Original Research Article

Effects of Aromatic Hydrocarbons and Marine Water from Niger Delta on the *β*-Galactosidase Activity of Mutant *Escherichia coli*

ABSTRACT

Aims: To determine effects of aromatic hydrocarbons and marine water from Niger Delta on the *β*-Galactosidase activity mutant *Escherichia coli.*

Study Design: Fifteen treatments and the control designs were set up in triplicates in microtitre plates containing 200 µL of the 100 % concentration of samples (three marine waters and distilled water spiked each with xylene, anthracene and pyrene). The fifteen treatments and control ($HgCl_2$) set ups designated as A, B, C, D, E, F and G were used to determine their median effective concentration (EC_{50}) for the inhibition of *β*-Galactosidase activity of mutant *Escherichia coli.*

Place and Duration of Study: Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli Nigeria between February, 2017 to July, 2017.

Methodology: A laboratory scale study was carried on the water samples from the three studied areas using physicochemical analyses and bacterial Toxi-chromo test.

Results: The findings revealed that the three sampling sites contain higher quantities of aromatic hydrocarbons, heavy metals and other physico-chemical parameters in the sediment samples than water samples. Xylene distillled water had the highest EC_{50} of 3.417 ± 0.094 mg/l while pyrene Onne water had the least EC_{50} of 0.015 ± 0.002 mg/l. Also, pyrene and anthracene are significantly (P = .05) highly toxic aromatic hydrocarbons (EC_{50} < 1 mg /L) while xylene is a significantly (P = .05) toxic aromatic hydrocarbon (1 mg /L < EC_{50} ≤ 10 mg /L) compare to the positive control ($HgCl_2$) (EC_{50} < 1 mg /L) indicating that enzyme inhibition among test samples were much different from the positive control.

Conclusion: Thus, the toxicity results (< 0.1 mg /L < EC_{50} ≤ 10 mg /L) in this study indicate that the potential eco-toxicity and environmental health effects of these toxicants should be given attention in order to get rid of their dangerous outcomes.

Keywords: Aromatic hydrocarbons, aquatic pollution, $EC_{50,}$ mutant *Escherichia coli, pubilc health, Niger Delta.*

1. INTRODUCTION

The Niger Delta ecosystem of Nigeria is subjected to man-induced changes and seriously threatened by increasing environmental deterioration. The aquatic ecosystem of the region faces increasing ecological and toxicological problems from the release of petroleum pollutants Besides this direct pollution, the occasionally pipeline leaks, transportation accidents, storage tank ruptures and refining petroleum is further intensifying the pollution of this area [1].

Environmental contaminants such as hydrocarbons, heavy metals and pesticides have been known to have direct toxic effects when released into the aquatic environment. There is a direct link between surface water and sediment contamination. Accumulated heavy metals or organic pollutants in sediment could be released back into the water with deleterious effects on human health [2]. These pollutants and untreated industrial effluents can pose a major risk to the environment and aquatic life. For these reasons, many assays especially chemical and biological, have been developed to meet the demand of screening for toxic substances. It is important to assess the risks of these pollutants for environmental policy. Ecological risk assessment (ERA) is a tool to estimate adverse effects on the environment from chemical or physical stressors. Toxicity bioassays are the important line of evidence in an ERA [3, 4].

Microbial toxicity tests are known to be fast, simple, and inexpensive. These properties of the tests have resulted in their ever-increasing use in environmental control, assessment of pollutants in waste, and so on. Toxicity test methods based on the reaction of microbes are useful in toxicity. In particular they can be a very valuable tool for the toxicity classification of samples from the same origin. Microbial tests can be performed using a pure culture of well-defined single species or a mixture of microbes. The variables measured in toxicity tests may be lethality, growth rate,

change in species diversity, decrease in degradation activity, and energy metabolism or activity of specific enzymes. The results are generally expressed as the dose– response concentration and the EC_{50} or EC_{10} value [4, 5, 6, 7, 8, 9].

