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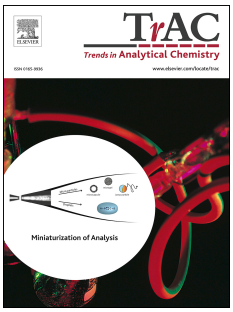

Analytical methodologies for arsenic speciation in macroalgae: a critical review

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AsO_4^{3-}
As-Sug
AsO_3^{3-}
AsO_4^{3-}
DMA
MMA

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Analytical methodologies for arsenic speciation in macroalgae: a critical review

Abstract

Marine and estuarine macroalgae are increasingly used for direct consumption, due to its high content of essential nutrients. However, the toxicity of chemical forms of arsenic (As) present in macroalgae are becoming of great concern and an important research topic.

This review will discuss the sophisticated analytical methodologies used for arsenic speciation, as well the investigation of cheaper and fit for purpose methods able to inform about the safety of macroalgae consumption. Furthermore, we will also discuss relevant speciation studies performed by various authors highlighting the methodologies and respective figures of merit for all components of As in macroalgae, not only inorganic As (iAs). Focusing on the steps prior to speciation, such as sample extraction and clean-up, it will be stressed the need to develop certified reference materials (CRMs) in order to assist in the validation of analytical methodologies for As speciation in macroalgae.

Keywords: Arsenic; arsenate; arsenite; macroalgae; speciation; toxicity

1. Introduction: aims and scope

According to FAO [1], in 2014, 28.4 million tonnes of seaweed were harvested specially for direct consumption and processed for food, but they are also increasingly used in pharmaceutical and cosmetic industries. Furthermore, marine and estuarine macroalgae are becoming sound candidates for the development of functional foods, due to its high content of essential nutrients. However, despite the associated nutritional properties, macroalgae may also contain harmful contaminants and other unwanted substances, such as arsenic (As).

Arsenic occurs in four oxidation states, –3 as in arsine gas or arsenic hydride (AsH_3), 0 as in crystalline As, +3 as in arsenite (AsO_3^{3-}), and +5 as in arsenate (AsO_4^{3-}), for a large variety of organic and inorganic As (iAs) compounds. In seawater, iAs is usually present under the form of either trivalent (arsenite; As(III)) or pentavalent (arsenate; (As(V)) [2], and these species may show toxic effects when present in food and water, even at low concentrations. The dominant organic As (oAs) species found in macroalgae are arsenosugars (As-Sug) (> 80% total As) and although these compounds are accumulated by algae in high concentrations [3], [4], there is controversy regarding their toxicity [5]. The organic methylated forms of As, monomethylarsonic acid (MMA; $CH_3AsO(OH)_2$) and dimethylarsinic acid (DMA; $(CH_3)_2AsO(OH)$),

have toxicity associated and occur as a minor fraction of As in macroalgae. The As compounds most commonly found in macroalgae are shown in Figure 1, as well as the As-Sug, differentiated by end groups containing glycerol (OH), phosphate (PO_4), sulfonate (SO_3), and sulphate (SO_4).

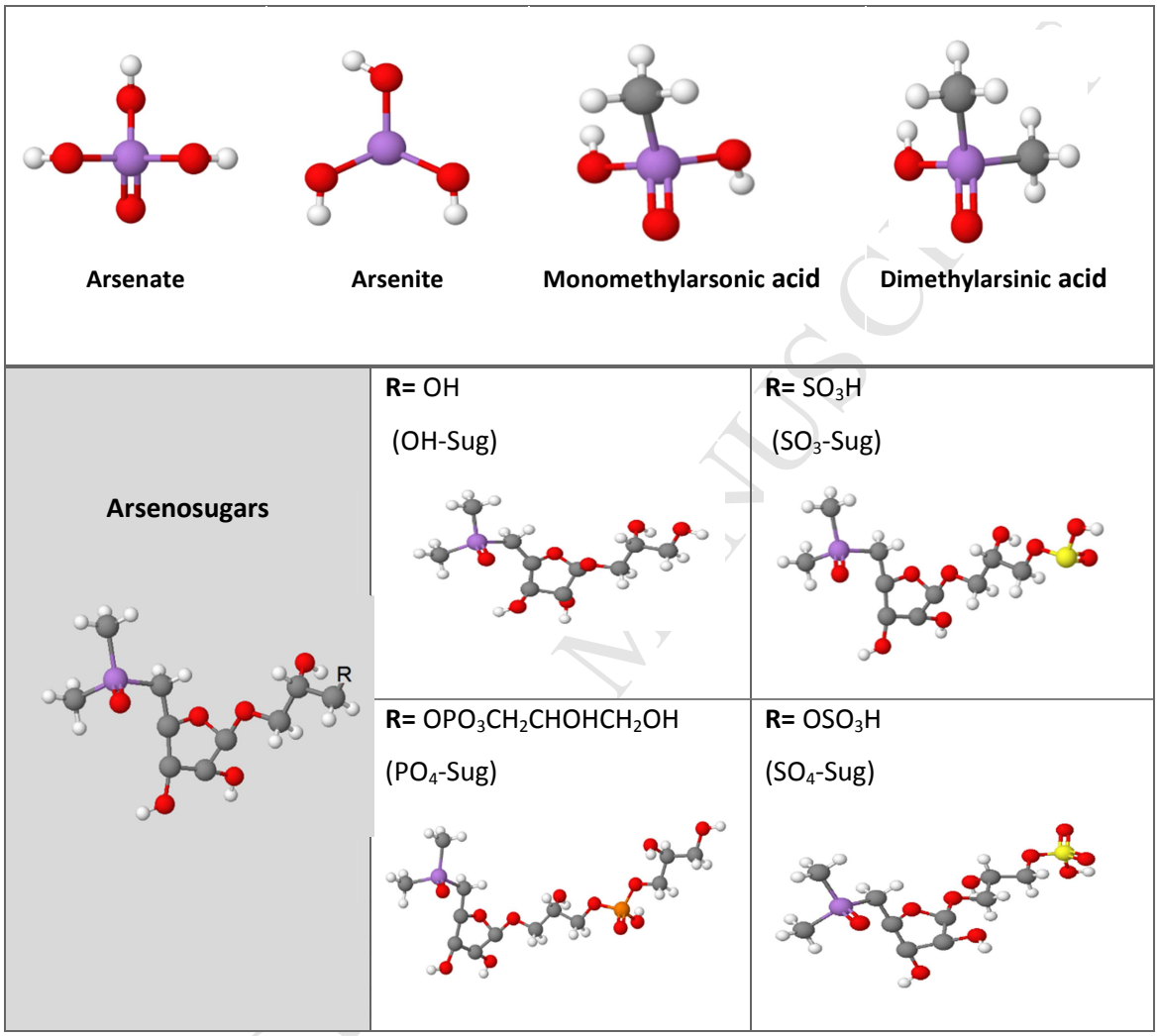

Figure 1*:* Structures of the most common As compounds in macroalgae. (obtained from http://molview.org/)

The occurrence of high concentration of total As in macroalgae was first reported by Jones [6] and later confirmed by Edmonds and Francesconi [7], who attempted, for the first time, the chemical speciation of As in macroalgae. This verdict associated with the ever increasing of macroalgae consumption triggered the alarm for the possibility of macroalgae to contain high concentrations of this metalloid. In 1979, Maugh [8] has attempted to explain the processes influencing As bioaccumulation in macroalgae and they have shown that the principal mechanism responsible for bioaccumulation is the competition between AsO_4^{3-} and PO_4^{3-} for absorption into the cells of algae. Arsenate is a chemical analogue of phosphate, in size,

geometry, and ability to enter biochemical reactions thus facilitating its entry into the cells by transport mechanisms, unable to discriminate between PO_4^{3-} and AsO_4^{3-} [9]. However, according to the most recent literature, the accumulation rate depends not only on the availability of As but also on biological, chemical, and environmental factors. Biotic and abiotic parameters can influence the bioavailability and bioaccumulation of As in algae [10], [11], [12].

Marine environment naturally contains 1 to 5 µg As L^{-1} [13]. However the ability for bioaccumulation in macroalgae, allows the As storage to become many times larger than those baseline values found in the environment. According to Francesconi and Edmonds [14] macroalgae obtain As from seawater as arsenate, possibly due to the incapacity to distinguish it from the essential macronutrient phosphate [8]. Consequently, some macroalgae accumulate large quantities of iAs which are after metabolized by the algae to a variety of organoarsenic species, especially As-Sug [14].

