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Risk Index Profile of Polycyclic Aromatic Hydrocarbons in Sediments of Manzala Lake for Protection of Benthic Life

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ABSTRACT

During the last few years, Lake Manzala, the largest Egyptian coastal lake, has suffered from the excessive discharge of industrial, agricultural, and municipal wastewater. This study aimed to identify the level of PAHs contamination in the sediments of Manzala Lake, investigate possible pollution sources, and address the associated ecological risk of PAHs on benthic communities. Sixteen priority PAHs recommended by the USEPA, including Naph, Ace, Acthy, F1, Phen, Ant, Flu, Pyr, BaA, Chr, BbF, BkF, BaP, DBA, BghiP, and IP, were monitored in this study. Surface sediment samples were collected from 11 sites in the lake. The results of $\sum$PAHs fluctuated between 15.92 and 12707.64 µg/Kg, while four-cyclic PAHs recorded 58.31% of $\sum$PAHs. The PAH source distribution was determined using different isomeric ratios (Ant/(Ant+Phen), Flu/Pyr, InP/(InP+BghiP), BaA/ (BaA+Chr) that suggested pyrolysis as the major input source of PAHs. We used different sediment quality guidelines (SQGs), effect range low (ERL) and effect range median (ERM), threshold effect concentration (TEC), and probable effect concentration (PEC) approaches to infer hazard risk caused by PAHs on benthic communities. Generally, $\sum$PAHs concentrations were lower than the ERM and PEC values at all sites under study. However, some compounds such as F1, Flu, Pyr and DBA exceeded ERL and TEC levels, which detected a moderate possibility of an ecological effect in the middle sector of the lake. Moreover, based on the toxicity indices (mPEC-mERM-q), the middle sector was the most contaminated and thus it is the most probable site to cause a risk for benthic organisms.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic pollutants widely distributed in the environment. PAHs may enter the aquatic system mainly by human activities; they are derived mainly from incomplete combustion and oil spills. Pyrogenic hydrocarbons predominate in many industrialized areas, entering the environment during the burning of wood, coal, garbage and petrogenic products **(Saleh *et al*., 2021)**. Natural sources of PAHs include volcanic eruptions, forest, grass and bushfires in addition to natural spills of petroleum and erosion of bituminous rocks. PAHs containing two to three benzene rings are known as low molecular weight PAHs (LMWPAHs), and those from 4 to 6 benzene rings are known as high molecular weight PAHs (HMW PAHs) **(Ghandourah, 2022)**. PAHs introduced into the aquatic environment move from the water to the sediment due to their hydrophobic properties; they are quickly bound to organic particles suspended in water columns and deposited on bottom sediments **(Stern *et al*., 2023)** in particular. The high-molecular-weight PAHs, consisting of several aromatic rings have a greater tendency to bind to the sediment. Organic matter represents the main factor influencing the PAHs sorption. Sediments represent the most important sink for PAHs in aquatic environments, but they can be released into water because of changes in environmental factors or bioturbation, posing toxicity risks to benthic organisms **(McGrath *et al.* 2019)**. Sediment quality guidelines (SQGs) were set up by the National Oceanic and Atmospheric Administration (NOAA). They are widely used for comparison with the detected concentrations in sediments to predict if there are adverse biological impacts on aquatic ecosystems. SQGs included effects range low (ERL), effects range median (ERM), threshold effect level (TEL), probable effect level (PEL), threshold effect concentration (TEC) and probable effect concentration (PEC).

Manzala Lake is the largest natural lake of the Egyptian northern lakes along the Mediterranean coast. The lake is exposed to high levels of pollutants from different sources: industrial, domestic and agricultural, including rapid municipal growth at Port Said, Damietta and Dakahlia Governorates in addition to the reduction in lake surface by illegal land reclamation of wetlands for agriculture **(Elshemy *et al*., 2016)**. Five major drains, which carry agricultural drainage water as well as wastewater from urban areas cross the Delta and discharge into the southern, eastern and western regions of the lake. Bahr El-Baqar is the largest drain that provides the largest flow rates and pollution loads, contributing much to the deteriorating water quality of the lake **(Elshemy, 2016)**. These drain inflows are also responsible for the elevated concentrations of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) **(Barakat *et al*., 2012; El Kady *et al*., 2017)**, PAHs **(Barakat *et al*., 2013; El-Kady *et al*., 2018)** and heavy metals **(Zahran *et al*., 2015; Redwan & Elhaddad, 2022)**, which have been identified in water, sediment and biota. Sixteen priority PAHs are recommended by the USEPA as dangerous organic pollutants. These compounds are naphthalene (Naph), acenaphthylene (Acthy), acenaphthene (Ace), fluorene (F1), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DBA), benzo[ghi]perylene (BghiP), and indeno[1,2,3-cd]pyrene (InP). This study aimed to evaluate the distribution pattern of PAHs in different sectors of Manzala Lake, identify the main sources whether pyrogenic or petrogenic, and finally estimate potential environmental risks associated with PAHs on benthic communities using some indexed SQGs.

MATERIALS AND METHODS

1. Study area

Manzala Lake is a shallow, brackish-water lake located on the northeastern edge of the Nile Delta. Geographically, it is located between longitudes 31° 45′ and 32° 22′ E and latitudes 31° 00′ and 31° 35′ N. The lake is bordered by the Mediterranean Sea to the North, Port Said to the north-east, Dakahlia to the southwest and Sharkia Provinces to the South, and Damietta Branch of the Nile to the West (Fig. 1). According to **Mahmoud *et al.* (2022)**, the surface area of Manzala Lake was about 1,709 km^{2}in 1990, 1,200 km^2 in the 1970s, 904,785 km2 in 1981, 600 km^2 in 2010, and 565.91 km^2 in 2016. After the efforts of the Egyptian government to develop the

Egyptian lakes, which started in 2016, the total area of the lake increased to 572.41 km^2 in 2020, with an open water area of about 75% of the whole lake area.

