Biological monitoring of heavy metals in blood of Kerman residents, southeast of Iran

Azam Rajabi¹, Majid Aghasi¹*Ali Akbar Roodbari²

*Corresponding author: Majid Aghasi, PhD

E-mail: az.rajabi20@gmail.com

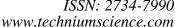
Abstract. Heavy metals, particularly lead, cadmium, chromium and arsenic, are toxic elements and can cause serious complications for human health. This research is a crosssectional descriptive study with the aim of biomonitoring heavy metals found in blood of residents in Kerman. Sampling was conducted by random sampling at 8 sites on a total of 80 people (10 per site). The samples were examined by acid digestion using ICP-OES to measure the heavy metals. The results of the present study confirmed a concentration of toxic heavy metals in the blood of residents of Kerman. The maximum concentration of heavy metals were observed in the blood of the residents of Moshtagh and Azadi districts, which were theheavy-traffic areas of the city. A statistically significant relationship was seen between the concentration of some of the above elements and occupation, education and smoking. Statistical tests showed a significant relationship between lead concentration and age. The results suggested that exposure to lead elements can cause some degrees of depression in humans. The presence of toxic heavy metals in the blood of residents can affect their health and also cause some complications.

Introduction

Fine particles with an aerodynamic diameter smaller than 2.5 µm have been identified as the causes of various diseases, including cancer, coronary and peripheral artery disease, diabetes, stroke, and respiratory diseases (1, 2). Generally, the high concentration of heavy metals in the environment can cause health hazards, such as incompatibility with nervous system, blood, cardiovascular system, kidney and reproduction systems. The other effects are reduced intelligence, lack of concentration, and behavioral disorders (3). Some metals, such as copper and zinc, are harmless in small amounts, but some metals, mainly lead, arsenic, mercury and cadmium that are known as toxic heavy metals, are toxic even at very low concentrations and can cause many diseases and cancers (4).

¹Department of Environmental Health, School of Public Health, Kerman University of Medical Sciences, Kerman, Iran

²Department of Environmental Health, School of Public Health, Shahrood University of Medical Sciences, Shahrood, Iran





Heavy metals and fine elements can be measured in blood, serum, urine, hair and other body tissues. In blood, metals are distributed in the intercellular (plasma/serum) and intracellular fluid (mainly erythrocytes) (5). Toxic heavy metals and the acute effects of toxicity of metals and trace elements have been widely studied. There are also effects caused by prolonged exposure (6). Age and sex are two intrinsic factors that determine the concentration of heavy metals in human blood (7). Generally, heavy metals enter the body through mouth, inhalation and skin contact, all of which have adverse effects on human health (8).

A study was performed by Thompson et al. (2008) in Ireland to investigate the toxic effects of cadmium on the reproductive system and embryo. The collected data showed that cadmium affects the reproductive system of males from fetal to adulthood periods and have adverse effects on the growth of the gonads (9). The findings of the study by Rosalie et al. in the United States showed that the amount of metal intake in smokers is different depending on the type of cigarettes (7).

In a study in Nigeria, Orisakwe reported that lead and cadmium are involved in human mental health (10). In a study by Bouchard et al., it was found that smoking can enhance the blood lead level, which increases depression in young Americans (11).

Accumulation of toxic heavy metals in the environment can lead to their entrance to the human body. No matter what route they enter the body, these elements enter the bloodstream and reach different organs. Toxic metals affect the target organs and finally damage the human health (12). Blood monitoring is one of the accepted methods for biomonitoring of exposure to toxic metals in environmental toxicology (13). This study aimed to measure the toxic heavy metals of arsenic, lead, cadmium, and mercury, in the blood of residents of Kerman, and evaluate the association between the concentration of these elements and socio-demographic and behavioral characteristics of the volunteers.

Methods and Materials

Study design

This study is a descriptive cross-sectional research and was conducted based on measuring the concentration of heavy metals in blood samples of residents in Kerman. Those over 18 years volunteered for participation in the present research.

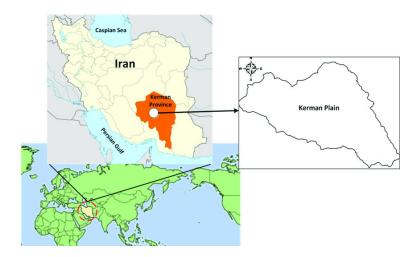
Ethics Committee approval

This research has been registered with code: IR.KMU.REC.1398.292 on 18 September, 2019, in Ethics Committee of Kerman University of Medical Sciences.

