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VERTICAL DISTRIBUTION OF PHOTOSYNTHETIC PIGMENTS CHARACTERIZED BY HPLC UNDER THE ICE OF SAROMA KO LAGOON

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Abstract: Composition of photosynthetic pigments (chlorophyll *a* and its derivatives) under the ice of Saroma Ko lagoon was quantified by high-performance liquid chromatography (HPLC). In ice algae, the relative percentage of chlorophyll *a*, early degradation products of chlorophyll *a* (chlorophyll *a*′-1, 2, 3 and chlorophyllide *a*) and pheopigments (pheophytin *a* and pheophorbide *a*) was found to be 74.4, 8.0 and 17.6%, respectively. In suspended and sinking particles, the percentage of chlorophyll *a* was lower than that of ice algae. Vertical profile of chlorophyll *a* in suspended particles showed high percentage at the surface and low percentage at the bottom, while chlorophyll *a* derivatives showed a reverse pattern to that of chlorophyll *a*. These results suggest that the degradation of chlorophyll *a* in suspended particles proceeded with increasing depth. The percentage composition of chlorophyll *a* in sinking particles did not show marked variation with depth, and closely resembled that of suspended particles in the bottom water. The absence of significant difference in the photosynthetic pigments composition of sinking particles between upper and lower layers indicated that the time spent for sinking was too short for more degradation to proceed with depth, because of the shallowness of the lagoon. From the results of the present study, it is suggested that the main component of sinking particles could be feces of herbivores and/or the senescent algal cells.

1. Introduction

Chlorophyll *a* is the only pigment commonly present in all phytoplankton and the main pigment governing photosynthetic electron transport in the cell. Therefore, the amount of chlorophyll *a* has been used as an indicator of phytoplankton biomass, and also for the estimation of productivity (ARUGA and MONSI, 1963). On the other hand, the degradation products of chlorophyll *a* are diagnostic indicators of the physiological condition, degradation and grazing processes of phytoplankton (LORENZEN, 1967; SHUMAN and LORENZEN, 1975; WELSCHMEYER *et al.*, 1984).

Since 1980, high-performance liquid chromatography (HPLC) analysis has been shown to be a powerful method for separating and quantifying photosynthetic pigments in lakes and oceans (*e.g.*, GIESKES and KRAAY, 1983; MANTOURA and

LLEWELLYN, 1983; HURLEY and ARMSTRONG, 1990).

Saroma Ko lagoon, which is known as the most southern area of seasonal sea ice distribution in the northern hemisphere, is located on the Okhotsk coast of Hokkaido, Japan. During the ice covered season, ice algae color the bottom ice (HOSHIAI and FUKUCHI, 1981), thus providing a source of organic carbon for benthic communities in the lagoon (SATOH *et al.*, 1989). In the present study, the photosynthetic pigment composition of ice algae, suspended particles and sinking particles were analyzed by using HPLC, in order to describe the extent of degradation of chlorophyll *a* both quantitatively and qualitatively in Saroma Ko lagoon.

2. Materials and Methods

Sampling station was located at the southeastern part of Saroma Ko lagoon (Fig. 1), and the depth was about 10 m. Water samples were collected at 1, 5 and 9 m depth on February 21, 1992. Sinking particles were collected by deploying sediment traps (diameter 10 cm; height 42 cm) at 0.5, 2.5 and 7.5 m from the undersurface of the ice for a period of 90 hrs from February 21 to 25, 1992. Temperature and salinity of the water column were measured by CTD sensor (Sea-Bird Model SBE-19) on February 21, 1992. Ice algae were collected from the undersurface of the sea ice by scraping with a metal plate, an area of 100 cm^2 (3 cm thickness) of the colored part of ice. These samples were filtered through Whatman GF/F glass-fiber filter, and the filters were stored at $-20°C$ until pigment analysis. Photosynthetic pigments were extracted from the thawed sample with 90% acetone by keeping overnight at 4°C and were fractionated by reverse-phase gradient elution HPLC (Type L-6000, Hitachi) and then detected by fluorescence ($\lambda_{ex}=440$ nm; $\lambda_{em}=660$ nm). As described below, the chromatographic separation was carried out

