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HAIR MINERAL CONTENT ANALYSIS IN CATS WITH DIFFERENT LIVER DISORDERS

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Abstract

The main objective of this study was to evaluate the levels of 20 potentially toxic metals and essential minerals in hair samples from cats with different liver disorders, compared to control samples. Analysis of the hair elemental content of the cats with liver failure (n=5), cats with liver abscess (n=4), cats with chronic hepatitis (n=6), and clinically healthy cats as control (n=15), were performed by inductively coupled plasma-optic emission spectrometry (ICP-OES). In this study, Ca and Mg levels registered very significant differences (p<0.001), and Cu, Na, and Zn concentrations registered significant differences (p<0.05) between their levels in hair samples from cats with different liver disorders and control samples. No significant differences have been registered for heavy metals present in hair samples from cats with different liver disorders and clinically healthy cats. Independent of the significant differences, the highest values were registered by all the elements in hair samples from cats with chronic hepatitis, excepting Ca, Mg, and Se levels, which were higher in clinically healthy cats. The current study presents one of the first investigations of the suitability of hair as an indicator for mineral status of cats with different liver disorders in an urban area of Romania. Hair mineral levels determined in the present research may be considered as a contribution to a base of reference concentrations of minerals in female cats in Romania.

Key words: *hair, cats, heavy metals, minerals, liver disorders.*

INTRODUCTION

The numerous functions of the liver, including but not limited to metabolism, storage, synthesis, and its implication in hematopoiesis, immunologic responses, digestion, and detoxification makes it one of the most important organs in the organism. Also, because of its capacity of regeneration, the hepatic injury has to be important or chronic in order to determine observable hepatic dysfunction or failure (Center, 2016a).

The liver role in xenobiotics excretion exposes it to high levels of toxic substances and their metabolites (Osweiler, 1996a).

Different toxins (e.g. mycotoxins, phytotoxins, phycotoxins) or prescription drugs (Goran and Crivineanu, 2016) or other toxic substances like heavy metals, certain herbicides, fungicides, insecticides, and rodenticides have been reported to be hepatotoxic (Center, 2016b).

Heavy metals are considered systemic toxicants, which induce multiple organ injuries, even at lower levels of exposure (Tchounwou et al., 2012).

Generally, after heavy metal absorption in the organism, they accumulate in one or more of the organs (liver, kidney, bone, and brain) (Goran and Crivineanu, 2016), metabolize, and are excreted via feces and urine. They are also excreted in sweat and accumulated in keratin-rich tissues, like hair and nails (Oostdam et al., 1999; Poon et al., 2004).

Hair or other keratinized skin structures samples were used for evaluating the mineral status of animals or humans, because of their easy and non-stressful sampling way. Also, unlike blood and tissue samples, the levels of most minerals in hair are higher (Combs et al., 1982; Combs, 1987; Batool et al., 2015). Hair mineral analysis as a screening and diagnostic tool has started to become routine since the early 1970s (Campbell, 1985; Foo, 1993, Kosla et al., 2011; Skibniewska et al., 2011). Many researchers have reported correlations between hair mineral content and blood or tissue mineral concentrations (Goran and Crivineanu, 2007; Crivineanu et al., 2008; Roug et al., 2015), and the use of hair samples in order to evaluate heavy metals pollution (Patra et al., 2006; Crivineanu et al., 2010; Filistowicz et al., 2011; de Almeida Curi et al., 2012; Baran and Wieczorek, 2013; Petukhova, 2013; Skibniewski et al., 2013).

Also, in recent years, more researchers have reported correlations between hair mineral content and different health conditions both in humans and animals, which makes mineral hair content evaluation a good option to both patients and veterinarians or physicians (Poon et al., 2004; Adams et al., 2006; Michalak et al., 2012; Hernández-Moreno et al., 2013; Badea et al., 2016a, Badea et al., 2016b).

Mineral elements have essential roles in organisms' biological processes, as enzyme cofactors, nervous system functions, and in redox processes, but both essential and nonessential minerals can be toxic in certain doses (Osweiler, 1996b). Some of them are implicated in etiology of hepatotoxic disorders (Center, 2016b), after their liver accumulation, which is influenced by problems of metal elimination from the liver. Copper and iron as transition metals play an important role in oxidative stress, and if they accumulate, they are known to lead to necroinflammatory liver disorders, where they enhance liver oxidant damage (Labuc, 2012).

