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Assessment of the Resource Potential of Baltic Sea Macroalgae

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Abstract: The excess biomass of drifting algae and their casting to the Baltic Sea coast imposes a significant environmental burden. The analysis of beach-cast algae showed that the dominant species are macroalgae *Ulva* sp., *Furcellaria lumbricalis*, *Cladophora* sp., and *Polysiphonia fucoides*. The biomass of *Furcellaria* and *Polysiphonia* algae, containing 25.6% and 19.98% sugars, respectively, has the greatest resource potential in terms of obtaining carbohydrates. Fucose, glucose, and galactose were found to be the most common carbohydrates. The lipid content did not exceed 4.3% (2.3–4.3%), while the fatty acid composition was represented by saturated fatty acids (palmitic, stearic, methyloleic, behenic, etc.). The highest content of crude protein was found in samples of macroalgae of the genus *Polysiphonia* and amounted to 28.2%. A study of the elemental composition of drifting algae revealed that they have a high carbon content (31.3–37.5%) and a low hydrogen (4.96–5.82%), and sulfur (1.75–3.00%) content. Red algal biomass has the most resource potential in terms of biofuel generation, as it has a high number of lipids and proteins that can produce melanoidins during hydrothermal liquefaction, enhancing the fuel yield. The study noted the feasibility of using the biomass of the studied algae taxa to produce polysaccharides and biofuels. The analyses of antioxidant properties, fat content, and fat composition do not provide convincing evidence of the viability of using the aforementioned macroalgae for their production.

Keywords: macroalgae; biomass; plastic; elemental composition; nutrients; resource potential

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1. Introduction

It is well understood that the formation of excess algae biomass is currently a serious environmental problem that requires a greater focus on finding solutions. The accumulation of algae biomass in coastal waters or beach-cast algae on the coast is linked not only to the release of foul-smelling substances and a decrease in the territory's recreational attractiveness, but also to the formation of greenhouse gas emissions [1]. According to recent studies [2], the impact of the rotting biomass of drifting algae and marine debris on climate change is underestimated. It has been established that approximately a billion tons of greenhouse gases are emitted annually during the process of their destruction [2].

Several scientists have noted that algae biomass has significant resource potential [3,4]. Macroalgae have been used to produce medicines [3,5–9], cosmetics [3,7–14], nutrient sources [15–20], fertilizers and agrochemicals [3,21–27], biochar [28–32], methane, synthesis gas, and hydrogen [33–35], liquid fuels [28,36–39], and bioethanol [28,40]. The processing of drifting macroalgae into value-added products will allow not only to solve climatic and environmental problems of cleaning up the coastal zone, but also to obtain a significant economic effect, including of multiplicative nature, for example, by increasing its tourist attractiveness.

The accepted concept of bioeconomy development calls for the development of technological schemes for the cascade processing of biomass, ensuring its maximum utilization [41]. This approach involves the stepwise extraction of valuable components from the most valuable to the least valuable. Obviously, developing a cascade of technologies without a comprehensive assessment of the resource potential of macroalgae is impossible. In this regard, assessing the resource potential of macroalgae is important, as it will allow for the development of scientific, theoretical, and technological foundations for the development of technologies for their cascade processing.

The goal of this study, the findings of which are presented in this article, is to assess the resource potential of the biomass of the dominant macroalgae species found on the Baltic Sea coast. An analysis of the physicochemical composition and antioxidant properties of macroalgae is used to identify promising areas of application.

The article discusses the prospects for using algae biomass as a source of nutrients and biologically active substances, as well as a raw material in the production of biofuels. The novelty of the study lies in a detailed description of the species diversity of beach-cast macroalgae on the coast of the Russian part of the Baltic Sea in the area, as well as information on the physicochemical composition of macroalgae selected in this part of the Baltic Sea.

2. Materials and Methods

2.1. Research Methodology for Assessing the Volume of Accumulation and Species Diversity of Macroalgae

The volumes of macroalgae biomass accumulation along the Baltic Sea coast were assessed from March 2019 to September 2021 [42–45]. Monthly observations were carried out at four model sites in two major locations of the Baltic Sea (in Filinsky Bay and on the beach of Zelenogradsk). During the survey, sampling and accounting (measurement and description) were carried out according to the developed standardized scheme with the geo-referencing of the results of beach-cast algae using GPS navigation and photo fixation. Beach-cast algae samples were weighed, then washed to remove sand to determine the wet and dry weights after drying in an oven for two weeks at 60 °C. The taxonomic affiliation of algae was visually determined using determinants [46–48].

2.2. Research Methodology for Assessing the Physicochemical Composition of Macroalgae

The content of the mass fraction of proteins, fats, and carbohydrates was studied to assess the potential resource attractiveness of drifting algae biomass as a source of nutrients in the feed and food industries.

The protein content was determined using two different methods: Bradford and Kjeldahl. The Kjeldahl method [49] consists of mineralizing organic matter with sulfuric acid in the presence of a catalyst, forming ammonium sulfate and destroying ammonium sulfate with alkali, releasing ammonia and removing ammonia with water vapor into a solution of sulfuric or boric acid, which is followed by titration. The mass fraction of nitrogen is calculated and converted into the crude protein content based on the titration results (by multiplication by a factor of 6.25).

When using the Bradford method [50], proteins were extracted using distilled water and NaOH solution. A total of 50 mg of dried microalgae biomass were mixed with 4 mL of distilled water and incubated at 4 °C for 12 h. The algae mixture was then ground in a mortar with a pestle for 5 min before being incubated at 4 °C for another hour. After grinding, the mixture was centrifuged for 20 min (at 4 °C, 75,456$\times$ g). The supernatant was collected and the algae pellet was re-extracted with 1 mL of 0.1 N NaOH with 0.5% β-mercaptoethanol (v/v). The mixture of algae extract and NaOH solution was kept at room temperature for 1 h, periodically shaking by hand. The mixture was then centrifuged for 20 min at 21 °C and 75,456× g. The supernatant was combined with the first one. Then, the protein content was determined using the Bradford method [50].