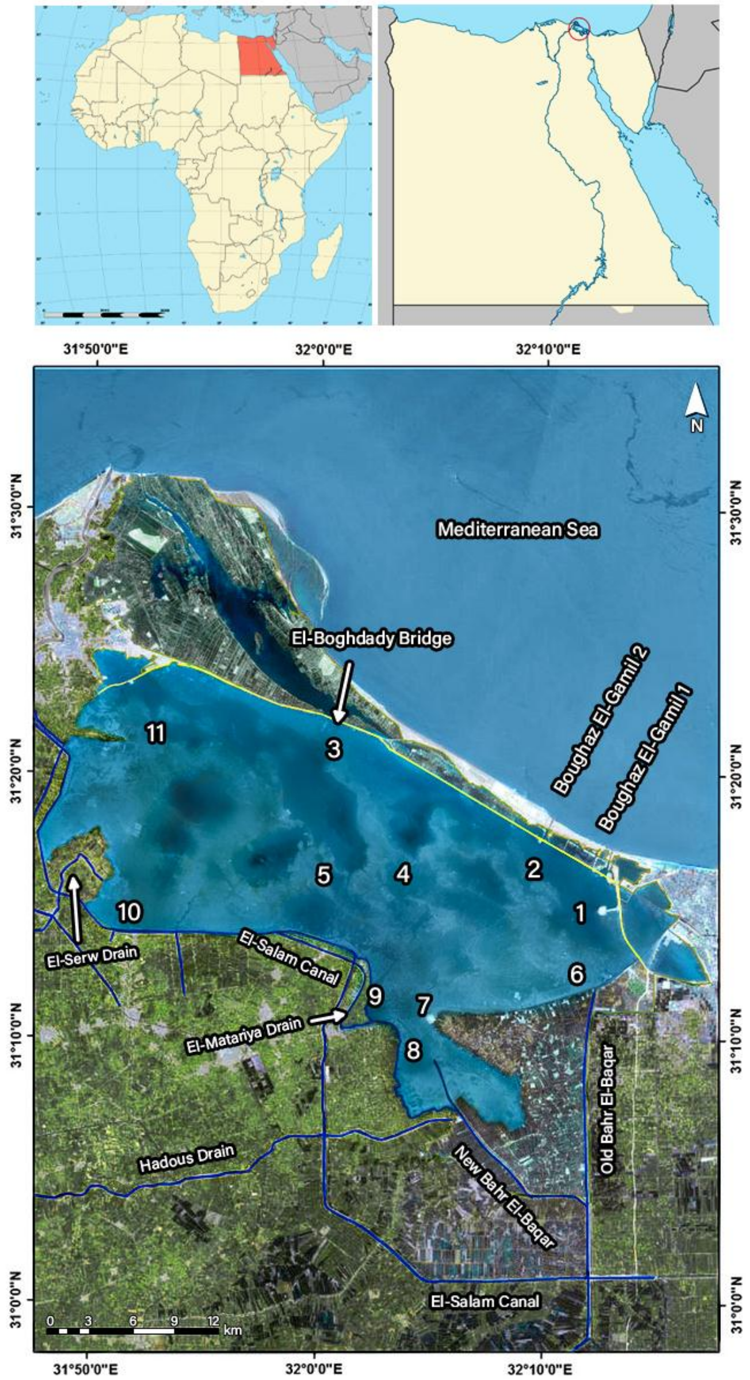

Fig. 1. Map of Manzala Lake showing sampling sites

2. Sampling

Sediment samples were collected from 11 sites in Manzala Lake during autumn 2021 (Fig. 1 & Table 1). The 11 sites represent the main four sectors of the lake; the northern sector (sites 1-3), the middle sector (sites 4 and 5), the east-south sector (sites 6-9), and the western sector (sites 10 and 11). Surface sediment samples (0–0.2 m) were manually collected using a Van–Veen grab. The samples were kept at $4^{\circ}C$ until analysis. Before analysis, samples were left to dry in room temperature and analyzed for polycyclic aromatic hydrocarbons according to **USEPA (1993)**.

Table 1. The details of the sampling locations

Site	Feature	Latitude	Longitude
1	**Boughaz El-Gmail 1**	**31°14'49.66" N**	**32°11'58.78" E**
2	**Boughaz Ashtoum El-Gmail (El-Gamil 2)**	**31°17'17.97" N**	**32° 9'53.49" E**
3	**Boughaz El-Boghdady**	**31°20'47.80" N**	**31°59'50.94" E**
4	**Bahr Kurmullus; in the middle of the lake**	**31°16'4.88" N**	**32° 3'42.61" E**
5	**Bahr El-Hamrah; in the middle of the lake**	**31°16'31.33" N**	**32° 0'49.55" E**
6	**Bahr El-Bashtir 1; in front of the old Bahr El-Bqar darin**	**31°12'2.64" N**	**32°12'11.01" E**
7	**Bahr El-Bashtir 2; south of the lake**	**31°11'5.18" N**	**32° 4'54.62" E**
8	**Bahr El-junaka (in front of the discharge point of several drains including new Bahr El-Bqar darin)**	**31°10'24.25" N**	**32° 4'28.71" E**
9	**Bahr Dishdi (El-Mataryia)**	**31°11'25.20" N**	**32° 2'21.13" E**
10	**Bahr El-Diju (El-Serw)**	**31°15'14.21" N**	**31° 51'29.45" E**
11	**Bahr El-Zarka**	**31°22'11.01" N**	**31° 53'17.53" E**

3. Preparation and extraction of samples

Each sample was blended with anhydrous sodium sulfate at a ratio of 2:1 in a mortar at high speed. The mixture was Soxhlet extracted by dichloromethane for about 8h, filtered and extracted twice more. Solvent fractions were combined and filtered through filter paper with 1g of anhydrous sodium sulfate. Extracts were concentrated using an evaporator. The extracted volumes were subjected to column chromatography packed with 10g of silica, 10g of alumina, and finally 1g of anhydrous Na_2SO_4, while the column was pre-eluted with 20ml dichloromethane. Extracts were then concentrated and collected in 2ml vials. Finally, fractions decreased to about 0.2ml under a stream of N_2. Activated copper was employed for desulfurization of the extract and all extracts were degassed for 10 min in an ultrasonic bath before injection **(Salem *et al.*, 2014)**. PAHs were analyzed by injecting 5µl of the extract into a Hewlett Packard (HP) 5890 Series II gas chromatograph, equipped with a flame ionization detector (GC-FID) according to instrument settings in Table (2).

