Lead, Cadmium and Other Metals in Serum of Pet Dogs from an Urban Area of NW Poland

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Abstract This study was designed to evaluate the degree of exposure of pet dogs from an urban area of NW Poland to selected metals, including toxic Cd and Pb. The study was conducted on a group of 48 healthy dogs. The serum concentration of the analysed elements followed the order Fe > Al > Zn > Cu > Mn > As > Sr > Pb > Cd > Cr > Ni > V. The presence of cadmium and lead was found in all the serum samples tested. The average contents of these elements were 0.309 and 0.489 μg/mL. The factors that played the greatest role in the intake of the analysed elements were diet and breed-dependent size of dogs. Small-sized dogs had higher concentrations of all elements compared with large dogs, with statistically significant differences noted for Cu, Pb, Cd and Sr. It was also found that dogs receiving commercial and mixed food had more metals in serum compared with dogs on homemade food (except strontium). The present study showed elevated concentrations of some heavy metals (Pb, Cd, Fe and Cu) in serum of pet dogs, which is probably due to the excess elemental load of this area. Given that no information is available on the concentrations of strontium, vanadium and aluminium in dogs, further research is necessary to determine certain reference values which would allow for an easier interpretation of results and evaluation of exposure to these elements.

Introduction

Chronic environmental exposure to even low concentrations of elements having no biological function and to elements necessary for normal body function (bioelements) that have a narrow range of physiological activity and the concentrations of which are higher than the geochemical background poses a health threat to humans and animals. In both cases, exposure is associated with specific health consequences, resulting, among others, from the mutagenic, carcinogenic or immunosuppressive properties of these elements [1–3]. Most metals easily cross the placental–foetal barrier to affect the central nervous system [2, 4]. Especially the organic forms of metals (e.g. Hg and Pb) have a high affinity for nervous tissue, whilst the inorganic forms (Hg, Pb, Cd and Ni) are nephrotoxic. In addition, metals can interact (chemically or biologically). An increased supply of one element may impair the absorption, metabolism or biological utilization of other elements [5].

Determining the concentrations of trace elements, including toxic metals, in abiotic components of the environment does not fully reflect the extent of threat to humans and animals because of their different bioavailability. For this reason, environmental studies often measure chemical

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residues in the organs or bodily fluids of animals that live in specific habitats [6–9]. The use of animals as bioindicators also makes it possible to determine the degree of environmental contamination and the extent of exposure to certain substances.

Animals living in close proximity with humans, such as dogs and cats, are beginning to play an increasingly important role in biomonitoring studies [10–12]. Companion animals are useful indicators of human exposure to various toxicants because they live in the same environment (air, water, often food) as their owners and are exposed to the same pollutants. Dogs are used not only as bioindicators of environmental contamination but also as sentinels, i.e. early warning indicators of threats and their consequences for human health [13]. This is possible because they show analogous responses to certain toxic agents as humans whilst having shorter latency periods [14]. Reif et al. [15] report that many forms of canine cancer resemble their human analogues in biologic behaviour, pathologic expression and risk factors.

Many studies have been published to date concerning the concentrations of metals, in particular toxic metals, in human blood and hair, and in critical organs (liver and kidneys) of animals. In ecotoxicological studies, liver and kidneys are used most often as indicators of long-term environmental exposure of animals due to the continuous bioaccumulation of xenobiotics in these organs. Meanwhile, blood serum is a short-term indicator of the body's exposure to certain elements and enables their current intake to be determined. Few data are available on the serum concentration of these elements in dogs, which share the human environment. In the available literature, there is also a paucity of information on the blood/serum concentrations of nickel, vanadium, arsenic and aluminium, which are attracting increasing interest from a toxicological point of view.

