Monitoring of Pesticide Residues in Water and Fish (Nile Tilapia) samples from El-Bahr El-Pharony Drain in Menoufia, Using QuEChERS Technique

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Abstract: The extent of pyrethroid and carbamate pesticides contamination in El-Bahr El-Pharony drain which is situated in Menoufia governorate, Nile Delta was investigated for the period from January 2015 to November 2015. The sample cruises included four sites (El-Khadra (KHD), Kafr-Fesha (KRF), Hozet-Menuf (HZM) and Shanshour (SNR)) alongside El-Bahr El-Pharony drain. To determine the levels of pesticide residues in selected sites, water and Nile Tilapia fish samples were collected from each location every three months starting from January 2015. Water and fish samples were processed for extraction and cleaning-up using QuEChERS –technique followed by Gas Chromatography with electron capture detector (GC-ECD) and high-performed liquid chromatography with UV diode array detection (HPLC-DAD) for detection of pyrethroid and carbamate residues in environmental waters, respectively. Validation of the analytical procedure indicated that the average recoveries for identified pyrethroid and carbamate pesticides ranged from 83.11 to 93.96% and from 86.19 to 98.1%, respectively. The detected pyrethroids found in Tilapia fish samples from El-Bahr El-Pharony drain were cypermethrin and lambda-cyhalothrin. Cypermethrin was detected in fish samples from all selected areas of El-Bahr El-Pharony except SNR area, whereas lambda-cyhalothrin was not detected in fish samples from KHD area. Among the analyzed carbamate pesticides, carbofuran residues (0.3- 1.06 mg/kg) were found in all fish samples from the selected sites. In general, it was obvious that the concentrations of both pyrethroid and carbamate pesticides detected in water samples from the study area were lower than these in fish samples. KRF and HZM sites seemed to be the most contaminated among the selected sites. It was also found that all detected pesticides were at lower levels than their corresponding maximum residue level (MRLs) values in all fish samples from the four selected sites around El-Bahr El-Pharony drain, and this could be an important source of pesticide transfer to humans via fish consumption. The preliminary risk assessment indicated also that the estimated health risk index (HRI) values for all detected pesticides were far lower than 1.0, thus indicating that the risk of pesticide intake for humans through fish consumption was relatively low and does not present an immediate risk. The highest health indices were found for carbofuran, 0.883, 0.667 and 0.563 in KRF, HZM and SNR areas, respectively, whereas the lowest indices were found for cypermethrin, 0.056, 0.033, and 0.033 mg/kg/day, in KHD, KRF and HZM, respectively. Therefore, regulatory and awareness means are needed to control the use and possible seepage of these pesticides into the freshwater systems. Such efforts may help us protect the quality of fish as a food for human beings of this and other regions for the foreseeable future.

Keywords: El-Bahr El-Pharony drain, Fish (*Oreochromis niloticus*), Pyrethroids, Carbamate pesticides, QuEChERS, GC and HPLC, Health risk.

1. Introduction

Contamination of surface waters by agricultural pesticides and fertilizers, as well as by industrial metals, is a cause of increasing public concern. It is well known that residues of persistent pesticides, especially those of organochloines are found in terrestrial and aquatic environments as well as in the organisms occupying these environments. Most conventional pesticides are highly lipid soluble and lengthy exposure to them results in their high accumulation in non-target organisms, all contributing to adverse effects on the ecosystem **(Miyamoto *et al.*, 1990)**. Fish are known to accumulate these pollutants directly from the polluted water and indirectly from the food chain **(Mohamed 2009; Chaudhry and Jabeen 2011)**. Like many other developing countries, pesticides are used extensively in Egypt to increase the crop yield. Due to the widespread use of pesticides, their residues are detected in various environmental matrices, like soil, water and air. Pesticide contaminations of surface waters have been well documented worldwide and cause a major issue that gives rise to concerns at local, regional, national and global scales due to the adverse effects of pesticide on the environment **(Planas *et al.* 1997; USGS 1999; Huber *et al.* 2000; Cerejeira *et al.* 2003)**. Pesticide residues reach the water body through direct runoff, leaching, careless disposal of empty containers, equipment washing etc. The amount of pesticides lost from agricultural fields and transported to surface waters depends on several factors, including soil characteristics, topography, weather, agricultural practices, and physico-chemical properties of

individual pesticides **(Wagenet 1987; Leonard 1990)**. Recently organophosphates are replaced by pyrethroid pesticides such as bifenthrin, cypermethrin, cyfluthrin, deltamethrin and lambda cyhalothrin **(Amweg *et al.* 2006)**. These are commonly used in landscape maintenance, structural pest control and residential gardens and homes. These pyrethroids are more toxic to mammals and birds at low than the high temperature and these are over 100 times more toxic for fish due to not only their high sensitivity to toxic agents via gills but also insufficient hydrolytic enzymes for pyrethroids in fish **(Aydin *et al.* 2005)**. Fish components can be used for environmental monitoring because they can accumulate the contaminants directly from diet and water **(Chaudhry and Jabeen 2011; Kafilzadeh *et al.* 2012)**.

