Izabela Michalak*, Łukasz Tuhy, Katarzyna Chojnacka

Seaweed extract by microwave assisted extraction as plant growth biostimulant

DOI: 10.1515/chem-2015-0132
received January 26, 2015; accepted July 27, 2015.

Abstract: Microwave Assisted Extraction (MAE) was used to obtain aqueous extracts of Baltic seaweeds. Three different temperatures: 25, 40, 60°C were examined. Algal extracts were characterized in terms of polyphenols, micro- and macroelements, lipids content and antibacterial properties. This is the first study that examines the effect of algal extract obtained by MAE in plant cultivation. The utilitarian properties were checked in the germination tests on Lepidium sativum for three dilutions of extract (0.5, 2.5 and 10%). Results showed that the content of polyphenols in extracts decreased with temperature, whereas the content of micro- and macroelements increased with temperature. The aqueous extracts did not contain fatty acids and did not show inhibitory effect on Escherichia coli and Staphylococcus aureus. Germination tests showed that plants in the experimental groups with an optimal concentration of extract had a higher height, weight, chlorophyll and micro- and macroelement content than plants in the control group.

Keywords: algae, MAE, biologically active compounds, biostimulants

1 Introduction

Microwave Assisted Extraction (MAE) is known to be a novel, green method of extraction of biologically active compounds from the algal biomass [1]. This method is an alternative to conventional liquid extraction which has some drawbacks, such as the use of high amounts of solvent and several extraction steps [2]. During MAE extraction, microwave radiation causes disruption of hydrogen bonds and the migration of dissolved ions. Therefore, the penetration of the solvent into the matrix is increased and the extraction of target compounds is facilitated [1]. MAE can be operated at high temperatures, thus increasing the diffusion rates of analytes from a solid sample into a solvent [3]. The main parameters in microwave assisted extraction are microwave power, time, algae type and temperature [2,4].

According to the literature data, MAE is used for the extraction of lipids (fatty acids, sterols), pigments: carotenoids (e.g., astaxanthin, fucoxanthin), chlorophylls and phenolic compounds. Organic solvents are used for their extraction. Water as a solvent is used for the extraction of polysaccharides (e.g., fucoidan, agar). Detailed information is presented in Table 1. It is evident that this method is used for the extraction of a wide variety of compounds, which possess bioactive properties: antibacterial, anti-fungal, anti-viral, anti-oxidative, anti-inflammatory and anti-tumor. These activities allow the use of algal extracts in many areas [5]. However, in the available literature algal extracts obtained by MAE were examined only in terms of anti-oxidative properties [6-9].

Microwave Assisted Extraction was selected as a method of production of algal extract for this paper. The biomass of marine seaweeds was used as a raw material for the production of extracts because it was an inexpensive resource along coastal areas of the Baltic Sea in Poland. The organic and inorganic composition of the obtained extract will determine its future application in plant (fertilizers, biostimulants, bioregulators), animal (feed additives) and human (food, cosmetics, pharmaceuticals) products. Deionized water was selected as a solvent for the extraction of biologically active compounds because it
Izabela Michalak, Łukasz Tuhy, Katarzyna Chojnacka

has a high dielectric constant (80.1), is characterized by a high heating rate and a high affinity for polar compounds [3,10]. Wang and Weller (2006) indicated that solvents with a high dielectric constant such as water and polar solvents, which can absorb high microwave energy, are usually better solvents than nonpolar solvents [11]; therefore water is a preferred solvent in MAE [3]. The use of organic solvents in MAE prevents the application of the obtained algal extracts directly in the plant cultivation.

In the present work, algal extracts obtained by MAE were characterized in terms of polyphenols, micro- and macroelements, lipids content and antibacterial properties. The utilitarian properties were examined in the germination tests on *Lepidium sativum*. The content of nutrients and chlorophyll in the cultivated plants was determined. Additionally, the effect of algal extracts on plant morphology was analyzed using Scanning Electron Microscopy.

### 2 Experimental Procedure

#### 2.1 Chemicals

Folin-Ciocalteu’s phenol reagent, gallic acid and nitric acid 69% m/m, spectrally pure (Suprapur) were purchased from Merck KGaA (Darmstadt, Germany). Sodium carbonate (Na₂CO₃), ethanol, methanol were purchased from POCH S.A. (Poland). All the reagents were of analytical grade and used without further purification.
2.2 Collection of algae

Algae biomass (Polysiphonia, Ulva, Cladophora) from the Baltic Sea near Sopot (Poland) was collected for the experiments in August 2013. Biomass was obtained using two methods: it was collected directly from the water with a specially prepared charger which minimized the contamination of the raw material, as in sand. After the collection, the biomass was rinsed with water to purify it from the salt and sand. Following this process, impurities such as stones, sand, shells, pieces of wood were separated. Finally, the biomass was dried to 15% of moisture. The prepared and purified algal marine biomass was subjected to grinding to the particle size < 0.3 mm [12].

2.3 Extract production

The algal powder and deionized water were placed in Teflon bombs in a microwave oven Milestone Start D (USA). Extraction conditions were as follows: 1000 W, liquid/solid ratio (15 mL/5.0 g of dry biomass), extraction time (30 min.) and three different extraction temperatures (25, 40 and 60°C). After extraction, each sample was centrifuged at 4250 rpm for five minutes and filtered using Whatman No.1 filter paper. The resulting supernatant was taken as 100% algal liquid extract. Algal liquid extracts were prepared with different doses viz., 0.5, 2.5 and 10% for germination tests on Lepidium sativum.

