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Original Research

Contents of Copper, Chromium, Nickel, Lead, and Zinc in Hair and Skin of Farm Foxes

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Abstract

The content of trace elements (Cu, Cr, Ni, Pb, and Zn) in hair and skin of silver fox (*Vulpes vulpes*) and arctic fox (*Alopex lagopus*) were determined. The content of elements were determined using inductively coupled plasma atomic emission spectrometry (ICP-OES). Samples of animal hair and skin were collected from two farms located in mid-western Poland: farm A, situated in a rural area and farm B, situated in a typically urbanized and industrial region. The influence of farm location on the concentration of Cr, Cu, and Zn in fur coat, and Cr and Ni in skin was observed.

Keywords: heavy metals, silver fox, arctic fox, hair, skin

Introduction

It is generally known that mammal tissues are good bioindicators of trace elements, including heavy metals. The content of metals in the internal organs of foxes such as kidney and liver was discussed in relation to many aspects in numerous reports [1-4]. In addition, animal hair appears to be a good bioindicator of heavy metal levels [5-7]. Chemical composition of hair reflects the maintenance system, nutrition level; and also the level of environmental pollutants [6, 8, 9]. However, numerous factors like breed, sex, age, season, physiology, and health may modify the chemical composition of animal hair. In various sheep breeds from Syria, Greece, and Poland, different trace element contents have been observed [10]. The low levels of Cu in hair and an excessive content of Fe in forage was noted in yaks (*Poëphagus mutus f. grunniens*) with anaemia symptoms [11]. Differences in chemical composition depending on age and season of the year were observed in the hair of horses [12, 13]. Dey et al. [14] in turn, noted different values of Pb, Cr, Hg, and Se in hair of wild animals (leopard, civet cat, flying squirrel) in northeastern India, a region with a deficiency of trace elements in soils and plants.

There is a lack of data concerning the content of toxic elements in raw skin of animals and their possible relationship with chemical composition of hair. Only information concerning metals content, including toxic ones, in tanned skins may be found in the literature. For example, Aslan [15] observed eleven chemical elements in tanned leathers with the amounts dependent on tanning technique. Cr, Al, and Zr showed the highest levels, while Hg, As, Sb, Cd, and Ni the lowest. Also, Basaran et al. [16] observed the highest amount of Cr, and considerablly lower content of Zn and Cu in tanned skins.

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There are not many papers concerning the content of chemical elements, including toxic metals in hair and raw skin of foxes kept in farm conditions for fur-producing purposes. The contents of various chemical elements are analyzed most often in internal organs of slaughtered foxes, mainly in kidneys and liver [1-3, 17, 18] and even in internal parasites [4]. Only Filistowicz et al. [19] determined the concentrations of some chemical elements in hair and skin of wild foxes as compared to farm silver ones.

The aim of the present study was a comparative assessment of the content of some chemical elements like lead, chromium, copper, nickel, and zinc in hair and skin of the two farm species of foxes: silver and arctic ones maintained in typical farm conditions.

Material and Methods

The research material was obtained from farm foxes: 9-month-old silver foxes (16 individuals) and 8-month-old arctic ones of blue variety (16 individuals) from two farms (A and B) situated in midwestern Poland. Farm A was located on a typically agricultural region, but close to a natural gas well and a national road near Wielichowo, while farm B was in an urbanized region, bordering a large city (Leszno, Wielkopolska district). The foxes were kept in cages under the roof, and fed with the homogenized fodder that contained typical components: meat and butchery wastes, groats, carrot, and vitamin-mineral premix "Norka/Lis 0.1%." The fodder was preserved with sodium pyrosulfate (2 kg/1000 kg of fodder). In particular seasons of the year, the foxes were fed according to nutritional standards established by the same nutrition consultant. The body mass of slaughtered silver foxes was about 7 kg, and in the case of arctic foxes it was about 12 kg.

The slaughter of foxes was conducted once, when they had a wholly mature fur coat. After the slaughter, the skins were pulled off the animals using the bag system. After the complete stiffening of subcutaneous fat on the removed skin, fleshing was conducted, i.e. scraping of fat, subcutaneous membranes, and eventually muscle fibres from skin surface. Next, in order to remove fat and other contaminations from the rough side and hair, the skins were subject to cleaning using sawdust in a rotating drum. Then, the skins were put on fixed boards with hair outside and subject to a drying process for 48 hours. After that they were removed from the fix boards and the fur coat was combed out. They were not subject to tanning.

