DISSERTATION

UNDERSTANDING TOXIC ELEMENTS IN AGRICULTURAL PRODUCTS: INTEGRATING INSIGHTS FROM IONOMICS AND ARSENIC SPECIATION

Submitted by

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ABSTRACT

UNDERSTANDING TOXIC ELEMENTS IN AGRICULTURAL PRODUCTS:
INTEGRATING INSIGHTS FROM IONOMICS AND ARSENIC SPECIATION

We humans are very unlikely to move through the world without encountering toxic elements. Throughout our history, we have viewed toxic elements through a variety of lenses: as medicines, poisons, plumbing material, pesticides, environmental threats. They are present in air, soil, water, and – as a result – food. Their geographic distribution is influenced by both natural geologic processes and our own actions, and the burden of exposure does not necessarily fall equitably across communities. Different toxic elements have different physiological effects, threshold concentrations of concern, and distributions in the food supply. Even a single toxic element can take on various chemical forms with varying toxicities. These elements continue to cause human suffering as they make their way into our diets, and we continue to learn about them. However, the large degree of chemical variation associated not only with toxic elements themselves, but also the food matrices they exist within, demand ongoing development of robust analytical methods.

This dissertation assesses current availability and operationalization of data on toxic metals in the food supply and discusses a study of many different elements – including multiple toxic elements – in diverse foods. This study illustrates the importance of both understanding the large variation in toxic metal concentrations that can exist between different foods, and the co-occurrence of toxic and nutritive elements in foods. The second half of the dissertation focuses more specifically on arsenic, which is acutely toxic in some chemical forms, much less worrying

in others, and of uncertain concern in others still. Understanding the distribution of these chemical forms in a high-arsenic matrix is critical for understanding the potential risk posed by the product. Analytical techniques capable of providing this data are known as speciation methods. After describing development of an arsenic speciation method in hemp, the dissertation concludes with a study applying the method to a panel of foods that may be high in total arsenic.

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CHAPTER 1

CONTEXTUALIZING TOXIC ELEMENTS IN THE DIET: A CASE FOR INTEGRATION OF TOXIC ELEMENT DATA INTO FOOD DATABASES[i]

Introduction

Food comprises a vast array of diverse chemicals, from carbohydrates to vitamins to fatty acids. Of these constituents, the structurally simplest are the chemical elements. However, the 118 elements currently in the periodic table still differ from one another along many axes. Elements are broadly grouped into (several types of) metals, metalloids, lanthanides, actinides, halogens, noble gases, and other nonmetals. Across these groupings, the concentrations of elements in foods can vary over at least 6 orders of magnitude, with macrominerals like sodium and potassium generally present at relatively high levels (10s of mg per g of food on a dry weight basis) and ultratrace elements like boron and nickel generally present at relatively low levels (10s of μg per g of food).[1] Considerations around dietary intake of elements also vary. While some elements, like zinc and iron, are essential for good nutrition, others, like arsenic and lead, can be toxic.

Consumers in the US are used to seeing four nutritive elements (sodium, calcium, iron, and potassium) on nutrition facts labels. These labels don't – and can't – communicate the vast chemical diversity contained in food. Nutritional databases, such as the United States Department of Agriculture's FoodData Central,[2] offer an expanded view of nutritionally important elements in commonly consumed foods by also including magnesium, phosphorus, zinc, copper, and manganese (among others). These data are frequently used to estimate average

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intakes of these nutrients across populations to evaluate health impacts and generate nutritional guidance. However, we argue that the absence of comprehensive, robust, and easily accessible information about toxic metals and metalloids (such as arsenic, cadmium, and lead) in food creates a significant shortfall in nutrition education both for professionals and for members of the public. While high acute exposure to toxic elements can cause poisoning and even death,[3-5] for the average consumer in the United States, food typically does not pose such acute concerns. (Significant contamination events can occur; for example, the US Food and Drug Administration (FDA) recently investigated cinnamon applesauce products high in lead and chromium[6]). However, the health risks of lower-dose chronic exposure to these metals from the diet is an important public health consideration. Inorganic arsenic (i.e., chemical forms in which the metalloid is not bound to carbon) is a known carcinogen,[4, 7] cadmium can damage kidney function,[3] and the impacts of chronic lead exposure can be both wide-ranging and particularly deleterious for children and pregnant individuals.[5] Importantly, depending on dietary intake, some metals can be passed from the body of a lactating individual to an infant.[8] It also seems likely that a variety of heavy metals can contribute to the etiology of cardiovascular disease.[9]

Beyond these established effects, research suggests potential links between numerous other diseases and toxic metals. Although the literature has not provided definitive conclusions on suggested connections between metabolic syndrome and intake of toxic metals,[10] correlations (both positive and negative) have been identified in several retrospective analyses of the US[11, 12] and Korean[13] National Health Examination Surveys. These studies have reported that the relationship between toxic metal exposure and metabolic syndrome may be nonlinear[12] and vary by metal[11], and that exposure to multiple metals may be more deleterious than the sum of risks for individual metals would suggest[13] (see also Liu et al.'s more recent work on this topic[14]).

Negative impacts on immune function have also been linked to several toxic elements[15] (for an in-depth review of cadmium's effects, see Wang et al.[16]).

Toxic metals in food come from the environments (soil, water, and air) where foods are produced. Toxic metals occur naturally throughout these environments and may also be introduced through industrial activity.[17] Concentrations of toxic metals vary substantially across landscapes and methods of food production, and crops differ from one another in their tendencies to accumulate these metals.[17] This variability can make it difficult to estimate population levels of toxic metal exposure through food consumption and to generate meaningful consumer guidance on how to minimize toxic metal exposure in their diets. Independent analyses by the US FDA[6] and the nonprofit organization Consumer Reports[18] have elevated concerns of toxic metal contamination in various foods into public discourse. However, interpreting the implications of these types of studies and directing future research in this space requires a nuanced approach. The ubiquity and variability of toxic metals across the food supply necessitates an evaluation of trade-offs between the health-promoting effects of certain foods and the risk associated with toxic metal exposure at the dietary level. Important questions that need to be answered more comprehensively include (Figure 1): *What sources of commonly consumed foods are associated with meaningful levels of toxic metals? How might different sourcing or production strategies mitigate the prevalence of toxic metals in the food supply? What are the potential health benefits and tradeoffs of consuming different types and amounts of foods, given the balance of beneficial and potentially harmful compounds contained therein?*

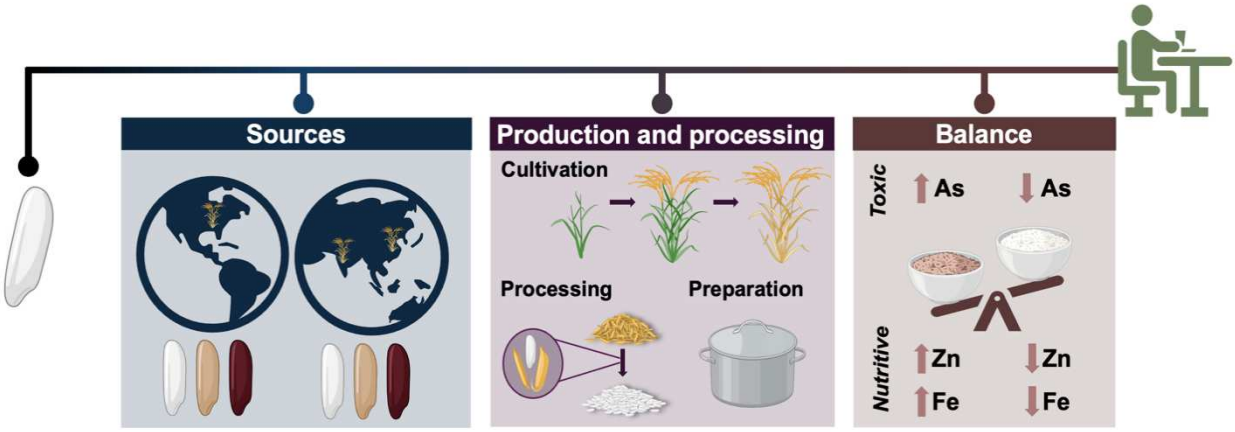

Figure 1: An overview of factors that may influence dietary considerations related to toxic element composition of food, using rice as an example. Different rice sources (geographic and genetic) may accumulate different levels of toxic elements. Cultivation practices, processing techniques, and preparation methods can also impact toxic element composition. The benefits of approaches that can reduce dietary toxic element intake, such as polishing brown rice to make white rice, must be weighed against their potential nutritional costs. (Created in BioRender. Prenni, J. (2024) BioRender.com/z86d400).

Availability and utility of data on toxic metals in food

For food composition data to inform nutritional guidance, the data must be available and accessible to professionals who provide consumer dietary advice, such as physicians and dietitians. To the best of our understanding, while training in the medical and dietetics fields may include some discussion of dietary toxic metals, there is a lack of emphasis on the integration of this knowledge into clinical practice. Further exacerbating the problem, of the primary food composition databases available to US nutrition professionals,[19] only the FDA Total Diet Study (TDS) includes acutely toxic elements such as arsenic and lead. (FoodData Central does incorporate a number of elements like selenium and copper that, though nutritionally relevant at low levels, may be concerning at high levels.[2]) Although the TDS is a wealth of valuable information and the FDA provides a helpful summary report,[20] if the user wishes to interact with all the food-level data it appears they must do so through a flat file that has approximately 69,000 rows.[21] Unless the user has coding or Excel programming experience, extracting information from these files would likely be challenging. Internationally, some large food databases do integrate information about acutely toxic elements. The Canadian FooDB project incorporates

arsenic, cadmium, and lead alongside a wide range of nutritive food components.[22] It is important to consider the sources of this data to evaluate its relevance across contexts. A few foods are linked with specific references to published literature; many foods seem to be associated with other databases. One key citation for quantitative arsenic, cadmium, and lead information is to a USDA plant database that, while rich, only offers insight into plant foods and seems to largely reference toxic element data that is now 25 or more years old.[23] To the best of our understanding, another key reference seems to be Frida, the Danish Food Composition Database, which does incorporate arsenic, cadmium, and lead values in a variety of foods and was last updated in May 2024.[24] The Australian Food Composition Database also includes arsenic, cadmium and lead.[25] The aforementioned influence of environmental factors on foods' toxic element content suggests that these example resources are unlikely to offer complete solutions for all nutrition professionals. However, such resources do demonstrate the feasibility of further expanding databases already familiar to many US providers.

Although the US FDA does test for toxic metals in the food supply and has a Toxic Elements Working Group,[26] currently another data challenge stems from an apparent lack of clear tolerable dietary intake levels within US policy for several acutely toxic metals. The Food and Agriculture Organization (FAO) / World Health Organization (WHO) Joint Expert Committee on Food Additives publishes a searchable database of food additives and contaminants, which provides provisional tolerable intakes for cadmium and (methyl)mercury, but such values are not currently available for inorganic arsenic and seem unlikely to be feasible for lead.[27] There are also some sources of food-level guidance around certain toxic metals. Among the toxic metals measured in the FDA TDS, only lead in apple juice and candy and inorganic arsenic in apple juice and infant rice cereal are assigned action levels.[20] The FAO and

WHO publish the Codex Alimentarius, which contains standards suggesting maximum allowable levels of (inorganic) arsenic, cadmium, lead, (methyl)mercury, and tin in a variety of foods that may be part of international trade.[28] Nutrition professionals should be aware of these important resources, but it is not clear how a provider should triangulate across them and integrate individual and social factors to determine the best advice for many patients.

Further complicating this calculation are cases in which the risk of harm from toxic metals must be balanced with the benefits provided by otherwise nutritious foods. For example, while spinach is widely understood to be a healthy food, this crop may also be particularly prone to accumulate cadmium.[1, 29] Similarly, in wheat, higher levels of iron, magnesium, and manganese may be associated with higher levels of bioavailable cadmium.[30] Conflicts can also arise around processing and preparation techniques. Polishing rice is an effective technique for removing arsenic, but converting brown rice to white also reduces the iron and zinc content of the grain,[31] decreases protein and fiber content, and may negatively alter the grain's glycemic effect on the body.[32] The US FDA suggests that most consumers manage the tension between nutrition and risk from arsenic in food by maintaining a diverse, balanced diet.[33] Though this is sensible and familiar advice, it lacks specificity, and economic realities may make following diverse and balanced dietary patterns difficult or impossible for many consumers.

Health equity concerns around nutrition could be amplified when we add toxic metals to the equation. A participatory research project in Santa Ana, California demonstrated that even before considering dietary intake, the burden of environmental exposure to arsenic, cadmium, and lead may fall disproportionately on lower-income and Latino/a/e or Hispanic consumers.[34] Disparities can also start to arise at the foundational level of drinking water. Many readers will recall the drinking water crisis that began in 2014 in the socioeconomically vulnerable,

predominantly Black city of Flint, Michigan and likely compounded inequities in childhood lead exposure.[35] Furthermore, consumers with less socioeconomic privilege may have limited choice and access to a wide variety of nutritious foods due to financial constraints. Urban gardening might help ameliorate this problem in some contexts, and consumers see a wide variety of benefits to these systems, including control over the addition of potentially toxic compounds like pesticides.[36, 37] Unfortunately, urban garden soils can be contaminated with toxic metals[38, 39] and these metals can be taken up into crops.[40] Consumers may suspect risks from soil contamination at the garden planning phase, and their concerns must be taken seriously.[41] Without location-specific testing, better information about toxic metals in the full diet, and tools to interpret this data, this promising public health strategy for improving access to fresh produce in urban areas may be compromised.

Conclusions and proposed solutions

To begin addressing the challenges described above and enhance consumers' protection from the effects of lower-dose chronic exposure to toxic elements, it is imperative to make data on toxic metals in foods more robust and accessible to provide actionable dietary guidance. Nutrition professionals require appropriate data and tools to help consumers determine which foods in which amounts are nutritious and safe to consume, balancing potentially competing dietary priorities as well as economic considerations. Although the scientific literature contains many reports on toxic metals in foods, the most readily available centralized and comprehensive survey of such information in the US seems to be the FDA TDS. Creating a more interactive, user-friendly web interface for this database could be a good starting point. Such a database should also integrate what information is available on acceptable metal levels in foods and

provisional tolerable intakes, though clearer guidance in these areas is needed. Although some links between chronic diseases and toxic elements are well-established (e.g., inorganic arsenic and cancer[4, 7]; cadmium and kidney disease[3]), additional work should be done to clarify and communicate potential interactions or additive effects of low-level dietary toxic elements across the lifespan. Ideally, this data would ultimately be integrated with existing food composition databases, such as USDA's FoodData Central, alongside nutrient information that is regularly accessed by nutrition professionals. However, such an effort may be met with reservations from companies in the food production space, which may be concerned about impacts on consumer perception and behavior. The health-focused rationale for expanding collection and use of data on toxic elements in food should be clearly communicated to corporations, and strategies to engage their support should be evaluated collaboratively. Although findings of elevated toxic element content in a given food could lead to negative press, perhaps rigorous practices within a company to monitor and mitigate toxic element content could win favor with consumers. Guidance from nutrition professionals about the continuum of risk from toxic elements and balancing the contributions of healthful food components may also help consumers make decisions based on a more nuanced assessment, rather than reacting solely out of fear to an increased awareness of dietary toxic elements.

As databases expand, it will be important to characterize variation in toxic metal content related to geographic origin, cultivation practices, and storage and processing conditions. A recent report on arsenic in rice suggests that this may be particularly important as climate change progresses and creates conditions that may impact uptake of toxic metals into foods.[42] As illustrated by the discussion of urban garden soils, it will be particularly important for this type of work to be designed and assessed through a health equity lens. This includes consideration of

the economic implications of characterizing particular foods or food sources as containing toxic elements – both for consumers who may not be able to afford alternatives, and to producers for whom sale of these foods is a sole or primary source of income. The prospect of adding spatial and social layers (and, ideally, their interactions) to the already-immense task of characterizing dietary chemical diversity naturally raises questions about analytical resources and data interpretation. There is no way around the need for real-world data. However, construction of a sufficiently large and robust dataset may eventually allow for development of tools (e.g., based on machine learning) that can "flag" foods of concern more rapidly. The time and materials needed for comprehensive analysis could be focused on foods where they are most likely to have the greatest impact within a given region or community.

In the longer term, information about the distribution of chemical forms of metals – i.e., speciation data – in foods should also be incorporated into food composition databases. Some chemical forms of toxic metals, such as arsenic[43], are more dangerous than others; similarly, bioavailability of nutritive elements, such as zinc[44], can vary among chemical forms. Nutrition professionals already have a grounding in this concept – for example, the heme iron in meat is more bioavailable than the nonheme iron prevalent in plant foods.[45] In the world of toxic metals, an analogous division exists between inorganic arsenic, which is classified as a known human carcinogen in the US EPA's Integrated Risk Information System,[7] and organic arsenic, which is not (note that the small organic species monomethylarsonic acid and dimethylarsinic acid are listed as possible human carcinogens by the WHO International Agency for Research on Cancer[46]). Speciation data is currently very limited in major US food composition databases (in

the FDA TDS, arsenic speciation is performed on a small subset of foods[20]). These considerations are summarized in Figure 2.

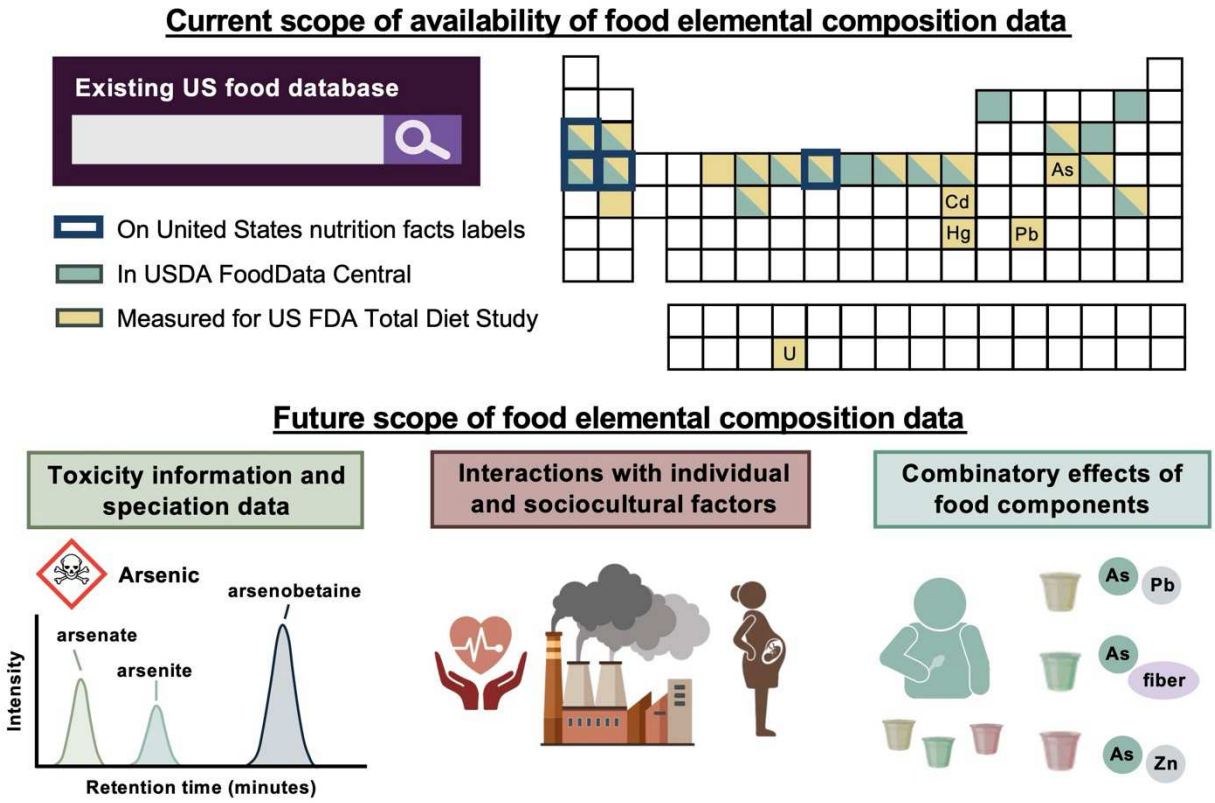

Figure 2: A comparison of the current and future scopes of food elemental composition data readily accessible to US nutrition professionals. Many toxic elements can be measured, but still need to be incorporated into interactive food composition databases that are widely used by US nutrition professionals. To make this information actionable, toxicity thresholds and speciation data, or information about the chemical forms of an element present in food, are required for a number of toxic elements. Further work is also needed to differentiate guidance according to consumers' environments and health statuses, and to understand interactions among food components for appropriately balanced recommendations. (USDA – United States Department of Agriculture; US FDA – United States Food and Drug Administration) (Created in BioRender. Prenni, J. (2024) BioRender.com/z86d400)

Expanding the knowledge base of toxic elements in food will take time and a coordinated global effort, as will understanding how to appropriately integrate the data into nutrition advice. To the best of our understanding, the history of national dietary toxic metal regulation for US consumers is relatively recent: The EPA set enforceable limits for various toxic metals in drinking water over the course of the early 1990s,[47, 48] and revised the allowable arsenic level to a lower value in 2001.[49] From a chemical analysis standpoint, food is much more complex than water, and the interface of food science and food policy is further complicated by the myriad

sociocultural dimensions of food. The US FDA only finalized its (non-binding) infant rice cereal inorganic arsenic action level guidance of 100 ppb in 2020.[50] However, this recent movement should be taken as an encouraging sign that researchers' continual improvement of techniques for food composition analysis – in tandem with epidemiological, toxicological, and nutrition-focused work – will allow further action in this space. Pressing concerns about escalating climate stress on global agricultural systems necessitates further advancement and broader application of these methods. The effort to better characterize and communicate about toxic metals in food is a critical investment in the promotion of a safer and more equitable understanding of nutrition.

CHAPTER 2

MULTIELEMENT PROFILING OF DIVERSE FOOD SAMPLES[ii]

Introduction

Minerals make up a critical portion of the micronutrients important to human health. In nutrition, minerals are incorporated as structural and functional components of metabolic enzymes and tissues. Unlike some nutrients, minerals are solely acquired through the diet, and proper mineral consumption is strongly linked to fundamental processes of human development, physiology, and disease. Mineral nutrient deficiency is recognized by the World Health Organization as a global health concern.[51] In particular, members of certain populations, including older adults who are institutionalized[52] and children and pregnant individuals in low- and middle-income countries,[53] are especially vulnerable. For example, zinc deficiency is a known factor in mortality among children younger than 5, and the effects of a range of nutritional deficiencies survived early in life can reverberate well into adulthood.[53, 54]

Just as lack of certain elements in food can lead to ill health, so can overabundance of others. The routes by which potentially toxic elements reach food products are numerous and encompass both geogenic and anthropogenic origins.[55] Concerning levels of toxic elements have been identified in common foods, including arsenic and cadmium in rice, cadmium and mercury in wheat,[56] and arsenic and lead in infant foods.[57, 58]

[ii] Published February 2023 in *ACS Food Science & Technology* (doi: 10.1021/acsfoodscitech.2c00396). Authors: Chaparro, Jacqueline M; Jones, Rachel R; Mitchell, Susan B; Broeckling, Corey D; Heuberger, Adam L; Shafizadeh, Tracy; Watkins, Steven; Prenni, Jessica E.

Additional information about this chapter is available in Appendix A.

This complex nature of mineral nutrition clearly motivates the need for a robust, flexible method for reliably quantifying a range of elements across a diversity of food types. Developing such a method presents notable analytical challenges: food matrices are very diverse and contain a wide range of lipid, protein, and carbohydrate levels. Furthermore, concentrations of biologically important elements in food vary over orders of magnitude both between elements and within elements across food types. On a dry weight basis, macroelements such as calcium and potassium can easily reach concentrations greater than 10,000 mg/kg in fruits, vegetables, and grains, whereas trace elements such as copper and manganese are often present at just a few mg/kg in these same food types.[59] The acutely toxic elements – arsenic, cadmium, chromium, and lead – have been measured at levels ranging from 10s-100s µg/kg in the major cereals.[56] Even within the categories of macro and trace elements, concentrations can vary substantially from one food to another – for example, avocado and banana each contain about 300 mg/kg calcium, but this element is present at 15,000-20,000 mg/kg in many leafy greens.[59]

Ionomics is a term used to describe powerful and broadly applicable methods of analysis to profile the elemental composition of living organisms.[60] In addition to immediate benefits for surveillance of nutritional quality and safety of food, ionomics methods can contribute to efforts in breeding or genetic engineering of crops that provide better mineral nutrition while excluding or safely sequestering potentially toxic elements. White et al. (2012) previously named a need for such crops and reviewed a number of relevant quantitative trait loci.[55] The potential of ionomics approaches to drive further gene discovery has been discussed in the literature for some time (for reviews, see Salt et al. 2008[60]; Huang & Salt 2016[61]) and recent work has successfully integrated ionomics and transcriptional analyses to investigate the genetic basis of plant responses to arsenic[62] and to mineral nutrient deprivation.[63]

Toward these endpoints there is a significant body of work utilizing ionomics approaches, primarily with inductively coupled plasma mass spectrometry (ICP-MS) technology, for the analysis of foods. However, these studies have largely employed a narrow methodological approach either in terms of the number of elements analyzed or the sample types. For example, Maillard et al. (2016) evaluated the effect of nutrient deprivation on interactions within the plant ionome limited to 13 elements.[63] Similarly, based on the analysis of 7 elements, González-Domínguez et al. (2016) identified a promising potential application of ionomics coupled with proteomics in environmental monitoring via analysis of clam digestive glands.[64] Other groups have employed methodologies that allow for quantification of a large number of elements but are applied only to a single food type, typically for purposes of geographical origin authentication. Notably, Nguyen-Quang et al. (2021) assessed 42 elements in an effort to distinguish pakchoi grown in different regions,[65] and Segelke et al (2020) evaluated 47 elements in globally sourced walnuts to develop a machine-learning-based classification model.[66]

A few studies have reported methods for assessing a substantial number of elements across a variety of food types. For example, Nardi et al. (2009) developed a method to quantify 16 elements in 18 food types.[67] Millour et al. (2011) reported validation of a method for quantification of 21 trace elements in a range of foods.[68] The focus on trace elements indicates a potential limitation of method dynamic range, which is important for nutritionally relevant elements in food. Husáková et al. (2011) analyzed 54 elements in 16 different foods and beverages.[69] While this study represents a robust and comprehensive approach, it utilizes advanced technology that is not commonly or readily available (ICP-oa-TOF-MS).