In Egypt, the pollution of River Nile system (main stem Nile, drains and canals) has increased in the past few decades because of increases in population; several new irrigated agriculture projects and other activities along the Nile **(APRP, 2002)**. El-Bahr El-Pherony is an important watershed and is a crucial source of irrigation water. It extends for more 20 km long, 100-300 m width, 3-7 m depth with total area of 2,500 feddans, it pours into Damietta Branch of the River Nile, at Kattamiya Village. It considers one of the most important sources of fisheries in Menoufia Governorate in which the annual fish production of El-Bahr El-Pharaony drain is about 2675 ton and the common fish species are carp **(ESIAF, 2010)**. This drain receives untreated domestic sewage from numerous towns and villages in addition to the agricultural wastes (salts, nutrient and pesticides) which had a significant impact on ambient water quality and the lack of fisheries **(GAFRD, 2011)**. However, contamination of El-Bahr El-Pharony water by pesticides has not been received an enough attention. Egypt is one of the largest producers of tilapia, with a production estimated at 600,000 MT in 2011 **(Tveteras, 2013)**. Nile tilapia, *Oreochromis niloticus*, is a very popular and tasty fish in Egypt. *O. niloticus* is native to Central and North Africa and the Middle East **(Boyd, 2004)**. It is a tropical freshwater and brackish water species. It prefers shallow, still waters on the edge of lakes and wide rivers with sufficient vegetation **(Picker and Griffiths, 2011)**. It is grown in agriculture drainage water than those grown in irrigation water.

A little work has been done to determine concentrations of pesticide residues in the muscles of Nile tilapia fish. There were wide difference in the number of the detected pesticides between pond water samples and that detected in the musculature tissue which reached to 6 and 18 pesticides respectively **(Abd El-Gawad *et al.*, 2012)**. This difference may be due to microbial degradation which analyzes organic pesticides as part of their food or mineralization of pesticides to carbon dioxide, ammonia, water and inorganic salts **(Muller and Korte, 1975)** or Photodegradation by (thermophilic temperatures, oxygen and hydrogen peroxide) that accelerate pesticides degradation **(Muszkat *et al.*, 2002)**. In addition, high density of phytoplankton in water could absorb a high quantity of most pesticides in the water **(El-Nemaki *et al.*, 2008)**. **Osman *et al.* (2010)** indicated that the concentrations of 13 organochlorine pesticides in water, sediment and fish tissues of two tilapia species (*Tilapia zillii* and *Oreochromis niloticus*) were present in non-detectable amounts except for *p, p*-dichlorodiphenyldichloroethylene was found in one sample (0.034 µg g-1) at inlet. Recently, water quality assessment in the North eastern part of Qarun Lake was investigated by **Ibrahim and Ramzy (2013)**. This study indicated that very low concentrations of organophosphorus and organochlorine pesticides were detected in the muscles, liver and brain of *Tilapia Zilli* and water samples. The concentrations of α-BHC, γ-BHC, hepta-epoxide, cadusaphos, Di-Syston, pirimiphos, fenitrothion, and profenofos in liver are depending on their concentration in water samples **(Ibrahim and Ramzy, 2013)**.

The main aim of this study was to determine the presence of pyrethroid and carbamate pesticide residues in *O. niloticus* fish and water samples collected from four regions of El-Bahr El-Pharony drain. Such determination of the chemical quality of freshwater fish is needed to avoid the possible risks to their meat quality and ultimately human health. This study may be helpful in determining the extent of accumulation of these compounds in the aquatic biota to help understand the behaviour and fate of these chemicals in fish specimens. This study, therefore, strives to provide crucial information on the levels of pyrethroid and carbamate residues in fish, and water for the first time from this study area around El-Bahr El-Pharony drain. Such information about pesticide residues should facilitate the scientific assessment of the impact of pesticides on fish composition, public health, agriculture and the environment in this area and beyond.

2. Materials and Methods

2.1. Site Selection

The study covered four sites around El-Bahr El-Pharony in Menoufia governorate called: El-Khadra (KHD), Kafr-Fesha (KRF), Hozet-Menouf (HZM) and Shanshour (SNR). These sites were receiving different types and amounts of pollutants from adjacent areas due to varying agricultural practices in the neighbourhood. The neighbouring farmers are regularly using conventional pesticides like pyrethroids, organophosphates and carbamates on field crops (cotton, maize and legumes) plus vegetables to control insects and other pests in order to get better crop production in the study areas. Therefore, it was logical to determine the presence of some pyrethroids (cypermethrin, lambda-cyhalothrin), carbamates (aldicarb, carbofuran, ethofumesate, fenoxycarb, kresoxim-methyl) in water and Nile tilapia fish from selected sites.

