RESIDUAL PESTICIDES AND TRACE/TOXIC METAL CONCENTRATIONS IN READY-TO-EAT KOLANUTS (*Cola nitida*)

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Abstract: This study evaluated the residual pesticides and trace/toxic metal levels in a world-wide traded kolanut species, *Cola nitida*, using Gas Chromatography (GC) and Atomic Absorption Spectroscopy (AAS), respectively. Results indicated that all the kolanuts analysed in this study contained oximes, endosulfan, diazenon, diazepam, propoxur, chlordane, lindane, atropine, and alachlor in different concentrations. Kolanuts obtained from Oyingbo market had the highest loads of all the pesticides relative to the ones obtained from Mushin and Lagos Island markets. The pesticide that occurred in highest concentration in kolanuts across the markets was chlordane, followed by lindane and endosulfan. On the other hand, oxime, diazenon, and diazepam were present in relatively lower concentrations of between 0.02 mg/kg and 0.07 mg/kg of kolanut samples. Zinc, Cu, Pb, and Cd were present in all the kolanuts that were analysed. The highest mean Zn, Cu, Pb, and Cd concentrations of 5.14 ± 2.04, 4.46 ± 2.38, 0.52 ± 0.37, and 0.42 ± 0.29 mg/kg, respectively occurred in kolanuts obtained from Oyingbo market. The differences in pesticide and metal concentrations in kolanuts sampled from the three markets were not statistically significant ($P > 0.05$). Governments, regulatory bodies and agencies are called upon to institute collaborative programmes that would arrest the practice of preserving kolanuts with harmful pesticides.

Keywords: Cadmium, caffeine, *Cola nitida*, contaminants, farmers, pollutants

Introduction

Kolanut tree is an economically important crop that is native to mainly the tropical rainforests of Africa, but now enjoys increasing cultivation in tropical America, West Indies, Sri Lanka, Malaya and other places (McIlroy, 1963; Tindall, 1998; Asogwa *et al*., 2012). Kolanut belongs to the family *Sterculiaceae* and genus *Cola*. Of the many species of kolanuts, *Cola acuminata* and *C. nitida* are of outstanding economic importance due their high caffeine content (Oladokun, 1982; Ndagi *et al*., 2012). Of these two, *C. nitida* is the main species that is more traded world-wide (Asogwa *et al*., 2012). *Cola acuminata* and *C. nitida* are differentiated physically by the number of cotyledon split; while *C. nitida* usually splits into two cotyledons, *C. acuminata* usually splits into more than two.

Kolanut is chewed in its fresh, raw form as a stimulant, to hold hunger, or just to satisfy a craving. In Africa, kolanut enjoys much use in social, religious, ritual and other ceremonial functions. It is reportedly the only stimulant muslims are allowed to take (Asogwa *et al*., 2006). Beyond the normal chewing and social usage, kolanut and its by-products are employed globally in the industrial production of caffeinated drinks, chocolates, medicine, wines, and animal feeds (Yahaya *et al*., 2002; Hamzat and Longe, 2002; Asogwa *et al*., 2012).

In the farm, a major sign that kolanut fruits (pods) are ripe and due for harvest is when these pods begin to drop off the parent plants. At this time, the farmer begins mass harvest by plucking the pods with appropriate wooden or metal devices. The harvested pods are pooled to a dedicated spot in the farm, and thereafter cut open one after the other with a knife or cutlass to pull out the kolanuts (seeds) which are embedded in the pod. The nuts are naturally covered with a thin, white, fleshy layer when they are pulled out of the parent pod. To remove this coat, the nuts are soaked in water to facilitate fermentation. When fermentation is completed, usually after 24 h, the coat or skin becomes soft and is easily pulled or washed away from the nuts with free hand. The skinned nuts are washed, air dried, and are now ready for consumption, sale, or storage. Because kolanuts are prone to attack by diseases (like moulds and rots) and insect pests (especially weevils), farmers and kolanut merchants sometimes immerse skinned kolanuts in dilute concentrations of pesticides, especially organochlorines like hexachlorocyclohexane (Gammalin 20 EC), before sale or storage, to extend their shelf life (Ndagi *et al*., 2012). In Nigeria and some other places, other organochlorine pesticides that are commonly used by farmers, wholesalers and retailers to preserve farm produce, including kolanuts, are dichlorodiphenyltrichloroethane (DDT), Dichlorvos, Chlorpyrifos, Endosufan and Aldrin. Although many of these pesticides are under ban, they still enjoy underground usage, sometimes under unknown trade names and labels (Sosan *et al*., 2008; Aiyesanmi and Idowu, 2012; Olufade *et al*., 2014). These pesticides may be used in excessive concentrations thereby exposing consumers to residual pesticide poisoning.

