

Free cupric ion concentrations and Cu complexation in selected Swiss lakes and rivers

Hanbin Xue¹, Andrea Oestreich², David Kistler² and Laura Sigg²

Research Centre for Limnology, Swiss Federal Institute for Environmental Science and Technology (EAWAG), CH-6047 Kastanienbaum¹ and CH-8600 Dübendorf², Switzerland

Key words: [Cu²⁺], Cu complexation, Cu speciation, Cu ligands, freshwater.

ABSTRACT

[Cu²⁺] and Cu complexation parameters in some selected freshwater systems in Switzerland were determined by the technique of ligand-exchange and DPCSV. Results from the water columns of some eutrophic and oligotrophic lakes are presented and compared to small acid lakes. Cu is strongly complexed by organic ligands which with very high stability constants at low concentrations are probably biologically produced, as indicated by the seasonal variations in the eutrophic lakes and by the relationship between Cu complexation and algal activity in the eutrophic (pCu=15–16), oligotrophic (pCu=13–14) and acidic (pCu=9–10) lakes. The extent of Cu complexation in river waters was generally lower than in the eutrophic lakes, at similar DOC levels. No obvious correlation between Cu complexation and DOC was observed, indicating that Cu complexing ligands are specific organic compounds.

Introduction

Trace metal ions are complexed in natural waters by a variety of inorganic and organic ligands and by particle surface groups; Cu tends to form stronger organic complexes than most other divalent metals (Whitfield and Turner, 1987; Bruland et al., 1991; Sigg and Xue, 1994). The free aqua metal ion concentration is a key parameter for the metal reactivities and biological effects; Cu toxicity to phytoplankton at elevated levels is related to free cupric ion concentration, not to total copper concentration (Sunda and Guillard, 1976; Brand et al., 1986). Phytoplankton may, in turn, affect trace metal chemistry via reactions of the metal ions with the cell surface or cell products (Bruland et al., 1991; Sunda, 1988/89; Xue and Sigg, 1993). It is, thus, essential to determine metal speciation and especially to evaluate the free aqua metal ion concentrations in natural waters.

Whereas Cu complexation in sea water has been extensively studied (e.g. Sunda and Hanson, 1987; Sunda and Huntsman, 1991; Moffett et al., 1990; Coale and Bruland, 1988, 1990; Donat et al., 1994; Miller and Bruland, 1994), few studies in freshwater systems are available, which involve the experimental determination of

speciation (Sunda and Hanson, 1979; Xue and Sigg, 1993; Xue et al., 1995). Some other studies in freshwater have relied on calculated speciation based on thermodynamic models (Balistrieri et al., 1992, 1994).

Recently, techniques have been developed which combine ligand-exchange and sensitive voltammetric analysis for determining free cupric ion concentrations (Hirose et al., 1982; Van den Berg, 1984; Donat and Van den Berg, 1992; Campos and Van den Berg, 1994; Scarano et al., 1992). A specific ligand-exchange technique, using catechol as a competing ligand, enables us to determine free cupric concentrations and to work at ambient total copper concentrations in freshwaters with high concentrations of strong ligands. The working conditions and the performance of the technique in freshwater samples have been carefully evaluated (Xue and Sigg, 1993).

Our recent study on Cu speciation in a eutrophic lake (Xue and Sigg, 1993) showed that in the water column Cu is strongly complexed by organic ligands, giving $\log [Cu^{2+}] = -16 - -14$; these ligands are probably biologically produced and may be released during algae blooms, as indicated by seasonal variations and correlation of Cu complexation and algal activity. We attempt in this paper to extend the observations made in Lake Greifen to other aquatic systems, especially to evaluate the relative importance and the origin of specific copper binding ligands. Various types of ligands could be significant for the complexation of copper in aquatic systems. Humic and fulvic acids, which are ubiquitous as degradation products of organic material, are known to bind strongly Cu(II) (e.g. Tipping and Hurley, 1992). Lower molecular weight organic ligands (e.g. amino acids) may also play a role. Furthermore, the significance of synthetic ligands, such as NTA (nitrilotriacetate) and EDTA (ethylenediaminetetraacetate) has also to be considered in rivers and lakes influenced by sewage inputs. Assuming that in addition to ligands originating from various sources, specific ligands are released by algae, we expect to find relationships between algal productivity and copper complexation. The following questions are therefore examined.

Can the observations in Lake Greifen be extended to other eutrophic lakes? Are there any differences in $[Cu^{2+}]$ and Cu complexation between eutrophic and oligotrophic lakes? Could any differences in $[Cu^{2+}]$ and complexation between acid and neutral lakes be interpreted by pH-dependence or/and by organism activities? Do industrial inputs and effluents of sewage treatment plants supply specific strong organic ligands for Cu complexation to river waters, in addition to decomposition processes of organic matter in the rivers, or are upstream lakes a major source of strong Cu ligands in rivers?

In this work, we selected some freshwater systems, mainly in Switzerland, including two eutrophic lakes (Lake Greifen and Sempach), one oligotrophic lake (Lake Lucerne), several alpine acid lakes and an industrially polluted and limed lake (Lago Orta), as well as a number of river sampling sites to compare the Cu complexation under different conditions. We determine free cupric ion concentrations by the technique of catechol ligand-exchange and DPCSV (differential pulse cathodic stripping voltammetry). The results on $[Cu^{2+}]$ are presented in function of depth and season in the water columns of the eutrophic and oligotrophic lakes, whereas less detailed informations are available for the other systems. Cu complexation is evaluated in terms of conditional

Free cupric ion concentrations

stability constants and concentrations of Cu-complexing ligands. All results in these various systems are compared, and the questions mentioned above are discussed.

Experimental

Sampling

The freshwater samples were taken from 7 rivers with 13 stations in Switzerland, from 7 Swiss lakes and from lake Orta in northern Italy. The sampling sites are located as in Figure 1. General description and morphometric parameters of the lakes are listed in Table 1 and of the rivers in Table 2.

