EFFECT OF ALKALINITY ON THE PLANKTON DIVERSITY IN BIOFLOC SYSTEMS.

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SUBMITTED BY,

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CERTIFICATE

This is to certify that the dissertation entitled ‘ **Effect of Alkalinity on the Plankton Diversity of Biofloc Systems** ’ submitted to St. Teresa’s College (Autonomous), Ernakulam, in partial fulfilment of the requirement of award of degree of Master of Science in Zoology is an authentic work carried out by **Ms. MEERA RAJEEV** (SM20ZOO006) in the academic year 2020 – 2022 under the guidance and supervision of Dr. Mithun Sukumar (External Guide) Assistant Professor, Department of Aquatic Biology and Fisheries, University of Kerala, Karyavattom Campus, Thiruvananthapuram and Ms. Tiya K.J. (Internal Guide), Assistant Professor, Department of Zoology, St. Teresa’s College, Ernakulam.

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DECLARATION

I hereby declare that the dissertation entitled ‘Effect of Alkalinity on the Plankton Diversity of Biofloc Systems ’submitted to St. Teresa’s College (Autonomous), Ernakulam in partial fulfilment of the requirements, for the award of the Degree of Master of Science in Zoology is a record of original research work done by me under the supervision and guidance of Dr Mithun Sukumar (External Guide) Assistant Professor, Department of Aquatic Biology and Fisheries, University of Kerala, Karyavattom Campus, Thiruvananthapuram during the period from 01-04-2022 to 30-04-2022 and Mrs. Tiya K.J. (Internal Guide), Assistant Professor, Department of Zoology, St. Teresa’s College, Ernakulam, to the best of my knowledge and belief, this project contains no material previously published or written by another person, except where due reference is made.

MEERA RAJEEV

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ABSTRACT

Aquaculture, the husbandry and farming of aquatic animals and plants, has expanded faster in recent decades than any other livestock sector. Increased human population, but more importantly increasing wealth and per capita consumption of fish, especially in wealthier Western countries, has driven incentives for aquaculture as an enterprise at a household and increasingly at the corporate level. Increasing the productivity of aquaculture in vertical and horizontal expansion leads to an excessive increase of pollutants in the surrounding environment. Biofloc technology (BFT) applications are one of the best aquaculture systems and contribute to the achievement of sustainable development and desired objectives for a clean environment. Water quality is a general term used to describe the physical, chemical, thermal, and/or biological properties of water. In broader terms the quality of water affects all components of the aquatic ecosystem. The alkalinity of water is its acid-neutralizing capacity comprised of the total of all titratable bases. Four biofloc systems of different levels of alkalinities were maintained using baking soda, and their algal and zooplanktonic diversity was studied. A total of six algal and four zooplanktonic species were obtained. The *Chlorella* sps. Showed varied morphology across increasing alkalinity, while *Pediastrum* sps. were abundant in lower alkalinities. *Paramecium* sps were the commonly found zooplankton in lower alkalinities, whereas higher alkalinities supported the presence of rotifers and *Moina* sps.

INTRODUCTION

Aquaculture, the husbandry and farming of aquatic animals and plants, has expanded faster in recent decades than any other livestock sector. It achieved a 7·5 % annual growth rate between 1990 and 2009, eclipsing the rapid growth rates of the pig (<2·5 %) and poultry (<5 %) sectors. In comparison, the overexploitation of wild fishery stocks has led to their contribution to world food stocks flat-lining. Approximately 30 % are overfished, more than 60 % fully fished and <10 % have remaining capacity. In response to expanding demand from growing and better off populations, the rise of aquaculture has been timely but its development has not been evenly distributed nor without criticism, especially regarding environmental and social impacts (Little.D.C., 2016).

The characteristics of aquaculture, growing rapidly from an artisanal and marginal activity, unknown in most of the World, to a position where there are now major complementarities and, potentially, resource allocation conflicts with terrestrial livestock and conventional fisheries are reviewed in the present paper. Aquatic products, 'fish', often remain neglected in the current discourse regarding food security despite its importance in world trade, human nutrition and support for livelihoods more broadly. Increased human population, but more importantly increasing wealth and per capita consumption of fish, especially in wealthier Western countries, has driven incentives for aquaculture as an enterprise at a household and increasingly at the corporate level. Urbanization hasmade self-sourcing impractical for most, fueling the trade in fish as a commodity.Historically fish culture has often been a peri-urban phenomenon, driven by easyaccess to inputs and markets (Little.D.C., 2016).

Since the end of World War II growth of global fish (fish + shellfish) supplies have outstripped population growth, effectively increasing annual per capita supplies from 9·9 kg in the 1960s to 18·4 kg in 2009. Growth has been fueled by rising demand for livestock and fish, the result of increased economic access, changes in trade policies and market liberalization, urbanization and marketing. During the first half of the post-war period increases in fish supply came from capture fisheries, thanks to massive private and public investments that resulted in a proliferation of larger, more robust and increasingly mechanized fishing craft and more effective means of locating, catching and preserving fish until landed. By the late 1970s, however, the majority of fish stocks was fully or overexploited. Today, capture fisheries is dominated in production and employment terms by small-scale artisanal tropical fisheries. While aquaculture accounted for only 3–6 % of global fish supplies in the 1970s in the subsequent decades it has consistently been among the fastest growing animal source food sectors, to the extent that one fish in two now consumed is farmed. Any future expansion of supplies must come from aquaculture (Little.D.C., 2016).

Increasing the productivity of aquaculture in vertical and horizontal expansion leads to an excessive increase of pollutants in the surrounding environment. Biofloc technology (BFT) applications are one of the best aquaculture systems and contribute to the achievement of sustainable development and desired objectives for a clean environment. Hargreaves (2013) defined the biofloc as “a mixture of algae, bacteria, protozoans and other kinds of particulate organic matter such as feces and uneaten feed in addition to some of zooplankton and nematodes, formed together to be an integrated and interdependent ecosystem”. Moreover, biofloc systems can operate with zero or low water exchange (0.5% to 1% per day) under high stocking density of fish or shrimp, assuring it to be an ideal system for saving water exchange. Biofloc is generated by adding organic carbon and high aeration, which reduces toxic dissolved nitrogen in the water, where internal waste treatment processes are emphasized and encouraged. Although it is a potential technology, the data on operational parameters of BFT is still inadequate. Hence, there is an urgent need for more applied research on operational parameters of BFT to optimize the system, such as immunological effects, microbial associated molecular patterns production, and nutrient recycling (Jamal, Mamdoh T *et al*., 2020).

Microbial biotechnology, which deals with applying microorganisms to ensure the security, safety, and usefulness of foods, yielding high-quality products, proper human nutrition, and defence against plant and animal diseases, is still rising with different new technologies for sustainable development in the agricultural field. These organisms are key agents of pollutant removal and recycling. BFT is one of such novel microbial biotechnologies that has been developed with an excellent eco-friendly technology not only for higher productivity but also for sustainable development (Jamal, Mamdoh T *et al*., 2020).

Water quality is a general term used to describe the physical, chemical, thermal, and/or biological properties of water. We often define it in terms of human usage for consumption, recreation, and aesthetics. In broader terms the quality of water affects all components of the aquatic ecosystem. The quality of water suitable and desirable for use by one organism may be completely unsuitable for another. Thus, water quality is a parameter that cannot be defined easily nor can standards be set that meet all uses and user needs. For example, physical, chemical, and biological parameters of water that are suitable for human consumption are quite different from those parameters suitable for a farmer irrigating a crop (Ritchie, J.C *et al*., 2000).

