Scientific Works. Series C. Veterinary Medicine. Vol. LXX (2), 2024
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

DOGS' HAIR AND TISSUES AS BIOINDICATORS FOR THE ASSESSMENT OF HEAVY METALS POLLUTION

Gheorghe Valentin GORAN[1], Emanuela BADEA[1], Cristina ȚOCA[2], Camille BELLI[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei Street, 050097, District 5, Bucharest, Romania
[2]Institute of Diagnosis and Animal Health, 63 Dr. Staicovici Street, 050557, District 5, Bucharest, Romania

Corresponding author email: gheorghe.goran@fmvb.usamv.ro

Abstract

The study aimed to examine the accumulation pattern and concentrations of heavy metals and minerals in different tissues of dogs (hair, kidney, brain, liver). Additionally, it explored the feasibility of utilizing these samples for identifying potential environmental impacts associated with these pollutants.
ICP-MS was used to analyse the samples for concentrations of heavy metals. The obtained values were assessed considering various factors that could impact the levels of minerals and metals in the organisms of animals, including age, gender, and habitat.
Generally, heavy metals recorded higher levels in the hair, liver and kidneys of dogs living outdoors compared to the ones living indoors. Of all types of samples lead had the highest levels in female dogs, in dogs younger than 5 years and in those living outdoors. The results also show that hair, among all samples, plays a significant role for the evaluation of heavy metals pollution.

Key words*: heavy metals, hair, liver, kidney, brain, dogs, ICP-MS.*

INTRODUCTION

Pollution is the undesirable physical, chemical and biological alterations that occur in air, water and soil following the action of anthropogenic and natural sources. With the alarming growth in global population, the release of potentially toxic substances continues to be one of the greatest challenges the global society has to face.

The widespread use of heavy metals across industrial, domestic, agricultural, medical, and technological sectors has led to their pervasive presence in the environment. Once released, these metals display resistance to natural degradation processes and persist indefinitely in certain organs and body systems, posing significant health risks.

Unlike organic pollutants, heavy metals once introduced into the environment cannot be biodegraded and they persist indefinitely in certain organs and body system causing serious health problems (Adams et al., 2006; Badea et al., 2016a; Hernández-Moreno et al., 2013; Michalak et al., 2012; Poon et al., 2004; Tchounwou et al., 2012). Heavy metals by definition are metals with relatively high densities and high atomic mass. Some of them are essential for human health but they can become toxic in larger amounts or forms. One of the reasons they are so toxic is related to their ability to stop the absorption, metabolism and use of essential minerals, leading to their deficiency (Jaishankar et al., 2014). The accumulation of xenobiotics depends on several factors, especially physiological conditions and habitats (Badea et al., 2017; Goran et al., 2017a; Goran et al., 2017b; Poon et al., 2004).

Dogs represent a very good indicator of the pollution load on the environment because they inhabit the same space with us and are exposed to the same pollutants (Nageeb Rashed & Soltan, 2005; Park et al., 2005). By analysing the different organs and their ability to accumulate heavy metals we can acquire a better understanding of their metabolism and kinetics (Badea et al., 2016b; Badea et al., 2018; Combs et al., 1982; Jafari, 2016; Kolachi et al., 2012; Roug et al., 2015; Singh et al., 2011).

The aim of this study is to investigate the pattern of accumulation and content of some heavy metals and minerals in various tissues of dogs (hair, kidney, brain, liver) taking into consideration their sex, age and habitat and the possibility of using such samples to biologically monitoring of the environmental pollution.

MATERIALS AND METHODS

Hair, liver, kidney and brain samples were collected from the cadavers of 12 dogs belonging to different breeds, the majority of them being mixed-breed that were raised in the Bucharest area (Table 1). Following the collection of anamnestic data such as sex, age, habitat and the pathological findings while they were still alive, all dogs were evaluated as clinically unhealthy.

