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[45]。

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 30s at −0.15V while the solution was stilled. The stirrer was stopped and 10s later the potential was scanned in the differential pulse stripping mode (pulse height 20mV) from −0.15 to −0.7V 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 (10ml) were diluted ten times with MQ and the diluted solutions were mixed with NN (final concentration 20μM), Tris (final concentration 10mM), and NaCl (final concentration 50mM). 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 (0–140μl) and 10ml of the mixture was pipetted into each vial (final added iron concentration increasing from 0 to 25nM). 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 10ml of the mixture. The iron complexed by the added NN was determined by CSV after purging and addition of potassium bromate (final concentration 10mM). 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:

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

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):

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

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:

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

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 $[FeL] = C_{Fe} - [\text{Fe labile}]$, 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:

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

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):

$$\log K'_{FeNN3} = -1.04 \log(\text{salinity}) + 30.12 \quad (10)$$

Here, a value for salinity of 2.9 was used (equivalent to 50 mM NaCl which was added to the sample, see above). Salinity derived from the original sample was negligible against the salinity derived from added NaCl because the salinity of the original sample was 0.16 practical salinity units (psu) and in addition to that, the sample was diluted ten times. Equation 10 was obtained for seawater at pH 6.9 but can be applied to cases at pH 8.1, such as in our experiment, because the conditional stability constant of $Fe(NN)_3$ for seawater at pH 8.1 is the same as for seawater at pH 6.9 (van den Berg 1995).

The free metal ion concentration $[Fe^{3+}]$ originally present in the sample was calculated from the following equation (Boye et al. 2001):

$$[Fe^{3+}]^2 \alpha'_{Fe} K'_{FeL} + [Fe^{3+}](\alpha'_{Fe} + K'_{FeL} C_L - K'_{FeL} C_{Fe}) - C_{Fe} = 0 \quad (11)$$

The concentration of inorganic iron [Fe′] originally present in the sample was calculated from $[Fe'] = \alpha'_{Fe}[Fe^{3+}]$, and pFe′ is defined as $-\log[Fe']$. The fraction of iron occurring as organic species was calculated as $\{(C_{Fe} - [Fe'])/C_{Fe}\} \times 100$.

Results and discussion

Method development

A standard addition voltammetric scan showed that the iron peak appeared at −0.5 V and that the peak heights increased linearly with increasing iron concentration until at least 40 nM (Fig. 1). The increase in the peak height became nonlinear and flattened out at iron concentrations higher than 40 nM because of saturation of the mercury drop electrode. Therefore, most of the freshwater samples needed to be diluted prior to analysis.

We used the differential pulse stripping mode for voltammetric scans and 50 mM of NaCl as electrolyte. Square-wave voltammetry is often used for trace metal analysis in seawater but is prone to contamination because this scan mode requires high concentrations of electrolyte. For this reason, square-wave voltammetry is suitable for seawater samples but not for freshwater samples.

We determined the optimum conditions of sample pretreatment for analysis of total dissolved iron. We examined the efficiency of our irradiation system for the UV digestion of typical lake water acidified to pH 2.5 with HCl. Figure 2 shows the effect of UV irradiation time on dissolved organic carbon (DOC) and absorbance at 260 nm of water from Lake Kasumigaura. The DOC decreased more slowly than the absorbance with irradiation time, and the organic compounds were completely digested within 60 min [Yokoi et al. (1999) reported that, with our irradiation system, all tested compounds having complexing ability or surface activity were 80% decomposed within 25 min]. An irradiation

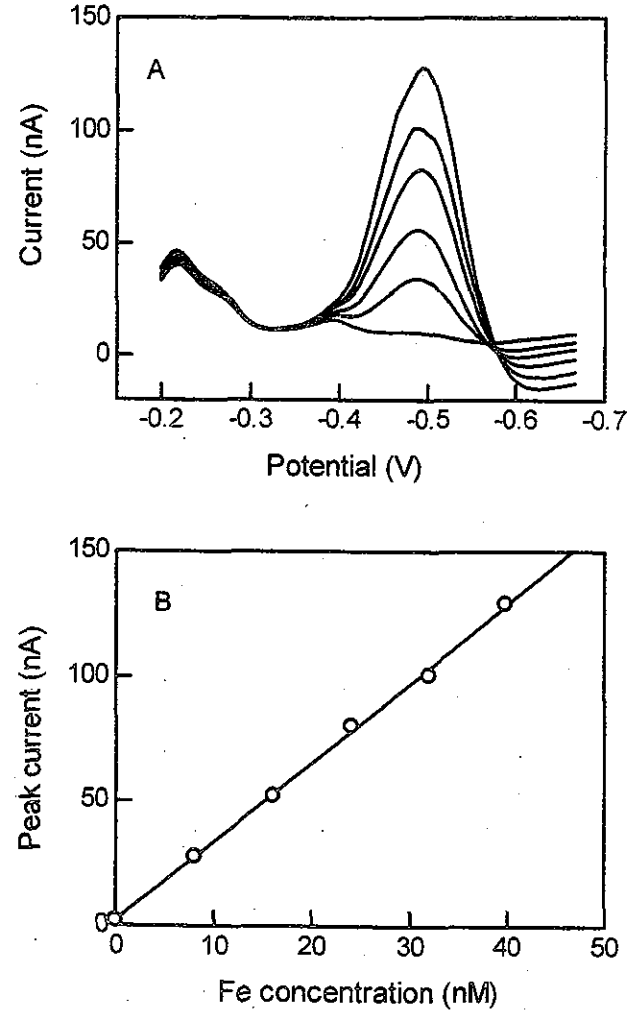

Fig. 1. Cathodic stripping voltammetry scans for 0, 8.5, 16.9, 25.3, 33.7, and 42.0 nM iron in Milli-Q water (A) and peak heights as a function of iron concentration (B)

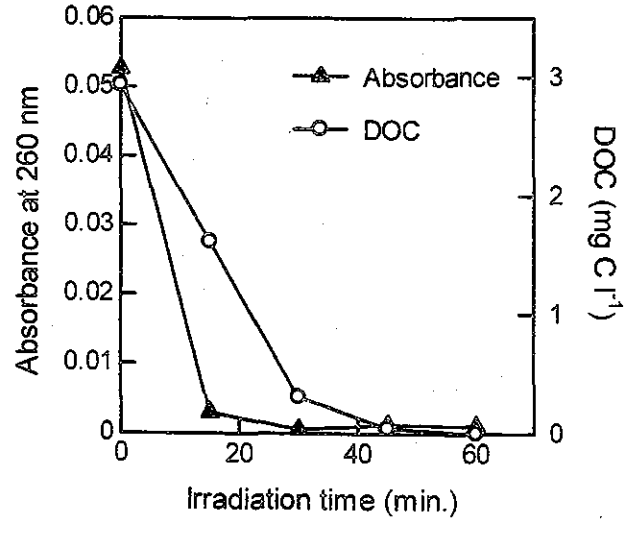

Fig. 2. Effect of ultraviolet (UV) irradiation time on absorbance at 260 nm and dissolved organic carbon (*DOC*) levels. Absorbance was measured with a Shimadzu UV-2500 UV/VIS spectrometer (Kyoto, Japan) and DOC was measured with a Shimadzu TOC 5000 total organic carbon analyzer

time of 60 min was used for all analyses. Samples were acidified to pH 2.5 with HCl before UV irradiation to prevent iron precipitation. HCl rather than HNO_3 was used for acidification because, during UV irradiation, nitrate ions are reduced to nitrite ions, which greatly interfere with the voltammetric scan. When samples were acidified to pH <2.5, more time was needed for complete UV digestion because chloride ions derived from HCl decreased the digestion efficiency significantly (data not shown). Therefore we concluded that the optimum pH for UV digestion was 2.5, which minimizes the risk of precipitation and sample evaporation.

In the speciation experiment, the problem of measurement instability caused by interfering organic compounds was overcome by using a high NN concentration (50 μM), dilution of the samples, and a well-conditioned voltammetric cell. Although seawater has been successfully analyzed by using NN concentrations of 2–13.6 μM, our attempts to analyze lake water using such NN concentrations were not successful. Surface active materials reduce the available surface area of the electrode and therefore reduce the linear range of the CSV response for iron. However, the linear range was extended by using a high NN concentration. Wu and Luther (1995) also reported a nonlinear signal response for iron concentrations greater than 4 nM when using NN at concentrations less than 6.8 μM. Dilution of the samples prevents saturation of the iron peak and reduces the concentration of dissolved organic matter. Conditioning the voltammetric cell improves the stability of the iron peak (van den Berg 1995; Wu and Luther 1995).

