ANNALS OF “DUNAREA DE JOS” UNIVERSITY OF GALATI
MATHEMATICS, PHYSICS, THEORETICAL MECHANICS
FASCICLE II, YEAR X (XLI) 2018, No. 1

Article DOI: https://doi.org/10.35219/ann-ugal-math-phys-mec.2018.1.06

ORGANOCHLORINATED COMPOUNDS IN THE PRUT RIVER SEDIMENTS

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Abstract
The River Prut is a transboundary river between Romania and Republic of Moldova. Numerous tributaries from both sides are bringing with their waters also wastewater from treatments plants and runoff from agricultural fields. Among the broad spectrum of pollutants in rivers resulted from human activity most attention is according to organochlorinated compounds. These persistent compounds refer to the group of substances that are not producing naturally into the environment (xenobiotics) and pose a threat to the biosphere. Organochlorinated compounds include organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) that are similar to pesticides by their physicochemical properties. Residual amounts of chlorinated hydrocarbons fed into rivers mainly due to washout from agricultural fields and large-scale atmospheric transport. Bottom sediments are an important component of water ecosystems, where most of the organic and inorganic substances are accumulating, including the most dangerous and toxic. Under certain conditions leading to a change hydrodynamic situation, composition and properties of water and other factors, they can become a source of secondary water pollution.
In this study, sediments samples were collected in summer 2016 from the Prut River to evaluate the contamination and ecological risks caused by several POPs. Sampling points were selected along the Moldovan side. Automated Soxhlet extraction technique was used for samples processing. The extracts were concentrated and treated with activated copper to remove the sulfur-containig substances, followed by cleaning up on silica gel column. The final solution was further concentrated up to 300 μL and analyzed using gas chromatography coupled to mass-spectrometry (GC-MS/MS). Strict quality assurance and controls were implemented throughout the whole analysis process. For every batch of 4 samples one solvent blank was run to check for interference and cross-contamination. Prior to extraction, each sample was spiked with internal standard (mix of DDX, PCBs) and recovery standard was added prior to injection in GC.

Keywords: organochlorinated pesticides, Prut River, gas chromatography.

1. Introduction

Persistent organic pollutants (POPs) is a group of organic chemicals that are semivolatile, bioaccumulative, persistent and toxic [12]. Organochlorinated pesticides (OC) and polychlorinated biphenyls represent an important group of POPs and cause a worldwide concern as toxic environmental contaminants. Although, Stockholm Convention (2009) restricted and/or banned their usage throughout the world, these chemicals are still being used in some developing nations for agricultural and aquacultural purposes [16]. However, due to their persistence and air transport these pollutants can be found in the environment even decades after being banned and detected in remote areas and in places where they have never been applied [7, 13].

In temperate regions, river sediments act as temporary or long-term sinks for different environmental pollutants [5]. In addition, organochlorinated compounds are strongly absorbed onto the surface of particles associated with the organic content of solid-phase matrix and can be deposited to the sediments. They remain very long in sediment due to their long half-life times [14]. From sediment, they can be taken up and retained in benthic organisms and consequently biomagnified through aquatic food chains to higher trophic levels [8, 3]. POPs enter the river environments be means of discharge of domestic sewage and industrial wastewater, runoff from nonpoint sources, and direct dumping of wastes into the river. Hence, the assessment of POP concentrations in aquatic environments is required to provide important information on anthropogenic effect on the environment and serve as an indicator of contaminant loading.

Thus, purpose of this study was to determine the concentrations of persistent organochlorinated compounds in river sediments and compare them with environmental quality standards for these pollutants in aquatic ecosystems.

2. Materials and methods

2.1. Reference substances and chemicals

The target compounds included in this study are DDT and its metabolites (*o,p*-DDT, *o,p*-DDE, *o,p*-DDD *p,p*-DDT, *p,p*-DDE, *p,p*-DDD). The following PCB congeners were targeted (IUPAC numbers): 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 170, 180, 189, 209. The internal standard (IS) mixture contained isotopically labeled standards ($^{13}C_{12}$ -18 PCBs and DDX).$^{13}C_{12}$ -PCB97 and $^{13}C_{12}$-PCB188 were used as a recovery standard and. Both mixtures had a concentration of 25 pg mL^{-1}. All compounds for IS and RS were purchased from Wellington Laboratories, Ontario, Canada. Dilutions were made in iso-octane, in order to cover the entire of POPs expected in the samples, and stored at -20°C. Glass wool, anhydrous sodium sulfate, silica gel high-purity grade (pore size 60 Å, 230-400 mesh particle size) were obtained from Sigma-Aldrich. Dichloromethane, Iso-octane, Acetone, Hexane (SupraSolv®), Sulfuric acid 98% (EMSURE®) were purchased from Merck (Darmstadt, Germany). Milli-Q water (Millipore) was produced in-house by filtration through MilliPak® 0,22 μm filter. Anhydrous sodium sulfate, and silica gel and were heated overnight at 400°C.

