^{210}Po and ^{210}Pb in the tissues of the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* from the Menez Gwen field (Mid-Atlantic Ridge)

Sabine Charmasson[a, *], Antoine Le Faouder[a], Jeanne Loyen[b], Richard P. Cosson[c] and Pierre-Marie Sarradin[d]

[a] Institut de Radioprotection et de Sûreté Nucléaire, IRSN-DEI Centre IFREMER, BP 330, F-83507 La Seyne Sur Mer Cedex, France
[b] IRSN-DEI, 31, rue de l'Ecluse, BP35, 78116 Le Vésinet Cedex, France
[c] MMS, Université de Nantes, BP 92208, 44322 Nantes Cedex 3, France
[d] IFREMER Centre de Brest, DEEP, B.P. 70, 29280 Plouzané, France

[*] Corresponding author : Sabine Charmasson, email address : Sabine.Charmasson@irsn.fr

Abstract:

The hydrothermal deep-sea vent fauna is naturally exposed to a highly specific environment enriched in potentially toxic species such as sulfides, metals and natural radionuclides due to the convective seawater circulation inside the oceanic crust and its interaction with basaltic or ultramafic host rocks. However, data on radionuclides in biota from such environment are very limited. An investigation was carried out on tissue partitioning of ^{210}Po and ^{210}Pb, two natural radionuclides within the ^{238}U decay chain, in *Bathymodiolus azoricus* specimens from the Mid-Atlantic Ridge (Menez Gwen field). These two elements showed different distributions with high ^{210}Pb levels in gills and high ^{210}Po levels in both gills and especially in the remaining parts of the body tissue (including the digestive gland). Various factors that may explain such partitioning are discussed. However, ^{210}Po levels encountered in *B. azoricus* were not exceptionally high, leading to weighted internal dose rate in the range 3 to 4 µGy h^{-1}. These levels are slightly higher than levels characterizing coastal mussels (~ 1 µGy h^{-1}).

1. Introduction

Hydrothermal vents provide hot, reduced and acidic fluids enriched in various compounds, such as hydrogen sulfide, methane, carbon dioxide and various metals (see review by German and Von Damm, 2007). These fluids are also enhanced in ^{238}U decay-chain nuclides such as ^{226}Ra, ^{222}Rn, ^{210}Pb and ^{210}Po (e.g. German et al., 1991; Kadko, 1996; German and Von Damm, 2007). Hydrothermal fluid composition is very variable on both the spatial and temporal scales. The starting fluid is mainly seawater which is modified by processes occurring within the oceanic crust along the hydrothermal flow path. Among these processes, water-rock interaction and phase separation are the most important. The overall influence of biological activity the vent-fluid geochemistry as well as the significance of magma degassing (due to volcanic eruptions and/or diking events) versus the more "steady-state" venting has yet to be established (German and Von Damm, 2007). In particular ^{210}Po-degassing has been reported during mid-ocean ridge and seamount volcano eruptions (e.g. Rubin, 1997).
In biota, ^{210}Po (half-life of 138.4 d) and its grandparent ^{210}Pb (22.3 y) present ratio generally greater than one (Cherry et al., 1992). Indeed, ^{210}Po accumulates to high levels in the tissue of various marine organisms (Heyraud and Cherry, 1979; Heyraud et al, 1988; Stepnowski and Skwarzec, 2000). In addition, ^{210}Po is an alpha-emitting radionuclide and is the main contributor to the natural internal radiation dose received by marine organisms (Cherry and Heyraud, 1982; Carvalho, 1988). The fact that polonium has been found to be correlated with sulfur-binding protein and other sulfur-seeking metals (e.g. Stewart et al., 2007) enhanced the interest of studying this element in sulfur-rich environments such as hydrothermal vents.

The peculiar and highly productive dense fauna colonizing hydrothermal vents is not dependant on photosynthesis but rely on microbial chemosynthetic primary producers using reduced chemicals present in the hydrothermal fluid (e.g. Fisher, 1990; Cavanaugh et al., 2006). The hydrothermal vent bivalve Bathymodiolus azoricus is an endemic and dominant species at various hydrothermal vent sites along the Mid-Atlantic spreading center where it can form extensive beds surrounding the active area (Desbruyères et al., 2000). The adaptive success of *B. azoricus* in such challenging environments is due to its mixotrophic nutrition. Indeed, this species hosts both methanotrophic and thiotrophic bacterial symbionts in its gill cells (Fiala-Médioni et al., 2002; Duperron et al., 2006). This dual symbiosis allows *B. azoricus* to cope with changes in geochemical regimes at the vent sites providing them a strong nutritional advantage. In addition, B. azoricus also derives its nutritional requirements by direct suspension-feeding (Riou et al., 2010)

While several studies on the presence and effects of metals on B. azoricus have been carried out (Geret et al., 1998; Company et al., 2008; Colaço et al., 2006; Kádár et al., 2006; Cravo et al., 2007; Cosson et al., 2008), to the best of our knowledge no data has been reported regarding ^{210}Po and ^{210}Pb in this species. Indeed, very little data exists on ^{210}Po and ^{210}Pb content in hydrothermal fauna (Cherry et al., 1992; Boisson et al., 2001; Charmasson et al., 2009). These authors have mentioned relatively high levels for these two elements in Alvinella and Paralvinella polychaetes from the East Pacific Rise (Cherry et al., 1992; Charmasson et al., 2009) and in the Cyclope gasteropod and Tellina bivalve in the Aegean Basin in the Mediterranean Sea (Boisson et al., 2001).

