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Study of Organic Pollutants in the Muscles of fish Collected from El-Mahmodia Stream at El-Beheira Governorate, Egypt

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Abstract

The purpose of this study is to evaluate the impact of organic pollution of EL-Mahmodia canal on the fish (*Oreochromis niloticus*) muscles tissues collected from two sites at EL-Mahmodia canal in summer and winter 2017. EL-Mahmodia canal is exposed to excessive of effluents which impact fish. The present results showed high concentrations of organic pollutants, particularly in winter. Organic pollutants were analyzed using the gas-chromatography-mass spectrometry (GC-MS). A variety of environmental screening studies concerning varieties of water pollutants in Egypt, the target of the present study was to scan the organic pollutants of El-Mahmodia stream at El-Beheira Governorate, Egypt within the muscles of Tilapia fish. Within the present study, it was found that; the analysis of fish muscles in summer season showed a high level of organic pollutants. The organic pollutants that are reported in the muscle of fish in the polluted site were reported as; Dimethomorph-(E), Hexestrol, Diisobutyl phthalate, Diamyl phthalate, Di-n-propyl phthalate, Chlorpyrifos, Phorate sulfoxide, Exaltolide [15-Pentadecanolide], Chlorfenapyr, Pyridate, Ethofumesate, Bis (2-ethylhexyl)phthalate, Dicyclohexyl phthalate, Di-n-octyl phthalate, Tricresylphosphate, meta-, XMC (3, 4-Dimethylphenyl N-methyl, XMC (3,5-Dimethylphenyl N-methyl, Hexestrol, Thymol, Kinoprene, Diisobutyl phthalate, Diisobutyl phthalate, Di-n-hexyl phthalate, Di-n-hexyl phthalate, Carbofuran-3-keto, Tefluthrin, cis-, Carbofuran-7-phenol, Carbofuran, Dicyclohexyl phthalate, Di-n-propyl phthalate, Di-n-propyl phthalate, Bis (2-ethylhexyl)phthalate, Ethofumesate, Hexestrol, Kinoprene, Di-n-hexyl phthalate, Exaltolide [15-Pentadecanolide], Spiroxamine metabolite (4-tert-b), Chlorfenapyr, Tricresylphosphate, para, Tricresylphosphate, meta-, Tricresylphosphate, ortho-, XMC (3, 5-Dimethylphenyl N-methyl, XMC (3,4-Dimethylphenyl N-methyl, Fluroxypyr-1-methylheptyl ester, Cashmeran, Propargite metabolite [Cyclohexa.], and Quinoclamine. The present results showed that Diisobutyl phthalate, Bis(2-ethylhexyl)phthalate, Pyridate and Ethofumesate were detected in winter season in the polluted site, whereas, Bis (2-ethylhexyl) phthalate, and Pyridate were the only organic pollutants that were found in winter in the reference site. The accumulation patterns of organic pollutants percentage in the polluted site in summer in the muscles of *O. niloticus* were in the following order: Chlorpyrifos> Diamyl phthalate> Diisobutylphthalate> Di-n-butylphthalate> Diamyl phthalate> Bis (2-ethylhexyl) phthalate, whereas, in the reference site in summer, it was Chlorpyrifos> Chlorfenapyr> Di-n-butylphthalate> Diisobutylphthalate> Hexestrol> Di-nhexyl phthalate. The accumulation patterns of organic pollutants in the polluted site in winter in the muscles of *O. niloticus*, were in the following order: Bis(2-ethylhexyl)phthalate> Pyridate> Ethofumesate, whereas in the reference site it was; Bis (2-ethylhexyl)phthalate> Pyridate.

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Introduction

The river Nile has been subjected to different sources of pollution that have an effect on its biological characteristics as industrial, agricultural, and municipal wastes [1, 2, 3, 4, 5, 6]. Organochlorine pesticides are washed into the aquatic system by water runoff and eroding.

Pesticides may drift throughout application and contaminate aquatic systems samples [7]. Organochlorine pesticides (OCPs) like hexa-chlorocyclohexane (HCH), dichloro-diphenyl and endosulfan are among the foremost wide applied chemicals within the world [8], Organophosphate (O, O -diethyl O -(3,5,6-trichloro-2-pyridinyl) phosphorothio-ate; CAS No. 2921-88-2; CPY) [9].

