ORIGINAL PAPER

PRELIMINARY STUDY ON THE MARINE ALGAE FROM THE ROMANIAN BLACK SEA COAST

EMIN CADAR[1], ELENA ROXANA AXINTE[2], MANUEL AMZOIU[3], SANDA JURJA[4], MELAT CHERIM[5]

Manuscript received: 07.10.2019; Accepted paper: 02.12.2019;
Published online: 30.12.2019.

Abstract. *Black Sea seaweed is an important marine resource that can bring many benefits. To use algae for various purposes, preliminary studies are required to know their composition. Preliminary studies are presented in this paper to identify bioactive compounds which hold therapeutic interest. Three species of algae were analysed: green algae represented by Ulva lactuca, brown algae represented by Cystoseira barbata and red algae represented by Ceramium rubrum. Seaweeds were harvested from four harvesting stations located along the Romanian Black Sea coast in two consecutive seasons (autumn 2018 and spring 2019). Following the analysis of the physico-chemical results, sulfate compounds, carbohydrates and total dietary fibers were obtained in appreciable quantities. Comparisons were made between the average values obtained in the two seasons. It is found that in the spring season the percentage values for sulfate and carbohydrates contained in all the algae tested are lower than those obtained in the autumn season, which is due to the influence of environmental conditions, but also of the stage of aquatic plant development. The phyto-chemical analysis performed in the preliminary studies allowed the identification of compounds with important actions for therapeutic purposes. Preliminary studies on seaweed confirm that permanent monitoring of seaweed compositions makes it possible to collect clean algae from their natural environment and use them for bioactive compounds with applications in the medical, pharmaceutical, cosmetic or nutritional industries.*

Keywords: *Ulva lactuca, Cystoseira barbata, Ceramium rubrum, marine macroalgae.*

1. INTRODUCTION

Sustainability has become a broad term that can be found in almost every aspect of life on Earth, locally or globally and at different times. The world's marine ecosystem is warming and patterns in atmospheric variability are changing, resulting in changes in marine stratification, circulation patterns, sea ice and light supply to the sea surface [1]. Biological responses to these effects are visible but uncertain. The diversity and productivity of marine ecosystems are extremely important in preserving the health of the marine and terrestrial environment, and provides important sources of food for humans and animals, for the

[1] Ovidius University of Constanta, Faculty of Pharmacy, 900527 Constanta, Romania.
E-mail: emin.cadar@gmail.com.
[2] "Carol Davila" University of Medicine and Pharmacy, 050474 Bucharest, Romania,
[3] University of Medicine and Pharmacy, Faculty of Pharmacy, Craiova, Romania.
**Corresponding author*: m_amzoiu@yahoo.com.
[4] Ovidius University of Constanta, Faculty of Medicine, 900527 Constanta, Romania.
E-mail: jurjasanda@yahoo.com.
[5] Ovidius University of Constanta, Department of Chemistry and Chemical Engineering, 900527 Constanta, Romania. E-mail: melat_kem@yahoo.com.

pharmaceutical industry, medicine, as well as for additives in the food industry and cosmetics, etc. [2, 3]. The Black Sea marine resource has been harnessed by isolating bioactive substances from both fauna and marine flora and using them for therapeutic [4-7], or nutritional purposes [8, 9]. The superior valorification of the marine biomass represents a highly important resource for the pharmaceutical industry, supplying raw materials for the extraction of bioactive substances (vitamins, sterols, and collagen) and various other substances, such as agar-agar, the purity of which is strongly connected to the state of the marine ecosystem [10, 11]. The seaweeds are found in their habitat as an extremely rich and dense vegetation in the rocky south on the Romanian coast up to a depth of 3-4 m. Black Sea algal habitats have been studied and monitored over the last 30 years [12-14]. It is obvious that habitats with minimal pollution risk are sought, so that the seaweed is clean, free from infestation with various polluting agents [15-19]. Contamination with pollutants can be found in both fish species and marine flora of coastal waters or in major tributaries such as the Danube for the Black Sea. Pollution from the marine ecosystem is a problem that must be taken into account, it can affect human health [20-25] and the coastal area [26, 27] in multiple aspects. In recent years there are studies carried out on marine algae harvested from different areas of the Romanian coast: for green algae [28, 29], brown algae [30] and red algae [31], [32], in which it was evaluated the antioxidant and antimicrobial activity of seaweeds [33-35]. The methods used in the analysis of seaweed are moderate physico-chemical methods, which are also applicable to the analysis of terrestrial plant resources [35-55]. Due to different methods of extraction of different bioactive compounds from different species of algae, due to the lack information on the multitude of compounds in the composition of the different Black Sea algae in the literature, a direct comparison between our results and other studies in other waters, is not possible [55]. Regarding the seasonal variations of the bio-activity, for all the types of algae tested, accompanied by a great variance of temperature led to different results for the compositions of different species of algae. Therefore, there is a need for permanent monitoring every year and session to collect information on the changes of composition for seaweed on the Romanian Black Sea coast. The paper presents three types of algae harvested from the Black Sea from the Romanian coast with representatives of the majority species: green algae (*Ulva lactuca*), brown algae (*Cystoseira barbata*) and red algae (*Ceramium rubrum).* Seaweeds are considered indicators for pollution of marine waters [56]. In this work a series of analyzes are included to perform preliminary studies on selected algae from the Black Sea. The marine algae investigated were collected in the autumn 2018 and spring 2019 to monitor the quality of the Black Sea waters, with the results obtained from Mamaia to Mangalia being selected as examples.

