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Biochemical Investigation and Heavy Metal Contents of *Cladophora dalmatica Kütz. and Ceramium ciliatum (Ellis) Ducl.* var. *robustum (J.Ag.)* from Aegean Sea (Turkish Coast)

Ege Denizi (Türkiye) Sahillerinde Yayılış Gösteren *Cladophora dalmatica Kütz.* ve *Ceramium ciliatum (Ellis) Ducl.* var. *robustum (J.Ag.)*'un Biokimyası ve Ağır Metal İçeriği

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Abstract

The levels of heavy metal accumulation and chemical composition of *Cladophora dalmatica* and *Ceramium ciliatum* var. *robustum* were analyzed in this investigation. The samples were collected at Yeni Şakran Bay at the north side of the gulf of Izmir.
It was found that the amounts of water, protein and fats of *C. ciliatum* var.*robustum* were higher than in *Cl. dalmatica*, grown in the same environment. The contents of ash, P, K and Ca were higher in *Cl. dalmatica* than in *C. ciliatum* var. *robustum* while the contents of Na and Mg of *C. ciliatum* var. *robustum* were higher than in *Cl. dalmatica*.
Although Fe, one of the heavy metals, accumulated similarly in both algae, Cu and Zn levels were higher in *Cl. dalmatica*, while Mn level higher in *C. ciliatum*

var.*robustum*. The accumulation of the heavy metals was ranked as Fe>Zn>Mn>Cu, for both groups.
Additionally, the physico-chemical parameters of environmental water were determined.

Keywords: *Cladophora dalmatica, Ceramium ciliatum* var. *robustum* biochemical analysis, heavy metal

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Introduction

Cladophora dalmatica Kütz. (Chlorophyta, Cladophoraceae) and *Ceramium ciliatum* (Ellis) Ducl. var. *robustum* (J.Ag.) G. Mazoyer (Rhodophyta, Ceramiaceae) are scattered on the rocks in intertidal zones of Yeni Şakran Bay (the northern coast of Izmir Bay, Aegean Sea). These two taxa adapt to some physical and chemical factors that have influence on their environment of growth.

Cladophora Kütz. and *Ceramium* Lyngbye are similar in that both have flamentous thallus. It seemed reasonable to compare the chemical compositions and the levels of heavy metal accumulation of the two taxa which belonged to different taxonomical groups while they were in the same group of functional form with intense or scarce branching.

Cl. dalmatica usually spreads in environments of either unpolluted or rich nutritive characteristics. Some research has been directed to chemical structure of *Cladophora* sp. (Munda and Gubensek, 1976; Gordon *et al.*,1980; Cirik *et al.*,1988; Türkan *et al.*,1989; Lavery *et al.*,1991; Peckol *et al.*, 1994; Elenkov *et al.*,1995; Rivers and Peckol, 1995).

C. ciliatum var. *robustum* grows superficially on various substrate of polluted or unpolluted regions. There are some studies concerned with the chemical composition and heavy metal content of *Ceramium* sp. (Munda, 1980a,1980b; Kiran *et al.*, 1980; Zavodnik and Juranic, 1982; Wallentinus, 1984; Munda and Gubensek, 1986; Munda, 1990; Güven *et al.*,1993; Ünlü *et al.*, 1995).

Both species, *Cl. dalmatica* and *C. ciliatum* var *robustum* grow in clear as well as in nutrient enriched waters. The aim of this study was to reveal the differences in chemical composition of both species considering morphological similarity caused by adaptation to similar environmental conditions.

Material

Cl. dalmatica and *C. ciliatum* var. *robustum* were collected from Yeni Şakran Bay in spring (19.04.1996) (Fig. 1).

