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Concentration of Selected Trace Elements in the Golden Jackal (*Canis aureus* L., 1758) Population from Serbia

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Abstract: Golden jackal is considered a top predator in many human dominated landscapes of South-eastern Europe. Concentrations of seven trace elements (Pb, Cd, Zn, Cu, Fe, Mn, and Ni) in livers of 129 specimens of golden jackals (*Canis aureus*) from six localities from Serbia were analysed. Both, sex and localities had no significant effects on concentrations of any metals. Lead concentrations, both average (9.59 mg/kg) and maximal values (23.00 mg/kg), were higher than those found in other mammal predator species. Furthermore, concentrations of essential trace elements (copper, iron and manganese) were also significantly higher than those reported by other researchers. Only zinc had significantly lower concentration (66.36 mg/kg), while cadmium was similar (14.89 mg/kg) with those reported in the literature. The jackal, being a wild omnivorous mammal at the top of the food chain with high adaptability and other supportive ecological features, is a good bioindicator of environmental contamination.

Key words: *Canis aureus,* golden jackal, trace elements, Serbia

Introduction

Industrial development leads to emissions of various pollutants, such as heavy metals (Fritsch *et al.* 2010). Heavy metals occur naturally but increased metal contents in the environmental can be attributed to atmospheric deposition from anthropogenic sources as well as mobilisation from soils, bottom sediments of lakes, and rivers. They persist in the environment and animal tissues over prolonged time. Some metals, like lead and cadmium can cause toxic effects even in small doses (Scheuhammer 1987, Burger 2008). The main sources of lead contamination are industrial plants, coal combustion, automotive industry and wastewater treatment plants. Long-term exposure to lead contributes to non-toxic phosphates accumulation of lead in bones, but in periods of increased demand of minerals lead can be released from bones leading to secondary poisoning. Cadmium concentrations in the environment increased due to industrial contamination, resulting from waste incineration and fertilizer production, often at a local scale. Others metals (e.g. copper, zinc, iron) are essential for various life forms at low levels, but in higher concentrations can disturb metabolism and growth of organisms (Bilandžić *et al*. 2012). Regular exposure to toxic metals results in chronic poisoning and poses serious threats, for instance nephrotoxic and hepatotoxic effects, which can produce remote effects manifested in successive generations (Piskorova *et al.* 2003) and influence entire populations (Lanszki *et al.* 2009).

High level of pollution in altered environments demand careful biomonitoring of soil, air, water, living organisms and food products (Kramarova *et al*. 2005). Animals respond differently to environmental

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contamination, and the presence of toxic substances in the environment and their accumulation in animal tissues need to be studied for a better understanding of the effects of pollutants on every element of the environment.

Golden jackal (*Canis aureus* L., 1758) is the largest and the only jackal outside Africa. It is the most widely distributed canid species, occurring in Northern and Eastern Africa, Asia Minor, Middle East, Central and Southern Asia, and South-eastern Europe (Jhala & Moehlman 2004). Golden jackal is believed to be a highly adaptable species due to its omnivorous diet consisted mainly of small mammals (Lanszki *et al.* 2010, Markov & Lanszki 2012), livestock remains and carcasses (Lanszki *et al*. 2010, Ćirović *et al*. 2014), and human refuse (Jaeger *et al*. 2007). Due to theirs opportunistic foraging behaviour, jackals are capable to survive in different environments, with high local densities (Jhala & Moehlman 2004, Šálek *et al*. 2014).

The aim of this study was to (i) determine the concentrations of lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) and nickel (Ni) in livers of golden jackals from various areas of Serbia; (ii); estimate the degree of environmental pollution in the examined area; and (iii) determine that golden jackal tissues can serve as bioindicator of metal pollution.

Material and Methods

Study area

This study was conducted on liver tissue samples obtained from six distinct areas in Serbia (Fig. 1). Localities where jackal carcasses were collected were along the rivers: Veliko Gradište – the Danube River, Negotin – the confluence of the Timok River with the Danube River, Svilajnac – the Resava River, Velika Plana – the Morava River, Smederevo – the confluence of the Morava River with the Danube River, Surčin – the Sava River. Surčin and Smederevo were located in the alluvial plains only, while the other localities (Veliko Gradište, Velika Plana, Svilajnac, and Negotin) extended to the surrounding foothills.

