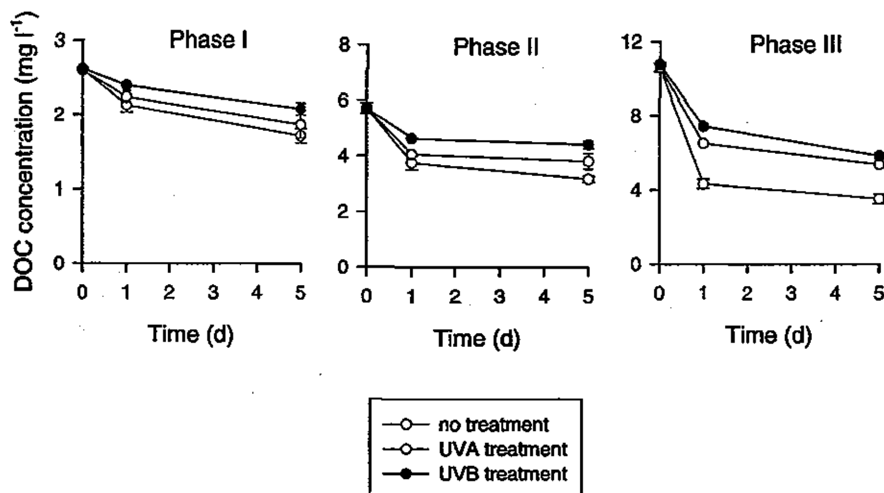

Fig. 2. Decomposition curves for algal DOM obtained from different growth phases and after UV treatment. Error bars represent standard deviations of means of triplicate treatment flasks (Errors less than the size of the symbols are not shown).

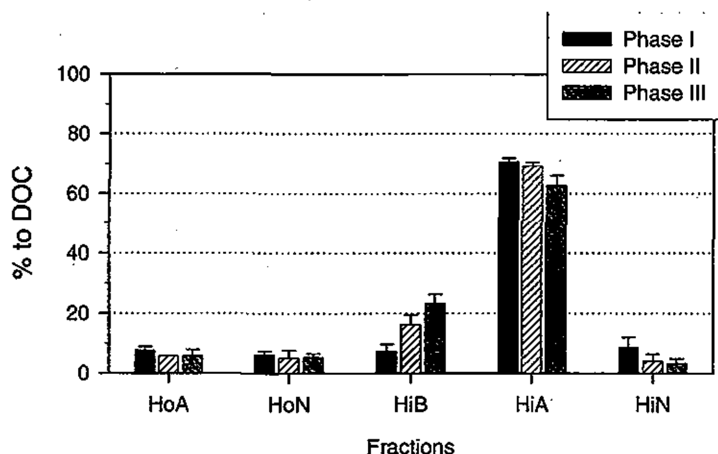

Fig. 3. Fractions of DOM released by *M. aeruginosa* in different growth phases (HoA: hydrophobic acids; HoN: hydrophobic neutral; HiB: hydrophilic bases; HiA: hydrophilic acids; HiN: hydrophilic neutrals). Error bars represent standard deviations of means of duplicate fractionation for each fraction.

phases of *M. aeruginosa*. Levels remained constant at 2.61 ± 0.01 mg/l in Phase I, 5.75 ± 0.05 mg/l in Phase II and 10.53 ± 0.07 mg/l in Phase III (Fig. 1). In contrast, exposure to UV made a considerable difference to the biodegradability of the algal DOM (Fig. 2). Biodegradability decreased after exposure,

depending on the UV treatment, and the decrease was largest in the oldest culture. For example, the microbial degradation (for 5 days) of the DOM from growth phase III decreased by up to 17.4 % after UVA treatment and by 21.1 % after UVB treatment, when compared to no UV treatment.

DOM-fraction distribution of algal DOM

Hydrophilic fractions of the DOM predominated in all growth phases of *M. aeruginosa* (Fig. 3). In particular, the HiA fraction was found to be the most abundant in all growth phases, representing 62.6–70.7 % of the algal DOM. The HiB fraction was the second most prominent, accounting for 7.2–23.2 %. Thus, the HiA and HiB fractions are likely to be the most significant fractions produced by *M. aeruginosa*. With aging of the algae, the proportions of the HiB fraction increased, while the HiA and HiN fractions decreased. The hydrophobic fractions (HoA and HoN) were consistently a minor proportion of the total in all growth phases, together accounting for less than 13 % of the algal DOM.

After exposure to UV radiation, however, the composition changed depending on the growth phase and the UV treatment (Fig. 4). The proportions of the HiB and HiA fractions were considerably changed compared to the other fractions. In all growth phases the proportion of the HiB fraction decreased after UV exposure (by 1.5–8.1 % after UVA treatment and by 5.3–15.8 % after UVB treatment). In contrast, the HiA fraction increased by as much as the decrease in the HiB fraction after UV exposure (by 4.7–8.7 % after UVA treatment and 9.3–16.3 % after UVB treatment). The changes in proportions of the two fractions after UV radiation increased with the age of the culture.

Evidence of photoalteration in two fractions, HiA and HiB, of algal DOM

To clarify the changes in the two fractions (HiB and HiA) after UV exposure, we measured the fluorescence at 270/350 nm, as an index of protein-like DOM for the HiB fraction, and several carboxylic acids for the HiA fraction.

The fluorescence (at 270/350 nm) of the algal DOM (whole DOM before fractionation) was high in Phase III (1.61) and low in Phase I (0.13) (Fig. 5 a). After exposure to UV, the fluorescence declined exponentially with UV exposure time in all growth phases, and the decrease was marked in Phase III after UVB treatment (Figs. 5 a, b).

Three carboxylic acids (oxalic, formic and acetic acids) were detected in all the growth phases of *M. aeruginosa* (Fig. 6). Oxalic acid occurred in low concentrations (below 10 µg/l in all phases) and showed little variation with the age of the culture. In contrast, formic and acetic acids had relatively high

