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Screening of chemical pollutants residues in Nile tilapia fish farmed from Lake Manzala region in relation to human health

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ABSTRACT

This study was undertaken to detect the bioaccumulation of different types of chemical pollutants (organochlorine and organophosphorus compounds) in fish musculature raised in culture facility filled with water of known polluted source. A total of 40 freshly caught Nile tilapia approximately within the same weight range, were collected from different fish farms in Lake Manzala during the summer season. Bioaccumulation analysis of fish musculature was performed using Gas chromatography-mass spectrometric (GC-MS) analysis. Results revealed the presence of harmful chemical pollutants such as biphenyl pesticides, herbicide phenols, organic pollutants, and hydrocarbons. Our findings revealed that fish can bio-accumulate many pollutants when raised in the polluted water source and these chemical pollutants not only affect fish health but it may impose public health concern to human as final consumers.

Keywords: chemical pollutants, fish, pesticides, Lake Manzala.

INTRODUCTION

Fish are considered nutritious food for humans, due to its high-quality protein, vitamins and other essential nutrients. Moreover, fish contains a higher content of two beneficial kinds of omega-3 polyunsaturated fatty acids (PUFAs): eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kris-Etherton et al., 2002). However, with high nutritive values of fish, there are several issues associated with their consumption. The most important concerning issues is the bioaccumulation of chemical pollutants in fish tissues raising a public health concern.

Fish are in intimate contact with their aquatic water, therefore, water sources for fish farms are critical factors in the incidence of infectious diseases and pollution (Adeyemo, 2007). With aquaculture expansion to meet the increased demand for fish production along with the limited renewable water resources, the reuse of wastewater is commonly practiced in Egypt (Mancy et al.,2000). Wastewater harbors many pathogens and pollutants that affect fish health with subsequent impact on humans. Additionally, anthropogenic activities such as mining, agricultural, domestic activities and urbanization also participate immensely in the higher pollution indicators and prohibit the availability of sustainable water supply.

Until recently, more attention was paid to the chemical contaminants such as methylmercury and polychlorinated biphenyls (PCBs) (Wilson, 2004). However, other contaminants of toxic importance could be accumulated in fish tissues and pose a potential source of human exposure; these contaminants include polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polycyclic 70 aromatic hydrocarbons, or pollutants such as polybrominated diphenyl ethers (PBDEs), polychlorinated diphenyl ethers (PCDEs), and polychlorinated naphthalenes (PCNs) as mentioned in several recent studies. Knowledge still lacked yet to fully understand the exposure and adverse effects of these contaminants in humans (Domingo, 2004 a, b, 2006). Consequently, to estimate the health risks of these chemicals is currently a very hard job.

Human health is largely influenced by the diet (Falco´ et al., 2006; Bocio et al., 2007). Among these, potentially chemical pollutants such as pesticides, phenol compounds and persistent organic pollutants (POPs: dioxins and furans, PCBs,

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etc.) are excessively spread into the environment (Dougherty et al., 2000; Odland et al., 2003; Charnley and Doull, 2005; Domingo et al., 2006; Llobet et al., 2007). It is known that persistent organic pollutants possess powerful endocrine-disrupting effects and interfere with the hormonal function of the body (Gavrilescu et al. 2015; Milic´ et al. 2013). The effects of exposure to emerging chemicals in aquatic species are of great interest (Fatoki and Opeolu, 2009). Currently, it has been established that serious general health effects on humans and wildlife areas due to continuous exposure to endocrine disruptors (Ferraz et al. 2007; Dmitruk et al. 2008).

A wide order of previously undetected compounds in a complex environmental matrix have been recognized and quantified at low microgram or nanogram-per-liter concentration with improvement in analytical techniques, such as high-performance liquid-chromatography-coupled mass spectrometry (HPLC–MS), liquid chromatography-mass spectrometry tandem mass spectrometry (LC-MS/MS) (Fawell and Ong, 2012). These groups of compounds have collectively been called emerging contaminants or emerging chemicals and have attracted significant research interest among the major international organizations such as United States Geological Survey (USGS), World Health Organization (WHO), United States Environmental Protection Agency (EPA) and the European Commission (EU). It should be known that while some previously used, harmful chemicals such as polychlorinated biphenyls, dichlorodiphenyltrichloroethane, chlordane, are being the withdrawal of circulation (Rivera-Utrilla et al., 2013).

