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Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b;Tristan, 1993,1995), (Kumasi et al., 2001)

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Ansell J, Hirsh J, Poller L (2004). The pharmacology and management of the vitamin K antagonists: the Seventh ACCP Conference on Antithrombotic and Thrombolytic. Therapy. 126:204-233

Ansell JE, Buttaro ML, Thomas VO (1997). Consensus guidelines for coordinated outpatient oral anti coagulation therapy management. Ann. Pharmacother. 31:604-615

Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for *Striga* suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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Full Length Research Paper

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its derivatives in marketed *Clarius wernerii* caught from Uganda's major urban wetlands

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The presence of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and derivatives in the *Clarius wernerii* of Uganda's major urban wetland ecosystems was investigated. Solid dispersion extraction method for extraction, florisil column method for clean-up, gas chromatograph equipped with an electron capture detector (GC-ECD) for analysis and gas chromatograph equipped with mass spectrophotometer (GC-MS) for confirmation of results were used in this study. The major DDT contaminants detected in the samples were *p,p'*-DDD, *p,p'*-DDE and *p,p'*-DDT which were found in 25, 22 and 21% of the samples, respectively. *o,p'*-DDD was detected in 19% and *o,p'*-DDT in 13% of the samples. For *o,p'*-DDE there were no measurable values since the levels were below limit of quantitation (LOQ). The concentrations of DDT derivatives ranged between ND-0.478 µg/kg for *p,p'*-DDE, ND-0.387 µg/kg for *o,p'*-DDD, ND-0.476 µg/kg for *p,p'*-DDD, ND-0.345 µg/kg *o,p'*-DDT and ND-0.556 µg/kg for *p,p'*-DDT. The concentration of total DDT in *C. wernerii* was in the range of 1.111 to 1.328 µg/kg dry wt. Generally, all the samples had DDT derivative levels below the maximum residue limit recommended by Food and Agriculture Organisation/World Health Organization (FAO/WHO) Codex Alimentarius Commission.

Key words: 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), DDT derivatives, *Clarius wernerii*, Uganda, wetlands.

INTRODUCTION

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) is a broad-spectrum insecticide that was once widely used in many countries in 1940's to control insects on agricultural crops and insects that carry diseases like malaria and typhus (Coulston, 1985), but was banned in the 1970's

because of its persistence in the environment (Shigeyuki et al., 2002). It is now used in only a few countries to control malaria (Agency for Toxic Substances and Disease Registry (ATSDR), 2002). DDT derivatives have entered all compartments of the world ecosystems due to

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their mobility, environmental stability and affinity for biological materials (ATSDR, 2002). When DDT enters the environment, most of it breaks down slowly into dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) generally by the action of microorganisms (ATSDR, 2002; Guilherme et al., 2000). DDE, the principle derivative of DDT generally resists further chemical and biological degradation (Guilherme et al., 2000). This phenomenon is usually used as an indicator for the time lapse of DDT usage (Montgomery, 2000). DDD and DDT are further oxidized to DDA, the major excreted derivative in animals (Gold and Brunk, 1982; Wallecave et al., 1974).

In Uganda, DDT had been heavily used for agricultural and public health purposes starting in the 1950's through the 1980's (Mitemi and Gitau, 1990). Although DDT was banned for agricultural and public health purposes in Uganda in the mid 1980's, its derivatives have been detected in sediments (Wasswa, 2009), water (Kasozi et al., 2006) and fish (Bimenya et al., 2007; Kasozi et al., 2006; Kyarimpa, 2007; Ogwok et al., 2009; Sebugere et al., 2008) from various aquatic ecosystems. These derivatives are still slowly being released into aquatic and terrestrial food chains and can reach significant concentrations in animals at higher trophic levels. The lipophilic nature, hydrophobicity and low chemical and biological degradation rates of DDT derivatives have led to their accumulation in biological tissues and subsequent magnification of concentrations in organisms progressing up the food chain (Ayas, 2007; Feng et al., 2003; Guzzella et al., 2005). DDT, DDE and DDD are believed to be possible carcinogens (ATSDR, 2000). DDT affects stages of neurological development (Ericksson et al., 1992; Rogan and Chen, 2005) and has been accused of being a potential endocrine disruptor (Frigo et al., 2002). DDE levels in the blood of pregnant women increase the chances of having a pre-term baby (ATSDR, 2002), also hyporeflexia in infants has been associated with DDE levels as low as 4 mg/kg milk fat in breast milk (Rogan and Chen, 2005).

