

# Bioaccumulation of $^{210}\text{Pb}$ and $^{210}\text{Po}$ in fish tissues in a radioactive naturally enhanced area: the Peníscola marsh (Castelló, Spain)

Author: López Castillo, E.

Directors: Jordi Garcia-Orellana,  
Núria Casacuberta Arola

Setembre 2011

- Llicenciatura de Ciències Ambientals, UAB-

## Abstract

$^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentration in fish tissues from Peníscola marsh were analyzed, being this area a radioactive naturally enhanced marsh located in the East coast of Spain. Results showed that  $^{210}\text{Po}$  accumulation in tissues could reach values ranging from  $28\pm 8$  Bq  $\text{kg}^{-1}$  in muscle of *Cyprinus carpio*, to  $8558\pm 6378$  Bq  $\text{kg}^{-1}$  in gut content of *Chelon labrosus*. On the other hand,  $^{210}\text{Pb}$  concentrations ranged from  $8\pm 4$  Bq  $\text{kg}^{-1}$  in muscle of *Cyprinus carpio*, to  $475\pm 481$  Bq  $\text{kg}^{-1}$  in gut content of *Chelon labrosus*. Bioaccumulation pattern is generally  $^{210}\text{Po} > ^{210}\text{Pb}$ , except in spine, where more  $^{210}\text{Pb}$  than  $^{210}\text{Po}$  is accumulated. When comparing our samples to those collected as blanks, individuals from Peníscola marsh showed an enrichment in  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  in their tissues compared to the blanks. Bioaccumulation factors showed that feeding is the major input route of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  into the fish body. Highest values of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentration in tissues were found on *Chelon labrosus* and *Carassius auratus*, being *Cyprinus carpio* the species with the lowest average values of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  accumulation.

Keywords:  $^{210}\text{Pb}$ ,  $^{210}\text{Po}$ , fish tissues, bioaccumulation.

## Resum

La concentració de  $^{210}\text{Pb}$  y  $^{210}\text{Po}$  present en diversos teixits de diferents espècies de peixos de la marjal de Peníscola va ser determinada. Aquesta àrea és una zona naturalment enriquida en radioactivitat, situada a la costa est d'Espanya. Els resultats mostren una acumulació de  $^{210}\text{Po}$  als teixits que va dels  $28\pm 8$  Bq  $\text{kg}^{-1}$  en el múscul de l'espècie *Cyprinus carpio* fins als  $8558\pm 6378$  Bq  $\text{kg}^{-1}$  al contingut intestinal de *Chelon labrosus* i pel  $^{210}\text{Pb}$ , de  $8\pm 4$  Bq  $\text{kg}^{-1}$  en el múscul de l'espècie *Cyprinus carpio* fins als  $475\pm 481$  Bq  $\text{kg}^{-1}$  al contingut intestinal de *Chelon labrosus*. El patró d'acumulació trobat sempre ha respost a  $^{210}\text{Po} > ^{210}\text{Pb}$ , excepte en el cas de les espines, teixit en el qual s'acumula sempre més  $^{210}\text{Pb}$  que  $^{210}\text{Po}$ . En comparació amb les mostres blanc analitzades, els individus de la marjal de Peníscola presenten un enriquiment en  $^{210}\text{Pb}$  i  $^{210}\text{Po}$  en els seus teixits. Els factors de bioacumulació mostren que la ingesta d'aliments és la font principal de  $^{210}\text{Pb}$  i  $^{210}\text{Po}$  als organismes. Els valors de bioacumulació més elevats van ser trobats a les espècies *Chelon labrosus* i *Carassius auratus*, sent *Cyprinus carpio* l'espècie que menys  $^{210}\text{Pb}$  i  $^{210}\text{Po}$  va bioacumular.

Paraules clau:  $^{210}\text{Pb}$ ,  $^{210}\text{Po}$ , bioacumulació, teixits de peixos,

## Resumen

La concentración de  $^{210}\text{Pb}$  y  $^{210}\text{Po}$  presente en diferentes tejidos de diversas especies de peces de la marjal de Peñíscola fue determinada. Este área es una zona naturalmente enriquecida en radioactividad, situada en el este de España. Los resultados muestran una acumulación de  $^{210}\text{Po}$  en los tejidos con valores entre  $28\pm 8$  Bq  $\text{kg}^{-1}$  en el músculo de la especie *Cyprinus carpio* hasta  $8558\pm 6378$  Bq  $\text{kg}^{-1}$  en el contenido intestinal de *Chelon labrosus*, y valores de  $^{210}\text{Pb}$  que van desde los  $8\pm 4$  Bq  $\text{kg}^{-1}$  en el músculo de la especie *Cyprinus carpio* hasta  $475\pm 481$  Bq  $\text{kg}^{-1}$  en el contenido intestinal de *Chelon labrosus*. El patrón de acumulación hallado siempre responde a  $^{210}\text{Po} > ^{210}\text{Pb}$ , excepto en el caso de la espina, tejido en el cual se acumula siempre más  $^{210}\text{Pb}$  que  $^{210}\text{Po}$ . En comparación con muestras blanco analizadas, los individuos de la marjal de Peñíscola presentan un enriquecimiento en  $^{210}\text{Pb}$  y  $^{210}\text{Po}$  en sus tejidos. Los factores de bioacumulación indican que la ingesta de alimentos es la fuente principal de  $^{210}\text{Pb}$  y  $^{210}\text{Po}$  en los organismos. Los valores de bioacumulación más elevados fueron encontrados en las especies *Chelon labrosus* y *Carassius auratus*, siendo *Cyprinus carpio* la especie que menos  $^{210}\text{Pb}$  y  $^{210}\text{Po}$  bioacumuló.

Palabras clave:  $^{210}\text{Pb}$ ,  $^{210}\text{Po}$ , bioacumulación, tejidos de peces.

## Introduction

Until recently, human health was the major focus of radiation protection practices, and it was understood that, if standards were set to protect human health, no other species would be threatened as a population even if individuals of the species were

harmed. However, awareness about the vulnerability of the marine and terrestrial environment has grown, also the need of protecting the environment against anthropogenic pollutants including radionuclides. Consequently, radiation protection philosophy has begun to evolve, increasing the emphasis on

protecting biotic populations other than man from the potential effects of radiation (Pentreath, 1999).

Some of the natural radionuclides of the  $^{238}\text{U}$  series ( $^{226}\text{Ra}$ ,  $^{210}\text{Pb}$  and  $^{210}\text{Po}$ ) and others of the  $^{232}\text{Th}$  series ( $^{228}\text{Ra}$ ) are considered crucial either for their toxicological significance or for their special accumulation behaviour in the environment (Shaheed *et al.*, 1997). The existing database regarding interactions of biota with naturally occurring radionuclides is slightly limited and considers a few isotopes whose half-lives and chemical characteristics make them interesting and suitable for different uses, such as tracers of productivity and carbon flux in the ocean (i.e. Murray *et al.*, 2005), which are scientifically used as chronometers of biogenic processes (i.e. Turekian *et al.*, 1979) or act as sources of ionising radiation for marine organisms (Cherry and Heyraud, 1982). Regarding this latest reason,  $^{210}\text{Po}$  is considered the most important source of internal radiation dose from natural sources to marine organisms (Cherry and Shannon, 1974; Cherry and Heyraud, 1982).

Alpha-emitting  $^{210}\text{Po}$  with a half-life of 138 days is a daughter of  $^{210}\text{Bi}$  and a granddaughter of  $^{210}\text{Pb}$  (Eisenbud and Gesell, 1997). The environmental  $^{210}\text{Pb}$ , with a half-life of 22.3 years, arises mainly due to the decay of  $^{222}\text{Rn}$  gas emanating from the earth's soil into the atmosphere.  $^{222}\text{Rn}$  gas decays to  $^{210}\text{Pb}$  via short-lived particulate nuclides ( $^{218}\text{Po}$ ,  $^{214}\text{Po}$ ,  $^{214}\text{Pb}$ ,  $^{214}\text{Bi}$ ) (Eisenbud and Gesell, 1997).

There is very few data regarding accumulation of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  in terrestrial ecosystems (i.e. Brown *et al.*, 2010) and there is even less data about interactions in freshwater or brackish environments (i.e. Clulow *et al.*, 1998; NKS, 2009). This pair of radionuclides has attracted the attention of scientists because of their relatively high concentrations in marine organisms in comparison with those in terrestrial organisms (Carvalho, 2011). Furthermore, it is of special relevance the greater accumulation of  $^{210}\text{Po}$  in marine biota compared to its grandparent  $^{210}\text{Pb}$ , as the former one might lead to greater human doses in case of ingestion of the organisms which accumulated it (Cherry and Shannon, 1974; Parfenov, 1974).

