EXPERIMENTAL STUDY

Study of the content of heavy metals related to environmental load in urban areas in Slovakia

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Abstract

It is natural for the man to take care of his environment so the negative influences of intensification of industrial and agricultural production and the entire process of pollution are kept at minimum or eliminated as early as possible. However, they must first be identified.
Background: **companion animals, for example dogs, are a very good indicator of the pollution load on the environment. They inhabit the same space as humans and are exposed to the action of the same pollutants. The most important role in contamination of the environment with heavy metals is played by emissions of different origin. For that reason it is appropriate and advantageous to evaluate the load on the man by parallel evaluation of the heavy metal load on dogs, particularly in large town agglomerations.**
Main purpose**: the aim of the study was to compare and evaluate the content of three heavy metals, cadmium, arsenic and lead, in the hair of dogs from two largest town agglomerations of Slovakia, Bratislava and Košice. In addition to that, an effort was made to compare the environmental load in Bratislava and Košice by using the *dog as a bioindicator* of environmental pollution with heavy metals and to quantify the content of heavy metals in dog' hair.**
Methods: **The generally low level of heavy metals in biological material makes it impossible to use common analytical methods. In our study the samples of dog hair were analysed using the *method of atomic absorption spectrometry*. The measurements were carried out on a Perkin-Elmer spectrometer, model 5000, using the following wavelengths: 228.8 nm for Cd, 193.7 nm for As and 283.3 nm for Pb. The results of analyses are presented as means. The concentrations of Cd, As and Pb are expressed in µg/kg dog hair. Statistical evaluation of results was carried out by means of Student's t-test.**
Results: **The mean values of heavy metals in the *set of dogs from Bratislava* reached the following levels: 38.09 µg/kg for Cd, 111.07 µg/kg for As, and 729.59 µg/kg for Pb. The *set of dogs from Košice* allowed us to detect the following mean values: 22.21 µg/kg for Cd, 79.87 µg/kg for As, and 542.49 µg/kg for Pb. *The mean values for the entire Slovak territory* reached 27.39 µg/kg for Cd, 90.06 µg/kg for As, and 601.96 µg/kg for Pb. The comparison of the first two experimental sets of data with the Slovak mean values showed highly significant differences in arithmetic means ($p<0.01$) for Cd and significant ($p<0.05$) for As with the Bratislava set reaching higher values. No statistically significant differences were observed with regard to Pb.**
Conclusion: **The study confirms the need for further research in this area with the dog as a bioindicator involving also other environmental loads not only that resulting from heavy metals. It indicates the importance of risk assessment in relation to environmental pollution. As the studies on animals are applicable also to the human population it appears very appropriate to use also this indirect way to point out to the negative impact of the polluted environment. Lead, cadmium and arsenic are the heavy metals that raise particular concern because of their wide-spectrum negative influences on live organisms. *(Tab. 4, Fig. 5, Ref. 28.)***
Key words: environment, heavy metals, cadmium, arsenic, lead, dog.

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The man with his activities puts increasing demands on nature and because of that environmental pollution remains still the topical issue of the present society. The principal sources of heavy metal pollution, which raises particular concern in Slovakia and in other countries, include electric power supply, industry, agriculture, transportation and tourism. Heavy metals are introduced into the environment in two main ways, by natural and anthropogenic processes. The *natural processes* include weathering, atmospheric deposition related to soil, processes in oceans and volcano eruptions. Heavy metals are a natural part of the Earth surface and because of that they occur in different levels throughout nature. The *anthropogenic sources* are related to burning of fossil fuels, industrial activities and use of various transportation means. The highest proportion of heavy metals is released to the atmosphere in the form of emissions. They are deposited subsequently as dust and precipitations.

Heavy metals are all metals with specific weight exceeding 5,000 kg/m^3. When focusing on environmental pollution, the group name *heavy metals* is commonly used for the following metals and semi-metals: lead, cadmium, mercury, chromium, arsenic, copper, nickel, selenium, vanadium, antimony, bismuth, uranium, cobalt, tellurium, silver, thallium, thorium, and tin. Their levels in soil, water and air are generally relatively low. It should be noted that the anthropogenic activities are the very source which can increase significantly their levels. Heavy metals are chemical elements and because of that they do not decompose in the environment and may accumulate excessively in its different parts. Metals are mostly bound in inorganic compounds but can also become a part of complex organic compounds. This very fact is highly important from the point of view of their effects on all organisms by means of affecting the enzymatic processes.

