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Original Research

Uptake of Polychlorinated Biphenyl Congeners in Freshwater Mussels *Anodonta complanata* from the Lower Odra River

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Abstract

The content of polychlorinated biphenyls (PCBs) in aquatic organisms reflects a degree of pollution in the environment in which they live. In the present study, the concentration of PCB congeners was examined in freshwater bivalve molluscs (*Anodonta complanata)*, bottom sediments and water from the lower Odra River. The bioconcentration factors (BCF) were lower than expected by about 30%. The BSAF in the mussel tissues exceeded the expected theoretical value (2.4) and ranged from 3.1 to 5.3. Mean PCB concentration in the mussels was highly correlated with the concentrations of these compounds in bottom sediments (r=0.82) and water (0.76).

Keywords: polychlorinated biphenyls (PCB), fresh-water mussels, BSAF, BCF

Introduction

The presence of polychlorinated biphenyls (PCBs) in the aquatic environment constitutes a severe threat for the hydrosphere due to their persistence, toxicity and bioaccumulation ability[1-3]. These compounds reach water reservoirs and watercourses mostly with municipal sewage and industrial wastes, with effluents from municipal landfill sites and post-process waste sites, as well as a result of air deposition [4]. Hydrobiota accumulate PCBs both from water, sediments and food, which leads to biomagnification of these compounds in successive links of the food chain, creating a risk for organisms at the top levels of the trophic pyramid [5]. It is assumed that PCBs are concentrated by passive diffusion and the rate of this process depends on concentration in the environment, being generally higher for hydrophobic pollutants [6]. A number of models were developed for assessing and forecasting a degree of bioaccumulation and bioconcentration of organic pollutants. The most well-known and frequently applied model for examining the fate of chemicals in aquatic ecosystems is the equilibrium partitioning theory (EPT) [6, 7]. This theory assumes that concentrations of a given substance in an organism reach maximum levels when its thermodynamic activity becomes equalized in the organism and the surrounding medium, e.g. in water and bottom sediments (a functional equilibrium state) [8]. The partitioning of compounds between the organism and the components of environment in which it lives is described by the bioconcentration factor (BCF, ratio PCBs in biota to PCBs in water) and biota-sediment accumulation factor (BSAF, ratio PCBs in biota to PCBs in sediment).

The Odra River is one of larger rivers of Europe and is the second largest river in Poland (total length 741.9

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km). Since 1993 the area called the Międzyodrze (Odra River Marsh; Oderbruch) has been under legal protection as the Lower Odra River Valley Landscape Park. It is the largest lowland bog in Europe and contains unique flora and fauna. The introduction of human sewage with differing degrees of effluent treatment into the river has had an adverse influence on the water quality of the Odra River and its biocenosis [9]. Pollution of the lower Odra River results both from the localization of large urban-industrial agglomerations in the river basin and the influx of considerable pollutant loads in its upper and middle sections, (i.e. from the heavily industrialized areas of the Czech Republic and Germany as well as Upper and Lower Silesia). Studies examining polychlorinated biphenyls in the Odra River estuary have been carried out since the 1970s [10-12]. Notwithstanding the restrictions being in force from several years and even total prohibition of using PCBs in some countries, these compounds are still found in the environment in substantial quantities. It is considered that from among synthetic compounds, the PCBs – as highly persistent – prevail in aquatic ecosystems [13, 11].

The aim of the present study was to determine bioconcentration factors (BCF) and biota-sediment accumulation factors (BSAF) of selected PCB congeners (PCB 28, 52, 101, 118, 138, 153, 180) based on their measured concentrations of these compounds in bottom sediments, water and freshwater mussels (*Anodonta complanata*) from the lower Odra River.

Experimental Procedures

The analyses included bottom sediments, water and soft tissue of mussels (*Anodonta complanata*) sampled from the lower section of the Odra River. Bottom sediments, water and mussels were collected in February, May, August and October 2001 from 5 sites. Sample collection sites are presented in Fig. 1.

Bottom Sediments

Bottom sediments were sampled with a Van Veen dredge and placed into glass containers. Desiccated at room temperature, sediments were ground in a mortar and passed through a 2 mm sieve. 30-g sediment samples were taken for analyses. The chlorobiphenyls were extracted according to the modified method of Cicero et al. [14]. The concentrated extracts were purified with 7% SO_3 in concentrated H_2SO_4, and then on 1 gram Florisil in 8 cm^3 LiChrolut® glass columns. Polychlorinated biphenyls adsorbed on Florisil were rinsed with a mixture of n-hexane and acetone (9:1) (v/v) (Restek Environmental Applications Note #59562). Organic carbon in bottom sediments were measured with Tiurin's method (www.ar.wroc.pl/~weber/an-c.htm).

