SEASONAL VARIATIONS IN THE CHEMICAL COMPOSITION OF SOME ADRIATIC *PHAEOPHYCEAE*

by Ivka MUNDA

The chemical composition of some Adriatic brown algae has been investigated only in regard to the iodine content (VIERTHALER 1867, KLAS 1932, CMELIK 1948, VOUK and CMELIK 1949). MOROVIC (1951) gives a historical survey of these investigations.

Some data on the mannitol and volatile oil content are available in the papers of CMELIK (1948) and CMELIK and MOROVIC (1950). Only scanty data are available, however, concerning the seasonal variations of the mannitol and iodine content.

The present work gives some informations about the chemical composition of some more frequent Adriatic *Phaeophyceae*, which may possibly be used as raw material (e. g. extraction of alginic acid, food industry). The content of ash, chlorine, iodine, mannitol, fat, protein and alginic acid and reducing compounds were followed in the course of one year (october 1958 to september 1959). Special attention was given to the seasonal variations of those compounds.

Materials.

The algal material was obtained from the Oceanographic Institute, Split. Single samples of the Punctariales and *Cladostephus verticillatus* originates from the Northern Adriatic (Rovinj, may) and *Laminaria rodriguezii* from the Southern Adriatic (Sika od Tresijavca, august).

FUCALES : *Fucus virsoides* J. AG., *Cystoseira spicata* ERC. (syn. *amentacea*), *Cystoseira barbata* J. AG., *Cystoseira abrotanifolia* C. AG., *Cystoseira crinita* BORY, *Cystoseira discors* C. AG., *Sargassum vulgare* C. AG.

DICTYOTALES : *Dictyota dichotoma* (HUDS.) LAM., *Dictyopteris polypodioides* (DESF.) LAM., *Padina pavonia* (L.) GAILL.

SPHACELARIALES : *Halopteris scoparia* (L.) LAUV., *Cladostephus verticillatus* (LIGHF.) LYNGB.

PUNCTARIALES : *Colpomenia sinuosa* (MERT.) DERB. et SOL., *Asperococcus bullosus* LAM,

LAMINARIALES : *Laminaria rodriguezii* BORN.

Méthods.

The algal material was obtained in the dry state. It was ground in a Wiley laboratory mill fitted with a 0,5 mm screen. The dry matter content was determined by heating a sample at 105°C for 24 hours, the ash content by combustion in a Heraeus laboratory oven at 400°C for 24 hours.

The amount of chlorine was determined according to Mohr in an aqueous extract of the meal.

The iodine content was determined by the method of BAGGESGAARD RASMUSSEN and BJERRESØ. Ether soluble substances were determined by means of Soxhlet extraction. For protein determination the KJELDAHL procedure was followed and for mannitol determination the method of CAMERON et al.

The sulfate content was determined turbidimetrically after BUTTERS and CHENERY (1959). The amount of reducing compounds was determined by the potentiometric method of HAUG and LARSEN (1958).

Alginic acid was determined by the Ca acetate method (HAUG and LARSEN — to be published). The cations were removed from the tissue by means of 0.2 N HCl and replaced with Ca ions (treating with 0.03 M Ca acetate for 24 hours). The Ca ions, bound to alginate, are liberated by means of 0.2 N HCl and determined by complexometric titration.

Results.

The results are given in tables and expressed as percentage of the dry matter, while the reducing power is expressed in milli-equivalents.

The *ash content* of all the Adriatic species investigated was rather high (table 1). The ash determination does not show a correct picture of the anorganic compounds of the algae, owing to the chemical reactions taking place during combustion. Our ash determinations were also disturbed, especially in the case of *Cystoseirae*, by epiphytic and epizooic organisms, which are difficult to remove.

