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Organophosphorous residue in *Liza aurata* and *Cyprinus carpio*

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ABSTRACT

Objective: To determine the amount of azinphos methyl and diazinon residues in two river fishes, *Liza aurata* and *Cyprinus carpio*, in the north of Iran. **Methods:** This study was done during 2006-2007. In this survey, 152 water and fish samples from Gorgan and Qarasu rivers, north of Iran, were investigated. Sampling was done in three predetermined stations along each river. Organophosphorus compounds (OPs) were extracted from the fishes and the water of rivers. After extraction, purification and concentration processes, the amount and type of insecticides in water and fish samples were determined by high performance thin layer chromatography (HPTLC). **Results:** There was a significant difference in the residue of the insecticides in the water and fish samples between summer and other seasons in the two rivers. The highest amount of insecticides residue was seen during summer. In both rivers, the amount of diazinon and azinphos methyl residues in the two fishes was more than 2 000 mg/L in summer. There was no significant difference in insecticides residue between the fishes in two rivers. The diazinon residue was higher than the standard limits in both rivers during the spring and the summer, but the residual amount of azinphos methyl was higher than the standard limits only during the summer and only in Qarasu River. **Conclusions:** It can be concluded that the amount of OPs in the water and the two fishes, *Liza aurata* and *Cyprinus carpio*, is higher than the permitted levels.

1. Introduction

Pesticides, especially organophosphorus compounds (OPs), are widely used in agriculture to reduce and control insects, nematodes, fungi, rodents and other pests which compete with humans in accessing food and threaten their health[1]. Despite this benefit, inappropriate use of pesticides can have unintended effects on the environment. Most of sprayed insecticides and herbicides reach destinations other than their target species, including non-target species, air, water, bottom sediments and food[2]. Pesticide residues have also been found in rain and groundwater[3]. Studies in England showed that pesticide concentrations exceeded permitted limits for drinking water in some samples of river water and groundwater[4]. Fishes and other aquatic biota may be harmed by the pesticide-contaminated water[5]. Pesticide surface runoff into rivers and streams can be highly lethal to the aquatic life and sometimes kill all the fishes in a particular stream[6]. The faster a given pesticide breaks down in the environment, the less threat it poses to the aquatic life. Insecticides are more toxic to the aquatic life than herbicides and fungicides[5]. Pesticides can enter the human body through inhalation of aerosols, dust and vapor that contain pesticides, through oral exposure by consuming food and water; and through dermal exposure by direct contact of pesticides with the skin[7]. OPs are used more than other pesticides in agricultural pest control[8,9]. Organophosphorus insecticides are known as a potential toxic pollutant contaminating aquatic ecosystems. Humans are exposed to OPs through many routs such as indoor dust inhalation, ingestion, eating fruits and fishes, *etc*[10]. Many studies have shown that the OPs exist in significant concentrations in different human tissues[11]. Aquatic animals, including fishes, are among the most important food sources of humans, especially in coastal areas. Therefore, contamination of the fish and its health in safety can gradually cause dangerous consequences which endanger the human health directly or indirectly. In fact, considerable amounts of pesticides are found in the flesh of the fishes of contaminated streams and rivers[12]. According to the unpublished agricultural department

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documents in Golestan Province in the north of Iran, more than 43 000 kg (liter) diazinon and 12 000 kg (liter) azinphos methyl are delivered to farmers for pest control annually, since these two pesticides are most commonly used by farmers in this area. Regarding the irregular and extensive use of insecticides, especially OPs, in gardens in the north of Iran and dangerous consequences of entering the residues of these toxins to rivers, the fishes and other aquatic animals are highly exposed to contamination.

This study was conducted to determine the levels of OPs in river fishes as potential toxic pollutants contaminating aquatic ecosystems.

2. Materials and methods

This descriptive cross-sectional study was conducted for about one year from May 2006 to March 2007 in Golestan Province, neighboring the Caspian Sea in the north of Iran.

2.1. Chemicals

The solvents which were used in this study, such as acetone, acetonitrly, methylene chloride, hexane and also silica gel (SiO_4) $60F_{254}$ plate (20 cm × 20 cm), were purchased from Merck Company. The azinphos methyl and diazinon standards were purchased from the representative of Accustandard Company, Switzerland.

