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Comparison of the Presence of DDT Metabolites in Sediments and Fish from Lakes Ohrid, Prespa and Dojran

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Abstract: The organochlorine pesticides have been detected in the samples of sediment and muscle tissue of barbell (*Barbus peloponnesius,* Valenciennes, (1842); *Barbus macedonicus,* Karaman (1924), collected from the three natural lake ecosystems in the Republic of Macedonia, i.e. Lake Ohrid, Lake Prespa and Lake Dojran, in the period from 2004 to 2006. Special attention has been paid to the presence of the insecticide 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), as dominant components in comparison to the other organochlorine pesticides.

The results that have been obtained indicated that dominant metabolic forms in the samples of sediment from Lake Ohrid and Lake Prespa is *p,p'*-DDE. The mean values for the content of this forms has been estimated at 0.84 µg kg^{-1} dry sediment in the samples collected from Ohrid and 1.56 µg kg^{-1} dry sediment in the samples from Lake Prespa. In the samples of sediment that have been collected from Lake Dojran, the dominant was the *p,p'*-DDT form, while it's content has been estimated at 4.06 µg kg^{-1} dry sediment. The highest values for the content of the sum of DDT metabolic forms in the fish muscle tissue have been evidenced in the samples of fish collected from Lake Dojran and the average value of this sum has been estimated at 16.38 µg kg^{-1} fresh tissue. The dominant metabolic forms for the analyzed muscle tissues of fish for all three lake ecosystems has been *p,p'*-DDE. The content of this form have been estimated at 5.98 µg kg^{-1}, 7.21 µg kg^{-1} and 9.77 µg kg^{-1} for the samples collected from Lake Ohrid, Lake Prespa and Lake Dojran, respectively. Thus, the highest evidenced value for the presence of *p,p'*-DDE has been recorded for the samples collected from Lake Dojran, i.e. 9,77 µg kg^{-1} fresh tissue.

Key words: organochlorine pesticides, DDT, persistent, lipophilic, trophy status

Introduction

One of the most important groups of compounds within the priority pollutants is the group of persistent organic pollutants, where as basic and most important group is the organochlorine pesticides. They are generally toxic organic compounds usually with synthetic heritage that are quite persistent to photolytic, biological and chemical disintegration. Besides the toxicity and persistency, additional concern in terms of serious potential risks upon the environment and the entire living world is their tendency to be accumulated in the fat tissue of the living organisms (lipophilicity) and thereafter to be bio-accumulated through the chain of nutrition (Kasozi *et al*. 2006), as well as the ability of the organochlorine pesticides to be transferred in an unchanged condition to distant areas where they have not been used at all (biomagnification).

Once introduced into the aquatic ecosystem, the persistent organic substances remain inside forever. They enter into the living world through the nutrition of the organisms or they are absorbed on the suspended particle, which is due to their high affinity to linking with the organic matter and in the end they are accumulated in the sediment, thus they are secondary source of pollution (Fytianos *et al.* 2006). The representatives of this group of pollutants remain for long time in the aquatic ecosystem in their natural form or in forms of its degradation (Vives *et al*. 2006).

Despite the fact that the insecticide dichlorodiphenyltrichloroethane (DDT) is not in use in a vast number of countries all around the world, including the Republic of Macedonia (banned for usage in the 1970s), still its presence in unchanged form or in some specific metabolic forms is noticeable in environmental (water, sediment, soil) as well as in fish muscle tissue (atsdr 2002; Barnhoorn *et al*. 2009; MOEPP 2004). This conclusion indicates to the fact that generally the living world is still affected by the mentioned insecticide and its effects, mainly due to the long period of persistency characteristic for this component and its lipophilic character (Jones, de Voogt 1999)

The biggest and most important aquatic resources in the Republic of Macedonia are the three natural lakes, i.e. Lake Ohrid, Lake Prespa and Lake Dojran. According to the data published in the literature by the National Institute for Health Prevention (MOEPP, 2004), in the territory of FYRMacedonia during the period 1947–1958 there have been used total of 23 910 kilograms of DDT as larvicide for procession of the larvae of the malaria mosquito. According to the same source, in Ohrid-Prespa region total of 4100 ha of area has been treated with DDT for final eradication of the malaria in 1973, which, in fact is the last date of usage of this pesticide in the country.

