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Photoalteration in Biodegradability and Chemical Compositions of Algae-derived Dissolved Organic Matter

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자외선에 의한 조류기원 용존유기물의 생분해도 및 화학조성변환. 최광순* · Akio Imai · Kazuo Matsushige · Takashi Nagai · 김용환 · 김범철[1] (일본 국립환경연구소, [1]강원대학교 환경과학과)

자외선에 의한 조류기원 용존유기물의 특성변화를 조사하기 위하여 배양한 남조류 두종 (*Microcystis aeruginosa, Oscillatoria agardhii*)의 체외배출용존유기물에 자외선 처리전과 처리후의 생분해도와 유기물의 화학적조성을 비교하였다. 자외선처리는 pyrex용기에 시료를 넣고 인공자외선램프(UVA: 40 W/m^2, UVB: 2 W/m^2)로 24시간 조사하였다. 유기물의 화학적조성은 XAD-8, 양이온, 을 이온수지를 이용하여 소수성 산 (hydrophobic acids; HoA), 소수성 중성 (hydrophobic neutrals; HoN), 친수성 산 (hydrophilic acids; HiA), 친수성 염기 (hydrophilic bases; HiB), 그리고 친수성 중성 (hydrophilic neutrals; HiN)의 5개 분획으로 분류하였다. 자외선처리동안 유기물의 농도변화는 거의 없었던 반면 생분해도는 자외선처리 전에 비해 현저히 감소하였다 (*M. aeruginosa*: 17%, *O. agardhii*: 28% 감소). 또한 자외선 처리 전과 후의 유기물의 화학적조성도 상당한 차이를 보였다. 자외선처리 후 HiB분획 (단백질, 아미노산류)은 감소한 반면 HiA분획 (카복실산류)은 증가하였다. 유기산분석에서도 3종류의 카복실산이 자외선처리 후 증가하는 것으로 나타났다. 일반적으로 수체의 유기물은 자외선에 의해 난분해성 유기물이 분해되거나 잘게 쪼개져 이분해성 유기물로 전환되는 것으로 알려졌다. 그러나 본 연구에서는 조류기원의 이분해성 유기물의 경우는 자외선에 의해 완전한 광분해는 보이지 않았지만 난분해성 유기물로 전환되었고 화학적조성도 바뀌었다. 이는 유기물의 기원과 종류에 따라 자외선에 대한 영향이 다르다는 것을 시사한다.

Key words : algal DOM, UV effects, photoalteration, biodegradability, chemical composition

INTRODUCTION

Dissolved organic matters (DOM), one of the major pools of organic carbon in aquatic ecosystems, can be an important source of carbon and energy for both heterotrophic microorganisms and higher trophic levels (Amon and Benner, 1994; Lampert and Sommer, 1997; Wetzel, 2001). However, only a minor portion of DOM is involved in a fast carbon cycle, while the remainder is resistant to microbial degradation (Søndergaard and Borch, 1992; Søndergaard *et al.*, 1995; Wetzel, 2001). Much attention has been paid to the role of ultraviolet (UV) radiation on the biological cycling of DOM in aquatic systems. The UV light can alter or cleave the DOM into smaller and more labile organic molecules

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(Miller and Zepp, 1995; Wetzel *et al.*, 1995; Amon and Benner, 1996; Moran and Zepp, 1997; Wetzel, 2000). As a result, UV radiation can increase the pool of labile DOM in aquatic systems and thereby enhance bacterial production.

However, these processes may be limited to the allochthonous DOM, which is mainly comprised of aromatic, recalcitrant and high molecular weight compounds. Recent studies noted that the autochthonous DOM (originating primarily from phytoplankton), which is composed of relatively labile compounds, is resistant to photodegradation (Thomas and Lara, 1995; Tranvik and Kokalj, 1998; Pausz and Herndl, 1999). Furthermore, labile DOM such as proteinaceous substrates and phytoplankton exudates can be transformed into recalcitrant forms after UV radiation exposure (Naganuma *et al.*, 1996; Tranvik and Kokalj, 1998; Pausz and Herndl, 1999; Obernosterer *et al.*, 2001). These results suggest that the autochthonous DOM seems to be resistant to photodegradation, but obviously altered in its characteristic. With progressing eutrophication autochthonous DOM becomes increasingly important. If lake waters displaying this feature are also exposed by UV radiation, this may affect the carbon cycling and pools of the aquatic systems. Nevertheless, there is little information on the photoalteration of the autochthonous DOM. Especially there have been very few studies on photochemical change in chemical composition of autochthonous DOM.

The purpose of this study was to examine the photoalteration of algal DOM produced from two blue-green axenic cultures by comparing their biodegradability and DOM fraction distributions before and after UV exposure. Bacterial degradation test was used as a measure of biodegradability of algal DOM. The algal DOM was fractionated into five classes using three kinds of resin adsorbents. Some organic acids newly produced during UV exposure also were analyzed with capillary electrophoresis system.

MATERIALS AND METHODS

Preparation of algal DOM

To obtain the algal-derived DOM, two axenic cultures of *Microcystis aeruginosa* and *Oscillatoria agardhii* that were isolated from Lake Kasumigaura (Japan) were grown axenically in 10 *l* polycarbonate bottles at 25°C and about 50 $\mu E/m^2/sec$ under a light/dark cycle of 12 h : 12 h on CB medium. The cultures were stirred by air bubbles provided from a pump equipped with a 0.2 μm sterilizing filter. Since the standard CB medium contains a high concentration of organic carbon, we modified the medium composition by substituting K_2HPO_4 for β-glycerophosphate and $NaHNO_3$ for Tris buffer. DOC concentrations in the medium after inoculation were below 0.5 mgC/l. When the cultures reached their stationary phase, they were filtered through pre-combusted (450°C for 4 h) Whatman GF/F glass-fiber filters. The filtrates were used as sources of the algae-derived DOM.

UV irradiation experiments

For the UV radiation treatment, one liter of DOM sample was put in 1.3 *l* pyrex bottle of a photo-reaction apparatus (USHIO, Japan) and the radiation experiments were conducted for 2, 4, 6, 8, 10, 12 and 24 h. UV light source was provided by UM-452 lamp which emitted the light of nearly all UV wavelength ranges (220 to 400 nm). Since pyrex bottles used in this experiment selectively cut off UV light shorter than 280 nm (the transmission of the pyrex was zero at 280 nm, and was 70% at 320 nm), short UV radiation (UV-C) was not included. UV radiation was measured with a radiometer (MI-340 UV meter, Eikoseiki, Japan), equipped with a UV-A sensor (316~400 nm) and a UV-B sensor (280~315 nm).

Biodegradability experiments

Biodegradability of algal DOM before and after UV exposure was quantified through a series of microbial degradation experiments. A portion (200 ml) of the algal DOM samples before and after UV exposure was poured into pre-combusted 300-ml glass bottles (550°C for 4 h), and then 1 ml of bacteria concentrate were added to give an initial bacterial abundance of around 10^5 cells/ml. Water for the bacteria inoculum was collected from Lake Kasumigaura. The bottles were then incubated in darkness at room temperature (ca 20°C) for 20 days. Ten milliliters of sub-samples for DOC determination were collected from the bottles after 0, 1, 2, 3, 4, 5, 7, 10, 15 and 20 days. The biodegradability experiments were performed in triplicate.

Table 1. Classification of organic compounds for dissolved organic matter in natural waters.

Fraction	Solute compound classes
hydrophobic acids (HoA)	humic substances (humic and fulvic acids)
hydrophobic neutrals (HoN)	hydrocarbons, carbonyl compounds
hydrophilic acids (HiA)	carboxylic acids (fatty and hydroxyl acids), sugar acids
hydrophilic bases (HiB)	protein, amino acids, aminosugars
hydrophilic neutrals (HiN)	oligosaccharides, polysaccharides

DOM fractionation

The DOM samples before and after UV treatment were fractionated into five fractions: hydrophobic acids (HoA), hydrophobic neutrals (HoN), hydrophilic acids (HiA), hydrophilic bases (HiB), and hydrophilic neutrals (HiN), based on their adsorption on a series of macroporous resin adsorbents. Nonionic Amberlite XAD-8 resin (20~60 mesh), strong cation exchange resin (Bio-Rad AG-MP-50, 50~100 mesh), and strong anion exchange resin (Bio-Rad AG-MP-1, 50~100 mesh) were used for fractionation. The fractionation procedure was according to Imai *et al.* (2002). Appropriate classification of organic compounds according to the DOM fraction is listed in Table 1 (Leenheer, 1981; Thurman, 1985).

Chemical analyses

Some carboxylic acids that were found to be major products formed during UV exposure were analyzed on a capillary electrophoresis (CE) system (Quanta 4,000, Waters). A 70 cm fused silica capillary (75 μm inner diameter), and a 100 mM sodium boric acid buffer containing 0.5 mM of an electro-osmotic flow modifier (OFM-BT, Waters) was used for the analyses. A separation voltage of 15 kV was applied and the analytes were detected by indirect UV detection at 185 nm. Standard curves (10~1,000 μg/l) were made for the three detected carboxylic acids (oxalic, formic, and acetic acids).

DOC was measured as non-purgeable DOC with a Shimadzu TOC-5,000 total organic carbon analyzer equipped with Pt catalyst on quartz wool. At least triplicate measurements were made for each sample and analytical precision was within 1% of coefficient of variance (CV). Potassium hydrogen phthalate (Kanto Chemical Co., Tokyo) was used as standard.

