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Full Length Research Paper

Dichloro-diphenyl-trichloroethane (DDT) residue levels in marketed Silver Fish (*Rastreneobola argentea*) caught from major water bodies in Uganda

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Silver fish (*Rastreneobola argentea*) samples from Lake Victoria and Lake Kyoga were analyzed for dichloro-diphenyl-trichloroethane (DDT) and metabolites using a Gas Chromatograph equipped with an Electron Capture Detector. Confirmation of results was by Gas Chromatography equipped with Mass Spectrophotometer (GC-MS). DDT and metabolites were detected in 90% of the total samples (63 out of 70). *pp'* DDT was the most frequently detected residues in the samples whereas *op'* DDE was detected in none of the samples. The mean ΣDDT concentrations in fish from Lake Victoria varied from 0.147 to 0.396 µg/kg while that in Lake Kyoga varied from 0.207 to 0.506 µg/kg dry wt of fish sample. The concentration of total DDT in the fish samples ranged from 0.147 to 0.506 µg/kg dry wt. This was far below the recommended FAO limit of 5000 µg/kg for fish (edible portion), implying that the fish are safe for human consumption.

Key words: Dichloro-diphenyl-trichloroethane (DDT) and metabolites, *Rastreneobola argentea*, Uganda water bodies.

INTRODUCTION

1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane dichloro-diphenyl-trichloroethane (DDT) is one of the "dirty dozen" persistent organic pollutants (UNEP, 2008) whose manufacture and use was banned in many countries worldwide in the 1970's (Tsuda, 2012) except in cases of public health emergency (ASTDR, 2002). In Uganda, the use of DDT was prohibited in the mid 1980's (Kasozi et al., 2006), however; it was used in the recent past for controlling malaria in the districts of Oyam and Apac in Northern Region. DDT residues are still circulating in Ugandan ecosystems that are simultaneously being exposed to increasing pollution loads resulting from rapid national population growth (Rusongoza, 2003) and urbanization. This is the case in the region surrounding Lake Victoria, a lake that is regarded as the most important natural resources in East Africa, with fishing as the main economic activity. Fish is an important object for research of water ecosystem contamination by substances due to its high position in the food chain.

Lake Kyoga on the other hand may receive DDT and metabolites through surface run-off and atmospheric drifts, being the nearest water body to the districts of

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Apac and Oyam, where indoor residual spray (IRS) was carried out. Uganda re-introduced the use of DDT for malaria control in the districts of Oyam and Apac in 2008 in accordance with the recommendations and guidelines of WHO and in line with the StockhoIm convection (UNEP, 2008). The Northern Region is the most vulnerable to malaria in the whole country and possibly in the whole world: it lies within the tropical Lake Kyoga basin which provides an ideal breeding ground for mosquitoes (Bimenya et al., 2009). In spite of the previous use of the pesticide in the districts of Oyam and Apac, no monitoring of its levels has been carried out. However, a recent study identified op' and pp' DDT isomers in vegetables grown in homes which participated in IRS (Mukasa, 2012), a clear indication of recent exposure.

Lake Victoria (UBOS/UDHS, 2006) and Lake Kyoga (LAKIMO, 2004) are the most important fisheries in Uganda. The fisheries sector contributes greatly to the Ugandan economy in terms of income, employment and export revenue. For example in 2001 fish products exports were valued at US $ 80.4 million, in 2002 at US $ 87.57 million (Nyombi and Bolwig, 2004) and in 2005 at US $ 142 million (UBOS/UDHS, 2006). Fish is a protein source for 24.7 million people in Uganda (UBOS, 2002) and other consumers in the East African Region. The demand for Ugandan fish has increased drastically over the last two decades due to rapid domestic population growth and the emergence of an export market for especially Nile perch and Nile tilapia (Odada et al., 2004). The decline in Nile perch and Nile tilapia has increased the demand for non-export small fish species on the domestic market. Silver fish, *Rastreneobola argentea* is the most abundant non-export small fish species found in Lakes. It is predominantly found in Lake Kyoga and to a lesser extent in Lake Victoria (Mbabazi and Wasswa, 2010). The silver fish is caught by light attraction, where Kerosene pressure lamps are floated on water attracting fishes to light and are pulled out of the water in their thousands with scoop-nets (FAO). They are then sun-dried directly on the beaches.

