Evaluation of Dog Bones in the Indirect Assessment of Environmental Contamination with Trace Elements

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Abstract The aim of this paper was to determine the level of five elements, two essential for life [zinc (Zn) and copper (Cu)] and three distinctly toxic [lead (Pb), cadmium (Cd), and mercury (Hg)], in four types of biological material in bones of the dog *Canis lupus familiaris*. The experiment was carried out on bones from the hip joints of dogs. The samples of cartilage, compact bone, spongy bone, and cartilage with adjacent compact bone came from 26 domestic dogs from northwestern Poland. Concentrations of Cu, Zn, Pb, and Cd were determined by ICP-AES (atomic absorption spectrophotometry) in inductively coupled argon plasma, using a Perkin-Elmer Optima 2000 DV. Determination of Hg concentration was performed by atomic absorption spectroscopy. In the examined bone material from the dog, the greatest concentrations (median) were observed for Zn and the lowest for Hg (98 mg Zn/kg and 0.0015 mg Hg/kg dw, respectively). In cartilage and spongy bone, metal concentrations could be arranged in the following descending order: Zn > Pb > Cu > Cd > Hg. In compact bone, the order was slightly different: Zn > Pb > Cd > Cu > Hg (from median 70 mg/kg dw to 0.002 mg/kg dw). The comparisons of metal concentrations between the examined bone materials showed distinct differences only in relation to Hg: between concentrations in spongy bone, compact bone, and in cartilage, being greater in cartilage than in compact bone, and lower again in spongy bone.

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Introduction

The dynamic development of manufacturing, the automotive industry, and energy sector since the late nineteenth century has resulted in a rapid increase in heavy metal contamination of natural ecosystems and the immediate surroundings of man, mainly with lead (Pb), cadmium (Cd), and mercury (Hg). These metals accumulate in living organisms along trophic chains [1]. Cadmium and mercury undergo biomagnification along aquatic trophic chains, with their greatest concentrations observed in animals that are the final links of those chains. Some authors suggest that biomagnification of lead may also occur at lower trophic levels [2, 3]. It is estimated that very low mercury concentrations detected in water may increase from a million to even 10 million times in piscivorous animals due to biomagnification [4].

One of the main methods of using bioindicators in the indirect evaluation of environmental contamination with toxic elements is in the determination of levels in living organisms [5, 6]. In ecotoxicology, comparative studies often use medium-sized and large free-living mammals (mainly those that are hunted by man) and domesticated mammals, including the dog, pig, sheep, cattle, and horse. Trace elements are most often determined in the kidney and liver as these organs play a crucial detoxification role.

However, in recent times, exposure to trace elements has been increasingly frequently evaluated from bone tissue examination. Knowing the concentrations of elements in bones, one may draw conclusions on the level of environmental contamination and long-term effects, for example the effect of heavy metals on free-living and domesticated

warm-blooded vertebrates. Processes of bone transformation span the entire life of the mammal and therefore can be considered as indicators of long-term accumulation of heavy metals.

Many works have been published on the neurotoxic, nephrotoxic, and hepatoxic effects of trace elements, yet there are much fewer papers on the accumulation of these elements [including copper (Cu), zinc (Zn), lead (Pb), and mercury (Hg)], and the correlations between them, in the elements of bones and cartilage of animals, for example *Canidae* [7–9]. Differences in the concentrations of heavy metals across individual components of the hip joint have only been examined in humans [7]. The elderly, who often undergo hip joint surgery, have for some time been subject of studies meant to analyze the mineral contents of their bones. The results of those studies indicate differences between the accumulation of metals in cartilage, spongy bone, and compact bone (including highly toxic, mainly Cd and Pb, and essential elements for warm-blooded organisms, such as Zn and Cu).

Therefore, the aim of this work was to determine the level of five elements (two essential for life—Zn and Cu) and three highly toxic (Pb, Cd, and Hg) in four types of biological materials from domestic dog bones *Canis lupus familiaris* (L., 1758) belonging to the order Carnivora. The aim was also to estimate intraspecies differences between the concentrations of the trace elements determined in cartilage and spongy bone and the transition layer between them, i.e., compact bone. Moreover, the aim was also to assess potential synergic and antagonist relationships between the analyzed metals in the bone material.