AIM AND OBJECTIVE

AIM

To identify and classify the phytoplanktons and zooplanktons obtained by maintaining biofloc systems at four different alkalinities.

OBJECTIVES

1. To prepare biofloc system with different alkalinity
2. To assess the diversity of algae in biofloc system
3. To assess the diversity of zooplankton in biofloc system

REVIEW OF LITERATURE

CURRENT TRENDS IN AQUACULTURE

According to the National Oceanic and Atmospheric Administration, Aquaculture is the breeding, rearing, and harvesting of fish, shellfish, algae, and other organisms in all types of water environments. As the demand for seafood has increased, technology has made it possible to grow food in coastal marine waters and the open ocean. Aquaculture is a method used to produce food and other commercial products, restore habitat and replenish wild stocks, and rebuild populations of threatened and endangered species. There are two main types of aquaculture—marine and freshwater (Glude, John B., 1977).

According to the latest worldwide statistics on aquaculture compiled by FAO, world aquaculture production attained another all-time record high of 114.5 million tonnes in live weight in 2018, with a total farmgate sale value of USD 263.6 billion. The total production consisted of 82.1 million tonnes of aquatic animals (USD 250.1 billion), 32.4 million tonnes of aquatic algae (USD 13.3 billion) and 26 000 tonnes of ornamental seashells and pearls (USD 179 000) (FAO, 2010).

World aquaculture production of farmed aquatic animals grew on average at 5.3 percent per year in the period 2001–2018, whereas the growth was only 4 percent in 2017 and 3.2 percent in 2018. The recent low growth rate was caused by the slowdown in China, the largest producer, where aquaculture production growth of only 2.2 percent in 2017 and 1.6 percent in 2018 were witnessed, while the combined production from the rest of the world still enjoyed moderate growth of 6.7 percent and 5.5 percent, respectively, in the same two years (FAO, 2010).

Global aquaculture production more than tripled in live-weight volume from34 Mt in 1997 to 112 Mt in 2017. The main species groups that contributed to thetop 75% of aquaculture production in 2017 included seaweeds, carps, bivalves, tilapia, and catfish. Although the production of marine and diadromous fish species and crustaceans has also grown rapidly during this period, it has been dwarfed by the live-weight volume of marine bivalves and seaweeds, and by the production of freshwater aquaculture. Freshwater fish account for 75% of global edible aquaculture volume, reflecting their favourable conversion from live to edible weight in comparison to molluscs and crustaceans, which have high shell weights (Naylor, R.L, 2021).

Aquaculture is more diverse today, with 40% more fish, shellfish, aquatic plant, and algal species cultivated in a wide variety of marine, brackish, and freshwater systems globally10. Global production remains concentrated, however, with only 22 of all 425 species groups farmed in 2017 (5%) accounting for over 75% of global live-weight production. A small fraction of the ‘aquatic plant and algae’ category (~32 Mt) consisted of aquatic plants (1,639 tonnes) in 20172. Aquatic plants are listed by the Food and Agriculture Organization (FAO) under ‘aquatic plants NEI’ and are underreported given the informal nature of the harvests for household and local consumption. Asia remains the largest aquaculture producer, accounting for 92% of the live-weight volume of animals and seaweeds in 20172. Aquaculture in Asia is also more diverse than other regions in terms of production systems and cultivated species11. Nine of the top-ten ranked countries for aquaculture species diversity are in Asia, with China leading by a wide margin. As an example, China cultivated 86 different species of aquatic organisms in a variety of production systems in 2017, whereas Norway cultivated 13 different species, mainly in marine cage system (Naylor, R.L, 2021).

AQUACULTURE IN INDIA

In India, the annual fisheries and aquaculture production increased from 0.75 million tonnes in 1950-51 to 9.6 million tonnes in 2013-2014. Globally the country now takes the second position, after China, with regard to annual fisheries and aquaculture production (FAO, 2010).

According to the FAO, the total aquaculture production in 2012-2013 was 4.21 million tonnes. This constituted over a third of the country’s total fish production. This quantity is almost fully consumed on the domestic market, except for shrimps and freshwater prawns, which are mainly exported. India is the largest exporter of shrimps to the Netherlands. Specifically freshwater aquaculture experienced over a tenfold growth in the past three decades, 0.37 million tonnes in 1980 to 4.03 million tonnes in 2010 (De Jong Jelte, 2017).

Over ten percent of the global fish diversity can be found on or near the Indian subcontinent and more than 14.5 million people depend on fisheries activities. Nevertheless, the national average annual consumption of fish and fish products in 2010 was 2.85 kg/capita. In the coastal state of Kerala, fish is consumed the most, with 22.7 kg/per capita and in the mountainous state of Himachal Pradesh consumption is with 0.03 kg/capita relatively low. About 40% of the Indian population does not eat fish since they are vegetarian and the remaining 60% only occasionally consumes fish. Lower income and rural families consume less fish than higher incomes or urban families (De Jong Jelte, 2017).

The state of Kerala is gifted with rich resources of freshwater bodies suitable for aquaculture. The state has a total freshwater area of 1, 58,358 ha, consisting of reservoirs (42,890 ha), private ponds (21,986 ha), irrigation tanks (2,835 ha), freshwater lakes (1,620 ha), panchayat ponds (1,487 ha), village ponds and other water holds (1,317 ha), and check dams, bunds, barriers or anicuts (1,138 ha).

The state has 41 west-flowing and 3 east-flowing rivers, constituting an area of 85,000 ha (Salin K, 2022).

Modern fish culture in India became prominent with the success and perfection of induced spawning techniques for the Indian major and exotic carps. *Catla catla* and *Labeo fimbriatus* were successfully bred at Malampuzha, and this centre became the focus of inland fisheries development in Kerala. Trials conducted at different parts of the country laid the scientific foundation of composite fish culture techniques. Commercial hatchery production of giant freshwater prawn, *Macrobrachium rosenbergii* was also achieved with the experiments conducted at the Fisheries College, Kochi in 1987 resulting in a cost-effective technology (Salin K., 2022).

At present, freshwater aquaculture system in Kerala remains restricted to carp culture in a few private ponds, prawn cum paddy culture in limited areas in Kuttanad and Kole lands, stocking of carps in a few irrigation reservoirs, and river ranching in a few rivers on a limited scale. No serious effort has been taken to develop coldwater fish culture, game fisheries, culture of indigenous fish species of Kerala, freshwater pearls, etc. The farming of Karimeen (*Etroplus suratenis*) has recently emerged as a popular practice in ponds, and often in cages set in open water bodies. Advanced farming practices such as cage culture; pen culture and running water culture are emerging in many places, and have great potential for utilizing vast areas of freshwater bodies in the State (Salin K., 2022).

