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DISTRIBUTION OF DDT METABOLITES IN THE SEDIMENT AND MUSCLE FISH TISSUE FROM AGRICULTURALLY IMPACTED LAKE DOJRAN (MACEDONIA/GREECE)

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ABSTRACT

In the period from 2004-2006, investigations of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) in littoral sediments and the muscle tissue of the bottom-dwelling fish (*Barbus macedonicus* Karaman) have been conducted in Lake Dojran, Republic of Macedonia. Special emphasis was given to the presence the DDT degradation products 1,1,1-trichloro-2,2- bis(p-chlorophenyl) ethane (*p,p'*–DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (*p,p'*–DDE) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (*p,p'*–DDD). Sediment samples showed a dominance of *p,p'*–DDT, ranging from 3.55 μg kg^{-1} to 5.01 μg kg^{-1} of sediment dry mass, whereas *p,p'*-DDE was dominant in the muscle tissue of the bottom-dwelling fish *Barbus macedonicus* Karaman, with contents between 8.98 μg kg^{-1} and 10.85 μg kg^{-1} fresh tissue. Although DDT concentrations were below international health standards they may increase anthropogenic pressure on the unique and vulnerable ecosystem of Lake Dojran. The general findings underline the long period of intensive usage of DDT in the Southern Balkans, as well as its great persistence in agriculturally impacted surface waters. Furthermore, DDT-ratios in the sediment indicated use after the official ban of the pesticide, a situation, which may be representative for a range of surface waters in southeastern Europe.

KEYWORDS: DDT, GC-ECD, Lake Dojran, sediment, fish muscle, *Barbus macedonicus*

1. INTRODUCTION

Despite the fact that the majority of the organochlorine pesticides, including the insecticide dichlorodiphenyltrichloroethane (DDT), in the Republic of Macedonia, as well as in a vast number of countries all around the world have been forbidden for usage since the 1970s century, their presence in unchanged form or in some metabolic forms is frequently detected in different matrices [1-4]. Due to their lipophilic nature and low solubility in water they are highly persistent in biota and sediments of lake and river ecosystems [5-7]. In particular, sediments can behave as reservoirs, as well as long-term sources of contamination in aquatic ecosystems [7], affecting both water quality as well as living organisms [8]. The level of sorption of pollutants has been observed to be in direct correlation to the sediment composition, i.e. to the content of organic materials in the sediment [9-12]. As a result, surface waters influenced by agriculture are expected to be particularly impacted, both from intense former use of DDT, as well as high organic pollution from agricultural nutrient loads. Significant DDT contamination of surface water sediments and biota has been described in America [13, 14], Africa [15, 16], Asia [17], Northern Europe [1, 18] and Southern Europe [2, 19, 20]. The aim of the present work is to assess the extent of the DDT legacy in agriculturally impacted surface waters in political transition countries of southeastern Europe, where DDT use has been generally banned in the 1970s but compliance with the law is widely unknown.

Transboundary Lake Dojran between Macedonia and Greece makes an ideal case study, given its location and predominantly agricultural watershed. Lake Dojran is a large (142 km^2), shallow lake, which harbors unique species [21] and was declared a wetland of international importance by Ramsar Convention in 2007 [22]. Moreover its fishery is of local economic importance and lake sediments are used for healing purposes [21]. As a result, important DDT contamination of lake fish or sediments could also be of great ecological and social significance.

This study presents and discusses measurements regarding the presence and distribution of DDT and some metabolites: 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (*p,p'*–DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene

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(*p,p'*–DDE) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (*p,p'*–DDD) in the samples of sediment collected from the littoral zone of Lake Dojran over a duration of three years (2004–2006), as well as in the muscle tissue of the regionally unique fish species *Barbus macedonicus*, Karaman (1924) living in the same ecosystem.

2. MATERIALS AND METHODS

2.1. Study site

Lake Dojran is located in the Balkan Peninsula in southeastern Europe (41°23' N; 22°45' E). It is a remainder of the ancient Lake (Again) Paionia, and was formed by seismic activity. Lake Dojran is situated in the deepest part of the Dojran valley (Fig. 1), on an altitude of 148 meters.