Environmental quality in urban areas depends primarily on city size, type of industry and the location of pollution emitters. Szczecin is one of Poland's largest cities. It is exposed to emissions from industrial (chemical) and power plants, low emissions from municipal operations (local boiler plants and home hearths), transport emissions and emissions from neighbouring areas (German oil refinery and waste incineration plant). Among the pollutants released into the environment by these emitters are heavy metals, including toxic elements, which are a potential threat to animals and humans living in this area.

Therefore, this study was designed to evaluate the degree of current exposure of pet dogs from an urban area of NW Poland to selected metals, including toxic Cd and Pb, based on their concentrations in serum and taking into account factors such as sex, age, diet and size of the dog.

Materials and Methods

Animals

The study was conducted on a group of 48 healthy dogs. The test animals and the data on their breed, health status, sex, age and diet were obtained from a veterinary clinic in Szczecin (northwestern Poland). There were 22 males, 20 females and 6 unrecorded, aged between 5 months and 12.5 years. Animals were divided into four groups according to age: <1 year ($n=6$), 1–5 years ($n=16$), 5–9 years ($n=12$) and over 9 years ($n=7$). To evaluate the effect of diet on the concentration of the analysed elements, dogs were divided into three groups, i.e. those receiving homemade ($n=12$), commercial ($n=9$) and mixed food ($n=21$). In addition, dogs were classified according to breed and the associated size into small ($n=14$, average body weight of 11.5 ± 5.1 kg) and large ($n=29$, average body weight of 36.8 ± 10.6 kg).

Animals originated from Szczecin and its surroundings. Szczecin (53.430° N, 14.559° E) lies in northwestern Poland, in the western part of the Zachodniopomorskie province near the Polish–German border. Two power plants, a heat and power station, cast iron works, aluminium foundry and a chemical plant are situated in the area from which the investigated dogs originated. The chemical plant specializes in the production of mineral (including phosphatic) fertilisers, which are manufactured from phosphorite, a rich source of both cadmium and lead. Coal burning in domestic hearths and local boiler plants, as well as transport, is also not without consequences on the degree of environmental contamination with heavy metals in the area under study.

Sampling

Blood was collected at a veterinary clinic during routine examinations. Blood samples were taken from the cephalic vein into tubes without an anticoagulant and left at room temperature to coagulate. After clotting, the samples were centrifuged and the serum obtained was separated and frozen at −20°C until chemical analysis.

Chemical Analysis

The serum samples were mineralized in 4.5 mL of 65 % nitric acid (Suprapure, Merck, Darmstadt, Germany) and 0.5 mL of 30 % hydrogen peroxide (Suprapure, Merck) in an Anton Paar Multiwave microwave (Anton Paar Ltd., Hertford, UK) that allows for temperature and pressure control in the vessels during mineralization. The oven programme was as follows: 0–5 min, generator power increased linearly from 100 to 600 W; 5–10 min, power kept constant at 600 W; 10 min, power increased to 1,000 W and

kept constant until 20 min (provided that pressure and temperature did not reach the threshold limit values of 75 MPa and 300°C, respectively); 20–35 min, the system was cooled.

The prepared samples were analysed by inductively coupled plasma–atomic emission spectrometry using an Optima 2000 DV spectrometer (Perkin Elmer Inc.). Measurements were made along the plasma in axial direction. The elements were quantified using calibration curves plotted from multi-element standard solution (ICP Multi-element Standard IV, Merck). The concentration of elements was determined directly from calibration plots after correction of the absorbance value obtained from blank sample. The limit of detection (3σ of digest blank solution) was as follows: Fe, 0.1 μg/L; Zn, 0.2 μg/L; Cu, 0.4 μg/L; Cd, 0.1 μg/L; Pb, 1.0 μg/L; Ni, 0.5 μg/L; Cr, 0.2 μg/L; Al, 1.0 μg/L; As, 1.5 μg/L; Sr, 0.05 μg/L; V, 0.5 μg/L; and Mn, 0.1 μg/L.

