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Research Article

Organophosphorus pesticides (diazinon, malathion and azinfos methyl) accumulation in three fish species, in south coasts of the Caspian Sea, Iran

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Abstract

In this study the distribution and accumulation of diazinon, malathion and azinfos methyl in mullet *Liza aurata*, Caspian white fish *Rutilus kutum* and common carp *Cyprinus carpio* from five estuaries along the Caspian Sea have been investigated. Also, the effects of pesticides concentrations on the acetylcholinesterase enzyme (AChE) in fish species were studies. Pesticides concentration varied with fish species, sampling station and toxins types. The results indicate that the pesticides concentrations varied from 0.01 to 0.16 mg kg^{-1} for diazinon, 0.01 to 0.15 mg kg^{-1} for malathion and 0.05 to 0.36 mg kg^{-1} for azinfos methyl in three fish species. There was significant difference between different toxins concentrations in fish species, (p<0.05), and the order of toxics concentrations was as follows: azinfos methyl>diazinon>malathion. There was significant difference in toxins concentrations between three fish species, and the highest toxins concentrations were absorbed in detritivores fish (*L. aurata*), followed by herbivorous fish (*C. carpio*) and carnivore fish (*R. kutum*). The results confirmed that toxins bioaccumulation in fish species is strongly controlled by habitat and feeding habits.

Keywords: Diazinon, Malathion, Azinfos methyl, Acetylcholinesterase, Caspian Sea

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Introduction

Pesticides play an important role in modern agriculture. Organophosphate pesticides (OPs) are used worldwide in agriculture, municipal hygiene, disease vector control and against household pests; they were also a group of compounds used historically as chemical warfare agents (Ebadi and Zare 2005). Organophosphate pesticides (OPs) are phosphorus-containing pesticides and the principal types are phosphotriesters, thiophosphotriesters, and phosphorothiolesters. Phosphotriesters contain a phosphate center with three O-linked groups, thiophosphotriesters have the phosphoryl oxygens replaced by sulfur and in phosphorothiolesters, and one or more of the ester oxygen are replaced by sulfur (Bavcon *et al*., 2003; Gholampour *et al*., 2007). Synthetic pesticides are recognized as a cost-effective method of controlling pests, improving productivity and food quality. However, while pesticides may have a beneficial effect on agricultural productivity, their indiscriminate use causes many serious problems to the environment and human health, since these compounds are toxic to non-target species (Adhikari *et al*., 2004; Davodi *et al*., 2011).

The organophosphate pesticides (OPs) in the environment are influenced by many processes (biological, chemical and physical) that determine their persistence and mobility (Fanta *et al*., 2003; Gabriel *et al*., 2012). Millions of tons of pesticides are applied annually, but it is believed that only a small fraction of these products effectively reaches the target organisms, and the remainder is deposited on the soil, contaminating non-target organisms and moving into the atmosphere and water (Malhat and Nasr, 2011; Shayeghi *et al*., 2012). Since many pesticide types are recalcitrant, they remain for a long time in soils and sediments, where they can enter the food chain directly or percolate into the groundwater (Colovic *et al*., 2011; Gabriel *et al*., 2012). Pesticides surface runoff and be used in agriculture into rivers, estuary and can be accumulate in the tissues of aquatic organisms. Fishes and other aquatic biota may be harmed or sometimes kill all the fishes by the pesticide-contaminated water, because OPs could with change in the level of the main and essential enzymes cause to be kill of the fish species (Dutta and Areids, 2003; Fanta *et al*., 2003; Kaur and Dhanju 2004; Gabriel *et al*., 2012; Hyde *et al*., 2013).

The Caspian Sea is bound to the northeast by Kazakhstan, to the northwest by Russia, to the west by Azerbaijan, to the southeast by Turkmenistan and to the south by Iran (Nasrabadi *et al*. 2011). Therefore, there is high population of farmer from different countries around the Caspian Sea that used pesticides for agricultural and public health purposes (Ebadi and Zare, 2005; Shayeghi *et al*., 2008). Pollution with these materials is a serious problem for aquatic organisms and also for consumers of fish. 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 fishes of contaminated streams and rivers (Khosravi-Katuli *et al*., 2014).

