

# Nutrient Enrichment Bioassay: A Study with Indigenous Phytoplankton of a Lagoon Receiving Sugar Mills Effluents

Taofikat Abosedede Adesalu (Corresponding author)

Department of Botany, University of Lagos, Nigeria

Tel: 234-802-312-7185 E-mail: bosededesalu@yahoo.com

Maria Abimbola Akindele

Department of Botany, University of Lagos, Nigeria

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## Abstract

The response of indigenous phytoplankton population to nutrients enrichment bioassay of phosphate-phosphorus and nitrate–nitrogen of different concentrations were studied for three locations created as “before the effluents discharge; point of effluents discharge and after the effluents discharge. For the present study, physico-chemical properties of sugar milling effluents recorded 1.50 mg/L of dissolved oxygen concentration; biological oxygen demand value was 150 mg/L while chemical oxygen demand recorded 59.0 mg/L with the pH of 4.82. Four divisions, Bacillariophyta, Cyanophyta, Chlorophyta and Euglenophyta were predominantly recorded for the combinations while the optimal growth was recorded for all the stations at 100<sup>P</sup>/50<sup>N</sup> nutrients combination.

**Keywords:** Nutrients, Bioassay, Phytoplankton, Effluents

## 1. Introduction

Phytoplankton constitute the bedrock or basis of grazing food chain and food web in surface water systems, they are recognized worldwide as bioindicator organisms in the aquatic environment (Yakubu *et al.*, 2000). Aquatic organisms have been used in comparative monitoring of pollution effects in different systems and to locate sources of toxicants (Philips 1989). According to Birge *et al.*, (1989) biomonitoring approach has proved to be promising as a reliable means of quantifying biological effects of complex effluents, although a large number of aquatic organisms have been used for assessment, some workers have recognized the importance of algae as indicator in assessment and evaluation of pollution (Prasad and Manjula, 1980; Nandan and Patel, 1986; Javed and Hayat, 1996).

In Nigeria, numerous workers have also shown that phytoplankton composition and population density are suitable to draw conclusions on the conditions present in the aquatic body. (Adesalu, 2007, Adesalu *et al* 2008; Kadiri and Azomani 1993; Nwankwo and Akinsoji, 1992). Aquatic ecosystems are affected by several health stressors that significantly deplete biodiversity. In the future, the loss of biodiversity and its effect are predicted to be greater for aquatic ecosystems than for terrestrial ecosystems (Sala *et al.*, 2000). The major known sources of water pollution are municipal, industrial and agricultural while the most polluting of them are sewage and industrial waste discharges into rivers. Alam *et al.*, (2007) reported that industrial effluents contain heavy metals, acid, hydrocarbon and atmospheric decomposition and the aquatic environment is becoming polluted not only because of a single source but a variety of sources include domestic and industrial wastes. As today's technology progresses, the natural environment suffers from the detrimental effects of pollution, the growth of human population and rapid industrialization led to increasing use of urban waters as sewers, compromising their other uses.

The discharge of industrial effluents has led inevitably, to alterations in the quality and ecology of receiving water bodies thus brings new challenges to both water resource managers and aquatic ecologists. Highly coloured water, besides being aesthetically unpleasing, limits light penetration, reducing production of phytoplankton and by association, zooplankton, fish and dissolved oxygen supply. Effluents with a high temperature can be of concern because high temperatures deplete dissolved oxygen levels in the water body and in general, sugar mill effluents contain acidic and alkaline compounds, a significant concentration of suspended solids, high Biological and Chemical Oxygen demand.

The significance of measuring algal growth potential in water samples is that differentiation can be made between the nutrients of a sample determined by chemical analysis and the nutrients that are actually available for algal growth. The addition of nutrients (usually nitrogen and phosphorus singly or in combination) to the sample can give an indication of which nutrients are limiting for algal growth ( Kadiri and Azomani, 1993; Miller *et al.*, 1974; Greene *et al.*, 1975, 1976, 1978; Payne, 1976; Gerhold, 1976). The objectives of algal growth potential tests might include the establishment of baseline data, determination of the growth controlling nutrients, the presence of a toxicant, the influence of an added nutrients or the source of a nutrient or toxicant when several inflows are involved.

