Organochlorine pesticide residue and heavy metals in leguminous food crops from selected markets in Ibadan, Nigeria

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Abstract
This study investigates the levels of organochlorine pesticides (OCPs) residues and potentially toxic metals in beans and cowpea. The OCPs and potentially toxic metals were analyzed using GC coupled with electron capture detector and atomic absorption spectrophotometer. The moisture content (%) and ash (%) were 6.0–8.0% and 6.0–10.0% for beans and 6.0–8.0% and 7.5–14.5% for cowpea, respectively. Cowpea Oje had the highest level of OCPs with dieldrin (20.14 ± 0.28), aldrin (7.81 ± 0.13), γ-benzene hexachloride (0.24 ± 0.11), pp-DDE (1.23 ± 0.16), endrin (1.82 ± 0.14), endosulfan II (16.90 ± 0.00), pp-DDT (1.82 ± 0.78), endrin CHO (12.89 ± 0.14), and endosulfan (13.49 ± 0.13), whereas beans Bodija had the lowest value of OCPs with dieldrin (0.99 ± 0.0), endosulfan II (1.02 ± 0.01), and endrin CHO (1.66 ± 0.12), which are all above the maximum residue limits. For the potentially toxic metals, lead was only found in cowpea Bodija and was below detection limit in other markets. Zn, Fe, Cu, and Cr were also detected in the samples.

KEYWORDS
beans, contamination, cowpea, persistent organic pollutants (POPs), toxicology

1 | INTRODUCTION

Persistent organic pollutants are organic compounds that are hazardous and resistant to environmental degradation; hence, they are not easily eliminated from the environment, and they accumulate in environmental media and fatty tissue of living organism and long-range transport (Ntow, 2001). Pesticides are poisons produced because they have toxic effects on one pest or the other (Banjo, Aina, & Rije, 2010); they are intentionally applied to the environment to control unwanted organisms such as fungi, weeds, and insects (Wendie et al., 2011). Organochlorine pesticides (OCPs) are chlorinated hydrocarbons used extensively from the 1940s to 1960s in agriculture, and representative compounds in this group include DDT, methoxycholor, dieldrin, chlordane, texaphene, mirex, kepone, lindane, and benzene hexachloride (BHC), all of which persist in the environment,
bioaccumulate, and magnify in the food chain, and their adverse toxicity to wildlife and humans is of global concern (Jones & de Voogt, 1999). Most of these pesticides and their active metabolites were found to cause severe health conditions such as neurological damage, hypertension, cardiovascular diseases and skin disorders in human, and cancer (Gerken, Suglo, & Braun, 2001; Mansour, 2004; Parbhoo, Rodgers, & Sullivan, 2009).

Cowpea (Vigna unguiculata L. Walp) and bean (Vigna umbellata) are important major staple food crops in sub-Saharan Africa (Adetiloye, Okeleye, & Olatunde, 2006). Their seeds are rich in protein and also with nutrient-rich leaves used as animal feed and help to enrich the soil via their capability to fix atmospheric nitrogen in the soil. They also help in improving total organic matter and develop soil structure in broad. The two major crops are known to constitute very important food items among the over 250 ethnic groups of Nigeria. Insect pests are major constraints to the production of these crops and subsequently lead to their low productivity.

