Kocatepe Veterinary Journal

Kocatepe Vet J. (2023):16(3):310-316

DOI: 10.30607/kvj.1290374

RESEARCH ARTICLE

Determination of Some Heavy Metal Concentrations in Serum of Young and Adult Cattle in the Şiran District of Gümüşhane by ICP-MS

Özkan ŞİMŞEK[1], Güngör Cağdaş DİNÇEL[2*]

[1]*Department of Physiology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye*
[2]*Eskil Vocational High School, Aksaray University, Aksaray, Türkiye*

ABSTRACT

The purpose of this study was to evaluate certain heavy metal concentrations such as selenium (Se), manganese (Mn), zinc (Zn), aluminium (Al), vanadium (V), tin (Sn), chromium (Cr), iron (Fe), nickel (Ni), cobalt (Co), copper (Cu), arsenic (As), cadmium (Cd), and lead (Pb) in young and adult cattle in the Şiran district of Gümüşhane. For this, a total of 100 blood samples were taken from 50 young (aged 1 to 3 years old) and 50 adult (aged 4 to 6 years old) cattle slaughtered at an abattoir in the Şiran. Serum was then separated from the blood samples by centrifugation, and analysed for certain heavy metals by using the inductively coupled plasma mass spectrometry (ICP-MS) method. The concentrations of serum heavy metals in the serum of young and adult cattle breed in the Şiran were determined. When comparing young and adult cattle, the adult cattle had significantly higher ($p<0.05$) concentrations of Al, V, Ni, and Sn. No age-related accumulation ($p>0.05$) was found for the other heavy metal species analysed (Se, Mn, Zn, Cr, Fe, Co, Cu, As, Cd, and Pb). As a result, in this study, serum heavy metal concentrations and age-related bioaccumulation in cattle farmed in the Şiran district were determined for the first time. The periodic monitoring of the concentrations of these heavy metals may be helpful in improving animal health and production.

Key Words: Aluminium, Bovine, Nickel, Serum, Tin, Vanadium

***

Gümüşhane'nin Şiran İlçesindeki Genç ve Yetişkin Sığırların Serumlarında Bazı Ağır Metal Konsantrasyonlarının ICP-MS ile Belirlenmesi

ÖZ

Bu çalışmanın amacı, Gümüşhane'nin Şiran ilçesindeki genç ve yetişkin sığırlarda selenyum (Se), manganez (Mn), çinko (Zn), alüminyum (Al), vanadyum (V), kalay (Sn), krom (Cr), demir (Fe), nikel (Ni), kobalt (Co), bakır (Cu), arsenik (As), kadmiyum (Cd) ve kurşun (Pb) gibi bazı ağır metal konsantrasyonlarının belirlenmesidir. Bunun için, Şiran mezbahanesinde kesilen 50 genç (1-3 yaş arası) ve 50 yetişkin (4-6 yaş arası) sığırdan toplam 100 kan örneği alınmıştır. Daha sonra kan örneklerinden santrifüj yoluyla serumlar ayrıştırılmış ve endüktif eşleşmiş plazma kütle spektrometresi (ICP-MS) yöntemi kullanılarak belirli ağır metaller açısından analiz edilmiştir. Şiran'da yetiştirilen genç ve yetişkin sığırların serumlarındaki ağır metal konsantrasyonları bu şekilde tespit edilmiştir. Genç ve yetişkin sığırlar karşılaştırıldığında, yetişkin sığırların Al, V, Ni ve Sn konsantrasyonlarının önemli ölçüde daha yüksek olduğu görülmüştür ($p<0.05$). Analizi yapılan diğer ağır metal türleri (Se, Mn, Zn, Cr, Fe, Co, Cu, As, Cd ve Pb) için ise yaşa bağlı bir birikim tespit edilmemiştir ($p>0.05$). Sonuç olarak, bu çalışmada Şiran ilçesinde yetiştirilen sığırlarda serum ağır metal konsantrasyonları ve yaşa bağlı biyoakümülasyonları ilk kez belirlenmiştir. Bu ağır metal düzeylerinin periyodik olarak izlenmesi, hayvan sağlığı ve üretimi açısından iyileştirici katkılar sağlayabilir.