RESULTS AND DISCUSSION

It is well recognized that UV radiation can alter the DOM pool in natural waters by complete degradation into CO_2, and by cleaving into more smaller and labile molecule enhancing the bacterial utilization. The photochemical removal of DOC into CO_2 in many natural waters shows a wide range of 0 to 60%, depending on the DOM sources, light sources, and time of light exposure (Wiegner and Seitzinger, 2001). In this study, no significant changes of dissolved organic carbon (DOC) were observed in algal DOM during UV radiation exposure, showing a constant levels of 12.34±0.08 mgC/l in *M. aeruginosa* and 8.68± 0.05 mgC/l in *O. agardhii* (Fig. 1). This implies that complete degradation of algal DOM to CO_2 did not occur during UV exposure. The UV treatment (for 24 h under 42 W/m^2) used in the present study corresponds to the level shown the photochemical effect in other natural waters. Hence, no change of algal DOC in this study is not due to the light treatment, but probably the DOM sources having resistant to UV radiation. Similar results were reported in other studies with phytoplankton exudates (Thomas and Lara, 1995; Tranvik and Kokalj, 1998).

On the other hand, there was a great difference

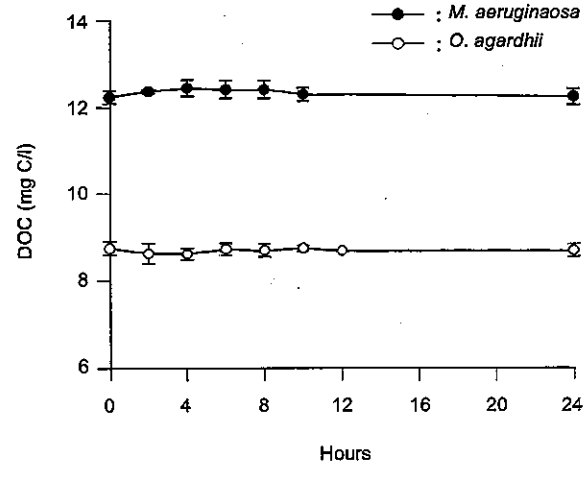

Fig. 1. Changes in concentrations of algal derived DOC with UV exposure times.

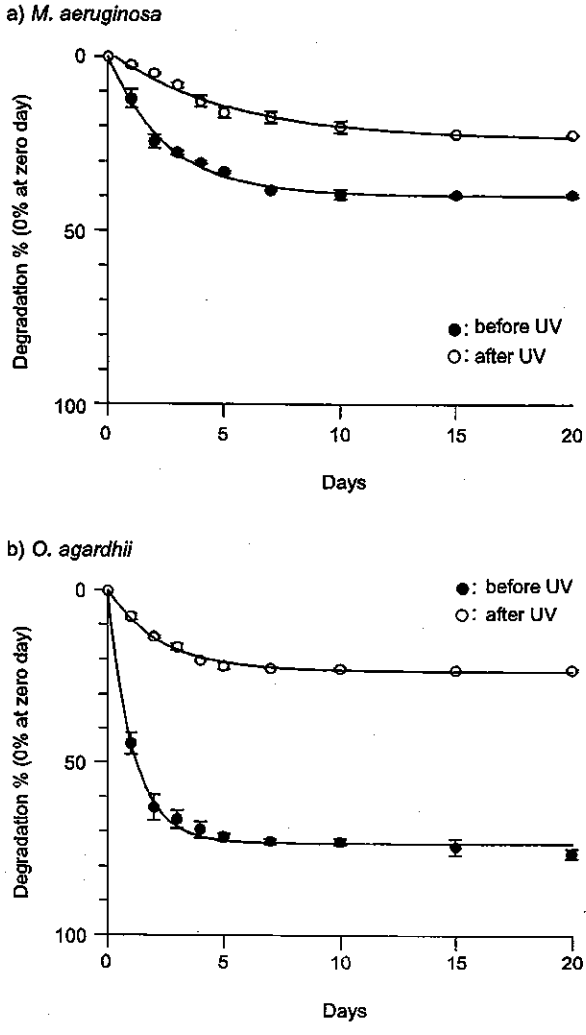

Fig. 2. Degradation curves of algal DOM before and after UV exposure. Bars represent standard deviation.

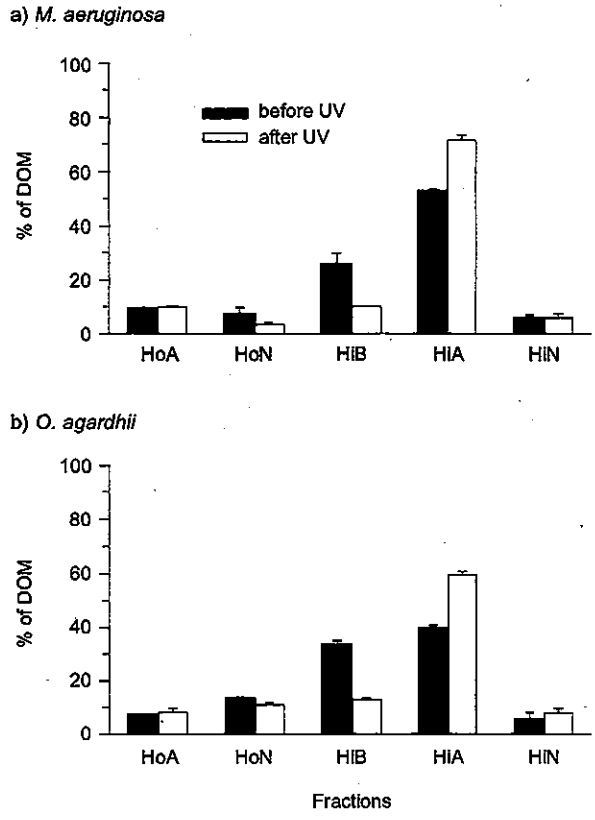

Fig. 3. Proportions (%) of DOM fractions for before and after UV treated samples. Bars represent standard deviation.

in the biodegradability between before and after UV exposure both in two algal DOM sources. Microbial degradations were reduced in the UV exposed algal DOM by 17% in *M. aeruginosa* and 53% in *O. agardhii*, respectively (Fig. 2). Decomposition rates also were two times lower in UV exposed algal DOM (0.20/day in *M. aeruginosa* and 0.45/day in *O. agardhii*, respectively) than in raw algal DOM (0.40/day in *M. aeruginosa* and 0.91/day in *O. agardhii*, respectively). The decreased bacterial activity on UV exposed algal DOM has also been reported in other studies (Tranvik and Kokalj, 1998; Pausz and Herndl, 1999). They found that microbial activity on the UV exposed algal DOM was inhibited by 15 to 20%, while the loss of DOC was less than 1% during the UV exposure. These results indicate that algal DOM can be altered qualitatively without complete degradation by UV radiation. Thus, we further tried to fractionate the algal DOM before and after UV exposure to understand the change of their chemical compositions caused by UV radiation.

The hydrophilic bases (HiB) and acids (HiA) were dominant fractions of the algal DOM (more than 70% both in *M. aeruginosa* and *O. agardhii*), although the proportion of each fraction differed with the sources of algal DOM (see black bars in Fig. 3). Hydrophobic fractions (HoA and HoN) contributed only 16% in *M. aeruginosa* and 20% in *O. agardhii*, respectively. After UV radiation exposure, the proportions of the HiB (protein-like DOM) and HiA (carboxylic acids-like DOM) fractions were considerably changed compared with other fractions (see white bars in Fig.

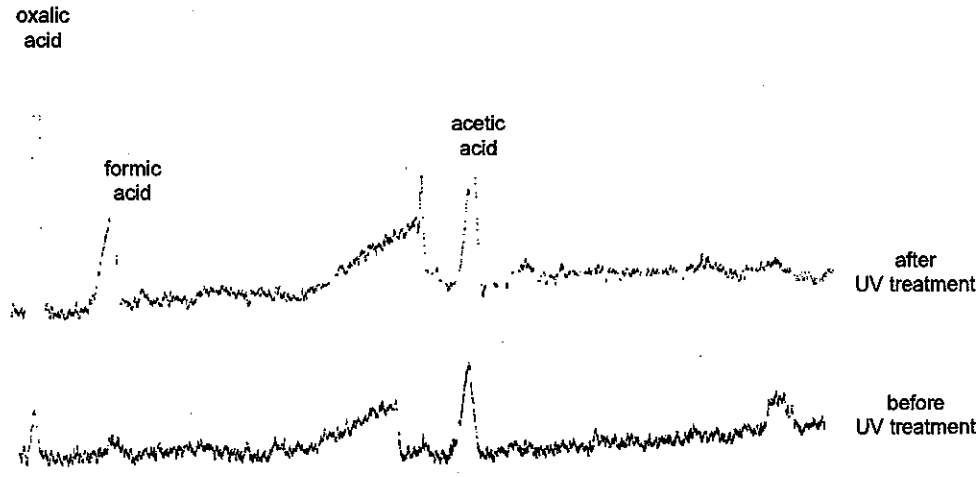

Fig. 4. Electropherograms for before and after UV treated samples (*M. aeruginosa*) indicating UV-induced increase of oxalic, formic and acetic acids.

3). In both algal DOM sources the fractions of the protein-like DOM decreased considerably (16.8% and 20.0% of DOM, respectively) after UV exposure. In contrast, the carboxylic acid-like DOM increased as much as the decrease of the protein-like DOM after UV exposure. Differences in the chemical compositions of algal DOM between before and after UV exposure also provided evidence that algal DOM changed qualitatively by UV radiation.

