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Toxic Effect of Refinery Industrial Effluent Using Three Toxicity Bioassays

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SOE, MUO and BOU designed the study. Author BOU performed the statistical analysis and wrote the protocol. Authors SOE and BOU wrote the first draft of the manuscript while authors SOE and MUO managed the analyses of the study. All authors managed the literature searches, read and approved the final manuscript.

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ABSTRACT

Aims: To determine the toxic effect of refinery industrial effluents using three toxicity bioassays.
Study Design: Five treatments and the controls designs were set up in triplicates containing 6.25%,12.5%, 25%, 50%, 100% and 0% of the industrial effluents and incubated at 24°C for 0 - 96 h. The five treatments and control set ups designated as PH, Warri and Control (Without effluent) were used to determine the toxic effect of industrial effluents.
Place and Duration of Study: Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Nigeria between September, 2019 and December, 2019.
Methodology: A laboratory scale study was carried on two composite samples of the produced water samples from the two studied areas using physicochemical analyses, microalgal toxicity test, mollusk toxicity and *Zea mays* test.
Results: The results revealed that Port Harcourt refinery effluent contains higher quantities of physicochemical parameters than the Warri effluent sample. Warri sample had the most harmful

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effects on *Selenastrum capricornutum*, *Lymnaea stagnalis* and *Zea mays*, with ErC_{50} values of 47.62%, LC_{50} of 51.86% and EC_{50} of-32.68%, respectively. Inhibition (%) and mortality (%) of all species used were found to be concentration dependent with a significant ($P < 0.05$) strong positive correlation at increasing concentrations of industrial effluents.
Conclusion: Thus, these raw industrial effluents from Port Harcourt and Warri refineries are toxic and induced growth inhibition, mortality and phytotoxicity and adequate measures should be taken by these industries to minimize their negative environmental impacts.

Keywords: Industrial effluents; growth inhibition; mortality; phytotoxicity and bioassay.

1. INTRODUCTION

Produced water (PW) is the waste usually generated in largest volume during production of oil and gas from offshore oil and gas wells [1]. Most PW is fossil water (formation water) that has accumulated over millions of years with fossil fuels in geologic formations deep in the earth. PW also may contain some surface water that has been injected into the formation for enhanced oil recovery. PW reaches the surface from natural oil seeps worldwide and during production of oil and gas from a well [1]. The chemical characteristics of PW are different for each production platform or formation from which the oil is extracted. It is typically highly saline and contains elevated levels of heavy metals, hydrocarbons (including polycyclic aromatic hydrocarbons (PAHs)), alkylphenols, ammonia and radionuclides compared to the receiving environment [2,3].

In Nigeria, the Department of Petroleum Resources (DPR), a Regulatory body of the Oil and Gas Industry in Nigeria has stipulated guidelines and standards for the management and discharge of Produced water and has set limits within which waste water generated from the activities of the petroleum industry in Nigeria must meet. This is prior to its discharge into the aquatic ecosystem (brackish and saline water). In an endeavour to operate within these stipulated regulatory limits, most oil companies treat their wastewater before they are discharged into the environment. Nevertheless, studies have revealed that some of the treated produced water do not meet the DPR limits with respect to some of the parameters, before being discharged into the surrounding [4].

Biotesting for environmental samples is integral to environmental regulations in many countries, including Nigeria. Lab testing, involving application of 'short-term bioassays' gives results that complement bioindication research data and improve credibility of biodiagnostics of the ecological quality of natural and man-made environments. A sufficiently broad range of freshwater toxicity bioassays have been devised and are to date used in global practice. The applicability of toxicity bioassays as efficient analytical tools should be supported by their standardization and validation [5].

Algae are often included among the species used in biotest batteries for hazard assessment of chemically contaminated wastes and leachates. As primary producers, 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. It is therefore crucial to assess the toxicity of chemicals to algae as the pollution is likely to end up in water bodies *via* industrial or household waste [6]. *Selenastrum capricornutum* was selected in this study because it is a model organism recommended by ISO [7].

Among aquatic organisms used in ecotoxicological studies, invertebrates have been employed due to their importance in trophic chains and greater sensitivity response to chemical pollutants. Although, mollusks are the second largest group in kingdom Animalia, they have not been considered in environmental risk assessment so far, mainly due to the lack of standardized protocols. In this sense, gastropods, the most abundant mollusks, have been successfully used as pollution indicators by different compound classes, such as, metals, pesticides and polluted waste water [8]. The use of *Lymnaea stagnalis* which are fresh water snails by several researchers have demonstrated that this species is a good model for laboratory and monitoring environmental studies, very common, readily available, cheap and globally distributed especially here in Nigeria [9–13].

Among the bioassays developed for detection of mutagenicity, genotoxicity, cytotoxicity, phytotoxicity and clastogenicity due to environmental pollutants, plant systems have

proven to be sensitive, cheap, and effective. Plant bioassays, which are mostly sensitive for the detection of phytotoxicity, may provide a warning of environmental hazards in the water [14]. *Zea mays* test has demonstrated that germination, root elongation and shoot length are the most authoritative parameters that indicate changes in environmental quality [15–17].

Several studies and literatures abound on the effects of refinery industrial effluents on microorganisms, invertebrates, plants and animals but there is paucity of information regarding their toxicities on *Selenastrum capricornutum*, *Lymnaea stagnalis* and *Zea mays* especially in our country Nigeria and hence, necessitated this study. So, this is the first study to be carried to the best of our knowledge here in our country Nigeria. This study was undertaken to determine the toxic effects of refinery industrial effluents on *Selenastrum capricornutum*, *Lymnaea stagnalis* and *Zea mays* using a battery of bioassays.

2. MATERIALS AND METHODS

2.1 Description of the Studied Area

The studied areas were Port Harcourt Refining Company (PHRC) located in Alesa-Eleme, in Eleme Local Government Area of Rivers State with coordinates 4^{o} 44'N and 6^{o} 49'E and Warri Refinery and Petrochemical Company (WRPC) located in Ubeji Community in Warri, Warri South Local Government Area of Delta State with coordinates 5^{o} 33'N and 5^{o} 42'E, respectively.

2.2 Sample Collection and Preparation

Ten random samples of the refinery industrial effluents (produced water) were collected from the ten (10) designated points of the two sampling sites. The samplings were done once in each of the two sampling sites in August, 2019. The samples were mixed together to obtain a total of two representative samples which were used for the analysis. The water samples were collected at the air-water interface by hand dipping of the sterile and clean 2 L plastic containers. The containers were aseptically rinsed with the samples thrice before collection. All the representative sample containers were labelled with sample type, date, time, and place of collection. They were placed into a sterile polythene bags in ice packed coolers and then transported to the Microbiology Laboratory, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Nigeria. The refinery industrial effluents samples were prepared by centrifuging and filtering the suspensions through glass fiber filters (d = 0.45 µm) using membrane filtration technique prior to the physico – chemical and toxicological assays [6,18–20].