The main objective of this study was to evaluate the levels of some potentially toxic metals and essential minerals in hair samples from cats with different liver disorders, compared to control samples, using inductively coupled plasma-optic emission spectrometry (ICP-OES).

MATERIALS AND METHODS

Sampling and samples preparation

Analysis of hair elemental content of cats with liver failure (n=5), cats with liver abscess (n=4), cats with chronic hepatitis (n=6), and clinically healthy cats as control (n=15), were performed by ICP-OES.

All cats with liver disorders in this study were above 8 years of age, and in the control group, 7 were above 8 years of age (5 females and 2 males). In this study, all cats with liver disorders were females except one (a male diagnosed with liver abscess), and in the control group, 11 cats were females and 4 were males.

The cats with liver disorders exhibited symptoms which led to the suspicion of a liver injury, the diseases being confirmed by ultrasound examination and biochemical blood tests. The male with liver abscess died, and the diagnostic was also confirmed by necropsy.

The hair samples were collected from all studied animals from the flank region, placed in plastic recipients, labeled, and transported to the laboratory. In the laboratory, the hair samples were stored in dark, dry places, with constant temperature. The samples were initially degreased, washed, rinsed, and then disaggregated. All hair samples were weighed and then digested using a Speedwave MWS-2 Berghof microwave oven as following: Step 1: 120^{o}C, power 50%; Step 2: 180^{o}C, power 75%; Step 3: 100^{o}C, power 40%. The samples were then analyzed to assay the presence of 20 mineral elements by ICP-OES.

Spectrometric analysis

Digested samples were treated with 5 mL HNO_3, 0.8 mL HCl and 1 mL H_2O_2, then diluted to 10 mL with ultrapure water and analyzed by a Thermo iCAP ICP-OES spectrometer (RF1100 W; reading time 30 s, washing time 30 s, nebulizer gas flow 0.5 L/min; auxiliary gas flow 0.5 L/min; sample injection pump flow 50 rpm). Calibration curves were developed using standard solutions of 0.001 ppm, 0.01 ppm, 0.1 ppm, 1 ppm, 5 ppm, 10 ppm, 50 ppm obtained by dilution from a multi-element ICP MERCK standard containing 1000 mL/L of Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr, and Zn.

Statistical analysis

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

RESULTS AND DISCUSSIONS

Because in this study cats with liver disorders were represented by a majority of females, the male with liver abscess was excluded from the study. Also, in order to make a correct interpretation of the results, all males and females below 8 years were excluded from the control group.

In this regard, this study evaluated total mineral content of hair samples in female cats over 8 years old with liver failure (n=5), liver abscess (n=3), and chronic hepatitis (n=6), compared to mineral levels in hair samples from healthy female cats over 8 years old (n=5), which represented the control samples.

Of the 20 elements that could be determined (Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr, and Zn), Ba, Be, Bi, Co, Cr, Li, Mn, and Sr levels were below the analysis method detection limit, and Al, Ca, Cd, Cu, Fe, K, Mg, Na, Ni, Pb, Se, and Zn levels were over the analysis method detection limit.

The mean mineral contents of hair samples from clinically healthy cats and those with different liver disorders are presented in Table 1 and expressed as *parts per million* (ppm).

Table 1. Mean heavy metal and mineral levels in cat hair samples (ppm)

Element	Health status				*p*-value
	LF	LA	CH	HA	
Al	10.75[a]	13.3[a]	40.95[a]	15.1[a]	0.16
Ca	**169.8[a]**	**135.3[a]**	**582.95[b]**	**811.2[b]**	**0.001**
Cu	**0.465[a]**	**0.38[a]**	**1.85[b]**	**0.78[b]**	**0.05**
Cd	0.028[a]	0.049[a]	0.343[a]	0.032[a]	0.41
Fe	170.3[a]	88.3[a]	417.7[a]	15.0[a]	0.50
K	26.95[a]	9.2[a]	104.6[a]	54.0[a]	0.24
Mg	**15.65[a]**	**11.4[a]**	**51.25[b]**	**72.3[b]**	**0.001**
Na	**252.25[a]**	**79.4[a]**	**1272.05[b]**	**414.9[a]**	**0.03**
Ni	0.35[a]	0.68[a]	1.785[a]	0.12[a]	0.24
Pb	0.092[a]	0.13[a]	0.42[a]	0.028[a]	0.33
Se	0.054[a]	BDL	0.062[a]	0.103[a]	0.36
Zn	**4.95[a]**	**2.7[a]**	**18.4[b]**	**10.1[ab]**	**0.05**

