ORIGINAL PAPER

STUDIES ON THE IDENTIFICATION OF BIOACTIVE COMPOUNDS IN ALGAE BIOMASS IN THE BLACK SEA WITH MAJOR THERAPEUTIC ACTIONS

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Abstract. *Seaweed are known and used from the most ancient times in food, in medicine and soil fertilization. Today the researchers from all over the world seek to solve the problems related to the exploration and exploitation of this precious marine flora. The studies regarding the chemistry and the biology of the kelp, or those focused on optimizing the processes of existing technology for recouping actually represents the measure the value for the use of these plants in the various fields of activity. The work presents the data on the distribution of algae biomass from new sources. Useful information on the composition in bioactive compounds in this algae biomass is highlighted. The chemical composition of the kelp is varied: terpenele, polysaccharides; alacaloids, halogenated compounds, phenolic compounds, vitamins, minerals.*
Keywords: *Ulva, Cladophora, Ceramium, algae biomass.*

1. INTRODUCTION

The Black Sea is one of the biggest semi-closed seas in the world with an area of 432.000 km^2, maxim depth of 2212 m and with the total volume of water at 547.000 km^3. The total length of the coastal area of the Black Sea is 4340 km and it belongs to 6 different countries Bulgaria (300 km), Georgia (310 km), România (225 km), Russia (475 km), Turkey (1400 km), Ukraine (1628 km) [1]. The Black Sea is vulnerable to different actions and pollution sources from the nearby grounds. Until the 1960's the Black Sea was known to be one of the richest seas with a luxuriant fauna containing many red algae from the Phylophora species, ideal places of food for different types of fishes emanant from the Mediteranean Sea. The Black Sea was widely mentioned as an example of eutrophicated ecosystem as a result of the permanent aflux of nutrients [2].

The process of eutrophication was defined by the European Comission in 2002 as a complex process with appears both in fresh water as in deep water when some types of algae (blue algae) are growing excessively and become a threat to both animal and human health. The prime cause for eutrophication is the high level concentration of nutrients. Today the term is used correlated to keeping a fine water status and conserving habitats [3]. Because of its effects eutophication became the most important quality problem nowadays for the marine ecosystems [5].

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Seaweed are known and utilised from the most ancient times in food, medicine and the soil ferilization. Since the growth of eutrophication important qualitative changes were observed in the structure and functionality of the macrophytobenthos starting from the oldest records to the newest ones. Because of the high quantity of suspended particles and plankton the transperancy of the sea lowered considerably. The position of the compensation point was modified so the flora which grew at lenghts bigger than 7-8 m became shadowed. This has been instrumental to the decline of macrophytes even with high concentrations of nutrients.The consequence of these changes is the change in multiannual and season dynamic of the algae community. The interaction between different antropogenic factors created different results from simplifying the structure to total disappearance. Today researchers from all over the world are looking to solve problems linked with exploration and exploitation of the marine biomass through different chemical and biological studies [6, 7]. There have been researches based on evolving biotechnology processes in order to maximize the exploitation of the marine biomass. The paper presents data on algae which present therapeutic actions for the pharmaceutical area [8-10].

2. MATERIALS AND METHODS

2.1. MATERIALS

The characteristics of the coastal waters were determined according to the framework directive. The sediment samples were collected by autonomous divers, from profiles situated in characteristic area on the 5 m, 10 m and 15 m isobathes, and was represented by sediments, analyzed from a chemical, biochemical and biological point of view. The following species stand out: *Cladophora vagabunda (L.), Enteromorpha intestinalis (L.),* and Species *Ulva Lactuca* (*Ulvae rigida*). From the red and brown algae we have *Ceramium rubrum* and *Cystoseira barbata*. From the green algae of the Black Sea, the systemic clasification of the Class *Chlorophyceae* was presented [7, 10, 11].

2.2. METHODS

The salinity has been measured in-situ with CTD. The dissolved oxygen was measured using the Winkler method, the pH using the potentiometric method and the nutrients from the water have been cuantified through spectrophotometric methods validated internally in the laboratory and having as reference the „Methods of Seawater Analysis" manual. The detection limits and the relative extented uncertainties, k = 2 , the coverage factor 95.45%. For measurements the UV-VIS Shimadzu spectrophotometer was used with the interval between 0-1000 nm.

The methods are from botanical phytochemical and physico-chemical area. The investigation of the coastal sector, especially between Vadu and Vama Veche (the area with the highest density of macrophyte algae), for the characterization of the coastal waters quality, throughout the year 2011-2012, was made through sample collecting from the integrated monitoring network for tranzition and coastal waters samples that were processed in laboratories, according to the international methodology, and interpreted by specialists.

Their processing was made in the laboratories belonging to the "Grigore Antipa" National Institute for Marine Research and Development, Constanta, approved by RENAR [8, 10]. The phytochemical studies were carried out in the laboratories of the Pharmacy Faculty, Ovidius University of Constanta [10-12].

