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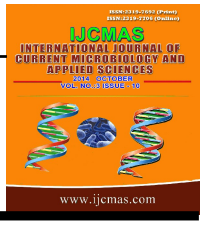

Original Research Article

Determination of organochlorine pesticide residues in Libyan fish

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K e y w o r d s

Organochlorine pesticide, Libyan fish, *Thunnus albacares*, *Sardinella aurita*, *Boops boops*

A B S T R A C T

The study was conducted to assess the level of contamination of marine fish persistent chemicals such as many organochlorine pesticide residues, where most countries suffer from the problems of pollution of marine fish persistent chemicals which have negative effects on human and animal health as cause of cancer, kidney failure, liver and fetal abnormalities as a result of accumulation in adipose tissue. However, the persistence of high levels of these pollutants with seafood is unknown accurately enough. And the aim of this study is to estimate organochlorine pesticide residues in Libyan fish where they were pulling samples of fish during the month (September, October, and November, 2013) from the local market to market the fish Port of Tripoli Sea, which is the main source of samples fresh. Samples were representative of the types of fatty fish include Round Sardinella (*Sardinella aurita*), European Pilchard (*Thunnus thynnus*), Yellow Fin Tuna (*Thunnus albacares*) and Bogue (*Boops boops*). The results showed that there was no concentrations higher than the Permissible limit, according to FAO, global in all tissues of the fish, which were estimated by organochlorine pesticides as for estimating vehicle organochlorine fats in fish has been found that some types of sardines contain high concentrations of endosulfan (5.5058) and Heptachlor epoxide (2.4366) and Methoxychlor (6.1312) and some types of mackerel contain a high concentration of Heptachlor epoxide (8.4513) and some types of tuna contain a high concentration of Dieldrin (9.1996) and these concentrations are higher than the Permissible limit, according to FAO where concentrations were calculated in mg / kg BW of fish.

Introduction

Organochlorine pesticides (OCPs), such as DDT and their metabolites, dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE) (sum of o,p_- and p,p_- DDT, DDD, and DDE is designated as DDTs), and hexachlorocyclohexanes ([HCHs]; including -HCH, the major constituent [60%] of the technical mixture; -HCH, with the highest toxicity and accumulation potential for

mammals; and -HCH, the only insecticidal isomer; sum of them represents more than 85% of the technical mixture), are ubiquitous in the environment and may continue to pose health threat to both wildlife and human beings, due to their persistency, bioaccumulative ability, and potential toxicity. In China, OCPs widely had been used in agriculture and sanitation for several decades until the official ban on their usage in 1983. The amounts of HCHs and DDTs produced in China were estimated at 4.9 and 0.4 million metric tons, accounting for 33 and 20%, respectively, of the total global production, Hua X and Shan Z (1996). More recent data showed that the average annual application of OCPs reached 37.2 kg per hectare from 1980 to 1995, four times higher than the country's average; Wong et al. (2005).

Organochlorine pesticides are among the agrochemicals that have been used extensively for long periods. They have been used widely in agriculture, as well as, in mosquito, termite and tsetse fly control programs, Guo et al. (2008). OC pesticides are characterized by low polarity, low aqueous solubility and high lipid solubility (lipophilicity) and as a result they have a potential for bioaccumulation in the food chain posing a great threat to human health and the environment globally, Afful et al. (2010). Residues and metabolites of many OC pesticides are very stable, with long half lives in the environment, El-Mekkawi et al. (2009). Studies have shown that DDT is still in its highest concentration in biota of some areas. It is a hydrophobic molecule which disrupts ionic channels like Na+-K+ pumps in nervous cell membrane leading to automatic stimulation of neurons and involuntary contraction of muscles, Esmaili Sari (2002). Many other recent works have indicated the presence of OC residues in surface waters, sediments, biota and vegetations, Afful et al. (2010) ; Darko et al. (2008) ; Ize-Iyamu et al. (2007). The persistent nature of organochlorine residues in the environment may pose the problem of chronic toxicity to animals and humans via air, water and foods intake. Many of these OC pesticides and their metabolites have been implicated in a wide range of adverse human and environmental effects including reproduction and birth defects, Edwards (1987), immune system dysfunction, endocrine disruptions and cancer, Adeyemi et al. (2008).

Fish are used extensively for environmental monitoring, Lanfranchi et al. (2006), because they uptake contaminants directly from water and diet. Generally the ability of fish to metabolize organochlorines is moderate; therefore, contaminant loading in fish is well reflective of the state of pollution in surrounding environments, Guo et al. (2008).

The determination of OC residues in fish, sediments and water may give indication of the extent of aquatic contamination and accumulation characteristics of these compounds in the tropical aquatic biota that will help in understanding the behaviour and fate of these persistent chemicals, Kannan et al. (1995).

