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IMPACTS OF PESTICIDES RESIDUES IN *OREOCHROMIS NILOTICUS* AND *MUGIL CEPHALUS* FROM SOME FISH FARMS IN EL-FAYOUM GOVERNORATE ON SOME BIOCHEMICAL PARAMETERS AND TISSUES STRUCTURES

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ABSTRACT

In the present study, the residue levels of some pesticides in water and tissues (muscles and liver) of *Oreochromis niloticus* and *Mugil cephalus* from three fish farms in El-Fayoum Governorate were measured during Spring 2006. The effects of the accumulated pesticides on the total protein and total lipid contents in the muscles and liver and on the histological structures of the muscles and liver were studied. In this study, organochlorine pesticides (γ-HCH, Chlordane, Endrin and *o,p´*-DDT) and pyrethroid pesticides (Thiram, Atrazine, Butachlor, Fenvalerate and Deltamethrin) were detected in the water of the fish farms. The third fish farm recorded the highest number of pesticides (six pesticides; γ-HCH, Chlordane, Endrin, *o,p´*-DDT, Butachlor and Deltamethrin). The muscles and liver of *O. niloticus* and *M. cephalus* showed the presence of a wide variety of organochlorine pesticides, including α-HCH, γ-HCH, Aldrin, Heptachlor, Chlordane, Dieldrin and Endrin, as well as pyrethroid pesticides (Thiram, Pencycuron, Atrazine, Butachlor, Diniconazole and Fenvalerate). In general, the residue levels of the organochlorine pesticides in the fish muscles (edible parts) were within the maximum permissible limit. The muscle and liver total protein content showed non significant changes in *O. niloticus* from fish farm I and significant decreases in *O. niloticus* from fish farms II and III and in *M. cephalus* from fish farm I. Otherwise, the total protein content in the muscles and liver of *M. cephalus* from fish farms II and III showed significant increases. On the other hand, the muscle and liver lipid content showed highly significant decreases in *O. niloticus* from the three fish farms. The muscle lipid content of *M. cephalus* showed non significant changes, except in *M. cephalus* from fish farm I, where a significant increase was observed. However, the liver lipid content of *M. cephalus* showed a highly significant decrease in fish farm II and a highly significant increase in fish farm III. Several histopathological alterations were observed in the muscles and liver of the studied fish collected from the three fish farms.

1. INTRODUCTION

In recent years, fish farming has become the main objective of the Egyptian government to achieve its animal protein production targets for rapidly increasing human population. However, fish can be affected directly through water or food by different kinds of pollutants. Pesticides are considered among the most environmentally hazardous chemicals. These toxic substances are extensively used all over the world for several decades to control pests, pathogens and weeds in agriculture, but their residues often reach either directly or indirectly to the aquatic environment. It has been estimated by Pimental and Goodman (1974) that only 5% of the pesticides reach the target pests.

Hence, about 95% of the used pesticides end up in other parts of the environment. This is the case particularly with aerial application techniques and uses of pesticides in the aquatic environment. Known that fish can store about 58-93% of these pesticides in their tissues (Elnemaki *et al.,* 2005).

Several fish farms are distributed around Lake Qarun in El-Fayoum Governorate which depends on agricultural drainage water. The most important widespread used pesticides are four classes: organochlorines, organophosphates, carbamates and pyrethroids. Organochlorine pesticides are ubiquitous anthropogenic environmental contaminants. In Egypt, they were used from the 1950s. Although the production and the use of organochlorine pesticides have been banned for some decades in most countries, they are still used in Africa for agricultural and public health purposes (Manirakiza *et al.,* 2002). This class of chemicals is characterized by its high activity against pests and slow chemical and biological degradation with persistence in the environment. Being lipophilic, organochlorine pesticides are characterized by a high bioaccumulation potential in food chains and therefore may pose a serious threat to higher trophic levels of aquatic communities (Ayas *et al.,* 2007). Gruzdyev *et al.* (1983) reported that organochlorines when entering an organism, they act on its nervous system, violating the lipoid equilibrium of the nerve cell membrane and preventing the transmission of nerve impulses. The natural and synthetic pyrethroids have emerged as a major class of highly active pesticides due to their high bio-efficacy and relatively low toxicity in comparison to organochlorine and organophosphorous pesticides are used worldwide in households, cereals, cotton, and other crops. Being well identified, the mode of action of the pyrethroids, like organophosphates and carbamates, is inhibition of acetylcholinesterase (Laji and El-Elaimy, 1991). Pyrethroids induced neurotoxicity by affecting sodium channel gating kinetics (Hutson and Roberts, 1985). These actions have been explained in terms of specific alterations of membrane permeability and conductance to the ions involved in axonic electrical events. Synthetic pyrethroides have been generally found to be neurotoxic and lethal to fish (Ural and Saglam, 2005).

Presence of organochlorine and pyrethroid pesticides in water has been extensively studied (Zhulidov *et al.,* 2002; Erkmen and Kolankaya, 2006; Kasozi *et al.,* 2006; Syakalima *et al.,* 2006 and Zhou, *et al.,* 2006). Aquatic contamination by organochlorine and pyrethroid pesticides used in crop protection has dangerous effects on fish which can accumulate pesticides residues directly from water through their respiratory processes and also from food (Teran and Sierra, 1987). Reports are available in plenty indicating the presence of organochlorine and pyrethroid pesticides in a variety of commercial fish species in many countries (Mourad *et al.,* 1999; Manirakiza *et al.,* 2002; Zhulidov *et al.,* 2002; El Nemr and Abd-Allah, 2004; Ribeiro *et al.,* 2005; El-Sikaily *et al.,* 2006; Kasozi *et al.,* 2006; Storelli and Marcotrigiano, 2006; Syakalima *et al.,* 2006; Orban *et al.,* 2007; Storelli *et al.,* 2007 and Yang *et al.*, 2007).

Accumulation of pesticides in the fish tissues may lead to high mortality rate or cause many biochemical and histological alterations in the survived members. Several investigations had concerned with the effect of pesticides on the levels of the tissue protein and lipid. Proteins seem to be suitable source for energy in fish species. Reduction in protein levels was noticed in the muscles and liver of fish exposed to pesticides (Murty and Devi, 1982; Ravinder *et al.,* 1988; Reddy *et al.,* 1991b; Reddy and Yellamma, 1991; Sastry and Das gupta, 1991; Begum, 2004; David *et al.,* 2004; Mohamed and Gad, 2004; Blanar *et al.,* 2005 and Durmaz *et al.,* 2006). However, Ghazaly (1994) observed increases in the muscle protein levels of *Tilapia nilotica* exposed to diedrin. Similarly, Philip

and Rajasree (1996) found significant increases in the liver and muscle protein contents of *Cyprinus carpio* exposed to cypermethrin. Oruc and Usta (2007) observed increases in the muscle protein contents of *Cyprinus carpio* exposed to diazinon.

Lipids are important transient energy source body materials and their level reflects the physiological capacity of fish. It was reported that pesticides affect the lipid metabolism in fish (Rao *et al.,* 1985). The influence of pesticides on the muscle and liver lipid content of fish has been studied by several authors (Murty and Devi, 1982; Rao *et al.,* 1985; Ganesan *et al.,* 1989; Prasada-Rao *et al*., 1990; Reddy *et al.,* 1991a; Ghazaly, 1994; Begum and Vijayaraghavan, 2001; Chandra *et al*., 2004; Mohamed and Gad, 2004 and Blanar *et al.,* 2005).

The histopathological alterations in the muscles of fish as a result of pesticides exposure have been studied by many authors (Sakr and Gabr, 1991; Abo Nour and Amer, 1995; Das and Mukherjee, 2000 and Elnemaki and Abuzinadah 2003). The histopathological effects of pesticides on the liver of fish were studied by many authors (Narayan and Singh, 1991; Radhaiah and Jayantha-Rao, 1992; Braunbeck and Appelbaum, 1999; Mourad *et al.,* 1999; Jiraungkoorskul *et al.,* 2003; Sarkar *et al.,* 2005 and Ayas *et al.,* 2007).

The aim of the present study was to assess the residue levels of some pesticides in water and tissues (muscles and liver) of *Oreochromis niloticus* and *Mugil cephalus* collected from three fish farms in El-Fayoum Governorate. Moreover, the study tends to evaluate the impact of such pesticides on the muscle and liver total protein and lipid contents and on the histological features of the muscles and liver of fish.

2. MATERIAL AND METHODS

2.1. Area of study

Several fish farms are distributed around Lake Qarun in El-Fayoum Governorate which depends on agricultural drainage water. Most of them are simple and primitive with wide variation of management. Three fish farms were included in the present study, the source of water for them is Dayer El-Berka Drain (agricultural drainage water):

- First fish farm with an area about 4200m^2, with 750kg *Mugil cephalus* and 1000kg tilapias fish production.
- Second fish farm with an area about 4200m^2, with unknown fish production.
- Third fish farm with an area about 8400m^2, with 850kg *Mugil cephalus* and 2000 kg tilapias fish production.

2.2. Collection of samples

2.2.1. Water samples

Water samples were collected from the three fish farms during Spring 2006, in polyethylene bottles. They were preserved with methylene chloride, transferred to the laboratory and kept refrigerated for later analysis.

