AN ECOTOXICOLOGICAL EVALUATION OF POLYCHLORINATED BIPHENYLS IN A COMMERCIALLY IMPORTANT CICHLID FISH SPECIES (Oreochromis niloticus)FROM OGBA RIVER, BENIN CITY, NIGERIA

*[1]Oiseoje M. Wangboje and[2]Jacob S.Ewutanure
[1]Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, P.M.B. 1154, Benin City, Nigeria
[2]Department of Fisheries and Aquaculture, Faculty of Environmental Management, Nigeria Maritime University, Okerenkoko, Nigeria
**Corresponding author: oiseoje.wangboje@uniben.edu*

ABSTRACT

Polychlorinated biphenyls (PCBs) are hazardous synthetic pollutants that are of relevance in ecotoxicological research. The Ogba River in Benin City, Nigeria, has been under anthropogenic onslaught such as the inflow of the Benin master drainage system and the paucity of PCB data for fish from this river warranted this research. The levels of PCBs in *Oreochromis niloticus* from the river were determined by Gas Chromatographic (GC) technique in order to ascertain their suitability for human consumption. The PCB profile consisted of both non-ortho PCBs (PCB 126 and PCB 169) and mono-ortho PCBs (PCB 156 and PCB 189). The concentration profile of the identified PCBs was PCB 126 > PCB 169 > PCB 156 > PCB 189. The mean values for PCBs (Mg/kg, wet weight) in *O. niloticus* ranged from 0.015 (PCB 189) to 0.074 (PCB 126) with a total weight of 0.143 mg/kg. The mean values for PCBs (mg/kg) in fish by station, ranged from 0.012 (PCB 189) at Unegbe to 0.0745 (PCB 126) at the Bridge station with a significant difference ($p<0.05$) observed in the mean concentrations of PCBs 169 and 156 respectively in fish between stations The hazard quotient (HQ) ranged from 0.0075 (PCB 189) to 0.037 (PCB 126) while a toxic equivalency (TEQ) value of 0.007723 was observed. *O. niloticus* was observed to be safe for human consumption as it was free from hazardous levels (2 Mg/kg) of PCBs However, continuous monitoring of the Ogba River ecosystem was suggested in other to mitigate the occurrence of PCBs.

Key words: Polychlorinated biphenyls, hazard quotient, *Oreochromis niloticus,* Toxic equivalency

INTRODUCTION

Polychlorinated Biphenyls (PCBs) are synthetic and persistent organic pollutants consisting of the biphenyl molecule that has been halogenated multiple times specifically with Chlorine atoms. These substances do not readily breakdown in the environment and are thus in constant circulation between environmental matrixes such as air, soil, flora, fauna and water. PCBs are easily taken up by hydrobionts including fish and readily accumulate in various organs and fatty tissue (Alaska Department of Environmental Conservation, 2023). Despite being banned over four decades ago, PCBs are still commonly found in natural aquatic media and their resources. PCBs can further bioaccumulate in tissues of fish which in turn may pose a health risk to end-of-line consumers such as man. Like perfluoroalkyl and polyfluoroalkyl substances, PCBs are classified as legacy pollutants by virtue of their lengthy half-life and their ability to accumulate in both fish and human tissue (Schultz *et. al.,* 2023). PCBs are known to increase the risk of developing cancer and also increase the risk of babies developing neurodevelopmental effects via their mothers (Ryu, 2023). Furthermore, PCBs have been categorized as endocrine disrupting chemicals or endocrine disrupting substances (Sakali *et al.,* 2023). Clearly, PCBs are hazardous to both wildlife and man and are thus extremely relevant in ecotoxicological studies. Ogba River in Benin City, Nigeria, was the focus of this research. The River is in receipt of a cocktail of effluents that come in directly via the Benin master drainage system and adjoining points. The scientific community has become interested in this ecosystem following the discovery of Mercury (Hg), a deleterious heavy metal, in fish and water (Wangboje and Oronsaye, 2001). Accordingly, studies abound on pollutants such as heavy metals (Obasohan *et al.,* 2006; Obasohan, 2008; Oronsaye *et al.,*2011; Wangboje and Duru, 2019; Wangboje and Braimah, 2022) and Polycyclic Aromatic Hydrocarbons (Wangboje *et al.,* 2016) especially the former, in this all important river. However, available ecotoxicological data on chemical contaminants in fish from the river has shown that there is a dearth of information on the PCB content in fish from this ecosystem. It is on this premise that this research becomes relevant in order to fill an existing gap in knowledge and to further guide potential consumers of natural aquatic resources. The target fish species, *Oreochromis niloticus* (Linnaeus, 1758), is a commercially important Cichlid species (Family: Cichlidae) that inhabits rivers, tributaries, Fadamas, lakes and large ponds. It can attain a total length of over 50cm weighing over 2 Kg. It has an omnivorous diet, feeding on algae, diatoms, insect larvae, detritus, fry of fish and fish eggs while it is widely distributed in Nigerian Freshwaters and is popularly known as the Nile Tilapia (Idodo-Umeh, 2003).

