IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 13, Issue 3 Ser. III (March. 2019), PP 30-36
www.iosrjournals.org

The Toxicity of Refinery wastewater effluent on Algae in Ekerekana Creek, Rivers state, Nigeria

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Abstract:*This study evaluated the effects of Refinery waste water discharge on the water quality and algae in Ekerekana creek, Rivers State, Nigeria. The sediment and water samples were collected from four sampling points in order to assess the concentration of industrial effluent in Ekerekana creek. Samples were analysed for physicochemical parameters in accordance with procedures outlined by the America Public Health Association (APHA). Ekerekana creek plays a significant ecological role as habitat to aquatic life. The results obtained are: pH (8.5), electrical conductivity (111.50-1118.97 µS/cm), DO (2.764-4.146, mg/l), BOD (6.863-1201 mg/l), Cu (0.952-2.478 mg/l), Ni (2.013-3.227 mg/l), Hg (<0.001mg/l), TSS (12.31-14.77 mg/l) and TDS (171.25-758.51 mg/l).Anabaena sp and Microcystis sp were cultured on BG11 media and isolated, the response of their mixed culture to refinery effluent was investigated using spectrophotometer to check the optical density. The result showed an increase in growth of the algae as dilution increased, hence an increase in optical density. Although, this did not follow the same pattern with respect to exposure as the percentage increase was higher in less dilutions when compared to exposure to higher dilutions of effluent, the response of algae to waste water may depend on specie. The Rural communities should not depend on the creek as a source of domestic water.*

Keywords:*Refinery effluent, Water quality, Algae, Ekerekana creek*

Date of Submission: 11-03-2019Date of acceptance: 28-03-2019

I. Introduction

Algae are the major primary producers in all types of water bodies and they contribute to water pollution in different ways [1]. Enrichments of the algal nutrients in water through organic effluents might selectively encourage the growth of algal species generating massive surface growths or 'blooms' that in turn decrease the quality of water, andaffect its use negatively. Some algae thrive in organic waste contaminated water, hence serve to purify self in water bodies. In fact, algae can play an important part in food chain of aquatic lives, and anything that disrupts the number and kinds of algae also affects all organisms in the chain including fishes[2].

The algae changed the atmosphere of the planet, gave rise to the land plants, provide half of the planet's annual oxygen supply, are directly responsible for all seafood, and indirectly responsible for all "land food," and they help supply fixed nitrogen to support life on the planet. The major gas and oil deposit came largely from Cretaceous algae. Various kinds of algae (but especially the blue–greens, reds, greens, and browns) are sources of new pharmaceutical compounds helpful in our battles against antibiotic-resistant bacterial strains plaguing our hospitals, against viral infections (including Herpes and AIDS), and against some forms of c The algae changed the atmosphere of the planet, gave rise to the land plants, provide half of the planet's annual oxygen supply, are directly responsible for all seafood, and indirectly responsible for all "land food," and they help supply fixed nitrogen to support life on the planet.. The major gas and oil deposits that we are so rapidly depleting came largely from Cretaceous algae deposits. Various kinds of algae (but especially the blue–greens, reds, greens, and browns) are sources of new pharmaceutical compounds helpful in our battles against antibiotic-resistant bacterial strains plaguing our hospitals, against viral infections (including Herpes and AIDS), and against some forms of cancer. the algae are a potential source of renewable biofuels—a source that from many perspectives is far more tractable than land plants (like corn or even sugar cane). The algae are efficient harvesters of sunlight They can grow in brackish or salt water and do not need precious crop lands for growth. The algae can strip nutrients from polluted waters and they do use lots of CO_2 to grow and prosper[3].

Water is a very essential for life and sustenance of the terrestrial and of aquatic organisms, pollution of water substance results. danger to human health, hindrance to aquatic activities, impairment of water quality with respect to its use in agricultural, industrial and often economic activities and reduction of amenities [4].

