ASSESSMENT OF OCCURRENCE AND CONCENTRATIONS OF XENOBIOTICS IN SELECTED FISH SPECIES FROM LAKE NAIVASHA, KENYA

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Abstract

Aquatic organisms are good bio-indicators of the environmental health in freshwater lakes; Xenobiotics are foreign materials to the organisms' body and do not occur naturally in nature. Xenobiotics accumulate in adipose tissues due to high affinity to fatty tissues. This paper reports the levels of Organochlorine pesticide (OCPs) residues, their metabolites and Polychlorinated Biphenyl (PCBs) congeners in selected fish species from Lake Naivasha, Kenya. PCBs and OCPs are persistent organic pollutants most of which are banned or under restricted usage. The levels of *p, p'*- DDT, *p, p'*- DDE, *p, p'*- DDD, heptachlor, heptachlor epoxide, aldrin, dieldrin, CB28, CB52 and CB105 were determined in Tilapia, *Oreochromis leucostictus,* Common carp, *Cyprinus carpio,* and Mirror carp, *Cuprinus spectacularlus,* during the months of September - December 2008. The concentrations ranged within Below Detection Limit (BDL) - 0.29 for Heptachlor epoxide, 0.42 - 4.19 µg/Kg, for Heptachlor, 0.43 - 4.73 Aldrin, BDL - 0.34 Dieldrin, *p, p'* - DDT, BDL - 6.69 *p, p'* - DDE, BDL - 27.15 *p, p'* - DDD, BDL - 28.87 methoxychlor, 0.05 - 5.56 CB28, 0.012 - 13.91 CB52 and BDL - 0.45 CB105. The OCPs and PCBs varied widely between and within species. *C. spectacularlus* showed high levels followed by *C. carpio* and *O. leucostictus* respectively. Trophic position, lipid contents, weight and age/size of fish were important parameters in Xenobiotics concentrations in specimens. The occurrences of the Xenobiotics in fish indicate recent use in the catchment. The mean values and ranges of residues found in fish were below the FAO/WHO maximum acceptable limits in fish and sea food however increased monitoring is recommended to detect any changes.

Key words***:*** *Pesticides, Organochlorine, Pollution, PCBs, Methoxychlor, DDT, Heptachlor*.

Introduction and Literature Review.

Polychlorinated organic pollutants (POPs) use has resulted in acute and chronic ecological damage, either by direct injury to both target and non-target organisms such as insects, primary producers, crustacea, anthropod, birds and fish, or by indirect effects such as elimination of natural enemies (UNEP, 1999). POPs are manmade and persistent in the environment, the hydrophobic nature lead to bio-accumulation in fatty tissues thus entering the food chain (UNEP, 1999). POPS find wide applications in agriculture in vector control and in industry in Kenya (Mitema and Gitau, 1992; Mugacia *et al*., 1992; Gitahi 2002). Once applied in the fields they are transported to aquatic bodies by rain runoff, rivers and streams and associate with biotic and abiotic macro-particles (Wandiga, 2001; Wandiga *et al*., 2002;

Getanga *et al*., 2004). They are removed from the water column to the benthic layers by settling of the particles into the water column (Jalili *et al*., 2007). The lipophilic nature, hydrophobicity, low chemical and biological degradation rates lead to their accumulation in biological tissues and subsequent magnification in organisms progressing up the food chain (Mugacia *et al*., 1992; Wandiga 2001; Gitahi *et al*., 2002; Mavura and Wangila, 2004). Consumption of fish from contaminated water bodies is considered to be an important route of exposure to persistent Organochlorine compounds (Mavura and Wangila, 2004; Mwevura *et al*., 2002). Detectable levels of pesticide residues have been reported in inland waters in Kenya (Mugacia *et al*., 1992 Wandiga, 2001; Gitahi *et al*., 2002; Getanga *et al*., 2004).

