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Trophic transfer of radioisotopes in Mediterranean sponges through bacteria consumption

Thomas Lacoue-Labarthe [a, b, *], Michel Warnau [a], Laureen Beaugeard [b], Pierre-Yves Pascal [c]

[a] International Atomic Energy Agency – Environment Laboratories, 4 Quai Antoine Ier, MC 98000 Monaco, Monaco
[b] Littoral Environnement et Sociétés, UMR 7266 CNRS - Université de La Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France
[c] Département de Biologie, Université des Antilles et de la Guyane, UMR 7138 UPMC-CNRS-MNHN-IRD, Equipe 'biologie de la mangrove', UFR des Sciences Exactes et Naturelles, BP 592, 97159 Pointe-à-Pitre, Guadeloupe, France

HIGHLIGHTS

- We study the transfer of radioisotopes in two sponge species fed with bacteria.
- We radiolabelled *Pseudomonas stutzeri* with seven metals and radionuclides.
- We examine the metal accumulation in bacteria and uptake and retention in sponge.
- Massive sponge assimilated less metal but retained 2-fold longer than erect species.
- Massive sponge could be pertinent candidate as biomonitor of chronic contamination.

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ABSTRACT

Numerous field studies highlighted the capacities of marine sponges to bioaccumulate trace elements and assessed their potential as biomonitors of the marine environment. Experimental works demonstrated that dissolved metals and radionuclides can be taken up directly by sponge tissues but, to the best of our knowledge, little is known on the contribution of the dietary pathway through the consumption of contaminated bacteria considered as one of the trophic source in sponge diet. Objectives of this work are to study trophic transfer of radiotracers ^{110m}Ag, ^{241}Am, ^{109}Cd, ^{57}Co, ^{134}Cs, ^{54}Mn and ^{65}Zn from the marine bacteria *Pseudomonas stutzeri* to the Mediterranean sponges *Aplysina cavernicola* and *Ircinia oros*. *P. stutzeri* efficiently bioaccumulated trace elements in our culture experimental conditions with CF comprised between 10^5 and 10^7 after 48 h of growth in radiolabeled medium. When fed with these radiolabelled bacteria, *A. cavernicola* took up around 60% of radiotracers accumulated in trophic source except ^{134}Cs for which only 8% has been transferred from bacteria to sponge. Contrasting to this, *I. oros* retained only 7% of ^{110m}Ag, ^{109}Cd and ^{65}Zn counted in bacteria, but retained 2-fold longer accumulated metals in its tissues. The sponge inter-specific differences of accumulation and depuration following a trophic exposure are discussed with respect to the structure and the clearance capacities of each species.

1. Introduction

Over the last 50 years, industrial and urban discharges considerably increased the input of foreign chemicals such as metals in coastal environment (Zhou et al., 2008). Heavy metals form complexes with organic compounds and lead to malfunction or death of affected cells. Consideration of this toxicity is essential in the management of coastal areas and the evaluation of heavy metal concentrations in marine environment is consequently a widespread concern. Measuring dissolved heavy metal presents analytical problems due to detection limits and contamination biases (Rainbow, 1995). Water contamination is difficult to infer from sediment as benthic accumulation is strongly linked with sediment characteristics such as grain size and organic content (Luoma, 1990). Analysis of heavy metal accumulated by marine organisms overcomes these disadvantages as tissues could be highly concentrated at rates proportional to environmental concentrations. Furthermore, only the bioavailable fraction of metal

* Corresponding author. UMR 7266 CNRS-Université de La Rochelle, Institut du Littoral et Environnement, 2 rue Olympe de Gouges, 17000 La Rochelle, France.
E-mail address: tlacouel@univ-lr.fr (T. Lacoue-Labarthe).

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presenting ecotoxicological relevance is measured and reflects metal availability over longtime period. For those different reasons, biota concentrations are routinely used to monitor environmental contamination and there is a continuous need for new marine biomonitors (Phillips and Rainbow, 1993; Conti and Iacobucci, 2008). Ideal biomonitor should be sedentary, abundant, easy to identify, widely dispersed, long lived and should accumulate heavy metal in his tissue at rate proportional to environmental contamination (Rainbow, 1995). Mussels have been the group most commonly used in heavy metal biomonitoring however sponge have been proposed as an additional group since they can dominate benthic community and are adapted to various ecological niches extending world widely from intertidal zones to deep sea (Patel et al., 1985; Pérez et al., 2005; Cebrian et al., 2007). By their filtering activity, sponges present high capacity to collect and concentrate heavy metals from dissolved and suspended phases (Hansen et al., 1995). Integration of heavy metal in sponge varies according to species (Patel et al., 1985) due to different amount of skeleton fibers and mesohyl in their tissues (Verdenal et al., 1990). Short and long life cycle sponge integrate contamination at different time scale and can be used simultaneously for a more effective monitoring of heavy metal pollution (Batista et al., 2014). As a result, knowledge about accumulation strategies of particular metals is clearly a prerequisite to use sponge as biomonitor.

