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Revealing of Polycyclic Aromatic Hydrocarbons (PAHs) in Temsah Lake, Egypt: A Case Study for Risk Assessment Associated with Bivalve's Consumption

Khaled Y. Abdel-Halim[1]*, Yosry M. Ahmed[b] , Amira A. Farahat[2]

[1]*Mammalian & Aquatic Toxicology Department, Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center (ARC), 12618-Dokki, Giza, Egypt*

[2]*Plant Protection Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt*

Abstract

The accumulation profiles of polycyclic aromatic hydrocarbons (PAHs) in bivalves of Temsah Lake and their risk on Ismailia residents were studied. The mean values of PAHs in sediment samples ranged from below detection limit (BDL) to 36.22 mg/kg and sum of PAHs was 102.60 mg/kg. However, the mean values of PAHs in bivalves were greater than the EU regulatory limits (30.0 µg/kg) of European Communities. The bioconcentration factor (BCF) values for ∑PAHs were in the following order: 8.83, 23.72, 12.37, and 13.37 in species: Comb circe, Surrclam, Grooved carpet shell, and Golden venus, respectively. Hazard index (HI) was 0.05 and 0.11 at 50 and 90th percentile of ingestion route. Congener benzo[a]pyrene (Bap) exhibited the greatest mean values of predicted cancer risk (CR) 0.098 and 0.065 for adults and children. However, benzo[a]anthracene (Baa) exhibited the mean values, 4.5E-05 and 1.2E-04) for adults and 3.6E-05 and 1.2E-04 for children at 50 and 90th percentile, respectively. Such accumulation resulting in impacted risk for local residents of Ismailia. Therefore, precautionary measures need to be taken in order to prevent further PAHs pollution.

Keywords: Polycyclic aromatic hydrocarbons; Temsah Lake; bivalves; risk assessment

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in environment. Anthropogenic supports of PAHs involve from the burning of fossil fuels, coal manufacture, oil industrial, oil falls, wood conservation, tobacco smoke and numerous forms of cooking. Natural causes contain volcanoes as well as forest fires [1]. Aromatic hydrocarbons include monocyclic aromatic hydrocarbons (MAHs), e.g. benzene, toluene, xylene, and alkylated benzene. PAHs are a wide class of organic compounds pervasive in sediments, since they are originated by different natural and anthropogenic processes. They are formed by two or more benzene rings and classified as persistent organic pollutants (POPs). They can represent between 0.2% to more than 7% of crude oil composition, in the most toxic fraction [2-4]. The significant forms of PAHs are benzo[a]pyrene (Bap), benzo[a]anthracene (Baa), and benzo[b]fluoranthene (Bbf) which distribute widely in atmosphere, surface water, sediments, soil, and groundwater. Regarding their potential effects as carcinogenic, teratogenicity and mutagenic effects, they can be an object of unlimited and permanent hazard to ecosystem care and human health [5, 6]. Several investigations recognized that, PAHs levels in the urban areas were greater than those in others [6, 7]. Furthermore, PAHs are released into water from burning of fossil fuel (pyrolytic source) and high-ring PAHs were more than that of low-ring PAHs. As mentioned previously in the literature, the uniform proportion was 1.21 and 12.9-folds during winter, and 3.4 and 8.3-folds during spring in water samples from great Bitter Lakes and Temsah Lake [8]. United States Environment Protection Agency (USEPA) stated that, pollutants sixteen PAHs were traveled into sediments, water and biota of Temsah Lake. The position of these compounds may explain a chemical sign of PAHs in a diverse foundation of PAHs trash in a carful region. Some PAHs, e.g. naphthalene (Naph), anthracene (Anth) and fluoranthene, (Flran) are registered as priority pollutants by USEPA and categorized as hazardous materials (WFD; Directive 2000/60/EC), attributing to their mutagenic (genotoxic) effects in vertebrates and invertebrates [9]. In addition, USEPA defined the key PAHs most frequently polluting the environment commonly known as the "16 USEPA PAHs". Eight of them are known carcinogens and/or

*Corresponding author e-mail khaled_yassen68@yahoo.com.; (**Khaled Y. Abdel-Halim**).