Thus, the aim of this study is to compare date presence and distribution of DDT and its products of degradation: 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) in the collected samples of sediment and fish tissue from the different points from three natural Lakes in FYRMacedonia, Ohrid, Prespa and Dojran.

Materials and Methods

Study site

In the period 2004-2006 a investigation in regards to the presence of total DDT through its metabolic forms *p,p'*–DDT, *p,p'*–DDE and *p,p'*–DDD in the sediment of the littoral zone and rivers of Lakes Ohrid, Prespa and Dojran, as well as from the muscle tissue of the barbel: *Barbus peloponnesius,* Valenciennes, (1842); *Barbus macedonicus,* Karaman (1924) from the same ecosystems has been conducted. From the littoral zone of Lake Ohrid there have been chosen seven localities, i.e. littoral near River Sateska, Daljan, Grasnica, Ohrid bay, St. Naum and littoral near River Cerava and Livadiste. From Lake Prespa, the samples have been collected from the three larger tributaries which supply the lake with water, i.e. River Golema, River Kranska and River Brajcanska and in the littoral near the lakes` deltas, i.e. Asamati, Krani and Nakolec. In Lake Dojran, the samples were collected from five localities, i.e. Partizan. Gradska plaza, Kaldrma, Acikot and Nikolik.

Sampling and analysis

Collection of sediment samples

Sediment samples were collected from the littoral zone of Lakes. The sampling was 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 ^{o}C, for a maximum of seven days.

Collection of fish muscle tissue

The fish samples of barbel, which is a benthic species fished also commercially, were collected from the region Граљница-Daljan (from Lake Ohrid). Fish samples of *Barbus peloponnesius,* Valenciennes, (1842) and *Barbus macedonicus,* Karaman (1924), were caught and their species determination was conducted by Velkova-Jordanoska (2008). Immediately after fish were caught, the muscle tissue was separated and shortly after it was frozen and kept at –20 ^{o}C until analysis.

Preparation of sediment samples

The determination of organochlorine pesticides in the sediment was conducted using the modified EPA 8081A method. 70 to 80 g of fresh and well-homogenized sediment were mixed with 50 mL of a 1:1 mixture of hexane and acetone (*V*/*V*) and 20 mL of methanol. Solid-liquid extraction was performed on a magnetic stirrer for 2 hours at room temperature. After the extraction of the sediment, the emulsion was transferred into two cuvettes and put in an ultracentrifuge (Niko Zelezniki; type LC-72) for the separation of three phases (organic, aqueous and solid). The organic extract was pipetted and the water contained in it was removed by transferring it through a layer of anhydrous sodium sulphate (Fluka, p.a.). The 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).

The cyclohexane extract was purified using a Florisil column in accordance with the EPA 3620B method. After loading the extract, three fractions were collected, eluted with suitable solvents, to leave interfering compounds on the column. Each of the fractions was dried and reconstructed with 0.5 mL hexane containing the internal standard pentachloronitrobenzene (PCNB at a concentration of 100 ng mL^{-1}) and 1 μL of the prepared sample was injected for GC/ECD analysis.

Prior to the process of extraction, the dry mass of the sediment was. The calculations included the presence of organic material in the sediment. The content of moisture in the sediment was determined by drying of the sediment at 105 °C for 6 h in a ceramic crucible. The content of organic material was determined by loss-on-ignition (LOI) method (ASTM Method D 2974-00).

Preparation of fish samples

The muscle tissue was fully thawed, cut into small pieces and homogenized. 10 g were placed in a glass and 30 mL of an extraction mixture hexane–acetone (1:1, *V*/*V*) and 10 mL methanol was added. The extraction was performed on a magnetic stirrer at room temperature for 2 h.