In Egypt fish are caught and collected from either wild sources or aquacultures distributed in different localities all over the country. Among these sources, Lake Manzala is considered one of the most productive lakes which is a brackish lake and located in north-eastern Nile Delta of Egypt near Port Said and a few miles from the ancient ruins at Tanis. It is bounded by Suez Canal from east, Damietta branch of Nile from the west and the Mediterranean Sea from the north. Lake Manzala receives the highly polluted untreated water from Bahr El-Baqr drains (which carry municipal and industrial sewage from eastern part of Cairo), Hadous, El-Serw, Ramsis, and Faraskour drains (agricultural effluents and urbanization), which have a contributing role to the higher accumulation of chemical pollutants inside the organs and edible muscle of fish reared in lakes with such poor quality water with subsequent great public health problems to the consumers (Osfor et al., 1998).

In contrast to many studies carried out in a wide variety of fish species from aquaculture in Egypt (Abumourad et al., 2013 and El-Moselhy et al., 2014), few studies focused on freshwater fish from the wild source like Lake Manzala which takes up an important position in the national fisheries in Egypt. Nile tilapia (*Oreochromis niloticus*), is one of the dominant fish species consumed in a wide range of Egypt and are most frequently cultured in Lake Manzala at Northeast Egypt. Therefore, the present study aimed to screen different chemical pollutants (organochlorine and organophosphorus compounds) residues in fish musculature cultured in a contaminated water source.

MATERIALS AND METHODS

Study area

Lake Manzala is the major northern delta lakes and the most productive one. It is a brackish lake and located in the northeastern Nile of Egypt near Port Said and a few miles from the ancient ruins at Tanis. It is bounded by Suez Canal from the east, Damietta branch of Nile from west and sand bar separates the lake from the

Mediterranean Sea from the north (Ayache et al. 2009). samples were collected from different fish farms in Lake Manzala region which receives highly polluted untreated water from Bahr El-Baqr drain carrying municipal, industrial sewage, and agricultural effluents from eastern part of Cairo, Hadous, El- Serw, Ramsis and Faraskour drains, which have a contributing role to the higher accumulation of chemical pollutants inside the organs and edible muscle of fish reared in such poor quality water with subsequent great public health problems to the consumers.

Collection of fish samples

A total of 40 freshly caught Nile tilapia of same weight range were collected from different fish farms in Lake Manzala, Egypt during summer seasons, Fish sampled were individually packed into a clean polyethylene bag then labeled and transferred at 4°C in icebox with a minimum of delay to the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University, Egypt. Each fish was dissected and fish skin was removed then musculature was collected, wrapped with aluminum foil, packed separately in a clean plastic polyethylene bag, labeled with identification number and date of collection, then the fish are kept frozen at -20 °C until sample preparation and digestion.

Sample preparation and extraction

Sample preparation and analysis were performed in Central Laboratories Network, National Research Centre, Cairo, Egypt according to (Anastassiades et al., 2003). Briefly, The procedure is based on an initial single-phase extraction using 10 or 15 g sample with acetonitrile at 1 ml acetonitrile per 1 g of sample. A liquid-liquid partition is created by inserting excess salts and buffers to the extract. The acetonitrile layer containing the pesticide is collected after centrifugation. The matrix can be extra cleaned and the spare water removed with a singled-SPE step by mixing acetonitrile extract with anhydrous MgSO4 and primary secondary amine (PSA) sorbents.

Gas chromatography-mass spectrometry analysis (GC-MS) for tissue bioaccumulation analysis:

The GC-MS system (Agilent Technologies) was equipped with gas chromatography (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, and Cairo, Egypt. The GC was equipped with an HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a splitless, injection volume of 2 µl and the following temperature program: 95 °C for 0.5 min; rising at 25 °C /min to 210 °C and held for 10 min; rising at 10 °C/min to 250 °C and held for 0.5 min; rising at 20 °C/min to 290 °C and held for 4.5 min. The injector and detector were held at 280 °C and 300 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50-550 and solvent delay 3 min. The identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

RESULTS AND DISCUSSION

In the current study, tissue bioaccumulation analysis revealed the presence of different hazardous compounds. Wastewater harbors many pathogens and different chemical pollutants that affect fish health with subsequent impacts on humans (Mancy et al., 2000). As observed, different chemical pollutants were detected in fish tissue classified as pesticides, phenolic compounds, biphenyl compounds, organic compounds, hydrocarbons and other chemicals with an adverse effect on fish and human health. Biphenyles pesticides such as [1, 1'-Biphenyl]-2, 3'-diol, 3, 4',5,6'-tetrakis(1,1-dimethylethyl) were detected in fish musculature sampled during summer season at peak area (997203.4) (Figure. 3).

