Studies on Cd, Pb, Hg and Cr Values in Dog Hairs from Urban Korea

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ABSTRACT : Dogs are a very good indicator of the pollution load on the environment. They share people's environment and are exposed to the action of the same pollutants. This study was to estimate the heavy metal contents in dog hairs in domestic districts, and to assess effects of age, sex, feed habits, living area, breeding environment and smoking habit of owner. The mean concentrations of heavy metals in 204 samples were 0.09±0.10 μg/g, 0.21±0.09 μg/g, 0.82±0.09 μg/g and 0.48±0.07 μg/g (Cadmium (Cd), Mercury (Hg), Lead (Pb) and Chromium (Cr)) in dog hairs, respectively. Concentrations of Pb, Cd, Hg and Cr in dog hairs in Yeongnam including Ulsan and Seoul, were higher than in Chungchong and Honam, and concentrations of Pb were significantly different ($p<0.01$). Concentrations of Cd, Hg, Pb and Cr in dog hairs increased with age ($p<0.05$). Only dogs fed commercial pet foods had significantly higher Cd and Cr concentrations in hairs than dogs fed dog feed and human diet ($p<0.01$ in Cd and $p<0.05$ in Cr). Cr concentrations of dog hairsfrom dogs kept on cement floors were the highest of the other environments ($p<0.01$). Heavy metal concentrations of dogs owned by smokers, were higher than dogs of non-smokers, but there were no significant differences. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 8 : 1135-1140)***

Key Words : Dog Hairs, Heavy Metal Ion, Age, Sex, Feed, Area, Breeding Environment

INTRODUCTION

Heavy metal pollution has become a serious health concern in recent years, because of industrial and agricultural development.

In general, heavy metals of industrial biowaste broadly contaminate the drinking water, food and air. Although certain heavy metals are essential for biological systems, and in some cases, provide beneficial effects to animals, excesses are usually associated with harmful effects in men and animals. The toxic heavy metals of greatest concern are Cd, Pb and Hg and the exposure to these heavy metals is a continuous daily process (Goyer, 1996; Mortada et al., 2002).

It is recognized that heavy metals may exercise a definite influence on the control of biological functions, affecting hormone system and growth of different body tissues (Teresa et al., 1997). Many heavy metals accumulate in one or more of the body organs (liver, kidney, bone and brain.) with differing half-lives (King, 1990). Furthermore, these heavy metals apart from acute or chronic poisoning can be transferred to the next generation and have potential toxicity from the viewpoint of a public health (Iyengar and Nair, 2000). However, hair gives a better estimate of the total body intake of certain elements than does blood or urine (Wilhelm et al., 1989). Zook (1978) considered dogs and children to be very similar in their susceptibility to lead and risk do exposure from environmental sources. Owing to the close association and common environment shared with humans, dogs are exposed to similar pollutants and have been suggested as sentinels for biohazards from toxic pollutants (Bernny et al., 1995; Swaup et al., 2000). Companion animals can be particularly valuable sentinels for human exposures because they share much of their environment (air, water and food) with people. Also, several heavy metals are added to animal diets for various purposes, resulting in a high concentration of heavy metals in animal waste (Ko et al., 2004). Pets may be even more exposed than their owners to some contaminants, such as soil or house dust. Animals respond to many toxic insults in ways analogous to humans, and they can develop similar environmentally induced diseases by the same pathogenic mechanisms. Because animals typically have shorter, physiologically compressed life spans when compared with people, latency periods for the development of some diseases are shorter in animals (Backer et al., 2001). Up to date, only a few attempt have so far been made at study of the content of heavy metals in companion animal (Berny et al., 1995; Kozak et al., 2002).

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is very useful for element analysis in hair due to extremely low instrumental detection limits, wide analytical range, negligible matrix effects and capacity for simultaneous, rapid, accurate and precise determination (Budtz-Jorgensen et al., 2004). Only a few attempts have so far been made at concentration of heavy metals in biological samples for dogs.

This study was to measure the heavy metal concentrations in dog hairs in Korea and to assess whether a correlation occurs between dog hairs, so as to provide that

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Table 1. Sample distribution of dog's hair by area, age and sex

Areas	Ages (years)	Male	Female	Total
Seoul	<1	5	9	14
	1-2	11	7	18
	>2	9	7	16
Yeongnam	<1	7	9	16
	1-2	11	7	18
	>2	9	7	16
Chungchong	<1	9	9	18
	1-2	11	7	18
	>2	9	7	16
Honam	<1	11	11	22
	1-2	7	9	16
	>2	9	7	16
Total		108	96	204

Take sample homogenized into vessel of MDS

↓

Add 20% nitric acid and stand for 30 mins

↓

Digest with microwave digestion system

↓

Cool in ice bath for 1 h

↓

Shake thoroughly for 5 mins

↓

Filter with 0.45 μm membrane filter

Fig. 1. Sample preparation for the analysis of heavy metals.

dogs can be used to monitor environmental quality of heavy metals.