2.2.2. Fish samples

Seventy samples of *O. niloticus* and *M. cephalus* were collected from the three fish farms during spring 2006. The collected fishes were measured to the nearest cm and weighed to the nearest g. The specimens used in this study ranged between 20.4 to 29.7 and 30.2 to 37.3cm in total length for *O. niloticus* and *M. cephalus*, respectively and 200.0 to 500.5 and 280.5 to 670.5g in weight for the same species, respectively. The muscles and liver of fishes were carefully removed for pesticides residues analysis, biochemical and histological studies. Another fish samples

were collected from Abbassa fish farm to be used as a control group.

2.3. Pesticide residue analysis

Multiresidue analysis of water and fish tissues was carried out in Pesticides Research and Analysis Laboratory in Environmental Poison Research Unit, Faculty of Agriculture, Ain Shams University. Extraction of pesticide residues in water samples (one liter sample) was carried out using the method described by Mann (1981). On the other hand, the extraction of pesticide residues in fish tissues (8g each sample) was carried out using acetonitrile-petroleum ether partitioning. Clean up was done on florisil column with three mixtures (6, 15 and 50% diethylether in petroleum ether) for elution, as described by Anonymous (1990). Gas chromatography apparatus [GC (Shimadzu, 12-A)] provided with FID (Flame Ionization Detector) and ECD (Electron Contraction Detector) detectors was used for separation and identification of the pesticides residues in water (mg/L) and fish tissues (mg/kg wet wt.).

2.4. Biochemical analysis

2.4.1. Total protein content in tissues

Sample of 0.1 g of muscle or liver was homogenized in a glass homogenizer for 3 minutes in 5 ml saline then centrifuged at 3000 r.p.m for 10 minutes. The supernatant was used for determination of total protein content. Total protein content was determined according to the method described by Doughaday *et al.* (1952).

2.4.2. Total lipid content in tissues

Sample of 0.1g of muscle or liver was homogenized in a glass homogenizer for 3 minutes in 5ml saline then centrifuged at 3000 r.p.m for 10 minutes. The supernatant was discarded and the pellet obtained was washed with ice cold 10% trichloroacetic acid (TCA). The mixture was centrifuged for 10 minutes at 600 r.p.m and the supernatant was discarded. This step was repeated twice with ice cold 5%TCA. The obtained dry pellet was extracted 3 times with a mixture of chloroform: ethanol: ether (1:2:2 [v/v/v]) (Little Field *et al.,* 1955). The combined extract was used for determination of total lipid content according to the method described by Knight *et al.* (1972).

2.5. Histological investigations

Immediately after isolation from the fish, the muscles and liver were fixed in Bouin's fluid for 24-48hrs. The fixed samples were washed several times in 70% ethyl alcohol and then dehydrated in ascending series of ethyl alcohol. The specimens were cleared in xylene for 15-20 min. and then embedded in paraffin wax. Sections of 4-6μm thickness were cut, mounted on glass slides and stained with Harris' haematoxylin and eosin (Bucke, 1972).

2.6. Statistical analyses

Values were expressed as means (M) $\pm$ Standard deviation (SD). Data were analyzed using t-test (Snedecor, 1962). The values were considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$.

3. RESULTS

3.1. Pesticides residues in water

Table (1) showed the chemical structure of the pesticides in water and fish. The results given in Table (2), revealed that one organochlorine pesticide (*o,p´*-DDT) was detected in the water of the first fish farm (0.092 mg/L) and second one (0.042 mg/L). On the other hand, the third fish farm showed the greatest number of organochlorine pesticides. These pesticides are γ-HCH,

Chlordane, Endrin and *o,p´*-DDT (0.017, 0.080, 0.016 and 0.021 mg/L, respectively). Concerning the pyrethroid pesticides residues, two pesticides were detected in the first fish farm, which were Fenvalerate (0.228 mg/L) and Deltamethrin (0.543 mg/L). The second fish farm showed the existence of Thiram and Atrazine, reaching 0.021 and 0.015 mg/L, respectively. Two pesticides were detected in the third fish farm, namely; Butachlor (0.076 mg/L) and Deltamethrin (1.377mg/L).

3.2. Pesticides residues in fish tissues

The residue levels of pesticides (mg/kg wet wt.) in the muscles and liver of *O. niloticus* and *M. cephalus* from the three fish farms are given in Table (3).

In the first fish farm, detectable levels of γ-HCH and Atrazine were identified in the muscles of *O. niloticus*, their concentrations were 0.251 and 0.013 mg/kg wet wt., respectively. On the other hand, residues of Pencycuron and Atrazine were found in the liver of *O. niloticus*, reaching 0.140 and 0.028 mg/kg wet wt., respectively. In *M. cephalus*, only Chlordane was detected in the muscles (0.039 mg/kg), however, α-HCH, Aldrin, Dieldrin and Endrin were detected in the liver (0.079, 0.068, 0.022 and 0.010 mg/kg, respectively).

In the second fish farm, residues of Chlordane, Thiram and Atrazine were found in the muscles of *O. niloticus*, reaching 0.013, 0.041 and 0.032 mg/kg, respectively. While, Pencycuron (0.022 mg/kg) and Butachlor (4.359 mg/kg) were detected in their liver. In the muscles of *M. cephalus,* α-HCH, Aldrin, Dieldrin and Endrin were recorded in concentrations of 0.045, 0.039, 0.083 and 0.082 mg/kg, respectively. However, only Atrazine was detected in the liver of *M. cephalus* (0.203 mg/kg).

In the third fish farm, in the muscles of *O. niloticus*, γ-HCH, Heptachlor, Endrin and Atrazine were detected, reaching 0.027, 0.080, 0.017 and 0.090 mg/kg, respectively. Dieldrin and Fenvalerate were found in the liver of *O. niloticus,* their concentrations were 0.046 and 0.583 mg/kg, respectively. Aldrin, Pencycuron, Diniconazole and Fenvalerate were identified in the muscles of *M. cephalus*, reaching 0.037, 0.092, 0.043 and 0.154 mg/kg, respectively. Butachlor (0.084 mg/kg) and Diniconazole (0.043 mg/kg) were detected in the liver of *M. cephalus.*

3.3. Biochemical findings

The biochemical data of *O. niloticus* and *M. cephalus* are presented in Table (4). The total protein and lipid contents in the muscles and liver of control fish are 18.27 $\pm$ 2.53, 16.10 $\pm$ 0.24, 0.64 $\pm$ 0.06 and 5.11 $\pm$ 0.45g/100g wet wt., respectively for *O. niloticus* and 18.20 $\pm$ 1.61, 17.67 $\pm$ 0.46, 0.69 $\pm$ 0.07 and 5.90 $\pm$ 0.18g/100g wet wt., respectively for *M. cephalus.*

In the first fish farm, the total protein content in the muscle and liver of *O. niloticus* showed insignificant ($P>0.05$) changes. Meanwhile, the muscle and liver lipid contents showed highly significant ($P \leq 0.01$) decreases (21.88% and 31.90%, respectively) in *O. niloticus*. In *M. cephalus,* the total protein content in the muscle and liver showed highly significant ($P \leq 0.01$) decreases (23.08% and 29.66%, respectively). In contrast, the muscle lipid content showed a significant increase (14.49%). The lipid content of the liver of *M. cephalus* did not reveal any significant difference.

In the second fish farm, the total protein and lipid contents in the muscle and liver of *O. niloticus* showed highly significant ($P \leq 0.01$) decreases (24.85%, 13.48%, 25.0% and 42.27%, respectively). In contrast, the muscle and liver protein contents of *M. cephalus* significantly increased (12.09% and 46.97%, respectively) and the muscle lipid content showed a slight insignificant increase. However, the liver lipid content of *M. cephalus* significantly decreased (35.59%).

As recorded in the second fish farm, the total protein and lipid contents in the muscle

and liver of *O. niloticus* from the third fish farm showed significant decreases (17.19%, 18.64%, 20.31% and 28.18%, respectively). In *M. cephalus*, the muscle and liver protein contents significantly increased (12.64% and 39.62%, respectively). Similarly, the liver lipid content showed a highly significant increase (14.07%). However, the muscle lipid content of *M. cephalus* did not show any significant difference.

Table (1): Chemical structure of the pesticides detected in the water and fish (*Oreochromis niloticus* and *Mugil cephalus*) muscles and liver collected from three fish farms at El-Fayoum Governorate.

Compound	Chemical structure
A- Organochlorine group:	
α -HCH	α - isomer of 1,2,3,4,5,6- hexachlorocyclohexane
γ -HCH (Lindane)	γ -isomer of 1,2,3,4,5,6- hexachlorocyclohexane
Aldrin	1,2,3,4,10,10-hexachloro-1,4,4 a, 5,8,8a-hexahydro-oxo-1,4-endo-5,8-dimethanonaphthalene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
Dieldrin	1,2,3,4,10,10-hexachloro-6,7-expoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene
Endrin	1,2,3,4,10,10-hexachloro-6,7-expoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,endo-5,8-dimethanonaphthalene
o,p´-DDT	1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)-ethane
B-Pyrethroid group:	
Thiram	bis (dimethylthiocarbamoyl) disulfide
Pencycuron	1-(4-chlorobenzyl)-1-cyclopentyl-3-phenylurea
Atrazine	6-chloro-N-ethyl-N´-(1-methylethyl)-1,3,5-triazine-2,4-diamine
Butachlor	N-(butoxymethyl)-2-chloro-N-(2,6-diethylphenyl) acetamide
Diniconazole	(β E)- β -[(2,4-dichlorophenyl) methylene]- α -(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol
Fenvaterate	(RS)- α -cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate
Deltamethrin	(S)- α -cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

Table (2): Pesticide residues (mg/L) in the three fish farms (El-Fayoum Governorate).