Animals, particularly the companion species, for example dogs, are a very good indicator of the environmental load because they live together with the man and are exposed to the same pollutants. Therefore determination of the environmental load of heavy metals on dogs can serve as a parallel indicator of their load on humans.

Emissions play the most important role in environmental pollution by heavy metals. The following percentage proportions of individual heavy metals have been identified in emissions: Pb 28.96 %, As 16.26 %, Zn 15.76 %, Cu 14.08 %, Cr 12.07 %, Ni 8.03 %, Se 2.18 %, Cd 1.91 %, Hg 0.76 %. Monitoring of contamination in Slovakia has been carried out particularly in industrial areas and large towns. The highest deterioration of environmental quality has been observed in the following industrial agglomerations: Bratislava, Banská Bystrica, Žilina, Horná Nitra, Ružomberok, Žiarska fold, Hnúšťa – Tisovec, Stredný Spiš, Jelšava – Lubeník, Košice, Prešov, and Strážske – Vranov – Humenné (Bíreš, 1993; Weissová, 1997; Kozák et al, 1998; Sokol et al, 1998; Kováč et al, 2001).

The aim of the study

The aim of the study was to compare and evaluate the content of three different heavy metals in the dog' hair in two largest town agglomerations of Slovakia, Bratislava and Košice, namely of lead, cadmium and arsenic. In addition to that, the extent of environmental load was to be assessed in Bratislava and Košice by using the dog as a bioindicator of contamination of the environment with heavy metals through evaluation of the respective heavy metals in dog' hair.

The output of the study was expected in the form of a set of data that could be used as a partial study focusing on evaluation of environmental load in large towns not only in Slovakia.

Materials and methods

The evaluation of environmental contamination with heavy metals was carried out in two groups of dogs, each constituting a representative sample of dogs living in Bratislava and Košice. We examined altogether 98 samples of dog hair, 32 from Bratislava and 66 from Košice. All dogs were kept consistently as companion animals, the majority of them in apartments and only few in family houses. Most of them were located in large housing developments. The age of dogs of both groups ranged from 1 to 11 and 1 to 13 years, resp., with dogs four to six years old being the largest category.

Before sampling the hair all dogs were bathed thoroughly to eliminate as much as possible the contamination with the deposited atmospheric dust. The hair was sampled in all the cases from the central dorsal area using an electric shaver, type Oster 5–55. Individual samples weighed approx. 2 g.

Samples were packed individually into marked plastic microtene bags. They were stored dry in a refrigerator until analysed.

The preparation of samples consisted in wrapping about 2 g quantity in a filter paper forming a small roll. The prepared rolls were rinsed thoroughly with distilled water, transferred to a Soxhlet apparatus and extracted with ether for 2 hours. About 0.5 l of dimethyl ethyl ether was used for one sample, the extract was collected and evaporated at 60 °C, and let dry in the air.

An important part of the analysis was the preparation of platinum dishes before the proper processing. It consisted in thorough mechanical cleaning, rinsing with distilled water and three rinsings with deionized water. The dishes were dried and weighed. Clean dry dog's hair (1–2 g) was weighed into the precleaned dishes to four decimal places using an electronic balance.

The samples were prepared and processed at the Institute of Physiology of Farm Animals of the Slovak Academy of Sciences. They were mineralised in a dry way. The procedure was based on drying the analysed sample, its burning and igniting at the access of air. The material investigated was burned in a muffle furnace at 450 °C for 12 h and then ignited in a similar muffle furnace at 550 °C for 4 h. The ignited samples were subjected to two-stage solubilization, first in 17 % HCl and subsequently in an acid mixture of the following composition: HCl, HNO_3, H_2O at the ratio of 1:2:7. The samples were then transferred quantitatively to individual 10 ml volumetric flasks and made up to the mark with the acidic mixture. This way prepared solutions were ready for the measurement of the content of heavy metals by the flame-free AAS method.

