Comparisons of N,N-Dimethylformamide and 90% Acetone as an Extraction Solvent for Fluorometric Determination of Chlorophyll *a* from Natural Phytoplankton Communities in the Southern Ocean

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南大洋の天然植物プランクトン群集を用いたクロロフィル*a*蛍光分析における抽出溶媒としてのN,N-ジメチルホルムアミドと90%アセトンの比較

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要旨: 南大洋で採集された天然植物プランクトン群集に対して，N,N-ジメチルホルムアミド (DMF) および 90% アセトンの2種類の溶媒を用いて色素の抽出を行い，クロロフィル*a*およびフェオ色素濃度を蛍光法によって求めた．前者の溶媒は第33次日本南極地域観測隊が用いたものであり，後者は従来の観測隊で用いられてきたものである．溶媒が異なってもクロロフィル*a*濃度には統計的に有意な差が認められなかった．フェオ色素に関しても溶媒の違いによる統計的有為差はみられなかった．しかしながら，フェオ色素の場合，クロロフィル*a*の場合に比べ相関が有意に低く，DMFで抽出したフェオ色素濃度の方が，90% アセトンで抽出した場合よりも高くなる傾向があった．クロロフィル*a*とフェオ色素を加えた全クロロフィル色素濃度の場合には，溶媒間の回帰直線の傾きを考慮すると，前者で抽出した方が後者よりも約10% ほど高かった．

Abstract: Algal chlorophyllous pigments were extracted with two different solvents, N,N-dimethylformamide (DMF) and 90% acetone, using natural phytoplankton communities in the Southern Ocean. Concentrations of chlorophyll *a* and phaeopigments were determined fluorometrically. The former solvent was employed during the 33rd Japanese Antarctic Research Expedition (JARE-33) while the latter has been used during previous JAREs. Statistical analyses showed that chlorophyll *a* concentrations were equivalent although the solvents were different. No significant difference was observed between concentrations of phaeopigments extracted with the different solvents either. The correlation coefficient obtained between phaeopigments concentrations was significantly lower than that between chlorophyll *a* concentrations. Phaeopigment concentrations extracted with DMF tended to be higher than those with 90% acetone. Total chlorophyllous pigments (chlorophyll *a* plus phaeopigments) were about 10% higher in the former solvent than in the latter, considering the slope of the regression line (= 1.088).

1. Introduction

Chlorophyll *a* has been determined during the cruise of the Japanese Antarctic Research Expedition (JARE) since 1965 (HOSHIAI, 1968), following the spectro-

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photometric (RICHARDS and THOMPSON, 1952) or fluorometric method (YENTSCH and MENZEL, 1963). That is, the known volume of seawater is filtered using a glass fiber filter. The filter is ground and chlorophyllous pigments from algal cells are extracted with 90% acetone. After the supernatant is obtained by centrifuge, the absorbance or fluorescence is measured. Since this method involves troublesome procedures, it is difficult to process many samples in a limited time.

BABA *et al.* (1979) and SATO *et al.* (1981) demonstrated that there is no difference between grinding and soaking of filters to extract pigments. SUZUKI and ISHIMARU (1990) introduced merits of an alternative solvent of N,N-dimethylformamide (DMF) in extraction efficiency of pigments and stability of the fluorescence. During the cruise of the JARE-33, DMF was employed as the solvent to extract chlorophyllous pigments from algal cells. In this paper the concentrations of chlorophyll *a* and phaeopigments extracted with DMF and 90% acetone are compared.

2. Materials and Methods

Water samples were collected during the cruise of JARE-33 (Fig. 1). At one Station (41°25′S, 110°07′E) (open circle in Fig. 1) samples were taken using Nansen bottles from depths of 10, 20, 30, 50, 75, 100, 125, 150, 175, and 200 m. The surface water was taken by a plastic bucket. Along the cruise track of the icebreaker SHIRASE, eighteen samples of surface water (closed circles in Fig. 1) pumped up from an intake on the hull (*ca.* 8 m depth) were collected (see FUKUCHI and HATTORI, 1987).

Two subsamples (100 m*l*) from the same seawater sample were filtered with Whatman GF/F glass fiber filters, respectively. One of the filters was put into a screw capped glass vial containing 6 m*l* of DMF and the other was put into another

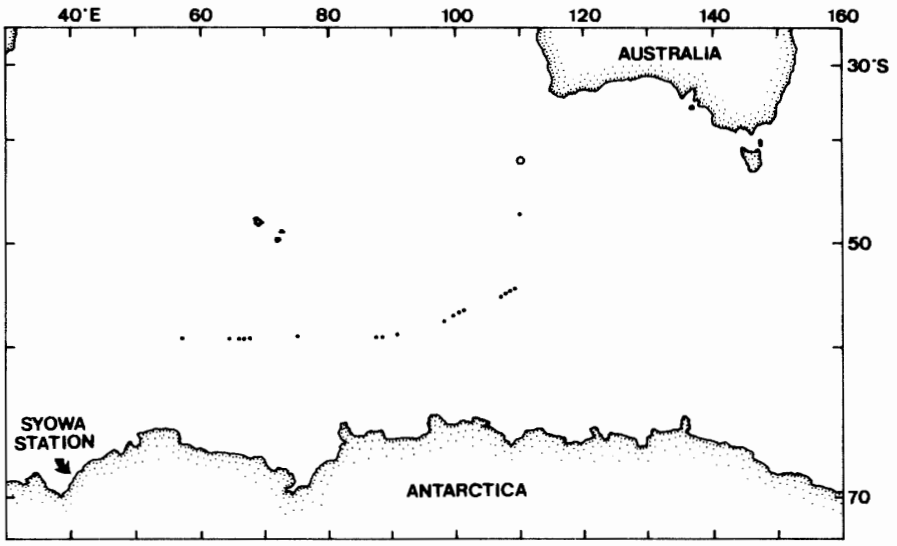

Fig. 1. Location of sampling stations during the cruise of the icebreaker SHIRASE (JARE-33).

glass vial containing the same volume of 90% acetone. Chlorophyllous pigments were extracted at −20°C in the dark without grinding. After 24 hours of extraction the fluorescence was measured with a Turner Design Fluorometer Model 10R, which had been calibrated against pure chlorophyll *a* (Sigma Chemical Co.). Acid factors obtained for the fluorometer were 2.04 for acetone and 1.98 for DMF.