MATERIALS AND METHODS

The study area

This research was carried out on Ogba River (Fig. 1) located in Benin City (Latitude 6°20’00’’N and Longitude of 5° 37’ 20’’E) in Edo state, Nigeria. It is one of the major sources of domestic water supply in Benin City (Wangboje and Oronsaye, 2001). The climate of the area is typical of the tropical rain forest belt with wet season (April-October) and dry season (November-March). Rainfall is bi-modal, usually peaking in July and September with a brief drop in August. Annual temperature ranges from 23° C to 34° C while annual humidity is between 67% and 96%. The marginal vegetation in the area includes *Commelina, Ipomea, Emilia* and *Sonchifolia* species while the dominant macrophytes are *Azolla* and *Ceratophyllum* species. The City is underlain by sedimentary formation.The formation is made up of top reddish clayey sand capping highly porous fresh water bearing loose pebble sands and sandstone with local thin clay and shale interbeds, which are considered to be of braided stream origin. The sand, sandstone and clay, vary in colour from reddish brown to pinkish yellow on weathered surfaces to white in deeper fresh surfaces. The formation is about 800 M thick and is covered with loose brownish sand known as the quaternary drift, almost all of which are water bearing with water levels varying from 20 M to 52 M. The Benin formation which is composed of coarse sand interspersed with lignite and lateritic clay, extends to a depth of about 763 M and is located on top of the Agbada formation, which is composed of sandstone and coarse sand (Asuen and Oronsaye, 1990) The study area is surrounded by arable farm land and some of the crops cultivated include cassava (*Manihot esculenta*), yam (*Discorea sp.*), maize (*Zea mays*), cocoyam (*Xanthosoma sagittifolium*) and melon (*Citrullus vulgaris*). Thickets of *Bambusa sp.* can be found along the banks of the River. The sampling zone was a stretch along the river consisting of three stations namely, Zoo station, Bridge station (approximately 1.5 km from the Zoo station) and Unegbe station (approximately 2 km from the Bridge station).Specific details of the aforesaid stations are presented in Table 1.

Collection of Samples

The research was conducted between August 2021 and January 2022. Both dry and wet seasons were captured in order to identify probable temporal variations in PCB levels. Fishes were captured with nylon nets from the respective established stations and were thereafter placed in labeled polythene bags and conveyed to the laboratory in a Thermolineo® ice box within 24 hours.

Laboratory procedures

The fish species were identified using field handbooks and guides (Idodo-Umeh, 2003; Adesulu, and Sydenham, 2007; Olaosebikan and Raji, 2013). Total length (cm) measurements of fish were taken using a measuring board while weight (g) of fish samples were ascertained using an electronic Scale (Mettler® PM4800 Delta Range). The mean total length was 34.62 ± 1.62 cm while the mean weight was 1.25 ± 0.67 kg (n=36) respectively. All reagents and chemicals used were of analytical grade (BDH, Poole, England and Sigma, USA). All glassware were soaked in detergent and then rinsed alternately with running tap water and distilled water. Ten (10) g of muscle tissue was excised from the flanks of the fish specimen with a stainless steel lancet and ground with anhydrous Sodium sulphate until a completely dry homogenate was obtained. The extraction of PCB in fish tissue was performed according to standard procedures (USEPA, 1996). The extract was concentrated to 2 ml with a rotary evaporator (rotovap) at 40°C. The concentrated extract was thereafter used for clean-up and for gravimetric lipid determination. Clean-up of extracts was done in line with the method by Kampire *et al.,* (2015) while a Perkin model 5890 gas chromatograph equipped with Ni 63 electron capture detector was used for quantification of PCBs. The quality control was performed by regular analyses of procedural blanks and blind duplicate samples along with random injection of standards and solvent blanks. All procedures conformed to standard scientific research guidelines including the ARRIVE® guidelines for experimental animals.

Hazard quotient (HQ) for PCBs

The Hazard Quotient (HQ) expresses the possibility of a contaminant being an ecological risk or a contaminant of potential ecological concern (Purchase, 2000). The HQ is expressed as follows:

HQ = *Measured concentration of contaminant* / *Toxic reference value for selected species* …………………………………Equation (1)

HQ ≥ 1 = Possibility of ecological risk indicated or a contaminant of potential ecological concern (i.e. COPEC).

Toxic equivalency (TEQ) for PCBs

Each PCB congener exhibits a different

gravity of toxicity. Therefore in order to have a summation of the toxicity of a host of congeners, toxic equivalency factors (TEFs) are applied to facilitate risk assessment and regulatory control (Commission Regulation, 2008).

TEQ = Σ Ti * TEF ………………………………………………….Equation (2)

where: TEQ = Toxic Equivalency

Ti = PCB concentration in fish

TEF = Toxic equivalency factor for PCB congener

Estimated annual intake (EAI) and Estimated daily intake (EDI) of PAHs

EAI (Mg/person/year) = Concentration of PAH in fish *Per capita figure/ 70 Kg adult body weight

………………………Equation (3)

where: Per capita figure is 13.3 kg/person/year for Nigeria (Word Fish Center, 2021)

EDI (Mg/person/day) $EAI/_{365\ Days}$……………………………………… Equation (4)

Statistical methods

A GenStat Release® computer software (Version 12.1 for PC/Windows Vista, VSN International Limited) was used for statistical analysis. Data were subjected to Analysis of variance (ANOVA) to determine significant differences between mean values of PCBs while significance means ($p<0.05$) were separated with New Duncan Multiple Range Test. Microsoft® Excel (for windows 2010) was used for all graphical features.