Pesticides	Fish farms		
	I	II	III
α -HCH	ND	ND	ND
γ -HCH (Lindane)	ND	ND	0.017
Aldrin	ND	ND	ND
Heptachlor	ND	ND	ND
Chlordane	ND	ND	0.080
Dieldrin	ND	ND	ND
Endrin	ND	ND	0.016
o,p´- DDT	0.092	0.042	0.021
Thiram	ND	0.021	ND
Pencycuron	ND	ND	ND
Atrazine	ND	0.015	ND
Butachlor	ND	ND	0.076
Diniconazole	ND	ND	ND
Fenvalerate	0.228	ND	ND
Deltamethrin	0.543	ND	1.377

ND= Not detected or the residues are existed in amounts below the limit of detection (0.01ppm).

Table 3. Pesticide residues (mg/kg wet weight) in the muscles and liver of *O. niloticus* and *M. cephalus* from the three fish farms (El-Fayoum Governorate).

Pesticides	Fish farms											
	I				II				III			
	O. niloticus		*M. cephalus*		*O. niloticus*		*M. cephalus*		*O. niloticus*		*M. cephalus*	
	Muscles	Liver	Muscles	Liver	Muscles	Liver	Muscles	Liver	Muscles	Liver	Muscles	Liver
α-HCH	ND	ND	ND	0.079	ND	ND	0.045	ND	ND	ND	ND	ND
γ-HCH (Lindane)	0.251	ND	ND	ND	ND	ND	ND	ND	0.027	ND	ND	ND
Aldrin	ND	ND	ND	0.068	ND	ND	0.039	ND	ND	ND	0.037	ND
Heptachlor	ND	ND	ND	ND	ND	ND	ND	ND	0.080	ND	ND	ND
Chlordane	ND	ND	0.039	ND	0.013	ND	ND	ND	ND	ND	ND	ND
Dieldrin	ND	ND	ND	0.022	ND	ND	0.083	ND	ND	0.046	ND	ND
Endrin	ND	ND	ND	0.010	ND	ND	0.082	ND	0.017	ND	ND	ND
o,p'-DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Thiram	ND	ND	ND	ND	0.041	ND	ND	ND	ND	ND	ND	ND
Pencycuron	ND	0.140	ND	ND	ND	0.022	ND	ND	ND	ND	0.092	ND
Atrazine	0.013	0.028	ND	ND	0.032	ND	ND	0.203	0.090	ND	ND	ND
Butachlor	ND	ND	ND	ND	ND	4.359	ND	ND	ND	ND	ND	0.084
Diniconazole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.043	0.043
Fenvalerate	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.583	0.154	ND
Deltamethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND= Not detected or the residues are existed in amounts below the limit of detection (0.01ppm).

Table 4. Total protein and lipid contents (g/100g wet weight) in the tissues of *O. niloticus* and *M. cephalus* from the three fish farms (El-Fayoum Governorate).

Fish	Site	Muscle protein content (g/100g wet weight)		Liver protein content (g/100g wet weight)		Muscle lipid content (g/100g wet weight)		Liver lipid content (g/100g wet weight)	
		M±SD	t-value	M±SD	t-value	M±SD	t-value	M±SD	t-value
O. niloticus	Control	18.27±2.53	----	16.10±0.24	----	0.64±0.06	----	5.11±0.45	----
	Fish farm I	17.55±1.97 (-3.94)	0.55	17.0±0.99 (+5.59)	2.15	0.50±0.06 (-21.88)	4.29**	3.48±0.51 (-31.90)	5.87**
	Fish farm II	13.73±1.83 (-24.85)	3.56**	13.93±0.85 (-13.48)	6.03**	0.48±0.08 (-25.0)	4.15**	2.95±0.22 (-42.27)	10.49**
	Fish farm III	15.13±1.08 (-17.19)	2.80*	13.10±0.91 (-18.64)	7.84**	0.51±0.06 (-20.31)	3.73**	3.67±0.19 (-28.18)	7.21**
M. cephalus	Control	18.20±1.61	----	17.67±0.46	----	0.69±0.07	----	5.90±0.18	----
	Fish farm I	14.00±1.20 (-23.08)	5.12**	12.43±0.70 (-29.66)	15.42**	0.79±0.07 (+14.49)	2.45*	5.37±0.97 (-8.98)	1.32
	Fish farm II	20.40±1.46 (+12.09)	2.48*	25.97±0.85 (+46.97)	21.05**	0.70±0.02 (+1.45)	0.32	3.80±0.50 (-35.59)	9.72**
	Fish farm III	20.50±0.94 (+12.64)	3.02*	24.67±2.25 (+39.62)	7.46**	0.69±0.04 (0)	0.0	6.73±0.51 (+14.07)	3.77**

M±SD = Mean ± Standard Deviation. Number of fish used (n) = 6

Figures between brackets are % change from control value. t-value = between control and fish farms values.

* significant differences at $P \leq 0.05$ ** Highly significant differences at $P \leq 0.01$

Table 5. Comparison of pesticide residues (mg/kg wet weight) in the muscles of *O. niloticus and M. cephalus* (present study) and some fish species from different Egyptian localities.

Location		Fish species	α-HCH	γ-HCH	Aldrin	Heptachlor	Chlordane	Dieldrin	Endrin	o,p'- DDT	Thiram	Atrazine	Butachlor	Fenvalerate	References
Lake Edku		*Mugil cephalus*	0.013	0.003	-	-	-	-	0.005	ND	-	-	-	-	Macklad *et al.* (1984)
		Tilapia galilea	0.002	0.0003	-	-	-	-	0.002	ND	-	-	-	-	
Shanawan drainage canal		*Oreochromis niloticus*	-	0.248	1.018	0.900	-	0.164	1.449	-	-	-	-	-	Khallaf *et al.* (1994)
Shebin El-Kanater (Kalubia Governorate)		*Bagrus bayad*	0.039	-	ND	ND	ND	-	ND	ND	ND	-	-	ND	Zidan *et al.* (2002)
Benha (Kalubia Governorate)		*Bagrus bayad*	0.053	-	0.036	0.075	ND	-	0.090	0.044	ND	-	-	0.089	
South Sinai Governorate		*Mugil sp.*	-	ND	-	ND	ND	0.013	-	-	-	-	-	-	El Nemr and Abd-Allah (2004)
		Boops boops	-	ND	-	ND	ND	0.003	-	-	-	-	-	-	
Demietta Governorate		*Mugil sp.*	-	0.030	-	ND	ND	0.019	-	-	-	-	-	-	
		Boops boops	-	0.007	-	ND	ND	0.004	-	-	-	-	-	-	
Fish farms (El-Fayoum Governorate)		*Mugil cephalus*	0.02-1.64	-	-	0.02-0.18	-	-	ND	ND	-	-	-	-	Sweilum (2004)
Fish farms (El-Fayoum Governorate)															
	1	*O. niloticus*	ND	0.251	ND	ND	ND	ND	ND	ND	ND	0.013	ND	ND	Present study
		M. cephalus	ND	ND	ND	ND	0.039	ND	ND	ND	ND	ND	ND	ND	
	2	*O. niloticus*	ND	ND	ND	ND	0.013	ND	ND	ND	0.041	0.032	ND	ND	
		M. cephalus	0.045	ND	0.039	ND	ND	0.083	0.082	ND	ND	ND	ND	ND	
	3	*O. niloticus*	ND	0.027	ND	0.080	ND	ND	0.017	ND	ND	0.090	ND	ND	
		M. cephalus	ND	ND	0.037	ND	ND	ND	ND	ND	ND	ND	ND	0.154	

ND= Not detected or the residues are existed in amounts below the limit of detection.

3.4. Histopathological alterations

The muscles of the control fish showed normal histological features (Figs. 1A, B, C). In general, the histopathological findings in the muscles of *O. niloticus* and *M. cephalus* from the three different fish farms were more or less similar. They included degeneration in muscle bundles (Figs. 1D, E, K, 2C, G, H, L, 3B, J) and focal areas of necrosis (myolysis) (Figs. 1F, G, L, 2C, G, H, 3B, C, K, L). Moreover, atrophy of muscle bundles (Figs. 1H, 2A, E, F, K, L, 3D, E, 4A, B) and vacuolar degeneration in muscle bundles (Figs. 1I, J, 2B, E, J, K, 3H, I, 4D) were observed. Splitting of muscle fibers (Figs. 2D, I, 3F, G, 4C) was seen. Also, edema between muscle bundles (Figs. 3A, G, 4A, B) was noticed.