Among the Fucales, *Fucus virsoides* showed the lowest values and slightest variations throughout the year. Among the *Cystoseirae*, *Cystoseira spicata* showed the highest values the winter-spring period. A sharp minimum in summer was followed by a steep increase in september. This maximum in early fall was common for all the observed species, with exception of *Fucus virsoides*. In the *Cystoseirae*, the results indicate that thallus growth and ash content may be correlated. The ash content was higher in the perennial basal parts and decreased with the progressive growth of the primary and secondary branches. In *Cystoseira spicata* the growth of the primary branches begins at the end of december (ERCEGOVIC 1952) and the minimum ash content coincides with the maximal size of the thallus. After the branches were rejected, the ash content increased. In *Cysteoseira barbata* an intensive vegetative growth takes place already in late fall. The ash content decreased slightly during the winter — spring period and reached a sharp minimum in june. *Cystoseira abrotanifolia* showed low values from january to july, without a sharp minimum, the thallus growth being diminished during summer. The ash content of the *Dictyotaceae*, *Sargassum* and *Halopteris* also reached high values in september. In the case of *Padina pavonia* the high ash content was due to calcareous incrustations of the tissue.

The *chlorine content* of the samples is given in table 4 as per cent of the dry matter. Table 3 gives the chlorinity (parts per thousand) of the sea water at the time of sampling. The high chlorine content observed in our samples is probably due to a relatively high content of water in the fresh plants and high chlorinity of the Adriatic water. At the end of the fructification period, a decrease took place in the chlorine content of the *Cystoseirae*. *Fucus virsoides* showed a maximum in february and *Halopteris scoparia* a progressive decrease from april to september.

When calculated as percentage of the ash, the chlorine content did not exceed the values usually observed in Atlantic Fucales. The existence of a correlation between the seasonal variation in the chlorine and ash contents is very uncertain, although the rapid increase in ash in early fall coincided with a pronounced decrease in the chlorine content. This fact may be due, in the case of the *Cystoseirae*, to the luxurious growth of epiphytic and epizooic organisms on the perennial basal parts.

The amount of *iodine* (table 5) was rather low in the *Cystoseirae*. The iodine content of *Fucus virsoides* was somewhat higher and it decreased progressively towards the autumn. Among the Fucales, the highest values were found for *Sargassum vulgare*, which had its maximum in april. Only negligible amounts were present in the *Dictyotaceae*. Among all the species investigated, *Laminaria rodriguezii* and *Halopteris scoparia* showed the highest values, the iodine content of the latter being higher in winter and spring. We find no obvious differences in comparing our data with those obtained by CMELIK. He found somewhat higher values for *Laminaria*, *Sargassum*, *Cystoseira abrotanifolia* and *Cystoseira discors*. This is quite probably due to the different localities used and to annual variations. On the contrary, KLAS did not get a positive reaction by means of the KYLIN method.

Date	10/10	30/12	31/1	3/3	31/3	5/5	8/6	5/7	1/9
Cystoseira barbata	39.8	43,5	42.5	45.1	44.6	37.0	33.7	30.2	46.2
Cystoseira spicata	36.2	39.0	38.5	31.1	31.5	32.9	33.4	35.3	47.1
Cystoseira abrotanifolia	40.8	38.0	37.1	34.0	34.6	34.1	29.5	39.9	44.4
Cystoseira crinita									40.8
Cystoseira discors									41.7
Fucus virsoides	33.7		29.1	28.4	25.1	28.0	25.5	24.5	24.6
Sargassum vulgare			31.0	33.5	38.5	36.0	37.4		46.1
Dictyota dichotoma			35.5	42.0	45.6	38.5	45.8	40.1	53.9
Dictyopteris polypodioides ...			36.9	41.2	50.0	52.2	50.2		
Padina pavonia	70.9								62.9
Halopteris scoparia			44.0	44.1	44.0	45.8	50.2	50.5	61.2
Laminaria rodriguezii, 10/8 .	53.9								

