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CONCENTRATION OF SELECTED HEAVY METALS IN BROWN HARE (*Lepus europaeus*) AND WILD BOAR (*Sus scrofa*) FROM CENTRAL TURKEY

Demirbaş Y. [1], *Erduran N.* [2]

Summary: In the present study, concentrations of heavy metals viz. chromium (Cr), manganese (Mn), copper (Cu), zinc (Zn), cadmium (Cd), mercury (Hg), lead (Pb), and a metalloid selenium (Se) were examined in kidney, liver and muscle tissues of 15 brown hares (*Lepus europaeus*) and 9 wild boars (*Sus scrofa*) obtained from Kırıkkale province located in central Turkey. Significant variation in the concentrations of the studied heavy metals in the selected tissues was recorded. Mean concentrations of Cr (1.02 mg/kg), Mn (6.00 mg/kg), Se (1.15 mg/kg) and Cd (4.49 mg/kg) were the highest in hare kidney compared to other tissues, whereas concentrations of Cu (2.34 mg/kg) and Zn (40.51 mg/kg) were the highest in hare liver, and Pb (7.83 mg/kg) was the highest in hare muscle. On the other hand, mean concentrations of Cr (1.82 mg/kg), Cu (1.25 mg/kg), Se (3.55 mg/kg) and Cd (3.05 mg/kg) were the highest in boar kidney compared to liver and muscle tissues, whereas concentrations of Mn (3.89 mg/kg) and Pb (0.75 mg/kg) were the highest in boar liver, and Zn (45.65 mg/kg) was the highest in boar muscle. Strong positive significant correlations were found between Cr and Hg, and between Cu and Pb concentrations in kidney in all wild boar samples. There were also strong positive significant correlations between Cd and Pb concentrations in liver and Cd and Pb concentrations in muscle in all hare samples. Our results revealed that Hg (1/9 boar kidney; 4/15 hare kidney), Cr (9/9 boar all three tissues; 9/15 hare kidney and 11/15 hare liver), Pb (4/9 boar muscle; 5/15 hare liver, 15/15 hare muscle) and Cd (9/9 boar all three tissues; 15/15 hare all three tissues) concentrations detected in wild boar and hare tissues in the present study were higher than the hygienic limits.

Key words: Lepus europaeus, *Sus scrofa*, heavy metals, environmental pollution, tissues, central Turkey

Introduction

The contaminants that have a toxic effect on organisms in ecosystems and disturb the balance among the living organisms are metals, pesticides, Polychloro Biphenyls (PCB) and Polyaromatic Hydrocarbons (PAH) (Webb et al., 2002). Heavy metals are among the most hazardous contaminants due to their extensive use. At the present time, as a result of increasing populations, urbanization, industrialization and intensive agricultural activities, increasing concentrations of heavy metals are found in all living organisms (Örün and Yalçın, 2011; Tunca et al., 2012).

[1] Demirbaş Yasin, PhD, assistant, University of Kırıkkale, Faculty of Science and Arts, Department of Biology, 71450 Yahsihan, Kırıkkale, Turkey;
[2] Erduran Nuran, PhD, assistant, University of Kırıkkale, Faculty of Science and Arts, Department of Chemistry, 71450 Yahsihan, Kırıkkale, Turkey.

Corresponding author: Demirbaş Yasin, email: ydemirbas71@hotmail.com

The leakage of heavy metals present in soil, water and air into the food chain is a significant environmental concern and leads to high risks for the living organisms in the ecosystem. It is reported that the high levels of some heavy metals could have lethal and sublethal effects on mammals (Iwegbue, 2008; Danieli et al., 2012). Game meat and offal widely consumed by humans are known to be a significant source of heavy metals. Toxic accumulation of heavy metals in soil and plants entails a high risk of transferring heavy metals to herbivorous/omnivorous wild mammals, game and farm animals. It is reported that the levels of contaminants in wild animals are higher than those in domestic animals (Chen et al., 2001; Bilandžić et al., 2010).

Mammals such as brown hare (*Lepus europeaus*), red fox *(Vulpes vulpes)*, wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), mink (*Mustela vison*), wood mouse (*Apodemus flavicolis*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*), and golden jackal (*Canis aureus*) are useful indicators of heavy metal pollution mainly due to their relatively longer lifespan (Medvedev, 1999; Wajdzik, 2006; Kramárová et al., 2005; Srebočan et al., 2011; Filistowicz et al., 2012; Ćirović et al., 2015). A long-term chemical accumulation in such animals provides an early warning of negative toxic effects for the general compartments of the ecosystem (Bilandzić et al., 2010). These studies, based on toxicity tests and bioaccumulation experiments, are highly significant for protection of ecosystems (Tunca, 2012).

