

Dreifing arsentegunda eftir þanglutum, sér í lagi arsenlíþíða

Distribution of arsenic species within the macroalgae - an emphasis on arsenolipids

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Ágríp á íslensku:	<p>Þörungar eru ríkir af steinefnum og eftirsóknarverðum lífvirkum efnum, en þeir geta einnig tekið upp mikið magn snefilefna, s.s. eittraðra þungmálma og þar á meðal frumefnið arsen. Arsen finnst sem ólífrænt arsen í sjónum og er tekið upp á því efnaformi af þörungunum. Í þörungunum greinist hins vegar arsen ekki einungis sem ólífrænt arsen heldur sem fjölbreytt úrval arsen-sambanda, svokölluð lífræn efnasambönd arsens t.d. arsenósykrur og arsenólípið. Enn leikur ýmislegt á huldu um uppruna þessara efnasambanda. Almennt hafa lífrænar arsenategundir verið taldar nokkuð hættulausar ólíkt ólífrænu arseni sem er þekktur krabbameinsvaldur. Hins vegar hafa nýlegar rannsóknir á arsenólípiðum sýnt að þau geta verið jafn frumudrepanði og ólífræna arsenið. Einnig er talið að arsenósykur geti mögulega haft langvarandi neikvæð áhrif við reglubundna neyslu. Magn arsenólípiða er að jafnaði ekki hátt í þörungum, en talið er að upphafspunktur framleiðslu þeirra eigi sér stað í þörungum. Þörungar eru hluti reglulegrar neyslu matvæla í austurlhuta heimsins og nýtur stöðugt meiri vinsælda á Vesturlöndum, því er brýn þörf á frekari upplýsingum um þessi efnasambönd til að meta til hlítar áhættuna sem fylgir neyslu þeirra sem og að tryggja að settar séu viðeigandi reglur um hámarks magn þeirra í matvælum. Til að skilja eiturefnafræðileg áhrif neyslu þörunga</p>		

	<p>er afar mikilvægt að fleiri gögnum sé safnað um öll mismunandi efnaform arsens, sér í lagi um arsenólípið en takmarkað af upplýsingum er til staðar í dag um þau. Sýnum af rauð-, græn- og brúnþörungum var safnað nálægt Grindavík og Kjalarnesi, á tveimur mismunandi tímapunktum. Sýnin voru ítarlega rannsökuð m.t.t. þungmálma og framkvæmd var tegundagreining arsens til að skilja betur á hvaða efnaformi arsenið var til staðar á. Valin sýni brún-, rauð- og grænþörungum voru mæld m.t.t. tegundargreiningar arsenólípiða með því að nota massagreiningu HPLC-ICP-M/ESI-MS/MS og HPLC-qToF-MS. Aukinheldur var brúnum stórþörungum skipt í líffræðilega hluta til að ákvarða hvort dreifing arsentegunda sé jöfn um þangið. Takmarkaðar upplýsingar eru til á heimsvísu um arsenólípið í þangi, svo þessi umfangsmikla prófílgreining þeirra í mismunandi tegundum þörungum mun styðja við að skýra hvernig þessi dularfullu lífrænu efnasambönd myndast og hvar þau eru geymd. Gögnin geta einnig nýst við áhættumat á arsentegundum í þangi til mannelis og geta því haft áhrif á framtíðarlöggjöf um matvælaöryggi.</p>
<i>Lykilorð á íslensku:</i>	þang, þungmálmar, arsen, arsen tegundagerð, matvælaöryggi
<i>Summary in English:</i>	<p>In recent years seaweed has gained popularity as a health food due to its high content of minerals and vitamins. However, seaweeds may also accumulate high levels of potentially toxic elements – in particular arsenic, which may become incorporated into larger biological molecules such as sugars and lipids. It is unclear how these organic arsenic compounds are formed/stored and if they may serve a biological purpose (i.e., detoxification or energy storage). However, toxicological studies into arsenic-containing lipids have demonstrated cytotoxicity comparable to that of arsenite, a known carcinogen, and arsenic-containing sugars are suspected to display toxicity with chronic exposure. This project aims to investigate variations in the distribution of arsenic compounds throughout several classes and species of seaweed. Samples of brown, red and green macroalgae were collected from two locations in Iceland across two different months and analysed for several potentially toxic elements as well as hydrophilic arsenic speciation using HPLC-ICP-MS. Brown macroalgae were additionally sectioned into anatomical parts to determine if the distribution of arsenic species differs throughout the thallus. Select samples were chosen for state-of-the-art lipophilic arsenic speciation using HPLC-ICP-MS/ESI-MS/MS and HPLC-qToF-MS. Limited information is available on arsenic speciation in seaweed thus it is hoped that this extensive profiling of several different species will help elucidate how these unusual compounds are formed and stored. The data from this project will also contribute to the necessary information needed for the risk assessment of arsenic species in seaweed for human consumption and may have an impact on future food safety legislations.</p>
<i>English keywords:</i>	seaweed, heavy metals, arsenic, arsenic speciation, food safety

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Introduction

Seaweed is becoming increasingly popular as a health food or supplement in Western countries and has been regarded by many as a 'superfood' due to its high content of minerals, vitamins, and antioxidants (1,2). Whilst seaweeds may be rich in essential elements such as iron and calcium, they may also accumulate high levels of arsenic – a toxic element widely associated with its use as a poison throughout history. Whilst arsenic is ubiquitous throughout the environment, it displays particularly unique behaviour in marine ecosystems where it may take on several chemical forms ranging from small, inorganic molecules to complex sugars and phospholipids. Seaweeds are thought to be the starting point for many of these organic arsenic compounds as algae serve as a primary food source for many higher marine organisms. However, the mechanisms by which these compounds are formed are still largely unknown and there is debate over whether they may be a product of detoxification or serve a biological function i.e., energy storage or conservation of phosphorous under limiting conditions (3,4). Investigating variations in arsenic compounds between seaweeds species may be useful to further our understanding of how and why these compounds are formed, as previous studies have demonstrated large variations in arsenic metabolism (4,5). Thallus sections within large brown macroalgae often differ in biological function, i.e., the holdfast provides support, and the meristem is responsible for new frond growth, therefore the distribution of arsenic species throughout the thallus was also investigated in order to uncover any possible links between the production of these compounds and biological function.

