

# To what extent acute exposure to volcanic gases affects cells of the respiratory epithelium, peripheral oxygen saturation and lung function?

Dissertação de Mestrado

Wilson Rodrigues Tavares

Mestrado em

**Ciências Biomédicas**



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## ABSTRACT

Furnas volcano is located in the eastern part of the island of São Miguel (Azores, Portugal), where volcanic activity is marked by several hydrothermal manifestations that continuously emit volcanic gases that are inhaled by the resident population (chronic exposure) or visitors to that village (acute exposure). Taking into account the characteristic atmosphere of Furnas and the lack of studies on the effects caused by volcanic gases at the cellular level and the effects from acute exposure to volcanic gases, this study was developed with the aim to answer three questions: (1<sup>st</sup>) What is the chemical constitution of Furnas volcanic gases condensate? (2<sup>nd</sup>) What are the effects resulting from the interaction between volcanic gases condensate and cells of the respiratory epithelium (abbr. CORE)? (3<sup>rd</sup>) What are the effects resulting from an acute exposure to volcanic gases on a group of volunteers, regarding heart rate and blood pressure, peripheral oxygen saturation and lung function parameters?

The results shows that the chemical constitution of Furnas fumarole condensate is mainly Ca, Na, Si and K. Regarding the exposure of CORE to the fumarole condensate the results are inconclusive. It appears that, with increasing time of exposure, cells tended to have more anomalies (*i.e.* DNA damage, cell morphological changes), as well as more difficulty to proliferate and to growth. These effects could have been caused by a consecutive exposure to both condensates, but it could also be caused by time itself, since with time cells tend to accumulate errors and lost their initial proliferative capacity and viability by themselves. The oximetry, spirometry, blood pressure and heart rate measurement results obtained from the acute exposure of a group of volunteers to the fumaroles showed that only the heart rate was significantly affected. The decrease in the heart rate value from the pre-exposure to the post-exposure indicates that the exposure to the fumarole, and the surrounding nature, had a calm and relaxing effect on the volunteers. As far as it was possible to determine, this is the first time that such a study was carried out.

**Key words:** cells of the respiratory epithelium; heart rate; oximetry; respiratory health; spirometry; volcanic environment; volcanic gas exposure.

## RESUMO

O vulcão das Furnas encontra-se localizado na parte oriental da ilha de São Miguel (Açores, Portugal), onde a actividade vulcânica é marcada por várias manifestações hidrotermais que emitem continuamente gases vulcânicos que são inalados pela população residente (exposição crónica) ou visitantes dessa freguesia (exposição aguda). Tendo em conta a atmosfera característica das Furnas e a falta de estudos sobre os efeitos causados pelos gases vulcânicos ao nível celular e os efeitos da exposição aguda aos gases vulcânicos, este estudo foi desenvolvido com o objetivo de responder a três questões: (1<sup>a</sup>) Qual a constituição química do condensado dos gases vulcânicos das Furnas? (2<sup>a</sup>) Quais são os efeitos resultantes da interação entre o condensado de gases vulcânicos e as células do epitélio respiratório (*cells of the respiratory epithelium*, abrev. CORE)? (3<sup>a</sup>) Quais são os efeitos decorrentes de uma exposição aguda a gases vulcânicos em um grupo de voluntários, em relação à frequência cardíaca e pressão arterial, saturação periférica de oxigênio e parâmetros da função pulmonar?

Os resultados mostram que o condensado das fumarolas das Furnas é maioritariamente constituído por Ca, Na, Si e K. Quanto à exposição do CORE ao condensado de fumarola, os resultados são inconclusivos. Parece que, com o aumento do tempo de exposição, as células tendem a ter mais anomalias (isto é, danos no ADN, alterações morfológicas das células), bem como mais dificuldade em proliferar e em crescer. Estes efeitos podem ter sido causados por uma exposição consecutiva a ambos os condensados, mas também podem ter sido causados pelo decorrer do tempo em si, uma vez que o passar do tempo, as células tendem a acumular erros e a perder a sua capacidade proliferativa e viabilidade inicial por elas próprias. Os resultados da oximetria, espirometria, pressão arterial e frequência cardíaca obtidos da exposição aguda de um grupo de voluntários às fumarolas mostraram que apenas a frequência cardíaca foi afetada significativamente. A diminuição do valor da frequência cardíaca desde a pré-exposição até a pós-exposição indica que a exposição à fumarola e à natureza circundante teve um efeito calmo e relaxante nos voluntários. Tanto quanto foi possível determinar, esta é a primeira vez que tal estudo foi realizado.