2.4 Characteristics of algal extract

2.4.1 Multielemental composition of algal extracts

The content of elements in algal extracts was determined by ICP-OES iCAP 6500 Duo, Thermo Scientific, USA. Quality assurance of the test results was achieved by using Combined Quality Control Standard from ULTRA SCIENTIFIC, USA. The samples were analyzed in three repetitions (the reported results were completed using the arithmetic mean, the relative standard deviation was < 5%). The samples of the algal biomass and cultivated plants (about 0.5 g) prior to the ICP-OES analysis were purified from organic matter with concentrated nitric acid (5 mL) in Teflon bombs in a microwave oven Milestone Start D (USA). After mineralization, samples were diluted with re-demineralized water (Millipore Simplicity) to 50 g.

2.4.1 Phenolic compounds in the algal extracts

Phenolic content of 100% MAE algal extracts was determined according to the modified procedure described by Sim et al. (2010) [13]. Gallic acid was used as a standard and a calibration curve was prepared with a range of concentration from 25 to 1000 mg L⁻¹. Different concentrations of the obtained gallic acid solution (0.1 mL) and negative control (methanol was used instead of gallic acid) were mixed with 7.9 mL of distilled water. Folin-Ciocalteu’s phenol reagent (0.5 mL) was added to each sample. After 3 minutes, 1.5 mL of saturated sodium carbonate (Na₂CO₃) solution was added to the mixture. The reaction mixtures were incubated for 30 minutes at 40°C. The blank contained only methanol. The absorbance was determined at 765 nm with a spectrophotometer – Varian Cary 50 Conc. Instrument (Victoria, Australia). The gallic acid calibration plot was obtained by plotting the absorbance against the gallic acid concentration (mg L⁻¹).

2.4.2 Antibacterial assay

Antibacterial activity was determined by the Kirby Bauer disk diffusion method. Two bacterial strains were used: gram-negative (Escherichia coli) and gram-positive (Staphylococcus aureus). The bacterial inocula were grown overnight in a nutrient broth (Muller-Hinton agar medium). A small amount of bacteria (about 1–2 × 10⁸ CFU mL⁻¹) was taken using the inoculation loop. The bacterial inoculum was suspended in test tubes with a saline solution to obtain a suspension that matched the turbidity of a 0.5 McFarland standard. The diluted bacterial culture was placed on a Muller-Hinton agar medium and spread throughout the sterile Petri dishes using a sterile glass “L” rod. This formed the bacterial lawn. The paper discs of 10 mm in diameter (prepared from Whatman No. 1 filter paper) were soaked with the 10 µL of 100% of algal extracts. Petri dishes were incubated for 20 h at 37°C. Antibacterial activity was recorded by measuring the diameter of the zone of inhibition. Gentamicin (concentration 10 mg mL⁻¹) was used as a positive reference.

2.5 Utilitarian properties of algal extracts

2.5.1 Germination tests – Petri dish tests

Germination tests were performed with Lepidium sativum to evaluate the utilitarian properties of Baltic seaweed extracts (concentrations: 0.5, 2.5 and 10%). Experiments
were designed and performed in three replicates in Petri dishes (50 seeds), under standardized conditions – isolated box with adjustable lighting and temperature (temperature fluctuations ± 4°C) – Jacobsen apparatus. Before germination tests, the dishes with seeds were put into the refrigerator for stratification (3 days). After this period, each dish was watered with 5 mL of appropriate algal extract, and the control group was watered with the same volume of distilled water. After three days, all dishes were watered with extract/water one more time. Plants in a seedlings phase were weighed, and height measurements of aerial parts were carried out.

2.5.2 Chlorophyll content in the cultivated plants
The above-ground parts of cultivated *Lepidium sativum* were subjected to a methanolic extraction process for 30 min. In this process, a colored solution was obtained and was further analyzed by a UV-VIS spectrophotometer (Varian Cary 50 Conc. Instrument, Victoria, Australia), to determine the plant pigments. Measurements were made at wavelengths of λ = 663 and 645 nm. The concentration of total chlorophyll (Total Chl), Chl(a) and Chl(b) was determined from the equations [14]:

\[
\text{Total Chl} = 8.02\cdot A(663) + 20.2\cdot A(645) \\
C_{\text{Chl(a)}} = 12.7\cdot A(663) - 2.69\cdot A(645) \\
C_{\text{Chl(b)}} = 22.9\cdot A(645) - 4.68\cdot A(663)
\]

2.5.3 SEM analysis of cultivated plants
Stalk, leaf – the internal and external parts of *Lepidium sativum* from the group MAE 40°C were examined using a Scanning Electron Microscope. The experiments were performed at Wroclaw University of Environmental and Life Sciences (Electron Microscope Laboratory). The samples were observed and photographed with a Scanning Electron Microscope – EVO LS 15 Zeiss (Oberkochen, Germany) operating at 20 kV. Plant samples were fixed in 4.0% glutaraldehyde (Sigma) (15 min., room temperature). All the samples were dehydrated by ethanol (from 30% to 100% concentration). Plant samples were mounted on an appropriate stub and thereafter gold-sputtered (using ScanCoat 2 – Oxford). For the test, a SE1 detector was used [15].

2.6 Statistical analysis
The results were elaborated statistically by *Statistica* ver. 10. Normality of distribution of experimental results was assessed by the Shapiro-Wilk test. On this basis, a statistical test was selected, which was used to investigate the significance of differences between the groups. The differences between the groups were investigated with a one-way analysis of variance (ANOVA) using the Tukey test. Results were considered significantly different when *p* < 0.05.

3 Results and Discussion
Characteristics of algal extracts obtained by MAE are presented in the present study. The presence of biologically active compounds in the examined extracts will determine their future application.

