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The Application of Homogenate and Filtrate from Baltic Seaweeds in Seedling Growth Tests

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Abstract: Algal filtrate and homogenate, obtained from Baltic seaweeds, were applied in seedling growth tests. Radish seeds were used in order to assess algal products phytotoxicity and their biostimulant effect on growth and nutrient uptake. Algal filtrate, at concentrations ranging from 5.0% to 100% was used for seed soaking and as a liquid biostimulant (soil and foliar application). Algal homogenate was developed for seed coating. Algal filtrate and homogenate were also enriched with Zn(II) ions in order to examine the influence on metal ion complexation. The optimal doses of algal filtrate and homogenate, as well as soaking time were established. Multi-elemental analyses of the raw biomass, filtrate, homogenate, and radish were also performed using ICP-OES (Inductively Coupled Plasma—Optical Emission Spectrometry). The best results in terms of seedlings' length and weight were obtained using clear filtrate at a concentration of 50% applied to the soil and for homogenate applied at a dose of 50 mg/g of seeds. Clear filtrate at a concentration of 50% used for seed soaking for one hour showed the best results. The applied algal products increased the content of elements in seedlings. Among the tested products, a concentration of 50% algal filtrate is recommended for future pot and field experiments.

Keywords: Baltic macroalgae; filtrate/homogenate; chelating properties; biostimulant/seed coating; soaking; growth enhancement/seedling growth test; analytical methods

1. Introduction

Many naturally-derived materials are recognized as biostimulants of plant growth, although their mechanism of action is still not completely understood [1]. Generally, five categories of biostimulants are distinguished: microbial inoculants, humic acids, fulvic acids, protein hydrolysates and amino acids, and seaweed extracts [2]. This paper focuses on the last category—algal extracts. Although the concept of the application of seaweed products in agriculture is still evolving, more literature on this subject has appeared, for example review articles by Khan et al. (2009) [3], Sharma et al. (2014) [4], Battacharyya et al. (2015) [5], Calvo et al. 2014 [2], Du Jardin 2015 [6]. Polysaccharides (alginates, carrageenans, laminarin), micro- and macronutrients, sterols, betaines, phytohormones (cytokinins, auxins, abscisic acid, gibberellins), proteins, amino acids, and lipids are the main biologically active compounds detected in algal extracts that contribute to the plant growth promotion [3,4,6]. Special attention should be paid to the content of phytohormones in green macroalgae [7,8]. Indoleacetic acid, which belongs to auxins, is responsible for the induction of elongation growth, apical dominance, and initiation of root formation; abscisic acid controls the stomatal apparatus function, gibberellic acid influences stem elongation and the initiation of seed germination, and polyamines regulate growth

and development at micromolar concentrations [8]. The advantage of biostimulants is their activity (enhancement of plant growth and development) at low concentrations. This response cannot be attributed to the application of traditional plant nutrients [4]. Some authors suggest that seaweed extracts are bioactive at low concentrations—diluted as 1:1000 or more [9].

Extracts can be applied on soils, in hydroponic solutions or as foliar treatments [4,6]. The first two application methods, increase water and low temperature stress resistance, improve nodulation and promote plant growth promoting rhizobacteria. Additionally, algal extracts suppress soil borne diseases and nematodes [3–5]. Arial application of seaweed extracts (e.g., seed treatment, foliar spray) (a) affects improved shoot and root growth, higher flowering and fruit set, better yield and (b) improves the resistance to biotic (e.g., fungal, bacteria, viral pathogens, insect pests) and abiotic stress (e.g., salt, drought, freezing, chilling tolerance, as well as enhanced photosynthesis) in plants [3,4].

Algal extracts can be obtained by different techniques, from classical solvent extraction with organic solvents, to the novel extraction techniques such as supercritical fluid extraction, as well as microwave-, enzyme- and ultrasound assisted extraction [10]. This paper focuses on algae-based products obtained with water as a solvent. Several methods of the isolation of active compounds from the algal biomass in aqueous environment can be distinguished, for example: boiling, autoclaving and homogenization (using blender) with distilled water [11]. In the literature it has been shown that such products have a positive effect on plant growth and development, for example in the works by Kavipriya et al. 2011 [12], Sridhar and Rengasamy 2011 [13], Sathya et al. 2010 [14]; Ahmed and Shalaby 2012 [7]; El Kaoaua et al. 2013 [15]; Kamaladhasan and Subramanian 2009 [16]; Gireesh et al. 2011 [17]. All the listed references concern green macroalgae (*Chlorophyta*), which were used in the present study as a raw material for the production of algal bio-products. Baltic macroalgae are known to be a rich source of proteins, minerals, essential amino acids, essential fatty acids, fiber, and carbohydrates [18,19].

In this work homogenates and filtrates were obtained from Baltic green seaweeds. The information on their production, properties and application is limited. Some of the examples are presented below. In the work of Pise and Sabale (2010), the active compounds from green macroalga *Ulva fasciata* were extracted using a blender, mortar and pestle and then the obtained extract was tested on fenugreek (*Trigonella foenum-graecum* L.) as a seaweed liquid fertilizer [20]. Sunarpi et al. (2010) for the production of algal filtrate used green macroalgae: *Ulva fasciata*, *Ulva ferticulata,* and *Chaetomorpha* sp. They examined its effect, as a liquid seaweed fertilizer, on the growth and yield of rice plants [21]. In the patent of Herve and Percehais (1990) it was found that the combination of four operations: (1) cryocomminution; (2) molecular milling; (3) turbodecantation; and (4) selective ultrafiltration made it possible to obtain a "physiological filtrate", characterized by certain properties such as being directly assimilable (it contains micro-elements, vitamins, micropeptides) and containing no residual alcohols originating from the extraction steps [22].

Another interesting approach is to use algal homogenate and filtrate not only as a biostimulant of plant growth, but also for seed soaking and coating. Seed soaking is a process in which seeds are immersed in appropriate solutions for an extended period of time in order to absorb nutrients, protectants, growth regulators, etc. [23]. Soaking of seeds (for example for 18–24 h) prior to sowing has been used in arid areas of the world to give cereal crops a "head start" in germination [4]. This seed treatment enhances fresh and dry weight, leaf area, plant height, leaf development [24], as well as seedling vigor and chlorophyll content and reduces harmful seed microflora and increases the level of plant defense enzymes [4].

In the case of seed coating, finely ground solids or liquids containing dissolved or suspended solids are used to form a more or less continuous layer that cover the natural seed coat [24,25]. Seed coating provides delivery of desired quantities of compounds, for example micro- and macronutrients, growth regulators. Coating materials can influence also the microenvironment during germination by holding water around the seed or providing a source of oxygen [24].

The aim of the present paper was to produce a filtrate and homogenate from Baltic green macroalgae, determine their physico-chemical properties, and assess phycotoxicity and utilitarian

characteristics in seedling growth tests on radish seeds. Radish was chosen because it can reach maturity in a short time period (up to 2 weeks). The method of the application of algal filtrate (soil and foliar) was examined. Algal filtrate was also used for seed soaking. The homogenate was applied for seed coating. Additionally, the obtained bio-products were enriched with Zn(II) ions in order to examine their ability to complex metal ions. Zinc was chosen as an example of metal ions, because it plays a crucial role in plant growth and metabolism [26]. This issue has not been discussed in details in the available literature yet. Finally, we chose the optimal dose and a method of application of the tested products and recommend them for future research.

2. Materials and Methods

2.1. Chemicals

All reagents were of analytical grade and used without further purification. We purchased 69% nitric acid, spectrally pure (Suprapur) from Merck KGaA (Darmstadt, Germany) and zinc sulphate ($ZnSO_4 \cdot 7H_2O$) from POCH S.A. (Gliwice, Poland).

2.2. Seaweed Biomass

The mixture of Baltic macroalgae (green algae *Cladophora glomerata* (L.) Kütz. (*Cladophoraceae*), *Ulva flexuosa subsp. pilifera* (Kütz.) M.J. Wynne (*Ulvaceae*), *Ulva clathrata* (Roth) Ag. and the red alga *Polysiphonia fucoides* (Hudson) Greville (*Rhodomelaceae*)) was collected from the sea in August 2013 in Sopot, Poland. Then macroalgae were transported to the laboratory and stored at −20 °C prior to extraction. Just before the homogenization process, algae were defrosted and rinsed with fresh water three times to remove salt, small insects, and epiphytes [27].

2.3. Homogenization of Algae

The homogenization process was performed using Thermomix equipment. About 150 g of fresh algal biomass was mixed with 500 mL of deionized water at room temperature, 100–500 rpm for 4 h. Then the obtained product was centrifuged 3 times for 10 min (4500 rpm). The solid residue was algal homogenate (H), whereas the obtained liquid was treated as algal filtrate (F)—100%.