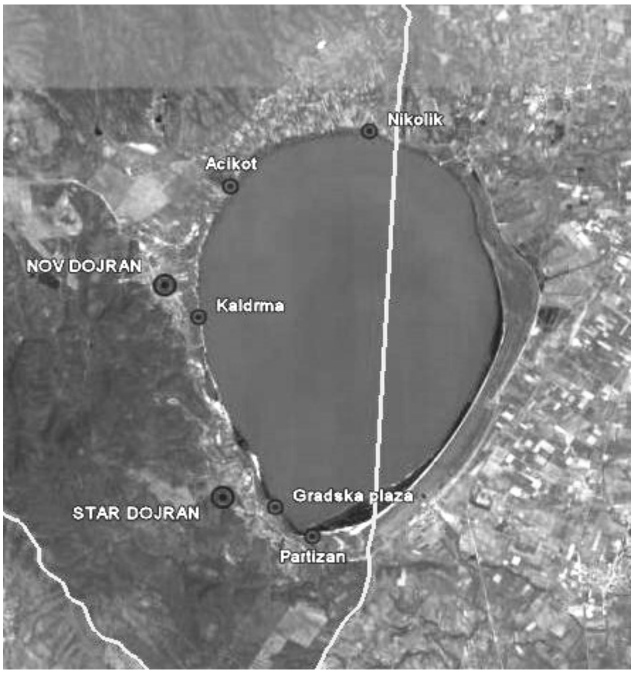

FIGURE 1 - Map of Lake Dojran with sampling locations

It has a surface of 42.5 km^2, with maximum length of 8.9 km and a maximum width of 7.1 km. The lake is shared between the Republic of Macedonia and the Republic of Greece with 26.58 km^2 (63 %) and 15.92 km^2 (37 %), respectively. Recharge of the lake is from direct runoff, small rivers, groundwater and atmospheric waters [23, 24]. Based on a 1991 bathymetric survey [25], the present average depth is between 3 and 4 meters.

Studies of the chemistry of the water and the sediment, as well as the flora and fauna of this typical eutrophic lake [26–30] placed Lake Dojran among very important water ecosystems as a result of the rich living world, characterized by several endemic taxa (e.g., *Spongilla carteri dojranensis* Hadzisce, 1953, *Candona paionica* Petkovski, 1958, *Niphargus pancici dojranensis* Karaman, 1960) [21]. The extremely rich plankton and periphyton production is the main reason due to which Lake Dojran is classified among the group of lakes which are the richest with fish in southeastern Europe [31].

Greece included Lake Dojran in the list Special Protected Areas (SPA Site Code: GR 1230003) within the framework of the European Ecological Network "Natura 2000" [32]. In Macedonia, Lake Dojran was declared as an Area of Special Conservation Interest – ASCI within the national Emerald network and is classified as natural rarity in accordance to the Decision Number 06-691/1. Moreover, Lake Dojran is entitled as an "Important Bird Area – IBA," on both the Macedonian as well as on the Greek side of the lake [21]. Finally, the Law for protection of Lakes Ohrid, Prespa and Dojran in 1977 and due to the specific characteristics (geological, geomorphologic, hydrological, hydro biological, limnological, cultural-esthetical, educational, health and recreational, as well as economical and commercial), the water, the shore region, the springs and the flows of Lake Dojran have been placed under special protection. This legislation act regulates the goal for protection of the Lakes with the main objective of their protection in their natural shape and condition.

In terms of economic use, Lake Dojran is of importance for fishery. In the 1960s, the annual fish catch was around 500 metric tons in Macedonia and 143,900 metric tons in Greece [21]. The fishery as an aspect of cultural heritage is characterised by a specific, traditional mode of fish catch called "mandri" with the assistance of the cormorants. In Macedonia, Lake Dojran is also a tourist attraction with health resorts focusing on the healing effects of lake sediments.

However, the extreme anthropogenic influence, long dry periods and uncoordinated agricultural development policy has resulted in a decrease in water level and water quality [21]. These changes led to in drastic effects to the composition of the microflora. Recent results on the phytoplankton and periphyton communities in Lake Dojran indicate that 109 algae taxa of 257 found in 1988 [33] may have disappeared [31]. The commercial activity is also endangered due to the anthropogenic impacts over the last 40 years. In the period from 1946 – 1986, the average annual fish catch in Lake Dojran, on the Macedonian side, decreased from 529 tons to 323 tons. After 1990, the annual catch of fish from the lake further decreased to only 25 tons per year in 2002. On the Greek side of the lake a decrease was also noted from 143,900 tons during the 1960s to 13,600 tons in the 1990s [21].

The usage of DDT has been banned in Macedonia since 1982. The last registered application of the insecticide was made in 1973 for the protection of the forests in the Republic of Macedonia [3].

2.2. Sampling and analysis

2.2.1. Collection of sediment samples

Sediment samples were collected at five sampling points from the costal region on the Macedonian side of Lake Dojran. Locations were selected at highly frequented tourist beaches: Nikolic, vacation spots Acikot, Kaldrma, Gradska Plaza and the auto-camp Partizan (Fig. 1). The sampling was been conducted with a Van-Veen grab sampler with a volume of 440 cm^3 (Hydro-bios, Kiel, Germany). Sediment samples were stored and transported in field – refrigerators. Before analysis samples were stored at 4°C, for a maximum of seven days.