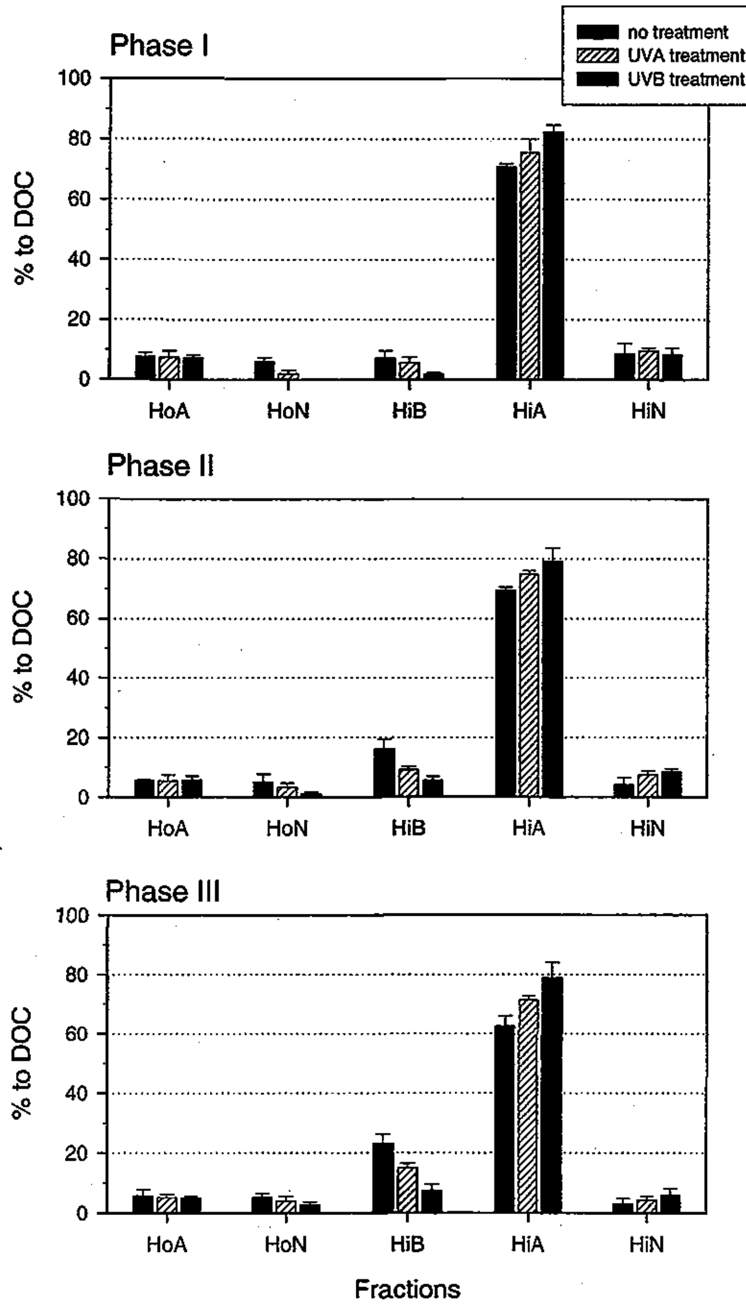

Fig. 4. Fractions of algal DOM obtained from different growth phases and after UV treatment (HoA: hydrophobic acids; HoN: hydrophobic neutral; HiB: hydrophilic bases; HiA: hydrophilic acids; HiN: hydrophilic neutrals). Error bars represent standard deviations of means of duplicate fractionation for each fraction.

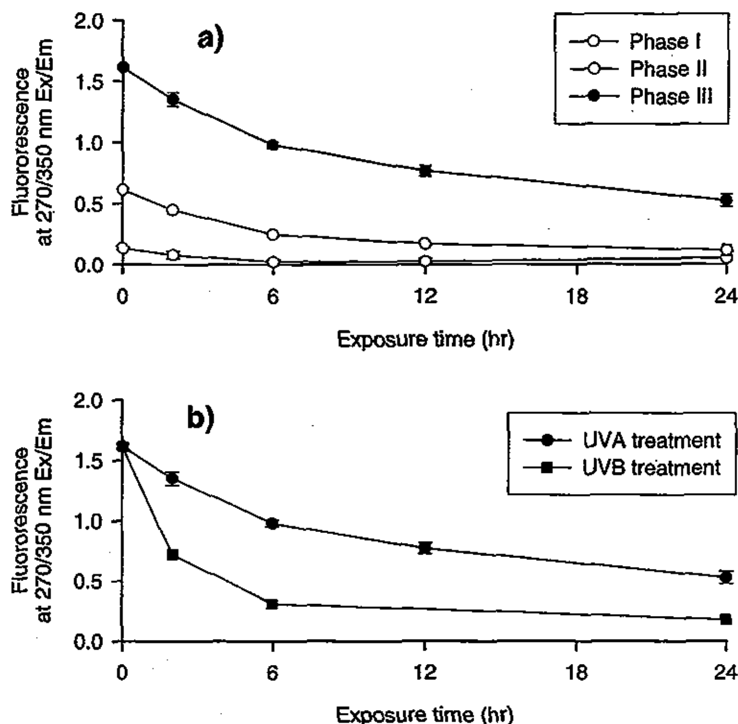

Fig. 5. Changes in fluorescence at 270/350 nm (Ex/Em) of algal DOM exposed to UV for various lengths of time; (a) for growth phases I, II and III after UVA treatment, (b) for Phase III algal DOM after UVA or UVB treatment. Error bars represent standard deviations of means of triplicate treatment flasks (Errors less than the size of the symbols are not shown).

concentrations (33–226 μg/l and 18–206 μg/l, respectively), and increased greatly with the age of the culture (see white bars in Fig. 6). After UV treatment, the three carboxylic acids greatly increased in all the DOM sources, indicating photochemical production of carboxylic acids from algal DOM. The increase in carboxylic acids was higher after UVB treatment than after UVA treatment in samples from all growth phases. For example, compared to no UV treatment, acetic acid increased up to 72 μg/l after UVA treatment and up to 153 μg/l after UVB treatment in Phase II.

Decreased biodegradability of HiB fraction after UV treatment

A different biodegradability (measured as the percentage of DOC utilized compared to the initial DOC in the fraction) was observed in the HiB fraction after UV treatment (Fig. 7). The HiB fraction produced by *M. aeruginosa* was initially very labile to bacterial degradation, showing a high biodegradation of

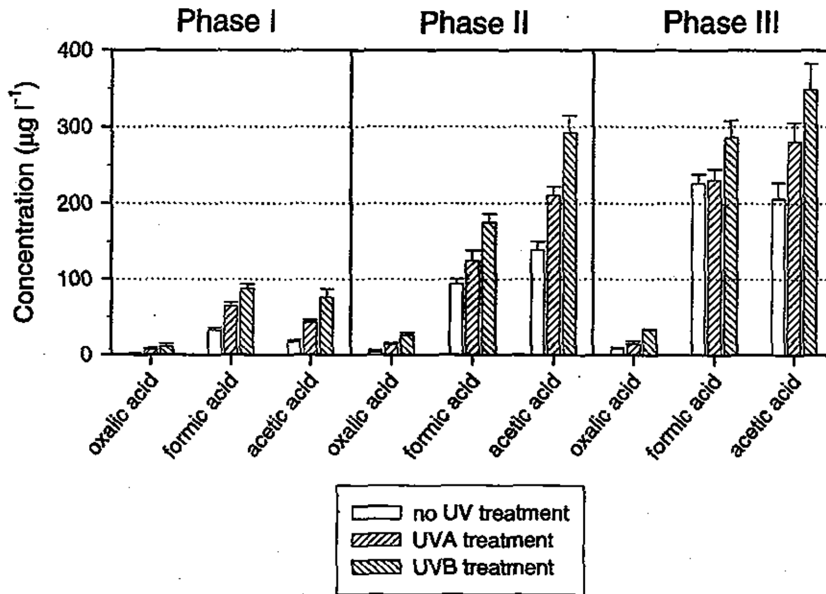

Fig. 6. Concentrations of three carboxylic acids in algal DOM obtained from different growth phases and after UV treatment, indicating UV-induced increase of oxalic, formic and acetic acids. Error bars represent standard deviations of means of triplicate treatment flasks.

