DETERMINATION OF THE CONCENTRATION OF ORGANOCHLORINE PESTICIDES IN LAKE OHRID FISH SALMOTHIMUS OHRIDANUS

Flakrim Aliu1*, Zehra Hajrullai2, Suzana Aliu1

1Faculty of Food Technology and Nutrition, State University of Tetovo, Marshal Tito nn, 1200 Tetovo, Macedonia
2Faculty of Veterinary and Medicine, University Ss. Cyril and Methodius, Lazar Pop Trajkov 5-7, 1000 Skopje, Macedonia

*email: flakrimaliu@hotmail.com

Abstract

Pesticides are chemicals used to eliminate or control a variety of agricultural pests that can damage crops and livestock and reduce farm productivity. They can also be found in fish. These contaminants may accumulate in fish in levels that can cause human health problems. The aim of this paper was analysis and determination of the concentration of pesticides in Lake Ohrid fish Salmonus ohridanus.

Examined fish were collected from 3 different spots on Lake Ohrid, from the depth of 15 to 20 meters. Fishes length was between 21.00 to 22.7 cm, and their weight between 112.5 to 139.5 grams. Organochlorine pesticides that have been determined are Aldrin, Dieldrin, Chlordane, 4,4-DDT, DDD, Heptachlor, Endrin, α- endosulfan, β- endosulfan, Hexachlorobenzene (HCB), α-Hexachlorocyclohexane (α-HCH), Lindan, Methoxychlor, and Polychlorinated biphenyls (PCB). Determination of an analyzed parameters was done with gas-chromatogram GC 7890A connected with mass spectrometer MS Triple Quad 7000A with multimode injector (Agilent, Waldbronn). Chromatographic split is done in columns HP-5MS (30 m × 0.25 mm, 0.25 μm thick, Agilent) following temperatures from 55 °C per 1.5 min., 200 °C with speed 6 °C/min., and 280 °C per 1.5 min. Carrier gas was Helium with constant flow of 1.2 mL/min.

Analyzed samples in our experimental results, are showing that in fish, organochlorine pesticides are not concentrated in high levels. The found amount of pesticides is under minimal value of < 10 micrograms/kg and maximal value of < 50 micrograms/kg. According to the results fish Salmonus ohridanus meets the standards to be used in daily consumption.

However, although the amount of pesticides found are under the allowed borders, still should be worked on their total elimination, as their long term effects are a cause of cancerous diseases.

Key words: Fish, Organochlorine pesticides, Human health, Lake Ohrid, Gas chromatography, Mass spectrometry.

1. Introduction

Lake Ohrid is one of Europe’s deepest and oldest lakes (Figure 1), preserving a unique aquatic ecosystem that is of worldwide importance, with more than 17 endemic fish species, 11 of these species have economic and commercial importance for the nearby living population [1].

The lake itself has very transparent waters with maximum transparency of 22 m in winter. This is a result to its oligotrophic state (low production of organic matter) and respectively to that relatively low fish yield [2]. Some of the endemic inhabitants of the lake, especially the round shaped sponge Ohridospongia rotunda represents a certain evidence that Lake Ohrid, unlike most of the lakes of the world has never been dried out since its formation.

In this lake are living many fish species, and one of them is Salmonothimus ohridanus (Figure 2). There are many names for this fish: Latin names as - Acanthioligua ohridana, or Salmo ohridanus. Salmo ohridanus, is also known by the local Macedonian name as the belvica. This is a relatively small fish, usually shorter than 30 cm with less than 0.5 kg weight. The fish is always moving and its weight gain is from 50 - 100 g per year [3, and 4].

It is a commercially exploited species subject to heavy fishing, and has been bred in fish farms for over 50 years. It has also been intentionally hybridized with another endemic species, the Ohrid trout (Salmoletnica). Although it is threatened by the hybridization, degradation of water quality and overfishing, the stock of this fish remains abundant [5].
Agricultural activity in the lakeshore of Lake Ohrid is high. Result of these activities is use of pesticides for crop “protection” and its uncontrolled use is the main reason of land and water pollution. Contribution to this pollution are rains and rivers tributary that are flowing to Lake Ohrid, where they are absorbed by living organisms. Big water contributor to Ohrid Lake are also Lake Prespa waters which are flowing into Ohrid Lake. However, Prespa area is known for the intensive apples cultivation and the high use of pesticides[6].

