

Assessment of Physicochemical Properties and Microbial Loading Index of Soil Samples from Mgbuka Market, Anambra State

Ezenwelu Chijioke O¹, Aribodor OB², Ezeonyejiaku CD², Okafor SI¹ and Oparaji EH^{3*}

¹Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

²Department of Zoology and Environmental Studies, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

³Department of Chemical Sciences, Spiritan University Nneochi, Abia State, Nigeria

*Corresponding author: Oparaji EH, Department of Chemical Sciences, Spiritan University Nneochi, Abia State, Nigeria, Tel: +2347034351552, E-mail: emeka.oparaji65@yahoo.com

Citation: Ezenwelu Chijioke O, Aribodor OB, Ezeonyejiaku CD, Okafor SI, Oparaji EH (2022) Assessment of Physicochemical Properties and Microbial Loading Index of Soil Samples from Mgbuka Market, Anambra State . J Environ Pollut and Manage. 4: 103

Abstract

Petroleum oil spilled soil from mgbuka market, onitsha axis of Anambra state showed some level of toxicological impact on agricultural soil. Contaminated soil showed pH of 4.24 In all, the control experiment maintained a pH of 7.6. Soil conductance ($\Omega^{-1}\text{cm}^{-1}$) peaked in the contaminated soil while the control experiment soil conductance was 610. Dissolved minerals of Cl, P, Mg, K, Ca and Mg were analyzed and they showed varying relativity in abundance and concentrations. Heavy metals analysis in the soil samples revealed the presence of the following: Fe, Cd, Pb and Cu. Hg and As were below detectable limits in all the polluted soil while in the control experiment, Pb, Cd, Hg and As were not detected. Total petroleum hydrocarbons (TPH) were increasingly high in the polluted soil compared to the control experiment. Total organic carbon (TOC) and organic matter (TOM) contents correlate with that of the TPH in bioaccumulation. Analyses of soil organisms showed the persistent of strains of *Pseudomonas* and *Klebsiella* sp in the polluted soil. Peak heterotrophic counts (CFU/g) of the organisms in all the polluted soil were seen at day 0 of which progressively decreases as the incubation days decreases from 0-21. The study has provided empirical the health related indices of contaminated soil with petroleum hydrocarbons.

Keywords: Petroleum oil; Physicochemical; Microbial; Heterotrophic Counts; Toxicology

Introduction

Environmental pollution accruing from petroleum and other petroleum derivatives such as allied petrochemicals are recognized as one of the most serious problems in developing countries due to their growing population [1]. Environmental pollution from oil and other oil recalcitrant related compounds have made a huge impact in all spheres of life including human health risk, damage to the ecosystem, disruption of lives and means of livelihood, health hazards to aquatic/terrestrial inhabitants, risk to food security and poverty [2, 3].

Over the years, anthropogenic activities of man towards industrialization, mechanizations etc in Nigeria have generated a myriad of pollutants in the form of gaseous emissions, oil spills, effluents and solid wastes [4] that have contaminated the environment beyond sustainability. Heightened navigational activities in inland and non-coastal regions of the country are among the anthropogenic sources of refined and spent petroleum pollution to the environment [5]. An investigation of the Polycyclic Aromatic Hydrocarbon (PAHs) concentrations in some south east region's sediment carried out by [6] revealed an elevated level of these pollutants in the sediments studied. These activities on several human mechanizations, trading and other artisanic craftsmanship generate solid waste and sludge composed of organic, inorganic compounds with chelated heavy metals. Effluents released by these industries are characterized by the presence of a large quantity of polycyclic aromatic hydrocarbons (PAHs), phenols, metal derivatives, surface active substances (surfactants), sulphides, naphthylenic acids and other chemicals [7, 8, 9].

With the increasing population pressure in Nigeria which resulted in increase in agricultural practices, very little attention has been paid to the agricultural soil quality in terms of fertility. Agriculture and its surrounding activities remain the foremost fulcrum of our nation's developmental strides and productivity to meet its exponential population demand [10].

With intensified farming activities around the southern/eastern coastline, agricultural soil fertility remains the force of breakthrough to every farmer in all cadre within these areas and the nation at large.