4. Quality control and assurance

The measurement of PAHs comprised the use of certified reference material for sediment samples, analytical duplicates and procedural blanks to control analytical reliability, ensure both recovery efficiency and result accuracy. The calibration curves and correlation coefficients for the measurement of PAHs using external standards were both higher than 0.99. The SRM 2974 PAHs mixed standard (purity > 99%) was provided by the National Institute of Standards and Technology of the United States. The 16 PAHs recovered on average on a scale of 81.3 to 111.7%, with a (RSD) range of 9.2 to 13.8 16%. The instrumental limit of detection (LOD) for a single PAH on spiked seawater samples is three times the standard deviation of eight independent experiments. The detection limits were 0.01 µg/L for naph, ace, f1, ant, flu, and phen, 0.02 µg/L for acthy, BaA, and BbF, and BaP, and 0.03 µg/L for BbF, pyr, InP, Chr, BghiP, and DBA. For every five samples, the blanks were inspected, and the LODs were determined using the results of the blank tests.

Table 2. GC Instrument settings for analysis of PAHs by HP 5890 Series II GC

GC parameter	Setting
Gas Chromatograph	HP 5890 Series II
Detector	FID
Column	100% dimethyl polysiloxane, 30 m length × 0.32 mm i.d. ×0.17 µm film thickness
Inlet mode	Splitless (5µL)
Inlet temperature	290 °C
Detector temperature	300 °C
Carrier gas	Nitrogen flowing at 1.2 ml/min
Oven program	60°C, 10°C/min to 300 °C
Total cycle time	29 min

5. Sediment quality guidelines and toxicity indices

The risk assessment of PAHs in sediments was carried out using sediment quality guidelines (SQGs). For each pollutant, there are two values of SQGs: the threshold effect levels (TELs), below which no detrimental consequences are anticipated, and the probable effect levels (PELs), at which adverse effects are anticipated. The first class establishes the effects range low (ERL) and the threshold effect concentration (TEC), whereas the second class establishes the effects range median (ERM) and the probable effect concentration (PEC) **(Macdonald *et al.*, 2000)**. In the same context, to assess the toxic effects of contaminated sediments on benthic-dwelling creatures, a variety of SQGs indices (toxic indices) have been proposed. Where, ERM quotient (ERM-q), PEC quotient(PECq), mean ERM quotient (mERM-q) and mean PEC quotient (mPECq) are useful tools to evaluate individual and combined toxicity of PAHs on benthic dwelling organisms, respectively. ERMq and PECq are calculated by diving each PAH concentration by its ERM and PEC values, while mERMq and mPECq are the mean values of ERMq and mPECq of different pollutants, respectively, as the following equations:

$$\mathbf{ERmq} = \frac{\mathrm{Ci}}{\mathrm{ERL}} \quad \textbf{and } \mathbf{mERMq} = \left[\frac{\sum \mathrm{Ci}}{\mathrm{ERM}}\right] / \mathbf{n}$$

$$\mathbf{PECq} = \frac{\mathrm{Ci}}{\mathrm{ERL}} \quad \textbf{and} \quad \mathbf{mPECq} = \left[\frac{\sum \mathrm{Ci}}{\mathrm{PEC}}\right] / \mathbf{n}$$

Where, C_i: the concentration of each PAH, and n: the number of hydrocarbons. The reference guideline values of mPEC and mERM are presented in Table (3).

6. Statistical analysis

Statistical analysis was performed by using Minitab 16® Statistical Software (Minitab Inc.). Concentrations below the MDLs were treated as zero. The level of statistical significance was set at $P<0.05$. Anderson Darling tests were performed for normal distribution. The differences between medians were determined using one sample sign rank t-test. Data were log-transformed when normality assumptions were not met. Potential relationships among target compounds and samples were evaluated by using Spearman’s rank correlation analysis and hierarchical cluster analysis (HCA) on PC-ORD 5 statistical program.

Table 3. Toxicity indices of sediment

Toxicity index	Value	non-adverse effect with a < 14% risk
PECq and mPECq	< 0.1	non-adverse effect with a < 14% risk
	>0.1 and < 1	slightly adverse effect with a 15-29% risk
	>1 and < 5	moderate effect with a 33-58% risk
	>5	High effect with a 75-81% risk,
ERMq and mERMq	≤0.1	Low priority site with a 9% probability of being toxic
	>0.1 and <0.5	medium–low priority site 21% probability of being toxic
	>0.5 and <1.5	high-medium priority site with 49% probability of being toxic
	>1.5	high priority site76% probability of being toxic

RESULTS AND DISCUSSION

1. PAHs distribution and composition

The total polycyclic aromatic hydrocarbons (ΣPAHs) concentration ranged from 15.55 to 12707.64μg/ kg dw, with a mean value of 1247.77μg/ kg. Sampling sites located in the middle sector exhibited the highest concentrations of PAHs (6572.33 μg/kg), especially site 5 that was 72 times more than the east southern sector of Manzala Lake (91.18 μg/kg), followed by the western region (45.48 μg/kg) and finally the north region of Manzala Lake (41.72 μg/kg), which represented the lowest concentrations of PAHs. The huge increase in the middle sector may be attributed to the fact that this region is the most open area, with no barriers and fewer islands. Therefore, this area is characterized by high fishing activity, which requires the use of motorized boats. This increases the emission and the spill of hydrocarbons into the lake, which settle on the bottom sediments.

The mean concentration of lower molecular weight PAHs (LPAHs) was 158.14μg/ kg, accounting for 9.83% of their total PAH content, while the mean concentration of higher molecular weight PAHs (HPAHs) was 1449.83μg/ kg, comprising about 90.17% of the ∑PAHs. The low levels of PAHs in the present study may be due to that they consist of few aromatic rings that degrade faster, and thus their concentrations in surface water and sediments are relatively low **(Han *et al.*, 2017)**. The PAHs compounds were in a decreasing order of BbF> Pyr> flu> Bkf> BaA> Chr> F1> DBA> Ant> BaP> phen>InP> Bghip> Ace> Acthy> Naph in sediments of Manzala Lake (Table 4). Pyr and BbF are components of fossil fuels, and a portion of them is associated with their combustion. BaP is usually emitted from catalyst and no catalyst automobiles. BaA and Chr are often resulted from the combustion of both diesel and natural gas **(Shreadah *et al.*, 2013)**. **Parra *et al.* (2020)** reported pyr, flu and phen as the most abundant compounds in sediments of Awotan-Asunle dumpsite area in the southwestern region of Nigeria. The major combustion compounds (COMB) are BkF, Pyr, Flu, BbF, BaA, InP, BaP, BghiP and Chr **(Al-Agroudy *et al.*, 2017)**. ∑COMB varied from 13.99 at site 7 and 12478.09 μg/kg at site 5. **USEPA (1993)** recorded 8 individual PAHs as carcinogenic PAHs (CAR PAHs) that are BaA, Chr, BkF, BaP, BbF, InP, DBA and BghiP. ∑CARPAHs fluctuated between 6.44 at site 10 and 10487.91μg/ kg at site 5. The relative contamination level of PAHs in sediment samples may be divided according to **Baumard *et al.* (1998)** into low level (0–100 μg/kg), moderate level (100–1000 μg/kg), high level (1000–5000 μg/kg) and very high level (>5000 μg/kg) of ∑PAH concentrations. According to this scale, 64 % of the lake's sites was low contaminated; 27 % was moderately contaminated, and 9% of sites was highly contaminated.

