



Genotoxicity and Environmental Implications of Crude Oil-related Pollutants in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author FOA managed the literature searches and wrote the first draft of the manuscript. All the authors participated fully in the production of this work. All read and approved the final manuscript.

Article Information

DOI:10.9734/AJARR/2019/v4i130103

Editor(s):

- (1) Dr. Azizur Rahman, Department of Chemical & Physical Sciences, University of Toronto, Davis Building, Canada.
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- (3) Dengxin Li, Donghua University, China.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/47233>

Review Article

Received 16 January 2019

Accepted 26 March 2019

Published 12 April 2019

ABSTRACT

This paper reviews the mechanistic and genetic effects of spilt crude oil and associated drill site chemicals on some Nigerian plants. Methods that range from morphological and physical observations, physico-chemical, anatomical, cuticular to cytological and cytogenetic analyses were reviewed. Quality data on various negative consequences of exposure of plants to pollution and contamination by crude oil-related pollutants were x-rayed. Proper legislation, monitoring and enforcement of sound environmental Laws by stakeholders were advised. Government collaboration with research institutions in order to keep abreast with new research findings was suggested as a way to help strengthen, manage and reinforce environmental Laws in order to protect our sources of food.

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Keywords: Pollution; plants; cytogenetics; toxic substances; environment.

1. INTRODUCTION

Nigeria is a country endowed with many natural mineral resources; one of these resources is crude oil. In the bid to harness this resource, a large extent of the environment has been negatively impacted due to poorly managed operational activities of the oil and gas industries. To examine and ameliorate this impact, the World Bank and other organisations have recommended Strategic Environmental Assessment (SEA) [1] in the oil exploration and exploitation locations. This programme helps to show the number of genotoxic substances released into the environment and also measures the devastation done to the environment.

Over the years, drilling operations have introduced oil and a wide range of other complex chemical compounds into the environment through drilling fluids and muds [1]. Oil exploration exercises generally have the tendency to pollute the environment through accidental discharges of chemicals, oil spillages and indiscriminate disposal of formation water. As these fluids find their way into the environment, the physical, chemical and microbiological properties of the soil are affected [2].

Pollution, caused by high industrialization has raised environmental and health concerns; this, has forced environmentalists to begin to assess the genotoxic effects of chemicals using plant as bioassay because of the possible genotoxic transfer from plants to humans by virtue of our food chain. To this effect, scientists around the globe have also geared their efforts towards ensuring environmental protection in order to support the sustainability of the earth. The environment is a life-supporting system and comprises the air, water, soil, plants, animals, micro-biota and humans living and interacting with each other. Introduction of any form of pollutant into the environment will automatically and detrimentally affect its biophysical (soil property, aquatic ecosystem, biodiversity [livestock, wildlife and plant life]) and socio-economic components (such as health, income), and ultimately diminish the livelihood of the populace [3,4,5,6].

Consequently, different genotoxic assays involving whole organisms, mammalian cells and

other biological entities have been developed over time; but, plant bioassays have been proven to have the highest sensitivity [7,8,9,10,11,12]. Genotoxic substances induce xenobiotic responses in the genetic system of an organism, thereby altering the molecular modes of action and the genetic framework of the organism. Several chemicals proven to have genotoxic abilities include: maleic acid hydrazine and N-methyl-N-nitroso-urea [13], boron [14], methyl tertiary-butyl ether [15]. Some metals also have genotoxic qualities, examples are: hexavalent chromium (Cr[VI]) [16], mercury (Hg), arsenic (As) and lead (Pb) [17], lead (Pb) and zinc (Zn) [18,19]. Moreover, industrial effluents [20,21], oilfield wastewater [7], crude oil [22,23], gasoline and diesel [24] and some medicinal plants [25,26,27] have been reported to have genotoxic effects on plants.

Juchimiuk et al. [13], stated that mutagens like maleic acid hydrazine and N-methyl-N-nitroso-urea have genotoxic effects on both plant and human cell nuclei. The expression of genotoxic attributes in plants therefore, means that humans are in danger of primary and/or secondary exposure to products of mutation(s) if polluted plants are consumed over a period of time. To this end, the expression of this mutation in humans has been reported to present itself in the form of cancer, neurological disorders and, malfunction of the kidney, liver and the central nervous system [28,19]. The relationship between human activities and the polluted environment has been strongly recognized hence, careful handling of chemicals and environmental monitoring have been advocated [7,29,30].

