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ORIGINAL ARTICLE

Bioaccumulation of Hydrocarbons in Freshwater Fish Species Cultured in a Shallow Coastal Lagoon, Egypt

Maha Ahmed Mohamed Abdallah[1]

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Abstract

Purpose Polycyclic aromatic hydrocarbons (The 15 parent) were determined in six freshwater fish species cultured in Lake Edku fish farm, one of the northern Nile Delta lakes in Egypt. That receives input from numerous anthropogenic activities through several huge drains, for the detection and evaluation of residues of PAHs in their edible parts.

Methods The soft tissue portions of the studied fish samples were analyzed for organochlorine following well-established techniques by UNEP/IOC/IAEA (1991 and 1992).

Results Total PAH concentrations ranged from 285.03 to 1008.73 ng/g wet wt, while aliphatic hydrocarbon levels ranged from 301.41 to 1268.11g/g wet wt. Anthracene was the most prevalent portion in all fish species. All the studied fish showed values higher than MRL (13.11–19.90 ng g^{-1}) except *T. galilaeus* was lower than MRL (2.10 ng g^{-1}). All samples showed benzo[a]pyrene concentration values lower than 3% of Σ15PAH, which may suppose some risk of human health. In all studied fish samples, total aliphatic hydrocarbons were found in higher concentrations than total PAHs; the edible parts of *Mugil capito*, *Tilapia galilaeus*, *Tilapia nilotica* and *Clarias lazera* had the highest total aliphatic, C-30 was the most prevalent in all fish samples except *Tilapia zilli* (the dominant is C-14).

Conclusion Considering the findings, we suggested that the major portion of the hydrocarbons retrieved from the fish were inherited from the hydrocarbons of the biological origin and a significant amount from fossil origin. The results indicated no adverse health effects (noncarcinogenic) are associated with the consumption of all fish species from Lake Edku with respect to PAHs.

✉ Maha Ahmed Mohamed Abdallah
mahaahmed7@ymail.com

[1] Marine Pollution Lab, National Institute of Oceanography and Fisheries, Alexandria, Egypt

Keywords Fish · Polycyclic aromatic hydrocarbon · Aliphatic hydrocarbon · Lake Edku

Introduction

Lake Edku is a shallow lagoon situated on the western margin of the Nile delta. The lake receives its main water budget from three agricultural drains (Berzik, Bousily, and Edku drains) located at its eastern and southeastern edges. About 980 × 10^6 m^3 of contaminated brackish water are transported annually by these drains to the lake. Berzik drain contributes the largest amounts of water, followed by Bousily and Edku drains (Abdel-Moati 1990). The current lake Edku map records many changes; for example, more than 50% of the lake's area has been isolated and converted to fish farms. About 300 small fish farms were located in the lake; in addition, there are three large fish farms. Two of these are located in the southeastern part of the lake, and one in the lake's northern Kom Bilag region. The Kom Bilag Fish Farm has a total area of 8400 m^2. About 30 fish farms are located at the western edge of Lake Edku; these belong to the Fishing Authority. Currently, the northwestern tip of the lake is converted to fish farms (Abdallah and Morsy 2013). The immediate area surrounding the lake is highly industrialized and includes chemical manufacturers, food processing and power producers, among other industries. In addition, the vehicular traffic is a year round source of contaminants to the lake and surrounding environment primarily from engine emissions.

Polycyclic aromatic hydrocarbons (PAHs) are a class of diverse organic compounds containing two or more fused aromatic rings of carbon and hydrogen atoms. They are ubiquitous pollutants formed from the combustion of fossil fuels and are always found as a mixture of individual compounds. PAHs, which are omnipresence environmental pollutants in urban areas, originate from a variety of anthropogenic sources that include thermal combustion processes (e.g., cooking and heating oils, and coal burning), vehicular emissions (e.g., automobiles and trucks), and biomass burning (e.g., Wreplaces and controlled burning) (Simoneit 1984). PAH generally occurs as complex mixtures and not as single compounds in environment. Some PAH such as benz[*a*]anthracene, benzo[*b*]Xuoranthene, benzo[*k*]Xuoranthene, benzo[*a*]pyrene (BaP), chrysene, dibenz[*a*,*h*]anthracene, and indeno[1,2,3-*c*,*d*] pyrene have mutagenic and genotoxic potential (WHO 1989). Chronic exposure to PAH poses a threat to marine biota and wildlife (Peterson et al. 2003).

