

## THE POTENTIAL USEFULNESS OF A NEW GENERATION OF AGRO-PRODUCTS BASED ON RAW MATERIALS OF BIOLOGICAL ORIGIN

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**Abstract.** The article discusses the possibility of the use of algal products, obtained by different methods – extraction (traditional solvent extraction, supercritical fluid extraction, extraction assisted by microwave) and homogenization, in the cultivation of plants. Algal extracts were discussed for their use as potential plant growth biostimulants and the homogenates as formulations for seed treatment. The work is focused on the possibility of using primarily macroalgae from the Baltic Sea basin (in many seaside resorts constitute a waste due to eutrophication) as raw material for the extraction/homogenization. Examples of the research (laboratory – germination tests and field trials) on the impact of algal preparations on the plant growth were presented. Literature data indicate that the algae based products increase the content of micro-, macroelements, chlorophyll in the cultivated plants, as well as their length and weight. Therefore, in the future, they may complement the range of products available on the market.

**Keywords:** algae, extracts, production, biostimulants, seed treatment

### INTRODUCTION

Commercially available algal based products have been already tested and applied in the agriculture. There is a wide range of algal based products on the market (tab. 1). For their production, the most abundant species of marine macroalgae are used, for example: brown seaweeds: *Laminaria digitata* (Agrocean B, Agrocean Ca, Agrocean Mg), *Ascophyllum nodosum* (Bio-algen S 90, Colorado, FoliqAscovigor, Goe-

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mar BM 86, GoemarGoteo, GoemarVerduro, Phytoamin, WuxalAscofol) and *Ecklonia maxima* (BasfoliarActiv).

The aim of our work is to produce fully standardised algal extract-based formulations that will be applied in the agriculture as biostimulants of plant growth. The raw material for their production is the biomass of green algae (Baltic macroalgae – *Chlorophyta*) and commercially available microalga *Spirulina* sp. Large amounts of beach-cast seaweed resulting from eutrophication present a nuisance (e.g. emission of odours due to decomposition) in Baltic coastal areas, especially in tourist resorts [Dederen 1992]. Therefore, it is important to examine methods of the removal and processing of algal biomass and to find a method for cost-effective utilization, enabling to obtain a value-added product.

According to the WAB Project (Wetlands Algae Biogas; <http://wabproject.pl>), only from the beach and sea in Sopot (Poland) in July, August and September 2011, almost 400 tons of algal biomass was collected – 164 tons from the beach and 231 tons from the water. It should be noted that the amount of algal biomass in different geographical areas depends on many factors, for example: seasonal, annual, environmental and physiological. Nevertheless, this excessive growth of algae is a serious problem in many countries, including Poland [Wilk et al. 2013a].

In the literature, several potential applications of algal biomass are proposed. Filipkowska et al. [2008] suggested that the biomass of macroalgae from the Sopot beach (the Baltic Sea) could be used as a bio-fertilizer. Hansen et al. [2003] found that macroalgae can be consumed directly from the beach shore by animals. Our work focuses on the production of algal extract-based products as plant growth biostimulants. According to Gawrońska [2008], this group of active natural products is efficiently used in enhancing the plant's endogenous resistance or tolerance to the biotic and abiotic stresses and has been intensively examined in the last two decades.

**Production of algae-based products for agriculture.** In the realized projects, algal products were obtained by different methods of extraction and homogenization. These processes should be preceded by the appropriate pre-treatment of biomass. The pre-treatment process of algae collected from natural water reservoirs (marine and freshwater) involved several steps, such as: harvesting of algal biomass, washing to remove sand particles and impurities, drying, shredding, milling to obtain homogenous sample and sieving. This process aims also to improve the extraction yield. Therefore, several methods: mechanical, thermal, physical, chemical and enzymatic are used in order to disrupt the cell wall of algal biomass and make the biologically active compounds more bioavailable [Michalak and Chojnacka 2014]. In the work of Wilk et al. [2014], technology for the preparation of Baltic seaweed biomass (species *Polysiphonia*, *Ulva* and *Cladophora*) for the extraction process was presented. Some economic data of the process were also included.

In our work, several extraction techniques were used to produce algal extracts: Supercritical Fluid Extraction – SFE [Wilk et al. 2013b, Chojnacka et al. 2014, Michalak et al. 2016], Microwave Assisted Extraction – MAE [Michalak et al. 2015], acidic extraction with water of pH 2 acidulated with H<sub>2</sub>SO<sub>4</sub> (final extract adjusted to the neutral pH) [Michalak and Chojnacka 2013], alkaline extraction with KOH (final extract

also adjusted to the neutral pH) [Michalak et al. 2014a], extract produced from composted macroalgae [Michalak et al. 2014b] – Figure 1. Among the examined methods, a special attention should be paid to SFE, which has an advantage over other methods, for example: fast extraction rate (allow to avoid degradation of labile compounds), high yield and minimal or no use of organic solvents [Michalak and Chojnacka 2014]. This new concept of production of supercritical algal extracts was tested on brown macroalgae – *Fucus* sp. [Wilk et al. 2013b]. Two extractions with or without ethanol as a co-solvent were performed at 40°C under 300–900 bar in a pilot plant scale (Supercritical Extraction Department, New Chemical Syntheses Institute in Puławy) to recover biologically active compounds. Algae are known to contain compounds such as carbohydrates, proteins, minerals, oils, fats, polyunsaturated fatty acids, as well as bioactive compounds such as antioxidants (polyphenols, vitamin E, vitamin C, mycosporine-like amino acids) and pigments, such as carotenoids (carotene xanthophyll), chlorophylls and phycobilins (phycocyanin, phycoerythrin), which possess antibacterial, antiviral, antifungal, antioxidative, antiinflammatory and antitumor properties [Thomas and Kim 2013, Michalak and Chojnacka 2015a]. With such a vast array of properties, algal extracts can find application in different branches of industry, for example: food, cosmetics, pharmaceuticals and agriculture. Additionally, in our work, algal homogenates were obtained as a second group of natural algal-based products for agriculture designed for the seed treatment [Dmytryk et al. 2014a, 2015].

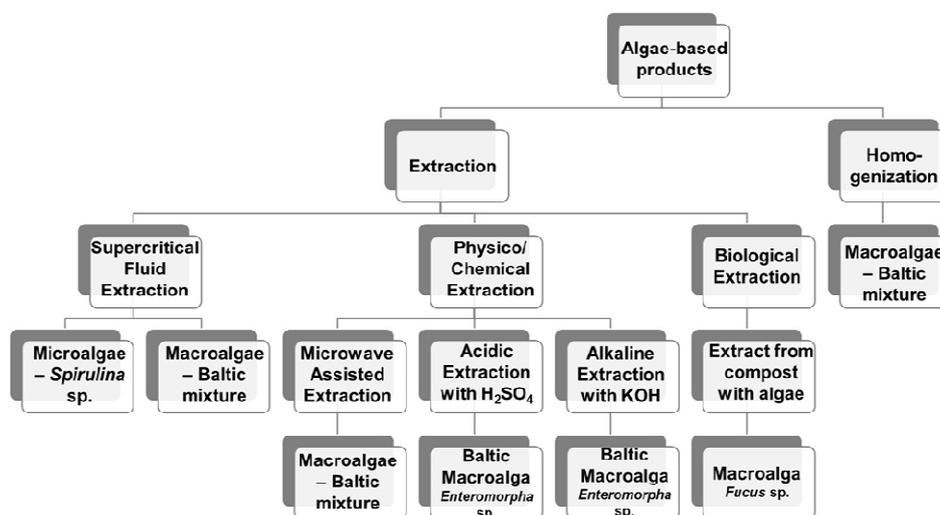


Fig. 1. Algae-based products obtained by different extraction methods and homogenization

