Antarctic Research, Vol. 6, No. 1, 73~78, June 1995

# The pigmental composition and the absorption spectral analysis of Ulothrix sp. and benthic diatoms in the intertidal zone of Great Wall Station, Antarctica

Mao Xinghua(毛兴华), Li Baohua(李宝华) and Huang Fengpeng (黄凤鹏)
*First Institute of Oceanography, SOA, Qingdao 266003, China*

Received June 3, 1994

**Abstract** This paper reports the results of the absorption spectral analysis of Ulothrix sp. attached to the rocks from the intertidal zone in front of Great Wall Station, Antarctica in February 1989, and the benthic diatoms growing on small stones and on the edge of swamps of that zone. Six kinds of pigments were separated and identified in benthic diatoms. They are carotene, chlorophyll-a, fucoxanthin, chlorophyin, neoxanthin and chlorophyll-c. Nine kinds of pigments were identified in Ulothrix sp.. They are carotinoid, pheophytin, chlorophyll-a, -b, chlorophyllin, chlorophyllide-a, neoxanthin, chlorophyll-c and pheophytin-c. The intertidal benthic diatoms and Ulothrix sp. have specific absorption spectra and there are also marked differences in the composition of algal pigments they contain. Since the pigments of marine algae show differences in their absorption spectra, the absorption spectrum can be used to identify the species of marine algae.

**Key words** Antarctica, Great Wall Station, intertidal zone, pigment, absorption spectrum.

## 1 Introduction

The living marine resources are very rich in the Southern Ocean, and the benthic algae are also most abundant in the intertidal zone nearby Great Wall Station, Antarctica. The chlorophyll-a content of the benthic diatoms in 1 $m^2$ of sea area is unexpectedly equal to that in 100 $m^2$ of ordinary sea area (the sea areas nearby Great Wall Station, Antarctica and Davis Station, Australia) (Zhang, *et al.* 1986). The benthic algae play a very important role in the intertidal ecosystem.

At present only a certain number of reports have been made on the absorption spectra of the marine phytoplankton pigments (Jeffrey, 1961; Balech and El-Sayed, 1965; Bunt, 1957; Bunt, 1963; Bunt, 1964; Bunt, 1966; Ealey and Chittleborough, 1965), but studies on the absorption spectra of benthic algae in the intertidal zone, Antarctica have not been reported as yet. This paper reports the results of analysis of Ulothrix sp. and benthic diatoms collected from the intertidal zone in front of Great Wall Station, Antarctica in February 1989.

## 2 Material and method

The benthic diatoms and Ulothrix sp. growing or attached on small stones (sample about $10\times10$ cm$^2$ in size) and on the margin of swamp respectively in the middle-tide line and the high-tide line of the intertidal zone in front of Great Wall Station, Antarctica were washed with filtered seawater. The whole volume of washing fluid was measured. A part of washing fluid was used for the identification and counting of species composition (Yu *et al.*, 1994), and another part of it (50～100 ml) was concentrated on a micro-pore filter membrane (0.8 μm), freeze-stored at −30°C, and was to be brought back home for analysis. First, the samples were extracted with 90% acetone solution and the optical density of the extract was determined. Then the extract was transferred to peroxide-free ether and was measured in absorption spectrum. The ether solution containing the algae was aerated with nitrogen and concentrated. The algae pigments were separated and identified by thin-layer chromatography. The developer was: acetic acid : ether : petroleum ether (30～60℃) = 1 : 25 : 25(v/v/v), the temperature of development: 19.5℃, and time of development: 7～10 min, the color and $R_f$ values (rates of migration) of the various pigmemts of the sample were obtained finally.

## 3 Results

The thin-layer chromatographs of the benthic diatoms and Ulothrix sp. in the intertidal zone in front of Great Wall Station were shown in Fig. 1 and the kinds of their pigments and $R_f$ values and color listed in Table 1.