Water

Surface water samples (10 dm^3 of each sample collection point) were collected into 2.5 dm^3 dark-glass bottles, containing 300 cm^3 n-hexane. The extraction of analyzed compounds was carried out in tap-funnels using n-hexane. The extracts were dried with anhydrous sodium sulfate (Na_2SO_4), concentrated in a rotary vacuum evaporator at 50°C up to 2 cm^3. Next, the extract was purified with 6 cm^3 fuming sulfuric acid (7% SO_3 in concentrated H_2SO_4). After separation of layers, the upper layer (n-hexane) was transferred quantitatively into 10 cm^3 test-tubes, then rinsed thrice with deionised water and dried with anhydrous Na_2SO_4 in 8 cm^3 LiChrolut® glass columns.

Freshwater Mussels

For analyses of mussel tissue, 7-gram pieces were sampled. The samples were spiked with 50 µl internal standard (Pesticides Surrogate Spike Mix) in 80 $\mu g \cdot dm^{-3}$ concentration, the whole lot ground in a mortar with anhydrous Na_2SO_4 to obtain a homogenous mass. The sample was then transferred quantitatively into an Erlenmeyer flask (300 cm^3) with glass ground-in stopper and extracted together with lipids with 50 cm^3 acetone and n-hexane mixture in 2.5:1 (v/v) and re-extracted in 50 cm^3 n-hexane and diethyl ether mixture (9:1). The obtained extracts were concentrated in a rotary vacuum evaporator at 50°C up to c. 2 cm^3 and transferred quantitatively with n-hexane to 10 cm^3 dried and weighed test-tubes with glass ground-in stoppers. To determine the percentage of lipids, the solvent was evaporated under a stream

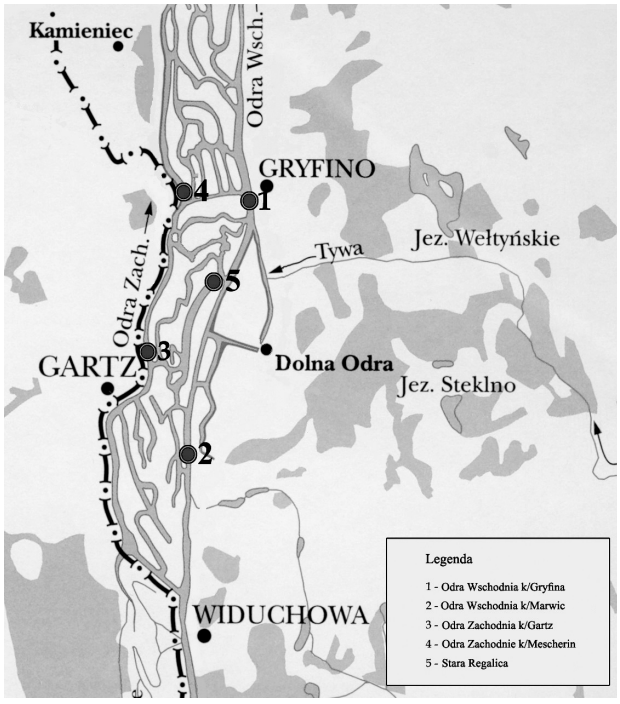

Fig. 1. Location of sample collection sites.

of nitrogen (N_2), and the residue was dried at 80°C to a solid mass. After determining the lipids mass, the content of test-tubes was dissolved in 2 cm^3 n-hexane and purified by adding 6 cm^3 fuming sulfuric acid (7% SO_3 in concentrated H_2SO_4). After separation of layers, the upper layer (n-hexane one) was rinsed with deionised water and dried with anhydrous Na_2SO_4.

Chromatographic Analysis

The extracts were concentrated with a dry nitrogen gas blowdown (N_2) to 0.5 cm^3 and submitted to gas-liquid chromatographic separation with the method of capillary gas chromatography with mass spectrometry in GC MSD HP 6890/5973. The accuracy of analyses was verified with the addition of internal standard Pesticides Surrogate Spike Mix, Supelko. The recovery of analyzed compounds in mussels, water and bottom sediments was: 71-90%, 73-82% and 72-91%, respectively. All determinations were conducted in triplicate, and the presented results are arithmetic means of the replicates.