*Levels not connected by the same letter are significantly different. The comparison can be made only between health statuses for the concentration of one element and not between different elements concentrations.
**LF – liver failure; LA – liver abscess; CH – chronic hepatitis; HA – healthy animals.
***BDL – below method detection limit.

In all samples that registered levels over the method detection limits, the highest values were observed in hair samples from cats with chronic hepatitis, with the exception of Ca, Mg, and Se levels, which were elevated in clinically healthy cats.

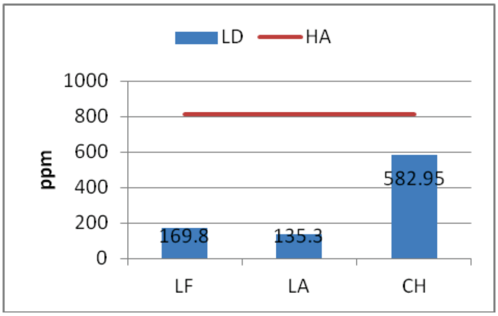
Another study on hair mineral content also reported that in the hair of clinically healthy cats above 5 years of age levels of Ca, Fe, K, and Mg were higher than in the study group (Badea et al., 2016a).

In this study, Ca and Mg levels registered very significant differences ($p<0.001$), and Cu, Na, and Zn concentrations registered significant differences ($p<0.05$) between their levels in hair samples from cats with different liver disorders and control samples.

Ca levels registered the highest concentrations in healthy animals hair samples (811.2 ppm, $p=0.001$), and very significant differences between its levels in hair samples from cats with liver failure and liver abscess, and those from cats with chronic hepatitis and clinically healthy cats ($p<.01$). The differences were not significant between Ca levels in cats with liver failure and liver abscess, and also between its levels in cats with chronic hepatitis and control group (figure 1).

Combs et al. (1982) have reported that Ca hair levels should not be able to influence dietary changes for calcium, as there is a constant homeostasis of blood calcium, and Ca levels increase or decrease only for short time after dietary changes.

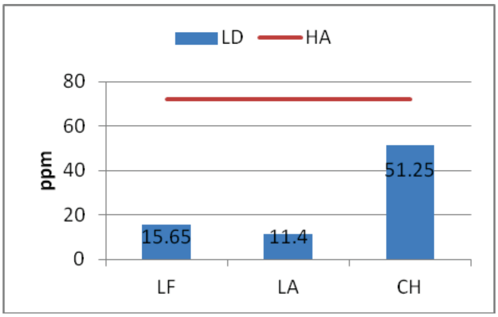
Other researchers have found that Cd effects on Ca metabolism develop gradually; as cadmium accumulates in the organism, Ca level decreases (Staessen et al., 1991).



*LF – liver failure, LA – liver abscess, CH – chronic hepatitis

Figure 1. Mean Ca levels in hair samples from cats with different liver disorders (LD) compared to clinically healthy animals (HA)

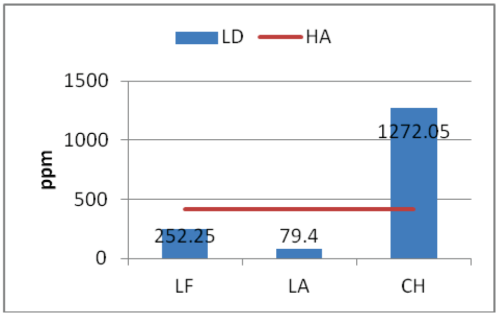
In the case of Mg, the differences registered were also very significant (p=0.001), with the same correlations pattern as that observed in the case of Ca (figure 2). The highest Mg level was found in hair samples from clinically healthy cats (72.3 ppm), followed by that in hair from cats with chronic hepatitis (51.25 ppm).



