Original Research Article

Effectsof Aromatic Hydrocarbons and Marine Sedimentsfrom Niger Delta on the Growth of Microalga *Phaeodactylumtricornutum*

ABSTRACT

Aims: To determine effects of aromatic hydrocarbons and marine sediments from Niger Delta on the growth of microalga *Phaeodactylumtricornutum*.
Study Design: Fifteen treatments and the controlwere designedin triplicatesin whichlong cells containing 25 mL of the algae-toxicant dilutions were supplemented with 0.0 mg /L, 1.0 mg /L, 1.8 mg /L, 3.2 mg /L, 5.6 mg /L, and 18.0 mg /L of xylene, anthracene and pyrene each; three sediments and potassium dichromate incubated for 3 daysat 20 ± 2 °C. The fifteentreatments and control ($K_2Cr_2O_7$) designated as C0, C1, C2, C3, C4 and C5 were used to determine their median effective concentration (ErC_{50})on the growth of microalga *Phaeodactylumtricornutum*.
Place and Duration of Study: Department of Microbiology, ChukwuemekaOdumegwuOjukwuUniversity, Uli Nigeria between February, 2018 to July, 2018.
Methodology:A laboratory scale study was carried on the sediment samples from the three studied areas using physiochemical analysis and marine microalga toxicity test.
Results: The findings revealed that the three sampling sites contain higher quantities of aromatic hydrocarbons, heavy metals and other physio-chemical parameters in the sediment samples than water samples. The $K_2Cr_2O_7$had the highest ErC_{50} value of 08.07 ± 0.03 mg /L with CV and r^2 values of 68.61 % and 0.99 while pyrenein Nembe sediment had the least ErC_{50} value of 04.63 ± 0.01 mg /L with CV and r^2 values of 78.27 % and 0.98 with very strong significant positive linear relationship between algal number and sample concentrations (P = .05).
Conclusion: Thus, the toxicity results (> 1 mg /L $<EC_{50} \leq$ 10 mg /L) in this studyare in line with other toxicity values for this type of toxicants, and are therefore considered to bescientifically relevant in ecotoxicological risk assessmentof Niger Delta, Nigeria.

Keywords: Aromatic hydrocarbons, aquatic pollution, $ErC_{50,}$ *Phaeodactylumtricornutum, pubilc health, Niger Delta.*

1. INTRODUCTION

In Nigeria, the Niger Delta region produces more than 80% of the country's crude oil. There is presently an unprecedented increase in the upstream and downstream activities of the oil and allied companies in this area [1]. Over the years, these oil companies have generated myriad of pollutants in the form of gaseous emissions, oil spills, effluents and solid waste [2, 3] that have polluted the marine environment beyond sustainability. Heightened navigational activities in inland and coastal waters of the Niger Delta region is another anthropogenic source of refined petroleum pollution of the aquatic system. An

investigation of the polycyclic aromatic hydrocarbons (PAHs) concentrations in some Niger Delta sediments carried out by Ezemonye and Ezemonye[4] revealed elevatedvalues of these priority pollutants in the sediments studied.

Environmental contaminants such as hydrocarbons, heavy metals and pesticides have been known to have direct toxic effects when released into aquatic systems. There is a direct link between surface water and sediment contamination. Accumulated metals or organic pollutants in sediment could be released back into the water with deleterious effects on human health [5]. These pollutants and untreated industrial effluents can pose a major risks to aquatic life. For these reasons, many assays especially chemical and biological, have been developed to meet the demand of screening for toxic substances. Unlike some other tests, Marine Algaltoxkit, biological assays detect active toxins and do not usually require a lengthy process of sample preparation [6].

As primary producers, planktonic microalgae are a key component of food chains in aquatic ecosystems. Many species serve directly as a food source for zooplanktonic organisms, which are subsequently consumed by other invertebrates, fish or birds. Changes in the structure and productivity of the algal community may induce direct structural changes in the rest of the ecosystem and/or indirectly affect the ecosystem by affecting water quality [7]. It is therefore crucial to assess the toxicity of chemicals especially aromatic hydrocarbons to algae as the pollution is likely to end up in water bodies *via* industrial or household waste[8].

A number of studies have found algae more sensitive to chemicals than fish [9, 10, 11]. According to Kahru and Dubourguier [11] algae and crustaceans were also the most sensitive environmentally relevant species for synthetic nanoparticles. This implies that reliable algal toxicity data may help to reduce the number of fish needed for regulatory toxicity testing [12]. The same tendency has been observed by Hutchinson *et al.* [10] for active pharmaceutical ingredients (APIs); for 73 of the 91 APIs, the algal median effect concentration (EC_{50}) and daphnid EC_{50} values were lower than or equal to the fish LC_{50} data. Thus,

for approximately 80% of these APIs, algal and daphnid acute EC_{50} data could have been used in the absence of fish LC_{50} data to derive PNEC (predicted-no-effect concentration) water values.

The Marine Algaltoxkit makes use of microalgae inoculum that can be stored for several months without losing its viability. Transfer of the algal inoculum in an adequate growth medium reactivates the microalgae leading - within 3 days – to a culture in the exponential growth phase ready for the bioassay. Optical density measurements of the algal suspensions and toxicant volumes at 670 nm wavelength in 10 cm long cells correlate very well with algal numbers and are hence in accordance with the prescription of ISO Guideline 10253 and other standard methods for determination of algal densities. A 72 hrs algal growth inhibition test is performed in long cell test vials, with the marine diatom *Phaeodactylumtricornutum*. The Marine Algaltoxkit test has been modelled on and follows the prescriptions of the ISO guideline "Water Quality - Marine Algal Growth Inhibition Tests with *Skeletonemacostatum*and *Phaeodactylumtricornutum*" (Guideline ISO/CD 10253) [6].Either cell density is monitored directly by cell counting during the test, or other parameters which are related to biomass are recorded. As a result, growth rate- or biomass based EC_{50}s, NOECS and LOECS can be calculated [8].

There is dearth of information regarding environmental hazard evaluation of chemicals and polluted sites using bioassays especially microalga toxicity assayin crude oil – impacted Niger Delta marine ecosystem owing to its increasing ecological and toxicological problems and hence necessitated and justifies this study. This study was undertaken to determine effects of aromatic hydrocarbons and marine sediments from Niger Delta on the growth of microalga *Phaeodactylumtricornutum*.

2. MATERIALS AND METHODS

2.1 Description of the Sampling Sites

The studied areas were Abonema Wharf Water Front (Plate 1) in Akuku-Toru Local Government Area, Nembe Water-side (Plate 2) in Port Harcourt Local Government Area and Onne Light Flow Terminal Seaport (Plate 3) located in Eleme Local Government Area of Rivers State. Abonema town is 53 km and Abonema Wharf Water Front is 3 - 5 km from Port Harcourt capital city; Nembe water side is located

within Port Harcourt capital city of Rivers State, while Onne Light Flow Terminal is about 35 km east from Port Harcourt capital city of Rivers State and 7 km from Onne town. These sites were geo - referenced using Handheld Global Positioning System (GPS) GPSMAP 76 sc with the coordinates obtained from the sampling points or positions Abonema Wharf Water Front, Nembe Water-side and Onne Light Flow Terminal Seaport were located between latitude 4°46'15.82"N to latitude 4°46'38.01"N and longitude 7°0'0.54"E to longitude 7°0'34.82"E with average elevation of 4.1 m , latitude 4°45'8.72"N to latitude 4°45'26.42"N and longitude 7°1'11.37"Eto longitude 7° 2'14.54"E with average elevation of 2.7 m and latitude 4°41'32.58"N and 4°41'58.18"N and longitude 7°9'26.34"E and 7°10'48.82"E with average elevation of 2.3 m, respectively. These water - ways are subjected to human - induced pressures resulting from urbanization, industrialization and intensive navigation. Abonema Wharf Water Front community is a popular and busy commercial but dangerous jetty area close to Portharcourt city inhabiting tens of thousands of different families living close to petroleum tank farms and tankers queue up daily to load refined petroleum products. Nembe Waterside is situated very close to Creek road market, Port Harcourt, Nigeria. It shares boundary with Bayelsa and links Port Harcourt city with Bonny Island where most of the oil installations in Rivers State arelocated. It also links the Island directly with the Atlantic ocean through which crude oil is exported by massive oil tankers [13]. Onne Light Flow Terminal Seaport is a port of Nigeria and the largest oil and gas free zone in the world supporting exploration and production for Nigerian activities. It is situated on the Bonny River Estuary along Ogu creek and account for over 65 % of the export cargo through the Nigerian Sea Port. Anthropological survey revealed the presence of human activities such as transportation of petrochemical products through tankers, canoes, boats and ships to neighboring villages, towns, cities, states and nations due to the presence of multinational petrochemical and oil servicing industries such as Chevron Nigeria Limited, Cameron Offshore services, Exxon Mobil Nigeria Limited, SocothermPipecoaters, Beker Hughes Oil Servicing Company, Aiteo Energy Resource, Sorelink Oil and Dozzy Oil and gas et cetra that generate the wastes that contaminate the sites above.