3.1 Characteristics of algal extract
Marine organisms are a valuable material for the extraction of biologically active compounds with biological properties. Baltic macroalgae are known to be rich in lipids, proteins, carbohydrates [16] and elements [17,18]. They constitute a valuable material for further processing. Initially, a suitable extraction technique should be selected. This selection must be carried out in accordance with the predicted nature of the extracted bioactive compounds. The extraction parameters that might have a significant influence on the isolated compounds should be also tested [19]. The choice of extraction temperature for MAE includes not only its extraction efficiency for target components but also its destructive effect [8]. As an example, the extraction of polyphenols from *Caulerpa racemosa* by MAE decreased rapidly when the temperature was higher than 40°C [9], whereas the extraction of polysaccharides from *Enteromorpha prolifera* by MAE increased with a rising temperature from 40°C to 70°C and gradually stabilized when the temperature was higher than 70°C [8].

3.1.1 Multielemental composition of algal extracts
In Table 2 a multielemental composition of raw algal biomass and extracts obtained by MAE in three different temperatures (25, 40 and 60°C) is presented. The extract obtained at 60°C was most favourable regarding the elemental composition. Generally, there is a correlation...
as in the lower temperature of the extraction, the lower concentration of the elements in the final extract. The concentration of microelements in MAE 60°C was much higher than in MAE at 25°C. As an example, the concentration for Fe and Si was four times higher, Zn two times higher, B 44% higher, and Mn and Ni 22% higher. The greatest difference in reference to the macroelements was that S had a 25% higher concentration, Na a 19% higher concentration, and K, a 10% higher concentration. It should be noted that toxic elements were extracted from the raw algal biomass in small amounts by MAE.

### Table 2: Multielemental composition of raw algal biomass and extracts obtained by MAE.

| Element | Algal biomass | MAE 25°C | MAE 40°C | MAE 60°C* |
|---------|---------------|----------|----------|----------|
| Microelements |               |          |          |          |
| B       | 97.8 ± 14.7   | 3.30 ± 0.5 | 3.44 ± 0.51 | 4.74 ± 0.71 |
| Co      | 2.84 ± 0.43   | 0.012 ± 0.003 | 0.012 ± 0.003 | 0.0135 ± 0.0034 |
| Cu      | 12.7 ± 1.9    | 0.117 ± 0.017 | 0.148 ± 0.022 | 0.108 ± 0.016 |
| Fe      | 6661 ± 1332   | 1.19 ± 0.2 | 2.17 ± 0.3 | 4.47 ± 0.70 |
| Mn      | 232 ± 35      | 2.52 ± 0.37 | 2.62 ± 0.39 | 3.07 ± 0.46 |
| Mo      | 0.310 ± 0.046 | 0.001 ± 0.000 | 0.179 ± 0.026 | 0.0108 ± 0.0027 |
| Ni      | 5.23 ± 0.78   | 0.108 ± 0.016 | 0.111 ± 0.016 | 0.132 ± 0.019 |
| Si      | 907 ± 136     | 3.10 ± 0.46 | 6.69 ± 1 | 11.9 ± 1.8 |
| Zn      | 64.9 ± 9.7    | 0.0746 ± 0.0112 | 0.201 ± 0.03 | 0.169 ± 0.025 |
| Macroelements |       |          |          |          |
| Ca      | 40292 ± 8058  | 354 ± 53 | 363 ± 54 | 365 ± 54 |
| K       | 5082 ± 1016   | 868 ± 130 | 901 ± 135 | 951 ± 142 |
| Mg      | 3181 ± 636    | 303 ± 45 | 311 ± 46 | 322 ± 48 |
| Na      | 6354 ± 1271   | 1050 ± 211 | 1200 ± 240 | 1250 ± 250 |
| P       | 1155 ± 231    | 9.52 ± 1.43 | 18.3 ± 2.7 | 32.9 ± 4.9 |
| S       | 8614 ± 1723   | 562 ± 84 | 582 ± 87 | 702 ± 105 |
| Toxic metals |        |          |          |          |
| As      | 3.90 ± 0.51   | 0.266 ± 0.032 | 0.150 ± 0.019 | 0.198 ± 0.025 |
| Cd      | 0.710 ± 0.092 | <LLD     | 0.001 ± 0.000 | 0.001 ± 0.000 |
| Pb      | 7.03 ± 0.91   | 0.0098 ± 0.0002 | 0.0104 ± 0.0021 | 0.032 ± 0.006 |

<LLD – below detection limit
* – the best variant

3.1.2 Polyphenols in algal extracts

In the literature it was shown that MAE extracts contain polyphenols that have antioxidant properties. Their extraction can be conducted with the use of water as an extractant. Tierney et al. (2013) found that the use of water as the solvent for extraction of phenolic compounds from Irish macroalgae (*Ascophyllum nodosum, Pelvetia canaliculata, Fucus spiralis* and *Ulva intestinalis*) resulted in the highest extraction yields, when compared with other solvents – acetone/water (80:20) and ethanol/water (80:20). This reflects the hydrophilic nature of the majority of components (e.g., polysaccharides) found within macroalgal cells [22]. This statement was confirmed...
in the work of Wang et al. (2011) who indicated that polysaccharides obtained by MAE from Enteromorpha prolifera possess antioxidant properties [8].

The present work shows the highest concentration of polyphenols being in MAE at 25°C (544 mg L\(^{-1}\)) followed by MAE at 40°C (337 mg L\(^{-1}\)) and finally by MAE at 60°C (165 mg L\(^{-1}\)). These results concur with other literature reports. Li et al. (2012) observed that the total phenolic content in ethanolic extracts obtained by MAE from Caulerpa racemosa increased between 20 and 40°C and decreased rapidly when the temperature was higher than 40°C [9]. These results indicated that lower temperatures enhanced the extraction yield to a certain level, and higher temperatures caused the decomposition of phenolic compounds [23].

