Aquatic Ecology
Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 71 (2), 2019: 235-240

Organic Pollutants in Water and Biota of River Ecosystems: a Case Study From Bulgaria

Gana M. Gecheva[1*], Vesela M. Genina[2], Marin I. Marinov[3] & Ivelin A. Mollov[1]

[1] Department of Ecology and Environmental Conservation, Faculty of Biology, University of Plovdiv "Paisii Hilendarski", 24 Tsar Asen Street, 4000 Plovdiv, Bulgaria
[2] Regional Laboratory Plovdiv, Environmental Executive Agency, 4000 Plovdiv, Bulgaria
[3] East Aegean River Basin Directorate, 4000 Plovdiv, Bulgaria

Abstract: Organic pollutants are dangerous toxicants in aquatic ecosystems. Priority substances enter aquatic organisms through water, food and sediments. In literature, data for organic pollutants in the aquatic biota mostly concern different fish species. Therefore, if focused on the protection of aquatic environment, the level of information of organic pollutants could be assumed as unsatisfactory. Our study aimed to reveal the relationships between the concentration of organic contaminants in water, macrophytes and macroinvertebrate and fish species of various families. Among the macrophyte taxa, the highest levels of accumulation showed *Leptodictyum riparium*. Macroinvertebrates were most effective in accumulation of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). When a representative quantity of a macroinvertebrate taxon could not be collected, total macrozoobethos samples were analysed. This is the first complex study of 37 organic pollutants based on four matrices (surface water, macrophytes, macroinvertebrates and fish). Most of the studied priority substances had higher concentrations in macrophytes and macroinvertebrates as compared to fish samples. Such biota monitoring is an alternative of measuring organic pollutants in fish.

Key words: Macrophytes, macroinvertebrates, fish, OCPs, PAHs, PCBs

Introduction

The European Water Framework Directive (WFD) requires the determination of chemical status in European waters into two classes based on monitoring of the list of priority substances (European Union 2000). According to the Directive 2008/105/EU on environmental quality standards in the field of water policy, good chemical status refers to a list of 41 pollutants: 33 priority substances (PSs) and eight other pollutants (European Union 2008). Directive 2013/39/EU requires action to newly identified substances and sets out a revised list of 45 PSs (European Union 2013).

In general, the available data for PSs in biota are very heterogeneous in terms of the analysed pollutants, taxa and regional distribution. The contamination in water, sediments and biota in the Ebro River Basin were overviewed (Navarro-Ortega & Barceló 2010). Study on 16 polycyclic aromatic hydrocarbons (PAHs) in stream water in Portugal and aquatic moss transplants (*Fontinalis antipyretica*) indicated that areas with activities of tertiary and industrial sectors had higher PAH concentrations in transplanted mosses (Augusto et al. 2011). A review of 38 articles published between 1997 and 2014, showed that several persistent organic pollutants were found in sediments and biota from coastal, estuarine and river areas of Portugal (Ribeiro et al. 2015). Additional studies concerning sediments and biota were found to be necessary to provide valuable information for assessing the fate of these pollutants in the environment.

Macroinvertebrates were chosen as the most appropriate organisms in active monitoring (Besse

*Corresponding author: ggecheva@mail.bg

et al. 2012). In addition, aquatic bryophytes (particularly widespread species *Fontinalis antipyretica* and *Platyhypnidium riparioides*) were proven to meet all criteria for chemical monitoring of biota (Gecheva & Yurukova 2014). Nevertheless, from the monitoring data for biota collected for the period 2006-2015, only 4.5% dealt with inland water bodies (Carvalho et al. 2016). Based on the above, activities on monitoring of PSs in biota are crucial in order to assess chemical status of aquatic ecosystems not only in surface water, but also for other matrices.

This study concentrates on the monitoring of PSs both in the water and biota in order to provide a valid data base for selection of the appropriate taxa from the groups of macrophytes, macroinvertebrates and fish.

