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▶ **To cite this version:**

Anna Maria Orani, Aurélie Barats, Wendy Zitte, Christine Morrow, Olivier P Thomas. Comparative study on the bioaccumulation and biotransformation of arsenic by some northeastern Atlantic and northwestern Mediterranean sponges. Chemosphere, 2018, 201, pp.826-839. 10.1016/j.chemosphere.2018.03.078 . hal-01852764

HAL Id: hal-01852764
https://hal.science/hal-01852764

Submitted on 28 Jan 2020

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ACCEPTED MANUSCRIPT

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^{-1} H_3PO_4 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

1. Introduction

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

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

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

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

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

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

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

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. Kilkieran 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*^{p} *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):

a) *Acanthella acuta* (Class: Demospongiae, Order: Axinellida, Family: Dictyonellidae) is a small erect sponge with rather small oscules.

b) *Cymbaxinella*^{p} *damicornis* (Class: Demospongiae, Order: Axinellida, Family: Axinellidae, ^{p}phylocode name), is a rather small, erectly branching sponge with short, compressed branches. Oscules are small and are located on the apices of the branches, the oscules are surrounded by a small triangular 'flap' of tissue. The genus *Axinella* is difficult to define on the basis of morphological characteristic but for the specie analyzed in this study the name *Cymbaxinella damicornis* was recently proposed by Gazave et al. (2010) following the *phylocode*.

c) *Chondrilla nucula* (Class: Demospongiae, Order: Chondrillida, Family: Chondrillidae) is amorphous, with globular lobes, or thickly incrusting, up to about 1 cm thick, spreading horizontally, with pronounced, deeply incised and lacunose, meandering lobes. The color is dark-brown to walnut-brown.

d) *Haliclona fulva* (Class: Demospongiae, Order: Haplosclerida, Family: Chalinidae), is a specimen with irregular and slightly hispid surface. The ectosomal and choanosomal skeletons have a regular, delicate, unispicular, and isotropic reticulation. Color is dark orange.

e) *Halichondria panicea* (Class: Demospongiae, Order: Suberitida, Family: Halichondriidae), is intertidal or shallow-subtidal, thickly encrusting, massive or occasionally branching, and presents typical volcanoe-shaped oscular chimneys. The surfaces of this sponge are smooth, consistency firm, texture crumb-of-bread. These sponges are basically light orange-yellow or pale yellowish green.

f) *Hymeniacidon perlevis (*Class: Demospongiae, Order: Suberitida, Family: Halichondriidae), is one of the most common species along the Atlantic coasts of Western Europe. It is orange and has an irregular surface, often with lower or higher irregular projections. Oscules are inconspicuous.

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

2.2. Sample preparation

All solutions were prepared with doubly deionized water obtained from Millipore water purification system (Elix & Synergy) (resistivity of 18.2 MΩ cm^{-1}, Total Organic Carbon <5 µg L^{-1} and microorganisms <0.1 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% 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 °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: 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 (≥30%, for trace analysis, Sigma-Aldrich) and H_2O_2 (≥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, ≥99.9%, Sigma-Aldrich) and H_3PO_4 (≥85 wt. % in H_2O, trace metals basis, Sigma-Aldrich). Extractions were performed the day before analyses and extracts were kept frozen during the