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mutagens and give rise to a serious health concern [10]. In 2002, the European Commission's Scientific Committee on Food (SCF) assessed 33 PAHs potentially occurring in food, confirmed the subset of eight PAHs from the USEPA list and identified other eight congeners as of major concern for consumers' health [11]. One aim for continuous health surveillance is to follow the development of morbidity and mortality patterns within various population groups. The function of an ecosystem is determined by the physical and chemical processes as well as by the relationships and interactions on the living organisms within the system. With the overall aim of the safe manufacture and use of chemicals, several objectives are covered by monitoring studies in natural environments. These can be described for instance as the monitoring of the levels of chemicals in various compartments within ecosystems. Furthermore, programs monitoring the fate and effects of chemical substances in the field are carried out in order to compare the results with existing information from similar studies carried out in the laboratory, such as the investigation of exposure-response relationships for risk assessment. In addition, monitoring of contamination levels in a selected environment is performed in order to evaluate compliance with specific environmental quality targets, for instance from regulatory point of view [1**2**]. In fact, PAHs in invertebrates such as bivalves have been extensively inspected as they are addressed bio-pointers of contamination due to their aptitude to filter the water [1**3**]. In Egypt, some studies demonstrated PAHs contamination in the Egyptian ecosystem. For example, distribution of 16 PAHs in 50 samples from the Nile River were investigated. ΣPAHs were ranged from 0.01 to 0.87 µg/l and fluoranthene was the dominant of PAHs [14]. In another investigation, analysis of 83 different oil samples from Egyptian markets showed that 16% were contaminated with benzo[a]pyrene (Bap) and 84% were not [15]. There is no national monitoring program for surface water in Egypt for the determination of POPs. So, there is lack of available data for the distribution of PAHs in the ecosystem of Egypt. This study was designed to evaluate the accumulation profiles of PAHs in bivalves of Temsah Lake and their risk on Ismailia residents as bivalves are being used as kind of seafood.

Material and methods

Chemicals

Multi-standards of PAHs: naphthalene [Naph], acenathylene [Acny], acenaphene [Acne], fluorene [Fluo], phenanthene [Phen], anthracene [Anth], fluoranthene [Flran], pyrene [Pyr], benzo[a]anthracene [Baa], chrysene [Chry], benzo[b]fluroranthene [Bbf], benzo[a]pyrene [Bap], benzo [g.h.i]perylene [Inp], and benzo[g.h.i]peryiene [Bgp] were supplied by Sigma Chem. Co. P.O. Box 14508 St. Louis MO 63178, USA. Solvents (acetonitrile, cyclohexane, ethanol, and ethyl acetate) were supplied by BDH AnalaR®, Laboratory Supplies, Poole, England. Meanwhile, $MgSO_4$, NaCl, sodium citrate and sodium hydrogen citrate were supplied by J.T. Baker Chemical Co., Phillipsburg, N.J. 08865. Primary Secondary Amine (PSA) and polytetrafluorethylene filter were supplied by Agilent Technologies, DE Germany.

Description of studied region

Five locations of Temsah Lake were chosen for the study. The Lake covers about 15 Km^2 between 32° 17' 30"; 32° 18' 30" E Latitude and 30° 32' 30"; 30° 40' 30" N Longitude, and is situated immediate the Suez Canal at a opinion distant 80 Km south of Port Said (Figure 1). Lake water ranges from 6 to 13 m after creation of the High Dam. Water quality changed from saline to freshwater associated with large amounts of gypsum and mud lamina rainfall. The visitors, tourism and fishing industries employ local residents and provide significant portions of the district revenues. In fact, increased number of brief and perpetual populations has also generated high volumes of wastes, including raw liquid, solid municipal sewage, agricultural runoff and industrial wastewater into the lake. It is also a basin for aliphatic, aromatic compounds and heavy metals (HMs) that have been created from shipping activities (accidental and incidental oil pollution), ballast water release and general vessel and facility maintenance [16].

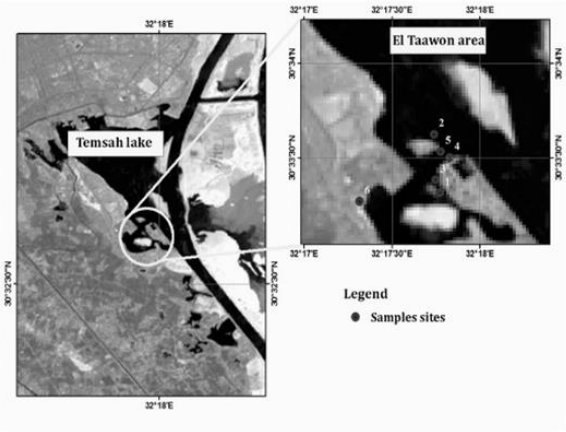

Figure 1: Google map of Temash Lake illustrates the sampling sites

Samples collection and preparation

Bivalve samples were captured from the studied sites during the period of 2017-2018. Each type of samples was packed in polyethylene bag, labeled and transferred to the laboratory in icebox for analysis. Sediment samples were reserved by using specific equipment at a depth of 5 cm of sediment. The samples were packed in a labeled polyethylene bag and

transferred to the laboratory in an icebox. They were air-dried in a dark place for 72 h, before analysis. Mollusca's species: Grooved carpet shell (*Ruditapes decussatus*), Surrclam (*Paphia undulata*), Golden venus (*Venerupis aurea*) and Comb circe (*Gafrarium pectinatum*) were caught and collected for the study.

Polycyclic aromatic hydrocarbons (PAHs) quantification.

Sediment samples.