2.2. Sampling of Water

Representative samples of about 1-L water were collected in glass bottles that were washed with distilled water and then with the drain water from the sampling sites. Four water samples from each sampling site were collected every three months starting from January 2015 to December 2015. Water samples were collected at midday as three replications from each selected site. Water samples were collected at two levels of the water surface: surface-water samples (at 0-5 cm depth) and subsurface samples (at 100-cm depth). Bottles were labeled and stored in icebox until transported to the Laboratory of Pesticide Analysis, Central Agricultural Pesticides Laboratory, Agricultural Research Center. The water samples were

filtered to remove sand and debris and then stored at +4º C prior to analysis.

A brief survey was also conducted around the selected sites of the samples collection for gathering information on the types of pesticide commonly used to verify the findings and also to determine if there was any unauthorized or authorized pesticide use by the farmers in these areas.

2.3. Sampling of Fish

Samples of the Nile Tilapia, *O. niloticus,* were collected concomitantly (around 2 kg) from each selected site with the samples of water. The fishing was randomly performed by using conventional gill nets in synchronization with the local fishermen. The total fish catches were harvested from three nets per site and transferred to large buckets filled with the drain water. The fish were sacrificed and transported to the research laboratory where each fish was dissected and muscles from mid dorsal side were stored at -20 °C until their use for chromatography analysis.

2.4. Chemicals Used

The main solvents used in the QuEChERS extraction procedure were analytical grade of acetone, methanol (Merck, Germany) and HPLC grade acetonitrile (Scharlau, Barcelona, Spain). Anhydrous magnesium sulfate, anhydrous sodium acetate (BDH, Poole, England), sodium chloride, trisodium citrate 5.5-hydrate, acetic acid were purchased from Sigma–Aldrich, Germany. Cypermethrin, lambda-cyhalothrin, aldicarb, carbofuran, ethofumesate, fenoxycarb, and kresoxim-methyl were of reference grade pesticide standards with purity from 98.5 to 99.5%.

2.5. QuEChERS Sample Extraction and Clean-Up Procedure

2.5.1. Water Sample Processing:

Extraction of samples for the analysis was according to the AOAC International Official method 2007.01 **(Lehotay, 2007)**. The solvent used for the analysis was 1% acetic acid in acetonitrile. 10 mL water sample was placed in 15 mL centrifuge tube and 2.5 mL solvent was added. 6 g anhydrous magnesium sulphate and 1.5 g anhydrous sodium acetate were added into the extraction tube. The tube was then capped and shaken immediately and vigorously for one minute. After vortex the tube was centrifuged for five minutes at 3000 rpm for phase separation. Using the extracts resulting from the phase separation, 1 ml of the supernatant was transferred into a 2 ml polypropylene tube containing pre-weighed dSPE sorbents: 50 mg primary secondary amine (PSA), 150 mg magnesium sulphate (MgSO4) and 50 mg graphitized carbon black (GCB).Each tube was labelled, capped, shaken vigorously for 2 minutes and then centrifuged for 5 minutes at 3000 rpm to separate the solid materials. Extracts were carefully transferred to amber coloured vials and kept in the refrigerator prior to chromatography analysis.

2.5.2. Fish Sample Processing:

The QuEChERS method **(Anastassiades *et al.*, 2003)** was used for the sample preparation of fish samples. Five grams of fish was transferred to a polypropylene tube of 50 mL (tube 1). Afterwards, 15 mL of acetonitrile acidified with 1% (v/v) of acetic acid was added, and the samples were manually shaken vigorously for 1 min. Next, the extract was centrifuged at 6900 g for 5 min. After that, an aliquot of 1 mL of the supernatant was transferred to a polypropylene tube of 2 mL (tube 2). The extract was shaken manually for 30 s and centrifuged for 5 min at 6900 g. In the end, 100 µL of the supernatant was transferred to a vial and became filled with 900 µL of mobile phase. The sample was filtered in a 0.20 µm Teflon filter into a vial for chromatography analysis.

2.6. Analysis of Pesticide Residues

2.6.1. GC Analysis:

The pyrethroid pesticides were analyzed on Hewlett Packard (HP) serial 6890, Gas Chromatograph, equipped with electron capture detector (GC-ECD). GC analysis was conducted on a HP-5 MS capillary column of 30 m length, 0.25 mm column ID, and 0.25 µm film thicknesses. The oven temperature was programmed from an initial temperature 80 ºC for 1 min, then increasing at 30 ºC min^{-1} up to 160 (2 min hold) then increasing to 260 ºC at a rate of 3 ºC min^{-1} and was maintained at 260 ºC for 12 min. Injector and detector temperature were maintained at 300 and 320 ºC, respectively. Nitrogen was used as a carrier at flow rate of 3 ml min^{-1}. 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.

