DE GRUYTER OPEN

doi: 10.1515/auoc-2016-0006

Ovidius University Annals of Chemistry Volume 27, Number 1, pp. 8-12, 2016

Investigation about the presence of organochlorine pollutants in mussels from the Black Sea, Bulgaria

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Abstract. The aim of this study was to investigate the presence of polychlorinated biphenyls (PCBs), organochlorine pesticides (HCB, DDT and its metabolites) and HCBD in mussels from Bulgarian Black Sea coast. Mussels *(Mytilus galloprovincialis)* are aquatic organisms which are immobile so that the concentration of pollutants should primarily be considered as an indication of local levels of organochlorine compounds. Samples were collected from three areas of Black Sea coast of Bulgaria in summer 2015.

The fifteen congeners of PCBs, HCB, HCBD, DDT and its metabolites DDE and DDD were performed by gas chromatography system with mass spectrometry detection. The metabolites DDE and DDD were found in all analyzed mussel samples, but PCBs were not detected in any sample. DDE concentrations were found in mussels from 1.09 to 1.63 ng/g wet weight. In mussel total DDT concentrations (2.14 ng/g ww) were found comparable to those in mussels, sampled in 2013 and 2014 (1.87 ng/g ww).

The levels of DDTs and polychlorinated biphenyls in mussels from the Black Sea were found comparable to levels measured in the same molluscs from neighbor seas - Mediterranean Sea and Adriatic Sea.

Keywords: mussels, polychlorinated biphenyls, organochlorine pesticides, Black Sea, Bulgaria

1. Introduction

Seafood provides essential fatty acids, high quality protein, fat-soluble vitamins and essential elements important for human health. However, seafood is also known to accumulate certain compounds, such as persistent organic pollutants (POPs), which can have harmful effects on aquatic organisms and on human health [1].

The presence of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) such as hexachlorobenzene, 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) and its metabolites is of great importance due to their persistence, bioaccumulation and toxicity to wildlife and humans. The levels of POPs in the environment are steadily declining [2], but they continue to bioaccumulate in animal tissues and bio-magnify in food chains, and may have potentially significant impacts on human health and the environment [3-5]. They accumulate in the lipid rich tissues in animals and become soluble in fatty tissues [6]. European Commission (EC) included them in the EU Priority Pollutants List [7].

Hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD) are named as priority substances under the EU Water Framework Directive [8]. HCB is a hydrophobic and highly persistent chemical [9]. The main sources of HCB today are incineration and chemical industry from which this compound can be emitted as a product in high-temperature processes [10].

HCBD was mainly used as a solvent in the production of rubber and other polymers. Other uses were in agriculture as a seed dressing, in hydraulic fluids and a number of other industrial processes [11].

Shellfish have been generally considered as possible pollution monitoring species to assess the environmental contamination in aquatic systems [12]. It is known that bivalve molluscs concentrate pollutants to levels above those present in marine water [9, 13, 14]. Advantages of using mussels to monitor organochlorine pollutants are their wide geographical distribution, sessile lifestyle, easy sampling, tolerance to a considerable range of salinity and rapid accumulation of toxic substances [7, 15, 16]. In this respect mussels should be considered the priority species to investigate pollutants, using natural populations or transplanted organisms [17]. Black Sea mussels *(Mytilus galloprovincialis)* are of commercial interest, they are important component of seafood dishes and a measure of chemical contaminants in them is of public interest. Shellfish farming increased in recent years in Bulgarian Black Sea coastline.

The present study aims to investigate the presence of polychlorinated biphenyls, DDT and its metabolites, hexachlorobenzene and hexachlorobutadiene in *Mytilus galloprovincialis* collected from Bulgarian Black Sea coast.

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2. Experimental

2.1. *Sampling and sample preparation*

Mussels were collected manually between May – October 2015 from north-east coastal areas of the Black Sea, Bulgaria – Kavana, Bulgarevo, Kranevo, Fichoza. Sampling was carried out in mussel natural and farmed populations. At each sampling site 2–3 kg of mussels of similar shell length were collected, placed in plastic bags, kept in ice and transported to the laboratory.

