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Bioaccumulation of Phenol, Guaiacol and Some Chlorophenols by Selected Freshwater Species of Leeches

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Abstract In the recent study, the bioaccumulation ability of phenolic substances was determined with field-collected specimens of three leech species, i.e., *Erpobdella octoculata* (Linnaeus), *Theromyzon tessulatum* (O. F. Műller) and *Glossiphonia complanata* (Linneaus). It was found that the examined leeches bioaccumulated phenol (0.03–27.10 mg/kg), 4-methylphenol (0.09–1.83 mg/kg), chlorophenols (0.03–14.90 mg/kg), guaiacol (0.22–2,941 mg/kg), tetrachloroguaiacol (0.06–1.98 mg/kg), 3-chlorosyringol (0.04–15.28 mg/kg) and chlorocatechols (0.33–23.24 mg/kg) present in the water (0.03–25.23 μg/L) and in the bottom sediments (0.75–760.5 μg/kg) of three ecosystems that were characterized by different contamination levels. Analysis of both the external mucous coat of the leeches and the tissue of the dermato-muscular sac showed that substantial quantities of the phenolic compounds may be accumulated in both the mucous (0.03–2,941 mg/kg) and the tissue (0.03–1,189.8 mg/kg).

Keywords Phenolic compounds · Leeches · Bioaccumulation ratio · Bioindicators

Phenolic compounds are widely spread in the environment because they are formed during natural and anthropogenic processes. Phenol and guaiacol are formed as a result of organic matter combustion and degradation of numerous aromatic compounds of natural origin (Kjallstrand et al. 2000). Some chlorinated phenols may also form naturally by bacterial biosynthesis from other organic compounds and inorganic chlorine with the participation of chloroperoxidases (Hoekstra et al. 1999).

However, the occurrence of phenols in the environment is mainly related to the activity of chemical industry, because these substances are used as components and precursors of chemical reagents, dyes, plastics and explosives (Michałowicz and Duda 2007). It was also proven that chlorophenols, chloroguaiacols and chlorosyringols enter aquatic environments with municipal sewage, as well as with wastewater derived from paper pulp processing plants (Michałowicz et al. 2005). Chlorophenols may also enter the environment through the production and use of phenoxy herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D) (Bukowska 2006). Phenols, and chlorophenols in particular, are significantly resistant to biodegradation. Thus, they may remain in aquatic environments for a long time negatively influencing aquatic organisms (Czaplicka 2004).

The toxicity of phenols has been well documented. Chlorophenols have been reported to be endocrine disruptors (Jung et al. 2004) and mutagenic agents (Michałowicz and Majsterek 2010). Some have reported to induce liver cancer, lymphomas, leukemias and hemangiosarcoma in rodents (NTP 1989). Moreover, there is some epidemiological evidence that 2,4,6-TCP and PCP are carcinogenic to humans, and were thus classified by The International Agency for Research on Cancer as probable and possible carcinogens, respectively (IARC 1995). It was also proven that phenols exhibit toxicity towards aquatic organisms (Breton et al. 2003; Zhong et al. 2010).

In some studies it has been proven that leeches have higher bioconcentrating ability than other aquatic

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organisms. For instance, it was shown that *Dina dubia* and *Erpobdella punctata* (Leidy) concentrated 1–3 orders of magnitude more chlorinated phenols than crustaceans and other benthic invertebrates (Metcalfe et al. 1984). Moreover, Hall and Jacob (1988) reported that leeches were effective in providing time-integrated monitoring information on the sporadic discharge of chlorophenols to aquatic ecosystem.

This study was conducted to determine the bioaccumulation of guaiacol, phenol, chlorinated phenols and their derivatives by three species of leeches, i.e., *Erpobdella octoculata*, *Theromyzon tessulatum* and *Glossiphonia complanata*, for the purpose of evaluating them as potential bioindicator species. The examined leech species have not yet been considered as potential bioindicators for estimating phenolic compounds presence in aquatic ecosystems, even though they commonly occur throughout European freshwater ecosystems (Brenzen et al. 2005; Neumann et al. 2002). Our study included samples collected from relatively unpolluted natural ecosystems ('Blue Springs' reserve), as well as from a river significantly polluted with organic substances (the Piasecznica River).