Cowpea is severely attacked at every stage of its growth by a myriad of insects that make the use of tolerant varieties and insecticides sprays imperative (Dugie, Omoigui, Ekeme, Kamara, & Ajaigbe, 2009). In storage, the crop is attacked by weevils (Callosobruchus maculatus Fab) regarded as the major insect pest on the crop (Singh, Zaidi, Saxena, & Ray, 1990). In order to prevent the attack of these crops by pests both on the field and in storage, pesticides are applied on them, and often times, the insecticides were applied indiscriminately by the farmers (for instance, Lowenberg-DeBoer & Ibro, 2008). These pesticides are often misused, overused, or unnecessarily used by farmers and retailers who have very limited or no information about how to apply them or their health implications when present in foodstuffs. Hence, harmful levels of pesticide residues or metabolites are left adsorbed unto foodstuffs to which they are applied. The most common route of exposure to insecticide residues is by ingestion of contaminated foods, inhalation, and dermal exposure, and each of these may pose health risk. Exposure to pesticide residues through ingestion is assumed to be five orders of magnitude higher than the other routes. Several cases of food poisoning after consumption of meals prepared from cowpea grains and bean that were suspected to contain appreciable amount of organochlorine insecticide residues have been reported (Adedoyin, Ojuawo, Adesiyun, Mark, & Anigilaje, 2008; Adeleke, 2009). For instance, in northern Nigeria, about 95% of stored cowpea and bean are treated with insecticides, and farmers use these pesticides even when they are not required (Lowenberg-DeBoer & Ibro, 2008).

Food and Agriculture Organization/World Health Organization (FAO/WHO; 1990) stated all over the world about 3 million people are poisoned and 220,000 die each year from insecticide poisoning, most of which occur in the developing countries, even though far greater quantities are used in the developed countries (Bhanti et al., 2004). For instance, in Nigeria, Shaibu (2008) reported that two children and 112 people were hospitalized after eating cowpea treated with pesticides in Cross Rivers state; 20 fast-food outlets were closed down in Nigeria as a result of fatalities traced to pesticide residue in their products (Chikwe, 2010); and 116 students of a school in Doma, Gombe State, fell ill and were hospitalized after eating contaminated by pesticide (Adegbola, Anugwon, Awagu, & Audu, 2012).

Soil has in many times been subjected to contamination at vary composition and concentrations (Kavamira & Esposito, 2010), perhaps as a result of wide range of actions such as deliberate application, poor residue disposal, inadvertent waste, and inaptness (Knaebel, Federle, McAvoi, & Vestal, 1994). Several human activities have increased the concentration of these metals in the environment (Dudka & Miller, 1999; Mishra, Tiwari, Shukla, & Seth, 2009), so when they are taken at high concentration, they are toxic to the body. The effects of heavy metal toxicity on animal health range from reduction in fitness to reproductive interference to carcinoma (Allison, 2007). This study aimed to assess the occurrence and levels of potentially toxic metals and OCPs residue in two leguminous food crops from selected markets in Ibadan, Nigeria.

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling

Cowpea and beans grains were collected from randomly selected wholesale traders in three major markets (Bodija, Oje, and Shasha) within the city of Ibadan, Nigeria. These markets were chosen because they are the biggest and most patronized markets in the study area. Foreign matters were removed manually from the samples, and they were dried to a constant weight and grinded to powdered form using an agate pestle and mortar; the powders were then stored in a labeled sealed polythene bags prior to analysis.

### 2.2 | Trace metal analysis

One gram of each of the samples was weighed into a beaker, 10 ml of nitric acid was added into each sample, and the beaker is placed on hot plate in the fume hood. One milliliter of perchloric acid was added after about 10 min of heating. The heating was continued until the
2.3 | Extraction, purification, and GC analysis for OCPs

Twenty-gram portion of each powdered sample was weighed into a pre-extracted Whatman extraction thimble. The extraction was carried out in a Soxhlet extractor for 4 hr using dichloromethane (DCM) as the extracting solvent. The extract was concentrated by distilling off the solvent (DCM) to about 2 ml. The concentrated extract is cooled down to room temperature. The reduced extract was then preserved for chromatographic cleanup.

A column of about 15 cm × 1 cm i.d. was packed with about 5-g activated silica gel prepared in a slurry form in n-hexane. About 0.5 cm of anhydrous sodium sulfate was packed at the top of the column to absorb any water in the sample or the solvent. The column is pre-eluted with 15 ml of n-hexane without the exposure of the sodium sulfate layer to air in order to prevent drying up and breaking of the adsorbent. The reduced extract is turned into the column and allowed to migrate below the sodium sulfate layer. Elution was done with 2 × 10 ml portions of the extracting solvent (DCM). The eluate was then collected, dried with anhydrous sodium sulfate, and evaporated to dryness.