The use of toxic pesticides to manage pest problems has become a common practice around the world. Pesticides are used almost everywhere - not only in agricultural fields, but also in homes, parks, schools, buildings, forests, and roads. It is difficult to find somewhere where pesticides aren’t used - from the can of bug spray under the kitchen sink to the airplane crop dusting acres of farmland, our world is filled with pesticides. In addition, pesticides can be found in the air we breathe, the food we eat, and the water we drink [7].

The goal of this paper is determination of organochlorine pesticides in the meat of the fish *Salmothimus ohridanus*, which is endemic specie in the Ohrid Lake. Even if the value of pesticides is in the allowed value for daily intake, there should be found other methods of protecting the agricultural fields, (e.g. injecting fungicide in the root of the apple tree), because the elimination of pesticides takes much longer time and its regular intake would threaten living organisms health through their deposit in the organism.

2. Materials and Methods

Analyzed samples were taken in 3 different locations nearby the city of Struga, at a depth of 15 to 20 m, or more precisely:
- Sample 1 (fish average size = 21.5 cm, average weight = 135.18 g) was fished nearby Euro Hotel Struga.
- Sample 2 (fish average size = 22.7 cm, average weight = 139.53 g) was fished nearby the “Male beach” of city of Struga.
- Sample 3 (fish average size = 21.0 cm, average weight = 112.51 g) was fished nearby restaurant Aquarius in Struga.

From each fish was taken 1 sample in total of 5 grams, each from fish tissue.
We analyzed following pesticides residues in fish tissue: Aldrin, Dieldrin, Chlordan, 4,4-DDT, DDD, Heptachlor, Endrin, α-endosulfan, β-endosulfan, Hexachlorobenzene (HCB), α-Hexachlorocyclohexane (α-HCH), Lindan, Methoxychlor, and Polychlorinated biphenyls, using MKS EN 1528.1,2,3 GC-MSD (A) method.

Determination of analyzed parameters was done with gaschromatogram GC 7890A connected with mass spectrometer MS Triple Quad 7000A with injector multimod (Agilent, Waldbronn) (Figure 3). Chromatographic split is done in columns HP-5MS (30m×0.25mm, 0.25μm thick, Agilent) following temperatures from 55°C per 1.5 min., 200°C with speed 60°C/min., and 280°C per 1.5 min. Carrier gas was helium with constant flow of 1.2 mL/min.

3. Results and Discussion

The presence and the negative effects of pesticides on the environment, to a large extent depend on whether and in what form they appear and how they are absorbed by flora and fauna. If they are present in aquatic ecosystem in increasing quantities they pose a risk to everyone in that living environment and close by.

For visual access, experimental results are represented in tables and figures.

