Competition of copper and zinc for strong ligands in a eutrophic lake

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Abstract

Free aquo copper ion and zinc ion concentrations were determined in water samples from eutrophic Lake Greifen by means of ligand exchange with catechol and cathodic stripping voltametry (for Cu) and ligand exchange with EDTA and anodic stripping voltametry (for Zn). The ratios of total dissolved Zn (10–40 nM) to dissolved Cu (7–20 nM) were [Zn]:[Cu] = 0.5–3 in samples taken at different seasons of a year; the ratios of the free aquo ion concentrations $[Zn^{2+}]:[Cu^{2+}]$ were $\sim 10^6$. pCu was in the range of 14.5–15.9 and pZn 8.6–9.5 at different times and depths. The release of Zn from electrochemically inert complexes upon addition of Cu suggested direct competition of Cu and Zn for ligands in all lake-water samples examined. The selectivity of the natural ligands for Cu over Zn was evaluated as the conditional constant for the reaction $Cu^{2+} + ZnL_1 = Zn^{2+} + CuL_1$; $K = (1.4\pm0.9) \times 10^6$ (average in the euphotic zone; pH 8). These highly selective ligands are probably of biological origin.

Cu and Zn are essential elements for biological activity, but they may be toxic at elevated concentrations, as has been demonstrated in marine phytoplankton (Brand et al. 1986; Sunda and Huntsman 1992). Phytoplankton may, in turn, affect trace metal chemistry by releasing metal-complexing ligands as well as by metal binding on surfaces and uptake and sedimentation in natural waters (Bruland et al. 1991; Sunda 1994; Xue and Sigg 1993). The free metal ion concentration—a key parameter for the reactivity, bioavailability, and effects of metals (Sunda 1994; Sunda and Guillard 1976)—is regulated by complex interactions between trace metal ions, ligands, and major ions and particles (Whitfield and Turner 1987; Bruland et al. 1991). To assess the fate and biological effects of trace metals in natural waters, it is thus essential to determine metal speciation and especially to evaluate the free aquo metal ion concentrations.

Cu and Zn complexation in seawater have been extensively studied (e.g. Sunda and Huntsman 1991; Coale and Bruland 1990; Donat and Bruland 1990), but few studies on freshwater systems are available. Cu is strongly complexed by organic ligands in seawater; Zn is also present mostly in organic complexes in the upper layers of the central Pacific (Bruland 1989; Bruland et al. 1991).

Our recent studies on Cu and Zn speciation in freshwater (Xue and Sigg 1993, 1994) showed that in the water column of a eutrophic lake (Lake Greifen), Cu is strongly complexed by organic ligands, which are probably biologically produced, giving $\log[Cu^{2+}] = -16$ to -14, whereas a substantial part of Zn is present as free Zn ions and weak organic complexes. The ratios of the free aquo ion concentrations $[Zn^{2+}]:[Cu^{2+}]$ were estimated to be in the range of 10^5–10^6 in this lake; however, the results

for Cu and Zn were not obtained in the same samples. These ratios may reflect the different tendency of Cu and Zn for binding to strong organic ligands. These findings raise questions about the selectivity of the ligands involved, their sources, and their possible significance in maintaining low levels of $[Cu^{2+}]$ and corresponding $[Zn^{2+}]$ in productive lakes.

Here we investigate whether direct competition between Cu and Zn for the strong natural ligands can be demonstrated and attempt to quantify the selectivity of these ligands for Cu in comparison to Zn. This study was carried out in Lake Greifen, a small eutrophic lake, because our previous investigations had shown the presence of high concentrations of ligands in the lake and because an extensive database on chemical and biological parameters is available for it. $[Cu^{2+}]$ and $[Zn^{2+}]$ from the same samples in the water column of the lake in different seasons of a year were determined by ligand exchange and voltametry. We compared the complexation characteristics of Cu and Zn. We used titrations of samples with Cu or with Zn to evaluate their competition for the natural ligands. We present the resulting exchange constants and discuss the possible sources of the strong organic ligands.

Theoretical background of the determination of $[Cu^{2+}]$ and $[Zn^{2+}]$ and of calculations of Cu-Zn exchange constants

$[Cu^{2+}]$ and $[Zn^{2+}]$—The methods used to determine $[Cu^{2+}]$ and $[Zn^{2+}]$ have been previously optimized for freshwater conditions; ligand-exchange theory and the $[Cu^{2+}]$ and $[Zn^{2+}]$ determinations have been described elsewhere (Van den Berg 1984; Xue and Sigg 1993, 1994).

No labile Cu can be detected by DPASV (differential pulse anodic stripping voltametry) in these samples; therefore, an indirect method must be used for Cu speciation. Briefly, $[Cu^{2+}]$ and complexation parameters for natural organic ligands are determined by competition

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between the natural organic ligands and the ligand catechol, which is added in excess. The concentration of Cu catechol complexes at equilibrium depends on the concentrations and stability constants of the natural ligands in the sample and can be measured by differential pulse cathodic stripping voltametry (DPCSV), e.g. the reduction current of copper-catechol complexes adsorbed onto the surface of a hanging mercury drop electrode (HMDE) is measured. $[Cu^{2+}]$ is calculated from equilibrium relationships with the catechol complexes.

Titrations of lake waters with Cu and determination of free cupric ion concentrations as a function of total Cu are interpreted with a two-ligand model. The direct results of these titration experiments are values of pCu and $\Sigma K_i[L_i]$, the product of the stability constants and of the ligand concentrations. The approximation of this complex system by fitting to a simple two-ligand model represents a simplification. The FITEQL program (Westall 1982) was used to estimate conditional stability constants (K_1 and K_2) and ligand concentrations (L_1 and L_2).

$[Zn^{2+}]$ was determined from measurements of labile Zn by DPASV, ligand exchange with EDTA (ethylenediaminetetraacetate), and equilibrium calculations (Xue and Sigg 1994). Total dissolved Zn in lake waters include labile and inert species with respect to voltametric methods. Inert Zn species are nonreducible complexes at the electrode surface or are not dissociated in the boundary layer at the chosen potential; these inert species include strong organic complexes and Zn adsorbed on colloidal particles. In interpreting the data, we considered the inert species to be strong organic complexes. The labile species include free aquo ions, inorganic, and weak organic complexes. The peak current obtained by DPASV (i_p) is regarded as a measure of the sum of all labile Zn species. The ZnEDTA complex is inert in terms of DPASV.

When EDTA is added to lake water under certain conditions, it competes with inorganic and labile organic ligands for Zn. The concentration of ZnEDTA complex is determined—at equilibrium with a given EDTA concentration—from the difference in labile Zn before and after adding EDTA. The concentration of free Zn ion in the original water can then be calculated from the equilibrium with EDTA. The results of the Zn speciation experiments are concentrations of inert complexes (ZnL_1), of weak organic (labile) complexes (ZnL_2), and of Zn^{2+}. Stability constants of the ZnL_1 complexes are not available from these experiments because only the exchange reaction of EDTA with the labile complexes is evaluated quantitatively. The complexing coefficients for L_2 (products $K_{2Zn}[L_2]$) are determined by titration with EDTA and measurement of labile Zn.

Conditional constants are used throughout this work for the calculations; the results are therefore expressed as concentrations of $[Cu^{2+}]$ and $[Zn^{2+}]$ under the conditions given.