Dissolved fraction of metal can be taken up directly by sponge tissues but also indirectly by their symbiotic bacteria that contribute to their nutrition and health and can represent 40% of sponge volume (Hentschel et al., 2006). Sponge species densely packed with symbiotic bacteria are known to extract considerable amount of Dissolved Organic Mater (DOM) from the water (Reiswig, 1981; Yahel et al., 2003; De Goeij et al., 2008) and this flux can be significant enough to structure marine environment at ecosystem scale (de Goeij et al., 2013). Assimilation of heavy metal associated with DOM (Iyer et al., 2005) could consequently be an important way of contamination for sponges (Webster et al., 2001). Sponge can also take up metals from the suspended particles collected during feeding. This filter feeding activity is based on pumping ambient water through an intricate system of aquiferous canals to choanocyte chambers where suspended particules are retained by sieving and then phagocyted (Riisgård and Larsen, 2010). Sponges can retains diatom, flagellate and ciliate (Coma et al., 2001) however this filtering system is rather specialized in retaining smaller preys (size range 0.1–2 μm) such as cyanobacteria, bacteria (Ribes et al., 1999b; Trussell et al., 2006; Yahel et al., 2007) and viruses (Hadas and Marie, 2006). The fact that sponges are able to retain smaller particles than most filter feeders is an adaptative advantage, particularly in oligotrophic environment (Ribes et al., 1999a). Bacteria have been considered as one of the primary source of energy in sponge diet (Pile et al., 1996; Kowalke, 2000).

Bacteria can take up heavy metals at rates high enough to be considered for remediation in polluted environments (Valls and Lorenzo, 2002; Malik, 2004). The cell surface of bacteria carries a net negative charge and can passively adsorb appreciable quantities of positively charged cationic metals (Brierley, 1990; Scott and Palmer, 1990). Metal can also actively pass across the cell membrane through the cell metabolic cycle and bind to intracellular compounds (Malik, 2004). Both modes of metal uptake give to bacteria good bioaccumulation properties.

The aim of the present study is to enrich bacteria *Pseudomonas stutzeri* with different metals and radionuclides (Mn, Co, Zn, Ag, Cd, ^{137}Cs, ^{241}Am) in order to feed two Mediterranean sponges species (*Ircinia oros* and *Aplysina cavernicola*) and to be able to evaluate their accumulation capacities of each element.

2. Materials and methods

2.1. Organisms

Specimens of *Aplysina cavernicola* and *Ircinia variabilis* were collected off the Fontvieille harbour at St Nicolas rocks in Monaco by SCUBA in March 2012 (lat: 43° 43′ 36.768″; long: 7° 25′ 25.248″). Immediately after collection, sponges were kept in seawater at constant temperature during their transfer to the aquaria within 30 min. At the IAEA premises, sponges were placed in a 20-L glass aquarium containing 1 μm filtered and UV sterilized natural seawater (seawater flux: 40 L h^{-1}; temperature 17 °C; 38 p.s.u.; light/dark cycle 12 h/12 h) and acclimated for one week before experiment and fed daily with live *Isochrysis galbana.*

A. cavernicola (Class: Demospongiae, Order: Verongida, Family: Aplysinidae) is a yellow digitate-shaped sponge, organized in one or more chimney-like structures. *I. oros* harboured abundant and phylogenetically diverse symbiont consortia including Gammaproteobacteria (Erwin et al., 2011). Numerous inhalant oscula pump water to the central atrium structure and then to the apical exhalent osculum (Pfannkuchen et al., 2009). The close species *Aplysina aerophoba* displays a clearance rate of 3–18 ml g^{-1} min^{-1} (Hoffmann et al., 2008).

I. variabilis (Class: Demospongiae, Order: Dictyoceratida, Family: Irciniidae) is a massive sponge. Among the large amounts of symbiotic bacteria hosted by *A. cavernicola*, gamma-proteobacteria are the second most abundant (Friedrich et al., 1999). Oscules are large and located at the top of the conical lobes. This species does not have mineral spicules but a dense fiber skeleton structurally diverse from classical spongins (Junqua et al., 1974). The clearance rate for this species has been estimated experimentally (data not shown) to *ca.* 0.8 ml g^{-1} min^{-1}.

2.2. Bacteria radiolabelling

The marine bacteria *Pseudomonas stutzeri,* belong to the class of gamma-proteobacteria and is distributed widely in the water column and in sediment (Lalucat et al., 2006). Gamma-proteobacteria is one of the most abundant bacterial taxa symbiotic of sponges (Webster and Taylor, 2012) and *P. stutzeri* has been previously identified in intertidal marine sponge (Zhang et al., 2013). In the present study, *P. stutzeri* have been cultured in a medium radiolabelled with ^{110m}Ag, ^{109}Cd (Amersham, UK) and ^{241}Am, ^{57}Co, ^{134}Cs, ^{54}Mn and ^{65}Zn (Isotope Product Laboratory, USA) and then used as trophic source of radioisotope to sponges specimen.

In detail, *P. stutzeri* isolates have been first grown for 48 h by adding bacteria in 5 ml of previously sterilized autoclaved bacterial liquid culture medium (Marine Broth 2216, Difco™) and kept in the dark at 4 °C. Then, the 5 ml culture was transferred in 300 ml of culture medium radiolabelled with 13 kBq l^{-1} of ^{110m}Ag, 7 kBq l^{-1} of ^{241}Am, 39 kBq l^{-1} of ^{109}Cd, 11 kBq l^{-1} of ^{57}Co, 20 kBq l^{-1} of ^{134}Cs, 11 kBq l^{-1} of ^{54}Mn and 11 kBq l^{-1} of ^{65}Zn and kept in the dark at 25 °C for 48 h. The culture media has been performed in triplicate.

