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Determination of concentration of organophosphate pesticides (diazinon – malathion – azinfos methyl) in mullet (*Liza aurata*) muscle tissue from Babolrud, Gorganrud and Tajan river estuaries.

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ABSTRACT: Pesticides are used extensively in agricultural lands in Northern provinces of Iran which makes river ecosystems more vulnerable to pesticides pollution. This study was conducted in three river estuaries (Babolrud, Tajanrud and Gorganrud), focusing mainly on the concentration levels of organophosphate pesticides (diazinon, malathion, azinphos-methyl) in the muscle tissue of golden mullet fish, an economically important and highly consumed fish in the region. The research was conducted in 2011-12. During the study, 27 samples of mullet fish were collected. After extraction and purification process, the average level of concentrations of diazinon, malathion in mullet fish muscle tissue were measured using Gas Chromatography. Based on the results, the highest average concentrations of azinphos-methyl belonged to samples taken from Babolrud river estuary (1.28 mg/kg), while these concentrations for Tajan and Gorganrud samples were respectively 0.76 and 0.04 mg/kg. The highest concentration of malathion in mullet fish muscle was found in the samples taken from Tajan River (0.19 mg/kg). The concentration of malathion in samples taken from Babolrud and Gorganrud rivers were also 0.17 and 0.07 mg/kg, respectively. The highest concentration of diazinon in mullet fish muscle was found in samples taken from Babolroud River (0.27 mg/kg), and then in samples from Tajan and Gorganroud rivers (respectively 0.09 and 0.08 mg/kg). It was concluded that the most contaminated river estuary was Babolrud river estuary.

Keywords: *mullet fish; diazinon; malathion; azinphos-methyl*

INTRODUCTION

Organophosphorous pesticides are derived from phosphoric acids, pyrophosphoric acid, fluorophosphoric acid, metaphosphoric acid isomer, thiophosphoric acid and dithiophosphoric acid which are indeed the most used pesticides in combating pests. Organophosphorous pesticides when combined with cholinesterase enzyme, make it convert to the phosphorylated enzyme in an almost irreversible reaction which takes several weeks to be hydrolyzed (Mayer, 1986). Organophosphorous pesticides (diazinon, malathion, azinphos-methyl) are indeed considered as organic pollutants (organic phosphates) which are non persistent and prone to be rapidly inactivated and degraded. The toxic effects of these pesticides are, however, on the sympathetic nerves as they inhibit the acetyl cholinesterase enzyme which will be impaired as a result of neurotransmission (Mayer, 1986).

Organophosphate pesticides are being used in agricultural fields and citrus gardens orchards in Northern provinces of Iran bordering the southern shores of the Caspian Sea. These pesticides can also be absorbed through human inhalation (Sheykhi-Gorjan et al., 2009). However, it is less than 0.1% of the used pesticides that reaches the target pests (Ewing, 1999). Pesticides can enter the river systems in two ways:

1) Direct charge of the pesticides into the aquatic eco-systems and,
2) Indirect charge of pesticides via precipitation and erosion resulting from wastewater of agricultural fields and industrial units (Ewing, 1999).

In other words, a large amount of pesticides is released to the environment contaminating the water and soil resources in Northern provinces of Iran. Inefficient control system on industrial waste-water and agricultural drainages are the major carriers of pollutant to the fresh water systems and eventually to the marine environment. These, in turn, feed into living organisms through muscle tissues of other organisms. Considering the importance of mullet fish as a major source of protein in the northern provinces of Iran, this important issue has to be investigated.

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MATERIALS AND METHODS

A total of 27 mullet fish (*Liza aurata*) were taken from river estuaries of Babolrud, Tajan and Gorganrud during the second half of the autumn 2011 (9 samples from each sampling station).

Since mullet fish does not migrate through the rivers, it could be used as an indicator of river pollution (Razavi Sayad, 1990). To sample the fish, the adjacent fishery units were used (Tajan: Jahan nama Fishery Co., Babolroud: Caspian Mazandaran Fishery Co. and Gorganroud: Abouhanifeh Fishery Co.). Net size used for fishing was between 30-33 mm in the fall and winter sampling and 33mm in spring sampling. .

After transferring the collected samples to the laboratory, biometric measurements were conducted (including length, total weight, standard length and density). The samples were then stored in -20°C. Prior to biochemical analysis, the fish were dressed and their muscle tissue was collected. Samples were then weighed (dry weight) and prepared based on a methodology suggested by Helrick (2000).Weighed samples were transferred to the appropriate laboratory glassware. Anhydrous sodium sulfate and 100 ml of ether de petrole were added and mixed for 30 minutes on a shaker (Stirring can be done for 5 minutes on high speed blender). Then the solution was filtered and were dried using a vacuum evaporation apparatus (Helrick, 2000).