Wastewaterfrom the crude oil-processing and petrochemical industries contain large amounts of crude oil products, polycyclic and aromatic hydrocarbons, phenols, metal by-products, surface-active substances (surfactants), sulphides, naphthenic acids and other chemicals [5]. Due to an inefficiency in the cleansing systems, wastewaters could beextremely harmful, and can lead to the build-up of poisonous products in the receiving water bodies with possiblysevere negative impacts on the ecosystem [6,7]. Wastewater discharge from sewage and industries are major component of water pollution, contributing to oxygen demand and nutrient loading of the water bodies, promoting toxic algal blooms and leading to a destabilized aquatic ecosystem [8]. [6] observed an increase in variation of physicochemical parameters of refinery effluent when compared to water bodies which indicates ineffective treatment systems and illegal waste disposal practices. These effluents were observed to have negative impact on the creek's ecosystem and pose a health risk to several rural communities which rely on the receiving water body as their source of domesticwater[6].

Ekerekana creek is located in Okrika, Rivers State and servesas the receiving water body of Port Harcourt Refinery Company limited (PHRC) wastewater effluents. Oil scums floatacross the surface of the creek, especially around mangroves and roots of aquatic plants.Refinery effluents have been categorized as harmful to public health, particularly where they contain petroleum component such as the aliphatic hydrocarbons [9][10][11]).This study is aimed at evaluating the effect of waste water (refinery effluent) on primary producers (algae) inEkerekana creek in Okrika (which is the body of water that receives effluent discharge from Port Harcourt Refinery) located in Niger Delta region of Nigeria.

II. Materials and Method

Sampling Site

Samples, both effluent and sediment, are randomly collected from four sampling points 100m away from each point in Ekerekana creek receiving the Port Harcourt refining company effluents.Sampling point 1 (S1), is the effluent discharge point. Sampling point 2 (S2) is 100m away from S1 and sampling site3 (S3) is 100m away from S2. The control was collected 200m upstream.