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

2.3. Sample analyses

A quadrupole ICP-MS (Elan DRCII, Perkin Elmer) was used as detection system for the determination of total As in sponge samples. ICP-MS operating parameters are summarized in Table 1. ^{75}As isotope may be interfered in saline matrices by Cl (by $^{40}Ar^{35}Cl^{+}$). The most common way to remove these spectral interferences is using the following correction equation: I(As) = I(m/z=75)-3.127×I(m/z=77)+2.733×I(m/z=82) where I stands for intensity (Potot et al., 2012; Barats et al., 2014). This mathematical correction accounts for about possible polyatomic interferences ($^{40}Ar^{35}Cl^{+}$ or $^{40}Ar^{37}Cl^{+}$) as well as for the occurrence of selenium ($^{77,82}Se$) in samples. Preparation of standard solutions and dilution of samples for analysis were carried out in a class 100 clean laboratory. External calibrations were performed with daily prepared standards obtained by proper dilution of multi-elemental standard for ICP-MS (ICP-MS standard N°3 Perkin Elmer®, ICP-MS standard N°2 from SCP SCIENCE®) or a mono-elemental As standard (PlasmaCal, SCP SCIENCE®). An internal standard solution (containing 10 and 1 µg L^{-1} of Ge and Tb respectively) was prepared by dilution of mono-elemental standards of Ge and Tb (PlasmaCal, SCP SCIENCE®). ^{74}Ge was chosen as an internal standard for As analyses to correct instrumental drifts. All sample analyses were preceded by a minimum of a five-point calibration curve spanning the entire concentration range of interest. All results were instrument blank corrected to account for any operational bias. The ongoing instrument performance was monitored by the analysis of continuing calibration 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}As averaged
200 30 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 µm, i.d. 4.1 mm).
207 Mobile phases were prepared by dissolving $(NH_4)_2CO_3$ 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 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 µg L^{-1} of As in diluted extracts, corresponding to 0.25 µg 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 $mg.L^{-1}$,
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_3 and then H_2O_2 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 conduced to a partial mineralization
249 of sponge samples. In Antarctic Demospongiae (*Sphaerotylus antarcticus*, *Kirkpatrikia 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 *Halicona 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_3/HF or HCl/HNO_3/HF mixtures. Nevertheless the use of
256 HNO_3/HF was preferred to prevent possible interferences onto the ^{75}As isotope coming from HCl. Finally, the
257 chosen sample preparation procedure involving a microwave digestion has been performed using 5 ml of HNO_3
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_2O, 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_3 was also used in chemical extraction for various aquatic organisms
271 (fishes, bivalves, crabs, shrimps) (Hong et al., 2014). H_3PO_4 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_2O in freshwater organisms (revealing low recovery of 30%)
274 (Schaeffer et al., 2006), or with a mixture H_2O/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 ± 5 mg kg^{-1} 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^{-1} H_3PO_4 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^{-1} H_3PO_4 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_3PO_4 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^{-1} H_3PO_4 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^{-1} H_3PO_4 solutions, AsB represent 64% of the extracted As with a better precision.

313 Speciation analyses were performed then on CRMs and conduced 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^{-1}
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 ± 5 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 ± 0.8 mg kg^{-1} and
319 another unknown As specie, named A, at 1.9 ± 0.6 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 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 mg kg^{-1} in *Haliclona sp.* white, 13 mg kg^{-1} in *Haliclona*
371 *permolis* (Yamaoka et al., 2001), 1.03 mg kg^{-1} in *Haliclona tenuiramosa* (Venkateswara Rao et al., 2009) and
372 between 1.5 and 8.5 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 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 mg kg^{-1}). Regarding *Halichondria panicea* , Vaskovsky et al. (1972) reported
376 similar As concentrations (6 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 ± 2 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 ± 2 and 2.9 ± 0.3 mg kg^{-1}
388 respectively. These values are below the limit imposed by the principal environmental authorities for As in
389 marine sediments (7.24 mg kg^{-1}, 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 ± 0.2 µg L^{-1} 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^{-1} 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^{-1}, 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 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 mg kg^{-1}, 1.1 µg L^{-1} and 3.6 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 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

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

3.3.2. As speciation in sponges

As speciation analyses were performed on extracts of sponge samples (Table 4). For each sponge species, speciation analyses were carried out on 3 sponge specimens. Significant differences on As speciation occur between the Irish and Mediterranean sponges (Fig. 2). Despite the fact that most of the As was successfully extracted in Irish sponges (>81 %), a large proportion of the extracted species was not identified/quantified (speciation recoveries ranged from 52 to 66 %). Vaskovsky et al. (1972) reported a similar As concentrations in *Halichondria panicea* sponges, but measured in lipid extracts. This result may prove the occurrence of As rather under organic As species, probably those not identified in our study. For Mediterranean sponges, although the extraction recovery was generally lower than Irish samples (especially for *C. damicornis*), almost the totality of extracted species were identified and quantified, with a speciation recovery between 79 and 119%. Within the same sponge species and the same sampling date, the variability of extracted As content and As speciation related to sampling site characteristics was also estimated (Fig. 1). ANOVA analysis revealed no difference on extracted As and As speciation for *C. nucula* sponges sampled at different depth; analogously no significant 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 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 µg L^{-1} of As(+V) and 0.2 µg L^{-1} of As(+III)
497 in the Mediterranean sea (Cabon and Cabon, 2000) and from 1.9 to 3 µg 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 ($4 < \log BAF < 5$).
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 occuring 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^{-1}.
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

567 **Acknowledgment**

568 The authors would like to express their deep gratitude to J.-P. Goudour for his technical assistance in
569 our daily laboratory work, to D. Rodrigues and M. Nicolas for sponge sampling in France. The authors are
570 grateful to F. Pannier and F. Séby for their precious advices for speciation analyses. The authors acknowledge
571 the University of Nice Sophia Antipolis where all analyses were performed. A. M. Orani has benefited from a
572 financial support from the University of Nice Sophia Antipolis and NUI Galway. The IAEA is grateful for the
573 support provided by the Government of the Principality of Monaco to its Environment Laboratories.

