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Pesticide Residues and Heavy Metals in Lake Victoria Nile Perch, *Lates niloticus*, Belly Flap Oil

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Abstract Oil was extracted from the belly flaps of varied sizes of Nile perch caught from Lake Victoria (Uganda). The oil was analyzed for pesticide residues and heavy metals. Total residual concentration of dichlorodiphenyltrichloroethane, endosulfan, hexachlorocyclohexane, hexachlorobenzene, heptachlor, chlordane, endrin, aldrin and chlorofenvinphos increased significantly ($p < 0.05$) with fish size. Mercury and lead were detected in most samples while arsenic and cadmium were below detection limits. Nile perch may, therefore, accumulate significant amount of chemical contaminants. Levels of contaminants in Nile perch oil were, in general, within limits considered acceptable by the stringent German Food Law for human consumption.

Keywords Lake Victoria Nile perch · Chemical contaminants

Introduction

Lake Victoria, the second largest freshwater body in the world and the largest in Africa, is shared by Kenya (6%), Uganda (43%) and Tanzania (51%). Nile perch (*Lates niloticus*) accounts for nearly two thirds of Lake Victoria's fish population (MAAIF 2006). Fish production in Uganda is estimated at 220,000 metric tonnes annually. Nile perch alone contributes ~60% of the total capture fisheries by volume. Frozen and chilled Nile perch fillets are a major export commodity for Uganda earning the country about USD 143 M yearly. However, there is limited value addition to processing byproducts such as belly flaps. Nile perch belly flaps are mainly made of lipids and therefore a potential source of fish oil (MAAIF 2006).

Heavy metals, organochlorine and organophosphorus pesticide residues may accumulate in fish tissue, especially if the source water contains their residues. However, Kasozi et al. (2006) studying Lake Victoria fish in 1996 reported presence of organochlorine pesticide residues in Nile perch tissue albeit at low levels. This study analyzed Lake Victoria Nile perch belly oil for organochlorine, organophosphates and heavy metals. Dichlorodiphenyltrichloroethane, endosulfan, hexachlorocyclohexane, hexachlorobenzene, heptachlor, chlordane, endrin, aldrin and chlorofenvinphos, mercury, cadmium, arsenic and lead are toxic to humans. It is therefore important to monitor their residue levels in food products. Given their capacity for biomagnifications along the food web, chemical contaminants are likely to be in higher concentrations in fish products from polluted waters (Storelli and Marcotrigiano 2001). Recent data meanwhile indicate that Lake Victoria is eutrophied with substantial rise in algal blooms (Ogutu-Ohwayo and Balirwa 2004). Nutrient enrichment in the lake is shown to be a consequence of increasing human activities along the catchment areas, untreated industrial effluents, and uncontrolled combustion processes (Ogutu-Ohwayo and Balirwa 2004). Nile perch, being a predator, is highly susceptible to accumulation of organic and inorganic contaminants and this may increase with age (Campbell et al. 2003). This study aimed at determining the levels of pesticide residues and heavy metals in Nile perch belly oil and investigating their variations with fish age, proxied by size.

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Materials and Methods

Nile perch (*Lates niloticus*) of three size categories (≤2, 10–30 and ≥40 kg) were obtained from four landing sites (Lambu, Kigungu, Kasenyi and Katosi) around Lake Victoria in September 2006. For each fish, fatty material was obtained from the visceral cavity after dissecting the fish. Nile perch weighing 1–2, 10–30 and 40–80 kg had average fatty tissue weights 0.10%, 0.03% and 0.03% body weight, respectively.

Fatty material from belly flaps was homogenized using a warring blender (Patterson Scientific, Blender 800E, USA) run for about 2 min. Crude oil was extracted by incubating the homogenate at 35°C for 1 h. Anhydrous sodium sulphate (30% w/w) was then added to the homogenate and centrifuged (1,150*g* for 20 min at 25°C). Extracted oil was stored at −20°C in 30 mL vials and analyzed within 2 weeks.