RESULTS AND DISCUSSION

As presented in Table 2, the mean values for PCBs (mg/kg) in *O. niloticus* ranged from 0.015 for PCB 189 to 0.074 for PCB 126, with a total of 0.143 mg/kg. In Table 3, the mean values for PCBs (mg/kg) in *O. niloticus* by station, ranged from 0.012 (PCB 189) at Unegbe to 0.0745 (PCB 126) at the Bridge station with a significant difference ($p<0.05$) observed in the mean concentrations of PCB 169 and PCB 156 in fish between stations while the mean values for PCBs (mg/kg) in *O. niloticus* by month, ranged from 0.0133 (PCB 169) in November to 0.0774 (PCB 126) in December with a significant difference ($p<0.05$) observed in the mean concentrations of all the PCB Congeners in fish between months (Table 4). The Hazard quotient (HQ) values for PCBs in fish ranged from 0.0075 (PCB 189) to 0.037 (PCB 126) as shown in Fig. 2 while a Toxic equivalency (TEQ) value of 0.007723 was observed in this research

(Fig. 3). The estimated annual intake (EAI) values (Mg/person/year) for PCBs ranged from 0.003 (PCB 189) to 0.0141 (PCB 126) while the estimated daily intake (EDI) values (Mg/person/day) for PCBs ranged from 0.0000082 (PCB 189) to 0.00004 (PCB 126) as presented in Figures 4 and 5 respectively.The spatial total values (Mg/kg) for PCBs ranged from 0.1228 at the Zoo station to 0.1328 at the Bridge station (Fig. 6) while the temporal total values (Mg/kg) ranged from 0.1327 in August to 0.1996 in December (Fig. 7).The quota of PCB congeners in *O. niloticus* according to stations ranged from 10.76% for PCB 156 at the Bridge station to 62.93% for PCB 126 at the Unegbe station as shown in Fig. 8.

In this research, four (4) PCB congeners were identified in the target fish species, *O. niloticus.* They consisted of both non-ortho PCBs (e.g. PCB 126 and PCB 169) and mono-ortho PCBs
(e.g. PCB 156 and PCB 189). The concentration profile of the identified PCBs took the order PCB 126 > PCB 169 > PCB 156 > PCB 189. Clearly, PCB 126 was the dominant congener accounting for more than half (51.74 %) of the observed total PCB content in fish. It was also the most dominant congener in the fish species according to stations, accounting for over 55 % of the spatial total in each case. Being lipophilic substances, PCBs bind easily to fatty tissues of animals and this attribute to a large extent, dictates the presence of these xenophobic substances in biota. Power *et al.,* (2023), carried out a preliminary investigation of persistent pollutants in Eurasian Curlew *Numenius arquata*eggs in Ireland and identified a total of 16 PCB congeners of both non-ortho and mono-ortho combination and attributed the high incidence of PCBs to anthropogenic impact. Wang *et al.,* (2023) observed that electrical waste is a primary source of PCB pollution especially in developing countries and that PCBs have been employed widely as insulators in capacitors, transformers and associated power systems. These substances are widely distributed in the environment and can be found in industrial, semi-industrial and urban regions (Arfaeinia, *et al.,* 2017). PCBs have also been found in fish sludge as reported by Brod *et al.,* (2023), further underscoring the ubiquity of these substances. Significant differences ($p<0.05$) were observed in the mean concentrations of PCB 169 and PCB 156 in fish between stations, giving a clear indication that the influx of the aforementioned PCBs was not uniform during the study period. It also indicates variations in the bioaccumulation pattern of PCBs by fish from one station to another. On the other hand, significant

differences ($p<0.05$) were observed in the mean concentrations of all the PCB congeners in fish between months, a clear indication of seasonal variation. Accordingly, the peak mean concentrations of PCB 126 and PCB 169 were observed to be in the month of December while the peak mean concentrations of PCB 156 and PCB 189 were observed to be in the months of September and October respectively. December is a dry month while September and October are wet months. In this research, the observed hazard quotient (HQ) values for all the PCB congeners were below unity, indicating that they do not present any oncological risk to potential consumers of the target fish species. However, PCB 126 is the congener that had the highest HQ rank profile (PCB 126 > PCB 169 > PCB 159 > PCB 189) and may thus become a problem in future if not checkmated. This assertion is further corroborated by the fact that PCB 126 accounted for a massive 95.82 % of the Toxic Equivalency (TEQ) compared to the other PCB congeners. It is also pertinent to note at this point that PCB 126 has a much higher toxic equivalent factor (TEF) of 0.1 compared to 0.01, 0.0005 and 0.0001 for PCBs 169, 156 and 189 respectively (Commission Regulation, 2008). PCB 126 has been described as the most potent dioxin-like PCB (DL-PCB), thus its presence in food and the environment must be taken seriously (Zhang *et al.,* 2012). Both the EAI and EDI figures were dominated by PCB 126 owing to the fact that the congener was more available in fish when compared to the other congeners. Although sub-zero figures were observed for both EAI and EDI, the numbers could add up over time arriving at more potent figures in the long run. The spatial total values for PCBs revealed a rank profile of Bridge > Zoo > Unegbe. This information gives an indication that there was seemingly more anthropogenic impact at the Bridge station compared to the other stations as shown in Table 1.With regard to the temporal total values for PCBs, the rank profile was December > January > November > October > September > August. Clearly, higher values were observed in the dry months of November, December and January compared to the wet months of August, September and October. This data gives an indication that the levels of PCBs in water were probably more concentrated in the dry months compared to the wet months as there was no rainfall to produce any dilution effect bearing in mind that fish can readily take up PCBs from water. Furthermore, higher air temperatures usually experienced in the dry season can increase the rate of evaporation of water thus making PCBs more concentrated. Fakhoury *et al.,* (2023), corroborated this assertion and further observed that industries in an area can severely affect the wildlife and overall health of waterways with regard to PCBs. Health-wise, it is important to note that both the individual mean concentrations of PCBs in *O. niloticus* and the total (Σ^4) of the means, fell below the threshold limit of 2 mg/kg as espoused by the Agency for Toxic Substances and Disease Registry (2020), implying that presently *O. niloticus* is safe for human consumption as it is free from hazardous levels of PCBs.