The bioaccumulation of  $^{210}\text{Pb}$  or  $^{210}\text{Po}$  refers to a process by which these radionuclides are accumulated in various tissues of a living organism. The level to which a radionuclide is accumulated in an organism depends on its chemical characteristics and speciation in water or sediment, as well as biological processes, including rates of uptake from water or diet, excretion, and metabolic transformation. These in turn, may be influenced directly by the physiology of the organism which is, of course, affected by diverse biological, physical and chemical factors,

such as habitat, feeding behaviour and species (Stewart *et al.*, 2008).

Neither lead nor polonium have any known biological function; hence, organisms would not actively be 'seeking' to incorporate them through enzymatic action or through specific membrane channels or other transport mechanisms, as happens with essential metals (Williams, 1981; Simkiss and Taylor, 1995). Besides, the concentrations of these radionuclides are generally so low that organisms would have to expend impractical amounts of energy to concentrate them from the surrounding water (Stewart *et al.*, 2008).

Lead and polonium, which speciate as cations in seawater, display very strong binding effects to particle surfaces, including organisms (Stewart *et al.*, 2008). Pb associates largely with dissolved carbonates (Bruland, 1983) and is an oxygen-seeking metal that frequently associates with mineral fractions of organisms (i.e. bone, shell and structure) (Nieboer and Richardson, 1980).

In living organisms, polonium associates with proteins (Cherry and Heyraud, 1981, Fisher *et al.*, 1983; Stewart and Fisher, 2003). Due to this reason and its position in group VI of the periodic table, it has been suggested that Po acts as a sulphur-analogue like Se or binding to sulphur ligands (Schwarz, 1976; Cherrier *et al.*, 1995; Church and Sarin, 2008).

Despite the numerous studies on  $^{210}\text{Po}$ , the specific mechanism of uptake remains unclear. Because the uptake is unaffected by light or temperature, and there is no biological requirement for this element, it appears that it is taken up inadvertently as an analogue of some needed element (Stewart and Fisher, 2003a).

However, ingestion is the main route of entry of this radionuclide (Carvalho and Fowler, 1994).  $^{210}\text{Po}$  is readily assimilated by marine primary producers (Fisher *et al.*, 1983) and further concentrated along the food chain, a behaviour that has been linked to sulphur uptake (Cherry and Shannon, 1974).

Unlike Pb, Po can penetrate into the cytoplasm of cells (Fisher *et al.*, 1983; Stewart and Fisher, 2003a).

Both radionuclides are particle reactive elements and therefore, once associated with single cells, there is the possibility that they can be assimilated into the tissues of animals that ingest those phytoplankton cells (i.e. molluscs, zooplankton). However, the efficiency with which ingested elements are assimilated in herbivores appears to be directly related to the extent to which they can penetrate into the cytoplasm of phytoplankton cells (Reinfelder and Fisher, 1991; Stewart and Fisher, 2003b). Assimilated Po pass through trophic chain, from herbivores to carnivores that consume them.

Then, Po is bioconcentrated in the tissues of diverse marine animals at higher trophic levels.

Regarding to terrestrial environments, Brown *et al.* (2010), studied activity concentrations of  $^{210}\text{Po}$  in fauna (invertebrates, mammals and birds). They found that concentrations ranged between 2 and 123 Bq kg<sup>-1</sup> dry weight and in plants and lichens between 20 and 138 Bq kg<sup>-1</sup> dry weight. Focusing on small mammals, activity concentrations fell within a range from 23 to 85 Bq kg<sup>-1</sup> dry weight.  $^{210}\text{Po}/^{210}\text{Pb}$  ratios were higher than ratios from other organisms studied, appearing to be indicative of a preferential uptake or prolonged retention of  $^{210}\text{Po}$  relative to  $^{210}\text{Pb}$  for this group of mammals in this particular environment.

NKS (2009) analyzed  $^{210}\text{Po}$  and other radionuclides in a terrestrial freshwater environment. Average concentrations of  $^{210}\text{Po}$  in lake waters was 1.9 mBq kg<sup>-1</sup>. Regarding to fishes, values of  $^{210}\text{Po}$  concentration in whole fish ranged from 1.0 to 6.5 Bq kg<sup>-1</sup> fresh weight. They analyzed edible parts and other parts separately, finding that in edible parts, concentration was one order of magnitude lower.

The main objective of this study was to determine the bioaccumulation in  $^{210}\text{Pb}$  and  $^{210}\text{Po}$ , in both, different fish species and fish tissues from samples collected in a Mediterranean coastal wetland (the Peníscola wetland, Castelló, Spain) characterised by having high levels of  $^{226}\text{Ra}$  ( $T_{1/2}= 1600$  y) and  $^{222}\text{Rn}$  ( $T_{1/2}= 3,8$  d) due to high values of radium in sediments and water ( $^{226}\text{Ra}=2 - 3 \cdot 10^3$  Bq·m<sup>-3</sup>,  $^{222}\text{Rn} =6.7 \cdot 10^2 - 6.2 \cdot 10^5$  Bq·m<sup>-3</sup> in water and  $^{226}\text{Ra}=2.2 \cdot 10^2 - 7.8 \cdot 10^2$  Bq·kg<sup>-1</sup> in sediments linked to the groundwater discharge from the Maestrat aquifer (Rodellas-Vila, 2009). Due to these high concentrations of natural radionuclides and because both  $^{226}\text{Ra}$  and  $^{222}\text{Rn}$  belong to the  $^{238}\text{U}$  decay chain so being  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  grandfathers, this area represents an ideal location for the study of the interaction between  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  and fishes of this brackish environment.

This main objective was divided into several specific objectives:

- Analyse the different accumulation of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  in tissues (i.e. kidney, muscle, gut, gills, spine and hepatopancreas).
- Identify the major route of entry of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  into the fish body.
- Study the dependence of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  accumulation with size and species.
- Analyse bioaccumulation factors (BAF), in order to determine the correlation between concentration of both radionuclides in tissues and its concentration water and feed.

## Materials and methods

Samples of *Chelon labrosus*, *Carassius auratus*, *Cyprinus carpio* and *Gambusia holbrooki* from Peníscola marsh and blank fish samples were fished. Water samples were collected in order to evaluate concentration factors (CF) from water to fish tissues.

The four species collected have several differences between them. While *C. labrosus* is an autochthonous and catadromus species, the other are introduced species. *G. holbrooki* is a small generalist predator and individuals caught had between 0 and 2 years. The other species have lifespan longer than 10 years. *C. auratus* is an omnivorous species. *C. labrosus* feed mainly on benthic diatoms, epiphytic algae, small invertebrates and detritus. *C. carpio* is selective benthic omnivorous that specialize on invertebrates that live in the sediments. Its feeding technique, of grubbing around in the sediment and straining food from the mud, has caused problems in areas where the carp has been introduced. As well as uprooting submerged vegetation, it also increases the turbidity of the water (Kottelat *et al.*, 2007).

Regarding biological samples, three stations were selected (St2, St3 and St6) under the hypothesis that  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  accumulation in organisms would be potentially different due to the different  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentrations in water (Rodellas-Vila, 2008).

Individuals were dissected and excepting *G. holbrooki*, from each individual, gonads, kidney, hepatopancreas and gut were obtained. Gut content was removed from the gut by squeezing and was deposited in a Petri dish and weighed. The gut was washed with water in order to remove any remaining content. After a portion of each tissue was obtained, samples were deposited in a Petri dish and dried at 60°C for 24h as to obtain fresh weight and dry weight.

$^{210}\text{Po}$  and  $^{210}\text{Pb}$  were determined by  $\alpha$ -spectrometry. For this purpose, 0,250 g. were weighed and transferred into teflon beakers, spiked with 0.1 mL of  $^{209}\text{Po}$  ( $0,703 \pm 0,014$  Bq mL<sup>-1</sup>) as a yield tracer and digested with 40 mL of nitric acid at 75°C of temperature overnight. The residue was dissolved by adding hydrogen peroxide. After a digestion, the solution was evaporated to dryness to remove the HNO<sub>3</sub> and subsequently converted to a hydrochloric form by adding 2-3 mL of concentrated HCl and evaporated to dryness. This step was repeated three times (García-Orellana, 2004).

The dried sample was dissolved in 80 mL of 1M HCl and placed on a magnetic stirrer with thermostat control at a temperature of 75°C. With the addition of ascorbic acid to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup>

(until the solution was colourless), thus eliminating interference in the deposition of polonium.

$^{210}\text{Po}$  and  $^{209}\text{Po}$  from the solution was spontaneously deposited onto a silver disc (25 mm diameter) suspended in the sample solution by means of a nylon thread taped to the beaker. One face of the disc was lacquered with urethane in order to avoid  $^{210}\text{Po}$  isotope to deposit into this face.

The silver disc was kept spinning at that temperature for a period of 6 hours with the aid of the stirrer. At the end of the plating period, the disc was taken out, rinsed with Milli-Q water and dried.

After plating, the solution was stored for 6 months to allow ingrowth of  $^{210}\text{Po}$  from  $^{210}\text{Pb}$ , and then the  $^{210}\text{Po}$  plating step was repeated.