This project will contribute significantly to current understanding by being the first to report a comprehensive study of the arsenic speciation in several species of brown, red and green seaweed collected in Iceland. Arsenic-containing sugars and arsenosugarphospholipids were both found to be most abundant in the reproductive tissues of the brown seaweeds, which may be evidence that the former are the starting product of the latter as both share a dimethylarsenoribose moiety. The lipids are perhaps produced by the binding of Arsenosugars to phosphatidic in the membrane which would provide an explanation as to why these lipids also contain a phosphate moiety and two acyl groups. Whether these species are produced accidentally as a result of biological infidelity or are produced with a biological purpose is unclear. It does not appear that arsenosugars serve as energy storage as is displayed with other sugars, however, their inclusion in the cell wall matrix may have yet to be discovered benefits (i.e., increased resistance to environmental changes).

Additionally, the results from this project may serve to address the lack of occurrence data currently available for arsenic speciation in seaweed – in particular the lipid-soluble species. Previously, the organic arsenic compounds were generally considered to be less toxic than the inorganic, but this has recently been disproven; Several arsenic-containing hydrocarbons have been demonstrated to exert cytotoxicity comparable to arsenite in vitro (6), and exert developmental toxicity in the

in vivo models *Drosophila melanogaster* and *Caenorhabditis elegans* (7,8). Thus, access to occurrence data will be crucial in the future for monitoring and regulatory purposes to ensure consumer safety.

Aims and Objectives

The aim of the project was to address several research questions regarding the arsenic speciation in macroalgae:

- In what proportions are the different arsenic species stored within the thalli?
- Does this relate to how complex arsenic compounds such as arsenosugars and arsenolipids are formed?
- Do other variables such as season and location have an impact on the arsenic speciation in macroalgae?
- Will Rhodophyta and Chlorophyta behave differently to Phaeophyta in terms of arsenic speciation?
- Are there different mechanisms for the formation of these compounds that are dependent on seaweed type?

To answer these research questions, a large sample set of brown, red and green seaweed specimens were collected from two locations in Iceland across two sampling months. The large brown thalli were divided into sections based on anatomy and function (i.e., sporophyll for reproductive tissues). This sample-set was then analysed for total element concentrations, water-soluble arsenic speciation and a select few for lipid-soluble speciation.

Sampling

A range of brown, red, and green seaweed species were collected from two locations in Iceland during May 2021 and February 2022, Table 1 and Figure 1. Fewer samples were collected in February due to the lack of availability of green and red species during the winter months. The samples were transported back to the lab in sterile plastic bags and were stored at -18°C until preparation. Samples were rinsed sparingly with tap water to remove sand, and any epiphytic species were removed manually using a stainless-steel blade rinsed with ultrapure water and a citric acid/ethylenediaminetetraacetic acid solution.

Table 1. Species of seaweed collected in May 2021 and February 2022.

	Grindavík (63°50'55.5"N 22°18'23.5"W)	Kjalarnes (64°13'34.2"N 21°48'50.7"W)
May 2021	<i>Fucus vesiculosus</i> , <i>Laminaria digitata</i> , <i>Ascophyllum nodosum</i> , <i>Alaria esculenta</i> <i>Palmaria palmata</i> , <i>Mastocarpus stellatus</i> <i>Ulva intestinalis</i> , <i>Acropsiphonia arcta</i>	<i>Fucus vesiculosus</i> , <i>Laminaria digitata</i> , <i>Ascophyllum nodosum</i> , <i>Saccharina latissima</i> <i>Palmaria palmata</i> , <i>Devaleraea ramentacea</i> , <i>Cystoclonium purpureum</i> , <i>Chondrus crispus</i> <i>Ulva prolifera</i> , <i>Ulva intestinalis</i> , <i>Acrosiphonia arcta</i>
February 2022	<i>Fucus vesiculosus</i> , <i>Laminaria digitata</i> , <i>Ascophyllum nodosum</i> , <i>Saccharina latissima</i> , <i>Alaria esculenta</i> <i>Palmaria palmata</i> , <i>Mastocarpus stellatus</i> , <i>Vertebrata lanosa</i> , <i>Chondrus crispus</i> , <i>Porphyra dioica</i> <i>Acrosiphonia arcta</i> or <i>coalita</i> *	<i>Fucus vesiculosus</i> , <i>Laminaria hyperborea</i> , <i>Ascophyllum nodosum</i> <i>Palmaria palmata</i> , <i>Chondrus crispus</i> -

*DNA results inconclusive.



Figure 1 – (top) The sampling location at Grindavík, Iceland. (bottom) The sampling location at Kjalarnes, Iceland.

Brown seaweed and red species with larger thalli were divided into anatomical sections such as meristem and young frond. The holdfast and stipe sections were pooled due to the small amount of material. The reproductive sori in the *Laminaria* and *Saccharina* species were only present in the February samples. Chlorophyta specimens were not sectioned as they are mostly annual, and the old and new parts are indistinguishable from one another. DNA testing was used to confirm the exact Chlorophyta species. After sectioning, parts from multiple thalli were pooled and freeze-dried until constant mass. Samples were then ground to a fine powder using an IKA tube mill and stored in airtight containers at room temperature, away from sunlight.

Summary of Results

All methods, results and an in-depth analysis of these results can be found in: Distribution of hydrophilic and lipophilic arsenic species within the macroalgae, Ph.D thesis, May 2024, University of Iceland. Rebecca Sim. (available open access on skemman.is after May 2024)

Paper I: Potentially toxic elements in Icelandic Seaweeds

<http://dx.doi.org/10.2139/ssrn.4743806>

Seaweed is becoming an increasingly popular food source due to its high mineral and antioxidant content, and the European demand for seaweed is expected to rise during the coming years. However, seaweeds may accumulate high levels of potentially toxic elements (PTEs) such as As, Cd and Pb, which may pose a threat to the safety of consumers. This study aims to investigate trends in PTEs in several species of brown, red and green seaweeds collected in Iceland, and how these elements may vary between thallus section, location, and season.

Brown seaweeds generally contained the highest levels of As, Cd and U, Figure 2, all of which were highest in the reproductive tissues during February. High levels of As may be of concern for *Laminaria digitata*, as a large portion may be in the form of carcinogenic inorganic species (9). As concentration was also negatively correlated with the concentration of several essential elements (Mn, Fe, Co and Cu) suggesting that As accumulation may have an impact on the uptake of other elements. Concentrations of Cd were also found to be elevated in commercially relevant species when compared with those grown in France and Norway – in particular *Alaria esculenta* and *Palmaria palmata* – and may attributed to the abundance of basalt rock found in Iceland. Green seaweeds were found to contain the highest concentrations of Cr and Fe, but seasonal comparisons could not be drawn due to the lack of specimens available during the winter months.