Table 1. Examples of algal based products for agriculture available on Polish market (20 August 2015)

| Product         | Composition   | Tested plants  | Action  | Producer                         |
|-----------------|---|--|---|----------------------------------|
|                 |   | <b>Biostimulants</b>   |   |                                  |
| Algex           | <ul style="list-style-type: none"> <li>– extract from <i>Ascophyllum nodosum</i>–10% (including vitamins, amino acids, phytohormones, polysaccharides, betaine)</li> <li>– N: 8.0% w/w, P: 3.6%, K: 7.0%, B: 0.036 %, Zn: 0.025%, Cu: 0.009%, Fe: 0.016 %, Mn: 0.036%, Mo: 0.0036%</li> </ul>   | <ul style="list-style-type: none"> <li>– apples, pears, cherry plum, strawberries, raspberries, currants</li> <li>– potatoes, corn, tomato, pepper, cucumber</li> <li>– rapeseed, sugar beets</li> </ul>   | <ul style="list-style-type: none"> <li>– stimulates the plant mineral nutrition, the process of Fertilization in flowering plants and fruit set</li> <li>– activates the life processes of plants in stressful situations</li> <li>– has a beneficial effect on cell division and growth of fruits</li> <li>– prevents deformation of fruits</li> </ul> | Rosier S.A., Belgium             |
| Bio-Algeen S-90 | <ul style="list-style-type: none"> <li>– extract from brown macroalgae</li> <li>– N: 0.02%, P (P<sub>2</sub>O<sub>5</sub>): 0.006%, K (K<sub>2</sub>O): 0.096%, Ca (CaO): 0.31%, Mg (MgO): 0.021%, B:16 mg·kg<sup>-1</sup>, Fe: 6.3 mg·kg<sup>-1</sup>, Cu: 0.2 mg·kg<sup>-1</sup>, Mn: 0.6 mg·kg<sup>-1</sup>, Zn:1.0 mg·kg<sup>-1</sup>, Mo, Se and Co</li> </ul> | <ul style="list-style-type: none"> <li>– seedbeds</li> <li>– green houses (ornamental, forest, etc.)</li> <li>– intensive crops (horticulture)</li> <li>– fruit trees (olives, citric, etc.)</li> <li>– in large extension farms (rice, corn, cereals, cotton, beetroots, etc.)</li> </ul> | <ul style="list-style-type: none"> <li>– assists rooting and encourages rooting system growth, promoting soil microbe life and fostering balanced plant growth</li> <li>– activates the plant's natural defences against pathogens</li> </ul>   | Schulze &Hermsen GmbH, Germany   |
| WuxalAscofol    | <ul style="list-style-type: none"> <li>– extract from marine algae – <i>Ascophyllum nodosum</i></li> <li>– B: 3.81%, Mn: 1.02 %, Zn: 0.64%, Fe, Cu</li> <li>– amino acids, plant hormones (cytokinins, auxin, gibberellins, betaine), vitamins</li> </ul>   | <ul style="list-style-type: none"> <li>– rapeseed, sugar beets</li> <li>– cereals</li> <li>– potatoes, corn</li> </ul>   | <ul style="list-style-type: none"> <li>– improves plant growth and development</li> <li>– increases the intensity of flowering</li> <li>– increases the quantity and quality of the crop</li> <li>– counteract the effects of physiological stress and increase the resistance</li> </ul>   | F&N Agro, Germany                |
| Phytoamin ®     | <ul style="list-style-type: none"> <li>– extract from marine algae – <i>Ascophyllum nodosum</i></li> <li>– N: 0.16%, K (K<sub>2</sub>O): 1.45%</li> </ul>   | <ul style="list-style-type: none"> <li>– berry plants</li> <li>– grapes</li> <li>– potatoes, onion, cabbage, asparagus</li> <li>– ornamental plants</li> </ul>   | <ul style="list-style-type: none"> <li>– increases plant resistance against diseases</li> <li>– improves plant growth and development and quality of leaves</li> </ul>  | AZELIS Poland sp. z o.o., Poland |

|             |  |  |   |                              |
|-------------|--|--|---|------------------------------|
| Agrocean B  | <ul style="list-style-type: none"> <li>- extract from marine algae <i>Lamina radigitata</i>: 47%</li> <li>- Mg: 5.0%, B: 2.5%</li> <li>- alginic acid, iodine, oligosaccharides, plant hormones (auxins, cytokinins, Indoleacetic acid, gibberellin, betaine), amino acids, mannitol, colloids</li> </ul>  | <ul style="list-style-type: none"> <li>- apples, pears, cherries, plums, currants, grapes</li> <li>- strawberries, raspberries</li> <li>- potatoes, corn, rapeseed, sugar beets</li> <li>- vegetables: cabbage, carrots, beets, beans, celery, tomato, pepper, cucumber</li> </ul> | <ul style="list-style-type: none"> <li>- provides the nutrients necessary for proper development of flowers in a period of limited activity of the root system</li> <li>- stimulates the synthesis of polyamines, which ensures proper development of flowers</li> <li>- improves the volatility of pollen germination and thereby increasing the number of fruit set</li> <li>- has a beneficial effect on cell division and growth of fruits</li> <li>- increases synthesis of chlorophyll in leaves</li> <li>- activates the life processes of plants in stressful situations</li> <li>- supplements the deficiency of nutrients at the beginning of the growing season</li> <li>- stimulates the uptake of fertilizers from the soil</li> <li>- increases the sugar content in the fruit</li> </ul> | Agrosimex sp. z o.o., Poland |
| Agrocean Ca | <ul style="list-style-type: none"> <li>- extract from marine algae <i>Lamina radigitata</i>: 47%</li> <li>- N: 8.77% (including- nitrates (NO<sub>3</sub>): 8.15% and NH<sub>3</sub>-N: 0.62%), CaO: 15%</li> <li>- alginic acid, iodine, oligosaccharides, plant hormones (auxins, cytokinins, Indoleacetic acid, gibberellin, betaine), amino acids, mannitol, colloids</li> </ul> | <ul style="list-style-type: none"> <li>- apples, pears</li> <li>- cherries, plums, currants, grapes</li> <li>- strawberries, raspberries</li> <li>- vegetables - tomato, pepper, cucumber</li> <li>- vegetables - cabbage, lettuce, celery</li> </ul>                              | <ul style="list-style-type: none"> <li>- increases the content of calcium in the fruits</li> <li>- improves the storage capacity of fruits</li> <li>- prevents: bitter pit, blossom-end rot of tomato, pepper, tip burn of cruciferous vegetables</li> <li>- improves firmness and hardness of the fruit</li> <li>- strawberries and raspberries</li> <li>- has a beneficial effect on cell division and growth of fruits</li> <li>- increases synthesis of chlorophyll in leaves</li> <li>- activates the life processes of plants in stressful situations</li> <li>- supplements the deficiency of nutrients at the beginning of the growing season</li> <li>- stimulates the uptake of fertilizers from the soil</li> <li>- increases the sugar content in the fruit</li> </ul>                      | Agrosimex sp. z o.o., Poland |