2.2 Sampling area and samples description

The Prut River is a transboundary river for Republic of Moldova and Romania. This river is a second largest river in Moldova and 695 km from 953 of it lengths the Prut flows in limits of the republic [16]. It has a catchment basin of 27 500 km^2 with numerous small affluents from both sides.

Surface sediments samples (n=6) were collected in summer 2016 during the expedition of Institute of Zoology of Moldova. The sampling covered 7 points situated on Moldavian side of river (Branişte, Sculeni, Leuşeni, Leova, Cahul, Cîşliţa, Girgiuleşti). Sediments samples were taken by means of Ekman grab sampler at 1 meter distance of the shore. The top layer (0-5 cm) from each replicate sample (n = 3) was taken and pooled to form a composite sample. Samples were dried at room temperature at the laboratory. Pebbles, shells and vegetable matter were manually removed. Samples were grounded and sieved, then pulverized using mortar grinding Pulverisette® 2 (Fritsch). Prior to extraction samples were sealed in polyethylene containers for storage at room temperature in dark place.

2.3. Sample extraction and cleaning-up

All procedures of extraction and clean-up were carried up in Laboratory of Persistent Organic Pollutants, Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden. The procedure of extraction was performed using an automatic extractor Soxtec® Avanti 2050 Soxhlet analyzer (Parameters are presented in Figure 1 and Table 1.). The extract was concentrated on TurboVap® II system (Biotage) with a water temperature of 40 °C and a nitrogen gas pressure of 5 bars. The activated copper was used to remove the sulfur-containing substances. Glass columns (i.d. - 18 mm, length - 250 mm) filled with acidified silica (50 %) and sodium sulfate anhydrous were prepared for further step of cleaning-up. Solution of hexane:dichloromethane 4:1 was used for elution. Final eluate was concentrated till 0,1 ml under a gentle nitrogen stream using Organomation N-EVAP® Nitrogen Evaporator (USA) and transferred to GC-vial.

Fig. 1. Map with sampling points.

2.4. Instrumental analysis

All compounds were analyzed using a gas chromatograph (GC; Agilent Technologies, 7890 A) coupled to a triple quadrupole mass spectrometer (Agilent Technologies, 7010, GC-MS/MS Triple Quad) and 7693 auto sampler (Agilent technologies). GC parameters were: injector temperature was held at 275 ºC and injection volume of 2 µL was used in splitless mode. A DB5 capillary column (60 m x 250 µm i.d. x 0.25 µm, Agilent Technologies) was used to separate the analytes. The column oven temperature was initially maintained at 190 ºC (held for 2 min), then 3 ºC/min to 250 ºC, then 6 ºC/min to 310 ºC (held for 1 min). Helium (He) was used as a carrier gas for analysis at a constant flow 2mL min^{-1}. 70 eV mode electron ionization (EI) was chosen for MS/MS operation. The ion source temperature was 300 ºC and the transfer line temperature 310ºC. Nitrogen (N_2) was used as collision gas and He as quench gas in the collision cell. The multiple reaction monitoring (MRM) mode was used for analysis with two transitions for each target analyte compound. Reference standards were used for identification and calculation of target compounds. Data acquisition and processing was controlled by Agilent MassHunter Quantitative Analysis for (QQQ) software.

Table 1. Parameters of extraction program

Parameters	Values
1st step (Boiling)	1 hour at 160 °C
2nd step (Rinsing)	1 hour at 160 °C
3rd step (Recovery)	2 minutes at 160 °C
4th step (Pre-drying)	5 minutes at 160 °C
Solvent	Acetone:hexane (1:1)
Volume of solvent	60 ml
Weight of sample	~2,5 gr
Extraction thimble	Micro-Glass fiber