Six silver and arctic foxes from each farm were randomly chosen for the study (32 individuals in total). Samples of skin (with hair) were collected once from dorsal (2 samples) and abdominal (4 samples) parts, each sample of about 10 cm^2. The hair was then carefully cut off, mixed and washed using detergents and distilled water (rinsing), and dried at room temperature. About 20 g of material in plastic bags was delivered for analysis. The skin (without hair) was cut with a scalpel into small pieces and carefully mixed. Weighted samples of 40 g in sterile plastic containers were delivered for analysis.

Samples of raw hair and raw skin were shortly stored in the fridge, and then were subject to laboratory analysis according to established and verified procedures. The samples were subject to the mineralization process in a MARS-4 (CEM, USA) microwave furnace. Spectrally pure nitric acid of MERCK company was used. The concentration of metals (Cu, Cr, Ni, Pb, and Zn) was determined using an ICP-OES (Varian, USA) emission spectrometer with plasma induction. Results are in wet weight.

Statistical assessment was conducted using SAS statistical packet. The assessment was done using two-component analysis of variance (GLM procedure) according to a constant model (object of study, species – fox variety). Differences between mean content of elements in skin and hair of foxes of different species and varieties, and from various objects were tested using Duncan's test. The relationship between element levels in skin and hair was determined using Pearson's straight correlation (CORR procedure).

Results and Discussion

The means, standard deviations, and correlation coefficients for all the analytical results are presented in Tables 1-4.

Significantly higher concentrations of Cr and Zn in hair of silver foxes as compared to arctic ones was observed on farm A, and similar concentrations of those elements in hair of both species on farm B. Maximum value of Cr was 0.268 mg/kg, and of Zn – 110.670 mg/kg. The hair of arctic foxes, in turn, contained significantly higher content of copper, while higher Cu concentrations were noted on farm A. Maximum Cu value was 4.092 mg/kg on farm A (arctic fox), and the lowest – 2.512 mg/kg on farm B (silver fox). No significant differences in Pb and Ni content in hair (Table 1) nor Pb in skin (Table 2) were observed in the case of both fox species. The skins of both species differed significantly as regards the concentration of Cr, Ni, Zn, and Cu (Table 2). Especially high content of Cr was noted in skin of silver foxes on farm A (0.687 mg/kg) and it was almost 2-fold higher than in skin of arctic foxes from farm A and over 4-fold higher from Cr content in skin of silver foxes on farm B. Over 2-fold higher content of Ni in skin of silver foxes from farm A as compared to silver ones from farm B and arctic foxes from both farms is noteworthy. Silver foxes from farm A were also characterized by the highest concentration of Zn in skin, but differed significantly with that element content only from arctic foxes from farm A. Arctic foxes on both farms in turn had significantly higher content of Cu in skin as compared to silver foxes. Generally, considerably higher content of heavy metals was found in skins than in hair, but the elements were placed in the same sequence, i.e. Zn>Cu>Pb>Ni>Cr, in hair and skin.

Positively significant correlations between the content of chromium and nickel (r=0.511) and chromium and zinc (r=0.512), negatively significant between chromium and copper (r=-0.363) and the lack of significant correlations between the content of elements in hair were observed

Table 1. The content of heavy metals ($\bar{x}\pm SD$) in hair of foxes.

Farm	Foxes	Cr	Cu	Ni	Pb	Zn
A	Silver (n=8)	$0.268^{a}\pm0.137$	$3.437^{Ab}\pm0.246$	0.505±0.359	0.640±0.087	$110.670^{a}\pm59.154$
	Arctic (n=8)	$0.193^{ab}\pm0.079$	$4.092^{Aa}\pm0.326$	0.560±0.362	0.543±0.062	$58.550^{b}\pm8.999$
B	Silver (n=8)	$0.145^{b}\pm0.033$	$2.512^{B}\pm0.343$	0.455±0.268	0.649±0.048	$79.867^{ab}\pm27.355$
	Arctic (n=8)	$0.147^{b}\pm0.032$	$3.762^{Aab}\pm0.681$	0.410±0.264	0.645±0.053	$73.417^{ab}\pm17.182$

A-B $p<0.01$, a-b $p<0.05$

Table 2. The content of heavy metals ($\bar{x}\pm SD$) in skin of foxes.