2. MATERIALS AND METHODS

The samples taken into the study were seaweed samples with representatives of different species: green algae *Clorophites* (*Ulva lactuca*), brown algae *Pheophites* (*Cystoseira barbata*) and red algae Rodophites (*Ceramium rubrum*). The investigated physico-chemical indicators were obtained from the sample analysis taken in September and April from two stations located on the 5 m and 20 m isobaths of 4 areas. Seaweeds constituents were quantified by spectrophotometric analytical methods, internally validated in the laboratory where the quality system is maintained according to SR EN ISO / IEC 17025: 2005 and referred to in the Methods of Seawater Analysis Manual [55].

Plant materials - The samples were collected from 4 areas and named: S1 to S4: S1 - Mamaia Nord, S2 - Constanta Casino, S3 - Constanta Port, S4 - Mangalia. Samples were collected in the fall (September) 2018 and spring (April) 2019. Each collected sample was washed thoroughly with seawater to remove all external matter such as epiphytes, sand particles, pebbles and shells. In the laboratory, the sample was cleaned with fresh water and distilled water, then dried and powdered using the grinder. The seaweed powder was passed through a 0.5 mm sieve to obtain a uniform powder which was kept in the freezer until it was extracted.

Preparation of algal extract - The extraction process was done by soaking the algae powder in alcoholic solvent (96% ethanol), (1:10 w / v) for 24 hours. It was centrifuged at 2800 rpm for 10 min. The received materials were concentrated in a rotary evaporator to reduce the volume. The extracts received were filtered through Whatman No.1 paper, three times, until clear extracts were obtained and then collected in borosilicate glass containers. The samples were placed in the refrigerator for storage for future use.

Macroscopic and microscopic examinations - Macroscopic and microscopic examinations were performed on the algae selected for analysis. The macroscopic examination was realized, which represents the first stage in the investigation of the known or untested vegetable products. This was done through the examination of the whole plant (rhizoid, celluloid and phylloid), with the human eye, as well as with a magnifying glass, in order to observe its appearance, dimension, colour, taste and smell [12, 28, 33]. The microscopic exam of the collected algae was realized through the use of specific pharmacobotanical researches [12, 33].

Methods of physico-chemical and phytochemical analysis - For the global chemical analysis, the extraction of the active principles is very important [30, 37, 38, 40]. Qualitative chemical analysis involves the successive and selective analysis of plant products, using solvents with opposite polarities and separation by chemical methods, followed by specific reactions that help to identify the different groups of active principles or certain chemical constituents [11, 28, 29, 33]. From the plant sample, the extractions are performed with a non-polar solvent (petroleum ether), with a medium polarity solvent (ethyl alcohol) and only with water and the following fractions are obtained: 1-etheric extraction solution (A), 2-alcoholic extraction solution (B), 3-water-based extraction solution (C) [12, 35-37]. Each extract is analysed to identify the active principles of pharmaceutical interest. To identify the chemical compounds in the three extracts, they are analysed separately, using methods appropriate to the physical and chemical properties of each group of active principles [38-41]. In the preliminary study on algae, a series of physico-chemical determinations will be made to analyse the chemical composition of the algae. We present below the methods of analysis used [42-46].

Determination of humidity and ash - The humidity was determined by drying the sample at 105 °C in the thermoregulator oven. The calculation of the sample calcination was done at 550 ± 10 °C after 12 hours. The organic substance (S.O.) was calculated by the difference between 100% and the sum of the values (%) of humidity and ash [28, 43].

Determination of the sulfate content - The determination of the sulfate content was performed by quantitative chemical analysis. The analysis for the determination of sulfates was performed spectrophotometrically according to the national standard STAS 3069-87 [28, 33, 55].

Determination of protein and total nitrogen - The protein content and total nitrogen of algae was determined by the Kjeldahl method, using the UdK DK6 digestor equipment. Mineralization was done in the presence of sulfuric acid under the catalytic action of mercury and selenium. Alkalization was then performed, and the ammonia is steam driven and captured in a boric acid solution which was titrated with hydrochloric acid. The results in relation to the amount of algae powder used were expressed as a percentage [28, 39, 46].

Determination of lipids - Lipids from the samples were determined by Soxhlet extraction method for 5 hours using dichloromethane as solvent. The lipid content was determined gravimetrically. The results were expressed as a percentage of the amount of algae powder tested [39, 41, 43, 55].

Determination of carbohydrates - Carbohydrate extraction was performed in 15% acetic acid solution. the carbohydrate content was determined spectrophotometrically, by the Dubois method (1956), at a maximum absorption at 490 nm. An Aquamate 8000 UV-VIS spectrophotometer was used (uses selectable wavelengths of 190-1100 nm and spectral bandwidth of 1.8 nm). The results were calculated based on a standard glucose calibration curve [36, 37, 47].

Determination of total dietary fiber - The term "dietary fiber" was coined by Hipsley [48] to cover indigestible constituents of plants that make up the cell wall of plants, known to include cellulose, hemicellulose and lignin. The purpose was to define some properties of food constituents that could be related to physiological behavior in the human small intestine. This definition of dietary fiber which has been extended by Trowell et al. [56] became primarily a physiological definition, based on edibility and resistance to digestion in the human small intestine; the definition included indigestible polysaccharides such as gums, modified cellulose, mucilages and pectin and non-digestible oligosaccharides (NDO). The method that evolved was the official method AOAC 985.29 "Total dietary fiber in food and the enzymatic-gravimetric method" [57]. Subsequently, the method was expanded to allow total measurement of soluble and insoluble fiber in foods (AOAC official method 991.43) and various other modified fiber methods approved by AOAC International [58].

3. RESULTS AND DISCUSSION

In Fig. 1 are presented the three representative algae harvested for: *Chlorophytes* class *Pheophyte* class and *Rhodophyte* class.

Ulva lactuca
***Chlorophytes* class**

Cystoseira barbata
***Pheophyte* class**

Ceramium rubrum
***Rhodophyte* class**

Figure 1. The three representative algae from Black Sea.