*LF – liver failure, LA – liver abscess, CH – chronic hepatitis

Figure 2. Mean Mg levels in hair samples from cats with different liver disorders (LD) compared to clinically healthy animals (HA)

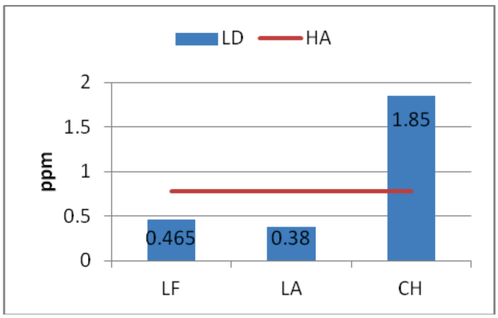
Na levels registered significant differences in hair samples from cats with different liver disorders compared to the control samples (p=0.03). The highest Na levels were observed in hair samples from cats with chronic hepatitis (1272.05 ppm), followed by a mean level almost 3 times lower registered in hair samples from clinically healthy cats (414.9 ppm) (figure 3). The differences were significant between its levels in hair samples from cats with chronic hepatitis and those in hair samples from cats with the other liver disorders and control samples (p<.05), and there were no differences between Na levels in hair from cats with liver failure, liver abscess, and in healthy animals, respectively.



*LF – liver failure, LA – liver abscess, CH – chronic hepatitis

Figure 3. Mean Na levels in hair samples from cats with different liver disorders (LD) compared to clinically healthy animals (HA)

Another element that registered significant differences between its levels, was the trace mineral Cu (p=0.05). Cu levels registered the highest concentrations in cats with chronic hepatitis hair samples (1.85 ppm), followed by those in clinically healthy cats (figure 4).



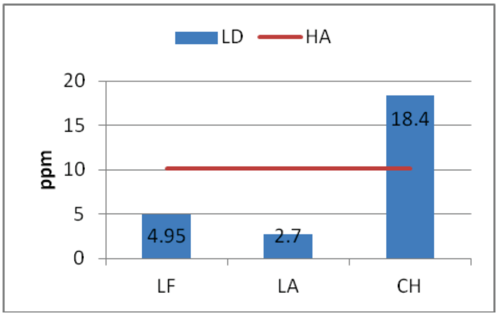
*LF – liver failure, LA – liver abscess, CH – chronic hepatitis

Figure 4. Mean Cu levels in hair samples from cats with different liver disorders (LD) compared to clinically healthy animals (HA)

The correlations of the significant differences between its levels could be made between hair samples from cats with liver failure and liver abscess vs. those from cats with chronic hepatitis and clinically healthy cats (p<.05). The differences were not significant between the levels in cats with liver failure and liver abscess, and also between the levels in cats with chronic hepatitis and those in healthy animals. Haynes and Wade (1995) have reported that liver degeneration in Siamese cats was due to excessive copper accumulation, which could also influence its levels in keratin-rich tissues. Other researchers have found that Cu levels in hair samples from dogs with atopic dermatitis were significantly decreased (p=0.01) in the study group compared to control samples (Badea et al., 2016b).

As it was reported by other researchers, that Cu accumulation lead to necroinflammatory liver disorders (Haynes and Wade, 1995; Labuc, 2012), the significant higher Cu mean levels in hair samples from cats with chronic hepatitis could also be positively correlated, in the present study, to this chronic disorder.

Zn also registered significant differences between its levels (p=0.05), the highest concentrations being found in cats with chronic hepatitis hair samples (18.4 ppm), followed by those in clinically healthy cats, which were almost 2 times lower (figure 5). The correlations of the significant differences between its levels could be made between hair samples from cats with liver failure and liver abscess vs. those from cats with chronic hepatitis (p<.05). The differences were not significant between the levels in cats with liver failure, liver abscess, and clinically healthy cats, and also between the levels in cats with chronic hepatitis and control group.



*LF – liver failure, LA – liver abscess, CH – chronic hepatitis

Figure 5. Mean Zn levels in hair samples from cats with different liver disorders (LD) compared to clinically healthy animals (HA)

Skibniewska et al. (2011) have reported that Zn registered higher levels in female feral cats (268.09 ppm), and lower in pet female cats (214.49 ppm), levels that were much higher than those registered in the presented study, independent of health status.

