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Metallothionein levels in Algerian mice (*Mus spretus*) exposed to elemental pollution: An ecophysiological approach

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Abstract

The potential use of metallothioneins (MTs) as biomarkers of trace metal contamination was evaluated for the first time in the Algerian mouse (*Mus spretus*). Mice were collected seasonally in an abandoned mining area (Aljustrel) and in a reference area, both located in southern Portugal. MT levels were quantified in liver and kidney by differential pulse polarography and hepatic elemental concentrations (Mn, Fe, Cu, Zn, Se) were determined by particle-induced X-ray emission. Hepatic iron and selenium concentrations were elevated in mice from Aljustrel mine when compared to reference animals. MTs levels were averagely higher in mice from Aljustrel than those originated from the reference area. A season-dependent significant effect was found on the hepatic and renal MT concentrations, characterized by higher levels in winter and lower in autumn. In contaminated mice positive relationship between liver elemental contents (Cu in autumn and Fe in winter) and MTs were found. The seasonal variation of MT suggests that probably physiological and environmental factors could influence hepatic and renal MT induction. Results seem to imply that some environmental disturbance occur in the vicinity of the Aljustrel mine. Therefore, for the management purposes MT levels should be followed in liver of *M. spretus*, especially in winter. Furthermore, other physiological factors that could influence MT expression and turnover in Algerian mouse should also be monitored.

Keywords: *Mus spretus*; Abandoned mines; Elemental contamination; Metallothionein

1. Introduction

In the latest years, several studies have stated that abandoned mining areas are potential sources of environmental pollution (Quevauviller et al., 1989; Grimalt et al., 1999; Pereira et al., 2004; Peplow and Edmonds, 2005), as a result of the accumulation of significant amounts of metal residues.

Currently, in Portugal, about 85 inactive mines have recently been deactivated by economic reasons and the great majority of them without any previous environmental plans (Santos Oliveira et al., 2002). Due to the long residence time of metals in mining areas, high levels of potential harmful elements may circulate and accumulate in their surroundings, with unpredictable consequences for living organisms, including man (Walker et al., 1997). An example of this is the Aljustrel mine, located in southern Portugal (Baixo Alentejo region). During labouring period of this mine from 1867 to 1996, approximately 50000000 t of metal residues were extracted.

Several authors have reported high metal body burdens in small mammals inhabiting mining areas (Andrews et al., 1984; Nunes et al., 2001; Ruiz-Laguna et al., 2001, 2006; Viegas-Crespo et al., 2003; Bonilla-Valverde et al., 2004;

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Pereira et al., 2006). However, by merely determining the metal accumulation in tissues of sentinel species its effects at the subcellular level cannot easily be assessed. Therefore, the use of biochemical markers such as metallothioneins (MTs) may be useful in evaluating metal exposure and predicting potentially harmful effects originated by metal contamination (Roesijadi, 1992).

MTs are a superfamily of ubiquitous low molecular weight cysteine-rich proteins involved in tissue metal-homeostasis and metal detoxification, present in most mammalian tissues (Kägi and Kojima, 1987). Thus, MT may protect cells against metal toxicity, and may provide zinc/copper ions to certain metallo-enzymes and transcription factors (Nartey et al., 1987; Sato and Brenner, 1993). Due to their high cysteine content MT also participate in the cellular defence against oxidative stress resulting, for instance, from metal exposure (Miura et al., 1997; Kiningham and Kasarskis, 1998). The induction of MT within some target tissues after exposure is well documented in marine organisms in wild conditions (Serafim and Bebianno, 2001; Bebianno and Serafim, 2003; Bebianno et al., 2003; Smaoui-Damak et al., 2004; Ivankovic et al., 2005) and in mice in experimental conditions (Wlostowski et al., 2000; Suzuki et al., 2002; Irato and Albergoni, 2005), but the same information on terrestrial wild individuals is scarce (Wlostowski, 1986; Rogival et al., 2007; Swiergosz-Kowalewska et al., 2007).

