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**Biomass of freshwater Cladophora as a raw material for agriculture and the cosmetic industry**

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**Abstract:** This study was undertaken to determine mineral content, amino acid and fatty acid composition of the freshwater macroalga – *Cladophora glomerata*. The studies were based on the content comparison in algal biomass collected from a lake and cultured in a laboratory. To determine the ability of copper cumulating by macroalgae, *Cladophora* was cultured in the medium supplemented with Cu ions. This study indicated that the relative abundance of metals in filaments decreased in the following order: Ca > K > Mg > Na > Fe > Cu > Zn > Pb > As > Ni > Cd > Mn > Cr > Co. Total protein content ranged from 14.45% in *Cladophora* from a lake to 26.55% in *Cladophora* from a laboratory. The main amino acids analyzed were aspartic and glutamic acid. The fatty acid content in the dry matter of the extract varied depending on the extraction method used: ethylene alcohol (19.0%), acetone (34.5%) or supercritical fluid extraction (62.5%). Freshwater *C. glomerata* due to the macrominerals, trace elements, amino and fatty acids composition in the extracts can be a valuable resource for nutritional and cosmetic applications.

**Keywords:** macroalgal biomass, amino acids, fatty acids, extraction, biosorption

1 **Introduction**

Extensive development of macroscopic algae in freshwater ecosystems more and more often form spatially large mats [1-3] and quickly create environmental and recreation problems in a reservoir. Therefore, there is an urgent need to exploit such biomass [4] due to the growing problem closely related to the development of the agricultural economy (intensification of fertilization increases the concentration of N and P in the water which efficiently accelerates algae growth) and tourism (preference for clean water without biomass of macroalgae). Filamentous green algae from the genus *Cladophora* from marine ecosystems have been the subject of numerous studies on the use of their biomass as a source of biofuel [5-7]. Such studies were carried out mainly on macroalgae forming a large biomass in marine ecosystems [8,9]. Many studies of marine green algae from the genus *Cladophora* indicate the occurrence in their cells of different bioactive compounds, such as unsaturated and saturated fatty acids [10-12], sterols, terpenoids [13], phenolic compounds [14], and others, that make them a very useful raw material for nutritional and pharmaceutical applications. So far, only a few studies have been conducted on freshwater *Cladophora* species and their use as a natural source of bioactive compounds. However, a wide range of primary and secondary metabolites were found in freshwater *Cladophora glomerata* (L.) Kütz, e.g.: carotenoids [15], minerals, vitamin A, C, E, B1, B2 [16], amino acids and proteins [17], fatty acids, sterols, terpenes, carbohydrates, glycosides [18], volatiles and enzymes [19].

Using such tests, systematic studies were carried out on algae in terms of their possible use in agriculture, animal nutrition and the food or cosmetics industry. Thus, one of the aims of this study was to analyze the elemental composition and to determine the amino acid and fatty
Biomass of freshwater Cladophora as a raw material

Acid content in the biomass of freshwater Cladophora. A negative factor for using macroscopic green algae, taking into account Cladophora biomass, can be Cladophora's ability to accumulate heavy metals [20,21]. In recent years, the biosorption process using Cladophora species has been widely studied including such metals as chromium [22], lead [23], cadmium [24], selenium [25] and others [26]. At the same time, the controlled concentration of elements could be induced in the biosorption process into the algal biomass that would be useful in cosmetics or as dietary supplements. In this paper, the possibility of obtaining ecological components for cosmetic purposes by enriching the filamentous biomass of freshwater Cladophora with copper is also presented. Most of the literature sources concern the biosorption of copper (II) by marine Cladophora in wastewater treatment [27,28]. However, several studies have been conducted referring to the use of freshwater Cladophora as a biosorbent to remove copper (II) [26,29,30]. There is a lack of research of such processes in freshwater C. glomerata. Copper ions presented in the studied material were selected for testing because they have anti-bacterial applications and can act as “free radical scavengers”. The question was asked whether the chemical composition of the biomass of freshwater macroscopic green algae, Cladophora, makes it a good raw material for commercial use.