The liver from the control fish showed normal histological features (Fig. 4E). Several histopathological alterations were seen in the liver of *O. niloticus* and *M. cephalus* from the three fish farms. They included severe vacuolar degeneration (Figs. 4F, G, H, 5H, I, 6B, K, 7G, H, 8A) with hypertrophy in the hepatocytes (Figs. 5H, I), focal areas of necrosis with necrotic cells between the hepatocytes (Figs. 4I, J, K, 5J, K, 6C, D, L, 7A, 8B), haemorrhage with fibrosis around it (Figs. 4L, 5A, 6E, 7A, B, I, 8C), dilation and thrombosis formation in central veins (Figs. 5B, 6J), dilation and congestion in hepatic (Fig. 5C) and hepatoportal (Figs. 5D, 7L) blood vessels and in blood sinusoids (Fig. 5E) and intravascular haemolysis in some hepatoportal (Fig. 5F) and hepatic (Figs. 6F, G) blood vessels. Also, small focal areas of coagulative necrosis (Fig. 5G) were seen. Thrombosis formation in hepatic blood vessels (Figs. 5L, 7E) and haemosiderin around some hepatic blood vessels (Fig. 6A) were observed. Moreover, aggregations of inflammatory cells (Figs. 7C, D, 8C) and haemosiderin (Fig. 8C) between the hepatocytes were noticed. Degeneration in the wall of hepatoportal blood vessels (Figs. 6H, I, 7L) was seen. In some cases, intravascular haemolysis and dilation in central veins (Figs. 7F, 8D) were noticed. Moreover, in *O. niloticus* from the third fish farm, shrinkage in central veins (Fig. 7J) and hepatic blood vessels (Fig. 7K) with separation from the surrounding hepatocytes were observed. In *M. cephalus* from the third fish farm, thrombosis formation and dilation in hepatic blood vessels with edema around them (Figs. 8E, F) were seen.

4. DISCUSSION

The extensive use of pesticides in Egypt contributes seriously in the contamination of the environment, especially the aquatic ecosystem which serves as a reservoir for tremendous quantities of these foreign organic chemicals. The exposure of fish to these chemicals is responsible for the great loss of a good source of animal proteins (Abd El-Aziz *et al.,* 1997). As the source of water for the studied fish farms is Dayer El-Berka Drain (agricultural drainage water), so several organochlorine and pyrethroid pesticides were detected in the water and tissues (muscles and liver) of fishes from the three fish farm in El-Fayoum governorate.

The present study indicated that the third fish farm revealed six pesticides, namely; γ-HCH (0.017 mg/L), Chlordane (0.080 mg/L), Endrin (0.016 mg/L), *o,p´*-DDT (0.021 mg/L), Butachlor (0.076 mg/L) and Deltamethrin (1.377 mg/L). However, the water samples from the first and second fish farms have lesser numbers of pesticides residues. Only *o,p´*-DDT (0.092 mg/L), Fenvalerate (0.228 mg/L) and Deltamethrin (0.543 mg/L) were detected in the first fish farm. The pesticides detected in the water of the second fish farm were *o,p´*-DDT (0.042 mg/L), Thiram (0.021 mg/L) and Atrazine (0.015 mg/L). Similarly, Abdel-Tawab (1999) detected some pesticides (carbaryl, dursban, fenitrothion and fenpropathrin) in the water of fish farms at El-Fayoum Governorate. Sweilum (2004) detected

several pesticides in the water of some fish farms at El-Fayoum Governorate. These pesticides included Endrin (0.004-0.03 mg/L), Heptachlor (0.03-0.20 mg/L), Reldan (0.03-1.01 mg/L) and Dursban (0.02-0.24 mg/L), however, DDT and α-HCH were not detected.

The present results showed that the muscles and liver of *O. niloticus* and *M. cephalus* contained a wide variety of organochlorine pesticides (α-HCH, γ-HCH, Aldrin, Heptachlor, Chlordane, Dieldrin and Endrin) as well as pyrethroid pesticides (Thiram, Pencycuron, Atrazine, Butachlor, Diniconazole and Fenvaterate). The existence of pesticide residues in the fish tissues found in this study was in agreement with those reported by several investigators (Mourad *et al.,* 1999; Manirakiza *et al.,* 2002; Zhulidov *et al.,* 2002; El Nemr and Abd-Allah, 2004; Kasozi *et al*., 2006; Syakalima *et al.,* 2006, Storelli *et al.,* 2007 and Yang *et al.,* 2007). By comparing the levels of pesticides residues in the studied fish muscles with those in other fish from different Egyptian localities (Table 5), it can be observed that most of pesticides residues in the present study were within the same range found in the literature.

In aquatic ecosystem, fish are located at the highest level of the biomagnification process for xenobiotics. So, they may accumulate significant concentration of pesticides even in waters in which those pesticides are blow the limit of detection in routine water samples (Ribeiro *et al.,* 2005). This explained the occurrence of pesticides in the tissues of the studied fish, however, these pesticides were not detected in the water. This finding is in agreement with the results of Sweilum (2004).

Habitat, physiological factors, lipid content, feeding behavior, rate and routes of biotransformation of pesticides, species, size, age and sex of fish are all important aspects that explain pesticides storage and elimination from the fish (El-Elaimy *et al*., 1994b and Ribeiro *et al.,* 2005).

The recommended levels of organochlorine pesticides in fish for the protection of piscivores are that DDT should not exceed 1 mg/kg and that of Aldrin, Dieldrin, Endrin, Chlordane and Lindane should not exceed 0.1 mg/kg (EPA, 1973). FDA (2001) recommended a level of 0.3 mg/kg for Heptachlor, Lindane, Endrin, Aldrin, Dieldrin and Chlordane for human consumption. FAO recommended a level of 0.3 g/kg in fish as maximum acceptable limit for DDT, Heptachlor, Lindane, Endrin, and Aldrin (FAO, 1983 and Mwevura *et al.,* 2002). Accordingly, the residue levels of the organochlorine pesticides in the muscles of the studied fish are still blow the permissible level.

The control values of the muscles and liver protein and lipid contents obtained in the present study for *O. niloticus* and *M. cephalus* are within the same range for *Labeo rohita* (Das and Mukherjee, 2003) and *O. niloticus* and *Clarias lazera* (Mohamed and Gad, 2005). In the present study, the detected pesticides residues in fish farm I did not exhibit any effect on the muscles and liver protein contents of *O. niloticus*. However, in *O. niloticus* from fish farms II and III and in *M. cephalus* from fish farm I, the muscles and liver protein contents showed significant decreases. On the other hand, the total protein in the muscles and liver of *M. cephalus* from fish farms II and III showed significant increases. According to Singh *et al.* (1996), tissue protein content depends on the dynamic equilibrium between the rates of synthesis and degradation. The quantity of protein may also be affected by impaired incorporation of amino acids into polypeptide chains. Abdel-Tawwab *et al.* (2007) reported that the reduction in tissue total protein may be due to the disturbances in tissue protein synthesis and/or protein breakdown.

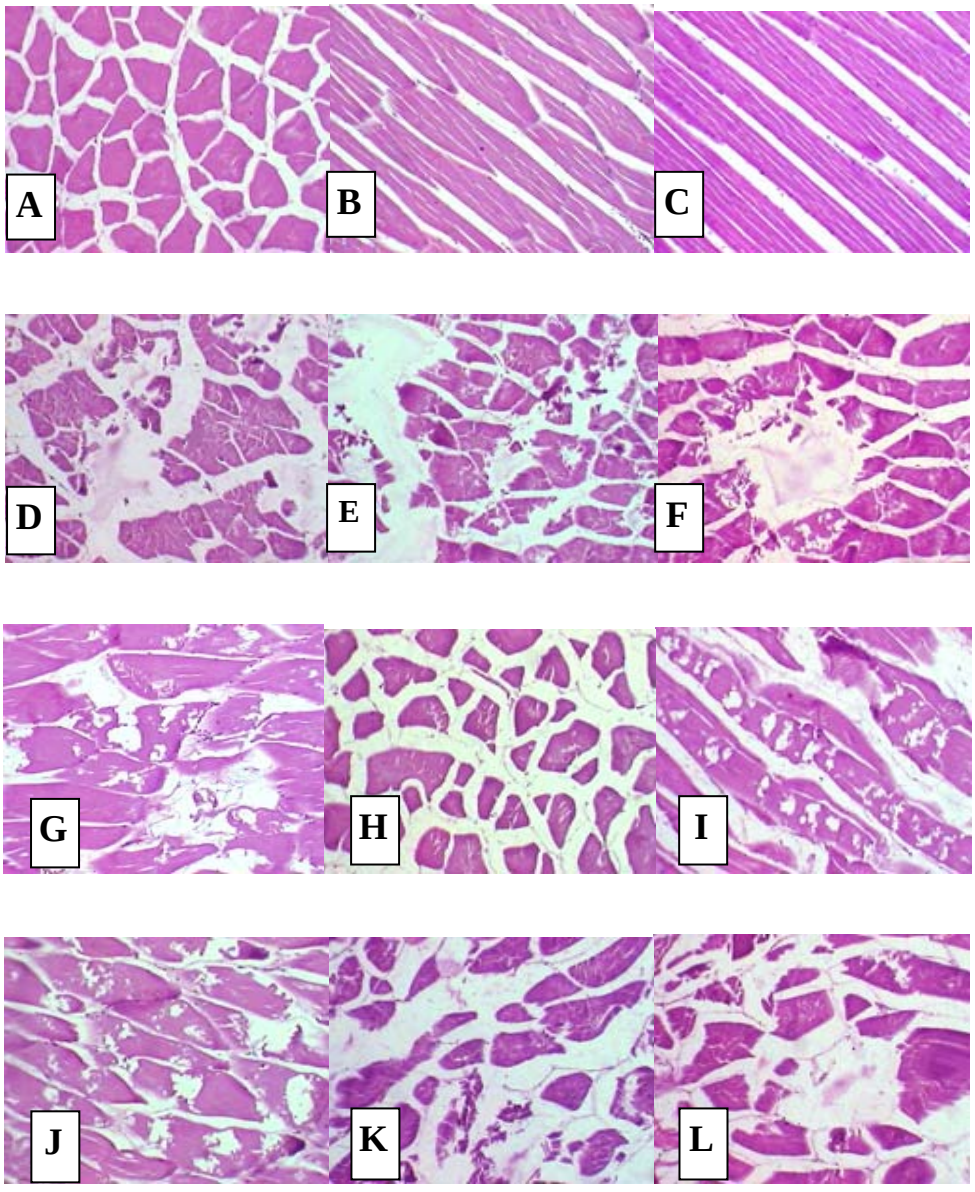

Fig. (1): Sections of muscles of fish showing the control (A,B&C), degeneration in muscle bundles (D&E), focal areas of necrosis (F&G), atrophy of muscle bundles (H), vacuolar degeneration in muscle bundles (I&J) (*O. niloticus*, 1st fish farm), degeneration in muscle bundles (K) and focal area of necrosis (L) (*M. cephalus*, 1st fish farm).

