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PRELIMINARY INVESTIGATION INTO THE BIOCHEMICAL COMPOSITION OF THE ADRIATIC PHYTOPLANKTON

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INTRODUCTION

Phytoplanktonic organisms as autotrophs are the main producers of organic matter, and also the basis of almost all the nutrition chains in the sea. To better characterize this first link in the nutrition chain it is necessary, in addition to others, to know their chemical composition.

Brandt (1898, 1919) was the first who examined the chemical composition of marine plankton. Other autors, among whom are Vinogradov (1927, 1938), Vitok (1964), Corner (1961) and Platt and Irwin (1973) also studied this problem. In the studied samples one phytoplanktonic species dominated, so the autors attributed all the characteristics of the sample to this one predominant species. The researches were carried out as to the carbohydrate, lipid, protein, and the inorganic constituents such as silicic acid, chlorine, phosphorus, calcium, iron et al. The seasonal and yearly changes of the chemical composition were also studied.

Recently, in order to better characterize proteins, many authors have examined the contents of amino acids in the phytoplankton cultures (Jeffries, 1969; Chau, Chuecas and Riley, 1967; Chuecas and Riley, 1969), and also in the marine phytoplankton samples (Cowey and Corner, 1962, 1963a, 1963b).

We have no data on the chemical composition of the phytoplankton in the Adriatic Sea. There are only the data of the preliminary examinations on the chemical composition of the zooplankton samples (Vučetić, Damjanić and Čubretović, 1969).

The main purpose of this work was to determine the biochemical composition of the phytoplanktonic populations from the Kaštela Bay. This was only a preliminary work, and the researches are to be continued in order to obtain more data on the seasonal changes in the biochemical composition in relation to the succesions of the phytoplanktonic populations.

METHODS AND TECHNIQUE

Samples were collected during the September 1969 in the Kaštela Bay near Split, 50—60 m from shore, by towing horizontally at 1—1,5 m depth a Nansen's net (smaller dimensions) with mesh size 50μ.

The microscopic analysis did not reveal any considerable quantities of zooplankton in the samples, so the obtained characteristics of the samples were considered to be phytoplanktonic exclusively.

Approximately one hour after sampling the samples were washed with destilled water and concentrated by centrifuging at 6,000 r.p.m. Thus obtained suspension of phytoplankton was stored in the dark at —20°C. After the

frozen samples had been lyophilized, the dry material was stored in sealed glass vials in a desiccator at 0—5°C.

Within the work on the determination of the biochemical composition the content of ash, water, lipid, carbohydrate, protein, free and total amino acids, pigment were determined.

The water content in the sample was determined by drying the sample in the oven at 105°C to the constant weight. Weighings were made after cooling in a desiccator.

To determine ash content phytoplankton sample was ashed to constant weight in a crucible at 500°C in a muffle furnace. Weighings were made after cooling in a desiccator.

The lipid content was determined according to the method of Bligh and Dyer, 1959, using the 1 : 2 : 0,8 chloroform — methanol-water mixture for the extraction.

The amount of carbohydrate was determined in the hydrolysed phytoplankton sample using the GOD-Perid method (Werner, Rey and Wielinger, 1970). Phytoplankton sample was hydrolysed by adding 10 ml conc. H_2SO_4 to one gramme of sample and refluxing for period of 10 hours. After the cooling the sample was neutralized with NaOH and filtered through a quantitative filter paper (blue-ribbon) to remove the remains of the cells. Yellowish colour was removed by boiling the sample with active carbon.

The content of free glucose in the cellular extract was determined. Cellular extract was obtained by crushing one gramme of the lyophylized sample with approximately two gramme wet aluminium oxide and 1 ml 0,1 M phosphate buffer pH = 7,0. Mixture was centrifuged at 0°C at 15,000 r.p.m. Aluminium oxide settled on the bottom of the centrifuging cuvette, above it a layer of uncrushed cells and cell membranes, and on top a viscous layer of the cell extract were formed. The cellular extract was separated and the aluminum oxide was washed twice with about 3 ml of phosphate buffer, and again centrifuged. The amount of free-glucose in cellular extract was determined using the GOD-Perid-method.

The protein content was calculated by multiplying the total nitrogen content with the 6.25 factor (Strickland, 1960). 15—25 mg of the lyophilized sample was used for the determination of total nitrogen content using the Markham semi-micro apparatus.

Free amino acids were determined according to Jeffries, 1969, using the Amino Acid Analyzer. About 3 g of the lyophilized sample or about 30 g of the suspension (3 g of dry weight) were used.

Total amino acids were determined in the hydrolysate of the sample according to the method of Moor, Spackman and Stein, 1958, using Amino Acid Analyzer. Acid hydrolysis was carried out by heating the samples with 6N hydrochloric acid for 24 hours in evacuated sealed vials at 105°C. The quantity of acid used was of the order of 3,000 times the weight of protein (as astimated from the nitrogen analysis). Hydrolysates were filtered, evaporated to dryness under reduced pressure at 60°C, then dissolved in 0.2N sodium citrate pH 2.2 (Moore and Stein, 1954) and made up to the

required volume. Two milliliters (0.03186 g dry weight) of this solution was put on the column of the Amino Analyzer.