Table 1. Studied dogs' samples depending on habitat, age and sex

Habitat	Indoor	4	M	3
			F	1
	Outdoor	8	M	3
			F	5
Age	< 5	6	M	3
			F	3
	> 5	6	M	3
			F	3

The hair samples were collected from the flank region, the liver samples were collected from each lobe, the kidney samples were collected from the medulla and cortical regions and the brain samples were collected in their whole. Each sample was packaged in plastic bags, labelled and transported to the laboratory where they were stored in the freezer. Each sample was numbered and the following data were noted: breed, sex, age, weight, habitat and pathology.

All tissues and hair samples were weighed to 0.01 g and placed in polypropylene tubes. Samples were then disintegrated by cold wet mineralisation, adding to each sample 5 ml of HNO_3 and 1 ml of HCl. After the complete disintegration, ultrapure water was added in each sample up to the volume of 10 ml. The disintegrated samples were analysed by ICP-MS.

Statistical analysis was performed using VassarStats software: Website for Statistical Computation (http://vassarstats.net/). For all samples' mineral concentrations One-Way ANOVA was performed, and when ANOVA generated $p \leq 0.05$, all-pair Tukey HSD Test was carried out for the comparison of the averages.

RESULTS AND DISCUSSIONS

The mean Zn, Pb and Cd contents of hair and tissues samples from the studied dogs depending on habitat, age and sex are presented in Table 2, Table 3 and Table 4, respectively, and expressed as ppm.

The hair of female dogs showed a significantly different higher mean level of Zn than the male dogs' hair samples. In the brain of male dogs, significantly high mean levels of Zn ($p=0.03$) were detected, compared to those in females (Figure 1).

Table 2. Mean mineral levels in dogs' hair, liver, kidney, and brain samples, depending on habitat (ppm)

Habitat	**Indoor**				**Outdoor**			
	H	**L**	**K**	**B**	**H**	**L**	**K**	**B**
Zn	79.823	18.407	11.803	6.265	84.539	25.728	14.186	6.136
Pb	0.699	0.051	0.052	0.013	2.002	0.609	0.109	0.069
Cd	0.044	0.074	0.323	0.005	0.071	0.046	0.169	0.005

H-hair, L-liver, K-kidney, B-brain

Table 3. Mean mineral levels in dogs' hair, liver, kidney, and brain samples, depending on age (ppm)

Age	**<5 years old**				**>5 years old**			
	H	**L**	**K**	**B**	**H**	**L**	**K**	**B**
Zn	6.113	82.751	83.183	6.245	14.058	23.528	21.142	12.725
Pb	0.102	2.077	1.059	0.012	0.139	0.763	0.082	0.041
Cd	0.006	0.079	0.045	0.004	0.150	0.039	0.071	0.290

H-hair, L-liver, K-kidney, B-brain

Table 4. Mean mineral levels in dogs' hair, liver, kidney, and brain samples, depending on sex (ppm)

Sex	Male				Female			
	H	L	K	B	H	L	K	B
Zn	14.340	21.836	12.600	6.530	83.435	25.360	13.515	5.828
Pb	1.202	0.101	0.058	0.017	1.934	0.744	0.122	0.084
Cd	0.069	0.069	0.293	0.006	0.056	0.041	0.147	0.004

H-hair, L-liver, K-kidney, B-brain

In both liver and kidney samples of female dogs, levels of Zn were not significantly different (p=0.8) compared to liver and kidney samples of males.

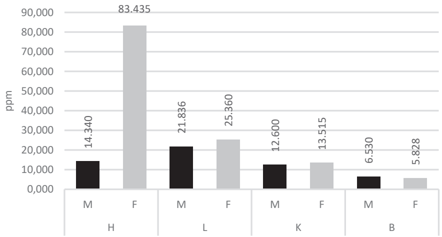

Figure 1. Mean Zn levels in dogs' hair, liver, kidney, and brain samples, depending on sex

In a study on heavy metals concentration in dogs' hair, Zn showed the highest levels, followed by Pb (Tomza-Marciniak et al., 2012). In correlation with the results reported in another study, Zn levels were higher in female dogs than in males (Hayashi et al., 1981), but not significantly different. Most of the minerals, such as Zn, are needed in small quantities in the brain, therefore lower levels of this mineral were detected in the brain compared to hair or the other tissues.