Materials and Methods

Material

The study was performed on bone elements from the hip joint from dogs—samples of articular cartilage, compact bone, spongy bone, and cartilage with adjacent compact bone from 26 domestic dogs from northwestern Poland (Fig. 1). The dogs came from veterinary practices where they had been put down due to respiratory deficiencies or tumors (they were not terminated specifically for study). This research was approved by the Local Veterinary Office in Szczecin and the Local Animal Research Ethics Committee (Resolution no. 4/2009).

The ages of the dogs were determined by the veterinary surgeons based on information in medical documentation and from the owners of the animals. The dogs were divided into two age categories: CF1 <9 years ($n=9$; age range, 4.1 ± 3.5) and CF2 >9 years ($n=17$; age range, 13.9 ± 2.6). Dogs older than 9 years of age are considered old and very old, and the results of this research using this classification can be used in comparison with the results of analogous studies on other warm-blooded vertebrates.

Preparation of Material for Analysis of Bone

Femoral heads were removed with a glass tool. Chemical analysis was performed on four materials: cartilage, compact bone, spongy bone, and cartilage together with directly adjacent compact bone. Bone material was dried to constant weight at 55°C and then at 105°C. This procedure was used to determine water content (gravimetric method). Dried samples were ground in an agate mortar.

Determination of Copper, Zinc, Lead, and Cadmium

The samples were divided into doses weighing from 0.5 to 1.0 g. Bone material was mineralized with wet digestion using a Velp Scientifica mineralizer (Italy) [8]. Concentrations of Cu, Zn, Pb, and Cd were determined by ICP-AES (atomic absorption spectrophotometry) in inductively coupled argon plasma, using a Perkin-Elmer Optima 2000 DV. The limits of detection of that device for Cu, Zn, Pb, Cd are 0.4, 0.2, 1.0, and 0.1 µg/l, respectively.

Determination of Mercury

Total mercury concentrations were determined from samples dried at 55°C using atomic absorption spectroscopy. The assays were run in an AMA 254 mercury analyzer (Altech Ltd, Czech Republic) based on flameless atomic absorption. For the analysis, 100 to 300 mg of each sample was collected and placed in the analyzer's nickel nacelle in which it was automatically weighed and dried. The sample was thermally decomposed in a stream of oxygen to obtain a gaseous form, and its degradation products were transferred to the amalgamator for the selective offtake of Hg. After the determination of the parameters of measurement, mercury vapor was released from the amalgamator by a brief heating. The amount of released mercury was measured by atomic absorption (detector in the AMA 254 analyzer is a silicon UV diode) at a wavelength of 254 nm in an arrangement of two measuring cells. The limit of detection for this method is 0.01 ng of Hg in the sample. For each sample, two or three repetitions were performed, and statistical analysis used the average of the data, expressed in milligrams per kilogram dry mass (dw).

Validation of Analytical Proceedings

The reliability of the analytical procedure was controlled by the determination of elements in two reference materials with known concentrations: National Institute of Standards and Technology SRM 1486 Bone Meal and

Fig. 1 Location of study areas in northwestern Poland

International Atomic Energy Agency-407 Trace Elements and Methylmercury in Fish. Concentrations of metals in the reference materials were provided by the equipment manufacturers.

Statistical Analysis

Analysis used Statistica 9.0. StatSoft software. In order to determine compliance with the expected normal distribution of results, a Kolmogorov–Smirnov test with Lilliefors correction ($p < 0.05$) was used. In order to compare the impact of various environmental factors on the concentration of metals in the bone material marrow test, a Kruskal–Wallis test was used, and in the case of significant differences, a Mann–Whitney (M–W) U test ($p < 0.05$) was performed. In addition, the Spearman rank correlation coefficients were determined between trace elements occurring in the material and between the different metals studied in different parts of the hip joint (cartilage, compact bone, and spongy bone, cartilage with bone compact).

