



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)

## Abnormal mortality of octopus after a storm water event: Accumulated lead and lead isotopes as fingerprints

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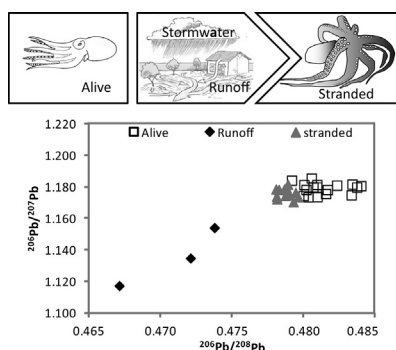
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### HIGHLIGHTS

- Short-term runoff event leads to sudden octopus mortality.
- Histology results showed severe lesions in the cells of the digestive gland.
- Pb isotopes as tracers for the rapid and high freshwater/runoff material input
- Different Pb isotopic signatures pointing to a change in Pb source with runoff
- Pb isotopes proved to be an adequate tool to confirm the cause of death.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 14 October 2016

Received in revised form 16 December 2016

Accepted 17 December 2016

Available online xxx

Editor: D. Barcelo

#### Keywords:

Octopus  
Digestive gland  
Pb isotopes  
Rainfall  
Runoff

### ABSTRACT

*Octopus vulgaris* is a sedentary organism that inhabits coastal waters being exposed to anthropogenic compounds. Lead concentration in coastal environments reflects many processes and activities namely weathering, industrial and domestic discharges, and atmospheric deposition. Since lead isotopic composition is little affected by kinetic processes occurring between source and sink, its signature has been used to identify different Pb sources. After a short-term heavy rainfall, hundreds of octopus appeared dead in two Portuguese coastal areas. Histopathology and Pb levels and its stable isotopes were determined in tissues, such as digestive gland, of stranded octopus and compared to alive specimens, sediments and runoff material from the same areas. Histology results showed severe damage in stranded octopus tissues suggesting that death was probably associated to multiple organ failure linked to hypertrophy and exudates input. In addition, Pb in stranded specimens reach concentrations up to one order of magnitude above the levels reported for alive octopus. Pb isotopic signatures in stranded organisms were closer to runoff material pointing to a similar origin of Pb. In summary, the results in this study showed that a short-term runoff event might change abruptly the salinity leading to the disruption of the osmoregulation function of octopus and consequently leading to its death. The analyses of stable isotopic Pb signature in octopus tissues corroborate these results and points to a change in the Pb source due to runoff after the storm water event. Pb stable isotopes in octopus proved to be an adequate tool to confirm the cause of death and linking it to the environment conditions.

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### 1. Introduction

Coastal environments are dynamic and complex ecosystems, and spatial-temporal variability associated with natural processes may

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mask the effect of anthropogenic pressures. Most of the existing large cities are located near the coast. Runoff from urban areas after heavy rain events, also known as storm water, often transport high concentrations of metals (Walker et al., 1999; Morrisey et al., 2003). Urban storm water runoff is known as an important source of contaminants to receiving waters (Wu et al., 1996, 1998; Sansalone and Buchberger, 1997; Davis et al., 2001). The sources of metals in urban storm water runoff are numerous and the metal release mechanisms complex (Davis et al., 2001), being transported attached to solids or dissolved. In urban environments worldwide, anthropogenic Pb originates from a variety of sources including vehicular exhaust, leaded paint, and industrial emissions. The identification of the potential sources to the environment is of extreme importance. The study of stable Pb isotopes provides a powerful tool in tracing Pb sources. The Pb isotope ratios vary with local geology and proximity to anthropogenic inputs, as well as temporally as pollution sources change. The ratios between isotopes provide an identification of different Pb sources, which have distinct isotopic signatures (Labonne et al., 1998, 2001). Physic-chemical and biological fractionation processes do not significantly alter these isotopic lead ratios. There are four stable Pb isotopes. The  $^{204}\text{Pb}$  isotope is non-radiogenic, while  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$  and  $^{208}\text{Pb}$  result from the decays of  $^{238}\text{U}$ ,  $^{235}\text{U}$  and  $^{232}\text{Th}$ , respectively (Labonne et al., 1998). Pb isotopes have been successfully applied in various studies using different matrices. For instance, Hoven et al. (1999) used isotopes ratios to distinguish between recent anthropogenic Pb inputs from background Pb in estuaries. In sediment cores, isotope Pb ratios were used as fingerprint of human activities, suggesting a change in Pb sources related to the increase of anthropogenic activities (Mil-Homens et al., 2013). The same authors also demonstrate that sediments contaminated with Pb were not constrained to estuarine-coastal areas and upper part of submarine canyons, but also to deeper parts of the Portuguese margin. By means of comparing Pb isotopes in sediments and organisms, the bioavailability of Pb to ragworms was assessed (Philippe et al., 2008). The same approach was used with octopus from different areas, and authors found that Pb isotopes could be used to distinguish octopus populations (Raimundo et al., 2009). The Pb isotope ratios were also done in bio-monitoring studies to determine the potential Pb sources to small cetaceans in European waters (Caurant et al., 2006).

