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Detection of Harmful Residues in Some Fish Species

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Abstract

Heavy metals (Cadmium Cd, Lead Pb and Mercury Hg), organochlorine pesticides and testesterone residue levels were assessed in some fish species sold in Cairo Governorate. A total of one hundred samples from five different fish species *Oreochromis niloticus* (Tilapia), *Clarias gariepinus* (Nile catfish), *Lates niloticus* (Bagrus Bayad) and, *Scomberomorus cavalla* (Mackerel), *Thunnus albacares* (Tuna) were purchased from vendors in Cairo governorate, Egypt. Samples were analyzed for Cd, Hg and Pb by Flame Atomic Absorption Spectrometer. The organochlorine pesticides were determined with Agilent gas chromatograph GC. Methyl testesterone hormone levels were determined wit Rida screen ELISA kit for tissue. Cd concentrations ranged from 0.0466 ppm to 0.0686 ppm for all fish species with highest levels in Tilapia. Total mercury ranged from 0.075 ppm to 0.151 ppm for all fish species with highest levels in Mackerel. Pb concentrations ranged from 0.06ppm to 0 .44 ppm with highest levels in Nile catfish. Organochlorine pesticides, such as (gamma-BCH, Alpha-BCH, delta- BCH, aldrin, p.p'-DDE, endosulfan, dieldrin, p,p'-DDT, p,p'-DDD, heptachlor, heptachlor epoxide, endosulfan, endrin, gama- chlordan and methoxychlor) were investigated. Five organochlorine (endrin, p,p'-DDD, methoxychlor, gamma-BCH and delta- BCH) pesticides were detected in all studied fish samples. Endrin and p,p'-DDD were the most abundant pesticide residue in the studied tissues of all fish species except Tuna. The highest values of Methyl testosterone residue were 2 ppb and found in (5) 25% of Tilapia fish samples.

Key words: Cadmium Cd- Lead Pb- Mercury Hg- organochlorine pesticides- methyl testesterone residue- fish.

Introduction

Fish had long been regarded as a desirable and nutritional source of high quality protein and generous supply of minerals and vitamins. During the last few decades, great attention has been paid to the possible dangers of many environmental pollutants due to the consumption of contaminated fish **(Patterson 2002).**

Various activities such as farming, fishing, forestry, construction, mining, urban development and land pollution occurring in or near the watershed of a reservoir could bring about water quality problems and disruption in fish **(Akan *et al.*, 2014).**

Pesticides are used widely to improve agricultural production and also to prevent arthropod-borne diseases. But they are used improperly due to the lake of appropriate knowledge about their applications and untoward effects. The excessive usage is harmful to ecosystem and they contaminate soil, surface and underground water resources. Chlorinated organic pesticides are very stable in both fresh and salt water and are resistant to photo degradation **(Shokrzadeh *et al.*, 2009).** They will disappear from the water with secondary mechanisms such as, absorption on sediment, biological breakdown by microflora and fauna, and absorption by fish through gills, skin and feeding. They are poorly hydrolyzed and slowly biodegrades in environment. Therefore, these compounds are persistent in food chains and are readily accumulated in animal tissues. Fish absorb these compounds directly by water or by ingesting contaminated food. In particular, Organochlorine pesticides are highly stable under different environmental conditions and persistent nature and chronic adverse effects on wildlife and humans **(Monirith *et al.*, 2000).**

Industrial and agricultural discharges such as coal and oil combustion, phosphate fertilizers, plastics and pesticides are considered the major sources of heavy metal pollutants of water. Fish absorbed heavy metals from water through the gills, skin and digestive tract. The heavy metals of the most wide spread concern to human health are lead, mercury and cadmium **(Kris- Etherton *et al.*, 2003; Chen *et al.*, 2007; Din *et al.*, 2008).** Heavy metals are recognized as toxic substances due to their low rate of elimination from the consumer body; either man or animals **(WHO, 1992; Wafaa *et al.*, 2003).**

Cadmium and Pb are two of the more toxic food chain contaminants. Cadmium damages the lungs and causes the painful Itai-Itai disease. Lead affects the blood, numerous organs, and the nervous system **(Malhat, 2011).**

Methylmercury may induce alterations in the normal development of the brain of infants and at higher levels may induce neurological changes in adults. Mercury contaminates mostly fish and fishery products. To protect public health, maximum levels of mercury in fishery products are laid down by (**Commission Decision 1993 No 93/351/EEC)**.

(Council Regulation 2001 (EC) No 466/2001) for total mercury sets a maximum level of 0.5 mg kg–1 for fishery products, with exception for certain listed fish species for which 1 mg kg–1 applies.