There are lots of reports on the organophosphates pesticides pollution in the fish species from Iran and other countries (Adhikari *et al*., 2004; Kaur and Dhanju, 2004; Li *et al*., 2004; Gholampour *et al*., 2007; Davodi *et al*., 2011; Malhat and Nasr, 2011; Shayeghi *et al*., 2008; Khosravi-Katuli *et al*., 2014) but information about effects of organophosphates pesticides on acetylcholinesterase enzyme is limited. The purpose of this study was to investigate the concentration of organophosphates pesticides (diazinon, malathion and azinfos methyl) in three fish species including *Liza aurata*, *Rutilus kutum* and *Cyprinus carpio* from five estuaries along the Caspian Sea(figure 1). Also, the effect of organophosphates pesticides on acetylcholinesterase enzyme in three fish species was investigated.

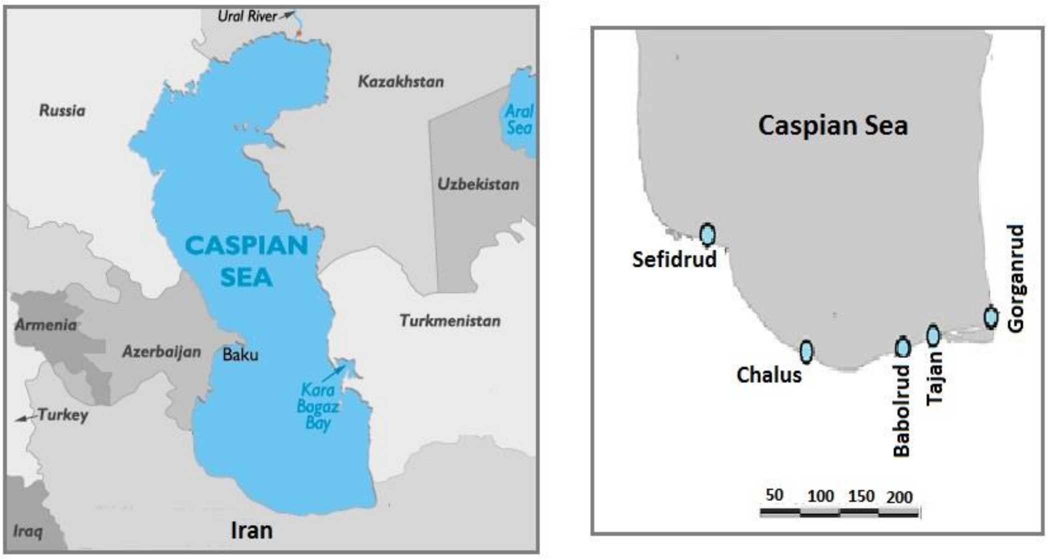

Figure 1: Map of Caspian Sea and sampling area.

Materials and methods

The fish species were collected from five estuaries including Gorganrud, Tajan, Babolrud, Chalus and Sefidrud, along the south coasts of Caspian Sea. The Samples were collected by trawling in summer and autumn of 2011. A total of 135 fishes were collected from study area. After sampling, the samples were wrapped in polyethylene bags, kept in an ice box and transported to the laboratory and stored at -80 $^{\circ C}$ for analysis (Sharma *et al*., 2010; Mashinchian Moradi and Golshani 2012). Each species was properly cleaned by rinsing with distilled water to remove debris, planktons and other external adherent, and morphological characteristics including length (cm) and total weight (g) were measured. The details of species characteristics are summarized in Table 1.

Table 1: Characteristics of the three fish species collected from Caspian Sea (mean±SD).

Scientific name	Common name	Food Ecology	n	Length (cm)	Weight (g)
Liza aurata	Mullet	Detritivores: consumes benthic organisms and detritus	45	38±0.1	955±20
Rutilus kutum	Caspian White	Omnivorous: consumes mollusks and crustacean	45	42±0.3	1120±35
Cyprinus carpio	Common Carp	Omnivorous: consumes alga, insects and benthic worms	45	39±0.2	1055±45

For tissues isolation, the body of fish samples was cut out and the muscle tissues were removed carefully. The muscle tissues samples (10 g) have been kept in aluminum foil and maintained in deep freezer until analysis. Anhydrous sodium sulfate and 100 mL of ether de petrole were added to fish samples and mixed for 30 minutes on a shaker. Then the solutions were filtered and dried using a vacuum evaporation apparatus. The filtrates were evaporated to dryness using rotary evaporator (Sharma *et al*., 2010; Sundkvist *et al*., 2010).

