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Arsenic speciation in animal feed and feed ingredients by ICP-MS and HPLC-ICP-MS methods

ERASMUS MUNDUS MASTER IN QUALITY IN ANALYTICAL LABORATORIES (EMQAL)

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Abstract

The increasing demand for food and the growth of aquaculture highlight the need for alternative feed ingredients, and arsenic (As) speciation analysis in feed is crucial for ensuring food chain safety. This study aims to obtain occurrence data for total arsenic (tAs), inorganic arsenic (iAs) and lipid-bound arsenic/arsenolipids (AsLipids) in animal feed, traditional and new feed ingredients using accredited methods according to ISO standard 17025: ICP-MS method for multielement analysis and HPLC-ICP-MS methods for iAs speciation, method for lipid-bound arsenic. In this study different matrices were analyzed including commercial feed samples (n=21), land animal samples (n=10), plant animal meal (n=15), fish meal (n=14), fish oil (n=10), microalgae (n=6), halophyte (n=3), insect meal (n=4), seaweed (n=2), hydrolyzed fish protein concentrates (n=8), tunicates (n=7), experimental feed containing seaweed (n=3), experimental feed for piglets containing yeast (n=2), experimental feed for chicken containing yeast (n=4), and experimental fish feed containing yeast (n=6).

Commercial fish feed showed total arsenic (tAs) levels ranging from 0.9 to 5.9 mg/kg ww, with lipid-bound As ranging from 0.6 to 2.8 mg/kg. For land animals, tAs varied from 0.03 to 0.08 mg/kg ww, iAs ranged from 0.02 to 0.05 mg/kg, and lipid-bound As ranged from 0.001 to 0.004 mg/kg. Plant meal showed tAs levels from 0.01 to 0.04 mg/kg, with iAs levels below the limit of quantification (LOQ). Fish meal showed tAs ranging from 2.3 to 10.7 mg/kg ww, iAs from below LOQ to 0.10 mg/kg, and lipid-bound As from 1.3 to 1.8 mg/kg. Fish oil had tAs ranging from 0.1 to 14.5 mg/kg ww, with lipid-bound As ranging between 0.1 and 13.2 mg/kg.

Microalgae showed tAs concentrations from 0.1 to 0.4 mg/kg ww, iAs from below LOQ to 0.08 mg/kg, and lipid-bound As from 0.03 to 0.2 mg/kg. Halophytes showed tAs ranging from 0.2 to 0.4 mg/kg ww, iAs from 0.09 to 0.26 mg/kg, and lipid-bound As from 0.004 to 0.008 mg/kg. Insect meal showed tAs ranging from 0.04 to 0.34 mg/kg ww, iAs from 0.02 to 0.21 mg/kg, and lipid-bound As from 0.005 to 0.007 mg/kg. Seaweed had tAs concentrations ranging from 8.2 to 14.4 mg/kg ww, with iAs at 5.71 mg/kg. Hydrolyzed fish protein concentrate (HFPC) exhibited tAs ranging from 1.1 to 19.5 mg/kg ww, iAs from 0.005 to 0.082 mg/kg, and lipid-bound As from 0.4 to 4.4 mg/kg. Tunicates showed tAs ranging from 9.4 to 43.0 mg/kg ww, iAs from 0.7 to 33.6 mg/kg, and lipid-bound As from 2.5 to 4.6 mg/kg. Experimental feeds containing seaweed had tAs from 3.7 to 4.4 mg/kg ww and iAs from 0.05

to 0.07 mg/kg. Experimental feeds for piglets, chickens, and fish containing yeast showed tAs ranging from 0.1 mg/kg ww in feed for piglets to 4.8 mg/kg ww in fish feed.

Our study showed that some samples, especially new feed ingredients such as HFPC, can have a high tAs concentration but low iAs concentration. While inorganic arsenic (iAs) is recognized as highly toxic, the toxicity of other forms, such as lipid-bound As or arsenolipids (AsLipids) remains less defined. Given that animal feed and its components play a critical role in the food chain, precise analysis of arsenic speciation within these elements is crucial.

Abbreviations

$[(CH_3)_3N]^+$	Quaternary ammonium ions
$[AsO(OH)_2]^-$	Arsenic oxide anions
As	Arsenic
As(III)	Arsenite
As(V)	Arsenate
As_2S_3	Arsenic trisulfide
AsB	Arsenobetaine
AsC	Arsenocholine
AsFA	As-containing fatty acids
AsHC	Arsenic containing hydrocarbons
AsLipids	Arsenolipids
AsSug	Arsenosugars
BCR	Tuna fish tissue certified reference material
BMDL	Benchmark dose lower confidence limit
bw	Body weight
Cd	Cadmium
CE	Capillary electrophoresis
CEN	The European Committee for Standardization
CONTAM	The Panel on Contaminants in the Food Chain
CRC	The collision/reaction cell
CRMs	Certified reference materials
DDEM	Discrete dynode electron multiplier
DMA	Dimethylarsinic acid
DMAA	Dimethyl arsenoacetate
DMAB	Dimethylarsenobutanoic acid
DMAE	Dimethyl arsenoethanol
DMAPr	Dimethylarsenopropanoic acid
dw	Dry weight
EFSA	The European Food Safety Authority
EPA	The Environmental Protection Agency
ERM BC 21	Rice certified reference material
EU	European Union
FAO	Food and Agriculture Organization
FPC	Fish protein concentrate
FPH	Fish protein hydrolysate
GC	Gas chromatography
Gly-sug	Glycerol sugar
H_2O	Water
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HFPC	Hydrolyzed fish protein concentrate
Hg	Mercury
HNO_3	Nitric acid

HPLC	High-performance liquid chromatography
IARC	The International Agency for Research on Cancer
iAs	Inorganic arsenic
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma mass spectrometry
IMR	The Institute of Marine Research
IPA	Isopropanol
ISO	The International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LB	The lower bound
LC	Liquid chromatography
LOQ	Limit of quantification
m/z	Mass-charge ratio
MAE	Microwave-assisted extraction
mg/kg	miligrams per kilogram
ML(s)	Maxium level(s)
MMA(III)	Monomethylarsonous acid
MMA(V)	Monomethylarsonic acid
MS	Mass spectrometry
MTBE	Methyl tert-butyl ether
NIST	National Institute of Standards and Technology
NMBU	the Norwegian University of Life Sciences
PAP	Processed animal protein
Pb	Lead
PO_4-sug	Phosphate sugar
PTEs	Potentially toxic elements
PTWI	The provisional tolerable weekly intake
RF	The radio frequency
RSD	The relative standard deviation
SAM	S-adenosylmethionine
SBM	Soybean meal
SD	Standard deviation
SO_3-sug	Sulphonate sugar
SO_4-sug	Sulphate sugar
TETRA	Tetramethylarsonium ion
thio-DMAB	Thio-dimethylarsenobutanoic acid
thio-DMAPr	Thio-dimethylarsenopropanoic acid
TMAO	Trimethylarsine oxide
TMAS	Trimethylarsine sulfide
TORT	Lobster Hepatopancreas certified reference material
ULPGC	The University of Las Palmas, Gran Canaria
UN	United Nations
WHO	The World Health Organization of the United Nations

ww	Wet weight
µg/kg	Micrograms per kilogram

Introduction

The human population is growing and this has led to an increased demand for food[1,2]. In order to meet this demand, the Food and Agricultural Organization (FAO) of the United Nations (UN) predicts that by 2050, global food production must increase by 70%[1]. The growing need for food is leading to a higher demand for farmland and pastures, specifically to produce food and animal feed[2,3]. As a result, animal farming has also expanded significantly around the world[4]. This increase helps meet the demand for various animal products, including meat and fish[5]. Seafood is a viable nutritional solution due to its high-quality protein content and other vital nutrients[6]. The limited projected increase of 1% in catch fisheries production highlights the significance of aquaculture as the primary driver of overall growth by 2025[7]. However, the advancement of aquaculture necessitates the exploration and utilization of novel feed and feed ingredients to support its development[8]. Fish meal and fish oil are crucial ingredients in aquafeeds but they are considered limited resources[9]. In the last decades, researchers have extensively explored alternative protein sources that match the nutritional value of fishmeal and fish oil, including essential amino acids, minerals, and fatty acids[9,10]. Plant-based materials such as soybeans, oil seeds, and cereal gluten are substitutes for fish meal and fish oils[9]. This is not considered optimal as they compete with food resources. Therefore, alternative feed ingredients with lower competition with human food resources are actively explored and developed. These include novel protein sources, such as microbial or insect-based, microalgae, and fish byproducts. The objective is to promote long-term economic viability, environmental sustainability, and social responsibility in aquaculture production[10].

Animal farming depends on the availability of high-quality feed ingredients, not only in terms of nutrition but also in terms of safety[11,12]. Contaminants such as heavy metals, mycotoxins, pesticide residues, and pathogens all pose potential hazards to aquafeeds[11,12]. Apart from the direct negative impact on the health of the cultured target species, there is a risk that contaminants will be passed along the food chain to consumers[11,12]. Therefore, the European Union (EU) has implemented comprehensive regulations to protect consumers, animals, and the environment concerning food and feed safety. The Directive 2002/32/EC and amendments have established maximum levels (MLs) for arsenic (As), mercury (Hg), cadmium (Cd), and lead (Pb) in feed and feed ingredients[13].

Among all known contaminants, As is one of the most studied[11]. As arsenic can exist in diverse chemical species and is commonly found in the environment[14]. Examples of As species that can be found in the environment are inorganic arsenic (iAs)[15], arsenobetaine (AsB)[16] and liposoluble arsenicals[17]. Identifying and quantifying of each chemical species (i.e., speciation analysis) in a sample is essential because properties such as toxicity and bioavailability depend on the specific chemical species present[18]. For instance, iAs stands as the most toxic species, while toxicity remains undetermined for many other forms including lipid-bound As (arsenolipids (AsLipids))[18–20]. Arsenic speciation analysis in feed and its components is pivotal, given their role as the primary link in the food chain[18,21].

Aims, objectives and hypotheses of the thesis

This master project is part of a larger project entitled MetMarRes (project number 15333, funded by the Norwegian ministry of Trade, Industry and Fisheries). The aim of MetMarRes was to document the presence of metals, As and As species in novel feed ingredients. This master project contributed with occurrence data for total arsenic (tAs), iAs and AsLipids in animal feed, traditional and novel feed ingredients.

Aims:

- To obtain occurrence data for tAs and iAs in fish feed, land animal feed, in traditional and novel fish feed materials.
- To obtain data on AsLipids for selected feeds and feed materials.

Objectives:

- Objective 1: Quantify the levels of tAs in commercial fish feed, land animal feed, fish meal, fish oils, plant meal, microalgae, halophyte, insect meal, seaweed, hydrolyzed fish protein concentrates, tunicates, and experimental feed.
- Objective 2: Quantify the levels of iAs and AsLipids in selected samples of commercial fish feed, land animal feed, fish meal, fish oils, microalgae, halophyte, insect meal, hydrolyzed fish protein concentrates and tunicates.
- Objective 3: Compare the obtained levels with the MLs given in the EU legislation.

Hypothesis:

1. Can new feed materials introduce high total and inorganic As levels into the complete feed?
2. Can new feed materials introduce lipid-bound As to the feed?
3. Are the levels of tAs and iAs in feed and feed ingredients under the MLs established by EU Commission?

1 Background

1.1 Arsenic – history, chemical properties, and environment

Arsenic (As) has had a long history of use in medicine, preservation, and poisoning since ancient times[14]. Albertus Magnus (1193–1280), a German Dominican friar, is credited with discovering As by heating As_2S_3 with soap to form elemental As[14]. However, Paracelsus (1493–1541), the father of modern toxicology, provided more detailed instructions for its production in his works[14]. In the late 1800s, As was a crucial chemical in pesticides and pigment products, but its exposure became a significant health concern and its use was restricted[22]. Nowadays, As is mainly characterized as a naturally occurring element widely distributed throughout the Earth's crust[23]. Classified as a metalloid, it exhibits properties of both metals and nonmetals[23,24]. Around 30 isotopes of As exist, but most have extremely short half-lives measured in microseconds or milliseconds. However, a few isotopes, such as ^{71}As, ^{72}As, ^{73}As, ^{74}As, and ^{75}As, have longer half-lives ranging from approximately 65 hours to 80 days. The only stable isotope is ^{75}As[25]. As also has several valence states, including -3, 0, +3, and +5[23,24,26], but the most common in environmental and biological systems are +3 and +5[26]. In general, As is commonly found combined with other elements as inorganic or organic compounds[23,24]. These white or colorless powders have no taste or smell, making them difficult to detect[23]. As can be found naturally in soil, minerals, and volcanic eruptions, entering the air, water, and land through wind-blown dust, runoff, and leaching[27,28]. Anthropogenic sources such as mining, smelting, and waste incineration also contribute to releasing As into the environment[23,27]. Arsenic can undergo various reactions in the environment, influenced by factors such as pH, temperature, and the distribution of biota[29]. It cannot be broken down and can only change its form or become attached or separated from particles[29]. This means that once As is released into the environment, it can persist for a long time and potentially affect living organisms for an extended period. The different chemical forms or compounds of As are known as As species.

1.2 Arsenic species

1.2.1 Definitions

Arsenic species can have different physical and chemical properties. In addition, their toxicity and environmental impact can vary depending on their chemical form and concentration. The

International Union of Pure and Applied Chemistry (IUPAC) published a set of definitions related to chemical speciation and element fractionation to provide a standardized framework for the analytical community[30]:

- ***"Chemical species:*** Specific form of an element defined as to isotopic composition electronic or oxidation state, and/or complex or molecular structure."
- ***"Speciation analysis:*** Analytical chemistry: analytical activities or identifying and/or measuring the quantities of one or more individual chemical species in a sample."
- ***"Speciation of an element:*** speciation: distribution of an element amongst defined chemical species in a system."
- ***"Fractionation:*** Process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties."

Arsenic species can be classified in several ways, depending on the criteria (chemical form, toxicity, environmental behavior) used for classification. One of the most common classifications is based on solubility. Figure 1 shows water-soluble inorganic species [arsenic acid (As(V)) and arsenous acid (As(III))] and water-soluble organic species [monomethylarsonic acid ((MMA(V)), monomethylarsonous acid (MMA(III)), dimethylarsinic acid (DMA(V)), dimethylarsinous acid (DMA(III)), tetramethylarsonium ion (TETRA), trimethylarsine oxide (TMAO), trimethylarsine sulfide (TMAS), dimethylarsinoyl ethanol (DMAE), dimethylarsinoylacetic acid (DMAA), arsenobetaine (AsB), arsenocholine (AsC), arsenosugars containing glycerol (Gly-sug), phosphate (PO_4-sug), sulphonate sugar (SO_3-sug), and sulphate sugar (SO_4-sug)]. The same figure includes liposoluble organic species [arsenic-containing hydrocarbons (AsHC), arsenic-containing fatty acids (AsFA), trimethylated arsenic-containing fatty alcohol].

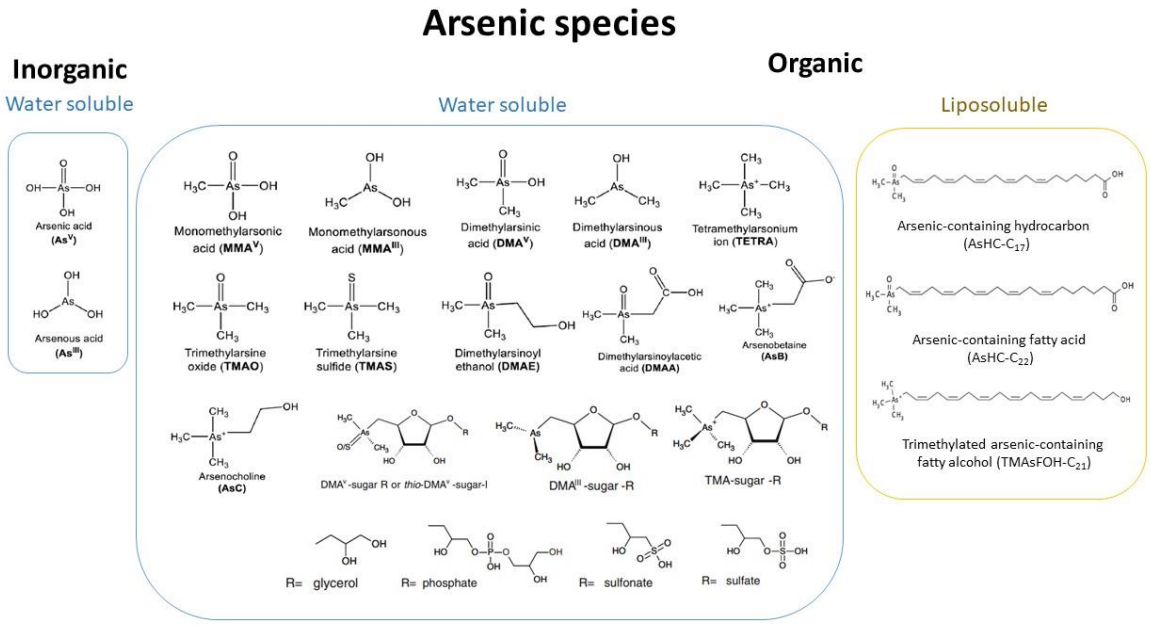

Figure 1. Chemical structures of common arsenic compounds. Figure adapted from Luvonga et al. (2020)[31], Sele et al. (2014)[32] and Feldmann et al. (2011)[33]

1.2.2 Comparison between terrestrial and marine environments

There are more than 100 species of As in the environment[34], with the most common ones found in terrestrial systems being As(III), As(V), DMA, and MMA[35]. However, the As forms in the marine environment are more diverse and complex[24]. In seawater, As is mainly present in inorganic forms, with As(V) being dominant in oxidizing conditions and As(III) in reducing conditions[36]. Conversely, organic forms are typically more prevalent in marine flora and fauna[37,38].

Of the water-soluble arsenicals, AsB is the major As species in most marine organisms[39]. Arsenobetaine is an organic compound containing As that displays zwitterionic properties[39], characterized by the presence of both positively charged quaternary ammonium ions $[(CH_3)_3N]^+$ and negatively charged As oxide anions $[AsO(OH)_2]^-$. It occurs in marine (finfish, shellfish)[40] and terrestrial organisms (mushrooms[41], lichens[42], earthworms[43], cattle or chicken[44]) and shares a structural similarity with glycine betaine, a nitrogen-containing compatible solute that aids in osmolytic processes[45]. The exact mechanisms of its biosynthesis are still being researched, but it is believed that the process involves a series of enzymatic

reactions [46]. Experimental findings have demonstrated that fish can bioaccumulate AsB in their muscle tissue, with a notable characteristic of slow and incomplete elimination[47]. However, variations in the elimination kinetics exist among different fish species[47].

Other methylated As compounds, such as MMA and DMA (Figure 1) are present in marine food at low concentrations and are primarily derived from the transformation of iAs by phytoplankton, bacteria, and microbial degradation of organic matter[31]. The metabolic pathway for As biotransformation has been proposed to involve enzymatic reduction of pentavalent iAs(V) and MMA(V) to their trivalent species, iAs(III) and MMA(III). This is followed by an oxidative methylation phase where S-adenosylmethionine (SAM) acts as a methyl donor to produce MMA(V) and DMA(V) as major metabolites. This pathway is commonly known as the Challenger pathway[48]. Additionally, other methylated As compounds such as TMAO, AsC, and TETRA (Figure 1) have also been identified in marine ecosystems.

Arsenosugars (AsSug) represent ribofuranosides commonly containing a dimethyl- or trimethylarsinoyl group[49] (Figure 1). They are commonly found in seafood, such as fish and shellfish, as well as in some seaweeds[31,50]. The metabolism of marine algae forms AsSug and can accumulate in the tissues of marine animals that consume them[50]. Over 20 different types of (AsSug) have been discovered[51] in seaweed, with the most common being glycerol sugar (Gly-sug), phosphate sugar (PO_4-sug), sulphonate sugar (SO_3-sug), and sulfate sugar (SO_4-sug)[52,53] (Figure 1).

Arsenolipids (AsLipids) or lipid-soluble As are a group of organic lipophilic (liposoluble) compounds containing As and are generally associated with fatty fish and fish oils[50]. Arsenolipids comprise structural groups, including AsHC, AsFA and trimethylated arsenic-containing fatty alcohol[50] (Figure 1). It is thought that AsLipids can move up the food chain from algae to fish, and there is a chance that the organism itself may produce them as the identified AsFAs resemble (non-As-containing) fatty acids commonly found in aquatic organisms[17]. Identifying AsLipids is an ongoing process, with over 50 currently known[50].

1.3 Animal feed and feed ingredients

1.3.1 Commercial feed and feed ingredients

In Norway, the livestock population consists of more than 800,000 cattle, exceeding one million sheep, surpassing 1.5 million fattening pigs, hosting over 4 million hens and over 62 million chicken[54]. Additionally, Norway is home to various farm animals, including deer, llamas, goats, rabbits, minks, foxes, ducks, geese, and turkeys[54]. Farmed fish are Norway's most important farm animals, and it is estimated that over 350 million fish are put out in fish cages in the sea[55]. Salmonids, including Atlantic salmon, rainbow trout, and sea trout, constitute approximately 97.5% of the total farmed fish production in Norway[56].

Animal feed is composed of different feed ingredients. Compound feed varies in its precise composition, depending on the specific animal it is intended for, the production needs, and the cost and availability of ingredients[56]. Some ingredients can be common to both land animal feed and fish feed. For example, soybean meal (SBM) is a commonly used protein source for fish[8,57], dairy cattle, pigs, and poultry[58]. Additional vegetable protein sources include wheat gluten, guar protein, sunflower, pea protein, and corn gluten[57]. Rapeseed or canola proteins have been utilized as a feed ingredient worldwide for years, catering to a wide range of animal species, including poultry, pigs, cattle, and various fish species, such as salmon, trout, tilapia, and prawns[8,59,60]. Within marine protein sources, there is fish meal derived from forage fish and cut-offs[57].

As illustrated in Figure 2, there has been a significant transformation in the composition of fish feed ingredients in Norway over the years. By 2020, plant-based materials, including protein sources and oils, became the leading contributors[57].

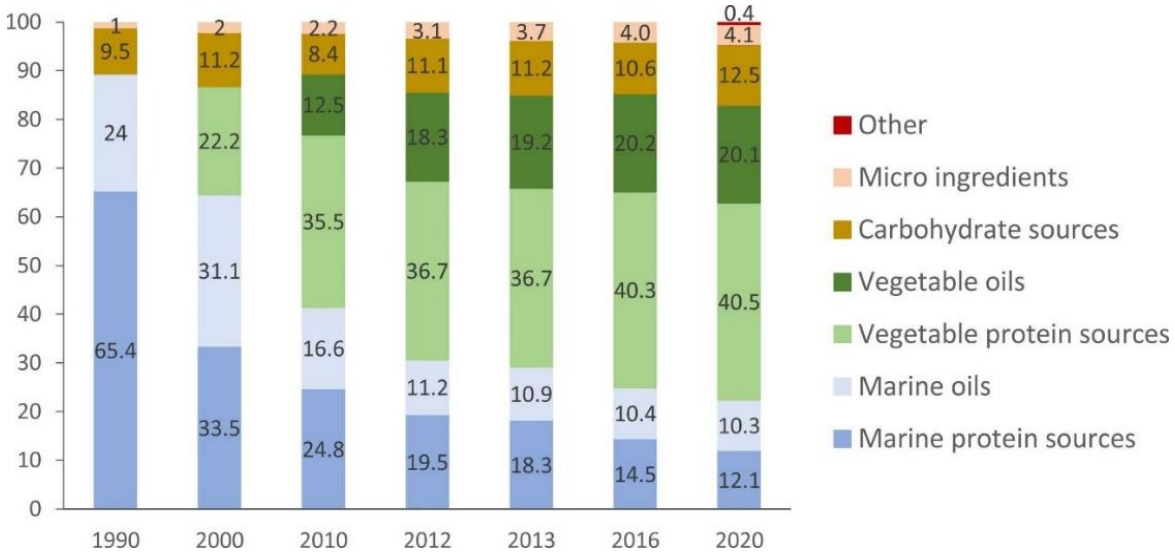

Figure 2. Comparison of feed ingredient sources (% of feed) in Norwegian salmon feed between 2020 and previous years. Reference: Aas et al., 2022

In 2020, the diet included plant-derived materials like proteins and oils, marine-based sources of proteins and oils, accounting for around 22%, carbohydrate sources at approximately 12%, and micro-ingredients at a 4% level[57]. Vegetable oils encompass rapeseed, linseed, soybean, camelia and coconut oil[57]. Fish oils consist of those obtained from forage fish and cut-offs[57]. Diverse carbohydrate sources comprise wheat, faba beans, and pea flour[57]. Micro-ingredients include vitamin- and mineral premixes, phosphorus sources, astaxanthin, and crystalline amino acids[57].

1.3.2 New feed ingredients

The 'Other' category (0.4%) (Figure 2) comprises new feed ingredients such as insect meal, single-cell protein, fermented products, and microalgae[57].

1.3.2.1 Insect meal

Insects, highly diverse animals, naturally serve as fish food, predominantly carnivorous and omnivorous species needing higher protein intake[8,61,62]. In the past two decades, intensive research explored insect meals as fishmeal alternatives, yielding promising substitution results. Challenges remain in costs and scaling insect production[10]. Most studies favorably replaced

some fishmeal with insect meals, though outcomes vary per fish and insect species[10]. However, exceeding 30% fishmeal replacement with insects often reduces fish growth[63,64].

1.3.2.2 Tunicates

Lately, the vase tunicate *Ciona intestinalis* has gained attention as a possible inclusion in aquafeeds due to its significant protein content, accounting for approximately 50% of the entire animal's ash-free dry weight[65]. Utilizing tunicates for feeds and feed components offers benefits of high yield without competing with human food production, potential environmental remediation, and the discovery that tunicates like *Ciona intestinalis* can be densely farmed for their rich nutritional content, fulfilling the need for ecologically sustainable, premium feed components[66,67].

1.3.2.3 Hydrolyzed fish protein concentrates (HFPC)

Hydrolyzed fish protein concentrates (HFPC), sourced from fishery by-products, introduce an innovative feed ingredient[68]. Various fish components such as skin, heads, frames, bones, and whole bodies have been explored for hydrolysate production across different species, offering protein and collagen sources[69–72]. These hydrolysates, especially those enriched with short-chain peptides, exhibit positive impacts in aquaculture, supporting growth, nutrient utilization, antioxidant activity, and immunity[73–76].

1.3.2.4 Microalgae and seaweed

Algae provide a natural and effective alternative to soybean in fish diets, offering economic and nutritional advantages. Studies comparing their profiles show algae better fulfill fish nutritional needs[8,77–79].

Historically, seaweed or marine algae, has been part of animal feed in coastal areas[79]. Green algae have been investigated as poultry feed, showing improved body weight gain with 3% supplementation[79]. Red seaweed holds promise from a prebiotic angle, enhancing broiler feed for growth and health[79]. Whether green, red, or brown, seaweed can enrich poultry eggs, improving quality and reducing yolk cholesterol while combating toxic bacteria[79]. Brown and red seaweed utilization in ruminant feed is limited, with few specific cases documented[79].

Challenges arise from low seaweed concentrations (1–2%) in meals[79]. However, this strategy could offer a healthier, cost-effective alternative[79].

Previously, organoarsenic compounds like roxarsone and p-arsanilic acid were common feed supplements, but their use is now restricted due to arsenic concerns[80–84].

Table 1 shows tAs concentrations in milligrams per kilogram dry weight (mg/kg dw) in some commonly used feed ingredients. Sources of As in the mentioned feed ingredients depend on the feed material and its origin.

Table 1. Total As concentrations in commonly used feed ingredients and animal feed. Adapted from López-Alonso et al. (2012)[85]

	Mean mg/kg dw	Median	Maximum	n
Complete feeds				
Ruminants — beef cattle	0.36	0.37	0.6	10
Poultry — unspecified	1.83	3	6.7	6
Pigs — unspecified	0.62	11.36	5	19
Traditional feed ingredients				
Fish meal	4.7	4.21	16.3	95
Fish oil	7.6	8.14	8.9	7
Oil seed meals	0.09	0.04	0.2	17
Maize grain and maize by-products	0.26	0.20	0.51	7
Other cereals and cereal by-products	0.06	0.01	1.08	47
Minerals and mineral supplements	6.8	3.05	15.7	42
Forages				
Grass silage	0.12		0.44	28
Hay	0.05		0.1	2
Maize silage	0.05		0.1	2
Straw	0.05		0.19	4

Microalgae serve as natural food sources for specific fish species and zooplankton within the food chain[8]. Foods derived from microalgae, such as *Chlorella vulgaris*, fall under the category of novel foods according to the novel food regulation[59]. These microalgae are commercially available and commonly used as nutritional supplements for both terrestrial and aquatic animals[59]. In general, Spirulina shows promise as a potential protein source in poultry and pork production, along with aquaculture[86]. *Chlorella vulgaris* is frequently used in aquaculture[87]. Incorporating 5% *Chlorella vulgaris* to finishing pig diets boosts nutritional value without harming growth[88].