The sex of fish can be significant in aquaculture because of differences between males and females in growth rate, size, behavior patterns, and breeding time. Administration of exogenous steroids as testosterone can be effective in controlling sexual development, this anabolic agent tend to leave residues in fish and thus cause some problems in consumer health **(Al-Ablani and Phelps, 2002).** Use of the hormone17α-Methyl Testosterone (MT) to induce sex reversal in farmed tilapias has become a common practice in many parts of the world. MT is a simple and reliable way to produce all-male tilapia stocks, which consistently grow to a larger/more uniform size than mixed sex or all-female stocks. Thus, MT usage in tilapia farming is expected to continue to increase rapidly as the global demand for large whole tilapia and tilapia fillets grows. Currently, tilapia is farmed in at least 85 countries, making it the most widely farmed finfish worldwide and second in volume only to carps **(FAO, 2006a).** The European Economic Community (EEC) banned the use of anabolic compounds as growth accelerators in food animals **(European Commission, 1999).**

The objective of this study was to evaluate the accumulation of some environmental pollutants such as some heavy metals (Cd, Hg and Pb), chlorinated pesticides and methyl testosterone hormone among common fish sold in the Egyptian markets.

Materials and Methods

Fish samples:

100 random samples of five different fish species (*Oreochromis niloticus* (Tilapia), *Clarias gariepinus* (Nile catfish), *Lates niloticus* (Bagrus Bayad) and, *Scomberomorus cavalla* (Mackerel), *Thunnus albacares* (Tuna) (20 of each) were collected from different fish markets in Cairo governorates. Each sample was kept in a separate sterile plastic bag and transferred to the laboratory in an insulated ice box as quickly as possible.

Determination of mercury (Silva *et al.*, 2006)

A small aliquot (0.5 g) of each sample was placed into a preweighed Teflon digestion vessel. Ultrapure nitric acid solution (25 mL of 70% v/v) was added to each vessel and the vessels tightly capped and reweighed. The samples were digested using a CEM microwave sample prepration system with advanced composite vessel accessory set. This system featured a 630-watt magnetron programmable at 40% power, at a pressure of 20, 40, 85, 135 and 175 PSI (Pressure Per Square Inch) for 10 minutes each and a temperature of 100˚ C. The vessels were reweighed to insure that no loss of liquid occurred during the digestion process. The contents of the vessels were then filtered using 47 mm Gelman Supor-450 filters and the filtrate transferred to 60 mL acid-leached storage bottles.

A Perkin Elmer Model 4100ZL (Boston, MA, USA) atomic absorption spectrometer equipped with a mercury hollow cathode lamp was used with the Model FIAS-100 flow injection analysis system and hydride generation accessories. The analytical wave length and slit width were 253.7 nm and 0.7 nm, respectively. All the instruments were controlled by Perkin Elmer AA-WinLab software.

Determination of Cd and Pb

One gram of tissue samples was transferred to a clean porcelain crucible. The furnace temperature was slowly increased from room temperature to 450˚ C in 1 h. The samples were ashed for about 4 h until a white or grey ash residue was obtained. The residue was dissolved in 5 ml of HNO3 (25% v/v) and the mixture, where necessary, was heated slowly to dissolve the residue. The solution was transferred to a 25 ml volumetric flask and made up to volume **(Vaidya and Rantala, 1996).** A blank digest was carried out in the same way. All metals were determined against aqueous standards using Flame Atomic Absorption Spectrometer *Thermo* (M series) controlled by a data station running SOLAAR software.

Extraction of pesticides from fish samples

Ten grams of fish muscles were homogenized with 20 g of anhydrous sodium sulfate with tissue homogenizer till a fine homogenate was obtained. The homogenate was extracted with 50 ml of n-hexane: acetone (2:1) using HPLC grade. Extraction was carried out using orbital shaker for 2 hours, and then the extract was filtered through anhydrous sodium sulfate and evaporated till dryness, using rotary evaporator under vacuum at 40 °C. **(Mosaad et al., 2008).**

Clean up

Cleaning up was carried out using 6g activated florisil (60-100 mesh) topped with 1 g anhydrous Na_2SO_4 then column was wet using 30 ml n-hexane then elution of sample was done with 200 ml of the following mixture dichloromethane : n-hexane: acetonitrile (50:48. 5:1.5) **(Mills *et al*., 1972).**

Determination of pesticide residues

The Agilent GC (6890), equipped with Ni^{63} – electron capture detector were use for the chromatographic separation and was achieved by using DB-17(J&W Scientific) capillary column (30m length x 0.32mm internal diameter x 0.25 µm film thickness), carrier gas: N_2 at a constant flow rate of 4 ml/min. The injector and detector temperature were programmed at 300˚C and 320˚C, respectively. The initial column temperature was 160˚C for 2 min, raised at 5˚C/min, and then held at 260˚C for 10 mins. The retention time, peak area and peak height of the sample were compared with those of the standards for quantization.

