Bulgarian Journal of Agricultural Science, 24 (Supplement 1) 2018, 119-124

HEAVY METALS CONCENTRATIONS IN ORGANS OF RED FOXES (*VULPES VULPES* LINNAEUS, 1758) AND GOLDEN JACKALS (*CANIS AUREUS* LINNAEUS, 1758) INHABITING THE "SARNENA SREDNA GORA" MOUNTAIN IN BULGARIA

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Abstract

GEORGIEV, D., E. RAICHEV, L. DOSPATLIEV, M. IVANOVA, S. PEEVA, S. KALCHEVA and K. GEORGIEVA, 2018. Heavy metals concentrations in organs of red foxes (*Vulpes vulpes* Linnaeus, 1758) and golden jackals (*Canis aureus* Linnaeus, 1758) inhabiting the "Sarnena Sredna gora" mountain in Bulgaria. *Bulg. J. Agric. Sci.*, 24 (Suppl. 1): 119–124

The purpose of the study is to determine the concentrations of the heavy metals lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), copper (Cu) and zinc (Zn) in the muscles, kidneys and liver samples taken from red foxes (n = 9) and jackals (n = 17) inhabiting the "Sarnena Sredna gora" mountain in Bulgaria and to compare the respective results. It was established that concentrations of heavy metals (mg/kg dry weight) in the muscles, kidneys and liver samples are as follows: in the red foxes' muscles: Pb – 0.170, Cd – 0.089, Ni – 2.312, Co – 0.074, Cu – 2.565, Zn – 41.382; in the red foxes' kidneys: Pb – 0.761, Cd – 15.522, Ni – 2.834, Co – 2.033, Cu – 8.470, Zn – 30.157; in the red foxes' livers: Pb – 0.953, Cd – 0.628, Ni – 2.480, Co – 1.664, Cu – 15.121, Zn – 30.106; in the jackal's muscles: Pb – 1.126, Cd – 1.742, Ni – 7.416, Co – 1.798, Cu – 6.466, Zn – 66.804; in the jackal's kidneys: Pb – 8.419, Cd – 11.185, Ni – 7.710, Co – 5.039, Cu – 33.861, Zn – 61.576; in the jackal's livers: Pb – 8.879, Cd – 11.569, Ni – 7.342, Co – 4.963, Cu – 56.319, Zn – 63.618. The red fox (*Vulpes vulpes* Linnaeus, 1758) and the jackal (*Canis aureus* Linnaeus, 1758), could be used as an effective biomarker for the assessment of the environmental pollution in their natural habitats.

Key words: golden jackal; red fox; heavy metals; muscles; kidneys; liver

Introduction

The development of the world economy has caused environmental pollution with multiple stable compounds. The exposure to some of them, like heavy metals (Cd, Hg, Pb, Cu, Zn, Cr, Ni, Co), continues nowadays worldwide. Since heavy metals are non-biodegradable, they could easily be accumulated up to some toxic levels (Adie and Osibanjo, 2009). The metals' concentration in the biota provides information about their transportation, accumulation in the

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environment and the potential toxicological effects (Torres and Johnson, 2001). Animals and especially mammals are useful indicators for the ecological monitoring of the contaminated ecosystems (Tataruch and Kierdorf, 2004). Most of the wild, carnivore mammals being on the top of the food chain, could be very sensitive to any kind of bioaccumulation processes (Markov and Lanszki, 2012; Ćirović et al., 2014; Ćirović et al., 2015). The red fox and the jackal are one of those predators that could be used in their natural habitats as an effective bioindicator in the assessment of the environmental pollution (Ansorge and Graeser, 1993; Corsolini et al., 1999; Dip et al., 2001; Kalisin'ska and Palczewska-Komsa, 2011; Šálek et al., 2014).