The Toxi-chromo test™ can be employed for qualitative measurements as well as experiments to quantify toxic potency. The assay is based on the ability of substances (toxicants) to inhibit the *de novo* synthesis of an inducible β-galactosidase enzyme in a highly permeable mutant of *Escherichia coli*. Addition of a chromogen after an exposure period produces as easy to read colorimetric endpoint that measures *β* -galactosidase activity in the affected bacteria. Results can be interpreted qualitatively using the naked eye or quantitatively with a spectrophotometer and the assay can be customized to meet client laboratory specifications. Comparing the amount of colour produced between a test sample and a reference sample provides a measure of toxic potency [3].

Several studies conducted emphasis the applications of Toxi-chromo test as a tool for the risk evaluation of contaminated sites, both in water and on land. Henda and van der Oost [10] reported that the sediments of three of the eight sites studied could be classified as toxic and one site even as very toxic. The outcome of the toxicity testing confirmed the results of the chemical analysis for only one site. Kwan [11] reported that highly permeable mutant of *E. coli was* responsive to a wide spectrum of pollutants including mycotoxins, pesticides and heavy metalsThere is dearth of information regarding assessment of ecotoxicological risks of contaminants and polluted sites using bacterial and enzymatic bioassays for toxicity testing in crude oil – impacted Niger Delta marine ecosystem; owing to its increasing ecological and toxicological problems and hence necessitates and justifies this study. This study was undertaken to determine effects of aromatic hydrocarbons and marine waste water from Niger Delta on the *β*-Galactosidase Activity mutant *Escherichia coli.*

2. MATERIALS AND METHODS

2.1 Description of the Sampling Sites

The studied areas were Abonema Wharf Water Front (Fig 1) in Akuku-Toru Local Government Area, Nembe Water-side (Fig 2) in Port Harcourt Local Government Area and Onne Light Flow Terminal Seaport (Fig 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"E to 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 are. It also links the Island directly with the Atlantic ocean through which crude oil is exported by massive oil tankers [12]. 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, Exon Mobil Nigeria Limited, Socotherm Pipecoaters, 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 (Fig 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 containers. The water samples were collected at the air-water interface by hand dipping the 95 % ethanol - sanitized cylindrical shaped 2 L plastic containers. 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, 12, 13, 14].