Results

In the examined dog bone samples, the lowest water content was observed in compact bone (12.4%) and the highest in spongy bone (20%). The bases of the statistical data describing the concentrations of trace elements in the bone elements constituting the dog hip joint are presented in Table 1. In three samples from two dogs (one cartilage and two spongy bone samples), the Cu concentrations were many times higher than in other individuals (42.2, 21.2, and 51.4 mg/kg dw, respectively). When these samples were included in the statistical analysis, the coefficient of variation was about 300%. Therefore, calculations were also performed excluding these abnormal samples. In the remaining samples, Cu concentrations did not exceed 6 mg/kg dw. The conformity of distribution of metal concentrations in the examined bone material with a normal distribution was examined using a Kolmogorov–Smirnov test with a Lilliefors correction. Only the distribution of Zn concentrations in the cartilage and spongy bone was normal, while

Table 1 Concentration of metals (milligrams per kilogram dry weight) in three types of bone material from dogs from northwestern Poland and differences between them

Metal	Parameter	Cartilage		Compact bone	Cartilage with compact bone		Spongy bone		K–W test significance
Zn	AM ± SD	86.40±46.80		80.70±53.20	83.54±49.73		87.90±50.30		NS
	Med	88.50		67.60	78.20		97.80		
	Range	5.50–197.70		4.80–278.20	4.80–278.20		5.90–209.40		
	CV	54.20		65.90	59.50		57.30		
Cu[a]		$n=26$	$n=25$	$n=26$	$n=26$	$n=25$	$n=26$	$n=24$	NS
	AM ± SD	2.64±8.14	1.06±1.04	0.91±1.22	1.78±5.83	0.89±0.74	3.64±10.52	0.92±0.73	
	Med	0.77	0.75	0.45	0.58	0.76	0.79	0.70	
	Range	0.07–42.25	0.07–3.82	0.04–5.44	0.04–42.26	0.10–3.11	0.01–51.43	0.01–2.75	
	CV	308.40	97.90	134.10	328.20	82.70	289.60	78.70	
Pb	AM±SD	1.90±2.17		1.54±1.62	1.72±1.90		2.69±3.38		NS
	Med	0.99		0.77	0.88		0.91		
	Range	0.18–9.38		0.13–6.71	0.13–9.38		0.17–12.68		
	CV	113.90		105.4	110.60		125.50		
Cd	AM ± SD	0.09±0.06		0.11±0.12	0.10±0.09		0.09±0.07		NS
	Med	0.09		0.05	0.09		0.05		
	Range	0.02–0.19		0.04–0.55	0.02–0.55		0.01–0.21		
	CV	63.00		105.20	90.00		79.70		
Hg	AM ± SD	0.0071±0.0146		0.0041±0.007	0.0056±0.0115		0.0037±0.0079		$p<0.05$
	Med	0.0023		0.0022	0.0023		0.0015		
	Range	0.0012–0.0651		0.0009–0.0400	0.0009–0.0651		0.0009–0.0418		
	CV	204.5		180.1	206.5		212.4		

AM arithmetic mean, *SD* standard deviation, *CV* coefficient of variation in percent, *K–W* Kruskal–Wallis test, *p* level of significance, *NS* nonsignificant

[a] Analysis for all specimens ($n=26$) and after removing samples with exceptionally high Cu concentrations

Table 2 Conformity of distributions of metal concentrations in the bone material from dogs with the expected normal distribution (Kolmogorov–Smirnov test with the Lilliefors correction)