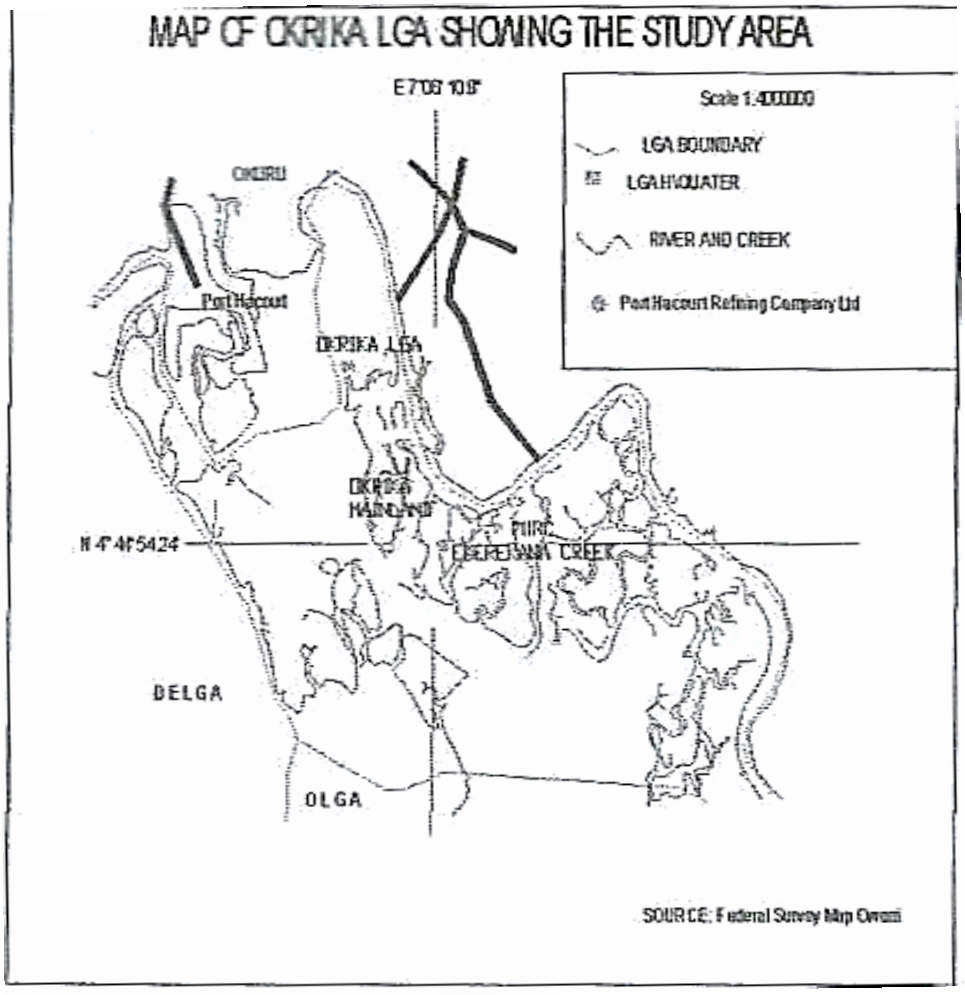

Fig. 1.Map of Okrika showing the study area

Sample collection

Acid washed, distilled water rinsed low density polyethylenebottles were used to collect sub-Surface water samples. The samples were labelled as follows: S1, S2 and S3 respectively.The collected samples were taken to the laboratory for analysis under standard conditions according to [12].

Physico-chemical Analysis

The pH, electrical conductivity, biological oxygen demand (BOD) and temperature measurements were done using HORIBA U-10 water checkerthat had been standardized with the phthalate auto calibration fluid. *In-situ* measurements were also made in order to determine the pH,temperature, total dissolved solids (TDS), turbidity, conductivity, dissolved oxygen (DO) and salinity using the HORIBA U- 10

Samples for the determination of the 5-day BOD (BOD_5) were also collected in BOD bottles and their initial DO contents determined with the HORIBA U- 10 water quality checker. Water samples for the determination of BOD_5 were left in a dark corner of the laboratory for five days incubation period at a temperature of 20 ± 2 °C [13]. On day 5, the dissolved oxygen (DO) of the samples was determined again with the HORIBA U-l0 Water Quality Checker.

Results were computed to determine BOD_5 according to the equation:

BOD_5 (mg/L) = $(DO_1 - DO_2)/P$ (1)

where DO_1 = Initial Dissolved Oxygen on Day 1,

DO_2 = Dissolved Oxygen after incubation on Day 5, and P = dilution factor.

Physiochemical parameters of water and sediment samples (Pd, Cu, Cd, Ni,Hg, TPH)

Lead, Stock Solution Corresponding to 1000mg/l of Pb

Weigh to the nearest + 0.0002gm, approx. 1.0000gm Pb metal (minimum purity 99.5%) and dilute in a covered 250ml glass beaker with 10ml HNO_3. Then add 100ml of water. Boil to expel nitrous fumes, cool, transfer to 1000ml volumetric flask and fill to the mark with water.

Lead, Standard Solution Corresponding to 10mg/ l of Pb

Pippette 10.0ml of Pb stock solution into a 1000ml volumetric flask. Add 20.0 ml of nitric acid, fill the mark with water and mix well. Prepare this solution on the day of use.

Heavy metals

The concentration of chromium, nickel, lead, mercury and copperwere done using the Atomic Absorption Spectrophotometer (AAS). A perklin Elmer 3100 atomic absorption spectrophotometer was used for the determination of heavy metals including nickel (Ni), lead (Pb), chromium (Cr),Mercury(Hg) and copper (cu).

Sediment Analysis

Sediment sample was air dried by thinly spreading on a clean laboratory bench surface at room temperature and brought to a relatively homogenous state by thoroughly mixing, and sieving with 2mm mesh before being treated. The pH and the conductivity of the sediments were determined by HACH pH conductivity meter. Total organic carbon was determined by the rapid wet oxidation method based on [14]. Nutritive salts (No-, Po42-) and exchangeable cations were determined by method outlined in [12].Total suspended solid (TSS) and TDS were measured using HACH water analysis kits (model DR2010) a 908AA spectrophotometer was used to determine dissolved oxygen, biochemical oxygen demand, nitrate, ammonia sulphate and phosphate. An HACH COD reaction was also carried out in line with the procedures in [12].