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

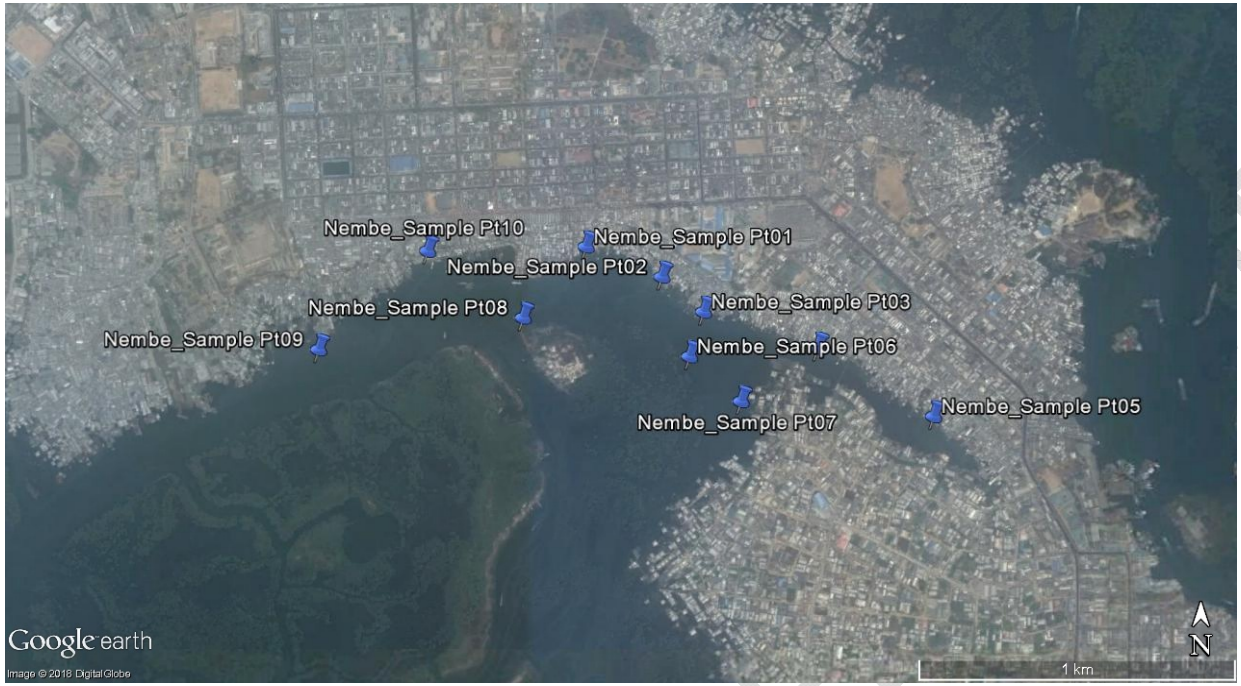


Fig 2. Geoeye satellite image (2016) showing the Nembe sample points

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

2.3 Sample Preparation and Extraction

Following the methods of Aruoja *et al.* [15] and Selivanovskaya *et al.* [4] with slight modifications, the sediment–water suspensions (10:100 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 concentrations and adequate light conditions for the growth of algae. Similarly, the marine water samples were prepared by filtering the

suspensions through glass fiber filters (d = 0.45 µm) using membrane filtration technique to separate suspended and dissolved solids in the samples before analyses

2.4 Physicochemical Analyses of Sediment and 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 [16].

2.4.2 Total heavy metal concentration analysis

Total heavy metal 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 [17].

2.4.3 General parameters analysis

Following the standard method described by AOAC [16] and APHA [16], the pH of the samples were 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 hrs. 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 Toxi - chromo test

The Toxi - chromotest was carried out according to the standard method of EBPI [3] toxi-chromotest procedure version 4.0 as follows:

2.5.2.1 Test bacterial strains

Lyophilized bacteria (Bottle B) with a highly permeable rough mutant of *E. coli* (3 units, 0.2g per vial)

2.5.2.2 Standard toxicant

4µg/ml mercury chloride (Bottle D) in water (1 unit, 2ml vial) was used as the positive control while diluent (Bottle G) for standard toxicant and samples (3 units, 10 ml vial) was used as the negative control.

2.5.2.3 Other parameters

Reaction mixture (Bottle A)- a cocktail containing an inducer for β-galactosidase and cofactors required for the recovery of the bacteria from their stressed condition. (3 units, 10 ml vial).

Rehydration solution (Bottle C)- a solution to rehydrate lyophilized bacteria prior to exposure. (3 units, 10 ml vial).

Chromogenic substrate (Botttle F) - blue chromogen (3 units, 12 ml vial).

2.5.2.4 Plate preparation

One hundred microlitres of diluent from Bottle G was dispensed to all other wells except row A containing undiluted sample and standard. Two hundred microlitres of standard toxicant (Bottle D) and 200 µL of the 100 % concentration of samples (marine water and distilled water spiked with xylene, anthracene and pyrene) were dispensed to the appropriate wells of Row A. The required two - fold serial dilutions of each samples and the standard toxicant ($HgCl_2$) were prepared by transferring 100 µL from well A of each column into the next well (B) and continued by serially transferring 100 µL until Well G and more dilutions starting in row A of the next column and down the column again were done to end with 13

dilutions per sample. One hundred microlitres of the diluent and highest sample concentration were dispensed to the wells in row H of the columns containing that sample to create reagent blanks. To the blank row (H), 100 µL of reaction mixture (bottled A) was dispensed to all wells of row H. These wells are the reagent blanks and contain no bacteria.

2.5.2.5 Bacterial preparation and addition

The rehydration solution in bottle C was transferred to bacteria in Bottle B, mixed and left at 25 ± 2 °C for 15 minutes for complete rehydration. One millilitre of rehydrated bacteria from Bottle B was transferred to reaction mixture in Bottle A. One hundred microlitres of reaction mixture including bacteria (from Bottle A) was dispensed to all wells in the microplate except the reagent blank (row H). The plates were incubated in the incubator (Kottermann D3165, West Germany) at 37 °C for 90 minutes. At this time, the chromogen (Bottle F) was warmed by placing it in the incubator.