Anahtar Kelimeler: Alüminyum, Kalay, Nikel, Serum, Sığır, Vanadyum

To cite this article: Şimşek Ö. Dinçel GÇ. Determination of Some Heavy Metal Concentrations in Serum of Young and Adult Cattle in the Şiran District of Gümüşhane by ICP-MS. (2023):16(3):310-316

Submission: 30.04.2023 **Accepted**: 22.08.2023 **Published Online**: 09.09.2023

ORCID ID; *ÖŞ: 0000-0003-3340-9382 GÇD: 0000-0002-6985-3197*

*Corresponding author e-mail: gcdincel@yahoo.com.tr

INTRODUCTION

Heavy metals are considered to be extremely biologically hazardous chemicals due to their bioaccumulative properties (Paksy et al. 1997). Industrial activities such as cement production, steel industry, combined heat and power generation, glass production, waste and sewage sludge incineration plants produce large quantities of heavy metals. These metals can enter the soil, air and water in various ways. They can also be transmitted to humans and animals through vegetables, fruits, and crops grown on contaminated farmland (Wu et al. 2010, Rajaganapathy et al. 2011). Therefore, it is important to consider the environment, soil and water in which food is grown to minimize exposure to heavy metals.

The toxicity of heavy metals depends on their mobility, their concentration in soil and water, the chemical composition of the parent material, the solubility of the composition and environmental factors (Arslanbas and Baydan, 2013). Studies have shown that the accumulation of heavy metals in biological tissues and body fluids such as liver, kidney, brain, etc. of animals fed plant-based feeds in heavily industrially polluted soils can lead to tissue damage and death (Karagül et al. 2000, Ergün 2001, Beşkaya et al. 2008, Saghaei et al. 2012).

Although certain heavy metals, such as Zn, Co, Fe and Ni are necessary for living organisms in low amounts, their bioaccumulation can cause toxic effects by disrupting enzyme systems in living organisms over time (Bigersson et al. 1988, Sözgen 2000, El-Demerdash et al. 2004). Heavy metals with no biological function, such as Pb, Cd, and mercury (Hg), have been found to have toxic effects in tissues even at low concentrations (Duffus and Worth 1996, Zheljazkov and Nielsen 1996, Al-Saleh et al. 2003, Dündar et al. 2012). Studies indicate that heavy metals such as As, Cr, Hg, Cd, Pb, and Ni exhibit carcinogenic, mutagenic, and teratogenic effects in addition to their toxic effects (Boffetta et al. 1998, Quayyum and Shah 2014, Romaniuk et al. 2014, Hsueh et al. 2017, Rhee et al. 2020).

Phytochelatins in plants and metallothionein proteins in mammals also play a key role in the accumulation of heavy metals. The function of these proteins is to contribute to homeostasis by binding essential elements. However, since they also bind heavy metals taken in various ways, they can build up high amounts of these metals in tissues over time (Kägi 1991, Cobbett and Goldsbrough 2002). Moreover, the accumulation of heavy metals can also vary depending on the effects of environmental factors, resulting in varying concentrations of heavy metals in humans and animals from region to region (Ekici et al. 2015, Horasan et al. 2019). Therefore, in the present study, 14 heavy metal species (Se, Mn, Zn, Al, V, Sn, Cr, Fe, Ni, Co, Cu, As, Cd, and Pb) commonly analysed in previous studies were selected and analysed in cattle's serum raised in the Şiran district of Gümüşhane province. Additionally, age-related bioaccumulation of these heavy metals were investigated.

MATERIAL and METHODS

Sample Collection

In this study, blood samples were collected from the hearts of a total of 100 slaughtered female cattle at an abattoir in Şiran district (Figure 1) of Gümüşhane, 50 young cattle aged 1 to 3 years (17 native breeds, 17 Simmental, 16 Holstein) and 50 adult cattle aged 4 to 6 years (21 native breeds, 15 Simmental, 14 Holstein). The samples were then brought to the laboratory under a cold chain at +4 °C. In the laboratory, these samples were scratched after clotting and then centrifuged (3000 rpm for 10 min) to separate the serum.

Afterwards, the serum was collected in Eppendorf tubes, and stored at -20 °C until heavy metal analysis.