For purifying, the dried residue extraction was solved in 15-20ml 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 2ml of ether de petrole solvent (Helrick, 2000).

At this stage the sample was prepared for injecting into the gas chromatography (GC).

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 (Helrick, 2000).

One-way analysis of variance (ANOVA) and Pearson correlation tests were used for evaluating the existence of a significant difference in level of pesticides in muscle tissue of mullet fish from different river systems.

Since there was no standard for pollution in terms of concentration of organo- phosphorus pesticides in the bodies of aquatic organisms at national level, results of this study were compared with the results of other similar studies inside or outside the country.

RESULTS AND DISCUSSION

In this study, levels of pesticides (diazinon, malathion, azinphos-methyl) in fish samples from three river estuaries were compared using ANOVA test. The results showed that:

1- There was a significant difference between the concentration of diazinon in samples from Babolrud and samples from the other two sites (Tajan and Gorganrud) (Table 1).

2- There was a significant difference between the concentrations of malathion in samples from Gorganrud and samples from the other two sites (Tajan and Babolrud) (Table 1).

3-There was a significant difference between the concentrations of azinphos-methyl in samples from Gorganrud and samples from the other two sites (Tajan and Babolrud) (Table 1).

Comparison of different pesticides levels at each site showed that:

1-The concentration of azinphos-methyl was significantly higher than the concentrations of two other pesticides in the samples from Babolrud river estuary (Fig. 1).

2-The concentration of azinphos-methyl was significantly lower than the concentrations of two other pesticides in the samples from Tajan river estuary (Fig. 1).

3-The concentration of azinphos-methyl was significantly lower in Gorganrud river samples when compared to the other pesticides (Fig. 1).

There was also a significant positive linear relation (correlation) between size of fish (length and weight) and the level of pesticides (diazinon, malathion and azinphos-methyl) in mullet fish muscle tissue (Table 1).

Based on the results of this study, samples from Babolrud river estuary contained higher levels of organophosphorous pesticides compared to samples from Tajan and Gorganrud rivers (Fig. 1).

The highest level of azinphos-methyl was observed in the samples from Babolrud and Tajan rivers. Azinphos-methyl is considered as a highly toxic

pesticide for fish (LC50 96h; for 0.03 to 0.12 mg/l) (Norouzian, 1999). Therefore, this pesticide was identified as the most hazardous studied pesticide (Fig. 1).

The results showed a significant positive correlation ($p < 0.05$) between the concentration of phosphorus pesticides and the size of fish (length and weight).

The highest average concentration of diazinon was found in samples from Babolrud with a concentration of 0.27 mg/kg. This concentration in samples from Gorganrud and Tajanrud river estuaries were respectively 0.09 and 0.08 mg/kg (Table 1).

The highest average concentration of malathion was found in the samples from Tajanrud with a concentration of 0.19 mg/kg. These concentrations in samples from Gorganroud and Babolroud were respectively 0.17 and 0.07 mg/kg (Table1). The highest average concentration of azinphos-mehyl was found in samples from Babolrud (1.28 mg/kg). These concentrations in samples from Gorganrud and Tajanroud were respectively 0.76 and 0.04 mg/kg (Table1).

CONCLUSION

The results of this study showed that there was a linear significant positive correlation between the size of fish (length and weight) and pesticide concentrations (diazinon, malathion, azinphos-methyl) in muscle tissue of mullet.

Also, comparison of results of this study with previous studies on determining diazinon and malathion concentrations in kutum and carp fish of the northern rivers of the country (Gholamipour *et al.*, 2007) shows higher levels of contamination in mullet fish. It can be related to the diet of mullet fish which is a detritus eater.

Also a significant correlation between the concentrations of pesticides in studied areas with the usage of these pesticides in the margins of these rivers was found.

Diazinon and azinphos-methyl are used for rice stem worm control (Sheikhigorjan *et al.*, 2009) in agricultural fields. These pesticides showed their highest concentration in samples from Babolrud river estuary. This could be explained by the presence of many agricultural rice fields along the Babolrud River in comparison with two other rivers (Mazandaran Province, 2010).

Highest average concentration of malathion was observed in samples from Tajan river estuary which reflects the presence of citrus orchards and farms along this river (Sheikhigorjan *et al.*, 2009). Furthermore, comparing the results of this study with a similar study performed on the concentrations of diazinon and malathion in kutum fish and carp (Gholamipour *et al.*, 2007) showed that mullet fish in the studied areas is more contaminated than kutum and carp, which is probably due to mullet fish diet as a detritus eater (Razavi Sayad, 1990) (Table 2).

