



Organochlorine pesticides in soils from south-western Uganda

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ABSTRACT

Organochlorine (OC) residues were analysed in soil samples collected from Kihiihi sub-county, Kanungu District, which is located in south-western Uganda. Mabira Central Forest Reserve which is located in central Uganda was used as a reference site in this study. The samples were collected at 15–20 cm depths below the soil surface and extracted with organic solvents. The extracts were cleaned using florisil, and were analysed using a gas chromatograph (GC) equipped with an electron capture detector (ECD). The results were confirmed using a GC equipped with a mass spectrometer (MS). The levels of the OC residues in soil from Kihiihi sub-county varied from non-detectable (ND) to 59 $\mu\text{g kg}^{-1}$ dry weight. The frequencies of detection for *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD in the total soil samples were 47%, 24% and 11%, respectively. The *o,p'* compounds were detected in the following frequencies: *o,p'*-DDT (23%), *o,p'*-DDE (19%) and *o,p'*-DDD (8%). The low *p,p'*-DDE/*p,p'*-DDT residue ratios (0.2–0.9) in Kihiihi samples suggest recent inputs of DDT in Kihiihi sub-county. The detection frequencies for dieldrin, endosulfan- α , - β and -sulphate in the soil samples were 21%, 26%, 31% and 19%, respectively. Mabira Forest Reserve soils showed detection frequencies of *p,p'*-DDT (5%) and *p,p'*-DDE (9% of the soil samples), varying from ND to 9 $\mu\text{g kg}^{-1}$. Although the use of OCPs has been banned, our results show that they can still be detected in the environment. Their presence may be attributed to adulteration of pesticides which are not banned and also atmospheric deposition.

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1. Introduction

Organochlorine pesticides (OCPs) were used from the 1940s in large quantities, but were banned in many countries in the 1970s because of their persistence in the environment (Shigeyuki et al., 2002). OCPs are semi-volatile and, therefore, can be transported over long trans-boundary distances via air and ocean currents, and have been detected in the Antarctic and other areas far from sites of use (Bouwman, 2004). OCPs are harmful to both humans and the environment (Hunter et al., 1997). Adverse health effects including reproductive failures, tumor induction, endocrine disruption and cancers can occur once living organisms are exposed to OCPs (Makris and Rowe, 1998). These chemicals pose a serious risk to the health, especially for infants in whom enzymatic and metabolic systems are not fully active (Garry, 2004). With regard to exposure to OCPs in early pregnancy, several epidemiological studies suggest that maternal employment in agriculture may be a risk factor for birth defects (Nurminen, 1995; Weidner et al., 1998; Engel et al., 2000).

The use of OCPs in Uganda dates back to the 1950s when they were first used in public health programmes and agriculture. DDT was widely used until the mid 1980s for pest control on crops, especially cotton, and in the control of tsetse flies and malaria-carrying mosquitoes (Kasozi et al., 2006). According to Aryamanya-Mugisha (1993), about 390 metric tonnes of dieldrin were used to control termites and cotton-ball worms per year, while an additional 30 tonnes of dieldrin were used for ground spraying and selective treatment of tree trunks for tsetse control in the country.

By 1973, the use of OCPs had been banned in most of the developed world, owing to pest resistance and increasing environmental concerns. However, in Uganda recent studies have revealed their presence in environmental samples such as fish (Ssebugere et al., 2009) and lake sediments (Wasswa, 2009). It is possible that the banned pesticides are secretly included in plant protection agents or in anti-mosquito chemicals to make them more efficient or they may be sold under other trade names. Although there have been studies aimed at determining the levels of OCPs in some parts of Uganda, only two studies have reported their levels in the south-western part of the country (Bimenya et al., 2007; Ssebugere et al., 2009). Yet indoor spray of DDT was carried out in this area, specifically in Kihiihi sub-county between the late 1950s and early 1960s. The study by Bimenya et al. (2007) reported residues of

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DDT/DDE in soils from Kihiihi, but their results were controversial firstly because their method of detection did not discriminate between DDT and its breakdown products. Secondly, the results were obtained using ELISA assay kits, and were not confirmed by any alternative method of analysis. The present study was aimed at confirming the presence of OCPs in soils from Kihiihi sub-county using alternative analytical methods.

