Residues of some organic pollutants, their bioaccumulation, and risk assessments profile in Lake Temsah, Ismailia, Egypt.

Almatari MS, Yousry M Ahmed, Laila Reda, Naglaa Loutfy and Tawfic Ahmed M*

Department of plant protection, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

Abstract

Residues of polycyclic aromatic hydrocarbons (pahs), polychlorinated biphenyls (pcbs) and organochlorine pesticides (ocps) were monitored in Lake Temsah ecosystem including fish and bivalve species, water and sediment. The concentration of these organic pollutants were in the order of pahs>ocps> pcbs. The mean total concentrations of pcbs (Σ 11 pcbs) were in the order of sediment (49.8 ng/g), biota (29.7- 44.7 ng/g), water (16.1 ng/ml. PCB118, a dioxin -like congener is detected in almost all samples, at concentrations not exceeding 4.5-24% of Σ 11 pcbs. Concentration of pcbs in sediment samples (49.8 ng/g) exceeded the Canadian pcbs threshold effect level (TEL) of 21.5 ng/g, but were below pcbs probable effect level (PEL) of 189 ng/g. The ocps concentration reported in biota were below FDA Regulatory Action Levels of 0.3 μg/g in Fish.

For biota, bioaccumulation factors (BAF) of ocps (23.7-560) were much higher than those of pcbs (0.1-12.9) and pahs (0.1-1.2), with heptachlor epoxide showing the highest mean bioaccumulation factor (BAF). Similar trend was reported for the biota−sediment accumulation factor (bsafs) with values of 0.33 to 5.57 for ocps, 0.11 to 4.53 for pcbs, and 0.16 to 4.47 for pahs. The greatest BSAF values were for DDT metabolites.

Keywords: Organochlorine pesticides, PAHS, PCBS, fish, bivalves, water, sediment, bioaccumulation, BAF, BSAF, Egypt.

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Introduction

Pollution has a dire impacts on ecosystems and water bodies are among the most vulnerable. Persistent organic pollutants (POPs) are semi-volatile compounds, persistent in the environment, and toxic to humans and wildlife. Being lipophilic, POPs tend to accumulate in food chains and therefore may pose serious threats to higher trophic levels of aquatic communities and humans [1-7]. Due to these characteristics, exposure to these pollutants can cause potential health damage [6,7]. Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) have been reported by Stockholm convention 2001 and The UNECE Protocol on POPs as a worldwide concern. PAHs are not included in the Stockholm Convention, but are listed in the UNECE (United Nations Economic Commission for Europe) Protocol on POPs and US EPA has reported sixteen PAHs as priority pollutants.

The persistence of many of the organochlorine pesticides in the environment has prompted continued studies aimed at evaluating their environmental impacts, including impact on wildlife and humans and their regulation worledwide. Although Egypt has been banneduse of organochlorine pesticides since the 1980s, they are still detected in various foods in the country [8]. Organochlorine pesticides are lipophilic and stable compounds. Their photo-oxidation, low vapour pressure, beside their low chemical and biological degradation rates have contributed to their accumulation in biological tissues and the subsequent magnification in organisms through food chains [9].

PCBs are priority pollutants, with wide impacts on man and his environment, including immunotoxicity, endocrine disruption and tumor [10]. Despite the fact that the production and sale of PCBs has been prohibited in most countries for many decades, they still pose potential threats to the health of humans and aquatic life. PCBs persist in nature due to the same high physical and chemical stabilities that made them attractive for industrial use [11].

PAHs are classified as environmentally hazardous pollutants due to their known hydrophobic, mutagenic, and carcinogenic characteristics [12,13], in addition to their endocrine disrupting activity [14-16]. In fish, PAHs have been found to exert their toxicity following biotransformation through toxic metabolites, which can be bound covalently to cellular macromolecules such as proteins, DNA and RNA, causing cell damage, mutagenesis, teratogenesis and carcinogenesis [17].

PAHs include two main groups: the Low Molecular Weight (LMW, 2-3 rings) PAHs and the High Molecular Weight (HMW, 4-6 rings) PAHs. While they can occur through natural processes, such as oil leaks or digenesis, the highest concentrations are mainly originated from human activities, and the primary sources are combustion products. The International Agency for Research on Cancer (IARC) has previously classified 14 out of 50 PAHs as potentially hazardous to mankind. PAHs anthropogenic sources include combustion of organic matter (pyrolytic PAHs) besides being present in oils (petrogenic PAHs). Pyrolytic PAHs are released into the atmosphere, followed by their deposition on water and soil. Petrogenic PAHs may be discharged directly into water as a result of oil spills or naval and offshore oil drilling activities [18]. The ratios of certain specific PAH isomers have been applied to infer the sources of PAHs [19].

Aquatic organisms are among major targets of POPs because of their vulnerability to lipophilic contaminants that tend to accumulate in their tissues, sometimes reaching alarming concentrations [20]. Aquatic organisms are also time integrating, since they can indicate the presence of contaminants that are no longer in the water, or those whose presence or use is intermittent [21]. Their ability to accumulate contaminants in their tissues to elevated levels reaching concentrations much higher than that of ambient water, makes these biota useful for assessment purposes.

Bioaccumulation is the ability of a pollutant to accumulate in living tissues at levels higher than those in the surrounding environment. It is a process in which a chemical pollutant enters the body and is not excreted but rather accumulates in the organism's adipose tissues. Bioaccumulation is the net result of competing processes of absorption, ingestion, digestion, and excretion [22]. Bioaccumulation of chemicals in biota is also a chief factor for adverse effects, and ecosystem degradation [23].

The distribution behavior of the contaminants between water and biota can be expressed as bioaccumulation factor (BAF) [24]. BAF reflects uptake of a substance by aquatic organisms exposed to the substance through all routes (i.e., ambient water and food), as would occur in nature. Sediment contaminants can be released into the overlying water, resulting in potential adverse health effects to aquatic organisms [25-27]. Biota Sediment Accumulation Factor (BSAF) incorporates all of the conditions and parameters influencing the bioaccumulation of the chemicals at the measurement site.

