Arch. Environ. Contam. Toxicol. 40, 551–556 (2001)
DOI: 10.1007/s002440010209

ARCHIVES OF
Environmental
Contamination
and Toxicology

Comparison of Heavy Metal Concentrations in Tissues of Red Foxes from Adjacent Urban, Suburban, and Rural Areas

R. Dip,[1] C. Stieger,[2] P. Deplazes,[2] D. Hegglin,[2,3] U. Müller,[4] O. Dafflon,[5] H. Koch,[5] H. Naegeli[1]

[1] Institute of Pharmacology and Toxicology, University of Zürich-Tierspital, August Forel-Strasse 1, 8008 Zürich, Switzerland
[2] Institute of Parasitology, University of Zürich, Winterthurerstrasse 266a, 8057 Zürich, Switzerland
[3] Work Group Urban Ecology and Wildlife Research, Wuhrstrasse 12, 8003 Zürich, Switzerland
[4] Swiss Rabies Center, University of Bern, Länggasstrasse 122, 3012 Bern, Switzerland
[5] Federal Veterinary Office, Schwarzenburgstrasse 161, 3003 Bern, Switzerland

Received: 25 July 2000/Accepted: 14 November 2000

Abstract. The red fox (*Vulpes vulpes*) is a representative of the canid family with wide distribution in the Northern Hemisphere and Australia. The increasing utilization of urbanized habitats by red foxes prompted us to test whether this species may be used to monitor the presence of anthropogenic pollutants in cities or suburbs. For that purpose, we compared the concentrations of heavy metals (Cd, Pb, Cu, Zn) in foxes from urban, suburban, and rural areas within the municipality of Zürich (Switzerland). The kidney and liver of suburban and rural foxes contained the highest Cd concentrations, whereas urban foxes contained the highest Pb levels. In the kidney of suburban foxes, Cd concentrations increased from a median value of 0.73 mg/kg in juvenile animals to 1.82 mg/kg in adults. Similarly, the liver of suburban foxes contained increasing Cd levels from a median of 0.21 mg/kg in juvenile animals to 0.94 mg/kg in adults. An age-dependent storage of Cd was also found in foxes from the rural surroundings, but no such accumulation occurred in urban foxes from the city center, where even adult animals contained very low Cd levels. Conversely, foxes from the urban center were characterized by elevated Pb concentrations during the first 2 years of life, but this transient Pb accumulation was absent in suburban or rural animals. The liver of juvenile foxes contained a median Pb concentration of 0.99 mg/kg in the city compared to only 0.47 and 0.37 mg/kg in the suburban and rural area, respectively. Thus, we found that animals from separate environmental compartments contain different patterns of tissue residues, implying that red foxes may serve as a bioindicator species to detect certain toxic hazards in urbanized habitats.

Life on our planet is threatened by anthropogenic pollutants that accumulate in the atmosphere, water, or soil, but exposure to such pollutants does not always produce toxic effects in living organisms. On the contrary, a potentially hazardous chemical becomes toxic only when it reaches an active site in the target species at sufficiently high concentrations to generate adverse reactions. Before establishing reliable risk assessment studies it is therefore necessary to determine the bioavailability of potentially toxic chemicals, *i.e.*, the efficiency by which such chemicals are transported from environmental matrices to biological receptors (Thibodeaux 1979; Hutton 1982; MacKay 1991; Eaton and Klaassen 1996).