A certain degree of variability can be expected with any assay or survey; once the baseline data are established, changes can be measured. Sustainability of lagoon fisheries is now threatened by coastal degradation for the great majority of species which spend their earliest stages near coast, estuarine, brackish or freshwater. The most noticeable hydrological features of lagoons and estuaries ecosystem in Nigeria is the diurnal and seasonal variations in salinity level of the water usually caused by tidal effects and influx of inshore waters (Ibe, 1988). In terms of biological production, the Nigeria lagoons have wide diversity of both flora and fauna (Amadi, 1990). Dublin-Green and Tobor (1992) classified the resources in the marine environment into two: renewable and non-renewable. They include the algae, some plants and finfish, marine mammals, reptiles and shell fishes.

About 80-85% of the industries in Nigeria are located in Lagos State and they all probably discharge their effluents into the Lagos lagoon complex. The effluents discharged are mainly untreated, while very few industries have any treatment plants. The proliferation of urban and industrial establishments along the shores of the lagoon has resulted in a complex mix of both domestic and industrial wastes.

## **2. Materials and Methods**

### *2.1 Study Area*

The study was carried out on the Liverpool creek around a point that receives effluents discharged from Sugar mill refineries located at Wharf Road Tincan Island Apapa, Lagos State. The company is responsible for the milling and production of sugar, the effluents of which are conveyed through an underground tunnel into Liverpool creek connecting to the Badagry creek and deposited into the Lagos lagoon. Lagos lagoon is one of the major Lagoonal systems in Nigeria, its an extremely important ecosystem and apart from high levels of biological productivity, it plays various important ecological roles such as transportation of nutrients and organic material to marine system through circulation. The Lagos lagoon is more than 50 km long and 3 to 13 km wide, separated from the Atlantic Ocean by long sand spit 2 to 5 km wide, which has swampy margins on the lagoon side. Its surface area is approximately 6,354.7 km<sup>2</sup>. There were no vegetations present around the study site, except for site of refuse dump and stream bathing.

### *2.2 Collection of Water Samples*

The methods described by Kadiri and Azomani (1993) were used for this experiment. In addition, the effluents were also collected directly from the tunnel before getting into the lagoon and analysed for physico-chemical parameters. Water samples were collected from three points “before effluents discharge”, (N 06<sup>0</sup> 26' 03.9”, E 003<sup>0</sup> 21' 44.9”), “at the point of effluents discharge” (N06<sup>0</sup> 26' 06.2”, E 003<sup>0</sup> 21' 42.5”) and “after the point of effluents discharge” (N 06<sup>0</sup> 26' 0.94”, E 003<sup>0</sup> 21' 40.2”), using Global positioning system. The samples were filtered using 100 µm plankton net to eliminate the presence of zooplankton and other objects.

### 2.3 Laboratory Analysis

In the laboratory, twenty-one uniform size conical flasks of 100ml capacity were used for the experiment. Into each conical flask, 50ml of the water samples were measured and spiked with phosphate and nitrate in the following combinations. The concentrations of the salts used were measured using Adventure™ Ohaus weighing balance:

PO <sub>4</sub> (µg l <sup>-1</sup> )		NO <sub>3</sub> (µg l <sup>-1</sup> )
0	+	0
100	+	0
100	+	50
100	+	200
25	+	50
50	+	50
0	+	200

The experiment was set up in triplicate for the three sampling sites. The flasks content were thoroughly mixed and arranged near a north-facing window where they received light intensity from the day light and aeration. All the cultures were maintained at room temperature of  $26 \pm 2^{\circ}\text{C}$  and continuous light intensity from a fluorescent tube of 40watts. The flasks were shaken manually daily to prevent clumping of phytoplankton cells. Growth of phytoplankton was measured as optical density at 680nm using the spectrophotometer machine CE2041 at two days intervals for a period of 14 days. Microscopic examination of the treatments was carried out using Olympus XSZ-N107 binocular microscope at the end of the experiment to determine which phytoplankton grew. Adesalu (2007), Olaniyan (1975), Whitford and Schumacher (1973), Wimpenny (1966), were adequately consulted to confirm identification.