Competition of Zn and Cu for strong organic ligands—
It is realistic to assume that competition between Cu and Zn for strong organic ligands can occur in lake water. To quantify the competition of Cu and Zn, we measure changes in labile Zn as a function of the total Cu con-

centration. Therefore, only the competition for ligands forming electrochemically inert complexes with Zn can be measured directly. We assume 1:1 complexation with the strong ligands for both metals at ambient level. The following mass balances can be written for dissolved Cu ($[Cu]_T$) and Zn ($[Zn]_T$), with a strong ligand, L_1 (for which competition between Cu and Zn is examined), and a weak a ligand, L_2,

$$[Cu]_T = [CuL_1] + [CuL_2] + \Sigma[Cu_{inorg}] + [Cu^{2+}] \quad (1)$$

$$[Zn]_T = [ZnL_1] + [ZnL_2] + \Sigma[Zn_{inorg}] + [Zn^{2+}]. \quad (2)$$

$[Cu_{inorg}]$ and $[Zn_{inorg}]$ are the inorganic complexes of Cu and Zn.

The competition of Cu and Zn for L_2 is not examined in detail; weak ligands are probably present in excess of the metals in lake water, so competitive effects are not as important for L_2 at the ambient levels. $[ZnL_1]$ is electrochemically inert, and labile Zn is

$$[Zn]_{lab} = [ZnL_2] + \Sigma[Zn_{inorg}] + [Zn^{2+}]. \quad (3)$$

The mass balance of the ligand L_1 is

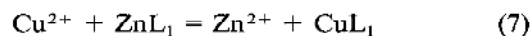$$[L_1]_T = [CuL_1] + [ZnL_1] + [L_1]. \quad (4)$$

The stability constants with L_1 (conditional for a given pH) are

$$Cu^{2+} + L_1 = CuL_1 \qquad K_{1Cu} = \frac{[CuL_1]}{[L_1][Cu^{2+}]} \quad (5)$$

and

$$Zn^{2+} + L_1 = ZnL_1 \qquad K_{1Zn} = \frac{[ZnL_1]}{[L_1][Zn^{2+}]}. \quad (6)$$

The exchange constant is defined for the reaction

$$Cu^{2+} + ZnL_1 = Zn^{2+} + CuL_1 \quad (7)$$

with the constant

$$K_{ex} = \frac{[CuL_1][Zn^{2+}]}{[ZnL_1][Cu^{2+}]} = \frac{K_{1Cu}}{K_{1Zn}}. \quad (8)$$

During titration of lake water with Cu, Cu replaces Zn in the complexes with L_1, and the labile Zn concentration increases. The following mass balances can thus be written (where $[ZnL_1]_0$ and $[CuL_1]_0$ are the concentrations of Zn and Cu bound to L_1 in the original water sample, and $\Delta[Zn]_{lab}$ is the increase of labile Zn at a given Cu addition):

$$[ZnL_1] = [Zn]_T - [Zn]_{lab} = [ZnL_1]_0 - \Delta[Zn]_{lab}; \quad (9)$$

$$[CuL_1] = [CuL_1]_0 + \Delta[Zn]_{lab}. \quad (10)$$

These equations are valid if the increase in $[CuL_1]$ is matched by the decrease in $[ZnL_1]$. The exchange constant can be written as

$$K_{ex} = \frac{([CuL_1]_0 + \Delta[Zn]_{lab})[Zn^{2+}]}{([Zn]_T - [Zn]_{lab})[Cu^{2+}]}. \quad (11)$$

After reorganization, we obtain the expression

$$\frac{([Zn]_T - [Zn]_{lab})[Cu^{2+}]}{[Zn^{2+}]} = \frac{[CuL_1]_0}{K_{ex}} + \frac{\Delta[Zn]_{lab}}{K_{ex}}. \quad (12)$$

When a water sample is titrated with Cu, $[Cu^{2+}]$ can be determined as indicated above at any point of the titration. $[Zn]_{lab}$ can also be measured at each point, and $[Zn^{2+}]$ is calculated by means of the complexing coefficient for the weak organic ligands and inorganic ligands. Plotting the left side of Eq. 12 as a function of $\Delta[Zn]_{lab}$ should yield a linear relationship. K_{ex} and $[CuL_1]_0$ can be evaluated from the slope and intercept of this function.

If most dissolved Cu is complexed with L_1 in lake water, the mass balance (Eq. 1) can be simplified to

$$[CuL_1] \approx [Cu]_T,$$

and the exchange constant can be written as

$$K_{ex} = \frac{[Cu]_T[Zn^{2+}]}{([Zn]_T - [Zn]_{lab})[Cu^{2+}]}. \tag{13}$$

The Cu-Zn exchange constant can be obtained from the parameters on the right side of Eq. 13 (determined in an original water sample) and from the first few points of a titration of lake water with Cu, for which the differences in labile Zn are larger and the values are therefore more precise than values in the later part of the titration curve.

From the exchange constant K_{ex} and the stability constant K_{1Cu} for the CuL_1 complex, the conditional stability constant K_{1Zn} for ZnL_1 (strong organic complexes) is readily obtained from Eq. 8.

It is difficult to distinguish between different ligands in lake-water samples. We therefore assume only one type of strong ligand in order to evaluate the competition between Cu and Zn. A one- or two-ligand model is in any case a strong simplification of the natural system, in which many different types of ligands are present.

Model calculations that use the parameters extracted from the experiments give insights into variations of the complex species; these calculations were performed with the MacμQL program (Müller 1993; Westall 1979).

Experimental

Water samples were collected from the water column of Lake Greifen, Switzerland, at its deepest point in different seasons of 1993 (four samplings over 1 yr). The lake is highly eutrophic and has a surface area of 8.5 km² and a volume of 150×10^6 m³. Its average depth is 17.7 m (max, 32.2 m). The tributaries of the lake are highly loaded with nutrients and pollutants from sewage and agriculture. The geochemical background of the lake is dominated by calcite. The major ion composition of Lake Griefen is characterized by Ca $\approx$ 1.2–1.6 mM, Mg $\approx$ 0.6 mM, and alkalinity $\approx$ 3.6–4.1 mM; pH is 7.5–8.5, with variations in time and depth. Dissolved organic C is 3–4 mg liter^{-1} in the water column. The lake is sampled regularly each month for routine parameters such as oxygen, nutrients, and chlorophyll (Limnology Department, EAWAG). Previous investigations of the depth profiles of dissolved Zn and Cu have been carried out over 1–2 full seasonal cycles (Sigg et al. 1995).

We used GoFlo bottles (General Oceanics, 5 liters) to collect samples from different depths (10–12 samples at different depths for nutrients and total concentrations and 2–4 samples for speciation experiments). The samples were transferred from the GoFlo to polyethylene bottles under N_2 pressure. All the bottles and GoFlo sampling bottles, as well as the filtration devices and filters (0.45 μm), were carefully cleaned with HNO_3 and double-distilled water. Samples were filtered and further treated just after transport to the laboratory. Filtration was performed under clean-bench conditions.

Total dissolved Cu was directly measured in acidified samples by graphite furnace atomic absorption spectrometry. Total dissolved Zn was determined in acidified samples by flame atomic absorption spectrometry after preconcentration, using the extraction of 8-hydroxyquinoline complexes on SepPak C_{18} resins (Sigg et al. 1995).

Ca and Mg were measured by inductively coupled plasma emission spectrometry. Total inorganic C and carbonate concentrations (used to calculate Zn and Cu speciation) were calculated from alkalinity (measured by Gran titration) and pH measurements. Phosphate and silicate were measured by standard spectrophotometric methods on an autoanalyzer. Chlorophyll *a* was measured by HPLC (Meyns et al. 1994).