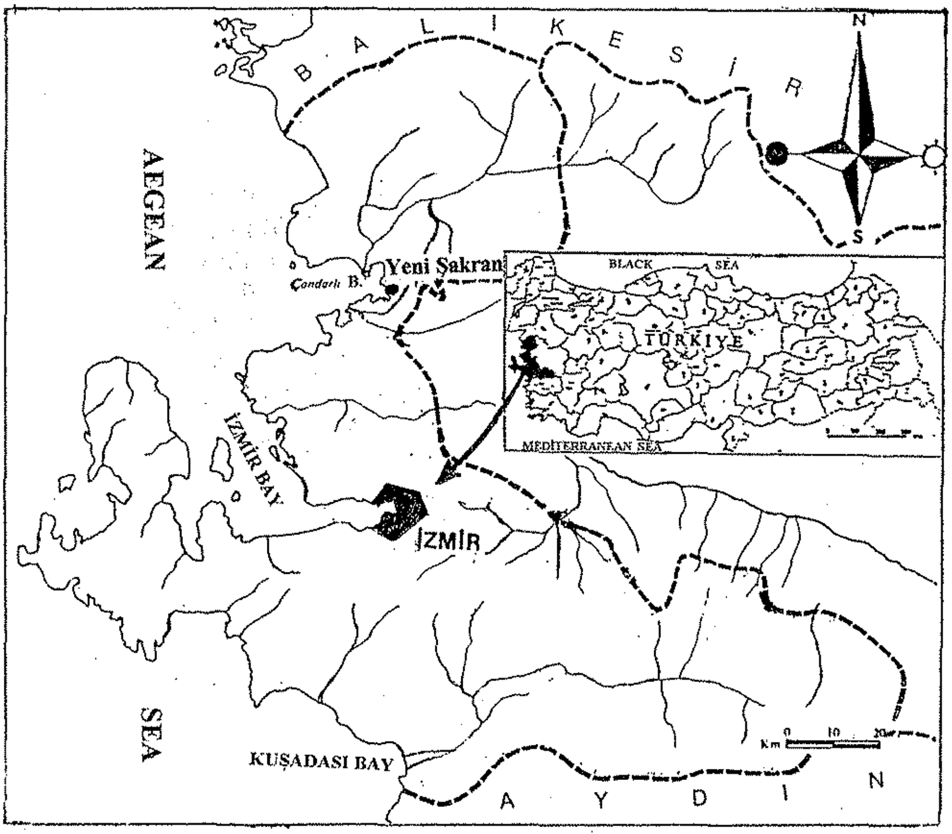

Figure.1.The map of research area

For biochemical analysis the two samples were left to dry at room temperature after they had been rinsed with water and subsequently with distilled water.

One gram of each sample was dried in an oven at 70 °C, for 8-10 h. Thus, the water contents of the samples were determined by calculating the difference between wet and dry weights (A.O.A.C.,1970). The ash contents of the dried samples were determined according to the standard methods, by burning 1 g of powdered algae at 600 °C, for 8 h. (A.O.A.C., 1970).

Total nitrogen (N) contents of the algae were determined using micro Kjeldahl method (Bremner, 1965). The determination of phosphorus (P), sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) was made by using the rest of the solution obtained by wet digestion of algae from N determination. To determine the P amounts, 5 ml of this solution was taken and diluted to 25 ml. The solution of 1 ml HNO_3 and 5 ml of special solution mixture (100 ml of HNO_3 was taken and added distilled water up to volume 200 ml, 1.25 g of ammonium vanadate was dissolved in hot water and cooled then added 4 ml of HNO_3 and distilled water up to volume 200 ml, 25 mg of ammonium molybdate was also dissolved in hot water, cooled and added distilled water up to volume 200 ml, The solutions in 1,2,3 steps were mixed for special solution) prepared as blow were added and after 15 min., the P content was determined in a spectrophotometre at 436 nm and calculated as P %. Flame photometry was used to determine Na, K, Ca and Mg contents of filtered solution obtained from wet digestion of algae (Pirdal 1989). The total protein contents were calculated by multiplying the total N value by 6.25 (Bremner, 1965).

