Journal of Environmental Radioactivity 102 (2011) 128–137

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

journal homepage: www.elsevier.com/locate/jenvrad

Factors affecting ^{210}Po and ^{210}Pb activity concentrations in mussels and implications for environmental bio-monitoring programmes

Fernando P. Carvalho*, João M. Oliveira, G. Alberto

Nuclear and Technological Institute (ITN), Department of Radiological Protection and Nuclear Safety, E.N. 10, 2686-953 Sacavém, Portugal

ARTICLE INFO

Article history:
Received 22 January 2010
Received in revised form
4 November 2010
Accepted 7 November 2010
Available online 8 December 2010

Keywords:
Polonium-210
Lead-210
Mussels
Condition index
Environmental monitoring
Mussel watch

ABSTRACT

The activity of ^{210}Po and ^{210}Pb was determined in mussels of the same size (3.5–4.0 cm shell length) sampled monthly over a 17-month period at the Atlantic coast of Portugal. Average radionuclide concentration values in mussels were 759 ± 277 Bq kg^{-1} for ^{210}Po (range 460–1470 Bq kg^{-1} dry weight), and 45 ± 19 Bq kg^{-1} for ^{210}Pb (range 23–96 Bq kg^{-1} dry weight). Environmental parameters and mussel biometric parameters were monitored during the same period. Although there was no seasonal variation of radionuclide concentrations in sea water during the study period, the concentration of radionuclide activity in mussels varied seasonally displaying peaks of high concentrations in winter and low concentrations in summer. Analysis of radionuclide data in relation to the physiological Condition Index of mussels revealed that ^{210}Po and ^{210}Pb activities in the mussel (average activity per individual) remained nearly constant during the investigation period, while mussel body weight fluctuated due to fat storage/expenditure in the soft tissues. Similar variation of radionuclide concentrations was observed in mussels transplanted from the sea coast into the Tejo Estuary. However, under estuarine environmental conditions and with higher food availability throughout the year, transplanted mussel Condition Index was higher than in coastal mussels and average radionuclide concentrations were 210 ± 75 Bq kg^{-1} (dry weight) for ^{210}Po and 10 ± 4 Bq kg^{-1} (dry weight) for ^{210}Pb, therefore lower than in coastal mussels with similar shell length. It is concluded that the apparent seasonal fluctuation and inter-site difference of radionuclide concentrations were mostly caused by mussel body weight fluctuation and not by radionuclide body burden fluctuation. This interpretation can be extended to the apparent seasonal fluctuation in concentrations of lipophilic and lipophobic contaminants in mussels, and provides an explanation for occasional high concentrations of ^{210}Po and man-made contaminants measured in mussels far from pollution sources.

1. Introduction

Mussels have been the most used multicellular organism as indicator species for environmental monitoring of radioactive and non radioactive pollutants in coastal environments around the world. In particular, the genus *Mytilus* has been used as a sentinel organism for pollution and as a test species in many toxicity and laboratory experiments (Gosling, 1992; Goldberg and Bertine, 2000; Francioni et al., 2007; Zorita et al., 2007). The monitoring of contaminants in coastal areas using mussels is generally accomplished through bulk sampling of mussels in order to obtain the amount of sample material required by the analytical method. Geographic trends of bio-available contaminant concentrations in the marine environment have been investigated in this manner, using concentrations measured in bulk mussel samples collected at various sites, whereas temporal trends in contaminant concentrations have been investigated through comparison of concentrations usually measured in bulk samples collected on different times at the same site (Cherry and Heyraud, 1991; Heyraud et al., 1994; Wildgust et al., 1998; Villeneuve et al., 1999; Ryan et al., 1999; Ugur et al., 2002; Bustamante et al., 2002; Monirith et al., 2003; Thébault and Rodriguez y Baena, 2007).

Uranium series radionuclides, such as polonium (^{210}Po) and radioactive lead (^{210}Pb) in the environment may be both naturally occurring and of anthropogenic origin and are significantly accumulated in mussels (Koster, 1989; McDonald et al., 1996; Carvalho, 1995). These radionuclides attracted attention because they are major contributors to the internal radiation dose received by humans through ingestion with the diet (Carvalho, 1995; Yamamoto et al., 2009). Discharges of phosphatic materials in estuaries and coastal areas are reported to increase the concentrations of those radionuclides in the aquatic environment, enhancing the risk of radiation exposure to seafood consumers (Koster, 1989; Camplin et al., 1996).

