International Journal of Biochemistry Research & Review
16(3): 1-9, 2017; Article no.IJBCRR.31376
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

Cytotoxicological Response of *Zea mays* to Crude Oil: The Ecological Effects of Exposure to Contaminants

A. C. Udebuani[1], O. Otitoju[2*], P. N. Abara[3], E. C. Eze[1] and M. C. Duru[3]

[1]Department of Biotechnology, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria.
[2]Department of Biochemistry, Federal University Wukari, P.M.B. 1020, Wukari, Taraba State, Nigeria.
[3]Department of Biology, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author ACU designed the study and wrote the protocol while author OO wrote the first draft of the manuscript and performed the statistical analysis. Authors PNA and ECE managed the analyses of the study. Author MCD managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/31376
Editor(s):
(1) Mohamed Fawzy Ramadan Hassanien, Biochemistry Department, Zagazig University, Egypt.
Reviewers:
(1) Merv Fingas, Alberta, Canada.
(2) Omega M. Immanuel, University of Port Haercourt, Rivers State, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/abstract/18665

Original Research Article

Received 3rd January 2017
Accepted 6th February 2017
Published 15th April 2017

ABSTRACT

Cytotoxicity of different concentrations of crude oil on *Zea mays* a widely cultivated crop in Nigeria was investigated for 21 days, using physicochemical and cytotoxicity assay. The experiment was conducted in green-house under controlled environmental conditions. The cytotoxic effects were determined based on the changes in growth rate and cellular morphology of the crop plant. Results obtained showed that pH of the polluted soils is in the range of 4.4 – 4.9, while the unpolluted sample was 7.0. The values obtained for exchangeable base, effective cation exchange capacity, organic carbon, total hydrocarbon were higher in the polluted soil samples compared to the unpolluted soil sample. However, nitrogen, phosphorus and conductivity values were higher in the unpolluted soil sample than in the polluted. Results of the investigation revealed that the crude oil polluted soil affected negatively the growth parameters of *Zea mays* as the number of leaves, plant

**Corresponding author: E-mail: otitojuo@fuwukari.edu, otitojuo@fuwukari.edu.ng;*

height, and total leaf area were reduced in the exposed plant sample compared to the unexposed. A disproportionate increase in cell size (hypertrophy) of new cells which later ruptures as the get cells get older was observed in the cells of plants exposed to crude oil than in the unexposed plant. The concentrations of heavy metals Pb, Cu, Cr, Ni, Zn and Cd in the polluted oil samples were higher in the polluted soil. However, the total hydrocarbon content of the polluted soil samples correlated positively with the heavy metal content and plant biomass of the polluted samples. This study therefore confirms the cytotoxicity effects of crude oil on a common plant, which produces one of the staple foods in Nigeria. Remediation measures should be adopted to reduce the impact of crude oil on the plant sample used especially in area where oil activities are going on.

Keywords: Zea mays; crude oil; pollution; cytotoxicity; heavy metals; agricultural soil.

1. INTRODUCTION

Crude oil exploration, transport and other activities related to its production has caused serious pollution in different environmental compartments, especially in the host communities. Nigeria has in recent times witnessed massive pollution from oil industries and the cause of this pollution has been well documented. Rim-Rukeh [1] reported the various means of release of crude oil into the environment to include; operational failure [2], corrosion of pipelines and storage tanks [3], loading and discharge operations [4]. Other forms of release such as blow-outs of production wells, is worsened by vandalization of oil pipelines [5,6]. Results of previous studies have identified diverse toxic effects of crude oil pollution on various environmental compartments [7,8,9,10]. However, soil as an essential part of the environment is directly affected by crude oil pollution and studies have shown that oil pollution is toxic to soil organisms and properties, rendering it unproductive [10,11,12,13]. Effects of oil pollution on plants have been reported to be mainly indirect effects, such as interference with nutrient uptake, which inhibits photosynthetic and other biochemical processes [14,15]. Plants exposed to oil pollution showed metabolic disorder, reduced growth, yield and complete mortality [16]. In as much as studies on the effects of oil on plants have been investigated, its potential cytotoxic impact on crops is yet to be fully assessed. Biological changes such as cytogenetic damage due to exposure to contaminants can serve as an important biological indicator or bio-monitors of crude oil pollution in the environment. *Zea mays* is an important crop food for human and livestock animals [17,18], apart from being a source of food, it serves as important raw material in the production of rubber, plastics and biofuel [19], alcohol fermentation, food additives [20]. As part of the evaluation of the toxicological impact of crude oil spillage on environment, we have taken the opportunity to investigate the growth performance, plant biomass and cytotoxicity in determining the effects of exposure of *Zea mays* to crude oil.

