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STUDY OF ORGANOCHLORINE PESTICIDE RESIDUES IN WATER, SEDIMENT AND FISH TISSUE IN LAKE OHRID (MACEDONIA/ALBANIA)

Elizabeta Veljanoska-Sarafiloska[1], Momčula Jordanoski[2], Trajče Stafilov[3]*, Marina Stefova[3]

[1] Hydrobiological Institute, Naum Ohridski Str. 50, 6000 Ohrid, Republic of Macedonia
[2] Faculty of Veterinary Medicine, St. Kliment Ohridski University, Prilepska b.b. Bitola, Republic of Macedonia
[3] Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, POB 162, MK-1001 Skopje, Republic of Macedonia
*e-mail: trajcest@pmf.ukim.mk

A systematic study has been performed for determination of a range of organochlorine pesticides in the water, sediments and fish samples in the Macedonian part of the Lake Ohrid and its larger tributaries. The obtained results give an overview of the contamination levels of these problematic compounds (i) at their potential sources in the river mouths, (ii) in the potentially affected, species-rich littoral section of the lake, and (iii) in the muscle tissue of one selected fish species *Barbus peloponnesius,* Valenciennes, collected near the rivers' deltas. The organochlorine pesticides measured in all three matrixes were: γ-HCH, ΣHCH (sum of α-isomer, β-isomer and δ-isomer), endosulfan (total of α and β endosulfan), DDT metabolites (*p,p*'-DDE, *p,p*'-DDD and *p,p*'-DDT). The range of the observed concentrations for *p,p'*-DDT was between 0.006 μg l^{-1} in the water samples from Daljan and St. Naum and 0.036 μg l^{-1} in the water from the Koselska River. The values for *p,p'*-DDT measured in dry sediment ranged from 0.121 μg kg^{-1} in the sample from St. Naum to 1.8 μg kg^{-1} in the dry sediment from the Velgoška River and between 0.553 μg kg^{-1} for total endosulfan and 5.982 μg kg^{-1} for *p,p'*-DDE of wet fish biomass. The most abundant of the detected organochlorine pesticides was the sum of DDT metabolic forms, i.e. *p,p*'-DDT, *p,p*'-DDE and *p,p*'-DDD. The detected concentrations are clearly below toxic thresholds and consequently severe effects on the endemic species of Lake Ohrid are not very likely.

Keywords: Lake Ohrid; organochlorine pesticides; *Barbus*; sediments; bioaccumulation

ИСПИТУВАЊЕ НА РЕЗИДУИ НА ОРГАНОХЛОРНИ ПЕСТИЦИДИ ВО ВОДАТА, СЕДИМЕНТОТ И ТКИВАТА ОД РИБА ОД ОХРИДСКО ЕЗЕРО (МАКЕДОНИЈА/АЛБАНИЈА)

Извршено е систематско испитување на застапеноста на органохлорните пестициди во примероци вода, седимент и риби од македонскиот дел на Охридското Езеро и неговите поголеми притоки. Добиените резултати даваат преглед на нивото на контаминација со овие штетни пестициди во потенцијалните нивни извори, односно во устијата на реките, во потенцијално загрозените литорални делови од езерото и во мускулното ткиво на посебно избран вид риба, *Barbus peloponnesius*, Valenciennes, колекционирани блиску до устијата на реките. Органохлорните пестициди кои се определувани во трите матрикси се: γ-HCH, ΣHCH (збир од α-изомерот, β-изомерот и δ-изомерот), ендосулфан (збир од α и β ендосулфан) и деградационите форми на DDT (*p,p*'-DDE, *p,p'*-DDD и

p,p'-DDT). Опсегот на добиените концентрации за *p,p*'-DDT е од 0,006 μg l^{-1} во примероците на вода од мерните места Далјан и Св. Наум до 0,036 μg l^{-1} во водата од Коселска Река. Вредностите на содржината на *p,p'*-DDT во седиментот се движат од 0,121 μg kg^{-1} во Св. Наум до 1,8 μg kg^{-1} во седиментот од Велгошка Река, додека оние за содржината на вкупниот ендосулфан од 0,553 μg kg^{-1} до 5,982 μg kg^{-1} за *p,p'*-DDE μg kg^{-1} во свежа риба. Најзастапени од детектираните органохлорни пестициди се деградационите форми на DDT, односно *p,p*'-DDT, *p,p'*-DDE и *p,p*'-DDD. Утврдените концентрации се под прагот на токсичност, па според тоа не се очекуваат некои посериозни влијанија врз ендемските видови од Охридското Езеро.

Клучни зборови: Охридско Езеро; органохлорни пестициди; мрена; седименти; биоакумулација

1. INTRODUCTION

Persistent organic pollutants (POPs), including organochlorine pesticides (OCPs), are of global concern because of their toxicity, resistance to degradation, potential for long-term transport and their tendency to accumulate in fatty tissues (lipophilicity), the latter of which renders them likely to bioaccumulate through food chain [1]. The potential risks they pose to the environment and to human health are so serious that international treaties, e.g. the United Nations Aarhus Protocol [2] and the Stockholm Convention [3] aimed at elimination or restriction of their production and use have been established. It should be mentioned that according to the Water Framework Directive (WFD) lindane is classified as a priority hazard substance due to its toxicity, persistence and bioaccumulation tendencies while cyclodiene pesticides and total DDT are not priority substances but other pollutants for which the environmental quality standards (EQS) for sediment and/or biota should be established at the national level and applied instead of the EQS values for water proposed by the Directive (*2008/105/EC*). In order to protect human health and environment from the influence of these pollutants, the Republic of Macedonia has also signed and implemented the Stockholm Convention in 2001.

Despite the fact that usage of the majority of organochlorine pesticides in the Republic of Macedonia has been forbidden since the seventies of the previous century, as well as in a vast number of countries in the world [4, 5], its presence in unchanged form or in some metabolic forms in different matrixes, water, sediment or animal tissue should be considered as a serious problem for the environment as a whole. Sediments represent the source of organochlorine components both for the water and the living organisms through their redistribution in the aquatic system, thus sediments can represent long-term pollutants [6]. The sediment stands for the habitat of the benthic fauna, a source and mechanism for removal of some specific contaminants from and to the aquatic ecosystem and transporters of contaminants in the ecosystems. The aquatic life represents an important source of food for the species living on the land and for the aquatic organisms such as the fish populations, which on the other side represent food for the people. Gas chromatography with different detectors, mainly electron capture detector (ECD), or more recently mass spectrometric detection (MS), is the most exploited technique for determination of OCPs in water, sediment and fish tissue [7–16].