The reconstructed extract in 2 mL of cyclohexane was then purified with an active Florisil column (Florisil cleanup, EPA 3620B method) producing the three fractions as described above. The three fractions were dried in test bottles and reconstructed in 0.5 mL hexane containing internal standard, IS (PCNB at a concentration of 100 ng mL^{-1}). The extracts were then analyzed by GC-ECD. The results are in terms of kg fresh tissue and represent the total of the contents in the three different fractions.

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 used for separation of OCP was VA-1701 (VA-123073-20) with the following characteristics: length 30 m; I.D. 0.32 mm; film thickness 0.25 μm, and temperature limits from – 20 °C to 280 °C (300 °C range). The GC temperature program conditions for the analysis on organochlorine pesticides were: initial oven temperature 70 °C, held for 1 min, ramped at 20 °C/min to 180, then at 10 °C/min to 230 °C, held for 3 min, and finally at 5 °C/min to 270 °C and held for 5 min. The nitrogen carrier gas had a constant flow (2 mL min^{-1}) and pressure (20.80 psi). Inlet: splitless with a total flow of 24.5 mL min^{-1};injector temperature 250 °C; detector temperature 300 °C.

The calibration curves were prepared using a standard mixture of organochlorine pesticides, Mix 2 from Dr. Ehrenstorfer GmbH, at a concentration of 2000 ng μL^{-1} in toluene/hexane (Lot: 20114TH) and the internal standard of pentachloronitrobenzene (PCNB, 5000 μg mL^{-1} in methanol, from Supelco). Working standard solutions were prepared in the concentration range from 0.01–1 μg mL^{-1}.

Laboratory quality control

In this study the method detection limit (MDL) for *p,p'*-DDE in sediments was 0.44 μg kg^{-1}, for *p,p'*-DDD 0.31 and for the *p,p'*-DDE 0.21. The practical quantification limit (PQL) was from 0.5 to 0.7 μg kg^{-1}. The mean values for recovery for p,p'-DDT in the sediment was 89 %, for *p,p'*-DDE was 83 % and for *p,p'*-DDD was 85 %.

Results and Discussion

Sediment sample

The average values of the content of degraded forms of the DDT and its sum in the sediment samples collected during the period 2004-2006 from the littoral zone of three natural Lakes in FYRMacedonia, are presented in Fig. 1. The obtained results, indicate to a presence of three metabolic forms of the DDT insecticide, i.e. *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD in the analyzed samples of sediment. It is characteristic that the most dominant degraded form in the samples of sediment collected from Lake Ohrid (Veljanoska-Sarafiloska *et al*. 2011) and Lake Prespa (Veljanoska-Sarafiloska *et al*. 2012 submittet) is *p,p*'-DDE, which average values were estimated in between 0.85 and 1.55 μg kg^{-1} dry sediment, respectively (Figure 1). For Lake Dojran (Veljanoska-Sarafiloska *et al*. 2011a) the average value for the composition of *p,p'*-DDE was 1.62 μg kg^{-1} dry sediment. In addition, according to the investigations of Wasswa *et al*. (2011) in the samples of sediment collected from the Lake Victoria, dominant degraded form is *p,p'*-DDE and it has been in the interval between 0.11 and 3.59 μg kg^{-1} dry sediment, while second best in terms of presence in this ecosystem has been the form *p,p'*- DDT (0.04 - 1.46 μg kg^{-1} dry sediment).

In the samples of sediment collected from Lake Dojran the highest composition is detected for the form *p,p'*-DDT with 4.06 μg kg^{-1} dry sediment. In the other two aquatic ecosystems (Lakes Ohrid and Prespa), the form *p,p'*-DDT is registered with quite lower contents. Hence, the average value that is registered for this form in the samples of sediment from Lake Ohrid was estimated at 0.36 μg kg^{-1} dry sediment, which is 11 times lower than the content evidenced in the sediment of Lake Dojran (Fig. 4). The average value for the content of *p,p'*-DDT in Prespa was estimated at 1.07 μg kg^{-1} dry sediment, which is four times lower than the evidenced content in Dojran. In the sediment of River Bojana and in numerous localities from Lake Scadar (in Albanian side), dominant is the degraded form *p,p'*-DDT. In the samples from the River Bojana the value of this form was estimated at 41.48 μg kg^{-1} dry sediment, while in the samples from Lake Scadar the value for this form is within the interval from 21.5 to 59.77 μg kg^{-1} dry sediment (Marku,Nuro 2005). According to the same authors, the main reasons for this condition are the global atmosphere deposition and the agricultural drainage waters. On the other hand, the dominance of the form *p,p'*-DDT in relation to the other degraded forms in the sediment collected from the River Kim Ngum, Vietnam, according to Hoai *et al*. (2010) is mainly due to the recent application of the insecticide DDT in the region where the river flows.