In the case of water samples, five stations of surface water were sampled (Wst2, Wst3, Wst4, Wst5 and Wst6). 3 L of water were collected and each container was properly labelled. Temperature, conductivity and salinity were measured in the field in all samples with a multi-parameter probe YSI 556. Samples were filtered at  $1\mu\text{m}$  pore by using a sandwich filter and a peristaltic pump, in order to separate particulate and dissolved fraction. Subsequently, samples were spiked with  $^{209}\text{Po}$  and  $\text{Pb}^{2+}$  as yield tracers and acidified to stabilize it. Once at the laboratory, 2 mL of  $\text{Fe}^{3+}$  carrier were added to the filtered and acidified sample. Po isotopes were pre-concentrated with iron hydroxides ( $\text{Fe}(\text{OH})_3$  precipitation) by slow addition of concentrated ammonium hydroxide with rapid stirring until the pH reached 9. The precipitate was evaporated and deposited following the same procedure as the one described for biological samples (Holm and Fukai, 1977).

Filters were transferred into teflon beakers, spiked with  $^{209}\text{Po}$  as a yield tracer and digested with 70 mL of concentrated  $\text{HNO}_3$  and 30 mL of  $\text{HCl}$ . Digestion and deposition was made following the same procedure as biological samples.

Po isotopes activities were measured with an alpha-spectrometer equipped with a silicon surface barrier and ion implanted silicon detector (active area:  $450\text{ mm}^2$ ) and a semiconductor silicon surface barrier detector. The Minimum Detectable Activity (MDA) was in the range of 0.50 - 5.58 mBq for  $^{210}\text{Po}$  for a 400,000 seconds counting time.

$^{210}\text{Pb}$  was measured through deposition of its grand-daughter  $^{210}\text{Po}$  after 6 months ingrowth. Ingrowth and decay corrections were applied to calculate activities of both,  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  at sampling date.

The quality assurance of radio-analytical measurements was ensured through analysis of certified reference materials.

## Results and discussion

### Concentrations of $^{210}\text{Pb}$ and $^{210}\text{Po}$ in Peníscola marsh water

Values of  $^{210}\text{Pb}$  in dissolved fraction ranged from  $13.3\pm 0.6$  to  $22.5\pm 0.9\text{ Bq m}^{-3}$ , and were higher than the values from  $^{210}\text{Po}$  in this fraction, that ranged from  $2.5\pm 0.3$  to  $4.6\pm 0.5\text{ Bq m}^{-3}$ . On the contrary, values of  $^{210}\text{Pb}$  in the particulate fraction ranged from  $2.5\pm 0.2$  to  $7.6\pm 0.6\text{ Bq m}^{-3}$  and  $^{210}\text{Po}$  ranged from  $0.8\pm 0.1$  to  $3.8\pm 0.4\text{ Bq m}^{-3}$ . In general terms, it is observed that there was more concentration of  $^{210}\text{Pb}$  in water rather than  $^{210}\text{Po}$ , and higher concentrations of both radionuclides in the dissolved fraction rather than in the particulate fraction, not reaching the secular equilibrium in any case.

These five samples were taken at different points according to the different radioactivity concentration observed in soils in previous studies (Rodellas-Vila, 2008; Rodellas-Vila, 2009). Results showed that there is no significant differences between stations and gradient between Wst 2, located at the northern part of the marsh, was supposed to be the sample with the lowest concentrations, and Wst 6 the one with the highest concentration, due to the levels of  $^{226}\text{Ra}$  and  $^{222}\text{Rn}$  in sediments and soils.

In comparison with the data, the values reported in this present research are higher than  $^{210}\text{Pb}$  activity concentrations in filtered seawater and suspended particulate matter in the North-East Atlantic Ocean, that ranged from 0.85 to  $2.27\text{ Bq m}^{-3}$ , and particulate  $^{210}\text{Pb}$  from 0.09 to  $1.00\text{ Bq m}^{-3}$  while  $^{210}\text{Po}$  activity concentrations in filtered seawater and suspended particulate matter in the North-East Atlantic Ocean ranged from 0.35 to  $1.70\text{ Bq m}^{-3}$  and particulate  $^{210}\text{Po}$  from 0.24 to  $1.12\text{ Bq m}^{-3}$  (Carvalho, 2011). With regards to  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentrations in freshwater, NKS (2009) reported values that oscillate around  $3.2\pm 0.5\text{ Bq m}^{-3}$  for  $^{210}\text{Pb}$  and  $1.9\pm 0.3\text{ Bq m}^{-3}$  for  $^{210}\text{Po}$ .

### Concentrations of $^{210}\text{Pb}$ and $^{210}\text{Po}$ in species

#### *G. holbrooki*

*G. holbrooki* showed  $^{210}\text{Pb}$  concentrations that ranged from  $12\pm 1$  to  $61\pm 3\text{ Bq kg}^{-1}$  and  $^{210}\text{Po}$  ranged from 91 to  $310\text{ Bq kg}^{-1}$ .  $^{210}\text{Pb}$  values were higher in fishes from the sampling station 3 (with an average value of  $35.3\pm 16.4\text{ Bq kg}^{-1}$ ) than those from the sampling stations 2 and 6 (with average values of  $17.0\pm 6.3$  and  $19.3\pm 3.6\text{ Bq kg}^{-1}$  respectively). These results agree with the higher concentration of  $^{210}\text{Pb}$  found in the water samples 3

and 5, collected in the same channel than the individuals of *G. holbrooki* from the sampling station 2.  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  did not show secular equilibrium in *G. holbrooki*, with greater values of  $^{210}\text{Po}$  compared with  $^{210}\text{Pb}$  concentration.

The  $^{210}\text{Po}/^{210}\text{Pb}$  was calculated and results showed that  $^{210}\text{Po}$  was accumulated with a range of 5.5 to 16.5 times more than  $^{210}\text{Pb}$ . The highest accumulation was found on the individuals from the sampling station 2. Those from St 3 and St 6 showed similar values although rather lower than the ones obtained in St 2. One hypothesis was that  $^{210}\text{Po}$  might accumulate depending on the size of weight of the organism. However,  $R^2$  values were calculated and the results obtained showed no relation (i.e.  $R^2$  ranging from 0 to 0,05 for both radionuclides, when considering SL and body weight, respectively).

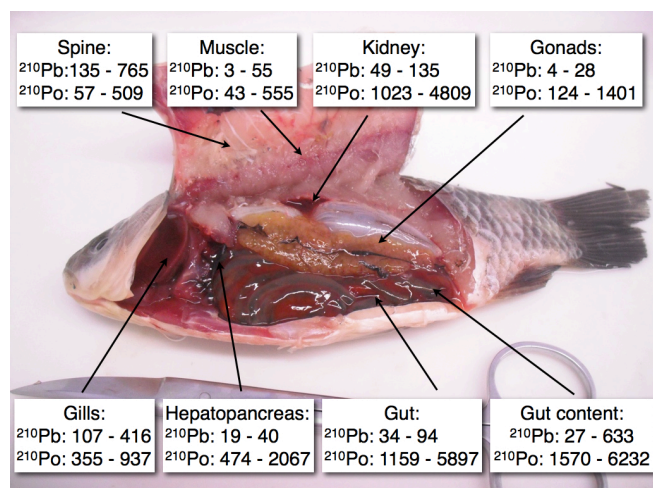
In comparison with results obtained from blank samples, the average concentration of  $^{210}\text{Pb}$  in marsh fish samples doubled the average concentration of  $^{210}\text{Pb}$  in blank samples ( $24\pm 13$  and  $11\pm 1$   $\text{Bq kg}^{-1}$ , respectively). Furthermore, concentrations of  $^{210}\text{Po}$  in marsh samples (with a mean value of  $188\pm 55$   $\text{Bq kg}^{-1}$ ) are 6 times higher than concentrations found in blank samples (with a mean value of  $33\pm 1$   $\text{Bq kg}^{-1}$ ). Therefore, it can be concluded that individuals of *G. holbrooki* from the Peníscola marsh show increased levels of concentrations of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  in their tissues.

