Bull Environ Contam Toxicol (2012) 89:910–914
DOI 10.1007/s00128-012-0755-z

Red Fox (*Vulpes vulpes* Linnaeus, 1758) as Biological Indicator for Environmental Pollution in Hungary

Miklós Heltai · Georgi Markov

Received: 29 February 2012 / Accepted: 17 July 2012 / Published online: 3 August 2012
© Springer Science+Business Media, LLC 2012

Abstract Our aim were to establish the metal (Cu, Ni, Zn, Co, Cd, and Pb) levels of red fox liver and the kidney samples (n = 10) deriving from central part of Hungary and compare the results with other countries' data. According to our results the concentrations of residues of the targeted elements (mg/kg dry weight) in liver and kidney samples were, respectively in liver: Cu: 21.418, Zn: 156.928, Ni: 2.079, Co: 1.611, Pb: 1.678 and Cd: 0.499; and kidney samples: Cu: 9.236; Zn: 87.159; Ni: 2.514; Co: 2.455; Pb: 2.63 and Cd: 0.818. Pb levels of Hungarian red fox liver samples significantly exceed the values of Italian specimens' samples, whilst the same element's concentrations of Hungarian red fox kidney samples were higher than the results published in Germany.

Keywords Red fox · *Vulpes vulpes* · Bioindicator · Metal · Agricultural area · Hungary

The development of industry and motorization, as well as the continuing over-intensive use of various chemical compounds in agriculture leads to a constant increase of the levels of metals in the environment; being non biodegradable, they readily accumulate to toxic levels (Adie and Osibanjo 2009). The anthropogenic heavy metal contamination affects nowadays large areas worldwide (Nicholson et al. 2003) and excessive levels of metals could be introduced into the agricultural ecosystems through industrial waste or fertilizers (Alloway 1990). Determining the concentrations of metals in the biota provides information concerning their movement through the environment, accumulation and potential toxicological effects (Torres and Johnson 2001). Animals, especially mammals, are useful bioindicators for environmental monitoring in ecosystems with pollution loads (Tataruch and Kierdorf 2004). Most wild carnivorous mammals, which are at the top of the food web, may be very sensitive to any biomagnification processes. One of these carnivores, the red fox (*Vulpes vulpes* Linnaeus, 1758), could be an effective bioindicator in estimating environmental pollution in its natural habitats because of the species' biological and ecological features (Ansorge et al. 1993; Corsolini et al. 1999; Dip et al. 2001; Kalisińska and Palczewska-Komsa 2011).

The lack of knowledge about bioaccumulation and actual values of the concentrations of main pollutants from the metals group in red fox (*V. vulpes*) specimens from natural populations inhabiting agricultural regions in Hungary determined the aim of the present investigation: (1) to evaluate the concentrations of priority pollutant residues from the metal group – elements with concentration dependant toxic effect (Cu, Ni, Zn, Co) and microelements with proven highly toxic effect on living organisms (Cd, Pb) – in samples from target organs of the red fox (liver and kidneys), originating from a natural population inhabiting the main agricultural region in Hungary; (2) to establish the initial norm of its variation in landscapes typical for agricultural territories in the country's plane region.

Materials and Methods

To find out metal concentrations in the red fox, samples (kidneys and livers, n = 10) were collected during the

M. Heltai (✉)
Institute for Wildlife Conservation, Szent István University, Páter K. u. 1, 2100 Gödöllő, Hungary
e-mail: heltai.miklos@gmail.com

G. Markov
Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bul. "Tzar Osvobiditel" No. 1, 1000 Sofia, Bulgaria

regular hunting season in 2008 from Jász-Nagykun-Szolnok County an agricultural region in the central part of Hungary, characterized by relatively intensive agriculture and well developed road network. All investigated red foxes were adults males in normal physiological condition. The liver and the kidneys were used as test systems for the analytic concentrations of studied toxicants in the red fox specimens.

