SPECIATION AND BIOAVAILABILITY
OF COPPER
IN LAKE TJEUKEMEER

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Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op woensdag 25 september 1991
des namiddags te vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

Stellingen

1. De door Van den Berg (1982) aanbevolen linearisatie van de Langmuir-vergelijking, voor gebruik bij de MnO_2-adsorptiemethode, is niet altijd de meest geschikte.

   Literatuur: - dit proefschrift (paragraaf 5.1);
   - Van den Berg, C.M.G. (1982). Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO_2. I. Theory. Marine Chemistry 11, 307 - 322.

2. Stroes-Gascoyne, Kramer & Snodgrass (1986) maken niet duidelijk waarom de drie door hen genoemde mechanismen voor de adsorptie van koper aan MnO_2 zich zouden moeten voordoen, en al helemaal niet waarom deze drie mechanismen in dezelfde mate zouden moeten optreden.

   Literatuur: Stroes-Gascoyne, S., J.R. Kramer & W.J. Snodgrass (1986). A new model describing the adsorption of copper on MnO_2. Environmental Science & Technology 20, 1047 - 1050.

3. Wanneer bij de SEP-PAK-ligand-kompetitie-techniek EDTA wordt toegevoegd, verschuift de kromme van geadsorbeerd versus totaal koper niet naar hogere koncentraties totaal koper, zoals Sunda & Hanson (1987) stellen, maar naar lagere koncentraties geadsorbeerd koper.

   Literatuur: Sunda, W.G. & A.K. Hanson (1987). Measurement of free cupric ion concentration in seawater by a ligand competition technique involving copper sorption onto C_{18} SEP-PAK cartridges. Limnology and Oceanography 32, 537 - 551.

4. Door *square-wave*-CSV-gegevens te tonen en *differential-pulse*-CSV gegevens te beschrijven, suggereren Huynh Ngoc & Whitehead (1986) een hogere gevoeligheid voor hun nikkel- en kobaltbepaling dan zij kunnen waarmaken.

   Literatuur: Huynh Ngoc, L. & N.E. Whitehead (1986). Nickel and cobalt determination in the North Western Mediterranean by differential pulse cathodic stripping voltammetry. Oceanologica Acta 9, 433 - 438.

5. De in het evenwichtsprogramma MINEQL (1976) opgenomen log(K) van -5.1 voor het Sn(IV)/Sn(II)-koppel is niet in overeenstemming met de standaard-reduktie-potentiaal zoals gegeven in Weast (1979), en zou +5.1 moeten bedragen.

   Literatuur: - Westall, J.C., J.L. Zachary & F.M.M. Morel (1976). MINEQL. A computer program for the calculation of chemical equilibrium composition of aqueous systems. Water quality Laboratory MIT Technical Note No. 18.
   - Weast, R.C. (ed.) (1979). CRC handbook of chemistry and physics, 59th edition. CRC Press, Boca Raton, Florida.

6. De formele lading van het arseen-atoom in mono- en dimethylarseenzuur bedraagt +5, en niet +3 resp. +1 zoals Wood (1974) beweert.

   Literatuur: Wood, J.M. (1974). Biological cycles for toxic elements in the environment. Science 83, 1049 - 1052.

7. De door Elkhatib, Bennett & Wright (1984) vermelde gegevens over de kinetiek van arseen(III)-adsorptie zijn in strijd met de door deze auteurs vermelde formule.

   Literatuur: Elkhatib, E.A., O.L. Bennett & R.J. Wright (1984). Arsenite sorption and desorptio in soils. Soil Science Society of America Journal 48, 1025 - 1030.

8. Een goede wetenschapper is niet altijd een goede manager.

9. Kunst is onbetaalbaar.

10. Alleen al het feit dat Veronica denkt *De wereld in een half uur* te kunnen behandelen, geeft aan hoe oppervlakkig deze omroep is.

11. Om het autogebruik te ontmoedigen verdient het aanbeveling een systeem te ontwikkelen waarbij wandelaars en fietsers voortdurend groen licht krijgen, tenzij automobilisten uit hun auto komen en via een "drukknopje" hun aanwezigheid kenbaar maken.

12. In het kader van het volksgezondheidsbeleid dient het gebruik van een walkman in openbare ruimten (inklusief het openbaar vervoer en musea) te worden verboden.

13. Aangezien de Nederlandse overheid zich bezorgder toont om de toekomst van de Nederlandse taal dan om die van de Friese, lijkt hier geen sprake van een principiële betrokkenheid bij bedreigde talen.

14. Efter dat allegearre, wat fatsoenlike lju ûnderinoar útmeitsje, leit dochs altyd it aventoer op'e loer (vrij naar Sigfrid Siwertz in de vertaling van Marten Sikkema).

15. Patstelling.

Stellingen behorend bij het proefschrift *Speciation and bioavailability of copper in Lake Tjeukemeer*. Wilko Verweij, 25 september 1991.

Alleen de zuivere verschijning der elementen, in evenwichtige verhouding, kan in leven en kunst de tragiek verminderen.

Piet Mondriaan

CONTENTS

DANKWOORD

Hierbij wil ik iedereen bedanken die op de één of andere manier heeft bijgedragen aan de totstandkoming van dit proefschrift. In de eerste plaats noem ik mijn kollega's: dank aan iedereen die geholpen heeft met bemonstering, analyses, diskussies over mijn arbeid(-somstandigheden), koffiepraat, ("basic"-)komputerwerk, typewerk, het verzamelen van literatuur, af en toe een verzetje, af en toe (nou ja...) een sjekje, enzovoort. Verder natuurlijk alle stagiair(e)s bedankt, die mij door hun leerzucht niet alleen veel werk hebben bezorgd, maar ook (en meer) werk uit handen hebben genomen. Thanks also to our foreign guests Roman, Luis and Tetsuo for interesting discussions (and not only on speciation subjects). Ook wil ik noemen vakgenotes (m/v) elders in den lande, op Texel, in Haren, Bilthoven, Den Helder, Yerseke, Amsterdam en niet te vergeten Wageningen. Verder dank aan alle vrienden (v/m) (benammen ek dy fan Progressyf Fryslân/Grien Links (Lofts?)) die er mede voor hebben gezorgd dat ik geen vakidioot geworden ben. Finally, thanks to Karen and Luis who corrected the English.