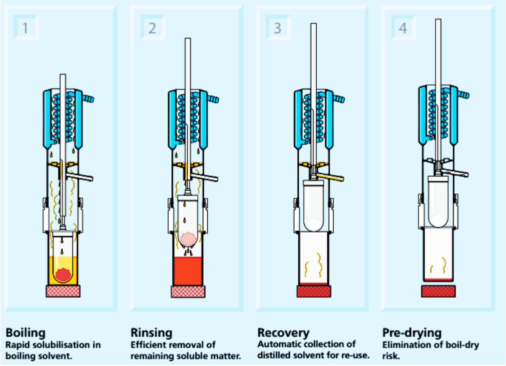

Fig. 2. Four steps of Soxtec analyzer

2.5. Quality Assurance

All samples were subjected to strict quality control procedures. Procedural and solvent blanks were analyzed using the same procedure of extraction and further cleaning-up. The instruments were calibrated with calibration standards before the GC–MS/MS analysis. In order to reduce the risk of any background contamination during sample preparation, all glassware, tools that were in direct contact with samples were cleaned, burned (~400°C), and rinsed with suitable solvents before use. Laboratory blanks were extracted and analyzed in parallel with every batch of 4 samples to check for potential background contamination of target compounds. If the analytes were not detected in any of the solvent blanks, the lowest point in the calibration curve was used as limit of quantification (LOQ). Concentrations those chromatographic peaks had a signal-to-noise ratio less than 3 (below the limit of detection - LOD) were excluded from calculation as well as those concentrations which were below the lowest calibration curve (LOQ). LOQ for individual PCBs and OCPs ranged between 0.1 and 0.86 ng g^{-1}.

3. Results and discussion

This study presents results obtained from assessment of the Prut River sediments for the levels of persistent organochlorinated compound, and reflects an attempt to understand the effects of the agricultural and industrial chemicals pollution and current contamination status in the area. As a result, the following interpretation and discussion will be focused on the organochlorine pesticide DDT, its metabolites, and 20 PCBs congeners in sediments.

3.1. Organochlorinated pesticides in sediments

Total concentrations of DDT metabolites (*o,p*-DDT, *o,p*-DDE, *o,p*-DDD, *p,p*-DDT, *p,p*-DDE, *p,p*-DDD) revealed a wide range of fluctuation from 3.68 (Braniște) to 21.85 ng g^{-1} (Sculeni) dry weight. The Swedish EPA’s environmental quality standards for sediment were used to compare found concentrations (Fig. 3). Detailed information about OC concentrations is presented in Table 1. Concentrations for total DDTs metabolites in sediments from the Prut River were up to two or three orders of magnitude greater than total DDTs observed in the same sampling sites (Braniște, Leova, Giurgiulești) in 2015 [10].

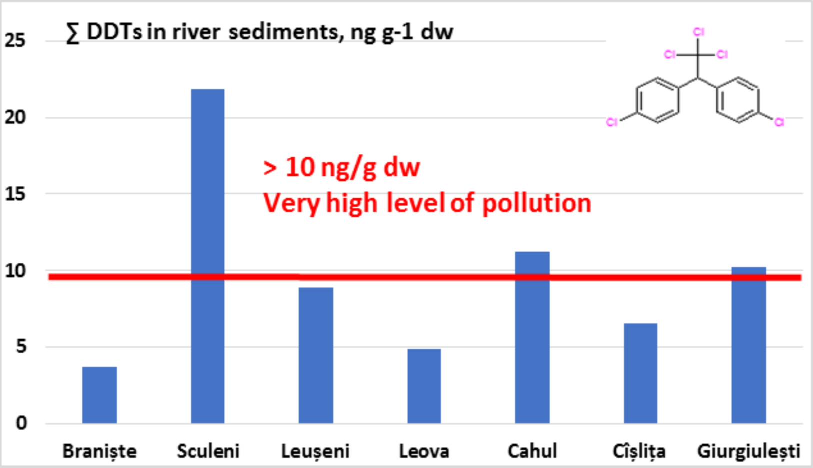

Fig. 3. Total DDTs concentrations, ng $^{g-1}$ dry weight.

DDTs compounds were detected in all sediments samples but the contribution of individual metabolites showed differences (Fig.4). The occurrence of DDT isomers is predominant in the following order: p,p-DDE > p,p-DDD > o,p-DDD > p,p-DDT > o,p-DDE > o,p-DDT. It is well known that the composition of organochlorine pesticides including some metabolites can help for a better understanding of origin and transport pathways [11]. DDD and DDE became the dominant compositions of DDTs in all sampling locations with the ratios of (DDE+DDD)/∑DDTs were greater then 0,5 (from 0,85 to 0,97), which can be explained by an old input of DDT in the region [9]. Since DDT was banned for use, other possible source should be considered. It has also been advised that DDT may be present as impurities in the acaricide dicofol, which is restricted and currently used widely in some states and may be another possible source of DDT in the environment [15]. In addition, a strong correlation (r = 0.88) between p,p-DDE and o,p-DDT may give an additive evidence for the possible contribution of dicofol. That is because a-chloro-DDT, another main impurity remains in the dicofol product, and it can be degraded to p,p-DDE by microorganism [1], organism [4], or photochemical reaction [2], which probably result in an elevated concentration of p,p-DDE in the sediments (Fig 4).