Lake Ohrid, which belongs to the group of old lakes, is characterized by high percentage of endemic and relict species which are alive even today. Due to this phenomenon the lake is also known as a "Museum of Living Fossils". Therefore, the percentage of endemism among the fish population is estimated at 60 %, among the water worms (*Oligochaeta*) is 70 % and among the snails and planaria – some 80 % [17]. These features of its living world have encouraged and still represent a challenge for many world famous scientists. In

addition, they are considered as an everlasting source for scientific information, especially in the sphere of limnology. Fish population in both qualitative and quantitative composition contributes immensely to this ecosystem and is also important from economic point of view for both countries sharing the lake. Moreover, tourism as an especially important commercial sphere is also to be taken into consideration.

All these characteristics indicate that Lake Ohrid is very important not only from a scientific but also an economic point of view, thus in 1979 the lake and the town of Ohrid were protected by the Programme for Prevention of the World's Cultural Heritage by UNESCO [18]. This kind of treatment enforces and encourages care for prevention of this ecosystem by every single individual, as well as by every single visitor. Unfortunately, a conclusion has been made that this is not true in reality. This is especially visible in the shoreline zone of the lake. The tributaries Velgoška, Koselska, Sateska and Čerava are the end recipients of all atmospheric, industrial and drainage waters arising from the agricultural areas, as well as the waste waters directly flowing into the lake, hence these waters by inflowing into the lake represent a great danger for disruption of the natural balance between the biotope and biocenosis by inflowing into the lake. The deltas of the mentioned tributaries present "hot spots" on the Ohrid shores.

The aim of this study was to characterize the nature and content of the persistent organic pollutants, i.e. the organochlorine pesticides during 2004 by analyzing the water and the sediment samples from the larger tributaries of the Lake Ohrid in its Macedonian part and from the littorals near their deltas. Special emphasis was placed on the influence of the rivers' waters on the lakes' waters, as direct contributors. Simultaneously, the analysis conducted on the muscle tissue of the barbel, *Barbus peloponnesius,* Valenciennes (1842) was assayed as another indicator for the presence of these components and their content for estimation of the their accumulation in the tissues. The organochlorine pesticides measured in all three matrixes were: *gamma*-HCH (γ-HCH), 1α,2α,3β,4α,5α,6β-hexachlorocyclohexane; ΣHCH [sum of α-isomer (1α,2α,3β,4α,5β,6β-hexachlorocyclohexane), β-isomer (1α,2β,3α,4β,5α,6β-hexachlorocyclohexane) and δ-isomer (1α,2α,3α,4β,5α,6β-hexachlorocyclohexane)], endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo(e)dioxathiepin-3-oxide) (sum of α and β endosulfan), some DDT metabolites [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (*p,p'*-DDE), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) – *p,p'*-DDD, and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane – *p,p'*-DDT].

2. MATERIALS AND METHODS

2.1. *Study site*

Lake Ohrid belongs to the group of lakes for which the rule for short-term existence does not apply due to the fact that it dates back to the geological times before the ice age. The lakes Tanganyika, Lake Baikal and Lake Nyasa belong to this group of lakes [17].

Lake Ohrid is a large (volume ~55 km^3), oligotrophic (total phosphorus ~4.5 μg P l^{-1}) lake situated in South-Eastern Europe on the border between Republic of Macedonia and Albania. Stable isotope measurements as well as recent tracer experiments have revealed that water from Lake Prespa flows into Lake Ohrid through the karst channels [19, 20]. It is one of the oldest lakes in the world (>3 million years) and harbours a variety of endemic and relict species [17]. Another peculiarity of Lake Ohrid is its slow water exchange rate (hydraulic residence time ~70 years), which is a result of the relatively dry, Mediterranean climate and the small drainage basin. Table 1 gives an overview of the key quantities of Lake Ohrid.

T a b l e 1

Characteristics of Lake Ohrid

Property	Value
Latitude	41° N
Longitude	20.7° E
Altitude	690 m asl
Surface area	358 km^2
Volume	55.4 km^3
Maximal depth	288.7 m
Average depth	155 m
Water residence time	70 years
Age	3–5 · 10^6 years
Endemism	> 150 species
Average phosphorus concentration	4.5 μg l^{-1}

Six points have been chosen for monitoring of OCPs in this study (Figure 1). Four of them are located in the littoral region of the shores of Lake Ohrid: Daljan, Grašnica, the littoral near River Čerava and the littoral near the monastery St. Naum (oposite of the outflow of River Crn Drim) and two points in the rivers Velgoška and Koselska close to the river mouths.

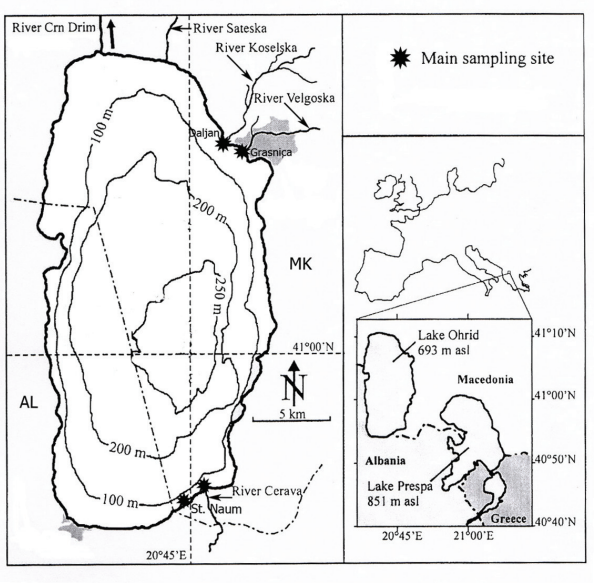

Fig. 1. Map of Lake Ohrid with the sampling locations

The rivers Velgoška and Koselska flow through settlements, agricultural areas and industrial zones. This results in organic overloading of the river waters as well as the lake waters in the regions where the rivers flow into Lake Ohrid.