Lake Temsah is the main brackish wetland ecosystem in governorate of Ismailia. The lake is the major source of fishes and bivalves consumed by the local population in the surrounding areas. The lake also embraces a number of other activities mainly related to fishing and shipping industries. The lake is the end point where some municipal, agricultural and industrial wastewaters are discharged (ECDG 2002). In addition, the corrosion of ships' hull coatings and the antifouling paints on ships awaiting berth would also contribute to lake contamination [28].

Monitoring is a repetitive observation for defined purposes of one or more chemical or biological elements over time and space, using comparable and standardized methods. A regular, systematic use of living organisms to evaluate changes in environmental or water quality, as in chemical monitoring and bioaccumulation monitoring, is called biological biomonitoring [29]. Aquatic organisms, including fish and bivalves are sound tool to reflect the quality of their environment.

Several monitoring studies have been carried out on the Temsah Lake to examine the pollution profile [30-35]. However, none of these studies dealt with bioaccumulation of POPs in the lake. Therefore the present study aimed to shed some light on the levels and profiles of OCPs, PCBs and PAHs in the Temsah lake ecosystem compartments (sediment, water and biota). The study has also meant to identify sources of contaminants based on their profile and to determine the bioaccumulation patterns of some of them.

Materials and Methods

Study area

Lake Temsah is a small water body (~15 km^2), lies on the Suez Canal at mid-way between Port Said and Suez. It lies between 30° 23' and 30° 36' N latitude and 30° 16' and 32° 21' E longitude. The lake has nearly a triangular shape with elongated sides extending roughly East-West. The lake receives high salinity water from the Suez Canal, mainly from the south, beside some drain and freshwater from surrounding areas.

Sampling

Water, sediment and biota samples were collected from four sites in the lake Temsah during November 2014.Selection of organisms was based on the different niches in which they normally thrive within the lake ecosystem.

Two bivalve species, pullet carpet shell (Venerupis pullastra) and textile venus clam (Paphia textile) and two fish species tilapia and mullet (Tilapia zilli and Mugil cephalus) were collected by fishermen stationed at different parts of the lake. Fishes had an average weight of 120 to 200 g respectively.

Sediment samples were collected from the top 10-cm layer of the lake's bottom. Samples were air-dried for 14 days, then shell and plant fragments were removed by passing the dried sample through a 2-mm sieve. The sieved sample was powdered and stored in the deep freeze until analysis. The total organic carbon (TOC) was determined by Walkley and Black method described by Jackson (1967).

Water samples were collected into brown glass bottles pre - washed with detergent, rinsed with water and pure acetone (99.9%) and then dried before samples collection. Samples were taken from 0.1 m below the water surface and transported directly to the lab. All samples were sent to central agricultural pesticides laboratory in Cairo and biota samples stored in deep-freezer at 4°C until the analysis.

Samples preparation

The edible parts of fish and bivalves were homogenized in food processor. Incremental samples were mixed and homogenized together to obtain an aggregate sample. Fat content was determined according to the method of Association of Official Analytical Chemists (AQAC) (1995). Organic matter determined by using Walkley and Black methods described by Jackson (1967).

Samples extraction and clean up

Water samples: Solid-phase extraction method was used to separate PAHs and organochlorine pesticides of water samples. The 360 mg C18 Sep-Pak cartridges were prepared with 10 ml methanol followed by 10 ml demonized water without allowing the cartridge to dry out. Then100 ml of water sample was added to the cartridge, allowing to pass through at a rate of 6 ml/min. The cartridges were then sucked dry for 5 minutes to remove all liquid, then the sample eluted with 5 ml ethyl acetate. The extract was evaporated with N2, adding 2 ml hexane and then transfer it to deep freezer until analysis.

Sediment samples: 10 g sediment, 10 ml of acetonitrile, 1 g of sodium chloride and 4 g of anhydrous magnesium sulfate ($MgSO_4$) was added to centrifuge tube (50 ml), the tube were closed and the tube vigorously shaken for 1 min using a vortex mixer, and centrifuged for 5 min at 4500 rpm and 4°C. An aliquot of 1 mL supernatant was transferred to new clean 15-mL centrifuge tube and cleaned up by dispersive solid-phase extraction with 25 mg PSA and 150 mg $MgSO_4$. The sample was again vortexed for 1 min and then centrifugation was carried out as mentioned above. Then, 1 mL of the supernatant were taken, filtered through a 0.22-μm PTFE filter (Millipore, Billerica, MA) and transferred into a glass vial for GC analysis.

Biota samples: 10 g homogenized fish, or bivalve, 10 ml of acetonitrile, 1 g of sodium chloride and 4 g of anhydrous magnesium sulfate was added to centrifuge tube (50 ml), the tube were closed and the tube vigorously shaken for 1 min using a vortex mixer, and centrifuged for 5 min at 4500 rpm and 4°C. An aliquot of 1 mL supernatant was transferred to new clean 15-mL centrifuge tube and cleaned up by dispersive solid-phase extraction with 25 mg PSA, 25 mg C18 and 150 mg $MgSO_4$. The sample was again vortexed for 1 min and then centrifugation was carried out as mentioned above. Then, 1 mL of the supernatant were taken, filtered through a 0.22-μm PTFE filter (Millipore, Billerica, MA) and transferred into a glass vial for GC analysis.

Determination of OCPs and PCBs

The extracts of OCPs and PCBs were concentrated and injected into Gas chromatography (GC) (Aglient 6890) equipped with a 63Ni ECD, a split/splitless injection inlet, capillary column capability, and a 7683A autosampler. Chemistation software was used for instrument control. GC analysis was conducted on a HP-5MS (Aglient, Folsom, CA) capillary column of 30 m, 0.25 mm id., 0.25 μm film thickness. The oven temperature was programmed between an initial temperature 160 (2 min hold) to 240°C at a rate of 5ºC min^{-1} and was maintained at 240°C for 20 min. Injector and detector temperature were maintained at 260 and 320ºC, respectively. Nitrogen was used as a carrier at flow rate of 3 ml min^{-1}. All OCPs and PCBs congener's reference standards were obtained from Dr. Ehrenstorfer, Augsburg in Germany. Method sensitivity and recovery were determined by using samples spiked with the tested compounds and congeners. Before analysis, relevant standards were run to check column performance, peak height, resolution, and limits of detection. Peaks were identified by comparison of sample retention time value with those of the corresponding of pure standard compounds. With each set of samples to be analyzed, a solvent blank, a standard mixture and a procedural blank were run in sequence to check for contamination, peak identification and quantification. The average recovery percentages of OCPs and PCBs for fortified samples at different levels were determined and calculated for all tested compounds in each aquatic system compartment. The average recovery percentages of OCPs and PCBs for fortified samples at different levels were determined and calculated for all tested compounds in each aquatic system compartment. Mean Recovery of organochlorine pollutants were 86.85 ± 5.4, 83.50 ± 5.12 and 84.71 ± 5.68 in water, sediment and fish sample, respectively. PCBs recovery percentage were 93, 88 and 91% for water, sediment and biota samples respectively.