DPCSV and DPASV measurements for speciation of Cu and Zn were carried out within a week of collection. The filtered samples were stored in the dark at 4°C until use. No detectable effects of storage and wall adsorption were observed (Xue and Sigg 1993, 1994).

DPCSV measurement of Cu-catechol complexes and titration curve with Cu—Details of free cupric ion concentration determination have been described elsewhere (Van den Berg 1984; Xue and Sigg 1993). Briefly, DPCSV was performed with a hanging mercury drop electrode, an Ag/AgCl reference, and a graphite counter electrode held in a Metrohm VA 663 stand combined with a 646 VA processor. Catechol was added to the samples after 5 min of purging with suprapure N_2. Equilibration was allowed for 5 min under N_2. A new Hg drop was made and the stirrer switched on simultaneously. The Cu-catechol complexes were collected on the electrode at 0 V for 2 min. After the collection period, the stirrer was turned off, and 15 s later the voltage scan was initiated in the negative direction.

To obtain a Cu titration curve of a lake-water sample, we spiked a series of subsamples with different concentrations of Cu. Subsamples (25 ml) of lake water were pipetted into a series of 50-ml high-density polyethylene beakers; 150 μl of HEPES buffer (a solution containing 1 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid and 0.5 M NaOH) was added to each beaker to give a final concentration of 6×10^{-3} M HEPES and a final pH of 8.0±0.1, which is close to the natural pH of the lake water. Cu was added to all beakers but one. The series was allowed to equilibrate at 20±2°C overnight. DPCSV analyses were performed in the same polyethylene beakers to minimize adsorption effects. The DPCSV sensitivity had to be calibrated for each individual lake-water

sample because of the effects of different organic compounds on the sensitivity, which varied between 0.25 and 0.40 A mol^{-1} liter^{-1}. Sensitivity was determined from the portion of the titration curve at high concentrations of Cu^{2+}, where stronger organic ligands are saturated and the increase of catechol complexes is proportional to added Cu. [Cu^{2+}] was indirectly determined by equilibrium calculations from the measured concentration of Cu-catechol complexes (Xue and Sigg 1993) using stability constants from Martell and Smith (1974–1989).

DPASV measurement of labile Zn and titrations with Zn or EDTA—DPASV measurements of labile Zn were performed with the same apparatus that was used for DPCSV determination of Cu-catechol complexes. After 10 min of purging with suprapure N$_2$, we made a new Hg drop and simultaneously switched on the stirrer. Labile Zn was deposited at −1.2 V for a period of 2 min under stirring followed by a 15-s rest; scanning then began in the positive direction (Xue and Sigg 1994).

To calibrate DPASV sensitivity of labile Zn, we titrated lake water with standard Zn ion solution. Aliquots (25 ml) of lake water were pipetted into a series of beakers with 6 × 10^{-3} M HEPES buffer at pH 8.0±0.1. Zn was added to all beakers but one for each series. All beakers were kept in the dark overnight until DPASV measurement. The DPASV sensitivity for Zn in lake water is in the range 0.045–0.052 A mol^{-1} liter^{-1} min^{-1}.

Free Zn ion concentration and Zn speciation were calculated from the labile Zn measurement at different concentrations of EDTA (with stability constants from Martell and Smith 1974–1989); [ZnEDTA] is calculated as the difference between initially labile Zn and measured labile Zn after addition of EDTA. Lake waters were thus titrated with EDTA in the same concentration range used for initially labile Zn. Aliquots (25 ml) of lake water were pipetted into a series of the beakers, 6 × 10^{-3} M of HEPES buffer was added, and the aliquots were spiked with different concentrations of EDTA (10–50 nM) in 3–5 steps. All the prepared samples were allowed to equilibrate in the dark overnight before DPASV measurement.

Competition of Cu and Zn for ligands—The competition of Cu with Zn for organic ligands was examined by adding Cu to samples in the concentration range 2 × 10^{-9}–3 × 10^{-7} M. The samples were equilibrated overnight (15–20 h). Labile Zn by DPASV and [Cu^{2+}] by exchange with catechol were measured as described above at each titration point; labile Zn was measured before addition of catechol. The instrument was set in DPASV mode for labile Zn after a sample was placed in the polarographic cell. After measurement of labile Zn, the instrument was switched to DPCSV mode. The desired concentration of catechol was added to the sample following 5 min of purging with N$_2$, and DPCSV measurement of Cu catechol was then performed.

In some experiments, lake-water samples were titrated with EDTA in the presence of added Cu. Titration series were equilibrated overnight. Labile Zn was then measured
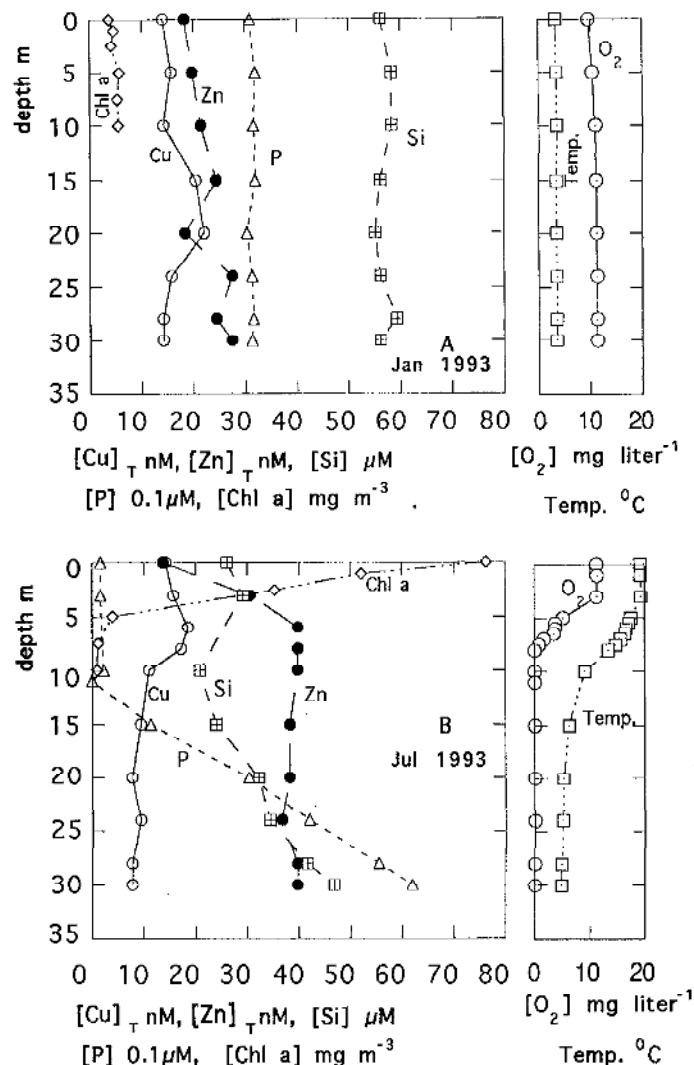

Fig. 1.   Vertical profiles of dissolved Cu, Zn, silicate, orthophosphate, and chlorophyll *a* concentrations, and O$_2$ and temperature in Lake Greifen in January and July 1993. (Chl *a* data provided by S. Meyns.)

by DPASV and free Zn^{2+} concentrations were calculated from equilibration with EDTA in order to evaluate changes in Zn speciation. Free [Cu^{2+}] was measured in another aliquot of the samples with added Cu.

