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Does gender influence the levels of heavy metals in liver of wild boar?

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ABSTRACT

The aim of this study was to determine heavy metal reference levels for risk assessment studies. For this purpose, the levels of lead, cadmium, copper and zinc were determined in liver tissues of wild boars sampled in NW Spain. The mean values were 0.383, 0.326, 23.50 and 56.86 mg/kg dried weight, respectively. In general, the levels detected were similar to or lower than the levels reported in literature. This study not only provides a useful baseline for biomonitoring the levels of the analyzed contaminants in wildlife in NW Spain, it also helps to understand the effects of gender on the levels of these elements. Similar to studies performed in other geographical regions, no significant gender-related differences could be detected. Although differences were not significant, the levels of zinc, cadmium and lead were modestly higher in males (55.78, 0.346 and 0.424 mg/kg, respectively) compared to females (45.25, 0.305 and 0.341 mg/kg). Our results indicate that, although gender did not significantly affect heavy metal uptake and toxicokinetics of contaminants in wild boars, these effects could vary between species, populations, organs, and elements. It is therefore essential to investigate gender-related differences for each species.

1. Introduction

Heavy metals, including lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn) are natural components of the Earth's crust. However, past and present industrial activities have resulted in a constant increase in their environmental concentrations, thereby potentially affecting wildlife adversely. Pb and Cd have no biological functions, are highly toxic, may induce both acute and chronic toxicological effects and have important ecotoxicological effects on wildlife and human health. Although Zn and Cu are essential for health and growth of animals, both reduced and excessive levels may have serious consequences on the ecosystem (Pérez-López et al., 2016). Due to their persistence and biomagnification through the food chain and potential toxicity, exposure to these chemical pollutants is of particular concern. The transfer of pollutants from the environment to biota is influenced by different environmental and biological parameters (Baker et al., 2003). Recently, the influence of gender on bioavailability, transfer and effects of contaminants has been demonstrated (Baker et al., 2003; Gonzalez et al., 2008; Fritsch et al., 2010; Tchounwou et al., 2012). Uptake, biokinetics and response to pollutants may differ significantly between male and female organisms due to differences in gene expression, germ cells, physiology and behavior (Burger, 2007). In addition, the reproductive status and period of the year may also affect gender-related patterns for metal accumulation (Robillard et al., 2002; Burger et al., 2007).

Studying wildlife populations in environments that could be potentially changed or damaged through anthropogenic activities provides relevant information about the viability and balance of those ecosystems. In addition, the use of natural populations as sentinels for environmental contamination helps to expand our knowledge of and to improve the response to environmental and human health concerns (Alleva et al., 2006). Species used for monitoring purposes should cover various levels of the food chain, such as primary (herbivorous) and secondary (carnivorous) consumers (Sánchez-Chardi et al., 2009). Game species can be used in biomonitoring studies (Froslie et al., 2001) since their tissues are known to be good bioindicators for toxic metal pollution (Santiago et al., 1998; Millan et al., 2008; Pérez-López et al., 2016). Wild boars (*Sus scrofa*) are considered particularly suitable

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as bioindicators due to their abundance in nearly all regions of Europe, both in agricultural and forest areas. In addition, wild boars are omnivorous animals. Although 80–90% of their diet comprises food collected from the ground (acorns, beech, nuts, herbs, grass, roots, rhizomes, or earth-worms), these animals also consume insects, frogs, eggs, chicks, rodents, and carrion (2–11%) (Schley and Roper, 2003; Baubet et al., 2004). The transfer of heavy metals to animal tissues proceeds predominantly through the digestive tract as a result of consumption of fodder that either contains heavy metals or is contaminated with soil. Heavy metals are, in part, taken up by wild boars due to consumption of earthworms, which are known to accumulate considerable amounts of lead as well as other heavy metals in their tissues (Latif et al., 2013). In addition, drawing the soil clods while grazing (in wild boar also known as rooting) may also play an important role in this process (Bakowska et al., 2016). Since home range size and feeding habits appear to be gender specific (Tataruch and Kierdorf, 2003), it is important to evaluate the effect of gender on heavy metal accumulation before using this species as a bioindicator.

In order to determine whether heavy metal exposure poses a threat to the environment in NW Spain, the levels of and correlations between Pb, Cd, Cu and Zn have been determined in livers of wild boars. In addition, in order to determine the suitability of this species as a bioindicator, the effect of gender on heavy metal levels has been investigated in this study.

2. Materials and methods

2.1. Field procedure and liver sampling

Wild boars ($n = 106$) were collected during the 2011–2012 hunting seasons in Galicia (NW Spain) (Fig. 1). The animals were hunted in rural areas consisting of forests and farmland with a sparse human population. Samples were grouped according to gender (53 males, 53 females). The number of young animals was too low to study the effect of age. This study therefore focused on adults between 2 and 5 years of age. To determine the age of the animals, tooth eruption and replacement was analyzed (Sáez-Royuela et al., 1989).

