

**AAPG European Region Annual Conference**  
**Paris-Malmaison, France**  
**23-24 November 2009**

**The MARINE BIOMASS from BLACK SEA COAST, COMPOSITION and  
CHARACTERISTICS, as an UNCONVENTIONAL RESOURCE**

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**Abstract** *The superior improvement of the marine biomass represents a highly important resource for the pharmaceutical industry, supplying raw material for the extraction of bioactive substances (vitamins, sterols and collagen). In the paper we present a comparative study regarding heavy metals content and antioxidative capacity of the main macrophyte algae, specific for Romanian Black Sea Coast, Cytoseira barbata, Ceramium rubrum and Ulvae lactuca. Heavy metals content had been determined by AAS and for antioxidant capacity of algae extracts, chemiluminescence method had been used. The results confirm that the heavy metal content is in accordance with Romanian Standard in force and could be correlated with the high level antioxidant activity of these species, which relieve the possibility to enlarge the options to use these natural vegetal resources from Black Sea Coast, in different degenerative diseases therapy.*

**Key words:** marine biomass, *Cytoseira barbata*, *Ceramium rubrum*, *Ulvae lactuca*, antioxidative activity, heavy metals content

## **INTRODUCTION**

The biologic role of macrophytes algae is well-known in all aquatic pool, contributing to maintaining the biologic equilibrium and representing the base of primary productivity in these pools. The paper presents comparative studies regarding the content of heavy metals and the antioxidant activity of three marine algae species, specific for the Romanian Black Sea seaside: *Cytoseira Barbata*, brown alga, *Ceramium rubrum*, red alga and *Ulvae rigida* Ag( syn. *Ulvae lactuca* L), green alga, as they are plant organisms which meet the requirements to be used in the toxicological monitoring programs, as bio-accumulative.

*Cytoseira barbata* is part of brown algae group, Phylum Phaeophyta, Cyclosporeae class, order Fucales, family Cystoseiraceae, *Cytoseira* Genus, Species *Barbata*, who represents a group of macroscopic algae, always multicellular, most of them marine and ocean (Fig. 1). The species is widespread in the Black Sea where the substrate forms a tough perennial pool, very valuable in terms of environment. Currently the association is very low, on the one hand due to frost in some prior periods and secondly because of pollution, increased water turbidity and substrate clogging.

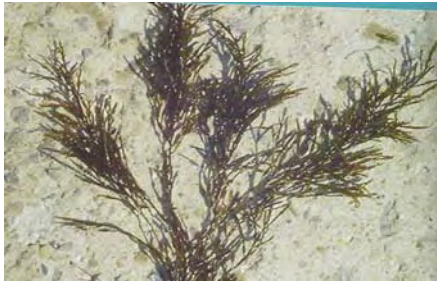


Fig. 1- *Cystoseira barbata*



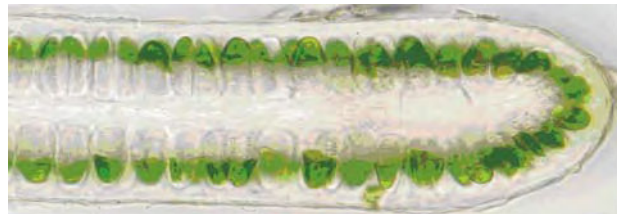
Fig. 2 - *Ceramium rubrum*

***Ceramium rubrum***, is part of red algae, Phylum Rhodophyta, Class Rodophyceae, including unicellular and multicellular algae that live in aquatic environment, most marine, few species are found only in freshwater (Fig. 2). Alga is widespread in Atlantic and Pacific Oceans, the Mediterranean and Black Sea. It is an annual species, large, that sometimes largely colonizes rocky substrates in the mid-and infra-shore in areas exposed to wave agitation. On the Black Sea seaside it is meet along the entire coastal areas, on rocks, at depths from 0.5 to 4-5 m, all year, growing more spring and summer. Literature data indicate the species *Ceramium rubrum* as producing a quality agar and , from the many species of red algae, *Ceramium rubrum* is the only with strong antibiotic properties over Gram positive bacteria, testing being done on *Bacillus subtilis*; but inhibitory properties of algae does not manifest also on Gram-negative bacteria.

