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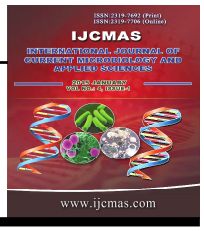

Original Research Article

Occurrence of Organophosphorous Pesticide Residues in some Fish Species Collected from Local Market in Tripoli, Libya

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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 Organophosphorus 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 Organophosphorus 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*), Madeiran Sardinella (*Sardinella maderensis*), Chub Mackerel (*Scomber japonicus*), Atlantic Mackerel (*Scomber scombrus*), Herring (*Clupea harengus*), and half of fatty fish include 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*). Dimethoate, Disulfaton, Famphur, Methyl parathion, O,O,O-Triethyl phosphoro thioate, Parathion and Phorate were detected in fish tissues and fat samples at level below the maximum residue limits (MRL). The results showed that there was no concentrations higher than the maximum residue limits, according to FAO and WHO, global in all tissues of the fish, which were estimated by Organophosphorus pesticides as for estimating vehicle Organophosphorus fats in fish has been found that some types of sardines and Grey Mullet contain high concentrations of Dimethoate (4.7611±0.02 , 1.1741±0.05) respectively, and some types of Tuna contain a high concentration of Famphur (1.0627±0.03), these concentrations are higher than the maximum residue limits, according to FAO and WHO where concentrations were calculated in mg / kg (µg/g , ppm) BW of fish.

Keywords

Organo-phosphorus , Pesticide residues, Libyan fish, and Marine fish

Introduction

In recent times, the extent of the use of pesticides, and their mode of application agriculture have been of much concern to environmental scientists. Alongside their

uses are also the residual effect of these pesticides and particularly their replicating effect on human health (Hurst *et al.,* 1991).

Pesticide residue as used in this research work is the residual amount of active components of a particular pesticide or group of pesticides found in a commodity (that is food or water) after the pesticide has accomplished the primary purpose of its application; or the residual amount of a pesticide found in a product which has been in the area of the pesticide application.

Though pesticides are often misunderstood to refer only to insecticides, the term pesticide also applies to herbicides, fungicides, acaricides and other substances used to control pest.

Under the US law, a pesticide may also refer to any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant (Hurst *et al.,* 1991).

The increasing population necessitates more agricultural products and food stuffs that consequently need more pesticide usage to destroy any pests (Khazaei *et al.,* 2010; Arjmandi *et al.,* 2010). The pesticide compounds include Organophosphorus, Organochlorine, Carbamate and Pyrethroid derivatives, (Samadi *et al.,* 2009). They have some side effects on living organisms such as organophosphorus inhibits cholinesterase activity and wit makes central nervous system (CNS) functional disturbances. Organochlorine accumulates in living organism bodies and also in food chain. Carbamate on man derivatives causes genetic mutations and CNS functional disturbances (Samadi *et al.,* 2009).

Pesticides are used widely to improve agricultural production and also to prevent arthropod-borne diseases. But they are used improperly due to the lake appropriate knowledge about their applications and ecosystem and they contaminate soil, surface and underground water resources (Khodadadi *et al.,* 2010; Srivastava *et al.,* 2010). Relevant poisoning in many countries, especially in developing countries is considered the second causes of mortalities after infectious diseases (Farshad, 2000). Pesticides cause untoward effects on man in two ways. Firstly, they have direct effects on the health of persons who use them; and secondly, their remnants accumulate in foodstuffs which also produce side effects on man (Yazgan and Tanik, 2010). The side effects include short term ones like abdominal cramps, vertigo, headaches, diplopia, nausea, ocular disturbances and dermatopathies. Long term adverse effects include increased likelihood of respiratory failures, depression, nervous defects, prostate cancer, leukemia and infertility. These problems are considered the major health problems in the world (Yazgan and Tanik, 2010; Ghasemi and Karam, 2009). The various investigations conducted on farmers in terms of their health status demonstrated that pesticides may increase from the likelihood of Parkinson disease (Hoek and Dawson, 2007; Bradman *et al.,* 2011). Environmental pollution by pesticides has been identified as one of the major environmental impacts from agriculture (Skinner *et al.,* 1997). Parent compounds as well as metabolites of pesticides have been identified in air (Rudel, 1997), water (Boonyatumanond *et al.,* 1997) and soil (Redondo *et al.,* 1997). The list of pesticide related compounds which have been identified in the environment and proved to be carcinogenic is growing as new methods of detection have been developed and sensitivities and specificities of assays have been improved.