Lake Greifen and Sempach are eutrophic. Samples were collected from the water column of Lake Greifen at the deepest point in different seasons of 1990–1991 and 1993–1994. The lake is highly eutrophic with a seasonally anoxic hypolimnion. The tributaries of the lake are highly loaded with nutrients and pollutants from sewage and agriculture. Lake Sempach is a pre-alpine lake situated in central Switzerland. The samples were collected from the water column at the deepest station, seasonally in 1994. Excessive nutrient inputs originating from farmland and waste water led to eutrophication of Lake Sempach for many years; O₂ is depleted in the

Table 1. Morphometric parameters and general description of the selected lakes (alk = alkalinity, range at different times of the year)

Lake	Surface (km ²)	Volume (m ³)	Depth max. (m)	Depth mean (m)	pH	alk mM	Remarks
Greifen	8.5	150 × 10 ⁶	32	17.7	7.3–8.5	3–4	eutrophic, seasonally anoxic
Sempach	14.4	662 × 10 ⁶	87	46	7.7–9.0	1.5–2.5	eutrophic, artificially oxygenated
Lucerne	114	11.8 × 10 ⁹	214	104	7.5–8.5	2	oligotrophic
Orta, Italy	18	1.3 × 10 ⁹	143	71	6–9	0.05–0.1	recovering from acidification and industrial pollution

Alpine lakes in Maggia Valley (Southern Swiss Alps)

	altitude (m)	Surface (km ²)	pH	alk μM	
Val Sabbia	2396	0.01	6.9	144	oligotrophic
Laghetto Inferiore	2070	0.05	6.0	15	oligotrophic
Zotta	2229	0.01	5.7	8	oligotrophic
Cristallina	2400	0.0075	5.9	8	oligotrophic



Figure 1. Location of the sampling sites

Table 2. Description of river sampling sites. The water discharge (measurements from stations of the Swiss National Hydrological and Geological Survey), pH and alkalinity (alk) are the values or ranges measured at the sampling dates

River	Site	Water discharge (m ³ /s)	pH	alk mM	Lake upstream
Aare	Eichholz, Berne	185	8.4	2.1	L. Thun, 30 km
Aare	Halenbrücke, Berne	185	8.4	2.1	L. Thun, 40 km
Aare	Brugg	306	8.2	2.7	L. Hallwil, 20 km
Birs	Münchenstein	6–7	8.4	4.3	none
Chriesbach	Dübendorf	≈ 1	8.3		none
Emme	Burgdorf	11	8.4		none
Glatt	Niederglatt	4–7	8.2	3.6	L. Greifen, 20 km
Glatt	Rheinsfelden	4–7	8.4	3.6	L. Greifen, 30 km
Rhine	Rekingen	370–780	8.4	2.5	L. Constance, 40 km
Rhine	Village-Neuf (below Basel)	900–1820	8.3	2.6	several, > 50 km
Thur	Andelfingen	45–60	8.3	3.6	none

hypolimnion during summer stagnation. The lake is treated by oxygenation of the hypolimnion. Since 1984, pure oxygen is introduced to its hypolimnion at a rate of 3 t/day during summer, and compressed air at 370 m³/h into the lake to intensify mixing during overturn. Due to this treatment, the oxygen concentration in the water column now always exceeds 4 mg/l (Stadelman, 1988; Gächter and Meyer, 1990).

Lake Lucerne is a large oligotrophic lake located in central Switzerland. It has been showing a distinct shift from mesotrophy to oligotrophy since the late seventies due to decrease of the P inputs (Ambühl, 1987; Bloesch and Ühlinger, 1990; Bossard unpubl. data, 1994). The primary productivity decreased from 250 gC m⁻² yr⁻¹ (1978–1980) to 150 gC m⁻² yr⁻¹ (1992) (P. Bossard, personal communication 1994). The samples were collected from the water column at the station Kreuztrichter (112 m deep) at various times of the year in 1991 and 1994.

Surface water samples were taken from acidic alpine lakes, Sabbia, Laghetto Inferiore, Zotta and Cristallina in the southern Alps of the Maggia valley, Tessin, Switzerland, during August 1992. These lakes are all situated within about 10 km distance; differences in pH and alkalinity are related to different geological background in the catchment areas (only crystalline rocks for Zotta and Cristallina, some dolomites and schists for Sabbia and Laghetto Inferiore). These alpine lakes have typically low alkalinity and low nutrient concentrations.

Lake Orta in Northern Italy was selected because of its history of having been over the years acidified and industrially polluted, and later restored by liming. The lake was heavily polluted in 1926 by ammonium sulphate and copper, later by other metals, leading to acidification and a consequent disappearance of all important organisms. It was limed with 10900 tons of CaCO₃ from May 1989 to June 1990, which led to pH increasing from 4.4 to 7–9 in the upper 25 m, total alkalinity to 50 µeq/L, decreasing Cu concentration from 35 µg/l to less than 10 µg/l. Various biological communities, e.g. diatoms, which had disappeared from the lake increased

again after liming from 1989 to 1993 (Camusso et al., 1991; Mosello et al., 1991; Calderoni et al., 1992). Two surface water samples were collected in the central basin and southern basin of the lake on 19 June 1994.

The river water samples were collected from 13 stations of 7 rivers, including river Rhine and its tributaries (Table 2 and Fig. 1). Some of the larger Swiss rivers were sampled, namely Aare and Rhine, as well as smaller rivers, such as Birs, Glatt, Emme, Thur and Chriesbach. All these rivers run through catchments dominated by carbonate rocks; alkalinities range about from 2–4 mM and typical pH from 7.5–8.5. Lakes and sewage treatment plants may be sources of strong ligands for copper, as well as biological activity and decomposition reactions in the river itself. In the cases of Birs, Emme, Thur and Chriesbach, there is no lake in the catchment area, whereas in the other cases lakes are located more or less far away upstream from the sampling sites. The sampling sites on the Birs in Münchenstein, Chriesbach in Dübendorf, Glatt at all sites, are strongly influenced by sewage effluents. In the Glatt river, samples were taken immediately up- and downstream of a sewage treatment plant. The site Aare in Halenbrücke is also immediately downstream of a sewage effluent, but with a much larger dilution factor by the river. An extensive data base of general chemical parameters exists for Aare, Birs, Glatt, Rhine and Thur (Jakob et al., 1994).

Go-Flo sampling bottles (General Oceanics, 5L) were used to collect samples from different depths in the water columns of the lakes. Surface water samples from the alpine lakes and from the small rivers were grabbed by a polyethylene spoon and transferred to clean polyethylene bottles. Samples from the larger rivers were pumped (from about 30 cm depth, 2–3 meters away from river bank) using a peristaltic pump. After transport to the laboratory, the samples were filtered under a clean bench within a few hours. DPCSV measurements for free $[\text{Cu}^{2+}]$ were carried out within a week after the collection of samples. The filtered samples were stored in the dark at 4 °C until use. Cu adsorption on the container walls and speciation change during 5 days storage of filtered samples were tested, and no obvious errors were observed, probably because of strong competition for adsorption by the natural ligands (Xue and Sigg, 1993).

All sampling, the filtration devices, bottles and filtering membranes (0.45 μm) were washed with 0.01 M HNO_3 and rinsed with bi-distilled water before use.