To evaluate the effects of added Zn on Cu speciation, we titrated the water samples with Zn and equilibrated them overnight. At the ambient Cu concentration, the concentrations of free cupric ions were too low to be precisely distinguished as a function of added Zn. Some titrations of lake water with Zn were therefore carried out in the presence of added Cu. Labile Zn was measured by DPASV, and free cupric ion concentrations were determined by DPCSV of Cu-catechol complexes after addition of catechol in excess. Complexation of Zn by the added catechol could have only a negligible effect; equilibrium computations indicate that free Zn ion concen-

Table 1. Cu speciations in the water column of lake Greifen. CL_1 and CL_2 are the total concentrations of organic ligands L_1 and L_2; $\log K_{1Cu}$ and $\log K_{2Cu}$ are the corresponding conditional stability constants of the Cu complexes, obtained from fitting the titration data to a two-ligand model (conditional for pH 8).

1993	Depth (m)	$[Cu]_T$* (nM)	$[Cu^{2+}]$† ($\times 10^{-15}$ M)	pCu	$[Cu]_T$: $[Cu^{2+}]$ ($\times 10^7$)	$\log K_{1Cu}$‡	$\log K_{2Cu}$‡	CL_1‡ (nM)	CL_2‡ (nM)
26 Jan	5	15.7	0.5	15.3	3.3	15.2	12.5	27	353
	30	14.2	3.2	14.5	0.4	13.8	11.8	99	463
5 Apr	5	11.0	0.4	15.4	2.6	15.3	13.2	24	275
	30	11.0	0.4	15.4	3.0	14.9	12.7	51	284
26 Jul	3	15.7	0.7	15.2	2.4	15.2	13.2	28	296
	6	18.6	1.6	14.8	1.2	15.1	13.3	19	260
	8	17.2	0.5	15.2	3.2	15.1	12.9	35	291
	30	7.9	0.1	15.9	7.1	15.6	13.4	28	279
13 Oct	2.5	12.6	0.6	15.2	2.0	14.6	13.0	57	333
	11	12.6	0.5	15.3	2.3	14.5	12.5	73	373
Year avg in the euphotic zone		13.8	0.6	15.3	2.6	15.1	13.0	34	314

* Measured.
† Calculated from ligand exchange.
‡ Two-ligand model fit.

trations would decrease by <20% in the presence of catechol.

Cu-Zn exchange and competition for an organic ligand have been demonstrated in a model solution with EDTA. The selectivity of EDTA for Cu over Zn gives an exchange constant $\log K_{ex} \approx 3$. Increase in labile Zn was observed when a solution containing 0.2 μM Zn and 0.1 μM EDTA was titrated with Cu. The experimental points were in agreement with a calculated curve (obtained by an equilibrium model with MacµQL). The good agreement between calculation and measurement indicates that Cu competition with Zn for a strong ligand leads to Zn release and to a detectable increase in labile Zn.

Results

Total dissolved Zn and Cu in the water column — Lake Greifen turns over during winter (December–January), and the thermocline develops in May–June. The deeper part of the lake becomes anoxic in May–June, and the hypolimnion below ~10-m depth remains anoxic from about June to November.

Total concentrations of dissolved Zn (<0.45 μm) in the water column of the lake were 10–40 nM; total concentrations of dissolved Cu were 7–20 nM. Systematic variations of total dissolved Zn over the seasonal cycle were observed in 1993; these variations were similar to those found in a more detailed study done in 1989–1990 (Sigg et al. 1995). A slight depletion of Zn from the epilimnion was observed during summer stagnation; during lake overturn, homogeneous concentrations of Zn were measured throughout the water column, as illustrated by the depth profiles in Fig. 1. Zn became depleted from the entire water column during late stagnation (October, data not shown).

Results on total dissolved Cu showed no depletion from the epilimnion in summer; in contrast, somewhat higher Cu concentrations were observed in the epilimnion than in the hypolimnion during summer stagnation.

The ratio of total dissolved Zn to Cu in the water column was thus Zn:Cu $\approx$ 2–3 (mol mol^{-1}) during lake mixing; this ratio remained rather constant in the hypolimnion, but it decreased to Zn:Cu $\approx$ 0.5–1 in the epilimnion during summer.

The differences between the depth profiles of Cu and Zn can be explained by the relative importance of inputs into the epilimnion and removal by sedimentation. During summer stagnation, the inputs occur into the epilimnion; the removal rates from the epilimnion by sedimentation are higher during summer for Zn than for Cu (Sigg et al. 1995). Zn is depleted in the epilimnion during summer stagnation, whereas Cu accumulates. The strong complexation of Cu probably affects the extent of removal by sedimentation; this effect is not as significant, however, as expected from the large difference in speciation between Cu and Zn. More data for 1993 than are available here would be needed to quantitatively evaluate the removal rates and mass balances of Cu and Zn.

[Cu^{2+}] and Cu complexation — The [Cu^{2+}] concentrations in the lake are very low, with an average pCu in the euphotic zone 15.3±0.1 (Table 1). The complexing parameters in the eutrophic zone and their average over the year are also listed in Table 1. The ratio of [Cu]$_T$ to [Cu^{2+}], a measure of the complexation of Cu-organic ligands, is very high (2.55±0.54)$\times 10^7$, close to the highest values observed in 1990. Most of the total dissolved Cu ($\gg$99%) must thus be present in the form of organic complexes. The concentrations of the strong ligands, L_1, obtained from fits of the titration data, always exceeded dissolved Cu; the weaker ligands, L_2, show an even larger excess.

Some differences in the samples are observed as a function of depth (Table 1). In the samples on 26 January 1993, Cu is much more strongly complexed in the 5-m sample than in the 30-m sample. This difference is less obvious in the April and July samplings. In the October samples, no Cu speciation measurement could be obtained in the 30-m sample, probably because of interferences by sulfide. The Cu speciation in the water column in July exhibited an interesting behavior related to stratification. The thermocline was observed at ~7–8-m depth; oxygen decreased from 11.3 mg liter^{-1} at 0–3 m to 3.8 at 6 m and <0.1 at 8 m. The total dissolved Cu concentration at 6 m was only a little more than that at 3 m, although the free cupric ion concentration was 2.5 times higher. [Cu^{2+}] decreased sharply from 6 to 8 m, which means over the oxic–anoxic interface. The stability constants for strong ligands obtained in the samples at 3, 6, and 8 m are very similar; the concentration of strong ligands is, however, lower in the 6-m sample than in the 3- and 8-m samples. These differences indicate that in the 6-m layer, mineralization processes, by which part of these ligands is decomposed, are predominant.

Differences between samples become clearer when the Cu titration curves rather than the overall results given in Table 1 are compared. Some titration data for [Cu^{2+}] and their fitted curves with a two-ligand model are plotted in Fig. 2. The data could be fitted well by using the stability constants and ligand concentrations (Table 1) obtained with the FITEQL program (Westall 1982). A side-by-side comparison of the titration curves, plotted as log[Cu^{2+}] vs. log[Cu]$_T$, shows seasonal variation of the complexation in the euphotic zone (Fig. 2A), as indicated by the shift in the curves along the x-axis (Sunda and Huntsman 1991). The titration curve of January is shifted to the left side of April, July, and October for the samples from the euphotic zone, which means to overall less strong complexation. Only the lower part of the titration curve of January overlapped the others, indicating low concentration of strong organic ligands. Figure 2B shows a side-by-side comparison of January water between the top and bottom layers. The titration curve of the bottom-layer water has a shape similar to other winter samples, and the titration from the upper layer indicates stronger complexation at lower concentrations of Cu.