Due to the different toxicity of chemical forms of As, the levels present in macroalgae are nowadays of great concern and investigation. Thus, the accurate identification of As species, through studies of speciation, has become one of the most important issues in order to evaluate the risk to human health associated with the consumption of algae. Speciation can be considered as the determination of the concentration of various chemical forms of an element in a matrix, and these species, together, constitute the total concentration of the element in the sample [15]. The mobility, bioavailability, toxicity, and even biological metabolism of As depend on its chemical forms and respective structure. Therefore, the development of analytical techniques for the selective determination of As compounds in algae matrix, becomes of extreme importance for assessing the effects and for the introduction of regulations associated with the consumption of algae and algae derived products.

The methods available for the speciation of chemicals in environmental and biological matrices are generally a combination of separation and detection techniques. The analytical methodologies (both separation and detection) for As speciation have been reported in various works for several decades with only minor improvements now and then. Separation of As species was first reported by Braman and Foreback [16], in 1973, based on the differences of boiling points of the different As species to form arsine. The development of chromatographic techniques, namely, gas chromatograph (GC), in 1975, by Talmi and Bostick [17]; high-performance liquid chromatography (HPLC), in 1977, by Brinckman et al. [18]; and, capillary zone electrophoresis (CZE), in 1992, by Morin et al. [19] led to a research boom in speciation studies. However, despite all the research potential of those analytical techniques, HPLC has been much more preferred than CZE and GC for separation of As species in macroalgae, mainly to problems of interfacing aqueous samples with different detection

systems. The detection techniques generally used are atomic spectrometry and mass spectrometry. The atomic spectrometric techniques most commonly used are hydride generation coupled to atomic absorption spectrometry (HG-AAS) [20], hydride generation coupled to atomic fluorescence spectrometry (HG-AFS) [21], and inductively coupled plasma atomic emission spectroscopy (ICP-AES)[22], while the mass spectrometric most commonly used are inductively coupled plasma mass spectrometry (ICP-MS) and electrospray mass spectrometry (ESI-MS) [23], [24]. Despite the very large differences in performance and cost, since 1988, the most widely used analytical method for arsenic speciation is high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) [25].

Regardless of the increasing sophistication in the analytical methods employed in As speciation, nowadays it is fundamental to advance strategies to guarantee the quality of results, including new developments and optimization in the steps that involve sample collection, sample processing, extraction, and clean-up of the extract. Although Quality Control (QC) and Quality Assurance (QA) are the support to ensure the reliability of the laboratorial work, such strategies have not been well developed to support the quality in the As speciation in algae matrix. The two most recent reviews [26] [27], published in 2017, emphasized essentially the need for assessment of actual iAs content in food and highlighted both the most common analytical methods in the As speciation and the gaps associated with the lack of legislation for iAs in food. Furthermore, as pointed by EFSA [28], in order to reduce the uncertainty on dietary exposure to iAs, more analytical data on actual iAs are needed, rather than obtaining such data by conversion factors from total As (tAs) content.

This review paper will focus only on macroalgae, including the separation and detection techniques used in As speciation, with particular focus on matrix clean-up that is often omitted from published scientific works. Furthermore, this review highlights both the less sophisticated and the most advanced analytical techniques, keeping in mind also both their purpose and their scope. There is also an emphasis on the need to develop Certified Reference Materials (CRM) to assist in the validation of analytical methods, especially in complex matrices, such as macroalgae.

2. Speciation and toxicity in algae

In general, the toxicity of As compounds depends on several factors such as oxidation number, physical state, particle size, rate of absorption into cells, and rate of elimination [29].

Usually the lower the oxidation number the higher the toxicity, and the higher the methylation the lower the toxicity, thus producing the following order of decreasing toxicity: arsenite > arsenate > monomethylarsonic acid > dimethylarsinic acid [30].

The As compounds are also toxic to humans and the effects depend primarily on the chemical specie, dose, and duration of exposure. Humans are directly exposed to various forms of As, mainly through food and water. Although the As bioavailability depends also on the type of matrix in which it is ingested, the daily intake is considered to be about 20-300 µg for this type of exposure [30].

International Agency for Research on Cancer (IARC) has classified iAs compounds as carcinogenic to humans (Group 1), while DMA and MMA are considered possibly carcinogenic to humans (Group 2B) [31].

The Commission of the European Communities by the Commission Directive 2003/40/EC of 16 May 2003 establish that the threshold level of total arsenic in drinking water is 10 µg L^{-1} [32], while the limit values for different As species has not been established. This limit refers only to total arsenic, which makes a compelling need for regulation based on the individual arsenic compounds. Moreover, there are no general accepted limits for algae based products. However, since the analysis of iAs is reliable for rice and rice based products, maximum levels were delimited and the European Union (EU) introduced regulations of 0.2 mg kg^{-1}, expressed as As, for iAs in white rice and 0.1 mg kg^{-1} for iAs in rice-based foods aimed at infants and children[33]. In 2009, the Scientific Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA) identified a range of benchmark dose lower confidence limit values between 0.3 and 8 µg kg^{-1} body weight per day for iAs species, but this range still is under revision [33].

There are a few regulations of maximum levels allowed in edible algae. France was the first and only European country to lay down specific regulations on the consumption of seaweed, stipulating maximum values of 3 mg kg^{-1} for iAs [34], while Australian and New Zealand [35] accepts a maximum level of 1 mg kg^{-1} edible seaweed. However, besides setting up worldwide limits for iAs, there is an emerging need to establish specific regulations for the relevant species of As by performing more studies to increase the availability of speciation data and risk assessment associated with consumption of As contaminated macroalgae.

3. Challenges in developing analytical methods for arsenic speciation

In order to ensure the reliability of analytical results with a view to their application it becomes necessary to take into consideration some issues in the following different steps of the methodologies for speciation: sample collection and processing; extraction; clean-up; separation; detection; and quantification of As species. Figure 2 shows schematically the procedure for As speciation in macroalgae and highlight the principal methods for each step while Table 1 and Table 2 includes an overview of procedures applied in the latest works in speciation.

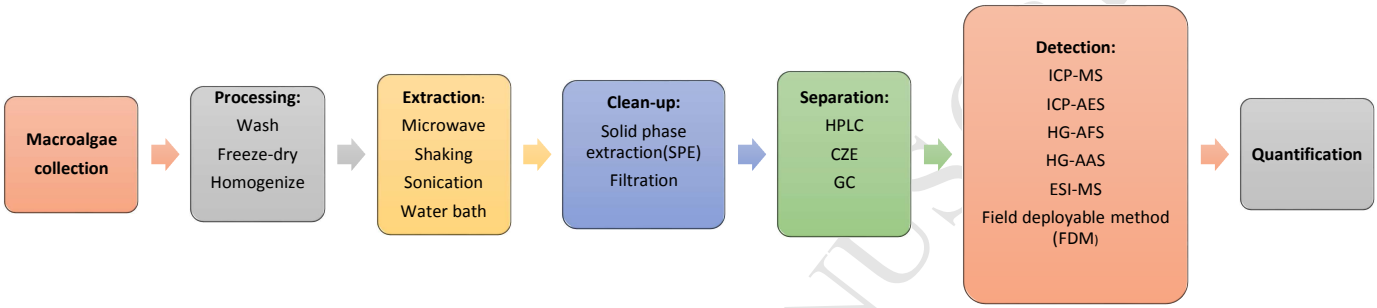

Figure 2: General analytical procedure for arsenic speciation in macroalgae

3.1. Highlighting the role of sampling and sample processing

The setup of procedures for sampling, processing and extraction is extremely important for the interpretation of results and there were well reviewed by Rubio et al. [36]. Sampling procedures often involve a risk of contaminating the sample itself because the transportation and storage of samples involve risks of overheating or freezing, contamination, and chemical changes of matrix.

The first step, sample processing, includes the removal of epiphytic communities living in symbiosis with algae and other substances, like sludge, that might interfere in further stages of the analytical methodology. Sample washing is often performed either with deionized and ultra-pure water or saline solutions similar to the medium where the samples grew. Many studies [37], [11], [38] report high concentrations of arsenobetaine (AB) in macroalgae, attributing the presence of this compound to the presence of epiphytes that were not correctly removed from the macroalgae during the washing process. According to Llorente-Mirandes et al. [11], despite the removal of visible epiphytic material, microorganisms, and microparticles remain in the samples. In addition, these authors highlight the importance of further studies in order to understand whether these microorganisms either accumulate AB or transform arsenosugars into AB.

After the algal material cleaning, freeze-dried and thermal treatments are the two main options used for removing water from samples, followed by homogenization until obtaining a powder. According to Michalke [39] and Rajakovic et al. [40] problems such as stability, contamination or loss of samples, are frequent and they need to be well thought of and solved in advance. Salgado et al. [41], investigated the stability of total arsenic, and iAs in *Sargassum fulvellum* and *Hizikia fusiformis*, and the results suggested that samples remained stable for at least a period of 12 months, stored in polystyrene containers at +20 °C, without showing any degradation of the analytical signals. This information becomes essential when it comes to establish the most suitable preservation conditions to ensure the accuracy of the analytical results.