2.5.2.6 Chromogen addition and colour development

The plates were removed from the incubator and 100 µL of the warmed chromogenic substrate (bottle F) was dispensed to all wells of the plates. The plates were incubated in an incubator at 37 °C for 30 minutes until a blue colour developed in the zero concentration wells or negative control wells (column 2).

2.5.2.7 Reading and analysis of results

The results were read quantitatively by measuring absorbance at 630 nm once sufficient blue colour development has occurred using a micro plate reader (MR – 96A MINDRAY, Germany). The absorbance values of each wells were measured and recorded.

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, toxicity factor (TF), coeffiecient of variation (CV) and median effective concentration (EC_{50}) were calculated and determined by the non – linear equation: Y=Bottom + (Top-Bottom)/ (1+10^ (($LogEC_{50}$-X)*HillSlope)). The results were considered statistically significant and valid if the probability is less than .05 (P = .05) and replicates CV ≤ 25 %

3. RESULTS

3.1 Physico - 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 Total heavy metal concentration analysis

The result of the total heavy metal concentration (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) heavy metal concentration 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 in 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 in 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 respectvely. 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 ppp 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

3.2 Acute Toxicity Testing

3.2.1 Toxi – chromo test

The result of the absorbance values of the toxicity of the aromatic hydrocarbons in distiiled water and wastewater of the three sampled locations using Toxi-Chromotest at different dilutions and as well as microplate test result for the Toxi – chromo testing of aromatic aromatic hydrocarbons and marine waste water samples are presented in Table 5 and Plate 1. From the result, pyrene and xylene hydrocabons in distilled water had the highest and lowest absorbance values of 0.398 ± 0.002 and 0.097 ± 0.002 as indicated in bold red coloured figures respectively. The result of the toxicity factor (TF) of the aromatic hydrocarbons in distilled water and wastewater of the three sampled locations is shown in Figure 1. From the Figure, pyrene in Nembe water had the highest toxicity factor of 273.200 ± 0.163 with CV and r^2 of 17.320 % and 0.097 and xylene in distilled water had the lowest toxicity factor of 07.300 ± 0.170 with CV and r^2 of 7.980 % and 0.256 showing no significant ($P > .05$) weak positive correlation respectively. In the same vein, the result of the mean 1.5 hr EC_{50} (mg /L) toxic response of mutant *E. coli* to the aromatic hydrocarbons in distilled and wastewater samples is shown in Figure 2. From the Figure, xylene distilled water had the highest EC_{50} of 3.417 ± 0.094 mg /L while pyrene Onne water had the least EC_{50} of 0.015 ± 0.002 mg /L.

4. DISCUSSION

The risk assessment of contaminated objects is mainly based on the chemical analyses of a priority list of toxic substances. This analytical approach does not allow for mixture toxicity, nor does it take into account the bioavailability of the pollutants present. In this respect, bioassays provide an alternative because they constitute a measure for environmentally relevant toxicity, that is, the effects of a bioavailable fraction of an interacting set of pollutants in a complex environmental matrix [4, 6]

In this study, an attempt was made to determine effects of aromatic hydrocarbons and marine sediments from Niger Delta on the growth of microalga *Phaeodactylum tricornutum* 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.* [14], 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.