2.2. Study site

Golestan Province (52 ° 25′N, 36 ° 50′E, with an area of 20 367 km^2) is situated in North-East of Iran. This province is adjacent to the Caspian Sea and Turkmenia Republic from North (Figure 1). This province has an appropriate climate and many rivers; therefore, it is one of the agricultural provinces of Iran. Many different plants can grow in this area. Some of the important agricultural products of Golestan include cotton, rice, wheat, different fruits and vegetables.

There are various and valuable reserves in the Caspian Sea. Among them, two fishes, *Cyprinus carpio* (*C. carpio*) (common crap) and *Liza aurata* (*L. aurata*) (golden grey mullet) are traditionally named as "Kapur" and "Kafal", respectively (Figure 2). This is high consumption both in North and other area of Iran, and theses two fishes were selected to be studied for the amount of the chosen pesticides' residue. "Gorgan" and "Qarasu" rivers which are two of the most important rivers of Golestan were also selected for the study (Figure 1). The Gorgan River is located at 53 km East of Gorgan. This river has a calm flow and its water is used to irrigate fields and gardens.

In this river, different types of warm water fishes including "Kafal" and "Kapur" live. The Qarasu River is also one of the most important rivers of the Caspian Sea basin in which many hydrothermal fishes can be found. According to the existing information, diazinon (60%) and azinphos methyl in emulsifiable concentrates (EC) formulation are the two most used insecticides in the fields and gardens of the Golestan Province. These fields and gardens are mainly located adjacent to Qarasu and Gorgan rivers, and therefore toxins can easily enter the water. For this reason, these rivers were selected for the study.

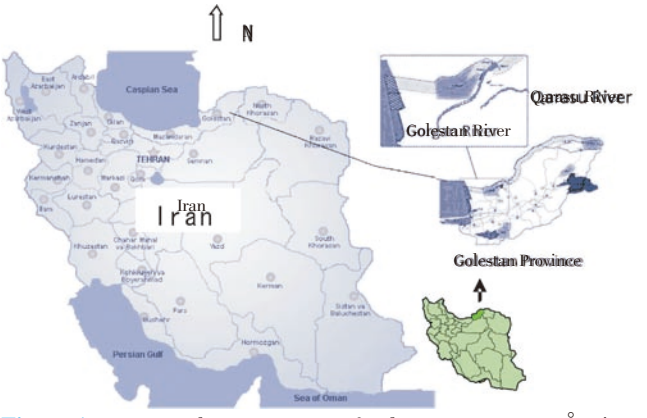

Figure 1. Gorgan and Qarasu Rivers of Golestan Province (52 ° 25′N, 36 ° 50′E, with an area of 20 367 km^2) in North of Iran.

a b

Figure 2. Two studied fishes.
(a) Liza aurata and (b) Cyprinus carpio

2.3. Sampling

In each river, three stations were chosen for water and fish sampling as the following: station 1 was the origin of the rivers; station 2 was the approximate midportion of the rivers; station 3 was the estuary of the rivers.

In each selected station, the samples, Kafal (*L. aurata*) and Kapur (*C. carpio*), were fished by hooks. In each sampling stage, 18 fishes were caught from each river. For more confidence, 9 of the fishes were caught from right bank, and the other 9 ones from the left bank of each river. It was because of non-proportional entering of feeder streams and school of fishes from the two sides of the rivers. Also, since male and female fishes have a similar diet pattern, sexuality of the sample fishes was not considered in this study.

For measuring the amount of the considered insecticides in the water of the two rivers, tests were done as the following. According to the standards, water sampling was done from 20 cm depth of the rivers. According to the calculations, in each stage, 18 samples were needed from each river. Samples were collected from different points of each station then mixed and tested for preventing breakdown of diazinon and azinphos methyl in water samples until extraction phase in which 50 mL methylene chloride solution was added to each water sample. Water sample containers were sealed by parafilm and the prepared samples were transferred to laboratory for extraction of the insecticides.