In the present study we determined ^{210}Po and ^{210}Pb levels in Bathymodiolus azoricus from the Menez Gwen field (Mid-Atlantic Ridge). We first present the concentration and ^{210}Po/^{210}Pb activity ratios variations of these naturally-occurring radionuclides and then discuss tissue distribution, the isotopes' likely trophic route, possible detoxification processes and radiation doses arising from internal exposure to ^{210}Po.

2. Materials and methods

2.1 Sampling

The mussels were sampled at the Menez Gwen hydrothermal vent field (Figure 1), which was discovered in 1994 during the DIVA cruise (Fouquet et al., 1994). This vent is a shallow hydrothermal site with a depth of 850 m along the Mid-Atlantic Ridge, southwest of the Azores Triple Junction. The site is characterized by small active hydrothermal structures venting clear with low mineral particle content. The faunal communities are dominated by large patches of the bivalve <u>Bathymodiolus azoricus</u> abundantly dispersed in the substratum with specimens abundance ranging from 400 to 700 individuals/m^2 (Colaço et al., 1998).

During the MoMARETO cruise (07/08/2006 – 06/09/2006), mussels were sampled using the grab of the ROV Victor6000 and stored in four acoustically retrievable cages. The cages were deployed on small vent outlets before their recovery. Mussels studied in this study come from the third cage, which was recovered in May 2007 (i.e. after 9 months) by the R.V. Arquipélago. All the samples were taken from the same area since they were within the same collecting cage. They are thus supposed to have been exposed to the same physical and chemical environment and so the range in measured concentrations should not be linked to environmental differences in these parameters.

Once the mussels were brought to the surface, they were immediately placed in cool particle-free seawater in the Azorean land-based hydrothermal vent laboratory, LabHorta (Colaço and Santos, 2003), for 3 to 4 days in order to depurate them of faeces and pseudo-faeces. The animals were in good state of health, as indicated by the color of their gills.

We selected hydrothermal mussels with almost similar size. This narrow size range was chosen in order to reduce age/size effects since allometric relationships with negative slope for both ^{210}Po and ^{210}Pb in marine organisms have been reported (e.g. Cherry and Heyraud, 1991). Organs were pooled in groups of five specimens with close shell size i.e. mean shell length (± 1 standard deviation) of 54.84 (±1.44), 54.04 (±0.96), 53.32 (±1.80) and 60.52 (±3.28) mm respectively for the A, B, C and D groups. An analysis of variance (ANOVA) revealed significant difference (P <0.001) between the means of the different groups. A multiple comparison test i.e. LSD (least significant difference) was then applied in order to determine which means differed from one another (Sokal and Rohlf, 1981). Group D is characterized by a mean significantly greater than groups A, B, and C, whose means do not differ from each other (P>0.05).

For each group of 5 individuals, gills, mantles, feet and the remaining parts of the soft tissue (including the digestive gland) were removed from the shells with sterile stainless steel razor blades and the byssus threads were discarded. These soft tissues were weighed, deep frozen (- 80°C) and lyophilized (Savant RVT400) at LabHorta. The samples were then homogenized using an agate mortar and pestle, in order to avoid any external contamination. These samples were processed to determine both ^{210}Po and ^{210}Pb content through alpha-counting following chemical purification.

In order to compare these concentrations with values encountered in coastal organisms, we performed ^{210}Po and ^{210}Pb analyses in the soft tissue of the Mediterranean coastal mussel, <u>Mytilus galloprovincialis</u>, sampled in Toulon (France). The mean size of this group was 67.25±3.38 mm (n=10) and was not statistically compared with the other data for <u>B. azoricus</u> since it is separate species (i.e. different growth rate and metabolism).