#### *C. auratus*

In *C. auratus*, results shown that highest concentration of  $^{210}\text{Pb}$  was found in gut content (ranging from  $228\pm 10$  to  $599\pm 20$   $\text{Bq kg}^{-1}$  with an average of  $384\pm 193$   $\text{Bq kg}^{-1}$ ), followed by spine (from  $194\pm 7$  to  $351\pm 359$   $\text{Bq kg}^{-1}$  with an average of  $253\pm 85$   $\text{Bq kg}^{-1}$ ) and gills (from  $169\pm 7$  to  $216\pm 174$   $\text{Bq kg}^{-1}$  with an average of  $194\pm 23$   $\text{Bq kg}^{-1}$ ). The lowest  $^{210}\text{Pb}$  concentration was found on muscle (ranging from  $6\pm 1$  to  $21\pm 29$   $\text{Bq kg}^{-1}$  with an average concentration of  $12\pm 8$   $\text{Bq kg}^{-1}$ ), gonads (from  $6\pm 2$  to  $22\pm 2$   $\text{Bq kg}^{-1}$  and the average value was  $14\pm 8$   $\text{Bq kg}^{-1}$ ) and hepatopancreas (from  $25\pm 5$  to  $40\pm 3$   $\text{Bq kg}^{-1}$ ). On the other hand, the highest  $^{210}\text{Po}$  concentration was found on gut content (ranging from  $2800\pm 1598$  to  $5494\pm 140$   $\text{Bq kg}^{-1}$  with an average of  $3833\pm 1452$   $\text{Bq kg}^{-1}$ ), followed by gut (from  $1386\pm 38$  to  $5621\pm 161$   $\text{Bq kg}^{-1}$  with an average value of  $3261\pm 2158$   $\text{Bq kg}^{-1}$ ) and kidney (from  $1814\pm 1033$  to  $3805\pm 137$   $\text{Bq kg}^{-1}$  with an average of  $2488\pm 1141$   $\text{Bq kg}^{-1}$ ). The lowest concentrations of  $^{210}\text{Po}$  were found on muscle (from  $69\pm 34$  to  $343\pm 9$   $\text{Bq kg}^{-1}$ ) and spine (from  $92\pm 9$  to  $245\pm 228$   $\text{Bq kg}^{-1}$  and the average of  $167\pm 77$   $\text{Bq kg}^{-1}$ ).

There was a high variation among the  $^{210}\text{Po}/^{210}\text{Pb}$  ratio calculated for the tissues analyzed. The lowest values (from  $0.4\pm 0.1$  to  $0.7\pm 1.4$ ) were

observed in spine, where  $^{210}\text{Pb}$  accumulated in greater proportion than  $^{210}\text{Po}$ . Furthermore, ratios on gills, and muscle of individuals in the three sampling stations were also low (from  $2.4\pm 0.9$  to  $9.6\pm 0.1$ ) in stations 3 and 6, where accumulation of  $^{210}\text{Po}$  was lower than ten times the concentration of  $^{210}\text{Pb}$ , in comparison to the other ratio values. Highest values (38 or more) corresponded to gonads, hepatopancreas and gut from sites 2 and 6, and also kidney in site 6.



**Figure 1.** Ranges of values of concentration of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  (in  $\text{Bq kg}^{-1}$  dry weight) in tissues of *C. auratus* ( $n=7$ ).

In general terms, concentrations of  $^{210}\text{Po}$  in tissues of fishes from the St 6 were higher than those from other sampling stations, doubling the values in most of the tissues. This result could be related to  $^{210}\text{Po}$  concentration in water from the Wst 6, which had the highest concentrations of  $^{210}\text{Po}$  both, particulate and dissolved fraction, between water samples. Concentration of  $^{210}\text{Po}$  was 1.3 and 1.8 times higher than concentration at St 2 and St 3 respectively.

Regarding fish size, there is no apparent relation between  $^{210}\text{Po}$  concentration and weight or length. Individuals from St 6, the ones with the highest  $^{210}\text{Po}$  concentrations in tissues coincide to be, generally, the smallest ones. Despite the high variability shown in TL and weight from individuals of St 2 and St 3, their  $^{210}\text{Po}$  concentration in tissues are very similar. More to this point, there are no significant differences between concentration of  $^{210}\text{Pb}$  in tissues among individuals of different size or weight.

Despite the blank sample was considered as a blank in the beginning, results show that this individual does not correspond to it, due to high concentrations of  $^{210}\text{Pb}$  found in hepatopancreas and  $^{210}\text{Po}$  in gut content, gut and hepatopancreas. This sample was collected from a little reservoir without any water input or output except rainwater and evaporation. Thus, the water in this little reservoir (and therefore organisms living there)



could be affected by the geology of the surrounding area. In this species, comparison between marsh individuals and blank samples can not be done.

### C. carpio

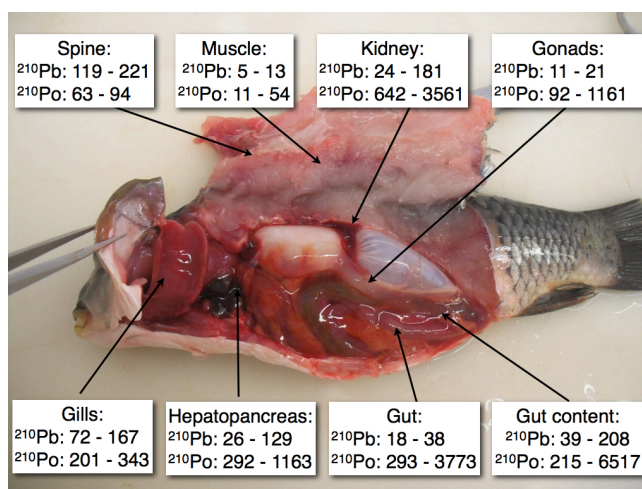
In *C. carpio*, results shown that highest concentration of  $^{210}\text{Pb}$  was found in gut content (ranging from  $60\pm 4$  to  $208\pm 11$   $\text{Bq kg}^{-1}$ , with an average of  $155\pm 82$   $\text{Bq kg}^{-1}$ ), followed by gills (from  $72\pm 3$  to  $167\pm 10$   $\text{Bq kg}^{-1}$  with an average of  $112\pm 49$   $\text{Bq kg}^{-1}$ ) and spine (from  $122\pm 6$  to  $221\pm 11$   $\text{Bq kg}^{-1}$  with an average of  $169\pm 50$   $\text{Bq kg}^{-1}$ ). The lowest  $^{210}\text{Pb}$  concentration was found on muscle (ranging from  $6\pm 0$  to  $13\pm 1$   $\text{Bq kg}^{-1}$  with an average concentration of  $8\pm 4$   $\text{Bq kg}^{-1}$ ) and gonads (from  $14\pm 1$  to  $18\pm 1$   $\text{Bq kg}^{-1}$  and the average value was  $16\pm 2$   $\text{Bq kg}^{-1}$ ). On the other hand, the highest  $^{210}\text{Po}$  concentration was found on gut content (from  $3288\pm 144$  to  $4691\pm 221$   $\text{Bq kg}^{-1}$  with an average of  $3781\pm 788$   $\text{Bq kg}^{-1}$ ), followed by gut (from  $1525\pm 64$  to  $2033\pm 65$   $\text{Bq kg}^{-1}$ ) and kidney (from  $1005\pm 53$  to  $2102\pm 64$   $\text{Bq kg}^{-1}$  with an average of  $1641\pm 569$   $\text{Bq kg}^{-1}$ ). The lowest concentrations of  $^{210}\text{Po}$  were found on muscle (ranging from  $19\pm 3$  to  $32\pm 2$   $\text{Bq kg}^{-1}$  with an average concentration of  $28\pm 8$   $\text{Bq kg}^{-1}$ ) and spine (from  $64\pm 7$  to  $94\pm 23$   $\text{Bq kg}^{-1}$  and the average of  $76\pm 16$   $\text{Bq kg}^{-1}$ ).

In this species there was also a high variation among the  $^{210}\text{Po}/^{210}\text{Pb}$  ratio calculated in the analyzed tissues. The lowest values were shown in spine (ranging from  $0.4\pm 0.1$  to  $0.6\pm 0.1$ ), where once again,  $^{210}\text{Pb}$  accumulated more than  $^{210}\text{Po}$ . Furthermore, ratios on gills and muscle of the three sampling stations and hepatopancreas from sites 1 and 3 were also low (ranging from  $1.4 \pm 0.1$  to  $5.7 \pm 0.1$ ), where accumulation of  $^{210}\text{Po}$  was more than ten times lower than the concentration of  $^{210}\text{Pb}$ , in comparison to the other ratio values. Highest values corresponded to gut (from the three sampling stations), gut content, and gonads from St 3 and kidney and gonads from individuals at St 6.

In general terms, concentrations of  $^{210}\text{Po}$  in tissues of fishes from the St 3 were higher than those from other sampling stations. If accumulation of  $^{210}\text{Po}$  had depended of concentrations in water, highest concentrations would have been seen on St 6. The same situation was repeated with  $^{210}\text{Pb}$  concentration. Highest values of  $^{210}\text{Pb}$  were shown in samples from St 2, where  $^{210}\text{Pb}$  concentration in water was the lowest.

Regarding fish size, there is no apparent relation between  $^{210}\text{Po}$  concentration and weight or length. This statement is confirmed by the individuals from St 3. Both individuals show high differences in length and weight, as it is shown in the average size and weight:  $31\pm 10$  cm and  $516\pm 460$  g. If accumulation of  $^{210}\text{Po}$  or  $^{210}\text{Pb}$  had depended of

size and length,  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  concentrations would have had high uncertainties in the different tissues.



**Figure 2.** Ranges of values of concentration of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  (in  $\text{Bq kg}^{-1}$  dry weight) in tissues of *C. carpio* ( $n=4$ ).

