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Monitoring of Pesticide Residues in Riyadh Cultured Farm Fish

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Abstract: Fish samples collected from different farms from the Riyadh region, Saudi Arabia were analyzed for the levels of pesticides residues in order to elucidate the status of these chemical contaminants in fish intended for human consumption. Thirty three pesticide residues related to the groups of insecticides (Organochlorine (OCPs), Organophsphorus (OPPs) and Pyrethroids), Herbicides, Acaricides and Fungicides were studied by Liquid Chromatography (LLC) coupled with Solid Phase Extraction (SPE) extraction techniques and determined by Gas Chromatography / Electron Capture Detector-Nitrogen Phosphorus Detector (GC/ ECD-NPD) in fish samples. Results indicated that the pesticide residues detected in fish samples were 5 members of Organochlorines pesticide, namely p,p-DDT, p,p-DDE, p,p,-DDD, γ-HCH and Heptachlor; and 3 members of Organophsphorus pesticide, namely α-Endosulfan, Diazinon and Chlorpyrifos with deferent concentrations levels. All detected pesticide residues were under the MRLs. Recovery % ranged from 94.2 ± 2.64 to 99.6 ± 1.88. Minimum Detection Limit also was determined to evaluate the efficiency of the extraction and analysis methods of current pesticide residues and it ranged from 0.001 ppm for OCPs and 0.002 ppm for other detected pesticides. Musa fish samples collected from farm showed the lowest detected pesticide residues. While, Tilapia fish from farm A was the highest contaminated by pesticide residues and then in B, C and D farms, respectively. It was concluded that the accumulation of the Organochlorine and organophosphorous in fish from Riyadh cultured farm fish in Saudi Arabia was studied. Results from this study validate that the pesticide residues detected in fish samples were 5 members of Organochlorines pesticide, namely p,p-DDT, p,p-DDE, p,p,-DDD, γ-HCH and Heptachlor; and 3 members of Organophsphorus pesticide, namely α-Endosulfan, Diazinon and Chlorpyrifos with deferent concentrations levels. Musa fish samples collected from farm D was lowest of detected pesticide residues. While, Tilapia fish from farm A was the highest contaminated by pesticide residues and then in B, C and D farms, respectively.

Key words: Pesticide · Residues · Riyadh · Fish · OPPs · OCPs.GC · GC/MS

INTRODUCTION

Several hundred pesticides of different chemical compositions are currently used for agricultural and vector control purposes all over the world. Because of their extensive use, they are detected in various environmental matrices, such as soil, water and air [1]. Due to their lipophilic nature, hydrophobicity and low chemical and biological degradation rates, organochlorine pesticides (OCPs) have accumuled in the biological tissues with subsequent magnification of concentrations in the organisms due to the progression up the food chain [2].

The increased use of various types of pesticides, particularly organochlorine pesticides (OCs), organophosphates (Ops) and pyrethroids, has lead to concerns regarding the potential for contamination of environmental media (i.e. water, sediment and biota) and associated effects on human health and wildlife. The hazards associated with the bio-accumulation of persistent and toxic pesticides were highlighted in Rachael Carson's book ''Silent Spring'' [3].

OCPs are considered as one of the most toxic and persistent pollutants in the environment, lipophilic in nature and readily absorbed into the tissues of non-target living organisms whereas they may have detrimental

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effects. These anthropogenic chemicals tend to be concentrated in marine environments in close proximity to urban centers and industrial sites [4, 5].

Identifying distribution and accumulation patterns of anthropogenic chemicals present in the marine environment, is an important first step in determining the extent to which these compounds are potentially available for uptake by aquatic organisms. Organochlorine and organophosphates pesticides residues are an important component of the chemical pollutants found in all parts of the global marine environment. They are potentially hazardous to living systems because of their inclination to bio-accumulate in the lipid component of biological species and their resistance to degradation [6]. It is known that a large portion of pesticide residues reaches the oceans through agricultural runoff, atmospheric transport and sewerage discharge. Although the use of OCs has been banned in the world for decades, residues of OCs are still being detected in lakes, rivers, water streams and fish to this date. In China, DDT residues in fish body ranged from 3.7 to 23.5 mg/kg and HCH ranged from 3.7 to 132 mg/kg [7]. Many other studies around the world [8-12]) showed the contamination of fish with OCs, however, the levels in most cases were below those permissible by WHO/FAO [13].

