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From Inundations to Golden Opportunity: Turning Holopelagic *Sargassum* spp. into a Valuable Feed Ingredient through Arsenic Removal

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Abstract: For over a decade, numerous Greater Caribbean and Western African coasts have received enormous masses of holopelagic *Sargassum* spp. (sargasso). A promising use of this beached biomass as a feed ingredient in the animal industry is restricted by its high arsenic (As) content. This proof of concept aimed to demonstrate that simple, low-cost processes involving hot water (either fresh or seawater) and/or citric acid can remove arsenic from the sargasso. Sargasso collected from a Mexican Caribbean beach in December 2023 had a total arsenic level of 62.2 mg/kg, which decreased to 7.2 mg/kg after treatment with hot freshwater (90 °C for 15 min), and then further decreased to 0.8 mg/kg when followed up with a citric acid treatment. Sargasso collected in March 2024 had total arsenic of 89 mg/kg, which was lowered to 2.6 mg/kg by applying hot freshwater and citric acid sequentially. Employing only citric acid reduced the arsenic concentration to 8.0 mg/kg, while treating the sargasso only with hot seawater reduced the As level to 10.1 mg/kg. Thus, simply using hot water, either fresh or seawater, lowered the arsenic levels to acceptable levels for the animal feeding sector. These straightforward and potentially cost-effective methods may transform the restraint of high arsenic contents into a valuable opportunity to use these seaweeds as animal feed.

Keywords: feed industry; sargasso; seaweed; toxic metal; valorization

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1. Introduction

Holopelagic *Sargassum* spp. (*S. fluitans* and *S. natans*, hereafter referred to as sargasso) are a potentially significant natural seaweed resource that has emerged as the world's largest recurring seaweed bloom since 2011 [1]. During a peak bloom of 2018, over 20 million wet tons of these seaweeds were found in the tropical Atlantic, Caribbean Sea, and Gulf of Mexico, collectively referred to as the Great Atlantic Sargassum Belt [1]. This biomass accounts for 95.8% of total seaweed produced by capture and aquaculture in 2010 and 57% in 2018 [1,2]. Periodic inundations of sargasso have impacted the Caribbean environment, economies, and societies. Beach cleanup efforts are costly [3,4], and valorizing the algal biomass may turn the problem into a valuable golden opportunity, and at the same time, mitigate the impacts of the inundations [4–6]. However, high total arsenic contents, even exceeding 200 mg/kg of dry weight, have been reported [7–11]. The inorganic component can contribute as much as 81.7% of total concentration [8,9].

Arsenic is an element found naturally in seawater in both inorganic and organic forms. Organic arsenic is less harmful to organisms than inorganic arsenic [12], and the primary chemical forms of inorganic arsenic in seawater are arsenate (As V) and arsenite (As III), with the latter being more toxic. Excessive arsenic is toxic when taken up from drinking water or food and is associated with various health complications, such as cancer, cardiovascular disease, and impaired cognitive development in children [13]. In aquatic systems, arsenic is taken up by phytoplankton [12] or other primary producers such as seaweed [7]. Bioaccumulation and transformation of arsenic during trophic transfers are of concern to human consumers of aquatic foods [14].

These concerns also apply if the aquatic resources are used as ingredients in the animal feed industry, where antinutritional factors or toxic elements may harm the animals [15] or consumers. Seaweed can contain considerable amounts of total arsenic, with the forms of arsenic varying depending on the species [6]. Brown seaweed tend to contain higher quantities of arsenic than green or red seaweeds [16]. For example, the brown seaweed *Sargassum fusiforme* (hijiki) had up to 147 mg/kg total arsenic and 69.5 mg/kg inorganic arsenic [16]. This seaweed has been used as food and medicine in Asia for generations, but due to its high arsenic levels, it is now deemed harmful and classified as a carcinogen type 1 [17]. As a result, methods for removing arsenic from this seaweed have been developed, ensuring that its arsenic levels fall within the maximum permissible amounts indicated by various regulations [17].

Sargasso biomass has potential as animal feed [18–20] because it contains valuable nutrients, including all the essential amino acids, polyunsaturated fatty acids, carotenoids, and bioactive compounds [18]. When sargasso is fed to shrimp or fish, their growth and immunological responses are increased [4]. Furthermore, sargasso feed can offer additional beneficial properties to the food products, such as milk or eggs presenting lower cholesterol and triglyceride levels [4]. Nevertheless, how much sargasso can be included in a diet will vary across the targeted animals depending on their tolerances to high fiber content, salts, or other antinutritional factors [4,18].