2.6.2. HPLC Analysis:

The HPLC analyses were carried out with Agilent 1100 system, consisting of a degasser, binary pump, auto sampler, column oven, UV- DAD and a fluorescence detector. The chromatographic separation was performed with the Zorbax EclipsePlus C18 (3.5 µm, 3.6 mm x 150 mm) chromatographic column. Carbamate residues, the mobile phase was demonized water containing 0.1% formic acid (mobile phase component A) and acetonitrile (component B) were employed for the gradient program, which started with 20% B for 3 min and was linearly increased to 100% B in 27 min (held for 3 min). The column was then re-equilibrated for 12 min back to 20% B. Thus, the total run time took 45 min. The flow rate was constant at 0.6 mL/min, and injection volume was 10 µL.

2.6.3. Validation of the Analytical Procedure:

The evaluated accuracy through the recoveries essay of pesticides was identified using the water and fish samples spiked with the analytes at three concentration levels: 0.05, 0.01 and 0.001 mg L^{-1} for water and 0.05, 0.01 and 0.001 mg kg^{-1} for fish. The validation of the proposed analytical method (GC-ECD) and (HPLC-DAD) were carried out according to **SANCO (2009)**. Linearity was evaluated by constructing matrix matched calibration curves in the range of 0.1–20 µg L^{-1} for (GC-ECD) and (HPLC-DAD). Fortified samples were extracted as described earlier and the average recovery percentages for fortified samples were determined. Limits of detection (LOD) and quantification (LOQ) were evaluated as the pesticide concentration that produces a peak signal-to-noise ratio of 3:1 and 10:1, respectively (**Table (1)**).

2.7. Health Risk Index (HRI) Assessment

To assess the risk of pesticide intake through fish meat consumption by the local population, it was assumed that one adult person would eat 100 g of fish per day. By multiplying the fish intake with the pesticide residue detected in micrograms per Kg of muscle yielded the estimated daily intake (EDI) per person for each pesticide in micrograms. Then EDI value was normalized for an average human weighing 60 kg **(WHO 1997)** to give intake values in micrograms per kilogram of body weight. Health risk index (HRI) was calculated using the equation:

Health risk Index (HRI) = EDI/ADI **(EFSA, 2007)**. An index more than 1 is considered as not safe for human health **(Darko and Akoto, 2008)**. The ADI values as defined by the World Health Organization **(WHO, 2004)** for lambda-cyhalothrin, cypermethrin, aldicarb, carbofuran and kresoxim-methyl pesticides are 2.5, 15, 3.0, 2.0 and 400 µg/kg/day, respectively.

3. Results

3.1. Validation of the Analysis Method for Water and Fish Samples

To evaluate the performance of analysis techniques of the selected pesticides in both water and fish samples, the method accuracy, precision, limits of detection (LOD) and quantification (LOQ) were measured. Average recoveries and standard deviations (SD) obtained for both water and fish muscle samples are presented in **Table (1)**. The recoveries obtained for water and fish samples were between 78.49-98.11% and 86.2-98.1%, and the relative standard deviation (RSD) values ranged from 11-18 and from 9-17, respectively. The detection limits were 0.003-0.01 mg/L and 0.003-0.05 mg/kg for spiked water and fish samples, respectively.

3.2. Pesticide Residues in El-Bahr El-Pharony Water

The concentrations range and mean values of pyrethroid and carbamate pesticide residues found in the analysed samples of water collected from surface and sub-surface of El-Bahr El-Pharoney drain are given in **Table (2)**. The results showed that cypermethrin residues (0.01 ~ 0.02 mg/L) were found in all water samples collected from selected sites. Water samples from selected sites were contaminated with cypermethrin by 25-50 % of collected samples. Lambda-cyhalothrin residues (0.02- 0.25 mg/L) were found only in samples from HZM and SNR areas. Among carbamate pesticides, aldicarb was detected in water samples from all sites at 0.01 mg/L. Samples were contaminated with aldicarb by 50-75 %. Carbofuran residues were also detected in water samples from three areas (KRF, HZM and SNR) at concentrations of 0.01 ~ 0.015 mg/L. Samples collected from both KRF and HZM areas were carbofuran-contaminated by 100%. The herbicide, ethofumesate was also detected in water samples from three areas (KHD, KRF and HZM) at concentrations 0.01 ~ 0.025 mg/L. Water samples from these areas were ethofumesate-contaminated with 50%. Residues for another herbicide, kresoxim-methyl, were detected in samples from only KHD (0.011 mg/L) and SNR (0.012 mg/L); whereas fenoxycarb was detected only in samples from HZM area (0.03 mg/L).

