Chemosphere 76 (2009) 212–215

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

DDT and metabolites in fish from Lake Edward, Uganda

Patrick Ssebugere [a], Bernard T. Kiremire [a,*], Michael Kishimba [b], Shem O. Wandiga [c], Steven A. Nyanzi [a], John Wasswa [a]

[a] Department of Chemistry, Makerere University, P.O. Box 7062, Kampala, Uganda
[b] Chemistry Department, Dar es Salaam University, P.O. Box 35061, Dar es Salaam, Tanzania
[c] Department of Chemistry, University of Nairobi, P.O. Box 30197, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 2 July 2008
Received in revised form 10 February 2009
Accepted 24 March 2009
Available online 26 April 2009

Keywords:
DDT
Metabolites
Fish
Lake Edward

ABSTRACT

This paper presents results based on determination of residue levels of 1,1,1-trichloro-2,2-bis[p-chlorophenyl]ethane (DDT) and its metabolites in five fish species from Rwenshama landing site on Lake Edward, Uganda. The residue levels were analysed by using a GC-ECD and confirmed by GC–MS. The DDT residues detected in fish samples from Lake Edward were *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDD and *o,p′*-DDD. All the analysed samples of fish presented mean DDT residues ranging from non-detectable levels to 68 μg kg^{-1} fresh weight. High levels of *p,p′*-DDT were detected in comparison to the metabolites (*p,p′*-DDE and *p,p′*-DDD). The detection of higher levels of *p,p′*-DDT than *p,p′*-DDE and *p,p′*-DDD, in most fish samples, suggests recent exposure of fish to DDT. Generally, most of the fish samples had residue levels below the maximum residue limits (MRL) recommended by FAO/WHO Codex Alimentarius Commission.

1. Introduction

DDT is among the 12 Persistent Organic Pollutants (POPs) that are restricted and targeted for ultimate elimination under the international treaty on POPs (UNEP, 2001). As a POP, DDT is characterised by high persistence, low polarity, low aqueous solubility and high lipophilicity, and as a result it has a potential to bioaccumulate in fatty tissues. DDT has been implicated in a broad range of adverse human health and environmental effects, including reproductive failures (Beard, 2005), endocrine disruption (Turusov et al., 2002), egg-shell thinning and, hence, poor reproductive success (Ratcliffe, 1967). DDT and some of its metabolites are mutagenic, potentially carcinogenic and are known to be environmental estrogens. They mimic hormones, binding to and activating the estrogen and androgen receptors, thereby, often producing estrogen-like effects (Robinson et al., 1985).

DDT became available in Uganda in the 1950s, and was widely used until the mid 1980s for pest management on crops, especially cotton, and control of malaria mosquitoes and tsetse flies (*Glossina* sp.) in public health programs (Kasozi et al., 2006). DDT was used in many parts of the country like in Kihiihi Sub County of Kanugu District to control malaria mosquitoes in the late 1950s and early 1960s, leading to the elimination of *Anopheles funestus* and reduction of the *Anopheles gambiae* mosquitoes (Rwakimari, 2007). DDT was also used in controlling *Simulium* flies in small rivers in Mt. Elgon and Budongo forests, and along the Victoria Nile Basin (Rwakimari, 2007).

In spite of the previous use of pesticides in Kanungu and other districts in Uganda, little or no monitoring of their levels has been carried out. However, a recent study by Bimenya et al. (2007) identified DDT/DDE in fish but the analytical method did not discriminate between DDT and its metabolites. This study was undertaken to confirm the presence of DDT by carrying out the extraction with non-polar solvents and by analysis of DDT and its metabolites in fish samples using a Gas Chromatograph (GC) equipped with an Electron Capture Detector (ECD) and fitted with semi-polar column. Confirmation of the results was carried out by fitting the GC with a non-polar column, as well as using a GC–MS.

2. Materials and methods

2.1. Study site

Fish samples were collected from Rwenshama landing site on Lake Edward north of Kihiihi in Rukungiri District (Fig. 1). Lake Edward is the nearest water body to Kihiihi, where DDT and metabolites may have been eroded over a number of years as surface runoffs from places where pest control was carried out, especially in 1950s and the 1960s. The geographical coordinates of Kihiihi are 0°57′27″ South and 29°47′23″ East.