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Extraction for methyl testosterone residue

Fat and connective tissue were removed from the muscle and 10g of the ground muscle was homogenized with 10mL of 67mM PBS buffer by mixer for 5min. 2g of homogenized sample were mixed with 5mL of tertiary butyl methyl ether (TBME) in a centrifugal screw cap vial and shaken vigorously by vortex for 30-60min. The contents were centrifuged at 3000rpm for 10min. The supernatant was kept and the extraction with TBME was repeated. The supernatants were combined and evaporated then the dried extract was dissolved in 1mL of 80% methanol. The methanolic solution was diluted with 2mL of 20mM PBS-buffer and applied to a RIDA C18 column (solid phase extraction column with C18 end-capped sorbent of an average particle size of 50µm) in the following manner: Column was rinsed by flowing of 3mL methanol (100%). Column was equilibrated by injection of 2mL PBS – Buffer (20mn). Three mL of sample was loaded on column. Column was rinsed by injection of 2mL methanol (40%). Column was dried by pressing nitrogen through it for 3min. Sample was eluted slowly by injection of 1mL methanol (80 %) An aliquot of the eluate was diluted with water, then 20µL per well of resulting solution was used in the test.

ELISA procedures

Ridascreen ELISA kits were obtained from RBio-pharm GmbH, Germany. Methyl testosterone standard solution used for the calibration curve were at levels of 4.5, 1.5, 0.5, 0.25, 0.125 and 0 ppb methyl testosterone in aqueous solution were all included in the ELISA test kit, as indicated by the manufacturer's literature. The standard and samples were analyzed in duplicate. To the marked microwells, 50µL of the diluted enzyme conjugate (peroxidase conjugated testosterone) was added. Then 20µL of standards or samples were added. After that 50µL of the diluted antibody solution was added and after mixing gently by rocking the plate manually, the contents were incubated at room temperature for 2h. The liquid poured out of the wells and after removal of liquid completely, all wells were filled with distilled water (250µL). After rinsing, the water was also discarded; washing was repeated two more times. Then, 50µL of substrate (urea peroxide) and 50µL of chromogen (tetramethylbenzidine) were added. After mixing thoroughly and incubating for 30min at room temperature and dark, 100µL of stop solution (1M sulfuric acid) was added. After mixing, the absorbance was read at 450nm. Color was stable for 60min **(Risto *et al.*, 2013**).

In order to obtain the methyl testosterone concentration in ppb present in the samples. The concentrations were read from the calibration curve for testosterone. For the construction of the calibration curve, the mean of the absorbance values obtained for the standards was divided by the absorbance value of the zero standard and multiplied

by 100 (percentage maximum absorbance). The absorbance is inversely proportional to the testosterone.

$$\frac{\text{O.D. standard (or sample)}}{\text{O.D. zero standard}} \times 100 = \% \text{ maximal absorbance.}$$

The values (% maximal absorbance) calculated for the standards were plotted (on the Y-axis) versus the testosterone equivalent concentration (ppb) on a logarithmic X-axis. The calibration curve was virtually linear in the 0 – 4.5 ppb range.

Statistical analysis:

A descriptive statistical analysis was performed to estimate the mean, minimum, maximum and standard error using the MEANS procedure of the Statistical Analysis System software **(SAS, 2004).**

Results and discussion

In developing countries, environmental protection laws have not been enforced, industrial and domestic wastes are dumped indiscriminately into water bodies. These wastes have been reported to contain toxic and hazardous substances including heavy metals and pesticides.

Heavy metals

The contamination of water resources, sediment, soil and fish by heavy metals is of important concern because of their toxicity, persistence and bioaccumulative nature **(Ikem *et al.* 2003).** Mercury toxicity may cause permanent harm to the central nervous system, such as behavioral disorders and deficiencies in the immune system and development. All forms of mercury can pass through the placenta to the fetus during pregnancy, where it may affect the developing central nervous system (CNS) **(Gochfeld, 2003).** However lead exposure can cause a wide spectrum of health problems, such as reduced cognitive development and intellectual performance in children aside from increased blood pressure, cardiovascular convulsions, coma and renal failure disease in adults (**Bilandžić *et al.*, 2011).** The half-life of lead varies from about a month in blood, 1–1.5 months in soft tissue, and to about 25–30 years in bone. Cadmium is an industrial and environmental pollutant that affects adversely human health, and progressively accumulates inside the body particularly kidneys. Cadmium is a cumulative toxic agent with a biological half – life of 10-30 years. Cadmium burden of the body increases with age and found to be greater in smokers than in non smokers. Accurately, cadmium acts on sulphhydryl groups of essential enzymes and also binds to albumin, phospholipids and nucleic acids, interferes with oxidative phosphorylation and replaces zinc in enzymes so changing their activities (**Bernard, 2004).** Concentrations of three elements in fish samples from the studying area are shown in **(Table 1)**.