3.2. Highlighting the extraction of arsenic species

The extractants most widely-used in the extraction process are water, methanol (MeOH), and mixtures of MeOH:water, although dilute solutions of nitric acid (HNO_3) or chloroform are also used. Water has been considered, up to now, the best solvent due to the polar nature of the As species, although as a soft extractant water cannot extract all As due to the presence of high concentrations of arsenolipids in some macroalgae species. As referred by Francesconi [42] and Han et al. [43], the use of severe extraction conditions and the application of high temperature could extract all As species and accelerate the process, but running the risk of decomposing some of the As compounds. Taylor et al. [44], report that most stability problems are associated with arsenite, because it is easily oxidized to arsenate, which may explain the high concentrations of arsenate in the speciation results while the arsenite sometimes goes undetected [43], [11]. Salgado et al. [41] studied the stability of total As and arsenate in *Sargassum fulvellum* and *Hizikia fusiformis* extracts and concluded that the Sargasso extract remain stable during 15 days at −18 °C while Hijiki extracts are stable for 10 days at +4 °C. In a similar report, Salgado and Quijano [21], studied the stability of *Hizikia fusiformis* after water extraction and concluded that the As species remained stable for 7 days under all storage conditions tested between +4 and -18 °C.

As reviewed by Rubio et al. [36], although this step is crucial for As speciation in macroalgae, it can vary significantly, for the following aspects: type of extractant and time of contact with the sample; ratio between extractant volume and sample mass; and method used in the extraction. As shown in Table 1 and Table 2, although the extraction yields were generally quite low for the different species of macroalgae, no extraction protocols have been further developed and optimized from 2010 onwards. The highest extraction percentages

were obtained by Narukawa et al. [45] and Salgado et al. [46], through aqueous extraction by ultrasound (72-93%) and microwave-assisted (49-98%), respectively. In spite of the above-mentioned aspects, the taxonomic differences between green, brown, and red algae play a decisive role to obtain higher percentages of extraction, as well as reliable results. Thereby, extraction process should involve the optimization of the extraction method for each species of macroalgae justifying a need for further research efforts in this direction.

3.3. Highlighting the clean-up of sample extracts

Besides the extraction process crucial importance, literature often ignores the relevance of clean-up of sample extracts prior to the injection into the HPLC system. This is, probably, a more crucial step in the whole analytical process because residual matrix components that are introduced to the column along with the analytes can be problematical and interfere heavily with both the separation and the detection steps, thus compromising the results. The extract clean-up procedure is particularly difficult for the biological samples, like macroalgae. The organic matter due to the presence of significant amounts of lipids, polysaccharides, proteins, pigments, and salt in the sample matrix can result in some deleterious effects, such as blockage, causing drastically shortening of overall life of the chromatographic column and loss of resolution between the chromatographic peaks.

Choi et al. [47], in order to achieve a cleaner extract, free of pigments, and to preserve the integrity of the chromatographic system, incorporated a single clean-up step in the extraction procedure by passing the final extract through SPE syringe cartridges with 50% (v/v) MeOH in 1% HNO_3. After clean-up, those authors verified that most of the seaweed pigments were removed and the eluate was clearer, concluding that this method provided a quick and suitable clean-up for the crude seaweed extract with good recoveries (104-120%). In a study of characterization and quantification of As-Sug in the CRM NMIJ 7405-a (Hijiki), Glabonjat et al. [48], showed that the data from HPLC- Electrospray Mass Spectrometry (ESMS) were strongly matrix-dependent. Thus, a cleanup step was incorporated, with a small silica column and the column was washed with MeOH/acetone (v/v) containing 1% formic acid, and then MeOH to remove matrix components. Moreda-Piñeiro et al. [49], in a study about pre-treatment for the As speciation in seafood, considered the use of a clean-up procedure based on C_{18} for preventing polar substances and salts to reach the analytical chromatographic column during the analysis of extracts, and verified that C_{18} produced the highest extraction efficiencies, with recoveries around 100%.

Low et al. [50], documented the presence of high chloride concentration in urine and seawater samples. They verified notorious consequences in the separation of As species by ion chromatography, leading to the splitting of a single peak into two or more discrete peaks, as a result of the large differences in the ionic strengths of the mobile phase and injected volume of sample. The authors concluded that the peak splitting can be minimized by inclusion, in the mobile phase, of a column surface modifier, di-n-butylamine phosphate. This modifier enhances the selectivity of As speciation by anchoring positively-charged amine molecules on the column surface, then allowing the modified surface to accommodate a larger concentration of anions.

Although there are few studies about the clean-up of macroalgae extracts, SPE is the method most used for this purpose, being also widely used in biological, clinical, and environmental sample cleaning. A detailed description of the effects of several variables in the clean-up step would most welcome for avoiding or even removing the analytical artifacts which may be found in the subsequent steps of the analytical process.

3.4. Separation of arsenic species

Unlike detection and quantification of total elemental As concentration, speciation involves a separation of individual chemical species. Comprehensive reviews on separation methods applied to biological tissues and environmental samples are available from McSheehy et al. [51] and Leermakers et al. [52]. The method most employed is HPLC due to the ease of coupling it with several detection systems, such as, HG-AFS, HG-AAS, ICP-AES, and ICP-MS and the vast majority of studies use an anion exchange PRPX-100 column to separate the anion species (As(III), As(V), DMA, MMA and As-sug: SO_3-Sug, SO_4-Sug, PO_4-Sug) with carbonate or phosphate as mobile phases. Less frequently, cation exchange chromatography can also be used for species, such as AB and OH-Sug, with pyridine as mobile phase.

Although capillary electrophoresis (CE) has not been frequently used for As speciation in macroalgae, Niegel et al. [53], developed a method for fast As speciation in marine macroalgae, using a CE coupled to electrospray ionization time-of-flight mass spectrometry (CE-ESI-TOF-MS). Separation was achieved by using short fused silica capillaries under high electrical field strength, and with TOF-MS was possible to verify the presence of the analytes without the need for standards due to the high mass accuracy provided by such an instrumentation. The authors obtained fast separations (about 2 min) by CE-ESI for four organic As species, namely OH-Sug SO4-Sug, AB, and arsenocholine (AC), despite AB and AC are not common in macroalgae. This technique is advantageous when the amount of sample is

low because allow injection volumes in the nL range. However, no results for iAs compounds were obtained, and according with authors is necessary a further optimization of the method in order to include all As species.

3.5. Detection and quantification of arsenic compounds

Regarding the detection and quantification of As, in the late 1980s emerges the major advance in the analysis of As compounds when the ICP-OES was introduced as detector for HPLC [54]. Later on, the ICP-MS coupled with HPLC became the most popular technique used because it is more efficient and provides very low quantification and detection limits, and high selectivity for several species of As, besides avoiding several issues associated with matrix effects. However, the major disadvantage associated with this technique is the high costs of instrumentation, maintenance, and operation for routine analyses, leading many companies to attempting to find alternative and suitable methods for As speciation.

The combination of HPLC either with HG-AAS or HG-AFS is increasingly gaining prominence giving such instrumentation being considered simple, fast, and relatively inexpensive for speciation of As. The main difference between HPLC-HG-AAS and HPLC-HG-AFS is the sensitivity of the method: the first attains detection limits in the order of μg L^{-1}, which sometimes are not low enough to detect the As species present in biological samples, while the second can reach limits of detection below μg L^{-1}, similar to ICP-MS detection [55].

As previously referred, speciation is usually associated with a previous separation step of the different species. However, there are methods based on direct measurements, without previous chromatographic separation, that have been widely used and are useful, in the determination of iAs species for purposes of food safety, although providing only a very limited speciation (iAs).

According to Welz [55] for samples containing only arsenite and arsenate the chromatographic separation is not necessary and the determination of these components can supposedly be performed only by HG-AAS. This technique is assumed to be efficient and consists on the determination of total inorganic arsenic (As(III) + As (V)) after pre-reduction of arsenate to arsenite generally with thiourea, ascorbic acid, or L-cysteine, while the determination of As(III) is conducted generally using hydride generation in controlled conditions, namely, HCl and $NaBH_4$ concentrations in order to inhibit the reduction of arsenate to arsine. Finally, the As(V) concentration is determined by the difference of both measurements. However, although this technique is accepted by various researchers [20] [56][12] , it is extremely laborious and the reaction conditions must be well controlled besides needing high amount of sample.

