

STUDIES ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF THE MARINE ALGAE ECOSYSTEM FROM THE ROMANIAN BLACK SEA

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Abstract. *Marine algae are an important resource for the marine ecosystem to be used for public health. Studying marine habitats is necessary to ensure the safety of a good marine biomass. In this paper the classification of protected areas with the specification of the six protected areas from the Romanian Black Sea coast is presented, according to European norms with Decision 2009 / 92 / EC on marine habitats. For marine algae habitats the following results for physico-chemical parameters studied on samples, such as dry matter, conductivity, pH and salinity are presented. The variables of nutrients concentrations recorded for 2016 (concentrations for nitrites and nitrates, ammonium and phosphates) are investigated as well. Marine algae, used in this study, were *Cystoseira barbata*, *Ceramium rubrum*, *Ulva lactuca*, and *Cladophora vagabunda* all gathered from Black Sea. The station network has remained steady since 2009, with sampling from Vadu to Vama Veche from depths between 0-3 m during the summer season in 2016.*

Keywords: *algae habitats, marine ecosystem, marine macroalgae, physico-chemical indicators.*

1. INTRODUCTION

The Romanian seashore situated in the pontic region, has a length of 244 km and is constituted from numerous marine, coastal and dune ecosystems. Considering only the territorial waters, it consists of a surface of approximately 5400 km². In the coastal part, from the total length of 244 km, approximately 68% is present in protected areas [1]. In Romanian legislation (i.e. OU 57/2007) regarding protected natural areas, conservation of natural habitats, flora and fauna, the protected area is defined as being an area in which natural values are protected, like various plant and animal species, biogeographical and landscape elements, geological, palaeontological, speological and others which have important ecologic and scientific value [2]. Black Sea ecological system presents certain characteristics which promote underwater development of life [3]. Coastal areas are a complex and dynamic systems, subject to natural and anthropogenic influences [4]. In terms of habitat diversity from coastal areas, they were classified in four water column types and eight per substrate [5]. The specific description of marine habitats highlights the fact that stony substrates are the ones who have the richest macroalgal vegetation [6]. The importance of the macrobenthic flora – algae and phanero-gammes – for the general productivity of the marine environment, especially in shallow waters, is becoming more and more obvious from the biological as well as from the economical point of view [7-14]. Marine resources offered by the Black Sea for

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therapeutic purposes have been exploited due to the quality of marine habitats along the Romanian seashore [15-16]. In recent years, marine algae have had a special value because they became an important resource for the pharmaceutical field [17-21]. Bioactive compounds with pharmaceutical importance have been studied in recent years [22-26]. The antioxidant activity of marine algae in the Black Sea was highlighted [27-30]. The quality of marine resources is conditioned by pollutant agents in marine habitats [31-35]. These important advances have made it necessary to take into account all legal aspects concerning protection against Black Sea pollutants that can infest the marine bioresources [36]. For using in the pharmaceutical field it was necessary to use only a non-contaminated resource of pollution agents [37, 38].

Comparative studies on the influence of pollutants on marine ecosystem contamination (e.g. polycyclic aromatic hydrocarbons from different sources) are known in literature [39, 40]. Methods for identifying existing pollutants in the marine ecosystem are classical methods used in specialized literature [41-43]. Marine ecosystem pollution indicators need to be monitored [44], to ensure the protection of human health [45]. The Black Sea marine biosource can be an important nutritional source [46, 47]. The paper presents a systematization of the information on the protected areas of importance as an integral part of the European ecological network "Natura 2000" in Romania, in accordance with the Decision 2009/92 / EC on marine habitats [48, 49]. The paper includes an analysis of the physico-chemical characteristics of algae habitats in the marine ecosystem of the Romanian Black Sea. Physical and chemical indicators were investigated in the years 2016 to monitor the quality of the Black Sea waters, with the results obtained from Vadu - Mangalia being selected as examples.