METERIALS AND METHODS

Collection of samples

Samples of dog hairs (1.5 g) and bloods (5 ml) were collected from apparently healthy dogs with no history of occupational exposure to Cd. Pb. Hg and Cr from different localities of Korea. After the skin was cleaned with 70% ethanol. whole blood was collected by vein puncture. About 5 ml of blood was drawn into a second syringe and transferred to a vacutainer (Brand. sterile interior SST® Gel and clot activator, USA) and immediately centrifuged at 800 g for 15 mins. The supernatant serum was transferred to an Ependorf tube (Becton Dickinson, USA) and frozen at -70°C until analysis. Approximately 1.5 g of dog hairs was collected from the nape of the neck using stainless steel scissors. and stored in polyethylene bag until analysis (Table 1).

Reagents and laboratory ware

Preparation of standards was carried out under clean conditions using deionized water of specific resistively of 16-18 MΩ (ELGA Ltd., UK). Analytical standards of Cd, Pb, Hg and Cr (Sigma Co., USA) were prepared from solutions (50% nitric acid. Merck Co., Germany) with a nominal concentration of 50, 100, 200 and 1.000 mg/L. All chemicals and reagents used were of ultra pure reagent grade.

Table 2. Operating conditions and data acquisition parameters of ICP-MS (HP 4500)

Analytical parameters	Conditions
Rf power (W)	1,200
Argon gas flow rates	
Plasma	16.0 L/min
Auxiliary	1.1 L/min
Carrier	1.0 L/min
Torch	Quartz, 2.5 mm
Nebulizer	Peek, Babington - type
Spray chamber	Glass, double pass
Sampler an skimmer cones	Nickel
Data acquisition	
Acquisition parameters	Quantitative
Points/mass	12
Integration time/mass	0.1 sec
Total acquisition time/replicate	7.17
Replicates	3
Total acquisition time/sample	21.5 sec

Sample preparation

In order to remove most of the adhering dust and oily materials and any loosely-bounded contaminants from perspiration and external source. dog hairs were given a pre-irradiation washing treatment. Dog hairs were cut into small pieces. manually homogenized into the vessel of microwave digestion system (MDS. Qlab 6000. Questron Co., USA). washed briefly with acetone. deionized water. again with acetone and then dried at 105°C. The dog hair samples were then transferred into a large weighing boat and placed in a drying box. The drying box contained desiccant for drying the samples. The samples were left overnight to dry and digested the next day.

Analysis

All of the sample (1 g dog hairs) was digested by weighing the sample directly into the Teflon vessel of MDS. adding 10 ml 20% HNO_3 (v/v) and then placing them into the microwave Teflon vessel of MDS. which was placed into the microwave oven and digested with MDS. On completion of the digestion program. the Teflon vessel was placed in a bath of cold water for 1 h before the vial was opened. The Teflon vessel was shaken thoroughly for 5 mins. filtered through a 0.45 μm membrane filter (Millipore Co., USA) (Figure 1).

Instrumentation

Cd, Hg, Pb and Cr were determined by ICP-MS (Hewlett-Packard 4500. USA). Operation conditions are outlined in Table 2.

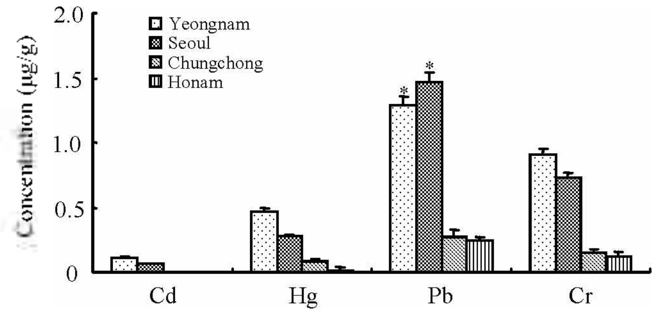

Figure 2. Effect of areas on metal concentration in dog hairs. * Significantly different between the indicated groups ($p<0.01$).