Analytical methods

Total dissolved Cu was directly measured in acidified filtrates by graphite furnace atomic absorption spectrometry (AAS). DOC was measured by high-temperature combustion on a Shimadzu TOC-500. pH was measured in the field with a combined electrode sensor (Lake Greifen, Sempach and Lucerne). In the acidic alpine lakes and in the rivers, pH was measured in the field, using a portable pH meter and a Ross electrode (Orion), which was carefully calibrated at the appropriate temperature; pH measurements in low ionic strength waters were carried out in samples adjusted with KCl to an ionic strength of 0.01 M. Alkalinity was determined by automated titration (Metrohm) to pH 4.5 in the samples from the rivers and Lakes Greifen, Sempach and Lucerne, and by Gran plot titrations in the samples from the

acidic lakes. Chlorophyll-a was determined by HPLC in the limnology department of EAWAG (Meyns et al., 1994).

DPCSV determination of copper-catechol complexes and titration with catechol or with Cu

Details of free cupric ion concentration determination have been described elsewhere (Van den Berg, 1984; Xue and Sigg, 1993). Briefly, the method is based on ligand exchange of added catechol with natural ligands, which are bound to Cu; the Cu catechol complexes formed are determined specifically by cathodic stripping voltammetry (DPCSV). $[Cu^{2+}]$ and the complexation parameters are determined from equilibrium calculations with the added catechol. All the stability constants were taken from Martell and Smith (1974) and corrected to an ionic strength of 0.01 M. The technique has been validated in some model solutions with EDTA and salicylate, and in an algal nutrient medium with known composition. Interferences by competition between Cu and other metals for natural ligands and catechol did not show obvious biases, because $[Cu^{2+}]$ was independent of the added catechol concentrations in the working range ($pCu \pm 0.1$ to ± 0.2) (Xue and Sigg, 1993). Interferences from humic acids were checked and found to be negligible under typical conditions.

All subsamples for Cu complexation measurements were buffered by a mixed solution of HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) and NaOH to keep pH constant. For neutral lake and river waters, a pH buffer stock (1 M HEPES and 0.5 M NaOH) was added to give a final pH of $(7.8-8.0) \pm 0.1$ with 6 mM HEPES. KNO_3 was used to adjust the ionic strength of the acidic lakes, and pH buffer stock solutions were prepared with different ratios of HEPES and NaOH, to examine the pH dependence of $[Cu^{2+}]$ and of copper complexation.

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 E506 polarecord or in a Metrohm 647 VA stand combined with a 646 VA processor. Catechol was added to the samples after 5 min purging with suprapure N_2 . Equilibration was allowed for 5 min under N_2 . The copper catechol complexes were collected on the electrode for 3 or 2 min. After the collection period and 15 s rest the voltage scan was initiated in negative direction. Scanning parameters were initial potential of 0 or 50 mV; pulse height of 50 mV; scan rate of 5 or 10 $mV s^{-1}$, depending on samples.

In order to obtain optimal catechol concentration ranges for the samples from different sources, titrations with catechol were carried out continuously in original samples (25 mL in the polarographic cell) by adding catechol increments. Few values within the optimal ranges, $5 \times 10^{-4} - 2 \times 10^{-3}$ M catechol in the samples from the neutral lakes and rivers, and $2 \times 10^{-5} - 2 \times 10^{-4}$ M catechol in the waters from acidic lakes, were used to calculate average $[Cu^{2+}]$.

The DPCSV sensitivity had to be calibrated for each individual lakewater sample by Cu titration. To obtain a Cu titration curve of a water sample, a series of subsamples were spiked with different Cu concentrations. 25 ml aliquots were pipetted into a series of 50 ml high density polyethylene beakers; 150 μ l of HEPES

buffer stock solution was added to each beaker. Copper was added to all beakers but one. The series was allowed to equilibrate at $20 \pm 2^\circ\text{C}$ overnight.

The direct results of these Cu titrations are values of $[\text{Cu}^{2+}]$ and $\sum K_i[\text{L}_i]$, a complexing coefficient which corresponds to the product of the stability constants and the ligand concentrations and approximately to the ratio of total dissolved copper to free copper ions $[\text{Cu}]_T/[\text{Cu}^{2+}]$, neglecting the inorganic species (Xue and Sigg, 1993). Because natural waters contain a wide range of different ligands with different stability constants, a simple discrete model with one or two ligands only represents an average, depending on the working window and on the model used (Van den Berg et al., 1990). For the sake of comparison, a two-ligand model was generally used to estimate conditional stability constants K_i and total ligand concentrations $T[\text{L}_i]$, using the FITEQL program (Westall, 1982). The values of K_i and $T[\text{L}_i]$ obtained in this manner are not independent of each other. For some samples, if a Scatchard plot was linear, a conditional stability constant and a ligand concentration were extracted from the plot with a one-ligand model.

Results and discussion

In a first part, we consider the effect of algal productivity on the complexation of copper by comparing two eutrophic lakes, Lake Sempach and Lake Greifen, with the oligotrophic Lake Lucerne. Seasonal variations within the lakes, as well as differences between epilimnion and hypolimnion, also give indications on the role of algae. Data on $[\text{Cu}^{2+}]$ and Cu complexation parameters from the eutrophic Lake Sempach, at different times and depths are presented in Table 3. $[\text{Cu}^{2+}]$ and Cu complexing parameters for the eutrophic Lake Greifen, have been presented elsewhere (Xue and Sigg, 1993, 1995); the results are summarized in comparison with the other lakes in Table 5. In the water column of lake Sempach, the free cupric ion concentrations are very low, with the range $\text{pCu} = 13.6\text{--}15.8$, and the ratios of dissolved copper to free ions ($[\text{Cu}]_T/[\text{Cu}^{2+}]$) are very high, $[\text{Cu}]_T/[\text{Cu}^{2+}] = 5.7 \times 10^5\text{--}3.8 \times 10^7$. Seasonal variations and a relationship between Cu complexation and algal activities were observed in Lake Sempach. The lowest $[\text{Cu}^{2+}]$ and the highest ratios $[\text{Cu}]_T/[\text{Cu}^{2+}]$ were found in the euphotic zone during summer. The ratio $[\text{Cu}]_T/[\text{Cu}^{2+}]$ and the conditional stability constants (K_i) of the strong organic ligands in summer time (July, October) were almost one order of magnitude higher than those in winter (February), for the samples from the euphotic zone. Cu complexation in the euphotic zone is much stronger than in the deeper layers during summer; in the samples from July the ratio $[\text{Cu}]_T/[\text{Cu}^{2+}]$ is much higher at 2.5 and 10 m depth than at 80 m depth. Titrations of lake water samples with Cu make the differences in complexation clearer (Fig. 2A); the titration curve with Cu for 80 m depth is shifted to the left side of that for 2.5 m depth, indicating weaker complexation in the 80 m sample. The differences in the complexation between epilimnion and hypolimnion samples are in this case obvious. In Lake Sempach with the maximal depth 87 m, epilimnion and hypolimnion are clearly separated. Due to the effect of artificial oxygenation, Cu-complexing ligands produced biologically in the productive layer may easily be degraded under the oxic conditions in the deeper water layers. The results observed in Lake Sempach are very similar to those in Lake Greifen

