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Bioaccumulation of Lead, Cadmium and Mercury in Roe Deer and Wild Boars from Areas with Different Levels of Toxic Metal Pollution

Durkalec, M.[1,2*], Szkoda, J.[2], Kolacz, R.[1], Opalinski, S.[1], Nawrocka, A.[2] and Zmudzki, J.[2]

[1]Department of Environment, Hygiene and Animal Welfare, Wroclaw University of Environmental and Life Sciences, Jozefa Chelmonskiego 38C, 51-630 Wroclaw, Poland

[2]Department of Pharmacology and Toxicology, The National Veterinary Research Institute, Aleja Partyzantow 57, 24-100 Pulawy, Poland

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ABSTRACT: We used wild boars and roe deer as biomonitors of lead (Pb), cadmium (Cd), and mercury (Hg) contamination in two major industrial sites in Poland with different levels of toxic metal pollution. Masurian Lakes District, located far away from industry, was used as the reference site. Levels of Pb, Cd, and Hg in liver, kidney and muscle samples and in the stomach content of the animals were determined using atomic absorption spectroscopy (AAS) methods. We calculated also the mean concentration factors in the animal tissues versus their concentration in the gastric or rumen content. Our results indicate that area affected by metal smelting was more contaminated than brown coal mining area and the reference site, as indicated by higher levels of Pb and Cd in tissues and stomach contents of the animals. High levels of those metals in the offal of game animals may pose a threat to consumers of venison.

Key words: Bioaccumulation, Roe deer, Wild boar, Toxic metals, Concentration factor

INTRODUCTION

Toxic metals including lead (Pb), cadmium (Cd), and mercury (Hg) are natural components of the earth's crust and their increased concentrations in the environment are mostly caused by anthropogenic activity. Industrial development in recent centuries has led to a sharp increase in the level of these contaminants in air, water, and soil. The main sources of toxic metal emissions are: combustion of fossil fuels, mining and smelting of non-ferrous metals, and waste incineration (Czaban *et al*. 2013; Norgate *et al*. 2007; Pacyna *et al*. 2007). Several studies have shown that metal mining and ore processing adversely impact the environment. Increased concentrations of toxic elements have been found in soil (Chrastny *et al*. 2012; Sajn *et al*. 2013), water, and sediments from areas of Pb-Zn smelters or ironworks across Europe (Alijagic & Sajn 2011; Krasnodebska-Ostrega *et al*. 2005). Combustion of coal, especially lignite, may cause metal contamination as well (Sucharova *et al*. 2011). Pb, Cd, and Hg taken up from water and soil can be transferred upward in plants (Reglero *et al*. 2008) or deposited with soil or dust particles on plant surfaces, then incorporated into the food chain (Gnamus *et al*. 2000). Important sources of toxic metals for herbivorous mammals may include intake of contaminated soil (Beyer *et al*. 2007) and direct inhalation of dust particles (Ma 1996). Smith *et al*. (2009) reported that the main source of Pb for grazing sheep is ingestion of soil, which ranged between 0.1 and 44% of dry matter intake. The diet is considered to be the most important pathway of metal transfer to the tissues of animals (Stankovic *et al*. 2013). Tissues of wild animals are known to be good bioindicators of toxic metal pollution (Gizejewska *et al*., 2014; Pokorny 2000; Wren 1986). Additionally, toxic metal analysis in the gastric or rumen content of animals may be a useful source of information about environmental pollution. The objective of our research was to assess the level of environmental contamination in two industrial sites in Poland: an area surrounding the zinc smelter "Miasteczko Slaskie", and the Turoszow Coal Basin. We examined Pb, Cd, and Hg concentrations in tissues of game animals (wild boars (*Sus scrofa* L.) and roe deer (*Capreolus capreolus* L.) as biomonitors of toxic metals. Masurian Lake District was used as the reference area.

