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Levels of organochlorine pesticide residues in *Mondia whitei*, a medicinal plant used in traditional medicine for erectile dysfunction in Ghana

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ABSTRACT

A study was conducted to determine fourteen organochlorine pesticide (OCP) residues in the root of *Mondia whitei* sampled from Kwahu-East and Biakoye Districts of Ghana using a Varian CP-3800 Gas Chromatograph equipped with a ^{63}Ni electron capture detector. The mean concentrations of the OCP residues were investigated at different season, of which seven were among the banned pesticides in Ghana. The mean OCP residue concentrations at dry season were found to range from 0.005 to 0.082 mg/kg and at rainy season the mean OCP residue concentrations ranged from not detected to 0.026 mg/kg. Mean OCP residue concentrations were much higher in the matrices collected in the dry season compared to those of the wet season. The total OCP mean residue concentrations in *M. whitei* also ranged from 0.038 to 0.528 mg/kg, with the highest total mean levels of 0.528 mg/kg recorded in the matrices collected from Kwahu-East District in the dry season while the lowest total OCP residue concentrations were detected in the matrices collected from the Biakoye District in the rainy season. With the exception of heptachlor in *M. whitei* from Kwahu-East District and residue levels obtained for the sum of aldrin and dieldrin in *M. whitei* from Biakoye and Kwahu-East Districts during the dry season, the mean values obtained were generally below maximum residue limits set forth by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Codex Alimentarius Commission and United States/European Pharmacopoeia.

INTRODUCTION

Mondia whitei L. (Hook. F) Skeel of the Family Periplocaceae and Subfamily Periplocoideae which is also commonly referred to as white's ginger, is a perennial, woody, robust and vigorous climber that grows from a large tuberous root stock. The roots are aromatic and apparently taste like ginger or have an aroma reminding one of vanilla (Figure 1). The leaves are attractive, large and opposite, with a deeply notched heart-shaped base with long stalks. The species are easily cultivated from seeds which are collected as the fruit starts to split open (Gelfand et al., 1985; Adjanohoun et al., 1996; Noumi et al., 1998).

M. whitei has for a long time been extensively and widely used in traditional medicine. It is used by some tribes in Zimbabwe for treating anorexia and bilharzia and as an antacid to treat indigestion, as a tonic and to stimulate appetite. Infusions of the root are also used by some tribes in Cameroon for the treatment of general pains and aches (Gelfand et al., 1985; Adjanohoun et al.,

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Figure 1. Showing fresh cut root of *M. whitei*.

1996; Noumi et al., 1998).

The plant is used in traditional medicine in Ghana for the management of male erectile dysfunction, also referred to as impotence which is; repeated failure to attain or sustain an erection firm enough for satisfactory sexual intercourse, a common condition in older men (Ofosuhene, 2005; Quasie et al., 2010).

M. whitei is endemic to South, Central, East and West Africa. It also grows in dense bush in a variety of woodland and forest habitats and has even been reported from shrubby swamp grassland (Adjanohoun et al., 1996; Noumi et al., 1998; Watcho et al., 2001, 2004, 2005, 2006, 2007).

In Ghana, the plant has been observed to be on the decline in several parts of the country, although the Eastern and Volta Regions specifically Kwahu-East and Biakoye Districts currently serve as the major sources. These two districts are also well known for the cultivation of cash and food crops where the use of chemical pesticides is an indispensable main means of controlling crop pests and diseases (MOFA 1990; EPA 1999).

Although in Ghana there are regulations as to the use of pesticides including synthetic ones, pesticide residues are unavoidably found in our food crops and the environment (Afful et al., 2010). While some pesticides have short residual action; others such as the organochlorine pesticide (OCPs) have long residual action and therefore persist in the environment. It is therefore not surprisingly to detect residues of pesticides in the environment after usage. Some of these residues may be taken up by the root of plants including *M. whitei* which is chewed raw and also used as a major ingredient in the production of most gin bitters in Ghana.