**Palavras-Chave:** ambiente vulcânico; espirometria; células do epitélio respiratório; exposição a gás vulcânico; frequência cardíaca; oximetria, saúde respiratória.

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## **ABBREVIATIONS**

%SpO<sub>2</sub> – Functional oxygen saturation of arterial haemoglobin

ATS – American Thoracic Society

BDL – Bellow detection limit

BMI – Body mass index

BMRCC – British Medical Research Council's Committee

CORE – Cells of the respiratory epithelium

D2% – Dulbecco's modified eagle medium supplemented with 2% fetal bovine serum

DMSO – Dimethylsulfoxide

DNA – Deoxyribonucleic acid

DPX – Distyrene plasticizer xylene

ERS – European Respiratory Society

FEV<sub>1</sub> – Forced expiratory volume in one-second

FVC – Forced vital capacity

ICP-MS – Inductively coupled plasma mass spectrometry

MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

NA – Not applicable

PEL – Permissible exposure limit

ROS – Reactive oxygen species

SD – Standard deviation

WHO – World Health Organization

# INTRODUCTION

## General Introduction

All around the globe, it is possible to find examples of geographic coexistence of volcanoes and human populations, as it happens, for example, in the subduction complexes of Greece and Italy, in the Southeast Asian arc systems, in the East African Rift system, in the “Ring of Fire” of the Pacific ocean and in the oceanic islands of “hotspot” volcanism (*e.g.* Azores, Iceland and Hawaii) (Blong, 1984; Small & Naumann, 2001). Volcanoes are well known for their capability of originating soils rich in nutrients, which result from rapid erosion of structures (made up by lava and ash) but also by their massive potential of destruction associated with eruptive episodes, which many times occurs without warning (Simkin & Siebert, 2000). In addition to these stunning events, more discrete events occur although also potentially lethal at long term, such as the released of toxic gases and fine particulates by active volcanoes (during or after eruptions), not only through the main crater but also through fumarolic fields and diffuse degassing of soils. Thus, on one hand, populations living in close proximity to active volcanoes may obtain benefits from volcanic activity, but on another hand, their lives and health may well be under threat on a daily basis (Amaral & Rodrigues, 2011).

## Volcanic Products and Hazards – Gases and Aerosols

Everyday several tons of different compounds are produced and ejected by active volcanoes. Depending on their chemical composition, the way they are expelled, their physical characteristics, and the proximity of humans to the volcano, these compounds may pose a hazard to humans (Blong, 1984). Volcanic products and hazards usually comprise tephra, pyroclastic density currents, volcanic landslides, volcanic mudflows (lahars), gases and aerosols (Amaral & Rodrigues, 2011), but for the matter of this work only the last two will be discussed.

A gas is a state of matter in which atoms of one element, or more in case of a gas mixture (*e.g.* the air that we breathe), are far apart, fast moving, and are not organized in any particular way (West, 2008). An aerosol is defined as a collection of solid or liquid particles suspended in a gas. Aerosols are two-phase systems, consisting of the particles and the gas in which they are suspended (Hinds, 1999).

The main volcanic gases and aerosols produced by an active or dormant volcano are carbon dioxide (CO<sub>2</sub>) and water vapour (H<sub>2</sub>O), which together constitute

more than 70% of the emissions. Volcanoes also release others gases in smaller amounts, such as hydrogen (H<sub>2</sub>), hydrogen chloride (HCl), hydrogen fluoride (HF), hydrogen sulfide (H<sub>2</sub>S), carbon monoxide (CO), methane (CH<sub>4</sub>), sulfur dioxide (SO<sub>2</sub>), sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and radon (Rn) (Delmelle & Stix, 2000; Amaral *et al.*, 2006; Amaral & Rodrigues, 2011).