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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.

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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^{-1}
	Auxiliary gas flow	1.1 mL min^{-1}
	Carrier gas flow	0.9 L min^{-1}
	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 $(NH_4)_2CO_3$ pH=9
	Mobile phase B	70 mM $(NH_4)_2CO_3$ pH=9
	Flow rate	2 mL min^{-1}
	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
Microwave digested solutions	Total As (mg kg-1)	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
Extracted solutions	Extracted As (mg kg-1)	23 ± 4 (n=3)	4.6 ± 0.3 (n=6)	
	Extraction recovery (%)	106	97	
	As from AsB (mg kg-1)	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-1)	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^{-1}.

	Extracted As (mg kg^{-1})	Extraction recovery (%)	As from AsB (mg kg^{-1})	As from AsB in extracts (%)
H_2O	21 ± 3	72	14 ± 3	67
MeOH 50%	21 ± 2	72	24 ± 3	114
MeOH 25%	25 ± 1	86	19 ± 3	76
H_3PO_4 0.3 mol L^{-1}	20 ± 3	69	13.0 ± 0.8	65
H_3PO_4 0.6 mol L^{-1}	23.5 ± 0.7	81	15.2 ± 0.8	64
H_3PO_4 1 mol L^{-1}	24.2 ± 0.5	83	13.9 ± 0.8	57

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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^{-1}	RSD (%)	mg kg^{-1}	RSD (%)		mg kg^{-1}	RSD (%)	mg kg^{-1}	RSD (%)	mg kg^{-1}	RSD (%)	mg kg^{-1}	RSD (%)	mg kg^{-1}	RSD (%)		
Villefranche sur Mer (France)	Feb. 2016	*H. fulva*	26	23	16	56	59	12	67	1,7	35	1,4	14	<0,1		1,5	33	106	66
		A. acuta	44	18	28	4	64	24	25	3,1	23	1,9	16	<0,1		1,9	21	109	69
		C. nucula	34	38	26	27	77	24	38	3	33	1,44	1	<0,1		1,3	8	116	88
		C. damicornis	74	15	36	22	49	23	35	3	33	1,6	25	<0,1		1,7	24	79	39
	Sep. 2016	*H. fulva*	29	17	20	25	69	16	13	1,9	5	0,1<c<0,4		<0,1		1	10	95	66
		A. acuta	33	6	17	12	51	16	25	2,1	5	0,6	33	<0,1		1,4	29	119	61
		C. nucula	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
		C. damicornis	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	*H. panicea*	7,3	11	6	2	83	2,5	8	<0,1		0,1<c<0,3		<0,1		0,89	9	57	47
		H. perlevis	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	*H. panicea*	5,7	9	4,7	9	81	1,7	18	<0,1		0,1<c<0,3		<0,1		0,8	25	52	43
		H. perlevis	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^{-1})	As concentration (mg kg^{-1})			Reference
			AsB	AsS	Others	
Hymeniacidon heliophila	Guanabara Bay, Brazil	4.6-8.1				Batista et al., 2014
Paraleucilla magna		1.2-2.6				
Haliclona oculata	Poole harbor, UK	1.5-8.5				Aly et al., 2013
Spheciospongia vagabunda	Darwin Harbour, Aurstralia	10.1-56.4				Padovan et al., 2012
Hyrtios erectus	Red Sea, Saudi Arabia	15-63.9				Pan et al., 2011
Hyrtios sp.		2.30				
Stylissa carteri		7.5-10.7				
Chalinula sp.		13.7-22.1				
Xestospongia testudinaria		20.7-42.2				
Phyllospongia papyracea		8.0				
Amphimedon sp.		11.2				
Spongia arabica		106.1				
Spheciospongia inconstans		15.0				
Haliclona tenuiramosa	Gulf of Mannar, India	0.32-1.09				Rao et al., 2009
Petrosia testudinaria	Gulf of Mannar, India	2.30				Rao et al., 2006
Thorecta sp.	Bohol Sea, Philippines	6.2*	3.0	2	1.2	Yamaoka et al., 2006 *water fraction
Dysidea sp.		24.8*	15.2	1.4	8.1	
Theonella sp.		157.0*	136.6	2.21	18.2	
Acanthella sp.		6.1 *	1.7	2.0	2.4	
Phyllospongia sp.		4.4*	1.5	2.12	0.7	
Aaptos sp.		112.5*	98.1	0.25	14.2	
Biemna fortis		1.0*	0.13	0.15	0.8	
Jaspis sp.		6.1*	0.6	3.7	1.7	
Subertes sp.		10.3*	1.7	7.4	1.1	
Haliclona permolis		13*	3.3	9.9	0.5	
Halichondria japonica		3.4*	1.1	1.6	0.8	
Haliclona sp. white		0.8*	0.2	0.5	0.2	
Halichondria okadai		5.5*	0.5	4.5	0.5	
Ephydatia fluviatilis	Danube river, Hungary	8.07	<0.02	0.29	0.12 (DMA)	Schaeffer et al., 2006
Spongia officinalis	Marseille, France	86.3-134.1				Perez et al., 2005