*Corresponding author E-mail:maciej.durkalec@piwet.pulawy.pl

MATERIALS & METHODS

A total of 169 wild boars and 110 roe deer were obtained in cooperation with local hunting authorities

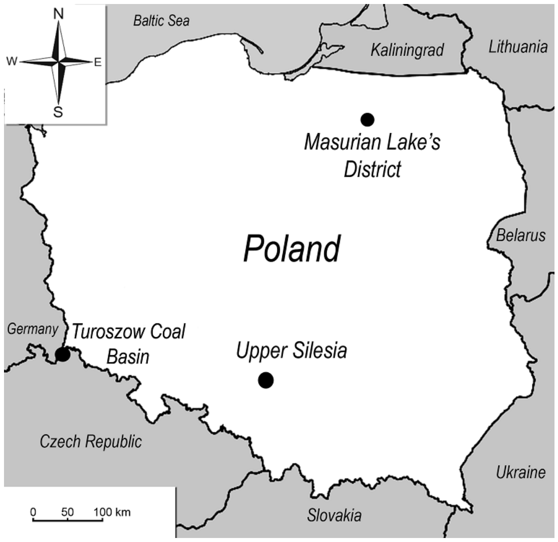

Fig. 1. Turoszow Coal Basin and Masurian Lake District

from Upper Silesia, Turoszow Coal Basin and Masurian Lake District (Fig. 1). Liver, kidney and muscle tissues were obtained from all animals, gastric content samples were taken from 118 wild boars and rumen content samples were taken from 69 roe deer. The hunting district surrounding the zinc smelter "Miasteczko Slaskie" in the Upper Silesia Region was chosen as the first study area. This area is considered to be the most polluted by toxic metals in the region (Bielinska & Mocek-Plocinniak 2010; Dudka *et al*. 1995; Pajak & Jasik 2010). The altitude of this area is approximately 290 m above sea level with a moderate climate (mean annual temperature 7–8°C, mean annual rainfall about 700 mm, and winds are mainly from the southwest). The landscape is woody and the vegetation is mainly *Leucobryo-pinetum*, *Quercobryo-pinetum*, and *Tilio carpinetum*. Another study site, the Turoszow Coal Basin, is located in the southwest part of Poland. The hunting zone in this site is situated close to the Turow Brown Coal Mine and the Turow Coal Power Plant – the third largest thermal power station in Poland, considered to be an important source of Pb pollution (Sucharova *et al*. 2011). The altitude in Turoszow Coal Basin ranges between 200 and 450 m above sea level, and the area is situated on artificial hills made of debris from open-pit mine and recultivated by afforestation. Natural vegetation in this site is mainly *Galio-carpinetum*. Mean annual temperature in this area ranges from 6 to 8°C, annual rainfall is about 1200 mm, and winds are mainly from the west. Masurian Lake District, situated far away from industrial areas, was chosen as a reference site; it is considered to be an unpolluted area (Falandysz *et al*. 2005). The climate is cooler than in other parts of Poland (5–6°C), mean rainfall is about 700–800 mm and winds are predominately from the west. The vegetation in the chosen hunting zone in Masuria is mainly *Tilio carpinetum*, *Stellario-carpinetum*, and *Querco-pinetum*. The mean altitude of the area in which animals were shot is 140 m above sea level. Samples of kidney, liver, and muscle tissue and gastric or rumen content of wild boars and roe deer were collected from September 2011 to the end of December 2013 in accordance with current hunting plans and regulations. Samples were collected carefully at a distance greater than 40 cm from the gunshot wound to avoid secondary contamination of Pb from bullets used by hunters (Danieli *et al*. 2012, Dobrowolska & Melosik 2008). Samples were packed separately in plastic bags and transported to the laboratory. All samples were frozen and stored at -20°C until analysis. The frozen samples were later thawed at 4°C for 24 h and homogenized using Buschi B-400 homogenizer (Buschi Labortechnik AG, Flavil, Switzerland). According to a previously described method (Szkoda & Zmudzki 2005), homogenized samples were mineralized in a muffle furnace at 450°C, and Pb and Cd concentrations were determined using graphite furnace atomic absorption spectroscopy (GF-AAS) with Zeeman background correction and an autosampler (Perkin Elmer PinAAcle 900T, Norwalk, CT, USA). Hg was measured directly in raw tissue using an AMA-254 mercury analyzer, Altec Ltd. (Szkoda *et al*. 2006). The limits of detection (LODs, mg/kg) were 0.002, 0.001 and 0.001 for Pb, Cd, and Hg, respectively. Quality control of analytical measurements was performed using the following certified reference materials: BCR-184, BCR-186 and ERM-CD281, and was regularly evaluated by participation in proficiency programs organized by Food Analyses Performance Assessment Scheme (FAPAS) and European Union Reference Laboratories comparisons. Recoveries of the metals are presented in Table 1. All statistics were performed using Statistica™ 10.0 PL software (StatSoft Inc., Tulsa, OK, USA). Concentrations below the limit of detection (LOD) were assigned as a half of the LOD values determined for each element. Prior to analysis data were tested using Shapiro-Wilk normality test to verify the distribution. Because the results did not present normal distribution all of the data were log transformed. Mean and SD were calculated and one-way analysis of variance (ANOVA) and the Tukey post-hoc test were applied to verify the significance of differences between metal levels in tissues of animals from different areas. Student t-test was applied to verify the difference between species. To calculate the mean concentration factor (CF) of toxic metals in the animal tissues versus their concentration in the gastric or rumen content,