Total dissolved iron

We estimated the detection limit of our voltammetric method from experiments using blank solutions. MQ was filtered through a 0.2-μm Nuclepore filter and then stored in a high-density polyethylene bottle after acidification to pH 2.5. The iron concentration was determined five times after UV irradiation. A detection limit of 1.8 nM was found (3 × the standard deviation of the measurement).

We verified the accuracy of our method by analyzing two standard river waters for which the iron concentration was certified (123.5 ± 9.0 nM for JAC 0031 and 1020.6 ± 35.8 nM for JAC 0032; standard samples obtained from The Japan Society for Analytical Chemistry). The iron concentration range between the two standards is in the normal range for freshwater, but the standards are not appropriate for our CSV method because they are acidified to pH <1.2 with nitric acid. To reduce the effect of nitrite ion produced from nitrate ions, we diluted the standards with MQ 20 times (for JAC 0031) and 50 times (for JAC 0032). The analytical parameters were the same as those for lake water samples, except that potassium bromate was added (final concentration 20 mM) to the JAC 0031 standard to increase sensitivity. The iron concentrations determined for JAC 0031 and JAC 0032 were 118 ± 5 nM (n = 4) and 1037 ± 56 nM (n = 5), respectively. These values are not significantly different from the certified values (verified by t test, α = 0.05). These results show the validity of our method for determining the total dissolved iron in freshwater.

The iron concentration in the water samples from Lake Kasumigaura was determined to be 47.8 ± 4.4 nM (n = 3). This level of iron is difficult to determine accurately by either graphite furnace atomic absorption spectrometry or inductively coupled plasma (ICP) emission spectrometry without preconcentration. We tried to determine the total dissolved iron concentration in the samples from Lake Kasumigaura by these spectrometric methods, but the iron concentration was too low. We also tried to determine the iron concentration by ICP mass spectrometry, but iron could not be detected because of interference from other elements. Therefore, our CSV method should be quite useful for the determination of total dissolved iron in lake water.

Iron speciation

Iron titrations were carried out for a filtrate sample and a UV-digested sample (UV irradiated for 60 min at natural pH). For the filtrate sample, the CSV peak height was suppressed at low iron concentrations, indicating the presence of excess iron complexing ligands (Fig. 3A). A linear relationship between the peak height and the iron concentration was obtained for the UV-digested sample, indicating that the complexing ligands had been successfully destroyed by UV irradiation and that they were organic in nature. Moreover, the slopes of the two titration curves at high iron concentrations were identical, indicating that all natural ligands were saturated with the added iron at high iron concentrations and that the titration range we used was appropriate. The effect of surface-active materials appeared to be small.

The ligand concentration and conditional stability constants for the lake water samples were determined from linear plots of [Fe labile]/[FeL] versus [Fe labile] (Fig. 3B). The ligand concentration was 80.0 ± 19.6 nM and the conditional stability constant was $10^{25.9\pm0.4}M^{-1}$ (n = 3). A pFe′ value of 13.4 was calculated, and the fraction of iron occurring as organic species was greater than 99.9%. These results indicate that virtually all dissolved iron in Lake Kasumigaura exists as organic complexes due to the very high conditional stability constants of the organic ligands in the lake water.

It should be noted that the pFe′ value (13.4) is only valid at pH 8.1. The pH of seawater is nearly constant at 8, whereas the pH in Lake Kasumigaura varies between 7 and 10 (Center for Global Environmental Research 2001). α'_{Fe} and K'_{FeL} are functions of pH, and thus pFe′ values should be greatly affected by pH. We did not evaluate changes in the K'_{FeL} value as a function of pH. Moreover, we did not determine the redox speciation of iron in this study. We calculated the iron speciation on the assumption that all iron in the sample exists as iron(III), because iron(II) is immediately oxidized to iron(III) in oxic water. However, there are a few studies reporting the existence of iron(II) in lake water despite the presence of oxygen (Aldrich et al. 2001; Sivan et al. 1998). This may be a result of photochemi-

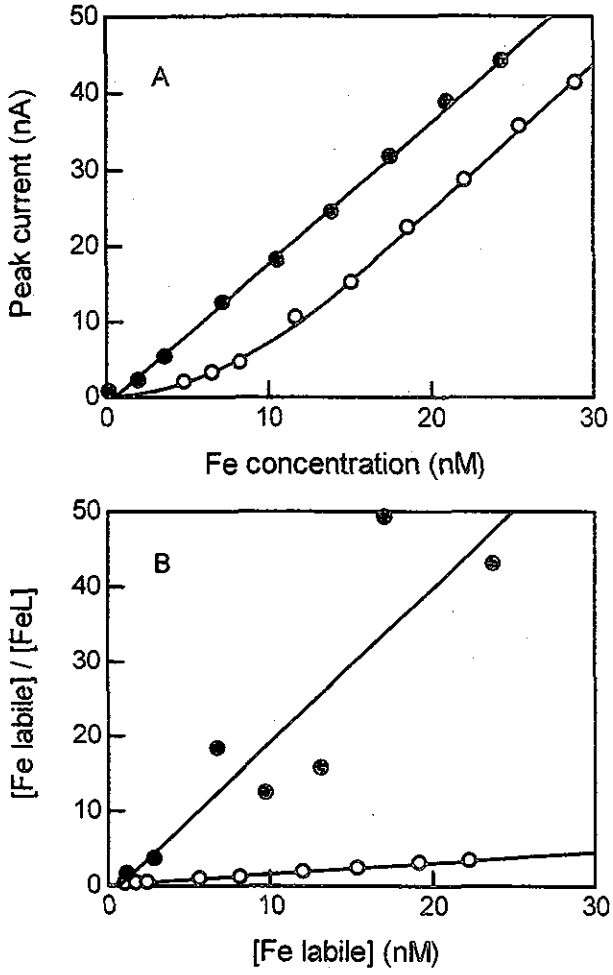

Fig. 3. Iron titrations of filtrate sample (O) and UV-digested sample (●) from Lake Kasumigaura: A, titration curves; B, linear plots. The UV digestion was performed without adjustment of pH to compare the two titration curves. As a result, iron was precipitated and lost in the UV-digested sample

cal reduction and biological activity (Emmenegger et al. 2001; Shaked et al. 2002). There is some possibility of changing the bioavailability of iron as a result of the production of iron(II). In this study, if iron(II) exists, the total iron concentration could contain both iron(III) and iron(II) species. In the iron speciation experiments, any iron(II) species in the samples could be oxidized to iron(III) during the equilibration period (van den Berg 1995).

In our analytical technique, it is important that appropriate ligand competition between natural ligands and NN occur: no iron is detected if the complexes with the natural ligands are much more stable than the complex with NN, whereas no complexation is detected if the complexes with natural ligands are much less stable (van den Berg 1995; Wu and Luther 1995). The degree of ligand competition could be estimated by comparing the α-coefficient values α'_{FeNN} and α_{FeL} (calculated from $K'_{FeNN}[NN]^3$ and $K'_{FeL}C_L$, respectively). The values were $10^{16.7}$ and $10^{17.8}$ (in the sample diluted ten times), respectively, indicating that the natural complexes were more stable than the complex with NN under the conditions of this study. Gledhill and van den Berg (1994) mentioned that the detection window is one order of magnitude either side of α'_{FeNN} ($10^{15.7}$–$10^{17.7}$ under our conditions) and that high values of α'_{FeL} that are outside the detection window result in a great error in the determination of $\log K'_{FeL}$ (±2). The α'_{FeL} value we obtained ($10^{17.8}$) was slightly outside the detection window, indicating that a higher NN concentration should have been used from the viewpoint of the detection window. Nevertheless, we used an NN concentration of 50 μM in our study because we did not obtain a great error in the value of $\log K'_{FeL}$ (±0.4), and using a higher concentration of NN carries a risk of iron contamination.