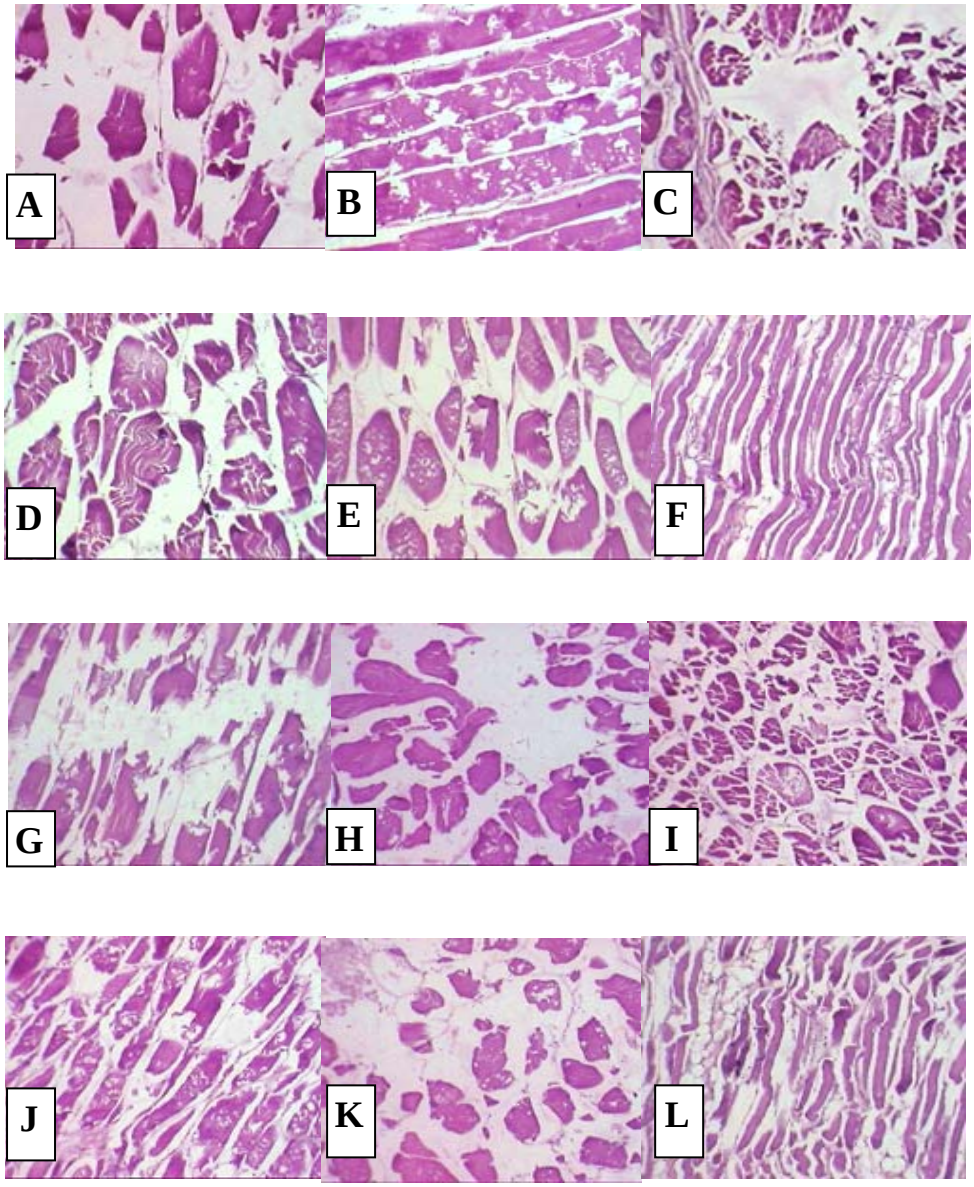

Fig. (2): Sections of muscles of fish showing atrophy (A) and vacuolar degeneration (B) in muscle bundles (*M. cephalus*, 1st fish farm), degeneration in muscle bundles and focal area of necrosis (C), splitting of muscle fibers (D), vacuolar degeneration and atrophy in muscle bundles (E), atrophy of muscle bundles (F) (*O. niloticus*, 2nd fish farm), degeneration in muscle bundles and focal areas of necrosis (G&H), splitting of muscle fibers (I), vacuolar degeneration in muscle bundles (J), vacuolar degeneration and atrophy in muscle bundles (K) and atrophy and degeneration in muscle bundles (L) (*M. cephalus*, 2nd fish farm).

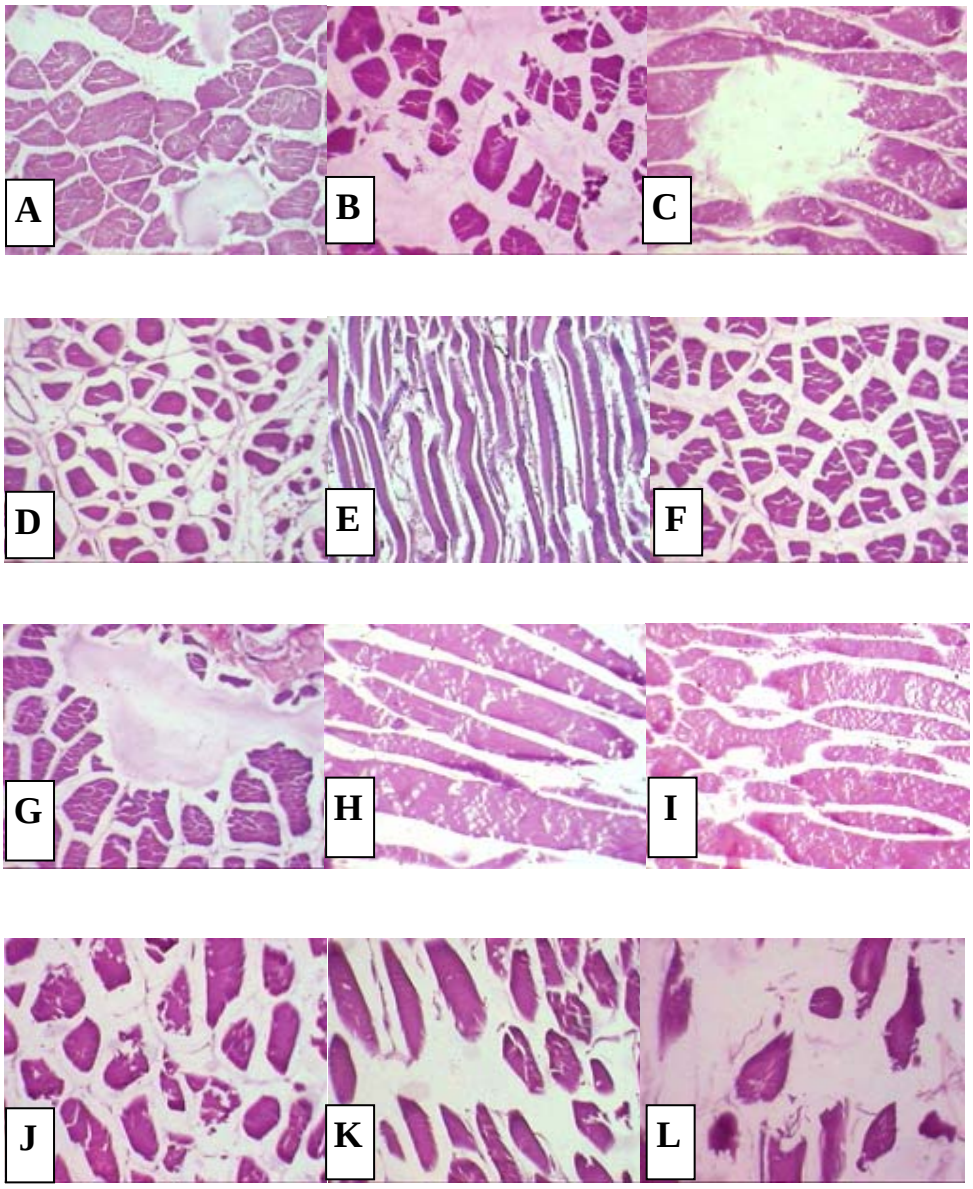

Fig. (3): Sections of muscles of fish showing edema between muscle bundles (A) (*M. cephalus*, 2nd fish farm), severe degeneration in muscle bundles and focal areas of necrosis (B), focal area of necrosis (C), severe atrophy of muscle bundles (D& E), splitting in muscle fibers (F), splitting in muscle fibers and edema between muscle bundles (G), vacuolar degeneration in muscle bundles (H&I) (*O. niloticus*, 3rd fish farm), degeneration in muscle bundles (J) and focal areas of necrosis (K&L) (*M. cephalus*, 3rd fish farm).