Tissue samples were taken from liver and kidneys in field conditions (none of the samples were contaminated with bullets), and were frozen for the subsequent laboratory analysis. The mineralized samples of its tissue from each investigated specimen were analyzed through Atomic Absorption method for determination of the content of Cu, Ni, Zn, Co, Cd and Pb (Havezov and Tsalev 1980). Concentrations of the above mentioned elements were expressed in mg/kg of dry analysed tissue.

Statistical analysis included the determination of the arithmetic mean, standard deviation from that mean and ±95 % confidence limits for means with regard to concentrations of investigated metals. The differences between the mean values of metals' residuals in the two studied test systems (liver and kidneys) were analysed using a non-parametric statistical method–Mann–Whitney U test. Differences were considered significant at $p < 0.01$. In addition, the significance of differences in metals' concentrations between red foxes from the agricultural region in the central part of Hungary and red foxes from other low-polluted regions in Europe were assessed. These comparisons were performed using the Student's t test. All calculations were performed using the statistical package STATISTICA 2008 version 8.0 (StatSoft Inc. 2008 www.statsoft.com).

Results and Discussion

The agricultural areas in Hungary cover the half of the country (5.5 million ha); 4.5 million ha of these are arable lands. On most of the cultivated areas (more than 95 %) wheat, rape, sunflower and corn are grown by intensive methods. The average size of a cultivated parcel is more than 75 ha, and more than 110 kg chemical fertilizers per hectare are used. Green-forage production (e.g.: alfalfa) is not typical because of the decline of livestock breeding, so rodent control on the arable lands is less practiced. The rodent populations on these sites are currently controlled only by natural processes (weather, nutrient-supply, and predation). That is why the biomass of the rodents may be significant, and might reach a level of 254 sp./1 ha (Lanszki et al. 2006). The comparative analysis of data from agricultural management in Jász-Nagykun-Szolnok County and the mean values from the whole country (Table 1) revealed the distinctiveness of this region, characterizing it as a typical agricultural territory in Hungary.

The average road density (motorways and major trough roads) is 2.02 km/100 km^2 in Jász-Nagykun-Szolnok County compared to 2.32 km/100 km^2 countrywide. In case of secondary (regional and local) roads, the road density is 5 km/100 km^2 in the County and 4.79 km/100 km^2 all over the country. The road density of all roads is 23.3 km/100 km^2 in Jász-Nagykun-Szolnok County and 34.05 km/100 km^2 in Hungary (National Road Database 2011 http://www.utadat.hu downloaded 19.10.2011/and the Hungarian Central Statistical Office 2011 http://www.ksh.hu/agriculture downloaded 19.10.2011), or it reaches 68.43 % of the average road density in the country and is typical for a region with predominantly agricultural land use. The descriptive information about the concentrations of metals in the liver and kidney in the Red fox in Jász-Nagykun-Szolnok County are presented in Table 2.

The concentrations of residues of priority pollutants of the metal group – existential elements with concentration dependant toxic effect (Cu, Ni, Zn, Co) and microelements with proven highly toxic effect on living organisms (Cd, Pb) found in samples from of target organs–liver and kidney of the Red fox, originating from natural population inhabiting the main agricultural region in Hungary revealed the following: (1) Zn and Cu had the highest accumulated levels among the existential elements in both studied organs; the quantities of the other two existential elements Co and Ni were low; the concentration of metals varied widely among analysed samples; the highest absolute variation of residuals' mean value was found in zinc, followed by copper and nickel; (2) the empirical mean values of copper and zinc residuals were higher in the liver, while the mean values of nickel, cobalt, lead and cadmium were higher in the kidneys; (3) the bioaccumulation potential (AP = mean value of metal in liver/mean value of metal in kidney) of the tissues of studied internal organs of the Red fox from the agricultural region of Jász-Nagykun-Szolnok County varies for the different metals. The AP values from the studied organs showed that the mean concentrations of existential elements (Cu and Zn) had higher levels in the liver, while the other two bioelements (Ni and Co with the high toxic effect Pb and Cd) had higher values in the kidneys; (4) the mean values of the concentrations of studied metals in the liver and kidneys were compared using the Mann–Whitney U-test that revealed statistically significant differences in copper and zinc ($p = 0.09$), in lead ($p = 0.39$) and only at $p = 0.67$ in cadmium and cobalt; (5) no significant differences were found in metal accumulation in both organs at $p < 0.01$.