A previous study indicated similar pCu values and seasonal variations of pCu that followed those of algal productivity in 1990 (Xue and Sigg 1993). Differences between winter and summer samplings are, however, less clear in the 1993 measurements. The stability constants of strong organic ligands, logK_{1Cu} = 15.3±0.1 in the euphotic zone in 1993, were an order of magnitude higher than the average calculated in 1990. This higher average may be related to the choice of sampling times in 1993, which coincided more often with algae blooms. Even the January sampling appears to have been influenced by an unusual occurrence of blue-greens during winter 1993 in comparison to other winters (H. R. Buergi pers. comm.). Lower values of logK_{1Cu} in the October samples and in the 30-m sample in January also support the hypothesis of the production of ligands by algae.
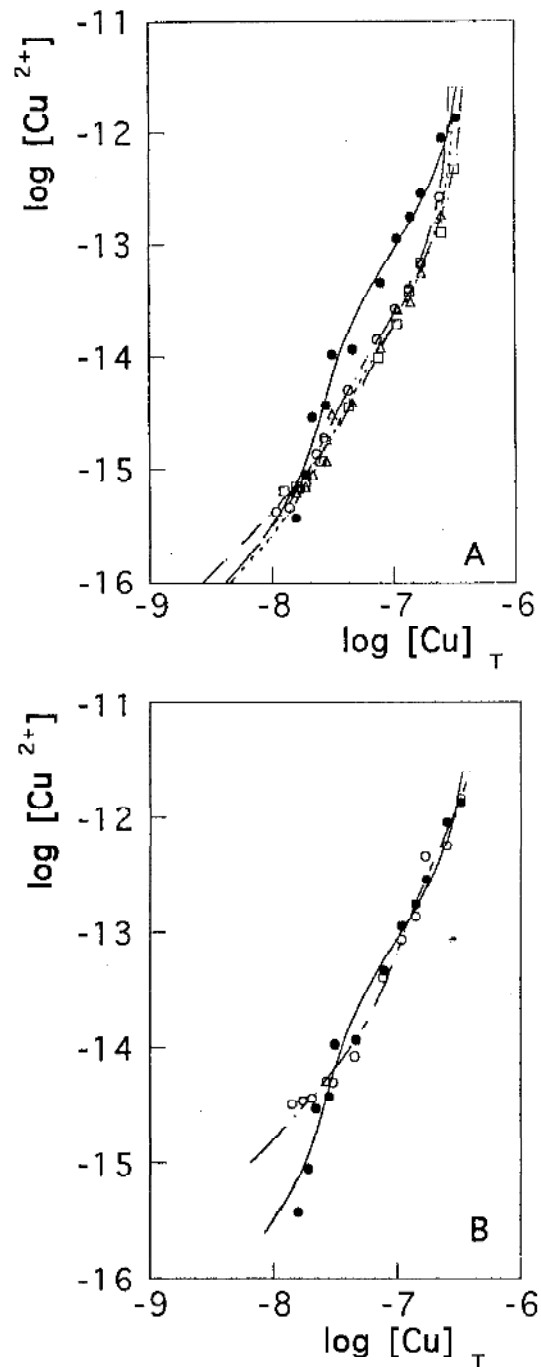

Fig. 2. Titration curves of Lake Greifen water samples with Cu in terms of log[Cu^{2+}] as a function of log[Cu]$_T$. The points were measured and the curves calculated with the stability constants and ligand concentrations listed in Table 1. A. Samples from the euphotic zone on different dates: (●——) 26 January 1993; (○—·—) 5 April 1993; (△·····) 26 July 1993; (□———) 13 October 1993. B. Samples taken on 26 January 1993: (●——) 5-m depth; (○—·—) 30-m depth.

[Zn^{2+}] and Zn speciation—The results obtained on the speciation of Zn indicate that, in contrast to Cu, a substantial part of Zn is present as free Zn ions and weak organic complexes, as shown in Table 2. Of the total

Table 2. Zn speciations in the water column of lake Greifen. Speciation results are conditional for pH 8. $[ZnL_1]$ and $[ZnL_2]$ are the concentrations of Zn in the strong (L_1) and weak (L_2) complexes.

1993	Depth (m)	$[Zn]_T$* (nM)	$[Zn^{2+}]$† (nM)	pZn	$[Zn]_T : [Zn^{2+}]$	$[ZnL_1]$* (nM)	$[ZnL_2]$† (nM)
26 Jan	5	19.9	0.6	9.2	32.6	7.7	11.0
	30	27.5	2.8	8.6	10.0	16.0	6.0
5 Apr	5	21.4	0.3	9.5	64.8	15.7	5.0
	30	29.1	0.4	9.4	69.3	20.3	7.9
26 Jul	3	30.6	1.2	8.9	24.9	19.8	8.6
	6	39.8	1.8	8.8	25.4	4.3	32.5
	8	39.8	2.8	8.6	14.5	6.6	27.7
	30	39.8	2.0	8.7	20.1	30.0	5.8
13 Oct	2.5	22.9	0.5	9.3	43.4	13.8	8.2
	11	36.8	2.3	8.7	16.3	12.3	20.4
Year avg in the euphotic zone							
		23.7	0.6	9.2	37.0	14.2	8.2

* Measured.
† Calculated from ligand exchange.

dissolved Zn, 1–10% was free Zn^{2+}; pZn was thus in the range 8.5–9.5. In the euphotic zone, 20–60% of free Zn^{2+} appeared to be present in weak organic complexes, while 40–80% was present in forms that were not labile to ASV determinations and were thus bound in stronger organic ligands or possibly in colloidal particles. The directly measured concentrations of ZnL_1 (nonlabile complexes) indicate that strong organic ligands for Zn are probably only available at lower concentrations than total dissolved Zn. The L_1 concentrations given in Table 1 (obtained from modeling the Cu titration data) correspond to the total available strong ligands; in view of the Cu-

Zn competition for them, they should be equal to the sum of $[ZnL_1]$ and $[CuL_1]$ ($\approx [Cu]_T$); L_1 concentrations (Table 1) are either close to this sum or in excess of it.

Competition of Cu and Zn for ligands—Addition of Zn seems to have no effect on Cu speciation (Fig. 3). There were no obvious variations of the peak current for [Cu-catechol] in titrations of original lake water with Zn, and thus no measurable differences in $[Cu^{2+}]$. As an example, free cupric ion concentrations in the presence of an additional 31.5 nM Cu are plotted in Fig. 3 as a function of added Zn. With added Zn_T in the range $1 \times 10^{-8} - 2 \times 10^{-7}$ M, the results show no detectable effect of Zn additions on Cu speciation or on $[Cu^{2+}]$ concentrations. Although the addition of Cu in this experiment would saturate the strong ligands, competition with Zn should be detectable if it were significant.