1.4 Toxicological aspects

1.4.1 Adverse effects of iAs on human health

The International Agency for Research on Cancer (IARC) and the Environmental Protection Agency (EPA) classify iAs as a known human carcinogen[89]. Exposure to iAs can lead to various adverse health effects, including cancer, skin lesions, and cardiovascular diseases[89]. The European Food Safety Authority (EFSA) reviewed available data for 2009, 2014 and 2021 and provided recommendations for the risk assessment of iAs[18,90,91]. Based on available data, EFSA determined that the provisional tolerable weekly intake (PTWI) of 15 µg/kg bw (micrograms per kilogram of body weight) established by the Joint Food and Agriculture Organization and the World Health Organization of the United Nations (FAO/WHO) Expert Committee on Food Additives (JECFA) was no longer suitable. The Panel found that iAs could cause lung and urinary bladder cancer, in addition to the skin, and that various adverse effects are associated with exposure levels lower than those previously reviewed by the JECFA. To arrive at this conclusion, the Panel conducted a dose-response analysis by modeling the data from key epidemiological studies and selecting a benchmark response of 1% extra risk. The analysis revealed benchmark dose lower confidence limit ($BMDL_{01}$) values between 0.3 and 8 µg/kg bw per day for cancers of the lung, skin, and bladder, as well as skin lesions. Later, JECFA identified a $BMDL_{05}$ of 3.0 µg/kg bw per day for an increased risk of lung cancer (range 2–7 µg/kg bw per day) based on epidemiological studies[90].

According to the latest CONTAM panel of EFSA findings, the highest dietary exposure to iAs was estimated in the young population (infants, toddlers, and other children)[90]. The main food groups contributing to iAs exposure in the European diet are rice and rice-based products, followed by drinking water, seafood, and other cereals[18,90,91].

1.4.2 Toxicity of other As species

Concerning the toxicity of organic As species, AsB is classified as non-toxic (Group 3) as it undergoes no metabolism in humans according to IARC[59]. The methylated As compounds MMA(V) and DMA(V) are categorized as potentially carcinogenic (Group 2B)[92]. However, the trivalent methylated As species, MMA(III) and DMA(III), exhibit greater toxicity than their pentavalent counterparts and iAs[31]. Compared to iAs and methylated As species, AsSug exhibits relatively reduced toxicity[31]. Nonetheless, AsSug can undergo metabolism upon

human ingestion, forming cytotoxic As species. The results of a preliminary cytotoxicity screening for AsSug metabolites indicate that the thio and oxo forms of dimethyl arsenoethanol (DMAE) and dimethyl arsenoacetate (DMAA) are less toxic than DMA(V), even though the thio derivatives are taken up readily by cells[93]. Initial assessments of AsLipids toxicity also demonstrated unfavorable outcomes. In an *in vitro* study using cultured human bladder and liver cells, AsHC displayed high potential cytotoxicity, and was reported to be five times more toxic than As(III), possibly causing neurotoxic effects. In contrast, AsFA was found to be less toxic than AsHC[94]. A study involving two human volunteers has demonstrated that the Aslipids found in cod liver are metabolized and excreted almost entirely in urine, primarily as DMA and four As-containing fatty acids (dimethylarsenopropanoic acid (DMAPr), dimethylarsenobutanoic acid (DMAB), and their thio-analogues (thio-DMAPr and DMAB)[95]. Currently, there are no specific regulations on the consumption of AsLipids in food, but research is ongoing to better understand their potential risks to human health[18,31,50].

1.4.3 Levels and sources of As in feed and feed ingredients

Certain heavy metals and potentially toxic elements (PTEs) such as Hg, Cd, Pb and As can be present in feed materials and animal feeds, posing a risk to animal and public health as they can come from various sources including the environment or production processes[96]. Arsenic, for instance, may arise from natural sources, industrial activities, or particular feed additives[21]. Terrestrial plants may also accumulate As from soil, groundwater, and airborne deposition on leaves[21]. Rapeseeds can accumulate heavy metals in the roots, plant, and seeds[59]. Analysis of plant materials has revealed the presence of various As species, including As(III), As(V), MMA, DMA, AsSug, and AsB, among others[97]. Research has shown that AsLipids are prevalent in marine oils and fats[16,21]. Arsenosugars are especially prevalent in marine microalgae (more than 80% of the tAs) and the taxonomic classification of the algae[98,99] influence their abundance. Larger algae can also contain AsSug and DMA(V), which may be re-converted into iAs during bioaccumulation[100]. Brown algae species have been found to accumulate high levels of As[101], especially AsLipids[102]. Brown seaweeds typically have higher As levels than red and green varieties[53,103]. DMA is often found in seaweeds, with MMA, AsC, and TMAO less common[53,104,105]. Microalgae *Spirulina platensis* cultivated using Zarrouk's medium containing different As(III) levels demonstrated the capacity to accumulate and methylate As[106]. Within the As species, As(V) was the predominant intracellular form, while traces of DMA(V) and MMA(V) were also identified[106]. Microalgae *Chlorella vulgaris*

maintains heavy metal levels within set limits by authorities[87,107] and no significant adverse effects are evident[87]. Its safety hinges on appropriate, uncontaminated cultivation environments[108]. *Caulerpa racemose,* algae collected from Indian Ocean beaches, showed the highest tAs concentration along with As(III), As(V), MMA, and DMA[109]. Fish meal is known to contribute primarily to water-soluble As species, while fish oil contributes to the presence of AsLipids in the complete fish feed[17].

1.4.4 Adverse effects of As on fish and animal

Toxic effects have been observed in fish exposed to iAs through diet[110,111]. These effects include elevated levels of hepatic metallothionein, histopathological changes in the liver and gall bladder, and decreased growth rate[110,111]. For example, rainbow trout exposed to iAs exhibited toxic responses such as altered feeding behavior and reduced growth[112]. However, when exposed to higher concentrations of certain organic As compounds, no toxic effects were observed[112]. This highlights the variation in toxicity depending on the chemical form of As. The presence of non-toxic organic As in feed materials suggests a minimal health risk to animals unless exposed to exceptionally high levels or specific contamination sources[85].

Although As is not widely recognized as an essential element, studies involving goats, chicks, hamsters, and rats have indicated that it may be beneficial or essential in very small amounts (μg/kg of diet)[85]. However, it is important to note that all livestock species can be susceptible to the toxic effects of iAs[85] at high concentrations. Chronic As intoxication in animals may manifest as reduced growth, feed intake, feed efficiency, and, in certain species, symptoms such as convulsions, uncoordinated gait, and decreased hemoglobin levels[113]. The tolerance level and toxicity of As in animal diets vary depending on the species and the specific form of As compound involved[13]. Toxic concentrations of As in the diet are generally 2-3 times higher than the typical levels found in animal feeds[85].

1.5 Analysis of arsenic

1.5.1 Sample pretreatment

The trace element analysis sampling process includes multiple stages, from source material collection to final analytical subsample extraction. Each level of subsampling aims to minimize material processed while maintaining representativity, ensuring a strong correlation between

subsamples and the primary sample's physical and chemical characteristics[114]. Particle size reduction can be achieved through various methods, including mechanical grinding, crushing, cutting in a Wiley or hammer mill, abrasion in a cyclonic mill, or crushing in a ball mill. Well-grinding the sample can result in a more homogeneous composition[114].

1.5.2 Sample digestion

The primary objective of sample digestion is to transform the physical form of the sample into a suitable state for chemical analysis. In determining As using ICP-MS, six primary digestion techniques are frequently documented in scientific literature. These techniques encompass electro-thermal vaporization, dry ashing in a standard oven, oxygen combustion, acid digestion in an open vessel, microwave digestion in a sealed vessel, and microwave digestion[114,115].

To analyze trace elements in organic material using ICP-MS, the samples were converted to a low-viscosity liquid by breaking down organic bonds with acids and releasing trace elements as ions. The process was accelerated by heating the samples using a microwave oven. Microwave-assisted extraction (MAE) is a technique that utilizes microwave energy to heat solid samples with solvent or solvent mixtures, allowing for the partitioning of compounds of interest from the sample to the solvent[63].

1.5.3 ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) is an analytical technique that allows for determining elemental composition in a sample. ICP-MS has become one of the most important techniques for multi-elemental analysis due to its low detection limits, high selectivity, and good precision and accuracy[116]. A commercial ICP-MS system, as shown in Figure 3, consists of several components, including the sample introduction system, inductively coupled plasma (ICP), interface, ion optics, mass analyzer and detector[117,118]. First, the liquid sample is pumped into the nebulizer and nebulized into a fine aerosol that is then introduced to the argon plasma. The high-temperature plasma ionizes and atomizes the sample, creating ions that are then extracted through the interface region and directed into a set of electrostatic lenses known as ion optics. The ion optics focus and guide the ion beam into the quadrupole mass analyzer, where ions are separated based on their mass-charge ratio (m/z) and measured at the detector[117,118].

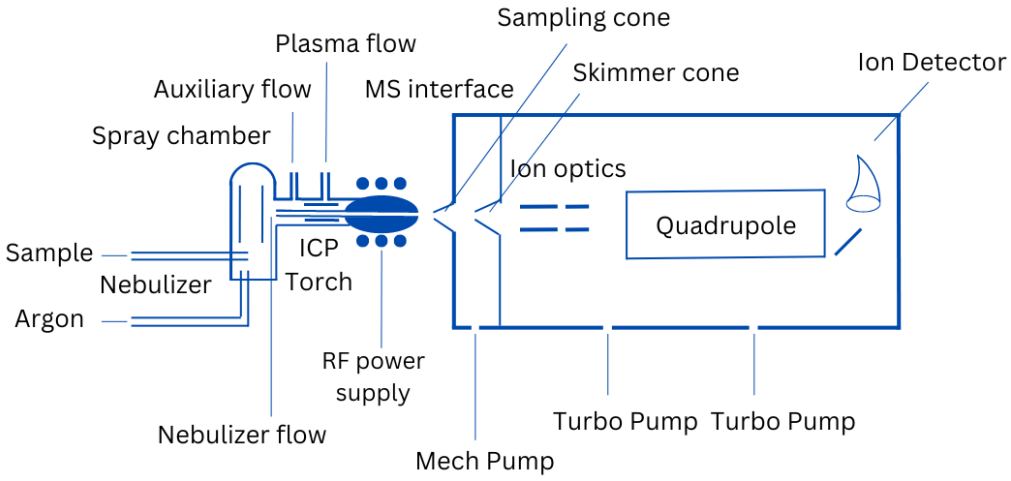

Figure 3. Schematic representation of a typical quadrupole ICP-MS instrument. Adapted from Brima et al. (2006)[119]

In ICP-MS, liquid samples are pumped into a nebulizer, converting them into a fine aerosol mist using argon gas[118,120]. The mist passes through a spray chamber to remove larger droplets and is then carried by the argon gas to the ICP plasma torch[118,120]. The argon is ionized using a radio frequency (RF) generator, creating a high-energy plasma[120]. The ionized argon gas reaches a temperature of 10,000 degrees Celsius and flows through the outer quartz tube, forming the plasma and cooling it[120]. The ionization of elements in the plasma depends on their ionization energy and the plasma temperature[118,120]. The plasma ion source and quadrupole mass spectrometer are linked by a vacuum interface[118,120]. This interface, consisting of cooled metal plates or cones with small holes, allows the ions to pass from the atmospheric pressure plasma to the mass spectrometer in the vacuum chamber[118,120].

ICP-MS systems introduced in the early 2000s commonly include a collision/reaction cell (CRC) to address spectral overlaps caused by unwanted ions[120]. Polyatomic ions, formed by combinations of atoms, can overlap with analyte ions of interest[120]. The CRC, typically an ion guide like an octopole or quadrupole, operates in a pressurized chamber and utilizes collision and reaction modes to mitigate interferences[120]. The mode choice depends on the gas (or gases) introduced to the cell, such as H_2, O_2, NH_3, CH_4, N_2O, or CH_3F, to enable specific reactions with interfering ions and resolve interferences[120]. The selection of the appropriate mode depends on the particular nature of the interferences and the desired analytical outcome[120].

The quadrupole is a mass analyzer that filters ions based on their m/z[120]. It consists of two pairs of rods with opposite electrical supplies[120]. The electric field between the rods determines the "set mass" of ions that can pass through the filter, while ions with different masses are rejected from the ion beam[120]. The quadrupole mass analyzer rapidly scans ions across a wide mass range by adjusting rod voltages[120]. It enables building mass spectra, time-resolved analysis, and single mass monitoring for specific measurements[120].

Most ICP-MS systems use a discrete dynode electron multiplier (DDEM) as the detector[120]. When ions from the quadrupole strike the first dynode, electrons are released, which then trigger a cascade of electron releases as they strike subsequent dynodes[120]. This electron cascade generates a pulse or count that is recorded by the DDEM electronics[120]. At high ion count rates, the detector switches to a low-gain mode to avoid overload and ensure accurate measurement of intense signals[120].

1.6 Arsenic speciation analysis

Speciation analysis typically involves three main steps: sample extraction, separation, and detection of chemical species. Sample extraction is commonly accomplished through hydrolysis procedures such as acid, alkaline, and enzymatic hydrolysis[121,122]. Alternatively, aqueous or organic solvents can be used to solubilize different compounds based on their specific physicochemical properties. When it comes to separation, liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis (CE) are the commonly employed techniques[123]. These techniques are often coupled with element-specific detection methods that offer high sensitivity, such as inductively coupled plasma mass spectrometry (ICP-MS)[19]. Precise quantification of contaminants in feed and food is important for assessing if samples comply with regulations for (element) As speciation.

1.6.1 Sample extraction

Most analytical instruments cannot handle solid samples. Thus, the target compounds must be transferred to a liquid phase. Solid-liquid extraction is one of the oldest techniques used in solid sample preparation and it is used to extract soluble compounds from a solid sample using

suitable solvents. Water, water-methanol, or water-methanol-chloroform mixtures are the most used solvents[124]. Other solvents, such as acetonitrile, have also been tested for efficacy in As species extraction[125]. Extraction methods based on solubilization with HCl and microwave-assisted distillation were developed to extract iAs from seafood products[126]. However, these methods were not suitable for determining As(III) and As(V) species separately because As(V) is converted to As(III) during the hydrolysis and extraction process[127].

To ensure accurate As speciation analysis, the extraction method employed must quantitatively extract all As species while preserving their original chemical forms, and the solvent used should not hinder the subsequent analysis[127].

1.6.2 Separation and detection

High-performance liquid chromatography (HPLC) is a separation technique that separates and isolates different chemical species in a sample based on their chemical and physical properties. Chromatography operates on a fundamental principle in which a mixture's molecules are introduced onto a solid surface. As they move, they separate from each other with the assistance of a mobile phase[128]. Several factors influence this separation process, encompassing molecular traits associated with adsorption (liquid-solid), partition (liquid-solid), and variations in molecular weights[128].

1.6.2.1 Ion-exchange Chromatography

Ion-exchange chromatography is a column-based LC technique designed to separate compounds with different charges or ionizable properties[129,130]. It consists of both mobile and stationary phases[129,130]. The mobile phase comprises an aqueous buffer system that serves as a solvent and eluent for the sample mixture[129,130]. The stationary phase is typically an inert organic matrix chemically modified with ionizable functional groups known as fixed ions. These fixed ions carry oppositely charged ions that are displaceable[129,130](see Figure 4).

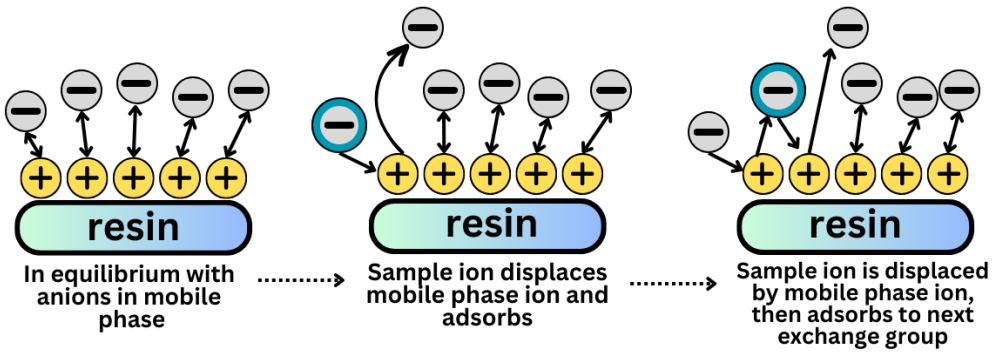

Figure 4. Ion exchange scheme. Adapted from Shimadzu: Inorganic anion detection. Accessed Sep 01,2023)[131]

In this chromatographic process, equilibrium exists between the mobile and stationary phases, resulting in two possible formats: anion exchange and cation exchange[130,132]. The counter ions, which are in equilibrium, can include protons (H^+), hydroxide groups (OH^-), monoatomic ions (such as Na^+, K^+, Cl^-), divalent monoatomic ions (such as Ca^{2+}, Mg^{2+}), polyatomic inorganic ions (such as SO_4^{2-}, PO_4^{3-}), as well as organic bases (NR_2H^+) and acids (COO^-)[129].

Cations are separated using a cation-exchange resin HPLC column, while anions are separated using an anion-exchange resin column[130,133]. The separation is based on the specific binding of analytes to positively or negatively charged groups that are fixed on the stationary phase[130,133]. These charged groups are in equilibrium with free counter ions present in the mobile phase[130,133]. The separation is achieved by exploiting the differences in the net surface charge of the analytes[130,133]. Both pH and ion strength will affect the separation[134].

During the journey of analytes through the column, the adsorption of analytes to the stationary phase and their desorption by eluent ions occur repeatedly due to ion-exchange interactions[130]. This process leads to the separation of analytes based on their ion-exchange characteristics[130].

In applications where all possible As species are to be analyzed, multiple separation modes are recommended. Tibon and co-workers combined using anion and cation exchange columns followed by ICP-MS detection, which detected 33 As species in marine-certified reference materials (CRMs)[16]. A similar approach by combining anion and cation exchange

chromatography was applied to measure As species in freshwater mussels and farmed freshwater fish[135,136].

Arsenic speciation analysis has been conducted using both anion and cation-exchange chromatography techniques[137]. Anion exchange is commonly used to analyze As(III), As(V), MMA(V), and DMA(V) based on the ionic characteristics of these As compounds. On the other hand, cation exchange is used to separate AsB, AsC, TMAO, and TETRA species[127].

1.6.3 Detection of chemical species

To analyze different chemical forms or species of a specific element, HPLC can be connected to ICP-MS. While ICP-MS is powerful for elemental analysis, it cannot differentiate between chemical forms[117]. Connecting the HPLC analytical column to the ICP-MS nebulizer via a capillary tube enables the separation and identification of different species while providing sensitive elemental detection[117]. An HPLC-ICP-MS is valuable for detecting low concentrations of contaminants in feed and food samples, ensuring compliance with regulations for specific elements[117].

The coupling of HPLC with ICP-MS (Figure 5) provides numerous advantages due to the high sensitivity, multi-element capability, large dynamic range, and isotope ratio measurement capability of the ICP-MS instrument[127]. HPLC-ICP-MS has been widely applied to As speciation[138].

The primary concern when conducting multi-elemental speciation analysis lies in identifying suitable HPLC-ICP-MS conditions that can ensure successful retention and elution of analytes within a reasonable timeframe, complete separation of analytical signals, symmetrical analytical signals, species stability throughout the entire analytical process, prevention of potential interferences, and attainment of sufficiently low detection limits[139].

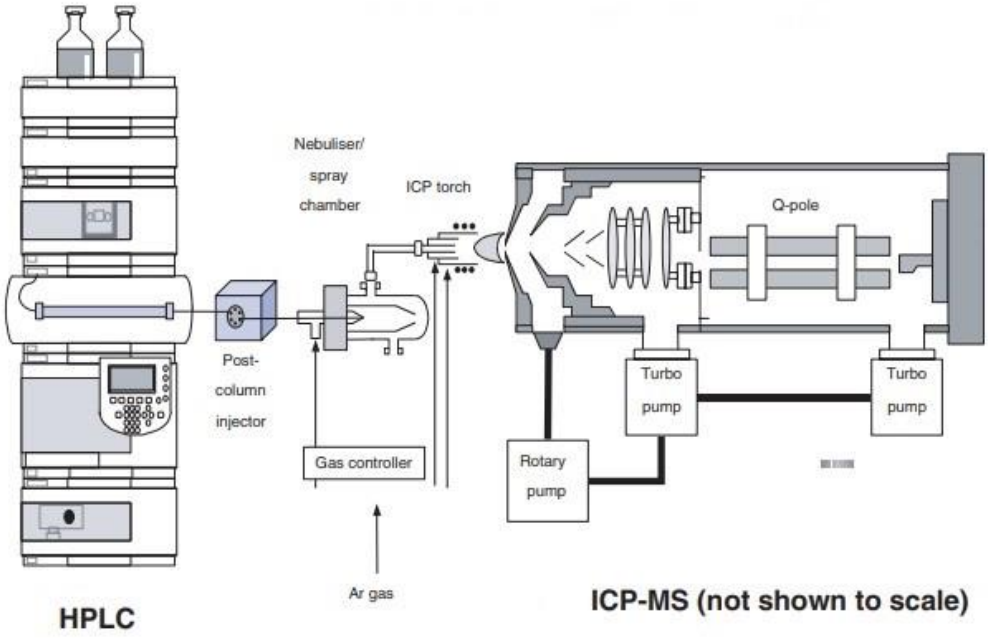

Figure 5. Schematic of elemental speciation setup: Analyte fractions from resolved LC are transformed through decomposition, atomization, and ionization within a high-temperature argon plasma. The resulting ions are then measured in a mass spectrometer, enabling calculation of species concentration. System software ensures synchronized operation between HPLC and ICP-MS. Taken from Fairman and Wahlen (2001)[140]

The concepts underlying HPLC-ICP-MS involve the utilization of resolved LC analyte fractions[140]. These fractions are introduced into a high-temperature argon plasma through a nebulizer, where they undergo decomposition, atomization, and ionization processes[140]. The resulting ions are then conveyed through a sampling interface and directed into a mass spectrometer for precise measurement[140]. By coordinating the chromatographic retention time of each peak with the elemental abundance detected in the MS, it becomes possible to calculate the concentration of the species[140]. A dedicated software system controls all aspects of the coupled setup, including the synchronization of HPLC and ICP-MS functions[140].

2 Experimental

2.1 Instruments

The list of instruments, equipment and chemicals can be seen in supplementary information (Supplementary Table 1 and Supplementary Table 2).

2.2 Samples

Table 2 shows the sample types and the number of samples used for each type.

Table 2.Overview of the samples for determination of tAs, iAs and lipid-bound As.

Sample type	Number of samples for tAs analysis	Number of samples for iAs analysis	Number of samples for lipid-bound As analysis
Fish feed	21	-	7
Land animal feed	10	5	2
Plant meal	15	7	-
Fish meal	14	4	4
Fish oil	10	-	9
Microalgae	6	6	3
Halophyte	3	3	2
Insect meal	4	4	3
Seaweed	2	1	-
Hydrolyzed fish protein concentrate	8	8	8
Tunicates	7	7	2
Experimental feed containing seaweed	3	3	-
Experimental feed for piglets containing yeast	2	-	-
Experimental feed for chicken containing yeast	4	-	-
Experimental fish feed containing yeast	6	-	-

In this study, samples of commercial fish feed (n=21), plant meal (n=15), fish meal (n=14), fish oil (n=10) and insect meal (n=4) were obtained from the Norwegian fish feed monitoring program (2021 - 2022). The plant meal samples (n=15) encompassed soya protein concentrate, guar meal, wheat gluten, pea flour, sunflower concentrate and meal. Additionally, 10 samples of land animal feed were obtained from the Norwegian monitoring program for land animal feeds (2021). Furthermore, six microalgae samples including *Tetraselmis chuii*, *Organic Spirulina platensis*, *Organic Chlorella vulgaris*, *Nannochloropsis oceanica* and *Tetraselmis striata* were purchased from Allmicroalgae – Natural Products S.A., Portugal. Three halophyte samples were provided by researchers at the Research Group of Aquaculture belonging to the University of Las Palmas (ULPGC), Gran Canaria, Spain. These halophyte samples included *Crithmum maritimum*, *Zygophyllum fontanesii (Tetraena fontanesii)*, and *Salicornia perennis*. Also, two seaweed samples - *Colpomenia sinuosa* and *Caulerpa racemose* – were part of our analysis. For our research work, we obtained hydrolyzed fish protein concentrates (8 samples) from various suppliers of fish products, including Pelagia in Norway (Table 3).

Table 3. Composition of hydrolyzed fish protein concentrates with the provided location and date of harvesting.

Sample	***Composition***	***Location***	***Date***
1	Whitefish	Lofoten, Norway	29/5-22
2	Whitefish, 20.8% cod, 75.3% haddock, 3.6% pollock, 0.3% other	Troms/Finnmark, Norway	30/3-22
3	Whitefish	Iceland	14/4-22
4	Whitefish	West-coast, Norway	13/6-22
5	Pelagic - swordfish	Troms, Norway	2/4-22
6	Pelagic - herring	Troms, Norway	16/1-22
7	10.4% herring, 30.5% cod, 59% pollock	N/A[1]	15/9-21

[1] Not available

8	37.6% herring, 39.4% cod (10.6 MSC/28.8 not MSC), 14.2% pollock, 8.1% capelin, 0.7% other	N/A	29/6-22

Tunicates (n=7) were obtained from Austevoll, Norway. The tunicates were sampled from areas close to the Research station of IMR, in Sauganeset (at position 60.087984, 5.262680) and in Ytrevagen (at position 60.129660, 5.903513). In Sauganeset the samples were collected by harvesting around 20 specimens from the floating elements on the pier and ropes, at approximately 0.5 to 1 meter depth. For the samples collected in Ytrevagen, around 20 specimens were collected from 7-meter-long ropes, collected specimens at around 3 m depth. All samples were frozen at -20 C until shipment in ice to the facilities in Bergen. After measuring the weight and length of the tunicates (Table 4), they were pooled into samples of approximately 20 specimens, weighted and freeze-dried (see *2.6.1 Freeze-drying*).

Table 4. Tunicate Sample Lengths (mm) - Minimum, Maximum, and Average Values

Sample	T1	T2	T3	T4	T5	T6	T7
Harvesting date	11/03/22	10/02/22	31/01/22	08/04/22	31/03/22	27/10/21	02/03/22
Location	-	Austevoll	Ytrevagen	Austevoll	Ytrevagen	Varaldsøy	Ytrevagen
n	20	20	8	20	20	6	20
min length (mm)	30.5	46.21	69.2	49.36	25.2	59.78	23.8
max length (mm)	76.23	100.02	128.21	90.16	53.78	107.89	42.53
average (mm)	51.8	61.7	90.9	71.2	37.5	89.1	32.5

The experimental diets, including piglet feed samples (n=2), salmon feed samples (n=6) and chicken feed samples (n=4), were provided by Foods of Norway, a Centre for Research-based Innovation at the Norwegian University of Life Sciences (NMBU), Norway. The samples of feed supplemented with seaweed (n=3) were given by the project HoLoFood which is supported by the European Union's Horizon 2020 (grant agreement No. 817729). The samples were promptly frozen at -18°C upon receipt in the laboratory and homogenized using a laboratory mill (Point) before analysis.

2.3 Total As determination by ICP-MS

To determine the tAs concentration in the samples, the method "Multielement determination with inductively coupled plasma (ICP-MS) after microwave-assisted digestion" as described by Julshamn et al. (2007)[141]. The method is accredited by the Norwegian Accreditation Authority, according to ISO standard 17025. This method can be applied to food, feed and solid/liquid biological material for the accredited elements As, Cd, copper (Cu), zinc (Zn), Hg, selenium (Se) and Pb. It can also determine the unaccredited elements silver (Ag), iron (Fe), cobalt (Co), manganese (Mn), vanadium (V), molybdenum (Mo), tin (Sn), chromium (Cr), nickel (Ni) and yttrium (Y).

2.3.1 Sample preparation for total As determination

A weight of 0.20 - 0.25 g (up to 0.5 g of wet materials) the sample material was taken in duplicate and placed in glass digestion tubes containing 0.5 mL of deionized and filtered Milli-Q water and 2 mL of concentrated nitric acid (HNO_3, 69% w/w). The wet samples were weighed directly into dry tubes. To ensure safety, the addition of concentrated acid was done in a fume hood. The tubes were then sealed with Teflon corks and placed in the UltraWave digester, previously filled with Milli-Q® water and 5 mL H_2O_2 to prevent excessive gas formation. After digestion, clear sample solutions were diluted with Milli-Q® water to a final volume of 25 mL, transferred to marked 50 mL falcon tubes and stored at room temperature until quantification on ICP-MS (up to one year).

The glassware and corks were thoroughly cleaned following each digestion. Tubes and small volumetric flasks were rinsed with Milli-Q water and then subjected to an acid steam cleaning protocol using concentrated HNO_3 at a temperature of 90ºC for 1 hour. Corks were soaked in a 10% HNO_3 solution for 24 hours (IMR, 2018). A second digestion with 2 mL of nitric acid was performed on the tubes used for a different matrix to ensure cleanliness.

2.3.2 Total As analysis by ICP-MS

The concentration of tAs in the samples was determined using an inductively coupled plasma mass spectrometer (ICP-MS) (Thermo iCap-Q) with a helium collision cell and FAST SC-4Q DX auto-sampler. The sample solution was nebulized and introduced into an argon plasma environment, vaporized by the plasma (at 7000°C) and the elements were ionized. The ions were then transported to a mass-sensitive detector, where the number of hits per second was recorded for the selected masses. Before analysis, the settings of the system were optimized by

injecting a tuning solution and ensuring that the signal met a control check. In Table 5 instrument settings applied are given. Isotopes of rhodium (Rh), germanium (Ge), and thulium (Tm) were used as internal standards to correct for any possible instrumental drift in the selected mass range.

Table 5. ICP-MS settings.

Parameter	**Value**
RF power	1550 W
Plasma gas flow	14.0 L/min
Carrier gas flow	1.02 L/min
Makeup gas flow	0.8 L/min
Dwell time and isotopes monitored	**0.05 s**: ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{95}Mo, ^{107}Ag, ^{111}Cd, ^{208}Pb **0.25 s**: ^{75}As, ^{78}Se, **0.1 s:** ^{202}Hg

The samples were then introduced into the auto-sampler of the ICP-MS and measured using a multielement external standard calibration curve. The calibration solutions were prepared daily by diluting a 1000 mg/L certified As stock solution with 5% (v/v) nitric acid. Data processing was performed using the software Q-Tegra ICP-MS (Thermo Scientific).