At the end of the incubation period, bacteria were concentrated by centrifugation (3000 rpm during 10 min at 25 °C) in order to remove the radiolabelled culture media, without causing destruction of bacteria. The supernatant was discarded and the bacteria were resuspended in 0.2 μm-filtered seawater and centrifugated again. The operation was repeated three times until no significant radioisotopes activities were detected in the supernatants. Finally, the pellet was resuspended in 50 ml of filtered seawater giving a suspension of living bacteria with a concentration of $2.10^7 \pm 3.10^5$ cell.ml^{-1}. One aliquot of this bacterial solution was radiocounted in order to assess the bioaccumulation of radiotracer in *P. stutzeri.*

2.3. Bacterial concentration assessment

The concentrations of radiolabelled cells in the bacterial suspension and in the experimental tanks were assessed through the optical density. Measured absorbance values (A_{680nm}) have been transformed in bacteria concentration (cell ml^{-1}) thanks to a calibration curve (Fig. 1) realized from successive dilutions of the bacterial suspension. The concentration of bacteria in diluted samples has been determined by cytometry. Briefly, bacterial suspensions were stained with Sybr Green I (1/10000 final concentration, Invitrogen) and incubated for 15 min in the dark. Samples were analysed with a FACS CantoII (BDBiosciences) equipped with an air-cooled, 20 mW solid state blue laser (488 nm) at low speed (~8 µL/min) during 1 min. Stained cells were discriminated according to green fluorescence (FL1) from Sybr Green I staining and side scatter properties (SSC) in log mode. Accurate cell concentrations were performed using TruCount beads (BDBiosceinces).

Feeding procedure: the dietary uptake of metals in sponges has been assessed using the pulse-chase feeding method (organisms were dietary exposed to radiotracers only once, in order to analyse metal assimilation and depuration from one ration; Warnau et al., 1996). For each sponge species, three individuals were placed individually in 450 ml plastic box (seawater flux: 20 L h^{-1}; temperature: 17 °C; salinity 38 p.s.u.; light/dark cycle 12 h/12 h) 24 h before the start of the experiment. Then, the water flow renewal has been closed and the radiolabelled bacteria have been resuspended in the seawater until reaching a cell density of 3.10^7 cell.ml^{-1}. Sponges were left for 1 h in closed-circuit tanks allowing them to feed with radiolabelled bacteria. After 20 and 60 min, the retention of bacteria by sponges has been assessed by measuring the bacteria concentration in seawater (A_{680nm}).

Following this one-hour exposure period, sponges were sampled, rinsed in clean seawater, and immediately counted. Then, sponges were placed in a 20-l tank with clean seawater (seawater flux: 40 L h^{-1}; temperature 17 °C; 38 p.s.u.; light/dark cycle 12 h/12 h) and the radiotracers loss in sponges has been followed by counting individuals every days for the first week and every third days during the next four weeks.

2.4. Radioanalyses and data treatment

Radioactivities were measured using a high-resolution γ-spectrometry system consisting of four coaxial Germanium (N- or P-type) detectors (EGNC 33–195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). The detectors were calibrated with an appropriate standard for each counting geometry used and measurements were corrected for background and physical decay of the radiotracers.

The bioaccumulation of each radiotracer in bacteria was expressed in concentration factors, which is the ratio between radiotracer activity in the bacteria – Bq g^{-1} – and the time-integrated activity in Marine Broth culture medium – Bq g^{-1}. The concentration of radiotracers expressed in Bq $cell^{-1}$ has been transformed using the bacteria mass value of *Pseudomonas putida* of 5.10^{-14} g dw $cell^{-1}$ (Troussellier et al., 1997).

The radiotracer depuration kinetics in sponges was expressed in terms of change in percentage of remaining activity and was best by either a single- or a double-exponential equation (see Lacoue-Labarthe et al., 2009 for detailed procedure).

Constants (and their statistics) of the best fitting uptake and depuration kinetic equations (decision based on ANOVA tables for two fitted model objects) were estimated by iterative adjustment of the models using the *nls* curve-fitting routine in R freeware (R Core Team, 2014). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results

3.1. Radiolabelling of Pseudomonas stutzeri

The culture of the bacteria *P. stutzeri* in a bacterial culture medium radiolabelled with dissolved radiotracers allowed a significant bioaccumulation in bacteria cells of ^{110m}Ag, ^{241}Am, ^{109}Cd, ^{57}Co, ^{134}Cs, ^{54}Mn and ^{65}Zn after 48 h of growth (Fig. 2). It is noteworthy that bioaccumulation efficiency varied among elements with the highest CF values observed for the non-essential metals ^{110m}Ag and ^{109}Cd ($1.10^7 \pm 4.10^5$ and $1.10^7 \pm 5.10^5$, respectively) and the radionuclide ^{241}Am ($5.10^6 \pm 3.10^5$). Contrasting to this, the essential elements ^{65}Zn, ^{57}Co and ^{54}Mn have been taken up 3, 20 and 30-fold lower, respectively, in bacteria than ^{110m}Ag and ^{109}Cd. Caesium reached a CF value of $2.10^6 \pm 6.10^4$.

