

The Concentration of Manganese, Iron and Strontium in Bone of Red Fox *Vulpes vulpes* (L. 1758)

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Received: 7 August 2013 / Accepted: 26 August 2013 / Published online: 7 September 2013
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Abstract The aims of the study were to determine manganese (Mn), iron (Fe) and strontium (Sr) concentrations in fox bone samples from north-western Poland and to examine the relationships between the bone Mn, Fe and Sr concentrations and the sex and age of the foxes. In the studied samples of fox cartilage, cartilage with adjacent compact bone, compact bone and spongy bone, the concentrations of the analysed metals had the following descending order: Fe > Sr > Mn. The only exception was in compact bone, in which the concentrations were arranged in the order Sr > Fe > Mn. Manganese concentrations were significantly higher in cartilage, compact bone and cartilage with compact bone than in spongy bone. Iron concentrations were higher in cartilage and spongy bone compared with compact bone. Strontium concentrations were greater in compact bone than in cartilage and spongy bone. The manganese, iron and strontium concentrations in the same type of bone material in many cases correlated with each other, with the strongest correlation ($r > 0.70$) between Mn and Fe in almost all types of samples. In addition, concentrations of the same metals in different bone materials were closely correlated for Mn and Fe in cartilage and cartilage with adjacent compact bone, and for Sr in compact bone and cartilage with compact bone. In the fox from NW Poland, there were no statistically significant differences in Mn, Fe and Sr in any of the types of bone material between the sexes and immature and adult foxes.

Keywords Iron · Manganese · Red fox · Strontium · Poland

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Introduction

Manganese (Mn), iron (Fe) and strontium (Sr) can have a positive or adverse effect on the mammalian organism, depending on the concentration [1–4]. The current state of knowledge on toxicity concentrations for these elements obliges supervision of their concentrations in food and the environment [4]. Monitoring of these elements in the abiotic environment implemented under State Environmental Monitoring (SEM) can be greatly complemented by measurement of concentrations in the bones of free-living animals, especially as SEM focuses mainly on toxic metals in the air, water and soil (lead, cadmium, mercury, arsenic). Data on other metals, especially in the biotic environment, come primarily from specialised scientific publications.

Iron (Fe) and manganese (Mn) are essential nutrients. Iron is a component of blood that participates in the transport of oxygen. It also takes part in erythropoiesis, the formation of white blood cells and immune reactions influencing humoral and cellular immunity. Little is known of the role of Fe in the processes of bone and cartilage formation. A few reports indicate that Fe is necessary for the correct functioning of osteoblasts, cells responsible for bone formation [5].

In an experimental study, Fe-deficient rats exhibited increased intestinal absorption of Mn [6]. Manganese is a component of many enzymes and participates in reproduction, growth, metabolism of carbohydrates and fats, functioning of the immune system and formation of cartilage and bones [2]. It takes part in the process of ossification and the synthesis of mucopolysaccharides in cartilage, which are especially intense in young organisms [7, 8].

Strontium has not been shown to be essential for mammals [9]. It stimulates bone formation, inhibits osteoclastic activity [10, 11] and increases the synthesis of collagen and non-collagenous proteins [12].

There have already been some attempts to indirectly assess the state of the environment based on the analysis of the

mineral composition of bones of warm-blooded vertebrates, more often from bird samples than from wild or domesticated mammals [13, 14].

Indirect evaluation of environmental pollution by various substances, including the determination of elements in living organisms, is one of two major methods of ecotoxicological bioindication. This type of research has usually focused on the liver and kidney, the main detoxification organs in warm-blooded vertebrates, but in recent years, bone has started to be considered as a much more relevant biomaterial in the evaluation of chronic and long-term environmental exposure to metals [15, 16].

In ecotoxicological research, bone has been used mainly in the determination of lead, but there are also a few studies on Mn concentrations in bone, such as by Bjora et al. [17] in the bone of moose (*Alces alces*) in Norway and by Michalska et al. [18] and Sołtysiak [19] in bone samples collected from dogs and cats in Poland.

Importantly, the evaluation of the long-term effect of various elements requires the bones of mammals that are long-lived, easy to collect and living in and around people. Next to the aforementioned animals, the wild fox is another good source of bone samples: this hunted mammal is found in agricultural areas and forests, and partly in the cities and their outskirts, i.e. areas highly contaminated by various substances, including metals.

