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Concentrations and speciation of arsenic in New England seaweed species harvested for food and agriculture

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Abstract

A survey of arsenic (As) concentrations and speciation was conducted on 10 species of seaweed from commercial harvesters and from collection at two sites in New England. Concentrations of As ranged from 4–106 mg/kg, mostly in the form of arsenosugars, with the distribution of arsenosugar analogs varying between taxa. In brown algae, As levels were correlated with phosphate concentrations, and arsenosugar speciation reflected differences in sulfur and phosphate concentrations between taxa. Several samples of the brown algae species *Laminaria digitata* contained significant levels of inorganic As (2.8–20 mg/kg), the most toxic form of As. A weak acid extraction with microwave heating was compared with a weaker methanol: water extraction method, and found to give slightly higher extraction efficiency with comparable relative concentrations of inorganic As, supporting the use of this faster and simpler extraction method for monitoring. Seaweed is a niche dietary item in the U.S. but its popularity is increasing; it is also used in agriculture and livestock farming which provide potential indirect routes for human exposure. The presence of occasional high concentrations of iAs, as well as the lack of toxicity studies on organic As species, suggest that monitoring of these high As foods is warranted.

Graphical Abstract

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Keywords

macroalgae; arsenic; extraction; speciation; human exposure

1. Introduction

Seaweed harvesting is an important industry in some coastal areas, and a potentially sustainable resource for maritime states, including New England. Seaweeds contain high concentrations of some nutrients (eg. amino acids, Vitamin K, iodine) and are increasingly marketed for consumption. They are also used as feed supplements for livestock and aquaculture, and as soil amendment for crops. Seaweeds also contain high concentrations of arsenic (As), mostly in organic forms (Francesconi and Edmonds, 1998; Tukai et al., 2002) which are generally considered less toxic than inorganic As (iAs) (NAS, 2014). Most human exposure studies and regulatory guidelines are based on iAs, yet the high concentrations of As in seaweeds are cause for further investigation into the distribution of As concentrations and species among macroalgae species from different regions.

Marine algae concentrate As^V, presumably due to chemical similarity to PO₄³⁻, from seawater where it is ubiquitously present at low concentrations (1–5 µg/L) (Morita and Shibata, 1990; Caumette et al., 2012). Concentrations of total As in seaweeds are typically in the range of 20–100 mg/kg (McSheehy et al., 2002; Rose et al., 2007), higher than most terrestrial foods, and 2–3 orders of magnitude higher than the As concentrations in rice, a primary source of As exposure. Arsenosugars make up >85% of the soluble As in most seaweeds (Rose et al., 2007), with four analogs of arsenosugars commonly identified: arsenosugar glycerol (OH), phosphate (PO₄), sulfonate (SO₃), sulfate (SO₄) (Madsen et al., 2000). Taxonomy plays a strong role in the concentration and distribution of As species and arsenosugar analogs (Edmonds et al., 1993; Tukai et al., 2002), although location (Duncan et al., 2014) and nutrient availability has been suggested to affect As uptake (Granchinho et al., 2004). The pathway of conversion of As^V to arsenosugars is unclear; a series of methylation and subsequent adenosylation reactions have been proposed (Geiszinger et al., 2001), although As glycopospholipids have also been suggested to play an intermediary role (Garcia-Salgado et al., 2012b). While most seaweeds convert iAs to arsenosugars, certain seaweeds, particularly the brown algae *Hijiki fusiforme* harvested in Asia, accumulate significant amounts of iAs (Almela et al., 2006).

The toxicity of As is dependent on its chemical form or speciation. Exposure to iAs from drinking water sources has been linked to lung and bladder cancer (EFSA, 2009; Steinmaus et al., 2013), but diet, and particularly rice, which can contain a significant proportion of iAs (Williams et al., 2007; Zhu et al., 2008), has been identified as a larger contributor to iAs exposure in U.S. (Xue et al., 2010) and European (EFSA, 2009) populations. Until recently, only drinking water was regulated for As (U.S. EPA, 2009; WHO-IARC, 2011), but monitoring programs for foods high in As have been implemented in the U.S. and Europe. The U.S. FDA have recently issued an action level of 10 µg/L iAs for apple juice (U.S. FDA, 2013b), and are conducting a risk assessment for regulating As in rice (U.S. FDA, 2013a). The EU introduced regulations of 0.2 mg/kg for iAs in white rice and 0.1 mg/kg for iAs for