As an indicator of environmental pollution, heavy metals are required to be constantly monitored. Though environmental pollution is recognized as one of the most important causes of the decrease in natural population, only one ecotoxic study has been conducted on wild mammals from southern region of Turkey until today (Yarsan et al., 2014). Turkish brown hare (*Lepus europeaus*) and wild boar (*Sus scrofa*) are among the most popular game animals which show large geographical distribution throughout Turkey. The purpose of this study was to determine the concentrations of the selected heavy metals (Cr, Mn, Cu, Zn, Cd, Hg, and Pb) and a metaloid Se in the kidney, liver, and muscle tissues of hares and wild boars from Kırıkkale province in the central region of Turkey. This monitoring is also important because the investigated animals are consumed by some people in Turkey

Material and Methods

Study area

This study was conducted on kidney, liver, and muscle tissue samples obtained from central part (approx. 1.500 km^2) of Kırıkkale province (39°49'N 33°30'E) in Turkey. The study area was covered mainly by cropland where wheat, barley, sunflower, sugar beet, watermelon, melon, and oak clover are cultivated. Also, there was a forest vegetation formed by oak scrubs and black pine in Balışeyh region and other forest vegetation formed by Turkey oak, downy oak and laurel-leaf cistus associations in Keskin region (Dönmez, 2002; Hamzaoğlu and Duran, 2004; Hamzaoğlu, 2005). Agriculture is a dominant economic activity at study area with usage of herbicides, pesticides, and fertilizers during production. In terms of transportation, Kırıkkale province is one of the most important crossroads in Turkey. On the other hand, there are large industrial facilities such as Mechanical and Chemical Industry and the Central Anatolian Oil Refinery. The mentioned industrial facilities located in west-central part of Kırıkkkale are in studied area.

Collected material

Kidney, liver, and muscle samples of adult hares (n=15) and wild boars (n=9) were collected in cooperation with a local hunting organization during the hunting season of 2013/2014 (September-February). Muscle samples were collected from the upper hind legs. The age determinations were identified according to the pronouncedness of sutures in the cranial bones

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(between the frontal and sagittal bones) and the morphological structure of processus supraorbitalis (Suchentrunk et al., 2000) in hares and the external and cranial characteristics (İnci 2003) in wild boars. Ammunition parts in the tissues were noticed and samplings were done far from these spots. Upon collection, all tissue samples were placed into labeled plastic bags and stored at -20 ºC until the analysis of the pronouncedness of sutures in the cranial bones (between the frontal and sagittal bones) and the morphological structure of processus supraorbitalis (Suchentrunk et al., 2000)

Determination of heavy metals

For the analysis, the samples of approximately 1 g were taken from kidney, liver and muscle tissues and those samples were put into glass vials. The samples were kept in an incubator (Nüve) set to 70 °C for 16 hours. In order to make the dehydrated samples soluble, 3 mL of concentrated HNO_3 (65% v/v Merck) was applied to the liver and muscle samples and 2.5 mL HNO_3 was applied to the kidney samples; and the results were kept in a shaking water bath for a night. In order to carry out the oxidation process, 2 mL H_2O_2 (35% v/v Merck) was applied to each sample, and each sample was kept in the incubator set to 110 °C for 3-4 hours until each sample turned clear. At the end of this period, the volumes of cooled liver and muscle samples were diluted to 30 mL by using purified water the volumes of kidney samples were diluted to 25 mL and the samples were put into capped polyethylene tubes.

Concentrations of heavy metals chromium (Cr), mangan (Mn), copper (Cu), zinc (Zn), cadmium (Cd), mercury (Hg), lead (Pb) and a metaloid selenium (Se) in the samples were measured on a wet weight basis. "Agilent 7500 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)" device was used to determine heavy metal concentrations in the samples. The results taken from the ICP-MS were multiplied with the dilution volume and the results were divided based on the mass of the samples and presented as mg kg^{-1}.

The hygienic limits of heavy metals were determined according to the Turkish Food Codex and Piskorovà et al. (2003).