Farm	Foxes	Cr	Cu	Ni	Pb	Zn
A	Silver (n=8)	$0.687^{A}\pm0.276$	$11.328^{A}\pm1.494$	$1.377^{a}\pm0.872$	0.630±0.037	$442.167^{a}\pm133.161$
	Arctic (n=8)	$0.372^{B}\pm0.288$	$17.317^{B}\pm2.543$	$0.523^{b}\pm0.313$	0.717±0.200	$275.833^{b}\pm48.118$
B	Silver (n=8)	$0.157^{B}\pm0.064$	$10.365^{A}\pm1.496$	$0.563^{b}\pm0.228$	1.023±0.603	$365.500^{ab}\pm128.472$
	Arctic (n=8)	$0.198^{B}\pm0.165$	$15.450^{B}\pm1.512$	$0.508^{b}\pm0.344$	0.675±0.106	$322.167^{ab}\pm47.254$

A-B $p<0.01$, a-b $p<0.05$

(Table 3). Also, high and significant relationships were noted between chromium content in skin and hair (r=0.559), nickel in the skin and hair (0.337), chromium in hair and zinc in skin (r=0.559), zinc in skin and hair (r=0.476), zinc in hair and chromium in skin (r=0.601), and between copper in skin and hair (r=0.621) were observed (Table 4). Negative significant correlations between the content of zinc and copper (r=-0.393), and lead and zinc (r=-0.354) also was observed.

The content of chemical elements in animal hair is conditioned by environmental influence, including metals content in feed, water, soil, and air. It is obvious that the raw skin the hair grows from may have an influence on the accumulation of chemical elements, but there are some not fully understood physiological problems connected to the growth process and mechanisms of hair exchange.

The results obtained are difficult to discuss, since there are not many data in this range in the literature available. For animals other than foxes, however, numerous data concerning metal accumulation (Cu, Cr, Ni, Pb, and Zn and others) in fur coat may be found.

The data concerning chromium (Cr) content in fur coat of animals are sparse. In the wool of Polish Merino sheep on uncontaminated areas the mean concentration of Cr was only 0.046 μg/g [7]. In wild cats (*Felis bengalensis*) in India its mean content in hair was as much as 78.4 μg/g [14], in hair of healthy racing horses in Japan it was 0.22 μg/g on average [13], and in dogs in an urban environment in Korea only 0.49 pg/g [20]. It is also worth mention that tanned leathers of animals contain from 0 to 36,000 ppm of Cr depending on tanning technique [15]. In the previous study the authors demonstrated that farmed red foxes and wild ones differed significantly in the content of Cr in skin (2.338 mg/kg, and 1.405 mg/kg, respectively) [19].

Moreover, the referee values of Cr for human hair are defined as 0.78-1.0 mg/kg [21].

Table 3. Pearson's straight correlations between metals concentration in skin (above diagonal) and in hair (below diagonal) of foxes (n=32) in both regions.

Metal	Cr	Cu	Ni	Pb	Zn
Cr		-0.363*	0.511**	0.043	0.512**
Cu	-0.209		-0.192	-0.130	-0.048
Ni	0.230	-0.125		0.038	0.206
Pb	0.229	-0.155	-0.051		0.164
Zn	0.222	-0.266	0.212	-0.292	

*$p<0.05$, **$p<0.01$

Table 4. Pearson's straight correlations between metals content in skin and hair of foxes (n=32) in both regions.

Hair	Skin				
	Cr	Cu	Ni	Pb	Zn
Cr	0.559***	0.058	0.302	-0.268	0.539**
Cu	0.070	0.621***	-0.042	-0.328	-0.184
Ni	0.043	0.139	0.337*	0.144	0.056
Pb	-0.117	0.011	0.254	0.218	-0.354*
Zn	0.601***	-0.393*	0.047	-0.125	0.476**

*$p<0.05$, **$p<0.01$, ***$p<0.001$

The concentration of copper (Cu) in hair depends to a high degree on its concentration in diet and in blood. In the case of sheep in Poland, various values are given, i.e. from 4.34 to 7.23 mg/kg depending on the state of the environment [10], while in Suffolk and Rambouillet breeds in Mexico see as much as 1.72 and 2.16 mg/kg [22]. Big differences also are observed in other animals, for example in hair of horses in Poland the content was 0.627-0.903 [12], while in Japan they found 4.8 mg/kg, on average [13]. In hair of cattle from an area of the copper industry the observed concentration was 6.04-26.53 mg/kg, depending if hair was washed or not [9]. In the previous study the authors observed that farm red foxes and wild ones differed highly significantly with the content of Cu in skin (10.847 mg/kg, and 7.680 mg/kg, respectively) [19].

The other authors [21, 23] in adult human hair observed that the concentration of Cu was 7.96-44.1 mg/kg, but the referee values were defined as 15-25 mgCu/kg [24].

