Effects of Dissolved Organic Matter and Iron Availability on Growth of Cyanobacteria in a Eutrophic Lake

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Introduction

Gradual accumulation of recalcitrant dissolved organic matter (DOM) in lakes

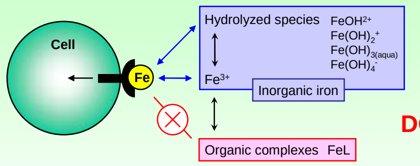
direct toxic effects → indirect effect of iron complexation

DOM can inhibit the algal growth

Imai et al. (1999) Can J Fish Aquat Sci 56: 1929-1937

The ambient level of fulvic acid in Lake Kasumigaura significantly inhibited the growth of *Microcystis aeruginosa* in artificial growth media because of complexation of Fe(III) with fulvic acid

Effect of iron speciation on iron availability



Organically complexed Fe is less available than inorganic Fe

DOM induces iron limitation

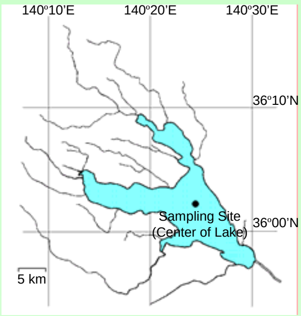
Our aims in this study

To assess the effects of iron complexation with DOM on algal growth in natural lake water samples from Lake Kasumigaura (In Japan) to understand the mechanisms of dominance of particular algal species

Methods

Study site : Lake Kasumigaura

In Lake Kasumigaura, a eutrophic lake in Japan, *Microcystis* blooms were frequently observed until 1986, but they disappeared in 1987, and thereafter *Planktothrix* spp. were instead dominant for a long time. Then, *Planktothrix* spp. became uncommon from 2001 onward, when diatoms such as *Cyclotella* spp. and *Skeltonema* spp. became dominant. The change of dominant species is difficult to explain reasonably (by N and P).



Basic approach for assessing the effect of DOM

UV (ultraviolet)-irradiation can decompose DOM in water sample without adding any chemicals. Both toxic organic compounds and iron-complexing organic ligands are destroyed by UV-irradiation, and consequently iron availability would be increased. The effects of DOM can be assessed by comparing the maximum growth of algae in AGP tests between UV-irradiated and unirradiated samples.

AGP test

Sample collection (July, August 2002, and January, April 2003)

Filtration

Standard / UV treatment:
- pH was adjusted to 2.5 with HCl
- UV-irradiation (60 min)
- Addition of Na_2EDTA (0.5 mg l^{-1})
- pH was adjusted to original value

Filter sterilization

10 ml of samples were pipetted into sterilized grass tubes

Addition of N, P, Fe, EDTA

KNO_3 - 10 mg N / l, $FeCl_3 \cdot 6H_2O$ - 0.1 mg Fe / l,
K_2HPO_4 - 1 mg P / l, $Na_2 \cdot EDTA$ - 0.5 mg / l

Incubation at 25 ºC, continuous light (20 mE·m^{-2}·s^{-1}) for 2 weeks

AGP was measured (absorbance at 750 nm)

Test species:
Microcystis aeruginosa strain NIES-44

Planktothrix agardhii strain NIES204

NOTE :
Water samples need to be acidified during UV-irradiation to prevent iron from precipitated out.

Addition of chelator (EDTA) is essential to keep iron dissolved near neutral pH before pH is adjusted to the original value

NO_3, NH_4, and PO_4 are solubilized from decomposed DOM in UV-samples. Therefore, it is impossible to distinguish whether the differences in AGPs between Standard-sample and UV-sample are a result of the effect of DOM or the increase in inorganic nutrient.

To overcome this drawback, we has come to the approach where the effect of DOM is assessed by comparing the AGPs in UV-irradiated and unirradiated samples after nutrient addition.