Fish grown in aquacultures in Saudi Arabia, facing a great threat. Growth retardation, decrease in the number of hatching eggs and signs of illness are noticed on cultured fish. Governmental efforts are being done to overcome this problem. One of the main causes to this problem could be the exposure of fish to environmental pollutants, especially pesticides. Spraying habits and practices of farmers can play an important role in contamination. Pesticides which run off from agricultural farms into aquaculture systems also potentially trigger the outbreak of diseases, as they cause deterioration of the ecosystem, as well as affecting the fish immune system. To ensure the safety of food for consumers, numerous legislations such as the EC directives (European Council Directives) have established maximum residue limits (MRLs) for pesticides in food. However, limited information is available regarding the contamination of pesticide residues in Saudi Arabia [14].

In Saudi Arabia, studies on the OCs contamination of fish are very scarce. OCs have been detected in other biological matrices such as human milk and found to contain dangerous residues of these persistent pesticides [15]. Both organophosphates and pyrethroids are the pesticides of choice for farmers in Saudi Arabia. They are extensively used on crops in fields and greenhouses in the same areas whereas fish farming is practiced. Pyrethroids have been shown to be up to 1000 times more toxic to fish than to mammals and birds at comparable concentrations [16].

Many products containing the pyrethroid cypermethrin are classified as ‘‘restricted use pesticides’’ by the US EPA because of cypermethrin’s toxicity to fish. Monitoring pesticide levels in fish would give us information about the extent of pollution and the safety of human consumption of fish. [17]. The objectives of this study were determination of pesticide residue in some fish cultured in the Riyadh area.

MATERIALS AND METHODS

Samples: Fish samples, including Tilapia, Catfish, Musa fish and Grey mullet were collected from different areas in Riyadh region i.e. (Al-karj, Al- mezahmia, Dirab and Tebraket) during summer and winter of 2006 to 2007 and summer of 2008. All samples were immediately transported to the laboratory and frozen at -20°C until analysis. The selection of the four species of fish samples was based on their different feeding habits. These fish are commonly observed in these habitats.

Standards and Reagents: The standard stock solutions of OCPs, OPPs and Pyrethroids including p,p-DDT, p,p-DDE, p,p,-DDD, γ-HCH and Heptachlor; and 3 Organophsphorus namely α-Endosulfan, Diazinon and Chlorpyrifos, respectively, were purchased from Chem Services, USA. Methylene chloride, cyclohexane, acetonitrile, hexane, methanol and acetone were of pesticide grade. The standard solutions were prepared from dilution of their stock standard solutions at concentrations of 0.001 to 2 ppm.

Sample Preparation: Fish samples of 25 g were homogenized using a tissue tearor and extracted 3 times with 50 ml of methylen chloride using an ultrasonic disrupter. The combined extracts were passed through funnels with sodium sulfate and evaporated to 0.5-0.75 ml. The concentrated extracts were transferred to the top of PrepSepTM Extraction Columns filled with 3-5 g of Florisil and 2 g of sodium sulfate. The columns were conditioned with hexane prior to clean-up. Pesticides were eluted with 10 ml of 6% ethyl ether in hexane, followed by 10 ml of 15% ethyl ether in hexane. The volumes of all cleaned extracts were reduced to 1 ml with the rotary evaporator and up to 0.75-0.25 ml in pure (99.5%) nitrogen stream prior to Gas Chromatographic (GC) analysis.

GC-ECD-NPD Analysis: Extracts were analyzed for 8 separate OCPs, OPPs and Pyrethroids utilized within the sampling area. GC analysis was performed using Agilent Technologies 6890N GC System equipped with Electron Capture Detector (ECD) and 5973 Mass Selective Detector. The capillary column was HP-5MS 30 m-0.25 mm, with 0.25 lm film thickness. Column temperature program started at 50°C held 1 min, ramped to 100°C with 25°C/min, followed by 5 C/min to 280°C. The mass selective detector was used in the Selective Ion Monitoring (SIM) mode for confirmative analysis. Analytic recoveries were quantified using fish tissues spiked with pesticides. Chromatographic columns employed for the analysis were: DB-1701, 30 m and 0.32 mm internal diameter with 1 ml film thickness as pre-elution column and HP-5MS, 30 m and 0.32 mm internal diameter with 1 lm film thickness as the second elution column.

GC-MS Analysis: The simplified two-dimensional gas chromatography apparatus (Agilent 6890N) equipped with a 7683 series auto-sampler, Deans switch and a 63Ni electron capture detector (ECD) was adopted in this investigation. It can transfer the interference fraction in the first chromatographic column to the second one for a further efficient separation with the heart cutting technique.

