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# Simple and Rapid Voltammetric Method Using a Gold Microwire Electrode to Measure Inorganic Arsenic in Holopelagic *Sargassum* (Fucales, Phaeophyceae)

T. Gobert<sup>a</sup>, S. Connan<sup>a</sup>, P. Salaün<sup>b</sup>, M. Eikelboom<sup>b</sup>, R. D. Riso<sup>a</sup>, V. Stiger-Pouvreau<sup>a</sup>, and M. Waeles<sup>a</sup>

<sup>a</sup>University of Brest, CNRS, IRD, Ifremer, LEMAR, Plouzane, France; <sup>b</sup>Department of Earth and Ocean Sciences, University of Liverpool, United Kingdom

## ABSTRACT

The valorization of massive strandings of holopelagic *Sargassum* spp. is strongly limited by high levels of inorganic arsenic (As<sub>i</sub>) that are potentially above the limit of current regulations. Monitoring As<sub>i</sub> in algal biomass is currently achieved using standard chromatographic separation followed by spectroscopic detection. Here, we propose an alternative simpler procedure based on the extraction of As<sub>i</sub> from the freeze-dried algal powder in deionized water and the electroanalytical detection of the diluted extract at a gold-microwire electrode. The protocol was optimized both in terms of extraction (powder/water ratio, extraction time, temperature) and electrolyte used for the voltammetric detection. Two electrolytes were tested: one composed of citric acid, sulfamic acid and KCl (pH 2.0) and another composed of an acetate buffer (pH 4.7) and NaCl. We demonstrate here that As<sub>i</sub> determination is possible with the first electrolyte but it is necessary to deal with a relative unstable signal. Measurement of As<sub>i</sub> was best achieved with the second electrolyte (acetate buffer and NaCl) with the following optimized electrochemical conditions: deposition potential of  $-1.2$  V, deposition time of 30 seconds and linear scan voltammetry. Voltammetric results were then compared to a reference method (HPLC-ICP-MS) using different morphotypes of holopelagic *Sargassum* spp. (*S. natans* VIII, *S. natans* I and *S. fluitans* III), using commercial extracts of brown seaweeds and using a *Hijiki* certified reference material. Very good agreement was obtained between our novel method and HPLC-ICP-MS. Both methods show that inorganic arsenic is almost entirely present as As(V) in *Sargassum* spp. extracts.

## ARTICLE HISTORY

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voltammetry; gold  
microwire

## Introduction

Arsenic (As) is recognized as a toxic element, but its toxicity depends mainly on its speciation, the organic forms being generally less toxic than inorganic forms, and As(III) being more toxic than As(V) (Karadjova, Slaveykova, and Tsalev 2008; McSheehy and Szpunar 2000; Rose et al. 2007). Typical As levels in oceanic water are relatively

homogeneous and range from 1 to  $3 \mu\text{g L}^{-1}$ , with an average level of  $1.7 \mu\text{g L}^{-1}$  (Andreae and Klumpp 1979). In seawater, arsenic occurs mainly as inorganic As species ( $\text{As}_i$ ), corresponding to arsenate oxyanion As(V) and arsenite oxyanion As(III) (Francesconi and Kuehnelt 2004; Wang et al. 2013). In marine organisms, organic forms of As, such as monomethylarsinite (MMA), dimethylarsinite (DMA), arsenobetaine (AB) or arsenosugars, are also present, in some cases representing the majority of total arsenic. These forms are the result of a biomethylation process of  $\text{As}_i$  (Lin et al. 2021; Taylor and Jackson 2016; Zhao et al. 2020).

The chemistry of As in the environment is complex and uptake pathways of this element differ from one organism to another. In the case of marine algae, especially brown macroalgae, As is probably taken up as arsenate As(V) *via* phosphate transporters due to the strong similarities in size and geometry of As(V) and P main chemical forms in seawater, i.e.,  $\text{HAsO}_4^{2-}$  and  $\text{HPO}_4^{2-}$  (Mamun et al. 2019; Taylor and Jackson 2016). Macroalgae, particularly brown seaweeds, are considered as arsenic hyper-accumulators with tissue As levels 1,000 to 50,000 times higher than in seawater (Borak and Hosgood 2007; Ma et al. 2018; Sartal, Alonso, and Barrera 2014). Although many marine algae generally convert  $\text{As}_i$  to organic As species through biomethylation processes (Geiszinger et al. 2001; Murray et al. 2003), some brown macroalgae such as the benthic *Sargassum piluliferum* can contain high  $\text{As}_i$  levels up to 50% of total As (Hirata and Toshimitsu 2007).

Since the 2010s, holopelagic *Sargassum* spp. (*S. natans* and *S. fluitans*) have been growing intensively in the tropical Atlantic Ocean (Franks, Johnson, and Ko 2016; Ody et al. 2019; Wang et al. 2019). Large quantities of holopelagic species drift under the influence of winds and ocean currents, subsequently causing massive stranding episodes on the coasts of the Caribbean islands, Mexico and also West Africa (Oviatt et al. 2019). These strandings are responsible for environmental, ecological, economic as well as health problems (Milledge and Harvey 2016; Resiere et al. 2021). Although many ways of valorization have been proposed, such as organic fertilizers or animal feed (Amador-Castro et al. 2021; Rushdi et al. 2020; Stiger-Pouvreau and Zubia 2020), the current regulations prevent the use of this biomass, especially due to high  $\text{As}_i$  levels. For example, in European countries, the maximum level of  $\text{As}_i$  is  $40 \text{ mg kg}^{-1}$  in organic fertilizer and  $2 \text{ mg kg}^{-1}$  in animal feed (EU 2015). Despite explicit legislation on  $\text{As}_i$ , it should be noted that all recent studies conducted on the composition of stranded holopelagic *Sargassum* spp. report only the total As content (Dassié et al. 2022; Fernández et al. 2017; Rodríguez-Martínez et al. 2019).