According to van den Berg and Donat (1992), several complexing ligands or sites can be detected by several detection windows. Using modeled CSV responses of a hypothetical seawater containing four iron complexing ligands, van den Berg (1995) suggested that a single titration experiment is insufficient to resolve the presence of several complexing ligands. In contrast, Rue and Bruland (1995) suggested that two ligands could be resolved from a single set of titration data by using the Scatchard transformation method to obtain C_L and K'_{FeL}. The Scatchard transformation method involves plotting the ratio [FeL]/[Fe labile] against [FeL]. For a single class of iron complexing ligands, the y-axis intercept of the resulting linear plot equals $K'_{FeL}C_L$ and the x-axis intercept equals C_L. If two classes of iron complexing ligands (a strong ligand and a weak ligand) exist, the linearized plots should yield a nonlinear curve. For such a case, Rue and Bruland (1995) suggested that two linear regions should be easy to separate using the Scatchard transformation rather than the Langmuir transformation. Langmuir and Scatchard plots of our titration data are compared in Fig. 4. Only one linear region was evident in the both plots. The total C_L and K'_{FeL} (single class of ligand) values were calculated from the linear plots. The Langmuir transformation gave a C_L value of 80.0 ± 19.6 nM and a K'_{FeL} value of $10^{25.9\pm0.4}M^{-1}$, whereas the Scatchard transformation gave a C_L value of 84.9 ± 18.9 nM and a K'_{FeL} value of $10^{25.6\pm0.2}M^{-1}$. Although the values of C_L and K'_{FeL} obtained from the Langmuir and Scatchard plots were not significantly different, the correlation coefficient for the Scatchard plot (average value 0.921) was lower than that for the Langmuir plot (average value 0.997). Therefore, we believe that the Langmuir transformation is more appropriate for the determination of total ligand concentration.

Field study

It is surprising that the value of the conditional stability constant for iron ligands (K'_{FeL}) in water from Lake Kasumigaura determined in this study was greater than any reported K'_{FeL} value for seawater (Table 1). These very strong ligands could be detected because a high NN concentration (equivalent to a high detection window) was used. Rue and Bruland (1997) and Boye et al. (2001) suggested that strong iron ligands ($\log K'_{FeL} = 22$–$23\,M^{-1}$) in seawater might be siderophores. Imai et al. (1999) calculated a conditional stability constant of greater than $10^{25}M^{-1}$ for Fe^{3+} complexed with fulvic acid isolated from Lake Kasumigaura. It can therefore be hypothesized that the iron ligands in Lake Kasumigaura are fulvic acid. Imai et al.

Table 1. Conditional stability constants (K'_{FeL}) of iron ligands and inorganic iron levels as determined by cathodic stripping voltammetry

Area	$\log K'_{FeL}(M^{-1})$	pFe'[a]	Reference
Lake Kasumigaura	25.9	13.4	This study
North Atlantic	18.8–19.7	10.6–12.0	Gledhill and van den Berg (1994)
Western Mediterranean	19.4–22.5	11.3–12.1	van den Berg (1995)
Northern North Sea	20.7–21.7	9.3–13.9	Gledhill et al. (1998)
Southern Ocean	21.0–23.0	11.5–13.2	Boye et al. (2001)
North Pacific	23.1	13.1–14	Rue and Bruland (1995)
Equatorial Pacific	22.7	14	Rue and Bruland (1997)

[a] pFe' = −log[Fe'], where [Fe'] is the concentration of inorganic iron

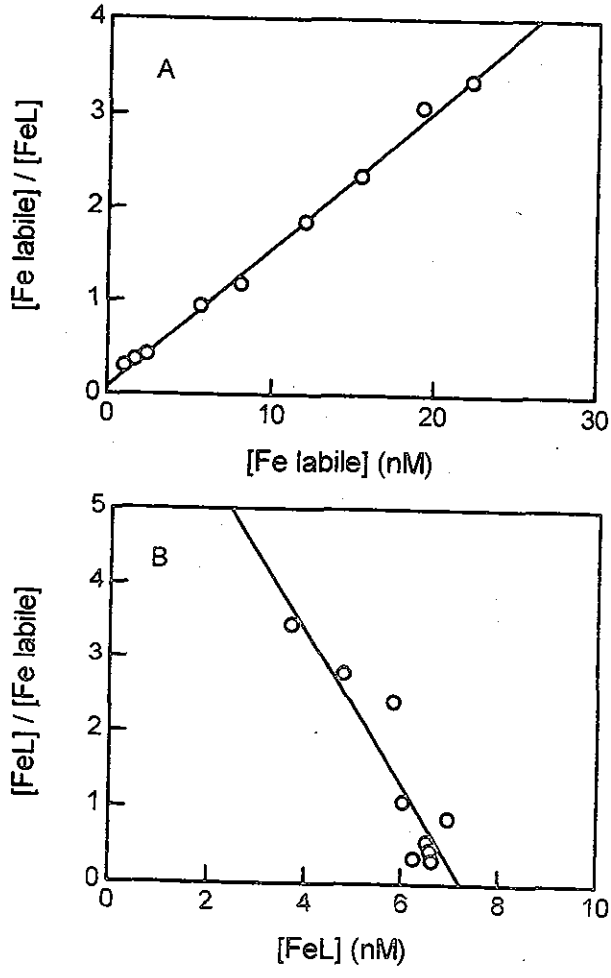

Fig. 4. Comparison of Langmuir transformation (**A**) and Scatchard transformation (**B**) when calculating ligand concentrations and conditional stability constants

(1999) also reported that the average concentration of fulvic acid in Lake Kasumigaura was 2.5 μM, which is about 30 times higher than the ligand concentration we measured (80 nM). However, fulvic acid is a nonspecific ligand, and thus if the iron ligand we detected is fulvic acid, the ligand concentration could be underestimated because of competitive interference from other trace metals or major divalent cations (Ca^{2+} and Mg^{2+}).

Whereas the stability constants of bivalent metal (such as copper) cation–fulvic acid complexes are well studied (e.g., Mantoura et al. 1978), there are only a few studies on stability constants of iron–fulvic acids complexes. Langford and Khan (1975) reported that conditional stability constants of iron–fulvic acid complexes were $10^{4.2}$–$10^{4.4}$ at pH 1.0–2.5. Pandeya also determined stability constants of iron–fulvic acid complexes using ligand exchange (Pandeya 1993), spectrocolorimetry (Pandeya and Singh 1997), and potentiometry (Pandeya and Singh 2000) and the values found were $10^{12.0}$–$10^{13.8}$, $10^{6.0}$–$10^{6.9}$, and $10^{5.6}$–$10^{7.6}$, respectively. However, they used fulvic acids extracted from soil, sludge, and manure. There are significant differences in physicochemical characteristics among fulvic acids of different origin. Therefore, these results cannot be simply compared with our result. Investigation of the interaction of iron with extracted fulvic acids using our CSV method is a subject for further study.

Comparison of the pFe' values (representative of inorganic iron) in Table 1 reveals that the pFe' value for Lake Kasumigaura water is similar to that found in the open ocean. Because the concentration of total dissolved iron in the open ocean is subnanomolar, the concentration in Lake Kasumigaura is 10–100 times higher. However, the bioavailability of iron in Lake Kasumigaura is very low, and thus the iron competition between phytoplankton that takes place in the open ocean may have occurred in the lake. Imai et al. (1999) reported the effect of iron limitation on the growth of *M. aeruginosa* in defined growth media. According to that study, *M. aeruginosa* could grow at a pFe' of higher than 12.9 but not at a pFe' of 13.9. Therefore, the pFe' value we obtained (13.4) was borderline for the growth of *M. aeruginosa*. Consequently, our result supports the hypothesis that the shift of the dominant species of phytoplankton in Lake Kasumigaura from *Microcystis* to *Oscillatoria* was caused by iron limitation.

Conclusion

We have presented a methodology for determining dissolved iron, its organic complexation, and its bioavailability in freshwater. We applied this method to lake water containing a large amount of interfering organic compounds and successfully determined the concentration of total dissolved iron and iron(III)–organic ligand complexation. We found that the level of inorganic iron in Lake Kasumigaura was similar to that in the open ocean. It is therefore possible that iron is a dominant selective factor in algal species suc-

cession and diversity in Lake Kasumigaura. We are currently using the methodology developed in this preliminary study to analyze additional freshwater samples and are investigating the following: (1) temporal and spatial changes of iron speciation in Lake Kasumigaura; (2) the effect of iron speciation on cyanobacterial blooms in a eutrophic lake; and (3) characterization of the iron ligand (i.e., fulvic acid or not).