Materials and Methods

pesticide standards were used in this study: dichlorodiphenyltrichloroethane, o, p'-dichlorodiphenyltrichloroethane), Benzene hexachloride (BHC) (Lindane), Chlordane, Aldrin, Dieldrin, Endrin, Heptachlor, Heptachlor epoxide, Endosulfan(1(α), 11(β)), Methoxychlor were used.

Fish Samples

Forty five samples of fish representing species of : Round Sardinella (*Sardinella aurita*), Euro Peqn Pilchard (*Thunnus*

thynnus), Madeiran Sardinella (*Sardinella maderensis*), Chub Mackerel (*Scomber japonicus*), Atlantic Mackerel (*Scomber scombrus*), Herring (*Clupea harengus*), Roving Grey Mullet (*Liza carinata*), Flathead Grey Mullet (*Mugil cephalus*),Thicklip Grey Mullet (*Chelon labrosus*), Boxlip Mullet (*Cdeachilus labeo*), Albacore (*Thunnus alalunya*), Bluefin Tuna (*Thunnus thynnus*), Yellowfin Tuna (*Thunnus albacares*) and Bogue (*Boops boops*) were collected from Tripoli market in Libya during (2013).

Methods

Instrument Conditions

The GC oven temperature was initiated at 140'C for 2 min, raised to 200°C at 4"Clmin, kept at 200°C for 5 min, raised to 230'C at S0C/min, kept at 230°C for 5 min, raised to 260°C at 2"CI min, and then kept at 260°C for 6 min. The temperatures of the injector and detector were 250 "C and 320"C, respectively. The injection volume was 1 pL. The flow rates of carrier gas (Nz) and makeup gas (N2) were kept at 1.9 and 30 mLImin, respectively.

Sample collection (sampling and processing)

The process of sampling is considered an important step and sensitive in research methodology, where take all scientific procedures and laboratory followed in sampling and taking into account all the circumstances that could affect or interfere in the readings obtained in the final results of the search, and this is a summary of the steps to withdraw the sample collection and processing: sampling was in the month (September, October, November, 2013 m) from the local market for the marketing of fish and sea port of Tripoli, which is the main source of fresh samples. Samples were representative of all types of fish mental include (sardines - Alkuala - Ranja), and fish half mental and include (mullet - tuna - Alboukh), according to the guide issued by the Research Center of Marine Biology (manual bony fish water Libyan, for the year 2009). The weight of the sample of each type of fish, ranging from (4 - 8 Kg). Process took place sample processing (cleaning and removing the viscera) and washing by deionized water (distilled water). Samples are placed in folders Ice (Ice Box) and transported to the laboratory. Process conducted chop and homogenized samples are placed in aluminum dishes. Samples stored at a temperature of not less than (- 20 ° C). The sample is divided into two parts is estimated pesticides organochlorine where: 1 - is the extraction of fat and pesticides organochlorine estimate. 2 - Determination of pesticides in organochlorine tissue sarcomas directly. conducted initial tests of the samples, the most important measurement of the proportion of mind using the device Sukshilt with solvent Butrollym ether (Petroleum ether), and then extract the largest possible size of the anointing of the samples the size of not less than (12-15 ml) of each sample with a rush out into account repeat 3 times to sample , according to the methods of internationally accredited (AOAC Methods), which rely on the extraction of the fat first sample in the detection and estimation of pesticide residues in fish organochlorine.

Determination of pesticide residues

Pesticide residues were determined according to AOAC (1995). Chromatographic analysis was performed with a Hewlett-Packard 5890 system with Ni 63 electron capture detector (ECD), fitted with HP- 1 capillary column (cross linked methyl silicon gum. 30m length x 0.25mm

diameter x 0.25 µm film thickness). The oven temperature was programmed from 160°C to 220 °C with rate of 5°C/min, and continued for total of 30 min. Injection and detector temperatures were 220 and 300°C, respectively. Recoveries of pesticides by this method were determined by fortification of the samples with definite concentrations of pesticides standards, and the recoveries ranged between 90 to 94 %. The limit of detection (in µg/g) was 0.02 for DDT derivatives and 0.01 for the other pesticides under investigation.

Gas chromatographic method for Chlorinated pesticides and polychlorinated biphenyl residues in fish

A. Principle

Chlorinated pesticides and polychlorinated biphenyls (PCBs) are extracted from prepared fish test portion with petroleum ether, cleaned up on Florisil column, and determined by GC against reference standards.

B. Apparatus

(a) Gas chromatograph.-With on-column injection system, 6 ft (1.8m) glass column (4 mm id), packed with 10% DC-200 on 80-100 mesh Chromosorb WHP, and electron capture detector. Other liquid phases such as 5% OV-101 on suitable supports may be substituted if known to give adequate resolution for compounds present in test samples. Linearized 6 3 ~deit ector capable of producing Y, scale deflection for 1 ng heptachlor epoxide is suggested; however, other equivalent electron capture detectors may be used. Operate GC in accordance with manufacturer's directions, adjusting to provide necessary response and resolution.