Since the late 1980s, Kampala city has developed rapidly. People from the countryside have migrated to the city and its suburbs to find work and livelihood based on trade and industry. This has caused the rapid population rise in these areas during the last three decades. There is increasing pollution load in urban wetland ecosystems due to population pressure (Rusongoza, 2003). Nakivubo and Lubigi channels are important pathways for pollutant transport into the urban wetland ecosystems. An important threat to these aquatic ecosystems is contamination by DDT derivatives, both directly and indirectly by rivers that feed them. Considering the duration and intensity of the application of DDT in Uganda, the richness of the country in wetlands and the biodiversity in these wetlands, studies on DDT and its derivatives are essential in these systems. Previous studies have collected data on DDT derivatives for various fish species

(Bimenya et al., 2007; Kasozi et al., 2006; Kyarimpa, 2007; Sebugere et al., 2008) from different aquatic ecosystems in Uganda. Substantial work has also been carried out on heavy metal contamination in urban wetlands, particularly in Nakivubo (Muwanga, 2006; Nabulo, 2008; Sekabira, 2010). Earlier studies on benthic feeders focused on *Clarias gariepinus* from Lake Edward (Bimenya et al., 2007; Sebugere et al., 2008) and Lake Victoria (Henry and Kishimba, 2006; Kasozi et al., 2006; Kyarimpa, 2007). Nonetheless, there is a lack of data pertaining to DDT derivatives in *Clarius wernerii* from wetland ecosystems. The present study identifies and quantifies these derivatives in marketed *C. wernerii* caught from Nakivubo and Lubigi wetlands. The results will serve in generating information needed for the assessment of DDT derivatives intake from this source.

MATERIALS AND METHODS

Study area

This study took place in Nakivubo and Lubigi wetlands. Nakivubo wetland is located in Kampala District at 0° 18' 13.32" N and 32° 37' 39.36" E whereas Lubigi is located in Wakiso District Central region, Uganda at 00° 19' 56" N and 32° 31' 34" E. Sampling stations in Nakivubo wetland were selected at Gaba and Bugolobi (Figure 1); this portion of the wetland is still intact with its natural vegetation, *Cyperus papyrus* and is the only part of the wetland where *C. wernerii* are found. This part of the wetland receives most of the waste water carried by the channel before it joins Lake Victoria (NEMA, 2011; Ssentongo, 1998). The selected section of Lubigi wetland was between Bombo and Mityana roads where the wetland is still intact. One sampling station was set along the main stream at Namungona, along Hoima road; this part of the wetland receives municipal wastes and other wastes from Bwaise and Kawempe slums (NEMA, 2011). Other sampling sites were set at points where Sentema and Mityana roads cut-across the wetland (Figure 2).

Sample collection

Sampling was conducted between March, 2012 and 2013. *C. wernerii* of length between 13 and 20 cm were collected every two months (from sites N1-N3 in the Nakivubo wetland and L1-L3 in the Lubigi wetland) by fishermen in basket like structures, dragged to the shores, killed and smoked. Because *C. wernerii* are consumed smoked by the local population, it was considered necessary to first smoke them. Samples were taken in triplicate per sampling site, wrapped in aluminium foil, placed in air tight bags and labeled. They were then kept in cold boxes containing ice during transportation to the laboratory where they were kept at -18°C until they were extracted for DDT derivatives.

Standards and chemicals

Certified pesticide standards, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDD, *p,p'*-DDD and parathion ethyl (internal standard) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Pesticide residue grade solvents: ethyl acetate, hexane and acetonitrile were supplied by the British Drug Houses (BDH, UK). These chemicals were used for extraction, analysis and