We used mussels as sentinel organisms to monitor these radionuclides in the coastal environment and to assess the enhancement of concentrations due to waste releases from phosphoric acid and

* Corresponding author. Tel.: +351 219946332; fax: +351 219941995.
E-mail address: carvalho@itn.pt (F.P. Carvalho).

0265-931X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jenvrad.2010.11.003

phosphate fertilizer production near Lisbon (Carvalho, 1995; Oliveira et al., 2005). During that work it was noticed that mussels from reference areas, i.e., far from industrial waste discharges, would sometimes display higher concentrations than mussels from polluted areas. Several authors have also reported results with higher ^{210}Po and ^{210}Pb concentrations in mussels collected far from waste discharges, or mussels from pristine areas, displaying higher ^{210}Po concentrations than those measured near industrial areas impinged by phosphate discharges (Germain et al., 1995; Ryan et al., 1999; Dahlgaard, 1996; Stepanowski and Skwarzec, 2000; Godoy et al., 2008).

It is known that in organisms of the same species, radionuclide concentrations may vary with body size (Cherry and Heyraud, 1991; Vives i Battle et al., 2009). Previous research had shown that radionuclide concentrations in mussels significantly decreased with mussel size, shell length or mussel weight (Cherry and Heyraud, 1991; Ryan et al., 1999; Carvalho and Oliveira, 2008; Carvalho et al., 2010). Based on such observations, we hypothesize that other factors, related to local environment conditions and to the season of the year, might also cause important variation in radionuclide concentrations in mussels. This paper reports ^{210}Po and ^{210}Pb radionuclide concentrations in mussel soft tissues collected periodically in a coastal site and in an estuarine site, as well as biological and environmental parameters that may help explain the seasonal fluctuation and inter-site variation of radionuclide concentrations in mussels.

2. Material and methods

2.1. Coastal sampling

2.1.1. Mussel sampling

In order to investigate the seasonal fluctuation of radionuclide concentrations, monthly samples of sea water and mussels were collected at Costa da Caparica, sampling site (A) (Fig. 1). This area, south of the Tejo River estuary not far from Lisbon, is a wide arch of sandy shore, with breakwaters offering a hard substratum for the development of sub-tidal mussel beds. The area is not affected by direct waste discharges or surface runoff from land based contaminant sources. Sampling and *in situ* measurements were made during low tide, by the middle of each month, during a 17-month period.

Bulk samples of mussels (*Mytilus galloprovincialis* Lmk) were detached from the rocks and transported to the laboratory on ice. In the laboratory, mussel clumps were disaggregated and 40 mussels with shell length between 35 and 40 mm, measured with a Calliper rule, were selected. Selected mussels were checked for clean shells (no attached biota), washed, and *in toto* mussel weight determined. Mussels were dissected, byssus threads were cut and discarded, pallial water drained and soft tissues of all 40 mussels removed and pooled in one sample. The wet weight of soft tissues was determined, then tissues were freeze dried and the dry weight determined. Dry soft tissues were homogenized prior to analyses.

The Condition Index (C_i) of mussels, a simple and robust measure of mussel physiological condition, was calculated as

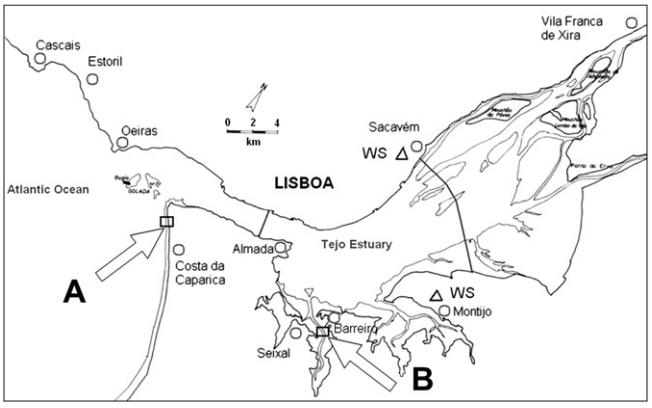

Fig. 1. The Tejo Estuary and location of sampling stations (squares) A, Costa da Caparica, and B, Barreiro Channel. Meteorological data was used from weather stations (WS, triangles) located in Montijo and Sacavém.

$$C_i = W \times L^{-3} \times 10^6$$

with W, average soft tissues dry weight (g) and L, average shell maximum length (mm) (Lucas and Beninger, 1985). In addition, observation notes were taken monthly on the development and colour of mussel gonad, that changes from grey when empty to yellow and voluminous when ripe.