***Ulvae rigida* Ag. (syn. *Ulvae lactuca* L.) – sea salad**, is part of Greening Chlorophytes Phylum, Class Chlorophyceae, Order Ulvaeles, Ulvaeceae family. The vegetative body of the alga has the appearance of irregular shaped foliaceus blade, sometimes with numerous breaks in the middle of a relatively firm texture connected with the substrate by a restraint system consisting of dark color rhizoids (Fig. 3 A, B).



A



B

Fig. 3. *Ulvae lactuca* L.; A- macroscopic appearance; B- microscopic appearance, section through the vegetative body

It is an tropical –Atlantic species ( Lower Boreal) which we can find on North-East and North-West Atlantic, Mediterranean Sea, Pacific Ocean and Black Sea ( Romanian shore in the southern part between Costinesti and Mangalia. It is met in polluted areas, sometimes even abundant in waters rich in nutrients [1-3].

## RAW MATERIAL AND METHODS

The algae were manually collected from the Romanian Black Sea Coast, Navodari-Mangalia area, during march – April period. Algal material was sorted, washed and weighed. For the determination of the heavy metal content, the samples were mineralized through the mineralization method according to the standardized methods [3]. The following quantities have been used:

Sample 1 – *Cystoseira barbata*=10.9201g; Sample 2- *Ceramium rubrum* =11.2702g; Sample3- *Ulvae Lactuca*=9.7628. The determination of the heavy metal content from the algal samples has been realized trough the flame atomic absorption spectrometry method (FAAS). The following materials have been determined: Zn ( $\lambda = 213$  nm), Cu ( $\lambda = 324$  nm), Pb ( $\lambda = 217$  nm) și Cd ( $\lambda = 228$  nm).

For the determination of the antioxidant capacity, the algae well dried have been ground, triturated until they became fine powder, and then there has been obtained a cold extract from each species of algae, utilizing 20mg of vegetal product dissolved in 1ml of ethyl alcohol, Merck. The extraction was effected for 24 hours, 72 respective, on the room temperature, in brown glass bowls, airtight closed. The mixtures were periodically shaken, and the separation of the extracts has been made through decantation, without filtering. For each algae extract has been determined the antioxidant capacity utilizing the standard method ACL (Antioxidant capacity of lipid soluble substances), by comparing with the standard substance Trolox (E vitamin), utilized for tracing the calibration curve, according to the standardized method Analytic Jena [4.5]. For the calibration curve have utilized the standard reagents kits Analytic Jena: R<sub>1</sub> (dilution solvent), R<sub>2</sub> (reagent buffoon), R<sub>3</sub> (photosensitive reagent), R<sub>4</sub> (calibration reagent). The calibration curve has been build by measuring a series of standard solutions which contain 0,5; 1,0; 2,0; 3,0 nmol Trolox (appropriate for 5 –30  $\mu$ L R<sub>4</sub>). By exposing at a radiation provided by the Hg lamp lined with phosphors which ensure maximum power at  $\lambda = 351\text{nm}$ , the photosensitive reagent, produces free radicals in the sample for analysis, having been a photochemical reaction. After releasing the free radicals, the antioxidants present in the sample annihilate them to some extent, some remaining in the sample and will combine with photosensitive reagent which, in the absence of external excitation source is acting as a detector of free radicals.

After the chemical reaction a quantum of light is delivered which is amplified and detected by the photo – multiplier. It is measured the total antioxidant capacity from the electric signal of the machine which is converted in concentration values.

Equipment used:

- CONTRAA 700 atomic absorption spectrophotometer Analytik Jena, Germany, 2008
- Photo-chemical- lightmeter PHOTOCHEM Analytik Jena, Germany, 2008

## RESULTS AND DISCUSSIONS

The results obtained after the determinations of heavy metals from the studied algal samples are presented in table 1.