|                       |  |   |   |                            |
|-----------------------|--|---|---|----------------------------|
| Agrocean Mg           | <ul style="list-style-type: none"> <li>– extract from marine algae <i>Lamina radiolata</i>: 47%</li> <li>– MgO: 13%</li> <li>– elginicacid, iodine, oligosaccharides, plant hormones (auxins, cytokinins, indoleacetic acid, gibberellin, betaine), amino acids, mannitol, colloids</li> </ul>   | <ul style="list-style-type: none"> <li>– apples, paer</li> <li>– cherries, plums, currants, grapes</li> <li>– strawberries, raspberries</li> <li>– vegetables – cabbage, lettuce, celery, carrots</li> <li>– rapeseed, sugar beets, corn</li> <li>– hop</li> <li>– potatoes</li> <li>– cereals</li> </ul> | <ul style="list-style-type: none"> <li>– effectively eliminates magnesium deficiency</li> <li>– affects cell division and growth of fruits</li> <li>– increases synthesis of chlorophyll in leaves</li> <li>– activates photosynthesis</li> <li>– activates the life processes of plants in stressful situations</li> <li>– increases the resistance of plants to adverse weather conditions, high temperatures, drought, excessive UV radiation</li> <li>– stimulates the uptake of fertilizers from the soil</li> <li>– increases the sugar content in the fruit</li> </ul> | Agrosimexp. z o.o., Poland |
| Basfoliar® Kelp P Max | <ul style="list-style-type: none"> <li>– extract from <i>Ecklonia maxima</i></li> <li>– N: 4.0 %, NH<sub>2</sub>nitrogen 4.0 %, P (P<sub>2</sub>O<sub>5</sub>): 6.0 %, K (K<sub>2</sub>O): 2.4 %</li> <li>– B: 0.25 mg·kg<sup>-1</sup>, Cu: 0.17, Fe: 0.61, Mn: 0.01, Zn: 0.56, Mo: 0.11</li> <li>– proteins 1.95 %, amino acids 0.2 %, ash 1.57 %, traces of vitamins (A, B, C, E)</li> </ul> | <ul style="list-style-type: none"> <li>– apple, apricot</li> <li>– rapeseed, sugar beets</li> <li>– wheat</li> <li>– potatoes, tomatoes, onion, corn, cabbage</li> <li>– flowering plants</li> </ul>  | <ul style="list-style-type: none"> <li>– enhances plant establishment, growth development and health</li> </ul>   | Compo Expert, Germany      |
| Basfoliar® Activ      | <ul style="list-style-type: none"> <li>– extract from <i>Ecklonia maxima</i></li> <li>– N: 3.0%, NH<sub>2</sub>nitrogen 3.0%, P (P<sub>2</sub>O<sub>5</sub>): 27%, K (K<sub>2</sub>O): 18%</li> <li>– B: 0.01%, Cu: 0.02%, Fe: 0.02%, Mn: 0.01%, Zn: 0.01%, Mo: 0.001%</li> <li>– proteins, amino acids, ash, traces of vitamins (A, B, C, E), phytohormones</li> </ul>                        | <ul style="list-style-type: none"> <li>– corn, rapeseed, sugar beets, potatoes</li> <li>– cereals</li> <li>– wine-grapes, strawberry</li> <li>– cucumber, tomatoes, onion, pepper, lettuce</li> </ul>   | <ul style="list-style-type: none"> <li>– enhances plant establishment, growth development and health</li> <li>– increases the concentration of all nutrients in the leaf</li> </ul>   | Compo Expert, Germany      |
| FoliQ® AscoVigor      | <ul style="list-style-type: none"> <li>– extract from marine algae – <i>Ascophyllum nodosum</i></li> <li>– N: 3.17%, K<sub>2</sub>O: 1.90%, CaO 0.18%, SO<sub>3</sub> 2.54%</li> <li>– B 3.0% w/w, Cu: 0.0003%, I: 0.003%, Fe: 0.005%, Mn: 0.8%, Zn: 0.5%</li> </ul>   | <ul style="list-style-type: none"> <li>– apples, paer</li> <li>– plums, cherries</li> <li>– strawberries</li> <li>– rapeseed</li> <li>– cereals</li> <li>– tomatoes, peppers, cucumbers, pumpkins</li> <li>– carrots, onions, leek</li> </ul>   | <ul style="list-style-type: none"> <li>– stimulates the life processes, growth and development of plants</li> <li>– develops the root system of plants</li> <li>– increases the plant resistance to stress conditions and fungal diseases</li> <li>– helps in the regeneration of frost damage in oilseed rape and winter cereals</li> <li>– improves the condition of crops</li> <li>– increases the amount and quality of the crop</li> </ul>   | Kazgodsp. z o.o., Poland   |

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|-----------|---|--|---|
| Multoleo® | – Filtrate from <i>Ascophyllum nodosum</i><br>– B: 9.9% w/w   | – rapeseed/canoll<br>– sunflower   | – activator of oilseed crop nutrition                     |
| BM 86     | – filtrate from <i>Ascophyllum nodosum</i><br>– B: 2.03% w/w, Mo: 0.02%, Mg: 4.8%                                       | – fruits: apple, pear, apricot,<br>cherry, nectarine, peach, plum,<br>blackcurrant, raspberry, kiwi<br>fruit, strawberry<br>– vegetables: tomato, pepper,<br>cucumber, green beans | – activator of fruitsetting                               |
| Forthial  | – filtrate from <i>Ascophyllum nodosum</i><br>– N: 6.2% w/w, Mg: 9.0%   | – straw cereals  | – activator of cereal physiology                          |
| Goteo     | – filtrate from <i>Ascophyllum nodosum</i><br>– P (P <sub>2</sub> O <sub>5</sub> ): 12% w/w, K (K <sub>2</sub> O): 5.0% | – vegetable crops plant nurseries<br>– vegetable crops open field and<br>greenhouses,<br>– vegetable crops glasshouses   | – activator of root growth and root activity              |
| Calibra®  | – filtrate from <i>Ascophyllum nodosum</i><br>– Mn: 1.0% w/w, Zn: 1.0%  | – rape<br>– fruits: apple, pear, apricot,<br>nectarine, peach, plum, cherry,<br>olive  | – activator of fruit calibre                              |
| Mag 20®   | – homogenate from <i>Ascophyllum nodosum</i><br>– Mg (MgO): 169 g·dm <sup>-3</sup>                                      | – vine<br>– pip fruits   | – activator of grape and fruit tree nutrition             |
| Folical®  | – homogenate from <i>Ascophyllum nodosum</i><br>– Ca (CaO): 15% w/w   | – fruits: apple, other fruit trees,<br>strawberry, raspberry,<br>– vegetables: tomato, pepper,<br>cucumber, lettuce, cabbage   | – activator of calcium nutrition of fruit trees           |
| Colorado  | – homogenate from <i>Ascophyllum nodosum</i><br>– Mn: 1.83% w/w, Zn: 1.9%   | – fruits: apple<br>– vegetables: tomato, pepper  | – activator of fruit staining                             |
| Rooter    | – filtrate from <i>Ascophyllum nodosum</i><br>– P (P <sub>2</sub> O <sub>5</sub> ): 13% w/w, K (K <sub>2</sub> O): 5.0% | – rapeseed<br>– cereals  | – biostimulator of root growth                            |
| Verduro   | – homogenate from <i>Ascophyllum nodosum</i><br>– Mn: 6.0% w/w, Zn: 3.0%  | – vegetables: onion, leafy<br>vegetables, green beans, potatoes  | – activator of yielding vegetables and potatoes           |
| Zeal      | – filtrate from <i>Ascophyllum nodosum</i><br>– P (P <sub>2</sub> O <sub>5</sub> ): 8.5% w/w, Zn: 1.98%, Mo: 0.02%      | – corn   | – biostimulator of mineral nutrition and yielding of corn |

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Plant protection products (pesticides)

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|              |                    |                          |   |   |
|--------------|--------------------|--------------------------|---|---|
| Plantivax    | – laminarine: 5.0% | – apple, pear            | – fungicide against fire blight ( <i>Erwinia amylovora</i> )  |   |
| Vaxiplant SL | – laminarine: 5.0% | – strawberry<br>– tomato | – biological preparation against noble rot ( <i>Botrytis cinerea</i> ), <i>Podosphaera macularis</i> , strawberry leaf scorch ( <i>Diplocarpon earliana</i> ), common leaf spot ( <i>Mycosphaerella fragariae</i> ) in strawberry and <i>Pseudomonas syringae</i> in tomatoes | ArystaLife-<br>Science<br>Polska Sp.<br>z o.o. Poland |

