

Assessment of Organochlorine Pesticide Residues in Vegetable Samples from Some Agricultural Areas in Togo

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Abstract

The study was conducted to monitor the concentration of pesticide residues in three staple vegetables (tomato, cabbage, lettuce) collected from 12 prefectures belonging to 4 agro-ecological regions of Togo. A total of 150 samples of ripe tomato, cabbage and lettuce were collected from the study areas and analyzed for 20 OCPs (organochlorine pesticides) residues, which can be grouped into DDTs, Drins, Heptachlors, Chlordanes and HCHs. The data revealed that 100% of the analyzed vegetable samples were contaminated by one or more pesticide residues; 83.32% and 100% were below the maximum residue limits (MRLs) set par European Union and FAO/WHO respectively whereas 16.68% were above the EU (MRLs) adopted values. Overall, cabbage contained the highest number of samples with pesticide residues above the MRLs followed by tomato, whilst none of lettuce samples contained pesticide residues above the MRLs. It is therefore proposed to perform continuous monitoring studies of pesticide residues in vegetables cultivated in Togo.

Keywords

Togo, Organochlorine Pesticides, Residues, Vegetables, Maximum Residue Limit (MRL)

1. Introduction

Pesticides are part of the control means available to farmers to combat insect pests. Today, it is estimated that as much as 45% of the world's crop is destroyed by insect pests and plant diseases. Thus in order to meet the food demand of the world, it is essential to use plant protection strategies, based on various approaches including among others the use of commercial pesticides [1].

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Organochlorine pesticides (OCPs) have been commonly used in agriculture in the past. But since the 1950s, the OCPs have become of much concern for human health and environment, and the Stockholm Convention on persistent organic pollutants (POPs) [2] listed most of them as banned (Annex A POPs) or at least severely restricted chemicals (Annex B POPs). In fact, because of their prolonged persistence, long-range transport nature, high toxicity, as well as bioaccumulative tendency, they can cause chronic toxicity in animals and human via air, water and food intake [3] [4]. Thus, exposure to OCPs has been associated with human health risk of arthritis, skin disease, bone disorder, endocrine disruption, developmental abnormalities, reproduction failure, cancer and nerve disorder. For humans, food is the major pathway for exposure to OCPs [5] [6].

Because of their harmful effects, OCPs are banned in developed countries since 1980s [7]-[9] and their monitoring in food has been conducted in several countries in order to study route exposure of human to OCPs through dietary intake [10]-[12]. However, control programs for pesticide residues in the developing countries are often limited if not absent because of the lack of adequate infrastructure and non-enforcement of legislation.

Recent monitoring in Ghana revealed contamination of vegetables with OCPs at worrying levels that exceeded the accepted maximum residue limit [13]-[15]. Even OCPs not registered for use on vegetables were found in food [16]. In addition, non-target crops were also contaminated with high levels of OCPs [17], indicating their widespread application in this country.

In Togo, the regulatory framework governing the use of organochlorine pesticides is the Decree N°. 31/APR/SG/DA of September 21, 2004 [18] that prohibits the import and use of organochlorines in Togo. Particularly the following POPs: Aldrin, endrin, dieldrin, DDT, mirex, toxaphene, hexachlorobenzene (HCB), chlordane and heptachlor. Lindane, chlordecone and endosulfan shall be added to the list. However, the banned OCPs may still continue entering the country since the local pesticide market for smallholder farmers is dominated by the informal sector that offers cheaper pesticides to farmers including those producing vegetables. Indeed the vegetables production sector in Togo is intensively consuming pesticides and it is therefore a public health concern to ensure that vegetables produced in the country are of good sanitary quality. In addition data on recent status of OCPs residue in vegetable from Togo are very scarce. Therefore, the aim of our study is to investigate the level of contamination of vegetables by OCPs in major vegetable production areas of Togo.

2. Materials and Methods

2.1. Study Areas

The study was conducted in 12 prefectures belonging to 4 agro-ecological regions of Togo (Figure 1). The areas selected for sampling are major areas with high vegetable production activity that develops around water sources (rivers, streams, garden wells) and extends throughout the year depending on demand and agro-ecological characteristics of crops.