Ecological impact assessment and other toxicological studies are efforts by environmentalists to bring to the fore the eco-dynamic nature of the environment to certain biotic and abiotic factors with accompanying data [11]. Several non-scientific speculations have been made on the impact of petroleum products on soil fertility with none seeming answerable to the problem. Mostly, annual and bi-annual crops are greatly affected by these petroleum products as reported by [11]. Mgbuka market has an expanse geographical locus on location with a very renowned reputation on automobile (in varieties) parts.

Huge anthropogenic activities are very common within the market with ecological implications on the receiving earth. The present study stands to fill the gap with the provision of empirical on the impact of petroleum effluents on certain soil physicochemical indices.

Materials and Methods

Materials

All chemicals/reagents and equipments used in the present were of analytical grade and products of the following companies: BDH chemical limited (England), Merck (Germany), May and Baker limited (England), Riedel-DeHaenHannaves (Germany), Hopkins and Williams Essex (England), Fluka chemical company (Germany), Kermel chemicals (China) and Lab. Tech Chemicals, Avighkar (India).

Soil sample

Soil sample was collected from the popular auto spare-part market colloquially known as Mgbuka, Anambra state, Nigeria. The soil was collected at four (4) stratified distance of 9m apart from each point of initial collection. An impression of 3cm is made through the soil at each designated collection point [9, 12]. The control experiment was sited at a distance of 1.04km from the test sample site.

Methods

Determination of Soil Physicochemical Properties

The following physicochemical properties of the polluted soil was determined in the presence of the non-polluted soil (control) as described by the journal of [13], they include:

- Soil pH Test
- Soil conductivity Test
- Determination of Macro and Micro Contents of the Soil

Mineral contents like soil electrolytes: K, Cl, Ca and Mg ions were determined

Potassium concentration of the soil was determined using spectrophotometer method; assay was carried out at wavelength of 610 nm [13].

Concentration of chloride ion in both mixtures is deduced from the formlar:

$A - B \times M \times 70900 \div \text{Vol. (ml)}$.

Where: A = titre volume of the blank

B = titre volume of the test sample

M = Molarity, Vol. of the waste pipetted.

$\text{CaCO}_3 \text{ content (mg/Kg)} = \frac{V \times E(\text{CaCO}_3) \times 100}{50}$

$\text{Calcium metal (mg/kg)} = \frac{T \times 400.5 \times 1.05}{50}$

T= titre value.

Magnesium hardness can be calculated from the determined total hardness and Calcium hardness

Formula;

$\text{Magnesium (as mg/l)} = (T - C) \times 0.243$

Where; T= total hardness mg/l (as CaCO_3)

C= Calcium hardness mg/l (as CaCO_3)

Total organic carbon content

$\text{TOC (mg)} = V_b - V_s \times 16,000 \div \text{vol. of sample used.}$

Total organic matter content (TOM) = 1.23 X TOC (EPA, 2010).

Heavy Metal Determination

Soil Digestion for Heavy Metal Analysis

10 ml from the dissolved soil solution was digested in 250 ml conical flask by adding 30 ml of aqua regia (HNO₃, HCl and HF in the ratio 3:2:1). It was further heated on a hot plate until volume remains about 7-12 ml. The digest was filtered using no 1 what-man filter paper and the volume was made up to the mark in a 50ml volumetric flask, and was then stored in a plastic container for atomic absorption spectra (AAS) analysis. Heavy metal standards were prepared accordingly as described by [14].

Total Petroleum Hydrocarbon (TPH) Contents

Total hydrocarbon (TPH) of the polluted soil was determined as described by ASTDR (2009).

Microbial Isolation from the polluted soil

Microbial ecology of the polluted soil was determined according to the method described by [15].

For the isolation process, 20g of soil sample were weighed into 40ml of distilled water in a clean conical flask and was shaken vigorously (stock solution). From the stock solution prepared, ten folds serial dilution were carried out and the 10⁻⁴ to 10⁻⁶ dilutions were plated out in a media plate.

Microscopic Features of the Isolated Bacteria

Three day old pure cultures were examined both under the microscope and by physical examinations. The colour, texture, spores and growth patterns were also to be observed.

