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## Comparative study on the bioaccumulation and biotransformation of arsenic by some Northeastern Atlantic and Northwestern Mediterranean sponges

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### Abstract

The bioaccumulation and biotransformation of arsenic (As) were studied in six representative marine sponges from the French Mediterranean and Irish Atlantic coasts. Methodologies were carefully optimized in one of the species on *Haliclona fulva* sponges for two critical steps: the sample mineralization for total As analysis by ICP-MS and the extraction of As species for HPLC-ICP-MS analysis. During the optimization, extractions performed with 0.6 mol L<sup>-1</sup> H<sub>3</sub>PO<sub>4</sub> were shown to be the most efficient. Extraction recovery of 81 % was obtained which represents the best results obtained until now in sponge samples. Total As analyses and As speciation were performed on certified reference materials and allow confirming the measurement quality both during the the sample preparation and analysis. Additionally, this study represents an environmental survey demonstrating a high variability of total As concentrations among the different species, probably related to different physiological or microbial features. As speciation results showed the predominance of arsenobetaine (AsB) regardless of the sponge species, as well as the occurrence of low amounts of dimethylarsinic acid (DMA), arsenate (As(+V)), and unknown As species in some samples. The process responsible for As transformation in sponges is most likely related to sponges metabolism itself or the action of symbiont organisms. AsB is supposed to be implied in the protection against osmolytic stress. This study demonstrates the ability of sponges to accumulate and bio-transform As, proving that sponges are relevant bio-monitors for As contamination in the marine environment, and potential tools in environmental bio-remediation.

**Keywords:** marine sponges; biomonitor; arsenic bioaccumulation; arsenic speciation; bioremediation

## 31 1. Introduction

32 Arsenic (As) is a ubiquitous element that ranks 20<sup>th</sup> in abundance in the earth's crust (1.5–3 mg kg<sup>-1</sup>). This  
33 metalloid occurs naturally in the environment, and it is also provided by some anthropogenic activities (Mandal  
34 and Suzuki, 2002). As is recognized to be toxic and one of the six most preoccupying pollutants on earth  
35 together with lead, mercury, chromium, some radionuclides and pesticides. The understanding of As cycle in  
36 marine ecosystem remains a challenging task since arsenic often occurs at very low concentrations (around 1  
37 µg L<sup>-1</sup> in seawater) and under one of the most toxic inorganic species (As(+V)) (Cabon and Cabon, 2000). Within  
38 this context, there is a clear need to gain a better understanding of the As cycle in the marine environment.

39 The quantification of total As in marine organisms and especially in seafood has been performed in  
40 numerous studies (Phillips, 1990; Kucuksezgin et al., 2014; Wu et al., 2014; Olmedo et al., 2013), but rarely in  
41 marine invertebrates. Among marine invertebrates, sponges are sessile filter feeders, capable of filtering every  
42 day a volume of seawater up to 50 000 times that of their body (Weisz et al., 2008). They have been recognized  
43 as excellent bio-monitors for trace element pollution (Perez et al., 2005; Cebrian et al., 2007) as they are  
44 capable to accumulate trace elements at concentrations higher than bivalves (Patel et al., 1985; Negri et al.,  
45 2006; Padovan et al., 2012). On hard substrata, sponges are one of the top spatial competitors (Bell, 2008) and  
46 have recently been proposed as model organisms to monitor aquatic contamination, in addition to the already  
47 existing and well known "Mussel Watch Program" (Genta-Jouve et al., 2012). The bioaccumulation of trace  
48 elements in sponges was suggested to differ according to sponge species and the element of interest (Batista  
49 et al., 2014; Mayzel et al., 2014; Cebrian et al., 2007; Patel et al., 1985). Only few studies focused on As  
50 bioaccumulation in sponges (Aly et al., 2013; Araújo et al., 2003; Batista et al., 2014; Denton et al., 2006; Keren  
51 et al., 2015; Keren et al., 2016; Keren et al., 2017; Padovan et al., 2012; Pan et al., 2011; Perez et al., 2005;  
52 Schaeffer et al., 2006; Shiomi et al., 1988; Vaskovsky et al., 1972; Venkateswara Rao et al., 2009; Yamaoka et  
53 al., 2001; Yamaoka et al., 2006). These studies revealed how As concentrations in sponges is variable,  
54 exceeding 100 mg kg<sup>-1</sup> in some cases, and usually higher than in other marine organisms (generally < 10 mg kg<sup>-1</sup>

55 in fish, algae and shellfish) (Llorente-Mirandes et al., 2010). Sponges seem to accumulate more As than other  
56 marine organisms, which makes them suitable models for biomonitoring studies, as already proposed by  
57 Genta-Jouve et al., 2012. As accumulation was previously demonstrated to be strongly related to sponge  
58 orders or species; for instance higher in demosponges than in calcareous sponges (Yamaoka et al., 2001).

59 The determination of total As concentration is however not sufficient as As toxicity and biological impact  
60 depend directly on its chemical form, *i.e.* its speciation (Cullen and Reimer, 1989; Hughes, 2002; Sakurai, 2002)  
61 . Among arsenic species, inorganic arsenite As(+III) and arsenate As(+V) are the most toxic forms. These two  
62 inorganic forms are carcinogenic and cause damage to the respiratory, cardiovascular, nervous, and  
63 hematopoietic systems, as well as lesions to skin and liver (Pershagen, 1981). The methylated forms  
64 monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are less toxic than the previously mentioned  
65 inorganic species, but they are recognized as cancer promoters (Brown et al., 1997) while arsenobetaine (AsB),  
66 the major species in marine animals, arsenocholine (AsC), trimethylarsine oxide (TMAO) and  
67 tetramethylarsonium ion (TMAs) are considered nontoxic (ATSDR, 2007). Other As species, like arsenosugars  
68 (AsS), often found in seafood and generally in marine organisms, have been recognized not to be acutely toxic;  
69 nevertheless a chronic toxicity is a possibility (Andrewes et al., 2004). The quantitation of the different As  
70 chemical forms, *i.e.* arsenic speciation, can be performed with different analytical techniques (Benramdane et  
71 al., 1999; Hsieh et al., 2010; Nearing et al., 2014; Tian et al., 2010). HPLC-ICP-MS represents the most used  
72 technique by far (Benramdane et al., 1999; Nearing et al., 2014) because this hyphenated technique combines  
73 a rapid, powerful and reproducible separation method with a very efficient detector due to its high sensitivity  
74 and large linear dynamic range (Beauchemin, 2008).

75 In most aquatic organisms, As speciation analysis has already revealed the occurrence of AsB, AsS, DMA and  
76 inorganic species. In freshwater organisms, the inorganic As(+III) species was reported as dominant for  
77 gastropods (Hong et al., 2014), two AsS species for microalgae, DMA for *Anguilla japonica* and more often AsB  
78 for other fish-species and crustaceans (Miyashita et al., 2009). AsB was suggested to contribute in a greater

79 proportion of total As in marine organisms than in freshwater ones, which represents the major difference in  
80 As speciation between aquatic organisms (Schaeffer et al., 2006; Hong et al., 2014). AsB is reported as the most  
81 abundant As-species in several organisms, such as shrimps, bivalves, crabs and fishes (Lai et al., 1999; Schaeffer  
82 et al., 2006; Taylor et al., 2012; Caumette et al., 2012; Hong et al., 2014), whereas some AsS species can also be  
83 dominant in clams and mussels (Grotti et al., 2010; Taylor et al., 2012). The relative proportions of these two  
84 major As-compounds (AsB and AsS) would depend on the position of the organism in the food chain: the  
85 percentage of AsB normally increases through the food web, whereas the AsS fraction decreases (Grotti et al.,  
86 2010). Concerning sponges, the inorganic As(+V) specie was reported as dominant in freshwater sponges from  
87 the Danube River in Hungary (Schaeffer et al., 2006) whereas in Japanese and Philippine marine sponges, AsS  
88 and AsB were found to be dominant, showing interesting variability among the studied species (Shiomi et al.,  
89 1988; Yamaoka et al., 2001; Yamaoka et al., 2006). Recently, the role of sponge-associated bacteria in As  
90 bioaccumulation was evaluated since bacteria are known as key contributors to important elemental cycling in  
91 sponges, specifically for carbon, nitrogen, sulfur as well as trace elements (Keren et al., 2015; Keren et al.,  
92 2016; Keren et al., 2017). The sponge symbiotic bacterium *Entotheonella* sp. was shown to constitute the As-  
93 accumulating entity within the holobiont.

94 Considering the lack of studies on As speciation in marine sponges a deeper knowledge of As speciation in  
95 marine sponges is needed. In this work, a methodology was first developed for total As analyses by ICP-MS and  
96 As speciation by HPLC-ICP-MS in sponges. This approach was then applied to different sponge species collected  
97 in distinct marine environments. This study focused on different sampling sites located in the French  
98 Mediterranean coast, but also in the Irish Atlantic coast. These sites were characterized by different natural  
99 and anthropogenic As inputs offering thus the opportunity to study possible differences in As bioaccumulation  
100 and biotransformation related to the element availability.

101

## 102 2. Materials and methods

## 2.1. Sampling and target species

The sponge samples analyzed in this study were collected either by SCUBA diving in the French Mediterranean coast in the Villefranche-sur-Mer Bay, or in the Irish Atlantic coast shore in Greenisland and in the Killkieran Bay. The French sampling area is densely populated and a well-known tourist destination, especially in summer time. Greenisland is a large inlet in the Belfast Lough, at the western end is the city and the port of Belfast, which sits at the mouth of the Lagan River. The lough opens into the North Channel and connects Belfast to the Irish Sea. Killkieran Bay is a large, complex inlet in southern Connemara, County Galway, on west coast of Ireland.