CONCLUSION

The paucity of data for PCBs in fish from Ogba River in Benin City, Nigeria warranted this research, against the backdrop of the hazardous nature of this persistent organic pollutant, employing *O. niloticus* as the target fish species. Health-wise, the individual mean concentrations of PCBs in *O. niloticus* and the total of the means, were below the threshold limit of 2 mg/kg indicating that presently *O. niloticus* is safe for human consumption as it is free from hazardous levels of PCBs.

RECOMMENDATIONS

It is recommended that continuous monitoring and surveillance of the Ogba River ecosystem is carried out by relevant government agencies in other to continue to keep PCB levels under hazardous limits. Future research could embrace a sedimentological approach to the evaluation of PCBs in this river which would further support the findings from this research.

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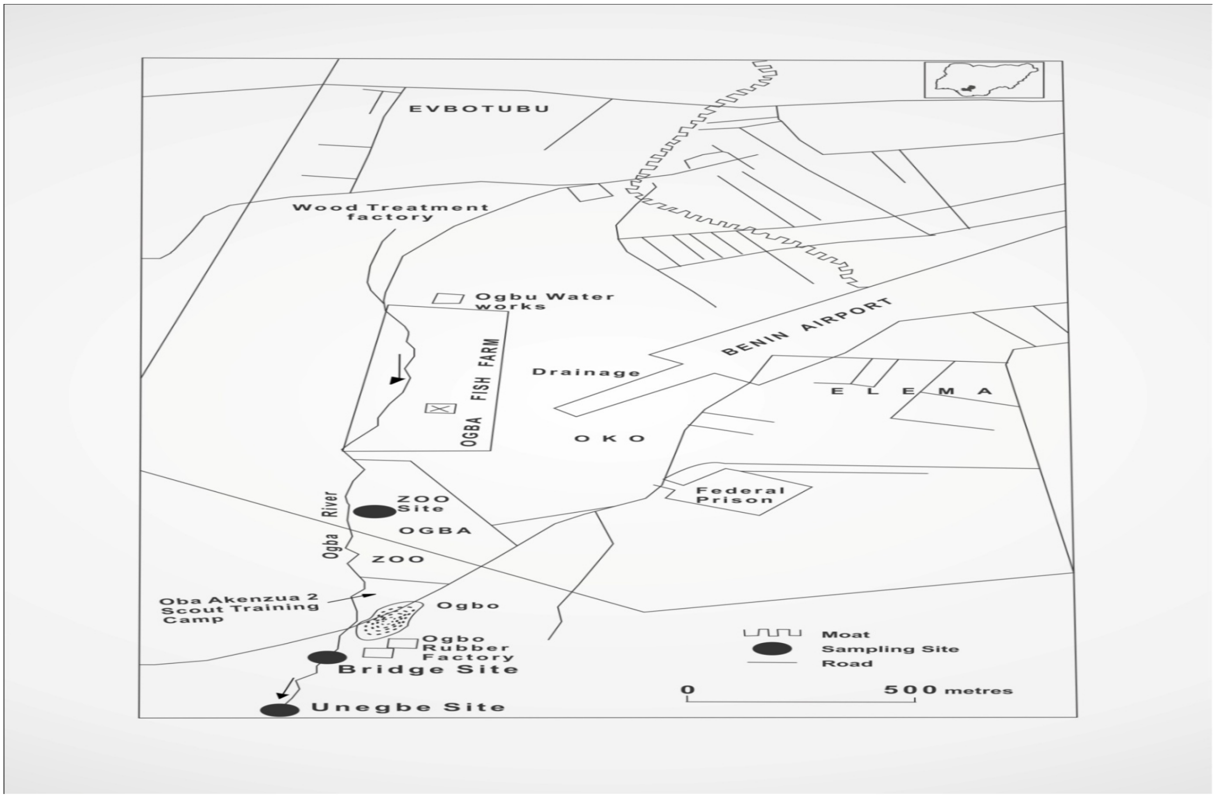

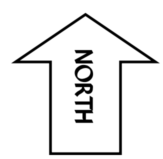

Fig. 1: Map of Ogba River showing sampling stations

Table 1: Details of sampling stations

Station code	Name of station	GPS co-ordinates	Description of station
01	Zoo station	N 06° 17.587' E 005° 34.933'	The station is located behind the Ogba Zoological garden. The zoological garden was established in 1971 by the Department of Agriculture and Natural Resources. It is in receipt of effluents from the Benin master drainage system which is located further upstream of this station. The river at this point also receives effluents emanating from the Zoo. Reddish-brown exudates of Iron are visible on the soil surface at this station especially around the riparian periphery. There is a boys' scout camp in the vicinity.
02	Bridge station	N 06° 17.095' E 005° 34.932'	The bridge is located along the Airport-Ogba road. Solid wastes can be sighted around the banks of the river at this point. Car washing and traditional worshiping activities can be observed. Fishing takes place here. There is a rubber processing factory in the vicinity.
03	Unegbe station	N 06° 14.380' E 005° 33.570'	Fishing activities take place here. There are also farming activities within the Unegbe community. The station is located downstream of the bridge station.