Depending on the type of gas emitted, the degree of hazard from volcanic gases varies. Some gases are poisonous while others are dangerous only if present in such high concentrations that they block oxygen respiration. From a health perspective, the most important volcanic gases and aerosols are CO<sub>2</sub>, SO<sub>2</sub>, Rn, H<sub>2</sub>S, HCl, HF, and H<sub>2</sub>SO<sub>4</sub> (see **Appendix 1** on APPENDIX section). Exposure to these has been the cause of the majority of volcanic gas-related pathologies and fatalities (Williams-Jones & Rymer, 2000).

### **Volcanic Products and Hazards – Heavy Metals**

Life, as we know it, has evolved with a complex interaction of organic (*i.e.* amino-acids, carbohydrates and nucleotides) and inorganic elements (*i.e.* metals). Heavy metals are natural components of the Earth soil. Their importance in biology is related to their role both as potential toxic compounds and also as essential nutrients (Vigneri *et al.*, 2017). The vital role of some metals in biology is well known: iron in hemoglobin (Gupta, 2014), zinc in nucleic acid conformation (*i.e.* zinc finger motifs) (Lai *et al.*, 2010) and selenium for thyroid deiodinases and other enzymes (Köhrle, 2005) are examples of metals essential to maintain regulated the metabolism of humans and other organisms. In contrast, higher concentrations of some metals can lead to poisoning. The toxic intracellular concentration may result from an acute exposure to a high concentration in the environment but also by progressively being exposed over time, resulting in an increased intracellular concentration compared to the environmental level, because metals may be taken up and stored in tissues faster than the compounds are metabolized and excreted (*i.e.* bioaccumulation) (Chojnacka & Mikulewicz, 2014). Chronic exposure to heavy metals results in its continuous bioaccumulation in tissues and organs, causing metal level deregulation that may result in both impaired cell functions (*e.g.* endocrine-disruptors) and also in malignant transformation (*i.e.* mutagenic, teratogenic and/or carcinogenic effects) (Järup, 2003; Rodrigues & Garcia, 2015). The work of Martín *et al.* (2011) showed that heavy metals could trigger the generation of reactive oxygen species (ROS), which can

damage deoxyribonucleic acid (DNA), lipids and proteins inducing malignant transformations in cells in terms of both initiation and cancer promotion.

Volcanic emissions are responsible for releasing several essential and nonessential metals and metalloids to the environment, the following elements being produced in higher amounts: aluminium (Al), arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), magnesium (Mg), mercury (Hg) and rubidium (Rb) (Delmelle & Stix, 2000; Amaral *et al.*, 2006; Amaral & Rodrigues, 2011). This volcanic environment is reason for concern since it contaminates the resident population and visitors via the polluted atmosphere, water and food (Abiye *et al.*, 2011; Dahal *et al.*, 2008; Queirolo *et al.*, 2000). The main threats from heavy metals to human health are associated with exposure to arsenic, cadmium, mercury and lead (Järup, 2003).

Long-term exposure to arsenic occurs mainly through consumption of polluted water (Dahal *et al.*, 2008) and it is not only related to increased risks of development of lung, kidney, bladder and skin cancer but also to some other skin lesions such as hyperkeratosis and pigmentation changes (WHO, 2001a). Short-exposure resulting from intake of large quantities of arsenic leads to severe disturbances of the gastrointestinal, cardiovascular and central nervous systems, and eventually death. Survivors from such events report several health problems like hemolysis, bone marrow depression, hepatomegaly, polyneuropathy, encephalopathy and melanosis (Järup, 2003).