RESULTS

The residual levels of pesticides in different fish, collected during 2006 to 2008 were shown in Tables 1-3. OCPs were detected in all the samples, but their concentrations were much below the MRL recommended for human consumption. Recovery % ranged from 94.2 ± 2.64 to 99.6 ± 1.88. Minimum Detection Limit also was determined to evaluate the efficiency of the extraction and analysis methodology of pesticide residues under this research and it was ranged from 0.001 ppm for OCPs and 0.002 ppm for OPPs. (Table 2).

Musa fish samples collected from farm D had the lowest detected pesticide residues. While, Tilapia fish from farm A was the highest contaminated by pesticide residues and then in B, C and D farms, respectively. Tilapia and Catfish samples collected from farm D was highest concentration of detected pesticide residues.

Table 1: Pesticide Residues (µg/ kg wet weight)in fish samples collected from Riyadh region during summer and winter 2006.

	Pesticides Rsidues (µg/ kg wet weight)in fish															
	p,p-DDT		p,p,-DDD		p,p-DDE		Heptachlor		γ -HCH		α-Endosulfan		Chlorpyrifos		Diazinon	
Fish sp	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W
Tilapia A	0.024	0.034	0.012	0.022	0.007	0.017	0.036	0.044	0.041	0.044	0.011	0.021	0.003	0.013	0.021	0.030
Tilapia B	0.005	0.008	0.004	0.014	0.002	0.019	0.017	0.027	0.027	0.030	ND	ND	0.008	0.018	0.016	0.031
Tilapia C	ND	ND	ND	ND	0.004	0.006	0.121	0.121	0.027	0.027	ND	ND	0.021	0.021	0.007	0.007
Tilapia D	0.001	0.005	0.004	0.009	ND	0.003	0.026	0.029	0.020	0.029	ND	ND	ND	ND	0.010	0.012
Catfish D	0.003	0.005	0.002	0.004	0.014	0.006	0.011	0.015	0.019	0.024	0.012	0.015	ND	ND	0.006	0.008
Musa D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Grey mullet D	ND	ND	ND	ND	ND	ND	0.011	0.014	ND	ND	ND	ND	0.009	0.011	ND	ND

S = Summer W= Winter
ND: Not Detected ±0.0003
α: Alpha β: Beta γ: Gamma

Table 2: Pesticide Residues (µg/ kg wet weight)in fish samples collected from Riyadh region during summer and winter 2007.

	Pesticides Rsidues (µg/ kg wet weight)in fish															
	p,p-DDT		p,p,-DDD		p,p-DDE		Heptachlor		γ -HCH		α-Endosulfan		Chlorpyrifos		Diazinon	
Fish sp	S	W	S	W	S	W	S	W	S	W	S	W	S	W	S	W
Tilapia A	0.021	0.044	0.011	0.032	0.009	0.052	0.026	0.049	0.031	0.051	0.015	0.029	0.008	0.032	ND	ND
Tilapia B	0.004	0.009	0.007	0.019	0.005	0.026	0.018	0.029	0.028	0.012	ND	ND	0.009	0.015	0.017	.022
Tilapia C	ND	ND	ND	ND	0.009	0.011	0.129	ND	0.029	0.021	ND	ND	0.027	0.029	0.009	0.011
Tilapia D	0.006	0.015	0.005	0.019	0.003	0.022	0.025	ND	0.026	0.023	ND	ND	ND	ND	0.011	0.016
Catfish D	0.031	0.008	0.006	0.011	0.018	0.014	0.018	0.011	0.006	0.010	0.010	0.025	ND	ND	ND	ND
Musa D	ND	ND	ND	0.005	ND	0.009	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Grey mullet D	ND	ND	0.003	0.004	0.002	0.008	0.011	0.014	ND	ND	ND	ND	ND	ND	0.007	ND

S = Summer W= Winter
α: Alpha β: Beta γ: Gamma

Table 3: Concentration of pesticides (μg/ kg wet weight)in fish collected from Riyadh region during summer 2008.