Table 2: Mean values of PCBs (Mg/kg) in *O. niloticus*

PCB Congener	Mean	Minimum	Maximum	% Dominance	Threshold*
PCB 126	0.074	0.071	0.088	51.74 %	2.0
PCB 169	0.031	0.026	0.045	21.67 %	2.0
PCB 156	0.023	0.025	0.028	16.08 %	2.0
PCB 189	0.015	0.014	0.021	10.49 %	2.0
Σ^4 PCB	**0.143**				

***** Agency for Toxic Substances and Disease Registry (2020).

Table 3: Mean values of PCBs (Mg/kg) in *O. niloticus* by Station

Station	PCB 126	PCB 169	PCB 156	PCB 189
Zoo	0.0734±0.0056[a]	0.0165±0.0016[b]	0.0179±0.0033[a]	0.015±0.0023[a]
Bridge	0.0745±0.005[a]	0.031±0.0032[a]	0.0143±0.0033[b]	0.013±0.002[a]
Unegbe	0.0678±0.006[a]	0.0155±0.0025[b]	0.0124±0.002[b]	0.012±0.0022[a]

Means with similar superscripts are not significantly different (p>0.05).Vertical comparisons only.

Table 4: Mean values of PCBs (Mg/kg) in *O. niloticus* by month

Month	PCB 126	PCB 169	PCB 156	PCB189
Aug.[w]	0.0772±0.004 [a]	0.0143±0.002 [b]	0.0142±0.001 [b]	0.0135±0.002 [a]
Sept.[w]	0.0743±0.003 [a]	0.0156±0.001 [b]	0.0196±0.002 [a]	0.0141±0.002 [a]
Oct.[w]	0.0765±0.001 [a]	0.0142±0.002 [b]	0.0185±0.002 [a]	0.0152±0.001 [b]
Nov.[d]	0.0655±0.003 [b]	0.0133±0.002 [b]	0.01742±0.001 [a]	0.0143±0.003 [ab]
Dec.[d]	0.0774±0.005 [a]	0.0167±0.001 [b]	0.01461±0.002 [b]	0.0135±0.001 [a]
Jan.[d]	0.0622±0.002 [a]	0.0234±0.002 [a]	0.01732±0.023 [a]	0.0143±0.0042 [a]

Means with similar superscripts are not significantly different (p>0.05).Vertical comparisons only. w=Wet month, d=Dry month