Heavy metal pollution in the marine environment is determined by measuring its concentration in water, sediment and living organisms [19]. The result in Table 6 revealed that all metals except iron were lower and within for water samples but higher for sediment samples in comparison to the WHO maximum permissible recommended limits. The studied elements are individually known to be mutagens and carcinogens. In other words, they are toxicants. The higher levels of these heavy metals in sediment 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 heavy metal pollution 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 heavy metal is 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 heavy metals analyzed in the water and sediments samples, it can be concluded that heavy metals are 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 8 revealed that Onne water and sediment 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. In the same vein, fish consumed from the river by humans will definitely have an adverse effect on them [26]. In the same vein, 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 result in Table 5 revealed that pyrene and xylene hydrocabons in distilled water had the highest and lowest absorbance values of 0.398 ± 0.002 and 0.097 ± 0.002 as indicated in bold red coloured figures respectively. The result in Figure 1 revealed that pyrene in Nembe water sample had very high significant (*P* = .05) acute toxicity potential than other samples. The trend of toxicity of the hydrocarbons is pyrene > anthracene > xylene showing that the increase in the number of benzine rings in aromatics hydrocarbons increases their level of toxicity as pyrene hydrocarbon possesses higher benzene ring than anthracene and xylene hydrocarbons respectively. The toxicity factor of aromatic hydrocarbon contaminated wastewaters were generally more than hydrocarbon contaminated distilled water. The reasons could be as a result of the interactions of these aromatic hydrocarbons, heavy metals and other organic and inorganic pollutants present in the sampled locations as they are generally known as toxicants. Adenike [26], reported that the genotoxic effect of the tobacco waste effluent as validated from the various tests of his study can be a result of the interactions of heavy metals which can be more deleterious than the individual effects. All the samples had their coefficient of variation (CV %) to less than 25 thereby proving the biological validity criterion of the test and which collaborate with guidelines of EBPI (2016) that for Toxi-ChromoTest™ quantitative results to be considered valid, the CV between the absorbance values of negative controls and sample replicates must be less than 25 percent. The result of the mean 1.5 hr EC_{50} (mg /L) toxic response of mutant *E. coli* to the aromatic hydrocarbons in distilled and wastewater samples is shown in Figure 2. From the Figure, xylene distillled water had the highest EC_{50} of 3.417 ± 0.094 mg/L while pyrene Onne water had the least EC_{50} of 0.015 ± 0.002 mg/L. The levels of toxicity class are drawn in accordance with International Regulations and National Legislative Program as followed: Highly toxic - LC_{50} / EC_{50} < 1mg /L; Toxic - 1mg /L< LC_{50}/ EC_{50} ≤ 10 mg /L; Harmful / hazardous for aquatic environment – 10 mg /L < LC_{50} / EC_{50} ≤ 100 mg/l; Very low toxic, non-toxic - LC_{50} / EC_{50} > 100 mg /L [28]. Generally, it could be deduced that pyrene and anthracene are significantly (*P* = .05) highly toxic aromatic hydrocarbons (EC_{50} < 1 mg /L) while xylene is a significantly (*P* = .05) toxic aromatic hydrocarbon (1 mg /L < EC_{50} ≤ 10 mg /L) compare to the positive control ($HgCl_2$) (EC_{50} < 1 mg /L) indicating that enzyme inhibition among test samples were much different from the positive control. These toxicity results (EC_{50}) are in line with other toxicity values for this type of

pollutants and are therefore consider being scientifically relevant and can be used in aquatic risk assessment.. Aruoja *et al.* (2011), also found out that the EC_{50} values of *V. fischeri* 15 minutes luminescence inhibition were experimentally determined for 28 aniline and 30 phenol compounds. Despite the fact that the analyzed molecules were structurally similar, the EC_{50} values spanned three orders of magnitude ranging from 0.37 mg /L (2, 3, 5 - trichlorophenol) to 491 mg /L (aniline). 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 in their EC_{50} values were clearly higher than our results and the reason could be due to the organism's responses which differ from our mutant *E. coli* used in our 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

O	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

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

Table 2. Total heavy metal concentration (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 4. Absorbance values of the toxicity of the aromatic hydrocarbons in distiiled water and wastewater of the three sampled locations using Toxi-chromotest at different dilutions