Polychlorinated biphenyls (CAS number 1336-36-3) are a class of organic compounds with 1 to 10 chlorine atoms attached to biphenyl, which is a molecule composed of two benzene rings. PCBs were used as coolants and insulating fluids ('transformer oil') for transformers and capacitors especially in components of early fluorescent light fittings, the locomotive's electrical transformers, plasticizers in paints and cements, stabilizing additives in flexible PVC coatings of electrical wiring and electronic components, pesticide extenders, cutting oils, reactive flame retardants, lubricating oils, hydraulic fluids, sealants (for caulking in schools and commercial buildings (Rudel *et al*., 2008), adhesives, wood floor finishes (such as *Fabulon* and other products of Halowax in the U.S.) (Rudel *et al*., 2008; Kathleen *et al*., 2009), paints, de-dusting agents, water-proofing compounds, casting agents, vacuum pump fluids, fixatives in microscopy, surgical implants, and in carbonless copy paper. Due to PCB's toxicity and classification as a persistent organic pollutant, PCB production was banned by the United States Congress in 1979 and by the Stockholm Convention on Persistent Organic Pollutants in 2001 (Kathleen *et al*., 2009).

Due to the undesirable effects on environmental quality and aquatic life, the production and usage of POPs were banned or severely restricted during the 1970s and 1980s in most developed countries. However, their demand has been increasing in some developing 124 countries in Africa, Latin America and Asia (Tanabe *et al*., 1993). There has been widespread use of chlorinated pesticides in Kenya in the last four decades because agriculture has been the mainstay of Kenya’s economy. Organochlorine pesticides have been extensively used and particularly, DDT and endosulfan for the control of maize and cotton pests. Lindane, dieldrin, aldrin, endrin and heptachlor have also had wide usage in Kenya, which has made their presence ubiquitous in the environment (Wandiga, 2001).

Lake Naivasha, Kenya’s second largest freshwater lake was recognized as a site of international significance and was awarded RAMSAR status in 1995 (RCW, 2010), however in recent years the lake has undergone significant ecological changes leading its classification as a RAMSAR site requiring urgent attention to save it from extinction.

The purpose of this study was to assess occurrence and concentrations of *p, p’*- DDT, *p, p’*- DDE, *p, p’*- DDD, heptachlor, heptachlor epoxide, aldrin, dieldrin, CB28, CB52 and CB105 in Tilapia *Oreochromis leucostictus,* Common carp, *Cyprinus carpio,* and Mirror carp, *Cuprinus spectacularlus*, from the Lake Naivasha basin, Kenya.

Materials and methods

Field sampling

Sampling was carried out November 2008 and May 2009. Fish net caught *O. leucostictus, C. carpio* and *C. spectacularlus* specimens were bought from licensed fishermen and identification done by the Kenya Marine and Fisheries Research Institute (KEMFRI) staff. Fish specimens were wrapped with aluminum foils and transported to the laboratories in cooler boxes under ice. The samples were extracted immediately and analyzed for *p, p'*- DDT, *p, p'*- DDE, *p, p'*- DDD, heptachlor, heptachlor epoxide, aldrin, dieldrin, methoxychlor, CB28, CB52 and CB105.

Extraction

Twenty grams samples were taken in triplicates and mixed with 20 g analytical grade anhydrous sodium sulfate in a mortar and crushed to give a homogeneous dry mixture. The mixtures were transferred into flasks and shaken for about 15 minutes with HPLC grade dichloromethane. The extracts were filtered through a glass wool plug into an evaporating flask and extraction repeated three times, with 50 cm^3 of dichloromethane. The extracts were pooled and evaporated completely at 40 ºC with a rotor vapor leaving only the lipid portion.

The pesticides were salted out through partitioning by dissolving in HPLC grade petroleum ether and 650 ml distilled water, 20 ml phosphate buffer pH 6.0, and shaken with hexane. 500 ml distilled water and 50 ml saturated sodium sulfate was then added and shaken vigorously. The aqueous layer was discarded and the hexane layers combined. The hexane extract was concentrated in rotary vapor.