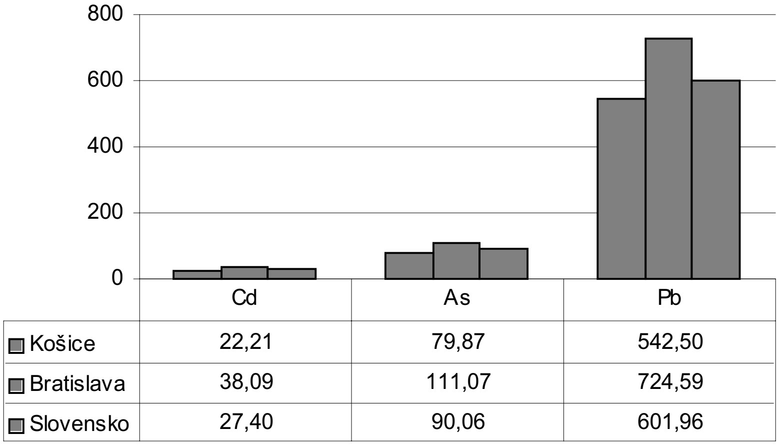


Fig. 1. The content of heavy metals in dog's hair in µg/kg. Slovensko = Slovakia.

With regard to the generally low level of heavy metals in biological materials, common analytical methods cannot be used. The detection limit of an applicable method should be at least 10-fold lower than the allowed concentration (Cibulka, 1991). This is the reason why the *method of atomic absorption spectrometry* was used. The measurements were carried out on a Perkin-Elmer spectrometer, model 5000. The atomisation took place in a graphite cell, type HGA 500, in an argon atmosphere using the wavelengths 228.8 nm for Cd, 193.7 nm for As and 283.3 nm for Pb.

The results of analyses were reported as mean values. The concentrations of Pb, Cd and As were expressed as µg/kg hair.

Tab. 1. The content of heavy metals in dogs in µg/kg.

	n	Cd	As	Pb
Košice	66	22,21	79,87	542,50
Bratislava	32	38,09	111,07	724,59
Slovakia	98	27,40	90,06	601,96

Tab. 2. Comparison of the level of heavy metals in dogs according to age in Bratislava (µg/kg).

Age	n	Cd	As	Pb
1	4	30,19	58,52	803,20
2	1	22,55	60,07	748,40
3	1	13,08	207,96	267,39
4	2	82,42	78,36	885,49
5	3	21,59	175,81	332,01
6	5	35,47	83,88	1255,04
7	3	16,28	111,54	555,99
8	3	64,80	128,97	387,56
9	1	30,66	196,85	656,23
10	6	41,86	102,42	723,00
11	2	45,47	88,72	721,62
12	x	x	x	x
13	1	39,68	238,38	648,02

Statistical evaluations were carried out by Student's t-test using a computer package MS OFFICE 2000 CZ Premium and a computer Pentium II Klamath.

Result

The mean levels of the heavy metals investigated in the hair of *dogs from Bratislava* reached 38.09 µg/kg for Cd, 111.07 µg/kg for As, and 729.59 µg/kg for Pb.

The mean levels reached in the hair of *dogs from Košice* were 22.21 µg/kg for Cd, 79.87 µg/kg for As, and 542.50 µg/kg for Pb.

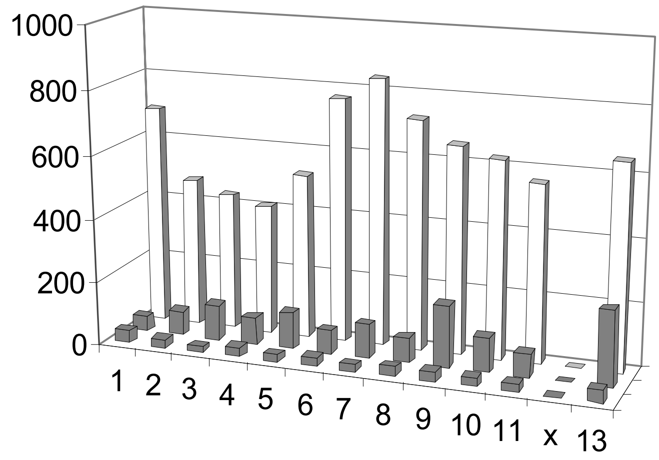
The mean values reported for Slovakia were 27.40 µg/kg for Cd, 90.06 µg/kg for As and 601.96 µg/kg for Pb (Tab. 1, Fig. 1). The comparison of both groups showed highly significant differences in arithmetic means ($p<0.01$) for Cd and significant ($p<0.05$) for As with higher values in the group from Bratislava. No statistical differences were observed in the values of Pb.