Table (1). Average percentage recoveries and detection limits for identified pyrethroid and carbamate pesticides in water and fish samples using HPLC-DAD and GC-ECD analysis methods.[*]

Pesticides	[a]Limits of detection (LOD)	[a]Limits of quantification (LOQ)	Relative standard deviation %	r^2	Average recovery %
		Water samples			
Cypermethrin	0.001	0.003	12	0.995	83.11
Lambda-cyhalothrin	0.001	0.003	17	0.998	92.92
Aldicarb	0.001	0.003	17	0.992	89.26
Carbofurn	0.010	0.030	12	0.994	91.24
Ethofumesate	0.020	0.050	11	0.994	95.24
Fenoxycarb	0.003	0.010	14	0.994	98.11
Kresoxim-methyl	0.001	0.003	18	0.991	78.49
		Fish samples			
Cypermethrin	0.001	0.003	11	0.998	86.19
Lambda-cyhalothrin	0.001	0.003	12	0.995	93.96
Aldicarb	0.001	0.003	14	0.992	87.32
Carbofurn	0.010	0.030	9	0.994	98.10
Ethofumesate	0.020	0.050	11	0.994	90.12
Fenoxycarb	0.003	0.010	17	0.994	86.20
Kresoxim-methyl	0.001	0.003	12	0.991	88.12

[*]HPLC-DAD refers to High Lquid *chromatography* with *Diode-Array Detector* used for analysis of selected pyrethroid pesticides; GC-ECD refers to *Gas Chromatography* with *Electron-Capture Detector used for analysis of selected carbamate pesticides.*
[a] LOD / LOQ values expressed as mg/L and mg/kg for water and fish muscle samples, respectively.

Table (2). Residue concentrations of identified pyrethroid and carbamate pesticide residues in water samples from selected sites of El-Bahr El-Pharony drain.

Pesticides	Mean concentrations ± SE (mg/L)[a]				Contaminated samples % (n)
	Surface water samples (0-5 cm depth)		Subsurface water samples (100-cm depth)		
	Mean Conc. (mg/L)	Conc. Range (mg/L)	Mean Conc. (mg/L)	Conc. Range (mg/L)	
			El-Khadra (KHD)		
Lambda-cyhalothrin	BDL[b]	-	BDL	-	-
Cypermethrin	0.02 ±0	BDL - 0.02	0.03±0	BDL - 0.03	25 (2)
Aldicarb	0.01 ±0	BDL - 0.01	0.01±0	BDL - 0.01	75 (6)
Carbofuran	BDL	-	BDL	-	-
Ethofumesate	0.025±0.007	0.02- 0.03	0.026±0.008	0.02- 0.032	50 (4)
Fenoxycarb	BDL	-	BDL	-	-
Kresoxim-methyl	0.011±0.0007	0.01- 0.011	0.013±0.0007	0.012-0.013	50 (4)
			Kafr-Fesha (KRF)		
Lambda-cyhalothrin	BDL	-	BDL	-	-
Cypermethrin	0.018±0.007	0.012-0.023	0.02±0.007	0.014-0.025	50 (4)
Aldicarb	0.01±0	BDL - 0.01	0.01±0	BDL - 0.01	75 (6)
Carbofuran	0.01±0	BDL - 0.01	0.01±0	BDL - 0.01	100 (8)
Ethofumesate	0.015±0.007	0.01- 0.02	0.018±0.008	0.012- 0.024	50 (4)
Fenoxycarb	BDL	-	BDL	-	-
Kresoxim-methyl	BDL	-	BDL	-	-
			Hozet-Menouf (HZM)		
Lambda-cyhalothrin	0.025±0.007	0.02-0.03	0.028±0.006	0.023-0.032	50 (4)
Cypermethrin	0.01±0	BDL - 0.01	0.015±0	BDL - 0.015	25 (2)
Aldicarb	0.01±0	BDL - 0.01	0.012±0.002	0.01-0.013	50 (4)
Carbofuran	0.015±0.003	0.012-0.02	0.018±0.002	0.015-0.021	100 (8)
Ethofumesate	0.01±0	BDL - 0.01	0.01±0	BDL - 0.01	50 (4)
Fenoxycarb	0.03±0	BDL - 0.03	0.034±0.001	0.033-0.035	50 (4)
Kresoxim-methyl	BDL	-	BDL	-	-
			Shanshour (SNR)		
Lambda-cyhalothrin	0.02±0	BDL - 0.02	0.021±0	BDL - 0.021	25 (2)
Cypermethrin	0.01±0	BDL - 0.01	0.012±0	BDL - 0.012	25 (2)
Aldicarb	0.01±0	BDL - 0.01	0.011±0.001	BDL - 0.012	50 (4)
Carbofuran	0.013±0.002	0.011-0.014	0.015±0.0007	0.014-0.015	50 (4)
Ethofumesate	BDL	-	BDL	-	-
Fenoxycarb	BDL	-	BDL	-	-
Kresoxim-methyl	0.012±0.002	0.01-0.014	0.013±0.002	0.011-0.015	50 (4)

[a]Mean values of two sets (water surface and sub-surface) of four samples each collected through the period from January 2015 to December 2015. [b]BDL= Below Detection Limit.