Organochlorine pesticides are persistent organic pollutants (POP) that are resistant to natural degradation (Darko and Acquaah, 2007). They can be toxic to humans and other animals even at low concentrations (Aiyesanmi and Idowu, 2012). A joint report by Food and Agricultural Organisation (FAO) and World Health Organisation (WHO) has it that as at 1990, about three million people suffered pesticide poisoning world wide, out of which 220,000 cases resulted in death annually (FAO/WHO 1990).

In addition to the problem of pesticide contamination, toxic metals in food and water have been a subject of concern due to the hazard they pose to human health. Metals can occur as residues in foods and crops like kolanuts through several sources like farming activities, food processing and storage. Excessive accumulation of these metals can result in many adverse health effects like hormone function disruption, renal and other system dysfunction (Lenntech, 2004; Flora and Mehta, 2008).

Nigeria is a major player in the global cultivation and export of kolanuts. Kolanut is sold in many towns and cities across the country. In Lagos, Nigeria, kolanut is sold by wholesalers in many major markets, from where retailers buy in small quantities and, in turn, sell to the final consumers. This study assessed the residual pesticide and metal concentrations in a kolanut species, *C. nitida*, obtained from three major markets in Lagos, Nigeria.

Materials and Methods

Sample collection

The kolanut species used for the study was *C. nitida*. Kolanuts (*C. nitida*) were bought from wholesalers in the foodstuff sections of three major open markets in Lagos, Nigeria; the markets were: (1) Ojuwoye market, Mushin (2) Oyingbo market, Oyingbo, and (3) Lagos Island (Fig. 1). From each market, twelve kolanuts (a dozen) were randomly selected from the basket stock, out which three were analysed for pesticides and metal levels in the laboratory. The mean fresh weight of the kolanuts was 20.0 ± 6.0 g. Samples of the kolanuts selected for analysis are shown in Fig. 2.

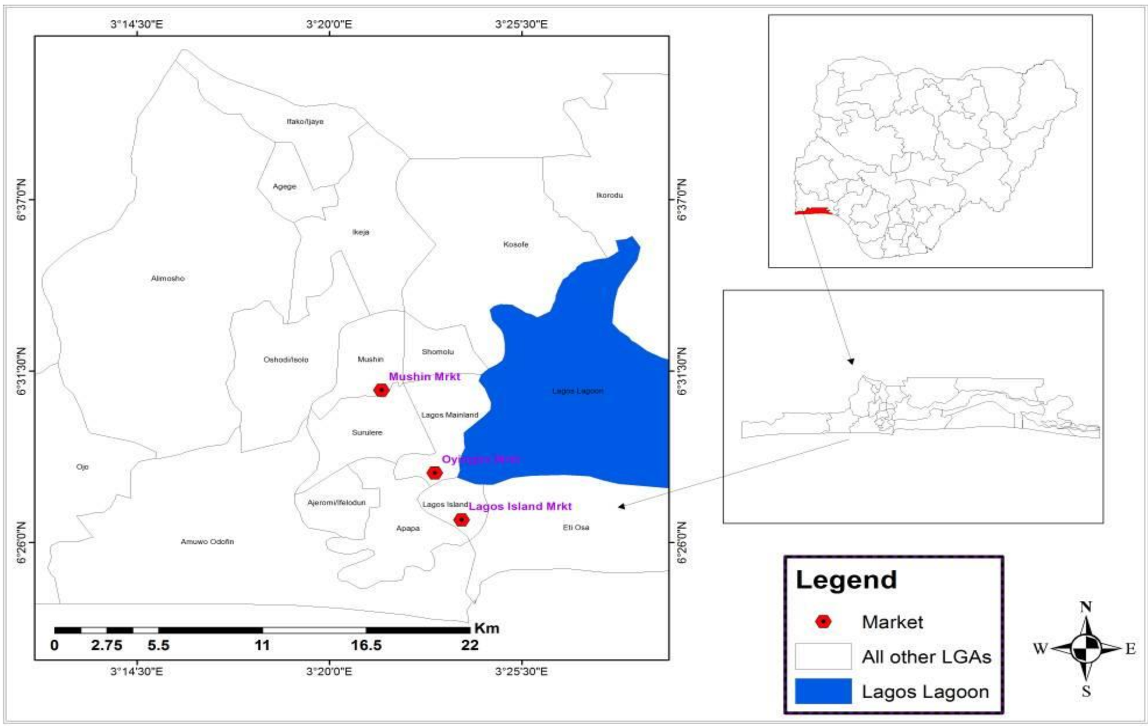

Fig. 1: Map of Nigeria (top right) showing the locations of sampling markets (left) in Lagos State (middle right)