All the other determined elements registered no significant differences (p>0.05) between the levels in hair from cats with different liver disorders and clinically healthy cats.

In the case of K, even if significant differences were not determined between hair samples from the healthy cats and those with liver disorders, its levels registered the same pattern as that found in Na. K levels were the highest in hair samples from cats with chronic hepatitis (104.6 ppm), and the lowest in hair samples from cats with liver abscess (9.2 ppm), which was almost 6 times lower than in the case of clinically healthy cats hair samples.

Fe also registered no significant differences between its levels in study groups and control hair samples (p=0.50). The highest mean level was observed in cats with chronic hepatitis hair samples (417.7 ppm), followed by liver failure and liver abscess cats hair samples, and the lowest mean levels in clinically healthy cats (15 ppm). Horiguchi et al. (2011) found that cadmium causes hemolysis, which could determine iron accumulation. Also, Fe accumulation as transition metal plays an important role in oxidative stress, leading to necroinflammatory liver disorders (Labuc, 2012).

The highest Se mean level was observed in hair samples from clinically healthy cats (0.103 ppm), followed by hair samples from cats with chronic hepatitis and liver failure. Se levels in hair samples from cats with liver abscess were below the method detection limit. Se also registered no significant differences between its levels in study groups and control hair samples (p=0.36).

The potential toxic metals have registered no significant differences between their levels, but almost the same pattern was observed in all these elements' mean levels. The highest levels of Al, Cd, Ni, and Pb were found in hair samples from cats with chronic hepatitis (40.95, 0.343, 1.785, and 0.42 ppm, respectively), followed by Ni and Pb levels in hair samples from cats with liver abscess and liver failure, by Al levels in healthy cats and cats with liver abscess, and Cd levels in liver abscess and clinically healthy cats.

Hyder et al. (2013) found that environmental cadmium exposure was associated with different liver disorders in humans.

Markiewicz-Górka et al. (2015) reported that exposure to low levels of Cd and Pb through food, water, and air is common in industrial and urban areas and is a real threat to the health of the general population, and could be evaluated using animal models.

Badea et al. (2016a) have reported that, in hair from clinically healthy cats over 5 years of age, Al, Cd, Ni, and Pb registered higher values than those in hair samples from the cats with renal failure, and, in the present study, the same elements registered levels almost 2 times lower in control hair samples.

Kosla et al. (2011) reported that higher Ni content in the coat of cats was found in hair samples from cats above 2 years of age (above 2 years - 0.87; below 2 years - 0.58 ppm). Another research reported that, in female cats with renal failure, Ni (study group - 0.22; control group - 0.19 ppm) and Pb (study group - 0.05; control group - 0.03 ppm) registered higher values compared to clinically healthy females. Filistowicz et al. (2011) have reported that farm fox hair registered higher levels for both Ni (farm foxes - 0.48; wild foxes - 0.3 ppm) and Pb (farm foxes - 0.64; wild foxes - 0.63 ppm). de Almeida Curi et al. (2012) have found traces of Pb in all species analyzed (maned wolf - 2.34 ppm; crab-eating fox - 2.45 ppm; hoary fox - 1.5 ppm), but Cd was not detected.

Skibniewski et al. (2013) have found that Pb content in hair samples from pet female cats registered lower levels (0.98 ppm) than feral female cats (3.58 ppm), which are much higher compared to the highest Pb levels determined in hair samples of both healthy animals and cats with different liver disorders analyzed in the present study.

Another research showed that in cats with renal failure, females registered higher levels of toxic metals like Al (10.56 ppm) and Ni (0.21 ppm) (Badea et al., 2016a), which could also be observed in the present study for the same elements, and also for Cd, Cu, Pb, and Zn in hair samples of cats with chronic hepatitis.

López Alonso et al. (2004) have reported that interactions between trace and toxic elements indicate that toxic elements compete with the essential metals, even at low levels of metal exposure, but in the same time the mineral status evaluation needs to be realized choosing a specific tissue or organ, also indicated by Elsenhans et al. (1987), who have reported that potential target organs for the evaluation of metal exposure need to be carefully analyzed for interfering metal-metal interactions.

CONCLUSIONS

The current study presents one of the first investigations of the suitability of hair as an indicator for the mineral status of cats with different liver disorders in an urban area of Romania.