In mammals, MTs are multi-regulated proteins (Kägi and Kojima, 1987) and in natural conditions many biotic and abiotic factors can differentially interfere with MT induction. Consequently, the influence of these factors on MT regulation cannot be forgotten, when assessing the potential use of MTs as a biomarker of metal contamination.

A variety of methods are currently available to quantify MT in tissues (Dabrio et al., 2002; Alhama et al., 2006). Differential pulse polarography is a simple, highly sensitive and low-cost method that can be used in routine analyses of MT contents. This technique has been successfully applied in marine organisms (Bebianno et al., 2003; Correia et al., 2004), and was first developed to be applied in mice after cadmium exposure (Olafson, 1981), but amongst wild terrestrial mammals, and rodents in particular, has rarely been used.

In this study, the Algerian mouse (*Mus spretus*, Lataste 1883), a common rodent species widely distributed in Portugal, was used as a model to assess the MT related-induction in terrestrial contaminated environments, by analyzing a population inhabiting an abandoned mining area. The influence of several factors, intrinsic and extrinsic, that may influence metal uptake and MT levels was also examined.

2. Materials and methods

2.1. Study areas

The study was carried out in the vicinity of an abandoned mining area (Aljustrel mine), located in Baixo Alentejo region in the Iberian Pyrite belt. The sampled area in Aljustrel (37°53′08″N; 08°08′32″W) is situated alongside the main watercourse (‘Água Forte’ stream), in which during rainy periods the stored acidic effluents are discharged. The climate is characterized by dry, hot summers and mild winters. Annual temperature ranged from 10.1 °C to 22.2 °C in the last decades. Maximum temperatures frequently exceed 40 °C during July and August and average total precipitation is approximately 586 mm (National Meteorological Service, Beja station, period 1961–1990). The surroundings of the study area are mainly covered by specimens of *Quercus rotundifolia* sparsely scattered. Shrubs and herbaceous species (*Rubus ulmifolius*, *Nerium oleander*, *Echium plantagineum*, *Bromus rigidus*, *Vulpia myunos* and *Phleum phleoides*) were also present.

For comparative purposes, an area with similar climate, vegetation and relief located 69 Km northeast from Aljustrel mine (38°2′15″N; 07°17′1″W), was chosen as reference, considering that no exogenous sources of metals were known.

A previous environmental survey was performed to assess the suitability of the selected reference area. This preliminary study confirmed the reduced levels of several metals in the soil and vegetation in the reference area in comparison with the mining area (Table 1).

2.2. Mice sampling and organs collection procedures

A total of 61 adult Algerian mice (reference area, $n = 30$, 19 males and 11 females; Aljustrel area, $n = 31$, 18 males and 13 females) were live-trapped on a seasonal basis, between October 2002 and August 2003, during 3-night sessions along 800 m transects. In both areas the total capture effort was 450 traps night^{-1}. At the time of capture mice were weighed, to the nearest 0.01 g, and sexed. In the laboratory, mice were sacrificed by cervical dislocation, according to legal and ethical recommendations. The liver was promptly removed, weighed (nearest 0.001 g) and divided in two fractions: one immediately stored at −20 °C for later quantification of elemental concentrations and the other frozen in liquid nitrogen for later determina-

Table 1
Elemental composition (μg g^{-1}) of soil samples[a] and a vegetation pool[b] from the reference and Aljustrel areas

	Mn	Fe	Ni	Cu	Zn	As	Rb	Pb
Reference								
Soil	173	7.4%	132	39.7	82.3	7.1	20.4	24.3
Vegetation	53.0	395	3.0	7.00	33.0	0.3	10.0	n.d.
Aljustrel								
Soil	487	5.1%	20.2	208	296	114	83.8	410
Vegetation	665	747	2.0	11.0	146	4.0	11.0	8.0

n.d., not detected.
[a] Determined by EDXRF (energy-dispersive X-ray fluorescence spectrometry).
[b] Determined by PIXE (particle-induced X-ray emission).