The gas chromatography conditions for the analysis were as follows: GC model was Agilent 7890A GC coupled with electron capture detector; injector and detector temperatures were 250 °C and 290 °C; the purge activation time was 30 s; inlet mode was splitless with flow rate of 2 ml/min; carrier gas was helium; make-up gas was nitrogen; inlet temperature was 250 °C; column type was DB-17 fused silica capillary column; column dimension was 30 m × 250 μm × 0.25 μm film thickness; and the oven condition was initial temperature at 150 °C and increase to 280 °C at 6 °C/min. The total run time was 21.67 min.

2.4 | Data analysis

The raw data were subjected to descriptive analysis, t test, and one-way analysis of variance using R statistics. Multiple Duncan range test was also conducted to determine significant difference between means. Pearson correlation coefficient (two-tailed) was conducted to determine the strength of association among OCPs compounds.

3 | RESULTS AND DISCUSSION

3.1 | Proximate analysis

Table 1 presents the results of the proximate analysis of the food samples. It showed that the moisture contents of beans and cowpea sample ranged between 6.0% and 8.0%, and this indicates that the samples can be stored favorably for a long period of time without deterioration. The moisture content of 14% was reported to have adverse effect during storage (Onimawo, Oteno, Orakpo, & Akubor, 2003). The ash content for cowpea ranged from 7.5% to 14.5% whereas that of beans ranged from 6% to 10%. This implies that the samples could be a source of mineral elements having nutritional significance.

3.2 | Trace metal analysis

Table 2 presents the level of trace metals in beans purchased from three selected markets in Ibadan. Pb was not detected in all the samples obtained from the three markets. The ranged (mg/kg) values of other metals are Zn (50.8–71.7), Fe (140–210), Cu (5.75–9.55), and Cr (12.1–66.5). All the metals detected in beans samples from these locations showed significant differences from each other.

Table 3 presents the mean concentrations of potentially toxic metals in cowpea. Pb (4.35 mg/kg) was only detected in cowpea purchased from Bodija market. Other metals concentrations (mg/kg) ranged as follows: Zn
(40.9–74.3), Fe (94.3–245), Cu (5.83–11.6), and Cr (not detected to 28.1). The mean values of these metals showed significant differences from one location to another.

Table 4 shows the total mean concentrations of these potentially toxic metals in both the two leguminous food crops. The *t*-test analysis showed no significant difference (*p* = 0.943) between the mean values of trace metals of the two leguminous crops. Pb was not detected in beans, whereas cowpea had mean concentration of 1.45 mg/kg. Cr showed significant difference between the two crops, whereas the other metals (Zn, Fe, and Cu) had no significant difference. Both the two crops had the same order of increasing concentrations: Fe > Zn > Cr > Cu > Pb.

Zinc is essential for normal growth, development, reproduction, and many other psychological functions in the body, although if it is beyond the limit, it will have adverse effect on the body such as anemia or reduced bone function. In this study, beans have higher concentration of zinc with a mean value of 59.6 ± 9.8, and this is above the 0.3 mg kg\(^{-1}\) day\(^{-1}\) recommended level by FAO/WHO (Hansson & Heiskala, 2014).

Lead is a highly toxic metal, and a high level of it in the body can affect the kidneys, gastrointestinal tract, joints, and reproductive system. In this study, lead was only detected in cowpea with the mean value (1.45 mg/kg), and this is above the recommended value of 0.004 mg kg\(^{-1}\) day\(^{-1}\) by FAO/WHO.

Iron is very essential for the proper functioning of the body. Most iron present in the body is found in the blood as hemoglobin that helps in oxygen transport. Excessive iron in the body can enlarge the liver and may provoke diabetes and cardiac failure. The mean value of iron (mg/kg) was higher in cowpea (179) than in beans (170). These values were above the 0.7 mg kg\(^{-1}\) day\(^{-1}\) recommended by FAO/WHO (2013).