The comparison of dogs from Bratislava and Košice, divided to subgroups according *to ages*, showed significant differences ($p<0.05$) in the content of lead in four years old dogs (Bratislava 885.49 µg/kg; Košice 345.28 µg/kg). Statistical differences in the content of Cd in 11-years old dogs were determined on the same level of significance with mean values of 45.47 µg/kg in Bratislava and 15.48 µg/kg in Košice (Tab. 2 and 3, Fig. 2).

The levels of heavy metals *in relation to age* are presented in Table 4 and Figs 3, 4 and 5. No unambiguous accumulation was observed in older animals although some tendency was noted. Maximum concentration of Cd recorded in young dogs less than 1 year of age was 39.35 µg/kg, of As in 5-year old dogs 115.16 µg/kg and the highest concentration of Pb in 7-years old dogs reached 839.77 µg/kg.

Discussion

The presence of heavy metals in the environment is undesirable. Their toxicity depends on the quantity accepted, exposure



	1	2	3	4	5	6	7	8	9	10	11	x	13
Cd	39,35	25,05	17,56	29,98	21,92	26,53	21,32	30,17	30,66	25,45	27,47	0	39,68
As	47,52	76,67	111,47	84,48	115,16	72,08	106,56	78,35	196,85	109,28	76,59	0	238,38
Pb	692,76	470,45	434,66	408,84	519,08	770,14	839,77	724,39	656,23	621,87	561,88	0	648,02

Fig. 2. Mean values of heavy metals in dog's hair according to age in µg/kg in Slovakia.

time, animal species, age, nutrition, stress factors and biological susceptibility. Toxicity also depends on chemical composition of their compounds, water solubility, pH of the environment, and interaction with other elements. **Lead** emissions originate mainly from transportation in its different forms, production of iron and steel, and production of glass and non-ferrous metals. The main source of **cadmium** is the production of glass and, to a lesser degree, production of non-ferrous metals and transportation. The majority of **arsenic** emissions has been ascribed to production of non-ferrous metals, burning of fossil fuels and the use of pesticides (Violová, Magulová, 1995). The principal sources of metal pollution are the local point sources that emit heavy metals to their immediate surroundings (Ondrašovič et al, 1996).

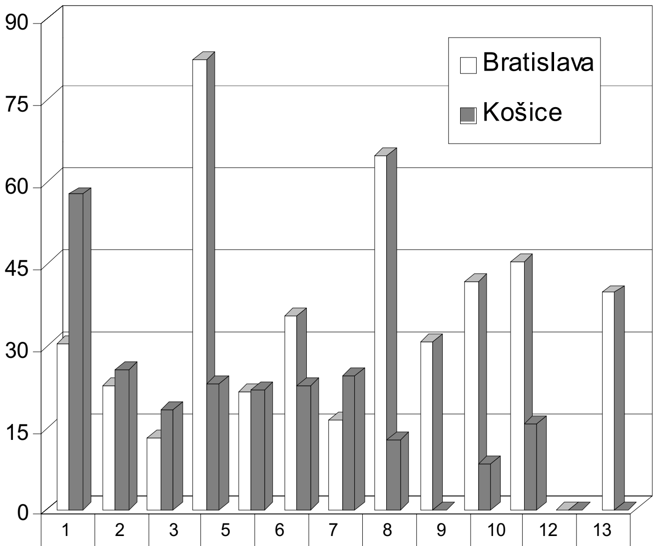
Tab. 3. Comparison of the level of heavy metals in dogs according to age in Košice (µg/kg).

