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The separation and identification of phytoplankton pigments from the adjacent waters of Great Wall Station, Antarctica

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Abstract This paper presents separation and identification of the pigments of the phytoplankton samples collected from the adjacent waters (62°12.5′S, 58°53′W～62°14.5′S, 58°57′W) of Great Wall Station, Antarctica during March 1988～February 1989 by using thin-layer chromatographic techniques. Of the 15 kinds of phytoplankton pigments separated, 13 were identified according to the R_f values of the various marine algal pigments. The features of seasonal variations of phytoplankton pigments in the studied area are also discussed.

Key words Great Wall Station, phytoplankton pigments.

1 Introduction

In the ocean, phytoplankton is the major primary producer and plays an extremely important role in the production of organic substances. It is indispensable for us to understand the dynamics of phytoplankton which reveals the carbon cycle and the energy flow of oceanic ecosystems. The photosynthesis of phytoplankton mainly depends on pigments within plankton chloroplasts (mainly chlorophyll). So the phytoplankton pigments attracted the interest of oceanographers in early time.

The research of the phytoplankton pigments begun early in 1920's. In 1906, Tswett published the monograph "Pigments of the Animal Kingdom"; Lind *et al* (1953) developed the chlorophyll extract with some solvents one after another on the first dimension carrier and with another solvent on the second dimension carrier and obtained six clearly separated zones of: carotene, xanthophyll, chlorophyll-a and -b, an unknown pigment and a colorless fluoresent substance; in the 1960'S, Jeffrey (1961) made a number of analyses and researches of oceanic phytoplankton pigment separation by using this method. Especially in recent years, very great progress has been made in both separation and analytical methods and high pressure liquid chromatography has been adopted in rapidly determining microgram quantities of pigments. At the same time remote sensing techniques have been used to carry out investigations on global primary productivity according to the principle that differences in chlorophyll concentration in sea waters will produce different reflectivity of seawater.

In this paper, thin-layer chromatography is used as the means of the separation of o-

ceanic phytoplankton pigments. This method needs a small quantity of samples, the development time is short, the method is simple and the rate of recovery is high, with the rate of recovery generally being over 95%. There have been a number of reports already on this aspect (Lind *et al.*, 1953; Jeffrey, 1968,1974,1981; El-Sayed and Mandelli, 1965). This paper reports the results of the separation and identification of the phytoplankton pigment samples from the adjacent waters of Great Wall Station, Antarctica.

2 Material and method

Four-five liters of surface sea water were collected from the adjacent shallow waters of the Great Wall Bay, Antarctica (62°12.5′S, 58°53′W～62°14.5′S, 58°57′W) from March 1988 to February 1989, and filtered with micropore filter membranes (0.8 μm) with suction. The phytoplankton samples were concentrated on the filter membranes, stored at −30°C and brought back home for analysis.

2.1 Preparation of the samples

The filter membranes which were fully loaded with phytoplankton samples were put into centrifugal tubes, and after addition of 90% acetone solution preserved in a refrigerator for extraction for 14 h, centrifuged at 3200 r.p.m. for 15 minutes, then the supernatant liquid was collected and then transferred to a separatory funnel containing equal volumes of peroxide-free ether and 10 ml (depending on the volume of the sample) of saturated magnesium carbonate ($MgCO_3$) solution in 10% sodium hydroxide solution, and shaken for a time. After the phases were completely separated (about 15 min. later) the supernatant solution was collected and washed two times using the same method and salt solution (the purpose is to transfer the phytoplankton pigments from 90% solution acetone to ether layer). The ether layer containing the phytoplankton pigments are transferred to a glass scintillation vial and finally, the ligroine was then evaporated by passing N_2 gas through the upper part of the vial. Then the sample was concentrated to 0.5 ml. If there were sediments, they were washed with a little 90% acetone solution to disolve them, then preserved for use in a refrigerator.

2.2 Preparation of thin-layer chromatographic plates

200-mesh silica gel G powder was mixed with distilled water in the proportion of 1 : 3 to make a paste (without containing air bubbles). The paste was evenly spread on a clean slide glass 8 cm long and 3 cm wide. After its surface dried it was put into an oven, and activated for 1 h at 110°C, then taken out and put in a desiccator containing silica gel for 24 h until use.

2.3 Sample application

The treated sample was pipetted with a capillary and applied in the form of a round

spot 1.2 cm from one side of the activated chromatographic plate. The spots were dried by lightly blowing cold air from a blower repeatedly until the color of the sample spots could be clearly seen, and the spots were controlled in a smallest range and then developed after they were completely dried.