Initial sampling of specimens of the sponge *Haliclona fulva* was carried out in the Bay of Villefranche sur Mer (N 43° 41' 59.0", E 07° 19' 31.5") on January 2014, in order to optimize the mineralization and extraction procedures for As analyses. Subsequent sampling was conducted in the Bay of Villefranche sur Mer on February and September 2016, in Killkieran Bay (N 53° 21' 22.625", O 9° 42' 17.153") in October 2016, and in Greenisland, Carrickfergus (N54°41'24.383", O 5° 51' 36.633") in November 2016. Sampling locations are shown in Fig. 1A (supplementary information). At least, three specimens of each sponge species were collected from each sampling site and period. In order to minimize differences due to sponge ages, organisms of a similar size were chosen. In the Mediterranean coast, samples were collected between 5 and 40 m depth, whereas in Ireland, they were collected from intertidal areas.

The following sponge species were collected for 2 sampling dates in the Mediterranean coast: *Acanthella acuta*; *Cymbaxinella<sup>p</sup> damicornis*; *Chondrilla nucula* and *Haliclona fulva*. From Greenisland and Killkieran Bay, the following species were collected: *Halichondria panicea* and *Hymeniacidon perlevis* (Fig. 2A, supplementary information). These species were selected because of their widespread occurrence but also for their different morphological characteristics (KeyToNature, 2015):

- 125 a) *Acanthella acuta* (Class: Demospongiae, Order: Axinellida, Family: Dictyonellidae) is a small erect  
126 sponge with rather small oscules.
- 127 b) *Cymbaxinella<sup>p</sup> damicornis* (Class: Demospongiae, Order: Axinellida, Family: Axinellidae, <sup>p</sup>phylocode  
128 name), is a rather small, erectly branching sponge with short, compressed branches. Oscules are small  
129 and are located on the apices of the branches, the oscules are surrounded by a small triangular ‘flap’ of  
130 tissue. The genus *Axinella* is difficult to define on the basis of morphological characteristic but for the  
131 specie analyzed in this study the name *Cymbaxinella damicornis* was recently proposed by Gazave et al.  
132 (2010) following the *phylocode*.
- 133 c) *Chondrilla nucula* (Class: Demospongiae, Order: Chondrillida, Family: Chondrillidae) is amorphous, with  
134 globular lobes, or thickly incrusting, up to about 1 cm thick, spreading horizontally, with pronounced,  
135 deeply incised and lacunose, meandering lobes. The color is dark-brown to walnut-brown.
- 136 d) *Haliclona fulva* (Class: Demospongiae, Order: Haplosclerida, Family: Chalinidae), is a specimen with  
137 irregular and slightly hispid surface. The ectosomal and choanosomal skeletons have a regular, delicate,  
138 unispicular, and isotropic reticulation. Color is dark orange.
- 139 e) *Halichondria panicea* (Class: Demospongiae, Order: Suberitida, Family: Halichondriidae), is intertidal or  
140 shallow-subtidal, thickly encrusting, massive or occasionally branching, and presents typical volcano-  
141 shaped oscular chimneys. The surfaces of this sponge are smooth, consistency firm, texture crumb-of-  
142 bread. These sponges are basically light orange-yellow or pale yellowish green.
- 143 f) *Hymeniacidon perlevis* (Class: Demospongiae, Order: Suberitida, Family: Halichondriidae), is one of the  
144 most common species along the Atlantic coasts of Western Europe. It is orange and has an irregular  
145 surface, often with lower or higher irregular projections. Oscules are inconspicuous.

146 Samples were kept frozen at -20°C. The sponge samples were then freeze-dried (Christ Martin™  
147 Alpha™ 1-2 Ldplus) and ground in an agate mortar in order to obtain a homogeneous powder. Samples  
148 were transferred into PTFE pre-cleaned tubes and kept in a desiccator.

149

## 2.2. Sample preparation

All solutions were prepared with doubly deionized water obtained from Millipore water purification system (Elix & Synergy) (resistivity of  $18.2 \text{ M}\Omega \text{ cm}^{-1}$ , Total Organic Carbon  $<5 \mu\text{g L}^{-1}$  and microorganisms  $<0.1 \text{ UFC ml}^{-1}$ ). All PTFE and Teflon containers used for sample preparation and/or analysis were pre-cleaned using a procedure consisting of 24 h bath in 10%  $\text{HNO}_3$  and careful rinsing with Milli-Q water. For total As quantification, samples were digested in a closed microwave system (Ethos One, Milestone) prior to ICP-MS analysis. The digestion program included a 20 min temperature ramp up to  $180^\circ\text{C}$  followed by 30 min isothermal step (power 2000 W). An aliquot of dried sponge sample was weighted in Teflon reactors. Chemical reagents were added and the microwave digestion was carried out. The sample mass and the use of different chemical reagents were optimized within this study. The following chemical reagents were used:  $\text{HNO}_3$  (Trace Metal Grade, 67 to 70% w/w, Fisher Chemical), HF (Ultra Trace Elemental Analysis 47-51% w/w, Optima, Fisher Chemical), HCl ( $\geq 30\%$ , for trace analysis, Sigma-Aldrich) and  $\text{H}_2\text{O}_2$  ( $\geq 30\%$ , for trace analysis, Sigma-Aldrich). The mineralized solution was then transferred to PE pre-cleaned tubes and gravimetrically diluted with Milli-Q water, up to a final volume of 50 mL. All samples were further diluted 15 times in Milli-Q water prior to ICP-MS analysis. At least one procedural blank and one quality control Certified Reference Materials (CRM) were included in each digestion run and analyzed with the rest of the samples. The following CRMs were used in this study: TORT-2 (lobster hepatopancreas, Institute for Environmental Chemistry, National Research Council Canada, Ottawa, Canada), BCR-627 (tuna fish powder, Institute for Reference Materials and Measurements, Geel, Belgium) and MESS-2 (estuarine sediment, Institute for Environmental Chemistry, National Research Council Canada, Ottawa, Canada). These CRM were prepared and analyzed along with the vent samples as an assessment of analytical accuracy.

For As speciation, the extraction method was optimized using different chemicals and/or proportion: Milli-Q water, methanol (MeOH, HPLC Plus,  $\geq 99.9\%$ , Sigma-Aldrich) and  $\text{H}_3\text{PO}_4$  ( $\geq 85 \text{ wt. \%}$  in  $\text{H}_2\text{O}$ , trace metals basis, Sigma-Aldrich). Extractions were performed the day before analyses and extracts were kept frozen during the



174 night. Since no sponge CRM is available on the market, the extraction performances were evaluated using the  
175 certified reference material BCR-627 (certified for As speciation) and TORT-2. For sponge samples, extractions  
176 were performed at least 3 times for each species to account for intra-specie variability. 10 mL of the selected  
177 solvent were added to about 50 mg of dried sample. The mixture was sonicated for one h. The extracts were  
178 then filtered with a single-use syringe through single-use syringe filters (0.45  $\mu\text{m}$ , Minisart® RC25, Sartorius)  
179 and gravimetrically diluted 10 times in Milli-Q water. The extracts were analyzed by HPLC-ICP-MS.

180

### 181 **2.3. Sample analyses**

182 A quadrupole ICP-MS (Elan DRCII, Perkin Elmer) was used as detection system for the determination of  
183 total As in sponge samples. ICP-MS operating parameters are summarized in Table 1.  $^{75}\text{As}$  isotope may be  
184 interfered in saline matrices by Cl (by  $^{40}\text{Ar}^{35}\text{Cl}^+$ ). The most common way to remove these spectral interferences  
185 is using the following correction equation:  $I(\text{As}) = I(m/z=75) - 3.127 \times I(m/z=77) + 2.733 \times I(m/z=82)$  where I stands  
186 for intensity (Potot et al., 2012; Barats et al., 2014). This mathematical correction accounts for about possible  
187 polyatomic interferences ( $^{40}\text{Ar}^{35}\text{Cl}^+$  or  $^{40}\text{Ar}^{37}\text{Cl}^+$ ) as well as for the occurrence of selenium ( $^{77,82}\text{Se}$ ) in samples.  
188 Preparation of standard solutions and dilution of samples for analysis were carried out in a class 100 clean  
189 laboratory. External calibrations were performed with daily prepared standards obtained by proper dilution of  
190 multi-elemental standard for ICP-MS (ICP-MS standard N°3 Perkin Elmer®, ICP-MS standard N°2 from SCP  
191 SCIENCE®) or a mono-elemental As standard (PlasmaCal, SCP SCIENCE®). An internal standard solution  
192 (containing 10 and 1  $\mu\text{g L}^{-1}$  of Ge and Tb respectively) was prepared by dilution of mono-elemental standards of  
193 Ge and Tb (PlasmaCal, SCP SCIENCE®).  $^{74}\text{Ge}$  was chosen as an internal standard for As analyses to correct  
194 instrumental drifts. All sample analyses were preceded by a minimum of a five-point calibration curve spanning  
195 the entire concentration range of interest. All results were instrument blank corrected to account for any  
196 operational bias. The ongoing instrument performance was monitored by the analysis of continuing calibration  
197 verification standards. Daily analyses of the certified reference natural river water SLRS-5 or SLRS-6 (National

198 Research Council, Canada) and analyses of the 3 solid CRM (MESS-2, TORT-2 and BCR-627) were carried out to  
199 check measurement accuracy and reproducibility of analytical calculations. Detection limits for  $^{75}\text{As}$  averaged  
200  $30 \text{ ng L}^{-1}$ .