2 Experimental procedure

2.1 Harvesting area of Cladophora biomass

The Oporzyn Lake is located near the village of Oporzyn, ca. 20 km north of Wagrowiec (in the northern part of the Wielkopolska region, Fig. 1). The lake is located in a hollow surrounded by hills with very steep slopes which facilitates the surface runoff from the surrounding farmland. It has an area of 15 ha and a mean depth of 1.2 m. The shore of the lake is covered with a wide
(to 10 m) and dense belt of rushes with Phragmites australis (Cav.) Trin ex Stued. and Typha latifolia L. as the dominant species. The lake is in the overgrowing phase and the pelagic zone is overgrown by Ceratophyllum demersum L. This submerged aquatic plant formed extremely dense layers that prevent access of light to the bottom of this lake and the development of other submerged plants.

Mats of filamentous green alga Cladophora glomerata (L.) Kütz. of macroscopic size formed a large surface mat which freely float on the water surface (macroalgae coating); mat formation regularly repeated every year. Freshwater green algae were collected in the first week of July 2013 when dense mats of Cladophora tightly covered the water column of the shallow parts of Oporzyn Lake. Algae were collected manually from the middle of the mats floating on the surface of the lake water. Due to the fact that the mats were also observed in the pelagic zone, the biomass was harvested using boats applying a strip, a cable, or a special rake.

After the collection, fresh algal biomass (FM) was weighed immediately per 1 m² of the water surface. Then, the material was dried in a specialized drying oven until a dry matter (DM) having a water content of < 15% was obtained.

The basic physico-chemical parameters of the water (temperature, conduction and oxygen concentration as well as pH) at the examined sites with Cladophora were measured with the use of the YSI Professional Plus handheld multiparameter meter.

2.2 Macroalgae culture in the medium supplemented with Cu ions

In order to determine the ability of copper accumulation by macrogreen algae, Cladophora glomerata from Oporzyn Lake was used. Filaments of Cladophora and water samples were collected in the natural habitat of the lake in September, 2013. In order to initiate the culture, samples of macroscopic green algae were carefully purified from organic matter and sand grains by hand. Then algal filaments (420 g of wet weight; one cell surface sorption area was 17.85 mm² and for a filament with 1 cm length this amounted to 119 cm²) were placed for 48 hours in distilled water at room temperature to acclimatize. Six liters of water from the lake and six liters of distilled water were filtered through Whatman GF/F glass fiber filters and sterilized. Glassware (1 liter flasks) were also sterilized. Algae were cultured in modified Wang’s medium for a period of 7 and 14 days, respectively, at a constant temperature of 21°C and light intensity of 250 µmol photons m⁻² sek⁻¹ in a phytothron (CONVIRON model CMP 6050) [31]. CuSO₄·5H₂O was used as the source of copper.

The experiment was composed of the control sample with water habitat (1) and five levels of enrichment of the medium with copper sulphate at different concentrations (0.01, 0.1, 1, 10 and 100 mg L⁻¹CuSO₄·5H₂O). Each level was studied in triplicate using glass containers with 500 mL of medium and 10 g of fresh algae biomass (Fig. 2). Two series of experiments were performed; the first lasted for 7 days, the second for 14 days.

Each time, after completion of these series of experiments, the FM of Cladophora filaments was determined, as well as their DM after drying in a laboratory oven for 2 hours at the temperature of 105°C. Next, the filaments were put into plastic 100 mL containers.

