Examination of Seasonal Variability of Indicator Polychlorinated Biphenyls in Nile Perch Products from Lake Victoria, Tanzania

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Abstract

In the current study, Nile perch products were analysed for seasonal variations in the levels of indicator polychlorinated biphenyls (PCBs). Samples of fish products were collected from Lake Victoria during the dry and rainy seasons and extracted using a common method referred to as QuEChERS (quick, easy, cheap, effective, rugged and safe) methodology. The residues of PCBs in fish samples were detected and quantified by using a Gas Chromatography equipped with Electron Capture Detectors and a Gas Chromatography equipped with Mass Spectrometer (GC-ECD/GC-MS). The concentrations of indicator PCBs in fish samples were generally greater during the rainy seasons than the dry seasons suggesting that seasonality has significant impacts on PCBs contamination in fish. However, the total PCBs in fish in the current study for both seasons were lower than the Maximum Recommended Limits of 75µg/kg that is set by the European Commission for fish and other fishery products. This indicates that the Nile perch products from Lake Victoria are safe for human consumption based on the studied toxicants.

Keywords: Indicator PCBs, Seasonality, fresh muscles, processed fish products, POPs.

Introduction

Polychlorinated biphenyls are a group of manmade chemicals and one of the original persistent organic pollutants that are covered by the Stockholm Convention. Studies have indicated that PCBs are very stable mixtures that are resistant to extreme temperature variations and pressures (Polder *et al.,* 2014; Witczak, 2012). In the past, PCBs were widely used in electrical equipment such as capacitors and transformers as flame retardants. When they enter the environment, such toxicants resist degradation by biological or chemical means and they are considered to be lipophilic in nature (Cork *et al.,* 2007; Bjermo *et al.,* 2013), a property that enables them accumulate in fatty tissues of different living organisms. They are also generally toxic and have been observed to travel longer distances (LRAT) before their final deposition in ecological media (Liu *et al.,* 2007). They further have the ability to accumulate in the aquatic biota and increase in concentration with time when they enter organisms above their trophic niches (Wenaty *et al.,* 2019a). PCBs are also referred to as endocrine disrupting agents (EDs) (Bell, 2014) because of having the ability of disrupting and altering the regular working of the endocrine system (Frouin *et al.,* 2013; Wenaty *et al.,* 2019a, b). These compounds have also been reported to be potentially carcinogenic to human beings due to the fact that they are associated with occurrence of different kinds of cancers such as breast, liver and testicular cancers. They are similarly reported to have negative reproductive effects such as low birth weights, small head circumferences, miscarriages, poor sperm quality as well as low sperm counts (Wenaty *et al.,* 2019a, c).

Seasons of the year have been implicated to have a significant impact on the levels of persistent organic pollutants including the indicator PCBs in the aquatic environment (Polder *et al.,* 2014). During the rainy season they are transported from one place to the other thus contaminating environmental matrices in areas where they have never been used or produced.

Moreover, in the recent years fisheries in Lake Victoria has been associated with abusive history of using different chemicals for fishing that could be important sources of PCBs in fish (Henry and Kishimba, 2006). Several studies to establish the levels of PCBs in various species of fish and other environmental compartments and the associated human health risks have been undertaken around the globe (Polder *et al.,* 2014; Ssebugere *et al.,* 2014; Oluoch – Otiego *et al.,* 2016; Wenaty *et al.,* 2019b). However, studies to establish seasonal variations of these toxic persistent organic pollutants in fish and fish products are very limited.

The current study was thus planned to evaluate the influence of seasons of the year on the concentrations of indictor PCBs in fresh muscles and processed Nile perch products that are widely consumed in the area in question. The main focus was on indicator PCBs since different studies have previously reported that they have more significant health impacts than other classes of PCBs (Polder *et al.,* 2014).

Materials and Methods

Description of the study area

This study involved four regions constituting the Tanzanian side of Lake Victoria namely Mwanza, Kagera, Mara and Geita. The regions were purposively selected for this study based on the fact that they are intensively involved in Nile perch fishing and processing for domestic, regional and international markets.

