

Altered inorganic composition of enamel and dentin in mice teeth chronically exposed to an enriched mineral environment at Furnas, São Miguel (Azores)

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Active volcanic environments pose a multifactorial stress challenge to local organisms, including the domestic mouse *Mus musculus*. Energy-dispersive X-ray microanalysis was used to determine the elemental composition of lower incisor enamel and dentin from *Mus musculus* inhabiting the hydrothermal field at Furnas volcano in the Azores (Portugal). Elemental concentration for Ca, P, Na and Cl was found to be significantly different in mice from the volcanic site when compared to an unexposed group. Enamel surface was differently coloured; animals from the volcanic site showed a prominent yellow/red coloration when compared to the unexposed group, probably due to mineral deposition in the former group. These results are discussed in relation to the environment in which specimens were caught.

Key words: volcanism, *Mus musculus*, geothermal, chemical composition, Azores

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INTRODUCTION

Geothermal biotopes are reducing environments with certain unique features, characterised by elevated soil, water, and atmospheric elemental composition, together with constant diffuse degassing and high temperatures (Cruz et al. 1999; Viveiros et al. 2008; Viveiros et al. 2009). Volcanic gases typically comprise water vapour, carbon dioxide, sulphur dioxide, hydrogen sulphide, and hydrogen chloride with lesser amounts of hydrogen fluoride (Ferreira & Oskarsson 1999). Rocks and volatiles of volcanic origin are enriched with metals/metalloids, such as Al, As, Cu,

Hg, Pb, and Zn (Aiuppa et al. 2000; Bagnato et al. 2007; Cruz et al. 1999). The diffusion of acidic volcanic gases through the rocks mobilise metals in associated soils and water bodies, enhancing a continuous availability of elements to biota (Cunha et al. 2008; Cunha et al. 2011; Tarasov et al. 2005).

Exposure to such environments is revealed by high levels of bioaccumulated elements such as heavy metals, modified cell cycles or even tissue/cell morphometric changes (Moore et al. 1995; Zaldibar et al. 2006). Furthermore, a number of human and domestic mammal diseases, such as fluorosis, several types of cancer and

chronic bronchitis seem to be more prevalent in volcanic environments or even increase after volcanic eruptions (Amaral & Rodrigues 2007; Araya et al. 1993).

The composition and structure of calcified tissues have long been used as disease markers (Emingil et al. 2000; Paunio et al. 1993) as well as indicators of environmental contamination (Kierdorf et al. 1993). Additionally, tooth discoloration has been widely used as a marker of fluorosis, revealed by the mottling of tooth enamel (Rubin et al. 1994).

This study aimed to investigate whether individuals of *Mus musculus* (Linnaeus, 1758) exposed to an extreme environment of volcanic origin revealed alterations in the mineral composition and surface coloration of a calcified matrix, such as permanent teeth.

MATERIAL AND METHODS

Two sample groups of the domestic mouse *Mus musculus*, were collected from two sites differing in the presence of volcanic activity on São Miguel Island, Azores: i) Furnas caldera, characterised by the presence of an active hydrothermal field with a mean CO₂ release of 734 td⁻¹ (Viveiros et al. 2010) and, ii) Rabo de Peixe, with no degassing activity.

The mice were trapped with live-catch mouse-traps. The individuals were transferred to the laboratory, anaesthetised with chloroform and sacrificed. They were housed in the lab for only the sufficient time necessary to sacrifice them. From each group, a set of 10 lower incisor teeth (left side) from adult male individuals were extracted post-mortem. The enamel surface colour was registered using repeated blind measurements by a single operator. Each tooth was then embedded in epoxy, grinded with abrasives powders and polished with various diamond pastes (Fig. 1). The polished surfaces of enamel and dentin were analysed with a JEOL JSM-5410 scanning electron microscope. Energy dispersive analyses were performed with an INCA X-sight microanalyser using an acquisition time of 120s, a current beam of 15 nA and an acceleration voltage of 15 kV.