The river Nile influences the public health, social life [10].Treated domestic waste material is being reused for irrigation with [11, 12]. Industrial waste products are the second of the most sources of river pollution due to the toxicant chemicals and organic loading [13]. All major pollution sources deteriorate river water. Indication of these pollutants has been reportable by microbiological quality measurements on several sections of the stream river [14, 13]. Sixteen organochlorine pesticides were detected within the drains and to less degree in canals [15]. The environmental pollution has a direct and indirect impact on natural resources, human activities [16-18].The unregulated application of pesticides will cause adverse effects to human health and to the ecosystems [19], [2, 3, 4, 5, 6].

Chlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) were habitually utilized in agricultural and industrial functions [20]. PCBs will still be unintentional byproducts, through the combustion of wastes and different industrial processes [21]. The World Health Organization (WHO) has declared that organochlorines are found to be malignant neoplastic disease compounds in animal models [22].

Material and Methods

The Study Sites

EL-Mahmodia Canal is a 45-mile-long sub canal from the river that starts at the Nile port of EL-Mahmodia and goes through Alexandria to the sea. This study was administrated in EL-Mahmodia province, El-Beheira governorate. Samples were collected from 2 web site areas throughout summer and winter 2017 as shown in Fig. (1). The site (1); The Rosetta branch of river on the point of Alatf village that thought-about as reference area free

Figure 1. The two sites of the study area at El- Mahmoudia (El Behaira, Egypt)

from the economic activities. The site (2); EL-Mahmodia canals contaminated area wherever the EL-Mahmodia station and a factory dump directly their effluents to the stream of the canal.

Sample of Tilapia fish (genus, *Oreochromis niloticus*) were collected from the mentioned areas throughout (summer, 2017) and (winter, 2017). Fish samples were caught by fisherman, the collected fish were with a median weight (120 g). When dissecting the fish, muscle tissues were separated for estimation of organic pollution.

GC-MS Running Conditions

Extracts and recovery samples (2 µl) were analyzed using the GC-MS equipped with a Varian 8200 auto sampler. An HP-5MS capillary column (30 m X 0.53 mm i.d. 0.25 um film thickness) was used to separate the components. Helium was used as the carrier gas. Separation conditions were as the following: initial column temperature set at 80° C for 6 min. It was increased to 215° C at 15° C/min (hold for 1 min), then to 230° C at 5° C/min and finally to 290° C at 5° C/min (hold for 2 min). The carrier gas was at a constant flow rate of 1.1 ml/min. The GC-MS was controlled by a computer system, which has EI-MS libraries (PEST spectral library). The target compounds were identified by their full mass spectra scans and retention time using the total ion current as a monitor to give a Total Ion Chromatogram (TIC). The use of the full scan mode allowed the contrast of the spectrum of obtained compounds with the EI-MS library [6].

Standard Materials

Acetonitrile and hexane were HPLC-grade and were obtained from local reputed chemical distributors. Pesticides reference standards (>99% purity) were purchased from Cornell Lab Scientific, Cairo, Egypt.

Pesticide Residue QuEChERS

The QuEChERS sample preparation EN method was employed using the extraction and dispersive SPE clean-up kits (Agilent Technologies catalogue # 5982-0650 and 5982-5056, respectively). The extraction process was conducted on 10 g of each sample: 10 mL of acidified acetonitrile (0.1and 100 mL of tri phenyl phosphate (TPP) as internal standard, then shaken vigorously for 1 min and frozen for 30 min. After that 1 g Na_3Citrate di hydrate, 0.5 g Na_2HCitrate sesquihydrate, 1 g NaCl and 4 g $MgSO_4$ were added, with the tube being shaken immediately after addition of the salts and then each sample was shaken vigorously for 1 min and centrifuged for 15 min at 4000 rpm by a Benchtop centrifuge (Hettick Lab Technology, Germany) centrifuge [6].