Arsenic speciation in seaweed is usually performed by coupling high-performance liquid chromatography (HPLC) with inductively coupled plasma-mass spectrometry (ICP-MS) (Cipolloni et al. 2022; Rubio et al. 1992; Sankararamakrishnan and Mishra 2018; Wolle and Conklin 2018a, 2018b) or with hydride generation-atomic fluorescence spectrometry (HG-AFS) (Camurati et al. 2021). Such coupling is a powerful analytical tool for arsenic speciation, allowing the separation and quantification of the main forms of arsenic (both inorganic and organic). However, these methods require expensive equipment and can be time-consuming. Electroanalysis is an alternative analytical method that is sensitive to total inorganic arsenic but can also differentiate As(III) and As(V) (Forsberg et al. 1975; Salaün, Planer-Friedrich, and van den Berg 2007;

Viltchinskaia et al. 1997). The method has the advantage to be portable, cheap and relatively fast while being sensitive. Many studies have reported electrochemical procedures to determine As<sub>i</sub> in natural waters (seawater, groundwater or rivers) (Mays and Hussam 2009; Rasul et al. 2002; Tupiti, Chandra, and Prasad 2018; Vandenhecke et al. 2007). However, no analytical development has been made to determine As<sub>i</sub> in macroalgae. If many metals (e.g. copper) or organic matter can potentially interfere with the electrochemical measurement of As<sub>i</sub>, recent advances in the design of gold electrodes and experimental procedures now allow robust and low-interference measurements of As<sub>i</sub> (Dugo et al. 2005; Feeney and Kounaves 2002). In this study, commercially available gold microwire electrodes were used and two different electrolytes suited for As<sub>i</sub> quantification in water (Metrohm, application bulletin 416/3) and groundwater (Eikelboom et al. 2023) were tested. Our objective was to implement a simple method, accessible to the greatest number of users (even to non-voltammetric experts), for As<sub>i</sub> quantification in holopelagic *Sargassum* spp.

## Materials and methods

### *Holopelagic Sargassum* spp. samples

Samples of the three morphotypes of holopelagic *Sargassum* spp., i.e., *S. natans* VIII, *S. natans* I and *S. fluitans* III, were collected at stations in the tropical Atlantic Ocean in June-July 2017. These samples corresponded to biomass collected at sea (“Caribbean” expedition, <https://doi.org/10.17600/17004300>) or to stranded biomass, the latter being collected on the Vauclin beach in Martinique (14.548°N, 60.838°W). Just after collection, seaweed samples were frozen as soon as possible after collection before being later freeze-dried and ground into a fine powder (MM400, Retsch, Germany).

### Chemicals

All chemicals and reagents were of analytical or suprapure quality and used without any purification. Ultrapure water (Milli-Q), with a resistivity > 18.2 MΩ cm<sup>-1</sup>, was used to prepare all solutions. From As(V) and As(III) standards stock solutions at 1,000 mg L<sup>-1</sup> (Chem-Lab, Belgium), working solutions at concentrations of about 1,000 μg L<sup>-1</sup> were prepared. Cacodylic acid sodium trihydrate (DMA, ≥ 97%) and methylarsonic acid sodium (MMA, ≥ 84.6%) were acquired from LGC Standards (Bury, UK). Arsenobetaine (AB, ≥ 95%) was supplied from Sigma-Aldrich (St. Louis, MO, USA). The standards were stored protected from light in a refrigerator, while the working solutions were wrapped in aluminum foil and regularly prepared to prevent photochemical oxidation of As(III). Sulfamic acid (H<sub>3</sub>NO<sub>3</sub>S, 99.3%), potassium chloride (KCl, 99.5%), potassium permanganate (KMnO<sub>4</sub>) and sodium chloride (NaCl, 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The other chemicals used were citric acid (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>, Fluka, Germany), sulfuric acid (H<sub>2</sub>SO<sub>4</sub> 96%, Chem-Lab, Belgium), sodium acetate (CH<sub>3</sub>COONa, AnalaR BDH Chemicals Ltd., England) and acetic acid (CH<sub>3</sub>COOH 99.5%, Fluka, Germany).

## **Instrumentation**

The main voltammetric sensor used in this work was the scTRACE Gold from Metrohm (Switzerland) which integrates a working electrode (gold microwire, 25  $\mu\text{m}$  diameter, length:  $\sim 1$  cm), a reference electrode (Ag/AgCl) and a carbon auxiliary electrode into one small single unit. The sensor was placed in a 663VA stand. An Autolab potentiostat type III was used with a IME663 interface controlled by the GPES software version 4.9. A Teflon cell ( $\sim 20$  mL) was used as well as a rotatory stirring system automatically controlled by the software.

## **Activation and cleaning of the gold electrode**

The activation/cleaning procedure of the ScTrace gold electrode was done along Metrohm recommendations (<https://www.metrohm.com/en/applications/ab-application-bulletins/ab-416.html>). The cleaning solution consisted of 0.5 M sulfuric acid (2.78 mL  $w(\text{H}_2\text{SO}_4) = 96\%$ ) and 0.05 M KCl (0.373 g) prepared in 100 mL ultrapure water. The first step consisted in running a cyclic voltammetry (CV) scan where the potential was varied 10 times between  $-1.5$  and  $1.0$  V at a scan rate of  $0.4 \text{ V s}^{-1}$ . The second step was performed by applying  $-1.0$  V for 10 s, followed by  $-0.3$  V for 5 s and a scan (in linear sweep mode) between  $-0.3$  and  $0.2$  V ( $0.4 \text{ V s}^{-1}$ , step potential of  $10 \text{ mV}$ ). A regular cleaning of the electrode system was also needed after several measurements. For this cleaning procedure, a CV between  $-1.0$  and  $1.0$  V was performed ten times followed by a scan, in cyclic voltammetry stripping mode, between  $-0.3$  and  $0.2$  V, with a step potential of  $0.01$  V and a scan rate of  $0.4 \text{ V s}^{-1}$ . The Au WireBond electrode was conditioned by imposing a low deposition potential ( $-2$  V, 10s) in  $0.5 \text{ M H}_2\text{SO}_4$ , followed by 3 CV scans from  $-0.2$  to  $1.5$  V ( $100 \text{ mV.s}^{-1}$ ).