2.3 Physicochemical Analysis

The following analysis were carried out on the samples: temperature, pH, conductivity and total dissolved solids, total suspended solids, total solids, turbidity, total hardness, total chloride, nitrate, phosphate, sulphate, total alkalinity, dissolved oxygen, biochemical oxygen demand (BOD_5)and chemical oxygen demand, oil and grease content according to the method of APHA [21]. Also, oil and grease content, total petroleum hydrocarbon (TPH), total hydrocarbon content (THC) and total phenol were determined by adopting the methods of Kiepper [22], Akpveta et al. [23], Nwineewii and Azuonwo [24] and Leouifoudi et al. [25]. The heavy metals such as iron, mercury, copper, chromium and lead) were determined by atomic absorption spectrometry following the method of APHA [21].

2.4 Microbiological Analysis

2.4.1 Determination of total cultural heterotrophic bacterial and fungal count (TCHBC/TCHFC)

Total heterotrophic bacterial and fungal counts for each effluent samples were enumerated using spread plate method as described by Willey et al. [26]. An aliquant (0.1 mL) of the dilution of 10^4 were aseptically transferred unto properly dried Nutrient Agar and Sabouraud Dextrose Agar plates containing antibiotic (tetracycline) to inhibit bacterial growth in duplicate, spread evenly using bent glass rod and incubate at 28°C for 24 h and at 28°C for 3 days, respectively. After incubation, the bacterial and fungal colonies that grew on the plates were counted and average counts were taken. The colony forming unit for the THBC of effluent samples were then calculated using the formula; THFC (CFU/mL) = Number of Colonies x Dilution factor (10^4) x volume plated (0.1 mL) [27].

2.4.2 Determination of hydrocarbon utilizing bacterial and fungal count (HUBC/HUFC)

The vapour phase method was used to determine the HUBC and HUFC on mineral salt agar containing 0.04 g MgSO4. $7H_2O$, 0.03 g KCl, 0.09 g KH_2PO4, 0.04 g $NaNO_3$, 0.13 g

K_2HPO4, 2.0 g NaCl, 15 g of agar powder, 100 mL of distilled water amended with 0.1 g of nystatin to inhibit the growth of fungi and tetracycline to inhibit bacteria [27]. Then, it was sterilized by autoclaving at 121°C and 15 psi for 15 min and allowed to cool to about 45°C. The already prepared medium was poured into Petri dishes and allowed to gel, then 0.1 mL of the inocula was spread on plates with rod aseptically. Filter paper (Whatman No 1) was saturated with crude oil and the crude oil impregnated papers were aseptically placed onto the Petri covers. The crude oil saturated filter papers supply diesel by vapour - phase transfer to the inocula. The plates were incubated by inversion for 7 to 10 days at 28°C. Plates yielding 30 to 300 colonies were enumerated from triplicates and mean values were recorded and calculated in CFU mL^{-1}.

2.5 Toxicity Bioassay

2.5.1 Algal growth inhibition test with unicellular green algae

The algal growth inhibition test was carried out according to the methods of ISO [7] with little modifications.

2.5.1.1 Preparation of growth medium

The growth medium was prepared by adding an appropriate volume of the nutrient stock solutions to water. Then, add to approximately 500 mL of water, 10 mL of stock solution 1 (NH_4Cl, $MgCl_2.6H_2O$, $MgSO_4.7H_2O$ and KH_2PO_4); 1 mL of stock solution 2 ($FeCl_3.6H_2O$, $Na_2EDTA.2H_2O$); 1 mL of stock solution 3 (H_3BO_3, $MnCl_2.4H_2O$, $ZnCl_2$,$CoCl_2.6H_2O$, $CuCl_2.2H_2O$, $Na_2MoO_4.2H_2O$) ; 1 mL of stock solution 4 ($NaHCO_3$) and finally made up to 1000 mL with water. Before use, the medium was equilibrated overnight, adjusted to 8.1 ± 0.2 using 1 mol/L sodium hydroxide solution and finally buffered with hydrogen carbonate.

2.5.1.2 Preparation of pre-culture and inoculum

One of the two tubes containing the microalga (*Selenastrum capricornutum*) inoculum was taken, shaken vigorously and the content was poured into 100 mL flask.The (same) tube was rinsed twice with 7.5 mL algal growth medium and the content was transferred into the flask to ensure the total transfer of the microalga inoculum. The flask was closed with the lid and incubated for 3 days in an incubator (Kottermann D3165, West Germany) at 23 ± 2°C under continuous white fluorescent lamps. After the 3 days of incubation, the suspension was placed in the spectrophotometer and the optical density (OD) was read at 700 nm after 10 seconds. The number of alga corresponding with OD was interpolated from the optical density/algal number (OD/N) standard curve and was used to determine the dilution factor needed to reach an optical density equal to OD2, corresponding to an algal density of 5 x 10^4 cells/mL. The algal suspension from the flask was transferred into a 100 mL sterile flask and the volume of algal growth medium needed to make up a 5.10^4 algal cells/mL suspension was added and the flask shaken thoroughly to distribute the algae evenly.

2.5.1.3 Choice of test sample concentration and preparation

The microalga cells were exposed to concentrations of the test sample in a geometric series with a ratio not exceeding 2.0 as follows: 0%, 6.25%, 12.5%, 25%, 50%, and 100%. The Port Harcourt and Warri samples were prepared as previously described in 3.2 above after which nutrient stock solutions 1, 2, 3, and 4 were added to the samples as prepared in accordance with 3.5.1.1. A quality control test was carried out with the reference chemical potassium dichromate ($K_2Cr_2O_7$) having toxicant dilution series: 1.0 mg/L, 10 mg/L, 18 mg/L, 32 mg/L, and 56 mg/L.

2.5.1.4 Preparation of test and control batches

The test batches were prepared by mixing the appropriate volumes of test sample, growth medium and inoculum in all the test vessels. Three replicate batches for each test sample concentration and six replicates control batches were prepared by adding the appropriate volume of inoculum to growth medium. The pH of a replicate batch at each test concentration and in one control replicate were measured.

