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PCDD/Fs and dioxin-like PCBs in fish species from Lake Victoria, East Africa

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HIGHLIGHTS

- Fish species collected from Lake Victoria were extracted for PCDD/Fs and dl-PCBs.
- Concentrations of the ∑PCDD/Fs in the muscles ranged from 0.06 to 0.59 pg g^{-1} fw.
- The ∑dl-PCBs in the fish muscles and livers ranged from 0.2 to 19.0 pg g^{-1} fw.
- The WHO_{2005}-TEQs varied from 0.001 to 0.74 pg TEQ g^{-1} for PCDD/Fs and dl-PCBs.
- Based on the calculated WHO-TEQs, the fish was fit for human consumption.

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ABSTRACT

Two commercially important fish species, Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) belonging to different trophic levels were collected from the Napoleon Gulf and Thurston Bay in Lake Victoria. Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) were extracted from the fish muscles and livers using the ^{13}C isotope dilution method, followed by multiple column chromatography clean-up. Analysis was achieved by a high resolution gas chromatography coupled with a high resolution mass spectrometer. The concentrations of analytes ranged from 0.07 to 0.59 pg g^{-1} fresh weight (fw) and 0.3–19.0 pg g^{-1} in *L. niloticus* and 0.06–0.18 and 0.2–15.7 pg g^{-1} in *O. niloticus*, for ∑PCDD/Fs and ∑dl-PCBs, respectively. Differences in congener concentrations were observed between the two fish species and study sites, and this was attributed to differences in feeding habits and trophic levels. World Health Organization-toxic equivalents (WHO-TEQs) were in the range 0.01–0.16 pg TEQ g^{-1} for the PCDD/Fs and 0.001–0.74 pg TEQ g^{-1} for the dl-PCBs. The TEQ values in the present study were lower compared to those of most fish samples reported in literature and were within permissible levels recommended by the European Union, implying that the fish was fit for human consumption.

1. Introduction

Lake Victoria has the largest fresh water fishery in East Africa with annual yields exceeding 800 000 metric tons (Odongkara et al., 2009). By 2008, increasing industrialization had been noted in the watershed together with a large human population of more than 30 million people that depend on the fish as a primary source of fats and proteins (Johnson, 2009). In spite of the importance of fish to the diet of the local population, as well as it being an important export commodity, a survey of literature shows no available data concerning the profiles of PCDD/Fs and dl-PCBs in the aquatic environment of Lake Victoria. Globally, these organic pollutants are widely spread in the environment and of concern especially because they are highly hydrophobic and, resistant to biotic and abiotic degradation (Laisi et al., 2008). The congeners of PCDD/Fs with chlorine substitution in the 2, 3, 7, 8 positions are thought to pose a risk to human health due to their toxicity, carcinogenic potency and potential effects on animal immunological and reproductive systems (Kumar et al., 2001). They tend to accumulate in top predators (Braune and Simon, 2003), and are thought to damage natural hormones (Minh et al., 2004).

Elsewhere in the World, concern about the health implications of PCDD/Fs and dl-PCBs has led to numerous surveys to determine their levels in fish products among other foods in order to assess human exposure (Zhang et al., 2008; Moon and Choi, 2009). The

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fish tend to accumulate amounts of such non-ionic organic chemicals at thousands of times their concentration in the surrounding medium through consumption of phytoplankton, insects, suspended particles and contaminated sediments (Bush and Kadlec, 1995). Consequently, human dietary intake of these chemicals is considered one of the major pathways, especially through consumption of fish (Bayarri et al., 2001). This study was aimed at obtaining preliminary information on the occurrence of PCDD/Fs and dl-PCBs in fish species from Lake Victoria. The generated data will be used as a benchmark to compare the levels of the pollutants over time.