The motivation for developing any aquaculture enterprise is increasingly driven by commercial objectives. Low input–low output, subsistence orientated aquaculture remains common in some parts of the world, especially where fish is everyday food, such as in much of Asia. Households still dependent on agriculture for much of their income typically use their aquatic resources as a ‘bank’ strategically; while selling or gifting some of their crop they will also continue buying in fish from the market and/or exploiting wild stocks. Growth in export-led markets from low- and medium-income countries-based production, initially for shrimp and more latterly for white fish species (tilapia and striped catfish), has often transformed geographical areas where it is concentrated. Clusters of production and processing have become relatively prosperous, generally related to growth in employment opportunities in the value chain as a whole. Such dynamism can also stimulate competition and quality improvement and the rise of larger-scale commercial aquaculture (Little D.C ,2016)

TYPES OF AQUACULTURE SYSTEMS

Owing to the great diversity of aquaculture operations, the description of types of aquaculture systems may be complex and sometimes confusing to the novice. According to water exchange there are two main systems:

Open system is the use of the environment as fish farm (e.g., cages), i.e. the culture organisms are confined or protected within the farm in a vast amount of water (e.g. a lake or an ocean) so that water quality is maintained by natural flows and processing. There is no artificial circulation of water through or within the system. Cage system classified as open systems when they are placed within a large body of water such as an ocean or an estuary. In these cage systems the fish are generally at high density and artificial feed is supplied. Water quality is, however, maintained by natural currents and tides. Therefore, these are intensive open systems. Open systems tend to have low operating costs, as there is no requirement for pumping. Capital costs vary greatly depending on the type of culture. Seasonal variation in environment result in large variation in growth rate and this is the most disadvantages of open system (Soltan Magdy.,2016).

Recirculating systems are usually characterized by minimal connection with the ambient environment and the original water source. These systems have minimal exchange of water during a production cycle, hence the description as ‘closed’ systems. Water is added to offset the effects of evaporation or incidental losses or, more frequently, to maintain water quality. Some water is dis-charged and replaced each day in most recirculating tank systems with intensive culture. This arises from aspects of the regular maintenance system, such as removing accumulated solids from filters. Water quality in completely closed tank systems with intensive culture is much more difficult to maintain than in systems in which there is a regular 5% or more replacement per day. Even with some limited water exchange each day, water quality within a recirculating tank system will only be maintained by artificial manipulation. The cost of construction and production in intensive recirculating tank systems has limited the commercial development of these systems for grow-out production. However, the possibility of high yields with year-round production close to markets drives their development (Soltan Magdy, 2016).

To support the rapidly growing human population, food production industries such as aquaculture also needs horizontal as well as vertical expansion. The rapid growth of global aquaculture industry cannot be over emphasized because environmental and economical limitations can hamper this growth. Intensification of the aquaculture activities generates an immense amount of excess organic pollutants that are likely to cause acute toxic effects and long-run environmental risks. The foremost common method of dealing with this problem has been the utilization of continuous replacement of the pond water through the exchange. The water volume required for even small to medium culture systems can reach up to several hundreds of cubic meters per day. Parenthetically 20 m3 of water is required for the production of 1 kg shrimp (Wang 2003).

A recirculating aquaculture system (RAS) is another approach for the removal of major toxic pollutants from the culture water without causing environmental concerns. The beneficial effect of this technology is that only 10% of the total water volume is needed to be replaced on a daily basis, but due to the high operational and maintenance cost, the adoption of RAS among the farming community especially in developing countries is low. Therefore, there was a wide search for years for a low cost, sustainable, and environment-friendly technology for large-scale adoption. Biofloc technology has gained attentions recently as a sustainable and eco-friendly method of aquaculture which controls water quality, along with the production of value added microbial proteinaceous feed for the aquatic organisms (Gutierrez-Wing and Malone, 2006).

BIOFLOC TECHNOLOGY

One of the major water quality problems in intensive aquaculture systems is the accumulation of toxic inorganic nitrogenous species NH_4^+ and NO in the water. Aquatic animals, such as fish and shrimp, excrete ammonium, which may accumulate in the pond. A major source of ammonium is the typically protein-rich feed. Aquatic animals need a high concentration of protein in the feed, because their energy production pathway depends, to a large extent, on the oxidation and catabolism of proteins. In highly aerated ponds, ammonium is oxidised by bacteria to nitrite and nitrate species. Unlike carbon dioxide which is released to the air by diffusion or forced aeration, there is no effective mechanism to release the nitrogenous metabolites out of the pond. Thus, intensification of aquaculture systems is inherently associated with the enrichment of the water with respect to ammonium and other inorganic nitrogenous species. The management of such systems depends on the developing methods to remove these compounds from the pond (Avnimelech, Yoram, 1999).

If carbon and nitrogen are well balanced in the solution, ammonium in addition to organic nitrogenous waste will be converted into bacterial biomass. By adding carbohydrates to the pond, heterotrophic bacterial growth is stimulated and nitrogen uptake through the production of microbial proteins takes place. Biofloc technology is a technique of enhancing water quality through the addition of extra carbon to the aquaculture system, through an external carbon source or elevated carbon content of the feed. This promoted nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification. Immobilization of ammonium by heterotrophic bacteria occurs much more rapidly because the growth rate and microbial biomass yield per unit substrate of heterotrophs are a factor 10 higher than that of nitrifying bacteria. The microbial biomass yield per unit substrate of heterotrophic bacteria is about 0.5 g biomass C/g substrate C used (Crab *et al*., 2012).

Under such conditions, dense microorganisms develop, functioning both as bioreactor controlling water quality and also acts as protein food source for the fishes and shrimps. Immobilization of toxic nitrogen species occurs much more rapidly in bioflocs because the growth rate and microbial biomass production per unit substrate of heterotrophs are 10 times higher than those of autotrophic nitrifying bacteria (Hargreaves, 2006).

The technology works on the basic principle of flocculation (co-culture of heterotrophic bacteria and algae) within the system. Biofloc technology (BFT) has been successfully implemented in aquaculture especially shrimp farming due to economical, environmental, and marketing advantages over a conventional culture system. Compared to conventional aquaculture techniques, biofloc technology provides more economical alternative and sustainable technique in terms of minimal water exchange and reduced feed input making it a low-cost sustainable technology for sustainable future aquaculture development (De Schryver *et al*., 2008).

Carbon-nitrogen ratio (C/N) in the aquatic environment plays an important role in the immobilization of toxic inorganic nitrogen compounds into useful bacterial cells (single-cell protein) that may act as a direct source of food for the cultured organisms. Immobilization of inorganic nitrogen takes place when the C/N ratio of the organic matter is higher than 10. Thus, alteration in the C/N ratio may result in a shift from an autotrophic to a heterotrophic system. Once a mature biofloc community is established, TAN and NO2-N concentrations can be effectively controlled by either heterotrophic assimilation or autotrophic nitrification that helps to maintain their concentrations at acceptable ranges for the cultured organisms even at higher stocking densities (Xu et al., 2016).

By adding a carbon source (direct or indirect C-sources) to the culture medium in limited-discharge systems (changing C/N ratio), it is possible to obtain a significant enhancement of useful microbial growth and the fixation of toxic nitrogen metabolites (Crab *et al*., 2010).

When C/N ratio is low in the feed, carbon becomes the limiting nutrient for the growth of heterotrophic bacterial populations in the aquaculture ponds and hence the heterotrophic bacterial population will not inflate beyond a certain point due to the limited availability of carbon in the system. The C/N ratio in an aquaculture system can be increased by adding different locally available cheap carbon sources (agricultural by-products) and also by the reduction of protein content in the feed. Different organic carbon sources (glucose, cassava, molasses, wheat, corn, sugar bagasse, sorghum meal, etc.) are used to enhance production and to improve the nutrient dynamics through altered C/N ratio in shrimp culture, and C/N ratio is also widely used as a guide for analyzing the decomposition of organic matter (Alexander and Ingram 1992).