2.4 Inorganic As speciation by HPLC-ICP-MS

The determination of iAs was carried out using anion-exchange HPLC coupled with ICP-MS, as previously described in Sloth et al. (2005)[15]. This method is per the standardized CEN method (NS-EN 16802:2016) for the determination of iAs in marine biological tissue and feed. The linearity of the method was determined in the concentration range from 0.0073 mg/kg to 0.04 mg/kg, with a limit of quantification (LOQ) of 0.0073 mg/kg. The method was validated over the range of 0.0073 mg/kg to 1 mg/kg dry sample. In cases where the iAs concentration exceeded the quantification range, the samples were further diluted before analysis. Empirical prediction of the concentration of iAs in the sample was carried out using the laboratory's database by comparing the results of previous analyses of the same matrix.

2.4.1 Sample preparation for iAs determination

In this study, a total of 0.20 g of freeze-dried or wet sample (0.8 g for fish proteins) was weighed and placed into 13 mL propylene centrifuge tubes in duplicate. To these tubes, 10 mL of an extraction solution of 0.1M HNO_3 in 3% (v/v) H_2O_2 was added using the dispenser. An

extraction solution was prepared by mixing 800 mL of H_2O, 6.5 mL of concentrated HNO_3 and 100 mL of H_2O_2 in a 1000 mL volumetric flask and then diluted to 1000 mL with H_2O. The samples were then agitated using a vortex mixer at 1800 *rpm* and left for 24 hours at room temperature. Afterward, the samples were heated in a water bath at 90 ± 2ºC for 60 minutes, shaken at 100 *rpm*, cooled to room temperature, and centrifuged at 3800 *rpm* for 10 minutes. Approximately 2 ml of the supernatant was removed with a 5 ml disposable syringe fitted with a long needle and then filtered through a 0.45 µm syringe filter (the needle is replaced with the filter) directly into a labeled 1 ml HPLC sample vial. The shelf-life of the analytes was one week after preparation.

2.4.2 Inorganic As determination by HPLC-ICP-MS

To quantify iAs, a 1260 Infinity HPLC and a 7900 ICP-MS (Agilent Technologies, Santa Clara, CA, USA) were used in conjunction with an anion-exchange column (IonPac AS7, 2 × 250 mm; Dionex, Sunnyvale, CA, USA). The elution process involved the use of 50 mM $(NH_4)_2CO_3$ in 3% CH_3OH, which was adjusted to pH 10.3 with NH_3 ($(NH_4)_2CO_3$, reagent grade; CH_3OH, ≥ 99.97%; NH_3, 25%; Merck, Darmstadt, Germany). Quantification relied on chromatographic peak areas and was determined using an external calibration curve derived from an arsenate (As(V)) standard solution (1000 mg L^{-1}; Spectrascan Teknolab, Ski, Norway).

For complete instrument settings, refer to the Table 6.

Table 6. HPLC-ICP-MS settings

Parameter	Value
RF power	1550 W
Carrier gas flow	1.15 – 1.25 L/min
Pump speed w/tuning	0.12 rps
Pump speed w/analysis	0.20 rps
Response	^{75}As ≥ 18 000 counts/second
RSD %	< 5
Injection volume	5 µL
Operating pressure	< 200 bar
Time of analysis	8 min
Separation method	isocratic
Mobile phase flow	0.300 mL/min

Mobile phase A	50 mM $(NH_4)_2CO_3$ pH 10.3

2.5 Determination of lipid-bound As

The lipid-bound As extraction was performed as described by Tibon et al (2022)[142] and Freitas et al (2020)[143]. Approximately 50 mg of oil or freeze-dried sample or 200 mg of wet material were weighed into a borosilicate glass tube (13 100 mm, DWK, Mainz, Germany) and vortex-mixed for 5 seconds with 1.5 mL of methanol. The mixture was then treated with 5 mL of methyl tert-butyl ether (MTBE, HPLC grade, Merck, Darmstadt, Germany). The tubes were sealed and placed in a test-tube rotator (LD-79, LABINCO, Breda, the Netherlands) for an hour to allow for optimal solvent-matrix interaction. Afterward, 1.25 mL of ultrapure water (18.2 MΩ*cm resistivity) was added to the tubes and left for 10 minutes. The tubes were then centrifuged for 10 minutes at 3500 rpm. Using polypropylene Pasteur pipettes, the upper organic layer was collected and transferred to quartz digestion tubes (ultraWAVE, Milestone, Sorisole, Italy). The residual layer and pellet were then extracted a second time with 2 mL of a 10:3:2.5 v/v/v mixture of MTBE, methanol, and water. After allowing the mixture to sit for 15 minutes, it was centrifuged at 3500 rpm for 10 minutes. The remaining organic phase was gathered and merged with the previously collected one. The tubes were then evaporated in a heated nitrogen evaporator (40°C, Reacti-Therm, Thermo Fisher Scientific, Waltham, MA, USA) until a lipid pellet was formed. These pellets were then analyzed for tAs as detailed in section 2.3.1 and 2.3.2.

2.6 Determination of dry matter

Dry matter content in the samples of tunicates and microalgae was determined through freeze-drying. For fish feed samples, land animal feed, plant meal, fish meal, halophyte, seaweed, fish protein concentrates, and experimental feed samples, the dry matter content was determined by drying them in an oven.

2.6.1 Freeze-drying

Samples of tunicates and microalgae were freeze-dried using a procedure following an accredited method (Method 377, IMR, 2016) with the following steps. Initially, the frozen samples were weighed and placed without a lid in a freeze dryer with a temperature of -20ºC.

The system was then pressurized to around 0.2 mbar, and the temperature was increased to 25ºC, facilitating the sublimation of the water in the samples. The freeze-drying process was performed for at least two days, and in some cases, a second round of freeze-drying was conducted to ensure complete dryness of the samples.

Following the freeze-drying process, the pooled samples were weighed, and their moisture content was calculated. The samples were homogenized in a grinder for approximately 10 seconds until the material reached a state of homogeneity in the form of a fine powder. To prevent any moisture absorption, the samples were stored in closed containers at room temperature until further analysis.

2.6.2 Determination of dry matter

The sample material was prepared for analysis by weighing two parallels of 3-5 g each of well-homogenized material into a tared aluminum pot. The sample was evenly distributed in the pot to increase the surface area. The parallels were dried for 16-18 hours at 104 ± 1°C in a drying cabinet to remove any moisture. After drying, the samples were cooled to room temperature for 30 minutes in a desiccator containing previously dried silica gel. The silica gel had been dried at 104°C until it changed color from transparent to orange. The cooled samples were then weighed again, and the measured weights were recorded.

2.7 Quality assurance

The accuracy and precision of the tAs analysis were performed using CRMs, including TORT-3, Lobster Hepatopancreas from the National Research Council of Canada (n=3), and NIST Oyster Tissue from the National Institute of Standards and Technology (n=3) in different sample series. "Sample series" refers to a group of samples that are analyzed on the same day, utilizing the same method, regardless of when they were prepared. To demonstrate specificity, one blank sample was included per digestion.

Here, iAs species, which includes both As(III) and As(V), were measured in the extracted sample as the sum of these two forms. This was accomplished by converting As(III) to As(V) during the extraction process. Two CRMs, tuna fish tissue (BCR 627) and rice (ERM BC 21),

were prepared in the same way as the rest of the samples and used for the quality control of the results.

Instrument verification and optimization were performed daily, using a tuning solution and procedure following the manufacturer's guidelines.

In this study, various matrices, including halophytes, plant meal, tunicates, fish protein concentrates, and land animal feed, were identified as challenging. Additional homogenization and reanalysis were conducted for most samples, leading to improved parallel differences, except for the land animal samples.

3 Results and Discussion

3.1 Total As in commercial animal feed and feed ingredients

In this study, total arsenic (tAs) concentration in commercial animal feed and feed ingredients was analyzed and the results are presented in Figure 6. The results are expressed in units of milligrams per kilogram of wet weight (mg/kg ww).

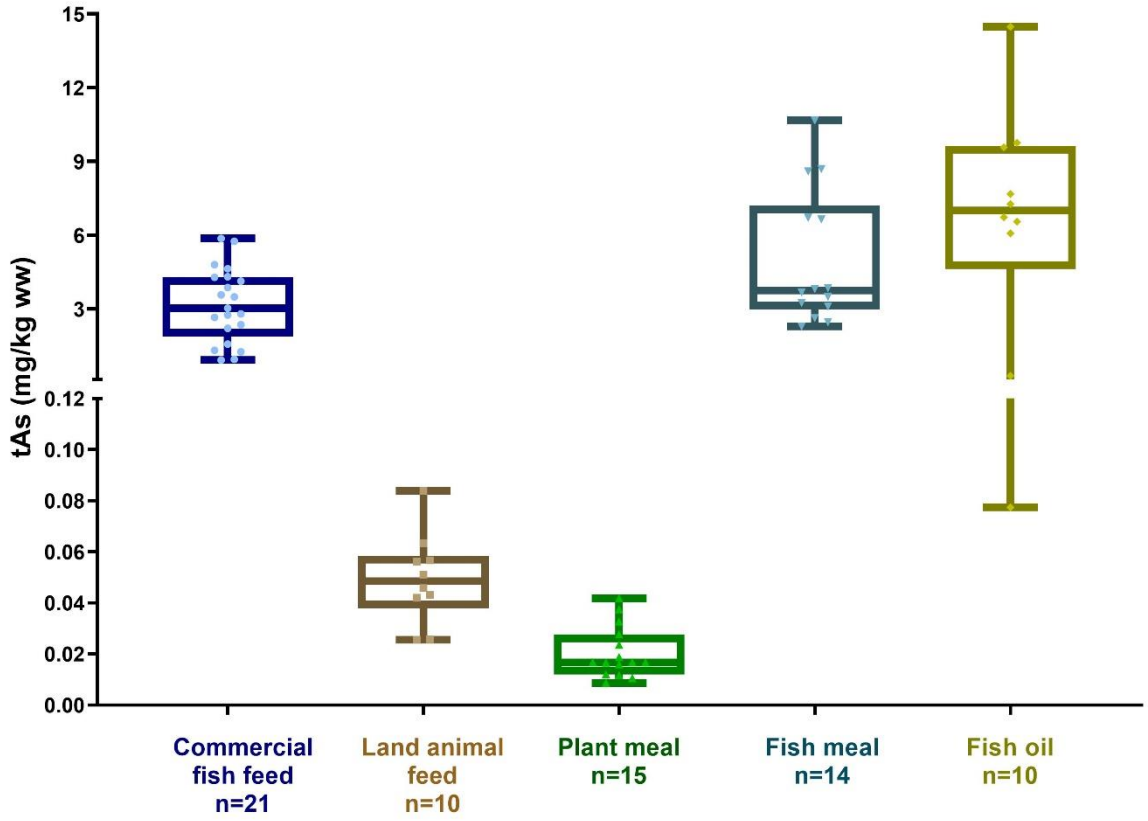

Figure 6. Boxplot of the tAs concentration in commercial feed and feed ingredients, including plant meal, fish meal and fish oil, with 'n' denoting the number of analyzed samples. The solid line inside the box is the median value; the dots represent individual samples and the horizontal lines show minimum and maximum.

3.1.1 Commercial fish feed

The results for the commercial fish feed samples analyzed show tAs concentrations ranging in concentrations from 0.92 to 5.87 mg/kg ww (n=21), whereas the results for the land animal feed samples analyzed show tAs concentrations ranging in concentrations from 0.02 to 0.09 mg/kg ww (n=10) (Figure 6). Comparatively, fish feed exhibited higher levels of As than land animal feed. This difference can be attributed to the inclusion of marine ingredients, such as

fish meal and fish oil, in fish feed composition[17]. The concentrations of tAs in marine fish used as ingredients in fish feed directly impact the overall As levels in the produced feed[15]. In addition to marine ingredients, fish feed typically contains plant meal components (like soya protein concentrate, wheat meal, and vegetable oils) and micro-ingredients[144]. In general, the levels of tAs in the fish feeds and the commercial feed ingredients (fish meal, plant meal and fish oil) align with the results reported in the national monitoring program for fish feed in Norway[58,81]. For fish feed, tAs concentrations ranged from 0.8 to 6.7 mg/kg ww in fish feeds (n=82) analyzed in 2021[145], and from 1.0 to 7.1 mg/kg ww in fish feeds (n=76) analyzed in 2018[146].

3.1.2 Plant meal

The results for the plant meal samples analyzed (n=15) show tAs concentrations ranging in concentrations from 0.01 to 0.04 mg/kg ww (Figure 6). The results for the fish meal samples analyzed (n=14) show tAs concentrations ranging from 2.29 to 10.68 mg/kg ww (Figure 6), while the results for the fish oil samples analyzed (n=10) show tAs concentrations ranging from 0.08 to 14.48 mg/kg ww (Figure 6). Terrestrial organisms typically contain lower concentrations of As than those found in the marine environment[147], which we can also see from the results given here. In this study, the tAs concentration in plant ingredients is consistent with previous reports of the national monitoring program for fish feed in Norway[145,146]. For example, vegetable feedstuffs analyzed exhibited tAs levels ranging from 0.01 to 0.04 mg/kg ww in 2021[145] and <0.09 to 0.1 mg/kg ww in 2018[146]. In comparison, uncontaminated terrestrial plants typically contain 0.2 to 0.4 mg/kg of As[148]. However, there are exceptions such as mushroom *Laccaria amethystina*[149] and plants grown in As-contaminated areas or those that accumulate As, which may contain high As levels comparable to those found in marine organisms[80]. The uptake of As by plants is influenced by various factors, including the availability of soluble As species in the soil, soil characteristics, redox and pH conditions, microbiological activity, and the specific plant species involved[21,85].

3.1.3 Fish meal and fish oil

The results of this study for the fish oil and fish meal are also in agreement with the results reported in the national monitoring program for fish feed in Norway. Samples of the commercial fish oils (n=10) analyzed in 2006[150], revealed average tAs concentration ranging from 7 to 16 mg/kg. The tAs concentration in fish meal was observed to range from 2.2 to 8.8 mg/kg ww in 2021[145], and from 2.6 to 12 mg/kg ww in 2018[146]. The variation in As levels

could be attributed to species differences, as the fish meal utilized in the feed surveillance program is primarily sourced from imports[151,152].

3.2 Total As in new feed ingredients

In this study, the concentration of tAs in new feed ingredients, including microalgae (n=6) (Figure 7), halophytes (n=3), insect meal (n=4), seaweed (n=2) (Table 7), tunicates samples (n=7) (Figure 9) and hydrolyzed fish protein concentrate (n=8) (Figure 7) were analyzed. The results are expressed in units of milligrams per kilogram of wet weight (mg/kg ww).

Table 7. Average of tAs concentration with the corresponding standard deviation in halophyte samples, insect meal and seaweed samples. Values are given in mg/kg ww.

	Mean tAs (mg/kg ww)	SD	No. replicates
Halophyte			
Crithmum maritimum	0.17	-	1
Zygophyllum fontanesii (Tetraena fontanesii)	0.418	0.004	2
Salicornia perennis	0.422	0.004	2
Insect meal			
Sample 1	0.047	0.005	2
Sample 2	0.0425	0.0004	2
Sample 3	0.34	0.01	2
Sample 4	0.039	0.001	2
Seaweed			
Colpomenia sinuosa (brown algae)	14.37	-	1
Caulerpa racemosa (edible green algae)	8.16	0.16	2

3.2.1 Microalgae

The analyzed microalgae samples comprise *Tetraselmis chuii*, *Spirulina platensis*, *Chlorella vulgaris*, *Nannochloropsis oceanica* and *Tetraselmis striata*. Figure 7 presents the results of tAs analysis in microalgae samples analyzed as average with their corresponding standard deviations (SD). In the microalgae samples (n=6), the lowest concentration was in the species of *Spirulina platensis* with a concentration of 0.11 ± 0.01 mg/kg ww (Figure 7), and the highest concentration measured in the sample of microalgae species *Chlorella vulgaris* with a concentration of 0.42 ± 0.01 mg/kg ww (Figure 7).

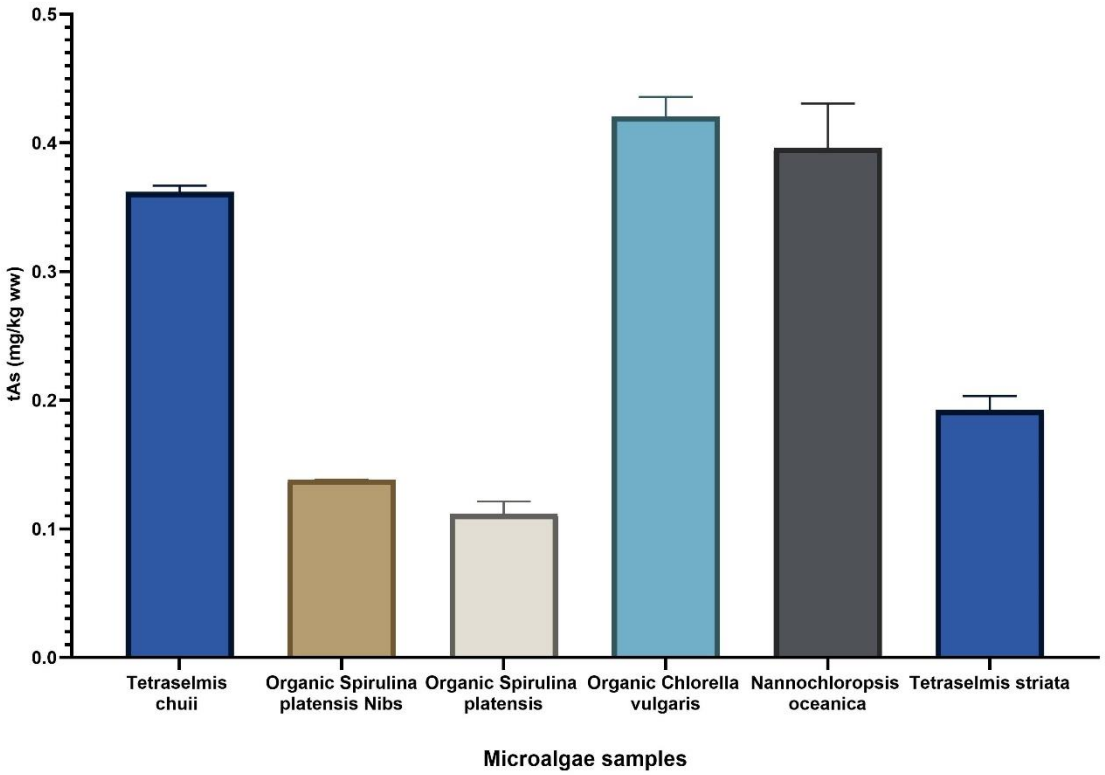

Figure 7. The average concentration of tAs in individual microalgae samples presented alongside their standard deviation (SD). Values are expressed in mg/kg wet weight (ww).

Microalgae have the ability to accumulate PTEs such as As, Cr, Hg, Pb, and Cd[153]. Accumulation of PTEs in microalgae may negatively impact food and feed safety as they form the base of the marine food chain and serve as vectors for the transfer of these substances to higher organisms such as zooplankton, shrimp, and shellfish[154]. *Tetraselmis chuii*, *Nannochloropsis oceanica* and *Tetraselmis striata* have not been previously studied for their tAs concentration. In a previous study, the microalgae species *Spirulina platensis* demonstrated the ability to accumulate As up to 4.1 mg/kg dw when exposed to As(III)[106]. In a study by S. García-Salgado et al.(2006)[155], commercial samples of *Chlorella vulgaris* exhibited a tAs concentration of 33 ± 5 mg/kg.

3.2.2 Halophytes

The As concentrations in halophyte ranged from 0.17 mg/kg ww for the *Crithmum maritimum* sample to 0.422 ± 0.004 mg/kg ww, for the *Salicornia perennis* sample (Table 7). *Crithmum maritimum*, commonly known as sea fennel, is a coastal plant that grows in salty, rocky habitats[156]. *Salicornia perennis*, a succulent halophyte that grows in saline habitats, including

salt marshes[157,158]. *Zygophyllum fontanesii* (formerly known as *Tetraena fontanesii)* is a halophytic shrub found in arid regions with saline soils[158]. Halophytes are currently being explored as a new feed material for animal/fish feed[159–161].

Halophytes are plants that can reproduce in environments with high salt concentrations and make up about 1% of the world's flora, with some growing best in saline conditions and others in non-saline conditions[162]. To our knowledge, there is no available literature data about the tAs content in *Crithmum maritimum* L. but previous studies have shown that under high salinity levels, the activity of the antioxidant system is reduced, and there is a higher accumulation of toxic ions[156]. *Salicornia perennis* has not yet been studied for its As content. However, a study conducted on a different species from the same family, *Salicornia patula* Duval-Jouve, revealed high levels of As, likely due to the plant's uptake of this element from the surrounding soil and water[163]. Recently, EFSA released a report[159] indicating that the average tAs concentrations (in µg/kg) in halophyte samples (n=7) ranged from 41 to 59 µg/kg, which are lower than those found in the samples analyzed in this study.

3.2.3 Insect meal

In the past few years, insects have gained significance in aquaculture as viable substitutes for protein[164]. The tAs in insect meal samples is shown in Table 7, where the concentrations ranged from 0.039 ± 0.001 mg/kg ww to 0.34 ± 0.01 mg/kg ww. The observed tAs concentrations in the analyzed samples were slightly higher than those reported in the report of the national monitoring program for fish feed in Norway, which ranged from 0.04 to 0.26 mg/kg ww[145].

In 2017, the EU Commission implemented regulation[88] (2017/893–24/05/2017)[165], allowing the utilization of processed animal protein (PAP) obtained from farmed insects in aquaculture feed. Currently, the types of insects that can be used for producing insect-based ingredients include the black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobious diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*), and field cricket (*Gryllus assimilis*)[165]. Among these, the black soldier fly, common housefly and yellow mealworm are considered the most significant species for producing insect ingredients for fish[8], poultry and pigs[166]. Presently, the aqua feed market accounts for around 50% of the animal feed in Europe derived from insects, and it is projected to increase to 75% by the year 2030[167].

3.2.4 Seaweed

The tAs concentration in seaweed samples is shown in Table 7, where the concentrations ranged from 8.16 ± 0.16 mg/kg ww in sample species *Caulerpa racemosa* to 14.37 mg/kg ww in sample species *Colpomenia sinuosa. Caulerpa racemosa* is edible green algae, while *Colpomenia sinuosa* belongs to brown algae.

It is generally observed that brown algae have higher As levels compared to red or green algae[103]. This pattern is also evident in the results of this study. The accumulation of heavy metals in seaweeds is dependent on factors such as their duration of growth in the sea and the concentration of heavy metals in the surrounding water[168,169]. The composition and levels of metals in seaweed vary based on the seaweed species, time of collection, growth phase, and collection location[169–171]. Previous studies have also shown that *Caulerpa racemosa* can accumulate high levels (8.85 ± 0.2 mg/kg) of As[109].

3.2.5 Hydrolyzed fish protein concentrate

Hydrolyzed fish protein concentrate (HFPC) is a valuable feed ingredient from unused fishery by-products. It stands out among the latest generation of feedstuffs due to its high protein content[68]. Figure 8 presents the results of tAs analysis in HFPC samples analyzed. Values are presented as average with their corresponding standard deviations (SD). The concentrations ranged from 1.14 ± 0.01 mg/kg ww (n=2, technical replicates) found in a sample containing whitefish (not specified fish species) from the West-coast of Norway to 19.5 ± 0.2 mg/kg ww in a sample containing a blend of herring (10.4%), cod (30.5%) and pollock (59%).

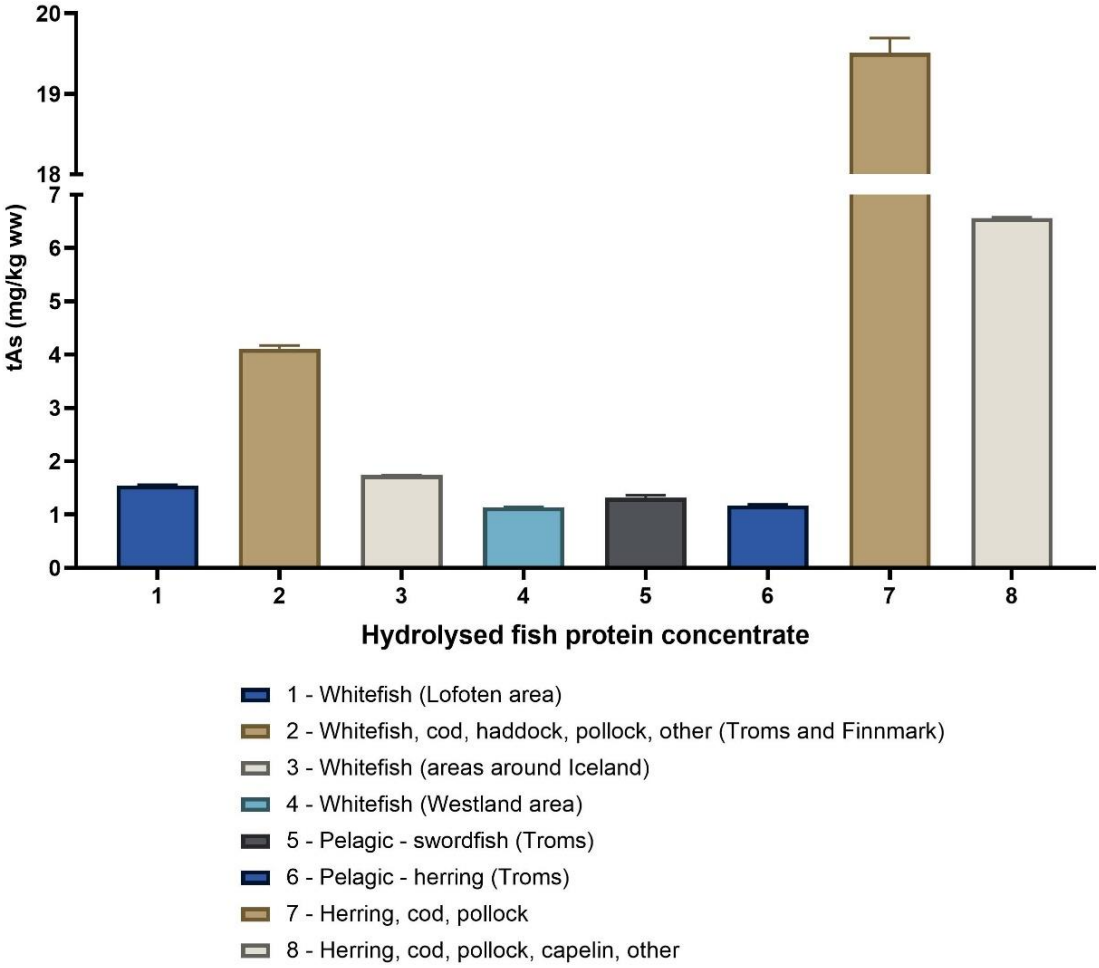

Figure 8. The average concentration of tAs in individual hydrolyzed fish protein concentrates (HFPC), with n=2 technical replicates, presented alongside their standard deviation (SD). Values are expressed in mg/kg wet weight (ww).

The obtained results in this study showed levels exceeding those previously documented in the national monitoring program for fish feed in Norway[151,152,172]. According to the most recent report from 2021[151], the tAs concentration in fish protein concentrates (FPC) samples ranged from 0.3 mg/kg ww to 4.5 mg/kg ww. Fish protein concentrate (FPC) is a concentrated protein product from fish, intended for human consumption, while HFPC is a fish waste-derived product with higher small peptide content due to hydrolysis, available in powder or liquid form[3]. The HFPCs are produced from fish and fish frames primarily from filleting plants[68]. A comprehensive analysis was conducted on fillet samples of various fish species, including Northeast Arctic cod (*Gadus morhua*), Norwegian spring spawning herring (*Clupea harengus*), mackerel (*Scomber scombrus*), Greenland halibut (*Reinhardtius hippoglossoides*), tusk (*Brosme brosme*), saithe (*Pollachius virens*), and Atlantic halibut (*Hippoglossus hippoglossus*),

sourced predominantly from open sea locations along the coast of Norway, with samples collected from 40 different positions, to determine the levels of tAs and iAs[173]. Northeast Arctic cod, Greenland halibut, Atlantic halibut, and tusk exhibited the highest concentrations of tAs among the studied species. The average concentrations of tAs in these species were 10.3 mg kg^{-1} ww, 11.2 mg kg^{-1} ww, 6.8 mg kg^{-1} ww, and 5.2 mg kg^{-1} ww, respectively. In contrast, Norwegian spring spawning herring, mackerel, and saithe displayed overall average concentrations of tAs below 3 mg kg^{-1} ww. Also, liver samples of Northeast Arctic cod were seen to contain high As levels[95,96]. These findings represent some of the highest recorded concentrations of As observed in marine organisms to date[174].

The selection of raw materials for HFPC is influenced by the geographical location of the processing plant[68]. In Europe, the predominant fish species utilized for HFPC manufacturing include cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), mackerel (*Scomber species*), pout (*Trisopterus luscus*), and whiting (*Merlangus merlangus*)[68]. The presence of As has also been detected in the fish protein hydrolysates (FPHs) and insoluble fractions[175]. The study by Liaset and Espe[175] showed that the cod FPH contained a high tAs concentration (37.6 mg/kg), while the cod insoluble fraction As level was lower, measuring 2.6 mg/kg. The variation in As content between different FPH could be attributed to the distinct tAs concentrations in fish species[176]. In this study, the tAs levels were seen to be highest for the samples containing herring, cod and pollock.