Clean up

The extracts were cleaned by passing through a column packed with analytical grade florisil from Florisin Company packed with a one inch layer of anhydrous sodium sulfate both at the top and below the florisil.

Analysis

Sample analysis was done using Varian CP 3800 Gas Chromatograph equipped with Electron Capture Detector. Separation was done using BPX 5 capillary column of dimensions 30 m x 0.25 mm x 0.25 µm film thickness. Confirmatory analysis was done using BPX35 capillary column of dimensions 50 m x 0.25 mm x 0.25 µm film thickness.

A temperature program was used starting from 90 ^{0}C (with hold time of 3 minutes), increased to 215 ^{0}C at 8 ^{0}C/min (with hold time of 25 min), then increased to 270 ^{0}C at 5 ^{0}C/min (with hold time of 5.37 min), and finally ramped to 275 ^{0}C at 5 ^{0}C/min (with hold time of 18.63 min). The carrier gas was high purity helium (99.9995%) with white spot nitrogen as the makeup gas. Quantification followed external calibration method using high purity pesticide reference standards mixture obtained from Ultra Scientific USA.

Quality Control and Quality Assurance

All sampling, extraction and analysis were done in triplicate to allow verification detected pesticide residues. The samples were spiked with PCB 155 during extraction and PCB 198 during analysis to minimize errors due to detector fluctuations. Recovery tests were also carried out using the reference pesticide standards to determine performance of the methodology. Quantification of pesticide residues was carried out using high purity pesticide reference standards.

Results

The average percentage recoveries were determined by spiking a known sample of lipid with standard and the sample treated using the analytical procedure, results are presented in Table 1. Precision and accuracy were also determined as measures of quality of analytical data and are presented.

Table 1. Quality control and assurance data for pesticide and PCB residues in fish.

OCPs	**Average recovery (%)**	**RSD**	**Accuracy**
p, p' - DDT	79.56 ± 2.89	9.1± 1.8	90.2± 2.3
Aldrin	80.23 ± 2.12	12.2± 0.9	88.8± 3.4
Heptachlor	76.12 ± 1.56	8.5± 2.5	87.2± 2.9
CB105	83.24± 2.56	6.7± 1.3	92.3± 3.1

Mean ± Standard Deviation, n = 3.

Lipid content (% wt/wt), length (cm), moisture (%), and weight (g) of Fish specimens were determined and are presented in Table 2.

Table 2. Lipid content (% wt/wt), length (cm), moisture (%), and weight (g) of Fish specimens

	O. leucostictus	***C. carpio***	***C. spectacularlus***
Lipid content (%)	1.87 ± 0.97	0.78 ± 0.01	0.92 ± 0.19
Moisture content (%)	78.89 ± 3.07	79.22 ± 3.81	79.78 ± 1.25
Weight (g)	202.69 ± 33.05	829.96 ± 196.67	765.13 ± 29.75
Length (cm)	22.57 ± 1.02	42.1 ± 3.94	41.66 ± 0.76

Mean ± Standard Deviation, n = 3.

The mean weight and length show similar trends and were highest for *C. carpio* followed by *C. spectacularlus* and *O. leucostictus* respectively. *O. leucostictus* had the highest percentage lipid content of 1.87 ± 0.97, 0.78 ± 0.01 *C. spectacularlus* and 0.92 ± 0.19 *C. carpio.* The moisture contents were within the range of 78.89 - 79.78 %, there was no significant difference at p=0.05. Organochlorine pesticides were determined in *O. leucostictus, C. carpio,* and *C. spectacularlus* data is presented in Table 3.