The potential of Ionomics approaches to support multiple lines of work aiming to ameliorate global concerns of mineral nutrient deficiency and elemental toxicity clearly

motivates the need for a reliable, readily adaptable method for quantification of numerous elements over a broad range of concentrations in diverse food matrices using commonly available instrumentation. To the best of our knowledge, such a method has not yet been described in the scientific literature. Here, we report an ICP-MS-based ionomics method for the quantification of 26 biologically relevant elements – ranging in abundance from macro-elements to trace elements – that can be easily adapted for any food type. The method, developed as part of the broader Periodic Table of Food Initiative (PTFI),[70] is demonstrated for the analysis of 100 diverse foods from both plant and animal sources.

Materials and Methods

Sample preparation: Food samples were sourced primarily from local grocery stores; additional foods were donated by agriculture researchers. Foods were processed according to typical home-cooking preparation methods. For example, most produce was rinsed with tap water and then a chef's knife was used to remove inedible portions; most grains were prepared in a pressure cooker using manufacturer instructions. After processing, foods were stored in zip-top plastic bags at -80˚C, lypophilized, and homogenized to a fine powder using a coffee grinder. The resulting powders were stored in zip-top plastic bags at -80˚C until microwave digestion. A complete list of foods, with corresponding sources and preparation notes, is available in Supplementary Data 1.

Food samples were randomized into batches of 12 for microwave digestion. For each sample, 325 mg (±25 mg) was weighed into a 75 mL Teflon microwave vessel. A blank sample (a vessel with no food added) was included in each digestion batch. Digestion was performed by adding 10 mL of redistilled concentrated nitric acid (HNO_3) spiked with indium (In, 562.5 ppb)

as an internal standard to each vessel. Vessels were allowed to sit for 10 minutes prior to sealing so that any pre-reactions could occur safely. Vessels were then sealed with a rupture disc and pressure seal and placed in a programmable Titan MPS microwave digestion system (PerkinElmer, Branford, CT). Samples were digested as follows, with pressure remaining constant at 30 bar throughout the run: (step 1) temperature was ramped for 5 minutes to 160°C, held for 5 min at 90% power; (step 2) temperature was ramped for 3 minutes to 190°C, then held for 30 min at 100% power; (step 3) temperature was ramped for 1 minute to 50°C, then held for 15 min at 0% power. Upon completion of the digestion, all samples were diluted with Milli-Q water (18.2 MΩ•cm at 25˚C) to a total volume of 15 mL. Samples were vortexed and subsequently 800 µL of sample was diluted to a final volume of 15 mL with Milli-Q water. This resulted in a sample matrix consisting of 20 ppb In and 2.5% HNO_3. Two prepared reference foods (eggplant and organic spinach) were independently digested 5 times. One mL of each fully diluted sample (excluding blanks but including all replicates of the reference food digestions) were combined to produce a pooled QC sample.

ICP-MS analysis: Concentrations of Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Sr, V, W, and Zn were measured using an NexION 350D mass spectrometer (PerkinElmer) connected to a PFA-ST (Elemental Scientific, Omaha, Nebraska) nebulizer and a Peltier-controlled (PC3x) quartz cyclonic spray chamber (Elemental Scientific) set at 4°C. Where multiple polyatomic interferences were present, certain elements were measured in multiple modes. Li, Be, B, Na, Co, Cu, Ni, P, S, Mg, K, Ca, W, and Pb were measured in standard mode. Se and As were measured in Dynamic Reaction Cell (DRC) mode using oxygen as the reactive gas. Al, V, Cr, Mn, Fe, Cd, Zn, Sr, Mo, and Ba were measured in DRC mode using ammonia as the reactive gas.

Prior to formal analysis, a fast TotalQuant™ screen was used to determine the approximate concentration of each element in representative foods (Supplemental Data 2 and corresponding README) from subjectively defined general food type groups (fish, meat, other animal products, vegetables, fruits, grains, seeds, fungi) within the full sample set. These screening results were used to optimize a custom standard 7-point curve to appropriately capture the concentration range of each individual element across the food types. The TotalQuant™ values were assumed to provide an estimate of the range of concentrations for each element across the general food type groups and therefore used to set the curve maximum (Standard 7) concentration for each element. Pre-determined dilution factors were then used to extrapolate down to Standard 1. This optimized calibration curve for the entire 100-food sample set was prepared by serial dilution of commercially available single-element standard stock solutions (Inorganic Ventures). The curve was prepared in 2.5% HNO_3 and 20 ppb In.

Samples were measured in a randomized order, with analysis of the pooled QC after every 9 samples and were introduced to the nebulizer using a prepFAST SC-2 (Elemental Scientific) autosampler. Each food was measured one time. Before analysis, the nebulizer gas flow and Quadrupole Ion Deflector (QID) were optimized for maximum In signal intensity. A daily performance check was run to ensure appropriately low formation rates of oxides (indicated by CeO^+:$Ce^+ < 0.025$) and doubly-charged species (indicated by Ce^{++}:$Ce^+ < 0.030$). For correction of instrument drift, an internal standard solution consisting of ^{6}Li, Rh and Ir was added to each sample online via the autosampler. All 5 replicates of each of the two reference food digestions were also measured once per food/sample. For quality control, 10 food samples were randomly selected and spiked with known concentrations of each element (corresponding to the midpoint of the calibration curve for each element, Supplemental Data 2).

ICP-MS data analysis and statistics: Data was processed using Microsoft Excel (Supplemental Data 3 and corresponding README), via the workflow described in Figure 3.[iii]

Figure 3: Flowchart describing the overall data analysis protocol for the method. For a detailed treatment of the data analysis workflow, please refer to Supplementary Data File 3 and the corresponding README.

First, each element was blank adjusted by subtracting the average signal for that element in the blanks from the signal for that element detected in each sample. Then, c orrection was performed by evaluating each internal standard (In, ^{6}Li, Rh, and Ir) and the most appropriate internal standard was selected by assessing coefficients of variance (CVs) of the QCs and standard curve. If the same internal standard minimized the CVs for both the QCs and standard curve, that internal standard was selected. If the internal standard that minimized the CVs for the QCs was different than the internal standard that minimized the CVs for the standard curve, then an

[iii] Since the publication of this article, I have developed an R script for a Shiny app that allows for a more streamlined, user-friendly implementation of this workflow. This app is described in detail in Appendix A.

average of the CVs including both the QCs and the standard curve was evaluated to determine the appropriate internal standard. This process was performed separately for each element.

Drift correction was then considered for each element. First, a drift correction factor was computed by (1) normalizing all QC values to QC1 for each element, then (2) fitting a simple linear regression to the normalized QC values for each element, and then (3) solving the model equation for each element-sample combination with the run order count as the predictor. Drift correction was then applied to each element-sample combination by multiplying the value selected at the internal standard correction step by its corresponding drift correction factor. The correction for each element (internal standard only *or* internal standard and drift) that minimized the CVs of the QCs was then chosen as the best correction. After the best correction was applied, the resulting values were used to calculate the concentration of the element in the unknown samples. Subsequently, the resulting concentrations were adjusted for the dilution factor. Limits of detection (LOD) and limits of quantification (LOQ) were calculated as 3 times and 10 times the standard deviation of the blank divided by the slope of the calibration curve, respectively. Final concentrations are reported as μg/g dry weight. Measured calculations below the LOQ were assigned to LOQ/2 for statistical analysis.

Hierarchical cluster analysis (HCA) was conducted on unit variance scaled data using SIMCA 17.0 (Sartorius AG, Goettingen, Germany). For generation of the HCA dendrogram, distances were calculated using the Ward similarity index and the tree was sorted by size. Scatter plots and bar charts were generated in Prism 9 (GraphPad, Version 9.3.1).

Results and Discussion

Standard curve R^2 values for all elements exceeded 0.996; this metric was >0.999 for all but four elements (Al, Be, Co, and S). The average CV across all elements for the pooled QC was 8% and did not exceed 20% for any element (Supplemental Data 3). Average CVs were higher for the reference foods; across all elements, the average CV for the eggplant reference food replicates was 15%, with CVs exceeding 20% for several low abundance elements (Al, Cr, Pb, V) and the difficult-to-ionize sulfur. The average CV across all elements for the organic spinach reference food replicates was 10%, with a similar problem-element profile as eggplant (CVs exceeded 20% for Al, Se, and W). Ninety six percent of the recoveries for spiked samples were within 20% of the spiked value (when spike concentration was > 3 times the background concentration for each element; Supplemental Data 1).

The described method allowed for simultaneous quantification of macrominerals (mg/g scale), and (ultra)trace elements (ng/g scale. For example, among the tested foods, spinach emerged as a rich source of magnesium (11,500 ± 250 μg/g) and calcium (14,300 ± 440 μg/g). However, spinach was also the highest-cadmium food assessed (2.1 ± 0.075 μg/g) and was additionally relatively high in lead (0.041 ± 0.0041 μg/g). With the exception of selenium, our method provides lower LODs and LOQs for such (ultra)trace elements than the limits reported for a matched set of foods in the 2017 FDA Total Diet Study analysis.[71] This increased sensitivity could allow for both more accurate risk assessment in the case of acutely toxic elements and improved dietary recommendations in the case of nutritive elements.

Hierarchical cluster analysis resulted in 5 groups (Figure 4). Although there was substantial diversity within some groups, the general divisions were as follows: Group 1 – animal products (11 foods); Group 2 – grains and fruits (19 foods); Group 3 – leafy greens (2

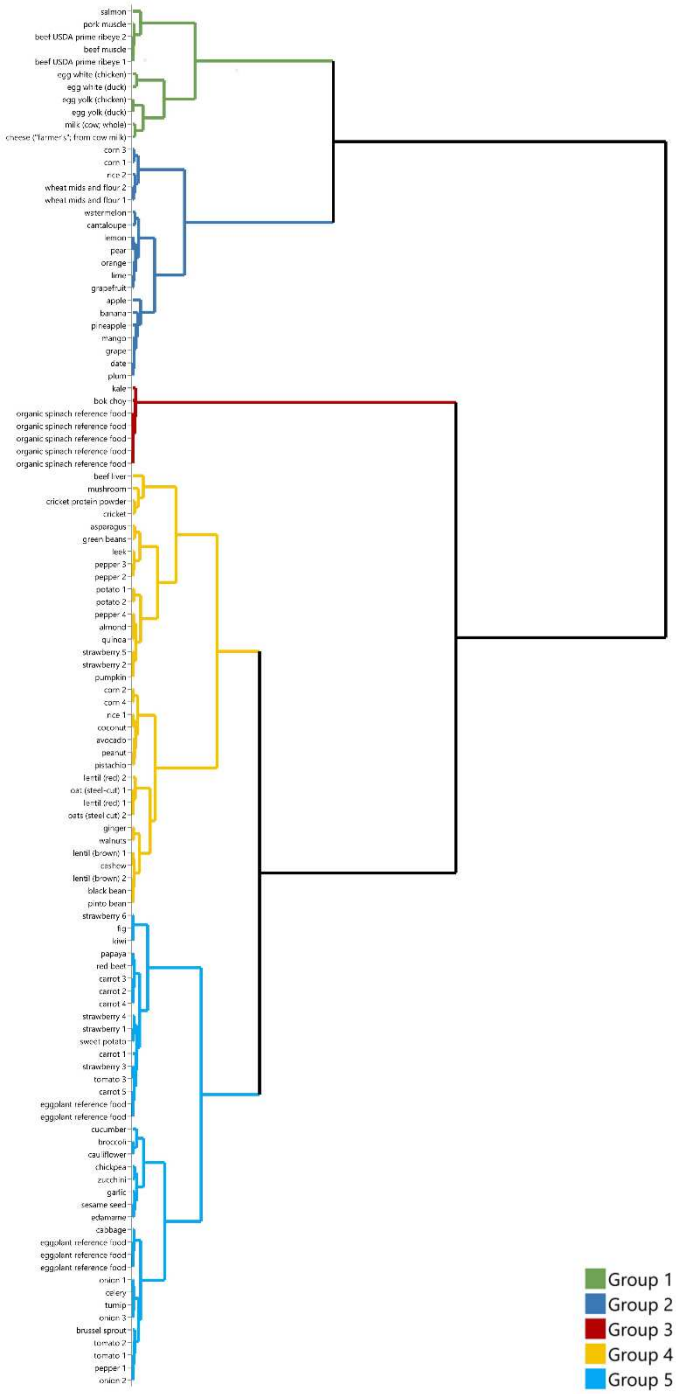

Figure 4: Dendrogram produced from hierarchical cluster analysis of unit variance scaled ICP-MS data for 100 foods. The analysis produced 5 main groups, which can broadly be described as: Group 1 – animal products (11 foods); Group 2 – grains and fruits (19 foods); Group 3 – leafy greens (2 foods); Group 4 – beans and seeds (35 foods); and Group 5 – fruits and vegetables (33 foods).

foods including 5 replicates of the organic spinach reference food); Group 4 – beans and seeds (35 foods); and Group 5 – fruits and vegetables (33 foods including the 5 replicates of the

eggplant reference food).

On average, concentrations of all element types (macrominerals, trace minerals, ultratrace elements, and other metals) were higher in Group 3 (leafy greens) than any of the other groups (Figure 5). However, foods notably high in nutritionally important elements occurred in all

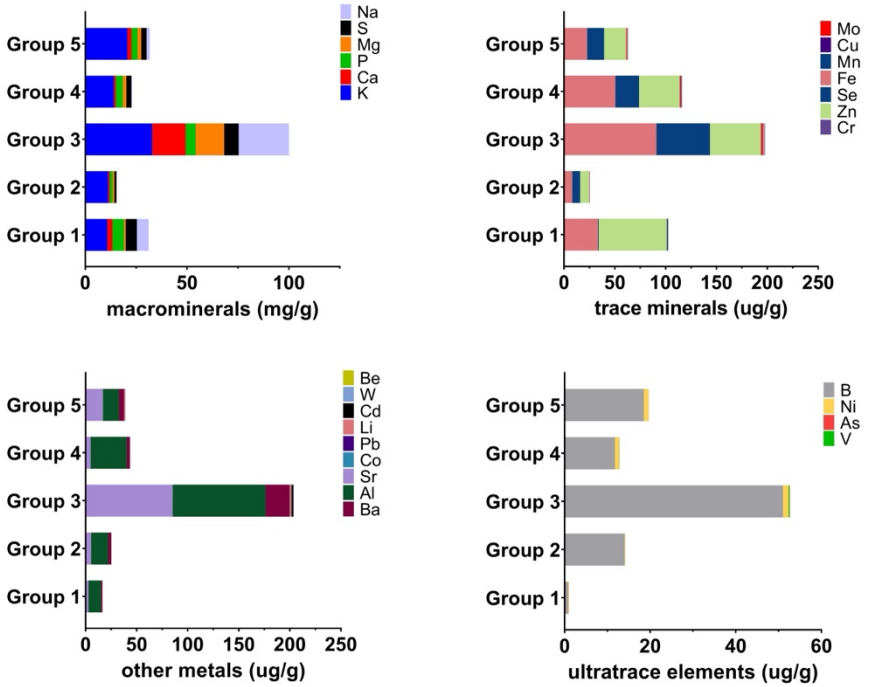

Figure 5: Bar charts illustrating the distribution of 26 elements in 100 foods assessed by ICP-MS. Food groups are as determined by dendrogram analysis. Concentrations of the simultaneously measured elements span 6 orders of magnitude (ultratrace to macro levels) in the diverse food matrices. Group 3 (leafy greens) tended to be highest in all elements across the concentration span.

groups (Figure 6) – for example, although kale (Group 3) had the highest calcium and iron concentrations of any food measured, cricket protein powder (Group 4) had the highest zinc concentration and duck egg whites (Group 1) had the highest selenium concentration. These findings highlight the utility of the method for high-throughput assessment of a wide range of foods that might be culturally and medically appropriate for alleviating nutritional deficiencies.

Foods high in toxic elements also occurred across the groups. Salmon was the highest-arsenic food (~0.36 µg/g). However, the predominant arsenic species in seafood tends to be

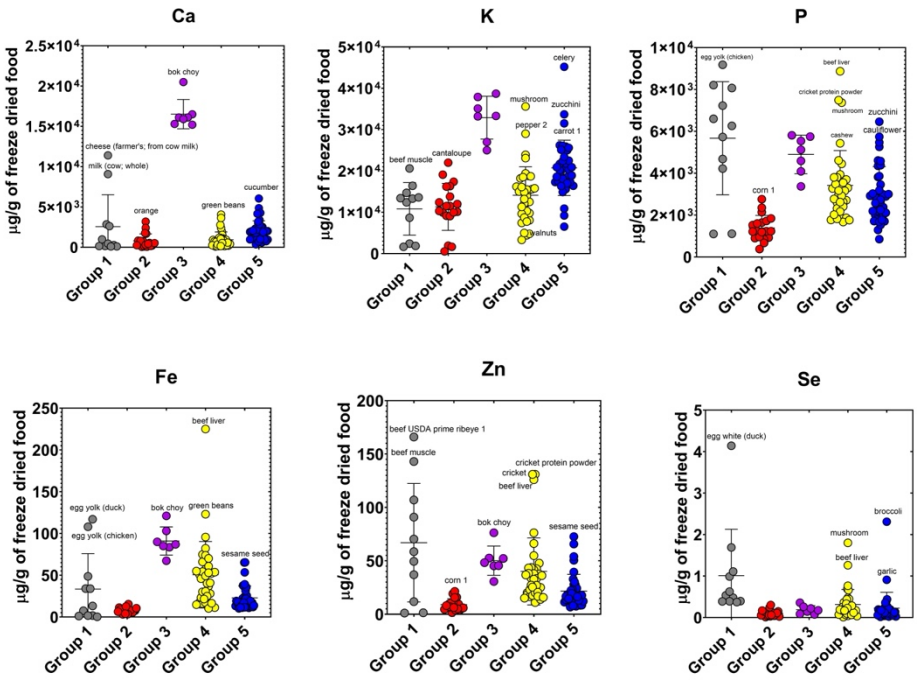

Figure 6: Scatter plots illustrating the distribution of concentrations of 6 nutritionally important elements assessed by ICP-MS in 100 foods. Foods are grouped and color-coded according to the main groups identified in HCA analysis (Figure 2), and foods particularly high in the selected elements are identified by labels. Within each group, the middle horizontal bar represents the mean and the upper and lower bars denote one standard deviation on each side of the mean. Minimum concentration values on the y-axes are fixed at 0 µg/g.

arsenobetaine, which, unlike inorganic arsenic compounds, seems to be minimally toxic.[72] A complementary analysis (HPLC-ICP-MS) would be necessary to speciate arsenic in the samples and make an accurate risk assessment. The current method can serve as a valuable screening assay to identify foods that should be assessed for speciation of arsenic and other metal(loid)s. Cucumber (~0.25 µg/g) and brown rice (~0.23 µg/g) were the second- and third-most arsenic-rich foods, respectively. Enriched white rice was much lower in arsenic (~0.11 µg/g) but was also lower in the micronutrients iron and manganese. Brown and white rice had similar zinc concentrations. Encouragingly, these results are all similar to the XRF findings reported by Lombi et al. (2009) for rice grain cross-sections.[73] Several types of onions had the second- and third-highest lead contents (~0.15 µg/g) in the sample set. Interestingly, although the lead level in uncooked red lentils (~0.0017 µg/g) was barely above the method LOQ, cooked red lentils had

the highest lead content (~0.30 μg/g) in the sample set. Lead levels were also higher in cooked (~0.014 μg/g) compared to uncooked (~0.0082 μg/g) brown lentils, and in cooked (~0.013 μg/g) versus uncooked (<LOD) oats. This pattern may indicate absorption of lead from the tap water used for cooking these foods.

In conclusion, we have demonstrated that the described method is a powerful tool for analysis of elemental composition of food. Testing the method on 100 foods from both plant and animal sources illustrates that (1) the method has a wide dynamic range, allowing for simultaneous quantification of 26 elements ranging from ultratrace to macro concentration levels (6 orders of magnitude), and (2) the method is robust to a wide range of sample matrices. Taken together, these qualities represent a substantial improvement on previously reported methods for ICP-MS analysis of foods. The method is promising for simultaneous identification of both nutritious and hazardous elemental properties of diverse foods.

CHAPTER 3

ARSENIC SPECIATION TO EXPLORE THE PHYTOMANAGEMENT POTENTIAL OF HEMP[iv]

Introduction

Arsenic's grim reputation in the societal conscience is well-earned – humans have used this metalloid as a poison for centuries, and it is a key component of several gases synthesized for chemical warfare.[74] However, the threat posed by arsenic is substantially dependent on the element's chemical form. As measured by acute oral LD_{50} in rats, the toxicity of arsenite (As(III), an inorganic form of arsenic) is ~15× that of the organic species dimethylarsinic acid (DMA); the LD50 of arsenobetaine (AsB; another organic arsenical) is over ~250× that of As(III).[75] Furthermore, the US Environmental Protection Agency has classified inorganic arsenic as a known human carcinogen,[7] a designation organic arsenic does not receive. Under the World Health Organization International Agency for Research on Cancer's system, DMA and monomethylarsonic (MMA) acid do carry a "possibly carcinogenic to humans" designation, while AsB is considered not classifiable.[46] Given these facts, it is not surprising that speciation of arsenic in food derived from plants (especially rice) has become an area of meaningful research interest (e.g., refs [76-80]).

Accurate analysis of arsenic speciation in plants presents several methodological challenges. Instrumentation for analysis by high-performance liquid chromatography in line with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) is readily accessible compared

[iv] The following individuals are recognized as co-authors of this work: Chaparro, Jacqueline M; Richards, Tyler J; Powell, Kit; Maloley, Kaitlyn; Prenni, Jessica E.

Additional information related to this chapter is available in Appendix B.

to alternatives such as x-ray absorption spectroscopy (XAS). Although XAS removes some experimental 'degrees of freedom' related to sample processing, it is less sensitive than HPLC-ICP-MS, which has become the technique of choice in arsenic speciation studies.[81] However, arsenic compounds must be extracted from plant samples prior to HPLC-ICP-MS analysis. The choice of extraction technique can affect not only extraction efficiency,[81, 82] but also the distribution of arsenic species in the sample.[83, 84] Furthermore, the HPLC mobile phase must be carefully tuned to achieve good separation of arsenic species.[85] In reverse-phase systems, solvent composition is particularly important for achieving full separation of As(III) from MMA and of DMA from arsenobetaine (AsB), possibly even more so over the course of a run in which numerous complex-matrix samples are analyzed.[86]

A broad range of extraction and chromatography techniques have been employed even within the selection of arsenic speciation literature cited in this document (e.g., [79, 80, 82, 84, 86-88]). We identified the need for a flexible, robust, and streamlined HPLC-ICP-MS method allowing for separation of diverse inorganic and organic arsenic species in plants. Ideally, such a method would employ a straightforward extraction protocol that can be adapted to various matrices and simple chromatography. Although ion exchange chromatography seems to be the most widely used technique, reverse-phase chromatography allows for use of a gentler mobile phase and so offers an interesting opportunity for additional methodological exploration. Here, we chose to evaluate our method in hemp (*Cannabis sativa*). This is an important commercial crop with a very wide array of consumer and industrial uses for both seeds (e.g., food and personal care products) and vegetative plant structures (e.g., textiles and construction materials)[89]. Hemp is also known to grow acceptably well on soils moderately contaminated with various toxic elements.[90]

The distribution of arsenic species in plant tissues has long been known to differ among different plant species broadly,[91] plants growing in different arsenic-contaminated soils,[87, 88] and arsenic (hyper)accumulator plants vs. non-accumulator plants.[81] The work of Lomax et al.[87] raises the additional point that plant-associated microbial communities can meaningfully influence arsenic speciation within plants. This diversity suggests a range of potential experimental questions to be addressed. For example, a robust arsenic speciation method could be applied to predict the safety of seeds and CBD products developed from a given crop of hemp; to investigate the role of hemp plant and soil microbiomes in arsenic speciation; or to identify particularly arsenic-tolerant hemp cultivars that may be suitable for phytomanagement (safe use of polluted areas to generate valuable products[92]) of arsenic-contaminated soils.