2. MATERIALS AND METHODS

2.1 Description of Study Site

This study was conducted at the Teaching and Research farm, School of agriculture and agricultural Technology (SAAT), Federal University of Technology Owerri, Southeastern Nigeria, between the months of June and August 2015. The area falls under the geographical coordinates between Latitude $5^\circ 20^1$ and $5^\circ 25^1$ and Longitude $7^\circ 00^1$ and $7^\circ 05^1$ E. It is a humid tropical climate with an average rainfall of about 2500 mm of which 80% of the rain falls between April and August. The land topography is a flat agricultural area that is converted to an institutional area, and its vegetation is typically a rainforest zone, characterized by multiple plant species which are rich in canopies.

2.2 Collection of Soil Samples

Soil samples were randomly collected from agricultural farm with no history of crude oil pollution from the depths of 0 – 20 cm using an auger of approximately 7.5 cm diameter and taken to the laboratory for studies. The samples were air-dried and sieved using a 2 mm sieve and the fine earth was used for the experiment. However, fifteen (15) core samples were collected for bulk density determination.

2.3 Procurement of Seeds of *Zea mays*

The seeds of *Zea mays* were purchased as a single batch from Ekeonunwa market in Owerri and were taken to the Green House facility of The School of Agriculture and Agricultural

Technology, Federal University of Technology Owerri for experiment analysis.

2.4 Contamination of Soil Samples with Crude Oil

Different quantities (0 ml, 1 ml, 2 ml, 3 ml and 4 ml) of crude oil were mixed in perforated plastic container, containing 140 g of unpolluted soil. Each quantity of crude oil added was thoroughly mixed with the soil using a spatula. The plastic container containing 0 ml treatment of crude oil served as the control. The containers were carefully labeled in triplicates all together we had 15 experimental plastic containers.

2.5 Growth of *Zea mays* in Crude Oil Polluted and Unpolluted Soil

Seedlings were raised from the *Zea mays* seeds on a sandy loamy nursery bed (1x3 m). The nursery bed was kept moist by the addition of adequate water. Seedlings were allowed to grow on the nursery for 2 weeks before they were transplanted into the crude oil treated containers.

Four seedlings were planted in each of the perforated plastic bucket representing each substrate and this was replicated three times. *Zea mays* seedlings of equal heights were planted in different concentrations crude oil polluted (PS1, PS2, PS3 and PS4) and unpolluted soil (USP), which serves as control. The experiment was laid out in a completely randomized block design (CDR). The temperature and relative humidity of the green house were maintained at 22±0.25℃ and 79.79 ±4.07% respectively. The growth parameters which include; leaf number, the plant height, leaf area, fresh matter weight and dry matter weight, were determined at 7 days' interval, starting from the day of transplant according to the method described by Odjegba and Ateba [21].

The plant height was obtained by measuring the plant from the soil level to the tip of the uppermost part of the plant. The leaf area was measured after multiplying the length and width of the leaf, the product was multiplied with a cofactor leaf (0.75).

2.6 Cytological Assessment of the Root Tips of *Zea mays*

The newly grown root tips of *Zea mays* were excised and placed in a petri dish and it was labeled appropriately. After which, 5 mls of 8-hydroxy quinoline was added to the petri dishes and they were left for 3 hours. This was transferred into other petri dishes containing 5 mls of carnoy's fluid. The root tips were allowed to fix in the carnoy's fluid for 24 hours before it was transferred to 70% ethanol. These were further hydrolyzed in 20% HCl for another 24 hours, and a single root tip was picked, dripped with FLP Orcein stain and covered with cover slip. It was then mounted on the microscope (x100) for examination.