In our previous study, the zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd) and mercury (Hg) concentrations were determined in the hip joint of red foxes (*Vulpes vulpes*) from north-western Poland [20]. In the fox cartilage, compact bone and spongy bone, concentrations of those studied metals could be arranged in the following descending order: Zn > Cu > Pb > Cd > Hg, with values ranging from 142 to 0.002 mg/kg dry weight (dw). The strongest synergistic relationships were observed in spongy bone between Zn and Cu, between Zn and Cd and between Cu and Cd. Statistically significant antagonistic relationships were detected between Zn and Pb in compact bone. In this study, the same bone samples were used to determine Mn, Fe and Sr concentrations. We also examined the relations between the Mn, Fe and Sr concentrations in the bone samples and the sex and age of the foxes.

Methods

Study Area

The material was collected in north-western Poland, in the West Pomeranian province including its capital, Szczecin (Fig. 1). Most of the province's area is agricultural (38 %) and forested (35 %), and several percent of the area is covered by water (numerous lakes, rivers, the Odra estuary with Dabie Lake and Szczecin Lagoon) (http://www.stat.gov.pl/cps/rde/xbcr/szczec/ASSETS_przepl_2.pdf).

Material

The material was collected in 2008–2009. Altogether, it consisted of 35 foxes from six districts of the West Pomeranian province (municipal Szczecin, Choszczno, Stargard, Gryfice, Kamień Pomorski and Mysłiborz, with three, eight, seven, one, two and two specimens, respectively, and seven remaining foxes from the Provincial Veterinary Inspectorate in Szczecin). In Poland, the fox is included in the list of animals for hunting (*Journal of Law* 2005, no. 45, pos. 433) and, according to the Minister of Environment, may be hunted from 1st July to 31st March (*Journal of Law* 2005, no. 48, pos. 459). The acquisition of biological material from the foxes was approved by the Local Ethics Committee for Research on Animals in Szczecin (Poland).

Fox age categories (*immaturus* lub *adultus*) were based on the examination of one single-root lower canine, with preserved anatomical crown according to the work of Lanocha et al. [20].

Determination of Mn, Fe and Sr

The crushed material was divided into samples of 0.5–1.0 g. The weighed samples were mineralised by wet digestion using a Velp Scientifica mineraliser (Italy) [13]. Mineralisation was carried out in a four-step heating regime, with a maximum temperature of 200 °C.

The concentrations of Mn, Fe and Sr were assessed by atomic absorption spectrophotometry emission in inductively coupled argon plasma (ICP-AES) with a Perkin Elmer Optima 2000 DV. The limits of detection of the apparatus were 0.1 mg/l (Mn and Fe) and 0.05 mg/l (Sr). The results were converted into dry weight.

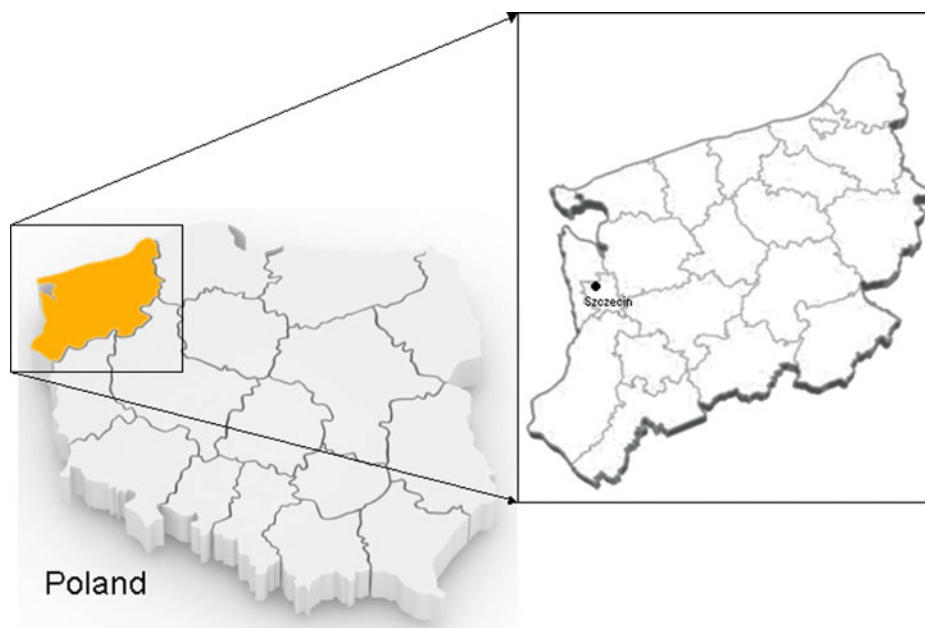
Validation of Analytical Proceedings

The accuracy of the analytical procedure was monitored by determination of the studied elements in two types of reference materials with known concentrations of Mn, Fe and Sr: NIST SRM 1486 Bone Meal (National Institute of Standards and Technology) and IAEA-407 Trace Elements and Methylmercury in Fish Tissue (International Atomic Energy Agency). The concentration values of the reference materials given by the manufacturers and our determinations are shown in Table 1. In order to determine the possible loss of analyte during the chemical process or the impact of other factors on the results of research, we conducted recovery testing.