Table 3. Metal concentration on sex in dog hairs

Element	Sex		Mean (204)[1]
	Male (108)	Female (96)	
Hair (μg/g)			
Cd	0.09±0.18[2]	0.05±0.09	0.07±0.13
Hg	0.25±0.19	1.08±0.52	0.66±0.35
Pb	0.98±0.06	1.06±0.04	1.02±0.05
Cr	1.97±0.09	2.86±0.47	2.41±0.28

[1] Number of sample. [2] Mean±SD.

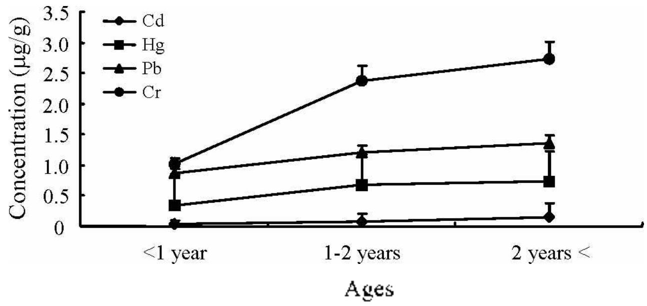

Figure 3. Effect of age on metal concentrations in dog hairs.

Table 4. Effect of smoking habit of owner on metal concentration in dog's hairs

Element	Smoking habit of owner		Mean (204)[1]
	Smoking habit owner (100)	Non-smoking habit owner (104)	
Hair(μg/g)			
Cd	0.09±0.21[2]	0.06±0.09	0.07±0.15
Hg	0.89±0.11	0.43±0.21	0.66±0.16
Pb	1.06±0.03	0.96±0.04	1.01±0.04
Cr	2.81±0.12	1.87±0.11	2.34±0.12

[1] Number of sample. [2] Mean±SD.

Statistical analysis

Statistical analyses were performed by use of SPSS (version 10). All data were expressed as mean ± SD and the level of significance was determined at $p<0.05$ and $p<0.01$. Analysis of variance (ANOVA) was used to determine the statistical significance of variables and concentration of heavy metals among the study area, age, weight and breeding environment.

Effect of areas on metal concentrations in dog hairs

In dog hairs, the mean Cd concentration was 0.11±0.17 μg/g in Yeongnam, 0.06±0.02 μg/g in Seoul and not detected in Chungchong and Honam, respectively. The mean Hg concentration was 0.47±0.21 μg/g, 0.28±0.44 μg/g, 0.08±0.07 μg/g and 0.01±0.02 μg/g. The mean Pb concentration was 1.28±0.09 μg/g, 1.47±0.08 μg/g, 0.26±0.13 μg/g and 0.24±0.02 μg/g, respectively. The mean Cr concentration was 0.91±0.12 μg/g, 0.73±0.11 μg/g, 0.14±0.13 μg/g, and 0.12±0.03 μg/g, respectively. There were statistically significant differences in Pb concentrations in dog hairs among the groups ($p<0.01$), but there were no statistically significant differences in Cd, Hg, and Cr (Figure 2).

Effect of sex on metal concentrations in dog hairs

In dog hairs, the mean Cd concentration was 0.09±0.18 μg/g for male and 0.05±0.09 μg/g for female, respectively. The mean Hg concentration was 0.25±0.19 μg/g and 1.08±0.52 μg/g. The mean Pb concentration was 0.98±0.06 μg/g and 1.06±0.04 μg/g. The mean Cr concentration was 1.97±0.09 μg/g and 2.86±0.47 μg/g. Consequently, males had higher in Cd than females, and females had higher in Cr, Pb and Hg than males. But, there were no statistically significant differences (Table 3).

Effect of each age on metal concentrations in dog hairs

In dog hairs, the mean Cd concentration was 0.03±0.01 μg/g for below one year, 0.07±0.02 μg/g for 1-2 years and 0.14±0.02 for over 2 years, respectively. The mean Hg concentration was 0.33±0.11 μg/g, 0.67±0.14 μg/g, and 0.73±0.02 μg/g, respectively. The mean Pb concentration was 0.85±0.09 μg/g, 1.21±0.08 μg/g, and 1.35±0.02 μg/g, respectively. The mean Cr concentration was 1.01±0.12 μg/g, 2.38±0.11 μg/g, and 2.73±0.03 μg/g, respectively. The concentration of heavy metals increased by age but there were no statistically significant differences in metal concentrations in dog hairs among the groups (Figure 3).

