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Assessment of Polycyclic Aromatic Hydrocarbons in *Lates niloticus, Oreochromis niloticus* and *Rastrineobola argentea* as Sources of Human Exposure in Kisumu Bay, Winam Gulf of Lake Victoria

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Abstract The concentrations of polycyclic aromatic hydrocarbons (PAHs) in *Lates niloticus*, *Oreochromis niloticus* and *Rastrineobola argentea* from three beaches were assessed to establish whether they are sources of human exposure in Kisumu Bay, Winam Gulf, Lake Victoria, Kenya. *O. niloticus* had 12 PAHs detected (TPAH 3.93 μg/kg); *L. niloticus* had 11 (TPAH 3.17 μg/kg). In both cases, the highest and lowest concentrations were Pyrene and Indeno(1,2,3-cd)pyrene, respectively, and the TPAHs were greater than the 2 μg/kg limit allowed by the European Commission although individually they were lower. *R. argentea* had 2 PAHs (TPAH 0.035 μg/kg). PAH pollution in the Winam Gulf, a potential source of exposure to human through contaminated fish, should be mitigated and safe management practices that reduce PAH levels should be adopted.

Keywords PAHs · Fish · Kisumu Bay · Winam Gulf · Human exposure

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Polycyclic aromatic hydrocarbons (PAHs) are of great concern in environmental monitoring because they are known or suspected carcinogens and/or toxicants. The United States Environmental Protection Agency has listed sixteen PAHs as priority pollutants that need to be periodically monitored in the environment because of their known carcinogenicity. Cancer incidence in Kenya is increasing and now numbers among the top 10 causes of mortality (American Society of Clinical Oncology website, accessed 21-11-2010). However, the cause of increase in cancer incidence and prevalence is not known. The population may be exposing themselves to carcinogens by eating contaminated fish (Takatsuki et al. 1985; Eisler 1987; Fent and Bätscher 2000).

Potential sources of PAHs exist in Kisumu Bay of Winam Gulf. The major point sources include petroleum fuel spillages, Kenya Pipeline Company depot runoff, car wash activities at the shore, oil spills from vessels at the Pier and Yatch Club, mechanical workshops (*Jua Kali* sheds) and petrol-station runoffs (Bowa et al. 2009). Mobile sources include motor vehicle exhaust (Lalah and Kaigwara 2005) and consumer products waste dumped into the lake. Diffuse sources include asphalt roads and road tar, fires of all types (municipal garbage incineration and burning of sugar cane from the surrounding sugar belt) and biomass energy combustion (Takatsuki et al. 1985; Gu et al. 2003; Lisouza et al. 2011), agricultural runoff, natural alteration of organic matter (Mitra and Bianchi 2003) following widespread infestation of the gulf by hyacinth. It is not known how much these sources contribute to the PAH burden in fish in the Winam Gulf and potential exposure to human since their levels are neither known nor periodically monitored. Although fish is an indicator of PAHs in aquatic environments and a source of exposure to humans, the contribution of fish to human PAH exposure in the region is not known.

Materials and Methods

General purpose grade (GPR) ethanol, methanol, and diethyl ether from Kobian (K) Ltd, Nairobi, were distilled in the laboratory prior to use for extraction. Cyclohexane, n-hexane, also from Kobian (K) Ltd, Nairobi, were 95% pure HPLC grade. Potassium hydroxide, sodium sulphide ($Na_2S.9H_2O$), anhydrous sodium sulphate (Na_2SO_4) and silica gel (230–400 mesh) were pure analytical grade and were obtained from Kobian (K) Ltd, Nairobi. Pure nitrogen and helium for gas chromatography–mass spectrometric (GC–MS) analysis were obtained from East African Oxygen Company, Kisumu, Kenya.

Samples of tilapia (*Oreochromis niloticus*), Nile perch *Lates niloticus* (wet weight ranging from 0.5 to 1.2 kg) and Omena (*Rastrineobola argentea*) (20–30 g wet weight) were collected in triplicate from the three main beaches that land fish in Kisumu Bay, Kenya (0°6′S, 34°45′E) i.e. Dunga Beach, Usoma Beach and Usare Beach, respectively, located along the Kisumu Bay. These species were chosen because they were the most important commercially and are consumed locally exported to other countries, including the EU market. *R. argentea* is popular and affordable for human consumption as well as for making animal feeds.

