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Accessing the Efficacy of *Sargassum*-Based Aqueous Phase Products Derived from Hydrothermal Carbonisation and Hydrothermal Liquefaction on Plant Growth

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Abstract: Mass *Sargassum* inundations have created opportunities for readily available biomass to be used as a crop enrichment application. However, the heavy metal contents of *Sargassum* pose serious concerns for crop administration and subsequent human consumption. Hydrothermal processing can break the feedstock components, allowing heavy metals to be partitioned, through the utilisation of high temperatures and pressures. As a result, seemingly nutrient-rich phases can be produced. Elemental analyses showed that *Sargassum*-derived fractions contain important macro- and micronutrients for plants, particularly ammonium, orthophosphate, and potassium, making them potential nutrient sources for plant growth. To date, no research has investigated the plant growth potential of hydrothermally processed *Sargassum* products from a bioavailability or biotoxicity perspective. We seek to determine if the aqueous phase products derived following *Sargassum* processing by hydrothermal carbonisation and liquefaction are toxic to higher plants, and if they can support plant growth. Aqueous phase products in $\geq$1% concentrations inhibit root growth and lateral root formation in *Arabidopsis* plants, likely from the presence of inhibitory compounds. However, aqueous phase products in $\leq$0.1% concentrations paired with an established nutrient mix may provide improved leaf and root growth. Both HTC and HTL were capable of eliciting improved foliage growth, while only HTC induced improved root growth. Conclusively, aqueous phase products lack nutrient potency to allow high dilutions for fertiliser application on their own and may contain inhibitory compounds that deter plant growth at high concentrations. However, they might have a purpose as an additive extract. The recovery of important elements needed for plant growth draws a promising path for future applications of hydrothermal processing with different feedstocks.

Keywords: hydrothermal liquefaction; hydrothermal carbonisation; HTC; HTL; *Sargassum*; fertiliser; phytohormones; seaweed; macroalgae; feedstock; heavy metals

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1. Introduction

Seaweeds are gaining increasing interest as a bio-resource, given the vast areas available for cultivation and their potential use as a natural source for numerous value-added recoverable products. Seaweeds' ability to sequester CO_2, coupled with the lack of competition for growing space with terrestrial plants and requirement for constant fresh water supply, provides an appealing option for mass cultivation. Moreover, with composition profiles featuring elemental and organic compounds, seaweed provides an interesting

opportunity for sustainable crop enrichment, potentially presenting an alternative to conventional synthetic petrochemical-derived fertilisers. Indeed, the application of seaweeds as natural fertilisers has been practised for centuries in many coastal communities around the world [1]. In more recent times, commercial seaweed extracts have become available for soil improvement, containing plant nutrients such as potassium, calcium, and iron, important for plant growth of crops, and plant hormones or 'phytohormones', such as abscisic acid, auxins, cytokinins and gibberellins [2,3]. In addition, seaweed extracts can promote the growth of beneficial soil fungi around plant roots and, consequently, improving the growth of higher plants [3].

Since 2011, mass abundances of pelagic *Sargassum* have been smothering beaches and coastlines in the Caribbean in what is colloquially known as 'Golden Tides'. Clear waters are turned murky and brown from decomposing inshore *Sargassum*, deterring tourism, vital to local communities' economies, and causing ecological problems to coral reefs and intertidal ecosystems [4,5]. Clean-up is costly and intensive; downstream solutions for utilising the removed biomass are often suggested to subsidise the process and range from bio-oil production to plastic precursors, animal feed, and fertiliser application. The existing research literature states *Sargassum* has valuable elemental properties required for plant growth and has shown bio-stimulatory effects through the modulation of phytohormones [6,7]. *Sargassum* extracts and enzymatic hydrolysates have been produced and used to treat crops in China for over 30 years. *Sargassum hemiphyllum* hydrolysates have been shown to trigger various immune responses against bacteria in tomato plants [8].

Despite possessing plant-growth-promoting properties, heavy metal contamination of seaweeds raises concerns for the intended purposes of crop enrichment, particularly regarding their arsenic, cadmium, mercury, and lead content [9,10]. The bioactive compounds of seaweeds such as *Sargassum* bind to metal ions, resulting in high affinity and bio-absorption of heavy metals [11–13]. Once applied to crops, heavy metals incorporated within seaweeds can readily transfer from seaweed-associated material to the soils [14]. Consequent heavy metal contamination can hinder plant growth and nutritional content of crops, and even inhibit important beneficial microorganisms such as nitrogen-fixing bacteria [15,16]. Additionally, heavy metal bioaccumulation of crops can pose serious health risks following human or animal consumption [17,18]. Ultimately, seaweeds' propensity for bio-absorption of heavy metals, and their potential ability to transfer from seaweed to crop, makes for a less attractive long-term crop enrichment option.