Material	Test and its significance for the conformity of distributions	Metal					
		Zn	Cu[a]		Pb	Cd	Hg
Cartilage			$n=26$	$n=25$			
	K–S	0.089	0.404	0.199	0.258	0.202	0.409
	p	>0.20	<0.01	>0.20	<0.05	<0.15	<0.01
	Lil. corr., *p*	<0.20	<0.01	<0.05	<0.01	<0.01	<0.01
	Distribution	Normal	Not normal	Not normal	Not normal	Not normal	Not normal
Compact bone	K–S	0.187	0.325		0.215	0.241	0.382
	p	>0.20	<0.01		>0.15	<0.05	<0.01
	Lil. corr., *p*	<0.01	<0.01		<0.01	<0.01	<0.01
	Distribution	Not normal	Not normal		Not normal	Not normal	Not normal
Spongy bone			$n=26$	$n=24$			
	K–S	0.141	0.386	0.163	0.283	0.237	0.382
	p	>0.20	<0.01	>0.20	<0.05	<0.10	<0.01
	Lil. corr., *p*	<0.15	<0.01	<0.15	<0.01	<0.01	<0.01
	Distribution	Normal	Not normal	Normal	Not normal	Not normal	Not normal

K–S Kolmogorov–Smirnov test, *Lill. corr.* with the Lilliefors correction, *p* level of significance

[a] Analysis for all specimens ($n=26$) and after removing samples with exceptionally high Cu concentrations

in the remaining cases, there was no such conformity (Table 2). The removal of samples with abnormally high Cu concentrations in spongy bone reverted the distribution to normal.

As in most cases, the distribution of data deviated from the expected normal distribution; for the comparison of mean concentrations of metals in the examined types of bone material, we used a nonparametric Kruskal–Wallis test. Only for mercury did it reveal the existence of statistically confirmed differences (Table 2). The next step was to compare concentrations of this metal between the pairs of means using a nonparametric Mann–Whitney U test.

Zinc concentration (median) in the four types of dog bone material ranged from ~70 to 100 mg/kg dw in compact bone and spongy bone, respectively. Medians showing the mean concentrations of copper in those materials did not exceed

Table 3 Comparison of metal concentrations in analogous dog bone materials between two age groups

Materiał	Parameter	Zn	Cu[a]	Pb	Cd	Hg
CF1, $n=9$						
			$n=8$			
Cartilage	AM ± SD	102.8±52.8	1.90±1.34	0.480±0.259	0.076±0.060	0.0161±0.0229
	Med	80.9	2.43	0.490	0.041	0.0048
	Range	39.7–197.8	0.16–3.82	0.176–1.031	0.022–0.170	0.0011–0.0651
	CV	51.4	70.4	53.9	82.7	42.2
Compact bone	AM ± SD	108.8±71.02	1.57±1.89	0.439±0.298	0.134±0.166	0.0072±0.0119
	Med	67.6	0.53	0.355	0.072	0.0029
	Range	60.0–278.2	0.19–5.44	0.130–1.106	0.024–0.550	0.0010–0.0380
	CV	65.3	120.7	67.9	123.8	165.3
			$n=7$			
Spongy bone	AM ± SD	79.5±65.8	0.99±1.04	1.708±4.122	0.055±0.057	0.0069±0.0133
	Med	67.8	0.56	0.427	0.039	0.0014
	Range	5.85–209.4	0.01–2.75	0.017–12.68	0.016–0.203	0.0009–0.0418
	CV	82.8	104.7	241.3	104.8	193.6
CF2, $n=17$						
			$n=16$			
Cartilage	AM ± SD	77.0±42.5	0.65±0.53	2.654±2.357	0.1001±0.055	0.0024±0.0009
	Med	90.8	0.431	1.568	0.098	0.0021
	Range	5.1–134.4	0.07–2.04	0.395–9.378	0.025–0.193	0.0013–0.0041
	CV	54.7	81.3	88.8	55.0	37.1
Compact bone	AM ± SD	65.8±34.9	0.57±0.43	2.12±21.740	0.096±0.079	0.0023±0.0013
	Med	67.6	0.37	1.745	0.092	0.0021
	Range	4.8–135.1	0.004–1.69	0.532–6.706	0.027–0.346	0.0009–0.0060
	CV	53.1	75.6	82.0	82.4	56.4
			$n=15$			
Spongy bone	AM ± SD	93.6±37.7	0.87±0.49	3.213±2.91	0.111±0.07	0.0021±0.0010
	Med	102.5	0.80	1.6800	0.095	0.0016
	Range	13.5–159.8	0.07–1.47	0.263–9.378	0.013–0.208	0.0010–0.0037
	CV	40.2	55.7	90.7	66.8	48.0
CF1 vs. CF2						
Cartilage	U	NS	28.0	13.0	NS	31.0
	p		0.02	0.001		0.02
Compact bone	U	NS	NS	8.0	NS	NS
	p			0.001		
Spongy bone	U	NS	NS	25.0	40.0	NS
	p			0.01	0.05	