All the metal concentrations were determined on a weight basis. Mean metal content in the all fish species followed the profile: lead > mercury > cadmium. The achieved results in **(Table 1)** declared that the mean detectable concentrations (ppm) of lead (Pb) levels in the examined muscle samples were 0.23±0.015, 0.25±0.014, 0.35±0.013, 0.20±0.019 and 0.17±0.015 in Bayad, Tilapia, Catfish , Mackerel and Tuna, respectively. The results recorded in **(Table 1)** illustrated that mean concentration of cadmium level (ppm) in the examined edible muscle samples were 0.057±0.0007, 0.0616±0.001, 0.056±0.0008, 0.057±0.0011and 0.058±0.001in Bayad, Tilapia, Catfish , Mackerel and Tuna, respectively. However the mean concentration of total mercury level (ppm) in the examined edible muscle samples were 0.115±0.004, 0.105±0.005, 0.092±0.0019, 0.133±0.003 and 0.106±0.0044 in Bayad, Tilapia, Catfish , Mackerel and Tuna, respectively. The highest concentration of lead was 0.44 ppm and was found in cat fish. While the highest concentration of Cadmium was 0.0686 ppm and was found in tilapia. However, Mackerel contains the highest concentration of total mercury which was 0.151 ppm. According to results shown in **(Table 2)** the maximum permissible level recommended by **E.O.S.Q.C. (1993)** was 0.5 ppm for Hg and 0.1 ppm for Pb in fish, while **E.O.S.Q.C, (2004)** recommended 0.05 ppm for Cd. According to theses legal standard 85%, 90%, 100%, 70% and 60% of Bayad, Tilapia, Catfish , Mackerel and Tuna, respectively were exceeded the permissible limits of Pb, while 80%, 100%, 85%, 80% and 90% of Bayad, Tilapia, Catfish , Mackerel and Tuna, respectively were exceeded the permissible limits of Cd. However none of the examined samples exceeded the permissible limits for mercury. Lead and cadmium were found in higher concentration than those recommended for fish, while Hg was almost found at the concentration below to those recommended by E.O.S. The obtained results for Pb similar to that obtained by **Lamada (2003),** who reported that mean concentration (ppm) was 0.219±0.011, while lower results obtained by **Seddek *et al.* (1996),** and **Hashim *et al.*, (2008).** While the obtained results for Cd were higher than that obtained by **Eletta *et al.* (2003)** and **Hashim *et al.*, (2008)** and similar to that obtained by **Laz and Abou El-Magd (2006).** However the obtained results for Hg were similar to those obtained by **Hashim *et al.*, (2008)** and **Eboh *et al.* (2006)**, while higher levels were recorded by **Daoud *et al.* (2007).**

Chlorinated pesticides

The mean concentrations of some organochlorine pesticides dichlorodiphenyldichloroethylene, (p,p'-DDE), 4,4-dichlorodiphenyldichloroethan (p,p'-DDD), 4,4- dichlorodiphenyltrichloroethane (p,p'-DDT), Alpha BHC, Gamma BHC, Delta BHC, methoxychlor, endrin, Endosulfansulphan, dieldrin, heptachlor epoxide, heptachlor and aldrin) residues in the flesh of Bayad, Tilapia, Catfish , Mackerel and

Tuna are presented in **Table (3)**. pp DDD was present in 60, 80, 40 and 20% in flesh of Bayad, Tilapia, Catfish and Mackerel respectively, above the MRL **(Table 4)** cited by **Codex 2009** as they set that the maximum residue limit (MRL) of 1.0 µg/kg for DDT and its metabolities, indicating contamination of the aquatic environment by pesticides. DDT and its DDE and DDD metabolites persist in the environment and are known to bioaccumulate in aquatic organism. DDT, DDD, and DDE have all been classified by NAFDAC as probable human carcinogens. DDT and its metabolites have been included as target pesticides residues in four species of fish; similar studies reported that there is a widespread of DDT and its metabolites in tissue of fish samples **Schmitt and Brumbaugh, (1990)** and **NOAA, (1987)**. The mean concentrations of endrin in the flesh of fish samples ranged between 0.04±0.01 ppm to 0.1±0.0003ppm. Endrine was present in 40, 100, 60 and 60% in flesh of Bayad Tilapia, Catfish and Mackerel respectively, above the MRL **(Table 4)** cited by Codex 1999 as they set that the maximum residue limit (MRL) of 100 ng/g for endrin, indicating contamination of the aquatic environment by pesticides. Delta BHC was found in15, 10. 35, 20% of Bayad, Tilapia, catfish and Mackerel fish respectively, above the MRL of 0.01 µg/kg by **Codex 2009**. Methoxychlor was found in 20% of Bayad fish samples. An MRL of 0.005 mg/kg/day for Methoxychlor has been derived for intermediate-duration oral exposure (15–364 days). Methoxychlor was intended to be a replacement for DDT, but has since been banned based on its acute toxicity, bioaccumulation, and endocrine disruption activity **(Akan et al., 2014).** Endrin had the highest frequency occurrence followed by pp- DDD, methoxychlor and gama BHC in that order.DDT metabolites were the second highest in frequency though no recent input from anthropogenic sources could be attributed to since p ,p'–DDD was much higher than the original compound p, p'-DDT. With a half life of 10-20 years in temperate regions, **Sericano et al., (1990),** DDT undergoes degradation to DDE and DDD. The DDE accounts for 50-70% of the DDT burden in the environment, **Newsome and Andrews (1993).** Although concentration of OC in most of the samples were within the regulatory limits, it must be emphasized that OC are inherently unmanageable and they bio-accumulate in living species. Therefore, the acceptable standard for any OC in any sample should ideally be zero **(Li et al., 2006).**