Wilko Verweij.
De Lemmer, januari 1991.

Chapter 1

Introduction

1.1. Actuation

In the nineteen sixties the Limnological Institute in the Netherlands started a long-term research project in Lake Tjeukemeer within the framework of the International Biological Programme (IBP). The research work was continued after the conclusion of the IBP, and until now physical, chemical and biological (algae, zooplankton, fish) parameters have been determined. In the nineteen eighties trace metals were included in the chemical monitoring program. Investigations were focused on a selected group of essential metals, with certain specific biological functions which can not be performed by other metals. The lack of such metals results in limited growth. Some of these metals may be toxic, however, if present in too high concentrations. Copper is such a metal which may be limiting and toxic.

Total copper concentration in Lake Tjeukemeer was found to be high enough to be toxic for algae, according to the literature. On the other hand, literature reports point at the fact that organic material is able to reduce algal copper toxicity by forming organic copper complexes, involving both synthetic organic ligands (Allen, Hall & Brisbin, 1980; Morel, Rueter & Morel, 1978) and natural organic ligands (Baccini & Suter, 1979; Toledo, D'Aquino & Tundisi, 1982). Lake Tjeukemeer contains high concentrations of organic material, and the possibility that it may complex copper reducing its availability to the extent of only limiting algal growth can not be ruled out. This apparent contradiction between high copper concentrations and non algal toxicity was the reason that prompted the start of this research project focused on the bioavailability of copper for algae in Lake Tjeukemeer in relation to copper speciation. This thesis reports the results of such study.

It should be emphasized that the chemical term "speciation" has several meanings. Before they can be discussed, it is useful to define the term "species" as a *molecular form (configuration) of atoms of an element or cluster of atoms of different elements* after Bernhard, Brinckman & Irgolic (1986). Based on this concept, "speciation" can then be defined as one of the following:

1. the analysis of species;
2. the description of the distribution of species of an element;
3. the reactivity of a species;
4. the transformation of certain species into other species.

In this thesis the term "speciation" refers to the distribution of an element over all possible species of that element.

1.2. Aim of this project and setup of this thesis

In order to investigate the relationship between copper speciation and its bioavailability in Lake Tjeukemeer, more insight into the environmental geochemistry of copper is required. First of all, it was not exactly known how the man-made hydrology of the lake affected total copper concentrations in the lake. Secondly, no information was available on the binding of copper to lake sediment.

Preliminary experiments indicated that copper complexing capacity in Lake Tjeukemeer is fairly high (several tens of µM of copper), but no additional information on copper speciation was available. There was also a lack of data on copper bioavailability, since no biological experiments had been performed. It was therefore chosen to investigate:

- the influence of Lake Tjeukemeer hydrology on its copper concentrations (described in sections 2.3 and 2.4);
- the binding of copper by lake sediments (section 2.5);
- the speciation of copper in Lake Tjeukemeer; one physical technique (ultrafiltration) was used (chapter 3) and several chemical techniques: anodic and cathodic stripping voltammetry (chapter 4), adsorption to manganese oxide (section 5.1), competition with a synthetic ligand in combination with voltammetry (section 5.2), and modelling (chapter 7);
- the fate of copper added to lake water by performing copper titrations (chapter 6);
- the bioavailability of copper for algae by carrying out bioassays (chapter 8).

The remaining sections in this chapter are devoted to the relationship between copper speciation and bioavailability, and the problems associated with investigating copper speciation at natural copper concentrations.

In section 2.1 and 2.2 the chemistry and hydrology of Lake Tjeukemeer are briefly discussed.

Finally, the information presented in each chapter is compiled and summarized in chapter 9. This is followed by summaries in English, Dutch and Frisian, and literature references.

1.3. Which species control copper availability?

In section 1.1 it has already been remarked that organic ligands could reduce the toxicity of copper by forming organic copper complexes. Apparently not all copper species are equally toxic. The question arises as to which copper species determine copper availability. Since copper predominantly exists in the divalent form under environmental conditions (Huntsman & Sunda, 1980), no attention was paid to monovalent copper. A literature review led to the conclusion that Cu^{2+}_{aq} (so-called "free" copper) controls copper availability, although lipid-soluble copper complexes may also be available and toxic too. This conclusion is discussed in the following paragraphs.

In 1976 Sunda & Guillard published a paper in which they described bioassays of two algal species in synthetic culture media. They varied the pH and the concentration of 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS) in order to control pH and free copper concentrations. The free copper activity was computed by chemical equilibrium calculations. The biological parameters examined by these authors (copper contents of the algal cells and growth rate inhibition) were found to be a function of free copper activity and not of total copper concentration.

Comparable experiments were also described by Anderson & Morel (1978). They monitored parameters such as fixation of $^{14}CO_2$ by a dinoflagellate and its loss of motility in artificial seawater media. TRIS and ethylenediaminetetraacetate (EDTA) were used to control the free copper concentrations. The mentioned parameters appeared to be a unique function of the calculated free copper activity rather than of total copper concentration.

Sunda & Gillespie (1979) described bacterial bioassays in diluted seawater in which the concentration of nitrilotriacetic acid (NTA) was varied to control free copper concentration. The uptake of ^{14}C-glucose again appeared to depend on free copper activity rather than on total copper concentration.

