INVESTIGATIVE STUDY OF ORGANOCHLORINE PESTICIDE RESIDUES IN MAIZE GRAINS, DRIED CASSAVA CHIPS AND AGRICULTURAL SOIL FROM ERINFUN FARM ADO-EKITI, SOUTHWESTERN NIGERIA

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ABSTRACT
The use of agrochemical in agriculture by farmers with little or no knowledge of the human and environmental dangers posed by the abuse and improper handling has been of great concern by human and environmental health over the years. However, pests and diseases activities render crops unattractive, causing a general decrease in productivity and hence loss to farmers has been of serious concern. This study investigated, using gas chromatography, the residue levels of organochlorine in cassava, maize, and soil of the Erinfun farm settlement in Ado-Ekiti, Nigeria. Organochlorine compounds detected at varying concentrations included Aldrin and Heptachlor occurring most frequently with the highest concentrations of 9.301 mg/kg and 0.168 mg/kg respectively. Other organochlorine compounds detected were Endosulfan and o,p-DDT. The concentrations of the organochlorine pesticides (mg/kg) measured in the soil samples showed a significant (p<0.05) correlation with the total organic matter contents of the soil. The findings from this research work thus, provided information on the current and health risk residue levels of organochlorine pesticides in soil and crop plants from this region with which future environmental performance on the use of pesticides could be progressively monitored.

Keywords: Organochlorine, Concentration, Contamination, Maize, Cassava, Soil
INTRODUCTION

In Nigeria and other West African countries, cassava is sold as a fresh tuber, processed flour (Garri), as dried chips, while maize is sold as fresh, roasted, or dried corn. Therefore, to overcome the high perishability due to their high moisture content and the seasonal nature of their production, cassava is processed into dried chips and flour, while maize is dried corn and flour, particularly in Nigeria and some other African countries. The semi-solid paste prepared from dried cassava chip flour, “Lafun”, and fried flour, “Eba” are popular and consumed in the rural and urban areas of Nigeria and other West African countries. Also, maize is prepared as a light paste called “Ogi”. Because of insect proliferation in cassava chips and maize corn on storage which has been a major problem by producers, wholesalers, and retailers of the product thus used insecticides regularly on the chips and corn during storage as well as during cultivation to prevent infestation of boring insects and weevils which cause considerable damage on the stored products as well as devalued it [Mestres et al., 2004].

In an attempt to reduce the infestation of insect pests on cassava and maize in the field and storage, the use of insecticides is usually relied upon. These insecticides are either misused, overused, or unnecessarily used by farmers and retailers that have low education levels, lack information and training on pesticide safety, poor spraying technology, and inadequate personal protection during pesticide application and the health implications when present in foodstuffs [Hurtig et al., 2003; Atreya, 2008]. Hence, harmful levels of pesticide residues or metabolites are left adsorbed unto the foodstuffs to which they are applied. In the storage of Cassava chips (in jute sacks), insecticides like Actellic, Phostoxin, or a mixture 162 of lindane and/or kerosene with water are applied [Ogunfowokan et al., 2012]. The attack on crops by scratching off the tissues and causing the completion of defoliation of the crops by insect pests leads to crop diseases and a decrease in yield. To meet the growing demands and as well as productivity, fertilizers are used to improve the yield whiles pesticides are used to control pests [Ntow, 2006].
Though these chemicals played a major role in enhancing the food grain production, the improvement in yield has resulted in the remains of their residue on foodstuffs, soil, and water [Ware and Whitacre, 2004] due to the intensive and indiscriminate use of pesticides. Pesticide residue according to [IUPAC, 1997 & European Communities, 2008] are substances or a mixture of substances in food for man or animals resulting from the use of pesticide including any specified derivatives, such as degradation and conversion products, metabolites, reaction products, and impurities considered to be of toxicological significance. These substances are highly dangerous in food products when present above the minimum residual limits. Pesticide residue found in foodstuffs is directly associated with the application on crops to attack vertebrate pests on farmland [Gwary et al., 2012]. Some pesticides contain organochlorine compounds, a wide group of chemicals, many of which persist in the environment with a half-life of more than ten years, because of which they are classified as Persistent Organic Pollutants (POPs). Most OCs are classified as environmental pollutants and as well as agricultural pesticides. Misapplication of these pesticides poses a serious health risk to consumers as a result of bioaccumulation of build-up of higher levels of these harmful compounds [Youdeowei, 2002]. However, the post-harvest application on storage of grains poses a great concern because in most cases it leads to acute toxicity upon consumption without adequate precautionary measures. If their production and use are not properly managed, the negative impact can be seen on human health and the ecosystem [Henry and Kishimba 2006]. There several research reports that have indicated the presence of different groups of pesticide residues in soils from several parts of the world [Manirakiza et al., 2003; Kannan et al., 2003; Dem et al., 2007]. This study was designed, as a preliminary survey, to investigate the occurrence and levels of organochlorine insecticide residues in maize grains and dried cassava chips in selected farm settlement, Erinfun, Ado Ekiti in Ekiti State, Nigeria. It is, therefore, worthwhile to investigate the level of contamination of the various environmental phases within the region by
organochlorine pesticides applied on the maize and cassava farms. Thus, the present study examined the level of Organochlorine Pesticides (OCPs) residues in the soil samples of some maize and cassava-producing areas of Ekiti State to complement the available data on pesticides residues levels in Nigerian soils.