Hydrothermal processing is recognised as an encouraging treatment for the breakdown of seaweed, recovery of bioavailable organic compounds, and the separation of heavy metals [19,20]. Hydrothermal treatments, which encompass both hydrothermal carbonisation (HTC) and hydrothermal liquefaction (HTL), are processes which heat liquid to elevated temperatures and pressures to break down biomass, producing valuable products rich in hydrocarbons known as 'bio-crude' oil; solid phase, known as 'biochar', containing metals; and an aqueous phase, comprising organic compounds and minerals [21]. Aqueous phase products are gaining increasing interest as a useful by-product, having have been found to cultivate microalgae using recovered nutrients from hydrothermal processing [22,23]. The fluid-based nature of hydrothermal processing allows for the use of wet feedstocks, and its subsequent disregard for energy-intensive drying processes, coupled with partitioning over 99% of metals to the solid phase [21,24], makes for an ideal seaweed nutrient recovery process, given an appropriate feedstock. In addition, the lack of a rinsing process for hydrothermal treatment allows for more nutrients to be retained [25].

While research efforts have focused on the suitability of seaweed as a feedstock for hydrothermal treatments and the suggestions of aqueous phase products to be utilised as fertilisers, no studies to our knowledge have tested the actual plant growth potential of hydrothermally processed products. In this study, we address this gap in knowledge and evaluate the efficacy of aqueous phase products derived from hydrothermal processing, via plant trials, using problematic *Sargassum* as the feedstock to help produce a sustainable, value-added fertiliser solution from the current mass inundations. The objectives of this

study are to determine (a) whether HTC and HTL aqueous phase products are toxic or inhibitory to *Arabidopsis* plants, (b) investigate if aqueous phase products can support plant growth both independently and as a supplement, and (c) to evaluate the most suitable hydrothermal treatment temperatures and dilutions for optimum results.

2. Materials and Methods

2.1. Collection of Sargassum and Hydrothermal Pre-Treatments

Samples of *Sargassum muticum* (UKSarg) were wild-harvested from Broadsands Beach, Paignton, UK multiple times between February and March 2020, frozen at −80 °C and then freeze-dried at −55 °C. Mexican samples (MexSarg) were collected from a beach in Cancún (June 2020), shipped to the Biorganix laboratory in Saltillo, Mexico, where they were cleaned of sand and non-*Sargassum* debris, and sundried for 48 h at temperatures between 25 °C and 35 °C during the day and 18 °C and 25 °C at night-time, with a constant flow of air from a ventilator. Mexican *Sargassum* samples collected were a mixture of different species (*Sargassum fluitans* and *Sargassum natans*) and were consequently referred to as *Sargassum* spp. Prior to processing, *Sargassum* were washed under running tap water to further remove any residual sand and salt and left to dry inside a walk-in fume hood for 24 h. HTC reactions were carried out using a bench top stirred reactor (Parr Instruments Company, Moline IL, USA), subject to temperatures of 180 °C and 250 °C, subsequently referred to as HTC180 and HTC250, respectively, with pressures of 40 bar for 1 h. Additional water was added to the reactor with the liquid-to-solid ratio kept constant at 4.7 (g/g) across all HTC reactions. The reactor was cooled via an in-built cooling coil connected to a water chiller, with the water temperature at −4 °C. HTL treatments were subject to temperatures of 300 °C and 350 °C and subsequently referred to as HTL300 and HTL350, respectively, with pressures of 250 bar over a 15 min duration in a 50 cm^3 reactor constructed using stainless steel Swagelok® (Solon, OH, USA) tube fittings. The body of the reactor consisted of a tubing capped at one end and connected at the other end to a pressure gauge, thermocouple, needle, and pressure relief valve. The HTL reactor was left to cool at room temperature. Cooling time for both HTC and HTL reactions was 20–25 min and, after cooling, products were separated by filtration using gravity. The resulting aqueous phase product was collected for intended use as a fertiliser product trial (Figure 1).