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CHARACTERIZATION OF DISSOLVED ORGANIC MATTER IN SHALLOW EUTROPHIC LAKE KASUMIGAURA

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SUMMARY – Dissolved organic matter (DOM) in water samples from the shallow eutrophic Lake Kasumigaura, the second largest lake in Japan, and its inflowing rivers was fractionated and characterized by using resin adsorbents into 5 classes: aquatic humic substances (AHS), hydrophobic neutrals (HoN), hydrophilic acids (HiA), bases (BaS) and hydrophilic neutrals (HiN). DOM-fraction distribution patterns were significantly different depending on the origin of the sample. AHS and HiA were found to be the dominant fractions in DOM in all samples studied. HiA prevailed over AHS in the lake water, whereas AHS were slightly more abundant than HiA in the river waters. AHS were in the great majority in forest streams and plowed-field percolates. HiA abounded in paddy-field outflow, domestic sewage, and sewage-treatment-plant effluent. Only domestic sewage contained a significant amount of HoN.

Furthermore, the trihalomethane formation potential (THMFP) and molecular size distribution of the lake-water DOM, AHS and hydrophilic fractions (HiF = HiA + BaS + HiN) were examined. The THMFP of HiF, normalized on a DOC basis, was found to be comparable to that of AHS (0.176 vs. 0.195 μmol THM $mg \cdot C^{-1}$, respectively). Molecular size distributions all exhibited a narrow size range and relatively low molecular weights. The weight-averaged molecular weights of DOM, AHS and HiF were 780, 957 and 606 $g \cdot mol^{-1}$, respectively.

1. INTRODUCTION

A steady increase in dissolved organic matter (DOM) has been observed in several lakes in Japan, even though extensive measures have been implemented to reduce organic pollutant loadings from their catchment areas. For instance, in Lake Biwa, the largest lake in Japan, the chemical oxygen demand (COD), an index for total organic matter, has been gradually increasing in the surface water since 1984. On the other hand, the biochemical oxygen demand (BOD), a parameter for easily biodegradable organic matter, has remained virtually constant. Since the concentration of chlorophyll-*a* does not exhibit any pattern of increase, phytoplankton activity is unlikely to relate directly to the COD increase. The dissolved fraction accounted for most of the COD. It is likely that some recalcitrant DOM has been accumulating in the water of Lake Biwa. This phenomenon is apparently new and has not been given any previous consideration.

The accumulation of recalcitrant DOM in lake water certainly influences the way in which lake environmental protection should be managed, because DOM is regarded as a source of organic pollution and, further, as an energy source for microbe-based aquatic food webs, as a factor in the cycling of trace elements, and as an influence on the biological activity of phytoplankton and bacteria. The increase in DOM also presents a serious challenge for drinking-water management because recalcitrant DOM can be a major precursor to carcinogenic trihalomethanes produced during chlorination in water treatment plants. Therefore, evaluation of the characteristics of DOM in lake water is urgently needed.

Nevertheless, DOM in natural waters is a heterogeneous mixture of organic compounds, and its physico-chemical characteristics are not clearly understood. In such a situation, the rational first step toward that understanding should be the evaluation of the characteristics of DOM in waters. An appropriate approach to this end is to separate DOM into well-defined macro-fractions and to examine their distribution and physico-chemical characteristics.

Aquatic humic substances (AHS), which are typical naturally occurring recalcitrant DOM, constitute 30% to 80% of DOM as dissolved organic carbon (DOC), and they constitute the largest fraction of natural organic matter in waters (Thurman, 1985). They are straw-colored, polar, hydrophobic organic acids derived from soil humus, terrestrial and aquatic plants, and plankton. For our purpose of evaluating the characteristics of DOM in waters, DOM fractionation methods based on the separation of AHS, namely, hydrophobic - hydrophilic and acidic-basic breaks, appear reasonable.

In this study, modifying the DOM fractionation method developed by Leenheer (1981) and Imai et al. (1998), we fractionated DOM using three kinds of resin adsorbents into five classes: hydrophobic acids (equivalent to AHS), hydrophobic neutrals, hydrophilic acids, hydrophilic neutrals, and bases (hydrophobic + hydrophilic bases). The objectives of this study were to (1) apply the DOM fractionation method to the waters of shallow eutrophic Lake Kasumigaura and its inflowing

rivers as well as to several DOM sources in its watershed, such as sewage-treatment-plant effluent, and so on, and to evaluate their DOM-fraction distributions, and (2) to determine the comparative significance of AHS and hydrophilic fractions of the lake-water DOM as trihalomethane precursors by measuring their trihalomethane formation potential (THMFP) and then to examine the relationships between their THMFP and physico-chemical characteristics.

2. MEHODS AND MATERIALS

2.1 Collection of water samples

Lake Kasumigaura, the second largest lake in Japan, is located in the eastern part of the Kanto Plain, 50 km northeast of Tokyo. The lake has two large bays, Takahamairi and Tsuchiurairi (**Fig. 1**). More than 900,000 people live in the lake's watershed (1,577 km^2). Land use in the watershed is 30% forest, 25% paddy field, 25% plowed field, 10% residential, and 10% other. The lake basin is smooth and shallow, with a surface area of 171 km^2, a mean depth of 4.0 m, and a maximum depth of 7.3 m. Because of its extremely high loads of organic matter and nutrients, this lake is well known for eutrophication.

The Koise and Sakura rivers are the main rivers influent to Takahamairi and Tsuchiurairi, respectively. Water flows through the lake largely from the northwest parts of Takahamairi and Tsuchiurairi southeast to the Hitachitone River. The average time needed for water to flow through the two bays to the center of lake may be around three months. In addition, Lake Kasumigaura is so shallow that vertical stratification is easily destroyed by a moderately strong wind.

Water samples were collected in a 1-liter glass bottle with a 2-m column sampler from the center of the lake monthly from May 1994 to February 1996. Samples for the determination of trihalomethane formation potential and molecular size distribution were collected from January 1997 to December 1997. The samples were immediately cooled in an ice cooler and brought back to our laboratory. The water was then filtered through a precombusted (450 °C for 4 h) Whatman GF/F filter (nominal pore size 0.7 μm). The filtrate was usually kept at 3 °C in a precombusted glass bottle until analysis.

Four large rivers, arranged in order of drainage-area size, were chosen for this study: the Koise, Sakura, Hanamuro, and Ono rivers. River-water samples were obtained in May, August, and November 1994 and in February 1995 at downstream stations on these four influent rivers (**Fig. 1**). Distinctive DOM sources chosen in the watershed were domestic sewage (DS), sewage treatment plant effluent (STPE), paddy-field inflow (PFI) and outflow (PFO), plowed-field percolate (PFP) and upland-forest stream (FS) (**Fig. 1**). PFI and PFO samples were collected only in May and August. Otherwise, samples were collected in May, August, and November 1995, and in February 1996. The samples of river water and other DOM sources were collected in HCl-washed polycarbonate containers and treated in the same manner as the lake-water samples.

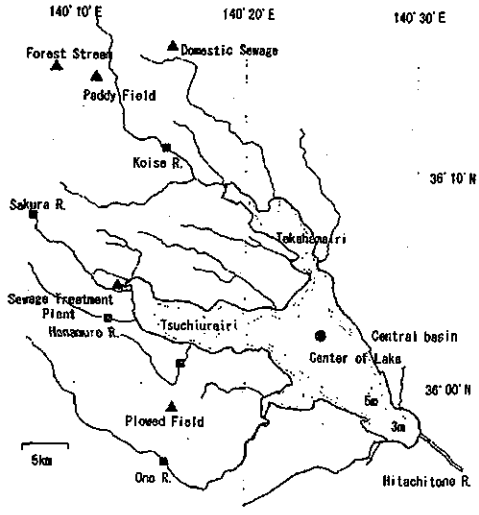

Fig.1. Sampling sites in Lake Kasumigaura, its inflowing rivers and other DOM sources in the lake catchment area.

2.2 DOM fractionation

Sample filtrates were fractionated into 5 fractions: aquatic humic substances (AHS), hydrophobic neutrals (HoN), hydrophilic acids (HiA), bases (BaS), and hydrophilic neutrals (HiN), based on their adsorption on a series of macroporous resin adsorbents. Nonionic Amberlite XAD-8 resin (20–60 mesh, Sigma Chemical Co., St. Louis, USA), strong cation-exchange resin (Bio-Rad AG-MP-50, 50–100 mesh, Nippon Bio-Rad Lab. KK, Tokyo, Japan), and strong anion-exchange resin (Bio-Rad AG-MP-1, 50–100 mesh) were used.