3.2.6 Tunicates

Tunicates, or sea squirts, are filter-feeding marine invertebrates in shallow ocean waters worldwide[177]. Recently, they have gained attention as a potential fish feed source, particularly for salmon feed[67]. In tunicates samples retrieved in this project, tAs concentrations ranged from 0.437 ± 0.003 mg/kg ww in samples harvested from Ytrevagen (Norway) in late March 2022 (Figure 9) to 1.74 ± 0.01 mg/kg ww in samples harvested from Varaldsøy (Norway) in late October 2021 (Figure 9).

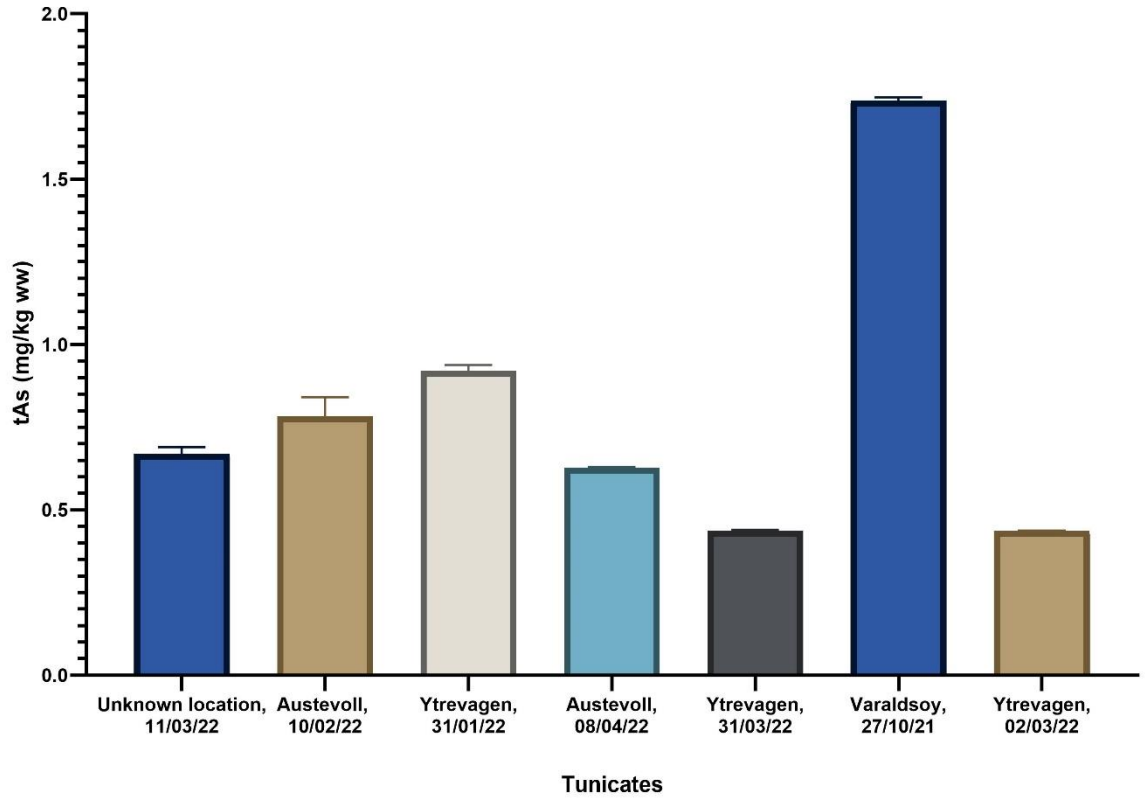

Figure 9. The average concentration of tAs in individual tunicates samples presented alongside their standard deviation (SD). Values are expressed in mg/kg wet weight (ww).

Given the exceptional water filtration capabilities of adult tunicates[178,179] there is a potential for the accumulation of pollutants, toxic microorganisms, and their byproducts[180]. This accumulation can result in unhealthy levels within the tunicates and may render their tissues toxic to predators, including humans[180–182]. Therefore, monitoring toxic components might be relevant in assessing tunicates' suitability as a fish feed ingredient. Certain species of tunicates can accumulate vanadium[183], a deterrent against predatory fish. Nevertheless, recent research has shown promising results when substituting 50% of dietary fish meal protein with tunicate meal at a dietary level of approximately 17%, even with the higher levels of vanadium[177]. This substitution reduced levels of contaminants, toxins, and heavy metals, such as toxaphene and As, in the overall composition of salmon[177].

Tunicates vary greatly in size[184]. Solitary adults of specific tunicate types can reach heights of 6-7 cm, while carnivorous species can grow as tall as 26 cm[184]. Table 4 provides detailed information on the precise length of tunicates samples analyzed in this study, including the number of individual tunicates, location of collection, and collection date. The samples were

collected throughout different seasons and at different locations. The study's findings indicate that the longest tunicates, specifically samples T3 and T6, showed the highest levels of tAs compared to other tunicate samples analyzed here (Figure 9).

3.3 Total As in experimental feed

3.3.1 Experimental feed containing fermented seaweed

The results for the experimental feed containing fermented seaweed samples analyzed (n=3) show tAs concentrations ranging in concentrations from 3.65 ± 0.02 mg/kg in the sample without seaweed inclusion to 4.41 ± 0.08 mg/kg in the sample with 2% of fermented seaweed (Figure 10). The results indicate that experimental feed samples containing seaweed had an increased tAs level compared to the control sample, containing no fermented seaweed. Samples with higher seaweed inclusion showed higher tAs concentrations.

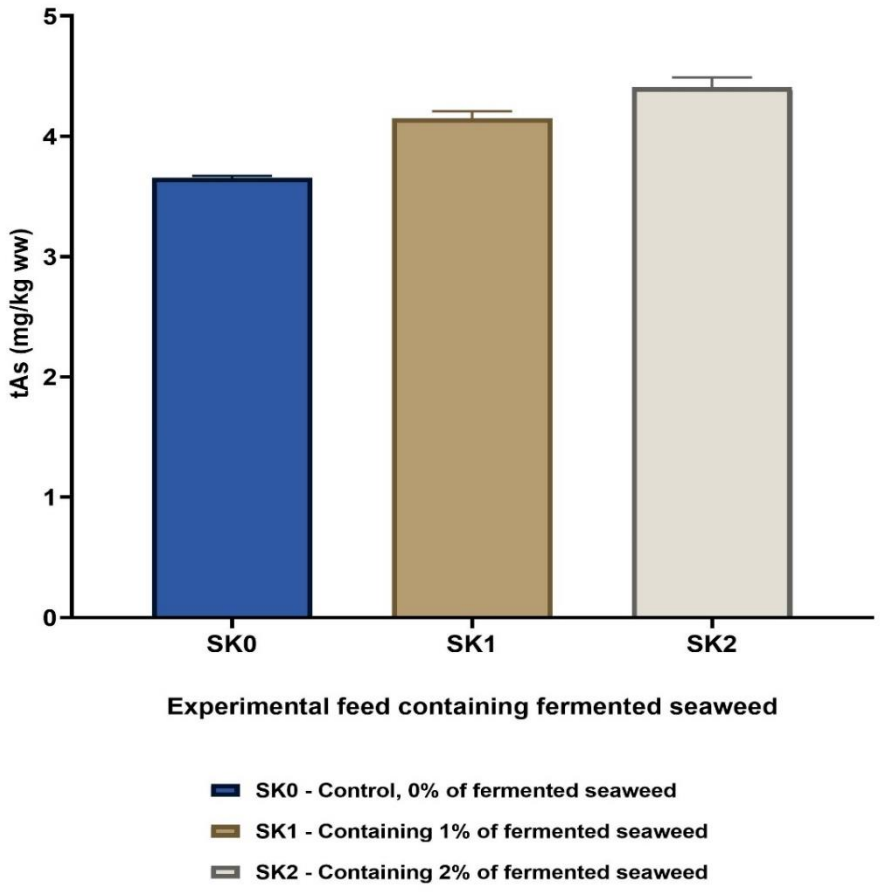

Figure 10. The average concentration of tAs in individual tunicates samples presented alongside their standard deviation (SD). Values are expressed in mg/kg wet weight (ww).

This aligns with a similar study investigating As speciation in diets containing up to 4% fermented kelp[185]. The utilization of feed ingredients derived from kelp or blue mussels resulted in elevated tAs concentrations in the experimental feeds, ranging from 3.4 mg/kg to 4.6 mg/kg ww[185]. However, it was found that less toxic arsenic forms were dominant species, accounting for 36% to 60% of tAs content[185]. This suggests that the choice of feed ingredients significantly influences both the tAs levels and the specific As species present in the feeds[185].

3.3.2 Experimental feed containing yeast

3.3.2.1 Piglet

Here, two samples of piglet feed containing yeast were analyzed (Figure 11). The sample with 14.6% inactivated *Cyberlindnera jadinii* yeast (replacing 40% of the protein ingredients in the control diet) had As concentration of 0.1129 ± 0.0003 mg/kg (n=2, technical replicates). Conversely, the sample with a mixture of wheat, barley, oats, soya bean meal, fish meal, potato protein concentrate, and rapeseed oil showed a concentration of 0.268 ± 0.005mg/kg (n=2, technical replicates). The elevated tAs value in the control sample could be linked to including fish meal and the tAs content in other utilized ingredients. In general, pig diets predominantly consist of vegetable sources, including cereals, soybean meal, sunflower and rapeseed meal, animal or plant fats, carbohydrates[58].

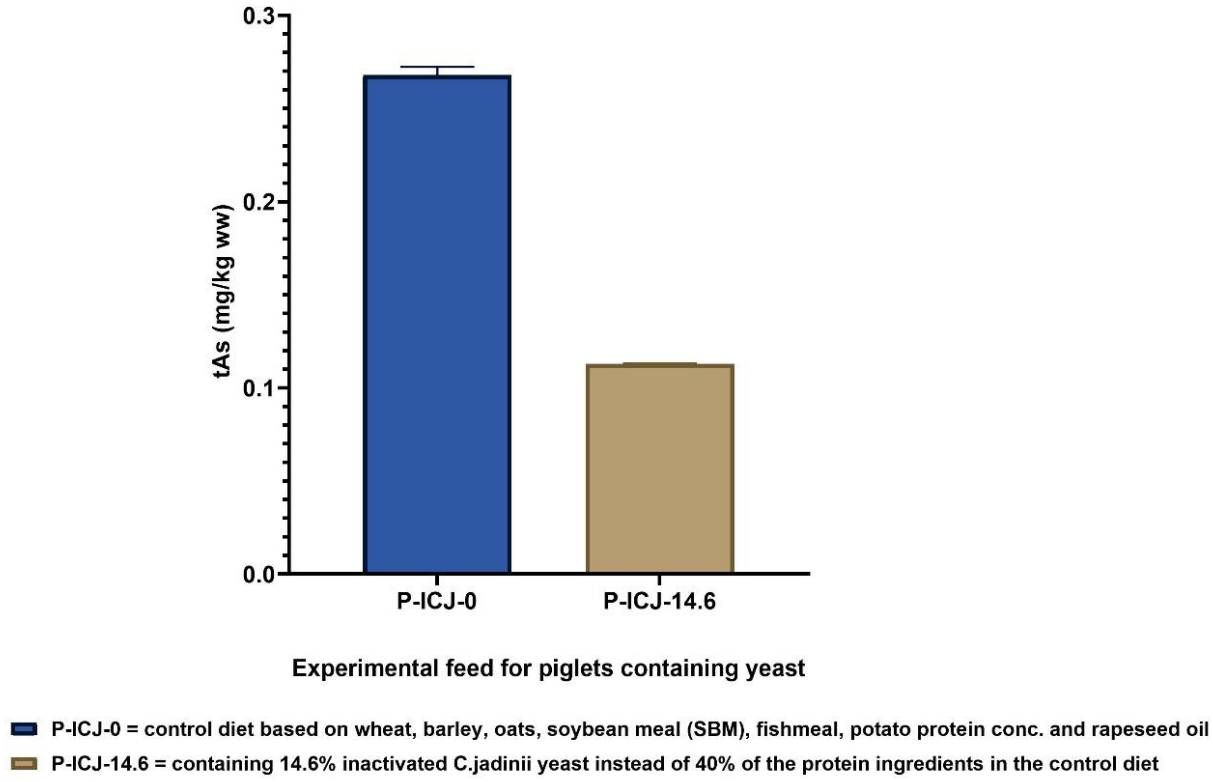

Figure 11. The average concentration of tAs in experimental feed for piglets containing yeast with corresponding standard deviation (SD). Values are expressed in mg/kg wet weight (ww).

3.3.2.2 Chicken

The results for the samples of experimental feed for chicken containing yeast analyzed (n=4) show tAs concentrations ranging in concentrations from 0.114 ± 0.008 mg/kg ww (n=2, technical replicates) in the sample with 30% of yeast to 0.15 ± 0.01 mg/kg ww (n=2, technical replicates) without yeast Cyberlindnera jadinii (Figure 12).

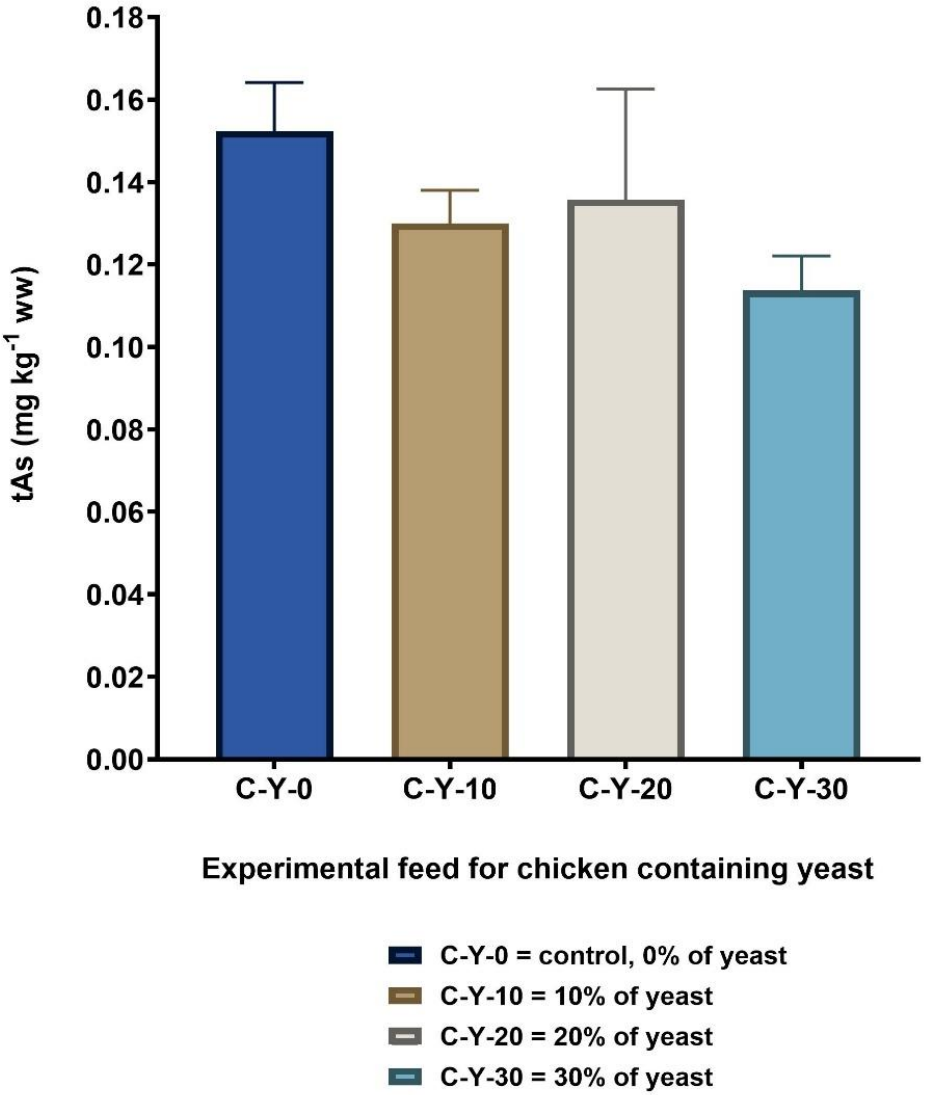

Figure 12. The average concentration of tAs in individual experimental feed for chicken containing yeast alongside their standard deviation (SD). Values are expressed in mg/kg wet weight (ww).

Throughout the production cycle, animals from various livestock species are fed different diets[58]. Poultry feeds are predominantly comprised of cereal grains, soybean meal, legumes animal by-product meals[186,187]. Yeast products and ingredients are commonly used as feed additives, improving animal growth performance and health[187,188]. However, limited information is available regarding the use of yeast as a protein source in animal feed[187]. Nutritional yeasts are valued for their high protein, amino acid, energy, and micronutrient content, making them a favorable alternative protein source to soybean meal and fish meal in poultry diets[188,189]. It was found that up to 10% *Cyberlindnera jadinii* crude protein can replace

soybean meal crude protein in broiler chicken diets without compromising growth and digestion. Still, higher levels (20% and 30%) may decrease bird performance[187].

According to our findings (Figure 12), the samples with yeast inclusion exhibited lower tAs levels than the control sample. The four treatments consisted of a wheat- and SBM-based diet (Control) and three diets in which 10 (CJ10), 20 (CJ20) and 30% (CJ30) of the crude protein were supplied by *Cyberlindnera jadinii* yeast, by gradually replacing SBM and other protein-rich ingredients.

3.3.2.3 Fish feed

The results for the samples of experimental fish feed containing yeast analyzed (n=6) show tAs concentrations ranging in concentrations from 2.45 ± 0.02 mg/kg (n=2, technical replicates) in the sample with 30% of soybean meal and 10% of inactivated *Cyberlindnera jadinii* to 4.81 ± 0.01 mg/kg (n=2, technical replicates) in the sample without soybean meal and yeast (Figure 13).

Figure 13 clearly indicates that the highest concentration of tAs was observed in the first fish feed (SBM-O Y-O). The second fish feed with the highest tAs concentration contained 30% soybean meal and 0% yeast (SBM-30 Y-0). The statistical difference in As concentration between these diets ($P<0.05$) is likely a result of the reduction in fish meal inclusion from approximately 433 g/kg to 208 g/kg.

On the other hand, the remaining diets (SBM 30 ICJ-10, SBM-30 ACJ-10, SBM-30 IWA-10, and SBM-30 AWA-10) exhibited similar concentrations of tAs. This observation suggests that the change in yeast species or type of treatment does not significantly influence the concentration of tAs in the feed.

The increasing demand for alternative protein feedstuffs has led to the consideration of using inactivated yeast *Cyberlindnera jadinii*, derived from sugars found in lignocellulosic biomass like the Norwegian spruce tree (*Picea abies*) as a potential feed ingredient[190]. *Cyberlindnera jadinii* is a potentially sustainable protein source with a lower carbon footprint than soy protein concentrate[191]. In recent studies, different yeast strains, such as *Cyberlindnera jadinii*, *Saccharomyces cerevisiae,* and *Kluyveromyces marxianus*, were assessed as substitutes for fish

meal in Atlantic salmon diets, with *Cyberlindnera jadinii*-based diets exhibiting superior growth performance attributed to their high crude protein content and digestibility[192].

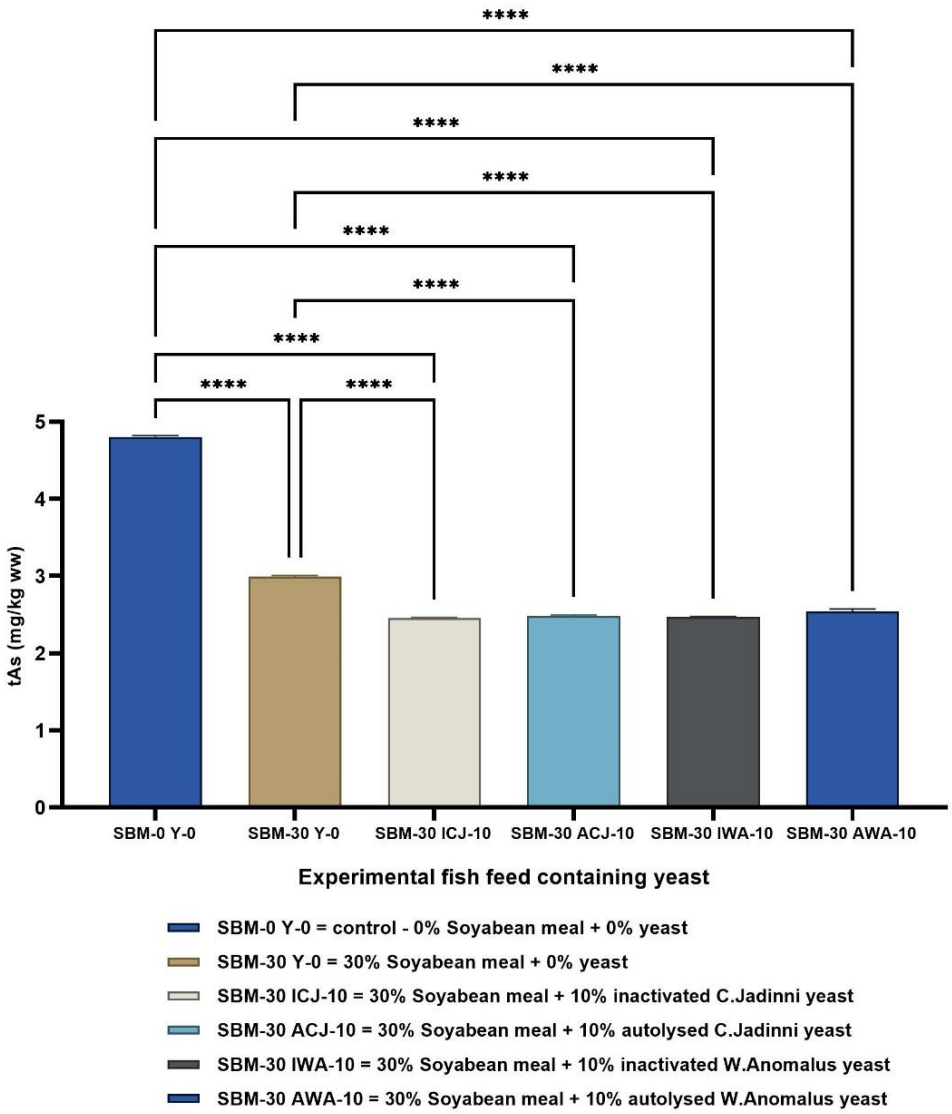

*Figure 13. Average concentration of tAs in individual experimental feed for salmon containing yeast alongside their standard deviation (SD). Values are expressed in mg/kg wet weight (ww). Statistical significance ($p < 0.0001$) indicated by **** according to Tukey's multiple comparisons test.*

Our findings (Figure 13) indicate that the samples with 30% soybean content and without yeast inclusion had lower tAs levels than the control sample. Additionally, all other samples

containing 30% soybean and 10% of either autolyzed or inactivated yeast showed lower tAs concentrations than those with 30% soybean and no yeast and the control sample.

3.4 Inorganic arsenic

Table 8 shows the median concentration with standard deviation, the concentration range (min-max) of iAs and the proportion of iAs to tAs (%) in land animal feed (n=5), plant meal (n=7), fish meal (n=4), microalgae (n=6), halophyte (n=3), insect meal (n=4), seaweed (n=1), fish protein concentrates (n=8), tunicates (n=7) and experimental feed containing seaweed (n=3).

Table 8. The results for iAs in traditional and new feed ingredients and experimental feed containing seaweed. The values are expressed in milligrams per kilogram (mg/kg) and include the median, minimum and maximum concentrations. The table provides the proportion of iAs compared to tAs (%) in the samples.

	Inorganic As						
Matrix	Median (mg/kg)	SD	Min	Max	n	n <LOQ	% iAs/tAs
Land animal feed	0.04	0.01	0.02	0.05	5	-	45.0 - 83.0
Plant meal	<LOQ[2]	<LOQ	<LOQ	<LOQ	7	7	*[3]
Fish meal	<LOQ	0.05	<LOQ	0.10	4	3	*
Fish oil[4]	-	-	-	-	-	-	-
Microalgae	0.02	0.03	<LOQ	0.08	6	2	up to 19.6
Halophyte	0.21	0.09	0.09	0.26	3	-	49.8 - 60.5
Insect meal	0.03	0.09	0.02	0.21	4	-	59.8 - 77.6
Seaweed	5.71	-	-	-	1	-	70.0
HFPC	0.017	0.025	0.005	0.082	8	-	0.3 - 2.2
Tunicates	1.91	12.1	0.7	33.6	7	-	6.7 - 78.2
Experimental feed containing seaweed	0.07	0.01	0.050	0.07	3	-	1.4 - 1.7

Some samples contained iAs below the limit of quantification (LOQ) for the method, which is set at 0.0073 mg/kg. The LOQ is the minimum concentration of a substance that can be measured with a given degree of uncertainty and varies depending on the sample type. Concentrations below the LOQ are denoted as "<LOQ". To include these values in median, average or sum calculations, concentrations below the LOQ were set to equal to the LOQ

[2] LOQ means concentrations below the limit of quantification (LOQ): LOQ=0.0073 mg/kg
[3] Not calculated due to values <LOQ
[4] Method limitation

concentration. This practice, called "upper bound summation," is the standard method for calculating the sum of dioxins[193,194]. In Table 8, the same principle is used for calculating median of iAs.

3.5 Inorganic As in commercial animal feed and feed ingredients

3.5.1 Land animal feed

In the land animal feed samples (n=5) the concentration of iAs ranged from 0.02 to 0.05 mg/kg, while the proportion of iAs to tAs ranged from 45.0% to 83.0% (Table 8).

This notable variation in the iAs to tAs ratio can potentially be elucidated by the composition of the land animal feed itself. The principal constituent of livestock feed is corn, constituting roughly 50% of the blend, followed by soybeans at 12%, and grains at 8%[195]. The predominant chemical form absorbed by the corn crop is As(V)[196,197], wherein the proportion of iAs has been found to range from 39% to 73% of the tAs concentration[196].

3.5.2 Plant meal samples

In the analyzed plant meal samples (n=7) the concentration of iAs was below the LOQ (Table 8). The uptake of metalloids by plants shows variation among different crops and cultivars [198]. In wheat plants, the lowest As accumulation occurs in the grains, while higher levels are observed in the leaf, stem, and roots[197,199]. In the bran or white flour extracts, only iAs species were detected, including both As(III) and As(V)[200]. Plant-based ingredients like peanut and soybean are considered non-hyperaccumulators, meaning their roots do not accumulate significant concentrations of metalloids like As[197]. In contrast, cereals such as maize and wheat are non-hyperaccumulator plants but have a higher tolerance to As[197]. Based on research conducted by Bianucci et al. (2020)[197], maize can withstand up to 30 m/L As, while wheat can tolerate up to 50 mg/L.

3.5.3 Fish meal

In the fish meal (n=4), the concentration of iAs was below the LOQ (<0.0073 mg/kg), as indicated in Table 8. These results align with a study conducted by Sloth et al.[15], which also analyzed fish meal samples (n=10) for iAs, and found that the concentration in all samples was below the detection limit (<0.007 mg/kg) of the method. Inorganic As, encompassing both

As(III) and As(V) forms[173,201], typically represents a minor fraction of tAs in marine organisms[51,202]. Usually, both fish meal and fish feed predominantly contain over 95% of the As in the form of organic As species, with low levels of iAs species[17,21]. The reason why iAs is so low in fish is the natural ability of most aquatic organisms to metabolize and excrete toxic iAs, whereas AsB is accumulated in fish muscle tissues [203].

3.6 Inorganic As in new feed ingredients

3.6.1 Microalgae

Among the lowest iAs concentrations were detected in microalgae (n=6), ranging from <LOQ to 0.08 mg/kg (Table 8). It was observed that the proportion of iAs to tAs was up to 20% in microalgae, including *Spirulina platensis*. In a previous study, *Spirulina platensis* cultured in Zarrouk's medium with varying concentrations of As(III) showed the ability to accumulate and methylate As[106]. In that study As(V) was the primary intracellular species, comprising 64% to 86% of tAs[106]. Other research on microalgae (*Diacronema lutheri*) exposed to As revealed that it could uptake and methylate As species, including AsSug, from seawater[204]. However, exposure to 10 µg/L As(V) led to increased iAs accumulation and reduced production of methylated As species in *Diacronema lutheri*[204]. This suggests the potential overwhelm of detoxification mechanisms due to the higher As exposure level[204].

3.6.2 Halophytes

Halophytes (n=3) ranged in iAs from 0.09 to 0.26 mg/kg (Table 8), with a high proportion of iAs to tAs ranging from 49% to 60% (Table 8). While halophytes are rich in nutrients and bioactive compounds[205–207], their high sodium content and potential heavy metal accumulation are concerns[208,209]. The European Commission requested in 2018[210] monitoring heavy metal and iodine levels in halophytes, seaweeds, and related products[159]. Exposure to iAs from halophytes and associated products was notable in adults (≥ 18 years to < 65 years old), ranging from 0.0039 to 0.0040 µg/kg body weight per day[159]. Information on halophytes is more limited compared to what is known about seaweeds[159].

Halophytes are the type of plants[162] and As uptake in plants varies with species, form, and environment[211]. As(V) mimicking phosphate enters roots[212], and As(III) is prevalent in reducing conditions, especially in flooded soils[213]. Arsenic uptake by plants occurs mainly as As(III), As(V), MMA, and DMA[212]. In a study, *Atriplex atacamensis* plants cultivated in As-

rich Chilean soil and water were exposed to As(V) and As(III) and exhibited distinct reactions to these As species[214]. As(III) was more toxic, hindering growth and impacting photosynthesis, with higher accumulation in the root compared to As(V)[214]. As(V) amassed in root cell walls, leaves demonstrated similar responses to both forms[214].