2.2.2. Collection of fish muscle tissue

The muscle tissue of the barbel has been analysed in previous studies of the fish population; this is a species which belongs to the Vardar watershed and it is a benthic species. This species is also fished commercially. Fish samples of *Barbus macedonicus,* Karaman (1924), were collected by catching. Species determination was conducted in the framework of the work of Velkova-Jordanoska [34]. Immediately after fish were caught, the muscle tissue was separated. Shortly after collection, the isolated muscle tissue was kept frozen until analysis.

2.2.3. Preparation of sediment samples

The determination of organochlorine pesticides (total DDT and its metabolic forms) in the sediment was conducted in accordance with the modified method of EPA 8081A [35].

70 to 80 grams of fresh and well-homogenised sediment were placed in a glass container and mixed with 50 ml of a 1:1 mixture of hexane and acetone and 20 ml of methanol. Solid-liquid extraction was performed on a magnetic stirrer, for 2 hours at room temperature without any thermal treatment of the sample.

After the extraction of the sediment, the emulsion was transferred in two cuvettes and put in an ultracentrifuge (Niko Zelezniki; type LC-72) for 10 minutes at 3000 cycles/ minute, disintegrating the organic phase. Water contained in the extract was removed by transferring it through a layer of anhydrous sodium sulphate (Fluka, anhydrous sodium sulphate, purum p.a.>99.0% (T); lot & filling code: 71650 14607C03) and into a 1:1 mixture of hexane and acetone for better transportation of the extract and elimination of material from the extract. The extract was then placed into a round-bottom flask (50 ml), for concentration in a rotary evaporator (Heidolph). Sulphur present in the sample was removed with an activated elementary powder (copper fine powder GR particle size 63 μm) and cyclohexane on a magnetic stirrer for 10 minutes (Sulfur cleanup, EPA 3660 B).

2.2.4. Purification of the extract (Florisil cleanup)

The extract was then purified using a Florisil column (Florisil for column chromatography 0.150-0.250 mm) in accordance with the method EPA 3620 B. The procedure separates organochlorine pesticides from aliphatic and aromatic compounds, as well as from compounds which contain nitrate. The Florisil was preheated for 6 hours at 600°C. The 10-15 cm high column with Florisil was prepared with hexane. After the transfer of the extract, three fractions were collected, each of which contained different organic solvents. Elution of the analytes was performed with suitable solvents, to leave interfering compounds on the column.

1. First fraction with 20 ml hexane; this fraction mainly contained *p,p*'-DDE, *p,p*'-DDT, heptachlor and aldrin,

2. Second fraction with 20 ml mixture of hexane/ dichloromethane (74/24 v/v); in this fraction almost all organochlorine pesticides were present except endosulphane sulphate and endosulphan II,

3. Third fraction with a mix of acetone/hexane (10/90 v/v); in this fraction endosulphane II, a part of endrin aldehyde and a fraction of the metoxychlor were present.

p,p'-DDT is present in all three fractions.

Each of the fractions was dried and reconstructed with 0.5 ml hexane and internal standard pentachloronitrobenzene (PCNB, 5000 μg ml^{-1} in methanol, Supelco, Lot: LB44785) and was injected in a volume of 1 μl in GC/ECD analysis. After the injection of the three fractions, all values for the pesticides which were found in each of the different fractions were summed up.

Prior to the process of extraction, the dry mass of the sediment was determined (i.e. the percentage of moisture), since the sediment was analysed fresh and the results were calculated with respect to dry mass. The calculations included the presence of organic material in the sediment. The presence of moisture in the sediment was determined by drying of the sediment at a temperature of 105°C for 6 hours in porcelain bottles.

The presence of organic material was determined by ashing the dry sediment for 3 hours at 550°C.

2.2.5. Preparation of fish samples

The muscle tissue was fully thawed, cut into small pieces and homogenised. A quantity of 10 g was placed in a glass and a mixture for extraction consisting 1:1 hexane–acetone (30 ml) and methanol (10 ml) was added for extraction. The extraction was conducted by a magnetic mixer without thermal processing. After separation the extracted layer was centrifuged, filtered through a layer of anhydrous sodium sulphate and dried by evaporation.

The purification of the extract was conducted with an active Florisil column (Florisil cleanup) separated the three fractions (3620B) as described above. The three fractions were dried with test bottles and a reconstruction was undertaken with 0.5 ml hexane and IS (PCNB at a concentration of 100 pg ml^{-1}). The extracts were then analysed by GC-ECD. The results are in terms of kg fresh tissue and represent the total of the contents of the three different fractions.