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Table 2. Algal products, their properties and applications

| Alga   | Extraction technique   | Tested properties of algal extracts  | Application   | Reference                   |
|--|--|--|---|-----------------------------|
| <i>Enteromorpha</i> sp.  | algae with distilled water with pH 2 at 65°C for 1 hour                          | – multielemental composition   | germination tests on garden cress ( <i>Lepidium sativum</i> ):<br>– multielemental composition of the cultivated plants<br>– length of the plants<br>– chlorophyll content (RGB based image analysis)                           | Michalak and Chojnacka 2013 |
| <i>Fucus</i> sp.   | filtration of algal compost with distilled water                                 | – multielemental composition   | germination tests on garden cress ( <i>Lepidium sativum</i> ):<br>– multielemental composition of the cultivated plants<br>– length and weight of the plants  | Michalak et al. 2014b       |
| <i>Spirulina</i> sp.   | supercritical Fluid Extraction (700 bar, 40°C)                                   | – content of polyphenols   | field trials on winter wheat ( <i>Triticum aestivum</i> ):<br>– yield (tonsha <sup>-1</sup> )<br>– length of plants, tillers number, ear number<br>– multielemental composition of the grain                                    | Chojnacka et al. 2014       |
| <i>Enteromorpha</i> sp.  | extraction with 1 M KOH, autoclaving   | – multielemental composition   | germination tests on garden cress ( <i>Lepidium sativum</i> ):<br>– multielemental composition of the cultivated plants<br>– length and weight of the plants  | Michalak et al. 2014a       |
| Mixture of Baltic macroalgae ( <i>Polysiphonia</i> , <i>Ulva</i> , <i>Cladophora</i> ) | microwave Assisted Extraction at three temperatures (25, 40 and 60°C) for 30 min | – content of polyphenols, micro- and macroelements, lipids<br>– antibacterial properties | germination tests on garden cress ( <i>Lepidium sativum</i> ):<br>– multielemental composition of the cultivated plants<br>– length and weight of the plants<br>– chlorophyll content in the plants<br>– plant morphology (SEM) | Michalak et al. 2015a       |

|  |   |   |   |                                   |
|--|---|---|---|-----------------------------------|
| Mixture of Baltic macroalgae<br>( <i>Polysiphonia</i> , <i>Ulva</i> ,<br><i>Cladophora</i> )                                 | supercritical Fluid Extraction<br>(500 bar, 40°C) | – multielemental<br>composition<br>– phenolic compounds<br>– plant hormones | germination tests on garden cress<br>( <i>Lepidium sativum</i> L.; Brassicaceae)<br>and wheat ( <i>Triticum aestivum</i> L.;<br>Poaceae):<br>– length and weight of the aerial parts of<br>plants<br>– length and weight of roots<br>– chlorophyll and carotenoid content in<br>cultivated plants | Michalak et al.<br>2016(in print) |
| <i>Spirulina</i> sp. and the mixture<br>of Baltic<br>macroalgae( <i>Polysiphonia</i> , <i>Ulva</i> ,<br><i>Cladophora</i> )  | supercritical Fluid Extraction<br>(800 bar, 40°C) | n.a.  | germination tests on cress ( <i>Lepidium<br/>sativum</i> ):<br>– length of the sprouts and roots<br>– fresh weight of the plants  | Dmytryk et al.<br>2014b           |
| <i>Spirulina</i> sp. and the mixture<br>of Baltic macroalgae<br>( <i>Polysiphonia</i> , <i>Ulva</i> ,<br><i>Cladophora</i> ) | supercritical Fluid Extraction<br>(800 bar, 40°C) | n.a.  | seed treatment of winter wheat<br>( <i>Triticum aestivum</i> ssp. <i>vulgare</i> ) –<br>germination tests:<br>– length of the sprouts and roots<br>– fresh weight of the plants   | Dmytryk et al.<br>2014a           |
| Mixture of Baltic macroalgae<br>( <i>Polysiphonia</i> , <i>Ulva</i> ,<br><i>Cladophora</i> )                                 | homogenization of algae                           | n.a.  | seed treatment of spring wheat<br>( <i>Triticum aestivum</i> ssp. <i>vulgare</i> ) –<br>germination tests:<br>– length of the sprouts and roots<br>– fresh weight of the plants   | Dmytryk et al.<br>2015            |

n.a. – not available  
Source: Own study

Table 3. Multielemental composition of 100% algal extracts obtained by different methods

| Element       | Extract from SFE                 | Extract obtained by alkaline extraction with KOH | Extract from algal compost | Extract obtained by MAE 60°C* | Extract obtained by acidic extraction with water of pH 2 |               |
|---------------|----------------------------------|--|----------------------------|-------------------------------|--|---------------|
|               | Michalak et al. 2016 (in print)  | Michalak et al. 2014a                            | Michalak et al. 2014b      | Michalak et al. 2015a         | Michalak and Chojnacka 2013                              |               |
|               | $\text{mg} \cdot \text{dm}^{-3}$ |  |                            |                               |  |               |
| Microelements | B                                | <LLD   | 19±3                       | 0.49±0.07                     | 4.7±0.7  | 16±2          |
|               | Co                               | 0.026±0.004                                      | <LLD                       | 0.57±0.09                     | 0.014±0.003  | 0.0075±0.0019 |
|               | Cu                               | 6.3±0.9  | 0.79±0.12                  | 0.27±0.04                     | 0.11±0.02  | 0.040±0.006   |
|               | Fe                               | 9.2±1.4  | 30±0.4                     | 2.2±0.3                       | 4.5±0.7  | 3.8±0.6       |
|               | Mn                               | 6.6±1.0  | 2.0±0.3                    | 1.0±0.2                       | 3.1±0.5  | 1.2±0.2       |
|               | Mo                               | 0.023±0.003                                      | <LLD                       | <LLD                          | 0.011±0.003  | <LLD          |
|               | Ni                               | 0.27±0.04  | <LLD                       | <LLD                          | 0.13±0.02  | 0.0068±0.0017 |
|               | Si                               | 83±12  | 565±8                      | 34±5                          | 12±2   | 2.3±0.3       |
|               | Zn                               | 5.2±0.8  | 6.9±1.0                    | 0.41±0.06                     | 0.17±0.03  | 0.65±0.10     |
| Macroelements | Ca                               | 1060±210   | 127±19                     | 730±110                       | 365±54   | 230±34        |
|               | K                                | 52±8   | 28260±5650                 | 4600±920                      | 950±140  | 143±21        |
|               | Mg                               | 406±61   | 34±5                       | 430±64                        | 322±48   | 29±4          |
|               | Na                               | 965±145  | 620±90                     | 2470±500                      | 1250±250   | 46±7          |
|               | P                                | 43±6   | 6050±1210                  | 590±89                        | 33±5   | 10±2          |
|               | S                                | 9300±1900  | 1350±270                   | 1750±350                      | 700±105  | 532±80        |

<LLD – below low limit of detection

\* – the best multielemental composition among tested extracts obtained by MAE at 25, 40 and 60°C

Source – own study

Table 4. Effect of the obtained algal products on the plants

| Algal product  | Plant tested  | Dose tested     | Action   | Reference                         |
|--|---|-----------------|--|-----------------------------------|
| Neutral algal extract<br>(acidic extraction with<br>H <sub>2</sub> SO <sub>4</sub> ) – <i>Enteromorpha</i> sp. | biostimulant– garden<br>cress<br>( <i>Lepidium sativum</i> )  | 50%, 100%       | – multielemental composition of plants (increase when compared with the control group (C)):<br>50%: K~3 times more, S – 40%, Ca – 26%, Mn – 77%, Zn – 10% more<br>100%: K~3 times more, S – 44%, Ca – 35%, Mn~2 times more<br>– average length of the stem:<br>C: 5.84±0.30 cm, 50%: 5.93±0.44 cm, 100%: 6.40±0.41 cm  | Michalak and<br>Chojnacka<br>2013 |
| Neutral algal extract<br>(alkaline extraction with<br>KOH) – <i>Enteromorpha</i> sp.                           | Biostimulant<br>– garden cress<br>( <i>Lepidium sativum</i> ) | 2.5%, 5.0%, 10% | – multielemental composition of plants (increase when compared with the control group):<br>2.5%: K~2 times more, Ca – 26%, P – 16%, B~8 times more, Cu – 39%, Fe – 20%, Zn – 11% more<br>5.0%: K~2.5 times more, P – 22%, Ca – 19%, B~3 times more<br>10%: K~2.5 times more, P – 26%, B~4 times more, Cu – 13%, Fe – 12% more<br>– average length of the stem:<br>C: 4.57±0.39 cm, 2.5%: 5.26±0.41 cm, 5.0%: 5.18±0.37 cm, 10%: 4.70±0.45 cm<br>– Average weight of the plants (dry biomass):<br>C: 0.0717±0.0111 g, 2.5%: 0.0672±0.0084 g, 5.0%: 0.0785±0.0026 g, 10%: 0.0753±0.0126g | Michalak et al.<br>2014a          |
| Extract from the compost<br>containing algae – <i>Fucus</i> sp.  | Biostimulant<br>– garden cress<br>( <i>Lepidium sativum</i> ) | 100%            | – multielemental composition of plants (increase when compared with the control group):<br>100%: S – 72%, Na – 51%, Mg – 40% more, B and Ca~2.5 times more, K~3 times more<br>– average length of the stem:<br>C: 3.01±0.80 cm, 100%: 5.45±0.34 cm<br>– average weight of the plants (fresh biomass):<br>C: 0.812±0.309 g 100%: 1.43±0.12 g  | Michalak et al.<br>2014b          |