Previous studies indicate that DDT residues are still detected in various compartments of the environment such as Lake Victoria sediments (Wasswa, 2009) and fish (Kasozi et al., 2006; Kyarimpa, 2007; Ogwok et al., 2009; Sebugere et al., 2008; Simon et al., 2004) from different water bodies in the country. DDT residues have previously been found in *Lates niloticus* and *Oreochromis nioticus* in Lake Victoria (Kasozi et al., 2006). Similarly, Bimenya et al. (2007) and Sebugere et al. (2008) found DDT residues in numerous fish species namely: *Bagnus docmac, Protopterus aethiopinus, Haplochromi nigripinnis, oreochromis niloticus* and *Clarius gariepinus* in Lake Edward. The presence of *op'* and *pp'* DDT isomers in Lake Victoria sediments (Wasswa, 2009) and fish from various water bodies in the country suggests that, Ugandan water bodies still receive considerable inputs of DDT residues from various anthropogenic sources such as direct discharges or indirectly from river flows and run-off, as well as from long distance transport through the atmosphere. Many studies have been conducted on the DDT residues contamination of the aquatic environment in Lake Victoria and Lake Edward. However, no studies have investigated DDT residues in non-export small fish species which are considered the most important protein source for the local population. The present work was undertaken to find out the status of DDT and its metabolites as well as to determine their residue levels in silver fish from Lake Victoria and Lake Kyoga. In this study, we incorporated modifications in the sample extraction procedure which necessitated method validation before application in the determination of DDT residues in fish samples. The validated method was then used to determine DDT residues in fish from Lake Victoria and Lake Kyoga so as to establish the extent of environmental contamination by this pesticide and also to evaluate the toxicological significance to the health of the consumers.

MATERIALS AND METHODS

Study area

The study area comprised of, seven landing sites, four on Lake Victoria and three on Lake Kyoga. Sampling was conducted for 1 year starting March 2012. Four stations (Gaba, Port Bell, Kasenyi and Kigungu) were selected on Lake Victoria and three (Namasale, Biko and Kayago) on Lake Kyoga. The various sampling stations on Lake Victoria and Lake Kyoga are shown in Figure 1. Lake Victoria is located along the equator at 0° 30' N, 3° 00' E and 31° 39' N, 34° 53' E, while Lake Kyoga is situated between longitudes 32° 10' and 34° 20' East, and between 1° 00' and 2° 00' North.

Samples

Silver fish, *R. argentea* were bought from the selected stations on Lake Victoria (V1-V4) and Lake Kyoga (K1-K3). Equal amounts of silver fish, were bought from four different people at each landing site and pooled to obtain a representative sample. Samples were then wrapped in aluminium foil, placed in air tight bags and appropriately 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 residues.

Chemicals and standards

Pesticide residue grade solvents: ethyl acetate, hexane and acetonitrile were supplied by the British Drug Houses (BDH, UK). The chemicals were used for extraction, analysis and confirmation. The purity of the solvents was checked by using a GC-ECD to ensure that no detectable traces of pesticides were contributed by solvents. The organochlorine pesticides standards (DDT and metabolites) as well as internal standard parathion ethyl were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Sample extraction and delipidation