Methyl Testosterone

Results achieved in **Table (5)** declared that methyl testosterone residue was detected only in (5) 25% in tilapia fish above the permissible limit. However the other fish species did not have any sample above the permissible limit according to **Gracey(1986)** which is 1ppb. The concentration of methyl testosterone in tilapia fish ranged from none detected to 2 ppb with mean value of 0.535±0.03 ppb. Higher levels obtained by **(El-Neklawey *et al.*, 2009)** who found testosterone in a value of 4.22±1.1 (ng/gm) in tilapia

farm fish and **Tag El-din *et al.*, (2009b)** recorded 7.52±0.67 (ng/gm) of testosterone in fresh water prawn tissues. The detection of hormonal residues in Tilapia may be attributed to widely use of synthetic androgen as methyl testosterone on fish production in Egypt for its anabolic and androgenic action in fish. This agrees with that stated by **(Mansour and Satyanareyana, 1989, Hegazy 2007).** Among scientific, public and political communities there has been a controversy and arguments about banning or allowing the regular use of such compounds in meat production, as some scientific reports indicated possible carcinogenicity and genotoxicity **(Andersson and Skakkebaek, 1999).** Also, it has been found that the highest rates of hormone-related cancer, including cancer of breast, ovary, prostate, testes and colon were found in North America ,where hormone-treated meat is consumed **(Sibbald,1999).** Moreover, after administration high dose of methyltestosteron in humans causes negative mood as irritability, mood swings, violent feelings, and hostility, then cognitive impairment as distractibility, forgetfulness, and confusion (**Risto *et al.*, 2013**). Other negative effects on the humans are increased risk of injury, increased blood pressure, gastrointestinal complications, benign and malignant liver tumours, Peliosis hepatis (blood- filled cysts), virilisation, clitoral hypertrophy, deepened voice, painful breast lumps in women, gynaecomastia and testicular atrophy in men, abnormalities of sperm count, motility and morphology, sterility, benign prostatic hypertrophy cutaneous striae **(Rashid *et al.*, 2007).**

MT treatment in tilapia farming could be safe if producers restrict tilapia MT treatment to the early fry stages, specifically to the first month from the time the fry are free-swimming/first-feeding. Also they should rear MT treated tilapia fry to adult size for at least five months after hormone treatment ends to ensure zero hormone residue remains in the fish. Moreover, avoid direct release of hatchery water used for MT treatment of tilapia fry into the environment. As a precautionary measure, tilapia hatcheries should utilize a gravel and sand filter, plus a shallow vegetated pond or an enclosed wetland, to receive and hold the hatchery wastewater for several days before discharge into the general environment.

Conclusion

In conclusion, the present study revealed that heavy metals, organochlorine pesticides and methyl testosterone residues are present in marketed Egyptian fish. Although most of these residues occurred at very low concentrations in the samples, they may accumulate to higher levels in human beings who consume these products.

The presence of contaminants, which are usually carcinogenic in nature, in fish of the Egyptian markets may pose serious health hazards to the local population. More detailed investigations, in terms of sampling network and sampling frequencies are required in view of increasing global concern for persistent pollutants and their hazardous impact on environmental and human health.

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Table (1): Heavy metal concentrations in examined fish species (N=20).

	Pb (ppm)			**Cd (ppm)**			**Hg (ppm)**		
Fish sp	**Min**	**Max**	**Mean± SE**	**Min**	**Max**	**Mean± SE**	**Min**	**Max**	**Mean± SE**
Bayad	0.09	0.32	0.23±0.015	0.05	0.0646	0.057±0.0007	0.085	0.138	0.115±0.004
Tilabia	0.08	0.32	0.25±0.014	0.0549	0.0686	0.0616±0.001	0.075	0.136	0.105±0.005
Catfish	0.26	0.44	0.35±0.013	0.05	0.061	0.056±0.0008	0.078	0.103	0.092±0.0019
Mackerel	0.06	0.34	0.20±0.019	0.048	0.062	0.057±0.0011	0.094	0.151	0.133±0.003
Tuna	0.1	0.28	0.17±0.015	0.0466	0.0666	0.058±0.001	0.083	0.115	0.106±0.0044

Table. (2): Samples exceeded MPL according to E.O.S (1993)*and E.O.S.(2004)**(n =20):-

Fish sp	Positive samples											
	Below permissible						Above permissible					
	Pb		Cd		Hg		Pb		Cd		Hg	
	No	%	No	%	No	%	No	%	No	%	No	%
Bayad	3	15	4	20	20	100	17	85	16	80	-	-
Tilabia	2	10	-	-	20	100	18	90	20	100	-	-
Catfish	-	-	3	15	20	100	20	100	17	85	-	-
Mackerel	6	30	4	20	20	100	14	70	16	80	-	-
Tuna	8	40	2	10	20	100	20	60	18	90	-	-

E.O.S. (1993)*:0.5 ppm for Hg and 0.1 ppm for Pb **E.O.S. (2004)** **:**0.05 ppm for Cd

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Table (3): The concentrations (ppm) of pesticide residues detected in tissue fish samples.