Results and Discussion

From the cited table it was found that; the analysis of fish muscles in summer season, 2017 showed a high level of organic pollutants in both the polluted site and the reference site with a high significance in the polluted site. The organic pollutant that are reported in the muscle of fish in the polluted site were reported as; Dimethomorph-(E), Hexestrol, Diisobutyl phthalate, Diamyl phthalate, Vamidothion,

Di-n-butylphthalate, Diamyl phthalate, Di-n-propyl phthalate, Chlorpyrifos, Phorate sulfoxide, Exaltolide [15-Pentadecanolide], Chlorfenapyr, Pyridate, Ethofumesate, Bis(2-ethylhexyl)phthalate, Dicyclohexyl phthalate, Di-n-octyl phthalate, Tricresylphosphate, meta-, XMC (3,4-Dimethylphenyl N-methyl, XMC (3,5-Dimethylphenyl N-methyl, Hexestrol, Thymol, Kinoprene, Diisobutyl phthalate, Diisobutyl phthalate, Di-n-hexyl phthalate, Di-n-hexyl phthalate, Carbofuran-3-keto,

Tefluthrin, cis-, Carbofuran-7-phenol, Carbofuran, Dicyclohexyl phthalate, Di-n-propyl phthalate, Di-n-propyl phthalate, Bis(2-ethylhexyl) phthalate, Ethofumesate, Hexestrol, Kinoprene, Di-n-hexyl phthalate, Exaltolide [15-Pentadecanolide], Spiroxamine metabolite (4-tert-b), Chlorfenapyr, Tricresylphosphate, para, Tricresylphosphate, meta-, Tricresylphosphate, ortho-, XMC (3,5-Dimethylphenyl N-methyl, XMC (3,4-Dimethylphenyl N-methyl, Fluroxypyr-1-methylheptyl ester, Cashmeran, Propargite metabolite [Cyclohexa.], and Quinoclamine. Table 1.

From the above cited table; results showed that Diisobutyl phthalate, Bis(2-ethylhexyl)phthalate, Pyridate and Ethofumesate were detected in winter season organic pollutant in the polluted site, whereas, Bis (2-ethylhexyl)phthalate, and Pyridate were the only organic pollutants that were found in winter in the reference site. The authors recommend that fishing in

Table 1. The organic pollutants in fish muscles collected 2017

I. The organic pollutants in fish tissue samples collected during summer season 2017			
	Carbofuran-7-phenol	572 001563-38-8	11
	Carbofuran	125 001563-66-2	11
	Pyridate	542 055512-33-9	23
	Dicyclohexyl phthalate	493 000084-61-7	38
	Di-n-propyl phthalate	143 000131-16-8	32
	Bis(2-ethylhexyl)phthalate	499 000117-81-7	27
	Ethofumesate	249 026225-79-6	12
X3(polluted site)	Hexestrol	443 000084-16-2	38
	Kinoprene	263 042588-37-4	10
	Diisobutyl phthalate	652 000084-69-5	90
	Diamyl phthalate	364 000131-18-0	59
	Di-n-propyl phthalate	143 000131-16-8	59
	Di-n-butylphthalate	254 000084-74-2	96
	Diisobutyl phthalate	652 000084-69-5	72
	Di-n-hexyl phthalate	786 000084-75-3	64
	Chlorpyrifos	269 002921-88-2	99
	Phorate sulfoxide	686 002588-03-6	12
	Exaltolide [15-Pentadecanolide]	638 000106-02-5	18
	Spiroxamine metabolite (4-tert-b...	882 000098-53-3	10
	Pyridate	542 055512-33-9	10
	Ethofumesate	249 026225-79-6	12
	Bis(2-ethylhexyl)phthalate	499 000117-81-7	43
	Di-n-propyl phthalate	143 000131-16-8	25
	Dicyclohexyl phthalate	493 000084-61-7	17
X1(referance site)	Diisobutyl phthalate	652 000084-69-5	90
	Di-n-propyl phthalate	143 000131-16-8	59
	Di-n-butylphthalate	254 000084-74-2	56
	Diisobutyl phthalate	652 000084-69-5	72
	Diamyl phthalate	364 000131-18-0	64
	Chlorpyrifos	269 002921-88-2	95
	Exaltolide [15-Pentadecanolide]	638 000106-02-5	14
	Chlorfenapyr	764 122453-73-0	99
	Ethofumesate	249 026225-79-6	16
	Pyridate	542 055512-33-9	12
	Bis(2-ethylhexyl)phthalate	499 000117-81-7	62
	Diamyl phthalate	364 000131-18-0	43
	Di-n-propyl phthalate	143 000131-16-8	43
X2 (reference site)	XMC (3,4-Dimethylphenyl N-methyl...	589 002525-10-7	12
	XMC (3,5-Dimethylphenyl N-methyl...	594 002655-14-3	12
	XMC (3,4-Dimethylphenyl N-methyl...	589 002525-10-7	10
	Di-n-butylphthalate	254 000084-74-2	72