3.2. Radiotracer trophic transfer from bacteria to sponges

During the one-hour pulse-chase feeding experiment, the monitoring of the absorbance (680 nm) of tank seawater allowed at determining the bacterial retention by both species. Thus, after one

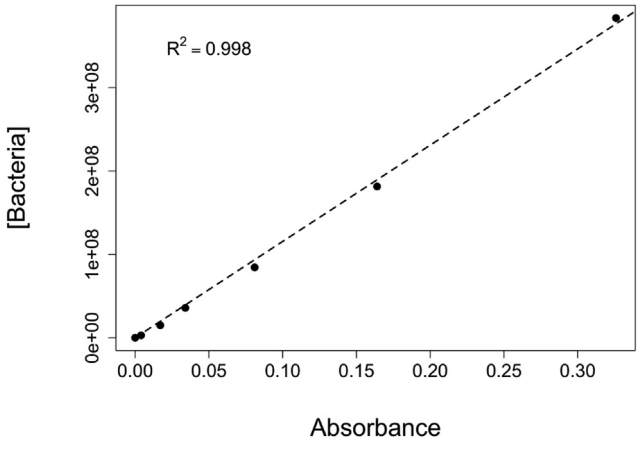

Fig. 1. Calibration curve relating the bacteria concentrations (cell/ml) according to the absorbance at 680 nm.

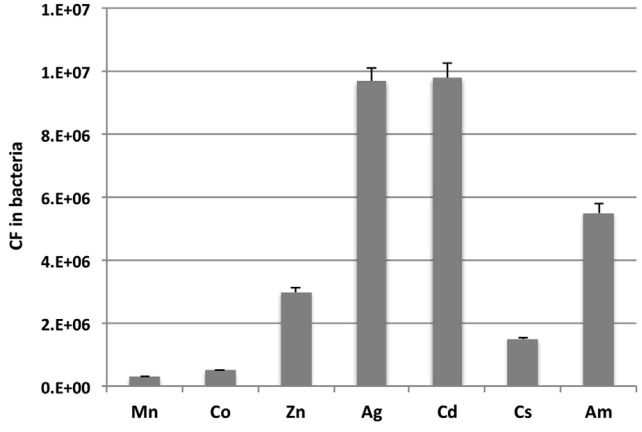

Fig. 2. Concentration factors (CF) of radiotracers in the bacteria *Pseudomonas stutzeri* cultured during 48 h in Marine Broth medium radiolabelled with ^{54}Mn (11 Bq ml^{-1}), ^{57}Co (11 Bq ml^{-1}), ^{65}Zn (11 Bq ml^{-1}), ^{110m}Ag (13 Bq ml^{-1}), ^{109}Cd (39 Bq ml^{-1}), ^{134}Cs (20 Bq ml^{-1}), ^{241}Am (7 Bq ml^{-1}).

hour, the three individuals of *Aplysina. cavernicola* removed 20, 45 and 65% of bacteria suspended in seawater, whereas no significant decrease of bacterial concentration was observed in *Ircinia oros* tanks, suggesting a lower filtration activity of this species in our experimental condition.

At the end of trophic exposure, the activities of radiotracers accumulated by *A. cavernicola* (Bq g^{-1} dw) were ranked as followed: ^{109}Cd >> ^{110m}Ag > ^{241}Am ≈ ^{65}Zn >> ^{57}Co > ^{134}Cs > ^{54}Mn (Fig. 3A), whereas only ^{109}Cd >> ^{110m}Ag > ^{65}Zn were significantly detected in *I. oros.* This pattern of bioaccumulation is congruent with the radioactivity levels measured in bacteria for each element, showing that *A. cavernicola* took up around 60% of radiotracers (Fig. 3B) accumulated in bacteria except ^{134}Cs for which only 8% has been transferred from bacteria to sponge. Contrasting to this, *I. oros* retained only 7% of ^{110m}Ag, ^{109}Cd and ^{65}Zn counted in bacteria.

Following the 1-h pulse chase feeding, the whole-body depuration for ^{110m}Ag, ^{241}Am, ^{109}Cd, ^{57}Co, ^{54}Mn and ^{65}Zn was followed for 35 days in both sponge species. The ^{134}Cs loss kinetic was not shown as this radionuclide activity has been found below the detection limit in sponge placed in clean seawater after 24 h, suggesting a rapid depuration of this element. The depuration kinetics in *A. cavernicola* were best fitted by a two-compartment model for all element except ^{54}Mn loss better fitted by a single-compartment model (Figs. 4 and 5; Table 1). Both ^{110m}Ag and ^{241}Am were the most rapidly depurated by sponge with 88 ± 3 and 79 ± 8% lost with a biological half-life ($T_{b½}$) of 1 and 1.5 d, respectively. The essential elements displayed the highest assimilation efficiency for the long lived components (A_{0l} = 55, 95 and 62% for ^{57}Co, ^{54}Mn and ^{65}Zn,