Determination of PAHs

A gas liquid chromatograph (Hewlett-Packard Model 5890N series II) with split/splitless injection system, capillary column capability, and flam ionization detector was used for analysis of PAHs. GC analysis for PAHs was conducted on a HP-608 (Agilent, Folsom, CA) fused silica capillary column of 30 m length, 0.53 mm id., 0.5 μm film thickness. The oven temperature was programmed between an initial temperature 70 (2 min hold) to 260°C at a rate of 6°C min^{-1} and was maintained at 260°C for 15 min. Injector and detector temperature were maintained at 280 and 300°C, respectively. Nitrogen was used as a carrier at flow rate of 4 ml/min. Chemstation software was used for instrument control and data analysis. Peak was identified by comparison of sample retention time value with those of the corresponding of pure standard compounds. The peak identities in samples with high PAH levels were further confirmed by GC/MS analysis.

Quality control

Preparation of blank solution: The same volume of solvents and anhydrous sodium sulfate used in extraction of OCPs and PCBs from water, sediment and fish samples were subjected to the same procedures as the examined samples to detect any possible traces of the studies pesticides or PCBs and its value was subtracted between the results.

Recoveries were carried out by the addition of PAHs standards mixture at different levels. All data were corrected according to the recovery percentage values. Compounds were identified by matching retention time against those of authentic standard.

For confirmation: Selected samples were analyzed by full scan GC-MS to confirm the GC-ECD results. The column used was HP-5MS (Aglient, Folsom, CA) capillary column of 30 m, 0.25 mm id., 0.25 μm film thickness. The carrier gas was helium at a flow rate of 0.5 ml min^{-1}. Inlet temperature was 225ºC with injection volume of 2 μl (splitless injector). The column temperature was set at 70ºC for 1min and then programmed at 10ºC min^{-1} to reach 200ºC. GC-MS interface was 280ºC. Chemistation software was used for instrument control and data analysis.

Accuracy and sensitivity: Method sensitivity and recovery were determined by using samples spiked with the tested compounds and congeners. Before analysis, relevant standards were run to check column performance, peak height, resolution, and limits of detection. Peak was identified by comparison of sample retention time value with those of the corresponding of pure standard compounds. With each set of samples to be analyzed, a solvent blank, a standard mixture and a procedural blank were run in sequence to check for contamination, peak identification and quantification. The average recovery percentages of OCPs and PCBs for fortified samples at different levels were determined and calculated for all tested compounds in each aquatic system compartment.

Bioaccumulation assessment

Bioaccumulation is measured with the bioaccumulation factor (BAF) which is defined as the ratio of the concentration of a chemical accumulated inside an organism to its concentration in the ambient environment at a steady state (U.S. Environmental

Protection Agency, 2010). Biota-Sediment Accumulation Factor (BSAF) is a parameter describing bioaccumulation of sediment-associated organic compounds into tissues of ecological receptors. In essence, it is a fugacity ratio for the chemical of interest between the organism and sediment. Because the contaminants concentration in water and /or sediment were mostly below the detection limit, it was only possible to calculate BAF and BSAF only for contaminants that mentioned in Tables 1 and 2. The BSAF are calculated by dividing the lipid-normalized tissue concentration by the organic carbon-normalized sediment concentration [36,37]. BAF was calculated according to the equation of Mackay and Fraser [38] as follows:

$$BAF = Cb(\mu g/kg) / Cw\ (\mu g/L)$$

$$BSAF = (Cb / R) / (Cs / oc)$$

Table 1. *Bioaccumulation factor (BAF) of reported contaminants, Lake Temsah.*

Compound	*Tilapia zilli*	*Mugil cephalus*	*Paphia textile*	*Venerupis pullastra*
		PCBs		
PCB44	2.2	3.1	0.4	0.7
PCB101	0.1	0.1	0.2	0.4
PCB152	12.9	4.5	4.3	0.9
PCB180	0.3	0.4	none	0.4
PCB192	0.5	0.7	none	0.5
		PAHs		
Pyrene	0.1	0.2	0.2	0.5
Benzo(b) fluoranthene	none	0.1	0.1	1.2
		Organochlorine pesticides		
α- HCH	74.4	none	none	none
Heptchlor epoxide	none	560	226.7	none
p,p-DDE	32.7	50.8	102.1	72.4
p,p-DDD	none	none	65.6	none
p,p-DDT	40	none	none	none
Heptachlor	none	none	123.3	none

Table 2. *Biota-sediment accumulation factor (BSAF) of reported contaminants, Lake Temsah.*

	Tilapia zilli	*Mugil cephalus*	*Paphia textile*	*Venerupis pullastra*
		PCBs		
PCB52	0.68	0.52	1	2.32
PCB44	4.53	3.26	2.21	2.21
PCB101	0.11	0.06	0.47	0.66
PCB118	0.26	0.14	1.09	0.23
PCB105	0.5	none	none	0.86
PCB138	0.13	none	1.96	none
PCB180	0.31	0.19	none	0.61
PCB192	0.62	0.42	none	0.81
		PAHs		
Naphthalene	0.16	none	0.35	none
Flourene	none	none	2.79	none
Pyrene	0.83	0.71	3.88	4.47
		Organochlorine pesticides		
γ-HCH	0.33	none	none	0.90
Heptchlor epoxide	none	0.75	1.8	none
Dieldrin	0.60	none	1.01	0.19
p,p-DDE	0.59	0.46	5.57	1.97
p,p-DDD	none	none	5.2	none

where Cw is the water chemical concentration, Cb is the organism chemical concentration (ng/g wet weight), R is the lipid content of the organism (g lipid/g wet weight), Cs is the surficial sediment chemical concentration (ng/g dry weight) and oc is the organic carbon content of the sediments (g organic carbon/g sediment dry w) respectively.