Wang et al. [17] described a technique to remove arsenic from *S. fusiforme*, which may also be applied to sargasso, as they belong to the same genus. This work was based on the methodology proposed by Wang et al. [17] with some modifications. This information will enable future consideration of sargasso as a potential feed ingredient for livestock and aquaculture.

2. Materials and Methods

2.1. Collection and Preparation

For this study, *S. fluitans* III, the dominant morphotype in the Mexican Caribbean [21], was used. The classical nomenclature of Parr [22] was used for homologation with the previous literature, but a new categorization has been proposed recently [23]. Two arsenic removal tests were applied. In December 2023, one sample of ~200 g wet weight of fresh *S. fluitans* III was collected from the beach of Puerto Morelos, Mexican Caribbean (20°52′5′′ N, 86°52′1′′ W). In March 2024, three samples of ~200 g wet weight, each of the same species, were obtained at the same site. Freshly stranded sargasso was collected from the beach and placed in a cooler for its transport to the laboratory (<20 min). In the laboratory, large debris and fauna were removed from the samples. The seaweed was then rinsed with distilled (first experimental test) or tap water (second experimental test), and a salad spinner was used to remove the excess water.

2.2. Arsenic Removal Treatments

Four treatments were performed for the first experimental test (Figure 1). The first treatment (Control) consisted of rinsing the sargasso with distilled water (1 L). The second treatment (Hot water) consisted of immersing sargasso in 300 mL of stirred distilled water at 90 °C for 15 min. The third [Hot water + reagent-grade citric acid (RG)] and fourth treatment [Hot water + food-grade citric acid (FG)] consisted of soaking the sargasso in stirred distilled water (300 mL) at 90 °C for 15 min, draining the resulting mass, and then immersing it in a 0.4% citric acid solution (500 mL) at 60 °C for 2 h. One subsample of 20 g wet weight was used per treatment as a preliminary evaluation of the efficacy of each treatment.