AM arithmetic mean, *SD* standard deviation, *CV* coefficient of variation in percent, *CF1* <9 years, *CF2* >9 years, *U* Mann–Whitney *U* test, *p* level of significance, *NS* statistically nonsignificant difference

[a] Analysis for all specimens ($n=26$) and after removing samples with exceptionally high Cu concentrations

Table 4 Spearman's rank correlation coefficient between metal concentrations in the examined dog bone materials ($n=26$)

Correlation	Cartilage	Compact bone	Cartilage with compact bone	Spongy bone
Zinc with:				
Cu	0.476*	0.566**	0.564***	0.631***
Cd	NS	NS	NS	0.458*
Pb	NS	NS	NS	0.482*
Hg	NS	NS	NS	0.420*
Copper with:				
Pb	NS	0.523***	NS	0.523**
Cd	NS	NS	NS	0.415*
Hg	NS	NS	NS	0.559**
Lead with:				
Cd	0.418*	NS	0.317**	0.842****
Hg	−0.486*	NS	−0.317*	NS
Cadmium with:				
Hg	NS	NS	NS	NS

NS statistically nonsignificant

*$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

0.8 mg/kg dw, but artithmetic means were a few times higher and reached >2.6 and >3.6 mg/kg dw in cartilage and in spongy bone, respectively. In both these cases, the Cu concentrations were calculated using all data (Table 1).

Among the three examined toxic metals—Pb, Cd, and Hg—in all the examined types of bone material, lead had the greatest concentration, followed by Cd and then Hg. The Mann–Whitney U test revealed distinct differences in two cases. Mercury concentration in compact bone was 47% greater than in spongy bone ($U=171.0$; $p<0.01$). In the cartilage, there was a more than 50% greater Hg concentration compared with spongy bone (0.0023 and 0.0015 mg Hg/kg dw), which was also confirmed by the M–W U test ($U=199.0$; $p<0.02$). In the cartilage and spongy bone, Hg concentrations were similar (about 0.0020 mg/kg dw).

Among the five examined elements, the greatest concentration (median) in the bone elements was observed for Zn, and the lowest for Hg. In the cartilage and spongy bone, metal concentrations could be arranged in the following descending order: Zn > Pb > Cu > Cd > Hg, and in compact bone in a slightly different series: Zn > Pb > Cd > Cu > Hg.

The collected samples were subjected to statistical analysis allowing for the sex and age category of the animals (two age categories). There were no statistically significant differences between the analogous bone samples from female and male dogs. There were some differences between the two age categories [CF1 <9 years ($n=9$) and CF2 >9 years ($n=17$)]. Table 3 presents the mean metal concentrations in three types of bone material and the results of comparative statistical analysis. In dogs older than 9 years (CF2), Pb concentrations in all types of bone material were greater than in younger dogs (CF1). The greatest and statistically significant differences ($p<0.001$) occurred in compact bone—in the CF2 dogs, it was about five times greater than in the corresponding materials from the CF1 dogs (<9 years) by 220% and over 290%, respectively (Table 3).

Such statistically confirmed differences existed also with regard to Cd concentrations in spongy bone ($p<0.05$) and Hg in cartilage ($p<0.02$). Cadmium concentration was more than 140% greater in older dogs than younger dogs, but Hg concentration was greater in younger dogs—by about 130%. In order to examine the relationship between the age of the dogs and the concentrations of individual metals in the cartilage, compact bone, and spongy bone, we used Spearman correlation coefficients and determined their significance. The greatest correlation coefficients were observed between age and lead concentration in the cartilage with compact bone ($p<0.001$) and slightly lower between age and Cd concentration in spongy bone ($r_s=0.511$; $p<0.01$). Mercury and copper negatively correlated with the age of the dogs: $r_s=-0.465$ ($p<0.02$) and -0.505 ($p<0.01$), respectively.