2.2 ^{210}Po-^{210}Pb determination

Concentrations of ^{210}Po and ^{210}Pb were measured using a classical technique based on two ^{210}Po measurements at a several month interval to allow ^{210}Po in-growth from the ^{210}Pb present in the samples. These analyses were performed at the IRSN – STEME laboratory (Le Vésinet – France). Dried samples of known weight (0.2-1.1g) were spiked with ^{209}Po (E$_\alpha$ = 4.9 MeV) as a tracer, in order to calculate the chemical recovery of polonium from the analyzed samples after the first chemical treatment. The samples were dissolved in a glass beaker using a 150 ml of a mixture of concentrated acids, HNO$_3$-HCl (2/3-1/3). They were then evaporated slowly, to near dryness, on a hot plate at a temperature of about 80°C. Then, 10ml H$_2$O$_2$ (30%) in 20ml of concentrated HNO$_3$ were added to whiten the sample residues. These were slowly dissolved with 35 ml of a mixture of concentrated HNO$_3$-HClO$_4$ (30ml/5ml) and again evaporated to near dryness. This step was performed 2 or 3 times until all the organic material was digested (Boisson et al, 2001). The sample was then dissolved in 20 ml of HCl (6M) (Matthews et al, 2007) and transferred into the deposition cell together with 2 rinses of the beaker using demineralised water, in order to obtain a total volume of approximately 100 ml in the Teflon deposition cell. Around 0.1 g of an ascorbic acid (C$_6$H$_8$O$_6$) salt was added as a reducing agent. The solution was stirred at room temperature in contact with a stainless steel disc (0.5 mm thick and 50 mm in diameter previously rinsed with ethanol (95%)) for 5 hours. Polonium spontaneously plated onto the disc (Miura et al, 1999). The plated disc was rinsed with demineralised water, dried and measured for 20,000 s using alpha spectrometry (CANBERRA grid chamber). ^{210}Po specific activity in the samples was calculated, using GENIE 2000 software, on the basis of the added ^{209}Po activity and expressed in the unit Bq.kg^{-1} dry weight (dw). The average value of the chemical recovery was 90% (individual values ranged from 71% to 100%).

The deposition solution was carefully transferred to a sealed bottle and stored at 3°C in a refrigerator to allow ^{210}Po in-growths from the ^{210}Pb of the sample. After 6 months, this remaining HCl solution was transferred to the Teflon deposition cell. About 0.1 g of an ascorbic acid (C$_6$H$_8$O$_6$) salt was added as a reducing agent and the sample was re-spiked with ^{208}Po (E$_\alpha$ = 5.1 MeV) as a yield tracer to calculate the deposition recovery of polonium during the second plating. The solution was stirred at room temperature in contact with a new stainless steel disc for 5 hours. ^{210}Po from the sample spontaneously plated onto the disc. The plated disc was rinsed with demineralised water, dried and measured for 20,000 s using grid chamber alpha spectrometry. ^{210}Po specific activity in the samples was calculated on the basis of the added ^{208}Po activity. The average chemical recovery reached 95% (individual values ranged from 85% to 100%).

In order to accurately estimate ^{210}Po supported by ^{210}Pb, the ^{210}Po activity determined from this second plating was corrected for the low remaining ^{210}Po activity in the solution after the first plating. This remaining ^{210}Po activity was calculated taking into account both the first chemical recovery and the activity decrease in the elapsed time between the 2 different depositions. The ^{210}Po and ^{210}Pb concentrations at the sampling time were calculated from these two separate counts and are expressed in the unit Bq.kg^{-1} dry weight (dw).

Analytical quality control measurements were regularly performed by reference sample analyses, blank sample analyses, and participation in international inter-comparison exercises such as ^{210}Po IAEA 2007-09.
In order to check that ^{210}Pb does not plate together with ^{210}Po onto stainless steel discs two tests were carried out. First a deposition disc, which had been in contact with a solution containing a known ^{210}Pb activity (and ^{210}Bi) in HCl (0.1M), was measured in a proportional beta counting detector (CANBERRA Serie5); this showed no detectable beta activity. Second the ^{210}Po deposition method was applied to 200 ml of a stable Pb solution prepared with 2 ml of a certified SPEX solution (9,985 mg / ml) diluted in HNO$_3$ 0.1M. A measurement of the Pb concentration was performed after the deposition step using an ICP-OES apparatus (Ultima Horiba Jobin Yvon). The Pb concentration measured was equal to the reference

concentration expected, demonstrating no detectable loss of Pb by deposition onto the stainless steel disc.

3. Results and discussion

3.1 ^{210}Po ^{210}Pb levels in Bathymodiolus azoricus
The entire set of radionuclide concentrations and activity ratios is presented in Table 1.

(table 1)

