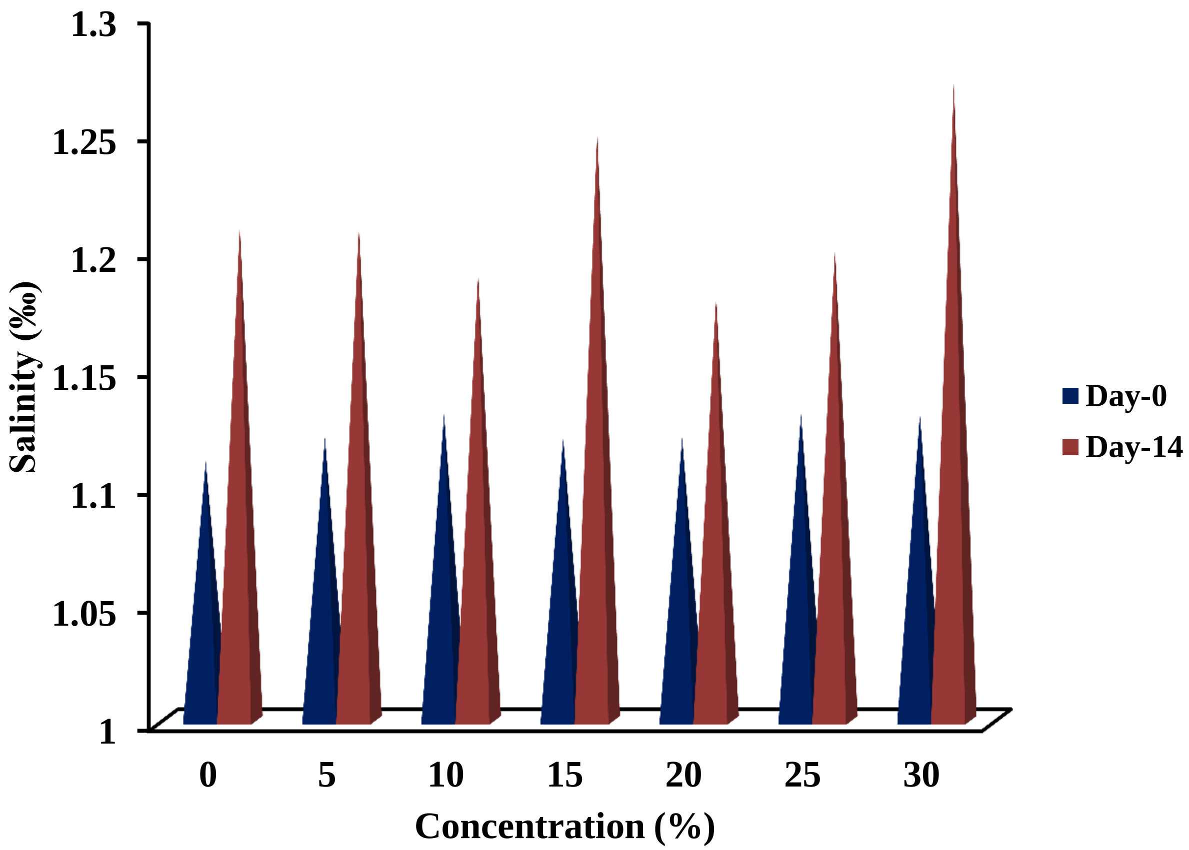

Figure 8b: Salinity of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

3.8 Nitrate of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically non-significant treatment effect, $t(6) = -2.142$, $p < .05$, with an increase in nitrate in all the concentrations from pre-treatment to post-treatment. After the experiment, 0% had the highest value followed by 15% and 10%. Concentration 30% had the least increase.