Table 1. Results of the analysis of the certified reference materials

Certified Reference Materials		Certified level mean ± SD, mg/kg dry wt	Analyzed level mean ± SD, mg/kg of dry wt)	Recovery (%)
ERM-CD281 Rye Grass	Pb	1.67 ± 0.11	1.53 ± 0.090	91.6
	Cd	0.120 ± 0.007	0.108 ± 0.009	90.0
	Hg	0.0164 ± 0.0022	0.018 ± 0.001	109.7
BCR-186 Pig kidney	Pb	0.306 ± 0.011	0.313 ± 0.018	102.2
	Cd	2.71 ± 0.15	2.59 ± 0.083	95.6
	Hg	1.97 ± 0.04	1.90 ± 0.076	96.4
BCR-185R Bovine Liver	Pb	0.172 ± 0.009	0.176 ± 0.015	102.3
	Cd	0.544 ± 0.017	0.477 ± 0.045	87.7

the following equation was used: $CF = \frac{C_T}{C_C}$, where C_T is the concentration of the toxic metal in the tissue of the singe individual and C_C is the content of the toxic metal in its gastric or rumen content. To verify the presence of secondary contamination of muscle samples by Pb from the rifle bullets, Pb levels in liver were analyzed versus muscle Pb levels using a linear regression (Taggart *et al*. 2011). Outlying results of Pb in muscle tissue obtained in this way were excluded from calculation of the mean. All concentrations of metals are given as mg/kg wet weight (wet wt).