In contrast, titration of lake water with Cu clearly influences Zn speciation (Table 3, Fig. 4). Labile Zn concentrations increased after addition of Cu (31.5 nM); free Zn ion concentrations increased in a similar manner (Table 3). It is likely that the added Cu replaced Zn in the strong organic complexes and that Zn was released to labile species. Labile Zn concentrations, as well as $[Zn^{2+}]$, increased in all samples upon addition of Cu ($1 \times 10^{-8} - 3 \times 10^{-7}$ M) and approached a plateau, as shown in Fig. 4. For some samples, maximal concentrations of labile Zn in titrations with Cu were close to total dissolved Zn (Table 4). The direct results of these experiments are the changes only in the electrochemically inert Zn species, which are considered to be the strong organic complexes (ZnL_1). The other changes in Zn speciation (relative fraction of inorganic and weak organic complexes) are obtained indirectly from the exchange with EDTA.

In the examples given in Table 3, the increase in labile Zn takes place mostly as an increase of the inorganic Zn complexes and of Zn^{2+} and only of a smaller fraction as ZnL_2. The results given in Table 3 represent the case of
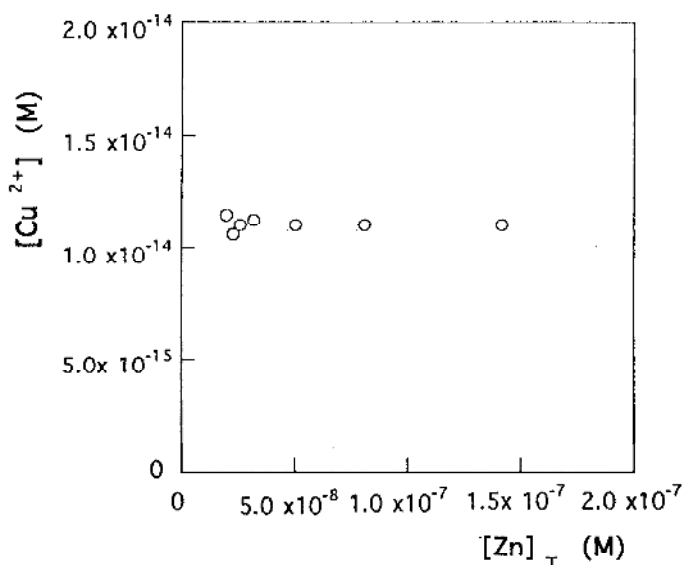

Fig. 3. Free cupric ion concentrations as a function of adding Zn. The sample was taken at 5-m depth on 26 January 1993, and the titration was carried out in the presence of added Cu (31.5 nM).

Table 3. Zn speciation in an original lake-water sample and after Cu addition. [Cu] added—31.5 nM; samples taken on 26 January 1993; pH 8; $[Zn]_T$—dissolved Zn (<0.45 μm); $[Zn]_{lab}$—labile Zn species by voltametry; $[Zn]_{inorg}$—inorganic Zn species; $[ZnL_1]$—complexes of Zn with strong organic ligands; $[ZnL_2]$—complexes of Zn with weak organic ligands.

	5-m depth		30-m depth	
	Orig.	+Cu	Orig.	+Cu
$[Cu]_T$ (nM)	15.7	47.2	14.2	45.6
pCu	15.3	13.9	14.5	14.1
$[Cu^{2+}]$ ($\times 10^{-15}$M)	0.5	11.5	3.2	8.3
$[Zn]_T$ (nM)	19.9	19.9	27.5	27.5
$[Zn]_{lab}$ (nM)	12.2	16.4	11.5	19.6
% of $[Zn]_T$	61.3	82.4	41.8	71.3
$[ZnL_1]$ (nM)	7.7	3.5	16.0	7.9
% of $[Zn]_T$	38.7	17.6	58.2	28.7
$[ZnL_2]$ (nM)	11.0	11.8	6.0	8.4
% of $[Zn]_T$	55.3	59.3	21.8	30.6
$[Zn]_{inorg}$ (nM)	0.6	2.3	2.8	5.6
% of $[Zn]_T$	3.1	11.6	10.2	20.4
$[Zn^{2+}]$ (nM)	0.6	2.3	2.8	5.6
% of $[Zn]_T$	3.1	11.6	10.0	20.4

a small addition of Cu; with higher additions of Cu, as in the higher range of the titrations in Fig. 4, the competition of Cu for L_2 probably also becomes more important. These results clearly indicate that competition between Cu and Zn for strong organic ligands takes place. However, it is difficult to interpret the differences from one sample to another and especially to understand in which cases the maximum labile Zn approaches total dissolved Zn. These differences may be related to variations of ligand properties and possibly to the occurrence of colloidally bound Zn.

Plots of $[ZnL_1]$ $[Cu^{2+}]/[Zn^{2+}]$ as a function of $\Delta[Zn]_{lab}$ from titrations of lake-water samples with Cu (Fig. 5) give, in most cases, linear curves according to Eq. 12. Values of $[CuL_1]_0$ obtained from these plots are usually lower than total dissolved Cu and thus lower than the CuL_1 concentrations obtained from Table 1. Figure 5 shows examples in which the first few points of the titrations with Cu were taken into account; the simplified exchange model may no longer be valid when the exchangeable ligands are nearly saturated by Cu in the later parts of the titrations. In addition, for some samples, variations of the function could not be systematically obtained because of small differences between total dissolved and initially labile Zn concentrations which resulted in low precision. Also, many simplifying assumptions are involved in deriving Eq. 12. Exchange constants obtained from these plots are log $K_{ex} = 5.4 \pm 0.7$ for samples from the euphotic zone.

As mentioned above, it is difficult to distinguish between different ligands in these waters. The competition between Cu and Zn can therefore also be calculated from the speciation in the original water samples under the assumption of the same ligands for both metals (Table 4). The $[Zn^{2+}]:[Cu^{2+}]$ ratios were quite constant around 10^6, especially for the euphotic zone, with an average of $(1.2 \pm 0.5) \times 10^6$. The exchange constants, K_{ex}, were calculated from the free ion and strong organic complex concentrations of Cu and Zn (Eq. 13) and are in the range of 10^6; the average K_{ex} for the euphotic zone is $(1.4 \pm 0.9) \times 10^6$. The conditional stability constants of complexes of Zn with organic ligands estimated from Eq. 8 range from 10^8 to 10^9, with an average log$K_{1Zn} = 9.0$ for the euphotic zone.

K_{ex} was also evaluated by Eq. 13 from the beginning part of the titrations of lake water with Cu. In this part, Cu probably is mostly bound to L_1, so that the assumption

Table 4. Cu-Zn exchange parameters in water samples from Lake Greifen. K_{ex}—exchange constant for the reaction: $Cu^{2+} + ZnL_1 = Zn^{2+} + CuL_1$, calculated from Eq. 13; K_{1Zn}—conditional stability constant of the complex ZnL_1; $[Zn]_{lab\ max}$—maximum labile Zn concentration measured after Cu addition; pH 8.