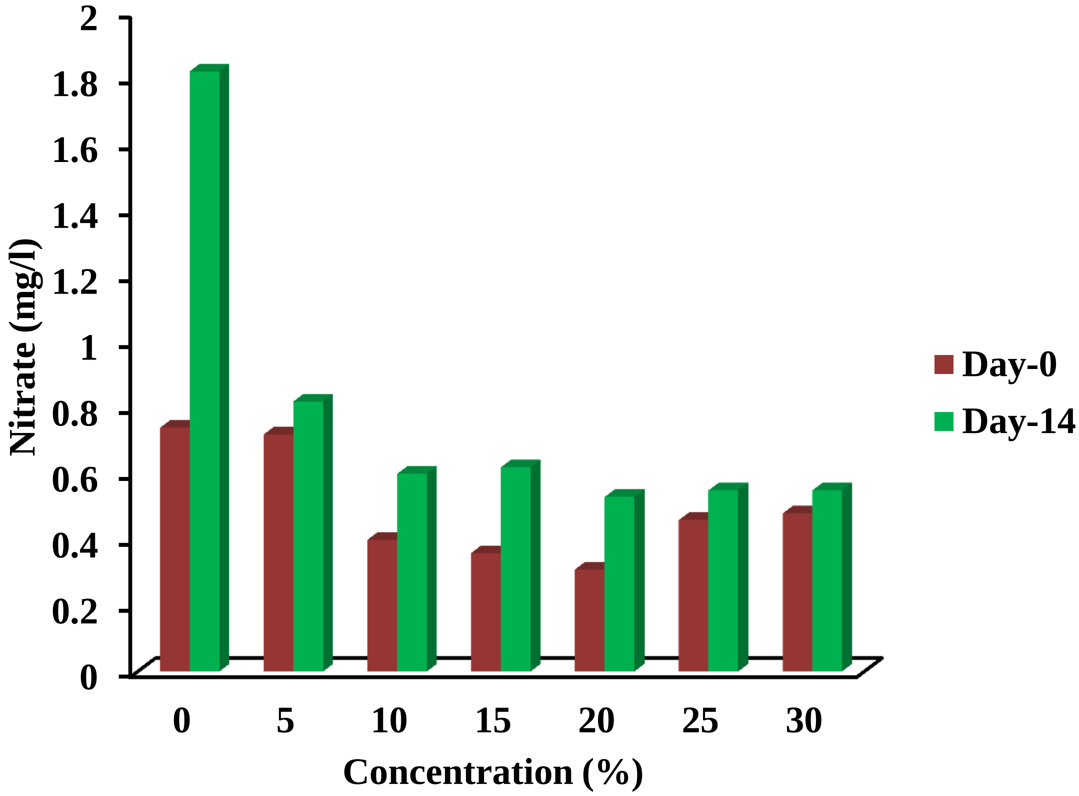

Figure 9a: Nitrate of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.8.1 Nitrate of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = -4.845$, $p < .05$, with an increase in nitrate in all the concentrations from pre-treatment to post-treatment. After the experiment, 0% had the highest increase followed by 5% and 10% while 25% had the least increase.

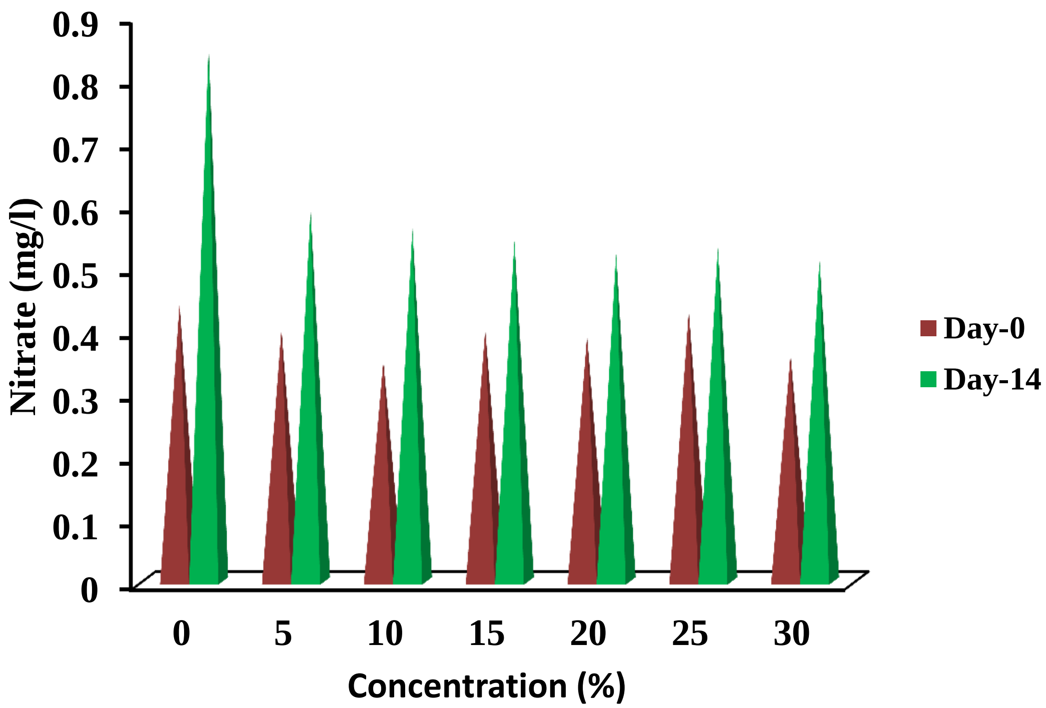

Figure 9b: Nitrate of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

3.9 Phosphate of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = 4.231$, $p < .05$, with an decrease in phosphate in all the concentrations from pre-treatment to post-treatment. 15% had the highest decrease followed by 5% and 10% with 30% having the least decrease.

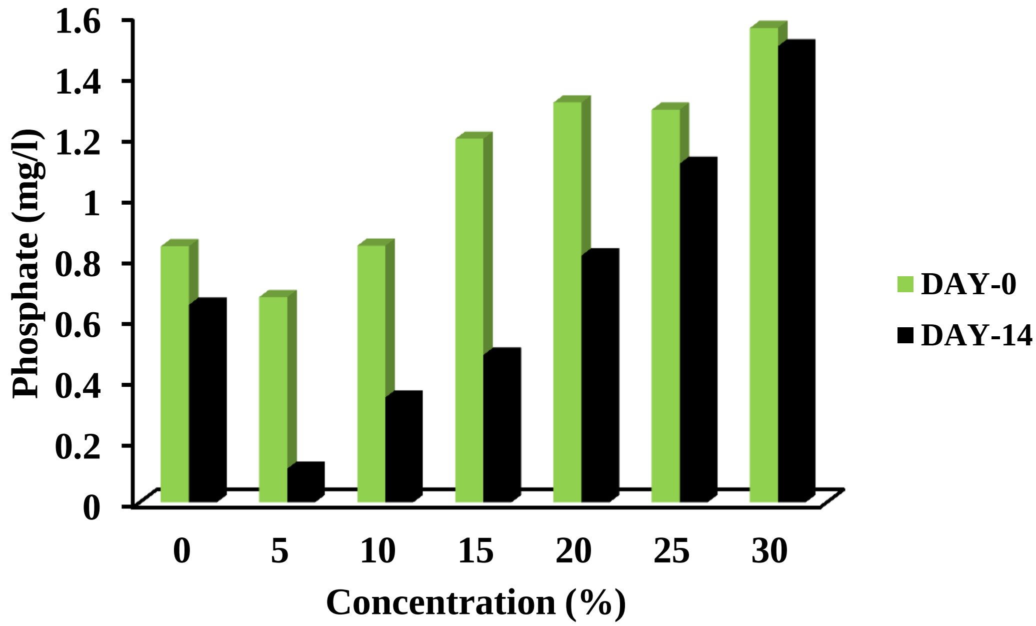

Figure 10a: Phosphate of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.9.1 Phosphate of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

The results of a paired-samples *t*-test indicated a statistically non-significant treatment effect, $t(6) = 1.999$, $p < .05$, with a decrease in phosphate in all the concentrations from pre-treatment to post-treatment with an exception of 25% and 0% concentration which had a slight increase. The highest decrease was observed in 20% concentration followed by 5% while 15% had the least decrease.