Fish is an essential food source, and the ever-increasing population typically has a strong influence on the demand for fish (Abdallah and Mohamed 2015; Abdallah 2008). Aquaculture is often presented as a potential alternative to the challenge caused by the stagnation in the fish catch (FAO 2007). Ponds that are closely connected to their surrounding watersheds and use as aquaculture, as a consequence, the fish produced may be contaminated by a large number of substances including PAHs (runoff and effluent) (Marielle et al. 2012).

This study aimed to provide baseline information on the levels polycyclic aromatic and aliphatic hydrocarbons in different fish species, information which was in turn used to further our understanding of the bioaccumulation of organochlorine pesticides (OCPs), to reflect the state of pollution in different fish farming environments, and identify potential input sources. The study extended to evaluate the possibility of adverse health effects due to consumption of fish species based on the current ingestion rate and to provide useful data for the environmental assessment initiatives for maritime sector for such future studies.

Materials and Methods

Study Area and Sampling

Edku lagoon is one of the northern Nile Delta lakes, west of the Rosetta branch of Nile River located between longitude 30°8′30″ and 30°23′00″E and latitude 31°11′00″ and 31°18′00″N. It is shallow, slightly brackish water lagoon on distance of 30 km to the northeast of Alexandria City. The water depth ranges from 40 to 150 cm, with an average of 85 cm, the lake has a total area of about 12,000 ha. The lake is connected to the Mediterranean Sea at its northwest end through a short channel called “El-Boughaz”. It receives agricultural drainage water at its eastern section from three main land drains. The morphometric data and selected chemical characteristics of the studied lake are shown in Table 1. The present study focused on six species of freshwater fish collected from fish farms inside the lake from three locations (Fig. 1). Nile tilapia (*Tilapia nilotica*, $n = 18$), Mango tilapia (*Tilapia galilaeus*, $n = 26$), Blue tilapia (*Tilapia aureus*, $n = 25$), Red belly tilapia (*Tilapia zilli*, $n = 30$), Thin lip gray mullet (*Mugil capito*, $n = 18$) and North African catfish (*Clarias lazera*, $n = 11$) were collected during the period September 2011 to February 2012. Body weight and length for each fish were measured prior to dissection (Table 1). Collected fish species were dissected; the soft tissue portion was wrapped in an aluminum foil, sealed in polythene bag and preserved at −20 °C until analysis.

Sample Preparation and Extraction

The samples were analyzed for organochlorine following well-established techniques by UNEP/IOC/IAEA (1991, 1992). The soft tissue portions of the studied fish samples were wrapped in an aluminum foil, sealed in polythene bag and preserved at −20 °C until analysis. The fish tissues were well homogenized (2–3 min) with sodium sulfate to ensure adequate dryness. The mixture was then transferred to a pre-cleaned extraction thimble, and the dehydrated tissue was extracted with 200 ml (1:1) of *n*-hexane-dichloromethane for 8 h in a Soxhlet apparatus cycling 5–6 times/h. Anhydrous sodium sulfate was extracted in the same fashion as the sample and used as the blank. The extracted solvents were concentrated with a rotary evaporator down to 2 ml (maximum temperature: 35 °C), followed by concentration with a pure nitrogen gas stream down to a volume of 2 ml.

Cleanup and fractionation were performed by passing the extract through a silica/alumina column. The first milliliter of the extracted volume was passed through the silica column prepared by slurry packing 20 ml (10 g) of silica, followed by 10 ml (10 g) of alumina and finally 1 g of anhydrous sodium sulfate. Elution was performed using 40 ml of hexane (aliphatic fractions, F1), then 40 ml of hexane/dichloromethane (90:10), followed by 20 ml of hexane/dichloromethane (50:50) (unsaturated and aromatic hydrocarbons fraction (F2). Finally, eluted samples were concentrated under a gentle stream of purified nitrogen to about 0.2 ml, prior to injection into GC/FID for PAHs analysis. Descriptive statistics (means and standard deviations) and correlation analyses were performed using the SPSS 12 statistical software (IBM, Armonk, NY, USA).