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

FIGURES

Figure 1

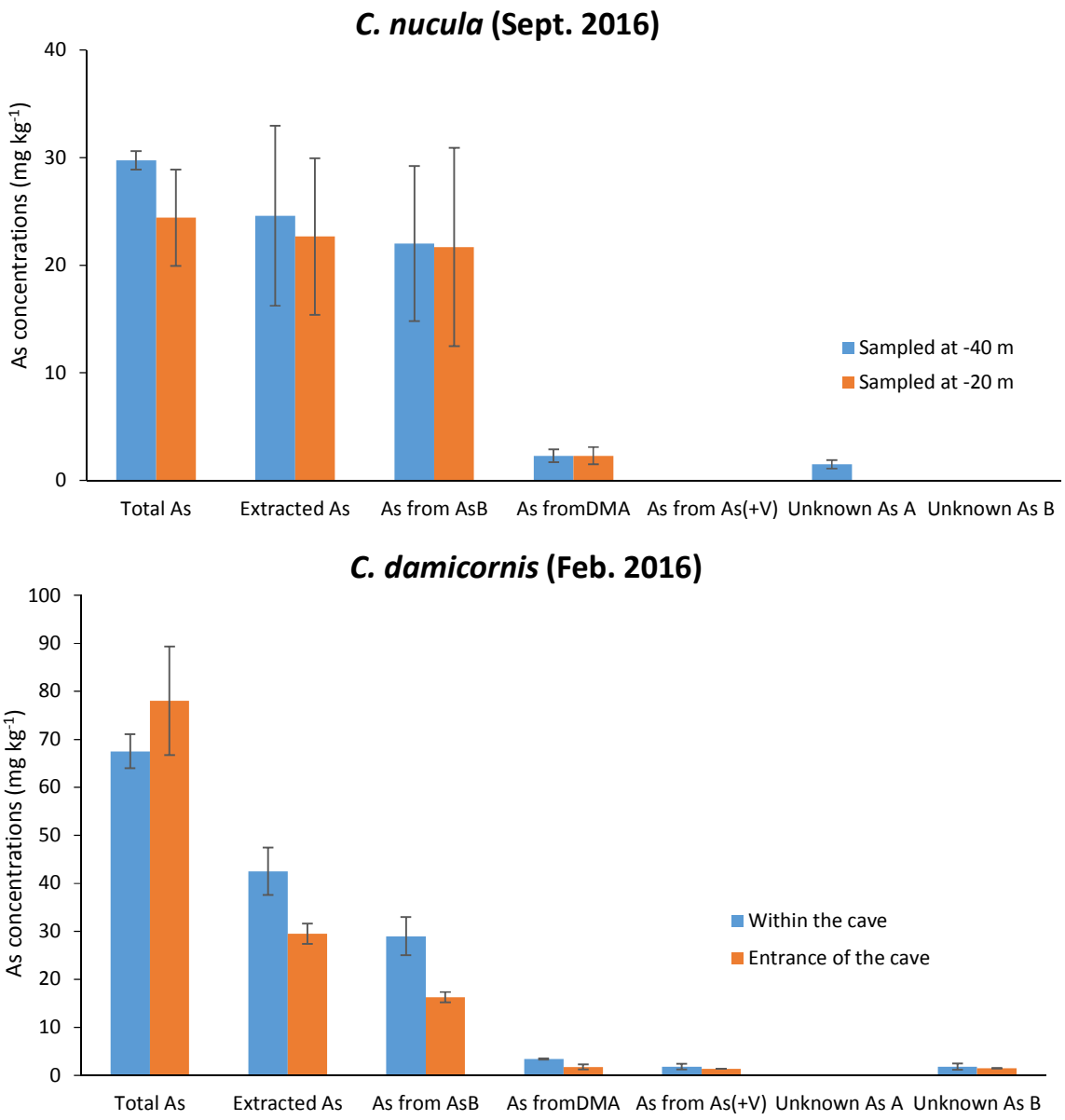