Two functional categories of bacterial populations are primarily responsible for water quality maintenance in minimal or zero water exchange systems (intensive systems) viz., heterotrophic ammonia-assimilative and chemoautotrophic nitrifying bacteria (Ebeling *et al*., 2006).

The color changes from green to brown which takes place as the culture progresses due to the transition from a mostly algal-dominant to a bacterial biofloc-dominant system. The number of bacteria in biofloc ponds can be between 106 and 109/ml of floc plug which contains between 10 and 30 mg dry matter making the pond a biotechnological industry (Avnimelech, 2007).

Microbial communities formed consist of phytoplankton, bacteria, and aggregates of living and dead particulate organic matter (Hargreaves, 2006).

According to Ju *et al*., (2008), bioflocs collected from *Litopenaeus vannamei* tanks contained 24.6% phytoplankton (dominated by diatoms like *Thalassiosira, Chaetoceros*, and *Navicula*), 3% bacterial biomass (two thirds was gram-negative and one third gram-positive), a small amount of protozoan community (98% flagellates, 1.5% rotifers, and 0.5% amoeba), and 33.2% detritus, and the remaining quantity was ash (39.25%). Only 2–20% of the organic fraction of sludge flocs is believed to be living (microbial cells) while the rest is of total organic matter (60–70%) and total inorganic matter (30–40%) (Wilen et al., 2003). Dominant bacterial species that are present in the bioflocs include *Proteobacterium, Bacillus* species, and *Actinobacterium*. Besides this, there are some other minor bacterial species such as *Roseobacter sp*. and *Cytophaga sp*. (Zhao *et al*., 2012).

The heterotrophic bacterial population utilizes the ammonium in addition to the organic nitrogenous wastes to synthesize single-cell microbial protein (Schneider *et al*., 2006) which act as natural feed for shrimps (Burford et al. 2004). The sinking rate of floc aggregate will be slow at a velocity of 1 to 3 m/h when densities of the microbial biomass move slightly above 1 g wet weight/ml (Sears *et al*., 2006).

In BFT, the microbes work for regulation of water quality through the control of nitrogen, resulting in the enhancement of the microbial proteins, which functions as a source of nutrition for aquaculture species. Moreover, the microbes in BFT play a vital role in biosecurity by inhibiting the pathogenic microorganisms' growth. For example, heterotrophic bacteria such as *Bacillus* spp. that were used as probiotics for bio-augmentation of biofloc enhanced immunity in the Indian white shrimp against pathogenic microorganisms resulting in better growth, survival, and productivity of the shrimp (Panigrahi *et al*., 2020).

Similarly, recent improvements in biofloc-dominated, super-intensive, limited-discharge systems for raising Pacific whitelegs prawn ensure higher biosecurity from viral and bacterial disease outbreaks (Jamal, Mamdoh T *et al*., 2020).

PARAMETERS OF WATER QUALITY

Water is the second most important need for life to exist after air. As a result, water quality has been described extensively in the scientific literature. The most popular definition of water quality is "it is the physical, chemical, and biological characteristics of water". Water quality is a measure of the condition of water relative to the requirements of one or more biotic species and/or to any human need or purpose (Alley, E. Roberts, 2007).

Based on its source, water can be divided into ground water and surface water. Both types of water can be exposed to contamination risks from agricultural, industrial, and domestic activities, which may include many types of pollutants such as heavy metals, pesticides, fertilizers, hazardous chemicals, and oils (Chatterjee, A. K. 1996).

Water quality can be classified into four types

Potable water: It is safe to drink, pleasant to taste, and usable for domestic purposes.

Palatable water: It is esthetically pleasing; it considers the presence of chemicals that do not cause a threat to human health.

Contaminated (polluted) water: It is that water containing unwanted physical, chemical, biological, or radiological substances, and it is unfit for drinking or domestic use.

Infected water: It is contaminated with pathogenic organism (Chatterjee, A. K. 1996).

There are three types of water quality parameters physical, chemical, and biological

Physical parameters of water quality:

Turbidity

Turbidity is the cloudiness of water. It is a measure of the ability of light to pass through water. It is caused by suspended material such as clay, silt, organic material, plankton, and other particulate materials in water. Turbidity in drinking water is esthetically unacceptable, which makes the water look unappetizing. It can increase the cost of water treatment for various uses. The particulates can provide hiding places for harmful microorganisms and thereby shield them from the disinfection process. Suspended materials can clog or damage fish gills, decreasing its resistance to diseases, reducing its growth rates, affecting egg and larval maturing, and affecting the efficiency of fish catching method.(Tarras-Wahlberg *et al*., 2003)

Temperature

Palatability, viscosity, solubility, odours, and chemical reactions are influenced by temperature. Thereby, the sedimentation and chlorination processes and biological oxygen demand (BOD) are temperature dependent. It also affects the biosorption process of the dissolved heavy metals in water. Most people find water at temperatures of 10–15°C most palatable (White, C., J. A. Sayer *et al*., 1997).

Chemical parameters of water quality

pH

pH is one of the most important parameters of water quality. It is defined as the negative logarithm of the hydrogen ion concentration. It is a dimensionless number indicating the strength of an acidic or a basic solution. Actually, pH of water is a measure of how acidic/basic water is. Acidic water contains extra hydrogen ions (H+) and basic water contains extra hydroxyl (OH−) ions (WHO, 2011).

pH ranges from 0 to 14, with 7 being neutral. pH of less than 7 indicates acidity, whereas a pH of greater than 7 indicates a base solution. Pure water is neutral, with a pH close to 7.0 at 25°C. Normal rainfall has a pH of approximately 5.6 (slightly acidic) owing to atmospheric carbon dioxide gas. Safe ranges of pH for drinking water are from 6.5 to 8.5 for domestic use and living organisms need (Cole, S *et al*., 2000).

Most aquatic animals and plants have adapted to life in water with a specific pH and may suffer from even a slight change. Even moderately acidic water (low pH) can decrease the number of hatched fish eggs, irritate fish and aquatic insectgills, and damage membranes. Water with very low or high pH is fatal. A pH below 4 or above 10 will kill most fish, and very few animals can endure water with a pH below 3 or above 11. Amphibians are extremely endangered by low pH because their skin is very sensitive to contaminants. Some scientists believe that the current decrease in amphibian population throughout the globe may be due to low pH levels induced by acid rain (Cole, S., *et al.,* 2000).

Acidity

Acidity is the measure of acids in a solution. The acidity of water is its quantitative capacity to neutralize a strong base to a selected pH level. Acidity in water is usually due to carbon dioxide, mineral acids, and hydrolyzed salts such as ferric and aluminum sulfates. Acids can influence many processes such as corrosion, chemical reactions and biological activities. Carbon dioxide from the atmosphere or from the respiration of aquatic organisms causes acidity when dissolved in water by forming carbonic acid (H2CO3) (Tomar, Mamta, 1999).

Chloride

Chloride occurs naturally in groundwater, streams, and lakes, but the presence of relatively high chloride concentration in freshwater (about 250 mg/L or more) may indicate wastewater pollution. Chlorides may enter surface water from several sources including chloride-containing rock, agricultural runoff, and wastewater (Chatterjee, A. K., 1996).