2.4. Extraction phase

2.4.1. Fish samples

The extraction of OPs from fishes was done as follows. In the first step, the flesh of the fish samples was detached and minced separately by a grinder. So, homogenate samples were prepared. It should be noted that the studied

fishes weighted about (500-750 g) because "Kafals" are smaller in size than "Kapurs". In the second step, 80 mL acetonitryl was added to 10 g of the grinded fish flesh. The mixture was shacked for one hour, so that the existing insecticides could be separated from tissues. In the third step, vacuum operation was done by vacuum pumps and filtration was implemented by paper filters (Whatman 125 mm cat No 1442125). In the fourth step, paper filters were repeatedly washed by 100 mL acetonitrly, so that the existing insecticides could be separated completely. In the fifth step, clean up operation was done using silica gel columns[13]. In the sixth step, the volume of the samples reached 50 mL (in laboratory environment at 30 ℃ temperature) and extra sultan was evaporated by a rotary device. In the last step, the containers of samples were washed by 1 mL acetone and the content was transferred to special leaded glass jars. The jars were sealed by para films. These samples were used for spotting.

2.4.2. Water samples

After settlement of water samples, 1 L of the homogenized sample was poured into the decanter funnel, and 50 mL of the saturated sodium chloride solution was added to it.

Extraction was done according to the common method of extracting insecticides from water samples[14,15].

Separating insecticides from the prepared solution was repeated three times by adding 25, 100 and 25 mL methylene chloride.

The organic phases were separated each time and then sodium anhydride was added to it for dehydrating samples. Clean up operation was done using prepared silica gel columns.

The separated organic solution was condensed using a vacuum evaporation rotary device at 30 ℃ and the volume reached 2 mL. The containers were washed by acetone for separating the remaining insecticides on their surfaces.

The extra acetone was evaporated in the laboratory temperature and the final volume of the condensed solution reached 1 mL. This final solution was used for spotting. Determining the efficiency of recovery operation was done by an empirical method through adding specified amounts of insecticides to the samples of pilot study and measuring their amounts in the samples. It showed that the efficiency of the recovery rate was more than 80 percent.

2.5. Determining phase

Extraction and separation of the phosphate insecticide was done by acetone and methylene chloride solutions using a decanter, which is a current method[14]. After separating and extracting azinphos methyl and diazinon insecticides from water samples, additional materials were deleted using a separator funnel and the insecticides were extracted by the acetone-methylene chloride solvent. The resulted solution was cleaned up in silica gel (SiO_4) columns. Then, the prepared solution was concentrated by an evaporation rotary device. The resulted solutions were tagged as unknown samples and their insecticide contents were determined.

Quantitative and qualitative analysis of insecticides and repellents is mostly done by gas chromatography (GC) and high performance liquid chromatography (HPLC)[15,16], but high performance thin layer chromatography (HPTLC) is inexpensive, faster and easier than HPLC and GC[13,17,18].

Spotting was done by an applicator and capillary on an aluminum plate containing silica gel (SiO_4) $60F_{254}$ as the stationary phase, using the standard solution and the unknown solutions of samples including extracted solutions of the water and fishes.

Multiple level methods were used for spotting of the standard sample. In this method, some different concentrations, or different volumes of a standard concentration, were used for spotting of the standard sample. These spots were allowed to dry before being placed to run in respective solvent systems. Then, the plates were put in a solvent tank (chamber tank) for development.

The organic solvent (as the mobile phase) for developing azinphos methyl and diazinon spot was a 80:20 acetone-hexane mixture[13,19].

This solvent was poured in the chamber tank and the prepared plates were put in it after saturation of the tank space (about 30 minutes).

The plates were removed from the tank and the spots were seen by the fluorescent light in a UV cabinet at 254 nm.

The retardation factor (R_f) value was calculated for each insecticide. The chromatographic zones corresponding to the spots of azinphos methyl and diazinon were scanned at 257 nm using TLC scanner 3 (V.1.14 S/N: 080320) (CAMAG company, Switzerland) and CATS4 software (version 4.06, S/N: 0805A007), in Reflection /Absorption measurement mode[13,17,18,20]. The source of radiation utilized was the deuterium lamp. At the end, the amounts of azinphos methyl and diazinon of each spot and their R_f values were determined. The position of a substance zone (spot) in a thin layer chromatogram can be described by R_f which is defined as the quotient obtained by dividing the distance between the substance zone and the starting line by the distance between the solvent front and the starting line[13].

Since there is no appropriate standard for the pollution of food and water by insecticides in Iran, the results of this study were compared to the international standards, including the standard of Germany. According to the standard of Germany, the acceptable amounts of diazinon and azinphos methyl in the drinking water are about 1 and 10 mg/L, respectively[14,21].