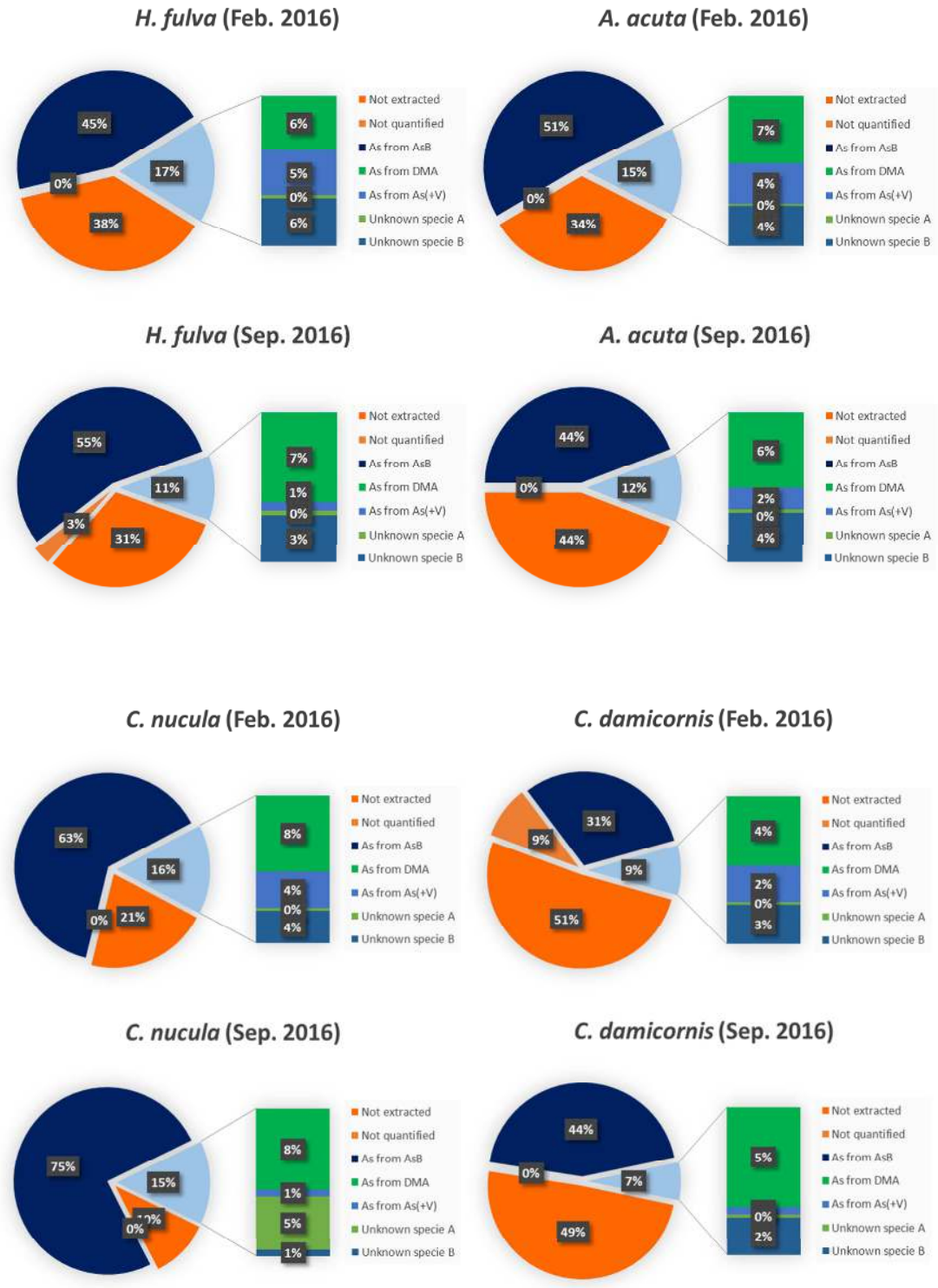
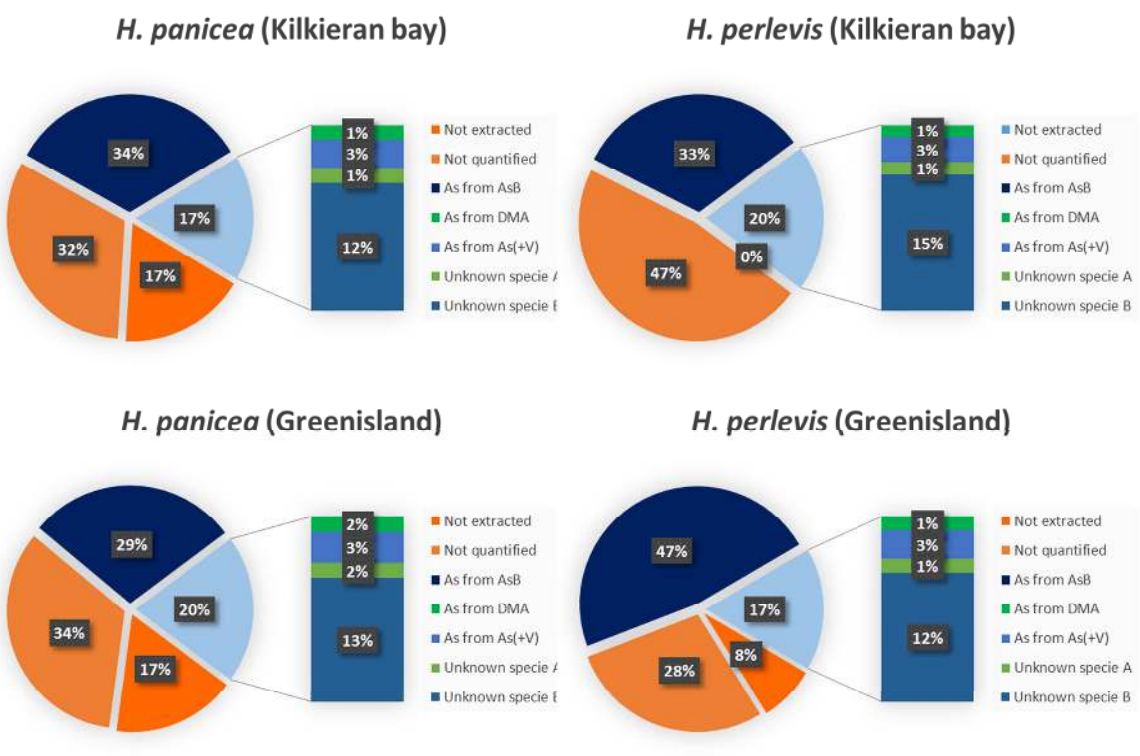