2.0 Experimental Details

2.1 Study Area

One sampling station was identified in Ado Ekiti town namely: Erinfun farm, an area well known for agricultural activities and farm market trading. This site was chosen for investigation, being one of the biggest farmland, closeness to Afe Babalola University farm, because of its long-term existence. Topsoil samples were taken at five randomly located points from each farm.

2.2 Sampling and Sample Preparation

A standard stainless steel hand auger was used to take the topsoil samples at a depth of 20 cm because nutrients uptake by plants is usually within this horizon, which is also most prone to surface runoff into water bodies. Forty-five (45) randomly selected points within the farmland were sampled. All sampling points were geographically referenced with a Global Positioning System (Garmin 12 Model). The soil samples were air-dried in the laboratory for 2 weeks, picked for obvious non-soil and extraneous materials, ground in an agate mortar, and sieved through a 2 mm mesh. These were stored in black polythene bags before analysis.

Ten samples of maize grains and ten samples of dried cassava chips were collected randomly selected from Erinfun farm settlement in Ado Ekiti in Ekiti State, Nigeria. This farm was chosen because they are the biggest and most patronized farm within the study area. Foreign matters such as grains, pebbles, and pod remnants were manually removed from the maize, and both maize and cassava chip samples were further dried to a constant weight. Each of the samples was thoroughly ground to powdered form using an agate pestle and mortar.

2.3 Extraction of Organochlorine Residues from Soil Samples

All the reagents used were of analytical grade and the glassware used for the study was
cleaned as prescribed [USEPA Method 1699, Ize-Iyamu et al., 2007]. Extraction of the soil samples was carried out by the method described by [ASTM 1979]. 10g of each sample and 20g of anhydrous sodium sulfate was grounded into dry powder. The ground sample was extracted with 150ml of a mixture of Acetone and n-Hexane (2:1). After extraction, the extract was transferred into a round bottomed-flask connected to a pre-weighed receiver through a Liebig condenser and concentrated to about 20 ml on a water bath maintained between 50 and 55°C. The remaining solvent in the concentrated extract was evaporated using a rotary evaporator. The almost-dry extracts were cleaned up in a micro-column as described in [Oyekunle et al., 2011]. 2 g of activated silica gel was packed into a chromatographic micro-column of 10 mm internal diameter and approximately 10 cm long. The silica gel was conditioned with 10 ml n-Hexane, while the sample extracts were dissolved in 5 ml n-Hexane before they were loaded onto the separate micro-column. Elution of each of the sample was done with 50 ml of ethyl-acetate:hexane mixture (9:1). The eluents were then concentrated on a rotary evaporator at about 45°C and under a gentle stream of nitrogen gas. The almost-dry concentrates were then dissolved in 2 ml acetone and were transferred into vials for subsequent injection into the Gas Chromatograph.