201 As speciation analysis was performed by HPLC-ICP-MS. Instrumental settings and chromatographic  
202 conditions used throughout this work were described in Table 1. Ion intensities at  $m/z = 75, 77$  and  $82$  were  
203 monitored using a 'time-resolved' method from Chromera software (version 2, Perkin Elmer). No mathematical  
204 correction equation was used, but signals at  $m/z = 77$  and  $82$  were thoroughly monitored to insure the absence  
205 of the polyatomic interference with Cl. The HPLC used in this study (Serie 200 Pump, Perkin Elmer) was  
206 equipped with an anion exchange column (Hamilton PRP-X100, length 25 cm, particle size  $10 \mu\text{m}$ , i.d.  $4.1 \text{ mm}$ ).  
207 Mobile phases were prepared by dissolving  $(\text{NH}_4)_2\text{CO}_3$  salt (Sigma Aldrich) in Milli-Q water. This salt is  
208 commonly used with this type of anion exchange column (Wahlen et al., 2004). The final pH of the solution was  
209 adjusted to 9, by adding small amounts of either nitric acid or ammonia. The HPLC injection loop was cleaned  
210 using a 10% MeOH solution. The outlet of the HPLC column was connected via PEEK capillary tubing ( $0.125 \text{ mm}$   
211 i.d.) to a Rheodyne switching valve (6 ports, 2 positions, purchased by Perkin), which was in turn connected to  
212 the ICP-MS cyclonic nebulizer. Chromatographic conditions were optimized (composition of mobile phase,  
213 gradient, flowrate) to reach a rapid and sensitive analysis of 5 As species in 5 min. For calibration, the  
214 procedural blank chromatogram was subtracted. External calibration curves were used to quantify AsB, As(+III),  
215 DMA, MMA, As(+V) with the corresponding standards. Limits of detection were estimated using peak  
216 maximum height, and averaged  $0.13 \mu\text{g L}^{-1}$  of As in diluted extracts, corresponding to  $0.25 \mu\text{g g}^{-1}$  in sponge  
217 samples. Arsenic speciation analyses were performed on diluted solutions obtained after a solid-liquid  
218 extraction, optimized in this study, lead on each dried sponge samples. For quantification in sponge samples  
219 and CRM, the chromatograms of the extraction blanks were systematically subtracted. In case of unknown As  
220 species, the calibration curves are similar for all As species analyzed in this work, as previously demonstrated  
221 (Francesconi and Sperling, 2005) and serves to quantify unknown As species when are detected. This approach

222 was previously used for algae (Llorente-Mirandes et al., 2010) and in sponge samples (Taylor et al., 2012).  
223 Standards used for As speciation were prepared daily from dilution of As(+III) and As(+V) stocks (1000 mg.L<sup>-1</sup>,  
224 Absolute standard, Inc.) and organic As-mono-species solutions. The latter were prepared from salts of  
225 arsenobetaine (AsB), sodium dimethylarsenite (DMA), disodium methylarsenate (MMA) (Sigma-Aldrich).  
226 Species were identified by their retention times compared with standard compounds. Concentrations were  
227 determined by comparing peak heights to known standards. The CRM BCR-627, certified for AsB and DMA, was  
228 analyzed to check the accuracy of extraction and analytical methods. The TORT-2 was also analyzed for As  
229 speciation and compared with literature data.

230 Statistical data treatments were carried out using XLSTAT (version 2014.05.5, Addinsoft, Paris, France).  
231 One-way ANOVA tests were performed with a significance level of  $p < 0.05$  to estimate the inter-species  
232 variability of As concentrations, between sites (Irish sponges), and between seasons (Mediterranean sponges).

233

### 234 **3. Results and discussion**

#### 235 **3.1. Optimizations of analytical methodologies**

##### 236 3.1.1. Development of the mineralization method for total As analysis in sponges

237 Nitric acid is often used to digest different types of marine organisms for trace elements analyses (shrimps,  
238 mussels, gastropods, worm, oysters) (Taylor et al., 2012; Zhang et al., 2015), even for freshwater sponges  
239 (Schaeffer et al., 2006). A two-steps method using HNO<sub>3</sub> and then H<sub>2</sub>O<sub>2</sub> was also reported in aquatic organisms  
240 such as algae, fishes, bivalves, crabs, and shrimps (Hong et al., 2014; Llorente-Mirandes et al., 2010), as well as  
241 in sponges and associated bacteria (Keren et al., 2017). In order to select the best digestion procedure for As  
242 determination in marine sponges, an optimization was carried out on a *Haliclona fulva* sample. Mineralization  
243 protocols were developed on about 100 mg of dried sponge sample. The different methods and their results  
244 are presented in Table 1A (supplementary information). Protocols without microwaves have been rapidly

245 abandoned because they resulted in jelly solutions, low As recoveries or results with a poor reproducibility.  
246 Acid digestions assisted by microwaves without HF usually resulted in slightly lower As recovery than those  
247 obtained with HF (around 20% less). Sample microscope observations revealed undigested spicules in the  
248 solution without HF (Fig. 3A, supplementary information). These protocols conducted to a partial mineralization  
249 of sponge samples. In Antarctic Demospongiae (*Sphaerotylus antarcticus*, *Kirkpatrickia coulmani* and *Haliclona*  
250 sp.) and in the Mediterranean species *Petrosia ficiformis*, the accumulation of pollutants (Cd, Pb and Cu) was  
251 demonstrated as being lower in the spicules than in the corresponding organic fraction even if spicules  
252 represent about 80% of the biomass (Illuminati et al., 2016). This observation is in full agreement with our  
253 results. Nevertheless, the As amount in *Haliclona fulva* spicules cannot be neglected because it represents  
254 around 20% of the total As. For sponges with siliceous skeletons, the use of HF is thus recommended. Higher As  
255 concentrations were obtained with the use of HNO<sub>3</sub>/HF or HCl/HNO<sub>3</sub>/HF mixtures. Nevertheless the use of  
256 HNO<sub>3</sub>/HF was preferred to prevent possible interferences onto the <sup>75</sup>As isotope coming from HCl. Finally, the  
257 chosen sample preparation procedure involving a microwave digestion has been performed using 5 ml of HNO<sub>3</sub>  
258 and 2 ml of HF.

259 The optimized mineralization protocol was then applied to BCR-627 and TORT-2 marine biota CRMs as well  
260 as to MESS-2 sediment CRM. The obtained results for total As were in all cases in good agreement with the  
261 certified values, as shown in Table 2. Further applications of the developed procedure were performed on  
262 about 50 mg of dried sponge.

### 263 3.1.2. Development of the extraction method for sponge samples

264 A very delicate step of the speciation analyses is the chemical extraction of As species as it has to  
265 guarantee the preservation of original chemical forms, avoiding oxidation, reduction or more generally any  
266 conversion in other chemical species. As reported by different authors (Lai et al., 1999; Schaeffer et al., 2006;  
267 Ciardullo et al., 2010; Llorente-Mirandes et al., 2010; Taylor et al., 2012; Zhang et al., 2015), the most common  
268 solid-liquid extraction for As species from biological materials include the use of H<sub>2</sub>O, MeOH or a mixture of

269 them, since AsB is the predominant specie in marine animals and it is soluble in both solvents (Leermakers et  
270 al., 2006). A solution made of 2% HNO<sub>3</sub> was also used in chemical extraction for various aquatic organisms  
271 (fishes, bivalves, crabs, shrimps) (Hong et al., 2014). H<sub>3</sub>PO<sub>4</sub> was more often used for As extraction from soils  
272 and sediments as it preserves the two redox states of As in these samples (Ellwood and Maher, 2003). In  
273 sponges, extractions were performed either with H<sub>2</sub>O in freshwater organisms (revealing low recovery of 30%)  
274 (Schaeffer et al., 2006), or with a mixture H<sub>2</sub>O/MeOH (Shiomi et al., 1988; Yamaoka et al., 2001; Yamaoka et al.,  
275 2006; Keren et al., 2017). Extraction recoveries were not estimated in these last studies using methanol. Due to  
276 lack of some data in these studies, an estimation of extraction recoveries obtained from sponges, can only be  
277 performed with the results from Shiomi et al., 1988, using the ratio of the total water-soluble As divided by the  
278 total As. These calculations gave extractions recoveries ranging from 19-56% for three sponge species. The aim  
279 of the present study was then to develop a more efficient extraction methods using different extracting  
280 solutions.

281 The optimization of the extraction procedure was performed on three replicates of *H. fulva* sponge  
282 samples, using 10 mL of a given solution and 50 mg of dried sponge sample. Extraction recoveries were  
283 calculated considering the total average As content of  $29 \pm 5$  mg kg<sup>-1</sup> previously measured by ICP-MS on  
284 digested samples. Regardless of the tested extracting solution, extraction recoveries obtained in the present  
285 study were higher than 72 % (Table 3) which are significantly higher than previous published results on  
286 sponges. The use of pure water as extracting solvent was not preferred because this method led to a lower  
287 extraction recovery (72%) than those obtained with the other methods. This result is in accordance with  
288 previous studies demonstrating that some As species are not soluble in water (Shiomi et al., 1988). The  
289 extraction procedures which gave the best recoveries of total As were those involving MeOH, reaching an  
290 extraction recovery of 86 %. Nevertheless the use of MeOH can lead to severe matrix effects, as already  
291 described in a previous study (Nam et al., 2010). Extraction methods using methanol revealed also a high  
292 variability of the results, as shown by high standard deviations (Table 3). Accounting uncertainties, similar

293 results were reached for extracted As with 25% MeOH or 0.6 mol L<sup>-1</sup> H<sub>3</sub>PO<sub>4</sub> as extractants. Even if extractions  
294 performed with 25% MeOH revealed a highest extraction recovery (86%), this method was not retained due to  
295 possible analytical discrepancies and higher variabilities of the results. The 0.6 mol L<sup>-1</sup> H<sub>3</sub>PO<sub>4</sub> solutions was  
296 selected because it appeared to be the best compromise: a good extraction recovery 81% (close to the 86%  
297 obtained with 25% of MeOH) and a good reproducibility of the results (RSD=3%). Considering that only a few  
298 studies have been published on As speciation in sponges, the choice of H<sub>3</sub>PO<sub>4</sub> solution was also made in order  
299 not to lose the possible inorganic As species (the most toxic ones) possibly occurring in sponges samples, and  
300 to be able to preserve their chemical forms. The chosen extraction method was therefore applied to the  
301 selected CRMs. The extraction recoveries were found to be quite good, 106 and 97 %, for TORT-2 and BCR 627  
302 respectively (Table 2). These results thus demonstrated the efficiency of the selected extraction solution.