The accuracy of the analytical procedure was tested by determining the analysed elements in reference material Seronorm (Trace Elements Serum L-1, Sero AS) together with the samples. The results of this study are presented in Table 1. The analytical procedure was also checked by analysis of the blank samples. Blank digests ($n=4$) were run with a series of serum samples, and no major interferences were found in the quantitative element analysis.

Statistical Data Analyses

Statistical analysis of the data was performed using Statistica, version 10 (Statsoft Inc.). Prior to analyses, data were investigated to determine their distribution using the Kolmogorov–Smirnov test. The concentrations of the elements

Table 1 Validation of the analytical procedure using Seronorm Trace Elements Serum L-1

Element	Certified level (μg/L)	Obtained level (μg/L)	% RSD	% Recovery
Fe	1,390	1,369	1.1	98.4±1.0
Zn	1,738	1,684	0.98	96.8±0.91
Cu	1,691	1,589	3.2	93.9±3.3
Ni	5.4	5.1	3.9	94.0±3.9
Al	33.6	32.2	5.9	95.8±5.6
Mn	15.0	15.8	3.5	105.0±3.2
Cr	1.5	1.4	1.7	92.8±1.6
Cd	0.126[a]	0.131	6.6	104.3±6.2
Pb	1.04[a]	1.10	3.6	106.1±3.3
As	0.47[a]	0.51	1.3	108.3±1.1
Sr	26.1[a]	24.7	3.0	94.4±2.92
V	0.96[a]	0.89	4.5	92.5±4.8

[a] Approximate concentration given by the manufacturer

were log-transformed to attain a normal distribution of the data. The effect of age, sex, diet and body size on the concentration of the analysed elements in the serum of dogs was determined using analysis of variance (general linear model). Where the effect of a certain factor was significant, statistical significance of differences between subgroups was tested. The relationships between the serum concentrations of the individual elements were calculated using Pearson's correlation analysis (r). All data are expressed throughout as an arithmetic mean, geometric mean (GM), median and standard error (SE).

Results

The results of determination of the analysed elements in serum of dogs are presented in Table 2. The presence of cadmium and lead was found in all the serum samples tested. Serum cadmium concentration ranged widely from 0.099 to 0.645 μg/mL and lead concentration from 0.191 to 0.910 μg/mL. The average contents of these elements were 0.309 and 0.489 μg/mL, respectively. Although serum cadmium concentration was higher in females than in males (0.591 vs. 0.419 μg/mL), statistical analysis did not show a significant difference ($p<0.05$). No differences between sexes were also found for cadmium. Iron concentration averaged 1.690 μg/mL, with males having a higher concentration of this element than females. There were large fluctuations in the Fe content (range=0.786–2.181 μg/mL). The contents of Al, Zn and Cu were at a level similar to that of iron. Manganese, strontium and arsenic concentrations were similar (0.683, 0.646 and 0.556 μg/mL, respectively). In the case of strontium, the concentration expressed as arithmetic mean is twice as high as the median. The maximum concentration of this element was as high as 10.200 μg/mL. The lowest serum concentrations were characteristic of nickel, chromium and vanadium (0.207, 0.249 and 0.247 μg/mL, respectively). In general, the serum concentration of the analysed elements followed the order Fe > Al > Zn > Cu > Mn > As > Sr > Pb > Cd > Cr > Ni > V.

Statistical analysis showed that sex had no effect on the metal concentrations in the serum of dogs and that age had a significant ($p<0.05$) effect only for strontium (Table 3 and Fig. 1). The factors that played the greatest role in the intake of the analysed elements were diet and breed-dependent size of dogs. Small-sized dogs had higher concentrations of all elements compared with large dogs, with statistically significant differences noted for Cu, Pb, Cd and Sr (Fig. 2). It was also found that dogs receiving commercial and mixed food had more metals in serum compared with dogs on home-made food (except strontium; Fig. 3). Dogs fed with a mixed diet had significantly ($p<0.05$) higher Fe, Mn, Cr, Pb and