The carbohydrate content of drifting algae biomass was determined in two forms, fiber and reducing sugars, which correspond to the difference in their potential areas of use. The 3,5-dinitrosalicylic acid method was used to determine the content of reducing sugars. When sugars interacted with 3,5-dinitrosalicylic acid, the latter was reduced to 3-amino-5-nitrosalicylic acid. Preliminary extraction was performed according to the method of Karemore and Sen [51]. For this, 50 mg of microalgae biomass were placed in a 100 mL flask and mixed with 50 mL of 2% H_2SO_4. The mixture was autoclaved at 121 °C and 1 bar for 30 min at pH 7. For separation, the resulting mixture was centrifuged at 4 °C at a speed of 764× g for 10 min.

The fiber content in algae samples was estimated by the weight method. The essence of the method is the sequential treatment of a weighed portion of the test sample dried to a constant weight with sulfuric acid and potassium hydroxide solutions, ashing, and the quantitative determination of the organic residue using the gravimetric method [52].

The total lipid content was determined by the Folch method [53]. For this, 20 mL of a solvent (a 2:1 (v/v) mixture of chloroform and methanol) was added to 1 g of dried biomass and stirred at 11 g for 20 min. Then, the algae biomass cells were destroyed in an ultrasonic disperser, homogenizer, and degasser. Next, the solvent containing the extracted lipids was centrifuged at 56,683× g and filtered under vacuum. The solvent was evaporated to dryness in a drying cabinet at 60 °C. Total lipids were quantified gravimetrically and expressed as a percentage of dry cell weight.

To determine the fatty acid composition, lipids were dissolved in 10 mL of hexane; then, 2 mL of the solution was transferred to a test tube, and 2 mL of a 10% sodium methoxide in methanol solution was added to the lipid solution. After vigorously shaking the mixture for 10 min, the top layer of hexane (1 mL) was removed and transferred to a vial, and the fractional composition was determined using GC/MS on an Agilent 5970B instrument. The following parameters were applied: Detection mode positive ions, electron energy 30 eV, He gas flow rate 1 mL/min, HP-5MS column, injector temperature 300 °C, thermostat temperature gradient of 80–280 °C, over 25 min.

To determine the content of individual carbohydrates by HPLC, 2 g of dry weight of algae was placed in a glass cup; then, 20 mL of a 10% sulfuric acid solution was added, and heated at 80 °C for 30 min. The resulting hydrolyzate was transferred into a 100 mL flask; 5 mL of Carrez solution 1 and 2 were added, stirred on a shaker for 30 min, and the volume was adjusted to the mark and filtered. A total of 1 mL of the filtrate was taken for HPLC and stabbed on a Shimadzu LC 20 AD HPLC system (Shimadzu Europa GmbH, Duisburg, Germany), flow rate 1 mL/min, Supelco 4.5 × 150 column (Shimadzu Europa GmbH, Duisburg, Germany), silica gel modified with amide phase, eluent water:acetonitrile, and gradient from 4% acetonitrile to 80% over 40 min.

The Elemental analysis of algae biomass was performed using an Elementar Analysensysteme (Elementar Analysensysteme GmbH, Langenselbold, Germany) CHNS-elemental analyzer Vario EL Cube model. Weighing was carried out on an analytical balance with an accuracy of 0.01 mg. The element content was calculated using a calibration straight line constructed from standard compounds based on the area of the chromatographic peaks of N_2, CO_2, H_2O, and SO_2. Each sample was analyzed with three independent measures, and the mean values are presented. The analytical data were processed and the element content of the sample was calculated using the instrument manufacturer's software.

2.3. Research Methodology for Assessing the Antioxidant Properties of Macroalgae

To assess the resource potential of algae biomass as natural antioxidants, the antioxidant activity of their alcohol extracts was examined, as well as the antioxidant activity of the major groups of substances separately (fats, carbohydrates, and proteins).

For antioxidant extraction, 0.1 g of crushed dry algae was mixed with 10 mL of 96% ethanol (diluted with distilled water). Extraction was carried out at room temperature for

24 h. The mixture was then filtered through a paper filter. The filtrate was collected and used as the crude extract.

The activity of the extract was evaluated by scavenging 2-2-Diphenyl-1-picrylhydrazyl (DPPH) free radicals. A 0.2 mM solution of DPPH (Sigma, Tokyo, Japan) in ethanol was prepared and added to 2 mL of the extracted samples at various concentrations (0.5, 1.0, and 2.0 mg/mL). After a 30 min incubation, the optical density was measured in a UV-3600 spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of 517 nm. Ascorbic acid at various concentrations (from 0.00125 to 0.008 mg/mL) was used as a control for antioxidant activity. The percentage of DPPH inhibition was calculated using the following equation:

$$\text{absorption effect (\%)} = (1 - \text{absorbance sample}/\text{absorbance control}) \times 100\%. \quad (1)$$

A plot of absorption activity versus sample concentration was created based on the measurement results.

The IC50, or effective concentration at which 50% of the DPPH radicals are absorbed, was determined using linear regression. The lower the IC50 value, the higher the antioxidant activity.

3. Results

3.1. Species and Qualitative Composition of Beach-Cast Macroalgae

When the composition of beach-cast macroalgae on the coast of the Baltic Sea within the Kaliningrad region was assessed, it was discovered that the casts of macroalgae and sea grasses on the coast have a complex, multicomponent composition, as they can contain various animal and plant objects for which they are edificatory organisms in habitats, food, or shelter while drifting at sea after separation from the substrate, as well as after being cast (Figure 1). In addition, beach-cast algae can accumulate debris and pollutants, such as plastic, petroleum hydrocarbons, and heavy metals [54].

(**a**) (**b**)

Figure 1. A fragment of beach-cast algae (**a**) 19 September 2020, near the village of Witlan; (**b**) 9 September 2021, cape Guardeisky (photo by J. Gorbunova).