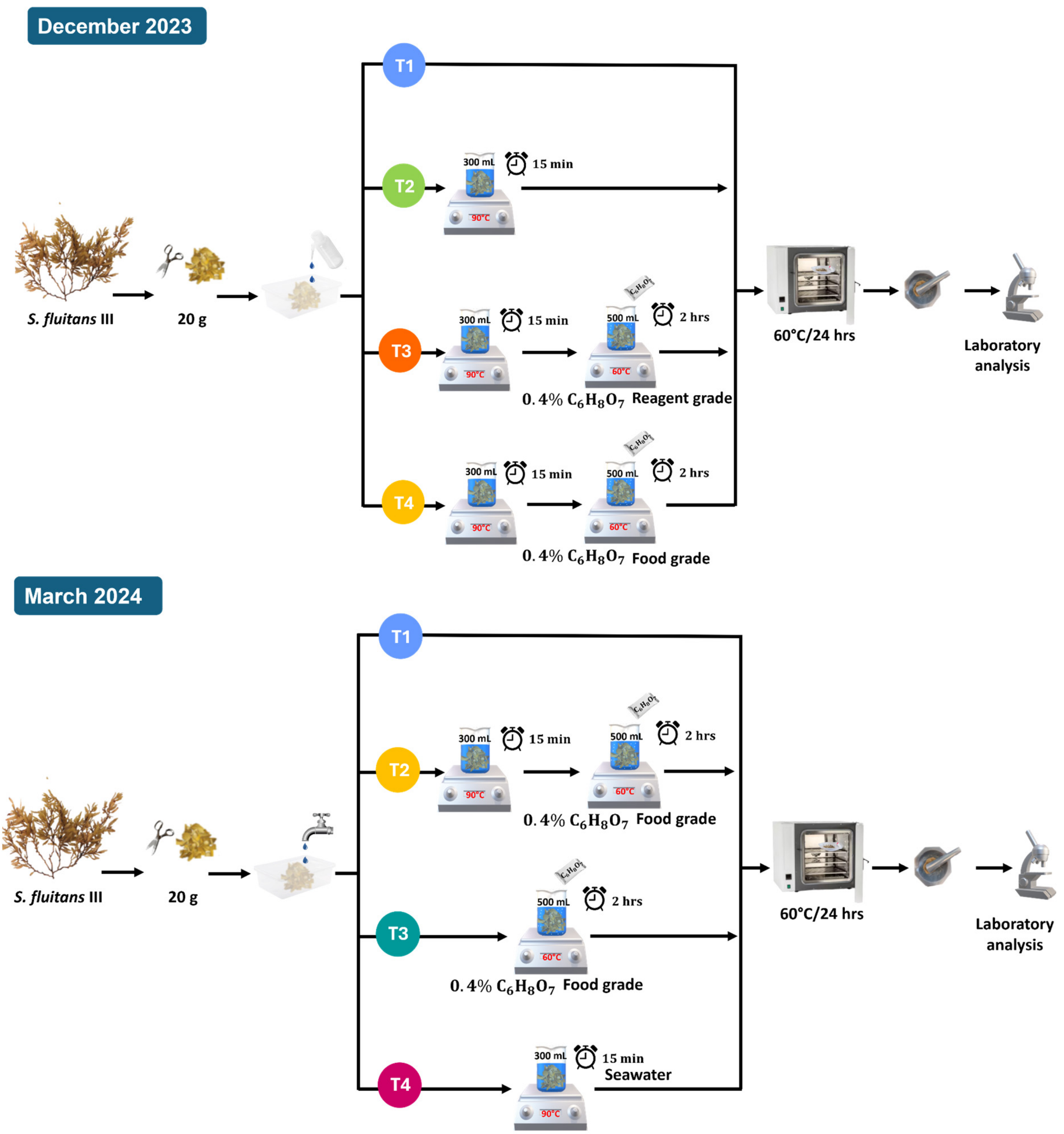

Figure 1. Flow diagram of the treatments. December 2023, T1: Fresh sargasso washed with distilled water (control), T2: Hot water (immersion in distilled water at 90 °C for 15 min), T3: Hot water + citric acid (immersion in hot water and subsequently reagent-grade citric acid at 60 °C for 2 h), T4: as T3 treatment, but with food-grade citric acid. March 2024, T1: Control, T2: as the T4 from the test of December 2023, T3: food grade citric acid (immersion for 2 h in a citric acid solution), T4: hot seawater (immersion in seawater at 90 °C for 15 min). After the application of each treatment, samples were dried, and total arsenic content was analyzed.

The second experimental test aimed to validate the results of the previous experiment and to test for more time- or water-use-efficient variants of the arsenic removal process. Each treatment was run in triplicate with 20 g wet-weight subsamples per replicate. The first treatment (Control) remained as the control from the first experimental test and consisted of rinsing with tap water. The second treatment (Hot water + citric acid FG) consisted of applying the fourth treatment from the first experimental test. The third treatment (Only citric acid FG) involved submerging the sargasso for two hours at 60 °C in a 0.4% citric acid solution (500 mL) to evaluate whether the 15 min of hot water at 90 °C could be avoided. For the last treatment, a single sample was used to determine whether seawater (300 mL) heated to 90 °C for 15 min could effectively remove arsenic.

Following each treatment from both experimental tests, the sargasso mass was rinsed with distilled water and drained. After oven-drying at 60 °C for 24 h, sargasso was ground with Agatha's mortar and pestle before total arsenic analysis.

2.3. Arsenic Determination

All samples were analyzed at the Institutional Chemistry Laboratory of ECOSUR in Chetumal, Quintana Roo, México, for total arsenic content (mg/kg). Samples were processed using an open-vessel wet ashing digestion procedure. A 0.5 g dry sample was placed in a digestion vessel (TECNAL®, Niort, France), to which 5 mL of concentrated HNO_3 (RG) was added. It was predigested for one night at room temperature in a fume hood and then heated at 95 °C for 2 h in a digester block (TECNAL®). Afterwards, the samples were cooled at room temperature, and 1.5 mL of HCl (RG) was added and heated again at 95 °C for 1 h. After cooling to room temperature, the resulting digest was diluted with deionized water with deionized water, filtered through Whatman #2 filter paper, and brought up to 50 mL final volume with deionized water after adding 0.2 mL of pre-reducing agent KI 20% (RG) to achieve a quantitative reduction to As (III). The mixture was left to react at room temperature for at least 4 h before analysis. The arsenic content was determined using a GBC Avanta PM atomic absorption spectrometer with a continuous flow hydride generator unit (HG 3000). It was used with a flame-heated quartz cell and an arsenic hollow cathode lamp (Photron®, San Diego, CA, USA). The instrumental parameters were as follows: wavelength, 193.7 nm; slit width, 1.0 nm; current lamp, 8.0 mA; flame air flow, 12 L/min; and acetylene, 1.5 L/min. The quality control accuracy of the employed procedure was confirmed by blanks and sample triplicate analysis as well as the calculation of recovery percentages of arsenic standard solutions (High Purity Standards, Charleston, SC, USA) and a certified reference material PACS-2 (Marine Sediment for Trace Metals) from the National Research Council Canada. Recovery percentages were between 80 and 120%.