Age	n	Cd	As	Pb
1	2	57,	67 25,50	471,88
2	6	25,47	79,44	424,12
3	6	18,31	95,38	462,54
4	15	22,99	85,30	345,28
5	11	22,02	98,62	570,10
6	11	22,47	66,73	549,73
7	5	24,35	103,57	1010,05
8	6	12,86	53,04	892,80
9	x	x	x	x
10	1	8,16	31,68	270,31
11	3	15,48	68,50	455,39

Animal organisms are most sensitive in the period of growth, gravidity and lactation. Differences between individuals affected by age and sex were recorded by Holm (1984), Tataruch (1984), Froslir et al (1986).

All animal species including the man have been used as bioindicators of environmental pollution. However, the evaluation of bioindication data requires unconditionally the sufficient knowledge about relationship between respective bioindicators and noxious substances and between bioindicators and other organisms. Many authors paid attention to dogs as bioindicators (Cattaned et al, 1985; Koh, 1985, 1986; Hansen, 1989; Hayashi and Tsukamoto, 1987; Hamir, 1986; Kučera, 1988; Von Stief, 1989).

Many biological materials can serve to such purpose. Maňkovská (1990) recommended also animal hair as it grows at relatively constant rate and can be used therefore for determination of the accepted quantity of metals in various periods in the past. Moreover, obtaining hair samples presents no problems. Holm (1984) observed correlation between the content of Pb, Cd and As between the liver and kidneys, kidneys and muscles, liver and hair and kidneys and hair in a rabbit. We based our observations on these facts and selected the dog as a suitable bioindicator. Another important factor of this approach is the possible parallel with the man from many different aspects. Many dogs that are not fed commercial dog feed become in fact the table companions of humans. They also drink water coming from the municipal water supply. It means that we can eliminate contamination of dogs with heavy metals originating from food and water, corresponding to the Codex Alimentorum. Therefore only contamination from the air can be considered. However, Ronneau et al (1983) stated that the most frequent source of contami-



	1	2	3	5	6	7	8	9	10	12	13
Bratislava	30,19	22,55	13,08	21,59	35,47	16,28	64,80	30,66	41,86	0,00	39,68
Košice	57,67	25,47	18,31	22,02	22,47	24,35	12,86	0,00	8,16	0,00	0,00

Fig. 3. Mean values of cadmium in dog's hair according to age in µg/kg.

nation of farm animals and also of dogs are toxic substances that can pass to them in the form of contaminated feed.

Arsenic is included in the group I of substances carcinogenic to man; Cd and Pb are potential carcinogens (according to IARC - International Agency for the Research of Cancer).

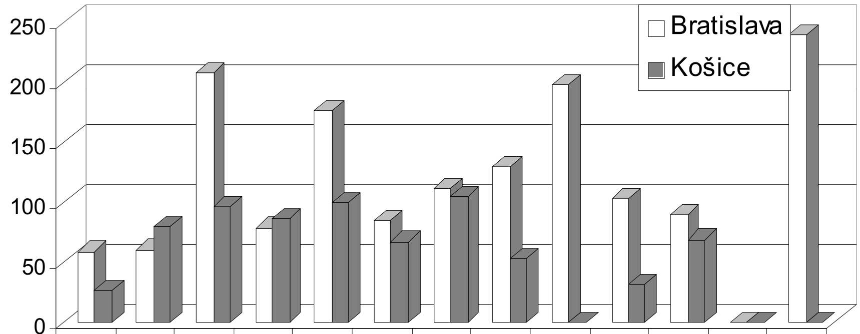
The level of Arsenic on the other hand, some authors indicated essentiality of arsenic which had been used even as a growth stimulator in pigs and poultry. If the recommended levels were not exceeded it did not accumulate extensively in the animal tissues (Underwood, 1977). Smith and Rongstad (1981) determined 0.65 $mg.kg^{-1}$ arsenic in the hair of healthy people. The study of 1000 samples of hair provided values ranging from 0.03 to 74 $mg.kg^{-1}$ with the mean value of 0.81 $mg.kg^{-1}$. According to this author, the level of 2–3 $mg.kg^{-1}$ raises suspicion of intoxication. Significantly higher were determined in men hair. The mean value for men was 0.62 $mg.kg^{-1}$ and for women 0.37 $mg.kg^{-1}$. According to WHO the present limit for human hair is 0.08–25 µg/kg. The information about the values in animals is scarce and because of that we present the human values. The levels determined in Slovakia, compared with those presented by Underwood (1977), are within acceptable limits with the value applied generally to town agglomerations in Slovakia reaching 27.395 µg/kg. The mean value for Bratislava was 111.074 µg/kg and for Košice 79.872 µg/kg. These values are lower in comparison with those published by other authors. Bíreš (1993) observed that the values in sheep reared close to the source of contamination (copper plant) reached 5.26 $mg.kg^{-1}$.