|  |   |   |  |                                |
|--|---|---|--|--------------------------------|
| Extract obtained by Microwave Assisted Extraction – Baltic macroalgae  | Biostimulant – garden cress ( <i>Lepidium sativum</i> ) | 25, 40 and 60°C: 0.5, 2.5 and 10%                   | <p>– multielemental composition of plants (increase when compared with the control group):<br/> 25°C, 0.5%: S – 12%, Zn – 30%, Cu – 28%, Mo – 16%, Mn – 14% more<br/> 25°C, 2.5%: S – 13%, Zn – 71%, Cu – 31%, Mn – 22%, Mo – 19% more<br/> 25°C, 10%: Zn – 41%, Cu – 12% more<br/> 40°C, 0.5%: Mn – 13%, Mo – 23%, Zn – 26% more<br/> 40°C, 2.5%: Mn – 14%, Mo – 22% more<br/> 40°C, 10%: Cu – 3 times more, Mo – 28% more<br/> 60°C, 0.5%: Mo – 23%, Mn – 15% more<br/> 60°C, 2.5%: Mo – 17%, Mn – 14% more<br/> 60°C, 10%: Mo – 56%, B – 47%, Cu – 12% more</p> <p>– average length of the stem:<br/> C: 11.9±2.5 cm<br/> MAE 60°C: 0.5% (12.2±3.0 cm), 2.5% (10.9±3.8 cm), 10% (10.2±3.8 cm)<br/> MAE 40°C: 0.5% (13.6±1.7 cm), 2.5% (13.7±1.7 cm), 10% (13.8±1.9 cm)<br/> MAE 25°C: 0.5% (13.3±1.9 cm), 2.5% (13.6±3.1 cm), 10% (11.4±3.2 cm)</p> <p>– average weight of the plants (dry biomass):<br/> MAE 60°C: 0.5% (0.0653 g), 2.5% (0.0652 g), 10% (0.0687 g)<br/> MAE 40°C: 0.5% (0.0648 g), 2.5% (0.0695 g), 10% (0.0648 g)<br/> MAE 25°C: 0.5% (0.06367 g), 2.5% (0.0635 g), 10% (0.0582 g)</p> | Michalak et al. 2015           |
| Extract obtained by Supercritical Fluid Extraction – Baltic macroalgae | Biostimulant – garden cress ( <i>Lepidium sativum</i> ) | 13.8 mg·dm <sup>-3</sup> , 27.6 mg·dm <sup>-3</sup> | <p>– average length of the stem:<br/> C: 2.50±0.2 cm, E (13.8 mg·dm<sup>-3</sup>): 2.54±0.2 cm, E (27.6 mg·dm<sup>-3</sup>): 2.50±0.2 cm</p> <p>– average weight of the plants (dry biomass):<br/> C: 0.178±0.004 g, E (13.8 mg·dm<sup>-3</sup>): 0.186±0.006 g, E (27.6 mg·dm<sup>-3</sup>): 0.183±0.005 g</p>  | Michalak et al. 2016(in print) |

|  |  |  |  |                      |
|--|--|--|--|----------------------|
|  |  | Extract (E) – Alga Premium 2 (doses: 0.009–0.045 cm <sup>3</sup> per Petri dish) | <p>– average length of the stem:<br/>C (water): 3.6±1.5 cm, C (Kelpak SL): 4.8±0.7 cm<br/>E(0.009): 4.0±1.0 cm, E(0.012): 4.1±1.0 cm, E(0.015): 4.3±1.1 cm, E(0.024): 4.2±1.0 cm, E(0.045): 4.8±0.5 cm</p> <p>– average weight of the plants (fresh biomass):<br/>C (water): 0.420±0.076 g, C (Kelpak SL): 0.637±0.161 g<br/>E(0.009): 0.468±0.099 g, E(0.012): 0.529±0.220 g, E(0.015): 0.654±0.043 g, E(0.024): 0.553±0.110 g, E(0.045): 0.758±0.077 g</p>   | Dmytryk et al. 2014b |
|  |  | Extract obtained by Supercritical Fluid Extraction – <i>Spirulina</i> sp.        | <p>extract (E)– Alga Sp. 2 (doses: 0.009–0.045 cm<sup>3</sup> per Petri dish)</p> <p>– average length of the stem:<br/>C (water): 3.6±1.5 cm, C (Kelpak SL): 4.8±0.7 cm<br/>E(0.009): 3.6±1.4 cm, E(0.012): 3.7±1.1 cm, E(0.015): 4.3±0.9 cm, E(0.024): 4.5±0.6 cm, E(0.045): 4.2±1.2 cm</p> <p>– average weight of the plants (fresh biomass):<br/>C (water): 0.420±0.076 g, C (Kelpak SL): 0.637±0.161 g<br/>E(0.009): 0.521±0.197 g, E(0.012): 0.530±0.119 g, E(0.015): 0.660±0.194 g, E(0.024): 0.636±0.142 g, E(0.045): 0.534±0.168 g</p> | Dmytryk et al. 2014b |
|  |  | Extract obtained by Supercritical Fluid Extraction – Baltic macroalgae           | <p>homogenate (H) – Alga Seed Premium (doses: 8.0, 14, 20 mm<sup>3</sup> per 1 g of seed)</p> <p>– average length of the stem:<br/>C: 13.1±1.1 cm, H(8.0): 12.3±0.9 cm, H(14): 12.2±1.4 cm, H(20): 13.0±1.0 cm</p> <p>– average root length:<br/>C: 12.8±2.9 cm, H(8.0): 14.8±3.9 cm, H(14): 13.7±3.1 cm, H(20): 13.7±3.9 cm</p> <p>– average weight of the plants (fresh biomass):<br/>C: 0.093±0.006 g, H(8.0): 0.0879±0.007 g, H(14): 0.092±0.009 g, H(20): 0.091±0.009 g</p>   | Dmytryk et al. 2014a |
|  | seed treatment – winter wheat ( <i>Triticum aestivum</i> ssp. <i>vulgare</i> ) |  | <p>homogenate (H) – Alga Seed Sp. 2 (doses: 8.0, 14, 20 mm<sup>3</sup> per 1 g of seed)</p> <p>– average length of the stem:<br/>C: 11.5±1.4 cm, H(8.0): 12.2±0.9 cm, H(14): 12.1±1.1 cm, H(20): 11.8±1.5 cm</p> <p>– average root length:<br/>C: 12.9±4.1 cm, H(8.0): 14.2±4.3 cm, H(14): 14.1±4.3 cm, H(20): 12.6±3.6 cm</p> <p>– average weight of the plants (fresh biomass):<br/>C: 0.0779±0.009 g, H(8.0): 0.0877±0.011 g, H(14): 0.0825±0.011 g, H(20): 0.0853±0.007 g</p>  | Dmytryk et al. 2014a |