^{210}Po and ^{210}Pb concentrations in <u>Bathymodiolus azoricus</u> tissue ranged from 134.8 to 990.9 Bq.kg^{-1} dw and from 21 to 331.4 Bq.kg^{-1} dw respectively. Whole organism (soft tissue) concentrations were calculated from the tissue concentrations and weight tissue proportions, these were from 551.4 to 669.2 Bq.kg^{-1} dw for ^{210}Po and 83.7 to 169.5 Bq.kg^{-1} dw for ^{210}Pb. The coastal <u>Mytilus galloprovincialis</u> showed lower concentrations and lower spread in the various tissues i.e. from 72.4to 318.1 Bq.kg^{-1} dw for ^{210}Po and from 3.5 to 34.4 Bq.kg^{-1} dw for ^{210}Pb. Whole organism concentrations were estimated to be 203.1 and 41.3 Bq.kg^{-1} dw for ^{210}Po and ^{210}Pb respectively. These levels are quite consistent with data previously reported for this species (McDonald et al., 1986; Uğur et al., 2002; Desideri et al., 2009). For various taxa, Cherry et al. (1992) reported median ^{210}Po and ^{210}Pb values nearing 267 Bq.kg^{-1} dw and 10 Bq.kg^{-1} dw (whole organisms) respectively, with ^{210}Pb concentrations showing a lower spread (Heyraud et al., 1988).
From these comparisons, it appears that coastal mussels exhibit lower activity levels and confirm the enrichment for both ^{210}Pb and ^{210}Po radionuclides in deep-sea hydrothermal vent mussels. However, these levels do not appear exceptionally high when compared, for example, to the ^{210}Po concentrations in the digestive gland of a coastal scallop ranging from 3,150 to 4,637 Bq.kg^{-1} dw (Bustamante et al., 2002), although our results refer to the remaining tissue, i.e., the digestive gland being only a part of it. Heyraud et al. (1988) reported high ^{210}Po concentrations in shrimps with a maximum of 25,863 Bq.kg^{-1} dw for whole organisms and 150,590 Bq.kg^{-1} dw in the hepatopancreas, the latter value being the highest level ever encountered in marine organisms.

In radioecology, it is common to use a concentration factor (i.e. the ratio of the concentration of a given radionuclide in an organism to that in the surrounding water) to assess the importance of bioaccumulation processes for a given species. For coastal bivalves, the recommended value given by IAEA (2004) is 5 10^4 for Pb and 2 10^4 for Po. We were not able to obtain seawater samples from the Bathymodiolus sampling area and to the best of our knowledge there are only few published sets of data on both ^{210}Po and ^{210}Pb in vent fluids, and they do not concern the Mid-Atlantic Ridge. It should also be underlined that CF is a parameter assuming that the organisms are in equilibrium with their ambient sea water (steady-state conditions), and this requirement is often far from fulfilled in vent systems since on the one hand the composition of vent fluids is highly variable in space and time and on the other hand strong chemical processes occur within the plumes. In our view, another major factor which lessens the usefulness of such a parameter is the importance of particulate forms in these vent environments. A more satisfactory approach would be to use bioenergetic-based kinetic models taking into account accumulation from both aqueaous and dietary phases, as have been developed for coastal mussels (Reinfelder et al., 1998), though such models allow estimates to be made of steady-state metal body or tissue concentration for a given exposure situation. Apart from the concentration of elements in the dissolved and particulate phases these models require the knowledge of a number of parameters such as the assimilation efficiency of the ingested element, the element efflux rate as well as physiological parameters such as the ingestion rate and the growth rate of the

organisms. These parameters are not yet quantified for B. azoricus but preliminary experimental work has begun on this species (M. Warnau, personal communication) and will be continued in the near future.

$^{210}Po/^{210}Pb$ activity ratios (A.R.) in B. azoricus highlighted excesses in ^{210}Po, and ranged mainly from 1.9 to 18.6, while for coastal mussels the range is narrow, i.e. 9.3 to 20.9. These values are in the lower ranges characterizing marine fauna, since Cherry et al. (1992) mentioned that, based on over 280 data obtained on non-vent organisms, this ratio is characterized by a median of about 32 and is less than 1.8 in only two cases. ^{210}Po excesses observed in B. azoricus are not consistent with previous data gathered in vent environments by Cherry et al. (1992) and Charmasson et al. (2009). Indeed, these authors reported high ^{210}Po and ^{210}Pb concentrations with AR close to 1 in Paralvinella grasslei polychaetes sampled on the East Pacific Rise. However, Boisson et al. (2001) found a $^{210}Po/^{210}Pb$ ratio of 48 in the bivalve Tellina tenuis collected in a vent zone off Milos Island (Mediterranean Sea). These various organisms are suspension-feeders, therefore these discrepancies are very likely due to different metabolism processes.

It is well known that ^{210}Po and ^{210}Pb associate with particles in the oceans (Bacon et al., 1976; Cochran et al., 1983) with preferential adsorption on organic particles for ^{210}Po (Stewart et al., 2007) and on fine inorganic particles for ^{210}Pb (Tateda et al., 2003). Indeed, within the water column above vent areas, radionuclide measurements indicate rapid ^{210}Po and ^{210}Pb uptake by plume particles from seawater i.e. hydrothermal scavenging (Kadko et al., 1986/1987; German et al., 1991; Yongliang et al., 2002). Kadko et al. (1986/1987) showed marked ^{210}Po deficiencies relative to ^{210}Pb within the plume, with $^{210}Po/^{210}Pb$ ratio values below 0.65, suggestive of Po removal from the water column. It has been suggested that ^{210}Po may associate preferentially with Mn-oxyhydroxide (Kadko et al. , 1986/1987) and that ^{210}Pb may have a greater affinity for Fe-oxyhydroxide particles (Kadko, 1993) hence leading to a fractionation of these two isotopes within the plume. In addition vent plumes are especially rich in bacteria which could be a key step in polonium transfer within hydrothermal vent communities. Indeed, Kim (2001) suggested that the significant deficiency of polonium in the Sargasso Sea was linked to its efficient trapping by bacteria and transfer to higher trophic levels. Therefore the ingestion of various kinds of particles by B. azoricus when feeding on suspended matter is certainly one of the pathways involved in the accumulation of these elements.