2.4 Extraction of Organochlorine Residues from cassava and maize Samples

Accurately weighed 20g portion of each powdered sample selected by coning and quartering method was weighed into a pre-extracted Whatman extraction thimble. Following the method described by [Williams 2013], the sample extraction was carried out in a Soxhlet extractor for 4 hours using dichloromethane (DCM) as the extracting solvent. The extract was concentrated by distilling off the solvent (DCM) to about 3 mL using a distillation kit operated at 39-41°C. The concentrated extract was cooled down to room temperature and then concentrated further to about 2 mL under a stream of high purity (99.99%) nitrogen. The reduced extract was then preserved for chromatographic clean-up before Gas Chromatographic analysis.

2.5 Clean-up Experiment
For the clean-up experiment, a column of about 15 cm x 1 cm was packed with about 5 g activated silica gel prepared in a slurry form in n3hexane. About 0.5 cm of anhydrous sodium sulfate was placed at the top of the column to absorb any water in the sample or the solvent. The column was pre-eluted with 15 mL of n-hexane without the exposure of the sodium sulfate layer to the air to prevent drying up and breaking of the adsorbent. The reduced extract was turned into the column and allowed to migrate below the sodium sulfate layer. Elution was done with 2 x 10 mL portions of the extracting solvent (DCM). The eluate was then collected, dried with anhydrous sodium sulfate, and evaporated to dryness under a stream of analytical grade nitrogen (99.99%).

2.6 Gas Chromatographic Analysis

The detection and determination of the insecticide residues were performed by dissolving the sample eluate in 1 mL n-hexane before injecting 1 µL of the 1.0 mL purified extract into the injection port of a Hewlett Packard 5890 63Series II Gas Chromatograph equipped with a Ni Electron Capture Detector (GC-ECD) and ChemStation software which was used for the identification and integration of peaks of the analytes. The column consisted of a DB-5 fused silica capillary column (30 m length × 0.32 mm i.d. × 0.25 µm film thickness). The column temperature was programmed from 60°C at a rate of 20°C/min to 140°C, held for 1 min, and then continued at a rate of 11°C/min to 280°C, held for 4 min to enhance good resolution at different boiling points. The temperatures of the injector and detector were 250°C and 280°C, respectively. The injection was carried out on a splitless injector at 250°C and the purge activation time was 30 s. The carrier gas was Nitrogen at 29.2 cm/sec., measured at 150°C. The run time was 23 minutes. Identification of insecticide residues was accomplished using reference standards and relative retention time techniques, while the concentration of the residues was determined by comparing the peak areas of the samples with the corresponding peak areas of the reference standards of known concentrations [ATSDR. 2007].

2.7 Sample Analysis for Organochlorine Pesticides Residues
Analyte Residues Analyzed All the samples were analyzed for their Aldrin, Heptachlor, \( \alpha, \rho \)-DDT and Endosulfan content. The average concentration of each insecticide was compared to the International Maximum residue limits (IRLs).

Residue levels (mg/l) were calculated using the equation [NRI, 1994]:

\[
\text{Residue Level} = \frac{\text{concentration of final extract} \times \text{dilution factor}}{\text{Weight of sample analyzed}}
\]

2.8 Statistical Analysis

Descriptive statistics (mean, range, and standard error) were used to analyze the results obtained using SAS version 9.2 software.

2.9 Quality Control Measures

With each set of samples, a procedural blank and a spiked matrix sample with known amounts of standards were run to check for contamination, peak identification, and quantification. The limits of detection (LOD) of OCPs were determined as the concentration of analytes in a sample that gives rise to a peak with a signal to noise ratio (S/N) of 3. The mean levels for each pesticide residue were calculated with the assumption of zero for undetected values. The level was designated as undetected (ND) if it was below LOD.