Seaweed grown in Iceland was found to be relatively high in essential trace elements such as Cu, Zn and Fe, and all samples contained low levels of toxic Hg – where all were below the ML of 0.1 mg kg^{-1} allowed in food supplements. With regards to other toxic elements, over half of the samples contained levels of As higher than the ML of 40 mg kg^{-1} for seaweed-derived animal feed, and 25 samples exceeded the 3 mg kg^{-1} ML for Cd in food supplements - levels of Cd in *A. esculenta* sporophyll were as high as 17 mg kg^{-1} . Seaweeds grown or cultivated in Iceland have the potential to provide a source of essential minerals and trace elements, however the levels of Cd should be monitored, and certain species avoided for human consumption (i.e., *A. esculenta*).

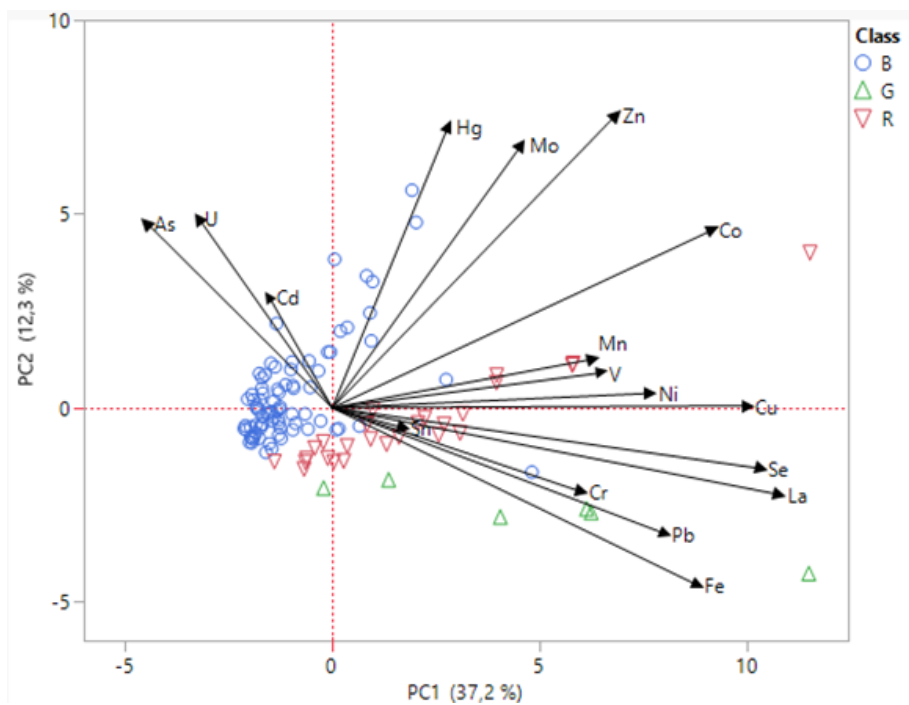


Figure 2 – Principal component analysis of the concentration of 17 elements in samples of Phaeophyta (B), Rhodophyta (R) and Chlorophyta (G) measured by ICP-MS.

Paper II: Inorganic arsenic in seaweed: a fast HPLC-ICPMS method without co-elution of arsenosugars

<https://link.springer.com/article/10.1007/s00216-024-05250-8>

Seaweed is known to accumulate high levels of As – which may be in the form of carcinogenic inorganic arsenic. If inorganic arsenic levels are to be regulated for this complex matrix, it is crucial that there are straightforward, fast methods available to ensure analysis is widely accessible. Here we propose a fast method for the routine measurement of inorganic arsenic in seaweed without co-elution of Arsenosugars that may complicate quantification. The addition of nitric acid and hydrogen peroxide to the extraction solution allows for a ‘partial speciation’ approach where inorganic arsenic is quantified as the sum of both inorganic species oxidised to As(V) and interferences from high levels of sulphur-containing Arsenosugars (AsSug-SO₃ and AsSug-SO₄) are removed by degradation to a dimethylarsenoribose with no side chain that elutes with AsSug-gly (10).

The developed method was optimised using a custom fractional factorial design of experiment (DOE) and tested on a range of reference materials including TORT-3 (0.36 ± 0.03 mg kg⁻¹), DORM-5 (0.02 ± 0.003 mg kg⁻¹), DOLT-5 (0.07 ± 0.007 mg kg⁻¹) which all showed good agreement with previously reported values (11,12). Additionally, for the reference material Hijiki 7405-b which has a certified value for inorganic arsenic (13), the recovery was found to be 99 ± 9%. The method was found to be suitable for high throughput analysis of inorganic arsenic in a range of food and feed matrices, Figure 3, including *Asparagopsis taxiformis* seaweed, grass silage and insect proteins, with an LOD of 0.006 mg kg⁻¹ and LOQ of 0.018 mg kg⁻¹.

The method requires minimal sample preparation and provides acceptable separation of inorganic arsenic from other analytes in under 7 minutes, Figure 3. However, it may be limited with regards to the quantification of DMA in seaweed, as the acidic extraction may cause overestimation of this analyte by causing degradation of lipid species that are typically more abundant in seaweed than other marine matrices such as Arsenosugarphospholipids. However, the concentrations of DMA quantified using this method may provide a better estimation with regards to exposure after ingestion and subsequent digestion of seaweed.