Bralatei et al. [57] developed recently a field deployable method (FDM) for determination of arsenate based on the Gutzeit [58] reaction:

$$2H_3AsO_4 + 2H_3O^+ + 2NaBH_4 \rightarrow 2AsH_3 + 2B(OH)_3 + 4H_2O + 2Na^+ \quad (1)$$

$$AsH_3 + HgBr_2 \rightarrow H_2As{-}HgBr + HBr \quad (2)$$

The method can be used either for speciation of iAs without separation or for determination of total iAs and allowed, in one hour, the determination of iAs in macroalgae with the aid of a field kit. Basically, this method consists in reduction of iAs present in samples to the corresponding volatile species (AsH_3) using sulfamic acid (H_3NSO_3) and sodium borohydride ($NaBH_4$). Arsine gas reacts with mercury bromide ($HgBr_2$) impregnated filter paper and AsH_3 is confined in the test paper giving a coloration proportional to iAs concentration (light yellow to dark brown), as a result of HgBr being replaced by AsH_2 group as shown in equation (2). The concentration range of iAs is obtained by comparison with a pre-existent color chart or by inserting the test paper in a digital photometric reader. Bralatai et al. [57] used the FDM to determine iAs concentration in macroalgae with an associated error of ± 19 %, when compared to HPLC-ICP-MS, thus showing that the methods usually available in laboratories for the determination of iAs are more precise and accurate than FDM. This kit can only provide an approximate estimate of the concentration of iAs from a predetermined color chart and the analyst must be aware that the test paper impregnated with a mercury compound may also pose some toxicity issues and safety precautions must be taken. However, this portable field kit shows a high potential as a tool for macroalgae monitoring *in situ*, allowing to check whether the concentration of iAs is above or below the regulated values set up by Australian, New Zealand and France for algae-products.

In addition to the importance of iAs due to its high toxicity, it is also crucial to know the concentration of other As species in the macroalgae, namely As-Sug. Niegel at al. [59] have overviewed the analytical methods for the determination of As-Sug, and the ESI-MS ionization technique associated with its improvement by the use of tandem mass spectrometry (MS/MS), was considered an advance for the quantification together with structural information of As-Sug, thus overcoming the issues associated with the co-elution with other species present in macroalga extracts.

Table 1: Overview of results and figures of merit of the analytical methods (including a separation step) applied for arsenic speciation in macroalgae (relevant works from 2007)

Algae, CRM, LOD and LOQ	As species (mg kg $^{-1}$)						% Extraction	Extraction method	Separation method	Detection method	Ref.
	tAs	As(III)	As(V)	MMA	DMA	AsSug					
Cladophora glomerata	18	0.62	–	0.05	0.35	(PO_4-Sug): 0.17 (OH-Sug): 1.70	16	0.01-0.02 g of sample MeOH: H_2O Sonication	HPLC, AE Shiseido, CAPCELL PAK C18 MGII	ICP-MS	[60]
NIES CRM No. 9 Sargasso tAs:115 ± 9	128 ± 4.4	–	–	–	–	–	–				
LOQ (ug L^{-1})	–	0.25				0.49	–				
Lessonia nigrescens	119 ± 1	105 ± 8	18.5 ± 2.0	<0.01	<0.01	<0.01	99	0.1 g of sample 10 mL of H_2O Shaking	HPLC, AE Hamilton, PRP-X100 HPLC, CE Agilent, Zorbax 300-SCX	ICP-MS	[61]
Durvillaea antarctica	15.7 ± 0.2	0.304 ± 0.090	0.117 ± 0.014	<0.01	0.10 ± 0.001	(PO_4-Sug): 0.095 ± 0.004 (SO_3-Sug): 6.13 ± 0.11 (OH-Sug): 2.53 ± 0.12	69				
NIES CRM No. 9 Sargasso tAs:115 ± 9	117 ± 2	<0.01	68.5 ± 6.6	<0.01	1.69 ± 0.16	(PO_4-Sug): 2.22 ± 0.36 (SO_4-Sug): 8.29 ± 0.54 (SO_3-Sug): 1.96 ± 0.50 (OH-Sug): 1.48 ± 0.13	86				
Sargassum sp. (12 samples)[a]	65.3 ± 0.6 - 90.3 ± 1.6	–	15 - 35	–	0.6 - 1.8	–	–	0.2 g of sample MeOH : H_2O Microwave-assisted	HPLC, AE Hamilton PRP-X100	ICP-MS	[43]
LOD (µg L^{-1})	–	0.4	0.6	0.7	0.6	–	–				
Caulerpa racemosa	0.77	<0.05	<0.05	<0.05	<0.05	–	–	–	HPLC	ICP-MS	[62]
Caulerpa taxifolia	0.77	0.14	0.12	<0.05	<0.05	–	–				
Sargassum sp.	8.68 ± 0.25	0.20	4.63	<0.2	0.16	–	–				
LOD (µg g^{-1})	0.05-0.2										

Laminaria digitata	41 ± 19	20 ± 1		–	–	–	–	0.5 g of sample Extraction solution ($0.1M\ HNO_3 + 3\%\ H_2O_2$) Water bath	HPLC IonPAC AS7	ICP-MS	[63]
Saccharina latissima	43 ± 0	0.39 ± 0		–	–	–	–				
NMIJ 7405-a (Hijiki) tAs:35.8 ± 0.9 As(V): 10.1 ± 0.5	–	–	9.5 ± 0.1	–	–	–	–				
LOD (μg g^{-1})	–	0.06	0.01	–	–	–	–				
Laminaria digitata[b]	36 ± 0.33 - 131 ± 3.1	2.2 - 87		–	–	–	–	–	HPLC Hamilton, PRPX-100	ICP-MS ESI-MS	[23]
Ascophyllum nodosum[b]	38 ± 6.4 - 111 ± 2.5	0.007 - 0.703		–	Detected by ESI-MS	Detected by ESI-MS	–				
LOD (μg g^{-1})	0.002	0.001		–	–	–	–				
LOQ (μg g^{-1})	0.02	0.01		–	–	–	–				
Hijiki[c]	–	0.08 - 0.79	8.94 - 70.52	–	0.16 - 2.72	(PO_4-Sug): 0.04 - 0.09 (SO_3-Sug): 0.12 - 0.56 (SO_4-Sug): 1.79 - 18.10 (OH-Sug): 0.32 - 1.50	As(III), As(V), DMA: 24.5 - 60.1 As-Sug: 3.7 - 27.6	–	HPLC	ICP-MS MS/MS	[64]
LOD (μg L^{-1})	–	0.8	1.1	0.2	0.3	–	–				

Cladophora prolifera	13.9 ± 0.8	<LOD	0.83 ± 0.03	<LOD	0.07 ± 0.01	(OH-Sug): 3.84 ± 0.05	53.4	0.1 g of sample 10 mL of H_2O Shaking	HPLC, AE Hamilton PRP-X100 HPLC, CE (Zorbax-SCX300))	ICP-MS	[11]
Enteromorpha compressa	6.2 ± 0.2	<LOD	0.14 ± 0.01	<LOD	0.03 ± 0.01	(OH-Sug): 1.56 ± 0.07	46.7				
Ulva rigida	5.3 ± 0.8	<LOD	0.11 ± 0.01	<LOD	0.07 ± 0.01	(PO_4-Sug): 0.06 ± 0.01 (OH-Sug): 0.84 ± 0.01	45.6				
Codium effusum	20.4 ± 0.7	<LOD	0.60 ± 0.03	<LOD	<LOD	(PO_4-Sug): 0.33 ± 0.06 (SO_4-Sug): 1.30 ± 0.13 (OH-Sug): 5.12 ± 0.16	69.4				
Codium vermilara	27.0 ± 2.3	1.36 ± 0.13	4.32 ± 0.23	0.22 ± 0.01	0.44 ± 0.01	(OH-Sug): 0.69 ± 0.03	47.4				
Halopteris filicina	9.4 ± 2.3	0.20 ± 0.09	1.50 ± 0.07	0.73 ± 0.04	<LOD	(SO_3-Sug): 1.45 ± 0.04 (OH-Sug): 0.99 ± 0.05	55.6				
Halopteris scoparia	9.1 ± 3.4	<LOD	0.87 ± 0.07	<LOD	0.09 ± 0.03	(SO_3-Sug): 2.48 ± 0.32 (OH-Sug): 1.28 ± 0.07	68.9				
Cystoseira mediterranea	39.0 ± 1.2	0.49 ± 0.19	1.17 ± 0.06	<LOD	0.26 ± 0.03	(PO_4-Sug): 0.39 ± 0.04 (SO_3-Sug): 19.6 ± 0.2 (OH-Sug): 2.21 ± 0.21	88.6				
Alsidium corallinum	11.0 ± 0.1	<LOD	1.25 ± 0.02	<LOD	0.15 ± 0.01	(PO_4-Sug): 0.13 ± 0.05 (SO_3-Sug): 4.64 ± 0.30 (OH-Sug): 0.84 ± 0.06	85.8				[65]
Jania rubens	2.0 ± 0.1	0.04 ± 0.01	0.12 ± 0.01	<LOD	<LOD	(OH-Sug): 0.50 ± 0.02	52.3				
Fucus serratus[d]	–	<LOD	<LOD	<LOD	0.01 ± 0.01	(PO_4-Sug): 0.09 ± 0.01 (SO_3-Sug): 0.64 ± 0.02 (SO_4-Sug): 0.40 ± 0.01 (OH-Sug): 0.10 ± 0.01	–				

