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Detection and analysis of the microdistribution of uranium in the gills of freshwater *Corbicula fluminea* by SIMS technique

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ABSTRACT

The microdistribution of uranium in the gills of freshwater bivalve *Corbicula fluminea* following chronic direct exposure to this radioelement has been investigated using the SIMS technique. Different exposure levels and exposure durations have been studied. The SIMS mass spectra and $^{238}U^+$ ion images produced with a SIMS CAMECA 4F-E7 show an U accumulation with the lower aqueous U concentration (20 μg/L) and the influence of the exposure levels on the bioaccumulation capacities. Furthermore, the ionic images display a heterogeneous distribution of uranium within the gill structure whatever the exposure conditions are. This study, in keeping with the ENVIRHOM French research program, was led to the conclusion that ion microscopy is an appropriate analytical method for trace elements and can give elemental cartography in a biological tissue section.

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1. Introduction

The ENVIRHOM research French program supported by the Institute for Radioprotection and Nuclear Safety (IRSN) is intended to improve assessment of the risks to the general public and the ecosystems associated with chronic exposure to low levels of radioactive contaminants. The main objectives are to study the speciation, transfer, biokinetic and accumulation processes of radionuclides and also the biological effects correlated with this exposure on the human model (rats and mice) and on environmental organisms (algae, molluscs, crustaceans, fish, plants, etc.). Uranium has been the first element studied as part of this program. Chronic exposure of uranium may occur naturally in contaminated areas (underground water) or as a result of human activity (nuclear fuel cycle, agricultural use, military use of depleted uranium).

Concerning the environmental aspect of the program, several biological responses have been studied in various living organisms to establish concentration-effects relationships: feeding strategies, ventilation of molluscs, reproduction, growth rate of living organisms and the effects on the ecosystems [1–3]. A thermodynamic equilibrium model of uranium in aqueous systems has been described to interpret the experimental data obtained on the bioavailability of uranium (VI) [4–6]. Simon and Garnier-Laplace [7,8] have studied the bioaccumulation of uranium in the freshwater bivalve *Corbicula fluminea*. They have shown a pH influence and a correlation between the exposure conditions (exposure durations and treatments) and the distribution of this actinide in the bivalve organs.

The aim of this paper is to present a study of the microdistribution of uranium in gills of the bivalve *C. fluminea* after chronic direct exposure of this radionuclide, by SIMS (Secondary Ion Mass Spectrometry) microscopy.

The SIMS technique introduced in the early sixties by Castaing and Slodzian [9] is used for the chemical characterization of solid surfaces mainly in geochemistry, microelectronics and material sciences.

However, few years ago, the study and analysis of biological samples by ion microscopy were developed in the biomedical [10–15], nuclear [16] and botanic fields [17–19]. In the literature, only a few papers describe uranium detection by this technique in biological samples [20,21]. In particular, Markich et al. [21] used SIMS to measure metal ratios such as U/Ca in freshwater bivalve shells (*Velesunio angasi*), showing that these clams can be used over their lifetime as archival indicators of metal pollution in surface waters of the Finniss River (in tropical northern Australia near a copper–uranium mine). Moreover, the isotope ratio of this long-lived radionuclide at very low concentration levels has been measured by SIMS technique for environmental monitoring in geoscience, cosmochemistry, and planetary and nuclear sciences [22–33].

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2. Materials and methods

2.1. SIMS microscopy

The aim of SIMS microscopy is the elemental and isotopic analysis of a solid surface by an ion beam coupled with a mass spectrometer. The principle of this technique is based upon the sputtering of a few atomic layers from the surface of a sample, induced by a bombardment of focused primary ions of sufficiently high energy (some keV). A more detailed description of the physical phenomenon is provided in the literature [9,34,35]. The SIMS analysis were performed on a CAMECA IMS 4F-E7 instrument.

For this study, O^{2+} beam bombardment was used to enhance the ionisation field of electropositive species such as uranium. In this scanning microscope, the primary beam is focused into a small spot (around 0.5 μm), which scans the sample surface. The collected secondary ions can be measured with an electron multiplier and also sequentially converted into an image. Mass resolution can reach $M/\Delta M = 10{,}000$ and the lateral resolution of the imaging is only 0.5 μm. The experimental conditions for this work are indicated in Table 1. For each area analysed, mass spectra at around the mass of isotope 238 of uranium, and ion images were obtained. $^{40}Ca^+$ images give the histological structure of the bivalve gills and $^{238}U^+$ images show uranium fixation within the structures.