OCPs are characterised by high persistence, diffusion in the environment, low polarity, low aqueous solubility, high lipid solubility (lipophilicity), bioaccumulation and biomagnification in the food chain (Lars, 2000; Fattore et al., 2002). The lipophilic nature of OCPs coupled with its low reactivity with respect to light and chemicals as well as its low biological degradation rates have led to OCPs accumulation in biological tissues and subsequent biomagnification in organisms progressing up the food chain (Baird, 1997; Fattore et al., 2002; Ritter et al., 2007). OCPs have adverse effects on wildlife and beneficial insects although it was favoured initially (Baird, 1997). OCPs have also been implicated in a broad range of adverse human health effects including reproductive failures, birth defects, immune system malfunction, endocrine disruptions and cancers (Hallberg, 1989; Garabrant et al., 1992; Baird, 1997). Although OCPs are classified as hazardous, farmers continue to use them because they are cheap and have broad-spectrum of activity (WHO, 1986; Gerken et al., 2000).

It is against this background that determination of OCPs residues in the root of *M. whitei* has become very relevant in updating data on OCP residue levels in medicinal plant parts used in traditional medicine in Ghana. Pesticide residue determination in medicinal plant parts used in traditional medicine ensures or guarantees the quality and safety of herbal medicines in terms of its pesticide residue contamination.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in the study were of high quality and of analytical grade. Hexane (99% +), Acetone (99.9% +) and Ethyl acetate (99.8% +) were bought from

Sigma-Aldrich, Ghana. Florisil adsorbent material (60-100 mesh) was purchased from Hopkin and William Limited, Ghana. The organochlorine pesticide standards used for the identification and quantification of OCPs residue were obtained from Dr. Ehrenstorfer GmbH of Augsburg, Germany. The internal standards used for the recovery experiment were obtained from United Nations Environmental Programme (UNEP) in sealed ampules.

Study area

The study was undertaken in two areas in Ghana, the Kwahu-East District in the Eastern Region and the Biakoye District in the Volta Region as shown in Figure 2. The criteria for the selection of the areas were the relatively high abundance and availability of the selected medicinal plant.

The sampling towns, Pepease and Abetifi in the Kwahu-East District lie between longitude 1° 0´ W and 0° 0´ and latitude 6° 0´ N and 7° 0´ N respectively as shown in Figure 2. In terms of climate, Kwahu East lies within the west semi-equatorial region. It experiences the double maxima rainfall pattern (major and minor raining seasons). The major raining season starts from April and ends in July while the minor raining season starts from September and end in October. Annual average rainfall is between 1580 and 1780 mm, with temperatures ranging between 26 and 30°C. The climate in the district coupled with the great irrigation potential of the Afram River favours the cultivation of food crops, fruits and vegetables. The soil belongs to the forest ochrosols.

The Biakoye District with its capital Nkonya Ahenkro also forms part of the districts and municipalities created in 2008. The sampling towns, Apesokubi and Worawora lie between longitude 0° 0´ and 1°0´ E and latitude 7° 0´ N and 8° 0´ N, respectively. The district falls within the wet equatorial zone and experiences a double maxima rainfall regime in May to July and September to November with peaks in June and October. The rainfall pattern averages between 1,250 to 1,750 mm per annum in the mountainous areas. The dry season is mostly manifested between December and February. The vegetation supports wildlife and the major animals found are monkeys, antelopes, bush pigs, pangolins, grass-cutters, chimpanzees and reptiles (www.ghanadistricts.com).

Sample collection

Sample collection and extraction were carried out according to the procedure described by Ansah and Gooderham (2002) and Therdteppitak and Yammeng (2003); while sample clean up and gas chromatography (GC) analysis of the pesticides was also carried out according to the procedure described by Darko et al. (2008) and Afful et al. (2010).

A total of 100 of each root samples of the herbal plant from each sampling site were collected during each season. The samples which were randomly collected were put into ice chests, well sealed and labelled with unique identity and then transported to the Plant Development Department of the Centre for Scientific Research into Plant Medicine (CSRPM) at Mampong Akuapem in Ghana for identification and classification.

Sample extraction

The fresh root samples were washed thoroughly with water, air dried for two weeks and milled. Approximately 10 g of the dried-powdered and homogenized samples were each transferred into an extraction thimble that had been pre-cleaned with n-hexane and acetone and oven dried. To each sample 5 µl of 0.5 ppm of internal standard (isodrin) was added to evaluate the recovery of the organochlorine compounds and soxhlet extracted with 160 ml of n-hexane/acetone (3:1) mixture for 12 h. Boiling chips were added to allow smooth boiling. The soxhlet was also monitored occasionally during the extraction period to ensure satisfactory recycling. Extracts as well as the blank were concentrated to about 20 ml using a rotary evaporator set at 40°C.