Bacteria Identification

The three days old pure cultures were used in preparing microscopic slides described by [15]. Prior to the microscopic examinations of the microbial isolates, gram staining was carried out on the colonies to distinguish the gram positive bacteria from the gram negative ones.

After the gram staining, an aliquote of the culture suspensions was dropped on the slide and a drop of safranin red was added to it. A cover slip was placed over it and examination was performed under the light microscope at X100 magnification. Microbial isolates identification was carried out by relating the microbial features and images of the micrographs to "Atlas of Bacteriology" by Barnett and Hunters (1972).

Biochemical Test

Biochemical test of each of the organisms were carried out as described by [15, 16,] Mamta *et al.* (2010) as follows:

- Catalase Test
- Citrate Utilization Test
- Indole Test
- Methy red-Voges Proskauer (MRVP) test
- Motility and Hydrogen sulphide production
- Sugar Fermentation Test

Heterotrophic Counting and Microbial Standardization

Total heterotrophic colonies (CFU/g) from both the nutrient, Mackonkey and the differential media were counted from the grown media plate as described as follow:

$$TCFU/ml = \text{microbial colonies observed} \times \text{innoculum dilution factor} \times \text{innoculum volume pipetted}$$

Where TFCU/ml is total coliform unit per volume of the innoculum in millilitres.

Results

The results of the physicochemical properties of soil samples from the mgbuka market located at Anambra state, Nigeria shows varying level of attributes which are significantly in variation from the control experiments. This variation owes to the fact on the impact of petroleum hydrocarbon in the soil of study.

Table 1 show the physicochemical properties of the soil sample from Mgbuka market located at Anambra state contaminated with petroleum oil.

Physiochemical parameters	Control experiment	Soil sample
pH	7.6	4.24
Soil Conductance	610	433
Chloride ion (Mg/g)	433	1151.614
Phosphorus (Mg/g)	1.78	1.23
Magnesium (Mg/g)	6.27	11.27
Potassium (Mg/g)	7.22	11.52
Calcium (Mg/g)	18.23	19.34
Iron(Mg/g)	3.52	38.7
Cadmium(Mg/g)	BDL	0.015
Mercury(Mg/g)	BDL	BDL
Arsenic(Mg/g)	BDL	BDL
Lead(Mg/g)	BDL	1.73
Copper(Mg/g)	1.08	25.49
Total petroleum hydrocarbon (TPH)(Mg/g)	0.34	1014.93
Total Organic Carbon (TOC) (Mg/g)	10.45	78.64
Total Organic Matter (TOM) (mg/g)	12.85	196.73

Table 1: Physicochemical properties of soil sample polluted with spent petroleum oil from mgbuka, Anambra state N=2

Total Heterotrophic Counts (CFU/g)	Day 0	Day 7	Day 14
Total Viable Counts (10 ⁻²)	5.1 X 10 ⁸	3.3 x 10 ⁷	3.9 X 10 ⁸
Total coliform Counts (10 ⁻²)	7.02 x10 ⁴	8.2 x 10 ³	3.5 x10 ⁴
Control (10 ⁻²) TVC	3.4 x 10 ⁵	TCC (10 ⁻²)	2.6 X 10 ³

Table 2: shows the heterotrophic counts of bacteria populations at day 0-14 of soil samples from Mgbuka market, Anambra state

Sample	Isolates	Shape	Colour	Cell shape	Gram stain
control	A1	Round, smooth, raised	Whitish	Short rod	+ve
	A2	Round, smooth, flat	Brilliant whitish	Short rod	+ve
	A3	Round, smooth, raised	Yellowish white- brilliant	Cocci in chains	+ve
polluted soil	H1	Round, rough, flat	Whitish	Spiral in sheath	-ve
	H2	Round, smooth, drop-like	Whitish	Large cocci	+ve
polluted soil	E1	Round, smooth, flat	Yellowish	Cocci in pairs	+ve
	E2	Round, smooth, flat	Whitish	Short rods in pairs	-ve
Nembe Polluted soil	F1	Round, smooth, raised	Whitish brilliant	Spiral without sheath	-ve
	F2	Round, smooth, drop-like	Purple (move)	Cocci in pairs	+ve
	E4	Round, rough, flat	Whitish	Short rods	-ve