A clearer understanding of the impact of crude oil-related pollutants on the environment could be achieved through an extensive look at various levels of the functional domains of exposed plants. Such domains could be cellular, tissue, organic or an entire organism. The level of pollution determines the methodology or approach that would be used to gain insight into the extent of damage done to the living organism.

This article is aimed at reviewing the genotoxic effects and environmental implications of crude oil-related pollutants on some Nigerian plants and ecosystems.

2. METHODOLOGY

Table 1 presents different methods adopted for genotoxic assessment of plants and soil. Each study followed an approach that gave access to the cytological or organic site of accumulation or action of the test pollutant(s).

3. RESULTS AND DISCUSSION

3.1 Plant Morphological Examination

This is the study of the physical attributes of plants; this includes plant height, leaf area, the shape of leaves, girth size, root length, fruit size and so on. Morphological variations are among the first visible signs observed in plants under a polluted environment; morphological assessments are therefore crucial when studying the effects of pollutants on plants. Many researchers have used morphological assessments to characterize the effects of chemicals on plants [42,9,33,6,1,43].

3.1.1 Growth inhibition and stimulation

Growth inhibition is the suppression of plant height; it occurs as reduced root growth, reduced root number, stunted shoots, reduced leaf number and size. Petroleum-related substances have been shown to significantly reduce seed germination, emergence of seedling, growth and production of seeds in plants. Olayinka and Arinde [37], reported suppressed growth of groundnut plants grown in spent engine oil compared with the control (Table 2). They also reported increased growth suppression with higher concentrations of spent engine oil.

Similar features were also reported by Odeigah et al. [7] with oilfield wastewater, Ogbemudia et al. [34] with whole and water saturated fractions of hydrocarbons, Nwakanma et al. [36] with diesel and gasoline, Olayinka and Arinde [37] with spent engine oil, Iyaba and Offor [22] and Osawaru et al. [23] with crude oil.

Table 1. Overview of different genotoxic studies using plants and soil bioassays

Study parameter	Method employed	References
Oilfield wastewater on onion	Cytology	[7]
Spent lubrication oil on groundnut	Physico-chemical analysis and plant morphology	[31]
Crude oil on beans	Cytology and physico-chemical analysis	[32]
Spent engine oil on maize and soil	Physico-chemical analyses on plant and soil samples	[9]
Diesel fuel and spent lubricating oil on maize	Plant morphology	[33]
Hexane on beans and soybeans	Plant morphology	[34]
Spent engine oil on beans	Plant morphology	[35]
Diesel and gasoline on bitter leaf	Cytology and plant morphology	[36]
Spent engine oil on groundnut	Plant morphology	[37]
Spent motor oil on groundnut	Physico-chemical analysis and plant morphology	[38]
Crude oil on <i>Jatropha curcas</i>	Physico-chemical and heavy metal analyses	[39]
Crude oil on maize and beans	Plant morphology	[22]
Crude oil on okra	Plant morphology and physicochemical analysis	[23]
Crude oil on the chilli pepper	Plant morphology	[12]
Spent engine oil and unused engine oil on beans	Plant morphology	[6]
Potassium chromate and sodium azide on cocoyam	Plant anatomy	[40]
Oilfield chemicals on cocoyam	Plant epidermis	[2]
Potassium chromate on beans	Plant morphology, epidermis and anatomy	[41]

Table 2. Effects of spent engine oil on plant height, number of leaves, leaf area and stem girth of *Arachis hypogea* (Groundnut) [Source: Olayinka and Arinde, 2012]

Treatment (mL)	Plant height (cm)	No of leaves plant ⁻¹	Stem girth (cm)	Leaf area (cm ²)
0	14.40	37.60	1.420	294.70
25	12.30	26.20	1.150	116.30
50	9.60	24.80	1.090	73.20
75	7.90	19.70	0.930	56.40
100	5.20	12.40	0.850	28.60

Oil-polluted environment also affects the chlorophyll contents of plants. Nwakanma et al. [36] reported that *Vernonia amygdalina* grown in diesel and gasoline polluted soil had lower chlorophyll content when compared to their controls; low chlorophyll and mineral contents of plants in polluted environment promote retarded growth. Osobor and Anoliefo [31] explained that polluted environment makes it difficult for plants to uptake basic mineral nutrients that aid in metabolic activities hence, inhibition of photosynthesis and respiration abound.