2.4. Products for Seedling Growth Tests

The algal filtrate (F 100%) was diluted to the concentrations of 5.0 (F 5.0%), 25 (F 25%), and 50% (F 50%). Each concentration of the tested filtrates was enriched with Zn(II) ions (as $ZnSO_4 \cdot 7H_2O$)—1% by weight (F 5.0%-Zn, F 25%-Zn, F 50%-Zn, F 100%-Zn). Zn(II) ions were chosen as an example. The amount of the added inorganic salt was based on the composition analysis of commercial biostimulants available on the market and on our previous experiments [28]. The algal homogenate after centrifugation (H 100%) was diluted 10-times (H 10%), following which it was enriched with Zn(II) ions (as $ZnSO_4 \cdot 7H_2O$)—1% by weight (H 10%-Zn). In the seedling growth tests, the control group contained distilled water (C-H_2O) and distilled water enriched with Zn(II) ions (as $ZnSO_4 \cdot 7H_2O$)—1% by weight (C-H_2O-Zn).

2.5. Seedling Growth Tests—Radish Seeds

The tests on seedlings were performed using radish seeds (*Raphanus sativus*), *Caro* variety (from TORSEED, Toruń, Poland) to assess the phytotoxicity of the obtained products. Experiments were performed on Petri dishes (diameter 85 mm) with cotton wool (approximately 5 g; Lohmann and Rauscher Company, Rengsdorf, Germany) soaked in distilled water. On each dish 25 seeds were placed. Before tests, the dishes with seeds were put into the fridge for stratification (3 days at 4 °C). The experiments were performed in standardized conditions—an isolated box with adjustable lighting and temperature (with fluctuations of ±4 °C) known as the Jacobsen Germination Table (type 5000–5300; Laborset, Lodz, Poland) according to the international norm (International Rules for

Seed Testing, 2011—International Seed Testing Association (Bassersdorf, Switzerland)) [29]. The tests lasted for 10 days. The general scheme of the conducted seedling growth tests is presented in Figure 1.

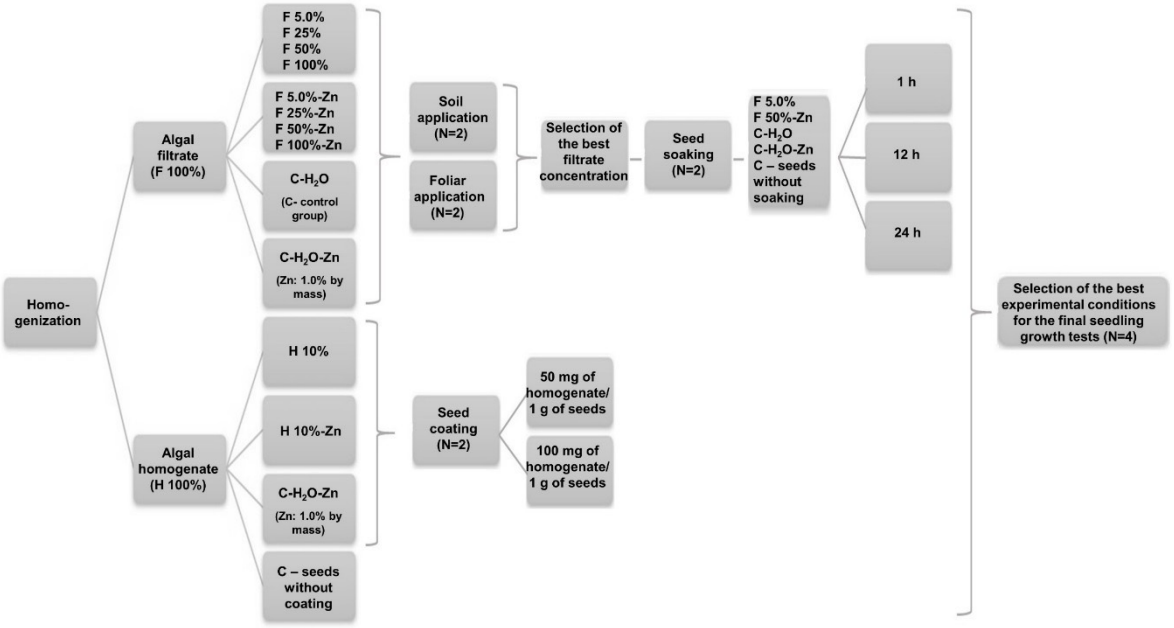


Figure 1. General scheme of the conducted experiments—selection of the best experimental conditions for the tests on radish seeds.

The preliminary experiments concerning the application method (soil/foliar), soaking time and seed coating dose were performed in two replicates. On this basis the best variant was chosen, and for these optimal conditions, four replicates for each tested group were applied in the proper tests on radish.

2.6. Method of the Application of the Obtained Products in the Seedling Growth Tests

2.6.1. Soil and Foliar Application

This experiment aimed at the choice of the best algal filtrate concentration. Each filtrate—clear (F 5.0%, F 25%, F 50%, F 100%) and enriched with Zn(II) ions (F 5.0%-Zn, F 25%-Zn, F 50%-Zn, F 100%-Zn)—was applied twice: 10 ml at the beginning of the experiment and 5 ml after the following 3 days, for the control group (C-H_2O)—10 and 5 mL of distilled water, respectively. For each group two replicates were performed (the choice of the optimal filtrate concentration). In the case of soil application, the cotton wool was evenly moistened with the appropriate filtrates. In the foliar application, the seeds/seedlings were sprayed with the same volume of the appropriate filtrate [27,30].

2.6.2. Seed Treatment

Seed coating was performed as two variants: 50 or 100 mg of 10 times diluted algal homogenate (H 10% and H 10%-Zn) per 1 g of seeds in a test tube shaker (Reax top; Heidolph, Germany) at 120 rpm for 15 min [31,32]. Radish seeds without coating and seeds treated with 50 or 100 mg of aqueous solution of $ZnSO_4 \cdot 7H_2O$ containing 1% of zinc by weight (C-H_2O-Zn) were used as a control group. For each group two replicates were performed. Following treatment, seeds were air-dried and subjected to vegetative tests as shown in Section 2.5.

Soaking of seeds was carried out in four groups. In each group, in plastic containers 25 radish seeds were treated with 15 mL of (1) clear filtrate (F 50%); (2) enriched filtrate (F 50%-Zn); (3) solution of $ZnSO_4 \cdot 7H_2O$ (C-H_2O-Zn); and (4) distilled water—control (C-H_2O), respectively. This selection was

made on the basis of the previously performed experiments concerning the choice of the best algal filtrate concentration (Section—Soil and foliar application). The best period of seed soaking (1, 12 or 24 h) was also chosen. After soaking, seeds were put directly into Petri dishes and were subjected to stratification. Seeds without soaking were used as a control group (they were also kept in a fridge for 1, 12 or 24 h). For each group two replicates were performed.

2.7. Length and Weight of Seedlings

Five seedlings randomly selected from each replicate (two replicates for each tested group) were measured. The average length of 10 seedlings (*N* of seedlings 2 × 5 = 10; 2—number of replicates, 5—number of measured seedlings) is shown in the present work. All seedlings (25) from each Petri dish (*N* = 2; two replicates) were dried and weighed. The result is presented as an average from two measurements of weight of 25 seedlings collected from each Petri dish.

2.8. Analytical Methods

2.8.1. Inorganic Composition of the Algal Biomass, Algal Products, and Seedlings

Organic matter was removed from samples (0.5 g of dry macroalgae and algal products) with 5 mL 69% spectrally pure concentrated nitric acid (Suprapur, Merck KGaA, Darmstadt, Germany) in Teflon bombs in a microwave oven (Milestone S.r.l., Sorisole, Italy). After mineralization, samples were diluted with demineralized water (Millipore Simplicity, Darmstadt, Germany) to 50 g. Digested samples underwent a multi-elemental analysis using ICP–OES iCAP (6500 Duo, Thermo Scientific, Waltham, MA, USA). Quality assurance of the test results was achieved by the Combined Quality Control Standard from ULTRA Scientific (LGC STANDARDS SP. Z O.O., Lomianki, Poland). Samples were analyzed three times (the reported results of the analyses were the arithmetic mean and the relative standard deviation was <5%), within the quality management system according to PN-EN ISO/IEC 17025:2005 [27].