### 303 3.1.3. As speciation analyses

304 Speciation analyses of the extracts allowed mainly AsB to be quantified whatever the extraction methods,  
305 AsB representing 57-114% of the extracted As (Table 3). The AsB concentration was overestimated using 50%  
306 MeOH as extractant, as shown by the anomalous high proportion of As from AsB in extracts (114%). As  
307 previously demonstrated, AsB concentrations can be overestimated using MeOH (Nam et al., 2010). Such as for  
308 extracted As, As from AsB concentrations revealed similar results with 25% MeOH or 0.6 mol L<sup>-1</sup> H<sub>3</sub>PO<sub>4</sub> as  
309 extractants, accounting uncertainties. The difference on extraction recovery obtained for As from AsB were  
310 thus not significant due to the high variability of the results obtained with 25% MeOH. Finally, the extraction  
311 method with 25% MeOH was not retained due to possible analytical discrepancies and higher variabilities of  
312 the results. With the 0.6 mol L<sup>-1</sup> H<sub>3</sub>PO<sub>4</sub> solutions, AsB represent 64% of the extracted As with a better precision.

313 Speciation analyses were performed then on CRMs and conducted to good speciation recoveries: 74% and  
314 98% for TORT-2 and BCR 627 respectively (Table 2), highlighting the efficiency of speciation analyses.  
315 Measurements on the certified reference material BCR-627 gave a mean value of AsB equal to 4.5 ± 0.5 mg kg<sup>-1</sup>  
316 (n=6) and the occurrence of DMA just above the detection limit, in agreement with certified values. In TORT-2,

317 the average AsB value was  $13 \pm 5 \text{ mg kg}^{-1}$  (n=4), in accordance with previously reported values (Suner et al.,  
318 2001; Wahlen et al., 2004). Two other As species occurred at low concentrations: DMA at  $1.2 \pm 0.8 \text{ mg kg}^{-1}$  and  
319 another unknown As specie, named A, at  $1.9 \pm 0.6 \text{ mg kg}^{-1}$ , eluting between DMA and MMA (retention time:  $t_R$   
320 = 2.5 min). The DMA result is in agreement with the indicative value. These results on the two CRMs confirm  
321 thus the efficiency of speciation analyses with accurate measurements of AsB.

322

### 323 **3.2. As bioaccumulation in sponges**

#### 324 3.2.1. Variability of As concentrations in sponges

325 The variability of As content in sponges was estimated within the same specimen (intra-specimen  
326 variability), within the same sponge species (inter-specimen or intra-species variability), between different  
327 sampling sites or seasons, as well between different sponge species (inter-species variability).

328 The intra-specimen variability was evaluated analyzing at least 3 replicates of each sponge specimen (Table  
329 2A, supplementary information). Each replicate underwent the whole sample preparation (i.e. microwave  
330 digestion and dilution). The total As content was highly variable within each specimen, with relative standard  
331 deviations (RSD) ranging from 8 to 36%. This result highlights a certain inhomogeneity which may be related to  
332 the low amount of sponge samples weighted for digestion (50 mg).

333 The inter-specimen (or intra-species) variability was evaluated by determination of total As in different  
334 specimens collected for each sponge species (Table 4). The variability, expressed with RSD, was usually around  
335 10%, but it reached 38% for *C. nucula* in Feb. 2016. In Irish sponges inter-specimen variability was lower than in  
336 French samples (Table 4). Different authors reported RSD higher than 30% when evaluating intra-species  
337 variability in total As and other trace elements found in sponge samples (Batista et al., 2014; Cebrian and Uriz,  
338 2007). A relevant natural variation shall further be considered for these marine invertebrates.

339 Variability of the total As content related to sampling site characteristics was also estimated. The variability of  
340 As content and its species were estimated according to light conditions and the depth of the sampling (Fig. 1).  
341 ANOVA tests revealed no significant difference between As content in *C. damicornis* specimens collected in the  
342 cave center and those collected at the cave entrance; analogously no significant differences were found  
343 between *C. nucula* specimens collected at different depths. For Irish sponges, ANOVA analyses revealed no  
344 significant differences in As contents for the same Irish specie collected in Belfast and Killkieran Bay. ANOVA  
345 analyses performed on Mediterranean sponges, showed no significant differences between As contents  
346 measured in samples collected in two different period, except for *C. damicornis* sponges which showed  
347 significant lower As content in September 2016. As-bioaccumulation is an integrative data over the sponge's  
348 life. This explains in part the low temporal variations of As content in Mediterranean sponges between the two  
349 sampling periods. Seasonal variations in As contents within the same species can be thus considered moderate  
350 taking into account the inter-specimen variability.

351 A significant inter-species variability of total As content was shown, regardless of the considered sampling  
352 period, and confirmed by ANOVA analysis. In France, *C. damicornis* sponges accumulate significantly higher As  
353 amounts compared to other Mediterranean species (up to two times more). In Irish samples, As concentrations  
354 were significantly lower than those measured in Mediterranean specimens (2 times lower). Differences in  
355 accumulation efficiency between sponge species can be related to differences in morphological characteristics  
356 but also to the age of the sponges. The different species have different growing rates. *H. panicea* and *H.*  
357 *perlevis* are very fast growing compared to *C. damicornis* and *C. nucula*. The samples collected in the Irish  
358 coasts belong to the same order (Suberitida), which could explain similar behavior in terms of As accumulation  
359 while the Mediterranean sponges belong to the different orders Axinellida (*C. damicornis*), Chondrosida (*C.*  
360 *nucula*), Haplosclerida (*H. fulva*), and Bubarida (*A. acuta*). This study confirms that As concentration is  
361 dependent on sponge species, as shown by the variable As contents found in the literature (Table 5). Sponges  
362 accumulate more As than other marine organisms, which makes them suitable models for biomonitoring



363 studies, as already proposed by Genta-Jouve et al., 2012. As accumulation was previously demonstrated to be  
364 higher in demosponges than in calcareous sponges (Yamaoka et al., 2001) and highly variable among different  
365 species of demosponges, as shown in the present study. Yamaoka et al. (2006) measured an As content equal  
366 to  $6.1 \text{ mg kg}^{-1}$  in *Acanthella* sp., which is slightly lower than values found in the present study. This difference is  
367 easily explained considering that the present study refers to As content in sponges measured after total  
368 digestion while Yamaoka et al. worked on water soluble fractions. Additionally, sponges may have very  
369 different morphological characteristics or microbiomes even when belonging to the same genus. In *Haliclona*  
370 sp., previous study revealed As concentrations of  $0.81 \text{ mg kg}^{-1}$  in *Haliclona* sp. white,  $13 \text{ mg kg}^{-1}$  in *Haliclona*  
371 *permolis* (Yamaoka et al., 2001),  $1.03 \text{ mg kg}^{-1}$  in *Haliclona tenuiramosa* (Venkateswara Rao et al., 2009) and  
372 between  $1.5$  and  $8.5 \text{ mg kg}^{-1}$  for *Haliclona oculata* (Aly et al., 2013). For these two species (*Acanthella* sp. and  
373 *Haliclona* sp.), the present study revealed higher As contents ranging from  $29$ - $44 \text{ mg kg}^{-1}$  (Table 4). Perez et al.  
374 (2005) measured also high As concentrations in another sponge species collected in the Mediterranean sea,  
375 *Spongia officinalis* ( $86.3$ - $134.1 \text{ mg kg}^{-1}$ ). Regarding *Halichondria panicea*, Vaskovsky et al. (1972) reported  
376 similar As concentrations ( $6 \text{ mg kg}^{-1}$ ). But these results refer to As concentration measured in lipid extracts  
377 which is only a part of the total As content determined in this study. The present study confirms thus that As  
378 concentrations in sponges depends on the specific properties related to a particular sponge species.

### 379 3.2.2. As bioaccumulation in sponges

380 The Bio-Concentration Factor (BCF) and the Bio-Accumulation Factor (BAF) are useful and powerful tools in  
381 the interpretation of such results, since they give an idea of sponge behavior related to the surrounding  
382 environment and feeding habits. BCF represents the ratio between the As concentration found in biota and in  
383 the habitat, in this case the sediment. BAF represents the ratio between the As concentration found in biota  
384 and in the organism's diet, in our case seawater, since sponges are filter feeders (Gobas, 2001).

385 In this work, sediment collected from the French coast showed a constant As content for both sampling  
386 period:  $7 \pm 2 \text{ mg kg}^{-1}$  of As (measured on three sediment samples for each sampling period) while sediment

387 samples collected from Kilkieran Bay and Greenisland show an As content of  $7 \pm 2$  and  $2.9 \pm 0.3$  mg kg<sup>-1</sup>  
388 respectively. These values are below the limit imposed by the principal environmental authorities for As in  
389 marine sediments ( $7.24$  mg kg<sup>-1</sup>, US EPA, 2006). Regarding sponges collected in Ireland, specimens coming from  
390 Kilkieran bay showed BCF close to 1, while specimens of *H. panicea* and *H. perlevis* sponges collected in Belfast  
391 showed BCF of 2.1 and 3.5 respectively. BCF in Mediterranean sponges range from 3.6 to 12. Similar values  
392 were reported for bivalves (6-19) (Negri et al., 2006) and were found to be higher than in other sponges (close  
393 to 1) (Mayzel et al., 2014). Extremely high As BCF of 477 was also previously measured in only one sponge  
394 species, *Theonella swinhoei*, which was explained by the presence of a particular bacterium (Mayzel et al.,  
395 2014). It is worth noticing that *Theonella swinhoei* is a slow growing and long lived sponge specie so As  
396 concentration may be also linked to the age of the sponge and thus responsible for the elevated BCF. The fact  
397 that most BCF were higher than 1 (except in Kilkieran Bay) proves that sponges are able to accumulate As at  
398 higher concentrations than their surrounding environment (sediment in our case), which means that there are  
399 additional biological processes involved in As accumulation.