Results and Discussion

Polycyclic aromatic hydrocarbons residues

Residual levels of 14 congeners of PAHs in biota, water and sediment samples from Lake Temsah are presented in Table 3. Generally, many of the PAHs congeners were below their limits of detection for all samples. Detected levels of PAHs had the descending order of sediment >Mugil cephalus (mallet)>Paphia textile>Tilapia zilli (tilapia)> water samples>Venerupis pullastra. Based on the lipid content biota samples are arranged in the order of Mugil cephalus (0.06), Tilapia zilli (0.03), Venerupis pullastra (0.02), and Paphia textile (0.01). Discrepancies in the lipid content and PAHs residue level is an indication that bioaccumulation is not only governed by lipid content but other factors might be also involved.

The mean total PAHs concentrations (14 congeners) in fish and bivalves samples ranged from 53.3 to 125.1 and from 42.6 to 90.8 ng /g respectively. In the current study lipid levels were not associated with PAHs concentration in bivalve's samples. Water samples showed contamination level of 45.8 ng/ml with Benzo (b)fluoranthene and pyrene as the only two detectable congeners. In case of the sediment samples, naphthalene, benzo(b)fluoranthene and pyrene were the only detectable congeners with mean total concentration of 259.4 ng/g . PAHs in aquatic systems tend to accumulate in sediments, resulting in long-term effects on benthic organisms [39]. After being accumulated in sediments, it is difficult for PAHs to decompose via photochemical degradation or microbial oxidation [40]. As a result, sediments serve as a major reservoir for PAHs contamination [41,42]. Humans can be directly or indirectly exposed to PAHs in sediments, and so studies of PAHs distributions in sediments are urgently needed [43,44].

Residues of PAHs detected in tilapia fish (53.3 ng/g) were less than those detected in mullet fish (125.1 ng/g). Such variation in the magnitude of PAHs accumulation could be explained in view of fat content and the ecological niche of each, with special reference to feeding habits, and position in the lake ecosystem. Mullet fish feed primarily on detritus residing on the sediment, and also on plankton and use gizzard - like stomach to aid with digestion [45,46]. With much of the persistent pollutants end up on the lake bottom, feeding on bottom detritus, sand and mud particles, would allow considerable amount of pollutants to move to fish through ingestion [47]. On the other hand, tilapia fish is a water column feeder that feed on PAHs while moving down the bottom through the water column.

The mean total concentration of PAHs in the bivalve Paphia textile (90.8 ng/g) was two times higher than the other bivalve (Venerupis pullastra) (42.6 ng/g) . This is possibly because Venerupis pullastra lives only few centimeters under the water surface while, Paphia textile is an in faunal filter feeding clam commonly found in sandy -muddy bottoms of the intertidal and

PAHs	***Tilapia zilli* (ng/g)**				***Mugil cephalus* (ng/g)**				***Paphia textile* (ng/g)**				***Venerupis pullastra* (ng/g)**				**Water(ng/ml)**				**Sediment (ng/g)**			
	min	Max	mean	S.D	min	Max	mean	S.D	min	max.	mean	S.D	min	max	mean	S.D	min	max	mean	S.D	min	max	mean	S.D
Naphthalene	nd	59.7	29.9	26.7	nd	nd	-	-	nd	44.5	22.3	19.9	nd	nd	-	-	nd	nd	-	-	150.6	229.8	190.2	102.1
Acenaphthylene	14.4	14.8	14.6	8	nd	53.8	26.9	24.1	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-
Acenaphthene	nd	nd	-	-	53.5	104.8	79.2	47	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-
Flourene	nd	nd	-	-	nd	nd	-	-	50.2.	69.5	59.9	33.5	nd	nd	-	-	nd	nd	-	-	56.6	72.1	64.4	34.1
Phenanthrene	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	3.8	3.8	3.8	2.1	nd	nd	-	-	nd	nd	-	-
Anthracene	nd	nd	-	-	nd	10.3	5.2	4.6	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-
Fluoranthene	2.8	4.8	3.8	2	3.2	3.8	3.5	1.6	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-
Pyrene	nd	7.9	4	3.5	nd	13.5	6.8	6	3.9	8.4	6.2	3.14	9.4	19.2	14.3	3.9	19.7	37.3	28.5	5.5	3.2	6.3	4.8	2.3
Chrysene	nd	2	1	0.9	nd	3.2	1.6	1.4	nd	1.5	0.75	0.7	1.1	2.1	1.6	0.9	nd	nd	-	-	nd	nd	-	-
Σ LOW	**17.2**	**89.2**	**53.3**	**-**	**56.7**	**189.4**	**123.2**	**-**	**54.1**	**123.9**	**89.2**	**-**	**14.3**	**25.1**	**19.7**	**-**	**19.7**	**37.3**	**28.5**	**-**	**210.4**	**308.2**	**259.4**	**-**
Dibenzo(a,h) anthrathene	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-
Benzo(b) fluoranthene	nd	nd	-	-	nd	3.7	1.9	1.6	nd	3.2	1.6	1.4	8.9	33.6	21.3	10.4	14.4	20.1	17.3	8.8	nd	nd	-	-
Benzo(k)fluorantnene	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-
Benzo(a)pyrene	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-
Benzo(ghi)perylene	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	3.1	1.6	1.4	nd	nd	-	-	nd	nd	-	-
Σ High	**-**	**-**	**-**	**-**	**-**	**3.7**	**1.9**	**-**	**-**	**3.2**	**1.6**	**1.4**	**8.9**	**36.7**	**22.8**	**-**	**14.4**	**20.1**	**17.3**	**-**	**-**	**-**	**-**	**-**
ΣPAHs	**17.2**	**89.2**	**53.3**	**-**	**56.7**	**193.1**	**125.1**		**54.1**	**132.1**	**90.8**		**23.2**	**61.8**	**42.6**	**-**	**34.1**	**57.4**	**45.8**	**-**	**210.4**	**308.2**	**259.4**	-
Σ LOW/ Σ High	-	-	-	-	-	-	64.8	-	-	-	55.8	-	-	-	0.87	-	-	-	1.65	-	-	-	-	-
Fluoranthene/ Pyrene	-	-	**0.95**	**-**	**-**	**-**	**0.51**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--

Table 3. *Polycyclic aromatic hydrocarbons (PAHs) residues in biota, water and sediment samples, lake Temsah*

sub littoral zones of the coastal environment [48], hence having the chance to accumulate much of persistent pollutants residing on the lakebed.