Stady area

Kerman is the capital of Kerman province and is located in the South-Eastern Iran. The city is located on a broad plain in a semi-arid area. Kerman is located at latitude 30.3 and longitude 57.1. This city is 1756 meters above sea level. The area of Kerman is 45401 km² and had a population of 634132 in the most recent census (14).





Sample Size Determination

Given the correlation calculated from the pilot study and the use of sample size formulas, a sample size of 80 was calculated(15).

Sample location

In Figure 2, the borders of eight blood sampling sites from individuals studied in Kerman are specified. At each site, the sampling was carried out at a radius of 5km. The names of sampling sites from 1-8 were as follows: Jahad Boulevard, beginning of Kuhpayeh Road, Havaniruz junction, Kowsar Square, Resalat Boulevard, Azadi Square, Moshtagh Square, and Khaju Square.



Collecting demographic information

Socio-demographic and behavioral information of the volunteers were recorded in a form prepared for this purpose. This information involves age, sex, occupation, education, history of residence, smoking habits, and etc.

SamplingBlood

Blood sampling was conducted based on the method used by similar studies (16, 17). Blood sampling was performed from 8 am to 10 am after a night without smoking or taking medication. 5 cc of fasting blood was collected in centrifuge tube without anticoagulant. Blood containing tubes were transferred to the laboratory to measure the desired parameters.



Preparation of blood samples

Blood samples were prepared based on the method proposed by Tan et al. (1). 5 cc of fasting blood was collected in a centrifuge tube without anticoagulant. In the shortest period after sampling, the blood samples were centrifuged at a speed of 5000 rpm for 10 minutes and supernatant was analyzed.

Sample analysis

Analysis of elements was carried out using inductively plasma spectroscopy method (ICP715-ES) (made in Australia) with a detection limit of ppm to ppb. In this method, wavelength calibration was performed using internal calibration by a mercury vapor lamp (18).

Data Analysis

Data analysis was conducted using SPSS software v.22. The central tendency and dispersion indices were used for data description, and correlation analysis was used for data analysis.

Results

Descriptive results of measurement of toxic heavy metals in blood are shown in Table 1. The results show that the highest concentration of lead and cadmium pollutants is in the blood of people in District 7, the highest concentration of arsenic is in the blood of people in District 6, and the lowest concentration of above-mentioned metals is in the blood of residents in District 2. Moreover, the mercury concentration in the blood of volunteers was lower than the detection limit of the device and was not measureable.

Table 1: Descriptive table of concentrations of toxic heavy metals in the blood

Sampling	number	Mean and standard deviation of heavy metal concentration							
Locations		measured in blood (mg/l)							
		Pb	Cd	AS					
1	10	0.0081±0.003	0.019±0.006	0.014 ± 0.002					
2	10	0.006±0.001	0.009 ± 0.002	0.008 ± 0.002					
3	10	0.186±0.047	0.011±0.003	0.272 ± 0.057					
4	10	0.186±0.050	0.015±0.002	0.311±0.057					
5	10	0.249±0.033	0.014 ± 0.004	0.300 ± 0.058					
6	10	0.274±0.106	0.014 ± 0.002	0.330±0.034					
7	10	0.288±0.049	0.019 ± 0.004	0.264 ± 0.039					
8	10	0.186±0.043	0.014 ± 0.002	0.266 ± 0.047					
Total	80	0.173±0.114	0.014 ± 0.005	0.221±0.130					
Ranng		0.003-0.372	0.004-0.032	0.005-0.372					

High concentration of heavy metals in districts 6 and 7 can be attributed to the heavy traffic of these areas. The low concentration of the referred elements in district 2 can also be attributed to the same issue (19). The presence of small workshops in district 7 can also exacerbate this issue (20). The results of a study by Schultze et al. on the heavy metals in the whole blood and serum in Sweden showed that the amount of metals in serum is lower than whole blood. The authors explained that this is due to the difference in distribution of various metals in blood and serum. This distribution in many heavy metals is related to physiological factors and mainly to renal function (16).



Association between blood lead, cadmium and arsenic concentration with socio demographic characteristics of the studied individualswas measured using one-way analysis of variance (ANOVA) at confidence level of 95%. The test results are shown in Table 2.