2.8.2. Determination of the Degree of Metal Ions Complexation in the Tested Products

The determination of the complexation degree of zinc ions by algal homogenates was based on the mass spectra analysis recorded in positive and negative regions of the used Mass Spectrometer with electrospray (ESI MS). Mass spectra (MS) were recorded on a ZQ Waters/Micromass Mass Spectrometer (Manchester, UK) with a quadruple analyzer at the following parameters: source potential ESI on capillaries—3 kV; voltage on focal plate—0.5 V; voltage on extractor—4 V; the cone voltage (*cv*)—30 V, ion fragmentation was examined with 10–150 V (*cv*); source temperature—120 °C; evaporation temperature—300 °C; nitrogen was used as a spraying and drying gas with a flow rate of 80 and 300 L/h. Negative and positive ions ESI mass spectra were recorded in the MCA mode (Multi Channel Acquisition) at an m/z = 100–1000 interval. The typical spectrum obtained is the average of 10 scans with a 0.6 s time interval. The solutions studied were introduced to the ionization source (at the flow rate 40 μL/min) through the Harvard's pump. In this method spectra recorded for $ZnSO_4 \cdot 7H_2O$ solutions were used as reference spectra. On the basis of the isotopic composition of zinc (64Zn 48.6%; 66Zn 27.9%; 67Zn 4.1%; 68Zn 18.8%), defined with the signals in the mass spectrum system, homogenates enriched with zinc ions, were attributed to the complexes not present in the spectrum of the solution $ZnSO_4 \cdot 7H_2O$—reference system. The degree of Zn(II) complexation in algal homogenate solutions was also determined by spectrophotometric techniques according to the methodology described by Karamać (2007) [33]. The percentage of bound metal ions Zn(II) in complexes was calculated using the following Equation (1):

$$\text{Bound Zn(II)}(\%) = [1 - \text{Concentration of free Zn(II)}/\text{Concentration of total Zn(II)}] \times 100 \quad (1)$$

Both methods gave similar results for zinc ions complexation level in the homogenate solutions.

2.9. Statistical Analysis

The obtained results were statistically elaborated with *Statistica 10* software (Cracow, Poland) and their distribution was tested with the normality test (Shapiro–Wilk). For normal distribution, homogeneity of variance was checked by means of the Brown–Forsythe test. When two groups were compared, statistically significant differences between them were investigated with the Independent T-Test for Two Groups. For more than two groups, the differences were investigated with the (RIR) Tukey test, which compares all pairs of means following one-way ANOVA. Results were considered significantly different when $p < 0.05$ (confidence level is 95%). If the distribution of the results was other than normal, the Kruskal-Wallis test was used (for more than two groups) or the Mann-Whitney test (when two groups were compared).

3. Results

3.1. Characteristics of Algal Products

The Multi-Elemental Composition of the Raw Algal Biomass, Algal Filtrate, and Homogenate

Table 1 presents the multi-elemental composition of the algal biomass from which algal bio-products (filtrate and homogenate) were obtained. The tested products showed that algal homogenate was the main source of micro- (B, Co, Fe, Mn, Zn) and macro-elements (K and P). In the case of the enriched products, the content of microelements decreased, whereas that of the macro-elements slightly increased. It is also worth noting that the content of toxic metals (mainly Cd, Pb) in the 100% filtrate (F 100%) and in diluted homogenate (H 10%) was at a low level when compared with the raw biomass.

Table 1. The multi-elemental composition (N = 3) of the algal biomass, algal filtrate and homogenate (average ± standard deviation; SD).

Element		Wavelength	Algal Biomass	F 100%	F 100%-Zn	H 10%	H 10%-Zn
		(nm)	(mg/kg d.w.)		(mg/L)		
Microelements	B	249.773	97.8 ± 14.7	2.67 ± 0.40	1.28 ± 0.19	5.18 ± 0.78	3.88 ± 0.58
	Cu	324.754	12.7 ± 1.9	2.86 ± 0.43	1.70 ± 0.25	1.40 ± 0.21	1.14 ± 0.17
	Fe	259.94	3760 ± 560	47.2 ± 7.1	42.0 ± 6.3	117 ± 17	114 ± 17
	Mn	257.61	232 ± 35	1.67 ± 0.25	1.68 ± 0.25	2.27 ± 0.34	2.32 ± 0.35
	Si	251.611	906 ± 136	97.7 ± 14.7	87.5 ± 13.1	50.6 ± 7.6	69.5 ± 10.4
	Zn	213.856	64.9 ± 9.7	2.27 ± 0.34	9060 ± 1810	3.55 ± 0.53	9340 ± 1870
Macroelements	Ca	315.887	24,800 ± 4950	619 ± 93	656 ± 98	608 ± 91	651 ± 98
	K	766.49	4150 ± 830	64.5 ± 9.7	55.2 ± 8.3	71.3 ± 10.7	30.9 ± 4.6
	Mg	279.079	1880 ± 280	236 ± 35	243 ± 36	190 ± 28	199 ± 30
	Na	588.995	6350 ± 1270	136 ± 20	119 ± 18	98.3 ± 14.7	86.0 ± 12.9
	P	213.618	881 ± 132	33.3 ± 5.0	29.2 ± 4.4	55.3 ± 8.3	40.2 ± 6.03
	S	182.034	6830 ± 1370	208 ± 31	4834 ± 725	205 ± 31	5070 ± 1010
Other elements	Al	308.215	1570 ± 236	62.5 ± 8.1	32.0 ± 4.2	43.4 ± 5.6	37.2 ± 4.8
	Ba	455.403	24.5 ± 3.7	6.09 ± 0.91	5.50 ± 0.82	4.66 ± 0.70	4.51 ± 0.68
	Cd	228.802	0.707 ± 0.092	<LOD	<LOD	0.119 ± 0.015	0.0498 ± 0.0100
	Cr	267.716	9.59 ± 1.44	0.585 ± 0.088	0.500 ± 0.075	1.05 ± 0.16	1.03 ± 0.15
	Ni	231.604	5.23 ± 0.78	0.790 ± 0.118	<LOD	2.33 ± 0.349	<LOD
	Pb	220.353	7.03 ± 0.91	0.522 ± 0.068	<LOD	0.267 ± 0.035	<LOD

where: <LOD below limit of detection; d.w.: dry weight; F 100%: initial algal filtrate (without dilution); F 100%-Zn: initial algal filtrate (without dilution) enriched with Zn(II) ions; H 10%: 10-times diluted initial algal homogenate (H 100%); H 10%-Zn: 10-times diluted initial algal homogenate (H 100%) enriched with Zn(II) ions.

3.2. Degree of Complexation of Metal Ions in the Tested Products

On the basis of the isotopic composition of zinc (64Zn 48.6%; 66Zn 27.9%; 67Zn 4.1%; 68Zn 18.8%), it was defined which of the signals in the mass spectrum systems of homogenates and filtrates enriched with zinc ions can be attributed to the complexes that are not present in the spectrum of the solution $ZnSO_4 \cdot 7H_2O$—reference system. Figure 2 presents the mass spectrum of the complexation degree of metal ions in the tested products: $ZnSO_4 \cdot 7H_2O$ solution (C-H_2O-Zn), H 10%, H 10%-Zn, F 100%, and

F 100%-Zn. It was found that in homogenate (H 10%-Zn), 45% of Zn(II) ions were complexed with the compounds that are present in the examined homogenate and in the filtrate (F 100%-Zn)—35% of Zn(II) ions.