Method precision and accuracy were tested by spiking experiments with all studied compounds at 3 different spiking levels. Pesticide recoveries were determined relative to the ratio of direct injection of extract and the working standards prepared in hexane. The mean recovery of OCPs was estimated at mean concentration levels. Recovery percentages ranged between 86.32 and 92.78. Triplicate samples were analyzed during each run to evaluate the reproducibility of the overall method. The relative standard deviations (RSD) for triplicate samples were less than ten percent. Procedural blanks, which consisted of water instead of sample, were included with each sample batch and analyte values obtained in the blanks were subtracted from values found in the sample extracts.
2.10 Percent Recovery (%R) Determination

Two samples of pulverized maize grains and dried cassava chips, each weighing 20 g were chosen. For each foodstuff, one sample was spiked with $10^{-1}$ mg kg standard mixtures consisting of some of the available organochlorine insecticides of interest. The mixture was thoroughly mixed to ensure maximum homogenization. The other sample was left unspiked. The two samples were extracted and clean up following the procedures of [Williams 2013]. Using an injection needle, 1.0 µL of the mixture was injected into the GC column for GC-ECD analysis. The recoveries of OCs were determined by comparing the peak areas of the OCs after spiking with those of the unspiked and the percentage recovery (%R) was evaluated based on the expression:

$$\%R = \frac{\text{Peak area of } A - \text{Peak area of } A^1}{\text{Peak area of OC in standard}} \times 100$$

Where $A = \text{OC in spiked sample and } A^1 = \text{OC in unspiked sample.}$

2.11 Determination of Limit of Detection (LOD)

The limits of detection (LOD) were evaluated by the determination of concentrations that gave signals equal to the blank signal plus three standard deviations of the blank based on the empirical and more specific definition using the relationship:

$$y_c = y_b + 3S_b$$

Where $y_c = \text{analyte signal equivalent to detection limit; } y_b = \text{blank signal; and } S_b = \text{standard deviation of the blank.}$ From the value of $y_c$ the analyte, concentration corresponding to the detection limit was evaluated for GC-ECD determination of OCs.
3.0 RESULTS AND DISCUSSION

The percentage recovery for the insecticides as shown in table 1 ranged from 86.32% (o,p-DDT) to 92.78% (Aldrin) in the food sample used while the LOD values were between 0.293 and 1.728 mg/kg for o,p-DDT, and Aldrin, respectively. Coupled with the LOD values of 0.293, 0.536, 0.973, 0.970, and 1.728 mg/kg for o,p-DDT, Endosulfan, Heptachlor, and Aldrin, respectively, the procedures outlined for OCs assessment in this study are adjudged reliable and efficient. A representative chromatogram of the analyzed maize crop, maize soil, cassava chips and cassava soil samples are shown in figures 1, 2, 3, and 4 respectively. The mean concentration of organochlorine insecticide residues in maize grains and dried cassava chips and their respective soil from Erinfun is presented in Table 2. The mean concentration of residues in maize grain samples ranged from 0.077 mg/kg ± 0.014 (Aldrin) to 0.168 mg/kg ± 0.050 (Heptachlor). The most predominant residue in maize grains from the farm was Aldrin while the least concentration was Heptachlor is used primarily by farmers to kill termites, ants and soil insects in seed grains and on crops, as well as by exterminators and homeowners to kill termites. However, in dry cassava chips, the mean concentration of residues detected in samples ranged from 0.102 ± 0.015 mg/kg (Heptachlor) to 9.301 mg/kg ± 0.114 (Aldrin). Thus, the most predominant residues detected in dried cassava chips sampled from the farm was Aldrin. This result further indicated that insecticides containing these two OC compounds are active ingredients of common insecticides likely to have been used in the control of insect pests of cassava and dried cassava chips. The mean concentration of each compound is illustrated in Figure 5. The relatively high concentration of Heptachlor in maize grains sampled from the farm implied that Heptachlor was an active ingredient in one of the common insecticides applied for maize insect control. The high concentration of its residues measured in the foodstuff was either a reflection of the past usage which resulted in bioaccumulation or probably arising from the cultivation of the crop on contaminated soils where the application of the insecticides was intense [ATSDR 2007]. According to [Adhikari 2010], the primary exposure of human beings to Heptachlor is
through contaminated foods. All the OC insecticide residues measured were detected in most of the samples. Heptachlor was the most predominant residue in all the maize samples while o,p-DDT, and Endosulfan were also recorded in the food item with a mean concentration of 0.005± 0.001mg kg and 0.274 ± 0.042mg/kg, respectively as the frequently detected insecticide from the foodstuff. The lowest levels of o,p-DDT measured in the foodstuffs indicated that the o,p-DDT residues present could be due to the decomposition of Aldrin.