Each sample was prepared from soft tissue of forty individual mussels of similar sizes (45–65 mm). The mussels were opened with stainless steel knives, and the soft tissues were removed and homogenized.

2.2. *Chemical analysis*

Samples were prepared according to a previously described method [18]. Briefly, twenty grams from homogenised tissue were taken for extraction. Each sample was spiked with internal standards PCB 30 and PCB 204. The compounds were extracted with hexane / dichloromethane (3/1) in Soxhlet Extractor.

An aliquot of the extract (1/5th) was taken for lipid determination. The solvent was carefully evaporated until dryness and the lipid content was determined gravimetrically.

After lipid determination, the extract was cleaned-up on a glass column packed with neutral and acid silica. Organochlorine compound were eluted with 50 cm^3 *n*-hexane followed by 50 cm^3 *n*-hexan / dichloromethane (80:20 v/v). The eluates were concentrated to near dryness and reconstituted in 0.5 cm^3 in *n*-hexane.

The simultaneous analysis was performed on gas chromatograph GC FOCUS (Thermo Electron Corporation, USA) using POLARIS Q Ion Trap mass spectrometer and equipped with an AI 3000 autosampler. Experimental mass spectrometry parameters are: the Ion source and Transfer line temperatures were 220ºC and 250ºC, respectively. The splitless Injector temperature was 250ºC. For HCB, HCBD and DDTs determination the oven was programmed as follows: 50ºC (1 min), 30ºC/min to 180ºC, 5ºC/min to 260ºC, 30ºC/min to 290ºC with a final hold for 3.0 min. The PCBs experimental temperature program - 90ºC for 1 min, then programmed 30ºC/min to 180ºC, 2ºC/min to 270ºC, 30ºC/min to 290ºC with a final hold for 3.0 min. Samples were injected in splitless mode in a TR-5ms capillary column with a length of 30 m, 0.25 mm ID and a film thickness of 0.25 μm with helium as carrier gas at a flow of 1 mL/min.

Pure reference standard solutions (EPA 625/CLP Pesticides Mix 2000 μg/mL - Supelco and PCB Mix 20 - Dr. Ehrenstorfer Laboratory) were used for instrument calibration, recovery determination and quantification of compounds.

In prepared extracts fifteen PCB congeners (IUPAC № 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170, 180), hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (*p,p*'-DDE), 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (*p,p*'-DDD) and 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (*p,p*'-DDT) were analyzed.

Each sample was analyzed three times and was taken an average of the results obtained. The limits of detection (LOD) varied for individual compounds from 0.02 to 0.05 ng/g ww.

2.3. *Quality control*

The quality control was performed by regular analyses of procedural blanks and certified reference materials: BCR - 598 (DDTs in Cod liver oil) and BB350 (PCBs in Fish oil) – Institute for Reference Materials and Measurements, European commission. Procedural blanks and a spiked sample with standards were analyzed between each 5 samples to monitor possible laboratory contamination. Blanks did not contain traces of contaminants.

3. Results and Discussions

A summary of the pollutant concentration ranges, measured in mussels in the current study is presented in Table 1.

The analyzed organochlorine pollutants were found in all samples in the range between 0.61 ng/g ww (DDD) and 1.60 ng/g ww (DDE). The dominant compound was DDE (1.17–1.60 ng/g ww), followed by DDD (0.61–1.00 ng/g ww) (Table 1). Lipid content of mussel samples ranged from 2.7 to 3.4 %.

The metabolites DDE and DDD were found in all analyzed mussel samples, but *p,p*'-DDT and PCBs were found below the analytical detection limits (Table 1).

Metabolite of DDT - *p,p*'- DDE represents a very slowly degradable compound and it was the most abundantly found in mussels. The levels of DDE were found in accordance with the concentration levels of mussels from the northern Adriatic Sea (1.6 - 3 ng/g fresh weight) [19] and from Croatian coast of the Adriatic Sea (in the range 0.57 - 2.61 ng/g ww) [3]. It's not surprising that *p,p*'-DDT were not found in mussels, as the use of these compounds in Bulgaria was prohibited in the late 1970s and the persistent half-life of DDT in aquatic environment has been suggested to be approximately 10–20 years [20]. DDT starts to degrade to DDE after discharge into the environment through photolysis or metabolic degradation by organisms. The ratio between the concentration of DDE and DDT has been used as a good indicator of the frequency of usage of pesticides whose active ingredient is DDT [21].