Sample	Positive Control	Negative control	Xylene Distilled water	Xylene Abonei water	Xylene Nembe water	Xylene Onne water	Anthra + Distilled water	Anthra + Abonei water	Anthra + Nembe water	Anthra e + Onne water	Pyren Distille water	Pyren Abone water	Pyren Nemb Wate	Pyren Onne water	Abone water	Nemb water	Onne wate
Undilut	0.146	0.156	0.142	0.121	0.146	0.159	0.155	0.188	0.183	0.294	**0.398**	0.332	0.268	0.167	0.270	0.183	0.25
½	0.127	0.156	0.122	0.146	0.128	0.135	0.155	0.236	0.150	0.235	0.240	0.330	0.230	0.178	0.258	0.190	0.24
¼	0.135	0.138	0.115	0.123	0.134	0.107	0.115	0.216	0.235	0.197	0.271	0.274	0.213	0.186	0.229	0.204	0.23
1/8	0.138	0.128	0.142	0.123	0.124	0.112	0.163	0.199	0.217	0.190	0.220	0.256	0.273	0.192	0.234	0.223	0.29
1/16	0.120	0.138	0.127	0.123	0.112	0.123	0.171	0.243	0.186	0.179	0.250	0.254	0.197	0.183	0.236	0.183	0.26
1/32	0.127	0.128	0.123	0.123	0.118	0.117	0.145	0.217	0.152	0.190	0.243	0.234	0.182	0.188	0.225	0.169	0.23
1/64	0.117	0.127	0.126	0.120	0.113	0.104	0.194	0.233	0.165	0.219	0.265	0.224	0.287	0.192	0.260	0.219	0.24
1/128			0.199	0.126	0.138	0.146	0.101	0.173	0.184	0.230	0.235	0.234	0.200	0.184	0.226	0.143	0.23
1/256			0.123	0.123	0.123	0.165	0.155	0.177	0.169	0.185	0.174	0.218	0.315	0.137	0.242	0.170	0.20
1/512			0.108	0.105	0.132	0.133	0.176	0.211	0.167	0.177	0.178	0.174	0.226	0.119	0.199	0.308	0.10
1/1024			0.113	0.111	0.138	0.161	0.156	0.182	0.155	0.155	0.218	0.185	0.276	0.144	0.289	0.196	0.31
1/2048			**0.097**	0.119	0.126	0.152	0.136	0.188	0.174	0.190	0.236	0.234	0.226	0.141	0.214	0.244	0.23
1/4096			0.123	0.112	0.131	0.153	0.175	0.189	0.166	0.181	0.259	0.208	0.308	0.161	0.308	0.238	0.28
Sample Blank			0.119	0.133	0.123	0.141	0.877	0.189	0.171	0.121	0.318	0.184	0.244	0.202	0.280	0.228	0.21
Blank		0.186	0.1755	0.195	0.2205	0.230	0.2325	0.259	0.228	0.252	0.306	0.570	0.286	0.220	0.297	0.250	0.51

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

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

Table 6. Mean values of cell algal density measurements (Cells/ml X 10^4) with their respective coefficient of variation, specific growth rates and percentage inhibition at different concentrations of anthracene

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 variation, specific growth rates and percentage inhibition at different concentrations of pyrene

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: 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

Plate 1. Microplate test result for Toxi - chromo testing of aromatic hydrocarbon compounds and marine water samples

