



# Levels of metals in kidney, liver and muscle tissue and their relation to the occurrence of parasites in the red fox in the Lower Silesian Forest in Europe



Łukasz J. Binkowski<sup>a, \*</sup>, Dorota Merta<sup>a</sup>, Anna Przystupińska<sup>a</sup>, Zenon Sołtysiak<sup>b</sup>, Jarosław Pacoń<sup>b</sup>, Robert Stawarz<sup>a</sup>

<sup>a</sup> Institute of Biology, Pedagogical University of Cracow, Podbrzezie 3, 31-054 Krakow, Poland

<sup>b</sup> Division of Parasitology, Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Wrocław University of Environmental and Life Sciences, Norwida 31, 50-375 Wrocław, Poland

## HIGHLIGHTS

- Sex and age factors were not generally significant for the concentration of metals.
- 34% of specimens examined were infected by parasites.
- No clear relationship between the occurrence of parasites and levels of metal found.
- Logistic regression revealed a trend linking parasites and metals.

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## ABSTRACT

Together with the occurrence of parasites, increased concentrations of xenobiotics, to which scavengers are greatly exposed, may significantly influence the physiology of red foxes. It is also suspected that these two factors interact. The accumulation of various metals (Ca, Cd, Cu, Fe, Hg, K, Mg, Ni, Pb, Zn) in kidney, liver and muscle tissue was investigated, as well as the occurrence of parasites, and the potential link to the presence of metals. Generally speaking, neither sex nor age influenced these concentrations. K, Mg and Fe were found in the highest concentrations and Hg was found in the lowest. Various relationships between the concentrations of metals were observed in the tissues. 34% of the specimens studied were hosts to parasites. No clear, significant connection between the concentrations and the occurrence of parasites was noted, but the discernible trend confirmed by the logistic regression, needs further study.

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## 1. Introduction

Bioaccumulation and biomagnification are important processes through which chemical compounds and elements may affect living organisms. Studies concerning these processes record the

extent to which changes in habitats have intensified and show the response of the organisms to environmental contamination which is strongly linked to metal emissions. Studies concerning concentrations of metals in animals from various kinds of urban areas show significant differences between habitats (including urban, non-urban, farmland, wetlands and forest habitats). However, there is no clear pattern of the bioaccumulation rate in various areas and the matter depends heavily on metal, species, diet and other factors, even including the interactions of other factors (Binkowski and Meissner, 2013; Dip et al., 2001; López-Alonso et al., 2007; Sawicka-Kapusta, 1979). There is a broad range of toxic effects from

\* Corresponding author.

E-mail addresses: [ljbinkowski@gmail.com](mailto:ljbinkowski@gmail.com) (Ł.J. Binkowski), [dorota-zbl@o2.pl](mailto:dorota-zbl@o2.pl) (D. Merta), [anna0543@o2.pl](mailto:anna0543@o2.pl) (A. Przystupińska), [zenon.soltysiak@up.wroc.pl](mailto:zenon.soltysiak@up.wroc.pl) (Z. Sołtysiak), [jaroslaw.pacon@up.wroc.pl](mailto:jaroslaw.pacon@up.wroc.pl) (J. Pacoń), [rms@up.krakow.pl](mailto:rms@up.krakow.pl) (R. Stawarz).

metals and metalloids, including carcinogenicity, impaired reproduction, teratogenicity, immunosuppression, cardiovascular and pulmonary diseases, nephrotoxicity and neurotoxicity (Wren, 1986; Goyer, 1996; Roychoudhury et al., 2010; Slivkova et al., 2010). Monitoring the concentration of metals in living organisms is thus essential and the best way of evaluating the exposition in the environment. The choice of the monitor species cannot be random because an efficient biomonitor must fulfill certain crucial demands such as its abundance, resistance to pollution and breadth of occurrence (Wren, 1986; Kalisińska et al., 2004).

As a group scavengers are very useful in ecotoxicological and biomonitoring studies (Tataruch and Kierdorf, 2003). They hold a position high on the food chain and thus accumulate a variety of substances that enter the body with food (Soulsbury et al., 2011). The red fox is a very valuable member of this group because it exhibits a measurable response to toxins, has a wide geographical distribution and is easy to acquire as wild game (Corsolini et al., 1998; Heltai and Marcov, 2012; Piskorová et al., 2003). The red fox has already been used in the biomonitoring of metals in a number of places, but still some metals (e.g. Hg) have yet to be comprehensively studied (Kalisińska et al., 2009). Since the red fox is omnivorous and occupies a position high in the trophic chain, exposure to a variety of substances, as well as to parasite infections may be substantial (Soulsbury et al., 2011; Willingham et al., 1996). It is still, however, unclear whether exposure to metals may influence the possibility of parasite infection in foxes. However, generally in ecotoxicoparasitology the connection between the mentioned variables is observed, but still in many cases not fully understood (McGrew et al., 2015). Due to the biomagnification of non-essential elements, intestinal helminthes may accumulate in higher concentrations in parasites than in the hosts (Sures, 2008).