Materials and Methods

The study was carried out between mid-July and early October of 2013-2014 during five sampling campaigns. Five monitoring stations in the Maritsa River Basin, Bulgaria (Table 1) were selected based on preliminary analysis of available data for point and diffuse sources of PSs, dominant substrate (stones, boulders, sand and silt), hydrological characteristics. *In situ,* we measured pH, electrical conductivity (C, µS cm-1), dissolved oxygen (mg L^{-1}) using calibrated WTW pH/Conductivity/Oxygen meter. Water sampling followed EN ISO 5667-6. Water sample preparation included solid-phase extraction (Thermo Scientific Dionex Auto Trace 280, USA).

The nomenclature followed Hill et al. (2006) for mosses, Delipavlov et al. (2003) for vascular plants, Fauna Europaea for macroinvertebrates (de Jong et al. 2014) and FishBase (Froese & Pauly 2017) for fish.

Microwave-assisted sample preparation was applied for biota (MARS 6, USA). The Directive 2013/39/EU (European Union 2013) does not specify whether whole fish or certain fish parts (e.g. filet) should be analysed. We used whole fish as a matrix for the protection of the aquatic environment. Thirty-seven PSs and other organic pollutants were analysed in extracted matrices (water and biota) with gas chromatography-mass spectrometry (Agilent 7890B/5977A): 13 organochlorine pesticides (OCPs), 18 polycyclic aromatic hydrocarbons (PAHs) and six polychlorinated biphenyls (PCBs) (Table 2). Quality control was assured using reference materials: Lake Michigan fish tissue (NIST1947), PCBs in mussel tissue (BCR 682-70G), organics in freeze dried mussel tissue (SRM 2974a), Pesticide mix 13 and PAH (Dr. Ehrenstorfer

Table 1. Coordinates, selected physical and chemical parameters and biota data at the river monitoring stations. Legend: *average from 5 measurements

	Site 1	Site 2	Site 3	Site 4	Site 5
Latitude/longitude	42.6902; 24.56917	42.1871; 24.91314	42.1457; 24.87722	42.1608; 24.95124	42.1529; 24.74322
pH*	8.3	7.9	8.1	8.32	8.14
C* µS cm^{-1}	420	316.4	292.9	267	291.3
DO* mg L^{-1}	9.1	8.3	8.5	9.03	9.2
Macrophytes	*Leptodictyum riparium*	*Platyhypnidium riparioides*	*Elodea canadensis*	*Fontinalis antipyretica*	*Myriophyllum spicatum*
			Elodea nuttallii		
Macroinvertebrates	*Asellus aquaticus*	*Asellus aquaticus*	*Baetis sp.*	*Ancylus fluviatilis*	*Baetis sp.*
	Baetis sp.	*Baetis sp.*	*Caenis sp.*	*Baetis sp.*	Ceratopogonidae
	Chironomidae	*Caenis sp.*	Ceratopogonidae	*Dicrotendipes nervosus*	Chironomidae
	Eiseniella tetraedra	Chironomidae	Chironomidae	*Dicrotendipes sp.*	*Ephemerella sp.*
	Gomphus sp.	Eiseniella tetraedra	Ecdyonurus sp.	Hydropsyche sp.	Hydropsyche sp.
	Hydropsyche sp.	Empididae	Elminthidae	*Leuctra pseudosignifera*	Naididae
	Naididae	Gammarus sp.	Ephemerella sp.		Simuliidae
	Radix sp.	Helobdella stagnalis	Hydropsyche sp.		
		Hydropsyche sp.	Simuliidae		
		Physa sp.			
		Naididae			
		Simuliidae			
Fish	*Barbus cyclolepis*	*Barbus cyclolepis*	*Barbus cyclolepis*	*Barbus cyclolepis*	*Barbus cyclolepis*
	Squalius orpheus	*Squalius orpheus*		Squalius orpheus	Squalius orpheus

Table 2. Maximum concentrations of analysed organic pollutants and biotic taxa/group. Legend: * single detected value