Table 4. PAHs concentrations (µg/Kg dw) in sediments of Manzala Lake during autumn 2021

Sediment	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S 10	S 11
Naph	0.18	0.12	0.14	3.26	5.92	0.4	Nd	3.06	0.21	0.19	0.11
Acthy	0.13	Nd	0.12	5.99	23.42	0.24	0.12	1.96	0.11	0.18	Nd
Ace	0.2	0.12	0.12	5.77	28.88	0.15	0.12	1.59	0.14	0.27	Nd
Fl	0.15	0.12	0.12	119.32	33.84	0.16	Nd	1.65	0.18	0.19	Nd
Phen	0.21	0.18	0.19	0.33	58.92	0.12	0.12	3.86	0.15	0.17	0.12
Ant	0.18	Nd	0.27	11.58	75.8	0.12	0.12	3.11	0.12	0.19	0.16
Flu	5.7	1.07	3.3	56.55	885.31	6.31	1.86	31.89	4.41	5.37	2.47
Pyr	56.37	5.59	9.84	185.29	1107.65	6.8	2.1	38.63	10.28	6.63	21.31
Chr	3.01	1.07	4.34	8.43	127.66	1.65	0.58	15.55	2.83	1.48	17.1
BaA	4.11	1.4	3.07	22.17	220.26	1.88	0.94	34.52	2.8	2.02	14.63
BbF	2.26	4.68	1.11	8.73	9534.49	0.65	5.52	17.02	0.91	1.46	2.14
BkF	1.48	0.68	0.34	1.76	512.82	0.25	0.94	2.48	0.12	0.33	0.51
BaP	0.11	0.12	0.1	0.3	86.15	0.12	0.12	0.65	0.12	0.12	0.13
BghiP	0.75	0.22	1.62	2.84	0.86	25.08	0.43	2.35	0.97	0.35	2.09
DBA	1.18	0.51	3.2	2.22	2.78	72.12	1.23	4.29	1.42	0.59	6.83
InP	1.22	0.32	4.23	2.49	2.9	39.03	1.57	5.24	1.92	0.68	3.27
∑PAHs	77.25	16.1	32.1	437.02	12707.65	155.07	15.78	167.85	26.7	20.21	70.87
∑LPAH	1.05	0.47	0.96	146.25	226.78	1.19	0.48	15.22	0.92	1.19	0.39
∑HPAHs	76.2	15.63	31.14	290.77	12480.87	153.88	15.29	152.63	25.78	19.02	70.49
∑CAR	14.13	8.98	18.01	48.93	10487.91	140.78	11.33	82.1	11.09	7.02	46.71
∑COMB	75.02	15.13	27.95	288.55	12478.09	81.76	14.06	148.34	24.36	18.42	63.65
%CAR	18.29	55.76	56.1	11.2	82.53	90.79	71.84	48.91	41.53	34.72	65.91
%COMB	97.11	93.96	87.06	66.03	98.19	52.73	89.12	88.37	91.25	91.18	89.81

Nd: not detected, concentrations below detection limit <0.1.

The 16 PAHs compounds could be divided based on the number of aromatic rings into two-ring PAHs (Naph); three-ring PAHs (Acthy, Ace, F1, Phen, Ant); four-ring PAHs (Flu, Pyr, BaA, Chr); five-ring PAHs (BbF, BkF, BaP, DbA) and six-ring PAHs (InP, BghiP) **(Edokpayi *et al.*, 2016)**. The number of aromatic rings in PAHs may determine their toxicity. The present results indicated that the %∑2C-rings PAHs, ∑3C- ring-PAHs, ∑4C-ring PAHs, ∑5C-ring PAHs and ∑6-ring PAHs presented (0-0.93), (0.32-33.12), (10.74-89.58), (3.1-79.75), (0.05-71.77) % of the total PAHs, respectively. The increase in the number of aromatic rings of PAHs increases both the hydrophobicity and chemical stability of the compounds and hence retard their degradation **(Pouch *et al.*, 2021)**. This suggests the cause of the high dominance of HPAHs over LPAHs at all the investigated sites. 4-rings carbon represented the highest abundant percentage in most sites due to high levels of Pyr, B[a]A, and Chr concentrations; however, sites 5 and 7 showed higher abundance of C5 due to the presence of high concentrations of BbF, BkF, and BaP at site 5 and the high levels of DBA at site 7. Whereas, site 6 showed a high abundance of C6 which may be attributed to the high concentrations of InP (fig). Site 6 is exposed to wastewater effluents discharged from Bahr El-Bashtir. In addition, **Zhao *et al*. (2012)** and **Okedeyi *et al.* (2013)** related the dominance of HPAHs in their studies around

Mai Po inner deep bay of Hong Kong and coal-fired power plants in South Africa, respectively, to vehicle emissions and fuel combustion.