2.4 Development

In a clean round chromatographic chamber 30 cm high and 20 cm in diameter was put a culture dish 9 cm in diameter and 4 cm in height, and the developer [acetic acid : ether : petroleum ether(30～60℃)=1 : 25 : 25 (v/v/v)] was poured into the dish and the chamber covered well with the lid and sealed air tight with vaseline to prevent light leak. After the gas inside the chamber reached saturation (about 10 min.), the thin-layer chromatographic plate spotted with the sample was placed obliquely in the culture dish in a gradient of 80°. The end of the plate spotted with the sample was put in the developer. The chromatographic chamber was then covered hermetically and the sample developed in the dark. The development temperature was 19℃ and development time 7～10 min. The development was stopped when the solvent front moved 2/3 of the length of the plate. The plate was then taken out and the results were recorded immediately.

3 Result and discussion

3.1 The kinds of phytoplankton pigments

Thin-layer chromatograms of phytoplankton pigments from the adjacent waters of Great Wall Station, Antarctica is shown in Fig. 1. The kinds, R_f values and colors of the phytoplankton pigments were listed in Table 1.

Fig. 1 and Table 1 show that of the 15 kinds of phytoplankton pigments isolated from the samples collected from the adjacent waters of Great Wall Station, Antarctica, 13 were identified. This paper reports the kinds of phytoplankton pigments identified according to the R_f values of various marine algae pigments as reported by Jeffrey(1961). Various kinds of phytoplankton pigments separated out indicate that in that sea area the phytoplanktonic organisms are abundant in kind and dense in distribution.

3.2 The seasonal variation of phytoplankton pigments

The kind of phytoplankton pigments (represented by number of spots) in different months is shown in Fig. 2.

The phytoplankton pigments in the adjacent sea area of Great Wall Station vary obviously in different seasons (Figs. 1,2). Temperature is one of important factors controlling the growth of phytoplankton, the number kind of phytoplankton is acturally shown by the presence of different pigments (number of spots). It can be seen from Fig. 2 that the content of chlorophyll-a and the number and kind of phytoplankton trend basically in

Table 1. Kinds, R_f values and colors of the phytoplankton pigments from the adjacent waters of Great Wall Station, Antarctica from Mar. 1988~Feb. 1989

Month	Mar.	Apr.	May.[1]	Jun.	Jul.[2]	Aug.	Sep.	Oct.	Nov.[3]	Dec.	Jan.	Feb.	Kind	Color
1	0.95	0.96	0.97	0.98	0.97	0.97	0.97	0.98	0.98	0.96	0.93	0.98	carotene	pale yellow
2		0.89		0.94	0.88	0.88	0.90	0.90	0.90	0.91		0.92	pheophytin-a	gray*
3	0.83	0.82		0.82	0.82	0.82	0.84	0.85	0.85	0.85	0.85	0.83	chlorophyll-a	blue-green
4							0.77				0.76		xanthophyll	yellow
5		0.70					0.71				0.70		diadinoxanthin	-
6				0.69				0.69	0.67				chlorophyll-b	pale yellow
7								0.54		0.59			derivative	-
8							0.48			0.52			fucoxanthin	yellow
9					0.36			0.37	0.38				chlorophyllide-a	-
10	0.30						0.30	0.33				0.33	derivative	pale brown
11		0.26					0.24	0.24		0.26			pheophorbide-a	pale yellow-green
12				0.22				0.20				0.21	pheophorbide	gray
13	0.15	0.15					0.16	0.13	0.15	0.17	0.14		neoxanthin	yellow
14		0.11		0.12	0.11	0.11	0.11	0.09	0.08	0.11	0.10	0.12	chlorophyll-c	pale yellow
15	0.06	0.06		0.04		0.05	0.06	0.05			0.05	0.07	pheophytin-c	-

* colorless light spot is gray. 1: a long zone. 2: No. 14 spot is big. 3: No. 9. spot is big.