## **Voltammetric procedures for arsenic speciation**

Two procedures using two electrolytes were tested in this study. All measurements were performed without deoxygenation of the solutions

The **Procedure A**, which is based on a procedure developed by Metrohm for As<sub>5</sub> measurements in water (<https://www.metrohm.com/en/applications/ab-application-bulletins/ab-416.html>), used an electrolytic solution (hereafter called CASA) that consisted of  $0.5 \text{ M}$  citric acid ( $10.51 \text{ g}$ ),  $1 \text{ M}$  sulfamic acid ( $9.71 \text{ g}$ ) and  $0.45 \text{ M}$  potassium chloride ( $3.35 \text{ g}$ ) dissolved in  $100 \text{ mL}$  of ultrapure water. This CASA solution was characterized by a pH of  $1.9$ . Electrochemical measurements using the CASA were done in the electrochemical cell using the following proportions:  $11 \text{ mL}$  of ultrapure water,  $2 \text{ mL}$  of CASA electrolyte and  $100 \mu\text{L}$  of a  $0.2 \text{ mM KMnO}_4$  stock solution (final concentration  $1.5 \mu\text{M}$ ).

The **Procedure B** is based on the development conducted by Eikelboom et al. (2023). The electrolytic solution (hereafter called CIAC) consisted of  $0.21 \text{ M}$  acetic acid ( $6 \text{ mL}$ ),  $0.6 \text{ M}$  sodium acetate ( $23.4 \text{ g}$ ) and  $2.5 \text{ M}$  sodium chloride ( $73 \text{ g}$ ) dissolved in  $500 \text{ mL}$  of ultrapure water. This electrolytic solution was characterized by a pH of  $4.7$ . Electrochemical measurements using the CIAC were done with the following

**Table 1.** Voltammetric conditions used in CASA and CIAC electrolytes.

Procedure	Electrolyte	pH	Deposition step	Stripping step	Observations
A	CASA: 0.5 M citric acid 1 M sulfamic acid 0.45 M KCl	1.9	−1.0 V (60s)	SWV from −0.3 to 0.4 V step: 8 mV, amplitude: 20 mV frequency: 100 Hz	For both procedure A and B, No purging required Any As(III) present is oxidized to As(V) due to addition by $\text{MnO}_4^-$ ions
B	CIAC: 0.21 M acetic acid 0.6 M sodium acetate 2.5 M NaCl	4.7	−1.2 V (30s)	LSV from −0.4 to 0.6 V, scan rate: $4.9 \text{ V s}^{-1}$ step: 2 mV, current range: 10 $\mu\text{A}$	

SWV: square wave voltammetry; LSV: linear scan voltammetry.

proportions: 11.8 mL of ultrapure water, 1.2 mL of CIAC electrolyte and 13  $\mu\text{L}$  of a 10 mM  $\text{KMnO}_4$  solution (final concentration 10  $\mu\text{M}$ ).

Voltammetric detection was carried out by anodic stripping voltammetry (ASV) in both electrolytes. Each ASV scan consists of a deposition step followed by a stripping step. Conditions for both electrolytes are given in Table 1.

After testing both procedures for the detection of arsenic in each electrolyte, they were used to determine  $\text{As}_i$  concentration in algal biomass. For that purpose, a small volume of algal extract (200  $\mu\text{L}$  or less) was added into the solution of the measuring cell (see extraction procedure).

### Total arsenic with ICP-MS

In order to determine the proportion of inorganic arsenic, total arsenic was quantified by ICP-MS. After weighing 50 mg of dried and powdered material, digestions were performed at 80 °C for 3 h in closed 15-mL Teflon screw-cap vials (Savillex, Minnetonka, MN, USA) with 1 mL suprapur 65% nitric acid (Merck, Darmstadt, Germany) and 0.25 mL suprapur 30% hydrogen peroxide (Merck, Darmstadt, Germany). Measurements of total arsenic concentrations were conducted on diluted mixtures (2.3%  $\text{HNO}_3$ ) using an ICP quadrupole mass spectrometer (X-series II, Thermo Scientific) operated at the Pole Spectrometry Ocean Brest (PSO, Brest, France). All concentrations shown in the present study were well above detection limits while digestion blanks were below detection limits.

### Arsenic speciation with HPLC-ICP-MS

For comparative purposes, arsenic speciation analysis, and more specifically As(V) determination, was achieved by coupling an anion-exchange liquid chromatograph (Model 655 A-12, Merck, Darmstadt, Germany) with a sector field ICP-MS (Thermo Scientific ELEMENT XR) which was used in high resolution mode. The HPLC column was a PRP-X100 column (250  $\times$  4.6 mm, 5  $\mu\text{m}$ , Hamilton, Reno, NV, USA). The analytical conditions applied corresponded to a method optimized by Lindeman (2016). The mobile phase was prepared with 2.6 mM of  $(\text{NH}_4)_2\text{HPO}_4$  and 1 mM of EDTA in aqueous 2% (v/v) methanol and adjusted to pH 8.2 with ammonia. *Sargassum* extracts were diluted according to their total As concentration in order to stay within the linear range of the HPLC-ICP-MS method. Chromatographic peaks for the sample extracts were identified by comparison of retention time with standards and quantified by standard

addition (see Figure S1, supplementary materials). HPLC-ICP-MS measurements were made at PSO (Pôle Spectrométrie Océan, IUEM, UBO), France.