Table 2: Heterotrophic counts (CFU/g) of bacteria population at day 0 of 10% different effluent pollution

Control	Biochemical test	Observation	Organism
A1	catalase	+VE	<i>Pseudomonas, lactobacillus, staphylococcus sp.</i>
	Methy red voges proskauer	+VE	
	Motility and hydrogen sulphide	-VE	
	Arabinose	-VE	
	glucose	+VE	
	lactose	+VE	
	mannose	-VE	
	indole	+VE	
	citrate	-VE	

A2	catalase	+VE	<i>E.coli, Bacillus, Streptococcus sp.</i>
	Methy red voges proskauer	-VE	
	Motility and hydrogen sulphide	-VE	
	Arabinose	-VE	
	Glucose	+VE	
	Lactose	+VE	
	Mannose	-VE	
	Indole	+VE	
	Citrate	-VE	
Polluted soil 1			
H1	Catalase	+VE	<i>Bacillus, Stapylococcus, Pseudomonas, Klebsiella, Azobacter, Lactobacillus, Vibro, Ecoli sp.</i>
	Methy red voges proskauer	+VE	
	Motility and hydrogen sulphide	-VE	
	Arabinose	-VE	
	Glucose	+VE	
	Lactose	+VE	
	Mannose	-VE	
	Indole	-VE	
	Citrate	-VE	
Polluted soil II			
EI	Catalase	+VE, -ve	<i>Ecoli, Salmonella, Bacillus, Pseudomonas, Klebsiella, Azobacter, Streptococcus sp.</i>
	Methy red voges proskauer	+VE	
	Motility and hydrogen sulphide	-VE	
	Arabinose	-VE	
	Glucose	+VE	
	Lactose	-VE	
	Mannose	-VE	
	Indole	+VE	
	Citrate	-VE	

Table 3: Biochemical Features of the Bacteria Isolates



Figure 1: Pure bacteria strain in the nutrient, manitol and *Salmonella-Shigella* broth