2.7 Laboratory Studies

Standard routine test were used to analyze the physicochemical parameters of the soil. Particle size analysis was determined by hydrometer method [22]. Bulk density was estimated by core procedure [23]. Soil pH was measured using a pH meter in a soil water ratio 1:2:5 [24], cation exchange capacity (CEC) was determined by ammonium acetate method [25]. Soil organic carbon was estimated by wet digestion method [26].

2.8 Data Analysis

Soil data was analyzed using SAS statistics package for analysis of variance (ANOVA). Significant difference between soil organic carbon stocks between land uses was analyzed using simple correlation analysis.

3. RESULTS AND DISCUSSION

The result of the physical properties of polluted soil (Table 1) showed that sand have the highest value in both polluted and unpolluted soil in the range of 88.15 – 89.18% compared to the silt and clay values which are almost the same. Bulk density increased with increase in concentration of crude oil, while porosity decreased with increase in concentration of crude oil. Moisture content was higher in the polluted soil compared to unpolluted. The soil textural class is sandy and it showed that crude oil pollution did not affect the textural class of the soil. Onweremadu et al [27] reported that the soil textural class of the region is not always affected by crude oil pollution.

Result of bulk density showed the polluted soils to have higher values of bulk density and lower values in soil porosity as the concentration of crude oil increases, compared with the unpolluted which has lower values of the two parameters (Table 1). Wali et al. [28] reported that the average bulk density of agricultural soil is in the range of 1.1 – 1.5 gm^{-3} for organic soil. Increase bulk density and decrease porosity is attributed to this crude oil pollution. The availability of water in soil depends on soil particle size, bulk density and porosity.

The pH values of polluted and unpolluted soil samples are shown in Table 2. The unpolluted soil samples have pH value of 7.0 while the pH values for the polluted soil samples ranges from 4.4 to 4.9, showing that the polluted soil samples were acidic. This result agrees with the finding of [29] that hydrocarbon polluted soils are acidic. Reduction in soil pH could be attributed to the production of organic acids due to microbial metabolism of the substrate [30]. Soil acidity may create chemical and biological conditions which could be harmful to some plants and animals either in the short or long run.

Similarly, higher values of nutrient elements, exchangeable base and conductivity in polluted soil than in the unpolluted soils were recorded (Table 2). The higher levels of nutrient elements are as a result of the underutilization of the mineral elements. One of the unfavorable conditions created by oil pollution on soil is that it makes nutrient elements especially macronutrient abundant in the soil but unavailable to plants. Reduction in nitrogen and available phosphorus were observed in this study. Lin et al. [31] reported that reduction in nitrogen is as a result of transformation and metabolites produced during microbial metabolism. Many researchers have reported deficiency of nitrogen and phosphorus in oil polluted soil. Deficiency of a single nutrient can slow the growth of plants and consequently affect the value chain of the affected plants. Soil pH and electrical conductivity are also important indicators of the availability of many nutrient ions for both plants and soil biota [32].

Table 1. Mean physical properties of polluted and unpolluted soils

Treatment	Sand →	Silt (%)	← Clay	TC	MC (%)	BD (gcm^{-3})	TP (%)
UPS	89.18	5.85	5.38	S	8.39	1.3333	48.31
PS1	89	4.72	6.2	S	10.683	1.34	51.0
PS2	89.14	4.68	6.19	S	15.69	1.38	47.9
PS3	88.49	5.85	5.66	S	15.633	1.51	43.0
PS4	88.15	5.85	6.2	S	18.8	1.63	38.5
LSD(0.05)	0.874	1.131	1.271	S	0.5468	0.04462	1.741
STDEV	0.616	0.775	0.654		1.3057	0.0597	2.331

Key: UPS: Unpolluted soil, PS: Polluted soil, TC: Textural class, MC: Moisture content, BD: Bulk density, TP: Total porosity, S: Sand

Table 2. Results of the chemical properties of crude oil polluted and unpolluted soil