2. Materials and methods

2.1. Study stations

The first study station was Kihiihi (00°57'27"S, 29°47'23"E), a sub-county located in Kinkizi county, Kanungu District, in south-western Uganda. Five sites (A, B, C, D and E) were located for collection of soil samples and were selected with the assistance of the residents based on areas where the sprayed homesteads were situated in the 1950s and 1960s. The sites were at a distance of approximately 700 m from one another (Fig. 1). The second study station was Mabira Forest Reserve (01°14'20.40"S, 30°56'42.00"E) and the sampling sites were 1, 2 and 3 (Fig. 2). Mabira has been gazetted as a Forest Reserve since 1932. It is one of Uganda's largest surviving Natural Forests, covering an area of 306 km² and located between Kampala and Jinja in Mukono District. The Forest was used as a reference in this study because the area, especially the soil, was considered to be in pristine condition with respect to

exposure to pesticides that have been used in malaria control and in the farming system.

2.2. Sampling

A total of 105 and 63 loamy soil samples were collected from Kihiihi sub-county and Mabira Forest, respectively. At each sampling site, 21 soil samples were randomly taken at approximately 25 m from one another. The samples were taken using an auger (tube sampler). Distilled water was used to clean the auger each time before the next sampling. Sampling was focused on top soil at a depth of 15–20 cm because such soils are expected to be the most contaminated, ecologically relevant and have the greatest potential for exchange of residues with plants. To avoid cross contamination, 5 cm length of the soil profile was taken from the lower end of the auger using a marked stick. The soil sample (200 g) was wrapped in aluminium foil, transferred into a plastic bag with a zip lock, labeled with a permanent marker and placed into a clean dry plastic bucket. The samples were then transported to the laboratory and kept in a freezer maintained at –18 °C till extraction.

2.3. Pesticide reference standards, solvents, reagents and glassware

Thirteen pesticide reference standards (*p,p'*-DDT, *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, dieldrin, aldrin, endrin, lindane, endosulfan- α , - β and -sulphate) were obtained from Dr. Ehrenstor-