Contradictory results were reported by Thomas and Lara (1995), who found no changes in chemical compositions as well as concentration of algal DOM after UV exposure. The differences between their and our results may be due to the algal DOM used in two experiments. We used freshly produced algal DOM, while the DOM used in their experiments had been aged in the presence of bacteria for 8 months. During the long incubation, bacteria would utilize initially labile constituents that also would be changeable by UV radiation. Thus, initially labile DOM might be not involved in their experiments in spite of the fact that they are important fraction of algal DOM.

To clarify the increase of the carboxylic acids-like fraction after UV exposure, we measured several carboxylic acids (oxalic, formic, and acetic acids) with capillary ion electrophoresis (CE) system. The three carboxylic acids increased after UV exposure in both algal DOM sources, although the extent of increase for each organic acid differed with the sources of algal DOM (Table 2). Especially, a substantial increase of oxalic acid (410 µg/l) and formic acid (530 µg/l) after the UV exposure was observed in DOM from *M. aeruginosa* (Fig. 4, Table 2). In general, carboxylic acids are easily decomposable materials for bacteria (Allard *et al.*, 1994; Bertilsson and Tranvik, 1998; Wetzel, 2000). Hence, the increased HiA fraction (probably produced as photo-product of HiB fraction) may not be linked to the recalcitrance of algal DOM by UV exposure. Further research is needed to clarify the mechanism of the photoalteration of algae-derived DOM in aquatic ecosystems.

Table 2. Results of the CIE analysis indicating an increase of carboxylic acids after UV exposure. The values are averages of duplicates. (Unit: µg/l)

Carboxylic acids	*M. aeruginosa*		*O. agardhii*	
	before	after	before	after
Oxalic acid	50	460	70	180
Formic acid	30	560	550	560
Acetic acid	150	200	30	150

In the present study, we presented that algal DOM can be photochemically altered in its chemical composition and biodegradability. The photoalteration of algal DOM is likely to have an influence on the carbon cycle and pool in aquatic systems, especially algal DOM is important carbon source, e.g. by making algal DOM unavail-

able for the production of bacteria.

ABSTRACT

The effect of ultraviolet (UV) radiation on the characteristics of algae-derived dissolved organic matter (DOM) was examined by comparing the biodegradability and DOM fraction distribution of algal DOM before and after UV exposure. Algal DOM from two axenic cultures of *Microcystis aeruginosa* and *Oscillatoria agardhii* were irradiated for 24 h at a UV intensity of 42 W/m^2. A complete degradation of algal DOM during the UV exposure did not occur, remaining at constant concentrations of dissolved organic carbon (DOC). After UV exposure, however, microbial degradations were reduced by 17% in *M. aeruginosa* and 53% in *O. agardhii*, respectively, and decomposition rates also were two times lower in UV exposed algal DOM. In addition, the chemical compositions of algal DOM altered substantially after UV radiation exposure. The proportions of hydrophilic bases (HiB; protein-like DOM) decreased considerably in both algal DOM sources after UV exposure (16.8% and 20.0% of DOM, respectively), whereas those of hydrophilic acids (HiA; carboxylic acids-like DOM) increased as much as the decrease of the HiB fraction. Capillary ion electrophoresis (CE) analysis showed that several carboxylic acids increased significantly after UV exposure, further confirming an increase in HiA fractions. The results of this study clearly indicate that algal DOM can be changed in its chemical composition as well as biodegradability without complete degradation by UV radiation.

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雲門湖水中の溶存有機物の特性

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Characteristics of Dissolved Organic Matter in Lake Unmun

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Abstract

Dissolved organic matter (DOM) in Lake Unmun (Korea) and its two inflowing rivers was fractionated using three resin adsorbents (XAD-8 resin, macroporous cation and anion exchange resins) into five classes : aquatic humic substances (hydrophobic acids), hydrophobic neutrals, hydrophilic acids, bases, and hydrophilic neutrals. The characteristics of DOM were investigated by evaluating DOM fraction distribution, ultraviolet ray absorbance of DOM fractions (UV absorbance at 260nm), and molecular size distribution of DOM. Aquatic humic substances (AHS) and hydrophilic acids (HiA) were found to be predominant in both lake and river waters. In particular, AHS were greater in percentage fraction than HiA. The UV to dissolved organic carbon (DOC) ratio (UV/DOC ratio) of AHS was two times higher than that of HiA. The UV/DOC ratios of the total DOM, AHS and HiA were 12.9-32.8, 7.2-29.4 and 5.3-17.1 [m ABS $cm^{-1} \cdot l \cdot g\ C^{-1}$] , respectively. The molecular weight distribution of the lake water DOM was determined by high-pressure size exclusion chromatography and found to exhibit a relatively narrow size range and low weight-averaged molecular weights ranging from 1,010 to 1,110 $g \cdot mole^{-1}$.

Key words : DOM fractionation, aquatic humic substances, hydrophilic acids, molecular weight distribution

1. はじめに

近年，社会，産業構造の高度化による水需要量の増加に伴い，河川自流水よりも湖水やダム湖水を水道水源水として利用する割合が増えている。一方，湖沼の水質に眼を転じると，幾つかの湖，例えば琵琶湖や霞ケ浦において，流域発生源対策が実施されているにもかかわらず，湖内の全有機物指標である化学的酸素要求量（Chemical Oxygen Demand, COD）が漸増する傾向が報告されている[1,2]。特に溶存態CODの増大がCOD濃度を押し上げている。水道水源としての湖水・ダム湖における有機物濃度の上昇は，浄水処理過程の塩素殺菌プロセスにおいて産生されるトリハロメタン等の消毒副生成物に係る健康リスクの増大を意味する。従って，水道水源保全の観点から，有機物，特に溶存有機物（dissolved organic matter, DOM）濃度が上昇する原因を明らかにすることが求められている。

湖水や河川水中のDOMは複雑で不均質な混合物であり，過去数十年間に渡り研究がされてきたが，その物理化学的特性，生態学的および地球化学的役割は未だに十分に理解されていない[3]。従って，このような状況においてDOMの特性や起源についての有益な情報を得るためには，最初に，DOMを明白な共通の化学的性質に基づいた切り口でマクロ的に分画して各分画成分の存在比や特性を評価する必要がある。分離・分画の切り口の基本となるDOM成分としては，代表的難分解性DOMであり天然水中のDOMの主要成分であるフミン物質（フミン酸＋フルボ酸）が最も適切と考えられる。フミン物質は疎水性の有機酸である，すなわち，分画の切り口は，疎水性—親水性，酸性—塩基性となる。Leenheer (1981)[4]は，この切り口に基づいて，DOMを3種類の樹脂を用いて6つの分画する分取手法を開発した。しかし，彼の手法はDOM濃度の比較的高いサンプルを対象とした樹脂脱着に基づく分取法であり，かつ操作が煩雑なため，多数のサンプル，少量のサンプル，DOM濃度の低いサンプルの場合にはその適用が困難である。

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本研究では，Leenheerの分取法を低いDOM濃度および少量のサンプルに適用でき多数のサンプルにも対応できるように改良された分析手法[2]を採用して，湖水DOMを疎水性—親水性，酸性—塩基性の違いに基づいて分画し，DOM分画分布および各画分の特性を評価する。本研究の目的は，ダム湖である雲門湖湖水および流入河川水を対象として，本DOM分画手法を適用し，フミン物質および各分画成分の存在比や吸光度特性およびDOMの分子量分布を把握し，ダム湖におけるDOMの特性や起源を評価することである。雲門湖においてもCODの漸増傾向が報告されている[5]。

2．実験方法

2.1 サンプル採取地点

雲門湖（Lake Unmun）は，韓国の東南に位置する琴湖江系統の広域上水道事業の一環として建設された人工のダム湖である（**Fig. 1**）。このダム湖の流域面積は301 km²で，1993年から湛水が開始され，その水は大邱広域市，慶山市，永川市や雲門郡地域の上水源，農業用水及び河川維持用水として利用されている。湖へ流入する主な河川は東倉川と雲門川の二つである。

水サンプルは，東倉川からの影響が大きいと考えられる地点(DL)，ダム湖心(DC)，雲門川からの影響が大きいと考えられる地点（UL），東倉川の流入地点（DR），雲門川の流入地点(UR)から採取した。1998年6月から1999年5月まで月1回のペースで，湖水ではバンドーン採水器を用いて表層水（1 m）を2 *l* 採水した。流入河川水も同様に2 *l* 採水した。

水サンプルは450°C 4時間熱処理したガラス瓶に採取した。ガラスボトルの密栓は全てテフロンライナー付きのものを用いた。試料は，クーラーボックスに入れ氷冷状態で実験室に持ち帰った。サンプルのろ過には熱処理したWhatman GF/Fフィルター（平均孔径0.7μm）を用いた。採水後，直ちに実験できない場合には4°C，冷暗所で保存した。

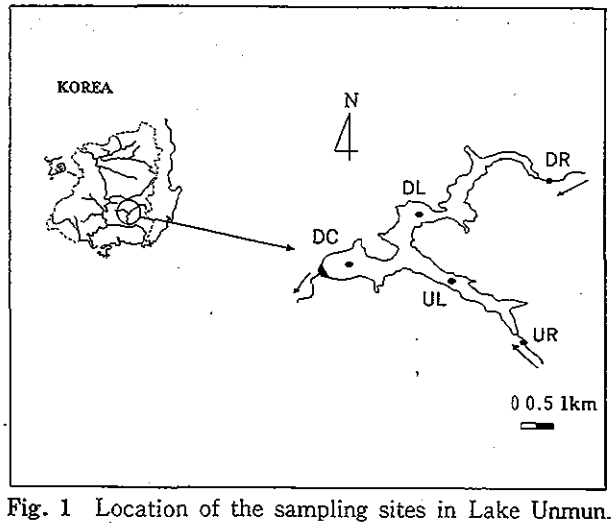

Fig. 1 Location of the sampling sites in Lake Unmun. DL: Dongchang Point; DC: Dam Center; UL: Unmoon Point; DR: Influent river, the Dongchang River, into Lake Unmun; UR: Influent river, the Unmoon River, into Lake Unmun.