2.2. *Sampling and analysis*

2.2.1. *Collection of water samples*

Water samples were collected at six sampling points, two in rivers Velgoška and Koselska and four from the costal region on the Macedonian side of Lake Ohrid near the river mouths (Figure 1). Samples were collected with Ruttner collector of 2.25 liters (Hydro-bios, Kiel, Germany) and were placed in glass bottles of 1 liter.

The samples reached the laboratory the same day after sampling, were acidified to pH 2.5 with hydrochloric acid (HCl, 6 mol l^{-1}) to inhibit biological activity, filtered through 0.45 μm glass fiber filters (Whatman GF/F) to remove sand and debris and stored in the dark at temperatures between 0 °C and 4 °C prior to extraction. Water samples were extracted in the course of seven days after their collection and the complete analysis was conducted in 40 days from their extraction. The content of organic matter in water samples, expressed as $KMnO_4$ consumption, was determined by volumetric method [21].

2.2.2. *Collection of sediment samples*

Sediment samples were collected at the same six sampling points (Figure 1). 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 °C, for a maximum of seven days.

2.2.3. *Collection of fish muscle tissue*

The fish samples of barbel, which is a benthic species fished also commercially, were collected from the region Grašnica–Daljan. Fish samples of *Barbus peloponnesius,* Valenciennes (1842) were caught and their species determination was conducted by Velkova-Jordanoska [22]. Immediately after fish were caught, the muscle tissue was separated and shortly after it was frozen and kept at –20 °C until analysis.

2.2.4. *Preparation of water samples*

The determination of the organochlorine pesticides in the water samples was conducted using the USA EPA 608 method.

One liter of previously acidified water sample was used for liquid extraction with methylene chloride. The procedure was repeated three times with fresh portions of 30 ml of methylene chloride and 15 min. After that, the sample was transferred to a separatory funnel and after 10–15 min the two layers were separated. The organic layer was transferred to a 250 ml round bottom flask. After the separation of the organic layer, the water layer was placed back into the balloon and the extracted two more times. The three methylene chloride extracts were combined and the whole extract was concentrated in a rotary evaporator (Heidolph, Laborota 4011-digital).

Depending on the purity of the sample, further purification of the extracts can follow. The extracts obtained from the water samples were relatively clean, no emulsions were obtained during the extraction and no further purification was necessary. The dry extract was reconstituted with addition of 1 ml hexane, containing the internal standard pentachloronitrobenzene (PCNB) with concentration of 100 ng ml^{-1}. Finally, 1 μl of the sample was injected into GC with ECD.

2.2.5. *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 placed in a glass container and 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 10 minutes at 3000 cycles min^{-1}, 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 (Florisil for column chromatography 0.150–0.250 mm) 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:

- First fraction eluted with 20 ml hexane containing mainly *p,p*'-DDE, *p,p*'-DDT, heptachlor and aldrin,
- Second fraction eluted with 20 ml mixture hexane/dichloromethane (74/24, *V*/*V*) containing almost all organochlorine pesticides except endosulphane sulphate and endosulphan II,
- Third fraction eluted with 20 ml mixture acetone/hexane (10/90, *V*/*V*) containing endosulphane II, part of endrin aldehyde and a fraction of the metoxychlor.

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. After the injection of the three fractions, the values for the quantities 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 analyzed fresh and the results were calculated with respect to dry mass. 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). This method involves heat destruction of all organic matter in the sediment. A known weight of sample is placed in a ceramic crucible which is then heated for 6 hours at 550 °C. Organic matter content is calculated as the difference between the initial and final sample weight divided by the initial sample weight times 100 %.

2.2.6. *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 extraction mixture was then centrifuged (for 10 minutes at 3000 cycles min^{-1}), the hexane layer separated, transferred through anhydrous sodium sulphate and dried by evaporation.

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 kilogram fresh tissue and represent the total of the contents in the three different fractions.

2.3. 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 length 30 m; I.D. 0.32 mm; film thickness 0.25 μm, and temperature limits from –20 °C to 280 °C (300 °C). The temperature program used for the analysis of the organochlorine pesticides is given in Table 2. The nitrogen carrier gas was with 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.

T a b l e 2

Temperature program

Temperature/ °C	Temperature increase per minute/ °C min^{-1}	Time/ min
70	–	1
180	20	0
230	10	3
270	5	5

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}, 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} and IS concentration of 100 ng ml^{-1}.

2.4. *Laboratory quality control*

For water samples, method detection limit (MDL) was 0.002 μg ml^{-1} and practical quantification limit (PQL) was 0.005 μg ml^{-1} for all pesticides, including HCH isomers, α- and β-endosulfan and DDT's (*p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD). For all investigated organochlorine pesticides in this study the method detection limit (MDL) for sediments was 0.2 μg kg^{-1}, and the practical quantification limit (PQL) was 0.5 μg kg^{-1}. The mean recovery for γ-HCH (lindan) in water was 96 %, for α-HCH, δ-HCH, α-endosulfan and *p,p'*-DDD was 91 %, for β-HCH 92 %, for β-endosulfan 90 %, for *p,p*'-DDT 94 % and for *p,p'*–DDE 90.5 %. Values for recovery for γ-HCH in the sediment was 84 %, α-HCH 73 %, β-HCH 82 %, δ-HCH 91 %, α-endosulfan 78 %, β-endosulfan 90 %, *p,p*'-DDT 89 %, *p,p*'-DDE 83 %, *p,p*'-DDD 85 %.

3. RESULTS AND DISCUSSION

3.1. *Water samples*

The average concentrations of organochlorine pesticides in the water samples collected in the respective locations in the four seasons in 2004 are presented in Figure 2. The results of the investigation have indicated the presence of certain organochlorine pesticides in the water samples collected at the investigation points, such as: HCH measured as α, β and δ isomers; γ-HCH (lindane); endosulfan measured as α and β endosulfan; *p,p'*-DDT; *p,p'*-DDE, and *p,p'*-DDD.