Fig. 2: Samples of kolanuts selected for analysis

Analysis and quantification of residual pesticides in kolanuts (C. nitida)

Extraction

Three kolanuts from each market were homogenised by an electric blending machine. Thereafter, 20.00 mg of the homogenised sample was weighed into an amber bottle and 20 ml of ethylacetate was added into it. The mixture was vigorously shaken for 30 min using a mechanical shaker. The mixture was then filtered into a glass beaker. The filtrate was allowed to concentrate atmospherically to 1 ml, transferred into a sample vail, and stored in a refrigerator (4°C) pending further analyses.

Analysis of pesticides in extracted sample

To analyse pesticides in the extracted kolanut sample, 1 ml of pure standard pesticides was first injected into the Gas Chromatography, GC, (Model HP5890 Series II) to obtain a standard chromatogram and peak area. This was used to calibrate the GC for the test sample. To calculate the concentration of each pesticide in the sample, the peak area of the test sample was compared to that of the standard, relative to the concentration of the standard as in the Equation 1 below (Dada and Ikeh, 2018).

$$\text{Concentration of pesticides in test sample} = \frac{\text{Sum of peak area} \times \text{concentration of standard}}{\text{Sum of peak area of standard}} \quad \text{................ Equation 1}$$

To obtain the actual values of the pesticide, the result obtained from the equation above was divided by the dilution factor.

Programming of GC

The programming of the GC was as follows: GC make: HP5890 Series II; Injector type: On-column injector; Column type: 0v-3 (length - 30 m; thickness – 3.2 µm; ID - 5.3 µm); Injector temperature: 220°C; Oven temperature: 240°C; Detector temperature: 270°C; Carrier gas: nitrogen; Combustion gas: hydrogen/compressed air; Hydrogen flow: 45 ml/min; Air flow: 450 ml/min; Nitrogen flow: 22 ml/min; Ramping range: 10°C/min; Holding time: 2 min; Initial temperature: 50°C.

Quantification of metals in kolanut samples

Fresh kolanut was homogenised by crushing and blending in an electric blending machine. The resultant homogenised kolanut was oven-dried at 70 - 80°C for 24 h. It was subsequently digested and analysed for Zn, Cu, Pb, and Cd using a flame atomic absorption spectrophotometer, AAS, (Perkin Elmer model 460) as in Dada *et al*. (2017).

Statistical analysis of data

The data obtained from the study were processed by univariate analysis of variance. The mean values were compared for statistical significance using the Least Significant Difference (LSD) post hoc analysis. All statistical analyses were carried out using the Statistical Package for Social Science (SPSS) version 20.

Results and Discussion

Concentrations of pesticide in kolanuts

Results indicated that all the kolanuts sampled in this study contained oximes, endosulfan, diazenon, diazepam, propoxur, chlordane, lindane, atropine, and alachlor in different concentrations (Table 1). Kolanuts obtained from Oyingbo market had the highest concentrations of all the pesticides relative to the ones obtained from Mushin and Lagos Island markets. The pesticide that occurred in the highest concentration across the markets was chlordane, followed by lindane and endosulfan. On the hand, oxime, diazenon, and diazepam were present in relatively low concentrations of between 0.02 and 0.07 mg/kg. The differences in pesticide concentrations in kolanuts sampled from the three markets were not statistically significant (P > 0.05). A representative chromatogram of GC analysis of residual pesticides in kolanuts is presented in Fig. 3.