400 As concentration in seawater used for the calculation of BAF in Mediterranean sponges was determined in  
401 a sample collected in Monaco and measured at the Environment Laboratories of the International Atomic  
402 Energy Agency. The average value found in seawater sample was  $1.5 \pm 0.2$  µg L<sup>-1</sup> and it was used for BAF  
403 calculations because of the vicinity of the sampling site. This As content is in agreement with total As content of  
404  $1.3$  µg L<sup>-1</sup> previously measured in surface seawater from Mediterranean Sea (Cabon and Cabon, 2000).  
405 Mediterranean sponges BAF of As ranged between 15000 and 60000, *i.e.* in the order of 4.2 to 4.8 for log BAF.  
406 For the calculation of BAF in Irish sponges, the average reference value for As in seawater was  $2.5$  µg L<sup>-1</sup>, as  
407 reported by Crompton and Crompton (1989). The BAF obtained were between 274 and 318 for samples  
408 collected in Kilkieran Bay and between 250 and 412 for samples collected in Greenisland, namely values  
409 between 2.4 and 2.6 in logarithmic scale. BAF calculations revealed thus significant higher values for

410 Mediterranean sponges than Irish ones, but both sufficiently high to indicate that these organisms are efficient  
411 bioaccumulators of As.

412 Other BAFs reported in the literature for freshwater or marine organisms are in the range of  $3 < \log \text{BAF} < 4$   
413 for fishes, bivalves, shrimps, gastropods and mussels (Hong et al., 2014; Giusti and Zhang, 2002) algae and  
414 plankton (Chen et al., 2000; Mitra et al., 2012). In uncontaminated environments, As concentrations were  
415 measured in freshwater sponges (*Ephydatia fluviatilis*), water and sediments by Schaeffer et al., (2006) showing  
416 values of about  $8.07 \text{ mg kg}^{-1}$ ,  $1.1 \mu\text{g L}^{-1}$  and  $3.6 \text{ mg kg}^{-1}$  respectively (which is lower As contents than our  
417 Mediterranean sampling site but comparable with the Irish one). With these previous measurements on  
418 freshwater sponges, BAF and BCF calculations performed for As revealed: a BCF of 2.24 and a BAF of 7336 (log  
419  $\text{BAF}=3.9$ ). These results were slightly lower than those determined in our Mediterranean marine sponges but  
420 higher than results obtained for Irish samples. Nevertheless, it is quite difficult to compare these results due to  
421 different environmental conditions (freshwater or seawater). Concentrations of As in water, sediments and  
422 biota were previously suggested to increase with increasing salinity and BAF for As in other aquatic organisms  
423 might be slightly inversely proportional to salinity (Hong et al., 2014). In this study, BAF for As were higher in  
424 samples coming from the Mediterranean Sea (average salinity of about 38‰) than those collected in Ireland  
425 (average salinity 34‰) (Salaün et al., 2007; Tsimplis and Baker, 2000). However, the comparison is not easy due  
426 to the presence different sponge species, and the fact that TEs bioaccumulation in sponges was suggested to  
427 differ according to sponge species (Batista et al., 2014; Mayzel et al., 2014). Factors affecting the differences in  
428 the bioaccumulation are suggested to be mainly related to sponge species due to morphological characters like  
429 spicules, fibers but also the microbial content.

430

### 431 **3.3. As biotransformation in sponges**

#### 432 3.3.1. Variability of extracted As according to sponge species

433 For all sponge species collected in this study, extraction recoveries vary from 49% to 105% (Table 4),  
434 demonstrating the efficiency of the optimized extraction method for As. The variability of As extracted  
435 amounts within the same species was quite moderate (ranging from 10 to 30%). It can be easily noticed that  
436 the extraction recoveries differ according to sponge species. The best As extraction recoveries (> 77 %) were  
437 obtained for *C. nucula*, *H. panicea* and *H. pervelis*. Extraction recoveries ranged between 51 and 69% for *H.*  
438 *fulva* and *A. acuta* sponge species. Lowest extraction recoveries were obtained for *C. damicornis* (46-49 %).  
439 These low extraction recoveries may be explained by the occurrence of As organic species (for example, AsS),  
440 not extracted with our method and which would be better extracted with methanol. For *C. damicornis* sponges,  
441 further development of the extraction procedure would be necessary to increase the extraction recovery.

#### 442 3.3.2. As speciation in sponges

443 As speciation analyses were performed on extracts of sponge samples (Table 4). For each sponge species,  
444 speciation analyses were carried out on 3 sponge specimens. Significant differences on As speciation occur  
445 between the Irish and Mediterranean sponges (Fig. 2). Despite the fact that most of the As was successfully  
446 extracted in Irish sponges (>81 %), a large proportion of the extracted species was not identified/quantified  
447 (speciation recoveries ranged from 52 to 66 %). Vaskovsky et al. (1972) reported a similar As concentrations in  
448 *Halichondria panicea* sponges, but measured in lipid extracts. This result may prove the occurrence of As rather  
449 under organic As species, probably those not identified in our study. For Mediterranean sponges, although the  
450 extraction recovery was generally lower than Irish samples (especially for *C. damicornis*), almost the totality of  
451 extracted species were identified and quantified, with a speciation recovery between 79 and 119%. Within the  
452 same sponge species and the same sampling date, the variability of extracted As content and As speciation  
453 related to sampling site characteristics was also estimated (Fig. 1). ANOVA analysis revealed no difference on  
454 extracted As and As speciation for *C. nucula* sponges sampled at different depth; analogously no significant  
455 difference in extracted As and As speciation was found for *C. damicornis* specimens collected at the center and

456 entrance of the cave. Such as As bioaccumulation, As speciation in sponges is suggested to be mainly related to  
457 sponge species.

458 As speciation analysis in sponge extracts reveals the large occurrence of AsB (>29% of the total As) and the  
459 presence of different As species at low concentrations: DMA, As(+V) (Fig. 2). Additionally, two unknown species  
460 were found: a first one eluting between DMA and MMA (only for *C. nucula* in Sep. 2016) and a second one  
461 eluting after As(+V). These results on As speciation in marine sponges are different from those obtained in  
462 freshwater sponge *Ephydatia fluviatilis* in which inorganic species were found to be dominant: 57 % of As(+V),  
463 20 % of As(+III)), AsS (11%), DMA (4%) and undetected AsB (<1%) (Schaeffer et al., 2006). In the present study,  
464 among minor As species, DMA accounted for about 10% of extracted As in Mediterranean sponges, (i.e. 4-8%  
465 of the total As content) whereas this specie was not detected in Irish sponges at all. As(+V) was detected in all  
466 sponges' species, but it was possible to quantify it only for some of the Mediterranean specimens. This species  
467 represents about 5-9 % of the extracted As content (i.e. 1-5 % of the total As content). The unknown As  
468 species, named B and eluting after As(+V) (retention time,  $t_R = 4.5$  min), presents a similar As concentration  
469 (close to  $1 \text{ mg kg}^{-1}$ ) regardless of the sponge species, representing between 5% and 15 % of extracted As in  
470 Mediterranean and Irish sponges respectively (i.e. 2 and 15% of the total As). The unknown As specie A, eluting  
471 between DMA and MMA ( $t_R=2.5$  min), occurs in *A. acuta* specimens but only in samples collected in September  
472 2016, representing 6% of extracted As (i.e. 5% of the total As). Even if AsB is the main As species in *H. fulva*, *A.*  
473 *acuta* and *C. nucula* sponge species, it occurs in a lesser proportion in *C. damicornis* (31-44 %), *H. panicea* (29-  
474 34 %), and *H. pervelis* (33-47 %) (Fig. 2). Even though *C. damicornis* accumulates more As than the other sponge  
475 species, this sponge specie does not accumulate more AsB. This higher As content is thus related to other As  
476 species, probably other organic forms not extracted or not detected with the methodology used in the present  
477 study. The AsB predominance in *A. acuta* (86-94% of As extracted or 48-55% of total As) is in accordance with  
478 the previous results on a sponge of the same genus (28% of the water soluble extract) (Yamaoka et al., 2006).  
479 Two AsS were also reported to occur in large amounts (22% oxo-AsS-phosphate, 11% oxo-AsS-glycerol, and

480 39% of unknown As species). These other As species may coincide with the occurrence of unknown As species  
481 found in our study or other As species not extracted with our method, specifically the As specie B representing  
482 7.5% of extracted As. In *H. fulva*, AsB was also the predominant As-species (75-80% of As extracted or 46-55%  
483 of total As), whereas in the same genus (*H. permolis*), the oxo-AsS-phosphate was identified (61% of the water  
484 soluble extract) (Yamaoka et al., 2001 and 2006). The same authors also reported AsS as predominant As  
485 species in *Halichondria* sp., and a content of AsB between 9 and 32% whereas AsB were reported dominant in  
486 another study (Shiomi et al., 1988). In the present study, *H. panicea* sponges from the same genus contained  
487 an AsB which accounts for 34 and 29% of total As, for samples collected in Kilkieran bay and Greenisland,  
488 respectively. All these results demonstrates that As speciation and the predominance of AsB or the AsS may  
489 not result only from sponge specie or genus characteristics. Yamaoka et al., (2001 and 2006) suggested that the  
490 different proportions of AsS may reflect the different symbionts living within sponges. This previous hypothesis  
491 would support: (1) the change of the predominant As-species within the same sponge species, and (2) the  
492 difference of As-bioaccumulation and biotransformation within a specific sponge species (*C. damicornis*, here)  
493 comparing to the others.