3.2 Feeding and metabolism

The ^{210}Po and ^{210}Pb tissue distributions appeared to be different between coastal and deep-sea mussels (table 1). In coastal mussels, both radionuclides exhibited concentrations which varied in the following manner: other tissue (including digestive gland)> gills > foot = mantle while in *B. azoricus*, these two isotopes showed a different tissue distribution. The highest ^{210}Po concentrations were found in the other tissue (including digestive gland) and in the gills, with slightly lower levels in the latter, while the highest ^{210}Pb concentrations were observed in the gills, and almost similar concentrations were observed for the mantle, the foot, and the other tissue (including digestive gland).

In Bathymodiolus azoricus, tissue distribution of both ^{210}Po and ^{210}Pb probably highlight different uptake routes and/or assimilation efficiencies. As mentioned above, B. azoricus is characterized by a mixotrophic nutrition via suspension-feeding and its association with symbiotic bacteria in the gills. The parameters that determine and control the participation of suspension-feeding to the total energy budget are currently being studied (Martins et al.,

2008). These authors have determined size-dependent variations of the nutritional pathway. They have considered shell length from 10 to 110 mm and revealed that small mussels are strongly dependent on suspension-feeding, while larger ones obtain a significant proportion of their energy from endosymbiosis, related to the variation of gill weight with total weight. Using their model, as our mussels have an average shell size of 56mm, the relative contribution of filter-feeding should be about 40%, under estimated Menez Gwen H_2S and CH_4 conditions. For chemoautotrophic symbionts, Fiala-Médioni et al. (2002) reported a greater dependency on methanotrophy in the Menez Gwen Bathymodiolus population in relation to environmental conditions. De Busseroles et al. (2009) analysing $\partial^{13}C$ and $\partial^{15}N$ signatures in B. azoricus from the Tour Eiffel vent (Lucky Strike), suggested that small mussels inhabiting the cooler microhabitats rely primarily on thiotrophy whereas larger mussels tend to colonize warmer areas and rely more on their methanotrophic symbionts. However, their work revealed a high degree of variability in isotopic signatures which is certainly linked to variations in the vent fluid characteristics at small spatial scales and thus variations in the local carbon sources. Indeed, as they underlined, evidence of the strong influence of hydrothermal fluid characteristics on both the diversity and activity of vent primary producers is growing.

Gills exhibited high levels of both radionuclides with quite low $^{210}Po/^{210}Pb$ ratios i.e. in the range 2 to 4. These organs are directly exposed to dissolved and particulate forms. Due to the sulfur-analog properties of polonium, it is possible that gills provide a preferential uptake route for this element given the fact that B. azoricus gills host sulfo-oxidizing bacteria. Harada et al. (1989) suggested that ^{210}Po in Florida groundwater could be fixed by sulfo-oxidizing bacteria. Cherrier et al. (1995) showed that once associated with the bacteria cell, polonium is dispersed between the cell walls, cytoplasm and protein in a manner similar to sulfur. Vent species harbouring sulfur-oxidizing endosymbionts have to supply their symbionts with sulfide which is highly toxic. Pruski et al. (1998) proposed that sulfur-amino acid (i.e.thiotaurine) synthesis inside the bacteriocytes could act as a trap for free sulfide protecting the cells against its toxicity and serve as a non-toxic reserve of reduced sulfur for the symbiont in various symbiotic species from hydrothermal vents and cold seeps.

The high ^{210}Pb levels can be linked to lead sequestration within the gill tissues under insoluble forms by their symbionts (Chassard-Bouchaud et al., 1986). They can also be due to microvillar transfer of particles as observed by Kádár et al (2008) in non-symbiotic ciliated gill cells in B. azoricus. In addition, it has been suggested that mucus excreted by gill cells plays a role in depuration processes for metals in B. azoricus (Kádár, 2007). In the same way Juniper et al. (1986) reported mucus enriched in various trace elements and sulfide minerals in vent alvinellid polychaete worm suggesting a role of mucus secretion in detoxification processes.