The pigments were determined according to Strickland and Parson, 1965. About 2 g of sample was extracted with 5 ml of 90% acetone. The characteristic extinctions were determined at several wavelengths and their values were introducted into the equations to calculate the concentrations of the pigments. The values of the chlorophyll »a« and »b« concentrations were expressed per 100 mg of dry weight.

The Samišev, 1970, method was applied for the determination of the calorific value of the sample.

RESULTS

Water and ash contents:

There is an average of 3% of water in the lyophilized sample in spite of lyophilization.

Total ash as percentage of dry weight was 74,5 ± 0,3%.

Total nitrogen and protein contents:

The value of total nitrogen as percentage of dry weight was 0,57 ± 0,05%. On the assumption that all the body nitrogen was present as protein, and that proteins contain on an average 16% (factor 6,25) nitrogen, the protein equivalent would be 3,6 ± 0,3%.

Free amino acids :

The content of free amino acids was too low therefore it was tried to determine them not only on the lyophilized sample but also on larger amounts of a fresh suspension. Using Amino Analyzer it was impossible to determine any of the free amino acids.

The results on total amino acids determinations are given in Table 1

Table 1: Amino acid content in the phytoplankton of Kaštela Bay.

	24 hour hydrolysis		
	mg amino acid/ 100 g dry weight	% amino acid/ 100 mg protein	% amino acid to leucine (1.000)
ASP	127.86	18.031	1.624
THR	45.26	6.383	0.575
SER	37.83	5.335	0.480
GLU	15.74	2.219	0.199
GLY	71.76	9.739	0.877
ALA	66.82	9.423	0.849
VAL	106.60	15.018	1.353
MET	19.39	2.729	0.245
ILE	51.15	7.213	0.649
LEU	78.70	11.098	1.000
TYR	36.23	5.109	0.460
PHE	49.55	6.522	0.587
LYS	61.40	8.658	0.780
HIS	17.06	2.362	0.212
ARG	47.03	6.632	0.597
Total	832.38		

The experimental results showed that 100 mg of dry weight of the sample contained: 832.38 μg amino acids, 709.21 μg proteins, 113.20 μg protein nitrogen, and 550 μg total nitrogen.

Lipid content:

By desiccating the sample extract above the phosphorus pentoxide in the desiccator the lipids in the amount of $1.33 \pm 0{,}4\%$ were found.

Carbohydrate content:

In the hydrolysate of the sample there was $11.5 \pm 0.8\%$ of glucose per dry weight, and in the cell extract $0.3 \pm 0.04\%$ of glucose per dry weight.

Pigments:

Table 2 shows the characteristics extinctions of the pigment extract.

Table 2: Extinction values of the pigment extract at some characteristic wavelengths.

Wavelengths (Å)	7500	6650	6450	6300	5100	4800
E	0.185	0.120	0.110	0.108	0.180	0.270

By introducing these values into the equations (Strickland and Parson, 1965) the values for the chlorophyll »a« and »b« were obtained, but the chlorophyll »c« could not be caluculated with this method.

(1) Chlyorophyll »a« concentration according to Richard = 0.963 mg/100 dry weight
(2) Chlorophyll »a« concentration according to Parson and Strickland = 0.758 mg/100 mg dry weight
(3) Chlorophyll »a« concentration according to Humphrey and Jeffrey = 0.880 mg/100 mg dry weight
(4) Chlorophyll »b« concentration according to Richard = 0.625 mg/100 mg dry weight
(5) Cholorophyll »b« concentration according to Parson and Strickland = 0.787 mg/100 mg dry weight
(6) Chlorophyll »b« concentration according to Humphrey and Jeffrey = 0,903 mg/100 mg dry weigt.

Calorific value:

The calorific value of the sample was 314 cal per 100 mg dry weight.

DISCUSSION

The examinations of the chemical composition of the lyophilized planktonic sample revealed the presence of great amount of inorganic matter (74,5%).

The high value of the inorganic matter in the sample was probably due to the presence of the traces of the electrolytes, that could not have been removed in spite of having been washed with distilled water. The content of the inorganic matter was increased also by the calcium and silicium content in the coccolithophores and diatoms. The quantitative analysis of the

collected phytoplankton samples showed that it consisted mainly of diatoms, which is characteristic of the area (Pucher-Petković, 1966).

One of the reasons for a high content of inorganic substances in the sample could be also the suspended material in the sea.

The examination of the net plankton samples, collected in the waters of the Plymouth Bay in September 1960, and filtered through a 0.5—1.0 μ membrane filter revealed the presence of 40—60% of inorganic matter (Corner, 1961). The values obtained by our examinations could not be compared with the above values, because nets of various mesh size were used. It is supposed that while sampling with a net of 50 μ mesh size a considerable amount of nannoplankton escaped and this probably resulted in a reduced content of the organic substances in the sample.