Iron speciation

Competitive ligand equilibration-cathodic stripping voltammetry
(Details of method was described in Nagai et al. (2004) Limnology, 5: 87-94)

↓

Natural ligand concentration and the conditional stability constant were determined

↓

Concentration of inorganic Fe (Fe') originally present in the sample was calculated

Results and Discussion

Comparisons of untreated samples (Standard-sample, STD) and samples after UV-irradiation (UV-sample, UV)

		Jul-2002		Oct-2002		Jan-2003		Apr-2003	
		STD	UV	STD	UV	STD	UV	STD	UV
DOC	mM	0.26	0.04	0.29	0.05	0.28	0.06	0.27	0.06
NO_3	μM	2.7	8.4	40.3	42.9	39.6	42.5	ND	3.9
NO_2	μM	1.5	0.1	0.1	0.2	0.4	0.1	ND	ND
NH_4	μM	3.9	14.6	0.8	13.0	0.2	11.5	ND	10.4
PO_4	μM	0.3	0.5	1.6	1.7	0.3	0.4	0.1	0.2
DTFe	nM	44	44	69	69	48	48	24	24
logFe'	M	-13.3	-9.5	-13.5	-9.9	-13.4	-9.5	-12.9	-8.8

DOC – Dissolved organic carbon

DOCs of UV-samples include organic carbon derived from added EDTA

DTFe – Dissolved total iron

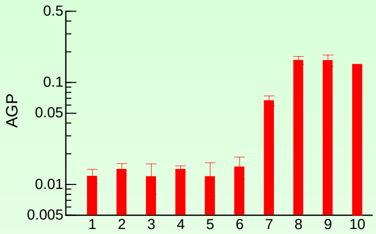
Fe' – Inorganic iron

ND means not detected (<0.1 μM)

>99.9% of dissolved iron presented as organic complexes in all lake water samples
Inorganic Fe (Fe') were very low (0.03 to 0.13 pM) compared with DTFe

The Fe' of the UV-samples were four orders of magnitude higher than those of the Standard-samples → Fe in the UV-samples was much more bioavailable than that in the Standard-samples

AGP test for *M. aeruginosa* (July, 2002)



1 Control
2 N
3 P
4 Fe
5 EDTA
6 N + P
7 UV + N + P
8 N + P + Fe + EDTA
9 UV + N + P + Fe
10 CB medium

The AGP did not increase with the addition of N and P (bars 2, 3, and 6) or the addition of Fe (bar 4) compared with Control (no addition, bar 1), but it increased greatly with the addition of N, P, Fe, and EDTA (bar 8), to the same level as in the CB medium (bar 10). These results show that N, P, and Fe simultaneously limited algal growth

The AGPs did not differ between Standard-samples and UV-samples after the addition of N, P, Fe, and EDTA (bars 8 and 9), indicating clearly no direct toxic effect of DOM

The AGP of the UV-sample after the addition of N and P (bar 7) was higher than that of the Standard-sample after addition of N and P (bar 6), indicating that DOM inhibited the growth through iron complexation

Summary for effects of DOM and limiting nutrients

		Effect of DOM		Limiting nutrient	
		Toxicity	Iron complexation	Primary	Secondary
M. aeruginosa	Jul-2002	–	○	N, P, Fe	
	Oct-2002	–	–	N, P, Fe	
	Jan-2003	–	–	N, P, Fe	
	Apr-2003	–	○	N	P, Fe
P. agardhii	Jul-2002	–	○	Fe	N, P
	Oct-2002	–	○	Fe	N, P
	Jan-2003	–	○	Fe	N, P
	Apr-2003	–	○	N, P	Fe

○ effect found

– effect not found

Effect of iron complexation with DOM and Iron was the primary limiting nutrient

Jul 2002 for *M. aeruginosa*
Jul, Oct-2002 and Jan-2003 for *P. agardhii*

DOM inhibited the algal growth in Lake Kasumigaura through iron complexation

Our results suggest

Effects of iron on algal growth cannot be disregarded even in a eutrophic lake that contains high concentration of total dissolved iron (effect of DOM is substantial)

Characteristics of DOM should vary among the water samples in Lake Kasumigaura, and therefore, physicochemical characteristics of DOM may be a key factor

Iron requirement and/or iron availability differ between *M. aeruginosa* and *P. agardhii*