Collection and extraction of fish samples

This current study involved five products of Nile perch including; fresh fish, salted-sundried, trims, processed by smoking and fried products which were collected from randomly selected landing sites and fish processors and sellers at different fish markets in the study area during the dry and rainy seasons between 2016 and 2019. Three hundred samples consisting of 60 samples of individual fish products were prepared for analysis. The extraction and clean-up of fish samples for analytes detection and quantification was done by a QuEChERS procedure with some modifications at the National Fish Quality Control Laboratory (NFQCL) in Mwanza. Based on this method, thirty grams of each sample was measured in triplicates and blended to obtain a sample homogenate. Thereafter, thirty grams of the homogenized samples were transferred into 200 mL centrifuge tubes. Furthermore, 2.5 g of sodium bicarbonate ($NaHCO_3$), 60 mL of ethyl acetate and 15 g of anhydrous Na_2SO_4 were added and homogenized together for 20 min. Then the supernatants were transferred into 15 mL centrifuge tubes containing 0.125 g of Primary Secondary Amine (PSA) and 0.75 g of anhydrous $MgSO_4$ (Anastassiades *et al.,* 2003; Wenaty *et al.,* 2019a, b, c). The mixture was then centrifuged at 2500 rpm for 10 min and left for further 5 min to enable separation process. The supernatants were thereafter transferred into vials for Gas Chromatographic analysis.

Recoveries and Analytical Quality Control

The recovery tests were performed for six indicator PCBs of interest to determine the efficacy of the extraction and clean-up processes. Blank samples were spiked with known concentrations of the indicator PCBs followed by extraction and analysis. Blanks and standards were run every after five samples for the purpose of maintaining the quality of analytical results. The percentage recoveries were determined using equation 1

$$\%Recovery = \frac{Spiked - Unspiked\ concentration}{Expected\ concentration} \times 100 \quad (1)$$

Chemical Analysis

The chemical analysis was carried out in Mwanza at the National Fish Quality Control Laboratory. The samples of fish were analysed for 7 indicator polychlorinated biphenyls namely: PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180. The selection of the congeners was based on their persistence in the environment and ultimate accumulation on the food chains.

Analysis of samples by a Gas Chromatographic method

The indicator PCBs in the fish samples were quantified chromatographically as described by Wenaty *et al.* (2019a, b, c). The analysis was carried out using a gas chromatography (GC-2010, Shimadzu) equipped with 63Ni Electron Capture Detector (ECD) and a non-polar (HP-

5MS) capillary column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness. Nitrogen was used as both a carrier and make-up gas and the flow rate was set at 23.7 mL min–1. The temperature programme was as follows: initial temperature of 120°C held for 2 min, then increased at a rate of 10°C min–1 to 270°C held for 1 min, and at a rate of 2°C min–1 to the final temperature of 290 °C held for 3 min. The injector and detector temperatures were 220°C and 290°C, respectively.

The Gas Chromatograph was operated in a splitless mode with an injection volume of 1 µL. The standard mixture was injected in the beginning and after every five samples. The samples were injected in duplicate. The findings were confirmed using gas chromatography- mass spectrometry (Shimadzu GC-MS QP 2010 Ultra equipped with a mass selective detector-MSD, fused silica capillary column Rtx-5MS of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness and an autosampler) as described by Mahugija *et al.* (2018) and Wenaty *et al.* (2019a). The GC-MS was performed in a split less injection mode and the mass spectrometer was operated in an electron impact (EI) ionization and full scan mode. The calibration/ working standard solutions were prepared by dissolving portions of the stock solutions in the same solvents as used for the samples. Calibration curves were prepared by running series of mixtures of standard solutions and plotting the peak areas against concentrations.
Identification of the compounds involved checking the matching of the retention times and the mass spectra of the PCBs in samples to those of external reference standards that were prepared and run at the same conditions as for the samples. Quantification was carried out by linear integration of the standards and sample data based on peak areas.

Data Analysis

The measured PCBs data were subjected to descriptive statistics to deduce the minimum, maximum, mean concentrations and standard deviations of the congeners. The data was further subjected to SPSS, Version 16.0 for analysis. The concentrations of the congeners were presented as mean ± SD. The one – way ANOVA was used to compare concentrations between products. In data processing, the concentrations of PCBs in samples established to be below the limit of detection (<LOD) were treated as zero. The separation of means was done using Duncan's Multiple Range Test. The significant difference was declared at $p<0.05$ for all analyses.