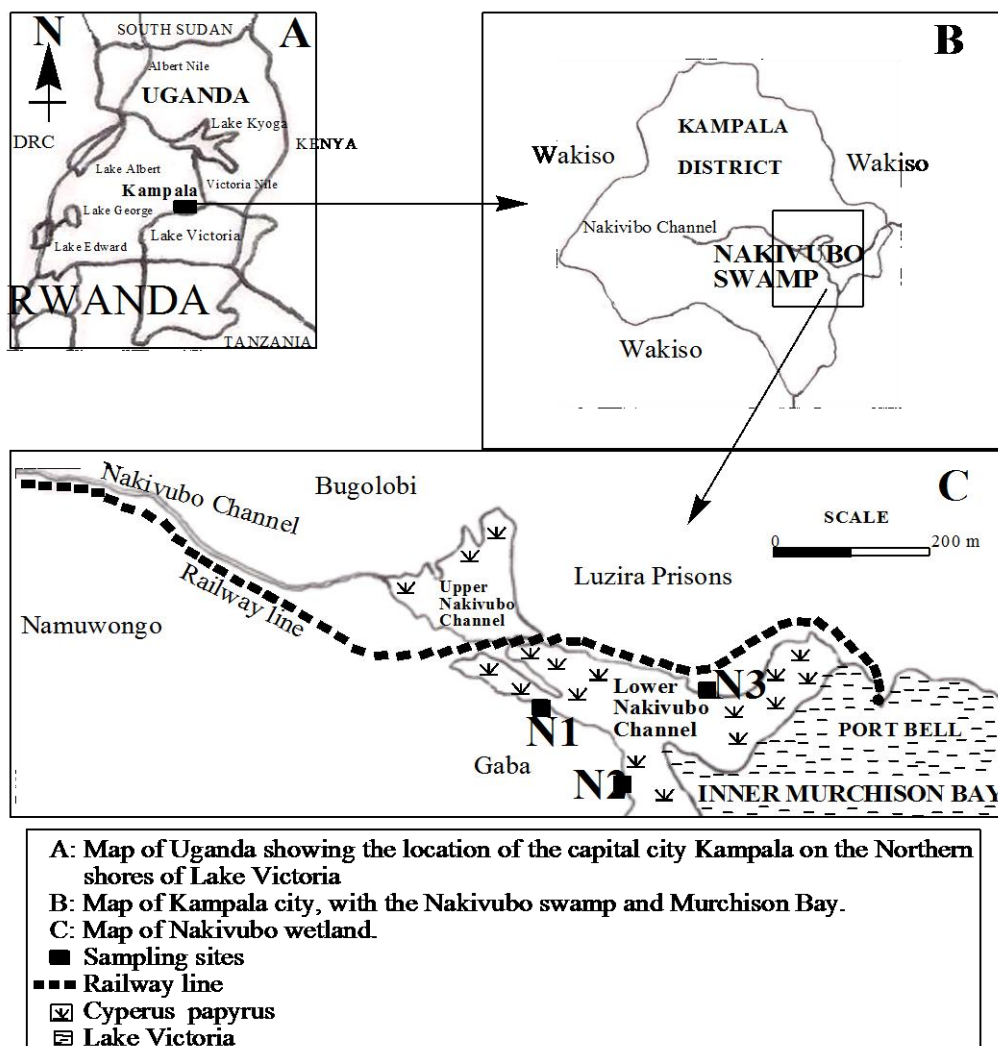


Figure 1. Map of Nakivubo wetland showing sampling stations.

confirmation. All solvents were of the highest purity commercially available.

Sample extraction

The sample was extracted using a method described by Åkerblom (1995). 10.0 g of smoked fish sample was weighed using analytical balance with a sensitivity of 0.1 mg in an Erlenmeyer. The sample was ground with a known quantity of sodium sulphate and parathion ethyl (internal standard) using a mortar and a pestle. An internal standard is a chemical compound that is similar but not identical to the chemical species of interest in the sample, it is added in a constant amount to the samples, blanks and calibration standards in a chemical analysis to correct for the loss of the analyte during sample preparation (Åkerblom, 1995). The mixture was then extracted with ethyl acetate (50, 20, 20 and 20 ml). The combined extract was concentrated using a rotary evaporator at a temperature of 40°C. The concentrated extract was then dissolved in 30 ml of acetonitrile stored in a freezer at -18°C and allowed to stand overnight for lipids precipitation and separation. The cold extract at -18°C was immediately filtered with glass wool to remove