Chondrus crispus	18.2 ± 0.5	0.5 ± 0.05	0.51	<LOD	–	(PO_4-Sug): 9.2 ± 0.3 (OH-Sug): 8.4 ± 0.2	99.5	0.1 g of sample 10 mL of H_2O Shaking	HPLC, AE Hamilton PRP-X100 HPLC, CE (Zorbax-SCX300))	ICP-MS	
Porphyra purpurea	40.7 ± 2.8	<LOD	<LOD	<LOD	0.53 ± 0.07	(PO_4-Sug): 27.6 ± 3 (OH-Sug): 2.02 ± 0.04	98.6				
Ulva rigida	5.8 ± 0.4	–	0.30	<LOD	0.05 ± 0.04	(PO_4-Sug): 0.29 ± 0.02 (OH-Sug): 1.45 ± 0.02	52.6				
Laminaria ochroleuca	56.8 ± 2.4	<LOD	<LOD	<LOD	0.26 ± 0.08	(PO_4-Sug): 6.2 ± 0.1 (SO_3-Sug): 39.4 ± 1.6 (OH-Sug): 2.71 ± 0.04	87.4				
Laminaria saccharina	52.4 ± 2.1	<LOD	<LOD	<LOD	0.67 ± 0.02	(PO_4-Sug): 6.9 ± 0.2 (SO_3-Sug): 30.7 ± 1.2 (OH-Sug): 2.9 ± 0.1	110.6				
Undaria pinnatifida	41.0 ± 2.6	<LOD	0.29 ± 0.03	<LOD	0.13 ± 0.03	(PO_4-Sug): 0.30 ± 0.02 (OH-Sug): 0.87 ± 0.03	29.1				
NIES No.9 (Sargasso) tAs:115 ± 9	110.3 ± 0.7	<LOD	69.9 ± 1	<LOD	2.1 ± 0.1	(PO_4-Sug): 2.2 ± 0.1 (SO_3-Sug): 1.8 ± 0.1 (SO_4-Sug): 9.0 ± 0.7 (OH-Sug): 1.2 ± 0.2	92.1				[11] [65]
LOD (μg g^{-1})	0.033	0.005	0.017	0.009	0.007	(PO_4-Sug): 0.015 (SO_3-Sug): 0.061 (SO_4-Sug): 0.089 (OH-Sug): 0.008	–				
LOQ (μg g^{-1})	0.109	0.016	0.058	0.031	0.025	(PO_4-Sug): 0.050 (SO_3-Sug): 0.205 (SO_4-Sug): 0.297 (OH-Sug): 0.028	–				

Undaria pinnatifida	24.4 ± 0.8	–	–	0.22 ± 0.02	1.30 ± 0.10	(OH-Sug):22.0 ± 0.3	–	MeOH : mobile phase Microwave	HPLC Hamilton PRP-X100	ICP-MS ESI-MS	[24]
Laminaria japonica	32.6 ± 0.2	–	–	–	0.571 ± 0.008	(PO_4-Sug): 7.06 ± 0.15 (OH-Sug): 23.7 ± 0.8	–				
Sargassum cristaefolium	4.73 ± 0.09	0.152 ± 0.01	0.53 ± 0.03	–	0.432 ± 0.022	(PO_4-Sug): 0.834 ± 0.044 (OH-Sug): 2.42 ± 0.08	–				
Porphyra dentata	31.6 ± 0.3	–	–	0.250 ± 0.003	0.235 ± 0.007	(PO_4-Sug): 0.235 ± 0.003 (OH-Sug): 0.343 ± 0.022	–				
BCR-279 (*Ulva lactuca*) tAs:3.09 ± 0.21	3.02 ± 0.12	0.065 ± 0.003	0.674 ± 0.022	0.234 ± 0.010	0.200 ± 0.004	(PO_4-Sug): 0.302 ± 0.008	–				
LOD (μg L^{-1})	–	0.015	0.011	0.009	0.006	–	–				
Laminaria japonica	49.27 ± 0.39	–	0.19	0.46	7.89	–	87.0	0.50 g of sample 10 mL of H_2O Ultrasonic bath	HPLC CAPCELL PAK C_{18} MG	ICP-MS	[45]
Undaria pinnatifida	49.07 ± 1.3	0.25	0.24	–	2.08	–	81.8				
Gelidum grinale	5.56 ± 0.06	–	0.11	–	0.13	–	72.4				
Hizikia fusiforme	46.4 ± 0.033	0.52	16.55	–	1.24	–	92.9				
NMIJ 7405-a (Hijiki) tAs:35.8 ± 0.9 As(V): 10.1 ± 0.5	35.9 ± 0.03	–	–	–	–	–	–				
LOD (ng g^{-1})	–	0.1				–	–				

Cladophora sp (Salado River)	11100 ± 300	2 ± 1	389 ± 7	–	–	–	5	0.10g of sample 10 mL of H_2O Shaking	HPLC, AE Hamilton PRP-X100 HPLC, CE (Zorbax-SCX300))	ICP-MS	[66]
Cladophora sp. (Middle Loa)	182 ± 7	4 ± 1	64 ± 4	0.31 ± 0.02	<LOD	–	53				
Chara sp.	341 ± 6	3.88 ± 0.09	28.2 ± 0.8	–	0.14 ± 0.01	(OH-Sug): 0.93 ± 0.02	13				
Fucus serratus[d]	–	–	–	–	0.01 ± 0.01	(PO_4-Sug): 0.07 ± 0.01 (SO_3-Sug): 0.56 ± 0.04 (SO_4-Sug): 0.37 ± 0.02 (OH-Sug): 0.07 ± 0.01	–				
BCR-279 (*Ulva lactuca*) tAs:3.09 ± 0.21	2.9 ± 0.3	0.06 ± 0.03	0.53 ± 0.04	0.04 ± 0.01	0.06 ± 0.03	(PO_4-Sug): 0.08 ± 0.01 (OH-Sug): 0.096 ± 0.004	57				
LOD (µg g^{-1})	0.003	0.02	0.08	0.05	0.03	(OH-Sug): 0.15	–				
LOQ (µg g^{-1})	0.01	0.07	0.28	0.15	0.11	(OH-Sug): 0.49	–				
Porphyra tenera	2.07 ± 0.53	<LOD	<LOD	<LOD	0.085 ± 0.079	–	–	1 g of sample 8mL of MeOH in 1% HNO_3 Sonicator bath	HPLC Hamilton PRP-X100	ICP-MS	[67]
Laminaria japonica	3.04 ± 1.47	<LOD	<LOD	<LOD	0.026 ± 0.020	–	–				
Undaria pinnatifida	1.84 ± 0.81	<LOD	<LOD	<LOD	0.24 ± 0.13	–	–				
Hizikia fusiforme	4.49 ± 0.65	0.220 ± 0.16	2.13 ± 1.39	<LOD	0.68 ± 0.33	–	–				
Sargassum fulvellum	6.48 ± 0.60	0.067 ± 0.005	5.28 ± 0.45	<LOD	<LOD	–	–				
NMIJ 7405-a (Hijiki) tAs:35.8 ± 0.9 As(V): 10.1 ± 0.5	34.6 ± 0.7	–	9.8 ± 0.8	–	–	–	–				
LOD (µg L^{-1})	0.028	0.061	0.025	0.018	0.028	–	–				