Table 2 Serum toxic and other metal concentration in the dogs considered in this study

	Concentrations (µg/mL)											
	Cu	Fe	Mn	Cr	Pb	Cd	Sr	Ni	Al	As	Zn	V
Total												
x	1.363	1.690	0.683	0.249	0.489	0.309	0.646	0.207	1.649	0.556	1.523	0.247
SE	0.051	0.077	0.043	0.016	0.065	0.048	0.287	0.013	0.098	0.142	0.056	0.077
Median	1.350	1.642	0.657	0.239	0.388	0.282	0.305	0.200	1.556	0.381	1.495	0.130
GM	1.330	1.614	0.642	0.234	0.409	0.296	0.335	0.196	1.559	0.385	1.485	0.151
Min	0.820	0.786	0.302	0.112	0.191	0.099	0.144	0.099	0.736	0.060	0.612	0.060
Max	2.305	2.181	1.645	0.595	0.910	0.645	10.200	0.497	3.760	5.913	2.584	2.648
Female												
x	1.419	1.491	0.721	0.260	0.591	0.336	1.136	0.208	1.775	0.485	1.616	0.138
SE	0.090	0.068	0.073	0.026	0.092	0.042	0.580	0.020	0.162	0.071	0.090	0.014
Median	1.356	1.234	0.654	0.239	0.377	0.274	0.308	0.197	1.593	0.363	1.551	0.124
GM	1.368	1.219	0.665	0.240	0.423	0.280	0.419	0.194	1.659	0.403	1.572	0.128
Min	0.830	0.786	0.309	0.112	0.191	0.099	0.173	0.099	0.927	0.125	1.108	0.060
Max	2.305	1.955	1.645	0.595	0.910	0.645	10.200	0.497	3.760	1.275	2.584	0.325
Male												
x	1.342	1.783	0.666	0.244	0.419	0.302	0.293	0.209	1.581	0.637	1.460	0.349
SE	0.045	0.094	0.043	0.016	0.027	0.049	0.016	0.013	0.100	0.227	0.058	0.124
Median	1.357	1.720	0.673	0.245	0.398	0.274	0.294	0.201	1.554	0.378	1.522	0.138
GM	1.323	1.702	0.635	0.233	0.401	0.285	0.283	0.200	1.510	0.378	1.426	0.177
Min	0.820	0.920	0.302	0.112	0.212	0.104	0.144	0.101	0.736	0.060	0.612	0.065
Max	1.740	2.181	1.312	0.472	0.823	0.645	0.508	0.393	3.004	5.913	1.963	2.648

Zn concentrations than dogs receiving homemade food. In the case of dogs receiving commercial food, these differences

Table 3 Results of statistical analysis on the effects of age, sex, diet and body size on the content of the analysed elements in the serum of dogs

	Factors			
	Age	Sex	Diet	Size
Cu	ns	ns	ns	$F=7.517; p<0.05$
Fe	ns	ns	ns	ns
Mn	ns	ns	ns	ns
Cr	ns	ns	ns	ns
Pb	ns	ns	ns	$F=5.004; p<0.05$
Cd	ns	ns	$F=7.396; p<0.005$	$F=30.338; p<0.001$
Sr	$F=4.111; p<0.05$	ns	$F=3.226; p<0.001$	$F=47.215; p<0.001$
Ni	ns	ns	ns	ns
Al	ns	ns	ns	ns
As	ns	ns	ns	ns
Zn	ns	ns	ns	ns
V	ns	ns	ns	ns

ns non-significant

concerned Mn, Cr, Ni, Cd and Zn. No significant differences were found between dogs receiving the commercial and mixed food.