Figure 2

a)


H. fulva (Feb. 2016)
45%
0%
17%
38%
6%
5%
0%
6%
A. acuta (Feb. 2016)
51%
0%
15%
34%
7%
4%
0%
4%
H. fulva (Sep. 2016)
55%
3%
11%
31%
7%
1%
0%
3%
A. acuta (Sep. 2016)
44%
0%
12%
44%
6%
2%
0%
4%
C. nucula (Feb. 2016)
63%
0%
16%
21%
8%
4%
0%
4%
C. damicornis (Feb. 2016)
9%
31%
9%
51%
4%
2%
0%
3%
C. nucula (Sep. 2016)
75%
0%
15%
8%
1%
5%
1%
C. damicornis (Sep. 2016)
44%
0%
7%
49%
5%
0%
2%
Not extracted
Not quantified
As from AsB
As from DMA
As from As(+V)
Unknown specie A
Unknown specie B

b)


H. panicea (Kilkieran bay)
34%
17%
32%
17%
1%
3%
1%
12%
H. perlevis (Kilkieran bay)
33%
20%
0%
47%
1%
3%
1%
15%
H. panicea (Greenisland)
29%
20%
34%
17%
2%
3%
2%
13%
H. perlevis (Greenisland)
47%
17%
8%
28%
1%
3%
1%
12%
Not extracted
Not quantified
As from AsB
As from DMA
As from As(+V)
Unknown specie A
Unknown specie B

ACCEPTED MANUSCRIPT

Highlights

- The efficiency of H_3PO_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