LOQ (μg L^{-1})	0.095	0.205	0.083	0.061	0.093	–	–				
Sargassum fulvellum	206.9 ± 11.2	1.7 ± 0.2[e]	112.4 ± 2.3	1.7 ± 0.2[e]	1.05 ± 0.40	(PO_4-Sug): 9.66 ± 0.67 (SO_3-Sug):2.71 ± 0.38 (SO_4-Sug): 11.9 ± 2.84	63.1[f]	0.1 g of sample 10 mL of H_2O Ultrasonication instead of microwaves	HPLC, RP Capcell Pak C18 ODS HPLC,AE Hamilton PRP-X100	ICP-MS ES-MS/MS	[37]
Sargassum piluliferum	288.0 ± 16.8	1.4 ± 0.2[e]	47.4 ± 3.3	1.4 ± 0.2[e]	2.7 ± 0.8	(PO_4-Sug): 5.62 ± 0.39 (SO_3-Sug):2.14 ± 0.30 (SO_4-Sug): 9.44 ± 2.26	21.4[f]				
Undaria pinnatifida	26.9 ± 7.3	–	1.7 ± 0.1	–	0.56 ± 0.03	(PO_4-Sug): 6.40 ± 0.04	91.2[f]				
Kelp	47.6 ± 1.2	35.1 ± 1.2[e]	0.78 ± 0.06	35.1 ± 1.2[e]	2.9 ± 0.4	(PO_4-Sug): 2.23 ± 0.52 (SO_3-Sug):14.4 ± 0.87	86.2[f]				
Myelophycus simplex	52.2 ± 4.9	0.8 ± 0.5[e]	4.2 ± 5.1	0.8 ± 0.5[e]	27.6 ± 4.6	(PO_4-Sug): 1.09 ± 0.23 (SO_3-Sug): 0.17 ± 0.07	94.6[f]				
Hizikia fusiformis	60.4 ± 0.4	0.5 ± 0.10[e]	16 ± 1.6	0.5 ± 0.10[e]	–	(PO_4-Sug): 1.09 ± 0.23 (SO_3-Sug): 0.17 ± 0.07 (SO_4-Sug): 1.17 ± 0.43	27.3[f]				
Pelvetia wrightii	14.5 ± 0.5	0.13 ± 0.05[e]	0.6 ± 0.4	0.13 ± 0.05[e]	0.3 ± 0.2	–	9.3[f]				
LOD (μg L^{-1})	–	0.03 ± 0.23				–	–				
Cystoseira barbata	55 ± 2	–	27 ± 0.8	–	–	(SO_3-Sug): 1.43 ± 0.08 (OH-Sug): 0.40 ± 0.06	69	H_2O	HPLC, AE Hamilton PRP-X100 HPLC, CE (Zorbax-SCX300)	ICP-MS	[68]
Cystoseira compressa	–	–	3.27 ± 0.07	–	0.072 ± 0.004	(SO_3-Sug): 1.3 ± 0.1 (OH-Sug): 0.35 ± 0.01	30				
Gracilaria sp.	7.1 ± 0.4	0.05 ± 0.01	0.50 ± 0.02	–		(SO_3-Sug): 1.21 ± 0.05 (OH-Sug): 1.27 ± 0.07	45				

Gracilaria gracilis	5.7 ± 0.2 - 12.2 ± 0.5	–	0.68 ±0.007	–	0.052 ± 0.006	(PO_4-Sug): 0.12 ± 0.01 - 0.53 ± 0.01 (SO_3-Sug): 1.26 ± 0.04 (OH-Sug): 0.164 ± 0.001 -1.10 ± 0.02	9-48				
Hypnea musciformis	4.6 ± 0.4 - 5.0 ±0.2	0.048 ± 0.009	–	–	–	(OH-Sug): 0.155 ± 0.006	25				
Codium fragile	–	7.4 ± 0.3 - 39.0 ± 0.4	–	–	0.059 ± 0.01- 0.062 ± 0.002	(PO_4-Sug): 0.26 ± 0.04 (OH-Sug): 0.7 ± 0.02 - 5 ±0.9	14-47				
Enteromorpha intestinalis	–	0.041 ± 0.004 0.049 ± 0.02	–	–	–	(OH-Sug): 0.044 ± 0.001 - 0.068 ± 0.001	21-30				
Ulva rigida	2.7 ± 0.03	–	–	–	–	(OH-Sug): 0.075 ± 0.002 - 0.433 ± 0.007	21-65				
Fucus serratus[d]	–	–	–	–	0.01 ± 0.01	(PO_4-Sug): 0.07 ± 0.01 (SO_3-Sug): 0.56 ± 0.04 (SO_4-Sug): 0.37 ± 0.02 (OH-Sug): 0.07 ± 0.01	–				
NIES No.9 (Sargasso) tAs:115 ± 9	99 ± 9	–	–	–	–	–	–				
LOD (µg g^{-1})	–	0.01	0.03	0.02	0.02	(PO_4-Sug): 0.03 (SO_3-Sug): 0.01 (OH-Sug):0.008	–				
LOQ (µg g^{-1})	–	0.04	0.1	0.06	0.05	(PO_4-Sug): 0.1 (SO_3-Sug): 0.3 (OH-Sug):0.03	–				
Colpomenia marina	16.12	0.05		–	0.42	(PO_4-Sug):0.72 (SO_3-Sug):0.74 (SO_4-Sug): 3.90 (OH-Sug):0.65	7-117	0.25 g of sample MeOH:H_2O, Sonication	HPLC AE, CE Hamilton PRP-X100 Supercosil SCX	ICP-MS	[44]
Ascophyllum nodosum	23.14 ± 6.82 - 23.68 ± 4.33	0.06 ± 0.08 - 0.08 ± 0.003		<LOD	0.21 ± 0.12 - 0.35 ± 0.11	(PO_4-Sug):0.50 ± 0.005 - 0.59 ± 0.10 (SO_3-Sug):1.10 ± 0.11 - 1.34 ± 0.42 (SO_4-Sug):5.18 ± 2.79 - 5.65 ±					

					1.11 (OH-Sug): 2.05 ± 0.77 - 2.34 ± 0.84					
Fucus spiralis	16.27 ± 1.413	0.04 ± 0.05	<LOD	0.19 ± 0.06	(PO_4-Sug):0.31 ± 0.04 (SO_3-Sug):3.63 ± 0.03 (SO_4-Sug):1.51 ± 0.49 (OH-Sug): 0.56 ± 0.45					
Fucus vesiculosus	28.89 ± 2.16 - 32.76 ± 3.73	0.06 ± 0.04	<LOD	0.18 ± 0.08 0.39 ± 0.11	(PO_4-Sug):0.42 ± 0.04 - 1.28 ± 1.07 (SO_3-Sug):7.90 ± 3.94- 14.84 ± 9.36 (SO_4-Sug):1.74 ± 0.65 - 2.72 ± 0.69 (OH-Sug): 0.50 ± 0.29 - 2.21 ± 1.62					
Agarum clathratum	61.84	<LOD	<LOD	0.89	(PO_4-Sug): 11.82 (SO_3-Sug): 24.92 (SO_4-Sug): 0.29 (OH-Sug): 2.44					
Alaria esculenta	34.46 ± 13.72	0.03 ± 0.04	<LOD	0.47 ± 0.28	(PO_4-Sug): 5.69 ± 3.92 (SO_3-Sug): 12.54 ± 8.29 (SO_4-Sug): 0.01 ± 0.03 (OH-Sug): 4.50 ± 1.81					
Laminaria digitata	106.73 - 50.38 ± 11.91	8.32 ± 7.67	0.06 ± 0.13	0.97 ± 0.41 - 0.94	(PO_4-Sug): 10.63 ± 8.71 - 8.20 (SO_3-Sug): 46.09 - 10.23 ± 7.61 (SO_4-Sug): 0.28 (OH-Sug): 5.10 ± 0.91- 3.43					
Laminaria longicruris	74.14 ± 8.01	0.12 ± 0.04	–	0.78 ± 0.18	(PO_4-Sug): 11.20 ± 2.06 (SO_3-Sug): 40.82 ± 3.20 (SO_4-Sug): 0.06 ± 0.10 (OH-Sug): 3.63 ± 1.91					
Saccharina latíssima	56.29	–	–	0.39	(PO_4-Sug): 2.79 (SO_3-Sug): 15.45 (OH-Sug): 0.28					

Porphyra umbilicalus	20.73	0.12	0.05	0.25	(PO_4-Sug): 24.98 (SO_3-Sug): 0.09 (OH-Sug): 1.95					
Heterosiphonia japonica	8.24	0.47	0.04	0.05	(PO_4-Sug): 0.65 (SO_3-Sug): 0.06 (OH-Sug): 0.56					
Polyiphonia lanosa	14.00	0.26	0.06	0.74	(PO_4-Sug): 2.10 (SO_3-Sug): 0.27 (SO_4-Sug): 1.46 (OH-Sug): 2.22					
Chondrus crispus	12.13 ± 0.97 - 6.10	0.06 - 0.07 ± 0.08	–	0.19 ± 0.18 - 0.29	(PO_4-Sug): 0.17 - 4.44 ± 4.15 (SO_3-Sug): 0.14 ± 0.12 - 0.20 (SO_4-Sug): 0.12 (OH-Sug): 1.21 ± 0.36 - 3.10					
Phyllophora pseudoceranoides	4.17	0.14	–	0.21	(PO_4-Sug):1.56 (SO_3-Sug):0.07 (SO_4-Sug): 0.14 (OH-Sug): 0.17					
Gracliaria vermiculophylla	11.80	0.23	0.09	0.06	(PO_4-Sug):1.24 (SO_3-Sug):0.07 (OH-Sug):1.13					
Palmaria palmata	8.95 ± 4.80	0.06 ± 0.11	0.03 ± 0.01	0.96 ± 0.35	(PO_4-Sug): 4.35 ± 3.22 (SO_3-Sug):0.16 ± 0.16 (SO_4-Sug): 0.01 ± 0.02 (OH-Sug):3.03 ± 1.50					
Chaetomorpha sp	6.69	–	–	–	(PO_4-Sug): 0.34 (SO_3-Sug):0.17 (OH-Sug): 0.87					
Gayralia oxysperma	12.68	0.17	–	0.04	(PO_4-Sug): 0.02 (SO_3-Sug):0.03 (OH-Sug):0.57					
Ulva lactuca	5.34 - 4.13	0.02-0.13	–	0.05	(PO_4-Sug): 0.02 - 0.15 (SO_3-Sug):0.01 - 0.06 (OH-Sug):0.06 - 0.07					