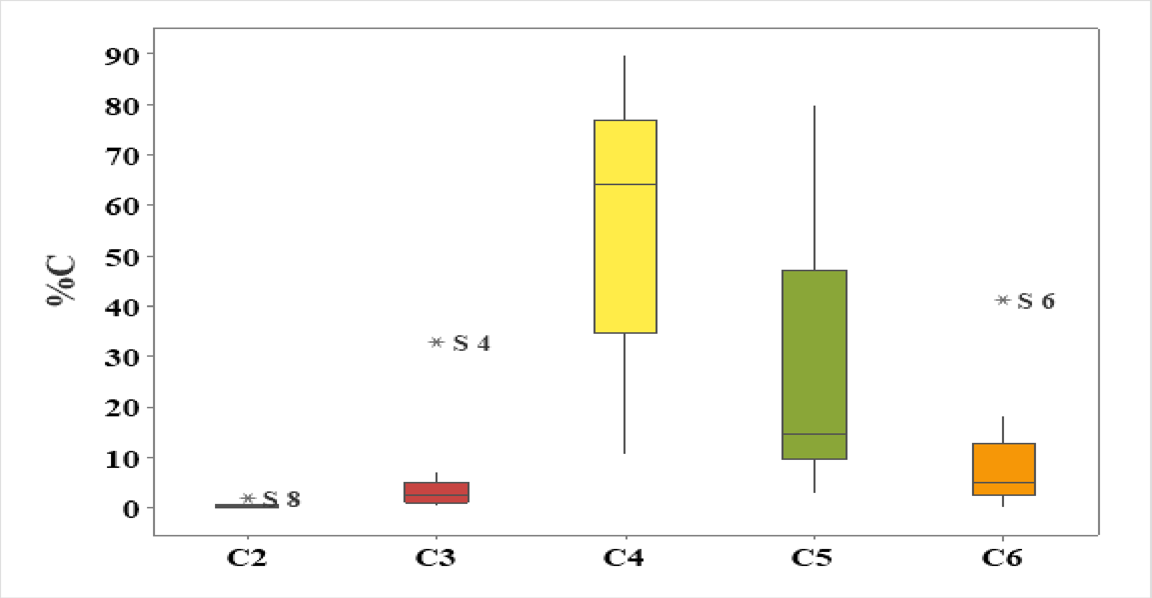

Fig. 2. Boxplot of distribution of different %C-rings in sediment of Manzala Lake
* represents sites with outlier values of C%

2. Source identification of PAHs

To predict the source of PAHs in sediments of Manzala lake, we used proportion analysis in addition to different isomeric ratios **(Edokpayi *et al*., 2016)**. High ratios of HPAHs are indicative of pyrolytic sources, while high LPAHs characterize petroleum sources, hence LPAHs/HPAHs ratios of less than one are indicative of pyrogenic sources, whereas petrogenic sources have a LPAHs/HPAHs > 1 **(Okedeyi *et al*., 2013)**. In the present study, LPAHs/HPAHs ratios were within values (0.01-0.5) lower than 1, suggesting pyrolytic sources of PAHs at all sites.

The ratios of some PAH compounds of the same molecular weight were used in this study as indices of their source, such as Phen/Ant, Ant/(Phen+Ant), Flu/(Flu + Pyr), InP/(InP+BghiP) and BaA/(BaA+Chr) **(Yunker *et al*., 2002)** (Table 7). The Phen/Ant ratio was extensively used to infer the nature of PAH pollution in sediments **(El-Sikaily *et al*., 2005)**. In petrogenic PAH pollution, the Phen/Ant ratio is very high (>10), but low ratios (<10) are indicative of the pyrogenic source. Our results recorded low values of phen/ant between 0-2.89 that inferred to pyrolytic sources at all sites of Manzala Lake. Flu/(Flu + Pyr) ratio of less than 0.4 indicates petroleum contamination, while ratios from 0.4 to 0.5 indicate fossil fuel combustion (crude oil and vehicle), and those greater than 0.5 suggest combustion of coal, wood and kerosene **(El-Naggar *et al*., 2018)**. Results of the Flu (Flu+Pyr) ratio were less than 0.4 at most sites S1, S2, S3, S4, S9 and S11 that indicated petrogenic pollution of PAHs at these sites; however, sites S5, S6, S7, S8 and S10 recorded Flu/(Flu+Pyr) ratios between 0.4 and 0.5, which are mainly originated from petroleum combustion (mixed source) (Table 5). A ratio BaA/(BaA + Chy) lower than 0.2 indicates a petroleum source, while that fluctuates from 0.2 to 0.35 indicates a combustion of petroleum origin and a value higher than 0.35 indicates combustion source (Sany *et* *al*., 2014). Ratios of BaA/(BaA + Chy) indicated that all sites of the present study had pyrogenic origins.