Copper is an important mineral element for plants and animals; high dosage of it can lead to anemia, liver and kidney damage, and stomach and intestinal irritation. The mean concentration of copper is higher in cowpea (8.23) than in beans (7.38). These values are above the 0.04 mg kg\(^{-1}\) day\(^{-1}\) recommended by FAO/WHO (2013).

Chromium is an important trace element essential for carbohydrate metabolism, and its deficiency can lead to impaired glucose tolerance. In this work, the mean value of chromium was higher in beans (44.7 ± 26) than in cowpea (10.3 ± 14). These values are above the 1.5 mg kg\(^{-1}\) day\(^{-1}\) recommended by FAO/WHO (2013).

### 3.3 OCPs residues

Figure 1a–c presents the chromatogram of the OCPs mix standard (2 ppm) and representative chromatograms of OCPs of cowpea and beans samples, respectively. The mean concentrations of cyclodienes isomers in cowpea are presented in Table 5. The result showed that aldrin, dieldrin, and endrin aldehyde were present in all the samples with value range 1.46–7.81 mg/kg, 2.43–20.14 mg/kg, and 3.74–12.89 mg/kg, respectively. Furthermore, the concentrations obtained in the samples from Oje were higher compared with other markets, and this may be as a result of pesticides added during storage.

Table 6 shows the mean concentrations of dichloro diphenylethene in cowpea; none of these isomers was found in samples from Shasha. Methoxychlor was not present in any of the samples. The residue levels of the isomers found range as follows: para, para-dichlordiphenyldichloroethane (p,p'-DDD; 0.62–1.81 mg/kg), p,p'-DDE (0.48–1.23 mg/kg), and p,p'-DDT (1.05–1.82 mg/kg). The values of each contaminant

### Table 3

Mean concentrations (mg/kg) in cowpea obtained from three markets

| Location | Zn     | Pb     | Fe   | Cu     | Cr    |
|----------|--------|--------|------|--------|-------|
| Bodija   | 74.3 ± 4.2\(\text{b}\) | 4.35 ± 1.1\(\text{a}\) | 245 ± 5.7\(\text{c}\) | 11.6 ± 1.4\(\text{b}\) | 2.70 ± 1.3\(\text{a}\) |
| Oje      | 54.2 ± 5.7\(\text{a}\) | ND     | 94.3 ± 2.5\(\text{a}\) | 5.83 ± 1.3\(\text{a}\) | 28.1 ± 1.7\(\text{b}\) |
| Shasha   | 40.9 ± 2.8\(\text{a}\) | ND     | 197 ± 4.2\(\text{b}\)  | 7.30 ± 1.3\(\text{a}\) | ND    |

*p* value .011 .011 .000 .029 .000

Note. Mean values with different subscript alphabets are significantly different at *p* < 0.05.

Abbreviation: ND, not detected.

### Table 4

Total mean values of potentially toxic metals in the sample

| Sample   | Zn     | Pb     | Fe   | Cu     | Cr    |
|----------|--------|--------|------|--------|-------|
| Beans    | 59.6 ± 9.8 | ND     | 170 ± 33 | 7.38 ± 2.1 | 44.7 ± 26 |
| Cowpea   | 56.5 ± 15 | 1.45 ± 2.3 | 179 ± 69 | 8.23 ± 2.8 | 10.3 ± 14 |

Abbreviation: ND, not detected.
obtained in Oje were higher than the corresponding values obtained at Bodija. These values were higher than the Federal Food, Drug and Cosmetic Act (FFDCA) MLRs of 0.02 mg/kg. The one-way analysis of variance revealed significant differences among the dichloro diphenylethene compounds in cowpea.