2. Materials and methods

2.1. Study area and sampling

Two sampling areas namely, the Napoleon Gulf and Thurston Bay in Jinja Municipality were selected for this study. Jinja Municipality has dense urban development and industrialization, and these land use activities could be sources of pollutants into Lake Victoria via runoff. The Napoleon Gulf is adjacent to Jinja Municipality on the northern shoreline of Lake Victoria, where River Nile (the only outlet of the lake) begins its journey to the Mediterranean Sea, while the Thurston Bay which is also located on the same shoreline is rather far from Jinja town (Fig. 1). A total of 64 fish of different species (*Lates niloticus* and *Oreochromis niloticus*) and ages 1–3 years were collected in March, 2011. The weights of *L. niloticus* varied from 1522.6 to 2876.9 g (mean 2262.9 g) while those of *O. niloticus* ranged from 274.8 to 552.4 g (mean 406.3 g). The mean length (±standard deviation) of the latter species was 27.6 ± 2.6 cm, whereas that for the former was 65.2 ± 4.7 cm. Specimens were individually rinsed with distilled water to remove any impurities. Subsequently, the muscles and livers of 4–5 fish of the same species and location were separately excised, pooled and macerated to obtain homogenized samples. The samples were transferred into clean glass bottles and sealed using aluminum caps. The bottles were kept in cooling boxes containing ice packs and transferred to the German Research Center for Environmental Health for laboratory chemical analysis, and frozen at −28 °C until analysis.

2.2. Chemical analysis

The tissues were allowed to thaw and a known mass was spiked with ^{13}C-labelled PCDD/F and dl-PCB surrogate standards. The spiked tissues were then mixed with hydromatrix™ (Varian) to remove any moisture. Extraction was carried out using an accelerated solvent extractor (Dionex 200, Sunnyvatem CA, USA) at a temperature of 120 °C and pressure of 120 bar, using a 3:1 v/v of *n*-hexane/acetone as extraction solvent. The resulting organic extract was passed through a drying funnel of anhydrous sodium sulfate and eluted with 100 mL of *n*-hexane. The extraction volume was concentrated to 2 mL on a rotary evaporator and kept for purification to remove interferences by two sequential liquid chromatography steps.

The first purification step was performed by adsorption chromatography using a multilayered sandwich glass column (packed successively from bottom to top with 2 g silica gel, 5 g 33% silica gel-sodium hydroxide, 2 g silica gel, 5 g 44% silica gel-sulfuric acid, 10 g 22% silica gel-sulfuric acid and 5 g sodium sulfate). The column was washed with 60 mL *n*-hexane before being connected to a reversible carbon column (Carboxen 1016, Supelco) that had been rinsed by an equivalent volume (25 mL) of toluene and *n*-hexane, respectively. The concentrated extract was then applied to the column and eluted with 100 mL of *n*-hexane. PCDD/Fs and non-*ortho* PCBs were retained in the carbon column, while the mono-*ortho* PCBs passed through both columns. The sandwich column was removed and the carbon column was further eluted with