rice-based foods aimed at infants and children (European Commission, 2016). The U.S. EPA uses an oral reference dose of 0.3 μg iAs/kg body weight/day (U.S. EPA, 1998), but are in the process of reviewing exposure recommendations, as are the European Food Safety Authority. Advisories against hijiki consumption have been issued by both Canada and the United Kingdom, but other seaweeds are not considered a significant source of iAs. There are currently no regulations for organic As compounds, and human exposure and toxicological data are insufficient to evaluate exposure risk. Arsenobetaine (AB), which is the major As species in finfish but not algae, is not toxic to, or metabolized by, humans (Kaise et al., 1985; Francesconi, 2010). Trace amounts of methylated As species, dimethyl and monomethyl As (DMA and MMA), are also found in seaweeds, and are also major (DMA) and trace (MMA) constituents of rice, and have much lower toxicity than iAs (Petrick et al., 2000; Williams et al., 2005). Studies on arsenosugar toxicity are limited, and only arsenosugars-OH and -SO₃ have been tested and shown to have low cytotoxicity (Sakurai et al., 1997; Andrewes et al., 2004; Leffers et al., 2013a). Unlike AB, arsenosugars are metabolized, although with high variability between individuals (Raml et al., 2009), and one of the metabolites has demonstrated significant cellular toxicity (Leffers et al., 2013b; Ebert et al., 2014). Pending further investigation of metabolic pathways and toxic effects of these compounds, it has been suggested that arsenosugars, along with all organic As compounds other than AB, be considered potentially toxic (Feldmann and Krupp, 2011).

Exposure to As from seaweed via other pathways, such as its use as fertilizer and animal feed, is not well understood. While no regulation exists for As applied in fertilizers, recommendations for land application of sewage sludge are based on total As concentration, and limited to 41 mg/kg (U.S. EPA, 1993). Arsenosugars from seaweed applications to soil have shown to decompose to iAs over time (Castlehouse et al., 2003) suggesting a total As limit is appropriate in this instance. Animal feed is not regulated for As in the USA, but in the EU, a maximum limit of 40 mg/kg As has been implemented for complete fish feed containing seaweed, which assumes a level of iAs less than 2 mg/kg. (European Commission Directive, 2003; Petursdottir et al., 2015)

Seaweed presents a challenge to monitoring As in food and assessing As exposure, due to its variable and complex As species distribution. While As concentrations in seaweeds are high, regulations based on total As or on iAs in foods with higher consumption rates may unduly restrict seaweed use, despite its many attributes. A further difficulty comes from reliably quantifying As species in marine algae; a round-robin study of iAs determination in wheat, spinach and algae found performance was poor (20%) for iAs quantification in algae, and found that most labs overestimated iAs (de la Calle et al., 2012), likely due to peak overlap or misidentification. Furthermore, extraction efficiencies can be low with the use of mild extraction conditions (methanol, water) whereas acidic extractions (Lai et al., 1998) can cause transformation of the arsenosugar analogs (Gamble et al., 2002).

In this study, As speciation was determined in seaweeds harvested from New England, to determine variation across taxa in this region. A partial speciation method, for determining just iAs and weak-acid extractable As was also applied to a subset of samples to evaluate this simpler analytical approach for seaweed monitoring. This data was used to assess exposure to As from seaweed consumption, and from pathways based on agricultural use.

2. Materials and Methods

2.1 Sample collection

Seaweed samples were obtained from 7 commercial harvesters in northern Maine and manually collected from near shore sites within the Great Bay Estuary in New Hampshire (Fig. 1). These samples encompassed a wide range of taxonomic groups present in New England (Table 1). Commercially harvested seaweed samples included nine different species, including red ($n = 6$), green ($n = 1$) and brown ($n = 19$) macroalgae. Samples of 10 macroalgae species were collected in December 2012 from two locations in Great Bay, NH: Kittery, ME ($n = 10$), at the mouth of the estuary, and Adam's Point, Durham, NH ($n = 10$), which is further inland; these samples also included species of red ($n = 10$), green ($n = 6$) and brown ($n = 4$) macroalgae, and two different species (*Zostera marina*: $n = 2$; *Spartina alterniflora*: $n = 1$) of aquatic flowering plants. In addition, 2 samples of mixed seaweed, and 2 samples of liquid seaweed fertilizer, were purchased from commercial harvesters. Samples were air dried, and homogenized using a stainless steel coffee grinder. Liquid samples were also dried, and wet and dry weights were obtained.