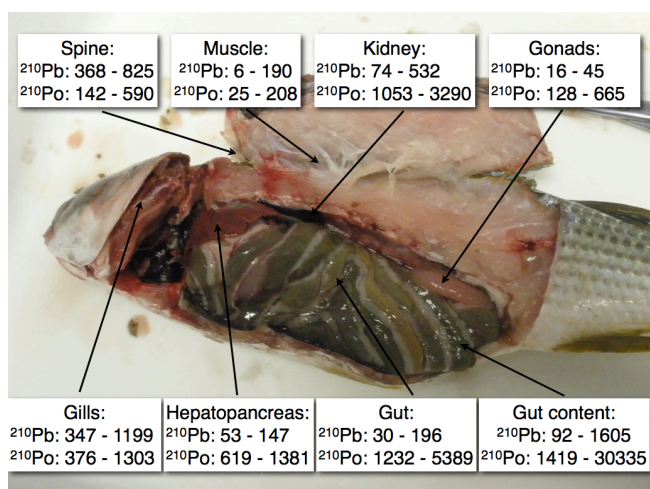
In comparison with results obtained from blank samples, average concentration of  $^{210}\text{Pb}$  in marsh samples ranges from 7 to 47 times higher than concentrations in blank sample. However,  $^{210}\text{Pb}$  concentration in gonads from marsh sample are only 1.7 times higher than blank samples ( $16\pm 2$   $\text{Bq kg}^{-1}$  in marsh sample and  $9\pm 3$   $\text{Bq kg}^{-1}$  in blank sample). Furthermore, concentrations of  $^{210}\text{Po}$  in marsh samples (average concentrations range from  $28\pm 8$   $\text{Bq kg}^{-1}$  in spine to  $3782\pm 789$   $\text{Bq kg}^{-1}$  in gut content) are from 8 to 245 (for spine and gut content, respectively) times higher than concentrations found in blank samples (concentrations of  $^{210}\text{Po}$   $2\pm 0$   $\text{Bq kg}^{-1}$  in muscle and  $15\pm 1$   $\text{Bq kg}^{-1}$  in gut content). Therefore, it can be said that individuals of *C. carpio* from Peníscola marsh show increased levels of concentrations of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  in their tissues.

### C. labrosus

In *C. labrosus*, highest concentration of  $^{210}\text{Pb}$  was found in spine (ranging from  $368\pm 14$  to  $622\pm 21$   $\text{Bq kg}^{-1}$  with an average of  $461\pm 140$   $\text{Bq kg}^{-1}$ ), followed by gut content (from  $92\pm 6$  to  $1014\pm 38$   $\text{Bq kg}^{-1}$ , with an average of  $475\pm 481$   $\text{Bq kg}^{-1}$ ) and gills (from  $347\pm 15$  to  $907\pm 28$   $\text{Bq kg}^{-1}$  with an average of  $706\pm 311$   $\text{Bq kg}^{-1}$ ). The lowest  $^{210}\text{Pb}$  concentration was found on muscle (from  $6\pm 0$  to  $98\pm 3$   $\text{Bq kg}^{-1}$  d.w. with an average concentration of  $38\pm 53$   $\text{Bq kg}^{-1}$ ) and gonads (from  $18\pm 1$  to  $34\pm 4$   $\text{Bq kg}^{-1}$  and the average value was  $26\pm 8$   $\text{Bq kg}^{-1}$ ). On the other hand, the highest  $^{210}\text{Po}$  concentration was found on gut content (from  $4685\pm 232$  to  $15920\pm 703$   $\text{Bq kg}^{-1}$  with an average of  $8558\pm 6378$   $\text{Bq kg}^{-1}$ ), followed by gut (from  $2542\pm 76$  to  $3311\pm 141$   $\text{Bq kg}^{-1}$ ) and kidney (from  $1195\pm 73$  to  $2205\pm 99$   $\text{Bq kg}^{-1}$  with an average of  $1544\pm 573$   $\text{Bq kg}^{-1}$ ). The lowest concentrations of  $^{210}\text{Po}$  were found on muscle (from

25±2 to 122±4 Bq kg<sup>-1</sup> with an average concentration of 61±54 Bq kg<sup>-1</sup>) and spine (from 258±41 to 366±20 Bq kg<sup>-1</sup> and the average of 310±55 Bq kg<sup>-1</sup>).

Great variation was found among the <sup>210</sup>Po/<sup>210</sup>Pb ratio calculated for the tissues analyzed. Spines showed higher accumulations of <sup>210</sup>Pb. In this species, the <sup>210</sup>Po/<sup>210</sup>Pb ratio was also lower for the gills (with values that range from 0.8±0.1 to 1.1±0.1), where in species from St 6 there was a greater accumulation of <sup>210</sup>Pb, instead of <sup>210</sup>Po as observed in spines and individuals collected at St 2 and St 3 where there was almost the same accumulation of <sup>210</sup>Pb than <sup>210</sup>Po. Values for muscle were low in comparison to the ratio of other tissues, concentrating only from 1.25 to 4.30 times more <sup>210</sup>Po than <sup>210</sup>Pb. Depending on the sampling station, values for gonads, kidney and hepatopancreas were also low. For instance, gonads from individuals in St 2 accumulated 4 times less <sup>210</sup>Po than gonads from the other sampling stations. Highest ratios were found on gut and gut contents where gut from individuals from St 3 accumulated 63 times more <sup>210</sup>Po than <sup>210</sup>Pb, gut contents from St 3 and St 6 accumulated almost 16 times more <sup>210</sup>Po and the ratio <sup>210</sup>Po/<sup>210</sup>Pb of the gut content from samples collected at St 2 was 51.



**Figure 3.** Ranges of values of concentration of <sup>210</sup>Pb and <sup>210</sup>Po (in Bq kg<sup>-1</sup> dry weight) in tissues of *C. labrosus* (n=5). Values are shown in dry weight.

In general terms, <sup>210</sup>Pb concentration is higher in fishes from Wst 3 and 6, but accumulation of <sup>210</sup>Po in tissues does not point higher in one specific sampling station. Furthermore, *C. labrosus* is a specie that moves within different waters, from sea to marsh water. It would be difficult to establish a relation between water concentrations of <sup>210</sup>Pb and <sup>210</sup>Po and concentration in tissues of this species.

Regarding fish size, there is no apparent relation between <sup>210</sup>Po and <sup>210</sup>Pb concentration and weight or length.

In comparison with results obtained from blank samples, average concentration of <sup>210</sup>Pb in marsh samples ranges from 4 to 201 times higher than concentrations in blank sample. Furthermore, concentrations of <sup>210</sup>Po in marsh samples (average concentrations range from 61±54 Bq kg<sup>-1</sup> in muscle to 8558±6378 Bq kg<sup>-1</sup> in gut content) are from 24 to 216 (for muscle and gut content, respectively) times higher than concentrations found in blank samples (concentrations of <sup>210</sup>Po 2±0 Bq kg<sup>-1</sup> in muscle and 40±2 Bq kg<sup>-1</sup> in gut content). Therefore, can be said that individuals of *C. labrosus* from Peníscola marsh show increased levels of concentrations of <sup>210</sup>Pb and <sup>210</sup>Po in their tissues.

### Bioaccumulation Factors (BAF)

BAF for <sup>210</sup>Pb in *G. holbrooki* had the same order of magnitude for all the individuals (10<sup>2</sup>), independently of the sampling station where they were taken. On the other hand, BAF for <sup>210</sup>Po ranged from 10<sup>3</sup> to 10<sup>4</sup> depending on the sampling station. Individuals from sampling station 1 showed higher BAF (one order of magnitude above samples from St 6) and individuals from St 3 showed variations on the BAF, having values which differ up to two orders of magnitude.

BAF factors for *C. auratus* showed high differences depending on the tissue and the sampling station where they were taken. BAF for <sup>210</sup>Pb displayed values ranging from 10<sup>1</sup> to 10<sup>3</sup>, with differences in two orders of magnitude, and so did BAF for <sup>210</sup>Po, where ranged from 10<sup>3</sup> to 10<sup>5</sup>. Lowest values of <sup>210</sup>Pb BAF were shown generally in muscle. Highest values of <sup>210</sup>Pb BAF were gut contents. Moreover, <sup>210</sup>Po BAF showed similar patterns, with lowest BAF in muscle and the highest in gut content.

In *C. carpio*, BAF for <sup>210</sup>Pb displayed differences of three levels of magnitude (from 10<sup>1</sup> to 10<sup>3</sup>). Once again, the lowest values for BAF were found in muscle. Highest BAF values were shown in spine, followed by kidney, hepatopancreas and gills in different order depending on the sampling station. BAF for <sup>210</sup>Po had also differences of three levels of magnitude. Lowest concentrations were found again in muscle, and the highest in gut content, followed by gut and kidney in all the sampling stations.