The samples were wrapped in precleaned aluminium foil, placed in an ice cooler box and transported to the laboratory in Maseno University. Extraction method followed was a modified one based on the method described by Takatsuki et al. (1985). Edible parts of fish were cut into small pieces and homogenized; 50 g was saponified using a mixture of ethanol (200 mL), 50% aqueous potassium hydroxide (35 mL) and sodium sulphide (2 g) and then refluxed on a water bath for 2 h. The mixture was cooled to 40°C, 150 mL of n-hexane added in small portions, with occasional swirling. The mixture was poured into 150 mL water in a 500-mL glass separation funnel, the flask rinsed twice with 10 mL ethanol and the rinses added to the mixture. The separation funnel was gently and adequately shaken and set to separate. The aqueous layer was extracted twice with 150 and 100 mL portions of n-hexane, respectively. All the n-hexane extracts were combined, washed with 100 mL deionized water, dried over anhydrous sodium sulphate, transferred to a rotary evaporator and then concentrated to about 3–5 mL.

For clean-up and separation, a chromatographic column (glass: 1 cm diameter) was packed with 8 g silica (in slurry) and 3 g anhydrous Na_2SO_4, added on top. The column was protected from light with aluminium foil, washed with 30 mL n-hexane; solvent drained to just top of column but column drying and contact of air with the packing was avoided. The concentrated extract was transferred to the column. Clean up and elution of PAHs was done slowly under gravity with 150 mL volume of 10% diethyl ether in n-hexane. The extract was evaporated to 1–2 mL, in rotary evaporator, then under mild stream of nitrogen to dryness with gentle warming and the residue was taken up in 1 mL with pentane (Sigma, St. Louis, MO, USA) and stored in sealed vials for analysis.

The samples were analyzed by GC–MS, a 7890A standalone GC (Agilent Technologies, Inc., Beijing, China) and a 5975 C MSD (Agilent Technologies, Inc., Santa Clara, CA, USA). The conditions were: inlet temp 270°C, transfer line temp of 280°C, and column oven temperature programmed from 35 to 285°C initially for 5 min then 10°C/min to 280°C for 10.5 min and then at 5°C/min to 285°C for 29.9 min. The GC had HP-5 MS low bleed capillary column (30 m × 0.25 mm i.d., 0.25-μm) (Restek, Bellefonte, PA, USA). Helium was the carrier gas (flow rate 1.25 mL/min). The MSD ion source temperature was 250°C and quadrupole temperature of 180°C. Electron impact mass spectra were obtained at acceleration energy of 70 eV. Fragment ions were analyzed over 40-550 m/z mass range in the full scan mode. A 1.0 μL aliquot of extract was automatically injected in the split/splitless mode using an auto sampler 7,683 (Agilent Technologies, Inc., Beijing, China). The filament delay time was set as 3.3 min. Library—MS searches using NIST/EPA/NIH Mass Spectral Library (NIST 05) and NIST Mass Spectral Search Program Version 2.0d.

External PAH standards (PAH Mix 9; Dr. Ehrenstorfer mbH, Augsburg, Germany) were used in different dilutions to come up with calibration curves which were used to relate the various concentrations to peak areas. To evaluate the recovery of PAH and to account for matrix effects in the GC–MS chromatograms, spiked control samples were analysed under the same conditions. The results obtained were then corrected based on percentage recovery for each PAH. The results were analysed for descriptive statistics using INSTAT and MSTAT-C.