An examination of the flora and fauna of beach-cast algae samples collected in the Kaliningrad zone of the Baltic Sea coast revealed that macroalgae thalli accounted for an average of 95% of the total mass of the sample cleaned from sand in the vast majority of cases. In total, 14 taxa of macroalgae and sea grasses were recorded in coastal casts. The species composition changed with the seasons and was also influenced by the sampling area. Macroalgae species found in coastal casts are presented in Table 1.

Table 1. List of macroalgae and seagrass species found in casts on the coast of the Baltic Sea within the Kaliningrad region.

Taxon	Species
Rhodophyta	*Ceramium tenuicorne* (Kutzing) Waern *Ceramium virgatum* Roth *Coccotylus truncatus* (Pallas) M.J. Wynne & J.N. Heine *Furcellaria lumbricalis* (Turner) Lamouroux *Polysiphonia fucoides* (Hudson) Greville
Chlorophyta	*Cladophora glomerata* (L.) Kützing *Cladophora rupestris* (L.) Kützing *Cladophora sericea* (Hudson) Kützing *Urospora penicilliformis* (A.W. Roth) J.E. Areschoug *Ulva intestinalis* L. *Ulva prolifera* O.F. Müller
Phaeophyta	*Fucus vesiculosus* L. *Pilayella littoralis* (L.) Kjellman
Plantae Vasculares	*Zostera marina* L.

Beach-cast algae, predominantly represented by the red alga *Furcellaria lumbricalis*, often contained epiphytic organisms (bivalve mollusks *Mytilus edulis*, bay barnacle *Amphibalanus improvisus*, and moss animals *Bryozoa*). At the same time, the biomass of the mussels *Mytilus edulis* was comparable to that of the algae in some cases. In the summer, old algae casts sometimes contained a large number of *Diptera* larvae and imago, but their biomass was very low.

The beach-cast algae tended to contain large amounts of sand (Table 2), averaging 39% of wet weight. The observed content of sand reached 87%.

Table 2. Main characteristics of beach-cast algae.

Indicator	Value		
	Medium	**Maximum**	**Minimum**
Sand content in beach-cast algae, % wet weight	39	87	0
Beach-cast algae weight (initial when sampling from the coast), kg/m^3	450	1072	95
Wet weight of beach-cast algae after sand removal by washing, kg/m^3	283	840	71
Dry weight of beach-cast algae after sand removal by washing, kg/m^3	35	70	13

The sand content was a significant factor in determining the specific weight of the beach-cast algae sampled on the coast. The weight of 1 m^3 of beach-cast algae was 450 kg on average, but it could reach 1072 kg in some cases. After sand removal by washing, the weight of 1 m^3 of beach-cast algae averaged 283 kg wet and 35 kg dry weight, respectively (Table 2). It should be noted that the same volume of beach-cast algae composed by different species could have significant differences in weight. In equal volumes, branched thallus algae (*Furcellaria lumbricalis*) had a lower mass than filamentous species (genus *Cladophora*).

Based on an examination of 109 samples of algae casts collected at various times in the Filinsky Bay, it was discovered that 28% of them contained mesoplastics (fragments of plastic 5–25 mm long or in diameter that are clearly visible to the naked eye). In these cases, 77% of the polyethylene fragments were 0.0001–0.0050 m^2 in size. Thus, 1 m^3 of beach-cast algae contained approximately 0.06 m^2 of polyethylene on average.

3.2. Spatial Distribution of Beach-Cast Macroalgae

An analysis of the features of the spatial distribution of beach-cast algae on the coast of the Baltic Sea within the Kaliningrad region made it possible to identify their significant spatial and seasonal heterogeneity. From March 2019 to September 2021, the coast of the Sambia Peninsula experienced 22 episodes of significant macroalgae cast in terms of area and volume.

In most cases, casts were local in nature and were mainly confined to six districts: Zaostrovye on the east side of Cape Gvardeisky (the most significant cast was recorded in August 2019, with a length (L) of 150 m, with a maximum layer thickness (h) of up to 0.35 m, (Figure 2a), west of the mouth of the Zabava River (August 2019, L = 200 m, h up to 0.45 m (Figure 2b), the settlement Otradnoe (June 2020, L = 250 m, h up to 0.50 m (Figure 2c), Filinsky Bay (July 2019, L = 250 m, h up to 0.35 m (Figure 2d), the western part of the beach in Baltiysk (July 2019, L = 70 m, h up to 1.0 m (Figure 2e), and the western part of the beach in Pionersky (July 2019, L = 50 m, h up to 0.30 m (Figure 2f)).