In *C. labrosus*, BAF for <sup>210</sup>Pb had differences of 4 orders of magnitude. Again, lowest BAF were found on muscle, but in sampling stations 2 and 3, lowest BAF were found also in gonads and hepatopancreas for the St 3 and gonads for St 6. For this radionuclide, highest concentrations were found on spine in all the sampling stations and in gills at St 2 and St 6 and in gut content at St 3. <sup>210</sup>Po BAF showed differences of 3 levels of magnitude, being muscle once again the tissue with the lowest BAF. The highest BAF were found on gut content, followed by kidney. At St 2 high values were

observed for hepatopancreas and at St 6 BAF was also high in gut.

### Comparison between species

As *G. holbrooki* differs totally from the other species in terms of metabolism, physiology, behavior, size, etc. This species can not be compared with other species, although it should be taken into consideration that *G. holbrooki*, despite its small size, it greater accumulates  $^{210}\text{Pb}$  and  $^{210}\text{Po}$ , in comparison with other species.

Generally speaking, the three species (*C. carpio*, *C. labrosus* and *C. auratus*) have high concentration levels of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  in their tissues. However, there are several differences. *C. labrosus* showed extremely high values of  $^{210}\text{Po}$  concentration in gut content ( $8558\pm6378$  Bq kg $^{-1}$ ) and also high levels of  $^{210}\text{Pb}$  in spine and gills ( $461\pm140$  and  $706\pm311$  Bq kg $^{-1}$ , respectively) in comparison to the other species. Both, *C. labrosus* and *C. auratus* show high levels of  $^{210}\text{Pb}$  in gut content ( $475\pm481$  and  $384\pm193$  Bq kg $^{-1}$ ) in comparison with *C. carpio*, whose levels are lower ( $155\pm83$  Bq kg $^{-1}$ ). The same pattern is observed with gut, where in *C. carpio* the average is  $28\pm8$  Bq kg $^{-1}$  while in *C. labrosus* and *C. auratus* the value is tripled and doubled, respectively.

Regarding accumulation of both radionuclides in hepatopancreas, *C. labrosus* and *C. auratus* show similar concentrations for  $^{210}\text{Po}$  ( $1103\pm95$  and  $1144\pm758$  Bq kg $^{-1}$ , respectively) while in *C. carpio* concentration is a half.  $^{210}\text{Pb}$  concentration in hepatopancreas from individuals of *C. auratus* is lower than those from *C. labrosus* and *C. carpio* ( $30\pm9$ ,  $93\pm36$  and  $65\pm56$  Bq kg $^{-1}$ , respectively).  $^{210}\text{Po}$  concentration in kidney from the three species show similar values, ranging from  $1544\pm573$  Bq kg $^{-1}$  the lowest, *C. labrosus*, to  $2488\pm1141$  Bq kg $^{-1}$  the highest, *C. auratus*.  $^{210}\text{Pb}$  concentration in kidney in *C. labrosus* triples the values from the other species, with values of  $248\pm154$  Bq kg $^{-1}$  in *C. labrosus* and  $90\pm82$  and  $88\pm21$  Bq kg $^{-1}$  in *C. carpio* and *C. auratus*, respectively.  $^{210}\text{Po}$  accumulation in gills was low (in comparison with other tissues) in the three species, with values ranging from  $682\pm303$  Bq kg $^{-1}$  the highest (*C. labrosus*) to  $261\pm73$  Bq kg $^{-1}$  the lowest (*C. carpio*). Levels of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  in gonads are lower than in gills in the three species, being  $^{210}\text{Pb}$  concentration always lower than  $^{210}\text{Po}$ . Levels of  $^{210}\text{Po}$  in gonads ranged from  $332\pm207$  Bq kg $^{-1}$  the lowest (*C. labrosus*) to  $635\pm481$  Bq kg $^{-1}$  the highest (*C. auratus*). Levels of  $^{210}\text{Pb}$  in gonads ranged from  $26\pm8$  Bq kg $^{-1}$  the highest (*C. labrosus*) to  $14\pm8$  Bq kg $^{-1}$  the lowest (*C. auratus*). Regarding to spine, there were high differences between the  $^{210}\text{Pb}$  content in the three species. The highest value, as said before, was  $461\pm140$  Bq kg $^{-1}$  from *C. labrosus*. The lowest value was for *C. carpio*, whose spines had an average concentration of

$^{210}\text{Pb}$  of  $169\pm50$  Bq kg $^{-1}$ , almost 4 times lower.  $^{210}\text{Po}$  concentrations in spines were low, ranging from  $76\pm16$  Bq kg $^{-1}$  the lowest (*C. carpio*) to  $310\pm55$  Bq kg $^{-1}$  the highest (*C. labrosus*). Finally, referring to muscle, concentrations of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  were so low, ranging from  $8\pm4$  Bq kg $^{-1}$  ( $^{210}\text{Pb}$ ) and  $28\pm8$  Bq kg $^{-1}$  ( $^{210}\text{Po}$ ) the lowest (both in *C. carpio*) to  $38\pm53$  Bq kg $^{-1}$  ( $^{210}\text{Pb}$ ) and  $164\pm155$  Bq kg $^{-1}$  ( $^{210}\text{Po}$ ) for *C. labrosus* and *C. auratus* respectively.

**Table 1.** Average concentrations of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  (in Bq kg $^{-1}$  dry weight) in tissues from *C. carpio* (n=4), *C. labrosus* (n=5) and *C. auratus* (n=7).

Species	Tissue	$^{210}\text{Pb}$	$^{210}\text{Po}$
<i>Cyprinus carpio</i>	Muscle	8 ± 4	28 ± 8
	Spine	169 ± 50	76 ± 16
	Gonads	16 ± 2	398 ± 310
	Gills	112 ± 49	261 ± 73
	Kidney	90 ± 82	1641 ± 569
	Hepatopancreas	65 ± 56	596 ± 266
	Gut	28 ± 8	1745 ± 261
	Gut content	155 ± 83	3782 ± 789
<i>Chelon labrosus</i>	Muscle	38 ± 53	61 ± 54
	Spine	461 ± 140	310 ± 55
	Gonads	26 ± 8	332 ± 207
	Gills	706 ± 311	682 ± 303
	Kidney	248 ± 154	1544 ± 573
	Hepatopancreas	93 ± 36	1103 ± 95
	Gut	94 ± 59	2927 ± 543
	Gut content	475 ± 481	8558 ± 6378
<i>Carassius auratus</i>	Muscle	12 ± 8	164 ± 155
	Spine	253 ± 85	167 ± 77
	Gonads	14 ± 8	635 ± 481
	Gills	194 ± 23	634 ± 183
	Kidney	88 ± 21	2488 ± 1141
	Hepatopancreas	30 ± 9	1144 ± 758
	Gut	65 ± 21	3261 ± 2159
	Gut content	384 ± 193	3833 ± 1453

The BAF values for organisms living in the same habitat vary by orders of magnitude (especially with  $^{210}\text{Pb}$  BAF) and demonstrate that  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  bioaccumulation is not from simple radionuclide absorption from water, underscoring the role of feeding as the cause for  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  accumulation in fishes. Therefore, *C. carpio*, who is an species characterized by being benthic omnivorous and uprooting macrophytes when feeds, did not show the highest values of  $^{210}\text{Po}$  nor  $^{210}\text{Pb}$  in gut content. As this behaviour increases turbidity in water, because the sediment is removed, it was expected that this specie would have shown the highest values, levels which *C. labrosus* shown. However, as in waters where *C.*



*carpio* lives, the level of particulate matter in water increases and when feeding, species ingest more particulate matter (the fraction where polonium is binded), the highest concentrations of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  in gut content in all the species could be explained.

### Comparison with bibliographical data

In general terms it can be concluded that almost all the values shown in all the species analyzed in this study are higher in comparison with the existing data. Nevertheless, resulting trends agree with the existing data regarding  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  accumulation in fish tissues.

According to Skwarzek (1988) the highest  $^{210}\text{Po}$  concentrations occur in organs involved in digestion and metabolism, such as intestine, stomach, spleen and pyloric caecal of fish. Yet in our research, the highest  $^{210}\text{Po}$  concentrations are found in organs involved in digestion and metabolism, such as gut, kidney and hepatopancreas.

We also observed that the concentration of  $^{210}\text{Po}$  in wet weight in the muscles of fish was always higher than in the spine, according with results observed by Iyengar *et al.* (1980).

Levels of  $^{210}\text{Po}$  found in fish digestive organs tend to correlate with the degree of stomach repletion and thus decrease if food is scarce (Skwarzek, 1988). The residence time of  $^{210}\text{Po}$  within the digestive system of fish is short, resulting in a rapid decrease in  $^{210}\text{Po}$  content in the liver and intestine when the stomach is empty (Lazorenko *et al.*, 2002). However, once  $^{210}\text{Po}$  is uptaken by the organism is subsequently distributed in fish in the following order: entrails  $\geq$  liver  $>$  skeleton  $>$  muscles (Lazorenko *et al.*, 2002). As observed in our study, the distribution pattern agree with the data found in bibliography. Furthermore, results from the accumulation of  $^{210}\text{Po}$  in liver are higher than those from spines, and in spines, were also higher than  $^{210}\text{Po}$  accumulation in muscles.