The limited information on the bioaccumulation processes and the real concentration of heavy metals in the red foxes and jackals in Bulgaria has determined the purpose of the present study: to determine the concentration of some heavy metals (Pb, Cd, Co, Ni, Cu, Zn) in the muscles, kidneys and liver samples of red foxes (n = 9) and jackals (n = 17) inhabiting the „Sarnena Sredna gora" mountain and to compare the respective results.

Material and methods

Samples

In order to determine the concentrations of heavy metals: lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), copper (Cu) and zinc (Zn) in the muscles, kidneys and livers of red foxes and jackals, we collected samples of red foxes (n = 9) and jackals (n = 17) from the "Sarnena Sredna gora" mountain within the regular hunting season 2011-2012 (Fig. 1).

The territory where the survey took place is situated between the Dalboshka River to the East and the villages Kazanka, Pastrovo and Sladuk Kladenec to the West. The location of the village "Starozagorski mineralni bani" could be pointed as a center of the respective area (42^{o}27' N and 25^{o}33' E). It's a hilly region with 350-800 m of altitude. The climate is transitional between moderate-continental and continental-Mediterranean. Winter is mild with an unstable snow blanket. The woodlands are followed by farmlands, abandoned vineyards and orchards. Foxes and jackals in the region live together with a large number of wandering dogs and cats, marten and wild cats. There are also wolves in the region.

The tissue samples from muscles, kidneys and livers have been taken on field and transported in freezer bags to the Central Research Laboratory of the Trakia University.

Reagents

The reagents are qualified "p.a". Stock standard solution (Merk, Germany) with a concentration of 1000 mg/l for the determination of Pb, Cd, Co, Ni, Cu and Zn was applied. Some double-distilled water has been used in all the procedures.

Mineralization of the samples

The mineralization of the samples has been carried out according to the EPA Method 3052 (Method EPA 3052, 1996). 1 g sample to the nearest 0.001 g in PTFE vessels was weighted. Some HNO_3, HF, HCl and H_2O_2, were added using a microwave system Multiwave 3000. The maximum power was 1400 W, and the maximum pressure in Teflon vessels - 40 bar.

Determination of the concentration of heavy metals in the samples

In order to determine the concentration of heavy metals in the samples, an atomic absorption spectrometry has been used – "Analyst 800 with graphite furnace HGA"; "Perkin Elmer", at wavelengths: Pb – 217.0 nm, Cd – 228.8 nm, Co – 240.7 nm, Ni – 232.0 nm, Cu – 324.8 nm and Zn – 213.9 nm.

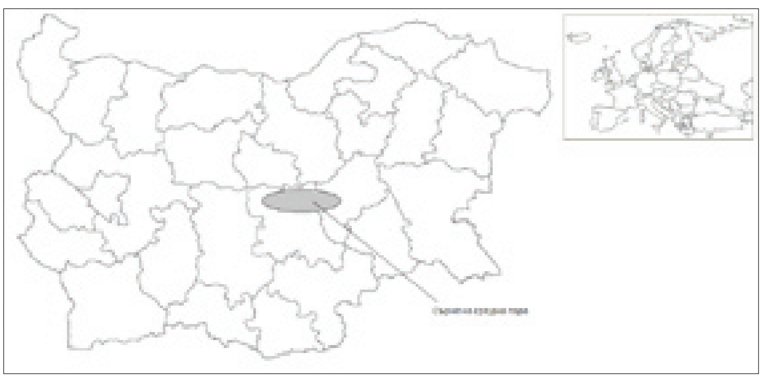

Fig. 1. Location of the "Sarnena Sredna gora" mountain on the map of Bulgaria

Statistical analyses

The SPSS program (Statistical Package for Social Science) has been used in the processing of the statistical data. The statistical analysis has involved the calculation of the average value, the standard deviation from the average value and ± 95% a safety margin of means influencing the concentrations of the studied metals.

Results

The results from the descriptive analysis of the concentration of Pb, Cd, Co, Ni, Cu and Zn in the muscles, kidneys and liver samples taken from red foxes and golden jackals are presented in Tables 1 and 2.

