An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

14SF860 - Arsenic in marine macroalgae and implications for commercial uses

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

The interdisciplinary AsMARA team established an analytical method for the determination of inorganic arsenic (As_{inorg}) seaweeds, and to characterise the levels of total arsenic (As_{TOT}) and As_{inorg}, including intra-plant variability, in commercially relevant seaweeds from Irish coastal waters. Environmental factors that govern As_{TOT} and As_{inorg} occurrence and the effects of processing and storage were investigated.

Brown seaweeds in particular, often contained high natural levels of As_{TOT} but very low levels of toxic As_{inorg}. A unique exception was

Laminaria digitata, which had high concentrations of As_{inorg}, increasing through the thallus. These novel results were published in Chemosphere. A seasonal study on levels in *L. digitata* and *Ascophyllum nodosum* found no temporal differences. The effects of drying method, storage conditions, soaking and cooking in *L. digitata*, *Palmaria palmata* and *Saccharina latissima* were investigated. Drying method did not alter the ratio of inorganic to total arsenic whereas differences were observed following 30 days' storage in air-dried L.

digitata. Soaking and boiling significantly decreased As_{TOT} content in the three species and As_{inorg} in *L. digitata*. While this may provide a mitigation strategy for consumers, the resulting soaking and cooking waters contained appreciable amounts of As_{TOT} for the three species, and As_{inorg} in *L. digitata*.

Results were disseminated in peer-reviewed publications, an end-of-project workshop presenting key findings to stakeholders from industry, academia, the FSAI, DAFM and BIM, and information leaflets for industry. The project provided findings that assist industry in considering future product-development strategies and policy makers with a remit for developing consumer protection regulation and advice. Selected findings of this project were presented to the European Food Safety Authority (EFSA) by the FSAI in 2018. Data derived from the project are also informing the FSAI's *Ad hoc* committee on seaweed, evaluating the risks of seaweed consumption in Ireland.

KEYWORDS

Arsenic, Macroalgae, Food

ACRONYM	AsMARA
PROJECT COORDINATOR, INSTITUTION	Dr. Evin McGovern, Marine Institute.
EMAIL	evin.mcgovern@marine.ie
COLLABORATORS, INSTITUTION	Dr. Dagmar Stengel, NUI Galway.
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Section 1 - Research Approach & Results

Start Date

01 May 2015

End Date

30 September 2018

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 1: Basic Principles Observed

NRPE Priority area

Food for Health

Total DAFM Award

€246,737.41

Total Project Expenditure

€246,737.41

Rationale for undertaking the Research

Seaweed has long been an important resource in Ireland, used as food, fertiliser and for animal feed. There is renewed interest in using this resource for a host of products in the food, feed, agricultural, cosmetics, and biotechnology sectors with a predicted estimated worth for the seaweed sector of €30million per annum by 2020 stated in SeaChange which was published by the Marine Institute in 2006. A barrier to developing this industry relates to high levels of arsenic found in many types of seaweed. Arsenic (As) is an element naturally present in seawater as well as introduced by human activities and can occur at high levels in marine biota. Arsenic may be present as inorganic arsenic (As_{inorg}) or bound in organic compounds with many different organo arsenics known. Inorganic arsenic is much more toxic than organic forms, many of which are considered to exhibit little or no toxicity to humans. Marine algae cannot discriminate in uptake between phosphate and arsenic in seawater but convert the As_{inorg} to arsenosugars. There are no EC regulatory limits for arsenic in seafood although new limits for As_{inorg} are anticipated. Directive 2003/100/EC amending of Directive 2002/32/EC sets a maximum limit for total arsenic (As_{TOT}) in seaweed meal and feed materials derived from seaweed of 40 mg/kg (12 % moisture). Frequent noncompliance with this limit presents a significant problem for the algal-based feed industry in Ireland. The requirement for a national baseline of As in commercially relevant macroalgae species from Irish waters was identified.