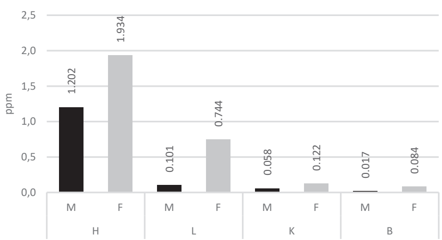

Figure 2. Mean Pb levels in dogs' hair, liver, kidney, and brain samples, depending on sex

In all studied hair and tissues samples of female dogs, mean Pb concentration were higher (p>0.05) than in male dogs, but not significantly different (Figure 2). In correlation with the results reported by Hayashi et al. (1981), Pb levels were higher in female dogs than in males. Due to slow brain excretion, excessive accumulation of Pb is observed in the brain of dogs, similar to what was detected in the rat's cerebral cortex (Li et al., 2015).

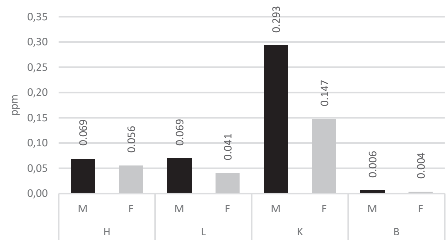

Figure 3. Mean Cd levels in dogs' hair, liver, kidney, and brain samples, depending on sex

The Cd mean levels in the hair and tissues samples from the male dogs were insignificantly different (p>0.05) than in female dogs' samples (Figure 3).

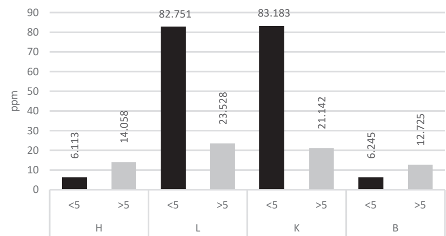

Figure 4. Mean Zn levels in dogs' hair, liver, kidney, and brain samples, depending on age

The hair and brain samples from dogs older than 5 years showed insignificantly higher mean levels of Zn (p>0.05) than younger dogs, while the Zn mean levels of dogs younger than 5 years in liver and kidney samples were insignificantly higher (p>0.05) than in those of dogs older than 5 years (Figure 4). Zn showed a negative correlation between the hair and the age of the dogs as previously reported by Park et al. (2005). The sex of the animals has only minor impact on the concentrations of the elements in the kidney, as has been observed by Hermoso de Mendoza et al. (2011), but differences depending on age of the animals are observed. Goran et al. (2020) reported that Zn mean levels in hair samples from female cats showed insignificant differences across various health statuses, and in all animals regardless of age or health condition.

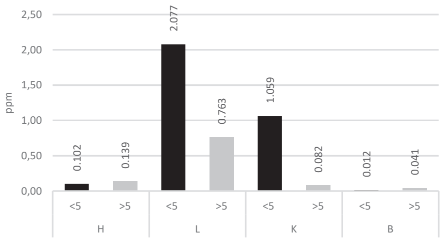

Figure 5. Mean Pb levels in dogs' hair, liver, kidney, and brain samples, depending on age

The hair and brain samples of dogs older than 5 years showed insignificantly higher Pb mean concentrations ($p>0.05$) than those of younger dogs (Figure 5). In the liver and kidney samples of younger dogs insignificantly higher Pb concentrations ($p>0.05$) were detected than in those from the older ones. In this study, the concentration of Pb in dog hair is slightly increased with the age as it has been previously observed by Park et al. (2005).