Prior literature has suggested this latter approach,[93] and supports its potential viability for industrial hemp cultivated on soils contaminated by cadmium and lead.[94] A recent thoughtful review considered a related approach: using hemp to phytoremediate, or clean up, polluted soil and then converting the resulting plant matter into biofuels.[90] However, this approach contains an inherent tension. If hemp takes up very high levels of toxic contaminants, this would support the goal of phytoremediation but may make production of biofuels unsafe due to the potential for distribution of arsenic back into the environment.[90] Reframing efforts around phytomanagement could mitigate this tension by focusing on safe, useful products, with the caveat that such sites will likely remain contaminated for a long time.[92] In the case of arsenic-contaminated sites, speciation analysis of harvested plant biomass may offer an important additional dimension to the utility consideration. Here we describe efforts toward developing a method that could support this aim.

Although the plant growth phase of this experiment has proven challenging, preliminary results are promising and support further investigation of hemp for arsenic phytomanagement. Additionally, the analytical method development phase of this work has laid the foundation for additional projects requiring arsenic speciation. Flexible and robust methods for arsenic speciation are of particular importance in the context of food safety analysis. As described in the next chapter, we have adapted the method described here to a wide range of sample matrices, allowing for speciation of arsenic in diverse foods that populations around the world rely on for nutrition.

Methods

Plant growth: The basic plant growth design was modified from Picchi et al.[95] Magenta™ GA-7 plant culture boxes were filled with approximately 250 g of accurately weighed Turface®, a calcined clay product (PROFILE Products). The Turface was then spiked with 500 ppm As(V) stock prepared in 18.2 MΩ·cm water. The appropriate volume of stock for this step was calculated using the Turface weight and water holding capacity, and the desired final concentration of arsenic: 15 mg/kg, 45 mg/kg, or 100 mg/kg. These treatments were selected to represent moderately elevated, high, and very high arsenic levels in US topsoil as reported by the United States Geological Survey.[96] Non-spiked controls (0 ppm treatment) were also prepared. The Turface was allowed to equilibrate for four days. Seeds of 3 different hemp cultivars (Lifter, Diploid Lifter, and Stem Cell) were sown directly on the Turface. One seed was sown per box, and five replicates of each cultivar × treatment combination were transferred, making for a total of 60 boxes, each housing one plant.

Plants were maintained in a greenhouse. Deionized water was applied as needed to keep the Turface moist. Nutrition was provided by three treatments of no-Fe ½ Hoagland's solution (replacing water at 12, 19, and 26 days post-sowing). Plants were harvested 30 days after sowing. Although this short growth period does not allow plants to reach maturity, it does allow for relatively efficient screening of potentially tolerant cultivars without generating the very large quantities of acutely hazardous arsenic waste that would result from an experimental design incorporating a full plant life cycle. At harvest, roots were gently shaken loose from the Turface, then roots and shoots were separated, and samples were stored in plastic zip-top bags at -80˚C until further processing.

Sample preparation: Harvested plants were quite small. To ensure that sufficient biomass would be available to yield detectable arsenic levels, pooled samples were created by combining the 5 individual plants of each cultivar, leaving 3 biological replicates (each a different cultivar) at each arsenic treatment level. Roots and shoots were separated, pooled per the description above, lyophilized to complete dryness, and homogenized using a bead beater. Extraction methods were adapted from the optimized protocol reported by Zhao et al.[84] All available biomass for each pooled sample was accurately weighed (in general, this came to around 15 mg for roots and 50 mg for shoots). All samples were sonicated in 25% ethanol (prepared in 18.2 MΩ·cm water). Roots were sonicated in 5 mL of 25% ethanol for 2 hours and shoots were sonicated in 10 mL of 25% ethanol for 30 minutes. The ethanol was evaporated under nitrogen gas, then the extracts were diluted (roots to 25 mL and shoots to 50 mL) with 18.2 MΩ·cm water and passed through a 0.22 µm polyvinylidene difluoride (PVDF) filter.

Speciation: Speciation of arsenic in the samples was achieved using reversed phase HPLC-ICP-MS. Six species were assessed: arsenite (As(III); Spex® CertiPrep Assurance®

grade), arsenate (As(V); Spex® CertiPrep Assurance® grade), monomethylarsonic acid (MMA; Toronto Research Chemicals), dimethylarsinic acid (DMA; Sigma-Aldrich), arsenobetaine (AsB; European Reference Material), and tetramethylarsonium (TETRA; Toronto Research Chemicals). The speciation method was adapted from Narukawa et al.[86] Separation of the six As species was performed on a NexSAR™ HPLC (PerkinElmer) equipped with a CAPCELL PAK C18 MG 100A column (5 µm, 250 mm × 4.6 mm; PerkinElmer). Chromatography was isocratic and the mobile phase consisted of 10 mM sodium-1-butanesulfonate, 4 mM malonic acid, 4mM tetramethylammonium hydroxide, 5 mM ammonium dihydrogen phosphate ($NH_4H_2PO_4$), and 0.05% acetonitrile, adjusted to pH 2.7. The flow rate was 0.75 mL/min and the injection volume was 10 µL.

Detection was provided by a NexION® 2000P ICP-MS (PerkinElmer). Post-column flow from the HPLC was fed directly into a PFA-ST nebulizer (Elemental Scientific) and then a 4˚C Peltier-controlled (PC3×) quartz cyclonic spray chamber (Elemental Scientific). To eliminate potential mismeasurement resulting from the interference of $^{40}Ar^{35}Cl$ on *m/z* 75, arsenic was measured at *m/z* 91 after reaction with oxygen in the dynamic reaction cell. The TotalQuant feature of Syngistix™ was used to determine the approximate range of total arsenic concentrations in the samples. Based on this information, a 10-point calibration curve in which each standard contained all 6 arsenic species standards in equal concentrations was developed. Standard 1 contained 0.39 ppb of each species and standard 10 contained 200 ppb of each species. The calibration curve was analyzed at the beginning of the run. Equal portions of all root and shoot sample extracts were combined to create separate root and shoot pooled quality control (QC) samples. Shoots were analyzed in a randomized order for the first half of the run and roots in the second half; 4 corresponding QCs were analyzed throughout each half of the run. A

mixture containing 10 ppb of all the standards was analyzed after the first 3 QC samples in each half of the run.

Data and statistical analysis: Peaks were picked in Clarity™ (DataApex). Raw data was read into R[97] 4.4.0 running within RStudio 2024.04.1+748 (Posit Software, PBC) on macOS 14. for all further analysis. The in-house R script is available on request and has the following package dependences: broom 1.0.6, coin 1.4-3, dunn.test 1.3.6, lubridate 1.9.3, and tidyverse 2.0.0. Signal-to-noise ratios of 3 and 10 were taken as the limit of detection (LOD) and limit of quantitation (LOQ), respectively, for individual peaks. Within each chromatogram, normalized peak areas were computed as the product of a given peak area and the average peak area, divided by the total peak area. Arsenic species were quantified on the basis of normalized peak area using weighted ($1/x$) linear regressions fit to the standard curve ($R^2 > 0.999$ for all regressions). Peak values below LOQ and "missing" peak values (e.g., MMA in a sample where this compound was not detected) were replaced with values equal to ½ the lowest fully quantified value for that species in the standard curve. All concentrations are reported on a dry-weight basis and as arsenic equivalents – e.g., values of 5 mg/kg As(III) and 5 mg/kg AsB should both be interpreted as 5 mg/kg of arsenic, and therefore are directly comparable even though these two species have different molecular masses.

The data did not appear to meet the ANOVA assumptions of normally distributed residuals (based on Q-Q plots of residuals) and equal variances (based on plots of residuals against fitted values). Therefore, to evaluate differences in arsenic species concentrations among experimental groups, we used Kruskal-Wallis tests. These tests were only performed for arsenic species with at least one peak >LOQ in the dataset. For roots, potential differences in concentrations of As(III), As(V), DMA, and AsB across treatment groups were tested. For

shoots, As(III), As(V), and MMA were tested. If the overall result was significant at the $\alpha = 0.05$ level, the Kruskal-Wallis test would be followed by pairwise comparisons using Dunn's test with a Bonferroni *p*-value adjustment for multiple testing. We evaluated differences in the concentrations of the two inorganic arsenic species (As(III) and As(V)) within experimental groups that had peaks >LOQ using exact Mann-Whitney U-tests with a Bonferroni *p*-value adjustment. We also used this latter method to test for differences in As(III) accumulation between roots and shoots within experimental groups.

Results and Discussion

Plant growth: Hemp seeds germinated and plants survived the entire experimental period in all experimental groups, albeit with some visible stress symptoms such as stunting and leaf chlorosis and necrosis (Figure 7). We found this resilience somewhat surprising for the 100

Figure 7: An image of a hemp plant in the 15 mg/kg arsenic treatment group exhibiting visible stress symptoms. Note the chlorosis and necrosis occurring over the distal portion of the true leaf closest to the viewer.

mg/kg treatment, but it generally aligns with the results reported by a number of prior authors (e.g., [90, 95, 98]). We cannot be certain that these stress symptoms are caused by arsenic alone,

particularly given the apparent presence of arsenic in the control group (discussed below). Contributions from potential nutrient imbalances or other environmental stressors cannot be ruled out. In particular, chlorosis of older leaves – as is visible in Figure 7 – could be associated with deficiencies of potassium, nitrogen, or magnesium.[99]

Speciation analysis: All six arsenic species in the calibration curve were baseline-resolved with minimal tailing (Figure 8), including As(III) and MMA, which can be difficult to

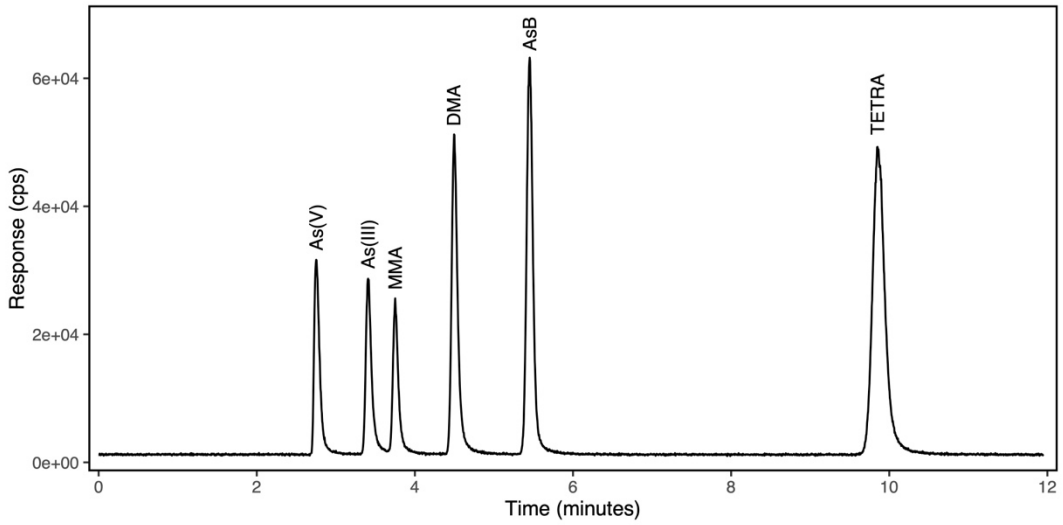

Figure 8: Chromatogram showing separation of all evaluated arsenic species in standard 6 of the calibration curve, which contained 12.5 ppb of each species. Arsenic species include the inorganic species arsenate (As(V)) and arsenite (As(III)), and the organic species monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB) and tetramethylarsonium (TETRA). The *y*-axis shows the detector response in counts per second (cps).

fully separate with reverse-phase chromatography.[86] Recoveries for arsenic species in the 10 ppb checks were very close to 100% (96% - 104%) for the first half of the run, but did decrease somewhat over the course of the run (Table 1). This latter observation is concordant with the anticipated reduction of detector sensitivity during extended use. However, recoveries later in the run stayed in an acceptable range (none fell below 80%), and CVs for As(III) and As(V) in the pooled root QCs were quite good (<5%). (All arsenic species were <LOQ in the shoot QC samples, and organic arsenic species were not observed in any QC samples.)

Table 1: Method quality information for six analyzed arsenic species. Recoveries are averaged values over all analyzed 10 ppb checks, and the range of values is given in parentheses. LODs and LOQs are calculated as 3× and 10× the standard deviation of the ^{91}AsO noise in blanks, respectively, divided by the slope of the calibration curve regression for each As species. Since roots and shoots have different dilution factors, the reported values are reported without dilution factor adjustment.

Arsenic species	10 ppb check recovery	10 ppb check CV	LOD (ppb)	LOQ (ppb)
As(V)	91.4% (81.8% - 101%)	9.97%	0.052	0.174
As(III)	90.6% (80.6% - 102%)	9.85%	0.055	0.184
MMA	91.8% (80.5% - 103%)	11.2%	0.070	0.232
DMA	91.6% (80.4% - 104%)	10.3%	0.030	0.100
AsB	92.6% (82.6% - 104%)	9.56%	0.023	0.076
TETRA	92.6% (80.8% - 101%)	7.59%	0.015	0.049

Very few organic arsenic peaks were detected in the samples. Among roots, MMA was detected at a trace level in one sample (45 mg/kg treatment); DMA was quantifiable (15.8 mg As/kg) in one sample (15 mg/kg treatment); AsB was quantifiable (8.25 mg As/kg) in one sample (control group) and at a trace level in another (100 mg/kg treatment). Among shoots, MMA was quantifiable (21.3 mg As/kg) in one sample (45 mg/kg treatment) and at a trace level in another (15 mg/kg treatment). The infrequent occurrence of organic arsenicals may be partially attributable to the fact that plants seem to acquire organic arsenic as a metabolite of microbes in the growth substrate, rather than producing organic arsenicals during endogenous metabolic processes.[87] The growth conditions in this experiment probably did not promote development of robust soil microbial communities, and thereby likely minimized the availability of organic arsenicals to the plants. Future work should explore the influence of rhizosphere microbial communities on the distribution of arsenic species in hemp. Given the previously discussed disparities in toxicity between organic and inorganic arsenic species, rhizosphere microbiomes could substantially shift the phytomanagement potential of hemp. A rhizosphere microbiome that efficiently methylates arsenic could theoretically contribute to more efficient phytomanagement than might be possible at a site that cannot support such a microbiome.

Inorganic arsenic species (As(III) and As(V)) were detected in both roots and shoots in all experimental groups, including the control. In shoot samples, inorganic arsenic peaks only

exceeded the LOQ in the 100 mg/kg arsenic treatment group. In root samples, inorganic arsenic peaks exceeded the LOQ in all experimental groups. However, no statistical tests for differences in arsenic species concentrations across experimental groups returned a statistically significant *p*-value (at the $\alpha = 0.05$ level), so no pairwise comparisons were completed. Similarly, no statistically significant differences were identified when comparing As(III) and As(V) concentrations within groups, nor when comparing As(III) concentrations between roots and shoots (even before *p*-value adjustment). These statistical findings are summarized in Table 2. It is likely that sample pooling ultimately left this experiment underpowered for detection of any true differences using inferential statistics, particularly given the unaccounted-for cultivar variability. The repeated occurrence of a $W = 9$ test statistic for the Mann-Whitney U-tests suggests the possibility that groups are being clearly separated by rank, but the per-group *n* is simply too small to achieve a *p*-value below 0.1. However, potentially interesting trends that suggest some meaningful differences are qualitatively observable in the data.

Table 2: Statistical tests and performed and resulting (unadjusted) *p*-values. (NS: not significant at the $\alpha = 0.05$ level)

Comparison	Statistical test	Result
Inorganic arsenic concentrations across experimental groups in roots	Kruskal-Wallis	As(III): $p = 0.56$ (NS) As(V): $p = 0.86$ (NS)
Inorganic arsenic concentrations across experimental groups in shoots	Kruskal-Wallis	As(III): $p = 0.10$ (NS) As(V): $p = 0.86$ (NS)
Concentration of As(III) vs As(V) within experimental groups	Mann-Whitney (exact)	All tests: $p = 0.1$ (NS)
Concentration of As(III) in roots vs shoots within experimental groups	Mann-Whitney (exact)	All tests: $p = 0.1$ (NS)

Concentrations of As(III) seemed to be generally higher than concentrations of As(V) in roots, and this pattern appears to hold for shoots from the 100 mg/kg treatment as well (Figure 9). This may indicate activation of a previously described putative arsenic tolerance mechanism: As(III) derived from reduction of As(V) in the roots is effluxed back to the growth medium.[100] Such behavior has been reported in tomato and rice,[101] the grass *Holcus lantanus,*[100] and the

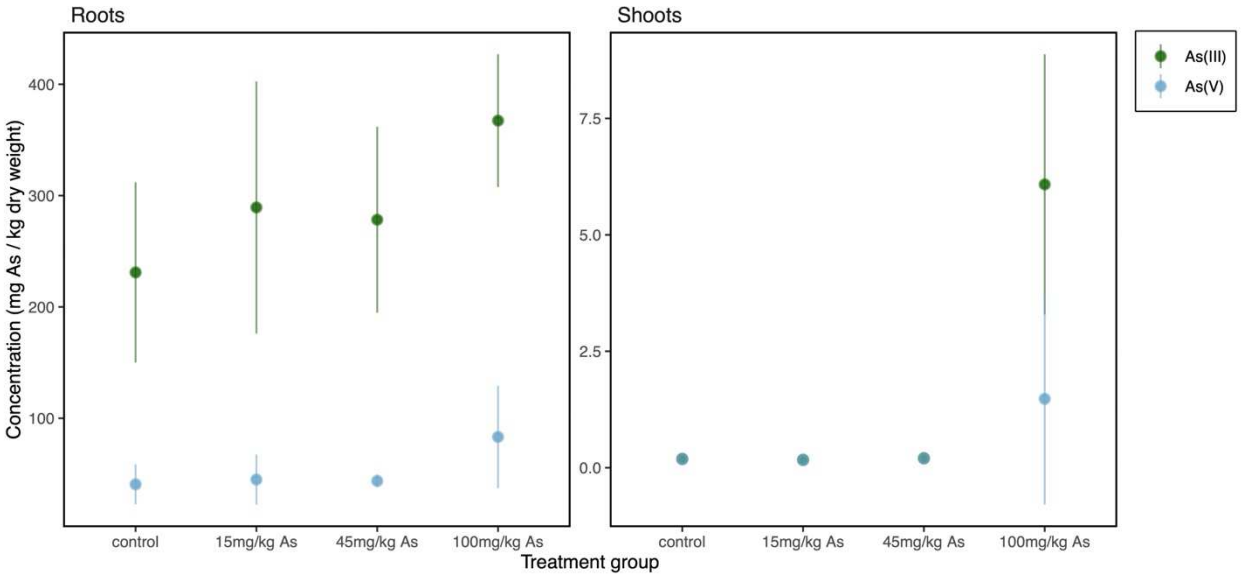

Figure 9: Measured concentrations of the inorganic arsenic species arsenite (As(III)) and arsenate (As(V)) in roots (left) and shoots (right) of hemp plants grown in substrate spiked with different concentrations of arsenic. Concentrations are reported as mg of arsenic (not molecular weight) in each species per kg of dry plant biomass. Treatment groups are the concentrations of arsenic originally added to the plants' growth substrate (note that no arsenic was added to the control group substrate). Points represent means in each experimental group and error bars extend one standard deviation above and below each mean. Error bars are not visible for most shoot data due to replacement of values below the LOQ.

model plant species *Arabidopsis thaliana*.[102] Reduction of As(V) can also allow plants to immobilize arsenic in the roots via chelation of As(III).[102] This tolerance mechanism, which has been reported in a variety of plant species,[91, 103, 104] may help explain the observation that shoot inorganic arsenic species concentrations appeared to be generally lower than corresponding levels in roots (Figure 10). Considering both tolerance mechanisms together, we could hypothesize the following: Root accumulation of inorganic arsenic doesn't appear to change much between the 15 mg/kg and 45 mg/kg arsenic treatments due to As(III) efflux, and chelation in roots results in minimal shoot inorganic arsenic levels. In the 100 mg/kg treatment, the plants' combined efflux and chelation capacities are overwhelmed and inorganic arsenic is apparently translocated to the shoots, while root accumulation seems only somewhat increased.

The presence of relatively high arsenic levels in the control samples is a puzzling finding that we observed not only in this study, but also in a second trial with very similar methods (data

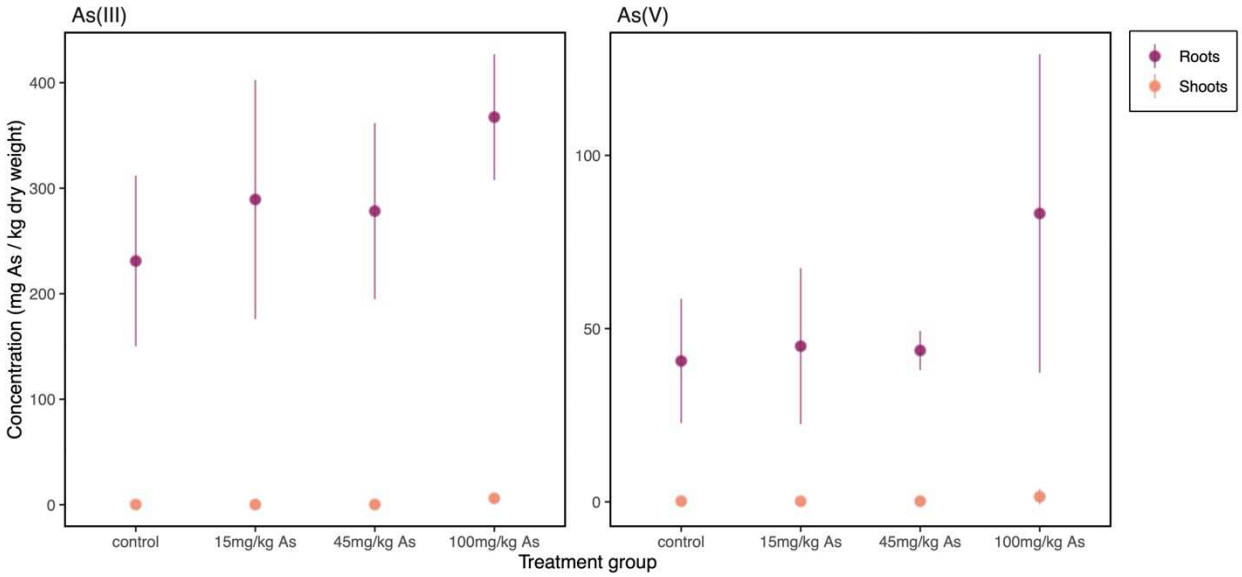

Figure 10: Measured concentrations of the inorganic arsenic species As(III) (arsenite; left) and As(V) (arsenate; right) in roots and shoots of hemp plants grown in substrate spiked with different concentrations of arsenic. Concentrations are reported as mg of arsenic (not molecular weight) in each species per kg of dry plant biomass. Treatment groups are the concentrations of arsenic originally added to the plants' growth substrate (note that no arsenic was added to the control group substrate). Points represent means in each experimental group and error bars extend one standard deviation above and below each mean. Error bars are not visible for shoot data in treatment groups below 100 mg/kg due to replacement of values below the LOQ; in the 100 mg/kg treatment group, error bars are present but appear very small due to the *y*-axis scale required to accommodate the root values.

not shown). Given the replicability of this result and the precautions taken to avoid arsenic cross-contamination, we do not believe that it arose from accidental transfer of arsenic from the treatment groups during preparation of the growth medium nor plant harvesting. We considered the hypothesis that additional arsenic was released from the Turface itself, but extraction of "clean" (non-spiked) Turface with nitric acid and subsequent ICP-MS analysis revealed negligible total arsenic levels (data not shown). Additionally, the Turface SDS[105] and spec sheet[106] do not describe any concerns related to potential arsenic contamination, and this product is more typically used on athletic fields, so we find it unlikely that the Turface is the source of the high arsenic levels in the control plants. At the time of this writing, we remain unable to explain this result.

Conclusion: Overall, these results may support potential utility of hemp for arsenic phytomanagement. The germination and survival of plants at all tested arsenic levels is certainly

notable. US EPA regulates inorganic arsenic (CAS no. 7440-38-2) as hazardous waste at the 5 ppm level (US Code of Federal Regulations 40 § 261.24).[107] In this trial, inorganic arsenic in hemp shoots only exceeded that threshold in the 100 mg/kg treatment (with a few scattered observations of higher organic arsenic levels in lower-arsenic treatment groups). Therefore, hemp may eventually prove to be a candidate crop for moderately arsenic-contaminated sites. However, though this study offers an interesting proof-of-concept, the insight it can offer into the phytomanagement question is limited. This experiment examined only the earliest stages of hemp plant development and took place under highly controlled conditions that do not reflect the realities of field production.