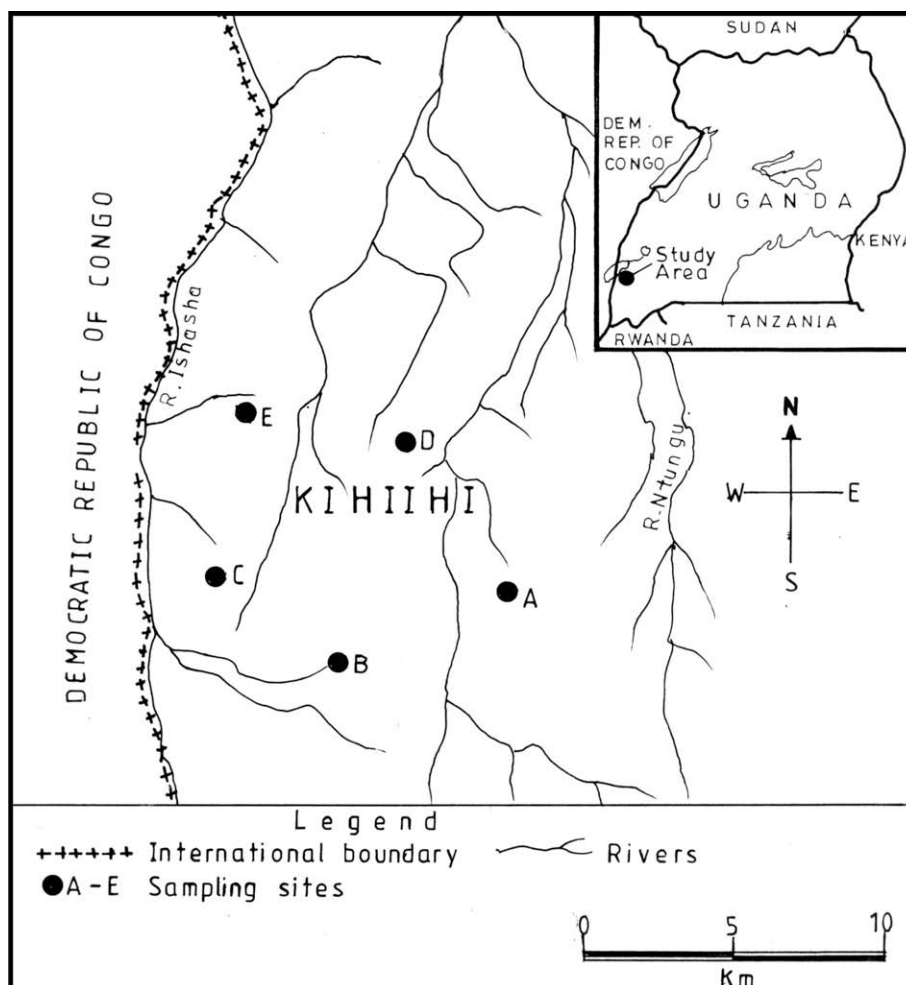


Fig. 1. Map showing the sampling sites in Kihiihi sub-county.

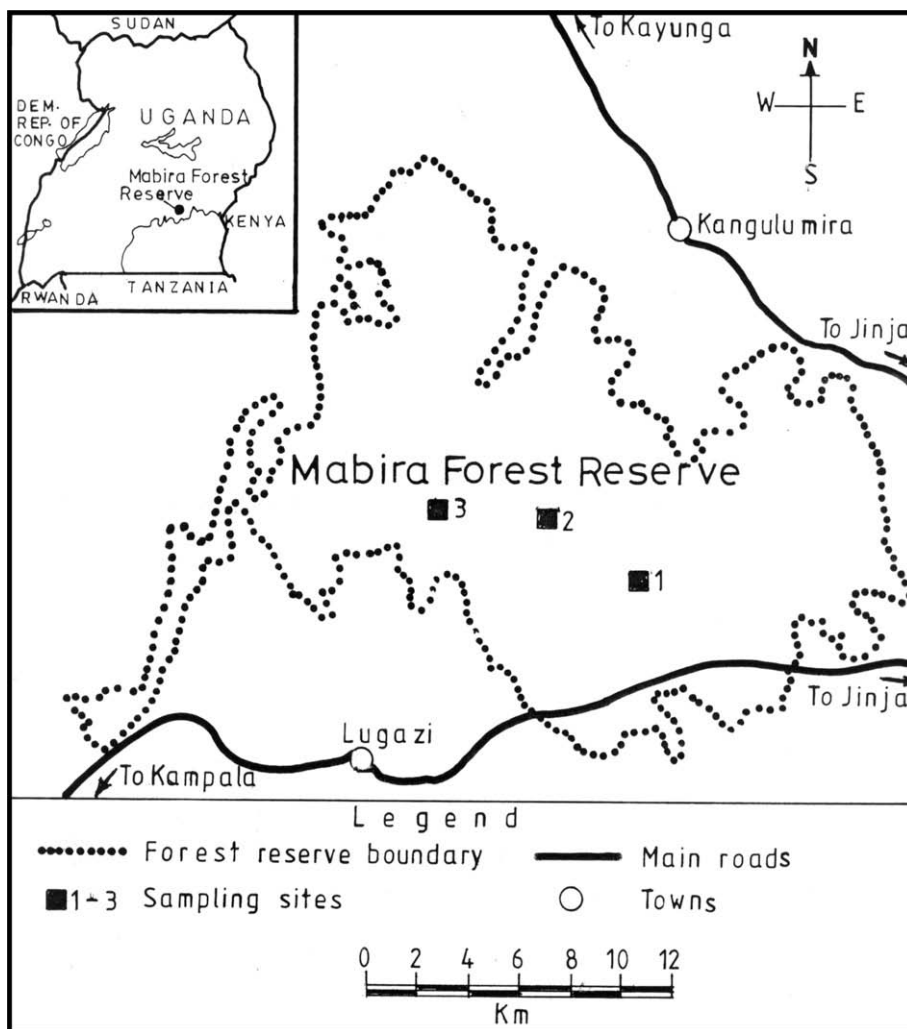


Fig. 2. Location of the sampling sites in Mabira Forest Reserve.

fer GmbH (Augsburg, Germany). All the standards were above 99% purity. The analytical stock solutions of each pesticide were prepared using *n*-hexane and stored in amber flasks maintained at -18°C . Ethyl acetate, acetone, cyclohexane and *n*-hexane were of pesticide residue grade. Florisil (PR grade 60–100 mesh), ammonium chloride, anhydrous sodium chloride and anhydrous sodium sulphate were of analytical grade (BDH, England). All glassware was cleaned by first soaking it for 2 h in tap water mixed with a detergent and rinsed with hot water followed by acetone. The glassware was then dried in an oven for 4 h at 105°C .