Results and Discussion

A number of PAHs were detected in concentrations ranging from 0.004 to 0.886 μg/kg while the total PAHs (TPAHs) for the three fish species ranged from 0.035 to 3.934 μg/kg (Table 1; Fig. 1a, b). *O. niloticus* had 12 priority PAHs ranging from 0.073 μg/kg (Indeno(1,2,3-cd)Pyrene) to 0.886 μg/kg (Pyrene at Dunga Beach) and the mean total PAH was found to be 3.934 μg/kg. *L. niloticus* had 11 priority PAHs with concentrations in the range of 0.133 μg/kg

Table 1 The mean PAH concentration (±SD) in fish (μg/kg ww)

PAH	*Oreochromis niloticus*	*Lates niloticus*	*Rastrineobola argentea*	PAH mean
Naphthalene	0.21 ± 0.016	0.19 ± 0.025	0.004 ± 0.00	0.17 ± 0.075
Acenaphthylene	nd	0.43 ± 0.193	0.0.03 ± 0.00	0.19 ± 0.248
Acenaphthene	0.11 ± 0.011	0.22 ± 0.021	nd	0.14 ± 0.08
Fluorene	0.45 ± 0.022	0.34 ± 0.010	nd	0.34 ± 0.151
Phenanthrene	0.23 ± 0.006	0.16 ± 0.008	nd	0.17 ± 0.077
Anthracene	0.80 ± 0.017	0.51 ± .015	nd	0.56 ± 0.271
Fluoranthene	0.41 ± 0.006	0.31 ± 0.008	nd	0.31 ± 0.137
Pyrene	0.89 ± 0.035	0.58 ± 0.007	nd	0.63 ± 0.301
Chrysene	0.32 ± 0.010	nd	nd	0.14 ± 0.165
Benz(k)fluoranthene	0.21 ± 0.009	0.16 ± 0.007	nd	0.16 ± 0.072
Indeno(1,2,3-cd)pyrene	0.07 ± 0.003	0.13 ± 0.015	nd	0.09 ± 0.048
Dibenzo(a,h)anthracene	0.09 ± 0.002	0.14 ± 0.008	nd	0.10 ± 0.046
Benz(g,hi)perylene	0.15 ± 0.012	nd	nd	0.06 ± 0.074
Total PAH	3.934	3.166	0.035	3.047

nd not detected/below detect limit (detection limit = 10exp − 12 g)

ww wet weight

(Indeno(1,2,3-cd)Pyrene) to 0.581 μg/kg (Pyrene at Dunga Beach) while the mean total PAH was found to be 3.166 μg/kg. *R. argentea* had only 2 priority PAHs, Naphthalene 0.004 μg/kg and Acenaphthylene 0.031 μg/kg detected in Dunga samples only and the mean total PAH was 0.035 μg/kg.

Similar studies in other locations have shown comparable to higher values. Muscles of 6 species of fish in Lake Ontario had 3–8 μg/kg ww of Total PAH (Eisler 1987). PAH levels in the liver and muscle tissues of English Sole from Vancouver Harbour had concentrations of 0.001–0.037 μg/g dw of low molecular weight PAH and trace-0.074 μg/g of high molecular weight PAH (Goyette and Boyd 1989). There were variations in the number and levels of PAHs from one species to another in this study. These variations can be attributed to species biological differences and their feeding modes and minor differences in sampling sites. The sources of these variations were, however, not investigated.

The recommended daily dietary protein allowances for adults (http://www.nutrition.com.sg/he/herda-adt.asp accessed 27/6/2011) are Men (68 g), Women (58 g), Pregnant Women (67 g) and Lactating Women (83 g first 6 months and 77 g after 6 months). These values were used to estimate the weekly (daily exposure × 7) exposure assuming all daily protein was provided by fish (Table 2).

Assuming that all the PAHs being taken in are not being broken down by natural processes in the body, it only takes 1 week for lactating women taking the daily protein requirement of 83 g from *O. niloticus* to reach the limit set by European Commission Regulation (2006) while the rest and those using *L. niloticus* would need just over a week. This shows that the potential human exposure in the region would be very high especially given that there are other sources of PAHs e.g. water and inhalation (Bowa et al. 2009) and smoke during cooking (Lisouza et al. 2011). Bowa et al. (2009) reported significantly high concentrations of the same USEPA priority PAHs in surface sediment and water in the same area where the fish samples for this study were obtained from. The mean concentrations of individual PAHs in surface sediment and water samples ranged from 0.38 to 11.77 μg/g (dry weight) and nd-15.66 μg/L, respectively. Bowa et al. (2009) also estimated weekly human exposures ranging from 0.05 to 20 mg through untreated drinking water from Winam Gulf. Lisouza et al. (2011) recently found varying concentrations of similar USEPA PAHs in soot collected from rural households in the Lake Victoria basin indicating that use of firewood in cooking in the rural areas could be contributing significantly to PAH human exposure in the region. The mean levels of individual PAHs in soot from the sampled kitchen roofs ranged from 0.23 to 4.75 μg/g, depending on the type of firewood and age of the kitchen.