Statistical Analysis

Statistical studies were performed using StatSoft STATISTICA 9.0 and Microsoft Excel 2007. We calculated the arithmetic means (AM), standard deviations of the AM (SD), median

Fig. 1 Location of study areas in north-western Poland



(Med), lower (Q_L) and upper quartile (Q_U). In order to determine the conformity of the distribution of the results with the expected normal distribution, we used a Kolmogorov–Smirnov test with Lilliefors correction ($p < 0.05$). When the distribution of empirical data conformed with the expected normal distribution, an ANOVA test was used, and in the case of statistically significant differences, Student’s t test was used. In the absence of conformity between the expected normal distribution and the empirical data, we used the nonparametric Kruskal–Wallis (K-W) test, and the significance of differences was confirmed by the Mann–Whitney U (MW-U) test. Finally, a Spearman rank correlation coefficient was calculated between trace elements occurring in the material, as well as between the different metals studied in different parts of the hip joint.

Results

In literature, bone concentrations of metals are presented in different ways—per wet weight, dry weight and in ash. Therefore, to facilitate comparisons with other papers, we determined the percentage of water content in the tested fox bone samples: cartilage (23.3 %), compact bone (25.5 %),

cartilage with compact bone (24.4 %) and spongy bone (11 %), and expressed results in milligrams per kilogram dry weight.

Table 2 shows the basic statistics for metal concentrations in the examined fox bone samples. The distribution of Mn, Fe and Sr concentrations in almost all types of bone material deviated from the expected normal distribution, which was verified by Kolmogorov–Smirnov tests ($p < 0.05$), with Lilliefors corrections ($p < 0.01$). Normal distributions were only observed for Mn concentrations in compact bone and Sr in spongy bone (Table 3). Taking this into account, nonparametric tests were used in further statistical analyses.

The concentrations of metals were arranged in the following descending series: Fe > Sr > Mn, except for compact bone, in which it was a little different: Sr > Fe > Mn.

Manganese

Manganese concentration in the analysed bone material ranged from 0.05 to 5.74 mg/kg dw. The highest median value was observed in compact bone (1.05 mg/kg dw) and the lowest in the spongy bone (0.69 mg/kg dw). The K-W test showed that there were statistically confirmed differences

Table 1 Concentration of selected elements in certified reference materials in milligrams per kilogram dry weight

| Metal | Bone meal SRM NIST 1486 | | OD/RV (%) | Fish tissue IAEA-407 | | OD/RV (%) |
|-------|-------------------------|--------------|-----------|----------------------|--------------|-----------|
| | RV | OD ($n=7$) | | RV | OD ($n=8$) | |
| Mn | 1.00 ^a | 0.90±0.04 | 90.00 | 3.52±0.32 | 3.33±0.31 | 94.6 |
| Fe | 99.00±8.00 | 106.00±17.00 | 107.00 | 146.00±14.00 | 141.25±16.00 | 96.7 |
| Sr | 264.00±7.00 | 226.00±8.00 | 85.60 | 130.00±11.00 | 115.76±26.00 | 89.0 |

RV reference value, OD own determination

^a Estimated value

Table 2 The concentration of Mn, Fe and Sr (in milligrams per kilogram dw) in four types of bone material coming from foxes in the vicinity of Szczecin

| Metal | Parameter | C (n=34) | CB (n=35) | C+CB (n=34) | SB (n=34) | Significance K-W (n=34) |
|-------|-----------------------------------------------|----------------|--------------|---------------|---------------|-------------------------|
| Mn | AM±SD | 1.39±1.22 | 0.93±0.43 | 1.14±0.66 | 0.92±0.81 | <i>p</i> < 0.05 |
| | CV | 87.90 | 46.10 | 57.60 | 87.60 | |
| | Range | 0.05–5.74 | 0.05–1.89 | 0.05–3.45 | 0.27–4.57 | |
| | Med | 0.79 | 1.05 | 0.96 | 0.69 | |
| | <i>Q</i> _L – <i>Q</i> _U | 0.62–2.03 | 0.68–1.17 | 0.75–1.42 | 0.51–0.96 | |
| Fe | AM±SD | 194.70±231.80 | 79.30±132.50 | 134.20±137.60 | 148.00±153.90 | <i>p</i> < 0.001 |
| | CV | 119.10 | 167.10 | 102.50 | 104.00 | |
| | Range | 18.90–1,194.50 | 1.60–708.80 | 6.60–693.90 | 20.40–766.00 | |
| | Med | 146.60 | 22.00 | 118.10 | 83.40 | |
| | <i>Q</i> _L – <i>Q</i> _U | 35.90–256.10 | 13.20–98.90 | 27.60–184.20 | 57.00–191.50 | |
| Sr | AM±SD | 48.90±27.60 | 68.80±49.90 | 58.20±34.10 | 55.40±40.00 | NS |
| | CV | 56.50 | 72.60 | 58.60 | 72.30 | |
| | Range | 0.83–148.3 | 4.62–241.20 | 13.20–194.80 | 16.10–197.6 | |
| | Med | 45.80 | 63.30 | 52.30 | 42.80 | |
| | <i>Q</i> _L – <i>Q</i> _U | 34.60–56.40 | 37.50–78.10 | 40.50–62.60 | 35.20–58.00 | |