Pesticide detected	**Concentrations of pesticide residues (ppm)**					**Permissible limits (Codex, 2009)**
	Mean and SE					
	Bayad	**Tilapia**	**Catfish**	**Mackerel**	**Tuna**	
Methoxychlor	0.0264±0.001	ND	ND	ND	ND	-
PP-DDT	ND	0.004±0.0008	ND	ND	ND	1.0 µg/kg
PP-DDD	0.06±0.01	0.08±0.008	0.04±0.01	0.02±0.009	0.0004±0.0001	1.0 µg/kg
PP-DDE	0.0044±0.001	0.0002±0.0004	ND	ND	ND	1.0 µg/kg
Endrin	0.04±0.01	0.1±0.0003	0.06±0.01	0.06±0.0004	ND	100 ng/g
dieldrin	ND	ND	ND	ND	ND	0.2 µg/kg
Endosulfan	0.0042±0.001	0.0012±0.0004	0.0003±0.0001	ND	ND	0.1 µg/kg
Delta-Chlordane	ND	0.0008±0.00041	0.0004±0.0001	ND	ND	-
Aldrin	ND	ND	ND	ND	ND	0.2 µg/kg
Heptachlor epoxide	ND	ND	ND	ND	ND	-
Heptachlor	ND	ND	0.00025±0.00005	ND	ND	0.2 mg/Kg
Delta- BHC	0.004±0.0001	0.0014±0.0006	0.0004±0.0001	0.02±0.009	ND	0.01 µg/kg
Gama-BHC	ND	ND	ND	ND	0.014±0.006	0.01 µg/kg
Alfa-BHC	ND	ND	ND	ND	ND	0.01 µg/kg

ND: Not detectable.

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Table (4) Occurrence level of peticides residues in flesh of fish samples above the MRL cited by Codex 2009 (n=20).

Fish	Endrin		PP-DDD		Delta- BHC	
	No	%	No	%	No	%
Bayad	8	40	12	60	3	15
Tilapia	20	100	16	80	2	10
cat fish	12	60	8	40	7	35
mackerel	12	60	4	20	4	20
Tuna	-	-			-	-

Table (5): Mean value of testosterone residues in the examined fish samples (ppb)(n=20).

Fish	Above MRL		Min	Max	Mean± SE
	No	%			
Bayad	Zero	zero	ND	0.05	0.025±0.03
Tilapia	5	25	ND	2	0.535±0.03
Cat Fish	Zero	Zero	ND	0.05	0.015±0.03
Mackerel	Zero	Zero	ND	0.7	0.365±0.03
Tuna	Zero	Zero	ND	0.15	0.05650±0.03

ND: Not detectable.

Note: The detection limit of the apparatus 0.1ng/gm.

M.R.L: less than 1ppb according to (Gracey, 1986).

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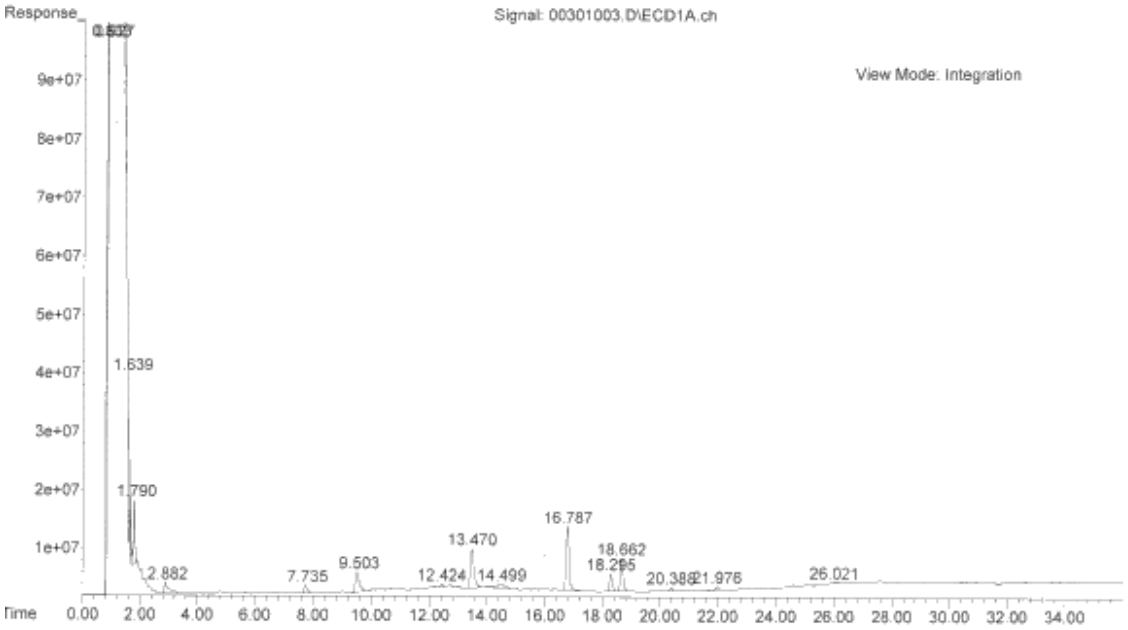

Fig (1): Detected pesticides sample of Bayad.