Table 2: Relationship between blood lead, cadmium and arsenic concentrations with sociodemographic characteristics of the subjects with ANOVA test

	Pb			Cd			As		
Specification	F	df	p-value	F	df	p-value	F	df	p-value
Age	2.46	6	0.032	1.90	6	0.09	2.67	6	0.021
Sex	2.30	1	0.133	2.10	1	0.15	2.65	4	0.220
Educational status	1.64	4	0.174	0.96	4	0.43	2.66	4	0.039
Job	0.54	3	0.655	4.50	3	0.006	0.46	3	0.711
marital status	0.12	1	0.729	4.50	1	0.037	0.34	1	0.559

There was a significant relationship between concentrations of arsenic and lead and age. The Tukey's test was used for multiple comparisons, and the results showed that there was a higher amount of lead and arsenic in the blood of people in the age range of 51-55 years. The results showed that there was no statistically significant relationship between the concentration of the above heavy metals and sex. There was also a significant relationship between cadmium concentration and marital status and occupation.

The results of the Tukey's test indicated that single people had higher cadmium concentration in their blood. Higher amount of cadmium was seen in the blood of the unemployed people. The results of Table 3 show that there is a significant association between arsenic concentration in blood and educational status. The results of Tukey's test showed that people with the educational level lower than junior high school had higher blood arsenic concentration.

In a study in northern India, Anitha et al., did not find the amount of heavy metals in the blood of residents to be a concern, but the level of heavy metals was statistically associated with age, sex, and dietary habits of the subjects (7). In some studies, a statistically significant association is reported between arsenic concentration in the blood and age, and with an increase in age, the arsenic concentration is also increased (21). In some studies, a relationship is reported between cadmium concentration and marital status, so that the cadmium concentration was higher in blood of the single people (22, 23).

Married people with a high cadmium concentration in blood had reducedchildbearing (23). In a study in Lithuania by Loreta et al., the relationship between cadmium concentration and marital status is reported, and single people had higher cadmium concentration in their blood (22). In other studies, a relationship is reported between unemployment and cadmium concentration in blood(24). In a study in India by Singh et al., a relationship between arsenic and educational status and age has been reported, and with an increase in age and lower education, a higher arsenic concentration has been found in their blood (25).

Relationship between blood lead, cadmium and arsenic concentration and number of children was measured by Pearson test at confidence level of 95%. The test results are shown in Table 3.



Table 3: Relationship between blood lead, cadmium and arsenic concentrations with number of children using Pearson test

	Pb		C	Cd	As		
Parameter studied	F	p-	F	p-	F	p-	
		value		value		value	
Number of Children	0.133	0.339	0.289	0.034	0.07	0.614	

Results showed that there is no significant relationship between blood lead and arsenic concentration and number of children. Due to the effect of some pollutants on infertility, the relationship between some factors and the number of children was evaluated. There is a significant relationship between the blood cadmium concentration and the number of children, and the number of children is increased with an increase in cadmium concentration.

Results of some studies are not consistent with the results of the present study; in these studies with an increase in cadmium content in blood, the number of children is reduced and childbearing is declined. This is due to the effect of cadmium on the genes of the studied individuals (26-28). It should not be forgotten that the population in the present research involves both men and women.

Relationship between blood lead, cadmium and arsenic concentration and behavioral characteristics of the studied individuals was measured by one-way analysis of variance (ANOVA) at confidence level of 95%. The test results are shown in Table 4.

Table 4: Relationship between blood lead, cadmium and arsenic concentrations with behavioral characteristics of the subjects using ANOVA test

Parameter	Pb			Cd			As		
Specification	F	df	p-value	F	df	p-value	F	df	p-value
smoking	4.74	3	0.004	3.30	3	0.025	2.06	3	0.113
Hookah consumption	0.80	2	0.454	0.83	2	0.438	0.86	2	0.427
Consume fruits and vegetables	9.87	1	0.002	1.23	1	0.270	8.77	1	0.004

Results show that concentration of metals such as cadmium and lead has a statistically significant relationship with smoking at 95% confidence level. Tukey's test showed that increased smoking is associated with increased concentration of metals. The results showed no significant relationship at 95% confidence level between the concentration of heavy metals and hookah use. According to the results, there is a statistically significant relationship between the intake of fruits and vegetables and blood lead and arsenic concentration at 95% confidence level. Tukey's test showed that people with less intake of fruits and vegetables had higher concentrations of above elements in their blood.