RESULTS & DISCUSSION

Mean concentrations of Pb in the liver, kidney and muscle tissue as well as in the gastric content of animals are summarized in Table 2. Levels of Pb and Cd in wild boar tissues from Upper Silesia were much higher (pd”0.01) than those in tissues from animals from the reference area and the Turoszow Coal Basin. A similar trend was observed in the gastric content samples of the animals. Numerous studies on Pb and Cd concentrations in wild boars have indicated lower tissue concentrations than those presented in our research. The average level of Cd in liver and kidney of wild boar from Upper Silesia was much higher than that found in a contaminated area of Croatia (Bilandzic *et al*. 2010; Suran *et al*. 2013), in the province of Viterbo, Central Italy (Amici *et al*. 2012; Danieli *et al*. 2012) in Slovakia (Gasparik *et al*. 2012), in southeastern Poland (Rudy 2010) and in central Poland (Dlugaszek & Kopczynski 2013). High concentrations of Pb and especially Cd in offal may pose a threat for consumers of venison, particularly hunters and their families, who consume meat of game animals more often than the general population (Danieli *et al*. 2012). Regulations on maximum levels of contaminants in foodstuffs are not provided for game animals. The maximum levels of toxic metals are specified only for slaughtered animals; these are for meat: 0.1, 0.05 liver: 0.5, 0.5 and kidney: 0.5, 1.0 mg/kg wet wt for Pb and Cd and respectively (EC, 2006). The maximum permissible level for Cd in kidney was exceeded in all samples from Upper Silesia and in the majority of samples obtained from the other sites (Table 2). Despite the extreme care taken during the collection of samples, high levels of Pb were found in muscles (up to 15 mg/kg). Therefore, the contamination of venison by Pb-based rifle bullets is an important issue in food safety and needs particular attention (Hunt *et al*. 2009; Mateo *et al*. 2006, Szkoda *et al*. 2012). As mentioned above, some of muscle samples (27% of muscle samples of wild boar and 20% of muscle samples of roe deer from all regions) were excluded from the calculation of mean Pb levels to avoid the influence of secondary contamination form rifle bullets. Concentration of Cd in muscle samples (excluding 5 samples from Upper Silesia) was below the maximum level for slaughtered animals (0.05 mg/kg) and was similar to concentrations measured in other European countries (Frosile *et al*. 2001; Srebocan *et al*. 2011). Hg was detected in almost all samples, which demonstrates that this element is still present in the environment and can accumulate in animal tissues (Szkoda *et al*. 2013B). The presented results were comparable to those of other researchers (Berzas Nevado *et al*. 2012; Srebocan *et al*. 2011; Suran *et al*. 2013; Szkoda & Zmudzki 2001). The diet of wild boar is based on bulbs, tubers, acorns, cereals, insect larvae, and even small mammals, which may be the reason that accumulation of Hg in their tissues is higher than in other game species (Berzas Nevado *et al*. 2012; Szkoda *et al*. 2013A). Our findings confirm a significant difference between Hg levels in the tissues of wild boar and roe deer (pd”0.05, Student t-test). The maximum level of Hg is not specified in the Commission Regulation (EC) no 1881/2006 setting maximum level for certain contaminants in foodstuffs (EC, 2006). The European Union Reference Laboratory for Chemical Elements in Food of Animal Origin in Rome (EU-RL CEFAO) suggests that the maximum level of Hg in the Commission Regulation (EC) no 149/2008 should be respected (EC, 2008). This regulation is specified for

Table 2. Concentrations of toxic metals in tissues and gastric contents of wild boar (*Sus scrofa*) and the number of samples exceeded maximum level of toxic metals for slaughtered animals