*Solea solea* and *Sparus* sp. are species that were investigated in other studies and results showed that low activities of  $^{210}\text{Po}$  were found in the gills, skins, bones and muscle (the latter one presenting the lowest activity) and the highest activities were observed in the livers and intestine (Connan *et al.*, 2007). However, results varied from the species due to their lifestyle (Connan *et al.*, 2007). Results obtained by Connan *et al.* (2007) agree with results found on the present study as  $^{210}\text{Po}$  concentration in tissues obtained herein match with the resulting pattern of their study.

Other studies focused on the  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  concentration in organisms and their transfer in marine food chains (Carvalho, 2011). This study reported that common intertidal fishes such as

those from the Blenniidae family displayed low  $^{210}\text{Po}$  concentrations in muscle tissue ( $1.5\text{-}2.8\text{ Bq kg}^{-1}$ ), and high concentrations in other internal organs. Yet in their study,  $^{210}\text{Po}/^{210}\text{Pb}$  ratios were always above 1. In coastal fishes, the general  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  distribution pattern was: lower concentrations in muscle tissue and higher concentrations in the gut, liver, gonad and spine. Carvalho (2011) also reported high inter-individual variations and often, apparent seasonal variations of  $^{210}\text{Po}$  activity concentrations. Comparison between values reported by Carvalho and values reported by this present study are shown in Table 2 and 3. Most of the highest values reported by Carvalho (2011) came from a specific species, *Sardina pilchardus*, which in his research, Carvalho (2011) found that it accumulates extremely high values of  $^{210}\text{Po}$  from the environment. In the present study, higher  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentrations were reported. Thus would mean that higher  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentrations in water may lead to higher  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentrations in tissues.

In comparison to NKS (2009), which studied  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentrations in fishes from a terrestrial and freshwater environment, results provided in this present research are higher. They analyzed edible parts (i.e. muscle) and other parts from fish separately. They found concentrations of  $^{210}\text{Pb}$  that ranged from  $0.014\pm 0.003$  to  $0.13\pm 0.02\text{ Bq kg}^{-1}$  w.w. for edible parts and from  $0.123\pm 0.021$  to  $1.507\pm 0.256\text{ Bq kg}^{-1}$  w.w. for other parts. On the other hand, they found concentrations of  $^{210}\text{Po}$  that ranged from  $0.079\pm 0.018$  to  $1.863\pm 0.35\text{ Bq kg}^{-1}$  w.w. for edible parts and ranges from  $1.492\pm 0.269$  to  $8.950\pm 1.611\text{ Bq kg}^{-1}$  w.w.. Concentration of both,  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  reported by this article are extremely low in comparison with results reported from this research, which the concentration of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  in the tissue equivalent to edible parts (muscle) ranged from  $1.1\pm 0.1$  to  $21.3\pm 0.8\text{ Bq kg}^{-1}$  w.w. for  $^{210}\text{Pb}$  and from  $4.4\pm 0.6$  to  $76.7\pm 2.0\text{ Bq kg}^{-1}$  w.w. for  $^{210}\text{Po}$ .

With regards to bioaccumulation factors (BAF), NKS (2009) reported BAF for edible parts ranging from  $5.0\cdot 10^1$  to  $1.2\cdot 10^3$  for  $^{210}\text{Po}$  and from  $9.6\cdot 10^0$  to  $2.4\cdot 10^2$  for  $^{210}\text{Pb}$ . In this present research, BAF calculated for muscle ranged from  $8.5\cdot 10^2$  to  $1.1\cdot 10^4$  for  $^{210}\text{Po}$  and from  $4.5\cdot 10^1$  to  $8.5\cdot 10^2$  for  $^{210}\text{Pb}$ . As it can be observed from the results, BAF in fishes from Peníscola marsh show, for each radionuclide, always an order of magnitude higher than BAF calculated for terrestrial and freshwater environments. Thus, in relation to  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentrations in water,  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  concentrations in fish tissues collected in Peníscola are higher than those from freshwater ecosystems. This result reflects that highest  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  concentration in water is not only the reason why this research has found higher bioaccumulations in

fish tissues, and highlights that the absorption with ingested food (gut transfer) as the main route of radionuclide uptake.

**Table 2.** Comparison between  $^{210}\text{Pb}$  concentrations (in  $\text{Bq kg}^{-1}$  w.w.) reported by Carvalho (2011) and  $^{210}\text{Pb}$  concentrations (in  $\text{Bq kg}^{-1}$  w.w.) reported by this present study.

	Carvalho (2011) $^{210}\text{Pb}$	Present study $^{210}\text{Pb}$
Muscle	0.15±0.07 to 2.1±1.3	1.1±0.1 to 21.3±0.8
Spine	0.65±9.1 to 31±1	4.0±1.5 to 362.5±12.0
Gonads	0.20±0.01 to 22±2	2.9±0.2 to 54.7±44.1
Hepato-pancreas	0.29±0.02 to 134±12	4.4±0.6 to 33.4±0.9
Gut	0.29±0.01 to 100±8	2.5±0.2 to 27.1±0.9

**Table 3.** Comparison between  $^{210}\text{Po}$  concentrations (in  $\text{Bq kg}^{-1}$  w.w.) reported by Carvalho (2011) and  $^{210}\text{Po}$  concentrations (in  $\text{Bq kg}^{-1}$  w.w.) reported by this present study.

	Carvalho (2011) $^{210}\text{Po}$	Present study $^{210}\text{Po}$
Muscle	0.52±0.01 to 66±2	4.4±0.6 to 76.7±2.0
Spine	5.9±0.2 to 197±16	8.6±1.0 to 705.7±20.1
Gonads	4.5±0.2 and 275±9	19.8±1.4 to 183.0±6.8
Hepato-pancreas	5.36±0.42 to 2140±60	56.3±7.1 to 476.9±11.4
Gut	9.86±0.3 to 28000±2000	187.3±5.2 to 666.0±19.0

## Conclusions

Tissues with the highest accumulation of  $^{210}\text{Po}$  were gut, kidney and hepatopancreas ranging from 1745±261 to 3261±2159  $\text{Bq kg}^{-1}$  d.w. in gut, from 1544±573 to 2488±1411  $\text{Bq kg}^{-1}$  d.w. in kidney and from 596±266 to 1144±758  $\text{Bq kg}^{-1}$  d.w. in hepatopancreas. High concentrations in gut can be justified by the fact that gut content resulted in enhanced concentrations of  $^{210}\text{Po}$  ranging from 782±789 to 8558±6378  $\text{Bq kg}^{-1}$  d.w. On the contrary, lowest concentrations of  $^{210}\text{Po}$  were found in muscle, spine, gonads and gills, with values ranging from 28±8 to 164±155  $\text{Bq kg}^{-1}$  d.w. in muscle, from 76±16 to 310±55  $\text{Bq kg}^{-1}$  d.w. in spine, from 332±207 to 635±481  $\text{Bq kg}^{-1}$  d.w. in gonads and from 261±73 to 682±303  $\text{Bq kg}^{-1}$  d.w. in gills.

Regarding  $^{210}\text{Pb}$  accumulation, results showed that the highest concentrations were found in gut content, gills, spine and kidney, with values ranging from 155±83 to 475±481  $\text{Bq kg}^{-1}$  d.w. in gut content, from 112±49 to 706±311  $\text{Bq kg}^{-1}$  d.w. in gills, from 169±50 to 461±140  $\text{Bq kg}^{-1}$  d.w. in spine and from 88±21 to 248±154  $\text{Bq kg}^{-1}$  d.w. in kidney. Lowest  $^{210}\text{Pb}$  concentrations found by this research were in muscle, gonads, hepatopancreas

and gut, with values ranging from 8±4 to 38±53  $\text{Bq kg}^{-1}$  d.w. in muscle, from 16±2 to 26±8  $\text{Bq kg}^{-1}$  d.w. in gonads, from 30±9 to 93±36  $\text{Bq kg}^{-1}$  d.w. in hepatopancreas and finally, from 28±8 to 94±59  $\text{Bq kg}^{-1}$  d.w. in gut.

The results showed that the distributions of both radionuclides are consistent with other studies and with the chemical properties of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  (i.e. Nieboer and Richardson, 1980).

The major input route of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  into the fish body pointed to be ingestion, due to the high levels of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  found in gut content as well as in the organs involved in digestion and metabolism (i.e. gut, kidney and hepatopancreas). This statement agrees with the literature regarding marine species of fish and invertebrates. On the other hand, breathing organs such as gills, although they could be an entry route of  $^{210}\text{Pb}$ , they are not for  $^{210}\text{Po}$ .

Bioaccumulation factors (BAF) of different tissues within the same individual showed differences that reached up to two and three orders of magnitude. Lowest BAF were generally found in muscle and the highest in gut and kidney. It was found that the BAF values for organisms living in the same habitat varied by orders of magnitude (especially with  $^{210}\text{Pb}$  BAF) and demonstrate that  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  bioaccumulation is not from simple radionuclide absorption from water, underscoring the role of feeding as the cause for  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  accumulation in fishes.