Chloride ions Cl^- in drinking water do not cause any harmful effects on public health, but high concentrations can cause an unpleasant salty taste for most people. Chlorides are not usually harmful to people; however, the sodium part of table salt has been connected to kidney and heart diseases. Small amounts of chlorides are essential for ordinary cell functions in animal and plant life (WHO, 2007)

Nitrogen

There are four forms of nitrogen in water and wastewater: organic nitrogen, ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen. If water is contaminated with sewage, most of the nitrogen is in the forms of organic and ammonia, which are transformed by microbes to form nitrites and nitrates. Nitrogen in the nitrate form is a basic nutrient to the growth of plants and can be a growth-limiting nutrient factor.A high concentration of nitrate in surface water can stimulate the rapid growth of the algae which degrades the water quality. Nitrates can enter the groundwater from chemical fertilizers used in the agricultural areas. Excessive nitrate concentration (more than 10 mg/L) in drinking water causes an immediate and severe health threat to infants. The nitrate ions react with blood hemoglobin, thereby reducing the blood’s ability to hold oxygen which leads to a disease called blue baby or methemoglobinemia. (Metcalf, Eddy, *et al.*, 2014).

Alkalinity

The alkalinity of water is its acid-neutralizing capacity comprised of the total of all titratable bases. The measurement of alkalinity of water is necessary to determine the amount of lime and soda needed for water softening (e.g., for corrosion control in conditioning the boiler feed water). Alkalinity of water is mainly caused by the presence of hydroxide ions (OH−), bicarbonate ions (HCO3−), and carbonate ions (CO32−), or a mixture of two of these ions in water. As stated in the following equation, the possibility of OH− and HCO3− ions together are not possible because they react together to produce CO32− ions:

$$OH^- + HCO_3{}^- \rightarrow CO_3{}^{2-} + H_2O$$

Alkalinity is determined by titration with a standard acid solution (H2SO4 of 0.02 N) using selective indicators (methyl orange or phenolphthalein).The high levels of either acidity or alkalinity in water may be an indication of industrial or chemical pollution. Alkalinity or acidity can also occur from natural sources such as volcanoes. The acidity and alkalinity in natural waters provide a buffering action that protects fish and other aquatic organisms from sudden changes in pH. For instance, if an acidic chemical has somehow contaminated a lake that had natural alkalinity, a neutralization reaction occurs between the acid and alkaline substances; the pH of the lake water remains unchanged. For the protection of aquatic life, the buffering capacity should be at least 20 mg/L as calcium carbonate (Davis ML, 2010).

The total alkalinity (TA) of water is defined as the concentration of titratable bases, mainly bicarbonates (HCO-3) and carbonates (CO3-2),expressed as CaCO3 equivalents. Water used for aquaculture should have a TA ≥ 20 mg L-1 CaCO3 (in freshwater)(de Holanda Cavalcante, Davi, *et al.*, 2014)

METHODOLOGY

Twelve tanks, each of 70L capacity was taken and was divided into four experimental setups each having triplicates. The experimental setup is that there will be four biofloc systems, with each of the triplicates having an alkalinity of 80ppm, 120ppm, 160ppm, 200ppm respectively. The tanks were arranged in a randomised way so that each tank of each setup has an equal probability of being affected by the weather conditions. The tanks were provided with aeration throughout the experiment.

Figure 1: Experimental Setup

The alkalinity of the water to be added into the tanks were measured prior to addition of any alkylating agents. The tanks were filled up and the alkylating agent (baking soda) was added gradually in increments so that the desired alkalinity in each setup was achieved. A period of 12 to 24 hours was given after each addition of baking soda for the carbonate ions to completely ionise in the water. The alkalinity of each system was accurately measured by titration using 0.02N H2SO4, with phenolphthalein and methyl orange as indicators.

Inoculum was added to each of the biofloc systems from the nearby Hymavathy pond. Approximately 200ml of pond water and 10ml of pond sediments were added to each tank.

Fish feed was taken as the nitrogen source. The required amount of feed is 30 grams per tonne of water. The feed was left to soak in water and then was crushed and added into each system. This induces the formation of ammonia due to decaying of fish feed.

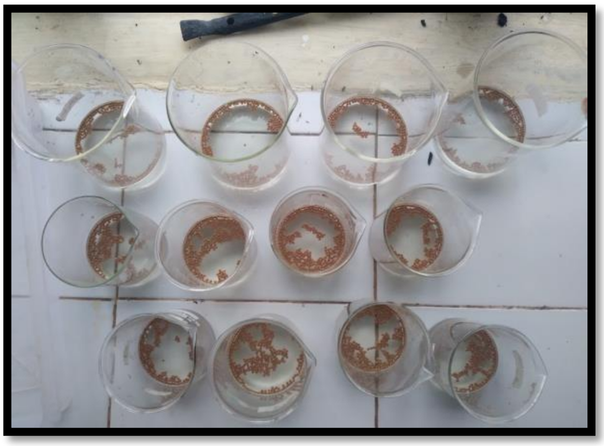

Figure 2: Fish feed used to induce ammonia formation in setup

Figure 3 :Imhoff Cone

Jaggery was taken as the carbon source. The required amount of carbon in the system must be 20 times the amount of nitrogen. Accordingly, jaggery is added. The addition of carbon and nitrogen sources was done according to the total ammonia in the system. Once the total ammonia reaches 1, only carbon source is added.

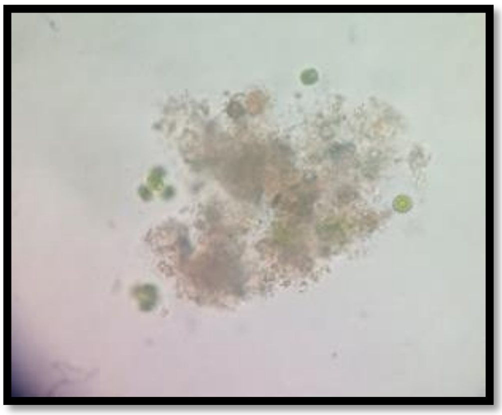

Figure 4 : formation of biofloc

Floc formation was noted every 3 days using an Imhoff cone. The total alkalinity was checked weekly and additions of the alkylating agent was added if necessary to maintain the experimental alkalinity.

Water samples from each biofloc system was taken and analysed under a stereo microscope and a compound microscope for the identification of algae and zooplankton. The identification was done by the procedure according to Edmondson (1959) and Patterson (1992).

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RESULT

1) Algal Taxonomy.