2.5.1.5 Incubation and measurement

The test vessels were covered with lids to avoid airborne contamination and to reduce water evaporation and then incubated at 23 ± 2°C under aeration and continuous white fluorescent lamps for 72 h. The cell density in each test batch (including the controls) were measured at 0, 24, 48 and 72 h by mixing the test batches prior measurement. At the end of the test, the pH of the samples of at least one replicate batch at

each test sample concentration and one control replicate were measured.

2.5.1.6 Calculation

2.5.1.6.1 Plotting of growth curve

The cell density measurements for each test batch was tabulated according to the concentration of the test sample and the duration of measurement. Growth curve for each test concentration and control was plotted as a graph of the logarithm of the mean cell density against time. The mean growth rate, specific growth rate and growth inhibition was calculated using the formula:

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

where: *t0* is the time of test start; *tL* is the time of test termination or the 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.

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

Where: *I*μi is the percentage inhibition (growth rate) for test concentration *I*; μi is the mean growth rate for test concentration *i*; μc 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.

2.5.2 Mollusc toxicity test

2.5.2.1 Laboratory animal

The freshwater snails, (*Lymnaea stagnalis*) were collected from Omor River in Ayamelum Local Governement Area of Anambra State, Nigeria (body weight = 23.73 ± 0.21 g) and transported in field water to the laboratory. The snails were kept after two weeks collection for adaptation in aerated and filtered tap water contained in 5 L per six 10 L aquarium containers (dimension: 43 cm X 32 cm x 25 cm). The snails were maintained under laboratory conditions (BOD = 11.30 mg/L, temperature = 28.30°C, pH = 7.90) with photoperiod 12 h light: 12 h dark. The culture was fed fresh carrots *ad libitum* and a balanced diet before toxicity studies according to the methods described by Sheir [28] and Atlia and Grosell [13].

2.5.2.2 Adult snail exposure assay

Static-renewal acute toxicity assays were performed with adult snails (*Lymnaea stagnalis*) at least four months old and with shell diameter of 20 mm were not fed 24 h before test start. The mortality before assay start did not exceed 10 percent. The animals were exposed to five Port Harcourt and Warri effluents concentrations of 6.25, 12.5, 25, 50 and 100% and a negative control group that was exposed to only tap water under the same experimental conditions. The chosen lethal concentrations were determined by previous work done by Tallarico et al. [8]. Exposures were performed for 96 h in triplicate for a total of 10 snails per treatment. After 24 h of exposure, snails were washed and observed daily (24, 48, 72 and 96 h). Mortality and abnormal responses, as retraction or extending of body and release of mucus and hemo- lymph, were recorded at each observed time. The median lethal concentration (LC_{50}) of the samples was thus determined.

2.5.3 *Zea mays* test

The modified guidelines for the testing of effluents on seeds of terrestrial plants according to the Organization for Economic Cooperation Development (OECD) [29] were used for this study. First, floatation method was adopted to determine the viability of the seeds. The seeds that sink represent the viable seeds while the seeds that float represent seeds the non – visible seeds. Surface sterilization of the viable seeds was then performed by immersing the seeds into 70% ethanol for 5 minutes to reduce contamination and finally washed thoroughly with water. Ten maize seedlings were placed in Petri dishes lined with tissue paper containing 30 mL each of the effluent (Port Harcourt and Warri) in different concentrations (6.25%, 12.5%, 25%, 50% and 100%), while the Petri dishes lined with tissue paper containing 30 mL of distilled water served as the control. The investigation of each concentration including the control was carried out in triplicates. The Petri dishes were covered with lids to prevent evaporation. The seeds were planted under room temperature for seven (7) days, after which the seed germination (%), shoot length (cm), root length (cm), relative root length percentage, germination index (%) and vigour index were measured and calculated [30]. The shoot length measurement was taken from

the base to the apical leaf of the plant using a transparent plastic ruler; while the root length was also measured by the same procedure after it was harvested and carefully washed with distilled water.

2.6 Analysis of Data

The data obtained in this study were subjected to descriptive statistics, one - or two-way ANOVA with Dunnet multiple comparison, paired two tailed T – test and Pearson coefficient of variation among the samples, controls and concentrations at 95% confidence intervals such that values lower than 0.05 probability level are considered statistically significant ($P < 0.05$).

3. RESULTS

3.1 Physicochemical Profile

The result of the physicochemical qualities of the refinery produced discharge water is presented in Table 1. From the result, the Port Harcourt (PH) produced water had the highest values of conductivity, TDS, turbidity, TS, total Chloride, nitrate, temperature, phosphate, sulphate, DO, BOD, COD, oil and grease, TPH, THC, TCHBC, TCHFC, copper, mercury, lead and phenol while Warri sample had the highest values of pH, total hardness, total alkalinity, TCHB, and iron. Both samples had too low to count (TLTC) in their hydrocarbon utilizing bacteria count (HUBC) and

Table 1. Physicochemical qualities of the refinery produced discharge water

Parameters	PH sample	Warri sample	DPR standard
PH	5.30	6.30	6.50 - 8.50
Conductivity (µS/cm)	460.00	350.00	-
Temperature (℃)	28.90	28.60	30.00
Turbidity (NTU)	1898.00	193.25	15.00
TDS (mg/L)	345.00	262.50	5000.00
TSS (mg/L)	39.50	42.70	50.00
TS (mg/L)	384.50	304.70 -	
Total hardness (mg/L)	420.00	465.00	-
Total Chloride (mg/L)	97.63	44.40	2000.00
Nitrate (mg/L)	26.96	10.02	-
Phosphate (mg/L)	3.91	3.69	-
Sulphate (mg/mL)	617.40	411.60	200.00
Total alkaline (mg/L)	340.00	490.00	-
DO (mg/L)	16.20	12.80	-
BOD (mg/L)	262.00	22.00	125.00
COD (mg/L)	393.00	33.00	125.00
Oil and grease (mg/L)	51,900.00	7,900.00	40.00
TPH (mg/L)	18,270.00	17,590.00	-
THC (mg/L)	11,390.00	9,296.00	40.00
Phenol (GAE)	594.52	441.85	-
TCHBC (CFU/mL)	$4.1x10^5$	$9.1X10^5$	-
TCHFC(CFU/mL)	$3.2x10^5$	TLTC	-
HUBC (CFU/mL)	TLTC	TLTC	-
HUFC (CFU/mL)	TLTC	TLTC	-
Iron (ppm)	3.13	3.77	1.00
Chromium (ppm)	0.07	0.03	0.50
Mercury (ppm)	0.03	0.02	0.00
Lead (ppm)	0.68	0.79	-
Copper (ppm)	0.98	0.94	-

Key: PH = Port Harcourt, TDS=Total dissolved solid, TSS = Total suspended solid, TS=Total solid, DO=Dissolved oxygen, BOD=Biochemical oxygen demand, COD=Chemical oxygen demand, TPH=Total petroleum hydrocarbon, THC=Total hydrocarbon content, TCHBC = Total culturable heterotrophic bacterial count, TCHFC = Total culturable heterotrophic fungal count, GAE= Gallic acid equivalent, ppm= Part per million, CFU/ml=Colony forming unit per milliliter, mg/L=Milligram per liter, µS/cm= micro Siemen per centimeter, °C = Degree centigrade, NTU=Nephelo transmitting unit, HUBC= Hydrocarbon utilizing bacterial count, HUFC = Hydrocarbon utilizing fungal count, DPR = Department of Petroleum Resources, TLTC = Too low to count

utilizing fungi count (HUFC) while total culturable heterotrophic fungi count (TCHFC) in Warri sample is TLTC.