3.3.Pesticide Residues in the Fish Tissues

The residue levels of identified pyrethroid and carbamate pesticides found in fish tissues and their maximum residue limits (MRLs) are presented in **Table (3).**The data showed that mean concentrations of pesticides found in muscles of fish were higher than these found in water samples. Cypermethrin was detected in muscles of fish samples from KHD (0.5 mg/kg), KRF (0.3 mg/kg) and HZM (0.3 mg/kg) sites. Cypermethrin was detected in only one sample (25%) from KRF and HZM. Lambda-cyhalothrin was also detected in fish from KRF (0.5 mg/kg), HZM (0.65 mg/kg) and SNR (0.4 mg/kg). It was detected in only one sample (25%) from KRF and SNR sites.

For the identified carbamates, carbofuran residues were detected in all fish samples from the four selected sites (**Table (3)**). It was realized at higher concentrations in fish samples from KRF (1.06 mg/kg) and HZM (0.8 mg/kg) than those found in samples from the other areas. Also, aldicarb was found at the concentrations of 0.78 and 0.73 mg/kg in fish samples from KHD and KRF, respectively. All fish samples obtained from KHD area were

contaminated (100%) with aldicarb. Residues of kresoxim-methyl were only found in samples from KHD area (0.3 mg/kg), where two samples (50%) were contaminated.

Pesticide residues of the present study were compared with MRL (**Table (3**)) established by **European Union (2012)** and **Codex Alimentarius Commission (2004)**. It was found that the detected pesticide residues were higher than MRL values in all fish samples from selected areas of El-Bahr El-Pharony drain.

3.4.Daily Intake and Health Risk Assessment Based On Residues in Fish Tissues

Estimated the health risk index (**HRI**) were obtained by dividing the estimated daily intake (**EDI**) (mg/kg body weight/day) by their corresponding values of acceptable daily intakes (**ADI**) for agricultural and veterinary chemicals **(Australian Government, 2005; WHO, 2012)**. **Table (4)** expresses the estimated daily intake values of the residues and their corresponding health risk index in the muscles of Tilapia fish. The results showed that the highest health indices were found for carbofuran (0.883, 0.667, and 0.563 mg/kg/day) in fish from KRF, HZM and SNR sites, respectively. Data also showed that estimated health risk indices for aldicarb were 0.433 and 0.406 in fish from KHD and KRF sites, respectively. For cypermethrin, it was obvious that it had the lowest health indices' values, 0.056, 0.033 and 033, in fish from KHD, KRF and HZM sites, respectively. Lambda-cyhalothrin had also low health hazard indices, 0.167, 0.217 and 0.133, in fish from KRF, HZM and SNR sites, respectively. It was obvious that all hazard indices for detected pesticides in fish muscles were less than 1.0 and belonged to no risk class (**Table (4**)). However, the main health risk may be posed by carbofuran followed by aldicarb where HRI values were close to 1.0; while rest of the pesticide residues were still far lower than 1.0 indicating that risk of detected pesticides intake for human through fish consumption was relative low.

4.Discussion

The current study is the first attempt to investigate the patterns of occurrence and distribution of synthetic pyrethroid and carbamate pesticide residues in water and Tilapia fish samples from El-Bahr El-Pharony drain, Menoufia and also to identify the possible risk of pesticide intake for humans through fish consumption. El-Bahr El-Pharony drain is considering an important watershed and crucial source of irrigation water, and so important source of fisheries in Menoufia governorate as well. This drain is surrounded by agricultural lands. A large amount of fertilizers and pesticides are used by agricultural farmers which can enter the drain through running waters and tributary canals. Also, garbage and untreated wastewaters are discharged into the river by neighbouring inhabitants. All these factors may lead to the contamination of this drain. No studies for determining the extent of pesticides or other agrochemicals contamination in El-Bahr El-Pharony drain have been done or/and published. However, there are some attempts to evaluate the levels of some environmental pollutants in El-Bahr El-Pharony drain. **Ghannam *et al.* (2014)** mentioned that water of this drain was contaminated by various kinds of heavy metals as a result of excessive use of fertilizers and pesticides applied in the surrounding village along the drain. Three heavy metals Cu, Pb, and Zn were found to be higher than the permissible limits.