Malformed leaves, change in the number of leaves per plant stand as well as plant deaths are some of the effects of crude oil-related pollutants on plants. These characters were reported by Adu et al. [6] with spent and unspent engine oil on *Vigna unguiculata*, Ajah and Obute [41] with potassium chromate (oilfield chemical) on *Vigna unguiculata* and Ajah and Osuji [44] with sodium thiosulphate and graphite powder (oilfield chemicals) on *Colocasia esculenta* and *Xanthosoma maffafa*. Ajah and Obute [41] reckoned that malformed leaves are caused by chromosome breakage, reduction in auxin level, changes in enzyme activities and variation in ascorbic acid concentration. Change in the number of leaves per plant stand has been attributed to be the cause of induction of metabolic disorder by pollutants [9]; this promotes the inability of seedlings to absorb water due to the change in the physical and chemical structures of the soil [39]. Plant death is opined to be caused by the destruction of microbes present in the soil due to insufficient aeration following a decrease in the air-filled pore space [38] by the oil-related pollutants.

Growth stimulation is the expression of more vegetative vigour in plants under polluted conditions when compared to plants in an unpolluted environment. Researchers like Anoliefo and Vwioko [42] with *Capsicum annum*, Bamidele and Sijuade [45] with *Colocasia esculenta* and, Eze and Dambo [46] with *Zea mays* reported that plants in polluted

environments were taller, had increased leaf area and higher yield than their respective controls. This phenomenon raises serious health concerns because it is believed that plants take up traces of these pollutants and, there will be genotoxic transfer when these polluted plants are consumed by animals and man; this, would eventually lead to increased incidences of terminal illnesses.

3.2 Plant Cytological Examination

Cytology is the study of cells [47] especially with reference to its sub-cellular structures [48]. Cytogenetics deals with genetic events within the cell, which is the basic building block of living organisms [49]. Cytogenetic studies provide information about the genetic architecture of plants, including the physical location of genes on chromosomes [50,51]. Chromosomal characters are important because chromosomes contain genetic information expressed in the phenotype [52]. Cytology and cytogenetic studies have proved invaluable in characterizing the mutagenic effects of chemicals on plants [36,18,15]. Cytological studies use the root tips of plants because the root systems of plants house the most vigorous cytological activity at the root tips, which are usually the first contact of any environmental pollutant [7]. To this end, scholars had used cytological examinations to study the cytotoxic potentials of many chemical compounds [53,54].

3.2.1 Chromosomal aberration

Chromosomal aberration is a shift from the normal karyomorphological view of the chromosome, particularly during different mitotic phases. The introduction of genotoxic substances poisons the spindle fibres causing mito-depressive (i.e. mitosis depressive) effects, decreased mitotic index, anti-mitotic activities, abnormal cell divisions, aberrant cells, nuclear lesions, anaphase bridges among others.

Odeigah et al. [7] on oilfield wastewater with *Allium cepa* reported decreased mitotic index

that further decreased with higher concentrations of the oilfield wastewater. Chromosomal aberrations were also reported to be significantly high; ranging from sticky chromosomes, vagrant chromosomes, multipolar anaphases to anaphase-telophase bridges with or without acentric fragments.

Related findings were reported by Nwakanma et al. [36] on *Vernonia amygdalina* with diesel and gasoline. They recorded decreased mitotic index, vagrant chromosomes, aberrant mitotic cells, chromosomal bridges and stickiness as well as decreased mitotic cells (Table 3) that further decreased with higher concentrations. In the same vein, Saikia et al. [16] studied the effects of hexavalent chromium on *Bacopa monnieri* and reported RNA damages. Furthermore, chromosomal aberration, decreased mitotic index and reduced fertility were also reported by Oladele et al. [18] in *Vigna subterranean* treated with lead and zinc. Similarly, Ismail and Morsi [15] reported chromosomal aberration, decreased mitotic index and anti-mitotic activities in *Vicia faba* using Methyl Tert-butyl Ether as a test agent.