Tissue	Element	Area	n	n>ML*	Mean ± SD	Median	Range
Muscle	Pb	Upper Silesia	34	17**	0.046 ^{A} ± 0.025	0.048	< 0.002 - 0.092
		Turoszow Coal Basin	47	9**	0.019 ^{B} ± 0.014	0.018	< 0.002 - 0.049
		Masurian Lake's District	42	19**	0.027 ^{B} ± 0.025	0.020	< 0.002-0.130
	Cd	Upper Silesia	51	14	0.040^{A} ± 0.031	0.028	0.008 - 0.140
		Turoszow Coal Basin	56	0	0.004^{B} ± 0.003	0.003	< 0.001 - 0.016
		Masurian Lake's District	61	0	0.004^{B} ± 0.004	0.003	< 0.001 - 0.022
	Hg	Upper Silesia	51	11	0.007 ± 0.006	0.006	< 0.001 - 0.035
		Turoszow Coal Basin	56	13	0.007 ± 0.005	0.005	< 0.001 - 0.023
		Masurian Lake's District	61	8	0.006 ± 0.004	0.005	< 0.001 - 0.020
Liver	Pb	Upper Silesia	52	23	0.904^{A} ± 1.107	0.446	0.036 - 6.660
		Turoszow Coal Basin	56	2	0.248^{B} ± 1.290	0.047	0.012 - 9.672
		Masurian Lake's District	59	2	0.083^{B} ± 0.141	0.051	0.015 - 0.956
	Cd	Upper Silesia	52	52	5.573^{A} ± 6.031	3.530	0.768 - 39.600
		Turoszow Coal Basin	56	22	0.542^{B} ± 0.365	0.368	0.094 - 1.657
		Masurian Lake's District	60	4	0.189^{B} ± 0.167	0.151	0.029 - 0.945
	Hg	Upper Silesia	52	42	0.027±0.033	0.018	< 0.001 - 0.231
		Turoszow Coal Basin	56	47	0.026±0.018	0.024	0.006 - 0.084
		Masurian Lake's District	60	42	0.019±0.013	0.015	0.003 - 0.028
Kidney	Pb	Upper Silesia	52	38	1.190^{A} ± 1.234	0.881	0.114 - 6.930
		Turoszow Coal Basin	56	2	0.102^{B} ± 0.150	0.067	0.013 - 0.851
		Masurian Lake's District	60	0	0.088^{B} ± 0.045	0.081	0.032 - 0.255
	Cd	Upper Silesia	52	52	49.50^{A} ± 45.75	40.1	10 - 245.50
		Turoszow Coal Basin	56	55	4.838^{B} ± 3.281	3.867	0.429 - 19.940
		Masurian Lake's District	60	49	2.113^{B} ± 1.239	2.115	0.422 - 8.155
	Hg	Upper Silesia	52	50	0.089^{A} ± 0.080	0.061	0.005 - 0.364
		Turoszow Coal Basin	56	56	0.095^{A} ± 0.054	0.087	0.022 - 0.274
		Masurian Lake's District	60	58	0.046^{B} ± 0.035	0.037	0.006 - 0.202
Gastric content	Pb	Upper Silesia	34	-	15.83^{a} ± 38.73	2.355	0.237 - 165.0
		Turoszow Coal Basin	31	-	0.266^{b} ± 0.262	0.163	0.010 - 1.030
		Masurian Lake's District	53	-	2.627^{b} ± 17.270	0.192	0.012 - 126.0
	Cd	Upper Silesia	34	-	2.082^{a} ± 6.923	0.389	0.024 - 39.22
		Turoszow Coal Basin	31	-	0.052 ± 0.071	0.027	< 0.001 - 0.312
		Masurian Lake's District	53	-	0.040^{b} ± 0.041	0.022	0.002 - 0.161
	Hg	Upper Silesia	34	-	0.006 ± 0.007	0.004	< 0.001 - 0.027
		Turoszow Coal Basin	31	-	0.004 ± 0.005	0.003	< 0.001 - 0.023
		Masurian Lake's District	53	-	0.004 ± 0.005	0.002	< 0.001 - 0.024

[AB] Means marked with different letters are significantly different between areas at p=0.01
[ab] Means marked with different letters are significantly different between areas at p=0.05 (Tukey test)
* ML - Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting maximum levels for certain contaminants in foodstuffs. Muscle - Pb 0.1, Cd 0.05; liver - Pb 0.5, Cd 0.5; kidney Pb 0.5 and Cd 1.0 mg/kg of wet wt
* ML - Commission Regulation (EC) No 149/2008 of 29 January 2008 amending Regulation (EC) No 396/2005 of the European Parliament and of the Council by establishing Annexes II, III and IV setting maximum residue levels for products covered by Annex I thereto (Text with EEA relevance). Maximum level of Hg in meat, liver and kidney: 0.01 mg/kg of wet wt
**Samples excluded from the calculation based on regression (with 95% confidence interval) for Pb levels in muscle and liver tissue of wild boars from studied areas