Nile perch belly oil was analyzed for chlorinated pesticides (DDT, aldrin, hexachlorocyclohexane [α-HCH], dieldrin, endrin, heptachlor and their metabolites, β-HCH, 2, 4′-dichlorodiphenyldichloroethane [2, 4′-DDD], 4, 4′-DDD, 4, 4′-DDE, 2, 4′-DDT, 4, 4′-DDT, heptachlor epoxide, lindane and nonacachlor) and chlorofenvinphos. Oil samples were purified according to the method described by Hollamby et al. (2004). Crude belly oil (10 g) was dehydrated by mixing with anhydrous sodium sulphate (100 g) in the beaker. Dehydrated sample was then transferred to a beaker and dissolved in a mixture of ethyl ether:petroleum ether, 1:1. The mixture was then warmed in a water-bath at 60°C for ∼5 min. The process of ether extraction was repeated thrice and the combined extract dried over a warm water-bath. Subsequently, the dry extract was dissolved in hexane and 1 mL of the solution purified by passing through a Silica Gel column (5 g Silica Gel 60, 9-mm column i.d.). A blank sample of refined Nile perch belly oil that was pre-tested and found to have no detectable pesticide residues was similarly prepared and spiked at 0.1 ppm.

Standard curve (area verses concentration) was prepared using organochlorine pesticides mixture containing 20 analytes in hexane:toluene (50:50) mixture at 2,000 μg/mL. Standard solutions was prepared by serial dilution of the standard reagents. Linear standard curves in the range of 0.002–0.5 ppm were used for quantification. Final duplicate extracts (1 μL) were analyzed on a fused silica capillary column (100% dimethylpolysiloxane 6′ × 0.25″ i.d., 0.52 μm) by gas chromatography (PerkinElmer, Norwalk, USA) coupled with high-resolution mass spectrometry.

Mercury, lead, arsenic and cadmium in Nile perch belly oil were determined using atomic absorption spectrometer (AAS; PerkinElmer, Norwalk, USA) as described by AOAC (1999) methods 971.21, 972.25, 986.15 and 973.34, respectively. Samples were digested using a mixture of concentrated nitric acid and sulphuric acids (1:1). Mercury was determined at 253.7 nm, lead at 283.3 nm, arsenic at 840 nm, and cadmium at 228.8 nm. Quantitative analysis of mercury was carried out using a cold vapour AAS. Mercury analysis was performed after reduction using tin (II) chloride. Mercury (II) chloride, lead nitrate, sodium arsenate and cadmium sulphate were used for preparing mercury, lead, arsenic and cadmium standards, respectively.

Data collected was analyzed using SPSS statistical package. Analysis of variance, ANOVA, was performed to compare the level of pollutants between the fish size categories. Differences between the means were obtained using the least significance difference (LSD).

Results and Discussion

Total DDT and DDE residue levels in Nile perch oil increased significantly ($p < 0.05$) with fish size category (Table 1). Belly oil of the small size fish (<2 kg) presented lower mean total DDT residue concentration (15.18 ± 1.03 μg kg^{-1} lipid) than the medium size (10–30 kg) fish (45.25 ± 3.78 μg kg^{-1} lipid). The large size fish (>40 kg)

Table 1 Residue levels (μg kg^{-1}) of DDT metabolites in oil extracted from Nile perch belly flap of varying sizes

Metabolite	Fish size (kg)			Mean (μg kg^{-1})
	Small (1–2)	Medium (10–30)	Large (40–80)	
O,P′-DDE	0.52 ± 0.02^{a}	1.10 ± 0.21^{b}	1.10 ± 0.03^{b}	0.91 ± 0.54
P,P′-DDE	12.53 ± 0.54^{a}	30.95 ± 5.26^{b}	58.55 ± 3.51^{c}	34.01 ± 1.62
O,P′-DDD	0.63 ± 0.15^{a}	3.05 ± 0.04^{c}	1.35 ± 0.14^{b}	1.68 ± 0.01
P,P′-DDD	0.17 ± 0.00^{a}	3.40 ± 0.76^{c}	0.40 ± 0.00^{b}	1.32 ± 0.18
O,P′-DDT	0.48 ± 0.08^{a}	3.05 ± 0.62^{b}	4.50 ± 1.02^{c}	2.68 ± 0. 36
P,P′-DDT	0.85 ± 0.20^{a}	3.70 ± 0.71^{b}	4.90 ± 0.03^{c}	3.15 ± 0.28
Total DDT	15.18 ± 1.03^{a}	45.25 ± 3.78^{b}	70.80 ± 4.62^{c}	43.74 ± 1.74

Values in rows followed by a different superscript are significantly different ($p < 0.05$)

DDT dichlorodiphenyltrichloroethane, *DDD* dichlorodiphenyldichloroethane, *DDE* dichlorodiphenylchloroethane