Previous monitoring programs have reported that the pollution level in Lake Temsah is much higher than other wetlands in Egypt of other lagoons around [49,33]. Mostafa, [33] reported that PAHs contamination level in clams had a mean value of 28.4 mg /kg for total PAHs and 24.4 mg /kg for carcinogenic PAHs. Indeno (1,2,3-cd) pyrene, was the most frequently detected congener, followed by benzo(a) pyrene, dibenzo(a,h)anthracene, and benzo(b)fluoranthene. Tundo et al. (2004), reported that PAHs concentrations for sediment samples from Temsah lake are (27.8-544.7 ng/g) with benzo (bkj) fluoranthene had the highest concentrations in almost all sampling stations. Ali et al. [50] reported Σ 16 PAHs, with concentrations ranging between 585.9-8592.8 µg/kg in sediment, 1694-4785.7 µg/kg in fish and 52.46-3393 µg/l in water samples with predominance of high molecular weight PAHs in all samples. Similarly, Said and EL Agroudy [51] reported a concentration mean of 10.78 µg/ l and 87.69 µg/g for water and Osteicthyes fish samples respectively, with benzo (a) pyrene as the most dominant congener in water samples with an average concentration of 3.8 µg/l.

The present results are also below the concentrations of 16 PAHs reported for water (192.5 to 2651 ng/l) and sediment (127.1 to 927.7 ng/g) from Tonghui River, Beijing varied by Zhang et al. [52].

The concentration of total PAHs reported in this study for the two fish species are close to the levels determined for the same species (tilabia: 19.7 ng/g and mullet: 154.3 ng/g) collected from the vicinity of the Temsah lake [53]. This would indicate that the concentration level of PAHs in Lake Temsah is almost stable for the last ten years.

The spectrum of PAHs in the lake ecosystem, including water, biota, and sediment, can provide some information about emission source. In the present study, the ratio of high molecular weight, to low molecular weight PAHs (HMW-PAHs to LMW-PAHs) has been used to characterize the origin of PAHs in the lake ecosystem (Table 4). The prevalence of LMW PAHs are typical for PAHs mixtures generated by petrogenic pollution [54].

On the other hand, PAHs emitted from combustion processes (pyrolytic origin) would often contain elevated concentrations of HMW (e.g., phenanthrene, fluoranthene, pyrene) and fewer LMW PAHs [55]. Therefore LMW/HMW>1 suggests a petrogenic origin, whereas LMW/HMW<1 indicates pyrolytic sources [56]. Likewise, ratios between individual PAHs compounds (like Phenanthrene/Anthracene and Flouranthene/pyrene) are used to identify the processes from which PAHs originate [57]. Baumard et al. [58] suggested that because pyrene is more stable than fluoranthene, hence pyrolytic products are usually characterized by a predominance of fluoranthene over pyrene at a ratio>1.

In the current study many of the PAHs congeners were below limits of detection, therefore it was not possible to analyze the congeners profile for all studied species. In addition, LMW PAHs were predominant in all samples with naphthalene as the main contributor, with a ratio of 70 %, 56%, 24%, of all PAHs for sediment, Tilapia zilli and Paphia textile samples respectively. US ATSDR considers naphthalene as a human carcinogen [59]. Naphthalene is widely used as an intermediate in the production of many surfactants and pesticides [60]. The ratio of LMW/HMW were higher than one in Mugil cephalus and Paphia textile indicating that the sources of these PAHs is petrogenic [61], while the ratio for the other bivalve species (Venerupis pullastra) was below one suggesting a pyrogenic source of pollution.

PAHs of petrogenic sources show characteristically higher proportion of LMW congeners, while pyrogenic PAHs have characteristically higher proportion of HWM PAHs (LMW / HMW<1) [61]. The available fluoranthene/pyrene ratio for fish species (Tilapia zilli and Mugil cephalus) was below one suggesting petrogenic source of PAHs pollution.

The low molecular weight PAHs (LMW PAHs) are acutely toxic to many aquatic organisms, whereas the high molecular weight PAHs (HMW PAHs) are strongly carcinogenic and mutagenic [62]. In the present study benzo (g,h,i) perylene was detected in only one sample of bivalve (Venerupis pullastra), while residues of benzo(b)fluoranthene were detected in the biota and water samples. Benzo (a) pyrene, commonly used as an indicator for PAHs in ambient air and food, has not been detected in any samples of water, biota or sediment. Meanwhile, benzo (b) fluoranthene and pyrene were the only congeners detected in all samples at detectable concentration.

In the present study, PAHs associated risk in sediments was assessed by applying the "US Sediment Quality Guidelines" (SQGs) [11,63]. SQGs provide two effects-based sediment guideline values: effects range-low (ERL) and effects range-median (ERM), which quantitatively assess the adverse biological effects in sediments [64]. Accordingly, PAHs will not be harmful to the environment and its biota when their concentrations are lower than ERL; while PAHs concentrations higher than ERM, will show negative impacts frequently. PAHs with concentrations between ERL and ERM are considered to be harmful occasionally [63,64]. Concentrations of ΣPAHs in sediments of Lake Temsah was less than the ERL value of 4749 ng/g dw. But mean concentration of naphthalene and flourene were 190.2 and 64.4 ng/g dw respectively, exceeding the ERL value of 160 and 19 ng/g dw. These results indicated that the probability of ecological risk associated with these PAHs was below 10% and the adverse biological toxicity effect would occur occasionally.