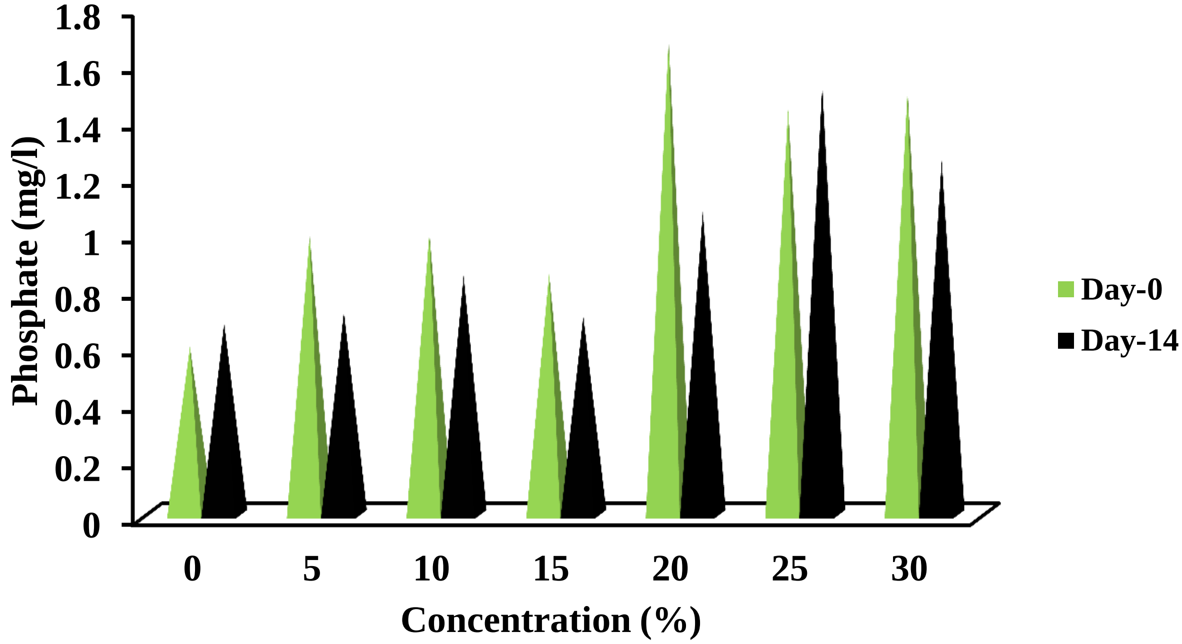

Figure 10b: Phosphate of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

3.10 TOC of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically significant treatment effect, $t(6) = 2.536$, $p < .05$, with a decrease in total organic carbon in all the concentrations from pre-treatment to post-treatment with an exception of 20%, 25% and 30% concentrations. From figure 11a below, a significant decrease occurred in low concentrations (0%, 5% and 10%) which became constant in higher concentration (20%, 25% and 30%).

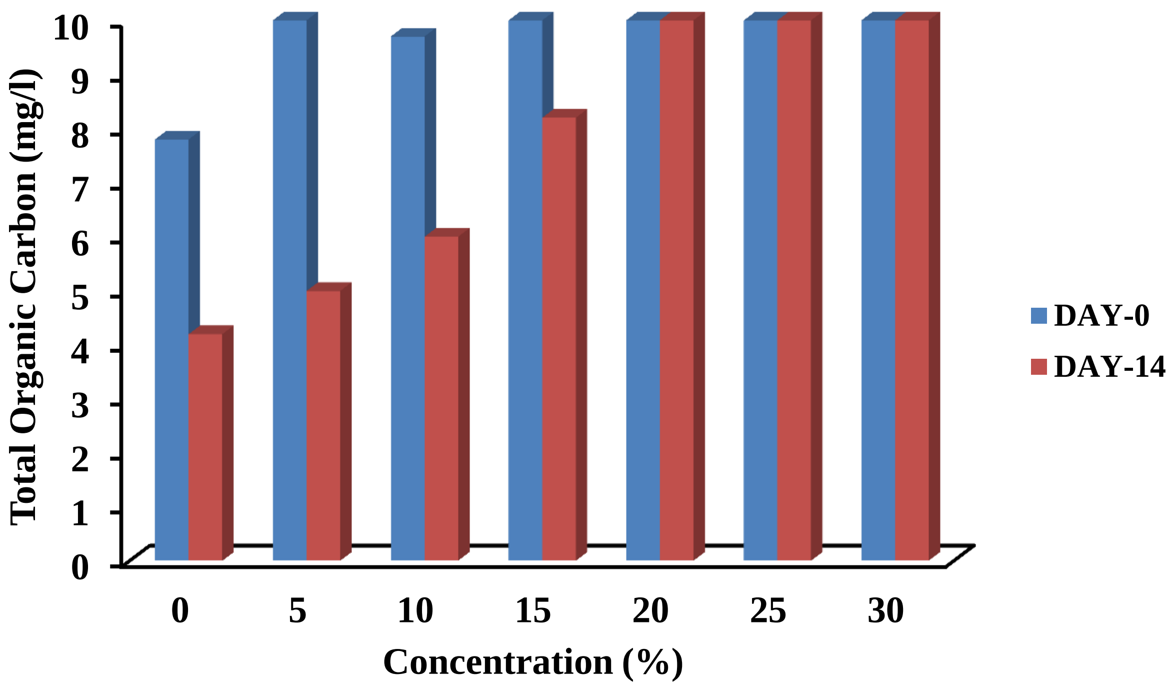

Figure 11a: TOC of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.10.1 TOC of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