Measuring of Total Dissolved Solids

Wash filter paper, dry evaporating dish and weigh, stir sample, pipette 50 ml while stirring, filter and wash three times, transfer filtrate to evaporating dish and dry, cool and weigh, calculate in mg/L.

Calculating total dissolved solids concentration:

Ml sample

(A-B)x1000 = mg dissolved solids/L (2)

where:

A = weight of dried residue + dish, mg

B= weight of dish, mg

Microbiological Analysis

Isolation and Enumeration of algae

Tenfold serial dilution was carried out using 1ml of the sediment and 9mls of peptone water, dilution was plated out through the spread plate technique into an algae medium, BG11, themedia was prepared according to the manufacturer's instructions and incubated

Microscopic examination of algae

A drop of normal saline was placed on a slide and inoculating needle was used to pick few strands and placed in the normal saline, it was covered with a cover slip and viewed microscopically.

Identification of Algae

The isolated microalgae were identified microscopically using the light microscope at X 40 ocular with a standard manual for algae.

Preparation of the BG11 Broth for Assay

The isolates were inoculated into a BGII broth which will also be used as an inoculum for toxicity test. The medium was incubated with light intensity for 7 days. After blooming, characteristic colonies (round green) were picked from the broth and purified by serial subcultures. The pure culture was harvested by centrifugation for 20 minutes at 2500 rpm whereby the supernatant are completely removed and cells washed off for the toxicity test. Algae was identified morphologically according to [15].

***Algae* Blooming**

The optical density of algal growth was determined using spectrophotometer, a sterile pipette was used to pick 0.1ml algal of culture and placed on a slide, it was covered with a cover slip and viewed on a microscope using x 40 objective lens.

Toxicity Test

10 mlof the effluent was placed in each beaker and distilled water was used to vary the concentration of the toxicant. The quantity of distilled water was increased by one (1) ml in each beaker except the control which had no water. The ratio of effluent to distilled water is (10:0 ml, 10:1 ml, 10:2ml, 10:3ml, 10:4ml, 10:5ml, 10:6ml, 10:7ml, 10:8ml, 10:9ml; 10:10 ml).The eleven (11) test tubes with the algae media and algae culture was placed 2ml each into the test tubes. The 2mls of the algae culture was collected from the beaker the beaker and placed into each of the test tubes and spectrophotometer was used to check for the optical density of each sample and the effect of waste water on the algae, which was done by checking turbidity if the dilution.

Biomass Determination

The efficiency of algae biomass was accessed by measuring the optical density (OD), it was carried out by using a UV-VIS spectrophotometer with wavelength at 540nm and 600nm.

III. Results and Discussions

The concentrations of the measured physico-chemical parameters represented in Table 1 shows that pH ranged between 6.58 to 8.02 and these values are within the limits of EGASPIN and DPR. Electrical conductivity (EC) was within the limit of DPR and EGASPIN.The presence of inorganic solid such as chlorides affect water conductivity, it also indicates salinity. High EC above the limit indicates pollution from cations and anions sources[8]. In water, the values of TSS varied among sampling points but it did not exceed WHO limits DO, TDS, Electrical conductivity, BOD_5, pH, turbidity and electricalconductivity were found to be higher when compared to the control. Thisshowed that effluent from industries contributes to the pollution of Ekerekana River. This result is similar to previous studiesby [16] and[17].

The higher values of TSS in the water body when compared with wastewater effluent means that other sources contribute to the addition of wastewater into the water body. These may include rainstorm deposition or agricultural run off as well as run off from surrounding creeks. Variation in TSS among sample sites were observed and this agrees with the report of [6].

The values of TDS ranged from 138.02 to 458.01 ppm. However, these values where within theEGASPIN limit for effluent, but it exceeded WHO standard for portable water. High amounts of TDS affect taste in water.