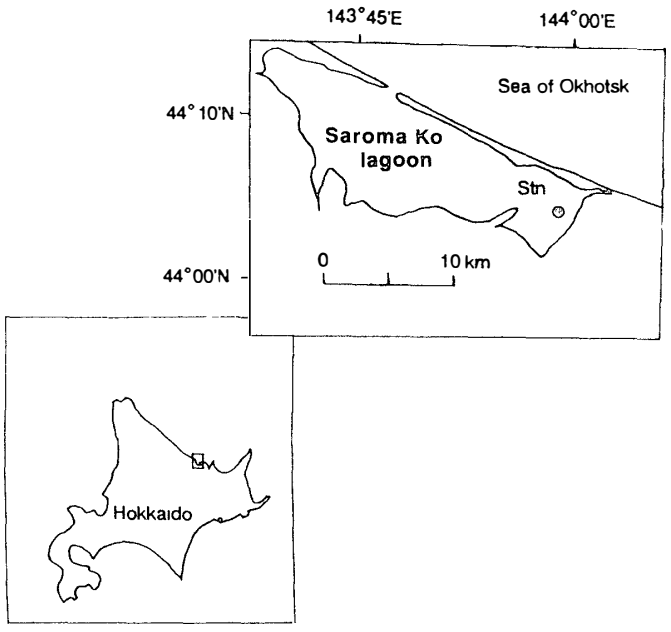

Fig. 1. Location of the sampling station in Saroma Ko lagoon.

following a method slightly modified from that of MANTOURA and LLEWELLYN (1983). The gradient consisted of an initial solvent of 1M ammonium acetate: methanol = 10 : 90 (v/v) with a 10-min linear gradient of 100% methanol. Flow rate was kept constant at 1.0 $ml\,min^{-1}$. Separations were performed on a E-Merck 18 RPe column and the column temperature was kept at 29±1°C.

Chlorophyll a and its derivatives were identified by comparing the retention times of their respective standard peaks, and classified into chlorophyll *a*, early degradation products of chlorophyll *a* (chlorophyll *a'*-1, 2, 3 and chlorophyllide *a*) and pheopigments (pheophytin *a* and pheophorbide *a*). Standards of chlorophyll *a*, pheophytin a and pheophorbide *a* were obtained from Wako Chem. Co., Tokyo. Chlorophyllide *a* was prepared according to the method of BARRET and JEFFREY (1971). Three more peaks present in the standard chromatogram of chlorophyll *a* were tentatively identified as the degradation products of chlorophyll *a* such as chlorophyll *a'*-1, 2, 3.

Chlorophyll *a*, pheophytin *a* and pheophorbide *a* were quantitatively evaluated by drawing the calibration curve using the amount of standards and their respective peak areas. The calibration curve for chlorophyll *a* was used to calculate the concentrations of chlorophyll *a'*-1, 2, 3 and chlorophyllide *a* in the samples.

3. Results and Discussion

Temperature did not show any marked variation in the water column, ranging from −1.3 to −1.1°C (Fig. 2). Salinity was low at the surface (29.8), but did not change from subsurface (2.5 m) to bottom (7.5 m) with the value of 31.7 (Fig. 2).

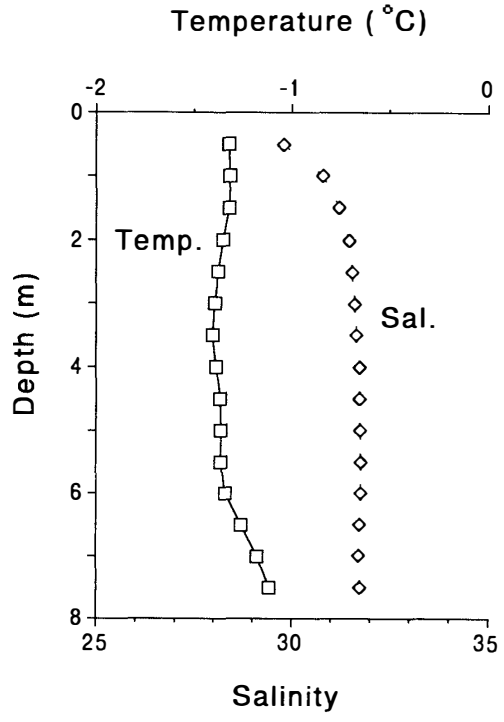

Fig. 2. Vertical profiles of temperature and salinity in Saroma Ko lagoon.

Table 1. Standing stock and pigment composition of ice algae in ice of Saroma Ko lagoon.

Pigments	Standing stock ($mg\,m^{-2}$)	Relative composition (%)
Chlorophyll *a*	12.0	74.4
Chlorophyllide *a*	0.1	0.5
Chlorophyll *a'*-1	0.9	5.9
Chlorophyll *a'*-2	0.3	1.6
Chlorophyll *a'*-3	0.1	0.1
Pheophytin *a*	0.6	3.9
Pheophorbide *a*	2.2	12.1

As a result, a weak density stratification was observed at 0.5 to 1.0 m depth.

The integrated chlorophyll *a* standing stock of ice algae in sea ice was 12 mg m^{-2} (Table 1) and the percentage of chlorophyll *a* in the total pigments was 74.4%. In the water column, the integrated chlorophyll *a* standing stock was 8.1 mg m^{-2} and the contribution of chlorophyll *a* to the total pigments was 60.1%. Among all samples analyzed, the percentage of chlorophyll *a* in ice algae was the highest and the degradation products of chlorophyll *a* in ice algae were the lowest. It could be suggested that the amount of living cells in ice algae was higher than that of suspended particles.