### 3.1.3 Fatty acids in algal extracts

Fatty acids were not extracted from the Baltic seaweed by MAE (GC analysis). They remained in the post-extraction residue. For the extraction of lipids, organic solvents should be used separately or as a mixture (e.g., chloroform/methanol, hexane, \(n\)-heptane/isopropanol, hexane/acetonitrile/methanol).

### 3.1.4 Antibacterial properties of algal extracts

The literature did not address algal extracts obtained by microwave assisted extraction in terms of antibacterial activity. The present study tested the antibacterial activity of MAE extracts against two bacterial strains: gram-negative (Escherichia coli) and gram-positive (Staphylococcus aureus). No inhibitory activities of aqueous extracts from Baltic algae were observed. The literature data concerning antibacterial properties of aqueous algal extracts are divergent. Christobel’s et al. (2011) work exhibited that a 100% aqueous extract of Ulva fasciata showed equal inhibitory action towards gram positive (10 mm zone of inhibition of Staphylococcus aureus) and gram negative bacteria (9 mm for Escherichia coli) [24]. Alghazeer et al. (2013) examined extracts obtained by extraction of dried algae powder (Ulva lactuca) with distilled water in a shaker incubator for 24 h at room temperature. The inhibition zone for both bacteria: Staphylococcus aureus and Escherichia coli was 12 mm [25]. Mansuaya et al. (2010) showed that aqueous extracts of Ulva reticulata (obtained by Soxhlet extraction) did not inhibit the growth of Escherichia coli [26]. The same observations were noted by Selvi et al. (2001). Aqueous extracts of Enteromorpha compressa, E. intestinalis, Ulva lactuca, U. fasciata showed trace antibacterial activity (for both strains: Staphylococcus aureus and Escherichia coli) [27]. Different antibacterial properties of algal extracts may have been due to the composition of a given alga, place and season of its collection and finally the used extraction method and parameters of extraction. For antibacterial properties, proteins, polyphenols, polysaccharides, pigments such as chlorophyll and carotenoids, the PUFAs extracted from biomass of algae are responsible [5].

### 3.2 Utilitarian properties of algal extracts

Seaweed products exhibit growth stimulating activities, and it is known that the use of seaweed formulations as nutrient supplements, biostimulants or biofertilizers in agriculture and horticulture increase plant growth and yield [28]. However, there are no data regarding the application of algal extracts obtained by microwave assisted extraction in plant cultivation. The present study investigates the effect of MAE algal extracts on total height, dry weight, content of chlorophyll and nutrients and morphology of Lepidium sativum. For the germination experiments on cress, dilutions of the raw extract obtained by MAE in different temperatures were prepared (0.5, 2.5 and 10%). According to the literature, seaweed extracts are bioactive at low concentrations (diluted as 1:1000 or more) [29,30]. The enhancement of the vegetative growth can be related to the composition of seaweed which is a rich source of macro- and microelement (Fe, Cu, Zn, Co, Mo, Mn and Ni), amino acid, vitamins, plant hormones (cytokinins, auxins, abscisic acid) that affect cellular metabolism of plants leading to enhanced growth and crop yield [29].

### 3.2.1 Total height of the cultivated cress

For each MAE extract (obtained in 25, 40, 60°C), the height of plants (\(N = 20\) from each group) was determined for all three dilutions (0.5, 2.5 and 10%). The studied extracts exhibited varying degrees of the stimulatory effect on the growth of cress seeds. In the case of extracts MAE 60°C and MAE 25°C it was found that with the decrease of algal extract concentration, the height of plant increased. The opposite observation was observed for the extract obtained by MAE at 40°C. The obtained results are as follows:

MAE 60°C: 0.5% (12.2 ± 3.0 cm) > 2.5% (10.9 ± 3.8 cm) > 10% (10.2 ± 3.8 cm)
MAE 40°C: 10% (13.8 ± 1.9 cm) > 2.5% (13.7 ± 1.7 cm) > 0.5% (13.6 ± 1.7 cm)

MAE 25°C: 2.5% (13.6 ± 3.1 cm) > 0.5% (13.3 ± 1.9 cm) > 10% (11.4 ± 3.2 cm)

Plants in the experimental groups with an optimal concentration of the extract were taller than the plants in the control group (11.9 ± 2.5 cm). The best results were obtained for plants in MAE at 40°C:10% group – plants were 16% longer than in the control group. However, the differences were not statistically significant (for \( p < 0.05 \)).

Similar results were obtained by Kavipriya et al. (2011), who tested extracts obtained from green alga Ulva lactuca that were autoclaved at 121°C with distilled water. It was found that the lower concentration (0.1, 0.2, 0.3, 0.4 and 0.5%) of the applied extract correlated with a taller green gram (Vigna radiata) [31].

Our previous experiments also confirmed that the lower concentration of algal extract obtained from Enteromorpha sp. by hydrolysis with 1 M KOH (10, 5 and 2.5%), the longer the plants of Lepidium sativum [32]. In the work of Latique et al. (2013), both treatments with 25% and 50% of Ulva rigida extract (obtained by boiling fresh biomass in distilled water) provided the significant effects on plant growth of bean plants (Phaseolus vulgaris L.); however, the maximum effect was found with 25% treatment [33]. The low concentration – 20% (selected from the series of concentrations: 5, 10, 20, 30, 40 and 50%) of aqueous seaweed extract, obtained by boiling Ulva lactuca in distilled water, promoted seedling growth in terms of shoot and root length of Vigna unguiculata L. Walp. Higher concentrations (≥ 40%) inhibited germination [20].