### 2.4 Physico-Chemical Analysis

#### 2.4.1 Surface Water

The surface water temperature was measured using mercury-in-glass thermometer while transparency was determined with a Secchi disc and salinity was determined using a hand refractometer.

#### 2.4.2 Analysis of Effluents

Total suspended solid and total dissolved solids were determined using the gravimetric method (APHA 1998). Total hardness was determined by EDTA titrimetric method, chloride using the argentometric method and conductivity of the effluents sample using a conductivity meter JENWAY (4071). The hydrogen ion concentration of the sample was obtained using a Cole Palmer Test 3 meter while alkalinity was measured by titration method. Dissolved Oxygen was determined using the titrimetric method; while Biological oxygen demand was done after the dissolved oxygen had been measured using the standard method of biochemical consumption of oxygen in 5 days at  $20^{\circ}\text{C}$ . With the use of closed reflux method Chemical Oxygen Demand was determined. The cations Calcium, Sodium, Magnesium and

Potassium were determined using the EDTA titrimetric method (APHA 1998). Atomic absorption spectrophotometer was used for the determination of heavy metals.

### 2.4.3 Nutrients Determination

Hach Cadmium reduction method was used for nitrate determination, (APHA 1998). Phosphate-phosphorus is known to be important in a number of ways, one being that it facilitates the uptake of nitrogen. It was determined by ascorbic acid method while gravimetric method was used to measure the sulphate, Oil and grease was determined using the carbon tetrachloride method and filtrate viewed at 450nm with a DR2000 spectrophotometer and the values obtained were recorded in milligrams per litre ( $\text{mgL}^{-1}$ ), (APHA 1998).

## 3. Results

### 3.1 Physico-Chemical Parameters

The surface water temperature at the three points of the study (before effluents, point of effluents and after effluents discharge) had a constant value of  $32^{\circ}\text{C}$  while the transparency for before the point of effluents was 9cm; point of effluents recorded the lowest value (5cm) and after the point of effluents had 29cm. The salinity values recorded 0.10‰, 0.19‰ and 0.20‰ for point of effluents, after effluents and before effluents respectively.

### 3.2 Effluents

The total suspended solid value was 130.0mg/L while total dissolved solids of the effluents had 496.0mg/L. The total hardness value recorded 988.0mg/L while the chlorine content and conductivity values were 60.0mg/L and  $1780\mu\text{S}/\text{cm}$  respectively and hydrogen ion concentration of the effluents was 4.82mg/L. Dissolved oxygen measured was 1.50mg/L and the effluents discharge had high biological oxygen demand value of 150.0mg/L. Chemical oxygen demand recorded 59.0 mg/L and the high content of Calcium and Magnesium (164.0mg/L and 824mg/L) were recorded respectively while Sodium ion recorded 23.59mg/L and Potassium ions measured was 10.50mg/L. The nutrients values of 3.20mg/L and 0.78mg/L were recorded for nitrate-nitrogen and phosphate-phosphorus respectively. Sulphate recorded same value (0.78mg/L) as phosphate-phosphorus while oil and grease value was 132.0mg/L neither Cadmium (Cd) nor Lead (Pb) was present ((Table 1).

Table 1. Results of physico-chemical parameters of Sugar mill effluents.

Parameters	Results obtained (mg/L)
Total Suspended Solid	130.0
Total Dissolved Solid	496.0
Alkalinity	ND
Total Hardness	988.0
Sulphate	0.78
Nitrate-nitrogen	3.20
Phosphate-phosphorus	0.78

Chlorine	60.0
Calcium	164.0
Magnesium	824.0
Sodium	23.59
Potassium	10.50
Cadmium	ND
Lead	ND
Biological Oxygen Demand	150.0
Chemical Oxygen Demand	59.0
Dissolved Oxygen	1.50
Hydrogen ion Concentration (pH)	4.82
Oil and Grease	132.0