	Diisobutyl phthalate	652 000084-69-5	72
	Diamyl phthalate	364 000131-18-0	59
	Diisobutyl phthalate	652 000084-69-5	72
	Exaltolide [15-Pentadecanolide]	638 000106-02-5	22
	Pyridate	542 055512-33-9	17
	Carbofuran-7-phenol	572 001563-38-8	11
	Carbofuran	125 001563-66-2	10
	Ethofumesate	249 026225-79-6	12
	Bis(2-ethylhexyl)phthalate	499 000117-81-7	22
	Diethyl phthalate	72 000084-66-2	10
	Tricresylphosphate, para	853 000078-32-0	14
	Tricresylphosphate, meta-	850 000563-04-2	10
	Tricresylphosphate, ortho-	846 000078-30-8	10
X3 (reference site)	XMC (3,5-Dimethylphenyl N-methyl...	594 002655-14-3	12
	XMC (3,4-Dimethylphenyl N-methyl...	589 002525-10-7	12
	Fluroxypyr-1-methylheptyl ester	456 081406-37-3	10
	Cashmeran	585 033704-61-9	10
	Thymol	568 000089-83-8	32
	Hexestrol	443 000084-16-2	59
	Propargite metabolite [Cyclohexa...	672 999004-03-4	50
	Diisobutyl phthalate	652 000084-69-5	90
	Diamyl phthalate	364 000131-18-0	59
	Di-n-propyl phthalate	143 000131-16-8	59
	Di-n-butylphthalate	254 000084-74-2	95
	Di-n-hexyl phthalate	786 000084-75-3	59
	Pyridate	542 055512-33-9	10
	Tefluthrin, cis-	637 079538-32-2	10
	Ethofumesate	249 026225-79-6	12
	Quinoclamine	244 002797-51-5	10
	Bis(2-ethylhexyl)phthalate	499 000117-81-7	43
	Dicyclohexyl phthalate	493 000084-61-7	38
	Di-n-propyl phthalate	143 000131-16-8	16
	Tricresylphosphate, meta-	850 000563-04-2	14
	Tricresylphosphate, para	853 000078-32-0	10
II. The organic pollutants in fish collected in winter:			
X1 (polluted site)	Diisobutyl phthalate	652 000084-69-5	74
	Bis(2-ethylhexyl)phthalate	499 000117-81-7	78
	Pyridate	542 055512-33-9	25
	Ethofumesate	249 026225-79-6	12
X2 (polluted site)	No hits were retrieved		
X3(polluted site)	Pyridate	542 055512-33-9	12
X1(reference site)	Bis(2-ethylhexyl)phthalate	499 000117-81-7	78
X2(reference site)	Pyridate	542 055512-33-9	23
X3(reference site)	Pyridate	542 055512-33-9	12

winter is better than that of summer and that fishing from the reference site is preferred. Table 2 and 3.

From the above cited table it is concluded that; the order of the % of organing pollutants which were found in summer in the muscle of fish collected from the polluted site followed the order of: Chlorpyrifos> Diamyl phthalate> Diisobutylphthalate> Di-n-butylphthalate> Diamyl phthalate> Bis(2-ethylhexyl)phthalate, whereas in the reference site in summer, it was Chlorpyrifos> Chlorfenapyr> Di-n-butylphthalate> Diisobutylphthalate> Hexestrol> Di-nhexyl phthalate. The accumulation patterns of organic pollutants in the polluted site in winter in the muscles of *O. niloticus*, were in the following order: Bis(2-ethylhexyl)phthalate> Pyridate> Ethofumesate, whereas in the reference site it was; Bis(2-ethylhexyl)phthalate> Pyridate.