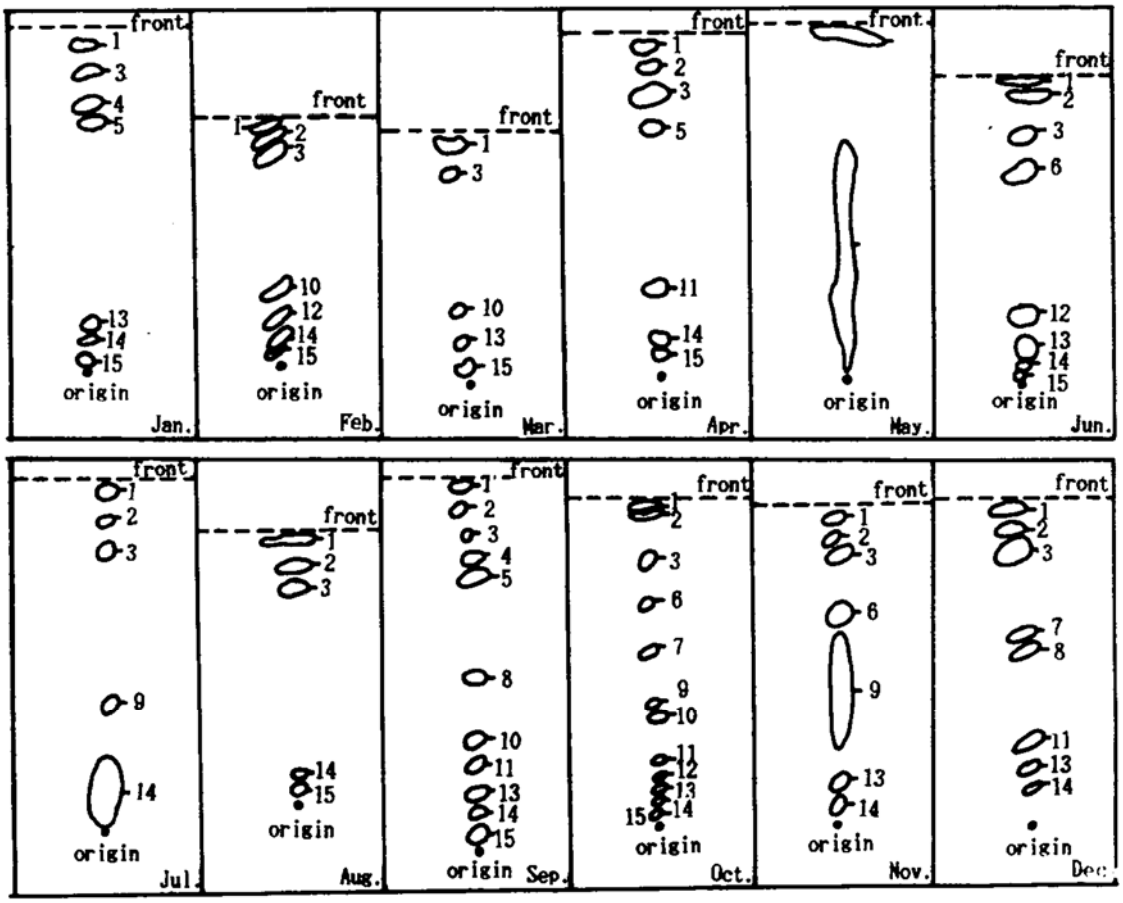

Fig. 1. Chromatomap of the phystplankton pigments from the adjacent waters of Great Wall Station, Antarctica.

conformity with seasonal variations, and the same kind of pigment differs greatly in different seasons. In summer the content of chlorophyll-a and the number and kind of phytoplankton are fairly high, whereas the kinds of phytoplankton pigments (or the number of spots) are relatively fewer. Furthermore, still in summer, the dominant species of phytoplankton prevail overwhelmingly, but they are simple in composition. The chlorophyll-a is very high in content and the pigments are relatively less in kind. Thus, the spots as the presence of pigments are also few in number but large in size. For example, the kinds of phytoplankton pigments (number of spots) separated out during December 1988～May 1989 were fewer, but the sizes of the spots were all large, in May except the spots at the front there was a long unseparated pigment zone. Several duplicate experiments gave the same results.