## Discussion

Serum is regarded as a short-term indicator of the body's status of certain elements, reflecting their current intake. However, in the available literature, there are few studies on toxic elements and other metals in the serum of dogs [14–16]. Most studies deal with the concentration of elements in liver and kidneys [10, 17, 18], hair [19–22] or blood [12, 23]. For this reason, it is difficult to evaluate our results, especially with regard to some elements, namely Ni, Sr, V, Al and As. Most studies concern a limited number of elements, with the primary focus on toxic metals.

The present study revealed that serum cadmium concentrations in dogs from NW Poland are higher than the values reported by Park et al. [16] for dogs from Korea and by Mert et al. [15] for dogs from Turkey (Table 4). These authors found that the serum concentration of this element was 0.22 ± 0.01 µg/mL. The average content of lead in our study was lower than that reported by Park et al. [16], who observed that serum Pb concentration averaged $0.68\pm$

Fig. 1 Effect of age on serum metal concentrations (arithmetic mean ± SE). *Different letters* denote statistically significant differences (at *p*<0.05)
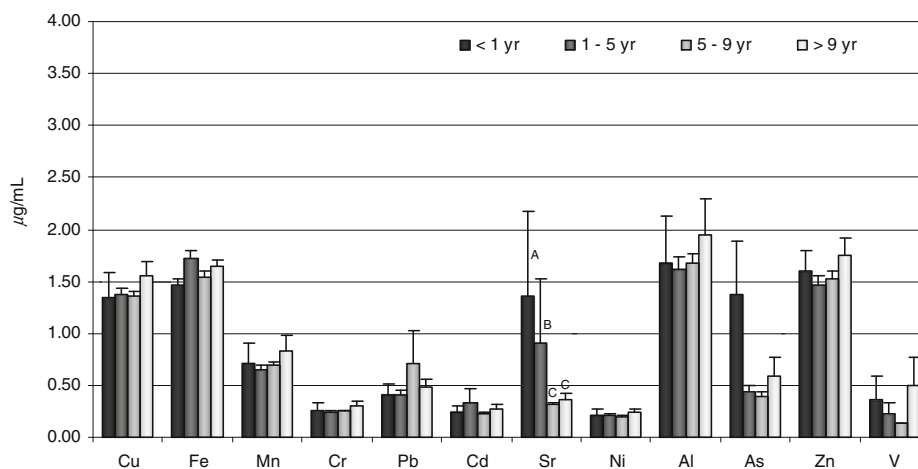
0.19 μg/mL and was >1 μg/mL for dogs over 1 year of age. In our study, the maximum concentration of this element was 0.945 μg/mL. According to Puls [24], Pb concentration of 0.3–0.8 μg/mL in whole blood of canids should be regarded as high. It is difficult to compare our results with these values because we made determinations in serum. However, approximately 69 % of the samples contained lead at a concentration exceeding 0.3 μg/mL, which is the upper limit of the normal range for dogs. In general, exceeding this limit does not produce toxicity symptoms [25]. Dogs from Canada [23] and the USA [12] were found to contain several-fold lower concentrations of this element in whole blood (0.03 and 0.15 μg/mL, respectively).

It is worth noting the elevated serum concentrations of Fe and Cu in dogs from NW Poland. Most of the analysed samples exceeded the normative values of 0.94–1.22 μg/mL for Fe and of 0.2–0.8 μg/mL for Cu [24]. In the case of iron, relatively high concentrations were also observed by Mert et al. [26].

The concentrations of manganese, nickel and zinc observed in these studies were also higher than the values reported in the literature (Table 4). The zinc content established in our study was also twice lower than that reported by Seyrek et al. [27] and Ural et al. [28]. However, a

comparison of our results with normative values for Zn [24] reveals that they were within standard limits. The concentration exceeded 2 μg/mL in only one sample.

There are scant data in the literature regarding chromium concentration in serum or blood of dogs. Research in this area was done by Park et al. [16] who found that the concentration of this element in dogs from Korea averaged 0.50±0.06 μg/mL. These values correspond to the maximum concentration observed in dogs from NW Poland.