In agreement with the fact that the specimens we studied relied partly on suspension-feeding and also that ^{210}Po has been found to concentrate in the hepatopancreas of several marine invertebrates (e.g. Heyraud and Cherry, 1979; Heyraud et al., 1988; Bustamante et al., 2002), we found high ^{210}Po concentrations and higher AR in the remaining tissue (including the digestive gland) of the mussels (Table 1). Indeed, the main ^{210}Po uptake route in marine animals is through food ingestion (Heyraud et al., 1988; Carvalho and Fowler, 1993, 1994; Charmasson et al., 1998), which makes it possible to use this isotope as an indicator of marine organism diet (Heyraud et al., 1988). The faster turnover of ^{210}Pb compared with ^{210}Po and the greater ^{210}Po digestive assimilation efficiency (e.g. Carvalho and Fowler, 1993; Stewart et al, 2007) account for the disequilibrium observed in the remaining tissue (including the digestive gland). High concentrations of ^{210}Po in the digestive gland is also certainly to be linked to its association with sulfur-rich, metal-binding proteins present in these organs as

demonstrated by Durand et al. (1999, 2002). Indeed, according to Wildgust et al. (1998), [210]Po behaves mainly like a class B metal (which readily bind with –SH, -S-S, -SR and –NH$_2$ functional groups) though they also reported correlation with borderline metals and class A metals such as calcium and aluminium (phosphate, carboxylic and carbonyl functional groups).

In the case of Bathymodiolus azoricus, the multi-contaminant context may be important for [210]Po bulk in tissue, especially in the gills and digestive organs. Indeed exposure to various metals in hydrothermal vent fauna is known to increase the amount of metal-binding proteins such as the metallothioneins (Geret et al., 1998) and, as suggested by Swift et al. (1995), this increase can reduce the elimination rate for [210]Po by increasing the number of binding sites. However, when Hardivillier et al. (2006) and Company et al. (2008) exposed Bathymodiolus specimen to various monometallic solutions, they did not observe a clear induction of metallothionein synthesis in gills and mantle. This could be due to high pre-existing metallothionein levels in hydrothermal biota since levels of metallothioneins were higher in hydrothermal vent mussels compared to coastal mussels.

3.3 Radiation dose estimates

Hydrothermal vent biota is continually exposed to toxic compounds (metals, sulfides, radionuclides and reactive oxygen species) which may cause DNA damage (Pruski and Dixon, 2003). Regarding radionuclides, toxicity is related to the amount of energy deposited in an organism over time i.e. dose rate expressed in Gray per hour (Gy h-1). The dose rate is radiation type dependent i.e. exposure to α-radiation is more effective than γ-rays and most β-radiation in producing biological damage. In the last years, method for assessing radiation dose factors to aquatic organisms per unit radionuclide concentration in the surrounding media and in the organism itself has been developed (Brown et al., 2004; Vives I Battle et al., 2004). Regarding the marine environment it is well known that the radiation doses are mainly due to internally incorporated [210]Po (Cherry and Heyraud, 1982; Carvalho, 1988). Therefore in order to estimate doses received from [210]Po in B. azoricus, weighted internal dose rates (D) were calculated using the simplified approach presented in Connan et al. (2007) by applying the following equation:

$$D = A * DPUC^{int} * R_{wf}$$

where A is the [210]Po specific activity in the organisms (whole body) Bq kg^{-1} ww, DPUC[int] is the dose rate per unit concentration factor for internal exposure for [210]Po (µGy h[-1] per Bq kg^{-1}), R$_{wf}$ is the radiation weighting factor (this factor is applied in order to take into account the radiation quality i.e.).
Data presented in Table 2 were calculated using DPUC[int] of 3.1 10^{-3} µGy h[-1] per Bq kg^{-1} and a R$_{wf}$ of 10 for alpha radiation according to Brown et al. (2004) and Vives i Battle et al. (2004). It has to be underlined that DPUC[int] were determined assuming that radionuclides are distributed uniformly through all tissues.

In B. azoricus the weighted internal dose rates arising from [210]Po varied from 3.2 to 3.8 µGy.h[-1] (Table 2) while in M. galloprovincialis the value is slightly lower i.e. 1.1 µGy.h[-1]. This latter dose rate corresponds to the value calculated by Brown et al. (2004) for marine molluscs and is consistent with the values calculated by Connan et al (2007) for another coastal mussel species, M. edulis.
(table 2)

At the present time, the development of a system ensuring the protection of the environment against ionising radiation is internationally debated (IAEA, 1999; ICRP, 2003; Pentreath, 2009). Therefore guidelines values, such as the PNEDR (Predicted-No-Effect Dose Rate) i.e. the potential value below which it is accepted that a chronic dose has no effect at the population level, have been recently derived (10 µGy.h^{-1} for aquatic ecosystems; Garnier-Laplace et al., 2006). But direct comparison of the PNEDR value with our estimated weighted dose rate is not correct since this value is benchmark designed to protect the ecosystem but not any one organisms in particular (Mathews et al., 2009).