2.2. Sample Collection

A total of one hundred fifty (150) samples of vegetable were collected from the study areas including 55 samples of cabbage, 55 samples of ripe tomato and 40 samples of lettuce. These vegetables were chosen because they are very common in Togolese diets and need little or virtually no processing before consumption. For each prefecture, 2 composite samples of 1 - 2 kg of vegetables were constituted by blending from vegetables samples collected from the main producing sites of localities where the vegetables were ready for sale. The composite samples were chopped and washed with distilled water, wrapped with aluminum foil, labeled, placed in an ice chest box and then were transported to the laboratory (Ghana Atomic Energy commission GAEC) and stored at -20°C for further analysis.

2.3. Pesticide Extraction and Clean up

Frozen chopped samples were kept at room temperature for about 3 hours and ground at full speed using a war-ring blender. Extraction of vegetable samples was carried out according to procedures described by Bempah *et al.* 2012 [15] with slight modification. Sub-samples (10 g) of homogenize sample was weighed into a clean, dry and labeled conical flask containing 30 g of anhydrous sodium sulfate (Na_2SO_4) and 5 g of sodium hydrogen carbonate (NaHCO_3) and thoroughly mixed. The mixture was extracted with $3 \times (30 \text{ ml})$ ethyl acetate-hexane (2:1 v/v) by shaking for 30 min using a sonicator (BRANSONIC 220). The supernatant was concentrated to near