The current study revealed that pesticide residue levels of the identified pyrethroid and carbamate pesticides in the fish samples were below the maximum residue limits (MRLs) at the four polluted sites of El-Bahr El-Pharony drain. The preliminary risk assessment indicated also that the estimated health risk index (HRI) values for all detected pesticides were far lower than 1.0, thus indicating that the risk of pesticide intake for humans through fish consumption was relatively low. However, carbofuran seemed to be the most dominated among the identified pesticides followed by cypermethrin. In general, KRF and KHD sites seemed to be the most contaminated among the selected sites. Data also showed that mean concentrations of pesticides found in muscles of fish were higher than these found in water samples collected from four variably polluted sites (KRF, HZM, KHD, and SNR). This may be because these pesticides were less soluble in water due to their hydrophobic nature that made their presence in water to be at very low level and so their precise determination was difficult. The adsorption of these compound to sediments and other particulate matter is an important mechanism for their removal from the water column and consequently the sediment component of aquatic ecosystems can be the ultimate sink of these pesticides **(Jabeen *et al.*, 2015)**. However, these pesticides can accumulate in fish tissues due to their lipophilic nature when they are discharged into water bodies. High concentration of pesticides in fish samples could be due to the fact that fish are mobile so the fish may have been exposed to compounds in their parts of the hydrologic system and also due to the presence of fat content in fish tissues, which is in good agreement with the findings of **Upadhi and Wokoma (2012)**. Six pesticide residues (endosulfan, carbofuran, cypermethrin, profenophos, triazophos and deltamethrin) were detected in muscle tissues of *Cyprinus carpio* fish samples collected from Head Balloki (BH) in the River Ravi **(Mahboob *et al.*, 2013)**. The endosulfan and profenofos were the most abundant pesticides recorded in the fish tissue. Profenofos and cypermethrin were dominant pesticides recorded in the water samples from the River Ravi at BH. The maximum and minimum concentration of cypermethrin in muscles o*f C. carpio* was observed as 0.52 and 0.21 mg kg^{-1}, respectively. The presence of carbofuran residues in all fish samples of *C. carpi* was observed and the highest concentration detected was 8.53 mg kg^{-1}) **(Mahboob *et al.*, 2013)**. In a recent study, **Jabeen *et al.* (2015)** examined the presence of pyrethroids, carbamates and neonicotinoids in fish for potential health risks to the consumers. Three pesticides (deltamethrin, carbofuran and cypermethrin) were detected in fish and sediment samples Deltamethrin in *Cyprinus carpio* ranged from 0.490-0.839 µg/g, mostly exceeding 0.5 µg/g as MRL suggested by FAO-WHO. This is worrying because these pesticides may pose health risks for the fish and people of the study area. However a preliminary risk assessment indicated that the calculated

Table (3). Residue concentrations of identified pyrethroid and carbamate pesticide residues in muscles of Tilapia fish samples from selected sites of El-Bahr El-Pharony drain.

Pesticides	Mean[a] ± SE concentration (mg/kg)	Concentration range (mg/kg)	Contaminated samples % (n)	MRLs[b] (mg/kg)
		El-Khadra (KHD)		
Lambda-cyhalothrin	BDL[c]	-	-	0.02
Cypermethrin	0.5±0.14	0.4 – 0.6	50 (2)	0.05
Aldicarb	0.78±0.20	0.6 – 1.0	100 (4)	0.01
Carbofuran	0.3±0.0	BDL - 0.3	25 (1)	0.02
Kresoxim-methyl	0.3±0.28	0.1 – 0.5	50 (2)	0.01
		Kafr-Fesha (KRF)		
Lambda-cyhalothrin	0.5±0.0	BDL – 0.50	25 (1)	0.02
Cypermethrin	0.3±0.0	BDL – 0.30	25 (1)	0.05
Aldicarb	0.73±0.21	0.50 – 0.93	75 (3)	0.01
Carbofuran	1.06±0.83	0.50 – 2.30	100 (4)	0.02
Kresoxim-methyl	BDL [a]		-	0.01
		Hozet-Menouf (HZM)		
Lambda-cyhalothrin	0.65±0.07	0.6 – 0.7	50 (2)	0.02
Cypermethrin	0.3±0.0	BDL – 0.3	25 (1)	0.05
Aldicarb	0.33±0.22	0.1 – 0.6	100 (4)	0.01
Carbofuran	0.8±0.53	0.5 – 1.6	100 (4)	0.02
Kresoxim-methyl	BDL	-	-	0.01
		Shanshour (SNR)		
Lambda-cyhalothrin	0.4±0.0	BLD – 0.4	25 (1)	0.02
Cypermethrin	BDL	-	-	0.05
Aldicarb	BDL	-	-	0.01
Carbofuran	0.68±0.41	0.4 – 1.3	100 (4)	0.02
Kresoxim-methyl	BDL	-	-	0.01

[a]Mean values of four samples each collected through the period from January 2015 to December 2015. [b]MRLs refer to Maximum Residue Limits. [c]BDL= Below Detection Limit.