The level of Cadmium in Slovakia (0.027 $mg.kg^{-1}$) is lower than that published in the majority of sources and is even below the lower limit. Levels of Cd in the hair reached 1.83 $mg.kg^{-1}$ in cattle (Piskáč et al, 1985), 0.04–0.15 $mg.kg^{-1}$ in calves (Cibulka, 1991) and 0.09–0.19 $mg.kg^{-1}$ in sheep (Horák and Helan, 1983). Píša and Cibulka (1989) found 0.04–0.15 $mg.kg^{-1}$ of Cd in cattle hair. Anke (1985) found 0.123 $mg.kg^{-1}$ of Cd in the hair of horses. The following levels were reported in wild-living animals: 0.06 $mg.kg^{-1}$ in roe-buck, 0.09 $mg.kg^{-1}$ in deer and 0.06 $mg.kg^{-1}$ in chamois (Chudík and Maňkovská, 1989). Underwood (1977) detected 2.76 $mg.kg^{-1}$ of cadmium in the hair of men and 1.77 $mg.kg^{-1}$ in women.

The levels of Lead determined in our study do not differ much from the majority of published values. The level determined in Bratislava was 724.59 µg/kg, in Košice 542.50 µg/kg and the nation-wide mean value reached 601.96 µg/kg. Underwood (1977) found that the hair of children contained 2–95 $mg.kg^{-1}$ of lead, the hair of men 17.8 $mg.kg^{-1}$ and of women 19 $mg.kg^{-1}$ of

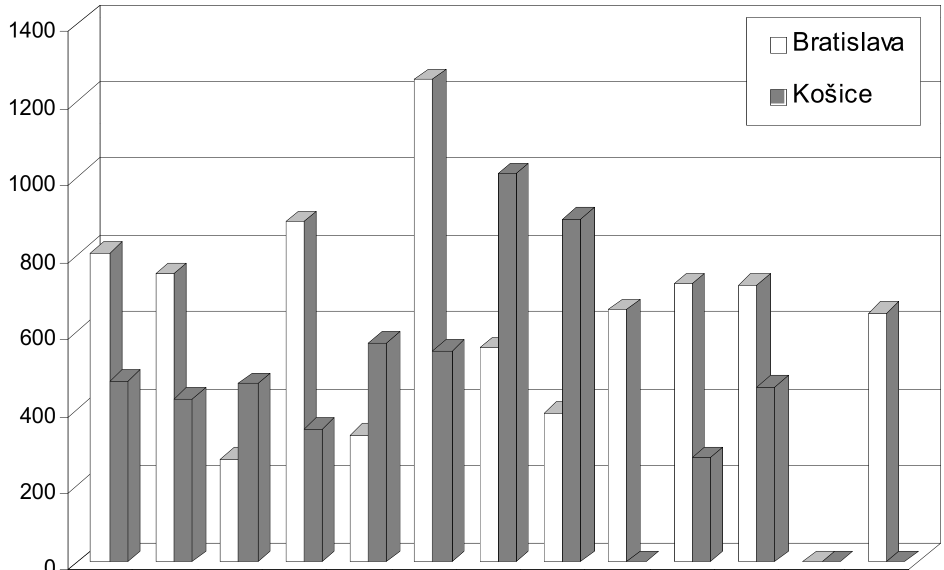
Tab. 4. Comparison of the level of heavy metals in dogs according to age in µg/kg.