Table 1: Concentrations of pesticides in kolanuts

Pesticide type	Mean concentrations of pesticides in kolanuts obtained from different sampling markets					
	Oyingbo market		Lagos Island market		Mushin market	
	Conc (mg/kg)	% of total pesticide	Conc (mg/kg)	% of total pesticide	Conc (mg/kg)	% of total pesticide
Oxime	0.05±0.05	0.48±0.77	0.05±0.05	0.48±0.78	0.05±0.05	0.63±0.90
Endosulfan	0.24±0.17	1.97±3.14	0.17±0.15	1.81±3.00	0.17±0.11	2.66±1.02
Diazenon	0.06± 0.07	0.61±1.00	0.03±0.05	0.48±0.84	0.02±0.03	0.01±0.00
Diazepam	0.07±0.07	0.66±1.08	0.04±0.07	0.62±1.07	0.04±0.04	0.53±0.75
Propoxur	0.13±0.15	1.62±2.73	0.10±0.08	1.02±1.66	0.09±0.05	1.55±0.10
Chlordane	3.82±1.25	24.45±36.64	3.68±0.85	26.21±39.87	3.71±0.74	88.44±7.62
Lindane	0.26±0.24	2.42±3.96	0.21±0.16	2.09±3.42	0.17±0.09	3.20±1.77
Atropine	0.19±0.21	1.92±3.19	0.17±0.09	1.52±2.44	0.11±0.13	0.85±1.20
Alachlor	0.21±0.22	2.06±3.41	0.09±0.16	1.45±2.51	0.12±0.12	1.48±2.09
TPK	5.04±2.39		4.55±1.66		4.50±1.33	

TPK = Total pesticide load in mg/kg of kolanut; Conc = concentration; Each value is the mean of triplicate analysis

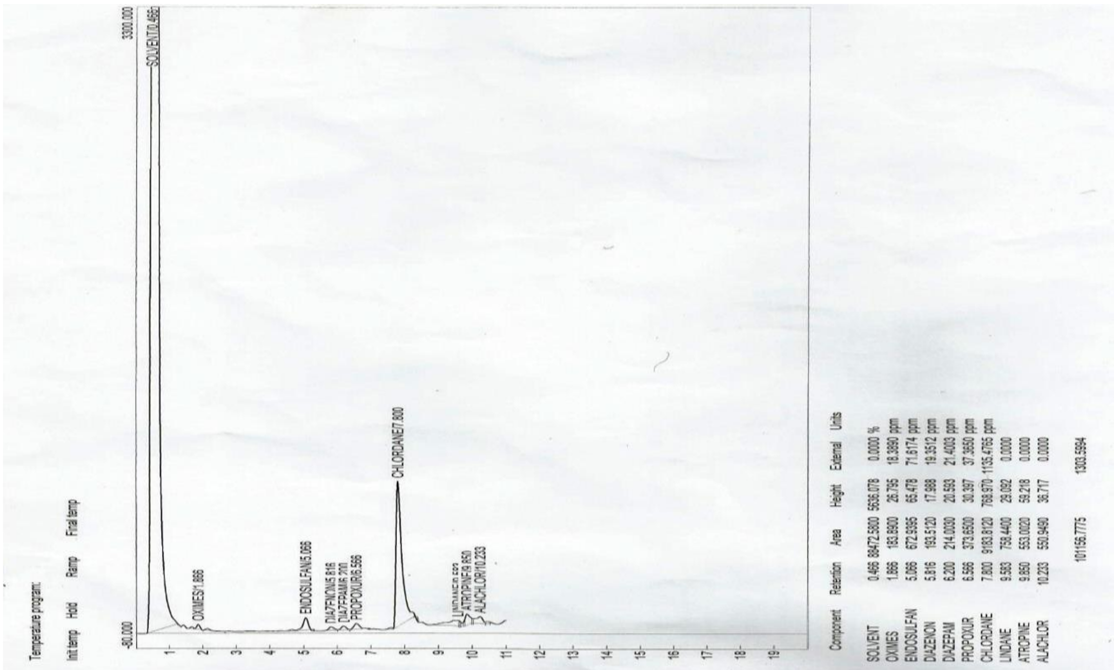

Fig. 3: Representative chromatogram of GC analysis of residual pesticides in kolanuts

The fact that all the kolanuts analysed in this study contained oximes, endosulfan, diazenon, diazepam, propoxur, chlordane, lindane, atropine, and alachlor in different concentrations is an indication that kolanut is a potential medium of pesticides ingestion. Many previous local studies have documented similar findings in other farm produce like cowpea grains (Olufade *et al*., 2014), vegetables, fresh yam tubers (Adeyeye and Osinbajo, 1999), maize grains (Ogah *et al*., 2011), beans (Ogah *et al*., 2012). From available records, the only attempt at assessing pesticides in kolanuts was four decades ago when Gamma-BHC was evaluated in *C. nitida* (Ivbijaro, 1977).