### 3.3 Biological Analysis

#### 3.3.1 Phytoplankton

The list of the phytoplankton identified for the bioassay study is presented on Table 2. *Oscillatoria* spp., *Navicula* sp. and *Nitzschia* sp. were fairly populated at the point of the sugarmill's effluents discharge. Comparing the nutrients concentrations of  $100^P/50^N$  and  $0^P/200^N$ , it was observed that  $0^P/200^N$  combination recorded the highest number of organisms population ( $\leq 100.3$ ) for after point of effluents followed by before point of effluents ( $\leq 99.6$ ) while the point of effluents discharge recorded ( $\leq 71.2$ ) population showing phosphate-phosphorus as the limiting factor (Fig.1). However, the combination  $100^P/50^N$  recorded an increase in population ( $\leq 88$ ) for the point of effluents, decrease ( $\leq 100.1$ ) for after point of effluents discharge and the same value ( $\leq 99.1$ ) for before the point of effluents (Fig.2). It was observed that growth dropped at day 8 and later start increasing in most nutrients combinations for before effluents point (Figs 3) while point of effluents and after effluents point followed almost same pattern in which growth increases from day 2 to 8 after which it started declining (Figs 4 and 5) respectively. Highest value of population growth for the entire study was recorded at  $50^P/50^N$  (Fig.6). The combinations  $25^P/50^N$  and  $50^P/50^N$  followed almost same pattern with the lowest values recorded for the point of effluents discharge (Figs 6a and 6b) while  $100^P/0^N$  combinations indicated the importance of phosphate-phosphorus over nitrate-nitrogen in aquatic environment (Fig.7).

Table 2. Phytoplankton identified at the Liverpool part of Lagos lagoon after nutrient enrichment bioassay.

Treatments	Before effluents	Point of effluents	After effluents
0PO <sub>4</sub> + 0NO <sub>3</sub>	<i>Amphiphora</i> sp. <i>Nitzschia</i> sp. <i>Oscillatoria bonnetti</i> kutz <i>Oscillatoria formosa</i> <i>Oscillatoria limosa</i> (C.A. Agardh) <i>Pinnularia</i> sp.	<i>Oscillatoria limosa</i> <i>Pinnularia</i> sp. <i>Cyclotella</i> sp. <i>Euglena</i> sp.	<i>Amphiphora</i> sp. <i>Cyclotella</i> sp. <i>Surirella</i> sp. <i>Tabellaria</i> sp. <i>Pinnularia</i> sp. <i>Cosmarium</i> sp.
100PO <sub>4</sub> + 0NO <sub>3</sub>	<i>Amphiphora</i> sp. <i>Gyrosigma scalproides</i> <i>Navicula</i> sp. <i>Oscillatoria bonnetti</i> kutz <i>Pinnularia</i> sp.	<i>Cyclotella</i> sp. <i>Lyngbya</i> sp. <i>Tabellaria</i> sp.	<i>Amphicora</i> sp. <i>Cymbella</i> sp. <i>Euglena spiroides</i> <i>G. scalpoides</i> <i>Spirulina</i> sp
100PO <sub>4</sub> + 50NO <sub>3</sub>	<i>Amphiphora</i> sp. <i>Coscinodiscus</i> sp. <i>Navicula</i> sp. <i>Nitzschia</i> sp. <i>Oscillatoria formosa</i> <i>Oscillatoria limosa</i> (C.A. Agardh) <i>Spirulina</i> sp.	<i>Coscinodiscus</i> sp. <i>Euglena</i> sp. <i>Navicula</i> sp. <i>Spirulina</i> sp.	<i>Amphiphora</i> sp. <i>Navicula</i> sp. <i>Nitzschia sigmoidea</i> <i>Oscillatoria limosa</i> <i>Synedra</i> sp. <i>Volvox</i> sp.
100PO <sub>4</sub> + 200NO <sub>3</sub>	<i>Amphiphora</i> sp. <i>Coscinodiscus</i> sp. <i>Cyclotella</i> sp. <i>Navicula</i> sp. <i>Oscillatoria</i> sp.	<i>Chlamydomonas</i> sp. <i>Cosmarium</i> sp. <i>Navicula</i> sp. <i>Pinnularia</i> sp. <i>Nitzschia thermalis</i>	<i>Amphicora</i> sp <i>Navicula</i> sp. <i>Nitzschia sigmoidea</i> <i>Oscillatoria</i> sp. <i>Pinnularia</i> sp.
25PO <sub>4</sub> + 50NO <sub>3</sub>	<i>Amphiphora</i> sp. <i>Coscinodiscus lineatus</i> Ehr <i>Navicula</i> sp. <i>Nitzschia</i> sp. <i>Oscillatoria</i> sp.	<i>Chlamydomonas</i> sp. <i>Cyclotella</i> sp. <i>Navicula</i> sp. <i>Oscillatoria</i> sp. <i>Spirulina</i> sp.	<i>Amphiphora</i> sp. <i>Cymbella</i> sp. <i>Cyclotella</i> sp. <i>Euglena</i> sp. <i>Oscillatoria formosa</i> <i>Oscillatoria limosa</i> (C.A. Agardh)
50 PO <sub>4</sub> + 50NO <sub>3</sub>	<i>Amphiphora</i> sp. <i>Euglena</i> sp.	<i>Chlamydomonas</i> sp.	<i>Amphiphora</i> sp. <i>Cyclotella</i> sp.