3.2 Acute Toxicity Profile

3.2.1 Algal toxicity studies

The results of the algal growth inhibition by refinery discharge effluents and potassium dichromate as well as 72 h effective growth rate concentration of refinery discharge effluents and potassium dichromate on *Selenastrum capricornutum* are shown in Figs. 1 and 2. From the results, 100% concentration of Warri sample had the highest percentage (%) inhibition (65.84%) while PH sample had the lowest percentage (%) inhibition (65.85%). Also, PH and Warri samples had the highest and lowest ErC_{10}, ErC_{50}, ErC_{90} values of 10.53%, 52.58%, 94.74% and 9.52%, 47.62%, 85.71%, respectively.

3.2.2 Mollusc toxicity profile

The toxic responses of *Lymnaea stagnalis* to PH and Warri samples refinery discharged effluents are shown in Tables 2 and 3. From the results, there was a higher mortality rate in PH sample at 100 % and 50 % concentrations with LC_{50} value of 85.47 % while Warri sample recorded an optimum mortality rate at all the concentrations with LC_{50} value of 51.86% after 72 h exposure. Also, the toxic effect of refinery discharged effluents to *Lymneae stagnalis* after 72 h exposure is shown in Fig. 3. From the result, Warri sample had higher percentage mortality at all the concentration while PH sample recorded optimum toxicity at increase concentrations (0 – 100%).

3.2.3 *Zea mays* toxicity profile

The results of the growth features of *Zea mays* seedlings exposed to Port Harcourt and Warri refinery discharged effluent are presented in Tables 4 and 5. From the results, 100% and 6.25% PH effluent sample had the lowest and highest GI and VI values of 13.11%, 86.94%, 1.10 and 12.53, respectively. Similarly, 100 % and 6.25% Warri effluent sample had the lowest and highest GI and VI values of 7.55%, 76.85%, 0.83 and 10.53, respectively. The result of the toxicity threshold concentrations of refinery discharged effluents on *Zea mays* seedlings is shown in Fig. 4. From the Figure, Warri and PH samples had the highest and lowest EC_{10}, EC_{50} and EC_{90} values of - 80.40%, - 32.68%, 15.04% and - 87.54%, - 37.56% and 12.43%, respectively.

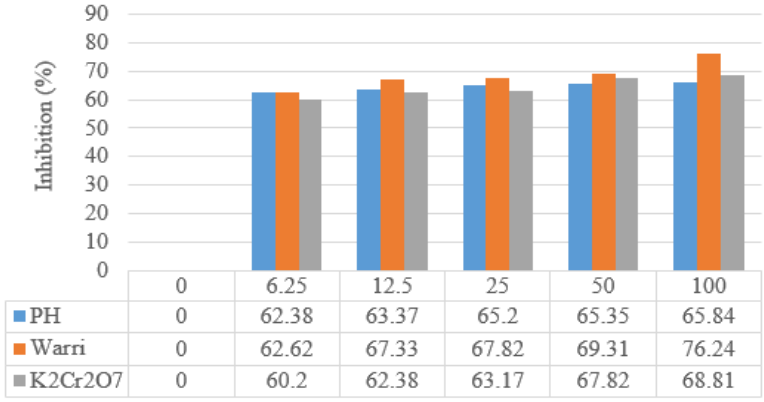

Fig. 1. Algal growth inhibition by refinery discharge effluents and potassium dichromate

Table 2. Toxic response of *Lymnaea stagnalis* to Port Harcourt refinery discharged effluent

Concentration (%)	Mortality rate					Total (%)	Mean ± S.D	LC_{50} (%) (CL)
	0 h	24 h	48 h	72 h	96 h			
100	0	3	3	4	4	14 (46.7)	3.5±0.27	
50	0	2	3	3	4	12 (40.0)	3±0.50	
25	0	1	2	2	4	9 (30.0)	2.25±51.11	85.47 (-50.3 - 29)
12.5	0	1	2	2	2	7 (23.3)	1.75±1.36	
6.25	0	0	1	1	2	4 (13.3)	1±1.92	
0	0	0	0	0	1	1 (3.33)	0.25±0.00	

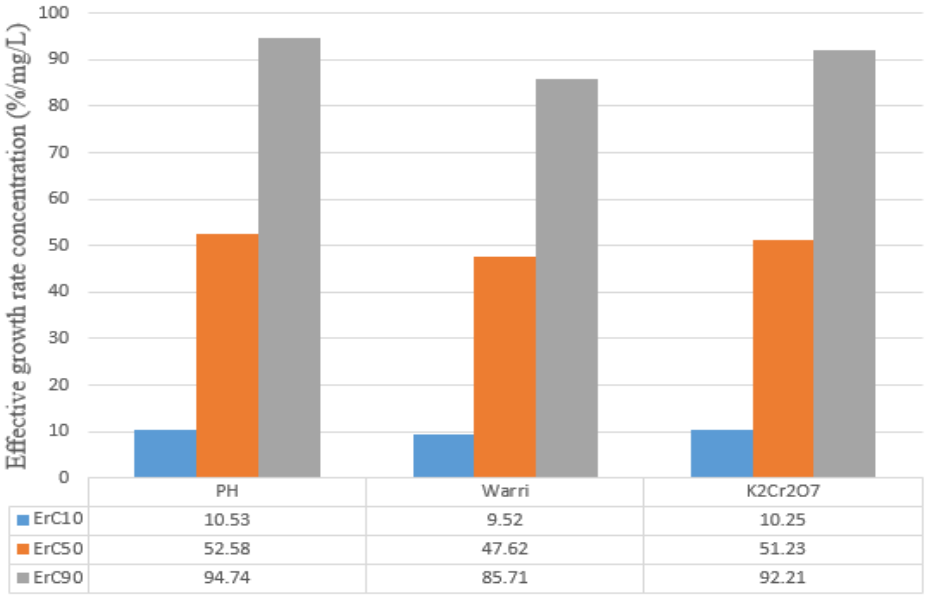

Fig. 2. 72 h effective growth rate concentration of refinery discharge effluents and potassium dichromate on *Selenastrum capricornutum*