Polychlorinated biphenyls residues levels

Concentration of eleven PCBs congeners are presented in Table 5. The levels of different chlorinated congeners varied significantly, ranging from ND to 34.6 ng/g in tilapia (Tilapia zilli), ND to 12.8 ng/g in mullet (Mugil cephalus), ND to 14.6 ng/g in Paphia textile, ND to 25 ng/g in Venerupis pullastra, ND to 11.5 ng/ml in water and ND to 22.3 ng/g in sediment samples. The mean concentrations of total PCBs (Σ 11 PCBs) reported in this study were in the order of: sediment (49.8 ng/g)>Tilapia zilli (44.7ng/g)>>Venerupis pullastra (33.1 ng/g)>Paphia textile (29.2 ng/g)>Mugil cephalus (27.8 ng/g)>water (16.1

	***Tilapia zilli* (ng/g)**				***Mugil cephalus* (ng/g)**				***Paphia textile* (ng/g)**				***Venerupis pullastra* (ng/g)**				**Water (ng/ml)**				**Sediment (ng/g)**			
	min	max.	mean	S.D	min	max	mean	S.D	min	max.	mean	S.D	min	max	mean	S.D	min	max	mean	S.D	min	max	mean	S.D
α- HCH	12	22.1	17.1	10	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	0.14	0.32	0.23	0.1	nd	nd	-	-
β- HCH	nd	nd	-	-	nd	5.3	2.7	2.4	nd	3.6	1.8	1.6	nd	11.2	5.6	5	nd	nd	-	-	nd	nd	-	-
γ-HCH	3.5	22	12.8	9.6	nd	nd	-	-	nd	nd	-	-	2.8	44	23.4	18.9	nd	nd	-	-	11.5	66.3	38.9	23.6
Heptachlor	nd	nd	-	-	nd	nd	-	-	nd	14.7	7.4	6.6	nd	nd	-	-	0.04	0.07	0.06	0.03	nd	nd	-	-
Heptchlor epoxide	nd	nd	-	-	11.2	22.3	16.8	10	2.3	11.2	6.8	4.9	nd	nd	-	-	0.02	0.03	0.03	0.01	nd	22.3	22.3	7.9
aldrin	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	0.02	0.04	0.03	0.02	nd	11.3	11.3	4
Endrin	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	0.23	0.12	0.08	nd..	nd	-	-
Dieldrin	5	55.3	30.16	24.6	nd	nd	-	-	nd	33.8	16.9	15.1	nd	12.3	6.2	5.5	nd	nd	-	-	11.7	88.3	50	30.6
γ- chlordane	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-
p,p-DDE	2.3	22.5	12.4	9.9	5.5	33	19.3	12.8	nd	77.5	38.8	34.7	11	44	27.5	19.1	0.05	0.71	0.38	0.3	18.9	22.8	20.85	9.7
p,p-DDD	nd	nd	-	-	nd	nd	-	-	nd	23.5	11.8	10.5	nd	nd	-	-	0.14	0.21	0.18	0.08	nd	13.5	13.5	4.8
p,p-DDT	nd	3.2	1.6	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	0.01	0.07	0.04	-	nd	nd	-	-
methoxychlor	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	0.12	0.35	0.24	-	nd	nd	-	-
Σ OCPs	**22.8**	**125.1**	**74.1**	**-**	**16.7**	**60.6**	**38.8**	**-**	**2.3**	**164.3**	**83.5**	**-**	**13.8**	**111.5**	**62.7**		**0.54**	**2.03**	**1.31**	**-**	**42.1**	**224.5**	**156.8**	**-**

Table 4. *Organochlorine pesticides (OCP) residues in biota, water and sediment samples, Lake Temsah.*

Compounds	***Tilapia zilli* (ng/g)**				***Mugil cephalus* (ng/g)**				***Paphia textile* (ng/g)**				***Venerupis pullastra* (ng/g)**				**Water (ng/ml)**				**Sediment (ng/g)**			
	min	Max	mean	S.D	min	Max	mean	S.D	min	max	mean	S.D	min	max	mean	S.D	min	max	mean	S.D	min	max	mean	S.D
PCB28	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-	nd	nd	-	-
PCB52	2.7	13.2	8	5.7	nd	6.7	3.4	3	3.6	8.1	5.9	3.6	11.2	25	18.1	11	nd	nd	-	-	3.9	19.5	11.7	6.8
PCB44	3.7	13.4	8.6	5.5	11.9	12.8	12.4	6.1	nd	2.8	1.4	1.3	nd	5.6	2.8	2.5	2.3	5.7	4	2.1	nd	3.7	1.9	1.3
PCB70	nd	2.1	1.1	0.9	nd	nd	-	-	nd	8.3	4.2	3.7	nd	3.9	2	1.7	nd	nd	-	-	nd	nd	-	-
PCB101	nd	1.1	0.6	0.5	nd	1.3	0.7	0.6	nd	1.8	0.9	0.8	nd	5	2.5	2.2	nd	11.5	5.8	4.1	5.4	6	5.7	2.6
PCB152	1.2	34.7	18	15.4	nd	12.5	6.3	5.6	nd	11.9	6	5.3	nd	2.3	1.2	1	nd	2.8	1.4	1	nd	nd	-	-
PCB118	2.3	2.7	2.5	1.4	2.5	2.8	2.7	1.5	nd	6.9	3.5	3.1	nd	2.9	1.5	1.3	nd	nd	-	-	2.8	16.3	9.6	5.7
PCB105	nd	5.2	2.6	2.3	nd	nd	-	-	nd	nd	-	-	nd	5.9	3	2.6	nd	nd	-	-	3.4	6.9	5.2	2.6
PCB138	nd	2.9	1.5	1.3	nd	nd	-	-	nd	14.6	7.3	6.5	nd	nd	-	-	nd	nd	-	-	nd	22.3	11.2	7.9
PCB180	nd	2	1	0.9	nd	2.3	1.2	1	nd	nd	-	-	nd	2.5	1.3	1.1	1.4	5.3	3.4	1.9	nd	6.3	3.2	2.2
PCB192	nd	1.6	0.8	0.7	nd	2.1	1.1	0.9	nd	nd	-	-	nd	1.3	0.7	0.6	nd	2.9	1.5	1	nd	2.6	1.3	0.9
Σ PCBs	9.9	78.9	44.7	-	14.4	40.5	27.8	-	3.6	54.4	29.2	-	11.2	54.4	33.1	-	3.7	28.2	16.1	-	15.5	83.6	49.8	-

Table 5. *Polychlorinated biphenyls (PCBs) residues in biota, water and sediment samples, Lake Temsah.*

ng/ml (PCBs are very lipophilic compounds and in many studies higher lipid content is associated with higher PCBs concentration [65,66]. However, in the current study lipid levels were not associated with PCBs concentration in case of fish samples. The amount of PCBs found in the biota samples are demonstrably not correlated with the sediment PCBs content.