Methodology

Analytical methods (Task 1): A robust reliable quality assured method for the determination of AsV following conversion of AsIII to AsV, dimethylarsinic acid (DMA), monomethylarsenous acid (MMA) and arsenobetaine was optimised in the Marine Institute in year 2. It was based on CEN method (I.S EN 16802:2016: Foodstuffs – Determination of elements and their chemical species. Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS), with some modifications. It was successfully used to analyse samples collected as part of this project in Tasks 2 to 4 inclusive. Analysis of total metals was conducted using the Marine
Institute's ISO17025 accredited test method for total metals in marine biota. Data quality assurance was of paramount importance hence the selection of European Standard methods and accredited methods where available. Certified reference materials were used where available and a subset of samples for full arsenic speciation were subcontracted to the Trace Element Speciation Laboratory Aberdeen
(TESLA) at the University of Aberdeen for confirmatory analysis. A macroalgae laboratory reference material was also prepared. Commercially relevant macroalgae species were sampled from four locations around the Irish coast (Donegal, Sligo, Galway Clare) to establish baseline levels on As_{TOT} and As_{inorg} (Task 2). Additional studies on intra-plant differences, seasonality and factors influencing arsenic uptake were undertaken with repeat sampling of macroalgae and water parameters at eight different sites along the coasts of Galway and Clare, collected in six sampling events between June 2016 and April 2018 (Task 3). The effects of drying method, storage conditions and duration, soaking and cooking on total and inorganic arsenic concentrations in L.
digitata, *Palmaria palmata* and *Saccharina latissima* were investigated in laboratory studies (Task 4). Data from each of the tasks is currently being entered into the Marine Institute's national database on contaminants in the Marine Environment.

Project Results

Ten different species of commercially relevant seaweeds were sampled at a number of locations. Results highlight that levels of total As (AsTOT) often exceeded the 40 mg/kg limit for seaweeds used in animal feed, with concentrations highest in brown seaweeds, followed by reds and then greens (for the species sampled). No clear spatial differences were observed. While the majority of seaweeds studied had low levels of inorganic arsenic (As_{inorg}), *Laminaria digitata* (sold as Kelp or Kombu) had appreciable amounts, with a high degree of variability both between and within thalli. There are few reports of As_{inorg} in *L. digitata* and such high concentrations and distribution have not been previously reported. Remarkably other kelp species tested, *Laminaria hyperborea*, which has strong morphological similarities to *L. digitata*, and *Saccharina latissima* did not show high As_{inorg}. Variations in As_{TOT} and As_{inorg} were observed in *L. digitata* and *A. nodosum*, during repeat sampling at eight sites along the Galway and Clare coasts and while there were slight elevations in As_{inorg} and As_{TOT} in *L. digitata* in March 2017, clear seasonal differences were not observed. Small amounts of As_{inorg} were detected in all *A. nodosum* samples, comprising less than 1% of the As_{TOT} content. Concentrations of As_{inorg} in *L. digitata* were significantly higher and as with previous work in the project, concentrations increased through the thallus with the highest concentrations detected in the decaying distal tips. The drying method utilised was found to have a small but significant effect on As_{TOT}

and As_{inorg} concentrations in *L. digitata*, and As_{TOT} and DMA in *Palmaria palmata* and *Saccharina latissima*, potentially related to differences in drying efficiency. There were significant decreases in As_{inorg} and increases in As_{TOT} and DMA in *L. digitata* following 30 days' storage, most pronounced in samples stored at room temperature. A greatly beneficial finding for the FSAI and industry is that soaking prior to cooking significantly reduced both As_{inorg} in *L. digitata*, and As_{TOT} content in *L. digitata*, P. palmata and *S. latissima*, with cooking further reducing this content. While this may provide a mitigation strategy for consumers, much of this transferred to the soaking and cooking water, thus the use of these seaweeds in broths or soups still presents a source of both As_{inorg} and As_{TOT}. Project data on the concentrations of As_{TOT} and As_{inorg} are being used by the FSAI to assess potential risks of seaweed consumption. The finding that *L. digitata* contains significant amounts of As_{inorg}, similar to Hijiki for which the FSAI recommend limited consumption, is of great importance. A number of retail sourced samples of macroalgae were analysed, with similar findings to wild samples collected in the project, including As_{inorg} in *Laminaria digitata* but not in the other species tested. Due to the nature of the Marine Institute's ICP-MS method for total metal analysis, data were also generated on levels of additional trace metals including cadmium and lead in seaweeds. These data are feeding into the FSAIs '*Ad hoc* Committee on Seaweed', of which the project team are members.