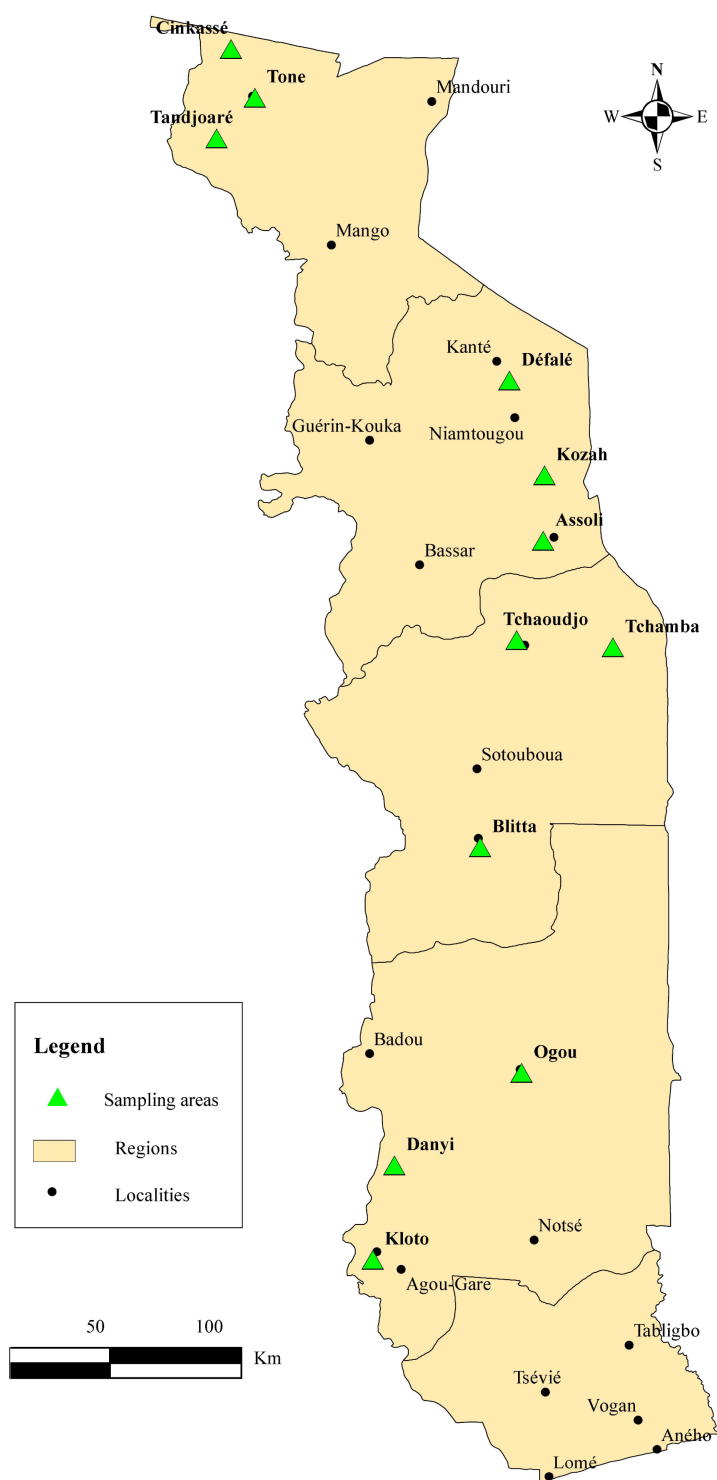


Figure 1. Map of Togo showing sampling areas.

dryness using a vacuum rotary evaporator (BÜCHI Heating Bath B-490) with a water bath at 45°C. The sample was purified on a complex of adsorbents (deactivated florisil, 4 g; anhydrous sodium sulfate, 1 g and activated charcoal, 2 g) and eluted with 40 ml portions of hexane mixtures and evaporated to dryness. The extract was picked in 2 ml ethyl acetate into a GC vial and sealed for subsequent quantification using gas chromatograph.

2.4. Pesticide Analysis

Measurements were carried out on a Shimadzu GC 2010, gas chromatograph equipped with an Electron Capture Detector. The chromatographic separation was done on an SGE BPX-5 of 60 m capillary column with 0.25 mm internal diameter and 0.25 μm film thickness and equipped with 1 m retention gap (0.53 mm, deactivated). The oven temperature program was set as follows: Initial temperature was set at 90°C for 3 min and ramped at 30°C/min to 200°C for 15 min and then to 265°C at a rate of 5°C/min for 5 min then to 275°C at the rate of 3°C/min and allowed to stay for 15 min giving a total run time of 58 min. The injector setting is a pulsed splitless mode with a temperature of 250°C at a pressure of 1.441 bar. Pulsed pressure was 4.5 bar, pulsed time 1.5 min, purge flow of 55.4 ml/min with a purge time of 1.4 min. The detector temperature was 300°C in “constant make-up flow” mode (30 ml/min of Nitrogen gas). The residue of pesticide was identified based on comparison of the measured relative retention times to those of known standards. The residue levels of organochlorine pesticides were quantitatively determined by the external standard method using peak area. Measurement was carried out within the linear range of the detector. The peak areas whose retention times coincide with the standards were extrapolated on their corresponding calibration curves to obtain the concentration.

2.5. Quality Control

The quality of organochlorine pesticides was assured through the analysis of solvent blanks, procedure blanks and duplicate samples. The method was optimized and validated using spiked together with the internal standard to evaluate the recovery of compounds [19]. The recoveries of internal standards ranged between 87% and 104% for all the organochlorine pesticide. The limit of detection was 0.001 $\text{ng}\cdot\text{g}^{-1}$.

3. Results and Discussion

The level of individual OCPs in vegetables is reported on **Figure 2** and **Table 1**. All concentrations of OCPs are expressed in $\text{ng}\cdot\text{g}^{-1}$ on a whole basis.

The different vegetables sampled (tomato, cabbage and lettuce) were analysed for the following Persistent OCPs compounds: HCHs, Drins, Heptachlors, Chlordanes, DDTs. Overall, total concentration of ΣOCPs ranged between <0.001 to 119.28 $\text{ng}\cdot\text{g}^{-1}$ with the mean of 6442 $\text{ng}\cdot\text{g}^{-1}$ (**Figure 2**). On the OCPs analyzed, the concentration of total HCHs was found to be higher with an average concentration of 5.847 $\text{ng}\cdot\text{g}^{-1}$ followed by total DDTs, Chlordanes, Drins and heptachlors which recorded concentrations of 0.27, 0.26, 0.25 and 0.012 $\text{ng}\cdot\text{g}^{-1}$ respectively (**Figure 2(a)**). Among the detected and quantified pesticides, Drins accounted 31.11% of the total OCPs, followed by HCHs and DDTs (23.3%), Chlordanes (16.50%) and Heptachlors (2.91%) (**Figure 2(b)**).