2.6. Data analysis

To compare organophosphorus insecticide residues in different stations, seasons and the two fishes, analysis of variance (ANOVA) and non-parametric Kruskal-Walis test were used.

3. Results

Analysis showed that there was a significant difference in insecticide residue between the summer and other seasons ($P<0.05$). The highest amounts of azinphos methyl and diazinon residue in both rivers were seen in the summer,

Table 1
Azinphos methyl residue in the water and fishes of Qarasu and Gorgan Rivers in 2007 (mean±SD) (mg/L).

Seasons	Rivers	Water	*C. carpio*	*L. aurata*
Spring	Qarasu	2.7±2.3	328.9±264.5	466.7±219.0
	Gorgan	1.8±2.5	352.6±298.8	531.8±159.6
Summer	Qarasu	14.6±10.6*	2095.6±36.1*	2123.0±111.6*
	Gorgan	14.9±11.7*	2060.7±114.4*	2017.8±101.6*
Autumn	Qarasu	0.2±0.5	374.0±111.6	251.1±121.4
	Gorgan	0.0±0.6	430.4±95.8	305.9±109.5
Winter	Qarasu	0.0±0.0	18.5±14.5	43.7±43.0
	Gorgan	0.0±0.0	13.3±19.2	54.8±45.3

*P<0.05 compared with other seasons.

Table 2
Diazinon residue in the water and fishes of Qarasu and Gorgan Rivers in 2007 (mean±SD) (mg/L).

Seasons	Rivers	Water	*C. carpio*	*L. aurata*
Spring	Qarasu	3.1±3.0	448.1± 272.8	584.4±184.4
	Gorgan	3.7±3.4	328.1±291.8	651.8±72.4
Summer	Qarasu	22.4±18.6*	2938.5±227.5*	2920.0±237.4*
	Gorgan	26.7±21.9*	2809.6±369.1*	3168.9±153.2*
Autumn	Qarasu	0.5±0.9	816.3±82.8	477.0±106.1
	Gorgan	0.6±1.1	794.8±98.7	791.6±103.2
Winter	Qarasu	0.0±0.0	19.2±20.4	47.4±34.7
	Gorgan	0.0±0.0	9.6±12.8	48.9±847.1

*P<0.05 compared with other seasons.

Table 3
Azinphos methyl residue in the water and fishes of Qarasu and Gorgan Rivers in different station (mean±SD) (mg/L).

Stations	Rivers	Water	Kapur fish (*C. carpio*)	Kafal fish (*L. aurata*)
Station 1	Qarasu	0.0±0.0*	701.1±843.8	677.2±907.3
	Gorgan	0.0±0.0*	715.0±831.1	717.8±767.0
Station 2	Qarasu	6.0±8.8	704.4±728.5	728.9±822.1
	Gorgan	5.3±8.1	695.0±804.6	677.8±806.1
Station 3	Qarasu	7.1±9.6	707.2±844.2	757.2±830.3
	Gorgan	7.3±11.4	732.8±825.6	782.2±769.8

*P<0.05 compared with other seasons.

Table 4
Diazinon residue in the water and fishes of Qarasu and Gorgan Rivers in different stations (mean±SD) (mg/L).

Stations	Rivers	Water	Kapur fish (*C. carpio*)	Kafal fish (*L. aurata*)
Station 1	Qarasu	0.0±0.0	1050.0±1173.0	1019.0±1217.7
	Gorgan	0.0±0.0	991.7±921.1	1165.0±1187.4
Station 2	Qarasu	6.6±9.9	328.1±1175.8	988.9±1081.5
	Gorgan	9.0±14.5	921.1±1100.7	1194.4±1239.3
Station 3	Qarasu	12.9±18.6	1043.3±1110.2	1013.3±1145.0
	Gorgan	14.3±20.6	1043.9±1133.6	1136.7±1205.2

*P<0.05 compared with other seasons.

but there was no significant difference in the amounts of insecticides residue between other seasons (spring, autumn and winter) (P>0.05) (Table 1 and 2).

The azinphos methyl residue in water samples of Qarasu and Gorgan Rivers in the summer were (14.6±10.6 mg/L) and (14.9±11.7 mg/L), respectively. But in winter, no amount of those insecticides was detected in the rivers (Table 1). The amount was (22.4±18.6 mg/L) and (26.7±21.9 mg/L) for diazinon in Qarasu and Gorgan Rivers, respectively in the summer. In the winter, the water of both Gorgan and Qarasu Rivers was free of diazinon (Table 2).

