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Exploring Contents of Lead and Cadmium in Tissues of Fat Dormouse *Glis glis* (Linnaeus, 1766) (Rodentia: Gliridae) for Use in Monitoring of Environmental Pollutants in the Southern Caspian Coast Forests, Iran

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Abstract: The levels of Pb and Cd in the liver and muscle samples of fat dormouse (*Glis glis*) were measured and compared in various localities in Hyrcanian forests of Northern Iran. We detected the highest Pb and Cd residues in sampled animals inhabiting forest patches closer to high-traffic roads and a decreasing trend was recorded ranging from polluted areas to far remote and high altitude areas. The results showed no significant differences between the sexes. We found positive correlation between lead concentration and age of the sampled animals as identified on the basis of their morphometric characteristics. The results of the present study as well as the relatively unique ecological and biological peculiarities of fat dormouse indicate this species as a potential bioindicator of the heavy metal contamination and forest ecosystems health.

Key words: bio-indicators, environmental pollution, forest ecosystems, Hyrcanian forests, Iran

Introduction

Fat dormouse, *Glis glis* (Linnaeus, 1766), is a nocturnal rodent inhabiting deciduous and mixed forest zone in Europe and southwestern Asia. It is a completely arboreal species, with a vast distribution in lowland forests, highly associated with tree and shrub layers. The fat dormouse is the only species belonging to the family Gliridae listed in the last checklist of mammalian species of Iran (Karami *et al.* 2008). It is distributed along the Hyrcanian ancient forests of Northern Iran. Its habitat selection characteristics, ecology and feeding behaviour make this species a potential bioindicator of environmental contaminants (Markov 2012). The main source of many heavy metals, including lead and cadmium, in arboreal species such as *G. glis*, is the amount of the pollutants deposited on the edible plant materials, mainly fruits, seeds, and nuts (Milika 2010). These metals accumulate mainly in bones, kidneys, liver and lungs (Friberg *et al.* 1986). High heavy metal concentration in free ranging wildlife species can result in pathological effects, poor health or even death (Lewis *et al.* 2001; Burco *et al.* 2012). Among them, lead and cadmium have long been documented as the source for serious environmental hazards to wildlife species, especially water birds (Binkowski 2013), carnivores (Burco *et al.* 2012), and small mammals (Lewis *et al.* 2001; Wijnhoven *et al.* 2007). Lead accumulation in small mammals can affect other trophic levels since these animals play an important role in food chain and many predatory species prey on them (Erlinge *et al.* 1983; Jongbloed *et al.* 1996). This issue can bring serious impacts, especially in cases of species facing conservation concerns. Lead is transferred from diverse sources such as soil, air, plants foliage, and shooting to animal bodies. Shore (1994) reported a positive correlation between soil Pb concentration and its amount

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in tissue samples from small mammals. Such correlation had been previously reported for small ground-living mammals such as voles (Martiniakova *et al.* 2011), shrews (Stansley & Roscoe 1995; Swiergosz-Kowalewska *et al.* 2005), and white footed mice (Clark 1979). Among anthropogenic activities, the heavy road traffic is considered the main source of heavy metal accumulation in small mammals' body (Martiniakova *et al.* 2011). Markov

With regard to the relatively unique biological and ecological peculiarities of the fat dormouse as a deeply divergent lineage in the Northern Iran Hyrcanian refugium (Naderi *et al.* 2013), we investigated its potential application as a model species to monitor environmental contaminants, especially in forest ecosystems. To clarify this potential role, we measured the concentration of lead and cadmium in liver and muscle tissues of the fat dormouse from various habitat patches close to high-traffic roads and in remote and high altitude areas.

Materials and Methods

In May 2011 – November 2013, we collected 61 specimens from six localities along the southern shores of the Caspian Sea (Fig. 1): Heiran col (38º23'N, 48º39'E), Kotah Koma water fall (38º19'N, 48º44'E), Lavandevil (38º18'N, 48º48'E), Noshadeh-Pellesara (37°02'N, 49°55'E), Ramsar (36º53'N, 50º39'E), and Nour (36°34'N, 52°07'E). All specimens were collected by native villagers during summer season to reduce the destroying effect of animals on garden products such as nuts. The criteria for choosing these habitats were the relatively higher fat dormouse abundance in these localities (Naderi *et al.* 2014) and their location based on the main aim of the study (Kota-Komah and Lavandevil are located in the remote areas and far from the main traffic routes, while others are crossed by high-traffic roads).