Baccini & Suter (1979) reported about chemical and biological conditions in limno-corrals. Copper bioavailability could be described as a function of the pH, the concentration of organic ligands and the conditional stability constant of the organic copper complexes. By using chemical equilibrium computations this relationship could be explained in terms of free copper controlling bioavailability.

In 1979 Wagemann & Barica published a commonly cited paper in which they suggested that other copper species besides free copper are suspected toxic. These authors described how lakes were treated with copper sulphate to reduce the growth of algae. They estimated free copper concentration by equilibrium calculations, but did not measure growth or any other biological parameter. However, they suggested that, in addition to Cu^{2+}, $Cu(OH)^+$ and $Cu(OH)_2$ are also toxic. No clear evidence was presented to support this hypothesis.

Petersen (1982) cultured a green alga in a synthetic medium, measured the growth rate and calculated the free copper concentration. Growth rate was a function of the free copper concentration rather than the total copper concentration.

Sunda & Ferguson (1983) examined the incorporation of ^3H-labelled amino acids by natural marine microbial communities. Copper and NTA concentrations were varied. Free copper concentration was calculated by comparing the incorporation in a copper addition series _without_ NTA with the incorporation in a copper addition series _with_ NTA, and by using appropriate equilibrium calculations. Amino acid incorporation was a function of free copper concentration and was independent of total copper concentration.

Florence, Lumsden & Fardy (1983) cultured a marine diatom in enriched sea water, and measured the cell density. Linoleic acid (an unsaturated fatty acid) reduced the growth of algae slightly, but its copper complex was more toxic. Two other ligands, 8-quinolinol and 2-methyl-8-quinolinol were also toxic, both as free ligand and as copper complex. These three mentioned ligands are all lipid-soluble. Comparable ligands whose copper complexes were not lipid-soluble, were not toxic. These authors concluded therefore that lipid-soluble copper complexes may be toxic for algae.

Sunda, Klaveness & Palumbo (1984) reported on bacterial bioassays in synthetic media in which the NTA-concentration was varied. Uptake of labelled amino acid or glucose was recorded to represent growth, which turned out to be a function of the free copper activity and not of the total copper concentration.

Anderson, Lively & Vaccaro (1984) used the same method as Sunda & Ferguson (1983) to determine free copper concentration. The uptake of labelled glucose by a bacterial clone was recorded, and appeared to be a function of the free copper concentration, not of the total concentration.

The conclusion from these studies is that the free copper ion Cu^{2+} and lipid-soluble copper complexes are the only species controlling copper availability for bacteria and algae. In this thesis, attention is focused on free copper, since the toxicity of lipid-soluble copper complexes is a result of the toxicity of copper and the toxicity of the ligands. The separate contributions can not be distinguished. Besides, the ecological relevance of the lipid-soluble copper complexes is not clear. Moreover, the free copper concentration was pointed to as the parameter controlling copper availability by many more authors than lipid-soluble copper complexes.

It should be stressed that, since none of these authors systematically varied the ionic strength, it is in fact not clear whether free copper _activity_ or free copper _concentration_ controls its bioavailability. Nevertheless, the fact that only one species is responsible for controlling bioavailability, allows one to focus attention on this particular species when investigating copper availability instead of having to take into account several possibilities. Besides, the activity of Cu^{2+}_{aq} is about 30% of the value of its concentration when the ionic strength is 0.5 M (as calculated using the Davies equation). This ratio is much smaller than the ratio between free and total copper concentration.

Two questions yet have to be answered in this section: why is copper essential for algae, and why can it be toxic. The reason copper is essential for algae stems from it being a necessary element in plastocyanin (a protein involved in photosynthesis) and a cofactor for several enzymes (Huntsman &

Sunda, 1980). Copper can not be replaced in these enzymes without disturbing their biological activity. When the copper concentration becomes too high, copper effectively competes with other metals for sites in numerous other enzymes whose biological activity is reduced when copper replaces those metals (Huntsman & Sunda, 1980). Besides, copper may react with functional groups in enzymes (Huntsman & Sunda, 1980; Stauber & Florence, 1986). Stauber & Florence (1985a, 1985b, 1987) demonstrated that trivalent metal ions (Al, Cr, Mn, Fe, Co) reduced copper toxicity, probably by forming copper-impermeable hydroxides on the outside of the cells, whereas divalent metal ions (Ni, Zn) did not.

1.4. How to determine [Cu^{2+}]

In all papers mentioned in the previous section, the free copper concentration or activity was calculated, not measured. The reason computations are used is that by means of currently available analytical techniques it is impossible to determine experimentally the free copper concentration at ambient levels. For open seawater samples, total copper concentration is typically between 10^{-9} and 10^{-8} M (Huntsman & Sunda, 1980), for inland water usually about one order of magnitude higher with extreme values up to 5 * 10^{-7} M for the highly polluted river Rhine (Salomons & Förstner, 1984).

Free copper is only a fraction of total copper and its concentration is therefore lower than the above mentioned values, thus reaching extremely low concentration levels. Yet many analytical chemists consider it a challenge to try and experimentally determine free copper concentrations at ambient levels. In this section several methods will be briefly described including their potentials and drawbacks. It will be shown that serious problems are associated with all methods mentioned.

Size fractionation techniques may be used to gain insight in the size of species present in a sample. Ultrafiltration, probably one of the most frequently applied size fractionation techniques in metal research, is not as straightforward as it sometimes seems to be. The results depend not only on the size of a molecule, but also on its shape and charge as well as the degree of hydrophobicity of the filter (Hoffmann et al., 1981). Besides, results may be complicated by adsorption of species onto the filter (Benes & Steinnes, 1974; Buffle, Deladoey & Haerdi, 1978), speciation changes due to the long time needed for the experiments and the gradually increasing concentration of species too large to be filtered off (Benes, Gjessing & Steinnes, 1976; Buffle & Staub, 1984; Hoffmann et al., 1981; Sojo & De Haan, 1991) or contamination (Benes & Steinnes, 1974; Giesy & Briese, 1977). Results of ultrafiltration experiments should therefore be interpreted with care.