Table 1. Composition, $R_f$ value and color of pigments from Ulothrix sp. and benthic diatom in intertidal zone of Great Wall Station

| Spot | Name of pigment | Color | $R_f$ values | | |
|---|---|---|---|---|---|
| | | | Standard chlorophyll-a | Benthic diatom | Ulothrix sp. |
| 1 | Carotene | Pale yellow | | 0.93 | 0.96 |
| 2 | Pheophytin-a | Gray | | | 0.90 |
| 3 | Chlorophyll-a | Yellow-green | 0.84 | 0.82 | 0.81 |
| 4 | Chlorophyll-b | Pale yellow | | | 0.63 |
| 5 | Fucoxanthin | Yellow | | 0.55 | |
| 7 | Chlorophyllin | — | | 0.36 | 0.38 |
| 8 | Chlorophyllide-a | Yellow green | | | 0.31 |
| 9 | Neoxanthin | Yellow | | 0.20 | 0.17 |
| 10 | Derivative of chlorophyll-c | Pale yellow | | | 0.14 |
| 11 | Chlorophyll-c | Pale yellow | | 0.13 | 0.09 |

Fig. 1 shows that different algae contain different kinds of pigments and have different $R_f$ values. In this paper the standard chlorophyll-a used was extracted from spinach. It can be seen from Table 1 that the $R_f$ value computed after separation of the standard chlorophyll-a is 0.84 and it is blue-green. This result is consistent with the $R_f$ value of the chlorophyll-a (0.84) determined by Jeffrey(1961) from the thin-layer chromatogram

of the various kinds of marine algal pigments.

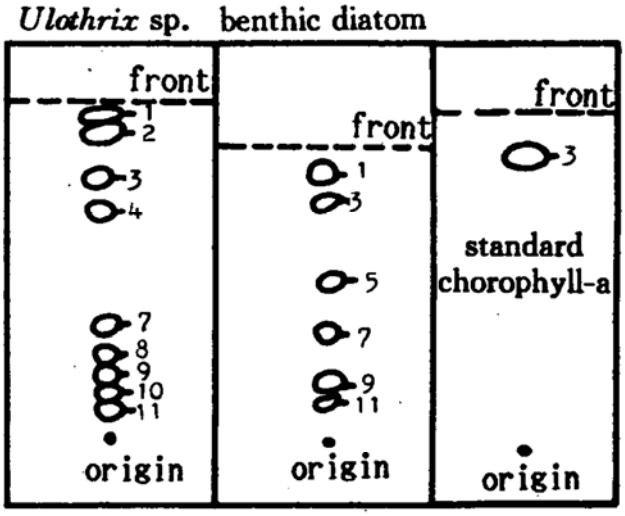


Fig. 1. Thin-layer chromatogram of pigments from Ulothrix sp. and benthic diatom in intertidal zone of Great Wall Station.

### *3. 1 The standard chlorophyll-a*

The absorption spectrum of the 90% acetone extract of the standard chlorophyll-a is shown in Fig. 2a, its absorption spectrum after being transferred into peroxide-free ether is shown in Fig. 2b.

Fig. 2a shows that the absorption peak (410 nm) of the standard chlorophyll-a at blue light portion is not in good consistence with those reported by scholars at home and abroad, which is mainly caused by insufficent purity of the chlorophyll-a; while the peak