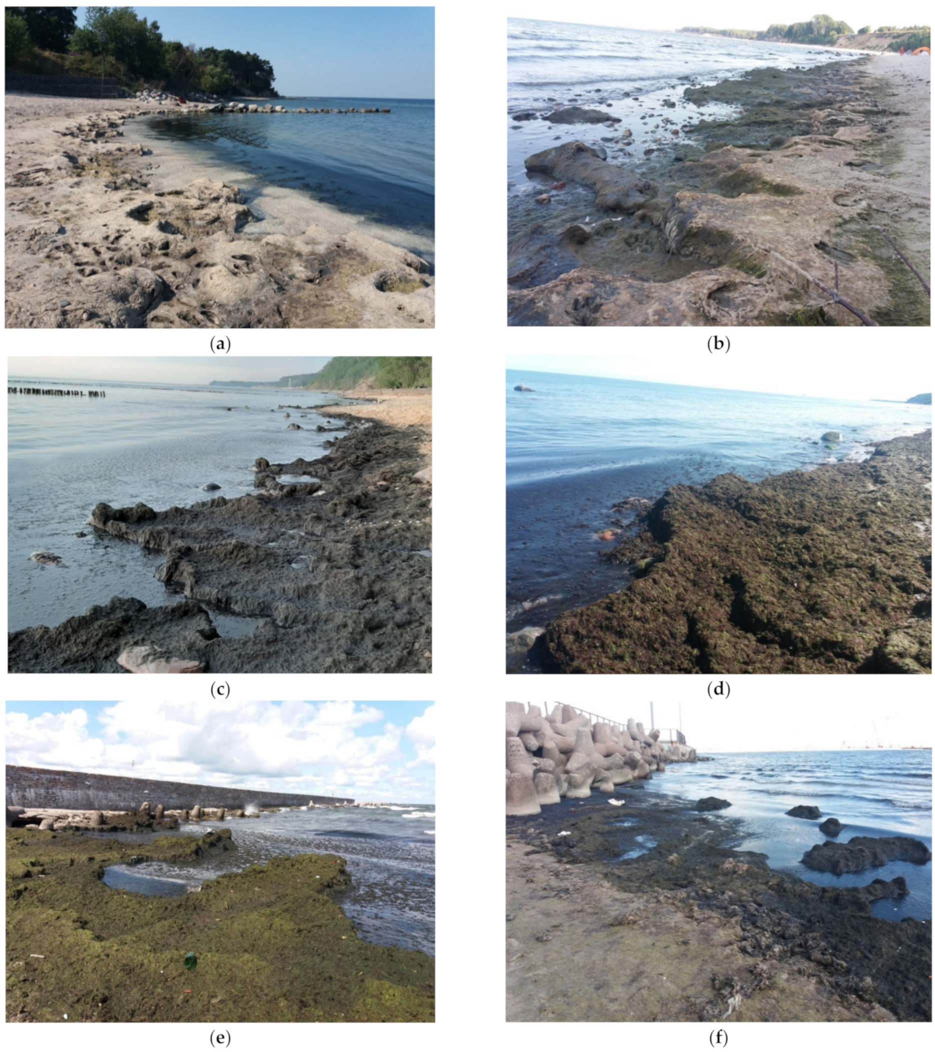

Figure 2. Algae casts of large volume, the layer thickness exceeds 0.15 m: (**a**) Zaostrovye settlement from the east side of Cape Gvardeisky, 28 August 2019; (**b**) coast, west of the mouth of the river Zabava, 31 August 2019; (**c**) Otradnoe settlement, 20 June 2020; (**d**) Filinsky Bay, 10 July 2019; (**e**) the western part of the beach in Baltiysk, 17 July 2019; (**f**) Western part of the Pionersky beach, 25 July 2019 (photos by J. Gorbunova).

Significant beach-cast algae occurrences were after stormy weather. So, in July 2019, after storm episod from 27 June–7 July 2019 with northwestern winds up to 20 m/s, algae casts layer exceed 0.15 m and were recorded in four local areas located on the northern coast of the Sambian Peninsula, as well as on the beach of Baltiysk. The maximum observed length of a continuous patch of beach-cast macroalgae was 250 m (Filinskaya Bay), the largest layer thickness was 1 m (western part of the beach in Baltiysk). The width (from the water edge deep into the beach) of continuous patches was 1–20 m. After storm lasted from 11 till 13 March 2020 with northwestern winds up to 20 m/s, macroalgae casts with layer thickness more than 0.15 m were observed at three sites along the northern coast in March 2020. The maximum observed length of a continuous patch of macroalgae cast was 300 m (the beach of the village of Otradnoe), and the largest layer thickness was 0.6 m (Filinskaya Bay).

During the warm season, June–September 2019 and 2021, in the absence of strong surf, fragments of beach-cast macroalgae and sea grasses were found almost throughout the Russian part of the Baltic southeastern coast.

Narrow cast bands, not exceeding 0.5–1.0 m wide, in which macroalgae did not form a continuous projective cover, were found along the entire seacoast of the Kaliningrad region and had a length of up to 1 km (the beaches of Yantarny and Zelenogradsk). Most of the continuous accumulations had an average layer thickness not exceeding 0.15 m. With the exception of a few isolated regions, most of the Russian sea shoreline had just single fragments of algal thalli that did not form clusters during the cold season.

Thus, on the northern coast of the Sambia Peninsula, large volumes of beach-cast algae were observed, in contrast to the western coast and the Vistula and Curonian Spit, especially in the period from late autumn to early spring, when the main biomass of the beach-cast algae was formed by species of the red algae *Furcellaria lumbricalis*. This is perhaps attributable to the presence of a belt of these algae in the Cape Taran area [55]. During the summer, representatives from the green algae division played a key role in casts along the whole length of the Kaliningrad region's seacoast, with the majority of the species being annuals growing on stones and groins [56]. However, even during this period, large concentrations were also confined to local areas, primarily the northern coast of the Sambian Peninsula.

3.3. Seasonal Dynamics of Beach-Cast Macroalgae

The seasonal dynamics of algae cast on the Baltic Sea coast within the Kaliningrad region were estimated at two model sites 100 m long with the maximum total annual cast, located in the Filinsky Bay, from March 2019 to August 2020. Model site No. 1 was located in the central part of the bay on the east side of the slipway for small boats, and site No. 2 was 200 m east of site No. 1. Both sites had a northern exposure to the coastline.

According to data analysis, on the model site 1, the highest levels of algae casts (up to 500–1700 kg dry weight/100 m of coastline) were observed primarily in the spring and summer months of 2019 and 2020. Significant amounts of algae (approximately 300 kg dry weight/100 m of coastline) were also observed in March 2019, during the phenological winter. On the model site 2, the dynamics were diverse.

The dynamics of casting show a general trend for both observed sites. However, despite their proximity to each other, the quantitative parameters differ significantly. The average annual indicator for section No. 1 was 197 kg dry wt./100 m of coast, while for section No. 2–66 kg dry wt./100 m of coast. This can be explained by the fact that, despite the identical northern exposure of both beaches, site No. 1 adjoins the slipway on the eastern side. As a result, a type of hydrodynamic trap forms, which aids in the accumulation of algae biomass under certain wind conditions.

Throughout the entire observation period, the qualitative composition of casts in both parts of the Filinsky Bay coast generally coincided. There was a clear seasonal dynamic of the species composition of beach-cast algae. In the cold period of the year, the biomass of red algae, primarily due to *Furcellaria lumbricalis*, reached 90–100% (Figure 3) and decreased to 30–50% in the warm period due to the massive vegetation of green algae of the *Cladophora* and *Ulva* genera.