The extraction of DDT residues from fish samples was performed

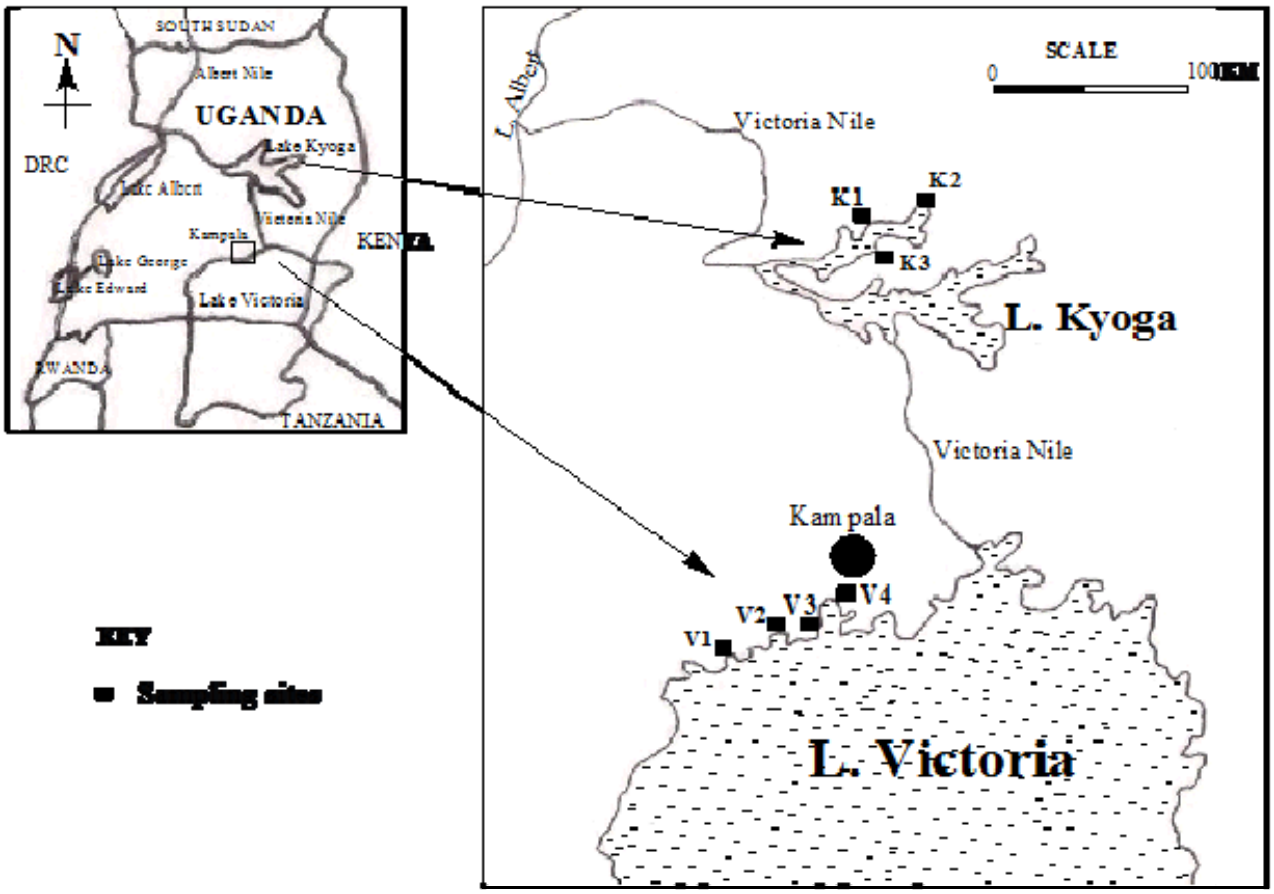


Figure 1. Map showing sampling sites on Lakes Victoria and Kyoga. Inset is the map of Uganda showing the location of Lake Victoria and Lake Kyoga.

according to the method described by Åkerblom (1995). Portions of 10 g of dry fish was weighed and ground with a known quantity of sodium sulphate using a mortar and a pestle. The powder 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 and 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 Florisil clean up procedure.

Sample clean up by florisil

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. The concentrated sample was applied to the column and eluted with 10 ml hexane/acetone 9:1 mixture. The eluate was then concentrated at 40°C with a rotary evaporator to about 1 ml and then to complete dryness by nitrogen stream. The residue was dissolved in hexane (1 ml) for GC-ECD analysis.