Materials and Methods

Standards of hydroxybenzene (phenol), 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), 2,3,6-trichlorophenol (2,3,6-TCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP), pentachlorophenol (PCP), 4-methylphenol (p-cresol), 1,2-dihydroxybenzene (catechol), 4-chlorocatechol (4-CC), 3,4,5-trichlorocatechol (3,4,5-TCC), tetrachlorocatechol (TeCC), 2-methoxyphenol (guaiacol), 4,6-dichloroguaiacol (4,6-DCG), 4,5,6-trichloroguaiacol (4,5,6-TCG), tetrachloroguaiacol (TeCG), 2,6-dimethoxyphenol (syringol), 3-chlorosyringol (3-CS), and trichlorosyringol (TCS), all above 99 % purity, were bought from Promochem (Germany) and Fluka AG (USA). Empore extraction discs as well as methanol, dichloromethane, diethyl ether, acetone, acetic anhydride and phosphoric acid of HPLC purity were obtained from Baker JT, (USA).

Water, sediment and the specimens of *E. octoculata*, *G. complanata* and *T. tessulatum* were collected from three surface water ecosystems situated in the administrative district of Tomaszów Mazowiecki town (Central Poland), i.e., an old course of the Piasecznica River in Ujazd locality (N: 51°35′57″; E: 19°55′22″), ponds within "Blue Springs" nature reserve (N: 51°30′49″; E: 20°1′42″), and a nursery situated near the reserve (N: 51°30′41″; E: 20°1′25″) (Fig. 1). Water depth ranged from 0.6 to 1 m at the sites of collection, and water was sampled at a depth of 0.5 m. Sediment samples were collected at a depth of 15 cm using a Kajak scoop, and then sieved in the field to remove solid particles larger than 2 mm.

The Piasecznica River is permanently polluted with sewage from municipal purification plants in Koluszki and Niewiadów towns, and also by several industrial facilities. Its water is strongly contaminated with excessive amounts of nitrates, phosphates and bacteria and it is characterized by high oxygen deficiency (Linowiecka 2007).

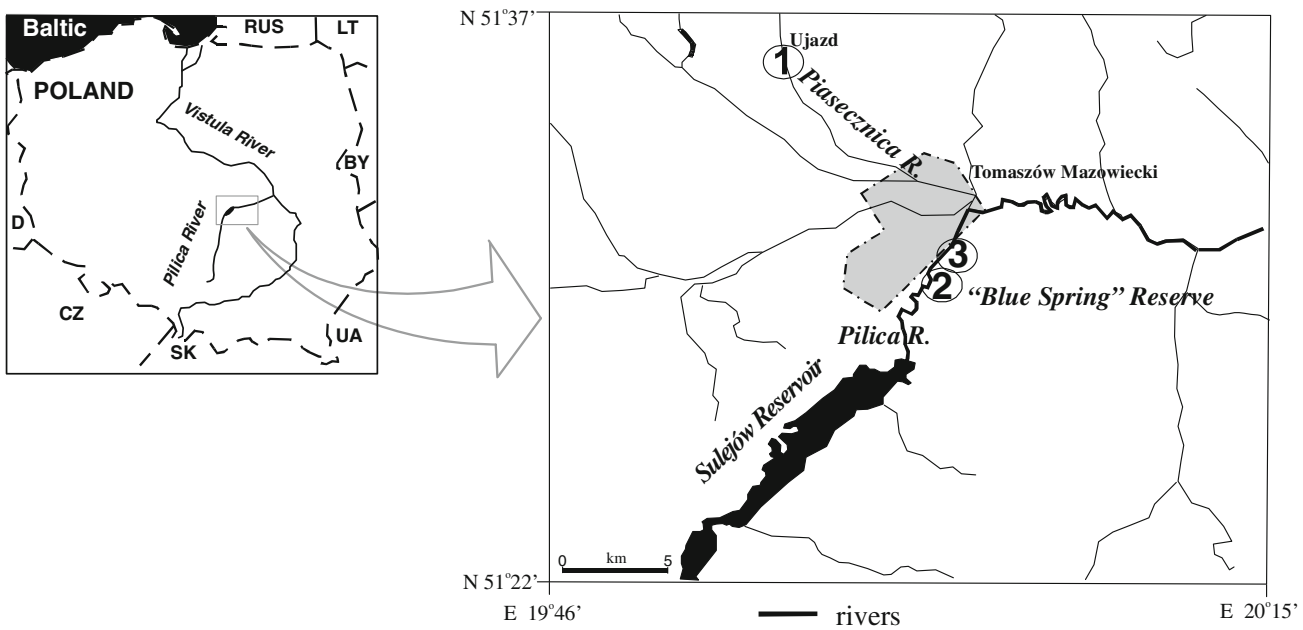

Fig. 1 The points of sample collection: *1* the Piasecznica River, *2* 'Blue Springs' Natural Reserve, *3* a nursery