2. MATERIALS AND METHODS

The samples taken into study were marine sediments and seaweed samples with representatives of different species: green algae (*Ulva lactuca* syn., *Ulva rigida*, *Enteromorpha Intestinalis*, *Cladophora vagabunda*), brown algae (*Cystoseira barbata*) and red algae (*Ceramium rubrum*). The investigated physico-chemical indicators were obtained from the sample analysis (N = 4) of surface water and water column (0-20 m) taken in June, from two stations located on the 5 m and 20 m isobaths. The main physico-chemical and status indicators that characterize and control the level of eutrophication were analyzed, namely: salinity, dissolved oxygen, anorganic nutrients. Salinity was measured in-situ with CTD (a salinometer equipped with a unit that includes sensors for conductivity, temperature and pressure to measure salinity). The seawater nutrients were quantified by spectrophotometric analytical methods, internally validated in the laboratory where the quality system is maintained according to SR EN ISO / CEI 17025: 2005 and referred to in the Methods of Seawater Analysis Manual [50].

3. RESULTS AND DISCUSSION

3.1. RESULTS FOR ANALYZING SPECIAL CONSERVATION AREAS AND ALGAE HABITATS

According to international and European Union directives, the Protected Marine Network must have a suitable surface to fulfill the assigned protection role and consists of protected areas linked through "green corridors" that provide natural conditions for movement, reproduction and refuge of species of marine flora and fauna. The primary delimitation of Marine Sites of Community Importance was made by Order of the Minister No. 1964/2007 on the establishment of the protected natural habitat regime of community importance as an integral part of the "Natura 2000" European ecological network in Romania. They became Special Areas of Conservation following the analysis of the European Commission at the Sibiu Biogeographical Seminar in June 2008, which led to Decision 2009/92 / EC. Six Special Areas of Marine Conservation were designated as follows [30]:

1. ROSCI0066 - Danube Delta Biosphere Reserve - Marine Area: Site of Community Importance, in accordance with the requirements of the Habitats Directive 92/43 / EEC, which overlaps the Danube Delta Biosphere Reserve - natural protected area of national and international interest - 121,697 ha.
2. ROSCI0094 - The Mangalia Sulfur Subsoil Sources: Site of Community Importance, in accordance with the requirements of the Habitats Directive 92/43 / EEC - 362 ha;
3. ROSCI0197 - Submarine beach Eforie Nord - Eforie Sud: site of Community importance, according to the requirements of the Habitats Directive 92/43 / EEC - 141 ha;
4. ROSCI0237 - Metanogene submarine structures at Sfântu Gheorghe: site of Community importance, in accordance with the requirements of the Habitats Directive 92/43 / EEC, - 6,122 ha;
5. ROSCI0269 - Vama Veche - 2 Mai: site of Community importance in accordance with the requirements of the Habitats Directive 92/43 / EEC, partially overlapping the Maritime Reserve 2 Mai - Vama Veche), protected area of national importance - 5,272 ha;
6. ROSCI0273 - Marine Head Area of Tuzla: site of Community importance in accordance with the requirements of the Habitats Directive 92/43 / EEC, - 1.738 ha.

Biodiversity and marine habitats on the Romanian seaside have been characterized by the values of the specific decision-making indicators. The state of biodiversity, defined by the total number of species identified at the Romanian seaside, is about 2,926 (bacteria - 113, microfite algae - 683, macrophyte algae - 138, invertebrates - 1,730, fish - 108, birds - 150 and mammals - 4) and 29 threatened species. Knowledge of biodiversity and coastal habitats specific to macrophyte algae is particularly important for both harvesting and monitoring of marine data [30].

These habitats are included in the priority European habitats (the Habitats Directive), [30]. By definition, according to the Habitats Interpretation Handbook "Natura 2000" - EUR 25 [29], habitats are subterranean and biogenic subsoil substrates, or exposed to low tides, concretions that occur in the seaside area but can extend to the coastal area where the uninterrupted use of animal and plant communities' area begins.