Section 2 - Research Outputs

Summary of Project Findings

The strong interdisciplinary team established during AsMARA developed national expertise in arsenic speciation in commercially important seaweeds. It laid the foundation for further research into mechanisms controlling As uptake and mitigation strategies to reduce As_{TOT} and As_{inorg} concentrations. A close working relationship was formed with Professor Joerg Feldmann and team (University of Aberdeen), a leading researcher in arsenic speciation, collaborating on two peer-reviewed publications with further work in discussion. Data on drying methods and storage demonstrates increases in As_{inorg} in air-dried *L. digitata* following storage, of interest to industry and policymakers. The observed reductions in As_{inorg} and As_{TOT} following soaking and cooking will help inform policy makers in defining advice for consumers, and may benefit industry when used as a mitigation measure, although consumption in broths and soups presents an exposure risk. The comprehensive HPLC-ICPMS method established in the Marine Institute will continue to be used beyond the life of AsMARA, testing marine biota (including fish) as part of routine monitoring. This infrastructure provides scope for further research into arsenic speciation. Levels of As_{TOT} often exceeded the limit set for seaweed in Directive 2003/100/EC, particularly in brown seaweeds. This poses potential challenges for industry although As_{inorg} was low in the majority of species studied. The advice and initial findings of this project have been presented to EFSA by the FSAI. Additional data on concentrations of trace metals was generated and will prove useful to regulators and industry given current discussions on establishing limits for metals such as cadmium and lead as well as arsenic in marine algae. Data from this project on the species studied is informing current policy makers via the FSAI's *Ad hoc* committee on seaweed (of which the team are members), supporting a national risk assessment and development of consumer advice on the consumption of marine algae.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
Post Doctorates	0	1	1
Other / Temporary	1	0	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	4	1. Ronan, J.M., Stengel, D.B., Raab, A., Feldman, J., O'Hea, L., Braletei, E, and McGovern, E. High proportions of inorganic arsenic in *Laminaria digitata* but not in *Ascophyllum nodosum* samples from Ireland. Chemosphere (2017); 186:17-23. DOI: 10.1016/j.chemosphere.2017.07.076 2. Bralatei E, Neksosiute K, Ronan JM., Raab A, McGovern E, Stengel DB, Krupp DM and Feldmann J. 2017. A field deployable method for a rapid screening analysis of inorganic arsenic in seaweed. Microchimica Acta. (2017) 184:1701– 1709 3. Smith, J.M., Gargan, S., O'Hea, L., Feldmann, J.F., McGovern, E., and Stengel, D.B. The effect of drying, storage, and processing on the concentrations of total and inorganic arsenic in macroalgae species used for human consumption and animal feed. In prep. 4. Smith, J.M., O'Hea, Crowley, R., L., Stengel, D.B., and McGovern, E. Seasonal and spatial variation in total and inorganic arsenic in *Ascophyllum nodosum* and *Laminaria digitata*. In prep.

Other	5	Scientific abstracts or articles including those presented at conferences: 5. Arsenic in Marine Macroalgae and Implications for Commercial Uses (AsMARA) - poster presentation. Authors: Jenny Ronan, Evin McGovern, Dagmar B Stengel 6. Arsenic in Marine Macroalgae and Implications for Commercial Uses (AsMARA) - poster presentation. International Seaweed Symposium, 2016. 19 to 24 June 2016. 7. Algae as a sustainable source of high value products. Oral presentation. World Seafood Congress, Reykjavík. 10-13 Sept 2017 8. Variations in total and inorganic arsenic in marine macroalgae – oral presentation by J. Ronan (Smith). Authors: Jenny Smith (Ronan), Evin McGovern, Dagmar B Stengel. Irish Algal Researchers Conference. 06/09/18 and 07/09/18 9. Total and inorganic arsenic in marine macroalgae – poster presentation. Authors: Jenny Smith (Ronan), Linda O'Hea, Joerg Feldmann, Dagmar B. Stengel, Evin McGovern. FSAI Science Conference August 21-22, 2019

Intellectual Property

Data are open access and publicly available. Due to the many current uncertainties of As and metal toxicity associated with seaweeds and seaweed products, the research undertaken here will represent essential fundamental knowledge which can in the future be used to develop new products or processes. Such processes may relate to storage, drying or extraction of seaweed biomass and related to changes in potential toxicity through the profiling of different arseno compounds.