residues of pesticides in or on food and feed, of plant and animal origin. Although this suggestion is a bit controversial, we have compared our results with the maximum level of Hg (0.01 mg/kg of wet wt) specified in that Regulation (Table 2). The levels of Pb, Cd, and Hg in kidneys, liver and muscles and in the rumen contents (mg/kg of wet wt) of roe deer are summarized in Table 4. Similarly, as in the case of wild boars, mean Cd concentrations in tissue samples of roe deer from Upper Silesia were higher (pd”0.01) than of animals from the Turoszow Coal Basin and Masurian Lake District. This trend was also observed for rumen content samples. Beiglbock *et al.* (2002) observed renal damage in roe deer environmentally exposed to Cd. The authors found a positive correlation between renal concentration of this metal and the presence of such abnormalities like vacuolic degeneration, pyknotic nuclei, karyolysis, and necrosis. Levels of Cd in the kidney of roe deer from the area of the zinc smelter "Miasteczko Slaskie" were about 20-fold higher than those observed by Beiglbock *et al.* (2002). High levels of toxic metals in wildlife were found in roe deer from industrial areas in Slovenia by Pokorny (2000). The highest mean concentration of Cd in kidney (22.7 mg/kg) was found in the Koroska region affected by lead smelting. This value was approximately half the level observed in Upper Silesia.

Table 3. Mean concentration factors (CFs) for average Pb, Cd, and Hg concentrations in tissues of wild boars (*Sus scrofa*) versus their diet (gastric content)

Research area	Pb			Cd			Hg		
	Muscle	Liver	Kidney	Muscle	Liver	Kidney	Muscle	Liver	Kidney
Upper Silesia	0,02	0,31	0,40	0,18	22,94	211,5	2,93	13,82	42,12
Turoszow Coal Basin	0,99	6,35	5,73	0,27	28,77	230,90	3,11	11,06	41,50
Masurian Lake's District	0,54	1,13	0,70	0,27	15,44	179,86	3,19	8,62	22,04

Table 4. Concentrations of toxic metals in tissues and gastric contents of roe deer (*Capreolus capreolus*) and number of samples exceeded maximum level of toxic metals for slaughtered animals