Several electrochemical techniques can also be applied to obtain information on free copper concentration. The cupric ion selective electrode (ISE) can be used to determine free copper activity but its high detection limit renders it unusable in unpolluted waters. Another disadvantage of the cupric ISE, is the interference of chloride ions. Although this does not represent a problem in freshwater, it does hinder its application in seawater (Sunda, Klaveness & Palumbo, 1984). Another electrochemical technique, anodic stripping voltammetry (ASV), has a sufficiently low detection limit to detect copper at ambient concentration levels, but beside free copper, several other important inorganic copper complexes and some organic complexes are determined simultaneously (Florence, 1986; Betti & Papoff, 1988). Recently cathodic stripping voltammetry has been introduced in metal speciation research (Van den Berg, 1984a, 1984b, 1984c, 1986). For copper the method is based on the competition of catechol (1,2-dihydroxybenzene, a synthetic ligand) with natural ligands complexing copper. Copper complexes that dissociate fast enough (typically within a few minutes) will therefore be detected together with free copper ions. This method can not be applied to samples containing high concentrations of organic carbon (Gerringa, 1990; this thesis).

Another group of methods also based on addition of a synthetic ligand, but requiring much longer equilibration times is ligand competition techniques. In these cases it may be assumed that true equilibrium exists, allowing the free copper concentration to be computed if certain conditions are fulfilled. Results of calculations have to be interpreted with care, however, because the data required for the equilibrium calculations are not always known accurately enough. Besides, sometimes extrapolation is necessary to obtain information on natural situations. Examples of ligand competition techniques are the MnO_2-adsorption method (Van den Berg & Kramer, 1979a, 1979b; Van den Berg, 1982a, 1982b, 1983) and the SEP-PAK C_{18} adsorption method (Sunda & Hanson, 1987).

Neutron activation analysis is a method with very low detection limits (Benes & Steinnes, 1974; Benes, Gjessing & Steinnes, 1976), but for copper no isotope exists with a suitable half-life time (Weast, 1979), which is a prerequisite for this technique.

This enumeration is not complete, since the number of techniques in use is so large that it is very hard to review them all. Apart from analytical techniques, chemical equilibrium calculations are sometimes used to estimate free copper concentrations. For simple, well defined, inorganic systems usually sufficient information is available to calculate the speciation. However, even in such simple systems problems arise, because the reported equilibrium constants sometimes differ by a factor 100 or 1000 (Giesy & Alberts, 1989). For natural systems it is usually not possible to calculate the speciation, unless detailed experiments are done to study the interactions of copper with organic matter.

Bioassays may yield ecologically interesting information on possible limitations in natural waters. Bioassays as such do not make clear which species is or are available, nor do they give specific quantitative information.

The conclusion thus must be that no ideal method exists for determining free copper concentrations in natural systems, except for highly polluted waters. The problem as described in section 1.2 must therefore be tackled by a careful interpretation of non-ideal methods and a combination of simultaneously used methods, e.g. bioassays with equilibrium computations (chapter 8).

1.5. Implications for materials and methods

The extremely low levels of total copper concentrations found in natural waters already imply that normal "good laboratory practice" is by far not enough to obtain reliable experimental results. For speciation research, demands on methods and procedures are obviously even more stringent. Because this holds for all experimental methods applied, the consequences of careful handling during experiments described here are general. By giving this information at this point, it becomes unnecessary to discuss this subject in detail in each individual chapter.

Collection and filtration of samples, as well as all subsequent handling may be a severe source of contamination (Mart, 1979a, 1979b; Mart, Nürnberg & Valenta, 1980). For this reason, all new sample containers and other new materials that might come in contact with samples were soaked with acid (usually 10% HNO_3) at least overnight, and flushed with Milli-Q water at least four times. The containers and other materials were kept in contact with Milli-Q water at least overnight, and this portion of water was measured for copper contents using a graphite furnace atomic absorption spectrophotometer (GFAAS). If the copper concentration did not exceed 3 nM (about the detection limit of the equipment), the materials were considered clean.

Polyethylene bottles were used throughout, except when explicitly stated otherwise. Bottles were, whenever possible, used for only one purpose (same matrix, same total copper concentration), and were in such cases rinsed only with Milli-Q water (four times) and not with acid. If bottles were to be used for a different matrix and/or total copper concentration, they were rinsed four times with Milli-Q water, equilibrated with Milli-Q water at least overnight, and the copper concentration of that portion of water was determined using GFAAS. Concentrations of 3 nM and lower were considered acceptable. When the copper concentration was higher, the bottles were treated as described for new materials.

All chemicals used were at least reagent grade. Milli-Q water was used for rinsing and preparing solutions. All critical experiments were carried out in a room only used for trace metal work. A slight positive pressure was applied to this room, and incoming air was being filtered to prevent contamination by dust.

All these measures were required in order to keep sample contamination to a minimum and under control throughout this project.

Chapter 2

Copper concentration in Lake Tjeukemeer and external influences

2.1. General description of Lake Tjeukemeer

Lake Tjeukemeer, the object of this study, is situated in the north of the Netherlands, in the province of Friesland (figure 2.1). It is a shallow, alkaline lake (average depth: 1.5 meter) with a peaty bottom. The water is eutrophic (1989 average: 0.22 mg $P \cdot L^{-1}$; 3.59 mg $N \cdot L^{-1}$) and has a brown colour caused by a high concentration of organic compounds (1989 average: 19.1 mg dissolved organic carbon $(DOC) \cdot L^{-1}$). Lake Tjeukemeer is connected with several other lakes by canals. This system of interconnected canals and lakes is called the Frisian "boezem".