2.2 溶存有機物（DOM）分画手法

本手法において，試料水DOMは3種類の樹脂（非イオン性網状アクリル系樹脂XAD-8［Amberlite 20-60メッシュ，平均比表面積450m²·g⁻¹］，強酸性マクロポーラス陽イオン交換樹脂［Bio-Rad AG-MP-50，50-100メッシュ，水素イオン形］，強塩基性マクロポーラス陰イオン交換樹脂［Bio-Rad AG-MP-1，50-100メッシュ，塩基イオン形］）により，疎水性酸（フミン物質），疎水性中性物質，親水性酸，塩基性物質，そして親水性中性物質の5つに分画される。本分画手法では，疎水性有機物と親水性有機物の分離はXAD-8樹脂への吸着あるいは溶出により操作的に定義した。この疎水性—親水性の分離は溶質の極性とサンプル量に対するXAD-8樹脂量の比によって規定される。カラム容量ファクター50の条件において，50%以上がXAD-8樹脂に吸着されるものを疎水性有機物，50%以上が溶出するものを親水性有機物とした[4]。pH 2でXAD-8樹脂に吸着しアルカリで溶出するものを疎水性酸(hydrophobic acids)，pHに関係なくXAD-8樹脂に吸着し，酸でもアルカリでも溶出しないものを疎水性中性物質（hydrophobic neutrals, HoN）と定義した。疎水性酸の操作的定義はフミン物質（aquatic humic substances, AHS）の分離条件と同一である。pH 2の条件でXAD-8樹脂カラムを通過し陽イオン交換樹脂に捕捉されるものを塩基性物質（bases, BaS）とした。一方，陽イオン交換樹脂を通過して陰イオン交換樹脂に捕捉されるものを親水性酸（hydrophilic acids, HiA），陽イオン交換樹脂にも陰イオン交換樹脂にも捕捉されないものを親水性中性物質（hydrophilic neutrals, HiN）とした。各画分に対応すると考えられる有機化合物を**Table 1**に示す。

XAD-8樹脂はThurmanとMalcolm[6]の方法に従って精製した。精製済みXAD-8樹脂は水とメタノールの混合液中に保存した。3 m*l* のXAD-8樹脂をガラスカラムに充填して，約200m*l* のMilli-Q水（Milli-Q SP. TOC，Millipore）で洗浄した後，0.1M NaOH溶液，次いで0.1M HCl溶液の順序で各々約10m*l* を通水する操作を3回繰り返した。0.1M HClの最終通水の際にブランクサンプルを採取した（B1）。

陽イオン交換樹脂と陰イオン交換樹脂は，メタノールで24時間ソックスレー洗浄した。さらに，陰イオン交換樹脂については樹脂量の10倍量の1 M NaOH溶液により水酸基イオン形に置換した。陽イオンおよび陰イオン交換樹脂をMilli-Q水で十分に洗浄した後，陽イオン交換樹脂6 m*l*，陰イオン樹脂12m*l* を各々ガラスカラムに充填し，陽イオン，陰イオン樹脂カラムの順序に連結し

Table 1 Classification of organic solutes for dissolved organic matter fractionation

Fraction	Solute Compound Classes
Hydrophobic Acids	Aquatic Humic Substances (humic and fulvic acids)
Hydrophobic Neutrals	Hydrocarbons, Pesticides, Carbonyl Compounds, LAS
Hydrophilic Acids	Sugar Acids, Fatty Acides, Hydroxyl Acids
Bases	Protein, Amino Acids, Aminosugars
Hydrophilic Neutrals	Oligosaccharides, Polysaccharides

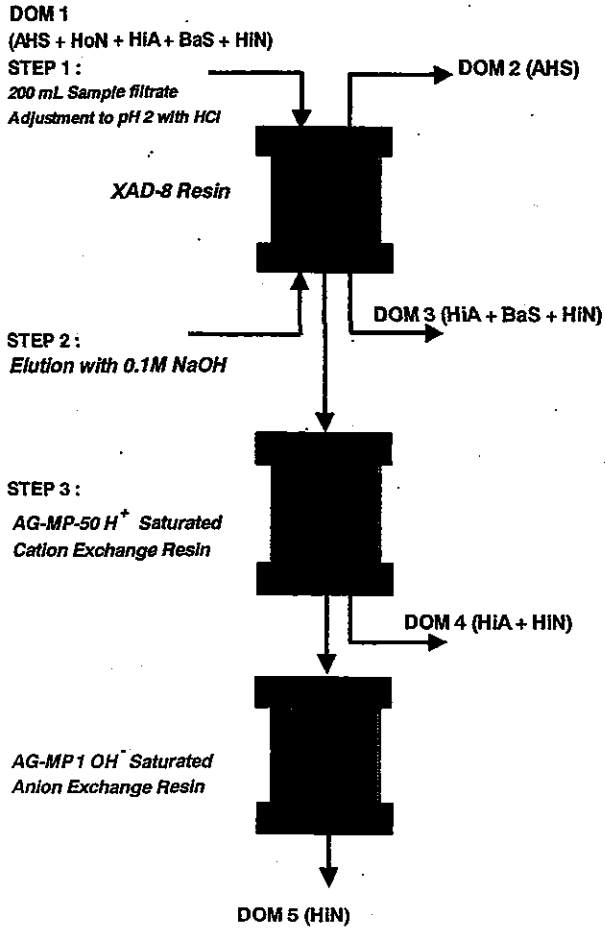

Fig. 2 Experimental procedure for dissolved organic matter (DOM) fractionation. DOM fractions are AHS, aquatic humic substances; HoN, hydrophobic neutrals; HiA, hydrophilic acids; BaS, bases; HiN, hydrophilic neutrals.

た後に約1 l のMilli-Q水を通水した。通水後,各々のカラムからブランクサンプルを採取した(B2, B3)。

DOM分画手法の手順を**Fig. 2**に示す[2]。HClでpH 2に調整したサンプル200ml (DOM 1)を3 mlのXAD-8樹脂カラムに通水した。約1ベッド容量の相当する0.01M HCl溶液を通水した後,約3ベッド容量に相当する0.1M NaOHを逆方向に通水し,溶出液量を測定した(DOM 2)。XAD-8樹脂カラム通過溶液(DOM 3)を,陽イオン—陰イオン交換樹脂の順序で連結された二つのカラムに通水した。陰イオン交換樹脂の3ベッド容量に相当するサンプルが通水した後,陽イオン交換樹脂カラム(DOM 4),次いで陰イオン交換樹脂カラム(DOM 5)の順でカラム通過液を採取した。各画分のDOM農度は以下に示す式(1)から(5)のように算出した。カラム通水はタイゴンチューブを用いてペリスタポンプで約1 ml·min^{-1},溶出は0.5ml·min^{-1}以下の流量で実施した。ポンプチューブ以外のチュービングは全てテフロンチューブを用いた。分画終了後に,DOM1～DOM5およびB1～B3のDOC濃度および紫外部吸光度(UV)を測定した。

$$AHS = DOM2 \times (\text{Elutant Volume}) / (\text{Sample Volume}) \quad (1)$$

$$HoN = DOM1 - AHS - (DOM3 - B1) \quad (2)$$

$$BaS = (DOM3 - B1) - (DOM4 - B2) \quad (3)$$

$$HiA = (DOM4 - B2) - (DOM5 - B3) \quad (4)$$

$$HiN = DOM5 - B3 \quad (5)$$

ここで,B1:XAD-8樹脂ブランク,B2:陽イオン交換樹脂ブランク,B3:陽イオン+陰イオン交換樹脂ブランク。XAD-8の樹脂ブランクの平均DOC濃度は0.23±0.08 (sd) mg·l^{-1} (n=59),陽イオン交換樹脂ブランクは0.05±0.04 (sd) mg·l^{-1} (n=59),陰イオン交換樹脂ブランクは0.04±0.03 (sd) mg·l^{-1} (n=59)であった。

2.3 分子量分布

湖水ろ過サンプル(DOM)の分子量分布および平均分子量を,ゲルろ過クロマトグラフィー(Hitachi L-6200ポンプ,Hitachi L-4000UV検出器,Hitachi D-2500データインテグレーター,Rheodyne7125サンプルインジェクター)により測定した[7]。ゲルろ過クロマトグラフィーカラムとしてはWater Protein Pak 125(ジオール結合したシリカゲル,直径10μm)を用いた。移動相としては,0.004Mリン酸バッファーでpH6.8, 4 M NaClを適量添加してイオン強度0.1Mに調整したものを用いた。移動相流速は1 ml·min^{-1},検出波長は260nm,サンプル量は100μlであった。サンプルのイオン強度は移動相と同じ程度になるように4 M NaClで0.1M程度に調整した。ゲルろ過クロマトグラフィの分子量スタンダードとしては,ポリスチレンスルホン酸ナトリウム(分子量35000, 18000, 8000, 5400, 1800)を用いた[8]。全浸透容量はアセトンを用いて求めた。湖沼・河川水中においてDOMのかなりの部分を占めるフミン物質は,ランダムコイル状態として存在すると考えられる[7,9]。従来,標準物質として使用されてきたタンパク質は球状であり,DOMの分子量を過大評価する恐れがある。従って,本研究では,球状タンパク質ではなくランダムコイル状態で存在するポリスチレンスルホン酸を分子量スタンダードとして使用した。