Typically, the bioavailabilty of environmental pollutants is assessed by measuring chemical residues in tissues or fluids of animals living in appropriate aquatic or terrestrial habitats. Many different species can be used to estimate the impact of toxic chemicals on aquatic organisms (Kendall *et al.* 1996), but the choice of suitable terrestrial indicators is more problematic. For example, wild animal species, such as chamois, European hare, moose, red deer, reindeer, or roe deer, have been proposed for biomonitoring studies (Doganoc and Gacnik 1995; Frøslie *et al.* 1986; Kottferová and Koréneková 1998; Markov 1996; Santiago *et al.* 1998). However, these species may not serve as representative indicators of pollution outside of their normal habitats (forests, farmland, or other rural areas). In addition, these herbivorous species often occupy very large territories and, therefore, may not be helpful in localizing specific sources of toxic hazards. In contrast, red foxes (*Vulpes vulpes*) adapt to a variety of environmental conditions and, in urbanized areas, occupy small territories of 0.5 km^2 or less (Harris 1977; Harris and Trewhella 1988; Doncaster and Macdonald 1991). The use of red foxes for biomonitoring purposes has been proposed before, but previous reports were limited to the analysis of foxes living in rural habitats (Brunn *et al.* 1991; Ansorge *et al.* 1993; Bukovjan 1997; Corsolini *et al.* 1999; Gunstheimer *et al.* 1997).

The present study was motivated by the observation that, in many European countries, red foxes have invaded suburban and even urban habitats (Harris 1977; McDonald and Newdick 1982; Christensen 1985; Schöffel *et al.* 1991; Willingham *et al.* 1996), where these animals are exposed, at least in part, to the same pollutants as the human population. During the last 10

Correspondence to: H. Naegeli

years, a considerable increase of the overall fox density was also observed in Switzerland (Breitenmoser *et al.* 1995). For example, the fox population permanently living in the city of Zürich is estimated to consist of 300–400 adult animals with cubs being bred in public parks and in private gardens (Hofer *et al.* 2000). The aim of this study was to explore whether red foxes living in cities may be used to monitor the presence of toxic hazards in their specific urban and suburban environments. For that purpose, we compared the tissue concentrations of various heavy metals in foxes from adjacent urban, suburban, and rural areas. Interestingly, we found that urban foxes accumulate a different mixture of toxic residues compared to the surrounding suburban and rural populations.

Materials and Methods

Study Area

The municipality of Zürich (92 km^2, 360,000 inhabitants) was divided into urban, suburban, and rural areas (Figure 1). The rural area consists mainly of forests, parks, farmland, and allotment gardens, in which the distance between neighboring buildings extends to over 100 m. In the urban area, the distance between buildings is reduced to less than 100 m. At the transition between these rural and urban compartments, we delineated a suburban ribbon that has a width of 0.5 km.

Foxes

A total of 100 red foxes were obtained between January 1997 and February 1998. The majority of animals (about 75%) were shot in the course of population control programs organized by the city forest service. The remaining animals were killed during road or traffic accidents. The carcasses were wrapped in plastic bags and stored at −20°C until necropsy. Tissues were carefully examined for postmortem degradation and for the presence of shots or shot injuries, and, if present, such samples were eliminated from further analysis. On the basis of these criteria, a total of 13 animals were excluded from this study. The age of each animal was determined by counting annuli in the cementum of one canine tooth of the lower jaw (Harris 1978; Grue and Jensen 1979; Kappeler 1985; Goodard and Reynolds 1993).

Sample Analysis

Metal concentrations were assayed in samples of homogenized tissue that were digested in the presence of nitric acid using an MDS-210 microwave (ProLab). Cd and Pb measurements were performed by atomic absorption spectrometry in combination with a graphite furnace using the 4100ZL spectometer from Perkin-Elmer. Cu and Zn was determined by flame atomic absorption spectrometry using the Aanalyst 300 spectometer from Perkin-Elmer. All results are given in mg/kg on a wet-weight basis. Each series of analysis included a blank, a standard calibration curve, and spiked specimens. The accepted recoveries for spiked samples ranged from 85% to 115%. The coefficient of variation on replicate samples was < 7%. The limit of detection for the different metals in tissue samples was 5 μg/kg (Cd), 50 μg/kg (Pb), 5 mg/kg (Cu), and 1 mg/kg (Zn).