FIGURE 1 (a) Chromatogram of the organochlorine pesticides mix standard, and (b and c) representative chromatograms of the cowpea and bean samples analyzed in this study. BHC, benzene hexachloride; p,p'-DDD, para, para-dichlorodiphenyldichloroethane
Table 7 shows the mean concentration of hexachloro cyclohexane in cowpea. It shows that γ-BHC was only detected in Oje sample (0.24 ± 0.11 mg/kg) and it is above the FFDCA MLRs value 0.02 mg/kg whereas α-BHC, β-BHC, and δ-BHC were not detected in the sample.

The mean concentrations of cyclodienes isomers in beans are presented in Table 8. The result showed that dieldrin, endrin aldehyde, and endosulfan II were present in all the samples and ranged 0.99–11.01 mg/kg, 1.66–6.70 mg/kg, and 0.85–1.37 mg/kg, respectively. The analysis of variance conducted showed varied significance among the cyclodienes compounds in beans.

Heptachlor and Hept-epoxide were not detected. Aldrin, endrin, and endosulfan I were only detected in Oje sample. The residual values detected were above the FFDCA maximum residue limits (MRLs).

Table 9 shows the mean concentrations of dichloro diphenylethane in cowpea; none of these isomers was found in samples from Bodija and Shasha. Methoxychlor was not present in any of the samples. The residue levels of the isomers found in Oje are as follows: p,p′-DDD, p,p′-DDE, and p,p′-DDT.

Table 10 shows the mean concentrations of hexachloro cyclohexane in beans.
(1.00 ± 0.00 mg/kg), p,p′-DDE (0.66 ± 0.14 mg/kg), and p,p′-DDT (0.72 ± 0.19 mg/kg). These values were higher than the FFDCA MLRs of 0.02 mg/kg. The analysis of variance indicated significant differences among the dichloro diphenylethane isomers in beans.

Table 10 shows that none of the hexachloro cyclohexane isomers was detected in the beans obtained from all the three markets.

The result of the total mean concentrations of the OCPs was presented in Table 11. It shows that the residues of all the isomers were above the FFDCA MLRs as shown on the table. Also, it is noteworthy that the pesticide residues were higher in cowpea than in beans. T-test analysis showed that there was significant difference (p = 0.05) between the OCPs mean values of the two crops. Similar observations were made in Togo by Mawussi et al. (2009) where levels of OCPs detected in cowpea grains exceeded the MRL. The MRL should not be exceeded in any foodstuff, and if this happens, such a foodstuff is said to be adulterated because it contains an unsafe or illegal amount of residue. Also, this study shows that the cyclodiene subgroup was mostly detected and this was also reported in Kolanuts from selected markets in Osun state, Nigeria (Sosan & Oyekunle, 2017). The predominance of γ-BHC (lindane) among the hexachlorohexanes detected in cowpea grain samples implies that the insecticides must have been used for treating the grains against insect pest attack either on the field or for storage. Lindane, the active ingredient in Gamalin 20EC, has been a household name in agricultural communities of Nigeria. High concentration of p,p′-DDD among the dichloro diphenylethane and its

| TABLE 11 | Total mean concentration (mg/kg) OCPs in the sample |
|-----------|--------------------------------------------------|
| OCPs      | Beans    | Cowpea    | MRLs    |
| Heptachlor| ND       | 0.64 ± 0.69| 0.01    |
| Aldrin    | 1.25 ± 1.94| 4.28 ± 2.89| 0.01    |
| γ-BHC     | ND       | 0.80 ± 0.13| 0.02    |
| p,p′-DDE  | 0.22 ± 0.35| 0.57 ± 0.56| 0.02    |
| Dieldrin  | 4.46 ± 5.07| 10.44 ± 8.02| 0.01    |
| Endrin    | 0.33 ± 0.52| 0.83 ± 0.83| 0.02    |
| p,p′-DDD  | 0.21 ± 0.34| 1.15 ± 0.54| 0.02    |
| Endosulfan II | 1.08 ± 0.25| 8.80 ± 7.6  | 0.02    |
| p,p′-DDT  | 0.24 ± 0.37| 0.96 ± 0.81| 0.02    |
| Endrin CHO| 3.54 ± 2.47| 7.04 ± 4.50| 0.02    |
| Endosulfan | 1.76 ± 2.72| 5.83 ± 6.25| 0.02    |

Source: FFDCA (2015).