The concentrations of total organochlorine pesticides in the analyzed water samples ranged between 0.084 μg l^{-1} in the water from the littoral of St. Naum, to 0.130 μg l^{-1} in the waters of Koselska River (Figure 2). The results clearly showed higher values for organochlorine pesticides in the river water compared to the samples collected from the littoral regions in their deltas. Hence, the concentration of total detected organochlorine pesticides in the water of the River Velgoška was 0.125 μg l^{-1} while in the littoral near its delta (Grašnica) the found concentration was 0.103 μg l^{-1}. In the water of River Koselska the concentration of the total detected organochlorine pesticides was estimated at 0.130 μg l^{-1} while the water from the region Daljan (delta of the River Koselska) – 0.094 μg l^{-1}. This condition indicates that there is a substantial influence of the rivers' water upon the quality of the water in the littoral regions near the river deltas.

The analysis has indicated that the lowest concentrations of organochlorine pesticides in most cases were measured in the water of the littoral of St. Naum while the maximum concentrations in the water of the littoral near the River Čerava.

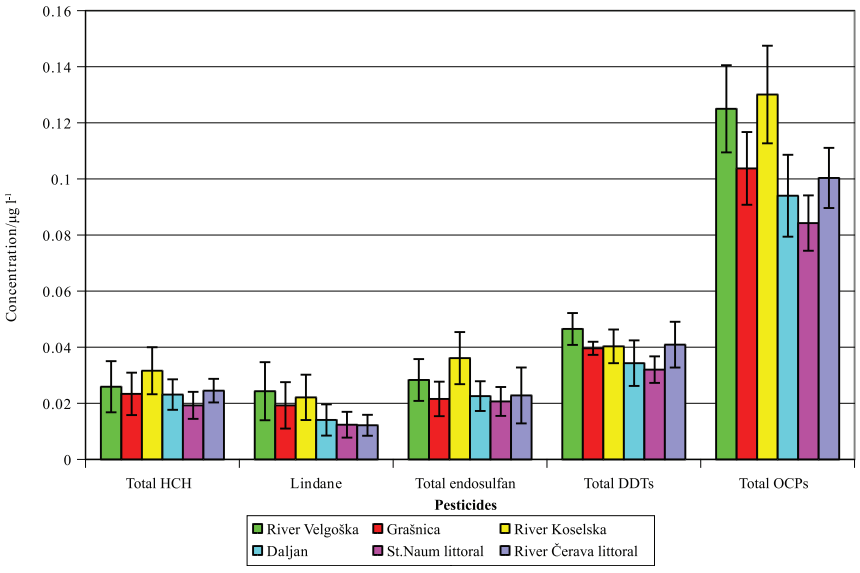

Fig. 2. Average concentrations of organochlorine pesticides in water samples in the four seasons in 2004 (n = 4)

The lowest concentration of lindane was found in the water of the littoral of St. Naum and River Čerava with value of 0.012 μg l^{-1} while its maximum value of 0.024 μg l^{-1} was found in the waters of the River Velgoška. The concentration of this pesticide was found to be 0.019 μg l^{-1} in the water samples from its delta. In addition, according to Golfinopoulos *et al.* [10], lindane has been determined in the River Axios (Vardar) with average values of 0.048 μg l^{-1} while in the water of the littoral of its delta 0.023 μg l^{-1} has been found. It should be mentioned that vast number of organochlorine pesticides in Greece are banned while lindane is still in use, only under specific circumstances.

The results of our investigation revealed the total concentration of the HCH isomers (α, β and δ-HCH) ranging from the lowest values in the littoral of St. Naum (0.019 μg l^{-1}) to the highest concentrations determined in the waters of the River Koselska – 0.032 μg l^{-1}. Comparable results have been obtained in the investigations of the rivers in northern Greece (Evros – Marica, Strimonas – Struma, and Pinios) for the presence of the three (α, β and δ) HCH isomers [23]. According to the author, the highest average value for the sum of HCH was evidenced in the water of River Evros – 0.069 μg l^{-1}. This author suggests the agricultural activities in the country in combination with the atmospheric bulks represent the source of pesticide residues in the investigated surface waters.

The investigations completed by Nestorovska-Krsteska *et al.* [24] which were undertaken on samples of water collected from the surface water in the Republic of Macedonia (including Ohrid Lake) indicated a presence of some pesticides. The registered pesticides were dimethoate, 2,4-dichlorophenoxy acetic acid, mecoprop and linuron. The levels of pesticides, which have been recorded in this study, were in the interval between 0.31 μg l^{-1} to 7.05 μg l^{-1}. According to Nestorovska–Krsteska *et al.* [24] pesticides, among which mostly herbicides and nematicides, are potential contaminants of natural waters because they are applied to soil and are transported to ground waters or leached directly in to the environmental waters.

Endosulfan measured as a sum of α and β isomers showed identical distribution as the sum of HCH. The minimum average concentration of endosulfan was found, again, in the littoral of St. Naum (0.021 μg l^{-1}). In the water of the two investigated rivers, higher concentrations were detected in comparison with their deltas. Highest concentration of 0.036 μg l^{-1} was measured in the River Koselska coming to 0.023 μg l^{-1} in its delta (Dalijan). In the water of the Velgoška River the concentration of endosulfan was 0.028 μg l^{-1}, while in its delta (Grašnica) – 0.022 μg l^{-1}.

In the study of Golfinoupoulos *et al.* [10], undertaken in certain rivers in northern Greece, a similar situation was demonstrated i.e. the measured concentrations of endosulfan were higher in the water samples collected directly from the river beds compared to the samples collected from the rivers' deltas. Hence, in the water of the River Evros the average concentration of endosulfan was 0.032 μg l^{-1}, while in its delta it was 0.024 μg l^{-1}. The corresponding concentrations in the River Strimonas were 0.043 μg l^{-1} and 0.022 μg l^{-1}.

The results of Laabs *et al*. [11] showed a substantial contamination with endosulfan (α and β endosulfan) in the analyzed samples of surface waters (0.008–0.034 μg l^{-1}) and especially of rain water (0.027–1.044 μg l^{-1}). According to these results, the inflow of pesticides through atmospheric deposits plays a great role for their presence in the aquatic ecosystems.