Furthermore, analysis concerned the relationships between the metal concentrations in the same type of material; Spearman correlation coefficients and their significances are presented in Table 4. It must be emphasized that in spongy bone, apart from Pb and Hg, all the examined metals significantly correlated with one another, while for Zn and Cu, there were significant correlations in all types of examined bone

Table 5 Spearman's rank correlation coefficient between the Zn and Cu concentrations in the examined dog bone materials ($n=26$)

	Zn C	Zn CB	Zn SB	Zn C + CB	Cu C	Cu CB	Cu SB	Cu C + CB
Zn C	–	NS	−0.530**	0.552**	0.525**	−0.389*	−0.480*	NS
Zn CB		–	NS	0.707****	NS	0.547**	NS	0.407*
Zn SB			–	NS	NS	NS	0.635***	NS
Zn C + CB				–	0.480*	NS	NS	0.453*
Cu C					–	NS	NS	0.830****
Cu CB						–	NS	0.409*
Cu SB							–	NS
Cu C + CB								–

C cartilage, *CB* compact bone, *C + CB* cartilage with compact bone, *SB* spongy bone, *NS* statistically nonsignificant

*$p<0.05$; **$p<0.01$; ***$p<0.001$****$p<0.0001$

material. These relationships were synergic in nature. Correlation coefficients were the greatest in spongy bone—between Pb and Cd (r_s=0.842; $p<0.0001$) and between Zn and Cu (r_s=0.631; $p<0.001$). For the remaining elements, it ranged from 0.415 to 0.559 (Table 4). In cartilage and in cartilage with compact bone, there were two positive correlations: between Zn and Cu and between Pb and Cd; there was one negative correlation between Pb and Hg. In compact bone, there were only two significant relationships—between Zn and Cu and between Cu and Pb.

A separate set of statistical analyses concerned relationships between essential and toxic elements in different types of bone material (Tables 5 and 6, respectively). The analyses revealed significant synergic and antagonist correlations (Table 5). The greatest positive correlation coefficients were observed between Cu concentrations in cartilage and in cartilage with compact bone (r_s=0.830; $p<0.0001$) and between Zn in compact bone and in cartilage with compact bone (r_s=0.707; $p<0.0001$). High negative correlation coefficients were observed between Zn in cartilage and in spongy bone (r_s=−0.530; $p<0.01$) and between Zn in cartilage and Cu in spongy bone (r_s=−0.480; $p<0.05$). Among correlations between highly toxic elements in the examined bone materials, the most notable are those for which Spearman correlation coefficients exceeded 0.80 (Table 6). These include synergic relations between Pb in cartilage and Pb in compact bone, cartilage with compact bone and spongy bone. Similar relations were also observed between Pb in compact bone and Pb in cartilage with compact bone, and between Pb in spongy bone and Pb in cartilage with compact bone. High values of r_s could also be observed between (1) Pb and Cd concentrations in spongy bone, (2) between Cd in spongy bone and Cd in cartilage with compact bone, (3) between Hg in cartilage and Hg in cartilage with compact bone, and (4) between Hg in compact bone and Hg in cartilage with compact bone. For the strongest four correlations between concentrations of toxic metals in the dog bone material, regression equations were calculated.

Discussion

In available literature, there is little information on Zn and Cu concentrations in the articular and bone elements of limbs of mammals, including Canidae (Table 7). Metal concentrations are usually expressed in either dry weight or wet weight (dw or ww). In order to facilitate a comparison of various reports of metal concentrations in bones, in our paper, we performed calculations assuming a standard water content of 12%.