(b) Chromatographic tube.-10 id x 300 mm column with Teflon stopcock, coarse fritted disk, standard taper 24/40 top joint (Kontes Glass Co. 420550, or equivalent).

(c) Kuderna-Danish (K-D) concentrators.-Snyder distilling column (Kontes 503000-0121); 125 mL K-D flask (Kontes 570001-9010) (special item) standard taper 19/22 lower joint; 10 rnL concentrator tube (Kontes K-570050-1025).

(d) Micro Snyder column.-Kontes 569251, standard taper 19/22.

C. Reagents

(a) Florisi1.-PR grade, 60-80 mesh . Must meet 970.52B (i).

(b) Solvents.-Petroleum ether, ethyl ether, hexane, and alcohol, known to be suitable for pesticide residue determination.

(c) Glass wool (Pyrex).-Must be free of interferer's with electron capture detection.

(d) Sodium sulfate.-Anhydrous, granular, reagent grade free of interference with electron capture detection.

D. Extraction

Weigh 20 g thoroughly ground and mixed test portion into metal blender CUP. Moisten 40 g granular Na2S04 with petroleum ether and add to sample. Mix test portion, using stirring rod, let stand 20 min, and mix again. Add 100 mL petroleum ether to test portion and blend in centrifuge bottle 1-2 min. Centrifuge balanced bottle 1-2 min at ca 2000 rpm to obtain clear petroleum ether extract. Place glass wool plug in funnel, overlay with 20 g granular Na2S04, and place funnel in 250 mL volumetric flask. Decant petroleum ether extract through Na2S04, into volumetric flask. Mix test portion again with stirring rod, add 100 mL petroleum ether, and extract as before. Repeat using 70 mL petroleum ether. Dilute to volume with petroleum ether. Transfer 25 mL aliquot to tared 100 mL flat bottom extraction flask. Place flask on steam bath to evaporate solvent, leave additional 30 min on steam bath, remove, and cool. Weigh

flask and determine % fat -in fish: For fish containing < lo% fat, transfer 25 mL aliquot to 125 mL K-D concentrator. For fish containing >lo% fat, take aliquot containing not >200 mg fat. Add several granules of 20-30 mesh carborundum and concentrate to ca 3 mL on steam bath. Let cool and remove Snyder column. Rinse concentrator with two 1 mL portions of petroleum ether and, using only current of air, concentrate sample to 3 mL for transfer to Florisil column.

E. Florisil Cleanup

Use 4 g Florisil adjusted for lauric acid value [JAOAC 51, 29(1968)]. Add Florisil to 300 x 10 mm id chromatographic tube and add Na2S04 to height 2 cm above Florisil. Completely open stopcock, tap tube to settle adsorbent, and mark tube 1 cm above Na2S04 layer. Add20-25 mL petroleum ether wash to Florisil column; as solvent level reaches mark, place 125 mL K-D flask under column. Using disposable Pasteur pipet, transfer 3 mL extract to column, and wash tube with 1 mL petroleum ether and add wash to column. Solvent level must not go below mark. Temporarily close stopcock if necessary. Add 35 mL petroleum ether-ether mixture (94 + 6) and elute PCBs and DDT and its analogs.

When solvent level reaches mark, change K-D flask, and add 35 mL petroleum ether-ether (85 + 15) to elute compounds such as dieldrin and endrin. Add several granules of carborundum to first concentrator, attach Snyder column, and carefully concentrate on steam bath. Let concentrator cool, remove Snyder column and evaporate solvent under air to appropriate volume for GC determination. Fractions containing mixture of PCBs and chlorinated compounds such as DDE may require additional separation techniques.

F. Additional Cleanup

Often additional cleanup is required for second fraction (85 + 15) to prevent deterioration of GC column.

G. Gas Chromatography

According to JAOAC 66,969(1983).

Analysis of the Total Fat Content in Fish

The method developed by Undeland et al (1998) was followed. Fish meat (10 g) was chopped and then mixed with 16 mL of isopropyl alcohol. The mixture was ground for 30 sec while kept in an ice hath. After adding 32 mL of n-hexane, the mixture was ground for another 30 sec and then centrifuged at 19,600 g (11500 rpm) for 15 min at 4°C. The n-hexane layer was collected and dried over nitrogen gas in a 28°C water bath. The fat content of the fish sample was thus calculated on the basis of the mass of dried residue.