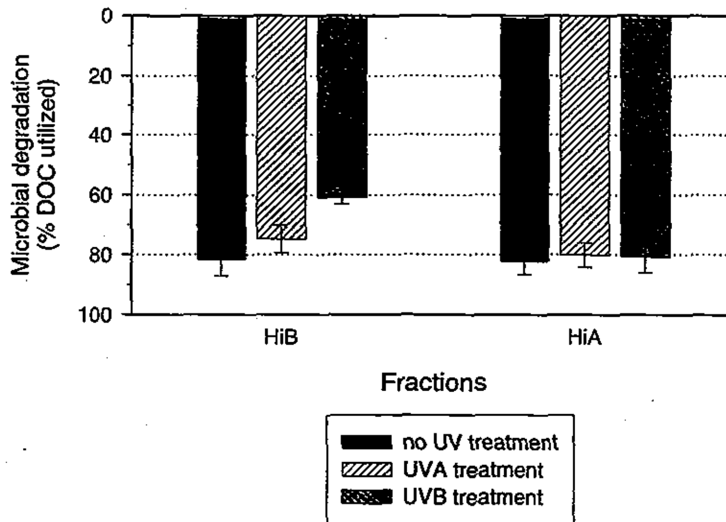

Fig. 7. Microbial degradation of the HiB and HiA fractions in algal DOM from Phase III before and after UV treatments. Samples were incubated in darkness at room temperature for 5 days. Error bars represent standard deviations of means of triplicate treatment flasks.

81.6 ± 5.6 % over 5 days. However, this biodegradability decreased significantly after UV treatment ($p = 0.009$ for UVA treatment and $p = 0.007$ for UVB treatment by paired t-test), and the decrease was greater in the UVB treatment than in the UVA treatment (74.7 ± 4.6 % after UVA treatment and 60.8 ± 2.3 % after UVB treatment). On the other hand, there was no difference in biodegradability of the HiA fraction between before and after UV treatments (Fig. 7).

Discussion

It is well known that UV radiation can alter the DOM pool by causing complete degradation into CO_2, and by cleaving the DOM into smaller and more labile molecules, enhancing bacterial activity (MILLER & ZEPP 1995, WETZEL et al. 1995, AMON & BENNER 1996, MORAN & ZEPP 1997, GARDNER et al. 1998, KIEBER et al. 1999, WETZEL 2000). However, the extent of photochemical transformation of DOM into CO_2 shows a wide range from 0 to 60 % in many natural waters (WIEGNER & SEITZINGER 2001). Clear photo-oxidation has been observed only in waters containing high levels of humic substances (HS), but with no or little algal DOM. Complete photo-oxidation may be limited to allochthonous DOM, high in HS, because HS strongly absorb short wavelength light (FRIMMEL 1994), and most HS are not derived from algae but rather higher plants (WETZEL 2001). In addition, recent studies have shown that initially labile algal-derived DOM becomes more recalcitrant after UV exposure (TRANVIK & KOKALJ 1998, PAUSZ & HERNDL 1999). These studies found that microbial activity in DOM which had been exposed to UV was inhibited by 15 to 20 %, while the loss of DOC was less than 1 % during UV exposure. Research to date has shown that the effects of UV radiation depend largely on the DOM source as well as the light source and length of exposure. In the present study, all the algal DOM produced from different growth phases of *M. aeruginosa* was transformed into more recalcitrant forms after UV exposure without photo-oxidation (Figs. 1 and 2). These results confirm several recent findings on the decreased biodegradability of algal DOM due to UV radiation, and indicate that these findings are common in algal DOM.

Furthermore, there was a difference in the distribution of DOM-fractions after UV exposure, especially in the two major fractions (HiB and HiA) of DOM produced by *M. aeruginosa* (Fig. 4), reflecting photoalteration in the fractional composition of algal DOM as well as the biodegradability. The changes in the two fractions after UV radiation were clear in the oldest culture. After UV exposure, the HiB fraction decreased, while the HiA fraction increased by as much as the decrease in HiB. In contrast, THOMAS & LARA (1995) showed that algal DOM was not changed in chemical composition or

concentration after UV exposure. The difference between their results and ours may be due to the sources of algal DOM used in the two studies. We used freshly produced algal DOM, while the DOM used by THOMAS & LARA (1995) had been aged in the presence of bacteria for 8 months. During this long incubation, bacteria would utilise initially labile constituents that could be changed by UV radiation. Thus, initially labile DOM was not involved in their experiments despite the fact that they are important fractions of algal DOM.

The classification of organic solutes by several researchers has suggested that the HiB and HiA fractions consist mainly of protein-like and carboxylic acid-like DOM, respectively (Table 1). Although the specific organic compounds of the HiB fraction were not identified in this study, the fluorescence at 270/350 nm of excitation/emission, used as an index for the protein-like DOM, supports the decrease in the HiB fraction due to UV radiation (Fig. 5). In addition, the increase of three carboxylic acids after exposure to UV supports the increase of the HiA fraction. Several studies suggest that the photochemical formation of carboxylic acids is linked to the presence of humic substances (ALLARD et al. 1994, BERTILSSON & TRANVIK 1998, WETZEL 2000). In this study, on the other hand, the photochemical production of carboxylic acid-like fractions may be related to the non-humic fractions of algal-derived DOM. However, the increased HiA fraction may not be linked to the recalcitrance of algal DOM caused by exposure to UV, since carboxylic acids are, in general, easily taken up by bacteria (BERTILSSON & TRANVIK 1998), and since the HiA fraction in this study was shown to have high biodegradability after UV treatment (Fig. 7).

Some studies indicate that labile proteinaceous substrates could be transformed into recalcitrant forms during UV exposure (NAGANUMA et al. 1996), or only after a long incubation (KEIL & KIRCHMAN 1994). In this study, the proportion of HiB increased with aging of the algae (Fig. 3), and the decreased biodegradability of algal DOM was more marked in the older phases (Phase II and III) than in the exponential phase (Phase I) (Fig. 2). Furthermore, the initially labile, protein-like HiB fraction became increasingly recalcitrant to bacterial degradation after exposure to UV (Fig. 7). These results indicate that the protein-like fraction may be important in the decrease of biodegradability of algal DOM by UV exposure.

The present study indicates that algal DOM can be photochemically altered in its chemical composition and biodegradability, and the photoalteration may be more important in older phases than in the exponential growth phase of the algae. Furthermore, our results suggest that the protein-like HiB fraction may be important in the formation of recalcitrant DOM.

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水環境におけるフミン物質の特徴と役割

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特集

水環境における腐植物質の役割と分析法の進歩

土壌などに含まれる有機成分である腐植（フミン）物質は，植物が腐朽してできた物質や微生物の代謝物などが複雑に反応してできた天然の高分子物質である。最近では，腐植（フミン）物質が環境中に放出された化学物質の運命に大きく関与している可能性が明らかにされつつあり，その機能が注目されている。本特集では，水環境において腐植（フミン）物質がどのような役割を演じているかについて概説するとともに，その挙動および特性の解明に向けた最新の分析方法の紹介と，土壌浄化を中心とした環境修復への利用について紹介する。（担当編集企画委員　独立行政法人産業技術総合研究所・市川廣保，兵庫県立健康環境科学研究センター・駒井幸雄）

水環境におけるフミン物質の特徴と役割*

今　井　章　雄

1．はじめに

フミン物質（腐植物質，humic substances）は水環境や陸上環境に遍在的に存在する，濃縮すると黄色から黒色を呈する，生物起源で不均質な難分解性の有機物である[1]。フミン物質は土壌有機物の主要な構成要素であり，植物や微生物への養分供給や土壌の団粒構造の保持等の機能を担っている。このためフミン物質の研究は土壌フミン物質を対象として開始され，今日まで2世紀に渡って行われてきた。