It can also be concluded that there is no apparent relationship between  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  accumulation and fish size or weight within the same species. Generally speaking, highest values of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentration in tissues were found on *Chelon labrosus* and *Carassius auratus*, being *Cyprinus carpio* the species with the lowest average values of accumulation. This result was not expected, because of the feeding habits of *C. carpio*, which uproots macrophytes and increases turbidity when feeding, it was expected that this species could show highest values of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentration in gut content. Hence, it is confirmed that the level to which a radionuclide is accumulated in an organism depends on a wide range of factors: its chemical characteristics and speciation in water or sediment, biological processes, including rates of uptake from water or diet, excretion, and metabolic transformation. These in turn, may be influenced directly by the physiology of the organism which is, of course, affected by diverse biological, physical and chemical factors, such as habitat, feeding behavior and species.

Both humans and other species live in a world with natural radioactivity. It is necessary to know our environment in order to understand the processes

that are occurring around us. For this reason it is evident that expanding the present database on  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  concentrations in different species and their tissues will greatly aid in refining estimates of dose and eventual assessments of the effects of ionizing radiation on biota.

## References

- Brown J.E., Gjelsvik R., Roos P., Kålås J.A., Outola I., Holm E., (2010). Levels and transfer of  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  in Nordic terrestrial ecosystems. *Journal of Environmental Radioactivity*, 102(5):430-7.
- Bruland, K. W. (1983). Trace elements in sea-water. In: Chemical Oceanography (Eds J. P. Riley and Chester). Academic Press, London, pp. 157–220.
- Bukovac, M.J., Wittwer, S.H., Tukey H.B., (1965) "Above ground plant parts as a pathway for entry of fission products into the food chain with special reference to 89-90Sr and 137Cs", Radioactive Fallout, Soils, Plants, Foods, Man (Flower, E., Ed.), Elsevier Press, New York, 82–109.
- Carvalho, F.P. and Fowler, S.W. (1994). A double-tracer technique to determine the relative importance of water and food as sources of polonium-210 to marine prawns and fish. *Marine Ecology Progress Series* 103: 251–264.
- Cherrier, J., Burnett, W. C., LaRock, P.A. (1995). Uptake of polonium and sulfur by bacteria. *Geomicrobiology Journal* 13, 103–115.
- Cherry, R.D. and Heyraud, M. (1981). Polonium-210 content of marine shrimp: Variation with biological and environmental factors. *Marine Biology* 65:165-175.
- Cherry, R. D. and Heyraud, M. (1982). Evidence of High Natural Radiation Doses in Certain Mid-Water Oceanic Organisms; *Science* 218.
- Cherry, R.D., Shannon, L.V., (1974). The alpha radioactivity of marine organisms. *Atomic Energy Review* 12, 3-45.
- Church, T.M. and Sarin M.M. (2008). "U and Th series radionuclides in the atmosphere: Supply, exchange, scavenging, and applications to aquatic systems" Chapter 2 (in:) *Radioactivity in the Environment*, Vol. 13. Krishnaswami, S. and J.K. Cochran (eds.). Elsevier.
- Clulow F.V., Dav N.K., Lim T.P., Avadhanula R. (1998). Radionuclides (lead-210, polonium-210, thorium-230, and thorium-232) and thorium and uranium in water, sediments, and fish from lakes near the city of Elliot Lake, Ontario, Canada. *Environmental Pollution* 99 199-213.
- Connan, O., Germain, P., Solier, L., Gouret, G. (2007). Variations of  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  in various marine organisms from Western English Channel: contribution of  $^{210}\text{Po}$  to the radiation dose. *Journal of Environmental Radioactivity* 97 (2-3): 168-88.
- Eisenbud, M. and Gesell, T. (1997). Environmental Radioactivity. From Natural, Industrial, and Military Sources. Fourth edition. Academic Press.
- Fisher, N. S., Burns, K. A., Cherry, R. D. and Heyraud, M. (1983). Accumulation and cellular distribution of  $^{241}\text{Am}$ ,  $^{210}\text{Po}$ , and  $^{210}\text{Pb}$  in two marine algae. *Marine Ecology Progress Series* 11: 233–237.
- Garcia-Orellana, J. (2004). Distribució i transferència de  $^{137}\text{Cs}$ ,  $^{239,240}\text{Pu}$  in  $^{210}\text{Pb}$  al Mar Mediterrani: La conca Alguero-Balear. PhD. Thesis.
- Holm, E. and Fukai, R. (1977). Method for multi-element alpha spectrometry of actinides and its application to environmental radioactivity studies. *Talanta*, 24: 659-664.
- Iyengar, M.A.R., Rajan, M.P., Ganapathy, S. and Kamath, P.R. (1980). Sources of natural radiation exposure in a low monazite environment. In: Proceedings of an International Symposium Held at Houston, Texas, Natural Radiation Environment III, vol. 2, pp. 1090-1106. USA, CONF-7804222.
- Kottelat, M. and Freyhof, J., (2007). Handbook of European freshwater fishes. Publications Kottelat, Cornol, Switzerland. 646 p.
- Krouglov, S.V., Filipas, A.S., Alexakhin, R.M., Arkhipov, N.P., (1997). Long-term study on the transfer of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  from Chernobyl-contaminated soils to grain crops, *J. Environ. Radioact.* 34 (3): 267–286.
- Lazorenko, G.E., Polikarpov, G.G. and Boltachev, A.R. (2002). Natural Radioelement Polonium in Primary Ecological Groups of Black Sea Fishes. *Russian Journal of Marine Biology* 28(1): 52–56.
- Murray, J. W., B. Paul, J. P. Dunne, and T. Chapin. (2005).  $^{234}\text{Th}$ ,  $^{234}\text{Pb}$ ,  $^{210}\text{Po}$  and stable Pb in the central equatorial Pacific: Tracers for particle cycling. *Deep-Sea Research I*, 52, 2109–2139
- Nieboer, E., and D. H. S. Richardson. (1980). The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. *Environmental Pollution*, 1, 3–26.
- NKS, (2009). Nordic Nuclear Safety Research, Po-210 and other radionuclides in terrestrial and freshwater environments, A Deliverable report for the NKS-B activity October 2008 GAPRAD
- Parfenov, Y., (1974). Polonium-210 in the environment and in the human organisms. *Atomic Energy Review* 12, 75-143.
- Pentreath, R. J. (1999). A system for radiological protection of the environment: Some initial thoughts and ideas. *Journal of Radiological Protection*, 19, 117–128.
- Reinfelder, J. R., and N. S. Fisher. (1991). The assimilation of elements ingested by marine copepods. *Science*, 251, 794–796.
- Rodellas-Vila, (2008). Distribució de radionúclids naturals en una marjal càrstica del Mediterrani Occidental: La Marjal de Penyíscola. Projecte de final de Carrera.
- Rodellas-Vila (2009). Characterization of natural radioactivity sources in a human-altered Mediterranean marsh. Projecte de Màster.
- Schwarz, K. (1976). Essentiality and metabolic functions of selenium. *Medical Clinics of North America*, 60, 745–767.
- Shaheed, K., Somasundaram, S.S.N., Shahul Hameed, P., Iyengar, M.A.R. (1997). A study of polonium-210 distribution aspects in the riverine ecosystem of Kaveri, Tiruchirappalli, India. *Environmental Pollution*, Vol. 95, No. 3, pp. 371-377, 1997
- Simkiss, K., and M. G. Taylor. (1995). Transport of metals across membranes. In: Metal Speciation and Bioavailability in Aquatic Systems (Eds A. Tessier and D. R. Turner). Wiley, Chichester, pp. 1–44.

Skwarzec, B., Falkowski, L. (1988). Accumulation of  $^{210}\text{Po}$  in Baltic Invertebrates, *J. Environ. Radioactivity* 8, 99-109.

Stewart, G. M., and N. S. Fisher. (2003a). Experimental studies on the accumulation of polonium-210 by marine phytoplankton. *Limnology and Oceanography*, 48, 1193–1201.

Stewart, G. M., and N. S. Fisher. (2003b). Bioaccumulation of polonium-210 in marine copepods. *Limnology and Oceanography*, 48, 2011–2019.

Stewart, G.M., Fowler, S.W., and Fisher, N.S. (2008) The Bioaccumulation of U- and Th-Series Radionuclides in

Marine Organisms. *Radioactivity in the Environment*, Volume 13, Elsevier.

Turekian, K. K., J. K. Cochran and Y. Nozaki. (1979). Growth rates of a clam from the Galapagos Rise hot spring field using natural radionuclide ratios. *Nature*, 280.385-387.

Williams, R. J. P. (1981). Physico-chemical aspects of inorganic element transfer through membranes. *Philosophical Transactions of the Royal Society of London B*, 294, 57–74.