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Short communication

USE OF CANINE HAIR SAMPLES AS INDICATORS OF LEAD AND CADMIUM POLLUTION IN THE REPUBLIC OF MACEDONIA

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Summary

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Different animal species have been used in environmental pollution biomonitoring studies in different habitats. Pets could prove to be good indicators of human exposure since they share the same environment as their owners, and therefore are exposed to the same pollutants. The aim of this study was to use dog's hair to evaluate and compare the content of two heavy metals: cadmium and lead in different areas, considering the influence of the age. For this purpose, hair samples were collected from 95 dogs from different localities and analyzed by atomic absorption spectrometry. The main lead level in dog hair was 763.71± 558.30 µg/kg, and that of cadmium – 69.51± 102.92 µg/kg. The comparison of the results between the different areas showed statistically significant differences in canine hair lead content between Veles and Prilep (P<0.001). Cadmium hair concentrations differed statistically significantly between Veles and Probistip (P<0.01) as well as between Veles and Prilep (P<0.01). The results of this study confirmed the potential of using dogs as bioindicators of heavy metal environmental pollution.

Key words: cadmium, dog, environment, hair, lead, pollution monitoring

Human activities contaminate the environment with toxic metals that have a wide range of health effects in human and animal populations. Lead (Pb) is a major environmental pollutant which has adverse health effects on exposed humans and animals. Besides the naturally occuring lead, a considerable amount of this metal enters the environment from anthropogenic sources as use of fossil fuels, automobile exhaust, mining and smelting of lead ores (Balagangatharathilagar *et al.*, 2006).

Cadmium (Cd) occurs naturally in high abundance in zinc and lead ores and phosphate fertilizers. Agricultural uses of phosphate fertilizers and sewage sludge and industrial use of cadmium have been identified as a major cause of wide-spread dispersion of the metal at trace levels into the environment in general and human foodstuffs (Soisungwan *et al.*, 2003).

Biomonitoring of toxic metals in the environment has allowed identification of many sources of pollutants. Typically, the bioavailability of environmental pollutants is assessed by measuring chemical residues in tissues or fluids of animals (Komarnicki, 2000).

Different animal species have been used in environmental pollution biomonitoring studies in different habitats. Pets could prove to be good indicators of human exposure since they share the same environment as their owners, and therefore are exposed to the same pollutants. Dogs living in mining, smelting, industrial and urban environment have been reported to have higher blood lead level than those in rural environment (Balagangatharathilagar *et al.*, 2006).

The aims of this study were to assay and to compare lead and cadmium levels in hair of dogs at different ages and inhabiting different areas in the Republic of Macedonia.

A total of 95 dog hair samples were collected from different areas of the Republic of Macedonia. Thirthy eight were taken from smaller towns (population < 20,000) – 18 from Delcevo and 20 from Probistip. The other 57 hair samples were taken from dogs in bigger towns (population > 60,000): Bitola, Prilep and Veles. All dogs were kept as companion animals. The age of the dogs was 1–10 years. The largest groups were dogs at the age of 1 and 2 years.

About 2 g of hair was taken from the lower neck area, cut near to the skin with stainless steel surgical scissors. The hair samples were packed in paper rolls and placed in little plastic bags. Then the samples were stored in refrigerator till the analyses.

Every hair sample was washed with distilled water, placed in Kjeldahl flasks and immersed with acetone. Then the sample was filtered trough filter paper, soaked in non-ionic detergent TRITON 100X (Merck), then washed at least 3 times with redistilled water, dried at 105°C for evaporation of the chemicals, and finally weighed on an analytic scale (Krejpdo *et al.*, 1999).

Thus prepared, every sample was submitted to wet mineralization with 0.25 mL nitric acid (Nitric Acid 69% Tracepur, Merck) and 0.1 mL 30% hydrogen peroxide. After these preparations the samples were placed in a digester on thermoregulated *Combiplac Selecta P* sand bath. Every sample was moderately warmed and evaporated to obtain a small amount of clean extract. This liquid extract was transferred to a 25-mL volumetric flask and filled to the mark redistilled water. Lead and cadmium levels in hair samples were assayed on an Perkin Elmer AAnalist 600 atomic absorption spectrometer. The atomization took place in a graphite tube type HGA 700, with an argon atmosphere at wavelengths 283.3 nm for Pb and 228.8 nm for Cd. Before every series of samples, lead and cadmium standard solutions with concentration 1000±2 mg/L (Merck) were run first. Then several blank samples were measured.

After the calibration, measuring of the samples was performed. The electrothermal atomic absorption spectrometry (ETAAS) technique was used, due to the low concentrations of heavy metals in samples.

Statistical data processing was performed by Student's *t* test by means of the STATISTICA 6.0 STATSOFT software.

Lead and cadmium content in the hair of dogs from Delcevo was between 66.38 and 2085.7 µg/kg and 12.32–266.50 µg/kg respectively (Table 1).

The highest mean heavy metal concentrations in dog hair originated from Veles: 1099.02±593.01 µg/kg for lead and 171.54±179.53 µg/kg for cadmium. Samples collected from Prilep contained on

Table 1. Lead and cadmium content in dog hair collected from different towns in the Republic of Macedonia. Data are presented as mean ± SD (range)

Town	n	Lead (μg/kg)	Cadmium (μg/kg)
Delcevo	18	579.00±478.29 (66.38–2085.70)	68.57±59.95 (12.32–266.50)
Probistip	20	1061.38±564.02 (180.21–1866.23)	26.86±23.30 (5.00–78.00)
Veles	17	1099.02±593.01 (220.26–2233.33)	171.54±179.53 (34.21–625.50)
Prilep	18	370.57±288.39 (50.04–987.54)	21.65±10.64 (2.61–38.10)
Bitola	21	687.05±482.82 (54.16–1827.27)	66.04±73.78 (4.38–267.07)
Total	95	763.71±558.30	69.51±102.92

Table 2. Lead and cadmium content in dog hair according to their age. Data are presented as mean ± SD (range).