### 494 **3.5. Pathways of As bioaccumulation and biotransformation**

495 In surface seawater, As usually occurs under toxic inorganic species, mainly As(+V). Arsenic speciation  
496 analyses in seawater reported in the literature revealed a content of  $1 \mu\text{g L}^{-1}$  of As(+V) and  $0.2 \mu\text{g L}^{-1}$  of As(+III)  
497 in the Mediterranean sea (Cabon and Cabon, 2000) and from  $1.9$  to  $3 \mu\text{g L}^{-1}$  of As(+V) and As(+III)<LOD in Irish  
498 seawater (Salaün et al., 2007). Sponges are efficient filter feeders, strongly accumulating As ( $4 < \log \text{BAF} < 5$ ).  
499 Due to As bioaccumulation and biotransformation (converting toxic inorganic As species in less-toxic organic  
500 species) within the sponge tissue, sponges may be a relevant tool for bioremediation of As-contaminated site,  
501 producing possible secondary metabolites of great interests (pharmaceuticals and bioactive compounds) under  
502 stressful environmental conditions (due to pollution). For instance, a polyarsenic organic compound showing  
503 antibacterial and antifungal properties was recently isolated from sponges (Mancini et al., 2006). The possible

504 application of sponges in the field of environmental bioremediation has been proposed in previous studies with  
505 different applications (Longo et al., 2010; Santos-Gandelman et al., 2014).

506 The main occurrence of AsB in osmoconformers such as sponges may be justified in waters characterized  
507 by high salinity because this molecule (as glycine-betaine) may serve to protect against osmolytic stress  
508 (Clowes and Francesconi, 2004; Caumette et al., 2012). Since AsB is also the least toxic form of arsenic, the  
509 production of such a molecule during a protecting process could be useful in the detoxification of As  
510 contaminated waters. Since no AsB occurs in seawater and low contents of inorganic As measured in sponges,  
511 biotransformation is responsible of these changes of As speciation. During their feeding process, sponges  
512 ingest water (where the dominant As-species is As(+V)), and microorganisms (potentially containing organic As  
513 species). Two different pathways may be responsible of the As bioaccumulation and biotransformation within  
514 sponges: (1) a dietary route, through the feeding of microorganisms (phytoplankton) enriched by organic  
515 species; (2) and/or a waterborne route, i.e. a direct uptake from seawater and biotransformation within  
516 sponges.

517 Phytoplankton is considered as a major food source for the organisms of higher trophic levels, such as  
518 sponges; this autotrophic organism plays an important role in the distribution and biotransformation of As  
519 species in the marine environment (Rahman et al., 2012). Microorganisms which constitute the sponge's food,  
520 are known as producers (the first link of the food chain), contain high concentrations of As. AsB retained first  
521 the attention because it is formed by organisms at low trophic levels and accumulated through the food chain  
522 (Edmonds et al., 1993; Edmonds et al., 1997; Cullen and Reimer, 1989; Francesconi, 2010). Phytoplankton,  
523 microalgae, bacteria or cyanobacteria are able to convert inorganic As into organic species through biological  
524 processes (Azizur Rahman et al., 2012; Wang et al., 2015; Miyashita et al., 2016). For example, in  
525 phytoplankton, inorganic arsenic is incorporated in cells where it is methylated and transformed into AsS via  
526 adenosylation steps, possibly as a detoxification process (Caumette et al., 2012). The direct uptake of AsB-  
527 enriched microorganisms by sponges seems thus to be unlikely because As speciation in producers (algae,

528 phytoplankton) revealed the main occurrence of AsS and a low content of AsB (Grotti et al., 2010; Llorente-  
529 Mirandes et al., 2010). Another dietary route would be the ingestion of AsS enriched microorganisms and the  
530 further conversion of AsS into AsB with sponge metabolisms or their symbionts organism. The unknown or  
531 undetected As species found in this study may be related to the occurrence of these AsS species.

532 The occurrence of AsB in sponges may be also related to microorganisms enriched with another As species, the  
533 (arsenoriboside) and ingested by the sponges. . As proposed by Caumette et al. (2012) for zooplankton, once  
534 sponges ingest phytoplankton, AsS may be degraded by associated bacterial communities, leading to the  
535 formation of AsB. The latter was also suggested to be produced within marine organisms from the  
536 transformation of arsenoribosides accumulated from their diet (Foster and Maher, 2016). Arsonioribosides  
537 would be thus another precursor of AsB formation. If the occurrence of As(+V) in seawater is recognized to be  
538 constant along time, the occurrence of micro-organisms is not continuous and can vary with seasons (e.g.  
539 phytoplankton dynamics vs nutrients in seawater). Because of the main occurrence of AsB within our sponge  
540 samples and the seasonal phytoplankton dynamic, the As biotransformation pathway via a dietary route it is  
541 rather unlikely to be the main process.

542 A waterborne route is the second hypothetic pathway for As bioaccumulation and transformation in  
543 sponges. After direct uptake of As(+V) from seawater, sponges might biotransform this inorganic As into AsB.  
544 Biological processes including methylation steps and conversion into AsB have previously been proposed for  
545 marine fishes (Zhang et al., 2016a; Zhang et al., 2016b) as well as for marine sponges (Yamaoka et al., 2006).  
546 For marine sponges, DMA was also proposed to be converted into AsS and further into AsB (Yamaoka et al.,  
547 2006). The unknown or undetected As species found in our sponge samples may account for the occurrence of  
548 AsS occurring in different proportions within sponges (34 - 60 %, orange parts in Fig. 2). The As  
549 biotransformation within sponges may be directly related to the sponge metabolism, but could also indirectly  
550 occur by the action of symbiotic micro-organisms (microalgae, bacteria). Symbiotic cyanobacteria can contain  
551 high As content and were already recognized as the source of the AsS in sponges (Yamaoka et al., 2001).



552 Recently, As cycle in a *Theonella swinhoei* sponges was demonstrated to be largely driven by symbiotic bacteria  
553 (Keren et al., 2017). This last result suggested rather the main implication of symbiont bacteria for As  
554 biotransformation within sponges; but it is not obvious to confirm this pathway with our results.

#### 555 **4. Conclusions**

556 For the first time, several sponge species from the northwestern Mediterranean, and the northeastern  
557 and western Irish coasts were investigated for their As bioaccumulation and biotransformation. Methodologies  
558 were optimized for As total determination and speciation in these marine organisms. The total As content was  
559 found to be very diverse according to sponge species and sampling sites, ranging between 6 and 77 mg kg<sup>-1</sup>.  
560 Bioaccumulation and bioconcentration factors revealed the great capabilities of sponges to accumulate this  
561 element with respect to concentration found in surrounding environment. As speciation showed predominance  
562 of AsB in all analyzed samples, in accordance with previous studies on marine organisms. The pathway for the  
563 conversion of As(+V) from seawater into AsB in sponges is likely to be associated to the sponge metabolism or  
564 symbiont organisms. Finally, the outcomes of this study contribute to a better understanding of the  
565 distribution and metabolism of arsenic compounds in marine sponges.

566

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**Figure captions**

Figure 1.

Results on total As in digested and extracted samples, and on As speciation for two sponge species (*C. nucula* and *C. damicornis*) collected under different environmental conditions (depth and light). The error bars represent the standard deviation calculated considering every replicate performed on each species (n=3).

Figure 2.

Distribution of different As species in a) different sponge species collected during two sampling periods in France and b) different sponge species collected in two sampling sites in Ireland.

## Tables

Table 1. ICP-MS and HPLC-ICP-MS settings used for total As analyses and As speciation in marine sponge samples

ICP-MS Settings		
RF power		1550 W
Plasma gas flow		15 L min <sup>-1</sup>
Auxiliary gas flow		1.1 mL min <sup>-1</sup>
Carrier gas flow		0.9 L min <sup>-1</sup>
Nebulizer		quartz concentric
Spray chamber		cyclonic
Interface		Pt sampling and skimmer cones
Analytical mass (m/z)		75, 77, 82, 74
HPLC conditions		
Column (anion exchange)		Hamilton PRP-X100
Column temperature		20°C
Injection volume		250 µL
Mobile phase A		5 mM (NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> pH=9
Mobile phase B		70 mM (NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> pH=9
Flow rate		2 mL min <sup>-1</sup>
Gradient programme		0-2 min: 100% A 3-6 min: 100% B 7-10 min: 100% A

Table 2. Total As and As speciation results for three CRMs: BCR-627, TORT-2 and MESS-2. Extracted As concentrations were determined by ICP-MS and AsB concentrations by HPLC-ICP-MS. \* and \*\* represent reference values given by \*Sunner et al., 2001 and \*\*Wahlen et al., 2004.