Heavy metal Analysis

The analysis of heavy metal concentrations (Se, Mn, Zn, Al, V, Sn, Cr, Fe, Ni, Co, Cu, As, Cd, and Pb) was performed using the ICP-MS (Agilent 7500a, Agilent Technologies, USA) device. The first step was the preparation of the samples for measurement. For this, 1 ml of serum from each sample was placed in Teflon cells in the microwave solubilization device. 5 ml of HNO3 (65%) was added, and after 20 minutes the device was turned on by closing the lid. After the solubilization process, the solutions were taken into 10 ml volumetric flasks and topped up with distilled water. Standards at increasing concentrations (0, 1, 5, 5, 10, 20, 20, 30, 40, and 50 ppm) were prepared from the samples ready for measurement and introduced to the ICP-MS device (Epa, 1994). After the analysis, the concentrations of heavy metals in the samples were read from the device, and the results were recorded in ppm. The method was validated by the parameters of accuracy and recovery (Se: 99.90%, Mn: 99.90%, Zn: 99.90%, Al: 99.80%, V: 99.90%, Sn: 99.90%, Cr: 99.90%, Fe: 98.20%, Ni: 99.90%, Co: 99.90%, Cu: 99.80%, As: 99.70%, Cd: 99.90%, and Pb: 99.90%), specificity, limit of detection (Se: 3.299 ppb, Mn: 0.480 ppb, Zn: 2.305 ppb, Al: 1.158 ppb, V: 0.120 ppb, Sn: 0.44 ppb, Cr: 0.730 ppb, Fe: 10.860 ppb, Ni :0.33 ppb, Co: 0.320 ppb, Cu: 1.363 ppb, As: 0.077 ppb, Cd: 0.070 ppb, and Pb: 0.200 ppb), and limit of quantitation (Se: 10.89 ppb, Mn: 1.58 ppb, Zn: 7.61 ppb, Al: 3.82 ppb, V: 0.40 ppb, Sn: 1.12 ppb, Cr: 2.41 ppb, Fe: 35.840 ppb, Ni: 1.09 ppb, Co: 1.06 ppb, Cu: 4.50 ppb, As: 0.25 ppb, Cd: 0.23 ppb, and Pb: 0.66 ppb). All chemicals used in heavy metal analysis were obtained from Sigma Chemical Company (Sigma-Aldrich, Co., Munich, Germany).

Statistical analysis

Statistical calculations were performed using SPSS 15.0 for Windows (SPSS Inc., USA). The results of this study are expressed as mean ± standard deviation (X±SD). Differences between parameters obtained from two groups (young and adult cattle) were analyzed using the Mann-Whitney U-test. A p-value of less than 0.05 was considered statistically significant.

Figure 1. The satellite image shows Şiran district of Gümüşhane

RESULTS

The concentrations of Se, Mn, Zn, Al, V, Sn, Cr, Fe, Ni, Co, Cu, As, Cd, and Pb in the blood samples collected from young and adult cattle were given in Table 1. The results revealed that the concentrations of Al, V, Ni, and Sn were considerably higher in adult cattle compared to young cattle ($p<0.05$). Specifically, the concentrations of Al in young cattle, were 1.682±0.951 (ranging from 0.355 to 1.884 ppm), V was 0.015±0.003 (ranging from 0.012 to 0.017 ppm), Ni was 0.022±0.016 (ranging from 0.009 to 0.038 ppm), Sn was 0.027±0.012 (ranging from 0.018 to 0.046 ppm). On the other hand, the concentrations of Al in adult cattle were 3.565±1.02 (ranging from 2.007 to 5.072 ppm), V was 0.046±0.015 (ranging from 0.026 to 0.062 ppm), Ni was 0.065±0.010 (ranging from 0.023 to 0.085 ppm), Sn was 0.066±0.029 (ranging from 0.022 to 0.103 ppm). Furthermore, there was no statistical difference between the two groups for the concentrations of Cr, Mn, Fe, Co, Cu, Zn, As, Se, Cd, and Pb in the blood serums.