For purification, the dried residue extraction was solved in 15-20 ml of ether de petrole and transferred to the glass column chromatography containing anhydride sodium sulfate, silica gel and florisiland. After opening the outlet valve of the column, the solvent was allowed to go out up to 0.5 mL above the surface of the contents of the fluid column, and then it were removed from the column with 150 mL of the remaining mixture of ether de petrole and ether ethylic. The solution was concentrated by vacuum distillation and the dried residue was dissolved in 2 mL of ether de petrole solvent (Sharma *et al*., 2010; Mashinchian Moradi and Golshani, 2012).

Gas chromatography (GC 6890N, AGILENT), with injection temperature of 250°^{C} and phosphorous pesticide-specific detector (TSD) at 280 °C and a sensitivity of 4×10-10 and the column temperature of 230 °C, was calibrated and prepared for receiving the sample. Since the basis of gas chromatography at the first stage is peaks identification and then amount determining, standards of the considered pesticides with a particular concentration were prepared and their retention time were identified. The prepared samples were then injected into the GC (Venkateswara Rao, 2006; Wang *et al*., 2011).

Significant differences between toxins concentrations in the different fish species were determined using One-Way analysis of variance (ANOVA) followed by Duncan post hoc test. Toxins concentrations were calculated in milligram per kilogram dry basis (mg/kg dry weight), a probability of p = 0.05 was set to indicate statistical significance. Standard deviation and recovery (%) for diazinon, malathion and azinfos methyl are summarized in Table 2.

Table 2: Standard deviation and recovery (%) for diazinon, Malathion and Azinfos methyl.

	Toxic		
	Diazinon	**Malathion**	**Azinfos methyl**
Standard deviation	0.02	0.04	0.15
recovery (%)	95	97.5	99

Results

The standard deviation (data variability) for fish species were ranged from ±0.01 to 0.05 for Diazinon, ±0.02 to 0.06 for Malathion, and ±0.05 to 0.15 for Azinfos methyl, respectively (Table 2). Organophosphorus pesticides (diazinon, malathion and azinfos methyl) concentrations and SD in the three fish species collected five estuaries along the Caspian Sea are shown in Table 3.

The results indicate that the pesticides concentrations varied from 0.05 to 0.16 with a mean 0.10 mg kg^{-1} for diazinon, 0.03 to 0.15 with a mean 0.08 mg kg^{-1} for malathion and 0.09 to 0.36 with a mean 0.20 mg kg^{-1} for azinfos methyl in the mullet, *L. aurata*. There was a significant difference between different toxins concentrations in mullet, *L. aurata* (p<0.05), and the order of toxic concentrations was as follows: azinfos methyl>diazinon> malathion.

The concentrations varied from 0.02 to 0.08 with a mean 0.04 mg kg^{-1} for diazinon, 0.01 to 0.09 with a mean 0.06 mg kg^{-1} for malathion and 0.05 to 0.20 with a mean 0.12 mg kg^{-1} for azinfos methyl in the Caspian white fish, *R. kutum*. There was a significant difference between different toxins concentrations in Caspian white fish, *L. aurata* (p<0.05), and the order of toxic concentrations was as follows: azinfos methyl> diazinon>malathion.

For common carp, *C. carpio*, the concentrations varied from 0.02 to 0.09 with a mean 0.05 mg kg^{-1} for diazinon, 0.01 to 0.08 with a mean 0.05 mg kg^{-1} for malathion and 0.04 to 0.18 with a mean 0.10 mg kg^{-1} for azinfos methyl. There was a significant difference between different toxins concentrations in common carp, *C. carpio* (p<0.05), and the order of toxic concentrations was as follows: azinfos methyl> diazinon>malathion.

Table 3: Mean concentration (mg kg^{-1}) of diazinon, malathion and azinfos methyl in muscle of the three fish species collected five estuaries along the Caspian Sea.