	<i>Oscillatoria bonnetti</i> <i>Navicula</i> sp. <i>Nitzschia</i> sp. <i>Pinnularia</i> sp.	<i>Cosmarium</i> sp <i>Euglena</i> sp.	<i>Microcystis</i> sp. <i>Navicula</i> sp. <i>Nitzschia</i> sp. <i>Pinnularia</i> sp. <i>Synedra</i> sp. <i>Microcystis</i> sp.
0PO <sub>4</sub> + 200 NO <sub>3</sub>	<i>Amphiphora</i> sp. <i>Cyclotella meneghiniana</i> Kutz <i>Navicula</i> sp. <i>Oscillatoria bonnetti</i> kutz <i>Oscillatoria formosa</i> <i>Oscillatoria limosa</i> (C.A. Agardh) <i>Pinnularia</i> sp.	<i>Chlamydomonas</i> sp. <i>Cosmarium</i> sp. <i>Cyclotella</i> sp. <i>Navicula</i> sp. <i>Pinnularia</i> sp.	<i>Amphiphora</i> sp. <i>Cymbella</i> sp. <i>G. scalpoides</i> <i>Spirulina</i> sp. <i>Tabellaria</i> sp. <i>Nitzschia</i> sp. <i>Navicula</i> sp. <i>Aulacoseira</i> sp. <i>Synedra</i> sp. <i>Microcystis</i> sp.