Statistics

Heavy metal concentrations in foxes from suburban, urban and rural environmental compartments were compared using the Mann-Whitney *U*-test. Differences were considered significant at $p < 0.05$.

Results

Sample Site, Fox Population, and Data Collection

The municipal territory of Zürich consists of a central urban area comprising 26 km^2. This urban center is surrounded by a suburban ribbon of 33 km^2 and several rural areas, mainly consisting of forests and farmland, located at the city border (Figure 1). Kidney and liver tissues were obtained from a total of 87 red foxes collected between January 1997 and February 1998. Eighteen animals were from the central urban area, 49 animals were collected in the suburban surroundings, and another 20 foxes were from the rural environments. Upon age determination, these animals were divided into three groups that included juvenile foxes with an age of 12 months or less ($n = 39$), young foxes that were between 13 and 24 months old ($n = 24$), and adult foxes with an age of more than 2 years ($n = 24$).

Heavy metal concentrations in the kidney and liver samples were determined by atomic absorption spectrometry. In Table 1, both arithmetic means and medians are given to facilitate comparisons with other reports in the literature. From the Cd concentrations of all animals we calculated median values of 0.97 mg/kg in the kidneys and 0.32 mg/kg in the liver. The median Pb concentrations in kidney and liver amounted to 0.37 and 0.58 mg/kg, respectively. Thus, the overall Cd and Pb concentrations were in a comparably low and nontoxic range as the values reported by Corsolini *et al.* (1999), who monitored residues of pollutants in a fox population from central Italy. On the other hand, we found higher Cd but lower Pb concentrations compared to the study of Ansorge *et al.* (1993), who monitored the residues in the kidney of a fox population from East Germany. Also, we observed similar Pb and Zn concentrations as in the study of Bukovjan (1997), who analyzed the heavy metal contamination of foxes in the Czech Republic. On the other hand, our analysis yielded considerably higher Cd and Cu concentrations than the study performed by Bukovjan (1997). Unlike previous reports (Ansorge *et al.* 1993; Corsolini *et al.* 1999), we were unable to detect a statistically significant difference in the level of heavy metal residues when the fox population of this study was separated according to sex.

Cd Residues

We observed a nearly 100-fold range of Cd concentrations in the kidney, from 0.08 mg/kg in one of the juvenile animals to over 7 mg/kg in some adult animals (Table 1). Even within the group of foxes that were older than 24 months, Cd concentrations in the kidney yielded a nearly 30-fold variability with values ranging from 0.26 to 7.33 mg/kg. Thus, the wide variation of Cd levels could be attributed only in part to the expected differences between juvenile and adult animals due to