Concentration of compound in sample

To calculate concentration of each compound in sample, follow the equation:

Concentration (µg/g) =

$$\frac{\textbf{Area sample} \times \textbf{concentration std. (0.5 µg)}}{\textbf{Area std.} \times \textbf{weight of sample (g)}}$$

Results and Discussion

Distribution concentrations (ppm) of pesticide residues detected in tissue fish samples collected from Tripoli

The result in Table (1) and illustrated figure (1) showed the distribution concentrations (ppm) of organochlorine pesticide residues detected in tissue fish samples collected

from Tripoli. Although the tissue fish samples of Round Sardinella (Sardinella aurita), European Pilchard (Thunnus thynnus), Madeiran Sardinella (Sardinella maderensis), Chub Mackerel (Scomber japonicus), Atlantic Mackerel (Scomber scombrus), Herring (Clupea harengus), Roving Grey Mullet (Liza carinata), Flathead Grey Mullet (Mugil cephalus),Thicklip Grey Mullet (Chelon labrosus), Boxlip Mullet (Cdeachilus labeo), Albacore (Thunnus alalunya), Bluefin Tuna (Thunnus thynnus), Yellow Fin Tuna (Thunnus albacares) and Bogue (Boops boops) had pesticide residues such as (Aldrin, Endrin, Endosulfan, Endosulfan sulfate, δ-Chlordane, α-Chlordane, Heptachlor epoxide, Heptachlor, p,p'-DDD, Methoxychlor, α-BHC, γ- BHC), but its concentration were lower than the permissible limits (ppm) (FAO, 1996).

Distribution concentrations (ppm) of pesticide residues detected in fat fish samples collected from Tripoli

The result in Table (2) and illustrated figure (2) showed the distribution concentrations (ppm) of pesticide residues detected in fat fish samples collected from Tripoli. Although some fat fish samples had pesticide residues and its concentration were lower than the permissible limits (ppm) (FAO, 1996), but Round Sardinella, Madeiran Sardinella and Atlantic Makerel had high concentration of Endosulfan (5.5058±0.4), Heptachlor epoxide (6.1311±0.4, 8.4513±0.4), respectively. These concentrations were higher than the permissible limits (ppm) (FAO, 1996).

From the above results it can be concluded that, although the incidences of pesticides were relatively high, mean concentrations were below the permissible limits proposed by FAO, 1996. On the other hand, the organochlorine pesticides were predominant in the fish samples, but in low concentrations, which might be attributed to the association of organochlorine pesticide residues with the fat phase in fish. However, some types of organophosphorus pesticides were found.

Other studies have been carried out in Egypt to detect pesticides in fish collected from different locations. It was found that the organochlorines were the main pesticides found in fish samples. DDT and its derivatives predominated, as reported by Hamza & Micheal (1979); Sharaf (1984); Abou-Donia (1990) and Dogheim et al. (1990), who found averages of 0.114, 0.061, 0.291 and 4.17 ppm, respectively. Lindane (mean 0.59 ppm), BBHC (mean 0.435 ppm) and chlordane (mean 0.059 ppm) were also detected by Abou-Donia (1990), while Dogheim et al. (1990) detected lindane (mean 0.07 ppm), heptachlor and heptachlor epoxide (means 0.56 and 0.14 ppm), aldrin and dieldrin (means 0.59 and 0.61 ppm) and endrin (mean 0.70 ppm).

It is worthy of note that, in studies in developed countries, the organochlorines were also the main pesticides detected in fish.

Stout (1980); Falandysz (1986) and Cocchieri & Arnese (1988) reported that DDT predominated and shown 0.18, 2.6 and a range of 0.0184, 0.153 ppm, respectively. Stout (1980) also surveyed residues of endrin and dieldrin in the Northwestern Atlantic Ocean and Northern Gulf of Mexico, reporting mean values of 0.008 and 0.007 ppm, respectively.

Table.1a The distribution concentrations (ppm) of pesticide residues detected in tissue fish samples collected from Tripoli (September, 2013)

Pesticides detected	**Concentrations of pesticide residues (ppm)**							**Permissible limits(ppm) (FAO, 1996)**
	Mean&SD							
	Sardine			**Mackerel**			**Herring**	
	Round Sardinella	European Pilchard	Madeiran Sardinella	Chub Mackerel	Atlantic Makerel	Mackerel		
Aldrin	-	-	-	0.0004±0.005	-	0.001±0.03	-	0.3
Endrin	-	-	-	-	-	0.0001±0.004	-	0.3
Endosulfan	-	-	-	0.0003±0.006	-	-	-	0.3
Endosulfan sulfate	-	-	-	0.0002	-	-	0.0039±0.01	0.3
δ-Chlordane	0.0001±0.005	-	-	-	0.0053±0.008	-	-	0.3
Heptachlor epoxide	-	0.0009±0.004	-	-	0.0002±0.006	-	-	0.3
p,p'-DDD	-	-	-	-	-	0.0021±0.05	-	5.0
Methoxychlor	-	-	-	-	-	0.0008±0.003	-	0.3