3.2.3 Multielemental composition of the cultivated cress

The literature showed that the application of algal extracts could increase the content of micro- and macronutrients in cultivated plants. Results in the work of Shaaban et al. (2010) showed that the best concentrations of macronutrients in wheat plants were achieved by the algal extract (Scenedesmus sp.) treatments or the higher dose of the micronutrient fertilizer. However, the best uptake, nutrient balance and dry matter accumulation was recorded with combined algal extract and micronutrient fertilizer treatment [34]. In the present study, the multielemental composition of Lepidium sativum was most affected by the application of the following extracts: MAE 60°C (10% extract), MAE 40°C (10%) MAE 25°C (2.5%) taking into account the content of micro- and macroelements (Table 3). Among these three extracts, the best properties had extract MAE at 25°C (2.5%). The content of B (0.55%), Cu (31%), Mn (22%), Mo (19%), Ni (23 times), Zn (71%), K (2%), Mg (1%), P (5%) and S (13%) was higher in this experimental group than in the control. The results showed that the obtained seaweed extract can act as a nontoxic and eco-friendly biostimulant which can constitute a supplement to chemical fertilizer. In places, where the use of inorganic fertilizers is not encouraged in agriculture farming, the application of algae derived products might be a good approach to replace the extensive use of inorganic fertilizers [30].

3.2.4 Chlorophyll content in the cultivated cress

In the present study, the dry mass of the cultivated plants was comparable in all groups, taking into account both the temperature of extraction and the dilutions of the extract. For MAE at 60°C, the average mass for all dilutions was 0.0664 ± 0.0020 g of dry mass, for MAE at 40°C 0.0664 ± 0.0027 g, for MAE at 25°C 0.0618 ± 0.0031 g. There was no observation of the algal extract concentration on the dry weight of Lepidium sativum. Gireesh et al. (2011) found that the low concentration (20%) of aqueous seaweed extract from Ulva lactuca promoted dry weight of Vigna unguiculata L. Walp. (above this concentration – 30, 40 and 50%, dry weight of plants was lowered). For the 20% concentration, the dry weight of V. unguiculata was 9% higher than in the control group [20]. Similarly, our work showed that algal extracts weakly affected the increase in plant biomass.

In the present study, the dry mass of the cultivated plants was comparable in all groups, taking into account both the temperature of extraction and the dilutions of the extract. For MAE at 60°C, the average mass for all dilutions was 0.0664 ± 0.0020 g of dry mass, for MAE at 40°C 0.0664 ± 0.0027 g, for MAE at 25°C 0.0618 ± 0.0031 g. There was no observation of the algal extract concentration on the dry weight of Lepidium sativum. Gireesh et al. (2011) found that the low concentration (20%) of aqueous seaweed extract from Ulva lactuca promoted dry weight of Vigna unguiculata L. Walp. (above this concentration – 30, 40 and 50%, dry weight of plants was lowered). For the 20% concentration, the dry weight of V. unguiculata was 9% higher than in the control group [20]. Similarly, our work showed that algal extracts weakly affected the increase in plant biomass.

3.2.4 Chlorophyll content in the cultivated cress

In the present study, the dry mass of the cultivated plants was comparable in all groups, taking into account both the temperature of extraction and the dilutions of the extract. For MAE at 60°C, the average mass for all dilutions was 0.0664 ± 0.0020 g of dry mass, for MAE at 40°C 0.0664 ± 0.0027 g, for MAE at 25°C 0.0618 ± 0.0031 g. There was no observation of the algal extract concentration on the dry weight of Lepidium sativum. Gireesh et al. (2011) found that the low concentration (20%) of aqueous seaweed extract from Ulva lactuca promoted dry weight of Vigna unguiculata L. Walp. (above this concentration – 30, 40 and 50%, dry weight of plants was lowered). For the 20% concentration, the dry weight of V. unguiculata was 9% higher than in the control group [20]. Similarly, our work showed that algal extracts weakly affected the increase in plant biomass.
Table 3: Multielemental composition of cultivated cress (mg kg\(^{-1}\) d.m.).