2.2 Standards and reagents

Nitric acid was sub-boil distilled in-house, using Fisher Trace Metal Grade HNO_3 . Calibration standards for total As were prepared daily by dilution of multi-element stocks (Inorganic Ventures, Christianburg, VA) in 1% HNO_3 . Speciation analysis was calibrated using inorganic As(III) and As(V) standards (Inorganic Ventures), and MMA, DMA and AB were prepared by dissolving salts of monosodium methane arsonate (Chem Services, West Chester, PA, USA), cacodylic acid and arsenobetaine (Sigma Aldrich, St Louis, MO). Four arsenosugar compounds (arsenosugar OH, PO_4 , SO_3 , SO_4) and TMAO were kindly donated by Jack Creed and Patricia Creed at the Environmental Protection Agency, Cincinnati, Ohio. Where possible, certified reference materials were used for method validation of total and speciated As concentrations. Reference materials included DORM-2 (NRC, Ottawa, Canada) which is certified for AB and TMAO, an in-house kelp standard from a Brooks Rand Laboratory interlaboratory comparison (Briscoe et al., 2015) for which a consensus value of iAs was reached, and the Antarctic krill MURST-ISS-A2 for which there are literature values of As species (Grotti et al., 2010), was generously provided by M. Grotti, Genova, Italy. A weak acidic extraction was also performed on the NIST Rice flour Standard Reference Material (SRM) 1568a (Gaithersburg, MD) for which consensus values have been reported by several studies *eg.* (Batista et al., 2011; Tyson, 2013).

2.3 Total As, S, Fe and P determination

All dilutions of samples for total digestion and extraction were done by mass. Samples were weighed (0.25 g) into 10 mL Teflon vessels and 5 mL concentrated HNO_3 (sub-boil distilled Fisher Trace Metal Grade) was added to each vessel. Samples were heated to 180 °C for 10 min by high pressure microwave digestion (MARS XPRESS, CEM, Mathews, NC). Samples were then transferred to pre-weighed 60 mL vials (Sarstedt, Germany), and diluted to 50 mL with ultrapure water (18 M Ω cm, EMD Millipore, Darmstadt, Germany). The samples were diluted another 30 $\times$ with 1% HNO_3 into 7 mL vials for analysis. Samples were analyzed for As by collision cell ICP-MS (7700x, Agilent, Santa Clara, CA) with a

collision gas flowrate of 5 ml/min He. Samples were also analyzed by magnetic sector ICP-MS (Element 2, Thermo Scientific, Bremen, Germany) for S, Fe and P, using medium resolution mode ($m/m = 4000$). Recoveries of As in digested SRM's were $95 \pm 3\%$ ($n = 3$) for DORM-2 (18 mg/kg), $86 \pm 2\%$ ($n = 3$) for MURST-ISS-A2 (5.02 mg/kg) and $111 \pm 6\%$ ($n = 3$) for NIST 1568a (0.290 mg/kg).

2.4 Extraction for As speciation

Approximately 0.25 g of each homogenized sample was accurately weighed into a pre-weighed 20 mL vial (Sarstedt). An aliquot of 10 mL of 1:1 v/v methanol:water was added to each vial, and samples were sonicated for 1 h, then left overnight. Vials were then uncapped and placed in an oven and evaporated to near-dryness at 50 °C, then re-diluted in 10 mL water. Finally, a portion of the sample extract was filtered through a 0.2 µm pore-size syringe filter (Whatman, Maidstone, UK), and transferred to a 1 mL PTFE septa vial (Agilent). Duplicate and spiked samples were prepared at least every 20 samples for quality control and peak identification.

A subset of 30 samples were also extracted by a second method: 0.25 g homogenized sample was weighed into 50 mL polypropylene tubes, and 10 mL 1% HNO₃ was added. Samples were sonicated for 0.5 h, then left overnight. Samples were then microwave heated through a program of 10 minutes at 55°C, 10 minutes at 75°C and 30 minutes at 95°C (Foster et al., 2007). An aliquot was then removed and spin-filtered through 10 KDa spin filters (VWR, Radnor, PA), then treated with 10% H₂O₂ and warmed (50°C) for 2h. For both extraction procedures, an aliquot of 0.1 ml of the final solution was removed and diluted with 5 mL 1% HNO₃ for determination of total extractable As by collision cell ICP-MS.

Speciation analysis was performed on an Agilent LC1120 liquid chromatograph coupled to collision cell ICP-MS, using an anion exchange column (Hamilton PRP-X100 10µm 4.6 × 250 mm; Reno, NV) with a mobile phase of 20 mM (NH₄)₂CO₃ at pH 9, a column temperature of 40°C and a flow rate of 1.5 ml min⁻¹. The ICP-MS was operated in collision mode with He as the collision gas at a flow rate 2 ml min⁻¹. An additional separation method was used to verify identification of cationic species which were not strongly retained on the anion exchange column. Species were separated on a Supercosil SCX (5 µm 4.6 × 250 mm; Sigma Aldrich, St. Louis, MO) column with a mobile phase of 20 mM pyridine at pH 2.5, and a column temperature of 40 °C.