3.6.3 Insect meal

Insect meal samples (n=4) showed iAs concentrations ranging from 0.02 to 0.21 mg/kg (Table 8). The proportion of iAs to tAs was approximately 60% to 78% (Table 8). This is consistent with previous data on iAs in insect meal, where the proportion of iAs to tAs in insect meal accounted for 55% to 76% of the tAs content[215]. While the uptake of metals by insects is well-known[216,217], they have been successfully employed as bioindicators for environmental pollution and heavy metal contamination[217]. However, limited data is available on these elements in farmed insects, such as mealworm (*Tenebrio molitor*) and the black soldier fly (*Hermetia illucens*)[218–222].

In the EU, the insects intended for feed or food, are regulated as a farmed animal[223,224]. The European insect industry has been utilizing various plant-based feeding media for rearing the black soldier fly larvae, which include vegetable and fruit residues, wheat bran, grass and brewery by-products, hay, and plant powders[225]. Additionally, European insect producers have recently shown interest in exploring other media for rearing insect larvae[225]. Among these potential resources, marine macroalgae or seaweed are being considered[216]. Seaweeds are listed as authorized feed materials for food-producing animals[226] and could be used as substrates for insect rearing[216]. In a study conducted on farmed black soldier fly larvae grown on seaweed-enriched media, the concentrations of heavy metals (Cd, Pb, Hg) and As increased in the larvae with higher inclusion of seaweed in the feeding media[216]. The black soldier fly larvae accumulated these metals (both total and inorganic forms) from the seaweed-enriched media[216]. Notably, less than 1% of the tAs in both the seaweed-enriched media and the larvae grown on such media was in the inorganic form, indicating that the larvae do not efficiently convert organic forms of As to iAs[216].

3.6.4 Seaweed

The green algae *Caulerpa racemosa* (n=1) showed iAs concentration of 5.71 mg/kg as presented in Table 8, with a high proportion of iAs to tAs of 70%.

Seaweed exhibits comparatively higher concentrations of iAs when compared to other marine matrices[227]. Mainly because some seaweeds do not convert iAs into organic As, they store As in various chemical forms, including substantial amounts of the toxic As(III) and As(V)[203]. Different explanations have been proposed to account for this phenomenon, including the possibilities that these seaweeds lack the genetic capacity for the conversion process, that it is energetically impractical to carry out the metabolic transformation, and that the accumulation of iAs within their cellular structure serves as a defense mechanism against predators[100]. In the 21 species collected from Norwegian waters, representing the three groups of red, green, and brown macroalgae, the levels of iAs were generally low, with most samples containing less than 0.5 mg/kg dw or constituting less than 7% of the tAs content[228]. This finding indicates that the As present in these seaweeds predominantly exists in the form of organic compounds[228]. However, within certain species of brown algae, the proportion of iAs relative to the tAs concentration can range from 20% to 80%[203,228].

3.6.5 HPFC

As seen in Table 8, iAs in HPFC samples analyzed ranged from 0.005 to 0.082 mg/kg (n=8), with the proportion of iAs relative to tAs ranging from 0.3% to 2%. In a study involving seven marine fish species (Northeast Artic cod, herring, mackerel, Greenland halibut, tusk, saithe and Atlantic halibut), the highest concentrations of iAs recorded were 0.015 mg/kg ww in saithe (n=22) and 0.006 mg/kg ww in cod, mackerel, and tusk[173]. Most samples (94%) had concentrations below the LOQ[173], ranging from 0.002 to 0.004 mg/kg. In comparison, other studies such as Sirot et al. (2009)[229] reported higher concentrations of iAs (ranging from 0.013 to 0.024 mg/kg ww) in fish species like cod, halibut, mackerel, and saithe. The proportion of iAs in fish fillets in the study by Sirot et al. ranged from 0.1% to 3.1% of the tAs content[229]. Although seafood products generally have higher tAs levels than terrestrial products, most As in seafood is non-toxic AsB[173]. Typically, fish fillets contain a low proportion of iAs, often constituting less than 1% of tAs content[173]. Conversely, higher iAs ratios have been found in various shellfish species[15,230]. Additionally, as the tAs content increases, the proportion of iAs in fish fillets tends to decrease[173].

3.6.6 Tunicates

Within the newly examined feed ingredients, tunicates (n=7) showed the highest observed iAs levels. These concentrations of iAs ranged from 0.7 to 33.6 mg/kg, as outlined in Table 8. The proportion of iAs to tAs exhibited a wide range, spanning from 7% to 78%. Contribution of iAs to tAs can be seen in Figure 14. Most of the samples demonstrated low iAs levels, except for the tunicate sample harvested from Varaldsøy (Norway) in late October 2021, where the iAs concentration was remarkably high at 33.6 mg/kg (Figure 14).

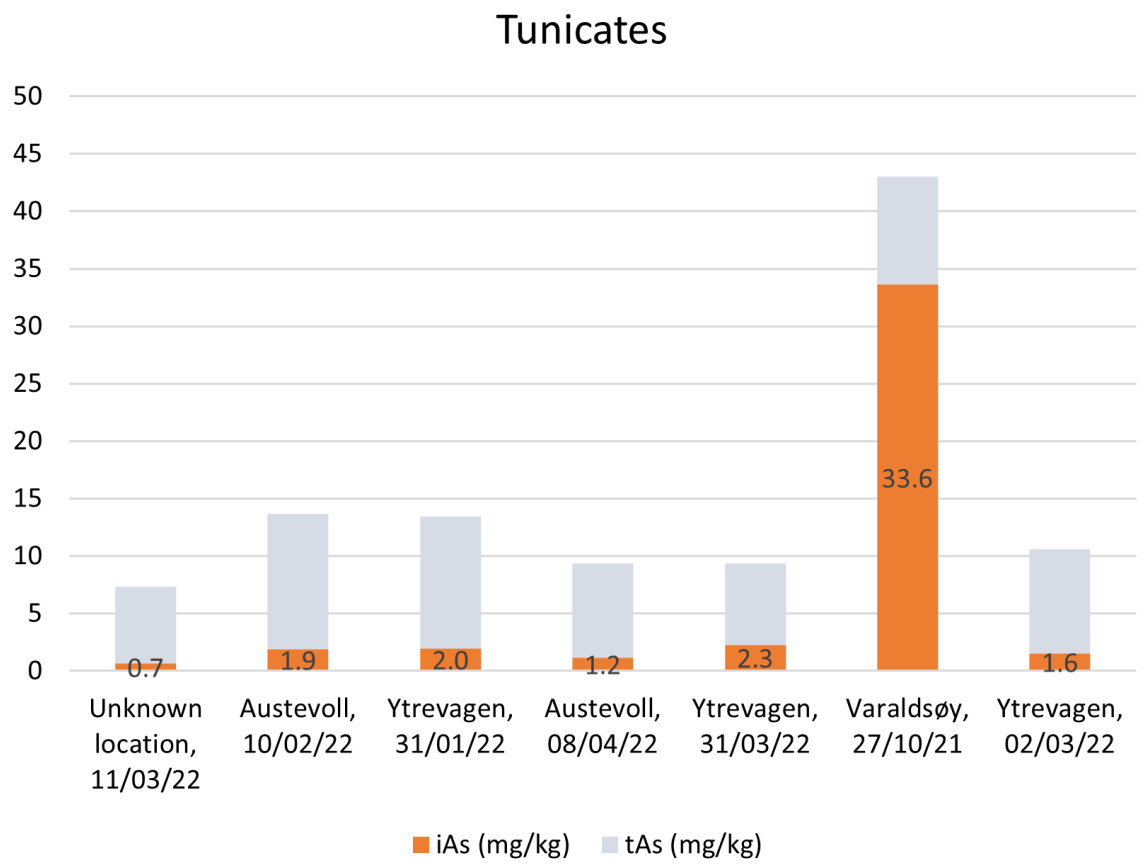

Figure 14. The proportion of iAs to tAs in individual tunicate samples harvested on different dates and locations is given in mg/kg.

The tunicate *Cionia intestinalis* has been found to efficiently accumulate iAs from seawater and convert it into mono-, di-, and trimethylated As[231]. In an exposure study, where tunicates were exposed to 1 ppm of iAs for five days, adults had the highest concentrations of total speciated As in their branchial sac (3705 mg/kg), followed by their heart (1019 mg/kg) and gastrointestinal tract (835 mg/kg)[231]. To our knowledge, there are few available data on the occurrence of iAs in tunicates in literature.

3.7 Inorganic As in experimental feed

3.7.1 Experimental feed containing fermented seaweed

In the experimental feeds with seaweed inclusion (n=3), the iAs concentrations ranged from 0.05 to 0.07 mg/kg (Table 8), compared to 0.05 mg/kg for the control sample. The proportion of iAs to tAs ranged from 1% to 2%, similar to that observed for land animal feed (Table 8). Promising findings have been observed in the utilization of seaweed species like *Ulva*, *Neopyropia/Porphyra/Pyropia*, *Gracilaria*, *Ascophyllum nodosum*, *Sargassum*, and *Padina* as fish feed ingredients[79,192,232]. The effectiveness seems to vary depending on the seaweed species, its inclusion rate, and the fish species for which the seaweed is used[79,192,232]. *Saccharina latissima* (known as kelp), a type of gigantic brown seaweed, has shown promise as a feed additive for rainbow trout (*Oncorhynchus mykiss*) at concentrations below 4%[233]. Seaweed cannot entirely replace typical animal feed[79]. The beneficial effects of seaweed in animal feed are generally observed at levels below 10% of the total concentration[79]. Beyond this threshold, negative effects have been demonstrated, with animals refusing to consume the feed provided[79].

3.8 Lipid-soluble arsenic/Arsenolipids

To estimate the concentration of arsenolipids (AsLipids), or lipid-bound As, the As content in the lipid fraction (mg/kg) of selected samples, including commercial fish feed, land animal feeds, fish meal, fish oil, microalgae, halophyte, insect meal, HFPC and tunicates were determined (Table 9). Additionally, the table shows insight into the proportion of tAs within the lipid fraction for each sample, expressed as a percentage (%).

Table 9. The results for lipid-bound As in traditional and new feed ingredients. The values are expressed in milligrams per kilogram (mg/kg) and include the median, standard deviation, minimum and maximum concentrations. The table provides the samples' proportion of tAs (%) in lipid fraction.

	Lipid bound As					
	Median (mg/kg)	SD	min	max	n	% of tAs in lipid fraction
Commercial fish feed	1.4	0.7	0.6	2.8	7	25 - 61
Land animal feed	0.003	0.002	0.001	0.004	2	3 - 9
Fish meal	1.5	0.3	1.3	1.8	4	12 - 48
Fish oil	6.2	4.1	0.1	13.2	9	84 - 94

Microalgae	0.04	0.08	0.03	0.2	3	20 - 45
Halophyte	0.006	0.003	0.004	0.008	2	1 - 2
Insect meal	0.005	0.002	0.005	0.007	3	2 - 12
HFPC	0.8	1.3	0.4	4.4	8	23 - 64
Tunicates	3.6	1.5	2.5	4.6	2	25 - 26

3.8.1 Lipid-bound As in commercial animal feed and feed ingredients

Commercial fish feeds (n=7) showed lipid-bound As concentrations ranging from 0.6 to 2.8 mg/kg (Table 9). In comparison, the land animal feed (n=2) showed a low content of lipid-bound As, ranging from 0.001 to 0.004 mg/kg (Table 9). The lipid fraction of commercial fish feed contained a higher proportion of tAs than land animal feed, with ratios of 25% to 61% and 3% to 9%, respectively (Table 9).

The higher lipid-bound As levels in fish feed were expected due to the inclusion of fish oil and fish meal as ingredients, both known to contain AsLipids[17,234]. These findings suggest that a significant portion of As in fish oils is lipid-bound. Fish oil is recognized for its relatively high As content, usually ranging from 0.2 to 16 mg/kg oil[17]. Moreover, it has been proposed that fatty fish might retain more AsLipids compared to leaner fish[235].

In the analysis of fish meal samples (n=4), the content of lipid-bound As ranged from 1.3 mg/kg to 1.8 mg/kg, as indicated in Table 9. Meanwhile, in fish oil samples (n=9), this range extended from 0.1 to 13.2 mg/kg, also specified in Table 9. The lipid fraction within the oils encompassed a substantial portion of tAs, varying from 84% to 94% (Table 9). In contrast, fish meal samples exhibited a range of lipid-bound tAs from 12% to 48% (Table 9).

Figure 15 shows a more detailed representation of lipid-bound tAs (mg/kg) contribution to tAs levels within fish meal and fish oil. Notably, as shown in Figure 15, nearly all tAs in fish oil samples were lipid-bound, while in fish meal samples, only approximately 1.5 mg/kg of tAs displayed lipid-bound characteristics.

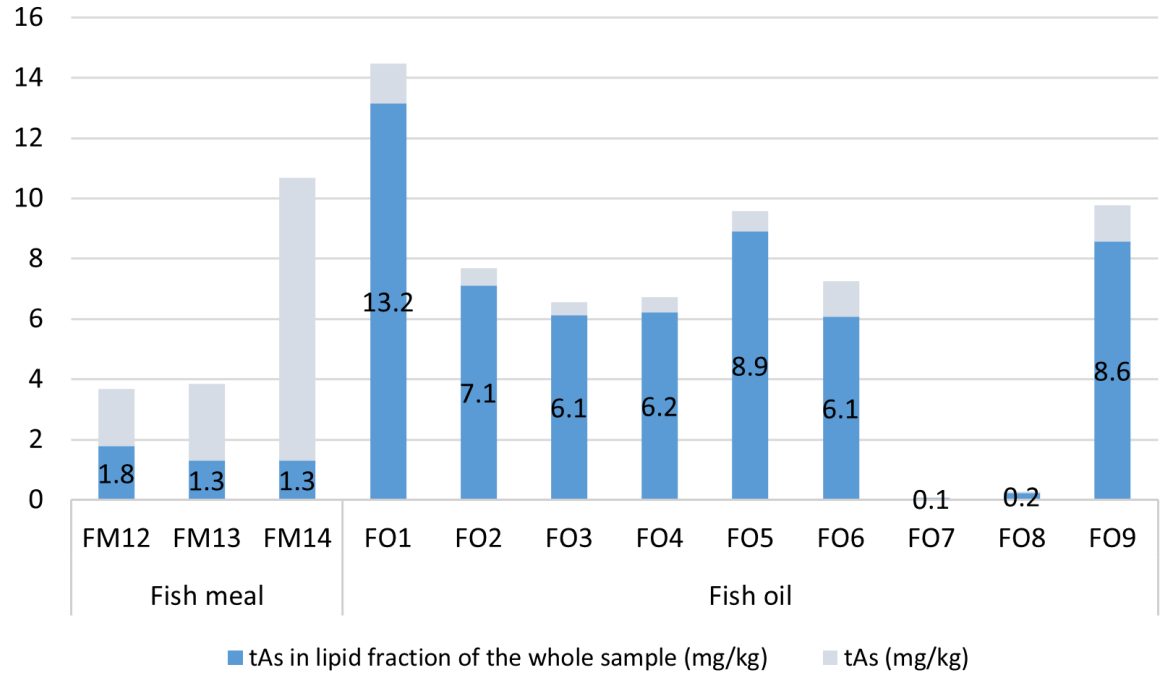

Figure 15. Contribution of the total As in lipid fraction to the whole As concentration of the sample in fish oils and fish meals. Values are expressed in mg/kg.

The As levels in the oils may depend on the fish species[17]. Furthermore, early studies indicated that the fish diet plays a role in the resulting As levels in the oils[235]. The fat fraction of marine fish organs, including livers, was rich in As, with concentrations ranging from 3 to 4.5 mg/kg oil, and sometimes even higher[17]. Lunde et al.[236] reported that oils obtained from marine fish and other marine organisms generally contain As levels ranging from 1 to 50 mg As/kg oil. In these organisms, lipid-soluble As compounds typically comprise around 10% to 30% of the tAs content[17,236].

Fish meal, which generally contains between 5% and 10% lipids, has been found to contain As levels ranging from 4.6 to 23.2 mg/kg oil[237]. The liver of a blue shark (*Prionace glaucus*) contained 60% of the tAs as lipid-soluble As[238]. Similarly, tuna fillets contained 87% of the tAs as lipid-soluble As[239], ringed seal blubber contained 90% of the tAs as lipid-soluble As[240] and cod liver samples contained between 25% and 77% of the tAs as lipid-soluble As[95].

These results show that certain samples of marine organisms have a predominance of lipid-soluble As.

3.8.2 Lipid-bound As in new feed ingredients

Lipid-bound As in microalgae (n=3) ranged from 0.03 to 0.2 mg/kg (Table 9) with a high proportion of tAs in lipid fraction ranging from 19.6% to 44.5%, whereas halophytes (n=2) showed lipid-bound As content ranging from 0.004 to 0.008 mg/kg (Table 9) with the low proportion of tAs in lipid fraction ranging from 1% to 2% (Table 9). Insect meal (n=3) showed lipid-bound As in the range of 0.005 to 0.007 mg/kg (Table 9), while lipid-bound As in hydrolyzed fish protein concentrates (HFPC) ranged from 0.4 to 4.4 mg/kg (n=8) presented in Table 9. Among the new feed ingredients, the highest levels of lipid-bound As were detected in tunicates (n=2), ranging from 2.5 to 4.6 mg/kg, with the proportion of tAs in lipid fraction ranging from 25% to 26%.

In algae, an unextracted As fraction potentially bound to compounds like lipids was found. This fraction could account for up to 50% of the total As content in algae[241]. Similar assumptions were made for insects. Approximately 13% of the overall As content in insect meal from black soldier fly larvae fed a diet containing 60% seaweed was not extracted[242]. This unextracted fraction possibly includes As-lipids, as the used As speciation method exclusively extracts water-soluble As species[242].

3.9 Feed safety

To address toxicity concerns, Directive 2002/32/EC[13], last updated for heavy metals by Regulation (EU) 2015/186[243], has set maximum limits for the presence of these substances in feed and feed materials[13]. Therefore, monitoring and mapping programs are carried out to obtain a situational picture of the feed area and identify potential risk factors to public health, animal health, and the environment. The Norwegian Food Safety Authority has carried out an annual program since 1996[145].

The commercially used fish feed and land animal samples belong to the category of "complete feed for fish and for animals" according to the EU regulations[13], with the ML for tAs concentration set at 10 mg/kg (Table 10). Plant meal belongs to the "feed materials" category,

with an ML of 2 mg/kg, while fish meal and fish oils fall under the category of "feed materials of fish, other aquatic animals, and products derived thereof," with an ML set at 25 mg/kg (Table 10). Regarding the new feed ingredient, microalgae samples were placed in the category of "feed materials of phosphates, calcareous marine algae" with an EU ML at 10 mg/kg, while halophytes are categorized as "feed materials" with an ML of 2 mg/kg (Table 10). Insect meal is also in the category of "feed materials" with an EU ML at 2 mg/kg. Seaweed samples are placed in the "feed materials of seaweed meal and feed materials derived from seaweed" category with an EU ML at 40 mg/kg (Table 10). Fish protein concentrates and tunicates are in the category of "feed materials of fish, other aquatic animals and products derived thereof," with an EU ML at 25 mg/kg (Table 10).

Experimental feed, including samples containing seaweed, experimental feed for piglets, chicken and fish containing yeast, are under "complete feed for fish and fur animals" with the ML for tAs concentration set at 10 mg/kg (Table 10).

Table 10. Mean total arsenic (tAs) values in mg/kg recalculated to a moisture content of 12% for commercial feed and feed ingredients (commercial and new). The table also categorizes new feed ingredients and provides maximum tAs levels (ML) set by Directive 2002/32/EC.

Matrix	Products intended for animal feed	Mean tAs (mg/kg dw) recalculated on 12% moisture content	ML in mg/kg relative to a feed with a moisture content of 12 %
Commercial fish feed	Complete feed for fish and fur animals	**2.99 ± 0.03**	**10**
Land animal feed	Complete feed for fish and fur animals	**0.048 ± 0.005**	**10**
Plant meal	Feed materials	**0.020 ± 0.002**	**2**
Fish meal	Feed materials of fish, other aquatic animals, and products derived thereof	**4.72 ± 0.06**	**25**
Microalgae	Feed materials of phosphates, calcareous marine algae	**0.25 ± 0.01**	**10**
Halophyte	Feed materials	**0.400 ± 0.004**	**2**
Insect meal	Feed materials	**0.109 ± 0.004**	**2**
Seaweed[5]	Feed materials of seaweed meal and feed materials derived from seaweed	**10.2 ± 3.6**	**40**
Fish protein conc.	Feed materials of fish, other aquatic animals, and products derived thereof	**12.3 ± 0.1**	**25**
Tunicates	Feed materials of fish, other aquatic animals, and products derived thereof	**14.3 ± 0.2**	**25**

[5] values given in mg/kg ww, not recalculated on 12% moisture content.

Experimental feed containing seaweed	Complete feed for fish and fur animals	**3.75 ± 0.05**	**10**
Experimental feed for piglets containing yeast	Complete feed for fish and fur animals	**0.19 ± 0.002**	**10**
Experimental feed for chicken containing yeast	Complete feed for fish and fur animals	**0.13 ± 0.01**	**10**
Experimental fish feed containing yeast	Complete feed for fish and fur animals	**2.91 ± 0.02**	**10**

In this study, most of the analyzed samples met the EU MLs for total arsenic (tAs) concentrations (Table 10). However, there were exceptions with some new feed ingredients, including one HFPC (Figure 17A, sample 7) and one tunicate sample (Figure 17B, sample 6), which exceeded the established limits for tAs.

There is a lack of recalculated data on 12% moisture content for seaweed samples due to the limited amount of available seaweed samples, but the value is given in mg/kg ww and it is below the EU's set limit.

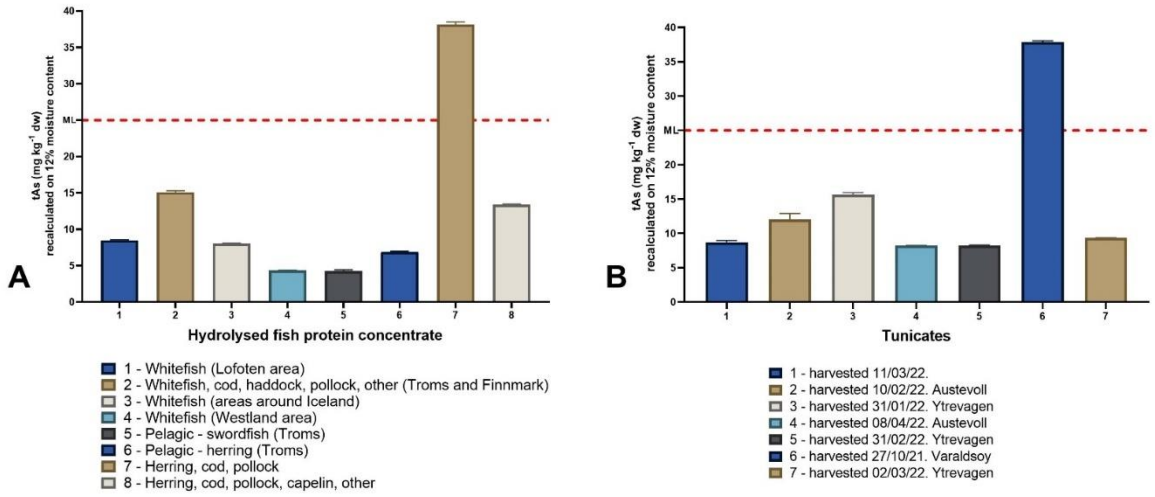

Figure 16. Recalculated tAs values at 12% moisture content for individual samples of hydrolyzed fish protein concentrate (A) and tunicates (B) with the EU-established maximum limit (ML). Values are expressed in mg/kg dry weight (dw).

Undesirable substances from both environmental sources and production processes can contaminate animal feed and its constituent materials[96]. These contaminants, when consumed by production animals, can subsequently transfer to animal-derived food products like liver, meat, and milk[96]. The commercial fish feed (n=21), land animal feed (n=10), and traditionally used feed ingredients like plant meal (n=15) and fish meal (n=14) analyzed in this work showed

tAs concentrations below the EU's MLs. Additionally, microalgae (n=6), halophyte (n=3), insect meal (n=4), seaweed (n=2) and experimental feeds containing seaweed (n=3), as well as experimental feed for piglets (n=2), chicken (n=4), and fish feed containing yeast (n=6), all met the EU's ML requirements.

For the category of "feed materials containing fish, other aquatic animals and products derived thereof" and "seaweed meal and feed materials derived from seaweed," there is a footnote in the regulations stating that the responsible operator must conduct an analysis upon request of competent authorities to demonstrate that the iAs content is below 2 ppm. The same rule applies to "complete feed for fish and fur animals".

3.10 Quality assurance of the results

3.10.1 Accuracy

For quality assurance of results, different CRMs were analyzed to control the accuracy of the methods. The analyzed concentrations for the CRMs included in this study aligned well with the certified values, as shown in Table 11. The results for all CRMs fell within two times the standard deviation of the certified values and the control charts established by the laboratory. There is currently a lack of CRMs for lipid-bound As species, which presents a challenge in accurately measuring and monitoring these specific forms of As in animal feed.

Table 11. Measured concentrations and certified values of certified reference materials (CRMs) for tAs and iAs (mean ± SD)

CRM	Sample type	Sample number	Analyte	Certified values	Measured concentration
SRM 1566b	Oyster tissue	3	tAs	7.65 ± 0.65 mg/kg dw	7.3 ± 0.3
TORT-3	Lobster hepatopancreas	3	tAs	59.5 ± 3.8 mg/kg dw	63.4 ± 2.4
ERM-BC211	Rice	6	iAs	124 ± 11 µg/kg	124 ± 3.79
BCR-627	Tuna fish tissue	6	iAs[6]	21 ± 3 µg/kg	19.02 ± 2.24

[6] Not certified for inorganic As, but an in-house established reference value.

3.10.2 Selectivity

The ICP-MS maintains a low and stable baseline. Chloride is recognized as a potential interference with As in ICP-MS, as chlorine (Cl) ions can combine with argon (Ar) ions to create $ArCl^-$. In multielement ICP-MS analysis, a collision gas is used to prevent this formation. However, for iAs, the chloride interference can be mitigated by monitoring Cl^- ions, a practice frequently used in this method.

For iAs, As(III) and As(V) are separated from other organic forms of As. Figure 18 shows a typical chromatogram for one of tunicate samples, showing a chromatographic broad peak eluting around and just after the void volume (r.t. 1.5 - 2 min), whereas iAs [As(III) and As(V)] in the sample elutes at approximately 5 min.

The organic As (e.g., AsB) species showed limited retention on an anion-exchange column.

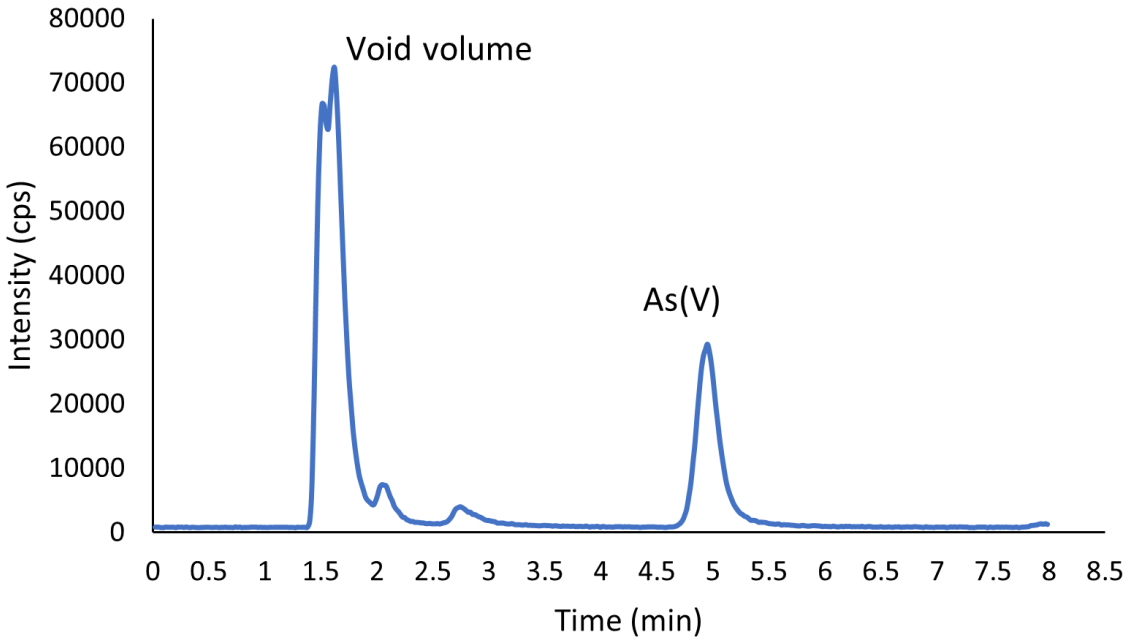

Figure 17. The anion exchange chromatogram of the tunicate sample shows the chromatographic separation of inorganic arsenic as As(V) from other arsenic compounds (AsB and other organoarsenicals), which exhibit low retention on the anion-exchange column (cps = counts s^{-1}) and they are primarily eluted in the void volume.

3.10.3 Source of errors

To ensure reliable results, it is important to address limitations and sources of uncertainty, including potential biases from sample preparation and analytical techniques. Fish feed

samples with high fat and protein content may have insufficient homogeneity, leading to significant variations in measured values. Reanalysis was performed when the deviations between replicates of samples exceeded 40% for tAs measurement and 10 or 20% (depending on the concentrations) for iAs. Differences in the composition of matrices can introduce errors due to the methods not being accredited for all sample types in this study. Additionally, biological factors like origin and harvesting season can also contribute to variability.