	Pollutant		Max (µg kg^{-1} w.w.)	Biota
OCPs	α-Hexachlorocyclohexane	(α-HCH)	15.34	Macroinvertebrates
	β-Hexachlorocyclohexane	(β-HCH)	51.16	*Fontinalis antipyretica*
	γ-Hexachlorocyclohexane	(γ-HCH)	39.23	Macroinvertebrates
	δ-Hexachlorocyclohexane	(δ-HCH)	19.05	Macroinvertebrates
	ε-Hexachlorocyclohexane	(ε-HCH)	6.31	Macroinvertebrates
	Aldrin		2.5*	*Barbus cyclolepis*
	Dieldrin		83.84	*Leptodictyum riparium*
	Endrin		40.61	*Squalius orpheus*
	Isodrin		148.50	Macroinvertebrates
	p,p-DDT		5.83*	*Leptodictyum riparium*
	o,p-DDT		5.73*	*Leptodictyum riparium*
	p,p-DDD		4.62	*Squalius orpheus*
	o,p-DDD		71.52	*Leptodictyum riparium*
PAHs	Naphthalene	(NAP)	119.34	Macroinvertebrates
	Acenaphthene	(ACE)	4.27*	*Barbus cyclolepis*
	Acenaphthylene	(ACY)	17.84*	Macroinvertebrates
	Fluoranthene	(FLU)	under LOQ	
	Phenanthrene	(PHE)	69.03	Macroinvertebrates
	Benzo(e)pyrene	(BeP)	4.64*	*Leptodictyum riparium*
	Benzo(j)fluoranthene	(BjF)	7.56*	Macroinvertebrates
	Fluoranthene	(FLA)	79.84	Macroinvertebrates
	Pyrene	(PYR)	17.47	*Elodea sp.*
	Benz(a)anthracene	(BaA)	2.69*	*Elodea sp.*
	Chrysene	(CHR)	2.51*	*Elodea sp.*
	Benzo(b)fluoranthene	(BbF)	7.06*	Macroinvertebrates
	Benzo(k)fluoranthene	(BkF)	1.72*	*Elodea sp.*
	Benzo(a)pyrene	(BaP)	10.51*	*Leptodictyum riparium*
	Indeno(1,2,3-cd)pyrene	(IND)	under LOQ	
	Dibenzo(a, h)anthracen	(DbahA)	under LOQ	
	Hexachlorobenzene	(HCB)	under LOQ	
	Benzo(g,h,i)perylene	(BghiP)	under LOQ	
PCBs	PCB 28		77.94	Macroinvertebrates
	PCB 52		4.49	*Fontinalis antipyretica*
	PCB 101		8.98	Macroinvertebrates
	PCB 138		7.32	Macroinvertebrates
	PCB 153		2.36	*Squalius orpheus*
	PCB 180		1.29	Macroinvertebrates

GmbH). The concentrations in biota were presented in µg kg^{-1}wet weight.

We summarised the distribution of accumulated PSs (by groups OCPs, PAHs and PCBs) in the three biota matrices (macrophytes, macroinvertebrates and fish) with Principal component analysis (PCA). The average data from the five sampling events were log-transformed (x' = log (x+1)), automatically cantered and standardised using Canoco v.5 (Smilauer & Budejovice 2014).

Results

Water

The measured physical and chemical variables were in agreement with good chemical status (Table 1). All 37 organic pollutants in the aquatic environment were below the limit of quantitation (LOQ; <0.01 µg L^{-1}).

Macrophytes

The registered macrophyte species (Table 1) had maximum values of five OCPs, six PAHs and one PCB (Table 2). *Leptodictyum riparium* (Hedw.) Warnst. had the highest number of maximum accumulated organic pollutants. From the OCPs group, maximum levels were registered for β-HCH, dieldrin, p,p-DDT, o,p-DDT and o,p-DDD. The pollutants β-HCH, dieldrin and o,p-DDD varied between twice and eight times between macrophyte samples from different sites (β-HCH 29.8-51.2 µg kg^{-1}; dieldrin 10.2-83.84 µg kg^{-1}; o,p-DDD 10.3-71.52 µg kg^{-1}), while maximums of p,p-DDT, o,p-DDT were single detected values. Five from the analysed maximums for polyaromatic hydrocarbons were registered once. Pyrene varied almost six times in macrophyte samples (2.97-17.47 µg kg^{-1}). PCB 52 values were in the range 0.62–4.49 µg kg^{-1} varying almost six times.