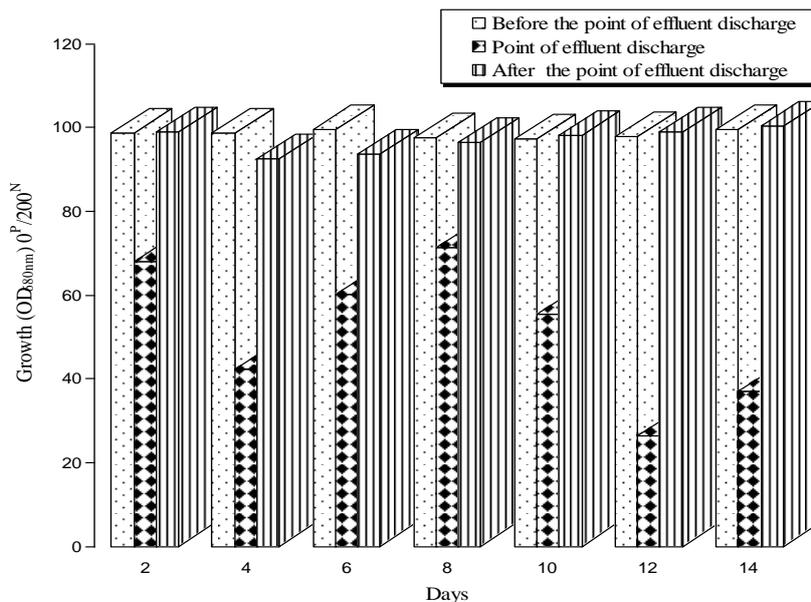


Fig.1: Growth pattern comparison for 0<sup>P</sup>/200<sup>N</sup> combination

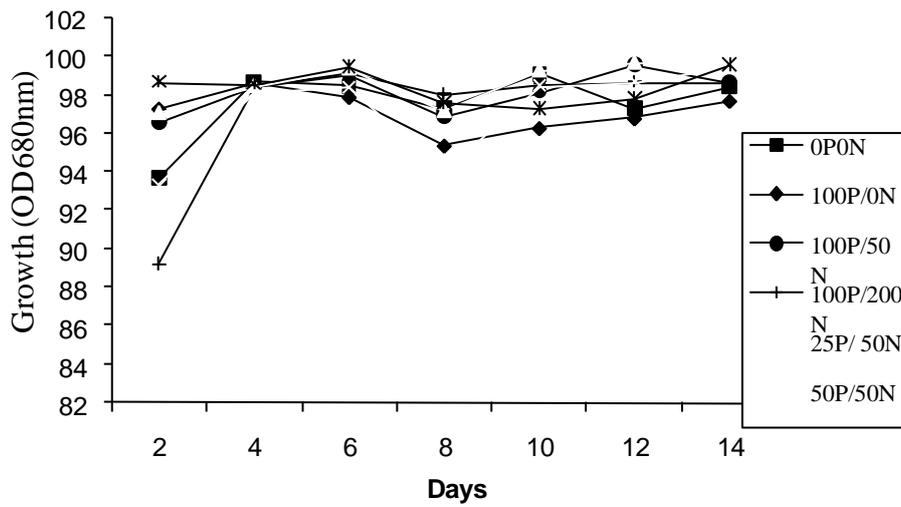


Fig.3: Growth response at "Before effluents discharge point" for all combinations.

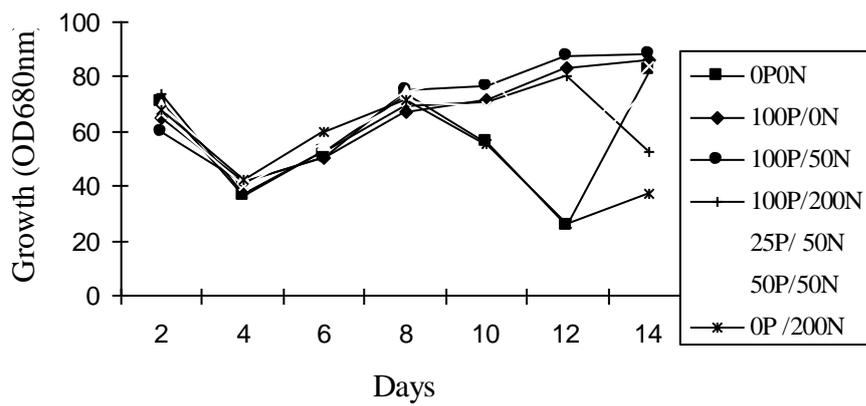


Fig. 4: Growth response at "Point of effluents discharge" for all combinations.

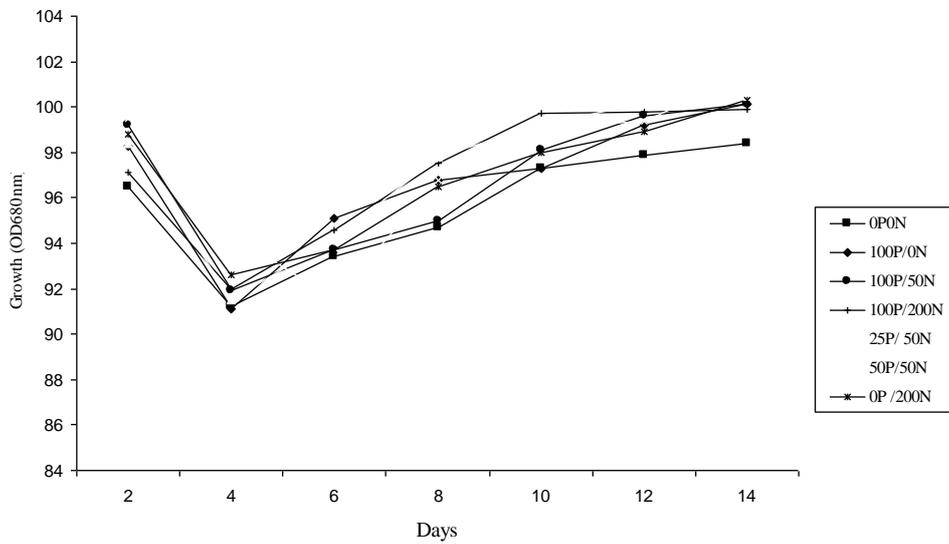


Fig. 5: Growth response at "After effluents discharge point" for all combinations.