3.1. MACROSCOPIC AND MICROSCOPIC ANALYSIS OF THE BLACK SEA ALGAE

***Ulva lactuca Ag. - syn. Ulva Rigida* (L.)** has a pale green to dark green thalle, with the aspect of an irregular leafy blade, sometimes with numerous breaks in the middle, with a relatively firm consistence, fixed on the substratum through a system formed of dark coloured rhizoids. It can reach dimensions of 5 to 30 cm, and sometimes more. Analysed on a macroscopical scale, the thalle is formed of two layers of cells, with a visible space between them, easily separable. The growth of the thalle is realized through cellular division, which always takes place perpendiculary on the surface of the thalle. The cells are placed in ordinate rows or randomly situated, rounded or long, and in this case they are 11-17 µm wide and 15-22 µm long. The cells have only one nucleus and a large cromatophor, located outside the cell, with a board aspect and 1-2 pirenoids. Between the cells of the thalle there are no plasmodesms. All cells are capable of breeding, as there are no breeding formations. They frequently present fine prolonged formations, especially on the lower side of the blade, visible with a magnifying glass. During the development circle, the gametophyte and the sporophyte are isomorphes, and the ganetophyte is dioic. *Ulva lactuca* vegetates throughout the whole year, with its peak in winter-spring, at low depths, close to the surface of the thalle [18, 28, 29].

***Cystoseira Barbata* (Good Et Wood) Ag.** is a brown monoic alga, with a thalle with various ramifications, fixed on rocks through a rhizoid as a disclike clip bolt. From this clip bolt stem various cilindric sticks, on which a large number of primary and secondary branches develop, cilindric or flat, along which chains of air-bearing vesicules, which help floating, grow. On the tip of the branches the receptacles develop (reproduction organs), shaped as a cilinder or as a conic cilinder. The receptacles shelter the conceptacles, on the bottom of which various eggs are found, each with a single eggsphere and a pedicel cell. On top, towards the opening of the conceptacle, uniocular anterides, each with 64 biflagellate anteroids. The eggspheres, eliminated into sea water, merge with the anteroids, and the resulting egg forms a new plant. *Cystoseira barbata* is a large brown alga, 1.5 – 2 m, which grows in the Black Sea on a rocky substratum, as multiannual associations [18, 28, 30].

***Ceramium rubrum* (Huds). Ag.** is a red algae with a philament like thalle, with ramifications, having a bushy aspect, fixed on the substratum through the rhizoid. The filaments have dichotomic ramifications, and are formed of a single row of connecting cells, which give it an articulate aspect. In the nodal area small cells form, called periaxial, which continuously divide and produce cortical cells, which cover the whole surface of the thalle. Each filament ends with two short twisted branches. In the transversal section of the thalle, in the centre there is a large cell, surrounded by 8 pericentral cells, which are also surrounded by another layer of cortical cells. In the central cell there are large strip-shaped plastides. Ceramium rubrum (Fig.1) has a trigenetic development circle, as most of red algae. On the Romanian coast, this annual red alga abundantly develops along the entire coastal area, on rocks, at depths of 0.5–4.5 m, all year long, but mainly in spring and summer [18, 28, 31, 32].

3.2. RESULTS FOR PHYTO-CHEMICAL STUDIES

As a follow up of the identification reactions previously discussed, the following results were obtained, summarized in Table 1 [7-11, 15].

Table 1. The selective reactions used in chemical analysis [18].

Analyzed solution	**Reactions used**	**Identified active principles**
Etheric extracts	Lieberman-Burchard	Steroles and triterpenes
	Fluorescent UV. (λ = 365 nm	Cumarines
Alcoholic extracts	Iron Chlorure reaction	Catehic Tanin
	Fehling	Reducing compounds
	Liebermann Bourchard	Triterpenic heterozides
	Borntrager	Antracenozide
	UV (λ = 365 nm)	Cumarines
Water based extracts	Fehling	Reducing compounds
	H_2SO_4 conc. + tymol	Ozes and poliozes
	Foaming	Soapozides
	$FeCl_3$	Catehic tanin

In etheric solution (A) (Table 2):

- a fraction of the etheric extract was evaporated up to dryness; the residuum obtained was dissolved in alcohol - the resulting solution does not have the specific smell of volatile oils for any of the algae species;

- the residuum obtained from the evaporation of the etheric extract was processed with HCl 2%; the solution undertook the Mayer and Bertrand reactions; the reactions were negative; the analysed algae does not contain basic alcaloids;
- the residuum obtained from the evaporation of the etheric extract was processed with methilic alcohol; the alcoholic solution undertook the Shibata reactions; the reaction was negative for all algae species, which do not contain flavonoic aglicones;
- the residuum obtained from the evaporation of the etheric extract was processed with amonium hydroxyde; the Borntrager reactive did not color the solution orange; the analysed algae does not contain endemoles;
- the Lieberman - Burchard reaction on the etheric extract was positive, indicating the presence of steroles and triterpenes in all analysed algae;
- the Carr – Price reaction was positive, thus they contain carotenoids, for the species Ulva rigida, and negative for the other species;
- the residuum obtained from the evaporation of the etheric extract was processed with amonium hydroxyde; the solution did not present an intense fluorescence on UV radiation, thus no species contains cumarines.