Free cupric ion concentrations

Table 3. $[\text{Cu}^{2+}]$ and complexation parameters from the water column of lake Sempach (pH 7.9)

Sampling date 1994	depth m	$[\text{Cu}]_T$ nM	pCu	$[\text{Cu}]_T/[\text{Cu}^{2+}]$	$\log K_1$	$\log K_2$	T[L ₁] nM	T[L ₂] nM	DOC mg/L
10 Oct.	2.5	13.5	15.1	1.7×10^7	15.5	12.8	19	182	4.2
10 Oct.	10	16.6	14.5	4.8×10^6	14.7	12.5	25	159	4.5
10 Oct.	80	14.0	14.7	7.4×10^6	14.8	12.3	23	179	3.5
18 Jul.	2.5	6.5	15.8	3.8×10^7	15.4	13.2	21	202	4.0
18 Jul.	10	6.9	15.1	9.1×10^6	14.7	13.1	23	232	3.6
18 Jul.	80	6.0	14.2	7.6×10^5		13.1		150	3.3
24 May	2.5	8.5	14.7	4.0×10^6					5.7
24 May	10	8.0	13.6	5.7×10^5					4.9
24 May	80	7.1	14.8	4.7×10^6					4.2
28 Feb.	2.5	3.5	15.0	3.7×10^6	14.3	12.4	40	201	2.9
28 Feb.	10	4.1	14.3	7.5×10^5	13.1	11.8	100	231	3.5
28 Feb.	80	4.4	14.1	5.7×10^5	14.6	12.4	9.7	213	3.4

(Table 5). In Lake Greifen seasonal dynamics of $[\text{Cu}^{2+}]$ and $[\text{Cu}]_T/[\text{Cu}^{2+}]$ exhibit the same pattern as the variation of chlorophyll over time (Xue and Sigg, 1993), even more obviously than in Lake Sempach. In both cases of these eutrophic lakes the ratios $[\text{Cu}]_T/[\text{Cu}^{2+}]$ are very high, and very low $[\text{Cu}^{2+}]$ are found in the euphotic layers; this means that in both cases copper is strongly bound by organic ligands with high conditional stability constants. The conditional stability constants of the ligands (K_1 and K_2) are also similar.

In comparison to the eutrophic lakes, Cu complexation in a oligotrophic lake, Lake Lucerne, is weaker. The data listed in Table 4 indicate that the total dissolved copper concentrations were comparable to those in the eutrophic lakes, but that $[\text{Cu}^{2+}]$ was generally higher. The ratio $[\text{Cu}]_T/[\text{Cu}^{2+}]$ and the conditional stability constants K_1 and K_2 were about one order of magnitude smaller than the respective values in the eutrophic lakes during summer. Seasonal variations of $[\text{Cu}^{2+}]$ and Cu complexation in this oligotrophic lake have not been observed, indicating that these parameters did not vary much over the year. In addition, differences in Cu complexation between upper and deeper layers were not obvious in the water column of Lake Lucerne, as shown by the overlap of the titration curves with Cu for 5 m and 110 m depth (Fig. 2B).

The similarity between the two eutrophic lakes and the differences in Cu complexation between the oligotrophic lake and eutrophic lakes can be clearly seen from Table 5, in which average Cu speciation parameters from the euphotic zone, as well as the average values of DOC and primary productivity are listed. The values of the ratios $[\text{Cu}]_T/[\text{Cu}^{2+}]$, corresponding to the complexing coefficients, in these three lakes increase in the same order as the primary productivity. The similarity of pCu and of the complexation parameters in the two eutrophic lakes, as well as the link between complexing coefficients and primary productivity, provide additional evidence to support that the very strong Cu-complexing ligands at low concentration are biologically produced and that the very low $[\text{Cu}^{2+}]$ are related to high productivity of phytoplankton in eutrophic lakes. The weaker ligands (L_2) with higher

Table 4. $[\text{Cu}^{2+}]$ and Cu complexation parameters from the water column of lake Lucerne (pH 7.8)

Sampling date	depth m	$[\text{Cu}]_T$ nM	pCu	$[\text{Cu}]_T/[\text{Cu}^{2+}]$	$\log K_1$	$\log K_2$	T[L ₁] nM	T[L ₂] nM
30 Jun. 94	2.0	11.6	13.7	7.2×10^5	13.9	11.9	16	185
21 Oct. 91	5.0	8.0	14.2	1.4×10^6	13.9	11.9	21	120
21 Oct. 91	20	6.1	14.3	1.2×10^6	13.9	11.5	19	168
21 Oct. 91	110	8.5	14.2	1.2×10^6	13.8	11.6	21	145
18 Jul. 91	5.0	11.8	13.4	3.1×10^5	13.2	11.3	34	156
18 Jul. 91	20	9.4	14.4	2.5×10^6	14.2	11.2	23	377
18 Jul. 91	110	5.8	14.3	1.8×10^6	14.3	11.8	16	157
1 Jul. 91	5.0	6.3	14.8	4.0×10^6	14.6	11.3	15	326
1 Jul. 91	20	5.7	14.2	8.3×10^5	13.8	11.3	15	154
1 Jul. 91	110	10.2	14.0	1.0×10^6	13.9	11.4	19	168
11 Feb. 91	5.0	7.9	13.9	6.0×10^5	14.3	11.0	10	737
11 Feb. 91	20	4.4	14.4	1.2×10^6	14.3	11.0	10	737
11 Feb. 91	110	4.4	14.4	9.8×10^5	13.0	11.3	9	149

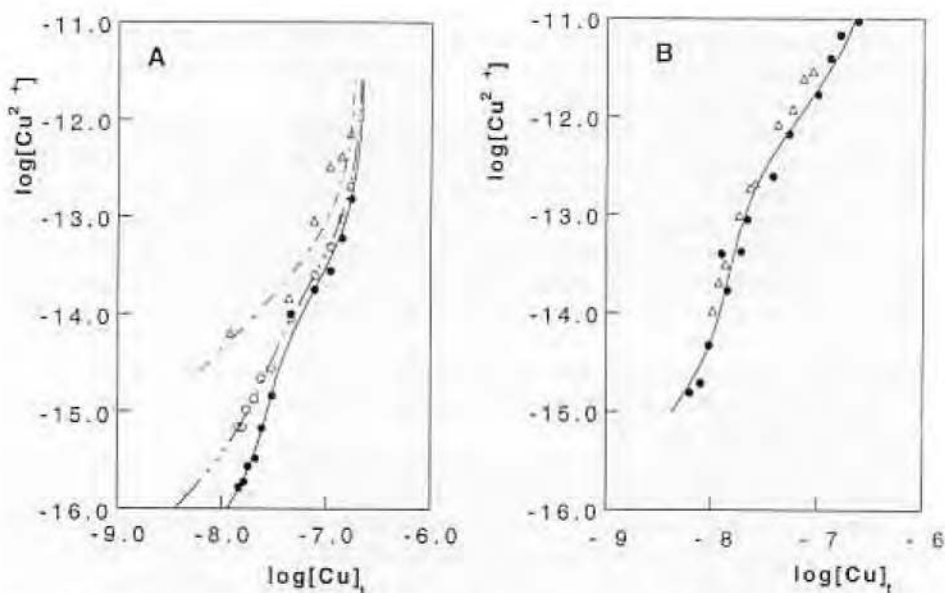


Figure 2. Titration of lake water samples with Cu, plotted as $\log [\text{Cu}^{2+}]$ versus $\log [\text{Cu}]_T$: A, Lake Sempach water samples, collected on 18 July 1994 at 2.5 m (●), 10 m (○) and 80 m (△) depth. B, Lake Lucerne water samples, collected on 1 July 1991 at 5 m (●) and 110 m (△), all curves are calculated with the parameters listed in Table 3 and 4