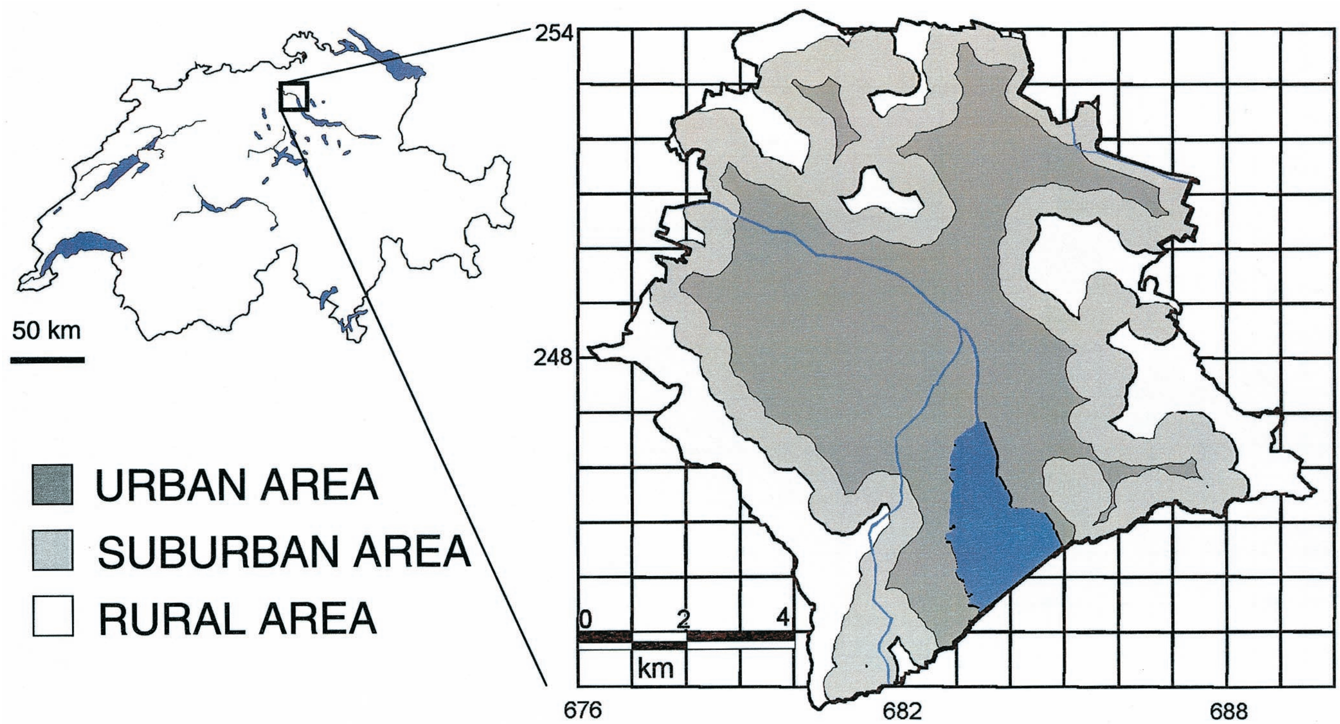

Fig. 1. Study area. The varying distances between neighboring buildings were used to divide the municipality of Zürich into a rural (distances > 100 m), suburban, and urban compartment (distances < 100 m). The suburban area constitutes a transition zone of 500 m between the rural and urban compartments. White: rural area; light gray: suburban area; dark gray: urban area; blue: lake and rivers

Table 1. Summary of heavy metal residues in the tested fox population (n = 87) within the municipality of Zürich

	Cd		Pb		Cu		Zn	
	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver
Median	0.97	0.32	0.37	0.58	4.8	16.2	20.2	41.8
Minimum	0.08	0.03	0.11	0.13	2.5	2.3	9.5	19.5
Maximum	7.33	2.59	1.33	2.59	20.7	157.7	58.0	116.6
Mean	1.45	0.52	0.57	1.20	6.3	20.2	21.2	44.9
SD[a]	1.43	0.51	0.90	3.61	4.1	18.8	7.8	16.7

All values are given in mg/kg on a wet-weight basis.
[a] SD, standard deviation.

continued retention of this metal in soft tissues (Klaassen *et al.* 1999). In fact, Figure 2A shows that only red foxes living in suburban and rural habitats accumulated Cd in an age-dependent manner. For example, the median Cd concentration of suburban foxes increased from 0.73 mg/kg in the kidney of juvenile foxes to 1.82 mg/kg in the kidney of adult animals. In contrast, there was no accumulation of Cd in urban foxes, and, in fact, even adult animals living in the city center contained very low levels of this metal in their kidneys (median value of 0.49 mg/kg). An age-dependent storage of Cd was also found in the corresponding liver tissue, but again exclusively in the animals obtained from suburban or rural areas, whereas the liver of urban foxes contained low amounts of this metal (Figure 2B). Thus, we found that foxes from distinct but adjacent environmental compartments contain significantly different levels of a potentially toxic pollutant, in this case Cd. Regardless of the mechanism underlying this difference, our finding supports the notion that urban and suburban/rural foxes constitute separate populations with little dispersal movement in either direction.