Pesticides differ in their mode of action, uptake by the body, metabolism and elimination from the body and ecological

toxicity potential. Because of these differences, some from the pesticides show acute short term effects, while others tend to accumulate in the body and with time demonstrate sublethal adverse health effects. Many of these compounds also persist in the environment and bioaccumulate in the animal and human tissues (El-Sebae, 1986). The degradation and transformation of the nonpersistent chemicals in the environment are dependent on their physicochemical properties, the environment in which they reside and the threshold levels of these chemicals in the environment. Degradation and transformation processes do not always result in decreased activity or dilution of the parent compound, for the degraded or transformed products are at times more toxic, resulting in biomagnification of the toxicity of the parent compound.

The presence of organophosphorus in sediment samples suggests that for these environmentally persistent compounds have been resident in the water bodies for a long time and may have contaminated the aquatic flora and fauna. The most striking effect of some of the pesticides in water is their ability to concentrate in the fatty tissue of successively higher components of the aquatic food chain.

The frequent presence of pesticides and their high toxicity along with considerable bioaccumulation in freshwater fishes make them toxicants that should be given due consideration in aquatic toxicology. Fish accumulate xenobiotic chemicals, especially those with poor water solubility because of the very intimate contact with the medium that carries the chemicals in solution or suspension and also because fish have to extract oxygen from the medium by passing enormous volumes of water over the gills. Fish kill or injury due to pesticides contamination is considered the primary cause of reducing fish populations and other animals including humans through the food chain, Akueshi *et al.* (2003). Behavioral avoidance of contaminants may be an additional cause of reduced fish populations, Akueshi *et al.* (2003).

Materials and Methods

Materials

All solvents were Pesticide Residue grade and were purchased from Sigma, USA. Dimethoate, Disulfaton, Famphur, Methyl parathion, O,O,O-Triethyl phosphoro thioate, Parathion and Phophorate were purchased from Sigma, USA. All other chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck.

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.

Instrument conditions

The GC oven temperature was initiated at 140°C for 2 min, raised to 200°C at 4 min, kept at 200°C for 5 min, raised to 230°C for 5 min, raised to 260°C at 2 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 ml min, 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 to 8 Kg. Each sample was cleaned (cleaning and removing the viscera) and washed 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). Sample was divided into two parts and the estimation of organophosphorus pesticides where done by 1. Extraction of fat and organophosphorus pesticides. 2. Determination of Organophosphorus pesticides in tissue sarcomas directly. Initial tests of the samples was conducted by the most important measurement of the proportion of mind using the device Sukshilt with solvent Petroleum ether, and then extracted the largest possible size of the samples not less than 12–15 ml of each sample with a rush out into account repeat 3 times to sample, according to the internationally accredited AOAC Methods, which rely on the extraction of the fat first sample in the detection and estimation of Organophosphorus pesticide residues in fish.

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 phosphorus pesticides residues in fish

Principle

Organophosphorus pesticides are extracted from prepared fish test portion with petroleum ether, cleaned up on Florisil column, and determined by GC against reference standards.

Apparatus

(a) Dispersing unit.-Ultra-Turrax® T-25 basic with a stainless dispersion tool S25N-10G (IKA® Labortechnik, Staufen, Germany).

(b) Suction filter system—Kiriyama funnel (Cat. No. SU-60; Nippon Rikagaku Kikai Co. Ltd., Tokyo, Japan) fitted with a filter paper (Cat. No. 5A-60; Nippon Rikagaku Kikai) was connected with a vacuum SPC joint (Cat. No. 3047-19; Sibata Scientific Technology Ltd., Tokyo, Japan) to a 250 mL graduated cylinder (Cat. No. 2355-250A; Sibata) as described previously (10).

(c) Rotary vacuum evaporator—Rotavapor with vacuum controller V-800 (Büchi Labortechnik AG, Flawil, Switzerland).