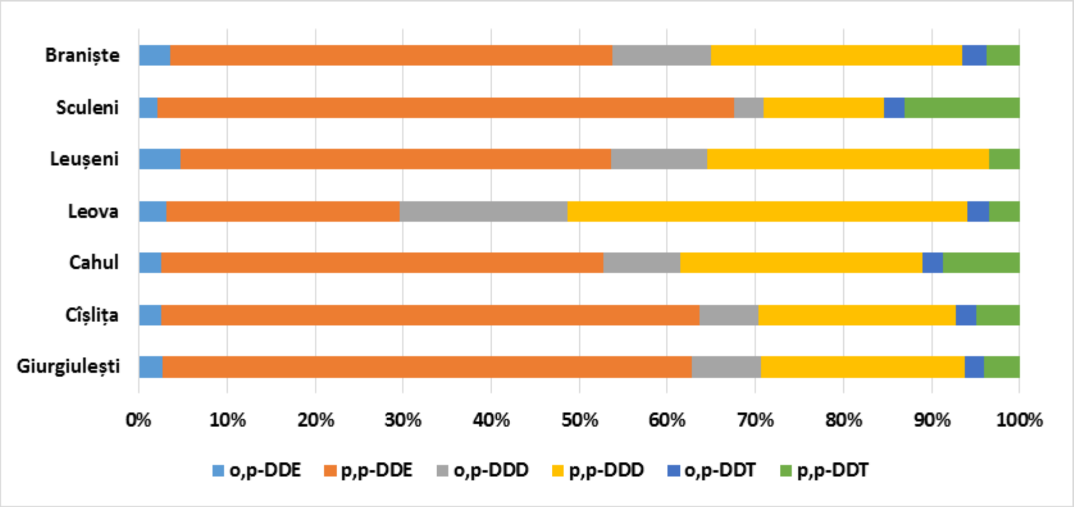

Fig. 4. DDT metabolites contributions to total DDTs concentrations in sediments.

Table 2. Summary of OC in sediments of Prut River, ng g^{-1} dw. (n.d. = not detected)

Compounds	Min	Max	Mean	Median	Range
o,p-DDE	0,13	0,46	0,27±0,13	0,27	0,33
p,p-DDE	1,29	14,29	5,36±4.33	4,34	13
o,p-DDD	0,41	0,98	0,75±0,24	0,8	0,57
p,p-DDD	1,05	3,09	2,29±0,78	2,36	2,04
o,p-DDT	n.d.	0,49	0,19±0,16	0,15	0,49
p,p-DDT	0,14	2,87	0,74±0,98	0,32	2,73
∑ DDTs	3,68	21,85	9,61±6,06	8,89	18,17
∑ PCBs	0,64	2,59	1,27±0.63	1,13	1,95

3.2. PCBs concentrations in sediments

Unlike to DDTs, PCBs were observed in relatively low concentrations in sediments of the Prut River. Total PCB concentrations of 20 congeners in sediments ranged from 0.64 ng g^{-1} in Cîșlița to 2.59 ng g^{-1} dry weigh in Leușeni (Figure 5).

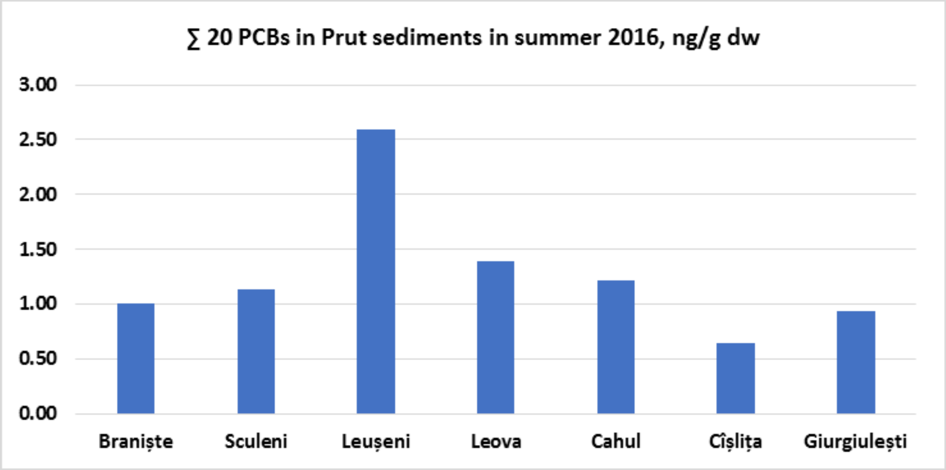

Fig. 5. Total concentrations of 20 PCB congeners in sediments, ng g^{-1} dry weight.