Pb Residues

A diametrically opposite pattern of contamination was observed when we measured the concentrations of Pb in the same tissue samples. First, we found that juvenile animals contained higher Pb levels than adult foxes. Second, this transient accumulation of Pb was detected only in the animals living under urban conditions. In the liver, there was a 20-fold range of Pb concentrations from 0.13 mg/kg in one of the suburban foxes to 2.59 mg/kg in one of the urban foxes (Table 1). Further analysis of these results revealed that the highest Pb levels were

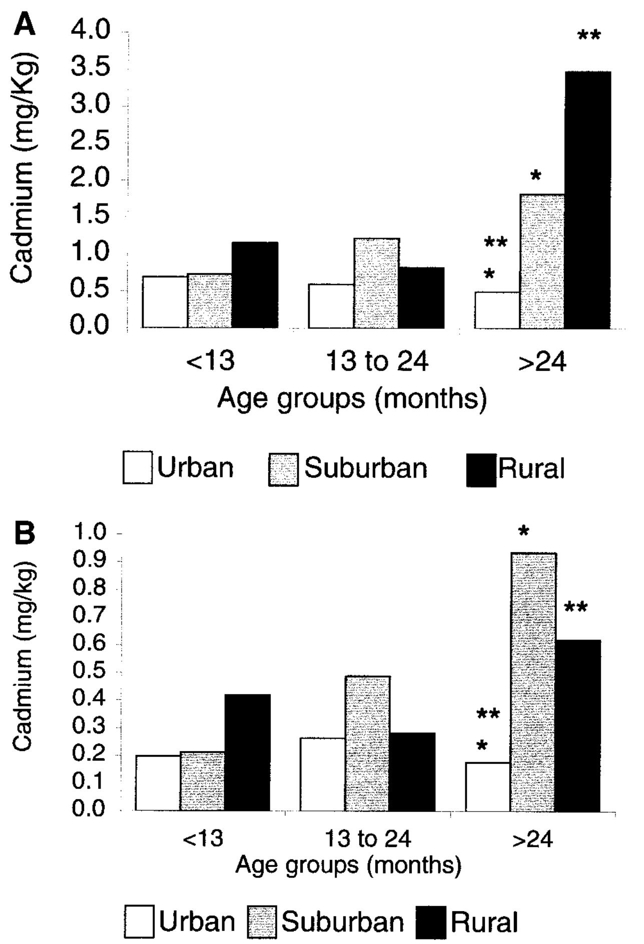

Fig. 2. Concentrations of Cd (medians) in the kidney (A) and liver (B) of foxes. All values are indicated in mg/kg on a wet-weight basis. The asterisks denote statistically significant differences between adult animals of different environmental compartments: *, $p < 0.05$ between the values of urban and suburban foxes; **, $p < 0.05$ between the values of urban and rural foxes

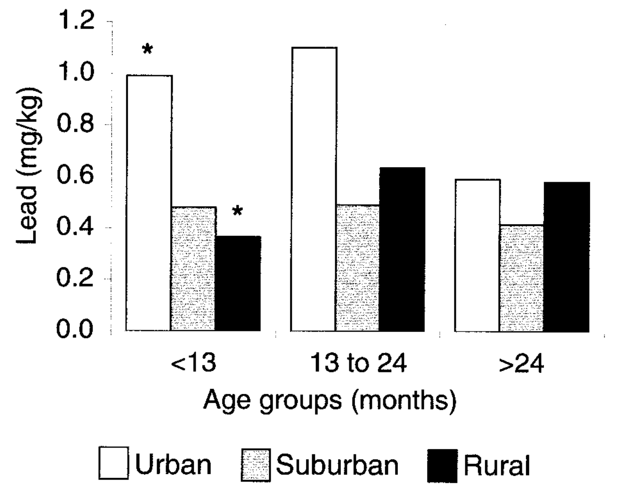

Fig. 3. Concentrations of Pb (medians) in the liver of foxes. All values are indicated in mg/kg on a wet-weight basis. The asterisk indicates a statistically significant ($p < 0.05$) difference between the Pb concentrations of urban and rural foxes