The following species were obtained from the biofloc system of 80ppm alkalinity

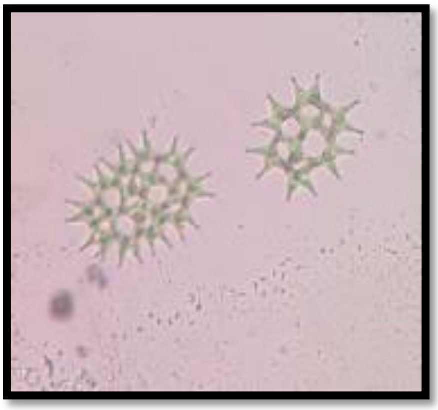

Figure 5: ***Pediastrum*** **sps**

Figure 6: ***Pediastrum*** **sps**

Figure 7 :***Selanastrum*** **sps.**

Figure 8 :***Scenedesmus*** **sps.**

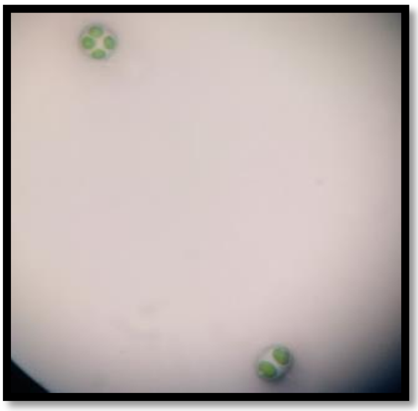

Figure 9: ***Chlorella*** **sps.**

Figure 10: ***Cosmarium*** **sps.**

The following species were obtained from the biofloc system of 120ppm alkalinity

Figure 11: ***Scenedesmus*** **sps**

Figure 12 :***Cosmarium*** **sps**

Figure 13 ***Ankistrodesmus*** **sps**

Figure 14 ***Scenedesmus*** **sps**

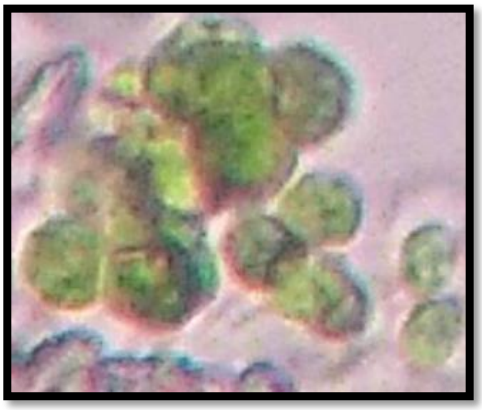

Figure 15: ***Chlorella*** **sps colony**

The following species were obtained from the biofloc system of 160ppm alkalinity

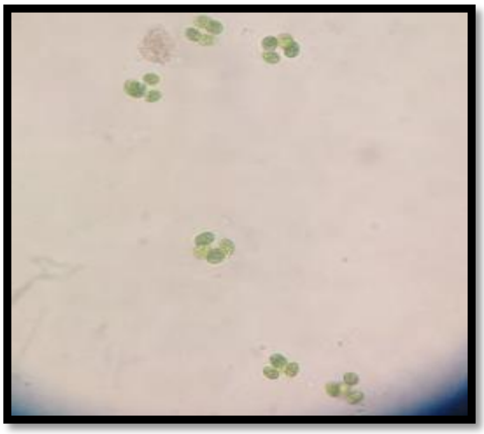

Figure 16: ***Chlorella*** **sps. colony**

The following species were obtained from the biofloc system of 200 ppm alkalinity

Figure 17: ***Cosmarium*** **sps**

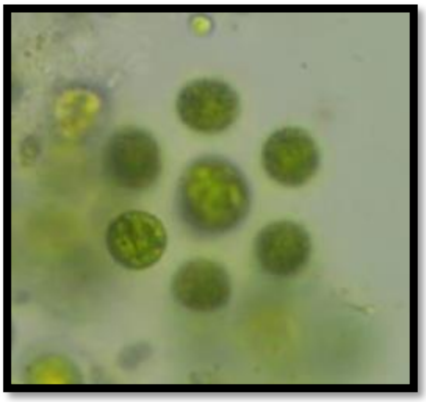

Figure 18: ***Chlorella vulgaris*** **colony**

2) Zooplankton Taxonomy

The following zooplankton were isolated from the biofloc system of 80 ppt alkalinity

Figure 19: ***Paramecium*** **sps.**

The following zooplankton were isolated from the biofloc system of 120 ppt alkalinity

Figure 20: *Paramecium* sps.

The following zooplankton were isolated from the biofloc system of 160 ppt alkalinity

Figure 21: *Rotifer* sps.

The following zooplankton were isolated from the biofloc system of 200 ppt alkalinity

Figure 22: ***Vorticella*** **sps.**

Figure 23: ***Moina*** **sps.**

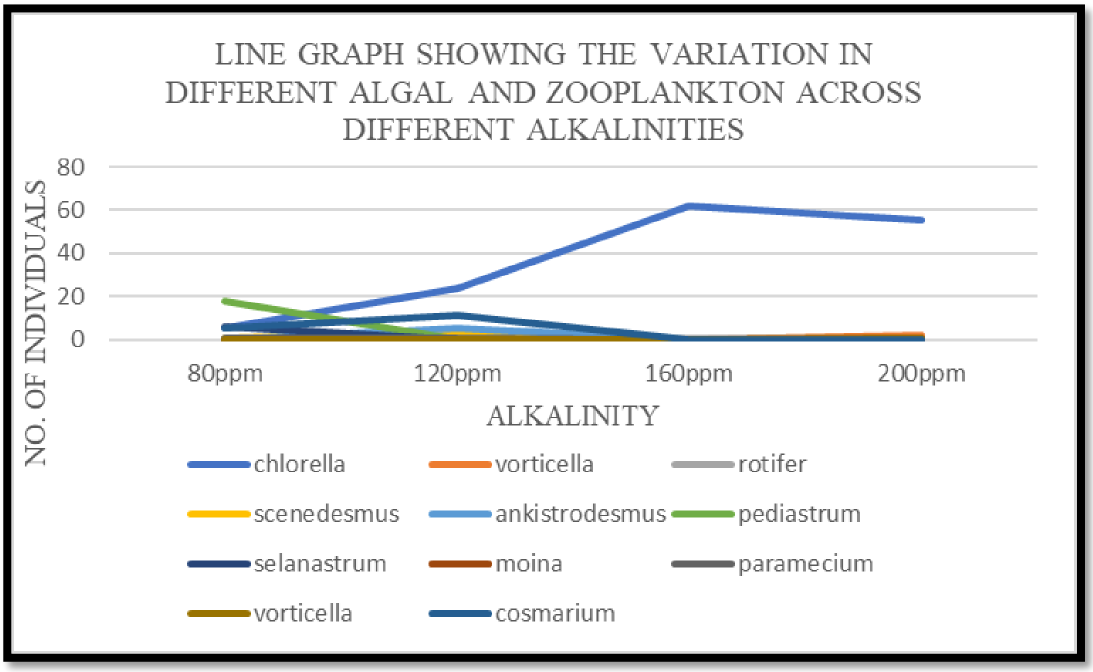

Figure 24: Line Graph Showing The Variation in Different Algal and Zooplankton Across Different Alkalinities

Experimental Setup	**80ppt**	**120ppt**	**160ppt**	**200ppt**
Pediastrum	18	0	0	0
Selenastrum	6	0	0	0
Scenedesmus	1	3	0	0
Ankistrodesmus	0	5	0	0
Chlorella	5	24	62	55
Cosmarium	5	11	0	0
Paramecium	1	1	0	0
Rotifer	0	0	1	0
Vorticella	0	0	0	1
Moina	0	0	0	1

Table 1: Table showing the Variation in number of different algal and zooplanktonic species in different alkalinities

S No	**Fig No**	**Kingdom**	**Phylum/Division**	**Class**	**Order**	**Family**	**Genus**
1	5,6	Plantae	Chlorophyta	Chlorophyceae	Sphaeropleales	Hydrodictyaceae	*Pediastrum*
2	7	Plantae	Chlorophyta	Chlorophyceae	Sphaeropleales	Selenastraceae	*Selenastrum*
3	13	Plantae	Chlorophyta	Chlorophyceae	Sphaeropleales	Selenastraceae	*Ankistrodesmus*
4	8,11,14	Plantae	Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	*Scenedesmus*
5	9,15,16,18	Plantae	Chlorophyta	Trebouxiophyceae	Chlorellales	Oocystaceae	*Chlorella*
6	10,12,17	Plantae	Charophyta	Conjugatophyceae	Desmidiales	Desmidiaceae	*Cosmarium*
7	19,20	Protozoa	Ciliophora	Ciliatea	Hymenostomatida	Parameciidae	*Paramecium*
8	22	Protozoa	Ciliophora	Ciliatea	Peritricha	Vorticellidae	*Vorticella*
9	21	Animalia	Rotifera				
10	23	Animalia	Arthropoda	Branchiopoda	Diplostraca	Moinidae	*Moina*

