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Ecotoxicological Evaluation of Pesticide Pollution in Ataturk Dam Lake (Euphrates River), Turkey

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Abstract

Residues of organochlorine pesticides (OCPs) were detected in water, sediment and liver tissue samples of the common carp (*Cyprinus carpio* Linnaeus, 1758) collected from the Ataturk Dam Lake. Ethoxyresorufin O-deethylase, glutathion S-transferase, glutathion reductase, superoxide dismutase, catalase and carboxylesterase activities have been evaluated in liver of *Cyprinus carpio*. The level of OCPs were determined by Gas Chromatography-Mass Spectrometry. No pesticide residue was determined in the water samples and residues in the sediments were higher than in the fish. In the wet season, the level of pesticides were higher than in the dry season. The concentrations of OCPs were highest in the Akyazı and Bozova areas. Enzyme analysis results showed that the activities were different from region to region and generally higher in Akyazı and Bozova than the other areas. This study is the first study that determines the levels of OCPs of sediment, water and fish in Ataturk Dam Lake and presents pesticide residue levels in the fish samples were above the maximum residue limits so could be a threat to the human health. The presence of OCPs indicates the need for continuous monitoring of the Lake fish population to safeguard the health of the consumers.

Keywords: Euphrates River; Organochlorine pesticides; GCMS; Biomarker; *Cyprinus carpio.*

Atatürk Baraj Gölü'nde (Fırat Nehri, Türkiye) Pestisit Kirliliğinin Ekotoksikolojik Yaklaşımla Değerlendirilmesi

Özet

Atatürk Baraj Gölü'nden toplanan su, sediment ve sazan balığının (*Cyprinus carpio* Linnaeus, 1758) karaciğer örneklerinde organoklorlu pestisit kalıntısı belirlenmiştir. *Cyprinus carpio*'nun karaciğerinde Ethoxyresorufin O-deethylase, glutathion S-transferase, glutathion reductase, superoxide dismutase, catalase ve carboxylesterase aktiviteleri de değerlendirilmiştir. Organoklorlu pestisit düzeyleri gaz kromatografisi kütle spektrometresi ile belirlenmiştir. Su örneklerinde pestisit kalıntısına rastlanmamıştır ve sedimentteki kalıntı düzeyi balıklardakinden yüksek çıkmıştır. Yağışlı dönemde pestisit düzeyi kurak döneme göre daha yüksek çıkmıştır. En yüksek pestisit konsantrasyonu Akyazı ve Bozova bölgelerinde bulunmuştur. Enzim analiz sonuçları, aktivitelerin bölgeden bölgeye farklı olduğunu ve genelde Akyazı ile Bozova'da diğer bölgelere göre daha yüksek olduğunu göstermiştir. Bu çalışma, Atatürk Baraj Gölü su, sediment ve balıklarındaki organoklorlu pestisit düzeyini belirleyen ilk çalışmadır ve balıklardaki pestisit kalıntı düzeylerinin maksimum kalıntı sınırının üstünde olduğunu böylece insan sağlığı üzerine bir tehdit oluşturabileceğini ortaya koymaktadır. Organoklorlu pestisit kalıntılarının varlığı, tüketicilerin sağlığını güvence altın almak için göldeki balık populasyonunun sürekli izlenmesi gerektiğini gösterir.

Anahtar Kelimeler: Fırat Nehri; Organoklorlu pestisitler; GCMS; Biyobelirteç; *Cyprinus carpio.*

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Introduction

Organochlorine pesticides (OCPs) are among the agrochemicals that have been used commonly for long periods (Guo et al., 2008). OCPs are very stable, with long half lives in the environment so they have a potential for bioaccumulation (El-Mekkawi et al., 2009).

The evaluation of the pesticides in water environments is very important for human health and local biota (Yang et al., 2013). Accumulation of pollutants in sediment is considered a great threat to aquatic biota and, consequently, to human health. Fish are good indicators for the prediction of pesticide residues in freshwater systems (Rashed, 2001). OCPs and other pollutants induce the intracellular generation of reactive oxygen species (ROS), which modify functions of antioxidant enzymes (Osburn and Kensler, 2008). Organisms possess enzymatic antioxidant defences such as catalase (CAT), superoxide dismutases (SOD) and glutathione peroxidases (GPX). Glutathione reductase (GR) is a widely used biomarker that may be indicator of oxidative stress (Stephensen et al., 2002). These antioxidant enzymes are used as common biomarkers in fish contaminated with pollutants (Oost et al., 2003). Xenobiotics such as OCPs are catalyzed by cytochrome P450 isoenzymes which are placed in Phase I biotransformation reactions. Induction of Ethoxyresorufin O-deethylase (EROD) activity shows exposure to xenobiotics (Mortensen et al., 2007). Phase II enzymes defend against free radicals by conjugation, one of them is glutathion S-transferase (GST) (Rahaman et al., 1999). Carboxylesterase (CaE) plays a significant role in the metabolism of many pesticides (Potter and Wadkins, 2006).