Table 1. Chemical pollutants residues detected in fish musculature by GC-MS

Peak	**RT**	**Area**	**Category**	**Uses**
Aliphatic hydrocarbon compounds				
1-Dodecene	4.81	1011339	aliphatic hydrocarbon	Detergent , petroleum
3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	14.48	1059479	aliphatic hydrocarbon	Petroleum
Aromatic hydrocarbon compound				
3-Pyridinecarboxamide	4.609	10474441	Aromatic hydrocarbon	Nutrient supplement
biphenyl compounds				
[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-	16.86	997203.4	Biphenyl compound	Pesticides
Chemical compounds				
n'-(2-fluoro-5-nitrobenzylidene)pyridine-4-carboxylic acid hydrazide	4.918	1977645	Chemical compond	Industerial pollutant
E-10-Methyl-11-tetradecen-1-ol propionate	6.835	579928.6	Chemical compound	Antiviral ,antibacterial
Acetamide, N-(9,10-dihydro-2-phenanthryl)-2,2,2-trifluoro	15.584	476410.9	Chemical compound	_
Pseudoephedrine	18.451	1878552	Chemical compound	Antiallergic
QUERCETIN 7,3',4'-TRIMETHOXY	21.38	2055459	Chemical compound	Food additive
Z-10-Methyl-11-tetradecen-1-ol propionate	6.818	329931.7	Chemical compound	Antiviral ,antibacterial
Organic compounds				
Aspidospermidin-17-ol, 1-acetyl-16-methoxy	14.508	520656.8	Organic compound	Antiparasite
Phenolic compounds				
2,4-Di-tert-butylphenol	5.021	2680384	Phenolic compound	Cosmetics ,plastics, herbicides
Benzonitrile, 2-(4-methylphenyl)-	6.595	719927	Phenolic compound	Cosmetics ,plastics, herbicides
Phenol, 2,4-bis(1,1-dimethylethyl)-	5.033	2620016	Phenolic compound	Cosmetics ,plastics, herbicides
Phenol, 3,5-bis(1,1-dimethylethyl)-	5.113	422078.8	Phenolic compound	Cosmetics ,plastics, herbicides

Fatty acids				
11,14-Octadecadienoic acid, methyl ester	10.08	2625026	Fatty acid	
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	13.862	1015295	Fatty acid	
6-Octadecenoic acid, methyl ester	10.211	2348249	Fatty acid	
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	10.079	884795.3	Fatty acid	
9-Octadecenamide, (Z)-	16.7	1853908	Fatty acid	
9-Octadecenoic acid (Z)-, methyl ester	10.268	474776.1	Fatty acid	
Arachidonic acid	13.805	810585.1	Fatty acid	
cis-5,8,11,14,17-Eicosapentaenoic acid	18.479	971979.5	Fatty acid	
Doconexent	18.445	1032431	Fatty acid	
Heptadecanoic acid, 16-methyl-, methyl ester	10.606	539316.5	Fatty acid	
Hexadecanoic acid	8.26	769814.2	Fatty acid	
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	19.338	1823769	Fatty acid	
Hexadecanoic acid, methyl ester	7.791	1168007	Fatty acid	
Methyl 10-trans,12-cis-octadecadienoate	10.114	2428517	Fatty acid	
Methyl 4,7,10,13,16,19-docosahexaenoate	18.451	1968146	Fatty acid	
Methyl hexadec-9-enoate	7.608	567806	Fatty acid	
Octadecanoic acid, methyl ester	10.612	705741.3	Fatty acid	
Oleic Acid	8.666	356365.8	Fatty acid	
Oleic acid,3 (octadecylox) propyl ester	18.542	993498.2	Fatty acid	
Phytochemical compounds				
Cholest-5-en-3-ol (3.beta.)-	25.403	40219878	phytochemical compound	_
Spirost-8-en-11-one,3-hydroxy-,(3β,5α,14β,20β,22β,25R)	25.409	34950611	phytochemical compound	_