Statistical analyses

As the heavy metal concentrations were not normally distributed, the nonparametric Mann-Whitney U-test was used for statistical comparisons between sexes and tissues of the two species, and Kruskal-Wallis test for comparisons between different tissues of the same species. Correlations between the heavy metal concentrations of the tissue samples belonging to hare and wild boar were calculated by using Pearson's correlation coefficient. Significant differences were accepted at the probability level of $P < 0.05$. Statistical analyses were conducted by using PASW Statistics 18 (SPSS 2010).

Results and discussion

Statistical analysis showed no significant difference between the sexes, and so the data were combined from both sexes in the subsequent analyses.

Table 1 showed mean concentrations of Cr, Mn, Cu, Zn, Se, Cd, Pb, and Hg in the kidney, liver and muscle samples of hares and wild boars in Kırıkkale province. While significant differences were found in Mn, Cu, Zn, Se, and Cd concentrations among tissues of wild boars, significant differences were found in Cr, Mn, Cu, Zn, Se, Cd, and Pb concentrations among tissues of hares ($P < 0.05$). On the other hand, there were significant differences between these animals in terms of Cr, Mn, Cu, and

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Se concentrations in kidney, Cr and Se concentrations in liver, and Cr, Mn, Zn, Cd, and Pb concentrations in muscle ($P < 0.05$) (Table 1).

Table 1. Concentrations (mean ± SD) (mg/kg) and the results of comparison of eight heavy metals in kidney, liver, and muscle of hares (*Lepus europaeus*) and wild boars (*Sus scrofa*) in Kırıkkale, 2013-2014.

Heavy metals	Species	Kidney	Liver	Muscle	P_{KW}
Cr	*L. europaeus*	1.02±0.90	1.00±0.78	0.24±0.06	0.00*
	S. scrofa	1.82±1.79	1.30±0.77	1.35±0.59	0.90
	P_{MW}	0.03*	0.02*	0.00*	
Mn	*L. europaeus*	6.00±3.27	4.80±1.23	1.51±0.40	0.00*
	S. scrofa	3.20±0.76	3.89±0.68	1.00±0.18	0.00*
	P_{MW}	0.02*	0.44	0.00*	
Cu	*L. europaeus*	1.91±2.33	2.34±2.81	0.63±0.14	0.00*
	S. scrofa	1.25±0.41	0.71±0.19	0.45±0.21	0.00*
	P_{MW}	0.00*	0.08	0.08	
Zn	*L. europaeus*	32.65±6.58	40.51±9.13	20.35±6.93	0.00*
	S. scrofa	32.15±4.85	38.85±5.54	45.65±13.72	0.03*
	P_{MW}	0.24	0.34	0.00*	
Se	*L. europaeus*	1.15±0.39	0.19±0.13	0.03±0.02	0.00*
	S. scrofa	3.55±0.74	0.42±0.09	0.08±0.03	0.00*
	P_{MW}	0.00*	0.00*	0.29	
Cd	*L. europaeus*	4.49±4.74	0.83±0.46	1.19±0.45	0.00*
	S. scrofa	3.05±0.99	0.61±0.15	0.51±0.02	0.00*
	P_{MW}	0.77	0.11	0.00*	
Pb	*L. europaeus*	1.23±1.22	2.19±2.87	7.83±5.72	0.00*
	S. scrofa	0.52±0.18	0.75±0.51	0.44±0.17	0.45
	P_{MW}	0.43	0.22	0.00*	
Hg	*L. europaeus*	0.10±0.12	0.06±0.03	-	0.97
	S. scrofa	0.12±0.17	-	-	-
	P_{MW}	0.37	-	-	-

(P_{MW}: Significance level of Mann-Whitney U-test, P_{KW}: Significance level of Kruskal-Wallis test)

Correlation analysis showed significant correlations between metal concentrations accumulated in different tissues. The significant correlation was found between Cu concentrations in kidney and liver of wild boars (r = -0.84, P = 0.011). A strong statistically significant correlation was found between Se concentrations in liver and muscle of wild boars (r = -0.994, P = 0.045). The significant correlations were found between Cr and Hg (r = 0.975, P = 0.0001), Mn and Cd (r = -0.800, P = 0.022) and Cu and Pb concentrations (r = 0.925, P = 0.002) in kidney in all wild boar samples. There were significant correlations between Cr and Mn (r = 0.682, P = 0.030), Cr and Cu (r = 0.688, P = 0.028), Cr and Hg (r = 0.832, P = 0.003) and Mn and Hg concentrations (r = 0.750, P = 0.013) in kidney; Cr and Zn (r = 0.724, P = 0.012), Cu and Se (r = 0.715, P = 0.013), Mn and Hg (r = - 0.638, P = 0.035), and Cd and Pb concentrations (r = 0.914, P = 0.0001) in liver; Cr and Mn (r = 0.778, P = 0.023) and Cd and Pb concentrations (r = 0.996, P = 0.0001) in muscle in all hare samples.