Table 2. The active principles traced as a follow up of chemical analyses on the etheric extracts

The seaweed species	Steroles and triterpenes	Cumarines
Ulva lactuca	++	++
Cystoseira barbata	++	-
Ceramium rubrum	++	-

In non-hydrolized alcoholic solution (B) (Table 3):

- the reaction with the Styassny was negative;
- the reaction with iron chlorure is dark green, thus positive form catehic tanin for the *Ulva rigida, Cystoseira barbata* species, and negative for *Ceramium rubrum*.
- In hydrolyzed alcoholic solution (B):
- through the Fehling reaction, a brick-red precipitate was obtained for all species. Reducing compounds are present in all species;
- the reaction with ninhydrine of the water-based solution obtained from the residuum of alcoholic solution was positive for *Ulva lactuca*, which contain aminoacids, and negative for *Cystoseira barbata, Ceramium rubrum:*
- the residuum obtained through the evaporation of the non-hydrolized alcoholic solution is processed with a water based solution of HCl 2%, then turned alcaline with amonium hydroxyde and extracted with ether; after the evaporation of the etheric solution and the recuperation of the HCl 2% residuum, the Mayer and Bertrand reactions are done; the reactions were negative for all species. The analysed specie does not contain salt alcaloids;
- the residuum obtained after the evaporation of the alcoholic hydrolized solution is processed with 50% methilic alcohol; the Shibata reaction on alcoholic solution was negative for all the species; none of the algae analysed contains flavonozides;
- the Liebermann – Bourchard reaction on the residuum of the alcoholic hydrolized solution caused a green-violet coloration, thus it signalled the presence of triterpenic heterozides in *Ulva species*;
- the Borntrager reaction was negative for all species. Antracenozides lack in all species;
- the acid solution is dark brown and does not indicate the presence of antocianozides in any of the species;
- the solution becomes fluorescent under UV radiations, thus cumarines are present in *Ulva* species.

Table 3. The active principles traced as a follow up of chemical analyses on the alcoholic extracts

The seaweed species	Catehic Tanin	Reducing compounds	Triterpenic heterozides	Antracenozides	Cumarines
Ulva lactuca	++	++	++	++	++
Cystoseira barbata	++	++	-	-	-
Ceramium rubrum	++	++	-	-	-

In the water based extractive solution (C) (Table 4):

- the reaction for the identification of starch was positive for *Ulva species*;
- it was obtained a flaky precipitate – poliuronides present in *Ulva lactuca*;
- the Fehling reaction was positive for all the species analysed – reducing compounds are found in all analysed species;
- the water based extractive solution evaporates into residuum, adding a few drops of concentrate sulphuric acid and tymol alcoholic solution – there results a red coloration, which demonstrates the presence of oozes and polioozes in all analysed species;
- the foaming reaction of soapozides was negative; soapozides lack in all analysed species;
- the water based extractive solution reacts with diluted $FeCl_2$ and a dark green coloration emerges, which confirms the presence of catehic tanin in all species, except *C. rubrum*;
- the Mayer and Bertrand reactions were negative – basic alcaloids lack in all analysed species.

Table 4. The active principles traced as a follow up of chemical analyses on the water-based extracts

The seaweed species	Reducing compounds	Ozes and poliozes	Soapozides	Catehic tanin
Ulva lactuca	+	+	++	+
Cystoseira barbata	+	+	-	+
Ceramium rubrum	+	+	-	+

It can make a summary and centralize in Table 5 bioactive compounds from seaweed composition and highlight the functions of these compounds and their role in therapeutic applications.

Table 5. Bioactive compounds in the composition of marine algels

Compound	Function	Application
Polysaccharides	Components from the cell wall (fucoidan, alginates, laminarin).	Provide strength, flexibility, maintain ionic balance, and prevent dehydration. Provide strength, flexibility, maintain ionic balance, prevent dehydration
Phenols and florotanines	Phenol rings in polyphenols they act as electronic traps for the destruction of radicals.	Antimicrobial compounds, antioxidants, antiviral care protect algae under abiotic and biotic stress, for example, florotanins are composed of structures of oligomers and floroglucinol.
Proteins, peptides and fatty acids and essential amino acids	Polyunsaturated fatty acids (PUFA) (ω-3 and ω-6)- higher level than terrestrial plants.	Antioxidants, but they are difficult to extract. Structural membrane lipids are important in the diet of humans and animals. Composed of glycerol, sugars, bases esterified with fatty acids (saturated or unsaturated C12-C22)
Terpenoid and sterols	Carotenoid, xanthophyl, fucoxanthin, astaxanthin.	Antioxidant activity, antiviral, anti-inflammatory, UV protection.
Minerals	Se, Zn, Mn, Cu - structural components of enzymes. Antioxidant.	
Vitamins	A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid. Pantothenic. Seasonally dependent level.	

3.3. RESULTS FOR PHYSICO-CHEMICAL STUDIES

The network of stations remained constant until 2019, with sampling points along the coast from S1 Mamaia to S 4 Mangalia in the autumn and spring season. From the analysis of the results, as in previous years, dominated the photophilic association of species of *Ulva* enriched with elements of the genus *Ceramium* (*Ceramium rubrum and Ceramium virgatum*) during the monitored autumn and spring periods. Similar to the previous years, in the autumn of 2018, the dominant species from a quantitative point of view was *Ulva* sp. *Ceramium rubrum* and *Ceramium virgatum* were representatives of *Rhodophyta* species 2018 and 2019, with a notable development, but biomass has been reduced compared to previous years [58]. In the specialty literature, Abdul Khalil [59], studyed the properties of algae proposes an orientation composition for seaweed, shown in Table 5

Table 6. Composite Chemistry of algels [59].

Components	**Compositions**
Water	80-90%
Carbohydrates	50% d.w.
Proteins	3–15% d.w. (brown algae) / 10–47% d.w. (ed or green algae)
Minerals	7–38% d.w.
Lipids	1–3% d.w.

The results obtained from the analysis of the algae harvested from the Black Sea reported as average values for the autumn-spring seasons are presented in Table 6.

Table 7. Chemical Composition of algae from Romanian Black Sea coast.