一方，水環境でのフミン物質の研究は，湖沼，河川，地下水に溶存しているフミン物質（溶存態フミン物質，aquatic humic substances）が対象であったため，技術的な困難さから，その研究の本格的な進展は米国地質調査研究所の研究者によって定量的分離手法が開発された1980年代初めまで待たねばならなかった[2]。当時，溶存フミン物質の研究が急速に進展した背景要因の一つは，フミン物質を含む水道原水を塩素殺菌処理した場合に有害な消毒副生成物が産生されることが明らかになった[3]ためと言われている。

本稿では，水環境中において溶存態として存在するフミン物質を対象として，その定義，特性（濃度・存在比，分子量等）や水環境における役割・機能について概説する。一般に，フミン物質（humic substances）は，酸やアルカリへの溶解性によって，フミン酸（酸不溶，アルカリ溶解），フルボ酸（酸とアルカリに溶解）およびヒューミン（酸とアルカリに不溶）に操作的に分類される。すなわち，本稿で対象とするフミン物質とは溶存態のフミン酸とフルボ酸ということになる。これ以降，本稿では，溶存態のフミン物質を“フミン物質”と称する。

2．水環境中のフミン物質の特徴

2.1　フミン物質の定義

土壌フミン物質の定義が操作的なものであるように[4]，水環境中のフミン物質も実験操作的に定義された

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ものである。天然水中からフミン物質を分離するために幾つかの手法が提案されてきたが，現時点で最も一般的なものは非イオン性樹脂（XAD-8等）を用いた吸着クロマトグラフィーによる分離手法と言える。この手法はフミン物質の持つ疎水性的性質と酸性官能基含有量に基づいたものである。Thurman と Malcolm[2]は，そのランドマーク的論文において，pH2で，カラム容量ファクター100の条件下で，50%が XAD-8樹脂カラムに吸着し，0.1M の NaOH で溶出するものをフミン物質（aquatic humic substances）と定義した。フミン物質のうち，濃度500mgC・l^{-1}以上，pH1で沈殿するものをフミン酸，溶存しているものをフルボ酸とした。この分離手法は国際腐植物学会（IHSS）[5]で推奨されている手法でもある。ただし，近年，カラム容量ファクターとして100ではなく50という値が使用されている[6,7]。

XAD-8樹脂吸着クロマトグラフィーによるフミン物質の分離手法には，落とし穴が一つある。肝心の XAD-8樹脂が生産中止となり樹脂の入手が極めて困難なことである。同じアクリルエステル系の XAD-7樹脂は市販されているが，0.1MNaOH でフミン物質を樹脂から溶出させると大量の溶存有機物が樹脂から漏れ出してしまう[8]。最近 XAD-8樹脂の代替として DAX-8樹脂（スペルコ社製）が使用され始めている。DAX-8樹脂は，少し吸着能が高いが，フミン物質分離に関して XAD-8樹脂とほとんど差はないようである[9]。

2.2 フミン物質の濃度と存在比（図1）

フミン物質の濃度は水環境によって異なる[10]。一般に地下水や海水では非常に低い（0.05－0.60mgC・l^{-1}）。渓流水・河川水や湖水では0.5－4.0mgC・l^{-1}，泥炭地や湿原水では10－30mgC・l^{-1}と非常に濃度が高い。このセクションでは，様々な水環境中のフミン物質の濃度や存在比について概説する。ここではフミン物質の組成にはほとんど言及しないが，存在する水環境が著しく異なるとフミン物質の組成・特性も顕著に異なることは既に認識されている[11]。

(a) 河川水・渓流水

米国における渓流水・河川水の溶存有機物（dissolved organic matter, DOM）の主要コンポーネントはフミン物質であり，平均で DOM の約50%を占めると報告されている[10]。河川水フミン物質のほとんどはフルボ酸であった（80-90%）。存在比に関する例外的ケースは，泥炭地や湿原を水源とする茶褐色を帯びた河川水である。例えば，湿原を水源とする米国スワニー河[7]や熱帯域に位置するブラジル・リオ・ネグロ川[12]では，DOM の70-90%はフミン物質である。湿原水中のフミン物質の存在比は非常に高いようである。サロベツや霧多布の湿原水を調査したところ，フミン物質の存在比は実際とても高かった（50-75%，今井未発表データ）。

わが国の河川水や渓流水中のフミン物質の存在比に関する報告例はそれほど多くない。琵琶湖北湖に流入する4河川水のフミン物質は，DOM の37-73%（0.32-0.71 mgC・l^{-1}）を占めていたと報告されている[13]。DOM 濃度が最も低い森林自然系河川水（安曇川）でフミン物質の存在比が最大となった。また霞ヶ浦に流入する主要4河川水のフミン物質は0.43-1.39mgC・l^{-1}で，その平均存在比は38.6%（31.1-42.7%）であった[14]。

(b) 地下水

地下水は表流水に比べてフミン物質の含有率が少ないようである。地下150m 以深の地下水では，フミン物質は DOM の12-33%であったとの報告がある[10]。Cronan と Aiken[15]は，浅い O/A 層土壌水では，フミン物質は DOM の約46%占めたが，深い B 層土壌水では27%で

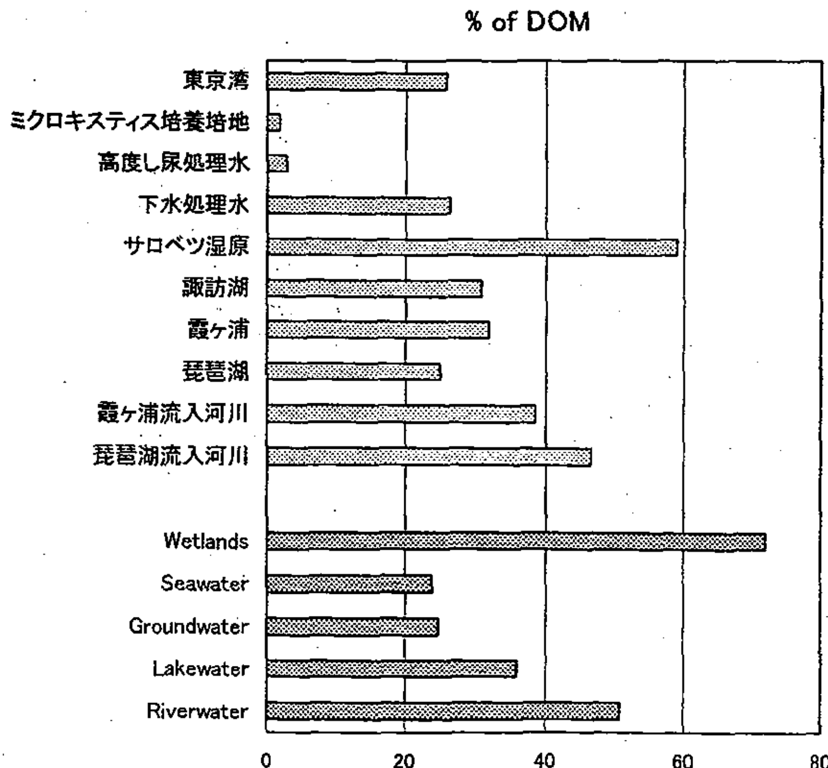

図1　水環境中におけるフミン物質の溶存有機物（DOM）に対する存在比（溶存有機炭素 DOC として）。英文表記は国外データを示す。

あったと報告している。存在比の深さ方向の減少は無機土壌によるフミン物質の選択的吸着のためと説明された。

(c) 湖 水

湖水フミン物質の存在比は，一般に，DOMの40%程度と言われている[10]。フミン物質の濃度は湖の栄養状態が高まると増大する（貧栄養湖：0.5-1.0，中栄養湖：1.0-1.5，富栄養湖：1.5-5.0mgC・l^{-1}）。河川水と同様に，湖水でもフルボ酸がフミン物質として優占する(85-90%)。