The lake is surrounded by polders that are predominantly used for agricultural purposes. The hydrological regimes of these polders determine by and large the composition of the water in Lake Tjeukemeer. In autumn and winter, the wet seasons, there is usually a surplus of water in the polders, which is pumped into the lake. Water from the polders is more eutrophic (1989 average at the sampling point: 0.26 mg $P \cdot L^{-1}$; 4.39 mg $N \cdot L^{-1}$) and browner by organics (1989 average: 29.5 mg $DOC \cdot L^{-1}$) than the lake's. As a consequence, in the wet seasons, the lake contains increasing concentrations of phosphorus, nitrogen and DOC. In the dry seasons, there is usually a need for water in the polders. Lake water is then let into the polders, and the lake is supplied (via the "boezem") with water from Lake IJsselmeer, which itself is not part of the "boezem". Lake IJsselmeer is fed by the river IJssel, a branch of the river Rhine, which contains high concentrations of sodium chloride. This results in increasing chloride concentrations in the lake and, because of dilution, decreasing DOC-concentrations in summer time. The chloride concentration decreases at the start of the wet season by dilution with water from the polders. Changes in meteorological conditions out of the average may obscure these changes.

The eutrophication of the lake results in huge algal blooms, usually dominated by diatoms in spring and by Cyanobacteria in summer. Cryptophyceae and green algae may also occur in large numbers, but they seldom dominate the phytoplankton community.

More information on Lake Tjeukemeer can be found in e.g. Leenen (1982), Moed & Hoogveld (1982) and Van Huet (1990).

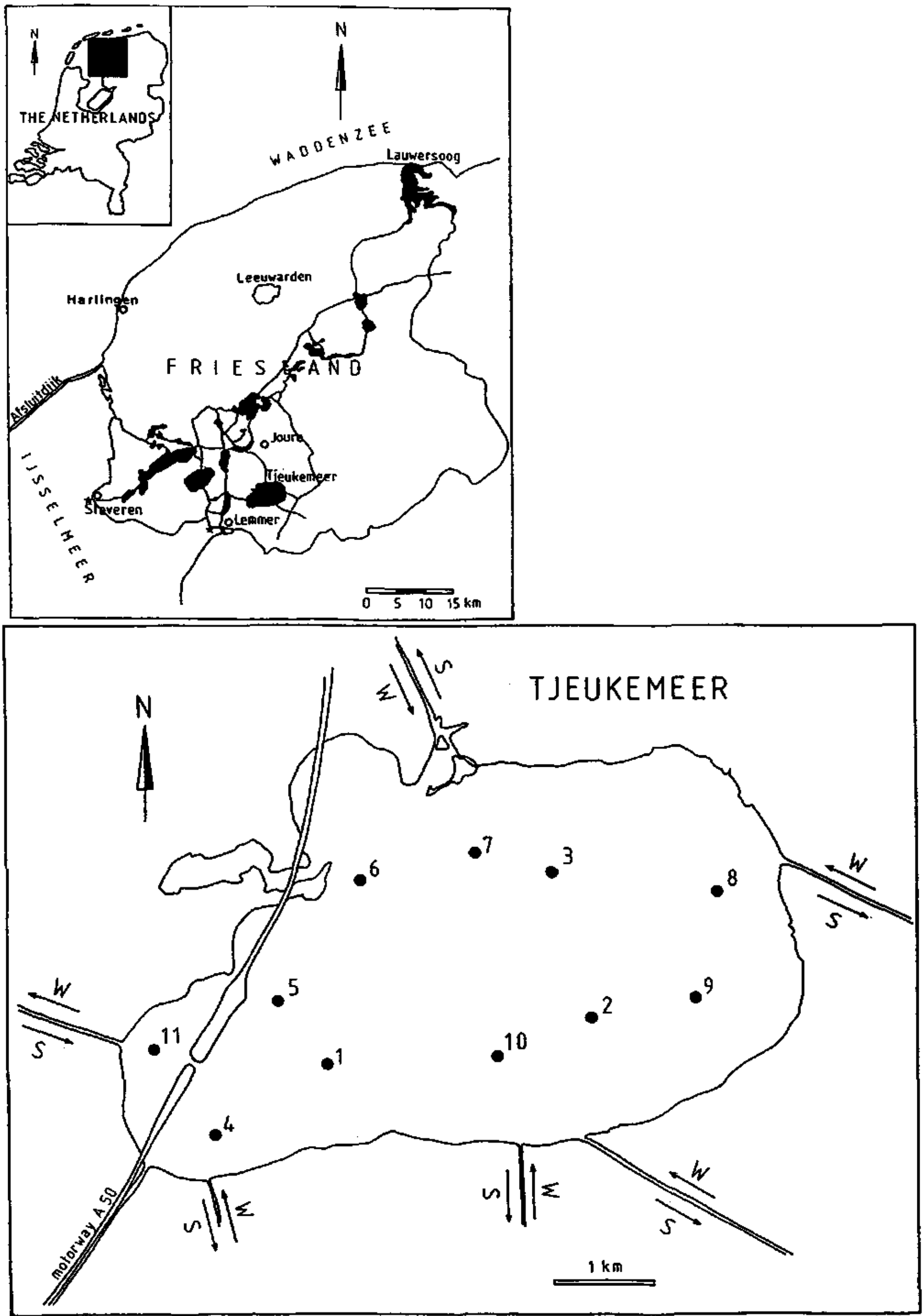

Figure 2.1. Map showing location of Lake Tjeukemeer and its sampling points. Arrows indicate water movement in <u>w</u>inter and <u>s</u>ummer.