The values of DO ranged from 2.98 to 5.27 ppm which are below the limit hence depletion of oxygen in water body. Increase in the level of waste discharged into the water body can reduce DO. Moderate DO level in water supports life. The values of BOD ranged from 6.28 to 8.46ppm. This is less than thespecified standards.Organic mattersare broken down by bacteria and oxygen is needed for the decomposition process, thereby reducing the DO. Increase in BOD results in reduced DO. BOD can increase because of organic

contamination which enter the water body, sewage discharge into the water body and organic matter decomposition of wastes. Decrease in DO concentration could be attributed to break of organic matter by aerobic microbes;as the oxygen required for this process is taken from the surrounding water this diminishes its total content.Low DO levels affect aquatic lives. The level of DO was also below WHO standard for drinking water. The increase in BOD value of effluent showed it did not comply with EGAPSIN and DPR limit, the lower value in treated effluent could be attributed to the presence of degradable organic matter. BOD also exceeded WHO limit for drinking water, hence, frequent discharge of refinery effluents into the water body affects the environment and health of the rural communities who depend on the creek as a major source water for domestic use. The above report is similar to the works of [6] and [8].

The concentrations of Pb, Cd, Cu and Ni in the effluent were within the DPR standard of <1.000. However, it was observed that the values at some distances away from discharge point increased above EGASPIN standard. Theselow levels maybe attributed to the capacity of the water body to purify itself]18}. The brackish water nature of the creek increases volatility of the element [19]. The concentrations of Pb and Ni exceeded EGASPIN limit and WHO limit for drinking water. However, Cd, Cu and Hg were within the limits. Most measured parameters were slightly higher in effluent and lower as distance increases from point of discharge. This is seen in the case of Cu and Cd. This suggest that there are other sources of pollution in the water body,as the metals may have entered the water body through other sources, for example, the leaching of pollutants from the waste dumpsite into the creek and subsequently into the sediment which agrees with the findings of [20] and [21]. The levels of Pb and Nickel exceeded the WHO limit for portable water but the concentration of copper in sample points .[22] also reported high concentrations of Nickel on sediments at Ntawogba river(Nigeria).

Table 1: Physiochemical Parameters

Parameter	**Control 200m upstream**	**Effluent**	**Sample Point 1 (S1)**	**Sample Point 2 (S2)**	**Sample Point 3 (S3)**	**EGASPIN Effluent Limit**	**WHO Limit (Portable Water)**	**FEPA** Effluent **Limit**
P^H	6.92	8.02	6.58	6.65	6.82	6.5-8.5	6.5-8.5	6-9
EC(µs/cm)	168.00	1118.97	126.48	168.08	111.50	1400	500.0	2500
TSS (ppm)	9.00	13.21	14.77	13.35	12.31	30	NA	30
TDS (ppm)	138.02	758.51	171.26	271.46	235.82	<2000	50	2000
DO (ppm)	5.275	2.764	3.764	4.146	3.45	4.0-5.0	8-10	8-10
BOD(ppm)	8.463	12.01	7.98	6.863	7.28.	10	<4.00	30
Pd (ppm)	0.014	0.667	0.37	0.025	0.048	0.05	0.01	<1.00
Ni (ppm)	1.581	3.227	2.142	2.013	3.0021	0.05	0.07	<1.00
Cu (ppm)	0.028	0.952	2.478	1.692	0.0972	1.5	2	<1.00
Cd (ppm)	0.004	0.0014	<0.001	0.0026	<0.001		0.003	<1.00
Hg (ppm)	0.00	<0.001	<0.001	<0.001	<0.001	0.1	0.006	<0.005

[23] EGASPIN: Environmental Guidelines and Standards for Petroleum Industries in Nigeria (2002) andWHO Drinking-water Quality 3rd edition (2008):[24] Source: FEPA, (1991).[25].