* Corresponding author. Tel.: +256 772 589313.
E-mail address: kiremire@chemistry.mak.ac.ug (B.T. Kiremire).

0045-6535/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2009.03.049

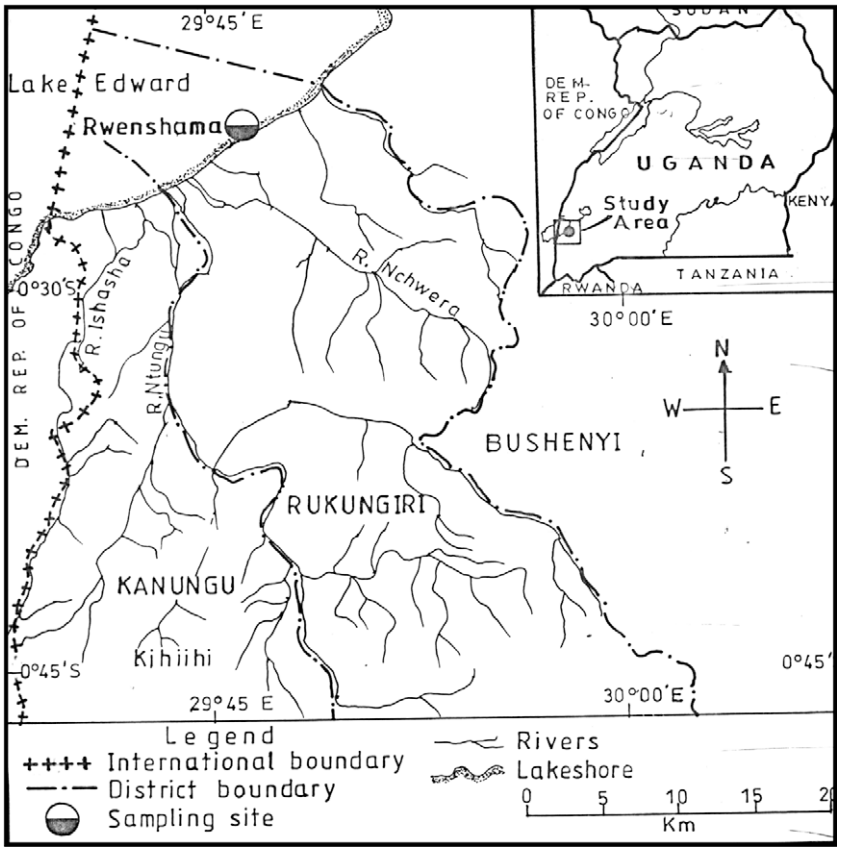

Fig. 1. Map showing the study area.

2.2. Sampling

A total of 68 representative fish samples: Semutundu (*Bagrus docmac*), Mamba (*Protopterus aethiopinus*), Enjunguri (*Haprochromis nigripinnis*), Nile tilapia (*Oreochromis niloticus*) and Male (*Clarias gariepinus*) were bought from local fishermen at Rwenshama landing site. The sampled fish had lengths of 24.5–78 cm from nose to tail fin and weighed between 178.38 and 2638.87 g. The samples were wrapped in aluminum foil, labeled using permanent stickers and were then placed in polythene bags. The fish samples were transported in cooling boxes containing ice packs to the laboratory for identification, and later frozen at −18 °C to stop microbial activities before extraction.

2.3. Chemicals and standards

Pesticide residue grade solvents (over 99% certified purity) were supplied by the British Drug House (BDH, UK). The chemicals used included ethyl acetate, cyclohexane and acetonitrile. 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 certified reference material CRM 430 (organochlorine pesticides) was obtained from the Institute for Reference Materials and Measurements (Geel, Belgium). Analytical standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The standards were kept separately in a freezer at −18 °C before use.