Reefs generally support benthic algae and animal communities, including concretions, inlaid and coral concretions. A classification of habitats at the Romanian coast takes into consideration only the types for macrophytes development [30]. It includes:

- 1170-2 Rock and boulder agglomerations,
- 1170-3 The supralittoral rock,
- 1170-4 The superior medium littoral rock,
- 1170-5 The inferior medium littoral rock.

The pressure on biodiversity and habitats was expressed by the existence of 14 exotic species, 8 commercially exploited species (6 fish and 2 molluscs) and 11 types of anthropogenic activities with impact on the conservation status of biodiversity and habitats. The area of wet lands was 586,188ha, out of which 6,188 ha in Constanta County.

The impact on biodiversity and marine habitats was assessed by the ratio of the number of endangered species / total number of species, ie 29/2926 and the number of missing species / total number of species, which was 13/2926.

From a qualitative point of view, in 1935, along the Romanian seaside, the aforementioned number of macrophytes decreased to 77. Between 1970 and 1980 only 69 species were recorded. According to other estimates, at the end of the 1990s, only 38 *Chlorophylls*, 1 *Xanthophite*, 14 *Feofite* and 41 *Roodophytes* were identified. These observations point to the strong qualitative decline of macrophytobenthos at the Romanian shore. In 1989 only the southern part of the Romanian seashore had a greater specific diversity. Here, 24 taxa were identified (1 *Cyanophyte*, 11 *Chlorophytes*, 2 *Feofites*, and 10 *Rhodophytes*). With the increase in eutrophication, significant changes in quality have been recorded in the structure and functionality of macrophytobenthos, starting with the earliest records and by the end of 2000. Thus, due to the high variability of ecological factors, these changes in the ecosystem and community structure have led to the replacement some phytocenosis with others. The consequence was a change in the seasonal and multiannual dynamics of algal communities.

3.2. RESULTS FOR DETERMINING PHYSICO-CHEMICAL PARAMETERS

The sediments were analyzed in 2016. They are deposits of processed substances from the sea but also generated by mineral and organic loading through the interface of the sediment. Regarding the distribution of concentration of the parameters analyzed, there are generally large variations along the two reference stations and at a different depth. Concerning the distribution in the investigated surface area of the dry suspension, the minimum value in Mangalia of 5 m ($65.20 \pm 0.38\%$) and the maximum at Vadu 10 m ($98.97 \pm 0.51\%$) was observed. The mineral residue showed the minimum value in the 15 m Vadu station (85.45 ± 1.32) and the maximum value in Mangalia (95.88 ± 1.28) see Table 1.

**Table 1 Physico-Chemical Parameters
(dried substance, mineral residue and organic substance).**

Profile	Dried substance [%]	Mineral residue [%/SU]	Organic substance [%/SU]
MANGALIA 5 m	65.20±0.38	91.22±1.23	9.82±0.25
10 m	75.03±0.42	95.88±1.28	4.10±0.09
15 m	95.55±0.25	93.66±1.37	7.37±0.16
COSTINESTI 5 m	78.26±0.41	90.26±1.33	8.84±0.12
AGIGEA 10 m	73.25±0.33	91.33±1.36	8.54±0.2
15 m	72.36±0.28	91.81±1.46	8.15±0.19
CONSTANTA 5 m	82.36±0.35	94.25±1.22	5.0±0.15
MIDIA 5 m	82.23±0.55	94.55±1.33	5,35±0.12
10 m	95.13±0.56	94.87±1.35	5,23±0.12
15 m	88.56±0.59	93.95±1.38	6.05±0.18
VADU 5 m	83.25±0.48	90.73±1.42	9.27±0.17
10 m	98.97±0.51	88.65±1.28	9,75±0.19
15 m	76.76±0.54	85,45±1.32	13.85±0.22

The variables of nutrients concentrations recorded for 2016 are presented in Figs.1-2. The highest values were recorded at Mangalia at 5 m for ammonium (9.04 µg/g) and for phosphates (6.25 µg/g), Costinesti at 5 m for nitrites (0.58 µg/g) and Vadu at 5 m for nitrates (0.25 µg/g).