Regardless of the sampling season, there was no significant difference in the amount of diazinon residue between the two studied rivers (P>0.05).

The residual amount of diazinon in the spring and summer was higher than the standard limits for both rivers.

The highest amount of azinphos methyl in *C. carpio* was (2095.6±36.1 mg/L) in the summer in the Qarasu River and the lowest amount was (13.3±19.2 mg/L), which was seen in the winter in the Gorgan River (Table 1).

In both rivers, there was a significant difference in the residual amount of azinphos methyl in *C. carpio* among various seasons (P<0.05) (Table 1).

The highest amounts of azinphos methyl in *L. aurata* were seen in the summer in both rivers and the lowest amounts were seen in the winter (Table 1). There was no significant difference in azinphos methyl residue between the two fishes (P>0.05) (Table 1). In both rivers, there was a significant

difference in azinphos methyl and diazinon residues in the fishes in different seasons ($P<0.05$) (Table 1 and 2).

The amount of diazinon residue in the two fishes was significantly different between all seasons ($P<0.05$), but there was no significant difference in diazinon residue between Kafal and Kapur in the two rivers ($P>0.05$) (Table 2).

Therefore, it was clear that the amounts of diazinon and azinphos methyl in the water of both studied rivers were different in various seasons, and also the amounts of these pesticides in the fishes were different in various seasons ($P<0.05$).

In both rivers, there was no significant difference in azinphos methyl residue in Kapur and Kafal fishes in 3 station samples ($P>0.05$) (Table 3).

Also in both rivers, there was no significant difference in diazinon among 3 station samples between Kapur and Kafal fishes ($P>0.05$) (Table 4). However, there was a significant difference in azinphos methyl and diazinon residue in the two fishes and 3 station samplings ($P<0.05$) (Table 3 and 4).

There was a significant difference in azinphos methyl and diazinon residue in the water of rivers on 3 station sampling ($P<0.05$). There was no significant difference between 2 and 3 station sampling ($P>0.05$).

There was a significant difference in OPs residues between the fishes and the water ($P<0.05$). Approximately, the amount of azinphos methyl and diazinon residues in the fishes was 100-150 times more than the water (Table 1-4).

4. Discussion

Our evaluation showed that the highest amounts of azinphos methyl and diazinon residue in both water and fish samples could be seen in the summer while the lowest amounts were detected in the winter which is in accordance with insecticide spraying programs. In fact, with the end of spraying in the late summer, the amount of insecticide residue in the water reduces during the autumn and reaches its minimum in the winter. So, insecticide spraying of fields and gardens can be the main source of rivers pollution.

Although it was the first report on the OPs pollution of the water and fishes of the two main rivers in the north of Iran, it had already similar reports from other parts of Iran. Another study has shown that the spillway pollution of the Amir-kabir Dam with diazinon and azinphos methyl was higher than the standard levels, especially in May and June[19]. In our study, the highest amounts of OPs were seen in the summer and the lowest amounts were detected in the winter. There are also a few other reports on the pollution of underground and surface waters with insecticides in Iran[22]. Studies in the north of Iran have shown that OPs residues are more than allowed limited in spraying seasons in Haraz River, surface water and rice paddies[23]. Other studies on the fishes and water contamination to different pesticides have reported in South of Iran[24,25].

Studies have shown that the amounts of insecticides in the water depend on factors like the extent of agricultural activities, distance between the fields and gardens and the rivers, geographical position of the fields and gardens, the amount of precipitation in different areas and washing insecticides from the surface of plants and soil, temperature of the environment, and the amount of insecticides used by farmers. In all of the studies, the pollution of sweet water sources with insecticides, especially OPs, has been determined[26-28]. In this study, the amount and duration of the rivers pollution with OP insecticides were more than the amounts reported by previous studies. Therefore, similar and follow-up studies should be performed in this area in future years.

Many studies have been performed all over the world on the residue of pesticides in water resources which are not entirely compatible with this study due to their different geographical conditions, but some similar cases will be mentioned hereunder. A study conducted in the Atoya River, Nicaragua, showed the highest amounts of diazinon (18 mg/L) and azinphos methyl (14 mg/L) were detected in the summer[29].