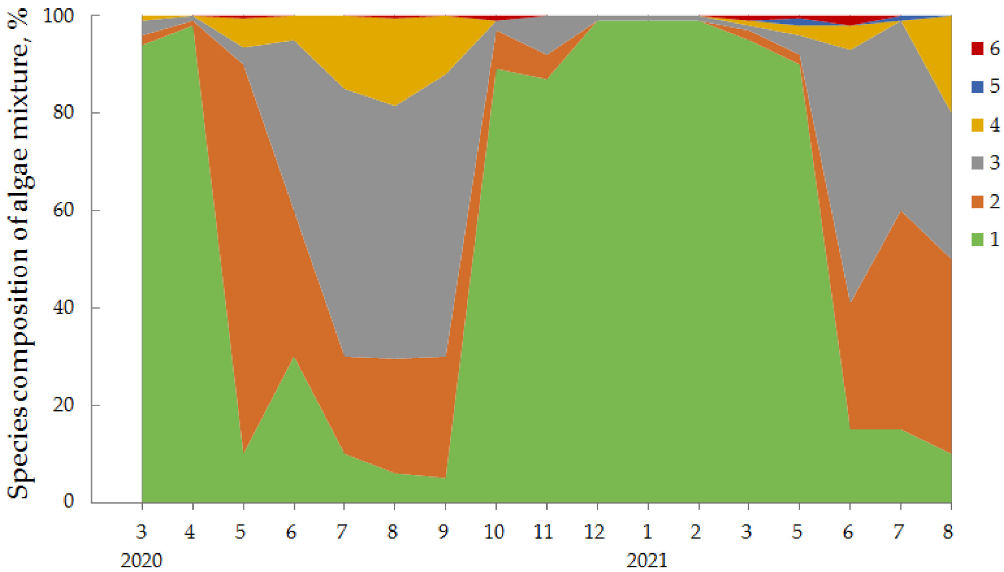

Figure 3. Taxonomic representation in beach-cast algae from Filinsky Bay in different seasons: 1—*Furcellaria*, 2—*Polysiphonia*, 3—*Cladophora*, 4—*Ulva*, 5—*Fucus*, 6—Others.

A preliminary assessment of the total annual volume of macroalgae casts on the Baltic Sea coast within the Kaliningrad region revealed significant spatial and seasonal unevenness in their distribution [56,57]. In most cases, casts significant in terms of volume were of a local nature and were mostly confined to areas of the northern coast of the Sambian Peninsula. In total, for the period from March 2019 to August 2020, 22 episodes of significant macroalgae casts in terms of area and volume with a layer thickness exceeding 0.25 m were recorded in six local areas of the coast. The recorded maximum length of the cast was 300 m and thickness was 1 m.

3.4. Content of Lipids, Proteins, Carbohydrates, and Biogenic Elements in the Biomass of the Studied Macroalgae

The highest content of crude protein was observed in samples of macroalgae of the genus *Polysiphonia* and amounted to 28.2% of absolute dry matter (a.d.m.). In samples of algae of the genus *Furcellaria*, the content of crude protein was 25.4% of a.d.m. For algae of the genus *Cladophora*, this indicator was 20.8% of a.d.m. The lowest content of crude protein was observed in samples of macroalgae *Ulva* sp., 17.3% of a.d.m. (Table 3).

It was demonstrated that the protein content determined by the Bradford method was significantly lower than that measured using the Kjeldahl method. This may be due to insufficient extraction of protein from the biomass, and also to the fact that the Kjeldahl method also determines non-protein nitrogen in the samples. According to the data obtained by the Bradford method, the highest amount of protein was found in macroalgae *Furcellaria* samples and amounted to 3.5%. In samples of macroalgae of the genera *Cladophora* and *Polysiphonia*, the protein content was 3.1% (by the Bradford method). The highest content of reducing sugars (Table 3) was found in samples of macroalgae *Furcellaria* (21.96%). The content of reducing sugars in algae samples from the genus *Polysiphonia* was 17.84%, while *Cladophora* had a content of 13.04%. The lowest content of reducing sugars was observed for *Ulva* sp., at 11.83%.

Table 3. Content of crude protein, lipids, and carbohydrates in macroalgae samples.

Indicator	Content, % of Absolute Dry Matter			
	Cladophora	*Polysiphonia*	*Ulva* sp.	*Furcellaria*
Protein (Kjeldahl)	20.8 ± 0.62	28.2 ± 0.84	17.3 ± 0.52	25.4 ± 0.76
Protein (Bradford)	3.1 ± 0.1	3.1 ± 0.1	2.8 ± 0.1	3.5 ± 0.1
Reducing sugars	13.04 ± 0.39	17.84 ± 0.53	11.83 ± 0.35	21.96 ± 0.65
Cellulose	4.63 ± 0.12	2.14 ± 0.06	2.14 ± 0.08	3.67 ± 0.11
Carbohydrates (total)	17.67 ± 0.51	19.98 ± 0.59	13.97 ± 0.43	25.63 ± 0.76
Carbohydrates (total) according to the literature data	34.7 [58]	25.8 [59]	31.34–45.5 [60–62]	55.4 [63,64]
Lipids	2.8 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	4.3 ± 0.1
The ratio of proteins and carbohydrates	1.18:1	1.41:1	1.24:1	1:1

The results of determining the content of individual carbohydrates by HPLC are presented in Table 4.

Table 4. Carbohydrate composition of macroalgae (% of the content of reducing carbohydrates).