Ataturk Dam Lake, situated in the Euphrates River Basin, is the largest dam lake in Turkey and ranks sixth amongst the largest earth-and-rock fill embankment dams in the world and is used for irrigation and electrical energy production. Pollution of Ataturk Dam Lake increased in recent years due to industry and agricultural activities improved around this lake. The economy of this district is mainly based on agricultural activity. Tobacco, cotton and pulses are the main crops of this district and polluted by urban, industrial and agricultural wastewater from Adiyaman and Sanliurfa cities around the dam lake. The possible contamination is important for Turkey, Syria and Iraq due to the path of Euphrates River (Karadede et al., 2004). There is no study about pesticide residue in Ataturk Dam Lake. The present study, therefore, provide baseline data on the quantity and distribution of some OCPs in fish (*Cyprinus carpio*), sediments and surface waters of Ataturk Dam Lake that will contribute to scientific evaluation of the effect of pesticides on health and the environment in Turkey. This study was the first attempt to identify and quantify some organochlorine pesticides in water, sediment and fish of the Ataturk Dam Lake (Euphrates River), Turkey.

Material and Methods

Sampling Sites

Six sampling points were selected and field of the study was Adiyaman and Sanliurfa basins of the Ataturk Dam Lake. The sites were chosen based on pollution in the main agricultural sector of this region. Water, sediment and fish samples were collected at six stations: four from the coastal region on the Adiyaman (Sitilce, Kahta, Oluklu, Samsat) and two from the side of Sanliurfa (Akyazı, Bozova) (Figure).

Sample Collection

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Collection of samples was done in both dry (November-December 2013) and wet (April-May 2014) seasons. Twelve water samples were collected and analyzed. The water samples were collected with a Ruttner water sampler (Hydro-Bios 2 L, 0.5 m long) and kept in icebox and carried to laboratory. Twelve sediment samples were collected. The sampling of sediment was perform with a Eckman grab sampler that surface area of 0.185 m^2 (Hydro-Bios, Kiel, Germany). Eight fish samples were collected from each sampling point based on dry and wet seasons so totally ninety-six fish were catched and analysed for OCP residues and enzymes. Catching of fish were done with gill nets that are used by fishermen in the area and they were anesthetized with MS222 containing 100 mg/L in a plastic gallon (Sigma, USA) for a few minute for sacrificing and then transported to the laboratory using ice boxes. The total length of fish ranged between 45 and 60 cm and the weights varied between 600 and 1000 g. Age determination was made by reading of scales and the maximum age was obtained as 9+ years. Animal capture was approved by the Ethic Committee of Inonu University, Turkey (Permissions no. 2014/A-25). All animal procedures were performed as described in the American Society for Testing and Materials guidelines (ASTM, E 1849). The liver tissues were taken for the OCP residues and enzyme analyses. Experimental studies were carried out in the central research laboratory of Adıyaman University.

Water Quality Analyses

Water temperature, pH, conductivity and dissolved oxygen concentrations were measured using mobing meters. BOD, COD, ammonium, nitrate, nitrite and phosphate values were determined by the spectrophotometer DR/2010 model Hachlange.