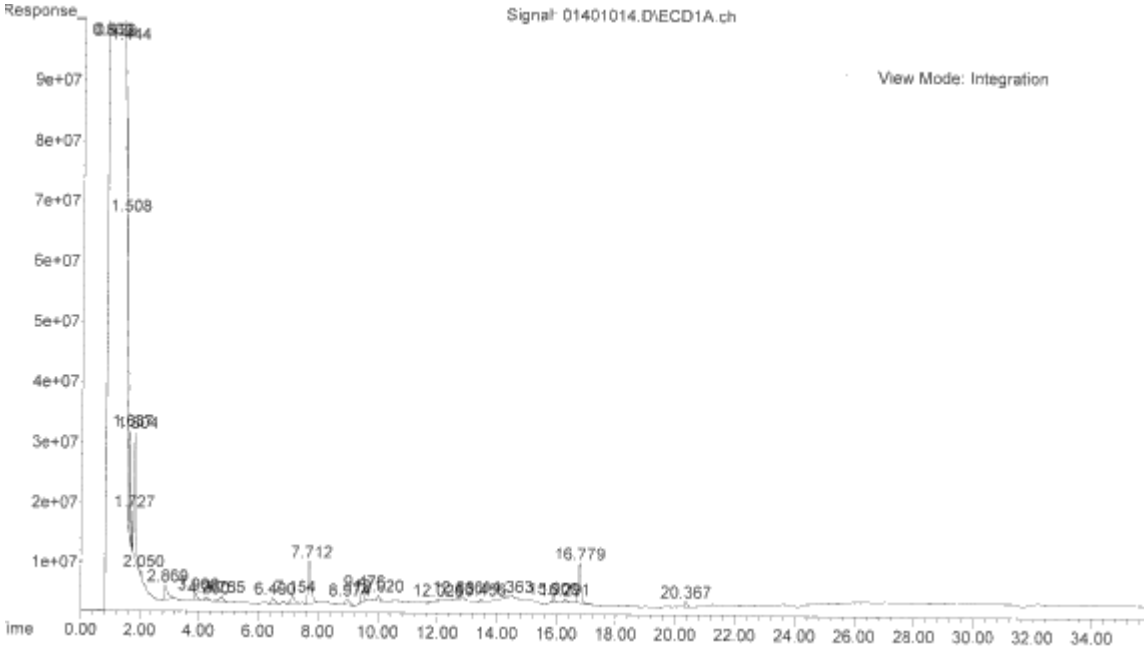

Fig (2): Detected pesticides sample of Catfish.

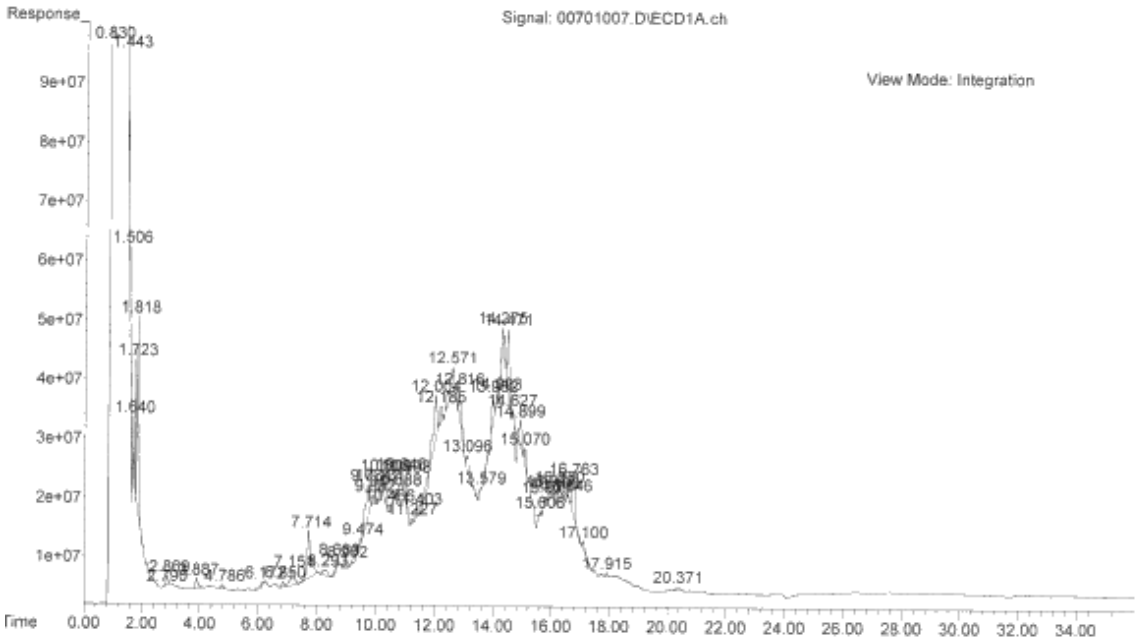

Fig (3): Detected pesticides sample of Tilapia.