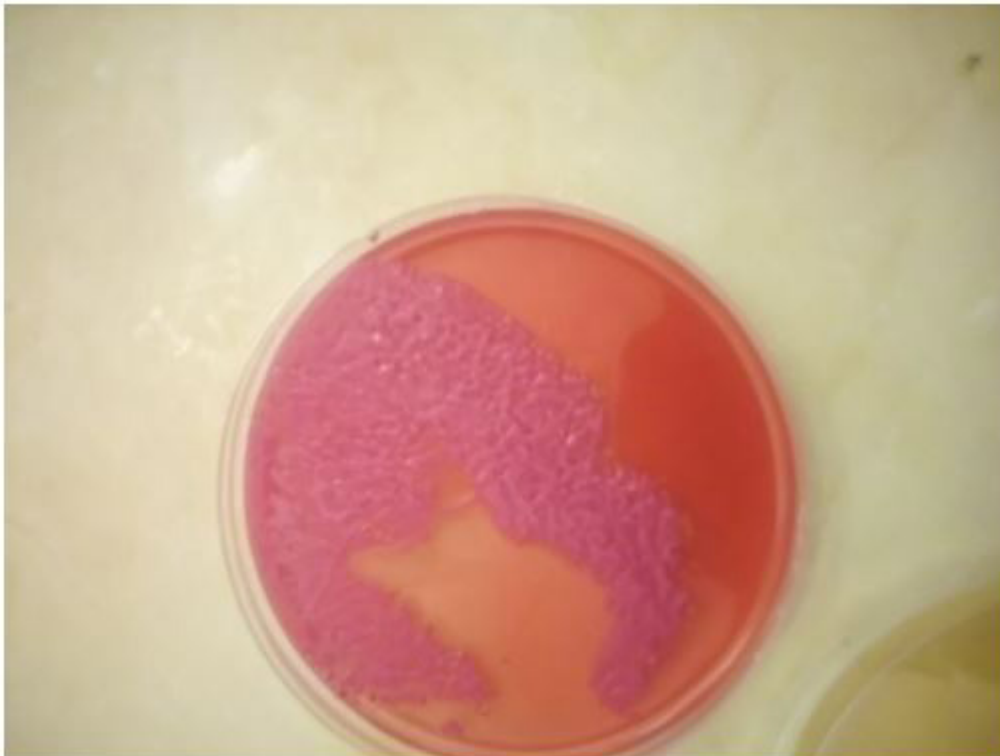


Figure 2: strains of bacteria on Mackonkey agar

Discussion

Oil pollution has been recognized as the most significant contamination problem (Allaiges *et al.*, 2006). These oil spills can cause severe damage to the land, sea and shoreline organisms (Pradect *et al.*, 2011). Most responsible for the contamination are service stations, garages, scrap yards, waste treatment plants, sawmills and wood impregnation plants (Pradect *et al.*, 2011). This study looked at assessing the impact of effluent from an automobile mechanic jetty site in Nigeria on the physicochemical properties of agricultural soil and related soil microorganisms. Soil samples were sampled and investigated. Physicochemical analysis of the soil showed pH of 4.24, in the presence of the control experiment which maintained a pH of 7.6 throughout the experiment. This can be attributed to the nature of the contaminant in the soil such as oil and other acidic compounds (oleic, benzoic acids) as stated in the proceedings of the ASTDR, 2009. Soil conductivity showed a progressive increase of soil conductivity. Dissolved mineral contents were found in the following order: Cl>Ca>Cu>Fe>Mg>K>PO₃> Pb while heavy metals of Hg, As and Cd were found at below detectable limit range (BDL) in both the contaminated and non-contaminated soil. The concentration of Cl, Ca, Mg, K, PO₃ and Pb increases. Fe showed an upward decrease in concentration. Petroleum effluents as stated by [9] contain numerous minerals which impound in any receiving body and increase the physicochemical properties of the body. The progressive decrease in the concentration of Fe can be attributed by the availability of masking elements such as oxygen, sulphur and other ligands which competitively bind to iron and diminishes their bioavailability.

Total organic matter (TOM) and total organic carbon (TOC) contents were found at 196.73 and 78.64. [17] in their study at the Eleme petrochemical jetting port site reported a similar correlation of ions concentrations in the contaminated Eleme port soil. They revealed a higher concentration of the mineral ions in the following order 2.28, 1.84, 5.22 and 1789.22 mg/g respectively for K, nitrate, magnesium and chloride ions.

Khalid *et al.* (2017) in their assessment study on soil pollution and Lead (Pb) accumulation revealed higher quotients of heavy metals like Fe, Pb and Cu in the soil while heavy metals of Hg, As, Cd were found below detectable limits in the soil.

The total petroleum hydrocarbon which depicts the entirety of petroleum hydrocarbons of poly aliphatic and polycyclic aromatic hydrocarbons (PAHs) contents are the major contents of crude oil spills implications [17]. Total petroleum hydrocarbon (TPH) of the contaminated soil was 1014.93 mg/g respectively. This showed a strong significant different from the control experiments in all the tested samples [18-20].

Soil organisms are responsible for many biogeochemical and metabolic activities in the soil, it is noteworthy to recall that organisms are like vessels carrying proteins known as enzymes. They are significant indicators in the soil against influx and out way of minerals in the soil. [21-25] Analysis of soil micorganism in the experimented soil samples contaminated with petroleum effluent showed a significant reduction of the soil organism as the effluent increases. However, there was a noticeable increase in the proliferation of the organisms contaminated with petroleum effluent incubated from day 0-7 but a decrease from 14 – 21 days of incubation.

Biochemical observation of bacteria isolates as depicted in table 3 shows much organismal presence of strains of *Bacillus*, *Lactobacillus* sp in both the polluted soil from Mgbuka and the control experiment respectively. However, there were recorded high proliferation of *Pseudomonas* and *Klebsiella* from the spent engine polluted soil. [25-28] It will be recalled that Adieze *et al* (2010) stated that strains of *Pseudomonas* sp and *Klebsiella* sp are rugged hydrocarbonolistic bacteria that survive in a crude oil polluted soil. They utilized the carbons of the petroleum compounds as their sole carbon source(s). As stated by [15], Adieze *et al.* (2010) soil physicochemical components including those of soil structures, texture affect the inhabitant organisms domicile in the soil and such influences their proliferation and doubling.