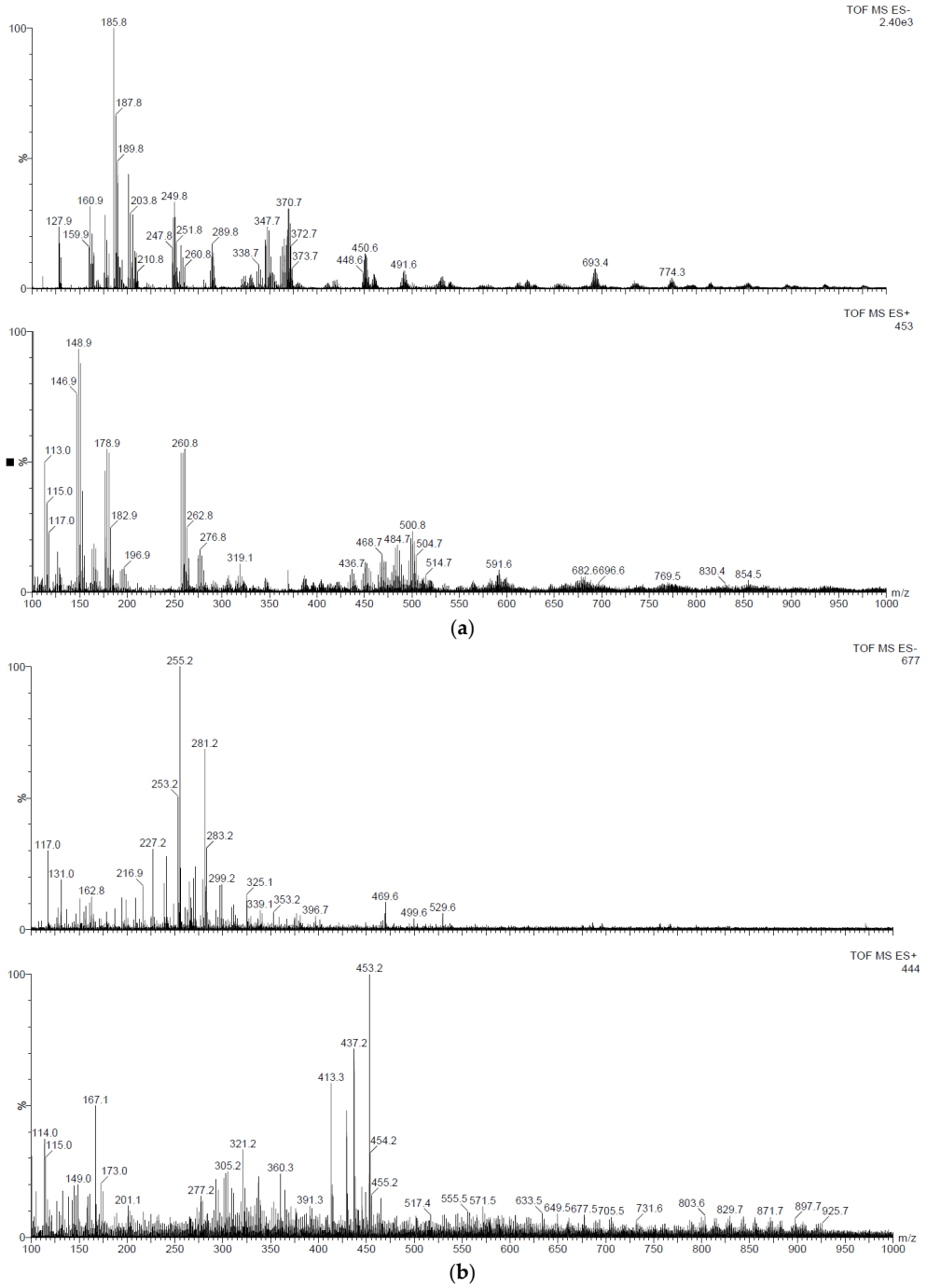
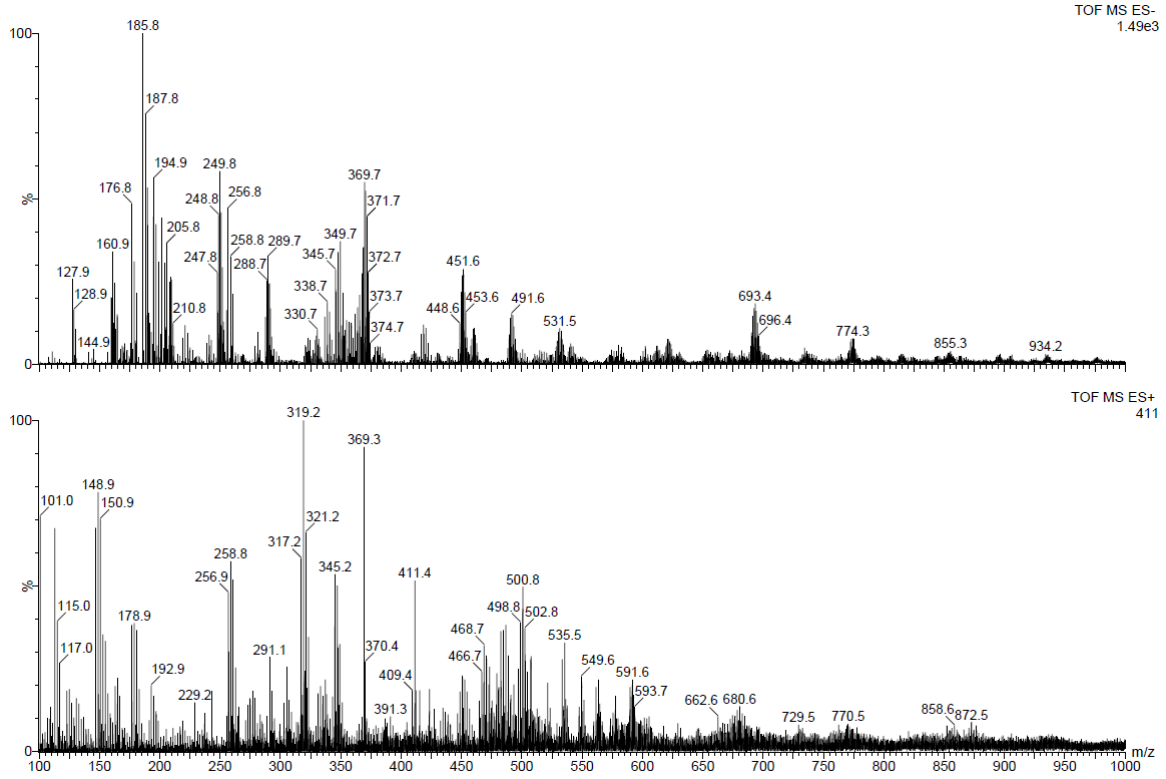
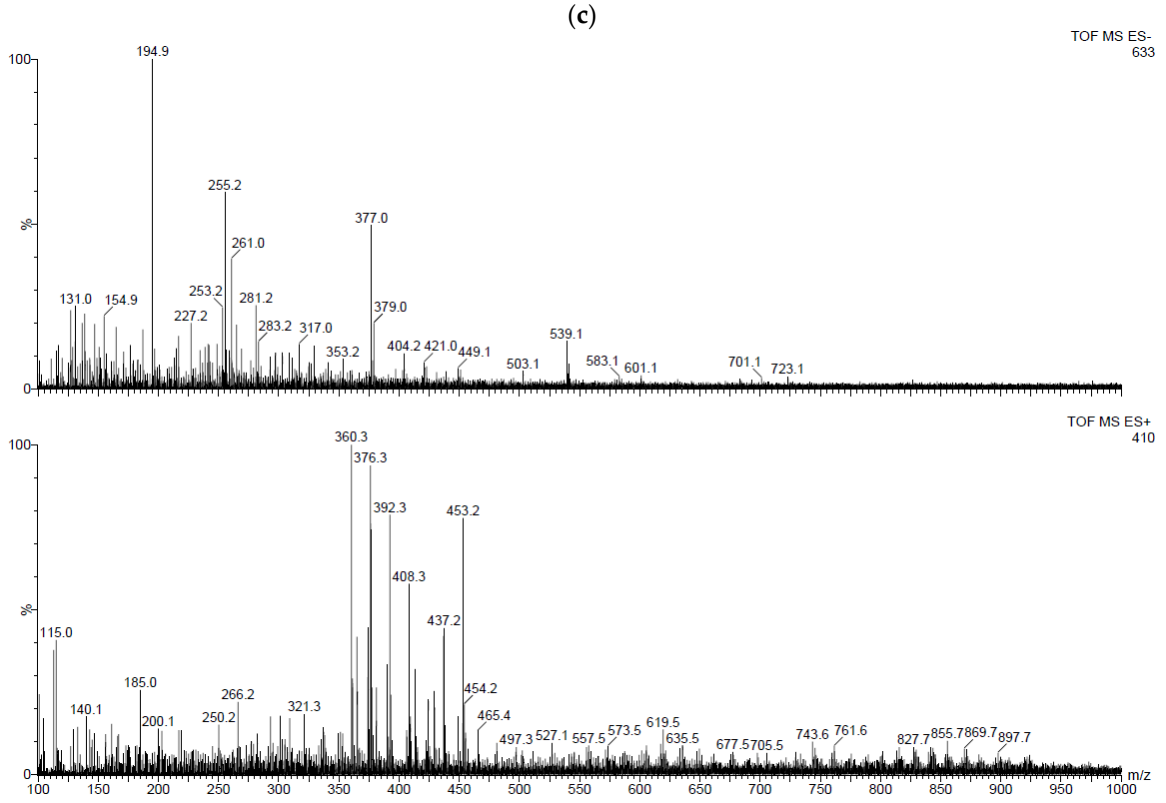