Table 1 Comparison of the concentrations (μg/L(kg)) of phenolic compounds determined in water and sediments (dry weight) of the Piasecznica River, 'Blue Springs' natural reserve and a nursery

Compound	Piasecznica River				"Blue Springs" reserve				Nursery			
	Water		Sediment		Water		Sediment		Water		Sediment	
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Phenol	0.19 ± 0.038	–	–	202.1 ± 1.829	0.31 ± 0.072	0.65 ± 0.057	14.34 ± 1.375	223.7 ± 2.71	–	–	–	152.75 ± 2.70
4-methylphenol	–	–	–	7.92 ± 0.199	–	–	–	7.13 ± 0.268	–	0.03 ± 0.000	–	7.03 ± 0.176
2-chlorophenol	–	–	2.03 ± 0.181	–	–	0.31 ± 0.215	–	7.76 ± 0.370	–	–	–	–
2,4-dichlorophenol	–	–	17.24 ± 0.506	–	0.13 ± 0.093	–	49.5 ± 0.948	19.06 ± 0.140	–	–	21.50 ± 11.00	5.77 ± 0.518
2,4,6-trichlorphenol	–	–	3.92 ± 0.160	7.70 ± 0.645	–	0.05 ± 0.000	2.06 ± 0.129	119.2 ± 0.814	0.04 ± 0.037	0.12 ± 0.077	0.75 ± 0.506	–
Tetrachlorophenol	–	0.18 ± 0.056	35.30 ± 1.449	–	–	0.12 ± 0.052	5.00 ± 0.000	40.14 ± 0.305	0.05 ± 0.033	0.29 ± 0.077	1.00 ± 0.092	–
Pentachlorophenol	–	0.35 ± 0.114	–	–	–	0.07 ± 0.000	–	–	0.09 ± 0.067	0.31 ± 0.081		–
Guaiacol	25.23 ± 0.290	–	708.4 ± 3.111	125.87 ± 0.267	2.91 ± 1.924	–	476 ± 3.17	760.5 ± 1.073	0.09 ± 0.016	–	430 ± 2.278	–
4,6-dichloroguaiacol	–	–	2.05 ± 0.135	–	–	–	–	–	–	0.10 ± 0.056	–	–
Tetrachloroguaiacol	–	0.39 ± 0.233	–	–	–	–	–	–	–	0.43 ± 0.006	–	–
3-chlorosyringol	–	–	187.3 ± 5.45	–	0.59 ± 0.133	–	–	–	–	–	–	–
Trichlorosyringol	–	–	4.01 ± 0.092	419.5 ± 3.00	1.28 ± 0.855	–	110.6 ± 1.20	15.99 ± 0.126	–	–	19.75 ± 8.502	47.32 ± 1.886
4-chlorokatechol	–	0.36 ± 0.625	–	–	–	–	–	–	–	–	–	–
Trichlorocatechol	–	8.12 ± 5.358	–	–	–	–	–	–	–	–	–	–
5,6-dichlorovanilin	–	–	–	–	–	–	–	–	–	0.12 ± 0.033	–	–

Mean ± SD values were calculated for 3 individual samples of water or sediments. Samples were collected in spring and autumn

–, not detected

Table 2 Comparison of the concentrations (mg/kg) of phenolic compounds determined in mucous and tissue (dermato-muscular sacs) of *E. octoculata*, *T. tessulatum* and *G. complanata*

