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

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Abstract :

The valorization of massive strandings of holopelagic Sargassum spp. is strongly limited by high levels of inorganic arsenic (Asi) that are potentially above the limit of current regulations. Monitoring Asi 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 Asi 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 Asi determination is possible with the first electrolyte but it is necessary to deal with a relative unstable signal. Measurement of Asi 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.

Keywords : Brown seaweed, holopelagic Sargassum spp, metals, inorganic arsenic, 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 et al., 2008; McSheehy and Szpunar, 2000; Rose et al., 2007). Typical As levels in oceanic water are relatively homogeneous and range from 1 to 3 $\mu g\ L^{-1}$, with an average level of 1.7 $\mu g\ L^{-1}$ (Andreae and Klumpp, 1979). In seawater, arsenic occurs mainly as inorganic As species (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 monomethylarsonite (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 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. $HAsO_4^{2-}$ and 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 et al., 2014). Although many marine algae generally convert 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 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 et al., 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 fertilisers 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 As_i levels. For example, in European countries, the maximum level of As_i is 40 mg kg^{-1} in organic fertiliser and 2 mg kg^{-1} in animal feed (EU. 2015). Despite explicit legislation on 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., 2021; 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 et al., 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_i in natural

waters (seawater, groundwater or rivers) (Mays and Hussam, 2009; Rasul et al., 2002; Tupiti et al., 2018; Vandenhecke et al., 2007). However, no analytical development has been made to determine As_i in macroalgae. If many metals (*e.g.* copper) or organic matter can potentially interfere with the electrochemical measurement of As_i, recent advances in the design of gold electrodes and experimental procedures now allow robust and low-interference measurements of As_i (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_i quantification in water (Metrohm, application bulletin 416/3) and groundwater (Eikelboom et al., 2023) were tested. Our objective here was to implement a simple method, accessible to the greatest number of users (even to non-voltammetric experts), for As_i quantification in holopelagic *Sargassum* spp.

Materials and methods

Holopelagic Sargassum spp. samples

Samples of the three different morphotypes of holopelagic *Sargassum* spp., i.e. *S. natans* VIII, *S. natans* I and *S. fluitans* III, were collected at different stations in the tropical Atlantic Ocean in June-July 2017. These samples corresponded to biomass collected at sea ("Caribbean" expedition, http://dx.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 (using a freeze-dryer (Christ, Germany)) and grounded 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^{-1}, was used to prepare all solutions. From As(V) and As(III) standards stock solutions at 1,000 mg L^{-1} (Chem-Lab, Belgium), working solutions at concentrations of about 1,000 µg L^{-1} 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 aluminium foil and regularly prepared to prevent photochemical oxidation of As(III). Sulfamic acid (H_3NO_3S, 99.3%), potassium chloride (KCl, 99.5%), potassium permanganate ($KMnO_4$) and sodium chloride (NaCl, 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The other chemicals used were citric acid ($C_6H_8O_7$, Fluka, Germany), sulfuric acid (H_2SO_4 96%, Chem-Lab,

Belgium), sodium acetate (CH_3COONa, AnalaR BDH Chemicals Ltd., England) and acetic acid (CH_3COOH 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 µm diameter, length: ~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 Autotlab potentiostat type III was used with a IME663 interface controlled by the GPES software version 4.9. A Teflon cell (~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 sulphuric acid (2.78 mL w(H_2SO_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 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 V s^{-1}, step potential of 10 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 V s^{-1}. The Au WireBond electrode was conditioned by imposing a low deposition potential (-2V, 10s) in 0.5 M H_2SO_4, followed by 3 CV scans from -0.2 to 1.5 V (100 $mV.s^{-1}$).

Voltammetric procedures for arsenic speciation

Two procedures using two different 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_i 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 M citric acid (10.51 g), 1 M sulphamic acid (9.71 g) and 0.45 M potassium chloride (3.35 g) dissolved in 100 mL of ultrapure water. This CASA solution was characterised by a pH of 1.9. Electrochemical measurements using the CASA were done in the electrochemical cell using the following proportions: 11 mL of ultrapure water, 2 mL of CASA electrolyte and 100 µL of a 0.2 mM $KMnO_4$ stock solution (final concentration 1.5 µM).

The **Procedure B** is based on the development conducted by (Eikelboom et al., 2023). The electrolytic solution (hereafter called ClAC) consisted of 0.21 M acetic acid (6 mL), 0.6 M sodium acetate (23.4 g) and 2.5 M sodium chloride (73 g) dissolved in 500 mL of ultrapure water. This electrolytic solution was characterised by a pH of 4.7. Electrochemical measurements using the ClAC were done with the following proportions: 11.8 mL of ultrapure water, 1.2 mL of ClAC electrolyte and 13 µL of a 10 mM $KMnO_4$ solution (final concentration 10 µM).