湖水フミン物質は外部由来フミン物質と湖内部で生産されるフミン物質の混合物と考えられる。外部由来と内部生産由来フミン物質の相対的な寄与率は，湖沼の物理的サイズや主要流入DOM源が土壌由来か，河川由来か，あるいは藻類由来かに関係している[10]。一般に，湖沼のサイズが大きいほど，藻類由来DOMの湖沼有機物プールへの寄与が大きくなる。ところが，藻類由来DOMに占めるフミン物質の割合はとても低い。Choiら[16]は3種の藍藻類を室内培養して培地中のフミン物質を測定したところ，その存在比は0.2%－16%の範囲にあったと報告している。すなわち，湖のサイズが大きいほど，藻類由来有機物が主要なDOM源であるほど，湖水フミン物質の存在比は低くなる傾向にある。

わが国における湖水フミン物質に関する情報を調べてみるとは，その存在比は琵琶湖北湖で約25%[13]，霞ヶ浦で32%[14]，諏訪湖で31%，手賀沼で30%（今井未発表データ）であった。琵琶湖でフミン物質の存在比が比較的低いのは，琵琶湖が他の湖沼に比較してサイズがとても大きく，DOM源としても藻類由来DOMが優占しているためと推測される。

(d) 海 水

海洋においてフミン物質はDOMの5%-25%(0.06-0.60mgC・l^{-1})を占めている[17]。そのほとんど(>90%)はフルボ酸である[18]。Fukushimaら[19]は広島湾のDOMの33%(0.40mgC・l^{-1})はフミン物質であったと報告している。東京湾表層水ではDOMの25-28%をフミン物質が占めていた（今井未発表データ）。外洋中に蓄積されているDOMのほとんどは藻類等の微生物由来と考えられる[20]。すなわち，陸地から離れるほどフミン物質のDOMに対する寄与は低下するだろうと推察される。

(e) 排水処理水

Imaiら[21]は様々な排水処理水中のフミン物質濃度を測定した。フミン物質の存在比は下水処理水で18-27%(0.6-1.1mgC・l^{-1})，し尿処理水で3-24%(0.1-8.7mgC・l^{-1})，合併処理浄化槽排出水で28%(1.7mgC・l^{-1})であった。下水処理水では処理場の規模が大きくなるほどフミン物質の存在比が大きくなった。限外沪過膜等を採用している高度し尿処理場処理水にはフミン物質はほとんど含まれていなかった。

2.3 分子量と分子構造

(a) 分子量

分子量とサイズ分布に関する情報は，フミン物質のバルク的な物理化学的特性を理解するうえでとても重要である。Thurmanら[22]は，小角X線散乱法により，水環境中のフルボ酸の分子量は500－2,000，フミン酸の分子量は10,000以下であり，当時考えられていた分子サイズよりもかなり小さいことを示した。フルボ酸は同じようなサイズの分子の集合体で，一方，フミン酸は著しく分子サイズが異なる分子の集合体であることも指摘された。Chinら[23]は，無関係電解質の溶離液への適正な添加とランダムコイル状の非タンパク質ポリマー（polystyrene sulfonates）を分子量スタンダードとして用いることにより，高速液体サイズ排除クロマトグラフィーによってフミン酸やフルボ酸の分子量を高い信頼性をもって測定できることを示した。水環境中に存在するフミン物質（主にフルボ酸）の分子量は概ね1,000－2,000の範囲にあることが示された。

(b) 分子構造

Leenheerら[24]は，IHSS標準サンプルである米国スワニー河フミン物質を対象とした研究成果を基に，フルボ酸に対する幾つかの構造モデルを提案した。フルボ酸は基本的に不均質であり，同じフルボ酸分子でも反応性に顕著な違いのあることが指摘された。MacCartyとRice[25]は，生態学的な観点から，フミン物質の持つ特性，(1)分子構造の不規則性と(2)微生物が関与する特異的な生成経路の欠如は，フミン物質の共通的な特徴である難分解性（残存性）と遍在性を説明していると主張した。つまり，規則正しい順序で配列できないモノマー（単量体）の複雑で不均質な混合物であるフミン物質は，微生物がその進化の過程において，わざわざ特異的な酵素を誘導して分解するには余りにも利益の少ない炭素基質とみなせる。従って，微生物に無視されたフミン物質は，難分解性物質として残存し続けてきたと言える。

一方，最近の分析機器の進展に伴い，これまで得ることができなかったフミン物質の分子レベルの構造特性がかなり明らかとなってきた。Leenheerら[26]は，電子スプレーイオン化多段式質量分析計によって，スワニー河フルボ酸に対する単一の前駆化合物の存在を示し，その構造を提案している。

3．水環境中におけるフミン物質の役割・機能

水環境中におけるフミン物質の役割は，基本的に“modifier”と表現されるような，何らかの作用を緩衝・修正する機能を担っていると考えられる。同時に，その機能は二元的（正と負），環境条件によって効果が逆転するような側面を持っている。

3.1 毒 性

フミン物質の毒性に関する情報は極めて少ないが，人の健康に直接関係しているとする2，3のケースが報告されている。Luら[27]は，台湾において，井戸水中のフミン物質と黒足病との因果関係を示唆している。Pengら[28]は，飲料水中のフルボ酸がカシンベック（Kasin-Beck）病におけるフリーラディカル生成メカニズムに直接的に関与すると報告している。

3.2 トリハロメタン生成能

フミン物質が浄水処理プロセスの塩素殺菌過程において産生される発ガン物質トリハロメタン等の消毒副生成物の主要な前駆物質であることは良く知られている[3,29]。フミン物質は，凝集沈殿や活性炭吸着の浄水処理によって良好に除去できるようである[30]。最新の研究では，フミン物質の存在比が低い水源では，フミン物質よりも非フミン物質（親水性DOM）の方がトリハロメタン

前駆物質として重要であることが示されている[31]。

3.3　フミン物質の相互作用：金属，有害化合物，リン

(a)　金　属

水環境中に存在する溶存有機物，特にフミン物質は微量金属と錯体を形成することにより，毒性と直接関係する“フリーな（水化した）”イオン濃度を変動させ，よって金属の生物に対する毒性や利用性を規定している[33]。一般に，フミン物質の金属に対する錯化反応の安定度は，土壌フミン物質と同様に，Irving-Williamsシリーズに従う[34]：$Hg^{2+}>Cu^{2+}>Ni^{2+}>Zn^{2+}>Co^{2+}>Mn^{2+}>Cd^{2+}>Ca^{2+}>Mg^{2+}$。フミン物質は3価イオンのFeやAlとも安定した錯体を形成する。