frozen lipids. The precipitated lipids on the flask surface were re-dissolved in 10 ml acetonitrile to perform filtration again by the same procedure. The filtered extract was concentrated to 1 ml by rotary evaporation and dried under nitrogen atmosphere to follow the Florisil clean-up procedure (Åkerblom, 1995). A 15 cm long glass column of 4 mm id was plugged with glass wool and washed with hexane (10 ml). It was then packed with florisil (10.0 g) followed by anhydrous sodium sulfate (4 g). The column was lightly tapped to compact the florisil bed and then rinsed with hexane (5 ml) to remove any impurities. The column was conditioned with 5 ml of hexane/acetone 9:1 and then with 5 ml hexane. Elution was done by hexane/acetone 9:1 mixture. The eluate was concentrated on a rotary vacuum evaporator and transferred quantitatively to a glass-stopper test tube. Solvents were completely evaporated under mild flow of pure nitrogen. The evaporated sample was dissolved in double distilled hexane (1 ml) for GC- μ ECD analysis.

Analysis of samples

GC- μ ECD analysis was performed on a Varian CP-3800 gas chromatograph equipped with a 63Ni electron capture detector

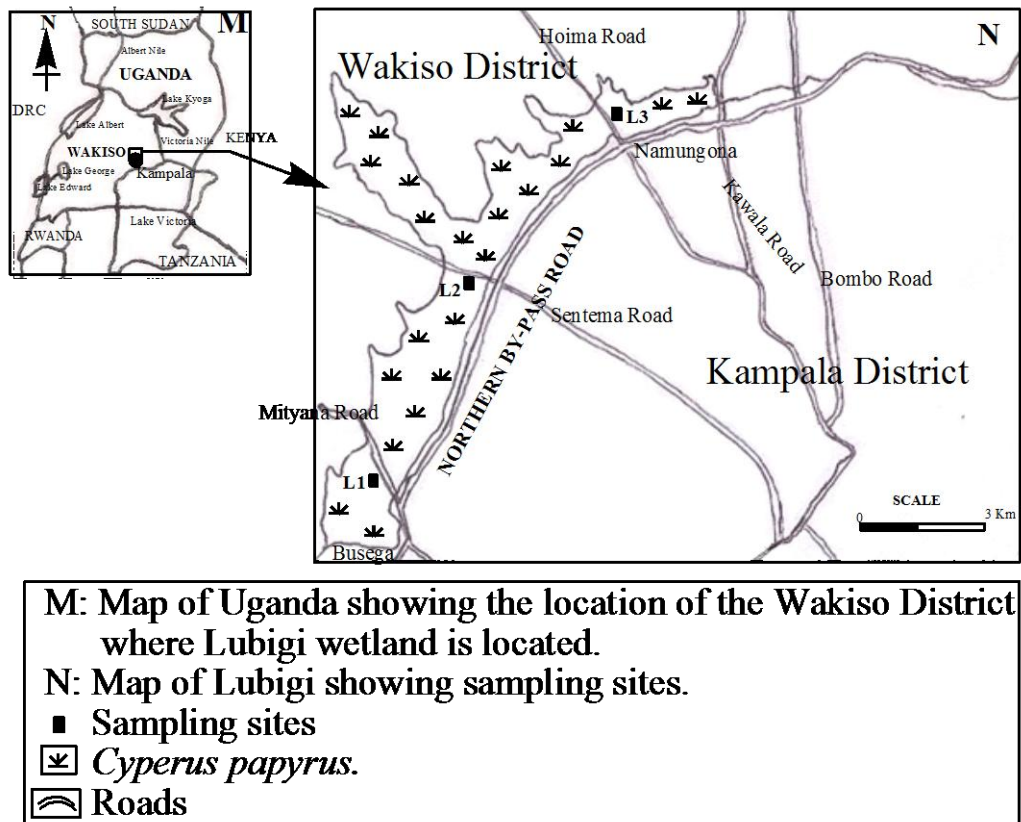


Figure 2. Map of Lubigi wetland showing sampling stations.