Results of the study by Alrobaian et al. in Saudi Arabia showed that the amount of heavy metals in the blood and hair of smokers was higher than non-smokers (29). The results of some studies, such as Ray in India, have shown that there is a relationship between blood lead concentration and hookah use (30). The contradiction in the results with the present study can be attributed to discontinuous hookah use in 34% of the studied individuals. Numerous studies have shown an association between concentrations of heavy metals and smoking (31-33).

In a study in Saudi Arabia, Ashraf reported that with an increase in smoking, the blood lead level is increased (34). Price in London (35), Bernhard et al. in Germany (36), and Zwayed et al. in Iraq (37)



showed a relationship between cadmium concentration in blood and smoking, such that with an increase in smoking, the blood cadmium level is increased. Blood cadmium concentration can cause health problems, including neurological disorders and cancer (38). The results of some studies are in contrast to the results of the current study. The authors of above articles have stated that increased consumption of vegetables leads to increased concentration of heavy metals in the blood. This is due to the irrigation of vegetables with wastewater (39) or contaminated water (40) or atmospheric absorption of metals (41).

Relationship between depression and concentration of heavy metals in blood was measured using oneway analysis of variance (ANOVA) at 95% confidence level. The results are shown in Table 5. The results presented in the table show that there is no statistical significant association between depression and cadmium and arsenic concentration, number of children and BMI, but there is a significant relationship between lead concentration in the blood and depression. Tukey's test was used for a multiple comparison and it was found that the highest lead concentration was in people with severe depression, so it can be said that with an increase in blood lead level, the depression rate is also increased.

Table 5: Relationship between depression and toxic heavy metal concentrations in blood, BMI, number of children using one way ANOVA

Parameter	F	p-value	df
Pb	3.86	0.007	4
Cd	0.78	0.541	4
As	2.84	0.850	4
BMI Number of Children	2.21 0.34	0.076 0.850	4 4

Discussion

Blood lead concentration measurement is the most commonly used method of proving its presence and complications in body (42). In the literature review, a relationship between lead concentration and depression is reported, such that with an increase in blood lead concentration, the incidence of depression is also increased (11). Blood lead concentration causes a series of mental problems such as low attention (43) and depression(11). In numerous studies, an association between blood lead concentration and depression is also shown (44, 45).

Conclusion

In the present study, the concentration of heavy metals in the blood of residents of Kerman was assessed, showing that the residents of Kerman are exposed to toxic heavy metals. The results of this study confirmed the relationship between traffic and absorbed concentration of toxic heavy metals in humans. In the statistical tests, a significant relationship was seen between lead concentration and age. This suggests that older people are more exposed to the lead. Moreover, exposure to lead causes some degrees of depression in humans.

Lower concentrations of elements such as lead and arsenic in people with less intake of fruits and vegetables show that the studied individuals are exposed to such metals by other methods such as smoking. The fact that single and unemployed people have higher blood cadmium concentration indicates



that these people have unhealthy lifestyles or leisure-time activities and marriage can improve their physical or perhaps psychological health.