2.3 Biomass, elements and amino acids analysis

The samples of Cladophora glomerata collected from the lake and cultured in a laboratory were analyzed to determine dry biomass, ash, total protein, crude fiber and crude fat content. The process of element analysis was started with mineralization of dry biomass. The samples of 0.2 to 0.3 g of the dried Cladophora biomass were placed in Teflon reactors, then 10 mL of 65% nitric acid (V) (Sigma) was added, and left for 24 hours. The mineralization was conducted in a CEM Mars Xpress microwave oven. Process parameters were as follows: a first stage at a power of 800 W, temperature of 100°C, and time of 30 minutes; was followed by a second stage, power 800 W, temperature 130°C, time 30 minutes. After the mineralization, the samples were diluted with distilled water to 25 mL. Afterwards, the content of each selected element Ca, Mg, Na, K, Fe, Zn, Cu, As, Cd, Ni, Pb, Cr, Mn and Co, was determined in the samples. The elemental analysis was carried out in Inductively Coupled Plasma-Optical Emission Spectrometer (Varian ICP-OES VISTA-MPX) by ICP-OES method. The concentration of each element was expressed as µg g⁻¹ or mg g⁻¹ DM.

The algae samples were analyzed according to the AOAC [32], protocol no. 934.01 for dry matter, and method no. 942.05 for ash. Crude protein was determined with a Kjel-Foss Automatic 16210 analyzer (method no. 976.05), crude fat with a Soxtec System HT analyzer (method no. 2003.05), and crude fiber with a Tecator Fibertec System I (method no. 978.10). The content of N-free extractives was calculated as 1000 – (crude protein + crude fat + ash + crude fibre) and expressed as g kg⁻¹ DM. The samples of ground algae were subjected to acid hydrolysis with 6 M
HCl at 110°C for 23 hours. Amino acids were determined using an AAA 400 amino acid analyser (INGOS, Czech Republic) with ion exchange chromatography. Post column ninhydrin-based detection in a sodium citrate buffer was used. The ninhydrin amino acid derivatives were detected using a column packing of OSTION Lg ANB (column height: 35 × 0.37 cm) at 570 nm for primary amino acids and at 440 nm for secondary amino acids.

2.4 Biomass extraction

The classical method of extraction (Soxhlet) was used. Separately, ethylene alcohol and acetone were used as solvents, in the proportion 1 g of dry *Cladophora* biomass for 25 ml of solvent. In the recent years, more and more attention has been paid to the applications of clean processes such as supercritical fluid extraction (SFE) which are used to isolate natural biologically active compounds from different raw materials, such as plants, food by-products, and micro- and macroscopic algae. Carbon dioxide is the most common solvent used as the supercritical fluid because of its moderate critical temperature (31.3°C) and pressure (72.9 atm); moreover it is considered to be a “green solvent” [33]. In the case of Supercritical Fluid Extraction of *Cladophora* biomass, the methods previously described in detailed by Roj et al. [34] and Kostrzewa et al. [35] were used. The extractions of *Cladophora* were carried out using the multi-purpose pilot unit for supercritical fluid extraction at three different pressures: 300 bar, 500 bar and 700 bar, at 45°C. A second extraction was performed at 700 bar and at 45°C. Pure CO₂ was used as solvent, and samples were taken every 30 minutes.