Ulva prolifera	14.65	0.12		–	0.04	(PO_4-Sug): 0.06 (SO_3-Sug):0.10 (OH-Sug):0.46					
MURST-ISS-A2 tAs:5.02 ± 0.44	–	–		–	0.25 ± 0.03	(PO_4-Sug): 0.33 ± 0.03 (SO_4-Sug): 0.16 ± 0.02 (OH-Sug): 0.22 ± 0.03					
Laminaria ochroleuca	56.4 ± 1.4	–	–	–	–	(PO_4-Sug): 3.56 ± 0.12 (SO_4-Sug):22.8 ± 0.0 (OH-Sug):8.12 ± 0.25	110 ± 1	0.05-0.1 g of sample H_2O Sonication	HPLC, AE Hamilton PRP-X 100	ICP-MS, ESI-MS/MS	[69]
Undaria pinnatifida	38.3 ± 1.7	–	–	–	–	(PO_4-Sug): 5.35 ± 0.14 (SO_4-Sug):5.68 ± 0.09 (OH-Sug): 3.17 ± 0.09	86 ± 1				
Porphyra umbilicalis	48.8 ± 2.5	–	–	–	–	(PO_4-Sug): 26.2 ± 0.4 (OH-Sug): 4.30 ± 0.09	102 ± 2				
Ulva rigida	4.82 ± 0.05	–	–	–	–	(PO_4-Sug): 0.29 ± 0.01 (OH-Sug): 0.56 ± 0.03	44 ± 2				
LOD (pg g^{-1})	–	–	–	–	–	20	–				
Eisenia arborea	26 ± 1	–	7.0 ± 0.1	–	0.48 ± 0.04	(PO_4-Sug): 2.04 ± 0.01 (OH-Sug): 1.5 ± 0.1	77 ± 8	0.2 g of sample H_2O microwave-assisted	HPLC, AE Hamilton PRP-X100 HPLC, CE Hamilton PRP-X 200	(UV)–HG–AFS	[46]
Fucus vesiculosus	47 ± 2	–	11 ± 1	–	0.55 ± 0.07	(PO_4-Sug): 0.8 ± 0.1 (SO_3-Sug): 0.6 ± 0.1 (SO_4-Sug): 7.2 ± 0.1 (OH-Sug): 2.6 ± 0.1	76 ± 5				
Himanthalia elongata	24 ± 2	–	2.0 ± 0.1	–		(PO_4-Sug): 0.11 ± 0.01 (SO_3-Sug): 4.2 ± 0.9 (OH-Sug): 4.5 ± 0.4	75 ± 9				
Hijiki fusiformis	98 ± 5	–	50.3 ± 0.4	–	0.44 ± 0.06	(PO_4-Sug): 0.4 ± 0.1 (SO_3-Sug): 0.7 ± 0.1 (SO_4-Sug): 2.7 ± 0.4 (OH-Sug): 1.05 ± 0.03	73 ± 4				

Laminaria ochroleuca	42 ± 5 - 97 ± 5	–	11±2 - 32 ± 3	–	0.40 ± 0.04	(PO_4-Sug): 1.9 ± 0.3 - 22 ± 1 (OH-Sug): 3.1 ± 0.11 - 11 ± 3	74 ± 5 - 93 ± 9				
Laminaria digiata	126 ± 5	–	77 ± 3	–	–	(PO_4-Sug): 3.5 ± 0.1 (OH-Sug): 10.2 ± 0.7	73				
Undaria pinnatifida	37 ± 1 - 37 ± 2	–	2.2 ± 0.1- 4.5 ± 0.3	–	0.025 ± 0.007	(PO_4-Sug): 1.5 ± 0.1- 10.10 ± 0.05 (OH-Sug): 2.68 ± 0.03 - 14.3 ± 0.2	49 ± 8 - 73 ± 8				
Porphyra umbilicalis	23 ± 3 - 34 ± 3	–	–	–	0.064 ± 0.005	(PO_4-Sug): 13 ± 1 - 20.1 ± 0.3 (OH-Sug): 1.02 ± 0.07 - 1.6 ± 0.1	61 ± 9 - 74 ± 9				
NIES No.9 (Sargasso) tAs:115 ± 9	109 ± 2	–	70 ± 1	–	0.9 ± 0.1	(PO_4-Sug): 1.4 ± 0.2 (SO_4-Sug): 7 ± 2 (OH-Sug): 1.0 ± 0.2	98 ± 2				
LOD (µg g^{-1})	–	0.019	0.028	0.027	0.007	(PO_4-Sug): 0.048 (SO_3-Sug): 0.062 (SO_4-Sug): 0.076 (OH-Sug): 0.030	–				
Hizikia fusiformis	–	–	50.6 ± 0.8	–	0.50 ±0.05	(PO_4-Sug): 0.5 ± 0.1 (SO_3-Sug): 0.6 ± 0.1 (SO_4-Sug): 2.4 ± 0.2 (OH-Sug): 1.17 ± 0.04	–	0.2 g of sample 10 mL of H_2O Microwave-assisted	HPLC	(UV)–HG–AFS	[21]
LOD (µg g^{-1})	–	–	0.028	–	0.007	(PO_4-Sug): 0.048 (SO_3-Sug): 0.062 (SO_4-Sug): 0.076 (OH-Sug):0.03	–				

–, data not available; AE, Anion Exchange; CE, Cation Exchange; LOD, Limit Of Detection LOQ, Limit Of Quantification; UV, Ultraviolet;

[a] minimum and maximum values obtained for a total of 12 Sargassum samples;

[b] distribution of total As and iAs was determined in thallus parts of the kelp *Laminaria digitata* and the intertidal fucoid *Ascophyllum nodosum;*

[c] minimum and maximum values obtained for a total of 9 hijiki samples;

[d] values for *F. serratus* extract are given as absolute amount for extract µg;

[e] DMA + As(III);

[f] percentages of extraction determined for all species, except for As-Sug.

Table 2: Overview of results and figures of merit of the analytical methods (without of separation step) applied for arsenic speciation in macroalgae (relevant works from 2007)

Specie, CRM, LOD and LOQ	tAs	iAs	% Extraction	Extraction method	Detection method	Ref.
Hizikia fusiformis	34.8	0.41	–	0.5 -1 g of sample Shaking 20 mL of HCl	HG-AAS	[20]
LOD (μg g^{-1})	0.031	0.025				
Callophyllis variegata	8.0 ± 0.2	0.15 ± 0.01	96-99	0.50g of sample 4.1 mL of H_2O 18.4 mL of HCl Chloroform	FI-HG-AAS	[12]
Chondracanthus chamissoi	9.4 ± 0.4	0.43 ± 0.04				
Gigartina skottsbergii	12.5 ± 4.5	0.81 ± 0.36				
Gracilariachilensis	7.5 ± 1.7	0.93 ± 0.63				
Gymnogongrus disciplinalis	6.6 ± 1.6	0.42 ± 0.17				
Iridaea spp.	13.4 ± 1.0	0.62 ± 0.03				
Iridaea laminarioides	13.4 ± 1.6	0.58 ± 0.13				
Mastocarpus papillatus	18.3 ± 0.1	0.54 ± 0.01				
Mazzaella laminaroides	14.4 ± 1.6	0.31 ± 0.01				
Porphyra columbina	23.8 ± 15.0	0.24 ± 0.04				
Durvillaea antarctica	49.0 ± 34.5	0.31 ± 0.08				
Lessonia nigrescens	57.1 ± 22.8	0.35 ± 0.34				
Macrocystis piryfera	68.0 ± 20.6	1.70 ± 0.80				
Ulva rigida	3.1 ± 0.8	0.40 ± 0.29				
IAEA-140 *Fucus* tAs: 44.3 ± 2.1	–	1.21–1.33				
BCR-279 (*Ulva lactuca*) tAs:3.09 ± 0.21	–	1.27–1.37				
LOD (μg g^{-1})	–	0.013				
Himantothallus grandifolius	152 ± 33	0.25 ± 0.01	–	0.5 of sample 4.1 mL of H_2O and 18.4 mL of HCl	FI–HG–AAS	[56]
Phaeurus antarcticus	88 ± 20	0.84 ± 0.01				
Adenocystis utricularis	40 ± 4	0.27 ± 0.01				
Ascoseira mirabilis	52 ± 7	0.29 ± 0.01				
Desmarestia antarctica	62 ± 7	0.48 ± 0.08				
Desmarestia anceps	33 ± 5	0.28 ± 0.01				
Iridaea cordata	28 ± 6	0.55 ± 0.01				
Myriogramme sp	6 ± 3	0.12 ± 0.01				
Palmaria decipiens	11 ± 4	0.41 ± 0.01				
BCR-279 (*Ulva lactuca*) tAs:3.09 ± 0.21	3.08 ± 0.27					
LOD (μg g^{-1})	0.33	0.014				
Hijiki	_	53 ± 7–76 ± 20	86	0.1-5 g of sample HNO_3 Water bath	FDM	[57]
Wakame	_	0.05 ± 0.02–0.06 ± 0.02	80			
Nori	_	0.06 ± 0.06–0.3 ± 0.01	80			
LOD (μg kg^{-1})	_	5	–			
LOQ (mg kg^{-1})	_	0.05	–			