Figure 2. *Cont.*

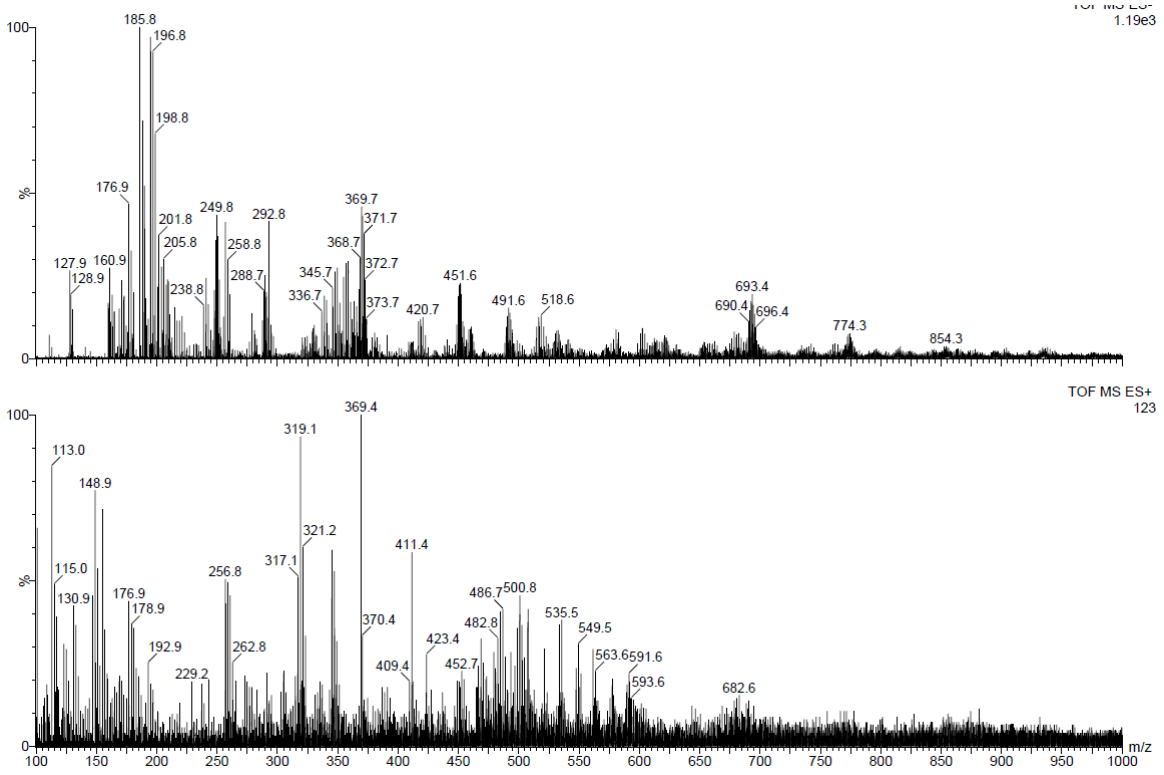


(**c**)



(**d**)

Figure 2. *Cont*.



(**e**)

Figure 2. Mass spectrum of degree of complexation of metal ions in the tested products: (**a**) $ZnSO_4 \cdot 7H_2O$ solution (C-H_2O-Zn); (**b**) H 10%; (**c**) H 10%-Zn; (**d**) F 100%; (**e**) F 100%-Zn.

3.3. Preliminary Seedling Growth Tests

3.3.1. The Choice of the Concentration of Algal Filtrate for Foliar and Soil Applications—Effect on Seedlings Length and Dry Weight

The obtained results showed that there were no phytotoxic effects of algal products on the germination of radish. Table 2 shows the results of the experiments (average length and weight of **s**eedling**s**) that concerned the selection of the best concentration of the clear and enriched with Zn(II) ions algal filtrate for the final seedling growth tests conducted at optimal conditions.

For all the tested products, applied into soil and foliarly, **s**eedling**s** length was arranged in the same order, taking into account the concentration of filtrate: F 50% > F 100% > F 25% > F 5.0% > C-H_2O. Many statistically significant differences were observed. The application of algal filtrates at all concentrations stimulated seedlings growth (seedlings length was higher than in the control group). The best concentration for both soil and foliar application was filtrate 50%—clear and enriched with Zn(II) ions. The average seedlings length treated with F 50% was ~4 and ~2.8 times higher than in the control group for the soil and foliar application respectively. For F 50%-Zn it was respectively ~3.2 and ~2.5 times higher than in the control group.

For both applications, soil and foliar, better results were obtained for clear rather than enriched filtrate used at the same concentration. For example, for soil application in the group treated with F 50%, seedlings length was 13% higher than for F 50%-Zn. For foliar application, the same difference was much higher—in F 50% seedlings length was two times higher than for F 50%-Zn.

Other observations concerned the application method of algal filtrate—soil or foliar. Generally, better results were obtained for soil application of clear filtrate; however, the differences were not statistically significant. Only for F 5.0%, the average length of seedlings treated foliarly was 68% higher than for soil application of filtrate (p 0.00973).

Table 2. The choice of the concentration of algal filtrate (F) for soil and foliar applications (N = 2).

Group	Soil Application [1]				Foliar Application [2]			
	Filtrate		Filtrate Enriched with Zn(II) Ions		Filtrate		Filtrate Enriched with Zn(II) Ions	
	Average Seedlings Length [3] (cm) ± SD	Average Seedlings Dry Weight [4] (g) ± SD	Average Seedlings Length [3] (cm) ± SD	Average Seedlings Dry Weight [4] (g) ± SD	Average Seedlings Length [3] (cm) ± SD	Average Seedlings Dry Weight [4] (g) ± SD	Average Seedlings Length [3] (cm) ± SD	Average Seedlings Dry Weight [4] (g) ± SD
C-H_2O	2.10 ± 0.88 ^{bd}	0.348 ± 0.012	2.35 ± 0.84 ^{bd}	0.346 ± 0.006	2.97 ± 1.08 ^{be}	0.228 ± 0.004	1.74 ± 0.95 ^{c}	0.218 ± 0.010
F 100%	6.15 ± 1.40 ^{ab}	0.305 ± 0.005	5.89 ± 1.21 ^{ab}	0.280 ± 0.006	5.81 ± 1.74 ^{ab}	0.282 ± 0.005	2.75 ± 1.59	0.248 ± 0.020
F 50%	8.46 ± 1.89 ^{cd}	0.361 ± 0.023	7.49 ± 2.12 ^{cd}	0.332 ± 0.013	8.39 ± 1.75 ^{acde}	0.337 ± 0.006	4.27 ± 0.87 ^{abc}	0.269 ± 0.002
F 25%	4.85 ± 2.07	0.295 ± 0.005	4.78 ± 1.33	0.282 ± 0.014	4.65 ± 1.56 ^{c}	0.267 ± 0.022	2.32 ± 0.94 ^{a}	0.260 ± 0.013
F 5.0%	2.70 ± 1.16 ^{ac}	0.303 ± 0.014	3.01 ± 1.42 ^{ac}	0.303 ± 0.014	4.53 ± 1.63 ^{d}	0.257 ± 0.022	2.04 ± 1.47 ^{b}	0.252 ± 0.013

where: 5.0%, 25%, 50%—concentration of filtrates prepared from the initial filtrate (100%); ^{a}, ^{b}, ^{c} … for $p < 0.05$; [1] the differences between the tested groups were determined by Kruskal-Wallis test (non-normal distribution); [2] the differences between the tested groups were determined by Tukey test (normal distribution); [3] N = 2 (two replicates for each group; from each replicate—5 randomly selected seedlings were measured; the result is an average length of 10 seedlings); [4] the average dry weight of 25 seedlings in both replicates (N = 2).

More differences were observed for the enriched filtrate. Better results were obtained in the case of soil application. For the group F 100%-Zn, the average seedlings length was twice as high (p 0.000096) as that for foliar application, for F 50%-Zn—75% higher (p 0.000316), for F 25%-Zn—twice as high (p 0.000156), for F 5.0%-Zn 47% higher (this difference was not statistically significant).

Summarizing the above experiments, it can be concluded that the best results in terms of seedlings length were obtained for clear 50% concentration filtrate applied to soil.

Table 2 also presents the weight of seedlings treated with clear and enriched filtrate. These results are in agreement with the data on seedlings' length. The best results were obtained for 50% algal filtrate, with the exception of 50% concentration filtrate enriched with Zn(II) ions (F 50%-Zn) applied to the soil. In this group, seedlings' weight from the control group was the highest, however higher only by 4.2% when compared with F 50%-Zn. F 50% applied to the soil influenced to the greatest extent the weight of the seedlings.

3.3.2. The Choice of the Dose of Algal Homogenate for Seed Coating

Table 3 presents the effect of the dose of algal homogenate (50 and 100 mg/g of seeds) on the length and weight of the seedlings. All tested products stimulated the growth of seedlings. No toxic effect was observed. The average seedlings' length in these groups was higher than in the control group, however these differences were not statistically significant. The best results were obtained for H 10% and H 10%-Zn, applied at the dose of 50 mg per 1 g of seeds. Seedlings' length in these two groups was 23% higher than in the control group (untreated seeds) and 9.3% than for the Zn solution (C-H_2O-Zn, 50 mg/g of seeds). Also the average weight of the seedlings was higher for the tested products than for the control group. The best results were obtained for the homogenate enriched with Zn(II) ions (H 10%-Zn) applied at doses of 50 and 100 mg. The average seedlings' dry weight was 48% higher than in the control group. Taking into account the average seedlings' length and weight obtained in the conducted experiments, the algal homogenate applied at the dose of 50 mg per 1 g of seeds is recommended for future work.

Table 3. The effect of the dose of algal homogenate (H) on the length and weight of the seedlings (N = 2).