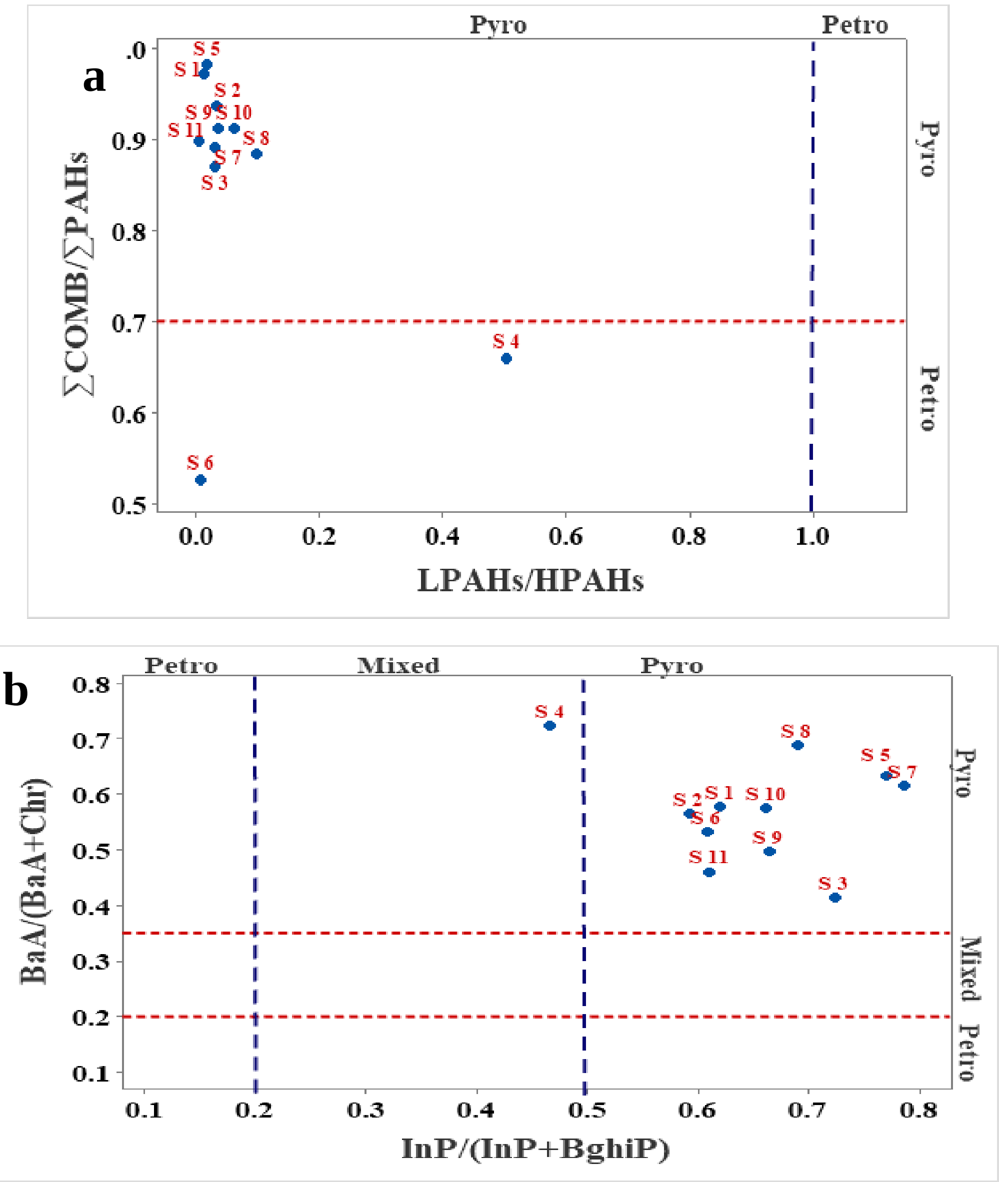

Fig. 3. Scatterplot ratios of a. ∑COMB/∑PAHs against LPAHs/HPAHs and b. BaA/(BaA+Chr) against InP/(InP+BghiP) in sediments of Manzala Lake

InP/(Inp+Bghip) ratio less than 0.2 indicates a petroleum source, while a ratio larger than 0.5 indicates combustion source, and a ratio between 0.2-0.5 indicates mixed source. Results of InP/(Inp+Bghip) indicated pyrolytic sources at all sites of the present study with the exception of site 4, which indicated a petroleum combustion source. A petrogenic source is indicated by a ΣCOMB/ΣPAHs ratio less than 0.3. A mixed source of ratio was between 0.3 and 0.7 and a pyrogenic origin of ratio larger than 0.7 **(Omokpariola *et al*., 2022)**. Results of ∑COMB/ ∑PAHs ratio indicated that most sites of the present study are those of pyrogenic origins, with the exception of sites 4 & 6 that suggested mixed sources. A good correlation was found between ∑PAHs and ∑COMB with [r= 0.99, n= 11, *P*< 0.001], suggesting pyrogenic sources of PAHs in the sediments of Manzala Lake.

Table 5. Sources of PAHs in Manzala Lake sediments according to different isomeric ratios

Ratio	**The present study**	**Reference guideline**			**Reference**
		Pyro	**Petro**	**Mixed**	
LPAHs/HPAHs	0.02±0.025*	<1	>1	-	Wang *et al.*, 2006
Phen/Ant	1.1 ±0.28	<10	>10	-	Sicre *et al.*, 1987
Ant/(phen+Ant)	0.47±0.08	>0.1	<0.1	-	
Flu/ (Flu+Pyr)	0.31±0.05	>0.5	<0.4	0.4-0.5	Yunker *et al.*, 2002
BaA/(BaA + Chry)	0.57±0.03	>0.35	<0.2	0.2-0.35	
InP/ (Inp+Bghip)	0.65±0.03	> 0.5	<0.2	0.2-0.5	
∑COMB/ ∑PAHs	0.9±0.04*	>0.7	<0.3	0.3-0.7	Liu *et al.*, 2017

*Presents non parametric data calculated as median ±SIQR. The others are parametric data calculated as mean±SD.

3. Statistical analysis

Two- way cluster analysis (TWCA) divided PAHs compounds into groups depending on their molecular weight and number of aromatic rings. TWCA is inferred to a different source of BghiP, DBA and InP in the lake, compared to the other compounds (Fig. 4). Cluster dendrogram divided sediments of Manzala Lake into five groups; Group A included sites 2 and 7, which were similar in moderate levels of BbF and BKF. Group B included sites 5 , 4 and 8 that contained the highest levels in Naph, Acthy, Ace, F1, Flu, Phen, Ant and Pyr that may be due to the fishing activities in the middle sector of the lake in addition to the effluent effect of the new Bahr El-Baqar drain at site 8. Bahr El-Baqar is a major source of contamination of the lake by the domestic waste. Group C contained sites 3 and 11, the farthest sites from the pollution source; Group D contained sites 6, 9 that are affected by the domestic activities and sewage from the El-Mataryia Town and the old Bahr El-Baqar drain, respectively, detecting high levels of BghiP, DBA and InP, especially at site 6, suggesting pyrogenic sources. Group E included sites 1, 10 that are affected by the wastewater treatment plant (WWTP) in the east of Port Said City and El-Serw Town, respectively, containing moderate sources of Naph, Flu, F1, Acthy and Ace, suggesting low petrogenic sources. TWCA predicted the relationships between sites and individual PAHs that mainly suggested pyrogenic sources of PAHs pollution. The spearman correlation coefficient indicated that Naph was positively correlated with Ace (r=0.902, n=11, P<0.05), Acthy (r=0.878), F1 (r=0.565), Phen (r=0.815), Ant (r=0.861), Flu(r=0.827), Pyr (r=0.855,), Chr (r=0.831), BaA (r=0.87), BbF (r=0.791), BkF (r=0,793) and BaP (r=0.794). On the other hand, these compounds have no correlation with BghiP, DBA or InP. This is attributed to the difference in the sources of PAHs. There was a positive correlation between BghiP and both DBA and InP (r=0.996 and 0.996); n=11, *P*<0.01) respectively.