It is classified by Environmental Protection Agency (EPA) as priority pollutants and this may be attributed to anthropogenic activities in this region. In another study by Yahia and El-Sharkawy (2013) pesticides detected in two types of Nile fish collected from Assiut, Egypt; were in form of different organophosphorus (OPs), organochlorine (OCs) and polychlorinated biphenyles (PCBs). Similarly, other types of PCBs were analyzed in fish sampled from Egypt's coastal areas (Abd-Allah et al., 1998). However, in Ghana lagoons, Essumang et al. (2009) detected other types of pesticide compounds such as diazinon and chlorpyrifos in fish samples. In agreement with our study, Salah El-Dein and Mahmoud (2011) reported that no organophosphorus pesticides were

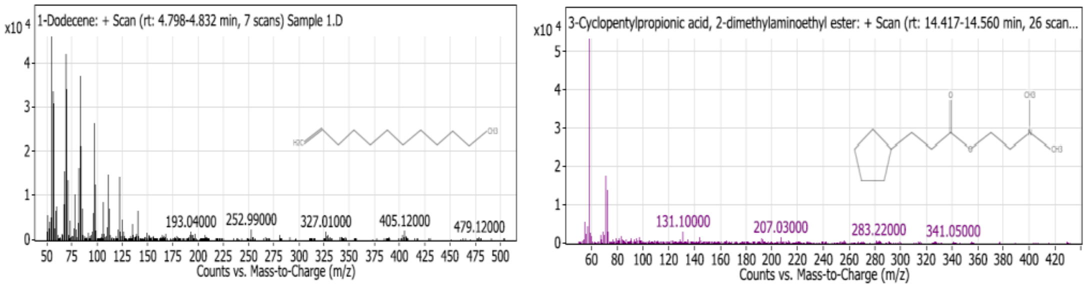

Figure 1. Aliphatic compounds

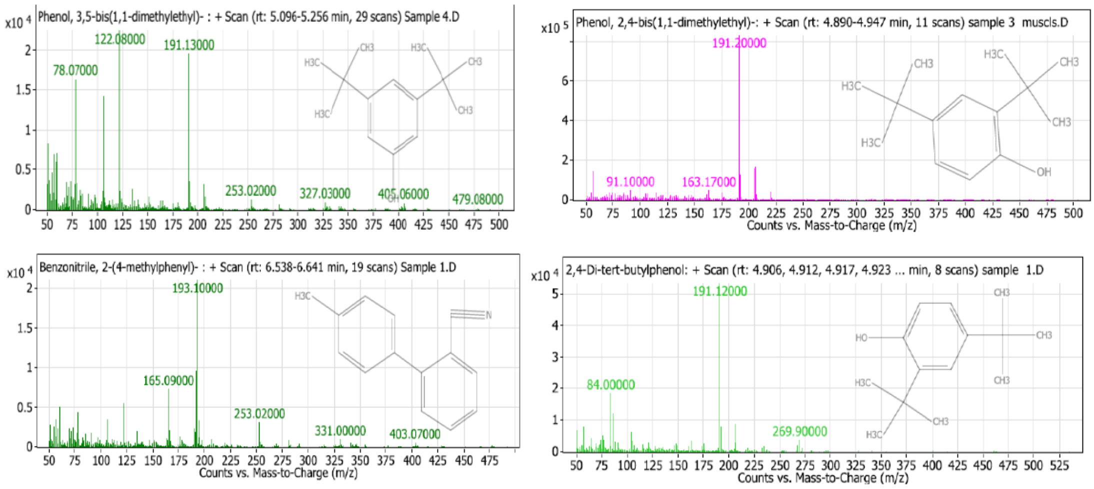

Figure 2. phenolic compounds

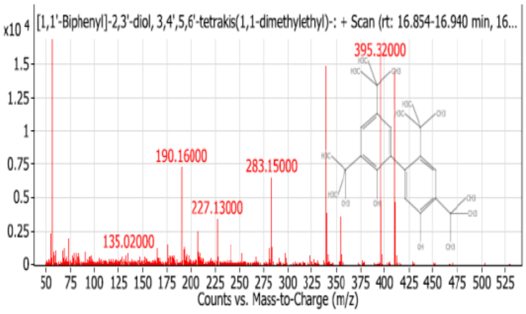

Figure 3. Biphenyls

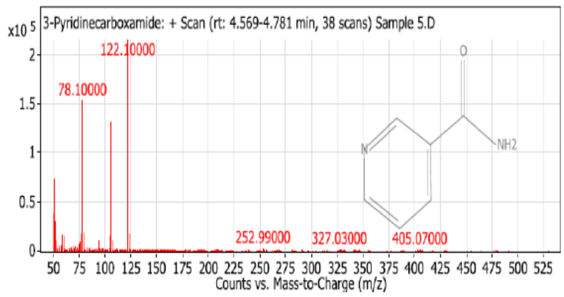

Figure 4. Aromatic hydrocarbon

detected in fish samples collected from district markets in Zagazig and Abo Kabeer, Sharkia Governate, Egypt.