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Processes	A close working relationship was formed with Professor Joerg Feldmann (University of Aberdeen), a leading researcher in the field of arsenic speciation in seaweeds, and his team at the Trace Element Speciation Laboratory (TESLA). Professor Feldmann acted as an advisor on the Project Advisory Committee. The teams collaborated on two peer reviewed publications and further work is in discussion. The collaboration with TESLA in trialling a cost-effective field kit for screening for inorganic arsenic in macroalgae is an additional element not foreseen in the project proposal. Developments of such novel techniques have significant potential for use by industry and regulators.	1
New Processes	The new HPLC-ICP-MS method for As_{inorg} in macroalgae is validated and operational for use in routine marine monitoring programmes, with potential to expand to other marine organisms where high levels of As are also known to occur.	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Socio-Economic	This project has developed national expertise in the analysis of arsenic speciation in commercially important seaweeds and laid a foundation for further research into the mechanisms controlling As uptake and mitigation strategies to reduce As_{TOT} and As_{inorg} in commercial products. A comprehensive HPLC-ICPMS method has been established in the Marine Institute, capable of identifying a number of different arsenic species in marine macroalgae. The method developed will continue to be used beyond the life of the project for testing of inorganic arsenic in marine biota (including fish) as part of routine monitoring. This is anticipated to be an EC monitoring requirement as new regulations/maximum limits for inorganic arsenic are under discussion at EC level. As_{TOT} and As_{inorg} data from the range of species studied will inform current policy makers and direct the focus of future research and highlight potential issues, such as the elevated As_{inorg} in *Laminaria digitata*. The scope of this method may be extended to include other arsenic species such as arsenosugars, prevalent in marine macroalgae, the toxicity of which is still being elucidated. This infrastructure provides scope for further research into the area of arsenic in marine macroalgae and fish.
Socio-Economic	The European Food Safety Authority (EFSA) is addressing risk of arsenic exposure to consumers and has a particular interest in macroalgae. The advice and initial findings of this project were presented to EFSA by the FSAI on behalf of the project team in 2018 with a further update provided in Nov 2018 by request of EFSA and FSAI. Data derived from the project is also being utilised by the FSAI in their *Ad hoc* committee on seaweed, which is assessing the risks to Irish consumers from seaweed consumption. The project team are members of this committee. The use of ICPMS enabled the measurement of concentrations of additional trace metals. This baseline data will prove useful to regulators and industry given current discussions on establishing limits for metals such as cadmium and lead as well as arsenic in marine algae.
Industry	An information leaflet for industry entitled 'Arsenic in Marine Macroalgae and Implications for Industry (AsMARA)' was published, sent to seaweed industry contacts and made available online in October 2015. It introduced the issue of arsenic in seaweeds and set out the aims of the AsMARA project. A second leaflet is currently in draft, highlighting the results of the project and is due for publication in late 2019.

Industry	Levels of As_{TOT} often exceeded the 40 mg/kg (12% moisture) limit set for seaweed in Directive 2003/100/EC, particularly in brown seaweeds including *Alaria esculenta*, *Fucus* spp, *Ascophyllum nodosum*, *Chondrus crispus*, *Laminaria digitata* and *Saccharina latissima*. This poses potential challenges for industry. Inorganic arsenic was low in the majority of species studied, with the exception of *Laminaria digitata*. This may pose a potential risk to consumers, however selective harvesting and processing strategies may minimise this risk, e.g., appropriate species selection or discarding the distal and decaying distal blades, which were shown in this research to contain the highest proportion of As_{inorg}.
Industry	Data on drying methods and storage is of use to industry and policy makers as it demonstrates that there may be increases in As_{inorg} concentrations in *L. digitata* following storage for air-dried material; however, this was a small-scale study which requires expansion. The observed reductions in As_{inorg} and As_{TOT} concentrations following soaking will help to inform policy makers in defining advice for consumers to mitigate the risks of consuming these species and may benefit industry when used as a mitigation measure, although consumption in broths and soups may still present a route of exposure.
Other	A strong interdisciplinary project team was established during this project combining complementary analytical and contaminant expertise of the Marine Institute with macroalgae expertise brought by NUIG. The combination of State Sector official laboratory with associated policy-links and academic partner is effective and the team continues to collaborate beyond the project.

Dissemination Activities

Activity	Details
Workshops at which results were presented	Arsenic in Marine Algae (AsMARA) – Implications for Industry, Consumers and Regulators. 3rd October at the Marine Institute, Rinville, Oranmore, Co Galway
Other	Project webpage: www.marine.ie/Home/AsMARA
Other	An information leaflet entitled 'Arsenic in Irish marine macroalgae - implications for industry' was published in October 2015. A second leaflet updated with findings and recommendations from the project is currently in draft and will be published in late 2019.