Total fats were determined by Soxhlet extraction. The extraction solvent was petroleum ether (150 ml.). The water bath temperature was 80°C. The extraction process was maintained continuously for 6-8 h. The extract was evaporated in rotary-evaporator and then dried at 105°C. The difference of the weights before and after extraction gave the content of total fats.

The samples were washed with distilled water and dried at room temperature. Each sample was ground and the wet digestion method was applied. 1 g of sample was mixed with a mixture of 12 ml. of HNO_3 and $HClO_4$ (5:1). The rates were prepared for analysis after being diluted up to 50 ml. with 0.1 N HCl and the heavy metal (Fe, Zn, Cu, Mn) contents were determined by using Varian Techtron Atomic Absorption Spectrophotometer (Model 1250) (Bernard, 1976).

The PVC bottles of one litre were filled with sea water and carried to the laboratory in boxes containing a mixture of salt and ice, for physico-chemical parameters. Beckman RS-7B Model Salinometer was used to assess salinity and pHep-pH Electronic Papier (HANNA Ins.) was used for pH measurement. Nutrient contents of seawater including nitrate (NO_3^- -N), ammonium (NH_4^+-N) and phosphate (PO_4^{-3}-P) were analyzed according to the method of Strikland and Parsons (1972) using spectrophotometer (Perkin-Elmer Model 35).

Na, K, Ca and Mg contents of sea water were determined by the method mentioned above.

Biochemical analysis and the content of heavy metals were calculated as percent and µg/g of dry weight.

The water contents of *Cl. dalmatica* and *C. ciliatum* var.*robustum* were found 85.08 % and 88.10 %, respectively. Their protein contents were 8.13 % and 14.81 %. The fat contents were low in *Cl. dalmatica* and *C. ciliatum* var.*robustum* contained more fat than *Cl. dalmatica*. The ratios of ash were 27.62 % and 23.76 %, for *Cl. dalmatica* and *C. ciliatum* var.*robustum*, respectively (Table 1).

Table 1.Biochemical analysis of *Cladophora dalmatica* Kütz and *Ceramium ciliatum* (Ellis) Ducl. var. *robustum* (J.Ag.) G. Mazoyer. The values are the means of three experiments (% of dry weight, * % of wet weight and µg/g of dry weight).

Algae / Content	*Cladophora dalmatica*	*Ceramium ciliatum* var.*robustum*
*Water (%)	85.28 ± 1.53	88.10 ± 0.72
Dry weight (%)	14.72 ± 1.00	11.90 ± 0.32
Ash (%)	27.62 ± 0.21	23.76 ± 0.70
Organic matter (%)	72.38 ± 0.76	76.24 ± 0.23
Protein (%)	8.13 ± 1.06	14.81 ± 0.96
Fats (%)	0.93 ± 0.11	2.79 ± 0.18
N (%)	1.30 ± 0.03	2.37 ± 0.21
P (%)	0.22 ± 0.06	0.11 ± 0.02
Na µg/g	2100 ± 14.53	3200 ± 11.02
K µg/g	30000 ± 574.37	20000 ± 480.14
Ca µg/g	2880 ± 32.15	1510 ± 30.55
Mg µg/g	590 ± 7.51	890 ± 12.77

The proportions of one of the inorganic components in both taxa, P, were 0.22 % in *Cl. dalmatica* and 0.11 % in *C. ciliatum* var.*robustum*. In *Cl. dalmatica*, the levels of Na, K, Ca and Mg were 2100 µg/g, 30000 µg/g, 2880 µg/g and 590 µg/g respectively, and in *C. ciliatum* var. *robustum*, the values were 3200 µg/g, 20000 µg/g, 1510 µg/g and 890 µg/g, respectively (Table 1).