Ultimately, studies that quantify and speciate arsenic in plants grown to industrial maturity, with exposure to endogenous rhizosphere microbiomes and environmental stressors, are necessary. Such studies present significant challenges that will require additional research expertise to overcome: The distribution of high-arsenic soils in the US is geographically heterogeneous,[108] creating potential location effects in field trials; meanwhile, growing hemp to maturity in containers of soil spiked with significant levels of arsenic would produce large quantities of acutely hazardous waste. The results presented here instead provide valuable proof-of-concept data and validate the arsenic speciation method which is further adapted and applied to diverse food matrices in the next chapter.

CHAPTER 4

SPECIATION OF ARSENIC IN FOOD MATRICES: EXPLORING VARIABILITY WITHIN AND ACROSS FOOD TYPES[v]

Introduction

Arsenic is present in soils and waters around the world. Arsenic levels in US topsoils vary significantly, with higher concentrations tending to occur in northern areas, along the Mississippi River, and in Nevada.[108] Elevated arsenic levels in agricultural soils sometimes result from natural geologic variation, but exogenous contamination can also occur, particularly from coal combustion, mining, and prior application of arsenic-based pesticides.[109] One recent modeling study suggested that arsenic could remain at problematic levels about 4× further from mine sites than other metals.[110] While some degree of exposure to arsenic from food is therefore inevitable, humans have long understood that this metalloid can be a potent toxin.[111] When present in inorganic forms, arsenic can be lethal upon high acute exposure and can cause cancers as well as nerve, skin, and organ damage with prolonged lower-dose exposure.[4] Conversely, organic arsenicals generally have lower acute toxicities, and some that are prevalent in seafood seem to pose minimal risk to humans.[72, 111] The literature contains suggestions that specific organic arsenicals, particularly dimethylarsinic acid, may be toxic or carcinogenic, though additional studies are needed.[43] As previous authors have noted,[112] the wide diversity of chemical structures and toxicity concerns across arsenic-containing molecules suggests that accurate prediction of the health impacts of dietary arsenic cannot be accomplished through measurement

[v] The following individuals are recognized as co-authors of this work: Chaparro, Jacqueline M; Maloley, Kaitlyn; Evans, Chris; Prenni, Jessica E.

Additional information related to this chapter is available in Appendix C.

of total arsenic alone. Analytical speciation methods, such as high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS), are necessary for distinguishing and quantifying the various chemical forms of arsenic present in foods.

Several nations perform total diet studies (TDSs) to examine the composition of their citizens' diets, and these studies often include analysis of elemental composition of food. Arsenic speciation analyses associated with TDSs from the USA,[21] Japan[113], Germany[114], Italy[115], and Hong Kong[116] have been reported. However, many of these applications have been limited in at least one of two key ways: (1) only a small number of arsenic species are measured, potentially creating an incomplete risk profile, or (2) foods are analyzed as composite samples, which may hide nuanced differences in risk. Furthermore, in the USA TDS, only a small subset of food types are considered for speciation.[21] Additionally, continued improvements to method sensitivity may be valuable; inorganic arsenic can be concerning at levels as low as 10 ppb in drinking water (US Code of Federal Regulations 40 § 141.62)[117] and 100 ppb in infant rice cereals[50] (US Food & Drug Administration industry guidance). Common TDS approaches therefore may restrict understanding of the real arsenic toxicity potential in foods and the potential variation in exposure for people eating the same food type from different sources. Market basket studies of particular high-arsenic food types (e.g., mushrooms[118] and seafood[119]) help fill some gaps around variation within food types, but generally have low sample diversity. The market basket approach can be adapted at the TDS level. For example, the USA TDS involves food sampling from different regions of the country at different times of the year, but samples of a given food for analysis are actually a mix of individual samples collected from different cities within the region.[21] While likely logistically necessary for such a large-scale

project, this approach may obscure more fine-grained variation in arsenic content related to specific sources and sub-types of some foods. Thus, a need exists for flexible, sensitive speciation analyses that integrate the sampling breadth of TDSs and the focused depth of food-specific market basket studies.

Toward this end, we developed a simple yet flexible workflow for arsenic speciation that is robust across sample matrices and achieves the required analytical limits for assessing arsenic in food. The method enables quantification of six arsenic species (two inorganic and four organic) as well as the ability to detect unknown species. Arsenicals of interest are shown in Figure 11. Here we report the application of this method to the analysis of panels of diverse high-

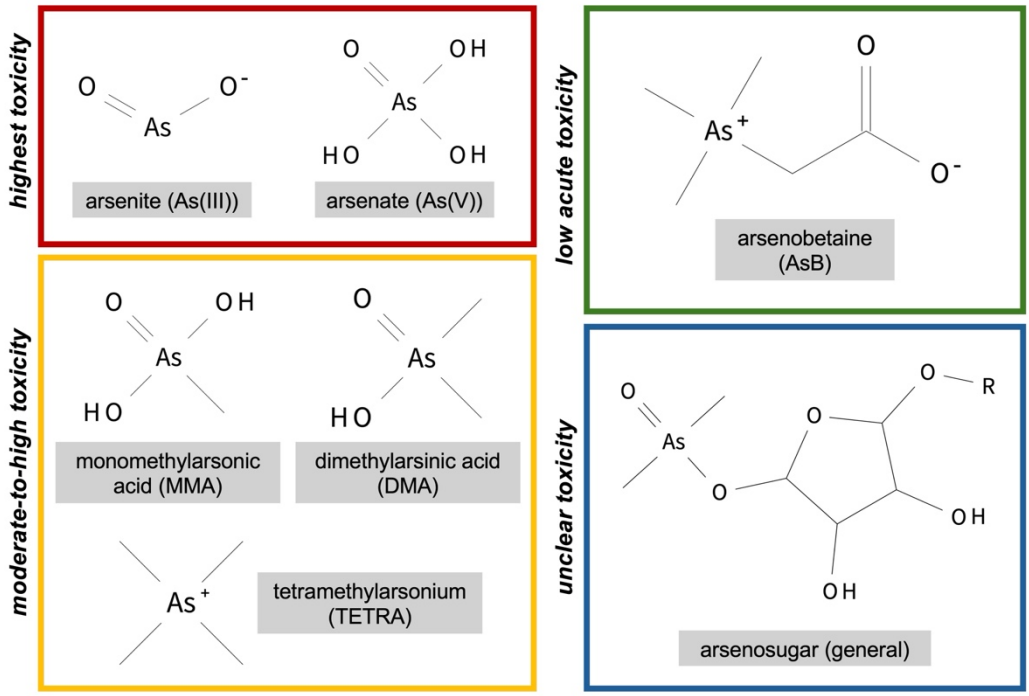

Figure 11: Arsenic species of interest, organized by broad toxicity categories. The inorganic species (As(III) and As(V)) are highly toxic, the organic species AsB seems to be minimally toxic, and the other species fall somewhere in between. The present method includes standards for As(III), As(V), MMA, DMA, and TETRA; arsenosugars are discussed later in the text.

arsenic food types. Our results demonstrate the potential utility of this workflow for large-scale speciation efforts to facilitate better characterization of arsenic risks presented by various dietary patterns.

Methods

Sampling: The 20 foods highest in total arsenic were selected from a previous ionomics survey of 100 foods.[1] These foods (in descending order of total arsenic concentration) included: salmon, cucumber, brown rice, white mushroom, papaya, asparagus, cricket, eggplant, kale, carrot, white rice, spinach, zucchini, turnip, bok choy, strawberry, cricket protein powder, sesame seed, celery, and onion. We then sourced panels of 2-5 different cultivars, production locations and methods, etc. for these food types primarily from grocery stores in Fort Collins, Colorado, USA (with the exception of cricket samples, which were obtained from a collaborator). Ultimately only one carrot sample was analyzed, and cricket and cricket protein powder were consolidated into one food type. Seven different varieties of seaweed (purchased from a commercial vendor; Maine, USA) were also included after ionomic analysis indicated very high total arsenic levels. This resulted in a final sample set comprising 77 foods across 19 food types. Full descriptions of each food in the panel are available in Appendix C (Table C1).

Sample preparation: Samples were transported to the lab under ambient conditions and then stored according to typical consumer patterns (e.g., salmon was kept in a 4˚C refrigerator and rice was kept at room temperature) until processing. Fruits and vegetables were rinsed and inedible portions were removed. Rice was prepared both in raw form and after cooking in an InstantPot® pressure cooker using the manufacturer's instructions. Seaweed was prepared both in dried form and after rehydration and sautéing. No other foods were cooked. After initial processing steps, all samples were stored in plastic bags and frozen at -80˚C. Samples were then lyophilized to complete dryness, homogenized using a coffee grinder, and then returned to -80˚C storage until extraction.

To extract arsenicals, approximately 250 mg of dried, homogenized sample was sonicated for two hours in 5 mL of a 1:3 ethanol:18.2 MΩ·cm water solution.[84] Samples were briefly removed from the sonicator and vortexed every 30 min, at which times the water in the sonicator was also exchanged for a new volume of ice-cold water. Upon completion of sonication, samples were diluted to a final volume of 15 mL with 18.2 MΩ·cm water. Analysis of a mix of arsenic standards indicated that a polyvinylidene difluoride (PVDF) filter may trap As(III) and TETRA, an effect not observed for a PTFE filter (data not shown), so the diluted extracts were filtered using a 0.22 µm polytetrafluoroethylene (PTFE) syringe filter. To reduce the potential for arsenic species interconversion in extracts over time, samples were randomized into two batches for extraction. Speciation of the first batch (40 foods) began while the second batch (37 foods) was undergoing extraction, with the analysis ultimately completed as one continuous run.

Speciation: Six known arsenic species were quantified: the inorganic arsenicals arsenite (As(III)) and arsenate (As(V)), and the organic arsenicals monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and tetramethylarsonium (TETRA). Standards were purchased from Spex® CertiPrep (As(III) and As(V), Assurance® grade), Toronto Research Chemicals (MMA and TETRA), Sigma-Aldrich (DMA), and as a European Reference Material (AsB). A calibration curve containing each of these species was analyzed at the start of the run, between the first and second extraction batches, and at the end of the run. Separation was achieved on a PerkinElmer NexSAR™ HPLC equipped with a CAPCELL PAK C18 MG 100A 5 µm column (250 mm ×4 .6 mm). Chromatography was reverse-phase and isocratic, with a mobile phase of 10mM sodium-1-butanesulfonate, 4 mM malonic acid, 4 mM tetramethyl ammonium hydroxide, 5 mM $NH_4H_2PO_4$, and 0.05% acetonitrile at pH 2.7.[86] A flow rate of 0.75

mL/min was maintained and 10 µL of sample was injected for analysis. Arsenic was measured as AsO at *m/z* 91 (to avoid the $^{40}Ar^{35}Cl$ interference at *m/z* 75) after reaction with oxygen gas in the dynamic reaction cell of a PerkinElmer NexION® 2000P ICP-MS.

The following measures were taken to improve and assess data quality: a pooled quality control (QC) sample was created by combining aliquots of all extraction batch 1 samples (40 foods) and run after every 6 food samples. Samples containing 5 ppb of each arsenic standard were run after every other QC (every 12 food samples). Sample preparation blanks as well as samples of tap water and 18.2 MΩ·cm water were also analyzed. For foods containing arsenic peaks that aligned to compounds in the calibration curve, the concentrations of these known compounds were summed and compared to the measured total arsenic concentration in the food (where available). Total arsenic was quantified using a previously described ionomics method (Chaparro et al. 2023).[1] Briefly, separate aliquots of the same food samples extracted for speciation were mineralized by microwave-assisted digestion in nitric acid. A total of 26 elements, including arsenic, were then simultaneously measured using ICP-MS in all but 12 of the food samples as part of a separate analysis project (data not yet published). In cases where chromatograms of these foods appeared unreliable, they were excluded from further analysis.

Data analysis: Peaks were picked in Clarity™ chromatography software (DataApex). Raw data was exported from Clarity and all further analysis was completed using an in-house R[97] 4.4.0 script running within RStudio 2024.04.1+748 (Posit™ Software, PBC) on macOS 14.5, with the following package dependencies: broom 1.0.4, lubridate 1.9.2, scales 1.2.1, and tidyverse 2.0.0. The script is available on request. Since some peaks were observed tailing into one another, quantitation was performed on the basis of peak height (instead of area). Limit of

detection (LOD) and limit of quantitation (LOQ) were defined as 3× and 10× the signal-to-noise ratio, respectively, for each peak.

Known arsenicals were quantified by weighted ($1/x$) linear regressions of the calibration curve. The method LOD and LOQ for each arsenic species were computed as 3× and 10× the quotient of the standard deviation of the ^{91}AsO noise in the blanks by the slope of the calibration curve regression, respectively. For statistical purposes, compound peaks <LOD (3× the signal-to-noise ratio) and "missing" compound peaks (i.e., those not detected in a given sample) were replaced by a value equal to ½ the method LOD. Compound peaks above LOD but below LOQ (10× the signal-to-noise ratio) were replaced by ½ the method LOQ. All concentrations of known arsenic compounds are reported as µg of arsenic per kg of fresh food. Therefore, the concentrations of, e.g., As(III) and AsB can be directly compared as arsenic equivalents, even though these molecules have substantially different molecular masses.

During peak picking, unknown arsenical peaks were observed in several chromatograms. Unknown peaks were sorted in descending order of height (independent of sample) and then aligned by retention time across the sample set. Retention time shifts between ~0.1 min and ~0.3 min were observed for known arsenicals over the course of the run, so a 0.2 min tolerance window (±0.1 min) was selected for alignment of unknowns. Unknown peaks were relatively quantified on the basis of normalized peak height. Peak heights were normalized within each chromatogram by multiplying the non-normalized peak height by the average peak height, then dividing by the total peak height. For each identified unknown, a normal distribution centered on one-tenth the lowest observed >LOQ normalized height value for the unknown, with a standard deviation equal to 10% of the center value, was randomly sampled to replace <LOQ and "missing" unknown values.

Results & Discussion

Method quality: Baseline chromatographic separation of the six arsenic species (As(V), As(III), MMA, DMA, AsB, and TETRA) was achieved in the calibration curve mixture (Figure 12). Quantitation was completed using an 8-point standard curve (individual arsenic species

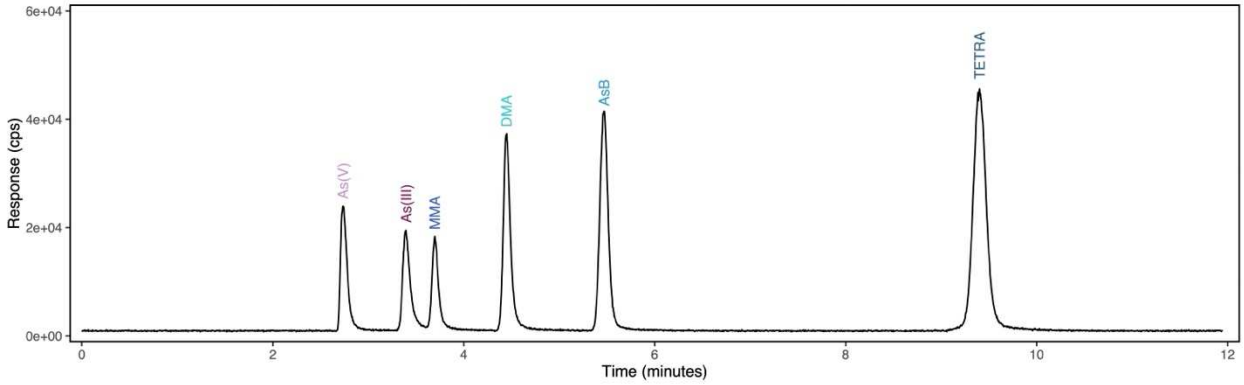

Figure 12: Chromatogram illustrating resolution of six arsenic species in the calibration curve mixture. Data is shown for a standard containing 6.25 ppb of each arsenic species. As(V) – arsenate; As(III) – arsenite; MMA – monomethylarsonic acid; DMA – dimethylarsinic acid; AsB – arsenobetaine; TETRA – tetramethylarsonium

concentrations from 0.39 ppb - 50 ppb); linear regressions all had $R^2 > 0.995$. Non-dilution-corrected LODs and LOQs for the arsenic species were between 0.02 - 0.04 ppb and 0.06 - 0.15 ppb, respectively; after adjusting for a typical dilution factor of 60, this corresponds to ranges from 1.01 - 2.62 ppb and 3.37 - 8.76 ppb in dried food (Table 3). This excellent sensitivity is

Table 3: Method quality metrics for six arsenic species included in the calibration curve. Recoveries in the 5 ppb checks are reported as average values across all analyzed injections. Note that method LODs and LOQs are calculated using a dilution factor of 60, which is typical in the food, and reported on a dry-weight basis.

Arsenic species	5 ppb check recovery	5 ppb check CV	LOD (ppb)	LOQ (ppb)
As(V)	104%	11.0%	1.94	6.48
As(III)	100%	13.5%	2.44	8.10
MMA	103%	11.4%	2.62	8.76
DMA	94.9%	12.1%	1.22	4.08
AsB	95.4%	11.2%	1.09	3.64
TETRA	95.5%	10.7%	1.01	3.37

critical for arsenic speciation methods as the US EPA requires that inorganic arsenic in drinking water not exceed 10 ppb.[117] Recovery of each arsenic species from the 5 ppb checks was close to 100% on average (Table 3) and adequately consistent (CVs < 15%).

Comparison of speciation data to recoveries of total arsenic in the food samples revealed interesting trends. In most foods, less than half the total arsenic measured by ICP-MS was recovered as known arsenicals in the speciation analysis (Appendix C, Table C5). This effect is partially attributable to the presence of unknown arsenicals, particularly in seaweeds (<1% total arsenic recovery from known species in many samples, with unknown arsenical peaks dominating chromatograms; see discussion below). However, in many cases, low recovery of total arsenic in the speciation data seems to be driven by non-quantitative extraction of arsenic. This is unsurprising as we employed a relatively gentle extraction method applied across very diverse matrix types. The simplicity and accessibility of the extraction technique offers an attractive option for screening out matrix types that tend to be high in (or have high variability in) particularly concerning arsenic species – i.e., inorganics and small organic acids – for further specialized method development.

Intriguingly, total arsenic was severely *over*estimated in three foods: portobello mushroom, coho salmon, and yellow onion. In the case of portobello mushroom and coho salmon, this effect was replicated in a second trial (data not shown). While enhanced detection of arsenic in carbon-rich matrices is a known complication in ICP-MS analyses,[120] it is not clear that extracts of coho salmon and portobello mushroom would be uniquely high in carbon compared to other food extracts. Notably, arsenic was not overestimated in other types of salmon and mushroom samples included in the speciation analysis. It is possible that our portobello mushroom and coho salmon samples contained elevated levels of zirconium; the ^{91}Zr isotope interferes on the *m/z* at which arsenic oxide is monitored. The potential for zirconium accumulation has been demonstrated in another species of fish eaten by humans (tilapia),[121] and this element was measured at variable concentrations among shiitake mushrooms produced on

diverse substrates.[122] However, preliminary ICP-MS analysis did not indicate high levels of zirconium in our coho salmon and portobello mushroom samples. Further work is required to clarify the source of this anomaly. For purposes of the present analysis, we removed the chromatograms that resulted in severe overestimation (and those that were otherwise unreliable).

Known arsenic species: Inorganic arsenic (As(III) and As(V)) was widespread at trace levels, but rarely reached quantifiable levels (Figure 13). Given the high toxicity of inorganic

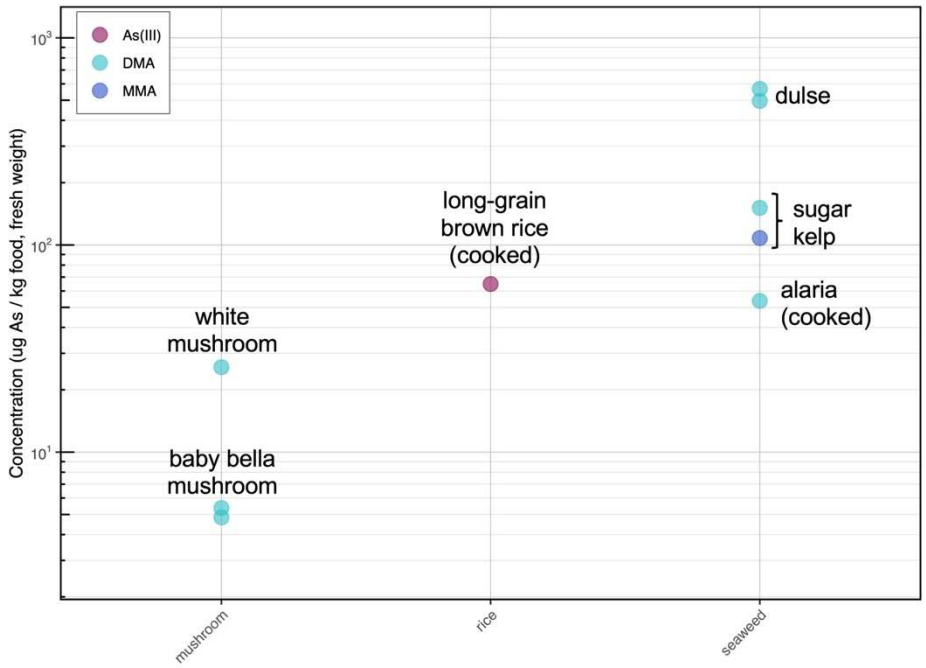

Figure 13: Concentrations of arsenic present as known arsenic species in 3 food types. Food types and individual samples for which no arsenic peak exceeded the LOQ are excluded (numerous peaks were observed at trace levels in seaweed, rice, and vegetables), as are foods with unreliable chromatograms. Peaks that initially aligned to arsenobetaine (AsB) were removed from this plot due to chromatographic retention time shifts (see discussion below). Tetramethylarsonium (TETRA) was not detected in any food. As(III) – arsenite; As(V) – arsenate; DMA – dimethylarsinic acid; MMA – monomethylarsonic acid

arsenicals,[4] this is a positive finding. As(III) was present at ~8 µg arsenic per kg fresh product in one cucumber sample, and at ~65 µg/kg in a sample of cooked long-grain brown rice (though in a replicate extraction analyzed later in the run, this peak missed the LOQ). When the cooked brown rice was dried, the As(III) concentration reached 165 µg/kg. This finding is notable for several reasons. First, the US FDA has published industry guidance recommending that

inorganic arsenic in infant rice cereal not exceed 100 ppb (equivalent to 100 µg/kg),[50] but the Codex Alimentarius recommended maximum level of inorganic arsenic in brown rice as a general commodity is 350 µg/kg.[28] Therefore, the question of the risk posed by this rice can only be answered in the context of its use case, and additional measurements should be completed to ensure accurate and precise quantitation. Second, this was the only rice sample in the dataset with notable levels of As(III). In organic brown rice, purple rice, long-grain white rice, and organic white rice samples, inorganic arsenic peaks exceeded the LOD, but not the LOQ. Arsenic concentrations and distributions of arsenic species in rice are known to vary by genotype and production location.[123-125] The present study supports the imperative of continuing to monitor the safety of rice and rice products at a granular level, as composite samples may provide insufficient information about geographically and socioculturally heterogenous risks.

Also consistent with prior work on rice,[123, 125] we found that when organic arsenic was detected in rice samples, it was in the form of DMA. This arsenic species was not quantifiable in any rice sample, but did exceed the method LOD in samples of purple rice and long-grain brown rice. DMA reached more notable levels in seaweed (up to ~500 µg/kg) and a white mushroom sample (up to ~25 µg/kg) (Figure 13). A baby bella mushroom sample purchased in a different location from the white mushroom sample also contained quantifiable levels of DMA. The white mushroom sample also contained trace levels of inorganic arsenic. A large survey of various market-purchased mushrooms in China also identified this pattern of arsenical distribution in some samples, and supports the implication that arsenical distribution can vary substantially within this food type.[118] However, the significance of this finding is somewhat difficult to discern, as very little regulatory guidance is available for organic arsenic species. The European Food Safety Authority (EFSA) recently published a risk assessment of DMA and MMA in food

which recognized significant gaps in the data, but ultimately concluded that, while MMA intake is generally low enough to avoid potential adverse effects, dietary DMA may present a cancer risk at high levels.[126] (In the present study, MMA only reached quantifiable levels in one sample of sugar kelp.) Genotype- and source-specific monitoring of DMA – along with inorganic arsenicals – in mushrooms and seaweeds may be prudent to allow for efficient regulatory action if and when toxicological data suggests such action is needed.