Sieved dried sample (10 g) was mixed with 20 ml of acidic acetonitrile in centrifuge tube (50 ml), 4 g of $MgSO_4$, 1 g of each NaCl and sodium citrate, and 0.5 g of sodium hydrogen citrate. The mixture then was shaken for 30 sec, sonicated for 10 min (ultrasonic bath at 50/60 Hz and 100 W, Barcelona, Spain) and centrifuged at 4000 rpm for 8 min. Ten ml of supernatant were added to 1.5 g of $MgSO_4$ and 0.250 g of primary secondary amine (PSA) in 15 ml centrifuge tube. The process was done as described before. The solvent was evaporated to dryness, dissolved in 1ml of cyclohexane and filtered on polytetrafluorethylene filters; 0.25 µm thickness [17].

The average recovery percentage of PAHs compounds for fortified samples at different levels were determined and calculated for all tested compounds in different types of samples. The used solvents were analytical grade and checked before used. The detection limits (LODs) for the examined compounds were determined.

Soft tissues

Quick, useful, cheap, effective and raged (QUCHERs) method was used to determine PAHs residues in soft tissues. An aliquot (10 g) was homogenized with 10 ml of acidic acetonitrile (1% glacial acetic acid), and shaken for 1 min. One g of NaCl and 3 g of $MgSO_4$ were added, vortexed for 1 min, followed by centrifugation at 4000 rpm for 5 min. An aliquot (1 ml) of the supernatant was transferred into micro centrifuge tube (2 ml) containing 150 mg of PSA and 200 mg $MgSO_4$, and prepared as described above. The supernatant was checked on gas chromatography [18].

Gas–Liquid Chromatographic determination of PAHs

The purified extract was injected into gas liquid chromatography (GLC) equipped with a flam ionization detector (FID). The instrument was equipped with PAS-5 fused silica capillary column (30 m×0.32 mm i.d, 0.25 µm film thickness). Oven temperature was automated at an initial degree 100 (2 min hold) to 260 ˚C at a rate of 5 ˚C/min, and conserved at 260 ˚C for 15 min. Injector and detector temperature were maintained at 280 and 300 ˚C, respectively. Nitrogen was used as a carrier gas (4 ml/min) [19].

GC–MS Confirmation for PAHs

Gas chromatograph instrument (Agilent 6890) connected to mass spectrometric detector, with a direct capillary interface was conducted. Pure samples were injected in fused silica capillary column PAS-5ms (30 m×0.32 mm; 0.25 µm film thickness) at flow rate of Helium (1.0 ml/min) under pulsed splitless mode. The solvent delay was 3 min and the injection size was 1.0 µl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v, for scan profile from 50 to 500 m/z. The ion source temperature was set at 230 ˚C. The electron multiplier voltage (EM voltage) was maintained at 1250 v. The system was automatically modified using perfluorotributyl amine (PFTBA). GC temperature program was started at 60 ˚C (2 min), and elevated to 280 ˚C, at rate 5 ˚C/min. The injector temperature was set at 280 ˚C. The mass spectral database was used to identify the separated peaks.

The limits of detection (LOD_s) of measured PAHs were planned as twofold of standard deviation of series of quantities of a solution against the blank absorbance [20]. Quality assurance procedures and precautions were followed to ensure reliability of the data.

Bioconcentration factor (BCF)

It is defined as the ratio between the chemical concentration in animal ($C_{org.}$) to the corresponding concentration in the closed media ($C_{med.}$) [21] as follows:

$$BCF = \frac{Corg.}{Cmed.}$$

Risk estimation

The risk of contaminated bivalves was assessed established to the guidelines of EPA [22-24], concentrations of PAHs, data of surveyed inhabitants of Ismailia region and some data of Integrated Risk Information System [25].

Chronic daily intake (CDI) as mg/kg/day of bivalve consumption was valued according to equation:

$$CDI = \frac{C \times IR \times ED \times EF}{BW \times AT}$$

Where, C is the concentration of the substance expressed as mg/kg, IR is the consumption rate (estimated for surveyed participants), ED is the average period (estimated), EF is the exposure frequency (meal/year), BW is the body weight (estimated) and AT is the averaging time (365 day/year) [26].

Calculation of cancer risks through ingestion route was assessed independent on availability of

cancer slope factors which are provided on USEPA web site [24, 25]. Cancer risk value was calculated as follows:

$$Risk_{oral} = CDI_{oral} \times SF_{oral}$$

Total cancer risk was assumed of risk values of all chemicals.

On the other hand, non-carcinogenic risk value was assessed independent on reference doses (RfDs) data. Target hazard Quotient (THQ) of the substances through ingestion pathway was assessed as follows:

$$THQ = \frac{CDI}{RfD}$$

Where, RfD is tabulated reference dose of the specified substances in website [24, 25]. Total THQ (TTHQ) or hazard index (HI) is the sum of more than one hazard quotient for multiple substances.