4 Conclusions

This study provides valuable data regarding tAs, iAs and lipid-bound As concentrations in various animal feeds, feed ingredients, and experimental feed formulations. The commercial animal feed and feed ingredients analysis revealed distinct patterns in tAs concentrations. Fish feed exhibited higher levels of tAs and lipid-bound As than land animal feed, attributed to marine ingredients such as fish meal and fish oil. Land animal feed showed high iAs concentrations, with proportions of iAs to total arsenic (tAs) varying between 45% and 83%. Fish meal showed comparable tAs concentrations to commercial fish feed, yet iAs levels were low, suggesting a predominant contribution of other As species to tAs. In contrast, tAs levels in fish oil samples were twice as high as those in commercial fish feed, with a significant proportion (84% to 94%) present in the lipid fraction. Plant meal samples indicate low tAs and iAs levels, aligning with prior findings.

One of the aims of this master project was to investigate As and As species in new feed ingredients, such as microalgae, halophytes, insect meal, seaweed, tunicates, and hydrolyzed fish protein concentrate (HFPC). These ingredients showed varying tAs levels, particularly evident in seaweed and tunicates. However, more data is required to gain a comprehensive understanding of the As species and a better overview of the contribution of iAs to tAs. The results from speciation analysis show that in seaweed, nearly 70% of tAs consisted of iAs, while in tunicates, this proportion ranged from 7% to 78%. Approximately 25% of tAs in tunicates was attributed to the lipid fraction. Microalgae demonstrated about 45% of tAs associated with lipids, and iAs remained below 20%. Inorganic As might be a significant fraction in some halophytes since its contribution in samples was up to 60% of tAs.

On the other hand, halophytes exhibited low tAs levels, suggesting that most tAs are water-soluble, as only 1% - 2% of tAs was identified in the lipid fraction. Insect meal showed low tAs. However, iAs contributed between 60% and 78% to tAs, highlighting the importance of monitoring iAs in feeds containing insect meal. The low concentration of iAs in HFPC suggests the prevalence of organic species, likely due to the incorporation of marine ingredients rich in less toxic organic forms. Although HFPC had tAs levels half that of commercial fish feed, they showed a significant tAs proportion in the lipid fraction ranging from 23% to 64%. Given the relatively high presence of lipid-bound As in HFPC, the unidentified As fraction will likely consist of organic water-soluble As.

The analysis of tAs in experimental feed containing fermented seaweed or yeast revealed that including specific ingredients could influence tAs levels. Although the inclusion of seaweed elevated tAs levels compared to the control sample (feed without seaweed), the experimental feeds containing seaweed showed an overall low proportion of iAs to tAs (1% - 2%). The substitution of some ingredients with yeast led to a reduction in the concentration of tAs. The feed with yeast inclusion was not assessed for iAs. The study shows the importance of considering the feed ingredients on both tAs and iAs levels.

The analyzed samples comply with EU maximum limits for tAs concentrations, except for some samples of HFPC and tunicates, which are considered as new feed ingredients. Furthermore, this study shows that elevated tAs levels do not always correlate with high concentrations of iAs, which is the As species of most concern regarding toxicity. For instance, in HFPC, tAs was elevated but low in iAs. This highlights the crucial role of speciation analysis.

Overall, this research enriches our understanding of tAs, iAs, and AsLipids (lipid-bound As) concentrations in various feed materials, benefiting feed manufacturers, regulatory bodies, and researchers striving for safe and high-quality animal feeds.

5 Future perspectives

While the current master project provides data on diverse feed and feed ingredients samples, it also opens new possibilities for further research. Expanding the scope of this study to include a more extensive and more varied set of samples would offer a comprehensive understanding of tAs and iAs concentrations across a broader range of animal feeds (e.g., shrimp feed, rabbit feed, sheep feed, etc.) and feed ingredients (e.g., blue mussel meal, krill, etc.). The data generated will benefit authorities dealing with risk assessment regarding feed safety.

Another possibility to expand the scope of this study to include more arsenic species (organoarsenic species). Presently, MLs are set for tA and iAs. Given recent studies emphasizing the toxicity of certain organoarsenic species (e.g., arsenosugars), future MLs might address these compounds. However, more data is needed. Implementing speciation analysis for all samples could yield insights into the distribution of specific arsenic species, allowing us to distinguish between different forms and their potential toxicities. This approach could also generate more particular data for lipid-bound As, moving beyond the collective "sum" presented in the current study. By incorporating a broader spectrum of samples and conducting thorough speciation analyses, trends and patterns in the presence of tAs and iAs across various feed materials can be identified. This analysis can encompass feed materials, considering factors such as seasonal and geographical variations and feed processing and handling. This, in turn, would enable the refinement of guidelines for including novel feed ingredients, ensuring compliance with safety standards and minimizing risks to both animal and human health. Furthermore, it's advisable to consider the inclusion of other undesirable substances in monitoring, such as metals, to ensure a comprehensive evaluation of feed safety and potential risks.

In summary, broadening the sample range and conducting comprehensive speciation for all samples would provide relevant data, facilitating more accurate risk assessments and safer feed formulation practices in the animal feed industry.

6 References

(1) FAO. How to Feed the World in 2050. Rome: High-Level Expert Forum. *2009*.

(2) *OECD-FAO Agricultural Outlook 2016-2025 | READ online*. oecd-ilibrary.org. https://read.oecd-ilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook-2016_agr_outlook-2016-en (accessed 2023-09-05).

(3) Saleh, N. E.; Wassef, E. A.; Abdel-Mohsen, H. H. Chapter Nine - Sustainable Fish and Seafood Production and Processing. In *Sustainable Fish Production and Processing*; Galanakis, C. M., Ed.; Academic Press, 2022; pp 259–291. https://doi.org/10.1016/B978-0-12-824296-4.00002-5.

(4) Mottet, A.; De Haan, C.; Falcucci, A.; Tempio, G.; Opio, C.; Gerber, P. Livestock: On Our Plates or Eating at Our Table? A New Analysis of the Feed/Food Debate. *Glob. Food Secur.* **2017**, *14*, 1–8. https://doi.org/10.1016/j.gfs.2017.01.001.

(5) Lubchenco, J.; Haugan, P. M. The Future of Food from the Sea. In *The Blue Compendium: From Knowledge to Action for a Sustainable Ocean Economy*; Lubchenco, J., Haugan, P. M., Eds.; Springer International Publishing: Cham, 2023; pp 1–13. https://doi.org/10.1007/978-3-031-16277-0_1.

(6) Caruso, G. Use of Plant Products as Candidate Fish Meal Substitutes: An Emerging Issue in Aquaculture Productions. *Fish. Aquac. J.* **2015**, *06* (03). https://doi.org/10.4172/2150-3508.1000e123.

(7) OECD. *Fish and Seafood*; OECD: Paris, 2016; pp 1–16. https://doi.org/10.1787/agr_outlook-2016-12-en.

(8) Sørensen, M.; Berge, G. M.; Thomassen, M.; Ruyter, B.; Hatlen, B.; Ytrestøyl, T.; Aas, T. S.; Åsgård, T. Today's and Tomorrow's Feed Ingredients in Norwegian Aquaculture.

(9) Daniel, N. A Review on Replacing Fish Meal in Aqua Feeds Using Plant Protein Sources. International Journal of Fisheries and Aquatic Studies. **2018**.

(10) Alfiko, Y.; Xie, D.; Astuti, R. T.; Wong, J.; Wang, L. Insects as a Feed Ingredient for Fish Culture: Status and Trends. *Aquac. Fish.* **2022**, *7* (2), 166–178. https://doi.org/10.1016/j.aaf.2021.10.004.

(11) Glencross, B. D.; Baily, J.; Berntssen, M. H. G.; Hardy, R.; MacKenzie, S.; Tocher, D. R. Risk Assessment of the Use of Alternative Animal and Plant Raw Material Resources in Aquaculture Feeds. *Rev. Aquac.* **2020**, *12* (2), 703–758. https://doi.org/10.1111/raq.12347.

(12) Tacon, A. G. J.; Metian, M. Aquaculture Feed and Food Safety. *Ann. N. Y. Acad. Sci.* **2008**, *1140* (1), 50–59. https://doi.org/10.1196/annals.1454.003.

(13) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on Undesirable Substances in Animal Feed - Council Statement. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002L0032-20191128&from=EN.

(14) Kapp, R. W. Arsenic. In *Encyclopedia of Toxicology*; Elsevier, 2014; pp 308–312. https://doi.org/10.1016/B978-0-12-386454-3.00817-4.

(15) Sloth, J. J.; Julshamn, K.; Lundebye, A.-K. Total Arsenic and Inorganic Arsenic Content in Norwegian Fish Feed Products. *Aquac. Nutr.* **2005**, *11* (1), 61–66. https://doi.org/10.1111/j.1365-2095.2004.00334.x.

(16) Tibon, J.; Silva, M.; Sloth, J. J.; Amlund, H.; Sele, V. Speciation Analysis of Organoarsenic Species in Marine Samples: Method Optimization Using Fractional Factorial Design and Method Validation. *Anal. Bioanal. Chem.* **2021**, *413* (15), 3909–3923. https://doi.org/10.1007/s00216-021-03341-4.

(17) Sele, V.; Sloth, J. J.; Lundebye, A.-K.; Larsen, E. H.; Berntssen, M. H. G.; Amlund, H. Arsenolipids in Marine Oils and Fats: A Review of Occurrence, Chemistry and Future Research Needs. *Food Chem.* **2012**, *133* (3), 618–630. https://doi.org/10.1016/j.foodchem.2012.02.004.

(18) Scientific Opinion on Arsenic in Food. *EFSA J.* **2009**, No. 10. https://doi.org/10.2903/j.efsa.2009.1351.

(19) Leermakers, M.; Baeyens, W.; De Gieter, M.; Smedts, B.; Meert, C.; De Bisschop, H. C.; Morabito, R.; Quevauviller, Ph. Toxic Arsenic Compounds in Environmental Samples: Speciation and Validation. *TrAC Trends Anal. Chem.* **2006**, *25* (1), 1–10. https://doi.org/10.1016/j.trac.2005.06.004.

(20) Xue, X.-M.; Xiong, C.; Yoshinaga, M.; Rosen, B.; Zhu, Y.-G. The Enigma of Environmental Organoarsenicals: Insights and Implications. *Crit. Rev. Environ. Sci. Technol.* **2022**, *52* (21), 3835–3862. https://doi.org/10.1080/10643389.2021.1947678.

(21) Authority (EFSA), E. F. S. Opinion of the Scientific Panel on Contaminants in the Food Chain [CONTAM] Related to Arsenic as Undesirable Substance in Animal Feed. *EFSA J.* **2005**, *3* (3), 180. https://doi.org/10.2903/j.efsa.2005.180.

(22) Hughes, M. F.; Beck, B. D.; Chen, Y.; Lewis, A. S.; Thomas, D. J. Arsenic Exposure and Toxicology: A Historical Perspective. *Toxicol. Sci. Off. J. Soc. Toxicol.* **2011**, *123* (2), 305–332. https://doi.org/10.1093/toxsci/kfr184.

(23) Tamaki, S.; Frankenberger, W. T. Environmental Biochemistry of Arsenic. In *Reviews of Environmental Contamination and Toxicology*; Ware, G. W., Ed.; Reviews of

Environmental Contamination and Toxicology; Springer New York: New York, NY, 1992; Vol. 124, pp 79–110. https://doi.org/10.1007/978-1-4612-2864-6_4.

(24) Scientific Opinion on Arsenic in Food. *EFSA J.* **2009**, No. 10. https://doi.org/10.2903/j.efsa.2009.1351.

(25) Arai, Y. Arsenic and Antimony. In *Trace Elements in Soils*; 2010; pp 381–407. https://doi.org/10.1002/9781444319477.ch16.

(26) Mandal, B. K.; Suzuki, K. T. Arsenic Round the World: A Review. *Talanta* **2002**, *58* (1), 201–235. https://doi.org/10.1016/S0039-9140(02)00268-0.

(27) Garelick, H.; Jones, H.; Dybowska, A.; Valsami-Jones, E. Arsenic Pollution Sources. In *Reviews of Environmental Contamination Volume 197*; Reviews of Environmental Contamination and Toxicology; Springer New York: New York, NY, 2009; Vol. 197, pp 17–60. https://doi.org/10.1007/978-0-387-79284-2_2.

(28) Signorelli, S. Arsenic in Volcanic Gases. *Environ. Geol.* **1997**, *32* (4), 239–244. https://doi.org/10.1007/s002540050212.

(29) Oakes, K. Membrane Filtration Processes for Removing Arsenic from Drinking Water. In *Impacts of Global Climate Change*; American Society of Civil Engineers: Anchorage, Alaska, United States, 2005; pp 1–7. https://doi.org/10.1061/40792(173)7.

(30) Templeton, D. M.; Ariese, F.; Cornelis, R.; Danielsson, L.-G.; Muntau, H.; Van Leeuwen, H. P.; Lobinski, R. Guidelines for Terms Related to Chemical Speciation and Fractionation of Elements. Definitions, Structural Aspects, and Methodological Approaches (IUPAC Recommendations 2000). *Pure Appl. Chem.* **2000**, *72* (8), 1453–1470. https://doi.org/10.1351/pac200072081453.

(31) Luvonga, C.; Rimmer, C. A.; Yu, L. L.; Lee, S. B. Organoarsenicals in Seafood: Occurrence, Dietary Exposure, Toxicity, and Risk Assessment Considerations – A Review. *J. Agric. Food Chem.* **2020**, *68* (4), 943–960. https://doi.org/10.1021/acs.jafc.9b07532.

(32) Sele, V. Method Development and Analysis of Arsenolipids in Marine Oils. Doctoral thesis, The University of Bergen, 2014. https://bora.uib.no/bora-xmlui/handle/1956/7983 (accessed 2023-08-27).

(33) Feldmann, J.; Krupp, E. M. Critical Review or Scientific Opinion Paper: Arsenosugars - a Class of Benign Arsenic Species or Justification for Developing Partly Speciated Arsenic Fractionation in Foodstuffs? *Anal. Bioanal. Chem.* **2011**, *399* (5), 1735–1741. https://doi.org/10.1007/s00216-010-4303-6.

(34) Reid, M. S.; Hoy, K. S.; Schofield, J. R. M.; Uppal, J. S.; Lin, Y.; Lu, X.; Peng, H.; Le, X. C. Arsenic Speciation Analysis: A Review with an Emphasis on Chromatographic

Separations. *TrAC Trends Anal. Chem.* **2020**, *123*, 115770. https://doi.org/10.1016/j.trac.2019.115770.

(35) Irgolic, K. J.; Goessler, W.; Kuehnelt, D. Arsenic Compounds in Terrestrial Biotaa. In *Arsenic Exposure and Health Effects III*; Chappell, W. R., Abernathy, C. O., Calderon, R. L., Eds.; Elsevier Science Ltd: Oxford, 1999; pp 61–68. https://doi.org/10.1016/B978-008043648-7/50009-1.

(36) Smedley, P. L.; Kinniburgh, D. G. A Review of the Source, Behaviour and Distribution of Arsenic in Natural Waters. *Appl. Geochem.* **2002**, *17* (5), 517–568. https://doi.org/10.1016/S0883-2927(02)00018-5.

(37) Edmonds, J. S.; Francesconi, K. A. Organoarsenic Compounds in the Marine Environment. In *Organometallic Compounds in the Environment*; John Wiley & Sons, Ltd, 2003; pp 195–222. https://doi.org/10.1002/0470867868.ch5.

(38) Francesconi, K. A.; Edmonds, J. S. Arsenic and Marine Organisms. In *Advances in Inorganic Chemistry*; Sykes, A. G., Ed.; Academic Press, 1996; Vol. 44, pp 147–189. https://doi.org/10.1016/S0898-8838(08)60130-0.

(39) Edmonds, J. S.; Francesconi, K. A. The Origin of Arsenobetaine in Marine Animals. *Appl. Organomet. Chem.* **1988**, *2* (4), 297–302. https://doi.org/10.1002/aoc.590020404.

(40) Krishnakumar, P. K.; Qurban, M. A.; Stiboller, M.; Nachman, K. E.; Joydas, T. V.; Manikandan, K. P.; Mushir, S. A.; Francesconi, K. A. Arsenic and Arsenic Species in Shellfish and Finfish from the Western Arabian Gulf and Consumer Health Risk Assessment. *Sci. Total Environ.* **2016**, *566–567*, 1235–1244. https://doi.org/10.1016/j.scitotenv.2016.05.180.

(41) Byrne, A.; Slejkovec, Z.; Stijve, T.; Fay, L.; Goessler, W.; Gailer, J.; Lrgolic, K. Arsenobetaine and Other Arsenic Species in Mushrooms. *Appl. Organomet. Chem.* **2004**, *9*, 305–313. https://doi.org/10.1002/aoc.590090403.

(42) Koch, I.; Wang, L.; Reimer, K.; Cullen, W. Arsenic Species in Terrestrial Fungi and Lichens from Yellowknife, NWT, Canada. *Appl. Organomet. Chem.* **2000**, *14*, 245–252. https://doi.org/10.1002/(SICI)1099-0739(200005)14:5<245::AID-AOC986>3.0.CO;2-K.

(43) Geiszinger, A.; Goessler, W.; Kuehnelt, D.; Francesconi, K.; Kosmus, W. Determination of Arsenic Compounds in Earthworms. *Environ. Sci. Technol.* **1998**, *32* (15), 2238–2243. https://doi.org/10.1021/es980018y.

(44) Dabeka, R. W.; McKenzie, A. D.; Lacroix, G. M.; Cleroux, C.; Bowe, S.; Graham, R. A.; Conacher, H. B.; Verdier, P. Survey of Arsenic in Total Diet Food Composites and Estimation of the Dietary Intake of Arsenic by Canadian Adults and Children. *J. AOAC Int.* **1993**, *76* (1), 14–25.

(45) Amlund, H.; Berntssen, M. H. G. Arsenobetaine in Atlantic Salmon (Salmo Salar L.): Influence of Seawater Adaptation. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2004**, *138* (4), 507–514. https://doi.org/10.1016/j.cca.2004.08.010.

(46) Popowich, A.; Zhang, Q.; Le, X. C. Arsenobetaine: The Ongoing Mystery. *Natl. Sci. Rev.* **2016**, *3* (4), 451–458. https://doi.org/10.1093/nsr/nww061.

(47) Amlund, H.; Francesconi, K. A.; Bethune, C.; Lundebye, A.-K.; Berntssen, M. H. G. Accumulation and Elimination of Dietary Arsenobetaine in Two Species of Fish, Atlantic Salmon (Salmo Salar L.) and Atlantic Cod (Gadus Morhua L.). *Environ. Toxicol. Chem.* **2006**, *25* (7), 1787–1794. https://doi.org/10.1897/05-107r1.1.

(48) Cullen, W. R. Chemical Mechanism of Arsenic Biomethylation. *Chem. Res. Toxicol.* **2014**, *27* (4), 457–461. https://doi.org/10.1021/tx400441h.

(49) Shibata, Y.; Morita, M. A Novel, Trimethylated Arseno-Sugar Isolated from the Brown Alga Sargassum Thunbergii. *Agric. Biol. Chem.* **1988**, *52* (4), 1087–1089. https://doi.org/10.1080/00021369.1988.10868781.

(50) Taylor, V.; Goodale, B.; Raab, A.; Schwerdtle, T.; Reimer, K.; Conklin, S.; Karagas, M. R.; Francesconi, K. A. Human Exposure to Organic Arsenic Species from Seafood. *Sci. Total Environ.* **2017**, *580*, 266–282. https://doi.org/10.1016/j.scitotenv.2016.12.113.

(51) Amlund, H.; Sele, V.; Sloth, J. J. Arsenic Exposure From Seafood Consumption☆. In *Encyclopedia of Environmental Health (Second Edition)*; Nriagu, J., Ed.; Elsevier: Oxford, 2019; pp 147–152. https://doi.org/10.1016/B978-0-12-409548-9.10929-7.

(52) Llorente-Mirandes, T.; Ruiz-Chancho, M. J.; Barbero, M.; Rubio, R.; López-Sánchez, J. F. Determination of Water-Soluble Arsenic Compounds in Commercial Edible Seaweed by LC-ICPMS. *J. Agric. Food Chem.* **2011**, *59* (24), 12963–12968. https://doi.org/10.1021/jf2040466.

(53) García-Salgado, S.; Quijano, M. A.; Bonilla, M. M. Arsenic Speciation in Edible Alga Samples by Microwave-Assisted Extraction and High Performance Liquid Chromatography Coupled to Atomic Fluorescence Spectrometry. *Anal. Chim. Acta* **2012**, *714*, 38–46. https://doi.org/10.1016/j.aca.2011.12.001.

(54) *Farm animals*. County Governor of Agder. https://www.statsforvalteren.no/en/Agder/Agriculture-and-food/Farming/Farm-animals/ (accessed 2023-07-30).

(55) *Fiskevelferd*. Institute of Marine Research. https://www.hi.no/en/hi/temasider/aquaculture/fish-welfare (accessed 2023-08-04).

(56) Osborg, G. A Path Tosustainable and Competitive Norwegian Protein Production for Aquaculture and Livestock Feed, 2022. https://nmbu.brage.unit.no/nmbu-xmlui/bitstream/handle/11250/3019205/osborg2022.pdf?sequence=1&isAllowed=y.

(57) Aas, T. S.; Åsgård, T.; Ytrestøyl, T. Utilization of Feed Resources in the Production of Atlantic Salmon (Salmo Salar) in Norway: An Update for 2020. *Aquac. Rep.* **2022**, *26*, 101316. https://doi.org/10.1016/j.aqrep.2022.101316.

(58) te Pas, M. F. W.; Veldkamp, T.; de Haas, Y.; Bannink, A.; Ellen, E. D. Adaptation of Livestock to New Diets Using Feed Components without Competition with Human Edible Protein Sources—A Review of the Possibilities and Recommendations. *Animals* **2021**, *11* (8), 2293. https://doi.org/10.3390/ani11082293.

(59) van der Spiegel, M.; Noordam, M. y.; van der Fels-Klerx, H. j. Safety of Novel Protein Sources (Insects, Microalgae, Seaweed, Duckweed, and Rapeseed) and Legislative Aspects for Their Application in Food and Feed Production. *Compr. Rev. Food Sci. Food Saf.* **2013**, *12* (6), 662–678. https://doi.org/10.1111/1541-4337.12032.

(60) Sehwag, S.; Das, M. A Brief Overview: Present Status on Utilization of Mustard Oil and Cake. **2015**, *14* (2).

(61) Nogales-Mérida, S.; Gobbi, P.; Józefiak, D.; Mazurkiewicz, J.; Dudek, K.; Rawski, M.; Kierończyk, B.; Józefiak, A. Insect Meals in Fish Nutrition. *Rev. Aquac.* **2019**, *11* (4), 1080–1103. https://doi.org/10.1111/raq.12281.

(62) *Insects as food and feed, a new emerging agricultural sector: a review*. https://doi.org/10.3920/JIFF2019.0017.

(63) Hua, K. A Meta-Analysis of the Effects of Replacing Fish Meals with Insect Meals on Growth Performance of Fish. *Aquaculture* **2021**, *530*, 735732. https://doi.org/10.1016/j.aquaculture.2020.735732.

(64) Liland, N. s.; Araujo, P.; Xu, X. x.; Lock, E.-J.; Radhakrishnan, G.; Prabhu, A. j. p.; Belghit, I. A Meta-Analysis on the Nutritional Value of Insects in Aquafeeds. *J. Insects Food Feed* **2021**, *7* (5), 743–759. https://doi.org/10.3920/JIFF2020.0147.

(65) Filgueira, R.; Strople, L. C.; Strohmeier, T.; Rastrick, S.; Strand, Ø. Mussels or Tunicates: That Is the Question. Evaluating Efficient and Sustainable Resource Use by Low-Trophic Species in Aquaculture Settings. *J. Clean. Prod.* **2019**, *231*, 132–143. https://doi.org/10.1016/j.jclepro.2019.05.173.

(66) Troedsson, C.; Thompson, E.; Bouquet, J.-M.; Magnesen, T.; Schander, C.; Li, J. Tunicate Extract for Use in Animal Feeds. WO2013088177A1, June 20, 2013. https://patents.google.com/patent/WO2013088177A1/en (accessed 2023-08-20).

(67) Samuelsen, T. A.; Haustveit, G.; Kousoulaki, K. The Use of Tunicate (Ciona Intestinalis) as a Sustainable Protein Source in Fish Feed – Effects on the Extrusion Process, Physical Pellet Quality and Microstructure. *Anim. Feed Sci. Technol.* **2022**, *284*, 115193. https://doi.org/10.1016/j.anifeedsci.2021.115193.

(68) Hertrampf, J. W.; Piedad-Pascual, F. Fish Protein Concentrate (Hydrolysed). In *Handbook on Ingredients for Aquaculture Feeds*; Hertrampf, J. W., Piedad-Pascual, F., Eds.; Springer Netherlands: Dordrecht, 2000; pp 192–197. https://doi.org/10.1007/978-94-011-4018-8_19.

(69) Blanco, M.; Vázquez, J. A.; Pérez-Martín, R. I.; Sotelo, C. G. Hydrolysates of Fish Skin Collagen: An Opportunity for Valorizing Fish Industry Byproducts. *Mar. Drugs* **2017**, *15* (5), 131. https://doi.org/10.3390/md15050131.

(70) Zhang, L.; Zhao, G.-X.; Zhao, Y.-Q.; Qiu, Y.-T.; Chi, C.-F.; Wang, B. Identification and Active Evaluation of Antioxidant Peptides from Protein Hydrolysates of Skipjack Tuna (Katsuwonus Pelamis) Head. *Antioxidants* **2019**, *8* (8), 318. https://doi.org/10.3390/antiox8080318.

(71) Villamil, O.; Váquiro, H.; Solanilla, J. F. Fish Viscera Protein Hydrolysates: Production, Potential Applications and Functional and Bioactive Properties. *Food Chem.* **2017**, *224*, 160–171. https://doi.org/10.1016/j.foodchem.2016.12.057.

(72) Ho, T. C. W.; Li-Chan, E. C. Y.; Skura, B. J.; Higgs, D. A.; Dosanjh, B. Pacific Hake (Merluccius Productus Ayres, 1855) Hydrolysates as Feed Attractants for Juvenile Chinook Salmon (Oncorhynchus Tshawytscha Walbaum, 1792). *Aquac. Res.* **2014**, *45* (7), 1140–1152. https://doi.org/10.1111/are.12056.

(73) Siddik, M. A. B.; Howieson, J.; Fotedar, R.; Partridge, G. J. Enzymatic Fish Protein Hydrolysates in Finfish Aquaculture: A Review. *Rev. Aquac.* **2021**, *13* (1), 406–430. https://doi.org/10.1111/raq.12481.

(74) Ospina-Salazar, G. H.; Ríos-Durán, M. G.; Toledo-Cuevas, E. M.; Martínez-Palacios, C. A. The Effects of Fish Hydrolysate and Soy Protein Isolate on the Growth Performance, Body Composition and Digestibility of Juvenile Pike Silverside, Chirostoma Estor. *Anim. Feed Sci. Technol.* **2016**, *220*, 168–179. https://doi.org/10.1016/j.anifeedsci.2016.08.011.

(75) Yaghoubzadeh, Z.; Peyravii Ghadikolaii, F.; Kaboosi, H.; Safari, R.; Fattahi, E. Antioxidant Activity and Anticancer Effect of Bioactive Peptides from Rainbow Trout (Oncorhynchus Mykiss) Skin Hydrolysate. *Int. J. Pept. Res. Ther.* **2020**, *26* (1), 625–632. https://doi.org/10.1007/s10989-019-09869-5.

(76) Wei, Y.; Liang, M.; Mu, Y.; Zheng, K.; Xu, H. The Effect of Ultrafiltered Fish Protein Hydrolysate Level on Growth Performance, Protein Digestibility and mRNA Expression of PepT1 in Juvenile Turbot (Scophthalmus Maximus L.). *Aquac. Nutr.* **2016**, *22* (5), 1006–1017. https://doi.org/10.1111/anu.12319.

(77) Lovell, R. T. 13 - Diet and Fish Husbandry. In *Fish Nutrition (Third Edition)*; Halver, J. E., Hardy, R. W., Eds.; Academic Press: San Diego, 2003; pp 703–754. https://doi.org/10.1016/B978-012319652-1/50014-8.

(78) Borquez, A.; Serrano, E.; Dantagnan, P.; Carrasco, J.; Hernandez, A. Feeding High Inclusion of Whole Grain White Lupin (Lupinus Albus) to Rainbow Trout (Oncorhynchus Mykiss): Effects on Growth, Nutrient Digestibility, Liver and Intestine Histology and Muscle Fatty Acid Composition. *Aquac. Res.* **2011**, *42* (8), 1067–1078. https://doi.org/10.1111/j.1365-2109.2010.02690.x.

(79) Morais, T.; Inácio, A.; Coutinho, T.; Ministro, M.; Cotas, J.; Pereira, L.; Bahcevandziev, K. Seaweed Potential in the Animal Feed: A Review. *J. Mar. Sci. Eng.* **2020**, *8* (8), 559. https://doi.org/10.3390/jmse8080559.

(80) Sapkota, A. R.; Lefferts, L. Y.; McKenzie, S.; Walker, P. What Do We Feed to Food-Production Animals? A Review of Animal Feed Ingredients and Their Potential Impacts on Human Health. *Environ. Health Perspect.* **2007**, *115* (5), 663–670. https://doi.org/10.1289/ehp.9760.

(81) Liu, X.; Zhang, W.; Hu, Y.; Cheng, H. Extraction and Detection of Organoarsenic Feed Additives and Common Arsenic Species in Environmental Matrices by HPLC–ICP-MS. *Microchem. J.* **2013**, *108*, 38–45. https://doi.org/10.1016/j.microc.2012.12.005.

(82) Hu, Y.; Cheng, H.; Tao, S.; Schnoor, J. L. China's Ban on Phenylarsonic Feed Additives, A Major Step toward Reducing the Human and Ecosystem Health Risk from Arsenic. *Environ. Sci. Technol.* **2019**, *53* (21), 12177–12187. https://doi.org/10.1021/acs.est.9b04296.