In 2007 in sediments from the Bahlui River total PCBs concentrated ranged from 24 ng g^{-1} to 158 ng g^{-1} dry weigh [6]. In 2015 Hydrometeorological State Service reported about quite high total PCBs PCB-6 and PCB118) concentrations in sampling locations Braniște, Leova which ranged from 2.3 to 13.2 ng g^{-1} dry weight [10]. All found concentrations of total PCBs in 2015 and 2016 with exception of concentrations found in the Bahlui River are lower then allowed concentrations (34.1 ng g^{-1} dry weight) for interim sediment quality guidelines (ISCGs) established by Canadian Ministry of the Environment, 1999.

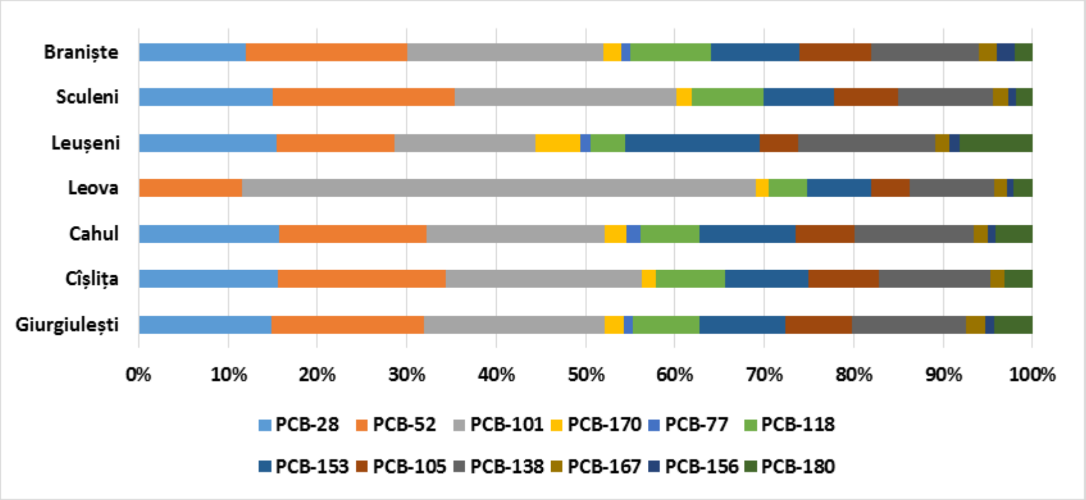

Fig. 6. Percentage distribution of the sum of PCB congeners in sediments from the Prut River.

Among 20 PCBs congeners analyzed in this study some "dioxin-like" compounds were detected (PCB77, PCB118, PCB105, PCB167, PCB156) and seven "non-dioxin-like" ones (PCB28, PCB52, PCB101, PCB153, PCB138, PCB180, PCB170) (Figure 6). Total concentrations of "dioxin-like" compounds ranged from 0,15 to 0,31 ng g^{-1} dry weigh. In addition, the total concentrations of PCBs were up to 7 times lower than total concentrations of DDTs in sediments of the Prut River, assuming significant agricultural rather than industrial pollution in the region.

Conclusions

Despite of prohibition for use of Persistent Organic Pollutants in Moldova some organochlorinated compounds such as DDTs and PCBs were detected in sediments samples from the Prut River. Generally, total DDTs concentrations showed recent agricultural pollution and total PCBs concentrations were not of concern in relevance with the global standards. Nevertheless, to understand the OC accumulation patterns in sediments, further detailed work with more samples is suggested. More extensive research would be needed in order to clarify the paths of inputs of DDTs and PCBs to the aquatic environment as well as to investigate further contamination by other organic pollutant groups such as dioxin-like compounds, polybrominated flame retardants.

Acknowledgement

The work of PhD student Anastasia Ivanova was supported by Project of the Institute of Zoology of Moldova AQUASYS 15.817.02.27A. This publication is a part of a PhD research work at Swedish University of Agricultural Sciences, funded by a Swedish Institute Scholarship. The author wishes to acknowledge Swedish project supervisor associate professor Lutz Ahrens for support and PhD Anna-Karin Dahlberg for instrumental and laboratory assistance.

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