Table 1. Lipid content (%) and concentrations of DDTs, HCB, HCBD and PCBs (ng/g ww) in cultured and wild mussels

	Cultured mussels		**Wild mussels**			
	Kavarna I	Kavarna II	Bulgarevo	Kavarna I	Kranevo	Fichoza
Lipids, %	3.4± 0.3	3.3± 0.2	2.7± 0.4	3.1± 0.2	3.2± 0.2	2.8± 0.2
p,p'-DDE	1.60± 0.10	1.36± 0.04	1.47± 0.04	1.26± 0.21	1.17± 0.06	1.21± 0.08
p,p'-DDD	1.00± 0.07	0.85± 0.03	0.68± 0.07	0.79± 0.04	0.61± 0.03	0.69± 0.03
p,p'-DDT	nd	nd	nd	nd	nd	nd
∑DDTs, ng/g ww	**2.60± 0.13**	**2.21± 0.07**	**2.15± 0.11**	**2.06± 0.14**	**1.87± 0.06**	**1.90± 0.05**
HCB, ng/g ww	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
HCBD, ng/g ww	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PCBs, ng/g ww	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

< LOD – below method detection limit

Among the sampling sites DDE concentrations in mussels varied between 1.17 and 1.60 ng/g ww. The highest concentrations of DDE were found in Kavarna I, whereas the lowest levels were reached in Kranevo (Table 1.) DDD showed very close values between 0.61 and 1.00 ng/g ww during period of study.

The maximum level of ∑DDTs in mussels was found at the Kavarna I (2.60 ng/g ww), while the minimum value was found at the Kranevo (1.87 ng/g ww). The experimental results for ∑DDTs in mussels from different sampling areas showed, however, no significant differences between different sampling area (statistical test - $p>0.05$).

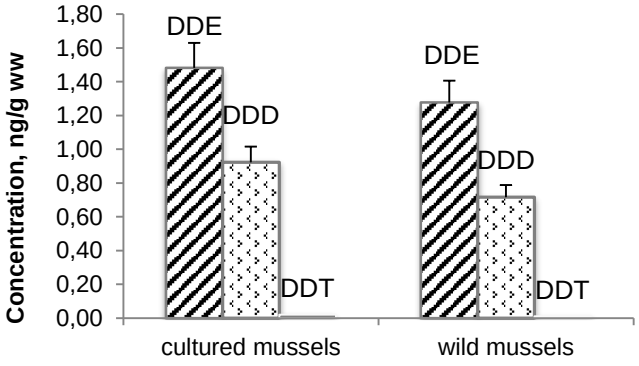

Figure 2. Comparison between DDTs concentration (ng/g ww) in cultured and wild mussels

The results for cultured mussels were compared with data from the study of wild mussels collected from the Black Sea in the same period (Fig. 2). In cultured mussels total DDT concentration (mean 2.42 ng/g ww) was found comparable to those in wild mussels (mean 2.00 ng/g ww). Statistical test ($p>0.05$) indicated that no statistically significant difference was observed between the wild and cultivated mussels.

Total DDT concentration (mean 2.14 ng/g ww) was found comparable to those in mussels, sampled in 2013 and 2014 (mean 1.87 ng/g ww).

The concentration of DDTs (like sum of DDE and DDD) is expressed also on a lipid weight basis (ng/g lw) in order to compare our results with data cited in the literature – Table 2. Nasso *et al*. [22] studied the blue mussels from the Gulf of Naples, Southern Italy and the levels of DDTs measured (sum of *p,p*'-DDT, *p,p*'-DDD and *p,p*'-DDE) were in the range 32.1-308.8 ng/g lipid weight. Sum DDTs found in mussels

from Black Sea, Bulgaria (65.7 ng/g lipid weight) were lower than levels observed by Stefanelli *et al*. [13] and Perugini *et al*. [23] in mussels from the Italian Adriatic Sea and central Adriatic Sea, respectively (Table 2).