The highest concentration of Pb has been found in the liver samples of *red foxes* (0.953 mg/kg), while much lower

Table 1
Descriptive analysis of the results for Pb, Cd, Co, Ni, Cu and Zn concentrations in the miscles, kidney and liver samples taken from red foxes (*V. vulpes*)

Element	Miscles				Kidneys				Liver			
	$\bar{x}$	SD	-95 % Confid.	+95 % Confid.	$\bar{x}$	SD	-95 % Confid.	+95 % Confid.	$\bar{x}$	SD	-95 % Confid.	+95 % Confid.
Pb	0,170	0,021	0,154	0,186	0,761	0,117	0,671	0,850	0,953	0,141	0,845	1,062
Cd	0,089	0,031	0,065	0,112	15,522	5,906	10,982	20,061	0,628	0,240	0,444	0,812
Ni	2,312	4,413	-1,080	5,703	2,834	0,098	2,759	2,909	2,480	0,090	2,410	2,549
Co	0,074	0,013	0,064	0,084	2,033	0,166	1,905	2,160	1,664	0,146	1,552	1,776
Cu	2,656	0,282	2,348	2,782	8,470	0,443	8,130	8,811	15,121	1,184	14,211	16,031
Zn	41,382	3,166	38,948	43,816	30,157	2,061	28,573	31,741	30,106	1,903	28,644	31,568

± 95% confidence limits for means (in mg/kg of dry weight analysed tissue) in muscles, kidneys and liver tissue samples of the red fox (*V. Vulpes*) from Stara Zagora region in the central part of Bulgaria (n = 9)

Table 2.
Descriptive analysis of the results for Pb, Cd, Co, Ni, Cu and Zn concentrations in the miscles, kidney and liver samples taken from golden jackals (*C. aureus*)

Element	Miscles				Kidneys				Liver			
	$\bar{x}$	SD	-95% Confid.	+95% Confid.	$\bar{x}$	SD	-95% Confid.	+95% Confid.	$\bar{x}$	SD	-95% Confid.	+95% Confid.
Pb	1,126	0,590	0,823	1,429	8,419	3,609	6,564	10,274	8,879	3,646	7,004	10,753
Cd	1,742	0,482	1,495	1,990	11,185	4,460	8,893	13,478	11,569	4,399	9,308	13,831
Ni	7,416	1,652	6,567	8,266	7,710	0,840	7,278	8,142	7,342	0,981	6,838	7,847
Co	1,798	0,381	1,602	1,994	5,039	0,659	4,700	5,738	4,963	0,644	4,632	5,295
Cu	6,466	1,811	5,534	7,397	33,861	7,703	29,901	37,822	56,319	14,399	48,916	63,723
Zn	66,804	18,064	57,517	76,092	61,576	17,331	52,665	70,487	63,618	18,315	54,201	73,034

±95% confidence limits for means (in mg/kg of dry weight analysed tissue) in muscles, kidneys and liver tissue samples of the of the golden jackal from Stara Zagora region in the central part of Bulgaria (n = 17) results have been obtained from the muscles samples (0.170 mg/kg). The Pb content in the kidneys is 0.761 mg/kg. With regard to the *golden jackal*, the highest concentration of Pb has been discovered in the liver samples (8.879 mg/kg), while the quantities measured in the muscles have been much lower (1.126 mg/kg). The Pb concentration in the kidneys is 8.419 mg/kg. In comparison with the Pb content, the highest concentration of Cd in the *red foxes'* samples has been found in the kidneys (15.522 mg/kg), and the lowest one in the muscles (0.089 mg/kg). The Cd concentration in the liver samples has been 0.628 mg/kg. In the samples taken from the *golden jackal*, the highest Cd concentration has been measured in the kidneys (11.185 mg/kg), while the lowest one in the muscles (1.742 mg/kg). The Cd concentration in the liver is 11.569 mg/kg. Despite the differences in the Cd concentration in the different tissues, the Cd has no significant effect on their condition.