Certified Reference Materials		TORT-2	BCR-627	MESS-2
<b>Microwave digested solutions</b>	Total As (mg kg <sup>-1</sup> )	22 ± 2 (n=6)	5.2 ± 0.5 (n=4)	19.2 ± 1.1 (n=4)
	Certified value	21.6 ± 1.8	4.8 ± 0.3	20.7 ± 0.8
	Recovery (%)	102	108	92
<b>Extracted solutions</b>	Extracted As (mg kg <sup>-1</sup> )	23 ± 4 (n=3)	4.6 ± 0.3 (n=6)	
	Extraction recovery (%)	106	97	
	As from AsB (mg kg <sup>-1</sup> )	13 ± 5 (n=4)	4.5 ± 0.5 (n=6)	
	Certified value	13.8 ± 0.2* 14.3 ± 1.1**	3.9 ± 0.2	
	As from DMA (mg kg <sup>-1</sup> )	1.2 ± 0.8 (n=4)	0.1 < c < 0.3	
	Certified value	0.97 ± 0.05* 0.84 ± 0.10**	0.15 ± 0.02	
	As from unknown peak	1.9 ± 0.6 (n=4)	-	
	Speciation recovery (%)	74	98	

Table 3. Extracted As concentrations and As speciation results obtained using different extraction methods on a pool of *H. fulva* sponges. Extracted As concentrations were determined by ICP-MS and AsB concentrations by HPLC-ICPMS. Extraction recoveries were calculated on the average total As content of 29 ± 5 mg kg<sup>-1</sup>.

	Extracted As (mg kg <sup>-1</sup> )			Extraction recovery (%)	As from AsB (mg kg <sup>-1</sup> )			As from AsB in extracts (%)
H <sub>2</sub> O	21	±	3	72	14	±	3	67
MeOH 50%	21	±	2	72	24	±	3	114
MeOH 25%	25	±	1	86	19	±	3	76
H <sub>3</sub> PO <sub>4</sub> 0.3 mol L <sup>-1</sup>	20	±	3	69	13.0	±	0.8	65
H <sub>3</sub> PO <sub>4</sub> 0.6 mol L <sup>-1</sup>	23.5	±	0.7	81	15.2	±	0.8	64
H <sub>3</sub> PO <sub>4</sub> 1 mol L <sup>-1</sup>	24.2	±	0.5	83	13.9	±	0.8	57

Table 4. Summary of As speciation results obtained for different sponges species sampled between February and November 2016 in three sites located in western Europe.

Site	Date	Sponge species	Total As		Extracted As		Extraction recovery (%)	As from AsB		As from DMA		As(V)		Unknown As specie A		Unknown As specie B		Speciation recovery (%)	Known As in sponge (%)
			mg kg <sup>-1</sup>	RSD (%)	mg kg <sup>-1</sup>	RSD (%)		mg kg <sup>-1</sup>	RSD (%)	mg kg <sup>-1</sup>	RSD (%)	mg kg <sup>-1</sup>	RSD (%)	mg kg <sup>-1</sup>	RSD (%)	mg kg <sup>-1</sup>	RSD (%)		
Villefranche sur Mer (France)	Feb. 2016	<i>H. fulva</i>	26	23	16	56	59	12	67	1,7	35	1,4	14	<0,1		1,5	33	106	66
		<i>A. acuta</i>	44	18	28	4	64	24	25	3,1	23	1,9	16	<0,1		1,9	21	109	69
		<i>C. nucula</i>	34	38	26	27	77	24	38	3	33	1,44	1	<0,1		1,3	8	116	88
		<i>C. damicornis</i>	74	15	36	22	49	23	35	3	33	1,6	25	<0,1		1,7	24	79	39
	Sep. 2016	<i>H. fulva</i>	29	17	20	25	69	16	13	1,9	5	0,1<c<0,4		<0,1		1	10	95	66
		<i>A. acuta</i>	33	6	17	12	51	16	25	2,1	5	0,6	33	<0,1		1,4	29	119	61
		<i>C. nucula</i>	27	15	24	29	87	22	32	2,3	30	0,1<c<0,3		1,5	27	0,1<c<0,3		104	92
		<i>C. damicornis</i>	55	13	25	12	46	27	48	2,7	37	0,1<c<0,3		<0,1		1	20	120	56
Kilkieran Bay (Ireland)	Oct. 2016	<i>H. panicea</i>	7,3	11	6	2	83	2,5	8	<0,1		0,1<c<0,3		<0,1		0,89	9	57	47
	<i>H. perlevis</i>	6,9	7	7,2	6	105	2,4	13	<0,1		0,1<c<0,3		<0,1		1,1	18	47	49	
Greenisland (Ireland)	Nov. 2016	<i>H. panicea</i>	5,7	9	4,7	9	81	1,7	18	<0,1		0,1<c<0,3		<0,1		0,8	25	52	43
	<i>H. perlevis</i>	7,6	4	7	29	88	3,7	30	<0,1		0,1<c<0,3		<0,1		0,91	3	66	61	

Table 5. Summary of literature data available on total As and its speciation in sponges. Total As results refer to total content in dried sample, unless otherwise stated.

Sponge specie	Sampling location	Total As (mg kg <sup>-1</sup> )	As concentration (mg kg <sup>-1</sup> )			Reference
			AsB	AsS	Others	
<i>Hymeniacidon heliophila</i>	Guanabara Bay, Brazil	4.6-8.1				Batista et al., 2014
<i>Paraleucilla magna</i>		1.2-2.6				
<i>Haliclona oculata</i>	Poole harbor, UK	1.5-8.5				Aly et al., 2013
<i>Sphaciospongia vagabunda</i>	Darwin Harbour, Australia	10.1-56.4				Padovan et al., 2012
<i>Hyrtios erectus</i>	Red Sea, Saudi Arabia	15-63.9				Pan et al., 2011
<i>Hyrtios</i> sp.		2.30				
<i>Stylissa carteri</i>		7.5-10.7				
<i>Chalinula</i> sp.		13.7-22.1				
<i>Xestospongia testudinaria</i>		20.7-42.2				
<i>Phyllospongia papyracea</i>		8.0				
<i>Amphimedon</i> sp.		11.2				
<i>Spongia arabica</i>		106.1				
<i>Sphaciospongia inconstans</i>		15.0				
<i>Haliclona tenuiramosa</i>	Gulf of Mannar, India	0.32-1.09				Rao et al., 2009
<i>Petrosia testudinaria</i>	Gulf of Mannar, India	2.30				Rao et al., 2006
<i>Thorecta</i> sp.	Bohol Sea, Philippines	6.2*	3.0	2	1.2	Yamaoka et al., 2006 *water fraction
<i>Dysidea</i> sp.		24.8*	15.2	1.4	8.1	
<i>Theonella</i> sp.		157.0*	136.6	2.21	18.2	
<i>Acanthella</i> sp.		6.1 *	1.7	2.0	2.4	
<i>Phyllospongia</i> sp.		4.4*	1.5	2.12	0.7	
<i>Aaptos</i> sp.		112.5*	98.1	0.25	14.2	
<i>Biemna fortis</i>		1.0*	0.13	0.15	0.8	
<i>Jaspis</i> sp.		6.1*	0.6	3.7	1.7	
<i>Subertes</i> sp.		10.3*	1.7	7.4	1.1	
<i>Haliclona permolis</i>		13*	3.3	9.9	0.5	
<i>Halichondria japonica</i>		3.4*	1.1	1.6	0.8	
<i>Haliclona</i> sp. white		0.8*	0.2	0.5	0.2	
<i>Halichondria okadai</i>		5.5*	0.5	4.5	0.5	
<i>Ephydatia fluviatilis</i>		Danube river, Hungary	8.07	<0.02	0.29	
<i>Spongia officinalis</i>	Marseille, France	86.3-134.1				Perez et al., 2005

<i>Callispongia diffusa</i>	Guam Island, Pacific Ocean	<0.01				Denton et al., 2006
<i>Cinachyra sp.</i>		<0.01				
<i>Clathria vulpina</i>		<0.01				
<i>Dysidea sp.</i>		0.01-10.5				
<i>Liosina cf. granularis</i>		39.7-47.7				
<i>Stylotella aurantium</i>		0.01-6.42				
Unidentified sponge 1		5.91-19.8				
Unidentified sponge 2		37.9				
Unidentified sponge 3		0.01-43.1				
<i>Cliona viridis</i>	Berlangas Islands, Portugal	25-32				Araujo et al., 2003
<i>Cliona celata</i>		12-47				
<i>Myriastr a ananoora</i>		73				
<i>Erylus discophorus</i>		60-99				
<i>Adocia sp.</i>		16-21				
<i>Spongia nitens</i>		33-65				
<i>Spongia agaricina</i>		47-83				
<i>Spongia officinalis</i>		47-64				
<i>Cacospongia scalaris</i>		64-119				
<i>Ircinia fasciculata</i>	31-65					
<i>Haliclona permolis</i>	Seto Inland Sea, Japan	13.00*	3.34	9.2	0.52	Yamaoka et al., 2001 *water fraction
<i>Halichondria japonica</i>		3.42*	1.10	1.54	0.78	
<i>Halichondria okadai</i>		5.50*	0.53	4.5	0.47	
<i>Haliclona sp. white</i>		0.81*	0.15	0.45	0.21	
<i>Halichondria okadai</i>	Chiba, Japan	6.80	n.q.			Shiomi et al., 1988 n.q. : not quantified
<i>Halichondria japonica</i>		6.40				
<i>Spirastrella insignis</i>		3.20	n.q.		n.q.	
<i>Halichondria panicea</i>	Posiet Bay Japan	6.4**				Vaskovsky et al., 1972 **lipid extract