Unknown arsenic species: While the identification of DMA in seaweed is consistent with prior findings,[126-128] this arsenic species accounted for a very small fraction of the total arsenic present in the seaweed samples evaluated in this study. The overwhelming majority of arsenic in seaweeds was present as unknown arsenic species which, based on their chromatographic retention times, are likely organic. Retention time alignment suggested the presence of approximately 10 unknown arsenical peaks that exceeded LOQ in at least two food samples. Of these unknowns, three seemed to be particularly widespread and abundant: a peak just before 5.0 minutes ("unknown 1"), a peak just after 6.0 minutes ("unknown 8"), and a peak at ~4.8 minutes ("unknown 11"). These unknowns are visible in an example chromatogram of alaria, a type of seaweed (Figure 14). Unknowns 1 and 11, like many of the less-abundant unknowns, were only found in seaweed, with substantial variation between different varieties of seaweed. Unknown 8 was identified in additional food types and is discussed in more detail below. It is likely that the seaweed-specific unknowns (1, 11, 70, 107, and 120) are arsenosugars, which have been reported in many edible seaweeds.[127-129] Confirming this conjecture is challenging, as arsenosugar standards are not readily commercially available. In the absence of authentic standards, HPLC-ESI-MS/MS might offer some confidence in the attribution of these peaks to arsenosugars.[129] However, even if arsenosugar identification is validated, additional toxicological data is required

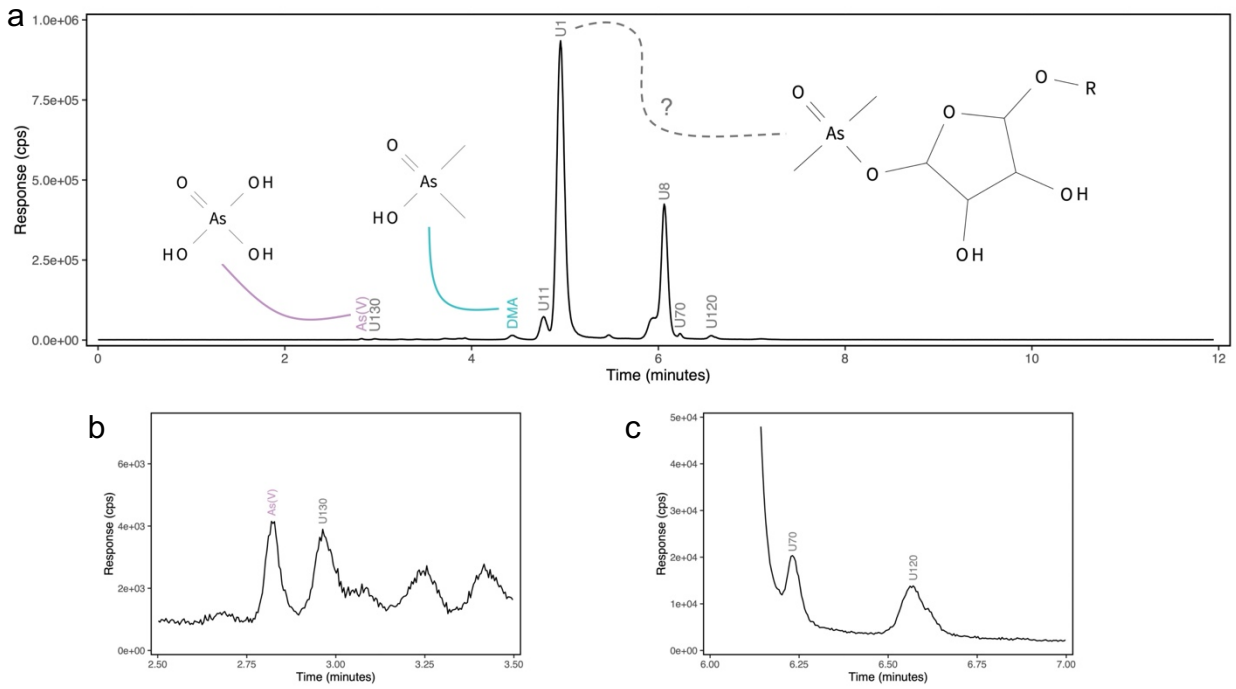

Figure 14: Chromatogram (a) and scaled subsets for visualization of lower-abundance peaks (b shows As(V) and an unknown peak, both present at trace levels; c shows two unknown peaks that both exceed LOQ) illustrating detection of various known and unknown arsenicals in alaria, a type of seaweed. Unknown organic compounds seem to be the primary contributors to the arsenic content of the sample. We hypothesize that unknown 1 is an arsenosugar. This trace shows raw, non-normalized data. As(V) – arsenate; DMA – dimethylarsinic acid; AsB – arsenobetaine; U – unknown

to understand how this finding might translate to human health guidance. The literature does not currently offer clear conclusions on this matter, suggesting both that whole arsenosugars do not seem to be of major toxicological concern *in vitro*, and also that some metabolic products of arsenosugars *in vivo* could pose a risk.[43, 130] This question is additionally complicated by the possibility that arsenosugar metabolism may differ widely among individuals.[131] Furthermore, as is clear from Figure 14, some of these unknown peaks are not baseline-resolved. Alternative chromatographic approaches, particularly ion exchange chromatography, may be required for a more targeted investigation of arsenosugars in seaweed samples.[112, 132] While the present method seems useful to screen for seaweeds enriched in inorganic arsenic and DMA, additional investigation focused on arsenosugars is essential.

Unknown 8, eluting at approximately 6 minutes, was not only common in seaweed but also appeared in two salmon samples, a cricket sample, and portobello mushroom. When the

standards were subjected to the sample preparation protocol, AsB eluted just after 6 min (rather than the 5.4 min observed here), and peaks at ~6 minutes generally aligned to AsB rather than aligning as unknowns (data not shown). AsB has been reported in all the food types in which unknown 8 was identified – salmon,[119] cricket,[133] mushroom,[118] and seaweed[127-129] – and it is generally understood that most of the arsenic in fish takes this minimally toxic form.[43, 72] Therefore, it is likely that unknown 8 represents AsB after sample processing. Unknown 17, which very narrowly missed alignment to unknown 8, occurred in another salmon sample as well as a different mushroom sample and replicate extractions of the same cricket and salmon samples, lending further support to this hypothesis. (In seaweed, potential co-elution with an arsenosugar cannot be ruled out – note the leading shoulder on the unknown 8 peak in Figure 14.) Additional testing indicated that proximate cause of this ~0.4 min retention time shift is the presence of ethanol in the matrix, rather than sonication or other elements of the extraction protocol. Accurate identification and quantification of AsB in the samples appears to require that the standards be at least minimally matrix-matched to the food extracts – the presence of food itself does not seem to be necessary, but the extraction solvent must be present. If unknown 8 is taken to be AsB, recovery of total arsenic in salmon would improve from about 1.5% to about 65% (total arsenic would still be overestimated several fold in wild-caught coho salmon).

Matrix effects were also observed for TETRA standards, but in this case, the change was much more dramatic than a retention time shift: The presence of ethanol dramatically reduced the intensity of the TETRA signal while producing a new arsenical peak at ~7 minutes (Figure 15). This product peak is clearly less polar than TETRA itself. Demethylation of TETRA to trimethylarsine oxide (TMAO) is possible, but literature reports involve either harsh

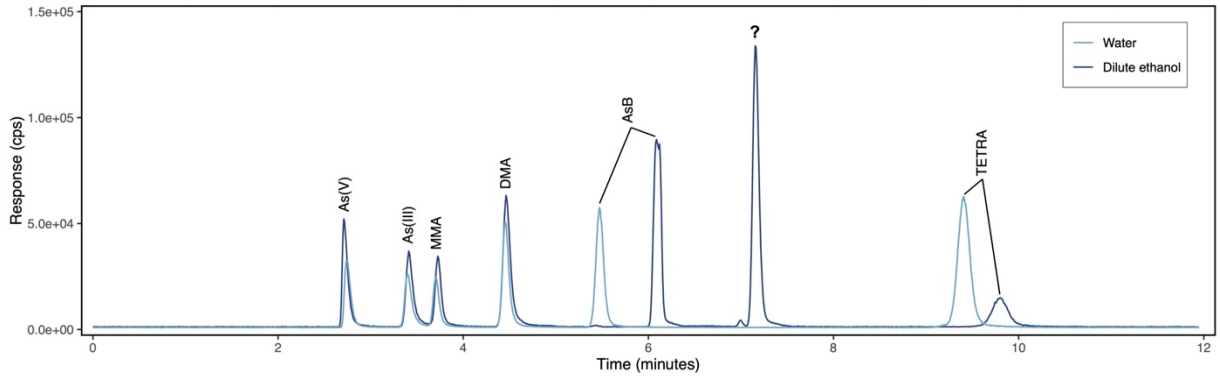

Figure 15: Overlaid chromatograms of a standard mix illustrating the chromatographic effects of dilute ethanol in the matrix. The chromatograms are for a standard that contained 12.5 ppb of each arsenic species. The peak at ~7 minutes was also observed when TETRA alone (but not AsB alone) was analyzed in dilute ethanol. As(V) – arsenate; As(III) – arsenite; DMA – dimethylarsinic acid; MMA – monomethylarsonic acid; AsB – arsenobetaine; TETRA – tetramethylarsonium

conditions[134] or (slow) microbial action.[135] It also appears that TMAO, if present, would likely elute later than the product peak under the chromatographic conditions used in this study.[86] TETRA, like AsB, contains an arsenic cation, suggesting the possibility of interaction with anions in the matrix. However, at the low pH of the mobile phase, substantial ethanol deprotonation is very unlikely. Given the lack of clarity around the chemistry at play, the method as currently proposed does not seem suitable for TETRA quantification. Potential TETRA peaks did not exceed LOD in any of our foods, and only two small peaks (both in seaweeds) aligned to the product peak, so this puzzle does not meaningfully impact our present conclusions. An inability to quantify TETRA could be significant for application of the method to mollusk samples, but this arsenical is thought to be of limited practical toxicological concern.[43, 72] (TETRA can also account for a small proportion of the arsenic in rice.[136]) Therefore, the method might be more efficiently improved by removing the late-eluting TETRA from the analysis to allow for better separation of major arsenosugars (once identified). However, if measurement of TETRA is critical and the analysis is not time- or resource-limited, drying the extracts down under nitrogen gas to remove the organic solvent may ameliorate the chromatographic concerns.

Conclusions: We conclude arsenic speciation results at the level of individual food samples, differentiated by source ontology, is likely important to accurately characterize the risk posed by rice, mushrooms, and seaweed. This is certainly not an exhaustive list; however, the present work presents a significant step toward the ability to perform regular, efficient, large-scale screening of arsenic species distribution in diverse foods. We envision that this method could be used to support a screening strategy that would strengthen protection of consumers from arsenic in the food supply (Figure 16): (1) Screen many diverse food types for arsenic content, as

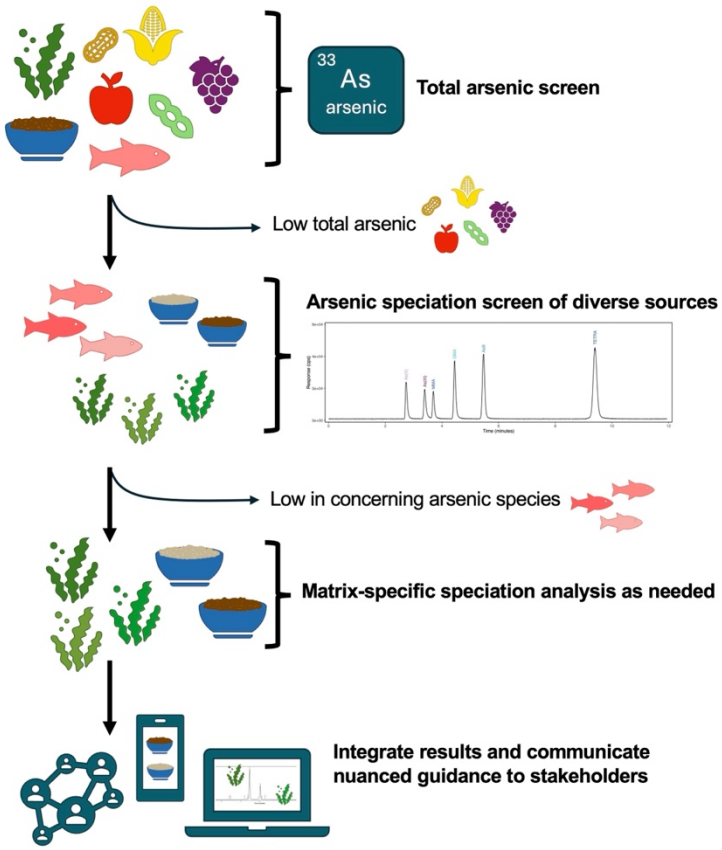

Figure 16: Diagram illustrating a potential screening strategy that strategically incorporates arsenic speciation methods to strengthen protection of consumers from arsenic in the food supply.

is already common in total diet studies. (2) Identify all high-arsenic foods and collect diverse sources of these foods to capture fine-grained variability in geographic and genetic origins. Employ a general arsenic speciation method, such as that described here, to identify samples that

may be enriched in arsenicals that pose human health risks. (3) If appropriate, use more focused and matrix-specific methods to achieve accurate quantitation of dangerous arsenicals where appropriate. (4) Integrate this information to provide nuanced guidance to consumers, regulators, and other stakeholders, emphasizing alternative sources of key foods that pose lower arsenic risks. The data should also be assessed for patterns of more- and less-concerning food sources, so that resources can be appropriately prioritized for consumers who may be at disproportionate risk. Ultimately, scientists, nutrition professionals, consumers, producers, and policymakers working together to implement such an approach may be able to meaningfully mitigate health risks from dietary arsenic.

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APPENDIX A

This Appendix provides additional information about the work described in Chapter 2 – Multielement profiling of diverse food samples.

Supporting information

For supporting information (Supplemental Data Files), the reader is referred to the published work at https://pubs.acs.org/doi/10.1021/acsfoodscitech.2c00396. Readers can access the article's supporting information at no cost. The supporting information is included via stable url (rather than direct incorporation into the text) because the "live" content of some Excel workbooks provided in the SI is essential. Reproducibility of the data analysis relies on a user being able to interact with formulas and linkages in the workbooks. Robust static documentation for the workbooks is also available in the published SI.

Shiny app

Motivation: The original data analysis workflow was programmed in an Excel workbook (example available at link above) by Dr. Jacqueline Chaparro. Though this format is valuable and is a key step, it comes with some drawbacks. We found that the workbook could be difficult for newer users to understand, as it contained many different interconnected sheets and does not have specific instructions in the user interface. These factors could slow users down and cause confusion. We recognized that translating the workflow into code could mitigate these concerns and potentially support enhanced reproducibility.

Development: Code was written in R[vi] and follows the same conceptual workflow described in the main text of the chapter. The workflow was initially translated to an R Markdown document that relied fully on algorithmic selections for internal standards and retained all standard curve points for each element. However, an analyst may at times identify alternate selections based on metrics not considered by the algorithm. For example, changing an internal standard selection from, say, indium (O_2 DRC mode) to rhodium (O_2 DRC mode) for a given analyte may slightly increase the coefficient of variance (CV) in the pooled quality control (QC) samples for that analyte, but substantially improve recoveries in the standard curve. In such a case, a reasonable analyst may wish to "overrule" the algorithm's selection of In (O_2 DRC) in favor of Rh (O_2 DRC). To accommodate analysts' best judgement, the fully automated R Markdown document was adapted into a Shiny app that suggests algorithmic internal standard selections, but allows for full customization of internal standard and curve point parameters (as is permitted by the Excel workbook).

During development, we recognized that the opportunity to adjust internal standard and curve point selections for each of the 26 elements in the panel (37 analytes, accounting for measurements taken at different isotopes and in multiple instrument modes) introduces potential concerns around reproducibility and analyst bias while increasing the time required to complete data analysis. To assess the extent of these issues and identify possibilities for mitigation, we used raw data from an ionomic analysis of 10 randomly selected, previously analyzed foods that were spiked with known quantities of all 26 elements. This data was analyzed using both the fully automated R Markdown document and the fully customizable Shiny app. Spike recoveries were calculated using a separate R script. For many element-food combinations, the opportunity

[vi] *R: A language and environment for statistical computing*; R Foundation for Statistical Computing: Vienna, Austria, 2024 (accessed June 2024).

for manual adjustment had a minimal effect on final computed spike recovery, with recovery differences between the two analysis methods in the 0-5 percentage point range (see zinc in Figure A1). However, manual adjustment consistently led to substantial recovery improvements for a small subset of elements across most or all foods: arsenic, boron, cadmium, cobalt, and vanadium (see arsenic in Figure A1). In many cases, manual adjustments for these elements shifted excessively low or high spike recovery values into an acceptable range (80% - 120%).

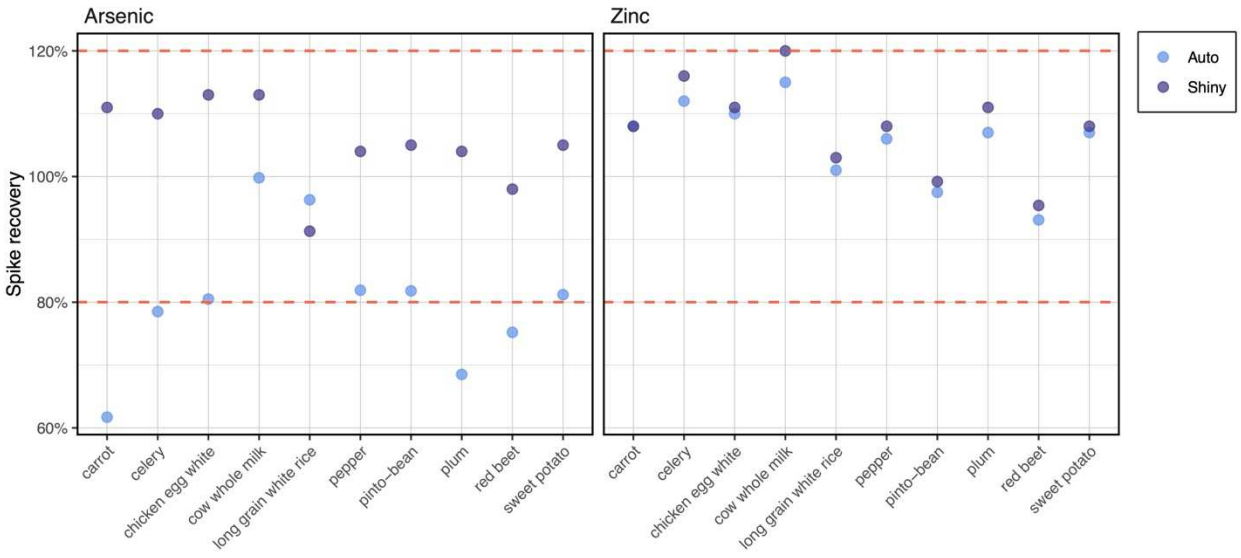

Figure A1: A plot illustrating spike recoveries for two different elements – arsenic (left) and zinc (right) – measured in 10 different foods, calculated from reports produced using two different data analysis workflows (auto and Shiny). Recoveries would ideally fall between 80% and 120% (dashed red lines). In the auto workflow, all selections are algorithmic; in the Shiny workflow, the analyst can make manual adjustments to algorithmic selections. Note that manual adjustments don't seem to have made much of a difference for zinc, but did improve arsenic recoveries in many foods.

Further testing indicated that automatic selections also may not consistently be adequate for selenium and sulfur. Therefore, a "mini" version of the Shiny app that allowed for manual adjustments to these 7 analytes (As, B, Cd, Co, S, Se, V), but retained algorithmic internal standard selections and all curve points for the other analytes in the panel, was also developed. For elements measured in more than one way (e.g., multiple isotopes or instrument modes), the mini app also selects the "best" analyte, defined as the measurement method that minimizes element concentration in the QCs. This theoretically represents the measurement least impacted

by interferents, as interferents are assumed to generally increase measured concentrations. In contrast, the full version of the app reports all results and allows the analyst to manually select the "best" analyte for multi-measurement elements. As a result of this automation, data analysis with the mini app could be faster and less influenced by bias compared to workflows using the full app. However, the mini app currently sees less practical use than the full app due to analyst preference and the wide variety of sample types analyzed by ICP-MS. The mini app may ultimately be more useful to analysts who are less experienced with ionomics methods. The remainder of this section will therefore focus on the full app.

As of submission of this dissertation, the app was last verified to run correctly in R version 4.4.0 (released 24 April 2024) within RStudio version 2024.04.1+748 (Posit Software, PBC). The app requires the following packages (last verified to run correctly using these versions): broom (1.0.6), DT (0.33), markdown (1.12), shiny (1.8.1.1), shinythemes (1.2.0), and tidyverse (2.0.0). Among core tidyverse packages, only forcats is *not* required. Individual tidyverse dependencies are: dplyr (1.1.4), ggplot2 (3.5.1), purrr (1.0.2), readr (2.1.5) stringr (1.5.1), tibble (3.2.1), and tidyr (1.3.1). The app was developed on macOS (last verified on Sonoma 14.5), but runs smoothly on modern Windows OS as well.

Product: The complete R script(s) for the app(s), plus data template files and a detailed SOP, are available from the author (GitHub: @rrjones). When making the same selections for a given dataset, output from the app is highly comparable to Excel output produced by the developer of the workbook. However, the app seems to often be more intuitive and user-friendly than the Excel workbook. App screenshots from key points in the analysis workflow follow below in Figures A2-A5.

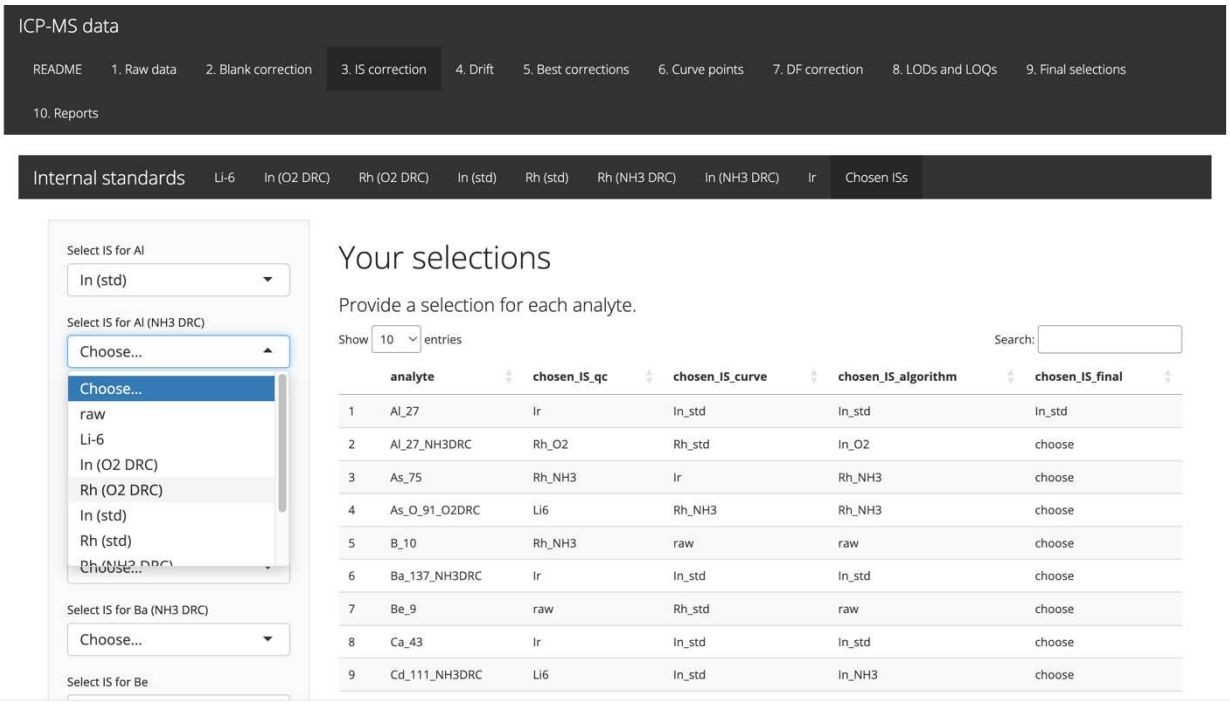

Figure A2: Screenshot of the internal standard correction tab of the Shiny ionomics data analysis app. On the interactive sub-tab (shown), a user can view the algorithmic internal standard recommendations for each analyte, then make appropriate manual selections. Other sub-tabs provide metrics on data quality for each analyte when corrected for each internal standard.