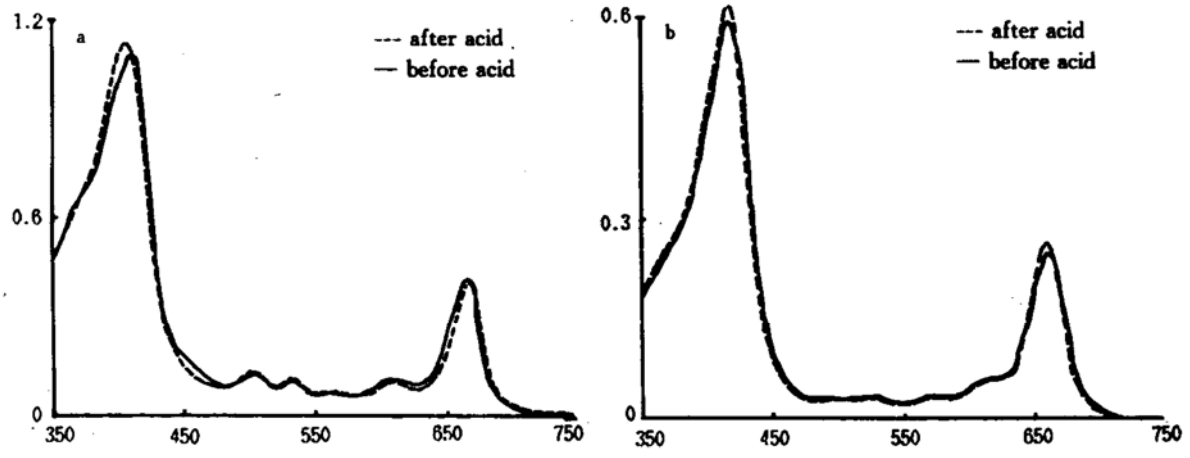


Fig. 2. Absorption spectrum of the standard chlorophyll-a. (a) In 90% acetone extract; (b) In ether extract after its transference from 90% acetone.

at 664 nm(red light portion) is basically the same as those reported by them. After being acidified with 0. 5 mol HCl, the chlorophyll was changed into pheophytin, which has

also two absorption peaks, only the one at blue-light portion moved 5 nm to the left, i. e. at 405 nm, and its optical density was 0. 034 higher than the original one, the absorption peak at 664 nm moved 2 nm to the right, at 666 nm, the optical density of the absorption peak was 0. 065 lower than the original one, yet it still could not reach the ratio of 1. 7 between the optical densities before and after acidification. The standard chlorophyll-a we used was stored in the form of paste but not in crystals, so it will change somewhat due to long storage time.

From Fig. 2a, 2b it can be seen that there is a great difference between the 90% acetone extract of the standard chlorophyll-a and its ether extract. The absorption spectrum of ether extract of stantard chlorophyll-a shows that one of the peaks originally at 410 nm moved to the right to 417 nm; after acidification, it was still at 417 nm, though the peak became higher, the other absorption peak originally at 664 nm moved to the left to 660 nm, and after acidification it moved 2 nm to 658 nm and was higher than that before acidification. This also shows that such a process of transference has led a change in the extinction character of the standard chlorophyll-a or a change in its structure. The molecular structure of the standard chlorophyll-a after its change remains to be further studied.

### *3. 2 The benthic diatoms*

The chromatogram of the benthic diatoms in Fig. 1 shows that after separation of the pigments from the benthic diatoms by thin-layer chromatographic techniques, 6 clear spots can be obtained. They were identified as the spots of: carotene, chlorophyll-a, fucoxanthin, chlorophyllin, neoxanthin and chlorophyll-c.

Fig. 3a is the absorption spectrum of 90% acetone extract of the benthic diatoms; Fig. 3b that of the benthic diatoms after being transferred to the ether solution.

Acetone solution has the function of penetrating through the cell walls of algae, setting free the chlorophyll and other pigments within the algal cells. This process is called extraction and the solution after the extraction is called extract. After measurement of the extract of marine algae with 90% acetone solution, a specific absorption spectrum can