2.2.6. Gas chromatography with an electron capture detector

Quantitative analysis was conducted with a gas chromatograph (Varian, USA), Model 3800, with an ECD detector and nitrogen as the carrier gas. The column which was used for the detection of OCP was VA-1701 (VA-123073-20), with the following characteristics: length, 30 m; I.D., 0.32 mm; film, 0.25 μm, with temperature limits from – 20°C to 280°C (300°C). A temperature program for the analysis of the organochlorine pesticides is given in Table 1.

TABLE 1 - Heating temperature program

Degree of increase of temperature, °C min^{-1}	Temperature, °C	Time, min
	70	1
20	180	0
10	230	3
5	270	5

The nitrogen carrier gas was set at constant flow of 2ml min^{-1}; pressure 20.80 psi; Inlet: splitless; 250°C; with a total flow of 24.5 ml min^{-1}. The temperature of the detector was 300°C.

The calibration curves were prepared with calibration standards and also used the internal standard of PCNB, and a standard mixture of organochlorine pesticide Mix 2 from Dr. Ehrenstorfer GmbH, at a concentration of 2000 ng μl^{-1} in toluene/hexane (Lot: 20114TH). Working mixtures were made with concentration range from 0.01–1 μg ml^{-1}, i.e. 0.01 μg ml^{-1}; 0.04 μg ml^{-1}; 0.1 μg ml^{-1}; 0.5 μg ml^{-1} and 1.0 μg ml^{-1}.

3. RESULTS AND DISCUSSION

3.1. Sediment samples

Table 2 presents the minimum and maximum contents of DDT and its metabolic forms (*p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD), during the period 2004 – 2006.

It can be seen in Table 2 that in all the sediment samples the three metabolic forms of DDT were present with values clearly beyond the detection limit (DL for sediment is 0.2 μg kg^{-1}). The minimum and maximum values for the specific forms were relatively close, especially for *p,p'*-DDD, where only small changes of the content of this form are observed over the three-year period. Generally, for all the sediment samples, the most dominant form was *p,p'*-DDT while the least present form was *p,p'*-DDD. The dominance of the least degraded form *p,p*'-DDT underlines the persistence of DDT in sediments.

This is in correlation with our previous measurements of water samples collected from the littoral zone of Lake Dojran [36] which indicated the presence of metabolic forms of DDT (*p,p*'-DDT, *p,p*'-DDE and *p,p*'-DDD) in the samples. The results of these analyses also indicate the domination of *p,p*-DDT (0.009–0.058 μg l^{-1}) in all samples of water collected from the investigated localities, if compared to the other two metabolic forms *p,p*'-DDE (0.008–0.039 μg l^{-1}) and *p,p*'-DDD (0,002–0.014 μg l^{-1}).

TABLE 2 - The range of the obtained contents of DDT and its metabolic forms in the sediments from five sampling localities (Fig. 1) for a three-years period

	p,p'-DDT min – max (μg kg^{-1}) n* = 9	*p,p'*-DDE	*p,p'*-DDD	Sum DDT
Nikolic	3.371 – 3.972	1.638 – 1.913	0.601 – 0.652	5.571 – 6.238
Acikot	4.102 – 5.044	1.367 – 1.738	0.547 – 0.651	6.447 – 7.112
Kaldrma	4.705 – 5.298	1.475 – 1.549	0.484 – 0.538	6.719 – 7.418
Gradska plaza	3.219 – 3.657	1.702 – 1.991	0.593 – 0.647	5.462 – 6.379
Partizan	4.082 – 4.889	1.503 – 1.892	0.454 – 0.529	6.363 – 7.037

n – number of sample

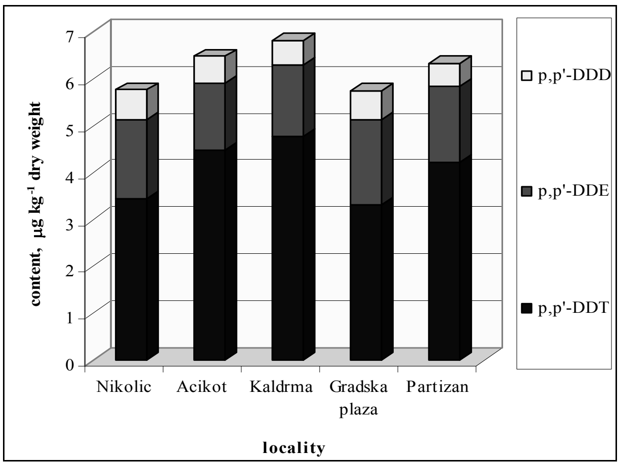

FIGURE 2 - Average three–year contents of DDT's metabolic forms in the sediment from different localities.

On the other hand, no residues of OC pesticides were found in water samples from the Province of Kayseri, Turkey [37].