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 As_i concentration in algal biomass. For that purpose, a small volume of algal extract (200 µL 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% 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 chromatography (Model 655A-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 × 4.6 mm, 5 µm, Hamilton, Reno, NV, USA). The analytical conditions applied corresponded to a method optimised by Lindeman et al. (2016). The mobile phase was prepared with 2.6 mM of $(NH_4)_2HPO_4$ 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 ClAC) in presence of 4 µg 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 mV, was found at +42 mV after 20 cycles. On the other hand, the As signal intensity (Figure 1A) was found stable in the ClAC solution (1.05 ± 0.03 µA.V^{-1}, relative standard deviation (RSD) 3%, n = 20), and so was the peak position (shifted only from -51 to -43 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_{dep} = -0.9 V while it was over ~40% at E_{dep}= -1.1 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 µg 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 important. This strong reduction current is counter balanced by an equally strong oxidation

current at the carbon auxiliary electrode, with a production of chlorine (Cl_2, g) and hypochlorous acid (HOCl) which may lead to physical damage/blockage of the auxiliary electrode (Khairy et al., 2010; Salaün et al., 2007).On the other hand, the detection of As(V) in the ClAC + $KMnO_4$ was stable, possibly due to the higher pH resulting in much lower reduction/oxidation current at the working/auxiliary electrode.

Overall, these different signal stability experiments demonstrate that any quantification of As_i in the CASA electrolyte (using a deposition at -1.0 V for 60s) cannot be achieved with more than 6 consecutives 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 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 ClAC, 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 As_i determination

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

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

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

As_i 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_3) must be avoided because an important part of the organic fraction of As is likely to be oxidised to As_i (Narukawa et al., 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 (Reis et al., 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 et al., 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 et al., 2008) and modify As speciation (Narukawa et al., 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 optimise 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 solubilisation 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 (e.g. 0.1 g of algal powder in 2 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 40 (16.0±2.0 mg kg^{-1}), 60 (16.0±1.0 mg kg^{-1}), 100 (13.5±0.7 mg kg^{-1}) and 200 (14.6±0.9 mg kg^{-1}), no significant differences in As_i concentration were found.

(2) No significant differences in As_i concentrations were observed between 30 min (13.5±0.7 mg kg^{-1}) and 120 min (14.7±1.0 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 analysed 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±0.7 mg kg^{-1}) or 24 h later (13.9±0.5 mg kg^{-1}) (Figure 2).

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 analysing the *Sargassum fusiforme* CRM (*Hijiki* 7405-b, National Metrology Institute of Japan) with satisfactory As_i recovery (see below).

Application to Sargassum extracts

As_i signals in the presence of a Sargassum extract

Although the presence of a small volume (100 or 200 µL) of a *Sargassum* extract in a CASA or a ClAC 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, i.e. the three different morphotypes (*S. natan*s 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 ClAC 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 et al., 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 Asi: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 different extractions from the same powder (*S. fluitans* III) and then 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±1.2 mg kg^{-1} corresponding to a relative standard deviation (RSD) of 5% (N = 24). For the method using procedure B (ClAC 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 found 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 Asi values (range 16-23 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 minutes are required for the proposed electrochemical method as compared ~ 3 hours for conventional HPLC-ICP-MS.

In the absence of certified reference material (CRM) for holopelagic *Sargassum* spp., the accuracy of the methods for As_i 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_i response was noisier, less sensitive (by a factor of ~2) and the quantification less reproducible than the response obtained with holopelagic *Sargassum* samples. Nevertheless, the results (22.5 ± 3.5 mg kg^{-1}, n = 3) were within 10% of the certified value (24.4 ± 0.7 mg kg^{-1}). For the method using procedure B (ClAC solution), a more reproducible signal was found as compared to the method using procedure A. Here, the As_i concentration found was 24.3 ± 1.7 mg kg^{-1} (n = 6).

The method using procedure A (CASA) was also tested on four different 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 analysed 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±0.7, 5.0±1.3, 5.4±0.8 and <0.3 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 mg kg^{-1}, respectively) demonstrating the potential of the electrochemical method to be used for wider applications such as As_i 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_i 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_i (Almela et al., 2006; Taylor and Jackson, 2016) .