Species		**Station**				
		Gorganrud	**Tajan**	**Babolrud**	**Chalus**	**Sefidrud**
	Diazinon	$0.05^{c}±0.02^{c}$	$0.14^{a}±0.02$	$0.16^{a}±0.02$	$0.08^{b}±0.02$	$0.06^{c}±0.02$
Liza aurata	Malathion	$0.06^{c}±0.03$	$0.13^{a}±0.03$	$0.15^{a}±0.02$	$0.04^{c}±0.06$	$0.03^{c}±0.05$
	Azinfos methyl	$0.09^{b}±0.15$	$0.24^{a}±0.12$	$0.36^{a}±0.14$	$0.18^{a}±0.13$	$0.13^{a}±0.13$
	Diazinon	$0.02^{c}±0.05$	0.04±0.01	$0.08^{b}±0.02$	$0.04^{c}±0.05$	$0.02^{c}±0.02$
Rutilus kutum	Malathion	$0.01^{c}±0.04$	0.06±0.04	$0.09^{b}±0.06$	$0.03^{c}±0.04$	$0.04^{c}±0.04$
	Azinfos methyl	$0.05^{c}±0.15$	$0.16^{a}±0.15$	$0.20^{a}±0.15$	$0.11^{a}±0.15$	$0.08^{b}±0.15$
	Diazinon	$0.03^{c}±0.02$	$0.08^{b}±0.05$	$0.09^{b}±0.02$	$0.05^{c}±0.03$	$0.02^{c}±0.02$
Cyprinus carpio	Malathion	$0.04^{c}±0.03$	$0.05^{c}±0.04$	$0.08^{b}±0.05$	$0.03^{c}±0.06$	$0.01^{c}±0.04$
	Azinfos methyl	$0.04^{b}±0.12$	$0.14^{a}±0.12$	$0.18^{a}±0.12$	$0.08^{a}±0.12$	$0.06^{b}±0.13$

In each line, different letters indicate significant differences (p<0.05) among the estuaries.

The results show there was a significant differences between the toxins concentrations in fish species ($p<0.05$), and the order of toxic concentrations in the all fish and estuaries was as follows: azinfos methyl > diazinon > malathion (Fig. 2a).

There was a significant difference in toxins concentrations between the three fish species ($p<0.05$) and the highest concentrations of pesticides were detected in the mullet, followed by common carp, and white fish, respectively (Fig. 2b).

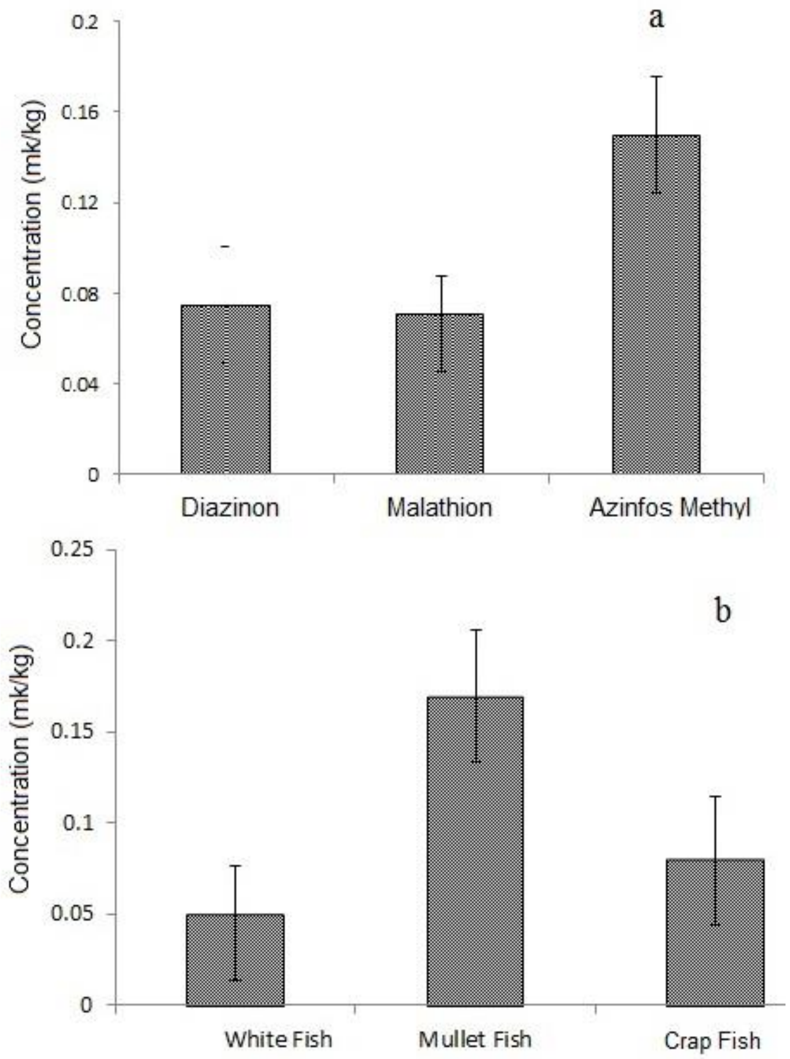

Figure 2: The comparison between toxins concentration in three fish species.