Extraction of OCPs from the Samples and Quantification

The standard pesticides were gotten from Sigma-Aldrich with 96.7% purity. Exraction of water samples was done following the method defined by Osibanjo and Adeyeye (1997). A rotary evaporator was used to intensify the exract to 10 ml at 45°C. The extract was dropped off 1 ml under nitrogen gas at 50°C and transferred into vial. The sediment samples were extracted according to Ize-Iyamu et al. (2007). The 20 g of anhydrous Sodium sulphate and 10 g of sediment was crushed powder using a mortar. The extraction of crushed sample was done with 150 ml of a mixture of n-Hexane and Acetone (1:2). The extract was concentrated to 20 ml in a water bath protected between 50 and 55°C and the remaining solvent was evaporated. Extraction of the liver tissue samples was done with QuEChERS method described by Brondi et al. (2011). The analyses consisted of the following steps: (a) putting the liver tissue about 10 g into a centrifuge tube; (b) supplementing the standarts of pesticides in the needed concentrations; (c) adding 1 g of NaCl, 10 mL of MeCN and 4 g of $MgSO_4$ in each tube, then centrifuging it at 3,000 *g* for 1 min; (d) transferring 5 mL of MeCN extract to a commercial SPE cartridge including 330 mg C18, 330 mg PSA, and 1 cm stratum of $MgSO_4$ (e) One milliliter extract was imported to a vial. The modern Shimadzu GCMSQP-2010 ULTRA was runned to analysing. Analysis was applied in triplicate. The conditions of GCMS were shown in Table 1. Recoveries of OCPs in the reference material were between 90% and 102% of certified concentrations. The limit of detection (LOD) value for all OCPs was 3 μg/kg and the limit of quantification (LOQ) was 9 μg/kg. The calibration curves showed a high level of linearity for all pesticides with correlation coefficients ranging between 0.985 and 0.999.

Determination of Enzyme Activities

Liver samples were weighed and then homogenized at 15000 *g* for 30 s (Ika T25 D) with seven volumes of ice-cold homogenization buffer (0.15 M KCl, 0.1 M KH_2PO_4, 0.05 mM DTT and 1 mM EDTA). Homogenate

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was centrifuged at 16000×*g* for 20 min at 4 °C (Hettich 460 R) and the supernatant was separated. The enzyme activities were measured with a microplate reader (Thermo, Varioscan Flash 2000) in triplicate. The total protein concentration in the supernatant was determined using the Bradford method with the BSA as a standard (0-1.4 mg BSA/ml) (Bradford 1976). Obtained protein values were used to calculate specific activity values of each enzyme. Glutathion reductase (GR), glutathion S-transferase (GST), Ethoxyresorufin O-deethylase (EROD), superoxide dismutase (SOD), catalase (CAT) and carboxylesterase (CaE) activities were determined. Activity of GR was measured according to Stephensen et al. (2002) with some modifications. Reaction mixture consist of 1.2 mM NADPH, 0.075 mM DTNB, and 20 µl of sample in a total volume of 190 µl. The reaction started with the addition of 20 µl of 3.25 mM GSSG. The GSSG converted to GSH by reducing the DTNB. The activity was measured through the use of extinction coefficient for DTNB (ε=14151 M^{-1} cm^{-1}). The GST activity was evaluated by the method defined by Habig et al. (1974). The reaction solution consist of 1 mM GSH, 0.1 M potassium phosphate buffer (pH 6.5), 1 mM CDNB and 10 µl of sample. The activity was determined by use of an extinction coefficient for CDNB (ε=9600 M^{-1} cm^{-1}). EROD activitiy was evaluated using a fluorescent spectrophotometer (Thermo, Varioscan Flash 2000) according to the method described by Flammarion et al. (1998). EROD was analysed in a last volume of 270 µL including a 0.1 M potassium phosphate buffer (pH 7.8), 3.7 µM of ethoxyresorufin, 0.37 mM of NADPH, and 20 µL of supernatant. The resorufin values were determined using a standard curve of resorufin. EROD activity was defined as pmol of resorufin created per min per mg protein. The activity of CAT was measured by the decomposition of 1 mmol H_2O_2 per minute per mg protein according to the method described by Luck (1963). SOD activity was calculated by the method of McCord and Fridovich (1969). The amount of enzyme that inhibits the rate of reduction of cytochrome C by 50% at 25℃ at 550 nm was described as one unit of SOD. The activity of CaE was analysed using PNPA as substrate. The method was used described by Santhosh Kumar and Shivanandappa (1999). The reaction mixture contained 250 ml 0.1 mM Trizma buffer (pH 7.4) and 5 mL of supernatant was incubated for 3 min at 25 °C. The activities of enzyme were measured by using the extinction coefficient of p-nitrophenol (ε=1830 $M^{-1}cm^{-1}$).

Statistical Analysis

Statistical analyses were carried out by analysis of variance (ANOVA) using SPSS 15 software. One-way analysis of variance (ANOVA) followed by The Duncan's Multiple Ranges, F-test was used to test for the level of significance at 0.05 level of probability for the pesticide residue levels and enzyme activities in sampling points and a Pearson correlation analysis (PCA) was used to determine the relationship among the pesticides and enzymes based on sampling points using XLSTAT 2016 programme.