Recoveries of AB (16.4 mg/kg) and TMAO (0.248 mg/kg) in DORM-2 ($n = 4$) were $111 \pm 13\%$ and $94 \pm 8\%$. Speciation of As in MURST-ISS-A2 found in this study (in mg/kg, $n = 4$) was comparable to literature values (Grotti et al, 2010), given in italics: AsSugar-OH: 0.22 ± 0.03 (*0.26*), AsSugar- PO₄ = 0.33 ± 0.03 (*0.3*), AsSugar- SO₄ = 0.16 ± 0.02 (*0.13*); AB = 2.26 ± 0.4 (*1.9*); DMA = 0.25 ± 0.03 (*0.22*). Grotti *et al.* reported a value of 0.4 mg/kg for the compound trimethylarsonio propionate (TMAP); while we do not have an analytical standard for this compound, an As species with a similar retention time by cation exchange to that reported for TMAP had a value of 0.49 ± 0.09 mg/kg. Concentrations of iAs in NIST 1568a extracted with the 1% HNO₃ method ($n = 2$) were comparable to literature values (Batista et al., 2011) (in italics): 0.083 ± 0.010 (*96*) mg/kg iAs and 0.171 ± 0.031 (*173*) DMA.

3. Results and discussion

3.1 As concentration and speciation

Concentrations and speciation of As in New England seaweeds are summarized in Table 1. Total As levels ranged from 4.1 to 106 mg/kg, which is similar to concentration ranges found in other regions (Tukai et al., 2002; Diaz et al., 2012). Macroalgae are taxonomically diverse, with brown algae being distinct from red and green algae at the kingdom level (belonging to Chromista and Plantae, respectively), and red and green algae being separated at the subkingdom level (belonging to Biliphyta and Viridiphyta, respectively). Taxonomy has a strong effect on As storage, and total As concentrations were significantly higher in brown algae (Ochrophyta) than in red (Rhodophyta) or green (Chlorophyta) algae, which has also been observed in studies from other regions. (Edmonds et al., 1993; Tukai et al., 2002) The brown algae of the order Laminariales had both the highest As and the largest range of As concentrations (13.9 – 106 mg/kg). Concentrations of As in flowering plants (*Spartina altiflora* and *Zostera marina*) from the same sites in New Hampshire were low (3.4–5.2 mg/kg) compared to macroalgae, and As uptake in plants is thought to occur by a different mechanism via roots (Thomson et al., 2007), than in macroalgae which are epiphytic. Sample sizes for most species in this study were not sufficient to decipher differences between commercial samples from Maine and those collected in New Hampshire. Similar ranges of total As in *Ascophyllum nodosum* were observed between the two regions (20.1 – 29.8 mg/kg in seaweeds from four different harvesters in Maine ($n = 4$) and 15.3 – 27.4 mg/kg in samples from two sites in New Hampshire ($n = 3$). Extraction with 1:1 methanol:water recovered 7 to 117% of the total As (Table 1). Extraction efficiency was variable even among the same species, and green algae had the lowest As recovery. Low and variable extraction efficiency is often observed in seaweeds using mild extractants, (Raab et al., 2005; van Elteren et al., 2007) however, these conditions prevent changes in arsenosugar speciation. Even within one seaweed species large seasonal differences in the relative amount of soluble As have been observed, which may be related to environmental conditions or plant age (Lai et al., 1998).

Arsenosugars were the dominant water-extractable species in all seaweeds, with small proportions of methylated As compounds present in all samples, mostly as DMA. Trace levels of AB were present in several samples. Inorganic As was a minor component (<1 mg/kg) across taxa, with the exception of the brown algae, *Laminaria digitata*, where higher concentrations (2.8 to 20.4 mg/kg) were found in five samples all from different harvesters in Maine, as well as in two samples of mixed, granulated seaweed products (6.6 and 10.9 mg/kg) containing this species. Concentrations of iAs were low (<0.01 mg/kg) in one sample of *L. digitata* collected in New Hampshire, and also in 3 samples of *Laminaria longicruris* from Maine. Other studies have found significant levels of iAs in macroalgae, particularly documented in hijiki seaweed (*Sargassum fusiforme*), eg. (Rose et al., 2007) but also across other taxa (Tukai et al., 2002; Llorente-Mirandes et al., 2011; Duncan et al., 2015). Several studies have reported elevated iAs in *Laminaria sp.*: appreciable levels of As^V (7.1 to 15.2 mg/kg) were found in 4 out of 12 commercial kelp samples, 3 of which were identified as *Laminaria* (Nischwitz and Pergantis, 2006); in a sample of granulated *L. digitata* from Iceland used in an inter-laboratory calibration study, a consensus value of iAs

(23.8 mg/kg) was reported, (Briscoe et al., 2015) and 77 mg/kg As^V was reported in a sample of *Laminaria sp.* from France (Garcia-Salgado et al., 2012a). Significant proportions of iAs (49 %) were also reported in *L. digitata* from Norway, whereas samples of the brown algae *S. latissimi* from the same site were not elevated in iAs (Maulvault et al., 2015). Studies of *L. digitata* in other regions have reported only minor components of iAs (Castlehouse et al., 2003).