The results of a paired-samples *t*-test indicated a statistically non-significant treatment effect, $t(6) = 2.105$, $p < .05$, with a decrease in total organic carbon in all the concentrations from pre-treatment to post-treatment with an exception of 25% and 30% concentrations. There was a slight increase in the control (0%), and a decrease in 5%, 10%, 15% and 20% with the highest occurring in 10% and 15% concentrations. At 25% and 30% treatments the values were constant.

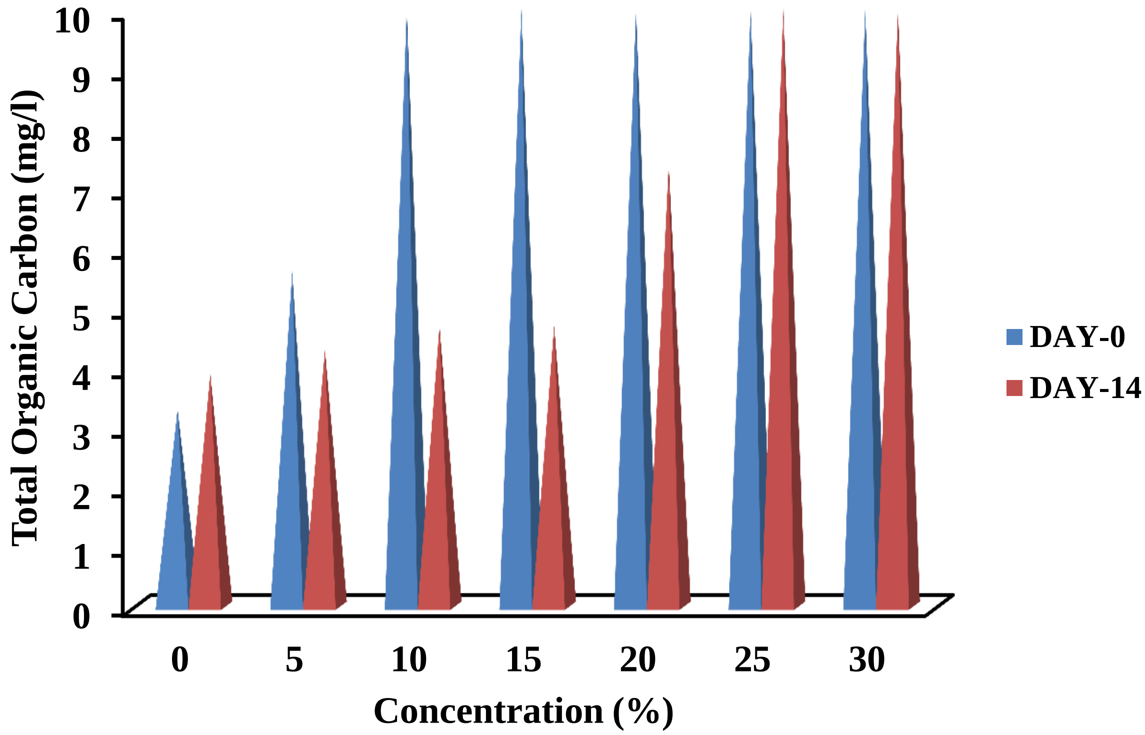

Figure 11b: TOC of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

3.11 DO of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically non-significant treatment effect, $t(6) = -0.284$, $p < .05$, with an increase in dissolved oxygen in 0%, 5%, 10% and 15% concentrations and a decrease in 20%, 25% and 30%from pre-treatment to post-treatment. The increase was observed in lower concentrations (0%, 5%, 10% and 15%) while the decrease occurred in higher concentrations (20%, 25% and 30%).