Compound	*E. octoculata*				*T. tessulatum*				*G. complanata*			
	Mucous		Tissue		Mucous		Tissue		Mucous		Tissue	
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Piasecznica River												
Phenol	0.03 ± 0.000	2.21 ± 0.185	0.67 ± 0.008	1.45 ± 0.074	0.84 ± 0.329	27.10 ± 0.637	0.46 ± 0.339	25.77 ± 0.569	0.75 ± 0.473	2.69 ± 0.000	0.52 ± 0.109	20.68 ± 1.239
4-methylphenol	–	–	–	–	–	–	0.57 ± 0.015	0.23 ± 0.000	0.09 ± 0.035	–	–	1.83 ± 0.140
2-chlorophenol	–	–	–	–	0.27 ± 0.189	1.23 ± 0.042	0.03 ± 0.000	–	0.04 ± 0.000	–	–	1.81 ± 0.208
2,4-dichlorophenol	–	–	0.84 ± 0.045	–	3.34 ± 2.177	14.90 ± 0.098	0.10 ± 0.044	0.49 ± 0.330	–	–	0.05 ± 0.000	–
2,4,6-trichlorphenol	–	–	–	0.40 ± 0.040	–	0.26 ± 0.090	–	1.25 ± 0.835	0.71 ± 0.118	–	0.05 ± 0.000	–
Tetrachlorophenol	0.03 ± 0.000	0.24 ± 0.168	–	–	–	–	–	1.25 ± 0.838	–	0.10 ± 0.000	–	1.83 ± 0.144
Pentachlorophenol	0.21 ± 0.032	–	–	–	–	–	–	–	0.11 ± 0.023	–	–	1.92 ± 0.067
Guaiacol	841.5 ± 6.568	19.22 ± 12.79	248.4 ± 3.46	–	6.75 ± 4.275	300.3 ± 1.312	6.40 ± 0.145	–	–	–	–	–
Tetrachloroguaiacol	–	–	–	–	–	0.21 ± 0.085	–	1.67 ± 0.037	–	–	–	1.98 ± 0.157
3-chlorosyringol	–	–	–	–	–	–	–	0.04 ± 0.000	–	–	–	–
4-chlorocatechol	6.68 ± 0.065	–	–	–	–	0.68 ± 0.126	–	–	–	–	–	–
Tetrachlorocatechol	–	–	–	–	–	–	–	–	0.40 ± 0.111	–	–	–
5,6-dichlorovanilin	–	–	–	–	–	0.27 ± 0.058	–	–	–	–	–	–
'Blue Spring' Natural Reserve												
Phenol	0.27 ± 0.076	–	1.78 ± 0.026	1.84 ± 0.045	1.87 ± 0.041	–	1.58 ± 0.017	–	1.13 ± 0.030	–	3.70 ± 0.020	–
2-chlorophenol	–	0.90 ± 0.116	0.20 ± 0.001	0.32 ± 0.020	–	0.37 ± 0.020	–	0.69 ± 0.020	0.18 ± 0.020	–	–	0.85 ± 0.010
2,4-dichlorophenol	0.14 ± 0.047	1.95 ± 0.213	1.32 ± 0.049	0.10 ± 0.011	–	0.63 ± 0.030	–	–	–	–	0.90 ± 0.030	0.21 ± 0.011
2,4,6-trichlorphenol	–	–	0.29 ± 0.025	0.37 ± 0.024	–	–	–	–	0.83 ± 0.020	0.28 ± 0.020	1.07 ± 0.020	0.25 ± 0.017
Tetrachlorophenol	–	0.41 ± 0.041	0.16 ± 0.026	0.27 ± 0.026	0.40 ± 0.017	–	0.43 ± 0.020	0.34 ± 0.036	–		–	–
Pentachlorophenol	0.66 ± 0.081	0.87 ± 0.134	–	–	–	–	0.46 ± 0.026		–	0.57 ± 0.030	3.27 ± 0.040	0.76 ± 0.036
Guaiacol	–	2,941 ± 41.2	–	1,189.8 ± 89.9	2.09 ± 0.015	0.84 ± 0.025	1.85 ± 0.030	0.27 ± 0.026	–	–	–	–
3-chlorosyringol	–	–	9.07 ± 0.24	15.28 ± 3.65	–	–	–	–	0.05 ± 0.000	–	–	–
4-chlorocatechol	–	23.24 ± 3.18	–	–	–	–	–	–	–	–	–	–
Trichlorocatechol	–	–	–	–	1.51 ± 0.020	–	–	–	–	–	–	–

Compound	*E. octoculata*				*G. complanata*			
	Mucous		Tissue		Mucous		Tissue	
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
A nursery								
Phenol	–	1. 73 ± 0.716	6.36 ± 0.170	0.98 ± 0.062	0.73 ± 0.080	0.43 ± 0.053	0.68 ± 0.053	2.45 ± 0.142
2-chlorophenol	0.06 ± 0.008	0.05 ± 0.000	0.15 ± 0.104	–	–	1.66 ± 0.080	–	0.06 ± 0.033
2,4,5-trichlorophenol	–	0.04 ± 0.015	–	–	–	–	–	0.07 ± 0.020
2,4,6-trichlorphenol	–	0.05 ± 0.008	–	–	–	–	–	0.07 ± 0.028
Tetrachlorophenol	–	0.09 ± 0.046	0.31 ± 0.207	0.56 ± 0.050	–	0.05 ± 0.016	0.04 ± 0.000	–
Pentachlorophenol	0.16 ± 0.047	9.20 ± 0.000	0.07 ± 0.046	–	–	–	–	–
Guaiacol	0.73 ± 0.111	1.18 ± 0.000	6.51 ± 0.000	0.22 ± 0.053	–	–	–	–
Tetrachloroguaiacol	–	–	–	–	–	–	0.06 ± 0.028	0.08 ± 0.060

