

Concentrations of heavy metals and PCBs in the tissues of European beavers (*Castor fiber*) captured in northeastern Poland

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Abstract European beavers (*Castor fiber*) from two regions were examined to identify exposure to persistent environmental contaminants. A reference group was comprised of six animals from the Forest Division of Srokowo, and an exposed group was comprised of five animals from the vicinity of a former military airport operated in 1918–1986—both from Warmia land in Poland. ^{137}Cs in beavers' muscles was considered negligible for the overall condition and reproduction of the animals. Elevated levels of Cd and Pb occurred in the kidneys and liver of beavers from a former military airport area. dl-PCBs were found in the liver of all

specimens, and pCB no. 118 was a dominant compound. Beavers as animals living both in water and land habitats can be good bioindicators of heavy metal environmental pollution caused by human activity. Despite the significant contamination of former military sites in northeastern Poland, the beaver population shows an upward trend, reflecting the excellent adaptation of this species to a changing environment.

Keywords European beaver (*Castor fiber*) · Heavy metals · Planar PCBs

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Introduction

The global economic development leads to environmental pollution with numerous persistent compounds while exposure to some of them, e.g., heavy metals (Cd, Hg, Pb, Cu, Zn, Cr), continues worldwide. Production and use of certain toxic and persistent organic compounds, e.g., polychlorinated biphenyls (PCBs), were banned (Falandysz 2007). The toxic impacts of heavy metals and persistent organic compounds relate both to acute and chronic effects and those that are chronic are more common (Coggins et al. 2006).

Animal populations reflect the health of the overall environment and are used as bioindicators while evaluating food safety (Food and Drug Administration 1993). Both domestic and wild species, including raptors and big game animals, have been successfully used as bioindicators of environmental pollution (Corsolini et al. 2000; Jorhem et al. 1991; Pokorny and Ribaric-Lasnik 2009; Szkoda and Żmudzki 2001; Szymczyk and Zalewski 2001).

Warmia and Mazury land in the northeastern region of Poland is largely a farmland, forestry, and great lakes region

that is away from heavy industry and urbanization. Nevertheless, due to the past use of organochlorine pesticides in agriculture and/or global atmospheric circulation, the compounds such as HCBs, DDTs, HCHs, CHLs remain as ultra-trace contaminants detected in wildlife and foods (Falandysz and Kannan 1992). Plant biomass from forests in Poland contains certain amounts of highly toxic and persistent PCBs, polychlorinated naphthalenes, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans due to local and global sources (Bochentin et al. 2007; Falandysz et al. 2012; Orlikowska et al. 2009).

Cd, Cu, and Zn were considered as risk factors to certain beaver (*Castor fiber*) populations in Europe (Fimreite et al. 2001; Nolet et al. 1994). In Polish beavers, some perfluorinated compounds were found as contaminants (Falandysz et al. 2007), but knowledge on their exposure and risk to many popular environmental contaminants is lacking.

After World War II, the beaver was a rare species in Europe. An increase in the beaver population size has been noted since the 1970s due to effective reintroduction and protection. Other factors that contributed to the abundance of beavers in Poland include a decrease in consumer demand for fur products and the construction of industrial and municipal wastewater treatment plants, which improved environmental quality. Today, there are approximately 2,230 beaver colonies in northeastern Poland. The total beaver population in this region, estimated at around 7,500–8,000 animals, is the largest in the country.

The main aim of this study was to assess exposure to Cd, Pb, Zn, and Cu and dioxin-like PCBs (dl-PCBs) and radiocesium (^{137}Cs) of European beavers inhabiting the “Beaver Mainstay” reserve that is under the direct influence of surface and groundwater inflowing from a contaminated former military airport (operated in 1918–1986) area in Warmia land in northeastern Poland.