生態システムの生産性の観点からみると，金属イオンとの錯化反応による金属毒性の低下は，フミン物質のとても“ポジティブ”な性質と言える。一方，植物プランクトンの増殖必須金属である鉄との錯化の場合には“ポジティブ”と“ネガティブ”，二元的な機能が認められる。Prakashら[35]は，室内培養実験において，フミン物質の存在は植物プランクトンによる鉄の取り込みを促進したとの報告している。一方，Imaiら[36]は，霞ヶ浦のフルボ酸を添加した培養実験で，湖水濃度レベルに匹敵するフルボ酸濃度で，アオコを形成する*Microcystis aeruginosa*の増殖が鉄不足のために著しく抑制されることを示した。従って，フミン物質の微生物の鉄利用性に対する影響は，その存在濃度に関連するトレード・オフ的なものと言える。

(b)　有害有機化合物

フミン物質は，収着反応（吸着や溶け込み）を介して，非イオン性有機化合物である農薬，有機塩素化合物（DDT，PCBs等）や多環式芳香族炭化水素（PAHs）と結合し，その溶解度を増大させることが知られている[37,38]。フミン物質との結合による溶解度の増大は，有機化合物の水中での移動性を高め，同時に大気相への揮散性を顕著に減少させる。また，金属の場合と同様に，非イオン性有機化合物の生物体への濃縮がフミン物質との結合によって減少する。例えば，PAHsの動物プランクトン（Daphnia）への生物濃縮はフミン物質との収着作用により低減される[39]。内分泌攪乱物質の水環境中の挙動・運命にもフミン物質との収着が大きく影響するとの報告もある[40]。

(c)　リ　ン

フミン物質は湖沼の表水層におけるリンの循環サイクルに深く関与していると示唆されている[41]。フミン物質と鉄の結合体（AHS−Fe）とオルソリン酸イオン（PO_4^{3-}）との間に化学的な平衡関係が存在する。リンの循環や生物利用性の観点からみると，$AHS-Fe-PO_4$結合体の存在は重要である。なぜならば，フミン物質の濃度が極めて低ければ，リンは鉄の酸化物や水酸化物に吸着されたり，リン酸鉄として沈殿してしまい，その生物利用性が極めて低くなるからである。$AHS-Fe-PO_4$結合体は，紫外線を吸収すると，光還元反応によりFe(III)がFe(II)に還元され，結果としてPO_4^{3-}が水中に放出される。水中でFe(II)が溶存酸素によってFe(III)に酸化されれば，再びPO_4^{3-}を取り込んで$AHS-Fe-PO_4$結合体に戻るらしい。

3.4　光吸収・光分解

フミン物質は紫外線をとても良く吸収する。フミン物質の光吸収能は波長が長くなると低下する特徴があり，UV-B域（290-320nm）で最大，UV-A域（320-420nm）で低くなり，PAR（光合成に利用される放射線）域（400−750nm）で最小となる[42]。

光吸収に関してもフミン物質の二元的（正と負の）影響が認められる。フミン物質濃度が非常に高い腐植栄養湖ではフミン物質のPAR吸収による光合成活性の低下が懸念される。一方，フミン物質は生物に有害な紫外線を効果的にブロックする役割を果たしている。

フミン物質が光吸収に伴って分解され，結果としてバクテリアの増殖（2次生産）を促進することが知られている[43]。フミン物質の光分解によって，生物利用性の高い低分子有機物（酢酸等のカルボキシル酸，アルデヒド等）や栄養塩（リン）が放出される。この増殖促進効果は，同時に，光化学的に生成されるCOやオキシダント（フリーラディカル等）によるDNAや細胞へのダメージによって抑制される[44]。腐植栄養湖での光放射線による増殖抑制効果は1−4％，フミン物質の光分解変質に伴う増殖促進効果は23-34％であったと報告されている[45]。

3.5　酸中和能

最近実施された湖沼調査によって，有機酸（フミン物質）は，酸中和能が0-50meq・l^{-1}の範囲では，表流水のpHを0.5-2.5ユニット低下させえることが示された[46]。この結果は，人為的な酸性化の影響を受けているpH緩衝能の低い湖においてでも，有機酸（フミン物質）の存在によって湖水pHが著しく低下する可能性を示している。一方，有機酸（フミン物質）は水環境の酸性化に正の効果も与えている。フミン物質は弱酸でpH緩衝能が高いため，強酸沈着によって酸性化された水域のpHの更なる低下を防ぐ役割を果たす。また，フミン物質は急性毒性の高いアルミニウムイオン等の金属イオンと強く錯化するため，毒性金属を無毒化する効果もある。腐植栄養湖で，着色していない湖沼よりも，pHが低くとも魚類等が生き残れるのは，この理由によると思われる[46]。

3.6　微生物生態系システムへの影響

“難分解性”と称されてきたフミン物質が，バクテリアにとって重要なエネルギーや栄養素の供給源となりえることが明らかとなってきている。従属栄養バクテリアを用いたフミン物質を唯一の炭素源とする条件の培養実験において，フミン物質がバクテリアに利用されることが明白に示された[47,48]。フミン物質の持つエネルギーや栄養素が従属栄養微生物にどのように利用されるか（例えば微生物ループへの関与）は，フミン物質の生態学的な役割に関する研究において重要なトピックと認識されている。

フミン物質の微生物利用性は多くの因子に関係している。例えば，光分解特性，分子サイズや栄養素（NやP）の存在状態等。3.4で記述したように光分解によって脂肪族有機物含量が高くなるとフミン物質はバクテリアにとって利用しやすくなる。

分子サイズは，フミン物質や溶存有機物（DOM）の生物利用性における重要な因子である。分子サイズと生物利用性の関係については，近年その認識が逆転した。高

分子分画は低分子分画よりも微生物利用性が低いと言われてきたが，最近では，反対に，高分子分画のほうが炭素源として容易に利用されることが示唆されている[49]。

フミン物質と結合して有機態として存在する窒素とリンの生物利用性は不明な部分が多い。おそらく，有機態リンのある部分はバクテリアや藻類に利用されるだろう。フミン物質の窒素含量は一般的に低いが[10]，量的な面を考えると重要な窒素プールとみなされる。さらに短波長の光吸収によって，フミン物質だけではなく有機態窒素やリンの利用性も顕著に増大するだろう[41,50,51]。

4．まとめ

本稿では，水環境中に存在するフミン物質の存在比や濃度，毒性，金属や有機化合物との相互作用，酸中和作用，生態系への影響等についてレビューを行った。

フミン物質は，基本的に毒性の無い，水環境中に遍在する，難分解性でありながら化学反応性のかなり高い有機酸である。また，フミン物質は全く分解しないというわけではなく，微生物に対する炭素(＋エネルギー)，リン，窒素の重要な供給源としての役割を果たしている。

水環境中でのフミン物質の遍在性や難分解性を考えると，フミン物質は，多面的な環境負荷の影響に対する緩和・調節機能を発揮することによって，水環境の地球化学的・生態学的な構造に高い安定性を与えていると言える。フミン物質の存在は，微生物生態系システムの複雑さを理解する上でとても重要である。

水環境中の有機物研究の現在までの流れをみると，明らかに，研究の焦点はフミン物質から溶存有機物(DOM)へとシフトしている。これは，最近，フミン物質とともに非フミン物質の重要性が認識されはじめたためである。今後のフミン物質研究は，DOM の主要コンポーネントとしての位置付けの下に進展するだろう。