The response of mixed culture of *Anabaena* sp and *Microcystis* sp to refinery effluent as represented in Table 2 shows that dilution has effect on algal growth. The higher the dilution (lower concentration of the effluent), the higher the growth of the algae, hence higher optical density. Therefore, the higher the intensity of scattered light, the higher the growth of algae, hence increase in turbidity. This agrees with the report of [6].Algal growth increased with dilution. Generally. It was observed that the growth of algae increased with time at all concentrations/dilutions this suggests the ability of algae to tolerate or survive in pollutant.

Table 2: Response of Algae to Toxicity of Refinery Effluent within 72 hours (3 days) at Optical Densities of 540 and 690(nm)

Sample	**Day 1**		**Day 2**		**Day 3**	
	540(nm)	690(nm)	540(nm)	690(nm)	540(nm)	690(nm)
T_1	0.038	0.029	0.061	0.051	0.070	0.065
T_2	0.056	0.033	0.064	0.061	0.086	0.073
T_3	0.057	0.040	0.096	0.062	0.114	0.079
T_4	0.060	0.048	0.103	0.069	0.118	0.097
T_5	0.065	0.050	0.108	0.084	0.129	0.099
T_6	0.127	0.089	0.158	0.117	0.162	0.135
T_7	0.147	0.098	0.174	0.133	0.178	0.139
T_8	0.158	0.100	0.224	0.159	0.231	0.163
T_9	0.172	0.111	0.229	0.163	0.238	0.172
T_{10}	0.175	0.118	0.232	0.164	0.243	0.175

T_{11}	0.231	0.239	0.250	0.241	0.301	0.294

The percentage differences in algae growth based on wavelength between the lowest (T_1)and highest dilution of effluent (T_{11}) after 24 hrs of exposure at 540 nm and 690nmwere 85.5%, and 87.8% respectively. Similarly, on day 2, percentage difference at 540nm and 690nm were 75.6% and 78.8% respectively, the percentage difference after 72 hours at 540 and 690nm were 76.7% and 77.9%. The percentage difference in growth between the lowest (T_1) and highest dilution (T_{11}) after 24 hrs, 48hrs and 72 hours at 540nm were 85.5%, 75.5%, and 76.7% respectively, while the percentage difference in growth after 24 hrs, 48hrs and 72 hours at 690 nm were 87.8%, 78.8%, and 77.9% respectively at the lowest(T_1) and highest dilutions (T_{11}) this is similar to the report of [26], he observed a growth gradual decrease on the *Microcystis* at increasing concentrations of crude oil. Similarly, [27] investigated an effluent receiving stream and reported an abundance of Phytoplankton members of the Families Chlorophyceae, Cyanophyceae, Euglenophyceae in the months of July and August with the highest abundance recorded in September and October this he reported could be attributed to the peak of rainfall and favourable physicochemical parameters due to dilution of water bodies which neutralised the toxic effect of the effluent.At T_1 without dilution, after 72 hours, the percentage growth was 45.7% and 55.4 % at 540mn and 690nm respectively while at $T_{11}$with most dilution, the percentage in concentration after 72 hours were 23.3% and 18.7% at 540nm and 690nm respectively. The 45.7% and 55.4% at 540nm and 690nm after 72hrs suggests that it could thrive in an effluent polluted environment, this agrees with the findings of [28] on the treatment of Sewage and Industrial Wastewater Effluents from salt and soda company by the Cyanobacteria *Nostocmuscorum* and *Anabaenasubcylinderica* and found that fruitful.[29] similarly reported an abundance of *Microcystisflos-aquioae*, *Chroococcusvarius*, *Aphanothecebullosa*, *Coelosphaeriumkuetzingianum*, *Cyanosarcinaburmensis*, *Merismopediapunctata* and *Oscillatoriatenuis* were recorded high at the sampling stations S1 to S4 which indicated their resistance to refinery effluent.