C cartilage, CB compact bone, C + CB cartilage with compact bone, SB spongy bone, AM arithmetic mean, SD standard deviation, CV coefficient of variation (in percent), Med median, *Q*_L lower, *Q*_U upper quartile, K-W Kruskal–Wallis test, *p* level of significance, NS difference non-significant

Table 3 The conformity of distributions of metal concentrations in the bone material from foxes with the expected normal distribution

| Material | Parameters | Test and its significance for the conformity of distributions | | |
|----------|----------------------|---------------------------------------------------------------|------------|------------|
| | | Mn | Fe | Sr |
| C | <i>n</i> | 34 | 34 | 34 |
| | K-S | 0.453 | 0.225 | 0.192 |
| | <i>p</i> | <0.01 | <0.01 | <0.15 |
| | Lil. corr., <i>p</i> | <0.01 | <0.01 | <0.01 |
| | Distribution | Non-normal | Non-normal | Non-normal |
| CB | <i>n</i> | 35 | 35 | 35 |
| | K-S | 0.140 | 0.281 | 0.208 |
| | <i>p</i> | NS | <0.01 | <0.10 |
| | Lil. corr., <i>p</i> | <0.10 | <0.01 | <0.01 |
| | Distribution | Normal | Non-normal | Non-normal |
| C+CB | <i>n</i> | 34 | 34 | 34 |
| | K-S | 0.239 | 0.188 | 0.188 |
| | <i>p</i> | <0.05 | <0.20 | <0.20 |
| | Lil. corr., <i>p</i> | <0.01 | <0.01 | <0.01 |
| | Distribution | Non-normal | Non-normal | Non-normal |
| SB | <i>n</i> | 34 | 34 | 34 |
| | K-S | 0.474 | 0.253 | 0.139 |
| | <i>p</i> | <0.01 | <0.05 | NS |
| | Lil. corr., <i>p</i> | <0.01 | <0.01 | <0.10 |
| | Distribution | Non-normal | Non-normal | Normal |

C cartilage, CB compact bone, C + CB cartilage with compact bone, SB spongy bone, K-S Kolmogorov–Smirnov test, Lil. corr. Lilliefors corrected Kolmogorov–Smirnov test, *p* level of significance, NS difference non-significant

between the medians calculated for the different types of fox bone material (Table 2). Pairwise comparison of medians, using MW-U tests, revealed a statistically confirmed difference in Mn concentrations (*p* < 0.005) between the cartilage with compact bone and the spongy bone (Table 4), being 39 % higher in the cartilage with compact bone. In addition, less pronounced differences in Mn concentrations (*p* < 0.07 or *p* < 0.06) were observed between cartilage and spongy bone (14.5 % lower in spongy bone) and between compact and spongy bone (52 % lower in spongy bone).

Iron

The highest Fe concentration was observed in cartilage (~147 mg/kg dw) while the lowest was observed in compact

Table 4 Significance of differences between metal concentrations in different fox bone materials

| Metal | Parameter | C vs CB | C vs SB | CB vs SB | C + CB vs SB |
|----------|-----------|---------|---------|----------|--------------|
| Mn, n=35 | MW-U | NS | 430.0 | 440.0 | 336.0 |
| | <i>p</i> | | 0.07 | 0.06 | 0.005 |
| Fe, n=35 | MW-U | 278.0 | NS | 284.0 | NS |
| | <i>p</i> | 0.001 | | 0.03 | |
| Sr, n=35 | MW-U | 404.0 | NS | 448.0 | NS |
| | <i>p</i> | 0.02 | | 0.08 | |

C cartilage, CB compact bone, C + CB cartilage with compact bone, SB spongy bone, MW-U Mann–Whitney U test, *p* level of significance, NS difference non-significant