| Element  | Control group | MAE 25°C | 10% | 2.5%* | 0.5% | MAE 40°C | 10% | 2.5% | 0.5% | MAE 60°C | 10% | 2.5% | 0.5% |
|----------|---------------|----------|-----|-------|------|----------|-----|------|------|----------|-----|------|------|
| Microelements |               |          |     |       |      |          |     |      |      |          |     |      |      |
| B        | 7.30 ± 1.10    | 6.95 ± 1.04 | 7.34 ± 1.10 | 5.83 ± 0.87 | 6.98 ± 1.05 | 7.07 ± 1.06 | 7.04 ± 1.06 | 10.7 ± 1.6 | 6.55 ± 0.98 | 5.54 ± 0.83 |
| Co       | 0.0755 ± 0.0189 | 0.0335 ± 0.0084 | 0.0689 ± 0.0172 | 0.0614 ± 0.0154 | 0.0340 ± 0.0085 | 0.0156 ± 0.0039 | 0.0271 ± 0.0068 | 0.0363 ± 0.0091 | 0.0730 ± 0.0183 | 0.0764 ± 0.0191 |
| Cu       | 4.24 ± 0.64    | 4.73 ± 0.71    | 5.55 ± 0.83    | 5.43 ± 0.81    | 9.99 ± 1.50    | 3.65 ± 0.55    | 4.08 ± 0.61    | 4.76 ± 0.71    | 4.26 ± 0.64    | 4.04 ± 0.61    |
| Fe       | 278 ± 42       | 190 ± 29       | 222 ± 33       | 190 ± 28       | 166 ± 25       | 173 ± 26       | 183 ± 27       | 182 ± 27       | 194 ± 29       | 211 ± 32       |
| Mn       | 41.9 ± 6.3     | 45.6 ± 6.8     | 51 ± 7.6       | 47.8 ± 7.2     | 45.2 ± 6.8     | 47.6 ± 7.1     | 47.2 ± 7.1     | 41.8 ± 6.3     | 47.6 ± 7.1     | 48.1 ± 7.2     |
| Mo       | 1.20 ± 0.18    | 1.3 ± 0.19     | 1.43 ± 0.21    | 1.39 ± 0.21    | 1.54 ± 0.23    | 1.47 ± 0.22    | 1.48 ± 0.22    | 1.87 ± 0.28    | 1.41 ± 0.21    | 1.48 ± 0.22    |
| Ni       | 0.630 ± 0.009  | 7.28 ± 0.11    | 14.7 ± 0.2     | 8.22 ± 0.12    | 5.26 ± 0.08    | 4.41 ± 0.07    | 12.2 ± 0.2     | 3.99 ± 0.06    | 2.22 ± 0.03    | 2.34 ± 0.4     |
| Si       | 182 ± 27       | 127 ± 19       | 133 ± 20       | 112 ± 17       | 113 ± 17       | 114 ± 17       | 126 ± 19       | 147 ± 22       | 127 ± 19       | 123 ± 18       |
| Zn       | 58.2 ± 8.7     | 82.3 ± 12.3    | 99.6 ± 14.9    | 75.8 ± 11.4    | 60.3 ± 9       | 59.7 ± 9       | 73.3 ± 11      | 60.8 ± 9.1     | 59.6 ± 8.9     | 59.7 ± 9       |
| Macroelements |          |          |     |       |      |          |     |      |      |          |     |      |      |
| Ca       | 11200 ± 2240   | 10500 ± 2110  | 9170 ± 1840    | 9100 ± 1820    | 9300 ± 1860    | 8190 ± 1640    | 9300 ± 1900    | 8900 ± 1800    | 8300 ± 1700    |
| K        | 58100 ± 11600  | 59200 ± 11800 | 59100 ± 11800  | 57900 ± 11600  | 55800 ± 11200  | 58900 ± 11800  | 55100 ± 11000  | 57500 ± 11500  | 60100 ± 1200   | 57600 ± 11500  |
| Mg       | 7000 ± 1400    | 7150 ± 1430   | 7070 ± 1410    | 7260 ± 1450    | 7560 ± 1510    | 7412 ± 1480    | 7000 ± 1400    | 7200 ± 1400    | 7300 ± 1450    | 7330 ± 1500    |
| Na       | 1850 ± 370     | 2830 ± 570    | 1800 ± 360     | 1300 ± 270     | 2340 ± 470     | 1550 ± 310     | 1390 ± 280     | 2700 ± 540     | 1580 ± 315     | 1200 ± 240     |
| P        | 16500 ± 3300   | 15900 ± 3180  | 17400 ± 3480   | 17700 ± 3530   | 16452 ± 3290   | 17600 ± 3520   | 17200 ± 3440   | 16100 ± 3200   | 17100 ± 3430   | 17700 ± 3500   |
| S        | 15500 ± 3100   | 13500 ± 2700  | 17500 ± 3500   | 17400 ± 3470   | 13900 ± 2790   | 16900 ± 3390   | 16200 ± 3230   | 14300 ± 2900   | 17900 ± 3600   | 17100 ± 3400   |
| Toxic metals |          |          |     |       |      |          |     |      |      |          |     |      |      |
| As       | 0.348 ± 0.045  | 0.591 ± 0.077 | 0.820 ± 0.107 | 0.560 ± 0.073 | 0.340 ± 0.044 | 0.348 ± 0.045 | 0.586 ± 0.076 | 0.430 ± 0.056 | 0.290 ± 0.038 | 0.400 ± 0.050 |
| Cd       | 0.258 ± 0.033  | 0.222 ± 0.029 | 0.203 ± 0.026 | 0.234 ± 0.030 | 0.192 ± 0.025 | 0.199 ± 0.026 | 0.203 ± 0.026 | 0.211 ± 0.027 | 0.221 ± 0.029 | 0.190 ± 0.025 |
| Pb       | 0.258 ± 0.033  | 0.954 ± 0.124 | 0.997 ± 0.13   | 0.821 ± 0.107 | 0.704 ± 0.092 | 0.557 ± 0.072 | 12.9 ± 1.7    | 1.09 ± 0.14   | 0.772 ± 0.100 | 0.711 ± 0.092 |

* – the best variant
In the present study a high Mg and Fe content in the Baltic algae (Mg: 3181 ± 63 mg kg\(^{-1}\) d.m., Fe: 6661 ± 1332 mg kg\(^{-1}\)) and consequently in extracts might have influenced the synthesis of chlorophyll.

Latique et al. (2013) also observed that the foliar application of a 25% aqueous extract of *Ulva rigida* enhanced chlorophyll content in leaves of plants when compared to plants in the control group, particularly for chlorophyll [33]. Pise and Sabale (2010) showed that extracts obtained from *Ulva* by three different methods (using blender and then mortar pestle, by boiling in distilled water for one hour and by soaking in distilled water for two days) influenced photosynthetic pigments percentage in fenugreek (*Trigonella foenum-graecum* L.) [35]. Gireesh et al. (2011) also noticed that lower concentrations of the aqueous *Ulva lactuca* extract promoted the chlorophyll content of *Vigna unguiculata* up to 20% when compared to the control, while higher concentrations (> 20%) decreased the chlorophyll content [20].