Abbreviations: BHC, benzene hexachloride; MRLs, maximum residue limits; ND, not detected; OCPs, organochlorine pesticides; p,p′-DDD, para, para-dichlorodiphenyldichloroethane.

| TABLE 12 | Correlation coefficient in beans and cowpea for individual OCPs from Ibadan markets |
|-----------|--------------------------------------------------|
| Heptachlor | Aldrin    | α-BHC | ppDDE | Dieldrin | ppDDD | Endosulfan II | Endrin CHO | Endosulfan | Endosulfan Sulfate |
| Heptachlor | 1        | −.176  | −.244 | −.372   | −.306   | −.366       | −.252      | −.301       | −.308        |
| Aldrin     | −.244    | 1      | −.215 | −.264   | −.308   | −.306       | −.252      | −.301       | −.308        |
| α-BHC     | −.372    | −.215  | 1     | −.264   | −.308   | −.306       | −.252      | −.301       | −.308        |
| ppDDE     | −.306    | −.306  | −.264 | 1       | −.215   | −.252       | −.301      | −.308       | −.308        |
| Dieldrin  | −.366    | −.306  | −.306 | −.264   | 1       | −.215       | −.252      | −.301       | −.308        |
| ppDDD     | −.252    | −.252  | −.252 | −.252   | −.215   | 1           | −.252      | −.301       | −.308        |
| Endosulfan II | −.301 | −.301  | −.301 | −.301   | −.301   | −.301       | 1          | −.301       | −.301        |
| Endrin CHO| −.252    | −.252  | −.252 | −.252   | −.252   | −.252       | −.252      | 1           | −.301        |
| Endosulfan | −.308   | −.308  | −.308 | −.308   | −.308   | −.308       | −.308      | −.301       | 1            |

*Correlation is significant at .05 level (two-tailed).
**Correlation is significant at .01 level (two-tailed).
predominance in the two foodstuffs indicated that the DDT is in insecticide applied for control of insect pests of cowpea and beans. According to ATSDR (2002), DDD enters the environment during the breakdown of DDT, which can undergo slow biodegradation through reductive dechlorination to form the product. Residues of DDT have been reported to be most frequently encountered OCP in beans sampled from Lagos markets in Nigeria (Ogah, Tettey, Coker, & Adepoju-Bello, 2012).

3.4 Correlation

Correlation analysis was carried out for OCPs using two-tailed Pearson correlation coefficient to determine the relationship between individual components as shown in Table 12. Detailed analysis of the data set revealed that there were significant positive correlations among all the individual OCPs compounds at \( p < 0.01 \) with exception of heptachlor. Heptachlor was found to be negatively correlated with all other OCPs but weakly positive correlated with p,p’-DDD. Positive correlation that exhibited among this OCPs is an indication that these compounds have a common source of origin and similar environmental behavior. Negative correlation might be attributed to different chemical structures among these compounds.

4 Conclusion

This study showed that the food samples had significant concentrations of the pesticide residue, the three classes of OCPs were identified, and the pesticide residues were more in cowpea than in beans. Also, the research showed that the samples from Oje market have higher concentration than others and the values obtained generally were greater than MRLs. It also showed that the levels of the heavy metals were above the recommended daily intake. The farmers should be educated against indiscriminate use of pesticides. The controlling body and custom official in Nigeria should engaged in strict control of importation, sales, use, and disposal of these persistent organic pollutants. There should be proper surveillance and adequate health records of sales agent of these chemicals, farmers that engage in the use of pesticides, and victims of food poison. Establishment of medical research center where studies on epidemiological and metabolic studies of people that engaged in the use and sales as well as victims of food poison are carried out is highly recommended.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study are included within the article.

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