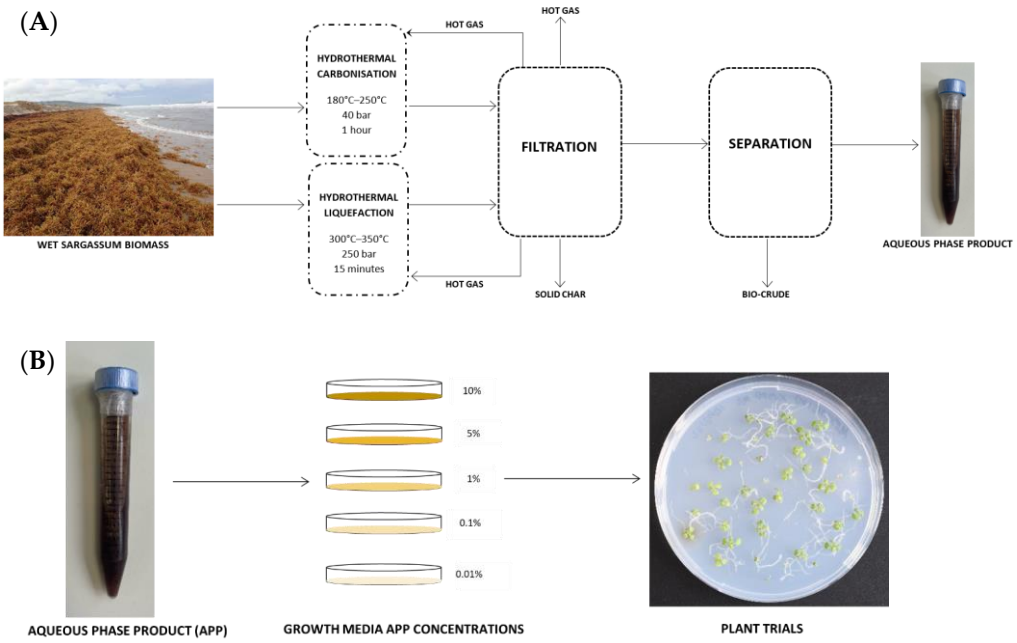

Figure 1. Diagrams demonstrating (**A**) HTC and HTL treatments processing *Sargassum* and associated by-products and (**B**) fertiliser trials using highly nutritious aqueous phase products on *Arabidopsis*.

2.2. Elemental Composition Analysis

HTL and HTC aqueous phase products were analysed for determining the content of $PO4^{3-}$-P, NH^{4+}-N, total nitrogen, and total organic carbon using test kits (Hach, Dusseldorf, Germany) and measured with a Hach DR3900 Spectrophotometer.

ICP-OES analysis of HTL and HTC aqueous phase material was performed as a service by MEDAC Ltd., Chobham, Surrey, UK and tested for the following elements: Al, As, B, Ba, Ca, Cu, Dy, Eu, Fe, Hg, K, Li, Lu, Mg, Mn, Na, Ni, P, Pb, Pt, Sc, Si, Sr, Y, Yb, and Zn. The minimum detectable limit of ICP-OES was 0.01 ppm.

2.3. Arabidopsis Seedling Growth Assays

Concentrations of aqueous phase product (10%, 5%, 1%, 0.1%, and 0.01%) were produced for both UKSarg and MexSarg for HTC180 using purified water from a Milli-Q purification system. Concentrations of 0.1% were made across all product treatments (HTC180, HTC250, HTL300, and HTL350). Media were set to pH 5.8 and mixed with 0.8% (*w/v*) agar. Control media treatments were made using half-strength Murashige and Skoog (MS½) to give a concentration of 2.2 g/L. Additional solutions were made with low-concentration aqueous phase products (APPs) (1%, 0.1%, and 0.01%) and MS½. Solutions were autoclaved to avoid any contamination. Under a laminar flow hood, 25 mL of each diluted product solution, control treatment, and diluted APP solution paired with MS½ were poured into individual Petri dishes and allowed to solidify in a biological safety cabinet.

Arabidopsis thaliana Columbia genotype (Col-0) seeds were sterilised with 70% ethanol + 0.5% triton × 100 for 1 min, centrifuged, decanted, and resuspended in 100% ethanol. Seeds were dried on sterilised filter paper under a laminar flow hood. Seeds were sown on solidified media plates maintaining generous uniform distances between each seed. Plates were sealed with Micropore tape and vernalised at 4 °C for 3 days. Plates were placed in growth cabinets with a relative humidity of 70% at 22 °C under a photoperiod of 16 h/8 h (light/dark) (100 μmol m^2 s^1). After 2–3 weeks, plates were removed and analysed.

2.4. Leaf Growth Analysis

Leaf growth was determined by photographing *Arabidopsis* seedings in plates directly from above with a ruler or known distance in frame to set the scale. Images were analysed using Fiji Image J (version 1.53f51) to first alter colour thresholds to select the foliage of each individual seedling and analysed using a ROI manager to calculate the foliage area of each seedling in cm^2. Control treatments were repeated 2–4 times.

2.5. Root Growth Analysis

Individual *Arabidopsis* seedings were taken out of agar plates carefully to avoid root breakages and placed on a black background to easily disseminate root growth. Pictures were taken from directly above with a ruler in frame for scale. Root length was determined using Fiji Image J by measuring the length of the main root and comparing to scale. Lateral root count was determined by counting occurrences of laterally growing roots. Control treatments were repeated 2–4 times.

2.6. Data Analysis

To determine if significant differences occurred between control and treatments, data were first tested for normality using the Shapiro–Wilk test. Consequently, either the two-tailed *t*-test (normally distributed data) or Mann–Whitney U test (non-normally distributed data) were implemented depending on their appropriation. Data analysis was carried out using SPSS statistics 28.0.