(83) Fisher, D. J.; Yonkos, L. T.; Staver, K. W. Environmental Concerns of Roxarsone in Broiler Poultry Feed and Litter in Maryland, USA. *Environ. Sci. Technol.* **2015**, *49* (4), 1999–2012. https://doi.org/10.1021/es504520w.

(84) Tang, R.; Wang, Y.; Yuan, S.; Wang, W.; Yue, Z.; Zhan, X.; Hu, Z.-H. Organoarsenic Feed Additives in Biological Wastewater Treatment Processes: Removal, Biotransformation, and Associated Impacts. *J. Hazard. Mater.* **2021**, *406*, 124789. https://doi.org/10.1016/j.jhazmat.2020.124789.

(85) López-Alonso, M. 9 - Animal Feed Contamination by Toxic Metals. In *Animal Feed Contamination*; Fink-Gremmels, J., Ed.; Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead Publishing, 2012; pp 183–204. https://doi.org/10.1533/9780857093615.2.183.

(86) Altmann, B. A.; Rosenau, S. Spirulina as Animal Feed: Opportunities and Challenges. *Foods* **2022**, *11* (7), 965. https://doi.org/10.3390/foods11070965.

(87) Ahmad, M. T.; Shariff, M.; Md. Yusoff, F.; Goh, Y. M.; Banerjee, S. Applications of Microalga Chlorella Vulgaris in Aquaculture. *Rev. Aquac.* **2020**, *12* (1), 328–346. https://doi.org/10.1111/raq.12320.

(88) Coelho, D.; Pestana, J.; Almeida, J. M.; Alfaia, C. M.; Fontes, C. M. G. A.; Moreira, O.; Prates, J. A. M. A High Dietary Incorporation Level of Chlorella Vulgaris Improves the Nutritional Value of Pork Fat without Impairing the Performance of Finishing Pigs. *Anim. Open Access J. MDPI* **2020**, *10* (12), 2384. https://doi.org/10.3390/ani10122384.

(89) ATSDR. Toxicological Profile: Arsenic. **2007**.

(90) European Food Safety Authority (EFSA); Arcella, D.; Cascio, C.; Gómez Ruiz, J. Á. Chronic Dietary Exposure to Inorganic Arsenic. *EFSA J.* **2021**, *19* (1). https://doi.org/10.2903/j.efsa.2021.6380.

(91) European Food Safety Authority. Dietary Exposure to Inorganic Arsenic in the European Population. *EFSA J.* **2014**, *12* (3). https://doi.org/10.2903/j.efsa.2014.3597.

(92) *A Review of Human Carcinogens*; Centre international de recherche sur le cancer, Ed.; IARC monographs on the evaluation of carcinogenic risks to humans; International agency for research on cancer: Lyon, 2012.

(93) Raml, R.; Goessler, W.; Traar, P.; Ochi, T.; Francesconi, K. A. Novel Thioarsenic Metabolites in Human Urine after Ingestion of an Arsenosugar, 2‘,3‘-Dihydroxypropyl 5-Deoxy-5-Dimethylarsinoyl-β-d-Riboside. *Chem. Res. Toxicol.* **2005**, *18* (9), 1444–1450. https://doi.org/10.1021/tx050111h.

(94) Müller, S. M.; Ebert, F.; Raber, G.; Meyer, S.; Bornhorst, J.; Hüwel, S.; Galla, H.-J.; Francesconi, K. A.; Schwerdtle, T. Effects of Arsenolipids on in Vitro Blood-Brain Barrier Model. *Arch. Toxicol.* **2018**, *92* (2), 823–832. https://doi.org/10.1007/s00204-017-2085-8.

(95) Schmeisser, E.; Goessler, W.; Francesconi, K. A. Human Metabolism of Arsenolipids Present in Cod Liver. *Anal. Bioanal. Chem.* **2006**, *385* (2), 367–376. https://doi.org/10.1007/s00216-006-0401-x.

(96) Adamse, P.; Fels-Klerx, H. J. van der; Jong, J. de. Arsenic, Lead, Cadmium and Mercury in Animal Feed and Feed Materials: Trend Analysis of Monitoring Result Collected in the Netherlands. **2017**. https://doi.org/10.18174/416680.

(97) Pizarro, I.; Gomez, M. M.; Cámara, C.; Palacios, M. A. Distribution of Arsenic Species in Environmental Samples Collected in Northern Chile. *Int. J. Environ. Anal. Chem.* **2003**, *83* (10), 879–890. https://doi.org/10.1080/0306731031000160330.

(98) Reis, V. A. T.; Duarte, A. C. Analytical Methodologies for Arsenic Speciation in Macroalgae: A Critical Review. *TrAC Trends Anal. Chem.* **2018**, *102*, 170–184. https://doi.org/10.1016/j.trac.2018.02.003.

(99) Wolle, M. M.; Todorov, T. I.; Conklin, S. D. Arsenic Species in Seaweeds Commercially Available in the United States. *ACS Food Sci. Technol.* **2021**, *1* (4), 511–523. https://doi.org/10.1021/acsfoodscitech.0c00120.

(100) Castlehouse, H.; Smith, C.; Raab, A.; Deacon, C.; Meharg, A. A.; Feldmann, J. Biotransformation and Accumulation of Arsenic in Soil Amended with Seaweed. *Environ. Sci. Technol.* **2003**, *37* (5), 951–957. https://doi.org/10.1021/es026110i.

(101) Makkar, H. P. S.; Tran, G.; Heuzé, V.; Giger-Reverdin, S.; Lessire, M.; Lebas, F.; Ankers, P. Seaweeds for Livestock Diets: A Review. *Anim. Feed Sci. Technol.* **2016**, *212*, 1–17. https://doi.org/10.1016/j.anifeedsci.2015.09.018.

(102) García-Salgado, S.; Raber, G.; Raml, R.; Magnes, C.; Francesconi, K. A.; García-Salgado, S.; Raber, G.; Raml, R.; Magnes, C.; Francesconi, K. A. Arsenosugar Phospholipids and Arsenic Hydrocarbons in Two Species of Brown Macroalgae. *Environ. Chem.* **2012**, *9* (1), 63–66. https://doi.org/10.1071/EN11164.

(103) Almela, C.; Algora, S.; Benito, V.; Clemente, M. J.; Devesa, V.; Súñer, M. A.; Vélez, D.; Montoro, R. Heavy Metal, Total Arsenic, and Inorganic Arsenic Contents of Algae Food Products. *J. Agric. Food Chem.* **2002**, *50* (4), 918–923. https://doi.org/10.1021/jf0110250.

(104) Llorente-Mirandes, T.; Ruiz-Chancho, M. J.; Barbero, M.; Rubio, R.; López-Sánchez, J. F. Measurement of Arsenic Compounds in Littoral Zone Algae from the Western Mediterranean Sea. Occurrence of Arsenobetaine. *Chemosphere* **2010**, *81* (7), 867–875. https://doi.org/10.1016/j.chemosphere.2010.08.007.

(105) Mac Monagail, M.; Morrison, L. Chapter Nine - Arsenic Speciation in a Variety of Seaweeds and Associated Food Products. In *Comprehensive Analytical Chemistry*; Duarte, A. C., Reis, V., Eds.; Arsenic Speciation in Algae; Elsevier, 2019; Vol. 85, pp 267–310. https://doi.org/10.1016/bs.coac.2019.03.005.

(106) Guo, Y.; Xue, X.; Yan, Y.; Zhu, Y.; Yang, G.; Ye, J. Arsenic Methylation by an Arsenite S-Adenosylmethionine Methyltransferase from Spirulina Platensis. *J. Environ. Sci.* **2016**, *49*, 162–168. https://doi.org/10.1016/j.jes.2016.06.013.

(107) Spherix Consulting Group, Inc. GRAS Notification for Chlorella Powder in Selected Conventional Food, GRAS Notice (GRN) No. 986 with Amendments. https://www.fda.gov/media/156121/download.

(108) Tang, G.; Suter, P. M. Vitamin A, Nutrition, and Health Values of Algae: Spirulina, Chlorella, and Dunaliella. *J. Pharm. Nutr. Sci.* **2011**, *1* (2), 111–118. https://doi.org/10.6000/1927-5951.2011.01.02.04.

(109) MISHEER, N.; KINDNESS, A.; JONNALAGADDA, S. B. Seaweeds Along KwaZulu-Natal Coast of South Africa—4: Elemental Uptake by Edible Seaweed Caulerpa Racemosa (Sea Grapes) and the Arsenic Speciation. *J. Environ. Sci. Health Part A* **2006**, *41* (7), 1217–1233. https://doi.org/10.1080/10934520600656489.

(110) Pedlar, R. M.; Ptashynski, M. D.; Evans, R.; Klaverkamp, J. F. Toxicological Effects of Dietary Arsenic Exposure in Lake Whitefish (Coregonus Clupeaformis). *Aquat. Toxicol. Amst. Neth.* **2002**, *57* (3), 167–189. https://doi.org/10.1016/s0166-445x(01)00198-9.

(111) Roy, S.; Bhattacharya, S. Arsenic-Induced Histopathology and Synthesis of Stress Proteins in Liver and Kidney of Channa Punctatus. *Ecotoxicol. Environ. Saf.* **2006**, *65* (2), 218–229. https://doi.org/10.1016/j.ecoenv.2005.07.005.

(112) Cockell, K. A.; Hilton, J. W. Preliminary Investigations on the Comparative Chronic Toxicity of Four Dietary Arsenicals to Juvenile Rainbow Trout (Salmo Gairdneri R.). *Aquat. Toxicol.* **1988**, *12* (1), 73–82. https://doi.org/10.1016/0166-445X(88)90021-5.

(113) NRC. *Mineral Tolerance of Animals: Second Revised Edition, 2005*; 2005. https://doi.org/10.17226/11309.

(114) El Hosry, L.; Sok, N.; Richa, R.; Al Mashtoub, L.; Cayot, P.; Bou-Maroun, E. Sample Preparation and Analytical Techniques in the Determination of Trace Elements in Food: A Review. *Foods* **2023**, *12* (4), 895. https://doi.org/10.3390/foods12040895.

(115) Zhang, N.; Shen, K.; Yang, X.; Li, Z.; Zhou, T.; Zhang, Y.; Sheng, Q.; Zheng, J. Simultaneous Determination of Arsenic, Cadmium and Lead in Plant Foods by ICP-MS Combined with Automated Focused Infrared Ashing and Cold Trap. *Food Chem.* **2018**, *264*, 462–470. https://doi.org/10.1016/j.foodchem.2018.05.058.

(116) Skoog, D. A.; Holler, F. J.; Crouch, S. R. *Principles of Instrumental Analysis*, Seventh edition.; Cengage Learning: Australia, 2018.

(117) Wilschefski, S. C.; Baxter, M. R. Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects. *Clin. Biochem. Rev.* **2019**, *40* (3), 115–133. https://doi.org/10.33176/AACB-19-00024.

(118) Thomas, R. *Practical Guide to ICP-MS: A Tutorial for Beginners, Third Edition*; CRC Press, 2013.

(119) Brima, E. I.; Jenkins, R. O.; Haris, P. I. Understanding Arsenic Metabolism through Spectroscopic Determination of Arsenic in Human Urine. *Spectroscopy* **2006**, *20* (3), 125–151. https://doi.org/10.1155/2006/759046.

(120) *A Beginner's Guide to ICP-MS, Mass Spectrometry basics | Agilent*. https://www.agilent.com/en/product/atomic-spectroscopy/inductively-coupled-plasma-mass-spectrometry-icp-ms/what-is-icp-ms-icp-ms-faqs (accessed 2023-07-15).

(121) Hai, T. D. Hydrolysis Methods for Protein Extraction from Seafood. *Am. J. Biomed. Sci. Res.* **2020**, *11* (2), 222–224. https://doi.org/10.34297/AJBSR.2020.11.001630.

(122) Reyes, L. H.; Mar, J. L. G.; Rahman, G. M. M.; Seybert, B.; Fahrenholz, T.; Kingston, H. M. S. Simultaneous Determination of Arsenic and Selenium Species in Fish Tissues Using Microwave-Assisted Enzymatic Extraction and Ion Chromatography–Inductively Coupled Plasma Mass Spectrometry. *Talanta* **2009**, *78* (3), 983–990. https://doi.org/10.1016/j.talanta.2009.01.003.

(123) Hu, B.; Chen, B.; He, M.; Nan, K.; Xu, Y.; Xu, C. Chapter Four - Separation Methods Applied to Arsenic Speciation. In *Comprehensive Analytical Chemistry*; Duarte, A. C., Reis, V., Eds.; Arsenic Speciation in Algae; Elsevier, 2019; Vol. 85, pp 89–144. https://doi.org/10.1016/bs.coac.2019.04.001.

(124) Ali, I.; Jain, C. K. Advances in Arsenic Speciation Techniques. *Int. J. Environ. Anal. Chem.* **2004**, *84* (12), 947–964. https://doi.org/10.1080/03067310410001729637.

(125) Caruso, J. A.; Heitkemper, D. T.; B'Hymer, C. An Evaluation of Extraction Techniques for Arsenic Species Fromfreeze-Dried Apple Samples. *Analyst* **2001**, *126* (2), 136–140. https://doi.org/10.1039/B009825F.

(126) Muñoz, O.; Vélez, D.; Montoro, R. Optimization of the Solubilization, Extraction and Determination of Inorganic Arsenic [As(III) + As(V)] in Seafood Products by Acid Digestion, Solvent Extraction and Hydride Generation Atomic Absorption Spectrometry. *Analyst* **1999**, *124* (4), 601–607. https://doi.org/10.1039/A809426H.

(127) Gong, Z.; Lu, X.; Ma, M.; Watt, C.; Le, X. C. Arsenic Speciation Analysis. *Talanta* **2002**, *58* (1), 77–96. https://doi.org/10.1016/S0039-9140(02)00258-8.

(128) Coskun, O. Separation Tecniques: CHROMATOGRAPHY. *North. Clin. Istanb.* **2016**. https://doi.org/10.14744/nci.2016.32757.

(129) Cummins, P. M.; Rochfort, K. D.; O'Connor, B. F. Ion-Exchange Chromatography: Basic Principles and Application. In *Protein Chromatography: Methods and Protocols*; Walls, D., Loughran, S. T., Eds.; Methods in Molecular Biology; Springer: New York, NY, 2017; pp 209–223. https://doi.org/10.1007/978-1-4939-6412-3_11.

(130) Acikara, Ö. B. Ion-Exchange Chromatography and Its Applications. In *Column Chromatography*; IntechOpen, 2013. https://doi.org/10.5772/55744.

(131) *Inorganic Anion Detection*. https://www.shimadzu.com/an/service-support/technical-support/analysis-basics/liquid-chromatography/ion/64intro/index.html (accessed 2023-09-01).

(132) *Ion Chromatography*; Elsevier, 1990.

(133) Kalidas, C.; Sangaranarayanan, M. V. Ion Exchange Chromatography and Its Applications in the Separation of Biomolecules. In *Biophysical Chemistry: Techniques and Applications*; Kalidas, C., Sangaranarayanan, M. V., Eds.; Springer Nature Switzerland: Cham, 2023; pp 421–427. https://doi.org/10.1007/978-3-031-37682-5_28.

(134) Khan, H. U. The Role of Ion Exchange Chromatography in Purification and Characterization of Molecules. In *Ion Exchange Technologies*; IntechOpen, 2012. https://doi.org/10.5772/52537.

(135) Soeroes, C.; Goessler, W.; A. Francesconi, K.; Schmeisser, E.; Raml, R.; Kienzl, N.; Kahn, M.; Fodor, P.; Kuehnelt, D. Thio Arsenosugars in Freshwater Mussels from the Danube in Hungary. *J. Environ. Monit.* **2005**, *7* (7), 688–692. https://doi.org/10.1039/B503897A.

(136) Soeroes, C.; Goessler, W.; Francesconi, K. A.; Kienzl, N.; Schaeffer, R.; Fodor, P.; Kuehnelt, D. Arsenic Speciation in Farmed Hungarian Freshwater Fish. *J. Agric. Food Chem.* **2005**, *53* (23), 9238–9243. https://doi.org/10.1021/jf0516639.

(137) Lintschinger, J.; Schramel, P.; Hatalak-Rauscher, A.; Wendler, I.; Michalke, B. A New Method for the Analysis of Arsenic Species in Urine by Using HPLC-ICP-MS. *Fresenius J. Anal. Chem.* **1998**, *362* (3), 313–318. https://doi.org/10.1007/s002160051080.

(138) Le, X. C.; Li, X. F.; Lai, V.; Ma, M.; Yalcin, S.; Feldmann, J. Simultaneous Speciation of Selenium and Arsenic Using Elevated Temperature Liquid Chromatography Separation with Inductively Coupled Plasma Mass Spectrometry Detection. *Spectrochim. Acta Part B At. Spectrosc.* **1998**, *53* (6–8), 899–909.

(139) Marcinkowska, M.; Barałkiewicz, D. Multielemental Speciation Analysis by Advanced Hyphenated Technique – HPLC/ICP-MS: A Review. *Talanta* **2016**, *161*, 177–204. https://doi.org/10.1016/j.talanta.2016.08.034.

(140) Fairman, B.; Wahlen, R. Speciation Analysis of Organotin Compounds by HPLC-ICP-MS. *At. Spectrosc.* **2001**.

(141) Julshamn, K.; Maage, A.; Norli, H. S.; Grobecker, K. H.; Jorhem, L.; Fecher, P. Determination of Arsenic, Cadmium, Mercury, and Lead by Inductively Coupled Plasma/Mass Spectrometry in Foods after Pressure Digestion: NMKL Interlaboratory Study. *J. AOAC Int.* **2007**, *90* (3), 844–856.

(142) Tibon, J.; Amlund, H.; Gomez-Delgado, A. I.; Berntssen, M. H. G.; Silva, M. S.; Wiech, M.; Sloth, J. J.; Sele, V. Arsenic Species in Mesopelagic Organisms and Their Fate during Aquafeed Processing. *Chemosphere* **2022**, *302*, 134906. https://doi.org/10.1016/j.chemosphere.2022.134906.

(143) Freitas, F. P.; Raber, G.; Jensen, K. B.; Nogueira, A. J. A.; Francesconi, K. A.; Freitas, F. P.; Raber, G.; Jensen, K. B.; Nogueira, A. J. A.; Francesconi, K. A. Lipids That Contain Arsenic in the Mediterranean Mussel, Mytilus Galloprovincialis. *Environ. Chem.* **2019**, *17* (3), 289–301. https://doi.org/10.1071/EN19213.

(144) Aas, T. S.; Åsgård, T.; Ytrestøyl, T. Utilization of Feed Resources in the Production of Atlantic Salmon (Salmo Salar) in Norway: An Update for 2020. *Aquac. Rep.* **2022**, *26*, 101316. https://doi.org/10.1016/j.aqrep.2022.101316.

(145) Sele, V.; Lundebye, A.-K.; Berntssen, M.; Storesund, J.; Lie, K. K.; Philip, A.; Nøstbakken, O. J.; Ørnsrud, R. PROGRAM FOR OVERVÅKING AV FISKEFÔR. *2021*.

(146) Sele, V.; Sanden, M.; Berntssen, M.; Storesund, J.; Lie, K. K.; Lundebye, A.-K.; Hemre, G.-I.; Waagbø, R.; Ørnsrud, R. PROGRAM FOR OVERVÅKING AV FISKEFÔR. *2018*.

(147) Kuehnelt, D.; Lintschinger, J.; Goessler, W. Arsenic Compounds in Terrestrial Organisms. IV. Green Plants and Lichens from an Old Arsenic Smelter Site in Austria. *Appl. Organomet. Chem.* **2000**, *14* (8), 411–420. https://doi.org/10.1002/1099-0739(200008)14:8<411::AID-AOC24>3.0.CO;2-M.

(148) Cullen, W. R.; Reimer, K. J. *Arsenic speciation in the environment*. ACS Publications. https://doi.org/10.1021/cr00094a002.

(149) Larsen, E. H.; Hansen, M.; Gössler, W. Speciation and Health Risk Considerations of Arsenic in the Edible Mushroom Laccaria Amethystina Collected from Contaminated and Uncontaminated Locations. *Appl. Organomet. Chem.* **1998**, *12* (4), 285–291. https://doi.org/10.1002/(SICI)1099-0739(199804)12:4<285::AID-AOC706>3.0.CO;2-#.

(150) Måge, A.; Julshamn, K.; Hemre, G.-I.; Lunestad, B. T. OVERVAKNINGSPROGRAM FOR FÔRVARER TIL FISK OG ANDRE AKVATISKE DYR.

(151) Lundebye, A.-K.; Bakkejord, J. A.; Jakob, O. PROGRAM FOR FREMMEDSTOFFER I FÔRMIDLER AV FISK. *2021*.

(152) Lundebye, A.-K.; Bakkejord, J. A.; Nøstbakken, O. J.; Ørnsrud, R. PROGRAM FOR FREMMEDSTOFFER I FÔRMIDLER AV FISK. *2018*.

(153) Leong, Y. K.; Chang, J.-S. Bioremediation of Heavy Metals Using Microalgae: Recent Advances and Mechanisms. *Bioresour. Technol.* **2020**, *303*, 122886. https://doi.org/10.1016/j.biortech.2020.122886.

(154) Duncan, E. G.; Maher, W. A.; Foster, S. D. Contribution of Arsenic Species in Unicellular Algae to the Cycling of Arsenic in Marine Ecosystems. *Environ. Sci. Technol.* **2015**, *49* (1), 33–50. https://doi.org/10.1021/es504074z.

(155) García Salgado, S.; Quijano Nieto, M. A.; Bonilla Simón, M. M. Determination of Soluble Toxic Arsenic Species in Alga Samples by Microwave-Assisted Extraction and High Performance Liquid Chromatography–Hydride Generation–Inductively Coupled Plasma-Atomic Emission Spectrometry. *J. Chromatogr. A* **2006**, *1129* (1), 54–60. https://doi.org/10.1016/j.chroma.2006.06.083.

(156) Renna, M. Reviewing the Prospects of Sea Fennel (Crithmum Maritimum L.) as Emerging Vegetable Crop. *Plants* **2018**, *7* (4), 92. https://doi.org/10.3390/plants7040092.

(157) Magni, N. N.; Veríssimo, A. C. S.; Silva, H.; Pinto, D. C. G. A. Metabolomic Profile of Salicornia Perennis Plant's Organs under Diverse In Situ Stress: The Ria de Aveiro Salt Marshes Case. *Metabolites* **2023**, *13* (2), 280. https://doi.org/10.3390/metabo13020280.

(158) Del Arco Aguilar, M. J.; Rodríguez Delgado, O. *Vegetation of the Canary Islands*; Plant and Vegetation; Springer International Publishing: Cham, 2018; Vol. 16. https://doi.org/10.1007/978-3-319-77255-4.

(159) Authority (EFSA), E. F. S.; Dujardin, B.; Ferreira de Sousa, R.; Gómez Ruiz, J. Á. Dietary Exposure to Heavy Metals and Iodine Intake via Consumption of Seaweeds and Halophytes in the European Population. *EFSA J.* **2023**, *21* (1), e07798. https://doi.org/10.2903/j.efsa.2023.7798.

(160) Attia-Ismail, S. A. Halophytes as Forages. In *New Perspectives in Forage Crops*; IntechOpen, 2018. https://doi.org/10.5772/intechopen.69616.

(161) Chu, Y.-T.; Brown, P. B. Optimal Dietary Crude Protein in Commercial Feeds for Shrimp and Halophytes in Marine Aquaponic Biofloc Systems. *Front. Mar. Sci.* **2022**, *9*.

(162) Flowers, T. J.; Colmer, T. D. Salinity Tolerance in Halophytes. *New Phytol.* **2008**, *179* (4), 945–963.

(163) Sánchez-Gavilán, I.; Rufo, L.; Rodríguez, N.; de la Fuente, V. On the Elemental Composition of the Mediterranean Euhalophyte Salicornia Patula Duval-Jouve (Chenopodiaceae) from Saline Habitats in Spain (Huelva, Toledo and Zamora). *Environ. Sci. Pollut. Res.* **2021**, *28* (3), 2719–2727. https://doi.org/10.1007/s11356-020-10663-w.

(164) Stamer, A. Insect Proteins—a New Source for Animal Feed. *EMBO Rep.* **2015**, *16* (6), 676–680. https://doi.org/10.15252/embr.201540528.

(165) COMMISSION REGULATION (EU) 2017/ 893 - of 24 May 2017 - Amending Annexes I and IV to Regulation (EC) No 999 / 2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142 / 2011 as Regards the Provisions on Processed Animal Protein.

(166) Veldkamp, T.; Bosch, G. Insects: A Protein-Rich Feed Ingredient in Pig and Poultry Diets. *Anim. Front.* **2015**, *5* (2).

(167) IPIFF. THE EUROPEAN INSECT SECTOR TODAY: CHALLENGES, OPPORTUNITIES AND REGULATORY LANDSCAPE. *2018*.

(168) Ortega-Calvo, J. J.; Mazuelos, C.; Hermosin, B.; Saiz-Jimenez, C. Chemical Composition ofSpirulina and Eukaryotic Algae Food Products Marketed in Spain. *J. Appl. Phycol.* **1993**, *5* (4), 425–435. https://doi.org/10.1007/BF02182735.

(169) Smith, J.; Summers, G.; Wong, R. Nutrient and Heavy Metal Content of Edible Seaweeds in New Zealand. *N. Z. J. Crop Hortic. Sci.* **2010**, *38* (1), 19–28. https://doi.org/10.1080/01140671003619290.

(170) Hou, X.; Yan, X. Study on the Concentration and Seasonal Variation of Inorganic Elements in 35 Species of Marine Algae. *Sci. Total Environ.* **1998**, *222* (3), 141–156. https://doi.org/10.1016/S0048-9697(98)00299-X.

(171) van Netten, C.; Hoption Cann, S. A.; Morley, D. R.; van Netten, J. P. Elemental and Radioactive Analysis of Commercially Available Seaweed. *Sci. Total Environ.* **2000**, *255* (1–3), 169–175. https://doi.org/10.1016/s0048-9697(00)00467-8.

(172) *Program for fremmedstoffer i fôrmidler av fisk*. Havforskningsinstituttet. https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2020-40 (accessed 2023-07-09).

(173) Julshamn, K.; Nilsen, B. M.; Frantzen, S.; Valdersnes, S.; Maage, A.; Nedreaas, K.; Sloth, J. J. Total and Inorganic Arsenic in Fish Samples from Norwegian Waters. *Food Addit. Contam. Part B* **2012**, *5* (4), 229–235. https://doi.org/10.1080/19393210.2012.698312.

(174) Sele, V.; Sloth, J. J.; Julshamn, K.; Skov, K.; Amlund, H. A Study of Lipid- and Water-Soluble Arsenic Species in Liver of Northeast Arctic Cod (Gadus Morhua) Containing High

Levels of Total Arsenic. *J. Trace Elem. Med. Biol.* **2015**, *30*, 171–179. https://doi.org/10.1016/j.jtemb.2014.12.010.

(175) Liaset, B.; Espe, M. Nutritional Composition of Soluble and Insoluble Fractions Obtained by Enzymatic Hydrolysis of Fish-Raw Materials. *Process Biochem.* **2008**, *43* (1), 42–48. https://doi.org/10.1016/j.procbio.2007.10.007.

(176) Julshamn, K.; Lundebye, A.-K.; Heggstad, K.; Berntssen, M. H. G.; Boe, B. Norwegian Monitoring Programme on the Inorganic and Organic Contaminants in Fish Caught in the Barents Sea, Norwegian Sea and North Sea, 1994-2001. *Food Addit. Contam.* **2004**, *21* (4), 365–376. https://doi.org/10.1080/02652030310001639512.

(177) Kousoulaki, K.; Sveen, L.; Norén, F.; Espmark, Å. Atlantic Salmon (Salmo Salar) Performance Fed Low Trophic Ingredients in a Fish Meal and Fish Oil Free Diet. *Front. Physiol.* **2022**, *13*, 884740. https://doi.org/10.3389/fphys.2022.884740.

(178) Petersen, J.; Riisgard, H. Filtration Capacity of the Ascidian Ciona Intestinalis and Its Grazing Impact in a Shallow Fjord. *Mar. Ecol. Prog. Ser.* **1992**, *88*, 9–17. https://doi.org/10.3354/meps088009.

(179) Draughon, L. D.; Scarpa, J.; Hartmann, J. X. Are Filtration Rates for the Rough Tunicate Styela Plicata Independent of Weight or Size? *J. Environ. Sci. Health Part A* **2010**, *45* (2), 168–176. https://doi.org/10.1080/10934520903429816.

(180) Lambert, G.; Karney, R.; Rhee, W.; Carman, M. Wild and Cultured Edible Tunicates: A Review. *Manag. Biol. Invasions* **2016**, *7* (1), 59–66. https://doi.org/10.3391/mbi.2016.7.1.08.

(181) Roje-Busatto, R.; Ujević, I. PSP Toxins Profile in Ascidian Microcosmus Vulgaris (Heller, 1877) after Human Poisoning in Croatia (Adriatic Sea). *Toxicon* **2014**, *79*, 28.

(182) Sekiguchi, K.; Sato, S.; Kaga, S.; Ogata, T.; Kodama, M. Accumulation of Paralytic Shellfish Poisoning Toxins in Bivalves and an Ascidian Fed on Alexandrium Tamarense Cells. *Fish. Sci.* **2001**, *67*. https://doi.org/10.1046/j.1444-2906.2001.00228.x.

(183) Treberg, J. R.; Stacey, J. E.; Driedzic, W. R. Vanadium Accumulation in Ascidian Coelomic Cells Is Associated with Enhanced Pentose Phosphate Pathway Capacity but Not Overall Aerobic or Anaerobic Metabolism. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2012**, *161* (4), 323–330. https://doi.org/10.1016/j.cbpb.2011.12.007.