2.2 Sample Collection and Processing

Ten samples were collected randomly at each designated points in the three particular sampling sites (Plate 1, 2 and 3) and mixed together after which a total of six representative sediment and water samples were taken for the analysis. The surface aerobic sediment samples were collected with a 95 % ethanol - sanitized plastic spatula at 5 cm depth inside 95 % ethanol - sanitized wide mouthed plastic containersafter evaporation. The water samples were collected at the air-water interface by hand dipping the 95 % ethanol - sanitized cylindrical shaped 2 L plastic containersafter evaporation. The containers were rinsed with the sediment and water samples before collecting the samples. All the composite or representative sediment and water samples containers were placed into a sterile polythene bag and then transported to the laboratory for physicochemical and algal toxicity analyses [1, 13, 17].

Plate 1. Geoeye satellite image (2016) showing the Abonema sample points

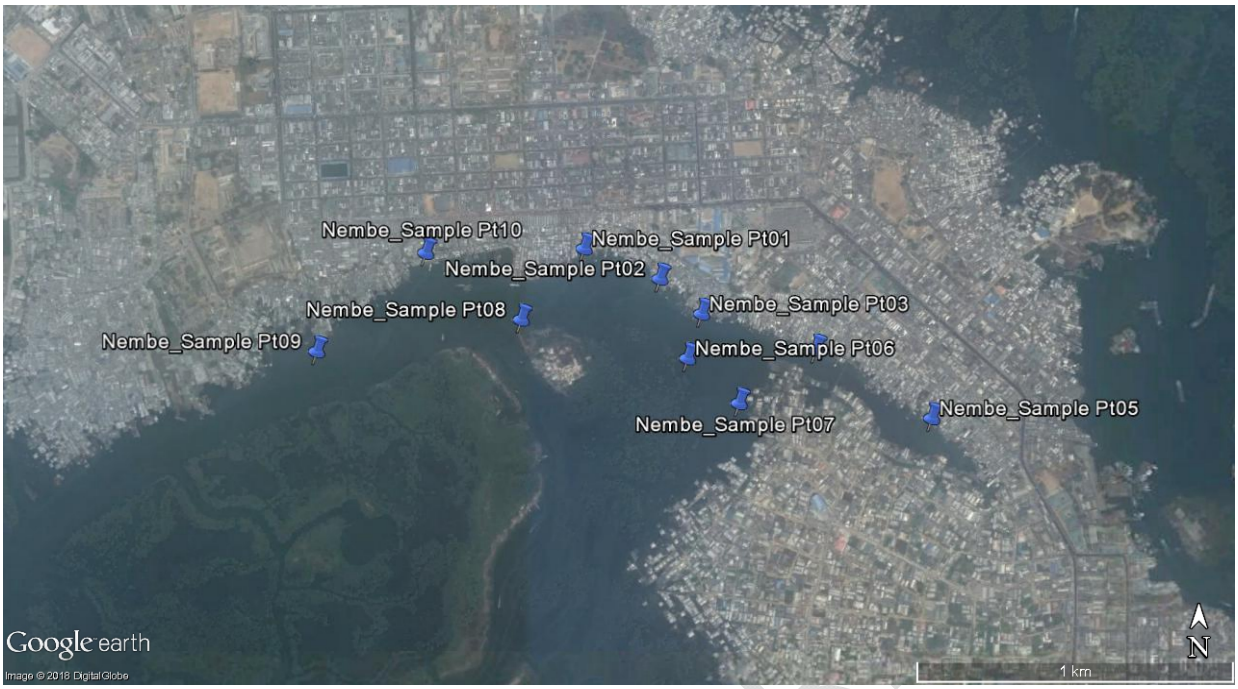

Plate 2. Geoeye satellite image (2016) showing the Nembe sample points

Plate 3. Geoeye satellite image (2016) showing the Onne sample points

2.3 Preparation of Sediment Suspensions and Extracts

The sediment–water suspensions (1:99 w/v) were prepared by shaking the sediments with sterile distilled water for 24 hrs at room temperature (25 ± 2 °C). The particle free extracts were obtained using membrane filtration technique by filtering the suspensions through glass fiber filters (d = 0.45 µm) and used for physicochemical and acute toxicity testing. The sediment:water ratio given above (1% sediment suspension) was chosen as a compromise between the expected toxic concentrationsand adequate light conditions for the growth of algae [8].

2.4Physicochemical Analyses of Sedimentand Water Samples

2.4.1 Total aromatic hydrocarbons (TAH) content analysis

TAH content was analyzed using scientific gas chromatographic system with flame ionization detector equipped with an on – column, automatic injector, mass spectroscopy, HP 88 capillary column (100 m x 0.25 µm film thickness) (M530 buck, CA USA) by adopting the standard method of AOAC [14].

2.4.2 Totalmetal and metalloid concentration analysis

Total concentration of iron (Fe), cobalt (Co), copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), zinc (Zn), nickel (Ni), Mercury (Hg) and arsenic (As) were analyzed using atomic absorption spectrophometer with oxidising air - acetylene flame(FS240AA- Agilent, USA) by adopting the standard method of APHA [15].

2.4.3 General parameters analysis

Following the standard method described by AOAC [14] and APHA [15], the pH of the sampleswere determined using a bench pH meter (PHS - 3CU, China); the conductivity and temperature were determined using a conductivity-temperature meter (DSS – 11A, China). The moisture content of the samples were determined by weighing Petri dish and sample before and after drying in the oven (DHG-9053AA, Life Assurance Scientific, UK) at 105 °C for 3 hrs. The percentage particle sizes were determined after sieving to dry weight. The soil porosity and bulk density were determined after oven-drying of the sediment soil samples at 105 °C for 2 hrsand the pore space (% porosity) was calculated

using the equation:Porosity = 1 – (particle density/bulk density) where particle density = oven - dry sediment weight / volume of sediment solids and bulk density = oven - dry sediment weight / volume of sediment solids and pores.The nitrogen content was determined using Kjedahl technique. The phosphorus content was determined using spectrophotometric technique (Astell, UV - Vis Grating, 752 W). The potassium and calcium contents were analyzed using atomic absorption spectrophometer with oxidising air - acetylene flame (FS240AA- Agilent, USA). The soil saturation was determined by measuring the time taken for the water to drop completely from the soil core. The total organic carbon was determined by titrating blank containing oxidant (potassium chromate) and sulphuric acid against the sample and the titre value was recorded. The soil texture (sediment type) were determined using soil texture triangular method. The total dissolved solids (TDS), Total suspended solids (TSS) and Total solids (TS) were determined using dry weight method on the water samples. The chemical oxygen demand (COD), dissolved oxygen (DO) and biological oxygen demand (BOD) were determined using titrimetric techniques on the water samples. Each of these analyses were carried out in triplicate determinations.

2.5 Acute Toxicity Testing

2.5.1 Analytical chemicals and reagents

Xylene of analytical grade was purchased from MERCK (PTY) Limited, South Africa (CAS NO: 1330 - 20 -7, C_8H_{10}, MW: 106.17 g/mol: MP: -34 °C, BP:136 °C, VP: 8.29 at 25 °C). The test chemical is greater than or equal to 98.5 % pure (HPLC).Anthracene of analytical grade was purchased from MERCK (PTY) Limited, South Africa (CAS: 129 - 00 - 0, $C_{14}H_{10}$, MW: 178.23 g/mol, MP: 213 - 216 ^{0}C, BP: 342 ^{0}C). The test chemical is greater than or equal to 96 % pure (HPLC). Pyrene of analytical grade was purchased from Sigma Aldrich, UK (CAS: 129 – 00 - 0, $C_{16}H_{10}$, MW: 202.25 g/mol, MP: 145-148 °C (lit.), BP: 404 °C). The test chemical is greater than or equal to 98 % pure (HPLC). Marine ALGALTOXKIT was purchased from MicroBiotests Inc. Belgium and used for toxicity testing study.

2.5.2 Marine algal toxicity test

The marine toxicity test for microalgae was carried out with marine algaltoxkit according to the standard method of EBPI [6] as follows:

2.5.2.1 Preparation of algal culturing medium (synthetic seawater)

Approximately 1,500 mL of deionized water was poured into 2 L volumetric flask and the contents of vial number 1 (NaCl) was poured into the flask and shaken until all the salt was dissolved. Similarly, the contents of the vials with concentrated salt solutions labelled number 2 (KCl), number 3 ($CaCl_2$), number 4 ($MgCl_2$), number 5 ($MgSO_4$), number 6 ($NaHCO_3$) and number 7 (H_3BO_3) were uncapped and poured into the 2 L volumetric flask.

2.5.2.2 Addition of nutrient stock solutions

Thirty millilitres (2 x 15 mL) of stock solution A, 1 mL of stock solution B and 2 mL of stock solution C were added to the 2 L volumetric flask. Five hundred millilitres of deionized water was added up to the 2, 000 mL mark and shaken to homogenize the medium.

