EUROPEAN ACADEMIC RESEARCH
Vol. V, Issue 10/ January 2018

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Occurrence of persistent pesticides in *Mullus barbatus*, Albanian coast

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Abstract:

Organochlorine pesticides (OCPs) have been extensively used in agriculture due to their insecticide activity. Due to their chemical stability and lipophilic behavior they have a high tendency for bioaccumulation in animal and human tissues through the food chain. During this study was investigated the presence of the organochlorines in red mullet (Mullus barbatus) fish from the Adriatic Sea. The fish samples were collected from products destined for domestic market and export as well. The instrumental analyses were carried out in GC/SM Triple Quadrupole in multiple reaction monitoring mode. From 16 organochlorine compounds, pesticides and its metabolites, screened in this study only the presence of DDT (found mostly in the form of its degradation product DDE) was detected and quantified. Its presence was confirmed at approx. 80 % of the samples in the range of 6 – 25 ηg/kg and in only one sample the DDT (DEE p,p` metabolite expressed as DDT) was found to be 106 μg/kg.

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Key words: *POPs, DTT, fish, environmental pollution*

AIMS AND BACKGROUND

The red mullet, *Mullus barbatus,* is commercially one of the most important demersal fish resources in the Mediterranean. It is uniformly distributed in all parts of the Adriatic (Jardas 1996).It is also one of the most important species in Albanian coast. Predominantly benthic species living on muddy bottoms between 5 and 250 m (Relini 1999). It prefers the more shallow waters of the northern and central Adriatic, i.e. depths above 100 m, while only few specimens may be caught in deeper waters. Red mullet prefers muddy and sandy bottoms, e.g. the regions with the highest availability of its food. It *is known as a* carnivorous specie, which feeds on polychaeta, bivalves and crustaceans, which makes it a very good biomarker of environmental contamination. It is nourished throughout the whole year, although to a greater extent during the summer and the autumn, probably in correlation to water temperature (JUKIĆ 1981). Organochlorine insecticides are ubiquitous pollutants in the terrestrial and aquatic environment. Persistent pesticides like organochlorine are well reported for their bioaccumulation and their presence worldwide will continue as result of environmental redistribution process for a long time after these substances are banned.

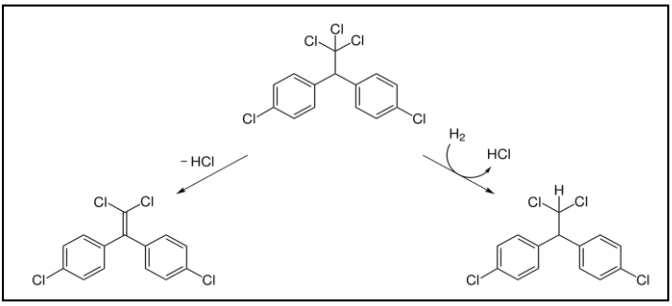

Figure 1: Schematic presentation of DDT break down

Different studies reveal that OCPs, at some critical growth periods, may generate severe health disturbances. Conclusively, the exposure to OCPs should be reduced so as to minimize the associated environmental and human health hazard (Zorawar Singh 2016). Recent studies has indicated their presence, still different agriculture areas in Albania (Mimoza Mukaj 2017). This presence seems to be due its agriculture use in the past. Figure 1 gives a schematic presentation of DDT break down in its main compound DDE and DDD. DDE is the widest speeded persistent pesticides worldwide.

EXPERIMENTAL

Sampling

All the samples were collected at the fish marked in different city of Albania. The sampling procedure was made in accordance with EC Regulation No 589/2014 of 2 June 2014 (Commission 2014). The laboratory sample was prepared in accordance with guidance (Technology n.d.) . Each one of the samples was handled independently in the Laboratory of Pesticides Residues in Food of Animal Origin at Food Safety and Veterinary Institute. All the fish samples were cleaned and after the offal's and skin were removed the muscle was homogenized by using Ultra-Turrax IKA-T50.

Chemicals

All organochlorine reference materials used were of pesticide grades and were purchased from Sigma Aldrich. Stock solution was prepared for each one of the Reference materials and appropriate dilutions were made in order to obtain proper dilution for the analysis. Calibration curve in matrix were prepared in every batch and extract from sepia was used as matrix blank due to low fat concentration of this marine product as well as the red mullet.