Results and Discussion

PAHs Levels in Fishes from Lake Edku Fish Farm

The PAH concentrations of the studied fish collected in September 2011 and February 2012 are shown in Table 2.

Table 1 Fish species examined and their ecological characteristics

English name	Scientific name	*N*	Mean ± SD		Feeding habits
			Length (cm)	Weight (g)	
Nile tilapia	*Tilapia nilotica*	18	19.1 ± 1.6	141 ± 41.0	Feed mainly on phytoplankton or benthic algae
Blue tilapia	*Tilapia aureus*	25	12.3 ± 1.0	32.5 ± 13.4	Feed on phytoplankton and small quantities of zooplankton
Red belly tilapia	*Tilapia zilli*	30	11.8 ± 0.5	31.6 ± 6.4	Feed on water plants and epiphyton, and some invertebrates
Mango tilapia	*Tilapia galilaeus*	26	10.5 ± 0.8	22.4 ± 2.9	Feed on algae and fine organic debris; bi-parental mouth brooder
Thin lip gray mullet	*Mugil capito*	18	24.7 ± 2.5	120.5 ± 30.5	Feed on epiphytic algae, detritus and small benthic or planktonic organisms, pelagic eggs and larvae
North African catfish	*Clarias lazera*	11	29.0 ± 7.4	176.9 ± 10.4	Feed on insects, plankton, invertebrates and fish but also takes young birds, rotting flesh and plants