Pearson correlation table for toxic concentration among species of fish shows a positive correlation between the same toxic in the fish species (Table 4). The highest correlation was between diazinon concentration in the *L. aurata* and *R. kutum* (r=0.47), azinfos methyl concentration in the *C. carpio* and *L. aurata* (r=0.39), malathion in the white fish with diazinon in the mullet (r=0.34), malathion in the *C. carpio* and *L. aurata* (r=0.34), and between azinfos methyl concentration in the *C. carpio* and *R. kutum* (r=0.32), that the correlation were significant.

Table 4: Correlation between mean concentrations of toxins in three fish species collected five estuaries along the Caspian Sea.

Species	Toxic	*Liza aurata* Diazinon	*Liza aurata* Malathion	*Liza aurata* Azinfos methyl	*Rutilus kutum* Diazinon	*Rutilus kutum* Malathion	*Rutilus kutum* Azinfos methyl	*Cyprinus carpio* Diazinon	*Cyprinus carpio* Malathion	*Cyprinus carpio* Azinfos methyl
Liza aurata	Diazinon	1	0.14	0.12	0.47**	0.21	0.17	0.088	0.18	0.27
	Malathion		1	0.21	0.19	0.34**	0.20	0.17	0.34	0.19
	Azinfos methyl			1	0.17	0.13	0.21	-0.023	0.29	0.39**
Rutilus kutum	Diazinon				1	0.093	0.26	0.31**	-0.18	-0.33
	Malathion					1	0.19	0.09	-0.19	0.028
	Azinfos methyl						1	0.26	-0.21	0.32**
Cyprinus carpio	Diazinon							1	0.19	0.24
	Malathion								1	0.17
	Azinfos methyl									1

*. Correlation is significant at the 0.05level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Discussion

Azinfos methyl toxin is very strong and has fast effects for destroying pests than the other toxins, because this toxin has high durability in environment and has not easily decomposed. Therefore, bioavailability in the marine environment for azinfos methyl toxin is higher than diazinon and malathion toxins. Li *et al*. (2004) and Venkateswara (2006) have found that azinfos methyl has high concentration among the organophosphorus pesticides in tissues of rainbow trout and tilapia. They suggest that azinfos methyl is a toxin more effective for destroing pests than the other toxins and most farmers used this toxin. Also, Shayeghi *et al*. (2012) and Khosravi-Katuli *et al*. (2014) have been reported that azinfos methyl toxic is very important and the most common pesticides in the agriculture around the Caspian Sea, so the concentration is higher than other pesticides. It can be concluded that higher concentration of the azinfos methyl than the diazinon and malathion may be related to used level, frequency of used, long effects, greater durability in the environment (Shayeghi *et al*. 2008).

The bioaccumulation of pollutants in the fish species might be a result of different ecological needs, metabolism, habitats and feeding habitats (Gholampour *et al*., 2007). In present study, we considered three groups of fish including mullet, (detritivores fish), Caspian white fish, (omnivorous fish prefer to carnivore) and common carp, (omnivorous fish prefer to herbivorous) as biological indicator~~s~~ candidates for evaluating the effects of trophic levels and habitats on toxins accumulation. Mullet lives in close association with sediments and feeds on small benthic organisms, detritus and occasionally insects and planktons. The second group is white fish, which is an omnivorous fish and feeds on mollusks, shrimps and amphipods. Third group is common carp, that is an omnivorous fish but more feeds on alga, seaweeds, insects, and benthic worms (Mashinchian Moradi and Golshani 2012).

The results of studying on mullet indicated high toxins accumulation in muscle tissues, because *L. aurata* feed on benthic organisms, detritus and ~~is~~ close to bottom sediments and receive more sediment-associated toxins. Hosseini *et al*. (2013) studied toxins contamination in three groups of fish species in trophic level. They found that detritivores fish feed on bottom detritus and receive high concentration of pollutants. According to Gewurtz *et al*. (2011), pesticides and DDT concentrations in the detritivores fish is higher than omnivorous, herbivores and carnivorous fishes.

The concentration of pesticides in the common carp may be related to alga and seaweeds eating habits of the fish species. Pesticides can readily accumulate within marine plants such as alga and seaweeds at much higher levels and transfer to top predators that fed them. Venkateswara Rao (2006) and Sundkvist *et al*. (2010) have been reported that heavy metals and PCB concentration in herbivores fish such as common carp that feed on alga and seaweeds was higher than carnivorous, piscivores and zooplanktivores fish species, because alga and seaweeds have high capacity for pollutants accumulate within tissues and herbivores fish eating plants and receive high concentration of pollutants.