2.4. Soil extraction procedure

The soil was extracted, following the method of Åkerblom (1995). A sample (20 g) was put into a 250 mL Erlenmeyer flask (E-flask). Ammonium chloride solution (0.2 M; 14 mL) was added; the mixture was swirled and then left to stand for 15 min. Cyclohexane/acetone (100 mL; 1:1 v/v) solvent mixture was added and the flask securely stoppered. The whole mixture was vigorously shaken for 1 min and then less vigorously so every 10 min for at least 1 h. The system was left to stand at 25°C overnight and subsequently shaken intermittently for another 2 h. The contents were allowed to settle and distilled water cautiously added until the upper organic layer reached the neck of the flask. Saturated sodium chloride solution (50 mL) was added, and the mixture shaken for 1 min to hasten complete separation of the layers. The upper or-

ganic layer was decanted into a fresh flask containing anhydrous sodium sulphate (20 g). The E-flask was swirled and left to stand for 15 min, and then re-swirled to ensure the free-flowing of the salt. The contents in the flask were allowed to pass through a plug of glass wool into an evaporation flask. The extract was concentrated to 1 mL on a rotary evaporator at 30°C and dissolved in cyclohexane (2 mL) for clean up.

2.5. Florisil clean up technique

A glass column (4 mm i.d. \times 15 cm) was plugged with glass wool previously washed with cyclohexane. It was then packed with florisil (2 g) followed by anhydrous sodium sulphate (10 g). The column was rinsed with acetone/hexane (2 mL; 1:9 v/v) and hexane (5 mL) to remove any impurities (Kegley and Laura, 1998). The concentrated soil extract was then added, eluted with the acetone/hexane mixture (10 mL), followed by hexane (10 mL). The eluate was further concentrated to near-dryness on a rotary evaporator at 40°C and reconstituted in hexane (2 mL) for GC analysis.

2.6. Gas chromatographic analysis

Organochlorine residues were analysed using a Varian (CP-3800, Palo Alto, CA, USA) GC-ECD fitted with both semi-polar (CP-Sil 19 CB, J & W Scientific, Folsom, CA, USA), and non-polar

(CP-Sil 5 CB, J & W Scientific, Folsom, CA, USA) fused-silica capillary columns of 30 m × 0.25 mm i.d. × 0.25 μm film thickness. The column temperature was programmed as follows: 90 °C for 1 min, 30 °C min⁻¹ to 180 °C, 4 °C min⁻¹ to 260 °C, and maintained at this temperature for 16 min. Hydrogen (99.99% purity) with a flow rate electronically set at 1.2 mL min⁻¹ was used as the carrier gas. Other GC operating conditions were: 230 °C and 300 °C injector and detector temperatures, respectively, together with a 30 mL min⁻¹ make-up nitrogen gas flow. A Turbochrom (Perkin-Elmer Corporation, 1989–1995, Norwalk, CT, USA) 4.0 chromatography station was used for chromatographic data processing. The GC was operated in a splitless mode and the injection volume was 1 μL for each injection. Reference standards of individual pesticides were used to identify and quantify the residues.

An Agilent 6890N GC–MS, USA version with an HP-5MS fused-silica capillary column 30 m × 0.25 mm i.d. × 0.25 μm was used for confirmation of the results. The MS used was equipped with a selective mass detector (Agilent 5975 inert XL Quadrupole, Palo Alto, CA, USA), initially set at 90 °C, held for 1 min and then increased at a rate of 30 °C min⁻¹ to 180 °C. The temperature was further increased at a rate of 4 °C min⁻¹ to 280 °C and kept there for 15 min. The injector temperature was 250 °C and the detector was maintained at 200 °C. Helium was used as the carrier gas at a flow rate of 1.0 mL min⁻¹. The GC–MS was operated in a splitless mode with a purge-off of 1 min and the injection volume was 1 μL for each injection. The MS solvent delay time was 3.57 min and the scanned mass range was 50–550 m/z. The full scan ion monitoring mode was used for the determination of the OCPs. The analytes were identified using the internal standards method. Data acquisition and processing was achieved using GC–MSD Chemstation Software (G1701dad.02.Osp1, JAS CWA, USA). After confirmation, quantitation was done using the Turbochrom chromatography station. The mean residue levels and their associated standard deviations were calculated from only those samples for which the OCPs were positively quantified.