Monitoring programs and research lines are focused in estuaries and coastal areas affected by relevant local emissions of contaminants (e.g. Islam and Tanaka, 2004). In recent years, mitigation measures have been implemented in various systems and, in particular, the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD) persuade managers and politicians to take actions to improve the quality status of transitional and coastal waters. The MSFD includes contaminants in commercial fishes as a key descriptor in line with the recognition of fish suitability for assessing the environmental status of marine regions (e.g. Van der Oost et al., 2003). Metal accumulation in fish tissues tends to be proportional to levels in water and food, although highly influenced by the biological role of the elements in metabolic mechanisms (Leland and Kuwabara, 1985).

Cephalopods represent an essential link in marine trophic chains. *O. vulgaris* have a short life span of 12 to 18 months, high metabolic rates and inhabit at coastal waters. This species is normally distributed on rocky, sandy and muddy bottoms (Mangold, 1983). Several studies have pointed that metal accumulation in octopus digestive gland can reflect elemental origin (Bustamante et al., 1998; Nessim and Riad, 2003; Raimundo et al., 2004; Napoleão et al., 2005; Seixas et al., 2005). In some cases, geographical variations of metal availability can overcome the biological differences (Nessim and Riad, 2003). Concentrations of Pb were showed to present contrasting geographic patterns in digestive gland of specimens collected in the Portuguese coast (Raimundo et al., 2004; Napoleão et al., 2005; Seixas and Pierce, 2005).

After a period of a heavy rainfall, hundreds of octopus died in two Portuguese coastal areas adjacent to rivers. Their arms presented symptoms of exposure to freshwater. The aims of this study were: (i) to

evaluate the impact of freshwater inputs in octopus after a high rainfall; and (ii) to trace the mechanism that caused the octopus death. These objectives were tested through: i) histological alterations in octopus tissues; and ii) determination of Pb concentrations and Pb stable isotopic signature in digestive gland, sediments and runoff particles. All biological and environmental data from the high runoff period was compared to data collected from “dry” environmental conditions. Digestive gland was selected since bioaccumulation studies have reported that storage of Pb in cephalopods occurs mainly in this tissue (e.g. Miramand and Bentley, 1992; Nessim and Riad, 2003; Seixas and Pierce, 2005; Bustamante et al., 2008; Raimundo and Vale, 2008). Although the application of Pb stable isotopes is scarce in marine organisms, their importance as a tool for environmental studies has been showed (e.g. Caurant et al., 2006; Philippe et al., 2008; Raimundo et al., 2009).

## 2. Material and methods

### 2.1. Samples

Seventy five common octopuses, *Octopus vulgaris*, were collected in two areas (Matosinhos and Cascais) located in the western Portuguese coastal zones that receive the discharge of the two major rivers of the Iberian Peninsula, Tagus and Douro, respectively (Fig. 1). Specimens were captured in two contrasting environmental conditions: i) octopuses were caught alive in November 2009; ii) dead octopuses were caught in January 2010, after a short period of heavy rains and runoff, hereafter identified as “Stranded”. Table 1 gives the number of specimens, gender proportion (males:females) and mantle length (ML, mm) and weight (g). Specimens were stored in individual plastic bags and frozen

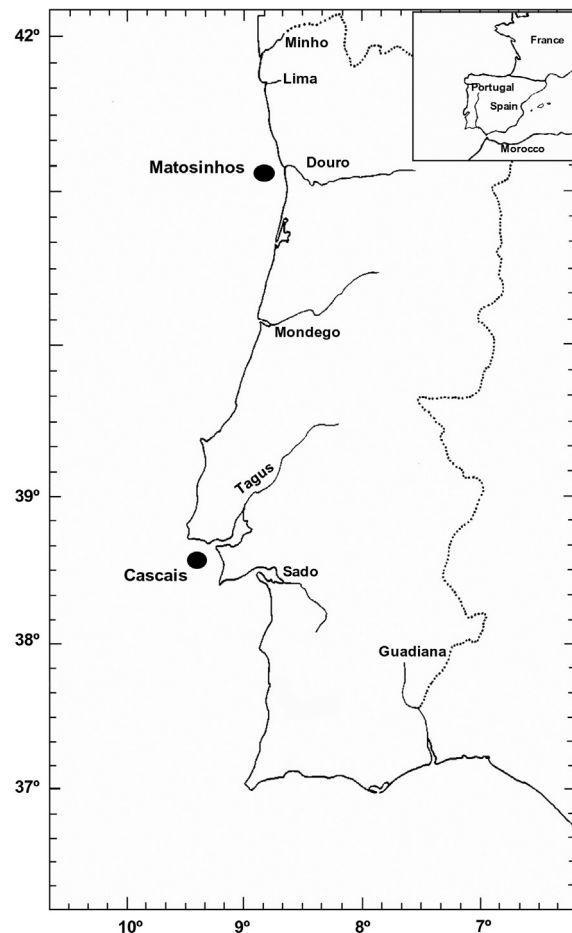


Fig. 1. Location of the two areas of capture of *Octopus vulgaris* in the Portuguese Coast: Matosinhos and Cascais.

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