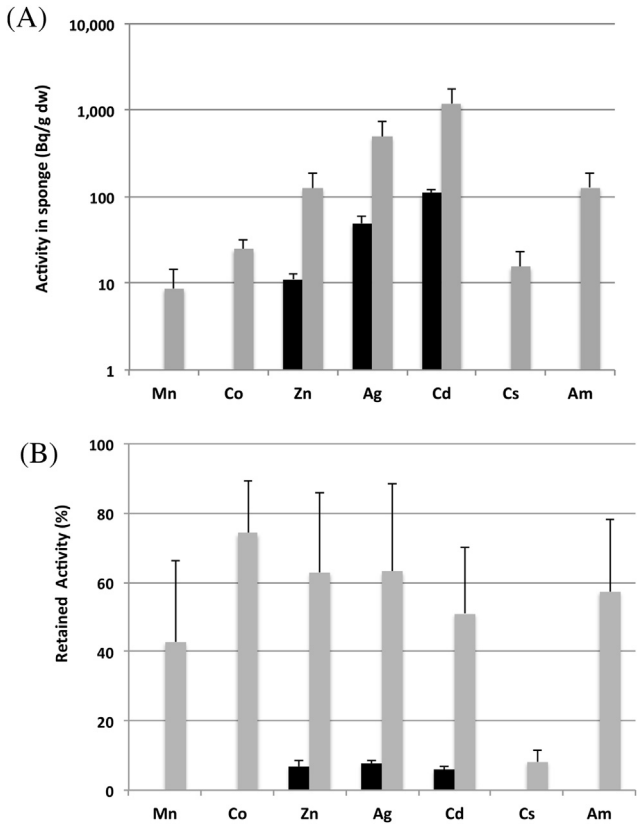

Fig. 3. (A) Radiotracers activities (Bq g^{-1} dw; n = 3 per sponge) and (B) retained activities (%) measured in sponges *Ircinia oros* (black histogram) and *Aplysina cavernicola* (grey histogram) after feeding with radiolabelled bacteria *Pseudomonas stutzeri* ($2.9.10^7$ bacteria ml^{-1}) for 1 h. Please note the logarithmic scale for the figure (A) and that ^{54}Mn, ^{57}Co, ^{134}Cs and ^{241}Am activities were under the limit detection in *I. oros.*

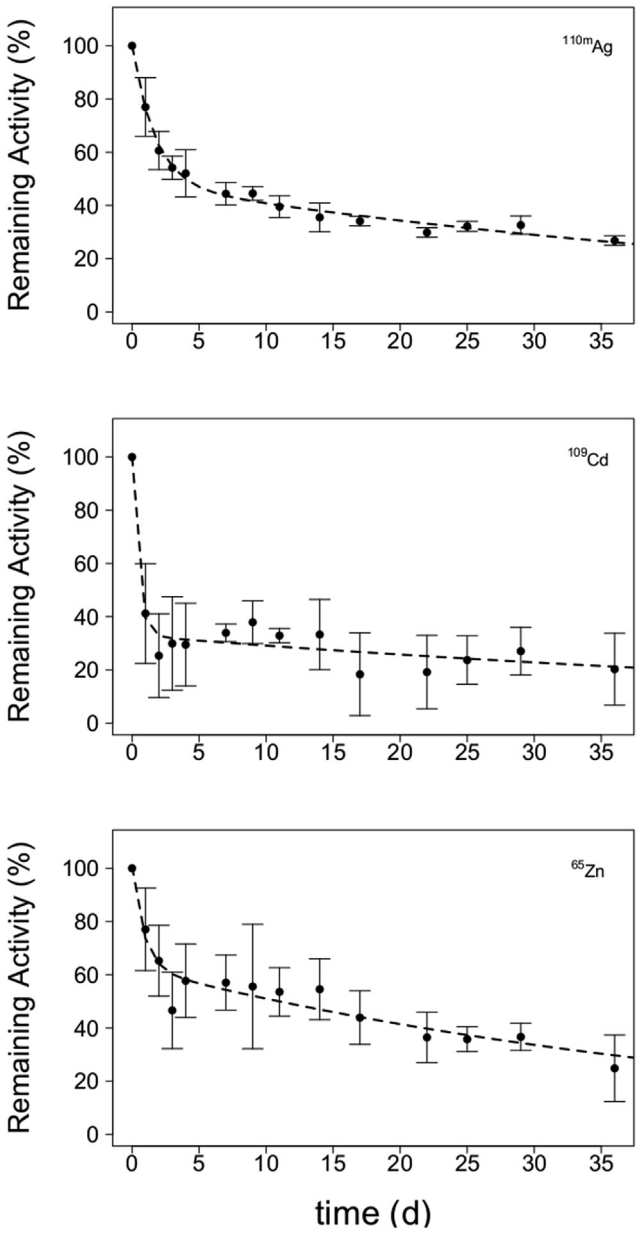

Fig. 4. *Ircinia oros*. Loss kinetics of ^{110m}Ag, ^{109}Cd and ^{65}Zn (% remaining activity [RA]; mean ± SD) in the sponge *Ircinia oros* (n = 3) previously fed with radiolabelled bacteria for 1 h. Parameters for the best fitting equations are given in Table 1.

respectively, with relatively short $Tb_{1/2}$ comprised between 12 and 18 d. Contrasting to this, 33% of ^{110m}Ag, 57% of ^{109}Cd and 63% of ^{65}Zn significantly accumulated by *I. oros* displayed longer $T_{b½}$ (41, 57 and 33 d, respectively), implying a better efficient and longer retention of these elements by sponge tissues or within their aquifer system.