Macroinvertebrates

Baetis sp. and *Hydropsyche* sp. were registered at all monitoring stations (Table 1). Macroinvertebrate samples had maximum values of five OCPs, six PAHs and four PCBs (Table 2). All isomers of hexachloro-

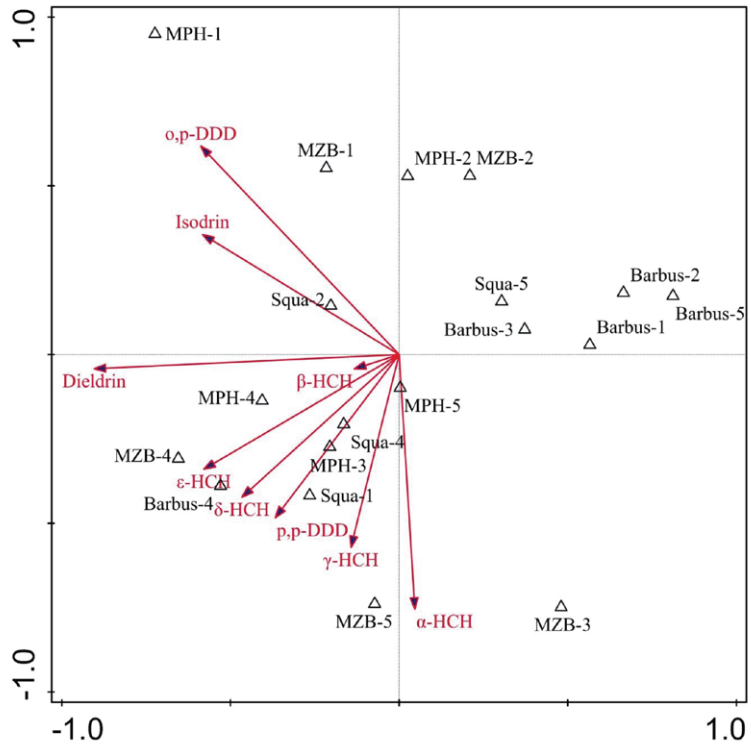

Fig. 1. PCA scatterplot of the four studied matrices (MPH – macrophytes; MZB – macrozoobenthos; Barbus – *Barbus cyclolepis*; Squa – *Squalius orpheus*; the numbers after groups' abbreviations correspond to the sampling site) in relation to the organochlorine pesticides (averaged data). See Table 2 for OCPs abbreviations

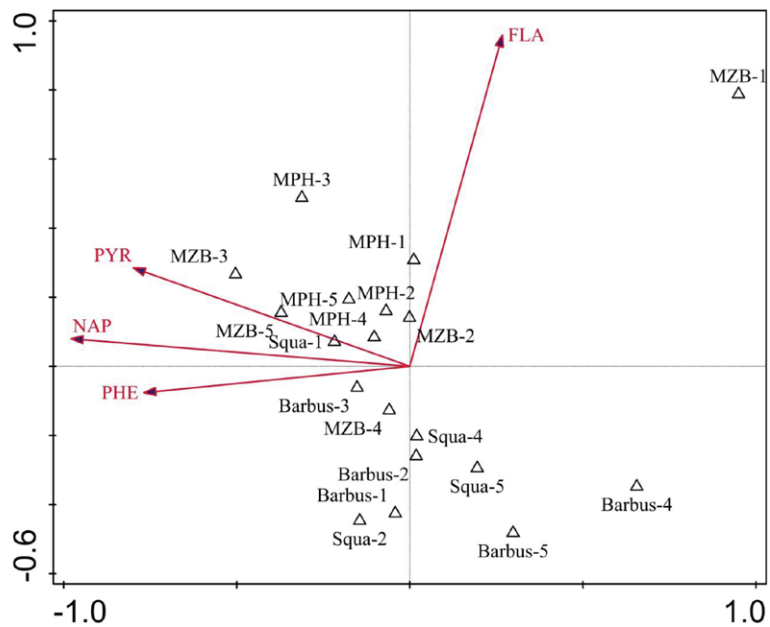

Fig. 2. PCA scatterplot of the four studied matrices (MPH – macrophytes; MZB – macrozoobenthos; Barbus – *Barbus cyclolepis*; Squa – *Squalius orpheus*; the numbers after groups' abbreviations correspond to the sampling site) in relation to the polyaromatic hydrocarbons (averaged data). See Table 2 for PAHs abbreviations