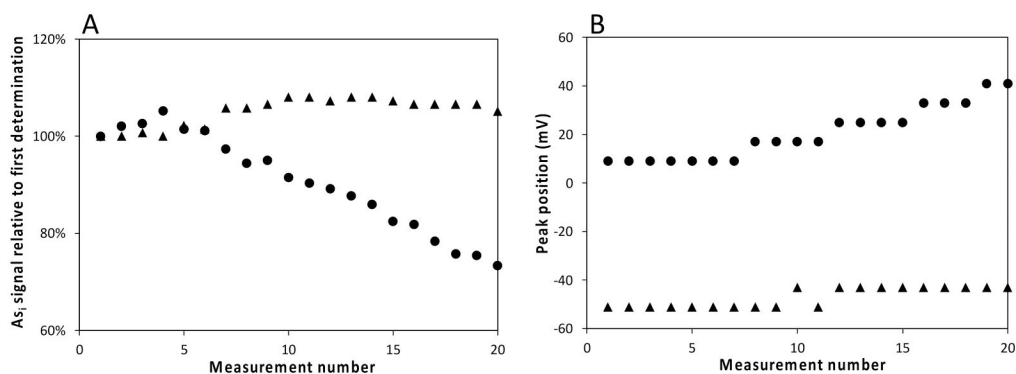
## Results and discussion

### Voltammetric detection of $As_i$

#### Peak instability

The  $As_i$  signal stability (Figure 1A) was first investigated by running successive stripping measurements using the scTRACE Gold sensor in both electrolytes (CASA and CIAC) in the presence of  $4 \mu\text{g L}^{-1}$  of  $As(V)$ . For the CASA solution, a clear decrease of the  $As$  peak intensity was observed, starting after six consecutive cycles. After ten cycles, the decrease was about 10%; and after twenty cycles, the decrease was 25%. This decrease or loss of sensitivity was concomitant with an anodic shift of the peak potential (Figure 1B). The  $As(V)$  peak, initially located at  $+9 \text{ mV}$ , was found at  $+42 \text{ mV}$  after 20 cycles. On the other hand, the  $As$  signal intensity (Figure 1A) was found stable in the CIAC solution ( $1.05 \pm 0.03 \mu\text{A.V}^{-1}$ , relative standard deviation (RSD) 3%,  $n = 20$ ), and so was the peak position (shifted only from  $-51$  to  $-43 \text{ mV}$ )

The cause of the decreasing  $As_i$  signal in CASA was further investigated by varying the deposition potential (Figure S2A) and deposition time (Figure S2B). The loss rate increased by decreasing the deposition potentials and increasing the deposition time. For instance, after twenty cycles, the decrease was less than 10% at  $E_{\text{dep}} = -0.9 \text{ V}$  while it was over  $\sim 40\%$  at  $E_{\text{dep}} = -1.1 \text{ V}$ . Similarly, using 30s deposition time, it took eighteen consecutive measurements for the signal to decrease more than 10% while it took only six measurements when using a deposition time of 120s. This loss of signal was found to be independent of the  $As$  concentration (same loss observed at 2, 5 and  $10 \mu\text{g L}^{-1}$ ). All these observations suggest that the deposition step carried out in the acidic CASA electrolyte triggers the production of a compound that affects the good functioning of the sensor. When using a strong reducing deposition potential in acidic conditions, production of  $H_2(g)$  from the reduction of protons at the working electrode can be



**Figure 1.** Evolution of the (A) voltammetric response of  $As_i$  (peak derivative) and (B)  $As_i$  peak position as a function of the number of measurements. The measurements were carried out in CASA, procedure A (round) with  $4 \mu\text{g L}^{-1}$  of  $As(V)$ ,  $E_{\text{dep}} = -1.0 \text{ V}$ ,  $t_{\text{dep}} = 60 \text{ s}$ , and in CIAC, procedure B (triangle) with  $5 \mu\text{g L}^{-1}$  of  $As(V)$ ,  $E_{\text{dep}} = -1.2 \text{ V}$ ,  $t_{\text{dep}} = 30 \text{ s}$ .

important. This strong reduction current is counter balanced by an equally strong oxidation current at the carbon auxiliary electrode, with a production of chlorine ( $\text{Cl}_2$ , g) and hypochlorous acid (HOCl) which may lead to physical damage/blockage of the auxiliary electrode (Khairy et al. 2010; Salaün, Planer-Friedrich, and van den Berg 2007). On the other hand, the detection of As(V) in the CIAC +  $\text{KMnO}_4$  was stable, possibly due to the higher pH resulting in much lower reduction/oxidation current at the working/auxiliary electrode.

Overall, these signal stability experiments demonstrate that any quantification of  $\text{As}_i$  in the CASA electrolyte (using a deposition at  $-1.0\text{ V}$  for 60s) cannot be achieved with more than 6 consecutive measurements. Due to this peak instability, the classical standard addition method (consisting of 4 repeated measurements of the sample, followed by a minimum of two standard additions with four repeated measurements for each addition) could not be applied. All  $\text{As}_i$  quantifications with the CASA solution were thus performed as follows: three independent determinations all conducted in a new solution and with two standard additions, with only one measurement for the sample and one for each standard addition. For quantifications with the CIAC, the classical standard addition method was used (four repeated measurements for sample and for each of the two standard additions). The next section shows that a good recovery was obtained using both procedures.

#### ***Linear range, detection limits and accuracy of $\text{As}_i$ determination***

The linear range of the  $\text{As}_i$  response at the gold electrode was examined with increasing additions of As(V) using procedure A (CASA solution,  $E_{\text{dep}} = -1.0\text{ V}$ ,  $t_{\text{dep}} = 60\text{ s}$ ) and procedure B (CIAC solution,  $E_{\text{dep}} = -1.2\text{ V}$ ,  $t_{\text{dep}} = 30\text{ s}$ ). For procedure A and procedure B, the signal increased linearly until 20 and 15  $\mu\text{g L}^{-1}$ , respectively. At higher concentrations, the As signal tends toward a plateau value due to progressive saturation of the gold active sites with non-conductive reduced As(0) (Khairy et al. 2010; Salaün, Planer-Friedrich, and van den Berg 2007). The linear range was found to be inversely correlated to the deposition time.

The limit of detection (LoD) for  $\text{As}_i$  quantification was determined, for both procedures, from 3 times the standard deviation ( $3\sigma$ ) of successive determinations. For procedure A (CASA solution,  $E_{\text{dep}} = -1.0\text{ V}$ ,  $t_{\text{dep}} = 60\text{ s}$ ), the LoD determined at 1.0  $\mu\text{g L}^{-1}$  of As(V), was 0.2  $\mu\text{g L}^{-1}$  ( $n=7$ , standard deviation (SD) = 5%). For procedure B (CIAC solution,  $E_{\text{dep}} = -1.2\text{ V}$ ,  $t_{\text{dep}} = 30\text{ s}$ ), the LoD determined at 1.5  $\mu\text{g L}^{-1}$  of As(V) was 0.3  $\mu\text{g L}^{-1}$  ( $n=7$ , SD = 6%).