Heavy metal concentrations in *Cl. dalmatica* and *C. ciliatum* var.*robustum* are shown in Table 2. It can be seen from the table, in *Cl.*

dalmatica and *C. ciliatum* var.*robustum* Fe concentrations were ranked between 1258 µg/g and 1231 µg/g, while Cu concentrations ranked 5.93-3.56 µg/g, Zn concentrations were found 136.61 µg/g in *Cl. dalmatica* and 74.50 µg/g in *C. ciliatum* var. *robustum*. Mn concentrations were determined as 49.66 µg/g in *Cl. dalmatica* and 72.65 µg/g in *C. ciliatum* var. *robustum* (Table 2)

Table 2. Heavy metal contents of *Cladophora dalmatica* Kütz and *Ceramium ciliatum* (Ellis) Ducl. var. *robustum* (J.Ag.) G. Mazoyer (µg/g dry weight). The values are the means of three experiments.

Algae / Content	*Cladophora dalmatica*	*Ceramium ciliatum* var. *robustum*
Fe	1258 ± 26.50	1231 ± 22.61
Cu	5.93 ± 0.06	3.56 ± 0.19
Zn	136.61 ± 2.33	74.50 ± 2.90
Mn	49.66 ± 1.37	72.65 ± 0.62

The results of the physico-chemical analysis of the sea water are shown in Table 3. The salinity, pH; NO_3^--N, NH_4^+-N, PO_4^{-3}-P were measured as 37.01 ‰, 7.6, 0.89 µg at l^{-1}, 1.21µg at l^{-1}, 0.18 µg at l^{-1}, however Na, K, Ca and Mg were found as 12000 mg/l, 400 mg/l, 440 mg/l and 6160 mg/l respectively.

Table 3. Chemical analysis of the sea water at Yeni Şakran Bay

pH	7.6
Na	12000 mg/l
K	400 mg/l
Ca	440 mg/l
Mg	6160 mg/l
Salinity	‰ 37.01
NO_3-N	0.89 µg at l^{-1}
NH_4-N	1.21 µg at l^{-1}
PO_4-P	0.18 µg at l^{-1}

Discussion

Although, the two taxa belong to the different taxonomical groups, they resemble to each other morphologically as flamentous structure and their distributions on hard substrata in an identical environment. However there are some differences between chemical characteristics from both morphologically different taxa. We believe that the differences in structural features would impact some changes in chemical results.

We found the water, protein, fats, Na and Mg contents of *C. ciliatum* var. *robustum* to be higher than those of *Cl. dalmatica.*

To date, there has not been much information about the chemical content of *Cl. dalmatica* and *C. ciliatum* var.*robustum*. Our findings show that these flamentous taxa contain, on average, 85-90 percent water and 10-15 percent solid material.

There is a correlation between the chemical content of the alga and the chemical features of the sea water (Zavodnik. 1973; 1979).

The taxa that are capable of living in both polluted and unpolluted regions adapt to the environment by losing water via permeability as a reaction due to high osmotic pressure (Chalaupka, 1939) especially due to the dissolved salts in the environmental water (Zavodnik, 1987).

Zavodnik (1973) showed that the amount of ash might increase as a result of abundance in environmental nutritive salts. Çetingül and Güner (1996) reported the irregular variations in the ash amount of green algae collected from polluted and unpolluted waters.

The protein content of *Cl. dalmatica* was found to be lower than that of *C. ciliatum* var.*robustum* (Table 1). Our findings demonstrate that the protein content of *Cl. dalmatica* (8.13 %) growing in natural sea water is lower than those of *Cl. rupestris* (L) Kütz. (28.56 %) (1) and *Cl.ruchingeri* (J.Ag.) Kütz. (3.2-12.3 %, for unpolluted environment and 22.5 % for polluted environment) (Munda, 1990).