Moreover, hair mineral levels determined in the present research may be considered as a contribution to a base of reference levels of minerals in female cats in Romania.

Ca, Cu, Mg, Na, and Zn registered significant differences between their levels in hair samples from cats with different liver disorders and clinically healthy cats.

In this research, hair samples from cats with chronic hepatitis registered higher levels of Cu, Zn, and Na, and Ca and Mg registered higher levels in control samples.

Cu mean levels in hair samples from cats with chronic hepatitis could be positively correlated to this chronic disorder.

No significant differences have been registered for heavy metals present in hair samples from cats with different liver disorders and clinically healthy cats.

Independent of the significant differences, the highest values were registered by all the elements in hair samples from cats with chronic hepatitis, excepting Ca, Mg and Se levels, which were higher in clinically healthy cats.

Mean hair concentrations of all toxic elements, Al, Cd, Ni, and Pb, were situated below the determined levels for cats reported by other authors.

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.

Badea E., Goran G.V., Matei E., Rotaru E., Crivineanu V., 2016a. Heavy metal and mineral content in the coat of cats in relationship with kidney failure.

Lucrari Stiintifice Medicina Veterinara Timisoara, XLIX(1):17-28.
Badea E., Goran G.V., Crivineanu V., 2016b. Hair mineral levels in dogs with atopic dermatitis. Bulletin UASVM-CN, 73(2): 451-453.
Baran A., Wieczorek J., 2013. Concentrations of heavy metals in hair as indicators of environmental pollution, E3S Web of Conferences 1:21005.
Batool F., Iqbal S., Chan K.W., Tariq M.I., Shah A., Mustaqeem M., 2015. Concentrations of heavy metals in hair and nails of young Pakistanis: correlation with dietary elements. Environmental Forensics, 16:1-6.
Campbell J.D., 1985. Hair analysis: a diagnostic tool for measuring mineral status in humans, Journal of orthomolecular psychiatry, 14(4):276-280.
Center S.A., 2016a. Overview of hepatic disease in small animals, Veterinary Manual, The Merck Manual, https://www.merckvetmanual.com/digestive-system/hepatic-disease-in-small-animals/overview-of-hepatic-disease-in-small-animals [accessed on 03.02.2017].
Center SA, 2016b. Hepatotoxins in small animals, Veterinary Manual, The Merck Manual, https://www.merckvetmanual.com/digestive-system/hepatic-disease-in-small-animals/hepatotoxins-in-small-animals [accessed on 03.02.2017].
Combs D.K., 1987. Hair analysis as an indicator of mineral status of livestock. J. Anim. Sci., 65:1753-1758.
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.
Crivineanu V., Leonidis A., Goran G.V., Codreanu I., 2010. Blood and wool lead levels in sheep farmed near roads from province of Thessaloniki. Lucrari Stiintifice Medicina Veterinara Timisoara, XLIII (2):140-146.
Crivineanu V., Tudoreanu L., Bianu E., Goran G.V., 2008. Stochastic – neuronal net models for cadmium accumulation in equines farmed in the Copsa Mica area, Buletin USAMV Veterinary Medicine, 65(2):274-282.
de Almeida Curi N.H., Brait C.H.H., Filho N.R.A., Talamoni S.A., 2012. Heavy metals in hair of wild canids from the Brazilian Cerrado. Biol Trace Elem Res, 147:97-102.
Elsenhans B., Schmolke G., Kolb K., Stokes J., Forth W., 1987. Metal-metal interactions among dietary toxic and essential trace metals in the rat. Ecotoxicol Environ Saf., 14(3):275-287.
Filistowicz A., Dobrzanski Z., Przysiecki P., Nowicki S., Filistowicz A., 2011. Concentration of heavy metals in hair and skin of silver and red foxes (*Vulpes vulpes*). Environmental Monitoring and Assessment, 182:477-484.
Foo S.C., Khoo N.Y., Heng A., Chua L.H., Chia S.E., Ong C.N., Ngim C.H., Jeyaratnam J., 1993. Metals in hair as biological indices for exposure. Int. Arch. Occup. Environ. Health. 65:S83-86.
Goran G.V., Crivineanu V. – Toxic actions on the liver. In: Goran G.V., Crivineanu V. (Eds) Toxicology, Printech Publ, Bucharest, 2016, 54-61 (in Romanian).
Goran G.V., Crivineanu V., 2007. Blood and wool copper concentration following supplementary feed copper administration in sheep. Lucrari Stiintifice Medicina Veterinara Iasi, 50(9):51-60.
Haynes J.S., Wade P.R., 1995, Hepatopathy associated with excessive hepatic copper in a Siamese cat. Vet Pathol., 32(4):427-429.
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.
Horiguchi H., Oguma E., Kayama F., 2011. Cadmium induces anemia through interdependent progress of hemolysis, body iron accumulation, and insufficient erythropoietin production in rats. Toxicol Sci, 122(1):198-210.
Hyder O., Chung M., Cosgrove D., Herman J.M., Li Z., Firoozmand A., Gurakar A., Koteish A., Timothy P.M., 2013. Cadmium exposure and liver disease among US adults. J Gastrointest Surg., 17(7):1265-1273.
Kosla T., Skibniewska E.M., Skibniewski M., 2011. Nickel content in the coat of cats depending on their keeping conditions. Bulletin of the Veterinary Institute in Pulawy, 55:149-153.
Labuc R., 2012. Hepato-biliary disease in dogs and cats, The 3rd Annual Vet Education Online Veterinary Conference - July 2012, Vet Education Pty Ltd, http://webinars.veteducation.com.au/wp-content/images/Hepato-Biliary-Disease-Notes-for-Distribution.pdf [accessed 04.02.2017].
López Alonso M., Prieto Montaña F., Miranda M., Castillo C., Hernández J., Luis Benedito J., 2004. Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. Biometals., 17(4):389-397.
Markiewicz-Górka I., Januszewska L., Michalak A., Prokopowicz A., Januszewska E., Pawlas N., Pawlas K., 2015. Effects of chronic exposure to lead, cadmium, and manganese mixtures on oxidative stress in rat liver and heart. Arh Hig Rada Toksikol, 66:51-62.
Michalak I., Chojnacka K., Saeid A., 2012. Relation between mineral composition of human hair and common illnesses. Chin Sci Bull, 57:3460-3465.
Oostdam, J.V., Gilman A., Dewailly E., Usher P., Wheatley B., Kuhnlein H., Neve S., Walker J., Tracy B., Feeley M., Jerome V., Kwavnick B., 1999. Human health implications of environmental comtaminants in Arctic Canada: review. Sci. Total. Environ. 230:1-82.
Osweiler G.D., 1996a. Toxicology of the liver and biliary system. In: Osweiler G.D. (Ed.) Toxicology: the national veterinary medical series. Williams & Wilkins, Philadelphia:87-99.
Osweiler G.D., 1996b. Metals and minerals. In: Osweiler G.D. (Ed.) Toxicology: the national veterinary medical series. Williams & Wilkins, Philadelphia:179-213.