Table 3. Organochlorine pesticide residues (µg/Kg, wet weight) in Fish specimens

	O. leucostictus	***C. carpio***	***C. spectacularlus***
p, p' - DDT	BDL - 1.91	BDL - 7.26	BDL - 0.72
p, p' - DDE	0.21 - 0.47	0.14 - 0.51	0.21 - 6.69
p, p' - DDD	BDL - 21.13	BDL - 27.15	0.23 - 4.33
Heptachlor	0.410 - 1.01	0.42 - 4.19	0.81 - 1.58
Heptachlor epoxide	BDL - 0.03	BDL - 0.14	0.14 - 0.22
Aldrin	0.43 - 1.63	0.17 - 2.89	1.35 - 4.73
Dieldrin	BDL	BDL - 1.19	BDL - 1.58

Mean ± Standard Deviation, n = 3, BDL - Below detection limit

p, p'- DDT and its metabolites *p, p'*- DDE and *p, p'*- DDD residues were analyzed and ranged within BDL - 7.26 µg/Kg, wet weight (Table). *p, p'*- DDE was detected in all specimens and ranged within 0.14 - 6.69 µg/Kg, wet weight. The highest concentration of *p, p'*- DDE was found in *C. spectacurlus*, whereas *C. carpio* recorded the highest concentrations for *p, p'*- DDD and *p, p'*- DDT. The concentration of *p, p'*- DDD were high in most of the cases, followed by *p, p'*- DDT and *p, p'*- DDE respectively. The high levels of *p, p'*- DDD compared to *p, p'*- DDT implied degradation of DDT to the *p, p'*- DDD metabolite and *p, p'*- DDE.

Among the screened pesticides, heptachlor was detected in all specimens; however heptachlor epoxide was not detected in all specimens analyzed (Table 3). Results indicate low concentrations of the epoxide compared to heptachlor indicating recent use of heptachlor in the catchment. The highest concentrations of heptachlor were recorded in *C. carpio* followed by *C. spectacurlus* and *O. leucostictus* respectively. Compared to its main metabolite heptachlor epoxide, heptachlor concentrations were 10 to 50 times higher for most specimens. The highest level of heptachlor epoxide is found in *C. spectacurlus* followed by *C. carpio* and *O. leucostictus* respectively. The fact that heptachlor was detected in fish samples indicate recent use of the pesticide in the catchment. In addition, the high levels of heptachlor compared to heptachlor epoxide imply the possibility of recent use of heptachlor.

Aldrin was detected in all specimens whereas dieldrin was detected in *C. carpio* and *C. spectacurlus* only (Table). Aldrin ranged within 0.17 - 4.73 µg/Kg, wet weight, the highest concentrations were found in *C. spectacurlus*, *C. carpio* and *O. leucostictus* respectively.

Aldrin is converted in the environment to dieldrin through epoxidation; the low dieldrin concentrations could indicate recent use of aldrin in the catchment. Dieldrin was not detected in *O. leucostictus* but higher levels were registered in *C. spectacurlus* compared to *C. carpio*.

The levels of CB28, CB52 and CB105 were determined in fish specimens and the results are presented in Table.

Table 4. Polychlorinated biphenyls concentrations (µg/Kg, wet weight) in Fish specimens

	O. leucostictus	***C. carpio***	***C. spectacularlus***
CB28	0.07 - 0.19	0.17 - 5.57	0.19 - 1.61
CB52	0.04 - 3.08	0.11 - 1.56	0.19 - 13.94
CB105	BDL - 0.43	BDL - 0.33	BDL - 0.45

Mean ± Standard Deviation, n = 3, Below detection limit - BDL

CB28 was detected in all samples analyzed (Table 4), this ranged within 0.07 - 5.57 µg/Kg, wet weight, the highest concentrations were found in *C. carpio* followed by *C. spectacurlus* and *O. leucostictus* respectively. CB28 concentrations are appreciably low in *O. leucostictus* compared to other species. CB52 was detected in all specimens and ranged within 0.04 - 13.94 µg/Kg, wet weight, the highest concentrations were recorded in *C. spectacurlus* and the lowest in *O. leucostictus* however CB52 was higher in *O. leucostictus* than in *C. carpio* unlike CB52. CB105 was not detected in all specimens. The study shows that the highest CB105 were recorded in *C. spectacurlus* followed by *O. leucostictus* and *C. carpio* respectively. The study show wide variations both between and within samples.