For benthic fauna there are three possible pathways of contaminant exposure: direct contact with sediment, organic and inorganic sediment ingestion, and contact with interstitial or overlying water [67]. According to Quensen et al. [68] and MacDonald et al. [69] differences in congener composition in the aquatic systems may be attributed to a decline in the proportion of less chlorinated PCBs that are more susceptible to losses through volatilization, sedimentation and possibly microbial degradation.

In the present study some variations in congener composition were observed between all studied samples. Generally PCBs 152, 44, 138 and 52 were the congeners that contributed more to total PCBs in the studied species, PCB28 was not detected in any of the samples, along with many other congeners, while PCB180 was detected in almost all samples except Paphia textile. PCB 180 is assumed to be the most persistent congeners due to its high chlorination level [70]. PCB 118 was the only dioxin- like PCB detected congener. The mean concentrations of PCB 118 were in the order of water: (0 ng/ml), Venerupis pullastra (1.5 ng/g), Tilapia zilli (2.5 ng/g), Mugil cephalus (2.7 ng/g) and Paphia textile (3.5 ng/g), while sediment presented a higher value of 9.6 ng/g., with a ratio not exceeding 4.5-24% of Σ11 PCBs at the different samples.

The presence and distribution of PCBs in edible fish is an important issue, not only for public health, but also from an ecological perspective. Sediment is a significant repository of environmental contaminants. Sediment analysis constitutes a tool of especial importance in aquatic ecosystem quality assessment, since sediments can reflect long-term contamination levels. Moreover, sediment is acting as reservoirs of all diversity of persistent pollutants and thus a source of potential contamination to benthic organisms [71,72]. The mean PCBs concentration (Σ11PCBs) reported for sediment in this study (49.8ng/g dry w) is much higher than PCBs concentration (Σ12 PCBs) of 2.6 ng/g dw for sediments of Montego estuary, Portugal [73].

Residues of PCBs in water samples reported in this study (Table 5) (16.1 ng/ml), are much higher than level (Σ PCB 5.2 to 190.8 ng/L) detected for surface water samples from Czech Republic [74], and water samples collected from the Houston Ship Channel in USA (0.49 to 12.49 ng/L for 209 PCB) [20], and PCB levels in surface water from Tonghui River of Beijing, China (31.58 to 344.9 ng/l) [52].

According to the USEPA guideline, the concentration of PCBs should be less than 14 ng/l for water, in order to be considered safe for aquatic and human health. Hence, the surface water of Temsah Lake is considered rather polluted by PCBs (ΣPCBs ranged from3.7 to 28.2 ng/ml) and would need the introduction of abatement measures to cut down the pollution level and improve its quality.

On the other hand, Σ 11 PCBs detected in the sediment of the lake (49.8 ng/g dry w) was comparable to sum of PCB 28, 52, 101, 118, 138, 153, and 180 that detected in the sediment of Brno reservoir, Czech Republic that ranged from <LOQ to 77.6 µg /kg dw [75].

In the present study the mean value of ∑ PCBs varied between of 27.8-44.7 ng/g wet weight, which is below the limit established for the muscle meat of fish and fishery products (75 ng/g wet weight) for Σ PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 set by European Commission [76] No. 1259/2011. Hence, there is no danger for human consuming fish and bivalves species from Lake Temsah.

Canadian sediment quality guidelines [77] can be used to assess the degree to which adverse biological effects are likely to occur as a consequence of exposure to PCBs in sediments. The present study revealed that concentrations detected in sediment samples from Temsah Lake (49.8 ng/g) exceeded the PCB threshold effect level (TEL), i.e. 21.5 ng/g, but were below PCB probable effect level (PEL), (189 ng/g.) Therefore, sediment of the Temsah Lake may be considered as a highly stressful environment, since PCB toxic effects may occur on benthic biota of the lake.

Organochlorine pesticides residues level

Thirteen OCP compounds were detected in the aquatic biota, water and sediment samples from Temsah Lake (Table 4). For biota, Σ OCPs residues ranged from 38.8 to 83.5 (ng/g). Water showed the lowest level of organochlorine pesticides residues (Σ OCPs 1.31 ng/ml), while sediment contained the highest, i.e. 156.8ng/g. This may indicate the effective removal of OCPs from water to sediments.

DDE was the dominant OCPs in biota and water samples. DDE also contributes significantly to the total OCPs (tilapia 16.7%, mullet 49.7, Paphia textile 46.5%, Venerupis pullastra 43%, and water 29%). While dieldrin was the dominant OCPs in sediment with a contribution of 31.9% of total OCPs residues. DDT was detected only in tilapia and water at concentration of 1.6 ng/g and 0.04ng/ml respectively. Methoxychlor was detected only in water with mean value of 0.24 ng/ml.

p,p-DDT was detected in water samples with concentration ranged from 0.01 ng/ml to 0.07 ng/ml and in tilapia samples with concentrations ranging from ND to 3.2 ng/g, suggesting recent input of this compound to the lake, Storm water might have carried DDTs from several sources such as agricultural lands or municipal areas which are sprayed for hygiene purposes and vector control.

DDT was widely used in Egypt on a variety of agricultural crops and for the control of disease vectors. Although its usage was banned in Egypt since 1970s, its detection along with its breakdown products, in sediments is expected because of its stability and long persistence estimated to be in the range of 10-20 years [78].

In general, the OCPs concentrations reported in biota samples in this study are far below FDA Regulatory Action Levels (Regulatory Values) of 0.3 µg/g in Fish for OCPs (Endrin, Dieldrin, Heptachlor, aldrin, heptchlor epoxide, chlordane and HCH) and 5 µg/g for total DDT in freshwater and marine fish [79].