Exposure to cadmium is associated with either kidney or bone tissue damages, since cadmium competes with calcium ion in physiological processes that occurs in these organs/structures (Rodrigues & Garcia, 2015). Long-term cadmium exposure, even at relatively low levels, may give rise to skeletal damage, evidenced by low bone mineral density (*i.e.* osteoporosis) and fractures (Alfvén *et al.*, 2000; Trzcinka-Ochocka *et al.*, 2010) and damage in the epithelial lining of the proximal convoluted tubules of the nephron, causing excessive excretion of proteins in urine (Nordberg *et al.*, 2002). A higher risk of kidney damage and the formation of kidney stones, possibly related to an increased excretion of calcium in urine following the initial tubular damage, has been shown in several studies (Järup, 2003; Liu *et al.*, 2016; Wang *et al.*, 2016; Avallone *et al.*, 2017). Furthermore there is data that indicates an association between cadmium exposure and developing kidney cancer (Song *et al.*, 2015) and lung cancer (WHO, 2007b).

Lead is a neurotoxic element, affecting particularly children during the

development of the nervous system, therefore, exposure to lead and its effects should be object of particular concern during pregnancy and/or during the first years of life (Rodrigues & Garcia, 2015). People who have been exposed to lead for a long time may suffer from kidney damage, anemia, prolonged reaction time, memory deterioration and reduced ability to understand anything (WHO, 2004). Acute psychosis, confusion and reduced consciousness are common in severe cases of lead encephalopathy (Järup, 2003). Also, there is a small risk of lung cancer, stomach cancer and gliomas development associated with prolonged exposure to lead (Steenland, & Boffetta, 2000). The symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system. In less serious cases, the most obvious sign of lead poisoning is disturbance of hemoglobin synthesis and proximal renal tubular damage (Järup, 2003; WHO, 2004).

The earliest symptoms of an acute exposure to mercury are paresthesia and numbness in the hands and feet. If the exposure continues, coordination difficulties, visual and auditory impairments may develop (WHO, 2007a). Chronic mercury poisoning is characterized by neurological and psychological symptoms related to nervous system damage, such as anxiety, changes in personality, depression, restlessness, sleep disturbance and tremors (Clarkson & Magos, 2006). Chronic exposure can lead also to kidney and lung damage (Järup, 2003). The most common route of poisoning by mercury is by food particularly when eating fish and marine mammals (Mieiro *et al.*, 2016), whereby a high fish based diet has been hypothesized to increase the risk of neurological damage (Freire *et al.*, 2010) and the risk of the occurrences of coronary heart disease such as myocardial infarction (Guallar *et al.*, 2002).

### **Volcanic Products and Hazards – Health Impact**

According to Cohen *et al.* (2004), exposure to air pollutants is directly responsible for about 800,000 cases of cancer and for the increase of various diseases, such as asthma, bronchitis, heart disease, low birth weight and birth defects. Among the natural sources of air pollution (*e.g.* forest fires, dust storms and biogenic pollutants) volcanic activity can be highlighted as the most relevant one (Rodrigues & Garcia, 2015). As already mentioned, during the potentially long periods between eruptions, volcanoes can continuously release gases to the surface through vents, fumaroles, hydrothermal systems, or diffusely through cracks in the soil (Durand *et*

*al.*, 2004). Compounds present in volcanic emissions can be transported at great distance by water and wind, although the surrounding environment is always the most polluted area (Vigneri *et al.*, 2017). So, for people that live near active volcanoes and for scientists and tourists that visit them, there exists the potentially serious hazard of toxic gases, aerosols and metals (Durand *et al.*, 2004). It is estimated that more than 600 million people worldwide live in the close proximity to some active or historically active volcano, either erupting or in a post-eruption phase, and thus are continuously exposed to volcanogenic pollutants (Rodrigues & Garcia, 2015).