Table 2 continued

Compound	*E. octoculata*				*G. complanata*			
	Mucous		Tissue		Mucous		Tissue	
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
3-chlorosyringol	–	0.20 ± 0.000	–	–	–	–	–	–
4-chlorocatechol	–	–	–	–	–	–	–	0.33 ± 0.065

Mean ± SD values were calculated for 6–7 specimens. Leeches were collected in the Piasecznica River, 'Blue Spring' natural reserve and a nursery in spring and autumn

–, not detected

Samples of water and fresh sediment (n = 3) were collected in spring (March) and autumn (October). Moreover, from 6 to 7 individuals of each leech species mainly of one-year-old (n = 6 or 7) were taken from each of the investigated ecosystems excluding the nursery site, in which no individuals of *T. tessulatum* were found. The individuals of *E. octoculata, G. complanata* and *T. tessulatum* were obtained from the littoral zone of reservoirs by viewing coastal, underwater and floating vegetation, branches, bark, shells of mollusks, and numerous discarded objects like tins, bottles, etc. The organisms were identified using Pawłowski keys (Pawłowski 1936). Samples of water, sediment and the specimens of leeches were taken at the same day in all the locations (ecosystems) studied.

Three samples of water in a volume of 1 L each were collected to brown borosilicate glass containers with Teflon-lined cap to analyze phenols. After the collection, 10 mL of methanol and 0.1 g of ascorbic acid were added to the samples immediately to inhibit oxidation processes and microbial development. Then, the containers were placed in ice (1–4°C) and transported to laboratory, in which they were stored for up 3 days at 4°C. In the laboratory, the internal standard solution (1.5 μg of 2,3,6-TCP dissolved in 1 mL of acetone) was added to 1 L water sample, followed by the addition of 200 g of sodium chloride and acidification with 10 % phosphoric acid to pH 2.0. Finally, the samples were mixed using a magnetic stirrer (750 rpm) for 30 min.

Wet sediment samples (30 g), after collection were transferred to brown borosilicate glass containers with Teflon-lined cap, which were stored in cold (1–4°C) during transport to laboratory. In the same day, they were dried at 37°C for 48 h. The extraction of phenolic compounds from sediments was performed by shaking 10 g of dried sample with two 20 mL volumes and then, two 10 mL volumes of methanol in the total time of 1 h. Sodium sulphite (1 g) was then added to the combined extracts to precipitate humus substances, which were then separated by filtration.

The study was conducted in accordance with national and institutional guidelines for the *protection of human subjects and animal welfare*. Living leeches were transferred to brown borosilicate glass containers (with Teflon-lined cap) containing water taken from individual ecosystem and transported in ice to laboratory. No mortality of the leeches occurred. Then, the leeches were anesthetized in ethanol. Mucus from the leeches was obtained by its extraction with three 5 mL volumes of ethanol, whereas the dermato-muscular sac was homogenized in three 5 mL volumes of methanol. The extracts obtained from the mucus and dermato-muscular sacs were filtered twice to obtain clear solutions.

Solid-phase extraction, sample derivatization, and analysis by gas chromatography–mass spectrometry were

Table 3 Comparison of bioaccumulation ratios of phenolic compounds determined in mucous and tissue (dermato-muscular sacs) of *E. octoculata*, *T. tessulatum* and *G. complanata* inhabited the Piasecznica River, 'Blue Springs' natural reserve and a nursery