2.5 Fatty acids analysis in extract

Analysis of fatty acids as methyl esters was carried out using gas chromatography (GC) combined with a mass detector from Thermo Fisher Scientific. In the analytic system, the chemical constituents were separated in a chromatography column and, then, were directed into the Mass Spectrometry Detector (MSD) where they were subjected to an electron beam and were fragmented. The resulting ion mixture was then monitored using one of the two techniques, SCAN or SIM. In the SCAN procedure, the detector swept a desired range of mass to charge ratio (m/z) successively until the total area was covered. To identify a peak shape, 5 to 10 scans of each constituent were necessary. The Selected Ion Monitoring (SIM) method can monitor only a few selected ions. In this case a SCAN technique was used to analyze the extract composition. The methyl esters of fatty acids were prepared in compliance with the PN-EN ISO 5509 standard for “Plant and animal oils and fats”. Preparation of methyl esters of fatty acids and the PN-EN ISO 5508 standard “Oils and plant and animal fats”. An analysis of the methyl esters was carried out by gas chromatography using the TMSH (trimethylsulfonium oxide) based method. 10 mg of liquid sample was solved in 500 μL of t-butyl-methyl ether and slightly heated, if necessary. Then 250 μL of TMSH was added and the solution obtained was shaken intensely. The prepared solution was ready to be injected into a chromatographic column.
Chemical identification of the mass spectral peaks was done by comparison of retention times of the obtained peaks with available pattern peaks. The mass spectrum contains a number of peaks that present a relationship of ionic current vs. m/z, and was used to determine the structure to identify the chemical compound. The m/z ratio provides information on formula mass weight or its part. The identification can be confirmed by comparison of retention times. The quantitative analysis was based on external standards. It is assumed that standard samples and analyte constituents generate identical output signals if they contain the same components.

3 Results

3.1 Habitat of freshwater Cladophora

The mass appearance of C. glomerata in the late June period may result from the great fertility of the Rudka river water flowing into the lake. Average concentrations of N-NO$^3_-$, N-NH$^+$, P-PO$^4_-$, and total iron in this period were 0.51 mg L$^{-1}$, 1.08 mg L$^{-1}$, and 0.19 mg L$^{-1}$, approximately. The value of the electrolytic conductivity fluctuated around 900 $\mu$S cm$^{-1}$, irrespective of the location and time of measurement. During Cladophora massive development, the pH value in the surface water ranged between 7.86 and 8.31. Concentrations of oxygen were changeable and they measured to 8.4 mg L$^{-1}$ in the littoral zone and 4.3 mg L$^{-1}$ in the central part of the lake.

Filamentous green alga Cladophora occurred generally in two forms − submerged and free-floating on the water surface. The submerged filaments of Cladophora consisted solely of juveniles, while the free-floating, on the water surface mats were formed by mature and dying forms. Submerged filaments of macroalgae were often tangled in Ceratophyllum demersum growing on more than 80% of the bottom of the lake. Cladophora biomass concentration appeared in a volume of 600 m$^3$ to 5000 m$^3$ tightly covering the water surface of the lake. In the period from May to July, quantitative proportions varied between submerged form (juvenile phase) and free-floating (mature phase and the dying). In May, the forms of the young was dominant and covered more than 85% of the lake. In June, during the decline in the share of submerged forms of Cladophora, the share of free-floating forms on the surface of the water increased at the same time.

3.2 Macrolegae culture in the medium supplemented with Cu ions

Table 1 presents the elemental composition observed in the Cladophora filaments (mg g$^{-1}$ DM) cultured in the laboratory during supplementation of copper ions. Generally, it was observed that, with the increase of copper content in the culture medium, the copper content in the cultivated biomass also increased. In excess of the concentration of 2.50 mg Cu L$^{-1}$ in the culture medium, a rapid increase in the concentration of copper in the biomass was observed, both in the case of 7 days and 14 days of exposure to copper. In the case of 7 days of exposure, copper content in biomass increased from 1.3 to 3.9 mg g$^{-1}$ of DM (from 2.50 to 24.96 mg Cu L$^{-1}$ of medium, respectively); while in the case of 14 days of exposure, copper content increased significantly more – from 1.3 to 8.5 mg g$^{-1}$ of DM (from 2.50 to 24.96 mg Cu L$^{-1}$ of medium, respectively).

3.3. Biomass analysis

The analysis of biomass was carried out to determine dry biomass, ash, total protein, crude fiber, crude fat, selected elements and amino acids content in C. glomerata. The results shown in Table 2 indicate that the dry biomass content was similar both in Cladophora from the lake and in Cladophora cultured in the laboratory. The ash content was higher in Cladophora biomass collected from the lake, whereas, the total protein, crude fiber and crude fat amount was higher in Cladophora cultured in the laboratory conditions.