Figure 4 shows typical HPLC-ICP-MS chromatograms obtained for holopelagic *Sargassum.* Two samples of Sargassum were analysed: one was collected from specimens stranded on the beach while the other was collected in the open sea. Extracts were obtained after extraction as described in section 3.2, 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 ng L^{-1} (Lindemann, 2016) and that DMA is 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 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 developed in this work for the determination of inorganic arsenic in holopelagic *Sargassum* by voltammetric detection. Two different procedures with different electrolytes were tested and best overall performance was obtained in a pH 4.7 acetate chloride containing solution (ClAC) where the signal was stable over time, allowing a standard addition procedure to be performed in one voltammetric cell. In the sulfamic-citric chloride containing solution (CASA), we optimised 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 solutions), this procedure also provided comparable results to the ClAC, which were in agreement with those obtained by the coupling of chromatographic and spectroscopic techniques. The extraction procedure and voltammetric procedures were validated on a *Hijiki* CRM but with better performances for the method using procedure B (ClAC 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 algaes by non-expert and is a reliable alternative to the usual chromatographic method.

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Declaration of competing interest

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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Table 1. Voltammetric conditions used in CASA and ClAC electrolytes (SWV: square wave voltammetry; LSV: linear scan voltammetry)

Procedure	Electrolyte	pH	Deposition step	Stripping step	Observations
A	CASA: 0.5 M citric acid 1 M sulphamic 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	No purging required Any As(III) present is oxidised to As(V) due to addition by MnO_4^- ions
B	ClAC: 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 V s^{-1} step: 2 mV, current range: 10 µA	

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

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

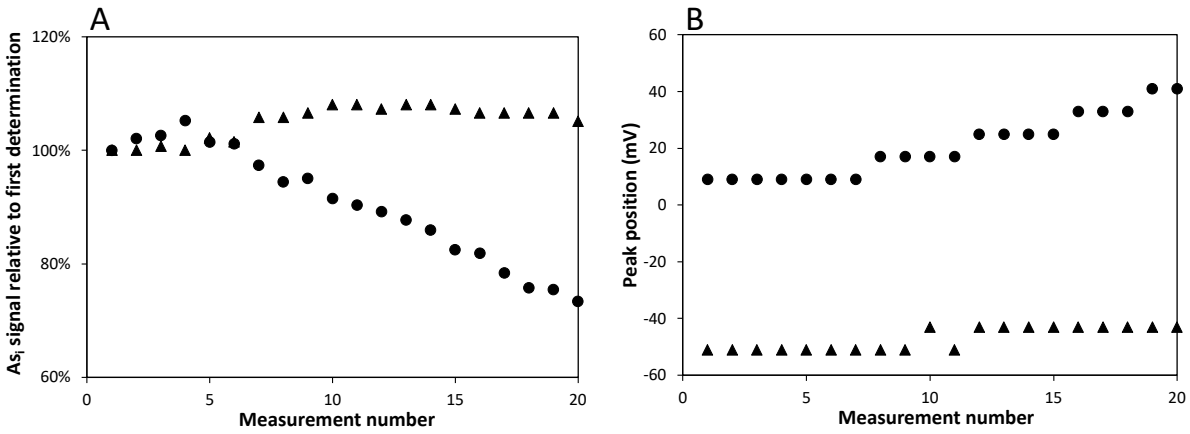

Figure 1. Evolution of the (A) voltammetric response of As_i (peak derivative) and (B) As_i peak position as a function of the number of measurements. The measurements were carried out with scTRACE Gold sensor, in CASA, procedure A (round) with 4 µg L^{-1} of As(V), E_{dep} = -1.0 V, t_{dep} = 60 s,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