Age	n	Cd	As	Pb
1	6	39,35	47,52	692,76
2	7	25,05	76,67	470,45
3	7	17,56	111,47	434,66
4	17	29,98	84,48	408,84
5	14	21,92	115,16	519,08
6	16	26,53	72,98	770,14
7	8	21,32	106,56	839,77
8	9	30,17	78,35	724,39
9	1	30,66	196,85	656,23
10	7	25,45	109,28	621,87
11	5	27,47	76,59	561,88
12	x	x	x	x
13	1	39,68	238,38	648,02



	1	2	3	4	5	6	7	8	9	10	11	x	13
Bratislava	58,52	60,07	207,9	78,36	175,8	83,88	111,5	128,9	196,8	102,4	88,72	0	238,3
Košice	25,50	79,44	95,38	85,30	98,62	66,73	103,5	53,04	0,00	31,68	68,50	0	0,00

Fig. 4. Mean values of arsenic in dog's hair according to age in µg/kg.



	1	2	3	4	5	6	7	8	9	10	11	x	13
Bratislava	803,20	748,40	267,39	885,49	332,01	1255,04	555,99	387,56	656,23	723,00	721,62	0	648,02
Košice	471,88	424,12	462,54	345,28	570,10	549,73	1010,05	892,80	0,00	270,31	455,39	0	0,00

Fig. 5. Mean values of lead in dog's hair according to age in µg/kg.

lead. The mean value in dog's hair reported by Piskáč et al (1985) was 21 $mg.kg^{-1}$. Sawicka and Kapusta (1987) detected 0.05–9.93 $mg.kg^{-1}$ of Pb in the hair of mice. Additional authors reported the following values: 0.25–0.34 $mg.kg^{-1}$ in sheep (Horák and Helán, 1983), 0.19–0.76 $mg.kg^{-1}$ in calves, 0.19–0.76 $mg.kg^{-1}$ in cattle (Píša and Cibulka, 1989), 3.45 $mg.kg^{-1}$ in wild boar (Maňkovská, 1990), 1.83 $mg.kg^{-1}$ in roe-buck, 2.09 $mg.kg^{-1}$ in deer, 2.06 $mg.kg^{-1}$ in chamois and 13.42 $mg.kg^{-1}$ in bear (Chudík and Maňkovská, 1989). According to Underwood (1977), the level of cadmium increases with age which was not observed in our dogs. The highest levels were recorded in young dogs while the older animals showed no marked accumulation.

The study investigated the levels of three heavy metals in the hair of dogs from large town agglomerations in Slovakia. The results obtained revealed the following:

Mean values for the entire Slovak territory reached 27.40 µg/kg for Cd, 90.06 µg/kg for As and 601.96 µg/kg for Pb. The levels measured in the group of dogs from Bratislava showed highly significant increase in the concentration of Cd (38.09 µg/kg) significantly higher concentrations of arsenic (111.07 µg/kg) and insignificantly higher concentrations of Pb (724.59 μg/kg) in comparison with the group of dogs from Košice which contained in their hair 22.21 μg/kg of Cd, 79.87 μg/kg of As, and 542.50 μg/kg of Pb.

No unambiguous increase in accumulation of heavy metals was observed with the age although the highest levels of As were determined in 9-year old dogs and a 13-year old dog and the lowest in a dog younger than 1-year. The highest level of Pb was observed in 7-year old dogs and the lowest in 4-year old animals. Cd values were the highest in 1-year old dogs and in old individuals (13 years) and the lowest in 3-year old dogs.

The study indicates the necessity of additional research using dogs as bioindicators of environmental load not only with regard to heavy metals. It indicates the importance and need of assessment of the potential risk associated with environmental pollution. As the results of studies on animals are applicable also to humans it appears appropriate to point also in this indirect way to the negative influence of environmental contamination. Lead, cadmium and arsenic are the very metals the effect of which is highly topical.

The problems arising from contamination with heavy metals are very urgent. The risk can only be removed by total improvement of the environment which is a long-term and very demanding process. When determining the risk resulting from contamination with heavy metals one should consider several important aspects, such as higher susceptibility of young and old animals and, at the same time, the „latent“ action of these contaminants, as their main toxic effect consists in inhibition of various enzymes and co-enzymes, their long-term accumulation in the bodies and persistence for decades in the environment. These are the reasons that make it more difficult to determine the respective risk and to reveal their negative and frequently irreversible action on live organisms.

In conclusion we feel necessary to mention that the man with his various activities contributes significantly to this pollution as it is confirmed by our study. The results obtained point to the relationship between human activities and the extent of environmental pollution.

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