Results and Discussions

The long usage of the pesticides in agriculture, due to their persistence in the nature, increases the possibility of detection of them in the water samples. This condition is probably a result of an increased influent of drainage waters to the rivers that flow from the agricultural areas. Table 2 shows data on water quality parameters of Ataturk Dam Lake. The values of water quality parameters were lower for Bozova, Akyazı, Sitilce than Kahta, Oluklu, Samsat (Table 2). Table 3 and 4 present the average concentrations of OCPs in sediment and fish samples, respectively. No pesticide residue was determined in the water samples of Ataturk Dam Lake. Seasonal concentration of pesticides in sediment of sampling points was shown in Table 3. The residue levels of pesticides were higher in sediment samples than in fish samples in both dry and wet season. These results because

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of that OCPs are not hydrophilic and tend to accumulate in sediment and subsequently in fatty tissue of organisms (Chau and Afghan 1982). No heptachlor, aldrin, heptachlor exo epoxide, alpha-HCH, beta-HCH, gamma-HCH, residues were observed in sediment. The other pesticides were found at appreciably higher concentration with the following ranges (μg/kg): p,p'-DDE>dieldrin>o,p'-DDD>p,p'-DDD>o,p'-DDT>p,p'-DDT. The lowest and highest mean concentration of pesticides residues were o,p'-DDT (6.05±0.04 µg/kg) and p,p'-DDE (177.08±1.96 µg/kg) respectively. Investigation of organochlorine pesticides in sediments was conducted to record of contamination levels in the Ataturk Dam Lake, especially in Akyazı and Bozova. The concentration of organochlorine pesticides from Akyazı was detected between 15.40±0.35 and 177.08±1.96 µg/kg dry weights, while in Bozova, concentration of OCPs between 21.42±0.36 and 64.97±1.41 µg/kg (Table 3). The area Akyazı and Bozova around Ataturk Dam Lake is famous for raising cottons, which is the main agricultural branch in the region and the main source of income for the people who live in the area; thus, the preparation process for this agricultural activity can be considered an additional reason for the presence of toxic pollutants in the water and sediment ecosystems. The high level of sensitivity of different fish species to pesticides makes it possible to use these organisms as indicators of water pollution. Generally, organochlorines are considered very toxic to fish (Murty 1986). Levels of OCP residue were in the following order; p,p'-DDE>dieldrin>p,p'-DDD>o,p'-DDD>p,p'-DDT>o,p'-DDT. The dominant form of OCPs found in fish-tissue samples was p,p'-DDE. The content of this metabolic form was within the range from 10.14±0.06 to 66.35±1.83 µg/kg fresh tissue. The second most dominant form in the analysed samples of liver tissue was the dieldrin, with a content that ranged from 12.72±0.07 to 41.37±1.65 µg/kg fresh tissue (Table 4). Charles et al. (2000) point out that fish are active so may have been exposed to pollutants in aquatic system and bioaccumulated the pesticides in their bodies. The reason of bioaccumulation was lipid content of fish (Kidwell et al., 1990). The levels of DDT and its metabolites were highest in all sample types. Reason of this result can be attributed to the separation and bioaccumulation of the DDT used in the past. Both DDD and DDE are degradation products of DDT but DDE is more stable than DDT (Ljiljana 2007). In this study, the percent distribution of DDE was higher than DDD can be attributed to historical usage of DDT (Sanpera et al. 2002). The second most dominant form in the analysed samples of sediment and liver tissue was the dieldrin. The increment of dieldrin showed rate of degradation of aldrin in the sediment samples (Doyle et al. 1994). The level of dieldrin in all fish samples was higher than the FAO and WHO set maximum residue limit of 0.2 µg/kg (Codex Alimentarius Commission, 2009). HCHs are considered as the less persistence OCPs. Regional HCH contaminats were predictable by measurement of HCH in water, soils, and sediments (Li 1999). None of the HCH types were detected in the water, sediment and fish samples.

In the wet season, the concentration of pesticides were generally higher than in the dry season. According to Ezemonye (2004), the pesticides entered the river up to 60 times in wet season than in the dry season. OC concentration of all the sediment samples were higher than fish samples. Due to the low water solubility of the OCPs, it is considered as OCPs concentrated in fish and sediment.