Parameters*	***Ulva lactuca***	***Cystoseira barbata***	***Ceramium sp.***	**Literature data values/References**
Moisture [%]	10.86±1.01	12.27 ± 0.42	11.01 ± 0.13	12.60 – 18.5[59]
Ash (%)	16.38±3.08	18.63 ± 1.73	15.83 ± 1.68	12.4– 29.9 [60]
Sulphates [%]	74.336± 1.66	71.79± 1.93	75.98 ± 1.94	65.3-70.5 [61]
Total nitrogen [%]	1.932±0.20	2.826 ± 0.34	2.19 ± 0.41	-
Protein [%]	16.58±1.30	18.13 ± 2.11	15.94 ± 2.56	8-17 [59]
Lipid [%]	1.71±0.06	1.63 ± 0.54	2.43 ± 0.25	1.5-3.6 [59]
Carbohydrate %)	55.19 ±1.41	61.95 ±1.06	67.697 ±1.05	59.10 - 61.50 [61]
Total dietary fibre [%]	59.05±2.05	61.075±1.66	57.455±1.7	50.3-60.50 [61]
Insoluble fibre [%]	20.45±0.56	30.62±1.26	35.81±2.65	24.2-32.6 [61]
Soluble fibre [%]	38.6±1.89	30.45±1.33	28.355±1.96	27.20-30.5 [61]

**All values show mean of three replicates, ± standard deviation*

However, it is necessary to analyze the results for each species of green, brown and red algae in each S1-S4 harvesting station for each season (autumn 2018 and spring 2019). In Figs. 2, 4 and 6 presented the average values obtained in the four harvesting stations S1, S2, S3 and S4, for the concentration of sulphated compounds in the two tested seasons (autumn and spring). From the analysis of the results it is found that all the algae recorded increased values in autumn 2018 compared to spring 2019. From the analysis of the algae compositions harvested on sampling stations the highest values for sulfated compounds are presented by the algae harvested from the S4 station (Mangalia), followed by S3 (Constanta Port), S1 (Mamaia Nord) and S2 (Constanta Casino). The highest values are the *Ceramium* species (78.5% in autumn 2018 in area S4) followed by the species *Ulva* (78.95% in autumn 2018 in Station S4) and *Cystoseira barbata* (72.3% in Station S4). In Figs. 3, 5 and 7 shows the average values obtained in the four harvesting stations S1, S2, S3 and S4 for the two seasons of autumn 2018 and spring 2019. The highest values are presented by *Ceramium rubrum* (68.9% in autumn 2018 at the S1 station - Mamaia Nord), followed by *Cystoseira barbata* (63.4% in autumn

2018 at station S4 - Mangalia), and *Ulva lactuca* (56.95% in autumn 2018 at station S1 - Mamaia Nord).

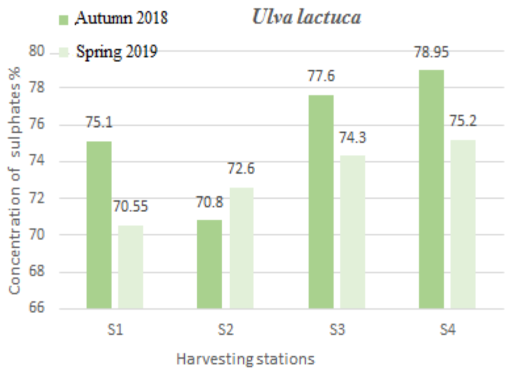

Figure 2. Variation of average values of sulphates for *Ulva species in* autumn 2018 and spring 2019.

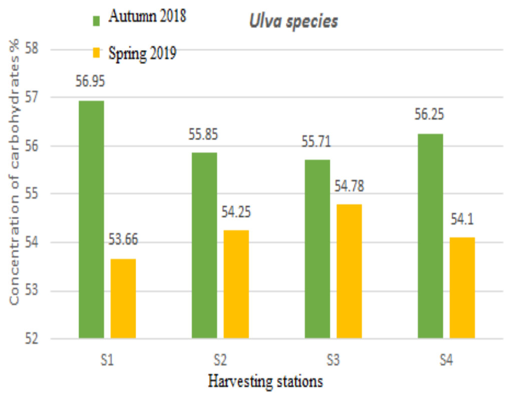

Figure 3. Variation of average values of carbohydrates for *Ulva species in* autumn 2018 and spring 2019.

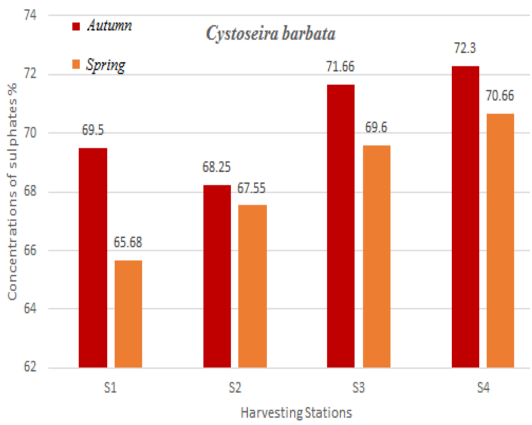

Figure 4. Variation of average values of sulphates for *Cystoseira species* in autumn 2018 and spring 2019.

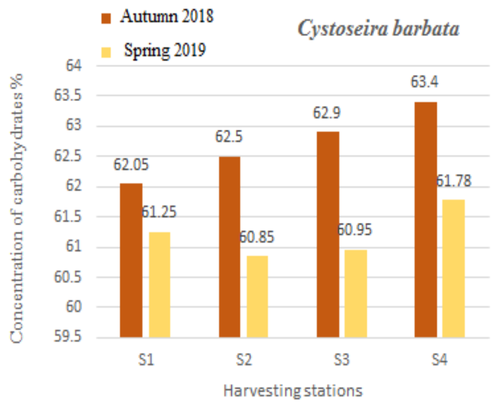

Figure 5. Variation of average values of carbohydrates for *Cystoseira barbata in* autumn 2018 and spring 2019.