|                                     |  |  |   |                     |
|-------------------------------------|--|--|---|---------------------|
| Homogenization of Baltic macroalgae | seed treatment – spring wheat ( <i>Triticum aestivum</i> ssp. <i>vulgare</i> ) | homogenate (H) – doses: 10, 25, 50 mm <sup>3</sup> per 1 g of seed           | – average length of the stem:<br>C: 10.5±2.7 cm, H(10): 11.5±2.8 cm, H(25): 12.2±3.4 cm, H(50): 10.3±2.2 cm<br>– average root length:<br>C: 10.7±4.1 cm, H(10): 11.5±3.9 cm, H(25): 11.7±3.8 cm, H(50): 12.4±4.1 cm<br>– average weight of the plants (fresh biomass):<br>C: 0.0317±0.0059 g, H(10): 0.0354±0.0119 g, H(25): 0.0429±0.0166 g, H(50): 0.0379±0.0089 g    | Dmytryk et al. 2015 |
|                                     |  | enriched homogenate (EH) – doses: 10, 25, 50 mm <sup>3</sup> per 1 g of seed | – average length of the stem:<br>C: 10.5±2.7 cm, EH(10): 11.4±1.8 cm, EH(25): 10.5±1.0 cm, EH(50): 8.8±2.0 cm<br>– average root length:<br>C: 10.7±4.1 cm, EH(10): 12.5±3.0 cm, EH(25): 13.0±3.3 cm, EH(50): 13.2±4.6 cm<br>– average weight of the plants (fresh biomass):<br>C: 0.0317±0.0059 g, H(10): 0.0404±0.0083g, H(25): 0.0376±0.0033g, H(50): 0.0361±0.0051 g | Dmytryk et al. 2015 |

Source – own study

**Utilitarian properties of the obtained algal products – germination tests.** Table 2 presents the general information about the production of the algal products with the use of different methods, their examined properties and applications. Usually, on the labels of the commercial biostimulants of plant growth, mainly mineral composition is presented (tab. 1). It should be noted that the commercial biostimulants are usually enriched with micro- and macroelements to the given level. Table 3 presents natural concentration of elements in the obtained extracts. However, enrichment with micronutrients is also possible. Among the presented extracts, the most interesting composition had the extract obtained by Supercritical Fluid Extraction.

Taking into account the application of algal extracts in the agriculture, in the first step, germination tests under laboratory conditions were performed on Petri dishes with a cotton as a support. As a model plant, mainly garden cress (*Lepidium sativum*) was chosen, because it can reach a mature state in a short period of time (about two weeks). The second tested plant was wheat (*Triticum aestivum*). In the experimental groups, plants were sprayed with the algal extracts, in the control group (C) with distilled water. The results from the germination tests were verified in the field trials on winter wheat (variety *Tacitus*). The measured parameters in the germination tests included: length and weight of the aerial parts of plants and roots, chlorophyll, carotenoid and elements content in the cultivated plants and plant morphology (Scanning Electron Microscopy) [Michalak et al. 2015]. In the field trials, yield, length of plants, tillers, ear number and multielemental composition of the grain were measured. The obtained results are presented in Table 4.

**Methodology of experiments on plants.** Germination tests were performed in Petri dishes (90 mm diameter), under standardized conditions – isolated box with adjustable lighting and temperature (temperature fluctuations  $\pm 4^{\circ}\text{C}$ ) in Jacobsen apparatus according to International Rules for Seed Testing (ISTA, 2011). Before germination tests, the dishes with untreated seeds were put to the refrigerator for the stratification. After this period, the seeds were sprayed with the appropriate volume of algal extracts in a specified time intervals [Michalak and Chojnacka 2013, Michalak et al. 2014a, Dmytryk et al. 2014b, Michalak et al. 2015]. In the case of seed treatment, the conditions of the germination tests were the same, however the seeds before tests were coated with algal homogenates. For this reason, vortex-type shaker was used. The process lasted 5 minutes. Then seeds coated with dense homogenate were placed directly on the Petri dishes [Dmytryk et al. 2014a, 2015].

**Multielemental composition of the cultivated plants.** On the basis of the conducted experiments, it can be stated out that the multielemental composition of the cultivated plants depended on the method of extraction and the dilution of the obtained algal extract (Table 4 – the differences higher than 10% were taken into account). In the work of Michalak and Chojnacka [2013], it was shown that the extract obtained by acidic extraction from *Enteromorpha* sp. increased mainly the content of K, Ca, S, Mn and decreased the content of Mg, Na, Cu and Si in the cultivated plants. The obtained results were comparable for both tested concentrations of algal extract – 50 and 100%. For the germination tests performed with the application of algal extract (10, 5.0 and 2.5%) produced by alkaline extraction with KOH, the best results in terms of the multi ele-

mental composition were obtained for the lowest concentration of extract – 2.5% [Michalak et al. 2014a]. Extract obtained from the compost that contained algae – *Fucus* sp. favoured accumulation in plants mainly macroelements (Ca, K, Mg, S). The content of microelements (Cu, Fe, Mn, Zn) was comparable in both groups [Michalak et al. 2014b]. In the next work, algal extracts were obtained using MAE in three different temperatures: 25, 40 and 60°C. Each algal extract was diluted to the following concentrations: 0.5, 2.5 and 10%. The multielemental composition of *Lepidium sativum* was affected in the highest extent by the application of the following extracts: MAE 60°C (10% extract), MAE 40°C (0.5%), MAE 25°C (2.5%) taking into account the content of micro- and macroelements. Among these three extracts, the best properties had the extract MAE 25°C (2.5%). For all tested extracts, there was no effect on the content of macroelements. Additionally, the decrease of Ca, Si and Fe in the cultivated plants from all tested groups was observed, when compared with the control group [Michalak et al. 2015a].

**Average length of the stem.** On the basis of the germination tests, it can be suggested that algal extracts and homogenates stimulated the growth of the plants – Table 4. In the case of the application of the extract obtained by acidic extraction, statistically significant differences ( $p < 0.05$ ) were observed between: control group (C) and 100% extract (higher for extract 100% by 9.6%) and between 100 and 50% extract (higher for extract 100% by 7.9%) [Michalak and Chojnacka 2013]. It was also observed that the average length of plants, treated with the extract obtained from the compost containing algae, was almost two times higher than in the control group [Michalak et al. 2014b]. The lowest concentration of the algal extract (2.5%) obtained by alkaline extraction influenced the average length of the stem of *Lepidium sativum*. The lower concentration of algal extract, the higher average length. Statistically significant differences ( $p < 0.05$ ) were observed between: experimental group 2.5% and the control group. Cress in the group with 2.5% extract was 15% longer than the control group and 12% than for extract 10% [Michalak et al. 2014a]. In the case of the extracts obtained by MAE, the best results were obtained for plants in MAE 40°C:10% group – plants were 16% longer than in the control group. However, the differences were not statistically significant ( $p < 0.05$ ) [Michalak et al. 2015].

In the next work, algal extracts obtained from the mixture of Baltic macroalgae using Supercritical Fluid Extraction were examined in the germination tests on cress. No statistically significant differences between experimental (plants growing from macerated seeds in 13.8 mg·dm<sup>-3</sup> SFE extract) and control (water) group were observed [Michalak et al. 2016]. In the work of Dmytryk et al. [2014b] it was found that algal extracts obtained from *Spirulina* sp. and the mixture of Baltic macroalgae using SFE stimulated growth of *Lepidium sativum*, similarly as the commercial product – Kelpak SL. Two preparations, tested in the doses 0.009–0.045 cm<sup>3</sup>, Alga Premium 2 (contains 25% of Baltic algae extract – dose 0.045 cm<sup>3</sup>) and Alga Sp. 2 (contains 25% of *Spirulina* extract – dose 0.024 cm<sup>3</sup>) increased the plant growth by 33 and 25%, respectively, when compared to the control group (treated with water). In the same conditions, Kelpak SL increased the average length of the stem also by 33%. These differences were statistically significant.