Tissue	Element	Area	n	n>ML*	Mean ± SD	Median	Range
Muscle	Pb	Upper Silesia	25	4^{**}	$0.057^{A} \pm 0.038$	0.042	0.009 - 0.154
		Turoszow Coal Basin	40	12^{**}	$0.026^{B} \pm 0.026$	0.017	< 0.001 - 0.099
		Masurian Lake's District	22	9^{**}	$0.012^{B} \pm 0.010$	0.009	< 0.001 - 0.039
	Cd	Upper Silesia	26	5	$0.043^{A} \pm 0.040$	0.028	0.003 - 0.151
		Turoszow Coal Basin	52	0	$0.005^{B} \pm 0.004$	0.004	< LOD - 0.018
		Masurian Lake's District	31	0	$0.007^{B} \pm 0.009$	0.003	< LOD - 0.040
	Hg	Upper Silesia	26	0	$0.001^{a} \pm 0.001$	0.001	< LOD-0.006
		Turoszow Coal Basin	52	4	$0.003^{b} \pm 0.003$	0.001	< LOD - 0.012
		Masurian Lake's District	31	0	$0.001^{a} \pm 0.002$	0.001	< LOD - 0.009
Liver	Pb	Upper Silesia	25	4	0.303 ± 0.311	0.199	0.101 - 1.616
		Turoszow Coal Basin	52	1	0.559 ± 0,064	0.031	0.090 - 0,345
		Masurian Lake's District	31	0	0.058 ± 0.076	0.032	0.011 - 0.384
	Cd	Upper Silesia	25	25	$6.435^{A} \pm 6.096$	4.561	0.938 - 20.61
		Turoszow Coal Basin	53	23	$0.492^{B} \pm 0.375$	0.433	0.063 - 1.613
		Masurian Lake's District	31	1	$0.115^{B} \pm 0.130$	0.083	0.015 - 0.514
	Hg	Upper Silesia	25	7	0.008 ± 0.009	0.004	0.001 - 0.033
		Turoszow Coal Basin	53	14	0.009 ± 0.012	0.004	< LOD - 0.061
		Masurian Lake's District	31	5	0.005 ± 0.007	0.002	< LOD - 0.025
Kidney	Pb	Upper Silesia	25	18	$0.906^{A} \pm 0.542$	0.912	0.215 - 2.150
		Turoszow Coal Basin	51	1	$0.099^{B} \pm 0.090$	0.061	0.023 - 0.454
		Masurian Lake's District	31	0	$0.102^{B} \pm 0.057$	0.091	0.034 - 0.284
	Cd	Upper Silesia	25	25	$39.60^{A} \pm 24.54$	32.032	1.851 - 89.88
		Turoszow Coal Basin	51	23	$4.653^{B} \pm 3.608$	3.716	0.764 - 17.29
		Masurian Lake's District	31	13	$1.582^{B} \pm 1.907$	0.953	0.233 - 9.026
	Hg	Upper Silesia	25	23	0.048 ± 0.045	0.038	0.009 - 0.233
		Turoszow Coal Basin	51	42	0.068 ± 0.146	0.022	0.005 - 0.998
		Masurian Lake's District	31	21	0.027 ± 0.028	0.015	0.003 - 0.114
Rumen content	Pb	Upper Silesia	11	-	$1.981^{A} \pm 1.881$	1.690	0.058 - 6.870
		Turoszow Coal Basin	34	-	$0.105^{B} \pm 0.104$	0.064	0.014 - 0.465
		Masurian Lake's District	24	-	$0.205^{B} \pm 0.178$	0.159	0.044 - 0.651
	Cd	Upper Silesia	11	-	$0.542^{A} \pm 0.331$	0.475	0.191 - 1.298
		Turoszow Coal Basin	34	-	$0.121^{B} \pm 0.068$	0.111	0.019 - 0.290
		Masurian Lake's District	24	-	$0.038^{B} \pm 0.022$	0.031	0.009 - 0.082
	Hg	Upper Silesia	11	-	0.005 ± 0.006	0.002	0.001 - 0.020
		Turoszow Coal Basin	34	-	0.005 ± 0.003	0.003	< LOD - 0.015
		Masurian Lake's District	24	-	0.004 ± 0.003	0.003	0.001 - 0.010

[AB] Means marked with different letters are significantly different between areas at p=0.01

[ab] Means marked with different letters are significantly different between areas at p=0.05 (Tukey test)

[*] ML - Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting maximum levels for certain contaminants in foodstuffs. Muscle - Pb 0.1, Cd 0.05; liver - Pb 0.5, Cd 0.5; kidney Pb 0.5 and Cd 1.0 mg/kg of wet wt

[*] ML - Commission Regulation (EC) No 149/2008 of 29 January 2008 amending Regulation (EC) No 396/2005 of the European Parliament and of the Council by establishing Annexes II, III and IV setting maximum residue levels for products covered by Annex I thereto (Text with EEA relevance). Maximum level of Hg in meat, liver and kidney: 0.01 mg/kg of wet wt

[**] Samples excluded from the calculation based on regression (with 95% confidence interval) for Pb levels in muscle and liver tissue of wild boars from studied areas

Table 5. Mean concentration factors (CFs) for average Pb, Cd, and Hg content in tissues of roe deer (*Capreolus capreolus*) versus their diet (rumen content)

Research area	Pb			Cd			Hg		
	Muscle	Liver	Kidney	Muscle	Liver	Kidney	Muscle	Liver	Kidney
Upper Silesia	0,06	0,95	0,82	0,09	10,51	71,38	0,35	1,88	16,11
Turoszow Coal Basin	0,74	1,14	1,94	0,07	5,31	64,07	0,66	1,92	11,83
Masurian Lake's District	0,29	0,79	1,24	0,21	4,02	60,82	0,42	1,63	7,42