Parameters	PS1	PS2	PS3	PS4	UPS
pH	4.9 ±0.01	4.5± 0.03	4.4±0.04	4.4±0.03	7.0
Organic carbon	2.55 ±0.01	2.95±1.61	3.55±0.01	3.69±0.001	1.54±0.01
Total nitrogen	0.37±0.002	0.24±1.18	0.18±0.001	0.16±0.002	0.614±0.001
Available phosphorus	17.12±1.55	20.77±0.20	18.28±0.57	17.78±0.05	28.52±1.05
Calcium	3.42±0.03	2.59±0.03	2.28±0.95	2.74±0.02	1.25±0.03
Magnesium	1.74±0.08	2.11±0.07	2.52±0.02	2.42±0.02	1.35±0.08
Potassium	0.21±0.001	0.23±0.001	0.25±0.002	0.28±0.002	0.152±0.001
Sodium	0.13±0.003	0.63±0.001	0.84±0.01	0.60±0.001	0.019±0.001
TEA	0.34±0.02	0.29±0.02	0.48±0.02	0.65±0.03	0.1±0.02
TEB	5.5±0.09	5.7±0.09	5.9±0.96	6.04±0.35	2.8±0.06
ECEC	5.8±0.11	5.99±0.10	6.38±0.50	6.69±0.49	2.9±0.06
% base saturation	94.2±0.31	95.2±1.10	92.5±5.52	90.3±0.48	96.5±0.34
Conductivity	$1.6x10^2$	$1.5x10^2$	$1.5x10^2$	$1.3x10^2$	7.7x103

Heavy metal concentrations in crude oil polluted soil samples are shown in Table 3. The results show that concentrations of heavy metals were found to be in the order, Cd>Ni>Pb>Cr>Cu>Zn. Although these heavy metals were obtained in this order, it shows that the polluted soil has high retention capacity for cadmium compared to other heavy metals. This is followed by nickel and the least retention capacity for zinc. In acidic soil, cadmium is more mobile and less likely to become strongly adsorbed to soil. Cadmium is highly toxic to plants, and its toxicity manifests in the form of chlorosis, necrosis, stunting and reduced photosynthetic potential [33]. In this study, stunting and necrosis were observed in the *Zea mays* exposed to crude oil. The level of nickel obtained in this study is similar to the finding of Osuji and Adesiyan [34]. Lead toxicity in plants and animals has also been reported [33, 35]. It has been reported that mobility and solubility of metals in the soil increases with decrease in pH, however in the present study, the observation is that the mobility and solubility of metals increase with decrease in pH.

3.1 Effects of Crude Oil on Growth Parameters of *Zea mays*

Physical appearance of *Zea mays* exposed to crude oil polluted soil showed some visible injury symptoms (Plates 1 and 2). Leaves showed some signs of discoloration and possible necrosis in the crude oil exposed groups than in the unexposed group (Plates 1 and 2). Also, the results of the growth parameters obtained (Tables 4a – 4c) showed a decrease in all the parameters analyzed, even as the day progresses. The result showed an increase in the number of leaves, fresh and dry matter weight, plant height and total leaf area in *Zea mays* plant that was not exposed to crude oil, compared to those exposed to crude oil. The growth parameters continued to show decrease in all parameters of plant exposed to crude oil while the unexposed continued to increase. These findings are in line with the work of many researchers [12,16,36]. Crude oil pollution covers the tips of the root hairs and prevents the normal root from absorbing nutrient elements and water, which is part of the food component. The plant growth dwindles and dies off due to lack of nutrients and water to manufacture its food.

Fig. 1a – c, shows the cellular morphology of *Zea mays* exposed to different concentrations of crude oil. While Fig. 1d is the cellular morphology of unexposed plant which is the control. Results obtained showed, a disproportionate increase in size (hypertrophy) of new cells formed in exposed plant, which later ruptured as the cells grow older. However, when compared with the control which is used as a standard for comparing Fig. 1a – c, which were exposed, it showed normal cell size. Studies have shown the cytotoxicity of crude oil on different species of plants [37,38,39].