The reason for high iAs in some seaweeds is unclear; the transformation of iAs to arsenosugars has been explained as a detoxification pathway allowing seaweeds to store high concentrations of As, or as an accidental by product of another biochemical process (Caumette et al., 2012). The high concentrations of iAs in *hijiki* has been suggested to be due to a lack of genetic capability, or that it is too energetically expensive to complete the conversion (Rose et al., 2007). Elevated fractions of iAs (36 – 43%) were found in *Fucus sp* from a contaminated site relative to a nearby control area (8–14%) (Koch et al., 2007), suggesting that transformation becomes inefficient under high exposure (Geiszinger et al., 2001). Elevated iAs in seawater is unlikely to be an explanation for iAs observed in samples in this study, as they were harvested from multiple sites, and the effects were not observed across species. Speciation changes during storage have been suggested to result in higher iAs (Nischwitz and Pergantis, 2006). In a study of the red algae *Palmaria palmata* samples from Denmark and Maine, slightly higher levels of iAs were observed in older versus younger dulse leaves, suggesting time of harvest in the algae's life cycle may play a role (Mouritsen et al., 2013).

3.2 Controls on species distribution

Taxonomy played a strong role in the distribution of arsenosugars. In green macroalgae, three arsenosugars (OH, PO₄ and SO₃) were present, whereas in red algae, ribosides OH and PO₄, were present with only trace levels of the SO₃ and SO₄ analogs. In brown algae, arsenosugars OH, PO₄ and SO₃ were present in kelp from the order Laminariales and in one sample of sea potato (order Ectocarpales), whereas all four arsenosugars were present in Fucales, with the SO₃ or SO₄ ribosides being the dominant form, depending on the species. Similar distributions of arsenosugars across taxa have been observed in other studies (Francesconi and Kuehnelt, 2002; Tukai et al., 2002). Low concentrations of arsenosugars were observed in flowering plant samples in this and other studies (Chanco et al., 2010), although these are suggested to be due to algal epiphytes attached to the samples, and not produced in the plants themselves (Thomson et al., 2007).

A correlation between As and P concentration was observed in brown algae (Fig. 2; $r^2 = 0.45$, $p < 0.001$), but not in red or green algae, although sample sizes were lower for those phyla. The ratio of As to P (Fig 2 slope) in brown algae is 0.015, which reflects the approximate ratio of As to P in seawater of 0.02 (As = 0.002 mg/L; P = 0.1 mg/L), suggesting they are taken up by the same mechanism without distinction. Uptake of As has been proposed to occur via active PO₄ uptake mechanism (Sanders and Windom, 1980), but there are conflicting reports on the effects of P on As uptake in algae. Incubation experiments with high *Fucus sp.* using high concentrations of As, showed a deficit of PO₄ in growth media promoted As uptake (Granchinho et al., 2004), suggesting As competes with

PO₄ for uptake, whereas an incubation study with *A. nodosum* found no common uptake mechanism (Klumpp, 1980). In brown algae, Tukai et al. found increased macroalgal P concentrations coincided with higher As concentrations, but also with elevated levels of other elements, suggesting uptake increases with stimulated metabolism and growth (Tukai et al., 2002). Other nutrients, such as N, Fe, Si, are also essential to growth and metabolic processes in algae, and may also influence the uptake and transformation of As (Duncan 2015). There was no correlation between As and Fe in this study, but relationships between As^V and Fe have been observed in marine plants, which take up As via their roots (Thomson et al., 2007). The presence of arsenosugar SO₄ in seaweeds of the order Fucales corresponded with significantly higher concentrations of S than in Laminariales, which had higher concentrations of arsenosugar SO₃ (Fig. 3), although the mechanism of S uptake and assimilation in algae is not well understood (Giordano and Raven, 2014). Similarly arsenosugar PO₄ was present in Laminariales but not in Fucales, which also had lower concentrations of P. Differences in arsenosugar composition between seaweeds have been suggested to be due to the presence of different enzyme systems capable of carrying out the glycosidation reaction (Francesconi et al., 1991).