Table 1 illustrates the level of pesticide concentration in the three vegetables analyzed. The average total concentration of OCPs in tomato, cabbage, and lettuce was $8 \pm 1.203 \text{ ng}\cdot\text{g}^{-1}$, $16.436 \pm 2.717 \text{ ng}\cdot\text{g}^{-1}$, $6.29 \pm 4.005 \text{ ng}\cdot\text{g}^{-1}$, respectively. ΣDrins , $\Sigma\text{Chlordanes}$, and ΣDDTs were detected in all three vegetables with the highest concentration below 3 $\text{ng}\cdot\text{g}^{-1}$. Similarly, Lindane was found in only tomato and lettuce. The $\Sigma\text{Heptachlors}$ were found in cabbage and lettuce in lower concentrations (below 1 $\text{ng}\cdot\text{g}^{-1}$). However, higher level was observed with ΣHCHs in tomato with the highest concentration of 11.307 $\text{ng}\cdot\text{g}^{-1}$ and in cabbage with the highest concentration

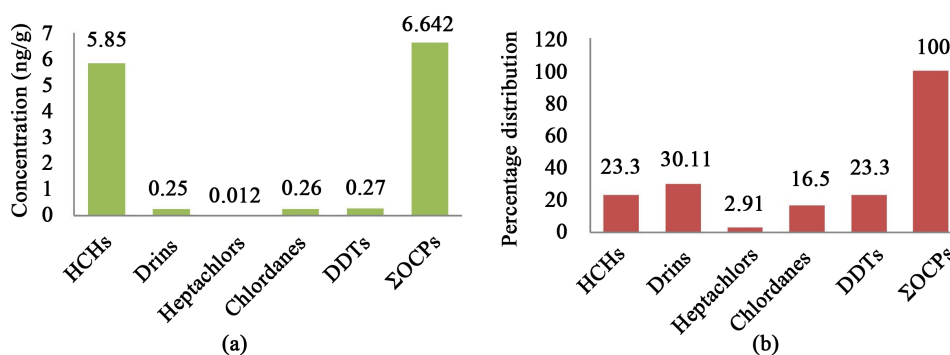


Figure 2. (a) Concentration ($\text{ng}\cdot\text{g}^{-1}$) of OCPs in studied vegetables. (b) Distribution (%) of OCPs in studied vegetables.

Table 1. Pesticide residue concentrations in the tested vegetables ($\text{ng}\cdot\text{g}^{-1}$).