Databases on radiation effects on biota have been developed in the last years within various European projects (e.g. the FREDERICA database; Copplestone et al.2008). However literature data on effects at the organism level are mainly dealing with short-time radiation exposures whilst in our case deep-sea vent organisms are exposed to lifetime radiation. Concerning this latter point, Sazykina (2005) presented interesting data on radiobiological effects of low-linear energy transfer radiation (gamma rays, x rays) for chronic/lifetime exposures observed on wildlife organisms in contaminated area within the former Soviet Union. She reported minor cytogenetic effects for sensitive vertebrate species within the dose range 4 to 20 µGy h^{-1}. Comparable effects have been found to occur in <u>Mytilus edulis</u> exposed experimentally to tritiated water (soft β- emitter) at low-dose range 12-500µGy h-1 for 96h for adults (Jha et al., 2005) and at dose rates down to 1-12µGy h-1 for 16,7-23h in the case of early life stages (embryo-larvae) (Hagger et al., 2005). However, as underlined by Gwynn et al. (2006) the extrapolation of biological effects observed at lower level of biological organisation (i.e. chromosomal, cellular) to a higher level (i.e., individual) is no simple matter.

Godoy et al. (2008) did not find any effect on micronuclei frequency and DNA breaks in <u>Perna perna</u> mussels showing ^{210}Po concentrations in the range 54-460 Bq kg^{-1} dry weight. It is therefore unexpected that ^{210}Po concentrations observed in <u>B. azoricus</u> lead to high radiation exposure. However we have to keep in mind that these organisms are exposed to a variety of toxic compounds and determined exposure levels they are subject to is certainly very complex.

4. Conclusion

These results provide a first step toward improving the data bank concerning natural radioactivity in the deep-sea environment and, more especially, in deep-sea hydrothermal vent ecosystems. ^{210}Po levels encountered in <u>B. azoricus</u> were not exceptionally high (552-670 Bq kg-1 dw whole organisms) leading to weighted internal dose rate in the range 3.2 to 3.8 µGy h^{-1}. These levels are however slightly higher than levels characterizing coastal mussels. Due to the fact that ^{210}Po is an alpha emitter, internal distribution of dose especially in tissues of possible importance to ecological endpoints, such as gonads, is of interest. Applying the same very simple approach to estimate the weighted dose rate to the various tissue, of which ^{210}Po contents are presented in table 1, would lead to weighted absorbed dose rates ranging from 0.8 - 2 µGy h^{-1} for the mantle, up to 5.7 -7.3 µGy h^{-1} for the remaining tissue (including the digestive gland), with gills around 3.1-3.7 µGy h^{-1}. However the biological and ecological significance of any elevation in doses to a specific organ is at present unclear (Brown et al., 2004).

In any case a lot remain to do for characterizing the radiation environment these organisms are subject to. Indeed, as underlined by Stewart et al (2008), if ^{210}Po and ^{210}Pb contents are quite well documented in marine organisms, there is a strong lack of data concerning all the other isotopes in the U-Th series.

Acknowledgements

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Tables

Table 1: ^{210}Po and ^{210}Pb concentrations (Bq.kg^{-1} dw ± 2σ propagated counting uncertainties) and ^{210}Po/^{210}Pb activity ratios in various tissue in Bathymodiolus azoricus (A-D) from the Menez Gwen site and Mytilus galloprovincialis (Coastal) from the Toulon harbor.
n= number of organisms; mean size ± 1 standard deviation; (size range in mm).

	Organ	^{210}Po	±	^{210}Pb	±	(^{210}Po/^{210}Pb)	dw[+] g	ww/dw[+]
A	Gills	826.4	102.4	242.3	41.4	3.4	1.01	6.97
n=5	Mantle	327.8	43.0	53.5	16.7	6.1	1.35	5.07
54.84±1.44	Foot	217.0	44.7	64.1	20.7	3.4	0.53	4.58
(53.4-57.2)	Other tissue (including digestive	990.9	85.9	67.2	18.9	14.7	1.34	4.20
	Whole organism *	643.1	71.0	104.3	23.8	6.2	4.23	5.19
B	Gills	751.7	102.2	189.3	40.8	4.0	0.99	7.05
n=5	Mantle	134.8	39.8	69.9	22.9	1.9	1.25	5.22
54.04±0.96	Foot	209.9	33.0	<29.1	-	-	0.37	4.68
(53-55.5)	Other tissue (including digestive	852.6	75.3	46.0	18.2	18.6	1.49	4.64
	Whole organism *	551.4	67.1	83.7	22.7	6.6	4.1	5.40
C	Gills	760.3	92.3	331.4	33.3	2.3	0.89	7.60
n=5	Mantle	261.6	34.3	89.8	14.0	2.9	1.26	5.18
53.32±1.80	Foot	234.1	46.8	62.9	19.6	3.7	0.36	4.58
(50.9-55.6)	Other tissue (including digestive	928.5	81.5	52.1	20.3	17.8	1.56	4.58
	Whole organism *	623.9	66.2	121.0	20.3	5.2	4.07	5.43
D	Gills	899.0	102.5	271.4	43.1	3.3	1.32	7.71
n=5	Mantle	264.3	35.5	101.1	15.4	2.6	1.48	5.97
60.52±3.28	Foot	186.0	37.6	21.0	13.4	8.8	0.47	4.91
(56.8-65.1)	Other tissue (including digestive	924.3	272.9	97.2	25.7	9.5	2.05	4.59