Table 3. Toxic response of *Lymnaea stagnalis* to Warri refinery discharge effluent

Concentration (%)	Mortality rate					Total (%)	Mean ± SD	LC_{50} (%) (CL)
	0 h	24 h	48 h	72 h	96 h			
100	0	1	1	9	9	20 (66.7)	5 ± 1.79	
50	0	1	1	9	9	20 (66.7)	5 ± 1.79	51.86 (- 624 - 136)
25	0	1	0	9	10	20 (66.7)	5 ± 1.69	
12.5	0	0	0	10	10	20 (66.7)	5 ± 1.58	
6.25	0	0	0	8	8	16 (53.3)	4 ± 1.06	
0	0	0	0	0	1	1 (3.3)	0.25 ± 0.00	

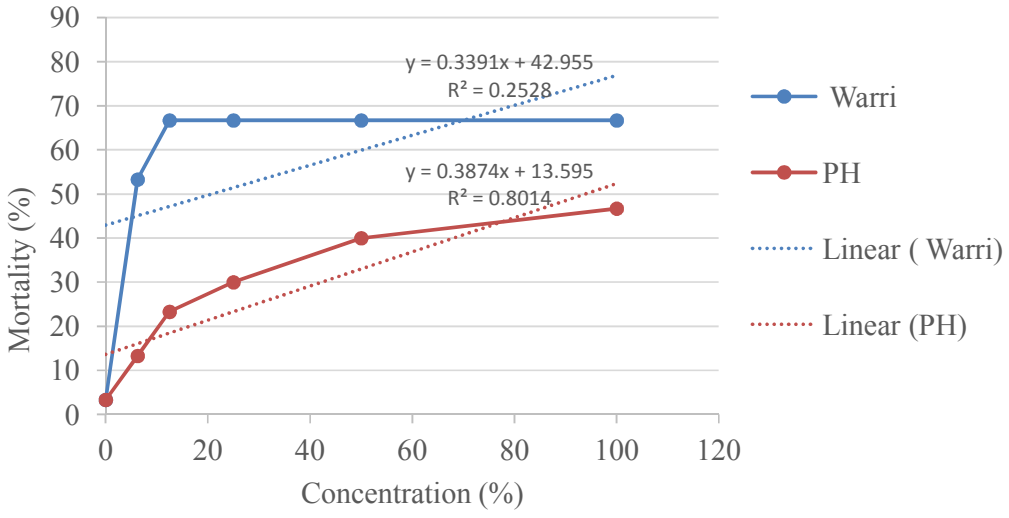

Fig. 3. Toxic effect of refinery discharge effluents to *Lymnae stagnalis* after 72 h of exposure

Table 4. Growth features of *Zea mays* seedlings exposed to Port Harcourt refinery discharged effluent

	Concentration (%)					
	Control	**6.25**	**12.5**	**25**	**50**	**100**
Number of seed germinated	9	8	6	5	3	2
Root length (cm)	5.00	4.94	4.30	4.01	3.50	2.95
Shoot length (cm)	10.00	9.30	7.00	6.00	3.7	2.00
% relative seed germination	-	88.00	66.67	55.56	33.33	22.22
% relative root growth	-	98.80	86.00	80.20	70.00	59.00
Germination Index (GI)	-	86.94	57.34	44.56	23.33	13.11
Vigour index (VI)	-	12.53	7.53	5.56	2.40	1.10

Table 5. Growth features of *Zea mays* seedlings exposed to Warri sample refinery discharged effluent

	Concentration (%)					
	Control	**6.25**	**12.5**	**25**	**50**	**100**
Number of seed germinated	9	7	6	4	2	1
Root length (cm)	5.00	4.94	4.70	4.20	3.95	3.40
Shoot length (cm)	10.00	8.60	6.30	6.30	4.70	4.07
% relative seed germination	-	77.78	66.67	44.44	22.22	11.11
% relative root growth	-	98.80	94.00	84.00	79.00	68.00
Germination Index (GI)	-	76.85	62.67	37.33	17.55	7.55
Vigour Index (VI)	-	10.53	7.53	7.33	1.92	0.83

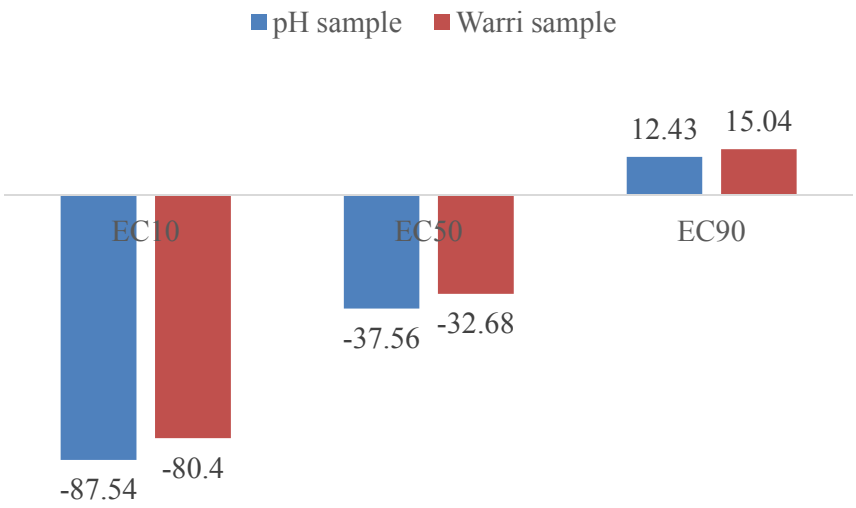

Fig. 4. Toxicity threshold concentrations of refinery discharged effluents on *Zea mays* seedlings