Enzymes are good indicators for monitoring the effects of pollutants on fish (Mdegela et al. 2006). We measured the activities of GST, GR, EROD, CAT, SOD and CaE in the liver samples of *Cyprinus carpio*. In this study, OCP pollution was significantly higher at Akyazı and Bozova stations consequently enzyme activities were higher. This result probably due to the activating of these enzymes by the pollutants to provide antioxidant conservation. EROD is a good indicator reflects the existence of contaminats in fish, providing indicate of receptor-

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mediated induction of cytochrome P450-dependant monooxygenases by xenobiotics (Cantrell et al. 1996). EROD activity was highest level in Akyazı (1.92±0.42 pmol/min/mg), whereas the lowest level was in Oluklu (0.65±0.05 pmol/min/mg) (Table 5). It was not found statistically significant differences between Akyazı, Bozova and Sitilce ($p \leq 0.05$). The EROD activity increases when fish exposed to certain pollutants (Gungordu and Ozmen 2011). Also, Ozmen et al. (2008) claimed that the increases of EROD activity may through bioaccumulation of various xenobiotics in fish. Glutathione is a very important detoxifying agent, facilitating the body removed toxins. The GST is a major antioxidant protects cells from free radicals. In this study the highest GST activity value was observed at Bozova station (216.83±15.54). The highest GR activity was found in Akyazı (19.32±1.76) but the differences between Akyazı, Sitilce and Bozova were not statistically different (p $p \leq 0.05$). Ozmen et al. (2008) found enhanced GST activity in liver of carp caughed from an area where OCP contamination was highest. GST activity increased when *Xenopus laevis* tadpoles exposed to six types of OP (C2, C3, C4, C5, C7, and C8) (Gungordu et al., 2013). SOD and CAT activities are the most widely used measures of oxidative stress. SOD catalyses the transformation of the superoxide anion radical to molecular oxygen and hydrogen peroxide (H_2O_2) could protect against superoxide-induced oxidative damage (Fridovich 1989). CAT is an antioxidative enzyme which protect the cell against H_2O_2. Generally the activities of SOD and CAT increase when exposed to pollutants (Dimitrova et al. 1994). Such coordination was shown in our study. The hepatic SOD and CAT activities of *C. carpio* were greatly rised at Bozova (0.63±0.08 for SOD, 26.65±1.45 for CAT) and Akyazı (0.59±0.07 for SOD, 24.36±1.12 for CAT) stations. Increased EROD levels may be reflect the induction of CYP1A by organochlorine compounds. Increased production of ROS by CYP1 activity also inceases levels of SOD and CAT. Carboxylesterases play an important role in detoxification of pesticides. Especially, carboxylesterases hydrolyze pyrethroids organophosphates and carbamates (Wheelock et al. 2004; Sogorb and Vilanova 2002; Casida and Quistad 2004). In this study, no significant differences were found statistically between locations. Differences in enzyme activity can be interpreted to accumulation of the OCPs and the other many xenobiotics on sediment and also in fish tissues. Similar studies with our study were done in terms of enzyme activity changes for carp in Turkey (Ozmen et al. 2006; Gungordu and Ozmen 2011; Karaca et al. 2014; Agus et al. 2015).

To evaluate which sampling variables were closely related, a plot of factor coordinates for all significant observations was constructed using the factors obtained from factor loading analysis (Table 6). Results of correlation analyses (bivariate correlations with Pearson correlations coefficients) among pesticide concentrations in fish and enzymes based on sampling points, showed that there were strong positive correlations among all the pesticides and enzyme species in Akyazı, Bozova and Sitilce areas (Figure 2). Based on the bi plots for PCA (Figure 2), the pesticide and enzyme parameters were reduced to 2 main factors (factors 1 and 2). The first factor corresponding to the largest eigenvalue accounts for approximately 77.97% of the total variance and the second factor accounts for approximately 14.18% of the total variance. Further analysis of factor loadings showed that o,p'-DDT, EROD and GR were the 3 major factors significating the pollution of Ataturk Dam Lake (Table 6). For factor 1, o,p'-DDT, o,p'-DDD, EROD, GR and SOD have the highest factor loading value (>0.96) and showed that these are the most effective variables for the principal component. For factor 2, there was no an effective factor loading value (>0.96). According to the PCA (Figure 2), the sampling sites that are clustered near each other have similar characteristics with respect to the factors for example values of o,p'-DDT, o,p'-DDD, p,p'-DDT, SOD, GR, EROD and CAT were close to each other for Akyazı and Bozova, but away from Kahta, Oluklu and Samsat.