Results and Discussion

Percentage recoveries of indicator PCBs for the extraction and clean-up procedures

The percentage recoveries of indicator PCBs are provided in Table 1. The mean percentage recoveries based on triplicate determinations of the analytes ranged from 71.3 to 92.5%. The percentage recoveries were carried out to determine the sensitivity, accuracy and suitability of analytical method. Different studies show that percentage recoveries from 70 to 120% are satisfactory and indicate good performance of the analytical method (Afful *et al.,* 2013a, b; Wenaty *et al.,* 2019a, b; Chamgenzi and Mugula, 2020). Thus the results herein suggest a good performance of a GC-MS/MS and that the extraction and clean-up

Table 1: Percentage recoveries of indicator PCBs to determine the sensitivity, accuracy and suitability of analytical method

PCBs	Amount spiked (µg/kg)	Amount calculated (µg/kg)	Recoveries (%)
PCB- 28	0.75	0.694±0.13	92.53±0.19
PCB- 52	0.50	0.410±0.24	82.00±0.45
PCB- 118	2.00	1.672±0.35	83.60±0.13
PCB- 138	2.60	2.034±0.47	78.23±0.27
PCB- 153	2.80	2.103±0.43	75.11±0.19
PCB- 180	3.90	2.780±0.93	71.28±0.23

processes were performed perfectly and that no corrections of the recoveries were needed.

Mean concentrations of the PCBs in fish products during the dry and rainy seasons

The results of the concentrations of indicator PCBs in µg/kg and their total concentrations that were measured in fresh muscles and processed products of Nile perch from the studied area are provided in Table 2. The results show that six indicator PCBs namely; PCB-28, PCB-52, PCB-118, PCB-138, PCB-153 and PCB-180 were discovered in different Nile perch products at quantifiable magnitudes in both the dry and rainy seasons during the study period whereas PCB- 101 was found to be below its limit of detection (<LOD) in any of the five Nile perch products that were considered in this study.

The results indicate further that the indicator PCBs were not detected (<LOD) in fresh Nile perch muscles during the dry seasons but three congeners namely; PCB-138, PCB-153 and PCB-180 were detected during the rainy seasons. The mean concentration of the three congeners that were detected in fresh Nile perch muscles were quantified to be 0.89, 1.93 and 2.34 µg/kg for PCB- 180, PCB- 138 and PCB- 153 respectively. For the salted- sundried Nile perch the levels ranged between 3.14 and 6.08 µg/kg during the dry season, while during the rainy season the average concentration of indicator PCBs ranged from 4.54 to 8.21 µg/kg. The results revealed further that, for trimmed fish products, the mean concentration of the detected indicator PCBs ranged from 1.88 to 7.83 µg/kg during the dry spell while during the rainy season the mean concentration was in the range between 2.79 and 10.48 µg/kg. On the other hand, the mean concentrations of indicator PCBs in smoked products ranged from 3.62 to 6.46 µg/kg and from 4.52 to 9.56 µg/kg during the dry and rainy seasons respectively.

Moreover, for deep fried Nile perch, the average amount of the congeners ranged between 1.25 and 3.40 µg/kg during the dry season and between 1.69 and 5.30 µg/kg during the rainy season. There were significantly high concentrations of the congeners in Nile perch during the rainy season compared to the dry season. This suggests that the rainy season has

Table 2: The mean concentrations in µg/kg of the congener PCBs and the sum of the PCBs in Nile perch products from the study area during dry and rainy seasons

Samples	Fresh fish		Salted- sundried		Trims		Smoked		Deep fried		MRL (EC, 2011)
Season	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	
PCBs											
PCB-28	<LOD	<LOD	6.08±1.95	8.21±0.97	4.92±1.38	5.64±0.93	5.20±1.58	6.90±1.43	1.75±0.35	2.27±0.51	
PCB-52	<LOD	<LOD	3.14±3.00	4.54±1.46	3.72±0.87	4.52±1.20	3.62±0.77	4.52±0.90	1.25±0.21	1.69±0.40	
PCB-101	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
PCB-118	<LOD	<LOD	4.24±3.04	5.80±2.73	1.88±0.76	2.79±0.87	5.50±0.98	6.70±1.32	3.40±0.57	4.37±0.73	
PCB-138	<LOD	1.93±0.32	5.43±3.58	7.25±1.80	7.13±3.48	9.61±2.17	5.81±1.86	7.31±1.52	3.00±1.84	5.30±2.21	
PCB-153	<LOD	2.34±0.70	5.74±5.18	8.34±2.90	7.83±4.65	10.48±2.77	6.46±4.05	9.56±3.12	3.30±0.14	3.69±0.83	
PCB-180	<LOD	0.89±0.13	3.93±3.37	5.63±1.74	6.07±5.15	8.89±3.49	4.08±2.55	6.39±2.30	3.35±0.07	5.25±1.72	
∑PCBs	**0.00**	**5.16**	**28.46**	**39.77**	**31.55**	**41.93**	**30.67**	**41.37**	**16.05**	**27.53**	**75**