**Table 1:** Percentage Recovery (%R) and Calculated Limits of Detection (LOD) for Some of the Insecticides

<table>
<thead>
<tr>
<th>Organochlorine Compound</th>
<th>%R</th>
<th>Calculated LOD (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>92.78 ± 2.30</td>
<td>1.728</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>91.56 ± 0.72</td>
<td>0.536</td>
</tr>
<tr>
<td>o,p- DDT</td>
<td>86.32 ± 1.30</td>
<td>0.293</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>91.48 ± 1.00</td>
<td>0.972</td>
</tr>
</tbody>
</table>

**Table 2:** Concentration (mg/kg) of organochlorine pesticide residue detected in samples from Erinfun farm Ado Ekiti.

<table>
<thead>
<tr>
<th>Organochlorine Compound</th>
<th>Maize</th>
<th>Maize soil</th>
<th>Cassava</th>
<th>Cassava soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>0.07734±0.014</td>
<td>0.90158±0.017</td>
<td>9.30087±0.114</td>
<td>3.25025±0.302</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>ND</td>
<td>ND</td>
<td>0.2743±0.042</td>
<td>N.D</td>
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<td></td>
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<td>--------------------</td>
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</tr>
<tr>
<td>o,p- DDT</td>
<td>ND</td>
<td>ND</td>
<td>N.D</td>
<td>0.00509±0.001</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>0.16775±0.050</td>
<td>ND</td>
<td>0.10163±0.015</td>
<td>0.07788±0.003</td>
</tr>
</tbody>
</table>

ND= Not detect

**Figure 1:** Chromatogram of organochlorine pesticide residue in maize crop.

**Figure 2:** Chromatogram of organochlorine pesticide residue in maize soil.
Figure 3: Chromatogram of organochlorine pesticide residue in cassava chips.

Figure 4: Chromatogram of organochlorine pesticide residue in cassava soil.
Figure 5: Mean and maximum concentrations detected for each compound

Table 3: Highest Insecticide Residues Found in Maize Grains and Dried Cassava Chips from Erinfun farm in (mg/kg)

<table>
<thead>
<tr>
<th>Organochlorine Compound</th>
<th>Highest amount found in Maize grains</th>
<th>Highest amount found in Cassava chips</th>
<th>Maximum residue limit for Maize grains</th>
<th>Maximum residue limit for Cassava chips</th>
<th>Maximum residue limit for Agricultural soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>0.07734</td>
<td>9.30087</td>
<td>0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>ND</td>
<td>0.2743</td>
<td>0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>o,p- DDT</td>
<td>ND</td>
<td>ND</td>
<td>0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.10&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Heptachlor though banned in Nigeria is suspected to still be sold under different names or labels as one of the active ingredients to other insecticides currently in use by Nigerian farmers. Farmers and consumers on exposure face immense risks to the use of toxic chemicals that are banned or restricted in other countries [Adeleke 2010]. Aldrin or Aldrex ®40EC has been sold in Nigeria, is an emulsifiable concentrate that has been used to control cocoa mirids, termites, locusts, ants, and other insect pests. Aldrin is used as soil insecticide for tuber beetles, termites, and crickets. The levels of Aldrin present in the maize grains were found to be above the FAO/WHO [2013] recommended MRLs value of 0.02 mg/kg. The predominance of Aldrin in dried cassava chips and maize from Erinfun farm, therefore, suggests non-observance of the waiting period or abuse of the insecticide because ordinarily, Dieldrin (a metabolite of Aldrin) was expected to be found as residues. The short interval between harvests to market and the likelihood of no testing for pesticide residues might contribute to high residual levels.