The products obtained from algal biomass were also used for the seed treatment. In the work of Dmytryk et al. [2014a], wheat seeds (*Triticum aestivum*ssp. *vulgare*) were coated separately with three different doses (8, 14 and 20 mm<sup>3</sup>) of formulation containing supercritical extract from *Spirulina* sp. or Baltic seaweeds. The best results of the sprout growth were achieved for seeds coated with 8 mm<sup>3</sup> of *Spirulina* sp. formulation. The coating resulted in the increased sprout height by approximately 6%. For both formulations in all tested doses, the average root length was also measured. The best results were obtained for the dose 8 mm<sup>3</sup> in both cases. The average root length was 10% higher in the group with *Spirulina* sp. extract and 16% higher for Baltic macroalgae, when compared with the control group. These differences were not statistically significant.

In the next work of Dmytryk et al. [2015], seeds of spring wheat were coated with two emulsion concentrates prepared by the homogenization of dried Baltic seaweeds (*Enteromorpha* sp. and *Cladophora* sp.). One of the emulsions was enriched with minerals: boron, copper, iron, manganese, molybdenum, zinc and magnesium. Each of the applied formulation dose (10, 25 and 50 mm<sup>3</sup> per 1 g of seed) was proved to affect mainly root development. The weakest stimulating effect of sprout height was noticed for the enriched with microelements algal homogenate. No statistically significant differences between experimental and control series were shown.

From the performed experiments, it can be seen that the best results were obtained for MAE extracts (however, in these experiments *Lepidium sativum* was cultivated on the Petri dishes with soil). On the basis of the comparison of the presented results, it can be concluded that water extract from *Enteromorpha* sp. influenced the average length of the stem in the highest extent.

**Average weight of cultivated plants.** Generally, the obtained results showed that the algal extracts influenced the weight of cultivated plants – Table 4. Michalak et al. [2014b] showed that the weight of wet biomass of *Lepidium sativum* was about 50% higher in the experimental group than in the control group. In the work of Michalak et al. [2014a], it was presented that higher concentrations of the algal extracts obtained by alkaline extraction (10 and 5%) slightly influenced the weight of *Lepidium sativum* – by 5 and 9.5% respectively, when compared to the control group. In the case of the application of algal extracts obtained by MAE, dry mass of *Lepidium sativum* was comparable in all groups, taking into account both temperature of extraction (25, 40, 60°C) and the dilutions of the extract (0.5, 2.5 and 10%). For MAE 60°C, the average weight for all dilutions was 0.0664±0.0020 g of dry mass, for MAE 40°C 0.0664±0.0027 g, for MAE 25°C 0.0618±0.0031g. No significant influence of algal extract concentration on dry weight of *Lepidium sativum* was observed [Michalak et al. 2015]. In the work of Michalak et al. [2015c], algal extracts obtained by SFE were tested on cress (*Lepidium sativum*) in two concentrations: 13.8 mg·dm<sup>-3</sup> and 27.6mg·dm<sup>-3</sup>. Plants growing from seeds macerated in 13.8 mg·dm<sup>-3</sup>SFE extract solution had an average weight only 4.5% higher than plants in the control group. There were no significant differences between the control and experimental groups for both tested concentrations of the algal extract [Michalak et al. 2016]. In the work of Dmytryk et al. [2014b], the effect of algal extracts obtained from *Spirulina* sp. and the mixture of Baltic macroalgae using SFE on the

weight of cultivated plants was determined. As a reference product, commercial Kelpak SL was used. The weight of plants for both extracts was higher than for the control group (treated with water). In the case of Baltic macroalgae extract, it ranged from 11% for the dose of extract 0.009 cm<sup>3</sup> until 80% for the dose 0.045 cm<sup>3</sup>. For *Spirulina* extract the range was from 24% for the dose 0.009 cm<sup>3</sup> to 57% for the dose 0.024 cm<sup>3</sup>. Better biostimulant properties than Kelpak SL showed Baltic macroalgae extract for doses 0.015 cm<sup>3</sup> (increase of the weight by 3.0%) and 0.045 cm<sup>3</sup> (increase by 19%) and *Spirulina* extract for the dose 0.015 cm<sup>3</sup> (slight increase by 4.0%).

Dmytryk et al. [2014a], examined the effect of seed treatment (*Triticum aestivum* ssp. *vulgare*) with formulations containing supercritical extracts from *Spirulina* sp. or Baltic seaweeds in three different doses (8, 14 and 20 mm<sup>3</sup>). Only *Spirulina* extract in all examined doses increased the fresh weight of plants by 13, 6.0 and 9.5%, respectively. However, the differences were not statistically significant. Seeds of spring wheat were also coated with two emulsion concentrates prepared by the homogenization of dried Baltic seaweeds (*Enteromorpha* sp. and *Cladophora* sp.), one of the emulsions was enriched with minerals. It was found that both preparations, in all applied doses (10, 25 and 50 mm<sup>3</sup> per 1 g of seed) affected positively the average fresh sprout mass. The best results were obtained for the pure homogenate formulation at a dose 25 – the fresh mass of spring wheat was 35% higher than in the control group (differences were not statistically significant) [Dmytryk et al. 2015].

**Chlorophyll content in the cultivated plants.** In the work of Michalak and Chojnacka [2013], RGB model (R red, G green and B blue) was used to assess the chlorophyll content. The water extract of *Enteromorpha* sp. Obtained by acidic extraction influenced mainly G parameter in the leaves of *Lepidium sativum* – the difference statistically significant ( $p < 0.05$ ) was observed between 100% algal extract and the control group. Chlorophyll content in *Lepidium sativum* also increased after the application of algal extracts obtained with MAE in 25, 40 and 60°C. In most cases, total chlorophyll content in *Lepidium sativum* from the experimental groups was higher than in the control group. The highest difference – 12.5% was noted between control group and MAE 60°C (concentration – 2.5%) [Michalak et al. 2015a]. In the work of Michalak et al. [2015b], algal extracts obtained by SFE were tested on cress (*Lepidium sativum*) and wheat (*Triticum aestivum* L.) in two concentrations: 13.8 mg·dm<sup>-3</sup> and 27.6 mg·dm<sup>-3</sup>. It was found that the statistically significant differences ( $p < 0.01$ ) were for the content of chlorophyll for both tested plants for the lower concentration of algal extract when compared to the control group. For wheat, treated with the 13.8 mg·dm<sup>-3</sup> concentration extract, chlorophyll contents was 14% higher than in the control group. Maceration of wheat seeds with higher concentrations of extract (27.6 mg·dm<sup>-3</sup>) adversely affected the chlorophyll content (28% lower).

**Utilitarian properties of the obtained algal products – field trials.** In the field trial on winter wheat (variety *Tacitus*), the algal extract form *Spirulina* sp., obtained by Supercritical Fluid Extraction, was compared with the commercial product – Asahi SL and the control group (untreated). All tested groups were performed in four replications. The trials were carried out without fertilization in order to induce the conditions of a biotic stress in the growing season 2012/2013. The dose of the *Spirulina* extract

(1.2 and 1.8 dm<sup>3</sup>·ha<sup>-1</sup>) was established on the basis of polyphenols content – the same as the content in Asahi SL applied in a dose 0.60 dm<sup>3</sup>·ha<sup>-1</sup>. The tested preparations were applied twice: on 23 April 2013 – crop growth stage BBCH 29–31 and on 30 May 2013 – crop growth stage BBCH: 43–45. The obtained results showed that there were no statistically significant differences concerning the crop yield, crop height and multi elemental composition. The supercritical algal extract showed slight impact in increasing crop yield per hectare in comparison with the control group – untreated (4.0%) and the reference product – Asahi SL (2.0%) [Chojnacka et al. 2014].