Table.1b The distribution concentrations (ppm) of pesticide residues detected in tissue fish samples collected from Tripoli (September, 2013)

Pesticides detected	**Concentrations of pesticide residues (ppm)**								**Permissible limits(ppm) (FAO, 1996)**
	Mean&SD								
	Mullet				**Tuna**			**Bogue**	
	Roving Grey Mullet	flathead Grey Mullet	Thicklip Grey Mullet	Boxlip Mullet	Albacore	Blue Fin Tuna	Yellow Fin Tuna		
Endrin	-	-	-	-	-	0.0037±0.02	-	-	0.3
Endosulfan	0.000010±0009	-	-	-	-	0.001±0.04	-	-	0.3
Endosulfan sulfate	-	-	0.004±0.07	-	-	-	-	-	0.3
α-Chlordane	0.0011±0.009	-	-	-	0.0001±0.003	-	-	0.0022±0.05	0.3
Heptachlor epoxide	-	0.0001±0.003	0.0019±0.02	-	0.0015±0.02	-	0.001±0.03	-	0.3
Heptachlor	-	-	-	0.0001±0.006	-	-	-	-	0.3
Methoxychlor	-	-	-	0.002±0.01	0.002±0.03	0.004±0.04	-	-	0.3
α-BHC	-	0.008±0.02	-	0.0034±0.007	-	0.277	-	-	0.3
γ- BHC	-	0.0001±0.008	-	-	-	-	-	-	0.3

Table.2a The distribution concentrations (ppm) of pesticide residues detected in fat fish samples collected from Tripoli (September, 2013)

Pesticides detected	**Concentrations of pesticide residues (ppm)**							**Permissible limits(ppm) (FAO, 1996)**
	Mean&SD							
	Sardine			**Mackerel**			**Herring**	
	Round Sardinella	European Pilchard	Madeiran Sardinella	Chub Mackerel	Atlantic Makerel	Mackerel		
Aldrin	0.0026±0.06	-	-	-	-	0.015±0.02	-	0.3
Endosulfan	5.5058±0.4	-	-	-	-	-	0.032±0.03	0.3
Endosulfan sulfate	-	-	-	-	0.0045±0.02	-	-	0.3
δ-Chlordane	-	-	0.0013±0.05	-	-	-	-	0.3
α-Chlordane		-	-	-	-	-	-	0.3
Heptachlor epoxide	2.4366±0.2	-	6.1311±0.4	-	8.4513±0.4	-	-	0.3
p,p'-DDE	1.386±0.7	-	0.9147±0.1	-	-	-	-	5.0
p,p'-DDD	-	-	-	-	-	-	-	5.0
Methoxychlor	-	0.0016±0.05	-	-	-	-	-	0.3

Table.2b The distribution concentrations (ppm) of pesticide residues detected in fat fish samples collected from Tripoli (September, 2013)

Pesticides detected	**Concentrations of pesticide residues (ppm)**								**Permissible limits(ppm) (FAO, 1996)**
	Mean&SD								
	Mullet				**Tuna**			**Bogue**	
	Roving Grey Mullet	flathead Grey Mullet	Thicklip Grey Mullet	Boxlip Mullet	Albacore	Blue Fin Tuna	Yellow Fin Tuna		
Aldrin	-	0.005±0.04	-	0.015±0.05	-	-	-	-	0.3
Endrin	-	-	-	-	-	-	-	-	0.3
Dieldrin	-	-	-	-	0.0031±0.007	-	-		0.3
Endosulfan	0.0011±0.03	-	-	-	-	-	-	-	0.3
Endosulfan sulfate	0.0011±0.03	-	-	-	-	-	-	-	0.3
Heptachlor epoxide	-	-	-	-	-	9.1996±0.6	-	-	0.3
Heptachlor	-	-	-	-	-	-	-	0.0012±0.04	0.3
β-BHC	0.0981±0.001	-	-	-	-	0.0034±0.06	-	-	0.3
Lindane	-	-	0.002±0.04	-	-	-	-	-	0.3