	Whole organism *	669.2	143.8	169.5	32.7	3.9	5.32	5.78
Coastal	Gills	165.6	22.8	10.6	6.6	15.6	1.25	7.93
n=10	Mantle	72.4	9.0	3.5	2.1	20.9	3.07	6.63
67.25±3.38	Foot	75.5	24.1	<43.6	-	-	0.21	4.26
(64-75)	Other tissue (including digestive	318.1	27.7	34.4	5.2	9.3	4.13	4.82
	Whole organism *	203.1	20.3	41.3	8.6	4.9	8.65	5.90

<: value below detection limit (main factors affecting the detection limit value are the blank activity of the detection system, the chemical yield, the size of the sample, the counting time, and the detection efficiency). * calculated from the ^{210}Po tissue concentrations and weight tissue proportions. $^{+}$ dw=dry weight, ww=wet weight

Table 2: Weighted internal dose rate to deep-sea hydrothermal vent mussel <u>Bathymodiolus azoricus</u> (A-D) and coastal mussel <u>Mytilus galloprovincialis</u> arising from ^{210}Po. (±2σ propagated counting uncertainties) dw: dry weight, ww: wet weight.

Organism	^{210}Po Whole-body concentration* Bq kg^{-}1 dw ± 2σ	^{210}Po Whole-body concentration* Bq kg^{-}1 ww ± 2σ	Weighted internal dose rate from ^{210}Po (μGy.h^{-1})
B. azoricus			
A	643.1 ± 71.0	123.9 ± 21.4	3.8
B	551.4 ± 67.1	102.1 ± 19.2	3.2
C	623.9 ± 66.2	114.9 ± 12.2	3.6
D	669.2 ± 143.8	115.9 ± 24.9	3.6
M. galloprovincialis	203.1 ± 20.3	34.4 ± 3.4	1.1

* calculated from the ^{210}Po tissue concentrations in table 1 and weight tissue proportions
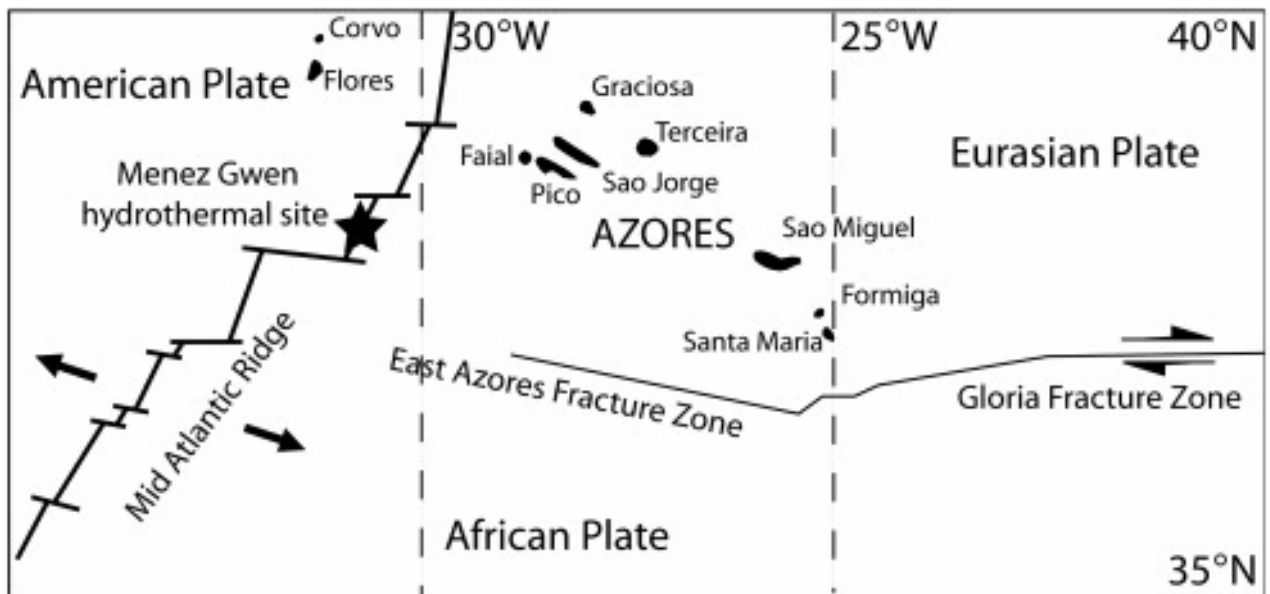
Figures

Figure 1: Location of the Menez Gwen hydrothermal field in the Mid-Atlantic Ridge. The Azores islands and the major tectonic structures are represented (map adapted from Fouquet et al., 1994).