De Mora et al. [38] found that the dominant form was *p,p*-DDT in the sediment samples from the Caspian Sea, with maximal values of 7.4 μg kg^{-1}.

Fig. 2 displays graphical representation of the average values of the contents of the metabolic forms of DDT (*p,p*'-DDT, *p,p*'-DDD and *p,p*'-DDE) in the mentioned period per locality, while Fig. 3 represents relative contents of the three forms.

Figs. 2 and 3 indicate that the maximum absolute concentrations and relative content of *p,p'*–DDT were found at the locality of Kaldrma with 5.01 μg kg^{-1} and 69 %, respectively. The lowest content for this metabolic form was 3.55 μg kg^{-1} at the locality of Gradska Plaza. Regarding the relative distribution it can be concluded that in all sampled localities this metabolic form (*p,p'*–DDT) was present as more than 50% of the metabolic forms of DDT (Fig. 3). Similar results were obtained by De Mora et al. [38] for the sediment samples from different localities in the Caspian Sea, which also indicated that the correlation among the three metabolic forms in the sum of DDT *(p,p'*–DDT, *p,p'*–DDE and *p,p'*–DDD), the form *p,p*'-DDT attained a values higher than 50%.

The other two products of the process of degradation *p,p*'–DDE and *p,p'*–DDD, were found in lower concentrations, i.e. with a smaller relative content of the total DDT. The average three-year concentrations for *p,p*–DDE were between 1.55 μg kg^{-1} for the locality of Acikot and 1.80 μg kg^{-1} for the locality of Gradska Plaza. The lowest relative content for *p,p'*–DDE was detected for the localities of Kaldrma and Acikot with 20 %, while the locality of Gradska Plaza showed the highest number with 29% (Fig. 3). The least present metabolic form in all localities was *p,p'*–DDD with values from 0.49 μg kg^{-1} for the locality of Partizan to 0.64 μg kg^{-1} for the locality of Nikolic.

According to other studies, the half-life of DDT can be attained up to 30 years [5, 39]. Given that the last known application of the DDT as an insecticide was in 1973 DDT concentrations may well have been twice the actual value 30 years ago [3]. On the other hand, unofficial information indicates illegal import and usage of DDT in the agricultural watershed of Lake Dojran [3]. The ratio of *p,p*'-DDT/*p,p*'-DDE, provides a useful index to assess whether the DDT at a given site is fresh or aged, with a value <0.33 generally indicating an aged input [38]. In the present study this ratio was between 1.3 and 2.1, indicating recent usage of the insecticide DDT in the Dojran region. In the investigations performed by Marku et al. [40] even higher values were obtained (DDT/DDE correlation in the interval from 9.62 to 33.0). According to the same authors, these results were due to the high content of *p,p*'-DDT, which was dominant in the aquatic ecosystem, if compared to its degradation products (*p,p*'-DDE and *p,p*'-DDD). In addition, according to Marku et al. [40], the high correlation of DDT/DDE, which was registered in sediment samples from the Lake Skadar, indicates to the recent usage of this insecticide in the surrounding agricultural areas.

An alternative explanation could be the fact that Lake Dojran is eutrophic [24, 41, 42]. Berglund [8], found a clear connection between the trophic state of aquatic ecosystems and the distribution of organochlorine components, i.e. a larger quantity of organochlorine components accumulates lates due to higher organic content and is "entrapped" due to reduced degradation in the sediment of eutrophic lakes,