2.4 分析方法

DOC濃度は,ろ液に2 M HClを添加しpH 2に調整したサンプルにキャリアガス(純空気)を通気し無機炭素を除去した後,Shimadzu TOC-5000により測定した。分析誤差は概ね1%以下であった。紫外部吸光度は,光路長1 cmの石英セルを用いて,波長260 nmでShimadzu UV-PC 2500により測定した。フミン物質のコンポーネントと考えられるフェノール化合物,安息香酸,多環芳香族化合物の極大紫外部吸収波長(π—π^*遷移)は200-290nmに存在する[10]。波長260nmの吸光度は硝酸イオン,臭素イオン等の影響か無視でき,難分解性有機物に高感度であると報告されている[11,12]。以上の理由で260nmを測定波長として選択した。サンプルのpHをHClでpH 2に調整し,Milli-Q水をブランクとして吸光度を測定した。生物化学的酸素要求量(BOD),COD,浮遊懸濁物濃度(SS),クロロフィル-a (Chl.-a)などの水質項目測定は,韓国の水質環境汚染公定試験法[13]およびStandard Methods[14]に準じて分析した。

3. 結果と考察

3.1 雲門湖水と流入河川水の水質およびDOM分画

雲門湖における水質(BOD, COD, SS, Chl.-a)の

1998年から1999年かけての変化を Fig. 3 に示す。5月から6月にかけて Chl. -a の濃度が8.13μg・l⁻¹まで上昇した。この時期には藻類の増殖が活発であったと思われる。8月と9月には，おのおの411mm, 364mm の激しい降雨が観測された。浮遊懸濁物質（SS）濃度が10月に急激に増加したのは，おそらく，8月と9月の降雨の影響による農耕地等からの懸濁物質の流出が1ヶ月遅れで湖水において発現したものではないかと推測される。易分解性有機物指標である BOD は年間を通して低い値を示した。一方，全有機物指標である COD の濃度は夏から秋にかけてわずかに増加する傾向を示した。

雲門湖内の3地点（東倉川側地点：DL，湖心：DC，雲門川側地点：UL）と2つの流入河川水（東倉川：DR，雲門川：UR）の DOC 濃度を Fig. 4 に表す。湖水の

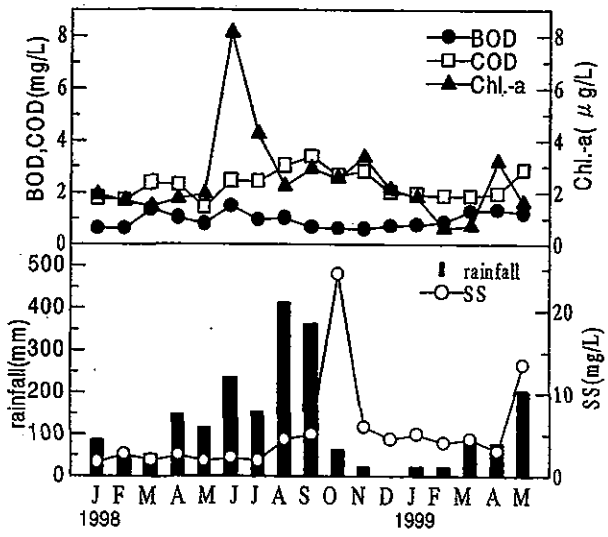

Fig. 3 Seasonal variations of water quality parameters (COD, BOD, Chl. -a, SS and rainfall) in Lake Unmun from January 1998 to May 1999. DC: Dam Center.

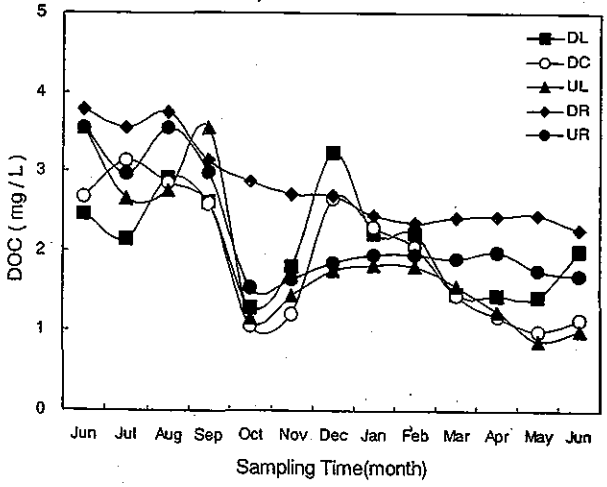

Fig. 4 Seasonal variations of dissolved organic carbon (DOC) in Lake Unmun water and rivers inflowing to Lake Unmun from July 1998 to May 1999. DL: Dongchang Point; DC: Dam Center; UL: Unmoon Point; DR: Influent river, the Dongchang River, into Lake Unmun; UR: Influent river, the Unmoon River, into Lake Unmun.

DOC 濃度は，本研究の調査期間に限って言えば，6月から9月まで高い濃度を示し，10月に急激に減少し，12月に再び上昇し，その後，翌年6月まで漸減する傾向を示した。流入河川水については，主要2河川で DOC 濃度のトレンドは顕著に異なった。両河川とも夏期に DOC 濃度が最大となったが，東倉川（DR）は高い DOC 濃度を維持しつつ漸減する傾向を示し，一方，雲門川（UR）では DOC 濃度が10月に急激に減少し，それ以降，11月—12月で少し上昇するが，ほぼ一定の値の値を示した。DR を除いて，湖内3地点および UR では DOC 濃度が10月に急激に減少した。8月と9月に大量の降雨があったことを考えると（Fig. 3），この DOC 濃度の低下は降雨による影響の結果と推定される。ただ，DR，すなわち東倉川の DOC 濃度は減少していないため，降雨の DOC 濃度への影響は UR，すなわち雲門川に顕著に発現したと言える。雲門川からの河川水流出はとても大きな影響を湖水 DOC 濃度に及ぼしていると推測される。

本調査期間においては，1998年9月と1999年12月を除いて，流入河川水の DOC 濃度のほうが湖水よりも高い値を示した。従って，湖内部生産に起因する DOM 寄与は相対的にそれほど大きいものではないと思われる。流入河川水 DOM の特性が湖水 DOM の特性を規定する可能性が高い。

3.2 雲門湖水と流入河川水の DOM 分画

湖内3地点および2つの流入河川で採取したサンプルに DOM 分画手法を適用して得られた DOM 分画分布（平均値）を Fig. 5 に示す。全てのサンプルにおいて有機酸，すなわちフミン物質（疎水性酸）と親水性酸の存在比が卓越していた。フミン物質が最も多く，次いで親水性酸，塩基性物質，親水性中性，疎水性中性物質の順に存在比は小さくなった。

フミン物質の平均存在比は，湖内の東倉川側地点（DL）地点で36%（0.88mg・l⁻¹），ダム湖心（DC）で39%（0.90 mg・l⁻¹），湖内の雲門川側地点（UL）地点で40%（0.89

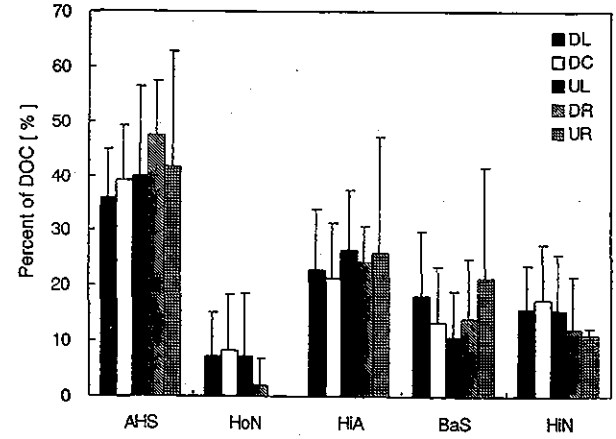

Fig. 5 DOM-fraction distributions in Lake Unmun. DOM fractions are AHS, aquatic humic substances; HoN, hydrophobic neutrals; BaS, bases; HiA, hydrophilic acids; HiN, hydrophilic neutral. Error bars represent±1standard deviation. DL: Dongchang Point; DC: Dam Center; UL: Unmoon Point; DR: Influent river, the Dongchang River, into Lake Unmun; UR: Influent river, the Unmoon River, into Lake Unmun.

mg·l^{-1})，東倉川 (DR) で47% (0.96mg·l^{-1})，雲門川 (UR) で42% (0.96mg·l^{-1}) であった。雲門湖水と河川水で顕著な差はなかった。親水性酸の存在比も，ダム湖心のDC部では21%，DL地点で23%，UL地点で27%であり，流入河川水における親水性酸の存在比とあまり変わらなかった (東倉川 (DR) 24%；雲門川 (UR) 26%)。塩基性物質については湖水で平均11-18%，河川水は平均14-21%を占め，親水性中性物質については湖水で平均15-18%，河川水平均11-12%を占めた。一方，疎水性中性物質の平均存在比は10%以下と低く，農薬や炭化水素化合物，カルボニル化合物のような有機化合物の湖水および河川水DOMへの寄与は低いと考えられた。