Materials and methods

Animals

Two groups of beavers of reproductive age were captured between 20 February and 5 March 2003 in the district of Olsztyn. One group (exposed animals) consisted of three males and two females from the Beaver Reserve on the Pasłęka river, situated in the close neighborhood of a former military airport and located at 54°06' N, 20°07' E. A second (reference) group consisted of six males captured in the Forest Division of Srokowo near the town of Kętrzyn. The number of animals available for research by license was relatively low because beavers are strictly protected and are listed in the Red Book of Threatened Species of Poland (IUCN 2008). Beavers were captured during the day using

nets and in accordance with the legal regulations and permissions issued by the local Environmental Conservator (OSR/OIII/6636/16/2002) and the local Animal Ethics Committee (1/N/04.01.2002). The animals were anesthetized with i.m. injection of 2.5 ml xylazine (Xylazine 10%, Biowet Puławy, Poland) at 2.5 h after capture. After 10 min, they were administered i.m. with 1 ml kethamine (Bioketan, Biowet Gorzow Wielkopolski, Poland). The animals were weighed on an electronic scale within an accuracy of 0.01 kg, and the following measurements were taken: body length from the tip of the nose to the end of the tail, the length and width of the head, the circumference of the thorax, and the length and width of the tail. The animals were bled and decapitated under full anesthesia. Twenty-gram samples of the biceps femoris muscle were taken with a scalpel. Liver samples of similar weight and the kidneys were collected immediately postmortem. The reproductive organs of the beavers were also collected for a fertility analysis described elsewhere (Bierła 2007).

Chemical analysis

Heavy metals

Samples were weighed (about 1 g) in PTEE (Teflon) vessels, and 5 ml of concentrated HNO_3 was added. After predigestion at room temperature overnight, the samples were digested in closed vessels in an Automatic Microwave System. Then, the samples were transferred to a 50-ml volumetric flask and diluted with ultrapure water.

Metals were determined using validated methods by inductively coupled plasma-mass spectrometry (PerkinElmer Elan 5000) using the external standard method with indium as an internal standard or with an inductively coupled plasma double focused high-resolution mass spectrometer Plasma Trace (VG Elemental, UK) equipped with an ultrasonic nebulizer USN (Applied Research Laboratories, Switzerland).

Radiocesium

^{137}Cs was determined in beavers' muscles by gamma spectrometry (Pietrzak-Flis and Krajewski 1994).

Polychlorinated biphenyls

dl-PCBs were determined by isotope dilution and capillary gas chromatography coupled to high-resolution mass spectrometry (HRGC/HRMS). Isotope labeled ($^{13}\text{C}_{12}$) non and mono-*ortho* chlorobiphenyl congeners were used as internal standards (PCB mixture—EC-4937). Details of PCB determination (extraction, cleanup and fractionation, multi-layer silica gel cleanup step, alumina layer cleanup and fractionation step,

hypercarb-HPLC fractionation of sub-step, and PYE-HPLC fractionation of sub-step) have been described previously (Hanari et al. 2004).

Statistical procedure

For all statistical procedures, STATISTICA 8.0 was used. Results were expressed as mean and standard deviation (SD). The difference was considered significant if *p* was below 0.05.

Results and discussion

Animals

The captured European beavers were examined in detail, including individual traits such as coloring, body weight, and body measurements. Based on the results, it was concluded that their body weight ranged from 13.29 to 23.94 kg, and it was correlated with body length and other measurements. The smallest animal was beaver 2 and the largest—beaver 8. In general, beavers from the Forest Division of Srokowo were larger and in better physical shape than those captured on the Pasłęka river. The most obese individuals were beavers 11 and 10.

Radiocesium

The ¹³⁷Cs content of muscles was low, below the detectability level in some animals (Table 1).

Heavy metals

The highest concentration of cadmium in beavers captured in Warmia and Mazury was found in the kidneys (from 0.47 to 12.43 mg/g fresh weight), whereas much lower levels were observed in the liver. The muscles of all beavers contained negligible quantities of cadmium. The cadmium content of all analyzed tissues of animals inhabiting the area in the vicinity of a former military airport was higher than in the control group (beavers captured in the Forest Division of Srokowo) over threefold in the kidneys. Despite differences in the cadmium content of tissues between animals, cadmium had no significant effect on their condition.