Sample cleanup

The florisil packed glass column which was ticked to allow the florisil to settle was conditioned by passing 12 ml n-hexane through the packed column. The sample was then transferred onto the florisil column and eluted three times each with 10 ml portions of n-hexane using Pasteur pipette. The eluate was collected into a round bottom flask with a ground-glass stopper and evaporated to dryness using a rotary evaporator fitted to a vacuum pump. The extract was recovered with 1 ml of ethyl acetate using Pasteur pipette and then transferred into glass vials for gas chromatograph analysis.

Gas chromatographic analysis

A Varian CP-3800 Gas Chromatograph (Varian Associates Inc. USA) equipped with ^{63}Ni electron capture detector was used for the analysis. A volume of 1 µl of the extracts was injected and the separation was achieved on a 40 m length fused silica gel capillary column coated with VF- 5 ms with internal diameter and film thickness of 0.25 mm and 0.25 µm, respectively. The carrier gas and make up gas was nitrogen at a flow rate of 1.0 and 29 ml/min, respectively. The injector and

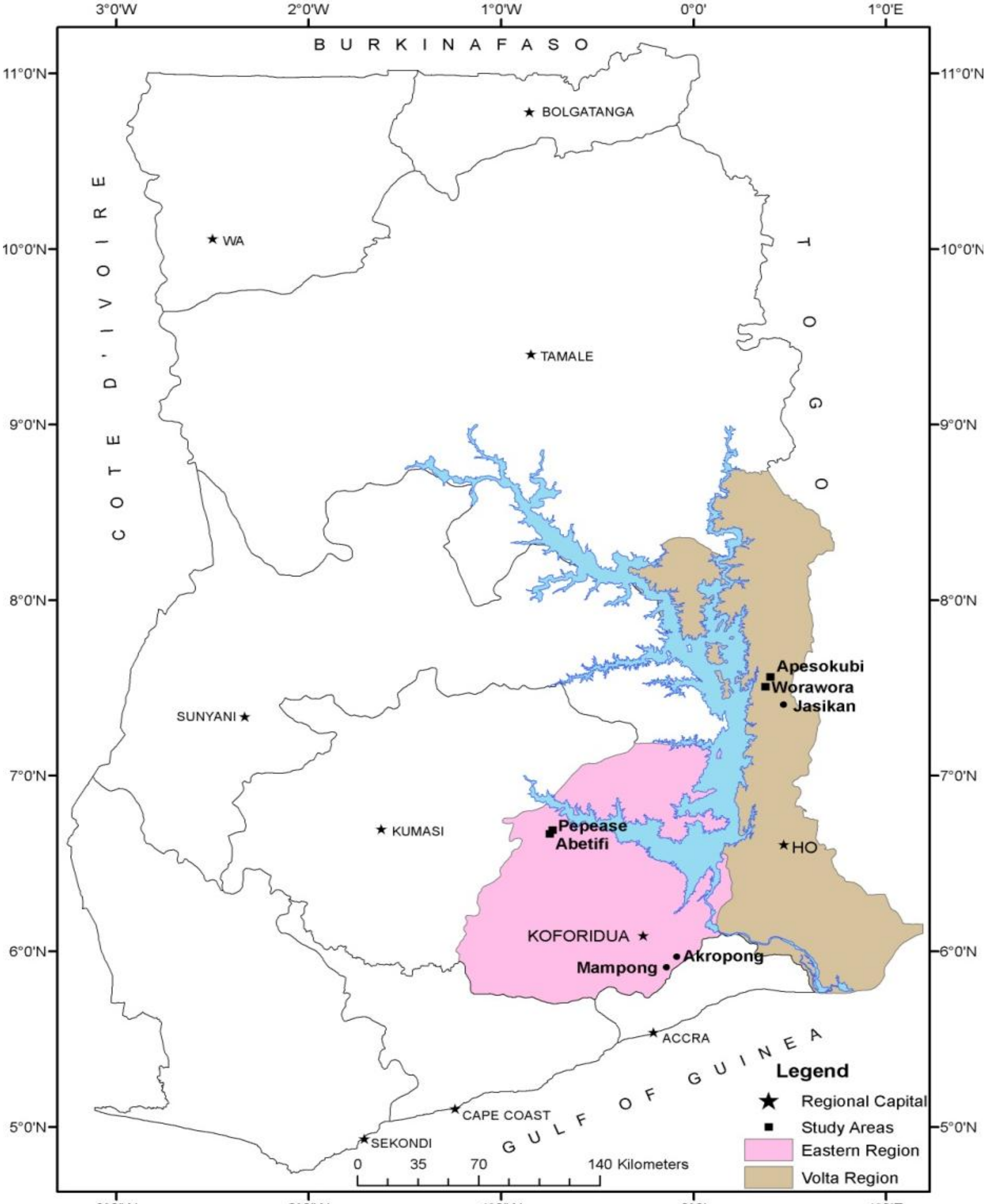

Figure 2. Map showing the study area and sampling site.

Table 1. Mean organochlorine pesticide residue in *M. whitei* (mg/kg) sampled from Kwahu-East and Biakoye Districts during dry and wet seasons.

Pesticide	Dry season				Rainy season			
	Biakoye Mn		Kwahu-East Mn		Biakoye Mn		Kwahu-East Mn	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Beta-HCH	0.010	0.005	0.023	0.011	0.001	0.002	0.001	0.001
Delta-HCH	0.066	0.023	0.068	0.063	nd		0.004	0.004
Gamma-HCH	0.082	0.021	0.066	0.013	0.001	0.001	0.005	0.004
Heptachlor	0.031	0.013	0.077	0.042	0.026	0.035	0.001	0.001
Aldrin	0.057	0.008	0.011	0.008	0.001	0.001	0.007	0.013
Gamma-chlordane	0.043	0.019	0.020	0.022	0.002	0.001	0.006	0.011
Alpha-endosulphan	0.010	0.007	0.029	0.007	0.001	0.001	0.005	0.007