3. Results and Discussion

3.1. Heavy Metal Content and Potential Toxicity

MexSarg products were found to have no detectable levels of cadmium and chromium, small amounts of mercury (<5 ppm) and negligible levels of lead (around 1 ppm) in HTL300 and HTL350 (Table 1). UKSarg products contained negligible amounts of cadmium and lead (<0.7 ppm) only in the HTC180 and HTC250 treatments, respectively (Table 2). Chromium and mercury were detected but only in small amounts of around 3 ppm in UKSarg. Concentrations of arsenic were significantly higher in UKSarg (up to 84.5 ppm) than MexSar (up to 23.85 ppm). MexSarg arsenic levels ranged from 5.45 to 23.85 ppm and were within natural levels found in soil which can be found up to 40 mg/kg and under the limit required for seaweed feed [26,27]. Such low arsenic levels are unlikely to cause concern for toxification when APPs are diluted as a plant feed. In addition, aluminium was found in all samples, with levels ranging from 16.01 to 76.66 ppm. Aluminium is known to accumulate in seaweeds [28], and despite not being an essential plant nutrient, it can provide beneficial anti-pathogenic properties to plants at low concentrations, though in higher concentrations, it can cause root inhibition and toxicity in plants [29].

Table 1. Elemental and organic compound analysis for HTC and HTL treatments of Mexican *Sargassum* aqueous phase product by colorimetric analysis (ppm) (Hach Lange) and ICP-OES (ppm) analysis. Quantities not detected are denoted with "-".

	HTC 180	HTC 250	HTL 300	HTL 350
Orthophosphate ($PO4^{3-}$-P)	292.4 ± 3.9 [a]	215.6 ± 6.2 [a]	316 ± 7.9 [a]	276 ± 33.9 [a]
Ammonium ($NH4^{+}$-N)	109 ± 2.1 [a]	398.4 ± 38.9 [a]	558.8 ± 10.7 [a]	407.2 ± 33 [a]
Total N	1720 ± 79.2 [a]	2792 ± 916.4 [a]	2328 ± 610 [a]	2362 ± 314 [a]
TOC	15,175 ± 35.4 [a]	10,030 ± 113.1 [a]	11,030 ± 594 [a]	13,245 ± 92 [a]
Al	57.92	18.4	16.35	26.92
B	569.68	488.93	470.87	498.13
Ba	28.28	51.79	42.93	40.89
Ca	63,604.77	26,197.61	36,514.48	22,446.34
Co	0.68	-	-	0.34
Cu	14.99	13.63	4.43	11.24
Fe	4.09	1.7	3.75	9.54
K	793.87	555.03	564.57	785.69
Mg	10,385.01	4494.04	5362.86	5284.50
Mn	8.52	1.36	5.79	12.27
Na	1641.91	1273.25	1382.96	1367.63
Ni	5.45	1.36	9.54	9.54
P	53.49	19.08	112.10	39.18
Si	482.11	97.79	314.48	139.69
Sr	2257.92	1579.22	1619.08	1168.31
Zn	3.06	1.36	28.22	5.78
As	23.85	17.38	19.08	5.45
Cd	-	-	-	-
Cr	-	-	-	-
Hg	-	4.77	2.04	3.41
Pb	-	-	0.34	1.02

[a] Analysed using Hach Lange.

3.2. Elemental and Organic Compounds Breakdown Analysis

NH^{4+}-N (ammonium) and $PO4^{3-}$-P (orthophosphate) were detected in higher concentrations in UKSarg treatments compared to MexSarg treatments (Tables 1 and 2). Potassium concentrations were over 100-fold higher in UKSarg products across all treatments compared to MexSarg products. Significant potassium recovery was consistent with aqueous phase recovery composition of previously tested HTL-processed *Sargassum* [24].

Table 2. Elemental and organic compound analysis for HTC and HTL treatments of British *Sargassum* aqueous phase product by colorimetric analysis (ppm) (Hach Lange) and ICP-OES (ppm). Quantities not detected are denoted with "-".

	HTC 180	HTC 250	HTL 300	HTL 350
Orthophosphate ($PO4^{3-}$-P)	790.8 ± 41.2 [a]	410.4 ± 46.4 [a]	428 ± 3 [a]	400 ± 100 [a]
Ammonium ($NH4^{+}$-N)	468 ± 0.8 [a]	1120 ± 24 [a]	1204 ± 17 [a]	1736 ± 45 [a]
Total N	3276 ± 116 [a]	3828 ± 28 [a]	3811 ± 39 [a]	4264 ± 124 [a]
TOC	15,725 ± 85 [a]	17,110 ± 20 [a]	14,725 ± 205 [a]	17,415 ± 106 [a]
Al	36.46	52.47	16.01	76.66
B	357.07	382.28	308.01	438.16
Ba	7.16	6.81	12.27	8.18
Ca	8190.80	5488.93	16,446.34	7921.64
Co	0.34	0.34	1.7	0.34
Cu	6.47	2.04	12.27	0.68
Fe	15.67	3.41	278.71	6.81
K	96,327.09	95,209.54	78,729.13	127,413.97
Mg	27,734.24	5921.64	6258.94	4603.07
Mn	31.35	5.11	33.73	4.77
Na	12,735.95	11,764.91	9359.45	13,638.84
Ni	2.73	-	97.44	1.7
P	2918.91	94.04	594.21	135.6
Si	463.71	129.81	108.01	97.10
Sr	933.22	202.73	1074.28	375.13
Zn	8.52	0.68	54.86	0.34
As	84.50	67.46	46.68	45.66
Cd	0.34	-	-	-
Cr	-	0.34	3.07	0.34
Hg	-	-	3.07	-
Pb	-	0.68	-	-

[a] Analysed using Hach Lange.