The protein content of *C. ciliatum* var.*robustum* was found as 14.81 % (Table 1). Munda (1990) found the protein contents of *C. ciliatum* var.*robustum* as 4.8-14.7 % in unpolluted regions and 16.1 % in polluted regions and the protein content of *C. diaphanum* (Roth.) Harv. in unpolluted regions were found to be 4.3-13.6 %.These findings are consistent with our results. However, Zavodnik and Juranic (1982) found the protein content of *C. diaphanum* to be 12.5 %. Wallentinus (1979a) noted the red algae had usually higher proportion of N than the green algae and the brown algae. Zavodnik and Juranic (1982) emphasized that some red algae had higher protein contents than green and brown algae. The protein content of red alga *C. ciliatum* var. *robustum* was found higher than that of the green alga, *Cl. dalmatica*. P and N are essential elements for algae to grow (Blinks, 1951). N, P and Si have an important role as essential elements for living things to maintain their lives.

Independently from the phylogenetic evolution of the taxa (Wallentinus, 1984), the algae growing in rich nutritive environment have high N and P contents (Wallentinus, 1979c).

The protein and P contents of benthic species change accordingly to the seasons, regions and sea water were reported by Zavodnik and Juranic (1982) and Zavodnik (1987).

Our study showed that *Cl. dalmatica* had a higher P contents (0.22 %) than *C. ciliatum* var. *robustum* (0.11 %) (Table 1). At the time the P element in the sea water was considerably high, Zavonik and Juranic (1982) found the P contents of *Cl. dalmatica* and *C. diaphanum* to be 0.30-0.40 % and 0.42 %, respectively.

The concentrations of nutrients in sea water influence the levels of N and P in algae (Provasoli, 1969).

The range of N/P ratio from 5/1 to 15/1 in sea water is essential for algae and phytoplankton to grow, and the researches have shown that a value beyond that range would have toxic effects (Topping, 1976).

As for the fat ratios of these two taxa collected from the supralittoral zone, *C. ciliatum* var.*robustum* contained more fats than *Cl. dalmatica* (Table 1). Similarly, some researchers have found the fats ratio in one of the red algae, *Gelidiella acerosa* (Forssk.) Feldm. *et* Hamel, much higher than that of *Ulva lactuca* Linn. a green alga (Murthy and Radia, 1978). Miller (1962) suggested that the fats production was stimulated by light.

For the contents of significant salts (Na, K, Ca and Mg) the comparison between the sea water and the two taxa produced interesting results (Table 1,3). The salts were usually in different amounts in algae and sea water. We observed that *C. ciliatum* var.*robustum* had accumulated more Ca than *Cl. dalmatica*. It has been reported that the changes in Ca contents of the algae may be resultant of the high concentrations of Ca in environmental water (Yang and Wang, 1983). Vinogradov (1953) suggested that *Ulva* and some members of Chlorophyceae accumulated and stored significant amounts of Ca. In this study, although Ca content of the sea water was higher than K content, both taxa were found to contain more K compared with Ca. Walker (1957) suggested that the over accumulation of these ions in the algae cells might result from the functioning of the plasmolemma as a diffusion barrier. In this research, K contents were found notably higher than Na and Ca contents in both algae. These findings are consistent with Vinogradov (1953) results. Pillai (1965) suggested that there was an inverse relation between Na and K contents of algae. However, Sitakararao and Tipnis (1967) failed to reveal such relation. Also, Mg content of *C. ciliatum* var.*robustum* was found higher than that of *Cl. dalmatica* and the Mg amount of the sea water was much higher than those in the algae (Table 1 and 3).