–, data not available; FI, Flow Injection

4. Analytical quality control and quality assurance

The quality control and quality assurance always play a dominant role in any study of chemical speciation if the results are intended for example in the assessment of environmental quality or food safety. The analytical performance of a method must be evaluated through validation protocols to demonstrating that the results will be close enough to the true value for the content of the analyte under study. The most important figures of merit for the speciation of As in macroalgae include the LOD (limit of detection) and the LOQ (limit of quantification). Through those limits, it is possible to define the lowest concentration of an analyte that can be reliably detected and quantified with acceptable degree of certainty. Table 1 and Table 2 comprises figures of merit from the latest works, and they show that sometimes the LODs and LOQs for the different methods of speciation are not comparable because they are expressed in different units (e.g. ug L^{-1} or ug g^{-1}).

The lowest LOD values were obtained with a system confirming the sensitivity of this analytical method. Narukawa et al. [45] found LODs of 0.1 ng g^{-1} for As(III), As(V), MMA, and DMA while Sartal et al. [69] described LODs of 20 pg g^{-1} for As-Sug. Although the LODs values obtained are generally between 0.001 and 0.2 ug g^{-1}, as shown in Table 1 and Table 2, the LODs values obtained by HPLC-ICP-MS are very similar to the detection limits obtained by HPLC-HG-AFS and HG-AAS that are methods considered to have less detection capability . Díaz et al. [12] used FI-HG-AAS for the quantification of inorganic species and achieved a variety of good analytical characteristics, including a low detection limit of 0.013 ug g^{-1}, a precision of 4%, an As(III) recovery of 99%, an As(V) recovery of 96% with a coefficient of variation less than 10%. Similarly, Salgado et al. [46] evaluated the analytical characteristics for the HPLC-HG-AFS and found that the precision, evaluated as relative standard deviation, varied between 2.6 and 4.6% and achieved LODs of 0.019, 0.028, 0.027, and 0.007 ug g^{-1} for As (III), As (V), MMA and DMA, respectively, and between 0.030 to 0.076 µg g^{-1} for As-Sug.

As equally important to maintain quality control, it is also critical to keep in place a quality assurance system in order to guarantee the quality of analytical data on a long term basis. One of the solutions to solving methodological and analytical problems in speciation studies is the use of CRMs. However, the main problem associated with the determination of As species in seaweeds and their toxicological evaluation is the unavailability of CRMs.

Taking as a basis the review by McSheehy et al. [51], one would expect noticeable developments in recent years in the field of assessment of quality control for As speciation in macroalgae. However, the review shows that in all the studies of speciation the main problem associated with the determination of As species and respective toxicological evaluation

remains on the lack of CRMs for the different chemical forms of As, thus hindering the validation of results.

Despite the recognized importance of the development of specific CRM, only in 2012 Narukawa et al. [45] developed the NMIJ 7405-a (*Sargassum fusiforme*) with certified values for arsenate. However, this material is very expensive, and its use unfortunately did not become widespread as it should. Also since 2012, for total As content in seaweed there have been made available two CRMs (NMIJ 7405-a *Sargassum fusiforme* and ERM-CD200 *Fucus vesiculosus*) which replaced the CRMs phased out (BCR-279 (*Ulva lactuca*) and Nies No.9 (Sargasso)). In Tables 1 and 2 there are records of values obtained for those reference materials.

Therefore, due to the lack of CRMs for As speciation, other strategies to support the accuracy of the results were used, such as spiked experiments or method comparison. Madsen et al. [70], performed the preparation of a homogeneous extract of *F. serratus* and used it as a strategy to promote the quality of analytical results and to check the proper quantification of As-Sug. Such algae extracts have been used, since 2000, for the identification and quantification of the major species of As-Sug (as displayed in Figure 1) assessing also the quality of the extraction process.

5. Speciation of As compounds: overview and general discussion of relevant works

As shown in Table 1 and Table 2, different algae taxonomy and location played a strong role in the distribution of As compounds. For example, in general, brown algae accumulate higher concentrations of total As, as well as of the different As species, namely iAs, relative to red and green algae. This fact may be due to the taxonomic differences, namely physiological and biochemical factors, allowing an easier accumulation of this element [71]. Concentrations determined for different species of macroalgae showed that for the most toxic components (iAs), brown algae (namely *Laminaria* sp. [63], [23]*; Lessonia* sp. [61]*; Hijiki* sp. [37], [64]**,** [45]; and *Sargassum* sp. [43], [37]) contain extremely high concentrations of iAs. For green algae, the highest concentrations of iAs were found in *Codium sp.* [68], [11], while for red algae the highest concentration (1.25 ± 0.02 mg kg^{-1}) was obtained for *Alsidium corallinum* [12]. Therefore, it is especially important to study the behavior of these species in order to understand why they accumulate large concentrations of iAs. Furthermore, those findings imply special care in the use of these algae for consumption purposes since they may have

potentially toxicological implications on health depending on the phyla (brown algae vs. green algae vs. red algae).

Relatively to the location role in the distribution of As compounds, an example is the green algae *Cladophora sp.*, because this algae have low concentrations of As species in Hayakawa River (Japan) [60] and in Western Mediterranean Sea [11] while in the Loa River Basin (Chile) have an extreme high concentration of As species [66].

It is also common to verify that the sum of the concentrations of the various species of As are below the sum total of As, as shown for example in Miyashita et al. [60], Pell et al. [68], Llorente-Mirandes et al. [11] studies. Possibly, this is due to the low percentages of extraction which compromised the analytical quality of the results

Relatively to As-Sug, OH-Sug was present in all samples while SO_4-Sug was present in few samples. Generally, brown algae contain mainly OH-Sug, PO_4-Sug, and SO_3, although OH-Sug is present in low concentrations relative to PO_4-Sug and SO_3-Sug [44], [21], [61]. In green macroalgae, common arsenosugars are OH-Sug and PO_4-Sug [60], [11], [24], while in red algae, OH-Sug was the dominant form, although PO_4-Sug and SO_3-Sug were also detected [11], [46], [24], [68].

6. Trends and future work

- Arsenic speciation in macroalgae is a hot topic nowadays due to the increase in consumption of macroalgae around the world. Considering the emerging use of macroalgae as feedstock's, besides a source of food products, and its importance for environmental monitoring and for the industrial community, the setup of specific regulations for each individual species of As should be prioritized in the near future.
- The implementation of less instrumentally demanding techniques in future studies, may be fit for the purpose of easily inform about the amount of the As species present,without discarding the importance of HPLC-ICP-MS, when higher detection capabilities are needed. Adequate decisions on consumption safety can be easily and quickly taken by using HPLC-HG-AFS or even methods not involving a chromatographic step, such as FDM and FI-HG-AAS.
- The complexity of algae matrix presents a challenge for the chromatographic separation of As species due to the high contents in organic matter causing deleterious effects for the chromatographic system and compromising the quality of the analytical results. It's important to highlight the necessity to the

development and optimization of extraction analytical strategies and extract clean-up procedures before separation process, in order to avoid erroneous results and problems in the chromatographic step. ..

- Finally, the main obstacle associated with the determination of As species and their toxicological evaluation in foodstuffs continues to be the unavailability of CRM for analysis of speciation, compromising the validation of results. Such validation studies are fundamental not only for the proper characterization of the method performance but also for providing a suitable framework for setting up limits of As for environmental and public health protection.

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Highlights

Importance of the application of cheaper and fit for purpose methods able to inform about the safety of macroalgae for consumption.

This review justifies the need for developing and improving the steps prior to speciation, such as extraction and clean-up that are crucial for the acquisition of reliable results.

Pinpointing the lack of certified reference materials as the main limitation for development of arsenic (As) speciation studies in macroalgae.