Table 2: Table showing the Taxonomic Classification of the species obtain up to genus level

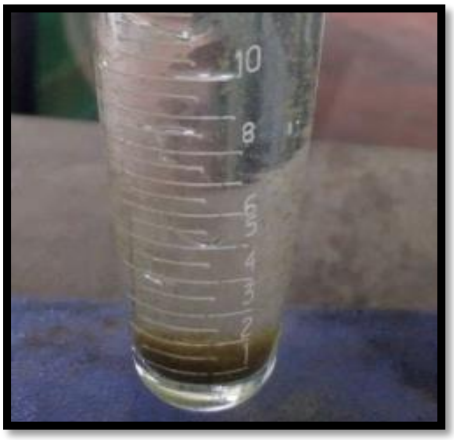

Figure 25: Floc Volume in Exp Setup 1

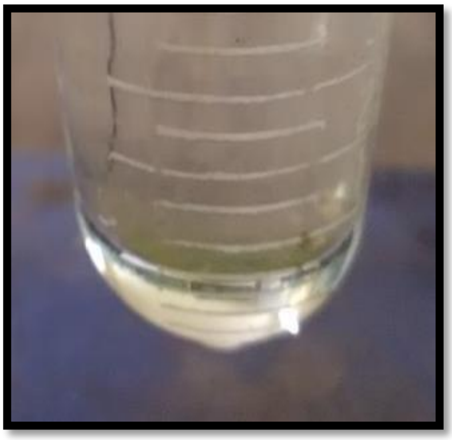

Figure 26: Floc Volume in Exp Setup 2

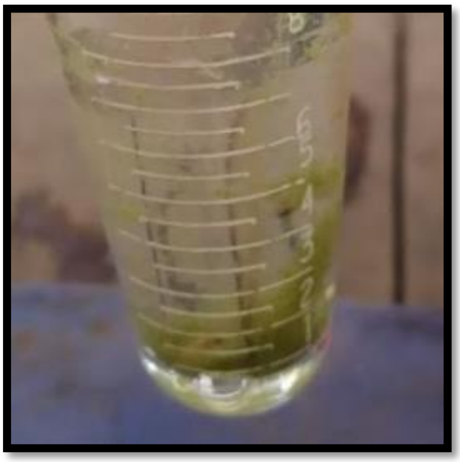

Figure 27: Floc Volume in Exp Setup 3

Figure 28: Floc Volume in Exp Setup 4

DISCUSSION

ALGAL DIVERSITY

Two distinct *Pediastrum* sps. were identified in biofloc of 80ppt alkalinity. The abundance of *Pediastrum* sps. was very high in the same experimental setup. According to Prasertsin *et al*., *Pediastrum* spp. are green algae belonging to the Division Chlorophyta, Class Chlorophyceae, Order Chlorococcales, Family Hydrodictyaceae. The dominant characteristics include the coenobia disc shape or stellate. The cells in the coenobia are usually arranged in a single layer, forming a flat circular plate. Rarely to the large coenobia have cell arranged in two layers in the center. There is one chloroplast per cells, parietal and massive and the chloroplasts contain one pyrenoid, which sometimes multiplies in older cells, particularly in the large adult coenobia. The recent genus Pediastrum was only found in freshwater (Prasertsin *et al.*,2014).

Pediastrum, genus of colonial green algae (family Hydrodictyaceae), comprising part of the freshwater plankton. *Pediastrum* colonies are disk-shaped and are characterized by peripheral hornlike projections. The number of cells per colony varies (2–128) depending on the species. Young cells are uninucleate, whereas mature cells may have up to eight nuclei. During asexual reproduction the cell contents divide and form motile spores that arrange themselves into colonies before being liberated. Sexual reproduction is by motile gametes Blokker, Peter, *et al*., 1998).

Selenastrum sps were also obtained in the biofloc systems of 80ppm and 120 ppm. In likeliness to the findings of Pugliese *et al*., *Selenastrum capricornutum* is an unicellular green algae belonging to the class of Chlorophyceae; it has a diameter comprised between 40 and 60 µm. This family includes the most common members of phytoplankton in nearly all types of inland waters. They can produce mass developments in lakes, ponds, pools and rivers. Although they commonly occur in freshwater habitats, some species tolerate moderate saline habitats as well, i.e. some taxa are reported from brackish and low saline areas of the Baltic Sea. The Selenastraceae are valuable indicator organisms for ecosystem health, and several species of this family are regularly used as indicator species, e.g. in the frame of the European Water Framework Directive. The morphology of this group comprises a variety of shapes: from coccoid to elongated, cylindricalto fusiform, sickle–shaped to spirally curved, with sharp or rounded ends, wherecell arrangements varies from the solitary to colonial forms. Their reproduction is exclusively by autospore formation, in which the cytokinesis of the mother cell protoplasm gives rise to 2–4–8 young cells. The combination of cell size, shape, solitary or colonial lifestyle, the releasing process of the autospores and special habitat preferences, are considered to be species–specific. Based on these criteria,up to 100 species were described in various genera and included in this family(Pugliese, Annarita, *et al*., 2020).

Chlorella sps was the most abundant among all the algal species. The colonies of *Chlorella* showed different morphologies as the alkalinities increased. According to Malis-Arad *et al*., *Chlorella*, genus of green algae (family Chlorellaceae) found either singly or clustered in fresh or salt water and in soil. *Chlorella* has been extensively used in photosynthetic studies, in mass cultivation experiments, and for purifying sewage effluents. Because the algae multiply rapidly and are rich in proteins and B-complex vitamins, several species have also been studied as a potential food product for humans both on Earth and in outer space. *Chlorella* is sometimes used as a vegan nutritional supplement.The algal cell is roughly spherical and features a cup-shaped chloroplast and numerous starch grains. Members of the genus reproduce asexually by nonmotile reproductive cells (autospores) that rupture through the mother cell. Those cells sometimes cling together to form a new colony (Malis-Arad, Shoshana, *et al*., 1980).

In *Chlorella vulgaris* cell aggregation, the clustering of single cells into groups is induced by an alkaline pH (9.5). The process of alkalinity-induced aggregation may be divided into two stages: the first stage (0–24 hr after exposure to the alkaline pH) is characterized by enhanced precipitation of cells from the medium, as well as by a seven fold increase in cell volume. The second stage (24–120 hr)is associated with a further increase in the extent of cell precipitation in the culture, which seems to result from the aggregation of clusters of enlarged cells.Electron micrographs reveal the existence, at this phase, of a number of autospores in the cells within a modified multi-layered mother cell wall. The pectin content of cells at this stage is twice that of control cells grown at pH 6.3.In addition, the relative content of the different pectin fractions is modified as a result of the exposure to alkalinity. It is suggested that the aggregates result fromthe repeated failure of the cells to detach from their original mother cell walls, thus forming clusters which represent several generations of cells(Malis-Arad, Shoshana, *et al*., 1980).