TABLE 1. — *Ash, g per 100 g dry matter.*

Date	10/10	30/12	31/1	3/3	31/3	5/5	8/6	5/7	1/9
Cystoseira spicata.........	20.1	29.4	30.0	30.0	34.2	26.4	32.8	25.8	13.5
Cystoseira barbata	19.2	31.2	31.8	27.7	26.4	21.1	30.3	18.0	11.8
Cystoseira abrotanifolia	22.9	23.7	27.5	40.4	34.6	35.5	26.0	32.5	15.6
Cystoseira crinita									16.8
Cystoseira discors									12.8
Fucus versoides	18.6		33.4	25.6	16.7	28.0	29.3	31.6	24.5
Sargassum vulgare			33.4	30.6	31.2	33.8	29.5		30.6
Dictyota dichotoma			29.4	28.0	31.8	26.1	29.9	31.5	20.5
Dictyopteris polypodioides ...			23.4	20.8	17.5	23.6	25.3		
Padina pavonia	13.4								11.1
Halopteris scoparia			21.2	28.4	31.2	25.6	18.2	12.3	10.3
Laminaria rodriguezii, 10/8 ..	23.3								

TABLE 2. — *Chlorine, g per 100 g ash.*

10/10	30/12	31/1	3/3	31/3	5/5	8/6	5/7	1/9
18.23	19.98	19.66	20.23	19.80	17.88	19.23	19.48	17.69

TABLE 3. — *Chlorine, parts per thousand, in the sea water at the time of sampling.*

The *sulfate content* was determined only in samples from june 1959. The results are given in table 11 as grams per 100 g dry matter. *Halopteris*, *Dictyota*, *Dictyopteris* and *Fucus* contained more sulfate than *Cystoseirae* and *Sargassum*.

Date	10/10	3/12	31/1	3/3	31/3	5/5	8/6	5/7	1/9
Cystoseira spicata	7.99	12.60	12.67	13.51	15.03	11.70	11.05	8.81	6.31
Cystoseira barbata	7.80	11.93	11.82	9.45	9.15	7.19	8.95	7.15	5.17
Cystoseira abrotanifolia	8.31	9.21	10.53	12.64	10.90	11.74	9.00	11.52	7.36
Cystoseira crinita									6.94
Cystoseira discors									5.35
Fucus virsoides	6.10		9.78	7.34	4.15	7.81	7.45	8.05	6.26
Sargassum vulgare			10.34	10.52	11.87	12.15	11.01		14.10
Dictyota dichotoma			10.39	11.76	14.32	9.95	13.74	12.75	11.01
Dictyopteris polypodioides			8.47	8.50	5.25	12.24	12.75		
Padina pavonia	9.52								6.92
Halopteris scoparia			9.32	12.59	13.70	11.58	9.16	6.21	6.21
Laminaria rodriguezii, 10/8	12,54								

TABLE 4. — *Chlorine, g per 100 g dry matter.*

Date	10/10	31/1	31/3	8/6	1/9
Cystoseira spicata	0.005	0.012	0.004	0.004	0.004
Cystoseira barbata	0.007	0.014	0.012	0.012	0.011
Cystoseira abrotanifolia	0.043	0.012	0.008	0.005	0.005
Cystoseira crinita					0.011
Cystoseira discors					0.019
Fucus virsoides	0.021	0.025	0.011	0.009	0.006
Sargassum vulgare		0.067	0.095	0.064	0.015
Dictyota dichotoma		0.002	0.002	0.014	0.003
Dictyopteris polypodioides		0.004	0.004	0.013	
Padina pavonia	0.017				0.018
Halopteris scoparia		0.170	0.170	0.093	0.099
Laminaria rodriguezii, 10/8	0.126				

TABLE 5. — *Iodine, g per 100 g dry matter.*

CMELIK (1950) found a maximum *mannitol content* for the same *Cystoseira* species and *Sargassum linifolium* in december, while the minima occurred at different seasons (*Cystoseira abrotanifolia* and *Sargassum* in july, *C. barbata* in october and *C. amentacea* in february).