Compound		*E. octoculata*								*T. tessulatum*								*G. complanata*							
		Spring				Autumn				Spring				Autumn				Spring				Autumn			
		Mucous		Tissue		Mucous		Tissue		Mucous		Tissue		Mucous		Tissue		Mucous		Tissue		Mucous		Tissue	
		Water	Sed	Water	Sed	Water	Sed	Water	Sed	Water	Sed	Water	Sed	Water	Sed	Water	Sed	Water	Sed	Water	Sed	Water	Sed	Water	Sed
Phenol	A	–	–	3,526	–	–	11	–	7	4,421	–	2,421	–	–	134	–	127	3,947	–	2,737	–	–	13. 3	–	102
	B	871	18.8	5,742	124	554	–	2,831	8.2	6,032	130	5,097	110	–	–	369	–	3,645	79	11,935	258	477	–	–	–
	C	–	–	–	–	–	11.3	–	6.4	–	–	–	–	–	–	–	–	–	–	–	–	–	2.8	–	16
Guaiacol	A	33.2	1,188	9.8	–	–	153	–	–	268	10	254	9	–	2,386	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	1,484	–	3,867	–	1,563	718	4.4	636	3.9	–	1.1	–	–	–	–	–	–	–	–	–	–
	C	8.1	1.7	72.3	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4-methylphenol	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	231
2-chlorophenol	A	–	–	–	–	–	–	–	–	–	133	–	–	–	–	–	–	–	20	–	–	–	–	–	–
	B	2,903	116	–	–	–	–	1,032	41.3	–	–	–	–	1,194	48	2,226	89	–	–	–	–	–	–	2,806	110
2,4-dichlorophenol	A	–	–	–	–	–	49	–	–	–	194	–	–	–	–	–	6	–	–	–	3	–	–	–	–
	B	1,077	2.8	10,154	27	–	102	–	5.2	–	–	–	–	–	33	–	–	–	–	6,923	18	–	–	–	11
	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2,4,6-trichlorophenol	A	–	–	–	–	–	–	–	–	–	–	–	–	–	34	–	163	–	182	–	–	–	–	–	–
	B	–	–	–	141	–	–	7,400	3.1	–	–	–	–	–	–	–	–	–	403	–	519	5,600	2.3	5,000	2.1
	C	–	–	–	–	417	–	–	–	–	–	–	–	–	–	–	–	–	–	583	–	–	–	–	–
Tetrachlorophenol	A	–	1,333	–	–	–	–	–	–	–	–	–	–	–	–	6,944	–	–	–	–	–	556	–	10,176	–
	B	–	–	–	32	4,100	10.2	1,350	6.7	–	80	–	86	–	–	2,833	8.5	–	–	–	–	–	–	–	–
	C	–	–	6,200	413	310	–	–	–	–	–	–	–	–	–	–		–	–	800	40	172	–	–	–
Pentachlorophenol	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5,486	–
	B	–	–	–	–	1,243	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8,143	–	10,857	–
	C	1,778	–	778	–	9,677	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4-chlorocatechol	A	–	–	–	–	–	–	–	–	–	–	–	–	1,889	–	–	–	–	–	–	–	–	–	–	–
4,6-dichloroguaiacol	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	24	–	–	–	–
Tetrachloroguaiacol	A	–	–	–	–	–	–	–	–	–	–	–	–	539	–	4,284	–	–	–	–	–	–	–	5,077	–
	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	186	–
3-chlorosyringol	B	–	–	15,373	–	–	–	–	–	–	–	–	–	–	–	–	–	85	–	–	–	–	–	–	–

–, no data; A, Piasecznica River, B, 'Blue Springs' natural reserve; C, a nursery

conducted according to previously published procedures (Michałowicz et al. 2011).

Statistical analysis was based on calculations carried out using the STATISTICA package (2000 StatSoft, Inc., Tulsa, OK, USA). Variables were log-transformed ($\log_{10}(x + 1)$), when necessary. Pearson correlation was calculated to determine the interdependence between phenolic compounds content in the environment (sediment, water) and their concentrations in mucous and tissue of the leeches.

Results and Discussion

Bioindicators function through tissue uptake of contaminants in amounts significantly higher than that present in aquatic ecosystems. Moreover, these organisms are effective time integrators of pollutant levels, since they constantly sample the ambient environment (Prahacs and Hall 1996). It has been proven that leeches may be useful bioindicators. For instance, Metcalfe and Hayton (Metcalfe et al. 1989) observed that a leech *Nephelopsis obscura* was able to bioaccumulate chlorophenols at levels 1–2 orders of magnitude greater than a frequently used biomonitoring organism, the freshwater mussel *Elliptio complanata*.

Our results showed that in the Piasecznica River the concentrations of phenolic substances ranged from 0.18 µg/L for TeCP to 25.23 µg/L for guaiacol; and in sediment from 2.03 µg/kg for 2-CP to 708.4 µg/kg for guaiacol. In the water of 'Blue Springs' reserve, concentrations ranged from 0.05 µg/L for 2,4,5-TCP to 2.91 µg/L for guaiacol, while in the sediment, they ranged from 2.06 µg/kg for 2,4,6-TCP to 760.5 µg/kg for guaiacol. The lowest concentrations of phenols were determined in a nursery adjacent to the 'Blue Springs'. Here, the concentrations ranged from 0.03 µg/L for 4-methylphenol to 0.43 µg/L for TeCG, while in the sediment, they ranged from 0.75 µg/kg for 2,4,6-TCP to 430 µg/kg for guaiacol (Table 1).