4. Discussion

The paper first reports that bacteria *P. stutzeri* efficiently bioaccumulated trace elements in our culture experimental conditions with CF comprised between 10^5 and 10^7 after 48 h of growth in radiolabeled medium. Similar high bioaccumulation efficiencies have been already reported in picoplankton *Synechococcus* sp (Fisher, 1985), under similar exposure conditions. The small size of bacteria and the subsequent high surface/volume ratio are argued

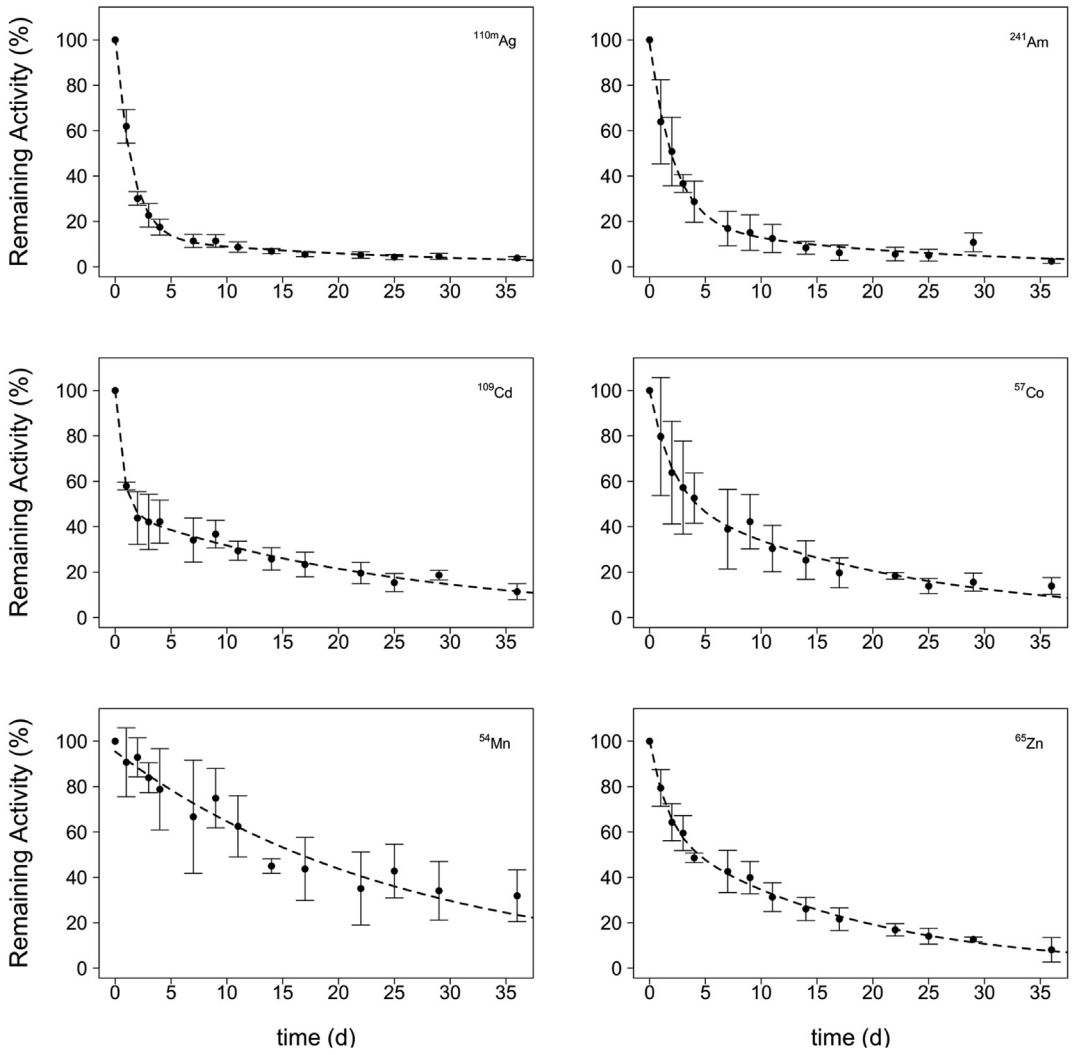

Fig. 5. *Aplysina cavernicola*. Loss kinetics of radiotracers (% remaining activity [RA]; mean ± SD) in the sponge *Aplysina cavernicola* (n = 3) previously fed with radiolabelled bacteria for 1 h. Parameters for the best fitting equations are given in Table 1.

as the main explanation fostering the uptake capacities. Basically, two main processes drive the trace element uptake in microorganisms, *i.e.* the adsorption of metal on the particle walls and external materials such as the exopolysaccharides, and the absorption in cells through ionic transporters (Gadd, 1990).

The gram-negative wall of *P. stutzeri* and its associated negative charges might favour the binding of metallic cations (Gadd et al., 1989). For each element, the adsorption on bacterial surface could

Table 1
Parameters and statistics of the depuration kinetics of radiotracers in two species of sponge, *Ircinia oros* and *Aplysina cavernicola*, fed for one hour with radiolabelled bacteria *Pseudomonas stutzeri* and kept for 36 days in depuration condition.

Metal	Species	Model	A_{0s} ± SE (%)	k_{es}	$T_{b½s}$ ± SE (d)	A_{0l} ± SE (%)	k_{el}	$T_{b½l}$ ± SE (d)	R^2
^{54}Mn	*I. oros*	–	–	–	–	–	–	–	
	A. cavernicola	O	–	–	–	95 ± 4	0.039***	18 ± 2	0.887
^{57}Co	*I. oros*	–	–	–	–	–	–		–
	A. cavernicola	T	45 ± 13	0.516	1.3 ± 0.7	55 ± 13	0.049**	14 ± 4	0.917
^{65}Zn	*I. oros*	T	38 ± 8	1.145	0.6 ± 0.3	63 ± 5	0.021***	33 ± 8	0.848
	A. cavernicola	T	38 ± 6	0.652***	1.1 ± 0.3	62 ± 5	0.058***	12 ± 1	0.982
^{110m}Ag	*I. oros*	T	52 ± 4	0.589***	1.2 ± 0.2	48 ± 3	0.017***	41 ± 8	0.974
	A. cavernicola	T	88 ± 3	0.681***	1.0 ± 0.1	13 ± 3	0.040**	17 ± 5	0.993
^{109}Cd	*I. oros*	T	67 ± 8	2.258*	0.3 ± 0.2	33 ± 3	0.012	57 ± 35	0.860
	A. cavernicola	T	53 ± 5	1.490***	0.5 ± 0.1	47 ± 3	0.039***	18 ± 2	0.966
^{241}Am	*I. oros*	–	–	–	–	–	–	–	–
	A. cavernicola	T	79 ± 8	0.470***	1.5 ± 0.3	19 ± 18	0.047	15 ± 8	0.969