The accuracy of the methods were evaluated by analyzing solutions spiked with known concentrations of As(V). The two concentrations tested were 2.1 and 5.1  $\mu\text{g L}^{-1}$ . Using procedure A (CASA solution,  $E_{\text{dep}} = -1.0\text{ V}$ ,  $t_{\text{dep}} = 60\text{ s}$ ), the concentrations obtained after three independent determinations with two standard additions were  $2.4 \pm 0.2$  and  $4.9 \pm 0.4\ \mu\text{g L}^{-1}$ , respectively. Using procedure B (CIAC solution,  $E_{\text{dep}} = -1.2\text{ V}$ ,  $t_{\text{dep}} = 30\text{ s}$ ), the concentrations obtained after one determination with two standard additions (and four consecutive measurements at each level) were  $2.0 \pm 0.1$  and  $5.2 \pm 0.3\ \mu\text{g L}^{-1}$ , respectively. Overall, these results show that the measurement of  $\text{As}_i$  using the proposed standard addition methods, which is specific for each procedure, can be conducted with a good accuracy.



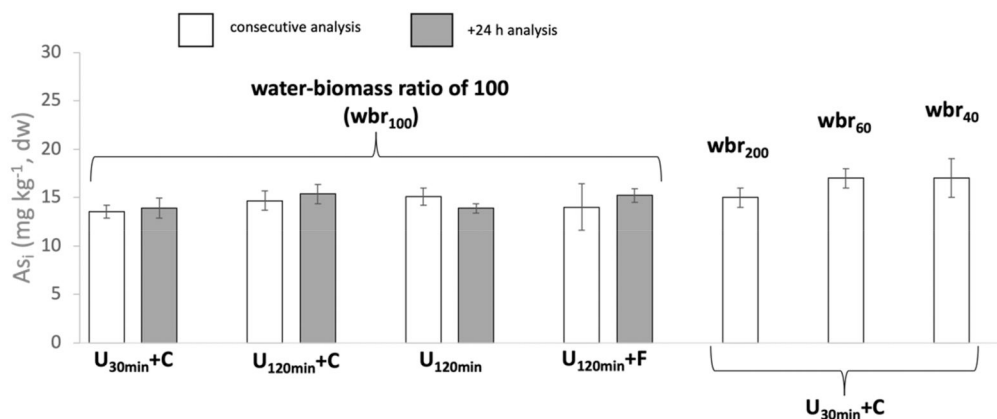
### ***As<sub>i</sub> extraction from Sargassum samples***

The choice of solvent for the As extraction from *Sargassum* powder is important. Indeed, the solvent should not modify the speciation of arsenic and acidic conditions (e.g., HNO<sub>3</sub>) must be avoided because an important part of the organic fraction of As is likely to be oxidized to As<sub>i</sub> (Narukawa, Hioki, and Chiba 2012). In most studies, water is the most recommended solvent (Rubio et al. 2010). It allows the extraction of the most polar and ionic As species including As(V) and As(III). It is worth noting that water is widely adopted by the community for the analysis of inorganic arsenic, as reported by Vanessa, Reis, and Duarte (2018). The addition of methanol in variable proportions is also possible in order to increase the solubility of the less polar species such as arsenolipids (Cui et al. 2018).

In the present study, whose objective was the analysis of the inorganic fraction, water was selected. The extraction with water can be assisted by different techniques such as ultrasound, microwave, or mechanical mixing (Rubio et al. 2010). In the context of the development of a simple method, we chose ultrasonication, whose yields are generally similar to those of microwaves (Narukawa, Hioki, and Chiba 2012). It should be noted that high temperature can accelerate the extraction of As species from seaweed (Tibon et al. 2021). However, high temperature can also decompose some As-containing components (e.g. arsenosugars) during extraction (Nakamura, Narukawa, and Yoshinaga 2008) and modify As speciation (Narukawa, Hioki, and Chiba 2012). Therefore, extraction was performed at room temperature (range 20-25 °C).

In addition to these fixed initial conditions (water, ultrasonication and room temperature), we examined the influence of four different parameters in order to optimize the extraction protocol: (1) water-biomass ratio, (2) ultrasonication time, (3) different ways to separate (or not) the liquid from the solid and (4) time between extraction and analysis.

1. Although many studies indicate that the water-biomass ratio (wbr) is not a parameter on which the solubilization of As is highly dependent (Narukawa et al. 2020; Tibon et al. 2021), we verified this statement by testing different water-biomass ratios ranging from 40 (i.e.. 0.1 g of algal powder in 4 mL of ultrapure water) to 200 on a *Sargassum* sample that had been ultrasonicated for 30 min and whose liquid phase was separated from the solid after centrifugation. Figure 2 shows that for the majority of the tested ratios, i.e. 40 ( $16.0 \pm 2.0 \text{ mg kg}^{-1}$ ), 60 ( $16.0 \pm 1.0 \text{ mg kg}^{-1}$ ), 100 ( $13.5 \pm 0.7 \text{ mg kg}^{-1}$ ) and 200 ( $14.6 \pm 0.9 \text{ mg kg}^{-1}$ ), no significant differences in As<sub>i</sub> concentration were found.
2. No significant differences in As<sub>i</sub> concentrations were observed between 30 min ( $13.5 \pm 0.7 \text{ mg kg}^{-1}$ ) and 120 min ( $14.7 \pm 1.0 \text{ mg kg}^{-1}$ ) ultrasonication time (Figure 2).
3. Centrifugation does not seem to be essential since analyses of a raw extract or analyses of a filtered extract gave similar results. However, we recommend centrifugation, because it is simpler than filtration, and because the solution obtained can be also analyzed in total As which gives access to the water soluble fraction of As (via ICP-MS measurements for example).
4. No significant differences were noted between analyses conducted immediately after extraction ( $13.5 \pm 0.7 \text{ mg kg}^{-1}$ ) or 24 h later ( $13.9 \pm 0.5 \text{ mg kg}^{-1}$ ) (Figure 2).