a significant impact on contaminant loading in the aquatic environment in the sense that some persistent organic compounds are carried out by rain from far distances thereby increasing in concentration in the aquatic biota.

The study revealed further a domination of three congener PCBs in all the Nile perch products that were considered in this study in both the dry and rainy seasons. In this study, the fish products were dominated by three PCBs; PCB-138, PCB-153 and PCB-180. This domination tendency has also been highlighted in other previous studies (Wenaty *et al.,* 2019a, c). The reason for this domination tendency could be due to the fact that the metabolism of PCB-138, PCB-153 and PCB-180 by certain organisms is hard compared to the rest of the congeners that have low degree of chlorination as reported by Ssebugere *et al.* (2014). This enables their easy detection in different environmental compartments (Wenaty *et al.,* 2019b).

The total indicator PCBs loading in Nile perch products during both the dry and rainy seasons are shown in Figure 1. For fresh Nile perch muscles the sum of the PCBs were below the detection limits (<LOD) during the dry season and 5.16 µg/kg during the rainy season. The loading of the congeners during the dry season for the salted- sundried Nile perch products were reported to be 28.46 µg/kg but 39.77 µg/kg during the rainy season. For the trimmed fish products, the sum of the indicator PCBs was 31.55 µg/kg during the dry season and 41.93 µg/kg during the rainy season. The study revealed further that the sum of the PCBs during the dry season for the smoked products of Nile perch were 30.67 µg/kg while during the rainy season the sum of the indicator PCBs was 41.37 µg/kg and for deep fried products, the sum of the PCBs were 16.05 µg/kg during the dry season and 27.53 µg/kg during the rainy season.

The percentage contribution of the three dominant congeners to the total loads of the congeners were 0 and 100% for fresh Nile perch muscles, 53.1 and 53.3% for salted-sundried fish products, 66.7 and 69.1% for trimmed fish products, 53.3 and 56.2% for products that were smoked and 60.1 and 51.7% for products that were deep fried during the dry and rainy seasons respectively. High percentage contribution of the three congeners of indicator PCBs were also reported by Wenaty *et al.* (2019a) and Ssebugere *et al.* (2014) in previous studies.

In this study, the deep fried Nile perch had low levels of PCBs compared to other fish products processed under different processing operations. This is probably because of high cooking temperatures of the oil that enables it to act as an extracting solvent. This phenomenon has also been reported in the previous findings (Witczak, 2009a, b; Wenaty *et al.,* 2019b). On the other hand, the trims of Nile perch had significantly higher PCBs loading compared to the rest of the products because of high amounts of fatty that makes them lipophilic in nature compared to other products (Wenaty *et al.,* 2019a).

The study revealed further predominantly high levels of PCBs in smoked fish products. This is due to a reduction in co-distillation process of the congeners with water vapour as suggested in the previous studies (Witczak and Ciereszko, 2006) and the removal of water from the products because the compounds are soluble in fat and lipids, therefore the removal of water concentrates other insoluble components as reported in some other previous studies (Wenaty *et al.,* 2019a, b, c).