Group	Applied Doses for Seed Coating	Average Seedlings Length [1] (cm) ± SD	Average Seedlings Dry Weight [2] (g) ± SD
C (Control)	-	5.75 ± 1.96	0.241 ± 0.013
C-H_2O-Zn	50 mg/g of seeds	6.45 ± 1.89	0.314 ± 0.038
	100 mg/g of seeds	6.30 ± 1.95	0.308 ± 0.021
H 10%	50 mg/g of seeds	7.05 ± 1.94	0.303 ± 0.117
	100 mg/g of seeds	5.76 ± 1.69	0.270 ± 0.057
H 10%-Zn	50 mg/g of seeds	7.05 ± 1.07	0.357 ± 0.021
	100 mg/g of seeds	6.90 ± 1.41	0.357 ± 0.041

where: C: control—seeds without coating; C-H_2O-Zn: control—water enriched with Zn(II) ions (1% by weight) as $ZnSO_4 \cdot 7H_2O$; H 10%: 10-times diluted initial algal homogenate (H 100%); H 10%-Zn: 10-times diluted initial algal homogenate (H 100%) enriched with Zn(II) ions; [1] N = 2 (two replicates for each group; from each replicate—5 randomly selected seedlings were measured; the result is an average length of 10 seedlings); [2] the average dry weight of 25 seedlings in both replicates (N = 2).

3.3.3. The Choice of Soaking Time

Table 4 presents the effect of different time of seed soaking (1, 12, and 24 h) on seedlings' weight and length. Generally, it was found that the average seedlings' length decreased when the soaking time of seeds increased. The best results were obtained for the control group, where seeds, before tests, were kept in a fridge for 1 h. The average seedlings' length in this group was 2.5 times higher than in C-H_2O-Zn, 46% higher than in F 50%-Zn, 24% higher than in C-H_2O and only 3.3% higher than in F 50% (for soaking that lasted 1 h).

Table 4. The effect of soaking time on the length and weight of the seedlings (N = 2).

Group	Soaking Time (h)	Average Seedlings Length [1] (cm) ± SD	Average Seedlings Dry Weight [2] (g) ± SD
C—seeds without soaking *	1	2.85 ± 1.03 $^{j–m}$	0.206 ± 0.001
C—seeds without soaking	12	1.95 ± 0.60	0.291 ± 0.004
C—seeds without soaking	24	2.45 ± 0.90 ^{n}	0.251 ± 0.023
C-H_2O	1	2.30 ± 0.79°	0.239 ± 0.013
C-H_2O	12	2.10 ± 0.97	0.246 ± 0.009
C-H_2O	24	2.05 ± 0.76	0.265 ± 0.001
C-H_2O-Zn	1	1.15 ± 0.63 ^{bgk}	0.292 ± 0.034
C-H_2O-Zn	12	1.10 ± 0.46 ^{chl}	0.285 ± 0.019
C-H_2O-Zn	24	0.720 ± 0.364 ^{deimno}	0.306 ± 0.013
F 50%	1	2.76 ± 0.75 $^{a–d}$	0.322 ± 0.048
F 50%	12	2.20 ± 0.86 ^{e}	0.254 ± 0.068
F 50%	24	2.75 ± 0.98 $^{f–i}$	0.263 ± 0.015
F 50%-Zn	1	1.95 ± 0.96	0.288 ± 0.018
F 50%-Zn	12	1.50 ± 0.47	0.292 ± 0.005
F 50%-Zn	24	1.00 ± 0.47 ^{afj}	0.318 ± 0.001

where: ^{a}, ^{b}, ^{c} ... for $p < 0.05$; the differences between the tested groups were determined by Kruskal-Wallis test; * seeds without soaking (C) were kept in a fridge respectively for 1, 12 or 24 h; [1] N = 2 (two replicates for each group; from each replicate—5 randomly selected seedlings were measured; the result is an average length of 10 seedlings); [2] the average dry weight of 25 seedlings in both replicates (N = 2).

The negative effect on seedlings' growth came from filtrate enriched with Zn(II) ions (F 50%-Zn) and $ZnSO_4 \cdot H_2O$ solution (C-H_2O-Zn). Seedlings' length in these groups for all tested soaking periods (1, 12 and 24 h) was much lower than for both control groups (i.e., without soaking (C) and soaking with distilled water—C-H_2O).

Summarizing these experiments, it can be concluded that comparable results were obtained for the group without soaking, and for the group in which seeds were macerated with F 50% for one hour. However, also taking into account the average weight of the seedlings, soaking of seeds with F 50% for one hour is recommended. The average weight of seedlings in this group was 12% higher than for F 50%-Zn, 10% higher than for $ZnSO_4 \cdot H_2O$ solution, 35% higher than for C-H_2O (seeds macerated for one hour) and 56% higher than control—without soaking.

3.4. Final Seedling Growth Tests

The final seedling growth tests were carried out for algal products that showed the best results in the preliminary tests, concerning the length and weight of the seedlings. For these tests, the following groups were chosen: F 50% and F 50%-Zn as experimental groups and C-H_2O-Zn and C-H_2O as the control groups. The tests in all groups were performed as four replicates. The obtained results are presented in Table 5. For both methods of application, foliar and soil, the best results were obtained for F 50%. The average seedlings' length was twice as high for soil and 1.7 for foliar application compared to the control group (C-H_2O). For this filtrate, seedlings length was 21% higher for soil than for foliar application; however, this difference was not statistically significant.

In the case of the soaking, the highest length was obtained for seed macerated with distilled water; however, the average length in this group was only 2% higher than for F 50%. Algal filtrate (F 50%) used for seeds soaking influenced radish length when compared to seeds without soaking by 24%. The average seedlings weight ranged from 0.204 g for C-H_2O-Zn to 0.136 g (seeds without soaking). The difference in average weight was only 0.068 g.

Algal homogenate (H 10%) influenced the average length of seedlings—it was 2.4 times higher than in the control group (seeds without coating, statistically significant difference for $p < 0.05$), 31% higher than for H 10%-Zn, 37% higher than for C-H_2O-Zn. The average weight was comparable in all tested groups. There were no statistically significant differences.

Table 5. The final tests on seedlings with the selected algal products: (**a**) method of application—soil and foliar; (**b**) seed treatment—soaking and coating (N = 4).

(**a**)				
Group	**Soil Application**		**Foliar Application**	
	Average Seedlings Length (cm) ± SD [1,3]	**Average Seedlings Weight (g) ± SD [2,4]**	**Average Seedlings Length (cm) ± SD [2,3]**	**Average Seedlings Weight (g) ± SD [2,4]**
C-H_2O	4.38 ± 1.28	0.255 ± 0.061 ^{cd}	4.38 ± 1.28 ^{cde}	0.255 ± 0.061 ^{cd}
C-H_2O-Zn	0.715 ± 0.134 ^{b}	0.329 ± 0.033 ^{bd}	1.38 ± 0.21 ^{be}	0.301 ± 0.022 ^{bd}
F 50%	8.85 ± 1.51 ^{ab}	0.226 ± 0.019 ^{ab}	7.33 ± 1.18 ^{abc}	0.201 ± 0.022 ^{ab}
F 50%-Zn	0.825 ± 0.072 ^{a}	0.282 ± 0.022 ^{ac}	1.35 ± 0.24 ^{ad}	0.303 ± 0.019 ^{ac}
(**b**)				
Seed Treatment				
Method	**Group**		**Average Seedlings Length (cm) ± SD [1,3]**	**Average Seedlings Weight (g) ± SD [2,4]**
Seed soaking (1 h)	C—seeds without soaking		4.78 ± 0.54	0.136 ± 0.005 ^{df}
	C-H_2O		6.03 ± 1.02 ^{b}	0.160 ± 0.022 ^{ce}
	C-H_2O-Zn		2.28 ± 0.25 ^{ab}	0.204 ± 0.018 ^{bef}
	F 50%		5.93 ± 1.65 ^{a}	0.157 ± 0.012 ^{ab}
	F 50%-Zn		3.25 ± 0.34	0.199 ± 0.009 ^{acd}
Seed coating (50 mg/g of seeds)	C—seeds without coating		1.30 ± 0.26 ^{c}	0.273 ± 0.019
	C-H_2O-Zn		2.30 ± 0.40	0.264 ± 0.032
	H 10%		3.15 ± 0.34 ^{c}	0.256 ± 0.012
	H 10%-Zn		2.40 ± 0.08	0.291 ± 0.023

where: ^{a}, ^{b}, ^{c} ... for $p < 0.05$; [1] Kruskal-Wallis test; [2] Tukey test; [3] N = 4 (four replicates for each group; from each replicate—5 randomly selected seedlings were measured; the result is an average length of 20 seedlings); [4] the average dry weight of 25 seedlings from four replicates (N = 4).