The cytotoxic effects of oilfield chemicals on plant roots imply that similar fate awaits humans and animals because of our link in the food chain. It is therefore important that we guard against the accidental or intentional release of chemical mutagens into the environment because their introduction will affect the physical, chemical and soil microbial properties. This will eventually lead to the buildup of essential, non-essential elements and heavy metals in the soil which will be taken up and translocated to different plant tissues [9].

3.3 Plant Cuticular Examination

This is the study of the epidermal architecture of plants. This study involves the use of epidermal tissues of leaves to study plant behaviour. Cuticular studies are important because they yield critical information about stomata, trichomes and epidermal cells of a plant [55]. Cuticular examinations of plants are important when investigating the effects of pollutants on plant species because they are sensitive to any shift in the environment [41,56]. Morphological variations are among the first signs observed in plants under polluted conditions but, studies have shown that some plants growing in polluted environment do not show any morphological defects and may even have more vegetative

growth than plants in unpolluted environments [35,57]. Based on this trend, many scholars resorted to using epidermal and anatomical characters of plants for environmental monitoring [58,59,60,61,62]; the reason being that these characters are seen as superior environmental bioindicators [63] and are believed to be of much value at the levels where other classical methods cannot be applied [64].

3.3.1 Epidermal modification

Epidermal characters are of high taxonomic value and are often altered when plants are assaulted with chemicals. Distortions in the sizes and numbers of stomata and trichomes affect the morphology, respiratory and fertility responses in plants. Consequently, Omosun et al. [4], reported that the shape of the epidermal cells changed from isodiametric to polygonal and irregular when *Amaranthus hybridus* was polluted with crude oil. Additionally, they reported a decrease in the stomatal index and changes in the nature of the anticlinal walls; these variations were reportedly more prominent with higher concentrations of the pollutant.

Ajah and Obute [41] with potassium chromate on *Vigna unguiculata* seedlings also reported reduced number of stomata as well as decreased stomatal indices and frequencies; they explained that with reduced number of stomata, the gaseous exchange and photosynthetic capacities of the polluted plants reduced. This confirmed the assertion by Lata et al. [65] that the degree of sensitivity and fertility of plant species depend on their nutritional status and environmental factors.

Change in the nature of anticlinal walls was also reported by Ajah et al. [56] with potassium chromate treatment on the abaxial epidermis of *Xanthosoma maffafa* (Plate 1). They explained that the change was due to the pressure exerted on the stomata in the course of the development of the plant. Generally, the ability of any substance to change the natural architecture of plants means that such substance has genotoxic potential hence; proper handling of such substance should be emphasized.

3.4 Plant Anatomical Examination

This is the study of the internal structures of plants. Anatomical characters have proved relevant in the recognition and identification of species [40,44]. Anatomical features are known

Table 3. Mitotic effects of diesel and gasoline polluted soil on the root tips of *Vernonia amygdalina* (Bitter leaf) [Source: Nwakanma et al. 2011]

Conc. (mL)	No of cells	No of dividing cells	No of dividing cells				Stickiness	C-mitosis	Bridges and Vagrant fragments	Binucleus	Mitotic index (%)
			P	M	A	T					
Control	500	37	6	13	7	11	0	0	0	0	7.40
5 (p)	335	10	2	3	2	3	1	0	1	5	2.99
10 (p)	319	10	2	4	2	2	2	0	2	2	3.13
15 (p)	291	12	0	5	3	4	4	0	3	3	4.12
20 (p)	265	9	0	3	2	4	3	0	2	3	3.40
25 (p)	213	7	0	2	3	2	2	0	2	2	3.29
5 (d)	317	10	1	3	3	3	3	0	2	3	3.15
10 (d)	313	8	1	3	2	2	1	0	2	3	2.56
15 (d)	275	8	0	3	2	3	2	0	2	2	2.91
20 (d)	251	5	0	2	1	2	1	0	2	2	1.99
25 (d)	207	5	0	3	1	1	1	0	2	2	2.42

Keys: [(p) = petrol, (d) = diesel, P = prophase, M = metaphase, A = anaphase, T = telophase]