The XAD-resin was cleaned and conditioned as described by Thurman and Malcolm (1981). Three milliliters (wet volume) of the XAD-8 resin was packed into a glass column and rinsed 3 times, alternating 0.1 M NaOH with 0.1 M HCl each time, just before application of the sample. A blank sample was collected from the final rinse with 0.1 M HCl (B1). Both the AG-MP-50 (hydrogen form) and AG-MP-1 (chloride form) were Soxhlet-extracted with methanol for 24 h. AG-MP-1 was then converted into its free-base form with 1 M NaOH and rinsed with Milli-Q water (Milli-Q SP.TOC, Nihon Millipore Ltd.,

Tokyo, Japan). Glass columns containing 6 ml (wet volume) of the cation resin and 12 ml (wet volume) of the anion resin were connected in series and conditioned by pumping about 1 l Milli-Q water through the resins. Blank samples were collected from each column after pumping 1–2 bed volumes of 0.01 M HCl solution just before and after application of the samples.

A flow schematic of the DOM fractionation procedure is shown in **Fig. 2**. Details of the DOM fractionation procedure can be found elsewhere (Imai et al., 2001). DOM fractionation was done in duplicate for each sample. After fractionation, dissolved organic carbon (DOC) was measured for DOM fractions 1 to 5 and for the blank samples. Each DOM fraction was calculated as follows:

$$\text{AHS} = \text{DOM2} \times (\text{elutant volume})/(\text{sample volume}) \quad (1)$$

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

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

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

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

The blank DOC from the XAD-8 column during 0.1 M NaOH elution contained less than 0.7 mg•C l^{-1}; its contribution to AHS would have been no more than 0.03 mg C•l^{-1}. Thus, the blank DOC contribution to AHS was neglected. The relative errors of the duplicated measurements for AHS, HoN, HiA, BaS, and HiN in the DOM fractionation were 4–8%, 20–53%, 4–35%, 20–48%, and 22–38%, respectively.

DOC measurements were conducted as non-purgeable DOC with a Shimadzu TOC-5000 total-organic-carbon analyzer (Shimadzu Co., Kyoto, Japan). At least 3 measurements were made for each sample, and analytical precision was typically less than ± 2%. Potassium hydrogen phthalate was used a standard.

2.3 Trihalomethane formation potential

Sample filtrates, AHS and the hydrophilic fractions (HiF, equivalent to DOM3 in **Fig. 2**) were diluted with Milli-Q water to produce a DOC concentration of 1 mgC•l^{-1} before chlorination. Freeze-dried AHS samples were reconstructed in Milli-Q water. The HiF samples, the pH of which remained at 2 after the DOM fractionation, were adjusted to about pH 7 with NaOH.

Trihalomethane formation potential (THMFP) was measured according to the Standard Methods for the Examination of Drinking Water (JWSA 1993). Samples were adjusted to pH 7 with phosphate buffer. Double-distilled sodium hypochlorite solution was added at a dose that produced a free chlorine concentration of 1 to 2 mg•l^{-1} as Cl_2 after a reaction time of 24 h at 20 °C. Reactions were performed in headspace-free containers and in the absence of light. Following the 24-h reaction period, free chlorine concentrations were determined by the *o*-trizine method. Excess chlorine was quenched with anhydrous sodium sulfite, and then concentrations of trihalomethanes were determined by the headspace method using gas-chromatography/ mass-spectrometry equipped with a headspace autosampler. Analytical precision was typically less than ± 2%.

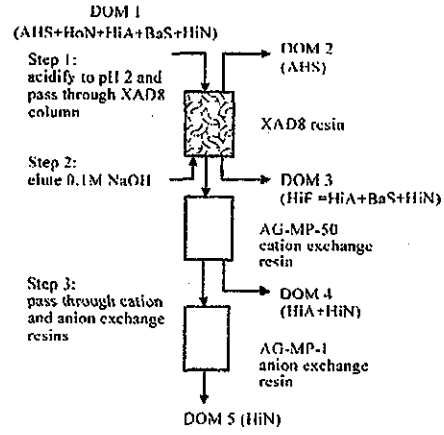

Fig. 2. Schematic diagram of the procedure for DOM fractionation. DOM fractions are AHS, aquatic humic substances; HoN, hydrophobic neutrals; HiA, hydrophilic acids; BaS, bases; HiN, hydrophilic neutrals.

2.4 Molecular size determination

High-pressure size exclusion chromatography (HPSEC) was performed at room temperature with a Hitachi L-6200 pump (Hitachi Ltd., Tokyo, Japan), a Hitachi L-4000 UV detector operating at 260 nm, a Hitachi D-2500 data integrator, and a Rheodyne rotary injection valve equipped with a 100-µl sample loop. A Waters Protein-Pak 125 modified silica column (Waters Co., Milford, USA) was used for this study (Chin et al. 1994). Mobile phases were composed of Milli-Q water buffered with phosphate to a pH of 6.8 and sodium chloride to yield an ionic strength of 0.1 M. Molecular weight standards were composed of sodium polystyrene sulfonates (35, 18, 8, 5.4, and 1.8 K) and acetone. The calibration curves were semi-log linear over the range defined by our standards, showing an excellent correlation (R^2 = 0.99), and were used to determine both number- and weight-averaged molecular weights. Sample filtrates were adjusted to an ionic strength of 0.1 M with the appropriate addition of 4 M NaCl, and to a pH of 6.8 with additions of phosphate buffer and HCl. AHS samples were prepared by dissolving the freeze-dried samples into the mobile phase solution. HiF samples were adjusted to an ionic strength of 0.1 M and a pH of 6.8 with additions of phosphate buffer, NaOH and NaCl.

Typical precision for the determination of averaged molecular weights was ± 3%.

3. RESULTS AND DISCUSSION

3.1 DOM fractionation distribution of lake and river waters

DOC concentrations at the center of Lake Kasumigaura ranged from 2.97 to 4.80 $mgC \cdot l^{-1}$ and averaged 4.08 $mgC \cdot l^{-1}$ during the sampling period (**Table 1**). The lake-water DOC exhibited a tendency to increase from spring to fall and then to decline gradually. DOC concentrations in the four rivers were found to vary from 1.45 to 3.28 $mgC \cdot l^{-1}$ with an average value of 2.76 $mgC \cdot l^{-1}$. DOC concentrations in all rivers exhibited the greatest values in May, decreased in August, and reached their lowest in November and February. The highest riverine DOC concentrations observed in May are likely to result from massive usage of river water to fill paddy fields with water in preparation for transplanting young rice plants.

Table 1. Average concentrations of dissolved organic carbon (DOC) in Lake Kasumigaura, its inflowing rivers, and other DOM sources in the lake catchment area

Sample name	DOC [$mgC \cdot l^{-1}$]	N
Lake water	4.08 (0.49)*	22
River water	2.76 (0.57)	16
Forest stream (FS)	0.47 (0.07)	4
Paddy field inflow (PDI)	3.12	2
Paddy field outflow (PDO)	7.26	2
Plowed field percolate (PFP)	0.28 (0.07)	4
Domestic sewage (DS)	16.62 (5.53)	4
Sewage treatment plant effluent (STPE)	4.93 (0.85)	4

*Standard deviation.

In Lake Kasumigaura, AHS and HiA dominated, collectively accounting for more than 70% of the DOM as DOC (Fig. 3). Thus, the lake-water DOM is predominantly acidic. HiA was dominant over AHS, amounting to 43% of the DOM against AHS being 32%. The HoN fraction was around 9%, indicating that hydrocarbons, pesticides, carbonyl compounds, and linear alkylbenzene sulfonate (LAS) do not contribute significantly to DOM in the lake. The average percentage of BaS and HiN was 10% and 4% of total DOM, respectively. Protein-like and carbohydrate-like DOMs are unlikely to be present in significant quantities as their free forms in lake water.

The DOM-fraction distribution pattern in the river waters was significantly different from the pattern in the lake water (**Fig. 3**). As in the lake water, AHS and HiA dominated in riverine DOM, accounting for more than 70%. However, the percentage of AHS was only slightly greater, 39% vs. 37%. The HoN, BaS, and HiN fractions accounted for 9%, 11%, and 6%, respectively. Riverine DOM appears to be more hydrophobic than lake DOM. Two-sample *t*-tests comparing the mean percentages of the DOM fractions revealed that only AHS and HiA were significantly different with respect to DOM-fraction distribution between the lake and river waters ($p < 0.0001$ for AHS, $p < 0.001$ for HiA). As far as DOM composition is concerned, the percentages of AHS and HiA were of importance in distinguishing between the DOM in Lake Kasumigaura and its inflowing rivers.