2.7. Detection limits and recovery experiments

The detection limits (DL), in μg kg⁻¹, calculated by a signal to noise ratio of three were *p,p'*-DDT (0.05), *o,p'*-DDT (0.06), *p,p'*-DDE (0.40), *o,p'*-DDE (0.05), *p,p'*-DDD (0.07), *o,p'*-DDD (0.08), aldrin (0.06), dieldrin (0.10), lindane (0.10), endosulfan-α (0.20), endosulfan-β (0.05) and endosulfan-sulphate (0.05). Samples were considered positive when their residue levels were ≥DL. Blank samples were spiked with 0.2 and 0.6 μg kg⁻¹ of individual OCP solutions to soil that had been pre-analysed and found to have no detectable residue levels. The recovery assays were replicated three times. The recovery ranges (in%) at 0.2 μg kg⁻¹ spiking level were 75–80, 73–82, 67–78, 59–71, 72–76, 69–74, 52–59, 75–84, 67–74, 69–85, 74–79 and 79–89 for *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, aldrin, dieldrin, lindane, endosulfan-α, -β and -sulphate, respectively. At the 0.6 μg kg⁻¹ spiking level the recovery ranges (in%) were 80–86, 83–93, 79–87, 69–74, 78–95, 75–89, 86–89, 84–91, 71–76, 86–90, 78–84 and 82–92, respectively, for the pesticides above. Generally, better recoveries (69–95%) were obtained at the 0.6 than 0.2 μg kg⁻¹ spiking level (52–89%). Consequently, no adjustment was made in the residue data since majority of the recoveries were ≥70%.

3. Results and discussion

3.1. Levels of OC residues in soil samples

Table 1 indicates that the *p,p'* isomer of DDT and its metabolites were present in higher levels than the associated *o,p'* compounds

in all the sampling sites in Kihiihi sub-county. The *p,p'*-DDT residues were detected in levels ranging from ND–59 μg kg⁻¹ dry weight (d.w.) in 47% of the total soil samples. The *p,p'*-DDE metabolite was detected in 24% frequency, with levels ND–46, and *p,p'*-DDD in 11% with levels ≤14 μg kg⁻¹. The high detection frequencies and residue values of *p,p'*-DDT compared to its metabolites (*p,p'*-DDE and *p,p'*-DDD) suggest a recent input of technical DDT. According to Strandberg and Hites (2001) a high DDE/DDT ratio indicates past input of DDT. Low DDE/DDT ratios, especially when <1, indicate recent input (Gonzalez et al., 2003). The low *p,p'*-DDE/*p,p'*-DDT residue ratios (0.2–0.9) in the Kihiihi soil samples suggest recent input of DDT in the area. This is in contrast to the findings of Bimenya et al. (2007) which reported past inputs of DDT. In addition, the latter authors reported DDT/DDE residues in soil from Kihiihi sub-county as 7.28 μg kg⁻¹, but their results could not be compared with those of the present study as they did not distinguish among DDT and its metabolites.

In Kihiihi study station, the levels of *o,p'*-DDT were higher than those of *o,p'*-DDE and *o,p'*-DDD in all the sampling locations. The detection frequencies were *o,p'*-DDT (23%), *o,p'*-DDE (19%) and *o,p'*-DDD (8%) in all the samples. The residue levels of *p,p'*-DDD at site C (Fig. 1) were in the range 3–14, with a mean of 7 ± 4 and median value of 6 μg kg⁻¹. The detection of DDD suggests a transformation process taking place on DDT, presumably due to aerobic or anaerobic degradation. In the environment, living organisms metabolise DDT to DDE and DDD via different pathways. The DDE metabolite is formed by dehydrochlorination of DDT in mammalian species, insects and micro-organisms (Fox et al., 1998). On the other hand, DDD is the product of reductive dechlorination in mammals, birds and insects (Hassau, 1972). DDT and DDE are further oxidised to DDA, the major excreted metabolite in animals (Gold and Brunk, 1982).