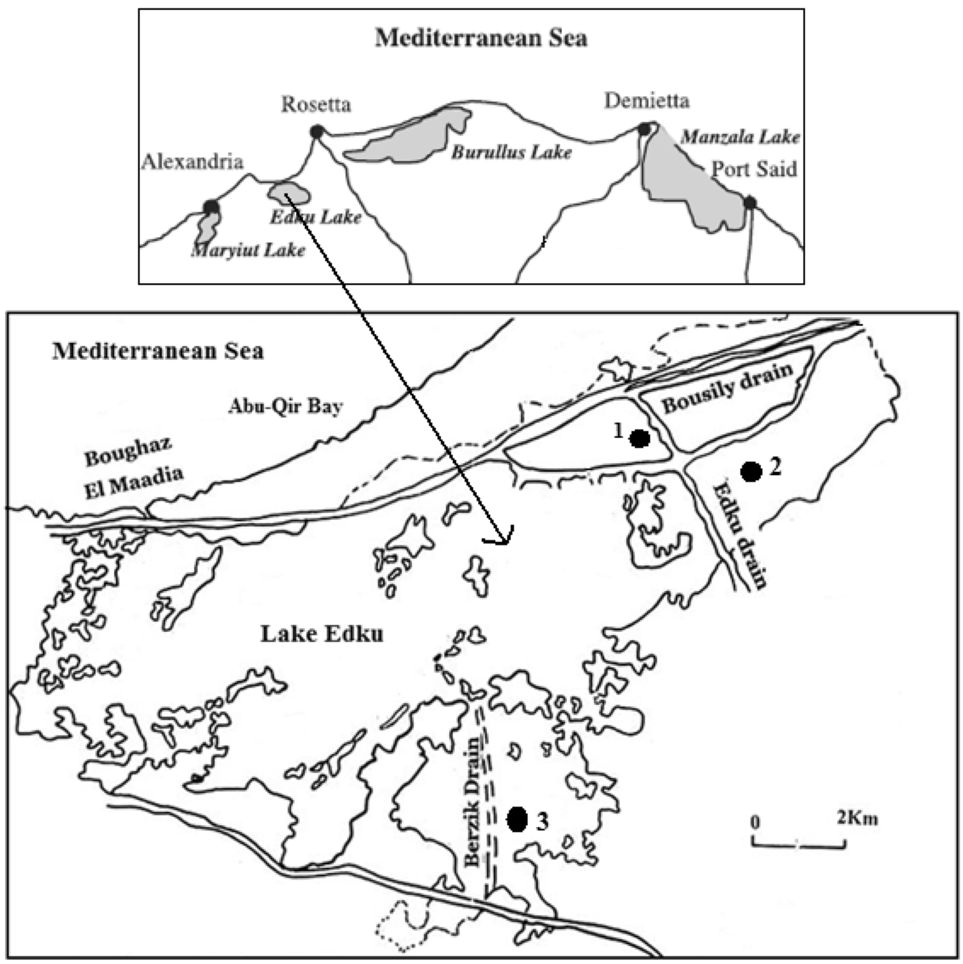

Fig. 1 Sampling sites of the study area of Lake Edku

Concentrations of PAHs in Lake Edku fish species can arrange in decreased order as *C. lazera* (1008.73 ng/g) > *T. nilotica* (843.70 ng/g) > *T. aureus* (705.44 ng/g) > *T. zilli* (647.97 ng/g) > *M. capito* (401.60 ng/g) > *T. galilaeus* (285.03 ng/g). The concentration of PAHs was ranged from 285.03 to 1008.73 ng/g of wet muscles samples. All studied fish accumulated high concentrations of low molecular PAHs mass (Nap, Acft, Ace, Fluo, Phen,

Table 2 Concentration (ng/g wet weight) of aromatic hydrocarbons in fish samples collected from Lake Edku

Chemical compound	*T. nilotica*	*T. aureus*	*T. zilli*	*T.galilaeus*	*M. capito*	*C. lazera*	Average	Max	Min
Acenaphthylene	23.60	14.60	15.20	2.40	13.40	26.32	15.92	26.32	2.40
Acenaphthene	63.70	48.20	45.30	15.21	23.80	85.50	46.95	85.50	15.21
Phenanthrene	119.60	72.90	85.10	33.10	21.36	156.23	81.38	156.23	21.36
Anthracene	258.30	231.10	189.20	101.30	144.80	278.25	200.49	278.25	101.30
Fluoranthene	125.60	114.50	126.10	43.50	65.70	145.36	103.46	145.36	43.50
Chrysene	2.10	3.52	3.27	1.02	3.05	4.23	2.87	4.23	1.02
Pyrene	149.20	140.10	112.50	66.30	74.25	185.47	121.30	185.47	66.30
Benzo(*a*)anthracene	8.20	7.80	7.51	2.10	6.50	10.35	7.08	10.35	2.10
Benzo(*b*)fluoranthene	29.52	31.50	24.19	11.60	18.65	33.52	24.83	33.52	11.60
Benzo(*k*)fluoranthene	39.40	21.40	14.50	2.20	11.54	49.40	23.07	49.40	2.20
Benzo(*a*)pyrene	14.50	13.20	16.80	2.10	13.11	19.90	13.27	19.90	2.10
Dibenzo(*a*,*h*)anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo(ghi)perylene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Indeno(1,2,3 cd)pyrene	ND	ND	1.88	ND	ND	ND	1.88	1.88	1.88
Total PAHs	843.70	705.44	647.97	285.03	401.60	1008.73	648.75	1008.73	285.03
Ant/(Ant + Phen)	0.348	0.398	0.347	0.315	0.527	0.354	0.382	0.527	0.347
BaA/(BaA + Chry)	0.796	0.689	0.697	0.673	0.681	0.710	0.708	0.796	0.673

and Ant) than that of high molecular mass PAHs (BaA, Chry, BbF, BkF, BaP, InP, DBA, and BghiP) in its tissues. Schnell et al. (1980) attributed this phenomenon to the metabolism; it is suspected that PAHs of high molecular weight (HMW) are more rapidly metabolized than low molecular weight (LMW) due to differences in enzyme affinity. Additionally, PAHs such as BaP may be metabolized at different rates, producing unexpected bioaccumulation patterns (Baumard et al. 1999).