Dieldrin	0.021	0.016	0.051	0.017	0.001	0.000	0.014	0.019
Endrin	0.015	0.005	0.020	0.011	0.001	0.000	nd	
Beta-endosulphan	0.077	0.015	0.055	0.013	nd		0.006	0.001
p,p'-DDE	0.023	0.010	0.032	0.021	0.002	0.001	0.002	0.001
p,p'-DDD	0.013	0.003	0.047	0.015	nd		nd	
P,P'-DDT	0.007	0.002	0.014	0.014	0.001	0.001	0.003	0.005
Methoxychlor	0.005	0.002	0.015	0.011	0.001	0.000	0.002	0.000
Total	0.46		0.528		0.038		0.056	

Mn, *Mondia whitei*; SD, standard deviation; nd, not detected.

detector temperatures were 270 and 300°C, respectively. The column oven temperature was programmed as follows: 80°C for 1 min to 180°C at 25°C/min up to 300°C at 5°C/min held for 1 min. Sample peaks were identified by their retention times compared to the corresponding retention times of the pesticide standards.

RESULTS AND DISCUSSION

The results for the types and levels of OCP residues in *M. whitei* collected from Kwahu-East and Biakoye Districts during the dry and rainy season are shown in Table 1.

Analysis of the pesticide samples revealed the presence of fourteen OCP residues. Detectable compounds were β-HCH, δ-HCH, γ-HCH, heptachlor, aldrin, γ-chlordane, α-endosulfan, p,p'-DDE, dieldrin, endrin, β-endosulfan, p,p'-DDD, p,p'-DDT and methoxychlor.

Spiked samples with 5 µl of 0.5 ppm of the internal standard were also determined with good recoveries. The recoveries of internal standard ranged between 75 and 113.5% for all the OCP analyzed.

Organochlorine pesticide residues contamination

The mean OCP residue concentrations in the samples collected in the dry season ranged from 0.005 to 0.082 mg/kg as shown in Table 1. The highest mean concentration of 0.082 mg/kg was obtained for γ-HCH in *M. whitei* samples collected from Biakoye District, while the lowest mean concentration of 0.005 mg/kg was recorded for methoxychlor in the samples from Biakoye District.