Out of the vegetal sample sprinkled with a non-polar solvent (ethilic ether, petroleum ether, benzen, hexan, clorophorm etc), then with a medium polarity solvent (ethanol, methanol), the following fractions were obtained, [7, 10, 11]:

1. etheric extractive solution (A)
2. alcoholic ectractive solution (B)
3. water based extractive solution (C)

Each extract is the analyzed for the identification of the active principles of pharmaceutical interest. For the identification of the chemical compounds of the three extracts, they are analyzed separately, using the methods fit for the physical and chemical properties of each group of active principles. In the etheric extract we can identify lipophyle chemical compounds, and in the other two extracts hydrophyle chemical compounds. The study material was represented by the collected vegetal products: Cladophora, Enteromorpha intestinalis, *Ulvae Lactuca* and *Ulvae Rigida*, *Cystoseira Barbata and Ceramum Rubrum.* The materials are characteristic for pharmaceutical researches.

3. RESULTS AND DISCUSSION

3.1. RESULTS

The characteristics of the local marine biomass of the Black Sea

The physico-chemical indicators have been investigated in 2015 in order to observe the quality of the Black Sea water. The main indicators who control the level of eutrophication have been analized: the salinity, the pH, the dissolved oxygen and the value of anorganic nutrients (see Table 1).

Table 1. General indicators.

Indicator	Registered values	Observations
Salinity	13.47 – 16.18 PSU	Specific values in the Black Sea, slightly lowered near the surface. The maximum value is registered in Vama Veche 20m
pH	8.39 – 8.81	Normal values which qualify in the admitted limits imposed by Order no: 161/2006
Oxygen	9.11-12.012 mg/dm^3	Coastal waters have been well oxygenated and qualify in the admitted limits imposed by Order no: 161/2006; 6.2 mg/dm^3 and 80%

From the completed analysis we see that the coastal waters are qualified in the quality limits imposed for the marine waters [13]. In Table 2 are presented the physico-chemical indicators and the limits for nutrients in sea water.

Table 2. Physico-chemical indicators and detection limits for determining the concentration of nutrients in sea water.

No.	The measured parameter	Obtained values (µM)	Detection limit ($\mu mol/dm^3$)	Observations
1.	$(NO_3)^-$	1.79 – 2.49	0.12	Low values which do not go over the maximum admitted concentration imposed by the Order161/2006 whichis1.5 mg/dm^3(107.14µM)
2.	$(NO_2)^-$	0.08 – 0.14	0.03	All values qualify in the accepted limit imposed by Order 161/2006: 0.03 mg/dm^3 (2.14 µM).
3.	$(NH_4)^+$	4.63 – 12.40	0.12	The maximum value was observed near the shore and it exceeds the admitted value imposed by Order 161/2006 0,1 mg/dm^3 (7.14 µM)

Table 2. Physico-chemical indicators and detection limits for determining the concentration of nutrients in sea water (continued).

4.	$(PO_4)^{3-}$	0.20 - 0.36	0.01	Slightly higher near the shore comparable to the ones from the 60's which is a reference period
5.	$(SiO_4)^{4-}$	2.90 - 6.70	0.30	The highest values have been determined at the water-sediment interface as a result of stock regeneration consumed by Spring activity

The marine algae distribution

In the romanian Black Sea shore, marine algae form communities in place with small depth being found between 0-10 m, as a consequence of local conditions rocky substrate and limited transparency. Because of the influence of antropogenic factors phytobentos elements can respond to marine flora changes by modifying their qualitative and quantitative composition. In this sense algae are good indicators for changes in marine ecosystems.

In 2015 the submerged macrophytic vegetation has been analized with observations on deposits and sample gathering across Navodari and Vama Veche shores.

A special attention has been given to species with high ecological value which targeted qualitative, quantitative and distribution areas. Data has been collected starting with May untill the start of October 2015, to highlight maximum growth periods of the submerged vegetation on the local shore.

In the phytoplankton structure there were identified 141 species of different varieties and forms, belonging to 7 taxonomic groups (Bacillariophyta, Dinoflagellata, Chlorophyta, Cyanobacteria, Chrysophyta, Euglenophyta şi Cryptophyta). The biggest diversity has been found in marine waters, where dinoflagellates have been dominant with 52 species followed by diatoms and clorophyte with 25 species each.