The concentrations of the three detected metabolic forms of DDT: *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD and their sum, presented as total DDT's, are given in Figure 3. Obviously, *p,p*'-DDE is the most abundant form in the analyzed water samples. Its concentrations were in the range from 0.018 μg l^{-1} near St. Naum to 0.024 μg l^{-1} in the waters of the River Velgoška. The concentrations of *p,p*'-DDT and *p,p*'-DDD in the water samples from the studied locations were relatively balanced, with the exception of River Velgoška and the littoral near River Čerava where *p,p*'-DDT was found in higher concentrations (0.014 μg l^{-1} and 0.011 μg l^{-1}) than *p,p*'-DDD (0.009 μg l^{-1} and 0.008 μg l^{-1}), respectively.

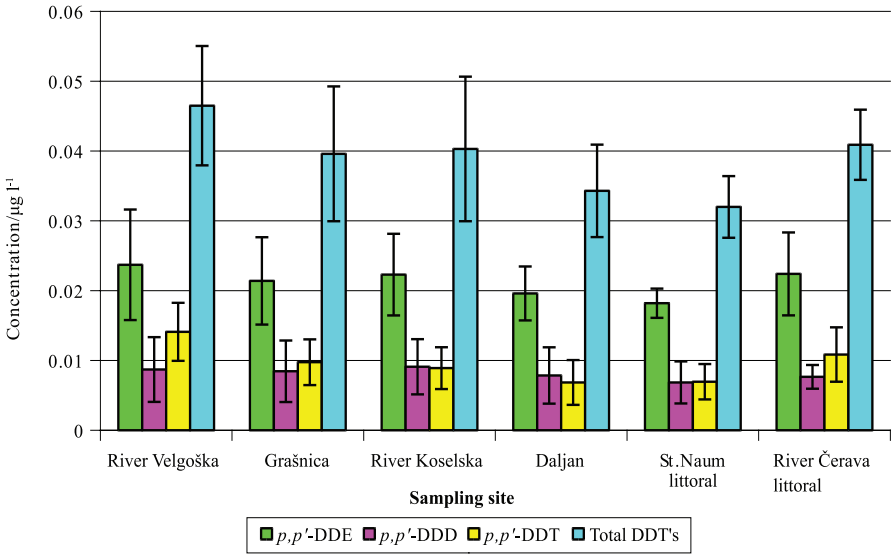

Fig. 3. Concentrations of *p,p'*-DDT; *p,p'*-DDE and *p,p'*-DDD and total DDTs in the water of the investigated locations

The characteristic of the organochlorine pesticides to be adsorbed on the organic matter enables their persistence and detection in the aquatic ecosystem [25]. Likewise, our results show that organochlorine components concentration evidenced in the water samples, follow the organic matter quantity (Figure 4).

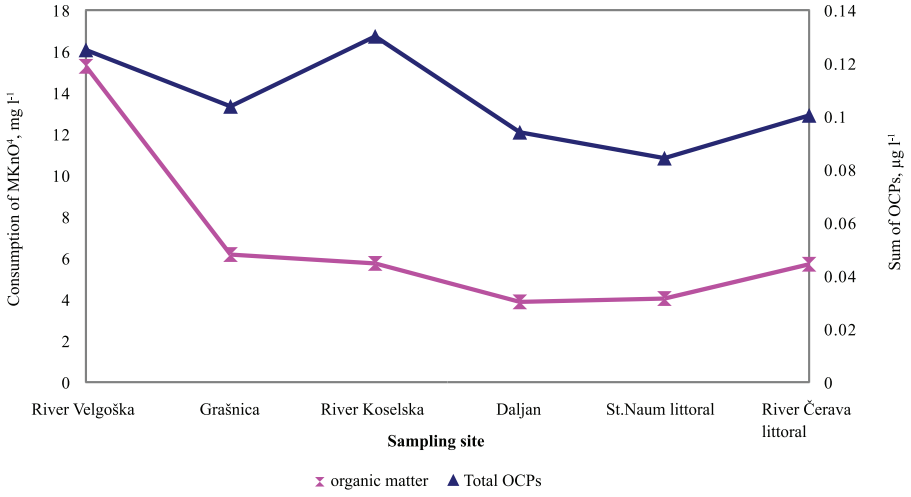

Fig. 4. Comparison of the concentration of the sum of the detected OCPs to the content of the organic matter in the water samples

3.2. *Sediment samples*

The residues that reach the hydrosphere are concentrated in certain parts of the aquatic ecosystem, i.e. they either remain in solution for extended periods, or are adsorbed on the particulate matter and thereby deposited in the sediments [25]. According to Bartolomeo *et al.* [26], the sediments represent a very important component of the lake ecosystem in which the toxic material is being accumulated through complex physical adsorption mechanisms

which are influenced by the sediment matrix and the characteristics of the material that is being accumulated.

The results of our study of the distribution of the organochlorine pesticides in the sediment, presented in Figure 5, indicate that their qualitative composition is identical with the one obtained for the water samples from the investigated locations. Out of the presented results in the figure, it can be seen that the content of total organochlorine pesticides was in the interval from 2.43 μg kg^{-1} in the sediment of the littoral St. Naum to 6.85 μg kg^{-1} in the sediment collected from the River Velgoška. Moreover, the graph also indicates that the content of organochlorine pesticides in the sediment of the rivers was higher compared to the content in the sediment in the littoral regions.

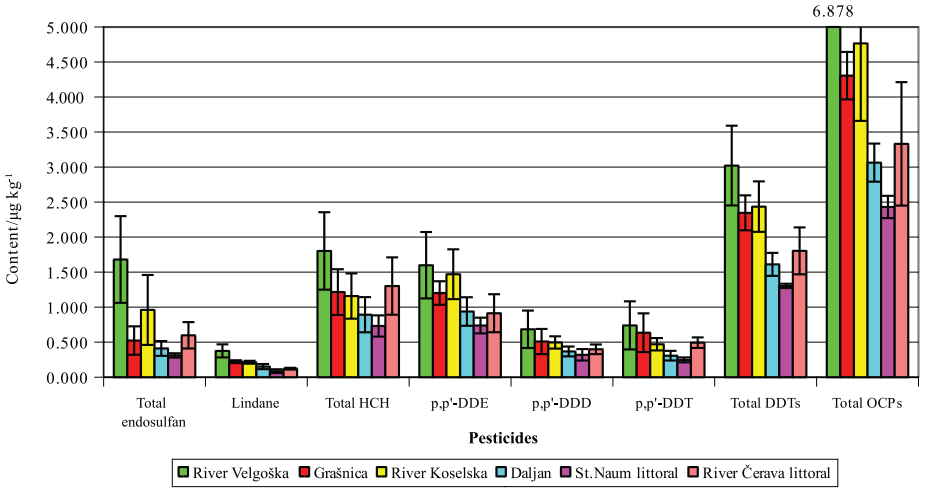

Fig. 5. Mean values for the content of organochlorine pesticides in sediment samples from the investigated locations (n = 3)