According to German standards for insecticide residues in the water (up to 10 mg/L and 1 mg/L for azinphos methyl and diazinon, respectively), the level of OP residues in the summer was higher than permitted levels in our study.

Other studies have also shown that organochlorine and organophosphorus insecticides are the main causes of the pollution of the drinking water supplies, especially in rural areas close to fruit gardens[30].

Due to irrigations in the spring which wash the insecticides and also further insecticide spraying by farmers in the summer, the high amounts of pesticide residues in the summers can be regarded as a logical case. Therefore, the fishes which live in such rivers can be exposed to toxins, especially considering the fact that the amount of azinphos methyl was higher than the standard limits in station 3 of the both rivers. The amounts of diazinon were also higher than the standard limits in the station 2 and 3 of the two studied rivers. At the end of the rivers, due to factors like adjacence of the rivers and the agricultural fields, the direction of rivers stream, washing of insecticides and their accumulation toward the end of rivers and the entrance of seas, it is logical to detect higher amounts of insecticides. So, the fishes which live in these parts of the rivers are the most polluted ones.

Our study showed the two fishes including Kafal (*L. aurata*) and Kapur (*C. carpio*) were contaminated with OPs (diazinon and azinphos methyl). Their pollution to OPs in spring and summer is more than allow limited.

In one study in Nile River in Egypt, OPs such as chlropyrifos, malathion and diazinon were determined in some fish samples[31].

A study in Lake Taabo in Côte d'Ivoire showed contamination of two fishes (tilapia and catfish) and a prawn with organochlorine pesticides, especially lindane and endosulfan[12].

Finally, the residual amounts of azinphos methyl and diazinon were higher than the standard limits in both fishes in all seasons because the acceptable amount in the fish is less than 2 mg/L. OPs such as diazinon and azinphos-methyl are widely used for agricultural pest control in many areas in Iran and other countries and are often used without necessary supervision and therefore pollute the environment and organisms. The fishes which are used as food and also