found in the liver of urban foxes with median concentrations of 0.99 mg/kg in juvenile animals of 12 months or less, and 1.10 mg/kg in young animals aged between 13 and 24 months (Figure 3). Such a transiently increased Pb concentration is compatible with the higher bioavailability of this heavy metal in young animals compared to adults (Coyer 1996). Consistent with subsequent elimination or immobilization into bones, Pb concentrations in the liver dropped with increasing age and reached a median value of only 0.58 mg/kg in adult urban foxes. We also observed that both suburban and rural animals contained significantly lower levels of Pb during the first 2 years of life compared to urban foxes. In fact, Pb concentrations in the liver of juvenile animals were 0.47 mg/kg in the suburbs and 0.36 mg/kg in the rural environment, and these low Pb contents were maintained in suburban and rural foxes throughout their life (Figure 3). The analysis of kidney samples yielded a similar distribution of Pb residues, *i.e.*, highest values in young urban animals and lowest values in adult suburban foxes, although in this case the differences did not reach the required degree of statistical significance (data not shown).

Cu and Zn Residues

In contrast to the results obtained by measurement of Cd and Pb concentrations, no significant difference between fox populations or age groups were found when we analyzed Cu and Zn levels. The arithmetic means and medians are again summarized in Table 1. Interestingly, a few single animals showed rather high levels of Cu or Zn relative to the overall values found in the general population. For example, a female fox (27 months old) from the suburban area contained Cu at concentrations of 157.7 mg/kg in the liver and 11.6 mg/kg in the kidney. The highest Cu concentration in the kidney (20.7 mg/kg) was found in a male urban fox of 11 months. On the other hand, the highest Zn concentration (116.6 mg/kg) was found in the liver of a male fox (23 months old) from the suburban area. The kidney of this animal contained Zn at a concentration of 51.1 mg/kg. Finally, the highest Zn value in kidney (58.0 mg/kg) was found in another animal (male, 11 months) from the urban center. These Cu and Zn concentrations reached values that were up to 8.4-fold higher than the arithmetic means or medians of Table 1. Recent ingestion of contaminated water or food is likely to be responsible for these single cases of increased Cu and Zn levels.

Discussion

The outstanding result of this study is that the urban fox population contains lower Cd but higher Pb tissue levels than the surrounding suburban and rural populations. A material flux analysis indicates that the major storage site for Cd in Switzerland is the soil, with over 4,000 t of Cd being located in the matrix of the upper soil layers (Kaufmann *et al.* 1997). Thus, the accumulation of Cd in suburban and rural foxes may reflect the mobilization of this heavy metal from the soil matrix and its subsequent transfer into mammalian organisms (Goyer 1996; Langgemach *et al.* 1995). It seems unlikely that the soil of parks and gardens in the city center is less contaminated with Cd than the soil of suburban or rural areas. Thus, the lower Cd concentration in urban foxes may not reflect different levels of environmental contamination but, because Cd is assimilated mainly through the diet, a different feeding behavior between urban and suburban/rural foxes. In fact, the analysis of stomach contents of the animals of this study indicates that the diet of urban foxes is dominated by food of anthropogenic origin found in household rubbish (D. Hegglin, unpublished data). These dietary components were originally intended for human consumption and, due to appropriate monitoring programs, contain low Cd concentrations that do not exceed the currently tolerated maximal residue level. In contrast, suburban and rural foxes rely on natural food sources including earthworms, rodents and other small mammals (D. Hegglin, unpublished data). Invertebrates living in the soil are particularly prone to Cd accumulation (see, for example, Hendriks *et al.* 1995), such that ingestion of these organisms (or ingestion of small mammals feeding on them) may account for the increased Cd uptake of suburban and rural foxes.