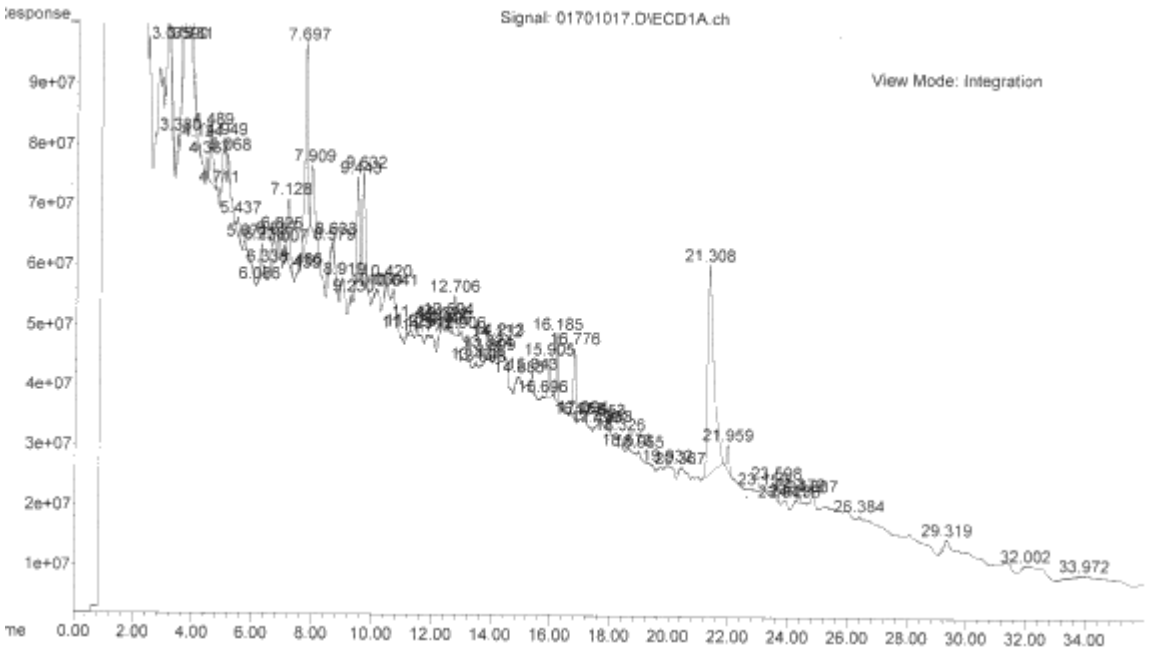

Fig (4): Detected pesticides sample of Mackerel.

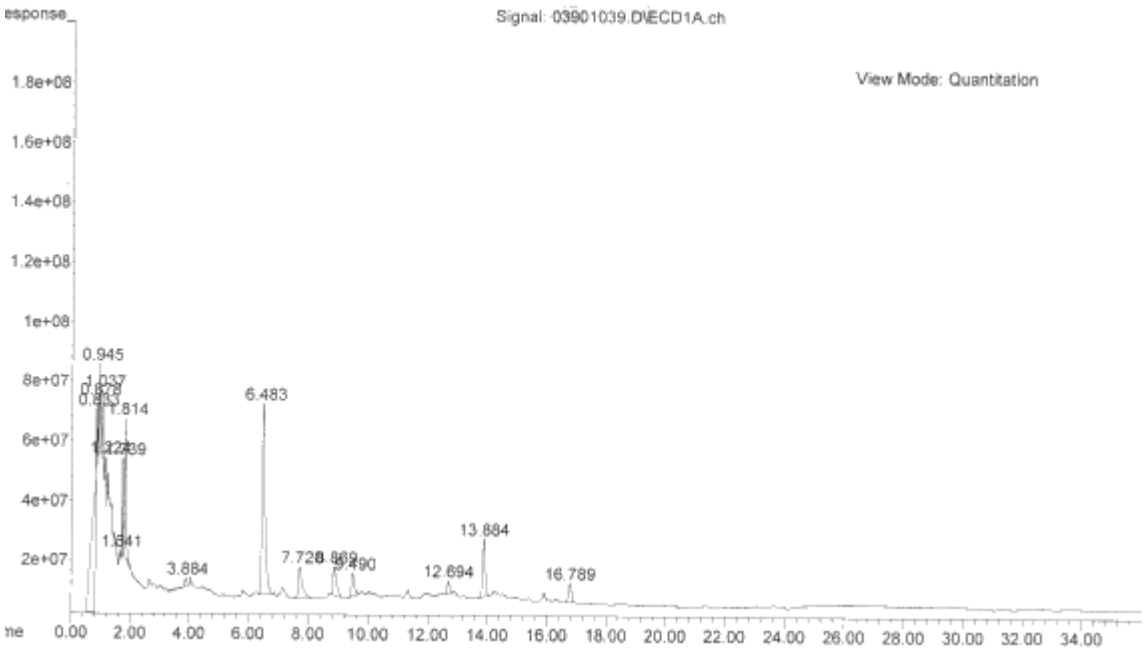

Fig (5): Detected pesticides sample of Tuna.

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