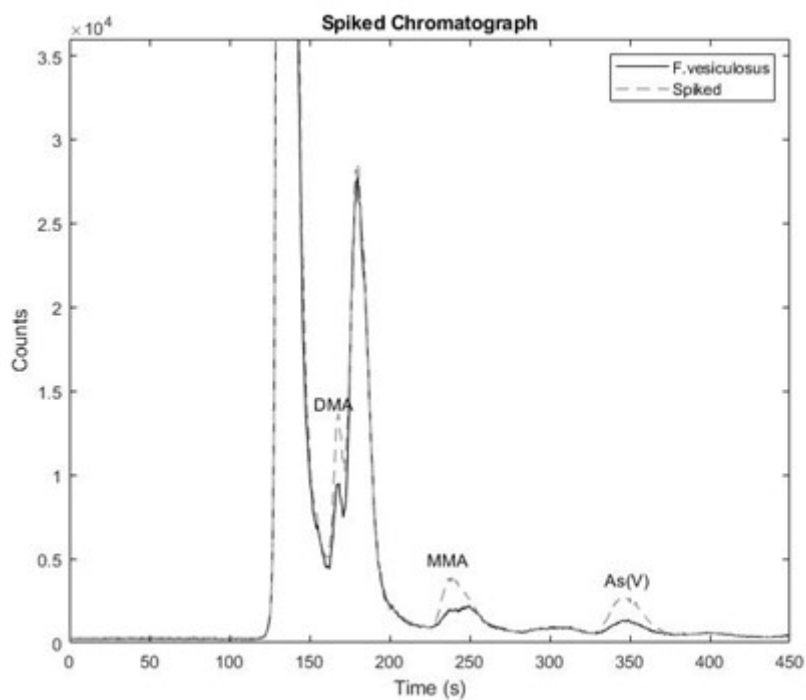


Figure 3 – The separation of inorganic arsenic (as As(V)) from other analytes using the developed HPLC-ICP-MS method. Extraction solution: 1% (v/v) nitric acid, 3% (v/v) hydrogen peroxide. Chromatography: 60 mM ammonium carbonate, 3% (v/v) methanol, isocratic elution. Column: PRP-X100 anion exchange, 250 x 4.1 mm, 10 μ m.

Paper III: The impact of different sample preparation methods on the arsenic speciation in Laminaria digitata

Brown seaweeds are known to accumulate high concentrations of As which may be metabolised to complex structures such as arsenic-containing sugars and lipids. Preservation of these large As compounds throughout sample preparation is crucial to ensure accurate results. The aim of this study was to investigate the impact of different drying methods on the water-soluble As speciation in *L. digitata*, as well as the fractionation of As before and after drying.

The distribution of As throughout the lipid-soluble, water-soluble, and residual fractions was changed significantly after drying, Table 2, where the polar lipid-soluble As accounted for 19.3% of the total As before drying and 1.1-3.8% after drying (t-test, $p < 0.03$). The residual As was also found to increase after drying depending on the method used, where oven drying at 110°C resulted in the highest concentration of non-extractable As ($9.12 \pm 1.81 \text{ mg kg}^{-1}$) compared to air-dried (7.0 mg kg^{-1}) and freeze-dried (6.7 mg kg^{-1}) samples. As has been demonstrated to be stored in the cell wall, with smaller amounts in the intracellular matrix in *L. digitata* (14). Perhaps the evaporation of water from the cytosol during drying does not allow cells to be opened sufficiently during extraction leading to an increase in residual As, particularly if residual As is bound as As(III) (or DMA(III)/MMA(III)) to sulphur-containing groups within the cell (15,16).

The water-soluble speciation was found to remain similar between drying methods, although lower levels of AsSug-gly were extracted from fresh material (10.5 mg kg^{-1}) than dried ($13.4\text{-}15.1 \text{ mg kg}^{-1}$). Freeze-drying removed higher levels of AsSug-PO₄ (32-42%) and AsSug-SO₃ (11-14%) than fresh, air-drying and oven-drying – suggesting that these analytes may be stored in the cell wall matrix which is damaged during freeze-drying due to the formation of crystals (17). AsSug-SO₄ was not detected. Oven-drying was found to increase DMA concentrations, likely due to thermal degradation of large As compounds (Arsenosugars or even AsLipids) to unknown, smaller arsenicals and DMA. As(V) concentrations were similar between fresh and dried samples ($22.1\text{-}25.4 \text{ mg kg}^{-1}$) - which means accurate results can be obtained for this carcinogenic species regardless of how the sample was prepared.

Table 2 – The arsenic fractionation in *Laminaria digitata* after sample preparation by freeze-drying, air-drying, oven-drying or no drying. Non-polar lipid soluble: hexane

Sample	Non-polar lipid-sol As (mg kg ⁻¹)	Polar lipid-sol As (mg kg ⁻¹)	Water-sol As (mg kg ⁻¹)	Non-extractable As (mg kg ⁻¹)	Total As (mg kg ⁻¹)	Recovery (%)
Fresh <i>L. digitata</i>	<LOQ (<0.1%)	12.3 ± 1.11 (19.3%)	51.1 ± 0.42 (80.3%)	2.61 ± 0.03 (4.1%)	63.6 ± 3.16	104 ± 2
Freeze-dried <i>L. digitata</i>	<LOQ (<0.1%)	2.39 ± 0.32 (3.8%)	51.9 ± 1.32 (81.6%)	6.71 ± 0.60 (10.6%)	63.6 ± 3.16	96 ± 1
Air-dried <i>L. digitata</i>	<LOQ (<0.1%)	1.41 ± 0.11 (2.3%)	57.1 ± 0.93 (93.5%)	7.04 ± 1.45 (11.5%)	61.1 ± 0.53	107 ± 1
Oven-dried <i>L. digitata</i>	<LOQ (<0.1%)	0.72 ± 0.15 (1.1%)	53.2 ± 0.68 (79.5%)	9.12 ± 1.81 (13.6%)	66.9 ± 4.61	94 ± 4
Hijiki 7405-b (<i>Sargassum fusiforme</i>)	<LOQ (<0.1%)	5.62 ± 0.17 (%)	21.5 ± 0.33 (%)	19.7 ± 0.91 (%)	50 ±	95 ±

extraction, polar-lipid soluble: DCM/methanol extraction, Water-soluble: water extraction, and Residual: non-extractable (digested). CRM Hijiki (7405-b) is also shown.

Paper IV: Temporal and intra-thallus variation in arsenic species in the brown macroalga Laminaria digitata

<https://www.publish.csiro.au/en/EN22123>

Arsenosugars account for the majority of total As in common seaweed species, yet it is unclear whether these are formed through some detoxification pathway for inorganic arsenic or are precursors/degradation products of arsenic-containing phospholipids in the cell wall. The aim of this study was to compare temporal and intra-thallus variations in water-soluble As and total non-polar and polar AsLipids in *L. digitata* to offer potential insight into the metabolism of As.