The size of the area where jackals' carcasses were collected varied from 50 km^2 (Smederevo) to 197 km^2 (Veliko Gradište). Predominantly the land was used for agriculture in all six areas, with crops (grain, maize, sunflower, soy bean) covering from 50% (Negotin) to 82% (Surčin) of the area. Meadows/pastures (1-13%) and orchards/vineyards (2-15%) were also present.

Forests were represented by small fragments within the agricultural matrix and covered from 6% (Surčin) to 23% (Negotin) of the study areas. Alluvial willow (*Salix sp.*) and white poplar (*Populus alba*) forests were present along the rivers. Away from the rivers, mixed forests consisted predominantly of oak (*Quercus sp.*), narrow leaved ash (*Fraxinus angustifolia*), and common hornbeam (*Carpinus betulus*). Agriculture is the dominant economic activity at all chosen localities with small amount of pesticides used during production. There are no large industrial facilities, except in Smederevo (steelworks) and a chemical factory in Negotin which was closed 20 years ago.

In Serbia the main areas of the golden jackal's distribution are along the north-eastern border with Bulgaria, and the Plain of Srem. In the northeast of the country large populations are present in the vicinity of Negotin and Bela Palanka (Šálek *et al*. 2014). From the surroundings of Negotin jackals have spread into Velika Morava River valley. Population from the Plain of Srem expanded along the banks of river Sava up to the slopes of Fruška Gora mountain. (Milenković & Paunović 2003).

Collected material

Bodies of 129 specimens of golden jackals were collected in cooperation with local hunting organizations. Most of these animals were legally hunted

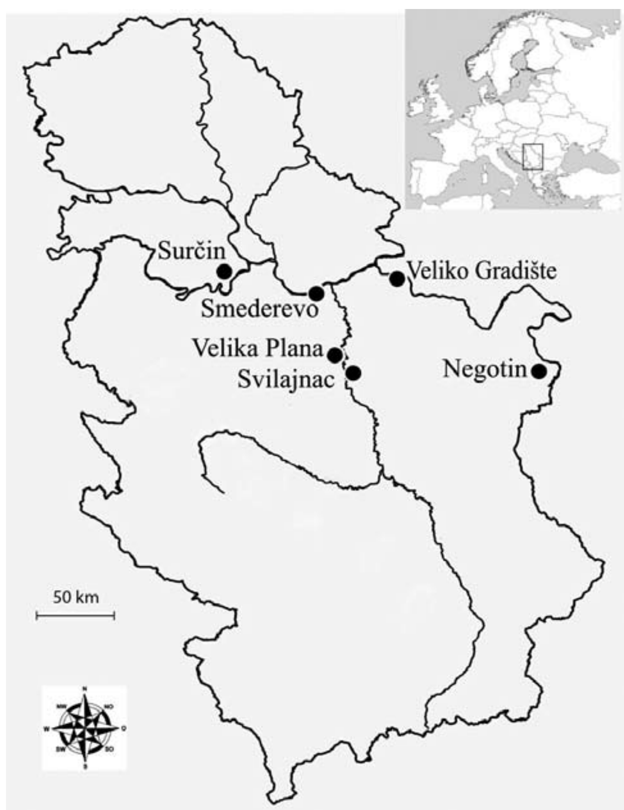

Fig. 1. Location of the six sampling sites for analysis of trace elements concentration in the liver of golden jackals in Serbia

(n=124), while the rest were collected as road kills (n=5). Samples were collected during the winter of 2009/2010 (December-January). Since jackals give birth during spring, in this part of the year there are no more pups in the population. After all the morphological measurements were taken skulls were cleaned and the shape and wear of maxillary incisors were examined (following Lombaard 1971), we determined that all the jackals were adults specimens. Livers were removed in the field, and frozen at -20°C for later analysis.

Determination of trace elements

Trace element contents were determined by atomic absorption spectrometry (Thermo Scientific Solaar S Series AA) (REF). All liver samples were dried using a Freeze Dryers Rotational-Vacuum-Concentrator (GAMMA 1–16 LSC, Germany). From each jackal a sample of 50 mg of dry liver was taken. Furthermore, samples of dry livers from each locality were pooled by sex. Samples were ground using porcelain mortar and a pestle and digested in 10 ml of a 2:1 mixture of concentrated HNO_3 and $HClO_4$ (Merck, Germany). Samples were heated until perchloric acid white fumes were no longer visible and the solution became clear. Samples were filtered after digestion and 50 ml of deionised water was added. The concentrations of the analysed metals were expressed in mg/kg dry weight.