The size of the fish has an important effect on bioaccumulation. The rates of feeding and respiration are allometric and consequently are influential on the rates of contaminant uptake. The uptake of PAHs are controlled externally by the partitioning behavior of the contaminant (between sediment, water, and food) and internally by the organism's behavior and physiology. The biological processes that can influence uptake include fish size, growth rate, membrane permeability, ventilator rate, extraction efficiency, ingestion rate, gut residence time, and osmoregulation. Some of these processes are effective to a species, others are interdependent (e.g., ingestion rate and growth rate), and many may be influenced by environmental factors such as temperature, oxygen content, pH, and salinity (Landrum 1988).

Several factors could affect the amount of PAHs accumulated by organisms, as organism behavior, organism size, and environmental inputs. Temperature and lipid content affect the rates of uptake, and elimination is not addressed separately (Meador 2003). However, it is considered that the organisms do not change their behaviors and feeders in Lake Edku fish farm.

However, it is important to keep attention with the levels of PAHs during rice straw burning periods, each year in autumn thousands tons of rice straw as well as garbage are get rid through burning steadily in rural areas around Lake Edku, as well as in El Maadia region that containing a fishing port, includes many types of activities that result amounts of exhaust fuel and may cause a rise in PAHs in the lake water and consequently to the Fish. The emission factor (EFs) of rice straw burning was (9.29–23.6 mg/kg) from 16 PAHs Lu et al. (2009). In waters of high turbidity and low depth, as Lake Edku, where particles are re-suspended regularly, fishes will contain a higher proportion of the more hydrophobic 4- to 6-ring compounds (HMWPAH) due to accumulation of PAHs from the sediment (Porte and Albaiges 1993). In most of the investigated samples of all species, LMWPAH was in most cases much lower than the HMWPAH that could be possibly related to the metabolic pathway in the investigated fish.

The total average concentration of PAHs from fish muscles in the present study (648.75 ng/g; wet weight) was much lower than the average of PAHs (12,112 ng/g; 57,980 and 87,690 ng/g wet weight) in fish and Osteicthyes fishes collected from the western coast of Alexandria, Great Bitter lakes and El Temsah lake (Suez Canal), reported by Said (2007) and Said and El Agroudy (2006), respectively. While it is higher than the average of PAHs (295 ng/g; wet weight) in mussels and clams reported by Law and Andrulewicz (1983) for the southern Baltic Sea, surprisingly, Anthracene was the most dominant PAHs fraction ranging from 101.30 to 278.25 ng/g with average

200.49 ng/g wet weight in all fish species not BaP. As the main source of Anthracene in the water is the manufacture of dyes for the textile processes, the relatively high concentrations in the studied fish may attributed to wastewater containing residues of dyes containing anthracene that flowing directly to Abu Qir Bay through "Tabia pump station" without any treatment, and it is possible that they may reach to the lake through the El Boughaze opening that connect the lake water with water Abu Qir Bay. In addition, the ability of fish species to adapt to changes plays an important role for living in polluted areas.

With regard to BaP, which represents the most potentially carcinogenic PAH, European Regulation 1881/2006/EC (2006) fixed at 10 ng/g of wet weight the MRL (maximum residual level) in bivalves mollusks. All the studied fish showed values higher than MRL (13.11–19.90 ng/g) except *T. galilaeus* showed value lower than MRL (2.10 ng/g). All samples showed BaP concentration values lower than 3% of Σ15PAH, which may suppose some risk for human health.

The PAH sources may be identified by individual PAH compound ratios based on the peculiarities in PAH composition and distribution patterns as a function of the emission sources (Guo et al. 2007). According to Guo et al. (2007) and Yunker et al. (2002), the Ant/(Ant + Phen), BaA/(BaA + Chry) ratios (Table 2) have been applied to distinguish petrogenic and pyrogenic inputs. Petrogenic inputs show Ant/(Ant + Phen) values lower than 0.10 and pyrogenic inputs exhibit higher values. BaA/(BaA + Chry) ratio of <0.2 suggests petrogenic input, and a ratio of >0.35 indicates pyrogenic processes, while a ratio between 0.2 and 0.35 is characteristic of combustion process. In the present study, PAH isomeric ratios from fish indicated the pyrogenic process as the main source. Although PAHs do not show extremely high acute toxicity to aquatic organisms, the lower molecular mass compounds tend to exhibit higher lethal toxicity than the larger PAHs (Law et al. 1997).