The highest Ni concentration in the red foxes' samples has been found in the kidneys (2.834 mg/kg) and the lowest one in the muscles (2.312 mg/kg). The Ni concentration in the liver has been 2.480 mg/kg.

In the *golden jackal's* samples the highest Ni concentration has been observed in the kidneys (7.710 mg/kg) and the lowest in the liver (7.342 mg/kg). The Ni concentration in the muscles samples has been 7.416 mg/kg.

The highest Co concentration in the *red foxes'* samples has been measured in the kidneys (2.033 mg/kg), while the lowest one has been measured in the muscles (0.074 mg/kg). The Co concentration in the liver samples has been 1.664 mg/kg.

The Co highest concentration in the *golden jackal's* samples has been discovered also in the kidneys (5.039 mg/kg) and the lowest one in the muscles (1.798 mg/kg). The Co concentration in the liver has been 4.963 mg/kg.

The highest Cu concentration has been observed in the samples taken from *red foxes'* livers (15.121 mg/kg) while the concentration found in the muscles has been much lower (2.565 mg/kg). The Cu concentration in the kidneys is 8.470 mg/kg.

The highest Cu concentration in the *golden jackal* samples has been found in the liver (15.121 mg/kg) and the lowest one in the muscles (6.466 mg/kg). The Cu concentration in the kidneys is 33.861 mg/kg.

The highest Zn concentration has been observed in the *red foxes'* muscles (41.382 mg/kg) and the lowest one in the liver samples (30.106 mg/kg). The Zn concentration in the kidneys is 30.157 mg/kg.

The highest Zn concentration in the *golden jackal's* samples has been measured in the muscles (66.804 mg/kg) and the lowest one in the kidneys (61.576 mg/kg). The Zn concentration in the liver is (63.618 mg/kg).

Discussion

The group of heavy metals observed in the present study includes both highly toxic non-essential metals such as Cd and Pb and essential metals which are characterised by their dependent toxicity. The essential trace elements like Cu, Zn, Ni and Co are substantial for growth and they have to be obtained by food. Their absorption depends on the body nutritional demand, the physiological shape and the presence of food which is a rich source of all those elements (Czajkowska et al., 2011). As we know, significant differences could be found in the metals' concentration in the tissues of individuals of one and the same species (Dip et al., 2001, Heltai and Markov, 2012), as well as in separate species inhabiting one and the same region (Friel et al., 1987).

The established average values and confidence intervals (Table 1, 2) expand the concept of the physiological and ecotoxicological characteristics of the red fox and the jackal and provide a solid basis for interpretation of the study's results.

The concentration of existential elements with an expected toxic effect like Cu, Ni, Zn, Co and the microelements with a proven high toxic effect on the living organisms (Cd, Pb) found in studied in *V. vulpes* and *C. aureus* shows the following trends:

1. The distribution of heavy metals in the foxes' organs is as follows:

- In muscles: Zn > Cu > Ni > Pb > Cd > Co;
- Kidneys: Zn > Cd > Cu > Ni > Co > Pb;
- Liver: Zn > Cu > Ni > Co > Pb> Cd.

2. The distribution of heavy metals in the jackal's organs is as follows:

- In muscles: Zn > Ni > Cu > Co > Cd > Pb
- Kidneys: Zn > Cu > Cd > Pb > Ni > Co
- Liver: Zn > Cu > Cd > Pb > Ni > Co

3. The existential elements Zn and Cu have had the highest levels of accumulation in the three studied organs besides the Cd concentration in the samples taken from foxes' kidneys and the Ni concentration in the samples taken from the jackal`s muscles.

4. The quantities of the other two essential elements - Cu and Ni have been lower.

5. The biggest absolute variation of the average values has been registered in the Zn concentration, followed by the Cu concentration.