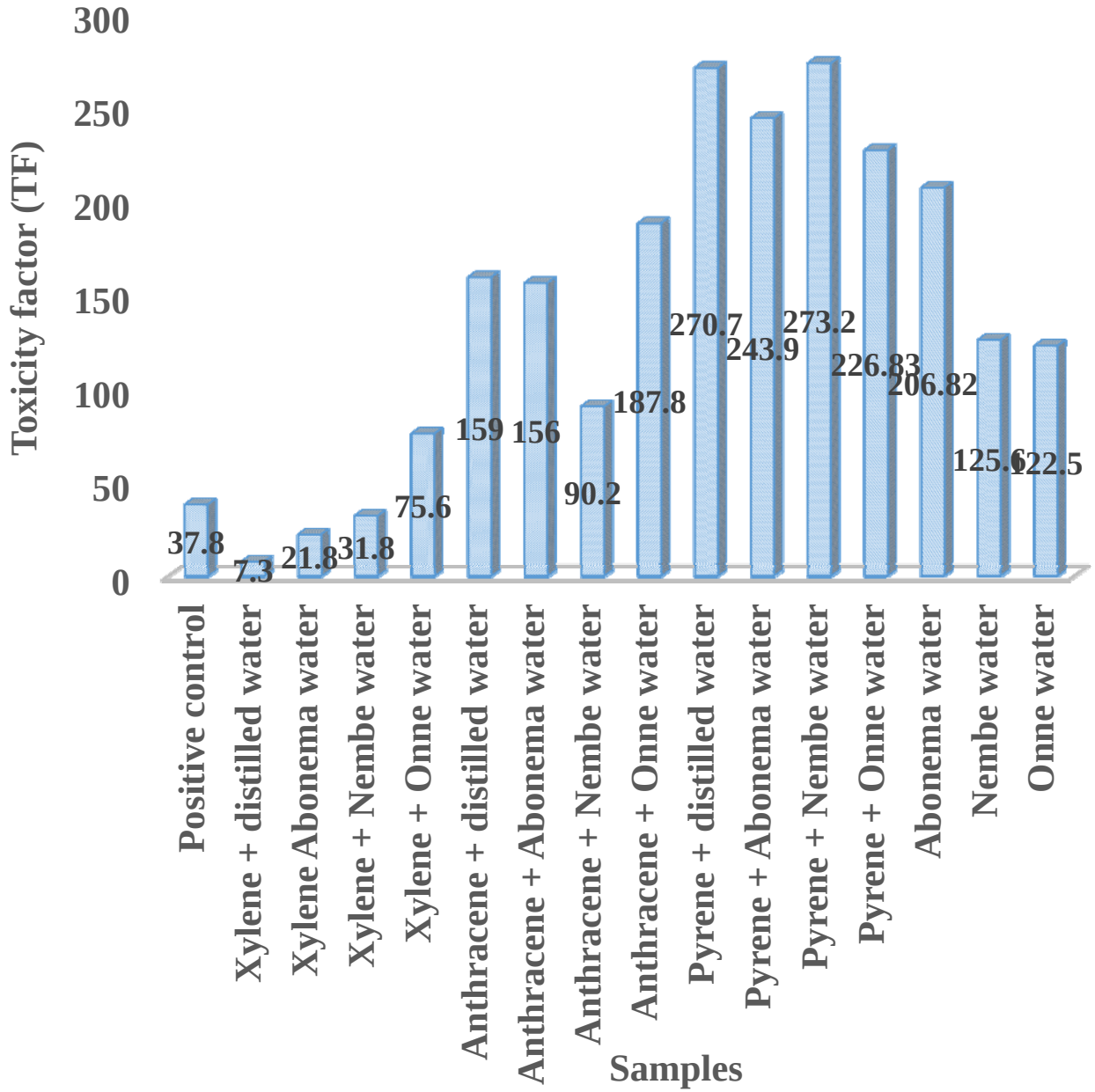
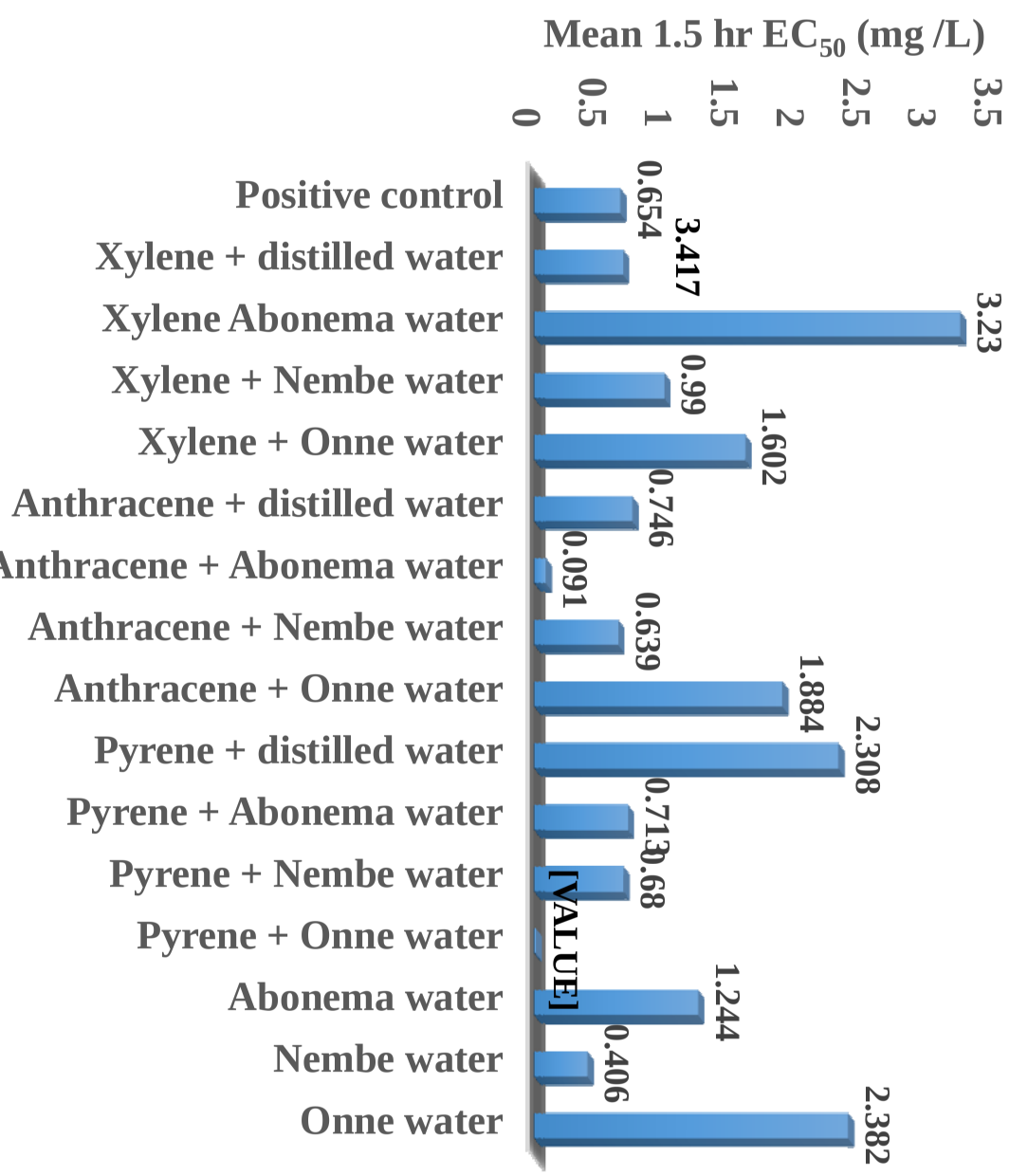