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RESEARCH PAPER

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Voltammetric determination of dissolved iron and its speciation in freshwater

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Abstract Analytical methods were developed to determine the concentration of total dissolved iron and its chemical speciation in freshwater using cathodic stripping voltammetry (CSV) with 1-nitroso-2-naphthol (NN) at pH 8.1. The concentrations of total dissolved iron in river water that iron concentration was certified and in natural water samples from Lake Kasumigaura were determined successfully. The natural iron ligand concentration and the conditional stability constant were determined by ligand competition between NN and the natural ligands present in the sample. In the water samples from Lake Kasumigaura, the concentrations of total dissolved iron and natural ligand were 47.8 ± 4.4 nM and 80.0 ± 19.6 nM and the conditional stability constant (K'_{FeL}) was $10^{25.9\pm0.4}$ M^{-1} ($n = 3$). The value of K'_{FeL} was greater than any reported K'_{FeL} for seawater. More than 99.9% of the dissolved iron existed as organic species due to the very high value of the conditional stability constant. The inorganic iron concentration calculated from these results was $10^{-13.4}$ M, indicating that the inorganic iron level in Lake Kasumigaura was similar to that in the open ocean and therefore that iron can be a limiting factor for algal growth in Lake Kasumigaura. This is the first report of the complexation of iron(III) and inorganic iron levels in lake water determined by CSV.

Key words Iron · Speciation · Organic complexation · Voltammetry

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Introduction

Iron is an essential micronutrient for algal growth, and cyanobacteria, in particular, have a higher cellular iron requirement than other algae (Brand 1991). Iron may be the limiting factor for primary production in parts of the open ocean (Martin and Fitzwater 1988; Martin et al. 1990; Martin et al. 1994). Although the concentration of dissolved iron in freshwater is generally greater than that in seawater, iron can be a limiting factor in the growth of bloom-forming cyanobacteria: the growth of *Microcystis aeruginosa* in filtrates of water samples from eutrophic Lake Kasumigaura was stimulated by the addition of $FeCl_3$ or ethylenediaminetetraacetic acid (EDTA) (Yagi et al. 1987); addition of iron was essential for the occurrence of a *Microcystis* bloom in outdoor experimental ponds (Aizaki and Aoyama 1995); the ambient level of fulvic acid in Lake Kasumigaura significantly inhibited the growth of *M. aeruginosa* in defined growth media because of complexation of Fe(III) with fulvic acid (Imai et al. 1999).

Several studies of dissolved iron concentration in lake water have been reported (Balistrieri et al. 1992; Achterberg et al. 1997; Inaba et al. 1997), but determining the concentration of dissolved iron in unpolluted natural lake water is difficult without preconcentration. Therefore, there is a need to develop a highly sensitive method requiring minimal sample pretreatment.

It is also important to determine the chemical speciation of iron as well as its concentration to examine the effect of iron on algal growth. The free hydrated and hydrolyzed ferric iron species [such as $FeOH^{2+}$, $Fe(OH)_2^+$, $Fe(OH)_3^0$, and $Fe(OH)_4^-$] are thought to be the biologically active species (Hudson et al. 1992). In the oceanic water column, most of the dissolved iron is strongly complexed with organic matter (Gledhill and van den Berg 1994; Rue and Bruland 1995; van den Berg 1995; Boye et al. 2001), suggesting that algal growth is limited not only by the general lack of iron but also by its low availability. On the other hand, in lake water, it has long been known that only a small part of the iron is available to phytoplankton (Hutchinson

1957). The existence of organic complexes of iron (Perdue et al. 1976), iron–fulvic acid complexes (Sojo and De Haan 1991), and stabilized colloidal iron (Cameron and Liss 1984) in freshwater has been reported. However, to date, no studies have been published that report the fraction of organic and inorganic (bioavailable for algae) iron in natural freshwater. Thus the methodology has not yet been established.

Cathodic stripping voltammetry (CSV) for determination of low levels of iron in seawater has been developed. This method relies on the specific adsorption of a complex of the metal ion with an added chelator onto a hanging mercury drop electrode followed by a voltammetric scan to determine the amount of adsorbed metal ion. An important advantage of this voltammetric method is that it can be used to determine the chemical speciation of iron as well as its concentration. A competitive ligand equilibration/CSV method using the competitive ligand 1-nitroso-2-naphthol (NN) (Gledhill and van den Berg 1994) or salicylaldoxime (SA) (Rue and Bruland 1995) has been used to study complexation of iron(III) by natural organic ligands in seawater. However, it is difficult to study metal complexation by CSV in lake water that contains a high concentration of organic matter, part of which is surface active. This organic matter, especially the surface-active material, is prone to interfere with voltammetric measurement. During measurement, the dissolved organic matter shields part of the electrode surface from the sample, which results in nonreproducible scatter in the data.

In this study, we modified the CSV method using NN with bromate and determined the dissolved iron concentration and its speciation in freshwater at pH 8.1. The CSV method using NN has advantages over that using SA: a short deposition time with a catalytic effect with H_2O_2 (Yokoi and van den Berg 1992) or bromate (Aldrich and van den Berg 1998) and a large linear range of the CSV response for iron, which is suitable for freshwater analysis. pH 8.1 was chosen because it is the appropriate value for freshwater and the conditional stability constant for the complexation of iron(III) by NN (needed for calculation of iron speciation) can be obtained from the literature (Gledhill and van den Berg 1994; van den Berg 1995). Here, we describe the method in detail and demonstrate its validity by determining the concentration of dissolved iron and its speciation in Lake Kasumigaura.

Methods

Materials

The voltammetric system consisted of a Princeton Applied Research (PAR; Oak Ridge, Tennessee, USA) 303A static mercury drop electrode connected to a PAR 394 voltammetric analyzer. The working electrode was a hanging mercury drop (medium size), the reference electrode was Ag/AgCl in 3-M KCl saturated with AgCl and the counter electrode was a platinum wire. Solutions in the Teflon voltammetric cell were stirred with a Teflon-coated magnetic stirring bar driven by a PAR 305 electric stirring motor.

Milli-Q water (MQ; Millipore, Billerica, USA; resistance 18.3 MΩ) was used for reagent and sample preparation. A 0.02-M stock solution of 1-nitroso-2-naphthol (NN) was prepared in methanol (Wako, Infinity Pure Grade, Osaka, Japan). A 1-M stock solution of tris (hydroxymethyl) aminomethane (Tris) was adjusted to pH 8 with HCl (Merck, suprapur grade, Darmstadt, Germany). A 0.4-M stock solution of potassium bromate and a 5-M stock solution of NaCl were prepared in MQ. Iron contaminants were removed from the Tris, potassium bromate, and NaCl stock solutions (the potassium bromate and NaCl stock solutions were buffered at pH 8 with 10-mM Tris) by adding 20 μM NN and passing the mixture through a Sep-Pak C18 cartridge (Waters, Milford, USA; precleaned with methanol, HCl, and then MQ). Iron standard solutions were prepared by diluting a 100-ppm-Fe standard (Wako) with MQ and acidifying to pH 2.5 with HCl.