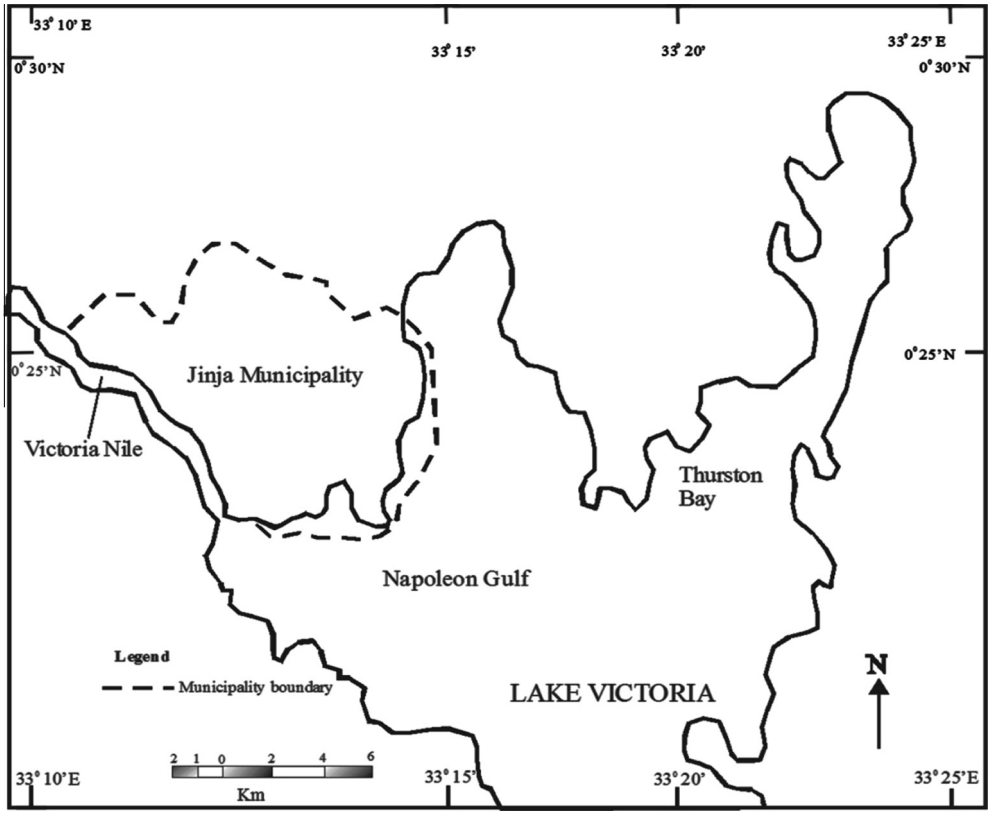

Fig. 1. Map showing the study areas (adopted from Ssebugere et al. (in press).

30 mL of 10% dichloromethane in *n*-hexane, and the resultant solution concentrated. The carbon column was reversed, eluted with 100 mL toluene and the resultant solution was concentrated to <2 mL. The second purification step was achieved by using a chromatographic column filled with 5 g alumina B super 1 as described in Çok et al. (2009). The purified fractions were transferred to sample vials followed by addition of internal standards containing ^{13}C-labelled PCDD/Fs and dl-PCBs prior to gas chromatographic determinations.

Sample extracts were analyzed for PCDD/Fs and non-*ortho* PCBs using an Agilent model 6890 gas chromatograph (GC) equipped with a high resolution mass spectrometer (HRMS) MAT 95S. Chromatographic separation was achieved by injecting 1 μL of extract in the pulsed splitless mode with a period of 120 s on a Restex Rtx-Dioxin 2 capillary column (40 m × 0.18 mm i.d. × 0.18 μm). The instrumental analysis of the mono-*ortho* PCBs was performed on an Agilent model 5890 GC series 11 coupled with a HRMS MAT 95S. The separation was achieved by injecting 1 μL of the extract on a Phenomenex MultiResidue-2 column (30 m × 0.25 mm i.d × 0.20 μm). The MS was operated at a dynamic mass resolution >10000 and quantification was achieved by isotope dilution method.

2.3. Quality assurance

Matrix spiking tests were run for every five samples. The limit of detection (LOD) for PCDD/Fs and dl-PCBs, defined by a signal to noise ratio (S/N) > 3 times the average variation of the baseline, ranged from 0.01 to 0.07 and 0.01 to 7.9 pg g^{-1} fw, respectively. The recoveries from spiked samples were between 42% and 109%. Concentrations of PCDD/Fs and dl-PCBs were calculated using HRMS software for only samples above the LOD. Samples below the LODs were not considered so as to eliminate artificial bias. All statistically significant correlations were expressed as $p < 0.05$.