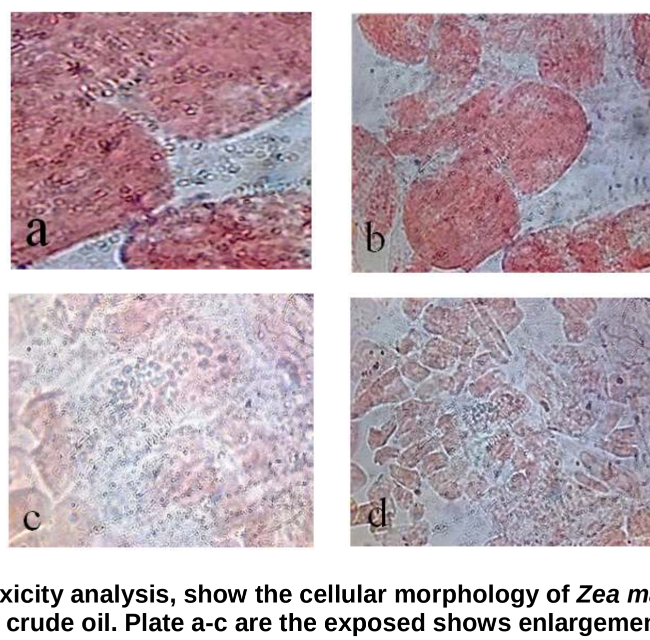

Fig. 1. Cytotoxicity analysis, show the cellular morphology of *Zea mays* exposed to concentrations of crude oil. Plate a-c are the exposed shows enlargement of the cells, while plate d is the control and it shows normal cell size

Table 3. Concentration of heavy metal in polluted and unpolluted soil

Heavy metal	PS1	PS2	PS3	PS4	UPS
Pb	1.07±0.012	1.09±0.01	1.24±0.12	1.49±0.01	0.75±0.02
Cu	3.02±0.02	3.17±0.04	3.28±0.03	3.89±0.10	0.19±0.05
Cr	0.25±0.01	0.28±0.005	0.34±0.01	0.47±0.002	0.13±0.002
Ni	1.29±0.24	1.55±0.02	1.55±0.002	1.67±0.04	0.14±0.004
Zn	0.087±0.001	0.06±0.003	0.021±0.001	0.055±0.007	0.081±0.001
Cd	0.35±0.09	0.36±0.04	0.38±0.005	0.42±0.004	0.36±0.008

Key: PS: Polluted soil; USP: Unpolluted soil sample

Table 4a. Growth and yield parameters of *Zea mays* plant at maturity after seven days of exposure

Parameters	PS1	PS2	PS3	PS4	USP
No. of leaf	5	4	4	4	5
Fresh matter weight (g)	0.18	0.15	0.14	0.13	1.3
Dry matter weight (g)	0.03	0.05	0.02	0.018	0.23
Plant height (cm)	6.8	7.3	7.0	5.9	13.9
Leaf area (cm)	9.5	7.4	6.5	6.4	12.5
Total leaf area	7.2	5.6	4.86	4.83	9.43

Key: PS: Polluted soil; USP: Unpolluted soil sample

Table 4b. Growth and yield parameters of *Zea mays* plant at maturity after fourteen days of exposure to different concentrations of crude oil

Parameters	PS1	PS2	PS3	PS4	USP
No. of leaf	6	5	4	5	9
Fresh matter weight (g)	0.21	0.25	0.22	0.16	1.8
Dry matter weight (g)	0.03	0.05	0.02	0.02	0.31
Plant height	7.1	7.6	7.1	6.3	18.7
Leaf area	11.8	9.9	9.0	9.4	79.3
Total leaf area	8.9	7.5	6.8	7.1	59.8

Key: PS: Polluted soil; USP: Unpolluted soil sample

Table 4c. Growth and yield parameters of *Zea mays* plant at maturity after twenty-one days of exposure to different concentrations of crude oil

Parameters	PS1	PS2	PS3	PS4	USP
No. of leaf	5	4	4	4	13
Fresh matter weight (g)	0.1	0.31	0.28	0.29	4.17
Dry matter weight (g)	0.03	0.04	0.04	0.04	0.29
Plant height (cm)	6.8	7.3	7.0	6.3	25.3
Leaf Area (cm)	11.8	9.9	9.0	9.5	133.66
Total leaf Area (cm)	8.9	7.5	6.8	7.1	100.8