Though farmers regularly use pesticides to control weevil, fungal and other forms of pest attack in kolanut farms, these will not likely get to the kolanut seeds because the seeds are enclosed by an outer, green, thick cover, and an inner thin, membranous layer. Therefore, the source of the residual pesticides detected in kolanuts in this study was likely the post-harvest pesticide treatment. It has been noted that the main source of human exposure to pesticides is intestinal ingestion (Hall, 1999; Centers for Disease Control and Prevention, CDC, 2016). Therefore, the lack of adequate attention to the practice of preserving kolanuts with harmful, outlawed pesticides, and the consequential consumption of the same pesticide-laden kolanuts portend immediate and long term health consequences.

Worth taking cognizance of, is the fact that while many other residual pesticide-bearing crops that have attracted attention (e.g. beans, vegetables, yam tubers, maize) are usually subjected to further processing and, or cooking before consumption (a situation that may likely reduce pesticide load), most often, a large proportion of kolanuts is eaten in its raw, fresh form without further processing, peeling, or cooking, and sometimes, without rinsing. The implication of this is that kolanut is potentially a more potent medium of direct pesticide ingestion.

The fact that chlordane, and to a lesser extent, lindane and endosulfan occurred in highest concentrations in kolanuts sampled from all the markets is an indication that they are more predominant in pesticide formulations used to preserve kolanuts. *Gammalin*, a formulation of lindane, has over the years, been a choice pesticide used by farmers, merchants, and vendors in Nigeria and some other West African countries to preserve food crops including kolanuts. Endosulfan, a broad spectrum insecticide, has also become a pesticide of choice used, most times, as a substitute for lindane (Olufade *et al.*, 2014). Chlordane, endosulfan, lindane, alachlor, together with other pesticides, detected in this study are organochlorine pesticides whose use in agricultural activities have been banned or severely restricted by United States (US) Environmental Protection Agency (EPA), European Union and some other regulatory bodies (Sustainable Agricultural Network, 2011). Though these organochlorine pesticides are effective against a wide array of pests, their use was banned or restricted due to their environmental persistence and adverse health effects in animals and humans (CDC, 2016). A joint report by the Food and Agricultural Organisation (FAO) and World Health Organisation (WHO) has it that as at 1990, about three million people suffered pesticide poisoning worldwide, out of which 220,000 cases resulted in death annually (FAO/WHO, 1990). In addition, some non-lethal effects of most organochlorine pesticides, including immune and reproductive system damages, can be very serious (Lemaire *et al*., 2004).

The toxicity of organochlorine pesticides varies according to their molecular size, volatility, and effects on the central nervous system (CNS). Generally, organochlorines will either stimulate or depress the CNS, depending on the type and dose. In humans, organochlorine pesticides' toxicity largely results from stimulation of the CNS (Pope *et al*., 2011). Chlordane and lindane which occurred in highest concentrations in kolanuts analysed in this study, are toxic organochlorines and endocrine disruptors which act by altering the electrophysiological and associated enzymatic properties of nerve cell membranes, causing a change in the kinetics of Na+ and K+ ion flow through the membrane. They may interrupt the uptake of chloride ions in the CNS by antagonizing the activity of the neurotransmitter, gamma-aminobutyric acid (GABA), which induces the uptake of chloride ions by neutrons. This results in partial repolarization of the neuron and a state of uncontrolled excitation, agitation, confusion, and seizures (Ratra *et al*., 2001; Pope *et al*., 2011).

Farmers should be encouraged to forsake the use of banned pesticides and adopt alternatives that are equally effective against pests, yet relatively harmless to ecological systems and man. Since cheap pricing is a major reason why farmers in developing countries stick to using banned pesticides (Osinbajo, 1994), crashing the prices of safe alternative pesticides to affordable levels will likely shift farmers' attention away from those harmful ones.