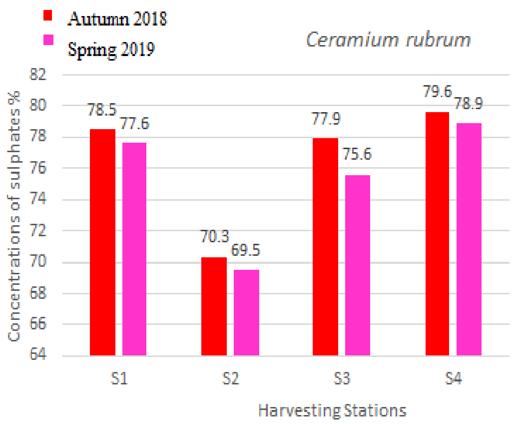

Figure 6. Variation of average values of sulphates for *Ceramium* species in autumn 2018 and spring 2019.

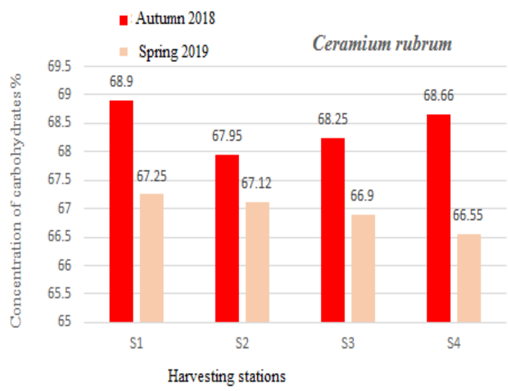

Figure 7. Variation of average values of carbohydrates for *Ceramium rubrum in* autumn 2018 and spring 2019.

In Fig. 8 Total dietary fibers [%] are presented in seaweeds for the seasons of autumn 2018 and spring 2019 for the three species of algae existing on the Romanian Black Sea coast. It is found that the highest percentages were presented by all algae in the spring season 2019.

Brown algae *Cystoseira barbata* has the highest percentage of total dietary fibers (62.65%) followed by green algae *Ulva lactuca* (60.5%) and red algae *Ceramium rubrum* (56.66%).

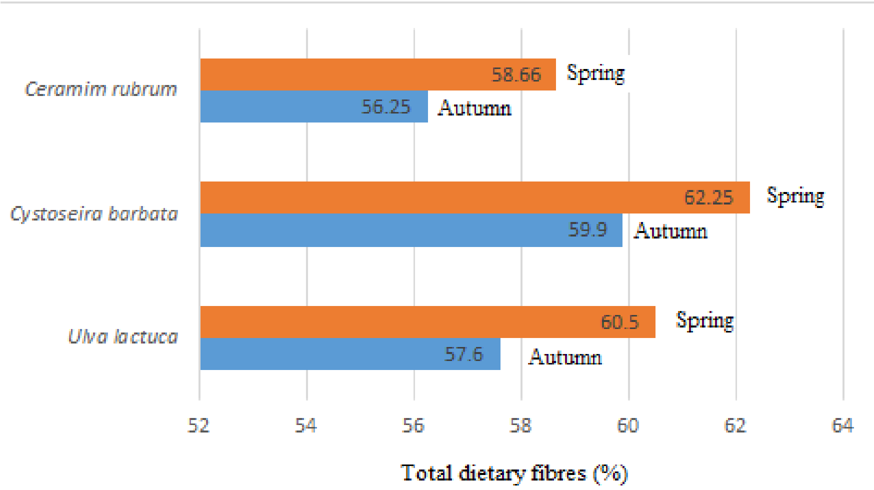

Figure 8. Total dietary fibres [%] in seaweeds from Black Sea.

4. CONCLUSIONS

The primary studies carried out on the seaweed harvested from four sampling stations along the romanian black seacoast are supported by the specialized literature that shows that the seaweed represent natural resources for further exploitation in other areas. From the phytochemical studies on the green, brown and red algae harvested, with the usual phytochemical methods it was identified the categories of compounds of therapeutic interest.

From the physico-chemical analyses carried out on the algae species, sulfate compounds, carbohydrates and total dietary fiber were shown in appreciable percentages, they were analysed on algae harvested in two consecutive seasons (autumn 2018 and spring 2019). There are different percentages for the two seasons and in the four different sampling stations. In spring 2019, the average values are lower compared to autumn 2018. This result is due to the influence of environmental factors, seasonal temperature and algal development stage.

The marine ecosystem along the Romanian coast of the black sea requires a permanent monitoring of the special marine conservation areas in accordance with the requirements of the legislative system, in line with the framework created by EC. Permanent monitoring of seaweed compositions makes it possible to collect clean algae from their natural environment to be used for extracting bioactive compounds with applications in the medical, pharmaceutical, cosmetic or nutritional industry.

REFERENCES

[1] Sands, P., *Principles of International Environmental Law*, 2nd Ed., Cambridge University Press, 2003.
[2] Sirbu, R., Zaharia, T., Bechir, A.M., Lilios, G., Nicolaev, S., Roncea, F.N, *Journal of Environmental Protection and Ecology*, **13**(3A), 1842, 2012.
[3] Sirbu, R., Mustafa, A., Tomescu, A., Stanciu, G., Cadar, E., *Materiale Plastice*, **56**(1), 148, 2019.
[4] Cherim, M., Sirbu, R., Tomescu, A., Popa, M.F., Cadar, E., *Materiale Plastice*, **56**(1), 179, 2019.