2.5.2.3 Preculturing of the alga

One of the two tubes containing the microalga inoculum was taken, handshaken vigorously and the content was poured into one of the pre - culturing cells.The (same) tube was rinsed twicewith 7.5 mL algal culturing medium and the content was transferred into the pre - culturing cell to ensure the total transfer of the microalga inoculum.The pre - culturing cell was closed with the lid and incubated for 3 daysin an incubator (Kottermann D3165, West Germany) at 20 ± 2 °C under aconstant uniform illumination supplied by cool white fluorescent lamps.

3.5.2.4 Preparation of concentrated algal inoculum

The long cell with the label "Calibration long cell" was filled with 25 mL algal culturing medium and closed with the lid. The cell was placed in the spectrophotometer (BA – 88A MINDRAY, Germany), and the instrument was zero - calibrated. The pre - culturing cell was taken and shaken to homogenize the algal suspension after the 3 days of incubation. The cell was placed in the spectrophotometer and the optical density (OD1) was read after 10 seconds. The number of alga (N1) corresponding with OD1 was interpolated from the optical density/algal number (OD/N) standard curve. The N1/N2 ratio was calculated

inorder to determine the dilution factor needed to reach an optical density equal to OD2, corresponding to an algal density of 1.10^6 cells /mL with N2 equal to 1.10^6 algal cells /mL. The algal suspension from the pre - culturing cell was transferred into a 100 mL sterile flask and the volume of algal culturing medium needed to make up a 1.10^6 algal cells /mL suspension was added and the flask shaken thoroughly to distribute the algae evenly. The pre - culturing cell was rinsed and 25 mLof the 1.10^6 algal cells/ml was transferred into this cell, the lid on the cell was placed, shaken gently and the OD was read after 10 seconds. The OD value was finally checked on the OD/N standard curve to know if the OD corresponds with the desired OD2 value (1.10^6 algal cells /mL).

2.5.2.5 Preparation of the toxicant dilution series

Eight (8) calibrated flasks of 100 mL contents were labelled as 'Stock 1' and 'Stock 2' while the others C0, C1 to C5. Hundred milligrams of potassium dichromate was weighed on an analytical balance and transferred into the 'Stock 1' flask. Algal culturing medium was added to the 100 mL mark and shaken to dissolve the chemical and obtained a 1 g /L (Stock 1) concentration. Ten mL from 'Stock 1' was transferred into 'Stock 2' flask and filled to the 100 mL mark with algal culturing medium. The contents were shaken to homogenize and obtained a 100 mg /L (Stock 2) toxicant concentration. The following volumes of toxicant solution was transferred from 'Stock 2' into the following flasks: - 18 mL to flask C1 (18 mg /mL), 5.6 ml to flask C2 (5.6 mg /mL), 3.2 mL to flask C3 (3.2 mg /mL, 1.8 mL to flask C4 (1.8 mg /mL) and 1.0 mL to flask C5 (1.0 mg /mL). The algal culturing medium was added up to the 100 mL mark in the C0, C1, C2, C3, C4 and C5 flasks. Zero point nine mLof the 1.10^6 cells /mL of algal stock in the tube was added to each flasks (except the stock flasks), in order to obtain an initial concentration of 1.10^4 algal cells /mL in each toxicant concentration. The flasks were closed and shaken thoroughly to distribute the algal suspension evenly.

2.5.2.6 Reference test

A quality control test was carried out with the reference chemical potassium dichromate ($K_2Cr_2O_7$) and all the steps inpreparation of the toxicant dilution series were repeated in like manner.

2.5.2.7 Transfer of the algal - toxicant dilutions into the test vials

The holding trays were taken, the rubber bands and the plastic strips were removed, and the long cells in sets of 3 (a, b, c) for each concentration (from CO to C5) were marked. All the cells were opened by lifting up one end of the lids. After thoroughly shaking, 25 mL of the algal - toxicant dilutions from each flasks were transferred into the corresponding 3 long cells. All cells were opened and shaken. The OD value of each long cell was determined in the spectrophotometer (BA – 88A MINDRAY, Germany) and expressed as 0 h (T0) OD values. The T0 data were recorded on the results sheet.

2.5.2.8 Incubation of the test vials

All the long cells were put back into their holding trays and all the lids on the same side were lifted up slightly and the plastic strips were slid over the open part of the long cells, taking care to leave an opening near the middle of the long cells for gas exchange. The long cells were placed in the holding tray in a random way (that is not in the sequence of C0 to C5, and not all three parallels next to each other), in order to compensate for possible small "site to site" differences during incubation. The holding trays were incubated for 3 daysin an incubator (Kottermann D3165, West Germany), at 20 ± 2 °C under aconstant uniform illumination supplied by cool white fluorescent lamps.

2.5.2.9 Scoring of the results

The inhibition of the algal growth relative to the control was determined by daily measurement of the OD of the algal suspensions in the long cells during the 3 days of the test, i.e. after 24 hrs, 48 hrs and 72 hrs exposure to the toxicant. The long cells were put back in the holding trays in a random way after the daily measurement. The daily results for each long cell were recorded on the results sheets.

2.5.2.10 Data treatment and expression of results

The mean daily OD values for the 3 replicate long cells of each toxicant dilution were calculated. The algal growth inhibition from these data was calculated by integrating the mean values from t0 to t72 hrs (termed as the "area under the curve") for each concentration tested, including the control. The growth curves for each test concentration and control as a graph of the logarithm of the mean cell density against

time was plotted. The average specific growth rate (μ) for each test culture was calculated using the equation:

$$\mu = \frac{\ln NL - \ln No}{tL - t0}$$

where: *t0* is thetime of test start; *tL*is the time of test termination or th time of the last measurement within the exponential growth period in the control; *No* is the nominal initial cell density; *NL* is the measured cell density at time *tL.* Alternatively, it was also calculated from the slope of the regression line.

The percentage growth inhibition for each test concentrations was calculated using the equation:

$$I\mu i = \frac{\overline{\mu c} - \overline{\mu i}}{\overline{\mu c}} \times 100$$

where: *I*μi is the percentage inhibition (growth rate) for test concentration *I*; $\overline{\mu i}$ is the mean growth rate for test concentration *i*; $\overline{\mu c}$ is the mean growth rate for control *c.*The effective concentration (E_rC_x)was determined by tabulating and plotting the normalized inhibition (*I*μi) against the test concentration on the logarithmic scale.

2.6 Data Analysis

The data were analyzed using Graph-Pad Prism statistical software version 7.00 (GraphPad software Inc. San Diego, California). All values were expressed as mean ± standard deviation. Ordinary one-way analysis of variance (ANOVA) followed by post Tukey's, multiple comparison test was performed on the data obtained. Also, a linear model was suitably fitted to the experimental data points by probitregressional analysis and the median effective concentration (E_rC_{50}) was determined by the equations:

Y= Slope X + Intercept Y and Antilog EC_{50}.

The results were considered statistically significant if the probability is less than .05 (*P* = .05).

3. RESULTS

3.1 Physio - Chemical Analyses

3.1.1 Total aromatic hydrocarbon (TAH) content analysis

The result of the total aromatic hydrocarbon fractions (ppb) of sediment and water samples of the three sampling locations is presented in Table 1. From the result, there are more non-significant (*P*> .05) hydrocarbon fractions in sediment than water samples with anthracene (116.40 ± 0.12 ppb) and pyrene (05.90 ± 0.30 ppb) hydrocarbons having the highest fractions in Nembe sediment and Onne water samples, respectively while 1, 2-benzoanthracene as well as benzo (b) fluoranthene and acenaphthylene were not detected in sediment and water samples.

3.1.2 Totalmetal and metalloidconcentration analysis

The result of the total metal and metalloidconcentration (ppm) of sediment and water samples of the three sampled locations is presented in Table 2. From the result, there are more non-significant (*P*> .05) metal and metalloidconcentrations in sediment than water samples with iron metal having the highest fractions in both Abonema sediment (110.24 ± 0.20 ppm) and water (03.27 ± 0.25 ppm) samples, respectively. The order of concentration abundance of these metals and metalloidsin sediments from the three locations is in the sequence: Fe > Zn > Cu > Ni > Cr > Hg > Cd > Co >Pb> As while the order of concentration abundance of these metals and metalloidsin water from the three locations is in the sequence: Fe > Zn > Hg > Ni > Cd > Co > Cr > Cu >Pb> As.