3. Results and discussion

3.1. Concentrations of PCDD/Fs in the fish muscle homogenates

The results from analysis of PCDD/Fs from fish homogenates on a fresh-weight (fw) basis are presented in Table 1. The highest concentration of PCDD/Fs in the muscles of *L. niloticus* was 0.21 pg g^{-1}. Congener 1,2,3,7,8-PeCDD was the only detected PCDD (range n.d to 0.1 pg g^{-1}) in the muscle homogenates of *L. niloticus* from the Napoleon Gulf. The concentration ranges of PCDFs in the muscle of the same fish species from the Gulf were 0.09–0.21, 0.08–0.09, n.d to 0.13, 0.12–0.17, 0.08–0.09, n.d to 0.08, n.d to 0.06, n.d to 0.15 pg g^{-1} for 1,2,3,4,6,7,8,9-OCDF, 1,2,3,6,7,8-HxCD, 1,2,3,4,7,8,9-HpCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,7,8-HxCDF, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDF, respectively. The PCDF ranges in *L. niloticus* from the Thurston Bay were n.d to 0.09 pg g^{-1} for 1,2,3,4,7,8-HxCDF, 0.07–0.14 pg g^{-1} for 1,2,3,4,6,7,8-HpCDF, n.d to 0.17 pg g^{-1} for 1,2,3,4,6,7,8,9-OCDF. The levels of PCDFs in most cases were higher than PCDDs suggesting combustion as a possible source of these pollutants. Baker and Hites (2000) pointed out that for given samples if PCDFs are greater than PCDDs, then combustion is the major source of dioxins and/or related compounds into the environment.

It was observed that OCDF was the major contributor to the ∑PCDD/Fs in most *L. niloticus* muscle homogenates (18.6–45.6%). The pattern of PCDD/Fs characterized by high OCDF in the present study is similar to that reported by Wan et al. (2005) in six fish species (*Lateolabras japonicus*, *Chaeturichthys sitgmatias*, *Platycephalus indicus*, *Nibea albiflora*, and *Liza so-iuy*) from Bohai Bay in northern China. The ∑PCDD/Fs in the muscles of *L. niloticus* in our study varied from 0.07 to 0.59 pg g^{-1} fw. The levels are lower than those reported by Bordajandi et al. (2003) in fresh water fish species (*Salmo trutta*, *Anguilla Anguilla* and *Barbus barbus*) from River Turia basin in Spain and Fang et al. (2007) in fish (*Ictalurus punctatus*, *Pampus agrenteus* and *Cyprinus carpio*) from Lake Dongting in the people's republic of China.

For *O. niloticus* from the Napoleon Gulf, the only detected PCDD/F in the muscle homogenates was 1,2,3,4,6,7,8-HpCDF, at a maximum concentration of 0.1 pg g^{-1}. Three congeners were detected in *O. niloticus* from the Thurston Bay, these included 1,2,3,4,7,8-HxCDF (range n.d to 0.06 pg g^{-1}), 1,2,3,6,7,8-HxCDF (range n.d to 0.05 pg g^{-1}) and 1,2,3,4,6,7,8-HpCDF (range 0.06–0.07 pg g^{-1}). On the whole, the concentrations of PCDD/Fs in *L. niloticus* were higher than those in *O. niloticus* from the Gulf and Bay area. The high levels of PCDD/Fs in *L. niloticus* could be attributed to the fact the species is a benthic feeder. Traunspurger and Drews (1996) revealed that fish living in benthic regions of aquatic habitats display higher whole body levels during their cycle due to their close contact with sediments. The levels of PCDD/Fs in *O. niloticus* in the study herein were 5–10-fold lower compared to those reported by El-Kady et al. (2007) in the same fish species from River Nile in Egypt.

The concentrations of low chlorinated 2,3,7,8-substituted-PCDD/Fs did not exhibit statistically significant trends ($p > 0.05$) with trophic levels while those of the high chlorinated 2,3,7,8-substituted-PCDD/Fs declined significantly with increasing trophic levels, implying that the congeners undergo trophic dilution. This observation is similar to that reported by Naito et al. (2003) in fish species (*Lateolabrax japonicus*, *Dasyatis akajel*, *Conger nyriaster*, *Konosirus punctatus*, *Kareius bicoloratus* and *Limanda yokohamae*)

Table 1
Mean concentrations (pg g^{-1} fw) of PCDD/Fs in fish muscle homogenates.