HTC180 treatments contained significantly more magnesium than any other treatments, while concentrations of calcium were most considerable in HTC180 of MexSarg and HTL300 of UKSarg. Notable concentrations of both magnesium and calcium indicate a promising sign for plant feed as both can work synergistically to facilitate improved plant growth performance [30]. In addition, concentrations of boron were found to be between 300 and 580 ppm across all treatments, which also works synergistically with calcium [31].

Na concentrations were nearly 10-fold higher in UKSarg than MexSarg APPs, but both were considerably lower than unprocessed *Sargassum* [26]. Similar levels of sodium to our analysis can be toxic and cause osmotic stress [32]; however, dilution of APPs is likely to nullify such stresses. In terms of the other essential micronutrients we found, copper, iron, manganese, and zinc were detected in small quantities across all treatments, all capable of providing positive effects on higher plant growth at low concentrations [33,34].

3.3. Arabidopsis Plate Trials

3.3.1. Leaf and Root Growth Inhibition in High Concentrations (10%, 5%, and 1%)

The application of high-concentration APPs (10%, 5% and 1%) resulted in a significant decline of foliage growth, root length growth, and lateral foot formation for both MexSarg and UKSarg treatments across all 10%, 5%, and 1% concentrations (Figure 2). Purple anthocyanin pigmentation, associated with an indication of being stressed, developed most notably in the 10% concentration treatment (Figure A1), indicating a clear sign of unfavourable growing conditions [35]. Pigmentation was less prominent at 5% and 1% concentrations, suggesting that nutrient-deficiency-induced stress was not the main cause of the anthocyanin pigmentation. Instead, production of anthocyanin pigmentation in higher concentrations could be a result of phytohormone-induced stress through ABAs in *Arabidopsis* [36].

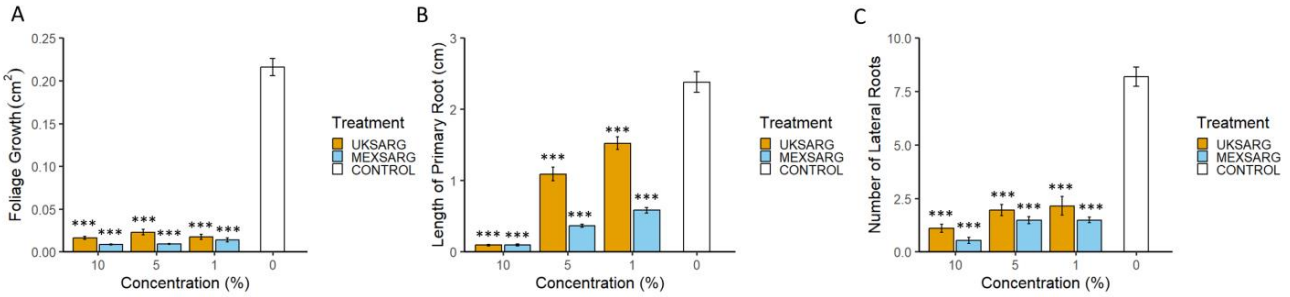

Figure 2. Effects of 0–10% HTC180 aqueous phase product on both UKSarg and MexSarg, with control (MS½) on 21-day-old seedlings. n = 21. (**A**) **Foliage growth**; significant inhibition between treatment and control (Mann–Whitney U) identified in UKSarg: 10% (p = 0.001), 5% (p = 0.001), and 1% (p = 0.001). MexSarg: 10% (p = 0.001), 5% (p = 0.001), and 1% (p = 0.001). (**B**) **Primary root length**; significant inhibition between treatment and control (Mann–Whitney U) identified in UKSarg: 10% (p = 0.001), 5% (p = 0.001), and 1% (p = 0.001). MexSarg: 10% (p = 0.001), 5% (p = 0.001), and 1% (p = 0.001). (**C**) **Number of lateral roots**; significant inhibition between treatment and control (t-test) identified in UKSarg: 10% (p = 0.001), 5% (p = 0.001), and 1% (p = 0.001). MexSarg: 10% (p = 0.001), 5% (p = 0.001), and 1% (p = 0.001). Asterisks signify differences compared to 0% control: *** $p < 0.001$ from the standard error derived from the mean.