cyclohexane, except β-HCH, were with the highest bioaccumulation levels in macrozoobenthos samples, varying about four to seven times. Eight times was the variation of isodrine between macroinvertebrate samples (25.5-148.5 μg kg^{-1}). Three of the polyaromatic hydrocarbons were detected once as maximums; NAP and FLA varied from eight to 41 times (NAP 15.5-119.3 μg kg^{-1}; FLA 1.94-79.8 μg kg^{-1}). PCBs varied from twice to three times, except for PCB 28 with 19 times variation (4.06-77.9 μg kg^{-1}).

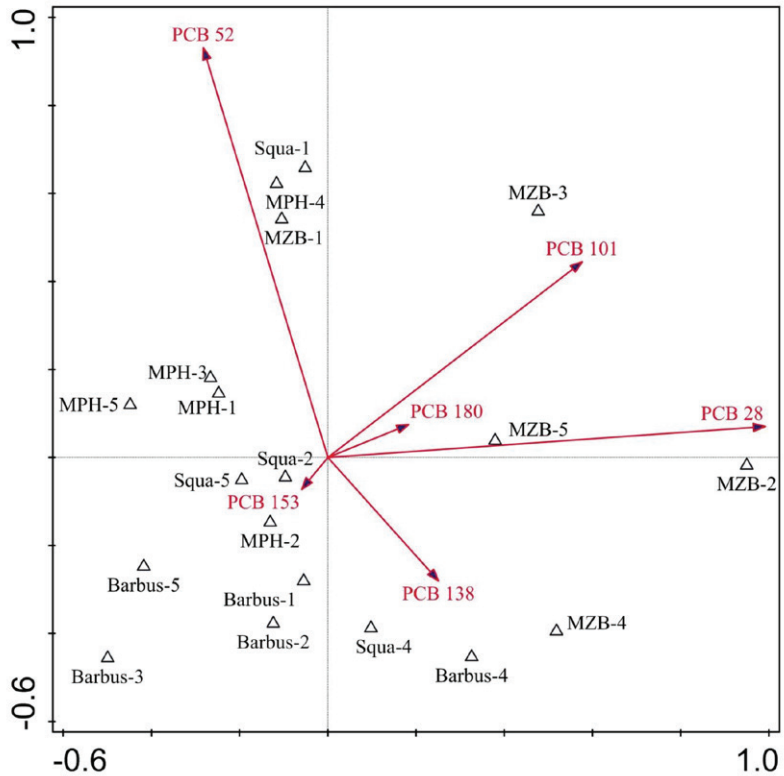

Fig. 3. PCA scatterplot of the four studied matrices (MPH – macrophytes; MZB – macrozoobenthos; Barbus – *Barbus cyclolepis*; Squa – *Squalius orpheus*; the numbers after groups' abbreviations correspond to the sampling site) in relation to the polychlorinated biphenyls (averaged data). See Table 2 for PCBs abbreviations

Fish

Maximum concentrations of aldrin and acenaphthene were recorded in *Barbus cyclolepis* (Heckel, 1837), while endrin, p,p-DDD and PCB 153 had maximum values in *Squalius orpheus* Kottelat & Economidis, 2006 (Table 2). Both maximums in *B. cyclolepis* were single detected for the biota matrices. Endrin for the total samples from *S. orpheus* was detected only as the maximum value. Five times was the variation of p,p-DDD (0.95-4.62 µg kg^{-1}), eight times of PCB 153 (0.29-2.36 µg kg^{-1}). Both fish taxa accumulated fluoranthene between 0.80 and 3.8 µg kg^{-1}, i.e. almost ten times below the EQS_{biota}. Hexachlorobenzene in all samples was below the LOQ (<1 µg kg^{-1}).