Component	Content, %			
	Ulva	*Cladophora*	*Polysiphonia*	*Furcellaria*
Glucose	4.51 ± 0.13	1.63 ± 0.04	2.31 ± 0.07	1.73 ± 0.04
Xylose	1.10 ± 0.03	0.69 ± 0.01	0.34 ± 0.01	0.44 ± 0.01
Fucose	2.32 ± 0.07	2.71 ± 0.10	4.13 ± 0.10	6.49 ± 0.10
Arabinose	1.24 ± 0.06	3.38 ± 0.08	0.64 ± 0.01	0.43 ± 0.02
Rhamnose	0.39 ± 0.01	1.22 ± 0.03	0.37 ± 0.01	0.32 ± 0.01
Galactose	1.66 ± 0.04	1.85 ± 0.05	1.18 ± 0.03	2.51 ± 0.08

The highest carbohydrate content in the biomass of the red algae *Furcellaria* and *Polysiphonia* was related to the differences in their development. These algae are perennial, while green algae are seasonal (vegetation period 3–6 months) and do not have a thick cell wall, resulting in a lower carbohydrate content in the biomass (*Ulva* sp. 11.83% and *Cladophora* 13.04%). Fucose, glucose, and galactose dominated among carbohydrates. Arabinoses were abundant only in the biomass of *Ulva* sp. samples. The content of cellulose in the biomass of algae was approximately the same and varied in the range of 2.14–4.63%.

The highest total lipid content was found in macroalgae samples from the genus *Furcellaria*, accounting for 4.3% of total dry biomass. The total lipid content in *Cladophora* algae samples was 2.8% (Table 3). The lipid content of macroalgae from the genus *Ulva* sp. was 2.4% of the total biomass. The algae of the genus *Polysiphonia* had the lowest lipid content (2.3%). The original HPLC data are presented in Supplementary Materials.

The next stage of research was focused on determining the fatty acid composition of lipids found in drifting algae in order to assess their potential for use in the nutraceutical, pharmaceutical, and cosmetic industries. The results of the HPLC analysis are presented in Table 5.

Table 5. Fatty acid composition of drifting algae.

Component	Release Time	Content, %			
		Polysiphonia	*Ulva* sp.	*Cladophora*	*Furcellaria*
Methyl palmitate	8.78	0.40 ± 0.08	0.60 ± 0.07	0.80 ± 0.09	1.20 ± 0.10
Methyl stearate	11.24	0.20 ± 0.04	0.30 ± 0.01	0.10 ± 0.01	0.20 ± 0.01
Tribehenate glycerate	12.90	0.70 ± 0.08	0.80 ± 0.06	0.60 ± 0.03	1.20 ± 0.11
Methyl oleate	13.43	0.052 ± 0.002	0.082 ± 0.005	0.10 ± 0.01	0.20 ± 0.04
Behenic acid	14.98	0.20 ± 0.01	0.10 ± 0.01	0.20 ± 0.02	0.08 ± 0.01
Trifumarylglycerate	17.82	0.05 ± 0.01	0.063 ± 0.005	0.07 ± 0.01	0.04 ± 0.01
Linolenic acid	19.41	0.063 ± 0.007	0.082 ± 0.007	0.032 ± 0.005	0.052 ± 0.007
Tetradecylic acid	19.74	0.041 ± 0.009	0.12 ± 0.01	0.073 ± 0.006	0.083 ± 0.007
Geronyl izovalerate	22.73	0.023 ± 0.001	0.041 ± 0.005	0.031 ± 0.008	0.051 ± 0.008

The predominant components of the fatty acid composition in the biomass of algae samples from the genera *Polysiphonia* and *Ulva* were tribehenate glycerate (content 0.70 ± 0.08% and 0.8 ± 0.06%, respectively) and methyl palmitate (0.40 ± 0.08% and 0.60 ± 0.07, respectively), and the content of behenic acid was at the level of 0.2%. The predominant components of *Furcellaria* biomass were methyl palmitate and tribehenate glycerate (content 1.2%), and methyl oleate and methyl stearate (about 0.2%). In *Cladophora* algae, a high content of methyl palmitate (0.80 ± 0.09%) and tribehenate glycerate (content at the level of 0.6%) was noted.

The content of C, H, N, S, and O is a significant biomass parameter that affects the quality of the resulting biofuel, which makes it possible to predict the gross formula of algae. A high content of sulfur, nitrogen and oxygen reduces the quality of fuel, increasing its corrosiveness, reducing calorific value, and changing consumer properties. In this regard, further studies were aimed to estimate the element composition of algae. The research results are presented in Table 6.

Table 6. Elemental analysis of algae biomass.

Sample	Source	Content, %				
		C	H	N	S	O (Calc.) [1]
Furcellaria	Experimental research	37.52 ± 1.12	5.82 ± 0.17	3.60 ± 0.10	3.00 ± 0.09	50.05 ± 1.50
Polysiphonia		33.80 ± 1.01	5.05 ± 0.14	3.65 ± 0.11	2.38 ± 0.07	55.12 ± 1.65
Ulva		32.08 ± 0.96	5.15 ± 0.15	2.06 ± 0.06	1.94 ± 0.06	58.77 ± 1.76
Cladophora		31.33 ± 0.93	4.96 ± 0.14	2.26 ± 0.06	1.75 ± 0.05	59.70 ± 1.79
Coniferous wood	[65]	48.56	11.84	0.7	0.06	38.85
Poplar		51.60	6.00	0.60	0.02	41.70
Rice hulls	[66]	49.40	6.20	0.30	0.40	43.70
Cotton stalk		49.80	5.70	0.69	0.22	43.10
Corn stalk		49.30	6.00	0.70	0.11	43.60

[1]—this value was obtained by calculation.

The ash content of biomass samples was measured in conjunction with elemental analysis, and the results are shown in Table 7. The ash content was estimated gravimetrically by calcining the biomass in a muffle cabinet at a temperature of 900 °C.

Table 7. The ash content assessment of macroalgae biomass.

Algae Species	Ash Content, % (wt.)
Furcellaria	7.80 ± 0.23
Polysiphonia	20.04 ± 0.60
Ulva	24.21 ± 0.72
Cladophora	28.30 ± 0.85

3.5. Studying of the Antioxidant Properties of Macroalgae

A solution of a known antioxidant, ascorbic acid, was used as a reference substance when evaluating the results of determining antioxidant properties. Table 8 shows the results of the antioxidant activity determination for algae extract samples.