Our data differ from results previously reported in other European countries (Srebocan *et al.* 2011; Pokorny & Ribaric-Lasnik 2002) and were higher than those reported in past decades. In a review paper, Frosile *et al.* (2001) indicates that toxic metal levels in roe deer from different areas of European countries in the past century ranged from 0.07 to 0.96 and 0.47 to 11.0 mg/kg wet wt in liver and kidney, respectively. Contents of Cd and Pb in liver and kidneys of roe deer from Upper Silesia were much higher than previously described in Polish industrial sites by Kucharczak *et al.* (2003), Wieczorek-Dabrowska *et al.* (2013), higher than mean levels observed in roe deer from central Poland (Dlugaszek & Kopczynski, 2013) and across Poland (Szkoda *et al.* 2013A). The results reported by Kucharczak *et al.* (2006) from the Turoszow Coal District were comparable to data presented in our work, with the exception of Cd in the kidneys of roe deer. It has been reported that accumulation of toxic metals in game animals, in particular Cd, is strongly correlated with their age (Falandysz *et al.* 2005; Garcia *et al.* 2011; Srebocan *et al.* 2012). Unfortunately, in this study it was not possible to divide animals into age groups because of an unequal number of individuals in respective age groups. However, our research in this area is continuing and the influence of age on metal concentrations in game animals will be investigated. Hg concentrations in muscle and liver of roe deer observed in this study were generally lower than those obtained by Albinska *et al.* (2011) in the province of Lodz in Poland. Mean Hg levels reported in that study were 0.0070, 0.0188 and 0.0523 mg/kg in muscle, liver and kidneys, respectively. As was mentioned above, we have compared our results with the maximum level of Hg set out in the Commission Regulation (EC, 2008). Maximum level of Hg in tissues of animals was exceeded in the majority of samples, especially in offal (Table 4). The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives has established provisional tolerable weekly intake (PTWI) of 4 μg of total Hg per 1 kg of body weight (JECFA 2010). Taking our results into account, the risk of Hg ingestion from meat of roe deer and wild boar is very low. However, offal may be an important source of Hg exposure. Concentration factors (CFs) of toxic metals in tissues of wild boars and roe deer versus their gastric and rumen contents are shown in Tables 3 and 5. Few studies have been published on trace elements transfer into game animals from environmental compartments or from their diet (Gnamus *et al.* 2000; Sheppard *et al.* 2010; Sheppard 2012). In our research, we used gastric or rumen content, which should reflect the animal's diet including soil ingestion. CFs reported by Sheppard (2010) in muscle tissue of Canadian cervids are 0.2 for Cd and 0.069 for Pb, which are similar to those observed in this study. Gnamus *et al.* (2000) calculated CFs using Hg concentration in a set of 42 plant species, collected twice a year, which represent the diet of roe deer. They reported that in muscle, liver, and kidney, the CF of Hg ranged from 0.1–0.13, 0.02–1.2 and 0.3–13.5 respectively. These results are similar to findings of this study for roe deer but are much lower than the values reported in this study for wild boar tissues and stomach contents.

CONCLUSIONS

This study has led us to conclude that the area of zinc smelter "Miasteczko Slaskie" located in the Upper Silesia district appears to be the most contaminated of the investigated sites. The majority of kidney and liver samples from both wild boar and roe deer exceeded the maximum levels imposed for slaughtered animals; this problem should be widely discussed with regard to the safety of human consumers of venison. We also found that venison contamination by Pb from standard rifle bullets used by hunters may adversely affect consumers' health. Our research may be a useful aid for decision makers to review existing food safety regulations. It is worth noting that the law should protect the welfare of all citizens. Research on the influence of industry on game animals in Poland is already in progress, and the levels of toxic metals in soil and plants, as well as in the hair of animals, are also under investigation.

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