In general, lower levels of total As were detected in the samples collected in May (39.2-74.5 mg kg⁻¹) compared to those collected in February (72.6-151 mg kg⁻¹). The concentration of As(V) was found to consistently increase along the thallus from the holdfast/stipe (0.78-1.82 mg kg⁻¹) to the decaying fronds (44.4-61.0 mg kg⁻¹) in both months, and AsSug-SO₃ was the dominant Arsenosugar in the majority of samples, Figure 4. AsSug-SO₄ was not detected in any samples. The extraction efficiency was lower in fresh samples (64-77%) than in freeze-dried (95-116%) from the same month.

Water-soluble, polar AsLipids, and residual As concentrations were generally highest in February, and the non-polar AsLipids accounted for <0.42% of total As in all samples. The levels of polar AsLipids were significantly higher during February than in May, which is in line with the higher levels of total lipid content found in edible seaweeds during colder months (18). The residual As was higher in February where a maximum of 26.1 mg kg⁻¹ was found in the old frond compared with maximum of 10.1 mg kg⁻¹ in the young frond in May. The water-soluble fraction followed a similar trend to the As(V) concentration, where levels increased from holdfast/stipe to decaying frond and were highest in February.

This study is the first to report not only temporal, but intra-thallus variations in the water-soluble As speciation and distribution of As in *L. digitata*. This dataset adds significantly to our understanding of As metabolism of *L. digitata*, and how this may fluctuate temporally and spatially within algal thalli.

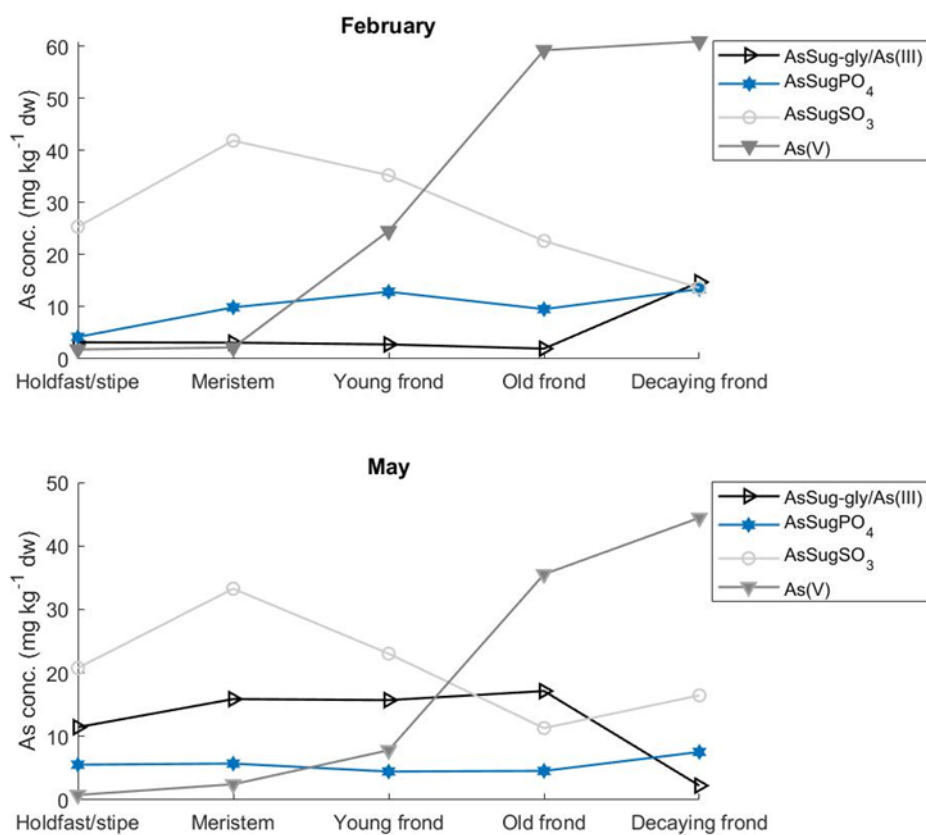


Figure 4 – The average concentrations of water-soluble As species in February and May 2022 in different sections of the *Laminaria digitata* thalli. Samples were analysed in duplicate (n=2) and all concentrations are expressed per kg of dry sample weight. Analysed by HPLC-ICP-MS following extraction with water.

Paper V: A comprehensive analysis of water-soluble arsenicals in Icelandic macroalgae

Seaweeds are abundant in arsenic-containing sugars, where they may comprise over half of the total As (4). The mechanism of Arsenosugars production in macroalgae is still largely unknown but has been shown to vary between algae species, thallus section and season. This study reports the As speciation of brown, red and green macroalgae collected in Iceland across two sampling months (May and February).

Arsenosugars were found to account for the majority of the extracted As in all seaweed samples (>50%), and only small amounts of DMA (<LOQ-0.41 mg kg⁻¹) and MMA (<LOQ-0.07 mg kg⁻¹) were present. All samples contained low levels of toxic, inorganic arsenic – where only in *Ulva intestinalis* (Chlorophyta) did concentrations exceed 1 mg kg⁻¹. The sulphate and sulfonate ribose derivatives were the dominant arsenicals in brown algae, whereas the phosphate ribose was generally the dominant As compound in red and green algae, Figure 5. The majority of seaweed species appear to have the ability to synthesise all four Arsenosugar derivatives, however it is unclear why some are produced to higher abundances than others. The glycerol and phosphate riboses were detected in every sample analysed, which might suggest that AsSug-gly is the starting point for AsSug-PO₄, which could be formed by AsSug-gly binding to phosphate-containing lipids in the cell membrane.

The production of Arsenosugars may be an efficient way of excluding As from intracellular matrix, or they may serve as useful components in the cell wall similar to polysaccharides in the cell wall matrix, as extremely high levels of Arsenosugars (<100 mg kg⁻¹) can be tolerated by the algae with no ill effects.