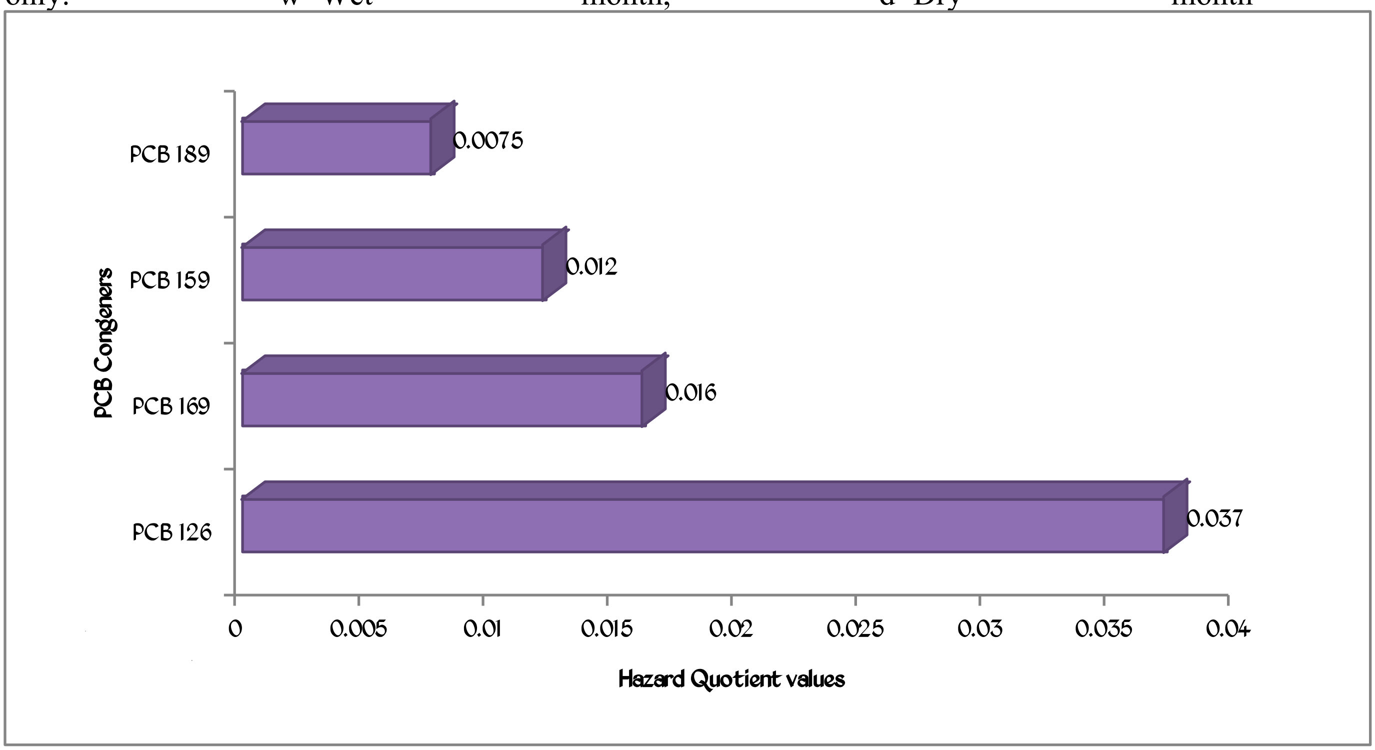

Fig.2: Hazard quotient (HQ) values for PCBs

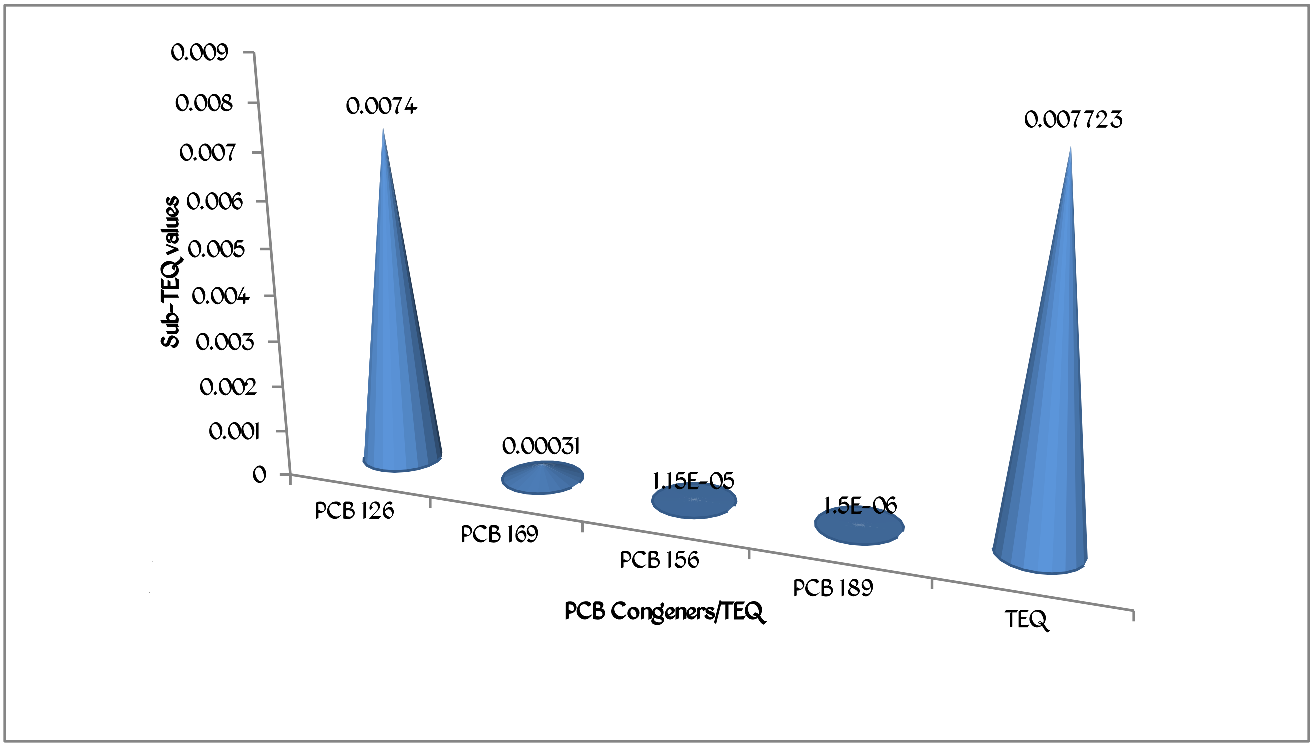

Fig. 3: Toxic equivalency (TEQ) values for PCBs

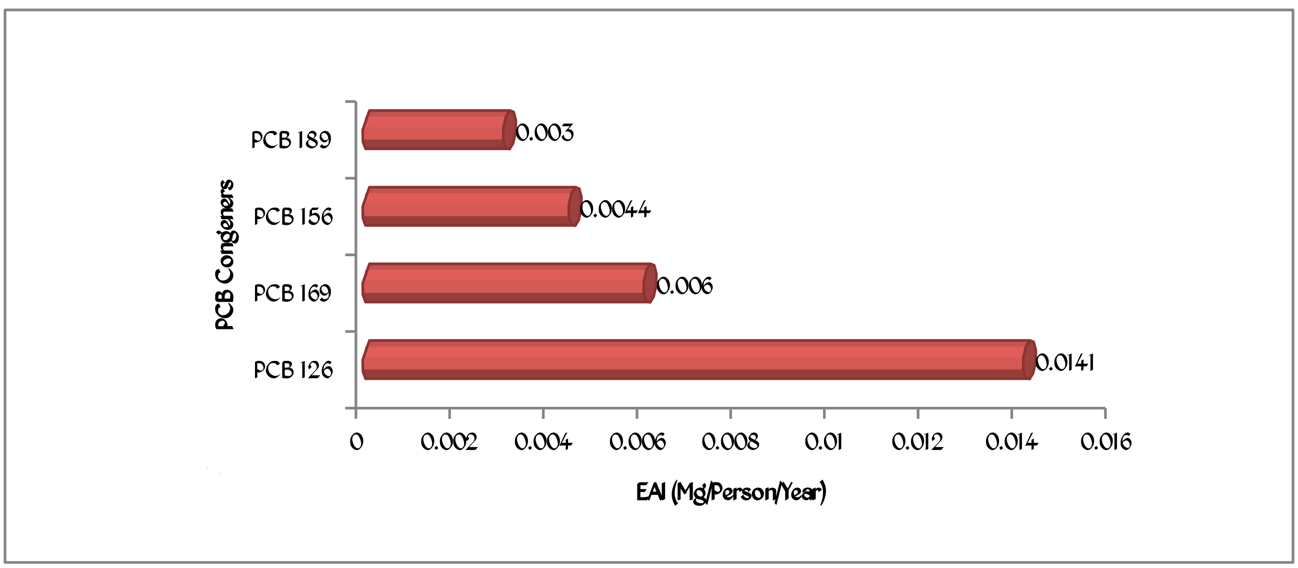