Table 5 Comparison of metal concentrations in analogous fox bone materials between two age groups

| Material | Parameter | Mn | Fe | Sr |
|-----------|--------------------------------|-----------|----------------|--------------|
| ad, n=12 | | | | |
| C | AM±SD | 1.37±0.97 | 188.50±135.50 | 43.40±25.70 |
| | CV | 71.20 | 71.90 | 59.20 |
| | Range | 0.05–3.20 | 20.00–450.40 | 0.84–85.50 |
| | Med | 1.10 | 153.30 | 39.50 |
| | Q _L –Q _U | 0.73–2.16 | 60.70–289.40 | 33.70–59.00 |
| CB | AM±SD | 0.75±0.52 | 83.60±49.30 | 53.10±33.10 |
| | CV | 70.10 | 59.00 | 62.30 |
| | Range | 0.05–1.89 | 33.60–191.50 | 5.98–107.20 |
| | Med | 0.77 | 62.30 | 51.20 |
| | Q _L –Q _U | 0.33–1.06 | 54.00–108.50 | 25.00–80.10 |
| C+CB | AM±SD | 1.00±0.58 | 139.80±100.80 | 46.40±21.90 |
| | CV | 57.80 | 72.10 | 47.30 |
| | Range | 0.05–2.18 | 6.60–370.40 | 13.20–83.60 |
| | Med | 0.94 | 126.30 | 43.60 |
| | Q _L –Q _U | 0.59–1.41 | 75.70–172.40 | 30.70–60.10 |
| SB | AM±SD | 0.63±0.22 | 106.70±204.00 | 46.60±24.20 |
| | CV | 35.00 | 191.20 | 51.90 |
| | Range | 0.27–0.98 | 4.26–708.80 | 16.20–96.30 |
| | Med | 0.66 | 22.70 | 41.90 |
| | Q _L –Q _U | 0.46–0.78 | 16.70–76.90 | 31.40–54.40 |
| im, n=18 | | | | |
| C | AM±SD | 1.62±1.42 | 240.90±290.30 | 49.20±29.50 |
| | CV | 86.40 | 120.50 | 60.00 |
| | Range | 0.33–5.70 | 18.90–1,194.50 | 0.83–148.30 |
| | Med | 1.07 | 175.50 | 45.30 |
| | Q _L –Q _U | 0.67–2.06 | 35.00–261.00 | 34.60–52.80 |
| CB | AM±SD | 1.03±0.39 | 78.80±80.20 | 76.60±52.90 |
| | CV | 37.80 | 101.20 | 69.10 |
| | Range | 0.09–1.64 | 1.60–227.60 | 4.62–241.20 |
| | Med | 1.12 | 33.00 | 65.80 |
| | Q _L –Q _U | 0.97–1.22 | 15.10–160.40 | 54.70–78.10 |
| C + CB | AM±SD | 1.30±0.70 | 159.80±165.20 | 62.90±36.10 |
| | CV | 55.30 | 103.30 | 57.50 |
| | Range | 0.40–3.40 | 14.00–693.90 | 25.10–194.80 |
| | Med | 1.12 | 129.20 | 55.70 |
| | Q _L –Q _U | 0.89–1.70 | 29.50–226.40 | 47.20–63.80 |
| SB | AM±SD | 1.20±1.03 | 210.50±187.70 | 63.20±50.70 |
| | CV | 85.40 | 89.20 | 80.32 |
| | Range | 0.40–4.57 | 27.60–766.00 | 23.40–197.60 |
| | Med | 0.81 | 122.80 | 44.40 |
| | Q _L –Q _U | 0.52–1.46 | 77.90–318.80 | 32.60–65.70 |
| ad vs. im | | | | |
| C | MW-U | NS | NS | NS |
| | <i>p</i> | | | |
| CB | MW-U | NS | 60.00 | NS |
| | <i>p</i> | | 0.05 | |
| C + CB | MW-U | NS | NS | NS |
| | <i>p</i> | | | |

Table 5 (continued)

| Material | Parameter | Mn | Fe | Sr |
|----------|-----------|----|-------|----|
| SB | <i>p</i> | | | |
| | MW-U | NS | 49,00 | NS |
| | <i>p</i> | | 0.02 | |

C cartilage, CB compact bone, C+CB cartilage with compact bone, SB spongy bone, AM arithmetic mean, SD standard deviation, CV coefficient of variation in percent, Med median, Q_L lower, Q_U upper quartile, ad adultus, im immaturus, MW-U Mann–Whitney U test, *p* level of significance, NS statistically non-significant difference

bone (22 mg/kg dw). The K-W test confirmed the statistical significance of these differences (*p* < 0.001), and further analysis was carried out using MW-U tests (Table 4). Iron concentrations did not differ significantly between cartilage and cartilage with compact bone, or between cartilage and spongy bone. Iron concentrations differed significantly between cartilage and compact bone (560 % greater in cartilage) and between spongy bone and compact bone (almost 180 % greater in spongy bone).