The most interesting finding was the fact that for almost all detected organochlorine pesticides in the sediment, the lowest values were detected in the littoral near St. Naum, a region characterized by sandy bottom, while the highest values were found in the sediment in the River Velgoška, a region characterized with mainly muddy consistency with presence of organic waste in a phase of disintegration. As for the contents in the sediment of the littoral regions, the maximum values for the organochlorine pesticides were measured in the region of Grašnica. This result for the total content of measured OCPs in the sediment of the littoral region of this river correlates to the highest content retained in the sediment of the river.

Figure 5 indicates that the content of total HCH (α, β and δ-HCH isomers) is higher than the content of endosulfan and lindane, ranging for littoral sediments from 0.731 μg kg^{-1} near St. Naum to 1.301 μg kg^{-1} in the littoral near River Čerava. The maximum value for this pesticide of 1.802 μg kg^{-1} dry weight was measured in the sediment of the River Velgoška.

Total endosulfan exhibited an analogous distribution as total HCH with lowest mean value found in the littoral of St. Naum (0.31 μg kg^{-1} dry weight) and highest value measured near River Čerava (0.59 μg kg^{-1} dry weight). By taking into consideration the fact that the contamination with endosulfan is evidenced in the environment even in substantial distances from the spot of its application [27, 28], many authors have detected its presence in the atmosphere, soils, sediments, estuaries, surface and rainwater, and food stuffs [10, 29–32]. Most probably, this characteristic of endosulfan for long-term transport as well as its usage as a plant protection agent in the Republic of Macedonia and in the neighboring regions is the main reason for its presence in the analyzed sediment during our study.

The obtained results clearly show that lindane is evidenced with the lowest contents in the sediment of all researched locations in comparison with the other organochlorine pesticides. The values of this pesticide are below the detection limit at St. Naum and Daljan. These low values can be explained by the fact that lindane is characterized by higher solubility compared to the rest of the pesticides [25].

In the investigations of the sediment of Lake Skadar, the sum of the three HCH isomers (α, β and δ-HCH) indicated substantially higher values compared to lindane and endosulfan. The mean value found for the sum of HCH in Lake Skadar was 6.25 μg kg^{-1} dry weight, for lindane it was 2.33 μg kg^{-1} dry weight, while for the sum of the isomers of the endosulfan, 3.23 μg kg^{-1} dry weight were measured [33].

The highest content from all analyzed organochlorine pesticides in the samples of sediment collected from the studied locations was measured for total DDT, calculated as sum of the detected three forms *p,p'*-DDT; *p,p*'-DDE and *p,p*'-DDD. The values were in the interval between 1.30 μg kg^{-1} dry weight for the sediment from the littoral of St. Naum to 3.02 μg kg^{-1} dry weight for the sediment of the River Velgoška (Figure 5). This is most probably a result of the great persistence of this insecticide, which is higher compared to the other substances form the group of organochlorine pesticides, and its long life in the ecosystems. It is known that the highest amount of DDT in relation to other organochlorine pesticides is a result of the fact that it is kept in the shallowest parts of the sediment and it stays unchanged for a longer period of time, due to its persistence, stability and resistance to the process of degradation [25, 34].

The diagram presented in Figure 6 illustrates the percentage distribution of each of the three forms of DDT (ratio of each isomer in total DDT content), which have been determined in the sediments of the studied locations.

The diagram shows that the dominant form in all analyzed samples was *p,p*'-DDE with percentage concentration in the total DDT content from 50 % in the sediment collected from the locality near River Čerava to 61 % in the sediment from the river bed of River Koselska. In the samples from the littoral near St. Naum, Koselska and the littoral Daljan, *p,p'*-DDT was present with 20 %, while its highest abundance (28 %) was found in the littoral Grašnica. The third form, *p,p*'-DDD, had small variation with highest percentage concentration of 24 % in the sediment of the littoral near St. Naum and lowest percentage presence of 20 % in the sediment of the River Koselska.

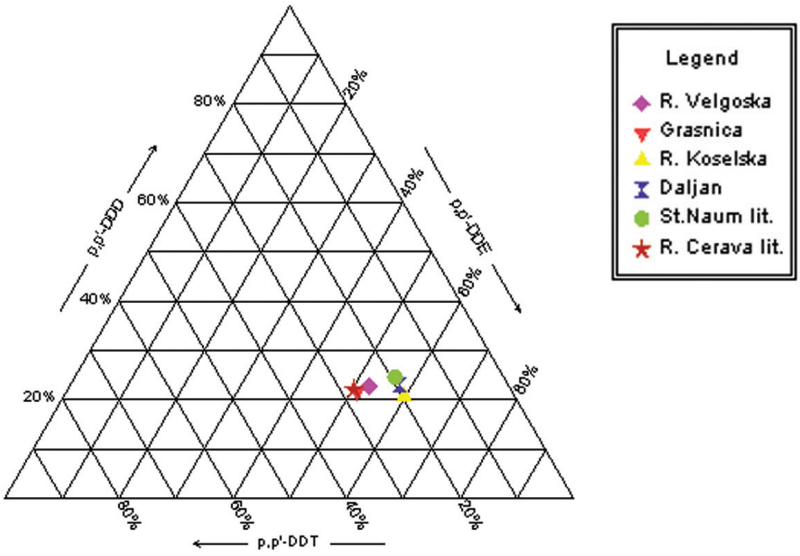

Fig. 6. Percentage distribution of *p,p'*-DDT; *p,p'*-DDE and *p,p'*-DDD in Lake Ohrid sediments from six locations

The ratio of the content of *p,p*'-DDT/*p,p*'-DDE provides a useful index to assess whether DDT at a given site is fresh or aged. A value below 0.33 generally indicates an aged input [35]. This ratio obtained in our study ranged between 0.31 and 0.54. In other words, for the majority of the assayed locations (the sediments of Koselska, the littoral Daljan and St. Naum) the values were lower than 0.33. The highest value was obtained in the analysis of the sediment of the littoral near the river Čerava (0.54). This result may be attributed to the fact that this river ecosystem flows in the Republic of Albania, too, where there is still a possibility for use of these pesticides despite the ban.