4. DISCUSSION

Many industries particularly refinery industries in Port Harcourt and Warri areas of Niger Delta produce effluents during production processes. These effluents are discharged most times indiscriminately into the aquatic and terrestrial ecosystems without adequate detoxification processes. This research is aimed at ascertaining the degree of toxicity of the effluents on *Selenastrum capricornutum*, *Lymnaea stagnalis* and *Zea mays* and to suggest ways to possibly curb the menace. From Table 1, the physicochemical analysis revealed that the samples are moderately acidic with a moderate temperature. This could be attributed to a high

content of acidic salts and compounds in the samples. The presence of these soluble organic salts and ions also made the samples to have high conductivity values. The concentration of DO is low and could results to respiratory malfunction in aquatic organisms due to stress caused by the effluents. The high BOD and COD levels showed that a high biological activities and chemical oxidation occurred in the samples resulting to decomposition of organic compounds. The BOD determines only the decomposable organics and needs a reasonably long period of time to obtain desired results. Also, the COD test processes the oxygen correspondent of the biological substances in waste water that can be oxidized chemically. The COD will always be greater than the BOD, because the COD checks materials that are both chemically and biologically oxidized. The ratio of COD: BOD gives a vital guide to the amount of organic material present in waste water [31,32]. Okereke et al. [33] also reported that the dissolve oxygen is beneficial in aerobic respiration of organisms which supports the observation made in this research work. The total hardness showed high presence of magnesium ion (Mg^{2+}) and calcium ion ($Ca^{2+)}$. The samples also contain linear and branched chains of aromatic and aliphatic hydrocarbon fragments as showed in the THC and TPH. The samples contain high oil and grease as well as phenol content which may be attributed to the high THC, TPH and phenolic compounds used and produced during refining processes. This increase had led to the selection of bacteria and fungi (though low counts) that can utilize or metabolize the hydrocarbons as carbon and energy sources known as hydrocarbon utilizers. The findings are similar to the works published by Okoro [34] and Ajuzieogu et al. [18] who both reported that the low microbial counts revealed that there is paucity of nutrients in oil produced water thereby supporting low population counts. The turbidity of the samples is high which shows that there is a high rate of impurities and dissolved substances. This is capable of increasing the BOD and COD of the samples as revealed above. The high TDS values of both samples correlated with the conductivity findings above as TDS is a surrogate value of salinity and conductivity. There were lower values of total chloride, nitrate, phosphate, sulphate, total alkalinity but higher values of the heavy metals analyzed. Comparatively, PH sample had a higher turbidity, conductivity, acidity, TDS, TS, total chloride, nitrate, phosphate, sulphate, DO, BOD, COD, oil and grease, TPH, THC, copper, chromium, mercury and phenol, while Warri sample had a higher TSS, total hardness, total alkalinity, TCHB, iron, and lead. From these results, it is obvious that the two samples are highly contaminated even though PH sample proved to be more contaminated than the Warri sample. These chemicals such as chloride, phosphate, nitrates and sulphates can result to pollution and make water hazardous for drinking, entertaining events and fishing activities when they are in high quantity [35]. They also encourage the growth of bacteria and substantial algae blooms [33]. Also, Okereke, and Nnoli [36] reported that high content of organic and inorganic phosphates results to eutrophication and either disappearance or shallowness of some surface water bodies. It is imperative to note that the samples exceeded most of the DPR standard limits for produced wastewater discharge which means that the environment where these effluents are discharged is unsafe for inhabitation of living organisms. The physicochemical results of this study revealed that the effluents contain chemicals mainly heavy metals and hydrocarbons which are capable of altering the normal concentration of a given habitat. Considering the standard limits for produced water by the Department of Petroleum Resources (DPR) [37], the physicochemical qualities of both samples (PH/Warri) vary tremendously but similar results were recorded by Ajuzieogu et al. [18]. The following parameters have their contents greater than the DPR standard limits: oil and grease, BOD, COD, turbidity and THC while TDS, TSS, total chloride, and chromium. From the result, the BOD and COD of Warri sample are lower than the DPR standard limits while that of PH is higher.

The results in Figs. 1 and 2 revealed that there was an exponential decrease in the algal cell counts which intensified as the time of exposure increases (24 - 72 h). The decrease in the cell number of algae when exposed to the samples showed that an unfavorable condition which decreased the rate of metabolism had been created. The cells were unable to withstand the dynamic nature of the effluents which eventually led to a distinct reduction in proliferation. This shows that the both samples (Warri / PH) sample had a high hydrocarbon and heavy metal contents even though PH sample was a bit higher. The specific growth rate decreases as the concentration of the samples increases which means that there is a decrease in cell density per time. This can be expressed in reciprocal days (day^{-1}) [7]. The average growth rate (3.61),

coefficient of variation (4.7 %) and pH (9.10) of our control replicates met the validity criteria of International Standard Organization [7] which states that the average growth rate, variation coefficient (CV) and pH in the control replicates shall be at least 1.4 d^{-1}, not more than 5% and 1.5 relative to the pH of the growth medium. This growth rate corresponds to an increase in cell density by a factor 67 in 72 h. The results further showed that Warri sample proved to be the most harmful effluent (ErC_{50} 47.62%) while other samples also had a harmful effects on the growth rate of the algal species according to GESAMP [38] and CSP [39] toxicity classification system which states that: highly toxic - $LC_{50}/EC_{50} < 1$ mg/L, toxic- 1 mg/L < $LC_{50}/EC_{50} \leq 1$ mg/L, harmful/hazardous for aquatic environment - 10 mg/L < $LC_{50}/EC_{50} \leq 100$ mg/L, very low toxic – non – toxic - $LC_{50}/EC_{50} > 100$ mg/L. There were statistically significant differences detected ($P < 0.05$) among the samples and control with R^2 value of 0.992. Similar observation was made by Yamagishi et al. [40] who reported that the growth of a green alga decreases in the presence of a toxic substance in high concentration but Prato et al. [41] discovered that some aquatic organisms such as *Sphaeroma serratum* can tolerate some toxicants (Cu, Cd and Hg).

Further study was carried out to ascertain the environmental effects of the effluents using *Lymnaea stagnalis* (freshwater snail) as bioindicators for water monitoring. The standardization and justification of this native species for ecotoxicological studies are applicable mainly for West Africans where dogmas and protocols concerning the environments are still quite primitive compared to other countries. The results in Tables 2 and 3 depict several changes which occurred in movement, retraction, production of mucus, and mortality. These features increased with time and concentration in Warri sample while PH sample recorded a moderate mortality rate at every time and concentration. When the molluscs were supplied with a vitamin source, similar mortality was recorded. This showed that the refinery industrial effluents contained a high hydrocarbon content which cannot be metabolized by the molluscs. The result in Fig. 3 demonstrated that the Warri sample exhibited the most harmful effect on 50% population of *Lymnaea stagnalis* with LC_{50} value of 51.86% in accordance with the GESAMP [38] and CSP [39] acute toxicity criterion. Statistically, there was no significant difference detected ($P > 0.05$) among the samples and control with R^2 value of 0.207. The likely reason behind this could be as a result of the presence of heavy metals especially chromium present in the both samples. Hexavalent chromium has been widely examined for toxicity and used as reference chemical compound in ecotoxicological studies to different aquatic organisms such as fresh water snails. Prato et al. [41] reported that mercury was more toxic to *Gammarus aequicauda*, *Corophium insidiosum*, *Idoteabaltica*, *Sphaeromaserratum*, and *Mytilus galloprovincialis* than copper and cadmium. Tallarico et al. [8] reported that WWWTP water samples showed acute toxicity to *B. glabata* adult snails in the samplings II and III; samplings II were toxic to snails (LC_{50} = 41.25 %) and sampling III was slightly toxic (LC_{50} = 84.16%) and no toxicity was observed in the sampling IV.