Strontium

Strontium concentrations were lowest in cartilage and in spongy bone and did not exceed 46 mg/kg dw while, in cartilage with adjacent compact bone, Sr concentrations assumed intermediate levels (~52 mg/kg dw) and was the highest in compact bone (>63 mg/kg dw). The differences between the four types of samples were not revealed by the K-W test. However, the MW-U test for a pairwise comparison showed differences in Sr concentrations between cartilage and compact bone (*p* < 0.02) and an almost statistically significant difference between compact bone and spongy bone (*p* < 0.08) (Table 4).

Sex and Bone Mn, Fe and Sr Concentrations

With one exception, we found no statistically confirmed differences between sexes and Mn, Fe and Sr concentrations in analogous types of bone material. The exception was Fe concentrations in the cartilage with adjacent compact bone which was 159.1 mg/kg dw in males, almost three times (~2.7) higher than in females at 58.3 mg/kg dw (MW-U test=90.0, *p* < 0.05).

Age and Bone Mn, Fe and Sr Concentrations

Comparing the concentrations of metals in the corresponding bone material between the immature foxes (im) and adult foxes (ad), we only found a statistically significant difference in Fe concentrations between spongy bone (MW-U=49.0, *p* < 0.015) and compact bone (MW-U=60.0, *p* < 0.05) (Table 5). Iron

concentration in im foxes was greater in the spongy bone and lower in the compact bone compared with the ad.

Correlations Between the Levels of Different Metals

The analysis of relationships between the pairs of metals in the same type of samples, as well as between the concentrations of the same metal in different types of samples, involved all foxes in the study ($n=35$). Spearman correlation coefficients (r_s) were positive in all cases, and their values and statistical significance are summarized in Table 6. Strong and moderately synergistic relationships (r_s from 0.580 to 0.800) were detected between the concentrations of Mn and Fe concentrations in the four types of materials: cartilage ($r_s=0.801$), spongy bone ($r_s=0.707$), compact bone ($r_s=0.581$) and the cartilage with compact bone ($r_s=0.754$). A weak correlation ($r_s=0.364$) was observed between Mn concentrations in compact bone and Fe in the cartilage with compact bone. There was a significant correlation between Mn and Sr concentrations in compact bone ($r_s=0.492$) and between Mn in compact bone and Sr in cartilage with compact bone ($r_s=0.445$). Iron and strontium concentrations showed moderate correlations in the spongy bone ($r_s=0.411$) and compact bone ($r_s=0.373$), and there was a correlation between Fe in compact bone and Sr in the cartilage with compact bone ($r_s=0.361$).

Correlations Between the Concentrations of the Same Metal in Two Different Types of Samples

Correlations were found between Mn concentrations in cartilage and spongy bone ($r_s=0.507$), cartilage and cartilage with

compact bone ($r_s=0.834$) and compact bone and cartilage with compact bone ($r_s=0.473$). We also found correlations between Fe concentrations in cartilage and spongy bone ($r_s=0.569$), cartilage and cartilage with compact bone ($r_s=0.819$), spongy bone and cartilage with compact bone ($r_s=0.461$) and compact bone and cartilage with compact bone ($r_s=0.659$). Correlations were also found between Sr concentrations in cartilage and spongy bone ($r_s=0.390$), cartilage and cartilage with compact bone ($r_s=0.573$), spongy bone and cartilage with compact bone ($r_s=0.457$) and between compact bone and cartilage with compact bone ($r_s=0.817$) (Table 6). None of the metals showed a significant statistical correlation between cartilage and spongy bone concentrations, or between spongy bone and compact bone.

Discussion

Scientific studies on the effects of various minerals on organisms often use a variety of warm-blooded vertebrates, with some mammals used as models. In bioindication studies, these are usually medium to large wild animals (mainly hunted) and domesticated animals, including fox, domestic dog, pig, sheep and cattle [17, 21–25].