In the long term, as result of chronic exposure to volcano-emitted products, several health problems can arise, particularly in the tissues most exposed to the toxic agents presented in a polluted volcanic environment (Amaral & Rodrigues, 2011; Williams-Jones & Rymer, 2000). Humans can be contaminated by contact (skin), inhalation (lungs) and ingestion of water/food (gastrointestinal tract). Besides that, tissues that are not directly exposed to toxic compounds can also be damaged through bioaccumulation (*e.g.* kidneys and liver, the two major organs that metabolize, filter and excrete xenobiotics) (Vigneri *et al.*, 2017). Exposure to volcanic emissions, even at low but constant concentrations, can cause health pathologies such as the development of chronic respiratory diseases like acute bronchitis (Amaral & Rodrigues, 2007; Iwasawa *et al.*, 2009; Longo & Yang, 2008), and even cancers, such as thyroid cancer (Russo *et al.*, 2015; Vigneri *et al.*, 2017), breast cancer (Amaral *et al.*, 2006; Kristbjornsdottir & Rafnsson, 2013; Russo 2015), lip, oral cavity and pharynx cancers (Amaral *et al.*, 2006) and prostate, stomach, kidney, lymphatic and haematopoietic tissue cancers (Kristbjornsdottir & Rafnsson, 2013; Russo *et al.*, 2015). These studies demonstrate that populations that are chronically exposed to volcanically active environments have a higher risk for cytotoxicity and genotoxicity on the upper respiratory tract tissues, as well of developing other respiratory pathologies and several types of cancer. Studies regarding the human health effects of acute exposure to volcanic gases are scarce, being to this date the work of Durand *et al.* (2004) the most notable in that area. Their study showed that the excretion rate and concentration of Al and Rb in urine were elevated following exposure of only 20 minutes to volcanic gas emissions.

## **Furnas Volcano Case**

The Azores is an archipelago in the centre of the Atlantic Ocean composed of nine islands situated about 1,500 km west of continental Portugal (Bentz *et al.*, 2013), where the African, Eurasian and American lithospheric plates meet (between 36°45'-39°43'N and 24°45'-31°17'W) (Searle, 1980), whereby seismic and volcanic activities are frequent in the archipelago (Ferreira *et al.*, 2005). The largest island of the archipelago is São Miguel and it is formed by three major active central volcanoes (*i.e.* Fogo, Furnas and Sete Cidades), linked by rift zones (Guest *et al.*, 1999). Furnas volcano is located in the eastern part of the island, where volcanic activity is marked by several hydrothermal manifestations consisting of active fumarolic fields, thermal and cold CO<sub>2</sub>-rich springs, and soil diffuse degassing areas (Ferreira *et al.*, 2005; Viveiros *et al.*, 2009). Gases released in these diffuse degassing areas are essentially carbon dioxide (CO<sub>2</sub>) and radon (<sup>222</sup>Rn), the last one a radioactive gas (Linhares *et al.*, 2015).

The work of Rodrigues and colleagues (2012) shows a higher risk of DNA damage in human buccal epithelial cells of Furnas inhabitants. Furthermore, in some other studies (Amaral *et al.*, 2006; Amaral & Rodrigues, 2007), evidence has been found that Furnas inhabitants have a high incidence of chronic bronchitis and of some cancer types (*e.g.* breast, lip, oral cavity and pharynx). Moreover, a study by Camarinho *et al.* (2013) showed that chronic exposure to volcanogenic air pollutants from Furnas volcano causes lung injury in wild mice and, a more recent study from Linhares *et al.* (2016) showed that individuals chronically exposed to indoor radon (<sup>222</sup>Rn) in a hydrothermal area had a higher level of DNA damage in their oral epithelial cells.

## **Objective**

Taking into account the studies mentioned above, the characteristic atmosphere of Furnas and the lack of studies on the effects caused by volcanic gases at the cellular level and the effects from acute exposure to volcanic gases, this study was developed with the aim to answer three questions: (1<sup>st</sup>) What is the chemical constitution of Furnas volcanic gases condensate? (2<sup>nd</sup>) What are the effects resulting from the interaction between volcanic gases condensate and cells of the respiratory epithelium (abbr. CORE)? (3<sup>rd</sup>) What are the effects resulting from an acute exposure to volcanic gases on a group of volunteers regarding heart rate and blood pressure,

peripheral oxygen saturation and lung function parameters?

This work aims to contribute to the assessment of the health risks from acute exposure to environments with intense secondary volcanic activity (*i.e.* diffuse soil degassing and fumaroles), particularly in cases of acute exposure to volcanic gases.

As far as it was possible to determine, this is the first time that such a study was carried out.