[5] Sirbu, R., Stanciu, G., Cadar, E., Tomescu, A., Cherim, M., *Revista de Chimie*, **70**(3), 835, 2019.
[6] Sirbu, R., Zaharia, T., Maximov, V., Bechir, A., Maris, M., Negreanu-Pirjol, B., Maris, D.A., Negreanu-Pirjol, T., Leca, M., Cadar, E., Stoicescu, R.M., Mocanu, L., Jurja, S, *Journal of Environmental Protection and Ecology*, **11**(2), 654, 2010,
[7] Bechir, A.M., Sirbu, R., Pacurar, M., Podariu, A.C.; Monea, M., Bechir, E.S., Ghergic, D.L., *Revista de Chimie*, **65**(3), 362, 2014.
[8] Sirbu, R., Bechir, A., Negreanu Pirjol, T., Sava, C., Negreanu Pirjol, B., Zaharia, T. Ursache, C., Stoicescu, R.M., *Scientific Study and Research-Chemistry and Chemical Engineering Biotechnology Food Industry*, **12**(3), 221, 2011.
[9] Sirbu, R., Ursache, C., Zaharia, T., Nicolaev, S., Negreanu-Pirjol, T., Negreanu-Pirjol, B., Stoicescu, R. M., *Journal of Environmental Protection and Ecology*, **13**(3A), 1865, 2012.
[10] Sirbu, R., Zaharia, T., Pirjol, B.S. N., Nicolaev, S., Bologa, A., Psegalinschi, I., *Journal of Environmental Protection and Ecology*, **11**(4), 1336, 2010.
[11] Sirbu, R., Zaharia, T., Nicolaev, S., Bologa, A., *2007 OCEANS Conference Proceedings*, **1-5**, 137, 2007.
[12] Sirbu, R., Zaharia, T., Bechir, A., Lilios, G., Nicolaev, S, *Journal of Environmental Protection and Ecology*, **13**(1), 190, 2012.
[13] Zaharia, T., Sirbu, R., Nicolaev, S., Micu, D., *2007 OCEANS Conference Proceedings*, **1-5**, 147, 2007.
[14] Lepadatu, C., Nitulescu (Amzoiu), E., *Acta Chimica Slovenica,* **50**, 539, 2003.
[15] Cadar E., Sirbu, R., Negreanu Pirjol, B.S., Ionescu, A.M., Negreanu Pirjol, T., *Revista de Chimie*, **70**(8), 3065, 2019.
[16] Cadar, E., Mustafa, A., Tomescu, A., Cherim, M., *Journal of Science and Arts,* **42**(1), 255, 2018.
[17] Albota, F., Caira, M. R.; Draghici, C., Dumitrascu, F., Dumitrescu, D.E., *Beilstein Journal of Organic Chemistry*, **12**, 2503, 2016.
[18] Cadar, E., Tomescu, A., Negreanu-Pirjol, B.S., *Journal of Science and Arts*, **3**, 533, 2017.
[19] Dumitrascu, F., Draghici, C., Caproiu, M.T., Dumitrescu D., Badoiu A., *Revue Roumaine de Chimie*, **51**(7-8), 643, 2006.
[20] Tomescu, A., Mocanu, L., Brezeanu, D., Stanciu, G., Sirbu, R., Tomescu, C., *Revista de Chimie*, **70**(5), 1731, 2019.
[21] Popa, M.M., Georgescu, E., Draghici, C., Georgescu, F., Dumitrascu, F., Dumitrescu D., *Monatshefte fur Chemie*, **146**(12), 2029, 2015.
[22] Tomescu, A., Sirbu, R., Stanciu, G., Tomescu, C.L., *Journal of Environmental Protection and Ecology,* **15**(1), 303, 2014.
[23] Cristea, O.M., Amzoiu, M., Ene, C., Amzoiu, E., Popescu, F., Popescu, F.D., Radu, L., Sirbu, R., *Journal of Science and Arts,* **2**(47), 425, 2019.
[24] Paris, S., Miresan, H., Margaritti, D., Stanciu, G., Eremia, C.L., Doicescu, D., *Journal of Environmental Protection and Ecology*, **15**(1), 61, 2014.
[25] Tomescu, A., Tomescu, C., Aschie, M., Stanciu, G., Sirbu, R., Tica, V., Brezeanu, D., *Revista de Chimie*, **70**(7), 2681, 2019.
[26] Negreanu-Pirjol, B., Negreanu-Pirjol, T., Tofan, L., Sirbu, R., Sava, C., Meghea, A, *Journal of Environmental Protection and Ecology*, **12**(4A), 2205, 2011.
[27] Sirbu, R., Negreanu-Pirjol, T., Sirbu, M., Cadar, E.M., *Journal of Environmental Protection and Ecology*, **7**(1), 59, 2006.
[28] Negreanu-Pirjol, B.S, Negreanu-Pirjol, T., Paraschiv, G., Bratu, M., Sirbu, R., Roncea, F., Meghea, A., *Scientific Study and Research-Chemistry and Chemical Engineering Biotechnology Food Industry*, **12**(2), 173, 2011.
[29] Sirbu, R., Sava, C., Ghergic, D.L., Passy, M.N.A.F., *Scientific Study and Research-Chemistry and Chemical Engineering Biotechnology Food Industry,* **7**(1), 193, 2006.
[30] Cadar, E., Sirbu, R., Ibram, A., Ionescu, A.M., *Revista de Chimie*, **70**(7), 2684, 2019.
[31] Sava, C., Sirbu, R., Dumitrescu, C., *Scientific Study and Research-Chemistry and Chemical Engineering Biotechnology Food Industry,* **7**(4), 785, 2006.

[32] Sava, C., Sirbu, R., Leon, A., *Journal of Environmental Protection and Ecology,* **13**(1), 289, 2012.