Data were registered as weight percentage (wt %) and arcsin transformed prior to analysis by means of a factorial ANOVA and Tukey post-hoc tests. Cochran's test for homogeneity of variances amongst groups was used prior to analyses to ensure operation at $\alpha=0.05$. The colour pattern was assessed using four rank criteria: 0, no coloration; 1, weak coloration; 2, strong coloration; 3, very strong coloration. Results were analysed by means of a t-test for independent samples (n=20) (ZAR 1999).



Fig. 1. Polished fracture of mouse lower incisor. Scale bar = 500 μ m

RESULTS AND DISCUSSION

Calcium and phosphorus (in both dentin and enamel) and sodium (in dentin) were found to be significantly higher in mice inhabiting the volcanic site (Fig. 2). Enamel showed a significantly higher ratio of Ca/P (measured as CaO/P₂O₅) when compared to dentin, which is normal for domestic mice (Engel 1981) and humans (Frank et al. 1966).

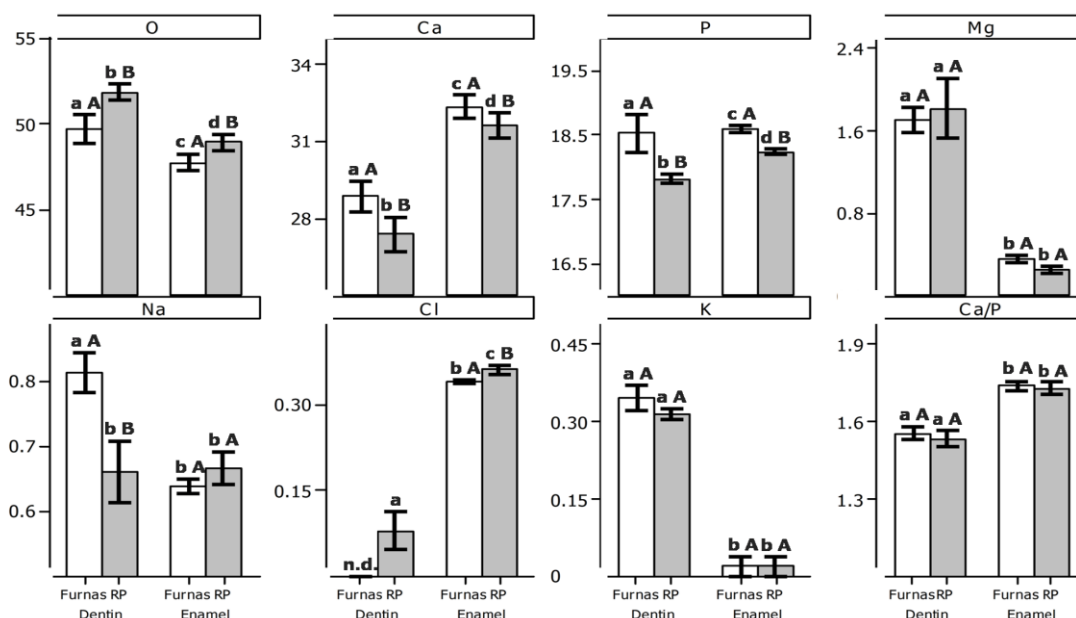


Fig. 2. Content in wt % ($\pm$ S.E.) of O, Ca, P, Mg, Na, Cl and K, and the ratio between Calcium and Phosphorus (Ca/P) in dentin and enamel of permanent teeth from *Mus musculus* inhabiting Furnas (with volcanic exposition) and RP (Rabo de Peixe) (with no volcanic exposition). Different capital letters (for site effect) and lower case letters (between structures) denote significant differences ($p < 0.05$).

Variations in dental elemental composition can reveal metabolic disorders during teeth development and after eruption (Hellwig & Lennon 2004; Kierdorf et al. 1993). Although these disorders may be of genetic origin, they are more frequently caused by variable environmental pressures (Rubin et al. 1994).