Table 2 Residue levels (μg kg^{-1}) of endosulfan metabolites in oil extracted from Nile perch belly flap of varying sizes

Metabolite	Fish size (kg)			Mean (μg kg^{-1})
	Small (1–2)	Medium (10–30)	Large (40–80)	
α-Endosulfan	1.30 ± 0.00[a]	1.35 ± 0.30[b]	3.70 ± 0.07[c]	2.14 ± 0.09
β-Endosulfan	ND	5.10 ± 0.63[a]	5.60 ± 1.16[b]	5.35 ± 0.18
Endosulfan sulphate	0.79 ± 0.14[a]	2.75 ± 0.07[b]	4.00 ± 0.16[c]	2.51 ± 0.02
Total endosulfan	2.09 ± 0.13[a]	9.20 ± 0.99[b]	13.36 ± 0.92[c]	8.22 ± 0.01
(α-/β-) Endosufan	-	0.26	0.67	0.40

Values in rows followed by a different superscript are significantly different ($p < 0.05$)

ND not detected

had the highest DDT residue concentration (70.80 ± 4.62 μg kg^{-1} lipid). All samples contained higher levels of P,P′-DDE (12.53–58.55 μg kg^{-1} lipid), the main DDT metabolite, than the parent P,P′ -DDT (0.85–4.90 μg kg^{-1} lipid). This is indicative of past exposure of Lake Victoria Nile perch to the persistent pesticide. Increase in the mean DDE concentration with fish size is indicative of bioaccumulation (Henry and Kishimba 2006). Data on DDT residue levels, in Nile perch from the Ugandan side of Lake Victoria, were in same range as values reported in this study, for the large size category (Kasozi et al. 2006). However, Kasozi et al. (2006) did not report the weight range of fish used for sample preparation. Meanwhile, studies from the Tanzanian side of Lake Victoria, reported much higher level of DDT residues (3,800 μg kg^{-1} lipid) in Nile perch tissue (Henry and Kishimba 2006). This was attributed to application of the pesticide shortly before the study. Higher DDT content has been reported for salmon, halibut and cod liver oils than values recorded for Nile perch belly oil in this study (Tsigouri and Tyrpenou 2000). Results of this study further demonstrate that DDT residue concentration in Lake Victoria Nile perch oil is still far below the recommended German maximum residue level (MRL) of 5,000 μg kg^{-1} for fish products (CREM/CBI 1998). The German food standard so far provides the most stringent requirement for exports of fish and fish products to European Union (EU) countries.

Endosulfan was found in trace concentration in Nile perch oil and varied significantly with fish size ($p < 0.05$; Table 2). In all samples, levels of β-endosulfan (not detected-5.60 μg kg^{-1} lipid) were higher than α-endosulfan (1.35–3.70 μg kg^{-1} lipid) except for the small fish category, where the β-isomer was not detected. The observed isomer distribution is expected since the α-isomer, which is more volatile than the β-isomer, is easily dissipated (Guerin 2001). However, beta-endosulfan is more readily metabolized than the α-isomer. This could therefore explain the lower concentration of the β-endosulfan in oil from the small size Nile perch than the α-isomer (Nowak et al. 1995). The lower β-endosulfan concentrations than α-endosulfan, may also be a consequence of their varied composition in commercial endosulfan (β:α = 30:70). According to Nowak et al. (1995), (α/β)-endosulfan ratio ranging from 0.5 to 1.0 is indicative of recent exposure of the fish to endosulfan. Therefore, Nile perch at the Ugandan side of Lake Victoria with (α/β)-endosulfan ratio of 0.67 shows recent application of endosulfan in the lake basin. Moreover, the higher concentrations of endosulfan sulphate observed in all the fish size categories confirm long time exposure of the fish to the pesticide (Henry and Kishimba 2006).

Nile perch belly oil content of endosulfan sulphate, the stable endosulfan metabolite, and total endosulfan generally increased ($p < 0.05$) with fish size. This may be attributed to bioaccumulation of the pesticide residues in the fish tissue stemming from its application around Lake Victoria basin. Consequently, the mean total endosulfan residues in large fish (13.36 ± 0.92 μg kg^{-1}) exceeded the prescribed MRL (10 μg kg^{-1}) for foods of animal origin (CREM/CBI 1998). However, total endosulfan residue levels in medium (9.20 ± 0.99 μg kg^{-1} lipid) and small (2.09 ± 0.13 μg kg^{-1} lipid) size fish were still within the food safety requirements for fish products (CREM/CBI 1998) and close to that reported by Kasozi et al. (2006). The content of endosulfan in a black bass (*Micropterus salmoides*), a fatty fish species, from Lake Naivasha (Kenya) was higher than that in this study (Gitahi et al. 2002).