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Data in Table 7 provide the correlation matrix of the parameters obtained from the PCA. Generally, the enzymes show strong corelation with pesticide kinds, so that most of the correlation coefficients are higher than 0.7 (absolute value) except GST and CaE. The correlation coefficients between EROD, GST, GR, CAT and SOD were strong however there was a weak correlation between the CaE and all of the other enzymes (Table 7). The results of PCA analysis were similar to the results of Duncan's Multiple Ranges.

Ataturk Dam Lake is on the Euphrates river in south-east Turkey. Built to supply water for irrigation and power generation, it is the largest dam in the country and ranks sixth amongst the largest earth and rock fill embankment dams in the world. It is important for fishery and irrigation. This study gives valuable data for the literature because it is the first study that determines the levels of organochlorine pesticides of sediment, water and fish in Ataturk Dam Lake and ensures useful data for the control of the pollutants of this region. The use of OC is banned in Turkey as it is in many countries. But they are still being used illegally in various areas of Turkey (Kolankaya 2006). Their use in past years resulted in residues observed in the bodies water, sediments, soil and organisms. Due to the presence of chlorinated pesticides in Ataturk Dam Lake, more monitoring work must be done to maintain the future of dam.

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Table 1. GCMS conditions

Column	Rxi-5ms, 30 m x 0.25 mm ID, 0.25 µm df
Oven temperature	70 °C hold time: 2 min , 25°C /min to 150 °C, 3°C / min to 200 °C, 8°C/min to 280 °C hold time: 6 min.
Injection temperature	250 °C
Injection mode	Splitless
Sampling time	1 min
Carrier gas-prim. Press.	Helium, 500-900
Flow control mode	Pressure
Pressure	145.4 kPa
Total flow	50.0 mL/min
Column flow	2.30 mL/min
Linear velocity	55.6 cm/sec
Purge flow	3.0 mL/min
Split ratio	-1.0
Scan range	Selected ion monitoring (SIM), 12 monitoring groups used
Ion source temperature	200 °C
Interface temperature	280 °C

Table 2. Some water quality parameters of Ataturk Dam Lake during the study period

Parameters	Kahta	Oluklu	Samsat	Bozova	Akyazı	Sitilce
Dissolved oxygen (mg/L)	8.8	8.7	8.6	7.9	7.8	4.2
Temperature (°C)	22.4	22.3	22.6	23.6	23.8	23
Conductivity (IS/cm)	342	310	332	353	356	337
pH	8.33	8.28	8.25	8.18	8.45	8.2
NH_4^+ (mg/L)	0.013	0.104	0.202	3.04	3.08	2.95
NO_3^- (mg/L)	0.353	0.356	0.344	1.15	0.358	1.31
NO_2^- (mg/L)	0.018	0.013	0.012	0.087	0.049	0.471
PO_4^{-3} (mg/L)	0.099	0.107	0.543	0.806	0.814	0.712
COD	83.5	41.4	39.7	51.1	65.2	2164
BOD	6	2	2	8	16	350

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Table 3. Seasonal (mean) concentration of pesticides in sediment (µg/kg) of sampling points

			Dieldrin	p,p'-DDE	p,p'-DDD	o,p'-DDD	o,p'-DDT	p,p'-DDT
Sediment	Kahta	D.S	22.56^{c}±0.22	29.19^{c,d}±0.25	12.25^{a}±0.27	13.52^{a,b}±0.08	ND	ND
		W.S	25.74^{c}±0.38	38.25^{d}±0.29	11.27^{a}±0.16	14.68^{b}±0.14	ND	ND
	Oluklu	D.S	ND	ND	ND	ND	ND	ND
		W.S	ND	ND	ND	ND	ND	ND
	Samsat	D.S	12.04^{a}±0.25	13.65^{a}±0.14	11.33^{a}±0.12	10.05^{a}±0.07	ND	ND
		W.S	15.26^{a,b}±0.32	16.42^{a,b}±0.36	12.84^{a}±0.13	11.43^{a}±0.13	ND	ND
	Bozova	D.S	45.22^{d}±1.19	47.36^{e}±1.08	31.15^{b}±1.12	32.93^{c}±1.13	25.44^{b}±0.55	21.42^{c}±0.36
		W.S	49.63^{d}±1.24	64.97^{f}±1.41	33.89^{b}±1.15	39.22^{c}±1.22	23.81^{b}±0.42	30.55^{d}±1.22
	Akyazı	D.S	72.37^{e}±1.56	106.15^{g}±1.72	34.21^{b}±1.44	37.56^{c}±1.15	26.72^{b}±0.63	17.83^{b}±0.48
		W.S	83.81^{f}±1.95	177.08^{h}±1.96	46.53^{c}±1.65	48.70^{d}±1.75	32.12^{c}±1.27	15.40^{b}±0.35
	Sitilce	D.S	16.25^{b}±0.27	18.19^{b}±0.23	13.65^{a}±0.76	15.33^{b}±0.42	6.05^{a}±0.04	10.76^{a}±0.53
		W.S	18.58^{b}±0.28	25.43^{c}±0.36	12.25^{a}±0.32	14.06^{b}±0.33	8.92^{a}±0.05	10.15^{a}±0.50