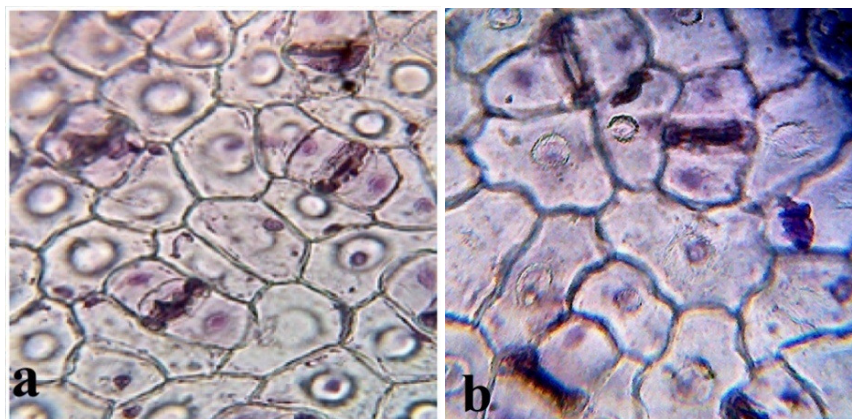


Plate 1. Nature of the anticlinal walls of the abaxial epidermis of *Xanthosoma maffafa*: a) Straight epidermal walls of the control b) Wavy epidermal walls of the treated plant [Source: Ajah et al. 2017c]

to be reliable in determining the effects of pollutants in plants because, the anatomical features are rarely affected by environmental factors [52]. The use of anatomical characters of plants for environmental monitoring have been carried out with *Cynodon dactylon* [58], *Gazania harlequin* [66], *Abutilon indicum*, *Croton sparsiflorus*, *Cassia occidentalis* [62], *Colocasia esculenta* and *Xanthosoma maffafa* [40,44].

Anatomical changes in plants are variable and differ from species to species. Leaf anatomical features have been observed to be informative about the physiological conditions of plants under adverse conditions. Dias *et al.* [67], believed that the anatomy of plants contributes to our understanding of the physiological and structural changes they undergo thereby, providing information on how they adapt to their dynamic environment.

3.4.1 Anatomical distortion

This is the modification of the internal structures of plants. Saikia *et al.* [16] working with hexavalent chromium reported different alterations in the cortical and parenchyma tissues of *Bacopa monnieri*. They observed intracellular accumulation of Cr (VI) in the cortical tissues, hypertrophied cortical cells and formation of dark-ringed structures within the cortical cells and intercellular locations. A strong reduction of the meristematic activity in the hypertrophy of cortical parenchyma was also reported. In the same vein, Omosun *et al.* [4] on the effects of crude oil on *Amaranthus hybridus* reported reduction in the parenchyma cells of the stem and, reduced intercellular air spaces that

further reduced with higher concentrations of crude oil. The cortical cells of the roots and stems of *A. hybridus* were also reported to flatten tangentially and were smaller in size when compared with the control. The parenchyma cells of both the stem and roots were reportedly smaller in size when compared with the control. These variations were purportedly more prominent with higher concentrations of crude oil.

Furthermore, Ajah and Osuji [40] on the effects of sodium azide and potassium chromate on *C. esculenta* and *X. maffafa* reported various changes in the treated plants, these changes included: distortions in the shape of the epidermal cells, thickness of the cuticle, presence of oxalate crystals, shape of vascular bundles, absence of medullary rays, occurrence of Siamese (contiguous) vascular bundles, vascular bundles with connecting tissues as well as change in the number of vascular bundles. According to the study, plants exposed to these chemicals have accelerated photosynthetic and growth abilities because of the change from barrel-shaped epidermal cells to convex shaped cells. Lata *et al.* [65] affirmed that convex shaped epidermal cells increase the ability of leaves to capture light energy and, this promotes photosynthetic capacities in plants. The treated plants were also reported to have thickened cuticles and higher oxalate crystals contents hence, have more defensive mechanism against herbivores and pathogenic attacks than the controls. Akin and Robinson [68] stated that thickened cuticles help build plant's defensive mechanisms against herbivores and pathogenic attacks while Osuji and Agogbua [69] concluded

that oxalate crystals help plants ward off herbivores.

The report of Ajah and Osuji [40] is worrisome as polluted plants tend to have more desirable qualities than the controls; this means that humans may constantly be exposed to polluted food sources and, prolonged consumption of such foods will build up toxins in the human system and will eventually lead to serious terminal diseases. To circumvent this, proper legislation must be put in place to ensure that farmlands undergo adequate evaluation so as to ensure the safety of our food sources.