Table 1. Determination of pesticides in fish tissue for Salmothimus ohridanus sample 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pesticides</th>
<th>Allowed pesticides in EU (μg/kg)</th>
<th>Amount of pesticides (μg/kg)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aldrin and Diaeldrin</td>
<td>200</td>
<td>&lt; 30</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
<tr>
<td>2</td>
<td>Total DDT (m)</td>
<td>1000</td>
<td>&lt; 50</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
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<tr>
<td>3</td>
<td>Heptachlor (m)</td>
<td>200</td>
<td>&lt; 20</td>
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<tr>
<td>4</td>
<td>Endrin (m)</td>
<td>50</td>
<td>&lt; 30</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
<tr>
<td>5</td>
<td>α-HCH (m)</td>
<td>200</td>
<td>&lt; 10</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
<tr>
<td>6</td>
<td>Lindan (m)</td>
<td>20</td>
<td>&lt; 10</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
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<tr>
<td>7</td>
<td>HCB (m)</td>
<td>200</td>
<td>&lt; 20</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
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<tr>
<td>8</td>
<td>Methoxychlor (m)</td>
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<td>&lt; 10</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
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<tr>
<td>9</td>
<td>Chlordan (m)</td>
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<tr>
<td>10</td>
<td>Endosulphan (m)</td>
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<td>&lt; 10</td>
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### Table 2. Determination of pesticides in fish tissue for *Salmothimus ohridanus* sample 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pesticides</th>
<th>Allowed pesticides in EU (µg/kg)</th>
<th>Amount of pesticides (µg/kg)</th>
<th>Method</th>
</tr>
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<td>Total DDT (m)</td>
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<td>&lt; 50</td>
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<tr>
<td>3</td>
<td>Heptachlor (m)</td>
<td>200</td>
<td>&lt; 20</td>
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</tr>
<tr>
<td>4</td>
<td>Endrin (m)</td>
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<tr>
<td>5</td>
<td>α-HCH (m)</td>
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<td>&lt; 10</td>
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<tr>
<td>6</td>
<td>Lindan (m)</td>
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<tr>
<td>7</td>
<td>HCB (m)</td>
<td>200</td>
<td>&lt; 20</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
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<tr>
<td>8</td>
<td>Metoxychlor (m)</td>
<td>10</td>
<td>&lt; 10</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
<tr>
<td>9</td>
<td>Chlordane (m)</td>
<td>50</td>
<td>&lt; 20</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
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<tr>
<td>10</td>
<td>Endosulphan (m)</td>
<td>50</td>
<td>&lt; 10</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
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### Table 3. Determination of pesticides in fish tissue for *Salmothimus ohridanus* sample 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pesticides</th>
<th>Allowed pesticides in EU (µg/kg)</th>
<th>Amount of pesticides (µg/kg)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aldrin and Dieldrin</td>
<td>200</td>
<td>&lt; 30</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
<tr>
<td>2</td>
<td>Total DDT (m)</td>
<td>1000</td>
<td>&lt; 50</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
<tr>
<td>3</td>
<td>Heptachlor (m)</td>
<td>200</td>
<td>&lt; 20</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
<tr>
<td>4</td>
<td>Endrin (m)</td>
<td>50</td>
<td>&lt; 30</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
<tr>
<td>5</td>
<td>α-HCH (m)</td>
<td>200</td>
<td>&lt; 10</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
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<tr>
<td>6</td>
<td>Lindan (m)</td>
<td>20</td>
<td>&lt; 10</td>
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<tr>
<td>7</td>
<td>HCB (m)</td>
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<td>&lt; 20</td>
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<tr>
<td>8</td>
<td>Metoxychlor (m)</td>
<td>10</td>
<td>&lt; 10</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
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<tr>
<td>9</td>
<td>Chlordane (m)</td>
<td>50</td>
<td>&lt; 20</td>
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<tr>
<td>10</td>
<td>Endosulphan (m)</td>
<td>50</td>
<td>&lt; 10</td>
<td>MKS EN 1528.1,2,3 GC-MSD (A)</td>
</tr>
</tbody>
</table>

Figure 4. Pesticides amounts in fish tissue for *Salmothimus ohridanus* samples 1, 2 and 3
All analyzed samples in our experimental results, are showing that in fish, organochlorine pesticides are not concentrated in high levels. The found amount of pesticides is under minimal value of < 10 micrograms/kg and maximal value of < 50 micrograms/kg. According to the results fish *Salmonus ohridanus* meets the standards to be used in daily consumption.

4. Conclusions
- Our conclusion regarding analyzed samples in experimental results, is that in fish, organochlorine pesticides are not concentrated in high levels. The found amount of pesticides is under minimal value of < 10 microgram/kg and maximal value of < 50 microgram/kg.
- According to the results fish *Salmonus ohridanus* meets the standards to be used in daily consumption. Although the amount of pesticides found are under the allowed borders, still should be worked on their total elimination, as their long term effects are a cause of cancerous diseases.
- Using of chemicals (pesticides) must be controlled in agriculture especially along watershed of the river’s which are Lake Ohrid tributary and Lake Prespa. It is necessary to adopt and apply strict legislation based on international and national laws.
- There is a need for international co-operation and agreement between Macedonia and Albania. This cooperation should be agreed among governmental (Ministries, local government) and non-governmental organizations.

5. References