The obtained results relating to the content of cadmium in the European beaver (Table 1) are similar to the percentage of this element in the tissues and organs of European beavers in Norway. Fimreite et al. (2001) studied a large population of beavers (92 animals) and reported the following cadmium concentrations in their tissues, as compared with our findings (micrograms per gram fresh material, FM): liver—1.03/0.58, kidneys—10.25/7.097,

Table 1 Cs (¹³⁷Cs, c.p.m. per kilogram FM), Cd, Pb, Zn, and Cu (micrograms per gram FM) concentration in the muscles, kidneys, and livers of the European beavers

Beaver no.	Muscles						Kidneys						Liver									
	¹³⁷ Cs	Cd	Pb	Zn	Cu		¹³⁷ Cs	Cd	Pb	Zn	Cu		¹³⁷ Cs	Cd	Pb	Zn	Cu					
1F	3.5±0.8	0.025±0.009	0.041±0.002	27±1	0.65±0.24	4.2±1.0	0.20±0.01	20.15±0.85	2.64±0.20	0.16±0.014	20.25±0.68	4.07±0.10	4.1±1.0	0.037±0.007	0.068±0.004	0.14±0.014	24.20±0.41	2.88±0.05	1.41±0.048	24.20±0.41	5.05±0.12	
2	< 1.0	0.057±0.003	0.025±0.005	35±1	1.20±0.31	11±1	0.10±0.003	31.32±2.16	3.04±0.03	0.55±0.044	25.18±0.04	3.22±0.08	6.1±4.3	0.044±0.005	0.011±0.002	0.10±0.005	26.15±1.38	3.28±0.14	0.32±0.055	27.06±1.90	4.29±0.15	
3	6.1±4.1	0.054±0.003	0.031±0.008	4±3	0.72±0.01	6.32±0.52	0.11±0.006	24.33±1.37	1.84±0.16	0.46±0.029	21.94±3.15	3.58±0.32	4.9±4.1	0.054±0.003	0.031±0.008	0.18±0.013	21.94±3.15	0.46±0.029	0.18±0.013	21.94±3.15	3.58±0.32	
4F	2.5±1.1	0.055±0.006	0.016±0.004	43±2	1.3±0.0	4.22±1.06	0.08±0.006	26.21±2.36	2.17±0.05	0.24±0.018	25.76±2.77	5.30±0.08	2.5±1.1	0.055±0.006	0.016±0.004	0.09±0.003	25.76±2.77	0.24±0.018	0.09±0.003	25.76±2.77	5.30±0.08	
5	10±5	0.023±0.004	0.019±0.001	38±1	0.68±0.04	2.870±0.583	0.07±0.003	26.02±1.23	4.37±0.04	0.10±0.010	24.32±1.02	4.29±0.17	3.0±2.2	0.030±0.003	0.011±0.001	0.07±0.005	26.02±1.23	4.37±0.04	0.10±0.010	24.32±1.02	4.29±0.17	
6	4.7±2.7	0.022±0.002	0.022±0.002	44±2	0.41±0.02	4.517±0.297	0.07±0.004	23.48±0.67	4.67±0.43	0.14±0.005	23.81±0.33	4.08±0.30	2.5±1.1	0.033±0.004	0.018±0.018	0.11±0.014	23.81±0.33	0.14±0.005	0.11±0.014	23.81±0.33	4.08±0.30	
7	24±7	0.033±0.004	0.028±0.004	49±4	1.9±0.2	1.587±0.091	0.11±0.016	24.14±1.09	2.53±0.06	0.18±0.018	25.91±0.11	4.56±0.23	3.0±2.2	0.022±0.002	0.028±0.004	0.27±0.021	25.91±0.11	0.18±0.018	0.27±0.021	25.91±0.11	4.56±0.23	
8	3.4±2.7	0.024±0.001	0.031±0.003	44±4	0.94±0.08	2.117±0.089	0.08±0.009	26.02±1.45	3.28±0.16	0.31±0.017	26.87±0.40	4.16±0.12	3.4±2.7	0.024±0.001	0.031±0.003	0.18±0.009	26.02±1.45	3.28±0.16	0.31±0.017	26.87±0.40	4.16±0.12	
9	<1.0	0.020	0.01	26	0.41	0.541±0.067	0.09±0.006	36.40±0.41	1.90±0.09	0.33±0.026	38.42±1.93	4.01±0.04	<1.0	0.020	0.01	0.07	36.40±0.41	1.90±0.09	0.33±0.026	38.42±1.93	4.01±0.04	
10	31	0.060	0.071	54	1.9	0.474	0.07	21.00	1.84	0.09	20.93	3.22	31	0.060	0.071	0.29	21.00	1.84	0.09	20.93	3.22	
11	3.9	0.043	0.035	37	0.86	12.426	0.20	36.81	4.67	1.46	40.35	5.30	3.9	0.043	0.035	0.19	36.81	4.67	1.46	40.35	5.30	
Min.	7.9	0.031	0.021	45	1.0	2.440	0.09	27.05	3.15	0.22	27.52	4.40	7.9	0.031	0.021	0.14	27.05	3.15	0.22	27.52	4.40	
Average, 1–5																						
Average, 6–11																						