Effect of smoking habit of owner on metal concentrations in dog hairs

In dog hairs, it was a somewhat difference in Cd concentration between dogs of smoking habit owner and non-smoking habit owner in the determined levels, was 0.09±0.21 μg/g and 0.06±0.09 μg/g, Hg were 0.89±0.11 μg/g and 0.43±0.21 μg/g, Pb were 1.06±0.03 μg/g and 0.96±0.04 μg/g, Cr were 2.81±0.12 μg/g and 1.87±0.11 μg/g, respectively. Little higher levels of heavy metals are in dogs of smoking habit owner, but there were no statistically significant differences (Table 4).

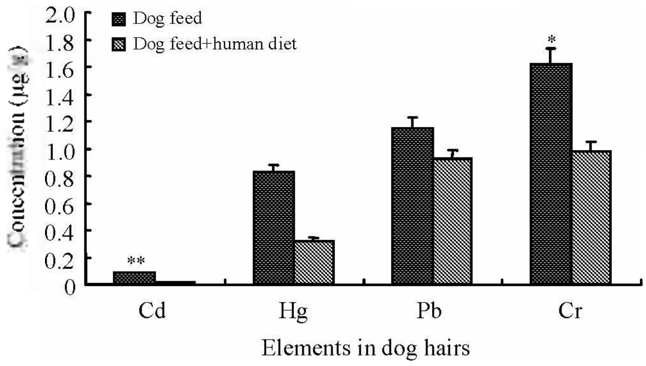

Figure 4. Metal concentration in dog hairs by feed styles. * Significantly different between the indicated groups (* p<0.01, ** p<0.01).

Effect of feed types on metal concentrations in dog hairs

In dog hairs, the mean Cd concentration was 0.09±0.06 μg/g for dog feed and 0.02±0.02 μg/g for dog feed+human diet. The mean Hg concentration was 0.83±0.11 μg/g and 0.32±0.12 μg/g. The mean Pb concentration was 1.15±0.06 μg/g and 0.93±0.14 μg/g. The mean Cr concentration was 1.62±0.01 μg/g and 0.98±0.05 μg/g, respectively. Dog feed had significantly higher levels of Cd and Cr in their dog hairs than the others (p<0.01 for Cd and p<0.05 for Cr) (Figure 4).

Effect of breeding environment on metal concentrations in dog hairs

In dog hairs, the mean Cd concentration was 0.05±0.01 μg/g for Indoor, 0.07±0.04 μg/g for outdoor (cement) and 0.15±0.02 μg/g for outdoor (sand). The mean Hg concentration was 0.75±0.03 μg/g, 0.17±0.01 μg/g, and 0.19±0.02 μg/g. The mean Pb concentration was 1.12±0.07 μg/g, 0.77±0.08 μg/g, and 0.85±0.01 μg/g. The mean Cr concentration was 1.76±0.02 μg/g, 8.86±0.04 μg/g, and 3.93±0.07 μg/g, respectively. Outdoor (cement) had significantly higher levels of Cr in there dog hairs than the others (p<0.01) (Table 5).

Correlation coefficients among parameters in dog hairs

By correlation coefficients between dog hairs of all subjects, a significant correlation was found between Hg (r = 0.594, p<0.01) and Pb (r = 0.556, p<0.01) in dog hairs and serum. But, in Cr (r = 0.097) and Cd (r = -0.050), there was no significant correlation between dog hairs and serum (Table 6).

Table 6. Correlation coefficients among parameters between dog hairs and serum

H \ S	Cr	Cd	Hg	Pb
Cr	0.097			
Cd		-0.050		
Hg			0.594*	
Pb				0.556*

* Pearson's correlation at p<0.01. S: Serum, H: Hairs.

DISCUSSION

In other previous studies (Motada et al., 2001), the determination of Cd, Pb and Hg levels in blood, urine, hair, and nails have proven to be of considerable use for the diagnosis of metal over exposure. Though a large number of studies have been made on heavy metal concentration of hairs and blood in human, little is known about that of hairs in dogs.

Backer et al. (2001) mentioned that companion animals, for example dogs, are a very good indicator of the pollution load on the environment. They inhabit in the same space as humans and are exposed to the same pollutants. The examination of animals is able to complete the information obtained by the examination of inhabitants. It may be even assumed that the changes of body burdens of environmental pollutions start earlier than those in man, because the animals are exposed to the impact of contamination more directly (Petering et al., 1973), and Bencko (1991) found virtually identical concentrations of arsenic in the hairs of children and hairs of rabbits living in the same locality. In another previous study (Muller and Anke, 1994), the determination of Pb levels in dog hairs have been carried. Our study documented the Pb values of dog hairs was 1.29±0.09 μg/g in Yeongnam, 1.47±0.08 μg/g in Seoul, 0.27±0.14 μg/g in Chungchong and 0.25±0.03 μg/g in Honam, respectively. This result demonstrated that dogs lived in rural area contained less heavy metal in their hair compared to metropolitan area like Seoul.