Fig. 4: Estimated annual intake (EAI) values for PCBs

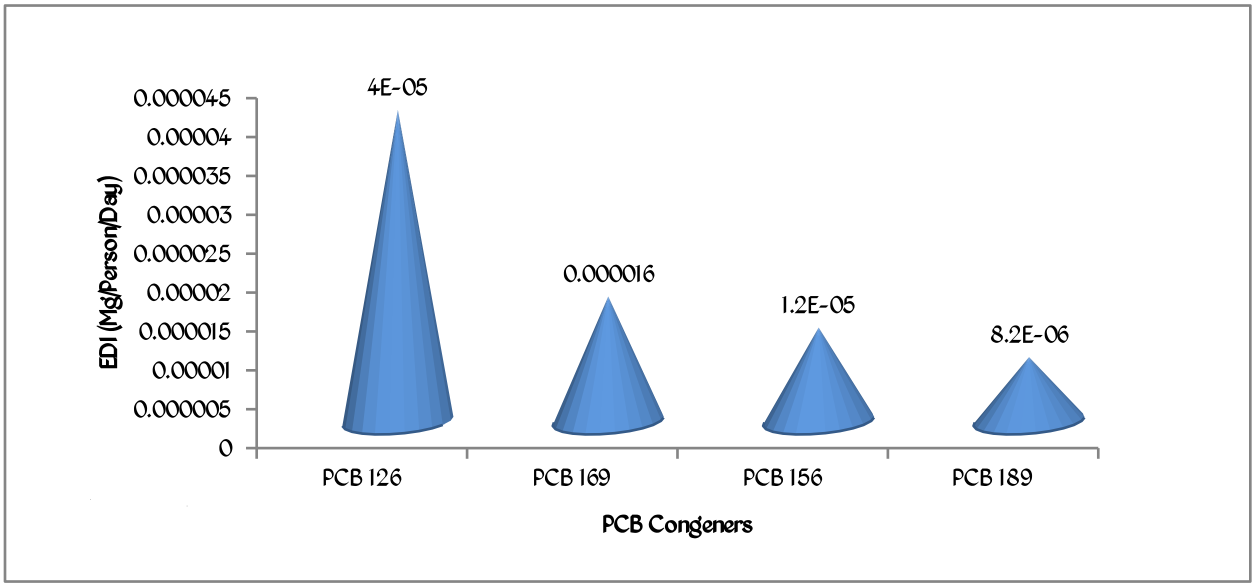

Fig. 5: Estimated daily intake (EDI) values for PCBs

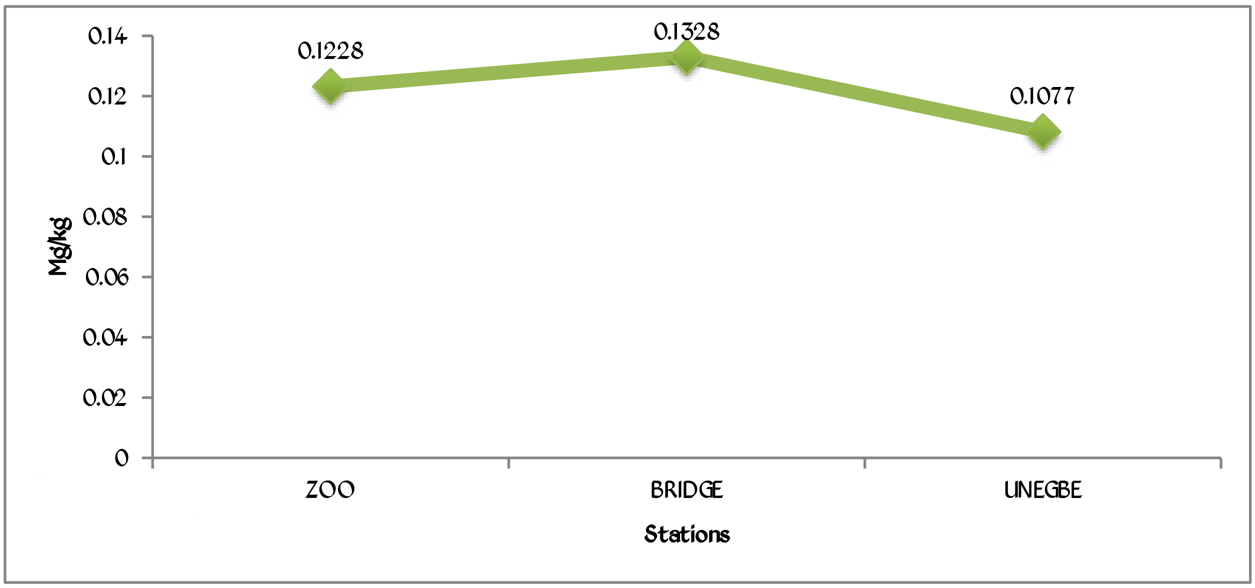