*Data in the same column followed by the same alphabets are not significantly different at $\alpha = 0.05$ using the new Duncan Multiple Range Test, ND: Not detected D.S: Dry season W.S: Wet season.

Table 4. Seasonal (mean) concentration of pesticides in fish liver (µg/kg) of sampling points

			Dieldrin	p,p'-DDE	p,p'-DDD	o,p'-DDD	o,p'-DDT	p,p'-DDT
Fish	Kahta	D.S	14.27^{a,b}±0.16	17.16^{b}±0.19	8.43^{a}±0.08	6.85^{a}±0.06	ND	ND
		W.S	16.23^{b}±0.26	12.46^{a}±0.08	ND	9.37^{a}±0.08	ND	ND
	Oluklu	D.S	ND	ND	ND	ND	ND	ND
		W.S	ND	ND	ND	ND	ND	ND
	Samsat	D.S	ND	ND	ND	ND	ND	ND
		W.S	ND	10.14^{a}±0.06	ND	7.42^{a}±0.05	4.64^{a}±0.04	ND
	Bozova	D.S	31.44^{c}±1.15	32.87^{c}±1.36	22.32^{b}±0.44	13.65^{b}±0.07	15.71^{b}±0.06	5.62^{a}±0.03
		W.S	30.09^{c}±1.12	50.27^{d}±1.58	21.15^{b}±0.42	14.37^{b}±0.08	6.42^{a}±0.03	7.43^{a}±0.05
	Akyazı	D.S	37.42^{c,d}±1.27	53.82^{d}±1.77	25.26^{b}±1.06	21.42^{c}±1.14	12.98^{b}±0.08	10.07^{b}±0.07
		W.S	41.37^{d}±1.65	66.35^{e}±1.83	31.77^{c}±1.13	32.09^{d}±1.39	14.11^{b}±0.09	18.89^{c}±0.15
	Sitilce	D.S	13.28^{a}±0.09	15.68^{a,b}±0.22	7.38^{a}±0.06	ND	ND	ND
		W.S	12.72^{a}±0.07	13.44^{a}±0.08	6.40^{a}±0.05	5.17^{a}±0.06	3.45^{a}±0.02	ND

*Data in the same column followed by the same alphabets are not significantly different at $\alpha = 0.05$ using the new Duncan Multiple Range Test, ND: Not detected D.S: Dry season W.S: Wet season.

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Table 5. Selected enzyme activities of liver tissues of *C. carpio* collected from different site of Ataturk Dam Lake

Biomarkers	Kahta	Oluklu	Samsat	Bozova	Akyazı	Sitilce
EROD (pmol/min/mg protein)	0.86^{a}±0.07	0.65^{a}±0.05	0.73^{a}±0.04	1.85^{b}±0.23	1.92^{b}±0.42	1.53^{b}±0.18
GST (nmol/min/mg protein)	103.91^{a}±4.53	95.72^{a}±2.12	105.66^{a}±4.22	216.83^{c}±15.54	145.34^{b}±6.35	197.57^{c}±9.85
GR (nmol/min/mg protein)	6.83^{a}±0.78	4.14^{a}±0.85	3.92^{a}±0.57	16.75^{b}±1.28	19.32^{b}±1.76	12.46^{b}±1.05
CAT (U/mg protein)	11.94^{a}±0.45	13.56^{a}±0.64	10.21^{a}±0.36	26.65^{b}±1.45	24.36^{b}±1.12	21.43^{b}±0.96
SOD (U/mg protein)	0.44^{b}±0.05	0.31^{a}±0.03	0.35^{a}±0.03	0.63^{c}±0.08	0.59^{c}±0.07	0.46^{b}±0.05
CaE (nmol/min/mg protein)	192.33±12.4	185.65±7.16	174.22±3.45	183.35±6.54	187.78±8.32	176.4±5.43