Furthermore, the fresh Nile perch muscles had no residual PCBs during the dry seasons but very small amounts (ΣPCBs of 5.16) of the dominant congeners were detected during the rainy seasons. This suggests that the aquatic environment in Lake Victoria is not alarmingly contaminated by PCBs and that seasons have a significant influence on the levels of PCBs in the aquatic biota. However, the mean concentration of ΣPCBs in both dry and rainy seasons as indicated in this study were about two folds lower than the maximum recommended limit of 75 µg/kg set by the European Commission for fish and other fishery products (EC, 2011). This is an indication of the safety of the Nile perch products from Lake Victoria. The study revealed further that, processed Nile perch products had higher levels of indicator PCBs than fresh fish muscles. This suggests further that apart from seasons having significant impacts on the levels

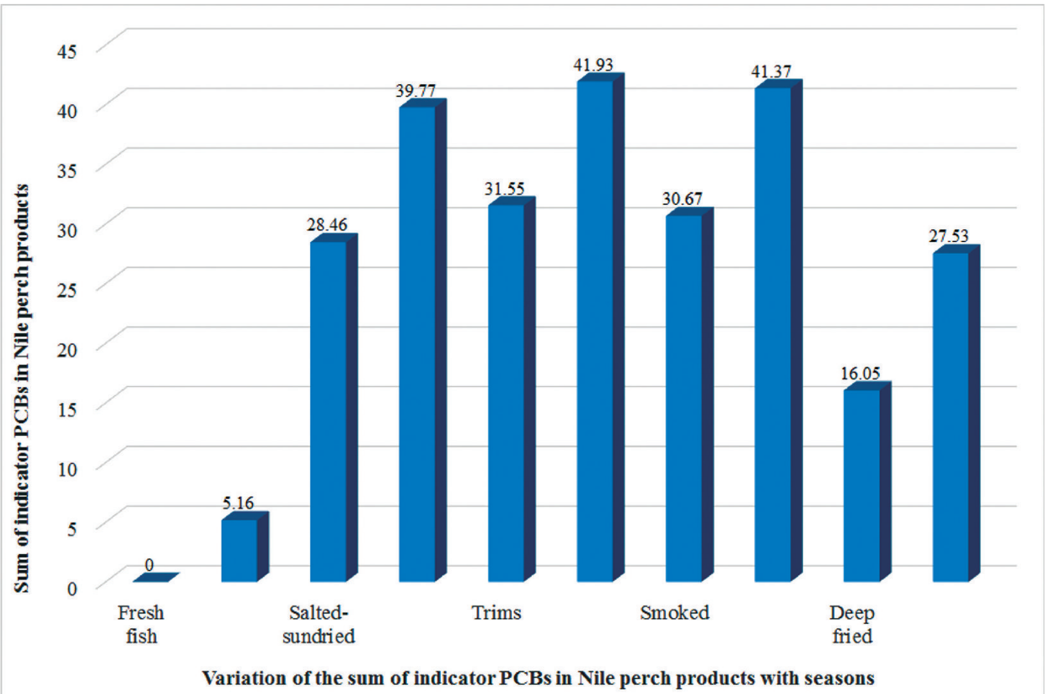

Figure 1: Variations of the total indicators PCBs in Nile perch products during with seasons

of PCBs, contaminations takes place in the Nile perch value chain.

Table 3 indicates the Analysis of Variance of the studied components that shows a significant difference in the levels of the studied indicator PCBs. This study revealed existence of significant differences in the levels of indicator PCBs between seasons with the rainy season reporting higher levels compared to the dry season. This suggests that the rain may serve as a media of transport of these toxic persistent organic pollutants from one place to the other.

Conclusion and recommendations

The current study assessed the seasonal variability of indicatory congeners of PCBs in different products of Nile perch from the study area that are intended for domestic, regional and international markets. The levels of the detected indicator PCBs varied with seasons with the rainy seasons having significantly higher levels than the dry seasons. This suggests that seasonality has a significant impact on the levels of PCBs in Nile perch products from Lake Victoria.

Table 3: Analysis of Variance of PCBs in processed fish products

	Sources of variation		
PCBs	**DF**	**F**	**P**
PCB-28	4	3.59	0.042**
PCB-52	4	3.32	0.039**
PCB-118	4	9.63	0.001**
PCB-138	4	2.92	0.018**
PCB-153	4	0.68	0.023**
PCB-180	4	0.59	0.637

*** Indicates that the means are significantly different at a level of 0.05*

DF: Stands for the degree of freedom, F: Stands for F-value and P: Stands for P–value

However, in both seasons the loadings of the congeners were below MRL suggesting safety of fish products from Lake Victoria. Yet, follow up studies to assess the influence of different fish processing technologies such as smoking on the levels of different congeners of PCBs need to be undertaken.

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Conflict of interest

The authors declare that there is no conflict of interest.

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