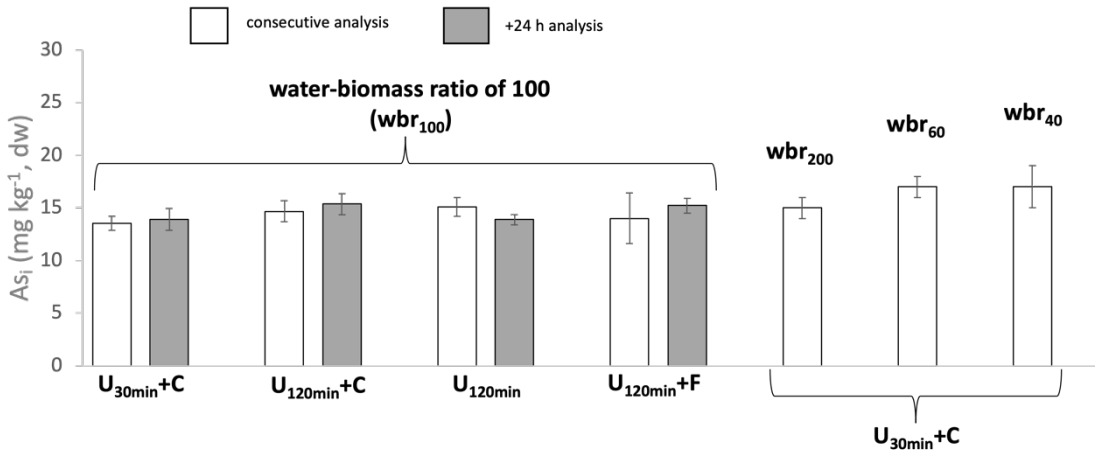

Figure 2. Analyses of the same holopelagic *Sargassum* extract using different water-biomass ratios (wbr), different ultrasonication times (U) (i.e. 30 min or 2 h) and different methods to separate (or not) the liquid phase from the solid phase (i.e. centrifugation - C or filtration - F). Analyses were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

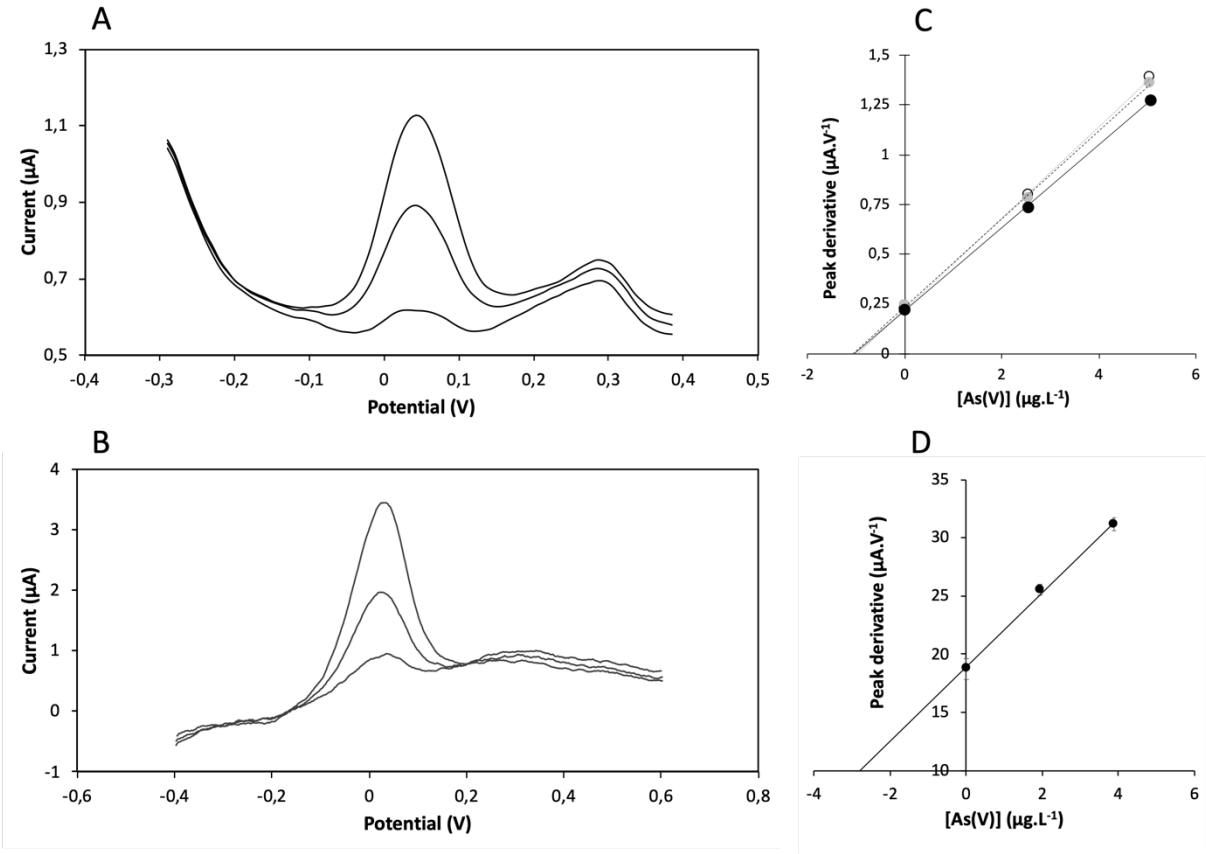

Figure 3. Example of voltammetric determination of As_i in seaweed extracts by the method of standard additions (A) SW voltammograms obtained with procedure A (CASA solution, E_{dep} = -1.0 V, t_{dep} = 60 s) in a 13.0 mL solution containing 100 µL of a *Sargassum* (*S. fluitans* III) extract. (B) LS voltammograms obtained with procedure B (ClAC solution, E_{dep} = -1.2 V, t_{dep} = 30 s) in a 13.0 mL solution containing 100 µL 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.