**Figure 2.** Analyses of the same holopelagic *Sargassum* extract using different water-biomass ratios (wbr), different ultrasonication times (U) (i.e., 30 min or 2 h) and different methods to separate (or not) the liquid phase from the solid phase (i.e., centrifugation - C or filtration - F). Analyses were performed shortly after extractions and after 24 h. All analyses were conducted after the addition of 100  $\mu\text{L}$  of extract in  $\sim 13$  mL solutions using procedure A (CASA solution,  $E_{\text{dep}} = -1.0$  V,  $t_{\text{dep}} = 60$  s). Each determination was done in triplicate with the standard addition method. Height of the bars represent the average while the error bars represent the standard deviation ( $\pm$ ) of these triplicate determinations.

Based on these different tests, a water-biomass ratio of 100 (typically 50 mg of algal powder in 5 mL of ultrapure water), an ultrasonication time of 30 min followed by centrifugation and an electrochemical determination within 24 h was thus applied on all further samples. This methodology was validated by analyzing the *Sargassum fusiforme* CRM (*Hijiki* 7405-b, National Metrology Institute of Japan) with satisfactory  $As_i$  recovery (see below).

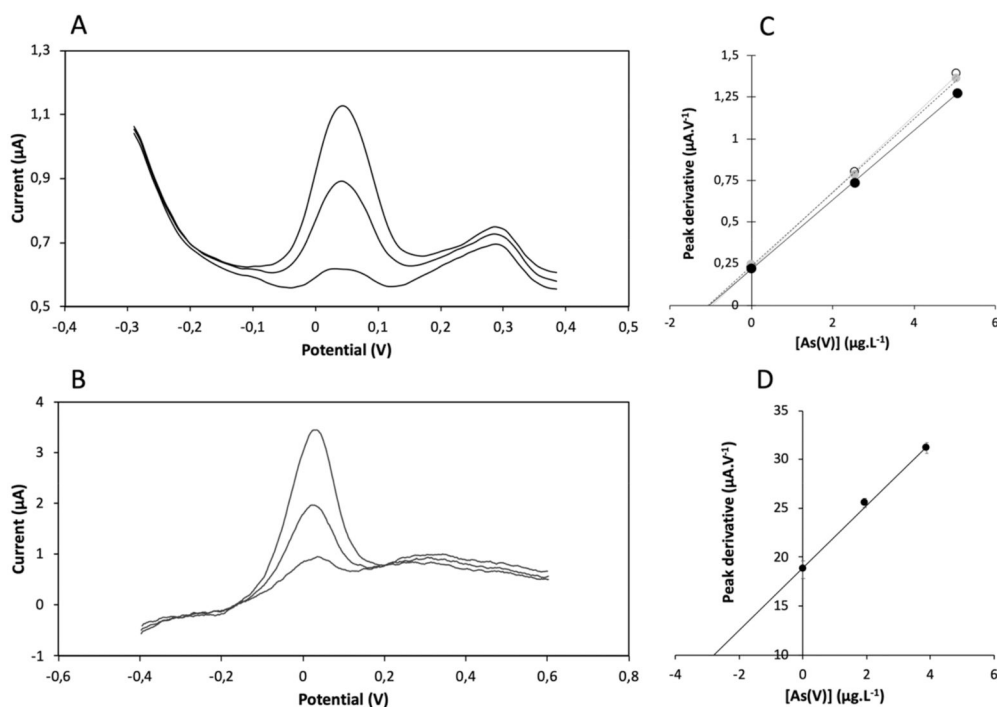
### Application to *Sargassum* extracts

#### *As<sub>i</sub>* signals in the presence of a *Sargassum* extract

Although the presence of a small volume (100 or 200  $\mu\text{L}$ ) of a *Sargassum* extract in a CASA or a CIAC solution substantially affects the shape of the voltammetric scan (Figure 3A, B), the  $As_i$  response was still well-defined and was found in the same potential range (i.e., 0–0.1 V) as compared to measurements conducted without algal extracts. Moreover, two additions of  $As(V)$  standard gave a strong linear increase for each procedure (Figure 3C,D) with similar sensitivity compared to those obtained without algal extracts. This strong linearity has been observed for all three morphotypes of holopelagic *Sargassum* (*S. natans* VIII, *S. natans* I and *S. fluitans* III).

#### Interferences

The extraction of  $As_i$  from *Sargassum* samples may also release other metallic elements or organic compounds, including organic As compounds, which could potentially interfere with the measurement of  $As_i$ . Copper (Cu) can be problematic on a gold electrode in acidic conditions (Feeney and Kounaves 2002), its stripping peak at +0.25 V possibly overlapping with that of As. However, for all samples of *Sargassum* spp. tested,



**Figure 3.** Example voltammetric determination of  $As_i$  in seaweed extracts by the method of standard addition. (A) SW voltammograms obtained with procedure A (CASA solution,  $E_{dep} = -1.0\text{ V}$ ,  $t_{dep} = 60\text{ s}$ ) in a 13.0 mL solution containing 100  $\mu\text{L}$  of a *Sargassum* (*S. fluitans* III) extract. (B) LS voltammograms obtained with procedure B (CIAC solution,  $E_{dep} = -1.2\text{ V}$ ,  $t_{dep} = 30\text{ s}$ ) in a 13.0 mL solution containing 100  $\mu\text{L}$  of a *Sargassum* (*S. fluitans* III) extract. (C)  $As_i$  response (peak derivative) as a function of  $As(V)$  additions (three independent determinations) for the solution corresponding to panel A (procedure a). (D)  $As_i$  response (peak derivative) as a function of  $As(V)$  additions for the solution corresponding to panel B (procedure B). for panel D, error bars represent the standard deviation of 4 measurements at each level.

corresponding to the three different morphotypes (*S. natans* VIII, *S. natans* I and *S. fluitans* III), the Cu peak was always sufficiently low and well separated from the As peak, in agreement with Cu levels in *Sargassum* spp. being at least one order of magnitude lower than total As levels (Ortega-Flores et al. 2022).