There is no information available in the literature about the concentrations of strontium, vanadium, aluminium and arsenic in the blood/serum/plasma of dogs. These elements are widespread in the human environment, especially in urban and industrialized areas. They originate from the liquid (V) and solid fuel combustion processes (Sr and As) and from many branches of industry (paper, chemical, metallurgical, cement and others). Some of these metals (Al and As) are also found in pharmaceutical preparations. It is estimated that approximately 90 % of vanadium in industrialized cities comes from combustion of liquid fuels. The industry located in the vicinity of Szczecin (NW Poland) includes an oil refinery (Schwedt, Germany), a conventional power station and a chemical fertiliser plant which produces

Fig. 2 Concentrations of analysed metals in the serum of small- and big-sized dogs (arithmetic mean ± SE). Statistically significant differences in the concentrations of individual elements in the serum between large and small dogs have been marked as *p*<0.05 and **p*<0.001
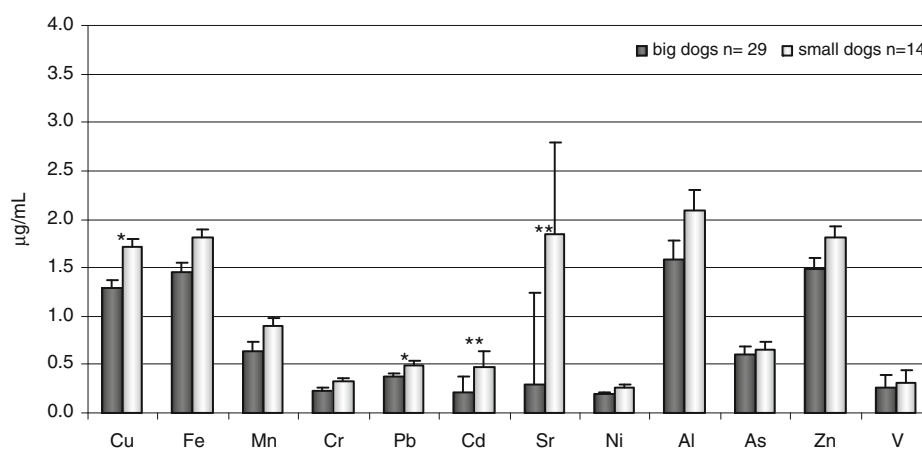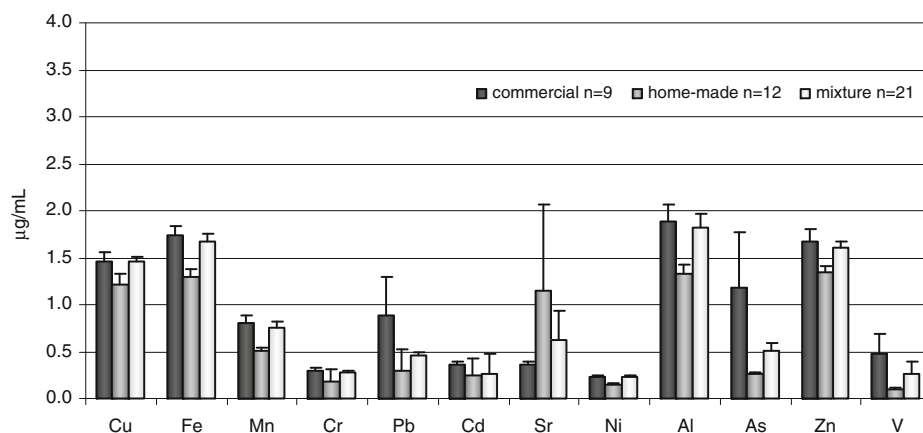
Fig. 3 Contents of the analysed elements in the serum of dogs depending on diet (arithmetic mean ± SE)