Or samples showed relatively high mannitol content in the autumn (table 6). This period was followed by a minimum in early spring and a pronounced increase during the summer. The mannitol content of all the species, with exception of *Cystoseira abrotanifolia* and *Fucus virsoides*, showed a slight decrease in autumn. In the case of *Halopteris scoparia*, we were not able to detect mannitol during the winter — spring period, while it was present in the samples collected in summer. It seems to be a general rule, that the mannitol content decreases during the winter period and reaches its minimum in spring. The

Date	10/10	30/12	31/1	3/3	31/3	5/5	8/6	5/7	1/9
Cystoseira barbata	5.91	4.71	4.30	2.31	1.82	5.53	11.51	11.00	7.71
Cystoseira abrotanifolia	6.28	6.40	5.15	3.33	5.40	9.50	8.69	8.81	9.23
Cystoseira spicata	5.50	1.60	1.55	1.22	3.31	4.91	8.49	10.00	7.31
Cystoseira crinita									3.51
Cystoseira discors									8.90
Fucus virsoides	8.57		3.62	1.51	3.32	9.14	6.69	5.72	8.30
Sargassum vulgare			4.01	2.92	0.46	6.35	5.45		8.73
Dictyota dichotoma			5.65	1.21	0.55	7.51	7.47	8.45	5.91
Dictyopteris polypodioides			5.15	2.00	0.91	4.76	4.28		
Padina pavonia	2.73								3.31
Halopteris scoparia	0.0		0.00	0.00	0.00	3.50	1.00	0.64	0.00
Laminaria rodriguezii, 10/8	9.65								
Colpomenia sinuosa, 14/5	3.61								
Asperococcus bullosus, 14/5	0.00								

TABLE 6. — *Mannitol, g per 100 g dry matter.*

minimum coincided with the sprouting of the *Cystoseira* branches. The summer maximum occurred in may in the *Fucus* and *Sargassum* and in the *Cystoseirae* in june - july. The relatively small decrease taking place in the autumn indicates that the seasonal variations are the same in the perennial basal portions and in the branches.

Date	10/10	30/12	31/1	3/3	31/3	5/5	8/6	5/7	1/9
Cystoseira spicata	6.9	9.6	10.5	10.1	10.0	9.1	6.5	7.2	7.0
Cystoseira barbata	8.3	8.7	9.2	7.0	7.5	7.3	5.9	6.0	5.4
Cystoseira abrotanifolia	6.8	6.5	7.7	7.9	9.4	7.8	5.4	5.3	4.8
Cystoseira crinita									7.8
Cystoseira discors									5.2
Fucus virsoides	5.1		11.8	8.5	8.5	9.0	7.9	5.4	5.5
Sargassum vulgare			13.7	13.5	11.5	10.4	7.9		6.5
Dictyota dichotoma			12.0	16.6	13.6	13.3	11.0	12.8	9.0
Dictyopteris polypodioides			12.5	14.2	13.9	11.5	10.3		
Halopteris scoparia			10.0	11.7	11.0	11.0	7.3	6.9	7.4
Padina pavonia	4.1								4.4
Laminaria rodriguezii, 10/8	7.2								

TABLE 7. — *Protein, g per 100 g dry matter.*

CMELIK determined mannitol gravimetrically as an insoluble tribenziliden derivative and found generally lower values.

The seasonal variations of the *protein content* (table 7) was generally controverse to the variations of the mannitol. Maximum values were observed when the thallus growth is the most intensive. Among the *Cystoseirae*, the highest values were found in *Cystoseira* *spicata*. In this species no pronounced maximum was observed, but high values were found from december to may. In *Cystoseira barbata* the branch growth starts earlier than in the other *Cystoseira* species. The protein maximum occurred already in february, and was followed by a slight decrease towards a minimum in the fall. *Cystoseira abrotanifolia* had an