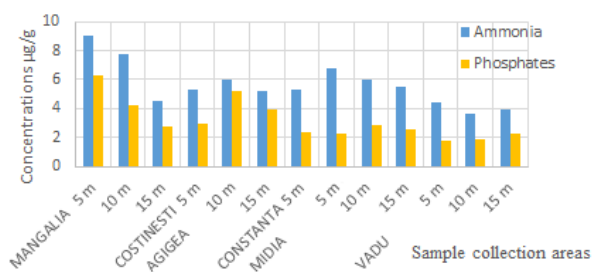


Figure 1. The variation of the ammonium and phosphate concentrations found in the sediments collected.

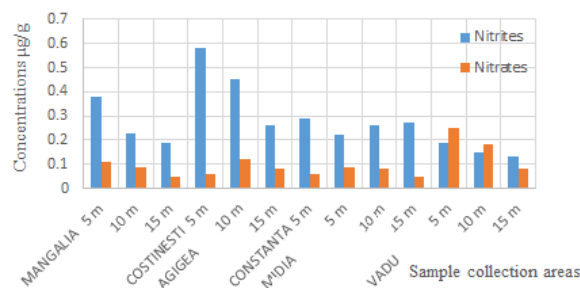


Figure 2. The variation in concentrations for nitrites and nitrates found in the sediment collected.

The lowest values were recorded at Vadu at 10 m for ammonium (3.65 µg/g) and at 5 m for phosphates (1.77 µg/g), at Vadu at 15 m for nitrites (0.13 µg/g). For nitrates, the lowest values were recorded at Mangalia at 15m (0.05 µg/g) and at Midia at 15 m (0.05 µg/g).

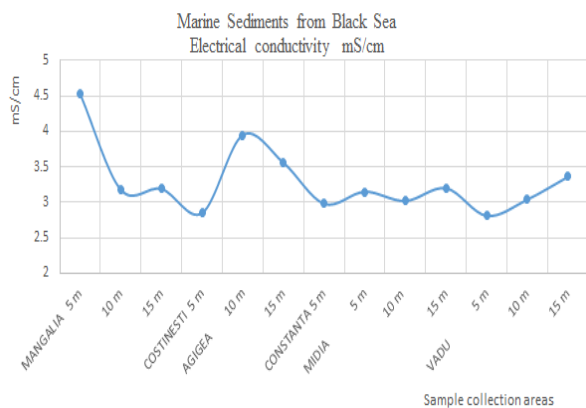


Figure 3. Variation of electrical conductivity found in harvested sediments.

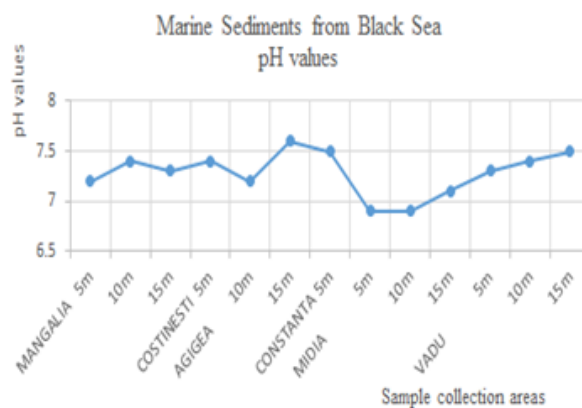


Figure 4. Variation of pH values recorded in harvested sediments.

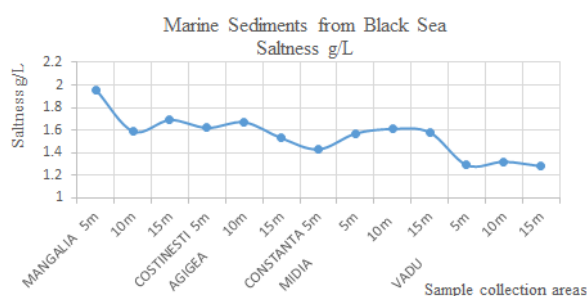


Figure 5. The salinity variation recorded in the sediments collected.