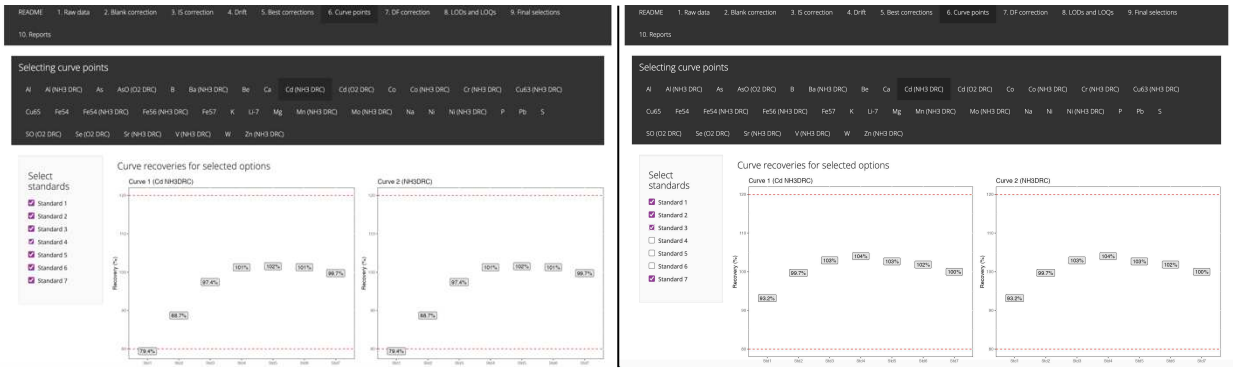

Figure A3: Screenshots of the curve point selection tab of the Shiny ionomics data analysis app. Users can select which curve points to use to fit linear regressions for each analyte independently. In some cases, such as the cadmium example shown, this manual input can meaningfully improve curve recoveries. In the left-hand image, all curve points are selected. The plots show recoveries of cadmium in the standard curve, with red dashed lines at 80% and 120%, and dynamically update with user input. Dropping some points from the curve (right-hand image) improves recoveries particularly at the low end of the curve.

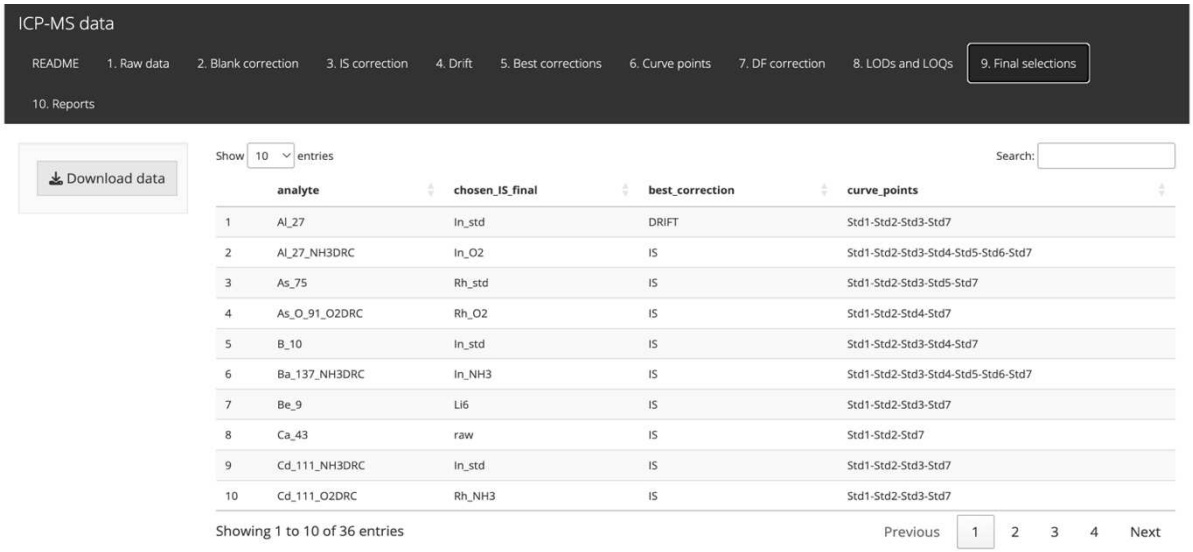

Figure A4: Screenshot of the Shiny ionomics data analysis app tab displaying selections made throughout the app. Content dynamically updates if the user decides to return to a prior tab and make changes. Users can download the table as a csv file to provide documentation for the analysis and support reproducibility.

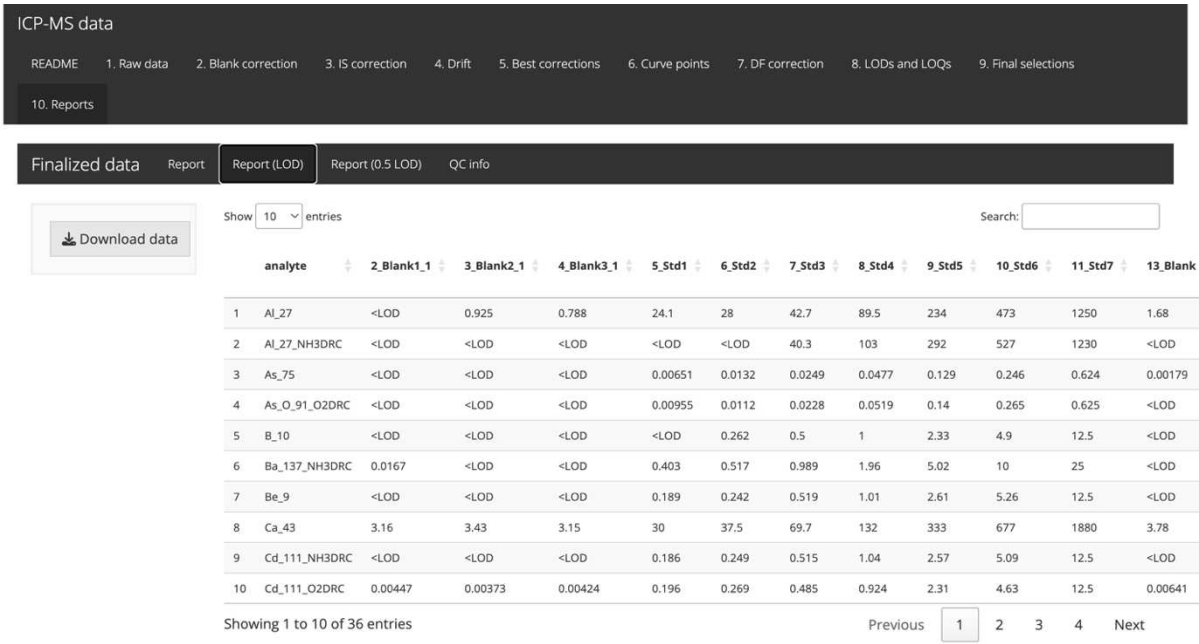

Figure A5: Screenshot of the final reports tab of the Shiny ionomics data analysis app. The app provides three different versions of the final report (shown: the version in which values below the limit of detection are replaced with "<LOD"), all of which can be downloaded as csv files. This tab also provides some final checks on QC data in the reports. If the user chooses to go back to any prior tab and make changes, the reports dynamically update.

The app in ongoing use for ionomics projects. It has proven stable for relatively large ionomics datasets (~100 samples plus ~40 standards, blanks, and QCs per run), such as those required for the Periodic Table of Food Initiative (PTFI). The script is written with durability in

mind and weathered a major R version update (4.3.0 to 4.4.0) with ease, requiring only very minimal updates. However, further work should make the code more concise and encourage the generation of more informative error messages in the user interface when issues do arise. One significant disadvantage of the app as currently written is its inflexibility relative to the analytical method. If a researcher adds or removes analytes or internal standards to the analytical method, updates to the app's source code are necessary to accommodate these changes. Ideally the app would have the capacity to accept any arbitrary set of input analytes. This may not be possible for the mini version of the app, but given sufficient time and expertise, I suspect it is technologically feasible for the full version.

APPENDIX B

This Appendix provides complete final analytical data for the work described in Chapter 3 – Speciation of arsenic in hemp. Sample identifiers should be interpreted as follows: "Shoots" and "roots" describe the aboveground and belowground segments of plants, respectively. Concentrations in ppm (e.g., "15 ppm") indicate the arsenic treatment concentration (i.e., experimental group) the plants are from. Replicate number (e.g., "rep 1") identifies the set of pooled plants within the treatment group to which the measurement corresponds. Root and shoot QCs are pooled quality control samples. Within roots and shoots, all QCs are aliquots of the same pool analyzed multiple times throughout the run (as indicated by different numerical identifiers).

Final report

Table B1: This table contains the final report from the speciation analysis. Each row represents one compound assessed in one sample. RT (min) is the retention time of the compound in minutes. SN ratio is the signal-to-noise ratio for the peak representing the compound. If a compound in a given row has an RT of "NA", the compound was not detected in the associated sample and the row has been introduced in code for statistical analysis purposes. The final three columns are absolute quantities in µg/kg. In the "µg/kg" column, no adjustments are made for compounds below the limits of detection and quantitation. In the "µg/kg (LOD)" column, values below the LOQ (SN = 10) but above the LOD (SN = 3) are replaced with "TRACE" and values below the LOD are replaced with "<LOD". In the "µg/kg (replaced)" column, values below the LOQ are replaced with arbitrarily small values (details in main text of chapter). Rows introduced by code for compounds not identified in a given sample will only have quantified values in this column.

Sample	RT (min)	Compound	SN ratio	µg/kg	µg/kg (LOD)	µg/kg (replaced)
Roots, 0 ppm rep 1	2.76	As(V)	105	48200	48200	48200
Roots, 0 ppm rep 1	3.421	As(III)	454	285000	285000	285000
Roots, 0 ppm rep 1	4.47	DMA	2.93	122	<LOD	248
Roots, 0 ppm rep 1	NA	MMA	NA	NA	<LOD	293
Roots, 0 ppm rep 1	NA	AsB	NA	NA	<LOD	207
Roots, 0 ppm rep 1	NA	TETRA	NA	NA	<LOD	269
Roots, 0 ppm rep 2	2.752	As(V)	93	53600	53600	53600
Roots, 0 ppm rep 2	3.412	As(III)	354	270000	270000	270000
Roots, 0 ppm rep 2	NA	MMA	NA	NA	<LOD	397

Sample	RT (min)	Compound	SN ratio	µg/kg	µg/kg (LOD)	µg/kg (replaced)
Roots, 0 ppm rep 2	NA	DMA	NA	NA	<LOD	336
Roots, 0 ppm rep 2	NA	AsB	NA	NA	<LOD	281
Roots, 0 ppm rep 2	NA	TETRA	NA	NA	<LOD	366
Roots, 0 ppm rep 3	2.76	As(V)	58.8	20200	20200	20200
Roots, 0 ppm rep 3	3.421	As(III)	309	138000	138000	138000
Roots, 0 ppm rep 3	5.41	AsB	46.2	8250	8250	8250
Roots, 0 ppm rep 3	NA	MMA	NA	NA	<LOD	287
Roots, 0 ppm rep 3	NA	DMA	NA	NA	<LOD	243
Roots, 0 ppm rep 3	NA	TETRA	NA	NA	<LOD	264
Roots, 15 ppm rep 1	2.752	As(V)	152	70800	70800	70800
Roots, 15 ppm rep 1	3.412	As(III)	687	420000	420000	420000
Roots, 15 ppm rep 1	NA	MMA	NA	NA	<LOD	273
Roots, 15 ppm rep 1	NA	DMA	NA	NA	<LOD	231
Roots, 15 ppm rep 1	NA	AsB	NA	NA	<LOD	193
Roots, 15 ppm rep 1	NA	TETRA	NA	NA	<LOD	251
Roots, 15 ppm rep 2	2.752	As(V)	112	30000	30000	30000
Roots, 15 ppm rep 2	3.412	As(III)	631	228000	228000	228000
Roots, 15 ppm rep 2	4.462	DMA	88.2	15800	15800	15800
Roots, 15 ppm rep 2	NA	MMA	NA	NA	<LOD	262
Roots, 15 ppm rep 2	NA	AsB	NA	NA	<LOD	185
Roots, 15 ppm rep 2	NA	TETRA	NA	NA	<LOD	241
Roots, 15 ppm rep 3	2.752	As(V)	86.6	34000	34000	34000
Roots, 15 ppm rep 3	3.421	As(III)	431	220000	220000	220000
Roots, 15 ppm rep 3	NA	MMA	NA	NA	<LOD	269
Roots, 15 ppm rep 3	NA	DMA	NA	NA	<LOD	228
Roots, 15 ppm rep 3	NA	AsB	NA	NA	<LOD	190
Roots, 15 ppm rep 3	NA	TETRA	NA	NA	<LOD	248
Roots, 45 ppm rep 1	2.752	As(V)	134	50200	50200	50200
Roots, 45 ppm rep 1	3.412	As(III)	773	371000	371000	371000
Roots, 45 ppm rep 1	NA	MMA	NA	NA	<LOD	237
Roots, 45 ppm rep 1	NA	DMA	NA	NA	<LOD	201
Roots, 45 ppm rep 1	NA	AsB	NA	NA	<LOD	168
Roots, 45 ppm rep 1	NA	TETRA	NA	NA	<LOD	218
Roots, 45 ppm rep 2	2.752	As(V)	90.3	40400	40400	40400
Roots, 45 ppm rep 2	3.412	As(III)	354	209000	209000	209000
Roots, 45 ppm rep 2	3.742	MMA	5.1	2280	TRACE	418
Roots, 45 ppm rep 2	NA	DMA	NA	NA	<LOD	354

Sample	RT (min)	Compound	SN ratio	µg/kg	µg/kg (LOD)	µg/kg (replaced)
Roots, 45 ppm rep 2	NA	AsB	NA	NA	<LOD	296
Roots, 45 ppm rep 2	NA	TETRA	NA	NA	<LOD	385
Roots, 45 ppm rep 3	2.76	As(V)	105	40500	40500	40500
Roots, 45 ppm rep 3	3.421	As(III)	508	255000	255000	255000
Roots, 45 ppm rep 3	NA	MMA	NA	NA	<LOD	228
Roots, 45 ppm rep 3	NA	DMA	NA	NA	<LOD	193
Roots, 45 ppm rep 3	NA	AsB	NA	NA	<LOD	161
Roots, 45 ppm rep 3	NA	TETRA	NA	NA	<LOD	210
Roots, 100 ppm rep 1	2.76	As(V)	147	77000	77000	77000
Roots, 100 ppm rep 1	3.421	As(III)	621	434000	434000	434000
Roots, 100 ppm rep 1	NA	MMA	NA	NA	<LOD	265
Roots, 100 ppm rep 1	NA	DMA	NA	NA	<LOD	224
Roots, 100 ppm rep 1	NA	AsB	NA	NA	<LOD	187
Roots, 100 ppm rep 1	NA	TETRA	NA	NA	<LOD	244
Roots, 100 ppm rep 2	2.752	As(V)	137	132000	132000	132000
Roots, 100 ppm rep 2	3.412	As(III)	282	349000	349000	349000
Roots, 100 ppm rep 2	NA	MMA	NA	NA	<LOD	548
Roots, 100 ppm rep 2	NA	DMA	NA	NA	<LOD	464
Roots, 100 ppm rep 2	NA	AsB	NA	NA	<LOD	387
Roots, 100 ppm rep 2	NA	TETRA	NA	NA	<LOD	504
Roots, 100 ppm rep 3	2.752	As(V)	163	40700	40700	40700
Roots, 100 ppm rep 3	3.412	As(III)	994	319000	319000	319000
Roots, 100 ppm rep 3	5.41	AsB	3.89	-71.2	TRACE	155
Roots, 100 ppm rep 3	NA	MMA	NA	NA	<LOD	220
Roots, 100 ppm rep 3	NA	DMA	NA	NA	<LOD	186
Roots, 100 ppm rep 3	NA	TETRA	NA	NA	<LOD	202
Roots, QC001	2.76	As(V)	92.7	25300	25300	25300
Roots, QC001	3.421	As(III)	481	170000	170000	170000
Roots, QC001	4.479	DMA	6.55	417	TRACE	243
Roots, QC001	5.419	AsB	4.18	-67.4	TRACE	203
Roots, QC001	NA	MMA	NA	NA	<LOD	287
Roots, QC001	NA	TETRA	NA	NA	<LOD	264
Roots, QC002	2.752	As(V)	109	25500	25500	25500
Roots, QC002	3.412	As(III)	560	170000	170000	170000
Roots, QC002	4.462	DMA	7.72	464	TRACE	243
Roots, QC002	5.385	AsB	4.81	-72.9	TRACE	203
Roots, QC002	NA	MMA	NA	NA	<LOD	287

Sample	RT (min)	Compound	SN ratio	µg/kg	µg/kg (LOD)	µg/kg (replaced)
Roots, QC002	NA	TETRA	NA	NA	<LOD	264
Roots, QC003	2.76	As(V)	107	25100	25100	25100
Roots, QC003	3.421	As(III)	545	170000	170000	170000
Roots, QC003	4.47	DMA	6.99	351	TRACE	243
Roots, QC003	5.402	AsB	4.66	-92.3	TRACE	203
Roots, QC003	NA	MMA	NA	NA	<LOD	287
Roots, QC003	NA	TETRA	NA	NA	<LOD	264
Roots, QC004	2.76	As(V)	98.4	24200	24200	24200
Roots, QC004	3.421	As(III)	485	160000	160000	160000
Roots, QC004	4.462	DMA	6.74	285	TRACE	243
Roots, QC004	5.402	AsB	4.23	-152	TRACE	203
Roots, QC004	NA	MMA	NA	NA	<LOD	287
Roots, QC004	NA	TETRA	NA	NA	<LOD	264
Shoots, 0 ppm rep 1	2.735	As(V)	6.33	3650	TRACE	224
Shoots, 0 ppm rep 1	3.395	As(III)	2.87	2190	<LOD	235
Shoots, 0 ppm rep 1	NA	MMA	NA	NA	<LOD	256
Shoots, 0 ppm rep 1	NA	DMA	NA	NA	<LOD	216
Shoots, 0 ppm rep 1	NA	AsB	NA	NA	<LOD	181
Shoots, 0 ppm rep 1	NA	TETRA	NA	NA	<LOD	235
Shoots, 0 ppm rep 2	2.752	As(V)	6.07	3060	TRACE	163
Shoots, 0 ppm rep 2	3.412	As(III)	1.89	807	<LOD	171
Shoots, 0 ppm rep 2	NA	MMA	NA	NA	<LOD	187
Shoots, 0 ppm rep 2	NA	DMA	NA	NA	<LOD	158
Shoots, 0 ppm rep 2	NA	AsB	NA	NA	<LOD	132
Shoots, 0 ppm rep 2	NA	TETRA	NA	NA	<LOD	172
Shoots, 0 ppm rep 3	2.735	As(V)	2.09	123	<LOD	156
Shoots, 0 ppm rep 3	3.429	As(III)	4.1	1690	TRACE	164
Shoots, 0 ppm rep 3	4.487	DMA	1.2	-129	<LOD	151
Shoots, 0 ppm rep 3	NA	MMA	NA	NA	<LOD	179
Shoots, 0 ppm rep 3	NA	AsB	NA	NA	<LOD	126
Shoots, 0 ppm rep 3	NA	TETRA	NA	NA	<LOD	164
Shoots, 15 ppm rep 1	2.743	As(V)	2.74	NA	<LOD	161
Shoots, 15 ppm rep 1	3.421	As(III)	2.99	NA	<LOD	169
Shoots, 15 ppm rep 1	4.47	DMA	2.67	NA	<LOD	156
Shoots, 15 ppm rep 1	NA	MMA	NA	NA	<LOD	184
Shoots, 15 ppm rep 1	NA	AsB	NA	NA	<LOD	130
Shoots, 15 ppm rep 1	NA	TETRA	NA	NA	<LOD	169

Sample	RT (min)	Compound	SN ratio	µg/kg	µg/kg (LOD)	µg/kg (replaced)
Shoots, 15 ppm rep 2	2.752	As(V)	4.15	277	TRACE	162
Shoots, 15 ppm rep 2	3.429	As(III)	8.54	1350	TRACE	170
Shoots, 15 ppm rep 2	3.742	MMA	5.27	761	TRACE	185
Shoots, 15 ppm rep 2	4.445	DMA	1.22	-324	<LOD	157
Shoots, 15 ppm rep 2	NA	AsB	NA	NA	<LOD	131
Shoots, 15 ppm rep 2	NA	TETRA	NA	NA	<LOD	170
Shoots, 15 ppm rep 3	2.752	As(V)	2.52	534	<LOD	164
Shoots, 15 ppm rep 3	3.421	As(III)	3.92	1900	TRACE	172
Shoots, 15 ppm rep 3	4.487	DMA	0.833	-294	<LOD	159
Shoots, 15 ppm rep 3	NA	MMA	NA	NA	<LOD	188
Shoots, 15 ppm rep 3	NA	AsB	NA	NA	<LOD	133
Shoots, 15 ppm rep 3	NA	TETRA	NA	NA	<LOD	173
Shoots, 45 ppm rep 1	2.743	As(V)	3.28	571	TRACE	157
Shoots, 45 ppm rep 1	3.429	As(III)	5.22	1540	TRACE	165
Shoots, 45 ppm rep 1	NA	MMA	NA	NA	<LOD	180
Shoots, 45 ppm rep 1	NA	DMA	NA	NA	<LOD	152
Shoots, 45 ppm rep 1	NA	AsB	NA	NA	<LOD	127
Shoots, 45 ppm rep 1	NA	TETRA	NA	NA	<LOD	165
Shoots, 45 ppm rep 2	2.743	As(V)	5.44	2060	TRACE	273
Shoots, 45 ppm rep 2	3.421	As(III)	4.21	2210	TRACE	286
Shoots, 45 ppm rep 2	NA	MMA	NA	NA	<LOD	312
Shoots, 45 ppm rep 2	NA	DMA	NA	NA	<LOD	264
Shoots, 45 ppm rep 2	NA	AsB	NA	NA	<LOD	220
Shoots, 45 ppm rep 2	NA	TETRA	NA	NA	<LOD	287
Shoots, 45 ppm rep 3	2.743	As(V)	3.63	289	TRACE	157
Shoots, 45 ppm rep 3	3.412	As(III)	4.29	579	TRACE	165
Shoots, 45 ppm rep 3	3.742	MMA	64.4	21300	21300	21300
Shoots, 45 ppm rep 3	NA	DMA	NA	NA	<LOD	152
Shoots, 45 ppm rep 3	NA	AsB	NA	NA	<LOD	127
Shoots, 45 ppm rep 3	NA	TETRA	NA	NA	<LOD	165
Shoots, 100 ppm rep 1	2.743	As(V)	13.3	4100	4100	4100
Shoots, 100 ppm rep 1	3.421	As(III)	11.5	4160	4160	4160
Shoots, 100 ppm rep 1	NA	MMA	NA	NA	<LOD	183
Shoots, 100 ppm rep 1	NA	DMA	NA	NA	<LOD	155
Shoots, 100 ppm rep 1	NA	AsB	NA	NA	<LOD	129
Shoots, 100 ppm rep 1	NA	TETRA	NA	NA	<LOD	168
Shoots, 100 ppm rep 2	2.752	As(V)	6.04	1170	TRACE	176

Sample	RT (min)	Compound	SN ratio	µg/kg	µg/kg (LOD)	µg/kg (replaced)
Shoots, 100 ppm rep 2	3.412	As(III)	13.7	4800	4800	4800
Shoots, 100 ppm rep 2	3.734	MMA	1.58	24.1	<LOD	201
Shoots, 100 ppm rep 2	NA	DMA	NA	NA	<LOD	170
Shoots, 100 ppm rep 2	NA	AsB	NA	NA	<LOD	142
Shoots, 100 ppm rep 2	NA	TETRA	NA	NA	<LOD	185
Shoots, 100 ppm rep 3	2.752	As(V)	7.21	2160	TRACE	159
Shoots, 100 ppm rep 3	3.421	As(III)	20.2	9290	9290	9290
Shoots, 100 ppm rep 3	NA	MMA	NA	NA	<LOD	181
Shoots, 100 ppm rep 3	NA	DMA	NA	NA	<LOD	153
Shoots, 100 ppm rep 3	NA	AsB	NA	NA	<LOD	128
Shoots, 100 ppm rep 3	NA	TETRA	NA	NA	<LOD	167
Shoots, QC001	2.752	As(V)	3.6	550	TRACE	171
Shoots, QC001	3.412	As(III)	5.73	1690	TRACE	180
Shoots, QC001	3.751	MMA	4.89	1890	TRACE	196
Shoots, QC001	NA	DMA	NA	NA	<LOD	166
Shoots, QC001	NA	AsB	NA	NA	<LOD	138
Shoots, QC001	NA	TETRA	NA	NA	<LOD	180
Shoots, QC002	2.752	As(V)	4.85	649	TRACE	171
Shoots, QC002	3.429	As(III)	7.39	1990	TRACE	180
Shoots, QC002	3.751	MMA	7.06	2190	TRACE	196
Shoots, QC002	NA	DMA	NA	NA	<LOD	166
Shoots, QC002	NA	AsB	NA	NA	<LOD	138
Shoots, QC002	NA	TETRA	NA	NA	<LOD	180
Shoots, QC003	2.752	As(V)	4.27	576	TRACE	171
Shoots, QC003	3.421	As(III)	7.27	1760	TRACE	180
Shoots, QC003	3.751	MMA	6.47	2010	TRACE	196
Shoots, QC003	NA	DMA	NA	NA	<LOD	166
Shoots, QC003	NA	AsB	NA	NA	<LOD	138
Shoots, QC003	NA	TETRA	NA	NA	<LOD	180
Shoots, QC004	2.752	As(V)	4.21	557	TRACE	171
Shoots, QC004	3.421	As(III)	6.48	1720	TRACE	180
Shoots, QC004	3.742	MMA	6.03	2060	TRACE	196
Shoots, QC004	NA	DMA	NA	NA	<LOD	166
Shoots, QC004	NA	AsB	NA	NA	<LOD	138
Shoots, QC004	NA	TETRA	NA	NA	<LOD	180

APPENDIX C

This Appendix provides data and metadata for the work described in Chapter 4 – Speciation of arsenic in diverse food matrices. Sample IDs can be used to connect foods across tables. This appendix also contains supplementary figures expanding on the discussion of seaweed in the main text, emphasizing potential future work.