Analysis of samples

For GC analysis, a Varian (CP-3800, Palo Alto, CA, USA) gas chromatograph equipped with a 63Ni ECD (Electron Capture Detector) 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) was used. 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, Norwak, 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 obtained from Dr.

Table 1. Mean values of slopes, intercepts and regression coefficients from calibration curves.

Pesticide	Slope	Intercept	Regression coefficient (r^2)
*pp'*DDE	1.001	-0.006	1.000
*op'*DDE	1.002	+0.102	0.998
*op'*DDD	0.990	+0.047	0.999
*pp'*DDD	1.014	-0.003	0.999
*pp'*DDT	0.971	+0.035	0.998
*op'*DDT	1.100	-0.005	0.997

EhrenstorferGmbH, Augsburg, Germany.

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 formula below.

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

Confirmation of results

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 was used for confirmation of the results. The GC-MS used was equipped with a selective mass detector (Agilent 5975 inert XL Quadrupole, Palo Alto, CA, USA). The operating conditions were: 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. The temperatures of the injector and MS detector were 250 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 µl 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 metabolites. Identification of the analytes was done using the internal standards method. Data acquisition and processing was achieved using GC-MSD Chemstation Software (G1701dad.02.0sp1, JAS CWA, USA).

Method validation

Linearity

The linearity of the method was evaluated by analyzing in triplicate, a set of 6 DDT and metabolites standard solutions in hexane ranging in concentration from 0.03 to2.7 µg/ml. Peak area ratios of the analytes to the internal standard were plotted against concentrations. The regression equations with the slopes, intercepts and correlation coefficient (r^2) were generated with Microsoft Excel software and are presented in Table 1.

Recovery and precision

Recovery and precision were determined by standard addition method (ICH, 1995) at 3 spiking levels of 0.05, 0.1 and 0.15 µg/ml of mixtures of *pp'* DDE, *op'* DDE, *op'* DDD, *pp'* DDD, *op'* DDT and *pp'* DDT and analyzing the samples in triplicates under repeatable and internal reproducible conditions. The mean recovery ranged between 81 to 124% and the coefficients of variation (CV) were ≤ 10%.

Limit of detection (LOD) and limit of quantification (LOQ)

In order to determine the sensitivity of the GC-ECD, nine independent fish samples (from control site) were fortified at three different low concentrations of each pesticide (ICH, 1995) and analyzed in triplicates. The values of LOD and LOQ were obtained by calibration curve method (they were based on the residual standard deviation and slope of the regression line) (ICH, 1994, 1995). The LOD and LOQ were calculated as 3.3 and 10SD/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 pesticide. The values of LOD obtained were 0.013, 0.021, 0.030, 0.021, 0.024 and 0.058 µg/ml for *pp'* DDE, *op'* DDE, *op'* DDD, *pp'* DDD, *op'* DDT and *pp'* DDT respectively; meanwhile the values of LOQ were 0.044 µg/ml (*pp'* DDE), 0.071 µg/ml (*op'* DDE), 0.099 µg/ml (*op'* DDD), 0.069 µg/ml (*pp'* DDD), 0.074 µg/ml (*op*' DDT) and 0.065 µg/ml (*pp*' DDT).

Statistical analysis

The data was subjected to Analysis of variance (one way, ANOVA) using Graph pad Instat package program to test site differences.