In the elemental analysis, there was detected the content of selected elements, such as: Ca, Mg, Na, K, Fe, Zn, Cu, As, Cd, Ni, Pb, Cr, Mn and Co in C. glomerata biomass. Table 3 shows the elemental composition

Table 1: Elemental composition observed in the freshwater Cladophora filaments (µg g$^{-1}$ or g$^{-1}$ DM).

| Element | The element content [g g$^{-1}$ DM] | Element | The element content [µg g$^{-1}$ DM] |
|---------|-----------------------------------|---------|-----------------------------------|
| Ca      | 146.2±3.4                         | As      | 0.53±0.05                         |
| Mg      | 3.63±0.02                         | Cd      | 0.07±0.01                         |
| Na      | 0.47±0.02                         | Ni      | 0.15±0.03                         |
| K       | 17.59±0.22                        | Pb      | 0.80±0.09                         |
| Fe      | 0.19±0.01                         | Cr      | 0.01±0.001                        |
| Zn      | 0.03±0.01                         | Mn      | 0.09±0.03                         |
| Cu      | 0.05±0.01                         | Co      | 0.01±0.001                        |
observed in the freshwater *Cladophora* filaments (µg g\(^{-1}\) or mg g\(^{-1}\) of DM). The results show that the studied biomass of freshwater *C. glomerata* collected from the lake was characterized by the highest calcium content of the detected metals. Furthermore, *Cladophora* biomass contained also large amounts of potassium, magnesium, sodium and iron. Lead, arsenic and nickel occurred in the highest concentrations between other unfavorable heavy metals. While chromium and cobalt were detected in the lowest amounts in the biomass.

As far as amino acids analysis is concerned, the content of amino acids in algal biomass (g/100 g of total protein) is presented in Table 4. It can be observed that glutamic acid and aspartic acid were detected in the highest content in *C. glomerata* collected from the lake. In addition, the alga was found to be a rich source of arginine, leucine, alanine, glycine and valine.

### 3.4 Fatty acids analysis in extract

Table 5 shows the changes in fatty acid concentrations in extracts of the freshwater *C. glomerata* biomass with respect to the type of extraction. The differences in fatty acids composition were noticed depending on the extraction method and solvent. It was observed that the highest amounts of detected fatty acids, both saturated and unsaturated, were obtained using supercritical fluid extraction. The saturated fatty acids ranged from C9:0...
Table 5: Changes in fatty acid concentrations in extract of the freshwater Cladophora biomass with respect to the type of extraction.

| Polyunsaturated/ saturated fatty acid | % weight of fatty acids in dry matter of the extract | Extraction Soxhlet method | Supercritical extraction-CO₂ |
|--------------------------------------|----------------------------------------------------|---------------------------|-----------------------------|
|                                      |                                                    | solvent: ETOH | solvent: Aceton         |
| C9:0                                 | 1.9 n.d.                                           | 1.6 n.d.      | n.d.                       |
| C10:0                                | n.d.                                               | n.d.          | n.d.                       |
| C11:0                                | n.d.                                               | n.d.          | n.d.                       |
| C12:0                                | 0.1 n.d.                                           | 0.2 n.d.      | n.d.                       |
| C14:0                                | 2.7 3.0 n.d.                                       | 12.5 n.d.     | n.d.                       |
| C16:0                                | 5.9 7.1 n.d.                                       | 17.4 n.d.     | n.d.                       |
| C18:0                                | 0.4 0.8 n.d.                                       | 0.6 n.d.      | n.d.                       |
| C20:0                                | n.d.                                               | n.d.          | n.d.                       |
| C22:0                                | n.d.                                               | n.d.          | 0.2 n.d.                  |
| C16:1 (n-7)                          | 1.9 2.8 n.d.                                       | 4.9 n.d.      | 9.3 n.d.                  |
| C18:1 (n-9)                          | 1.0 1.4 n.d.                                       | 5.2 n.d.      | 9.3 n.d.                  |
| C18:2 (n-6)                          | 1.9 3.0 n.d.                                       | 6.5 n.d.      | 9.3 n.d.                  |
| C18:3 (n-3)                          | 1.7 2.2 n.d.                                       | 5.2 n.d.      | 9.3 n.d.                  |
| C18:4 (n-3)                          | 1.5 3.0 n.d.                                       | 3.1 n.d.      | 9.3 n.d.                  |
| C20:4 (n-6)                          | n.d.                                               | n.d.          | 0.4 n.d.                  |
| C20:5 (n-3)                          | n.d.                                               | n.d.          | 0.4 n.d.                  |
| C22:1 (n-9)                          | n.d.                                               | n.d.          | n.d.                       |
| C22:6 (n-3)                          | n.d.                                               | n.d.          | n.d.                       |