(d) GPC system—Automated GPC cleanup system (Shimadzu Corp., Kyoto, Japan) equipped with a CLNpak EV-2000 column (300 × 20 mm id) and a CLNpak EV-G guard column (100 × 20mmid; ShowaDenko, Tokyo, Japan). The UV detector was set at 254 nm. The mobile phase was acetone–

cyclohexane (2 + 8) at a flow rate of 5 mL/min.

(e) Minicolumn suction system (Figure 1).—Glass reservoir (available on special order from Asahi Techniglass Co. Ltd., Nagoya, Japan), stopcock valve (Waters), syringe needle (Cat. No. 875-18; Nippon Rikagaku Kikai), and vacuum joint (Cat. No. 072-1515; NipponRikagaku Kikai). These 4 units were supported by a metallic rack, and 4 vacuum joints were connected with Teflon tubing to a metallic manifold (Cat. No. SKB-04; Nippon Rikagaku Kikai) that was connected to an aspirator.

(f) Dual-column GC system—HP5890 Series II gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with a split-splitless injector installed with a Siltek splitless liner with wool and (Part No. 22406-213; Restek, Bellefonte, PA), HP7673 autosampler, a 30 m × 0.32 mm id, 0.5 µm film thickness, DB-5ms column (J&W Scientific, Folsom, CA) with nitrogen–phosphorus detector and a 30m× 0.32 mm id, 0.25 µm film thickness, DB-1701P column (J&W)with a flame photometric detector with phosphorus filter. The inlets of both columns were connected with a Siltek "Y" press-tight connector (Part No. 20486; Restek), and a 2 m × 0.53 mm id, Siltek guard column (Part No. 10028; Restek) was installed in a single injection port.

The GC conditions were as follows: oven temperature program, initial 60°C (1 min hold), to 200°C at 10°C/min, and then to 280°C at 5°C/min (7min hold) carrier gas, He; carrier gas pressure program, initial 130 kPa (1min hold), to 204 kPa at 2 kPa/min; injection temperature, 240°C; temperature of both detectors, 300°C; injection volume, 2 _L; splitless injection

Reagents

(a) Solvents and chemicals—Acetonitrile, sodium chloride, ethyl acetate, anhydrous sodium sulfate, acetone, cyclohexane, and petroleum ether (Wako Pure Chemical Industries Ltd., Osaka, Japan) were pesticide-analysis grade. Phosphoric acid, dipotassium hydrogen phosphate, and potassium dihydrogen phosphate were analytical grade from Wako. Water was obtained by using a Puric-Z system (Organo Co. Ltd., Tokyo, Japan). Phosphate buffer (1M) was prepared by dissolving 105 g dipotassium hydrogen phosphate and 61 g potassium dihydrogen phosphate in water and diluting to 1 L.

(b) Analytical standards.—Pesticide standards were purchased from Wako and Hayashi Pure Chemical Industries Ltd. (Osaka, Japan). Individual stock standard solutions (1000 mg/L) were prepared in acetone. Quinalphos standard solution was stored at –20°C, and the others were stored at 4°C. Working standard solutions of each pesticide at 1–5 mg/L, depending on the sensitivity of the detector in the recovery tests, were prepared in acetone by dilution of the stock standard solutions. Calibration standard solutions were freshly prepared by diluting the working standard solutions by a factor of 5–10 with acetone.

(c) Minicolumns.—Silica-gel 690 mg Sep-Pak Plus Silica and Florisil 910 mg Sep-Pak Plus Florisil (both Waters, Milford, MA) were preconditioned with 10 mL petroleum ether followed by 10 mL acetone before use.

Extraction

A55 g portion of chopped sample was extracted with 150mL acetonitrile for 2min by using a dispersing unit. The extract was filtered into a 250 mL graduated cylinder containing 25 g NaCl and 25mL 1Mphosphate buffer by using a suction filter system. The graduated cylinder fitted with a cap was shaken, and the acetonitrile layer was cleaned up by a salting-out step. Half of the acetonitrile layer, equivalent to 25 g sample, was transferred to a 500 mL round-bottom flask and evaporated to near dryness by using a rotary vacuum evaporator. To the residue added 50 mL ethyl acetate and 20 g anhydrous sodium sulfate, with sonication for 1min, and the mixture was filtered without suction through a filter paper into a 200 mL round-bottom flask. The 500 mL round- bottom flask was rinsed with two 20 mL portions of ethyl acetate, and the rinses were decanted through the filter paper. The filtrate was concentrated to near dryness by using a rotary vacuum evaporator. The residue was dissolved in acetone–cyclohexane (2 + 8), the volume was adjusted to 5mL, and the mixture was centrifuged for 5 min at 3000 rpm. The supernatant was ready for cleanup.