Table (4). Estimated Daily Intake (EDI) and Health Risk Index (HRI) of identified pyrethroid and carbamate pesticide residues in muscles of Tilapia fish samples from selected sites of El-Bahr El-Pharony drain.

Pesticides	ADI* (mg/kg/day)	EDI* (mg/kg/day)	HRI*	Health Risk
		El-Khadra (KHD)		
Lambda-cyhalothrin	0.005	BDL *	-	-
Cypermethrin	0.015	$8.3x10^{-4}$	0.056	No
Aldicarb	0.003	$1.3x10^{-3}$	0.433	No
Carbofuran	0.002	$5.0x10^{-4}$	0.250	No
Kresoxim-methyl	0.400	$5.0x10^{-4}$	0.0013	No
		Kafr-Fesha (KRF)		
Lambda-cyhalothrin	0.005	$8.3x10^{-4}$	0.167	No
Cypermethrin	0.015	$5.0x10^{-4}$	0.033	No
Aldicarb	0.003	$1.22x10^{-3}$	0.406	No
Carbofuran	0.002	$1.77x10^{-3}$	0.883	No
Kresoxim-methyl	0.400	BDL	-	-
		Hozet-Menouf (HZM)		
Lambda-cyhalothrin	0.005	$1.08x10^{-3}$	0.217	No
Cypermethrin	0.015	$5.0x10^{-4}$	0.033	No
Aldicarb	0.003	$5.42x10^{-4}$	0.181	No
Carbofuran	0.002	$1.33x10^{-3}$	0.667	No
Kresoxim-methyl	0.400	BDL		No
		Shanshour (SNR)		
Lambda-cyhalothrin	0.005	$6.67x10^{-4}$	0.133	No
Cypermethrin	0.015	BDL	-	No
Aldicarb	0.003	BDL	-	No
Carbofuran	0.002	$1.13x10^{-3}$	0.563	No
Kresoxim-methyl	0.400	BDL	-	

*Source: Joint Meeting on Pesticide Residues (JMPR), 2006; ADI= Acceptable Daily Intake (mg/kg b.w./day); EDI= Estimated Daily Intake(mg/kg b.w./day); HRI= Health Risk Index; BDL = Below Detection Limit.

daily intake of detected pesticides by people consuming fish from the Indus River was low and did not present an immediate risk to the fish consuming people **(Jabeen *et al.*, 2015)**. Another recent study has been done by **Corcellas *et al.* (2015)** reporting for the first time the pyrethroid bioaccumulation in wild river fish in Iberian river basins (Spain). For the first time, this work described pyrethroid bioaccumulation in edible river fish samples collected from 4 different Iberian rivers. All samples were found positive to these insecticides. Levels of

concentration ranged from12 to 4938 ng g^{-1} lipid weight (lw). Also, isomers of cyhalothrin, cyfluthrin and cypermethrin were evaluated in this work for the first time

in not exposed biota samples. **Corcellas *et al.* (2015)**. All these results remark the importance of including pyrethroids in environmental quality and monitoring studies, given that, even at non-lethal doses, pyrethroids are known as stressors **(Forsgren *et al.*, 2013)**.

Conclusion

From our study, the identified pyrethroids found in Tilapia fish samples from El-Bahr El-Pharony drain were cypermethrin and lambda-cyhalothrin. Cypermethrin was detected in fish samples from all selected areas of El-Bahr El-Pharony except SNR area, whereas cyhalothrin was not detected in fish samples from KHD area. The carbamate pesticides, aldicarb and carbofuran were the two most commonly identified in fish. It was obvious that the concentrations of both pyrethroid and carbamate pesticides detected in water samples from the study area were lower than that of fish samples. Pesticide residue levels in fish of the present study were compared with the maximum residue limits (MRLs) established by **European Union Pesticides Database (2010)**. It was found that all detected pesticides were at lower levels than their corresponding MRLs values in all fish samples from the four selected sites around El-Bahr El-Pharony drain, and this could be an important source of pesticide transfer to humans via fish consumption. However a preliminary risk assessment indicated that the daily intake of detected pesticides by the fish consuming people around El-Bahr El-Phaony is low and does not present an immediate risk. While the pesticide use for pest control may be unavoidable, its unlimited use may have implications for the fish and its consumers. Therefore, regulatory and awareness means are needed to control the use and possible seepage of these pesticides into the freshwater systems. Such efforts may help us protect the quality of fish as a food for human beings of this and other regions for the foreseeable future.

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