Pesticide	Tomato (n = 55) ^a		Cabbage (n = 55)		Lettuce (n = 40)	
	Range	Mean \pm SE ^b	Range	Mean \pm SE	Range	Mean \pm SE
Alpha-HCH	<0.001 - 0.156	0.073 \pm 0.006	<0.001 - 0.176	0.064 \pm 0.005	<0.001 - 0.170	0.069 \pm 0.023
Beta-HCH	<0.001 - 10.832	1.626 \pm 0.337	<0.001 - 90.673	14.544 \pm 2.507	<0.001 - 2.087	0.330 \pm 0.26
Gamma-HCH	<0.001 - 0.026	0.002 \pm 0.000	<0.001	<0.001	<0.001 - 0.053	0.017 \pm 0.008
Delta-HCH	<0.001 - 0.313	0.089 \pm 0.010	<0.001 - 2.983	0.507 \pm 0.079	0.150 - 0.324	0.201 \pm 0.019
ΣHCHs	<0.001 - 11.307	1.79 \pm 0.354	<0.001 - 93.832	15.115 \pm 2.592	0.150 - 2.634	0.617 \pm 0.312
Aldrin	<0.001 - 0.263	0.166 \pm 0.010	<0.001 - 0.352	0.177 \pm 0.010	0.183 - 0.380	0.262 \pm 0.022
Dieldrin	<0.001 - 0.194	0.018 \pm 0.005	<0.001 - 0.086	0.011 \pm 0.002	<0.001 - 0.010	0.001 \pm 0.001
Endrin	<0.001 - 0.161	0.023 \pm 0.004	<0.001 - 0.239	0.055 \pm 0.008	<0.001 - 0.310	0.039 \pm 0.038
ΣDrins	<0.001 - 0.618	0.207 \pm 0.015	<0.001 - 0.677	0.243 \pm 0.02	0.183 - 0.7	0.302 \pm 0.061
Heptachlor	<0.001	<0.001	<0.001 - 0.081	0.007 \pm 0.002	<0.001 - 0.254	0.032 \pm 0.031
Cis heptachlor	<0.001	<0.001	<0.001 - 0.154	0.014 \pm 0.004	<0.001	<0.001
Trans-heptachlor	<0.001	<0.001	<0.001 - 0.013	0.001 \pm 0.000	<0.001	<0.001
ΣHeptachlors	<0.001	<0.001	<0.001 - 0.248	0.022 \pm 0.006	<0.001 - 0.254	0.032 \pm 0.031
Cis-Chlordane	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trans-Chlordane	<0.001	<0.001	<0.001 - 0.292	0.052 \pm 0.008	<0.001 - 0.081	0.014 \pm 0.01
Trans-Nonachlor	<0.001 - 0.367	0.152 \pm 0.016	<0.001 - 1.294	0.463 \pm 0.038	<0.001 - 0.454	0.101 \pm 0.066
ΣChlordanes	<0.001 - 0.367	0.152 \pm 0.016	<0.001 - 1.586	0.515 \pm 0.047	<0.001 - 0.535	0.115 \pm 0.077
p,p-DDT	<0.001 - 0.527	0.165 \pm 0.021	<0.001 - 0.643	0.190 \pm 0.000	<0.001 - 0.681	0.232 \pm 0.1
o,p-DDT	<0.001	<0.001	<0.001 - 0.010	0.001 \pm 0.000	<0.001	<0.001
p,p-DDE	<0.001 - 0.253	0.080 \pm 0.010	<0.001 - 0.595	0.215 \pm 0.021	<0.001 - 0.368	0.081 \pm 0.047
p,p-DDD	<0.001	<0.001	<0.001 - 0.034	0.003 \pm 0.001	<0.001	<0.001
o,p-DDD	<0.001	<0.001	<0.001 - 0.236	0.042 \pm 0.008	<0.001 - 0.187	0.023 \pm 0.023
o,p-DDE	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ΣDDTs	<0.001 - 0.78	0.245 \pm 0.031	<0.001 - 1.518	0.451 \pm 0.052	<0.001 - 1.236	0.104 \pm 0.171
ΣOCPs	<0.001 - 13.072	2.394 \pm 0.33	<0.001 - 97.861	16.436 \pm 2.717	0.333 - 5.08	1.17 \pm 0.653

^an: number of samples; ^bSE: standard error = SD/\sqrt{n} .

of $93.382 \text{ ng}\cdot\text{g}^{-1}$ and at low level in lettuce (highest concentration of $2.634 \text{ ng}\cdot\text{g}^{-1}$). Within the HCHs group, β -HCH was found in the highest level in all three vegetables analyzed. Similarly Aldrin, Heptachlor, Trans-Nonachlor, p,p-DDT, respectively among Drins, Heptachlors, Chlordanes and DDT groups, had the highest concentrations when present in the tested vegetables.