Conclusion

The present research has shown with analysed empirical the presence of certain recalcitrants in petroleum oil implicated contamination of soil from mgbuka market, Anambra state, Nigeria on agricultural soil. Petroleum hydrocarbon compounds of polycyclic aromatic hydrocarbons were estimated in the respective soil samples and at; The afore with data will provide the health and risk assessment of inhabitants of concerned community moreso the present study has shown the level of pollution of the community agricultural soil.

Funding Information

The present research on Mgbuka soil in Anambra state was fostered courtesy of funds from Tertiary Education Trust fund (TETF fund) through institution based research grant (IBR) intervention funds. Ref: (TETF/DR&D/CE/UNIV/AWKA/IBR/2020/VOL II).

Author's Contributions

Ezenwelu Chijioke O (PhD) (co.ezenwelu@unizik.edu.ng): Conceived and designed the experiments, performed the experiment and processed the data, analyzed the data and wrote the manuscript.

Aribodo O (og.aribodo@unizik.edu.ng): Revised the manuscript and performed the experiment

Ezeonyejiaku C (cd.ezeonyejiaku@unizik.edu.ng): Guided the experimental design and processed the data.

Okafor, S (si.okafor@stu.unizik.edu.ng): Performed the experiment and the guide experimental design

Oparaji Emeka H: Processed the data and wrote the manuscript.

Ethics

Authors declared no ethical issues that may arise after the publication of this manuscript.

Appreciation

The authors appreciate the staff and management of tertiary education trust fund (TETFUND) and Applied Biochemistry, Nnamdi Azikiwe University Awka Anambra state for providing the enabling laboratory where the analysis and further experimentations were carried out

References

1. Adieze I (2012) Preliminary assessment of hydrocarbon-polluted soil bacterial isolates for bio surfactant production. *J Niger Environ Soc* 7: 77-87.
2. Alexander LQC, Richard HQC, Daniel L, Claire M (2012) Particulars of claim between The Bodo community, Gokana Local Government Area, Rivers State, Nigeria and The Shell Petroleum Development Company of Nigeria Ltd in the High Court of Justice Queen's Bench Division; Claim No. HQ11X01280.
3. Duran R, Cravo-Laureau C (2016) Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment. *FEMS Microbiol Rev* 40: 814-30.
4. Adieze I, Nwabueze R, Onyeze G (2004) Effect of poultry manure on the microbial utilization of hydrocarbons in oil-polluted soil. *Niger J Microbiol* 17: 12-6.
5. Chikere C, Chikere B (2012) Bioreactor based bioremediation of hydrocarbon polluted Niger delta marine sediments. *Nigeria* 2: 53-66.
6. Ezemoye N, Ezemoye E (2005) Polychlorinated biphenyls (PCBs) levels and distributions in Ethiopia and Benin Rivers of the Niger Delta, Nigeria: surface water and sediment. *Int J Environ Stud* 62: 491-504.
7. Procópio L, de Cassia Pereira e Silva M, van Elsas J, Seldin L (2010) EPA Method 8270D (SW-846) Semivolatile Organic Compounds by Gas Chromatography/mass Spectrometry (GC/MS), Revision 4.
8. Ajiboye O, Yakubu F, Adams E (2011) A Review of Polycyclic Aromatic Hydrocarbons and Heavy Metal Contamination of Fish from Fish Farms. *J Appl Sci and Environ Manage* 15: 235-8.
9. Valerio D (2010) *Environmental biotechnology: A Biosystems approach*. 4th edition. 1245-453.
10. Chukwu M, Adams E (2016) Effect of Generator (Exhaust) Fumes on the Growth and Development of *Lycopersicon esculentus* (Tomato). *J Appl Environ Sci and Manage* 20: 335-40.
11. Chang H, Holland R, Bumpus J, Churchwell M, Doerge D (2019) Inactivation of *Coprinus cinereus* peroxidase by 4-chloroaniline during turnover: Comparison with horseradish peroxidase and bovine lactoperoxidase. *Chem Biol Int J* 123: 197-217.
12. Oparaji E, Nweze E, Agbo K, Arinzechukwu E, Anosike J, et al. (2017) Estimations of polycyclic aliphatic hydrocarbon and total petroleum hydrocarbon in aquatic faunas found in foccardos terminal river in port harcourt, rivers state. *J Environ and Analytic Toxicol* 7: 519-66
13. Agency for Toxic Substance Development and Disease Registry (ATSDR) (2010) *Documentary on Toxicological Profile of Total Petroleum Hydrocarbon Contaminations*. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Toxicology Information Branch, Atlanta, Georgia.
14. Oyibo J, Wegwu M, Uwakwe A, Osuoha J (2018) Analysis of total petroleum hydrocarbons, polycyclic aromatic hydrocarbons and risk assessment of heavy metals in some selected finfishes at Forcados terminal, Delta State, Nigeria. *Environmental Nanotechnology, Monitoring and Management* 9: 128-35.