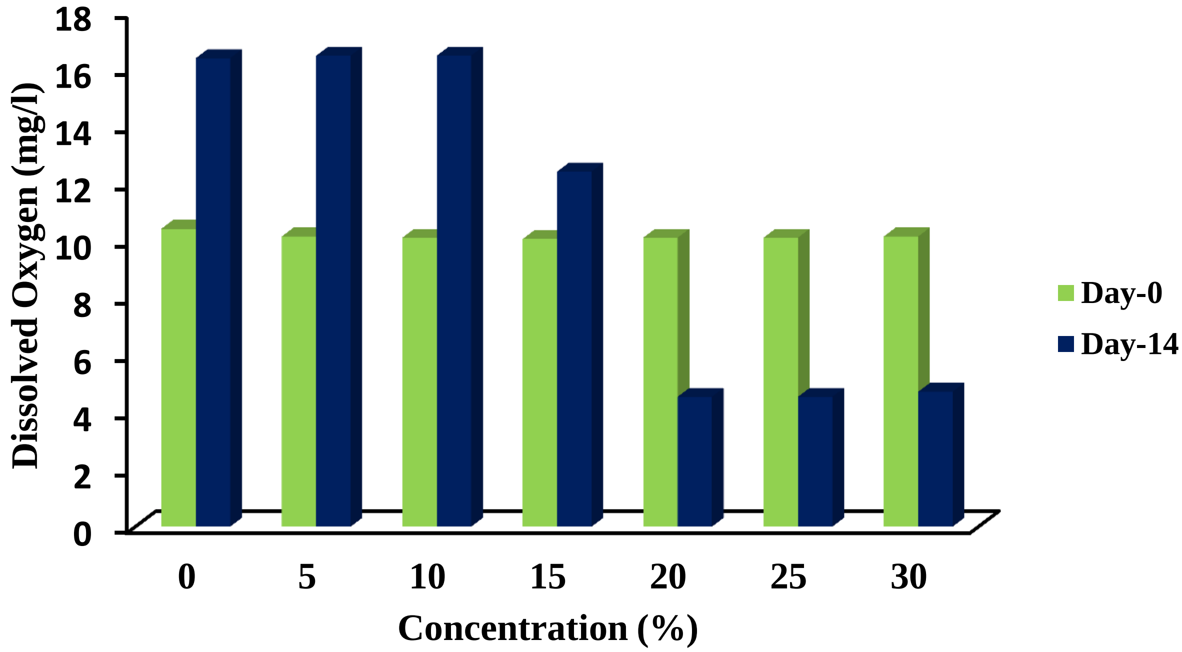

Figure 12a: DO of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.11.1 DO of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

The results of a paired-samples *t*-test indicated a statistically non-significant treatment effect, $t(6) = -72.908$, $p < .05$, with an increase in dissolved oxygen in all the concentrations from pre-treatment to post-treatment.

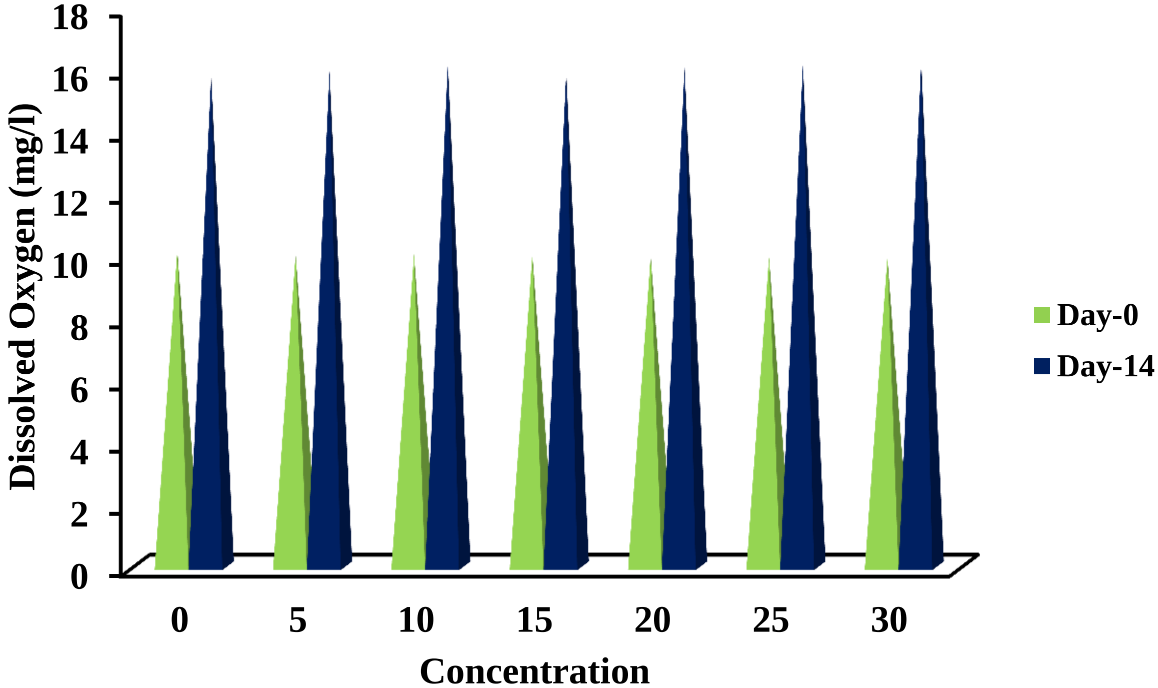

Figure 12b: DO of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

3.12 COD of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

The results of a paired-samples *t*-test indicated a statistically non-significant treatment effect, $t(6) = 1.925$, $p < .05$, with a decrease in chemical oxygen demand in 0%, 5%, and 10% concentrations (lower concentrations) from pre-treatment to post-treatment. At higher concentrations (20%, 25% and 30%), the values became constant.