2.2. Copper concentration in Lake Tjeukemeer

During this study, the copper concentration in water from Lake Tjeukemeer was measured fortnightly in both raw and filtered (< 0.2 µm) water. Samples were taken from ten sites (numbered 1 - 10 in figure 2.1), using a perspex tube (length 1 meter). The samples were mixed upon arrival at the laboratory. Since copper concentrations were sometimes close to the detection limit of our equipment, a 100 ml aliquot of the mixed sample was concentrated by freeze-drying, redissolving in 10.6 ml 0.79 M HNO_3 and heating overnight at 110°C. Copper concentrations were subsequently determined on an atomic absorption spectrophotometer (Perkin-Elmer 2380) equipped with a graphite furnace (HGA-400) and autosampler (AS-40). Background correction was performed by using a deuterium lamp.

The most common pore size used for separating "dissolved" species and particles is 0.45 µm (Salomons & Förstner, 1984). Yet for this study 0.2 µm was chosen because it is the pore size which has been used for over twenty years at our institute, allowing to compare results obtained in this study with those for other elements. However, some material which is not truly dissolved may be smaller than 0.2 µm (Stumm & Morgan, 1981), so neither 0.45 µm nor 0.2 µm really distinguish between the dissolved and the particulate fraction.

De Haan et al. (1990) showed that the total concentration of copper in

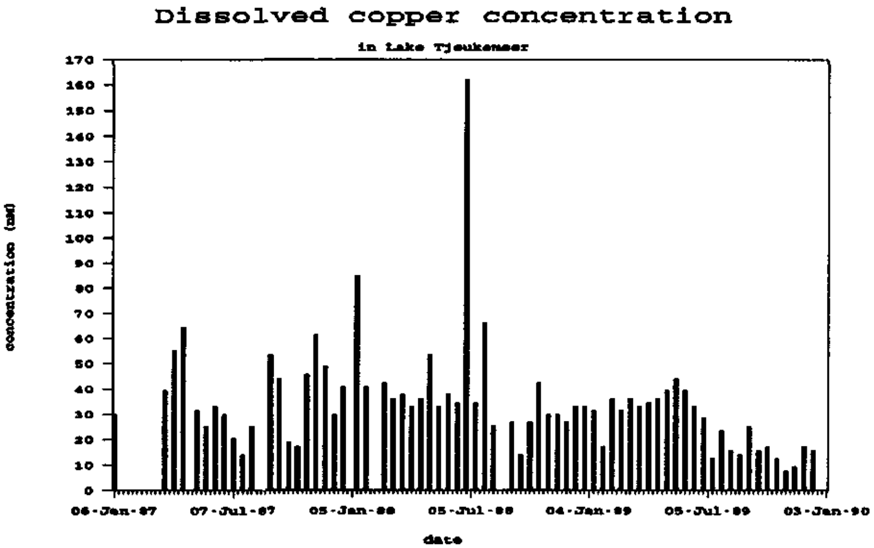

Figure 2.2. Dissolved (< 0.2 µm) copper concentration in Lake Tjeukemeer (in nM).

Lake Tjeukemeer was strongly correlated with wind speed. Wind-induced resuspension in this shallow lake was therefore supposed to control the strongly fluctuating total copper. For this reason, all experiments described in this thesis were done in filtered (< 0.2 µm) samples to exclude wind effects, unless explicitly stated otherwise. Using filtered samples also has a disadvantage, since during the process of filtration the sample may become contaminated (see also section 3.1). However, contamination of the "dissolved" copper fraction by filtration was at most a few tens of percents, while wind influenced total copper concentration by as much as a factor three (De Haan et al., 1990).

Figure 2.2 shows the "dissolved" copper concentration in Lake Tjeukemeer during the term of the experimental part of this project. With the exception of one contaminated sample half-way 1988 containing more than 160 nM, "dissolved" copper concentration was never higher than 85 nM.

2.3. Influence of water from Lake IJsselmeer

One might wonder if the water quality in Lake IJsselmeer, indirectly fed by the highly polluted river Rhine, may increase copper concentration in Lake Tjeukemeer, when water from Lake IJsselmeer is let into the Frisian "boezem". This is not a purely hypothetical situation, since usually 30% of the water let in at Lemmer (near Lake Tjeukemeer) reaches Lake Tjeukemeer (Van Huet, De Haan & Claassen, 1987). At present it is not easy however, to answer this question using the available data.

Measurements taken by other researchers showing that copper concentrations in Lake IJsselmeer are much higher than in Lake Tjeukemeer, as described by De Haan et al. (1990), may shed some light on the subject. The difference between copper concentrations in both lakes must be analyzed with caution, since the methods and techniques used to determine copper in Lake IJsselmeer may not be compatible with those used in Lake Tjeukemeer. At any rate, the assumption that water from Lake IJsselmeer entering the Frisian "boezem" contains more copper than that of Lake Tjeukemeer, does not necessarily imply that the copper load let in at Lemmer reaches Lake Tjeukemeer.

One way of testing this latter statement is to look at the copper concentrations at an eleventh sampling point (called "11") situated in the west of Lake Tjeukemeer, close to where water from Lake IJsselmeer enters Lake Tjeukemeer (see figure 2.1). Unfortunately, only copper measurements in unfiltered samples are available for this sampling point. Comparison with copper concentrations in unfiltered samples taken from the rest of the lake is hardly useful because of the wind effects that probably work out differently in the small, nearly enclosed, western part of Lake Tjeukemeer than in the much larger rest of the lake. If this comparison nevertheless is

Average copper concentration (nM)		
year	Lake Tjeukemeer	point "11"
1987	36 (20)	34 (20)
1988	38 (25)	35 (25)
1989	27 (24)	27 (24)

Table 2.1. Average copper concentration in nM during three years in Lake Tjeukemeer and at point "11", the sampling station close to where water from Lake IJsselmeer enters Lake Tjeukemeer; between brackets: n.

made, hardly any difference is found, as demonstrated by table 2.1. Even in dates when the chloride concentration exceeded the year average (i.e. when water from Lake IJsselmeer was let in), the same conclusion is reached. It is therefore not possible to conclude unambiguously that letting in water from Lake IJsselmeer increased the copper concentration in Lake Tjeukemeer.