3.1.3 General parameters analysis

The result of the general parameters of the sediment and water samples of the three sampled locations are presented in Table 3 and Table 4. From the tables, conductivity at 25 °C (EC25) for Onne, Abonema and Nembe sediments are 24.10 ± 0.25 µS/cm, 20.60 ± 0.31 µS/cm and 19.91 ± 0.02 µS/cm while Onne, Abonema and Nembe waters are 69.90 ± 0.27 µS/cm, 19.74 ± 0.03 µS/cm and 13.22 ± 0.13 µS/cm respectively. Onne sediment and water samples exhibited lower pH values (06.72 ± 0.02 and 06.41 ± 0.02) followed by Abonema (07.47 ± 0.02 and 06.55 ± 0.02) and then Nembe (07.16 ± 0.02 and 07.31 ± 0.16) respectively. All the sediment samples had greater percentage of sand followed by clay and then silt with Onne sediment having the highest sand percentage of 59.72 ± 0.02 %. Grain size measurements of superficial sediments revealed that Abonema and Nembe contained clayey loam whereas Onne contained sandy loam. Nembe had the highest moisture content, porosity, bulk density and total organic

carbon of 85.94 ± 33.11 %, 0.04 ± 0.00, 01.72 ± 0.00 g/mL and 17.82 ± 0.03 % and lowest soil saturation time of 18.00 ± 0.03 minutes which contained clayey loam sediment type. Nembe had highest total nitrogen (TN) of 03.81 ± 0.01 % and 0.97 ± 0.01 % whereas Onne and Abonema had highest total phosphorus (TP), potassium (K) and calcium (Ca) content of 05.50 ± 0.00 mg/L and 03.72 ± 0.02 mg/L; 11.45 ± 0.00 ppm and 04.60 ± 0.01 ppm; and 12.78 ± 0.02 ppm and 06.30 ± 0.01 ppm for both sediment and water samples respectively. Furthermore, Abonema water having the highest COD (106.60 ± 65.56 mg/L), DO (25.30 ± 0.08 mg/L), BOD (210.00 ± 2.52 mg/mL), TDS (07.48 ± 0.01 mg/L), TSS (0.15 ± 0.00 mg/L) and TS (07.63 ± 0.01 mg) followed by Nembe and Onne water samples. The COD and BOD values were found to be higher than WHO standards except TDS, TSS and TS that were found below the maximum recommended limits. Non-significant differences (*P*> .05) were detected in all the sampled parameters

4.2 Acute Toxicity Testing

4.2.1 Marine algal toxicity assay

The results of the mean values of cell algal density measurements (Cells /mL X 10^4) with their respective coefficient of variations, specific growth rates and percentage inhibitions at different concentrations of the aromatic hydrocarbon contaminated distilled water and sediment samples during 0 -72 hrs are presented in Tables 5, 6, 7 and 8. From the Tables, there were exponential growth patterns in the control test cultures (μ = 0.083 hr^1) while continuous inhibitions were observed at the varying test concentrations of the test samples.The result of the inhibition of growth rate of *Phaeodactylumtricornutum* by aromatic hydrocarbon contaminated distilled water and sediments of the three sampled locations is shown in Figure 1. From the result, there were drastic reductions in their respective specific growth rates leading to remarkable increase in the percentage inhibition values of all the test concentrations of the test samples with pyreneNembe sediment having the highest inhibition of 98.19 ± 0.07 % and μ = 0.200 hr^{-1} during 0 – 72 hrs. Moreso, the result of the mean 72 hr ErC_{50} (mg /L) toxic response of *Phaeodactylumtricornutum* to aromatic hydrocarbon contaminated distilled water and sediments of the three sampled locationsis shown in Figure 2. From the Figure, positive control ($K_2Cr_2O_7$) had the highest ErC_{50} value of 08.07 ± 0.03 mg /L with CV and r^2 values of 68.61 % and 0.99 while pyreneNembe sediment had the least ErC_{50} value of

04.63 ± 0.01 mg /L with CV and r^2 values of 78.27 % and 0.98 with very strong significant positive linear relationship between algal number and sample concentrations (P = .05).

4. DISCUSSION

Aquatic ecotoxicology has rapidly matured into a practical discipline since its official beginnings in the 1970's [16]. Integrated biological/chemical ecotoxicological strategies and assessment schemes have been generally favoured since the 1980's to better comprehend the acute and chronic insults that chemical agents can have on biological integrity. However, the experience gained with the bioassay of solid or slime like wastes is as yet inadequate [16].

In this study, an attempt was made to determine effects of aromatic hydrocarbons and marine sediments from Niger Delta on the growth of microalga *Phaeodactylumtricornutum*and the result in Table 1 showed that the detectable hydrocarbons values were above the WHO standard for PAHs (50 ng/l) in water. The reason may be due to the hydrophobic and insoluble nature of aromatic hydrocarbons to water molecules making them to adsorb more on the surface of sediments than water samples. Also, this result suggest that Nembe water side is more polluted than other sampled locations probably due higher particle sizes, higher total organic contents and the numerous anthropogenic activities that go on there, as a result of introducing and absorbing more aromatic hydrocabons. The result is similar to the work done by Gorleku*et al.* [17], who reported that total mean concentrations of the PAHs in the sea water are generally less than concentrations in sea sediments. Polycyclic aromatic hydrocarbons are non - polar, hydrophobic compounds which do not ionize. They have a relatively low solubility in water, but are highly lipophilic. Dissolved and colloidal organic fractions also enhance the solubility of PAHs which are incorporated into micelles. Due to their hydrophobic nature, PAHs entering the aquatic environment exhibit a high affinity for suspended particulates in the water column. As PAHs tend to adsorb to these particles, they are eventually settled out of the water column onto the bottom sediments. Thus, the PAH concentrations in water are usually quite low relative to the concentrations in the bottom sediments. Emoyan [18], reported the concentration range of 0.2309 to 1.0468 mg L^{-1} for PAHs in surface water due to contamination from

Kokori - oil field in the Niger-Delta. Fluorene was the dominant of the 16 PAHs priority pollutants investigated. The source of water contamination was identified to be mainly petrogenic.

Metal and metalloidpollution in marine systems is determined by measuring its concentration in water, sediment and living organisms [19]. The result in Table 6 revealed that all metals except iron, cadmium nickeland mercurywere lower and within for water samples but higher for sediment samples in comparison to the Federal Ministry of Environment (FME)water standards for aquatic life fresh (FWA) Nigeria,maximum permissible recommended limits(MPL)and agreed with the finding of Olusola and Festus [19]. The studied elements are individually known to be mutagens and carcinogens. In other words, they are toxicants. The higher levels of thesemetals and metalloidin sediments of the coastal water could be attributed to industrial and agricultural discharges, iron, steel and sewage materials from vessels and residential area and possible spills of petrol petroleum products from fishing boat, speed boat and ships used as means of transportation over the years. Similar observations were made by Obiajunwa*et al*. [20], who reported that the enrichment factors for Sr, Zn, Pb, Ba, and Fe were very high for every soil, sediment and solid waste samples in Niger Delta, Nigeria. The study summarize that there is significant relationship between metal and metalloidpollutions and crude oil production industry which may be spillage have occurred in the process of production. This is very harmful because the high contamination of metal and metalloidis very dangerous to both aquatic environment and human health. Owamah [21], reported that the enrichment factors for Cd, Cr, Cu, Fe, Ni, and Pb were very high for water and sediment samples in Niger Delta, Nigeria. Olusola and Festus [19], reported that the concentrations of Cd, Cu, Cr, Pb and Zn in the sediment samples of coastal waters of Ondo State Niger Delta were much below the probable effect concentration of sediment metals levels. Also, by comparing the concentrations of metaland metalloidanalyzed in the water and sediments samples, it can be concluded that metal and metalloidare highly accumulated in sediments than water confirming what have earlier been reported that sediments act as reservoir for all contaminants and dead organic matter [19, 22].

The result in Table 7 and 8revealed thatOnne water and sediments have greater content ions, carry more current and more salty than other sampling locations although there can be natural variability such as

temperature, tidal and seasonal flushing. Also, they give surrogate values of levels of salinities and total dissolved solids, (TDS) and the samples were found to be acidic and neutral.Hassanshahian*et al.* (2010), reported that the electrical conductivity of the Persian Gulf sediments was 6.5 (ds m^{-1}) in comparison with 8.4 (ds m^{-1}) value in the Caspian Sea sediments. Interestingly, the Persian Gulf is located in the south of Iran with warm weather and oil production area while the Caspian Sea is located in the north of Iran with rainy and temperate weather. The acidic pH value of the sandy loam sample could be interconnected with buildup of acidic metabolites and low mineral content of the soil [23]. All the sediment samples had greater percentage of sand followed by clay and then silt. This observation is similar to the work carried out by Amer*et al.* [24] that the sediments collected from the stations P, Q, and R located in El-Max district bay Mediterranian Sea Egypt are mainly composed of sand (85.82 – 95.62 %) while the sediment of station S displayed a different composition, containing approximately the same percentage of sand (39.41 %) and silt (34.39 %) and a higher proportion of clay (26.20 %) compared to the rest of the stations (0 – 5.49 %). The result also revealed that the differences in the water content, porosity, bulk density, TOC, soil saturation and grain size as they are known to influence the solubility of elements and nutrients in marine sediments, ultimately affecting the distribution of metals and other pollutants that preferentially bind to fine particles [24], determining as a consequence that the three sampled locations analyzed constitute different environmental niches. Both particle size and total organic content of sediments have been shown to be important factors in sediment PAH distribution, suggesting a particle size effect due to differences in adsorptive surface area [17]. All the sampled locations showed high content of inorganic nutrients and exchangeable bases and the possible reasons for these occurrences may be due to pH and human activities observed along the study area which include agricultural land use and farming operation, anthropogenic activities and industrialization. This report slightly contradicts the findings of Amer*et al*. [24] who reported that all the stations showed total nitrogen content below 0.2 % w/v. Stations R and S showed a high content of total phosphorous with 0.83 and 0.59 ppm respectively. Oyedele*et al*. [25] reported that low pH (acidic) favours the abundance of exchangeable anions, but reduced cation, while high pH (basic) favours the abundance of exchangeable cations, but reduced anions in soils. Plants growing around the river in which the water has been discharged may experience excessive growth due to these nutrients. River fish could potentially have adverse effects to humans if consumed[26].