The least present degraded form of DDT in the sediment from our investigation localities is the form *p,p'*-DDD (Fig. 1). The average value of this form for Ohrid has been estimated at 0.40 μg kg^{-1} dry sediment, 0.62 μg kg^{-1} dry sediment for Prespa and 0.56 μg kg^{-1} dry sediment for Dojran.

According to the obtained results of our investigations and from the results obtained from the investigation of Marku, Nuro (2005), a difference in regards to the mass contribution of the metabolic form of DDT can be noticed in the sediment of the natural aquatic ecosystems. This condition, presumably is due to the collation of their trophic state (Veljanoska-Sarafiloska 2011; Veljanoska-Sarafiloska, Patceva, 2012; Marku, Nuro 2005). Lake Ohrid is with an oligotrophic character, Lake Prespa with mesotrophic character while Lake Dojran and Lake Scadar are ecosystems with highest trophic condition and according to some parameters indicate to eutrophic character. The investigations of Berglund *et al*. (2001), regarding the relationship between the trophic state of the aquatic ecosystems and the distribution of organochlorine components indicated that the concentration of these components in the lake sediments was positively correlated to the lake trophy, i.e. larger quantity of organochlorine components is accumulated and 'entrapped' in the sediment of the eutrophic lakes, if compared to the oligotrophic lakes.

The three basic degraded forms of the insecticide DDT, i.e. *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD, can be used for determination of the chronology of entrance of DDT in the aquatic ecosystems (Hoai *et al*. 2010). 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 (De Mora *et al*. 2004). The comparison values obtained for the ratio *p,p'*- DDT/ *p,p'*-DDE for the three aquatic ecosystems which were subject of the investigations of this study, indicate that this ration in the sediment of Lake Dojran (Veljanoska-Sarafiloska *et al*. 2011a) is with the highest values and is in between 1.85 and 3.17, which indicated to recent entrance or usage of the insecticide DDT in the region. The obtained values of this ratio for the samples of Prespa are substantially lower and are in the interval from 0.49 to 0.84. The lowest values are recorded in the samples from Lake Ohrid (Veljanoska-Sarafiloska *et al*. 2011), which are within the interval from 0.31 and 0.52. These obtained values, for the last two aquatic ecosystems, also indicate to a relatively recent entrance of this component.

In the investigations conducted by Marku , Nuro (2005) in Lake Scadar, Republic of Albania, the values of the ratio *p,p'*-DDT/*p,p'*-DDE are within the interval from 9.62 to 33. This condition is linked by the authors to the fact that dominant degraded form in the investigated samples of sediment from Lake Scadar is *p,p'*-DDT, same as the case with the collected and analyzed samples from Dojran. Unlike this two ecosystems, in the samples of sediment collected from Ohrid and Prespa, dominant degraded form is *p,p'*-DDE.

Knowing the fact that the sorption of the pesticides is directly affected by the composition of the organic matter in the environment (Aboul-Kassim, Simoneit 2001; Kumar, Philip 2006), a correlation between the contents of these compounds in the sediment of the investigated locations is expected. Thus, the good linear correlation ($R^2 = 0.816$) between the content of total DDT's in the sediment, *w*(DDTs), given in μg kg^{-1}, and the percentage of the total organic material in the sediment, *w*(Organic matters), for Lake Ohrid is represented by the equation:

$w(\text{OCPs}) = 0.401 \cdot w(\text{Organic matters}) + 0.452$

The very good linear correlation ($R^2 = 0.965$) for sediment samples from Lake Prespa, can be expressed by the equation:

$w(\text{OCPs}) = 0.2868 \cdot w(\text{Organic matters}) + 2.0747$

Linear correlation ($R^2 = 0.906$) for sediment samples from Lake Dojran can be expressed by the equation:

$w(\text{OCPs}) = 0.225 \cdot w(\text{Organic matters}) + 5.089$

According to Kumar, Philip (2006) the organic

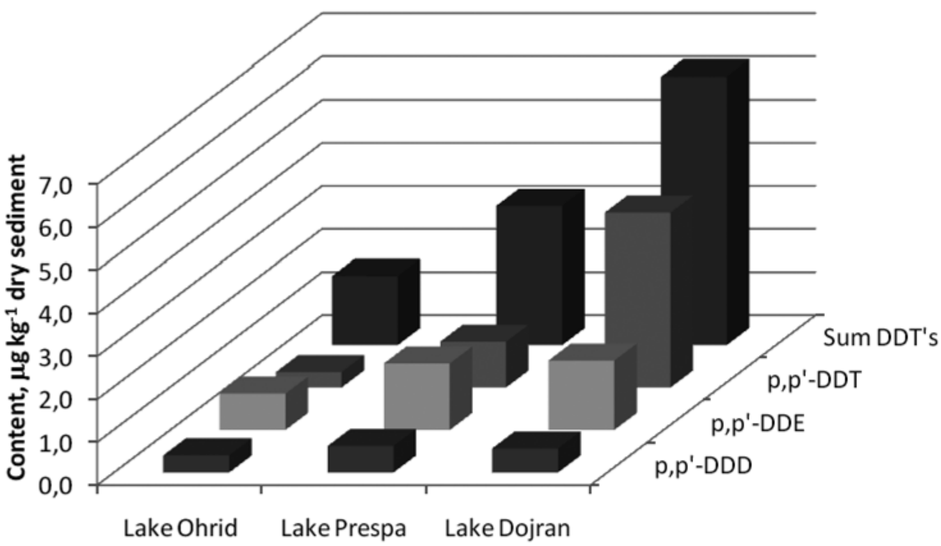

Fig. 1. Content of DDT metabolites in sediment sample from Lakes Ohrid, Prespa and Dojran.

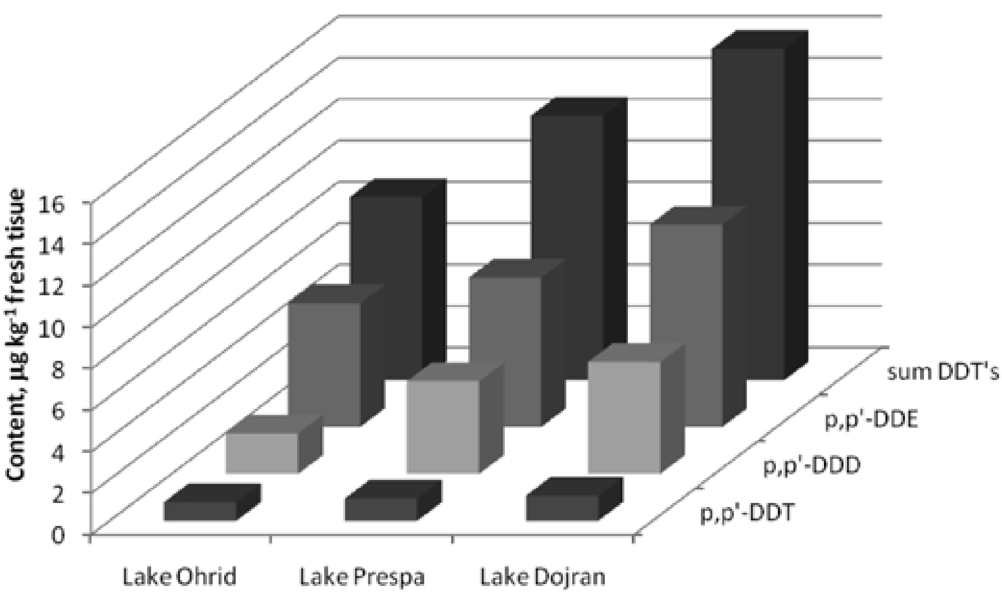

Fig. 2. Content of DDT metabolites in fish tissue.

matter in the sediment and sand plays a very important role in the processes of adsorption and desorption of persistent organic pollutants. It has been shown in his work that the bottom with the highest percent of organic matter has the highest adsorption power, as well.