Mean values of α- and γ-HCH residue concentrations (1.29 ± 0.06 and 0.69 ± 0.00 μg kg^{-1} lipid, respectively) in Nile perch belly oil were significantly lower ($p < 0.05$) than that of β-isomer (1.36 ± 0.14 μg kg^{-1} lipid; Table 3). HCH is predominantly converted to beta-HCH, which is the most persistent and bioaccumulative isomer in the animal tissue. A recent study on Nile perch from the Napoleon gulf of Lake Victoria (Uganda), reported higher γ-HCH (lindane) levels than that reported in this study (Kasozi et al. 2006). Proximity of the sampling site to the source of the parent pesticide as well as time lag in the pesticide application is probably the reason for the difference in the residue levels. The HCH in Nile perch belly oil may be due

Table 3 Levels (μg kg^{-1}) of organic pollutants in oil extracted from Nile perch belly flap of varying sizes

Compound	Fish size (kg)			Mean (μg kg^{-1})	MRL
	Small (1–2)	Medium (10–30)	Large (40–80)		
DDT	15.18 ± 1.03[a]	45.25 ± 3.78[b]	70.80 ± 4.62[c]	43.74 ± 1.74	5,000
Endosulfan	2.09 ± 0.13[a]	9.20 ± 0.99[b]	13.36 ± 0.92[c]	8.22 ± 0.01	10
α-HCH	1.29 ± 0.06[a]	ND	ND	1.29 ± 0.06	200
γ-HCH	0.43 ± 0.04[a]	0.65 ± 0.08[b]	1.00 ± 0.05[c]	0.69 ± 0.00	500
β-HCH	0.69 ± 0.06[a]	2.55 ± 0.01[c]	0.85 ± 0.17[b]	1.36 ± 0.14	100
Aldrin	0.33 ± 0.03[a]	6.00 ± 1.52[c]	2.45 ± 0.43[b]	2.93 ± 0.46	200
Dieldrin	ND	ND	ND	ND	200
Endrin	1.07 ± 0.07[a]	3.50 ± 0.63[b]	3.70 ± 0.32[c]	2.76 ± 0.24	10
Heptachlor	1.86 ± 0.15[a]	4.10 ± 0.74[b]	5.40 ± 1.16[c]	3.79 ± 0.48	10
Chlordane	1.38 ± 0.16[a]	3.65 ± 0.17[b]	3.70 ± 0.95[b]	2.91 ± 0.22	10
HCB	0.14 ± 0.02[a]	0.90 ± 0.08[c]	0.60 ± 0.05[b]	0.55 ± 0.01	500
Chlorofenvinphos	0.35 ± 0.00[a]	0.50 ± 0.05[b]	0.48 ± 0.00[b]	0.38 ± 0.01	NS

Values in rows followed by a different superscript are significantly different ($p < 0.05$)

DDT dichlorodiphenyltrichloroethane, *HCB* hexachlorobenzene, *HCH* hexachlorocyclohexane, *MRL* German maximum residue level, *ND* not detected, *NS* no standard

to its high affinity for fatty tissue leading to higher levels in samples from the larger size fish. These values are, however, lower than α- and γ-HCH recorded for a carnivorous sole fish (*Cynoglossus abbreviatus*) and silver carp (*Hypophthalmichthys molitrix*; Zhou et al. 2007). In addition, residual levels of the different isomeric forms of HCH in belly oil from Nile perch of varying sizes were below recommended limits for human consumption (Table 3).