Concentrations of suspended chlorophyll *a* and its derivatives decreased from the surface to bottom. Maximum concentrations of chlorophyll *a* and pheophorbide *a* were 4.0 mg m^{-3} and 1.6 mg m^{-3} at the surface (Fig. 3). Pheophytin *a* was not detected in suspended particles. The concentration of chlorophyll *a* (4.0 mg m^{-3}) at the surface was comparable to the concentration (3.0 mg m^{-3}) reported by FUKUCHI *et al.* (1989) in the same lagoon during a bloom period which occurred later in the season (from the middle to the end of March). Also, the presence of pheophorbide *a* in the samples can be interpreted as a more advanced degradation state of chlorophyll *a*, as reported by VERNET and LORENZEN (1987).

The highest and lowest percentages of chlorophyll *a* in suspended particles were found in the surface (65.6%) and bottom layers (46.1%), respectively. On the other hand, pheophorbide *a* in suspended particles showed a reverse distribution pattern to chlorophyll *a*, with the highest contribution (46.1%) at the bottom and the lowest (27.0%) in surface waters, respectively. Increase in the proportion of chlorophyll *a* derivatives in parallel with a decrease in that of chlorophyll *a* with depth indicated that the decomposition of suspended particles occurred between the surface and the bottom. Besides, SATOH *et al.* (1989) suggested that the light intensity at the undersurface of the ice was insufficient for photosynthesis during winter season in Saroma Ko lagoon because of thick ice and snow covered on the surface. Obvious decrease in phytoplankton biomass (Fig. 3) and the change in the chlorophyll *a* composition in the bottom layer may be due to the limited availability of light near the bottom.

The percentages of chlorophyll *a* and pheophorbide *a* in sinking particles were 34.2–39.0% and 40.6–48.7%, respectively (Table 2). Percentages of chlorophyllide

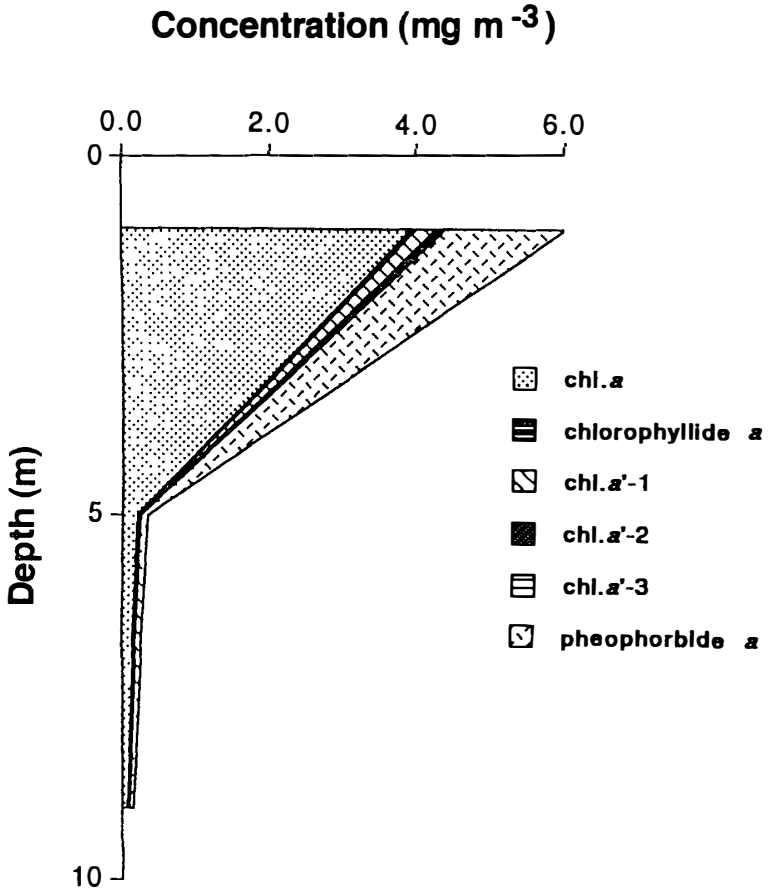

Fig. 3. Vertical distribution of chlorophyll a *and its derivatives in the water column of Saroma Ko lagoon.*

Table 2. Pigment composition of sinking particles in Saroma Ko lagoon.