Unlike Lake Ohrid, where our results implied *p,p*'-DDE as the dominant form of DDT and relatively low values of the ratio DDT/DDE, in the studies of the Lake Skadar, values between 9.62 and 33.00 for this ratio have been obtained for sediment samples [33]. These values were a result of the high content of *p,p*'-DDT as the dominant form compared to its degradation products. These high values of the DDT/DDE ratio in the sediment samples from Lake Skadar indicate the recent usage of this insecticide in the surrounding agricultural areas.

On the basis of the obtained results from our research as well as the results of Marku *et al.* [33] a difference can be observed in the content of organochlorine pesticides in the sediment samples collected in the two aquatic ecosystems. This condition most probably is due, and is in correlation, to their trophic state. Lake Ohrid is a lake with an oligotrophic character [36–39], while Lake Skadar is of mezotrophic type [40].

The presence of organochlorine pesticides in the sediment and their mass distribution can be linked to the trophic state of the ecosystem. The investigations of Berglund *et al.* [6] 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.

Knowing the fact that the sorption of the pesticides is directly affected by the composition of the organic matter in the environment [41–42], a correlation between the contents of these compounds in the sediment of the investigated locations is expected. Thus, the correlation between the composition of total organochlorine pesticides, γ(OCPs), detected in this study and the percentage of the total organic material in the sediment, *w*(organic matter), is presented in Figure 7. The very good linear correlation ($R^2 = 0.967$) can be expressed by the equation:

$$\gamma(\text{OCPs}) = 0.776 \cdot w(\text{organic matter}) + 1.686$$

The graph indicates that the maximum values for both parameters were found in the sediment of River Velgoška while in the locations Daljan and St. Naum the lowest values in relation to these parameters were noted. The two latter locations are both characterized by sandy bottoms.

According to Kumar *et al.* [43] the organic matter in the sediment and sand plays a very important role in the processes of adsorption

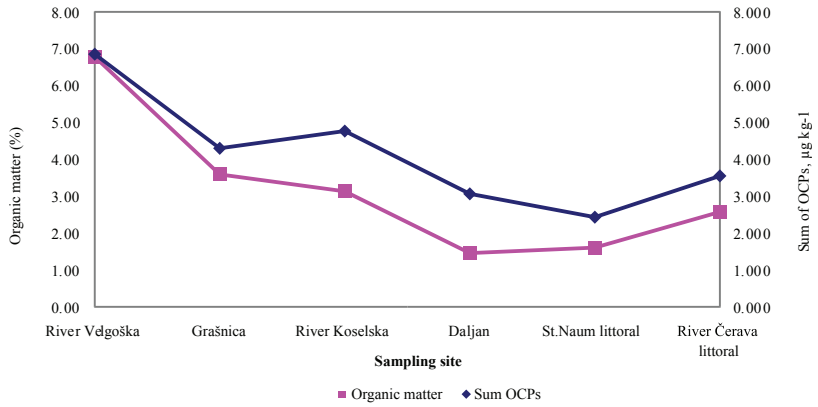

Fig. 7. Comparison of the average value of the sum of OCPs to the percent of the organic matter in the sediment from investigated locations

and desorption of persistent organic pollutants. It has been shown in his work that the bottom with the highest percentage of organic matter has the highest adsorption power, as well. He points out that the adsorption of the isomers of the endosulfan (α and β endosulfan), which were the main interest of his research, in sandy bottom was insignificant, that is, the mobility of endosulfan is greatest in sandy bottoms and they are retained relatively shortly in such consistency.

3.3. *Fish samples*

The great sensitivity of the fish fauna on the chemical environment enables its use as an indicator for the level of the pollution of the water. The most important issue is the fact that fish are getting in touch with their surrounding with the entire body and species living close to the bottom of the flowing waters and the water accumulations are interacting with greater quantities of the chemical components mixed in the mud [25]. The fish predators which are in a higher level of the trophic pyramid consume greater quantities of the active matter of the chemicals with the food which, on the other hand, are already accumulated in the organisms which represent their food.

The research of the muscle tissue of the barbel *Barbus peloponnesius,* Valenciennes (1842) [22] have indicated the presence of organochlorine pesticides, which has been evidenced in the water and in the sediment of this ecosystem, too. The mean values for the detected organochlorine pesticides in the total number of samples of barbel are presented in the graph in Figure 8 expressed as μg kg^{-1} wet weight (w.w.).

The graph clearly shows that total DDT (sum of *p,p*-DDT; *p,p*-DDE and *p,p*-DDD) was present with the highest average values, among

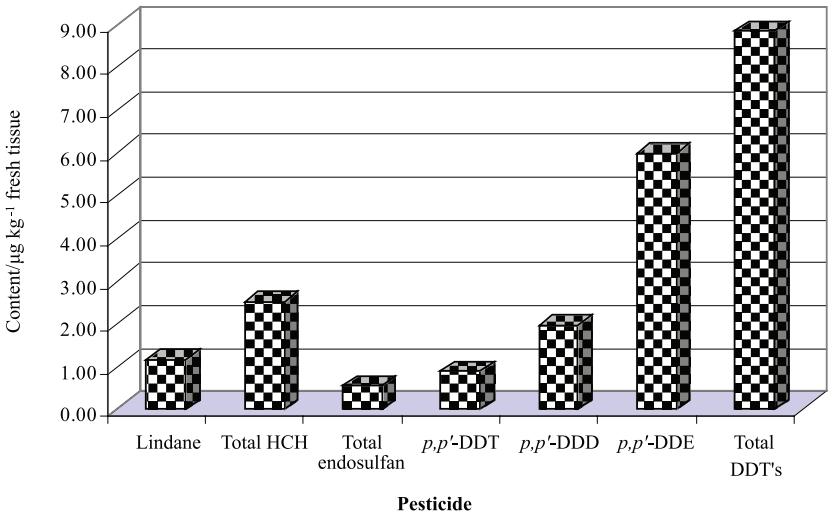

Fig. 8. Average values for the content of organochlorine pesticides in fish tissue from Lake Ohrid

which *p,p'*-DDE was most abundant (5.981 μg kg^{-1} w.w.). The higher quantity of this form could be due to, according to Vives *et al.* [44], the biodegradation of *p,p*'-DDT into *p,p*'-DDE occurring when the first one enters into the organism of the fish and with aging. The same author suggests that the increase of the concentration of *p,p*'-DDE is a result of its difficult elimination from the organism or inability to be metabolized.