Table 5. Comparison of Cu complexation in eutrophic and oligotrophic lakes. Data for Cu speciation are average values from the euphotic zone of these lakes; pCu and $[Cu]_T/[Cu^{2+}]$ are calculated from the average of $[Cu^{2+}]$ and average $[Cu]_T$

Lake	$[Cu]_T$ nM	$[Cu^{2+}]$ $\times 10^{-15}M$	pCu	$[Cu]_T/[Cu^{2+}]$	$\log K_1$	$\log K_2$	T[L ₁] nM	T[L ₂] nM	Productivity gCm ⁻² yr ⁻¹	DOC mg/L
Lucerne	9.1	14.9	13.8	6.1×10^5	13.9	11.6	21	197	150	1.0
Greifen	15.8	1.7	14.8	9.2×10^6	14.6	12.6	54	366	500	3.6
Sempach	6.4	1.0	15.0	6.4×10^6	15.1	12.8	27	195	350	4.0

concentrations may correspond to less specific complexing material, like fulvic and humic acids. It is however difficult to decide whether the ligands present in these three lakes actually have the same stability constants, because the stability constants and concentrations of ligands, obtained by fitting the titrations with Cu, are not independent of each other.

The alpine lakes represent a different situation, mainly because of a much lower pH range (pH 5–7); the algal productivity is low in these lakes, both because of low nutrient levels and because of the low pH. The DOC concentrations are also low, providing only small concentrations of ligands. The extent of complexation of copper is expected to decrease with decreasing pH.

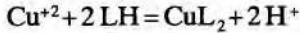
Free cupric ion concentrations in the studied acidic alpine lakes are much higher than those in the neutral lakes at the same level of total dissolved Cu, with ratios of $[Cu]_T/[Cu^{2+}] \approx 10$ –40, pCu was 9–10, $[Cu^{2+}]$ between 1–10% of total dissolved copper concentrations at the original pH (Table 6). The low extent of complexation can be attributed to low pH (5–7), low algal productivity (chlorophyll a < 2 µg/L) and low DOC (< 1 mg/L). The chlorophyll a concentration in lake Zotta with pH < 6 was higher than those in Lake Sabbia and Laghetto Inferiore with pH 6–7, probably due to algae adapted to these acidic conditions. A stronger complexation of Cu(II) in Lake Zotta ($[Cu]_T/[Cu^{2+}] \approx 40$ at pH 5.3) was observed, which may be attributed to ligands originating from these algae. Using again the FITEQL program with a two ligand model in the samples of the alpine lakes (buffered at pH 7.5), the stability constants for the stronger ligands are in the range $\log K_1 = 10$ –12, thus being similar to $\log K_2$ for weaker ligands in the neutral lakes.

Lake Orta represents a somewhat different case, in which the relatively low pH and high total copper concentration are due to industrial pollution inputs. The lake history is mentioned in the experimental part; pH now is nearly neutral (≈ 7), the lakewater is still poorly buffered (ANC ≈ 50 µeq/L). As shown in Table 6, $[Cu^{2+}]$ was $1.5 \times 10^{-10}M$ at the original pH ~ 7 , with very similar results at the two stations; the ratio $[Cu]_T/[Cu^{2+}]$ is 4.7×10^2 . The extent of complexation is therefore somewhat higher than in the alpine lakes, but still much lower than in Lake Lucerne or Sempach. Here again the effects of relatively low pH and of low algal productivity are evident. Comparing to Cu complexation in 1987 (Camusso et al., 1991), the total dissolved Cu has decreased by one order of magnitude ($5 \times 10^{-7}M$ – $7 \times 10^{-8}M$), whereas $[Cu^{2+}]$ decreased by a factor of 3000. This strongly enhanced complexation may originate not only from the increase of pH in the lake during that time, but also from an increase of organic ligands biologically generated.

Table 6. Cu speciation and complexation in selected acid lakes

Lake	date Sampling	pH original	pH buffered	[Cu] _T nM	[Cu ²⁺] nM	pCu	[Cu] _T / [Cu ²⁺]	log K ₁	log K ₂	T[L ₁] nM	T[L ₂] nM	Chloro- phyll a µg/L	DOC mg/L
Sabbia	Aug. 92	6.9	6.0	2.8	1.7×10^{-10}	9.8	16.5					0.4	0.8
	Aug. 92	6.9	7.5	2.8	2.3×10^{-12}	11.7	1.2×10^3	11.0	9.8	21	100	0.4	0.8
L. Inferiore	Aug. 92	6.0	6.0	5.4	5.9×10^{-10}	9.3	8.6					0.2	0.4
		6.0	7.5	5.4	8.0×10^{-13}	12.1	6.3×10^3	11.2	9.5	14	100	0.2	0.4
Zotta	Aug. 92	5.7	5.3	5.5	1.5×10^{-10}	9.9	35.7					1.9	0.3
		5.7	7.5	5.5	1.1×10^{-12}	12.0	5.0×10^3	12.2	9.2	18	93	1.9	0.3
Cristallina	Jul. 90	5.3	7.5	7.6	2.5×10^{-11}	10.6	3.0×10^2	10.2	8.6	35	138		0.5
Orta	Jun. 94	7.0	7.0	70.8	1.5×10^{-10}	9.8	4.7×10^2		9.5		233		
		7.0	7.5	70.8	2.7×10^{-11}	10.6	2.7×10^4		10.2		233		1.1

The pH-dependence of complexation was examined in more detail in the samples from Lake Orta, because it was in this case easy to change and maintain the pH within a desired range by pH buffers. $[\text{Cu}^{2+}]$ of surface waters from central basin and southern basin of lake Orta were determined at different pH (after buffering) (5.8–7.5). From the titration curves of lake water with Cu, we found that the Scatchard plots are linear and that the data could be fitted by a one-ligand model. Fig. 3 shows the plots of pCu and log K vs pH, which give very good linear relationships with slope 2. The value of this slope may reflect the exchange of one metal with 2 protons of ligands and the formation of bidentate complexes:



This plot illustrates the pH dependence of the Cu complexation, with strongly increasing $[\text{Cu}^{2+}]$ at lower pH. An extrapolation can be done from this plot, for example, $\log K \approx 11.3$ and $\text{pCu} \approx 11.7$ would be obtained at pH 8.0, giving a value of log K similar to that of the weaker organic ligands in Lake Lucerne. The presence of stronger ligands could not be excluded, since low concentrations of strong ligands may be saturated with Cu at the high level of total Cu.