Pigments	Relative composition (%) Depth (m)		
	0.5	2.5	7.5
Chlorophyll *a*	39.0	34.2	34.9
Chlorophyllide *a*	0.0	2.9	4.1
Chlorophyll *a'*-1	9.5	6.1	7.3
Chlorophyll *a'*-2	11.0	8.1	6.9
Pheophorbide *a*	40.6	48.7	46.8

a, chlorophyll *a'*-1 and chlorophyll *a'*-2 were low, while chlorophyll *a'*-3 and pheophytin *a* were not detected in sinking particles (Table 2).

Only ice algae revealed the presence of pheophytin *a*, this pigment being absent from both the suspended and sinking particles. DENANT *et al.* (1991) reported that pheophytin *a* was not detected in the water column of the Krka Estuary, Adriatic Sea. They proposed two hypotheses to explain the absence of pheophytin *a*; 1) there may be no formation of pheophytin *a* in natural conditions, and 2) there may be a rapid transformation into pheophorbide *a*. Significant amount of pheophorbide *a* in these samples seemed to indicate that pheophytin *a* was rapidly degraded into

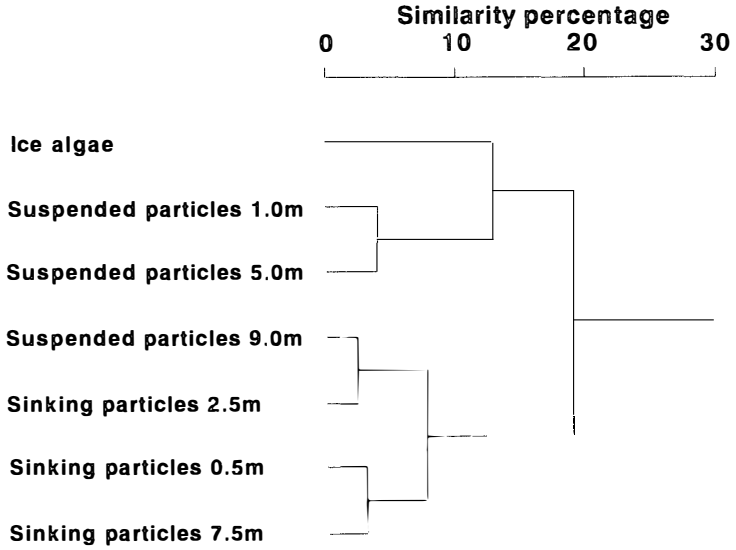

Fig. 4. Dendrogram of cluster analysis of photosynthetic pigments in ice algae, suspended particles and sinking particles collected from Saroma Ko lagoon.

pheophorbide *a*. Again, this suggests that some decomposition occurred in the water column, and agrees with the chlorophyll *a* degradation pathway proposed by VERNET and LORENZEN (1987).

To evaluate the extent of pigment degradation, similarity analysis was carried out for the pigment composition of ice algae, sinking particles and suspended particles. Data were clustered into 2 major groups using the group average clustering procedure (Fig. 4). Ice algae and suspended particles at 1.0 and 5.0 m depth formed into one group, and suspended particles at 9.0 m and sinking particles from different depths were classified into another group.

Although the pigment composition of sinking particles was similar at all depths, the pigment composition of sinking particles and ice algae were not similar, as it can be seen from the chlorophyll *a* content of sinking particles (34.2–38.5%) and ice algae (74.4%). Chlorophyll *a* can be degraded by grazing of micro/macrozooplankton or during senescence of algal cells. Microscopic examination of the trap material revealed a mixed assemblage of dinoflagellates and other flagellates, and diatoms, as well as the presence of fecal pellets. The pigment signatures in the traps emerge from these various contributors. The degradation of chlorophyll *a* in sinking particle might reflect heterotrophic feeding (DALEY, 1973; STROM, 1993), macrozooplankton production of feces and/or senescence of algae. It is known that some copepods could feed on ice algae (CONOVER *et al.*, 1986; HOSHIAI *et al.*, 1990). Also, dying algae are thought to produce a pheophorbide *a*-like pigment, which have been reported in the water column and sediment traps (HEAD and HORNE, 1993; HEAD *et al.*, 1994). From these results, it could be suggested that the main component of sinking particles might not be fresh algal cells, but fecal pellets and/or senescent algal cells.

In conclusion, the results obtained in the present study suggested that the degradation of chlorophyll *a* in suspended particles proceeded with increasing

depth. The absence of statistically significant variation in the pigment composition of sinking particles between upper and lower layers indicated that the time taken for the sinking is too short to degrade the chlorophyll *a* containing particles because of the shallowness of the lagoon. However, the percentages of chlorophyll *a* in sinking particles were distinctly lower than that in ice algae, indicating that the sinking particles comprised originally degraded chlorophyll *a*. It could also be suggested that fecal pellets of zooplankton and/or senescent algal cells are the main component of sinking particles under the ice of Saroma Ko lagoon during winter season.

Acknowledgments

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