Manganese in canine bone has been studied in the farm raccoon dog from Poland by Mertin et al. [26] and in dogs in the USA [27]. In bones of the raccoon dog, Mn concentration was 2 mg/kg dw while, in rib cartilage of two breeds of dogs, it was 0.62 and 0.77 mg/kg dw (Table 7). Although the latter study was conducted on material harvested from only two individuals—a dachshund with chondrodystrophy and a

Table 6 Spearman correlation coefficients between toxic metal concentrations (Mn, Fe and Sr) in different bone materials (cartilage, spongy bone, compact bone, cartilage with compact bone) of the fox

| | Mn C | Mn SB | Mn CB | Mn C+CB | Fe C | Fe SB | Fe CB | Fe C+CB | Sr C | Sr SB | Sr CB | Sr C+CB |
|---------|------|---------|-------|----------|----------|----------|----------|----------|------|--------|---------|----------|
| Mn C | – | 0.507** | NS | 0.834*** | 0.801*** | 0.442* | NS | 0.618*** | NS | NS | NS | NS |
| Mn SB | | – | NS | 0.460** | 0.638*** | 0.707*** | NS | 0.490** | NS | NS | NS | NS |
| Mn CB | | | – | 0.473** | NS | NS | 0.581*** | 0.364* | NS | NS | 0.492** | 0.445** |
| Mn C+CB | | | | – | 0.729*** | 0.423* | 0.545*** | 0.754*** | NS | NS | NS | NS |
| Fe C | | | | | – | 0.569*** | NS | 0.819*** | NS | NS | NS | NS |
| Fe SB | | | | | | – | NS | 0.461** | NS | 0.411* | NS | NS |
| Fe CB | | | | | | | – | 0.659*** | NS | NS | 0.373* | 0.361* |
| Fe C+CB | | | | | | | | – | NS | NS | NS | NS |
| Sr C | | | | | | | | | – | 0.390* | NS | 0.573*** |
| Sr SB | | | | | | | | | | – | NS | 0.457** |
| Sr CB | | | | | | | | | | | – | 0.817*** |
| Sr C+CB | | | | | | | | | | | | – |

C cartilage, SB spongy bone, CB compact bone, C+CB cartilage with compact bone, NS statistically non-significant difference

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, levels of significance

Table 7 Concentrations of trace elements (in milligrams per kilogram) in animal bone material from various parts of the world

| Place | Species | Tissue | Age | Sex | n | dw or ww | Mn | Fe | Sr | Source |
|--------------------------|----------------------------------------------|-----------------|--------|-----|-------|----------|-------------------------------------------|---------------------------------------------|-------------------------------------------|------------|
| Order Carnivora, Canidae | | | | | | | | | | |
| Poland, Szczecin | Dog, <i>Canis lupus familiaris</i> | Femur | ad | F+M | 24 | dw | 0.59 ^{C+CB} , 0.35 ^{SB} | 88.2 ^{C+CB} , 82.2 ^{SB} | 53.4 ^{C+CB} , 84.6 ^{SB} | This study |
| | | | im | F+M | 2 | dw | 0.96 ^{C+CB} , 1.65 ^{SB} | 113.5 ^{C+CB} , 109.1 ^{SB} | 16.4 ^{C+CB} , 41.1 ^{SB} | |
| Poland, Krakow | Red fox, <i>Vulpes vulpes</i> | Femur | im, ad | F+M | 34–35 | dw | 1.14 ^{C+CB} , 0.92 ^{SB} | 134.2 ^{C+CB} , 148.0 ^{SB} | 58.2 ^{C+CB} , 55.4 ^{SB} | |
| Poland, Krakow | Raccoon dog, <i>Nyctereutes procyonoides</i> | Bone | subad | F+M | 18 | dw | 2.0 | 55.7 | – | [26] |
| USA, San Francisco | Dog, <i>Canis lupus familiaris</i> | Rib (cartilage) | im | – | 1 | dw | Dachshund 0.62 | – | – | [27] |
| | | | im | – | 1 | dw | Beagle 0.77 | – | – | |
| Denmark | Dog, <i>Canis lupus familiaris</i> | Femur | im | F+M | 24 | dw | – | – | 76 ^{CB} | [37] |

C cartilage, CB compact bone, C+CB cartilage with compact bone, SB spongy bone, F female, M male, ad adultus, im immaturus, subad subadultus, dw dry weight

healthy beagle—a similar concentration was observed in cartilage in the population of foxes in north-western Poland (0.79 mg/kg dw).

Several studies have found that Mn concentrations in mammals change over the course of ontogenesis. Laboratory studies on mice showed that the Mn concentrations in the parietal bone is greatest from 5 to 43 days after birth (mean 0.61 mg/kg dw) and lowest in newborns aged 2 days (0.09 mg/kg dw). In mouse fetuses, Mn concentration was 0.29 mg/kg dw [28]. These observations are not consistent with our results, as the fox from NW Poland showed no statistically significant age-related differences in Mn in any of the types of bone material between the immature and adult groups.