3.3 Partial speciation comparison

Slightly higher extraction efficiencies (Fig. 4a and b) for total and inorganic As were achieved using a 1% HNO₃ extractant with microwave heating relative to the methanol:water method. Similar extraction conditions (2% HNO₃, 90°C) were found to give optimal recoveries in a study of marine plants (Foster et al., 2007). With the 1% HNO₃ method, peaks for arsenosugars PO₄, SO₃, and SO₄ were not observed, whereas a large peak eluted with the void volume by anion exchange chromatography. With neutral extraction conditions, all four arsenosugars are preserved, whereas under the heated, acidic extraction, cleavage of the long R-side chains (R= glycerol, PO₄, SO₃, SO₄) of each of these arsenosugars occurs, forming a ribose molecule with an –OH side chain (m/z = 254), which co-elutes with arsenosugar glycerol by anion chromatography (Gamble et al., 2002). Oxidation of As^{III} to As^V also occurs under these conditions, as well as conversion of thio-analogs of arsenosugars to their oxo-counterparts. This “partial speciation” approach (Feldmann and Krupp, 2011) provides a simpler analytical separation and omits the need to evaporate the methanol from the sample prior to analysis while still obtaining an accurate total iAs measurement, making this method appealing for food monitoring purposes.

3.4 Implications for As exposure from seaweed

Several studies have assessed the bioaccessibility of As from seaweed using *in vitro* simulated digestion techniques, and found that, similar to extraction efficiency, bioaccessibility is variable between samples, with taxa playing some role in As solubility (Sartal et al., 2012). While relatively low levels of As bioaccessibility have been reported (11–29 %; (Sartal et al., 2012), several studies have found the majority of both iAs and total As, which is predominantly arsenosugars, in seaweed are solubilized by simulated digestion (Laparra et al., 2003; Almela et al., 2005; Koch et al., 2007; Brandon et al., 2014). Using a caco-2 cells as a model for the intestinal barrier, iAs has been found to have much higher permeability (62%) across the intestinal wall than arsenosugars (1.7–2.8%) (Leffers et al., 2013a), although following consumption of a pure arsenosugar compound by human

subjects, up to 98% was recovered in urine (Raml et al., 2009). The bioavailability of arsenosugars to bladders cells is also low relative to iAs, however uptake of thio-DMA^V, a cytotoxic metabolite of arsenosugars, is higher than As^{III} in these cells (Leffers et al., 2013a). The high bioaccessibility of iAs from seaweed, suggests it should be considered completely bioavailable when assessing exposure, similar to drinking water (Brandon et al., 2014). While the metabolism, distribution in the body, and toxicological behavior of organic As compounds require further study, arsenosugars have been shown to be metabolized, and are therefore also considered bioavailable to humans.

Currently, human exposure risk can only be evaluated based on iAs concentrations (NAS, 2014), and levels of iAs found in some *L. digitata* samples were significant relative to current guidelines. Consumption of a 5 g portion of this seaweed, which contain 2.8 to 20.4 mg/kg iAs, exceeds the exposure from drinking 1L of water at the safe drinking limit (10 µg/L iAs)(U.S. EPA, 2009; WHO-IARC, 2011). For a 75 kg adult, this results in a dose of 0.19 – 1.4 µg iAs/kg body wt., which makes up half to more than four times the current reference dose of 0.3 µg iAs/kg body wt. per day. Seaweed consumption in Japanese populations have been estimated at 4–7 g per day (Zava and Zava, 2011), but is not well documented in the USA or Europe, and is presumed to be much lower, with the exception of specific subpopulations (Brandon et al., 2014). However, changing trends in aquaculture and food consumption, as well as the use of seaweed products in food and dietary supplements could lead to significant increases in iAs exposure. While the consistently high concentrations of iAs in *hijiki* can be addressed by consumption guidelines, spuriously elevated levels of iAs found in other seaweeds are difficult address, and assessment from regular monitoring may be needed evaluate the causes of iAs and their consumption risk.

There are currently no guidelines for exposure to organic As, which are less toxic and therefore a lower priority for regulation than iAs. Yet, because arsenosugars are present at high concentrations particularly in brown algae, and are retained and metabolized in the humans with the formation of a cytotoxic intermediate (thio-DMA^V), further research into the exposure risk from these compounds is needed.

Aside from direct consumption, seaweed is used as a feed supplement in agriculture and aquaculture, and as a fertilizer for crops. In the Northeast U.S., *A. nodosum* is most commonly used for these purposes, due to its high biomass availability. Average total As concentrations in commercially harvested samples of this species were 20.1 – 29.8 mg/kg, as well as in seaweed-based liquid fertilizers (2.3 and 5.7 mg/kg), which are lower than the EPA guideline for sewage sludge added to crops (41 mg/kg). *A. nodosum* harvested in the North Atlantic and applied as fertilizer to golf courses was found to contain higher levels of As (43–92 mg/kg) (O'Neill et al., 2014). Other seaweeds, including *Laminaria sp.* and *Fucus sp.*, were used as fertilizer in the Scottish Isles (Castlehouse et al., 2003). Total As in *Laminaria* in this study exceeds the sewage sludge guideline, and is higher than the recommended level for As in complete fish feed (Petursdottir et al., 2015). In Scottish sheep which graze on seaweed, concentrations of As in tissue and organs were significantly elevated relative to grass-fed sheep (Feldmann et al., 2000).