Our studies contribute to a new area of research into various elements (including Hg) and a full statistical inquiry into the potential connection between exposure to metals and parasite infections. The main aim of the studies is to ascertain the distributions and concentrations of cadmium (Cd), calcium (Ca), copper (Cu), iron (Fe), mercury (Hg), magnesium (Mg), nickel (Ni), potassium (K), lead (Pb) and zinc (Zn) in kidney, liver and muscle tissue in red foxes (including the influences of sex and age) from the Lower Silesian Forest in southern Poland. The analysis of the occurrence of parasites and their potential connection to metal concentrations were also fully evaluated, as were the relationships between metal concentrations in tissue of the red fox.

## 2. Materials and method

81 red foxes (*Vulpes vulpes*), 41 males and 40 females, were collected by hunters during the hunting season of 2012/2013 in the Lower Silesian Forest in southern Poland (Fig. 1). After the foxes were shot and sexed the carcasses were frozen (at  $-18\text{ }^{\circ}\text{C}$ ) and transported to the laboratory of the Division of Parasitology (Wrocław University of Environmental and Life Sciences). On the basis of dental analysis (Roulichowa and Andrea, 2007) the carcasses were divided into two groups according to age: below and above one year of age (35 young and 46 adult).

### 2.1. Study site

The study area is located in the western part of the Lower Silesian Forest, a continuous lowland forest area (140–180 m a.s.l.) covering 1650 km<sup>2</sup> (51°21'N, 150°7'E). The average annual temperature for this region is 8.3 °C; average annual precipitation is 550 mm; and there are only approximately 40 days of snow cover (Bena, 2005). The forested area is covered predominantly by fresh coniferous and mixed coniferous forests comprising 82.6% of the

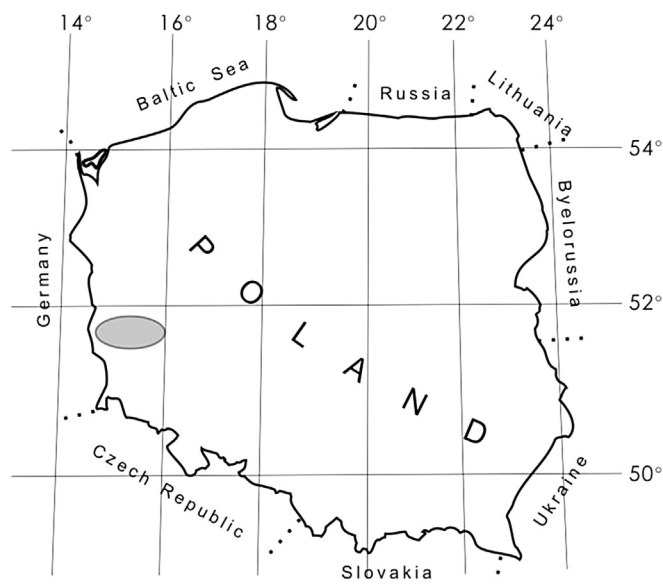


Fig. 1. Site of the research –the Lower Silesia Forest area (grey ellipse) in western Poland, central Europe.

total area (Kobielski et al., 2007). The forest is commercially exploited and dominated by even-aged stands of Scots pine (*Pinus sylvestris*), constituting 93% of the forest stand, with undergrowth consisting of berries such as *Vaccinium* spp. Pine stands are supplemented by birch (*Betula pendula*), spruce (*Picea abies*), oak (*Quercus robur*), and beech (*Fagus sylvatica*). Red fox, marten, raccoon and wolf constitute the wild predators in the area. The main sources of metal pollution in the studied area are small local emitters, such as households, and transport (Jerz et al., 2012). In specific weather and wind conditions, the influence of bigger, more distant emitters (including those from the Czech Republic and Germany) may be suspected.

### 2.2. Metal analyses

During the section, samples of kidney, liver and muscle tissue (*quadriceps femoris muscle*) were collected in the laboratory. They were then packed separately in polyethylene bags, frozen at  $-18\text{ }^{\circ}\text{C}$  and transported to the laboratory in the Institute of Biology (Pedagogical University of Cracow). Then approximately 1 g wet weight (w.w.) of each sample was dried at 60 °C (using a SUP-100W dryer, WAMED), mineralized with hot nitric acid (65%, Baker Analyzed, JT Baker) in the open mineralizer system (DK20, VelpScientifica) and diluted up to 10 mL (with ultrapure water, 18.2 MΩ cm at 25 °C, Direct-Q 3, Merck-Millipore). Thus prepared, solutions were analyzed for Ca, Cd, Cu, Fe, K, Mg, Ni, Pb and Zn with the flame AA spectrometer (AAAnalyst 200, PerkinElmer). The final results were presented as μg/g of the dry weight (d.w.) of the sample.

Mercury concentrations were measured in the wet weight samples, without the drying and external mineralization processes, with a mercury analyzer (MA-2, Nippon Instruments). The results were recalculated on the basis of water content in the samples to the concentrations in the d.w.

For each metal a limit of quantification was calculated and the whole procedure was checked against the analysis of the certified reference materials. All the recoveries and the quality of the measurements were satisfactory (Table 1). In the result section we also present the water content in each tissue sample to allow the reader

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