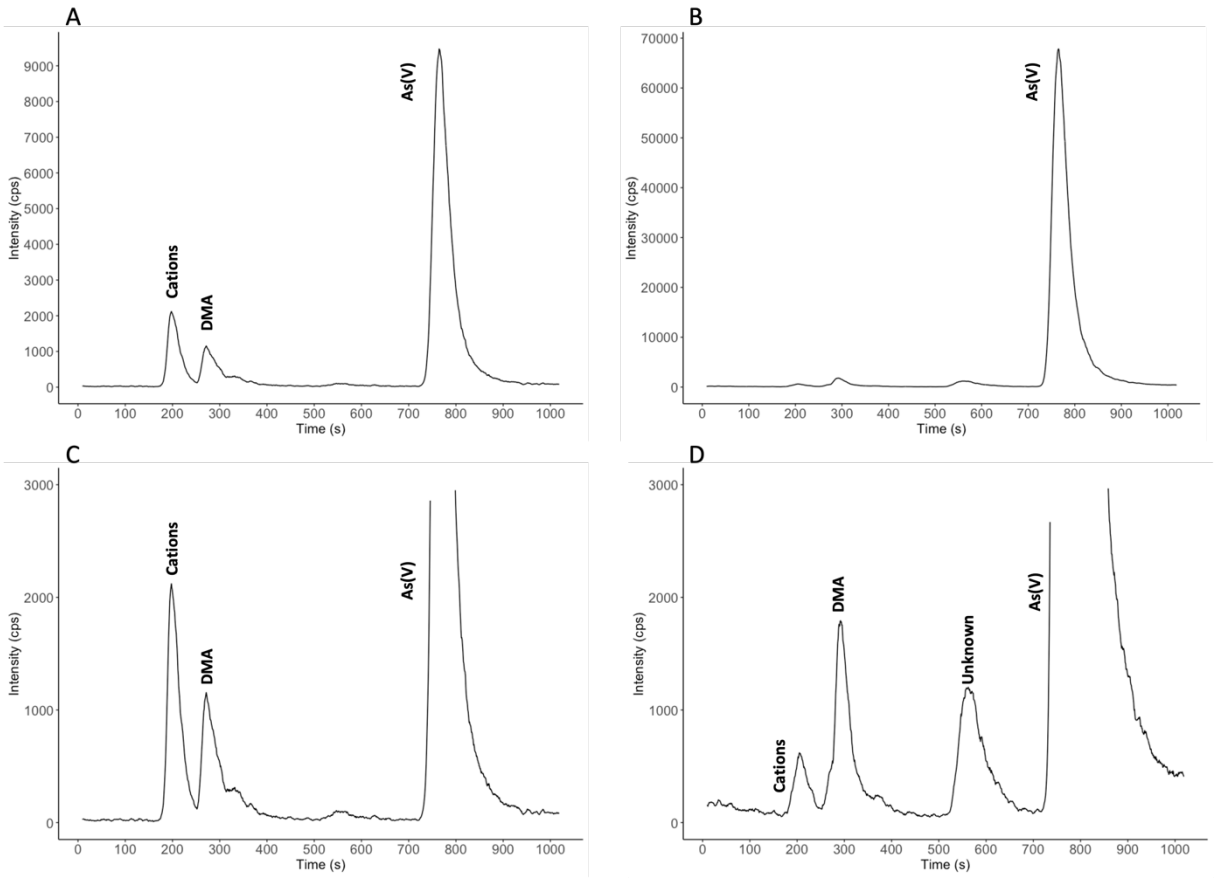

Figure 4. Chromatograms of anion-exchange separation of As species in (A) a stranded holopelagic *Sargassum* and (B) collected at sea. Enlarged chromatograms of (C) panel A and (D) panel B. Column, Hamilton PRP-X 100 (250 x 4.6 mm, 4 µm), with guard column (8 x 3 mm); mobile phase, 2.6 mM $(NH_4)_2HPO_4$; pH = 8.2; flow rate 1.0 mL min^{-1}; injected volume 100 µL.