Amounts of dieldrin in samples from all fish size categories were below detectable level (Table 3). Despite its high persistence in the environment and previous application in Lake Victoria basin (Ejobi et al. 1996a), residual concentration was below the limit of detection of 0.001 μg kg^{-1} lipid. Studies on avifauna, from the Ugandan side of Lake Victoria catchment area, also showed similar results with no dieldrin residues detected in both fish and bird tissues (Hollamby et al. 2004). Kasozi et al (2006), on the contrary, reported higher concentrations of dieldrin residues in Lake Victoria Nile perch captured in 1998. Reduced level of dieldrin residue in fish tissue is perhaps associated with controlled application of the pesticide. Dieldrin was reportedly applied in 1970s in Lake Victoria basin for vector control, particularly against banana weevils and tsetse flies (Ejobi et al. 1996 a). Surprisingly, the levels of aldrin (0.33–2.45 μg kg^{-1} lipid), the parent dieldrin, observed in this study was similar to that reported by Kasozi et al. (2006). Aldrin levels in the present study increased significantly ($p < 0.05$) with fish size probably because of bioaccumulation. However, pesticide residue level in Nile perch belly oil is still within the German acceptable limit. Aldrin content of Nile perch oil was in addition close to that reported for red swamp crayfish (*Procambarus clarkii*) from Lake Naivasha (Kenya; Gitahi et al. 2002).

Total heptachlor, chlordane, endrin and HCB residue concentrations in Nile perch oil increased ($p < 0.05$) with fish size category (Table 3). Heptachlor epoxide and γ-chlordane levels ranged from 1.21–4.60 to 0.05–2.05 μg kg^{-1} lipid, respectively. Higher levels of these stable metabolites of chlordane and heptachlor, than their parent compounds, confirm pesticide application in the lake region. Notably, HCB and endrin conversion products are presently not included in the MRLs under German Food Law (CREM/CBI 1998). Observed trace amounts of total heptachlor, chlordane, endrin and HCB in oils derived from fish of different sizes may be due to pesticide accumulation in the fatty tissue. Unfortunately, previous studies on pesticide residues in Nile perch did not provide data for comparison of these pesticides. However, the amounts of heptachlor and endrin in this study were lower than those reported for silver carp (*Hypophthalmichthys molitrix*; Zhou et al. 2007). Total chlordane and HCB concentrations in Nile perch were also found far lower than values reported for yellow perch (*Perca flavescens*) from Lake Winnipeg (Stewart et al. 2003). Trace amounts of these xenobiotics in Nile perch belly oils are so far below the recommended MRL of 10 μg kg^{-1} under German Food Law (CREM/CBI 1998). Mean HCB (0.55 ± 0.01 μg kg^{-1}) were also found far below the extraneous limit of 500 μg kg^{-1} by German standard.

Chlorofenvinphos, an organophosphorus insecticide was detected in significant amounts in all Nile perch samples (Table 3). Concentrations of chlorofenvinphos in belly oils from medium size fish and large size fish were not

significantly different ($p < 0.05$). Content of chlorofenvinphos in belly oils from the small size fish were significantly lower than that from the medium and large fish size categories. Henry and Kishimba (2006) detected higher amounts of organophosphorus pesticide in Nile perch from the Tanzanian side of Lake Victoria than those observed in this study. However, Henry and Kishimba (2006) mainly looked at fenitrothion residues, which is a phenyl organothiophosphate insecticide.

Concentrations of mercury (Hg) and lead (Pb) in Nile perch belly oil increased ($p < 0.05$) with fish size. Hg and Pb amounts in belly oil ranged from not detected-5.99 ng g^{-1} and 159.90–217.45 ng g^{-1}, respectively. Higher amount of Hg and Pb in oil from the large fish category is indicative of bioaccumulation (Storelli and Marcotrigiano 2001). Inappropriate disposal of lead-based paints, industrial and municipal wastes and burning of fossil fuels, especially lead-based gasoline, have been reported as a major source of Pb in Lake Victoria (Muwanga and Birifaijo 2006). Battery and metal fabricating industries, located along Lake Victoria basin, may be responsible for discharging significant amount of Pb into the environment. Nile perch, being a predator, ultimately accumulates significant amount of Pb. Meanwhile, Campbell et al. (2003) has demonstrated that biomass burning and soil erosion contributes significantly to Hg deposition in Lake Victoria. Consequently, Hg having high potential for biomagnification accumulates rapidly in the large predatory Nile perch. Mercury content in Nile perch belly oil was however, much lower than values reported by Storelli and Marcotrigiano (2001) for swordfish (*Xiphias gladius*) and bluefin tuna (*Thunnus thynnus*) from the Mediterranean Sea. On the other hand, arsenic and cadmium concentrations were below detection limits in Nile perch belly oil. This is consistent with the very low amounts of these metals in industrial waste waters (<0.001 mg/L), soil and sediments (<0.001 ppm) around Lake Victoria (Muwanga and Birifaijo 2006). Mean heavy metal concentrations were, in general, within limits acceptable by German Food Law for voracious fish (CREM/CBI 1998).

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