Statistical analysis

Personally collected data were analyzed in MSTAT-C2.1 program. Exposure estimation data were tabulated according to differences in ages of the target population, particularly children. Exposure and risk estimates were performed according to 50 and 90th percentile of probability among different age categories. All data were processed by Microsoft Excel (Microsoft, 2000) and statistical analysis was conducted using the program of SAS Release 6.12 [27].

Results

Standards of 14 PAHs were examined on GC-FID. The response of GC was conducted at limits of detection (LODs) ranged from 0.6 to 5.3 ppb (Table1). So, the samples were extracted, cleaned up and determined using GC-FID as mentioned in the experimental section. The concentrations were corrected for 100 recoveries. Output pattern (Peaks was identified by comparison of sample retention time (Rt) with that of corresponding pure standard. The recovery percentages were calculated arising the ranges (32.0-105.0%) and (87.0-120.0%) for tissue and sediment samples, respectively.

Table 1:Recovery percentages and limits of detection (LODs) of PAHs in samples of bivalves and sediments.

Congener	Abbr.	Recovery %		LODs (ppb)
		Tissue	Sediment	
Naphthalene	Naph	39.0	100.0	3.0
Acenathylene	Acny	36.0	87.0	1.5
Acenaphene	Acne	55.0	107.0	2.0
Fluorene	Fluo	55.0	98.0	0.6
Phenanthene	Phen	69.0	105.0	2.5
Anthracene	Anth	65.0	89.0	1.9
Fluoranthene	Flran	92.0	90.0	1.7
Pyrene	Pyr	91.5	90.0	1.6
Benzo[a]anthracene	Baa	100.0	105.0	2.3
Chrysene	Chry	100.0	100.0	2.0
Benzo[b]fluroranthene	Bbf	100.0	110.0	5.0
Benzo[a]pyrene	Bap	97.0	102.0	5.3
Benzo[g.h.i]perylene	Inp	105.0	120.0	5.0
Benzo[g.h.i]peryiene	Bgp	100.0	100.0	5.0

GC- mass spectrometric analysis

Pure standards of PAHs and some measured samples were confirmed by using GC-MS analysis. The desirable compounds were scanned after electron ionization (EI) and selective ion monitoring (SIM) modes. Ion mass program was used to quantify congeners in details (Table 2), where they were established by retention time and abundance of quantification/confirmation ions in dependable PAHs standards. Based on main five isotopic isomers, 16 PAHs were monitored [19]. Naphthalene-d_8 with a retention time of 6.94 min was employed for Naph. Acenaphthylene- d_{10} with a retention time of 10.06 min was employed for Acny and Fluo within retention time in range of 10.06, and 26.08 min. Phenanthene-d_{10} with a retention time of 31.22 min was employed for congeners within retention time range of 31.22-and 38.24-min. Chrysene-d_{12} was employed for Chry and Baa, but Perylene-d_{12} was subjected for the remaining congeners

Table 2: Congeners of PAHs conducted in the study including their abbreviations (Abbr.), formula, molecular weights (M.W), retention times on GC-MS and ion mass.

Congener	Formula	Abbr.	GC-MS (Rt) min	M.W	m/z window
Naphthalene	$C_{10}H_8$	Naph	6.941	128	127, 128, 129, 136, 172
Acenathylene	$C_{12}H_8$	Acny	10.057	152	151, 152, 153, 154, 164, 166, 167
Acenaphene	$C_{12}H_{10}$	Acne	22.077	154	
Fluorene	$C_{13}H_{10}$	Fluo	26.084	166	
Phenanthene	$C_{14}H_{10}$	Phen	31.220	178	101, 176, 178, 179, 188, 200, 202, 203
Anthracene	$C_{14}H_{10}$	Anth	31.967	178	
Fluoranthene	$C_{16}H_{10}$	Flran	32.179	202	
Pyrene	$C_{16}H_{10}$	Pyr	38.104	202	
Benzo[a]anthracene	$C_{18}H_{10}$	Baa	38.240	228	
Chrysene	$C_{18}H_{10}$	Chry	42.875	228	226, 228, 229, 240, 244
Benzo[b]fluoranthene	$C_{20}H_{12}$	Bbf	42.985	252	
Benzo[a]pyrene	$C_{20}H_{12}$	Bap	43.121	252	
Benzo[g.h.i]perylene	$C_{22}H_{12}$	Inp	44.408	276	
Benzo[g.h.i]peryiene	$C_{22}H_{12}$	Bgp	44.705	276	

Residue levels

Accumulation pattern of PAHs in sediments and bivalve tissues of selected sites from Temsah Lake is showed in Table (3). The mean values of PAHs in sediments ranged from BDL to 36.22 mg/kg. Congener Anth exhibited the greatest mean value (36.22 mg/kg), followed by congener Flan (25.22 mg/kg) and Acne (8.83 mg/kg). Sum of PAHs was found to be 102.60 mg/kg.