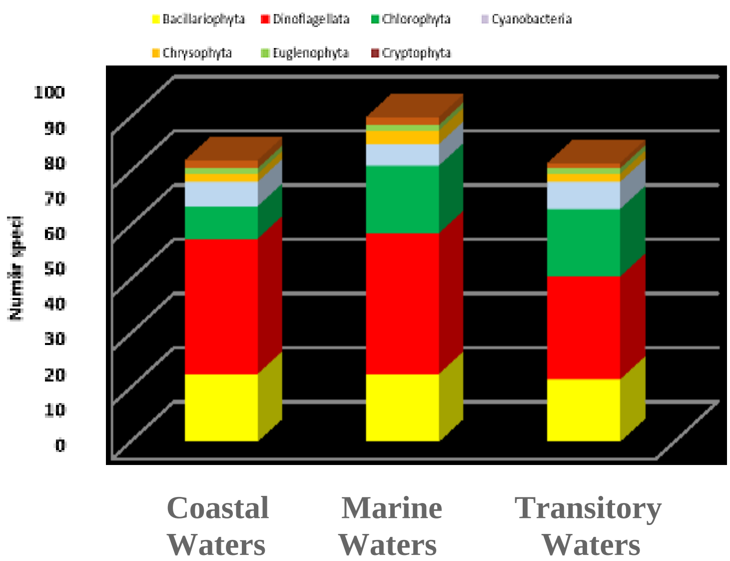

Figure 1. Phytoplankton distribution in 2015.

In coastal and transitory waters the number of species was relatively equal dinoflagellates have been dominant (48,37 %) followed by diatoms with 24% in coastal waters and clorophyte 24% in transitory waters. Fig. 1 presents the distribution of the phytoplankton on Romanian shores in 2015.

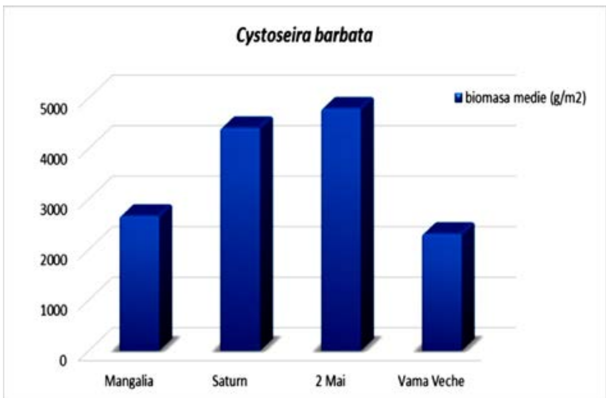

Figure 2. ***Cystoseira barbata*** **– Biomass variance in 2015.**

Chemical Composition in active principle from Seaweeds

As a follow up of the identification reactions previously discussed, the following results were obtained, summarized in Table 3.

Table 3 The selective reactions used in chemical analysis and identified active principles from seaweeds.

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

3.2. DISCUSSION

The highest values of phyoplanktonic densities in transitory waters is found in Portita station on the isobath of 5 and 20 m ($2.9\cdot10^6$ şi $3\cdot10^6$ cel/L), and in coastal waters in Gura Buhaz, Constanta N and Casino stations on the 20m isobath ($1.9\cdot10^6$, $2.3\cdot10^6$ şi $2\cdot10^6$ cel/L). Concerning biomass the maximum values of this month are found at Gura Buhaz and Portita station on the 5 m isobaths (1.4 and 1.3 g/m^3).

In marine waters most of the total phytoplankton values are maintained between $100\cdot10^3$ - 1 million cel/L in 28 % of the samples collected. Values over 1 million are rarely encountered in 12 % of the samples. The abundancy maximum for marine waters is found in Portita 4, Sulina 3, Saint Gheorghe 4, Casino 3 and Mangalia 3 stations (between $1.2\cdot10^6$ and $2.4\cdot10^6$ cel/L). During the hot season the following associations were dominant *Ulva - Cladophora - Ceramium*, with Ulva rigida a constant presence in all the stations between 1 and 3 m depth with biomasses varying between 100-700 g/m^2 with the maximum value found in Costinesti in July. With regards to the perennial species especially Cystoseira these are found in the south shores of the Romanian shores. Analysing the areas on the south shores (through observations and sample gatherings) the high values of fresh biomass gathered support the idea formulated in the previous years of regenerating the Cystoseira flora in the marine biomass. Fields of Cystoseira have been found in the southern areas of the shores in Mangalia, Jupiter-Saturn, 2 Mai, Vama Veche. Fresh biomass has been gathered from all over the stations varying from (2.300 and 4.700 g/m^2), only the size being different therefore in Mangalia and 2 Mai has found big samples while in Vama Veche the samples were smaller.

For the identification of the chemical compounds of the three extracts, they are analyzed separately, using the methods fit for the physical and chemical properties of each group of active principles. In the etheric extract we can identify lipophyle chemical compounds, and in the other two extracts hydrophyle chemical compounds. Each extract is the analyzed for the identification of the active principles of pharmaceutical interest.

4. CONCLUSIONS

The general conclusions of the studies are the following:

– As a follow up of the pharmaceutical studies, we have identified and dosed compounds such as flavonoic aglicoles, cumarines, streroles and triterpenes, which are valuable active principles for the pharmaceutical industry.

– In addition, we have identified ozes and poliozes, catehic tanin and reduction compounds.

– The results obtained enhance the possibility of opening new directions in the process of valorification of the resources offered by the Black Sea, in the research of medicines produced from natural resources.

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