The heavy metals such as Fe, Cu, Zn and Mn, which have significant impacts on nutrition as well as on pollution, were not as high (Table 2). In our study, the Fe levels were approximately the same in the two algae. In the gulf of Izmir, the Fe levels in *Cladophora* sp. were found to be 9.1 µg/g wet weight (Cirik *et al.*,1988), 580 µg/g dry weight (Kesgin *et al.*,1987), 2671-3125 µg/g dry weight (Yüksel *et al.*, 1988) and 670-1578 µg/g dry weight (Türkan *et al.*,1989). Then Fe level of *C. rubrum* (Huds.) C. Ag. of Bosphorus was found to be 1750-2879 µg/g dry weight by Güven *et al.* (1993). We found that *Cl. dalmatica* contained more Cu than *C. ciliatum* var. *robustum* (Table 2) and Cirik *et al.*, (1988), Yüksel *et al.*,(1988) and Türkan *et al.*,1989 found the Cu levels in *Cladophora* sp. to be 5.543 µg/g wet weight, 113-194 µg/g dry weight, and 22-45 µg/g dry weight respectively. Also Güven *et al.*, (1993) found the Cu level in *C. rubrum* as 10.80-22.44 µg/g dry weight. Again, we found that *Cl. dalmatica* contained more Zn than *C. ciliatum* var.*robustum* (Table 2). Kesgin *et al.*, (1987), Cirik *et al.*, (1988), Yüksel *et al.*, (1988) and Türkan *et al.* (1989) found the levels of Cu in *Cl. dalmatica* as 40 µg/g dry weight, 7.795 µg/g wet weight, 34-284 µg/g dry weight and 25-100 µg/g dry weight respectively. Güven *et al.*, (1993) found the Cu level in *C. rubrum* as 77.61-31.37 µg/g dry weight. In our study, the Mn level was higher in *C. ciliatum* var.*robustum,* compared to *Cl. dalmatica* (Table 2). Kesgin *et al.*, (1987), Cirik *et al.*, (1988) and Yüksel *et al.*, (1988) demonstrated that the Mn levels in *Cladophora* sp. were 70 µg/g dry weight, 41.146 µg/g wet weight and 81-243 µg/g dry weight respectively. The Mn level in *C. rubrum* was found to be 14.03-28.79 µg/g dry weight by Güven *et al.*, (1993). The amounts of accumulation of heavy metals have changed depending on the species and the regions.

The order of accumulation of heavy metal contents of algae was found as Fe>Zn>Mn>Cu.

Although they are in different taxonomical groups, *Cl. dalmatica. C. ciliatum* var.*robustum* grow in identical settings, and they have similar flamentous structures, morphologically. In this frame, we determined the anatomical and chemical differences. Anatomically, both taxa show dichotomous branching, and the discriminative features are that the taxa belong to the different groups and that *C. ciliatum* var.*robustum* has additional cortex cells around the central cell (in node) and it has carposporangia as reproductive organs. There are significant differences in protein, fats and P contents, also.

The dominant parameters influencing the growth of *Cl. dalmatica* and *C. ciliatum* var. *robustum* are nutrients.

Özet

Bu çalışmada, İzmir Körfezi'nin (Ege Denizi, Türkiye) kuzeyinde yer alan Yeni Şakran koyunun bentik alglerinden *Cladophora dalmatica* ve *Ceramium ciliatum* var. *robustum*'un kimyasal kompozisyonları ve ağır metal birikim düzeyleri incelenerek karşılaştırılması yapılmıştır.

Aynı ortam şartlarında gelişme gösteren bu iki algden *C. ciliatum* var. *robustum*'un su, protein, yağ miktarları *Cl. dalmatica*'nın miktarlarından fazla bulunmuştur. Kül, P, K ve Ca miktarları *Cl. dalmatica*'da *C. ciliatum* var. *robustum*'a göre daha fazla, Na ve Mg miktarları ise *C. ciliatum* var. *robustum*'da *Cl. dalmatica*'dan daha fazla saptanmıştır.

Ağır metallerden Fe'in birikim düzeyi her iki algde yakın olmakla birlikte, Cu ve Zn düzeyleri *Cl. dalmatica*'da *C. ciliatum* var. *robustum*'a oranla daha fazla, Mn düzeyi ise *C. ciliatum* var. *robustum*'da *Cl. dalmatica*'ya oranla daha fazla bulunmuştur. Bununla beraber, her iki grubun ağır metal birikimleri Fe>Zn>Mn>Cu olarak sıralanmaktadır.

Ayrıca, ortam suyunun fiziko-kimyasal parametreleri tayin edilmiştir.

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