The variation of the parameters: electrical conductivity (Fig. 3), pH (Fig.4) and salinity (Fig. 5) were monitored for 2016 and are presented as average values for all harvesting stations along the Romanian seashore between Vadu and Vama Veche. Higher values at Mangalia were recorded at 5 m for electrical conductivity and salinity. The pH values range around the neutral range (6.6 at Midia and 7.6 at Agigea).

Values analyzed on the samples collected in 2016 are comparable to those presented in the Marine Environment Status Report 2016 [31]. The variation of these parameters influences the development of marine macroalgae, which make the phytobenthos. Thus, annual correlations can be made regarding the quality of the phytobenthos characteristic of the Black Sea.

3.3. RESULTS FOR PHYTOBENTHOS STUDIED

As shown in the Marine Environment Status Report 2016 [31], the monitoring of the phytobenthic component continued with the study of previous years, with submerged vegetation undergoing a complex assessment, both qualitative and quantitative. Also, because the macroalgae element and marine phanerogam is considered a good indicator of changes in the marine environment due to anthropogenic disturbances or natural causes [31]. The station network has remained steady since 2009, with sampling from Navodari to Vama Veche from depths between 0-3 m during the summer season. From the analysis of the results, as for previous years, *Ulva-Cladophora photophilic* association enriched with elements of the *Ceramium* genus (*C. virgatum* mainly) dominated the associations over the monitored period. Similar to the previous years, in the summer of 2016 the dominant species in terms of quantity was *Ulva rigida*, followed by

species of *Cladophora* (*C. vagabunda* in association with *C. sericea*). For *Ulva rigida*, a maximum biomass was recorded at Agigea (1030 g/m^2 - Fig. 6.a), and for *Cladophora sp.* at Fishing (816 g/m^2 - Fig. 6.b). *Ceramium virgatum* was the only representative of the *Rhodophyta* groves in 2016 with a notable development but, however, the biomass was reduced (a maximum of only 125 g/m^2 in Costinesti - Fig. 7) compared to 2015 when the maximum biomass was about 500 g/m^2 [31]. Average biomass variation for dominant opportunistic species in 2016 are presented in Fig.6, Fig.7 and Fig.8

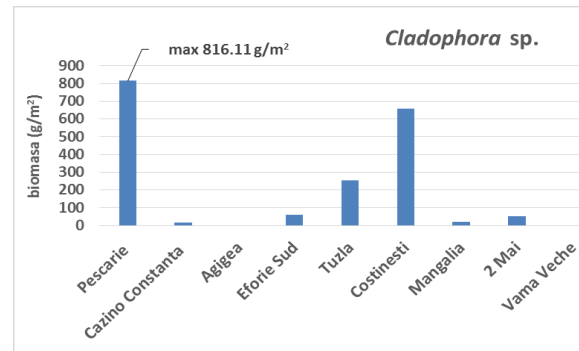
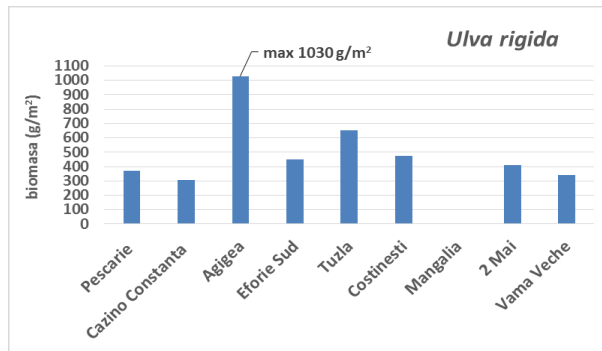


Figure 6. Average biomass variation for *Ulva rigida* (a) and *Cladophora* species in 2016.