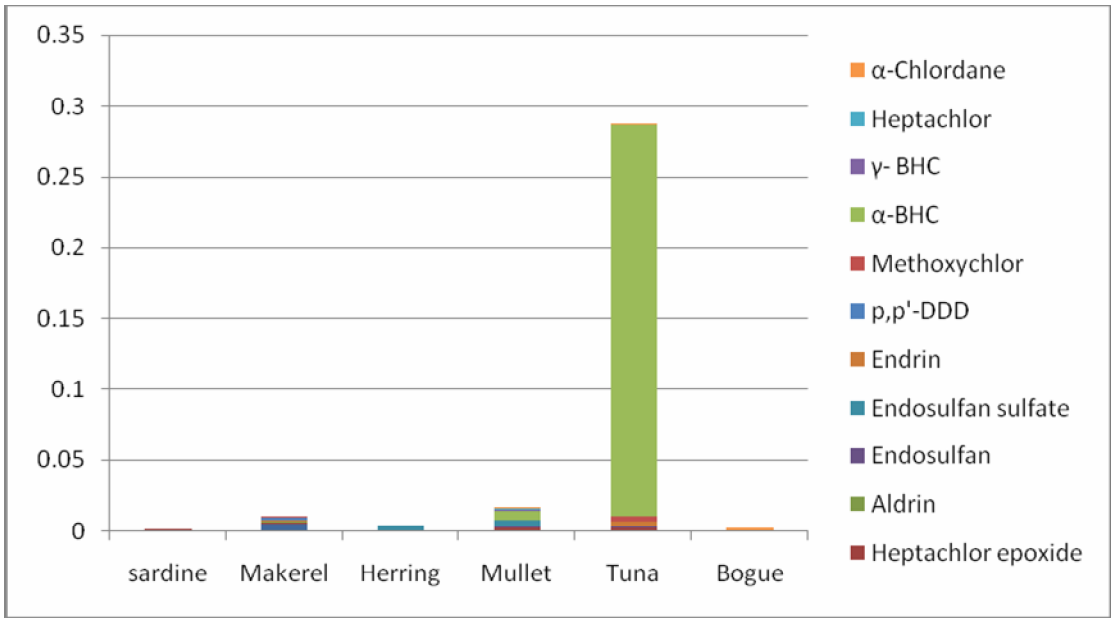

Fig.1 Survey of organochlorine in Libyan tissue fish isolated from Tripoli market during (2013)

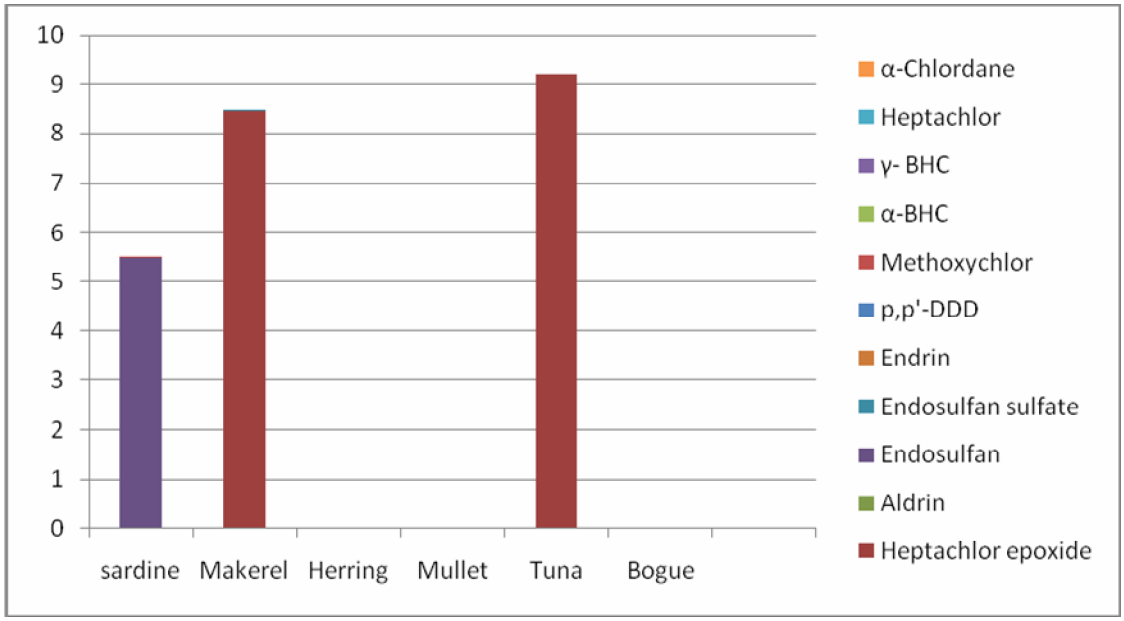

Fig.2 Survey of organochlorine in Libyan fat fish isolated from Tripoli market during (2013)