The BSAF (biota-sediment accumulation factors) and BCF (bioconcentration factors) were calculated according to the following equations [17, 6]:

$$BSAF = \frac{[PCB]_{organism\ (lipids)}}{[PCB]_{sediment\ (C_{org})}}$$

$$BCF = \frac{[PCB]_{organism\ (lipids)}}{[PCB]_{water}}$$

Based on the equilibrium partitioning theory (EPT), the bioaccumulation (BSAFs) and bioconcentration (log BCFs) factors were estimated for each PCB congener. The calculations were made using the following relationships [18, 17, 6]:

$$BCF = K_{ow}$$

$$BASF = \frac{BCF}{K_{OC}}$$

$$K_{OC} = 0.41 \cdot K_{OW}$$

where: K_{OC} – is the organic-carbon normalized sediment/water distribution coefficient.

The expected BSAF level for all analysed PCB con-

Table 1. Mean level of selected PCB congeners in bottom sediments, water and freshwater mussels (n = 60).

Sample collection point	Sediments, µg/kg d.m.						
	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180
1.	0.166±0.017	0.301±0.053	0.331±0.086	0.409±0.067	0.391±0.066	0.465±0.051	0.552±0.060
2.	0.523±0.041	0.529±0.100	0.747±0.269	0.665±0.109	0.776±0.182	0.758±0.274	0.900±0.326
3.	1.013±0.078	1.107±0.177	1.652±0.262	1.452±0.200	1.419±0.526	2.129±0.557	2.528±0.662
4.	1.182±0.202	1.225±0.184	1.656±0.636	1.722±0.256	1.550±0.200	2.218±0.945	2.634±0.123
5.	1.741±0.476	2.037±0.703	2.691±0.548	2.404±0.553	2.084±0.225	3.185±0.466	3.782±0.553
Sample collection point	Water, ng/dm^3						
	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180
1.	0.209±0.016	0.212±0.040	0.187±0.067	0.166±0.027	0.194±0.046	0.189±0.069	0.225±0.081
2.	0.249±0.025	0.452±0.079	0.364±0.095	0.450 ±0.073	0.431±0.072	0.155±0.017	0.184±0.020
3.	0.405±0.031	0.443±0.071	0.413±0.066	0.363±0.050	0.355±0.131	0.532±0.139	0.632±0.165
4.	0.355±0.061	0.368±0.055	0.370±0.107	0.344±0.051	0.310±0.040	0.444±0.189	0.527±0.225
5.	0.348±0.100	0.407±0.144	0.538±0.103	0.481±0.099	0.417±0.016	0.319±0.049	0.378±0.059
Sample collection point	Mussels, µg/kg lipids						
	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180
1.	2.149±0.132	3.916±0.689	4.043±1.047	5.074±0.829	5.040±0.848	5.698±0.623	6.856±0.749
2.	4.252±0.321	4.799±0.766	6.816±1.030	6.008±0.828	6.091±2.256	8.692±2.276	10.459±2.739
3.	5.380±0.997	5.818±0.853	7.266±2.792	7.757 ±1.103	7.153 ±0.905	9.752±4.085	11.675±4.977
4.	6.597±0.518	6.878±1.300	9.206±3.282	8.251±1.359	9.986±2.347	9.625±4.033	11.169±4.042
5.	6.466±1.766	7.919±2.940	9.650±2.079	8.776±2.019	7.891±0.852	11.475±1.678	13.808±2.020

geners is predicted to be 2.4. On the other hand, log BCF value is equal to partition coefficient n-octanol/water (log K_{OW}) and should equal 6.5 to 7.0.

Results and Discussion

The results of PCB congener concentrations in bottom sediments are presented in Table 1. The determined concentrations differed considerably. The highest degree of pollution found in the sediments was from the Stara Regalica River (Old Regalica) (sampling site 5), in which the level of analyzed compounds ranged from 1.741 ± 0.476 µg/kg d.w. (PCB 28) to 3.782 ± 0.553.µg/kg d.w. (PCB 180), whereas the lowest concentration in the sediments was sampled near Gryfino (sampling site 1). In the latter case, the analyzed PCB concentration averaged from 0.166 ± 0.017 µg/kg d.w. in the case of PCB 28 to 0.552 ± 0.060 µg/kg d.w. in the case of PCB 180. It was also observed that bottom sediments from the Odra Zachodnia River (Western Odra) (sampling sites 3 and 4) were more polluted than those from the Odra Wschodnia River (Eastern Odra). This finding is in agreement with the studies of Protasowicki *et al.* [12] and Ciereszko et al. [10].