In collected bivalves, Acny exhibited the greatest values as follows: 7.09, 9.26, 17.58, and 8.79 mg/kg in species; Comb circe, Surrclam, Grooved carpet shell, and Golden venus, respectively, with mean value (10.68 mg/kg). However, congener Fluo exhibited the least values (0.1 mg/kg). Sum of PAHs was in the following order: 13.110, 24.094, 24.338, and 16.512 mg/kg for the caught species, respectively, with a mean value of 19.514 mg/kg.

The mean values of PAHs in the caught bivalves were greater than the rough of permissible limits of European Communities [28]. Regarding congener Bap*, the mean value was 329 µg/kg with 54.8-folds of the permissible limit (6.0 µg/kg). However, $\sum PAH_4$ (Baa*, Chry*, Bbf*, and Bap*) in bivalve tissues was 2195 µg/kg with 62.7-folds of that of permissible limit (35.0 µg/kg). On the other hand, the bioaccumulation pattern of the examined PAHs was estimated as a ratio of their concentration in whole body of bivalve to the respective concentration in sediments. BCF obtained from ratio sediment/tissue for all detected congeners did not exceed 100. In bivalve Surrclam, congener Bbf exhibited the greatest ratio (23.00) followed by Acny (8.79) in Golden venus and Grooved carpet shell. Congener Acny exhibited high values in the caught species. However, congener Fluo was absent in these species, except in Comb circe (0.204). The values of BCF for ∑PAHs were in the following order: 8.825, 23.715, 12.367 and 13.367 in the collected species (Comb circe, Surrclam, Grooved carpet shell, and Golden venus, respectively) (Table 4).

Risk estimation.

Table 3:Residue levels of PAHs (mg kg^{-1}) in sediments and bivalves collected from Temsah Lake, Ismailia.

Congener	Sediments			Bivalve				Mean	C.V %
	Range	Mean	C.V %	Comb circe	Surrclam	Grooved carpet shell	Golden venus		
Naph	(BDL-21.83)	4.37	51.36	3.370	2.720	3.050	BDL	**2.285**	52.38
Acny	-	BDL	-	7.090	9.260	17.580	8.790	**10.680**	53.25
Acne	(BDL-18.18)	8.83	50.88	BDL	0.016	0.110	0.230	**0.100**	47.66
Fluo	(BDL-0.75)	0.49	38.63	0.100	BDL	BDL	BDL	**0.025**	28.99
Phen	(BDL-0.73)	0.33	49.05	0.007	0.110	0.810	0.007	**0.234**	47.95
Anth	(BDL-181.08)	36.22	43.43	1.760	3.030	2.041	7.105	**3.484**	53.27
Flran	(BDL-126.11)	25.22	37.18	BDL	0.097	BDL	0.002	**0.025**	35.60
Pyr	(BDL-1.01)	0.20	37.18	0.010	0.010	0.030	0.056	**0.027**	43.37
Baa	(BDL-0.22)	0.04	49.95	0.010	0.001	0.033	0.015	**0.015**	32.44
Chry	(BDL-2.22)	0.59	46.05	0.003	0.010	0.056	0.015	**0.021**	44.95
Bbf	(BDL-0.78)	0.29	39.32	0.570	6.670	0.065	0.052	**1.839**	45.30
Bap	-	BDL	-	0.160	0.610	0.531	0.015	**0.329**	34.87
Inp	(BDL-0.76)	0.15	45.06	0.030	BDL	0.032	0.225	**0.072**	49.39
Bgp	-	BDL	-	BDL	1.560	BDL	BDL	**0.390**	47.66
∑PAHs	(BDL-181.08)	102.60	50.00	13.110	24.094	24.338	16.512	**19.514**	37.80

Table 4:Bioaccumulation factor (BCF) of PAHs in bivalves collected from Temsah Lake and their permissible limits (µg/Kg).

Congener	BCF				[a]MRLs
	Comb circe	Surrclam	Grooved carpet shell	Golden venus	(µg/Kg)
Naph	0.771	0.622	0.000	0.000	
Acny	4.727	6.173	8.790	8.790	
Acne	0.227	0.002	0.013	0.026	
Fluo	0.204	0.000	0.000	0.000	
Phen	0.021	0.333	2.455	0.021	
Anth	0.049	0.084	0.056	0.196	
Flran	0.067	0.004	0.010	7.9×10^{-5}	
Pyr	0.050	0.050	0.150	0.280	
Baa*	0.250	0.025	0.002	0.375	
Chry*	0.005	0.017	0.075	0.025	
Bbf*	1.966	23.00	0.375	0.179	
Bap*	0.160	0.610	0.025	0.050	6.0
Inp	0.200	0.000	0.179	1.500	
Bgp	0.000	1.560	0.000	0.000	
∑PAHs	8.825	23.715	12.367	13.367	
*∑PAH4					35.0

-[a]Source= (EC, 2006) -*∑PAH4=benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene

Table 5:Personal information data of Ismailia residents as bivalve consumers.