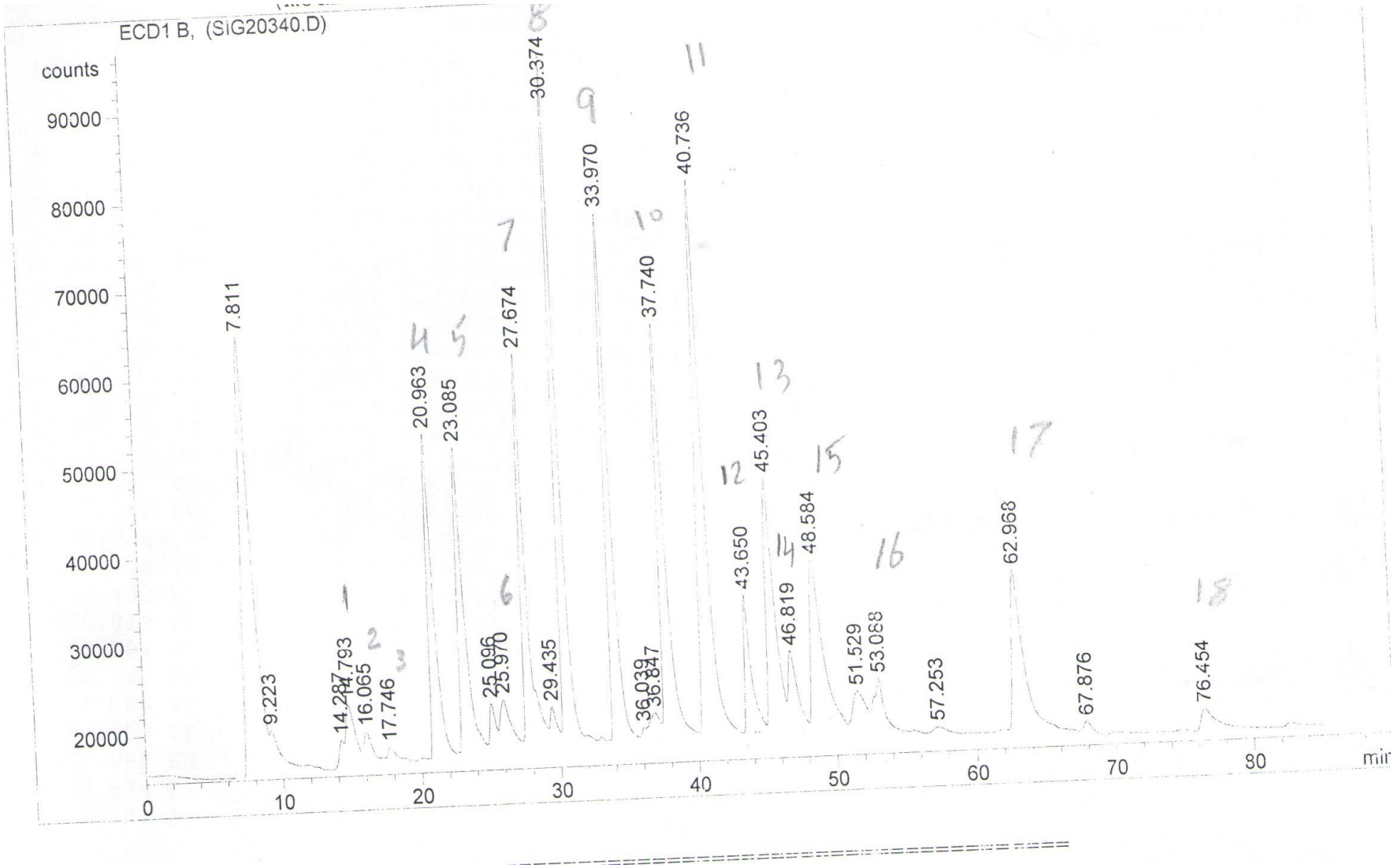

Fig.3 standard samples

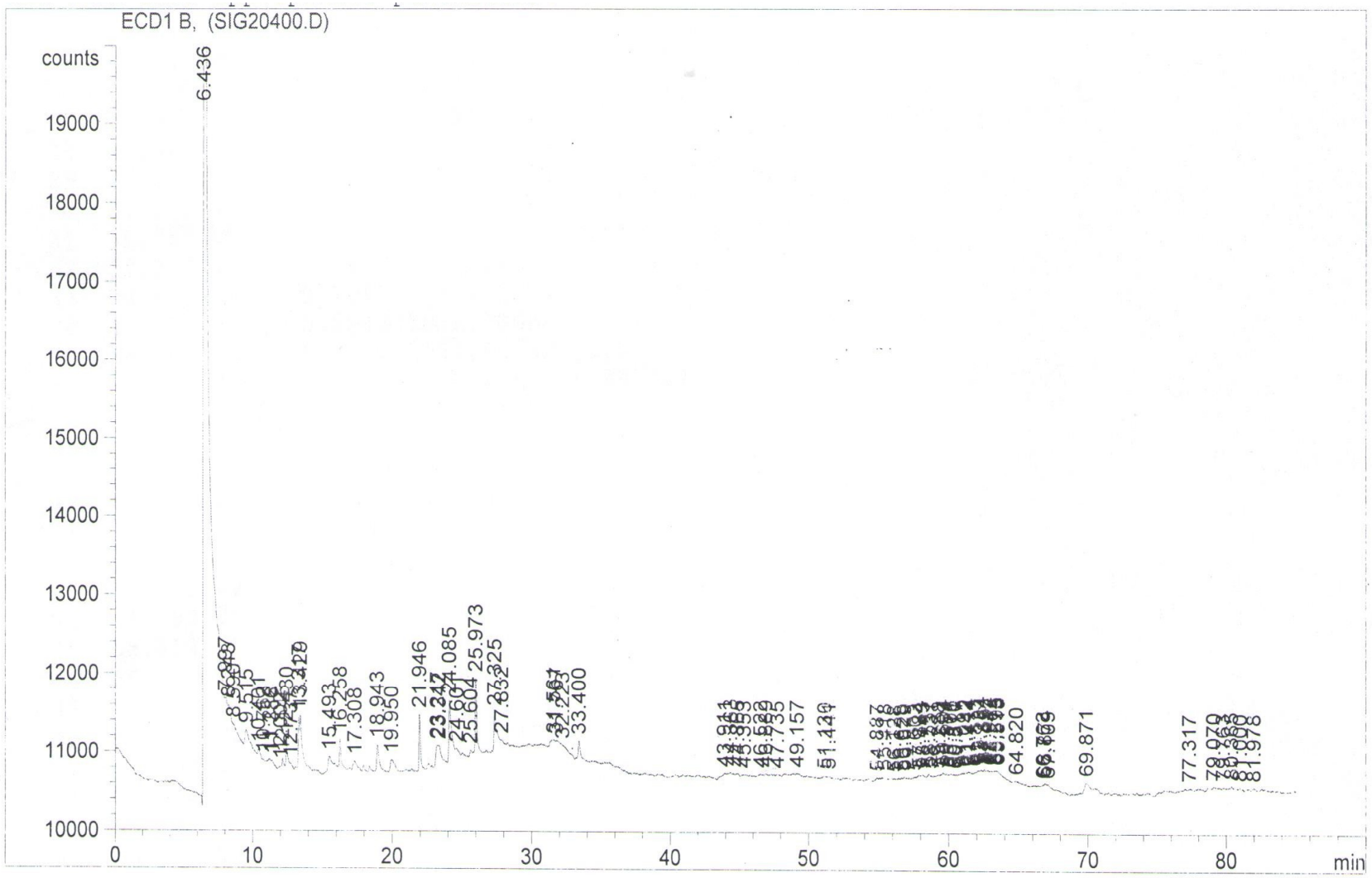

Fig.4. Detected sample

Arzi et al (2011) reported that concentrations of hexachlorocyclohexane isomers (β,γ,δ HCH), dichlorodiphenyl trichloro ethane (pp, opDDT) and its metabolites (ppDDE, ppDDD), aldrin, dieldrin, heptachlor , heptachlor epoxide, endosulfan isomers (α, β) and methoxychlor were determined in Benni fish collected from Shadegan, Mahshahr and susangerd cities at Khozestan province in Iran. All the collected fish were contaminated by 14 investigated organochlorine pesticides. The highest and lowest mean concentrations of organochlorine pesticides belonged to β-HCH (65.36 µg/kg) and opDDT (0.13 µg/kg) and were found in Mahshahr and Shadegan Benni fish respectively.

Abdallah et al (1990) collected one hundred random samples representing 34 species of marketable fish from 8 Egyptian governorates during March 1986-88 and analysed for organochlorine pesticides. β-BHC [β-HCH] and lindane predominated, with maximum levels of 435.3 and 59.0 µg/kg, respectively, for fish samples obtained from Damietta governorate. The corresponding levels of aldrin and o,p′-DDT were 34.27 and 734.1 µg/kg, respectively, for fish from the Red Sea governorate. γ-Chlordane, p,p′-DDE and p,p′-DDT were found in fish from Ismailia governorate at respective maximum levels of 36.17, 234.4 and 57.19 µg/kg. Heptachlor was identified at 8.5 µg/kg in Port Said governorate fish and o,p′-DDE at 10.59 µg/kg in Suez governorate fish. Mirex was not detected at all.

To reducing the Risk of Pesticides: Prior to using a pesticide, consider the following:

1. Use a Pesticide Only When Necessary. Is the problem bad enough to justify the use of a toxic chemical? Are there alternative ways of treating the problem? Landowners should consider the costs and consequences of pesticide treatment relative to the problem.

2. Use Less Toxic Pesticides. One way to reduce the effects of pesticides on aquatic systems is to use those chemicals that are least poisonous to aquatic life. Select the least toxic material.

3. Use Safe/Sensible Application Methods. The first rule of responsible pesticide use is to read and then reread the pesticide label and follow the directions precisely.

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