Cleanup and GC Analysis

A 2 mL aliquot of the extract, equivalent to 10 g sample, was loaded into the automated GPC cleanup system. The first 65 mL of GPC eluate was discarded, and the next 20 mL was collected in a 32 mL collection tube as pesticide fraction 1; an additional 40 mL was collected in 2 more collection tubes as pesticide fraction 2 (Figure 2). As shown in Figure 1, the pesticide fractions were purified by the 2-step minicolumn cleanup without concentration. First, pesticide fraction 2 was loaded on a silica-gel minicolumn, and the eluate was collected in a 100 mL round-bottom flask by using the minicolumn suction system. After a Florisil minicolumn was inserted on the silica-gel minicolumn, pesticide fraction 1 was loaded on the tandem minicolumn, which was then eluted with 15 mL acetone–petroleum ether (3 + 7). The combined eluate was concentrated to near dryness by using a rotary vacuum evaporator. The residue was dissolved in acetone containing triphenyl phosphate at 0.2 mg/L as a retention index standard to a volume of 5 mL. The test solution (1 mL) corresponded to 2 g sample.A2 µL aliquot of the test solution was injected into the dual-column gas chromatograph equipped with nitrogen–phosphorus and FPD.

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) =

$$\frac{\text{Area sample} \times \text{concentration standard}}{\text{Area standard} \times \text{weight of sample}}$$

Where, concentration standard was 10 µg and weight of sample was 2g.

Data handling

Data collected were subjected to one-way analysis of variance (ANOVA) were used to assess whether pesticide residues varied significantly between fish and organs samples, possibilities less than 0.05 ($p<0.05$) were considered statistically significant.

Results and Discussion

There are many factors which may affect the contamination levels of OPs in drainage water such as the presences of most minerals and salts, Schlauch (1989), photosensitizers, temperature, pH, radiation, metal cations, Meikle and Youngson (1970), as well as micro-organisms, Haven and Rase (1990). Dimethoate, Disulfaton, Famphur, Methyl parathion, O,O,O-Triethyl phosphoro thioate, Parathion and Phorate were detected in fish tissue and fat samples (Tables 1 and 2) and Figures (1 and 2).

Distribution concentrations (µg/g) of pesticide residues detected in tissue fish samples collected from Tripoli

The results in Table 1 and Figure 1 showed the distribution concentrations (ppm) of organophosphorus pesticide residues detected in tissue fish samples collected from Tripoli. Although some tissue fish samples of Mackerel and Mullet had pesticide residues such as (Famphur, Parathion, Methyl parathion and Dimethoate but its concentration were lower than the maximum residue limits (µg/g) (FAO and WHO 2013).

The tissue fish samples of Sardine, Herring, Tuna and Bogue were free from these organophosphorus pesticide residues.

Distribution concentrations (µg/g) of pesticide residues detected in fat fish samples collected from Tripoli

The result in Table 2 and 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 maximum residue limits (µg/g) (FAO and WHO 2013). such as some types of Mullet and Tuna, but some Sardine, Mullet and Tuna had high concentration of Dimethoate (4.7611±0.02, 1.1741±0.05), Famphur (1.0627±0.03), respectively. These concentrations were higher than the maximum residue limits (mg/kg) (FAO and WHO 2013). The fat fish samples of Mackerel, Herring and Bogue were free from these organophosphorus pesticide residues.