2.4. Statistical Analysis

Differences in total arsenic contents among the March 2024 treatments were assessed using Kruskal–Wallis followed by Dunn's post hoc test as the data did not follow a normal distribution. Analyses were performed using R Studio 2024.04.2 + 764.

3. Results

The sargasso collected in December 2023 had a total As content of 62.2 mg/kg. After 15 min of hot water application at 90 °C to the sargasso, the total As content was reduced to 7.2 mg/kg (Figure 2A). Following the successive application of hot water and citric acid, total arsenic levels were down to 0.8 mg/kg with both the reagent-grade and food-grade citric acid (Figure 2A).

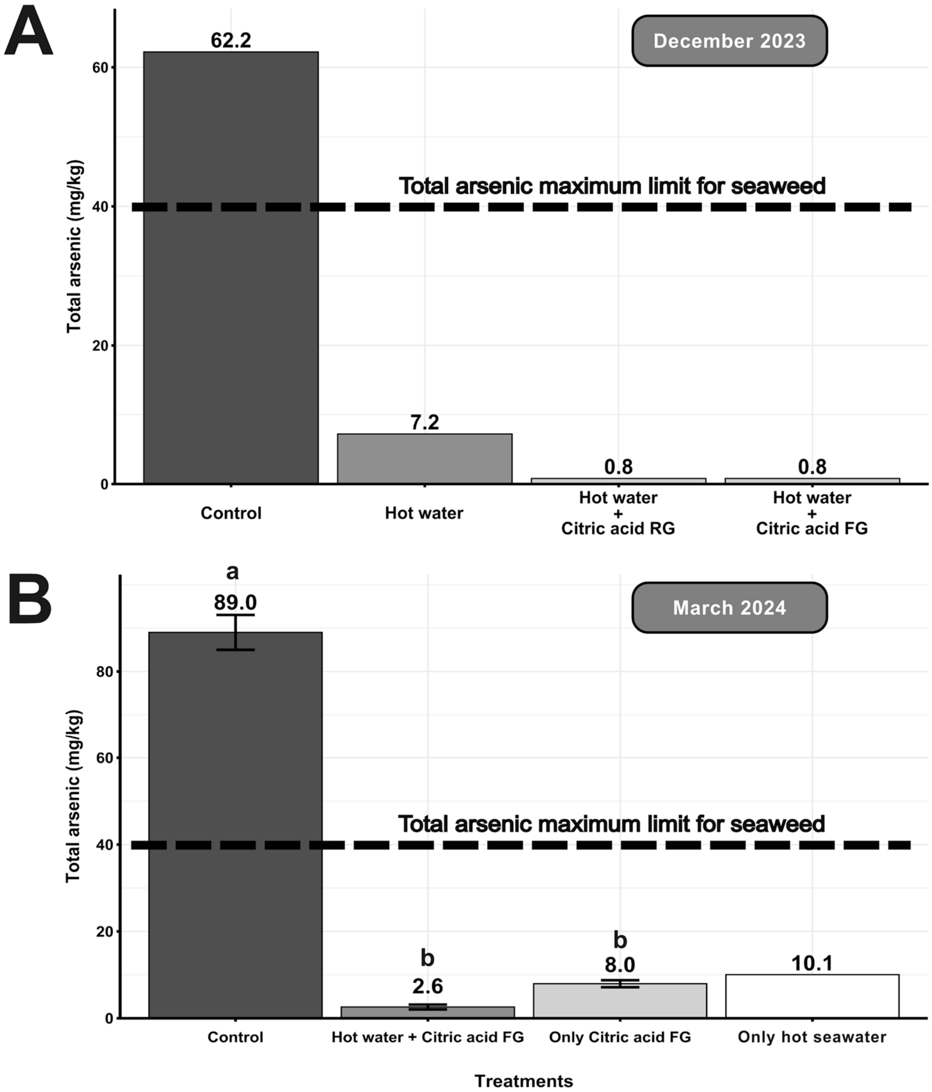

Figure 2. Total arsenic concentration (mg/kg, dry weight) in *Sargassum fluitans* III after application of various treatments. (**A**) Experiment 1, (**B**) Experiment 2, different small letters denote significant differences between the treatments with Dunn's test. RG reagent grade, FG food grade. The horizontal dashed according to European regulations [24].