### 3.2.5 SEM analysis of cultivated plants

In order to evaluate the effect of MAE extracts on plant morphology, stalk, leaf – the internal and external part of *Lepidium sativum* were examined by using a Scanning Electron Microscope. SEM observations for two magnifications (500 and 2000) are presented in Fig. 1. As an example, MAE at 40°C was selected. Morphological studies showed a shrinkage of the surface layer of the stalk treated with the aqueous extract (Fig. 1a). There was no effect of the MAE extract on the morphology of the internal part of leaf (stoma) (Fig. 1b). SEM photographs of the external part of leaf showed shrinkage of cuticle (Fig. 1c). The observed changes had no influence on the growth of the plants.

### 4 Conclusions

In the present paper, microwave assisted extraction at three different temperatures (25, 40, 60°C) was used to obtain natural, non-toxic extracts from the biomass of Baltic macroalge. The extracts were characterized using different analytical techniques. The extraction temperature influenced the composition of the obtained products. It was observed that there was a correlation of the lower temperature of the extraction showing a lower concentration of the micro- and macroelements in the final extract. Low temperatures favored the extraction of polyphenols (higher temperatures caused the decomposition of phenolic compounds). Algal extracts obtained by MAE did not contain fatty acids and antibacterial activity.

More promising results were obtained for germination tests with the use of algal extracts. It was shown that the obtained products positively influenced height, weight, multielemental composition and the content of chlorophyll in the cultivated *Lepidium sativum* when compared with the control group. However, it is difficult to clearly state which extraction temperature (25, 40, 60°C) and which dilution of the initial extract (0.5, 2.5 and 10%) was the best in terms of plant growth parameters and the composition of the biomass. Plants were the

| Sample       | Total chlorophyll concentration | Concentration of chlorophyll a | Concentration of chlorophyll a |
|--------------|--------------------------------|-------------------------------|-------------------------------|
| Control      | 30.4                           | 22.2                          | 8.25                          |
| MAE 25°C     |                                |                               |                               |
| 10%          | 30.5                           | 22.1                          | 8.46                          |
| 2.5%         | 27.7                           | 19.6                          | 8.12                          |
| 0.5%         | 28.2                           | 19.8                          | 8.37                          |
| MAE 40°C     |                                |                               |                               |
| 10%          | 29.7                           | 21.7                          | 8.06                          |
| 2.5%         | 32.9                           | 23.0                          | 9.95                          |
| 0.5%         | 32.9                           | 24.0                          | 8.94                          |
| MAE 60°C     |                                |                               |                               |
| 10%          | 33.0                           | 23.3                          | 9.70                          |
| 2.5%         | 34.2                           | 24.1                          | 10.1                          |
| 0.5%         | 33.5                           | 24.0                          | 9.55                          |
Figure 1: The influence of algal extracts produced by MAE on plant morphology: (a) stalk, (b) leaf – internal part, (c) leaf – external part of plant
Figure 1: The influence of algal extracts produced by MAE on plant morphology: (a) stalk, (b) leaf – internal part, (c) leaf – external part of plant
highest for 10% MAE at 40°C. The heaviest plants were observed in the group with an extract obtained by MAE at 40 and 60°C, dilution had no effect. Taking into account the multielemental composition of *Lepidium sativum* the best option was the application of 2.5% MAE at 25°C. Chlorophyll content in the plants was influenced to the highest extent by 2.5% MAE at 60°C.

The presented results suggested that MAE algal extracts can induce stronger seed germination and plant growth parameters. Seaweed extract obtained from *Polysiphonia, Ulva, Cladophora* rich in nutrients and polyphenols can be recommended to the growers as a natural product which will increase germination, growth and yield of cultivable plants.

**Acknowledgments:** This project is financed in the framework of a grant entitled – Biologically active compounds in extracts from Baltic seaweeds (2012/05/D/ST5/03379) attributed by The National Science Centre, and grant entitled – Innovative technology of seaweed extracts – components of fertilizers, feed and cosmetics (PBS/1/AI/2/2012) attributed by The National Centre for Research and Development in Poland.

**Conflict of interest:** The authors have declared no conflict of interest

### Abbreviations

- DHA: docosahexaenoic acid
- MAE: microwave assisted extraction
- MTBE: tert-butyl methyl ether
- PLE: pressurised liquid extraction
- PUFA: polyunsaturated fatty acids
- SLE: solid–liquid extraction

### References

[1] Kadam S.U., Tiwari B.K., O’Donnell C.P., Application of novel extraction technologies for bioactives from marine algae, J. Agric. Food Chem., 2013, 61, 4667–4675.

[2] Mäki-Arvela P., Hachemi I., Murzin D.Y., Comparative study of the extraction methods for recovery of carotenoids from algae: extraction kinetics and effect of different extraction parameters, J. Chem. Technol. Biotechnol., 2014, 89, 1607–1626.

[3] Wang Y. PhD thesis: Sample preparation/concentration for trace analysis in GC/MS. Faculty of the Virginia Polytechnic Institute and State University, 1997.

[4] Pasquet V., Chéroutrier J.-R., Farhat F., Thiéry V., Piot J.-M., Bérard J.-B, Kaas R., Serive B., Patrice T., Cadoret J.-P., Picot L., Study on the microalgal pigments extraction process: Performance of microwave assisted extraction, Process Biochem., 2011, 46, 59–67.

[5] Michalak I., Chojnacka K., Algae as production systems of bioactive compounds, Eng. Life Sci., 2015, 15, 160–176.

[6] Zhao L., Chen G., Zhao G., Hu X., Optimization of Microwave-Assisted extraction of astaxanthin from *Haematococcus Pluvialis* by Response Surface Methodology and antioxidant activities of the extracts, Sep. Sci. Technol., 2009, 44, 263–262.