Fish tissue

The effect of the presence of organochlorine pesticides in the water and the sediment of the aquatic ecosystems is reflected upon the living world which inhabits them, too. According to IZE-IYAMU *et al.* (2007), the concentrations of some specific organochlorine pesticides are higher in the organisms which are concentrated in the bordering layer sediment – water.

Fig. 2 shows the average values for the content of DDT degraded forms in the samples of fish collected from the three aquatic ecosystems, which indicate to a presence of the three metabolic forms.

The highest content of the insecticide DDT (16.38 μg kg^{-1} fresh tissue) is registered in the samples of collected fish from Lake Dojran (Fig. 2), which is the same as the case with the collected sediment (VELJANOSKA-SARAFILOSKA *et al*. 2011a). In the other two aquatic ecosystems, there have been registered relatively lower values for the sum of DDT (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD) in the samples of collected fish (VELJANOSKA-SARAFILOSKA *et al*. 2011; VELJANOSKA-SARAFILOSKA *et al*. 2012 submitted), i.e. 8.84 μg kg^{-1} fresh tissue for Lake Ohrid and 12.76 μg kg^{-1} fresh tissue for Lake Prespa (Fig. 2), which is in correlation with the lower average values of the sum of DDT in the samples of sediment. The transfer of contaminants from the sediment through the benthic organisms to the fish, which usually live and are finding food in the contact layer sediment–water is more dominant in the polluted aquatic ecosystems (VARANSI *et al*. 1992). As supportive arguments for this notion are the data of STANGE *et al*. (1997), for Barents Sea where the contamination of the sediment is low, hence the benthic fish do not have additional source of contamination, thus the values for the composition of the determined organochlorine components in them are relatively low.

In the investigations of TOPI *et al*. (2006), in the muscle tissue of all investigated samples of fish in the Albanian side of Lake Ohrid, out of the determined organochlorine pesticides, the dominant is the sum of DDT. Therefore, in the analyzed muscle tissue of samples of carp (*Cyprinus carpio*), the average value for this component is 19.09 μg kg^{-1} fresh tissue, while in the samples of trout (*Salmo letnica*) the value is 50.02 μg kg^{-1} fresh tissue. According to the same authors, the main reason for the presence of the degraded form of the insecticide DDT in the analyzed samples is the recent usage of the insecticide in the region.

It is quite characteristic that in the samples of the three ecosystems the most present degraded form is *p,p'*-DDE (VELJANOSKA-SARAFILOSKA *et al*. 2011; VELJANOSKA-SARAFILOSKA *et al.* 2011a; VELJANOSKA-SARAFILOSKA *et al*. 2012 submitted) . With lowest value of this form registered in the samples collected from Ohrid is 5.98 μg kg^{-1} fresh tissue, in the samples collected from Prespa the average value of *p,p'*-DDE is 7.21 μg kg^{-1} fresh tissue, while in Dojran 9.77 μg kg^{-1} fresh tissue (Fig. 2).

The other two forms (*p,p'*-DDT and *p,p'*-DDD) with lowest values are registered in the samples collected from Lake Ohrid and they are estimated at 0.90 μg kg^{-1} fresh tissue for *p,p`*-DDT and 1.96 μg kg^{-1} fresh tissue for *p,p'*-DDD. For the collected samples of fish from Lake Prespa, the average value for *p,p'*-DDT has been estimated at 1.07 μg kg^{-1} fresh tissue, while for the *p,p'*-DDD, the average value has been estimated at 4.48 μg kg^{-1} fresh tissue.