Several researchers have demonstrated that germination, root elongation and shoot length are the most authoritative parameters that indicate changes in environmental quality [42–44] and the results in Tables 4 and 5 showed that there was general and remarkable decrease in all the growth features of the *Zea mays* seedlings exposed to both effluents. The inhibition of root and shoot growth was concentration dependent and statistically significant ($P < 0.05$) at the tested concentrations of PH and Warri samples in comparison with their controls with R^2 values of 0.6256 and 0.7067, respectively. The reason for these decreases and differences could be due to the accumulations and magnifications of heavy metals and hydrocarbon components present in the samples by the root cells of these bio – indicators which further affect the growth and structures of the seed embryos negatively. The result in Fig. 4 revealed that Warri sample exhibited the most growth inhibitory effect on 50% population of *Z. mays* with EC_{50} value of -32.68%. Using two tailed paired T-TEST, there was no significant difference ($P > 0.05$) between PH and Warri samples revealing that both samples had equal and significant harmful effects on the root and shoot growths of the *Zea mays* seedlings. Our result was validated by the research work of Odutayo et al. [44] who reported that rate of growth and length of roots differed according to effluent concentrations of industrial effluent with the lowest shoot and root growth occurring at 100% concentration and the highest growth seen at the control. A similar study was been conducted by Gvozdenac et al. [45] where germination, root and shoot length of selected plants were used as indicators of water

quality and also by Orhue et al. [46] where they studied the effect of brewery effluents on the growth of maize crop.

5. CONCLUSION

This research study has shown that untreated industrial effluents from refiners are highly toxic to plants, invertebrates and microorganisms due to their high hydrocarbon, heavy metals and other pollutant contents. The toxicity values > 1 EC50/ECr50/LC50 < 100 obtained in this study are in line with other toxicity data on untreated refinery industrial effluents. Also, appropriate care should be employed because effect observed particularly in *Zea mays* can also occur in plants when exposed to the effluents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Neff JM, Johnsen S, Frost TK, Utvik TIR, Durell GS. Oil well produced water discharges to the North Sea. part II: comparison of deployed mussels (*Mytilus edulis*) and the DREAM model to predict ecological risk. Mar Environ Res. 2006; 62:224–246.
2. Lee K, Azetsu-Scott K, Cobanli SE, Dalziel J, Niven S, Wohlgeschaffen G, Yeats P. Overview of potential impacts from produced water discharges in Atlantic Canada. In: Armsworthy SL, Cranford PJ, Lee K, editors. Offshore oil and gas environmental effects monitoring approaches and technologies. Battelle Memorial Institute, Columbus, OH; 2005.
3. Sturve J, Hasselburg L, Fälth H, Celander M, Förlin L. Effects of North Sea oil and alkylphenols on biomarker responses in juvenile Atlantic cod (*Gadusmorhua*). Aqua Toxicol. 2006;78S:S73–S78.
4. Isehunwa SA, Onovae S. Evaluation of Produced water discharge in the Niger Delta. J Eng Appl Sci. 2011;6(8):66-72.
5. Terekhova VA, Wadhia K, Fedoseeva EV, Uchanov PV. Bioassay standardization issues in freshwater ecosystem assessment: test cultures and test conditions. Know. M. Aqua. Ecosys. 2018; 419:32.
6. Aruoja V, Sihtmäe M, Dubourguier HC, Kahru A. Toxicity of 58 substituted anilines and phenols to algae *Pseudokirchneriella subcapitata* and bacteria *Vibrio fischeri*: Comparison with published data and QSARs. Chem. 2011;84(10):1310–1320.
7. ISO. Water quality- Fresh water algal growth inhibition test with unicellular green algae, ISO (2012) 8692:2012 (E), Case postale 56.CH-1211. Geneva, Switzerland: International Standard Organization; 2012.
8. Tallarico LD, Borrely S, Hamada N, Grazeffe VS, Ohlweiler FP, Okazaki K, Granatelli AT, Pereira IW, Pereira CA and Nakano E. Developmental toxicity, acute toxicity and mutagenicity testing in freshwater snails *Biomphalaria glabrata* (Mollusca: Gastropoda) exposed to chromium and water samples. Ecotox Environ Saf. 2014;110:208–215.
9. Pyatt AJ, Pyatt FB, Pentreath VW. Lead toxicity, locomotion, and feedingin the freshwater snail, *Lymnaeastagnalis* (L.). Invertebr. Neurosci. 2002;4:135–140.
10. Siwela AH, Nyathi CB, Naik YS. A comparison of metal levels and antioxidant enzymes in freshwater snails, *Lymnaeanatalensis*, exposed to sediment and water collected from Wright Dam and Lower Mguza Dam, Bulawayo, Zimbabwe. Ecotox Environ Saf. 2010;73:1728–1732.
11. Brix KV, Esbaugh AJ, Grosell M. The toxicity and physiological effects of copper on the freshwater pulmonate snail, *Lymnaeastagnalis.* Comp. Biochem. Physio. C. 2011;154:261–267.
12. Grosell M, Brix KV. High net calcium uptake explains the hypersensitivity of the freshwater pulmonate snail *Lymnaeastagnalis*, to chronic lead exposure. Aquat. Toxicol. 2009;91:302–311.
13. Atlia G, Grosell M. Characterization and response of antioxidant systems in the tissues of the freshwater pond snail (*Lymnaeastagnalis*) during acute copper exposure. Aqua. Toxic. 2016;176:38–44.
14. Barbério A, Barros L, Voltolini JC, Mello MLS. Evaluation of the cytotoxic and genotoxic potential of water from the Brazilian river Paraíba do Sul with the *Allium cepa*test. Braz. J. Biol. 2009;69(3): 837-842.
15. Wang X, Sun C, Gao S, Wang L, Shokui H. Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*. Chem. 2001;44:1711-1721.