Item	Male	Female	SD	P value	F-test
Survey (%)	40.0	60.0	14.4	0.0005	***
Age (yr)					
29-40	40.0	46.67	4.7	0.0021	**
40-70	60	53.33	14.14	0.00062	***
Weight (kg)					
60-90	82.5	83.33	47.14	0.0047	**
90-110	17.5	16.67	45.96	0.00080	***
Frequency (meal/yr)					
12		70.00	31.90	0.043	*
6		18.00			
4		12.00			
Consumption rate (g/meal)					
250		4.00	25.48	0.046	*
500		46.00			
700		50.00			
Children					
Age (yr)					
6-10		52.00	2.83	0.085	-
10-18		48.00			
Rate (g/meal)					
50-175 g at 50 and 90th percentile					
Family (No.)					
2-5		86.00	50.91	0.00068	***
5-10		14.00			
Bivalve species					
Golden venus		35.77	20.37	0.038	*
Grooved carpet shell		47.15			
Surrclam		15.45			
Comb circe		1.63			
Cooking type					
boiled		88.00	53.74	0.00073	***
grilled		12.00			

-Each value represents the mean of 100 participants. -F-test at * represents significant at 0.05, ** means significant at 0.01, and *** means significant at 0.0001 level of probability.

Each value is the mean of the measured sample during 4 seasons. -BDL= below detection limit. After the attained residue levels of PAHs and special information documents concerning consumers in Ismailia (Table 5), the risks were considered for adults and children classes as described in the section of material and methods. Risk calculation depended on chronic daily intake CDI, cancer risk and non-cancer risk values.

The mean CDI values of PAHs congeners; Naph, Acny, Acne, Fluo, Phen, Anth, Flran, Pyr, Baa, Chry, Bbf, Bap, Inp and Bgp for adults were 0.015, 0.125, 1.5E-03, 7.0E-05, 2.0E-03, 3.5E-02, 2.5E-04, 7.0E-05, 7.0E-05, 2.0E-04, 0.02, 0.0135, 6.5E-04 and 4.0E-03 mg/kg/day, respectively at 50^{th} and 90^{th} percentile of ingestion. Congener Acny exhibited the greatest values of CDI, 0.06 and 0.19 mg/kg/day at 50^{th} and 90^{th} percentile of ingestion, while congeners Pyr, Flran and Bbf exhibited the lowest value of CDI at the same rank of ingestion. Regarding children's categories, ∑CDI ranged from 0.007 to 0.023 mg/kg/day, for 50^{th} and 90^{th} percentile of ingestion pathway. The positively detected PAHs showed CDI values in the following order: Acny> Inp> Baa> Fluo> Bap> Anth (Table 6).

The results of non-cancer risk (HQ) of PAHs associated with bivalve's consumption in Ismailia are illustrated in Figure 2. The mean concentration values of PAHs congeners; Naph, Fluo, Flran, Acne, Anth and Pyr for adults were 0.75, 0.00157, 6.25E-03, 0.2485, 0.117 and 0.008, respectively, at 50^{th} and 90^{th} percentile of ingestion route. Congener Naph exhibited the highest values; 0.5 and 1.0 at 50^{th} and 90^{th}, while Fluo forced the lowest values; 0.001 and 0.0025 at the same rank of ingestion pathway. Regarding children categories, hazard index (HI) ranged from 0.044875 to 0.10495, respectively at 50^{th} and 90^{th} percentile of ingestion route. The positively detected PAHs congeners showed HQ values in the following order: Naph> Anth> Acne> Fluo> Flran> Pyr.

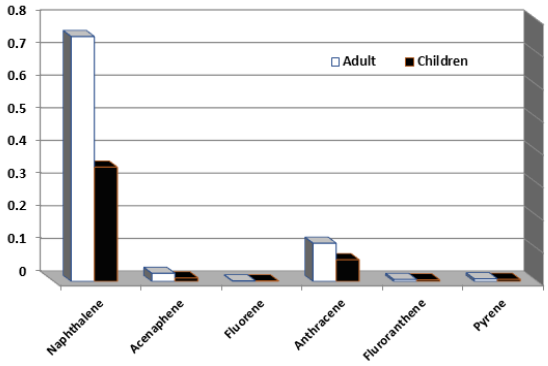

Figure 2: Estimated HQ of PAHs associated with bivalve's consumption for Ismailia residents.

Predicted cancer risk (CR) values of PAHs associated with bivalve′s consumption are presented in Figure (3). Baa imposed CR values of 4.5E-05 & 1.2E-04 for adult and 3.6E-05 & 1.2E-04 for children at 50 and 90^{th} percentile, respectively. Congener Bbf imposed the values of 0.12 & 0.36 for adult and 6.0E-03 & 2.4E-02 for children at the same rank of ingestion pathway. However, congener Bap exhibited the highest mean values of 0.0988 and 0.065 for adult and children, respectively. These congeners are employed for weight of evidence cited by EPA, where Baa, Bbf, and Bap are classified as B2 (probable carcinogenic), while other congeners are classified as D (not classified as carcinogenic).