phosphate fertilisers. They are made from phosphorites, which contain large amounts of vanadium [29]. Increasing attention has recently been given to aluminium, which, for many years, was considered to be of low toxicity to humans. The availability of Al and the ongoing acidification of the environment contribute to the increased mobility of this metal and its toxic effects on the human body. It is now believed, however, that Al is toxic to mammals and accumulates in tissues. The anthropogenic sources of Al are particulate emissions from coal combustion and the cement industry [30]. It is safe to assume that the concentration of these elements will be strictly dependent on the place of origin of animals, on their location in relation to industrial plants and transport routes and on possible treatment. For the dogs in the study, the main source of this element seems to be heat and power station and aluminium foundry.

In unpolluted areas, diet should be the main determinant of metal intake and accumulation. Statistical analysis of our results showed, however, that this factor had no significant effect on the serum concentration of most metals, except cadmium and strontium. Likewise, López-Alonso et al. [18] concluded that the diet of dogs did not significantly affect the metal content of their organs (except lead in liver). However, certain differences were observed in the content of the analysed elements according to the type of food provided. Our study showed that dogs receiving commercial and mixed foods had higher serum concentrations of metals (including toxic metals) compared with dogs on homemade food (Fig. 3). These differences can probably be attributed

to the greater proportion of dry foods in the diet of the first two groups of dogs. Atkins et al. [31] provided evidence that dry dog food contains the highest concentrations of toxic elemental contamination in comparison with wet dog food. They also found that most of the trace element contaminant levels in the dog and cat foods are significantly higher than in the canned food samples made for human consumption. Higher cadmium and chromium concentrations in dry food were also noted by Duran et al. [32], although the differences were not so pronounced. López-Alonso et al. [18] also observed that dogs receiving commercial food were characterized by higher lead concentrations in the liver compared with dogs receiving homemade or mixed foods.

In general, the concentration of metals in different animal organs increases with age, which is related to their tendency for bioaccumulation. In such a case, age has a significant effect on increasing the concentration of most trace elements in internal organs and hard tissues [10]. However, this relationship is not observed in serum, which reflects current intake of metals. In our study, we noticed that neither age nor sex had a significant effect on elemental levels in serum of dogs, except strontium, the concentration of which was significantly higher in young dogs than in older dogs. The observed differences may result from the fact that the diet of young dogs contains more dairy products, which are rich in this element, and young animals have a better intestinal absorption of Sr, as reported by Sugihira [33]. Perhaps the higher concentration of strontium in the young animals may be connected also with more intense osteogenesis.

Table 4 Metal concentrations (in micrograms per milliliter) in serum and whole blood

	Cu	Fe	Mn	Cr	Pb	Cd	Ni	Zn	References
Serum	–	–	–	0.50	0.68	0.22	–	–	Park et al. [16]
Serum	0.84	1.32	0.009			0.003	0.014	0.74	Mert et al. [26]
Blood	–	–	–	–	0.25	–	–	–	Balagangatharathilagar et al. [25]
Blood	–	–	–	–	0.15	–	–	–	Bischoff et al. [12]
Blood	–	–	–	–	0.03	–	–	–	Kucera [23]

Our study assumed that dogs of small and large breeds must differ in the intake of various elements and that small dogs will be more vulnerable. Such inference follows from the fact that small dogs are lower to the ground and inhale larger amounts of dust, soil particles and deposited particulates, which carry heavy metals. It turned out that of all the factors analysed, body size had the largest effect on the concentration of elements (Cu, Pb, Cd and Sr).

Pet animals living in urban areas may be indicators of urban environment pollution whilst providing information about the potential exposure of humans to various toxic substances. The present study showed elevated concentrations of some heavy metals (Pb, Cd, Fe and Cu) in serum of pet dogs, which is probably due to excess elemental load of this area. Given that no information is available about the concentrations of strontium, vanadium and aluminium in dogs, further research is necessary to determine certain reference values, which would allow for an easier interpretation of results and evaluation of exposure to these elements.

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