According to VIVES *et al*. (2005), the dominance of *p,p'*-DDE is as a result of the transformation of *p,p'*-DDT into *p,p'*-DDE, which is a process that occurs after the entrance of the metabolite in the organism (fish), while according to BARNHOORN *et al*. (2009), the metabolism of *p,p'*-DDE in the mass tissue is with decreased intensity, a characteristic which results in domination of this metabolic form in the organism. The mass tissue is a reservoir (warehouse) for the metabolic form *p,p'*-DDE, which is the main and most persistent metabolite in the environment (ATSDR, 2002).

Conclusion

The results of the investigations conducted and presented in this study indicate that the DDT metabolic forms (*p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD) are present in the analyzed matrixes, i.e. sediment and fish muscle tissue collected from the three natural Lakes in FYRMacedonia, Ohrid, Prespa and Dojran. This condition is mainly due to the persistency of this group of compounds, even though their usage has been banned in the Republic of Macedonia (and in most of the world's countries) in the 1970s of the XX century.

According to the obtained results from our investigations, the different mass presence of the detected metabolic forms of the insecticide DDT in the analyzed samples from the three aquatic ecosystems, as well as the favorable correlation between the organic matter and the composition of the sum of DDT, it can be concluded that all of those are in interconnection with the trophic state of the lakes. Lake Ohrid is an ecosystem with an oligotrophic character; Lake Prespa is a mesotrophic lake; while Lake Dojran, for which there are evidenced the highest values for all analyzed parameters, is a lake with eutrophic character.

The detection of the DDT and metabolites in the analyzed matrixes (sediment and fish tissue), as well as the correlation of *p,p'*-DDT/*p,p'*-DDE indicate to the long-term effect of the agricultural and other anthropogenic activities in the surrounding agricultural areas not only in Macedonian, but in Albanian and Greek side of the Lakes, as well.

The domination of the evidenced metabolic forms of DDT insecticide is noticed in the samples of fish muscle tissue, as a result of their lipophilic character and bioaccumulation. The highest average value for the sum of the three evidenced metabolic forms of DDT is noticed in the samples of collected fish from the Lake Dojran. The concentration 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.

References

ATSDR 2002. Toxicological profile for DDT, DDE and DDD.U.S. Department of health and human services, Public Health Service Agency for Toxic Substances and Disease Registry, Atlanta, Georgia.

Aboul-Kassim, T. A. T., B. R. T. Simoneit 2001. Pollutant-Solid Phase Interactions: Mechanisms, Chemistry and Modeling. – In: The Handbook of Environmental Chemistry, Water Pollution Series. Vol. 5/Part E, Springer-Verlag: Heidelberg.

Barnhoorn I. E. J., M. S. Bornman, C. Jansen van Rensburg and H. Bouwman 2009. DDT residues in water, sediment, domestic and indigenous biota from a currently DDT-sprayed srea. – *Chemosphere*, **77**, 1236-1241.

Berglund O., P. Larsson., G.Ewald and L. Okla 2001. Influence of trophic status on PCB distribution in lake sediments and biota. -*Environ. Pollut*. **113**, 199-201.

De Mora S. J-P. Villeneuve, M.R. Sheikholeslami, C. Cattini and I. Tolosa 2004. Organochlorinated compounds in Caspian Sea sediments. – *Marine Pollution Bulletin* **48** (1-2), 30-43.

Fytianos K., R. J. W. Meesters, H. Fr. Schröder, B. Gouliarmou, and N. Gantidis 2006. Concentration and distribution of organochlorine pesticides in surface water and sediments in Lake Volvi (N. Greece). – *International Journal of Environmental Analytical chemistry*, **86** (1&2), 109-118.

Hoai P. M., N. T. Ngoc, N. H. Minh, P. H. Viet, M. Berg, A. C. Alder and W. Giger 2010. Recent levels of organochlorine pesticides and polychlorinated biphenyls in sediments of the sewer system in Hanoi, Vietnam. – *Environmental Pollution*, **158**: 913-920.

Ize-Iyamu O. K., I. O. Asia and P. A. Egwakhide 2007. Concentrations of residues from organochlorine pesticide in water and fish from some rivers in Edo State Nigeria. – *International Journal of Physical Sciences*, **2**, 237-241.