Table 6:CDI values (mg/kg/day) of PAHs associated with bivalve's consumption.

Congener	Age categories					
	Adult			Children		
	50^{th}	90^{th}	Mean	50^{th}	90^{th}	Mean
Naph	0.10	0.02	0.015	0.004	0.01	0.0025
Acny	0.06	0.19	0.125	0.040	0.12	4.5E-04
Acne	0.001	0.002	0.0015	3.0E-04	1.0E-03	0.090
Fluo	4.0E-05	1.0E-04	7.0E-05	3.0E-04	1.0E-04	0.0125
Phen	0.001	0.003	2.0E-03	0.001	0.002	1.5E-04
Anth	0.02	0.05	3.5E-02	0.01	0.03	6.5E-05
Flran	1.0E-04	4.0E-04	2.5E-04	1.0E-04	3.0E-04	6.5E-05
Pyr	4.0E-05	1.0E-04	7.0E-05	3.0E-05	1.0E-04	0.0002
Baa	4.0E-05	1.0E-04	7.0E-05	3.0E-05	1.0E-04	0.020
Chry	1.0E-04	3.0E-04	2.0E-04	1.0E-04	2.0E-04	0.0015
Bbf	0.01	0.030	0.020	0.005	0.020	6.5E-05
Bap	0.007	0.020	0.0135	0.040	0.14	6.5E-04
Inp	3.0E-04	1.0E-03	6.5E-04	2.0E-04	7.0E-04	0.080
Bgp	2.0E-03	6.0E-03	4.0E-03	0.001	0.004	0.007
∑CDI	0.012	0.035	0.0235	0.007	0.023	0.015

-EPA getting risk threshold= person per 1.0×10^{6}

Discussion

The present study focused on the accumulation of PAHs on four species of bivalves in Temsah Lake. Additionally, the predicted risks associated with this kind of consumption for local residents was assessed. The major contributors to pollution in marine coastal environments as result of the shipping and oil exploitation activities are PAHs and other POPs. Due to their resilience in nature, PAHs are considered to have long-term effects in the environment and are longer retained in marine environments due to their stable structures [29, 30]. In addition, produced waters for oil and gasoline activities [31] and fossil fuels lead to increase concentrations of some PAHs congeners into environment, such as naphthalene, phenanthrene and fluoranthene [32]. As documented in Arctic environment, AMSA (2009) [33] stated that oil shipping lead to oil spill and wonted discharge into marine, so, the release of oil redialed pollutants could lead to acute and long-term consequences for marine environment. The distribution of such compounds may be addressed in ecosystem components which are highly lipid contents e.g. mollusks, fish and sediment.

Bivalves are considered as hyperaccumulators of contaminants, where they are addressed bio-pointers of contamination due to their aptitude to filter the water. The presented data show that PAHs concentrations in mollusks tissues were greater than in sediments. These concepts are in accordance with that previously obtained by May et al. (1978) [34] where PAHs are highly photosensitive and thermolabile in the present of light and oxygen resulting to their quick degradation. Once they reach an aquatic medium, they become less susceptible to solar radiation. In the water, they can be incorporated into sediments, where they may remain undisturbed for long periods of time. The PAH cycle in the aquatic environment is relatively simple, where PAHs of high molecular weight (HMW) are quickly adsorbed on the surface of organic and inorganic particles and settle down. They may be remobilized into the water column by biological activity bioturbation and automatic processes. Congeners with low molecular weight (LMW) have a tendency to persist in solution, where they are freely offered to marine organisms through ingestion or respiration. Solubility of congener increases as temperature increases. This concept was recognized in the present study, where PAHs proportions in summer were greater than in the winter. As mentioned in the literature, the solubility of congener Anth ($C_{14}H_{10}$; M. W=178.24) increased from 12.7 to 55.7 mg/L from 5.2℃ to 28℃ [30]. In fact, bioavailability of LMW PAHs increases in winter seasons, because dissolved congener is more freely uptake by organism than those adsorbed on sediments. Moreover, LMW PAHs are more toxic for aquatic organisms [35, 36].

Few studies were carried out and focused on PAHs monitoring in ecosystem. For example, Marsili et al. (2001) [37] monitored the concentrations of PAHs in Mediterrean cetaceans *Balaenoptera physalus* and *Stenella coeruleoalba*, where patterns of 14 PAHs were attained for both species. On the other hand, distribution of PAHs in some tissues of edible fish from the largest freshwater lake in China was conducted by Zhao et al. (2014) [38], where the data indicated that the hepatobiliary system stored higher proportions of PAHs than extrahepatic tissues.