From this experimental pH dependence we may infer that in previous years with lower pH in Lake Orta, Cu^{2+} may have been much higher than now. A prediction

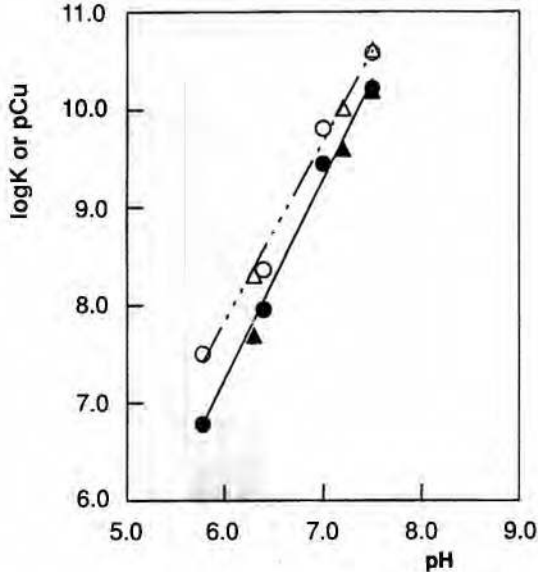


Figure 3. pCu and the conditional stability constant log K of the ligands in function of pH in Lake Orta water (● log K of sample from the central basin, ▲ log K of samples in the South basin, ○ pCu, central basin, △ pCu, South basin; the solid line is for log K with slope 2.05, the dashed line for pCu with slope 1.85)

Table 7. $[\text{Cu}^{2+}]$ and Cu complexation parameters in selected Swiss river waters, 1993

River	Station	date Sampling	$[\text{Cu}]_r$ nM	pCu	$[\text{Cu}]_r/[\text{Cu}^{2+}]$	$\log K_1$	$\log K_2$	T[L ₁] nM	T[L ₂] nM	DOC mg/L					
Aare	Eichholz, Berne	9 Jun.	16.9	14.5	5.3×10^6	14.1	11.6	37	541	0.9					
	Halenbrucke, Berne	9 Jun.	19.4	14.2	2.7×10^6					1.0					
	Brugg	9 Jun.	17.5	14.8	1.2×10^7					2.0					
Birs	Münchenstein	18 May	28.2	13.9	2.2×10^6					2.0					
		6 Jun.	47.4	13.3	8.6×10^5					2.2					
Chriesbach	Dübendorf	15 Jun.	74.3	12.9	6.0×10^5		12.3		200						
		15 Jul.	38.0	14.1	5.1×10^6										
Emme	Burgdorf	9 Jun.	25.4	15.1	2.9×10^7					1.9					
Glatt	Niederglatt: downstream ARA	24 May	31.3	13.1	4.1×10^5						3.6				
		28 Jun.	21.4	13.1	2.7×10^5						3.9				
		28 Jun.	20.0	13.8	1.3×10^6						13.3	11.8	106	525	3.5
	Rümlang	5 Mar.	29.9	14.4	7.3×10^6	14.6	12.3	58	521	4.2					
		6 Mar.	23.6	14.4	5.7×10^6	14.4	12.1	45	561	5.8					
		24 May	34.8	15.1	4.1×10^7	14.8	13.1	88	525	3.2					
Rhine	Rekingen	18 May	10.0	15.0	9.0×10^6						1.8				
		6 Jul.	43.2	13.5	1.4×10^6						2.7				
	Village-Neuf	18 May	10.0	15.1	1.2×10^7						14.4	12.6	98	457	1.8
		6 Jul.	15.7	13.1	1.8×10^5						2.3				
Thur	Andelfingen	16 Jun.	34.4	13.5	1.2×10^6						3.6				
		2 Aug.	68.4	14.4	1.8×10^7						2.4				

of complexation from the pH-dependence is however only valid when the nature and concentration of the organic ligands keep constant. The evolution of Cu complexation in the lake during lake restoration can not be predicted from this plot, as more organic ligands may be produced by phytoplankton if pH increases. The actual level of $[Cu^{2+}]$ now is tolerable to some freshwater algae (Knauer et al., 1995), but the levels in previous years with lower pH and higher total copper were very probably toxic to most species. It is known that, following chemical changes in the lake during its restoration, various biological communities are increasing (Calderoni et al., 1992).

In river water, it is expected that various ligands of different origin may be present and that the influence of less specific ligands like humic and fulvic acids may be more important than in the previous examples of the lakes. In the case of less specific ligands, a relationship between the DOC concentrations and the extent of complexation is expected.

Table 7 lists free cupric ion concentrations and Cu complexing parameters in the various river water samples. Total dissolved Cu varies between 10–70 nM and $[Cu^{2+}]$ is in the range 10^{-13} – 10^{-15} . Free $[Cu^{2+}]$ are generally higher and ratios $[Cu]_T/[Cu^{2+}]$ lower in the river waters than in the lake waters, at comparable pH and DOC concentrations. Systematic tendencies are somewhat difficult to recognize. DOC varies in these river waters in the range 1–6 mg/L. A plot of $[Cu]_T/[Cu^{2+}]$ versus DOC shows no obvious correlation, implying that the ligand concentration is not directly related to DOC (Fig. 4). This fact implies that not all organic carbon gets involved in Cu complexing with the same affinity and that the strong Cu-com-