Preparation of Standards

Sixteen stock standard solutions were prepared separately at a concentration around 1000 µg mL−1, using isooctane as a solvent. Intermediate standard mixtures in ethyl acetate, containing 10 µg mL−1 of each compound, were prepared by mixing appropriate quantities of the individual stock solutions. A working standard mixture of 10 ml at concentration of 1 µg mL−1 was prepared by dilution 1 ml of intermediate standard mixture with 9 ml sepia extract. This solution was used for the quantitative measurements and the preparation of the quality control samples. The solution was stored in amber glassware − 20 °C until the time of analysis.

Extraction and clean-up

The muscle extraction and clean-up was performed following the "SweET" method (Pihlström n.d.). From each fish sample 5 ± 0.2 gram of muscle tissue was weighted in a falcon tube and 10 ml of distilled water was added in each sample. For the cleanup 0. 25 mg of PSA and octadecyl silane-bonded silica (C_{18}) were added on each sample. For the extraction was used 10 ml ethyl acetate followed by 20-minute extraction which was performed in horizontal shaker. After the first extraction a second extraction was performed for another 10 minutes in the shaker after adding the 5 gram of Na_2SO_4 in each sample tube. After extraction the samples were centrifuged for 10 minutes in 3200 rpm. A flow diagram of "SweEt" method used in this study is given in the figure 2. (Pihlström n.d.).

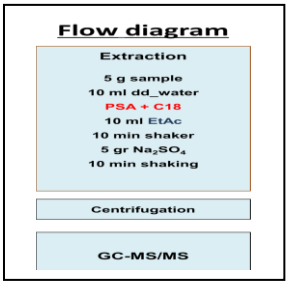

Figure 2: Flow diagram of SweEt method.

1 ml from the extract was transferred in a 2 ml glass vial and injected in GC/MS/MS.

Identification/ quantification work condition

The identification and quantification of organochlorine pesticides in the extracts were performed in GC/MS triple Quad, in Multiple Reaction Monitoring mode.

Table 1: Multiple reaction monitoring (MRM) transitions, collision energy (CE) for each transition for each analyte.

Compound name	Parameter in GC-MS/MS		
	Precursor ion	Product ion	CE
Hexachlorocyclo-hexane α, γ, δ	181	145	15
	181	109	30
Hexachloro-benzene	283.9	248.8	25
	283.9	213.9	35
Heptachlor	274	239	20
	272.1	143	50
	271.9	236.8	25
Aldrin	298	263	8
	263	191	40
DDE o, p`	318	248	30
	246	211	20
	235	199.1	20
	235	165	25
Dieldrin	263	193	40
	263	191	35
Endrin	263	193	40
	263	191	35
DDE p, p`	318	248	30
	246	211	20
	235	199.1	20
	235	165	25
DDD o, p`	237	165	20
	235	199.1	20
DDD p, p`/ DDT o,p`	237	165	20
	235	199.1	15
	234.9	165.1	20
DDT o, p`	237	165	20
	235	199.1	15
	234.9	165.1	20
Methoxychlor	227	169	30
	227	141.1	40
Mirex	272	237	20
	272	235	25

The injected volume was 1 µl. The column used for separation was HP-5 MS (30 m x 250 µm x 0.2 µm). Mass spectrometer was operating Electron Impact Ionization (EI) mode, the electron energy was 70 eV and the temperature source at 290°C. The initial oven condition was 50°C for 0.6 minute following by a temperature increase up to 180°C at a rate of 15°C/min held for one minute. Then the temperature was increased at 230°C by 7°C/min and the last step was up to 280 °C by 3 °C/min. All the analysis were performed in MS/MS mode. Quantifications were performed by using matrix matched calibration curves, from 0 to 200 ηg/kg, and sepia extract was used as blank material.

Quality assurance/quality control

QA/QC was performed in each batch of analysis as follow: one laboratory blank sample, two spike samples at concentration of 10 µg/kg, bracketing calibration of 5 points was prepared in matrix bulk extract. All the calculations were performed by MassHunter workstation software Quantitative Analysis B.04.00.

RESULTS AND DISCUSSION

The red mullet fish samples were sampled at the sailing point in different cities of Albania. All the sample belong to the Adriatic Sea harvesting area (Dimitrios Moutopoulos 2015), figure 3.

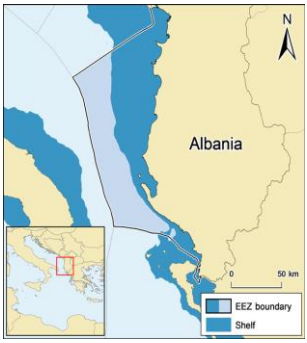

Figure 3: Definition Map of Albania and its Exclusive Economic Zone (EEZ).

After the removal of offal's, the length and weight of fish samples was measured according to Anderson and Neumann (Anderson n.d.). In the figure 4 is shown the variation of the range of length and weight of fish during this study. According to the literature these results show that the approximate age of the samples were approx. 2 years (Rakaj 1995).