Congener	Napoleon Gulf		Thurston Bay	
	L. niloticus (*n* = 4)	*O. niloticus* (*n* = 3)	*L. niloticus* (*n* = 3)	*O. niloticus* (*n* = 4)
PCDDs				
1,2,3,7,8-PeCDD	0.10	<0.09	<0.08	<0.07
1,2,3,4,7,8-HxCDD	<0.06	<0.05	0.15	<0.04
PCDFs				
1,2,3,7,8-PeCDF	0.15	<0.01	<0.01	<0.01
2,3,4,7,8-PeCDF	0.06	<0.01	<0.01	<0.01
1,2,3,4,7,8-HxCDF	0.08	<0.01	0.09	0.06
1,2,3,6,7,8-HxCDF	0.09	<0.01	<0.01	0.05
1,2,3,4,6,7,8-HpCDF	0.17	0.11	0.21	0.07
1,2,3,4,7,8,9-HpCDF	<0.02	0.13	<0.02	<0.01
OCDF	0.16	0.09	0.17	<0.02
∑PCDD/Fs	0.81	0.33	0.62	0.18
WHO_{2005}-TEQ	0.07	0.01	0.03	0.02

n – number of fish muscle homogenates.

Table 2
Mean concentrations (pg g^{-1} fw) of dl-PCBs in fish tissues.

Congener	Napoleon Gulf				Thurston Bay			
	L. niloticus		*O. niloticus*		*L. niloticus*		*O. niloticus*	
	Muscle (n = 4)	Liver (n = 4)	Muscle (n = 3)	Liver (n = 3)	Muscle (n = 3)	Liver (n = 3)	Muscle (n = 4)	Liver (n = 4)
Non-ortho PCBs								
PCB #77	2.2	<2.0	0.4	<0.2	<0.2	<4.4	<3.7	7.8
PCB #126	<0.1	<1.6	<0.4	<0.1	0.3	<0.1	0.2	7.2
Mono-ortho PCBs								
PCB #123	<0.3	<2.6	<0.2	14.1	0.8	<7.9	<0.2	<3.3
PCB #157	<0.2	5.1	<0.2	6.9	0.5	<2.1	<0.1	6.6
PCB #167	<0.3	11.8	<0.2	7.6	0.9	19.0	<0.1	15.7
∑PCBs	2.2	16.9	0.4	28.6	2.5	19.0	0.2	37.3
WHO_{2005}-TEQ	0.001	0.02	0.001	0.01	0.01	0.02	0.02	0.74

n – number of fish tissue homogenates.

from the aquatic food web of Tokyo Bay. None of the fish liver homogenate in the current study had detectable levels of PCDD/Fs. This could be attributable to poor limits of quantification observed in our laboratory for the livers. Toxic equivalents quotients (TEQs) were calculated for each homogeneous sample using 2,3,7,8-TCDD toxic equivalent factors (TEFs) reported by WHO in 2005 for fish (van den Berg et al., 2006). The WHO_{2005}-TEQs for PCDD/Fs in the present study ranged from 0.01 to 0.17 pg TEQ g^{-1} fw. Our TEQs were lower than those reported in different fish species from water bodies elsewhere in the world (Matthews et al., 2008; Storelli et al., 2011; Pacini et al., 2013).