Inhibited root growth of primary length and lateral root formation from high-concentration APP treatments were consistent with previous research utilising 10% seaweed extracts [37,38]. Possible causes for root inhibition could be from Na stress at high concentrations [37], but were unlikely to be from ammonium toxicity as other forms of nitrogen besides ammonium were present in our analysis (Tables 1 and 2) [39]. Instead, the presence of cytokinins could contribute towards Al^{3+}-mediated growth inhibition of roots [38]. Similarly, ABA and cytokinin can inhibit lateral root formation and growth [40,41], both of which have been found in *Sargassum* [42].

3.3.2. Inhibitory Effects of 1% Concentration with MS½ on Lateral Root Formation

Following the unsuccessful growth of higher-concentration APPs, lower-concentration APPs (1%, 0.1%, and 0.01%) paired with an additional nutrient mix (MS½) grew successfully, exhibiting no purple pigmentation or leaf chlorosis (Figures 3 and 4). Lower-concentration APPs applied on their own produced significantly stunted foliage growth (Figure A2), likely from severe nutrient deficiency caused by high dilution.

Primary root length showed significant increases with 0.1% concentration paired with MS½ in both MexSarg (2.23 cm ± 0.12) and UKSarg (2.56 cm ± 0.16) samples compared to the control (1.47 cm ± 0.11). This is possibly due to potential auxins promoting root growth in *Sargassum*, though small concentrations of ABA have been found to promote elongation of primary roots in *Arabidopsis*. Furthermore, the additional macro- and micronutrients of APPs could provide enhanced growth with MS½ nutrients, along with the additional vitamins and amino acids found in *Sargassum* [26].

Interestingly, lateral root formation and primary root length at 1% APP concentration with the addition of MS½ nutrient mix were reduced significantly in both MexSarg and UKSarg, suggesting that root inhibition is related to a toxic inhibitory substance, rather than a lack of nutrient availability at APP concentrations as low as 1%. Meanwhile, more dilute concentrations of 0.1% and 0.01% indicate a sufficient dilution of APP that does not inhibit growth with a paired nutrient mix (Figure 3). These findings indicate concentrations of 0.1% may be the most suitable for plant growth (Figure A3).

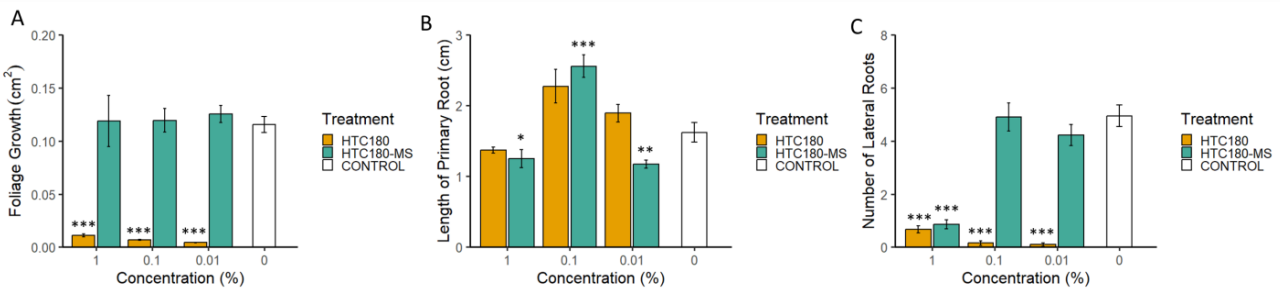

Figure 3. Effects of 0–1% HTC180 aqueous phase product paired with and without MS½ on UKSarg, with control (MS½) on 14-day-old seedlings. n = 20–24. (**A**) **Foliage growth**; significant difference between treatment and control (Mann–Whitney U) identified in HTC180: 1% (p = 0.001), 0.1% (p = 0.001), and 0.01% (p = 0.001). (**B**) **Primary root length**; significant difference between treatment and control (t-test) identified in HTC180: 0.1% (0.003) and 0.01% (0.014). HTC180-MS: 0.1% (p = 0.001) and 0.01% (p = 0.015). (**C**) **Number of lateral roots**; significant difference between treatment and control (t-test) identified in HTC180: 1% (p = 0.001), 0.1% (p = 0.001), and 0.01% (p = 0.001). HTC180-MS: 1% (p = 0.001). Asterisks signify differences compared to 0% control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ from the standard error derived from the mean.