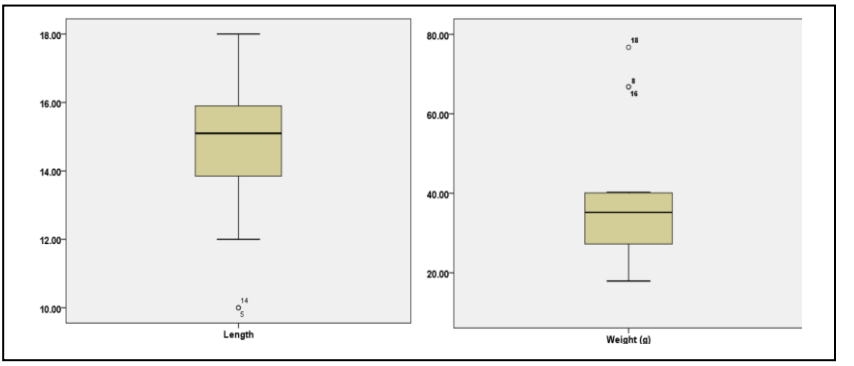

Figure 4: Range weight of red mullet samples in gram, Range length of red mullet samples in gram.

After the homogenization, each sample was screened for the presence of compounds containing 16 organochlorine pesticides and their metabolites, which has been categorized as Persistent Organic Pollutants at Stockholm Convention. Screening test of organochlorine compounds has shown no presence of Hexachlorocyclohexane α, γ, δ, Hexachlorobenzene, Heptachlor, Aldrin, Endrin, Dieldrin, Mirex and Methoxychlor in the fish muscle.

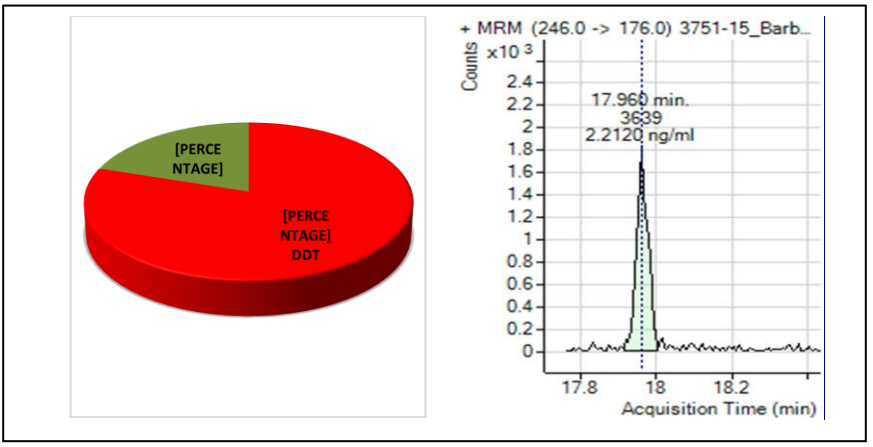

Figure 5: The percentage of samples showing DDT presence (on the left), Chromatogram of sample showing the presence of DDEp,p` the main DDT metabolite (on the right).

On the other hand, from all the samples analyzed in this study, measurable amounts of DDE p,p` were found in 80 % (16 samples out of twenty). The figure clearly shows the widespread presence of DDTs in fish samples. The concentration level of DDE varied from 6 to 25 ηg/kg. Only in one sample the value was very high, the concentration measured was 106 ηg/kg. Porte et al. has reported the presence of DDTs residues in red mullet in the range of 17 to 172 ng/g w.w.. (ALBAIGÉS 2001). The high occurrence of DDE in fish tissue has been attributed to a specific input of this compound. The results found in this study, although they far from the maximum values reported for DDT (1 μg/g) for seafood consumption adopted in certain countries (WHO/UNEP. 1995), has shown one more time that bioaccumulation of pesticide, listed as Persistent Organic Pollutant, still remains in nature.

CONCLUSION

From 16 compounds (organochlorine pesticides and their metabolites) investigated in red mullet from Albanian cost area, only DDT, in its metabolite form of DDE pp` was found.

Measurable amounts of DDE p,p` were found in 80 % of the samples. Its concentration level of DDE varied from 6 to 25 ηg/kg. The highest concentration measured was 106 ηg/kg. The low concentration of DDTs does not represent a high risk to human health but exposure to other sources in the food chain should be taken into consideration. Further investigation should be taken to monitor the occurrence of pesticides and its degradation products in other fish species and the aquatic environment, and the final consequences for public health.

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