(ECD) fitted with both semi-polar (CP-Sil 19 CB, J & W Scientific, Folsom, CA, USA), and non-polar (CP-Sil 8 CB, J & W Scientific, Folsom, CA, USA) fused-silica capillary columns (30 m length, 0.25 mm i.d. and 0.25 µm film thickness). Hydrogen (99.9% purity) with a flow rate electronically set at 1.2 ml min⁻¹ was used as the carrier gas, while nitrogen was used as an auxiliary gas for the ECD at a flow rate of 30 ml min⁻¹. An oven 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 for both categories of columns. The temperatures of the injector and ECD detector were 250 and 300°C, respectively. 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. Identification and quantification were accomplished by comparison with reference standards. The concentration (µg/kg) of each component in the sample was calculated from its chromatogram using the peak area and concentration of the reference standards using the following formula:

Concentration (µg/kg) =

$$\frac{1000 \times \text{Concentration of standard} \times \text{Peak area of sample} \times \text{Volume of concentrated extract}}{\text{Peak area of standard} \times \text{Weight extracted}}$$

Quality control

Calibration curves using the internal mixed standard of DDT and derivatives was performed for each compound to be quantified at concentrations of 5, 10, 20, 50, and 100 ng/ml. Calibration standards were run every 10 samples and all measurements were performed in the ranges of linearity found for each compound. The limit of detections (LODs) and the limit of quantifications (LOQs) were calculated from DDT and derivatives mixed standard as 3.3 and 10 SD/b, respectively; where SD is the standard deviation of the GC chromatographic area (response) and b is the slope of the regression line of each derivative and the results are shown in Table 1. Accuracy and precision were determined by the standard addition method (ICH, 1994, 1995) at three different fortification

levels (0.05, 0.1 and 0.15 µg/ml). The samples were analysed in triplicates and recoveries and precision calculated for each derivative. The validation data showed quantitative recoveries in the range of 81 to 124%. The precision of the matrix was in the range of 1.87 to 9.7% CV. We considered the method to be reliable to quantify the concentration of DDT and derivatives.

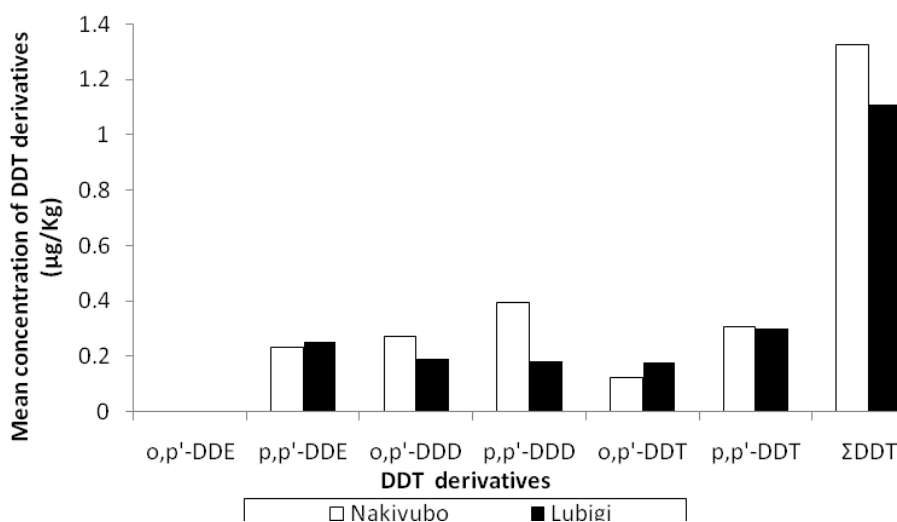
Confirmation of results

Confirmation of results was carried out using an Agilent 6890N GC-MS, USA version with a fused silica capillary column (HP-5MS) of 30 m length, 0.25 mm i.d. and 0.25 µm film thickness. The GC-MS used was equipped with a selective mass detector (Agilent 5975 inert XL Quadrupole, Palo Alto, CA, USA). The operating

Table 1. Mean values of LODs and LOQs ($\mu\text{g}/\text{Kg}$) of DDT and derivatives determined using GC-ECD technique.