	Pesticide Residues (μg/ kg wet weight) in fish							
Fish sp	p,p-DDT	p,p,-DDD	p,p-DDE	Heptachlor	γ -HCH	αEndosulfan	Chlorpyrifos	Diazinon
Tilapia A	0.028	0.022	0.017	0.030	0.061	0.018	0.009	0.024
Tilapia B	0.009	0.014	0.012	0.013	0.021	0.009	0.003	0.012
Tilapia C	ND	ND	0.003	0.126	0.020	0.005	0.023	0.002
Tilapia D	0.002	0.007	0.002	0.026	0.020	ND	ND	0.010
Catfish D	0.023	0.008	0.019	0.012	0.013	0.016	ND	0.002
Musa D	ND	ND	0.005	ND	ND	ND	0.002	0.018
Grey mullet D	ND	0.005	0.008	0.011	ND	ND	0.004	ND

S = Summer ND: Not Detected ±0.0003

α: Alpha β: Beta γ: Gamma

The data in Tables 1-3 showed that, all detected pesticide residues in fish samples was higher in winter seasons than summer seasons. The appearing of DDTs compounds and there derivatives were lower than the MRLs in some fish samples indicated that it was used in same agriculture areas and transferred from soils to ground water and then to fish. The results imply that the bioaccumulation of OCPs in fish is species - specific due to their ecological characteristics such as feeding habits and habitat. Data in Table 1 showed that concentrations of OCPs in the Balti of farm A and B were in the range 0.002 to 0.036 μg/ kg. In the winter seasons, all concentrations of OCPs were increased up to 0.044 μg/ kg. DDT was recorded high concentration in Balti of farm A and B in all summer and winter seasons. The detection of DDE in all samples in summer seasons was 0.004 and in winter 0.006. DDT and DDT were not detected in Balti fish of farm C. The use of organochlorine pesticides has been restricted or even banned throughout the EC, nevertheless, they are still present in animal tissues. In the present study, residues of the lindane, o, p'-DDT and p, p'-DDT have been observed in 31, 35 and 30 of 36 samples, respectively, but these residues were not higher than the EC MRLs. In spite of the ban, the existence of lindane and DDT in the wheat samples confirms that these residues tend to accumulate in food chain.

DISCUSSION

DDT and its metabolites DDE and DDD are very highly persistent in the environment, with reported half lives of up to 15 years [18]. According to WHO [19], these compounds are also very highly toxic to many aquatic species. DDTs were frequently detected within fish tissues of the Organochlorine and some organophosphorus pesticides have lipophilic tendencies and tend to accumulate in tissues of high lipid content [20-22]. Despite the observed concentrations of DDT in fish tissues, Threshold Effect Concentrations (TEC) for total DDTs in fish were not exceeded [23]. Other organochlorine pesticides such as lindane and chlordane were also detected in fish tissues collected from Riyadh. Lindane and chlordane are considered very highly toxic to fish and aquatic invertebrate species [24] and moderately toxic to bird species [25]. Lindane is an organochlorine insecticide and fumigant, which has been used in lotions, creams and shampoos for the control of lice and mites (scabies) in humans [26]. Nevertheless, neither lindane, nor chlordane exceeded Threshold Effect Concentrations for fish [27]. OCPs which contained in farmland run-off can persist for long period underground, they can therefore be absorbed in the sediment and onto suspended particulate matter, transferred into food chains, accumulated in the fatty tissues of fish and finally reach human beings. Data revealed that, farm D which Balti, Mousa, Bori and Grey mullet were grown, DDts only were detected in Grey mullet and Balti with 0.002 to 0.014 and 0.001 to 0.002 μg/ kg, respectively. Lindane and Diazinon were not detected in Mousa and Bori in farm D. The average of Diazinon was ranged in 0.006 to 0.021 μg/ kg in summer season while, it was increased in winter season and it reported 0.007 to 0.030 μg/ kg. Kong *et al.* [28] reported that, carnivore fish showed significantly higher levels of DDTs in its muscle than other fresh-water fish with different feeding modes. Also, presence of DDT and its metabolites in fish and other aquatic life have been reported from other parts of the world as well [29]. The DDT and its metabolites bio-accumulate and are reported to be probable human carcinogens [30]. The use of lindane (γ-HCH) and DDT had been banned in Turkey in 1985 [31].

CONCLUSION

Accumulation of the Organochlorine and organophosphorous in fish from Riyadh cultured farm fish in Saudi Arabia was studied. Results from this study validate that the pesticide residues detected in fish samples were 5 members of Organochlorines pesticide, namely p,p-DDT, p,p-DDE, p,p,-DDD, γ-HCH and Heptachlor; and 3 members of Organophsphorus pesticide, namely α-Endosulfan, Diazinon and Chlorpyrifos with deferent concentrations levels. Musa fish samples collected from farm D was lowest of detected pesticide residues. While, Tilapia fish from farm A was the highest contaminated by pesticide residues and then in B, C and D farms, respectively.

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