The freshwater samples were collected from the center of Lake Kasumigaura, a shallow, eutrophic lake in Japan (Lake Kasumigaura is the second largest lake in Japan). Surface-water samples were collected directly into 250-ml high-density polyethylene bottles on January 10, 2003. The samples were immediately cooled in an ice cooler, brought back to the laboratory, and filtered through a 0.2-μm-pore-size polycarbonate membrane filter (Nuclepore, Whatman, Brentford, UK). The filtrates were stored frozen (−20°C) in high-density polyethylene bottles until analysis of the iron speciation. Separate samples were stored at 3°C in Teflon vials after acidification to pH 2.5 with HCl for the determination of total dissolved iron. The high-density polyethylene bottles were cleaned by soaking in 3-M HCl for 3 days and then rinsing with MQ. The Teflon voltammetric cells and Teflon vials were cleaned by soaking in 3-M HCl for 3 days, then soaking in 2-M HNO_3 for 3 days, and finally rinsing with MQ.

Determination of total dissolved iron

Samples for the determination of total dissolved iron were ultraviolet (UV) irradiated prior to analysis to decompose interfering organic compounds. Samples (10 ml) were placed in acid-washed quartz tubes and then UV irradiated with a 400-W low-pressure Hg lamp for 60 min. Details of the UV irradiation system were previously described (Yokoi et al. 1999). UV-irradiated samples were diluted with an appropriate amount of MQ (usually ten times), and 10-ml aliquots of the diluted solutions were pipetted into the Teflon vials. Ten microliters of a 0.02-M NN solution (final concentration 20 μM) and 100 μl of a 5-M NaCl solution (final concentration 50 mM) were added to the samples. The pH was made approximately neutral using ammonia solution, and 100 μl of a 1-M Tris solution (final concentration 10 mM) was added (final pH 8.1). The solution was deaerated by purging for 4 min with nitrogen gas, and 250 μl of a 0.4-M potassium bromate solution (final concentration 10 mM) was added prior to the voltammetric

scan. Deposition onto a fresh mercury drop was carried out for 30 s at −0.15 V while the solution was stilled. The stirrer was stopped and 10 s later the potential was scanned in the differential pulse stripping mode (pulse height 20 mV) from −0.15 to −0.7 V at a scan rate of $20\,mV\,s^{-1}$. This measurement was repeated with three standard additions of iron to the sample sufficient to double the peak height, and quantification was made by the standard addition method.

Determination of iron(III) complexation by natural organic ligands

The conditional stability constants and complexation capacities of the natural iron(III) complexing ligands in the freshwater samples were determined by a competitive ligand equilibration method (Gledhill and van den Berg 1994). Samples (10 ml) were diluted ten times with MQ and the diluted solutions were mixed with NN (final concentration 20 μM), Tris (final concentration 10 mM), and NaCl (final concentration 50 mM). The final pH of the mixture was 8.1. Appropriate amounts of a 1.79-μM (100-ppb) iron standard solution were pipetted into the Teflon vials in 9 increments (0–140 μl) and 10 ml of the mixture was pipetted into each vial (final added iron concentration increasing from 0 to 25 nM). The added iron, NN, and natural complexing ligands were allowed to equilibrate overnight with gentle shaking. At the same time, the voltammetric cell was conditioned in the remaining 10 ml of the mixture. The iron complexed by the added NN was determined by CSV after purging and addition of potassium bromate (final concentration 10 mM). The voltammetric procedure was the same as that described above.

Theory

Ligand concentrations (C_L) and conditional stability constants (K'_{FeL}) are defined as follows:

$$K'_{FeL} = [FeL]/[Fe^{3+}][L'] \quad (1)$$

$$C_L = [FeL] + [L'] \quad (2)$$

where [FeL] is the concentration of iron complexed by natural organic ligand, L, and [L′] is the concentration of ligand L not complexed by iron. We made the assumption that iron is complexed with L in the ratio of one to one. $[Fe^{3+}]$ is directly related to the labile iron concentration ([Fe labile], the concentration of iron complexed by the added NN as well as all inorganic iron) as follows:

$$[Fe^{3+}] = [Fe\ labile]/(\alpha'_{Fe} + \alpha'_{FeNN}) \quad (3)$$

where α'_{Fe} is the α-coefficient for the inorganic complexation of iron and α'_{FeNN} is the α-coefficient for the complexation of Fe^{3+} by NN (see below).

C_L and K'_{FeL} were calculated from the slope and the y-axis intercept of the following equation based on the Langmuir transformation (Ruzic 1982; Gledhill and van den Berg 1994):

$$[Fe\ labile]/[FeL] = [Fe\ labile]/C_L + (\alpha'_{Fe} + \alpha'_{FeNN})/(C_L K'_{Fel}) \quad (4)$$

Equation 4 is given by substituting Eq. 2 and Eq. 3 into Eq. 1. The data were fitted to Eq. 4 by linear least-squares regression from plotting the ratio [Fe labile]/[FeL] against [Fe labile]. [Fe labile] is related to the CSV peak height (i_p) via sensitivity S:

$$[Fe\ labile] = i_p/S \quad (5)$$

where S is obtained from the slope of the linear part of the titration curve where all organic ligand L is saturated. [FeL] was calculated from [FeL] = C_{Fe} − [Fe labile], where C_{Fe} is the total dissolved iron concentration, including the added and originally present iron.

The value for α'_{Fe} (defined as $\alpha'_{Fe} = [Fe']/[Fe^{3+}]$, where [Fe′] is the concentration of inorganic iron) in freshwater is different from that in seawater. A value for α'_{Fe} of $10^{12.3}$ was calculated for freshwater at pH 8.1 as follows. Because the inorganic iron in freshwater at pH 8.1 exists mostly as hydrolyzed species, the mass balance of inorganic iron (Fe′) is given by:

$$[Fe'] = [Fe^{3+}] + [FeOH^{2+}] + [Fe(OH)_2^+] + [Fe(OH)_3^0] + [Fe(OH)_4^-] \quad (6)$$

where the multimeric species $Fe_2(OH)_2^{4+}$ and $Fe_3(OH)_4^{5+}$ can be neglected because the inorganic iron concentration is very low (see below); thus α'_{Fe} is expressed by:

$$\begin{aligned}\alpha'_{Fe} &= [Fe']/[Fe^{3+}] \\ &= 1 + [FeOH^{2+}]/[Fe^{3+}] + [Fe(OH)_2^+]/[Fe^{3+}] + [Fe(OH)_3^0]/[Fe^{3+}] + [Fe(OH)_4^-]/[Fe^{3+}] \\ &= 1 + K_1/[H^+] + \beta_2/[H^+]^2 + \beta_3/[H^+]^3 + \beta_4/[H^+]^4\end{aligned} \quad (7)$$

where K_1, β_2, β_3, and β_4 are the stability constants for each hydrolyzed species and Eq. 7 is solved using the stability constants from Turner et al. (1981).

Iron(III) and NN form a complex of the type of $Fe(NN)_3$. Therefore, α'_{FeNN} is described by:

$$\alpha'_{FeNN} = [Fe(NN)_3]/[Fe^{3+}] = K'_{FeNN3}[NN]^3 \quad (8)$$

where K'_{FeNN3} is the conditional stability constant for complexation of iron(III) by NN and is defined by:

$$K'_{FeNN3} = [Fe(NN)_3]/[Fe][NN]^3 \quad (9)$$

A value for α'_{FeNN} of $10^{16.7}$ was calculated from the NN concentration and K'_{FeNN3}. A value for K'_{FeNN3} of $10^{29.6}$ was derived using the following equation (Gledhill and van den Berg 1994):