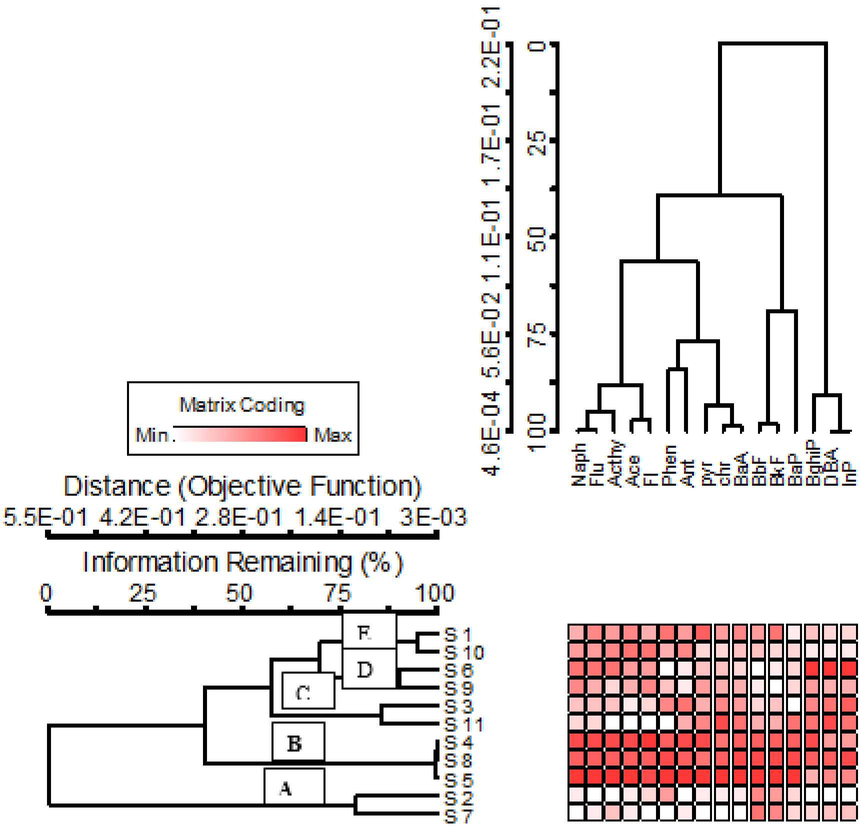

Fig. 4. Two- way cluster analysis of PAHs in sediments of Manzala Lake

Shapiro Wilk probability was used to test the normality of individual PAHs and ∑PAHs. All the investigated PAHs failed in the normality test and thus 1- sample signed rank t-test was used to explore the significance or non-significance of data between the investigated sites. There was a significant variation in the concentrations of Naph ($P<0.001$), Ace ($P<0.01$), Acthy ($P<0.05$), and BghiP ($P<0.05$) between the different sites. This confirmed the presence of different sources of PAHs pollution in the lake. On the other hand, the other compounds and ∑PAHs showed no significant variation between the sites ($P>0.05$).

The results of the present study were compared to some previous studies in Egypt and other areas around the world (Table 4). The present results of ∑PAHs (15.55 – 12707 µg/kg dw) in Manzala Lake recorded higher levels than those (246–9910 µg/kg dw) recorded in the study of **El-Kady *et al.* (2018)** regarding the same lake. In addition, the present values are higher than those recorded for Edku and Burullus Lakes, with levels of 13–183 and 18.1–672, respectively **(Barakat *et al.*, 2011)** and those recorded for the Suez Gulf, Aqaba Gulf and the Red Sea coast **(Salem *et al.*, 2014)**. On the other hand, the current results are lower than those of **Barakat *et al.* (2011)** for the Egyptian Mediterranean coastal and those reported for the Huaihe River, China (210-39,000) in the study of **Yuna *et al.* (2022)**.

4. Sediment quality guidelines

PAHs in sediments can pose harm to the benthic community. Numerous SQGs are available for the protection of benthic life. They are compound- (e.g., naphthalene) or class-specific (total PAHs) estimates of concentrations in sediments that are intended to protect sediment- dwelling organisms from adverse effects. ERL and

ERM, as well as TEC and PEC are widely used in the ecological risk assessment of PAHs in aquatic sediments **(Liu *et al*., 2017)**. PAH concentrations less than the ERL or TEC values indicate that an incidence of adverse effect on benthic organisms is rare, while the concentrations exceeding the ERM or PEC values suggest that adverse effects are likely to frequently occur, and values between the ERL and ERM or TEC and PEC values imply that there will be an occasional adverse effect **(Long *et al*., 1995; MacDonald *et al*., 2000)**. We compared our results with SQGs to assess the potential toxicity of PAHs in sediments on benthic organisms between different sites under study. According to SQGs, site 5 exceeded the ERL value of Ace, F1, Flu and Pyr. Moreover, F1 and DBA exceeded the ERL value at sites 4 and 6, respectively. On the other hand, F1, Ant, Flu, Pyr and BaA have values above their TEC at site 5, while DBA exceeded its ERL only at site 6 (Table 6). These findings inferred the probability of incidence of adverse effects on the sediment biota of the middle sector of the lake. However, ERM and PEC thresholds did not exceed at any sampling site in Manzala Lake. The obtained results inferred the necessity of continuous monitoring of sites 4, 5 and 6 to avoid excess levels of ∑PAHs than ERM and PEC values.

Table 5. Comparison of ∑PAHs concentrations (µg/kg dw) in sediments of Manzala Lake with other areas in Egypt and other water bodies

Water body	Country	∑16 PAHs	Reference
Manzala Lake	Egypt	15.55 – 12,707	The present study
Manzala Lake		246–9,910	El-Kady *et al*., 2018
Edku Lake		13–183	Barakat *et al*., 2011
Burullus Lake		18.1–672	
Mediterranean Coastal		3.5–14,100	
Suez Bay		9.1-981.10	Soliman *et al*., 2023
Suez Gulf		1,667.02-2,671.27	Younis *et al*., 2018
El Max Bay		1,123 and 8,654	Mohamed *et al*., 2016
Suez Gulf		19-97	Salem *et al*., 2014
Aqaba Gulf		0.37–0.74	
Red Sea Coast		0.3-0.57	
Xiangxi Bay	China	5.26 - 1325.25	Li *et al*., 2022
Huaihe River		210-39,000	Yuan *et al*., 2022
Lake Taihu		8.99 to 199.2	He *et al*., 2023
Erhai Lake		32–559	Yuan *et al*. 2016
Fuxian Lake		83.2–261.7	Gu *et al*., 2017
Dianchi Lake		210–11,070	Zhao *et al*., 2014
Northeastern		89–749	Liu *et al*. 2013
Al-Arabeen Lgoon	Saudia Arabia	5.4-2,543.3	Rasiq *et al*., 2018
Al-Shabab Lagoon		39.3-1,729.1	
Brunei Bay, East	Malaysia	10.4-376	Pang *et al*., 2022
Rijeka Bay area	Croatia, USA	0.032–13.681	Alebic-Juretic, 2011