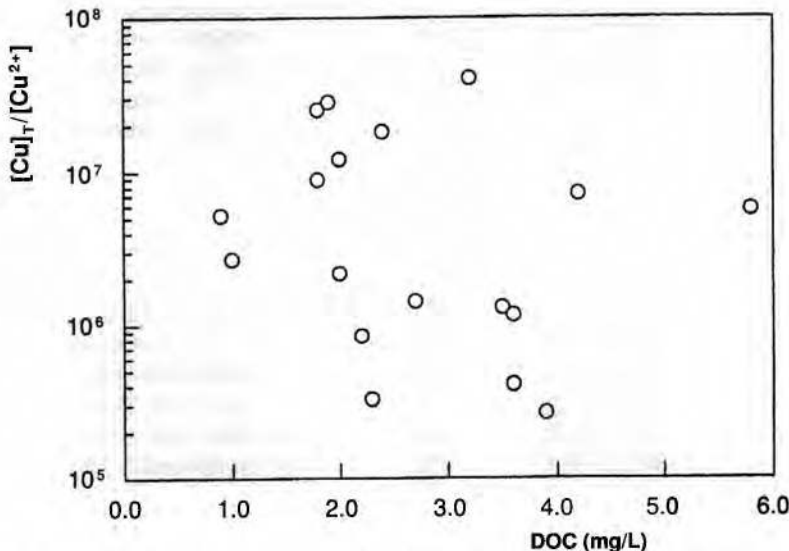


Figure 4. Ratios of $[Cu]_T/[Cu^{2+}]$ in function of DOC in various river water samples

plexing ligands are specific organic compounds. Strong complexation was observed for example in river Emme, and on one date at both sampling sites in the Rhine (Rekingen and Village-Neuf), with relatively low DOC in all these cases. These three cases also have no obvious relationship to material originating from a lake. Colloidal particles, especially under high discharge conditions with high concentrations of suspended solids, may also play a role as ligands. The effects of upstream lakes are not clear in the river samples. The samples from river Glatt, for example, are probably more strongly influenced by the sewage effluents than by material from Lake Greifen.

Relatively low ratios of $[Cu]_T/[Cu^{2+}]$ and high $[Cu^{2+}]$ are observed downstream of sewage treatment plants, as in river Glatt and in the small river Chriesbach. It appears therefore that sewage treatment plants are not major sources of strong ligands. In addition to natural organic ligands, effluents of sewage may contain strong synthetic ligands such as NTA and EDTA (Kari, 1994). Although the EDTA inputs into e.g. the Glatt river are not negligible (resulting in EDTA concentrations in the range 0.05–0.5 μM), they appear not to influence strongly the Cu complexation, probably because of the competition by other ligand and metal ions and of the presence of FeEDTA complexes, which would exchange very slowly with other cations (Kari, 1994; Xue et al., 1995). A decrease of Cu complexation downstream of sewage treatment plants may be due to inputs of other metals, which could compete with Cu for the same ligands.

The speciation of Cu has been extensively studied in seawater, in which the presence of strong ligands has been attributed to biological activity (Sunda, 1988/89, Bruland et al., 1991). The Cu complexation in the lakes at neutral pH seems to be even stronger than in seawater. In seawater, pCu in the range of about 11–13.5 have been reported, and stability constants of the stronger ligands of $\log K = 11 - > 13.5$ (Sunda and Huntsman, 1991; Moffett et al., 1990; Coale and Bruland, 1988, 1990; Van den Berg et al., 1987; Donat et al., 1994). It must be however considered that the data are conditional for the respective ionic strengths and composition of freshwater and of seawater. The data presented here with stability constants for the strong ligands $\log K \approx 13-15$ (pH 7.8–8, $I \approx 0.01 \text{ M}$) may therefore be compatible with those observed in marine systems, if competition of major cations (Ca, Mg) and correction of ionic strength are taken into account.

Conclusions

The free copper aqua ion concentrations $[Cu^{2+}]$, determined in this study by an indirect ligand-exchange method, appear to be several orders of magnitude lower than total dissolved copper, in lakes and rivers with circumneutral pH. The ratios of total dissolved Cu to free copper ions are in the range $[Cu]_T/[Cu^{2+}] \approx 1 \times 10^5$ to $> 1 \times 10^7$ at pH 8. The speciation of copper in these lakes and rivers is therefore dominated by organic complexes, in which $> 99\%$ of Cu is bound. The presence of strong organic ligands is not related in a simple manner to the total concentrations of dissolved organic matter, indicating the presence of specific strong ligands. The strongest complexation is found in the euphotic layers of eutrophic lakes, in comparison to an oligotrophic lake and to various rivers. These findings indicate the

presence of low levels of very specific ligands, especially in lakes, which appears to be linked to high algal productivity. Cu ligands in the lakes may thus be either directly produced by living algae actively releasing extracellular compounds, or by dying algae leaching intracellular products and their debris. Specific strong ligands are less important in rivers than in lakes, and fulvic or humic acids play probably a more important role in binding Cu in river waters.

The extent of Cu complexation obviously depends on pH. Data collected in some alpine acidic lakes provide direct evidence for the strong pH dependence of Cu complexation and for the increase in free Cu^{2+} with decreasing pH.

With regard to the effects of Cu as a toxic element on algae, the very strong complexation in eutrophic lakes would provide a rather efficient protection against toxicity. Even under conditions with somewhat less strong complexation, such as in an oligotrophic, but neutral lake, and in the river waters examined, the measured $[\text{Cu}^{2+}]$ appear to be in a low range, in comparison to toxic ranges observed for freshwater algae, and even to those for marine algae (Knauer et al., 1995; Brand et al., 1986). It appears thus that the presence of strong ligands, which are possibly released by algae, has a beneficial effect in maintaining $[\text{Cu}^{2+}]$ at low levels. Toxic effects of Cu^{2+} may be expected under acidic conditions, especially if the total concentrations of copper are at the same time elevated. These combined effects are illustrated by the pollution history of Lake Orta (Calderoni et al., 1992).

ACKNOWLEDGEMENTS

We thank Don Adams for samples from Lake Orta, Silke Meyns for chlorophyll a measurements, Thomas Rüttimann for DOC and alkalinity measurements, Daniel Steiner for sampling.

REFERENCES

- Ambühl, H., 1987. Seenrestaurierung in Theorie und Praxis: Eine Aufgabe des modernen Gewässerschutzes. *Gas Wasser Abwasser* 67:433–438.
- Bloesch, J. and U. Uehlinger, 1990. Epilimnetic carbon flux and turnover of different particle size classes in oligo-mesotrophic lake Lucerne, Switzerland. *Arch. Hydrobiol.* 118:403–419.
- Balistreri, L.S., J.W. Murray and B. Paul, 1992. The biogeochemical cycling of trace metals in the water column of Lake Sammamish, Washington: response to seasonally anoxic conditions. *Limnol. Oceanogr.* 37:529–548.
- Balistreri, L.S., J.W. Murray and B. Paul, 1994. The geochemical cycling of trace elements in a biogenic meromictic lake. *Geochim. cosmochim. Acta* 58:3993–4008.
- Brand, L.E., W.G. Sunda and R.R.L. Guillard, 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* 96:225–250.
- Bruland, K.W., J.R. Donat and D.A. Hutchins, 1991. Interactive influences of bioactive trace metal on biological production in oceanic water. *Limnol. Oceanogr.* 36:1555–1577.
- Calderoni, A., R. Mosello and D. Ruggiu, 1992. Sixty years of limnology on lago d'Orta: a case history of recovery from heavy pollution. *Mem. Ist. ital. Idrobiol.* 50:201–223.
- Campos, M.L.A.M. and C.M.G. van den Berg, 1994. Determination of copper complexation in sea water by cathodic stripping voltammetry and ligand competition with salicylaldehyde. *Anal. Chim. Acta* 284:481–496.
- Camusso, M., G. Tatari and A. Zirino, 1991. Measurement and prediction of copper ion activity in Lake Orta, Italy. *Environ. Sci. Technol.* 25:678–683.