Biota matrices

Principal component analysis (PCA) was applied to relate the biota matrices to the accumulated organic pollutants. The PCA of the OCPs (eigenvalues of the first two axes were 0.302 and 0.216, together explaining 51.8% of the taxa-pollutants relation) indicated that in general macrophytes, macroinvertebrates and *S. orpheus* had similar level of pollutant accumulation (Fig. 1). The barbell had lower levels of analysed OCPs (on the right side). Although both fish species feed mainly on aquatic invertebrates, plant detritus was found to be the most important nutrient for the barbell in summer (Rozdina et al. 2008).

The PCA analysis of the PAHs (eigenvalues of the first two axes were 0.613 and 0.280, together explaining 89.3% of the taxa-pollutants relation) showed that all samples (except for Squa-1) of both fish species showed the lowest accumulation levels for all four PAHs (Fig. 2). On the other hand, high concentrations of PYR and FLA were found in macrophyte samples. The macrozoobenthos samples (except for MZB-4) showed large amounts of NAP, PYR and FLA.

Three biota matrices formed distinguished groups as regarding the PCBs (eigenvalues of the first two axes were 0.541 and 0.193, together explaining 73.5% of the taxa-pollutants relation; Fig. 3). The macrozoobenthos exhibited a clear pattern of high bioaccumulation (on the right of the diagram). High bioaccumulation values of PCB 52 were detected in macrophytes (on the upper left of the diagram), while both fish species (bottom left) showed low bioaccumulation potential to PCBs.

Regarding the allocation of pollutants at the monitoring stations, samples from site 1 and 2 (Figs. 1-3) showed high accumulation of OCPs, PAHs and PCBs. Both river stations suffered from serious anthropogenic pressures, e.g. industrial discharges, agricultural runoff, untreated sewage effluents.

Discussion

Concerning macrophytes, the comparison of our results with data from previous studies on polychlorinated biphenyls and hexachlorocyclohexanes (Mouvet et al. 1985) and on polycyclic aromatic hydrocarbons (Roy et al. 1996), we found lower levels in aquatic mosses from the Maritsa River Basin. Contrary to previous research of organic pollutants with moss bags (Mouvet et al. 1985), our results showed that OCPs were accumulated in higher amounts than PCBs. Regarding the examination of fish tissues, the comparison with previous results for aldrinacenaphthene, endrin and PCB 153 in muscle from the same species and sampling sites showed that they were below the LOQ (Genina & Marinov 2017). Only p,p-DDD was in higher concentration in muscle samples.

All analysed organic pollutants were below the LOQ in water samples, while the highest values were measured in macrophytes and macroinvertebrates. Most of the maximum accumulated organic pollutants in macrophytes were detected in the aquatic moss *Leptodictyum riparium*. Macroinvertebrates could be recommended for monitoring of organic pollutants and especially of PAHs and PCBs as they have accumulated in higher amounts in invertebrate bottom fauna than in other organism groups. The results indicate that when it is not possible to provide representative samples from single macroinvertebrate taxa at a sampling site, the sum of sampled macrozoobethos species could be analysed for pollutants.

Monitoring of priority substances in fish with respect to protection of aquatic environments should be based on whole fish analysis instead of muscle as edible parts. Based on the results, between the two used fish species, *S. orpheus* should be selected as a matrix, especially for the group of OCPs.

The results indicated that aquatic macrophytes and macroinvertebrates could be monitored as alternative groups along fish. Moreover, levels of organic pollutants are higher both in macrophytes and macroinvertebrates and long-term monitoring might provide data for equivalent EQSs for protection.

References

Augusto S., Gonzalez C., Vieira R., Máguas C. & Branquinho C. 2011. Evaluating sources of PAHs in urban streams based on land use and biomonitors. Environmental Science & Technology 45: 3731-3738.

Besse J.-P., Geffard O. & Coquery M. 2012. Relevance and applicability of active biomonitoring in continental waters under the Water Framework Directive. Trends in Analytical Chemistry 36: 113-127.