Table 1 The content of heavy metal

| Alga type                 | Pb (mg/kg) | Cd (mg/kg) | Cu (mg/kg) | Zn (mg/kg) |
|---------------------------|------------|------------|------------|------------|
| <i>Cystoseira barbata</i> | 2,15       | 0,195      | 3,905      | 1,82       |
| <i>Ceramium rubrum</i>    | 4,95       | 0,206      | 5,43       | 5,51       |
| <i>Ulvae lactuca</i>      | 1,98       | 0,179      | 3,65       | 18,25      |

It is observed a higher content in heavy metals for the red alga *Ceramium rubrum* then the brown alga *Cystoseira barbata* especially because of the bio-geographical disposal of these algae at the sea level. The *Cystoseira barbata* alga lives in the seaside area, at small depths, where the sun light do interfere and, therefore, part of these metals enter in the bio-geo-chemical circuit and are used at photosynthesis, while the red alga *Ceramium rubrum* lives at big depths, where the sun light doesn't penetrate and there is no photosynthesis and because of the specific currents from the Black Sea they depose and form sediments of heavy metals.

In comparison with the other two species, the green alga *Ulvae Lactuca* which is very resistant in the polluted environments, presents the lowest content of Pb, Cd, Cu metals, but also the highest content of Zn. We observed that the concentration limits for heavy metals on the algae processed in this

study are comparable with the data of specific literature and are in concordance with the existing standards which regulates the concentration of heavy metals in marine waters.

The antioxidant capacity of algae samples, related to the extraction time and the sample volume utilized in the experiment, according to ACL method quantified through comparison with Trolox standard substance, and the results are expressed in nmol/sample, Trolox equivalent units, according to Table 2.

The antioxidant capacity related to Trolox (ACL method)

| Sample (Alga type)        | Extraction time (hours) | Used sample volume ( $\mu\text{L}$ ) | Analyses Time (sec.) | Trolox equivalent units (nmol/ sample volume) | Trolox equivalent units (nmol/ g dried sample) |
|---------------------------|-------------------------|--------------------------------------|----------------------|---|--|
| <i>Cystoseira barbata</i> | 24                      | 20                                   | 120                  | 1,058   | 52,9   |
| <i>Ceramium rubrum</i>    | 24                      | 20                                   | 120                  | 1,042   | 52,1   |
| <i>Ulvae lactuca</i>      | 24                      | 20                                   | 120                  | 0,681   | 34,05  |
| <i>Cystoseira barbata</i> | 72                      | 20                                   | 120                  | 2,83  | 141,5  |
| <i>Ceramium rubrum</i>    | 72                      | 20                                   | 120                  | 2,64  | 132  |
| <i>Ulvae lactuca</i>      | 72                      | 20                                   | 120                  | 1,24  | 62   |

The highest antioxidant capacity was registered at *Cystoseira barbata*, at both 24 and 72 hours, compared with values obtained for algae *Ceramium rubrum* and *Ulvae Lactuca*. The lowest antioxidant capacity it is found at *Ulvae Lactuca* alga, at 24 hours and 72 hours.

## CONCLUSIONS

- In the paper have been performed comparative studies regarding the heavy metals content of algae and their antioxidant capacity. For the study have been used three species of macrophytes algae from the Romanian Black Sea seaside : brown alga *Cystoseira barbata*, red alga *Ceramium rubrum* and green alga *Ulvae lactuca*, which meet the organisms requirements to be used in monitoring programs.
- It was found that concentrations of heavy metals (Pb, Cd, Cu, Zn) in algae studied are within the limits permitted by the applicable standards. Of the three species studied, the algae *Ceramium rubrum* presents the highest content of heavy metals.
- All three algae show high antioxidant activity, the most intense being the species *Cystoseira barbata*, activity that could be used as the capitalization of these species in the therapy of degenerative diseases.

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