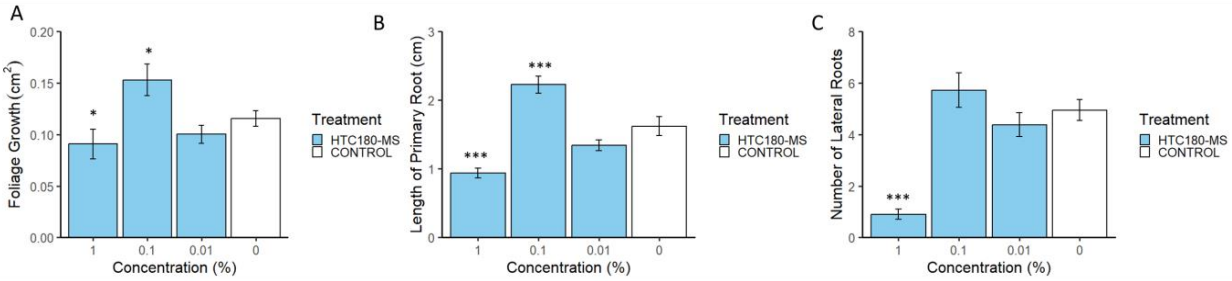

Figure 4. Effects of 0–1% HTC180 aqueous phase product paired with MS½ of MexSarg with control (MS½) on 14-day-old seedlings. n = 20–22. (**A**) **Foliage growth**; significant difference between treatment and control (Mann–Whitney U) identified in 1% (0.046). (**B**) **Primary root length**; significant difference between treatment and control (t-test) identified in 1% (0.001) and 0.1% (0.001). (**C**) **Number of lateral roots**; significant difference between treatment and control (t-test) identified in 1% (p = 0.001). Asterisks signify differences compared to 0% control: * $p < 0.05$, *** $p < 0.001$ from the standard error derived from the mean.

3.3.3. Comparison across HTC and HTL Treatments at 0.1%

After establishing a suitable concentration of 0.1% APP, all HTC and HTL treatments (HTC180, HTC250, HTL300, and HTL350) were examined to determine which treatment could provide the best performance for plant growth. Foliage growth significantly improved in the HTC180 (0.153 $cm^3 \pm 0.02$), HTC250 (0.147 $cm^3 \pm 0.01$), and HTL350 (0.151 $cm^3 \pm 0.01$) treatments of MexSarg compared to the control group (0.115 $cm^3 \pm 0.01$), while no UKSarg treatments showed significant improvements across treatments (Figure 5). Despite this, HTL350 treatments produced the most foliage growth for MexSarg and UKSarg, while HTL300 produced the fewest foliage of all treatments for MexSarg and UKSarg (Figure 5).

HTC180 exhibited the most significant primary root length growth in both MexSarg and UKSarg treatments compared to the control, possibly from the high magnesium content of HTC180 which can promote root growth. Conversely, HTL300 treatments experienced the least root growth of all treatments for both MexSarg and UKSarg. Lateral root formation did not significantly increase across all treatments compared to the control but did show significant reduction in lateral root formation in the HTL300 UKSarg treatment.

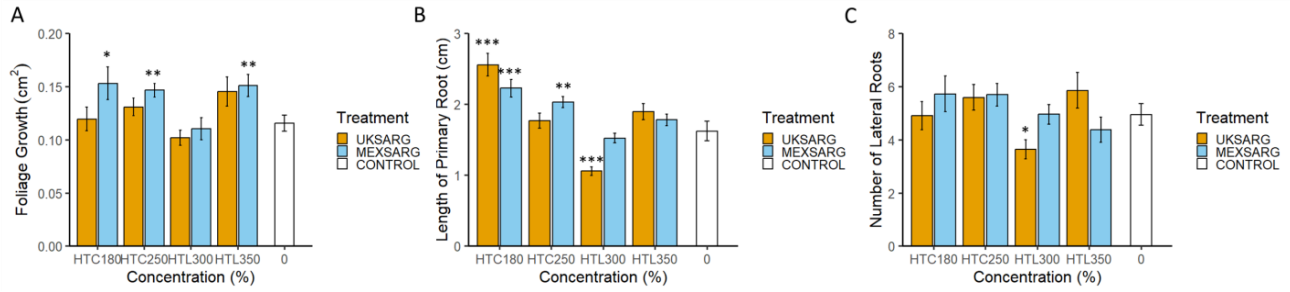

Figure 5. Effects of 0.1% aqueous phase product paired with MS½ across all products (HTC180, HTC250, HTL300, HTL350) on both UKSarg and MexSarg with control (MS½) on 14-day-old seedlings. n = 20–23. (**A**) **Foliage growth**; significant difference between treatment and control (*t*-test) in MexSarg: HTC180 ($p = 0.04$), HTC250 ($p = 0.003$), and HTL350 ($p = 0.008$). (**B**) **Primary root length**; significant difference between treatment and control (*t*-test) in UKSarg HTC180 ($p = 0.001$), HTL300 ($p = 0.001$), and HTL350 ($p = 0.01$). MexSarg: HTC180 (0.001), HTC250 (0.001), and HTL350 (0.028). (**C**) **Number of lateral roots**. Asterisks signify differences compared to 0% control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ from the standard error derived from the mean.