2.1.2. Sea water sampling and determination of environmental parameters

Determinations of sea water physical parameters were made *in situ* with a portable probe, Horiba U-22. Sea water samples for chemical analyses were filtered on the beach, using several filtration systems and vacuum pumps, powered with a portable electric generator (Honda).

For radionuclide analysis, sea water samples of about 50 L were filtered through 0.45 μm pore size, 14.2 cm diameter, pre-weighed membrane filters. Filtered sea water was split in two samples of similar volume in polyethylene drums, acidified with HNO_3 to pH < 2, and transported to the laboratory for analysis of dissolved ^{210}Po and ^{210}Pb. Filters with suspended particulate matter were folded and placed into individual tagged plastic bags. In the laboratory, filters were oven dried at 60 °C, and the dry weight of suspended matter samples determined prior to their use for radionuclide analysis. Results for ^{210}Po and ^{210}Pb in filtered water and suspended particulate matter in replicate samples were in good agreement (<5% difference) and were averaged for reporting.

On the shore, other sea water samples were filtered for chemical analyses as follows. Three replicate 5 L sea water samples were filtered through pre-weighed and pre-combusted 45 mm diameter Whatman GF/F glass microfiber filters (average pore size 0.7 μm) for determination of suspended particulate matter load. In the laboratory these filters were dried at 60 °C in the oven, the dry weight of suspended matter determined, and filters used for determination of 450 °C combustible organic matter.

Triplicate 1 L sea water samples were filtered through pre-combusted and pre-weighted Whatman GF/F filters also for determination of organic carbon in particulate suspended matter by the potassium dichromate wet oxidation method (Strickland and Parsons, 1968). Standardization of the method was made with glucose solution standards and determinations made by titration.

Three replicate sea water samples of 0.5 L and 1 L were filtered through Millipore AA membrane filters, 0.45 μm pore size, respectively for determination of chlorophyll-a and phaeopigments by spectrophotometry according to the UNESCO method using a Shimadzu UV-2100 (Strickland and Parsons, 1968).

Records of air temperature and rainfall were provided by two National Meteorological Office weather stations located near the study area (Fig. 1).

2.2. Transplanted mussels

In order to compare the effect of environmental conditions on the accumulation of ^{210}Po and ^{210}Pb by mussels, another study area (B) was selected inside the Tejo Estuary, in the Barreiro channel (Fig. 1). From previous work carried out in this estuary it had been observed that food availability to mussels and radionuclide concentrations in the water and in the mussels could differ from those at coastal sites. In this work, instead of using mussels from the estuarine population that could be different in genetic and physiological characteristics, we collected mussels from the sampling station A on the coast and transplanted them into the estuary. For this purpose, specimens with a shell length in the 35–40 mm range were selected and grouped in 18 samples of about 60 mussels each. Each sample was put in a nylon net bag (1.5 cm mesh size) and attached to piers by the low tide mark in Barreiro channel. Sample bags were prepared in quantity to last for an 18-month period with a monthly sampling rate, however several bags disappeared and the final sample number remaining for analysis was less than planned.

Transplanted mussels were allowed to acclimate to estuarine conditions for a 1 month period before the beginning of sampling. No mortality occurred in the transplanted mussels and the sample bags were collected during a 15 month period. Mussel samples for analysis, containing 40 selected specimens each, were prepared as described above for coastal mussel samples. On the sampling day, water physical–chemical parameter measurements were performed *in situ* and water samples collected for analysis of radionuclides, algae pigments, combustible organic matter and particulate organic carbon as described under Section 2.1.2 for coastal sea water.

2.3. Radioanalytical determinations

Analyses of radionuclides in sea water were performed after ^{209}Po addition as an internal tracer for radiochemical yield and according to tested methods (Carvalho, 1995). Briefly, after thorough mixing of the tracer with bubbling nitrogen gas, radionuclides were co-precipitated from water with MnO_2, and the precipitate allowed to settle overnight, recuperated and dissolved with HCl and H_2O_2 (Carvalho, 1988, 1995). Po-210 and ^{210}Pb were co-precipitated quantitatively from sea water by this method. A first plating of polonium isotopes onto a silver disc allows the determination of ^{210}Po in water. After immediate cleaning of polonium traces from the solution with an Ag foil scrap, and following storage of sample solutions for about 6 months to allow radioactive decay of ^{210}Pb and ^{210}Po in-growth, another ^{209}Po spike is added and a second polonium plating performed in order to determine ^{210}Pb through ^{210}Po. Radionuclides dissolved in water were determined in replicate