The ratios of the parent DDT to its metabolites provide useful information on the identification of pollution source in biota, water and sediment. Concentrations reported in this study for all samples showed a common concentration sequence of DDE>DDD>DDT. In the present study, the concentration of DDE and DDD are much higher than that of DDT in all samples. This in turn would indicate a long-term biotransformation process whereby DDT is converted to DDE and DDD. Moreover, this would also indicate that there is no fresh input of DDT into the lake [80].

BAF and BSAF

The bioaccumulation factor is used to describe the process by which an organism absorbs contaminants from its environment and food [81]. Another factor frequently used to evaluate contaminant accumulation is the BSAF. Together, the BAF and BSAF represent bioaccumulation from dissolved and suspended contaminants in the water.

BAF and BSAF are commonly used as an index of the extent of bioaccumulation at a particular site because they represent the ratio of the chemical concentration in the organism to the concentration in the environmental medium (i.e., tissue residue divided by the sediment or water). In some cases like the present study, BSAF are normalized to site-specific conditions such as lipid content of the organism and the organic matter content of sediment. Organic matter content is particularly relevant in the context of accumulation of hydrophobic organic contaminants since it represents a factor directly influencing the concentration of PCBs in bottom sediments. Tomza et al. [7] reported that sediments rich in organic matter (muddy deposits) accumulated more PCBs than sandy ones. The BSAF were calculated by dividing lipid-normalized chemical concentration in the organism by that in the sediment on an organic carbon basis (mean value was used).

The organic carbon content reported in this study for sediment samples was 0.03 g/g, and the biota fat content were 0.06, 0.03, 0.02 and 0.01 (g/g) for mullet, tilapia, Venerupis pullastra and Paphia textile respectively.

Table 1 shows the bioaccumulation factor (BAF) for the contaminants that have been reported in biota and water. BAF for five PCBs congeners in fish and bivalves are present in Table 1. BAF of PCBs ranged from 0.1 to 12.9. The congener PCB152 had the highest BAF value among all studied biota. PCBs are very lipophilic and in many studies higher lipid content is associated with higher PCB concentration [65,66]. However, in the current study lipid levels were not correlated with pollutants concentration.

On the other hand out of the 14 measured PAHs, only benzo(b) fluoranthene and pyrene congeners were reported in water samples as well as in biota samples. PAHs are one of the major categories of pollutants entering the aquatic environment and finally accumulating in the sediments. The BAF reported for these PAHs congeners was the lowest in this study, ranging from 0.1 to 0.2 for fish and from 0.2 to 1.2 for bivalves. The bioaccumulation factors of PAHs in different species vary greatly [82,83]. Species that do not metabolize PAH at all or to only a limited extent, such as algae, oligochaetes and mollusks, and the more primitive invertebrates (protozoans, porifers and cnidaria) accumulate high concentrations of PAHs, as would be expected from their high log Kow values. However, organisms that metabolize PAHs such as fish and higher invertebrates, accumulate little or no PAHs (Ololade and Lajide 2010) [85]. PAHs accumulate in animals located at lower levels of the food chain because they are poorly metabolized in these species. For fish and bivalves, BAF of OCPs (23.7-560) were extremely greater than those of PCBs (0.1-12.9) and PAHs (0.1-1.2). Heptchlor epoxide that poses potential human health risks had the highest BAF within this study with a value of (560).

BSAF values of OCPs individual compounds in two fish species were less than 1, ranging from 0.33 to 0.75 while BSAF values for bivalves were higher than 1, ranging between 0.19 - 5.57. Generally BSAF reported for p,p-DDD and p,p-DDE in in bivalve Paphia textile had the highest BSAF in this study (Table 2).

The present BSAF for the sum of five OCPs reported for fish in this study are many times lower than the national-scale value reported for fish in USA (0.7-8.6 for eight different OCPs) [84] and the range reported for feral eel (Anguilla anguilla) (approximately1-70) by Van der Oost et al. [81].

BSAF values reported for PAHs were higher in bivalves than fish samples, with only three congeners (Naphthalene, Flourene and pyrene) accumulated in the studied biota BSAF for fish and bivalves were in the range of 0.16-4.47, with pyrene as the dominant accumulated congener. Eight PCBs congeners were detected in biota and sediment as well. The BSAF values for PCBs congeners ranged from 0.11 to 4.53 in fish samples. PCB105 and PCB138 were not accumulated in mullet fish. The BSAF values in the present study were lower than the fish-sediment ratios obtained from the Pearl River Estuary [85].

According to Corl, [86] bioaccumulation is rather a process that merits considerable attention because of its bearing on environmental risk assessment of chemical pollutants. Occurrence of bioaccumulative chemicals is not necessarily a proof of ecological risk, but may indicate that the need to assess the ecological evaluation of these accumulative contaminants [87-90].

Conclusions

As far as the authors are aware, the present study is the first to shed some light on the residue level of some priority pollutants beside their bioaccumulation profile in the Temsah Lake. The concentrations of all studied pollutants in biota samples, i.e. fish and bivalves were consistently lower than that detected in their surrounding sediment.

The results obtained in this study demonstrate that the levels of organic pollutants in fish taken from the Temsah Lake were relatively high, however, they were lower than the pollutants levels published in earlier studies. This in turn would reflect efforts exerted by regulatory bodies to improve the quality of Lake Ecosystem. Nevertheless, continuous efforts should be put on place in order to maintain a manageable level of these organic pollutants. By comparing the present study results with the US SQGs values, probability of ecological risk associated with these PAHs is not expected; however further attention should be paid for naphthalene and flourene congeners since their concentrations exceeded their corresponding ERLs value.

The levels of PAHs, OCPs and PCBs that reported in the present study for the Temsah lake reflects the remarkable improvements by the government and the Egyptian Environmental Affaires Agency (EEAA) to save the lake ecosystem. Some actions and legal measures toward the reduction of contaminants use have been taken to decrease the contamination load for the lake [49]. Finally the present study provides useful information to the local administration about contaminants level in lake ecosystem [90-110].

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***Correspondence to:**
Tawfic Ahmed M
Department of plant protection
Suez Canal University
Egypt
Tel: 01000149668
E-mail: motawfic@tedata.net.eg