3.5 Plant Physico-chemical Examination

This can simply mean the quantitative and qualitative analyses of plant extracts; it involves the extraction, isolation, identification and characterization of extracts from parts (leaves, stem, flowers, fruits, roots) of a photosynthetic plant. Physico-chemical analyses are usually carried out before and after pollution occurs, results are then compared to evaluate the presence of substances that were not present before the occurrence of pollution; they are known to be reliable environmental bioindicators [9,23,31,32].

3.5.1 Presence of heavy metals

Ogbuehi et al. [70] reported that diesel oil contaminated soil significantly reduced the macro-elements composition (nitrogen, phosphorus and potassium) of soybeans; the decrease in the macro-elements contents of this plant was reportedly as a result of the inhibitory properties of diesel oil which consequently affected the quality of food materials produced

by this plant. In the same vein, Agbogidi et al. [39] reported significant accumulation of heavy metals in the tissues of *Jatropha curcas* when compared to their controls; they believed that this accumulation is gradual, but with time, could rise to a dangerous or lethal level. He stated that contamination of soil with heavy metals causes changes in the composition of soil microbial community and, other biological and biochemical properties of the soil thereby making the soil acidic and unfit for plant propagation.

The presence of heavy metals in our food sources even at small doses is dangerous because continuous consumption of such polluted food materials will lead to heavy metal accumulation in the human system. Lead has been found to be a causative agent of cancer [28]. Long-time exposure to chromium VI can cause damage of kidney, liver and nerve tissues. Nickel has been shown to be injurious to human health [71], while the accumulation of cadmium is said to cause tubular dysfunction and disturbances in calcium homeostasis and metabolism [30]. Iron accumulation has also been linked to an increased risk of colorectal cancer [71].

Furthermore, Nkwocha et al. [72] investigated the impact of oil pollution on some plants and soil of some communities in Imo State; they reported higher levels of heavy metals in the impacted soil and plants when compared to their controls. The presence of lead, chromium, nickel and cadmium reported by these researchers in the soil and leaves of pumpkin and cassava (Table 4) calls for further and urgent toxicological studies in areas where oil exploration activities take place.

Table 4. Average concentrations of heavy metals in the soils and leaves of plants in some communities in Imo State [Source: Nkwocha and Duru, 2010]

Sites	Parameter	Cadmium (mg/Kg)	Chromium (mg/Kg)	Nickel (mg/Kg)	Lead (mg/Kg)
Awara (polluted)	Cassava	0.16	0.57	0.03	0.11
	Pumpkin	0.07	0.70	0.04	0.22
	Soil	1.50	10.75	14.77	48.20
Awara (control)	Cassava	0.01	0.01	0.01	0.00
	Pumpkin	0.00	0.00	0.02	0.00
	Soil	0.10	1.50	2.03	5.21
Izomba (polluted)	Cassava	0.17	0.70	0.04	0.16
	Pumpkin	0.06	0.85	0.03	0.20
	Soil	1.98	9.81	14.80	46.73
Izomba (control)	Cassava	0.00	0.01	0.01	0.02
	Pumpkin	0.01	0.00	0.01	0.00
	Soil	0.08	1.69	1.62	5.28

4. CONCLUSION

The study looked at how crude oil-related pollutants negatively affect our environment especially plants, our major food source. Polluted food sources have direct link to the increased rates of terminal illnesses like cancer, diabetes, liver and kidney failures in Nigeria. This translates to heavy medical tourism abroad in search of treatments and also affects both the economy and the life expectancy ratio in Nigeria. To this end, the authors wish to recommend that every farm produce must thoroughly be analyzed by appropriate agencies to ensure that they pass safety test before they are released to the public. Therefore, the Ministry of Environment, as well as Environmental Protection Agencies of various States across Nigeria, must ensure that there is total compliance to environmental legislation and regulations. Government should also ensure that environmental and agricultural extension officers are trained on how to monitor and enforce the International Standard Organization (ISO) on Environmental management. Lastly, Government should also continue to collaborate with research institutions in order to keep abreast with new research findings that would help in strengthening our environmental Laws, so as to protect our sources of food.

ACKNOWLEDGEMENTS

The authors wish to thank the World Bank African Centre of Excellence for Oil Field Chemicals Research, University of Port Harcourt for their financial assistance during the course of this project work. The authors also wish to thank Pastor Mark Uwaya for paying the publication fee of this article.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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