Conclusions

The detection of xenobiotics in fish indicate recent use of the chemicals or mixtures in the catchment. The determined xenobiotics are banned or under restricted use in Kenya, this calls for regular monitoring to indentify sources and to detect changes. Though the chemicals were in low concentrations they are likely to adversely affect the health and well being of the aquatic life and should thus be banned.

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References

Getenga, Z. M., Kengara, F. O., and Wandiga, S. O., (2004). Determination of organochlorine pesticides in soil and water from River Nyando drainage system within Lake Victoria Basin, (Kenya), *Bull. Environ. Contam. Toxicol*, **72**:335-342.

Gitahi, S.M., Harper, D.M., Muchiri, S.M., Tole, M.P., and Ng'ang'a, R.N., (2002). Organochlorine and organophosphorus Pesticides concentrations in water, sediment, and selected organisms in Lake Naivasha (Kenya), *Hydrobiologia* **488**:123-128.

Jalili, S.H., Ilkhanipour, M., Heydari, R., Farshid, A.A., and Salehi, S., (2007). The Effects of Vitamin E on Endosulfan–Induced Oxidative Stress in Rat Heart. *Pakistan Journal of Nutrition*, **6:**375-380.

Kairu, J. K., (1994). Pesticide residues in birds at Lake Nakuru, Kenya. *International Journal of Salt Lake Research* **3**:31-48.

Kathleen E., Zhao, C.Y., and Kane, C.M., (2009). Organochlorine Pesticides, PCBs, Dioxins, and Metals in Postterm Peregrine Falcon (*Falco peregrinus*) Eggs from the Mid-Atlantic States, 1993–1999, *Arch. Environ Contam. Toxicol*. **57**:174-184.

Mavura W.J., and Wangila, P.T., (2004). *Distribution of Pesticide Residues in Various Lake Matrices: Water, Sediment, Fish And Algae, The Case of Lake Nakuru, Kenya*. The african network for chemical analysis of pesticides, Arusha International Conference Centre 8th – 11th August.

Mitema, E.S., and Gitau, F.K. (1990). Organochlorine residues in fish from Lake Victoria, (Kenya), *Afri J. Ecol.* **28**:234-239.

Mugacia, J.C., Kanja, L., and Gitau, F., (1992). Organochlorine pesticide residues in fish from Lake Naivasha and Tana River, Kenya, *Bull. of Environ. Contam. Toxicol*. **49**:207-210.

Mwevura, H., Othman, C., and Mhehe, L., (2002).Organochlorine Pesticide Residues in Edible Biota from the Coastal Area of Dar Es Salaam City *Western Indian Ocean J. Mar. Sci*., **1**:91–96.

Wandiga, S.O., (2001). Use and distribution of Organochlorine pesticides, The future in Africa. *Pure Appl. Chem*. **73**:1147-1155.

Wandiga, S.O., Yugi, M.W., Barasa, M.W., Jumba, I.O., and Lalah, J.O., (2002).The distribution of organochlorine pesticides in Marine samples along the Indian Ocean Coast of Kenya, *Environmental Toxicology*, **23**:1235-1246.

RCW (Ramsar convention on wetlands), 2010. *Update on the status of sites in the Ramsar. List of Wetlands of International Importance* 41st meeting of the standing committee, Kobuleti, Georgia, 30th April - 1st May 2010.

UNEP Chemicals (1999). *Guidelines for the Identification of PCBs and Materials Containing PCBs*. United Nations Environment Programme. pp. 2. http://www.chem.unep.ch/pops/pdf/PCBident/pcbid1.pdf. Retrieved 2007-11-07.

Wasswa, J., and Kiremire, B. T., (2004). *Pesticide residue distribution in sediment and fish Samples from the Ugandan side of Lake Victoria*, The African network for chemical analysis of pesticides Arusha International Conference Centre, 8th - 11th August