This study has revealed that the total PAHs concentration levels in fish from Kisumu city Bay in Winam Gulf are higher than maximum allowable limits indicative of fish being a significant potential human exposure to PAHs. *O. niloticus* is a more potential source of exposure followed by *L. niloticus* while *R. argentea* is the least potential source of exposure. The values of individual PAHs in the fish are lower than the maximum limits set by Commission Regulation (EC) No 1881/2006 which is 2 μg/kg (EU 2006). However, the upper values for total PAHs showed higher figures than this limit. The population in the area

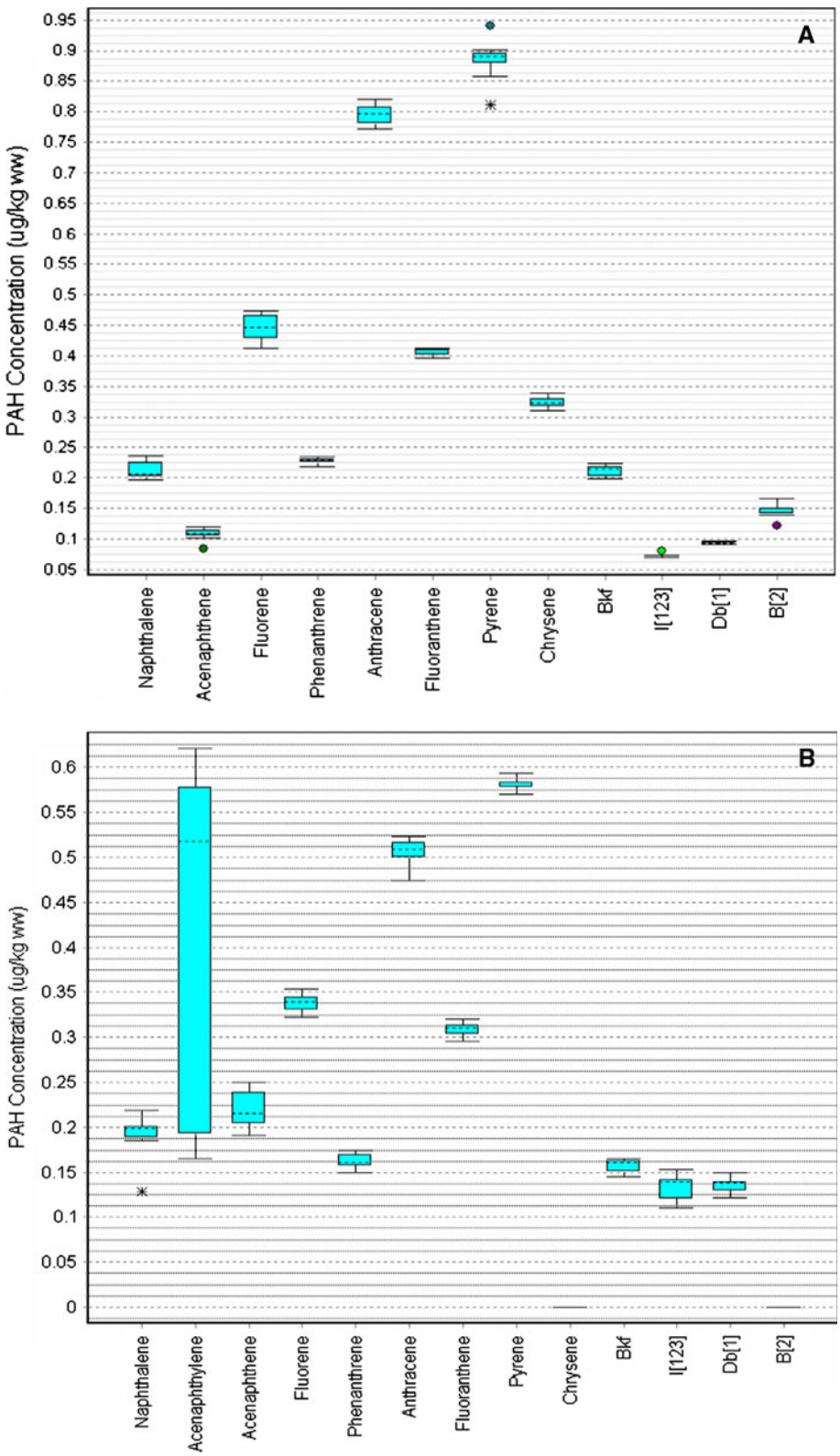

Fig. 1 **a** Means, ranges, minima and maxima of PAH concentration in *Oreochromis niloticus* (µg/kg ww). **b** Means, ranges, minima and maxima of PAH concentration in *Lates niloticus* (ug/kg ww)

should be encouraged to eat *R. argentea* while consumption of *O. niloticus* and *L. niloticus* should not be very frequent because of the high potential toxicity they pose due to PAH exposure. However investigations should be carried out to investigate the effect of cooking methods used on PAH removal from edible fish portions.

Table 2 Weekly exposure to total PAHs assuming all daily dietary protein allowance is provided by the given foods (μg/kg)

Group	FISH		
	O. niloticus (3.934 μg/kg)	*L. niloticus* (3.166 μg/kg)	*R. argentea* (0.035 μg/kg)
Men	1.873	1.507	0.017
Women	1.597	1.285	0.014
Pregnant women	1.845	1.485	0.016
Lactating women (first 6 months)	2.286	1.839	0.020
Lactating women (after 6 months)	2.120	1.706	0.019

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References

American Society of Clinical Oncology (ASCO) http://pda.asco.org/anf/Past+Issues/January+2008/Cancer+in+Kenya?cpsextcurrchannel=1. Accessed 21-11-2010