Table 5. Metal concentrations on breeding environment in dog hairs

Element	Breeding environment			Mean
	Indoor (140)	Outdoor-cement (40)	Outdoor-sand (24)	(204)[1]
Hair (μg/g)				
Cd	0.05±0.01[2]	0.07±0.04	0.15±0.02	0.09±0.02
Hg	0.75±0.03	0.17±0.01	0.19±0.02	0.37±0.02
Pb	1.12±0.07	0.77±0.08	0.85±0.01	0.91±0.05
Cr**	1.76±0.02	8.86±0.04	3.93±0.07	4.85±0.04

[1] Number of sample. [2] Mean±SD.

* Significantly different between the indicated groups (p<0.05).

** Significantly different between the indicated groups (p<0.01).

Comparisons between our results for dog hairs Pb with those recently published include 0.73 μg/g for Pb in Bratislava, Slovakia (Kozak et al., 2002). These findings imply that the Pb exposure of the dog in Seoul and Yeongnam is more than that in the others, also more than Slovakia. This may be due to dense traffic in Seoul, which may lead to more Pb emissions from automobile exhaust as well as increasing industrial emission of Ulsan surrounding Yeocheon Petroleum Chemistry Complex. Koh and Babidge (1986) compared blood Pb levels in dogs from a lead-mining (1.05 μmol/g), lead-smelting (0.80 μmol/g), urban (0.38 μmol/g) and rural island (0.32 μmol/g) environment. Ward et al. (1977) described results of a 1976 survey in New Zealand of 1,142 domestic animals, including 271 dogs. The mean Pb concentrations of dogs in New Zealand were reported as 0.23 μg/ml, with a range of 0.08-0.36 μg/ml. Rural sheepdogs had a mean blood lead value roughly half that of urban dogs (0.15 vs. 0.27 μg/ml), and this difference was reported as being highly significant. Ostrowski et al. (1990) described results of blood lead values in dogs from a rural area in 1987, the mean lead concentrations of dogs were reported as 0.05 μg/ml.

Our results for Cd levels in dogs were 0.09±0.10 μg/g in dog hairs. Comparisons between our results for hairs Cd with that of other study (0.03 μg/g for Cd in Bratislava, Slovakia, Kozak et al., 2002), 0.11±0.17 μg/g in Yeongnam and 0.06±0.02 μg/g in Seoul suggest that the levels of hairs Cd among dogs vary by environment. The result demonstrated that industrial pollution (extensive use of Cd for anti-corrosion plation of metals, construction of batteries, pigments, stabilizer for PCV etc) using Cd in this area. All things considered, variations in the level of hairs Cd are related to different environments. But no supportive recent data were reported in the literature to present the normal or toxic levels of Cd in dog hairs. The present study showed that Cr concentration of dog hairs breed in outdoor-cement was higher than the other environment. It is because that Cr is used to make cement production. So, it seems reasonable to conclude that Cr affected dogs rolling in the cement in cement.

It was concluded that levels of heavy metal in dog hairs are affected by geographic factors. Especially, Pb levels in dog hairs were significantly different ($p<0.01$). We found that dogs owned by smoking habit, had higher heavy metal concentration in dog hairs than those by non-smoking habit owner. But there were no significant differences. In addition to this, there are studies concerning correlation between trace element concentrations in hairs (Folin et al., 1991). And Hodkins et al. (1991) point to high correlation coefficients for Pb concentrations in blood and hair: in children ($r = 0.85$ for $p<0.1$), and in adults ($r = 0.72$ for $p<0.1$), respectively (Bergoni et al., 1989; Cabeza et al., 1991; Hodkins et al., 1991). In humans, blood-Hg and hair-Hg levels are generally used as indicators of exposure and have been correlated to both total body burden and brain levels, and hair analyses can be correlated with blood-Hg values at the time of hair growth (Phelps et al., 1980). But no studies have ever tried to assess whether a correlation occurs between the heavy metal concentrations in the dog hairs in Korea.

Considering this, a conclusion may be drawn that high positive correlations were observed between dog hairs in Pb and Hg. The results indicate that dog hairs are good indicator of exposure to levels of Hg and Pb reciprocally.

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