Derivative	LOD	LOQ
<i>p,p'</i> -DDE	0.013	0.044
<i>o,p'</i> -DDE	0.021	0.071
<i>o,p'</i> -DDD	0.030	0.099
<i>p,p'</i> -DDD	0.021	0.069
<i>p,p'</i> -DDT	0.024	0.074
<i>o,p'</i> -DDT	0.058	0.065

Result for n=10

**Figure 3.** Variation in mean DDT derivatives in *C. wernerii* of Nakivubo and Lubigi wetlands.

conditions included a series of conditions: The oven temperature was programmed to: 90°C for 1 min, $30^{\circ}\text{C min}^{-1}$ to 180°C , $4^{\circ}\text{C min}^{-1}$ to 260°C , and maintained at this temperature for 16 min. The temperatures of the injector and MS detector were 250°C and 300°C , respectively. Helium was used as the carrier gas at 1 ml min^{-1} flow rate. The GC-MS was operated in a splitless mode with a purge-off of 1 min and the injection volume was $1 \mu\text{l}$ for each injection. The MS solvent delay time was 3.57 min and the scanned mass range was 50 to 550 m/z. The full scan ion monitoring mode was used for the determination of DDT and derivatives. Identification of the derivatives was done using the internal standards method (Åkerblom, 1995).

RESULTS AND DISCUSSION

Derivatives of DDT in *C. wernerii* from Nakivubo and Lubigi wetlands

Out of all the samples analysed, DDT derivatives were detected in 85% (34 out of 40) of the samples. The major DDT derivatives detected in the samples were *p,p'*-DDD, *p,p'*-DDE and *p,p'*-DDT which were found in 25, 22 and 21% of the samples, respectively. *o,p'*-DDD was detected in 19% while *o,p'*-DDT was found in 13% of the samples. For *o,p'*-DDE there were no measurable values since the

levels were below LOQ. Presence of *p,p'*-DDE and *p,p'*-DDD derivatives in most of the samples indicates historical usage. The detection of *p,p'*-DDT and *o,p'*-DDT in more than 30% of the fish samples suggests recent exposure of the fish to DDT which may be due to some illegal usage or dumping. The presence of DDT and derivatives in fish may be due to run-off and atmospheric depositions as a result of their use in Uganda. Fish tend to concentrate DDT derivatives from the surrounding water and diet into their tissues (Bryan et al., 1979). *C. wernerii* being a benthic organism accumulates high concentrations of DDT derivatives due to its feeding habits and habitats. In addition, DDT derivatives have a high affinity for the un-dissolved organic matter in the benthic zone and the organisms living in the benthos are more exposed to these contaminants (Ayas, 2007).

Composition of DDT in *C. wernerii* from the urban wetlands

The contribution of all the detected metabolites showed slight differences between Nakivubo and Lubigi wetlands (Figure 3). Generally, fish from Nakivubo wetland contained

slightly higher concentrations of all the derivatives except *o,p'*-DDT, *p,p'*-DDE. This indicates that human activities which lead to waste discharge into this wetland are more intense. The Nakivubo channel passes through relatively dense industrial and residential areas where expired pesticides and wastes could be discharged directly or indirectly into the wetland. Among the derivatives, *o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDE and *p,p'*-DDT were found to be in notable amounts in both wetlands. This may be attributed to slow degradation or recent inputs of the pesticide in the environment. The high percentage composition of *o,p'*-DDT and *p,p'*-DDT with respect to total DDT in both wetlands clearly illustrates that DDT usage has not been eradicated yet in the country, and there might be new inputs of the pesticide to the aquatic ecosystems as asserted by (Kyarimpa, 2007; Sebugere et al., 2008; Wasswa, 2009). This situation suggests that, although it was restricted, the illegal use of DDT still continues. The sources of contamination to both wetlands are closely related to human activities such as domestic and industrial discharge, street runoff as well as atmospheric transport.