Thurman (1985) reported that AHS and HiA generally account for 40% and 41%, respectively, of DOM as DOC in lakes. He also states that AHS is the major component of DOM in rivers, accounting for about 50% of DOC, with HiA being about 25%. McKnight et al. (1994), studying lakes where DOM is mostly derived from phytoplankton, found that AHS accounts for 13% - 20% of DOM. These results suggest that (1) river waters are rich in AHS but poor in HiA, since most DOM in rivers is allochthonous and (2) lake waters contain more HiA compared with AHS since autochthonous DOM sources outweigh allochthonous ones in lakes. Our results for the waters of Lake Kasumigaura and its inflowing rivers are consistent with these reported findings. It appears, however, that in Lake Kasumigaura DOM is more likely to be algal derived than is typical, but its inflowing rivers may have less allochthonous or pedogenic DOM.

3.1 DOM-fraction distribution in DOM sources

DOM-fraction distributions in the other DOM sources also showed an interesting feature. AHS and HiA also dominated in all the other DOM sources as they did in the river and lake DOM (**Fig. 3**). Nevertheless, their distribution exhibited more widely different patterns, depending on the

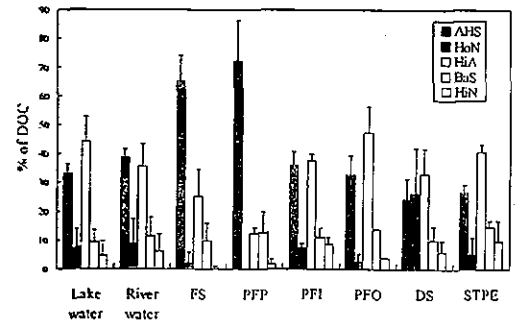

Fig. 3. DOM-fraction distributions of water samples from the center of Lake Kasumigaura, its inflowing rivers and other DOM sources in the lake catchment area. DOM fractions are AHS, aquatic humic substances; HoN, hydrophobic neutrals; HiA, hydrophilic acids; BaS, bases; HiN, hydrophilic neutrals. DOM sources are FS, forest stream; PFP, plowed-field percolate; PFI, paddy-field inflow; PFO, paddy-field outflow; DS, domestic sewage; STPE, sewage treatment plant effluent. Error bars represent ±1 standard deviation of the mean except for PFI and PFO. Bars for PFI and PFO represent relative deviation from the mean.

origin of the sample. Compared with the lake and

river waters, FS and PFP were by far dominated by AHS, which accounted for more than 60% and 70% of DOM, respectively. Both FS and PFP have only allochthonous DOM; thus, such high percentages of AHS are understandable. The DOM-fraction distribution of PFI was similar to that of the river waters, as expected. PFO was very different from PFI and exhibited the greatest percentage of HiA among the samples. The muddy sediment in paddy fields is likely to diffuse DOM containing much more HiA than AHS.

The DOM-fraction distribution of DS was noticeably different from that of the lake water. DS, like the lake water, had a considerably greater proportion of HiA than of AHS; however, its HoN fraction was distinctively greater, accounting for 25% of the DOM. Since DS samples were foamy when they were collected, DS should contain a large amount of synthetic detergent such as LAS, which is categorized as a HoN.

STPE contained more HiA (45%) than AHS (27%), like the lake water. DOM in STPE is considered to be mostly of microbial origin and should be refractory to bacterial degradation since it is what remains after extensive biodegradation. This finding suggests that not only AHS but also HiA may accumulate as recalcitrant DOM when STPE is discharged into a lake either directly or indirectly.

3.2 Molecular size distribution

All HPSEC chromatograms of DOM, AHS and HiF showed broad, monomodal size distributions with subtle shoulders and small sub-peaks. Typical HPSEC chromatograms of DOM, AHS, and HiF are shown in **Fig. 4**. The weight-averaged to number-averaged molecular weight ratio (i.e., the analyte's polydispersity) was less than 2 in all cases, indicating that the DOM, AHS, and HiF molecules all occupy a relatively narrow size range and do not possess molecular weights that vary by orders of magnitude. The weight-averaged molecular weights of DOM were relatively low, having an average of 780 g•mol^{-1}. AHS had a greater weight-averaged molecular weight than HiF, exhibiting an average value of 957 g•mol^{-1}, compared with 606 g•mol^{-1} for HiF. AHS and HiF appeared to contribute mainly to the high- and low-molecular-weight fractions, respectively, of the DOM in Lake Kasumigaura. Seasonal variations in the weight-averaged molecular weights of DOM, AHS and HiF were not very pronounced, varying within ± 13%, ± 11% and ± 24% of the monthly average values of DOM, AHS and HiF, respectively.

The weight-averaged molecular weights of DOM, AHS and HiF in Lake Kasumigaura were found to be small, showing the average values of 780, 957 and 606 g•mol^{-1}, respectively. Thurman et al. (1982) measured the molecular weight of AHS from various environments and concluded that an average molecular weight for humic substances from surface water was 1000 to 2000 g•mol^{-1}. The molecular weights of AHS determined in this study were around the lowest end of that range. The molecular weight of HiF has not been reported before as far as we know; thus, the measured values cannot be compared with those in the literature. Nevertheless, it can be suggested that the molecular size of DOM belonging to HiF is very small.

3.3 Trihalomethane formation potential

When normalized on a DOC basis, the specific THMFP (STHMFP) of AHS in Lake Kasumigaura was found to be slightly greater that of HiF. The average STHMFP of the AHS samples was 0.195 μmol THM mg•C^{-1}, while the average HiF STHMFP was comparable at 0.176 μmol THM mg•C^{-1} (**Fig. 5**). The paired *t*-test on the comparison of STHMFP between AHS and HiF showed a significant difference at $P < 0.05$ (n = 12); however, when we excluded one pair of data observed in July, which showed the largest difference, the *t*-test did not give a significant result. The DOM samples exhibited STHMFP values somewhere between those of HiF and AHS (0.188 μmol THM mg•C^{-1}).

The STHMFP of the non-humic fraction, namely HiF, was found to be comparable to that of AHS. This finding appears to be somewhat contrary to the conventional belief that AHS behave as the principal THM precursor material. Nevertheless, this result is consistent with those reported by Owen et al. (1995). They showed in studying THMFP of different waters from a reservoir, river and groundwater that the non-humic fraction reacted with chlorine and produced THM per unit DOC at an extent comparable to the humic fraction.

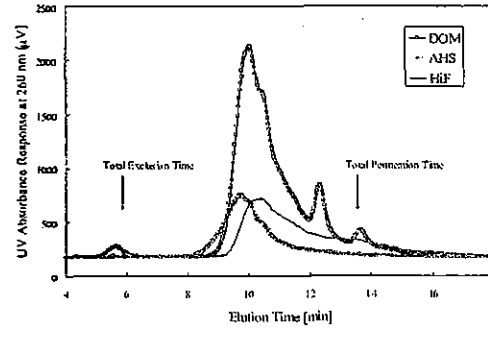

Fig. 4. Typical high-pressure size exclusion chromatograms of DOM, AHS and HiF in water from Lake Kasumigaura. The water sample was collected on September 10, 1997 at a point near the intake of a water treatment plant located on the shore of the lake.

The importance of HiF over AHS as THM

precursors becomes more pronounced when THM formation is evaluated in terms of concentration, that is, when THMFP is assessed in $\mu mol \cdot l^{-1}$. The THMFP of HiF is much greater than that of AHS: the average values for HiF and AHS are 0.374 and 0.229 μmol THM• l^{-1}, respectively. This comparison reveals that HiF account for, on average, 57% of the THMFP of DOM, while AHS account for 35%. Therefore, we concluded that when waters from eutrophic lakes such as Lake Kasumigaura are used as sources of drinking water, lower-molecular-weight hydrophilic DOM, in particular HiA, should be of more concern as a THM precursor than AHS, from the viewpoint of water treatment practices.