Table 6. Sediment guideline values (µg/kg) of 16 PAHs to protect benthic life

	ERL	**ERM**	**ERL<S<ERM**	**S>ERM**	**TEC**	**PEC**	**TEC<S<PEC**	**S>PEC**
Source	**Long *et al*., 1995**				**MacDonald *et al*., 2000**			
Naph	160	2100	-		176	561	-	
Acthy	44	640						
Ace	16	500	S 5					
F1	19	540	S4, S5		77.4	540	S 5	
Phen	240	1500	-	-	204	1170	-	-
Ant	85.3	1100	-	-	57.2	845	S5	-
Flu	600	5100	S5	-	423	2200	S5	-
Pyr	665	2600	S5	-	195	1520	S 5	-
Chr	384	2800	-	-	166	1290	-	-
BaA	261	1600	-	-	108	1050	S5	-
BbF								
BkF								
BaP	430	1600	-	-	150	1450	-	-
BghiP								
DBA	63.4	260	S6	-	33		S6	
InP								
Total	4022	44792	S5	-	1610	22800	S5	-

5. Toxicity Indices

In the present study, SQGs indices (PECq, mPECq, ERMq, and mERMq**)** were used to assess the potential toxicity of the different PAHs on the benthic organisms in the Manzala Lake. It is clear that Naph, Phen and Ant showed a low potential risk (PEC-q ≤0.1; with a 14 % probability of being toxic) at all sites, while F1, Flu and BaA showed a moderate risk (0.1 < PEC-q < 1; with a 15-29% probability of being toxic) at site 5. Meanwhile, Pyr was at a moderate risk at 18 % of the sites (S4 and S5). Based on the average value, the descending order of PEC-q of the different hydrocarbons was Pyr>Flu>BaA>Chr>Ant>BaP>Phen>F1>Naph, respectively, while there is no available data about the PEC value of Ace, Acthy, BbF, BkF, BghiP, DBA and InP. Based on the mPEC-q values, all sites of Manzala Lake were at a low risk of toxicity, with the exception of S5 that was determined to be in a moderate risk (Fig. 5). In a related vein, Naph, Acthy, Ace, Phen, Ant, Chr and BaP showed a low potential risk (ERM-q ≤0.1; with a 9% probability of being toxic) at all sites. F1, Flu, Pyr, BaA and DBA showed a moderate to low risk (0.1<ERM-q <0.5; with a 21 % probability of being toxic) at 9% of sites. There is no available data about ERM values of BbF, BkF, InP and BghiP. The sequence of ERMq between the individual PAHs was Pyr> DBA> F1> Flu> BaA> Ant> Ace> Chr> BaP> Acthy> Phen >Naph. The results of mERM-q declared that all sites of Manzala Lake were at low risk (Fig. 5), with a higher value at site 5. According to mPEC-q and mERM-q values, the middle sector of the lake showed a higher toxicity risk of ∑PAHs on sediment-dwelling organisms, followed by the eastern south sector and the western and the northern sectors of the lake. The east-south sector is influenced by industrial and sewage effluents, especially Bahr El-Baqar effluent. **El-Kady *et al.* (2018)** reported very high contamination of sediments of Manzala Lake at sites close to Bahr El-Baqar. The northern sector represented the lowest risk as it is subjected to open water entering into the lake from the Mediterranean Sea through Boughaz El-Gmail and Boughaz Ashtoum El-Gmail that renewed water of the lake and also diluted the concentrations of PAHs in samples of the northern sector.

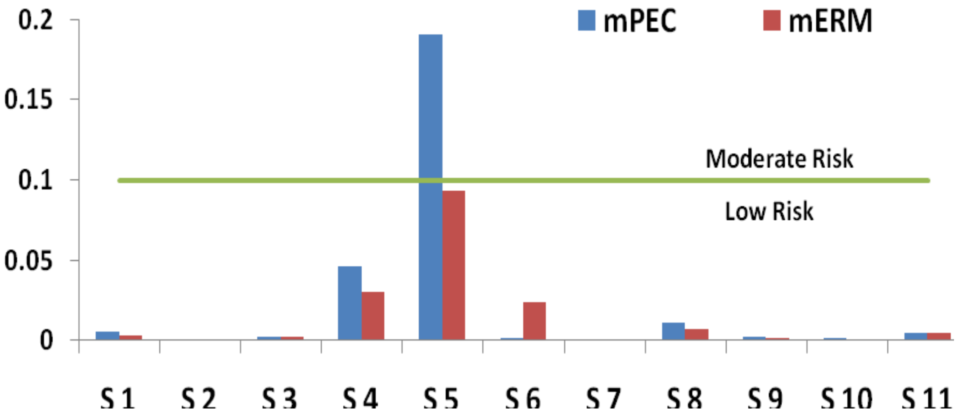

Fig. 5. mPEC and mERM indices of PAHs in sediments of Manzala Lake

CONCLUSION

Distributions of 16 PAHs compounds were examined in sediments of the Manzala Lake, Egypt to evaluate the pollution status and eco-toxicological aspects. The toxic, carcinogenic and mutagenic properties of these compounds may present a threat to benthic-dwelling organisms of the aquatic environment. ∑PAHs, ∑COMB, and ∑CAR PAHs varied between (15.55 - 12707.64); (13.99-12478.09), and (6.44-10487.91); µg/Kg dw, respectively. According to the Baumard scale, 64, 27 and 9 % of sites were classified as low, moderate and highly contaminated by PAHs; respectively. The middle and the eastern south sectors of the lake were highly contaminated by PAHs. Different diagnostic ratios; LPAHs/HPAHs; (Ant/(Ant+Phen), Flu/Pyr, InP/(InP+BghiP), BaA/(BaA+Chr) were used in this study. A combination of these indices inferred pyrolysis (combustion sources) as the major source of PAHs in the lake. The data of SQGs revealed lower ∑PAHs than ERL and TEC guideline values. On the other hand, Ace, F1, Flu, Pyr and DBA exceeded the ERL value, while Ant, F1, Flu, Pyr, BaA and DBA exceeded the TEC value. The toxicity indices (mERMq and mPECq) indicated a low toxicity risk by PAHs at all the sites, with the exception of site 5. This site is in the middle sector of the lake; it is influenced by fishing activities and vehicular emissions, and its content of PAHs may cause a moderate toxicity risk on sediment-dwelling organisms. Thus, great attention and integrated risk assessment approaches are needed to reduce the pollution risk by PAHs and maintain ecological sustainability.

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