Hg, Cr, Pb, and Cd concentrations found in the tissues were compared to the hygienic limits viz; Hg- 0.1 mg/kg in kidney and liver, 0.03 mg/kg in muscle; Cr- 0.5 mg/kg in all tissues; Pb- 1 mg/kg in kidney and liver, 0.4 mg/kg, in muscle; Cd- 1 mg/kg in kidney, 0.5 mg/kg in liver, 0.1

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mg/kg, in muscle. Our results revealed that Hg (1/9 boar kidney; 4/15 hare kidney), Cr (9/9 boar all three tissues; 9/15 hare kidney and 11/15 hare liver), Pb (4/9 boar muscle; 5/15 hare liver, 15/15 hare muscle) concentrations detected in wild boar and hare tissues in the present study were higher than the hygienic limits. The values of Cd concentrations exceed the hygienic limits in all tissues from both animals.

Discussion

The major path of heavy metal exposure in animals is oral consumption. Both age of the animal and the tissue type are important biological factors that affect the level of metal concentration Massanyi et al. (2003). Heavy metals target heart, kidney, liver, immune and nervous systems; however, kidney and liver are the most effected organs in mammals (Kolesarova et al., 2008). There were significant variation patterns of heavy metal concentrations among organs (kidney, liver and muscle) of both animals (hares vs. wild boars) in the present study. Mean concentrations of Cr (1.02 mg/kg), Mn (6.00 mg/kg), Se (1.15 mg/kg) and Cd (4.49 mg/kg) were the highest in hare kidney as compared to other organs, whereas concentrations of Cu (2.34 mg/kg) and Zn (40.51 mg/kg) were the highest in hare liver and Pb (7.83 mg/kg) was the highest in hare muscle. It is worth noting that many authors stated that Cd was considerably accumulated in kidney and liver compared to other body organs (Toman and Massányi, 1996; Toman and Massányi, 2002; Toman et al., 2005; Shahid et al. 2013). In the present study, higher Cd concentration was recorded in kidney than in liver and muscles of *Lepus europaeus,* but slightly higher concentration of Cd was recorded in muscle (1.19 mg/kg) than in liver (0.83 mg/kg) of *L. europaeus*. This might probably be affected by random individual variations, age structure of the collected animals or a small sample size. On the other hand, mean concentrations of Cr (1.82 mg/kg), Cu (1.25 mg/kg), Se (3.55 mg/kg) and Cd (3.05 mg/kg) were the highest in boar kidney as compared to liver and muscle tissues, whereas concentrations of Mn (3.89 mg/kg) and Pb (0.75 mg/kg) were the highest in boar liver, and Zn (45.65 mg/kg) was the highest in boar muscle. Moreover, strong positive significant correlations were found between Cr and Hg, and between Cu and Pb concentrations in kidney in all wild boar samples. There were also strong positive significant correlations between Cd and Pb concentrations in liver, and Cd and Pb concentrations in muscle in all hare samples.

Researchers have conducted many studies about the potential negative effects and accumulation of heavy metals in hares (*Lepus europaeus*) and wild boars (*Sus scrofa*) in various countries (Kramàrovà et al., 2005; Wajdzik, 2006; Bilandžić et al., 2010; Filistowicz et al., 2012; Danieli et al., 2012; Amici et al. 2012; Petrović et al., 2013). It is recorded that meat and offal of game species, such as hare and wild boar, are not suitable for human consumption because of high Cd and Pb concentrations in their bodies (Bilandzić et al., 2010; Shahid et al., 2013). Our results revealed that $Pb_{liv.}$, $Zn_{kidn.}$, and $Zn_{liv.}$ concentrations in hares, and $Cd_{kidn.}$, $Pb_{kidn.}$, $Pb_{liv.}$, $Cr_{kidn.}$, and $Cr_{liv.}$ concentrations in wild boars were higher than those reported in previous studies conducted on two animals in other European countries (Table 2, 3).