Bowa KO, Lalah JO, Wandiga SO (2009) Spatial and seasonal variations in concentrations of polycyclic aromatic hydrocarbons in water and sediment of Kisumu City Bay of Winam Gulf, Lake Victoria-Kenya. Bull Environ Contam Toxicol 83:722–733

Eisler R (1987) Polycyclic aromatic hydrocarbon hazards to fish, wildlife, and invertebrates: a synoptic review. US fish and wildlife service biological report 85(1.11), p 81

EU (2006) European Commision: commission regulation (EC) no 1881/2006

Fent K, Bätscher R (2000) Cytochtome P450 1A induction potencies of polycyclic aromatic compounds in a fish hepatoma cell line: demonstration of additive interactions. Environ Toxicol Chem 19:2047–2058

Goyette D, Boyd J (1989). Distribution and environmental impact of selected benthic contaminants in Vancouver harbour, British Columbia: appendices 1985–1987. Environmental Protection Service Regional Program Report 89-02, p 99

Gu S, Kralovec AC, Christensen ER, VanCamp RP (2003) Source apportionment of polycyclic aromatic hydrocarbons in dated sediments from Black River Ohio. Water Res 37:2149–2161

http://www.nutrition.com.sg/he/herda-adt.asp. Accessed 27/6/2011

Lalah JO, Kaigwara PN (2005) Polynuclear aromatic compounds in kerosene, diesel and unmodified sunflower oil and in respective engine exhaust particulate emissions. Toxicol Environ Chem 10(1):1–17

Lisouza FA, Owuor PO, Lalah JO (2011) Variation in indoor levels of polycyclic aromatic hydrocarbons from burning various biomass types in the traditional grass-roofed households in Western Kenya. Environ Pollut 159:1810–1815

Mitra S, Bianchi TS (2003) A preliminary assessment of polycyclic aromatic hydrocarbons distribution in the lower Mississippi River and Gulf of Mexico. Marine Chem 82:273–288

Takatsuki K, Suzuki S, Sato N, Ushizawa I (1985) Liquid chromatographic determination of polycyclic aromatic hydrocarbons in fish and shellfish. J Assoc Anal Chem 68:231–233