Despite the benefits of a seafood-based diet, regular consumption of seafood has proven to be a health risk due to the exposure of these food sources to pollutants. This is exacerbated by the fact that mollusks possess the highest metabolic capacity to metabolize and accumulate pollutants in their tissues. This concept was detailed, where the blood cockle's capability to performance as a sentinel species and a bio-indicator is well described. For these explanations, blood cockles are viewed as exceptional bio-indicators of global pollutant dissimilarities, as the pollutant attentions in their soft tissues is meditative to the pollutant differences of their marine and coastal waters over a quite short time scale [39, 40]. USEPA has categorized for PAHs into Group B2 (carcinogenic PAHs) to assess their cancer potency and toxicity [41, 42]. The diet remains to be the main source of PAHs for humans, contributing to more than 70% of the total exposure. In September 2012, European Commission (EC) and the European Food Safety Authority (EFSA) approved the regulation 835/2011 and established the permitted levels of Bap and the sum of Chry, Bap, Baa and Bbf. These regulations forbid the use of these compounds as the only PAH marker in seafood [28, 42].

Although several studies have been conducted on PAHs risk assessment in different regions of world [43], no background and updated databases are available on PAHs and their related risk in most countries. In view of that, the present study was aimed to study about the concentrations of PAHs and also to understand the magnitude of accumulation and possible risk in some of the selected edible mollusks in special community (Ismailia governorate). Moreover, the results of the present study are in accordance with those obtained by Nozar et al. (2013) [44] who stated that PAHs concentrations in marketed fish and seafood in Iran were greater than the permissible levels. Also, Obiakor et al. (2014) [45] stated that dietary intakes of PAHs of contaminated fish in Anambra River (Canada) were higher than restricted values of situation edible tissues of fish and/or shellfish for human consumptions. In another study, different tissues of edible fishes from Poyang Lake (China) were found to be intense with PAH

residues. Regarding the human health risk by means of Bap potency equivalent concentration (PEC) as well as the incremental lifetime cancer risk (ILCR), it was reported that PAHs in fish would induce potential carcinogenic effects [38].
Public health risk from consumption of aquatic food such as fish cannot be extremely excepted to be low. The continuous daily intake of seafood and fish is potential health alarm as long-term to low acting proportions is likely to be active or more potent. In addition, mollusks and other invertebrates freely accumulated high level of the contaminant since, they are in capable of metabolism and excretion of PAHs [46, 47]. With increasing human exposure to environmental organic contaminant that are potentially carcinogenic such as PAHs, further studies are needed on different food groups other than fish to delineate the extent of human toxicant exposure.

In fact, approximately PAHs and their metabolic products are of a great worry associated with their documented carcinogenicity, but information of their health risk assessment is rather limited [48]. Congeners, Baa, Bbf and Bap are the only PAHs sufficient as carcinogenic potency factors, respect to other congeners. Additionally, biotransformation of PAHs in fish and aquatic organisms commonly attended by crosswise properties resulting from the formation of carcinogenic intermediates [49, 50]. PAHs metabolites are formed by photochemical/chemical reactions which known to be more toxic than their parent compounds according to tumorgenicity studies on mice [51]. So, the potential carcinogenic risk of PAHs ingestion pathway of bivalves' intakes in this study may be underestimated for some congeners without considering the proportions of their metabolites. Also, numerous PAHs and their metabolites are aryl hydrocarbons receptor (AhR) ligands and may activate estrogen receptors (ER), where Bap, Baa and Bbf could induce significant estrogenic effects *in vivo*, and thus might affect their toxic as carcinogenic substances [52].

Uncertainty refers to a lack of knowledge about specific factors, whereas variability refers to factor heterogeneity attributable to natural random processes. The present data are exhibiting wide range of values and variability of residues may be results of combining samples collected from known areas of contamination and samples collected randomly. Combining samples provide a more accurate representation of the site wide contamination than either sampling scheme by itself. A significant source of uncertainty is the variation of consumption rates and frequency due to variability in age categories and residential habits. Unfortunately, full determination and toxicity values are not available for all compounds. Therefore, health risks/hazardous cannot be quantitatively assessed for all contaminants and the total risks of the region may be underestimated in such circumstances. Sum of risks for individual compounds may also lead to some uncertainty. Antagonistic or synergistic effects are not accurate for this characterization, resulting in potential over-or-underestimation of risks. In addition, the assumption of HI suggests that all substances induce the same effect by the same mode of action. So, the use of HI equation to a number of substances that are not likely to induce the same action will overrate the potential for adverse effects.

Conclusion

The recently presented findings showed that aquatic contamination with PAHs induced bio-accumulation from sediment of Temsah Lake to the tissues of bivalves. Such accumulation resulting in impacted risk for local consumers (Ismailia residents). In view, precautionary measures e.g. ecosystem re-management, wastewater treatments, and monitoring programs need to be taken in order to prevent further PAHs pollution. It also improves the baseline data and health risk impaction of these contaminants in bivalves commonly consumed as seafood in Ismailia region. Such data provide valuable information on safety of bivalves commonly consumed.

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