Date	10/10	30/12	31/1	3/3	31/3	5/5	8/6	5/7	1/9
Cystoseira spicata	0.058	0.052	0.077	0.072	0.093	0.046	0.041	0.036	0.041
Cystoseira barbata	0.097	0.154	0.103	0.123	0.140	0.067	0.067	0.077	0.072
Cystoseira abrotanifolia	0.059	0.051	0.124	0.051	0.082	0.061	0.067	0.052	0.067
Cystoseira crinita									0.041
Cystoseira discors									0.062
Fucus virsoides	0.095		0.231	0.125	0.149	0.311	0.293	0.237	0.360
Sargassum vulgare			0.067	0.062	0.067	0.036	0.025		0.036
Dictyota dichotoma			0.041	0.056	0.056	0.043	0.026	0.25	0.031
Dictyopteris polypodioides ...			0.041	0.041	0.036	0.023	0.020		
Padina pavonia	0.020								0.005
Halopteris scoparia			0.067	0.051	0.056	0.035	0.007	0.010	0.025
Laminaria rodriguezii, 10/8.	0.005								

TABLE 8. — *Reducing power, mequiv, per g dry matter.*

expressed maximum in april. *Fucus vrsoides* showed a maximum in february, coinciding with the most luxurious growth. Rather high values, but no pronounced maximum, were observed in *Sargassum*, followed by a rapid decrease towards summer. *Halopteris* had a rather high protein content from february to may and a sharp drop during summer and autumn. The *Dictyotaceae* contained more protein than the *Fucaceae* and had the maximum content in march.

Date	10/10	30/12	31/1	3/3	31/3	5/5	8/6	5/7	1/9
Cystoseira spicata	0.89	0.78	2.11	2.00	1.57	1.90	0.80	0.94	1.05
Cystoseira barbata	0.89	0.81	2.58	1.85	2.20	0.92	0.33	0.20	0.51
Cystoseira abrotanifolia	0.97	0.51	1.50	0.76	0.66	0.80	0.90	0.82	0.55
Cystoserira crinita									0.82
Cystoseira discors									0.48
Fucus virsoides	6.68		3.51	2.86	2.21	4.10	3.85	4.08	4.34
Sargassum vulgare			1.22	0.97	0.89	1.22	0.96		0.57
Dictyota dichotoma			4.35	5.08	4.38	6.28	4.96	5.30	3.49
Dictyopteris polypodioides ...			1.19	2.03	1.19	2.00	1.65		
Padina pavonia	2.73								1.05
Halopteris scoparia			1.35	2.00	1.35	2.03	0.88	0.64	0.70
Laminaria rodriguezii, 10/8.	0.89								

TABLE 9. — *Fat, g per 100 g dry matter.*

The amount of the reducing compounds present in the Adriatic algae was very low compared to Atlantic Fucales (table 8). The highest amounts were found in *Fucus virsoides*, which contained up to 0.30 mequiv. per g dry matter, the content being higher in summer and autumn. Among the *Cystoseirae* the relatively highest values were noticed in *Cystoseira barbata*. Only slight seasonal variations could be detected.

The *ether soluble substances* (table 9) were most abundant in *Dictyota dichotoma*, with the highest values in summer. *Dictyopteris polypodioides* and *Halopteris scoparia* showed lower values, without any prominent seasonal variations, and the same was true for Sargassum vulgare. The fat content of *Fucus virsoides* was low from february to may and high in summer and autumn. The seasonal variation of the amount of ether soluble substances in *Cystoseira spicata* and *Cystoseira barbata* was opposite to that of *Fucus*, while *Cystoseira abrotanifolia* showed only slight variations.