4. Conclusions

Despite the promising nutrient profiles of HTC and HTL aqueous phase products, and their low heavy metal contents, we conclude that such products are not suitable for use as fertilisers on their own, as they appear to deter plant growth at concentrations greater than 1% and lack sufficient nutrients required for supporting basic plant growth at lower concentrations. Presumably, the hinderance of plant growth at high concentration of aqueous phase products is caused by the presence of phytohormones such as ABA or cytokinins or toxic organic products generated during hydrothermal treatment. Consequently, we suggest that a more suitable application of high-concentration HTC and HTL aqueous phase products may be for use as a herbicide in deterring the growth of young seedlings. However, we conclude that both HTC and HTL products paired with sufficient nutrient mix could improve plant growth performance in low concentrations of 0.1% as an extract additive. HTL and HTC treatments pave a promising path for heavy metal partitioning and nutrient recovery and may present more successful applications utilising other biomass as feedstocks in the future for bio-fertiliser production.

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Abbreviations

APP	Aqueous phase product
HTC	Hydrothermal carbonisation
HTL	Hydrothermal liquefaction
UKSarg	British-sourced *Sargassum*
MexSarg	Mexican-sourced *Sargassum*
MS½	Murashige and Skoog medium at half-strength concentration
ABA	Abscisic acid

Appendix A

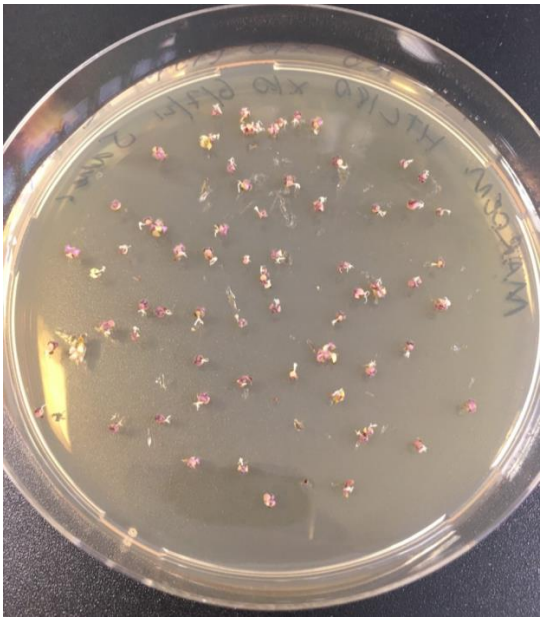

Figure A1. UKSarg HTC180 10% concentration with anthocyanin production in foliage.

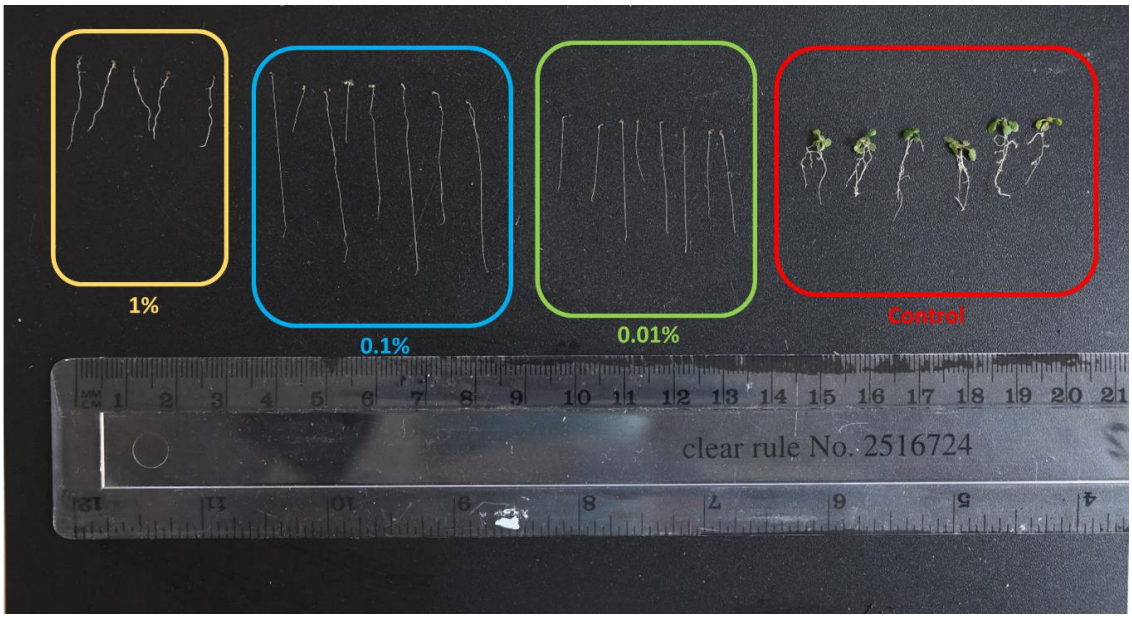

Figure A2. UKSarg HTC180 concentrations of 1% (Yellow), 0.1% (Blue), 0.01% (Green), and Control (Red).

Figure A3. MexSarg HTC180 0.1% concentration with M/S.

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