In our previous research on 15 dogs from NW Poland, Zn concentration in cartilage and spongy bone was ~80 mg/kg, and Cu concentration was significantly higher in cartilage than

Table 6 Spearman's correlation rank coefficient between the concentration of Pb, Cd, and Hg in different dog bone materials (n=26)

	Pb C	Pb CB	Pb SB	Pb C + CB	Cd C	Cd CB	Cd SB	Cd C + CB	Hg C	Hg CB	Hg SB	Hg C + CB
Pb C	–	0.836****	0.876****	0.975****	0.487*	NS	0.707***	NS	−0.448*	NS	NS	−0.418*
Pb CB		–	0.716***	0.921****	0.397*	NS	0.571	NS	−0.410*	NS	NS	NS
Pb SB			–	0.839****	NS	NS	0.830****	NS	NS	NS	NS	NS
Pb C + CB				–	0.488*	NS	0.661***	NS	−0.429*	NS	NS	−0.413*
Cd C					–	0.577**	0.475*	0.722****	NS	NS	−0.422*	NS
Cd CB						–	0.479*	0.936****	NS	NS	NS	NS
Cd SB							–	0.471*	NS	NS	NS	NS
Cd C + CB								–	NS	NS	NS	NS
Hg C									–	0.621***	0.449*	0.924****
Hg CB										–	0.605***	0.818****
Hg SB											–	0.593**
Hg C + CB												–

C cartilage, *CB* compact bone, *C + CB* cartilage with compact bone, *SB* spongy bone, *NS* statistically nonsignificant

*$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

Table 7 Comparison of trace elements concentrations (milligrams per kilogram) in dry weight and wet weight in Canidae from various parts of the world

Species	Place	Material	Zn	Cu	Pb	Cd	Hg	Age	Sex	n	dw or ww	Source
Domestic dog *Canis lupus familiaris*	Poland, Zachodniopomorskie province	Cartilage, spongy bone	81 80	2.3 1.8	–	–	–	–	F + M	15	dw	[10]
		Cartilage	–	–	2.28	0.13	–	–	F + M	15	dw	[22]
Wild red fox *Vulpes vulpes* (L., 1758)	Poland, Zachodniopomorskie province	Compact bone, spongy bone	–	2.8 0.9	–	–	–	–	F + M	24	dw	[11]
		Cartilage	–	–	1.96	0.16	–	–	F + M	24	dw	[22]
	Hiszpania, Donana Park	Bone	142.1	4.2	0.385	nd	0.012	imm ad	F + M	17	dw	[12]
	USA, San Diego, Zoo	Rib	138.0 (121.4)	–	–	–	–	–	F + M		ww (dw)	[13]
Arctic wolves *Canis lupus arctos*	Canada, Yukon	Bone	–	–	2.12	–	–	imm ad	F + M	13	dw	[16]

F female, *M* male, *imm* immaturus, *ad* adultus, *nd* not detected, *dw* dry weight, *ww* wet weight

in the spongy bone (2.3 and 1.8 mg/kg dw, respectively) [10]. In this current paper, Zn concentration was ~22% lower, and Cu concentrations in the analagous materials were similar, yet distinctly lower compared to the results obtained by Lanocha et al. [10].

In the fox from northwestern Poland, Cu concentration in the compact bone of the hip joint was three times greater than in domestic dogs in the current study, while in spongy bone, the Cu concentration was similar [11]. In bones of the fox from Spain [12], Cu concentration was about five times greater than in the compact bone of domestic dogs from NW Poland. Donana Park in Spain, where the animals examined by Millan et al. [12] came from, was the site of an environmental disaster in 1998.

Damage in containers with mine waste (situated above the park) leaked water highly contaminated with heavy metals, which permeated to surface and ground waters. The transfer to the food chains resulted in a significant increase in trace element concentrations. In the ribs of foxes from the San Diego Zoo (California, USA), Zn concentration was about 1.5 greater [13] than in cartilage of domestic dogs from NW Poland.

In our previous study, Lanocha et al. [10] determined Pb and Cd in hip joint cartilages of the dog and fox. Lead concentration in the cartilage of dogs was about 1.2 greater than in the dogs from this current study. Cadmium concentration in the cartilage of the dog was 1.4 greater than in the current study. In comparison, in the fox from southern Spain (Donana Park), Pb concentration in the bones of the fox was lower than in the cartilage, compact, and spongy bones of the domestic dogs from NW Poland (five, four, and seven times, respectively) [12]. It could indicate greater lead contamination in NW Poland.