Metadata

Table C1: This table contains metadata for all the food samples analyzed in the study described in Chapter 4. Unless otherwise noted, foods were *not* cooked. Except where otherwise noted, locations in food names indicate where foods were purchased, not necessarily where they were produced. Foods marked with an asterisk (*) are excluded from the reports of known compounds due to overestimation of total arsenic and/or chromatographic concerns.

Food	Sample ID	Source	Approximate water content
alaria	GGB101517	commercial vendor; ME, USA	unknown
alaria (cooked)	GGB101530	commercial vendor; ME, USA	67%
asparagus (Fort Collins, CO)	GGB100073	grocery store; CO, USA	90%
asparagus (Windsor, CO)	GGB102187	grocery store; CO, USA	92%
asparagus (organic)*	GGB102200*	grocery store; CO, USA	92%
bladderwrack	GGB101516	commercial vendor; ME, USA	8.5%
bok choy	GGB100026	grocery store; CO, USA	92%
bok choy (mei qing)	GGB102190	grocery store; CO, USA	93%
bok choy (baby)	GGB102204	grocery store; CO, USA	95%
bok choy (organic)	GGB102205	grocery store; CO, USA	94%
carrot	GGB100030	grocery store; CO, USA	53%
celery (Fort Collins, CO)	GGB100053	grocery store; CO, USA	96%
celery (organic)	GGB102206	grocery store; CO, USA	95%
celery (organic)	GGB102402	grocery store; CO, USA	95%
celery hearts	GGB102188	grocery store; CO, USA	95%
cricket (protein powder)	GGB100024	researcher; CO, USA	37%
cricket (protein powder)	GGB102396	researcher; WI, USA	39%
cricket (protein powder)	GGB102397	researcher; WI, USA	39%
cricket (whole)	GGB100025	researcher; CO, USA	39%
cucumber (Fort Collins, CO)*	GGB100038*	grocery store; CO, USA	95%
cucumber (Windsor, CO)	GGB102191	grocery store; CO, USA	95%

Food	Sample ID	Source	Approximate water content
dulse	GGB101515	commercial vendor; ME, USA	14%
eggplant (Windsor, CO)	GGB102197	grocery store; CO, USA	91%
eggplant (Fort Collins, CO)	GGB102201	grocery store; CO, USA	92%
eggplant (Fort Collins, CO)	GGB100867	grocery store; CO, USA	93%
Irish moss	GGB101514	commercial vendor; ME, USA	8.1%
Irish moss (cooked)	GGB101538	commercial vendor; ME, USA	95%
kale (Fort Collins, CO)	GGB100128	grocery store; CO, USA	84%
kale (Windsor, CO)	GGB102189	grocery store; CO, USA	84%
kale (lacinato)	GGB102192	grocery store; CO, USA	90%
kale (baby, organic)	GGB102203	grocery store; CO, USA	91%
kale (red, organic)	GGB102202	grocery store; CO, USA	85%
laver	GGB101512	commercial vendor; ME, USA	13%
laver (sauteed)	GGB101528	commercial vendor; ME, USA	74%
mushroom (baby bella)	GGB102196	grocery store; CO, USA	93%
mushroom (portobello)*	GGB102194*	grocery store; CO, USA	90%
mushroom (white)	GGB100028	grocery store; CO, USA	67%
onion (Peruvian gold)	GGB102198	grocery store; CO, USA	91%
onion (yellow, cv "Vaquero")*	GGB100012*	researcher; CO, USA	91%
onion (yellow, sweet)	GGB102398	grocery store; CO, USA	90%
papaya	GGB100155	grocery store; CO, USA	91%
papaya (golden)	GGB102199	grocery store; CO, USA	91%
rice (brown, long-grain, cooked)	GGB100040	grocery store; CO, USA	61%
rice (brown, organic, cooked)	GGB102208	grocery store; CO, USA	69%
rice (brown, organic)	GGB102207	grocery store; CO, USA	10%
rice (purple, cooked)	GGB102210	grocery store; CO, USA	6.2%
rice (purple)	GGB102209	grocery store; CO, USA	60%
rice (white, cooked)	GGB102212	grocery store; CO, USA	70%
rice (white, long-grain, cooked)	GGB100041	grocery store; CO, USA	58%
rice (white, organic, cooked)	GGB102214	grocery store; CO, USA	67%
rice (white, organic)	GGB102213	grocery store; CO, USA	8.5%
rice (white)	GGB102211	grocery store; CO, USA	8.8%
rockweed	GGB101511	commercial vendor; ME, USA	4.0%
salmon	GGB100133	grocery store; CO, USA	68%
salmon (coho, wild)*	GGB102216*	grocery store; CO, USA	77%
salmon (farmed, Atlantic)	GGB102215	grocery store; CO, USA	63%
sea lettuce	GGB101510	commercial vendor; ME, USA	20%
sesame seed	GGB100061	grocery store; CO, USA	2.8%

Food	Sample ID	Source	Approximate water content
sesame seed (black, organic)*	GGB102217*	grocery store; CO, USA	2.8%
sesame seed (organic)	GGB102218	grocery store; CO, USA	2.6%
spinach	GGB102185	grocery store; CO, USA	95%
spinach (baby, organic)	GGB102184	grocery store; CO, USA	94%
spinach (baby)	GGB102183	grocery store; CO, USA	93%
spinach (organic)	GGB102186	grocery store; CO, USA	95%
strawberry	GGB102400	grocery store; CO, USA	unknown
strawberry (organic)	GGB102399	grocery store; CO, USA	89%
sugar kelp	GGB101513	commercial vendor; ME, USA	21%
sugar kelp (cooked)	GGB101529	commercial vendor; ME, USA	89%
tahini	GGB102219	grocery store; CO, USA	0.3%
turnip (unknown origin)	GGB100154	grocery store; CO, USA	87%
turnip (US origin)	GGB102220	grocery store; CO, USA	90%
turnip (organic, white)	GGB102401	grocery store; CO, USA	89%
zucchini (Fort Collins, CO)	GGB100027	grocery store; CO, USA	94%
zucchini (Windsor, CO)	GGB102195	grocery store; CO, USA	94%
zucchini (organic)	GGB102193	grocery store; CO, USA	94%

Final report: Known compounds

Table C2: This table contains a final report from the speciation analysis. This report only includes information about the known arsenic compounds (i.e., those in the calibration curve). It provides concentrations on the basis of arsenic content (µg arsenic, rather than µg whole compound) per kg *dry* food. Values for peaks below the LOQ (SN = 10) but above the LOD (SN = 3) are replaced with "TRACE" and values below the LOD are replaced with "<LOD".

Food	Sample ID	As(V) (µg/kg DW)	As(III) (µg/kg DW)	MMA (µg/kg DW)	DMA (µg/kg DW)	AsB (µg/kg DW)	TETRA (µg/kg DW)
alaria	GGB101517	<LOD	843	<LOD	<LOD	<LOD	<LOD
alaria (cooked)	GGB101530	TRACE	<LOD	<LOD	164	138	<LOD
asparagus	GGB102187	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
asparagus	GGB100073	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
bladderwrack	GGB101516	<LOD	<LOD	TRACE	TRACE	TRACE	<LOD
bladderwrack	GGB101516	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
bok choy	GGB100026	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
bok choy	GGB102190	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
bok choy (baby)	GGB102204	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
bok choy (organic)	GGB102205	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
carrot	GGB100030	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD

Food	Sample ID	As(V) (µg/kg DW)	As(III) (µg/kg DW)	MMA (µg/kg DW)	DMA (µg/kg DW)	AsB (µg/kg DW)	TETRA (µg/kg DW)
celery	GGB100053	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
celery (organic)	GGB102402	TRACE	<LOD	TRACE	<LOD	<LOD	<LOD
celery (organic)	GGB102206	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
celery hearts	GGB102188	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
cricket	GGB100025	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
cricket (powder)	GGB102397	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
cricket (powder)	GGB102396	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
cricket (powder)	GGB100024	TRACE	TRACE	<LOD	<LOD	<LOD	<LOD
cricket (whole)	GGB100025	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
cucumber	GGB102191	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
dulse	GGB101515	<LOD	<LOD	TRACE	579	75	<LOD
dulse	GGB101515	<LOD	<LOD	TRACE	662	84.5	<LOD
eggplant	GGB102201	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
eggplant	GGB102197	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD
eggplant (reference)	GGB100867	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Irish moss	GGB101514	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
Irish moss (cooked)	GGB101538	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
kale	GGB102192	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD
kale	GGB102189	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD
kale	GGB100128	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
kale (baby, organic)	GGB102203	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
kale (red, organic)	GGB102202	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
laver	GGB101512	TRACE	<LOD	TRACE	TRACE	<LOD	<LOD
laver (cooked)	GGB101528	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
mushroom	GGB102196	<LOD	<LOD	<LOD	72	<LOD	<LOD
mushroom	GGB100028	TRACE	TRACE	<LOD	77.5	<LOD	<LOD
mushroom	GGB102196	<LOD	<LOD	<LOD	79.9	<LOD	<LOD
onion (Peruvian gold)	GGB102198	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
onion (yellow, sweet)	GGB102398	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
papaya	GGB100155	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD
papaya (gold)	GGB102199	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Food	Sample ID	As(V) (µg/kg DW)	As(III) (µg/kg DW)	MMA (µg/kg DW)	DMA (µg/kg DW)	AsB (µg/kg DW)	TETRA (µg/kg DW)
rice (brown, long grain, cooked)	GGB100040	TRACE	165	<LOD	TRACE	<LOD	<LOD
rice (brown, long grain, cooked)	GGB100040	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD
rice (brown, organic, cooked)	GGB102208	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD
rice (brown, organic)	GGB102207	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
rice (purple, cooked)	GGB102210	TRACE	TRACE	<LOD	TRACE	<LOD	<LOD
rice (purple)	GGB102209	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
rice (white, cooked)	GGB102212	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
rice (white, long grain, cooked)	GGB100041	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD
rice (white, long grain, cooked)	GGB100041	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD
rice (white, organic, cooked)	GGB102214	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
rice (white, organic)	GGB102213	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
rice (white)	GGB102211	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
rockweed	GGB101511	<LOD	<LOD	TRACE	TRACE	TRACE	<LOD
salmon	GGB100133	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
salmon	GGB100133	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
salmon (farmed, Atlantic)	GGB102215	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
sea lettuce	GGB101510	TRACE	<LOD	<LOD	TRACE	<LOD	<LOD
sesame seed	GGB100061	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
sesame seed (organic)	GGB102218	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
spinach	GGB102185	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
spinach (baby, organic)	GGB102184	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
spinach (baby)	GGB102183	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
spinach (organic, reference)	NA	TRACE	<LOD	TRACE	<LOD	<LOD	<LOD
spinach (organic)	GGB102186	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
strawberry	GGB100029	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Food	Sample ID	As(V) (µg/kg DW)	As(III) (µg/kg DW)	MMA (µg/kg DW)	DMA (µg/kg DW)	AsB (µg/kg DW)	TETRA (µg/kg DW)
strawberry	GGB102400	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
strawberry	GGB102400	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
strawberry (organic)	GGB102399	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
sugar kelp	GGB101513	TRACE	<LOD	137	191	<LOD	<LOD
sugar kelp (cooked)	GGB101529	TRACE	<LOD	TRACE	TRACE	<LOD	<LOD
tahini	GGB102219	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
turnip	GGB102220	<LOD	TRACE	<LOD	<LOD	<LOD	<LOD
turnip	GGB100154	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
turnip (white, organic)	GGB102401	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
zucchini	GGB100027	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
zucchini	GGB102195	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
zucchini (organic)	GGB102193	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
QC001	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC002	NA	182	<LOD	214	TRACE	<LOD	<LOD
QC003	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC004	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC005	NA	82.3	<LOD	TRACE	TRACE	<LOD	<LOD
QC006	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC007	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC008	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC008A	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC009	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC010	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC011	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC012	NA	<LOD	348	<LOD	TRACE	<LOD	<LOD
QC013	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC014	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC015	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC016	NA	<LOD	<LOD	<LOD	TRACE	<LOD	<LOD
QC017	NA	<LOD	<LOD	207	TRACE	<LOD	<LOD

Table C3: This table contains another final report from the speciation analysis. This report only includes information about the known arsenic compounds (i.e., those in the calibration curve). It provides concentrations on the basis of arsenic content (µg arsenic, rather than µg whole compound) per kg *fresh* food. Values for peaks below the LOQ were replaced with appropriate small values during an earlier workflow step (details in main text of chapter). Two strawberry samples, one spinach sample, and one alaria sample are excluded due to lack of fresh weight data.

Food	Sample ID	As(V) (µg/kg FW)	As(III) (µg/kg FW)	MMA (µg/kg FW)	DMA (µg/kg FW)	AsB (µg/kg FW)	TETRA (µg/kg FW)
alaria	GGB101517	NA	NA	NA	NA	NA	NA
alaria (cooked)	GGB101530	1.05	0.396	0.425	53.7	45.2	0.165
asparagus	GGB102187	0.0741	0.0931	0.101	0.0468	0.0418	0.0387
asparagus	GGB100073	0.0978	0.124	0.133	0.207	0.0551	0.0511
bladderwrack	GGB101516	0.887	1.12	4	1.87	1.67	0.463
bladderwrack	GGB101516	0.887	1.12	1.2	1.87	0.5	0.463
bok choy	GGB100026	0.0747	0.0943	0.101	0.0472	0.0421	0.039
bok choy	GGB102190	0.0695	0.0879	0.0944	0.0439	0.0391	0.0363
bok choy (baby)	GGB102204	0.0495	0.0623	0.067	0.0313	0.0279	0.0259
bok choy (organic)	GGB102205	0.0534	0.0673	0.0722	0.0337	0.0301	0.0279
carrot	GGB100030	0.449	1.88	0.606	0.284	0.253	0.235
celery	GGB100053	0.0427	0.0537	0.0577	0.027	0.0241	0.0223
celery hearts	GGB102188	0.0446	0.056	0.0606	0.0282	0.0252	0.0233
celery (organic)	GGB102402	0.148	0.056	0.201	0.0281	0.0251	0.0232
celery (organic)	GGB102206	0.0444	0.056	0.0601	0.0281	0.025	0.0232
cricket (whole)	GGB100025	0.587	0.737	0.798	0.371	0.331	0.307
cricket (whole)	GGB100025	0.598	0.755	0.81	0.378	0.337	0.312
cricket (powder)	GGB102397	0.592	0.748	0.804	0.374	0.334	0.309
cricket (powder)	GGB102396	0.587	0.742	0.798	0.371	0.331	0.307
cricket (powder)	GGB100024	2.04	2.57	0.832	0.387	0.345	0.319
cucumber	GGB102191	0.0518	0.0654	0.0702	0.0327	0.0292	0.0271
cucumber	GGB100038	0.0451	0.189	0.0609	0.0285	0.0254	0.0236
dulse	GGB101515	0.825	1.04	3.73	496	64.3	0.431
dulse	GGB101515	0.825	1.04	3.72	567	72.4	0.431
eggplant	GGB102201	0.0788	0.0996	0.107	0.0499	0.0444	0.0412
eggplant	GGB102197	0.0874	0.366	0.118	0.0552	0.0492	0.0456
eggplant (reference)	GGB100867	0.066	0.0832	0.0894	0.0417	0.0372	0.0345
Irish moss	GGB101514	0.887	1.12	1.2	1.87	0.5	0.463
Irish moss (cooked)	GGB101538	0.0472	0.0593	0.0637	0.0299	0.0266	0.0247
Irish moss (cooked)	GGB101538	0.0472	0.0593	0.0637	0.0299	0.0266	0.0247
Irish moss (cooked)	GGB101538	0.0472	0.0593	0.0637	0.0299	0.0266	0.0247
kale	GGB102192	0.1	0.421	0.136	0.0634	0.0566	0.0524

Food	Sample ID	As(V) (µg/kg FW)	As(III) (µg/kg FW)	MMA (µg/kg FW)	DMA (µg/kg FW)	AsB (µg/kg FW)	TETRA (µg/kg FW)
kale	GGB102189	0.154	0.648	0.209	0.0976	0.087	0.0806
kale	GGB100128	0.153	0.193	0.207	0.0969	0.0863	0.0801
kale (baby, organic)	GGB102203	0.0851	0.108	0.115	0.0538	0.0479	0.0444
kale (red, organic)	GGB102202	0.144	0.182	0.196	0.0913	0.0814	0.0754
laver	GGB101512	2.77	1.04	3.75	1.75	0.468	0.434
laver (cooked)	GGB101528	0.247	0.31	0.333	0.519	0.139	0.129
mushroom (baby bella)	GGB102196	0.0651	0.082	0.0881	4.84	0.0367	0.034
mushroom (white)	GGB100028	1.07	1.35	0.435	25.7	0.181	0.168
mushroom (baby bella)	GGB102196	0.0659	0.0827	0.0894	5.37	0.0371	0.0344
onion (Peruvian gold)	GGB102198	0.0846	0.107	0.114	0.0534	0.0476	0.0441
onion (yellow, sweet)	GGB102398	0.0955	0.121	0.129	0.0604	0.0539	0.0499
papaya	GGB100155	0.085	0.357	0.115	0.0537	0.0479	0.0444
papaya (gold)	GGB102199	0.0835	0.105	0.113	0.0528	0.047	0.0436
rice (brown, long grain, cooked)	GGB100040	1.26	64.8	0.511	0.797	0.213	0.198
rice (brown, long grain, cooked)	GGB100040	0.382	1.6	0.518	0.242	0.215	0.2
rice (brown, organic)	GGB102207	0.868	1.09	1.18	0.549	0.489	0.453
rice (brown, organic, cooked)	GGB102208	0.296	1.24	0.401	0.187	0.167	0.155
rice (brown, organic, cooked)	GGB102208	0.296	1.24	0.401	0.187	0.167	0.155
rice (brown, organic, cooked)	GGB102208	0.296	1.24	0.401	0.187	0.167	0.155
rice (purple)	GGB102209	0.386	0.487	0.523	0.244	0.217	0.202
rice (purple, cooked)	GGB102210	2.99	3.76	1.21	1.89	0.505	0.468
rice (white)	GGB102211	0.872	1.09	1.19	0.551	0.492	0.455
rice (white, cooked)	GGB102212	0.286	0.358	0.385	0.18	0.161	0.149
rice (white, long grain, cooked)	GGB100041	0.402	1.69	0.546	0.254	0.226	0.21
rice (white, long grain, cooked)	GGB100041	0.407	1.71	0.55	0.257	0.229	0.212
rice (white, organic)	GGB102213	0.873	1.1	1.18	0.552	0.492	0.457
rice (white, organic, cooked)	GGB102214	0.322	0.407	0.437	0.204	0.182	0.168
rockweed	GGB101511	0.925	1.16	4.18	1.95	1.74	0.483
salmon	GGB100133	0.311	0.39	0.422	0.196	0.175	0.162
salmon	GGB100133	0.307	0.387	0.415	0.194	0.173	0.16

Food	Sample ID	As(V) (µg/kg FW)	As(III) (µg/kg FW)	MMA (µg/kg FW)	DMA (µg/kg FW)	AsB (µg/kg FW)	TETRA (µg/kg FW)
salmon (Atlantic, farmed)	GGB102215	0.355	0.449	0.482	0.224	0.2	0.185
sea lettuce	GGB101510	2.53	0.957	1.03	1.59	0.427	0.396
sesame seed	GGB100061	0.933	1.18	1.26	0.59	0.526	0.487
sesame seed (organic)	GGB102218	0.931	1.17	1.26	0.588	0.524	0.486
spinach	GGB102185	0.0504	0.0634	0.0681	0.0319	0.0284	0.0263
spinach (baby)	GGB102183	0.0671	0.0843	0.0912	0.0424	0.0378	0.035
spinach (baby, organic)	GGB102184	0.0546	0.0686	0.0737	0.0345	0.0307	0.0285
spinach (organic)	GGB102186	0.0524	0.0659	0.0708	0.0331	0.0295	0.0274
spinach (organic, reference)	NA	NA	NA	NA	NA	NA	NA
strawberry	GGB100029	NA	NA	NA	NA	NA	NA
strawberry	GGB102400	NA	NA	NA	NA	NA	NA
strawberry	GGB102400	NA	NA	NA	NA	NA	NA
strawberry (organic)	GGB102399	0.101	0.127	0.138	0.0641	0.0572	0.0531
sugar kelp	GGB101513	2.52	0.954	108	151	0.425	0.394
sugar kelp (cooked)	GGB101529	0.359	0.136	0.487	0.227	0.0607	0.0563
tahini	GGB102219	0.953	1.2	1.29	0.602	0.537	0.497
turnip	GGB102220	0.097	0.407	0.131	0.0612	0.0546	0.0507
turnip	GGB100154	0.126	0.16	0.171	0.0798	0.0711	0.0659
turnip (white, organic)	GGB102401	0.106	0.134	0.144	0.0671	0.0599	0.0555
zucchini	GGB100027	0.0536	0.0675	0.0725	0.0339	0.0302	0.028
zucchini	GGB102195	0.0626	0.0787	0.0852	0.0396	0.0353	0.0327
zucchini (organic)	GGB102193	0.0536	0.0675	0.0725	0.0339	0.0302	0.028

Final report: Unknown compounds

Table C4: This table contains a final report from the speciation analysis. Each row represents one compound assessed in one sample. RT (min) is the retention time of the compound in minutes. "Matched to" indicates the peak ID of the unknown a given peak in a given food matched to (see more details in the main text of the chapter). These peaks were selected first and used for subsequent matching because they were larger than their matches in other samples. SN ratio is the signal-to-noise ratio for the peak. The final three columns are relative quantities based on peak height (cps = counts per second). The "Height (cps)" column contains raw data, and the "Height (norm)" column contains data normalized TIC (details in the main text). In the "Height (replaced)" column, values below the LOQ (SN = 10) are replaced with a small value randomly sampled from an appropriate normal distribution (details in the main text). This table contains only peaks that exceeded the LODs.