Since there are no standards for the allowable amount of organophosphate pesticides in fish residues, the results of this study, when compared to another studies, reflect that these levels are much higher than health limit and may be considered as highly toxic level (Collier, 2002; Ewing, 1999) (Table 3).

It seems that these excessive amounts of pesticides may be due to uncontrolled use of pesticides in the study areas and the lack of an appropriate treatment system for controlling sewage and different wastewaters entering into the river systems in the Northern provinces of Iran.

Table 1: obtained concentrations of pesticides in mullet fish body in the studied areas (mg/kg)

Station	Diazinon average Concentration	Malation average Concentration	Azinphos-methyl average Concentration	Average of total length (cm)	Average of total weight (g)
Babolroud	0.27	0.17	1.28	39.2	**465.2**
Tajan	0.09	0.19	0.76	29.3	**297.5**
Gorganroud	0.08	0.07	0.04	27.5	**249.3**

Table 2: comparison between the obtained results and the previous studies

Babolroud studied area	
Results of the present study	**Results of the previous study (Gholamipour)**
Average concentration of diazinon in mullet fish 0.27 mg/kg	Average concentration of diazinon in white fish 0.23 mg/kg
Average concentration of malation in mullet fish 0.15 mg/kg	Average concentration of malation in carp fish 0.15 mg/kg
Average concentration of azinphos-methyl in mullet fish 1.28 mg/kg	----

Table 3: lethal dose of 50% of diazinon, malathion, azinphos-methyl

Pesticide	(mg/L) Concentration
Diazinon	**2.6 – 23.4**
Malathion	**0.1 – 0.28**
Azinphos-methyl	**0.03 – 0.12**

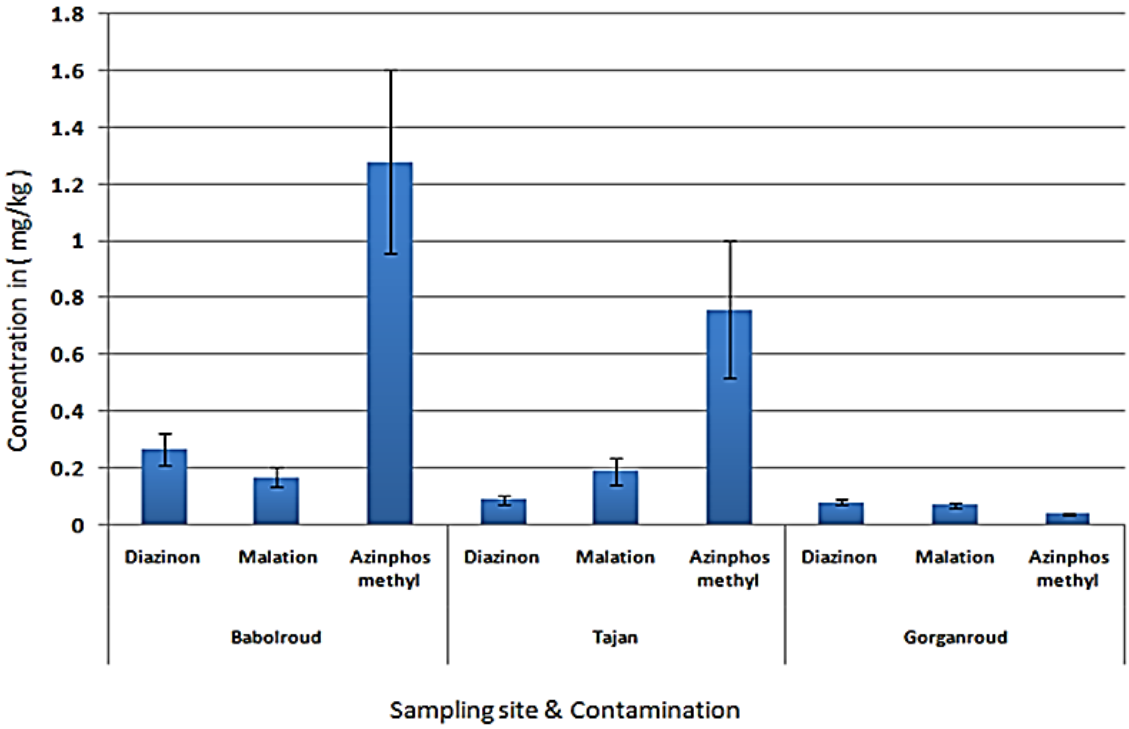

Fig. 1: diagram of general comparison of pesticides concentrations in mullet fish in studied areas

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