Key: PS: Polluted soil; USP: Unpolluted soil sample

Plate 1. Physical appearance of *Zea mays* grown in crude oil polluted soil

Plate 2. Physical appearance of *Zea mays* grown in crude oil unpolluted soil

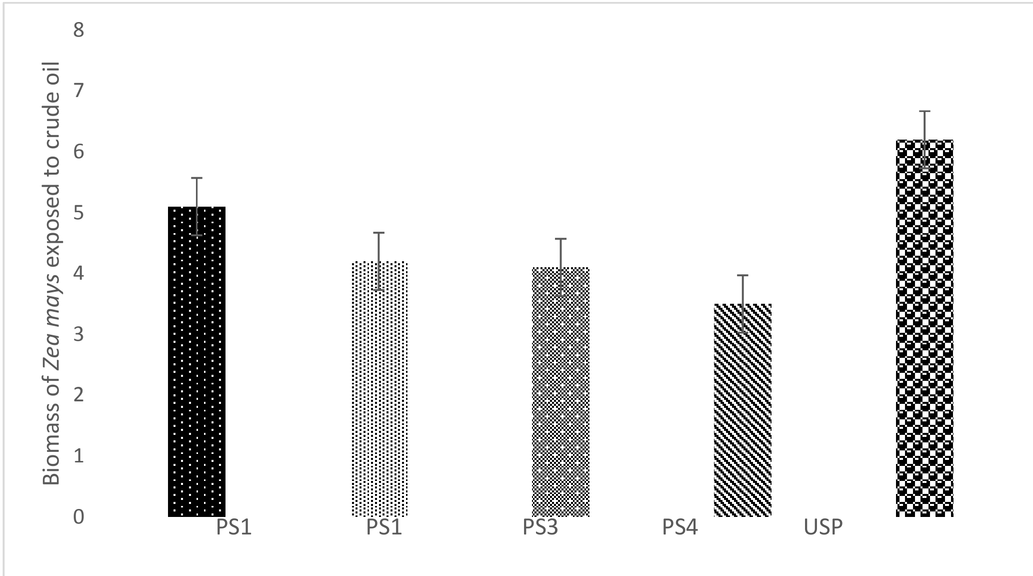

Fig. 2. Effect of crude oil pollution on biomass of *Zea mays*

Key: PS: Crude oil polluted soil, USP: Crude oil unpolluted soil

The present study showed that crude oil in soil affected negatively the growth of *Zea mays*; this was obvious in the treatment PS4. These plants exposed to crude oil showed significant reduction in biomass compared to the control which showed higher values (Fig. 2 above). Statistically, the plant biomass correlated positively with all the plant parameters analyzed. This is followed by treatment with the lowest concentration of crude oil and the highest concentration of crude had the lowest biomass. Plant biomass reduction in crude oil polluted soil has been reported [36,40,41,42].

4. CONCLUSION

The ability of *Zea mays* to tolerate stress due to exposure to crude oil has affected its cellular morphology. Such plants which are sensitive to pollutants can produce highly sensitive symptoms like reduced growth rate, necrosis, and enlarged cells can be used to monitor crude oil pollution in environment. This may suggest that the release of crude oil and its related compounds into the soil environment may subtly affect agricultural production of staple food. Therefore, effective clean-up process should be initiated in crude oil impacted environments to ensure environmental safety and sustainability.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rim-Rukeh A. Oil spill management in Nigeria: SWOT analysis of the Joint Investigation Visit (JIV) process. Journal of Environmental Protection. 2015;6:259-271.
2. Cacciabue PC. Human factors impact on risk analysis of complex systems. Journal of Hazardous Materials. 2000;71(1-3):101.
3. Osam MU, Wegwu MO, Ayalogu EO. Physicochemical and biological assessment of the efficacy of some wild type legumes in the remediation of crude oil contaminated soils. Archives of applied Science Research. 2011;3(3):470–480.
4. Yip T, Sun I, Liu JJ. Group and individual heterogeneity in stochastic frontier model: Container terminal operations. European Journal of Operational Research. 2011; 213:517–525.
5. Otitoloju AA, Don-Pedro KN, Oyewo EO. Assessment of potential ecological disruption based on PTM toxicity, accumulation and distribution in media of Lagos Lagoon. Afr. J. Ecol. 2007;45:454–463.
6. Iturbe R, Castro A, Perez G, Flores C, Torres LG. TPH and PAH concentrations in the subsoil of polyduct segments, oil pipeline pumping stations, and right-of-way pipelines from Central Mexico. Environmental Geology. 2008;55(8):1785–1795.