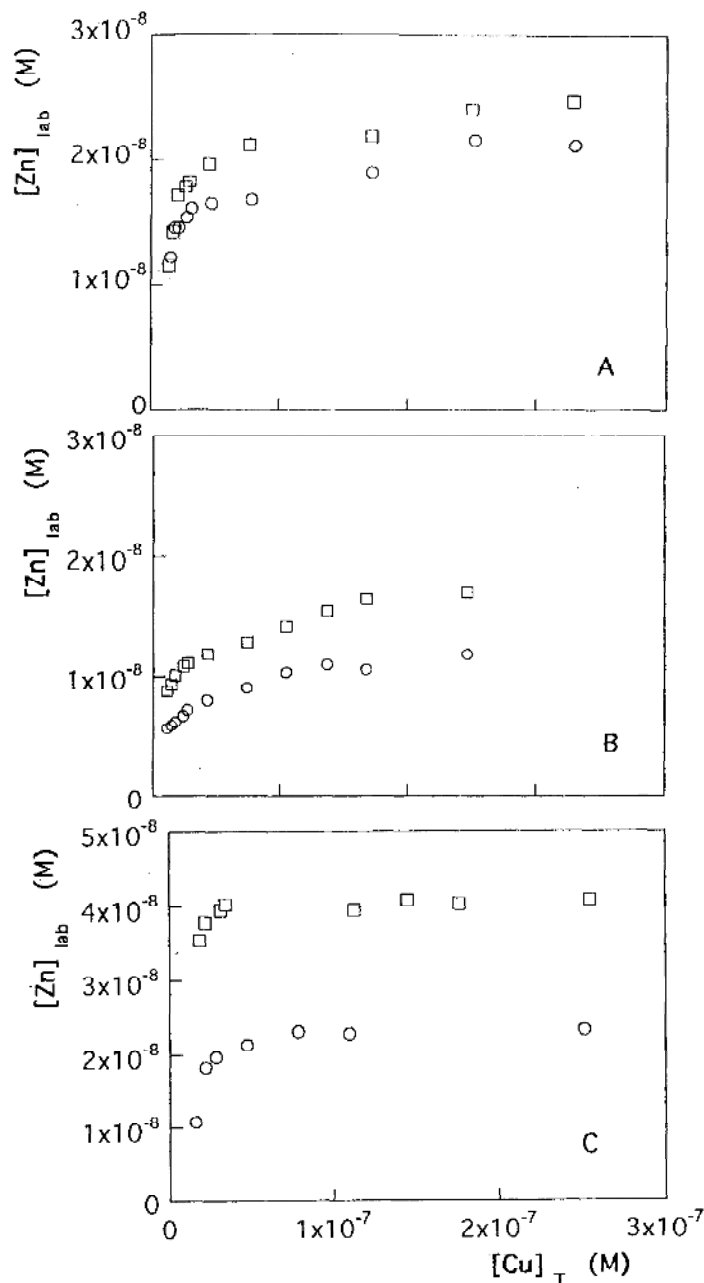| 1993	Depth (m)	$[Zn^{2+}]:[Cu^{2+}]$ ($\times 10^6$ mol mol^{-1})	K_{ex} ($\times 10^6$)	K_{1Zn} ($\times 10^8$ M^{-1})	logK_{1Zn}	$[Zn]_{lab\ max}$ (nM)
26 Jan	5	1.3	2.6	6.1	8.8	20.9
	30	0.9	0.8	0.8	7.9	24.5
5 Apr	5	0.8	0.5	37.0	9.6	11.8
	30	1.1	0.6	13.0	9.1	17.1
26 Jul	3	1.9	1.5	10.6	9.0	23.0
	6	1.0	4.3	2.9	8.5	40.0
	8	5.2	13.4	0.9	8.0	40.0
	30	17.8	4.7	8.5	9.0	12.0
13 Oct	2.5	0.8	0.8	5.8	8.8	18.4
	11	4.2	4.3	0.7	7.8	36.8
Year avg in the euphotic zone						
		1.2	1.4	9.0	9.0	

Fig. 4. Labile Zn as a function of adding Cu. A. Samples taken on 26 January at 5-m depth (O) and at 30-m depth (□). B. Samples taken on 5 April at 5-m depth (O) and at 30-m depth (□). C. Samples taken on 16 July at 3-m depth (O) and at 6-m depth (□).

Fig. 5. Linear relationship of $[ZnL_1] [Cu^{2+}]/[Zn^{2+}]$ vs. $\Delta[Zn]_{lab}$ (Eq. 12) in titrations of Lake Greifen water with Cu. Samples taken at 5-m depth on 26 January (O) and on 5 April (●) 1993.

$[Cu]_T \approx [CuL_1]$ is justified. The weak organic complexing coefficients for Zn remain constant at the beginning of the titration, so free Zn ion concentrations are estimated from these coefficients and from the measured labile Zn. The average K_{ex} values from the titrations were similar to those directly calculated from original samples, resulting in average $\log K_{1Zn} = 8.8$.

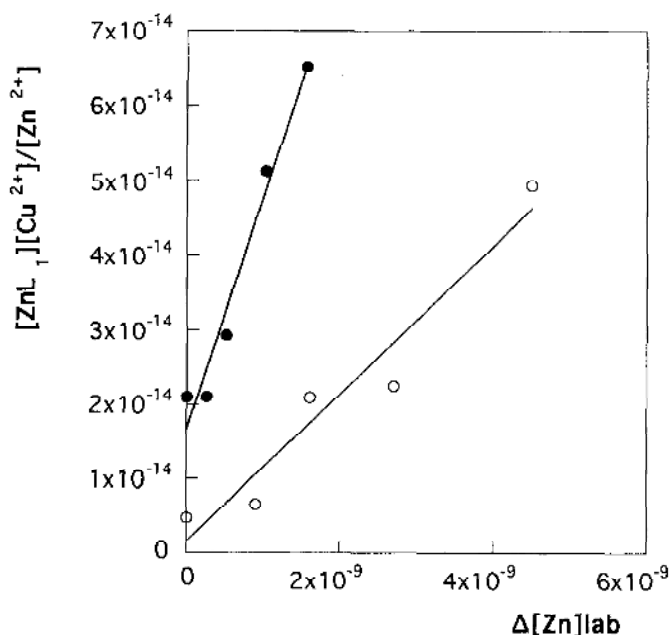All conditional constants obtained are at pH 8. The errors in the exchange constants were estimated to $< \pm 0.4$

for $\log K_{ex}$ and $\log K_{1Zn}$ from the precision of the pCu, $[Zn^{2+}]$, and labile Zn determinations.

Figure 6 illustrates model calculations of titrations of lake water with Cu from the experimentally determined complexation parameters. K_{1Zn} is as indicated in Table 4; L_1 is taken from the Cu titrations in the corresponding samples. It is assumed that competition between Cu and Zn takes place in a similar manner for L_2 as for L_1. The stability constant for the weaker Zn complexes was evaluated from the complexing coefficients $K_{2Zn}L_2$ and the concentrations of L_2 from the Cu titrations; this gives $\log K_{2Zn} \approx 7$ and thus a similar selectivity of L_2 for Cu over Zn as was found for L_1. Figure 6 shows that the general shape of the increase in labile Zn can be modeled in this way. Calculation of the different species during a titration with Cu shows the increase in CuL_1 and the decrease in ZnL_1 at the beginning of the titration. ZnL_2 decreases at higher Cu concentrations because L_2 becomes saturated with Cu.

Discussion

All these findings, especially the release of Zn from electrochemically inert complexes upon addition of Cu, indicate that competition for strong organic ligands takes place between Cu and Zn. A fraction of the dissolved Zn is originally bound to ligands, which actually have a high selectivity for Cu. The difference in speciation between Cu and Zn, as well as the ratios of their aquo ion concentrations, are in line with the generally much higher tendency of Cu for complexation with organic ligands (e.g. as derived from the Irving-Williams series, Stumm and Morgan 1981). The conditional constants obtained here

for the exchange of Cu with Zn, as well as those for the complexation of Cu and Zn by organic ligands, depend on the models used for their calculation and are average values over probably a number of different ligands. The concentrations of strong ligands, calculated from the titrations with Cu, are also dependent on the model used; they indicate, however, an excess of strong ligands over the original Cu concentration, but in most cases not over the sum of Cu and Zn. These concentrations are consistent with the observed speciation of Zn, which includes a fraction of Zn bound in strong complexes and another fraction in weak complexes and as free ions. Small colloidal particles might play a role in the binding of both Zn and Cu in these samples; nonlabile strong ligands may be in colloidal form. Exchange of Cu with Zn, as well as exchange of Cu with catechol, are possible, however, indicating that Zn and Cu can be released from these compounds.