Section 3 – Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€24,581.45	The Marine Institute's Agilent 7700x ICPMS and additional laboratory/equipment was utilised for the project work. Specifically, for delivery of this project the Marine Institute procured an additional HPLC for hyphenation with Agilent 7700x ICPMS for development of a speciation method.
Additional Staff	€100,000.00	Additionally, 2 MI staff and the senior lecturer in NUIG were directly involved in the delivery of this project, each giving 10 % of their time to the project over the three years and four months of AsMARA.

Future Strategies

Through AsMARA, the project team has become established as the key team in Ireland and developed relevant international connections with leading researchers in the field of arsenic speciation. The team members are advisors on the FSAI's *Ad hoc* Committee on Seaweed, assessing the risks to Irish consumers posed by seaweed consumption, with data from the project feeding into its assessment report. Dr. Wayne Anderson, FSAI, presented the work of AsMARA to the EFSA Working Group on Emerging Risks in 2017 and 2018. The team is now in a position to further this research and address questions that have arisen, such as what controls As_{inorg} in *L. digitata* in uncontaminated waters around Ireland? There are also knowledge gaps on occurrence, fate, controls and toxicology of arsenic in seaweed, which may warrant further research. Further monitoring and enhanced datasets will enable Irish authorities to support EFSA risk assessments that may be undertaken relating to use of marine algae in food and feed. These data will support ground-truthing any proposals for EC maximum limits; and provide data supporting national risk assessments and consumer-advice on consumption of marine algae.

Project Publications

Peer-reviewed publications, International Journal/Book chapters:

1. Ronan, J.M., Stengel, D.B., Raab, A., Feldman, J., O'Hea, L., Braletei, E, and McGovern, E. High proportions of inorganic arsenic in *Laminaria digitata* but not in *Ascophyllum nodosum* samples from Ireland. Chemosphere (2017); 186:17-23. DOI: 10.1016/j.chemosphere.2017.07.076
2. Bralatei E, Neksosiute K, Ronan JM., Raab A, McGovern E, Stengel DB, Krupp DM and Feldmann J. 2017. A field deployable method for a rapid screening analysis of inorganic arsenic in seaweed. Microchimica Acta. (2017) 184:1701– 1709
3. Smith, J.M., Gargan, S., O'Hea, L., Feldmann, J.F., McGovern, E., and Stengel, D.B. The effect of drying, storage, and processing on the concentrations of total and inorganic arsenic in macroalgae species used for human consumption and animal feed. In prep.
4. Smith, J.M., O'Hea, Crowley, R., L., Stengel, D.B., and McGovern, E. Seasonal and spatial variation in total and inorganic arsenic in *Ascophyllum nodosum* and *Laminaria digitata*. In prep.

Scientific abstracts or articles including those presented at conferences:

1. Arsenic in Marine Macroalgae and Implications for Commercial Uses (AsMARA) - poster presentation. Authors: Jenny Ronan, Evin McGovern, Dagmar B Stengel
2. Arsenic in Marine Macroalgae and Implications for Commercial Uses (AsMARA) - poster presentation. International Seaweed Symposium, 2016. 19 to 24th June 2016
3. Algae as a sustainable source of high value products. World Seafood Congress, Reykjavík. 10-13 Sept 2017
4. Variations in total and inorganic arsenic in marine macroalgae - oral presentation by J. Ronan (Smith). Authors: Jenny Smith (Ronan), Evin McGovern, Dagmar B Stengel. Irish Algal Researchers Conference. 06/09/18 and 07/09/18
5. Total and inorganic arsenic in marine macroalgae – poster presentation. Authors: Jenny Smith (Ronan), Linda O'Hea, Joerg Feldmann, Dagmar B. Stengel, Evin McGovern. FSAI Science Conference August 21-22, 2019

Popular non-scientific publications:

1. www.marine.ie/Home/AsMARA
2. Information leaflet entitled 'Arsenic in Irish marine macroalgae - implications for industry' published in October 2015
3. A second leaflet updated with findings and recommendations from the project is currently in draft and will be published in 2019.

Workshops/seminars at which results were presented:

1. Arsenic in Marine Algae (AsMARA) – Implications for Industry, Consumers and Regulators. 3rd October at the Marine Institute, Rinville, Oranmore, Co Galway