2.4. Sample extraction and clean-up

2.4.1. Fish extraction procedure

Fish muscle (10 g) was weighed and ground with a known quantity of sand and sodium sulphate to a free floating powder using a mortar and a pestle. The powder was then extracted by shaking with ethyl acetate (50, 20, 20 and 20 mL), respectively. The combined extracts were concentrated using a rotary evaporator at 30 °C to give fat solution. The fat solution was then dissolved in 3 mL of cyclohexane. The latter was transferred into an extralut-3 column and left to equilibrate for 10 min. The column was then eluted with four portions of 5 mL of acetonitrile saturated with cyclohexane. The extract was then kept for clean up.

2.4.2. Clean-up of fish samples

Gel Permeation Chromatography (GPC) clean-up was performed using 50 cm × 1 cm i.d. chromatographic tube, with two adapters, six-way valve with 1 mL sample injector loop and teflon tubing. The extract (1 mL) was loaded in the sample loop by the use of a syringe, while the GPC valve was at the loading position. The loop was turned to the injection point, the extract was conveyed through the column and eluted with cyclohexane/ethyl acetate (1:1 v/v). Initially, the GPC was calibrated using a mixture of *p,p′*-DDD and *p,p′*-DDT. The pesticides were eluted in the volume range 17–36 mL. During the clean up, the first fraction (16 mL) was discarded. The second fraction (17–36 mL) was collected and concentrated for analysis. The column was cleaned with 20 mL of cyclohexane/ethylacetate (1:1 v/v) before injection of the next sample. The extract was evaporated and the residue dissolved in cyclohexane (2 mL) for GC-ECD and GC–MS analysis (Åkerblom, 1995).

2.5. Gas chromatographic analysis

Analysis of DDT and its metabolites was performed using a Varian (CP-3800, Palo Alto, CA, USA) GC equipped with an Electron Capture Detector (ECD) and 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 hold for 16 min. The carrier gas was hydrogen (99.999% purity) with electronic flow control at 1.2 mL min^{-1}. Other GC operating conditions were 230 °C and 300 °C injector and detector temperatures, respectively, and 30 mL min^{-1} make-up gas flow (nitrogen). 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 standards.

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. The MS used was equipped with a selective mass detector (Agilent 5975 inert XL Duadrupole, Palo Alto, CA, USA). The initial temperature was 90 °C and it was 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 held 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 1.0 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 50–550 *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).

2.6. Analytical quality assurance

To ensure quality of residue data, a certified reference material (CRM 430), blanks and spikes were included in the analysis. Individual reference standards were used to identify and quantify the levels of residues. Arithmetic means and standard deviations were calculated from positive quantifiable samples only, and in all cases, the differences were considered significant, if the exact *p* value was $\alpha \leqslant 0.05$.

2.6.1. Limits of detection (LOD)

Limits of detection were calculated as the concentrations that produced a signal equal to 3-times the background noise level. The limits of detection in μg kg^{-1} 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) and *o,p′*-DDD (0.08). Samples were considered positive when their residue levels were ⩾LOD.

2.6.2. Average percentage recoveries

Satisfactory recoveries with the great majority above 70% were obtained from spiked representative fish samples in duplicate at 0.2 and 0.5 μg kg^{-1} (Table 1). The mean recoveries of the detected residues in fish samples varied 64–88% at 0.2 and 79–93% at 0.5 μg kg^{-1}, respectively. The majority of the standard deviations were below 10%, reflecting stability of DDT and degradation products, especially during sample preparation and GC analysis. Recoveries of DDT and metabolites were 85% for *p,p′*-DDT and 90% for *p,p′*-DDE. The recoveries served to ensure that the performance of the method for each of the samples was within acceptable limits (70–120%) for routine analysis (Åkerblom, 1995). Since most recoveries were above 70%, the results were not corrected for recovery.

Table 1
Average percentage recoveries of DDT and metabolites from certified reference material (CRM 430) and spiked fish samples.