Furthermore, all the sampled locations showed high content of chemical oxygen demand (COD), oxygen demand (OD) and biochemical oxygen demand (BOD) but low content of total dissolved solids (TDS), total suspended solids (TSS) and total solids (TS). The COD and BOD were found to be higher than WHO standards except TDS, TSS and TS that were found below the maximum recommended limits.Ogunfowokan*et al.* [27], observed significant elevation of water indices such as pH, BOD, nitrate, phosphate and TSS. It is well known that oxygen depletion in water bodies could cause fish death while increase in BOD signifies high load of organic matter. Also, organic matter decomposition in surface water produced inorganic nutrients such as ammonia, nitrate and phosphorus with resultant effects of eutrophication and other serious ecological problems of such water body.

The results in Tables 5 – 8 revealed that there were exponential growth patterns in the control test cultures (μ = 0.083 h^{-1}) while continuous inhibitions were observed at the varying test concentrations of the test samples. The control growth rate (0.038 h^{-1}) is comparable to the minimal cell multiplication factor of 16 during a three day test given in the Guideline (corresponding to a growth rate of 0.038 h^{-1}) which corresponds to EBPI [6] guideline on validity criteria of the assay. The reasons for the significant growth in the control could be due to the relatively high start cell density and absence of test substances in the test cultures while cell death could be the reason for the drastic reduction and decline in the test cultures with different concentrations of the test samples. Similarly, the result in Figure 1 showed that there were drastic inhibitions in all test samples and the reasons for the significant growth in the control could be due to the relatively high start cell density and absence of test substances in the test cultures while cell death could be the reason for the drastic reduction and decline in the test cultures with different concentrations of the test samples. The fact that higher percentage of inhibitions were obtained showed that the test substances increasingly affected the growth at higher concentrations. Moreso, the result in Figure 2 revealed that positive control ($K_2Cr_2O_7$) had the highernon-significant (P>.05) ErC_{50} value than the test samples indicating that inhibition effects among test samples were not much different from the control.The levels of toxicity class are drawn in accordance with international regulations and national legislative program as followed: Highly toxic - LC_{50} / ErC_{50}< 1mg/L; Toxic - 1mg/L< LC_{50}/ ErC_{50} ≤ 10 mg/L; Harmful / hazardous for aquatic system– 10 mg/L< LC_{50} / ErC_{50} ≤ 100 mg/L; Very low toxic, non-toxic -

LC_{50} / ErC_{50} > 100 mg/L [28]. Generally, it could be deduced that the aromatic hydrocarbons and sediment samples possess acute aquatic toxicity (1 mg /L<ErC_{50} ≤ 10 mg/L) than the individual test samples with very strong significant positive correlation between increasing test concentrations (0 - 18 mg /L) and growth inhibitions to the marine microalga (*P*=.05) which indicates that inhibition is concentration dependent. Our finding was similar to the work published by Oldersma*et al*. [29], who reported that the statistical endpoints included in the model calculation, demonstrated a significant effect on the growth rate, and little or no effect on the growth of *Selenastrumcapricornutum*(expressed as the area under the growth curve). The difference between the ErC_{50} and the EbC_{50} values therefore has no toxicological relevance. The ErC_{50} value of 4.4 mg $L^{-1}$was considered to provide the best expression of toxicity of *p* - xylene to algae. Aruoja*et al.* [8], also found out that the EC_{50} values of *P. subcapitata*72 hrs growth inhibition were experimentally determined for all 58 compounds. Despite the fact that the analyzed molecules were structurally similar, the EC_{50} values spanned two orders of magnitude ranging from 1.43 mg /L (3, 4, 5 - trichloroaniline) to 197 mg /L (phenol). The toxicity of the studied compounds was dependent on the type (chloro -, methyl -, ethyl -), number (mono -, di -, tri -) and position (ortho -, meta -, para -) of the substituents. As a rule, the higher the number of substituents the higher the toxicity. The chloro - substituted molecules were generally more toxic than alkyl - substituted ones. The findings of this author supports the result of the present study.

Table 1. Total aromatic hydrocarbon fractions (ppb) of sediment and water samples of the three sampled locations

Hydrocarbon	ABSE	NESE	ONSE	ABW	NEW	ONW
Acenaphthene	11.10 ±0.20	06.90 ± 0.20	09.70 ± 04.97	Nd	05.10 ± 0.25	Nd
Acenaphthylene	0.60 ± 0.20	0.40 ± 0.20	1.20 ± 0.25	Nd	Nd	Nd
Phenanthrene	08.70 ± 0.12	11.60 ± 0.20	02.10 ± 0.25	02.20 ± 0.31	03.10 ± 0.25	02.00 ± 0.10
Anthracene	01.10 ± 0.27	116.40 ± 0.12	01.30 ± 04.40	Nd	03.80 ± 0.25	Nd
Flouranthene	01.10 ± 0.12	04.90 ± 0.25	01.30 ± 4.40	01.10 ± 0.15	03.10 ± 1.50	01.10 ± 0.20
Benzo(k)pyrene	03.10 ± 0.25	Nd	03.10 ± 0.30	01.50 ± 0.20	Nd	01.20 ± 0.20
Benzo(a)pyrene	0.60 ± 0.12	0.20 ± 0.31	0.40 ± 2.00	0.10 ± 0.02	0.30 ± 0.02	0.20 ± 0.10

Xylene	58.90 ± 0.21	103.60 ± 0.20	6.20 ± 2.00	1.70 ± 0.20	Nd	01.80 ± 0.02
Benzo(b) flouranthene	5.00 ± 0.03	02.60 ± 0.20	03.60 ± 0.20	Nd	Nd	Nd
Pyrene	03.50 ± 0.24	40.50 ± 0.20	03.70 ± 0.20	03.90 ± 0.20	Nd	05.90 ± 0.30
Benzo (g,h,i) perylene	08.60 ± 0.20	12.00 ± 0.20	08.40 ± 0.25	03.00 ± 0.20	01.10 ± 0.20	03.00 ± 0.25
Dibenzyl (a,h) anthracene	03.00 ± 0.20	04.80 ± 0.25	04.30 ± 0.25	Nd	04.00 ± 0.21	Nd
1,2-benzoanthracene	Nd	07.70 ± 0.25	Nd	Nd	Nd	Nd
Flourene	Nd	11.20 ± 0.20	Nd	01.10 ± 0.02	0.80 ± 0.02	01.10 ± 0.25

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NSE= Onne sediment; ABSE = Abonema sediment; NESE = Nembe sediment; ONW = Onne water, ABW = Abonema water, NEW = Nembe water; Nd = Not determined (below detectable limit); ppb = part per billion; values are mean ± Standard deviation of triplicate determination.

Table 2. Total metal and metalloidconcentration (ppm) of sediment and water samples of the three sampled locations

Metals	ABSE	NESE	ONSE	ABW	NEW	ONW
Iron (Fe)	110.24 ± 0.20	72.02 ± 0.12	104.44 ± 0.23	03.27 ± 0.25	01.11 ± 0.105	02.20 ± 0.20
Cobalt (Co)	0.13 ± 0.03	0.00 ± 0.00	0.05 ± 0.02	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Copper (Cu)	0.79 ± 0.122	0.20 ± 0.02	01.31 ± 0.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lead (Pb)	0.48 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cadmium (Cd)	0.02 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Chromium (Cr)	0.58 ± 0.02	0.16 ± 0.02	0.31 ± 0.02	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Zinc (Zn)	11.72 ± 0.20	2.64 ± 0.02	4.14 ± 0.02	0.06 ± 0.02	0.13 ± 0.00	0.10 ± 0.01
Nickel (Ni)	0.58 ± 0.02	0.32 ± 0.02	0.3 ± 0.00	0.00 ± 0.00	0.11 ± 0.02	0.05 ± 0.02

Mercury (Hg)	0.14 ± 0.02	0.34 ± 0.02	0.10 ± 0.01	0.13 ± 0.02	0.05 ± 0.02	0.05 ± 0.02
Arsenic (As)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

ONSE = Onne sediment; ABSE = Abonema sediment; NESE = Nembe sediment; ONW = Onne water, ABW = Abonema water, NEW = Nembe water; ppm = part per million; values are mean ± Standard deviation of triplicate determination.