## FIGURES

Figure 1

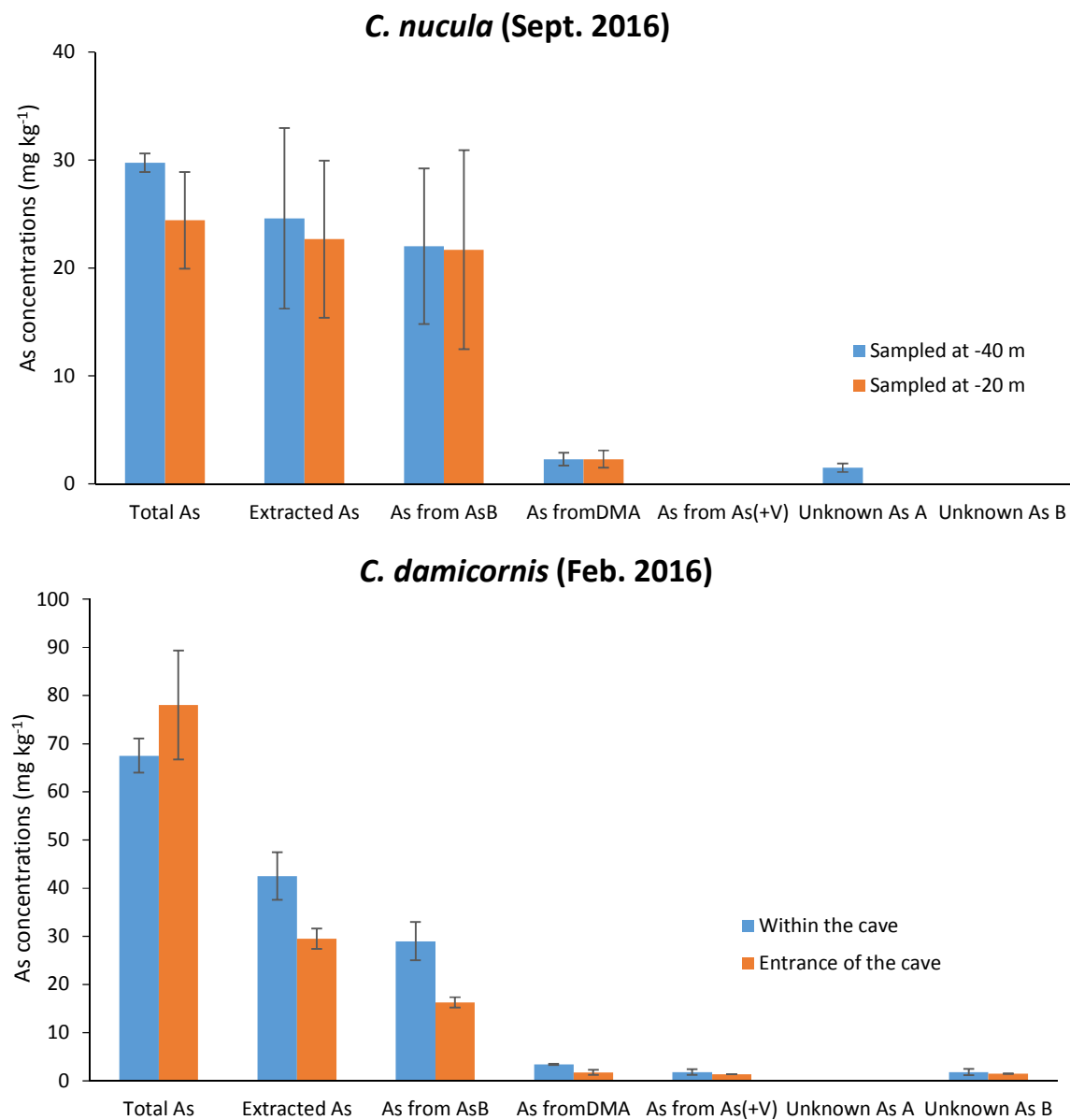
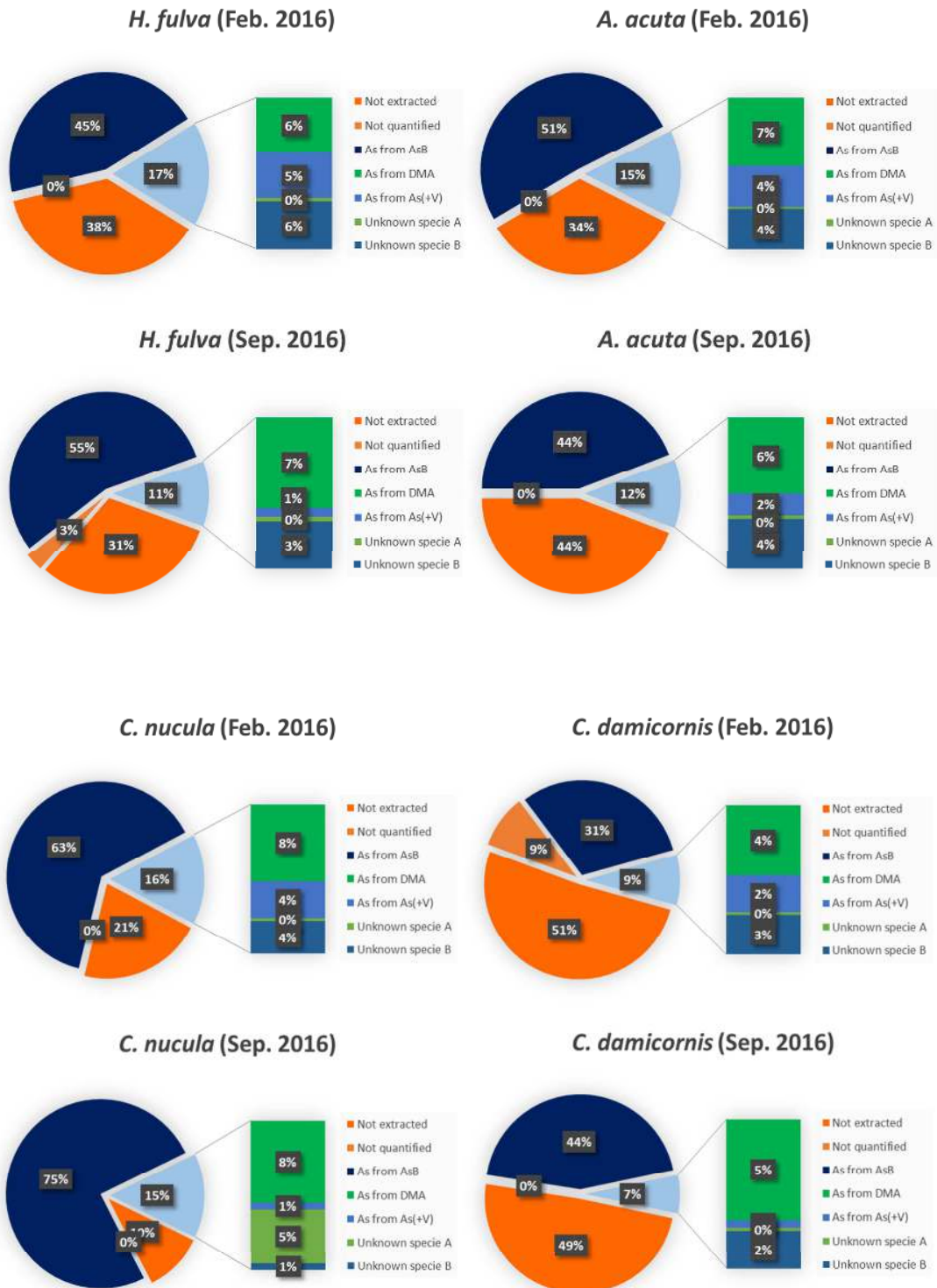


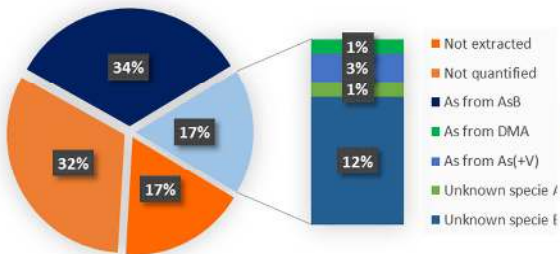
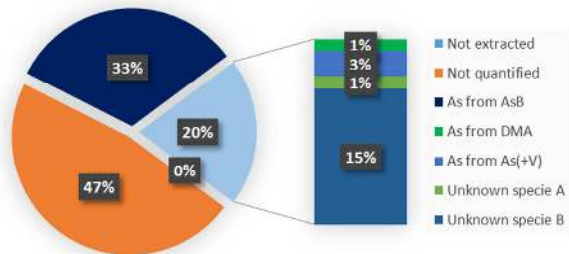
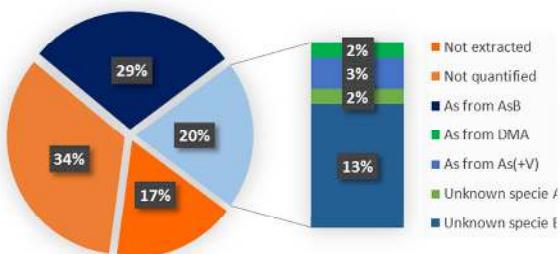
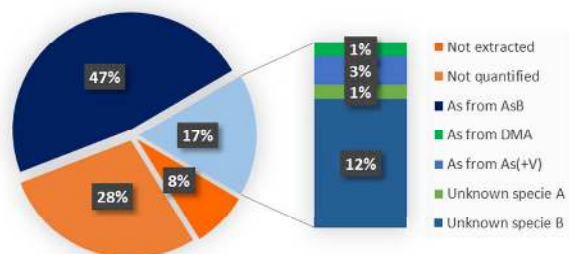
Figure 2

a)





b)

*H. panicea* (Kilkieran bay)*H. perlevis* (Kilkieran bay)*H. panicea* (Greenisland)*H. perlevis* (Greenisland)

**Highlights**

- The efficiency of  $\text{H}_3\text{PO}_4$  as extractant for As speciation in sponges was demonstrated
- As bioaccumulation and speciation mainly depend on sponge species
- As speciation in sponges revealed the predominance of AsB
- Sponge itself or symbiont organisms are responsible of AsB formation
- Sponges are efficient tools for biomonitoring and bioremediation studies