Aliphatic Hydrocarbon Levels in Fishes from Lake Edku Fish Farm

In all of the samples, the aliphatic fraction of the hydrocarbons was higher than the aromatic fraction (Table 3). The basic statistics for total and aliphatic hydrocarbon contents in farmed fish samples from Lake Edku were showed in Table 3. Quantitative determinations of aliphatic HC were based on comparisons of peak heights with those obtained from the reference standard (ASTM PS 18–44N Supelco Inc. Bellefonte, PA).The results represent the concentrations of 10 even detectable aliphatic hydrocarbons from C-12 to C-30, with total values ranged from 301.41 to 1268.11 ng/g (wet weight), indicating bioaccumulation of *n*-alkanes in some individuals of tilapia species from Lake Edku. Higher concentrations occurred in *C. lazera*, while the lower concentrations were found in *T. aureus.* Variations in hydrocarbon content of different fish species from the same location are and may be attributed to feeding patterns, different age and sizes of the specimens analyzed, and fat content. Mean total hydrocarbon concentrations (THC) were 1063.5 ng/g (range 501.7–1714.3 ng/g) in the studied samples. The concentrations of total aliphatic were generally lower than 1 µg/g wet weight in all Tilapia species, and higher than 1 µg/g in *M. capito* and *C. lazera* in Edku Lake. The predominant hydrocarbon in all samples was C-30, followed by C-14 in all Tilapia species, C-28 in *M. capito*, and C-26 in *C. lazera*. The data were generally slightly above the detection level of 0.01 µg/g (all over range of all aliphatic hydrocarbons was 2.04–682.50 ng/g). The THC contents observed in the present study are quite lower than to those in Gulf of Naples (Italy) and the zone of Taranto (Italy) reported by Amodio-Cocchieri and Cirillo (2003) and UNEP (1988), respectively.

It appears that for all species except *T. aureus* and *T. zilli*, the concentrations of n-C12 to n-C20 hydrocarbons were low concentrated than the longer chain (>20) *n*-alkanes. The traces of these higher alkanes are the residues of the hydrocarbons taken up by the fish either from petroleum pollution or from other fossil hydrocarbons dissolved in the lake water. Considering the findings, it may be identified that a major portion of the hydrocarbons retrieved from the fish were inherited from the hydrocarbons of the biological origin and a significant amount from fossil origin.

The limitations of using *n*-alkanes to isoprenoid ratios, such as nC18/phytane, for monitoring biodegradation were demonstrated in this study. The ratio of nC18/phytane is often used as qualitative measures of biodegradation (Ezra et al. 2000), because phytane is relatively persistent biomarkers. As the fuel contaminant is biodegraded, bacteria preferentially consume the nC17 and nC18 compounds, resulting in a relative enrichment of phytane in the residue. The relative nC18/Phy ratio at all species ranged from 0.60 to 1.23.

Toxicity, Risk Assessment, and Deduction of MAC Based on RfD for PAHs and Aliphatic Hydrocarbon Pollutants

The assessment of PAHs toxicity in the present study was performed based on the BaP toxic equivalency factors (TEFs). Among ΣPAHs are of high concern due to their potentially carcinogenic toxicities. In order to estimate the toxicity of PAHs, toxic equivalent quantity (TEQ) was calculated by multiplying the concentration of ΣPAHs by

Table 3 Concentration (ng/g wet weight) of aliphatic hydrocarbons in fish samples collected from Lake Edku