Chlorine was found in all enamel samples at higher concentration when compared to dentin composition, while potassium and magnesium were found in higher concentrations in dentin. The enriched mineralisation and trace element bioavailability in soil and water at Furnas, together with diffuse degassing phenomena, which can alter the surrounding atmosphere, may influence and perturb the normal input of elements and their metabolic pathways in animals (Baxter et al. 1999; Cunha et al. 2008; Cunha et al. 2011). Cell cycle and morphology are known to be altered under such extreme and abnormal environments (Cunha et al. 2008; Rodrigues et al. 2008), which

may also influence the development of calcified structures.

High metal levels in different organs in mice from Furnas have been documented (Amaral et al. 2007). Additionally, the effect of fluorine-rich waters, such as the water found at the Furnas springs (Cruz et al. 1999; Notcutt & Davies 1999) on teeth surface, is defined as a solid-state transformation of hydroxyapatite into fluorapatite. The first mineral species has weak Ca-P bonds in acidic environments, caused by bacteria typically living in the oral cavity and determines Ca depletion of the whole structure (Araya et al. 1993; Hellwig & Lennon 2004; Levy et al. 2010). Fluorapatite, with stronger Ca-P bonds, has a more resistant structure to acidic environments (Hellwig & Lennon 2004; Vieira et al. 2008). However, the microanalyser's detection limit is above fluorine detection and therefore it was not possible to detect and quantify the fluorine present. Nonetheless, the action of fluorine is proba-

bly the reason for the conserved higher mineral content in teeth, revealed by the Ca/P variation (Vieira et al., 2008).

The external enamel surface of mice inhabiting near the hydrothermal field showed a conspicuous and strong orange/red coloration (Fig. 3), with an average rank of 2.4 ± 0.13 ($\pm$ S.E.). This value was found to be significantly higher than mice from the non-volcanic site 0.9 ± 0.19 ($\pm$ S.E.) (T-test: T-value = -2.24; $p = 0.03$), and may indicate distinct diets of both populations. The higher iron and fluoride content of mineral water at Furnas springs may also be one of the causes; iron and fluorine are normally deposited on the surface of teeth, conferring a brownish yellow/orange colour and mottling of teeth, respectively (Halse & Selvig 1974; Rubin et al. 1994).

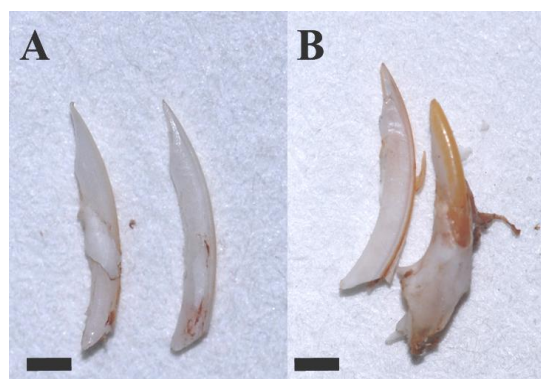


Fig. 3. Typical lower incisor of *Mus musculus* inhabiting a place without volcanic activity (A) and inside the geothermal field (B), Scale bar = 2mm.

In normal circumstances it would seem imperative to attempt dissecting the effects of individual chemical and physical stressors. This could be done by performing controlled laboratory exposures and monitoring the induced changes with a suit of morphological, physiological and molecular observations. Yet, active volcanic atmospheres comprise a range of dynamic and, in some cases, volatile constituents that are more or less continuously expelled.

The mice inhabiting the active volcanic site showed conspicuous variations in enamel mineral composition and colouration, most probably due to the surrounding extreme environment. Taking into account the continuous growth of mice dental

structures (Halse & Selvig 1974) and their living habits, in close proximity to humans, these mammals may be promising models for risk assessment under such extreme environments.

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