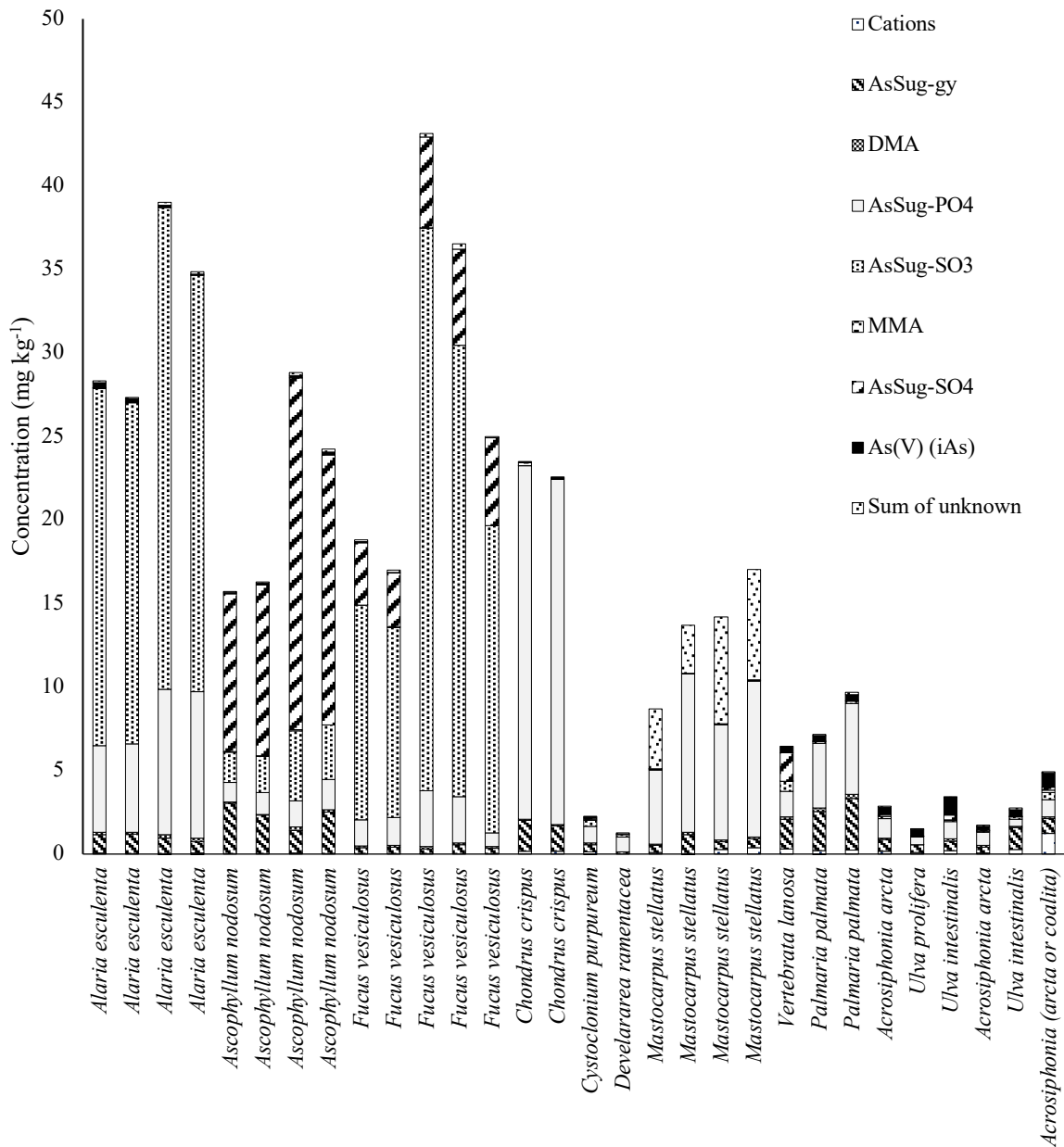


Figure 5 – The water-soluble composition of several species of seaweed collected in Iceland. Phaeophyta: *Ascophyllum nodosum*, *Alaria esculenta* and *Fucus vesiculosus*. Rhodophyta: *Cystoclonium purpureum*, *Devaleraea ramentacea*, *Vertebrata lanosa*, *Chondrus crispus*, *Mastocarpus stellatus*, *Palmaria palmata* and *Porphyra dioica*. Chlorophyta: *Ulva prolifera*, *Ulva intestinalis* and *Acrosiphonia arcta*.

Paper VI: Analysis of arsenic-containing lipids in Icelandic seaweeds

Seaweeds are known to sequester high levels of As - a potentially toxic metalloid - which may be metabolised to an array of complex lipid structures including hydrocarbons and phospholipids. The mechanisms of formation of these lipids are largely unknown but are thought to vary between brown, red and green seaweeds – where the latter two have been infrequently investigated. This study aims to investigate the distribution of AsLipids throughout marine macroalgae to help aid in understanding their formation.

In total, 35 different AsLipid species were identified across all samples, including 2 novel compounds (AsHC380 and AsFA398) identified in *A. nodosum*. Arsenosugarphospholipids were found to dominate in brown algae where Arsenosugarphospholipids958 (C16:0/C16:0) was most abundant in all except *L. hyperborea* where Arsenosugarphospholipids984 (C16:0/C18:1) dominated, Figure 6. Arsenosugarphospholipids were most concentrated in the reproductive tissues of the brown algae, where concentrations of Arsenosugarphospholipids958 ranged from 0.38-2.3 mg kg⁻¹. AsHC360 was also detected in relatively high concentrations in several samples where levels were highest in *F. vesiculosus* (0.16-0.64 mg kg⁻¹) and *Devaleraea ramentacea* (0.23 mg kg⁻¹).

Two ArsenosugarPhytol compounds were detected in the green seaweeds (AsSugPhytol546 and AsSugPhytol562), where the AsSugPhytol546 compound was generally the most abundant species found in *Ulva prolifera*, *U. intestinalis* and *Acrosiphonia arcta* but showed a dependence on sampling location for *U. intestinalis*. This arsenosugars phytol was also detected at trace levels in several samples of red seaweed including *Chondrus crispus* and *Porphyra dioica*, but it is unclear if this is produced by the algae or some epiphytic species that may reside on the surface of the seaweeds. These findings may have significant implications on the current understanding of As metabolism in Chlorophyta, as this is the first instance of ArsenosugarPhytols being reported in macroalgae.