[33] Negreanu-Pirjol, T, Negreanu-Pirjol, B., Sirbu, R., Paraschiv, G.M., Meghea, A., *Journal of Environmental Protection and Ecology,* **13**(3A), 1744, 2012.

[34] Sirbu, R., Stanciu, G., Tomescu, A., Ionescu, A. M., Cadar, E., *Revista de Chimie,* **70**(4), 1197, 2019.

[35] Sirbu, R., Bechir, A., Negreanu-Pirjol, T., Sava, C., Negreanu-Pirjol, B., Zaharia, T., Ursache, C., Stoicescu, R., *Scientific Study and Research-Chemistry and Chemical Engineering Biotechnology Food Industry* **12**(3), 221, 2011.

[36] Stoicescu, I., Popescu, A., Sirbu, R., Rosca, C., Doicescu, D.N., Bendic, V., Bala, C., *Revista de Chimie*, **63**(9), 865, 2012.

[37] Stoicescu, I., Popescu, A., Sirbu, R., Bala, C., *Analytical Letters*, **45**(17), 2519, 2012.

[38] Bintintan, A., Gligor, M., Dulama, I.D., Teodorescu, S., Stirbescu, R.M., Radulescu C., *Revista de Chimie,* **68**(4), 847, 2017.

[39] Buruleanu, L.C., Radulescu, C., Georgescu, A.A., Danet, F.A., Olteanu, R.L., Nicolescu, C.M., Dulama, I.D., *Analytical Letters,* **51**(7), 1039, 2018.

[40] Radulescu, C., Stihi, C., Ilie, M., Lazurcă, D., Gruia, R., Olaru, O., T., Bute, C., O., Dulama, I., D., Stirbescu, R., M., Teodorescu, S., Florescu, M., *Analytical Letters*, **50**(17), 2839, 2017.

[41] Buruleanu, L.C., Radulescu, C., Georgescu, A.A., Dulama, I.D., Nicolescu, C.M., Olteanu, R.L., Stanescu, S.G., *Analytical Letters*, **52**(8), 1195, 2019.

[42] Radulescu, C., Olteanu, R.L., Stihi, C., Florescu, M., Lazurca, D., Dulama, I.D., Stirbescu, R.M., Teodorescu, S., *Analytical Letters*, **52**(15), 2393, 2019.

[43] Nicolescu, C.M., Bumbac, M., Olteanu, R.L., Alecu (Holban), G.C., Boboaca-Mihaescu, D.N., Necula, C., Radulescu, C., *Journal of Science and Arts*, **1**(46), 201, 2019.

[44] State, G., Popescu, I.V., Radulescu, C., Macris, C., Stihi, C., Gheboianu, A., Dulama, I.D., Nitescu, O., *Bulletin of Environmental Contamination and Toxicology*, **89**(3), 580, 2012.

[45] State, G., Popescu, I.V, Gheboianu, A., Radulescu, C., Dulama, I., Bancuta, I., Stirbescu, R., *Romanian Journal of Physics,* **56**(1-2), 240, 2011.

[46] Sirbu, R., *Thermochimica Acta*, **352**, 1, 2000.

[47] Stanciu, G, Lupsor, S., Tomescu, A., Sirbu, R., *Revista de Chimie,* **70**(2), 373, 2019.

[48] Amzoiu, E., Amzoiu, M., Munteanu, M., Popescu, S., Cristea, O., Sirbu, R., *Journal of Science and Arts,* **1**(46), 177, 2019.

[49] Stanciu, G., Aonofriesei, F., Lupsor, S., Popescu, A., Sirbu, R., *Revista de Chimie,* **70**(5), 1800, 2019.

[50] Amzoiu, E., Amzoiu, M., Sirbu, R., Stanciu, G., Erand, M., Sandulovici, R., *Journal of Science and Arts,* **1**(42), 191, 2018.

[51] Stanciu, G., Lupsor, S., Sirbu, R., *Journal of Science and Arts*, **1**(38), 141, 2017.

[52] Mircioiu, I., Anuta, V., Stanciu, G., Sirbu, R., Sandulovici, R., *Journal of Science and Arts,* **1**(42), 203, 2018.

[53] Stoicescu, I., Sirbu, R., Negreanu-Pirjol, T., Cociasu, M., Balaban, D.P., Bala, C, *Revue Roumaine de Chimie*, **57**(7-8), 729, 2012.

[54] Oancea, I.A., Stanciu, T., Barbu, A., Vlad, A., *Journal of Science and Arts*, **1**(38), 113, 2017.

[55] Grasshoff, K., Ehrhardt, M., Kremling, K. (Eds.), *Methods of seawater analysis,* 1983.

[56] McCleary, B.V., Sloane, N., Draga, A., *Starch,* **67**, 860, 2015.

[57] *** *Official Methods of Analysis of AOAC International,* Methods 925.10, 985.29, 991.42, 991.43, 993.19, 994.13, 996.01, 2001.03, 2002.01, 2002.02, 2009.01, 2011.25, *19th Edition., AOAC International, Rockville, Maryland* 2012.

[58] Bologa, A.S., Sava, D., *Research Marine,* **36**, 5, 2006.

[59] Abdul Khalil, H.P.S., Ying Ying, T., Su Ting, C., Chaturbhuj, K.S., Paridah, M.T., Rudi, D., Mahummad, Izzuddin, S., *Bioresource,* **12**(1), 29, 2017.

[60] Rohani-Ghadikolaei, K., Abdulalian, E., Ng, W.K., *Journal of Food Science and Technology,* **49**, 774, 2012.

[61] Yaich, H., Garna, H., Bchir, B., Besbes, S., Paquot, M., Richel, A., Blecker, C., Attia, H., *Algal Research* **9**, 65, 2015.