It is possible that some geochemical mechanism is responsible for removing copper from Lake IJsselmeer water on its way to Lake Tjeukemeer. This mechanism could be coprecipitation with calcium carbonate. Salomons & Mook (1980) have shown the importance of calcium carbonate precipitation in the geochemistry of Lake IJsselmeer, especially in summer. It is likely that this precipitation reaction also occurred after water from Lake IJsselmeer was let into the Frisian "boezem". Possibly copper coprecipitated with calcium as a mixed carbonate, thus lowering the copper concentration. Another explanation is physical settling of particles (e.g. carbonate colloids or suspended matter with adsorbed copper) already formed in Lake IJsselmeer.

Summarizing, no reliable indications were found that the copper concentrations in Lake Tjeukemeer were increased by letting in water from Lake IJsselmeer into the Frisian "boezem".

2.4. Influence of water from the polders

It is interesting to investigate the possibility that water from the polders around Lake Tjeukemeer controlled the total concentration of copper in the lake, now that is has been established that water from Lake IJsselmeer did not demonstrably do so. Average copper concentrations in Lake Tjeukemeer and at "Echten/Bantega" (a sampling station in the "Veenpolder van Echten", a polder south of the lake), displayed in table 2.2, suggest this might be indeed the case. If this hypothesis is true, it should be possible to show that the copper concentrations in the polder water increased locally by either leaching, or surface run-off, or both. Two types of experiments were used to test the relevance of leaching (percolation experiments and sampling of ground

Average copper concentration (nM)		
year	Lake Tjeukemeer	point "Echten/Bantega"
1987	36 (20)	51 (20)
1988	38 (25)	42 (26)
1989	27 (24)	28 (24)

Table 2.2. Average copper concentration in nM during three years in Lake Tjeukemeer and at point "Echten/Bantega", a sampling station in the "Veenpolder van Echten", south of the lake; between brackets: n.

water), while a third type (sampling of field-drains and ditches[1], and comparing of results) was used for testing the importance of surface run-off.

Percolation experiments were used to investigate leaching in the upper ten to twenty cm of the soil column. Samples from meadows in the "Veenpolder van Echten" were taken in the wet season, by pressing a perspex tube into the soil, and were eluted with a solution of artificial rain water. Preliminary experiments with artificial rain water were carried out at a pH of 3 to test for potential copper leaching. Since this appeared to be the case, subsequent experiments were run at a pH of 4.7, equal to the local rain water pH. At both pH's, typical copper concentrations in the unfiltered eluate, as determined by flame AAS, ranged from 800 to 1600 nM. The pH of the artificial rain water solution appeared to have little effect on the eluate pH, since this was always alkaline. This indicates that the buffer capacity of the soil was not exceeded. The time during which the artificial rain water was kept into contact with the soil column appeared to have a greater effect than the pH of the solution. When the outlet of the column was shut off overnight, the copper concentration in the eluate was much higher the next morning than shortly before shutting the column off. This may be due to diffusion controlled kinetics of copper leaching or slow ion exchange kinetics.

A few experiments were also conducted with samples taken at a site where, until shortly before sampling, a dung-hill of cow-dung had been located. The copper concentrations in eluates of these soil columns were typically around 16000 nM. The conclusion from these experiments was that copper may leach from the upper ten to twenty cm of the soil column, provided the contact time is long enough.

To find out if copper may also leach from deeper soil layers, tubes of two meter in length were placed in the meadows at the "Veenpolder van Echten" to sample the ground water between one and two meter below the surface. The lower meter of the tube was perforated to let the ground water through, and

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[1] "ditches" as meant here are typically 0.5 to 1.5 m wide, and around 0.5 m deep.

covered with nylon gauze to prevent particles from entering the tube. The ground water was pumped up through a plastic tube and collected in polyethylene bottles. Copper concentrations in the unfiltered samples were determined using graphite furnace AAS. The average of 85 samples taken from eight different tubes during ten weeks in the wet season was 21 nM of Cu, which was a lower concentration than that of the polder water at the sampling site (cf. table 2.2). Apparently, copper may leach under certain conditions, but the copper concentrations in ground water between one and two meter below the surface did not reach values as high as in surface water. Leaching of copper is therefore not likely to have increased its concentration in surface water in the polder and in Lake Tjeukemeer.

Another possible explanation for the high copper concentrations in polder water, compared to Lake Tjeukemeer, might be surface run-off. To find out the validity of this assumption, samples were taken from field-drains, and from small drains collecting water from these field-drains. Copper concentrations in the unfiltered samples were determined using graphite furnace AAS. In the field-drains the average copper concentration was 127 nM (standard deviation 127 nM; median[1] 61 nM; 25 samples), in the small ditches 46 nM (standard deviation 54 nM; median[1] 26 nM; 41 samples). The highest concentrations were measured during rainy spells. This means that in the field-drains copper concentration was much higher than at the sampling site "Echten/Bantega", and slightly higher in the small ditches. Since these small ditches in turn, drain into larger ones which transport the superfluous water via sampling point "Echten/Bantega" to Lake Tjeukemeer, it is likely that surface run-off, especially during rainy weather, increased copper concentration in Lake Tjeukemeer.

2.5. Release of copper from the sediments to the water

It is also important to have an idea if copper may be released from the sediment into the water column. Extraction of sediments may give some indication about the binding of metals to sediment. In a typical extraction experiment, a portion of sediment is mixed with a solution of an extracting solution. After equilibration, the sediment is physically separated from the supernatant solution, and the concentration of the element under study is measured in this solution. In some cases, extraction schemes involving various extractant solutions of increasing degree of "aggressiveness" are used. Synthetic chelators are also used in the extraction process.

The interpretation of results of sediments extractions is not easy. E.g.