(184) Holland, L. Z. Tunicates. *Curr. Biol.* **2016**, *26* (4), R146–R152. https://doi.org/10.1016/j.cub.2015.12.024.

(185) Silva, M.; Tibon, J.; Sartipiyarahmadi, S.; Remø, S. C.; Sele, V.; Søfteland, L.; Sveier, H.; Wiech, M.; Philip, A. J. P.; Berntssen, M. Arsenic Speciation and Arsenic Feed-to-Fish

Transfer in Atlantic Salmon Fed Marine Low Trophic Feeds Based Blue Mussel and Kelp. Rochester, NY June 20, 2023. https://doi.org/10.2139/ssrn.4480971.

(186) Józefiak, D.; Józefiak, A.; Kierończyk, B.; Rawski, M.; Świątkiewicz, S.; Długosz, J.; Engberg, R. M. 1. Insects – A Natural Nutrient Source for Poultry – A Review. *Ann. Anim. Sci.* **2016**, *16* (2), 297–313. https://doi.org/10.1515/aoas-2016-0010.

(187) Cruz, A.; Sterten, H.; Steinhoff, F. S.; Mydland, L. T.; Øverland, M. Cyberlindnera Jadinii Yeast as a Protein Source for Broiler Chickens: Effects on Growth Performance and Digestive Function from Hatching to 30 Days of Age. *Poult. Sci.* **2020**, *99* (6), 3168–3178. https://doi.org/10.1016/j.psj.2020.01.023.

(188) Shurson, G. C. Yeast and Yeast Derivatives in Feed Additives and Ingredients: Sources, Characteristics, Animal Responses, and Quantification Methods. *Anim. Feed Sci. Technol.* **2018**, *235*, 60–76. https://doi.org/10.1016/j.anifeedsci.2017.11.010.

(189) Fathima, S.; Shanmugasundaram, R.; Sifri, M.; Selvaraj, R. Yeasts and Yeast-Based Products in Poultry Nutrition. *J. Appl. Poult. Res.* **2023**, *32* (2), 100345. https://doi.org/10.1016/j.japr.2023.100345.

(190) Øverland, M.; Skrede, A. Yeast Derived from Lignocellulosic Biomass as a Sustainable Feed Resource for Use in Aquaculture. *J. Sci. Food Agric.* **2017**, *97* (3), 733–742. https://doi.org/10.1002/jsfa.8007.

(191) Couture, J. L.; Geyer, R.; Hansen, J. Ø.; Kuczenski, B.; Øverland, M.; Palazzo, J.; Sahlmann, C.; Lenihan, H. Environmental Benefits of Novel Nonhuman Food Inputs to Salmon Feeds. *Environ. Sci. Technol.* **2019**, *53* (4), 1967–1975. https://doi.org/10.1021/acs.est.8b03832.

(192) Øverland, M.; Karlsson, A.; Mydland, L. T.; Romarheim, O. H.; Skrede, A. Evaluation of Candida Utilis, Kluyveromyces Marxianus and Saccharomyces Cerevisiae Yeasts as Protein Sources in Diets for Atlantic Salmon (Salmo Salar). *Aquaculture* **2013**, *402–403*, 1–7. https://doi.org/10.1016/j.aquaculture.2013.03.016.

(193) Joint Research Centre (European Commission); Robouch, P.; Stroka, J.; Haedrich, J.; Schaechtele, A.; Wenzl, T. *Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food*; Publications Office of the European Union: LU, 2016.

(194) FAO. CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF DIOXINS, DIOXIN-LIKE PCBs AND NON-DIOXIN-LIKE PCBs IN FOOD AND FEED CXC 62-2006 Adopted in 2006. Revised in 2018. https://www.fao.org/fao-who-codexalimentarius/sh-

proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B62-2006%252FCXC_062e.pdf.

(195) Lapingcao, C. *10 Main Ingredients in Farm Animal Feed*. The Happy Chicken Coop. https://www.thehappychickencoop.com/main-ingredients-in-farm-animal-feed/ (accessed 2023-08-28).

(196) Rosas-Castor, J. M.; Guzmán-Mar, J. L.; Hernández-Ramírez, A.; Garza-González, M. T.; Hinojosa-Reyes, L. Arsenic Accumulation in Maize Crop (Zea Mays): A Review. *Sci. Total Environ.* **2014**, *488–489*, 176–187. https://doi.org/10.1016/j.scitotenv.2014.04.075.

(197) Bianucci, E.; Peralta, J. M.; Furlan, A.; Hernández, L. E.; Castro, S. Arsenic in Wheat, Maize, and Other Crops. In *Arsenic in Drinking Water and Food*; Srivastava, S., Ed.; Springer: Singapore, 2020; pp 279–306. https://doi.org/10.1007/978-981-13-8587-2_9.

(198) Kundu, R.; Majumder, Dr. A.; Pal, S. Arsenic Accumulation Pattern of Different Potato Cultivars under Arsenic Contaminated Zone of India. *Res. J. Agric. Sci.* **2012**, *3*, 135–137.

(199) Kamrozzaman, M. M.; Khan, M. a. H.; Ahmed, S.; Sultana, N.; Quddus, A. F. M. R. Evaluation of Five Wheat Varieties on Arsenic Contaminated Soils of Bangladesh. *Int. J. Appl. Sci. Biotechnol.* **2016**, *4* (3), 330–337. https://doi.org/10.3126/ijasbt.v4i3.15761.

(200) Zhao, F.-J.; Stroud, J. L.; Eagling, T.; Dunham, S. J.; McGrath, S. P.; Shewry, P. R. Accumulation, Distribution, and Speciation of Arsenic in Wheat Grain. *Environ. Sci. Technol.* **2010**, *44* (14), 5464–5468. https://doi.org/10.1021/es100765g.

(201) Greene, R.; Crecelius, E. Total and Inorganic Arsenic in Mid-Atlantic Marine Fish and Shellfish and Implications for Fish Advisories. *Integr. Environ. Assess. Manag.* **2006**, *2* (4), 344–354. https://doi.org/10.1002/ieam.5630020406.

(202) Molin, M.; Ulven, S. M.; Meltzer, H. M.; Alexander, J. Arsenic in the Human Food Chain, Biotransformation and Toxicology – Review Focusing on Seafood Arsenic. *J. Trace Elem. Med. Biol.* **2015**, *31*, 249–259. https://doi.org/10.1016/j.jtemb.2015.01.010.

(203) Rose, M.; Lewis, J.; Langford, N.; Baxter, M.; Origgi, S.; Barber, M.; MacBain, H.; Thomas, K. Arsenic in Seaweed—Forms, Concentration and Dietary Exposure. *Food Chem. Toxicol.* **2007**, *45* (7), 1263–1267. https://doi.org/10.1016/j.fct.2007.01.007.

(204) Tibon, J.; Gomez-Delgado, A. I.; Agüera, A.; Strohmeier, T.; Silva, M. S.; Lundebye, A.-K.; Larsen, M. M.; Sloth, J. J.; Amlund, H.; Sele, V. Arsenic Speciation in Low-Trophic Marine Food Chain – An Arsenic Exposure Study on Microalgae (Diacronema Lutheri) and Blue Mussels (Mytilus Edulis L.). *Environ. Pollut.* **2023**, *334*, 122176. https://doi.org/10.1016/j.envpol.2023.122176.

(205) Petropoulos, S. A.; Karkanis, A.; Martins, N.; Ferreira, I. C. F. R. Edible Halophytes of the Mediterranean Basin: Potential Candidates for Novel Food Products. *Trends Food Sci. Technol.* **2018**, *74*, 69–84. https://doi.org/10.1016/j.tifs.2018.02.006.

(206) Barreira, L.; Resek, E.; Rodrigues, M. J.; Rocha, M. I.; Pereira, H.; Bandarra, N.; da Silva, M. M.; Varela, J.; Custódio, L. Halophytes: Gourmet Food with Nutritional Health Benefits? *J. Food Compos. Anal.* **2017**, *59*, 35–42. https://doi.org/10.1016/j.jfca.2017.02.003.

(207) Agudelo, A.; Carvajal, M.; Martinez-Ballesta, M. del C. Halophytes of the Mediterranean Basin—Underutilized Species with the Potential to Be Nutritious Crops in the Scenario of the Climate Change. *Foods* **2021**, *10* (1), 119. https://doi.org/10.3390/foods10010119.

(208) El-Said, G. F.; El-Sikaily, A. Chemical Composition of Some Seaweed from Mediterranean Sea Coast, Egypt. *Environ. Monit. Assess.* **2013**, *185* (7), 6089–6099. https://doi.org/10.1007/s10661-012-3009-y.

(209) Yang, H.; Deng, Y.; Wang, M.-W.; Zhu, M.; Liu, C.; Ge, Z.-J.; Xing, J.-C. Risk of Heavy Metal Contamination in Three Seawater-Cultivated Vegetables. *Pol. J. Environ. Stud.* **2019**, *29* (1), 961–967. https://doi.org/10.15244/pjoes/104457.

(210) COMMISSION RECOMMENDATION (EU) 2018/ 464 - of 19 March 2018 - on the Monitoring of Metals and Iodine in Seaweed, Halophytes and Products Based on Seaweed.

(211) González de las Torres, A. I.; Giráldez, I.; Martínez, F.; Palencia, P.; Corns, W. T.; Sánchez-Rodas, D. Arsenic Accumulation and Speciation in Strawberry Plants Exposed to Inorganic Arsenic Enriched Irrigation. *Food Chem.* **2020**, *315*, 126215. https://doi.org/10.1016/j.foodchem.2020.126215.

(212) Bali, A. S.; Sidhu, G. P. S. Arsenic Acquisition, Toxicity and Tolerance in Plants - From Physiology to Remediation: A Review. *Chemosphere* **2021**, *283*, 131050. https://doi.org/10.1016/j.chemosphere.2021.131050.

(213) Das, S.; Chou, M.-L.; Jean, J.-S.; Liu, C.-C.; Yang, H.-J. Water Management Impacts on Arsenic Behavior and Rhizosphere Bacterial Communities and Activities in a Rice Agro-Ecosystem. *Sci. Total Environ.* **2016**, *542* (Pt A), 642–652. https://doi.org/10.1016/j.scitotenv.2015.10.122.

(214) Vromman, D.; Martínez, J.-P.; Kumar, M.; Šlejkovec, Z.; Lutts, S. Comparative Effects of Arsenite (As(III)) and Arsenate (As(V)) on Whole Plants and Cell Lines of the Arsenic-Resistant Halophyte Plant Species Atriplex Atacamensis. *Environ. Sci. Pollut. Res. Int.* **2018**, *25* (34), 34473–34486. https://doi.org/10.1007/s11356-018-3351-x.

(215) Sele, V.; Berntssen, M.; Philip, A.; Lundebye, A.-K.; Lie, K. K.; Espe, M. PROGRAM FOR OVERVÅKING AV FISKEFÔR. *2020*.

(216) Biancarosa, I.; Liland, N. S.; Biemans, D.; Araujo, P.; Bruckner, C. G.; Waagbø, R.; Torstensen, B. E.; Lock, E.-J.; Amlund, H. Uptake of Heavy Metals and Arsenic in Black Soldier Fly (Hermetia Illucens) Larvae Grown on Seaweed-Enriched Media. *J. Sci. Food Agric.* **2018**, *98* (6), 2176–2183. https://doi.org/10.1002/jsfa.8702.

(217) Azam, I.; Afsheen, S.; Zia, A.; Javed, M.; Saeed, R.; Sarwar, M. K.; Munir, B. Evaluating Insects as Bioindicators of Heavy Metal Contamination and Accumulation near Industrial Area of Gujrat, Pakistan. *BioMed Res. Int.* **2015**, *2015*, 942751. https://doi.org/10.1155/2015/942751.

(218) Charlton, A. j.; Dickinson, M.; Wakefield, M. e.; Fitches, E.; Kenis, M.; Han, R.; Zhu, F.; Kone, N.; Grant, M.; Devic, E.; Bruggeman, G.; Prior, R.; Smith, R. Exploring the Chemical Safety of Fly Larvae as a Source of Protein for Animal Feed. *J. Insects Food Feed* **2015**, *1* (1), 7–16. https://doi.org/10.3920/JIFF2014.0020.

(219) Diener, S.; Zurbrügg, C.; Tockner, K. Bioaccumulation of Heavy Metals in the Black Soldier Fly, *Hermetia Illucens* and Effects on Its Life Cycle. *J. Insects Food Feed* **2015**, *1* (4), 261–270. https://doi.org/10.3920/JIFF2015.0030.

(220) Gao, Q.; Wang, X.; Wang, W.; Lei, C.; Zhu, F. Influences of Chromium and Cadmium on the Development of Black Soldier Fly Larvae. *Environ. Sci. Pollut. Res. Int.* **2017**, *24* (9), 8637–8644. https://doi.org/10.1007/s11356-017-8550-3.

(221) *Uptake of Cadmium, Lead and Arsenic by Tenebrio molitor and Hermetia illucens from Contaminated Substrates | PLOS ONE*. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0166186 (accessed 2023-08-05).

(222) Vijver, M.; Jager, T.; Posthuma, L.; Peijnenburg, W. Metal Uptake from Soils and Soil-Sediment Mixtures by Larvae of Tenebrio Molitor (L.) (Coleoptera). *Ecotoxicol. Environ. Saf.* **2003**, *54* (3), 277–289. https://doi.org/10.1016/s0147-6513(02)00027-1.

(223) Żuk-Gołaszewska, K.; Gałęcki, R.; Obremski, K.; Smetana, S.; Figiel, S.; Gołaszewski, J. Edible Insect Farming in the Context of the EU Regulations and Marketing—An Overview. *Insects* **2022**, *13* (5), 446. https://doi.org/10.3390/insects13050446.

(224) *Insects As Feed EU Legislation – Aquaculture, Poultry & Pig Species*. International Platform of Insects for Food and Feed, Brussels. https://ipiff.org/insects-eu-legislation/ (accessed 2023-08-26).

(225) *Risk profile related to production and consumption of insects as food and feed | EFSA*. https://www.efsa.europa.eu/en/efsajournal/pub/4257 (accessed 2023-08-05).

(226) Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of Feed materialsText with EEA Relevance.

(227) Tibon, J. S. *Development of Analytical Methods for Arsenic Speciation and Their Application to Novel Marine Feed Resources*; Technical University of Denmark: Kgs. Lyngby, 2022.

(228) Biancarosa, I.; Belghit, I.; Bruckner, C. G.; Liland, N. S.; Waagbø, R.; Amlund, H.; Heesch, S.; Lock, E. Chemical Characterization of 21 Species of Marine Macroalgae Common in Norwegian Waters: Benefits of and Limitations to Their Potential Use in Food and Feed. *J. Sci. Food Agric.* **2018**, *98* (5), 2035–2042. https://doi.org/10.1002/jsfa.8798.

(229) Sirot, V.; Guérin, T.; Volatier, J.-L.; Leblanc, J.-C. Dietary Exposure and Biomarkers of Arsenic in Consumers of Fish and Shellfish from France. *Sci. Total Environ.* **2009**, *407* (6), 1875–1885. https://doi.org/10.1016/j.scitotenv.2008.11.050.

(230) Sloth, J. J.; Julshamn, K. Survey of Total and Inorganic Arsenic Content in Blue Mussels (Mytilus Edulis L.) from Norwegian Fiords: Revelation of Unusual High Levels of Inorganic Arsenic. *J. Agric. Food Chem.* **2008**, *56* (4), 1269–1273. https://doi.org/10.1021/jf073174+.

(231) Thomas, D. J.; Nava, G. M.; Cai, S.-Y.; Boyer, J. L.; Hernández-Zavala, A.; Gaskins, H. R. Arsenic (+ 3 Oxidation State) Methyltransferase and the Methylation of Arsenicals in the Invertebrate Chordate Ciona Intestinalis. *Toxicol. Sci.* **2010**, *113* (1), 70–76. https://doi.org/10.1093/toxsci/kfp250.

(232) Kalla, A.; Yoshimatsu, T.; Araki, T.; Zhang, D.-M.; Yamamoto, T.; Sakamoto, S. Use of Porphyra Spheroplasts as Feed Additive for Red Sea Bream. *Fish. Sci.* **2008**, *74* (1), 104–108. https://doi.org/10.1111/j.1444-2906.2007.01501.x.

(233) Kamunde, C.; Sappal, R.; Melegy, T. M. Brown Seaweed (AquaArom) Supplementation Increases Food Intake and Improves Growth, Antioxidant Status and Resistance to Temperature Stress in Atlantic Salmon, Salmo Salar. *PloS One* **2019**, *14* (7), e0219792. https://doi.org/10.1371/journal.pone.0219792.

(234) Pétursdóttir, Á. H.; Jesus, J. R. de; Gunnlaugsdóttir, H.; Feldmann, J. Quantification of Labile and Stable Non-Polar Arsenolipids in Commercial Fish Meals and Edible Seaweed Samples. *J. Anal. At. Spectrom.* **2018**, *33* (1), 102–110. https://doi.org/10.1039/C7JA00333A.

(235) Lunde, G. Activation Analysis of Bromine, Iodine and Arsenic in Oils from Fishes, Whales, Phyto- and Zooplankton of Marine and Limnetic Biotopes. *Int. Rev. Gesamten Hydrobiol. Hydrogr.* **1967**, *52* (2), 265–279. https://doi.org/10.1002/iroh.19670520207.

(236) Lunde, G. Occurrence and Transformation of Arsenic in the Marine Environment. *Environ. Health Perspect.* **1977**, *19*, 47–52.

(237) Lunde, G. Trace Metal Contents of Fish Meal and of the Lipid Phase Extracted from Fish Meal. *J. Sci. Food Agric.* **1973**, *24* (4), 413–419. https://doi.org/10.1002/jsfa.2740240410.

(238) Kurosawa, S.; Yasuda, K.; Taguchi, M.; Yamazaki, S.; Toda, S.; Morita, M.; Uehiro, T.; Fuwa, K. Identification of Arsenobetaine, a Water Soluble Organo-Arsenic Compound in Muscle and Liver of a Shark, Prionace Glaucus. *Agric. Biol. Chem.* **1980**, *44* (8), 1993–1994. https://doi.org/10.1080/00021369.1980.10864258.

(239) Taleshi, M. S.; Jensen, K. B.; Raber, G.; Edmonds, J. S.; Gunnlaugsdottir, H.; Francesconi, K. A. Arsenic-Containing Hydrocarbons: Natural Compounds in Oil from the Fish Capelin, Mallotus Villosus. *Chem. Commun.* **2008**, No. 39, 4706–4707. https://doi.org/10.1039/B808049F.

(240) Ebisuda, K.; Kunito, T.; Kubota, R.; Tanabe, S. Arsenic Concentrations and Speciation in the Tissues of Ringed Seals (Phoca Hispida) from Pangnirtung, Canada. *Appl. Organomet. Chem.* **2002**, *16* (8), 451–457. https://doi.org/10.1002/aoc.319.

(241) Francesconi, K. A. Working Methods: Complete Extraction of Arsenic Species: A Worthwhile Goal? *Appl. Organomet. Chem.* **2003**, *17* (9), 682–683. https://doi.org/10.1002/aoc.499.

(242) Biancarosa, I. Insects Reared on Seaweed as Novel Feed Ingredients for Atlantic Salmon (Salmo Salar) : Investigating the Transfer of Essential Nutrients and Undesirable Substances along the Seaweed-Insect-Fish Food Chain. Doctoral thesis, The University of Bergen, 2020. https://bora.uib.no/bora-xmlui/handle/11250/2761076 (accessed 2023-08-08).

(243) COMMISSION REGULATION (EU) 2015/ 186 - of 6 February 2015 - Amending Annex I to Directive 2002/ 32/ EC of the European Parliament and of the Council as Regards Maximum Levels for Arsenic, Fluorine, Lead, Mercury, Endosulfan and Ambrosia Seeds.

7 Supplementary information

Supplementary Table 12. List of instruments and equipment used

Model	Name of the manufacturer	Application
Milestone UltraWave Microwave Digestion System with control unit	Milestone, Italy	Sample preparation for method 197
Analytical balance, 4 decimals when weighing in grams	Mettler Toledo, Switzerland	Method 197, 261
Centrifugal tube (quartz) with screw cork (Teflon), 50ml and 15ml with 15-positions sample rack	Milestone, Italy	Sample preparation for method 197
10, 25, 50 and 100ml volumetric flasks	Brand PMP, Germany Vitlab, Germany	Method 197, Extraction of arsenolipids
50 ml polyethylene falcon tubes	Greiner bio-one, Germany	Sample preparation for method 197
Labsystems Finnpipettes with tips	Thermo Fisher Scientific Inc., USA	Method 197, 261
ICPMS with collision cell and FAST auto sampler (Thermo iCapQ)	Thermo Fisher Scientific Inc., USA	Method 197
traceCLEAN	Milestone, Italy	Method 197
Water bath (temperature requirement 90 ± 2°C) Grant OLS200	Grant, UK	Sample preparation for method 261
Centrifuge 5702	Eppendorf, Germany	Sample preparation for method 261
Milipore RiOs - 18.2 MΩcm, EMD (Filter: Progard NP2)	Merck, Germany	Method 197, Method 261, Column cleaning procedure
Vortex mixer, MS1 Minishaker	IKA, USA	Sample preparation for method 261
Magnetic stirrers with Teflon-coated magnet (RCT S 23)	Kinematica, Switzerland	Preparation of the mobile phase for method 261
pH meter (WTW SenTix 81 pH electrode with WTW InoLab)	InoLab, Germany	Preparation of the mobile phase for method 261
10 mL ± 0.08 mL plastic volumetric flasks (PMP)	Brand, Germany	Method 261
10 mL dispenser - Dispensette® Organic Bottletop	Brandtech, USA	Sample preparation for method 261
1 L acid/base tolerant bottle for HPLC mobile phase	Duran, Germany	Preparation of the mobile phase for method 261
Centrifuge tube with round bottom, 13 mL, 101x16.5mm PP	Sarstedt, Germany	Sample preparation for method 261
Syringe filter, RC 0.45 µm	Agilent, UK	Sample preparation for method 261

Membrane filter, regenerated cellulose 47 mm, pore size 0.45 µm (Part #5191-4337)	Agilent, UK	Preparation of the mobile phase for method 261
Vacuum filtering system with flask, filter holder and glass funnel, InifinityLab	Agilent, UK	Preparation of the mobile phase for method 261
Vacuum pump, N816.3 KT18, 50Hz, 100W	KNF Laboport	Preparation of the mobile phase for method 261
5 mL disposable needle syringe (SOFT-JECT 5)	Henke Sass Wolf, Germany	Sample preparation for method 261
Needles 18Gx2" (1.2x50mm) w/luer fitting	Becton Dickinson, Spain	Sample preparation for method 261
HPLC Sample Vial, 1 mL PP (Part #5182-0567)	Agilent, UK	Method 261
Stopper for sample vials, PTFE/red silicone septa, 11 mm (Part #5182-0550)	Agilent, UK	Method 261
Polymer PEEK tubing, Red 1/16 x .005 x 5ft, IDEX	Teknolab	Method 261, Column cleaning procedure
Nuts for PEEK, FTG, BOLT, 10-32, DBL CONE, HP	Teknolab	Method 261, Column cleaning procedure
Ferrules for PEEK, FTG, FERRULE, DBL CONE, HP	Teknolab	Method 261, Column cleaning procedure
HPLC column; IonPac AS7 (2x250mm)	Dionex, USA	Method 261
HPLC pre-column; IonPac AG7 (2x50mm)	Dionex, USA	Method 261
HPLC: Agilent 1260 Infinity I Quaternary LC System	Agilent, UK	Method 261
ICPMS: Agilent 7900 ICP-MS with PC (HP) and Agilent Masshunter 4.6.	Agilent, UK	Method 197, method 261
Dionex AXP / MX010PFT3ADX - 10ML PK SF PXD STF FB-10, MX-Class Pump - High Pressure Piston Pump for HPLC and High-Performance Metering	Thermo Scientific / Teledyne SSI	Column cleaning procedure
250, 500 and 1000 mL glass bottles	Duran, Germany	Column cleaning procedure
Disposable syringes, 20 mL w/luer fitting (SOFT-JECT)	Hennke Sass Wolf, Germany	Column cleaning procedure

Parafilm	Bemis, USA	Column cleaning procedure
Borosilicate glass tube (13 × 100 mm)	DWK, Mainz, Germany	Extraction of AsLipids
Vortex mixer (MS1 Minishaker)	IKA, Germany	Extraction of AsLipids
Test-tube rotator (LD-79, LABINCO)	Breda, the Netherlands	Extraction of AsLipids
Centrifuge 5702	Eppendorf, Germany	Extraction of AsLipids
Polypropylene Pasteur pipettes 1 ml, small bulb, non-sterile	VWR International bvba, Belgium	Extraction of AsLipids
Quartz digestion tubes (ultraWAVE, Milestone)	Sorisole, Italy	Extraction of AsLipids
Nitrogen evaporator (40 ◦C; Reacti-Therm III)	Thermo Fisher Scientific, Waltham, MA, USA	Extraction of AsLipids

Supplementary Table 13. List of chemicals used

Total As

Name	Supplier
Deionized and filtrated water	>17 MX cm^{-1}, Nanopure System, Nanopure, Barnstead, UK
Concentrated nitric acid, ≥69% suprapur HNO_3 or self-distilled acid of similar quality	Merck, Germany
30% w/w H_2O_2 p.a. quality ISO (H_2O_2)	Merck, Germany
5% (v/v) nitric acid	prepared at IMR in the lab
Certified Reference Materials (CRM): Oyster tissue, OT, CRM 1566, NIST	National Institute of Standards and Technology (NIST), USA
Certified Reference Materials (CRM): Lobster hepatopancreas, TORT 3, NRC	National Research Council, Canada (NRC)
Mercury (Hg) 1005±6 mg/l in 5% HNO_3	Spectrascan, Norway
Rhodium (Rh) 1012±2mg/l in 5% HCl	Spectrascan, Norway
Thulium (Tm), 1000±3mg/l in 2-3% HNO_3	Merck SertiPUR
Germanium (Ge), 1000±3µg/l in H_2O	Spectrascan, Norway
Gold (Au) 1005±6mg/l in 2% HNO_3	Spectrascan, Norway
Multielement standard stock solution (1000mg/l Al, Fe, Mg, Zn, 50mg/l As, Ba, Cu, Mn, Se, Sr and 10mg/l Ag, Cd, Co, Cr, Mo, Ni, Pb. U, V)	Spectrascan, Norway

10mg/l Hg/Au-solution	Prepared in the lab
100mg/l Au-solution	Prepared in the lab
Stock solution for the calibration curve: 0,1ml of the multi-element standard (4.10) and 0,1ml 10mg/l Hg-solution (5.3), diluted to 10ml with 5% HNO_3 (5.5) in a volumetric flask	Prepared in the lab
Internal standard stock solution for analysis with iCapQ: 0,05ml 1000mg/l Rh (4.6), 0,02ml 1000mg/l Tm (4.7), 1ml 1000mg/l Ge (4.8) and 0,5ml 1000mg/l Au (4.9) to a 10ml with 5% HNO_3	Prepared in the lab
Internal standard for analysis with iCapQ: 0,25ml stock solution (5.5) diluted to 250ml with 5% HNO_3	Prepared in the lab
Nitrogen gas ultra-5.0	Nippon Gases Norge AS, Norway
Argon ultrapure 5.0 (99.999%)	Nippon Gases Norge AS, Norway
Helium ultra plus 6.0	Nippon Gases Norge AS, Norway

Inorganic As

Name	Supplier
CRM: ERM-BC211 Rice (reference value inorganic arsenic; 124±11 µg/kg)	ERM, European Commission, JRC, IRMM, Belgium
BCR-627 Tuna fish tissue (uses calculated average)	BCR, European Commission, Belgium
Concentrated nitric acid (HNO_3), min. 37% - Supelco®	Merck, Germany
Hydrogen peroxide (H_2O_2), min. 30% - Supelco®	Merck, Germany
Methanol (MeOH) >99.9% CHROMASOLV HPLC grade - Supelco®	Merck, Germany
Ammonia (NH_3), 25% aq - Supelco®	Merck, Germany
MilliQ water (H_2O), deionized and filtered (nano pure)	Milipor
Ammonium carbonate, powder ($(NH_4)_2CO_3$), HPLC grade, ≥NH3 base - Supelco®	Merck, Germany
Arsenic standard (As(V)) 1005±5 µg/ml in H_2O	Spectrascan, TeknoLab, Drøbak, Norway
ICP-MS stock tuning solution (Part #5188-6564, Agilent): Li, Y, Ce, Tl, and Co (10 mg/L in 2% HNO_3	Agilent

AsLipids

Name	Supplier

Methanol (MeOH) >99.9%, CHROMASOLV HPLC grade - Supelco®	Merck, Germany
Methyl tert-butyl ether (MTBE, HPLC grade)	Merck, Germany
MilliQ water (H_2O), deionized and filtered (nano pure)	Milipor
10:3:2.5 v/v/v mixture of MTBE, methanol, and water	Prepared in the lab

Column cleaning

Name	Supplier
MilliQ water (H2O)	
2-propanol (isopropanol, IPA), hypergrade for LC-MS LiChorosolv - Supelco®	Merck, Germany
Concentrated nitric acid (HNO3), min. 37% (laboratory distilled) - Supelco®	Merck, Germany
Methanol (MeOH) >99.9%, CHROMASOLV HPLC grade - Supelco®	Merck, Germany
Lye (sodium hydroxide, NaOH), 30%, Suprapur - Supelco®	Merck, Germany
~20% isopropanol (IPA)	Merck, Germany
~200mM HNO3 with 5% MeOH, 200mL	Prepared in the lab
~2M HNO3, 500mL	Prepared in the lab
100mM NaOH	Prepared in the lab