2.2. Corbicula fluminea exposure conditions

The experimental exposure conditions of bivalves, *C. fluminea*, have been widely described by Simon and Garnier-Laplace [7,8]. The freshwater bivalves, collected from Lake Sanguinet (Gironde, France), underwent an acclimatisation phase for at least 1 month under laboratory conditions (ambient temperature: 19–20 °C; artificial water in mg/L: $Ca^{2+} = 11.5$, $Mg^{2+} = 8$, $Na^+ = 11.6$, $K^+ = 6.2$, $Cl^- = 13.5$, $NO_3^- = 6.3$, $SO_4^- = 8.1$, $HCO_3^- = 71$, photoperiod: 12 h/12 h) in a storage tank containing quartz sand. The organisms were fed with an algae suspension (*Chlamydomonas reinhardii*). After the acclimatisation period they were exposed to aqueous uranium under laboratory conditions and they were not fed until the end of the experiments. The experimental system consists of two connected tanks: the first one maintains a constant pH and a constant U concentration in water and the second tank contains sand, aerated water and the bivalves. The uranium used for the contamination is a solution of uranyl nitrate. Two levels of exposure were used $[U]_{water} = 500$ μg/L and 20 μg/L, with different exposure durations. Table 2 indicates the main parameters of each treatment.

C. fluminea controls were prepared in the same way without the addition of uranium to the water.

2.3. Preparation of the biological samples for SIMS analysis

After the exposure phase, the gills of the clams were collected and underwent a classic chemical fixation procedure. They were fixed in a solution containing 6% glutaraldehyde in a sodium cacodylate buffer one day at 4 °C, then dehydrated in various propylene oxide and ethanol baths and permeated with a propylene oxide/Epon mixture. Finally, they were embedded in pure EPON-type resin.

Serial thin sections (0.5 μm) embedded in resin were cut and laid on polished ultra pure gold holders for SIMS analysis (to avoid relief effects and minimize charge effects) or on glass slides for histological controls with an optical microscope.

Table 1
Experimental analysis conditions by SIMS technique.

SIMS experimental conditions	1	2
Primary ion beam	$O2^+$	$O2^+$
Primary beam energy	12.5 keV	12.5 keV
Primary beam intensity	7×10^{-9} A	2×10^{-9} A
Secondary beam energy	4.5 keV	4.5 keV
Primary beam raster	200×200 μm^2	100×100 μm^2
Mass resolution	$M/\Delta M = 300$	$M/\Delta M = 300$

Table 2
Treatment conditions of contaminated *Corbicula*.

Total U concentration in water (μg/L)	Exposure duration (days)	pH
500	10	7
20	10	7
20	40	7
20	90	7

3. Results and discussion

The paired gills consist of two plate-like flaps (the outer demibranch and inner demibranch). They are attached to the two sides of the visceral mass and to the proximal part of the foot, and hang inside the mantle cavity on each side of the foot. Each demibranch contains an ascending limb and a descending limb (Fig. 1). The gill structure consists mainly of filaments. All the filaments are parallel and are arranged in series connected by interlamellar septa (Fig. 2). Around the gills filaments, we distinguish cilia.

The function of the gills is to transport water and gather food. The water passes between the gill filaments, which keep the respiratory exchanges steady. The ciliary and mucous cells draw, collect and transfer the food particles to the palps, the mouth and the digestive tract [37–39].

3.1. Corbicula fluminea controls

The morphology of the analysed gills filaments of the bivalves is shown in an optical microscope image and a $^{40}Ca^+$ ion image (Fig. 3). In the SIMS image, the hot colours represent the highest Ca concentrations.

Under these SIMS experimental conditions, the mass spectra of the gills of the clams that were not exposed to uranium, recorded at around the mass of isotope 238 of uranium at a low mass resolution ($M/\Delta M = 300$), do not show the presence of a significant peak at mass 238 (Fig. 4). This result suggests that natural uranium is not detected by SIMS and no polyatomic ions are superimposed on the element of interest at a low mass resolution. In this case, working at high mass resolution is not essential, which will improve secondary ion transmission and therefore also the detection limits.

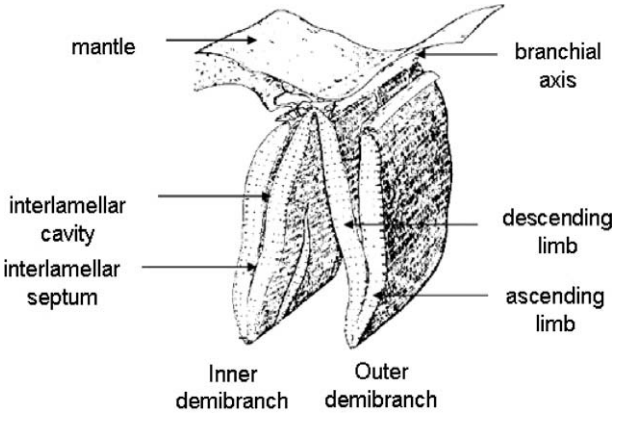

Fig. 1. Schematic representation of bivalve branchial [36].

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