The values of ±95 % confidence interval of the mean values of studied metal residuals (Table 1) found in the liver and kidneys of Red foxes from Jász-Nagykun-

Table 1 Data of agricultural management in Jász-Nagykun-Szolnok County

	1	2	3	4	5	6	7	8	9
A	352.3	50.8	46.2	24	28	46,173	6,290	70,921	2,680
B	4,501.60	1,004.20	1,895.60	17	366	1,191,804	7,470	549,804	2,670

Jász-Nagykun-Szolnok County (A) and Hungary (B): (1) cultivated land × 1,000 ha; (2) grassland × 1,000 ha; (3) forested area ha × 1,000 ha; (4) manure (organic fertilizer) t/ha; (5) fertilizer kg/ha; (6) zea mays area ha; (7) zea mays crops kg/ha; (8) sunflower area ha; (9) sunflower crops kg/ha

Source: Hungarian Central Statistical Office http://www.ksh.hu/agriculture downloaded 19.10.2011

Table 2 Descriptive statistics for metal residuals

Element	Liver				Kidney			
	X	SD	−95 % Confid.	+95 % Confid.	X	SD	−95 % Confid.	+95 % Confid.
Cu	21.418	5.087	8.781	34.054	9.236	1.175	6.319	12.154
Zn	156.928	44.774	45.703	268.154	87.159	24.851	25.424	148.895
Ni	2.079	0.984	–	4.523	2.514	2.222	–	8.033
Co	1.611	0.216	1.075	2.146	2.455	1.811	–	6.952
Pb	1.678	0.383	0.725	2.63	2.63	1.235	–	5.697
Cd	0.499	0.137	0.159	0.839	0.818	0.604	0.681	2.318

±95 % confidence limits for means (in mg/kg of dry weight analysed tissue) in liver and kidney tissue samples of the red fox (*V. vulpes*) from agricultural region (Jász-Nagykun-Szolnok County) in the central part of Hungary (n = 10)

Szolnok County revealed that: (1) in the liver, the zinc had the highest ratio between the maximum and minimum values among the existential elements, followed by nickel, copper and cobalt; among the elements with highly toxic effect this ratio was higher in cadmium than in lead; (2) in the kidneys, the nickel had the highest ratio between the maximum and minimum values among the existential elements, followed by the cobalt, zinc and copper, while among the elements with highly toxic effect this ratio was higher in lead than in cadmium.

The red fox is one of the most widespread and in many aspects, the most important predator of Hungary. It is well adapted to almost each type of forested and agricultural environments in the country. From 1988 to 2002 the Hungarian red fox populations increased steadily, but later a process of reduction has begun. At the beginning of the period, the estimated red fox population density was 4.4 sp./1,000 ha (1988), the highest density was 9.47 sp./1,000 ha (2002). After a remarkable fall recently it grows again. According to the National Game Management Database the hunting bag has more than doubled since 1988. In 1988 the hunting bag was 29,491, while in 2009 it was 66,671, with a maximum of 75,571 in 2003. Diet-composition researches of the red fox in Hungary showed that the most important base elements of the red fox's diet composition, depending on the type of the area and on the season (according to biomass counting 46 %–85 %), are small rodents (Heltai 2010). In open agriculture landscapes the red fox's most important prey is the common vole, which is considered as a pest in agriculture, and in forested landscapes, the bank vole plays the same role. According to the diet studies presented in this article, the proportion of small mammals in the diet of the red fox varied between 52 % and 73 %. The information about the range width of the established values of ±95 % confidence interval of the mean values of studied metal residuals (Table 2) expands the notion of physiological and eco-toxicological characteristics of red fox as zoo monitor and provides a substantial biological basis for interpretation of the obtained results from biological monitoring of the agricultural areas in Hungary. The accumulation of metals in foxes may reflect their mobilization from the soil matrix and their subsequent transfer into mammalian organisms (Goyer 1996). The rodents are foxes' main food sources in agricultural ecosystems, and being able to accumulate various toxic compounds, especially metals from the environment in their body (muscles, liver, kidneys, bones, etc.) (Goyer 1996). They could be considered as one of the main sources of exposure of red fox in agricultural regions to the action of various toxic compounds together with the atmospheric pollution from motor vehicles and introduced industrial waste or fertilizers.