The highest contamination frequency (Figure 3) of Σ HCHs was found in lettuce (100%) followed by tomato and cabbage that recorded the same contamination rate (72.72%) (Figure 3(a)). The same pattern of contamination was observed with Σ DDTs (Figure 3(e)). Within the Σ HCH, α -HCH (54.5%) was found to be dominant contaminant in tomato, while α -HCH and δ -HCH were dominant contaminants in cabbage with contamination frequency of 63.6%, and δ -HCH (100%) in lettuce. Alongside, p,p-DDT (50%) and pp-DDE (54.5%) were found to be dominant contaminant in lettuce and in cabbage respectively, and pp-DDT and pp-DDE were the most frequent pesticide residues found in tomato with a contamination rate of 36.4%. Lettuce, tomato and cabbage were contaminated by Σ Drins at the contamination rate of 100%, 90% and 81.81% respectively with Aldrin the most frequent residue found in the three vegetables (87.5%, 81.8% and 72.72% respectively for lettuce, cabbage and tomato) (Figure 3(b)). Σ Chlordanes was found at higher frequency in cabbage (90.90%), at a

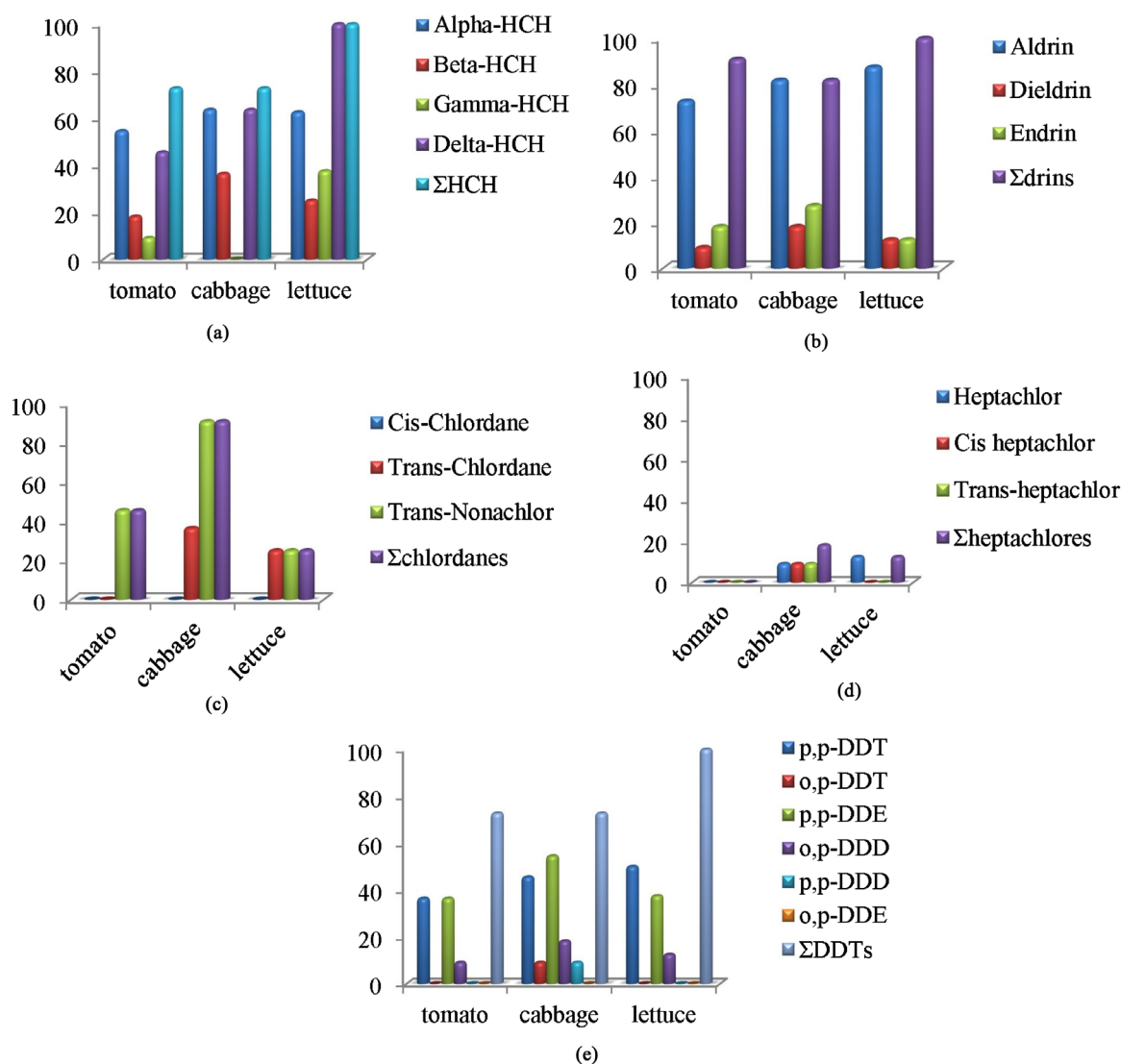


Figure 3. Pesticide residues frequency in tested vegetables. (a) HCHs; (b) Drins; (c) Heptachlores; (d) Chlordanes; (e) DDTs.

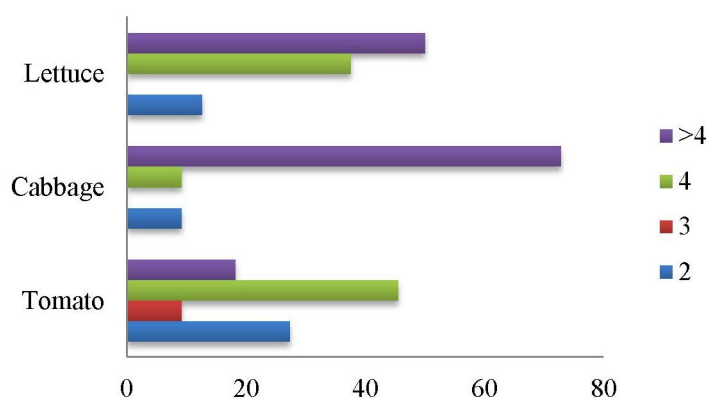
relatively medium frequency in tomato (45.5%) and at a low frequency in lettuce (25%) and the same pattern contamination was observed with the dominant residue of this OCP (**Figure 3(c)**). ΣHeptachlores was found only in cabbage and lettuce at low contamination rate (18.18% and 12.5% respectively) (**Figure 3(d)**).