In likeliness to Voltolina Domenico *et al., Scenedesmus,* genus of about 70 species of colonial green algae (family Scenedesmaceae), a common component of freshwater plankton. *Scenedesmus* species are used experimentally to study pollution and photosynthesis and are a potential source of biodiesel. In sewage purification processes, the algae provide oxygen for the bacterial breakdown of organic matter and thereby help to destroy other harmful substances. *Scenedesmus* species are nonmotile and usually consist of 4, 8, 16, or 32 cells arranged in a row. Some species are spiny or feature bristles. Reproduction is by nonmotile spores called autospores (Voltolina, Domenico, *et al*., 1999).

According to Belcher J. H., The cells of cultured material of *Ankistrodesmus arcuatus* are 30-50/t long and about 2-3/z across the centre. They are strongly curved into a semi-circle, not spiral, and terminate in long fine points. The parietal chromatophore occupies almost the whole of the cell, sometimes continuing into the ends. There is no pyrenoid, and a central paler area indicates the position of the nucleus. Cell division results in the formation of four or eight autospores. For their release the mother cell wall splits longitudinally down the convex side. The split mostly stops a short distance from the tips of the cell, as shown in the illustration. The sides of the cast walls curl up irregularly, but the entire ends often remain visible. Sometimes the unsplit portion of the cell wall is considerably longer at one end of the cell than at the other, approaching the condition found in *A. pseudomirabilis* (Belcher, J. H., and E. M. F. Swale.,1962).

In accordance to Zarina A, *Cosmarium* is one of the most common genera of the desmids, individuals are single celled, with a very obvious constriction in the center, two semi-cells are connected by a central part, the isthmus, containing the single nucleus. Each semi-cell has one or two chloroplasts, and with a prominent pyrenoid. There is much variation in shape, size of cell and wall surface. Sexual reproduction is by conjugation tube. *Cosmarium* is a single-celled placoderm desmid. The cells are deeply divided in the middle by a short isthmus that contains the nucleus. The two semicells are rounded in front view and flattened, oval, or elliptic in side view. The older half of the cell wall secretes mucilage, while the younger half is lifted off of the substrate at a slight angle. The mucilage swells as it absorbs water and propels the cell forward. The cell wall may be smooth with pores or ornamented with granules, pits, or warts. The vegetative cells usually do not have spines. Each semicell has at least one central chloroplast (usually 2 or 4) with pyrenoids. *Cosmarium* sometimes produces thick-walled resting cells. Triradiate cells with three semicells have sometimes grown in collections or cultures (Zarina, A., Fariha Naz, and F. Shameel., 2014).

ZOOPLANKTON DIVERSITY

According to Fokin, knowledge about distribution and composition of the *Paramecium* genus has evolved during over two and a half centuries. More than 40 descriptions of representatives were published in the literature for these very well-known ciliates. Members of the genus *Paramecium* look very typical and distinctive and may be characterized as follows. They are mostly elongate ciliates of cigar or slipper shape (50-300 µm) with dorsal and ventral surface, a distinct oral groove or depression running from anterior left to middle right of the body on the ventral surface. The oral groove leads into the vestibulum, which terminates in an opening, the buccal overture, leading into a buccal cavity. Buccal overture is located around the middle of the ventral surface (in most *Paramecium*spp.), or could be shifted forward from the cell's equator, considerably (*P. bursaria, P. putrinum* and *P. polycaryum*) or just a little (*P. schewiakoffi* and *Paramecium* sp. from Norway). A buccal cavity has special buccal ciliature including peniculi (2 sets of ciliary structures each consisting of four converging rows), quadrulus (again four ciliaty rows, but located in some distance from eachother) and endoral kinety (membrane), a zigzag row of cilia. This peculiar buccalciliature is very stable in structure, but can be larger or smaller for different *Paramecium* spp (Fokin., 2010).

According to Segers Hendrik, Rotifera is a Phylum of primary freshwater Metazoa containing two major groups: the heterogonic Monogononta and the exclusively parthenogenetic Bdelloidea. Monogononta contains 1,570 species-level taxa, of which a majority (1,488) are free-living fresh or inland water taxa. Bdelloidea contains 461 "species," only one of which is marine, but with many limnoterrestrial representatives or animals of unknown ecology. Rotifers, mostly monogononts, occur in all types of water bodies, worldwide. They are particularly diverse in the littoral zone of stagnant waterbodies with soft, slightly acidic water and under oligo- to mesotrophic conditions (Segers, Hendrik., 2007).

Rotifers comprise a modestly sized phylum (≈1,850 species) of tiny (ca. 50–2,000 µm), bilaterally symmetrical, eutelic metazoans, traditionally grouped within the pseudocoelomates or Aschelminthes. These saccate to cylindrically shaped protostomes possess three prominent regions (corona, trunk, foot). They are distinguished by a ciliated, anterior corona (used in locomotion and food gathering) and a pharynx equipped with a complex set of jaws (Wallace, Robert Lee.,2002).

In accordance to Elias-Guiterrez, *Moina* (*Baird*, 1850) (Crustacea: Anomopoda: Moinidae) is a cladoceran genus closely related to Daphniidae (Goulden 1968, Fryer 1995). They are opportunistic cladocerans typically occurring in temporary pools, saline lakes or other waters with extreme conditions (e.g., with high temperature fluctuations) (Petrusek, A. D. A. M., 2002). The genus has a worldwide distribution (except Antarctica). The majority of the species can be found in areas with a relatively warm climate, from tropical to temperate zones. All *Moina* are small or medium-sized cladocerans. The body length of adult parthenogenetic females varies between 0.5 and 1.8 mm, depending on the species. Males are always smaller than females (Elias-Gutierrez, Manuel, *et al*., 2019).

According to Ryu *et al., Vorticella* is the largest genus of sessile peritrich ciliates and its members live in a wide assortment of marine, freshwater and terrestrial environments worldwide. Their extraordinary variety of habitats and prodigious abilities as suspension feeders suit them to be used widely as biological indicators for assessing the quality of natural bodies of water (Sun, Ping, *et al*., 2013). Vorticella is a protozoon (protist) that belongs to the phylum Ciliophora. Vorticella has two motile organelles: the oral cilia of the zooid and the contractilespasmoneme in the stalk. The oral cilia beat periodically, generating a water flowthat translates food particles toward the animal at speeds in the order of 0.1–1 mm/s (Ryu, Sangjin, *et al*., 2016).

CONCLUSION

Aquaculture is the breeding, rearing, and harvesting of fish, shellfish, algae, and other organisms in all types of water environments. . Aquaculture is a method used to produce food and other commercial products, restore habitat and replenish wild stocks, and rebuild populations of threatened and endangered species.Owing to the great diversity of aquaculture operations, the description of types of aquaculture systems may be complex and sometimes confusing to the novice. One of the major water quality problems in intensive aquaculture systems is the accumulation of toxic inorganic nitrogenous species NH4+ and NO in the water. Biofloc technology is a technique of enhancing water quality through the addition of extra carbon to the aquaculture system, through an external carbon source or elevated carbon content of the feed. Four biofloc systems were created with varying levels of alkalinity. The algal and zooplanktonic diversity of each experimental setup was studied. A total of ten planktonic species were obtained, out of which six were algal species or phytoplanktons and four were zooplanktons. The future scope of this study can be extended towards microbial taxonomy and molecular characterisation.

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