We also tested the potential interferences of different organic As species on  $As_i$  response. Methylarsonite (MMA), dimethylarsinite (DMA) and arsenobetaine (AB) were added at concentrations ten times in excess relative to  $As(V)$ . For DMA and AB, the  $As_i$  signal was unaffected. For MMA, a relatively important increase of about two times of the  $As_i$  response was observed. Calibration curves of MMA compared to  $As(V)$  done at low concentrations in both CASA and CIAC showed that MMA is detected in both solutions, with a sensitivity close to 50% that of  $As(V)$ , in agreement with a previous study (Salaün, Planer-Friedrich, and van den Berg 2007). The shape of the signal and the peak potential are the same as for  $As(V)$ . Determination of  $As(V)$  without any interference from MMA does not currently seem to be possible. However, this interference is likely to be largely negligible for most brown seaweeds since  $As_i:MMA$  ratio is about 300-1000 in Hijiki (*Sargassum fusiforme*) (Hirata and Toshimitsu 2007; Wolle and

Conklin 2018a), 60 in *Sargassum fulvellum* and 30 in *S. piluliferum* (Cui et al. 2018). Moreover, our HPLC-ICP-MS measurements of arsenic speciation in the three morphotypes of holopelagic *Sargassum* (*S. natans* VIII, *S. natans* I and *S. fluitans* III), showed  $As_i:MMA$  ratios all above 100. Other organic As species that could interfere with the measurement of  $As_i$  are arsenosugars. Unfortunately, there are no standards commercially available for these arsenosugars to be tested by voltammetry. The chemical structure of arsenosugars have a common structure with DMA (Francesconi and Kuehnelt 2004) and the latter being voltammetrically inert (Salaün et al. 2012), it is likely that those arsenosugars should not interfere with the voltammetric determination of inorganic arsenic.

### Validation of the electrochemical methods for $As_i$ quantification in brown seaweed extracts

The validation of the two proposed methods (procedure A or B) was carried out with different experiments. The reproducibility of the method using procedure A (CASA solution) was evaluated after eight extractions from the same powder (*S. fluitans* III) and three independent determinations of  $As_i$  in each of the eight preparations (representing a total of 24 determinations). The results gave a mean concentration of  $17.8 \pm 1.2 \text{ mg kg}^{-1}$  corresponding to a relative standard deviation (RSD) of 5% ( $N=24$ ). For the method using procedure B (CIAC solution), a similar 5% reproducibility was also obtained, albeit with a lower number of determinations ( $N=8$ ).

The  $As_i$  concentrations determined by voltammetry were compared with those obtained by HPLC-ICP-MS (Table 2) for three samples of the different morphotypes of holopelagic *Sargassum* spp. (*S. natans* VIII, *S. natans* I and *S. fluitans* III). The obtained results were in good agreement with similar standard deviations for both methods. It should be noted here that the 3 samples, collected on the same beach, display fairly similar  $As_i$  values (range 16–23  $\text{mg kg}^{-1}$ ) representing between 46 and 55% of total arsenic. If voltammetry is a reliable alternative to the chromatographic method, it is worth mentioning here that in addition to its very limited cost, the analysis of a sample by electrochemistry is much faster. In the case of a quantification based on 3 replicates of analysis carried out by standard addition, 20 min are required for the proposed electrochemical method as compared  $\sim 3$  h for conventional HPLC-ICP-MS.

**Table 2.** Comparative analysis of inorganic arsenic ( $As_i$ ) (mean  $\pm$  standard-deviation, in  $\text{mg kg}^{-1}$ ) in various holopelagic *Sargassum* spp. water extracts by using the reported electrochemical methods using a gold microwire electrode in comparison with the commonly used anion exchange HPLC-ICP-MS method.

	$As_i$ Electrochemical methods ( $n=3$ )		$As_i$ HPLC-ICP-MS ( $n=2$ )	Total As ICP-MS ( $n=3$ )	%As <sub>i</sub>
	CASA	CIAC			
<i>S. natans</i> VIII	$17.4 \pm 2.0$	$15.9 \pm 1.7$	$16.2 \pm 1.8$	$35.5 \pm 2.7$	$46 \pm 3$
<i>S. natans</i> I	$20.2 \pm 0.4$	$22.9 \pm 1.9$	$21.1 \pm 0.9$	$39.0 \pm 3.7$	$55 \pm 4$
<i>S. fluitans</i> III	$17.8 \pm 1.2$	$21.4 \pm 1.3$	$21.0 \pm 0.9$	$38.8 \pm 0.6$	$52 \pm 6$

%As<sub>i</sub> corresponds to the ratio between  $As_i$  and total arsenic, which was calculated here from the average concentration obtained with the three methods of analysis.