RESULTS AND DISCUSSION

Levels of DDT residues in fish samples

70 samples of *R. argentea* from Lake Victoria and Lake Kyoga were analyzed for DDT and metabolites. Residues of DDT and its metabolites were detected in 90% (63 out of 70) of all the samples analyzed. Figures 2 and 3 show some of the selected GC chromatograms of DDT and metabolites detected in *R. argentea* from Lake Victoria and Lake Kyoga respectively. The mean concentrations on dry weight basis of *R. argentea* of *op'* DDE, *pp'* DDE, *op'* DDD, *pp'* DDD, *op'* DDT, *pp'* DDT and ΣDDT in the fish samples in different parts of Lake Victoria and Lake Kyoga are shown in Table 2. The proportion of DDT and its metabolites in the fish samples showed predominance of *pp'* DDT (39.4%), followed by *pp'* DDD (24%), *pp'* DDE (21%), *op'* DDT (8.3%), *op'* DDD (7.3%) and *op'* DDE was detected in none of the samples. Predominance of *p,p'*-DDT in the fish samples suggests recent exposure of the aquatic ecosystems to DDT, may be due some illegal usage or dumping. This pattern is similar to that reported in fish from Lake Songkhla, Thailand (Kumblad et al., 2001). The moderate percentage composition of *pp'*DDD

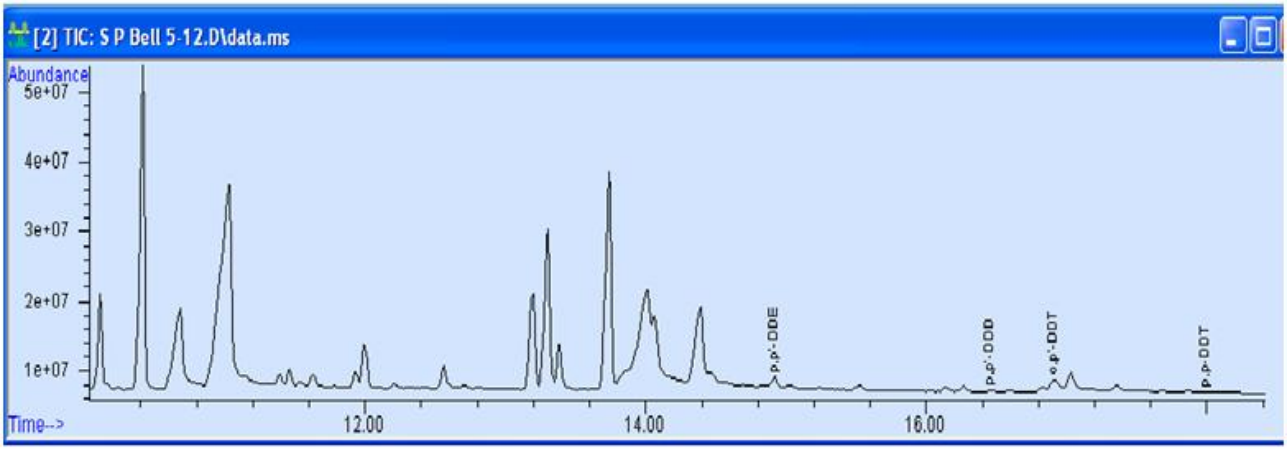


Figure 2. GC Chromatogram of DDT and metabolites in *R. argentea* from Lake Victoria.