O and T: 1- and 2-component exponential models, respectively; A_{0s} and A_{0l}: assimilation efficiency of the short- and long-lived component, respectively; SE: standard error; kes and kel: depuration rate of the short- and long-lived component, respectively; $T_{b½s}$ and $T_{b½l}$: biological half-life of the short- and long-lived component, respectively; R^2: determination coefficient; p-values: < 0.001 (***), <0.01 (**), <0.05 (*), >0.5 (ns).

be estimated through the partition coefficient between particles and seawater (K_d) reported in the literature. According to available data on particles or phytoplankton, the particle reactivity among studied elements decreases according to ^{241}Am > Mn > Zn > Ag > Co > Cd > Cs (Wang et al., 1996; Keung et al., 2008; Metian et al., 2008a, 2009). This ranking is not congruent with the CF values observed in this study, especially in the case of Ag and Cd supposed to be less bound on bacterial surface, but here highly accumulated on *P. stutzeri*.

In cells of *Pseudomonas aeruginosa*, high Hg uptake in strain has been attributed to cysteine-rich transport proteins carrying metal from external medium to cytoplasm (Chang and Hong, 1994). Similar mechanisms might be expected to contribute to the high accumulation of Ag, and to a lesser extent Cd, according to the affinity of these metals for sulfhydryl-rich group (Bell and Kramer, 1999). Moreover, some bacteria strains are known for their high resistance to silver toxicity (Haefeli et al., 1984; Li et al., 1997). While this resistance seems to be associated with energy-dependent ion efflux (Li et al., 1997) in most of studied bacteria (*e.g. Escherichia coli*), it has been demonstrated that the strain *P. stutzeri* AG259 accumulated Ag under a metal-based single crystal, precipitating then high Ag concentration under non-metabolically available form (Klaus et al., 1999).

Regarding Cd, Chang et al. (1997) highlighted a rapid adsorption of the element on cells, but a difference of biosorption capacity between inactivated and free cells, suggesting that a metabolic-dependent absorption occurs in this strain. Furthermore, same authors showed that cells at exponential growth phase exhibited higher Cd adsorption capacities, suggesting that in our radiolabelling conditions, the culture of *P. stutzeri* in a ^{109}Cd radiolabelled medium could contribute to reach high CF value.

In the present study, it is noteworthy that essential elements (*i.e.* ^{57}Co, ^{54}Mn, and ^{65}Zn) displayed lower CF values than ^{110m}Ag, ^{241}Am and ^{109}Cd. The microorganisms such as bacteria are known to synthesize secondary metabolites, called “siderophores”, that play a key role in the essential metal homeostasis (Lalucat et al., 2006). Indeed, some bacteria strains produce low-molecular-weight chelators with a high affinity for Fe and other transition metals to bind them in the extracellular environment and transport them inside the cells (Zawadzka et al., 2006). In *Pseudomonas aerunginosa*, salicylate-derived pyochelin seems to have a key role in supplying divalent metals such as Co or Ni, but displays a low affinity for Mn (Visca et al., 1992), suggesting that siderophore unlikely contribute to the uptake of this element. Some *P. stutzeri* strains produce deferrioxamine or PDTC (pyridine-2,6-bisthiocarboxylate) siderophores (Lewis et al., 2004) also known for their role in transition element nutrition as demonstrated for the Zn (Leach et al., 2007). However, information are lacking on the various affinity of siderophores for the essential transition metals to better assess their role in metal metabolism.

Additionally, it is important to remind that *P. stutzeri* were cultured in a medium (Marine Broth 2216; Zobell, 1941), which contains essential compounds and nutrients for an optimal bacteria growth. Yeast extract supplies trace element and may contribute to lower the bioaccumulation efficiency of essential metals. According to Grant and Pramer (1962), Mn and Co concentrations are almost one order of magnitude lower than those of Zn (3 and 3.5 $\mu g\ g^{-1}$ vs. 75 $\mu g\ g^{-1}$ dw) but similar to the Cd concentration (~1.5 $\mu g\ g^{-1}$ dw). Considering that addition of stable element in culture medium may lowers concentration factor (Metian et al., 2008b), the yeast extract composition would contribute to explain surprising lower CF values observed for essential elements such as Co and Mn, compared to non-essential element or radionuclide, such as Cs, known to be poorly accumulated in marine invertebrates and microorganisms (Wang et al., 1996; Pouil et al., 2015).