References

- Tan C, Wang Y, Lin M, Wang Z, He L, Li Z, et al. Long-term high air pollution exposure 1. induced metabolic adaptations in traffic policemen. Environmental Toxicology and Pharmacology. 2018;(58):156-62.
- Brunekreef B, Beelen R, Hoek G, Schouten L, Bausch-Goldbohm S, Fischer P, et al. Effects of long-term exposure to traffic-related air pollution on respiratory and cardiovascular mortality in the Netherlands: the NLCS-AIR study. Research report (Health Effects Institute). 2009(139):5-71; discussion 3-89.
- 3. Inyang HI, Bae S. Impacts of dust on environmental systems and human health. Journal of hazardous materials. 2006;132(1):v-vi.
- Willers S, Gerhardsson L, Lundh T. Environmental tobacco smoke (ETS) exposure in children with asthma—relation between lead and cadmium, and cotinine concentrations in urine. Respiratory Medicine. 2005;99(12):1521-7.
- Gaffney JS, Marley NA. The impacts of combustion emissions on air quality and climate From coal to biofuels and beyond. Atmospheric Environment. 2009;43(1):23-36.
- Messner B, Knoflach M, Seubert A, Ritsch A, Pfaller K, Henderson B, et al. Cadmium is a novel and independent risk factor for early atherosclerosis mechanisms and in vivo relevance. Arteriosclerosis, thrombosis, and vascular biology. 2009;29(9):1392-8.
- Jose A, Ray JG. Toxic heavy metals in human blood in relation to certain food and environmental samples in Kerala, South India. 2018;25(8):7946-53.
- Shao T, Pan L, Chen Z, Wang R, Li W, Qin Q, et al. Content of Heavy Metal in the Dust of Leisure Squares and Its Health Risk Assessment—A Case Study of Yanta District in Xi'an. International journal of environmental research and public health. 2018;15:394.
- Thompson J, Bannigan J. Cadmium: toxic effects on the reproductive system and the embryo. Reproductive toxicology (Elmsford, NY). 2008;25(3):304-15.
- 10. Orisakwe OE. The role of lead and cadmium in psychiatry. N Am J Med Sci. 2014;6(8):370-6.
- 11. Bouchard MF, Bellinger DC, Weuve J, Matthews-Bellinger J, Gilman SE, Wright RO, et al. Blood lead levels and major depressive disorder, panic disorder, and generalized anxiety disorder in US young adults. Archives of general psychiatry. 2009;66(12):1313-9.
- Salih N, Jaafar M. Heavy metals in blood and urine impact on the woman fertility. Chem Mat 12. Res. 2013:3:81-9.
- Madiha B KSA, Zahidqureshi, Nida M and Nimra. Determination of Heavy Metal Toxicity in Blood and Health Effect

by AAS (Detection of Heavy Metals and its Toxicity in Human Blood). 2018: 1(2).

- 15. Garcia Asuero A, Sayago A, González G. The Correlation Coefficient: An Overview. Critical Reviews in Analytical Chemistry - CRIT REV ANAL CHEM. 2006;36:41-59.
- Schultze B, Lind PM, Larsson A, Lind L. Whole blood and serum concentrations of metals in a Swedish population-based sample. Scandinavian journal of clinical and laboratory investigation. 2014;74(2):143-8.
- Lind PM, Olsen L, Lind L. Elevated circulating levels of copper and nickel are found in elderly subjects with left ventricular hypertrophy. Ecotoxicology and environmental safety. 2012;86:66-72.
- (NIOSH) TNIfOSaH. ELEMENTS by ICP (Microwave Digestion) 2014. 18.
- Zhang F, Yan X, Zeng C, Zhang M, Shrestha S, Devkota LP, et al. Influence of traffic activity on heavy metal concentrations of roadside farmland soil in mountainous areas. International journal of environmental research and public health, 2012;9(5):1715-31.