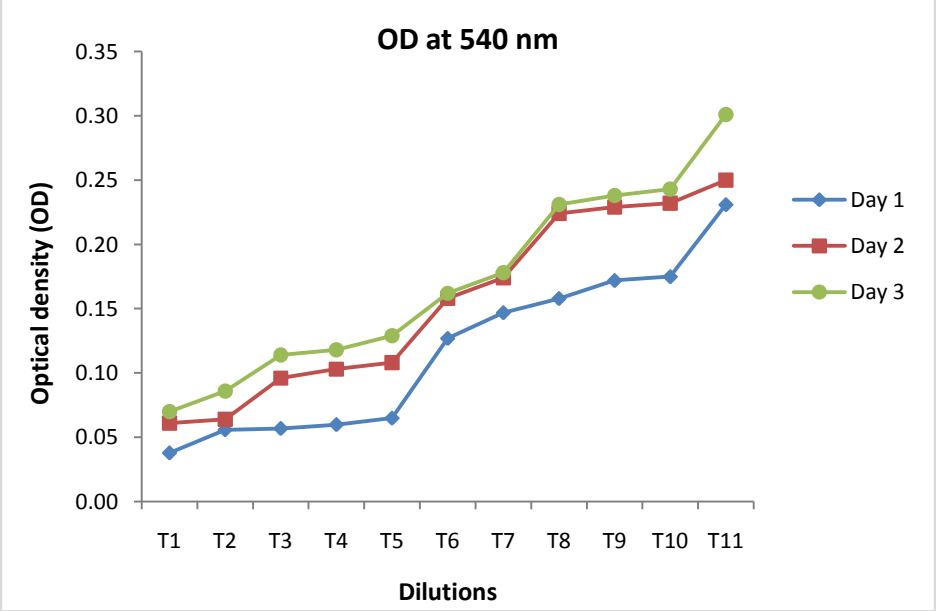

Figure 1: Variation in Algal growth for a 3-day period @540 nm OD

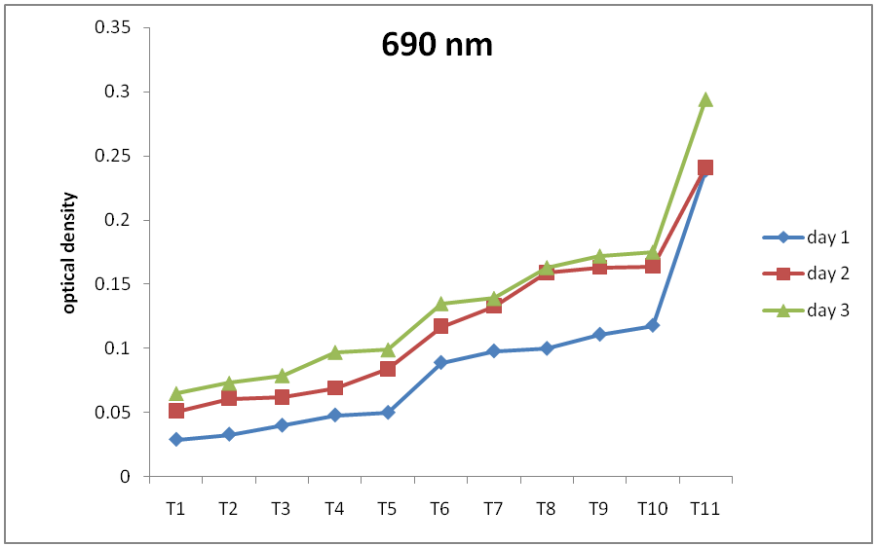

Figure 2: Variation in Algal growth for a 3-day period @690 nm OD

IV. Conclusion

Higher concentrations of most of the measured parameters from the water sample from the downstream of the creek suggests input of industrial effluents and other sources of wastes into the water Improper treatment of effluents result to marine toxicity and inhibit the survival of aquatic organisms, therefore, refinery waste water should be properly treated before discharging into the river. This study revealed that rural respondents in the community should not rely on the receiving water as their source of domestic water purpose without treatment. Waste water might influence the growth of algae, though this may depend on specie.

V. Recommendation

Toxicity testing should be carried out on individual cultures of *Mysticytis* and *Anabaena*sp rather than mixed culture to ascertain the individual responses to refinery effluent.

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