[7] Luo H.-Y, Wang B., Yu C.-G, Xu Y.-F., Optimization of microwave-assisted extraction of polyphenols from *Enteromorpha prolifera* by orthogonal test, Chinese Her. Med., 2010, 2, 321–325.

[8] Wang B., Tong G.-Z., Qu Y.-L, Li L., Microwave-assisted extraction and *in vitro* antioxidant evaluation of polysaccharides from *Enteromorpha prolifera*, Appl. Mech. Mat., 2011, 79, 204–209.

[9] Li Z., Wang B., Zhang Q., Qu Y., Xu H., Li G., Preparation and antioxidant property of extract and semipurified fractions of *Caulerpa racemosa*, J. Appl. Phycol., 2012, 24, 1527–1536.

[10] Leonelli C., Mason T.J., Microwave and ultrasonic processing: Now a realistic option for industry, Chem. Eng. Process., 2010, 49, 885–900.

[11] Wang L., Weller C., Recent advances in extraction of nutraceuticals from plants, Trends Food Sci. Technol., 2006, 17, 300–312.

[12] Wilk R., Chojnacka K., Rój E., Górecki H., Technology for preparation of algae extract. Part 1. Raw material, Przem. Chem., 2014, 93(7), 1215–1218 (in Polish).

[13] Sim K.S., Sri Nuresstri A.M., Norhanom A.W., Phenolic content and antioxidant activity of crude and fractionated extracts of *Pereszia bleo* (Kunth) DC. (*Cactaceae*), Afr. J. Pharmacy Pharmacol., 2010, 4, 193–201.

[14] Arnon D.I., Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*, Plant Physiol., 1949, 24, 1–15.

[15] Michalak I., Marycz K., Basinska K., Chojnacka K., Using SEM-EDX and ICP-OES to investigate the elemental composition of green macroalgae *Vaucheria sessilis*, Sci. World J., 2014, Article ID 891928, http://dx.doi.org/10.1155/2014/891928.

[16] Haroon A.M., Szaniawska A., Normant M., Janas U., The biochemical composition of *Enteromorpha spp.* from the Gulf of Gdański coast on the southern Baltic Sea, Oceanologica, 2000, 42, 19–28.

[17] Szefer P., Skwarzec B., Concentration of elements in some seaweeds from coastal region of the southern Baltic and in the Żarnowiec Lake, Oceanologica, 1988, 25, 87–98.

[18] Michalak I., Chojnacka K. Multielemental analysis of the biomass of macroalgae from the Baltic Sea by ICP-OES to monitor environmental pollution and establish potential uses of algae, Int. J. Environ. Anal. Chem., 2009, 89, 583–596.

[19] Ibañez, E., Herrero, M., Mendiola, J. A., Castro-Puyana, M., Extraction and characterization of bioactive compounds with health benefits from marine resources: Macro and micro algae, cyanobacteria, and invertebrates, in: Hayes, M. (Ed.), Marine Bioactive Compounds: Sources, Characterization and Applications, Springer Science+BusinessMedia, LLC, New York 2012, pp. 55–98.
Seaweed extract by microwave assisted extraction as plant growth biostimulant

[20] Gireesh R., Haridevi C.K., Saliikutty J., Effect of Ulva lactuca extract on growth and proximate composition of Vigna unguiculata L. Walm., J. Res. Biol., 2011, 8, 624–630.

[21] Möller M., Smith M.L., The significance of the mineral component of seaweed suspensions on lettuce (Lactuca sativa L.) seedling growth, J. Plant Physiol., 1998, 153, 658–663.

[22] Tierney M.S., Smyth T.J., Hayes M., Soler-Vila A., Croft A.K., Brunton N., Influence of pressured liquid extraction and solid–liquid extraction methods on the phenolic content and antioxidant activities of Irish macroalgae, Int. J. Food Sci. Technol., 2013, 48, 860–869.

[23] Hayat K., Hussain S., Abbasi S., Farooq U., Abbas S., Abbas S., Farooq U., Ding B.M., Xia S.Q., Jia C.Q., Zhang X.M., Xia W.S., Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro, Sep. Purif. Technol., 2009, 70, 63–70.

[24] Christobel J.G., Lipton A.P., Aishwarya M.S., Sarika A.R., Udayakumar A., Antibacterial activity of aqueous extract from selected macroalgae of southwest coast of India, Seaweed Res. Utiln., 2011, 33, 67–75.

[25] Alghazeer R., Whida F., Abduelrhman E., Gammoudi F., Azwai S., Screening of antibacterial activity in marine green, red and brown macroalgae from the western coast of Libya, Natural Sci., 2013, 5, 7–14.

[26] Mansuya P., Aruna P., Sridhar S., Kumar J. S., Babu S., Antibacterial activity and qualitative phytochemical analysis of selected seaweeds from Gulf of Mannar region, J. Exper. Sci., 2010, 1, 23–26.

[27] Selvi M., Selvaraj R., Chidambaram A., Screening for antibacterial activity of macro algae, Seaweed Res. Utiln., 2001, 23, 59–63.

[28] Rodríguez-Jasso R.M., Mussatto S.I., Pastrana L., Aguilar C.N., Teixeira J.A., Microwave-assisted extraction of sulfated polysaccharides (fucoidan) from brown seaweed, Carbohydr. Pol., 2011, 86, 1137–1144.

[29] Sousa A.M.M., Alves V.D., Morais S., Delerue-Matos C., Gonçalves M.P., Agar extraction from integrated multitrophic aquacultured Gracilaria vermiculophylla: Evaluation of a microwave-assisted process using response surface methodology, Bioresource Technol., 2010, 101, 3258–3267.