Metal (Zn, Cu, Pb, Cd) concentrations in kolanuts

Zinc, Cu, Pb and Cd were all present in the kolanuts analysed in this study (Fig. 4). Zinc and Cu occurred in highest concentrations while Pb and Cd occurred in least. The highest mean Zn, Cu, Pb, and Cd concentrations of 5.14 ± 2.04, 4.46 ± 2.38, 0.52 ± 0.37, and 0.42 ± 0.29 mg/kg, respectively occurred in kolanuts obtained from Oyingbo market. Conversely, the least mean Zn, Cu, Pb, and Cd concentrations of 5.08 ± 1.94, 4.34 ± 2.38, 0.38 ± 0.37, and 0.36 ± 0.27 mg/kg, respectively occurred in kolanuts obtained from the market in Lagos Island. The differences in the concentrations of Zn, Cu, Pb, and Cd in kolanuts obtained from the three markets were not significant ($P > 0.05$).

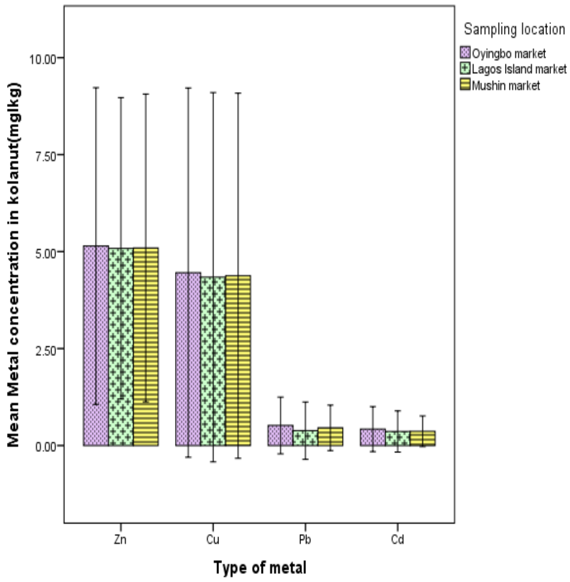

Fig. 4: Metal concentrations in kolanuts obtained from the three markets

The low metal concentrations found in the sampled kolanuts should not be taken for granted. Some of these metals, especially Cd, can produce toxic effects in animal systems even at low concentrations (Codex Alimentarius Commission, 2011). Moreover, an average person is potentially exposed to metal contaminants from a wide range of sources, the cumulative effects of which can be significant. The synergistic effects of metals and pesticides may even produce effects that are worse than their individual effects.

Conclusion

All the kolanuts analysed in this study contained varied concentrations of oximes, endosulfan, diazenon, diazepam, propoxur, chlordane, lindane, atropine, and alachlor; the presence of which can be attributed to post-harvest storage treatment. This is a problem that requires immediate attention and intervention. Among other adverse effects, pesticides, especially organochlorines, are prone to accumulating in vital organs like the brain, liver, and kidney, producing lethal and non-lethal effects. Regulatory and health bodies should, as a matter of urgency, put in place a workable action plan that would halt the practice of preserving kolanuts with harmful, outlawed organochlorine pesticides.

References

Adeyeye A & Osinbajo O 1999. Residues of organochlorine pesticides in fruits, vegetables and tubers from Nigeria markets. *The Sci. Total Environ.*, 231: 227-233.

Aiyesanmi AF & Idowu GA 2012. Organochlorine pesticides residues in soil of cocoa farms in Ondo State Central District, Nigeria. *Environ Natural Resources Res.*, 2(2): 65-73.

Asogwa EU, Agbongiarhuoyi AE, Mokwunye FC, Ndagi I, Adebiyi S & Ndubuaku TCN 2012. The challenges of kolanuts processing, trade and export from Nigeria and other sub-Saharan African countries. *Int. J. Sci. Nature*, 3(1): 06-11.

Asogwa EU, Anikwe JC & Mokwunye FC 2006. Kola production and utilization for economic development. *Afri. Scientist*, 7(4): 217-222.

Centers for Disease Control and Prevention (CDC) 2016. Organochlorine pesticides overview. National biomonitoring program summary report, USA. http://www.cdc.gov/biomonitoring/DDT_Biomonitoring Summary.html.

Codex Alimentarius Commission 2011. Joint FAO/WHO standards programme Codex committee on contaminants in foods, fifth session. Working document for information and use in discussions related to contaminants and toxins in the GSCTFF Codex Alimentary Comission, Viale delle Terme di Caracalla Rome, Italy, 90 pp. *www.codexalimentarius.net*

Dada EO, Ojo ON, Njoku KL & Akinola MO 2017. Assessing the levels of Pb, Cd, Zn and Cu in biscuits and home-made snacks obtained from vendors in two tertiary institutions in Lagos, Nigeria. *J. Appl. Sci. Environ Manage*, 21(3): 521-524.