Table 3. General parameters of sediment samples of the three sampled locations

Parameters	Sediment sampling locations		
	Abonema	Nembe	Onne
Conductivity at 25 %	20.60 ± 0.31	19.91 ± 0.02	24.10 ± 0.25
pH	07.47 ± 0.02	07.16 ± 0.02	06.72 ± 0.02
Sand (%)	43.79 ± 0.25	47.63 ± 0.02	59.72 ± 0.02
Clay (%)	32.59 ± 0.05	26.51 ± 0.00	18.56 ± 0.05
Silt (%)	23.62 ± 0.23	25.94 ± 0.04	21.72 ± 0.01
Moisture (%)	49.75 ± 19.01	85.94 ± 33.11	21.80 ± 0.30
Porosity	0.04 ± 0.00	0.04 ± 0.00	0.01 ± 0.00
Bulk density (g /mL)	01.36 ± 0.00	01.72 ± 0.00	01.58 ± 0.00
TN (%)	03.30 ± 0.00	03.80 ± 0.01	02.69 ± 0.00
TP (mg /L)	05.33 ± 0.00	04.70 ± 0.10	5.50 ± 0.00
Potassium (ppm)	09.24 ± 0.02	08.45 ± 0.03	11.45 ± 0.00
Calcium (ppm)	10.68 ± 0.01	11.99 ± 0.01	12.78 ± 0.02
Soil saturation (min.)	22.48 ± 0.03	18.00 ± 0.31	28.32 ± 0.02
TOC (%)	12.65 ± 0.05	17.82 ± 0.03	14.56 ± 0.03
Sediment type	Clay loam	Clay loam	Sandy loam

TN = Total nitrogen; TP = Total phosphorus; TOC = Total organic carbon

Table 8: General parameters of water samples of the three sampled locations

Parameters	Water sampling locations		
	Abonema	Nembe	Onne
Conductivity at 25 %	19.74 ± 0.03	13.22 ± 0.13	69.90 ± 0.27
pH	06.55 ± 0.02	07.31 ± 0.16	06.41 ± 0.02
TDS (mg /L)	07.48 ± 0.01	06.56 ± 0.02	05.76 ± 0.62

TSS (mg /L)	0.15 ± 0.00	0.01 ± 0.00	0.07 ± 0.00
TS (mg)	07.63 ± 0.01	06.56 ± 0.00	05.83 ± 0.21
COD (mg /L)	106.60 ± 65.56	80.00 ± 46.84	66.67 ± 31.41
DO (mg /L)	22.50 ± 0.05	25.30 ± 0.08	19.30 ± 0.15
BOD (mg /L)	210.00 ± 2.52	146.00 ± 0.20	46.00 ± 0.20
TN (%)	0.28 ± 0.01	0.97 ± 0.01	0.55 ± 0.03
TP (mg /L)	03.72 ± 0.02	02.16 ± 0.01	02.07 ± 0.02
Potassium (ppm)	04.64 ± 0.01	03.88 ± 0.01	04.28 ± 0.00
Calcium (ppm)	06.35 ± 0.01	05.78 ± 0.00	04.10 ± 0.00

TDS = Total dissolved solid; TSS = Total suspended solids; TS = Total solids; COD = Chemical oxygen demand; DO = Dissolved oxygen; BOD = Biological oxygen demand; TN = Total nitrogen; TP = Total phosphorus

Table 5.Mean values of cell algal density measurements (Cells/ml X 10^4) with their respective coefficient of variations, specific growth rates and percentage inhibitions at different concentrations of xyleneand sediments

Parameters	Concentration					
	0 mg /L	1.0 mg /L	1.8 mg /L	3.2 mg /L	5.6 mg /L	18 mg /L
Time (hr)			Xylene + Distilled water			
0	70.00	52.50	49.00	28.00	28.00	26.00
24	160.00	28.00	26.00	20.00	12.00	49.00
48	265.00	20.00	20.00	12.00	6.25	12.00
72	625.00	12.00	12.00	6.25	6.25	1.00
CV (%)	86.93	62.27	59.45	57.26	78.33	94.11
Specific growth rate	8.30	3.30	1.60	1.30	1.00	0.80
% Inhibition	1.33	30.24	50.72	54.34	57.95	60.36
Time (hr)			Xylene + Abonema sediment			
0	70.00	125.00	61.50	49.00	27.00	20.00
24	160.00	72.00	52.50	47.00	22.00	82.50
48	265.00	26.50	30.00	22.00	14.00	6.25
72	625.00	14.00	14.00	8.25	6.25	2.25
CV (%)	86.93	84.80	54.56	62.76	52.64	134.35
Specific growth rate	8.30	1.80	1.40	1.00	0.70	0.40
% Inhibition	1.33	78.31	83.13	87.95	91.57	95.18
Time (hr)			Xylene + Nembe sediment			
0	70.00	40.00	20.00	13.00	10.00	6.25
24	160.00	30.00	13.00	8.25	8.25	6.25
48	265.00	22.50	8.50	6.25	6.25	1.00
72	625.00	8.40	6.25	6.25	6.25	1.00
CV (%)	86.93	52.77	50.79	37.74	23.51	83.62
Specific growth rate	8.30	3.30	1.80	1.10	0.80	0.40
% Inhibition	1.33	60.20	78.31	86.75	90.36	95.18
Time (hr)			Xylene + Onne sediment			
0	70.00	26.00	24.00	20.00	14.00	6.25

24	160.00	26.00	20.00	85.00	8.25	6.25
48	265.00	15.00	8.50	6.25	6.25	6.25
72	625.00	1.00	6.25	6.25	6.25	1.00
CV (%)	86.93	69.77	58.89	128.16	42.19	53.16
Specific growth rate	8.30	1.70	1.50	1.10	0.90	0.50
% Inhibition	1.33	79.52	81.93	86.75	89.16	93.97

Table6.Mean values of cell algal density measurements (Cells/ml X 10^4) with their respective coefficient of variations, specific growth rates and percentage inhibitions at different concentrations of anthracene and sediments

Parameters	Concentration					
	0 mg /L	1.0 mg /L	1.8 mg /L	3.2 mg /L	5.6 mg /L	18 mg /L
Time (hr)		Anthracene + Distilled water				
0	71.00	265.00	22.00	22.00	13.00	13.00
24	164.00	262.00	22.00	13.00	6.25	6.25
48	268.00	250.00	6.25	6.25	1.00	0.90
72	626.00	6.25	1.00	1.00	0.90	0.90
CV (%)	86.05	64.62	84.47	85.8	108.12	109.11
Specific growth rate	8.40	2.90	1.70	1.30	0.90	0.50
% Inhibition	1.34	40.06	54.52	59.34	64.16	66.57
Time (hr)		Anthracene + Abonema sediment				
0	71.00	32.00	22.00	13.00	6.25	8.25
24	164.00	30.00	50.00	8.50	1.00	6.25
48	268.00	15.00	6.25	6.25	1.00	1.00
72	626.00	6.25	6.25	6.25	1.00	1.00
CV (%)	86.05	59.20	97.67	37.44	113.51	89.69
Specific growth rate	8.40	1.90	1.50	1.00	0.60	0.30
% Inhibition	1.34	77.11	81.93	87.97	92.77	95.39
Time (hr)		Anthracene + Nembe sediment				
0	71.00	32.00	24.00	20.00	13.00	10.00
24	164.00	15.00	13.00	10.00	8.00	6.25
48	268.00	8.50	6.25	6.25	6.25	1.00
72	626.00	6.25	6.25	6.25	6.25	0.00
CV (%)	86.05	75.45	67.70	61.13	38.11	108.49
Specific growth rate	8.40	2.10	1.50	1.20	0.80	0.30
% Inhibition	1.34	74.70	81.93	85.54	93.60	96.39
Time (hr)		Anthracene + Onne sediment				

0	71.00	32.00	30.00	24.00	14.00	14.00
24	164.00	20.00	14.00	8.25	6.25	6.25
48	268.00	8.50	8.25	6.25	6.25	6.25
72	626.00	6.25	6.25	6.25	6.25	0.01
CV (%)	86.05	71.02	73.60	76.81	47.33	86.43
Specific growth rate	8.40	1.90	1.40	1.00	0.70	0.30
% Inhibition	1.34	77.11	83.13	87.95	91.57	96.39

Table 7. Mean values of cell algal density measurements (Cells/ml X 10^4) with their respective coefficient of variations, specific growth rates and percentage inhibitions at different concentrations of pyreneand sediments