**Bio-based agrochemicals in the scientific literature.** Nowadays, new algal extracts are mainly tested as plant growth biostimulants under laboratory conditions (germination tests in Petri dishes or pot experiments), rather than in field trials. In field experiments, mainly commercially available algal biostimulants are tested, for example: Kelpak SL, AlgaminoPlant, Bio-algeen S 90, Goëmar BM 86, Seasol Commercial, Biovita, Acadian<sup>TM</sup>. In the germination tests, the most often examined algal extracts are those obtained by the extraction with distilled water by boiling and autoclaving and by homogenization with the use of blender or mortar and pestle. The effect of algal extracts obtained from different species of algae on plants in germination tests was described in details by Michalak and Chojnacka [2015b]. The new aspect of the present work was to present the effect of extracts obtained also by supercritical fluid extraction on plants. Usually, this method is used to extract biologically active compounds from algae that find applications in the pharmaceutical or cosmetic industry [Thomas and Kim 2013, Michalak and Chojnacka 2014]. At present, there are no literature data that presents the results on the application of algal extracts obtained by supercritical fluid extraction in the plant cultivation.

The results obtained in our experiments, when algal extracts were used as plant growth biostimulants, have found the confirmation in the literature data, where commercial biostimulants based on algal biomass were applied in the plant cultivation. The most common preparation tested in Polish conditions is the extract from marine brown algae *Ecklonia maxima* – Kelpak SL. In the work of Matysiak et al. [2012], field trials on winter oilseed rape using Kelpak SL were conducted. It was found that Kelpak had a positive influence on weight, number of seeds per silique, plant height and chlorophyll content in the leaves, however it did not change the quality parameters of seeds: protein, fat content and moisture. Field experiments on the winter oilseed rape with the use of Kelpak SL were carried out by Matysiak et al. [2014]. It was shown that the application of algal biostimulant did not influence the plant height, but the winter rape was characterized by a greater leaf chlorophyll content. The examined product considerably increased crop yield. Matysiak et al. [2010] compared the effect of seaweeds extracts: *Ecklonia maxima* – Kelpak SL and *Sargassums* pp. – AlgaminoPlant on the germination and early growth of winter oilseed rape (*Brassica napus* L.). The lowest dose of both preparations induced the seed germination stronger – for Kelpak SL: 1.5 cm<sup>3</sup>/200 cm<sup>3</sup> H<sub>2</sub>O (tested also 2.0 and 3.0), for AlgaminoPlant: 0.5 cm<sup>3</sup>/200 cm<sup>3</sup> H<sub>2</sub>O (tested also 1.0) and weight of shoots. Root mass did not differ from controls. Seaweeds did not influence the chlorophyll content in plants. Similar results were obtained for these two preparations in the glasshouse and laboratory experiments carried out on maize

(*Zea mays* L.) [Matysiak et al. 2011]. Seaweed extracts improved the germination efficiency of maize seeds and promoted shoot and root growth. Dobrzański et al. [2008] examined the influence of biostimulants from seaweed – *Sargassum* sp. (Algamino-Plant) in the cultivation of carrots. Two years experiments showed the tendency of the yield increase and positive changes in the chemical composition. The content of nitrates decreased and carotenoids slightly increased. Soaking carrot and parsley seeds in 2.0% AlgaminoPlant water solution accelerated germination and improved germinability of seeds. Kwiatkowski [2011] tested the effect of different growth stimulators (including Bio-algeen S 90 (1.0%)) on the raw material quality and yield of garden thyme (*Thymus vulgaris* L.). It was found that the foliar applications of Bio-algeen S 90 had a positive effect on some biometric traits of garden thyme plants (plant height, number of lateral branches). It was also found that the lack of the application of the growth stimulators was the most beneficial for the chemical composition of thyme. The application of other growth stimulators (Asahi SL and Tytanit) in this study had the most beneficial effect on garden thyme productivity. Gajc-Wolska et al. [2013] showed that the application of physio activators (Goëmar BM 86) in the cultivation of broccoli increased the marketable and the first-class yield, caused an increase in the average weight of broccoli curds, limited the occurrence of hollow stem and increased the content of macro- and micronutrients in broccoli curds. In the work of Alam et al. [2013], Acadian™ (water soluble alkaline extract from *Ascophyllum nodosum*) was applied in greenhouse and field experiments on strawberries. It was shown that the application of the extract increased strawberry root and shoot growth, berry yield and rhizosphere microbial diversity and physiological activity. The extract from *Ascophyllum nodosum*, known commercially as Bio-vita, was also found to significantly improve the growth, yield and protein content of wheat when applied together with N, P and K. Additionally it was found that the extract stimulated the bacterial activity in the soil. Therefore, the extract could be used for the fast and efficient decomposition of crop residues left on the soil surface [Sen et al. 2015]. Mattner et al. [2013] examined the effect of Seasol Commercial (kelp extract obtained from *Durvillaea potatorum* and *Ascophyllum nodosum*) on the growth of broccoli in a greenhouse and field experiments. It was found that the tested preparation significantly increased the leaf number, stem diameter and leaf area of establishing broccoli seedlings.

To sum up, the effect of the algal extract on the plants depends on many factors, for example: method of production, its composition, type of the cultivated plant and method of application etc. [Matysiak et al. 2010, 2011, 2014, Michalak et al. 2016]. The utilitarian properties of the algal extracts and homogenates examined in the germination tests and in the field trials confirmed their biostimulating activity. The next step involved detailed characteristics of the algal extracts, in terms of bioactive compounds which are responsible for the stimulation of plant growth. Our results showed that algal extracts contained plant hormones, polyphenols, micro- and macroelements [Michalak et al. 2016]. The last step will involve the preparations for product registration and marketing trade.

## CONCLUSIONS

In our work it was shown that it was possible to produce algal extracts/homogenates (from micro- and macroalgae) which can be candidates for the new biostimulants of plant growth or preparation for seeds treatment. There are many methods – traditional (solvent extraction) and novel (e.g., Supercritical Fluid Extraction, Microwave Assisted Extraction) that can be used to produce them. In the present paper it was shown that the obtained algae-based products contained micro- and macroelements, polyphenols, plant hormones that can be responsible for the stimulation of the plant growth. Germination tests and field trials showed that products based on micro- and macroalgae increased the content of micro- and macroelements in the cultivated plants, their length (aerial part and root) and weight, as well as the content of chlorophyll and carotenoids. These action of the examined extracts/homogenates is similar to those which are available on the market. In order to confirm the examined properties of the bio-products it is necessary to repeat the field trials on different plant species. Detailed economic analysis should be performed what determines market success.

## ACKNOWLEDGMENTS

This project is financed in the framework of 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/A1/2/2012) attributed by The National Centre for Research and Development in Poland.

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## POTENCJALNA PRZYDATNOŚĆ NOWEJ GENERACJI AGROPRODUKTÓW WYTWARZANYCH NA BAZIE SUROWCÓW POCHODZENIA BIOLOGICZNEGO

**Streszczenie.** W artykule podjęto temat możliwości wykorzystania produktów z alg wytworzonych na drodze ekstrakcji (tradycyjna ekstrakcja rozpuszczalnikowa, ekstrakcja nadkrytyczna, ekstrakcja wspomagana mikrofalami) oraz homogenizacji w uprawie roślin. Ekstrakty algowe omówiono pod kątem ich zastosowania jako potencjalnych biostymulatorów wzrostu roślin, zaś homogenaty jako preparaty do zaprawiania nasion. W pracy przedyskutowano możliwość wykorzystania przede wszystkim makroalg pochodzących z akwenu Morza Bałtyckiego (w wielu kurortach nadmorskich stanowią kłopotliwy odpad) jako surowca do procesu ekstrakcji/homogenizacji. Przedstawiono przykładowe badania laboratoryjne (testy kiełkowania oraz polowe) dotyczące wpływu preparatów algowych na wzrost roślin. Dane literaturowe wskazują, że produkty wytworzone na bazie alg zwiększają zawartość mikro-, makroelementów i chlorofilu w uprawianych roślinach, a także ich długość i masę. W przyszłości mogą więc stanowić uzupełnienie asortymentu produktów dostępnych na rynku.

**Słowa kluczowe:** algi, ekstrakty, wytwarzanie, biostymulator wzrostu roślin, zaprawianie nasion

Accepted for print: 16.06.2016

For citation: Michalak, I., Chojnacka, K. (2016). The potential usefulness of a new generation of agro-products based on raw materials of biological origin. *Acta Sci. Pol. Hortorum Cultus*, 15(6), 97–120.