Concentrations of total DDTs measured in the present study were much lower than those reported in mussels from seven mariculture zones in Hong Kong, China – 11 -1400 ng/g lw [24].

European Union and Bulgarian legislation are not regulated concentrations of DDTs in aquatic organisms yet.

Table 2. Comparison between levels of DDTs and HCBs (ng/g lw) from this study and from other studies

Location	∑DDTs, ng/g lw	HCB, ng/g lw	References
Adriatic Sea, Central	208	-	[23] Perugini *et al*. 2004
Adriatic Sea, Italy	142	< 6.62	[13] Stefanelli *et al*. 2004
Tyrrhenian Sea, Italy	177	< 25.7	[22] Naso *et al*. 2005
Black Sea, Bulgaria	65.7	< LOD	Present study
Hong Kong, China	11 -1400	26-430	[24] So *et al*., 2005

In relation to other organochlorine compounds normally present in coastal areas, hexachlorobenzene and hexachlorobutadiene were found below detectable levels and did not exceed the European EQS of 10 μg/kg and 55 μg/kg, respectively. HCB is known as volatile and practically insoluble in water compound which leads to a low bioavailability of this contaminant in marine organisms. Carro *et al*. [14] studied the present of HCB in wild mussels collected from Galician Rias and values of HCBs were fond below 0.25 ng/g ww. Nasso *et al*. [22] studied the blue mussels from the Gulf of Naples, Southern Italy and concentrations of HCB measured were < 2.7 ng/g lipid weight and So *et al*. [24] reported levels of HCB in the range 26-430 ng/g lw.

It can be seen that the mean levels of DDTs and HCB detected in mussels from the Bulgarian Black Sea coast are generally lower to those reported for specimens from other aquatic areas (Table 2).

PCBs are usually divided into two groups according to their toxicological properties: dioxin-like PCBs (dl-PCBs) and non dioxin-like PCBs (ndl-PCBs).

The six PCB congeners (IUPAC № 28, 52, 101, 138, 153 and 180) have been selected by the International Council for the Exploration of the Sea (ICES) for their abundance in environmental samples and have also been recommended by the European Union as indicators of PCB contamination [20].

The sum of the six indicator PCBs comprises about half of the amount of total non dioxin-like PCB present in feed and food [25]. That sum is considered as an appropriate marker for occurrence and human exposure ndl-PCB and therefore EU has set a maximum level in fish and mussels 75 ng/g fresh weight [26].

Polychlorinated biphenyls were found below detectable limits in all analyzed samples. The levels found in the present study are in accordance with the results for mussels from the Mid-Black Sea Coast of Turkey, where no PCBs were detected in any samples [27]. Okay *et al*. [15] were measured levels of PCBs in mussels of the Istanbul strait, Turkey during the period of January-February 2007 and their results ranged from 1026 to 35 983 pg/g wet weight.

4. Conclusions

Residues of organochlorine pollutants were analysed in wild and cultured mussels collected from north-east coastal areas of the Black Sea, Bulgaria. DDTs metabolites (*p*,*p*'-DDE and *p*,*p*'-DDD) were the major compounds in all the studied samples. The concentration order was DDE > DDD. The overall lack of *p*,*p*'-DDT in samples from all sampling areas would suggest the absence of recent input of technical DDT from the Bulgarian Black Sea coast. The results obtained in this study indicate that mussel contamination by organochlorine compounds were lower than levels measured in mollusks from neighbor seas - Adriatic Sea and Mediterranean Sea. The monitoring of priority organochlorine pollutants in seafood is of paramount importance in order to protect marine ecosystem.

Acknowledgments

This study was financed by EEA Grants and Ministry of Environment and Water of Bulgaria (Project D-33-49/2015).

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Received: 20 April 2016
Received in revised form: 08 May 2016
Accepted: 09 May 2016