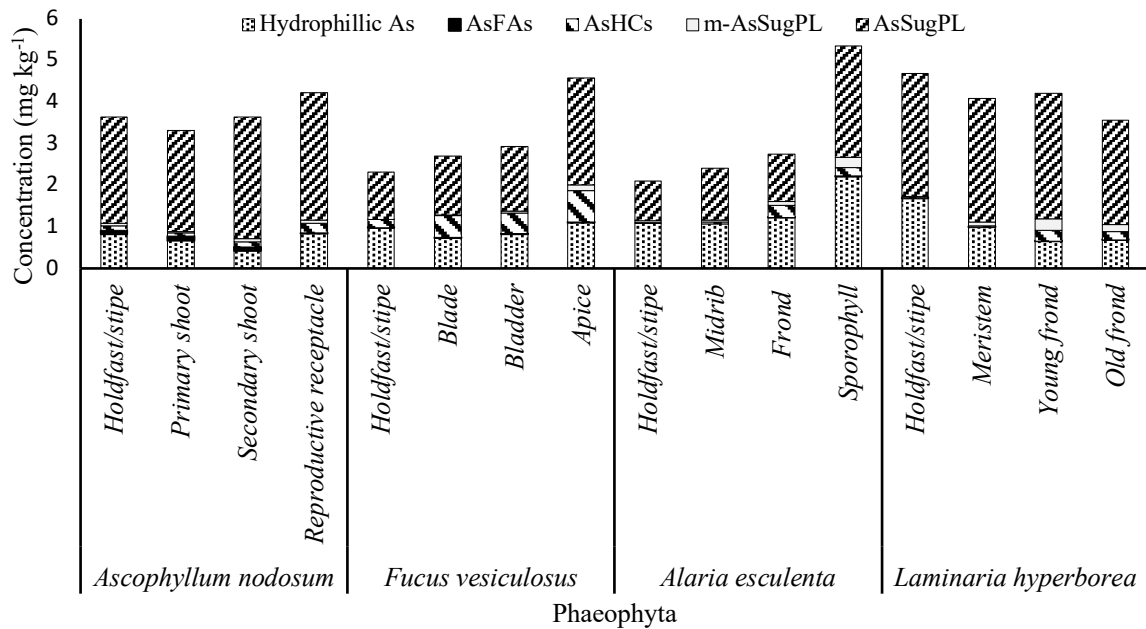
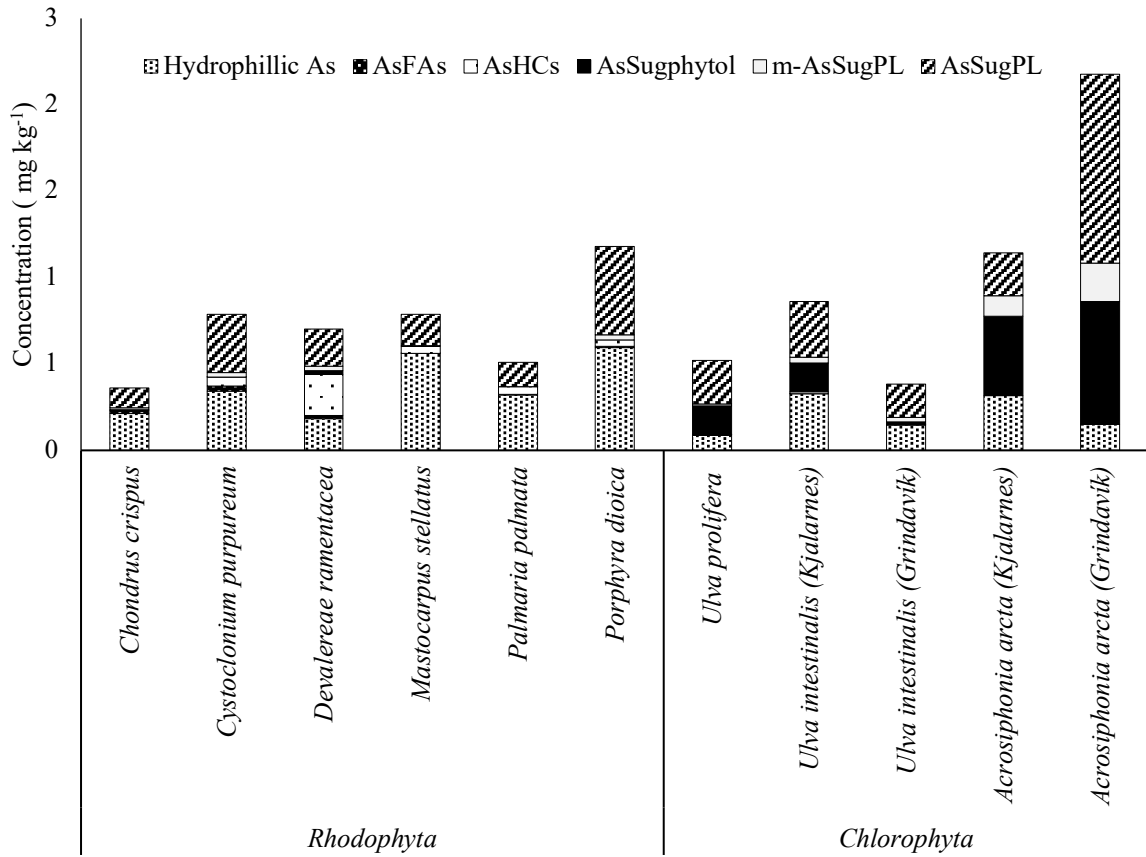


Figure 6 – The distribution of arsenic species based on retention time region, extracted using DCM/methanol in several samples of Rhodophyta, Chlorophyta and Phaeophyta collected in Iceland and analysed with HPLC-ICP-MS/ESI-MS and HPLC-qToF-MS. AsFAs: arsenofatty acids, AsHCs: arsenohydrocarbons, mArsenosugarphospholipids: mono-acylarsenosugarphospholipid, Arsenosugarphospholipids: di-acylarsenosugarphospholipid.

Conclusions

The aim of this project was to investigate differences in As speciation between several species of Phaeophyta, Rhodophyta and Chlorophyta. Hydrophilic As species were shown to vary significantly between seaweeds – where high concentrations of inorganic arsenic were only detected in *L. digitata* and Arsenosugar composition was highly dependent on species and season. All macroalgae appear to have the ability to synthesise all four Arsenosugar derivatives, but their production may be influenced by external factors. Lipophilic species additionally varied between species; However, differences were more pronounced between classes of seaweeds where Arsenosugarphospholipids were dominant in Phaeophyta and Rhodophyta (albeit in lower concentrations than Phaeophyta) and AsSugPhytols were most abundant in Chlorophyta. This would imply that the cycling of As is highly dependent on algal species, where different seaweed classes may have evolved different mechanisms and pathways for the metabolism of As.