Phenolic compounds were detected in fish tissue such as benzonitrile, 2-(4-methylphenyl), phenol, 3,5-bis(1,1-dimethylethyl) , 2,4-di-tert-butylphenol and phenol, 2,4-bis(1,1-dimethylethyl) at peak area (719927), (422078.8), (290441.7) and (10842746), respectively (figure 2). These compounds act as herbicides and endocrine disturbing chemicals and are involved in plastics and cosmetics (Table 1). Similarly, Ramirez-Sosa et al. (2013) detected 2,4-di-tert-butylphenol herbicide in different environmental compartments such as leachates, also Chen et al. (2016) detected 2,4-di-tert-butylphenol with an adverse effect on human health and aquatic environments.

Additonaly, phenol 2,6-bis(1,1-dimethylethyl)-4-(1-methyl-1- phenylethyl) ,2,4 (dimethylbenzyl-6-t-butylphenol) are phenolic compounds and considered as industrial pollutants detected by Zaman et al. (2012) in fish sampled from Mat lab flood plain areas, Bangladesh. This may be attributed to plenty of municipal wastewater, organic industrial byproducts and bad use of agricultural pesticides. The Egyptian national standards have a relatively zero tolerance to phenol and phenolic compounds (Subject66/Egyptian National decree 8/1983) in the drainage water due to its harmful effects on living cells including humans, animals, and fish.

The organic compound, aspidospermidin-17-ol, 1-acetyl-16-methoxy, was detected in the summer season at peak (520656.8). alongside with other organic pollutants which are very harmful and carcinogenic to human. Most of these compounds are discharged from industrial effluent and used for making fungicides, pesticides, plasticizers, pharmaceuticals, dyes, chemicals, personal care products, disinfectants, etc. and many of them are not anymore officially registered for use because of their toxicity. Due to particular toxicity, tumorigenicity, mutagenicity, reproductive toxicity, flammability, corrosively or reactivity of these substances, they cause a special health and safety hazard (The Pennsylvania Code 2011).

Moreover, aliphatic hydrocarbon compounds detected in summer were in form of 1-dodecene and 3- cyclopentylpropionic acid, 2-dimethylaminoethyl ester at area peak of (1011339) and (1059479) (Table. 1, Figure. 1). Most of these compounds act as nematicides, pesticides, and are involved in different products like personal care, cosmetics, paints, petroleum, plastics. Also, aromatic hydrocarbon compound such as 3-pyridinecarboxamide was detected in fish musculature at area peak of (10474441) (figure 4). In addition, other chemical pollutants were detected are N'-(2-fluoro-5-nitrobenzyledene) pyridine-4- carboxylic acid hydrazide, E-10-methyl-11-tetradecen-1-ol propionate, acetamide, N-(9,10- dihydro-2-phenanthryl)-2,2,2-trifluoro, pseudoephedrine, quercetin 7, 3',4'-trimethoxy, Z-10- methyl-11-tetradecen-1-ol propionate. Most of these chemicals have different activities such as antimalarial, antifungal, antibacterial, antiallergic and antiviral and are involved in personal care products and plastics.

CONCLUTION

To conclude, different types of chemical pollutant residues were detected in fish musculature as biphenyl compound, herbicides phenolic compounds; besides, other organic pollutants such as aliphatic and aromatic hydrocarbons. These compounds confirm the contamination of the water source of Bahr El-Baqar drain of Lake Manzala that directly fill fish farms in this area and subsequently bioaccumulate in their muscles and pose a health hazard to the final consumers. These findings warrant new policies and strategies for monitoring and controlling pollution in the aquatic ecosystem. Additionally, action plans to be taken to ensure the use of good quality water for raising fish not only to protect the fish and aquaculture industry but also to save human lives

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