and muscles—0.06–0.043. Similar values were observed in the tissues of fish in northern Luxembourg (Boscher et al. 2010) and in the tissues of otters in Hungary (Lanszki et al. 2009). The highest concentration of cadmium in the kidneys of herbivorous animals was observed in European beavers captured on the Mulde river in Germany—467 mg/kg dry matter, i.e., 93.4 µg/g fresh tissue weight (Nolet et al. 1994).

In the light of Polish and European Union legal standards for animal products, the maximum allowable cadmium content is 0.05 mg/kg for muscles, 0.5 mg/kg for the liver, and 1 mg/kg for the kidneys of mammals and poultry. According to Table 1, the Cd content of muscles was minimally higher than the above standards in only three beavers, while the Cd concentration in the kidneys was very high, particularly in beavers 2, 3, and 4 captured in the Beaver Mainstay in the close neighborhood of a former military airport. Statistical analysis (Student's *t* test) showed that the content of Cd in the kidneys is statistically significantly higher than the control group measurement.

Cadmium also induces changes in the concentration of sex hormones. It has a particularly profound effect on androgens, which play an important role in prostate cancerogenesis and acute testicular necrosis (Zeng et al. 2003). In the testicles, cadmium induces the production of high quantities of free radicals which causes morphological changes, such as damage to the seminiferous epithelium of sperm tubules and spermatogenesis problems (Oteiza et al. 1999). A detailed evaluation of spermatogenesis was presented by Bierła et al. (2007) who identified 27 morphological categories, of which only 12 were recognized as healthy sperm. The authors presented various sperm forms in the beavers examined in this study. The highest quantity of abnormal sperm (58.5%) was observed in beaver 5. In the other animals, the share of abnormal sperm ranged between 33% and 47%, reaching the lowest level in beaver 2 (Bierła

2007). It is interesting that the testes of non-scrotal mammals, for example *Suncus murinus* (Insectivora, Soricidae), are cadmium resistant. Dragen and McAllister (1970) proved that s.c. injections of CdCl₂ had a less significant impact on seminiferous tubule diameter in the shrew than in the mouse. According to these authors, the temperature differential between the rectum and cremaster sac in shrews is considerably lower than the rectal scrotal differential in mice. It has not been proved yet, but it is also highly possible in the case of beavers. Nonetheless, the above results do not support a clear conclusion due to the absence of research making a reference to beaver populations in other regions.

The high cadmium content of beaver kidneys could be explained by a high concentration of cadmium in their food in the autumn and winter (Brekken and Steinnes 2004; Fimreite et al. 2001; Hillis and Parker 1993; Kalas and Lierhagen 1992; Madland 1997; Nolet et al. 1994).

In comparison with cadmium content, a similar concentration of lead was observed in beaver muscles, but in the kidneys and liver, it was several times lower than Cd content (Table 1). The lead content of the tissues of beavers captured in the Beaver Mainstay was higher in comparison with animals of the control group. The discrepancies in the results obtained for every individual were very high, reaching up to 300% in the liver and more than 400% in muscles. The copper content of the liver ranged from 3.25 to 5.32 µg/g FM, and the average content in both analyzed groups was similar, also in the kidneys and muscles (Table 1).