The observed concentration of OCP compounds in this study was compared with recommended maximum residue limits (MRLs) set by European commission (2015) and WHO (2015) (**Table 2**). The comparison of obtained results with MRLs indicated that studied vegetables in this study had residue levels of OCPs far much below MRLs except for ΣHCHs for which 9.1% of tomato samples and 36.4% of cabbage samples exceeded EU MRLs.

The occurrence of pesticide residues is listed in **Figure 4**. None of the analyzed vegetable samples was residue-free. All of them were contaminated with at least one OCP; most samples had two or more residues. Among all three vegetables studied, cabbage was the most contaminated and it had multiple OCPs; hence 72.72% of cabbage samples collected were contaminated with more than 4 residues, whereas 9.09% of samples were contaminated with 4 residues and 2 residues respectively. Following cabbage was lettuce with 50%, 37.5% and 12.5% of samples being contaminated with more than 4 residues, 4 residues and 2 residues respectively. As for tomato, 18.18%, 45.45%, 9.09% and 27.27% of samples were respectively contaminated with more than 4 residues, 4 residues, 3 residues and 2 residues (**Figure 4**).

Table 2. Maximum residue limits (MRLs) of OCPs in vegetables.

Pesticide	Tomato (n = 55) ^a				Cabbage (n = 55) ^a				Lettuce (n = 40) ^a			
	MRL (EU)	MRL (FAO)	>MRL (EU)	>MRL (FAO)	MRL	MRL (FAO)	>MRL (EU)	>MRL (FAO)	MRL	MRL (FAO)	>MRL (EU)	>MRL (FAO)
Lindane	10	2000	0	0	10	-	0	-	10	-	0	-
ΣHCHs	10	-	9.1%	-	10	-	36.4%	-	10	-	0	0
Aldrin + Dieldrin	10	100	0	0	10	50	0	0	10	50	0	0
Endrin	10	50	0	0	10	50	0	0	10	50	0	0
ΣHeptachlors	10	20	0	0	10	50	0	0	10	50	0	0
ΣChlordanes	10	20	0	0	10	20	0	0	10	20	0	0
ΣDDTs	50	1000	0	0	50	1000	0	0	50	1000	0	0

^an: number of samples.**Figure 4.** Vegetables with multiple pesticide residues in samples analyzed.