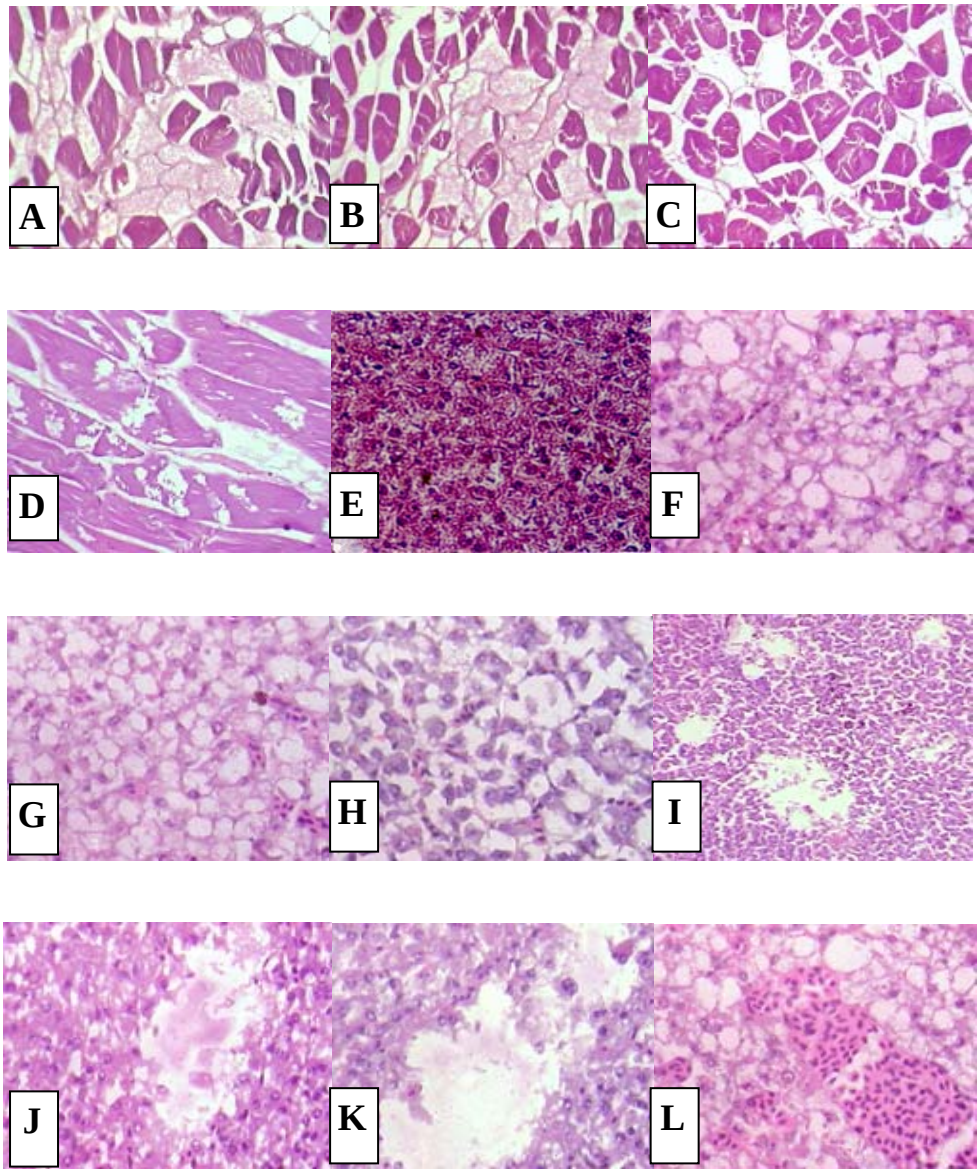

Fig. (4): Sections of muscles and liver of fish showing atrophy in muscle bundles and edema between muscle bundles (A&B), splitting in muscle fibers (C), vacuolar degeneration in muscle bundles (D) (*M. cephalus*, 3rd fish farm), control liver (E), severe vacuolar degeneration in the hepatocytes (F,G&H), focal areas of necrosis (I,J& K) and haemorrhage in hepatic tissue (L) (*O. niloticus*, 1st fish farm).

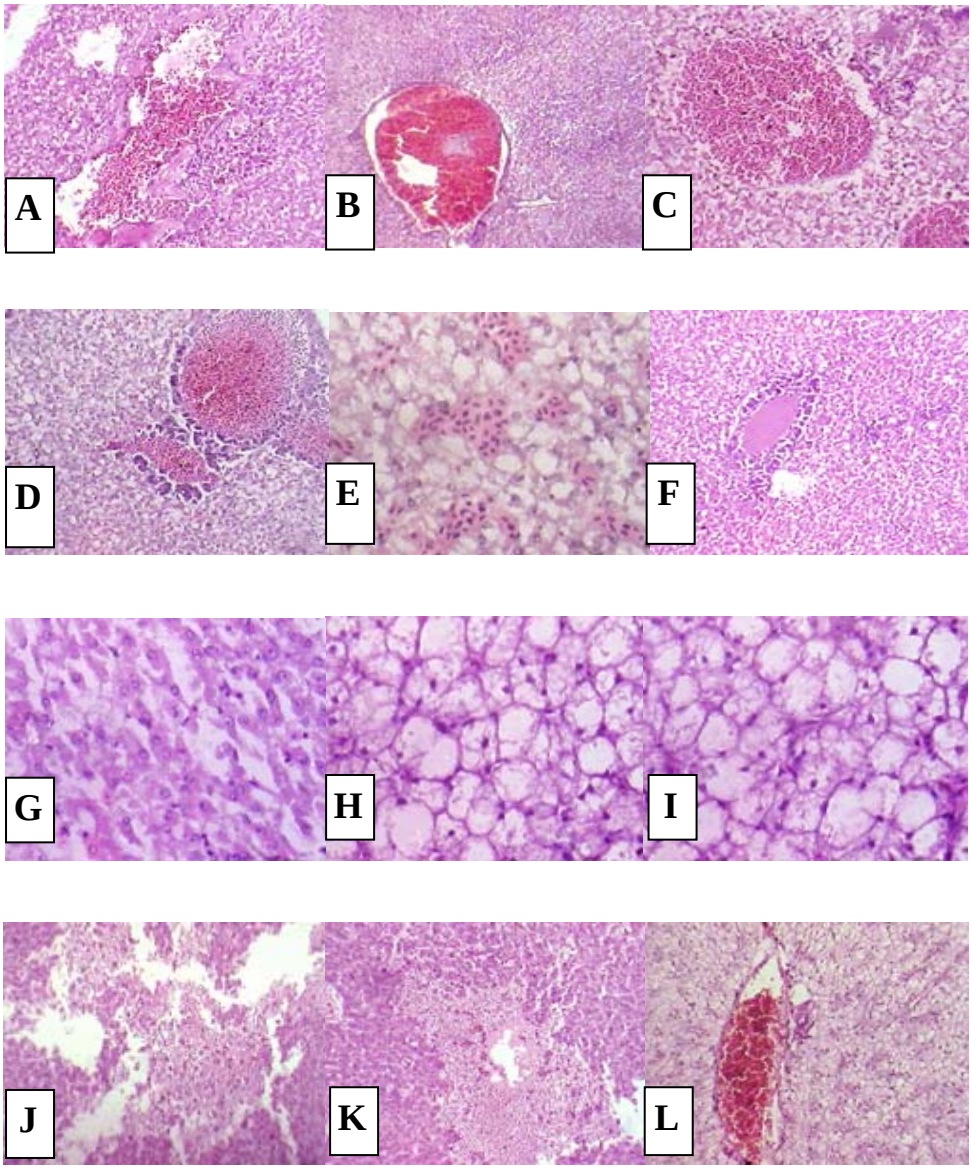

Fig. (5): Sections of liver of fish showing haemorrhage in hepatic tissue with fibrosis around it (A), dilation and thrombosis formation in central vein (B), dilation and congestion in hepatic (C), hepatoportal (D) blood vessels and in blood sinusoids (E), intravascular haemolysis in hepatoportal blood vessel (F), coagulative necrosis (G) (*O. niloticus*, 1st fish farm), vacuolar degeneration with hypertrophy in the hepatocytes (H & I), focal areas of necrosis with necrotic cells between the hepatocytes (J& K) and thrombosis formation in hepatic blood vessel (L) (*M. cephalus*, 1st fish farm).