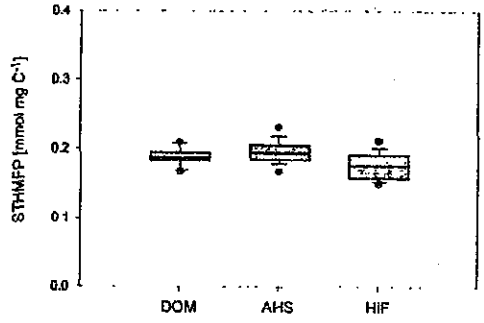

Fig. 5. Comparison of specific trihalomethane formation potential (STHMFP) between DOM, AHS and HiF in water samples taken from Lake Kasumigaura 1997. The lower boundary of each box indicates the 25th percentile, a line within the box marks the mean, and the upper boundary of the box indicates the 75th percentile. Whiskers indicate the 90th and 10th percentiles, and solid dots indicate outlying points. Abbreviations are explained in the text.

4. SUMMARY

DOM in water samples from Lake Kasumigaura, its inflowing rivers and several other DOM sources in the lake watershed was fractionated into five classes: AHS, HoN, HiA, BaS, and HiN. DOM-fraction distribution was found to be very useful parameters for evaluating the characteristics of DOM in waters.

DOM-fraction distribution patterns were significantly different depending on the origin of the sample. AHS and HiA were found dominantly in both the lake and the rivers. HiA prevailed over AHS in the lake water, while AHS was present in a slightly greater percentage in the river water. AHS and HiA were also dominant in all other DOM sources. AHS was the most abundant fraction in FS and PFP. HiA abounded in PFO, DS, and STPE. Only DS contained a significant amount of HoN, indicating the presence of LAS-like DOM.

The THMFP of HiF normalized on a DOC basis was found to be comparable to that of AHS (0.176 vs. 0.195 μmol THM $mg \cdot C^{-1}$, respectively). When THMFP is evaluated in terms of concentration (i.e., μmol THM$\cdot l^{-1}$), the THMFP of HiF was by far greater than that of AHS (mean 0.374 vs. 0.229 $\mu mol \cdot l^{-1}$, respectively).

Molecular size distributions of lake-water DOM, AHS and HiF were found to exhibit a narrow size range and relatively low molecular weight. The weight-averaged molecular weights of DOM, AHS and HiF in the waters of Lake Kasumigaura were, on average, 780, 957, and 606 g mol^{-1}, respectively.

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〈論 文—Original Paper〉

河川水中の溶存有機物分画データと流域特性の関係

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Relationships between Fractionation Data in River Water and Watershed Characteristics

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Abstract

In the basin of Lake Biwa and Lake Kasumigaura, relationships between fractionation data of dissolved organic matter (DOM) and in inflowing river water and watershed characteristics were investigated by regression analysis and specific unit modeling. Most hydrophobic neutrals were found to originate from domestic sewage. Domestic wastewater treatment plant effluent was found to significantly contribute to the load of hydrophilic acids in river water. Specific unit modeling based on the load of DOM fraction and the ratio of land use contribution in watershed enabled source identification for each DOM fraction including refractory dissolved organic matter. In the north basin of Lake Biwa, forest outflow significantly contributed to the load of aquatic humic substances and refractory fractions. The loads of other DOM fractions were found to be dominated mainly by urban drainage.

Key words: dissolved organic matter, fractionations, watershed characteristics, regression analysis, specific unit modeling

1．はじめに

溶存有機物に関する調査研究については，地球規模の炭素循環における海洋の溶存有機物の役割の重要性や水域の物質代謝過程でのバクテリアの役割と溶存有機物の関係に関する知見[1]などにより近年注目を集めている。湖沼における溶存有機物の問題が指摘されるようになってきたのは，1980年代後半から琵琶湖北湖においてBOD濃度が減少しているにもかかわらずCOD濃度が上昇するという「水質乖離現象[2]」が観測されるようになってからである。

この乖離現象の原因としては，湖内のCOD成分の大部分が溶存有機物であることから溶存有機物に何らかの変化が起こっていると考えるのが一般的である。琵琶湖表層では特に春季と冬期にCODの増加が目立ち，深層では季節によらずCODの増加が報告されている[3]。植物プランクトンの指標であるクロロフィルaには一定の増加傾向がみられないことから，内部生産の増大によるCODの増加は考え難く[2,4,5]，BODが易分解性有機物の代表的指標であることからも，BODには反映されないがCODに反映される難分解性溶存有機物が蓄積しているのではないかという推測[2,3]も説得力を持っている。

本研究では，湖沼流域における河川由来の有機汚濁負荷の量的な変化や質的な変化がどのような土地利用に起因するのかを解明することを目的として，日本の代表的湖沼である琵琶湖と霞ヶ浦に流入する河川水中の溶存有機物分画データと流域特性の関係を解析する。具体的には，難分解性溶存有機物と流域発生源の関係を整理することで，有機汚濁発生源の総合的な管理に資する基礎情報の提示を目指す。主たる解析方法は，一般的な回帰分析と土地利用形態別汚濁負荷発生原単位解析である。

まず，溶存有機物分画データと流域特性の単相関を考察した上で，変数増減法による重回帰分析を試みる。さらに，土地利用データと溶存有機物分画に基づいた比負荷量データを用いて，土地利用形態別流出原単位の推定を行う。最後に，琵琶湖と霞ヶ浦に流入する河川データから推定された土地利用形態別流出原単位により発生源ごとの溶存有機物中の各分画成分の存在比を考察するとともに，難分解性有機物の蓄積が懸念されている琵琶湖北湖流域の溶存有機物の流入負荷発生源寄与率を分画別に推定する。

2．溶存有機物分画データ

本研究で使用する溶存有機物分画データは，1994年度か

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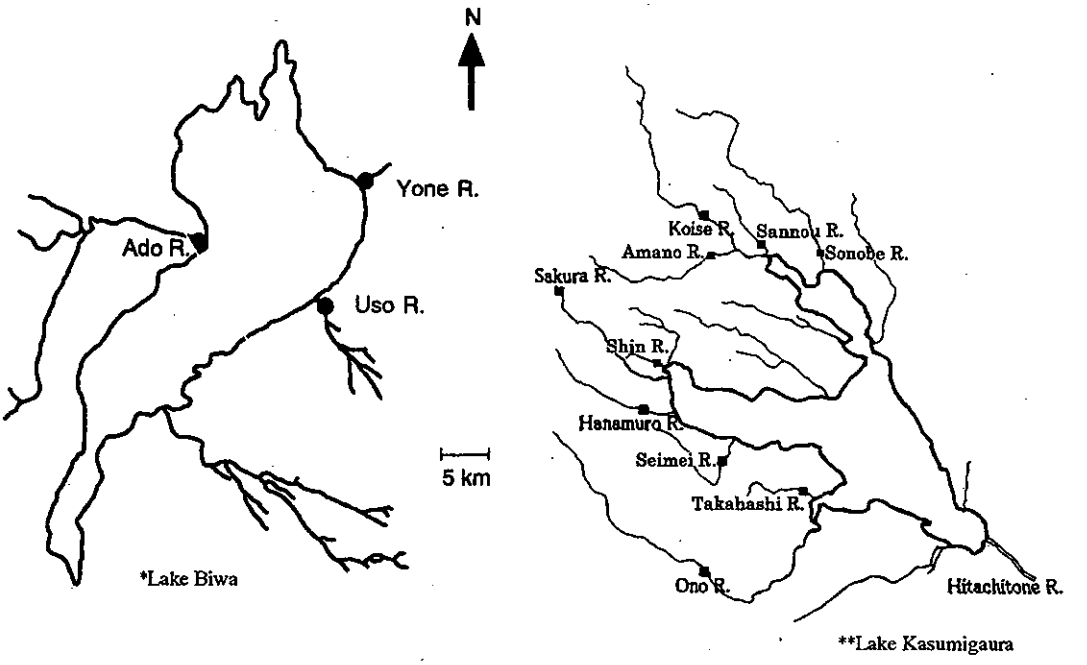

Fig.1 Location of sampling sites in Lake Biwa* and Lake Kasumigaura** basin

ら1996年度にかけて琵琶湖[5,6]と霞ヶ浦[1,8]において測定されたものである。**Fig. 1**に測定地点を示す。琵琶湖流入河川では，宇曽川，米川，安曇川の3河川において1995年度と1996年度に各年度4回測定された溶存有機物分画データ(易分解性－難分解性の分画[6]については1995年度のみ)を用いた。同時期に日野川でも分画データを測定してあるが，流量データが無く比負荷量が算定できなかったため，本研究の対象にはしていない。霞ヶ浦流入河川については，園部川，山王川，天野川，恋瀬川，桜川，新川，花室川，清明川，小野川，高橋川の10河川で1994年度に年4回の頻度で測定された溶存有機物分画データを用いた。