3.5. Characteristics of the Seedlings

Multi-Elemental Composition

Table 6 summarizes the multi-elemental composition of the seedlings in the examined groups. Table 6a presents the comparison of the composition of radish after soil and foliar application of tested products. In the case of both applications, only F 50% influenced the multi-elemental composition of radish. For soil application, the increase in the content of B (2.2 times), Na (~2 times), K (by 77%), Zn (by 58%), Mn (by 16%), Ca (by 11%) was observed when compared with the control group (C-H_2O). It was also found that the content of micro- and macro-elements in seedlings was generally arranged in the order: F 50% > C-H_2O > F 50%-Zn > C-H_2O-Zn. The products with Zn(II) ions generally decreased the content of all micro- and macro-elements in the biomass of seedlings after soil and foliar application. High content of zinc in plants can inhibit uptake of other micro-elements. Seedlings treated with water with Zn(II) ions were characterized by lower content of the elements. In the case of foliar application, the increase of elements after application of 50% filtrate was as follows: Zn (2.1 times), B (1.9 times), K (by 79%), Na (by 46%), Ca (by 16%), Mg (by 15%), Mn (by 11%) when compared to the control group (C-H_2O).

Algal homogenate used for seed coating had a beneficial impact on the composition of radish when compared with the control group—seeds without coating. For H 10%, the increase was observed for: B (by 31%), Fe (by 26%), Zn (by 18%), Na (by 36%). For H 10%-Zn and C-H_2O-Zn, the impact was insignificant. In the case of soaking of seeds, the best results in terms of multi-elemental composition were obtained for F 50%. The increase of microelements (mainly B—by 11% and Zn—by 30%) was observed when compared with the control group (without soaking)—Table 6b.

Table 6. Multi-elemental composition of the cultivated radish—(**a**) after soil and foliar application of tested preparations; and (**b**) grown from coated and soaked seeds in tested preparations.

(a)

Element		Average Content (mg/kg d.w. ± SD) *N* = 4								
		Soil Application				Element	Foliar Application			
		F 50%	F 50%-Zn	C-H_2O-Zn	C-H_2O		F 50%	F 50%-Zn	C-H_2O-Zn	C-H_2O
Microelements	B **	28.9 ± 3.5 ^{a}	10.9 ± 1.4	8.18 ± 0.31 ^{a}	12.9 ± 2.2	B **	24.0 ± 1.3 ^{a}	13.0 ± 0.5	10.1 ± 0.4 ^{a}	12.9 ± 2.2
	Cu	9.67 ± 1.63	7.62 ± 1.20	7.38 ± 0.66	9.14 ± 0.76	Cu	9.62 ± 0.61	8.19 ± 0.68	9.07 ± 2.37	9.14 ± 0.76
	Fe *	109 ± 5.0 ^{cd}	83.7 ± 5.5 ^{ac}	82.4 ± 2.9 ^{bd}	104 ± 3.0 ^{ab}	Fe *	113 ± 8.0 ^{bc}	99.0 ± 3.3 ^{b}	88.5 ± 4.1 ^{ac}	104 ± 3.0 ^{a}
	Mn **	48.7 ± 3.8 ^{a}	24.3 ± 4.2 ^{a}	24.0 ± 2.0	42.1 ± 4.3	Mn *	46.6 ± 4.7 ^{cd}	27.2 ± 2.9 ^{ac}	25.9 ± 1.3 ^{bd}	42.1 ± 4.3 ^{ab}
	Zn **	223 ± 43	14,600 ± 1140 ^{a}	12,000 ± 810	141 ± 18 ^{a}	Zn *	291 ± 115 ^{cd}	26,600 ± 2290 ^{ac}	26,400 ± 2030 ^{bd}	141 ± 18 ^{ab}
Macroelements	Ca *	9410 ± 441 ^{cd}	4500 ± 689 ^{ac}	4129 ± 213 ^{bd}	8460 ± 720 ^{ab}	Ca *	9810 ± 566 ^{ade}	5070 ± 395 ^{bd}	4750 ± 258 ^{ce}	8460 ± 720 ^{abc}
	K *	16,100 ± 1050 ^{ade}	6290 ± 199 ^{bd}	6550 ± 220 ^{ce}	9120 ± 1370 ^{abc}	K *	16,300 ± 1000 ^{ade}	6360 ± 82 ^{bd}	6400 ± 230 ^{ce}	9120 ± 1370 ^{abc}
	Mg *	4810 ± 203 ^{ade}	2810 ± 95 ^{bd}	2930 ± 76 ^{ce}	4430 ± 168 ^{abc}	Mg *	5080 ± 93 ^{ade}	3130 ± 90 ^{bd}	3130 ± 48 ^{ce}	4430 ± 168 ^{abc}
	Na **	17,080 ± 4580 ^{a}	849 ± 27	610 ± 117 ^{a}	8700 ± 3880	Na *	12,700 ± 2450 ^{cd}	1470 ± 87 ^{ac}	1280 ± 130 ^{bd}	8700 ± 3880 ^{ab}
	P *	12,900 ± 870 ^{ade}	11,100 ± 383 ^{bd}	11,000 ± 344 ^{ce}	14,300 ± 444 ^{abc}	P *	14,700 ± 331 ^{cd}	11,000 ± 169 ^{ac}	11,000 ± 188 ^{bd}	14,300 ± 444 ^{ab}
	S **	20,700 ± 1 210	19,700 ± 297	19,000 ± 259 ^{a}	22,500 ± 2550 ^{a}	S *	23,000 ± 927 ^{cd}	27,900 ± 1640 ^{ac}	28,500 ± 819 ^{bd}	22,500 ± 2550 ^{ab}

(b)

Element		Average Content (mg/kg d.w. ± SD) *N* = 4									
		Seed Coating				Element	Seed Soaking				
		H 10%	H 10%-Zn	C-H_2O-Zn	C—seeds without coating		F 50%	F 50%-Zn	C-H_2O-Zn	C-H_2O	C—seeds without soaking
Microelements	B *	11.8 ± 0.4 ^{ab}	7.87 ± 0.40 ^{a}	8.05 ± 0.24 ^{b}	8.98 ± 2.80	B	10.8 ± 2.7	8.04 ± 0.69	7.20 ± 1.49	10.8 ± 0.9	9.52 ± 0.87
	Cu	7.94 ± 1.10	7.25 ± 0.77	12.2 ± 6.2	7.53 ± 0.48	Cu **	7.02 ± 0.34 ^{a}	5.57 ± 0.32 ^{a}	6.84 ± 1.07	6.69 ± 0.61	6.45 ± 0.37
	Fe **	103 ± 7 ^{a}	86.6 ± 1.5	83.7 ± 6.6	81.8 ± 5.2 ^{a}	Fe	101 ± 4.0	97.8 ± 14.4	95.9 ± 7.8	107 ± 6	102 ± 3
	Mn	27.3 ± 1.7	28.0 ± 3.8	27.7 ± 2.7	27.5 ± 2.4	Mn **	39.2 ± 9.8	26.7 ± 4.7 ^{a}	28.2 ± 6.0 ^{b}	41.8 ± 6.5 ^{ab}	36.1 ± 1.6
	Zn **	83.5 ± 4.8	458 ± 45	540 ± 51 ^{a}	70.5 ± 4.5 ^{a}	Zn **	98.3 ± 17.4	3590 ± 797 ^{a}	5040 ± 649 ^{b}	102 ± 18	75.8 ± 3.7 ^{ab}
Macroelements	Ca	6350 ± 391	5980 ± 108	6460 ± 263	6190 ± 196	Ca *	8410 ± 975 ^{ef}	5570 ± 343 ^{ace}	5070 ± 869 ^{bdf}	9020 ± 430 ^{ab}	8290 ± 320 ^{cd}
	K	6590 ± 572	6480 ± 358	6590 ± 317	6750 ± 351	K	8540 ± 2004	6790 ± 494	6820 ± 1380	10,400 ± 820	10,600 ± 950
	Mg	3350 ± 164	3340 ± 64	3400 ± 131	3300 ± 129	Mg *	4570 ± 210 ^{ef}	3480 ± 192 ^{ace}	3610 ± 651 ^{bdf}	4950 ± 220 ^{ab}	4850 ± 200 ^{cd}
	Na *	1970 ± 190 ^{a}	1630 ± 163	1820 ± 163	1450 ± 240 ^{a}	Na **	7720 ± 4050	2310 ± 930	1110 ± 41 ^{ab}	9850 ± 1244 ^{a}	9570 ± 1780 ^{b}
	P	11,400 ± 367	11,400 ± 291	11,300 ± 390	11,300 ± 842	P *	11,800 ± 496	10,600 ± 376 ^{ab}	10,880 ± 1540	12,600 ± 520 ^{a}	12,400 ± 600 ^{b}
	S	17,100 ± 1320	17,800 ± 1170	18,900 ± 652	18,200 ± 761	S	16,900 ± 1530	15,800 ± 857	15,200 ± 2060	16,760 ± 950	16,600 ± 1130

where: ^{a}, ^{b}, ^{c} … for $p < 0.05$; * Tukey test for $p < 0.05$; ** Kruskal-Wallis test for $p < 0.05$.