Generally, sampling site E showed high mean residue levels of DDT and its metabolites compared to the other four sites (Table 1). This could be due to the fact that it is located in a valley and that surface run-off could have deposited residues at E from areas where it had been used in agriculture and vector control. The close proximity of neighbouring tea farms seems to support this assumption. Site D exhibited lower residue levels compared to all the other sampling sites, which suggested relatively minor deposition of DDT formulations in the area. Similar studies have also reported detection of DDT and metabolite residues in soil. In India, Dua et al. (1996) reported mean concentration of 3680 μg kg⁻¹ DDT in soil from Bahadrabad District. The residue levels in Bahadrabad were over 60 times higher than those in the present study. Kishimba et al. (2003) documented levels of up to 282,000,000 μg kg⁻¹ d.w. total DDT in top soil at Vikuge, a former storage site for pesticides. The results of the above researchers further showed residues of up to 500,000 μg kg⁻¹ in agricultural areas with large-scale pesticide use (Dar es Salaam coast, Mahonda–Makoba basin in Zanzibar, Sugar Plantations in Kilimanjaro region, Southern Lake Victoria and its basin). The levels in the two reported investigations are notably higher than those in the present study. In China, Gong et al. (2004) reported concentrations of *p,p'*-DDT and *p,p'*-DDE as 27.5 and 18.8 μg kg⁻¹, respectively. The results by Gong et al. (2004) further indicated that *p,p'*-DDT and *p,p'*-DDE were the predominant contaminant compounds in the surface soil samples. The findings are similar to those in the present study.

Soils from site 2 at the Mabira Forest Reserve showed frequencies of *p,p'*-DDT and *p,p'*-DDE of 5% and 9%, respectively, which exhibited residue levels between 2 and 9 μg kg⁻¹. The residue ratio of *p,p'*-DDE/*p,p'*-DDT in Mabira was 0.4. The presence of *p,p'*-DDT and *p,p'*-DDE residues in Mabira, the reference of this study, may be attributed to atmospheric transport from other places where it had been used (Ssebugere, 2008), probably from Lugazi tea estates located not far from the natural forest.

Table 1
Means, medians and ranges of DDT and its metabolites in soils from Kihiihi and Mabira Forest.

Compound	Kihiihi sampling sites					Mabira sampling sites		
	A	B	C	D	E	1	2	3
<i>p,p'</i> -DDT	20 ± 9	17 ± 6	18 ± 5	10 ± 7	46 ± 10	ND	6 ± 3	ND
Median	17	18	18	10	49	–	6	–
Range	7–34	10–23	11–25	3–18	32–59	–	3–9	–
<i>o,p'</i> -DDT	16 ± 4	32 ± 7	16 ± 5	ND	20 ± 5	ND	ND	ND
Median	17	33	15	–	19	–	–	–
Range	11–20	24–38	12–18	–	14–27	–	–	–
<i>p,p'</i> -DDE	5 ± 3	14 ± 6	9 ± 3	4 ± 2	33 ± 10	ND	3 ± 1	ND
Median	5	17	7	3	35	–	4	–
Range	2–10	9–20	6–13	2–7	19–46	–	2–4	–
<i>o,p'</i> -DDE	9 ± 4	11 ± 3	10 ± 4	ND	19 ± 5	ND	ND	ND
Median	8	12	10	–	21	–	–	–
Range	6–14	8–16	6–15	–	14–23	–	–	–
<i>p,p'</i> -DDD	ND	ND	7 ± 4	ND	ND	ND	ND	ND
Median	–	–	6	–	–	–	–	–
Range	–	–	3–14	–	–	–	–	–
<i>o,p'</i> -DDD	ND	7 ± 4	5 ± 3	ND	ND	ND	ND	ND
Median	–	6	6	–	–	–	–	–
Range	–	4–13	2–7	–	–	–	–	–
Total <i>p,p'</i> -DDE/total <i>p,p'</i> -DDT	0.2	0.9	0.5	0.5	0.7	–	0.4	–

The residue levels are expressed in $\mu\text{g kg}^{-1}$ dry weight. Results are presented as mean values of positive quantifiable samples only \pm standard deviation; ND – non-detectable.