Thurman[15]は，フミン物質は天然水中のDOCの40-60%を占め，湖水では40%程度と報告している。また，河川水の場合にはフミン物質が優占しDOCの約50%を占め，親水性酸は約25%程度と報告している。DavidとVance[16]は，米国メイン州の河川水においてフミン物質はDOCの平均57%，30%であったと報告している。また，今井ら[2,17]は琵琶湖と霞ヶ浦の湖水および流入河川水のDOM分画分布を測定して，湖水では親水性酸が，河川水ではフミン物質が優占することを示した。以上の既報研究の報告から，本研究の対象である雲門湖水とその流入河川水においてもDOM分画分布に関して同様な傾向が現れると予想したが，湖水と河川水ではフミン物質および親水性酸の存在比には顕著な差が見受けられなかった。湖水でもフミン物質が優占していることから，雲門ダム湖では湖水特有の内部生産等によるDOMへの寄与が少ないのでは推測される。これは雲門ダム湖が完成して間もないダム湖であることに起因している可能性がある。

雲門ダム湖3地点におけるDOM分画分布の季節変化を **Fig. 6** に示す。フミン物質の存在比は秋に最も高い値を示した。これはSS濃度の急激な上昇に対応しており (**Fig. 1**)，激しい降雨による水流出の際に，流域からフミン物質を多く含む水が湖水に流れ込んだものと考えられる。一方，親水性酸は夏にその存在比が大きくなった。藻類由来DOMの寄与が夏に大きくなったと思われる[17,18]。塩基性物質は，春と冬に増大した。親水性中性物質は8-20%の存在比を示し，春に顕著に低い値を示した。疎水性中性物質は夏に存在比が大きかった。

3.3 雲門湖水及び流入河川水のUV/DOC比

雲門湖水と流入河川水中の地点別UV/DOC比を **Fig. 7** に示す。湖水中DOMの平均UV/DOC比は16-21[m ABS cm^{-1}·l·g C^{-1}]，河川水中DOMの平均UV/DOC比は23-28 [m ABS cm^{-1}·l·g C^{-1}] であった。DL地点の湖水DOMのUV/DOC比は12-29 [m ABS cm^{-1}·l·g C^{-1}]，ダム湖心DC地点におけるDOMのUV/DOC比は11-38 [m ABS cm^{-1}·l·g C^{-1}]，UL地点DOMのUV/DOC比は13-43 [m ABS cm^{-1}·l·g C^{-1}] であった。河川水である東倉川 (DR) と雲門川 (UR) のDOMの平均UV/DOC比は，各々23, 28[m ABS cm^{-1}·l·g C^{-1}] で，河川水のほうが湖水より高い値を示した。湖水DOMは外部負荷によるDOMと内部負荷によるDOMからなると考えられる[19]。生物活動から生産されるDOMは主に炭水化物とタンパク質等の脂肪族性有機物であり，そのUV/DOC比は低いと報告されている。すなわち外部から流入するDOMのUV/DOC比は比較的に高く，内部生産性DOMのそれは低いと考えられる。従って，流入河川水のDOM UV/DOC比は湖水のそれよりも大きくなると考えられる。雲門湖水と流入河川水のDOM UV/DOC比の関係についても同様な傾向が見受けられた。

フミン物質の平均UV/DOC比は湖水で24 [m ABS cm^{-1}·l·g C^{-1}] で，流入河川の東倉川 (DR) と雲門川 (UR) で各々29 [m ABS cm^{-1}·l·g C^{-1}]，24 [m ABS cm^{-1}·l·g C^{-1}] の値を示した。雲門川に比較してDOM濃度が高い東倉川 (DR) のほうがわずかに高いUV/DOC比を表していた。湖水親水性酸の場合，UV/DOC比

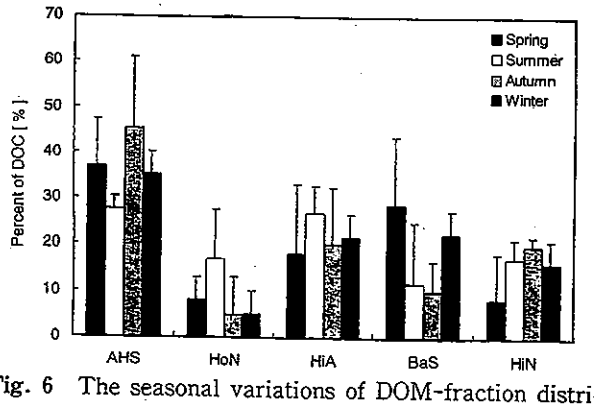

Fig. 6 The seasonal variations of DOM-fraction distributions in Lake Unmun. DOM fractions are AHS, aquatic humic substances; HoN, hydrophobic neutrals; HiA, hydrophilic acids; BaS, bases; HiN, hydrophilic neutral. Spring indicates the data in March; summer does from June to August; autumn does from September to November; winter does from December to February. Error bars represent±1standard deviation of the mean.

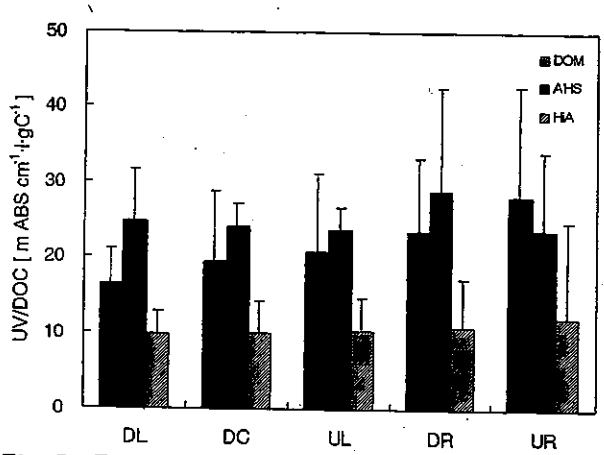

Fig. 7 The ratios of ultraviolet absorbance to dissolved organic carbon (UV/DOC) in Lake Unmun and its inflowing river waters. DOM, dissolved organic matter; AHS, aquatic humic substances; HiA, hydrophilic acids. Error bars represent±1 standard deviation. DL: Dongchang Point; DC: Dam Center; UL: Unmoon Point; DR: Influent river, the Dongchang River, into Lake Unmun, UR: Influent river, the Unmoon River, into Lake Unmun.

はDL地点，ダム湖心(DC)，ULの地点でほぼ同じで約10 [m ABS $cm^{-1}\cdot l\cdot g\ C^{-1}$] であった。一方，河川水の場合，東倉川 (DR) 地点で11 [m ABS $cm^{-1}\cdot l\cdot g\ C^{-1}$]，雲門川 (UR) 地点で12 [m ABS $cm^{-1}\cdot l\cdot g\ C^{-1}$] であった。

3.4 ゲルクロマトグラフィーによる分子量分布測定

雲門ダム湖の3地点，すなわち東倉川側 (DL) 地点，ダム湖心 (DC)，雲門川側 (UL) 地点で採取されたサンプルのゲルクロマトグラムを **Fig. 8** に表す。流入河川水の影響が大きいと考えられるDL地点やUL地点でのクロマトグラムに比較すると，ダム湖心であるDC地点のクロマトグラムは高分子域で少し低い値を示していた。DL地点のクロマトグラムにおいてのみ，排除限界付近(分子量200万以上）に大きなピークが認められた。このような非常に高分子のDOMに相当するピークは，湖沼底泥間隙水，生活雑排水，下水初沈水等のDOMクロマトグラムにおいて観察されているが（今井，未発表データ)，なぜ湖水，それもDL地点のみに発現したかは現時点で不明である。

Fig. 8 のクロマトグラムから求めた数平均 (Mn) および重量平均分子量 (Mw) を **Table 2** に表す。なお，DL地点のクロマトグラムで排除限界付近に出現したピークは無視した。雲門湖水DOMの Mw はかなり低く

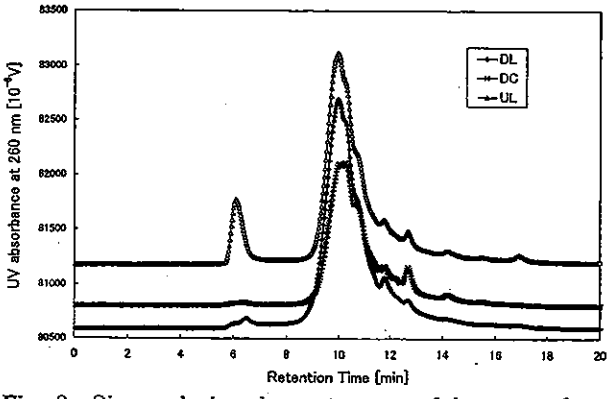

Fig. 8 Size exclusion chromatograms of the waters from DL, DC and UL in Lake Unmun from June 1998 to July 1998. DL : Dongchang Point ; DC : Dam Center ; UL : Unmoon Point. Retention times for PSS molecular weight standards of 35×10^3, 18×10^3, 5.4×10^3, and 1.8×10^3 dalton are 6.7, 7.19, 8.00, 8.49, and 9.63 min, respectively. The retention time of acetone, which corresponds with the total permeation time, is 13.04 min.