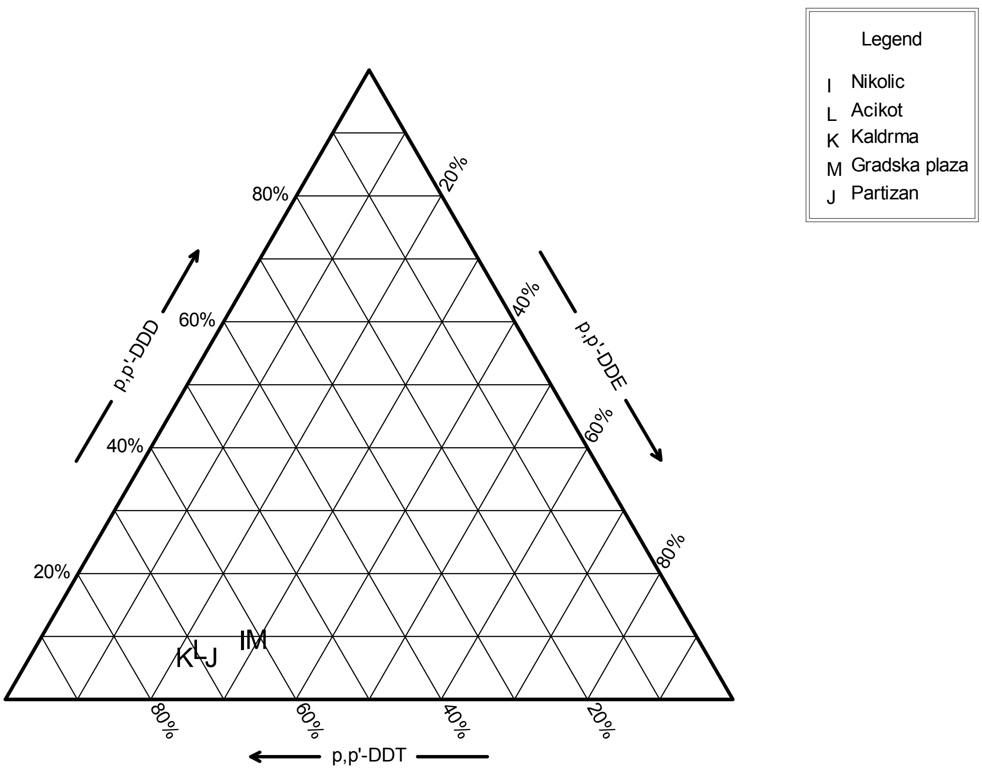

FIGURE 3 - Relative content of DDT and its metabolic forms in the sediment.

compared to oligotrophic lakes. The analysed samples of water from the littoral zone of Lake Dojran, in terms of quantity of the sum of DDT metabolites indicated that in the localities of Acikot, Partizan and Kaldrma, the sum of DDT was with higher concentrations in comparison to the localities of Nikolic and Gradska Plaza [36]. From the presented results obtained for the content of the sum of DDT in sediment of from the littoral zone of Lake Dojran (Figure 4 and Table 2), it may be noted that this insecticide with the highest values was recorded in the same localities as well as water samples [36]. In the study of Fytianos et al. [2], the distribution and concentration of the organochlorine pesticides in the surface water and the sediment were recorded at higher values in the sediment of Lake Volvi, northern Greece, compared to the surface water at the same sites. Therefore, sediments are a source of organochlorine components to the water and the living world through their redistribution in the aquatic system and, due to this, the sediments can be considered as long-term pollutants in aquatic ecosystems [8].

However, temporal variations of the concentrations of total DDT at the five sampling stations through the investigated period show a consistent, small decreasing tendency (Fig. 4). This tendency was most evident for *p,p*'-DDT (up to 20 % reduction in three years at the Acikot station), whereas *p,p*'-DDE showed an increasing tendency, in line with expected degradation. Though the tendency over a three year monitoring should not be overinterpreted, the data indicate (i) that degradation seems to occur despite high organic sediment content and (ii) that a relatively recent input of DDT, well after 1973, is likely to have occurred.

The observed distribution among the sampling locations (Figs. 2-4) may be the result of the composition of the sediment which in the localities of Nikolic and Gradska Plaza comprises of sand, while in the other localities (Kaldrma, Acikot and Partizan) it is more muddy and mixed with decaying plant and animal material. Given that the sorption of pesticides is directly affected by the organic matter content of environmental compartments [9, 10, 12, 43] a similar connection was expected for the presence of these compounds in sediment of the investigated localities.

Fig. 5 shows a good correlation between the average three-year contents of the sum of DDT metabolites (*p,p'*-DDT + *p,p'*-DDE + *p,p'*-DDD) and the percentage (three year period) of the total organic material in the sediment of the littoral region of Lake Dojran. Therefore, the lowest percentage of organic sediment content in the localities of Nikolic (3.42 %) and Gradska Plaza (2.16 %) coincided with the lowest evidenced concentrations of the sum of DDTs of 6.11 μg kg^{-1} in the locality of Nikolic and 5.89 μg kg^{-1} in the locality of Gradska Plaza (Fig. 5).

3.2. Fish samples

Pollutants or the toxic materials affect the health of the fish population even when they are sequestered in the sediments with an especially strong impact on species which, via the food chain are connected to the benthos. Therefore, Ize-Iyamu [15] has pointed out that the concentrations of detected organochlorine pesticides may be higher in organisms which live and feed near the sediment-water boundary in comparison to those that feed in the sediment or in the water.