From each animal, liver samples were collected (approximately 25 g) and stored at −80 °C. All samples were carefully examined for presence of shot injuries, and in that case (n < 20), affected samples were eliminated from further analysis. Metal contamination was avoided as much as possible during sample preparation by cleaning or replacing surgical tools between samples.

2.2. Determination of metal levels

Three aliquots were made of each liver sample (approx. 4 g of wet tissue). The aliquots were dried at 65 °C for 72 h and weighed, to determine the moisture percentage. Samples were subsequently mineralized by adding 6 mL of an acidic solution (perchloric, nitric, and sulphuric acids, 8:8:1 by volume, trace analysis quality, Scharlau, Barcelona, Spain) and transferred to digestion tubes that were prewashed with a 10% nitric acid solution. An automatic digester (Bloc Digest 20, Selecta, Barcelona, Spain) was used to mineralize 0.5 g of dry tissue. The temperature was increased from room temperature to 370 °C in 5.5 h, according to the method described by García-Fernández (1994). Digested samples were subsequently mixed with 200 mL of HCl (trace analysis quality, Merck, Darmstadt, Germany) and diluted with deionized water to a final volume of 20 mL. Calibration standards were included in all individual analyses.

A differential pulse anodic stripping voltammetric method was used for quantitative analyses (standard addition procedure) (Hernández-Moreno et al., 2013). The instrumental detection limits were 0.005 mg/kg for Cd and Pb, and 1.25 mg/kg for Cu and Zn. A certified sample of lyophilized bovine liver was used for quality control of the analytical procedure (BCR, ref 185R, Community Bureau of Reference, EU). The recovery yields ranged from 84% for Cd to 96% for Zn, and the coefficients of variation of certified samples were always below 10%. Moreover, all samples were analyzed in batches that included analytical blanks.

Metal concentrations are expressed as mg/kg dry weight (dw), since dry values are considered to be more reliable and consistent compared to wet weight values (ww) (Adrian and Stevens, 1979). However, to compare concentrations measured in this study with published data, metal levels were recalculated using an average dryness value of 0.30 instead of 0.32 (Jankovska et al., 2010).

2.3. Statistical analysis

Data were analyzed using statistical software Prism 5 (version 5.03) for Windows (GraphPad Software Inc., La Jolla, CA, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to determine whether the data were normally distributed. A Levene's test was performed to assess the homoscedasticity of the data. Since the data were not normally distributed, a non-parametric Mann Whitney *U*-test was used to evaluate the influence of gender. The concentrations of heavy metals were presented as mean values accompanied by SEM, median, and range. Finally, a Spearman test was performed to determine the correlations among metal levels. In addition to statistically significant correlations ($p < 0.05$), correlations were classified as strong ($r > 0.5$), moderate ($r = 0.3–0.5$) or weak ($r = 0.1–0.3$) (Cohen, 1977). A value of 50% of the limit of detection (LOD) was assigned to samples with metal concentrations below LOD. These values were included in the data-set for statistical testing, a technique that minimizes nominal type I error rates (Clarke, 1998).

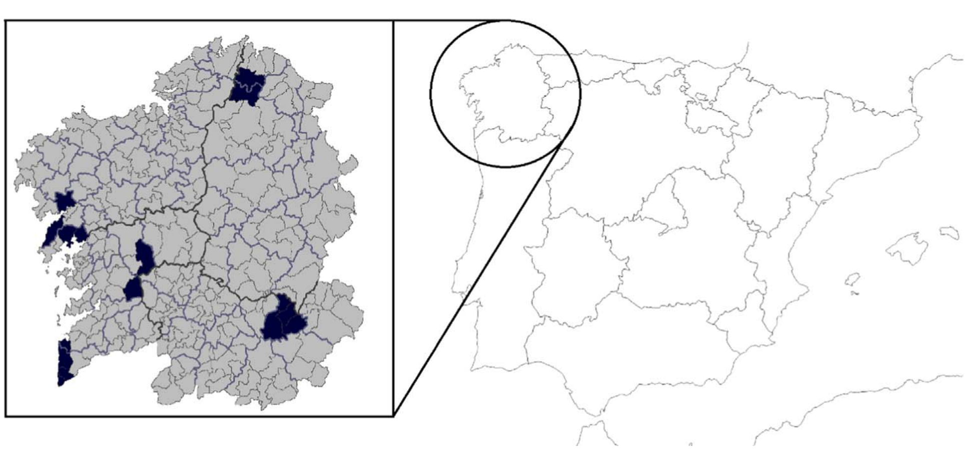

Fig. 1. Map showing the hunting areas where animals were sampled in Galicia (NW Spain).