Tissues of Mediterranean sponges fed with radiolabeled bacteria during one hour present an activity pattern of the seven radiotracers similar to bacterial pattern (Fig. 3A). *A. cavernicola* displayed around 60% of the radioactivities (no significant difference among radiotracers except ^{134}Cs; U-test; $p > 0.05$) added through bacteria whereas *I. oros* only accumulated around 7%. This contrasting bacterial metal transfer between both species is likely due to the low clearance rate of the massive *I. oros* being 4–22 fold lower than this of the branching erect *A. cavernicola* (Hoffmann et al., 2008). Thus, this estimation is congruent with the 9-fold difference of radiotracers activities counted in both species. As a result, only the elements highly accumulated in *P. stutzeri*, *i.e.* Ag, Cd and Zn, reached activities levels in sponge above the detection threshold, limiting the assessment of trophic transfer behaviour for the others radionuclides.

It is noteworthy that, in *A. cavernicola*, a low fraction of ^{134}Cs contained in bacteria, *i.e.* 8 ± 3% *vs.* 43 ± 24%, 74 ± 15%, 63 ± 23%, 63 ± 25%, 51 ± 19% and 57 ± 21% for ^{54}Mn, ^{57}Co, ^{65}Zn, ^{110m}Ag, ^{109}Cd, and ^{241}Am, respectively; U-test; $p < 0.05$) has been transferred to the sponge, suggesting a rapid depuration of this element by bacteria and sponge during the one hour pulse feeding chase. Indeed, Cs is a soluble element, known as a mimetic of K (Wang et al., 2000), implying both rapid uptake in cells and then loss in seawater, following water movements between both compartments (Lacoue-Labarthe et al., 2010).

Kinetic parameters following pulse-chase feeding (Figs. 4 and 5; Table 1) suggest that depurations of all element except Mn were best fitted by a two-compartment models, implying that 1) a fraction is rapidly lost likely depending on the water renewal in the sponge aquifer system, and that 2) another fraction of metals remained longer associated to sponge tissue through retention of bacteria in the aquifer system or assimilation of elements in sponge tissues. Results clearly show that the longest $T_{b½}$ is around 2 weeks in *A. cavernicola* whatever the metal, highlighting a poor retention of metals following particulate contamination. In comparison, $T_{b½}$ of elements accumulated from waterborne pathway were between 2 and 10-fold longer (with 35 and 157 days for ^{241}Am and ^{109}Cd, respectively) in the branch erect sponge *Axinella reniformis* (Genta-Jouve et al., 2012). Moreover, calculated $T_{b½}$ in this study for all elements exceeds one month in *I. oros* for Ag, Cd and Zn. Again, the contrasting clearance rates between both species could be one explanation of this difference, implying that the water renewal in the aquifer system drive the depuration rate for absorbed or assimilated trace elements in sponge. Thus, this work highlighted that the high pumping flow reported in erect sponge *A. cavernicola* contributed to a rapid accumulation of radiolabelled particles compared to the slow filter feeder massive sponge *I. oros* (Turon et al., 1997), but foster also a rapid depuration of trace elements, suggesting a limited net accumulation of metals from trophic route for this species. This observation is congruent with the bioaccumulation capacities of dissolved metals in Mediterranean sponges showing that with massive sponges (*Chondrosia reniformis*, *Agelas oroides* and *Ircinia variabilis*) display the highest uptake and retention capacities compared to erect species (*Acanthella acuta*, *Axinella damicornis*, *Axinella verrucosa*). These massive species possess higher choanocyte chamber volumes and consequently a much higher area to bind trace elements or retained contaminated particles as suggested by Cebrian et al. (2007).

This study demonstrated experimentally that filtered marine bacteria by Mediterranean sponges could be considered as a biotic vector of metal transfer along the trophic web. However, many questions merged from these results for enhancing our understanding of the observed metal transfer from microoganisms to sponges and the interspecific differences of bioaccumulation capacities among sponges. Further experiments including subcellular

fractionation could help to describe the trace element bioaccumulation pathways in bacteria (adsorption *vs.* absorption) and thus provide some indications on the metal bioaccessibility for the next trophic level. Moreover, the bioaccumulation efficiencies through particulate pathway differs between the two studied species, likely linked to their massive *vs.* erect structure and their respective pumping and clearance capacities (de Mestre et al., 2012). Considering the potential use of sponges as "biomonitors", the massive *I. oros* requests a prolonged metal input to reveal a contamination of bacterial resources, but retains more longer this information compared to erect *A. cavernicola.* In other terms, the first species seems to be appropriate to detect chronic contamination compared to the erect sponge. Nevertheless, beyond these characteristics, the role of symbiotic microorganisms in the particle retention, digestion and assimilation of nutrients and trace elements remains unknown. Both *A. cavernicola* and *I. oros* are considered as HMA (High Microbial Abundance) sponge species (Taylor et al., 2007; Weisz et al., 2008; Erwin et al., 2011). Thus, the interspecific bioaccumulation differences cannot be inferred to microbial density but rather potentially to distinct microbial communities according to sponge species (Selvin et al., 2009). Additionally, only one bacteria strain has been used in this work, implying that only one particle size has been investigated as a potential vector for metal trophic transfer. Sponges however show adaptive interspecific variation with respect to clearance rate and particle size selection (Turon et al., 1997). Further studies investigating trophic bioaccumulation on a large range of particle size using other bacterial strains or picoplankton could be valuable to define the contribution of filtered particles in the bioaccumulation of metals in these biomonitors of growing interest (Patel et al., 1984; Pérez et al., 2005; Cebrian et al., 2007). Finally, we hope that this study will revive some interest on the bacterivory as a pathway for metal integration in trophic web.

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