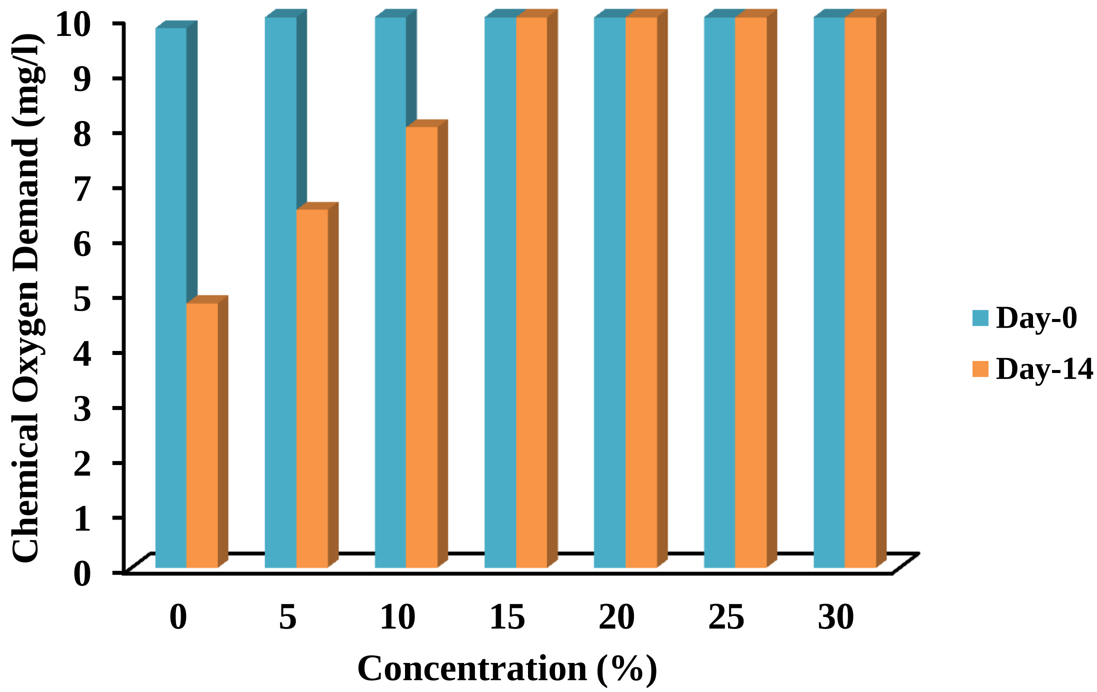

Figure 13a: COD of brewery effluent pre-treatment and post-treatment by *Thallassiosira* sp.

3.12.1 COD of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

The results of a paired-samples *t*-test indicated a statistically non-significant treatment effect, $t(6) = 1.759$, $p < .05$, with a decrease in chemical oxygen demand in 5%, and 10% concentrations from pre-treatment to post-treatment. From the graph below, there was a slight increase in 0% treatment (control) and a decrease in treatments 5%, 10%, 15% and 20% with the exception of 25% and 30% treatment which had constant values.

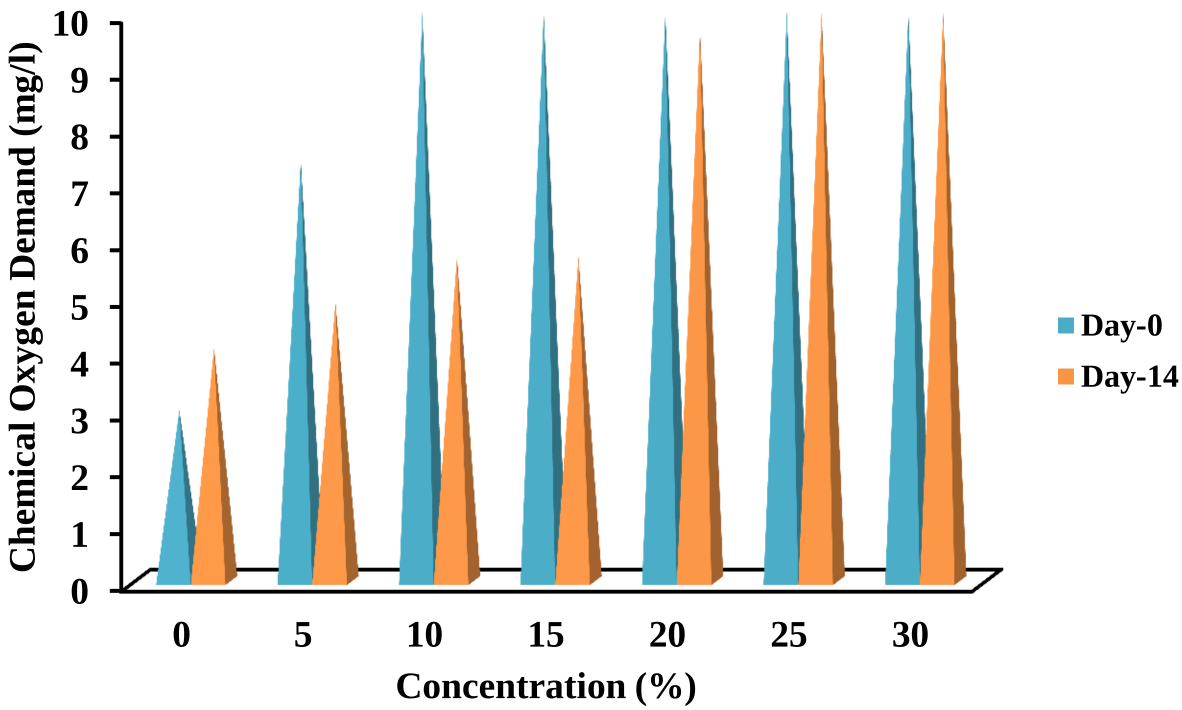

Figure 13b: COD of brewery effluent pre-treatment and post-treatment by *Chaetoceros gracilis*.

CHAPTER FOUR

DISCUSSION

The effect of different concentration of the brewery effluent on *Thalassiosira* sp. and *Chaetoceros gracilis* shows that growth was stimulatory. The most prominent growth was observed at day 4 in all concentrations used. Due to supply of nutrients present in the brewery effluent. Similar result and observation was made by Ansari *et al.* (2017); Arbib and Garridope (2013).