7. Isirimah NO, Zuofa K, Longanathan P. Effect of crude oil on maize performance and soil chemical properties in the humid forest zone of Nigeria. Discovery and Innovation. 1989;1(3):95–97.
8. Hong J, Feng H, Wang F, Ranjan A, Chen J, Jiang J, Ghirlando R, Xiao TS, Wu C, Bai Y. The catalytic subunit of the SWRI Remodeler is a histone chaperone for the H2A. Z-H2B Dimer. Mol. Cell. 2014;53(3): 498–505.
9. Kochhann KGD, Lopes FM, Krahl G, Aguiar E, Fauth G. Late Cretaceous- Early Paleogene (Turonian? to Early Danian) planktic foraminifera from DSDP site 356: A biostratigraphic reappraisal. Revista Brasileira de Paleontologia. 2014;17(2): 157-168.
10. Moses EA, Uwa EI. The effect of crude oil pollution on some soil fertility parameters in Ikot Oboreyin, Ikot Abasi, Akwa Ibom State, Nigeria. Merit Research Journal of Environmental Science and Toxicology. 2015;3(2):5–8.
11. Otitoju O, Ezeonu CS, Onwurah INE. Decreased biomass concentration on crude oil impacted soil; measurable indices in climate change. International Research Journal of Plant Science. 2014;5(3):37-42.
12. Njoku KL, Akinola MO, Busari TO. Effect of time of application of spent oil on the growth and performance of maize (*Zea mays*). African J Environ Sci Technolol. 2012;6:67-71.
13. Ewetola EA. Effect of crude oil pollution on some soil physical properties. J. Agric. and Veteri. Sci. 2013;6(3):14-17.
14. Bamidele IF, Agbogidi OM, Ohenbor OM. Phyto-toxic effects of gasoline on Ischaemum rusosum; a wet land species. American Journal of Plant Physiology. 2007;2(4):276-281.
15. Lopes A, Rosa-Osman SM, Piedade MTF. Effects of Crude oil on survival, morphology and anatomy of two aquatic macrophytes from Amazon Floodplains. Hydrobiologia. 2009;636:295-305.
16. Agbogidi MO, Edema NE. Effects of crude oil and its water soluble fraction on the growth parameter of *Panicum repens* “Linn*”.* Niger. J. Ecol. 2003;6:1-4.
17. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat. Rev. Mol. Cell. Biol. 2007; 8:519-529.
18. Qamar Z, Aaliya K, Nasir IA, Farooq AM, Tabassum B, Ali Q, Awan MF, Ali A, Tariq M, Husnain T. An overview of genetic transformation of glyphosate resistant gene in *Zea mays*. Nat Sci. 2015;13(3):80-90.
19. Bant MU. Tropical maize for biofuels; 2007. Available:http://www.aces.uiuc.edu/new/stories/new4169.html (Accessed: 25/05/2016)
20. Abbas HK, Cartwright RD, Xie W, Shier WT. Aflatoxin and fumonisin contamination of maize (*Zea mays* L.) hybrids in Arkansas. Crop Protection. 2006;25:1-9.
21. Odjegba VJ, Sadiq AO. Effect of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus* L. Environ. 2002;22: 23-28.
22. Gee WG, Or D. Particle-Size Analysis. In: Dane, J., and G.C. Topp (eds.). Methods of soil analysis. Book Series: 5. Part 4. Soil Science Society of America. USA. 2002; 255–293.
23. Grossman RB, Reinsch TG. Bulk density and linear extensibility. In J.H. Dane and G.C. Topp (ed.) Methods of soil analysis. Part 4. SSSA Book Ser. 5. SSSA, Madison, WI. 2002;201–228.
24. Hendershot WH, Lalande H, Duquette M. Ion exchange and exchangeable cations. *In:* M.R. Carter (ed.), Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, Fla. 1993;167–176.
25. Soil Survey Staff. Soil survey laboratory methods manual. Soil Surv. Invest. Rep. 42. Version 3.0. U.S. Gov. Print. Office, Washington, DC; 1996.
26. Nelson DW, Sommers LE. Total carbon, organic carbon, and organic matter. In: Methods of soil analysis, Part 2, 2^{nd} ed., A.L. Page et al., Ed. Agronomy. 9:961-1010. Am. Soc. of Agron., Inc. Madison, WI; 1996.
27. Onweremadu EU, Ndukwu BN, Ohiri KU. Variability in particle- size distribution of soils as affected by crude oil pillage. International Journal of Soil Science. 2009;1(1):104-109.
28. Wali MK, Evrendilek F, Fennessy SM. Biogeochemical cycling of material; in the environment science, issues and solutions. CRC press, New York. 2010;8–103.
29. Yu M, Tsunoda H, Tsunoda M. Environmental toxicology: Biological and health effects of pollutants. (Edn 3) CRC press; N.Y, United States of America. 2012;148-150.