We tested this hypothesis for Lake Dojran by analysing the muscle tissue of the species *Barbus barbus*, which is mainly a benthic inhabitant. Fig. 6 indicates that in all analysed fish samples the most present form of DDT form was the *p,p'*–DDE metabolite, with concentrations between 8.98 μg kg^{-1} and 10.85 μg kg^{-1} fresh tissue. The *p,p'*-DDT form was found at the lowest concentrations, between 1.16 μg kg^{-1} and 1.38 μg kg^{-1} fresh tissue. If we assume a simplified wet to dry mass ratio of five, *p,p*'-DDT per dry mass values of barbel are in the same range as in the sediments, whereas *p,p'*-DDE contents are up to 25 times

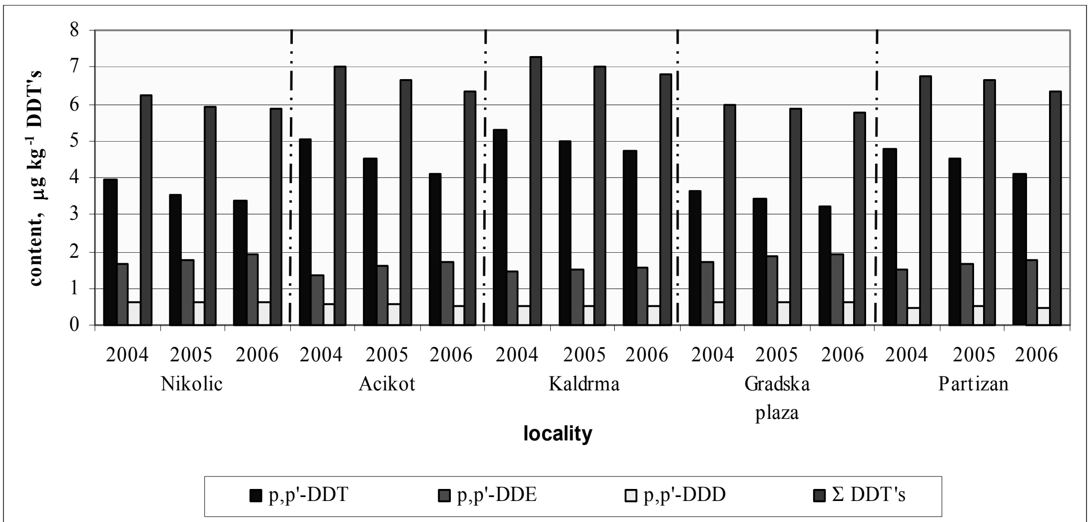

FIGURE 4 - Average annual values of DDTs for the period 2004 - 2006

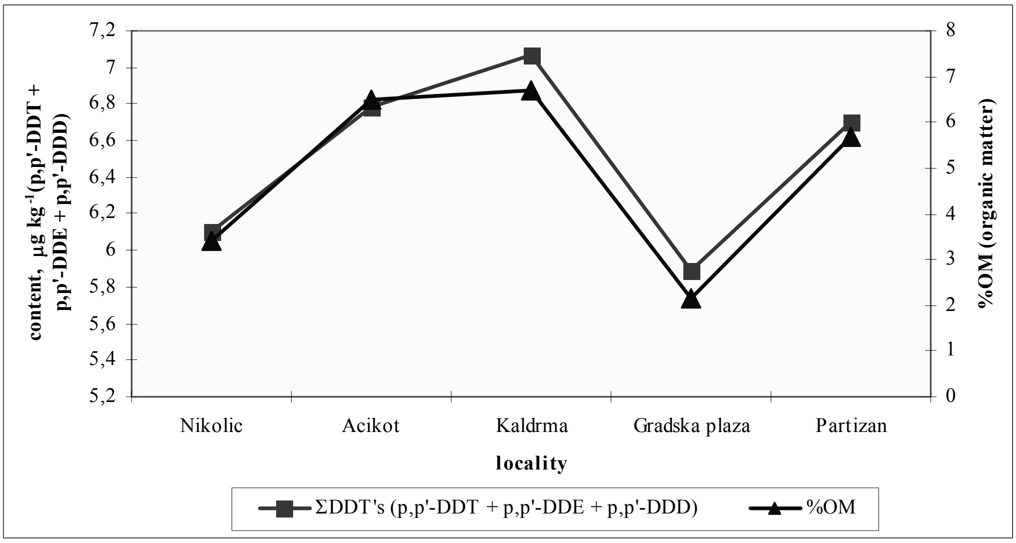

FIGURE 5 - Common correlation between the average value of the sum of DDTs and the percent of the total organic material in the sediment of Lake Dojran