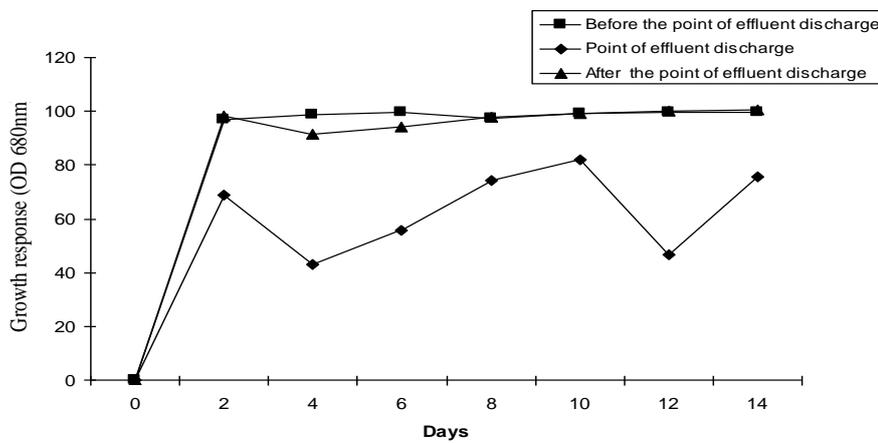


Fig.6a: Indigenous phytoplankton population growth response to 25<sup>P</sup>/ 50<sup>N</sup> nutrient enrichment at the three points.

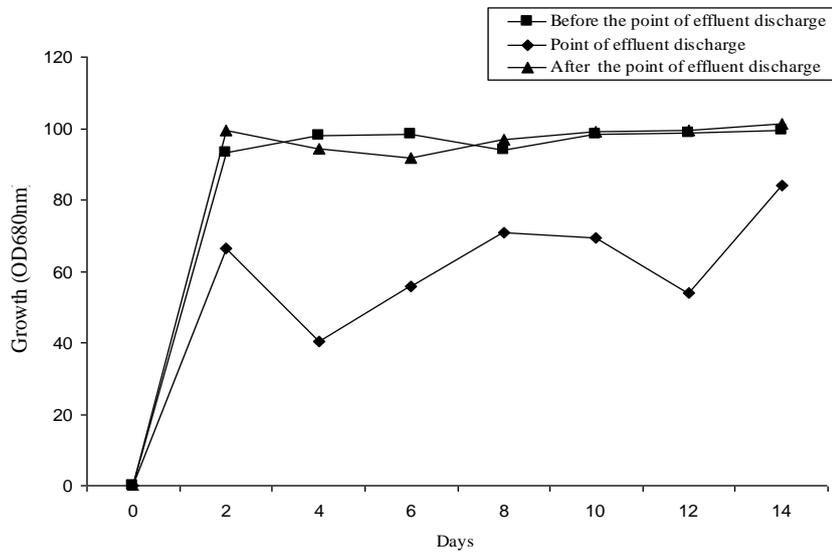


Fig.6b: Phytoplankton growth pattern for 50<sup>P</sup>/50<sup>N</sup> combinations for all the three points..