The organic matter in the sample consists mainly of protein, carbohydrate and lipid, so that their quantities can be compared with the values obtained by some other authors (Table 3).

Table 3: Organic components in the phytoplankton samples expresed in percent per dry weight.

Sample	protein	carbohydrate	lipid
Kaštela Bay Sept. 1969	21.6	70.0	8.4
Plymouth Corner, 1961	10.1	66.5	23.4
Diatoms (Chaetoceros sp.) Brandt, 1898	—	63.8	29.0
Dinoflagelata (Ceratium sp.) Ketchum and Redfield, 1949	13.6	85.0	1.4

It can be seen from Table 3 that the content of carbohydrate in the examined sample is similar to the sample of Plymouth sea water, however, it contains considerably less lipid and more protein.

The protein content was determined from the total nitrogen by Kjeldahl and amino acid nitrogen. The disparity between Kjeldahl nitrogen (550 μg/100 mg dry weight) and amino acid nitrogen (113.20 μg/100 mg dry weight) was obtained. It may be due to the presence of nonprotein compounds in the analyzed sample and because no examinations of cystine and tryptophan were conducted. The amount of the experimental material was not sufficient for separate chemical methods for their estimations. During the hydrolysis cystin is partly and tryptophan is almost completely destroyed.

The content of the free amino acids in the sample was below the detectable limit of the analitical method used so it could not be determined. According to the literature data phytoplankton has a very small content of free amino acids. It was found in unicellular alga *Scenedesmus* that about 93% of total nitrogen was present in proteins, 4% in various low mollecular

compounds soluble in water and only 3% of total nitrogen belonged to peptides and free amino acids (Strickland, 1960).

The content of amino acids determined in the hydrolysate of the sample can be compared with the results obtained by Cowey and Corner, 1962, who examined the phytoplankton samples from the Plymouth sea water. Table 4 shows the comparison of the relative molar quantities of amino acids (leucine 1.000) in the Kaštela Bay sample and Plymouth samples.

Table 4: Relative molar quantities of amino acids in the phytoplankton samples (leucine 1.000).

Amino acid	Sample from the Kaštela Bay, Sept. 1969	Samples of Plymouth sea water Cowey and Corner, 1962 Aug.—Sept. 1960	Aug., 1961
ASP	1.624	1.02	0.95
THR	0.575	0.83	0.64
SER	0.480	1.12	1.00
GLU	0.199	1.04	0.95
GLY	0.877	1.51	1.86
ALA	0.849	1.24	1.27
VAL	1.353	0.94	0.80
MET	0.245	—	—
ILE	0.649	0.67	0.61
LEU	1.000	—	—
TYR	0.460	0.38	0.21
PHE	0.587	0.62	0.53
LYS	0.780	1.06	0.62
HIS	0.212	0.13	0.04
ARG	0.597	0.57	0.52

It can be seeen from Table 4 that the amino acid composition of the sample of the Kaštela Bay show certain differences from the amino acids composition of the Plymouth samples.

The obtained calorific value (314 cal/100 mg ash-free dry weight) is in accordance with the recently published values (206—374 cal/100 mg ash-free dry weight) Platt and Irwin, 1973. For example, the calorific value for the zooplankton is much higher (500 cal/100 mg ash-free dry weight) Vitok, 1964.

CONCLUSIONS

From the obtained results it can be concluded that the examined phytoplankton sample consists of a large quantity of inorganic matter and a very small quantity of organic matter.

Considering the ratio of the basic organic chemical constituents it can be noticed that the content of the carbohydrate in the examined sample is

similar to that in the sample from the Plymouth sea water, however, it contains less lipid and more protein.

In the protein of the sample there are mainly all the assential amino acids and the content of the amino acids in the examined sample shows certain differences from the same content of the sample of the Plymouth sea water in the same period of the year.

The calorific value was similar to the published values.

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KRATAK SADRŽAJ

Ovaj rad je prvi pokušaj određivanja osnovnog organskog kemijskog sastava fitoplanktona Jadrana.

Ispitivan je liofilizirani uzorak dobijen horizontalnim povlačenjem planktonske mreže (veličina oka 50μ) u Kaštelanskom zaljevu, septembra 1969. U uzorku su prevladavale dijatomeje.

Uzorak je sadržavao veliku količinu anorganske tvari (74,5%). Organski dio uzorka se sastojao od 21,6% proteina, 70,0% ugljikohidrata i 8,4% lipida.

U hidrolizatu proteina, nakon 24-satne hidrolize, ustanovljeno je: 932,38μg amino kiselina, 709,21 μg proteina, 113,20 μg proteinskog dušika i 550,00 μg ukupnog dušika na 100 mg suhe težine uzorka.

Kalorična vrijednost je 314 cal na 100 mg suhe težine uzorka.