Pesticide	CRM 430	Fish ($N = 3$)	
		0.20 (μg kg^{-1})	0.50 (μg kg^{-1})
p,p′-DDT	85 ± 5	76 ± 3	79 ± 5
o,p′-DDT	NA	83 ± 8	88 ± 7
p,p′-DDE	90 ± 3	79 ± 4	80 ± 4
o,p′-DDE	NA	78 ± 4	83 ± 7
p,p′-DDD	NA	88 ± 3	93 ± 5
o,p′-DDD	NA	64 ± 9	88 ± 1

Results are presented as mean values of triplicate determinations ($N = 3$) ± standard deviation, NA – no data were available.

3. Results and discussion

3.1. Levels of DDT and metabolite residues in fish samples

The compounds *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD were detected in 40% 33% and 21% of the fish samples, while *o,p′*-DDT, *o,p′*-DDE and *o,p′*-DDD were found in 10%, 31% and 21% of the samples, respectively. The levels of DDT and metabolites were low in most of the analysed fish species. The bioaccumulation process of these contaminants depends on a number of factors including sex, age, position in the tropic level, etc. (Crosly et al., 1998). The mean levels of *p,p′*-DDT ranged from ND to 15 μg kg^{-1} (fresh-weight basis). The highest *p,p′*-DDT levels were found in *Bagrus docmac* (15 μg kg^{-1}). The mean concentrations of *Protopterus aethiopinus* and *Oreochromis niloticus* were 13 and 14 μg kg^{-1} fresh weight. Statistical analysis showed no significant difference ($\alpha = 0.05$) in *p,p′*-DDT levels between the two species, although *O. niloticus* in this study had greater *p,p′*-DDT levels than *P. aethiopinus*. Residue levels for *o,p′*-DDT ranged from ND to 11 μg kg^{-1}, with *B. docmac* species having residue levels of 11 μg kg^{-1}. The respective mean concentration of *p,p′*-DDE were 16 and 19 μg kg^{-1} for *B. docmac* and *P. aethiopinus*.

Higher *o,p′*-DDE residues (68 μg kg^{-1}) were detected in most *P. aethiopinus* compared to the other fish species, and this was attributed to their age. Generally, on the average this species was longer than the other species from nose to tail fin (their lengths were 25–27, 27–31, 53–62, 65–69 and 72–78 cm for *H. nigripinnis*, *O. niloticus*, *B. docmac*, *C. gariepinus* and *P. aethiopinus*, respectively). The mean levels of *o,p′*-DDE in *B. docmac*, *C. gariepinus* and *O. niloticus* are shown in Table 2. *o,p′*-DDD residues were detected in less than 25% of all the samples at levels of up to 6 μg kg^{-1} fresh weight. The samples of *H. nigripinnis* analysed had no detectable levels of DDT and its metabolites, and this was most likely because all the sampled specimens were young in which case their diet mainly consisted of plankton. The presence of DDT isomers in the other fish species is due to the fact that they feed mainly on aquatic insects, invertebrates, fishes, terrestrial invertebrates, phyto- and zooplankton. Zooplankton became more important with increasing fish size and predominated in the diet of the largest fish.

The DDE/DDT ratio can be used to establish whether degradation of DDT is significant or not, and also whether its input occurred recently or in the past. Generally, a high ratio indicates past input of DDT, which has at the time of analysis been largely converted to DDE. A low ratio indicates recent inputs of DDT or inputs of non-degraded DDT (Strandberg and Hites, 2001). The low DDE/DDT residue ratio (0.4–0.9) in fish suggests recent input of DDT formulations in Lake Edward. The detection of higher levels of *p,p′*-DDT than *p,p′*-DDE and *p,p′*-DDD, in most fish samples, also suggests recent exposure of fish to DDT, which has with time been degraded by sunlight, bacteria, etc. The presence of DDT and metabolites in fish may be due to run-off and atmospheric deposi-

Table 2
Levels of DDT and metabolites in fish samples from Lake Edward.