It was observed that the leeches studied bioaccumulated several chlorophenols including 2-CP, 2,4-DCP, 2,4,6-TCP, TeCP, PCP as well as some of their derivatives. High concentrations of phenols including PCP (mucous—0.16–9.20 mg/kg), 2,4-DCP (tissue—0.10–1.32 mg/kg; mucous—0.14–1.95 mg/kg), TeCP (tissue—0.31–0.56 mg/kg; mucous—0.03–0.27 mg/kg), 4-CC (mucous—6.68–23.24 mg/kg) and 3-CS (tissue—9.07–15.28 mg/kg) were detected in the specimens of *E. octoculata*. Moreover, considerable concentrations of 2,4-DCP (tissue—0.10–0.49 mg/kg; mucous—0.63–14.90 mg) and TeCG (tissue—0.21 mg/kg; mucous—1.67 mg/kg) were determined in the specimens of *T. tessulatum*, while notable amounts of PCP (tissue—0.76–3.27 mg/kg; mucous—0.11–0.57 mg/kg), 2,4,6-TCP (tissue—0.05–1.07 mg/kg; mucous—0.28–0.83 mg/kg), TeCP (tissue—0.04–1.83 mg/kg) and TeCG (tissue—0.06–1.98 mg/kg) were found in the specimens of *G. complanata* (Table 2).

Bioaccumulation ratios were calculated for the above phenols based on their simultaneous concentrations in leech tissues and water and/or sediments. The ratios were: 2-CP (41.3–116 for sediments; 1,032–2,903 for water), 2,4-DCP (1.4–102 for sediments; 1,077–10,154 for water), 2,4,6-TCP (2.1–519 for sediments; 417–7,400 for water), TeCP (1.4–1,333 for sediments; 172–10,176 for water), PCP (788–29,677 for water), TeCG (186–5,077 for water), and 3-CS (85–15,373 for water). Efficient bioaccumulation of phenols was additionally confirmed by statistically significant correlations between their content in the environment (water or sediment) and in the mucous or dermato-muscular sac of the leeches studied (Table 4).

These findings are in agreement with results obtained by Metcalfe et al. (1984, 1988), who observed effective bioaccumulation of chlorophenols by the leeches *D. dubia*, *Helobdella stagnalis* and *E. punctata*. They suggested that the high body burdens in the leeches might have been associated with a very slow elimination of di-, tri- and tetrachlorophenols from their bodies, which had half-lives of 25–40 days. In contrast, Ernst (1979) observed a fast elimination rate of PCP in the mussel, *Mytilus edulis*, with a half-life of 2–3 days.

It is also known that fate and transport of a chemical compound in the natural environment depends on its partition coefficient (K_{ow}) value in the octanol–water system. It was proven that K_{ow} values of phenols are associated with their chlorine number, and thus strongly determine accumulation of these substances in living organisms (Czaplicka 2004). We observed that almost all bioaccumulation ratios calculated for PCP were high, which may be connected with high log K_{ow} value (5.01–5.86) of this substance. Similarly, high bioaccumulation ratios found for TeCP may be connected with relatively high K_{ow} value (4.21–5.16) of this phenol (Czaplicka 2004). For dermato-muscular sac tissue, there was an increase in bioaccumulation ratio with chlorination (with the exception of 2,4-DCP). The mean bioaccumulation ratios from water for all three leech species combined were 2,021, 4,328, 4,717 and 5,707 for 2-CP, 2,4,6-TCP, TeCP and PCP, respectively. Efficient bioaccumulation of 2,4-DCP and 2,4,6-TCP by the leeches examined may be additionally connected with relatively high dissociation constant (pK_a) values of these substances (2,4-DCP 7.5–8.1; 2,4,6-TCP 6.0–7.4). It was proven that high pK_a value determines the existence of phenol in water mainly in un-ionized, biologically available form (Jones 1981). For instance, Ernst and Weber (1978) found high bioaccumulation ratios for low chlorinated isomers of phenols in the polychaete *Lanis conchilega*, and explained these results by relatively high pK_a values of these compounds.

Table 4 Pearson correlation calculated to determine the interdependence between the content of phenolic compounds determined in the environment (sediment, water) and mucous or dermato-muscular sac of the leeches in spring and autumn