The content of fatty acid (% weight) in the dry matter of the extract

| Polyunsaturated/ saturated fatty acid ratio in the dry matter of the extract | 0.72 | 0.83 | 0.91 |

n.d. = not detected. Limit detection 0.1% weight

4 Discussion

Formed macroalgae coating covered almost the entire surface of the water of the lake. The biomass of filamentous mats of Cladophora developed in 2013 (June, July) at a relatively constant level on the Oporzyn Lake and was 674 g DM m⁻² (5896 g FMM⁻²). The importance of Cladophora as a raw material for bioactive compounds is well documented for marine ecosystems [9-12], but our present studies of using a natural source of primary and secondary metabolites are based on macroalgal freshwater biomass. The result of significant copper increase in the case of 14 days of exposure may be explained due to the limited concentration of 2.50 mg Cu L⁻¹ in the medium, above which there was significantly higher biosorption of copper by Cladophora. According to our observations, excessively high concentrations of Cu may cause disturbances in the synthesis of chlorophyll and discoloration algae thalli. Further exposure of the algal culture to high levels of copper above 2.50 mg Cu L⁻¹ acted lethally on the thalli of Cladophora. The biosorption of copper using Cladophora biomass has been widely investigated in recent years. Deng et al. [27] analyzed the ability of marine green alga Cladophora fascicularis (Mertens ex Ag.) Kütz. to absorb copper (II) from aqueous solutions and showed that this alga was characterized by one of the highest adsorption capacities for Cu⁰ between others algal biosorbents [28]. Our studies confirmed the ability of Cladophora's effective copper biosorption, with the highest copper adsorption capacity of 8.5 mg g⁻¹, which corresponds to 0.13 mmol g⁻¹. Apart from studies on marine Cladophora species, there are also a few literature data concerning freshwater Cladophora as a biosorbents. Thus, in one of them, it was found that the freshwater Cladophora fracta (O.F. Müller ex Vahl) Kütz. may remove Cu with the highest efficiency (up to 2.388 mg g⁻¹) compared to other investigated metals: Zn, Cd and Hg [26]. Studies on another freshwater alga, Cladophora crispata (Roth) Kütz, exhibited the highest Cu (II) uptake capacity of 1.03 mmol g⁻¹ of biomass [30]. Afterwards, the effect of different Cu (II) concentrations on the adsorption yield using freshwater Cladophora was also evaluated. The results show that the adsorption rates increased with the increasing copper concentrations up to 150–200 mg Cu L⁻¹. The maximum equilibrium adsorption rate was estimated as 16.73 mg g⁻¹ at copper concentration of 204.2 mg Cu L⁻¹. In comparison to our results, the same group obtained adsorption capacity of 3.27 mg g⁻¹ Cladophora biomass at the concentration of 25 mg Cu L⁻¹ [32], whereas we noticed 8.5 mg Cu g⁻¹ biomass at the same concentration of Cu in the medium after the period of 14 days.
On the other hand, we noticed that the different copper concentrations in the culture medium affected the content of other elements in algae biomass. The effect of supplementation with various substances to bioactive compounds in freshwater Cladophora has been studied in several publications. Nechev et al. [36] reported differences in lipid and fatty acid content in freshwater C. glomerata after treatment with lead ions. Other research showed that the addition of phosphate to the medium enhanced the production of carotenoids and chlorophylls in freshwater Cladophora [15]. The same group demonstrated the influence of increased phosphorus concentration to increase in protein, vitamin A, P, β-carotene, lutein and