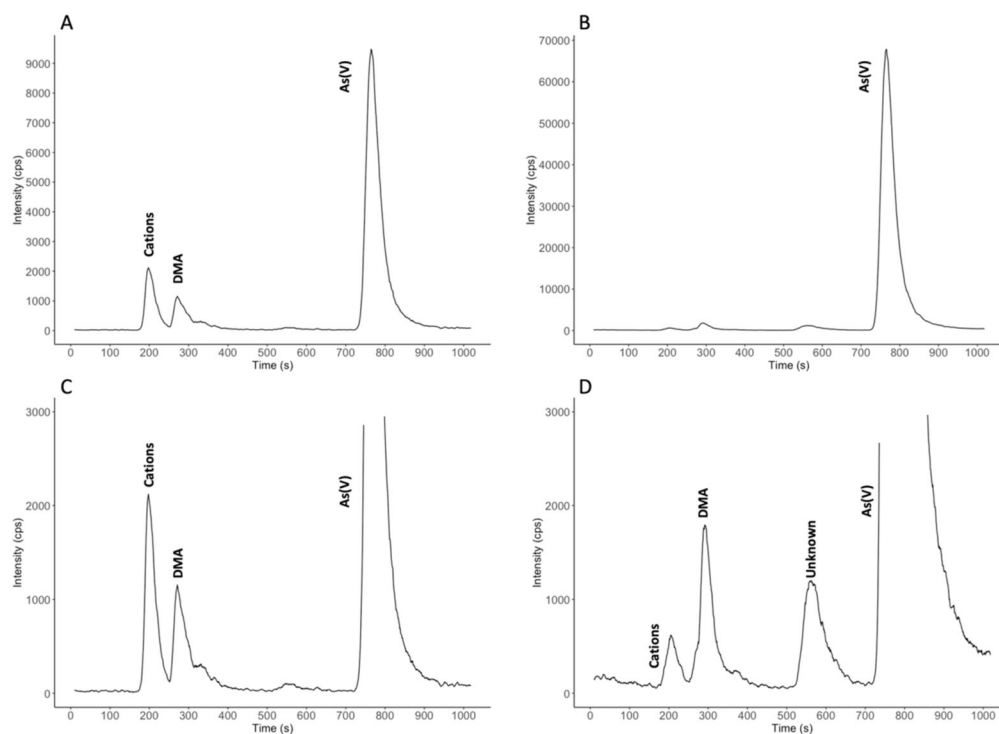
In the absence of certified reference material (CRM) for holopelagic *Sargassum* spp., the accuracy of the methods for As<sub>i</sub> quantification was evaluated on a *Sargassum fusiforme* CRM (Hijiki 7405-b, National Metrology Institute of Japan) (Narukawa et al. 2020). For the method using procedure A (CASA solution), the As<sub>i</sub> response was noisier, less sensitive (by a factor of  $\sim 2$ ) and the quantification less reproducible than the response obtained with holopelagic *Sargassum* samples. Nevertheless, the results ( $22.5 \pm 3.5 \text{ mg kg}^{-1}$ ,  $n=3$ ) were within 10% of the certified value ( $24.4 \pm 0.7 \text{ mg kg}^{-1}$ ). For the method using procedure B (CIAC solution), a more reproducible signal was found as compared to the method using procedure A. Here, the As<sub>i</sub> concentration found was  $24.3 \pm 1.7 \text{ mg kg}^{-1}$  ( $n=6$ ).

The method using procedure A (CASA) was also tested on four types of brown seaweed extracts provided by the ALGAIA company (Lannilis, France). These samples, which corresponded to intermediates of manufacturing process for food from *Laminaria digitata* and *Ascophyllum nodosum*, were analyzed and the results were compared, using a blind approach, to those obtained independently by the laboratory Phytocontrol (Rennes, France) which used a HPLC-ICP-AES method (AES: Atomic emission spectroscopy). The different results obtained with our electrochemical method (procedure A, CASA electrolyte) ( $6.2 \pm 0.7$ ,  $5.0 \pm 1.3$ ,  $5.4 \pm 0.8$  and  $<0.3 \text{ mg kg}^{-1}$  for the four different samples) were in good agreement with those obtained by HPLC-ICP-AES ( $5.8$ ,  $5.4$ ,  $6.2$  and  $<0.1 \text{ mg kg}^{-1}$ , respectively) demonstrating the potential of the electrochemical method to be used for wider applications such as As<sub>i</sub> in different types of brown seaweed extracts.

### **Arsenic speciation in holopelagic *Sargassum***

Seaweeds are known to contain high concentrations of organic As (Taylor and Jackson 2016). Arsenosugars are usually the dominant species in seaweeds, with small proportions of methylated As compounds, mostly as DMA (Rose et al. 2007). In contrast to most seaweeds, for which As<sub>i</sub> is present in small concentrations, some brown macroalgae, such as *Laminaria digitata* or *Sargassum fusiforme* can contain relatively high amounts and high proportions of As<sub>i</sub> (Almela et al. 2006; Taylor and Jackson 2016).

Figure 4 shows typical HPLC-ICP-MS chromatograms obtained for holopelagic *Sargassum*. Two samples of holopelagic *Sargassum* were analyzed: one was collected from specimens stranded on the Vauclin beach while the other was collected in the open sea. Extracts were obtained as described above, with a water/biomass ratio of 100, followed by a dilution adapted to respect the linearity range of HPLC-ICP-MS. In both cases, analysis of the extracts shows that the main arsenic species is As(V), that As(III) is below detection limit ( $< 1.6 \text{ ng L}^{-1}$  (Lindemann 2016) and that DMA can be also extracted in water. Much higher intensities of As(V) were observed in the samples collected at sea versus those stranded. This suggests that during strandings a large proportion of As(V) is released into the environment, as no other form of arsenic dominates (despite a slight increase in DMA). These preliminary results on As speciation in holopelagic *Sargassum* suggest that classical detoxification mechanisms such as biomethylation do not seem to be actively used by these algae, which can probably withstand high concentrations of As(V) by sequestering it in the cell walls. However, it should be noted



**Figure 4.** Chromatograms of anion-exchange separation of as species in (A) a stranded holopelagic *Sargassum* and (B) collected at sea. Enlarged chromatograms of (C) panel a and (D) panel B. Mobile phase, 2.6 mM  $(\text{NH}_4)_2\text{HPO}_4$ ; pH 8.2; flow rate  $1.0 \text{ mL min}^{-1}$ ; injected volume  $100 \mu\text{L}$ .

that our study presents a limited number of samples. Analyses of larger sample sets need to be conducted, with biomass collected in different environmental conditions and in different physiological status, in order to provide a better understanding of the As pathways in these brown macroalgae.

## Conclusion

A simple extraction procedure has been reported for the determination of inorganic arsenic in holopelagic *Sargassum* by voltammetric detection. Two procedures with different electrolytes were tested and best overall performance was obtained in a pH 4.7 acetate chloride containing solution (CIAC) where the signal was stable over time, allowing a standard addition procedure to be performed in the same aliquot. In the sulfamic-citric chloride containing solution (CASA), we optimized a procedure to deal with the instability of the peak with successive measurements. While being less convivial to use (standard additions had to be done in different aliquots), this procedure also provided comparable results to the CIAC, which were in agreement with those obtained by the coupling of chromatographic and spectroscopic techniques. The extraction and voltammetric procedures were validated on a *Hijiki* CRM but with better performances for the method using procedure B (CIAC solution). Our data indicate that As(V) is the main form of inorganic arsenic in holopelagic *Sargassum*, with no quantifiable amount

of As(III). We believe that the use of the integrated ScTrace gold electrode is simple enough to allow voltammetric quantification of inorganic arsenic in brown algae by non-expert and is a reliable alternative to the usual chromatographic method.

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## Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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