有機物分画分析手法の詳細については既報[2,5,7]を参照されたいが，XAD樹脂・イオン交換樹脂によるフミン質の分離に基づいて，溶存有機物をフミン物質（AHS），疎水性中性物質（HoN），親水性酸（HiA），塩基物質（BaS），親水性中性物質（HiN）の5つに分画したものである。また，難分解性画分は，100日間生分解性試験[6,8]による難分解－易分解性の分離に基づいている。本報告における有機物分画データの略称は**Table 1**に示す通りであるが，各々の分画データはDOCの内訳となり，難分解性画分（略称先頭にRを付加）はDOCを始め各分画成分合計に含まれる。

Table 1 Acronyms of Organic Solutes for Dissolved Organic Matter

acronym	Fraction
DOC	Dissolved Organic Carbon
├AHS	Hydrophobic Acids
├HoN	Hydrophobic Neutrals
├HiA	Hydrophilic Acids
├BaS	Bases
└HiN	Hydrophilic Neutrals
R-DOC	Recalcitrant Dissolved Organic Carbon
├R-AHS	Recalcitrant Hydrophobic Acids
├R-HoN	Recalcitrant Hydrophobic Neutrals
├R-HiA	Recalcitrant Hydrophilic Acids
├R-BaS	Recalcitrant Bases
└R-HiN	Recalcitrant Hydrophilic Neutrals

3．流域諸特性と溶存有機物分画データの関係について

3.1 使用するデータの概要

ここでは，河川流域における土地利用面積比率（市街地，田，畑，山林）および排水処理形態別人口密度（公共下水道，農村下水道，合併浄化槽，単独浄化槽，屎尿処理）と河川水中の溶存有機物分画データの比負荷量年度平均値（各年度とも，4回の測定日に直近の流量データ公表値を濃度データに乗じた負荷量の算術平均値）の関係を考察する。一般的に，流域発生源と河川水質あるいは負荷量の関係を検討する際には，発生源から採水地点までの自浄作用等を考慮しなければならない。本研究では，特に難分解性の溶存有機物の主要な起源に着目しているため，流下過程における生分解や懸濁態の沈降による水質変化は副次的な要因として扱い，流域特性と分画データの直接的な関係に焦点を当てながら，気象条件による季節変化が大きい濃度データよりも年間代表値的な扱いが妥当と考えられる比負荷量データを中心に流域諸特性と関連付けた。

対象河川流域は，流量データおよび流域単位の土地利用面積比率と排水処理形態別人口密度の両方が各種統計資料[1,10]より引用可能な琵琶湖流域3河川（宇曽川，米川，安曇川）と霞ヶ浦流域3河川（新川，花室川，清明川）である。琵琶湖流域3河川の土地利用面積比率と排水処理形態別人口密度は1995年度および1996年度の各々の年度単位に集計されているため，これら3河川の溶存有機物分画データの比負荷量については年度別の算術平均値を採用

した。土壌や植生など自然条件のバックグラウンドが異なる流域の河川データを同一母集団として統計解析することに関しては，既存報告の極めて少ない実河川における溶存有機物分画データのフィールド特性を考察する主旨で重要と考えた。対象河川における有機物分画濃度の平均値と標準偏差を**Table 2**に示す。

3.2 単相関分析による考察

溶存有機物分画データの比負荷量年間平均値と単位面積あたりの流域特性値との間の相関係数を**Table 3**に示す。DOC, HoN, HiA, HiNなど項目において，合併浄化処理人口，単独浄化処理人口との相関が高い。特に，HiAと単独浄化処理人口および合併浄化処理人口の間の相関係数が0.9以上という高い値を示している。下水処理水中ではHiAの占める比率が大きく生活雑排水ではHiAに加えて合成洗剤成分由来のHoNの存在比が高いという報告[8]もあり，合併浄化槽や単独浄化槽の処理水において親水性酸（HiA）や疎

Table 2 Mean values (standard deviations) of DOMfractions in the objected rives

mg/L		DOC	AHS	HoN	HiA	BaS	HiN	R-DOC	R-AHS	R-HoN	R-HiA	R-BaS	R-HiN
Uso R.	1995	1.60	0.54	0.25	0.30	0.21	0.32	0.83	0.40	0.07	0.25	0.04	0.06
		(0.59)	(0.12)	(0.29)	(0.04)	(0.08)	(0.37)	(0.24)	(0.12)	(0.06)	(0.09)	(0.04)	(0.04)
Uso R.	1996	1.33	0.49	0.23	0.52	0.06	0.04						
		(0.49)	(0.14)	(0.21)	(0.17)	(0.03)	(0.03)						
Ado R.	1995	0.38	0.24	0.00	0.06	0.04	0.04	0.30	0.20	0.01	0.07	0.00	0.01
		(0.05)	(0.06)	0.00	(0.04)	(0.03)	(0.03)	(0.06)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)
Ado R.	1996	0.37	0.17	0.07	0.05	0.01	0.06						
		(0.02)	(0.04)	(0.07)	(0.03)	(0.02)	(0.04)						
Yone R.	1995	1.63	0.52	0.23	0.38	0.23	0.26	0.83	0.36	0.09	0.26	0.04	0.09
		(0.52)	(0.06)	(0.20)	(0.10)	(0.17)	(0.28)	(0.10)	(0.14)	(0.12)	(0.05)	(0.04)	(0.06)
Yone R.	1996	1.23	0.37	0.29	0.39	0.04	0.15						
		(0.36)	(0.10)	(0.25)	(0.18)	(0.04)	(0.20)						
Shin R.	1994	4.84	1.57	0.73	1.97	0.43	0.13	3.23	1.13	0.39	1.34	0.36	N.D.
		(1.09)	(0.24)	(0.48)	(0.41)	(0.06)	(0.09)	(0.75)	(0.15)	(0.46)	(0.34)	(0.19)	
Hanamuro R.	1994	3.07	1.14	0.13	1.32	0.35	0.12	2.32	0.96	0.13	0.81	0.27	0.15
		(0.19)	(0.06)	(0.11)	(0.28)	(0.19)	(0.09)	(0.55)	(0.19)	(0.15)	(0.41)	(0.18)	(0.12)
Seimei R.	1994	3.09	1.24	0.17	1.28	0.16	0.24	2.43	0.96	0.31	0.91	0.12	0.13
		(1.04)	(0.27)	(0.25)	(0.51)	(0.12)	(0.16)	(0.79)	(0.23)	(0.42)	(0.32)	(0.14)	(0.10)

Table 3 Correlation coefficients between DOMfraction load in river and watershed characteristics

	DOC	AHS	HoN	HiA	BaS	HiN
urban sewer system	0.01	−0.14	0.10	0.22	0.02	−0.05
rural sewer system	−0.40	−0.44	−0.34	−0.41	−0.21	−0.34
domestic wastewater treatment	0.80 **	0.56	0.88 **	0.95 **	0.63	0.78 *
septic tank treatment	0.73 *	0.46	0.78 *	0.92 **	0.62	0.70 *
night soil collection	0.61	0.34	0.70 *	0.81 **	0.53	0.58
urban area	0.04	−0.17	0.12	0.31	0.08	−0.02
paddy field	0.46	0.22	0.59	0.53	0.42	0.50
field	−0.34	−0.41	−0.37	−0.14	−0.26	−0.38
forest	−0.23	0.10	−0.37	−0.51	−0.26	−0.20

*: $p<0.05$ **: $p<0.01$

Table 4 Correlation coefficients between refractory DOMfraction load in river and watershed characteristics

	R-DOC	R-AHS	R-HoN	R-HiA	R-BaS	R-HiN
urban sewer system	−0.19	−0.32	0.02	−0.02	0.41	−0.25
rural sewer system	−0.38	−0.36	−0.31	−0.44	−0.42	−0.10
domestic wastewater treatment	0.74	0.47	0.91 *	0.88 *	0.95 **	0.84 *
septic tank treatment	0.61	0.31	0.89 *	0.79	0.90 *	0.74
night soil collection	0.44	0.15	0.74	0.63	0.90 *	0.53
urban area	−0.18	−0.39	0.14	0.04	0.51	−0.18
paddy field	0.40	0.21	0.63	0.43	0.41	0.73
field	−0.43	−0.54	−0.09	−0.28	−0.14	−0.41
forest	0.04	0.37	−0.51	−0.21	−0.60	−0.20

*: $p<0.05$ **: $p<0.01$