Table 1. The concentrations of some heavy metals in serum of young and adult cattle in the Şiran district of Gümüşhane (n=50).*

	Young (1-3 years old)		Adult (4-6 years old)		
Elements	**Mean±SD**	**Min–Max**	**Mean±SD**	**Min–Max**	**p value**
Al	1.682±0.951	0.355–1.884	3.565±1.02	2.007–5.072	**<0.05**
V	0.015±0.003	0.012–0.017	0.046±0.015	0.026–0.062	**<0.01**
Cr	0.580±0.016	0.037–0.090	0.069±0.028	0.051–0.094	**NS**
Mn	0.010±0.002	0.004–0.017	0.012±0.003	0.007–0.019	**NS**
Fe	1.855±0.030	1.346–2.838	1.750±0.041	1.320–3.154	**NS**
Ni	0.022±0.016	0.009–0.038	0.065±0.010	0.023–0.085	**<0.01**
Co	0.002±0.0023	0.001–0.002	0.003±0.0037	0.002–0.004	**NS**
Cu	0.573±0.273	0.347–0.935	0.679±0.208	0.509–0.911	**NS**
Zn	0.626±0.182	0.382–0.843	0.651±0.196	0.504–0.927	**NS**
As	0.018±0.009	0.013–0.040	0.021±0.011	0.014–0.050	**NS**
Se	0.134±0.039	0.023–0.266	0.150±0.061	0.032–0.428	**NS**
Cd	0.003±0.002	0.002–0.005	0.004±0.001	0.003–0.007	**NS**
Pb	0.008±0.002	0.000–0.016	0.010±0.003	0.000–0.019	**NS**
Sn	0.027±0.012	0.018–0.046	0.066±0.029	0.022–0.103	**<0.05**

***** : as ppm **NS** : not significant

DISCUSSION

Heavy metals are known to pollute the environment through their release into water, soil, and air as waste products of industrial activities. Living organisms that consume water or consume products grown in contaminated soil may be exposed to heavy metals. In this way, heavy metals collected from the external environment mostly bind to proteins and are transmitted to the body's liver through the blood. Heavy metals processed in the liver are stored there, sent to bile or returned to the bloodstream for excretion by the kidneys (Kıvrakdal 2010, Rajaganapathy et al. 2011).

Heavy metals are known to exhibit bioaccumulation characteristics, and studies on this topic are still being carried out. The extent of accumulation in living organisms varies depending on the concentration of environmental contamination and duration of exposure. Therefore, the concentration of this heavy metal accumulation may differ from region to region and from age to age. In a study conducted by Simsek et al. (2015) in Çankırı province, it was found that Al, V, Mn, Ni, As, and Sn concentrations were higher in adults Angora goats than in young ones. The present study was found the similar results regarding the age-related accumulation of Al, V, Ni and Sn in cattle of Şiran district.

Aluminium, which has a neurotoxic effect, can accumulate in cattle through various routes such as the equipment used in the barns, feed additives, drinking water, and consumed vegetable feeds (Allowaw, 2013). The high concentrations of Al observed in adult cattle in this study may be related to

There are studies investigating the toxic effects of many heavy metals and their connection with cancer the high exposure to the above conditions.

Vanadium, which is an essential element in all mammalian, has been found to act like insulin in all the tissues mainly targeted by the hormone, namely skeletal muscle and fat (Chasteen 1983, Goldwaser et al. 2000). In our study, adult cattle was found to have a higher concentration of vanadium than young cattle, and the reason for this may be that the muscles of adult cattle are much more developed.

Nickel is a heavy metal that is present in coal, petroleum, copper and steel industries, mining and combustion of fossil fuels. It can easily pass into the soil, water and atmosphere with the use of wastewater in agricultural areas. It is then absorbed into the body through inhalation, drinking water and food contaminated with this metal (Chau and Kulikovsky-Cordeiro 1995, Seven et al. 2008). In this regard, housing the animals in conditions with air, drinking water and feed rich in this metal may have resulted in a higher concentration of Ni in adult cattle of Şiran district.

Tin is found in soil and plants. Its main use is in the coating of steel to protect it and in the manufacture of biocides, which are widely used in agriculture (Alloway, 2013). In our study, the accumulation of Sn in adult cattle was found to be significantly higher than in young cattle, which may be due to their longer exposure to an environment containing Sn.

The other heavy metals (Co, Cr, Mn, Fe, Cu, Cd Zn, As, Se, and Pb) investigated in the study, are not caused significant age-related bioaccumulation in animals. This could be due to the absence of intensive industrial activity such as mining in Şiran, unlike other districts of Gümüşhane.