Percentage yield of *Thalassiosira* sp. was higher than *Chaetoceros gracilis* during algae culture experiment as a result higher metabolic process obtained by *Thalassiosira* sp. The major metabolites of microalgal biomass are protein, lipid, and carbohydrate; the relative composition of these metabolites could vary among strains. This is also in line with the study of Tripathi *et al.* (2019) who reported that microalgae yield was higher in the nutritive medium f2 than in the other treatments, reaching average values of 2,363µg/ml for *Chaetoceros gracilis*.

The temperature of the brewery effluent with the treatment of *Chaetoceros gracilis* and *Thalassiosira* sp. showed that there was a decreased in temperature in all the treatment. This had no effect in the microalgal growth according to Dickinson *et al.* (2013) decrease in temperature had no effect on the growth of microalgal growth, because when the temperature reduced, it was still close to the optimal temperature required for microalgae growth. Metabolic activities of microalgae are regulated by varied kinds of enzymes and enzymes in turn are influenced by temperature (Shahid *et al.*, 2020).

The pH of the medium during *Thalassiosira* sp. and *Chaetoceros gracilis* microalgae growth varied across all concentrations at day 0. The optimum growth pH is the most favourable pH for the growth of microalgae, which was observed at day 14 of the experiment. Udaiyappan *et al.* (2017) the pH of microalgal cultures rises gradually during the day due to the uptake of inorganic carbon by microalgae. Higher pH limits the availability of CO_2, thus, inhibiting cell growth.

The total dissolved solids during *Thalassiosira* sp. and *Chaetoceros gracilis* microalgae growth varied in all concentrations used. Similar observations was made by Mohd-Udaiyappan *et al.* (2017); and reported that total dissolved solids in untreated and treated dairy industrial effluents were in the range of 2426 to 890 mg/l and 318 to 520 mg/l respectively.

Total solids of wastewater are mainly composed of carbonates, bicarbonates, chlorides, sulfate, phosphate, nitrate, Ca, Mg, Na, K, Mn and organic matter (Li *et al.*, 2019). In the results obtained there was an increase in TDS at 30% treatment with *Thalassiosira* sp. and increase in all the treatment with *Chaetoceros gracilis.* The increase in TDS at the end of the experiment is due the declination of algal growth, as such adding up to the TDS content present in the brewery wastewater. This is in accordance with the work of Hu *et al.* (2019) who reported that the high algal density would lead to accumulation of autoinhibitors, and a reduction in photosynthetic efficiency which lead to a reduction in algae growth.

Conductivity was examined and results showed that an increase occurred in all concentrations of brewery effluent using *Thalassiosira* sp. and *Chaetoceros gracilis*. This is due to the increase in salinity and TDS. Salinity and TDS influences conductivity because salinity is the total concentration of all dissolved salts and the salts also form ionic particles as they dissolve. The more ions that are present in the culture the higher the conductivity (Gupta *et al.*, 2016).

Salinity was examined before and after the experiment during the treatment of the brewery effluent using *Thalassiosira* sp. and *Chaetoceros gracilis* and results showed that there was increase in both algae. This may be as a result of low salt tolerant level of both microalgae causing the alga cell to suffer from osmotic and ionic imbalance (Miazek *et al.,* 2015).

The phosphate content of brewery effluent treatment by *Thalassiosira* sp and *Chaetoceros gracilis* reduced in all treatments due to the uptake of phosphorus which is one of the essential elements needed by microalgae for their growth. Similar observation made by Nie *et al.* (2020) who studied the ability of Chlorella vulgaris in nutrients removal and reported a nutrient removal efficiency of 86% for inorganic nitrogen and 78% for inorganic phosphorus.

Microalgae have the ability to take up nitrogen especially in inorganic forms such as nitrate, nitrite and ammonia ions (Darpito *et al.*, 2014). However in the treatment of different concentrations of brewery effluent using *Thalassiosira* sp. and *Chaetoceros gracilis*, an increase was observed at the end of the experiment although this is not in line with the work of Darpito *et al.* (2014) due to the decline in the growth of both microalgae at day 14 of the experiment.

Dissolved oxygen was measured before and after the experiment. Results showed that the DO increased at lower concentrations and reduced at higher concentrations of the brewery effluent containing *Thalassiosira* sp. This is because the microalgal growth was more in higher concentrations than lower concentrations (Shahid *et al.,* 2020). The results of DO of different concentrations of the brewery effluent using *Chaetoceros gracilis* showed that an increase in DO was observed in all concentrations due to the decline in growth of the microalga on day 14 of the experiment.

The chemical oxygen demand (COD) values during the treatment of brewery effluent using *Thalassiosira* sp. and *Chaetoceros gracilis* showed that there was a decrease in lower concentrations of the treatments and stable values occurred at higher concentrations. The stable values were as a result of the amount of the organic compound that were not affected by the breaking down process of microalgae. Nagajaran and Ramachandramororthy (2002) reported that COD increases according to the high amount of organic compounds that are not decomposed by the microalgae and this finding is in line with that of Alvaraz-Bernal *et al.* (2006).

The Total organic carbon (TOC) values during the treatment of brewery effluent using *Thalassiosira* sp. and *Chaetoceros gracilis* showed that there was a decrease in lower concentrations of the treatments and stable values occurred at higher concentrations.This is in line with the work of Latif *et al.* (2013) who evaluated that TOC of wastewater treated with microalgae range from 11.51 – 22.36 mg/l and that the highest TOC was observed at 20% treatment. High organic content means an increase in the growth of microalgae which contribute to the depletion of oxygen supplies in water bodies (Bello *et al.,* 2013).