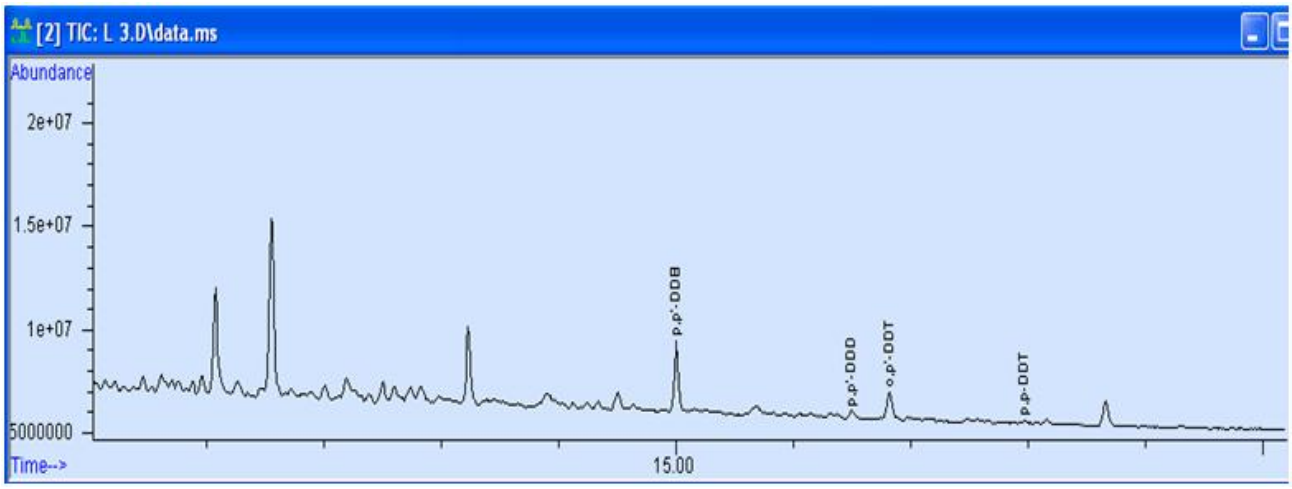


Figure 3. GC Chromatogram of DDT and metabolites in *R. argentea* from Lake Kyoga.

Table 2. Concentrations of DDT residues (µg/kg) in fish collected from different water bodies in Uganda.

Sampling location	*op'*DDE	*pp'*DDE	*op'*DDD	*pp'*DDD	*op'*DDT	*pp'*DDT	ΣDDT
Lake Victoria landing sites							
Kasenyi	ND	ND	ND	0.091±0.008	ND	0.077±0.007	0.168
Port Bell	ND	0.057±0.018	0.082±0.004	0.088±0.022	0.076±0.026	0.094±0.031	0.397
Gaba	ND	ND	0.062±0.013	ND	ND	0.085±0.009	0.147
Kigungu	ND	0.072±0.089	ND	ND	ND	0.097±0.024	0.169
Lake Kyoga landing sites							
Namasale	ND	ND	ND	ND	0.087±0.088	0.120±0.031	0.207
Biko	ND	0.197±0.018	ND	0.202±0.108	ND	0.107±0.048	0.506
Kayago	ND	0.083±0.008	ND	0.093±0.058	ND	0.193±0.056	0.369

Concentrations of DDT residues (µg/kg) expressed as Mean ± SD, ΣDDT (µg/kg) = *op'*DDE + *pp'*DDE + *op'*DDD + *pp'*DDD + *op'*DDT + *pp'*DDT, ND- Not Detected.

and *pp'* DDE metabolites may indicate slow biotransformation of DDT (ASTDR, 2002). The absence of *op'* DDE in all the samples may be due to the quantification level (0.099 µg/ml) which was relatively high.

The concentrations of DDT metabolites in *R. argentea* from Lake Victoria ranged between ND-0.090 µg/kg, ND-0.095 µg/kg, ND-0.099 µg/kg, ND-0.156 µg/kg, and ND-0.136 µg/kg for *pp'*DDE, *op'*DDD, *pp'*DDD, *op'*DDT and *pp'* DDT respectively, meanwhile their concentrations in the same fish species from Lake Kyoga ranged between ND-0.234 µg/kg (*pp'*DDE), ND-0.422 µg/kg (*pp'*DDD), ND-0.288 µg/kg (*op'*DDT), ND-0.244 µg/kg (*pp'*DDT). The mean ΣDDT concentrations in Lake Victoria varied from 0.147 to 0.396 µg/kg while that in Lake Kyoga varied from 0.207 to 0.506 µg/kg dry weight of fish sample. There was no significant difference in mean ΣDDT concentration between Lake Victoria and Lake Kyoga. The mean ΣDDT concentrations in the fish samples were far below the FAO action levels for fish (edible portion) of 5000 µg/kg (FAO-WHO, 1997). The maximum mean ΣDDT concentration in the sampled fish was 1.328 µg/kg, with a mean concentration of 0.266 µg/kg; this cannot be regarded as hazardous. The low residue concentrations might be attributed to high temperatures and solar radiation resulting in high degradation rates (Samuel and Pillai, 1989) and increased volatilization of the pesticide residues (Larsson et al., 1995). There were small differences in residue concentrations between locations. Spatial variations are to be expected since DDT was used extensively until mid 1980's (Kasozi et al., 2006) and thereafter for malaria control by the Ministry of Health in Northern Uganda (Bimenya et al., 2009).