The pesticides concentration in the white fish, *R. kutum* is lower than the mullet and carp, because these species live in the bottom water with low sources pollution and feed on mollusks and crustacean (shrimps and amphipods) (Gholampour *et al*., 2007). Molluscs and crustacean have not high capacity to accumulate contaminants in their tissues, and could not be transfer contaminants to fishes that eat them (Abdolahpur Monikh *et al*., 2012). According to the results of the study of Turkmen *et al*. (2005), Navarro *et al*. (2006) and Abdolahpur Monikh *et al*. (2012), pesticides and heavy metals concentrations in omnivorous fishes that feed~~s~~ on mollusks and crustacean is lower than herbivores, piscivores and phytoplanktivores fish species. Also, Ghaenim *et al*. (2015) reported fish species that consumes mainly mollusks or crustacean receive low concentration of PCB, PAH and heavy metals. Thus, in terms of pesticides accumulation, the expected ranking in our study is detritivores (mullet)>herbivorous (common carp)>carnivore (Caspian white fish). Therefore, this finding could confirm that toxins bioaccumulation in fish species is heavily controlled by habitat and feeding habits.

There were highly significant differences among the estuaries from accumulation of the pesticides point of view ($p<0.05$). The results showed that the highest concentration of diazinon, malathion and azinfos methyl in three fish species were detected in Babolrud, followed by Tajan, Chalus, Sefidrud and Gorganrud, respectively. Therefore, the order of the estuaries for the diazinon, malathion and azinfos methyl accumulation was as follows: Babolrud>Tajan>Chalus>Sefidrud>Gorganrud.

There are many pollution sources such as agricultural activities, farms and paddies around the Babolrud and Tajan estuaries than the other estuaries, therefore both estuaries receive the high concentration of pesticides used by farmers (Khosravi-Katuli *et al*., 2014). There are low pollution source around the Sefidrud and Gorganrud estuaries and receive the low concentration of pesticides than the other estuaries. Therefore, the concentration of pesticides in the aquatic environment depend on factors like the extent of agricultural activities, distance between the fields, farms and the rivers, geographical position of the fields and farms, the amount of precipitation in different areas and washing out insecticides from the surface of plants and soil, and the amount of pesticides used by farmers. So, pesticides spraying for fields, farms and agricultural activities can be the main source of estuaries pollution along the Caspian Sea (Nasrabadi *et al*., 2011; Shayeghi *et al*., 2012).

Pearson Correlation table (Table 4) for toxic concentration among species of fish shows a positive correlation between the same toxics in the fish species. There was positive correlation between same toxics in three seabirds, but there are no significant correlations between different toxics in the fish species.

Baser *et al*. (2003) found correlations between toxics concentrations in same tissues in different fish species. Also, Adhikari *et al*. (2004) observed relationships between the same toxins in the same tissues in different fish species. They suggested that there are significant correlations between same toxics in different fish species, but there are no significant correlations between different toxics in the different fish species. All previous studied shows the high correlation could be accounted ~~for~~ by the geology of the area, detoxification process, species type, tissue properties, chemical form of toxic and direction of detoxification mechanisms (Yamashita *et al*., 2000; Yildirim *et al*., 2006). Also, field and experimental studies in fish species suggest that tissue distribution and accumulation of pollutants also varies depending on growth stages, breeding status, molting and migration (Sundkvist *et al*., 2010; Abdolahpur *et al*., 2012; Hosseini *et al*., 2013)

Accumulation of organophosphorus pesticides (diazinon, malathion and azinfos methyl) in three fish species including mullet *L. aurata*, Caspian white fish *R. kutum* and common carp *C. carpio* were investigated. The results showed that the highest toxins concentrations were absorbed in detritivores fish (*L. aurata*), followed by herbivorous fish (*C. carpio*) and carnivore fish (*R. kutum*). The results confirmed that the toxins bioaccumulation in fish species is strongly controlled by habitat and feeding habits. The results showed that the highest concentrations of pesticides in three fish species were detected in Babolrud, followed by Tajan, Chalus, Sefidrud and Gorganrud, respectively.

Because there are many pollutant sources around the Babolrud and Tajan estuaries than the other estuaries, therefore both estuaries receive the high concentration of pesticides used by farmers. Finally, the concentration of pesticides in the aquatic environment 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 and the amount of pesticides used by farmers.

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