3.2. Levels of dl-PCBs in the fish tissues

The analyzed categories were the non-*ortho* and mono-*ortho* PCBs. Total PCB concentrations (as the sum of all the PCB congeners) varied largely between fish species with values ranging from 0.2 to 37.3 pg g^{-1} fw. For *L. niloticus* from the Napoleon Gulf, PCB 77 was the only detected non-*ortho* congener in the muscle homogenates (mean 2.1, range 1.8–2.4 pg g^{-1}). The high levels of PCB 77 could be attributed to the fact that it is subject to greater specific contaminant exposure in comparison to other congeners. Adu-Kumi et al. (2010) have also reported high levels of PCB 77 (up to 56 pg g^{-1}) compared to 81, 126 and 169 in fish from Lakes; Volta, Bosumtwi and Weija in Ghana. Only two mono-*ortho* PCB congeners (157 and 167) were detected in the livers of *L. niloticus* at concentrations of 5.1 ± 0.1 and 11.8 ± 0.1 pg g^{-1}, respectively. The muscles of *L. niloticus* from the Thurston Bay presented four dl-PCB congeners, these included 167 (mean 0.9 pg g^{-1}), 123 (0.8 pg g^{-1}), 157 (0.5 pg g^{-1}) and 126 (0.3 pg g^{-1}). The low levels of PCB 126 could be due to the ability of the two fish species to eliminate this pollutant via urinary excretion and/or during respiration. McFarland and Clarke (1989) reported that PCB congeners with low chlorination grade are more readily metabolized and eliminated from tissues than the highly chlorinated congeners. For *O. niloticus*, only the non-*ortho* congeners were detected in the muscles of fish from the Napoleon Gulf and Thurston Bay. The detected congeners were PCB 77 (mean concentration 0.4 ± 0.1 pg g^{-1}) and 126 (0.2 ± 0.1 pg g^{-1}). The dl-PCB concentrations in the livers of *O. niloticus* from the Gulf were higher than in the muscles (Table 2). The mean concentrations of PCB 77, 126, 157 and 167 in the liver homogenates of *O. niloticus* from the Bay were 7.8, 7.2, 6.6, 15.7 pg g^{-1}, respectively.

In most cases, the dl-PCBs reflected higher contaminant levels in *L. niloticus* compared to *O. niloticus,* although the opposite was true for PCDD/Fs, possibly due to differences in relative accumulation rates. The accumulation of pollutants by a particular species could be attributed to differences in exposure route via trophic transfer. This may have played part in the observed differential response of the fish species. The dl-PCBs in the fish livers were 2–3 folds higher than those in the muscle and this could be attributed to differences in uptake between the two tissues. The tissue homogenates of *O. niloticus* from the Thurston Bay had higher dl-PCB levels compared to *L. niloticus* which is at a high trophic level in the food-web, and this could be due to differences in desorption kinetics of the compounds and/or the diet of the fish. Barron (1990) revealed that the diet and feeding habits coupled with physiological and biochemical processes within the organism play an important role in the accumulation of contaminants.

The PCDD/F and dl-PCB levels in the fish tissues from the Thurston Bay were less consistent and no statistical differences (α = 0.05) were observed between species. The small catchment area of the Thurston Bay, its vegetation cover and processes in the catchment could probably affect contaminant transfer and deposition. The WHO_{2005}-TEQ values for PCBs in fish from the Gulf and Bay lay in the range of 0.001–0.74 pg g^{-1} fw. The mono-*ortho* PCBs contributed the highest percentage to the total TEQ content than the non-*ortho* PCBs in both *L. niloticus* and *O. niloticus*. Similar studies by Bordajandi et al. (2003) and Tanabe et al. (2004) have also reported the domination of the mono-*ortho* PCBs in comparison to the non-*ortho* PCBs. The TEQ values in our study were lower compared to those reported literature (Baeyens et al., 2007; Bocio et al., 2007; Moon and Choi, 2009) and were also within the permissible level of 4 pg g^{-1} fw set by the European Union (EU, 2001).

4. Conclusion

PCDD/F and dl-PCB congeners were detected in fish homogenates from Lake Victoria. The concentrations for the pollutants were in the descending order of mono-*ortho* PCBs, non-*ortho* PCBs, PCDFs and PCDDs. The congener specific contribution to WHO-TEQs showed mono-*ortho* PCBs as the most abundant congeners compared to the non-*ortho* PCBs. The concentrations and WHO_{2005}-TEQs in our study were lower compared those reported in literature and were below the maximum residues limit recommended by the European Union. Therefore, based on our results the consumption of fresh fish from Lake Victoria does not represent a risk to human health.

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