Pesticide	Fish species				
	Bagrus docmac ($\mu g\ kg^{-1}$)	*Protopterus aethiopinus* ($\mu g\ kg^{-1}$)	*Haplochromi nigripinnis* ($\mu g\ kg^{-1}$)	*Oreochromis niloticus* ($\mu g\ kg^{-1}$)	*Clarius gariepinus* ($\mu g\ kg^{-1}$)
p,p′-DDT	15 ± 4	13 ± 4	ND	14 ± 4	ND
o,p′-DDT	11 ± 3	7 ± 2	ND	ND	ND
p,p′-DDE	13 ± 2	9 ± 3	ND	6 ± 2	ND
o,p′-DDE	8 ± 1	68 ± 3	ND	12 ± 1	19 ± 3
p,p′-DDD	5 ± 3	7 ± 5	ND	13 ± 3	ND
o,p′-DDD	ND	ND	ND	6 ± 3	ND
Total DDE/total DDT	0.9	0.7	_	0.4	_

Results are presented as mean ± standard deviation, ND – not detectable.

tion from places where DDT had been used in malaria control campaigns and in agriculture.

A similar study on Lake Edward by Bimenya et al. (2007) reported residue levels of DDT/DDE in *B. docmac*, *P. aethiopinus*, *H. nigripinnis*, *O. niloticus* and *C. gariepinus* fish species as 22.98, 4.92, 3.94, 0.12 and ND $\mu g\ kg^{-1}$, respectively. The above residues could not be compared with the results of the present study, because the authors did not discriminate between DDT and its breakdown products. Furthermore, though no quantifiable levels of DDT/DDE were detected in *C. gariepinus* by the authors, the present study reports the mean concentrations of *o,p′*-DDE as 19 $\mu g\ kg^{-1}$ fresh weight in *C. gariepinus*. DDT is broken down by sunlight to DDE, while microorganisms degrade it to DDD. Hence, species where DDD was detected may be benthic feeders. Generally, the levels of DDT and its metabolites in fish from Lake Edward are similar to those of previous studies on Lake Victoria found in literature (Kasozi et al., 2006; Henry and Kishimba, 2006; Kyarimpa, 2007). In both lakes, the levels of DDT and its metabolites are below the extraneous residue limit of 5000 $\mu g\ kg^{-1}$ set by the Codex Alimentarius Commission of FAO–WHO, 1997.

Similar studies on determination of pesticide residues in the Kenyan freshwater reported *p,p′*-DDT levels in *Tilapia zilli* from River Tana as <0.003 $\mu g\ kg^{-1}$, whereas its metabolites *p,p′*-DDE and *p,p′*-DDD were 140.62 and <0.009 $\mu g\ kg^{-1}$, respectively (Mugachia et al., 1992). In comparison with the current study, the reported *p,p′*-DDT levels in fish from Tana River are lower than the residues detected in fish samples from Lake Edward. However, the *p,p′*-DDE reported in the Kenyan study was more than an order of magnitude higher than that reported in this study. Recent studies of DDT and metabolites in the fish from Kenya side of lake Victoria have reported high levels of *p,p′*-DDD from 10.81–415.95 $\mu g\ kg^{-1}$, whereas DDT and DDE were between 6.63–104.89 $\mu g\ kg^{-1}$ and 9.99–18.95 $\mu g\ kg^{-1}$, respectively (Madadi, 2005).

Studies outside Africa have also reported DDT residues in fish. In Spain levels of DDT and metabolites ranging from non-detectable to 2098 $\mu g\ kg^{-1}$ were detected in *Alburnus alburnus* from River Cinca (Agustina de la et al., 2007). Generally, higher levels were reported than those obtained in this study. The levels, however, were below the set maximum residue limits. A study carried out on *Liza aurata* from Lake Ganzirri in Italy showed mean concentrations of *p,p′*-DDE in the muscles and gills as 3.8 and 25.9 $\mu g\ kg^{-1}$, respectively (Patrizia et al., 2003). The concentrations are similar to those detected in the current study.

4. Conclusions

The DDT residues detected in fish samples from Lake Edward were *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDD and *o,p′*-DDD. The mean concentration of DDT in fish from Lake Edward ranged from ND levels to 68 $\mu g\ kg^{-1}$ fresh weight, but the mean residue levels were below the maximum residue limits recommended by FAO/WHO Codex Alimentarius Commission indicating that fish was safe to consume.