Parameters		Concentration				
	0 mg /L	1.0 mg /L	1.8 mg /L	3.2 mg /L	5.6 mg /L	18 mg /L
Time (hr)		Pyrene + Distilled water				
0	69.00	26.00	13.00	13.00	6.25	6.25
24	159.00	13.00	6.25	6.25	6.25	6.25
48	269.00	6.25	6.25	6.25	1.00	1.00
72	627.00	1.00	1.00	1.00	0.80	0.01
CV (%)	87.09	93.46	74.24	74.24	86.43	98.93
Specific growth rate	8.20	2.50	1.50	1.10	0.90	0.60
% Inhibition	1.31	49.88	61.93	66.75	69.16	72.77
Time (hr)		Pyrene + Abonema sediment				
0	69.00	63.00	52.00	32.00	20.00	14.00
24	159.00	54.00	30.00	22.50	13.00	6.25
48	269.00	30.00	24.00	1.00	6.25	6.25
72	627.00	13.00	10.00	6.25	6.25	0.04
CV (%)	87.09	56.90	60.25	92.90	57.77	86.16
Specific growth rate	8.20	1.70	1.30	1.00	0.60	0.30
% Inhibition	1.31	80.72	84.34	87.95	92.77	96.39
Time (hr)		Pyrene + Nembe sediment				
0	69.00	27.00	24.00	20.00	14.00	13.00
24	159.00	24.00	18.50	8.50	8.25	8.25
48	269.00	15.00	8.40	8.25	6.25	6.25
72	627.00	8.25	6.25	6.25	6.25	0.01
CV (%)	87.09	46.11	58.75	58.12	42.19	78.27
Specific growth rate	8.20	1.60	1.40	0.80	0.50	0.20
% Inhibition	1.31	80.72	83.13	90.36	93.97	98.19
Time (hr)		Pyrene + Onne sediment				

0	69.00	26.00	24.00	24.00	14.00	13.00
24	159.00	22.50	14.00	14.00	13.00	13.00
48	269.00	1.00	8.25	6.25	6.25	6.25
72	627.00	6.25	6.25	6.25	6.25	0.03
CV (%)	87.09	87.41	60.64	66.67	42.59	77.24
Specific growth rate	8.20	1.50	1.20	0.80	0.50	0.10
% Inhibition	1.31	81.93	85.54	90.36	93.97	97.59

Table 8. Mean values of cell algal density measurements (Cells/ml X 10^4) with their respective coefficient of variations, specific growth rates and percentage inhibitions at different concentrations of positive control ($K_2Cr_2O_7$)andcontaminated sediment samples

Parameters	Concentration					
	0 mg /L	1.0 mg /L	1.8 mg /L	3.2 mg /L	5.6 mg /L	18 mg /L
Time (hr)			Positive control($K_2Cr_2O_7$)			
0	72.00	262.00	60.00	49.00	28.00	26.00
24	162.00	259.00	49.00	26.00	26.00	22.00
48	267.00	252.00	26.00	28.00	22.00	6.25
72	628.00	22.00	13.00	13.00	6.25	6.25
CV (%)	86.41	59.32	57.76	51.38	47.96	68.61
Specific growth rate	8.10	1.60	1.40	1.10	1.0	0.40
% Inhibition	1.29	35.72	38.13	41.75	45.36	50.18
Time (hr)		Abonema sediment				
0	72.00	220.00	185.00	26.00	15.00	28.00
24	162.00	185.00	165.00	15.00	6.25	20.00
48	267.00	130.00	87.50	6.25	6.25	6.25
72	628.00	1.00	70.00	6.25	5.00	4.00
CV (%)	86.41	71.71	44.63	70.08	56.87	78.38
Specific growth rate	8.10	1.30	1.10	1.00	1.00	0.75
% Inhibition	1.29	56.75	57.95	57.95	58.77	60.95
Time (hr)		Nembe sediment				
0	72.00	100.00	82.50	50.00	52.50	60.00
24	162.00	70.00	62.50	12.00	28.00	26.00
48	267.00	52.50	49.00	12.00	12.00	13.00
72	628.00	15.00	12.00	6.25	2.00	1.00
CV (%)	86.41	59.78	57.69	100.39	93.24	101.88
Specific growth rate	8.10	2.00	1.60	1.00	0.70	0.50
% Inhibition	1.29	45.9	50.72	57.95	61.57	63.97
Time (hr)		Onne sediment				

0	72.00	300.00	165.00	82.00	82.50	52.50
24	162.00	175.00	62.50	60.50	52.50	28.00
48	267.00	82.50	49.00	26.00	20.00	12.00
72	628.00	28.00	20.00	13.00	6.25	3.25
CV (%)	86.41	81.33	85.16	69.62	84.74	90.34
Specific growth rate	8.10	1.30	1.10	1.00	1.00	0.60
% Inhibition	1.29	54.34	56.75	57.95	57.95	62.77

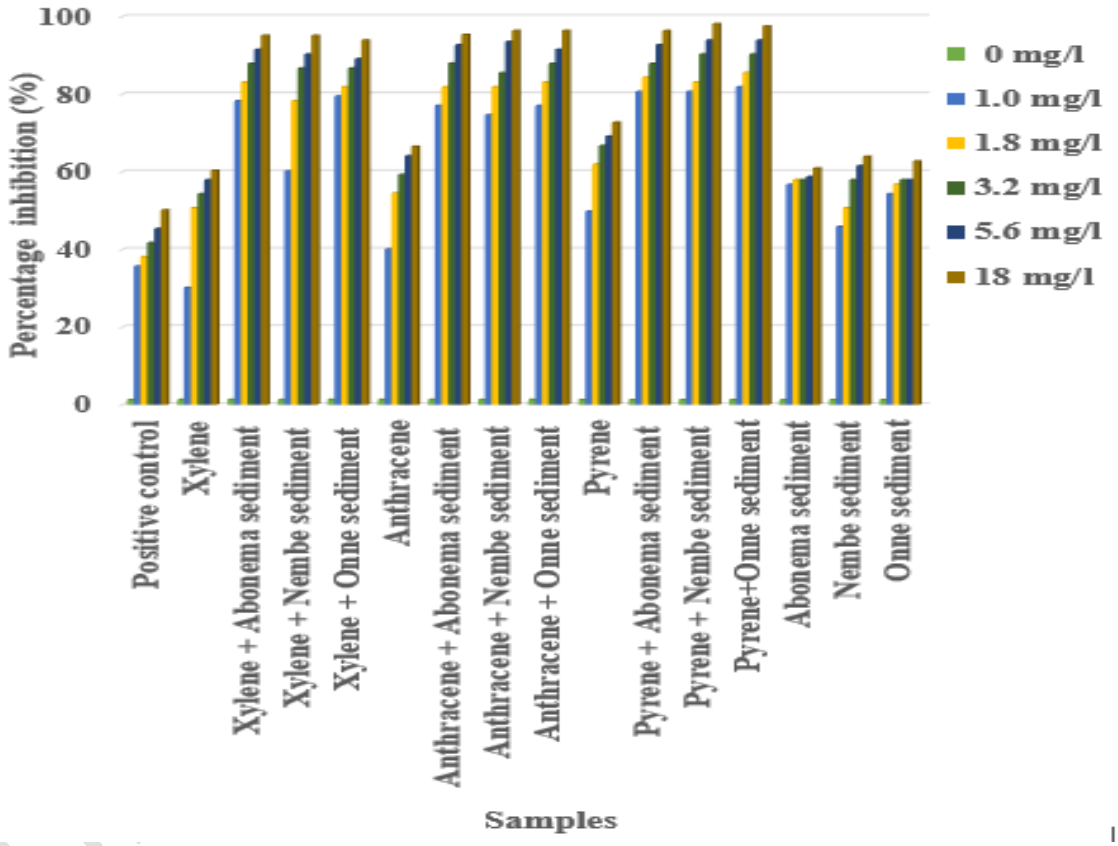

Fig. 1. Inhibition of growth rate of *Phaeodactylumtricornutum* by aromatic hydrocarbon contaminated distilled water and sediments of the three sampled locations

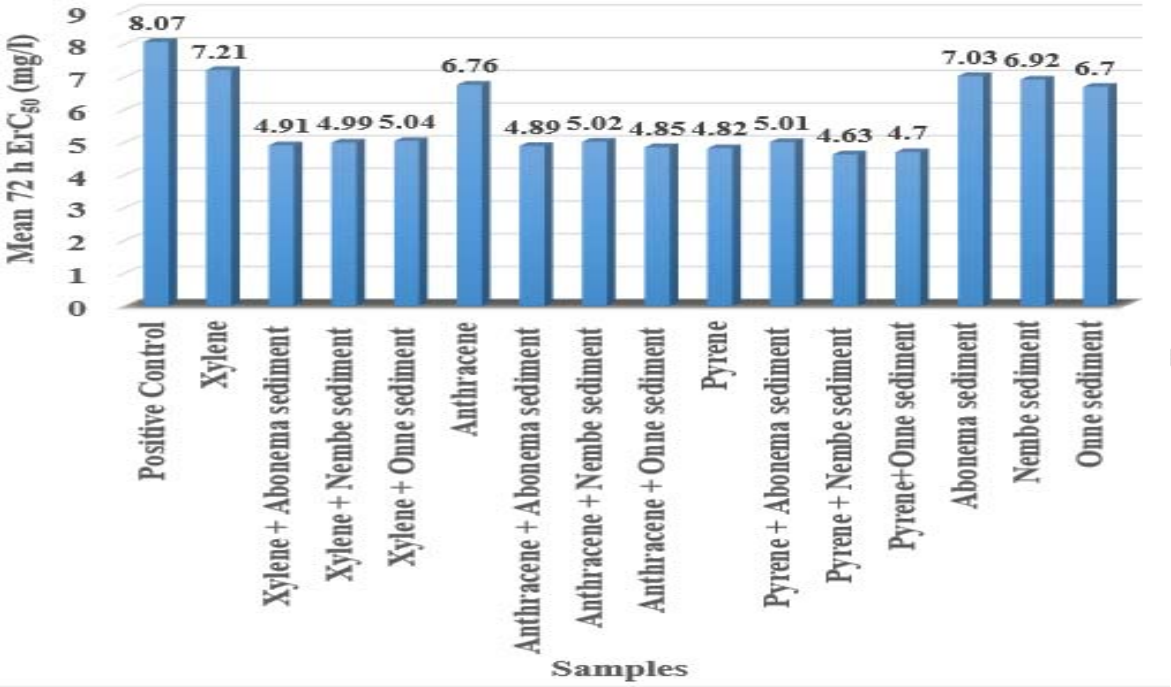

Fig. 2. Mean 72 hr ErC_{50} (mg/l) toxic response of *Phaeodactylumtricornutum* to aromatic hydrocarbon contaminated distilled water and sediments of the three sampled locations