The residuals of Pb and Cd (metals with highly toxic effect) in air-dry tissues of the liver and kidneys from adult red fox males from agricultural area in Hungary were compared with data (Table 3) about foxes from Italy and

Table 3 Comparison of metal concentrations in liver and kidney tissue samples

Element	Liver						Kidney					
	Hungary		Italy		*t*	*p*	Hungary		Germany		*t*	*p*
	X	SD	X	SD			X	SD	X	SD		
Pb	1.678	0.383	0.600	0.270	7.00	<0.01	2.630	1.235	0.800	0.230	4.67	<0.01
Cd	0.499	0.137	0.580	0.460	0.48	>0.01	0.818	0.604	1.110	0.910	−1.30	>0.01

Red fox (*V. vulpes*) from Hungary, Italy and Germany

X arithmetic mean in mg/kg dry weight, *SD* standard deviation, *t* student's *t* value, *p* significance level

Germany. The data from Italy concern red foxes from a rural and relatively undisturbed environment in Siena (Corsolini et al. 1999). The German data (Ansorge et al. 1993) refer to red foxes from the Obcrlausitz region in eastern part of Germany. This region has an area of about 3,000 km^2 including a variety of natural territories with slight anthropogenic changes, urban areas and human settlements, as well as areas under intensive agricultural management. Ansorge et al. (1993) found that the level of studied metals was not very high.

The comparative analysis of these three sets of data (Table 3) showed that: (1) The mean values of Pb assayed in samples of liver tissue from Hungarian and Italian foxes, as well as the mean values of Pb in kidneys of Hungarian and German foxes show statistical differences at $p < 0.01$; residues of lead are of high value in Hungarian foxes; (2) The mean values of Cd assayed in samples of liver tissue from Hungarian and Italian foxes, as well as the mean values of Cd in kidneys of Hungarian and German foxes didn't show statistical differences at $p < 0.01$.

As shown by the results of this study and by data reported by other authors (Ansorge et al. 1999; Dip et al. 2001) metal concentrations in red fox occasionally show a wide population intraspecific variability, and the accumulation of metals in foxes may reflect their mobilization from the soil matrix and subsequent transfer into mammalian organisms (Goyer 1996). Explanations for such wide population intraspecific variability of metal concentrations in red fox in European ecosystems, where these animals are positioned on the top of the local food chain, may be sought both in differences in the degree of local anthropogenic contamination of the environment, and in local variability of natural geochemical background (Dehn et al. 2006; Driscoll et al. 2007), but the possibility for remote cross-border pollution should also be considered. The examination of the ±95 % confidence interval of the mean values defined for studied metal residuals in the liver and kidneys of the red foxes from Jász-Nagykun-Szolnok County, which is considered to be affected by agricultural activities, established the initial norm of their variation and allows using this species as zoo monitor in comparative biomonitoring investigations in agricultural regions in Hungary.

The presence of highly toxic metals, such as lead and cadmium (Lucy and Venugopal 1977), considered as due to anthropogenic pollution of the environment (Sawicka and Kapusta 1979), in the liver and kidneys of free living red foxes from the studied agricultural region, shows that regular observations for assessment and forecasting of accumulation of toxic metals in free living animals in agroecosystems in Hungary are necessary. Regular monitoring of metal concentrations in the tissues of the foxes from agricultural areas is therefore highly needed in the nearest future in order to evaluate the natural pollution not only as a result of using of chemicals in agriculture, but with respect to probable transmission of pollutant emissions to long distances, as well. It should aid in expanding knowledge relevant to the problem of anthropogenic impact on agricultural lands.