Concentrations of chlorophyll *a* and phaeopigments were calculated (PARSONS *et al.*, 1984), although some investigators suggest that the concentration of phaeopigments measured with the conventional fluorometric method reflects the abundance of chlorophyll *b* containing phytoplankton (*e.g.* GIESKES, 1991).

3. Results

The concentrations of chlorophyll *a* extracted with DMF, which varied between 0.004 and 2.10 $\mu g/l$, were compared with those extracted with 90% acetone. A significant correlation ($r=0.994$, $P<0.001$, $n=29$) for chlorophyll *a* was obtained (Fig. 2a). The slope of the regression line was not significantly different from 1.0 ($ts=0.34$, $P>0.10$). The slope of 1.007 indicated that chlorophyll *a* concentrations were equivalent although different solvents were employed.

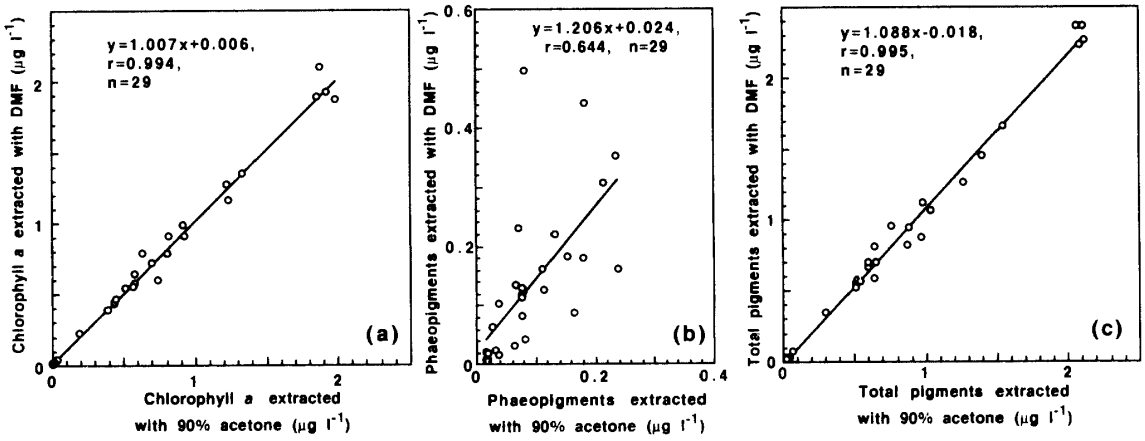

Fig. 2. Relationships between concentrations of pigments extracted with 90% acetone and N,N-dimethylformamide (DMF). Chlorophyll a *(a), phaeopigments (b), and total chlorophyllous pigment (c).*

Similarly, phaeopigment concentration extracted with DMF significantly correlated with that extracted with 90% acetone ($r=0.644$, $P<0.001$, $n=29$) (Fig. 2b). The correlation coefficient was, however, significantly smaller than that obtained for chlorophyll *a* ($ts=7.72$, $P<0.001$). Although the difference between the slope of the regression line and 1.0 was not significant ($ts=0.75$, $P>0.10$), the slope of 1.206, which is larger than 1.0, suggested that phaeopigment concentrations tended to be higher in DMF than in 90% acetone.

Significant correlation ($r=0.995$, $P<0.001$, $n=29$) was observed between concentrations of total chlorophyllous pigments (chlorophyll *a* plus phaeopigments) (Fig. 2c). The correlation coefficient was significantly larger than that for phaeo-

pigments ($ts=8.05$, $P<0.001$), but almost the same as that for chlorophyll *a* ($ts=0.33$, $P>0.10$). The slope of the regression ($=1.088$) was significantly larger than 1.0 ($ts=4.24$, $P<0.001$). Considering the slope of the regression, the total chlorophyllous pigments extracted with DMF is about 10% higher than that extracted with 90% acetone.

4. Discussion

The statistical analyses conducted in the present study show that there is no significant difference between concentrations of chlorophyll *a* extracted with DMF and 90% acetone based on natural phytoplankton communities collected in the Southern Ocean. Therefore, direct comparison between chlorophyll *a* data obtained by the JARE-33 with DMF extraction and the previous JARE with 90% acetone extraction is permitted.

On the other hand, phaeopigment concentrations seem to be higher in DMF than in 90% acetone, although the statistical difference was not significant between them. Total chlorophyllous pigment concentration extracted with the former solvent is about 10% higher than that extracted with the latter. These trends have also been reported by SUZUKI and ISHIMARU (1990). They compared extraction abilities of 90% acetone and DMF based on concentrations of chlorophyllous pigments from the natural phytoplankton community in Tokyo Bay. They showed that DMF extractions gave 10 to 30% higher concentrations of total chlorophyllous pigments and the increase of pigments extracted with DMF was greater in phaeopigments than in chlorophyll *a* (SUZUKI and ISHIMARU, 1990).

The present study has shown that data on chlorophyll *a* concentration collected during the cruise of the JARE-33 are compatible with data taken by the previous JAREs. The distribution of surface chlorophyll *a* along the cruise track of the icebreaker SHIRASE during the JARE-33 will be described elsewhere.

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