The present study had been undertaken so as to screen the pollutants content in fish muscles collected from EL-Mahmodia stream, El-Beheira Governorate. Egypt is that the second largest producer of Tilapia, that thought-about the foremost common fish presently being, refined commercially [23, 24]. GC–MS analysis of fish muscles collected from EL-Mahmodia stream water showed the presence of organic chemicals, fungicide, fatty acids and carboxyl acids. Most of the organic pollutants detected at the peaks in GC–MS knowledge analysis were known as endocrine disrupting phthalate esters, fatty acids, phenolic resin acids, carcinogens, and aquatic toxicants [25]. Phthalates like acid, chemical group tetradecyl organic compound, acid, octadecyl organic compound acid, chemical group 2-ethylbutyl organic compound, acid, di(2-propylpentyl) are discharged together with industrial wastewaters cause pollution and disturb the ecology of the receiving water bodies by making serious toxicity to aquatic organisms as results of bioaccumulation and therefore cause unhealthful, genotoxic effects also as disturb the inhibitor defense system [26, 27, 28], [4, 5, 6]. Phthalates are reportable to cause endocrine disruption in humans and animals upon long run exposure [29, 25].

The standard of river water worsened greatly within the past few years [12]. The main pollution sources of river and main canals are effluents from agricultural drains and treated or part treated industrial and municipal waste waters, as well as oil like monounsaturated fatty acid and wastes from traveler and watercourse boats [30, 2, 3, 4, 31, 6]. The water contains dissolved salts that washed from agricultural lands also as residues of pesticides and fertilizers, at the tip these pesticides collected in EL-Mahmodia stream water [12, 2, 3, 4, 5, 6]. In El-Mahmodia stream there is quantity of organochlorine pesticides detected within the stream water samples like Dieldrin [32], As DDD detected in stream water samples. These elevated values are beyond natural occurring and at some spots of river are higher than allowable limits for healthy water streams [33, 2, 3, 4, 5, 6]. Pollution of the aquatic surroundings by inorganic and organic chemicals may be a major issue movement a heavy threat to the survival of aquatic organisms [34].

The aquatic surroundings is subjected to varied varieties of pollutants that enter water bodies this can be a results of human activities that are associated with industrial, domestic and agricultural wastes that are drop into water bodies [16, 2, 3, 4, 5, 6]. Industrial waste water is taken into account as the most sources of river pollution owing to the unhealthful chemicals and organic loading during this waste water [35, 2, 3, 4, 5, 6]. The quantities and quality of effluent from agricultural lands are extremely variable. It contains animal wastes, plant nutrients [36]. The persistence of the organochlorine compounds and their metabolites, that are typically that are typically additional unhealthful [37, 38].

Azab *et al.* [39] reported that in summer season, organochlorines were considerably higher in water samples. The results of this study are in agreement there with of Azab *et al.* [39] as there was a high important totally different of organic pollutants within the muscles of fish that were collected in summer quite that were collected in winter.

The authors extremely suggest eating fish that were collected from El-Mahodia stream in winter quite that in summer as they are less polluted. Bioaccumulation of such compounds causes mutagenic effects to humans, animals, and aquatic

Table 2. The list of organic pollutants detected in water samples (n=3) collected from El-Mahmodia canal on Summer (Su), and Winter (W) from the two sites (polluted, and reference site) in 2017

no	Chemical	Summer season (Su)		Winter season (W)	
		Polluted site	Reference site	Polluted site	Reference site
1	Dimethomorph-(E)	D[1]	--[2]	--	--
2	Hexestrol	D	--	--	--
3	Diisobutylphthalate	D	D	D	--
4	Diamyl phthalate	D	D	--	--
5	Vamidothion	D	D	--	--
6	Di-n-butylphthalate	D	D	--	--
7	Diamyl phthalate	D	--	--	--
8	Di-n-propyl phthalate	D	D	--	--
9	Chlorpyrifos	D	D	--	--
10	Phorate sulfoxide	D	--	--	--
11	Exaltolide[15-Pentadecanolide]	D	D	--	--
12	Chlorfenapyr	D	D	--	--
13	Pyridate	D	D	D	D
14	Ethofumesate	D	D	D	--
15	Bis(2-ethylhexyl)phthalate	D	D	D	D
16	Dicyclohexyl phthalate	D	D	--	--
17	Di-n-octyl phthalate	D	--	--	--
18	Tricresylphosphate, meta	D	D	--	--
19	Tricresylphosphate, para	--	D	--	--
20	XMC(3,4-Dimethylphenyl-N methyl	D	D	--	--
21	XMC(3,5-Dimethylphenyl-N methyl	D	D	--	--
22	Hexestrol	D	D	--	--
23	Thymol	D	D	--	--
24	Kinoprene	D	--	--	--
25	Vamidothion	D	--	--	--
26	Di-nhexyl phthalate	D	D	--	--
27	Carbofuran-3-keto	D	--	--	--
28	Tefluthrin, cis	D	--	--	--
29	Carbofuran-7-phenol	D	D	--	--
30	Carbofuran	D	D	--	--
31	di-n-propyl phthalate	D	D	--	--
32	Tricresylphosphate, ortho	--	--	--	--
33	Phorate sulfoxide	D	--	--	--
34	Spiroxamine metabolite(4-tert-b)	D	--	--	--
35	Diethyl phthate	--	D	--	--
36	Fluroxypyr-1-methylheptyl ester	--	D	--	--
37	Cashmeran	--	D	--	--
38	Propargite metabolite[cyclohexa]	--	D	--	--
39	Teflothrin, cis	--	D	--	--
40	Quinoclamine	--	D	--	--