Age	n	Lead (μg/kg)	Cadmium (μg/kg)
1	27	743.20±544.73 (95.64–2085.70)	80.59±71.09 (2.61–253.64)
2	26	796.02±601.50 (50.04–2233.33)	78.97±27.19 (5.00–266.50)
3	14	1076.66±564.00 (65.20–1878.00)	66.71±60.39 (14.70–254.50)
4	10	769.88±513.03 (66.38–1730.77)	83.07±27.39 (7.74–267.07)
5	5	581.53±296.92 (140.00–925.00)	42.13±16.62 (26.10–60.80)
6	5	611.20±396.76 (199.40–1050.00)	40.93±22.89 (19.80–67.60)
>7	8	801.61±450.76 (220.30–1618.75)	72.84±56.55 (33.90–162.90)

the average 370.57±288.39 μg/kg lead and 21.65±10.64 μg/kg cadmium (Table 1).

The distribution of dog hair content of investigated heavy metals by age is presented on Table 2.

The comparison between the different areas showed a statistically significant difference between Veles and Prilep ($P<0.001$) for Pb. Cadmium hair concentrations differed statistically significantly between Veles and Probistip ($P<0.01$) as well as Veles and Prilep ($P<0.01$).

In general, main sources of accumulation of heavy metals in the environment are local point sources which emit heavy metals in their environment (Ondrašovič *et al.*, 1996). Lead emissions originate from burning fossil fuels that are used for transport, iron and steel production, metallurgic industry and others. As for Cd, the main sources of its emissions occur during the production of glass and ceramic, and in lower degree by production in non-ferrous metallurgy and transport.

All animal species are more sensitive of heavy metal exposure in the period of growth, pregnancy and lactation. Numerous animal species, as well as men have been used in environmental pollution studies.

Dogs were also reported to be bioindicators of pollution (Kozak *et al.*, 2002; López-Alonso *et al.*, 2002; Balagangatharathilagar *et al.*, 2003). For this purpose, different biological materials from dogs have been used but animal hair has been recommended as biological material for determination of heavy metal exposure (López-Alonso *et al.*, 2002; Balagangatharathilagar *et al.*, 2006) because of its constant rate of growing and the relatively easy collection of samples.

The average level of lead for all Macedonian towns studied (763.71±558.30 µg/kg) in this study was lower then the results obtained by Masatoshi *et al.* (1981): 1890 µg/kg in Tokyo and by Park *et al.* (2005) from Yeongnam, Chungchong and Honam (urban Korea) – 820 µg/kg. Our results were however higher than those reported by Kozak *et al.* (2002): 601.90 µg/kg from Košice and Bratislava (Slovakia).

The level of cadmium (69.51±102.92 µg/kg) was also lower than those in other studies on dogs carried out by Masatoshi *et al.* (1981) and and by Park *et al.* (2005) (390 µg/kg and 90 µg/kg, respectively). Same as for lead, our results for cadmium were higher than those presented by Kozak *et al.* (2002) – 27.40 µg/kg.

The dog hair samples collected from the regions where lead has been deposited in the environment had higher mean levels, like in samples from Probistip and Veles. Near Probistip there is a functioning lead ore Zletovo mine. Moreover, in 1976 an old tailing dam had collapsed and contaminated the soil and two regional rivers. At about 5 km from Veles, there is a lead and zinc smelter which had worked over 20 years till 2004.

There was a difference in the cadmium level in these two towns. The hair samples from Probistip were with the lowest content of cadmium (26.8 µg/kg), while those taken from Veles contained 171.50 µg/kg Cd, which was the highest mean level from all the areas.

The lowest mean cadmium level was that in hair samples from Prilep (21.60 µg/kg), while samples from Delcevo and Bitola had higher mean levels: 68.57 µg/kg and 66.04 µg/kg respectively. The hair samples from Prilep have been taken from dogs kept as pets in apartments, fed with home prepared food. The hair samples from Delcevo were mostly hunting dogs fed with home prepared food. On the other side the hair samples taken from Delcevo and Bitola have almost same contamination with cadmium.

One half of hair samples from Bitola were from dogs from villages of the Pelagonija used for hunting. The Mining Power Complex (TPP Bitola) in the region could probably play a role in pollution of the environment. This may be the reason for higher lead and cadmium levels in Bitola as compared to Prilep despite the closeness of these two towns (45 km). Mean lead and cadmium levels in Prilep were the lowest from all areas studied.

The highest mean lead level was measured in 3-year-old animals while the lowest was in 5-year-old dogs. The highest mean cadmium level was observed in 4-year-old dogs, while the lowest: in 6-year-old dogs. It is interesting that the lead level gradually increased from 1- to 3-year-old dogs (Table 2)

The results of this study confirmed the potential of using dogs as bioindicators of heavy metal environmental pollution. It aimed to encourage further similar research on the environmental pollution in the Republic of Macedonia and elsewhere. Heavy metals in hair samples are only some of the parameters that can be examined for evaluation of the environmental pollution. Water and soil examinations together with the presence of heavy metals in animals can give a complex evaluation of the environmental pollution.

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