Pb has been recognized as the most widespread metal pollutant in cities (Goyer 1996). The increased Pb concentration detected in the liver of urban foxes compared to their suburban or rural counterparts supports the conclusion that urban and suburban/rural foxes constitute separate populations characterized by a different composition of tissue residues. Also, the increased Pb level of urban foxes is reminiscent of the higher Pb exposure of children reported in certain urban communities. In the affected areas, even low levels of Pb uptake in children have been associated with neurobehavioral and cognitive deficits (Marecek *et al.* 1983; Baghurst *et al.* 1992; Pocock *et al.* 1994). Thus, the analysis of urban foxes may reveal the presence of Pb or certain other toxic hazards, possibly warning us of the impact of pollution before signs of toxicity appear in the human population. Future monitoring studies will be devoted to the analysis of other hazardous pollutants, including chlorinated hydrocarbon insecticides, polychlorinated biphenyls, or dioxins.

References

Ansorge H, Graeser K, Fink H-G (1993) Schwermetallr??ckstände beim Rotfuchs (*Vulpes vulpes*). Beiträge zur Jagd- und Wildforschung 18:79–82

Baghurst PA, McMichael AJ, Wigg NP, Vimpani GV, Robertson EF, Roberts RJ, Tong SL (1992) Environmental exposure to lead and children's intelligence at the age of seven years. The Post Pirie cohort study. N Engl J Med 327:1279–1284

Breitenmoser C, Kaphegyi T, Kappeler A, Zanoni R (1995) Significance of young foxes for the persistence of rabies in northwestern Switzerland. In: Schwyzer M, Ackermann M, Bertoni G, Kocherhans R, McCullough K, Wittek R, Zanoni R (eds) Proceedings of the 3rd Congress of the European Society of Veterinary Virology: Immunobiology of Viral Infections, pp 391–396

Brunn H, Georgii S, Bachour G, Elmadfa I (1991) Free-living animals as an indicator of environmental contamination. Tierärztl Umschau 46:549–553

Bukovjan K (1997) Concentration of selected foreign substances in the tissues of fox *Vulpes vulpes* L. Folia Venatoria 26/27:107–112

Christensen H (1985) Urban fox population in Oslo. Terre Vie 40: 185–186

Corsolini S, Focardi S, Leonzio C, 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

Doganoc DZ, Gacnik KS (1995) Lead and cadmium in meat and organs in Slovenia. Arch Environ Contain Toxicol 54:166–170

Doncaster CP, Macdonald DW (1991) Ecology and ranging behaviour of red foxes in the city of Oxford. Hystrix 3:11–20

Eaton DL, Klaassen CD (1996) Principles of toxicology. In: Klaassen CD (ed) Casarett & Doull's Toxicology, 5th ed. McGraw-Hill, New York, p 13–33

Frφslie A, Haugen A, Holt G, Norheim G (1986) Levels of cadmium in liver and kidneys from Norwegian cervides. Bull Environ Contam Toxicol 37:453–460

Goodard HN, Reynolds JC (1993) Age determination in the red fox (*Vulpes vulpes* L.) from tooth cementum lines. Gibier Faune Sauvage 10:173–187

Goyer RA (1996) Toxic effects of metals. In: Klaassen CD (ed) Casarett & Doull's Toxicology, 5th ed. McGraw-Hill, New York, pp 691–736

Grue H, Jensen B (1979) Review of the formation of incremental lines in tooth of terrestrial mammals. Danish Rev Game Biol 11:1–48

Gunstheimer G, Gunstheimer U, Anke M (1996) The red fox as a bioindicator of mercury exposure. In: Botev N (ed) Proceedings of the International Union of Game Biologists (IUGB) XXII Congress, pp 439–443

Harris S (1977) Distribution, habitat utilization and age structure of a suburban fox (*Vulpes vulpes*) population. Mammal Rev 7:25–39

Harris S (1978) Age determination in the red fox (*Vulpes vulpes*)—an evaluation of technique efficiency as applied to a sample of suburban foxes. J Zool 184:91–117