30. Oyem ILR, Oyem IL. Effect of crude oil spillage on soil physic-chemical properties in Ugborodo community. International Journal of Modern Engineering Research. 2013;3(6):3336–3342.
31. Debojit B, Jitu B, Sarada KS. Certain physicochemical changes in the soil brought about by contamination of crude oil in two oil field of Assam, NE India. European Journal of Experimental Biology. 2011;1(3):154–161.
32. Lin X, Tfaily MM, Green SJ, Steinweg MJ, Chanton P, Imvittaya A, Chanton JP, Cooper W, Schadt C, Kostka JE. Microbial metabolic potential for carbon degradation and nutrient (Nitrogen and phosphorus) acquisition in an ombrotrophic peatland. Applied Environmental Microbiology. 2014;80(11):3531–3540.
33. Hussion O. Redox potential (EH) and pH as drivers of soil/plant/microorganism systems: A trans-disciplinary overview pointing to integrative opportunities for agronomy. Plant Soil. 2013;362(1):389–417.
34. Yu M, Tsunoda H, Tsunoda M. Environmental toxicology: Biological and health effects of pollutants. (Edn 3) CRC press; N.Y, United States of America. 2012;148-150.
35. Osuji LC, Adesiyan SO. Extractable hydrocarbons, nickel and vanadium contents of Ogbodo-Isiokpo oil spill polluted soils in Niger Delta, Nigeria. Environ Monit Assess. 2005;110(1-3):129–139.
36. Stouthart AJHX, Spaning RAC, Lock SE. Effects of low water pH on Lead toxicity to early life stages of the common carp (*Cyprinus carpio*). Aquat. Toxicol. 1994;30: 137–151.
37. Merkl N, Schultze-Kraft R, Infante C. Phytoremediation in the tropics – The effect of oil on the growth of tropical plants. Biorem. J. 2004;8:177-184.
38. Ogboghodo JA, Iruafa EK, Osemwota IO, Chokor JU. An assessment of the effect of crude oil pollution on soil properties germination and growth of maize (*Zea mays*) using two crude oil types– Forcados light and Escravos light. Environ. Monitoring Assess. 2004;96:143-151.
39. Howell WM, Keller GE, Kirkpatrick JD, Jenkins RL, Hunsinger RN, McLaughlin EW. Effects of the plant steroidal hormone, 24-epibrassinolide, on the mitotic index and growth of onion (*Allium cepa*) root tips. Genet Mol Res. 2007;6(1):50–58.
40. Akintunde OM, Knapp C, Heffner DM, Knapp JH, Shafer J. Reinterpretation of the "J" basalt reflector from seismic data reprocessing across the coastal plain of South Eastern Georgia: Potential implications for long-term CO2 sequestration. AAPG, Annual Convention and Exhibition, Houston, Texas, AAPG Search and Discovery Article #80174; 2011.
41. ChaÎneau CH, Morel JL, Oudot J. Phytotoxicity and plant uptake of fuel oil hydrocarbons. J. Environ. Qual. 1997;26: 1478-1483.
42. Bona C, Mendonca de Rezende I, Santos G, Antonio de Souza L. Effect of soil contamination by diesel oil on the germination of seeds and the growth of *Schinus terebinthifolius* Raddi (Anacardiaceae) seedlings. Brazilian Archives of Biology and Technology. 2011; 54(6):1379 –1387.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18665