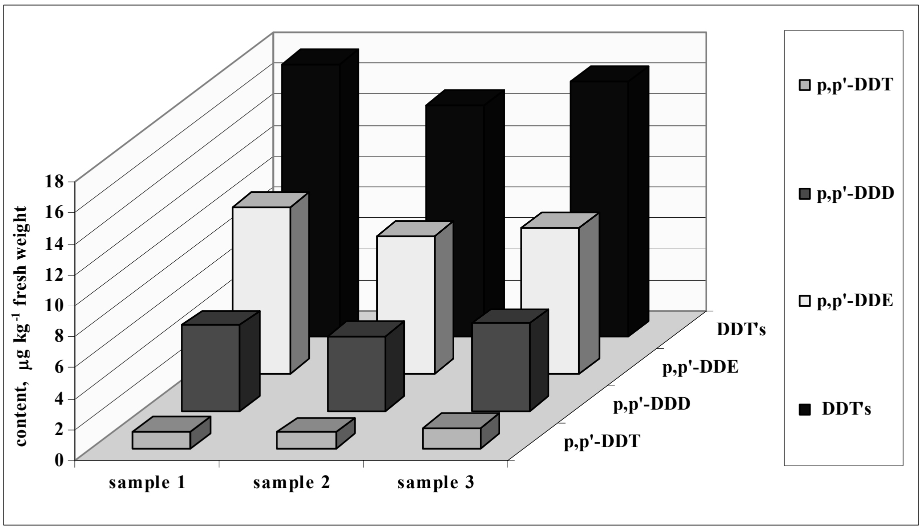

FIGURE 6 - Contents of the sum of DDT's (*p,p'*-DDT+ *p,p'*-DDE+ *p,p'*-DDD) and some metabolites forms in the tissue *Barbus macedonicus* tissue from Lake Dojran

higher, in agreement with the findings of Ize-Iyamu [15] above. In an analysis conducted by Caldas et al. [13], higher levels have been recorded for the content of the sum of DDT and its metabolic in fish samples in comparison to samples from the sediment. The sum of DDT was recorded in 98% of the samples of fish with a content which attained up to 77.7 µg kg^{-1}. Kiziewicz et al. [1] detected different contents of DDT and metabolites that to a great extent were dependant above all on the species of fish and to the parts that were taken as samples for extraction, such as the liver, muscle and brain. In the muscle tissue of different species of fish, the average value for the content of the total DDT was in the range from 2.7 to 53.07 µg kg^{-1}. Higher contents were detected for the brain and the liver (1165.9 and 646.90 µg kg^{-1}, respectively. Kiziewicz et al. [1] pointed out that this was due to the different accumulating ability of diverse animal tissues, as well as to different levels of accumulation of the pesticides in dissimilar species. Kayhan et al. [44] were found OC residues in all samples in the tissues (muscle and liver) of atlantic bluefin tuna from the Mediterranean Sea at mean concentrations under the permissible limits proposed by FAO/WHO.

According to Vives [20], the greater quantity of the form *p,p'*-DDE form can be explained by the transformation of the form *p,p'*-DDT form into *p,p'*-DDE which is occurs immediately after its entrance into the fish and accumulates with age. Similarly, multiyear research for the detection of organochlorine pesticides in many different fish species in US streams by Schmitt [14] indicated that in the period from 1970 to 1995, 70-74% of the total DDT was from *p,p'*-DDE. The explanation provided by him for this increase in the percent of this metabolite form in relation to the *p,p*'-DDT is that this is as a result of the decreased input of new quantities of the insecticide DDT. In contrast, Caldas [13] concluded that a high relative content of the most stable isomer *p,p'*–DDE (the mean value of 79.6% for the total DDT complex) may represent an indicator for recent exposure to DDT in the environment.

4. CONCLUSIONS

The results of our investigation of the sediment collected from the Lake Dojran, indicate the presence of the metabolic forms *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE, out of which the most highly represented form was *p,p'*-DDT. The results further indicate that higher concentrations of the detected DDT metabolic forms are evidenced in the samples of muscle tissue from *Barbus macedonicus,* Karaman in comparison to the samples from the sediment. In the muscle tissue, the dominant form was *p,p'*-DDE.

The concentrations of DDT found in fish are clearly below acceptable daily intake levels stipulated by the World Health Organization of 0.02 mg kg^{-1} of body weight, and are about 10 times lower than action level of 5 ppm proposed by the US Food and Drug Administration.

Nevertheless, the study indicates the presence of DDT in an ecosystem for which the anthropogenic influence is already intense. Moreover, the strong correlation between organic material and DDT indicates that sediment contamination is probably significantly higher, because of high trophy from agricultural effluents. Thus, agriculture seems to have a double effect on the lake ecosystem. Finally, given the presence of DDT, the usage of contemporary pesticides may represent additional pressure on the ecosystem.

Regarding the use of DDT, this study provides circumstantial evidence from the *p,p'*-DDT to *p,p'*-DDE ratio in the sediment, supported by apparent degradation in the sediment, that the last input to the lake ecosystem may have been much more recent than the official last use in 1973 or the official ban in 1982. It must be feared that the situation may be similar for other bodies of in south-eastern Europe.

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FEB/ Vol 20/ No 8a/ 2011 – pages 2027 - 2035