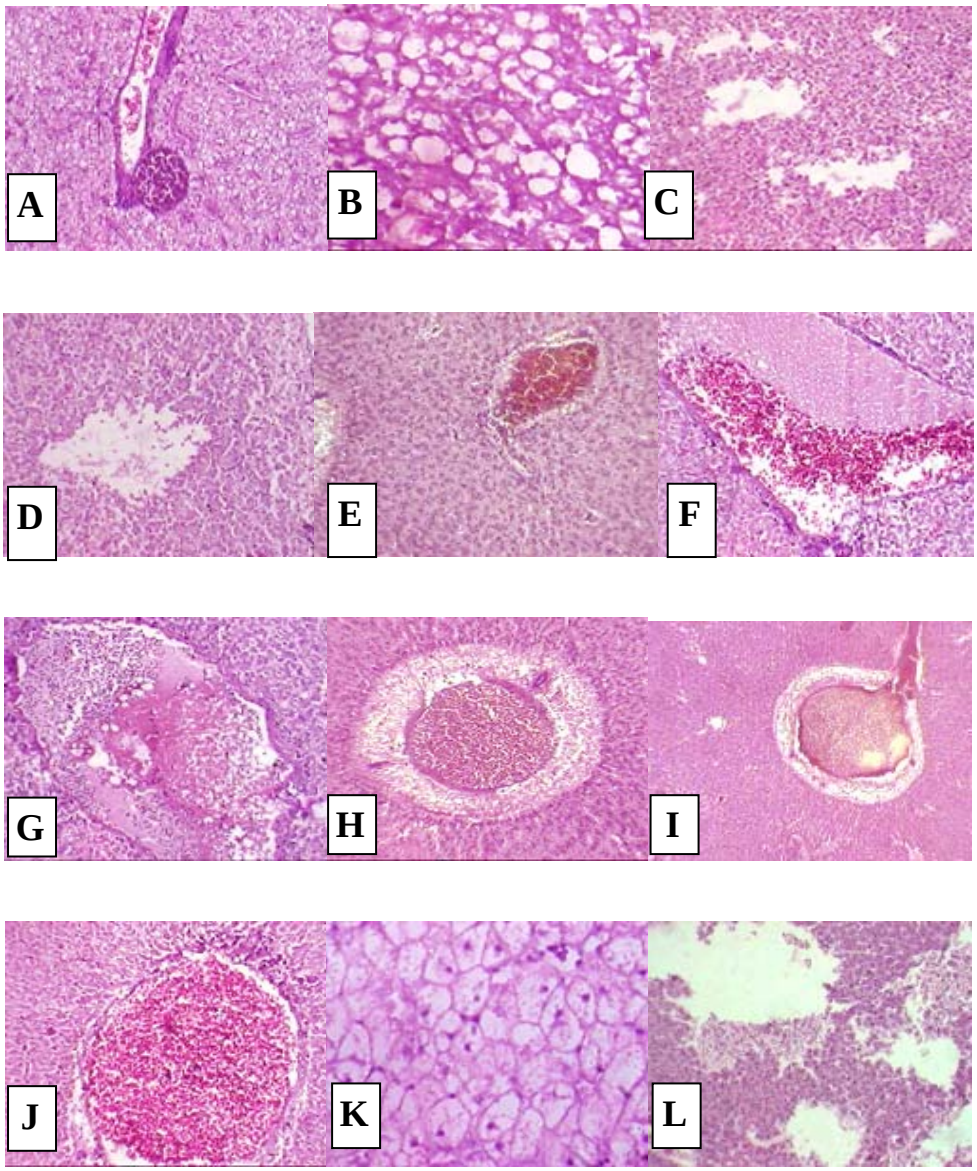

Fig. (6): Sections of liver of fish showing haemosiderin around hepatic blood vessel (A) (*M. cephalus*, 1st fish farm), severe vacuolar degeneration in the hepatocytes (B), focal areas of necrosis (C&D), focal haemorrhage in hepatic tissue (E), intravascular haemolysis and dilation in hepatic blood vessels (F&G), degeneration in the wall of hepatoportal blood vessel (H), degeneration in the wall of hepatoportal blood vessel with intravascular haemolysis in it (I), thrombosis and dilation in central vein (J) (*O. niloticus*, 2nd fish farm), severe vacuolar degeneration in the hepatocytes (K) and focal areas of necrosis (L) (*M. cephalus*, 2nd fish farm).

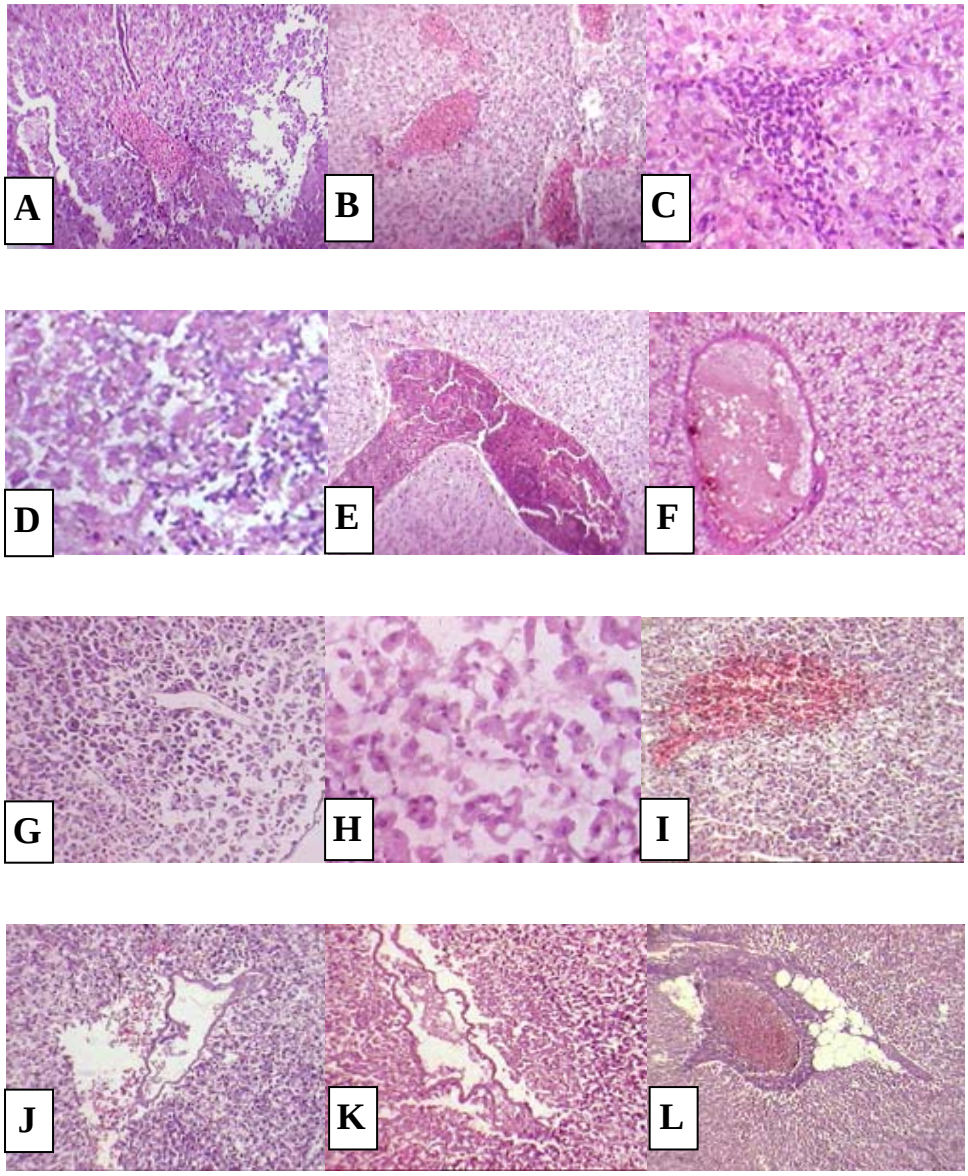

Fig. (7): Sections of liver of fish showing focal areas of necrosis and haemorrhage between the hepatocytes (A), severe haemorrhage (B) and aggregations of inflammatory cells (C&D) between the hepatocytes, thrombosis formation and dilation in hepatic blood vessel (E), dilation and intravascular haemolysis in central vein (F) (*M. cephalus*, 2nd fish farm), severe vacuolar degeneration in the hepatocytes (G&H), focal haemorrhage in hepatic tissue (I), shrinkage in central vein (J) and hepatic blood vessel (K) with separation from the surrounding hepatocytes and congestion in hepatoportal blood vessel with degeneration in its wall (*O. niloticus*, 3rd fish farm).

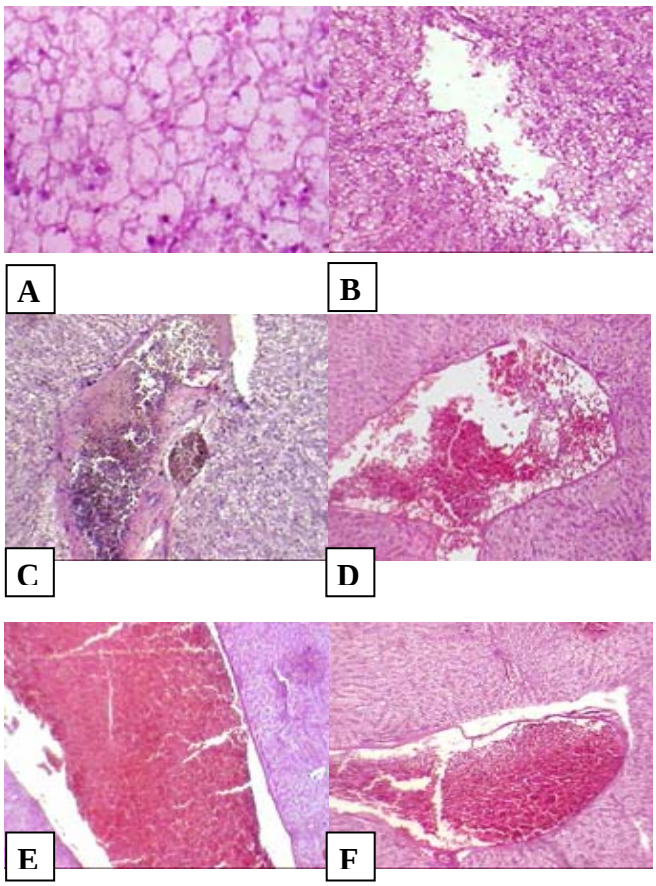

Fig. (8): Sections of liver of fish showing vacuolar degeneration in the hepatocytes (A), focal area of necrosis (B), haemorrhage, aggregation of inflammatory cells and haemosiderin between the hepatocytes (C), dilation and intravascular haemolysis in central vein (D), dilation and thrombosis formation in hepatic blood vessel (E) and dilation and thrombosis formation in hepatic blood vessel with edema around it (F) (*M. cephalus*, 3rd fish farm).