The second arsenic removal test (March 2024, N = 3 per treatment) revealed significant effects of the treatments (Kruskall–Wallis, $p < 0.05$; Figure 2A). According to Dunn's test, both the hot water with food-grade citric acid treatment and the citric acid alone for two hours at 60 °C significantly reduced total arsenic concentrations compared to the control (Figure 2B). Treating sargasso with hot seawater lowered the total arsenic content from 89.0 mg/kg to 10.1 mg/kg (Figure 2B).

4. Discussion

This work demonstrated that the methods (with small modifications) implemented by Wang et al. [17] to remove arsenic from a benthic *Sargassum* species (*S. fusiforme*) can also be applied to treat the holopelagic congeneric species *S. fluitans* III. All the methods tested in this work removed the arsenic in sargasso well below 40 mg/kg, the level considered generally safe as an animal feed ingredient [24]. In Mexico, no regulations exist for arsenic content in animal feed. The maximum permissible limit in Mexico's drinking water is 25 µg As/L [25]. The World Health Organization [26] stipulated the safe limits of a maximum of 10 μg/L for drinking water and a tolerable intake of 2–7 μg/kg body weight per day for human consumption.

The collected *S. fluitans* III from the beach at Puerto Morelos, Mexico, had a total arsenic content of 62.2 mg/kg in December 2023 and of 89.0 mg/kg in March 2024 (Figure 2). These contents are in the same range to those often reported for the sargasso collected from the beach or near-shore waters, although the arsenic concentrations vary among holopelagic *Sargassum* species, morphotypes [10,11], and spatiotemporally (Table 1). Therefore, lower and higher values can be found in the sargasso from other localities during different times of the year.

Table 1. Arsenic content in beached or near-shore sargasso (mixed morphotypes, or *Sargassum fluitans* III) in different localities. DR Dominican Republic, NA not available.

Country/Territory	Month and Year	Locality	As Content (mg/kg)	Reference
DR	October 2015	Ojeda Beach, Boca Chica Beach, Guayacanes	13.6–42.3	[27]
Barbados	June 2018	Consett Bay	35.2	[28]
Mexico	June 2018–May 2019	Puerto Morelos	48.2–175.0	[10]
Mexico	August 2018–June 2019	Contoy Island, Puerto Morelos, Cozumel, Mahahual, Chinchorro, Xahuayxol, Xcalak	24.0–172.0	[11]
Mexico	September 2018	Tulum, Akumal, Playa del Carmen, Puerto Morelos, Cancún	29.0–65.7	[29]
Turks & Caicos	June 2019	Shark Bay	123.9	[30]
Guadeloupe	May 2020–September 2021	Petit Cul-du-Sac Marin	53.5–145.2	[31]
Mexico	July 2020	Cancun, Puerto Morelos	55.9	[30]
Jamaica	August 2020	Hellshire bay	86.6	[30]
Mexico	January 2021	Cancun, Puerto Morelos	53.9	[30]
DR	February 2021	Punta Cana	21.4	[30]
Barbados	February–August 2021	Consett Bay	18.3–64.5	[8]
DR	March–August 2021	Bávaro, Punta Cana, Juan Dolio, Guayacanes, San Andrés, Nigua, Enriquillo, Juancho	35.0–101.0	[32]
Mexico	December 2023, March 2024	Puerto Morelos	62.2–89.0	This study
Ghana	NA	Western coastline	13.0–53.5	[33]