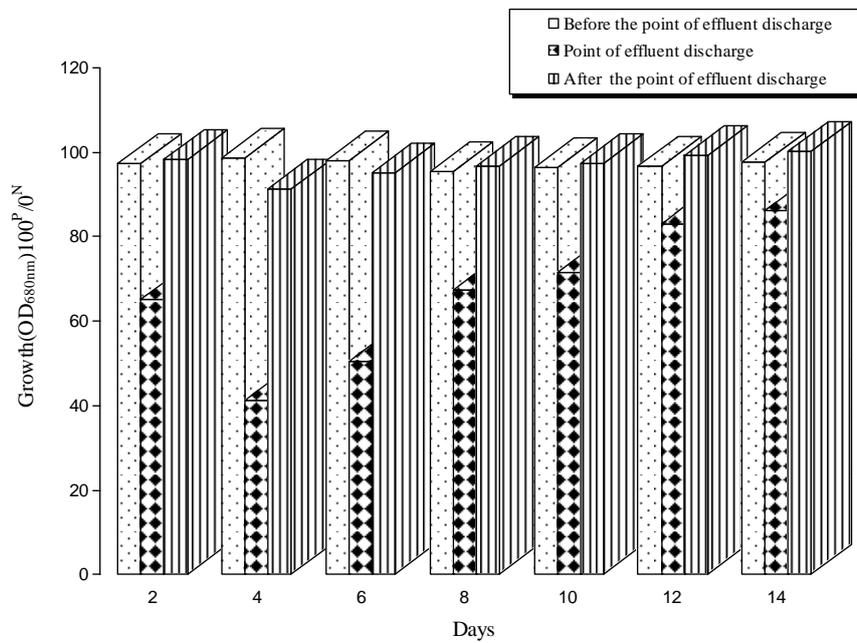


Fig.7: Phytoplankton growth pattern for 100<sup>P</sup>/0<sup>N</sup> combinations for all the three points

#### 4. Discussion

Results of the physiochemical analysis obtained for this study indicated that the effluents do not maintain the NEQS for pH, Biological oxygen demand and total dissolved solids. The nature of sugar mill effluents poses the most critical danger to the natural ecology of the local water bodies absorbing the wastewaters, with more muted impacts also likely for the fertility of the agricultural land irrigated with the effluents and the populations exposed through using contaminated groundwater. The most notable revelation of the chemical analysis, the presence of an extremely high biological oxygen demand and chemical oxygen demand levels, revealed adversity for the survival of aquatic life through a diminished oxygen supply and the production of toxic hydrogen sulphide.

The resultant foul-smelling septic conditions around the study site would also render the water body a nuisance for local residents and spoil any aesthetic value of the natural environment. These high total suspended solids levels will further endanger aquatic life as it limits light penetration and thereby, photosynthesis by phytoplankton, the primary blocks in the aquatic food chain. High total suspended solids and oil and grease can cause immediate fish kills through clogging fish gills. Agricultural yields may also be impaired through irrigation with effluents high in oil and grease content. The abundance of cyanophytes, chlorophytes and diatoms in sampling sites may probably due to favourable contents of oxidizable organic matter, rich calcium and abundant nutrients such as nitrates and phosphates with less dissolved oxygen. Rai and Kumar (1976) reported that the genus *Oscillatoria* has been found to be very tolerant to pollution which frequently inhabits the aquatic environment.

The algal growth potential principle is based on Liebig's Law of the minimum which recognizes that the development of a population is essentially regulated by the substance occurring in minimal quantity relative to the requirements of the population. Algal growth potential measurements thus are used frequently to derive information on the required nutrients which are limiting algal growth. This type of measurement is usually designed to examine in detail only a few nutrients which preliminary testing indicates may be limiting or in short supply. The approach is to observe the effects that nutrients have upon the algae. For this study, at the point of effluents the presence of phosphate nutrients probably helped in boosting the growth of organisms. Different views are held on the nutrients limiting the growth of algae and Phosphate-phosphorus has been implicated as the limiting nutrient in aquatic ecosystems (Toerien and Steyn.1973).

For  $0^P/200^N$ , the increase in population may be probably because there was a higher concentration of nitrate present which may have boosted the growth of organisms. The combinations,  $25^P/50^N$ ,  $50^P/50^N$  and  $0^P/200^N$  showed a significant differences and the great importance of nitrate over phosphate. This study conformed with the generally accepted view that phosphate limitation is related to its scarcity in most natural fresh water and their underlying geochemical substrate, relative to other chemical constituents of algae (Hutchinson 1967).

This work also in line that phosphate and nitrate are the most important in limiting primary

productivity and algal growth (Hutchinson, 1967, Schindler, 1977, Toerien *et.al.*, 1975). Some algal growth potential studies have shown correlations between orthophosphate and algal growth (Wang *et al.*, 1972), while correlations also are likely for different nutrients in other aquatic environments. This study supported Kadiri and Azomani (1993) who stated that response to nutrients enrichment by different phytoplankton divisions is somewhat dependent on the background level of the nutrient(s) in question i e limiting nutrient(s) as well as the nutrients combination.

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