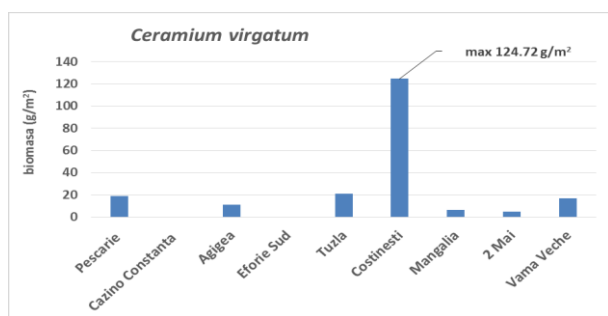


Figure 7. Variation of average biomass for *Ceramium* species in 2016.

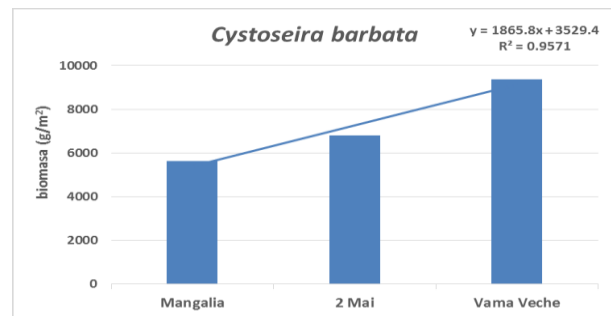


Figure 8. *Cystoseira barbata* - average biomass variation in 2016.

The monitoring of perennial species has focused on the southern sector of the Romanian seaside, from Mangalia to Vama Veche, where the development areas for *Cystoseira barbata* brown algae are found. *Cystoseira* varieties of varying sizes were identified at Mangalia, Jupiter-Saturn, May 2 and Vama Veche, with high average biomass ranging from 5000 to 9000 g/m^2 , with a maximum at Vama Veche (Fig. 8).

From the qualitative point of view, since 1935, along the Romanian sea shore, the macro – algae number mentioned above dropped to 77. Between 1970 and 1980, only 69 species were recorded. According to some other estimation, at the end of the '90, only 38 *Chlorophytaes*, 1 *Xanthophytaes*, 14 *Phaeophytaes* and 41 *Rhodophytaes* were identified. These observations outline the major qualitative decline of the macrophytobenthos at the Romanian shore. In 1989 only the south part of the Romanian littoral presented a higher specific diversity. 24 species were identified here (1 *Cyanophytaes*, 11 *Chlorophytaes*, 2 *Phaeophytaes* and 10 *Rhodophytaes*). With the increasing of the eutrophication, important qualitative changes were recorded in the structure and functioning of the macrophytobenthos, starting with the oldest recordings and until the end of the year 2000. So, due to the big variability of the ecological factors, these changes of the ecosystem and communities structure lead to the replacement of some phytocoenoses with others. The consequence was a change in the seasonal and multianual dynamics of the algal communities. The distribution of algae is directly influenced by many factors: substrate, climate (temperature), light penetration, water

chemistry (salinity); the modification of each of these factors (of the water quality) induces changes of the algal macro – flora.

From a quantitative point of view, green algae have dominated the whole period investigations (1996-2016), with a peak of development in 1997 and 1998, as well as large spawned biomass in 2003 and 2004. Biomasses of the genus *Ulva*, *Enteromorpha* were the highest. Considerable biomasses also had *Ceramium* species, other red algae having a low contribution.

4. CONCLUSIONS

The following conclusions can be drawn from this study: marine ecosystem along the Romanian Black Sea seaside varies and requires permanent monitoring for the special marine conservation areas required by the legislative system in accordance with the European Community framework; improving the status of the marine ecosystem could have beneficial consequences on the algal vegetation on the Black Sea coast, such as the reappearance of some species that have been missing for a long time, being considered missing; through the studies, recognized in the literature, marine algae are a rich natural resource for later exploitation in various fields; this paper advocates the knowledge of marine habitats in which those significant algae can form and their composition in valuable bioactive compounds; knowledge of algal marine habitats makes it possible to collect some algae from their natural environment to be used for the extraction of bioactive compounds with applications in the medical, pharmaceutical, cosmetic or nutritional industries. Areas and ways of collecting algae will be analyzed in a future work, as it requires complex investigations.

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