Table 2 Weight-averaged molecular weight, number-averaged molecular weight and polydispersity of dissolved organic matter in Lake Unmun

Sampling Station	HPSEC (Detector Wavelength : 260 nm)		
	Mn	Mw	Polydispersity Mw / Mn
DL	760	1110	1.45
DC	710	1010	1.43
UL	760	1090	1.44

1,010-1,110g·$mole^{-1}$であった。予測通りDC地点の分子量が最も低かったが，湖内3地点 (DL，DC，UL) では平均分子量についての顕著な差は示されなかった。Mn と Mw の比，すなわちポリディスパーシティ (Mw/Mn) は，DL地点で1.45，ダム湖心DCで1.43，UL地点で1.44であった。従って，雲門湖水DOMは比較的単分散系 ($Mw/Mn<2$) であり，同じような分子量を持つ有機物の集合体と示唆される[20]。雲門湖水DOMの分子量は，Chinらが報告[7]したFryxell湖水DOMの平均分子量 (Mn：713，Mw：1,080) や，Zhouらの報告[21]によるMissouri川DOMの平均分子量 (Mn：780，Mw：1,188) の値に匹敵しており，ポリシスパーシティ (Mw/Mn) についても同様な値を示しており，既存報告と整合していた。

ここで注意を要する点は，上記の検討はUV吸収を有するDOMを対象としてのみ有効である点である。サンプルDOM中にUVを吸収しない物質が多量に含まれている場合には，本研究で得られたクロマトグラムは“真の”のDOM分子量分布と一致しない可能性がある。

4．ま と め

雲門ダム湖および流入河川を対象として，疎水性—親水性，酸性—塩基性の違いに基づいた溶存有機物(DOM)分画法によって，湖水および河川水DOMをフミン物質，疎水性中性と親水性酸，中性及び塩基性物質の5つに分画し，DOM分画分布，吸光度特性，分子量分布を評価した。

雲門湖水と流入河川水ではDOM分画分布に顕著な違いは認められなかった。湖水および河川水ともに，DOM成分として有機酸，すなわちフミン物質と親水性酸が卓越し，特にフミン物質が優占していた。

湖水のDOM分画分布が季節的に変化する傾向が認められた。フミン物質の存在比は夏に低下し，秋に増大した。一方，親水性酸の存在比は夏に最大となった。塩基性物質の存在比は春に，疎水性中性物質の存在比は夏に高い値を示した。

湖水中の紫外部吸光度 (UV)/溶存有機炭素 (DOC) 比の値はフミン物質，DOM，親水性酸の順で増加し，フミン物質のUV/DOC比は親水性酸のUV/DOC比より約2倍程度高かった。

湖水DOMの重量平均分子量は1,010—1,110 (g·$mole^{-1}$) の範囲にあった。ポリシスパーシティ（重量平均分子量/数平均分子量）は1.43—1.45の比較的小さな値を示し，湖水DOMは比較的に同じサイズの分子からなる集合体であることが示された。

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Photoalteration of dissolved organic matter (DOM) released from *Microcystis aeruginosa* in different growth phases: DOM-fraction distribution and biodegradability

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With 7 figures and 1 table

Abstract: The photoalteration of dissolved organic matter (DOM) produced in different growth phases of the blue-green alga, *Microcystis aeruginosa*, was investigated by comparing the biodegradability and distribution of fractions of algal DOM after different ultraviolet (UV) treatments. The distribution of DOM-fractions (based on hydrophobic-hydrophilic and acidic-basic breaks) showed that two of the fractions, hydrophilic acids (HiA) and bases (HiB), were more abundant in all growth phases of *M. aeruginosa* than the other three fractions, hydrophobic acids (HoA), hydrophobic neutrals (HoN), and hydrophilic neutrals (HiN). The proportion of HiB increased, while the HiA fraction decreased with aging of the algae. After UV treatment, all algal DOM became recalcitrant to bacterial degradation without complete photo-degradation. This was more pronounced in DOM from older cultures (stationary phase) as compared to DOM from the exponential growth phase. The DOM distribution was also significantly different after UV exposure, implying photoalteration to the chemical composition of algal DOM. The proportions of the HiB fraction decreased as a percent of the total dissolved organic carbon pool by 1.5–8.1 % after UVA treatment and by 5.3–15.8 % after UVB treatment. In contrast, the HiA fraction increased by similar amounts. Analyses of fluorescent properties and some carboxylic acids confirmed the changes to the HiB and HiA fractions. However, the increased HiA fraction may not be linked to the recalcitrance of algal DOM after UV exposure, since there was no difference in the biodegradability of this fraction before and after UV treatments. The ini-

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tially labile HiB fraction, however, became less available to bacteria after UV exposure depending on intensity and thus may be linked to the recalcitrance of algal DOM after UV exposure. Our results confirm earlier reports that algal DOM can be changed in its chemical composition as well as biodegradability by UV radiation, and suggest that the HiB fraction may be important in the formation of recalcitrant algal DOM.

Key words: algal DOM, growth phase, UV effects, photoalteration, biodegradability, chemical composition.

Introduction

Dissolved organic matter (DOM) can play a major role as a source of carbon for heterotrophic bacteria in freshwater ecosystems (WETZEL et al. 1972, AMON & BENNER 1994, CARLSON et al. 1994, LAMPERT & SOMMER 1997, WETZEL 2001). There are two major sources of DOM in lake waters: allochthonous, i.e. derived from the catchment area, and autochthonous, i.e. produced within lakes. Allochthonous DOM is composed of primarily terrestrial humic substances (HS) which are recalcitrant to bacterial degradation, while autochthonous DOM is composed of relatively more labile compounds (MUNSTER & CHROST 1990, WETZEL 2001). In most lakes, where allochthonous material is the dominant DOM source, most of the pool is comprised of recalcitrant DOM (WETZEL 2001). However, even in eutrophic lakes and oceanic waters, where the majority of DOM is autochthonous, much of the DOM pool is resistant to microbial degradation (SØNDERGAARD & MIDDELBOE 1995, CHOI et al. 2001).

Recent studies have noted that autochthonous DOM can be transformed into recalcitrant forms, without complete degradation to CO_2, after exposure to UV radiation, implying that photoalteration is manifest in chemical characteristics (TRANVIK & KOKALJ 1998, PAUSZ & HERNDL 1999, OBERNOSTERER et al. 2001). However, there is little information on the changes induced by UV radiation in the chemical characteristics of autochthonous DOM and, thus, a relevant approach to evaluating photoalteration of algal DOM is needed. The first step may be to separate DOM into well-defined macromolecular fractions and to compare their distribution before and after UV exposure.

In pelagic waters, one of the most important sources of autochthonous DOM is extracellular organic matter (EOM) released from phytoplankton. This EOM may occur as a result of active excretion of photosynthetic products and/or leakage from senescent and dead algal cells, and its chemical composition varies with the physiological state of the algae (NALEWAJKO & LEAN 1972, SHARP 1977, FOGG 1982, CHROST & FAUST 1983, HAMA & HANDA 1987, BAINES & PACE 1991). It therefore seems reasonable to consider the physiological state of the algae in an examination of photoalteration of algal DOM.

The objective of this study was to examine the photoalteration of algal DOM produced from different growth phases of *Microcystis aeruginosa* by comparing the biodegradability and distribution of DOM-fractions, before and after UV exposure. The algal DOM was fractionated into five classes: hydrophobic acids, hydrophobic neutrals, hydrophilic acids, hydrophilic neutrals, and hydrophilic bases, using three kinds of resin adsorbents. To confirm the changes in the distribution of these fractions after UV exposure, we also examined their fluorescent properties and some of the organic acids of algal DOM. A bacterial degradation test was used as a measure of biodegradability of the algal DOM.

Materials and methods

Preparation of algal DOM

To obtain the algal-derived DOM, an axenic culture of *M. aeruginosa* (NIES-843), isolated from Lake Kasumigaura (Japan), was grown in ten litre (10 l) polycarbonate bottles at 25 °C and about 50 $\mu E\,m^{-2}/s^{-1}$ under a 12 h : 12 h light/dark cycle on CB medium. The culture was stirred by air bubbles from a pump equipped with a 0.2 µm sterilising filter. Since the standard CB medium contains a high concentration of organic carbon, we modified the medium by substituting K_2HPO_4 for B-glycerophosphate and $NaHNO_3$ for Tris buffer. The concentration of dissolved organic carbon (DOC) in the medium after inoculation was below 0.5 mg l^{-1}.

To determine the growth phases of the culture, its optical density (OD) was measured with a Shimadzu UV-2500 UV/VIS spectrophotometer at a wavelength of 550 nm using a 1 cm long quartz cell. The growth period was divided into one exponential and two stationary phases. When OD doubled within 24 h, growth was considered to be exponential (Phase I on day 7), and the following stages, in which OD remained more or less constant, were considered to be stationary phases (Phase II on day 10 and Phase III on day 13). In each growth phase, cultures were collected and then filtered through pre-combusted (450 °C for 4 h) Whatman GF/F glass-fibre filters. The filtrates were used as the source of algal-derived DOM.