Chemical compound	*T. nilotica*	*T. aureus*	*T. zilli*	*T.galilaeus*	*M. capito*	*C. lazera*	Average	Max	Min
C-16	54.37	42.30	55.80	50.40	41.90	54.20	49.83	55.80	41.90
C-18	27.30	19.20	38.90	26.30	20.970	24.70	26.23	38.90	19.20
C-20	24.70	21.40	31.70	28.00	35.20	41.15	30.36	41.15	21.40
C-22	6.15	3.18	9.92	7.50	15.27	23.60	10.94	23.60	3.18
C-24	24.00	7.32	2.04	4.32	3.32	14.60	9.27	24.00	2.04
C-26	23.60	11.23	19.70	19.80	201.87	158.95	72.53	201.87	11.23
C-28	57.60	42.80	24.90	19.20	119.20	139.42	67.19	139.42	19.20
C-30	426.30	58.30	44.89	485.85	526.28	682.50	370.69	682.50	44.89
Total aliphatic	753.02	301.41	344.88	757.81	1078.93	1268.11	750.69	1268.11	301.41
Total PAHs	843.70	705.44	647.97	285.03	401.60	1008.73	648.75	1008.73	285.03
Total hydrocarbon (THC)	1596.72	1006.85	992.85	1042.84	1480.53	2276.84	1399.44	1596.72	992.85

Table 4 Calculated HQs risks in the performed human health risk assessment in species from Lake Edku

Species	HQs		
	Total aliphatic	Total PAHs	Total hydrocarbons
T. nilotica	0.0014	0.10	0.11
T. aureus	0.0006	0.09	0.09
T. zilli	0.0006	0.08	0.08
T. galilaeus	0.0013	0.04	0.04
M. capito	0.0037	0.05	0.05
C. lazera	0.0023	0.12	0.13

the corresponding *TEF* (USEPA 2012). According to USEPA (2012), *TEFs* for BaA, Chr, BbF, BkF,BaP, InP, and DahA were 0.1, 0.01, 1, 0.1, 0.1, 0.1, and 1, respectively. The TEQ for all ΣPAHs in each site was calculated using the following equation:

$$\mathrm{TEQ_{PAHs}} = \Sigma \mathrm{TEF}_i \times \mathrm{C_{PAHs}}.$$

The reference dose—RfD (mg/kg wet weight/day), represents a safe dose for protection against threshold (noncarcinogenic) health effects. For dose–response assessment, the purpose was to derive a maximum allowable concentration (MAC) for a specific chemical in the fish tissue that represents the highest concentration of each toxicant that can occur in the fish without causing harm to the human beings.

The maximum permitted concentration in fish based on the RfD (MACRfD) is calculated based on the following equation (US/EPA 1989, 1996).

$$\mathrm{CDI} = \frac{\mathrm{C_{tis}} \times \mathrm{Rf} \times \mathrm{IR} \times \mathrm{Cf} \times \mathrm{ABC} \times \mathrm{Ef} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}},$$

where CDI is the chronic daily intake (mg/kg day), $\mathrm{C_{tis}}$ is the concentrations (mg/kg wet weight) of the hydrocarbons in fish tissues, Rf is the reduction factors (unitless): (1), IR is the ingestion rate (g/day): (FAO 2002), Cf is the conversion factor, ABS is the ingestion absorption factor: 100% (conservative), Ef is the exposure frequency (days/year): 350 days/year for noncarcinogenic effects (US/EPA 1997), ED is the exposure duration (years): 30 year for noncarcinogenic effects (US/EPA 1997), BW is the body weight (kg): 70 kg, AT is the average time of exposure (days): 365 × 30 for noncarcinogenic effects (US/EPA 1997).

$$\mathrm{HQ} = \frac{\mathrm{CDI}}{\mathrm{RfD}}.$$

As the RfD is a threshold dose or intake, which is conservatively chosen so that if the estimated intake is less than the reference dose ($\mathrm{HQ} < 0.1$), there is almost no possibility of an adverse health effect. However, if the intake exceeds the reference dose ($\mathrm{HQ} > 0.1$), this does not indicate that adverse health effect is expected, only that a conservative threshold is exceeded.

Calculated HQs for the investigated PAHs in all species are summarized in Table 4. It was found that no adverse health effects (noncarcinogenic) are associated with the consumption of all fish species from Lake Edku with respect to PAHs.

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