6. It has been found that the Pb concentration in the muscles, kidneys and liver samples of the jackal significantly exceeds the Pb concentration in samples taken from red foxes. At the same time the Cd concentration in the kidneys samples of red foxes is higher than the one in the jackal samples.

As we can see from the study's results and from the data reported by some other authors (Dip et al., 2001, Arnold et al., 2012), the concentration of metals in red foxes and jackals shows a serious intraspecific variability. The accumulation of metals in the fox and the jackal could represent their transfer from the soil to the mammals' organisms (Goyer, 1996).

The explanation for the wide intraspecific variability of metals in red foxes and jackals in the European ecosystems, where these animals are on the top of the food chain, could be found both in the different level of anthropogenic pollution and the local variability of the natural geochemical background (Dehn et al., 2006; Driscoll et al. 2007; Brožová et al., 2015). At the same time, the option of dealing with cross-border pollution needs also to be taken into consideration. Not only the atmospheric pollution, but also the chemical pollution of waters and soils as well as the balance of micronutrients are very important. A contamination with anthropogenic pollutants such as nitrates, pesticides, heavy metals, arsenic, petroleum products and trihalomethanes has been detected in the drinking water.

During our research we have found that the studied animals use a diverse range of food resources: rodents like brown rat (*Rattus norvegicus* Berk), wood mouse (*Silvimus sylvaticus* L.), yellow-necked mouse (*Silvimus flavicollis* Melch.), common vole (*Microtus arvalis* Pall.), house mouse (*Mus musculus* L.); wild birds like blackbird (*Turdus merula* L.), jay (*Glarrulus glandarius* L.), tit (Paridae sp.), partridge (*Perdix perdix* L.); some agricultural and domestic mammals like domestic pig (*Sus scrofa* domestica L.), sheep (*Ovis aries* L.), rabbit (*Oryctolagus cuniculus* L.), cat (*Felis catus* L.), dog (*Canis familiaris* L.), poultry (*Gallus domesticus* L.) and also some wild mammals.

The described food spectrum shows the multilevel and complicated relations that foxes and jackals have in the Biocoenoses. After a year-round study of the feeding habits of the fox and the jackal in Bulgaria, Atanasov (1958) has found around 350 animals usually consumed as food. All of them could be treated as a separate source of heavy metals for the red fox and the jackal. Before reaching the predators at the studied area, all these animals are also exposed to various toxic compounds, for example, air pollution caused by the motor vehicles, industrial wastes and fertilizers used in the agriculture.

Some wild animals like the carnivores and the omnivores usually have habitats near the human settlements. Such strategy provides them with some additional food sources, but also exposes them to the anthropogenic pollutants such as pesticides, effluents and fluel gas emissions (Bilandžić et al., 2010).

The presence of highly toxic metals like lead and cadmium in muscles, kidneys and livers of wild red foxes and jackals in the region of "Sarnena Sredna gora" proves that a regular observation is needed for evaluation and forecasting of the accumulation of toxic metals in the free-living animals. Therefore the regular monitoring of the concentration of heavy metals in foxes and jackals' tissues will be extremely important in the near future in order to assess the pollution level resulting not only from the usage of chemicals in the agriculture but also from the transmission of pollutants at a long distance – the cross-border pollution. The red fox (*Vulpes vulpes* Linnaeus, 1758) and the jackal (*Canis aureus* Linnaeus, 1758), could be used as an effective biomarker for the assessment of the environmental pollution in their natural habitats.

Acknowledgments

The present work has been financially supported by the Norwegian Collaboration Program, INNOVATION NORWAY, Subject: "Assessment, reduction and prevention of air, water and soil pollution in Stara Zagora Region" ref. no. 2011/115236, Trakia University, AF. The authors express their gratitude to the members of the Hunters and fishermen association - Stara Zagora and to the employees of the State Forestry Stara Zagora.

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