Date	10/10	30/12	31/1	3/3	31/3	5/5	8/6	5/7	1/9
Cystoseira spicata	21.1	19.2	19.1	16.8	19.6	24.1	23.4	23.4	15.2
Cystoseira barbata	24.0	21.3	20.8	20.6	20.9	24.7	18.6	17.7	17.9
Cystoseira abrotanifolia	23.2	20.1	20.3	19.3	20.7	21.7	23.3	20.7	18.4
Cystoseira crinita									19.7
Cystoseira discors									19.6
Fucus virsoides	22.1		22.9	23.5	24.3	24.3	18.4	18.3	17.6
Sargassum vulgare			21.5	19.5	19.6	21.3	22.0		13.9
Dictyota dichotoma			9.3	12.7	12.1	11.3	11.4	11.7	8.6
Dictyopteris polypodioides			12.7	12.9	12.6	13.1	15.9		
Padina pavonia	9.7								6.9
Halopteris scoparia			14.2	14.4	14.2	14.3	14.3	12.8	8.7
Laminaria rodriguezii, 10/7	30.3								
Cladostephus verticillatus, 14/5	21.9								
Asperococcus bullosus, 14/5	8.8								
Colpomenia sinuosa, 14/5	16.5								

TABLE 10. — *Alginic acid, g per 100 g dry matter.*

The amount of *alginic acid* was higher in the *Fucaceae* and *Cladostephus* than in *Dictyotaceae* and *Halopteris*. Lower values were observed in Punctariales. With 30,3 % alginic acid *Laminaria rodriguezii* had the highest content of all the species investigated. (table 10).

Cystoseira spicata	2.6
Cystoseira barbata	3.4
Cystoseira abrotanifolia	3.4
Fucus virsoides	4.7
Sargassum vulgare	2.6
Dictyota dichotoma	4.3
Dictyopteris polypodioides	3.9
Halopteris scoparia	5.9

TABLE 11. — *Sulfate, g per 100 g dry matter at the* 8/6.

Cystoseira spicata	19.5
Cystoserira barbata	16.9
Cystoseira abrotanifolia	23.9
Fucus virsoides	27.2
Sargassum vulgare	21.1
Dictyota dichotoma	21.3
Dictyopteris polypodioides	21.7
Halopteris scoparia	21.2

TABLE 12. — *Dry weight, g per 100 g fresh weight at the* 31/1.

In *Cystoseira* species the alginic acid content was low during winter and early spring. A marked increase took place in may, when the vegetative development is the most intensive, and the content remained high during the summer, when the branches had their maximum length. When the branches were rejected, the alginic acid content dropped, indicating that the amount of alginic acid is lower in the perennial basal parts of the thallus than in the branches. The alginic acid content in *Fucus* showed high values in winter and spring, and in *Sargassum* a minimum occurred during spring. Rather low values were observed in *Dictyotaceae* and *Halopteris*, without any prominent seasonal variations.

Comparing the seasonal variation of the alginic acid with the variations of the ash, an expressed negative correlation was obvious. In *Cystoseirae* and in *Fucus* the protein maximum corresponded to relatively low values in alginic acid. It seems possible that the observed variations in alginic acid represent only a secondary effect, due to the variations in the ash and protein content.

There is no obvious correlation between the protein and alginic acid content in *Halopteris* and *Dictyotaceae*.

The variation in the mannitol and alginic acid content showed the same trend in *Cystoseirae*, while in *Fucus virsoides* an expressed negative correlation was found. No relations were obvious in the case of *Dictyotaceae* and *Halopteris*.

Conclusion.

Among the compounds investigated, mannitol shows the most prominent seasonal variations. In all species investigated, the mannitol content is high in summer and fall and low in winter and early spring. The protein content seems generally to be higher in the period from january to may than the rest of the year, the protein maximum coinciding with the period of growth.

In the *Cystoseirae* the variation in chemical composition is probably closely connected with the vegetative cycle of these plants. The ash content is lowest in the period when the thallus is fully grown and show a maximum when only the perennial basal part is left, this maximum coinciding with a minimum in the chlorine content. Alginic acid shows the opposite trend. To some extent, this difference in chemical composition may be due to the marked growth of epiphytic and epizooic organism on the perennial parts.

All the species investigated contain very small amounts of reducing compounds compared to Atlantic Fucales. The highest reducing power was found in *Fucus virsoides*.

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