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Bioaccumulation of metals and effects of a landfill in small mammals Part III: Structural alterations ☆

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ABSTRACT

The leachates from the Garraf landfill located in a protected site (NE Spain) contain several potentially toxic substances such as heavy metals. Here we report the histopathological alterations produced by this pollution in wild specimens of an omnivorous species, the wood mouse, *Apodemus sylvaticus*, and an insectivorous species, the greater white-toothed shrew, *Crocidura russula*. Hepatic tissue presented the most severe alterations in both the species, namely cell cycle arrest (apoptosis and necrosis), inflammation, preneoplasic nodules, vacuolation and microsteatosis. The kidneys were altered more in the mice (presenting tubular necrosis and dilatation, inflammation, and cylinders) than in the shrews, suggesting that different metabolic pathways render shrews more tolerant to renal toxicity induced by pollutants. No pollution-related alterations were observed in lung, spleen, pancreas, gonads, oesophagus, intestine, or adrenals. We conclude that the two species could be used in conjunction as bioindicators to assess the effects of environmental pollution at different trophic levels.

1. Introduction

Wild populations inhabiting polluted sites are often exposed to a mixture of chemical pollutants which are mainly taken in with the food. These pollutants result in multiple stresses and affect biological systems at virtually all levels, from molecules to ecosystems. The assessment of the risks of the diversity of pollutants to natural populations is difficult as this depends on a variety of abiotic (e.g. temperature, pluviosity) and biotic (e.g. age, gender, species) factors. However, studies under field conditions provide crucial ecotoxicological data on bioindicator species that can provide information on the quality of the environment (e.g. Pereira et al., 2006) and how to manage protected areas (e.g. Sánchez-Chardi and Nadal, 2007; Sánchez-Chardi et al., 2007, 2009). The species used for monitoring purposes should cover various levels of the food chain, like primary (herbivorous) and secondary (carnivorous) consumers. Thus, terrestrial small mammals (rodents and insectivores) have often been used as bioindicators of physical and chemical pollution (revisions in Ma and Talmage, 2001; Sheffield et al., 2001; Talmage and Walton, 1991). The wood mouse, *Apodemus sylvaticus* (Rodentia, Mammalia), is a widespread species often abundantly present in nature areas. Although occasionally showing carnivorous behaviour, this mouse is mainly a primary consumer and as such is often used as bioindicator of pollution (e.g. González et al., 2008; Rogival et al., 2007). The greater white-toothed shrew, *Crocidura russula* (Soricomorpha, Mammalia), is a relatively common secondary consumer in South Europe, predating on arthropods, molluscs and earthworms. It has recently been reported as a suitable species for ecotoxicological studies (González et al., 2008, 2009; Sánchez-Chardi and Nadal, 2007; Sánchez-Chardi et al., 2007, 2008, 2009; Wijnhoven et al., 2007). The use of wild specimens in ecotoxicological studies includes aspects of bioavailability, toxicity and detoxification mechanisms, and specific or individual exposure and susceptibility as determining factors for environmental risk under natural conditions. However, most information concerning the toxic effects of pollutants, such as heavy metals, on the structure and function of organs have derived from laboratory experiments on animals exposed to a single compound (Damek-Poprawa and Sawicka-Kapusta, 2004; Hoffmann et al., 1975; Koyu et al., 2006; Włostowski et al., 2000) and, less frequently, to multiple compounds (Jadhav et al., 2007; Silva de

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Assis et al., 2005) or complex mixtures such as landfill leachates (Li et al., 2006a, b; Świergosz-Kowalewska et al., 2006; Talorete et al., 2008). This controlled assessment of individual health status is the basis for further assessments of the true effects of pollutants on wild populations and dynamic natural systems.

Landfills are a considerable source of pollution in Mediterranean countries (Loukidou and Zouboulis, 2001), including Spain. The decomposition of disposed waste or the interaction between water and waste leads to the formation of liquid effluents named leachates. If landfills are not adequately controlled and sealed, leachates enter natural systems and cause toxic effects in plants and animals (Li et al., 2006a, b; Wilke et al., 2008). The quantity, composition and toxicity of leachates vary depending on the nature and age of wastes, the method of disposal, dump depth and climatic factors (see references in Sánchez-Chardi et al., 2007). Landfill effluents often contain a wide variety of organic and inorganic pollutants, including potentially toxic metals such as Pb, Cd, Fe, Zn, Cu, Mn, Mo, and Cr (see references in Sánchez-Chardi et al., 2007). These elements may be responsible for oxidative stress and a number of genotoxic effects reported in culture cells and laboratory rodents (Bakare et al., 2005; Li et al., 2006a, b; Talorete et al., 2008; Thomas et al., 2009) and wild mice from a waste site (Tull-Singleton et al., 1994). The induction of oxidative stress as a result of the production of reactive oxygen species (ROS) can damage lipids, thiol-proteins and nucleic acids (Jadhav et al., 2007 and references herein) thereby producing large alterations to tissues and ending in cell cycle arrest (Damek-Poprawa and Sawicka-Kapusta, 2003, 2004; Goyer, 1997; Sánchez-Chardi et al., 2008; Świergosz-Kowalewska et al., 2006). Although hepatic and renal tissues are the primary targets of pollutants ingested with the diet (e.g. Koyu et al., 2006; Włostowski et al., 2000), metals can also affect other organs and tissues, such as blood, gonads, spleen, lung, brain and bones (Damek-Poprawa and Sawicka-Kapusta, 2003; Jadhav et al., 2007; Li et al., 2006a, b), as reported in histopathological evaluations for wild rodents inhabiting near metallurgical industries (Damek-Poprawa and Sawicka-Kapusta, 2004) and abandoned mines (Pereira et al., 2006). To our knowledge, the present study is the first to compare the histopathological alterations caused by environmental pollution in two species of small mammals placed at different trophic position; a murid (wood mouse) and a crocidurine (greater white-toothed shrew). Despite the scarce information on structural alterations in wild mammals exposed to landfill leachates, previous studies in the Garraf landfill (NE Spain) reported the bioaccumulation of highly toxic metals such as Cd, Zn, Cu, and Cr and also morphological, biochemical and genotoxic alterations in *A. sylvaticus* and *C. russula* exposed to leachates (Sánchez-Chardi and Nadal, 2007; Sánchez-Chardi et al., 2007). From 1974 to 2006, the Garraf landfill served as a disposal site for domestic and industrial wastes and solid sludge from the metropole of Barcelona, accumulating about 25 million tonnes of waste. In 1986, the karstic area of Garraf granted protection status in recognition of its singular habitats and the endangered species found there. Therefore, the need to biomonitor of the landfill site increased.