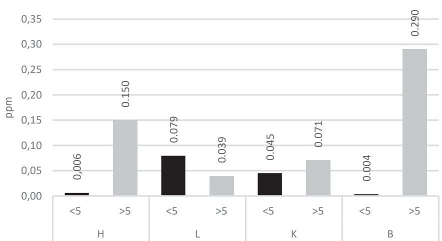

Figure 6. Mean Cd levels in dogs' hair, liver, kidney, and brain samples, depending on age

Cd concentrations were higher in liver samples in young dogs compared to old dogs, and higher in hair, kidney, and brain samples in older dogs compared to young dogs, however the differences were not statistically significant ($p>0.05$) (Figure 6). In this study, the concentration of Cd in dog hair increases with the age as it has been previously observed by Park et al. (2005). Paßlack et al. (2014) reported that Cd content in the feline liver doesn't show an increase with the animals' age. The hair of dogs living outdoor showed an insignificantly different higher mean level of Zn ($p>0.05$) than the hair of dogs living indoor. In addition, the highest Zn mean concentration was detected, among all the studied samples types, in hair samples, independent of the habitat (Figure 7). The liver and kidney of outdoor dogs showed insignificant higher Zn levels ($p>0.05$) than those of indoor dogs. In the brain of dogs living indoor insignificantly different mean levels of Zn ($p>0.05$) were detected compared to those in the brain of dogs living outdoor, and the lowest Zn mean concentration was detected in this type of sample independent of habitat.

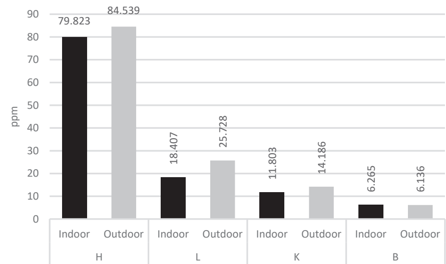

Figure 7. Mean Zn levels in dogs' hair, liver, kidney, and brain samples, depending on habitat

The results from this study show a higher accumulation of Zn in the kidneys compared to Pb and Cd as observed in a previous study on fish from the Arabian Gulf by Ashraf (2005).
In the study on felines by Paßlack et al. (2014), Zn was detected with the highest levels in the liver, followed by the kidneys.

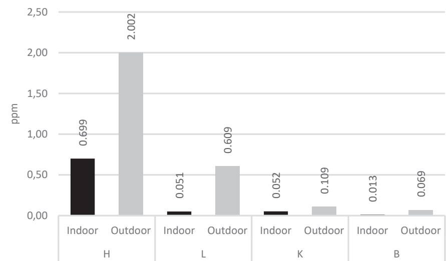

Figure 8. Mean Pb levels in dogs' hair, liver, kidney, and brain samples, depending on habitat

In all types of samples of dogs living outdoor insignificantly different higher Pb mean concentrations ($p>0.05$) were detected than in dogs living indoor, and, independent of the habitat, the highest Pb mean concentration was detected in hair (Figure 8).

The hair of dogs living outdoor showed insignificantly different Cd mean levels ($p>0.05$) than indoor dogs. In the liver and kidney samples of indoor dogs an insignificantly different Cd higher mean concentration was detected than in outdoor dogs' liver and kidney samples, and, independent of habitat, the highest Cd mean concentration was detected in kidney samples (Figure 9).

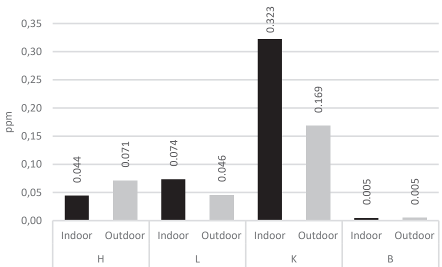

Figure 9. Mean Cd levels in dogs' hair, liver, kidney, and brain samples, depending on habitat

Both indoor and outdoor dogs showed the same Cd mean concentrations in the brain samples.