A factor influencing the concentrations of PCBs in bottom sediments was their organic matter content. It was found that sediments rich in organic matter (muddy deposits) accumulated more PCB than sandy ones (r = 0.80 – 0.86) (Fig. 2). This relationship has also been confirmed in earlier studies [18, 12, 17].

Polychlorinated biphenyls accumulated in sediments may be resuspended into water [19]. When comparing the PCB levels in water with those in bottom sediments from the Odra River we found that the concentrations in water were significantly lower than those in bottom sediments. The ratio of analyzed PCB congeners in sediments (µg·kg^{-1} d.w.) to their concentration in water (ng·dm^{-3}) was approximately 10^3:1. The obtained results correspond with the study of Wolska et al. [20].

Our study showed that Odra River water pollution was characterized by low area differentiation. The highest concentration of PCB 28, PCB 52, PCB 153 and 180 was found in the water sampled near Gartz (sampling site 3): 0.405 ± 0.031, 0.443 ± 0.071, 0.532 ± 0.139 and 0.632 ± 0.165 ng/dm^3, respectively. Conversely, the lowest PCB concentrations were found in the Odra Wschodnia River (Eastern Odra) near Gryfino (sampling site 1), in which the average level of analyzed congeners ranged from 0.166 ± 0.027 ng/dm^3 (PCB 118) to 0.225 ± 0.081 ng/dm^3 (PCB 180).

The water flowing throughout the mollusc's gills is a source of both nutrients and potentially harmful compounds, including PCBs [21, 22]. The average PCB levels in the mussels was highly correlated with the content of these compounds in bottom sediments (r = 0.82) and water (0.76). The predominant compounds in mussel tissues were PCB 180 and 153. Depending on the sample collection point, the concentration of these congeners was 6.856 ± 0.749 to 13.808 ± 2.020 µg/kg lipids and 5.698 ± 0.623 to 11.475 ± 1.678 µg/kg lipids, respectively. Statistical analysis showed that the mussels sampled near Gryfino (sampling site #1) were characterized by significantly lower levels of all examined compounds ($p \leq 0.05$) than those sampled from other sample collection points. The average PCB concentrations ranged from 2.149 ± 0.132 µg/kg lipids (PCB 28) to 6.856 ± 0.749 µg/kg lipids (PCB 180). Conversely, the levels of analyzed congeners determined in the mussels sampled from the Stara Regalica River (Old Regalica) and the Odra Wschodnia River (Eastern Odra) were higher: 5.380 ± 0.997 µg/kg lipids (PCB 28, sampling site 3) to 13.808 ± 2.020 µg/kg lipids (PCB 180, sampling site #5).

The calculated BCFs, expressed by a logarithm from the ratio of PCBs in mussel tissue to PCBs in water, helped to estimate their bioconcentration degree in particular exposure conditions. The obtained BCF values are presented in Fig. 3. Based on our results, these values are lower (by 30) than those expected based on the partition coefficient n-octanol/water (K_{OW}) calculat-

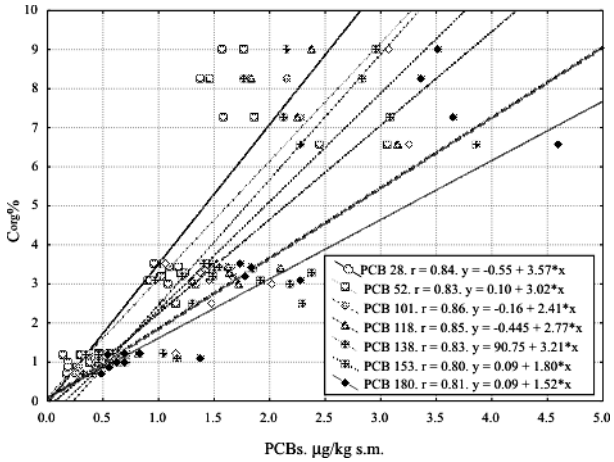

Fig. 2. Relationship between the PCBs concentration and content of organic carbon (C_{org}) in bottom sediments.