Toxicity factor (TF)
300
250
200
150
100
50
0
37.8
7.3
21.8
31.8
75.6
159
156
90.2
187.8
270.7
243.9
273.2
226.83
206.82
125.6
122.5
Positive control
Xylene + distilled water
Xylene Abonema water
Xylene + Nembe water
Xylene + Onne water
Anthracene + distilled water
Anthracene + Abonema water
Anthracene + Nembe water
Anthracene + Onne water
Pyrene + distilled water
Pyrene + Abonema water
Pyrene + Nembe water
Pyrene + Onne water
Abonema water
Nembe water
Onne water
Samples

Fig. 1.Toxicity factor (TF) of the aromatic hydrocarbons in distilled water and wastewater of the three sampled locations


Mean 1.5 hr EC50 (mg /L)
0
0.5
1
1.5
2
2.5
3
3.5
Positive control
Xylene + distilled water
Xylene Abonema water
Xylene + Nembe water
Xylene + Onne water
Anthracene + distilled water
Anthracene + Abonema water
Anthracene + Nembe water
Anthracene + Onne water
Pyrene + distilled water
Pyrene + Abonema water
Pyrene + Nembe water
Pyrene + Onne water
Abonema water
Nembe water
Onne water
0.654
3.417
3.23
0.99
1.602
0.746
0.091
0.639
1.884
2.308
0.713
0.68
[VALUE]
1.244
0.406
2.382

5. CONCLUSION

To our knowledge, this is one of the first systematic studies on effects of marine water on bacterial enzyme inhibition and first or description of toxic effects of aromatic hydrocarbons towards bacteria activities in 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 physico-chemical parameters in the sediment samples than water samples.

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

- Pyrene in Nembe water had the highest toxicity factor while xylene in distilled water had the lowest toxicity factor.

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

- The toxicity results (< 0.1 mg /L $< EC_{50} \leq 10$ mg /L) in this study indicated that the potential ecotoxicity and environmental health effects of these toxicants should be given attention in order to get rid of the dangerous outcome.

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