The complexity of As speciation in seaweeds present a challenge to monitoring: based on a total As, the concentrations of As in all seaweeds are likely to exceed any regulatory limit set for other foods such as rice, which may unnecessarily limit their consumption. The need for As speciation analysis in food is clear (Francesconi, 2010), and a modified speciation approach provides a simpler analytical approach to seaweed monitoring. The presence of significant iAs in *Laminaria digitata* suggests periodic testing of at least this species is needed. With recent interest in the seaweed as a sustainable resource in the Northeast and its use in food and agriculture, a robust monitoring of seaweed products is warranted, along with further assessment of the toxicity and metabolic breakdown of organic As compounds.

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Highlights

- As concentrations were correlated with P in brown algae
- Elevated inorganic As was found in the kelp species *Laminaria digitata*.
- Inorganic As concentration was comparable between two extraction methods.
- Taxonomy and nutrient concentrations played a role in As species distribution.

Fig. 1.
Location of seaweed harvesting region in ME, and Great Bay NH, where samples were manually collected.

Fig. 2.

Concentration of As (mg/kg) vs P (%) in brown, red and green algae.

Fig. 3. Concentrations of arsenosugars (PO₄, SO₃, and SO₄ in mg/kg), P and S (in %) in macroalgae of the order Fucales and

Fig. 4.
a) Total extracted As and b) iAs concentration by 1% HNO₃ and methanol: water extraction.

Table 1

Concentrations of As species (mg/kg) in macroalgae, flowering plants, and fertilizer products from New England.