According to our findings, 88.5% of the arsenic was removed using only hot water (fresh or seawater), while hot water combined with citric acid (food or reagent grade) removed 97.9%. These findings agree with those published for *S. fusiforme*; when this seaweed was treated with hot water, the total arsenic content was reduced from 76.2 mg/kg to 30.5 mg/kg. But when treated with hot water and 0.4% citric acid for 2 h at 60 °C, the arsenic content decreased to 2.2 mg/kg [17]. In this study, the total arsenic content was reduced in both samples to levels well below the European norm (Figure 2), indicating that the methods effectively reduced arsenic regardless of the initial arsenic concentration. Even though the arsenic removal method was evaluated in only one sargasso species (*S. fluitans* III) from a single location (Puerto Morelos, Mexico), sargasso is dispersed across the tropical Atlantic and this approach might be applied in other places. Beach-collected sargasso can contain a total As content as high as 255.2 mg/kg [17]. But even such high levels may be reduced to below the European norm of 40 mg/kg. If this removal efficiency would remain linear until 255 mg/kg of total arsenic, then applying only hot water would reduce the total arsenic to 28.9 mg/kg.

Although the total arsenic content of the seaweed after the treatments was below the maximum limit stated by the European Union, the inorganic arsenic level must be less than 2 mg/kg [12]. According to Alleyne et al. [8] and Ortega-Flores et al. [9], inorganic arsenic accounts for, respectively, 62% and 14.1% to 81.7% of the total arsenic content. Based on these values, low concentrations of inorganic arsenic can be achieved after arsenic removal treatment (Table 2). However, the inorganic arsenic content may not always be below the norm of 2 mg/kg, as the proportional inorganic arsenic of total arsenic vary seasonally, according to [9] (but see [8]). Considering the potential variations in total and inorganic arsenic contents, we recommend mixing batches of sargasso collected at different occasions or places to ensure that maximum arsenic levels allowed will not be exceeded. Subsequent treatment with *Lactobacillus rhamnosus* as reported for *S. fusiforme* [17], may further reduce the As content.

Table 2. Calculated inorganic arsenic content (mg/kg) in sargasso, after applying the removing arsenic treatments, assuming that 14% [9], 62% [8], or 82% [9] of the total arsenic was in inorganic form. ND not determined.

	December 2023			March 2024		
	14%	62%	82%	14%	62%	82%
Control	8.7	38.6	50.8	12.5	55.2	72.7
Hot (sea) water	1.0	4.5	5.9	1.4	6.8	8.2
Only acid citric food-grade	ND	ND	ND	1.1	4.9	6.5
Hot water + citric acid reagent-grade	0.1	0.5	0.7	ND	ND	ND
Hot water + citric acid food-grade	0.1	0.5	0.7	1.1	1.6	2.1

Employing citric acid as a natural chelating agent offers substantial benefits at an affordable cost and is environmentally friendly. Its mechanism involves forming stable complexes with metal ions, such as arsenic, through its carboxyl and hydroxyl groups, thereby preventing precipitation and enhancing solubility [34–36]. However, this process could impact specific nutritional properties, such as the precipitation of proteins, but the lipid solubility is maintained [37]. Heating could also alter the nutritional properties of sargasso [38] and more research is needed to determine this.

Here, we demonstrated a proof of principle of arsenic removal in sargasso. The availability of freshwater can be a limitation in many Caribbean regions. The finding of this study demonstrating the feasibility of the use of seawater for arsenic removal and achieving total arsenic-safe levels in animal feed by European legislation are promising, but the effectiveness of the combination of hot seawater and citric acid in seawater should be further studied. As a next step, it is important to perform further trials to determine whether this method is cost-effective and can be implemented on a larger scale. For scaling up, solar energy heating of the water may be considered to reduce costs. In addition, developing a treatment to remove metals and reagents from the residual water must be considered, and the use of filtering systems based on sargasso [39] could offer a promising alternative to address this issue. The methods employed in this work may reduce the concentrations of other potentially toxic metals, such as cadmium or lead, which could be determined in future studies. The promising results in this work merit scaling-up trials before application on an industrial scale, without losing sight of potential variations in geographical, social, and economic conditions across localities.

5. Conclusions

This proof-of-concept study has demonstrated that simple methods can effectively reduce the total arsenic content in sargasso to levels acceptable under the European standards for animal feed. Both hot fresh or seawater equally reduced the total arsenic contents in sargasso. Combining this treatment with citric acid achieved acceptable inorganic arsenic levels in the studied samples. Implementing these methods to significantly lower arsenic levels in sargasso not only improves the quality of this resource but also expands its potential applications across various industries, including food production and the manufacturing of derived products. Utilizing freshly beached sargasso for this purpose can contribute to addressing issues related to sargasso inundations.

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