- Coale, K.H. and K.W. Bruland, 1988. Copper complexation in the Northeast Pacific. *Limnol. Oceanogr.* 33:1084–1101.
- Coale, K.H. and K.W. Bruland, 1990. Spatial and temporal variability in copper complexation in the North Pacific. *Deep-Sea Res.* 37:317–336.
- Donat, J.R., K.A. Lao and K.W. Bruland, 1994. Speciation of dissolved copper and nickel in south San Francisco bay: a multi-method approach. *Anal. Chim. Acta* 284:547–571.
- Donat, J.R. and C.M.G. Van den Berg, 1992. A new cathodic stripping voltammetric method for determining organic copper complexation in seawater. *Mar. Chem.* 38:69–90.
- Gächter, R. and J.S. Meyer, 1990. Mechanisms controlling fluxes of nutrients across the sediment/water interface in a eutrophic lake. In: R. Baudo, J. Giesy and H. Muntau (eds.), *Sediments: Chemistry and Toxicity of In-Place Pollutions*. Lewis Publishers, pp. 131–162.
- Hirose, K., Y. Dokiya and Y. Sugimura, 1982. Determination of conditional stability constants of organic copper and zinc complexes dissolved in seawater using ligand exchange method with EDTA. *Mar. Chem.* 11:343–354.
- Jakob, A., J. Zobrist, J.S. Davis, P. Liechti and L. Sigg, 1994. NADUF-Langzeitbeobachtung des chemisch-physikalischen Gewässerzustandes. *Gas, Wasser, Abwasser* 171–186.
- Kari, F.G., 1994. Umweltverhalten von Ethylendiamintetraacetat (EDTA) unter spezieller Berücksichtigung des photochemischen Abbaus. Ph.D. Thesis No. 10698, Swiss Federal Institute of Technology, ETH Zürich.
- Knauer, K., R. Behra and L. Sigg, 1995. Interactions of copper and zinc with freshwater algae. Submitted.
- Martell, A.E. and R.M. Smith, 1974–1989. Critical stability constants. V. 1–6. Plenum.
- Meyns, S., R. Illi and B. Ribi, 1994. Comparison of chlorophyll a analysis by HPLC and spectrophotometry: where do the differences come from? *Arch. Hydrobiol.* 132:129–139.
- Miller, L.A. and K.W. Bruland, 1994. Determination of copper speciation in marine waters by competitive ligand equilibration/liquid-liquid extraction: an evaluation of the technique. *Anal. Chim. Acta* 284:573–586.
- Moffett, J.W., R.G. Zika and L.E. Brand, 1990. Distribution and potential sources and sinks of copper chelators in the Sargasso sea. *Deep-Sea Res.* 37:27–36.
- Mosello, R., A. Calderoni and R. de Bernardi, 1991. Mass budget as a tool predicting the response to liming of the acidified, ammonium polluted Lake Orta. *Verh. Internat. Verein. Limnol.* 24:1044–1048.
- Scarano, G., E. Bramanti and A. Zirino, 1992. Determination of copper complexation in sea water by a ligand competition technique with voltammetric measurement of the labile metal fraction. *Anal. chim. Acta* 264:153–162.
- Sigg, L. and H.B. Xue, 1994. Metal speciation: concepts, analysis and effects. In: G. Bidoglio and W. Stumm (Eds.), *Chemistry of aquatic systems: local and global perspectives*, Kluwer, pp. 153–181.
- Stadelmann, P., 1988. Zustand des Sempachersees vor und nach Inbetriebnahme der seeinternen Massnahmen: Künstlicher Sauerstoffeintrag und Zwangszirkulation 1980–1987. *Wasser, Energie, Luft* 80:81–96.
- Sunda, W.G., 1988/89. Trace metal interactions with marine phytoplankton. *Biological Oceanogr.* 6:411–442.
- Sunda, W.G. and R.R.L. Guillard, 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. Mar. Res.* 34:511–529.
- Sunda, W.G. and P.J. Hanson, 1979. Chemical speciation of copper in river water. In: E.A. Jenne (ed.), *Chemical modeling in aqueous systems*. American Chemical Society, Washington pp. 147–180.
- Sunda, W.G. and A.K. Hanson, 1987. Measurement of free cupric ion concentration in seawater by a ligand competition technique involving copper sorption onto C₁₈ SEP-PAK cartridges. *Limnol. Oceanogr.* 32:537–551.
- Sunda, W.G. and S.A. Huntsman, 1991. The use of chemiluminescence and ligand competition with EDTA to measure copper concentration and speciation in seawater. *Mar. Chem.* 36:137–163.
- Tipping, E. and M.A. Hurley, 1992. A unifying model of cation binding by humic substances. *Geochim. Cosmochim. Acta* 56:3627–3641.

- Van den Berg, C.M.G., 1984. Determination of the complexing capacity and conditional stability constants of complexes of copper(II) with natural organic ligands in seawater by cathodic stripping voltammetry of copper-catechol complex ions. *Mar. Chem.* 15:1–18.
- Van den Berg, C.M.G., A.G.A. Merks and E.K. Dursma, 1987. Organic complexation and its control of the dissolved concentration of copper and zinc in the Scheldt estuary. *Estuarine Coastal Shelf Sci.* 24:785–797.
- Van den Berg, C.M.G., M. Nimmo, P. Daly and D. R. Turner, 1990. Effects of the detection window on the determination of organic copper speciation in estuarine waters. *Anal. Chim. Acta* 232:149–159.
- Westall, J.C., 1982. FITEQL. A program for the determination of chemical equilibrium constants from experimental data. Oregon State Univ., Corvallis.
- Whitfield, M. and D.R. Turner, 1987. The role of particles in regulating the composition of seawater. In: W. Stumm (ed.), *Aquatic surface chemistry*. Wiley-Interscience.
- Xue, H.B. and L. Sigg, 1993. Free cupric ion concentration and Cu(II) speciation in a eutrophic lake. *Limnol. Oceanogr.* 38:1200–1213.
- Xue, H.B., F.G. Kari and L. Sigg, 1995. Speciation of EDTA in natural waters: Exchange kinetics of Fe-EDTA in river water. *Environ. Sci. Technol.* 29:59–68.
- Xue, H.B., D. Kistler and L. Sigg, 1995. Competition of copper and zinc for strong ligands in a eutrophic lake. *Limnol. Oceanogr.* 40:1142–1152.

Received 6 June 1995;

revised manuscript accepted 13 November 1995.