Dada EO & Ikeh RK 2018. Phthalate and metal concentrations in drinking water in Lagos, Nigeria. *J. Health Pollution*, 8(18): 180603.

Darko G & Acquaah SO 2007. Levels of organochlorine pesticide residues in meat. *Int. J. Environ Sci. Tech.*, 4(4): 521-524.

Flora S J S MM & Mehta A 2008. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J. Med. Res.*, 128: 501-523.

Food and Agricultural Organisation (FAO)/World Health Organisation (WHO) 1990. Pesticide residues in food. FAO Plant Protection Paper 102, Geneva.

Hall RCW 1999. Long term psychological and neurological complications of lindane poisoning. *Psychosomatics*, 40(6): 513-517.

Hamzat RA & Longe OG 2002. Potentials of kola testa as a sole feed for *Archachatina marginata* snails raised under kola plantation. *Nigeria J. Tree Crops Res.*, 4(2): 1-19.

Ivbijaro MF 1977. Gamma-BHC residues in kolanut Cola nitida and control of the kolanut weevil Balanogastris kolae (Desbr.). *Indian J. Exp. Bio.*, 15(12): 1236-1238.

Lemaire G, Terouanne B, Mauvais P, Michel S & Rahmania R 2004. Effect of organochloorine pesticides on human androgen receptor activation in-vitro. *Toxicol Appl Pharmacol*, 196: 235-246.

Lenntech Water Treatment and Air Purification 2004. Water treatment. Bulletin of Lenntech, Rotterdamseweg, Netherlands, 3 pp.

McIlroy RJ 1963. An Introduction to Tropical Cash Crops. Ibadan University Press. 163 pp.

Ndagi I, Babalola FD, Mokwunye IU, Anaagbogu CF, Aderolu IA, Ugioro O, Asogwa EU, Idrisu M & Mokwunye FC 2012. Potentials and challenges of kolanut production in Niger State, Nigeria. Int Scholarly Res Net 2012. Article ID 4922394, doi: 10.5402/2012/492394, 9 pp.

Ogah CO, Coker HA & Adepoju-Bello AA 2011. Pesticide residue levels in maize samples from markets in Lagos State, Nigeria. *Nigeria Quart. J. Hosp. Med.*, 21(2): 169-174.

Ogah CO, Tettey J, Coker HB & Adepoju-Bello AA 2012. Analysis of organochlorine residues in beans from markets in Lagos State, Nigeria. *W. Afri. J Pharm.*, 23(1): 60-68.

Oladokun MAO 1982. Morpho-physiological aspects of germination, rooting and seedling growth of in kola. Ph.D. Thesis, University of Ibadan, Nigeria, 230 pp.

Olufade YA, Sosan MB & Oyekunle JAO 2014. Levels of organochlorine insecticides residues in cowpea grains and dried yam chips from markets in Ile-Ife, Southwestern Nigeria: A preliminary survey. *Ife J. Sci.*, 16(2): 161-170.

Osinbanjo O 1994. Review of chlorinated hydrocarbon substances in African aquatic environment. *FAO Fish Report*, 502: 37-45.

Pope JV, Skurky-Thomas M & Rosen CL 2011. Toxicity, organochlorine pesticides. Medscape. https://www.medscape.org/viewarticle/730492.

Ratra GS, Kamita SG & Casida JE 2001. Role of human GABAa Receptor beta3 Subunit in insecticide toxicity. *Toxicol Appl Pharmacol*, 172: 233-240.

Sosan MB, Akingbohungbe AE, Ojo IAO & Durosinmi MA 2008. Insecticide residues in the blood serum and domestic water source of cocoa farmers in Southwestern Nigeria. *Chemosphere*, 72: 781-784.

Sustainable Agriculture Network 2011. List of prohibited Pesticides. Probibited Pesticide List (Nov. 2011), Sustainable Agriculture Network, Costa Rica. 41 pp. www.sanstandards.org.

Tindall R 1998. The culture of Cola: social and economic aspects of a West African domesticate. *Ethnobotanical Leaflets*, 2: 1-3.

Yahaya L E, Hamzat RA, Aroyeun SO & Odufuwa MI 2002. Production of liquid soap detergents from the pod husk of kola (*Cola nitida*). *Moor J. Agric. Res.*, 3(2): 252-256.