Jones K. C., P. de Voogt 1999. Persistent pollutants (POP's): State of the science. – *Environmental Pollution*, **100:** 209-221.

Kasozi G. N., B. T. Kiremire, F. W. B. Bugenyi, N. H. Kirsch, P. Nkedi-Kizza 2006. Organochlorine residues in fish and water samples from Lake Victoria, Uganda. – *Journal of Environmental Quality*, **35** (2): 584-589.

Kumar M. and L. Philip 2006. Adsorption and desorption characteristics of hydrophobic pesticide endosulfan in four Indian soils. – *Chemosphere*, **62**: 1064-1077.

Marku E. and A. Nuro 2005. Chlorinated pesticides in the sediments and fish species of Shkodra Lake. – *Journal of Environmental Protection and Ecology*, **6** (3): 539-549.

National implementation plan on reduction & elimination of POP's in the Republic of Macedonia. 2004. Ministry of Environment and Physical Planning (MOEPP),Skopje.

Stange K. and J. Klungsøyr 1997. Organochlorine contaminants in fish and polycyclic aromatic hydrocarbons in sediments from the Barents Sea. – *ICES Journal of Marine Science*, **54**: 318-332.

Topi D., P. Troja, K. Koci, E. Marku and A. Nuro 2006. Same experimental data about the levels of chlorinated pesticides and PCBs in the biota of Ohrid Lake. Proceedings on Conference on water observation and information system for decision support, Ohrid, Macedonia. (www.balwois.net).

Varanasi U., J. E. Stein, W. L. Reichert, K. L. Tilbury, M. M. Krahn and S. L. Chan 1992. Chlorinated and aromatic hydrocarbons in bottom sediments, fish and marine mammals in US coastal waters: Laboratory and field studies of metabolism and accumulation. – In: C. H. Walker and D. R. Livingstone (Ed.): Persistent Pollutants in Marine Ecosystems, 83-119. Pergamon Press. 272 p.

Veljanoska-Sarafiloska E., M. Jordanoski, T. Stafilov and M. Stefova 2011. Study of organochlorine pesticide residues in water, sediment and fish tissue in Lake Ohrid (Macedonia/Albania). – *Macedonian Journal of Chemistry and Chemical Engineering*, **30** (2): 163-179.

Veljanoska-Sarafiloska E., T. Stafilov and M. Jordanoski. 2011a. Distribution of DDT metabolites in the sediment and the muscle fish tissue from agriculturally impacted Lake Dojran (Macedonia/Greece). – *Fresenius Environmental Bulletin*, **20** (8a): 2027-2035.

Veljanoska-Sarafiloska E. and S. Patceva. 2012. Trophic Status of Lakes Ohrid and Prespa during 2004-2006. – *Journal of International Environmental Application & Science*, **7** (2):291-299.

Veljanoska-Sarafiloska E., M. Jordanoski and T. Stafilov. 2012. Presence of DDT metabolites in water, sediment and muscle tissue of fish from Lake Prespa, Republic of Macedonia. – *Journal of Environmental science and health*. Submitted.

Velkova–Jordanoska L. 2008. Populaciono-geneticka struktura vo vidot *Barbus genus* (pisces, cyprinidae) vo Republika Makedonija determinirana so alozimski i molekularni (RAPD) marker. [Population-genetic structure in species of the *Barbus genus* (pisces, cyprinidae) in Republic of Macedonia determined by allozymes and molecular (RAPD) markers]. Ph.D. Thesis, St. Cyril and Methodius University, Skopje. (In Macedonian).

Vives I., J. O. Grimalt, M. Ventura, J. Catalan and B.O. Rosseland. 2005. Age dependance of the accumulation of organochlorine pollutants in brown trout (Salmo trutta) from a remote high mountain lake (Redó, Pyrenees). – *International Journal of Environmental Pollution*, **133:** 343-350.

Wasswa J., B. T. Kiremire, P. Nkedi-Kizza, J. Mbabazi and P. Ssebugere. 2011. Organochlorine pesticide residues in sediments from the Uganda side of Lake Victoria. – *Chemosphere*, **82** (1): 130-136.