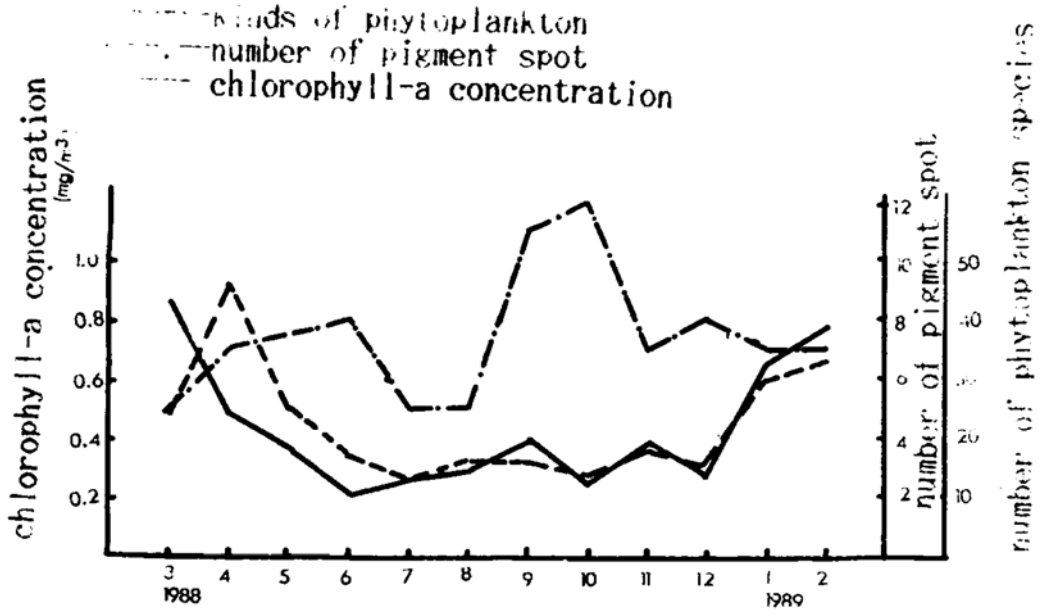

Fig. 2. Number of phytoplankton-pigments (spots), number and kind of phytoplankton, and seasonal variation of chlorophyll-a in the adjacent waters of the Great Wall Bay (Mar. 1988～Feb. 1989).

Diatoms are dominant species in the ocean (e. g. the Bohai Sea, the Yellow Sea, the Davis Sea of Antarctica; the East China Sea and the Southern Ocean). They are many in taxon and are great in quantity, eurythermal and euryhaline. So they are the dominant species in various seas, but dinoflagellates and green algae appeared only in some months. Chlorophyll-b was separated from the phytoplankton from the adjacent waters of the Great Wall Bay, Antarctica only in June, October and November, which shows that green algae existed in these three months but this kind of pigment was not separated out during January～May.

In the adjacent waters of Great Wall Station, Antarctica, sea water begins to freeze in June and the antarctic cold season sets in and continues until October. From Fig. 1 and Fig. 2, it can be seen that the peak of the kinds of plankton pigments appeared in

September and October, The highest amount of the spots of phytoplankton pigments was separated in October, which reveals two points: First, this shows the complex composition of the phytoplankton population and the existence of a fair number of species in the adjacent waters of Great Wall Station, Antarctica in winter, and in this period, the standing stock (chlorophyll-a) of the phytoplankton also showed a relatively high value in that seawater from September to November. Second, the temperature of the sea water was moderate, generally below −1 C. Most of the populations of the phytoplankton in the Antarctic seas are cryophilic. From Fig. 2 it can be clearly seen that the seasonal variations of the phytoplankton pigments in the adjacent waters of Great Wall Station, Antarctica mainly show the periodic double-peak regularity, which is in accord with the general law of the seasonal variations of the number of phytoplankton cells in the waters of the frigid zone. The succession of phytoplankton community causes seasonal variations in the composition of the kinds of the phytoplankton pigments.

There are a number of factors affecting the seasonal variations of phytoplankton pigments (including chlorophyll-a), such as, light, temperature, nutrient salts, pH value, stableness of water, etc. Among these factors, light and temperature are considered as the two most important ones for the Antarctic waters (El-Sayed and Mandelli, 1965). It has been found by researches that with the decrease of daily sunlight time in the Great Wall Bay, the decrease of chlorophyll-a content and quantity of phytoplankton may be fairly well correlated with the seawater temperture (because phytoplankton pigments are tested only qualitatively but not quantitatively, so they have no quantity and the correlations among them are calculated with chlorophyll-a (Wu *et al.* 1992).

4 Summary

(1) Of the 15 kinds of the pigments separated from the phytoplanktons collected from the adjacent waters of Great Wall Station, Antarctica. 13 have been identified.

(2) Carotene, chlorophyll-a, pheophytin-a and chlorophyll-c were found almost in the various months in the year, other pigments (e. g. lutein. neoxanthin) appeared only in some months, too. which indicates that there are definite differences in the composition of the kinds of phytoplankton pigments in different seasons in that sea area.

(3) The composition of phytoplankton pigments in that sea area has notable seasonal variations. the laws of their variations were not in good accord with the seasonal variations of the standing crop (chlorophyll-a) of phytoplankton and the kind and number of phytoplankton. It seems that the chlorophyll-a has a positive or negative correlation with the temperature and light intensity respectively.

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