*Data in the same line followed by the same alphabets are not significantly different at $\alpha = 0.05$ using the new Duncan Multiple Range Test

Table 6. Factor loadings of variables on principal components

Variables	F1	F2
Dieldrin	0.919	0.355
p,p'-DDE	0.842	0.473
p,p'-DDD	0.947	0.203
o,p'-DDD	0.966	0.183
o,p'-DDT	0.980	0.071
p,p'-DDT	0.877	-0.356
EROD	0.973	-0.179
GST	0.711	-0.669
GR	0.987	-0.041
CAT	0.924	-0.287
SOD	0.967	-0.033
CaE	0.179	0.761

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422
423 **Table 7.** Correlation matrix (Pearson (n))
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Variables	Dieldrin	p,p'-DDE	p,p'-DDD	o,p'-DDD	o,p'-DDT	p,p'-DDT	EROD	GST	GR	CAT	SOD	CaE
Dieldrin	**1**	0.974	0.981	0.976	0.929	0.647	0.828	0.392	0.884	0.711	0.864	0.334
p,p'-DDE	0.974	**1**	0.926	0.906	0.886	0.498	0.749	0.246	0.823	0.627	0.745	0.371
p,p'-DDD	0.981	0.926	**1**	0.994	0.954	0.744	0.866	0.495	0.901	0.764	0.894	0.189
o,p'-DDD	0.976	0.906	0.994	**1**	0.956	0.787	0.888	0.543	0.922	0.796	0.935	0.240
o,p'-DDT	0.929	0.886	0.954	0.956	**1**	0.826	0.938	0.616	0.964	0.899	0.901	0.175
p,p'-DDT	0.647	0.498	0.744	0.787	0.826	**1**	0.880	0.871	0.848	0.925	0.901	0.003
EROD	0.828	0.749	0.866	0.888	0.938	0.880	**1**	0.824	0.990	0.965	0.937	0.045
GST	0.392	0.246	0.495	0.543	0.616	0.871	0.824	**1**	0.743	0.867	0.749	-0.271
GR	0.884	0.823	0.901	0.922	0.964	0.848	0.990	0.743	**1**	0.948	0.944	0.167
CAT	0.711	0.627	0.764	0.796	0.899	0.925	0.965	0.867	0.948	**1**	0.888	0.045
SOD	0.864	0.745	0.894	0.935	0.901	0.901	0.937	0.749	0.944	0.888	**1**	0.242
CaE	0.334	0.371	0.189	0.240	0.175	0.003	0.045	-0.271	0.167	0.045	0.242	**1**

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428 **Figure 1.** The studied sites (Kahta, Oluklu, Samsat, Sitilce, Akyazı, Bozova) in the Ataturk Dam Lake, Turkey.

429
430 **Figure 2.** Bi plots for principal component analysis 1+2 of pesticides and enzymes of sampling points

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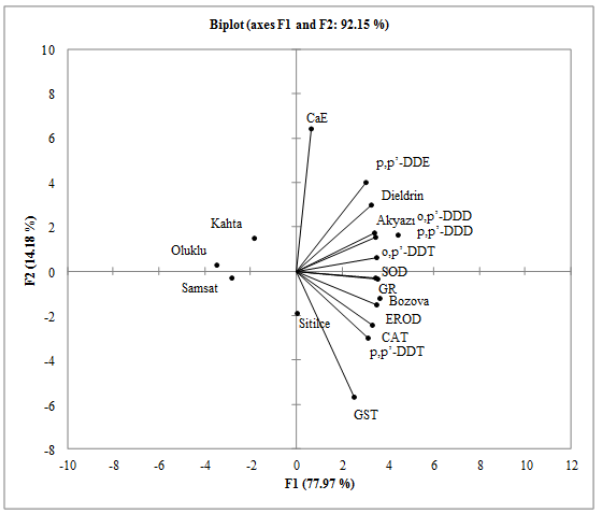

433
434 **Figure 2.** Bi plots for principal component analysis 1+2 of pesticides and enzymes of sampling points

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