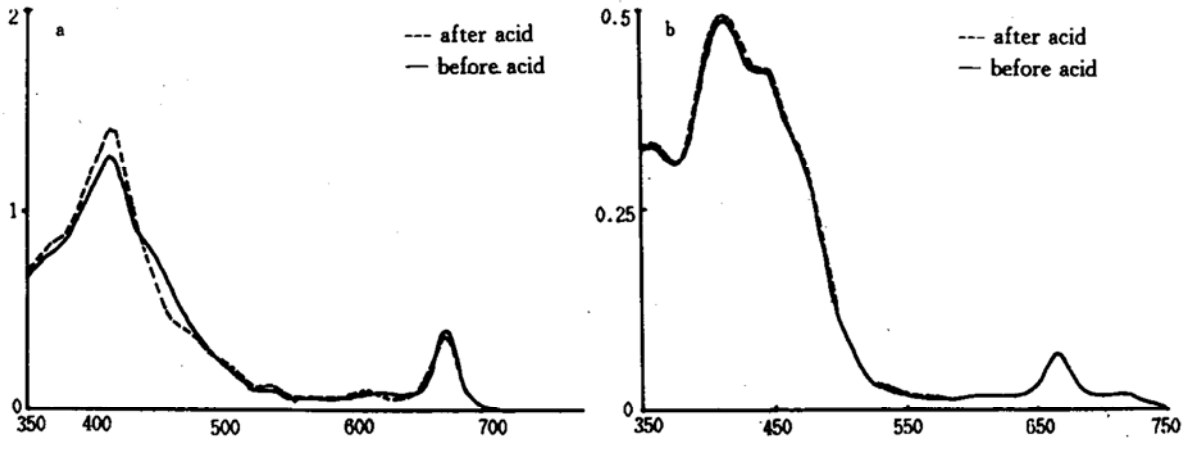


Fig. 3. Absorption spectrum of the extract of the benthic diatom. (a) In 90% acetone; (b) In ether after its transference from 90% acetone.

be obtained. The absorption spectrum of the 90% acetone extract of the benthic diatoms from that sea area is characterized by having two absorption peaks:one at 413 nm (blue light portion) and the other at 666 nm (red light portion) (Fig. 3a, solid line) and the peak at 413 nm has two low but not quite distinct shoulder peaks. The absorption peak (dotted line) of 90% acetone extract of the benthic diatoms after acidification and that before acidification did not show big change in shape basically, the two absorption peaks show the same location, at 413 nm the optical density value of the absorption peak is about 0.1 higher than the original one, whereas that at 666 nm is 0.03 lower than the original.

From Fig. 3b can be seen the characteristics of the absorption spectra of the benthic diatoms after the transference to the ether solution. It is different from the absorption spectrum of the acetone extract in that after transference to the ether, the two shoulder peaks at 413～415 nm are obviously low at the left and high at the right. At 715 nm there is a small absorption peak. In the 90% acetone extract there is not such a peak, the source of which remains to be studied.

### *3.3 Ulothrix sp.*

Ulothrix sp. is an alga which is attached to the rocks in the high-tide-line. After washing with filtered seawater, the samples were quantitatively treated. After separation with thin-layer chromatographic techniques, 9 distinct spots were obtained, which were identified as following 9 pigments: carotene, pheophytin, chlorophyll-a, chlorophyll-b, chlorophyllin, chlorophyllide, neoxanthin, chlorophyll-c and pheophytin-c.

The absorption spectrum of Ulothrix sp. (Fig. 4a, 4b) shows that the two absorption peaks of the 90% acetone extract are at 433 nm and 664 nm. The absorption peak at 433 nm has two distinct shoulder peaks and that at 664 nm is smooth. After acidation with 0.5 mol HCl, the absorption peak (dotted line) of Ulothrix sp. changed a lot. At 433 nm it moves 20 nm to the left and at 413 nm the shape of its absorption peak was identical with that before its acidation. The absorption peak at 664 nm moves 2 nm to the right to 666 nm after acidation.

The absorption spectrum of Ulothrix sp. shows that after being transferred from the 90% acetone, the ether extract also has two absorption peaks. The one at 420 nm is flat, the other at 664 nm was narrow and high and as compared with the 90% acetone extract there is much difference between the absorption peaks (Fig. 4a, 4b). At 433 nm the absorption peak moves left to 420 nm after the transference. Besides, the absorption peak of the 90% acetone extract of Ulothrix sp. is fairly narrow in shape and after transference the top of peak of Ulothrix sp. is fairly flat and smooth.