UV treatments

For the UV treatments, triplicate 400 ml sub-samples of filtrate were transferred to 500-ml quartz tubes with silicon stoppers penetrated by three glass tubes. To estimate the effect of different UV radiation levels on the algal DOM, two artificial UV lamps were used throughout the experiments. UVB treatment (2.4 W/m^2 of UVB and 2.0 W/m^2 of UVA) was provided using two Philips TL 40 W/12 RS lamps with a wavelength range of 280 to 400 nm (maximum emission: 300 nm). UVA treatment (13.6 W/m^2 of UVA) was provided using four Q-Panel UVA-340 lamps (wavelength range: 300 to 400 nm, maximum emission: 340 nm) and UVB cutting film with zero transmission at 320 nm (C. I. Kasei, Japan). The quartz tubes containing the filtrate were irradiated for

24 h at 25 °C under the two different UV regimes. Sub-samples (20 ml) for analyses of DOC and fluorescence were taken at 2, 6, 12 and 24 h. All incubation during the UV treatments was conducted under sterile conditions by using a 0.2 µm sterilising filter. UV radiation was measured with a radiometer (MI-340 UV meter, Eikoseiki, Japan), equipped with a UV-A sensor (316–400 nm) and a UV-B sensor (280–315 nm).

Biodegradability experiments

The biodegradability of the algal DOM before and after UV exposure was quantified through a series of microbial degradation experiments. Before and after UV exposure a portion (200 ml) of each algal DOM sample was poured into pre-combusted 300-ml glass bottles (550 °C for 4 h), and 1 ml of bacterial concentrate was added to give an initial bacterial count of around 10^5 cells/ml. Water for the bacterial inoculum was collected from the hyper-eutrophic Furuike Pond, Japan. The bottles were then incubated in darkness at room temperature (ca 20 °C) for five days. Sub-samples (10 ml) for DOC determination were collected from the bottles after 0, 1 and 5 days. The biodegradability experiments were performed in triplicate.

DOM fractionation

Before and after the UV treatment and the biodegradation tests, the DOM samples were fractionated into five classes: hydrophobic acids (HoA), hydrophobic neutrals (HoN), hydrophilic acids (HiA), hydrophilic bases (HiB), and hydrophilic neutrals (HiN), based on their adsorption on to a series of macroporous resin adsorbents. The original fractionation method described by LEENHEER (1981) produced six fractions, including hydrophobic bases (HoB), but we disregarded the HoB fraction since it is known to be very small (IMAI et al. 1998).

Nonionic Amberlite XAD-8 resin (20–60 mesh), strong cation exchange resin (Bio-Rad AG-MP-50, 50–100 mesh), and strong anion exchange resin (Bio-Rad AG-MP-1, 50–100 mesh) were used for the fractionation. The column capacity factor, k', for separating hydrophobic acids through the XAD-8 resin column was 50. Appropriate classification of organic compounds according to the DOM fractions is listed in Table 1 (LEENHEER 1981, THURMAN 1985).

The XAD-8 resin was cleaned and conditioned as described by THURMAN & MALCOLM (1981). Three millilitres (3 ml wet volume) of the XAD-8 resin was packed into a glass column and rinsed three times, alternating from 0.1 M NaOH to 0.1 M HCl, just

Table 1. Classification of organic solutes for dissolved organic carbon (LEENHEER 1981, THURMAN 1985).

Fraction	Solute compound classes
hydrophobic acids (HoA)	humic substances (humic and fulvic acids)
hydrophobic neutrals (HoN)	hydrocarbons, carbonyl compounds
hydrophilic acids (HiA)	carboxylic acids (fatty and hydroxyl acids), sugar acids
hydrophilic bases (HiB)	protein, amino acids, aminosugars
hydrophilic neutrals (HiN)	oligosaccharides, polysaccharides

before application of the sample. A blank sample was collected in the final rinse with 0.1 M HCl (B1). Both AG-MP-50 (hydrogen-form) and AG-MP-1 (chloride-form) resins were Soxhlet-extracted with methanol for 24 h. AG-MP-1 was then converted into the free base-form with 1 M NaOH and rinsed with Milli-Q water (Milli-Q SP. TOC, Millipore). Glass columns containing 6 ml (wet volume) of the cation exchange resin and 12 ml (wet volume) of the anion exchange resin were connected in series and conditioned by pumping about one litre of Milli-Q water through the resins. Blank samples (B2 and B3) were collected from each column after conditioning.

The flow scheme of the DOC fractionation procedure was as follows (IMAI et al. 1998).

Step 1: Acidify filtrates (DOC1) to pH 2.0 with 6 M HCl, pass 200 ml of the filtrate through the XAD-8 column by a peristaltic pump with Tygon tubing at a flow rate of about 1 ml/min, and rinse the column with 1–2 bed volumes of 0.1 M HCl.

Step 2: Elute the column in the reverse direction with more than 3 bed volumes of 0.1 M NaOH at a flow rate not exceeding 0.5 ml/min (DOC2), and measure the elutant volume.

Step 3: Pump the effluent from the XAD-8 column (DOC3) through a series of cation-anion resin columns at a flow rate of about 1 ml/min, and after pumping 1–2 bed volumes of the sample, collect elutant samples from the anion resin column (DOC5) and then from the cation resin column (DOC4).

DOC fractionation was performed in duplicate. After the fractionation, DOC was measured for DOCs 1–5 and for the blank samples. Each DOC fraction was calculated as follows:

$$\mathrm{HoA} = \mathrm{DOC2} \times (\text{elutant volume})/(\text{sample volume}) \quad (1)$$

$$\mathrm{HoN} = (\mathrm{DOC1} - \mathrm{B1}) - \mathrm{HoA} - \mathrm{DOC3} \quad (2)$$

$$\mathrm{HiB} = (\mathrm{DOC3} - \mathrm{B1}) - (\mathrm{DOC4} - \mathrm{B2}) \quad (3)$$

$$\mathrm{HiA} = (\mathrm{DOC4} - \mathrm{B2}) - (\mathrm{DOC5} - \mathrm{B3}) \quad (4)$$

$$\mathrm{HiN} = \mathrm{DOC5} - \mathrm{B3} \quad (5)$$

In order to examine the recovery efficiency of the DOM fractionation method, the relative standard deviation (RSD) of the duplicated measurement for each fraction was estimated according to Standard Methods (APHA 1998). The RSD values were less than 10 % for the determination of the HoA, HiA and HiB fractions and 20 % for the HoN and HiN fractions.

Chemical analyses

Some carboxylic acids that were found to be major products formed during UV exposure (BERTILSSON & TRANVIK 1998, WETZEL 2000), were analysed on a capillary ion electrophoresis (CIE) system (Quanta 4000 E, Waters). Two millilitres of each algal DOM sample were collected in polypropylene vials and 20 µl of octansulfonate was added to a final concentration of 70 µM to obtain isotachophoretic conditions during the electromigrative sample introduction (30 s at 5 kV). Duplicate sub-samples of 0.5 ml were added to polypropylene vials for analysis. A 60 cm fused silica capillary

(75 µm inner diameter), and a 100 mM sodium boric acid buffer containing 0.5 mM of an electro-osmotic flow modifier (OFM-BT, Waters) were used for the analysis. A separation voltage of 10 kV was applied and detection of carboxylic acids was accomplished by indirect UV detection at 185 nm. Standard curves (10–500 µg/l) were made for the three carboxylic acids (oxalic, formic, and acetic acids) detected.

Fluorescence can provide rapid and sensitive analysis of DOM, such as humic-type and protein-like DOM (MAYER et al. 1999). In the present study, we measured the fluorescence at 270/350 nm of excitation/emission because the excitation/emission wavelength is used as an index of protein-like DOM in natural water and phytoplankton exudates (MAYER et al. 1999, FUKUSHIMA et al. 2001). A fluorescence spectrophotometer (Shimadzu RF-5300 PC) equipped with a 150 W xenon lamp was used for the fluorescence measurements. The fluorescence of Milli-Q water was used as a blank.

DOC was measured as non-purgeable DOC with a Shimadzu TOC-5000A total organic carbon analyser equipped with Pt catalyst on quartz wool. Triplicate measurements were made for each sample and analytical precision was within 1 % of the coefficient of variance. Potassium hydrogen phthalate (Kanto Chemical Co., Tokyo) was used as the standard.

Results

Photoalteration of algal DOM produced from *M. aeruginosa*

Exposure to UV radiation made no significant changes to the amounts of dissolved organic carbon (DOC) in the DOM produced from different growth

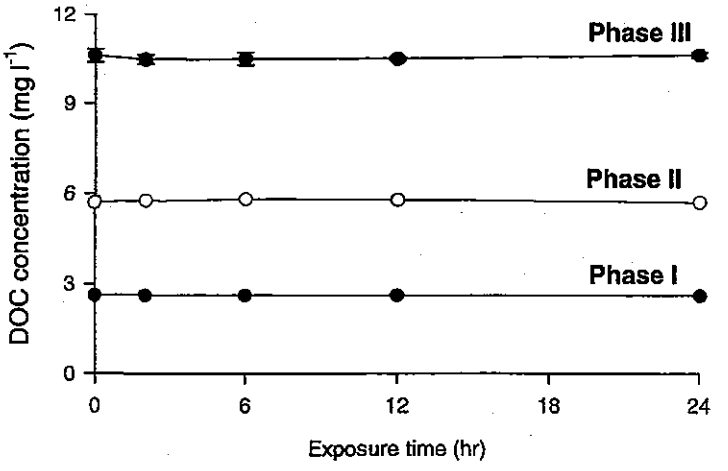

Fig. 1. Changes in DOC concentrations of algal DOM exposed to UV for various lengths of time (Phase I: exponential growth phase on day 7, Phase II: stationary phase on day 10, Phase II: stationary phase on day 13). Error bars represent the standard deviation of the mean of triplicate treatment flasks (Errors less than the size of the symbols are not shown).