Table.1 The distribution concentrations (µg/g) of pesticide residues detected in tissue fish samples collected from Tripoli (September, October, and November, 2013)

Pesticides detected	Concentrations of pesticide residues (µg/g)							MRL (µg/g)
	Mean&SD							
	Sardine			Mackerel			Herring	
	Round Sardinella	European Pilchard	Madeiran Sardinella	Chub Mackerel	Atlantic Makerel	Mackerel		
Dimethoate	ND	ND	ND	ND	ND	ND	ND	0.05
Disulfoton	ND	ND	ND	ND	ND	ND	ND	0.02
Famphur	ND	ND	ND	0.0343±0.06	ND	ND	ND	0.05
Methyl parathion	ND	ND	ND	ND	ND	ND	ND	0.05
O,o,o-triethyl phosphorothioate	ND	ND	ND	ND	ND	ND	ND	0.05
parathion	ND	ND	ND	ND	ND	ND	ND	0.1
Phorate	ND	ND	ND	ND	ND	ND	ND	0.05

Pesticides detected	Concentrations of pesticide residues (µg/g)								MRL (µg/g)
	Mean&SD								
	Mullet				Tuna			Bogue	
	Roving Grey Mullet	flathead Grey Mullet	Thicklip Grey Mullet	Boxlip Mullet	Albacore	Blue Fin Tuna	Yellow Fin Tuna		
Dimethoate	ND	ND	0.0242±0.09	ND	ND	ND	ND	ND	0.05
Disulfaton	ND	ND	ND	ND	ND	ND	ND	ND	0.02
Famphur	ND	ND	ND	ND	ND	ND	ND	ND	0.05
Methyl parathion	ND	0.0032±0.04	ND	ND	ND	ND	ND	ND	0.05
O,o,o-triethyl phosphorothioate	ND	ND	ND	ND	ND	ND	ND	ND	0.05
parathion	0.0051±0.07	ND	ND	ND	ND	ND	ND	ND	0.1
phorate	ND	ND	ND	ND	ND	ND	ND	ND	0.05

ND Not Detectable, MRL the maximum residue limits

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

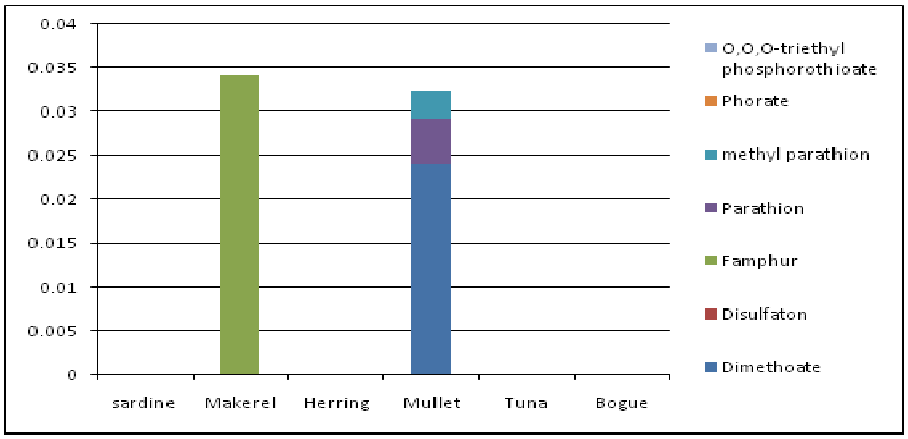


Table.2 The distribution concentrations (µg/g) of pesticide residues detected in fat fish samples collected from Tripoli (September, October, and November, 2013)

Pesticides detected	Concentrations of pesticide residues (µg/g)							MRL (µg/g)
	Mean&SD							
	Sardine			Mackerel			Herring	
	Round Sardinella	European Pilchard	Madeiran Sardinella	Chub Mackerel	Atlantic Makerel	Mackerel		
Dimethoate	4.7611±0.02	ND	ND	ND	ND	ND	ND	0.05
Disulfaton	ND	ND	ND	ND	ND	ND	ND	0.02
Famphur	ND	ND	ND	ND	ND	ND	ND	0.05
Methyl parathion	ND	ND	ND	ND	ND	ND	ND	0.05
O,o,o-triethyl phosphorothioate	ND	ND	ND	ND	ND	ND	ND	0.05
parathion	ND	ND	ND	ND	ND	ND	ND	0.1
Phorate	ND	ND	ND	ND	ND	ND	ND	0.05