Statistical analyses

As the heavy metals concentrations were not normally distributed, the nonparametric Mann-Whitney U-test was used for statistical comparisons between genders and Kruskal-Wallis test for comparisons between localities. Significant differences were accepted at level of probability $p < 0.05$. Statistical analyses were conducted using Statistica 5.1 software (Statsoft, Tulsa, OK, USA).

Results

Average concentrations of Pb and Cd in golden jackal liver samples at the six localities were 9.59 mg/kg and 14.89 mg/kg, respectively (Table 1). The highest Pb concentration was recorded from female jackals from Negotin (23.00 mg/kg). This group was also characterised by the highest concentration of Cd (37.67 mg/kg). No traces of Pb were found at Surčin, and the lowest Pb concentrations were detected from male jackals from Negotin (3.46 mg/kg). Cadmium concentrations were the lowest at Veliko Gradište, both from males and females (1.75 mg/kg and 1.65 mg/kg, respectively, Table 1).

Average concentration of Zn was 66.36 mg/kg. Female jackals from Negotin had the highest Zn concentrations (107.00 mg/kg), while the lowest was recorded in female jackals from Svilajnac (49.56 mg/kg, Table 1).

The average concentration of Cu was 57.85 mg/kg. The highest Cu level of 104.33 mg/kg was found from female group inhabiting the area of Negotin, while the lowest was reached in male jackals harvested in Svilajnac (39.63 mg/kg). The highest average concentration in general was determined for Fe (1017.27 mg/kg) with highest levels found in females inhabiting the area of Negotin (1546.67 mg/kg; Table 1).

The Mn concentrations were highest in female jackals from Negotin area (28.67 mg/kg). Average levels of Mn and Ni were 16.93 mg/kg and 13.43

Table 1. Mean concentrations of seven trace elements (mg/kg) in the liver of golden jackals at six study sites in Serbia, 2009-2010 (N - sample size; F – female, M -male)

Study site	Sex	N	Pb	Cd	Zn	Cu	Fe	Mn	Ni
Surčin	F	5	-	15.71	54.88	70.38	1361.09	17.75	14.69
	M	15	5.93	6.53	59.07	46.33	882.13	14.60	7.27
Negotin	F	3	23.00	37.67	107.00	104.33	1546.67	28.67	40.67
	M	13	3.46	9.23	52.23	48.92	560.08	15.39	7.08
Velika Plana	F	5	19.00	30.00	82.8	91.2	1612.6	25.40	35.80
	M	12	6.08	12.5	50.17	41.58	1294.5	16.17	10.58
Svilainac	F	9	5.33	18.22	49.56	47.78	760.00	17.33	11.33
	M	8	7.75	21.25	51.12	39.63	1042.5	18.13	14.13
Smederevo	F	15	4.40	12.07	53.07	54.27	886.6	15.33	8.73
	M	15	5.87	12.13	59.67	44.07	843.6	18.27	10.93
Veliko Gradište	F	17	15.06	1.65	62.47	41.35	437.18	4.77	-
	M	12	19.25	1.75	114.33	64.33	980.33	11.33	-
Average		**129**	9.59	14.89	66.36	57.85	1017.27	16.93	13.43

mg/kg, respectively. The lowest Mn level of 4.77 mg/kg was registered for the female group harvested in Veliko Gradište. Female jackals from Negotin had the highest Ni concentrations (40.67 mg/kg) and the lowest was found from males from Negotin (7.08 mg/kg). Traces of Ni were not found in Veliko Gradište.

Statistical analysis showed no significant difference between the sexes (Pb: U=18, p=0.963; Cd: U=11, p=0.262; Zn: U=15, p=0.631; Cu: U=8, p=0.109; Fe: U=16, p=0.749; Mn: U=14, p=0.522; Ni: U=6, p=0.065). Significant statistical differences in concentrations of analysed metals were not present among localities either.

Discussion

Both mammalian and avian wild predators' high position in food chain and wide-ranging behaviour make them prone to accumulate pollutants from wider areas. Common wild birds are also a potential source of contamination, in particular with Pb (Gasparik *et al.* 2010). Wild animals, mostly carnivores and omnivores, adapt to habitats near human settlements. This strategy provides them with additional food sources, but it also exposes animals to anthropogenic pollutants such as pesticides, effluents and fluel gas emissions (Bilandžić *et al.* 2010).