Patra R.C., Swarup D., Sharma M.C., Naresh R., 2006. Trace mineral profile in blood and hair from cattle environmentally exposed to lead and cadmium around different industrial units. Journal of Veterinary Medicine Series A, 53:511-517.

Petukhova T.V., 2013. Content of heavy metals in the muscle tissue of cattle. E3S Web of Conferences 1:15002.

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.

Skibniewska E.M., Skibniewski M., Kosla T., Urbanska-Slomka G., 2011. Hair zinc levels in pet and feral cats (*Felis catus*). J. Elem., 16(3):481-488.

Skibniewski M., Kosla T., Skibniewska E.M., 2013. Domestic cat (*Felis catus*) as a bioindicator of environmental lead contamination. Environmental Protection and Natural Resources, 4(58):47-50.

Staessen J., Amery A., Bernard A., Bruaux P., Buchet J.P., Claeys F, De Plaen P., Ducoffre G., Fagard R., Lauwerys R.R., Lijnen P., Nick L., Saint Annie R., Roels H., Rondia D., Sartor F., Thijs L., 1991. Effects of exposure to cadmium on calcium metabolism: a population study, Br J Ind Med, 48(10):710-714.

Tchounwou P.B., Yedjou C.G., Patlolla A.K., Sutton D.J., 2012. Heavy metals toxicity and the environment, EXS, 101:133-164.