-
15. Ezeonu M, Okafor J, Ogbonna J (2013) *Laboratory Exercises in Microbiology*. 1st edn. Ephrata Publishing and Printing Company, Nsukka 100-17.
16. Chessbrough M (2006) *Laboratory practice in tropical countries*. 2nd Edn., Cambridge university press, Cambridge, UK., ISBN-13: 9781139449298.
17. Brooijmans R, Pastink M, Siezen R (2009) Hydrocarbon-degrading bacteria: the oil-spill clean-up crew. *Microbial Biotechnol* 2: 587-94.
18. Anderson J (1979) An Assessment of Knowledge Concerning the Fate and Effects of Petroleum Hydrocarbons in the Marine Environment. In W., Vernberg, F., Vernberg, A., Calabrese and F., Thurberg (Eds.). *Marine Pollution: Functional Responses*. Academic Press, NY, pp. 3-22.
19. Antizar-Ladislao B (2009) Polycyclic Aromatic Hydrocarbons, Polychlorinated Biphenyls, Phthalates and Organotins in Northern Atlantic Spain's Coastal Marine Sediments. *J Environ Monitor* 11: 85-91.
20. Bánfalvi G (2011) "Heavy Metals, Trace Elements and their Cellular Effects". In Bánfalvi, G. *Cellular Effects of Heavy Metals*. Springer, 3-28.
21. Barbee C, Duncan B, Bickham W, Matson W, Hintze J, et al. (2008) In situ biomonitoring of PAH Contaminated Sediments Using Juvenile Coho Salmon (*Oncorhynchus kisutch*). *Ecotoxicol and Environ Safety* 71: 454-64.
22. Boag B, Palmer L, Neilson R, Legg R, Chambers S (2017) Distribution, prevalence and intensity of earthworm populations in arable land and grassland in Scotland. *Annals of Appl Biol* 130: 153-65.
23. Briones M (2014) Soil fauna and soil functions: A jigsaw puzzle. *Front. Environ. Sci* 2: 45.
24. Burkhard B, Kroll F, Nedkov S, Müller F (2012) Mapping ecosystem service supply, demand and budgets. *Ecosyst. Serv* 21: 17-29.
25. Callot H, Ocampo R (2009) Wetland and water pollution. *Boston Collection on Environmental Affairs and Law Reviews* 23: 885-919.
26. Chikere C, Okpokwasili G, Chikere B (2006) Bacterial Diversity in Typical Crude oil Polluted Soil Undergoing Bioremediation. *Afr J Biotechnol* 8: 2535-40.
27. Chikere C, Okpokwasili G, Chikere B (2009) Bacterial diversity in typical crude oil polluted soil undergoing bioremediation. *Afr J Biotechnol* 8: 2535-40.
28. Crossman N, Burkhard B, Nedkov S, Willemen L, Petz K, et al. (2013) A blueprint for mapping and modelling ecosystem services. *Ecosyst. Serv* 4: 4-14.