The heterogenic distribution pattern of carbon-hydrogen (H-C-H) isomers for all the samples analysed from the two districts during the dry and wet periods reflects the use of technical mixtures of HCHs. The high value recorded in the dry period also suggests isomerization of HCHs during the transformation process in the soil, owing to the action by microorganisms. This further suggested that lindane (γ-HCH) is being used extensively in the Ghanaian agricultural sector on vegetable cultivation and on food crop production in Ghana (Bempah and Donkor, 2010). The study also had a high mean concentration of lindane when compared with mean concentration of 0.02 µg/g detected in tomato by Bempah and Donkor (2010) and mean concentrations of 0.002 and 0.004 µg/g reported in tomato sampled from Nigeria and Indian markets (Adeyeye and Osibanjo, 1999; Bhanti and Taneja, 2005). This high mean value of lindane also supports the findings of pesticide use in Ghana by Awumbila and Bokuma (1994). This study revealed that 20 different pesticides were in use, with lindane being the most widely distributed and applied pesticides in Ghana. These were used on cocoa plantations, on vegetables farms and for the control of maize stem-borers. Lindane was marketed in Ghana as

Table 2. Ratio of dichlorodiphenyltrichloroethane (DDT) metabolites to parent dichlorodiphenyltrichloroethane (DDT) in the *M. whitei* samples collected from Kwahu-East and Biakoye Districts in the dry and wet seasons.

District	DDE/DDT (dry season)	DDE/DDT (wet season)	DDD/DDT (dry season)	DDD/DDT (wet season)
Biakoye	3.29	2.00	1.86	-- --
Kwahu-East	2.29	0.67	3.36	-- --

Gammalin 20 and until 2007 when its use was discontinued.

The high mean value of aldrin detected in the matrices sampled from Biakoye District during the dry period (Table 1) suggest less photodecomposition and less microbial degradation as well as less metabolism of the parent aldrin into dieldrin (ATSDR, 2002). The use of aldrin in Ghana was marketed under the trade name Aldrex 40 (Nollet, 2000). The mean values of endrin detected in the dried period (Table 1) may be as result of recent input into the environment. This also suggests less photodecomposition or less microbial degradation of endrin to endrin ketone and endrin aldehyde. The results also show endrin persistence in the environment (Bempah and Donkor, 2010).

Technical grade endosulfan products consist of a mixture of two isomers, α and β-endosulfan present in a 7:3 ratio. α-endosulfan is the more thermodynamically stable of the two, thus β-endosulfan irreversibly converts to form α-endosulfan. But in this study, the results suggests slow conversion. Endosulfan was considered for restriction in December 2008 from the registered pesticides in Ghana (Afful et al., 2010). This might have accounted for relatively high concentrations of β-endosulfan detected in the study.

The mean values of methoxychlor may be either as a result of historical use of DDT of which technically Methoxychlor contains about 88% of the p,p'-isomer (WHO, 1996) or recent DDT input into the environment. These values were lower than mean concentration of 0.03 µg/g detected in pawpaw by Bempah and Donkor (2010). The decrease perhaps, may be due to anaerobic biodegradation of methoxychlor which results mainly in dimethoxydiphenyldichloroethane (DMDD) as well as mono and dihydroxy or demethylated derivatives of methoxychlor (Nollet, 2000; Kuranchie-Mensah, 2009).

The study had high heptachlor mean concentrations of 0.026, 0.031 and 0.077 mg/kg, respectively as shown in (Table 1). These values were however higher than mean concentration of 0.01 µg/g detected in pawpaw by Bempah and Donkor (2010). Comparing the study with a similar one carried out in Romania where heptachlor concentration in coffee samples ranged from not detected to 0.011 mg/kg (Stanciu et al., 2008), then the medicinal plants in Ghana appear highly contaminated.

It is of interest to note that unlike the trend in the dry season, the wet season recorded comparatively low OCP mean residue concentrations in the samples investigated. This result could possibly be explained from the fact that during the wet season some of the pesticide residues might have been carried away from the soil through surface run-off. The mean OCP residues levels for all the samples investigated in the wet season ranged from not detected to 0.026 mg/kg, with the highest residue concentration of 0.026 mg/kg obtained for heptachlor in samples from Biakoye District as shown in (Table 1) below.