It must however be emphasized that lead accumulates more intensely in the cartilage than in the bones of warm-blooded vertebrates, which is indicated by many reports [8, 14, 15]. In literature, we have found only one report of more lead in the bones of Canidae—the wolf from Canada [16]. This predator, preying on medium- and large-sized mammals, often already shot by hunters, has been shown to have lead concentration at 2.12 mg/kg ww, roughly 2.6 mg/kg dw. This concentration was similar to that observed in the bones of the dog in Poland [10]. In the spongy bone of the dog in West Pomeranian province, lead concentration ranged from 0.01 to 12.68 mg/kg dw. The results of research on lead concentration in predatory mammals show differences between species resulting from environmental lead concentration and the risk of intoxication with lead shot present in the bodies of prey shot by hunters.

Mercury in bones is very rarely determined in literature, and the process of Hg accumulation in bones is rather poorly known. In dogs from NW Poland, Hg concentrations were one order of magnitude lower than concentrations determined by Spanish researchers (Table 7).

In the current study, the age of the dogs affected the concentration of Cu, Pb, Cd, and Hg in the bones of the hip joint. Similarly, humans have also been observed to have increased concentration of toxic metals in bones with age [17, 18]. In this work, we observed that the statistically significant Cu concentration in the cartilage was lower in older specimens (>9 years) than in younger (<9 years) ones. In younger dogs (<9 years), lead concentration in those materials was about five times less than in older dogs (>9 years), also confirmed statistically. Zaichick et al. [18], in their research in Russia, observed higher Pb and Cd concentrations in humans under 35 years of age, compared to the older group (from 35 to 55 years). Kuo et al [17] also observed a difference in Pb concentration in the bones of the hip joint between the groups of patients from Taiwan below and above 60 years of age (6.13 and 7.62 mg Pb/kg dw, respectively). During ossification, lead accumulation is

enhanced in the spongy bone, and in 50+ in the compact bone. The elderly often suffer from calcium metabolism disorders which make it more difficult for Pb to accumulate in the spongy bone [15].

In this current research, maximum Pb concentration in the bone material from domestic dogs older than 9 years was more than 12 mg/kg dw. It can result from the environmental contamination with lead, coming mainly from car fumes, as in Poland non-leaded petrol was first introduced as late as mid-1990s. A complete ban on gasoline with antiknock substances was introduced in 2005 [19, 20]. It is estimated that each kilometer of urban road in Poland produced about 8 kg of lead, and the range of its deposition beyond the road was even 100. The examined dogs came from the urban city of Szczecin, they were mostly old, and some were even 19 years old.

It seems possible that the lead from petrol could have accumulated in their osseous system throughout their entire life, with the highest intensity in the times when petrol contained high amounts of lead. Dogs from Canadian cities in the 1980s were considered a good bioindicator of lead contamination, mainly resulting from car fumes. It was shown that Pb blood levels in dogs were very similar to those determined in children—35 μg Pb/100 ml [21].

Conclusions

1. In the bone elements of the hip joint of the dog, the greatest medians of concentrations were observed for zinc, and the lowest for mercury (98 mg Zn/kg and 0.0015 mg Hg/kg dw, respectively).
2. In the cartilage and spongy bone of the dog hip joint, the concentrations of the metals could be arranged in the following descending order: Zn > Pb > Cu > Cd > Hg, and slightly differently in the compact bone: Zn > Pb > Cd > Cu > Hg (medians for Zn and Hg were from about 70 to 0.002 mg/kg dw, respectively).
3. A comparison of the metal concentrations in the obtained bone materials from all the specimens ($n=26$) showed distinct differences only in mercury concentration—between the spongy and compact bones (greater in the compact bone) and between Hg concentrations in the cartilage and spongy bone of the dog (greater in cartilage).

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