Heavy metals when accumulated over time can cause diseases by disrupting various physiological mechanisms (Sözgen 2000, Romaniuk et al. 2015). (Llobet et al. 2003, Hsueh et al. 2017, Rhee et al. 2020). Studies in rats and humans have also shown that certain heavy metals, such as Al, Ni, and Cd, disrupt the oxidant and antioxidant balance by increasing the production of free oxygen radicals (Gurer et al. 1998, El-Demerdash et al. 2004, Ranjbar et al. 2008).

CONCLUSION

In conclusion, this study determined for the first time heavy metals concentrations in young and adult cattle raised in the Şiran district of Gümüşhane province. Additionally, it was revealed that the serum Al, V, Ni, and Sn concentrations changed with age, and heavy metal accumulation was higher in adult cattle. As previous studies have shown, this consequently may lead to health problems in cattle and consequent a reduction of livestock production. Therefore, a regular monitoring system for heavy metal concentrations should be established, and ranchers should be knowledgeable about the threat of heavy metal contamination sources. Thanks to these precautions, heavy metal accumulation can be significantly reduced and kept under control in Şiran district. Indirectly, it will also contribute to the health of people who consume the meat of these animals. Additionally, It will be a reference for future heavy metal studies to be carried out in this region.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: The authors declared that they contributed equally to the article.

Ethical approval: This study is not subject to the permission of HADYEK in accordance with the

"Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

REFERENCES

Alloway BJ. (2013). Heavy metals in soils trace metals and metalloids in soils and their bioavailability. Environ Pollut, Netherlands: Springer. pp. 551-558.

Al-Saleh I, Shinwari N, Mashhour A. (2003). Heavy Metal Concentrations in the Breast Milk of Saudi Women. Biol Trace Elem Res, 2003; 96: 21-37.

Arslanbas E, Baydan E. (2013). Metal levels in organically and conventionally produced animal and vegetable products in Turkey. Food Addit Contam Part B Surveill. 6: 130-133.

Beşkaya A, Yıldız K, Başalan M, Us MF. (2008). Kırıkkale'de endüstri bölgesi civarında toprak, yem, su ve bu yörede yetiştirilen koyunlar ile parazitlerinde bazı ağır metallerin (Cd, Cu, Pb, Zn) belirlenmesi. Etlik Vet Mikrobiyol Derg. 19: 39-46.

Bigersson B, Sterner O, Zimerson E. (1988). Eine verständliche Einführung in die Toxikologie, Wiley-VCH, ISBN 3-527-26455-8.

Boffetta P, Garcia-Gómez M, Pompe-Kirn V, Zaridze D, Bellander T, Bulbulyan M, Caballero JD, Ceccarelli F, Colin D, Dizdarevic T, Español S, Kobal A, Petrova N, Sällsten G, Merler E. (1998). Cancer occurrence among European mercury miners. Cancer Causes and Control. 9: 591-599.

Chau YK, Kulikovsky-Cordeiro OTR. (1995). Occurrence of nickel in the Canadian environment. Environ Rev. 3: 95-120.

Chasteen ND. (1983). Copper, molybdenum, and vanadium in biological systems, Springer Berlin Heidelberg, pp. 105-138.

Cobbett C, Goldsbrough P. (2002). Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol. 53: 159-182.

Cooke M, Dennis AJ. (1985). Polynuclear aromatic hydrocarbons: mechanism, method and metabolism. Ohio Battelle Press, Columbus, USA.

Duffus JH, Worth HGJ. Fundamental toxicology for chemists, Royal Society of Chemistry Information Services, Cambridge, UK, 1996.

Dündar MŞ, Altundağ H, Kaygaldurak S, Şar V, Acar A. Çeşitli endüstriyel atik sularda ağır metal düzeylerinin belirlenmesi. SAÜ Fen Bil Enstit Derg, 2012; 16: 6-12.

Ekici H, Şimşek Ö, Arıkan Ş, Eren M, Güner B. Comparing levels of certain heavy metals and minerals and antioxidativemetabolism in cows raised near and away from highways. Turk J Vet Anim Sci, 2015; 39: 322-327.

El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β-carotene. Food Chem Toxicol, 2004; 42: 1563-1571.

EPA. Methods for the determination of metals in environmental samples. Method 200.8 EMMC version, Revision 5. 4. EMSL Cincinnati OH 45268, 1994.

Ergün A. (2001). Mineral elementler. In, Ergün A, Tuncer DŞ (Ed): Hayvan Besleme ve Beslenme Hastalıkları. Medipress, 77-91, Ankara.