Name of the species	Spring		Autumn	
	Water	Sediment	Water	Sediment
E. octoculata				
Mucous	Phenol**, 2,4-DCP***, Guaiacol***	Phenol***, 2,4-DCP***, Guaiacol***	Phenol***, 2-CP***, 2,4,6-TCP***, TeCP*	2-CP***, 2,4-DCP***, TeCP*, Guaiacol***
Tissue	Phenol*, 2,4-DCP*	Phenol***, 2-CP***	Phenol**, 2-CP***, 2,4,6-TCP***, TeCP*	Phenol***, 2-CP***, 2,4-DCP***, Guaiacol***
T. tessulatum				
Mucous	2,4-DCP*, Guaiacol***	Phenol***, 2-CP*** 2,4-DCP***, TeCP***, Guaiacol***	Phenol***, 2-CP***, TeCG***	Phenol***, 2-CP***, 2,4-DCP***, 2,4,6-TCP* Guaiacol***
Tissue	Guaiacol***	Phenol***, 2,4-DCP*, TeCP***, Guaiacol***	Phenol***, 2-CP**, TeCG***	Phenol***, 2-CP***, 2,4-DCP***, 2,4,6-TCP**, TeCP***, Guaiacol***
G. complanata				
Mucous	Phenol***, 2,4,6-TCP***	Phenol*, 2,4,6-TCP*	PCP***	2,4,6-TCP***, TeCP**
Tissue	Phenol*, 2,4-DCP***	Phenol***, 2,4-DCP***	Phenol**, TeCG***	2,4-DCP***, 2,4,6-TCP***, Guaiacol*

Significance level of the correlation coefficient: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

2-CP 2-chlorophenol, *2,4-DCP* 2,4-dichlorophenol, *2,4,6-TCP* 2,4,6-trchlorophenol, *TeCP* tetrachlorophenol, *PCP* pentachlorophenol, *TeCG* tetrachloroguaiacol

Phenol and 2-methoxyphenol (guaiacol) are widely spread both in natural and polluted environments (Kjallstrand et al. 2000; Michałowicz et al. 2008). However, bioaccumulation of these substances by leeches had not been previously reported. We determined high concentrations of phenol (tissue—0.67–6.36 mg/kg; mucous—0.03–0.21 mg/kg) and huge amounts of guaiacol (tissue—0.22–1,189.8 mg/kg; mucous—0.73–2,941 mg/kg) in the specimens of *E. octoculata* as well as very high concentrations of phenol (tissue—0.46–25.77 mg/kg; mucous 0.84–27.10 mg/kg) and guaiacol (tissue—0.27–6.40 mg/kg; mucous 0.84–300.3 mg/kg) in the specimens of *T. tessulatum* (Table 2). Moreover, we have calculated high bioaccumulation ratios and statistically significant correlations for phenol (2.8–134 for sediments; 2,421–11,953 for water) and guaiacol (1.1–3,867 for sediments; 8.1–718 for water).

It should be noted that the high bioaccumulation ratio (tissue/water) calculated for phenol in spring may be due to its low concentrations determined in water in that season (Table 1). Taking into consideration that phenol has good solubility in water (that determines its high mobility in aquatic ecosystems) and it is also degraded relatively fast in aquatic environments (US EPA 2002), its content could possibly fluctuate more significantly than in the case of chlorophenols, and often may have been higher than that detected in our study. As a consequence, calculated values of bioaccumulation ratios for phenol may be overestimated.

Toxic concentrations of phenols for invertebrates differ greatly with phenol structure and the species of exposed animal. For instance, TCP was toxic for cladocerans at 2 μg/L (Caceres et al. 2007). On the other hand, chlorophenols were toxic to the worm *Lumbricus variegatus* at higher concentrations ranging from 0.14 to 12 mg/L (Kukkonen 2002). Moreover, it was shown that phenol is toxic to various invertebrate species at concentrations ranging from 15.5 to 780 mg/L (Green et al. 1985). In another study, phenol was toxic for the leech *Hirudinea medicinalis* only at the very high concentration of 290 mg/L, (48-h test, LC_{50}) (Bengston and Henshel 1996). The above results suggest that the measured concentrations of phenols determined in the studied ecosystems should not have affected the viability of the leeches or their vital functions, including the uptake and depuration of phenolic compounds.

It has been revealed that chlorinated organics are bioconcentrated in solid tissues of various aquatic organisms, which is mainly connected with their dense structure and the presence of lipids facilitating dissolution of various xenobiotics (Egeler et al. 2001; Moremod et al. 2007). It is also known that some aquatic invertebrates including

leeches secrete mucous to protect themselves against attack by pathogens (Lent 1974). This mucous coat becomes the first target for contact with substances in water or sediment. Our results showed that all of the leech species efficiently accumulated phenolic substances in the mucous, which was confirmed by calculation of numerous bioaccumulation ratios (Table 3) and statistically significant correlations between concentration of phenols in the mucous with concentrations in either water or sediment (Table 4).

In conclusion, this study revealed that the investigated leech species, e.g., *E. octoculata*, *T. tessulatum* and *G. complanata* bioaccumulated phenol, guaiacol and chlorophenols that were present in their aquatic environments. These compounds were found to bioaccumulate in both the dermato-muscular sac tissue and the external mucous coat of the leeches.

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