Table 2
Means, medians and ranges of OC residues ($\mu\text{g kg}^{-1}$ d.w.) in soils from Kihiihi and Mabira Forest.

Compound	Kihiihi sampling sites					Mabira sampling sites		
	A	B	C	D	E	1	2	3
Dieldrin	ND	ND	ND	ND	4 ± 2	ND	ND	ND
Median	–	–	–	–	4	–	–	–
Range	–	–	–	–	2–6	–	–	–
Endosulfan- α	6 ± 3	13 ± 4	5 ± 2	7 ± 2	5 ± 2	ND	ND	ND
Median	6	12	5	6	6	–	–	–
Range	4–8	10–16	4–6	5–9	3–7	–	–	–
Endosulfan- β	8 ± 2	17 ± 3	ND	9 ± 3	12 ± 4	ND	ND	3 ± 1
Median	8	16	–	10	11	–	–	3
Range	5–10	14–20	–	6–11	10–15	–	–	2–4
Endosulfan-sulphate	4 ± 2	ND	3 ± 2	6 ± 3	ND	ND	ND	ND
Median	4	–	3	6	–	–	–	–
Range	3–5	–	2–5	4–7	–	–	–	–
Endosulfan- α /endosulfan- β	0.5	0.8	–	0.7	0.4	–	–	–

Means are calculated from positive quantifiable samples only. ND – non-detectable.

In addition to DDT and its various metabolites, pesticides such as aldrin, dieldrin, lindane, endosulfan- α , - β and -sulphate that have not been previously reported in Kihiihi soils were also identified (Table 2). The detection frequencies for endosulfan- α , - β and -sulphate were 28%, 37% and 19%, respectively. The mean levels of endosulfan were relatively similar at all sites except B, which had a moderately higher mean value of $17 \pm 3 \mu\text{g kg}^{-1}$. The median values of endosulfan are shown in Table 2. The endosulfan- α and - β in the analysed samples were found to be more dominant than the sulphate with the α/β ratio in the range 0.4–0.8, which indicates recent input of the pesticide (Nowak et al., 1995). The variation in ratios at the different sites could be attributed to differences in soil texture, pH, application techniques, dose rate or the presence of sensitive biological species. In a related study on analysis of soil samples from a village in Kasargode District, Kerala, India, concentrations of total endosulfan residues in the range $<1\text{--}10 \mu\text{g kg}^{-1}$ were reported (Atmakuru and Ambalatharasu, 2002). The results of the current study are in agreement with the ones reported in the above study.

Dieldrin was only detected in 21% of the soil samples at site E with a mean concentration of 4 ± 2 and a range of $2\text{--}6 \mu\text{g kg}^{-1}$. The detection of dieldrin reflects exposure to either aldrin or dieldrin, since dieldrin is a metabolite of aldrin (Hayes, 1976). Sunlight and bacteria alter aldrin to dieldrin, which degrades slowly in the soil (ATSDR, 2002). In a study carried out in neighbouring Kenya, Getenga et al. (2004) reported residues of aldrin and dieldrin as 4088 and $3512 \mu\text{g kg}^{-1}$ in the soil from the different fields in the sugar belt along the Lake Victoria basin. In our study no such residues were detected.

4. Conclusions

The results of this study have shown that OCPs banned in the country may still be in use in Kihiihi sub-county and its surroundings as they show considerable concentrations in the soil. The levels of OCPs in soil samples from Kihiihi varied from ND to $59 \mu\text{g kg}^{-1}$. Soils from Mabira Forest Reserve showed residues of

the *p,p'* isomer (*p,p'*-DDT, *p,p'*-DDE) and endosulfan- β up to $9 \mu\text{g kg}^{-1}$. In spite of the low residue levels, the presence of OCPs in the soil is of concern, because they may be absorbed by plant roots and later consumed by living organisms, especially human beings. Indeed, Bimenya et al. (2007) reported DDT/DDE in various food crops. In view of the fact that the use of OCPs has been banned, there is a possibility that pesticides imported for use may be adulterated.

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