- 20. Femi A. Heavy metal pollution of auto-mechanic workshop soils within Okitipupa, Ondo State, Nigeria2018.
- 21. Tyler CR, Allan AM. The Effects of Arsenic Exposure on Neurological and Cognitive Dysfunction in Human and Rodent Studies: A Review. Curr Environ Health Rep. 2014;1(2):132-47.
- Strumylaite L, Kregzdyte R, Bogusevicius A, Poskiene L, Baranauskiene D, Pranys D. Cadmium Exposure and Risk of Breast Cancer by Histological and Tumor Receptor Subtype in White Caucasian Women: A Hospital-Based Case-Control Study. Int J Mol Sci. 2019;20(12).
- McElroy JA, Kruse RL, Guthrie J, Gangnon RE, Robertson JD. Cadmium exposure and endometrial cancer risk: A large midwestern U.S. population-based case-control study. PLoS One. 2017;12(7):e0179360.
- Burke PDF, Hamza S, Naseem S, Nawaz-Ul-Huda S, Azam M, Khan I. Impact of Cadmium Polluted Groundwater on Human Health: Winder Balochistan. SAGE Open. 2016;6:1-8.
- Singh N, Kumar D, Sahu AP. Arsenic in the environment: effects on human health and possible prevention. Journal of environmental biology. 2007;28(2 Suppl):359-65.
- Pollack AZ, Ranasinghe S, Sjaarda LA, Mumford SL. Cadmium and Reproductive Health in Women: A Systematic Review of the Epidemiologic Evidence. Curr Environ Health Rep. 2014;1(2):172-84.
- Alaee S, Talaiekhozani A, Rezaei S, Alaei Jahromi K, Yousefian E. Cadmium and male infertility. Journal of Infertility and Reproductive Biology. 2014;2:62-9.
- Weller BK, Atan SU, Metin MS. Effects of cadmium on female fertility parameters: an experimental study. Studies on Ethno-Medicine. 2017;11(1):63-9.
- Alrobaian M, Arida H. Assessment of Heavy and Toxic Metals in the Blood and Hair of Saudi 29. Arabia Smokers Using Modern Analytical Techniques. International Journal of Analytical Chemistry. 2019;2019:1-8.
- Ray C. The hookah the Indian waterpipe. Current Science. 2009;96:1319-23. 30.
- 31. Jedrychowski W, Flak E, Mroz E, Rauh V, Caldwell K, Jones R, et al. Exposure to environmental tobacco smoke in pregnancy and lead level in maternal blood at delivery. International journal of occupational medicine and environmental health. 2006;(19)(4):205-10.
- Richter PA, Bishop EE, Wang J, Kaufmann R. Trends in tobacco smoke exposure and blood lead levels among youths and adults in the United States: the National Health and Nutrition Examination Survey, 1999-2008. Preventing chronic disease. 2013;10:E213.
- Apostolou A, Garcia-Esquinas E, Fadrowski JJ, McLain P, Weaver VM, Navas-Acien A. 33. Secondhand tobacco smoke: a source of lead exposure in US children and adolescents. Am J Public Health. 2012;102(4):714-22.
- Ashraf MW. Levels of heavy metals in popular cigarette brands and exposure to these metals via smoking. ScientificWorldJournal. 2012;2012:729430-.
- Price R. Cadmium Nephropathy and Smoking. Clinical Medicine Insights: Urology. 2017;10. 35.
- Bernhard D, Rossmann A, Henderson B, Kind M, Seubert A, Wick G. Increased serum cadmium and strontium levels in young smokers: effects on arterial endothelial cell gene transcription. Arteriosclerosis, thrombosis, and vascular biology. 2006;26(4):833-8.
- Zwayed K, Radhwan F, Ibrahim R. Estimation of Blood Cadmium Level Among Adults Smokers 37. In Comparative With Non Smokers2018.
- Rafati Rahimzadeh M, Rafati Rahimzadeh M, Kazemi S, Moghadamnia A-A. Cadmium toxicity and treatment: An update. Caspian J Intern Med. 2017;8(3):135-45.
- Oteef M, F. Fawy K, Abd-Rabboh H, Idris A. Levels of zinc, copper, cadmium, and lead in fruits and vegetables grown and consumed in Aseer Region, Saudi Arabia. Environmental Monitoring and Assessment. 2015;187.
- Banerjee D, Bairagi H, Mukhopadhyay S, Pal A, Bera D, Ray L. Heavy metal contamination in fruits and vegetables in two districts of West Bengal, India. Electronic journal of environmental, agricultural and food chemistry. 2010;9:1423-32.



- 41. Zhou H, Yang W-T, Zhou X, Liu L, Gu J-F, Wang W-L, et al. Accumulation of Heavy Metals in Vegetable Species Planted in Contaminated Soils and the Health Risk Assessment. International journal of environmental research and public health. 2016;13(3):289.
- 42. Kim H-C, Jang T-W, Chae H-J, Choi W-J, Ha M-N, Ye B-J, et al. Evaluation and management of lead exposure. Ann Occup Environ Med. 2015;27:30-.
- 43. Daneshparvar M, Mostafavi S-A, Zare Jeddi M, Yunesian M, Mesdaghinia A, Mahvi AH, et al. The Role of Lead Exposure on Attention-Deficit/ Hyperactivity Disorder in Children: A Systematic Review. Iran J Psychiatry. 2016;11(1):1-14.
- 44. Cahen L, Eberhardt P, Geiss J, Houtermans FG, Jedwab J, Signer P. On a correlation between the common lead model age and the trace-element content of galenas. Geochimica et Cosmochimica Acta. 1958;14(1):134-49.
- 45. Blagojević J, Jovanović V, Stamenković G, Jojić V, Bugarski-Stanojević V, Adnađević T, et al. Age Differences in Bioaccumulation of Heavy Metals in Populations of the Black-Striped Field Mouse, Apodemusagrarius(Rodentia, Mammalia). International Journal of Environmental Research. 2012;6(4):1045-52.