Furcellaria samples had the highest antioxidant activity of all the macroalgae alcoholic extracts tested. The IC50, or concentration of half-maximal binding of the DPPH radical, for this genus of algae was 90.38 mg/mL. The IC50 value for 33 *Cladophora* macroalgae samples was 136.61 mg/m; for the species *Ulva* sp., the concentration of half-maximal binding of the DPPH radical was 314.74 mg/mL. The samples of macroalgae *Polysiphonia* had the lowest antioxidant activity as the IC50 value was 1069.21 mg/mL.

Table 8. Antioxidant activity indicators of algae alcohol extracts.

Algae Biomass Used	DPPH (IC_{50}), mg/mL	
	Whole Algae Biomass Alcohol Extract	Acetone Solution of Lipids
Cladophora	136.61 ± 4.09	0.0167 ± 0.0005
Polysiphonia	1069.21 ± 32.07	0.0394 ± 0.0011
Ulva sp.	314.74 ± 9.43	0.0026 ± 0.0001
Furcellaria	90.38 ± 2.71	0.0739 ± 0.0022
Ascorbic acid (control)	0.0084 ± 0.0002	

The alcohol extracts of the studied macroalgae species did not possess significant antioxidant properties. Further research into the selection of other types of solvents should be conducted using samples of the biomass of algae from the genus *Furcellaria*, which demonstrated to have the highest antioxidant activity among other types of algae (Table 8).

The antioxidant activity of the isolated lipids was also assessed by scavenging 2-2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. Lipids isolated from samples of macroalgae of the species *Ulva* sp. had the highest antioxidant activity: the IC50 for this genus of algae was 0.0026 mg/mL. The concentration of half-maximal DPPH radical binding in the lipid samples of *Cladophora* macroalgae was 0.0167 mg/mL. Lipids isolated from samples of macroalgae of the genus *Furcellaria* had the lowest ability to bind DPPH radicals, at 0.0739 (Table 8). However, the lipid content in samples of algae from the genus *Furcellaria* was 4.3%, compared to 2.3–2.8% percent in other types of algae, which compensated for the moderate antioxidant activity of fats and made the biomass of this algae species the most promising source of antioxidants.

It was discovered that extracts of isolated carbohydrates and proteins lacked 2-2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging antioxidant activity due to negative DPPH radical scavenging effect values.

4. Discussion

The composition of beach-cast algae samples collected in the Kaliningrad zone of the Baltic Sea coast revealed that drifting algae thalli accounted for an average of 95% of the total mass of the sample cleaned from sand in the vast majority of cases. The share of anthropogenic waste in casts did not exceed 2%. Anthropogenic waste was represented by polymers, textiles with a small proportion of metals and composite materials. The composition of the waste also contained broken glass and cigarette butts. Casts were significantly contaminated with natural mineral impurities (up to 39% of wet weight). This considerably reduces the variety of conceivable applications for biomass, as contaminants decrease the quality of the resulting polysaccharides, proteins, and lipids, making their use in the pharmaceutical, cosmetic, and food industries both practically and technologically problematic.

Most casts were local in nature and were observed along the northern coast of the Sambian Peninsula (from Cape Taran to Zelenogradsk), which is very convenient for resolving logistical issues associated with collecting and delivering biomass for processing.

The species composition was quite diverse; during the study, 14 species of drifting algae and sea grasses were found. Two species of green algae (*Cladophora* and *Ulva*) and two species of red algae (*Furcellaria lumbricalis* and *Polysiphonia fucoides*) were dominant. Following the seasonal dynamics of the species, the composition of algae casting was established. In the cold period (from September to the end of May), the red algae *Furcellaria lumbricalis* and *Polysiphonia fucoides* (up to 90–100%) prevailed; in the summer period, green algae of the genera *Cladophora* and *Ulva* prevail. Such species diversity requires the development of two technological cascade processing scenarios (summer and winter, with a change in regimes and the range of products).

An analysis of the fatty acid composition of algae biomass led to the conclusion that the most significant components include unsaturated fatty acids (linolenic acid) and their esters (geronyl izovalerate). Linolenic acid belongs to essential fatty acids and is of great interest in connection with its use in pharmacology and medicine as part of anti-inflammatory, immunostimulating agents, in dietology, and biologically active food supplements [67–69].

It has now been scientifically proven that products containing linolenic acid can be used for weight management, to control food intake, appetite and/or weight loss [68]. Foods and drugs containing linolenic acid can be used to normalize cardiovascular activity [69].

High molecular weight saturated fatty acids (methyl palmitate, methyl stearate, behenic acid methyl oleate, and tribehenoate glycerate) are not so valuable from the point of view of applications in cosmetology and pharmaceuticals. However, for example, methyl palmitate is successfully used as an emulsifier and flavoring agent in the food industry [70]. Methyl stearate is used in the cosmetic industry [71,72]. Tribehenoate glycerate is used in cosmetics and as an adjuvant in the manufacturing of drugs [73,74]. Behenic acid, the main component of rapeseed oil, is used for the production of fuels [75].

Given the relatively low total fat content of the considered algae, 2.3–4.3 percent per a.d.w., and the relatively low content of the most valuable unsaturated fatty acids (for example, linolenic has 0.03–0.08%), it is not recommended to use their biomass as a raw material for the extraction of commercial lipids for use in pharmacology and cosmetology. However, even a 2–4% content of saturated fatty acids may indicate the possibility of obtaining significant amounts of biodiesel during thermochemical or hydrothermal processing [51].

When the content of carbohydrates in algae biomass was compared to the literature data (25.8–55.4%), it was discovered that the content established in the course of the studies was significantly lower, possibly due to differences in the methods of determination and seasonal variability of biomass composition. In this study, the biomass sampled at the end of September 2021 was subjected to analysis. The maximum sugar content was found in algae of the genus *Furcellaria*, which correlates with known data [63]. These algae are widely used to obtain valuable polysaccharides [64].

The analysis of the carbohydrate composition of macroalgae revealed a high content of fucose (2.3–6.5%, Table 4). Fucose is another rare sugar that is currently used in anticarcinogenic and anti-inflammatory drugs, hepatoprotectors, creams for accelerated wound healing, and as moisturizing and anti-aging supplements [76,77]. It has been proven that the addition of fucose to infant formulas contributes to a more complete mental development and formation of immunity in infants [77,78].