## 4 Summary

(1) After separation of the pigments of the benthic diatoms in the intertidal zone in front of Great Wall Station, Antarctica. 6 kinds of pigments were preliminarily identified. They are respectively carotene, chlorophyll-a, fucoxanthin, chlorophyllin, neoxan-

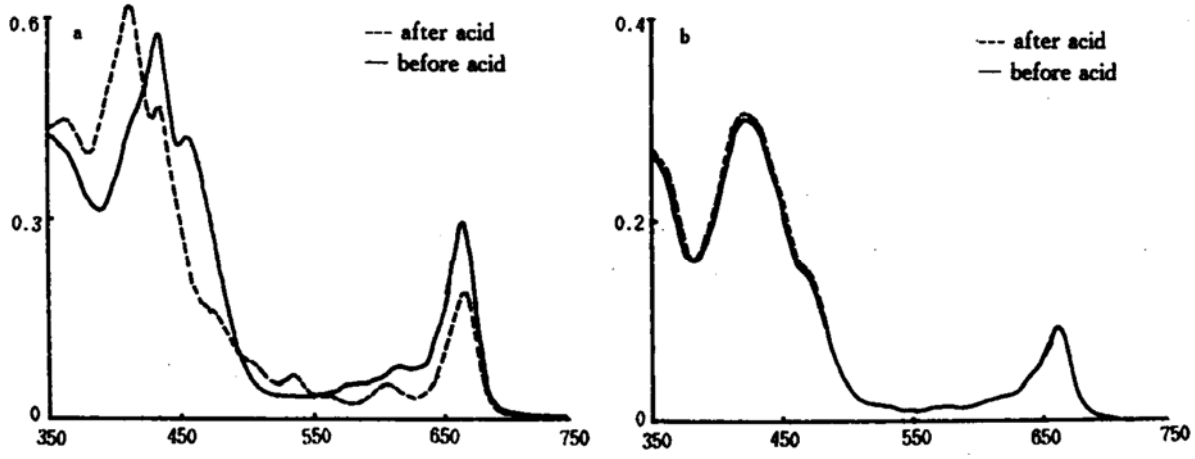


Fig. 4. Absorption spectrum of the extract of Ulothrix sp.. (a) In 90% acetone; (b)In ether-layer after its trasference from 90% acetone.

thin and chlorophyll-c. After separation of the pigments of Ulothrix sp., 9 kinds of the pigments were preliminarily identified. They are, respectively, carotene, pheophytin-a, chlorophyll-a, chlorophyll-b, chlorophyllin, chlorophyllide, neoxanthin, chlorophyll-c and pheophyllin-c.

(2) The results of analysis show the absorption spectra of the intertidal benthic diatoms and Ulothrix sp. each had its own specific absorption spectrum, and there were marked differences in the kinds of pigments contained in them. Each marine alga has its own specific pigmental composition, and each kind of the pigments has its own specific $R_f$ value. The absorption wave lengths of the various kinds of algal pigments are different.

## References

Balech, E. and El-Sayed, S. Z. (1965): Microplankton of the Weddell Sea. *Ant. Res. Series*, 5, 107—124.

Bunt, J. S. (1957): Introductory studies: hydrology and plankton, Mawston, June 1956—February 1957. *ANARE. Reps, Series B.*, 1960.

Bunt, J. S. (1963): Diatoms of antarctic sea ice asagents of primary production. *Nature*, 199, 1255—1257.

Bunt, J. S. (1964): Primary productivity under sea ice in antarctic waters. *Ant. Res. Series*, 1, 13—26.

Bunt, J. S. (1966): Microalage of antarctic park ice zone. Symposium on antarctic oceanography, Scott Polar Research Institute, Cambridge, 193—213.

Elaey, E. H. M. and Chittleborough, R. G. (1965): Plankton, hydrology and marine fouling at heard island. *Aust. Nal. Res. Exp. Rep.*, 15.

Jeffrey, S. W. (1961): Paper chromatographic separation of chlorophylls and carotenoids from marine algae. *Biochem. J.*, 80, 336—342.

Yu Jianluan, Li Ruixiang and Huang Fengpeng (1994): The preliminary study of the phytoplankton ecology in the Great Wall Bay, Antarctica. *Antarctic Research*, 5(2), 26—32.

Zhang Kuncheng and Lü Peiding (1986): Some ecological observations on Antarctic Ice Algae. A Collection of Anyarctic Scientific Explorations. No. 3, China Ocena Press, Beijing, 49—59 (in Chinese).