Zinc concentration in the muscular tissue of the beavers ranged from 27.13 to 50.59 µg/g of fresh tissue weight (Table 1). When compared with muscles, the kidneys and liver were characterized by lower Zn concentration per fresh weight unit. In the control group (beavers 6–11), zinc concentration was insignificantly higher than in beavers captured in

Table 2 Content of non- and mono-*ortho* planar chlorobiphenyls in the liver of the European beaver (picograms per gram wet weight)

PCB no. IUPAC	Beaver 1F	Beaver 2	Beaver 3	Beaver 5	Beaver 6	Beaver 7	Beaver 8	Beaver 9	Beaver 10	Beaver 11
81	0	0	0	11.3	84.1	22.6	29.5	9.2	37.9	16.0
77	18.8	65.0	22.8	20.2	40.1	124	83.6	33.3	268.1	87.9
123	2.3	19.8	14.7	19.5	6.2	36.6	57.8	7.0	41.2	10.7
118	87.3	198.5	92.9	87.3	57.9	280	216	72.4	517.7	285
114	0	4.2	3.5	1.8	2.2	10.2	4.3	0	14.2	4.2
105	57.6	139.4	54.3	49.6	54.6	145.1	113	57.7	331	100
126	9.8	7.4	9.8	16.7	106.1	5.9	10.2	0	8.1	5.4
167	10.9	27.7	4.5	9.6	9.4	94.1	58.7	15.7	72.6	49.0
156	19.3	58.9	12.7	17.2	20.6	136.2	103.2	36.8	255.9	95.3
157	7.8	17.2	3.3	7.6	4.5	46.6	8.8	5.1	22.8	12.0
169	4.2	0	0	6.2	10.6	1.6	1.6	0	2.1	0
189	3.0	5.9	1.5	2.3	2.5	16.0	12.2	4.0	17.1	12.2
Σ	221	533	201	249	399	919	699	241	1,589	678

the military area. High zinc concentration in tree leaves, bark, and cambium is due to the intensive uptake of this element (Zn^{+2}) by both the root system and the leaves. Soil zinc deficiency was not reported in the area where beavers were captured, and the soil pH (5–6) supports the optimal availability of Zn ions for plants.

Zn concentration of 37–45 $\mu\text{g/g}$ fresh weight in the muscles of beavers and a lower Zn content in the kidneys and liver are similar to the Zn concentration in the muscles and organs of other herbivores—the red deer and roe deer from the region inhabited by the examined beavers (Szymczyk and Zalewski 2001; Falandysz et al. 2005). A study of the tissues of herbivorous animals in central and southern Poland and in Germany in the 1990s provided similar results.

In view of the above results, the concentrations of zinc and copper in the tissues of beavers of both groups are comparable to the concentration of these elements in the tissues of other herbivores and do not pose a threat to the health of the studied animals.

PCBs

Data on dl-PCBs in the liver of beavers are given in Table 2. Total dl-PCB concentration varied between 221 and 1,589.7 pg/g wet weight. Up to 12 different dl-PCBs were identified in livers and at greater concentration were found in older animals (nos. 10, 11, and 7) (Table 2).

In this study, the examined individuals in the reproductive age could be grouped into three categories (the age of animals being roughly approximated): young animals (over 2.5 years), animals in their prime (3 to 10 years), and older animals (over 11 years). Nevertheless, data on dl-PCBs in wildlife other than beavers in Poland are lacking. Also lacking are data on dl-PCBs in beavers outside of Poland. A recent study of pine needles (*Pinus silvestris*) collected across Poland showed low contamination with total PCBs of vegetation in the northeastern part of Poland—an area where the examined beavers live, i.e., reported concentration was 4.1 ng/g wet weight (ww) in Kudypy, 3.7 ng/g ww in Olecko, and 5.7 ng PCBs/g ww in Pasłek sites, while in densely urbanized and industrialized regions of the country were up to 49 ng PCBs/g (Falandysz et al. 2012).

Conclusions

It is highly possible that the greatest changes in sperm morphology, observed in beaver 5, were caused by a relatively high content of Pb and Cd in the tissues and organs of this animal. The results of a detailed histopathological analysis of this beaver and the other ten animals were published in a doctoral dissertation (Bierła 2007). The conclusions

formulated with respect to beaver 5 cannot be extrapolated to the entire population. Further investigations are needed with the involvement of animals inhabiting regions characterized by higher environmental contamination levels than Warmia and Mazury, e.g., beavers from Silesia. The tissues of all beavers captured in the vicinity of a former military airport contained substantially larger quantities of the above elements than the tissues of control group animals. The concentrations of zinc, copper, and radioactive cesium in the tissues of beavers of both groups, captured in northeastern Poland, were low and did not pose a threat to the health and reproduction of the animals. Beavers can easily adapt to changing environmental conditions, and therefore, they colonize new habitats across Poland.

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