have an economic value can be hazardous for health of human and make economic losses if they are polluted to agricultural insecticides. Pollution with insecticides can result in acute or chronic poisoning of the inhabitants and also pose long-term damages to the environment.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Gupta RC. *Reproductive and developmental toxicology*. USA: Elsevier Inc; 2011, p. 17-18, 471-487.
[2] Whitacre DM. *Review of environmental contamination and toxicology*. London, New York: Springer Heidelberg Dordrecht; 2011, p. 8-22.
[3] Li C, Yang T, Huangfu W, Wu Y. Residues and dynamics of pymetrozine in rice field ecosystem. *Chemosphere* 2011; **82**(6): 901-904.
[4] Bingham S. *Pesticides in rivers and groundwater*. UK: Environment Agency; 2007, p. 10-12.
[5] Coats JR, Bradbury SP. Aquatic toxicology of synthetic pyrethroid insecticides. *Environ Toxicol Chem* 2009; **8**(5): 359-362.
[6] Toughill K. *The summer the rivers died: toxic runoff from potato farms is poisoning P.E.I*. Sydney: Toronto Star Atlantic Canada Bureau; 2007, p. 14.
[7] WHO. *The WHO recommended classification of pesticides by hazard and guidelines to classification*. Stuttgart, Germany: Winsseuchofttliche Verlagsgesllschat mbH; 2009, p. 45-66, 120-124.
[8] Mulvaney D. *Green food: an A to Z guide*. California. USA: SAGE Publication Inc; 2011, p. 12-18.
[9] Rawn Df, Quade SC, Shield JB, Conca G, Sun WF, Lacroix GM, et al. Oraganophosphate levels in apple composites and individual apples from a treated *Canadian orchard*. *J Agric Food Chem* 2006; **54**(5): 1943-1948.
[10] Sundkvist AM. Olofsson U, Haglund P. Organophosphorus flame retardants and plasticizers in marine and fresh water biota and in human milk. *J Environ Monit* 2010; **12**(4): 943-951.
[11] Buratti FM, D'Aniello A, Volpe M, Meneguz A, Testai E. Malathion bioactivation in the human liver: the contribution of different cytochrome P_{450} isoforms. *Drug Metab Dispos* 2005; **33**(3): 295-302.
[12] Roche H, Tidou A. First ecotoxicological assessment assay in a hydroelectric reservoir: the Lake Taabo (Côte d'Ivoire). *Bull Environ Contam Toxicol* 2009; **82**(3): 322-326.
[13] Denistrop HE. *Applied thin layer chromatography: best practice and voidance of mistakes*. Wiley-VCH; 2000, p. 1-304.
[14] Butz S, Stan HJ. Screening of 265 pesticides in water by thin layer chromatography with AMD. *Anal Chem* 1995; **67**: 620-630.
[15] Sharma D, Nagpal A, Pakade YB, Katnoria JK. Analytical methods for estimation of organophosphorus pesticide residues in fruits and vegetables: a review. *Talanta* 2010; **82**(4): 1077-1089.
[16] Chen ZM, Wang YH. Chromatographic methods for the dertermination of pyrethrin and pyrethroid pesticide residues in crops, food and environmental samples. *J Chromatogr A* 1996; **754**: 367-395.
[17] Srivastava MM. *High performance thin-layer chromatography (HPTLC)*. London, New York: Springer Heidelberg Dordrecht; 2011, p. 202-216.
[18] Khoobdel M, Jonaidi N, Sharif B. Quantitative and qualitative determination of dimethyl phthalate and N, N-diethyl-m-toluamide in depellents dommercial dormulations by high performance thin layer chromatography (HPTLC). *Pak J Biol Sci* 2007; **10**(20): 3678-3682.
[19] Shayeghi M, Khoobdel M, Vatandoost H. Determination of organophosphorus insecticides (malathion and diazinon) residue in the drinking water. *Pak J Biol Sci* 2007: **10**(17): 2900-2904.
[20] Spangenberg B, Poole CF, Weins Ch. *Quantitative thin-layer chromatography: a practical survey*. London, New York: Springer Heidelberg Dordrecht; 2011, p. 289-313.
[21] WHO. *Guide line for drinking water quality*. 4th ed. Geneva: WHO; 2011, p. 29-31, 434-441. [Online] Available from: http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/index.html. [Accessed on 10 May, 2011]
[22] Shayeghi M, Khoobdel M, Bagheri F, Abtahi M. The residues of azinphosmethyl and diazinon in Garaso and Gorgan rivers in Golestan Province. *J School Public Health Inst Public Health Res* 2008; **1**(6): 75-82.
[23] Nasrabadi T, Bidhendi GN, Karbassi A, Grathwohl P, Mehrdadi N. Impact of major organophosphate pesticides used in agriculture to surface water and sediment quality (Southern Caspian Sea basin, Haraz River). *Environ Earth Sci* 2011; **63**: 873-883.
[24] Arjmandi R, Tavakol M, Shayeghi M. Determination of organophosphorus insecticide residues in the rice paddies. *Int J Environ Sci Technol* 2010; **7**(1): 175-182.
[25] Davodi M, Esmaili-Sari A, Bahramifarr N. Concentration of polychlorinated biphenyls and organochlorine pesticides in some edible fish species from the Shadegan Marshes (Iran). *Ecotoxicol Environ Saf* 2011; **74**(3): 294-300.
[26] Chen Q, Fung Y. Capillary electrophoresis with immobilized quantum dot fluorescence detection for rapid determination of organophosphorus pesticides in vegetables. *Electrophoresis* 2010; **31**(18): 3107-3114.
[27] Kumari B, Madan VK, Kathpal TS. Status of insecticide contamination of soil and water in Haryana, India. *Environ Monit Assess* 2008; **136**(1-3): 239-244.
[28] Sivasankaran MA, Reddy SS, Govindaradjane S, Ramesh R. Organochlorine residuals in groundwater of Pondicherry region. *J Environ Sci Eng* 2007; **49**(1): 7-12.
[29] Castilho JAA, Fenz N. Organochlorine and organophosphorus pesticide residue in the Atoya river basin Chinandega, Nicaragua. *Environ Pollut* 1999; **110**: 523-533.
[30] Wang C, Wu Q, Wu C, Wang Z. Determination of some organophosphorus pesticides in water and watermelon samples by microextraction prior to high-performance liquid chromatography. *J Sep Sci* 2011; **34**(22): 3231-3239.
[31] Malhat F, Nasr I. Organophosphorus pesticides residues in fish samples from the river Nile tributaries in Egypt. *Bull Environ Contam Toxicol* 2011; **87**(6): 689-692.