4. Discussion

In the present paper, algae bio-products were obtained by their homogenization. Mechanical processing of macroalgae avoids the use of organic solvent, acid or alkali to produce extracts with properties that are significantly different from products obtained by chemical methods [34,35]. Therefore, these products are safe for application in agriculture.

In the present study we examined the multi-elemental composition of raw algae biomass, as well as the obtained filtrate and homogenate. Algal products are rarely studied in terms of the content of elements. The results showed that the tested products can be a source of micro- and macro-elements in plant cultivation, especially algal homogenate that was rich in B, Fe, Mn, Zn, K, P, and S in contrary to algal filtrate. The results from the present study were compared with multi-elemental composition of algal extract obtained from the same algae using microwave assisted extraction (MAE) [30] and chemical hydrolysis with acidified water (pH 2) [36]. It was found that especially micro-elements were extracted more efficiently using mechanical processing than the mentioned physico-chemical methods.

The filtrate and homogenate were also examined in terms of their ability to complex Zn(II) ions. It was assumed, that algal products enriched with microelement ions can be used as liquid fertilizers or organic fertilizers/soil amendments that will supply plants with a highly bio-available form of elements. It was shown that the amount of Zn(II) ions complexed by homogenate was higher than by filtrate. This can result from the physical form of the examined products (solid residue and liquid). Algal homogenate may contain many more macromolecules, such as polysaccharides (e.g., ulvan in green seaweeds) rich in functional groups capable of binding micronutrient ions in a reversible process [37]. Usually, as it is reported in the literature, the chelating properties of algal extracts are tested for ferrous ions when the antioxidant properties of extracts were examined [10].

The aim of the present study was also to examine the utilitarian properties of algal homogenate and filtrate in the tests on radish. We studied the effect of the method of application of algal filtrate (soil and foliar), as well as the effect of seed treatment with algal filtrate and homogenate (seed soaking and coating, respectively) on the length, weight, and multi-elemental composition of seedlings. In the literature, there are some papers that describe the effect of algal products obtained by mechanical processes on plant growth. Sunarpi et al. (2010) examined the effect of 15% algal filtrate obtained from green macroalgae *Ulva ferticulata* and *Chaetomorpha* sp., by homogenization in a blender, on the growth and yield of rice plants. Seaweed extract treatment was done by spraying the whole plant. The examined extracts did not give positive response to the rice plant height or the weight of stems and roots, but influenced the number of leaves, tillers, and the weight of rice panicle [21]. Trinchera et al. (2014) used a seaweed filtrate from *Ascophyllum nodosum* (produced after micro-grinding, flocculation, and filtration) in greenhouse trials on lettuce. The addition of filtrate directly to the irrigation water increased the lettuce shoot dry weight [1]. DeLucia and Vecchietti (2012) indicated that depending on the application method of biostimulants, different effects on plants can be observed, for example the drenching application method enhances the stem height, whereas foliar application increases the flower buds number and promotes the fresh weight of bulb roots [38]. In our research, it was found that better results in terms of seedlings' length were obtained for soil application of algal filtrate (50%) than foliar (by 21% longer). Soil application of algal extracts is known to improve shoot growth, root development, and mineral absorption [4]. It was also found that among the tested different algal filtrate concentrations (5.0%, 25%, 50%, and 100%), the best results concerning seedlings' length and weight were obtained for 50% algal filtrate. The stimulatory effects of algal extracts depend not only on application time and frequency of application, but also on their concentration. Lower doses often elicit the best growth responses [4]. The increased germination percentage at low concentrations may be due to the presence of some growth promoting substances such as phytohormones and micronutrients. Higher concentrations of the extracts can inhibit the germination [39].

In the present study, we also examined seed treatment with algal homogenate/filtrate. At present the majority of the research on biostimulants is conducted using foliar applications while little work has been done on biostimulants as seed treatments. Amirkhani et al. (2016) suggests that combining

the attributes of biostimulants and applying them to seeds via coating may have tremendous potential to enhance early plant growth and development [24]. The seed treatment in our study concerned the use of algal homogenate for seed coating and filtrate for seed soaking. It was found that seed soaking for 1 h with 50% algal filtrate is a better option to increase seedlings' length than seed coating. However, for both seed treatment methods, better results were obtained for soaked and coated seeds than for non-treated seeds. Seed treatment increases the resistance to biotic and abiotic stress, as well as being responsible for growth responses of plants (e.g., improved root and shoot growth) [3]. For example, Pise and Sable (2010) produced seaweed liquid fertilizer from *Ulva fasciata*, using a blender followed by a mortar and pestle. Several concentrations were prepared from the initial filtrate: 10%, 25%, and 50%. Fenugreek seeds were soaked for each concentration of aqueous extracts for 1 h. The maximum value of height and weight was recorded for the 50% extract. This extract also influenced positively the photosynthetic pigments' percentage, carbohydrate, proteins, free amino acids, polyphenols, and nitrogen content in the treated plants [20]. Seeds of cow pea soaked with *Sargassum wightii* and *Caulerpa chemnitzia* extracts performed better when compared to the water soaked controls and showed improved seedling growth, dry matter, chlorophyll, protein, amino acid, and sugar content [39]. Tomato seeds treated with *Ulva lactuca* extracts reduced fungus *Fusarium oxysporum* incidence (resistance to biotic stress) [40], whereas seeds of sweet pepper and aubergine primed with *Cystoseira barbata* extract were characterized by improved germination even at low temperatures (resistance to abiotic stress) [35]. The application of algal homogenate/filtrate as formulations with biostimulant properties for seed coating/soaking seems to be a promising option for a sustainable agriculture that aims at reducing the use of mineral fertilizers and pesticides. The biostimulant—a natural plant material, being a component of a seed coating blend—could be adopted for organic crop production and may also reduce the need for high levels of N fertilizer since the biostimulant can enhance N uptake efficiency [24].

In the present paper, we also found that the algal products enriched with Zn(II) ions (both filtrate and homogenate), as well as zinc supplied as inorganic salt in the cultivation of radish inhibited its growth. Although zinc is an essential nutrient for plant growth, elevated concentrations can result in growth inhibition and toxicity symptoms [26].

The application of algal products in plant cultivation can not only increase the height and weight of plants, but also their multi-elemental composition. Our experiments revealed that algal filtrate (50%) applied to the soil or foliarly increased the content of some elements, such as: Zn, B, K, Na, Ca, Mg, Mn. This is supported by results presented by Sosnowski et al. (2014), who found that spraying hybrid alfalfa with the seaweed extract from *Ecklonia maxima* resulted in an increase in the content of P, K, Zn, and Mn in aerial parts of this plant [41]. An increased content of P and K in lettuce was observed in the work of Trinchera et al. (2014) after application of seaweed filtrate from *Ascophyllum nodosum* [1]. Ahmed and Shalaby (2012) reported higher content of N and K in cucumber treated with extract from *Enteromorpha intestinalis* [7]. Trinchera et al. (2014) suggested that seaweed filtrates appeared to be a suitable product for promoting the early-stage development of vegetable plants [1]. Ahmed and Shalaby (2012) indicated that seaweed extracts enhance not only seed germination, but also the growth, yield, and uptake of nutrients by the plants [7].

5. Conclusions

In the present paper, the chemical and utilitarian properties of bio-products obtained by the homogenization of algae were described. We found that both algal filtrate and homogenate have good complexing properties of Zn(II) ions. In homogenate 45% of Zn(II) ions were complexed, in filtrate (F 100%-Zn)—35% of Zn(II) ions. Algal homogenate was a richer source of micro- (B, Co, Fe, Mn, Zn) and macro-elements (K and P) than filtrate. This paper shows that the diluted algal bio-products influenced the growth parameters of radish. Seedling growth tests indicated that a clear algal filtrate at a concentration of 50% is recommended for soil application (the highest influence on seedlings' length and weight). Application of algal homogenate at 50 mg per 1 g of seeds gave the best results in terms

of growth parameters. Experiments concerning the soaking time showed that soaking of seeds with F 50% for one hour is beneficial for seedlings' growth. Both algal products increased the content of micro- and macro-elements in the radish. We also found that the products enriched with Zn(II) ions inhibited seedlings' growth. Therefore, in the future the experiments should be carried out using lower zinc concentrations. It will be also necessary to conduct more advanced experiments on the content of organic compounds in the algal filtrates and homogenates that are responsible for plant growth. Natural homogenates and filtrates obtained from Baltic macroalgae through mechanical processing can be recommended to growers as an eco-friendly, effective, and cheap preparations stimulating plant growth and yield.

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