The group of trace elements whose presence was investigated in this study contains both highly toxic non-essential metals such as Cd and Pb, as well as essential metals which are characterised by dose-dependent toxicity. Essential trace elements, such as Cu, Zn, Fe and Mn, are required for growth and development and have to be supplied with food. Their absorption is determined by the body's nutritional demand, physiological condition and the availability of food which is a rich source of those elements (Czajkowska *et al.* 2011).

It is known that significant differences in metal concentrations in animal tissues could occur between individuals of the same species (Dip *et al.* 2001, Heltai & Markov 2012), as well as in different species inhabiting the same region (Friel *et al.* 1987). Gender effect in heavy metals accumulation in this study is determined most probably by the biology of the species. The size of golden jackal`s home range depends on resource distribution, extent of human pressure, type of ecosystem, and is estimated to be in the range from 1.1 to 20 km^2 (Jhala & Moehlman 2004). In general, male jackals have wider home ranges than females (Giannatos, 2004). Additionally the elimination rate of foreign compounds per unit body weight in mammals decreases as body weight increases (Prater 1980). Thus, males as heavier and more mobile are more susceptible to accumulating pollutants, including heavy metals. Although variation in concentration of different metals between gender exists, we found no significant sex-related differences in heavy metal accumulation, as opposed to results of similar studies by Passlack *et al.* (2014) in horses and Vahter *et al.* (2007) in humans.

Similar environmental conditions of human modified habitats were present at all the analysed localities and that could be the cause of the lack of statistical difference in concentrations of analysed metals among localities. Main source of pollution were agricultural chemicals which were widely used at all the localities. Although the steelwork near Smederevo and chemical industry near Negotin were not working during this study period, it is known that most metals do not undergo microbial or chemical degradation and their total concentration in soils persists for a long time after their introduction (Adriano 2003). Moreover, heavy metals can be carried by air and water across significant distances, and they are found even in regions that are situated remotely from sources of industrial pollution.

Golden jackals were characterised by high Cd and Pb levels which could affect zinc metabolism. Diets deficient in Fe, Zn and Cu intensify Cd absorption (Burger 2008) and accumulation in bodily tissues (Chrobaczyńska *et al.* 2011). Diet rich in Cu reduced resorption of Pb from the digestive system (Chrobaczyńska *et al.* 2011).

Small mammals preyed upon golden jackals absorb trace elements, including highly toxic metals, from soil, water, plants and air, and they are a potential source of contamination for predatory animals inhabiting natural and agricultural ecosystems (Fritsch *et al.* 2010).

Although metal concentrations in food sources typically consumed by golden jackals (e.g. small mammals) were not examined at the six study sites, heavy metals concentrations in the bones of black-striped field mouse (*Apodemus agrarius)* in two areas in Serbia showed similar concentrations of Cu (54.118 mg/kg) and Zn (74.256 mg/kg) (Blagojević *et al.* 2012) as compared to our study. In the same study concentrations of Fe (5.629 mg/kg), Mn (1.129 mg/kg) and Cd (0.213 mg/kg) were lower, while Pb (25.965 mg/kg) and Ni (28.677 mg/kg) concentrations were higher than in this study. In toxicological study on hare (*Lepus europaeus*) from Serbia the concentrations Cd and Zn were lower than in golden jackal (Petrović *et al.* 2013). High levels of trace elements in jackal livers could be attributed to the transfer of heavy metals from the food chain, in particular for animals inhabiting polluted regions or feeding on the dumps in vicinity of human settlements (Ćirović *et al*. 2014). Mammals inhabiting suburban habitats are already recognised as good bioindicators of environmental pollution (Bilandžić *et al*. 2012).

It is known that if food resources are abundant enough, golden jackals can survive in habitats that are highly altered, including large urban areas (Jaeger et al. 2007). Currently, jackals are spreading across the European continent (Arnold *et al*. 2012), reaching high local densities (Šálek *et al*. 2014). As a wild omnivorous mammal at the top of the food chain which is characterised by high adaptability and other supportive ecological features, the jackal is a good bioindicator of environmental contamination (Bilandžić *et al*. 2010). However, we acknowledge that the great mobility of golden jackal hamper conclusion about specific pollution at the studied locations. Due to commensalism with humans, omnivorous diet and high position in food chain, golden jackal could be an appropriate bioindicator for environmental pollution.

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