Pesticides detected	Concentrations of pesticide residues (µg/g)								MRL (µg/g)
	Mean&SD								
	Mullet				Tuna			Bogue	
	Roving Grey Mullet	flathead Grey Mullet	Thicklip Grey Mullet	Boxlip Mullet	Albacore	Blue Fin Tuna	Yellow Fin Tuna		
Dimethoate	ND	ND	ND	1.1741±0.05	ND	ND	ND	ND	0.05
Disulfaton	ND	ND	ND	ND	ND	ND	ND	ND	0.02
Famphur	ND	0.0195±0.2	ND	ND	1.0627±0.03	ND	ND	ND	0.05
Methyl parathion	ND	0.0132	ND	ND	ND	ND	ND	ND	0.05
O,o,o-triethyl phosphorothioate	ND	ND	ND	ND	ND	ND	0.0185±0.2	ND	0.05
parathion	ND	ND	ND	ND	ND	ND	ND	ND	0.1
phorate	ND	ND	ND	ND	ND	ND	ND	ND	0.05

ND Not Detectable, MRL the maximum residue limits

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

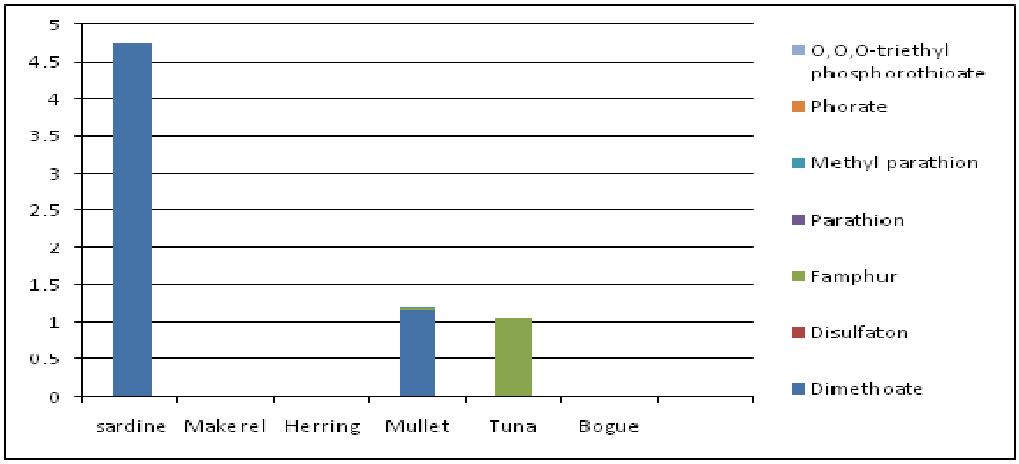
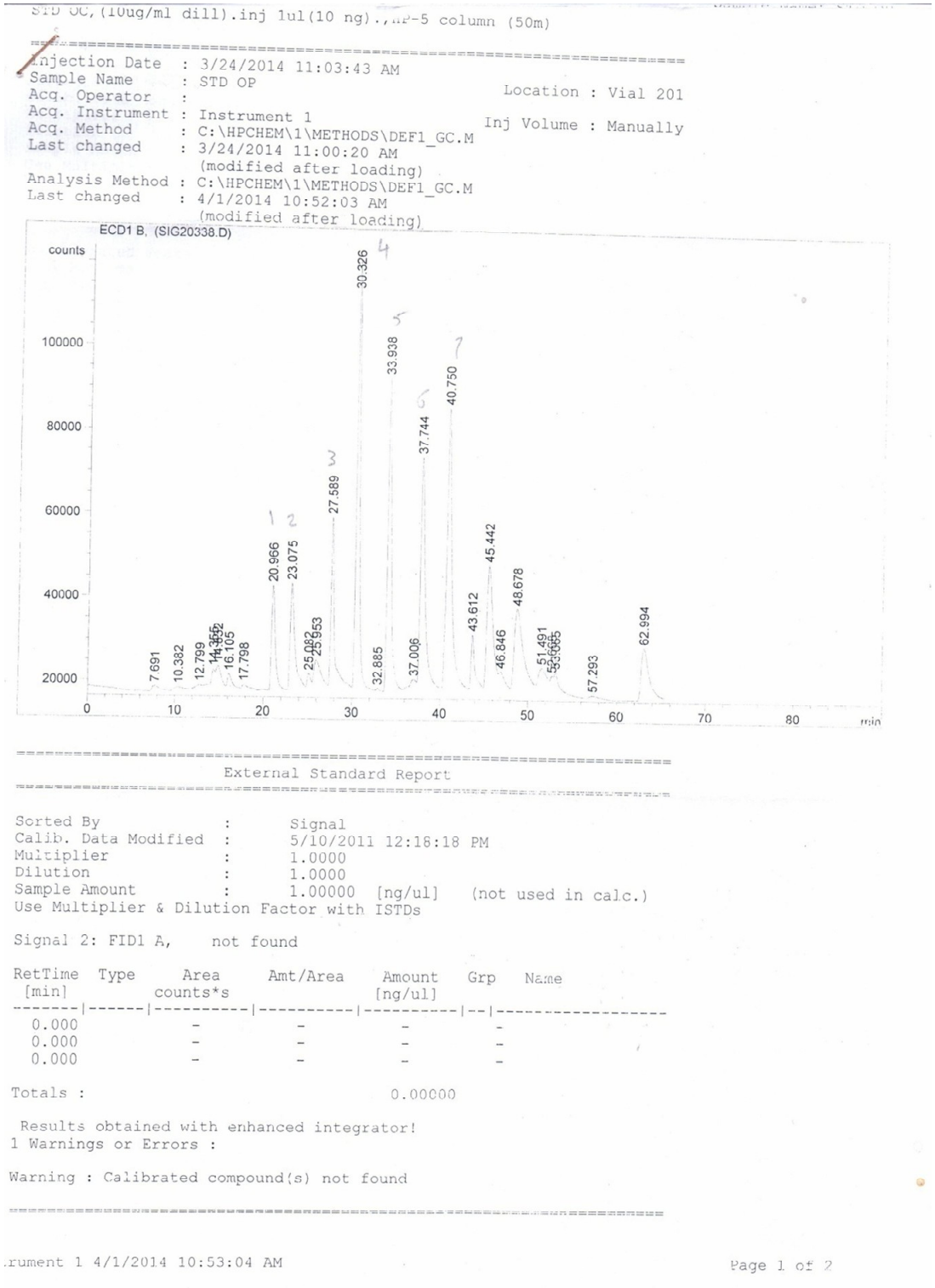