The results of the present study on pesticide residues revealed that a broad range of OCPs contaminated vegetables indicating a wide use of OCPs in Togo. However the level of OCPs residues in the tested vegetables, were found to be low in most vegetables samples as concentrations were below MRLs set par the European commission (2015) [20] and WHO (2015) [21]. In comparison, the levels of the residues in the tested vegetables were far below those previously reported in Ghana [13] [15] [17] and in India [22] [23] indicating that in the case of Togo, OCPs might probably originate from past uses instead of current application on cultivated vegetables [7]. Concentration of ΣHCHs in tomato samples from the Prefecture de Tchoudjo and in cabbage samples from Préfecture de Tchoudjo, Préfecture de l'Ogou, Préfecture de Kloti and Préfecture de Danyi was however found to be relatively high, exceeding EU MRL. These vegetables would have been grown on high polluted site or contaminated by leaching from a heavily polluted nearby site. This indicates that HCH was used in Togo extensively before its use in the agricultural sector was banned. HCHs are among the most widely used and most readily detected OCPs in environmental samples [24] and they were found at relatively high proportion (88.08% of ΣOCPs) in studied vegetables. When detectable, individual α , β , γ and δ isomers of HCH were found to contribute ~0% - 11%, 53% - 96%, 0% - 2% and 2% - 4% respectively. β -HCH has the lowest water solubility and vapor pressure, and is the most stable and relatively resistant to microbial degradation [25]. It has, as well higher affinity towards the lipidic fraction of plants cell walls and the most likely mechanisms of HCH accumulation in plants is sorption of soil HCH on roots [26]. In addition, after a long period of weathering, α - and γ -HCHs could be transformed into β -HCH [27]. It is these properties of β -HCH relative to other HCHs that most likely accounted for the greater proportion of β -HCH in the samples. The relatively high proportion of β -HCH observed indicated that the HCH as well as other OCPs found, was mainly due to historical use as a pesticide [28].

Because of their effectiveness, OCPs have been widely used as agricultural pesticides whether for food or

cash crops [29] [30]. In West Africa, OCPs were mostly used on cocoa plantations, on vegetable farms, and for the control of stemborers in maize [31]. These pesticides are still being used clandestinely in some developing countries in vegetable growing [15]-[17] though they have been banned in most countries [29] [30]. This can be explained by the fact that farmers in most developing countries have low levels of education and disregard the harmful effects of these pesticides on the environment. Having experienced the effectiveness of OCPs in the past, they would therefore be inclined to resort to clandestine supply of these products. A wide awareness on the negative impact of these pesticides on health and the environment is therefore necessary and must be continuously implemented within the farmer population of developing countries to deter and enable broad adoption of the regulation tests in this respect in these countries. It also calls for improved tighter regulation in the sale of pesticides as well as for education of farmers and the implementation of integrated pest management methods.

4. Conclusion

In Togo, apparently the regulation in this area is in force, preventing the entry of OCPs from neighboring countries. What characterizes the quite low levels of OCPs observed in most studied vegetables which never exceeded the residue levels of OCPs set by EU and FAO/WHO, indicating minimal risk to the consumers except for relatively high residue levels of Σ HCHs detected in tomato samples from the Prefecture de Tchoudjo and in cabbage samples from Préfecture de Tchoudjo, Préfecture de l'Ogou, Préfecture de Kloto and Préfecture de Danyi exceeding EU MRL and that could be supportive of a possible health risk for consumers. Thus strengthening the regulation, continuous monitoring of vegetables and extending to other vegetables are highly recommended in order to ensure consumers protection.

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