Harris S, Trewhella WJ (1988) An analysis of some of the factors affecting dispersal in an urban fox (Vulpes vulpes) population. J Appl Ecol 25:409–422

Hendriks AJ, Ma WC, Brouns JJ, de Ruiter Dijkman, EM, Gast R (1995) Modelling and monitoring organochlorine and heavy metal accumulation in soils, earthworms, and shrews in Rhine-delta foodplains. Arch Environ Contam Toxicol 29:115–127

Hofer S, Gloor S, M??ller U, Mathis A, Hegglin D, Deplazes P (2000) Urban cycle of *Echinococcus multilocularis* in the city of Zürich, Switzerland. Parasitol 120:135–142

Hutton M (1982) The role of wildlife species in the assessment of biological impact from chronic exposure to persistent chemicals. Ecotoxicol Environ Safety 6:471–478

Kappeler A (1985) Untersuchung zur Altersbestimmung und zur Altersstruktur verschiedener Stichproben aus Rotfuchs-Populationen (*Vulpes vulpes* L.) in der Schweiz. Diploma thesis, University of Bern

Kaufmann M, von Schulthess C, Ziegler M (1997) Cadmium. Stoffflussanalyse. BUWAL, Bern

Kendall RJ, Bens CM, Cobb GP, Dickerson RL, Dixon KR, *et al.* (1996) Aquatic and terrestrial ecotoxicology. In: Klaassen CD (ed) Casarett & Doull's Toxicology, 5th ed. McGraw-Hill, New York, pp 883–905

Klaassen CD, Liu J, Choudhuri S (1999) Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu Rev Pharmacol Toxicol 39:267–294

Kottferová J, Koréneková B (1998) Distribution of Cd and Pb in the tissues and organs of free-living animals in the territory of Slovakia. Bull Environ Contam Toxicol 60:171–176

Langgemach T, Deutrich V, Metz R (1995) Investigation of heavy metal concentrations in game animals in areas treated with human sewage. Tierärztl Umschau 50:480–490

Macdonald SW, Newdick MT (1982) The distribution and ecology of foxes, *Vulpes vulpes* (L.), in urban areas. In: Bornkamm R, Lee JA, Seeward MR (eds) Proceedings of the 2nd European Ecological Symposium: Urban, Blackwell, Oxford pp 123–135

MacKay D (1991) Multimedia environmental models: the fugacity approach. Lewis, Chelsea

Markov G (1996) Roe deer (*Capreolus capreolus* L., 1758), a zoo-monitor for the estimation of the natural environment quality in Bulgaria. In: Botev N (ed) Proceedings of the International Union of Game Biologists (IUGB) XXII Congress, pp 444–447

Marecek J, Shapiro IM, Burke A, Katz SH, Hediger ML (1983) Low-level lead exposure in childhood influences neuropsychological performance. Arch Environ Health 38:355–359

Pocock SJ, Smith M, Baghurst P (1994) Environmental lead and children's intelligence: a systematic review of the epidemiological evidence. Br Med J 309:1189–1197

Santiago D, Motas-Guzman M, Reja A, Maria-Mojica P, Rodero B, Garcia-Fernández AJ (1998) Lead and cadmium in red deer and wild boar from Sierra Morena Mountains (Andalusia, Spania). Bull Environ Contam Toxicol 61:730–737

Schöffel I, Schein E, Wittstadt U, Hentsche J (1991) Zur Parasitenfauna des Rotfuchses in Berlin (West). Berl M??nch Tierärztlich Wochenschrift 104:153–157

Thibodeaux LJ (1979) Chemodynamics: environmental movement of chemicals in air, water, and soil. Wiley, New York

Willingham AL, Ockens NW, Kapel, CM, Monrad J (1996) A helminthological survey of wild red foxes (Vulpes vulpes) from the metropolitan area of Copenhagen. J Helminth 70:259–263