In the present study, the reported decrease in the protein content of fish may reflect physiological adaptability of the fish to compensate for the pesticide-induced stress. To overcome the stress, the animals require more energy. This energy demand might have led to the stimulation of protein catabolism to provide substrate for gluconeogensis in order to produce glucose, the main fuel source, as previously reported by Sancho *et al.* (1998) and Durmaz *et al.* (2006). Ravinder *et al.* (1988) and Singh and Singh (2002) suggested that the depletion in protein content may be due to augmented proteolysis and possible use of the products for metabolic purposes. Das and Mukherjee (2003) reported that the reduction in the protein content in *Labeo rohita* exposed to cypermethrin may be due to proteolysis and retardation of protein synthesis. The reduction in protein content noted in the present investigation may be also due to blocking of the metabolism of amino acids by the pesticides and the cells may become incapable of synthesizing proteins, causing a decrease in their levels as reported by Goel and Agrawal (1981). El-Elaimy *et al.* (1994a) stated that the decrease in uptake of amino acids into the polypeptide chain was among the main causes of the protein depletion in fish treated with dimethoate. Oruc and Usta (2007) reported that pesticides exposure can inhibit protein synthesis and induce metabolic processes, which lead to protein deficiency.

According to Singh and Sing (2002), the synthesis of RNA plays an important role in protein synthesis. The inhibition of RNA synthesis at transcriptional level may affect the protein level. In the present study, the depletion in the protein content may also be due to decrease in RNA level in fish by the pesticides. Moreover, the recorded decrease in the protein content may be also attributed to the observed destruction and necrosis of the muscles and liver cells and subsequent impairment in protein synthesis machinery as reported by Bradbury *et al.* (1987). The observed decrease in the muscle and liver protein contents of the studied fish is in agreement with that recorded by Murty and Devi (1982) in the liver of *C. punctatus* exposed to endosulfan; Ravinder *et al*. (1988) in the muscle of *C. batrachus* exposed to decis; Reddy *et al.* (1991b) in the muscle and liver of *C. carpio* exposed to fenvalerate; Reddy and Yellamma (1991) in the liver of *T. mossambica* exposed to cypermethrin; David *et al.* (2004) in the muscle and liver of *C. carpio* exposed to cypermethrin; Blanar *et al.* (2005) in the muscle of *S. alpinus* exposed to toxaphene and Durmaz *et al.* (2006) in the muscle of *O. niloticus* exposed to diazinon.

On the other hand, the recorded increase in the muscle and liver protein contents of *M. cephalus* from fish farms II and III may be due to increase in protein synthesis in these tissues. Gill *et al*. (1990) reported that following phosphamidon exposure in *Puntis conchonius*, greater amount of proteins is synthesized by the liver which is needed for repair of damaged cell organelle and tissue regeneration. It is also stated that a compensatory production of enzymes lost as a result of tissue necrosis or to meet increased demand to detoxify the pesticides, may necessitate enhanced synthesis of enzyme proteins. Joshi *et al.* (2003) revealed that the increases in total protein induced by pesticides exposure might be due to stimulation of growth proteins and RNA synthesis. The observed increase in the muscle and liver protein content is in agreement with that recorded by Ghazaly (1994); Philip and Rajasree (1996); Das and Mukherjee (2003) and Oruc and Usta (2007).

Concerning the effects of the detected pesticides on the muscle and liver lipid content of the studied fish, the obtained data showed highly significant decreases in *O. niloticus* from the three fish farms. The increased secretion of catecholamines and corticosteroids in the blood after toxicant stress produces an enhanced metabolic rate which in turn reduces metabolic reserves (as proteins and lipids) (Marie, 1994). The decrease in the muscle and liver lipid content

recorded in the present study suggested the use of energy-rich lipids for energy production during toxic stress as previously reported by Sancho *et al.* (1998). The decrease in the lipid content observed in the present investigation is in accordance with that recorded in the muscles and liver of fish exposed to different pesticides (Murty and Devi, 1982; Ghazaly, 1994; Chandra *et al.,* 2004; Mohamed and Gad, 2004 and Blanar *et al.,* 2005).

The muscle lipid content of *M. cephalus* showed insignificant changes, except in the fish from fish farm I, where a significant increase was recorded. However, the liver lipid content of *M. cephalus* showed a highly significant decrease in fish farm II and a highly significant increase in fish farm III. The elevation in the lipid content may be attributed to enhanced lipid synthesis and/or reduced lipid catabolism (Woo and Tong, 1982). A similar increase in the lipid content was recorded in the muscles and liver of fish exposed to different pesticides (Prasada-Rao *et al.*, 1990; Reddy *et al.,* 1991a; Begum and Vijayaraghavan, 2001 and Mohamed and Gad, 2004).

Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the muscles and liver. The present study showed several histopathological alterations in the muscles of the studied fish. These alterations included degeneration in muscle bundles, focal areas of necrosis (myolysis), atrophy of muscle bundles, splitting of muscle fibers and edema between muscle bundles. The present results are in agreement with those observed by many investigators who have studied the effects of different pesticides on fish muscles. In this concern, Saker and Gabr (1991) observed cytoplasmic vacuoles in the skeletal muscles of *T. nilotica* exposed to diazinon. Exposure of *C. lazera* to sublethal dose of hostathion was found to induce atrophy and splitting of muscle fibers as well as damage and loss of architecture of muscle fibers (Abo Nour and Amer, 1995). Das and Mukherjee (2000) found severe intramuscular odema in addition to separation of muscle bundles in the muscles of carp exposed to hexachlorocyclohexane (HCH). Exposure of *O. spilurus* to sublethal concentrations of contra/insect 500/50 E.C. was found to induce focal areas of myolysis in the muscles (Elnemaki and Abuzinadah, 2003).

The liver is a detoxification organ and essential for both the metabolism and excretion of toxic substances. Exposure to toxicants may cause histological changes in the liver, which in turn could be used as a biomarker to indicate prior exposure. The present study showed several histopathological alterations in the liver of the studied fish. These alterations included vacuolar degeneration with hypertrophy in the hepatocytes, focal areas of necrosis and haemorrhage, aggregations of inflammatory cells and haemosiderin between the hepatocytes. Dilation, congestion, thrombosis formation and intravascular haemolysis were noticed in central veins and hepatic and hepatoportal blood vessels. Moreover, dilation and congestion in blood sinusoids were observed. Also, degeneration was seen in the wall of hepatoportal blood vessels. The cellular degeneration observed in the liver of the studied fish is most probably due to vascular dilation, intravascular haemolysis and thrombosis formation in the blood vessels with subsequent stasis of blood. The necrotic changes detected in hepatic cells were most probably a consequence of the cellular degeneration (Kadry and Abdel Mageid, 1995). The observed degeneration in the liver may be also due to disruption in the lysosomal membrane, which is very sensitive to toxicants as pesticides, and thus their enzymes released and caused degeneration and vacuolation of cytoplasm of hepatocytes (Mohamed and Gad, 2005).

According to Walter and Israel (1974), the presence of haemorrhagic areas and aggregations of inflammatory cells in the liver of the studied fish could be explained

that the necrotic cells lead to release of different chemostatic factors which attract different inflammatory cells. However, Ram and Singh (1988) reported that the rupturing of blood vessels causing invasive infiltration of inflammatory cells and detrimental focal necrosis. The present results are in agreement with those observed by many investigators who have studied the effects of different pesticides on fish liver (Radhaiah and Jayantha-Rao, 1992; Kadry and Abdel Mageid, 1995 and Mourad *et al.,* 1999). Das and Mukherjee (2000) noticed swelling of the hepatocytes with diffuse necrosis and marked swelling of blood vessels in the liver of *L. rohita* exposed to hexachlorocyclohexane (HCH). Moreover, sinusoids were distended and central veins appeared damaged due to marked swelling. Also, Jiraungkoorskul *et al.* (2003) observed vacuolation of hepatocytes in the liver of *O. niloticus* exposed to sublethal concentrations of roundup for 3 months. Sarkar *et al.* (2005) found necrosis and cordal disarrangement in the liver of *L. rohita* exposed to carbofuran, however, cypermethrin induced focal areas of coagulative necrosis in the fish liver. Yildirim *et al.* (2006) observed hydropic degeneration in the hepatocytes of *O. niloticus* fingerlings exposed to deltamethrin.

In conclusion, the results indicated that the detected organochlorine and pyrethroid pesticides in the water and in *O. niloticus* and *M. cephalus* tissues (muscles and liver) from the three fish farms (at El-Fayoum Governorate) induced changes in the total protein and total lipid contents in the muscles and liver of the studied fish. Moreover, these pesticides caused several histopathological alterations in the muscles and liver of the fish. Thus, for public health concern, the agriculture drainage water (the source of water for the fish farms) should be treated before being used in aquaculture by applying new approaches such as granular activated carbon.

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