4.1 Conclusion

In this study, the effect of different concentration of brewery effluent using the microalgae *Thalassiosira* sp and *Chaetoceros gracilis* was investigated. After the experiment, the different concentrations of brewery effluent used had a stimulatory effect on both microalgae which was more at higher concentrations than the lower concentrations. *Thalassiosira* sp had more stimulatory effect than *Chaetoceros gracilis* making it a better option in the bioremediation of brewery effluent in the bioremediation of brewery wastewater when applied in higher concentrations. Therefore more detailed studies spawning longer period of time is recommended to ascertain the full effect of brewery effluent on Thalassiosira sp and Chaetoceros gracilis and other microagal species should be employed in the treatment of brewery wastewater to determine the best option for microalgal bioremediation.

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APPENDEX

One way ANOVA Repeated Measures
***Thallassiosira* sp.**

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Day	Sphericity Assumed	9.705	7	1.386	85.774	.000	.860
	Greenhouse-Geisser	9.705	4.134	2.347	85.774	.000	.860
	Huynh-Feldt	9.705	7.000	1.386	85.774	.000	.860
	Lower-bound	9.705	1.000	9.705	85.774	.000	.860
Day * Concentration	Sphericity Assumed	5.479	42	.130	8.071	.000	.776
	Greenhouse-Geisser	5.479	24.806	.221	8.071	.000	.776
	Huynh-Feldt	5.479	42.000	.130	8.071	.000	.776
	Lower-bound	5.479	6.000	.913	8.071	.001	.776
Error(Day)	Sphericity Assumed	1.584	98	.016			
	Greenhouse-Geisser	1.584	57.880	.027			
	Huynh-Feldt	1.584	98.000	.016			
	Lower-bound	1.584	14.000	.113			

Chaetoceros gracilis.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Day	Sphericity Assumed	2.364	7	.338	25.435	.000	.645
	Greenhouse-Geisser	2.364	3.594	.658	25.435	.000	.645
	Huynh-Feldt	2.364	7.000	.338	25.435	.000	.645
	Lower-bound	2.364	1.000	2.364	25.435	.000	.645

Day * Concentration	Sphericity Assumed	1.998	42	.048	3.582	.000	.606
	Greenhouse-Geisser	1.998	21.564	.093	3.582	.000	.606
	Huynh-Feldt	1.998	42.000	.048	3.582	.000	.606
	Lower-bound	1.998	6.000	.333	3.582	.023	.606
Error(Day)	Sphericity Assumed	1.301	98	.013			
	Greenhouse-Geisser	1.301	50.317	.026			
	Huynh-Feldt	1.301	98.000	.013			
	Lower-bound	1.301	14.000	.093			

Temperature

Thallassiosira sp.

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 Day_0 - Day_14	.20000	.08165	.03086	.12449	.27551	6.481	6	.001

Chaetoceros gracilis.

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 Day_0 - Day_14	1.00000	.11547	.04364	.89321	1.10679	22.913	6	.000

pH

Thallassiosira sp.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Day_0 - Day_14	-1.29429	.40607	.15348	-1.66984	-.91873	-8.433	6	.000

Chaetoceros gracilis.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Day_0 - Day_14	-1.15571	.66913	.25291	-1.77455	-.53688	-4.570	6	.004

Total Dissolved Solids

Thallassiosira sp.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Day_0 - Day_14	-334.85714	653.05473	246.83149	-938.83203	269.11774	-1.357	6	.224

Chaetoceros gracilis.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Day_0 - Day_14	-621.42857	132.42464	50.05181	-743.90094	-498.95620	-12.416	6	.000

Conductivity

Thallassiosira sp.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Day_0 - Day_14	-.59857	1.24641	.47110	-1.75131	.55417	-1.271	6	.251

Chaetoceros gracilis.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Day_0 - Day_14	-1.17000	.26789	.10125	-1.41776	-.92224	-11.555	6	.000

Salinity

Thallassiosira sp.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Day_0 - Day_14	-.03286	.06601	.02495	-.09390	.02819	-1.317	6	.236

Chaetoceros gracilis

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Day_0 - Day_14	-.09286	.03251	.01229	-.12293	-.06279	-7.556	6	.000

Nitrate

Thallassiosira sp.

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			

	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1 Day_0 - Day_14	-.28857	.35648	.13474	-.61826	.04112	-2.142	6	.076

Chaetoceros gracilis.

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1 Day_0 - Day_14	-.19000	.10376	.03922	-.28596	-.09404	-4.845	6	.003

Phosphate

Thallassiosira sp.

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1 Day_0 - Day_14	.38586	.24126	.09119	.16272	.60899	4.231	6	.005

Chaetoceros gracilis.

Paired Samples Test

	Paired Differences	t	df	Sig. (2-

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			tailed)
Pair 1	Day_0 - Day_14	.17886	.23678	.08949	-.04012	.39784	1.999	6	.093

Total Organic Carbon (TOC)

Thallassiosira sp.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Day_0 - Day_14	2.01429	2.10113	.79415	.07106	3.95751	2.536	6	.044

Chaetoceros gracilis.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Day_0 - Day_14	1.98571	2.49628	.94351	-.32296	4.29439	2.105	6	.080

Dissolved Oxygen (DO)

Thallassiosira sp.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Day_0 - Day_14	-.63547	5.91480	2.23558	-6.10574	4.83481	-.284	6	.786

Chaetoceros gracilis.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Day_0 - Day_14	-6.00517	.21792	.08237	-6.20671	-5.80363	-72.908	6	.000

Chemical Oxygen Demand (COD)

Thallassiosira sp.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Day_0 - Day_14	1.50000	2.06155	.77919	-.40662	3.40662	1.925	6	.103

Chaetoceros gracilis.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Day_0 - Day_14	1.47143	2.21263	.83629	-.57491	3.51777	1.759	6	.129