Fig.3 Standard samples



STD OC,(10ug/ml dill).inj 1ul(10 ng).,HP-5 column (50m)

Injection Date : 3/24/2014 11:03:43 AM
Sample Name : STD OP
Acq. Operator :
Acq. Instrument : Instrument 1
Acq. Method : C:\HPCHEM\1\METHODS\DEF1_GC.M
Last changed : 3/24/2014 11:00:20 AM
(modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\DEF1_GC.M
Last changed : 4/1/2014 10:52:03 AM
(modified after loading)

Location : Vial 201
Inj Volume : Manually

ECD1 B, (SIG20338.D)

counts

External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/10/2011 12:18:18 PM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 2: FID1 A, not found

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
0.000		-	-	-		-
0.000		-	-	-		-
0.000		-	-	-		-

Totals : 0.00000

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

rument 1 4/1/2014 10:53:04 AM Page 1 of 2

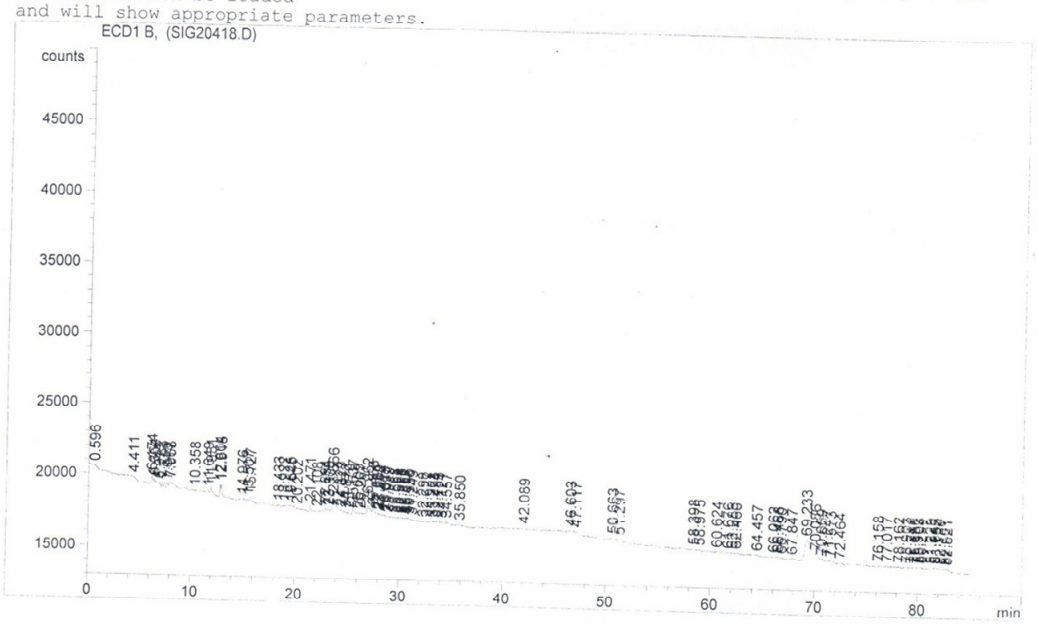
Fig.4 Detected sample

Data File C:\HPCHEM\1\DATA\SIG20418.D Sample Name: DM2A

DM2A ,1ml dil., 1ulinj.

Injection Date : 6/19/2014 8:38:20 AM
Sample Name : DM2A Location : Vial 201
Acq. Operator : M.SEIF
Acq. Instrument : Instrument 1 Inj Volume : Manually
Acq. Method : C:\HPCHEM\1\METHODS\NPD_ECD.M
Last changed : 6/19/2014 8:19:51 AM by M.SEIF
(modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\NPD_ECD.M
Last changed : 6/19/2014 1:10:51 PM by M.SEIF
(modified after loading)
Method to demo HP 6890 parameter editing for Split/Splitless & Cool On-Column inlets plus NPD and ECD. This method is for the purpose of OFFLINE demos only!

In order to load this method use the macro "6890demo.mac' or copy the file 'IQ1.MTH' from this methods' subdirectory to the instrument subdirectory and rename it 'IQ1.cfg'. The method can then be loaded
and will show appropriate parameters.



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: ECD1 B,

Peak #	RetTime [min]	Type	Width [min]	Area counts*s	Height [counts]	Area %
1	0.596	VV	0.1471	1711.09216	149.85959	0.38175
2	4.411	VV	0.1878	2465.75049	157.05714	0.55012
3	6.174	VV	0.0978	5140.08936	721.15521	1.14678
4	6.364	VV	0.1042	3656.35645	460.55630	0.81575
5	6.508	VV	0.0742	2242.76270	402.05057	0.50037
6	6.923	VV	0.1155	2895.91846	339.43271	0.64609

nstrument 1 6/19/2014 1:10:59 PM M.SEIF

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From the above results it can be concluded that, although the incidences of pesticides were relatively high, mean concentrations were below the maximum residue limits proposed by FAO and WHO 2013. On the other hand, the Organophosphorus pesticides were predominant in the fish samples, but in low concentrations, which might be attributed to the association of Organophosphorus pesticide residues with the fat phase in fish.

The presence of Dimethoate, Disulfaton, Famphur, Methyl parathion, O,O,O-Triethyl phosphoro thioate, Parathion and Phorate in fish samples from the studying area could be attributed to the intense agricultural activity in the area (cotton, maize and potatoes planted) and chemical application for control of agricultural pests. The contamination of fish and other aquatic organisms by OPs pesticides and their oxidation products was shown to be very low, Tsuda *et al.* (1997). The discharge and runoff of most minerals and salts promote photodecomposition of dissolved compounds, Schlauch (1989).

Similarly, the chemical manufacturing effluent may be exposed to degradation in the environment resulting in formation of smaller molecular entities through biotrans-formation, hydrolysis, photolysis and elimination as a result of mineralization. The component in the waste effluents can also undergo photodegradation if they are exposed to natural sunlight. They can also undergo indirect photodegradation when one or more of the chemical components (sensitizers) present in the waste effluent. Degradation is done with different mechanisms such as photoionization and electron transfer, Schlauch (1989) and Lymann *et al.* (1990).

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