Goldwaser I, Gefel D, Gershonov E, Fridkin M, Shechter Y. (2000). Insulin-like effects of vanadium: basic and clinical implications. J Inorg Biochem. 80: 21-25.

Gurer H, Özgüneş H, Neal R, Spitz DR, Ercal N. (1998). Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. Toxicol. 128: 181-189.

Horasan BY, Arık F. (2019). Assessing heavy metal pollution in the surface soils of Central Anatolia Region of Turkey. Carpathian J Earth Environ Sci. 14: 107-118.

Hsueh YM, Su CT, Shiue HS, Chen WJ, Pu YS, Lin YC, Tsai CS, Huang CY. (2017). Levels of plasma selenium and urinary total arsenic interact to affect the risk for prostate cancer. Food Chem Toxicol. 107:167-175.

Kägi JH. (1991). Overview of metallothionein. Methods Enzymol. 205: 613-626.

Karagül H, Altıntaş A, Fidancı UR, Sel T. (2000). Klinik Biyokimya, Medisan Yayın Serisi, 45: Ankara.

Kıvrakdal A. (2010). Farklı sertlik değerlerindeki sularda metal (Cu, Cr) etkisinde kalan farklı büyüklükteki balıkların (Oreochromis niloticus) ATPaz tepkilerin belirlenmesi. Yüksek Lisans Tezi. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Adana.

Llobet JM, Falco G, Casas C, Teixido A, Domingo JL. (2003). Concentrations of Arsenic, Cadmium, Mercury and Lead in common foods and estimated daily Intake by children, adolescents, adult and seniors of Catalonia, Spain. J Agric Food Chem. 51: 838-842.

Quayyum MA, Shah MH. (2014). Comparative study of trace elements in blood, scalp hair and nails of prostate cancer patients in relation to healthy donors. Biol Trace Elem Res. 162: 46-57.

Paksy K, Rajczy K, Forgács Z, Lázár, P, Bernard A, Gáti I, Kaáli GS. (1997). Effect of cadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells. J Appl Toxicol. 17: 321-327.

Ranjbar A, Khani-Jazani R, Sedighi A, Jalali-Mashayekhi F, Ghazi-Khansari M, Abdollahi M. (2008). Alteration of body totalantioxidant capacity and thiol molecules in human chronic exposure to aluminum. Toxicol Environ Chem. 90: 707-713.

Rajaganapathy V, Xavier F, Sreekumar D, Mandal PK. (2011). Heavy Metal Contamination in Soil, Water and Fodder and their Presence in Livestock and Products: A Review. J Environ Sci Technol. 4: 234-249.

Rhee J, Vance TM, Lim R, Christiani DC, Qureshi AA, Cho E. (2020). Association of blood mercury levels with nonmelanoma skin cancer in the U.S.A. using National Health and Nutrition Examination Survey data (2003–2016). Br J Dermatol. 183: 480-487.

Romaniuk A, Lyndin M, Moskalenko R, Kuzenko Y, Gladchenko O, Lyndina Y. (2015). Pathogenetic mechanisms of heavy metals effect on proapoptotic and proliferative potential of breast cancer. Interv Med Appl Sci. 7: 63-68.

Saghaei S, Ekici H, Demirbas M, Yarsan E, Tumer I. (2012). Determination of the Metal Contents of Honey Samples from Orumieh in Iran. Kafkas Univ Vet Fak Derg, 18: 281-284.

Seven T, Can B, Darende BN, Ocak S. (2008). Hava ve Toprakta Ağır Metal Kirliliği. UCBAD. 1: 91-103.

Simsek O, Ekici H, Cinar M, Atmaca N, Arikan S, Guner B. (2015). Heavy metal accumulation and oxidative-antioxidative status in angora goats depending on age. Fresenius Environ Bull. 24: 2659-2663.

Sözgen K. (2000). Ters Faz Likit Kromatografisi ile Ağır Metal Analizi. İ.Ü. Fen Bilimleri Enstitüsü Doktora Tezi. İstanbul.

Wu G, Kang H, Zhang X, Shao H, Chu L, Ruan C. (2010). A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, ecoenvironmental concerns and opportunities. J Hazard Mater. 174:1-8.

Zheljazkov VD, Nielsen NE. (1996). Effect of heavy metals on peppermint and cornmint. Plant Soil. 178: 59-66.