Fig. 6: Spatial total values for PCBs

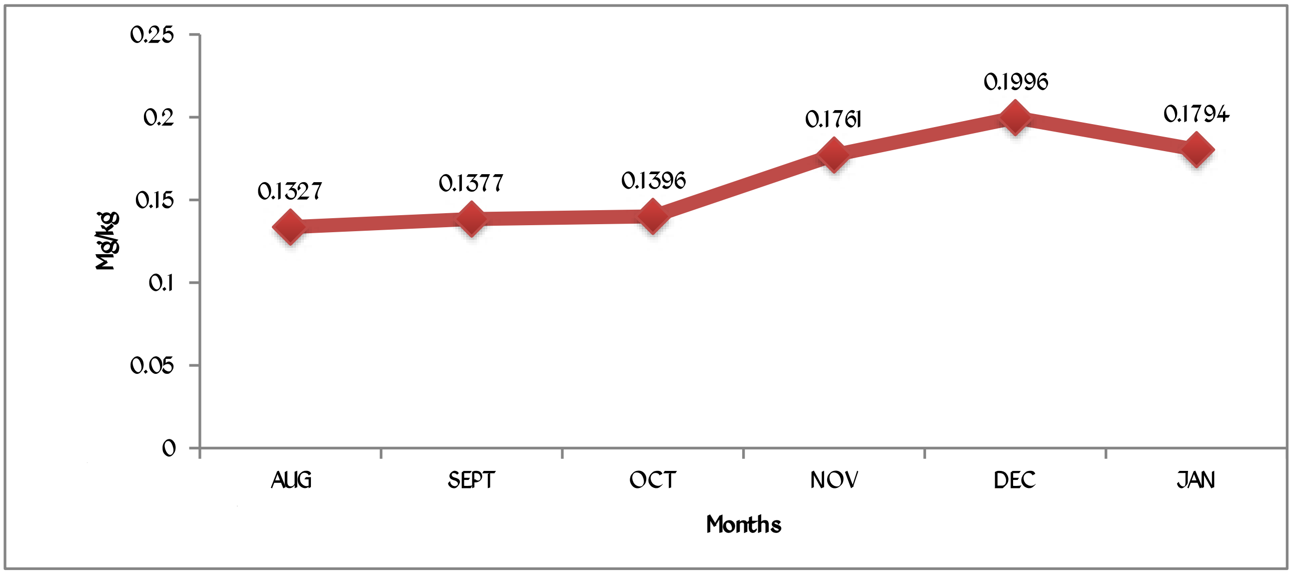

Fig. 7: Temporal total values for PCBs

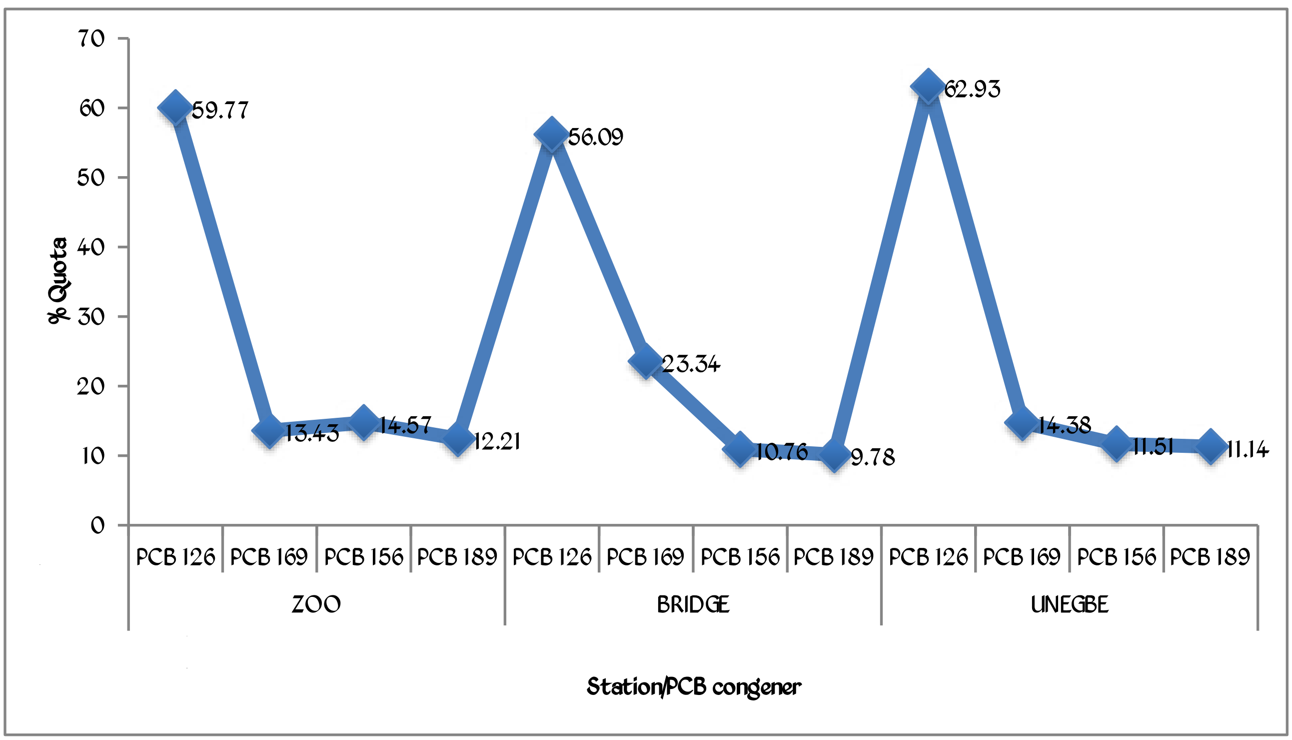

Fig. 8: Quota of PCB congeners in *O. niloticus* according to station