Total DDT reported in this study is less than that reported in earlier studies in fish from other aquatic ecosystems in Uganda and other African countries. Σ DDT concentrations of 33 $\mu\text{g}/\text{kg}$ in *Bagrus docman*, 20 $\mu\text{g}/\text{kg}$ in *Protopterus gethiopinus* and 33 $\mu\text{g}/\text{kg}$ in *Oreochromis niloticus* from Lake Edward were reported (Sebugere et al., 2008), on the other hand (Kasozi et al., 2006) found total DDT of 11.67 $\mu\text{g}/\text{kg}$ wet wt in Nile perch and 20 $\mu\text{g}/\text{kg}$ wet wt in Nile tilapia from Lake Victoria. Similarly, Henry and Kishimba (2006) reported 20 $\mu\text{g}/\text{kg}$ wet wt in Nile tilapia from Lake Victoria. Similar studies on determination of DDT derivatives in Tanzania's freshwater reported Σ DDT of 60.7 $\mu\text{g}/\text{kg}$, in *Limnothrissa miodon*, 95.7 $\mu\text{g}/\text{kg}$ in *Stolothrissa tanganyikae* and 794.7 $\mu\text{g}/\text{kg}$ fat wt in *Boulengerochromis microlepis* (Manirakiza et al., 2002). In a related study Kidd et al. (2001) found 13.1, 13.4 and 11.4 $\mu\text{g}/\text{kg}$ wet wt of total DDT derivatives in *Bagrus meridionale*, *Buccochromis nototaenia* and a variety of *Clarius sp.* of Lake Malawi, respectively. The total DDT found in a survey by Said et al. (2008) was 5.13 $\mu\text{g}/\text{kg}$ wet wt in *O. niloticus* and 12.54 $\mu\text{g}/\text{kg}$ wet wt *Clarie sp.* from Lake Burullus, Egypt. Organochlorine pesticides have also been studied in Tilapia Zilli and Catfish caught from river Ogba, Nigeria and found to contain total DDT of 56 and 106 $\mu\text{g}/\text{kg}$ wet wt, respectively. In all these studies, *p,p'*-DDT was detected in high percentages (34 to 88%), suggesting that DDT was used in those countries in the recent years. The species in these studies are at higher trophic levels in the food chain and are larger than *C. wernerii*. The low concentrations of Σ DDT reported in the present study could be attributed to the size (Feng et al., 2003) and bottom position of *C. wernerii* in the food web (Feng et al., 2003; Kumblad et al., 2001) since bioaccumulation of these pollutants depends on these two factors. In addition, high

temperatures and solar radiation could have resulted in high degradation rates (Samuel and Pillai, 1989) and increased volatilization of the pesticide derivatives (Larsson et al., 1995) leading to low levels detected in the fish. The mean Σ DDT concentrations in fish samples were far below the FAO action levels for fish (edible portion) of 5000 $\mu\text{g}/\text{kg}$ (FAO–WHO, 1997), implying that the fish are safe for human consumption.

However, over consumption of *C. wernerii* from those water bodies may lead to increased levels of the pesticide derivatives over time. Increased levels of DDE in the blood of pregnant women increase the chances of having a pre-term baby. Long-term exposure to small amounts of DDT may affect the liver. Children being smaller than adults would accumulate a higher dose of DDT derivatives (amount of DDT ingested per kilogram of body weight) than adults once exposed over the same period, implying that those effects would be more severe in the former (ATSDR, 2002). Studies outside Africa have also reported DDT derivatives in fish, with some Σ DDT values higher while others were lower than the ones found in the present study. Σ DDT magnitudes: 1010 $\mu\text{g}/\text{kg}$ fat wt in pelagic from Lake Como Italy (Bettinetti et al., 2008), 60 $\mu\text{g}/\text{kg}$ wet wt in Archid char from Lake Oyangen (Evenset et al., 2004) and 0.35 $\mu\text{g}/\text{kg}$ wet wt in white fish from Lake Stuorajavri, Norway (Christensen and Savinova, 2007). In all these studies low (1 ~ 6%) percentage of *p,p'*-DDT was detected and derivatives of *p,p'*-DDT (*p,p'*-DDE and *p,p'*-DDD) were detected at high percentage and long-term no use of DDT was presumed in the countries.

Conclusion

Five out of the six DDT metabolites were detected namely: *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, however their levels were still below the FAO/WHO recommended limit. The detection of notable amounts of *o,p'*-DDT and *p,p'*-DDT suggests that DDT is being used or was used in the country in the recent years. From the present findings, we suggest that fish are a good bio-indicator of environmental contamination by DDT derivatives. Periodic bio-monitoring of these derivatives in the environment should be carried out so as to assess the trends of environmental contamination by these chemicals.

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Conflicts of interest

No competing interests exist.

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The background of the entire page is a photograph of an outdoor setting. In the upper portion, there is a building with a grey metal facade and a corrugated metal roof, partially obscured by green trees. In the lower portion, a green trash bin with a black wheel is visible on a concrete pad next to a grassy area. The text is overlaid on this background.

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