The magnitude of mean ΣDDT concentration reported in this study is lower than that reported in earlier studies in Uganda. Sebugere et al. (2008) reported ΣDDT concentrations of 33 µg/kg in *Bagrus docman*, 20 µg/kg in *Protopterus gethiopinus* and 33 µg/kg in *Oreochromis niloticus* from Lake Edward. Kasozi et al. (2006) on the other hand found ΣDDT of 11.67 µg/kg wet weight in Nile perch and 20 µg/kg wet weight in Nile tilapia from Lake Victoria. The species in these studies are at higher trophic levels in the food chain and have large fat content compared to *R. argentea*. Low concentrations reported in this study could be attributed to the bottom positions of the studied species in the trophic level, small fish species lay at the bottom of the food chain, implying that they feed mainly on plant material and small insects from which they accumulate the pollutants. Many factors determine bioaccumulation of organic pollutants in aquatic organisms, position in trophic level being one of them (Feng et al., 2003; Kumblad et al., 2001)

Although the levels of total DDT in fish in the present study appear to be within the acceptable FAO-WHO limits, over consumption of silver fish from those water bodies may lead to increased levels of the pesticide residues 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 residues (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 (ASTDR, 2002).

In general, the concentrations of ∑DDT observed in this study of Ugandan fish were lower than, and in other cases comparable with those reported in edible and marketed fish collected from other parts of the world as shown in Table 3. Fish sampled from different locations presented varying relative compositions of the different DDT residues as shown in the Figure 4.

The most striking feature is the high relative amount of *p,p'* DDT found in fish samples from all sampling locations, actually it was the major constituent of ΣDDT in *R. argentea.* This implies that DDT products are still circulating in the Ugandan environment. There was notable relative amount of *pp'*DDD concentration in fish samples from Lake Kyoga. The same pattern has been reported for fish from Lake Songkha, Thailand in nutrient rich areas close to the coast (Kumblad et al., 2001). In line with the results discussed by Kumblad et al. (2001), it can be suggested that the Lake Kyoga, ecosystem is extreme in the amount of microbial activity leading to fast biological degradation of pp'DDT to its metabolites in this water body. Comparing the composition of DDT constituents in *R. argentea* from Lake Victoria and Lake Kyoga, the former has a much lower relative contribution of the most DDT metabolites. This is likely to be either due to dilution effect since Lake Victoria has a larger volume of water or high organic matter that might result in removal and binding of contaminants to the sediments. High organic matter in sediments has by several authors been reported to drastically increase the adsorption of contaminants on to sediments (Kumblad et al., 2001; Wasswa, 2009).

Conclusion

Only five DDT metabolites*: pp'*DDE, *op'*DDD, *pp'*DDD, *op'*DDT, *pp'* DDT were detected in the fish samples caught from the different parts of Lake Victoria and Lake Kyoga. The present study indicates that mean ΣDDT concentrations in the fish were far below the FAO standards. This implies that the fish species studied is safe for human consumption. However, over consumption or long time exposure may lead to health effects especially in pregnant women and children. The detection of notable relative amounts of *op'* DDT and *pp'* DDT suggests that Ugandan ecosystems still receive considerable inputs of DDT residues from various anthropogenic sources. This could be due to weak laws leading to inadequate management, use, distribution and disposal of the pesticides.

Table 3. ∑DDT concentration (µg/kg) in fish collected from different parts of the world.