All the collected specimens were weighed to the nearest gram and their external characters including hind foot length, total body length and tail length were measured. Liver and tissue samples were frozen after their sectioning in −18°C. After defrosting in the laboratory, each sample was weighed to the nearest 0.0001 g, put onto a petri dish, and placed in the dryer at 105ºC. After obtaining the stable weight, the samples were then turned into ashes at 450ºC and dissolved in 2 mL of 65% nitric acid; the entire solution was diluted in 10 mL of deionized water. The samples thus prepared were analysed with the flame atomic absorption spectrometer. The final results were presented as concentrations in µg/g of dry weight. The normal distribution of the data was checked by Kolmogorov-Smirnov test. In case of non-normal data distribution, we used Mann-Whitney U and Kruskall-Wallis H tests to investigate intra-population and inter-population heavy metal concentration, respectively. To explore the significant difference of metal concentration between sexes, student's T test was used. Spearman rank correlation coefficient analysis was applied to test significant relationships between heavy metal concentration and morphological peculiarities such as sex, age, weight, and total body length. The specimens were sexed (by examining baculum in males) and aged by their average weight and body length (Krystufek 2010). *SPSS* software package v. 19 was *used* for statistical *analysis.*

Results

Kruskall-Wallis test indicated that both Pb (H=21.7, $p<0.01$) and Cd (H=17.2, $p<0.01$) concentrations were significantly different in the studied populations. The average amount of the studied contaminants was positively correlated with their distance to traffic roads. The highest amount of the metals measured was recorded in areas very close to high-traffic roads ($r=0.75$, $p<0.05$; Heiran col and forest stands in Ramsar), while remote areas such as Kotah-koma waterfall and Noshadeh-Pellesara showed significantly lower amount of the metals. Table 1 shows the average of the quantitative analyses of lead and cadmium concentration in the liver and muscle tissues of populations studied. We found no significant dif-

Fig. 1. Sampling localities along the Caspian Sea forests of Northern Iran. The numbers correspond to the information presented in Table 1

ference between the average amount of both metals in the liver and muscle tissues in all specimens (Fig. 2). A positive correlation of contamination with age (weight and total body length) in this species was indicated ($r=0.84$, $p<0.05$). Student's T test showed no significant difference between males and females regarding metal concentration ($p>0.05$).

The mean lead concentration in the liver and tissue samples of the trapped specimens decreased from 8.94 µg/g dry weight closer to the high-traffic sites to 0.98 µg/g dry weight in remote areas at the higher elevation belts of the Hyrcanian forests. The same result was also recorded for Cd, with the lowest amount of cadmium found in the Kota-Komah area located in the remote areas at 800 m above sea level (Table 1). Kruskall-Wallis H test showed that the concentration of lead in different localities was significantly different for both liver and muscle tissue samples ($H=24.2$, $p<0.05$), while cadmium concentration was not significantly different among localities ($p>0.05$).