Ecotoxicological studies often compare material derived from animals of the same or related species inhabiting different environments, such as near factories and relatively unpolluted areas. Such studies have been conducted by Sanchez-Chardi and Lopez-Fuster [29] in the north-eastern part of Spain: the Ebro Delta (contaminated area) and the Medes Islands (uncontaminated areas).

The aforementioned researchers used bone material derived from the greater white-toothed shrew (*Crocidura russula*), representative of carnivorous Soricidae. Bone Mn concentrations in the animals in the Ebro delta and the Medes Islands amounted to 7.8 and 4.6 mg/kg dm. The greater white-toothed shrew living in a more contaminated area accumulated about 70 % more Mn than individuals living in the relatively clean area.

In the fox from NW Poland, Mn was considerably lower (from 0.92 to 1.14 mg/kg dw) than in the greater white-toothed shrew. Although, due to significant differences in biology (faster metabolic rate and a much lower life expectancy of insectivorous mammals compared to canines), these comparisons should be treated with caution, the increased Mn in these animals may not necessarily result from higher Mn concentrations in the abiotic environment.

Bones of various species accumulate from several milligrams to several hundred milligrams of iron per kilogram dry weight. The highest Fe concentrations (≥ 200 mg/kg dw) in wild animals have been found in small mammals, micromammalia, and among domesticated warm-blooded vertebrates—cattle [29–32]. The lowest Fe concentrations (<15 mg/kg) were observed in free-living elk and domesticated sheep [17, 33]. Iron concentration in animal bone samples usually ranges from 30 to 150 mg/kg dw.

There are few papers on canine bone Fe. Only Mertin et al. [26] analysed the concentrations of this metal in the bones of the farm raccoon dog in Poland. In this representative of the family Canidae, Fe concentration was nearly 60 mg/kg dm. In our study, bone samples from wild foxes from NW Poland showed Fe concentration ranging from about 80 to 195 mg/kg dw, depending on the type of bone material, considerably higher than in the farm raccoon dog.

Sanchez-Chardi and Lopez-Fuster [29] determined Fe concentrations in the bone of the greater white-toothed shrew and found significant differences between individuals living in the contaminated Ebro Delta and the uncontaminated Medes Islands. Bones of the greater white-toothed shrews from the contaminated area accumulated 40 % more Fe (~230 compared with 160 mg/kg dm in the uncontaminated area).

Studies on laboratory animals have shown that Fe supplementation increases the concentration in bone material. Iron levels in the bones of Sprague Dawley rats receiving feed enriched with iron (II) sulfate were 76 % higher in comparison to the bones of animals in the control group with standard feed without Fe supplementation at 48.7 and 27.6 mg/kg dm, respectively [34]. Such significant differences were not observed with respect to domesticated ungulates (sheep) in which Fe concentrations in bone were similar regardless of Fe supplementation or not (30 to 35 mg/kg dm) [35]. We have found no such comparisons carried out on canids. The wild foxes from NW Poland examined in this study accumulated from 22 to 147 mg Fe/kg dw, i.e. much more than that in small laboratory mammals and sheep.

Rheingold et al. [36] studied bone Sr concentrations in mammals from different trophic groups. Carnivorous animals accumulated the lowest Sr concentrations (from 114 to 331 mg/kg dm); omnivores accumulated from 170 to 455 mg/kg dm, and herbivores accumulated the greatest concentrations (from 455 to 570 mg/kg dm). Available scientific literature contains just one paper on Sr concentration in canine bone. Raffalt et al. [37] determined Sr in the compact bone of dogs from Denmark that had been given feed containing different amounts of strontium malonate (from 300 to 3,000 mg/kg/day). Bone Sr concentrations were two orders of magnitude greater in the samples from animals fed with the additive (bone Sr from 7,200 to 9,800 mg/kg) compared with the control group which received no additive (bone Sr 76 mg/kg dm). Compared with this study, Sr concentrations in the same material in wild foxes from NW Poland were about 69 mg/kg dw, so similar to the control group of dogs from Denmark, i.e. those not supplemented with strontium.

Based on the aforementioned information, it can be concluded that, in most cases, the Mn, Fe and Sr concentrations in the bones of wild foxes from NW Poland were similar to those found in other canids. The results confirm previous differences in bone concentrations of these metals between carnivores with some ungulates (sheep) and small mammals (micromammalia). However, due to the low number of papers in this field, it is advisable to conduct further studies on bone samples from animals of different taxa living in different environments. Any comparative studies on Mn, Fe, Sr and other metals should take into account corresponding types of bone samples and provide unified or unilateral units of measure.

Acknowledgments The study was financed as research project no. NN 304 361838 by the Polish Ministry of Education from the resources for the years 2010–2011.

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