zeaxanthin contents and decrease in carbohydrate content [16]. In our studies, in the case of 7 days of exposure to copper, the sodium content in biomass increased with increasing copper concentration in the culture medium up to the concentration of 0.25 mg Cu L\(^{-1}\), and afterwards the concentration of the sodium content decreased and increased again. Whereas, after the period of 14 days of exposure to copper, the amount of sodium in biomass increased up to a concentration of 0.02 mg Cu L\(^{-1}\), and the it decreased. Furthermore, during the 7 days of exposure to copper, the potassium content in algae biomass was at a similar level up to the concentration of 0.02 mg Cu L\(^{-1}\), and then, it decreased. In the case of 14 days of exposure to copper, the potassium amount in biomass increased up to the concentration of 0.02 mg Cu L\(^{-1}\), and then, it decreased. However, there was no correlation between the copper content in the culture medium and the calcium content in algae biomass. Finally, the magnesium amount in biomass increased up to the concentration of 0.02 mg Cu L\(^{-1}\), and it decreased after exceeding that concentration during a 7 day exposure to copper. A similar correlation for magnesium was observed in the case of 14 days of exposure to copper. To summarize, in most cases, the investigated element content in algae biomass increased up to the concentration of 0.02 mg Cu L\(^{-1}\) in the culture medium, and in excess of the concentration, the element content decreased with the highest copper concentration. Thus, we demonstrated that copper may affect freshwater C. glomerata metabolism indicating, that supplementation with the appropriate amount of Cu could enhance macro- and microelements production in this alga. Since heavy metals are common in the natural environment, trace amounts of contamination may be present as some components of cosmetics. European Union legislation does not regulate, in detail, what the maximum amounts of heavy metals are allowed. Each cosmetic must be safe and, therefore, the heavy metal content is taken into account when assessing the safety of specific cosmetic. As the copper in cosmetics acts as a disinfectant and accelerates wound healing, its content in cosmetics do not pose a risk to health. The results of our study indicate that the bioaccumulation of copper in the culture of Cladophora biomass is at a level enabling the use of these algae in cosmetics. However, in the case of products of plant origin, the copper content in food products should not exceed 1.0 mg kg\(^{-1}\) of the product.

Study of biomass of freshwater macroalgae as a source of fatty acids, amino acids and other bioactives is not very popular in contrast to marine algae. Chemical composition was well studied, for example, among brown algae – Padina pavonica (L.) Thivy [37], red algae – Ceramium virgatum Roth [38] and green algae – Cladophora vagabunda (L.) Hoek [39]. Different genus of marine green algae, e.g.: Cladophora, Codium, Chaetomorpha and Ulva, which are rich in fatty acids (especially: palmitic, myristic, behenic, palmitoleic, oleic and linoleic acids), may be potentially used for nutritional and pharmaceutical applications [40]. However, few sources refer to fatty acids content in freshwater C. glomerata [18].

To sum up, the contents of the studied metals decreased in the following order: Ca > K > Mg > Na > Fe > Cu > Zn > Pb > As > Ni > Cd > Mn > Cr > Co. In contrast, Khuantrairong and Traichaiyaporn [16] reported the highest content of magnesium in freshwater Cladophora. They evaluated the following content of studied elements: Mg > Fe > Ca > K > Zn in Cladophora biomass.