Pilarczyk et al. (2010) reported that the adult wild boars (age > 2 years) from north western Poland had Se deficiency. Se concentrations detected in liver and kidney tissues of wild boars in the present study were higher than those determined by Pilarczyk et al. (2010). However, it is difficult to draw conclusions from these findings because there are restricted reference values of Se concentration in liver and kidney tissues of wild boars in other European countries.

Yarsan et al. (2014) stated that lead (Pb) concentrations detected in hair tissues of wild boars in southern Turkey were higher than those detected in liver, kidney, and muscle tissues of wild boars in other European countries. This finding is consistent with the results obtained from both hare and wild boar samples in the present study. Also, the value of high lead concentration in hare musle was remarkable in the present study. It may sometimes depend on ammunition parts in tissue

Table 2. Concentrations (mg kg^{-1} w.w.) of Cd, Pb, Cu, and Zn in brown hares (*Lepus europaeus*) from various European countries

Country/Year	Tissue	Cd	Pb	Cu	Zn	References
Poland/ 1993-2001	kidney	26.10	1.50	-	-	Wajdzik (2006)
	liver	2.27	1.67	-	-	
Serbia/ 2010-2011	kidney	2.80	-	-	22.2	Petrović et al. (2013)
	liver	0.26	-	-	24.9	
Slovakya/ 2005	kidney	1.57	-	-	-	Kramàrovà et al. (2005)
	liver	0.16	-	-	-	
Finland/ 1992-1993	kidney	1.91	<0.50	4.49	28.8	Venäläinen (2007)
	liver	0.62	<0.50	4.64	28.9	
Turkey/ 2013-2014	kidney	4.49	1.23	1.91	32.65	This study
	liver	0.83	2.19	2.34	40.51	

Table 3. Concentrations (mg kg^{-1} w.w.) of Cd, Pb, Cr, and Hg in wild boar (*Sus scrofa*) from various European countries

Country/Year	Tissue	Cd	Pb	Cr	Hg	References
Slovakia/ 1998-1999	kidney	0.56	0.39	0.19	0.52	Piskorova et al. (2003)
	liver	0.28	0.24	0.15	0.24	
Croatia/ 2008-2009	kidney	2.84	0.18	-	0.07	Bilandžić et al. (2010)
	liver	-	-	-	-	
Italy/ 2005-2006	kidney	-	-	-	-	Danieli et al. (2012)
	liver	0.08	0.33	0.15	-	
Italy/ 2004-2010	kidney	1.05	0.29	-	-	Amici et al. (2012)
	liver	0.08	0.31	-	-	
Turkey/ 2013-2014	kidney	3.05	0.52	1.82	0.12	This study
	liver	0.61	0.75	1.30	-	

However, samplings in this study were done far from these spots. Hence, the cause of the excess lead in hare muscle should be reevaluated in more comprehensive physiological and ecotoxicological studies. Heavy metals such as Cd, Hg, and Pb are highly toxic and may induce impaired reproduction and teratogenicity in animals and human (Telisman et al., 2010; Gunnarsson et al., 2004; Kolesarova et al., 2008). A high intake of Cr depending on oxidation state can be toxic and carcinogenic (Tarley et al., 2001; Bielicka et al., 2005). Hg, Cr, and Pb concentrations detected in hare and wild boar tissues in the present study were higher than the hygienic limits for some individuals, whereas the values of Cd concentrations exceeded the hygienic limits in all tissues of both animals. Namely, our findings showed that meat and offal of hares and wild boars contain some metals above the hygienic limits. This might be associated with the fact that Kırıkkale province has various industrial facilities and it is an important crossroads in terms of transportation. Moreover, herbicides, pesticides and fertilizers containing potentially toxic trace elements are used in agricultural production in the studied area.

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Conclusion

In conclusion, the presented results suggest that high concentrations of some heavy metals might be an environmental problem because most species of wild animals are components of the food chain of humans. Therefore, determining the concentration of such heavy metals should be undertaken as primary study topics. In addition to these studies, combined scientific studies on the level of anthropogenic pollution and chemical composition of soil, water and air in studied areas will help scientists to get more information about the current status of environmental pollution.

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