The high conditional stability constants for Cu and high selectivity for Cu over Zn give some information about the ligand types that might be involved. Natural ligands may be either specific ligands released by organisms (e.g. during growth or upon decay of algal material) or degradation products of biological material. In all cases, these natural ligands may include some chelating structures (e.g. amino and sulfidic functional groups). By considering which known ligands achieve a similar selectivity for Cu, we may learn about plausible structures. Several organic chelating ligands like EDTA have stability constants for Cu in the range of $\log K = 12–18$. An example is ethylene-diiminodibutanedioic acid (EDDS), which has a stability constant with Cu $\log K = 18.4$ and with Zn $\log K = 13.5$; the conditional stability constant for Cu in the presence of Ca is calculated as $\log K = 15.8$. High selectivity for Cu over Zn is mostly found in chelating ligands with several amino groups. Ethylenediamine is an example of a simple ligand with a very high selectivity for Cu over Zn [$\log K$ (Cu en) $= 10.48$; $\log K$ (Zn en) $= 5.66$]. Other diamine ligands and those including three and more amino groups have similar selectivities and high stability constants for Cu [e.g. ethylenedinitrilo-tetrakis(2-ethyl-amine) has $\log K$ 22.1 for Cu and 16.1 for Zn]. Some peptides, including histidine, are also highly selective for Cu (e.g. Gly-L-His-L-Lys, $\log K_{Cu} = 16.4$, $\log K_{Zn} = 8.2$) (all stability constants are from Martell and Smith 1974–1989). A structure including several amino groups, which can chelate Cu, may occur in the natural ligands (e.g. in peptides). Very selective structures are also found in phytochelatins, the internal detoxifying ligands of algae, which may be released upon decay of algal material (Gekeler et al. 1988; Ahner et al. 1994).

It seems likely that the ligands in Lake Greifen are of biological origin. Seasonal variations and a relationship between Cu complexation and algal activity have been observed (Xue and Sigg 1993). The results presented here do not show the seasonal dependence as clearly because most samplings were performed during periods with high algal activity. The presence of high concentrations of selective ligands in the lake may be linked to the high algal productivity (~ 500 g C m^{-2} yr^{-1}, Bührer and Wasmer
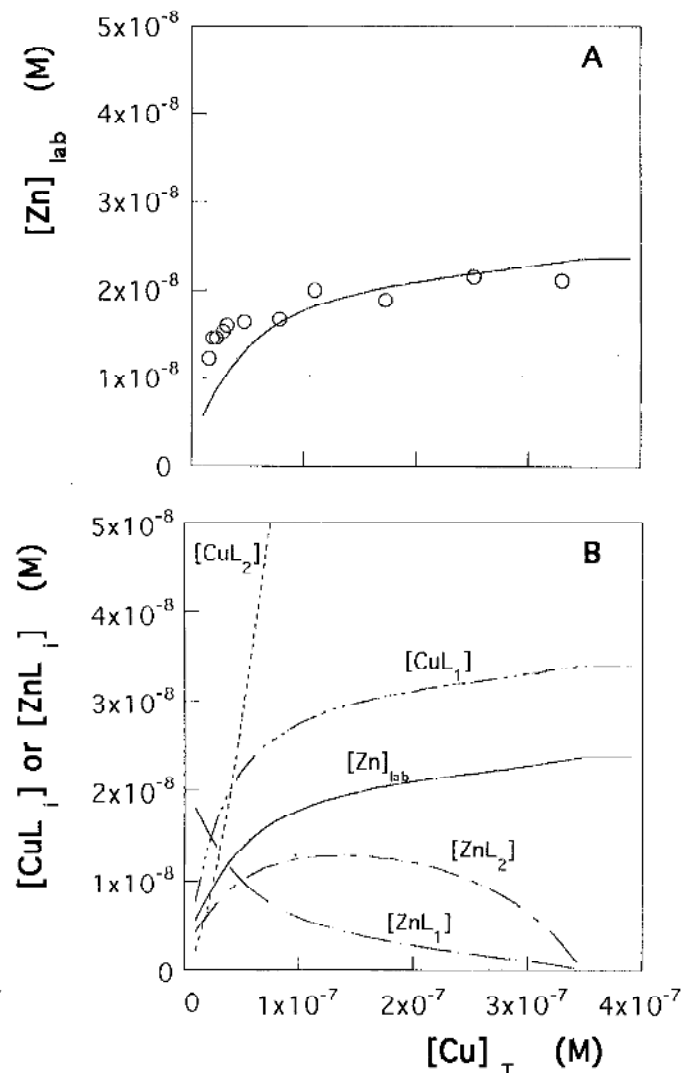

Fig. 6. Labile Zn concentration as a function of total dissolved Cu concentration (A) and calculated concentrations of Cu and Zn complexes with increasing Cu (B). In panel A, the points were measured for the sample taken at 5-m depth on 26 January 1993 and the curve was calculated with the same two ligands for Cu and Zn, using the average values in the euphotic zone for the complexation parameters: $[Cu]_T = 13.8$ nM, $[Zn]_T = 23.7$ nM, $CL_1 = 34$ nM, $CL_2 = 314$ nM, $\log K_{1Cu} = 15.1$ and $\log K_{2Cu} = 13.0$, $\log K_{1Zn} = 9.2$ and $\log K_{2Zn} = 7$. In panel B, all curves were calculated as in panel A.

1992). This hypothesis is also supported by comparing Lake Greifen with less eutrophic lakes; our unpublished results on oligotrophic lakes show a lower extent of Cu complexation. In a similar way, only weaker complexation of Cu was observed in rivers downstream of sewage inputs (unpubl. results).

Because Zn is complexed only to a small extent and the selectivity of the ligand for Cu over Zn (e.g. K_{ex}) is very high, these ligands contribute to the reduction of free cupric ion concentrations and may contribute to sustaining optimal pCu, pZn, and $[Zn^{2+}]:[Cu^{2+}]$ ratios. Toxicity

of [Cu^{2+}] to algae has been observed at low levels (10^{-12}–10^{-10} M); higher concentrations of Cu may induce the release of strong ligands and thus an increase in complexing capacity, as has been observed for green algae in defined ionic media and in lake water (Xue and Sigg 1990; Knauer et al. in prep.). Higher levels of [Zn^{2+}] are tolerated by algae, so that high [Zn^{2+}]:[Cu^{2+}] ratios may be favorable. In comparison with the toxic and limiting levels observed for marine algae (Sunda and Huntsman 1992; Brand et al. 1986; Sunda and Guillard 1976) and for some freshwater algae in culture media (Knauer et al. in prep.), both pCu and pZn in Lake Greifen would be lower than toxic levels.

More work is needed to characterize the strong and selective complexing ligands in lake water in order to understand the sources and nature of the strong ligands and their significance in sustaining low levels of [Cu^{2+}], optimal [Zn^{2+}], and [Zn^{2+}]:[Cu^{2+}] ratios in eutrophic lakes.

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