Acknowledgements

The authors gratefully acknowledge financial support from International Program for Chemical Sciences (IPCS) Sweden and the German Academic Exchange Service (DAAD). We thank Kenneth Arinaitwe, Steven Mulinda, Edward Mubiru, Christopher Bitainesha, Stella Nannyonga and Hasifa Nakayima for the GC-ECD and GC–MS sample analysis.

References

Agustina de la, C., Ethel, E., Demetrio, R., Concha, D., Damià, B., 2007. Spatial variation of DDT and its metabolites in fish and sediment from Cinca River, a tributary of Ebro River (Spain). Chemosphere 70, 1182–1189.
Åkerblom, M., 1995. Environmental Monitoring of Pesticide Residues: Guideline for SADAC Region. SADC/ELMS, Monitoring Techniques Series, Sweden.
Beard, J., 2005. DDT and human health. Sci. Total Environ. 355, 78–89.
Bimenya, S.G., Byarugaba, W., Baterana, B.B., Lugemwa, M., Okwi, A.L., 2007. The case for spraying with DDT as a strategy against malaria. In: Bimenya, S.G. (Ed.), Malaria Control and Prevention Strategies and Policy Issues. Forum on Health and Nutrition,. Uganda National Academy of Sciences, pp. 83–96.
Crosly, R.W., Donald, D.B., Block, H.O., 1998. Trends and seasonality in α and γ-hexachlorocyclohexane in western Canadian surface waters (1975–1994). Environ. Pollut. 103, 277–285.
FAO–WHO, 1997. Codex Maximum Residue Limits for Pesticides. FAO, Rome.
Henry, L., Kishimba, M.A., 2006. Pesticide residues in Nile tilapia (*Oreochromis niloticus*) and Nile perch (*Lates niloticus*) from Southern Lake Victoria, Tanzania. Environ. Pollut. 140, 348–354.
Kasozi, G.W., Kiremire, B.T., Bugenyi, W.B., Kirsch, N.H., Nkedi-Kizza, P., 2006. Organochlorine residues in fish and water samples from Lake Victoria, Uganda. J. Environ. Qual. 35, 584–589.
Kyarimpa, C., 2007. Distribution of pesticide residues in parts of fish species *(oreochromis niloticus and lates niloticus)* from murchison bay – Lake Victoria. MSc thesis, Department of Chemistry, Makerere University, Kampala, Uganda.
Madadi, V., 2005. Chemodynamic studies and assessment of pesticide residues in Lake Victoria catchment area for Rivers Sio and Nzoia. MSc thesis, Department of Chemistry, University of Nairobi, Nairobi, Kenya.
Mugachia, J.C., Kanja, L., Gitau, F., 1992. Organochlorine pesticide residues in fish from Lake Naivasha and Tana river, Kenya. Bull. Environ. Contam. Toxicol. 492, 207–210.
Patrizia, L., Giuseppa, D.B., Giacomo, D., Francesco, N., 2003. Organochlorine pesticides, PCBs and heavy metals in tissues of the mullet Liza aurata in Lake Ganzirri and Straits of Messina (Sicily, Italy). Chemosphere 52, 231–238.
Ratcliffe, D.A., 1967. Decrease in eggshell weight in certain birds of prey. Nature 215, 208–210.
Robinson, A.K., Schmidt, W.A., Stancel, G.M., 1985. Estrogenic activity of DDT: estrogen–receptor profiles and the responses of individual uterine types following *o,p′*-DDT administration. J. Toxicol. Environ. Health 16, 493–508.
Rwakimari, B.J., 2007. National policies and strategies for malaria prevention and control. In: Malaria Control and Prevention Strategies and Policy Issues. Forum on Health and Nutrition, Uganda National Academy of Sciences, pp. 34–43.
Strandberg, B., Hites, R.A., 2001. Concentration of organochlorine pesticides in wine corks. Chemosphere 44, 729–735.
Turusov, V., Rakitsky, V., Tomatis, L., 2002. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistences and risks. Environ. Health Perspect. 110 (2), 125–128.
UNEP, 2001. The Stockholm Convention on Persistent Organic Pollutants, Geneva, Switzerland. United Nations Environmental Programme (UNEP) Chemicals (June, 2001).