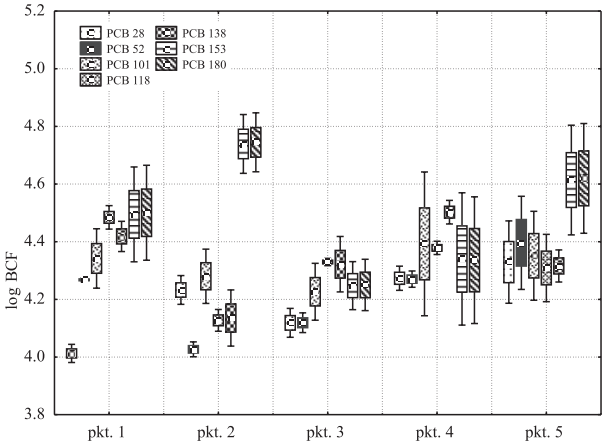

Fig.3. Mean values of PCB bioconcentration factors (BCFs) in tissues of fresh-water mussels (*Anodonta complanata*).

ed for each congener. Wang et al. [23] found that BCF values calculated based on K_{OW} may be lower or higher from the values obtained in studies, and there is no simple explanation of this fact. Thomann [19] suspects the reason of these deviations is due to lipid content differences which result from the growth of organism, seasonal changes in diet composition and different stages of sexual maturity.

The highest bioconcentration factors were obtained for the highest chlorinated congeners, i.e., for PCB 153 and PCB 180. The average value of log BCF was at the level of 4.25 ± 0.08 (PCB 153, sampling point 3) to 4.74 ± 0.10 (PCB 153, sampling point 2) and 4.24 ± 0.04 (PCB 180, sampling point 3) to 4.38 ± 0.05 (PCB180, sampling point 1).

Furthermore, it was observed that the mussels sampled near Gryfino (sampling point 1) were characterized by significantly lower bioconcetration factors for PCB 28 than those sampled in other sample collection points.

The BSAF obtained for all analyzed compounds were higher than their expected level (2.4) (Fig. 4). In the mussels from the Odra Wschodnia River (sampling points 1 and 2) and the Stara Regalica River, the highest bioaccumulation factors were found for PCB 28 and PCB 52, respectively: 3.25 ± 0.17 to 4.71 ± 0.44 and 3.32 ± 0.09 to 4.67 ± 0.30.

However, these values were considerably lower than the maximum that was found in mussels from sample collection point 4 (Mescherin), in which the bioaccumulation factor for PCB 138 averaged 5.32 ± 0.52. High BSAF values were also obtained for the remaining congeners, ranging from 3.92 ± 1.67 to 4.76 ± 0.94.

Conclusions

1. The presence of analyzed polychlorinated biphenyls was documented in all components of aquatic environment examined.
2. Sediments rich in organic matter accumulated larger amounts of PCBs than the sandy sediments. The highest degree of pollution was found in sediments from the Stara Regalica River, and the lowest was in those from the Odra Wschodnia River near Gryfino.
3. The calculated bioconcentration factors (log BCF) of select PCB congeners were lower than the expected values calculated based on equilibrium partitioning theory (EPT) by about 30%.
4. The bioaccumulation factors (BSAF) of the analyzed compounds in mussel tissue exceeded considerably the expected theoretical level (2.4) and ranged from 3.09 ± 0.09 to 5.32 ± 0.52.
5. The highest PCB bioaccumulation factors were found in mussels from the Odra Zachodnia River (sampling point 4), and the lowest in those from the Odra Wschodnia River (sampling point 1).

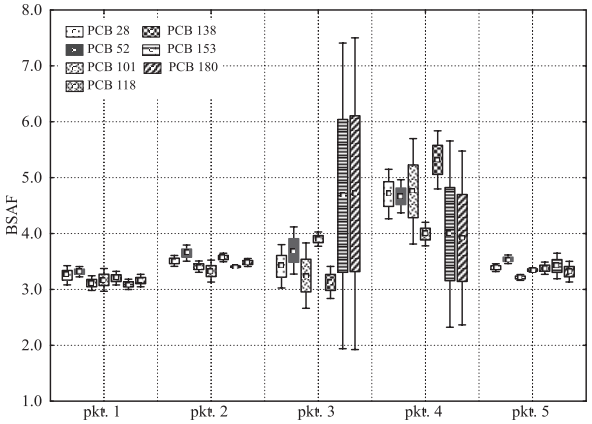

Fig. 4. Mean values of PBC bioaccumulation factors in soft tissues of freshwater mussels (*Anodonta complanata*).

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