1Detected and 2Not detected

Table 3. The mean % values of organic pollutants in the fish muscles collected from El-Mahmoudia 2017

no	Chemicals	Summer, 2017		Winter, 2017	
		Polluted site	Reference site	Polluted site	Reference site
1	Dimethomorph-(E)	11.0	--	--	--
2	Hexestrol	17.0	--	--	--
3	Diisobutylphthalate	76.2	79.2	74.0	--
4	Diamyl phthalate	50.5	60.6	--	--
5	Vamidothion	10	43	--	--
6	Di-n-butylphthalate	63.0	81.0	--	--
7	Diamyl phthalate	78.0	--	--	--
8	Di-n-propyl phthalate	35.2	53.6	--	--
9	Chlorpyrifos	97.6	95.0	--	--
10	Phorate sulfoxide	12	--	--	--
11	Exaltolide[15-Pentadecanolide]	14	18	--	--
12	Chlorfenapyr	38	89	--	--
13	Pyridate	14.3	13	18.5	17.5
14	Ethofumesate	13.6	13.3	12	--
15	Bis(2-ethylhexyl)phthalate	51	42	78	78
16	Dicyclohexyl phthalate	38	38	--	--
17	Di-n-octyl phthalate	53	--	--	--
18	Tricresylphosphate, meta	10	12	--	--
19	Tricresylphosphate, para	--	12	--	--
20	XMC(3,4-Dimethylphenyl-N methyl	12	14.5	--	--
21	XMC(3,5-Dimethylphenyl-N methyl	10	12	--	--
22	Hexestrol	40	59	--	--
23	Thymol	28	32	--	--
24	Kinoprene	20	--	--	--
25	Vamidothion	10	--	--	--
26	Di-nhexyl phthalate		59	--	--
27	Carbofuran-3-keto	17	--	--	--
28	Tefluthrin, cis	14	--	--	--
29	Carbofuran-7-phenol	11	11	--	--
30	Carbofuran	11	10	--	--
31	di-n-propyl phthalate		16	--	--
32	Tricresylphosphate, ortho	--	10	--	--
33	Phorate sulfoxide		--	--	--
34	Spiroxamine metabolite(4-tert-b)	10	--	--	--
35	Diethyl phthate	--	10	--	--
36	Fluroxypyr-1-methylheptyl ester	--	10	--	--
37	Cashmeran	--	10	--	--
38	Propargite metabolite[cyclohexa]	--	50	--	--
39	Teflothrin, cis	--	10	--	--
40	Quinoclamine	--	10	--	--

organisms [40, 41, 42, 26, 27, 28]. The 2, 6-dihydroxybenzoic acids, those were detected within the water samples, are the key metabolites of the polyaromatic hydrocarbons throughout effluent treatment [43, 44, 29, 45].

Conclusion

In EL-Behira Governorate, pesticides are used along a large scale; organochlorine and organophosphate are persistent pesticides which leave residues in drinking water that remain for days to many years. Organophosphates are found in high rate in the stream, Chloropyrifos as an Organophosphates pesticides was found in the stream water. The current study revealed that the water quality of El-Mahmodia canal is contaminated with elevated levels of organic pollutants: pesticides, plasticizers, plant residues compared to standard safety permissible guidelines.

Conflict of Interest

The authors declare no conflict of interest

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