Analysis of the variations in arsenic speciation between species, thallus sections and seasons was hoped to offer additional insight into how these compounds may be formed. Arsenosugars and Arsenosugarphospholipids were both most concentrated in the reproductive tissues of the brown seaweeds, which may be evidence that Arsenosugars are the starting product of Arsenosugarphospholipids as both share a dimethylarsenoribose moiety. The lipids are perhaps produced by the binding of Arsenosugars to phosphatidic in the membrane which would provide an explanation as to why these lipids also contain a phosphate moiety and two acyl groups. Whether these species are produced accidentally as a result of biological infidelity or are produced with a biological purpose is unclear. It does not appear that Arsenosugars serve as energy storage as is displayed with other sugars, however, their inclusion in the cell wall matrix may have yet to be discovered benefits (i.e., increased resistance to environmental changes).

This study has contributed significantly to understanding the occurrence of these compounds in marine macroalgae. Further research should focus on the use of imaging techniques to investigate the localisation of arsenic within cells, and the use of genetic techniques (i.e., gene 'knockout') in combination with speciation measurements to understand if algae have developed dedicated pathways for arsenic metabolism.

References

1. Ichikawa N. Is This Superfood the Secret to Living to 100 (And Good Hair)? [Internet]. 2023. Available from: <https://www.vogue.com/article/seaweed-superfood>
2. Gupta E, Mishra P. No Title. *Curr Nutr Food Sci*. 2021;17(2):144–66.
3. Petursdottir AH, Fletcher K, Gunnlaugsdottir H, Krupp E, Küpper F, Feldmann JJ, et al. Environmental effects on arsenosugars and arsenolipids in *Ectocarpus* (Phaeophyta). *Environ Chem*. 2015 Jan 1;13(1):21–33.
4. Taylor VF, Jackson BP. Concentrations and speciation of arsenic in New England seaweed species harvested for food and agriculture. *Chemosphere*. 2016 Nov;163:6–13.
5. Pétursdóttir ÁH, Blagden J, Gunnarsson K, Raab A, Stengel DB, Feldmann J, et al. Arsenolipids are not uniformly distributed within two brown macroalgal species *Saccharina latissima* and *Alaria esculenta*. *Anal Bioanal Chem* [Internet]. 2019/05/31. 2019 Jul;411(19):4973–85. Available from: <https://pubmed.ncbi.nlm.nih.gov/31152227>
6. Meyer S, Matissek M, Müller SM, Taleshi MS, Ebert F, Francesconi KA, et al. In vitro toxicological characterisation of three arsenic-containing hydrocarbons. *Metallomics* [Internet]. 2014 May 1;6(5):1023–33. Available from: <https://doi.org/10.1039/c4mt00061g>
7. Meyer S, Schulz J, Jeibmann A, Taleshi MS, Ebert F, Francesconi KA, et al. Arsenic-containing hydrocarbons are toxic in the in vivo model *Drosophila melanogaster*†. *Metallomics* [Internet]. 2014 Nov 1;6(11):2010–4. Available from: <https://doi.org/10.1039/c4mt00249k>
8. Bornhorst J, Ebert F, Meyer S, Ziemann V, Xiong C, Guttenberger N, et al. Toxicity of three types of arsenolipids: species-specific effects in *Caenorhabditis elegans*†. *Metallomics* [Internet]. 2020 May 1;12(5):794–8. Available from: <https://doi.org/10.1039/d0mt00039f>
9. Sim R, Feldmann J, Stengel DB, Pétursdóttir ÁH. Temporal and intra-thallus variation in arsenic species in the brown macroalga *Laminaria digitata*. *Environ Chem* [Internet]. 2023;20(2):55–65. Available from: <https://doi.org/10.1071/EN22123>
10. Gamble BM, Gallagher PA, Shoemaker JA, Wei X, Schwegel CA, Creed JT. An investigation of the chemical stability of arsenosugars in simulated gastric juice and acidic environments using IC-ICP-MS and IC-ESI-MS/MS. *Analyst*. 2002 Jun;127(6):781–5.
11. Gajdosechova Z, Grinberg P, Kubachka K, Wolle M, Raab A, Feldmann J, et al. Determination of inorganic As, DMA and MMA in marine and terrestrial tissue samples: a consensus extraction approach. *Environ Chem* [Internet]. 2023;20(2):5–17. Available from: <https://doi.org/10.1071/EN23006>
12. Marschner K, Pétursdóttir ÁH, Bücker P, Raab A, Feldmann J, Mester Z, et al. Validation and inter-laboratory study of selective hydride generation for fast

- screening of inorganic arsenic in seafood. *Anal Chim Acta* [Internet]. 2019;1049:20–8. Available from: <https://www.sciencedirect.com/science/article/pii/S0003267018313928>
13. Narukawa T, Raber G, Itoh N, Inagaki K. A New Candidate Reference Material for Inorganic Arsenic and Arsenosugars in Hijiki Seaweed: First Results from an Interlaboratory Study. *Anal Sci* [Internet]. 2020;36(2):233–7. Available from: <https://doi.org/10.2116/analsci.19P306>
 14. Ender E, Subirana MA, Raab A, Krupp EM, Schaumlöffel D, Feldmann J. Why is NanoSIMS elemental imaging of arsenic in seaweed (*Laminaria digitata*) important for understanding of arsenic biochemistry in addition to speciation information? *J Anal At Spectrom* [Internet]. 2019;34(11):2295–302. Available from: <http://dx.doi.org/10.1039/C9JA00187E>
 15. Navratilova J, Raber G, Fisher SJ, Francesconi KA. Arsenic cycling in marine systems: degradation of arsenosugars to arsenate in decomposing algae, and preliminary evidence for the formation of recalcitrant arsenic. *Environ Chem* [Internet]. 2011;8(1):44–51. Available from: <https://doi.org/10.1071/EN10107>
 16. Glabonjat RA, Blum JS, Miller LG, Webb SM, Stolz JF, Francesconi KA, et al. Arsenolipids in Cultured Picocystis Strain ML and Their Occurrence in Biota and Sediment from Mono Lake, California. *Life* (Basel, Switzerland). 2020 Jun;10(6).
 17. Kumar KN, Mallik S, Sarkar K. Role of freeze-drying in the presence of mannitol on the echogenicity of echogenic liposomes. *J Acoust Soc Am*. 2017 Dec;142(6):3670.
 18. Schmid M, Guihéneuf F, Stengel DB. Ecological and commercial implications of temporal and spatial variability in the composition of pigments and fatty acids in five Irish macroalgae. *Mar Biol* [Internet]. 2017;164(8):158. Available from: <https://doi.org/10.1007/s00227-017-3188-8>