Country	Fish species	∑DDT (µg/kg)	References
Burundi	*Limnothrissa miodon*	95.7	Manirakiza et al. (2002)
(L. Tanganyika)	*Stolothrissa tanganyikae*	794.7	
Malawi	*Bagrus meridional*	13.1	Kidd et al. (2001)
(L. Malawi)	*Buccochromis nototaenia*	3.4	
	Clarius sp.	1.4	
Egypt	*Oreochromis niloticus*	5.13	Said et al. (2008)
(L. Burullus)	*Clarius sp.*	12.54	
Italy			
(L.Como como)	Shad	1010	Bettinetti et al. (2008)
Norway			
(L. Oyangeu)	Archit char	60	Evenset et al. (2004)
(L. Stuorajavri)	White fish	0.35	Christensen et al. (2007)
USA			
(L.Washington)	Cutthroat trout	168	McIntyre and Beauchamp (2007)
Canada			
(L. Kusawa)	Lake trout	40.85	Ryan et al. (2005)
Uganda			
(L.Victoria)	*Rasteneobola argentea*	0.88	Present study
(L.Kyoga)		1.08	

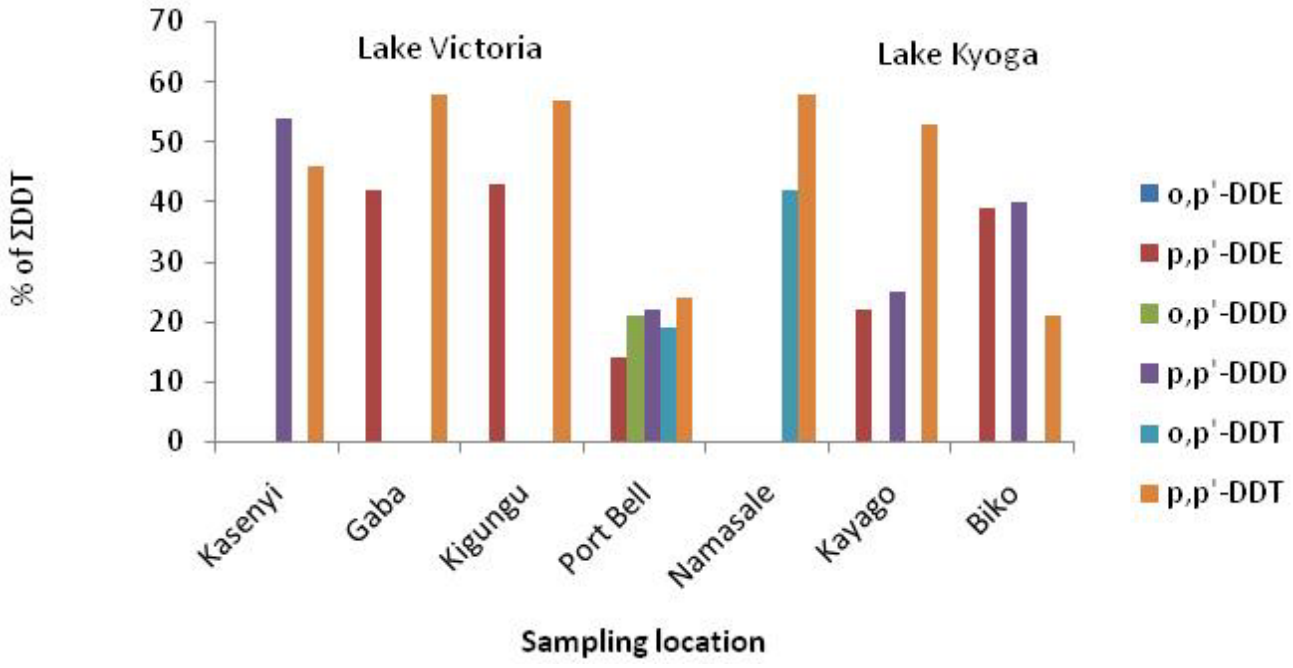


Figure 4. Relative composition of DDT metabolites in the fish samples from different locations in Lake Victoria and Lake Kyoga.

Conflict of Interests

There were no conflicts of interest which influenced the study.

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