The main objectives of this study are: (i) to qualify and quantify histological alterations in target tissues of two sympatric species of small mammals (a rodent and an insectivore) exposed to landfill leachates; (ii) to compare toxicity in these two species, which differ in their metabolism and trophic position; (iii) to identify the influence of sex and age as source of intra-species variation; (iv) to correlate histological alterations with metal bioaccumulation and other biomarkers; and (v) to assess the environmental consequences of landfill pollution particularly in protected areas.

2. Materials and methods

2.1. Study sites

The two study sites are located on the karstic massif of Garraf (NE Spain), a coastal system formed by hills of about 700 m, traversed by several valleys and covered by Mediterranean xerophytic vegetation. The area is located 30 km south of Barcelona city, and has granted partial protection as "Parc del Garraf". Using Sherman live traps, 49 specimens of the wood mouse, *A. sylvaticus*, and 28 specimens of the greater white-toothed shrew, *C. russula*, were trapped at two sites from February to May 1998. One polluted site called "Vall d'En Joan" is in the vicinity of the pool of the leachates, placed at the lower end of a valley. The reference site ("Olesa de Bonesvalls") is also a valley close to the landfill. No sources of pollution are known for this area.

Specimens were transported to the laboratory for dissection, following all ethical procedures for experimental animals. Sex was determined during dissection and relative age was determined on the basis of the tooth wear (see references in Sánchez-Chardi and Nadal, 2007; Sánchez-Chardi et al., 2007). The number of specimens by species, capture site, sex and age is described in Table 1. Concentrations of potentially toxic metals in liver and kidneys are reported in Table 2.

2.2. Histopathological evaluation

After dissection, the gonads, the adrenals and the left kidney and a small portion of lung, oesophagus, spleen, liver, intestine, and pancreas of all specimens were immediately fixed in 10% neutral-buffered formaldehyde. Samples of all lesions observed macroscopically were also taken and fixed. Tissues were individually dehydrated in ethanol series, cleared in toluene and embedded in Paraplast Histocomp (Vogel). Sections of 5–10 μm were stained with conventional hematoxylin and eosin and mounted in DPX. A Leitz DMRB light microscope with a camera Leica DC 500 was used for observations.

The incidence of alterations was reported in a qualitative evaluation. For statistical purposes, the alterations were measured on a semi-quantitative scale. A global score for each tissue was assigned to each specimen in a conventional scale on the basis of the severity and/or extent of lesions: without alterations (−), slightly altered (+), intermediately altered (++), and strongly altered (+++). A global score was given to each sample (0, 1, 2, 3). All results are expressed as mean and standard error of the mean ($M \pm SEM$).

2.3. Statistical analyses

For each tissue and species, the results of the semi-quantitative assessment were compared by site, age and sex using Mann–Whitney tests (U). To detect relations between histopathology and other biomarkers, Spearman's correlation coefficients (r) were calculated between the histopathological evaluation and the bioaccumulation of metals and morphometric, plasmatic, and genotoxic parameters for each species. Significant differences were accepted at $p < 0.05$. For all sequential tests, p-values were corrected using the Bonferroni adjustment. All statistical procedures were performed with the SPSS package (version 15.0 for Windows, SPSS Inc.).

3. Results

In general, the organs of specimens of *A. sylvaticus* and *C. russula* from the reference site showed healthy aspect. Livers had a compact structure and hepatocytes had a normal shape (Fig. 1A and F). Kidneys had a well developed cortex and medulla

Table 1
Number of animals captured in the reference and landfill site by species, site, age and sex (in brackets: males, females).

Species	Site	Age		Total
		Juveniles	Adults	
A. sylvaticus	**Reference**	5 (2, 3)	20 (12, 8)	25 (14, 11)
	Landfill	11 (6, 5)	13 (9, 4)	24 (15, 9)
C. russula	**Reference**	1 (1, 0)	15 (11, 4)	16 (12, 4)
	Landfill	3 (1, 2)	9 (9, 0)	12 (10, 2)