Algae	Order	Species	Site	n	As T	Extractable As	iAs	DMA	MMA	AB	AsSugOH	AsSugPO ₄	AsSugSO ₃	AsSugSO ₄
Ochrophyta	Ectocarpales	Colpomenia marina	NH	1	16.12	6.58	0.05	0.42	<MDL	<MDL	0.65	0.72	3.90	0.74
Ochrophyta	Fucales	Ascophyllum nodosum	NH	3	23.14 ± 6.82	10.43 ± 3.06	0.08 ± 0.03	0.21 ± 0.12	<MDL	<MDL	2.05 ± 0.77	0.59 ± 0.10	1.10 ± 0.11	5.18 ± 2.79
Ochrophyta	Fucales	Ascophyllum nodosum	ME	4	23.68 ± 4.33	12.53 ± 2.22	0.06 ± 0.08	0.35 ± 0.11	<MDL	<MDL	2.34 ± 0.84	0.50 ± 0.05	1.34 ± 0.42	5.65 ± 1.11
Ochrophyta	Fucales	Fucus spiralis	NH	2	16.27 ± 1.41	7.68 ± 1.26	0.04 ± 0.05	0.19 ± 0.06	<MDL	<MDL	0.56 ± 0.45	0.31 ± 0.04	3.63 ± 0.03	1.51 ± 0.49
Ochrophyta	Fucales	Fucus vesiculosus	NH	2	28.89 ± 2.16	12.90 ± 5.84	0.06 ± 0.04	0.18 ± 0.08	<MDL	<MDL	0.50 ± 0.29	0.42 ± 0.04	7.90 ± 3.94	1.74 ± 0.65
Ochrophyta	Fucales	Fucus vesiculosus	ME	2	32.76 ± 3.73	20.05 ± 5.87	<MDL	0.39 ± 0.11	<MDL	0.03 ± 0.03	2.21 ± 1.62	1.28 ± 1.07	14.84 ± 9.36	2.72 ± 0.69
Ochrophyta	Laminariales	Agarum clathratum	NH	1	61.84	44.32	<MDL	0.89	<MDL	<MDL	2.44	11.82	24.92	0.29
Ochrophyta	Laminariales	Alaria esculenta	ME	4	34.46 ± 13.72	24.03 ± 10.84	0.03 ± 0.04	0.47 ± 0.28	<MDL	<MDL	4.50 ± 1.81	5.69 ± 3.92	12.54 ± 8.29	0.01 ± 0.03
Ochrophyta	Laminariales	Laminaria digitata	NH	1	106.73	63.03	<MDL	0.94	<MDL	<MDL	3.43	8.20	46.09	0.28
Ochrophyta	Laminariales	Laminaria digitata	ME	5	50.38 ± 11.91	32.38 ± 15.24	8.32 ± 7.67	0.97 ± 0.41	0.06 ± 0.13	<MDL	5.10 ± 0.91	10.63 ± 8.71	10.23 ± 7.61	<MDL
Ochrophyta	Laminariales	Laminaria longicuris	ME	3	74.14 ± 8.01	52.40 ± 6.58	0.12 ± 0.04	0.78 ± 0.18	<MDL	0.02 ± 0.03	3.63 ± 1.91	11.20 ± 2.06	40.82 ± 3.20	0.06 ± 0.10
Ochrophyta	Laminariales	Saccharina latissima	ME	1	56.29	25.30	<MDL	0.39	<MDL	<MDL	0.28	2.79	15.45	<MDL
Rhodophyta	Bangiales	Porphyra umbilicalus	ME	1	20.73	24.20	0.12	0.25	0.05	<MDL	1.95	24.98	0.09	<MDL
Rhodophyta	Ceramiales	Heterosiphonia japonica	NH	1	8.24	1.75	0.47	0.05	0.04	<MDL	0.56	0.65	0.06	<MDL
Rhodophyta	Ceramiales	Polyphonia lanosa	NH	1	14.00	7.58	0.26	0.74	0.06	<MDL	2.22	2.10	0.27	1.46
Rhodophyta	Gigartinales	Chondrus crispus	NH	2	12.13 ± 0.97	5.83 ± 3.47	0.07 ± 0.08	0.19 ± 0.18	<MDL	0.02 ± 0.03	1.21 ± 0.36	4.44 ± 4.15	0.14 ± 0.12	<MDL
Rhodophyta	Gigartinales	Chondrus crispus	ME	1	6.10	4.20	0.06	0.29	<MDL	0.11	3.10	0.17	0.20	0.12
Rhodophyta	Gigartinales	Phyllophora pseudoceranoides	NH	1	4.17	2.52	0.14	0.21	0.06	0.19	0.17	1.56	0.07	0.14
Rhodophyta	Gracilariales	Gracilaria vermiculophylla	NH	1	11.80	2.85	0.23	0.06	0.09	<MDL	1.13	1.24	0.07	<MDL
Rhodophyta	Palmariales	Palmaria palmata	ME	4	8.95 ± 4.80	7.43 ± 3.34	0.06 ± 0.11	0.96 ± 0.35	0.03 ± 0.01	<MDL	3.03 ± 1.50	4.35 ± 3.22	0.16 ± 0.16	0.01 ± 0.02
Chlorophyta	Cladophorales	Chaetomorpha picquotiana	NH	1	6.69	2.41	0.09	0.22	<MDL	0.10	0.87	0.34	0.17	<MDL
Chlorophyta	Ulotrichales	Gayralia oxysperma	NH	1	12.68	1.25	0.17	0.04	<MDL	<MDL	0.57	0.02	0.03	<MDL
Chlorophyta	Ulvales	Ulva lactuca	NH	1	5.34	0.35	0.13	<MDL	<MDL	0.03	0.07	0.02	0.01	<MDL
Chlorophyta	Ulvales	Ulva lactuca	ME	1	4.13	1.00	0.02	0.05	<MDL	0.05	0.06	0.15	0.06	<MDL
Chlorophyta	Ulvales	Ulva prolifera	NH	1	14.65	1.06	0.12	0.04	<MDL	<MDL	0.46	0.06	0.10	<MDL
Tracheophyta	Alismatales	Zostera marina	NH	2	4.42 ± 1.12	1.48 ± 1.27	0.27 ± 0.08	0.21 ± 0.29	<MDL	0.02 ± 0.03	0.28 ± 0.27	0.04 ± 0.02	0.46 ± 0.07	0.08 ± 0.11

Algae	Order	Species	Site	n	As T	Extractable As	iAs	DMA	MMA	AB	AsSugOH	AsSugPO ₄	AsSugSO ₃	AsSugSO ₄
Spermatophyta	Cyperales	Spartina alterniflora	NH	1	3.40	0.54	0.10	0.02	<MDL	<MDL	0.08	0.05	0.17	<MDL
Mix 1		soup1	ME	1	57.29	40.00	6.56	0.85	<MDL	0.07	3.69	8.96	17.37	<MDL
Mix 2		soup2	ME	1	49.03	36.40	10.92	0.94	<MDL	0.03	3.83	8.24	13.87	<MDL
Fertilizer	Fucales	Ascophyllum nodosum - fert	ME	1	2.29	2.60	0.08	1.42	<MDL	<MDL	0.78	<MDL	0.29	<MDL
Fertilizer	Fucales	Ascophyllum nodosum - fert fish	ME	1	5.76	4.00	0.58	0.80	<MDL	2.98	<MDL	<MDL	0.04	<MDL