CONCLUSIONS

Generally, heavy metals recorded higher levels in the hair, liver and kidneys of dogs living outdoors compared to the ones living indoors.

Pb showed higher levels in the hair, liver and kidneys of dogs living outdoor compared to the ones living indoors.

The highest levels of Zn were detected in the liver, kidney and brain of dogs younger than 5 years compared to older dogs, and in the hair of dogs living outdoor.

Zn levels were higher in the hair of female dogs and in the brain samples of male dogs.

Of all types of samples, Pb had the highest levels in female dogs, in dogs younger than 5 years and in those living outdoors.

In all types of samples Pb had the highest levels in female dogs, in dogs younger than 5 years and in those living outdoor.

Male dogs showed higher accumulation of Cd in all studied samples compared to female dogs.

Independent of habitat, the highest Cd mean concentration was detected in kidney samples.

Analysing heavy metals especially in hair, of all the studied samples, may be valuable as a means of biologically monitoring the metallic elements pollution.

REFERENCES

Adams, J. B., Holloway, C. E., George, F., & Quig, D. (2006). Analyses of toxic metals and essential minerals in the hair of Arizona children with autism and associated conditions, and their mothers. *Biological Trace Element Research, 110*, 193-209.

Ashraf, W. (2005). Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. *Environ Monit Assess., 101*(1-3), 311-316. doi:10.1007/s10661-005-0298-4

Badea, E., Goran, G., & Crivineanu, V. (2016a). Hair Mineral Levels in Dogs with Atopic Dermatitis. *Bulletin UASVM Veterinary Medicine, 73*(2), 451-453.

Badea, E., Goran, G., Matei, E., Rotaru, E., & Crivineanu, V. (2016b). Heavy metal and mineral content in the coat of cats in relationship with kidney failure. *Lucrări Stiinţifice Medicină Veterinară Timişoara, XLIX*(1), 17-28.

Badea, E., Goran, G., Ţoca, C., & Crivineanu, V. (2017). Heavy metals in cat hair depending on keeping conditions. *Lucrări Ştiinţifice Medicină Veterinară Iaşi, 60*(19), 160-166.

Badea, E., Goran, G., Toca, C., & Crivineanu, V. (2018). Assessment of heavy metal and mineral levels in hair samples from dogs with mammary neoplasms. *Bulletin UASVM Food Science and Technology, 75*(1), 1-7.

Combs, D. K., Goodrich, R. D., & Meiske, J. C. (1982). Mineral concentrations in hair as indicators of mineral status: a review. *Journal of Animal Science, 54*(2), 391-398.

Goran, G., Badea, E., & Crivineanu, V. (2017a). Hair mineral content analysis in cats with different liver disorders. *Scientific Works. Series C. Veterinary Medicine, LXIII*(1), 101-108.

Goran, G., Badea, E., Toca, C., & Crivineanu, V. (2017b). The effect of habitat on hair copper, molybdenum, and selenium levels in cats. *Lucrări Ştiinţifice Medicină Veterinară, 60*(3), 325-329.

Goran, G., Badea, E., & Crivineanu, V. (2020). Comparison of zinc hair mineral levels in cats with liver and kidneys disorders. *Scientific Works. Series C. Veterinary Medicine, LXVI*(1), 11-16.

Hayashi, M., Okada, I., Tate, H., Miura, Y., Ohhira, S., & Yamada, Y. (1981). Distribution of environmental pollutants in pet animals. VI. Heavy metals in hair of house-dogs. *Bull Environ Contam Toxicol., 26*(1), 60-64. doi:10.1007/BF01622055

Hermoso de Mendoza García, M., Hernández Moreno, D., Soler Rodríguez, F., López Beceiro, A., Fidalgo Alvarez, L., & Pérez López, M. (2011). Sex- and age-dependent accumulation of heavy metals (Cd, Pb and Zn) in liver, kidney and muscle of roe deer (*Capreolus capreolus*) from NW Spain. *J Environ Sci Health A Tox Hazard Subst Environ Eng., 46*(2), 109-116. doi:10.1080/10934529.2011.532422