Carvalho R. N., Marinov D., Loos R., Napierska D., Chirico N., de Jong Y., Verbeek M., Michelsen V., Bjørn P., Los W., Steeman F., Bailly N., Basire C., Chylarecki P., Stloukal E., Hagedorn G., Wetzel F.T., Glöckler F., Kroupa A., Korb G., Hoffmann A., Häuser C., Kohlbecker A., Müller A., Güntsch A., Stoev P. & Penev L. 2014. Fauna Europaea – all European animal species on the web. BiodivData J 2: e4034. doi: 10.3897/BDJ.2.e4034. Available at: https://fauna-eu.org (Last accessed: 01/2018)

Delipavlov D., Cheshmedjiev I., Popova M., Terziyski D. & Kovachev I. 2013. Key to the Plants in Bulgaria. Delipavlov D, Cheshmedjiev I (Eds.) Agrarian Univ. Acad. Press, Plovdiv, 591 pp. (in Bulgarian)

European Union. 2000. Directive 2000/60/EC of the European Parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. OJEC L 327: 1–72

European Union. 2008. Directive 2008/105/EC of the European Parliament and of the council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Official Journal of the European Communities L 348: 84–97

European Union. 2013. Directive 2013/39/EU of the European parliament and of the council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. OJEC L 226: 1–17

Froese R. & Pauly D. (Eds.). 2017.FishBase. World Wide Web electronic publication. Available at: www.fishbase.org, (Last accessed: 01/2018)

Gecheva G. & Yurukova L. 2014. Water pollutant monitoring with aquatic bryophytes: a review. Environmental Chemistry Letters 12(1): 49-61.

Genina V. & Marinov M. 2017. Assessment of organic pollutants in fish from Maritsa River basin (Bulgaria). EcologiaBalkanica 9(1): 19-27.

Hill M.O., Bell N., Bruggeman-Nannenga M. A., Brugués M., Cano M. J., Enroth J., Flatberg K. I., Frahm J.-P., Gallego M. T., Garilleti R., Guerra J., Hedenäs L., Holyoak D., Hyvönen, T., Ignatov M. S., Lara F., Mazimpaka V., Muñoz J. & Söderström L.2006. An annotated checklist of the mosses of Europe and Macaronesia. Journal of Bryology28: 198-267.

Lettieri T. 2016. Monitoring-based Exercise: Second Review of the Priority Substances List under the Water Framework Directive. Report: https://circabc.europa.eu/w/browse/52c8d8d3-906c-48b5-a75e-53013702b20a

Mouvet C., Galoux M. & Bernes A. 1985. Monitoring of polychlorinated biphenyls (PCBs) and hexachlorocyclohexanes (HCH) in freshwater using aquatic moss *Cinclidotus danubicus*. Science of the Total Environment 44: 253-267.

Navarro-Ortega A. & Barceló D. 2010. Persistent Organic Pollutants in Water, Sediments, and Biota in the Ebro River Basin. In: Barceló D. & Petrovic M. (Eds.) The Ebro River Basin. The Handbook of Environmental Chemistry, 13. Springer, Berlin, Heidelberg

Ribeiro C., Ribeiro A.R. & Tiritan M. E. 2015. Occurrence of persistent organic pollutants in sediments and biota from Portugal versus European incidence: A critical overview. Journal of Environmental Science and Health Part B: 1-11.

Roy S., Sen C. K. & Hanninen O. 1996. Monitoring of polycyclic aromatic hydrocarbons using moss bags: Bioaccumulation and responses of antioxidant enzymes in *Fontinalis antipyretica* Hedw. Chemosphere 32: 2305-2315.

Rozdina D., Raikova G., Marinova R. & Uzunova E. 2008. Food spectrum and feeding of *Barbus cyclolepis* Heckel from the middle stream of Maritza River (Bulgaria). Bulgarian Journal of Agricultural Sciences 14(2): 209-213.

Smilauer P. & Budejovice C. 2014. CANOCO 5, Ecological multivariate data ordination program. Biometris Wageningen-UR. http://microcomputerpower.com

Received: 25.04.2018
Accepted: 21.08.2018