Ratio of DDT metabolites to parent DDT

The ratio of dichlorodiphenyldichloroethylene (DDE) or dichlorodiphenyldichloroethane (DDD) to dichlorodiphenyltrichloroethane (DDT) in the *M. whitei* samples analyzed were mostly greater than one. Generally the high ratio of DDE plus DDD to DDT mean values support the assumption that current DDT exposure levels primarily originate from previous contamination and environmental persistency (Ntow, 2005; Bempah and Donkor, 2010) as shown in Table 2. The high mean concentrations of p,p'- DDE and p,p'-DDD may be due to metabolic conversion and dehydrochlorination of p,p'- DDT. The high mean values also suggest the exposure of the matrices to intense sunlight experienced during the dry season and therefore possible conversions and isomerisation of p,p´- DDT by solar radiation to p,p'- DDE and p,p'-DDD (Barriada-Pereira et al., 2005) as in Table 2. DDE is generally more persistent in the environment than DDT, as a result of this, when the input levels of DDT in the environment ceases, the levels of its metabolite DDE will be higher than the parent DDT (Ntow, 2001, 2005).

Generally, the mean levels of the OCP residues in the plant species were below maximum residue limits set by FAO/WHO Codex Alimentarius Commission and United States/European Pharmacopoeia (WHO, 2007) as shown in Table 3. However, in the dry season, residue levels obtained for heptachlor in *M. whitei* from Kwahu-East District as well as residue levels obtained for the sum of aldrin and dieldrin in *M. whitei* from Biakoye and Kwahu-East Districts were higher than WHO and FAO set limits as shown in Table 3.

Conclusion

The results of this work indicate that OCP residues were

Table 3. Comparison of levels of organochlorine pesticide residue in plants collected in the dry and wet seasons with Codex Alimentarius Commission and United States Pharmacopoeia/European Pharmacopoeia extraneous residue limit.

Pesticide	Mean organochlorine pesticide residue in *M. whitei* (mg/kg)				Standard limits in mg/kg	
	Dry season		Wet season			
	Kwahu-East	Biakoye	Kwahu-East	Biakoye	Ph. Eur and USP	Codex Alimentarius Commission
Beta-HCH	0.023	0.010	0.001	0.001	++0.300	
Delta-HCH	0.068	0.066	0.004	nd	++0.300	
Gamma-HCH	0.066	0.082	0.005	0.001	0.600	
Heptachlor	0.077	0.031	0.001	0.026	+0.050	
Aldrin	0.011	0.057	0.007	0.001	+0.050	
Gamma-chlordane	0.020	0.043	0.006	0.002	+0.050	+0.500
Alpha-endosulphan	0.029	0.010	0.005	0.001	+3.000	
p,p'-DDE	0.032	0.023	0.002	0.002	+1.000	
Dieldrin	0.051	0.021	0.014	0.001	+0.050	
Endrin	0.020	0.015	nd	0.001	0.050	
Beta-endosulphan	0.055	0.077	0.006	nd	+3.000	+0.500
p,p'-DDD	0.047	0.013	nd	nd	+1.000	
P,P'-DDT	0.014	0.007	0.003	0.001	+1.000	
Methoxychlor	0.015	0.005	0.002	0.001	-	

USP, United States Pharmacopoeia; Ph. Eur, European Pharmacopoeia; +, sum of isomers and metabolites; ++, sum of isomers other than Gamma-HCH.

present in the matrices sampled from the two districts during the wet and dry season. The detection of fourteen OCP of which seven were among the banned pesticides of the Environmental Protection Agency (EPA) of Ghana indicates either wide use of these chemicals or environmental transport of these chemicals from other places. These banned organochlorines detected include; aldrin, dieldrin, endrin, heptachlor, lindane, DDT and gamma-chlordane (EPA, 1999; Afful et al., 2010).

DDT has been banned from agricultural use and restricted for public health purposes under the Stockholm convention in which Ghana is a signatory. The present investigation therefore confirms the banned of DDT from agriculture purposes in Ghana. Long term persistence in the environment of this pesticide and its metabolites has been reported in various publications. The work, thus, seeks to provide information on levels of pesticide residues in medicinal plant parts used in traditional medicine in Ghana that will assist in a scientific assessment of the impact of pesticides on public health and the environment in general.

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