Hernández-Moreno, D., de la Casa Resino, I., Fidalgo, L. E., Llaneza, L., Rodríguez, F. S., Pérez-López, M., & López-Beceiro, A. (2013). Noninvasive heavy metal pollution assessment by means of Iberian wolf (*Canis lupus signatus*) hair from Galicia (NW Spain): a comparison with invasive samples. *Environ Monit Assess, 185*, 10421-10430.

Jafari, S. (2016). *Use of pets as indicators of heavy metal exposure across Sydney.* (Thesis), Macquarie University.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B., & Beeregowda, K. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol., 7*(2), 60-72. doi:10.2478/intox-2014-0009

Kolachi, N. F., Kazi, T. G., Afridi, H. I., Kazi, N. G., & Khan, S. (2012). Investigation of essential trace and toxic elements in biological samples (blood, serum and scalp hair) of liver cirrhotic/cancer female patients before and after mineral supplementation. *Clin Nutr., 31*(6), 967-973.

Li, Q., Liu, H., Alattar, M., Jiang, S., Han, J., Ma, Y., & Jiang, C. (2015). The preferential accumulation of heavy metals in different tissues following frequent respiratory exposure to PM2.5 in rats. *Sci Rep., 5*, 16936. doi:10.1038/srep16936

Michalak, I., Chojnacka, K., & Saeid, A. (2012). Relation between mineral composition of human hair and common illnesses. *Chin Sci Bull, 57*, 3460-3465.

Nageeb Rashed, M., & Soltan, M. E. (2005). Animal Hair as Biological Indicator for Heavy Metal Pollution in Urban and Rural Areas. *Environmental Monitoring and Assessment, 110*, 41–53.

Park, S., Lee, M., & Kim, S. (2005). Studies on Cd, Pb, Hg and Cr Values in Dog Hairs from Urban Korea. *Asian-Aust. J. Anim. Sci., 18*(8), 1135-1140. doi:10.5713/ajas.2005.1135

Paßlack, N., Mainzer, B., Lahrssen-Wiederholt, M., Schafft, H., Palavinskas, R., Breithaupt, A., . . . Zentek, J. (2014). Concentrations of strontium, barium, cadmium, copper, zinc, manganese, chromium, antimony, selenium and lead in the equine liver and kidneys. *Springerplus, 3*, 343. doi:10.1186/2193-1801-3-343

Poon, W. T., Ling, S. C., Chan, A. Y. W., & Mak, T. W. L. (2004). Use of hair analysis in the diagnosis of heavy metal poisoning: report of three cases. *Hong Kong Med J, 10*, 197-200.

Roug, A., Swift, P. K., Gerstenberg, G., Woods, L. W., Kreuder-Johnson, C., Torres, S. G., & Puschner, B. (2015). Comparison of trace mineral concentrations in tail hair, body hair, blood, and liver of mule deer (*Odocoileus hemionus*) in California. *Journal of Veterinary Diagnostic Investigation, 27*(3), 295-305.

Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy metals and living systems: An overview. *Indian J Pharmacol., 43*(3), 246-253. doi:10.4103/0253-7613.81505

Tchounwou, P., Yedjou, C., Patlolla, A., & Sutton, D. (2012). Heavy Metals Toxicity and the Environment. *Exp Suppl., 101*, 133-164. doi:10.1007/978-3-7643-8340-4_6

Tomza-Marciniak, A., Pilarczyk, B., Bąkowska, M., Ligocki, M., & Gaik, M. (2012). Lead, cadmium and other metals in serum of pet dogs from an urban area of NW Poland. *Biol Trace Elem Res., 149*(3), 345-351. doi:10.1007/s12011-012-9433-6