From the other detected organochlorine pesticides, γ-HCH (lindane) was characterized by higher mean value (1.163 μg kg^{-1} w.w). In the research of Spirkovski *et al.* [45], lindane and DDT were found in the tissue of numerous fish species in Lake Ohrid. According to

this study, the fish species, age, its position in the trophic pyramid and the fats content in the tissue are the important factors affecting the bioaccumulation of the organic pollutants including pesticides into the fish tissues.

The presence of total DDT and lindane in the fish population has also been studied by researchers from the Albanian side of the Lake Ohrid. Thus, in the tissue of the carp (*Cyprinus carpio*), lindane concentration was found to be 5.19 μg kg^{-1} w.w. and the sum of DDT metabolic forms 17.29 μg kg^{-1} w.w. On the other side, in the trout (*Salmo letnica*), the lindane content was 10.09 μg kg^{-1} w.w., and the sum of DDT metabolic forms was 50.2 μg kg^{-1} w.w. In the tissue of the *Alburnus belvica*, the sum of DDT content was found to be 48.33 μg kg^{-1} w.w. [46].

The presence of lindane has been documented in the fish samples from the Lake Skadar, as well. In the results of Marku *et. al.* [33], it was pointed out that in the *Alosa falax nilotica* the content of lindane was 1.08 μg kg^{-1} w.w., whereas in the carp (*Cyprinus carpio*) it was 9.98 μg kg^{-1} w.w.

In the analyzed samples of the muscle tissue of the barbel during our investigations, the average value of the sum of HCH isomers was found to be 2.513 μg kg^{-1} w.w.

HCH isomers were also studied by Topi *et al.* [46], who found much higher content of the sum of α, β and δ-HCH in *Salmo letnica* (28.33 μg kg^{-1} w.w) and slightly lower in *Cyprinus carpio* (15,69 μg kg^{-1} w.w.), which is still higher than the values obtained in our study.

Figure 8 clearly indicates that the lowest average value of all analyzed OCPs in the tissue of the barbel was measured for endosulfan as sum of α and β isomers (0.553 μg kg^{-1} w.w).

3.4. *Comparison between the three analyzed matrixes*

An overview for the found minimum and maximum values for the determined organochlorine pesticides in the three types of samples from the selected locations in the Lake Ohrid obtained in this study is given in Table 3.

T a b l e 3

Results from the determined organochlorine pesticides in the samples of water, sediment and fish muscle tissue from selected locations from the Lake Ohrid

Samples	Lake Ohrid		
	Water samples	Sediment samples	Fish muscle tissue
Organochlorine pesticides	min–max μg l^{-1}	min–max μg kg^{-1} (dry mass)	min–max μg kg^{-1} (wet weight)
γ-HCH (lindane)	0.012–0.024	MDL–0.215	0.951–1.510
Total HCH (α+β+δ-HCH)	0.019–0.032	0.731–1.301	1.927–2.821
Total endosulfan (α+β endosulfan)	0.021–0.036	0.311–0.598	0.452–0.653
p,p'-DDT	0.006–0.014	0.247–0.635	0.868–0.937
p,p'-DDD	0.006–0.009	0.319–0.509	1.515–2.203
p,p'-DDE	0.018–0.023	0.738–1.202	5.213–6.601
Total DDTs	0.032–0.046	2.430–4.299	7.597–9.742

The presented results imply differences by order of magnitude in the content of the measured organochlorine pesticides in the water, sediment and fish muscle tissue. Lowest values were found in the water samples, much higher in the sediment samples due to their accumulation there and the highest values were observed in the muscle tissue of the barbel. This is as a result of the bioaccumulation, the lipophilic character of these compounds and their slow elimination from fish tissues [25].

According to the obtained results in this study, the variation in the content of organochlorine pesticides in the sediment follows the dynamics of their presence in the water, but with substantially higher content in the sediment. The research of Ezemonye *et al.* [47], also suggested substantially higher levels of organochlorine pesticides in the sediment compared to the water, a fact which this author explained by the concept that the sediment can be generally considered as a "purifier" for the pollutants.

In addition, the obtained results show that the most abundant group of OCPs in all three analyzed matrixes is total DDT (sum of *p,p'*-DDT; *p,p'*-DDE and *p,p'*-DDD). This apparently is a result of its long–time usage, persistency and (bio)accumulation. Although the usage of DDT is already banned in vast number of countries [7, 9, 10, 14, 23], including Republic of Macedonia [5], there are still some remains in the different matrices in environment due to the global pollution of the biosphere with this medium, which has been massively used in the past.

4. CONCLUSIONS

The analyses undertaken during this study for detection of organochlorine pesticides in different matrixes (water, sediment and muscle tissue of barbel) collected in Lake Ohrid represent the first round of a systematic research taking place in our laboratory. They are a prerequisite with regards to a thorough and long-term study of the persistent organic pollutants in the region of Ohrid and are a solid starting point for indication of the risks associated with this group of compounds upon the entire living world.

From the gained results, a conclusion can be made that higher content of organochlorine pesticides was found in fish muscle tissue than in sediment samples as a result of the bioaccumulation and the lipophilic character of this group of pollutants. Moreover, as an additional reason for this is the benthic way of living and nutrition of this fish species.

The detection of the organochlorine pesticides in the three analyzed matrixes, as well as the correlation of DDT/DDE indicate to the long-term effect of the agricultural and other anthropogenic activities in the surrounding agricultural areas not only in the Macedonian, but in the Albanian side of the Lake Ohrid, as well.

Even though the detected organochlorine pesticides are not evidenced in dangerous concentrations, still as organic pollutants with high persistency, bioaccumulation and toxicity, they represent quite high risk for the biotope and biocenose, and through them for the human as the highest level in the trophic pyramid.

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