In the literature, there is no evident interest in amino acid content in freshwater Cladophora. However, marine C. vagabunda (L.) Hoek was investigated, and it was characterized by a similar amino acid composition (glutamic acid, aspartic acid, glycine, valine, lysine, histidine, arginine) [17] as the freshwater C. glomerata. Moreover, our results show that amino acid content varies in Cladophora biomass from the lake and in Cladophora biomass cultured in the laboratory. However, in most cases, amino acid concentration in biomass was higher in Cladophora collected from the lake. The exceptions were aspartic acid, proline, cysteine, tyrosine, histidine, lysine and arginine, which were detected in higher amounts in Cladophora cultured under laboratory conditions. As a result, freshwater Cladophora from its natural habitat may be a potentially better amino acid source for cosmetic raw material than the same alga cultured in the laboratory. This is a very important aspect from the economical point of view, because cultivating of algae biomass in a laboratory is much more expensive than the harvesting of algae from their natural habitat.

In extracts from algae biomass, fatty acids ranged from 9 to 22 carbons in length and were determined
both as saturated and unsaturated compounds [41]. The number of double bonds in the fatty acid chains, however, never exceeds 6 and almost all of the unsaturated fatty acids are cis isomers [42]. The fatty acids are recovered from the biomass algae by an extraction process, which can be carried out using solvents in the Soxhlet method or supercritical fluid extraction using supercritical CO$_2$ [43,44]. In our studies, it was observed that the highest amounts of detected saturated and unsaturated fatty acids were obtained using supercritical fluid extraction. Higher efficiency of supercritical fluid extraction in obtaining bioactive substances from a natural sources as compared to other extraction methods has been proven in many studies [45,46]. Our results indicate that the content of fatty acids in extracts from Cladophora can be used as a significant supplement in animal feed.

It is interesting to observe a relatively large amount of the palmitoleic acid (C16:1), both in extracts obtained by supercritical fluid extraction and by solvent extraction, which rarely occurs in algae and is characterized by its strong antioxidative and anti free radical properties. We also noticed relatively high content of essential fatty acids – linoleic (C18:2; n-6) and α-linoleic acid (C18:3; n-3) in obtained extracts, which make these extracts potentially valuable sources for food, feed and cosmetics production. According to others studies concerning fatty acid composition in extracts from marine Cladophora, the alga contained the most amount of palmitic acid (C16:0) among others fatty acids [11-13] as in our research. C. vagabunda was found to be a rich source of C20:4 (n-6) fatty acid, which was the main unsaturated fatty acid in contrast to the freshwater C. glomerata, where we detected this acid in traces, and where the C18:1 acid was the dominant unsaturated fatty acid. What is more, we noticed the higher content of C16:1 acid, and essential fatty acids: C18:2 and C18:3 in C. glomerata in comparison to our results. Other differences were found in content of C16:1, C18:1 and C18:3 fatty acids, which were not detected [18], in comparison to our results. Summarizing, the changes in fatty acid composition in Cladophora extracts are evident and may depend on such factors as: extraction technique, solvents used, algae species and their natural habitat.

5 Conclusions

1. Lake Oporzyn in Poland is a convenient place for harvesting macroalgae of the Cladophora genus which is very important for obtaining organic biomass with commercial applications.
2. Amino acid content in Cladophora biomass indicates a very interesting new material which could be potentially used in animal feed as a feed supplement.
3. As a result of bioaccumulation, it is possible to obtain very high copper concentration in the culture of algae biomass, far in excess of the amount necessary for animal nutrition in this biomass. Using this technology, it is absolutely essential to control the amount of copper in the biomass because it can be a toxic.
4. The number and type of fatty acids and the ratio of unsaturated to saturated fatty acids in the extract of freshwater Cladophora biomass indicate that the extract can be a valuable supplement in human food, as well as a great supplement in animal feed. The extract obtained with the supercritical extraction process may also be used as a component in cosmetics: masks, creams and scrubs.

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