Galactose, whose content in algae biomass varied from 1.2–2.5% (Table 4), has proven immunomodulatory [79–82], antioxidant [83], and antiviral [84–89] effects. Rhamnose is a valuable carbohydrate that is used in cosmetology for skin regeneration and the stimulation of collagen production [90]. However, its content was quite low in the algae biomass (0.3–1.2%).

Common sugars (glucose, xylose, and arabinose) do not have significant pharmaceutical potential, but are excellent raw materials for biotechnological processes—yeast cultivation, bioethanol production, etc. We can draw conclusions about the feasibility of using algal biomass for the extraction of marketable carbohydrates since there is a significant amount of readily available, soluble, and hydrolysable sugars in the biomass. The biomass of *Furcellaria* algae has a particularly high potential, containing up to 22% of a.d.m. reducing sugars. The high potential of using *Furcellaria lumbricalis* as a source of polysaccharides, including agar and furcelloran, is also confirmed by a number of publications [91].

Thus, a detailed analysis of the content of carbohydrates indicates the potential expediency of extracting commercial polysaccharides from the biomass of *Furcellaria lumbricalis* algae, as well as the possibility of using the biomass of the considered algae as a source of polysaccharides for the implementation of biotechnological processes for obtaining liquid (bioethanol) and gaseous fuels.

The elemental composition was analyzed to determine whether algae biomass might be converted to fuel using thermochemical methods. The algae biomass exhibited a high carbon content (31.3–37.5%), but a low hydrogen concentration (4.96–5.82%), which was

2.2 times lower than the content in coniferous wood, according to elemental analysis. A distinctive feature of the biomass of red algae (*Polysiphonia* and *Furcellaria*) was a high nitrogen content associated with the presence of a significant amount of proteins in the composition. For comparison, the nitrogen content in the other types of biomass under consideration does not exceed 0.7%, while its concentration in microalgae is 2.06–3.6%. In the application of hydrothermal liquefaction and biomass pyrolysis processes, a high nitrogen concentration can obviously become a difficulty on the route to high-quality liquid fuel (Table 6).

However, scientists are beginning to believe that a high protein content is not a problem, because polycondensation reactions in the process of hydrothermal liquefaction between carbohydrates and proteins, as described by the Maillard reaction, result in the formation of complex melonoidins, which increases the total biofuel yield [92]. Thus, the ratio of proteins:carbohydrates at the level of 3:1 is considered optimal [93–95]. In the case of macroalgae, this ratio is 1.18:1 for *Cladophora* algae, 1.41:1 for *Polysiphonia*, 1.24:1 for *Ulva* sp., and 1:1 *Furcellaria*. As a result, we may conclude that adding a protein supply to the process of hydrothermal liquefaction is a good idea in order to maximize the Maillard reaction's potential (sludge from the primary settling tanks of biological wastewater treatment plants, soybean production waste, etc.). The low content of fats and proteins in the biomass of algae allows to conclude that the yield of fuel during hydrothermal liquefaction will not be great. At the same time, a high content of carbohydrates and fiber will lead to the formation of a significant amount of carboxylic acids [92], which will lead to a decrease in pH and a shift in the reaction towards the formation of coal. To avoid this problem, it is necessary to provide for the introduction of alkaline additives into the reaction medium.

The sulfur content in the biomass of algae was 1.75–3.0%, which is on average 11 times higher than in the biomass of herbaceous land plants and 47 times higher than in wood. The maximum sulfur content was observed in samples of the algae *Furcellaria*—3.0%, which is obviously associated with a high proportion of protein compounds. The high sulfur content should also be considered when implementing the hydrothermal liquefaction process, through the preliminary extraction of proteins.

In general, algal biomass is ideal for thermochemical conversion to produce liquid fuels, but the necessity for co-processing with other types of biomass rich in proteins and/or fats, which will improve the total fuel production, should be considered. Without a doubt, the resulting fuel will have a high content of sulfur and nitrogenous compounds, which will require the development of conditioning technologies for the resulting fuel products. The biomass of red algae, particularly *Furcellaria* algae, has the highest total maximum resource potential in terms of obtaining liquid fuels by thermochemical methods, because it has a higher concentration of organic carbon with a moderate proportion of oxygen-containing compounds (the proportion of oxygen in biomass is the lowest of all species, analyzed macroalgae—50.03%) and the lowest ash content, at 7.8%. External sources can be used to compensate for the shortage of protein molecules required for polycondensation between carbohydrates and proteins.

A comprehensive analysis of the results of determining antioxidant properties revealed that lipids isolated from samples of macroalgae of the species *Ulva* sp. had the highest antioxidant activity. The ability to bind DPPH radicals in *Furcellaria* macroalgae samples is 28 times lower (0.0739). At the same time, the whole biomass of *Furcellaria* algae samples had a high antioxidant activity (the high fat content compensated for the moderate antioxidant activity of fats). The proteins and carbohydrates of the studied macroalgae samples did not have significant antioxidant properties.

5. Conclusions

The study determined the places of localization of beach cast algae and the dominant species of algae in their composition. An analysis of the degree of contamination with impurities and the physicochemical composition of dominant species allows us to conclude that the most promising direction for using their biomass is thermochemical conversion to produce liquid, solid, or gaseous fuels. The biomass of *Furcellaria* algae samples is of practical interest for achieving a high degree of purity to produce polysaccharides, including furcellaran. The high protein content of the algae samples (17.3–28.2%) shows that their extraction as a target product has potential, but further research is needed to determine their nomenclature and the impact of contaminants on the quality of the protein products obtained.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12073599/s1, Figure S1: HPLC data to lipid content analysis of *Polysiphonia* algae biomass; Figure S2: HPLC data to lipid content analysis of *Ulva* algae biomass; Figure S3: HPLC data to lipid content analysis of *Cladophora* algae biomass; Figure S4: HPLC data to lipid content analysis of *Furcellaria* algae biomass.

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