Sample	Sample ID	RT (min)	Matched To	SN Ratio	Height (cps)	Height (norm)	Height (replaced)
alaria	NA	4.974	unknown1	1140	434150	86830	86800
alaria	NA	6.066	unknown8	544	207119	41424	41400

Sample	Sample ID	RT (min)	Matched To	SN Ratio	Height (cps)	Height (norm)	Height (replaced)
alaria	NA	4.788	unknown11	84.8	32282	6456	6460
alaria	NA	3.014	unknown130	66.2	25220	5044	5040
alaria (cooked)	GGB101530	4.953	unknown1	2010	931142	93114	93100
alaria (cooked)	GGB101530	6.062	unknown8	907	420158	42016	42000
alaria (cooked)	GGB101530	4.771	unknown11	150	69587	6959	6960
alaria (cooked)	GGB101530	6.231	unknown70	33.6	15561	1556	1560
alaria (cooked)	GGB101530	6.57	unknown120	20.9	9703	970	970
alaria (cooked)	GGB101530	3.924	none	8.82	4087	409	409
alaria (cooked)	GGB101530	2.963	unknown130	5.33	2467	247	560
asparagus (organic)	GGB102200	1.753	none	15	5970	1990	1990
bladderwrack	GGB101516	4.957	unknown1	800	667961	110740	111000
bladderwrack	GGB101516	6.244	unknown70	388	324061	53725	53700
bladderwrack	GGB101516	6.079	unknown8	121	100650	16687	16700
bladderwrack	GGB101516	4.995	unknown1	962	447580	89206	89200
bladderwrack	GGB101516	6.342	unknown107	277	129029	25717	25700
bladderwrack	GGB101516	6.117	unknown8	129	60132	11985	12000
bladderwrack	GGB101516	3.941	none	6.6	3070	612	612
cricket (whole)	GGB100025	5.982	unknown8	7.52	2866	2344	201
cricket (whole)	GGB100025	5.935	unknown17	10.2	4122	4122	4120
cucumber	GGB100038	2.955	unknown130	7.58	2709	1354	500
dulse	GGB101515	6.066	unknown8	514	187291	26673	26700
dulse	GGB101515	6.244	unknown70	21.2	7733	1101	1100
dulse	GGB101515	6.638	unknown120	10.4	3803	542	542
dulse	GGB101515	4.771	unknown11	7.12	2593	369	104
dulse	GGB101515	6.011	unknown8	373	118976	16997	17000
dulse	GGB101515	6.261	unknown70	17.8	5684	812	812
dulse	GGB101515	6.629	unknown120	9.49	3030	433	57.2
dulse	GGB101515	4.746	unknown11	6.64	2122	303	91.1
Irish moss	GGB101514	6.062	unknown8	429	233519	58380	58400
Irish moss	GGB101514	6.253	unknown70	11	5989	1497	1500
Irish moss	GGB101514	7.065	none	5.67	3087	772	772
Irish moss (cooked)	GGB101538	6.121	unknown8	9.54	3497	2959	207
Irish moss (cooked)	GGB101538	6.109	unknown8	8.41	3181	3181	183
Irish moss (cooked)	GGB101538	6.113	unknown8	8.75	3177	3177	165
kale	GGB102189	2.388	unknown63	4.95	1789	894	506
laver	GGB101512	5.918	unknown17	375	204067	51017	51000

Sample	Sample ID	RT (min)	Matched To	SN Ratio	Height (cps)	Height (norm)	Height (replaced)
laver (cooked)	GGB101528	5.922	unknown17	362	159608	53203	53200
laver (cooked)	GGB101528	6.121	unknown8	208	91747	30582	30600
mushroom (baby bella)	GGB102196	5.939	unknown17	5.72	1994	878	149
mushroom (white)	GGB100028	5.918	unknown17	3.2	1163	291	169
mushroom (baby bella)	GGB102196	5.914	unknown17	7.15	2467	1104	140
mushroom (portobello)	GGB102194	6.007	unknown8	4.11	1495	374	209
onion (“Vaquero)	GGB100012	7.815	none	5.68	1774	887	887
rice (brown, long grain, cooked)	GGB100040	2.159	none	8.43	2430	564	564
rockweed	GGB101511	6.316	unknown107	1650	689916	98442	98400
rockweed	GGB101511	4.801	unknown11	147	61175	8729	8730
rockweed	GGB101511	6.088	unknown8	111	46159	6586	6590
rockweed	GGB101511	7.116	none	14.2	5905	843	843
salmo (farmed, Atlantic)	GGB102215	6.024	unknown8	561	209302	209302	209000
salmon	GGB100133	5.977	unknown8	30.7	12570	12570	12600
salmon	GGB100133	5.939	unknown17	31.4	11318	11318	11300
salmon (coho, wild)	GGB102216	5.91	unknown17	93.1	34188	8547	8550
salmon (coho, wild)	GGB102216	2.455	unknown63	54.2	19912	4978	4980
salmon (coho, wild)	GGB102216	5.914	unknown17	94.3	31627	15814	15800
sea lettuce	GGB101510	5.889	unknown17	27.3	8413	1857	1860
sea lettuce	GGB101510	6.54	unknown120	4.28	1316	291	44.1
spinach (organic, reference)	NA	2.646	unknown137	5.36	2422	807	807
sugar kelp	GGB101513	4.97	unknown1	2170	938200	134029	134000
sugar kelp	GGB101513	6.092	unknown8	778	336374	48053	48100
sugar kelp	GGB101513	4.792	unknown11	596	257683	36812	36800
sugar kelp	GGB101513	6.604	unknown120	11.5	4984	712	712
sugar kelp (cooked)	GGB101529	5.033	unknown1	1560	508343	63543	63500
sugar kelp (cooked)	GGB101529	4.851	unknown11	320	103925	12991	13000
sugar kelp (cooked)	GGB101529	6.147	unknown8	264	85745	10718	10700
sugar kelp (cooked)	GGB101529	5.961	unknown17	187	60835	7604	7600
sugar kelp (cooked)	GGB101529	6.617	unknown120	4.11	1336	167	50.5
QC001	NA	4.961	unknown1	219	77416	15483	15500

Sample	Sample ID	RT (min)	Matched To	SN Ratio	Height (cps)	Height (norm)	Height (replaced)
QC001	NA	6.088	unknown8	85.3	30198	6040	6040
QC001	NA	4.784	unknown11	30.4	10745	2149	2150
QC001	NA	6.261	unknown70	28.3	10019	2004	2000
QC002	NA	4.953	unknown1	207	83798	11971	12000
QC002	NA	6.058	unknown8	77.9	31583	4512	4510
QC002	NA	4.775	unknown11	28.8	11688	1670	1670
QC002	NA	6.244	unknown70	26	10546	1507	1510
QC003	NA	4.957	unknown1	246	95236	18952	19000
QC003	NA	6.062	unknown8	92.9	35937	7152	7150
QC003	NA	4.775	unknown11	34.5	13355	2658	2660
QC003	NA	6.21	unknown70	33.9	13119	2611	2610
QC004	NA	4.961	unknown1	267	106725	17692	17700
QC004	NA	6.049	unknown8	97	38820	6435	6440
QC004	NA	6.172	unknown70	37.9	15181	2517	2520
QC004	NA	4.779	unknown11	37.3	14931	2475	2480
QC004	NA	5.914	unknown17	23.7	9490	1573	1570
QC005	NA	4.961	unknown1	200	75208	10744	10700
QC005	NA	6.075	unknown8	78.4	29500	4214	4210
QC005	NA	4.771	unknown11	28.1	10588	1513	1510
QC005	NA	6.261	unknown70	24.9	9379	1340	1340
QC006	NA	4.97	unknown1	201	75730	15146	15100
QC006	NA	6.088	unknown8	83.4	31414	6283	6280
QC006	NA	6.261	unknown70	28	10545	2109	2110
QC006	NA	4.788	unknown11	27.7	10455	2091	2090
QC007	NA	4.957	unknown1	202	77703	15541	15500
QC007	NA	6.075	unknown8	83.7	32194	6439	6440
QC007	NA	4.779	unknown11	29.4	11307	2261	2260
QC007	NA	6.244	unknown70	27.7	10665	2133	2130
QC008	NA	4.961	unknown1	254	85060	17012	17000
QC008	NA	6.071	unknown8	120	40197	8039	8040
QC008	NA	4.784	unknown11	34.4	11527	2305	2310
QC008	NA	6.215	unknown70	34	11380	2276	2280
QC008A	NA	4.987	unknown1	261	82776	16555	16600
QC008A	NA	6.104	unknown8	114	36160	7232	7230
QC008A	NA	6.223	unknown70	37.6	11936	2387	2390
QC008A	NA	4.805	unknown11	36.5	11564	2313	2310
QC009	NA	4.932	unknown1	193	67715	13543	13500

Sample	Sample ID	RT (min)	Matched To	SN Ratio	Height (cps)	Height (norm)	Height (replaced)
QC009	NA	6.032	unknown8	72.4	25407	5081	5080
QC009	NA	4.754	unknown11	27.1	9523	1905	1900
QC009	NA	6.248	unknown70	24.6	8640	1728	1730
QC010	NA	4.911	unknown1	221	82713	16543	16500
QC010	NA	6.007	unknown8	76.8	28731	5746	5750
QC010	NA	4.746	unknown11	29.4	10992	2198	2200
QC010	NA	6.202	unknown70	28.4	10638	2128	2130
QC011	NA	4.894	unknown1	172	58183	11637	11600
QC011	NA	5.948	unknown17	57.6	19501	3900	3900
QC011	NA	4.724	unknown11	24.1	8164	1633	1630
QC011	NA	6.27	unknown70	21.9	7430	1486	1490
QC012	NA	4.906	unknown1	143	58238	8320	8320
QC012	NA	5.99	unknown8	45.9	18757	2680	2680
QC012	NA	4.733	unknown11	20.5	8382	1197	1200
QC012	NA	2.256	unknown147	17.9	7309	1044	1040
QC012	NA	6.27	unknown70	17.8	7273	1039	1040
QC013	NA	4.928	unknown1	168	58238	11648	11600
QC013	NA	6.028	unknown8	56.9	19778	3956	3960
QC013	NA	4.75	unknown11	22.1	7695	1539	1540
QC013	NA	6.278	unknown107	20.8	7237	1447	1450
QC014	NA	4.945	unknown1	177	58040	11608	11600
QC014	NA	6.062	unknown8	64.9	21264	4253	4250
QC014	NA	4.771	unknown11	25.1	8242	1648	1650
QC014	NA	6.282	unknown107	21.9	7188	1438	1440
QC015	NA	4.966	unknown1	155	45793	9159	9160
QC015	NA	6.062	unknown8	55.3	16314	3263	3260
QC015	NA	4.788	unknown11	23.2	6846	1369	1370
QC015	NA	6.333	unknown107	11.9	3501	700	700
QC016	NA	4.966	unknown1	150	52310	10462	10500
QC016	NA	6.066	unknown8	51.8	18102	3620	3620
QC016	NA	4.771	unknown11	20.5	7182	1436	1440
QC016	NA	6.312	unknown107	15.2	5320	1064	1060
QC017	NA	4.995	unknown1	134	46025	6575	6580
QC017	NA	6.088	unknown8	42	14436	2062	2060
QC017	NA	4.813	unknown11	19.4	6658	951	951
QC017	NA	6.358	unknown107	11.9	4100	586	586
QC017	NA	2.942	unknown130	8.2	2819	403	540

Recovery of total arsenic

Table C5: This table contains data on the amount of total arsenic recovered in the speciation analysis. Recoveries are calculated based on the sum of all known arsenic species – As(V), As(III), MMA, DMA, AsB, TETRA – and the total arsenic measured by ICP-MS. Samples for which ICP-MS data is not yet available are excluded from this table. Except where otherwise noted, locations in food names indicate where foods were purchased, not necessarily where they were produced.

*If unknown 8 is taken to be AsB (see details in main text of chapter), recovery for this sample improves to ~65%.

Food	Sample ID	Arsenic recovery
mushroom (portobello)	GGB102194	2890%
onion ("Vaquero")	GGB100012	2528%
salmon (coho, wild)	GGB102216	511%
cucumber (Fort Collins, CO)	GGB100038	73.5%
rice (brown, long grain, cooked)	GGB100040	73.1%
strawberry (organic)	GGB102399	57.2%
cricket (protein powder)	GGB100024	53.5%
mushroom (white)	GGB100028	44.0%
onion (yellow, sweet)	GGB102398	40.9%
rice (white, long grain, cooked)	GGB100041	38.1%
rice (white, long grain, cooked)	GGB100041	37.6%
papaya (gold)	GGB102199	32.2%
mushroom (baby bella)	GGB102196	30.4%
mushroom (baby bella)	GGB102196	27.5%
turnip (US origin)	GGB102220	26.8%
dulse	GGB101515	26.3%
turnip (white, organic)	GGB102401	23.9%
kale (Windsor, CO)	GGB102189	23.2%
dulse	GGB101515	23.0%
rice (white, organic)	GGB102213	20.9%
rice (white, cooked)	GGB102212	20.3%
celery hearts	GGB102188	18.8%
cricket (protein powder)	GGB102396	18.6%
zucchini (organic)	GGB102193	18.6%
cricket (protein powder)	GGB102397	17.4%
rice (white, organic, cooked)	GGB102214	17.2%
bok choy (mei qing)	GGB102190	16.4%
rice (white)	GGB102211	16.4%
rice (purple, cooked)	GGB102210	15.2%
celery (Fort Collins, CO)	GGB100053	13.8%
eggplant (Windsor, CO)	GGB102197	12.4%
bok choy (organic)	GGB102205	12.2%

Food	Sample ID	Arsenic recovery
sesame seed	GGB100061	11.7%
rice (brown, organic, cooked)	GGB102208	10.9%
rice (brown, organic, cooked)	GGB102208	10.9%
rice (brown, organic, cooked)	GGB102208	10.9%
bok choy	GGB100026	10.7%
turnip (unknown origin)	GGB100154	10.6%
rice (purple)	GGB102209	10.0%
cricket (whole)	GGB100025	9.8%
cricket (whole)	GGB100025	9.6%
carrot	GGB100030	8.30%
zucchini (Fort Collins, CO)	GGB100027	8.12%
kale (red, organic)	GGB102202	7.83%
spinach (baby)	GGB102183	7.82%
alaria	GGB101517	7.53%
rice (brown, organic)	GGB102207	6.97%
spinach (baby, organic)	GGB102184	6.94%
spinach	GGB102185	6.81%
spinach (organic)	GGB102186	6.00%
asparagus (Windsor, CO)	GGB102187	5.92%
celery (organic)	GGB102402	5.88%
kale (baby, organic)	GGB102203	5.86%
kale (Fort Collins, CO)	GGB100128	5.27%
kale (lacinato)	GGB102192	5.06%
bok choy (baby)	GGB102204	4.93%
eggplant (Fort Collins, CO)	GGB100867	4.92%
celery (organic)	GGB102206	4.41%
papaya	GGB100155	4.06%
salmon (coho, wild)	GGB102216	3.73%
asparagus (Fort Collins, CO)	GGB100073	3.61%
rice, brown, long grain, cooked	GGB100040	3.41%
cucumber (Fort Collins, CO)	GGB100038	3.22%
alaria (cooked)	GGB101530	2.73%
sugar kelp	GGB101513	1.98%
salmon*	GGB100133	1.52%
salmon*	GGB100133	1.51%
sea lettuce	GGB101510	0.769%
Irish moss (cooked)	GGB101538	0.597%
Irish moss (cooked)	GGB101538	0.597%

Food	Sample ID	Arsenic recovery
Irish moss (cooked)	GGB101538	0.597%
laver	GGB101512	0.376%
Irish moss	GGB101514	0.168%
laver (cooked)	GGB101528	0.147%
rockweed	GGB101511	0.146%
sugar kelp (cooked)	GGB101529	0.123%
bladderwrack	GGB101516	0.117%
bladderwrack	GGB101516	0.070%

Seaweed

Chromatograms for seaweed samples included in this analysis often had a distinctive appearance – compared to other food types, seaweeds seemed more likely to (but did not always) contain a higher total number of arsenic peaks, more unknown arsenic peaks, or both. The main text of the chapter describes likely driving factors and implications of these observations. However, the main text does not expand on some potentially notable areas of nuance: First, two "modes" of arsenic species distribution seemed to differentiate two of the four seaweed varieties analyzed – alaria (*Alaria esculenta*) and sugar kelp (*Saccharina latissima*) – from the other two varieties – Irish moss (*Chondrus crispus*) and laver (*Porphyra umbilicalis*). Second, each of these seaweed varieties were analyzed both in dried and cooked form, and the impacts of cooking seemed to vary across varieties. This final section of the final appendix explores some interesting trends, contrasts, and avenues for further exploration of the literature and analysis of arsenicals in seaweed.

Seaweed samples were analyzed in dried form, as shipped by the vendor, and after cooking. Cooked seaweed samples were rehydrated and then sautéed. In alaria, we observed numerous

arsenic peaks, on which cooking seemed to have little impact (Figure C1a). In sugar kelp, we

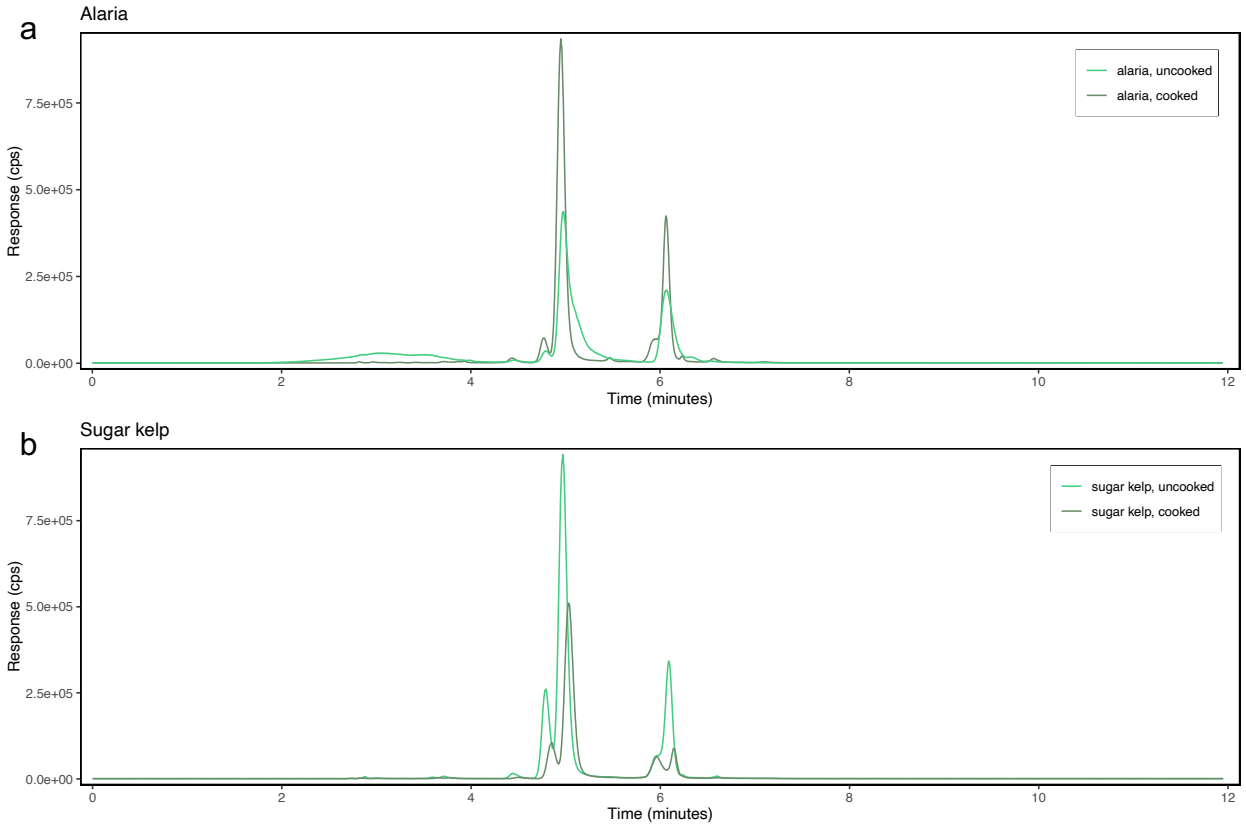

Figure C1: Chromatograms illustrating detection of arsenic species in (a) alaria and (b) sugar kelp. Each chromatogram contains two traces: one for the dried, uncooked product and one for the rehydrated, sautéed product. Note that the chromatograms display raw data, so differences in peak height cannot necessarily be used to directly infer differences in absolute concentrations. However, a positive retention time shift does seem to occur in cooked vs uncooked sugar kelp, a trend not observed in alaria. Given the proximity of the cooked and uncooked sugar kelp samples in the analytical runorder, this effect likely cannot be explained by drift.

observed a pattern of arsenic species distribution not too dissimilar from that in alaria, but cooking seemed to shift retention times later in sugar kelp (Figure C1b). The cooked and uncooked sugar kelp samples were run quite close to one another, so we think it is unlikely that this retention time shift is attributable to drift.

The impact of cooking on arsenicals in laver appeared minimal, but we observed considerably fewer peaks in this seaweed compared to alaria or sugar kelp (Figure C2a). Irish moss samples also produced few arsenic peaks, but cooking seemed to have a major effect on this seaweed variety: cooking greatly reduced the large peak observed at ~6 minutes in the uncooked samples (Figure C2b). Although the data shown in the figures is not normalized, the

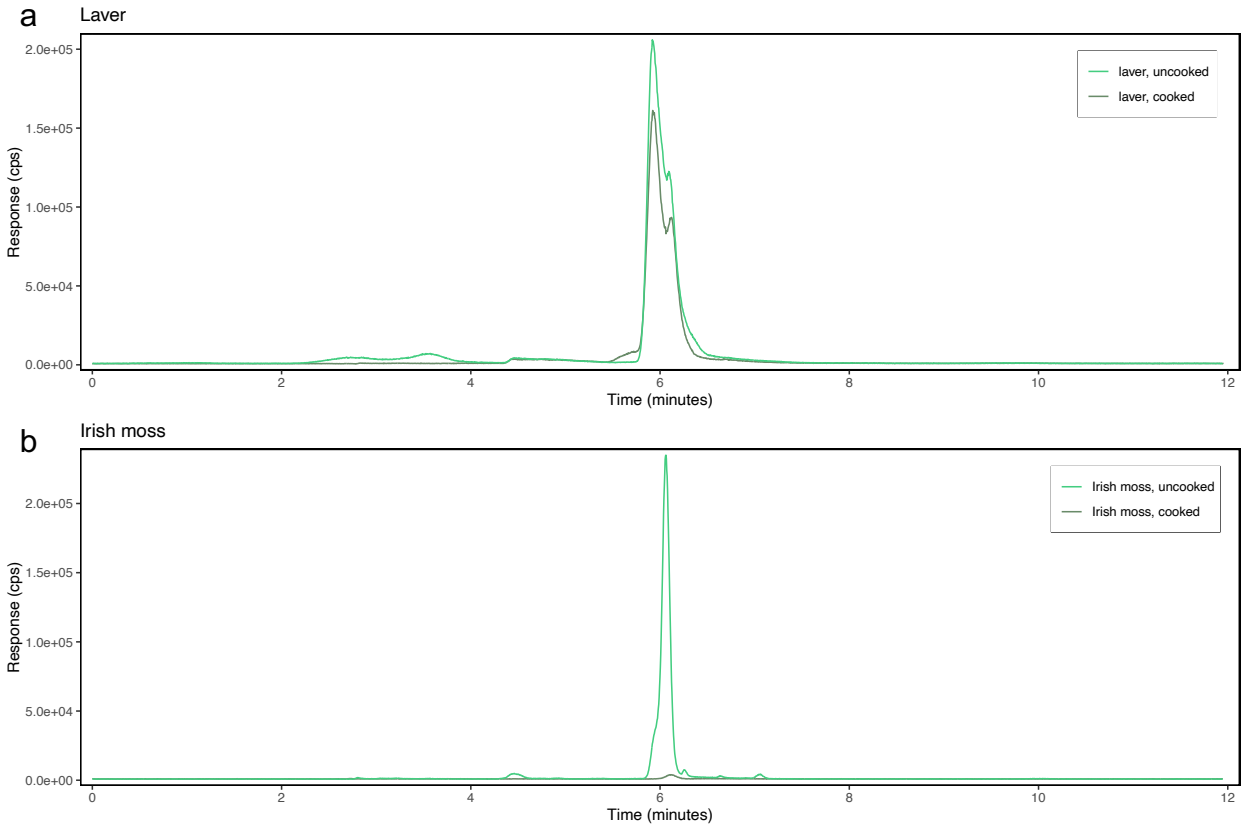

Figure C2: Chromatograms illustrating detection of arsenic species in (a) laver and (b) Irish moss. Each chromatogram contains two traces: one for the dried, uncooked product and one for the rehydrated, sautéed product. Note that the chromatograms display raw data, so differences in peak height cannot necessarily be used to directly infer differences in absolute concentrations. However, given the magnitude of the height difference between the ~6-minute peaks in uncooked vs cooked Irish moss, as well as their relative positions in the runorder, it seems likely that cooking had a true effect on the concentration of the compound represented by this peak.

magnitude of this difference is indicative of a true difference in the arsenic concentrations present in the respective extracts. Additionally, uncooked Irish moss was analyzed after the cooked aliquot of this seaweed variety, so the effect cannot be explained by typical reduction of detector sensitivity.

Based on the observations above, we hypothesize that matrix effects arising from the cooking process influence arsenic speciation results in sugar kelp and Irish moss, but not alaria or laver. In Irish moss particularly, it is possible that cooking induces changes that make key arsenicals more challenging to extract. However, the present analysis is not designed to adequately test this hypothesis, and focused bioavailability work would be required to

understand whether any true effect is meaningful for the risk profile of arsenic in these Irish moss samples.

LIST OF ABBREVIATIONS

As(III) – arsenite

As(V) – arsenate

AsB – arsenobetaine

CV – coefficient of variation (also known as relative standard deviation, RSD)

DMA – dimethylarsinic acid

DRC – dynamic reaction cell (part of some ICP-MS instruments)

EFSA – European Food Safety Authority

g – grams

ESI-MS/MS – electrospray ionization tandem mass spectrometry

HPLC – high-performance liquid chromatography

ICP-MS – inductively coupled plasma mass spectrometry

kg – kilograms

LD_{50} – lethal dose, 50%

LOD – limit of detection

LOQ – limit of quantitation

mg – milligrams

min – minutes

mL – milliliters

mm – millimeters

m/z – mass-to-charge ratio

MΩ·cm – megaohm centimeters (resistivity)

MMA – monomethylarsonic acid

ppb – parts per billion

ppm – parts per million

PTFE – polytetrafluoroethylene

PTFI – Periodic Table of Food Initiative

PVDF – polyvinylidene fluoride

QC – quality control (sample)

RT – retention time (on an HPLC system)

TDS – total diet study

TETRA – tetramethylarsonium

TMAO – trimethylarsine oxide

USDA – United States Department of Agriculture

US EPA – United States Environmental Protection Agency

US FDA – United States Food and Drug Administration

µg – micrograms

µL – microliters

µm – micrometers

For element symbols not otherwise specified, please refer to a periodic table.