5. CONCLUSION

To our knowledge, this is one of the first systematic studies on effects of marine water on algal growth and first or description of toxic effects of aromatic hydrocarbons towards algaein the Niger Delta, Nigeria. Based on the experimental results in this research, it can be concluded that:

- There were higher quantities of aromatic hydrocarbons, heavy metals and other physio-chemical parameters in the sediment samples than water samples.

- Aromatic hydrocarbons and sediment samples combination had acute dose dependent eco-toxicological effects on *Phaeodactylumtricornutum*than the individual test samples.

- Pyrene in Nembe sediment had the highest toxicity while positive control ($K_2Cr_2O_7$) had the lowest toxicity.

- The toxic response to *Phaeodactylumtricornutum*is in the order: Pyrene > anthracene > xylene and Onne water >Nembe water>Abonema water.

- The toxicity results (> 1 mg /L <EC_{50} ≤ 10 mg /L) in this study are in line with other toxicity values for this type of toxicants, and are therefore consider being scientifically relevant in ecotoxicological risk assessmentof Niger Delta, Nigeria.

REFERENCES

1. Abu GO, Chikere BO. Cell surface properties of hydrocarbon utilizing bacterial isolates from the Port Harcourt marine environment. Nig J Microbiol. 2006;20(1):809-16.
2. Odeyemi O, Ogunseitan OA. Petroleum industry and its pollution potential in Nigeria. Oil Petrochem. Pollut. 1985;2: 223-9.
3. Nweke CO, Okpokwasili GC. Effects of bioremediation treatments on the bacterial populations of soil at different depths. Nig. J. Microbiol. 2004;18:363-72.
4. Ezemonye LIN, Ezemonye NN. Polycyclic aromatic hydrocarbons in surface water and sediments of Niger Delta rivers of Nigeria. J. Nig. Environ. Soc. 2005;2:348-58.
5. Taiwo AM, Olujimi OO, Bamgbose O, Arowolo TA. Surface water quality monitoring in Nigeria: Situational analysis and future management strategy, water quality monitoring and asse9ssment. Dr. Voudouris (Ed.). InTech. 2012; 978-953-51-0486-5. Accessed 05 April 2017. Available from: http://www.intechopen.com/books/waterquality-monitoring-and-assessment/surface-water-quality-monitoring-in-nigeria-situational-analysis-and-futuremanagement- strategy.
6. Environmental Bio-detection Products Incorporation. Biotoxicity/microbiotest products handout and manual. Antario Canada: Environmental Bio-detection Products Incorporation;2016.
7. Nyholm N, Petersen HG. Laboratory bioassays with microalgae. In: Wang W, Gorsuch JW, Hughes JS, editors. Plants for environmental studies. Boca Raton (Florida): Lewis Publishers; 1997.
8. Aruoja V, Sihtmäe M, Dubourguier HC, Kahru A. Toxicity of 58 substituted anilines and phenols to algae *Pseudokirchneriellasubcapitata*and bacteria *Vibrio fischeri*: Comparison with published data and QSARs. Chem.2011;84(10):1310-20.
9. Weyer A, Sokull-Klüttgen B, Baraibair-Fentanes J, Vollmer A. Acute toxicity data: A comprehensive comparison of results from fish, *Daphnia* and algae tests with new substances notified in the European Union. Environ Toxicol Chem. 2000;19:1931-3.
10. Hutchinson TH, Barrett S, Buzby M, Constable D, Hartmann A, Hayes E, et al. A strategy to reduce the numbers of fish used in acute ecotoxicity testing of pharmaceuticals. Environ Toxicol Chem. 2003;22(12):3031-6.
11.Kahru A, Dubourguier HC. From ecotoxicology to nanoecotoxicology. Toxicol. 2010;269:105-19.
12. Jeram S, RiegoSintes JM, Halder M, BaraibarFentanes J, Sokull-Klüttgen B, Hutchinson TH. A strategy to reduce the use of fish in acute ecotoxicity testing of new chemical substances notified in the European Union. RegToxicol Pharm. 2005;42:218-24.
13. Chikere CB, Okpokwasili GC, Ichiakor O. Characterization of hydrocarbon utilizing bacteria in tropical marine sediments.AfrJ Biotech. 2009;8(11):2541-4.
14. Association of Official Analytical Chemists. Official method of analysis. 19th edn. Washington DC: Association of Official Analytical Chemists; 2012.
15. American Public Health Association. Standard methods for examination of water and wastewater. 22nd edn. Washington DC (USA): American Public Health Association, American Water Works Association and Water Environment Federation; 2012.
16. Selivanovskaya SY, Latypova VZ, Stepanova NY, Hung YT. Bioassay of industrial waste pollutants. In: Wang LK, Hung YT, Lo HH, Yapijakis C, editors. Waste treatment in the process industries. LCC, USA: CRC Press Taylor & Francis Group; 2006.
17. Gorleku MA, Carboo D, Palm LMN, Quasie WJ, Armah AK. Polycyclic aromatic hydrocarbons (PAHs) pollution in marine waters and sediments at the TemaHarbour, Ghana. Acad J Environ Sci. 2014;2 (7):108-15.
18. Emoyan OO. Quantification and distribution of polynuclear aromatic hydrocarbons (PAHs) in surface waters in the vicinity of Kokori Oil Field, Nigeria. JApplSci Environ Manag.2009;13(4):39-42.

19. Olusola JO. Festus AA. Assessment of heavy metals in some marine fish species relevant to their concentration in water and sediment from coastal waters of Ondo State, Nigeria. J Mar Sci Res Dev. 2015;5:163.
20. Obiajunwa EI, Pelemo DA, Owolabi SA, Fasasi MK, Johnson-Fatokun FO. Characterisation of heavy metal pollutants of soils and sediments around a crude-oil production terminal using EDXRF. NuclInstrum Meth Phys Res.2002;194(1):61-4.
21. Owamah HI. Heavy metals determination and assessment in a petroleum impacted River in the Niger Delta Region of Nigeria. J Petr Environ Biotech.2013;4(1):1-4.
22. Bazzi AO. Heavy metals in seawater, sediments and marine organisms in the Gulf of Chabahar, Oman Sea. J OceanMari Sci., 2014;5(3):20-9.
23. Ijah UJ, Abioye OP. Assessment of physicochemical and microbiological properties of soil 30 months after kerosene spill. Internat JResSciManagt.2003;1(1):24-30.
24. Amer RA, Mapelli F, El-Gendi HM, Barbato M, Goda DA, Corsini A et al. Bacterial diversity and bioremediation potential of the highly contaminated marine sediments at El-Max District (Egypt, Mediterranean Sea). Biomed Res Internat 2015;2015:981-9.
25. Oyedele DJ, Gasu MB, Awotoye OO. Changes in soil properties and plant uptake of heavy metals on selected municipal solid waste dump site in Ile-Ife, Nigeria. *Afr J Environ Sci Tech.* 2008;3(5):107-15.
26.Adenike A. Microbiological, physicochemical and genotoxicological assessment of tobacco wastewater. AdvApplSci Res.2014;5(3):423-38.
27. Ogunfowokan AO, Okoh EK, Adenuga AA. Asubiojo OI. An assessment of the impact of point source pollution from a university sewage treatment oxidation pond on a receiving stream – a preliminary study. J ApplSci. 2005;5(1):36-43.
28. Gheorghe S, Lucaciu I, Paun L, Stoica C, Stanescu E. Biodegradation- life science: Ecotoxicological behavior of some cationic and amphoteric surfactants (Biodegradation, Toxicity and Risk Assessment). London, UK: INTECH; 2013.
29. Oldersma H, van Dijk NRM, Hanstveit AO. Determination of the effect of p-xylene (CAS #106 – 42 – 3) on the growth of the fresh water green alga *Selenastrumcapricornutum*: TNO Report | V5819/01 | final. Netherlands: Netherlands Organisationfor Applied Scientific Research; 2004.