Nickel (Ni) is seldom determined in hair of animals. Its content in hair of goats, sheep, and camels in Egypt was in the range of 0.71-2.11, 0.6-1.75, 0.45-1.75 µg/g, respectively, and in healthy racing horses in Japan 0.26 µg/g on average [13]. It is interesting, thus, that in red foxes in Czech the content of Ni in parasites of alimentary tract (*Cestoda* and *Nematoda*) was on the level of 0.037-0.831 µg/g d.w. [4]. It is also worth mentioning that tanned leathers of animals contained 2.26-3.27 ppm of Ni [16]. The authors demonstrated in the previous study that farm red foxes and wild ones differed significantly as regards the content of Ni in skin (0.803 mg/kg and 0.447 mg/kg, respectively) [19].

It is also known that the Ni values in human hair are stated on a lower level <1.6 mg/kg [21].

Numerous authors reveal the wide range of Pb concentration in hair of animals that result from environmental predispositions. Patra et al. [6] observed the range of 1.82-15.09 mg/kg in hair of cows from industrial-urban areas of India, and 2.99 mg/kg on average in unpolluted areas. Asano et al. [13], in the case of racing horses in Japan, demonstrated a low range of Pb, i.e. from 0.71 to 1.13 mg/kg (0.93 on average). Age and sex of horses did not influence the concentration of that metal. In hair of goats, sheep and camels kept on farms in southern Egypt, the observed content of Pb was 0.35-12, 0.01-8.9, and 0.9-13 µg/g, respectively. In hair of dogs in an urban environment in Korea in turn, that was only 0.82 pg/g [20]. Tanned leathers of animals contained from 0 to 14.37 ppm of Pb depending on tanning technique [15]. In the previous study the authors observed that farm red foxes and wild ones differed significantly with the content of Pb in skin (0.827 mg/kg, and 1.445 mg/kg, respectively) [19].

It was also observed that in human hair, in different countries the content of Pb was 0.96-12.5 mg/kg [21, 25], but the propositions of the referee values are 0.31-1.66 mg/kg [23] or 0.5-2.0 mg/kg [24].

The content of zinc (Zn) is usually high in hair due to its physiological function. In wool of sheep from Greece, Mexico, Poland, and Syria its observed values were from 73.62 to 244.73 mg/kg [10, 22]. The concentration of Zn in horses ranged from 19.56 to 86.0 mg/kg [12, 13], and in cattle in an industrialized region from 98.5 to 333.9 mg/g [9]. In hair of Eurasian beaver (*Castor fiber*) in Norway the average value was 110.4 ppm of Zn [26], and quite other values were given for hair of opossum and grey fox in USA (California) by Arnhold et al. [27]. Tanned leathers of animals in Turkey contained 4.19-27.35 ppm of Zn [16], while the maximum values are even up to 102.56 ppm [15]. In the previous study the authors [19] demonstrated that farmed red foxes and wild ones differed significantly with Zn content in skin (403.833 and 151.500 mg/kg, respectively), and significantly in hair (95.267 and 42.050 mg/kg, respectively).

It also has been observed that human hair in different countries shows Zn content of 128.9-162 mg/kg [21, 23, 25], but the propositions of the referee values are 150-250 mg/kg [24].

Higher levels of Cr, Cu, and Ni in skin of both fox species were noted on farm A, situated in an agricultural region, but in the vicinity of natural gas well and a national road. An elevated Zn level was observed also in skin of silver foxes from farm A, and arctic foxes from farm B, located on the city boundary (second biggest in Wielkopolskie district). Similarly in the case of fur coats of both fox species on farm A, higher levels of Cr, Cu, and Ni were observed as compared to farm B, and like in skin, higher levels in hair of silver fox on farm A and arctic fox on farm B. Also, in the case of Cu, as in skin, higher levels were noted in fur coats of arctic fox as compared to silver ones.

Conclusions

It should be stated in the summary that the obtained results of five heavy metal assessments (Cu, Cr, Ni, Pb, Zn) in hair and skin of silver and arctic foxes differed from values observed in the cases of other animals' hair and skin, but they were within wide ranges of concentrations, especially for zinc and lead. Generally, considerably higher concentration of heavy metals were observed in skin as compared to hair, but the elements were placed in the same order, i.e. Zn>Cu>Pb>Ni>Cr in hair and skin of both animal species. The positive and significant correlations between chromium and nickel concentrations and chromium and zinc in skin and hair is worth noting, and demonstrates the synergism of those elements. The influence of farm location on content of Cr, Cu, Ni, and Zn in skin and hair of farm foxes was demonstrated, and a lack of such an influence in the case of Pb. The species influence was reflected in a higher accumulation of Cr, Ni, and Zn in skin and hair of silver fox, and Cu in skin and fur coat of arctic fox.

The usefulness of skin and hair of farm foxes in bioindication and ecotoxicological studies has been started, but the most prefered bioindicators are the tissues and organs of wild animals.

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