Acknowledgments Thanks to the two anonymous reviewers for their helpful comments as well as Judit Galló and Ferenc Markolt for the language revision of the manuscript. This work was supported by the Department of Game Management and Fishery of the Ministry of Agriculture and Rural Development, the Hungarian Academy of Sciences, and the Bulgarian Academy of Sciences.

References

Adie GU, Osibanjo O (2009) Assessment of soil-pollution by slag from an automobile battery manufacturing plant in Nigeria. Afr J Environ Sci Technol 3(9):239–250

Alloway BJ (ed) (1990) Heavy metals in soils. Blackie Academic & Professional, Glasgow-London, p 339

Ansorge HK, Graeser H, Fink G (1993) Schwermetallruckstande beim Rotfuchs (*Vulpes vulpes*). Beitr Jagd-Wildforsch 18:79–82

Corsolini S, Focardi S, Leonzio S, Lovari S, Monaci F, Romeo G (1999) Heavy metals and chlorinated hydrocarbon concentrations in the red fox in relation to some biological parameters. Environ Monit Assess 54:87–100

Dehn LA, Follmann EH, Thomas DL, Sheffield GG, Rosa C, Duffy LK, O'Hara TM (2006) Trophic relationships in an Arctic food web and implications for trace metal transfer. Sci Total Environ 362:103–123

Dip R, Stieger C, Deplazes P, Hegglin D, Muller U, Dafflon O, Koch H, Naegeli H (2001) Comparison of heavy metal concentration in tissues of red foxes from adjacent urban, suburban and rural areas. Arch Environ Contam Toxicol 40:551–556

Driscoll CT, Han YJ, Chen CY, Evers DC, Lambert KF, Holsen TM, Kamman NC, Munson RK (2007) Mercury contamination in forest and freshwater ecosystems in the northwestern United States. Bioscience 57:17–28

Goyer R (1996) Toxic effects of metals. In: Klaassen CD (ed) Casarett & Doull's toxicology: the basic science of poisons. McGraw-Hill, New York, pp 691–736

Heltai, M (ed) (2010) Emlős ragadozók Magyarországon. Mezőgazda Kiadó, Budapest 240 pp. ISBN: 978-963-286-593-5 Mammal predators in Hungary (in Hungarian)

Kalisińska E, Palczewska-Komsa M (2011) Teeth of the red fox *Vulpes vulpes* (L., 1758) as a bioindicator. Acta Theriol 56: 343–351

Lanszki J, Heltai M, Szabó L (2006) Feeding habits and trophic niche overlap between sympatric golden jackal (*Canis aureus*) and red fox (*Vulpes vulpes*) in the Pannonian ecoregion (Hungary). Can J Zool 84(11):1647–1656

Lucy T, Venugopal B (1977) Metal toxicity in mammals. In: Physiology and Chemical basis for metal Toxicity, vol 1. Plenum Press, New York-London. p 238

Nicholson FA, Smith SR, Alloway BJ, Carlton- Smith C, Chambers BJ (2003) An inventory of heavy metals inputs to agricultural soils in England and Wales. Sci Total Environ 311:205–219

Sawicka-Kapusta K (1979) Roe deer antlers as bioindicators of environmental polution in Southern Poland. Int J Environ Pollut 19(4):283–293

Tataruch F, Kierdorf H (2004) Mammals as biomornitors. In: Markert AA, Breure AM, Zechmeister HG (eds) Bioindicators and biomonitors. Elsevier, Amsterdam, pp 737–772

Torres KC, Johnson ML (2001) Bioaccumulation of metals in plants, arthropods, and mice at a seasonal wetland. Environ Toxicol Chem 20:2617–2626

Хавезов И and Цалев Д (1980) Атомно-абсорбционен анализ, Наука и изкуство. София. 188 pp. (Havezov, I, D Tsalev. 1980. Atomic absorption analysis, the Science and Art. Sofia. 188 pp)