Aldrin was the insecticide commonly used by farmers to preserve dried yam chips in areas where yam flour was produced [Ogah 2012]. Endosulfan which is marketed under various trade names such as Endocel 35EC is a broad-spectrum insecticide acting as a contact and stomach poison used for the control of bean aphids. Gammalin ®20 EC is another organochlorine marketed in Nigeria, which has been for a long time a popular household trade name in most agricultural communities of Nigeria.

The mean concentrations of the residues in both maize and dried cassava chips were found to be above the MRL for the foodstuffs. The level of Heptachlor detected in maize was higher than the

| Heptachlor | 0.16775 | 0.10163 | 0.01^b | 0.01^{ac} | 0.03^d |

MRL. The concentrations of Aldrin in cassava chips and maize were higher than the Codex [2016]. The comparison of the insecticide residues concentration observed from this study with US FDA [2016], Codex [2016], and GSO [2013] indicated that all OC residues in all the samples were far above [Ogah 2011] reported in Lagos, Nigeria that OC exceeds their maximum permissible intakes (MPIs).

The maximum permissible concentrations of organochlorine pesticide residues in contaminated soils in which grains and tubers are cultivated are 0.05 mg/kg for Aldrin, 0.05 mg/kg for Endosulfan 0.10 mg/kg for DDT, and 0.03 mg/kg for Heptachlor (FAO 2000). The concentrations of Aldrin (3.2503 ± 0.302 mg/kg), DDT (0.00509 ± 0.001 mg/kg), and Heptachlor (0.07788± 0.003 mg/kg) found in studied soils were within the range of FAO permissible exposure level for contaminated soils 0.1 mg/kg and 0.2 mg/kg, respectively except for Aldrin which was higher (Table 3). The maximum permissible concentrations of organochlorine pesticides in contaminated soil varied from 0.1 mg/kg to 8mg/kg (FAO 2000). For this reason, all the pesticide residue levels recorded in the studied soils were within permissible maximum residue limits recommended by FAO (2000). The result of the OCPs in the soil determined agreed with those of sample crops determined in this present study. The study by Joseph et al., [2015] shows the effect of soil as a pathway of pesticide transport to contaminate plants that constitute the human food chain.

With the mean levels of OCs in the foodstuffs higher, in most cases, than the recommended MRLs, this study has established the presence of insecticide residues in the food items at levels that should give cause for concern. This is because the foodstuffs might not be safe for human consumption all the time as the continued exposure to sub-lethal quantities of the insecticides for a prolonged period may result in chronic illness in humans of which the symptoms are not immediately apparent but manifest later. All the pesticides used are mostly synthetic organic compounds. The soil may act as an important sink for persistent organic pollutants including many pesticides used presently or in the past. They are relatively insoluble in water and
are retained strongly by the soil. Soil acts as a filter buffer and degradation of pollutants concerning storage of pollutants with the help of soil organic carbon. Persistent pesticides slowly break down into the soil and lead to contamination which is closely correlated to human activities agricultural applications and deforestation which leads to soil erosion.

CONCLUSION

The study revealed the presence of persistent, bioaccumulative, and toxic organochlorine insecticides in maize and dried cassava chips at levels generally above the FAO/WHO guideline values that raise public health concerns. Banned organochlorine residues originated from the use of pesticides by farmers in Erinfun farm to protect their crop from pests attack due to their persistent and lipophillic nature and bioaccumulative behaviour. All pesticide components occurred at higher concentrations in maize and dried cassava chips and were statistically significant (p < 0.05) Aldrin, Heptachlor, o,p-DDT and Endosulfan. Leaching of organochlorine pesticides into soil might have caused detection of the presence in the soil samples from the farmland. The current practice which may probably be due to lack of adequate knowledge of the human and environmental dangers posed by improper handling and general abuse of organochlorine needs to be controlled to prevent the build-up of appreciable residues levels of organochlorine in maize and dried cassava chips over time with its attendant hazard to human and environmental health.

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Conflict of Interests

The authors declare that they have no competing interests.

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