16. Mahmood S, Hussain A, Saeed Z, Athar M. Germination of seedling growth of corn (*Zea mays* L.) under varying levels of copper and zinc. Internat J Environ Sci Tech. 2005;2(3):269–274.
17. Odutayo FOI, Oyetade OB, Esan EB, Adaramola FB. Effects of industrial effluent on the environment using *Allium cepa*and *Zea mays* as bioindicators. Internat J Environ Poll Res. 2016;4(3):28–39.
18. Ajuzieogu CA, Odokuma LO, Blaise CC. Toxicity assessment of produced water using microtox rapid bioassay. S Asi J. Res. Microbiol. 2018;1(4):1–9.
19. Uba BO. Effects of aromatic hydrocarbons and marine water from Niger delta on the *β* - galactosidase activity of mutant *Escherichia coli*. Arch. Curr. Res. Internat. 2019;16(3):1–16.
20. Ekaye S, Uwagie-Ero EA, Aghayedo CO. Ameliorative effects of coconut oil on the ovaries of refinery effluent intoxicated Norwegian rats.Nig. J Environ. Sci. Tech. 2019;33(1):1–8.
21. APHA. Standard methods for examination of water and wastewater. 22nd edition. Washington, DC, USA: American Public Health Association; 2012.
22. Kiepper B. Understanding laboratory wastewater tests. USA: University of Georgia Cooperative Extension Circular 922; 2013.
23. Akpoveta OV, Egharevba F, Medjor O, Weltime O, Ize-iyamu K, Enyemike ED. Microbial degradation and its kinetics on crude oil polluted soil. Res. J. Chem. Sci. 2011;1(6):8–14.
24. Nwineewii JD, Azuonwo K. Total hydrocarbon concentrations (THC) in surface water, sediments and biota from Otamiri River, Rivers State, Nigeria. Internat J. Chem. Stud. 2018;6(3):2743–2748.
25. Leouifoudi I, Zyad A, Amechrouq A, Oukerrou MA, Mouse HA, Mbarki M. Identification and characterisation of phenolic compounds extracted from Moroccan olive mill wastewater. F. Sci. Tech. 2014;34(2):249–257.
26. Willey JM, Sherwood IM, Woolverton CJ. Prescott, Harley and Kleins Microbiology, 7th Edition, New York: McGraw-Hill companies; 2008.
27. Chikere CB, Okpokwasili GC, Ichiakor O. Characterization of hydrocarbon utilizing bacteria in tropical marine sediments. Afr. J. Biotech. 2009;8(11):2541–2544.
28. Sheir SK. The role of Caselio (plant fertilizer) exposure on digestive gland histology and heavy metals accumulation in the freshwater snail *Lanistescarinatus*. J.Biosci. Appl. Res. 2015;1(5):223–233.
29. OECD. Guidelines for testing of chemicals 208. Terrestrial plants growth test: Seedling emergence and seedling growth test. Paris: Organization of Economic Cooperation and Development Publishing; 1984.
30. Gopinathan M, Thirumurthy M. Evaluation of phytotoxicity for compost from organic fraction of municipal solid waste and paper and pulp mill sludge. Environ. Res. Eng. Manag. 2012;1(59):47–51.
31. Gray FN. Water technology: An introduction for environmental scientists andengineers. Oxford: Butterworth-Heinemann; 2002.
32. Metcalf X, Eddy X. Wastewater engineering: Treatment and reuse. In: Wastewater engineering, treatment, disposal and reuse. Techobanoglous G, Burton FL and Stensel HD (Editors), 4th edition. New Delhi, India: Tata McGraw - Hill Publishing Company Limited; 2003.
33. Okereke JN, Ogidi OI, Obasi KO. Environmental and health impact ofindustrial waste water effluents in Nigeria. Internat. J. Adv. Res. Biol. Sci. 2016;3(6):55–67.
34. Okoro CC. Microbiological impacts of produced water discharges in nearshore shallow marine waters near Chevron's Escravos tank farm, Nigeria. Afr J. Microbiol. Res. 2010;4(13):1400–1407.
35. Woryi JT, Ebere N, Ekweozor IKE, Moslen M. Acute toxic effects ofpetroleum refinery effluents on fingerlings of Tilapia *Oreochromis niloticus* and *Clariasgariepinus* Catfish. J. Toxic. Dig. 2017;1(2):148–159.
36. Okereke JN, Nnoli MC. Nature, distribution and usage of available watersources in Imo State. Internat J. Biotech. All. Sci. 2010;5(1):650–654.
37. Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN). Revised Edition Issued by the Department of Petroleum Resources (DPR); 2002.
38. GESAMP. Towards safe and effective use of chemicals in coastal aquaculture: Reports and studies. 65th edition. Rome: Group of Experts on the Scientific Aspects of Marine Environmental Protection; 1997.

39. CSP. Chemical risk Assessment: Aquatic toxicity. Pro L. (Editor) ChemSafetyPro Publication; 2016.
[Accessed on 01/03/1997]
40. Yamagishi T, Yamaguchi H, Suzuki S, Horie Y and Tatarazako N. Cellreproductive patterns in the green alga *Selenastrum capricornutum* and their variations under exposure to the typical toxicants potassium dichromate and 3, 5 – DCP. J. Environ. Biol. Ecosys. 2017;12(2): 6.
41. Prato E, Biandolino F, Scardicchio C. Test for acute toxicity of copper, cadmium, and mercury in five marine species. J. Inst. Coas. Mar. Environ. 2006;30:285–290.
42. Wang X, Sun C, Gao S, Wang L, Shokui H. Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*. Chem. 2001;44:1711-1721.
43. Mahmood S, Hussain A, Saeed Z, Athar M. Germination of seedling growth of corn (*Zea mays* L.) under varying levels of copper and zinc. Internat. J. Environ. Sci. Tech. 2005;2(3):269–274.
44. Odutayo FOI, Oyetade OB, Esan EB, Adaramola FB. Effects of industrial effluent on the environment using *Allium cepa*and *Zea mays* as bioindicators. Internat. J. Environ. Poll. Res. 2016;4(3):28–39.
45. Gvozdenac S, Inđić D, Vuković S, Grahovac M, Vrhovac M, Bošković Z, Marinković N. Germination, root and shoot length as indicators of water quality. Acta Agric. Serb. 2011;16:33–41.
46. Orhue ER, Osaigbovo AU, Vwioko DE. Growth of maize (*Zea mays* L) and changes in some chemical properties of an ultisol amended with brewery effluent. African Journal of Biotechnology. 2005; 4(9):973–978.

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