Discussion

The results once again prove that anthropogenic activities near or within the wildlife habitats are threatening the wildlife by exposing them to a variety of environmental contaminants. In this case, the concentration of heavy metals in animal tissues also depends on age, physiological state, and homeostatic mechanisms of an individual (Sawicka-Kapusta *et al.* 1995). The food consumption is one of the main routes of exposure of small mammals to heavy metals in contaminated environments (Ma *et al.* 1991). All these characteristics of zoomonitor species should be taken into account when they are analysed for the evaluation of the quality of the environment. Previous studies claimed that ecological bioindicators are those species with high abundance and widespread in a certain type of environment, restricted mobility, clear position in the trophic system, clear feeding strategy, medium to long generation as well as good knowledge about their ecology and physiology. Because of the wide-range distribution of the fat dormouse along the Hyrcanian forests of Northern Iran (more than 700 km), it can be an ideal model of environmental pollutants concentrated in the animal bodies (Gerhardt 2002). Based on such characteristics, it seems that fat dormouse is one of the suitable candidates for such role with regard to the wide distributional range, high abundance, and known ecology and physiology (Naderi *et al.* 2013). The only negative point regarding the candidate's characteristics can be its unequal metabolic rate, since the species have relatively long hibernation period (Krystufek 2010). Anyway, intensive feeding of about five months in forest canopy, along with its unique and dynamic population, gives an advantage to the species. These characteristics made this species to be introduced as a potential bioindicator by some investigators. Markov (2012), e.g., recorded 2.147 mg/kg dry weight of Pb and lower than 0.001 mg/kg dry weight of Cd in the examined fat dormouse in Bulgaria. He also suggested that the fat dormouse could be used as a bioindicator in forest and agricultural ecosystems provided that the harmless level of the metals could be established for this species. Markov (2012) also suggested that the fat dormouse could be introduced as a zoomonitor species to detect toxic anthropogenic hazards in other re-

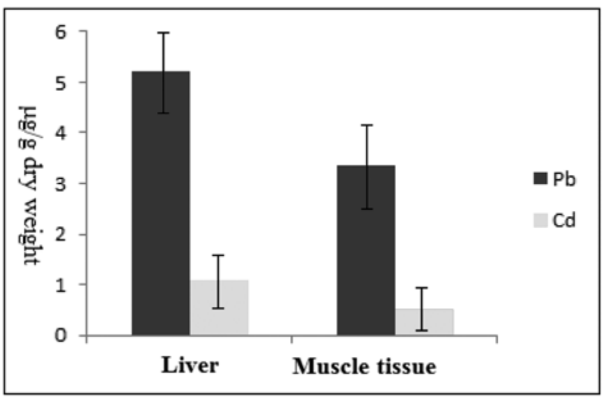

Fig. 2. Mean concentration value of Pb and Cd in liver and muscle tissue samples of fat dormouse in forests along Caspian coast of Iran

Table 1. Mean value of the measured heavy metals (± SD) in liver and muscle tissue samples (µg/g dry weight) in different localities and the number of studied specimens (n)

Locality		Elevation (m a.s.l.)	n	Lead		Cadmium	
				Liver	Muscle tissue	Liver	Muscle tissue
1	Heiran Col	250	7	8.94 (±0.70)	4.65 (±0.43)	1.95 (±0.03)	1.05 (±0.65)
2	Kota-Komah waterfall	740	9	0.98 (±0.23)	0.0	0.03 (±0.01)	0.0
3	Lavandevil	218	12	4.50 (±0.35)	3.25 (±0.12)	0.95 (±0.75)	0.24 (±0.15)
4	Noshadeh-Pellesara	495	11	2.31 (±0.15)	1.50 (±0. 3)	0.65 (±0.40)	0.08 (±0.06)
5	Ramsar	25	9	8.21 (±0.85)	5.45 (±0.43)	1.58 (±0.93)	0.90 (±0.59)
6	Nour	-9	13	6.45 (±0.48)	5.30 (±0.39)	1.35 (±0.77)	0.78 (±0.65)

gions of its wide area of distribution in Europe. Forest dormouse is also introduced as a potential bioindicator of environment status (Markov 2014). Some studies have been carried out on rodents in Iran as a potential environmental pollution bioindicators such as Norway rat (*Rattus norvegicus*) in the metropolitans (Hazratian *et al.* 2014), Persian Jird in the desert and semi-desert areas (Okti & Rezaee 2013; Khazaei *et al.* 2015) but there are no published documents about forest dwelling rodents. Our initial assessment of fat dormouse as a potential bioindicator of environmental pollution is the first study in the country and the second one worldwide after Markov's (2012, 2014) studies. Our results regarding the concentration of cadmium and lead as well as their relations with distance to high-traffic roads provided initial steps towards introducing fat dormouse as a potential zoomonitor and bioindicator species in forest ecosystems. Investigation on other metals such as Zn, Cu, Fe, Mn and Ni can provide more data to confirm our claim.

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