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[1] Because the standard deviation is about as high as the average, the median is also given.

Tessier, Campbell & Bisson (1979) developed an extraction scheme for metal studies consisting of five steps:

1. extraction with either a magnesium chloride solution or a sodium acetate solution to extract exchangeable metals;
2. extraction with an acidified (pH = 5) sodium acetate solution to release carbonate bound metals;
3. extraction with hot hydroxyl amine in 25% acetic acid to extract metals bound to iron and manganese oxides;
4. extraction with hot, acidified (pH = 2) hydrogen peroxide solution to release metals bound to organic matter;
5. finally digestion with a mixture of hydrogen fluoride and hydrogen perchlorate to release the remainder of the metals.

Although this method appeared to have a fairly high selectivity, some overlap occurs among extracted fractions. Care should therefore be taken in interpreting the metals released in one of these steps as belonging to a specific geochemical class of compounds. Rapin et al. (1986) pointed to other potential artefacts. Anoxic sediments displayed quite different extraction patterns after exposure to air for the first, second and third step in the procedure described above. They also showed that the pretreatment of the sediment samples influences the extraction results. De Groot, Zschuppe & Salomons (1982) emphasized the importance of controlling the grain size composition of the samples subjected to the extraction, as different grain size compositions may display quite different results. They proposed to use the fraction < 63 µm for extractions. It is therefore clear that the results of extractions have to be interpreted carefully; nevertheless interesting information may be obtained (Gerringa, 1990).

Samples were taken at six sites in the lake. Site A was situated near the inlet of water from the "Veenpolder van Echten", site B in the south east of the lake, site C in the north east, site D in the north, site E in the west (at the same location as the water sampling point "11"; see also section 2.3 and figure 2.1) and site F in the south west, near the water sampling point 1 (see figure 2.1). Cores of at least ten cm in length were taken using perspex tubes. All cores consisted of mainly peaty material. After arrival at the laboratory, the cores were split in three layers, of 0 - 2, 2 - 5 and 5 - 10 cm depth. The portions were sieved over 63 µm nylon gauze (UGB Panissières, France) with as little Milli-Q water as possible. Usually around 100 ml was required. The sediment was subsequently dried at 40° C as recommended by De Groot, Zschuppe & Salomons (1982) until dryness (usually a few weeks).

In this study two types of extractions were used. The first type followed the extraction scheme recommended by Salomons & Förstner (1984) which is a modification of the scheme developed by Tessier, Campbell & Bisson (1979). The second type of extraction uses EDTA and was developed by Keizer, Van Riemsdijk & De Haan (unpublished results).

The sequential extraction scheme as recommended by Salomons & Förstner (1984) was used as follows: about 0.1 g of dried sediment was weighed in a centrifuge tube and subjected to the extraction procedure.

1. 1 ml of 1 M ammonium acetate (pH = 7) was added (to release exchangeable metals) and the suspension was shaken for 2 hours. The centrifuge tubes were centrifuged for 30 minutes at 3000 rpm. The liquid phase was decanted, and stored in plastic tubes to measure copper. To prevent carry-over into the next extraction solution, 5 ml of Milli-Q water were added, the suspension was shaken for 30 minutes and centrifuged for 30 minutes. The liquid phase was decanted and discarded.
2. 1 ml of 1 M sodium acetate buffer (pH = 5) was added (to release carbonate bound metals) and the suspension was shaken for 5 hours. The suspension was subsequently separated and decanted as described for the first step.
3. 5 ml of hydroxyl amine, brought to pH = 2 with nitric acid were added and shaken for 12 hours to extract metals bound to easily reducible phases. For further treatment see the first step.
4. 5 ml of oxalate buffer (0.2 M of ammonium oxalate and 0.2 M oxalic acid; pH = 3) was added to extract metals bound to moderately reducible phases and shaken for 24 hours. Further treatment was as described for the first step.
5. 5 ml of hydrogen peroxide, acidified with nitric acid to pH = 2, were added to release organically bound metals. The centrifuge tubes were heated to 85°C until dryness. Ammonium acetate was added (5 ml; pH = 7) and after 2 hours of shaking the above described procedure was used.
6. 5 ml of concentrated nitric acid were added to extract the residual metals. The tubes were heated to 120°C until dryness. 5.4 ml of 1 M nitric acid was used to redissolve the residue. The concentration of copper was measured in the liquid phase without further treatment.

Copper concentrations were determined using graphite furnace AAS. Standards were prepared in the same matrix as the solutions. To check the reliability of the procedure, total copper concentrations were determined by destruction of the sediments by a mixture of equal volumes of nitric acid, hydrochloric acid and hydrofluoric acid. The total concentrations in sediments determined in this method, were compared to the sum of the concentrations obtained from each individual step in the extraction procedure.

Preliminary extractions had shown that during the first and second step no measurable amounts of copper were released; during the third step small amounts were extracted in only a few samples. Since, as far as known, changes in the extraction pattern by contact with air do not occur for the remaining steps of the extraction scheme, exposure to air was not avoided.

The sum of the concentrations released in each individual step was in

	depth (cm)		
sample	0 - 2	2 - 5	5 - 10
A	0.3/56.3/18.9/24.5 (286)	0.0/51.5/36.7/11.8 (78)	0.0/32.2/55.4/12.5 (69)
B	0.2/65.5/ 9.1/25.3 (393)	0.2/56.7/10.2/32.9 (277)	0.0/55.6/24.1/20.3 (163)
C	0.5/55.6/ 2.9/41.0 (345)	5.1/48.0/24.7/22.2 (146)	0.0/36.9/43.5/19.6 (225)
D	0.6/59.2/12.5/27.7 (605)	8.6/51.9/11.9/27.6 (424)	4.3/47.2/ 5.2/43.3 (243)
E	0.1/64.8/17.1/17.9 (577)	0.0/65.3/13.9/20.9 (485)	0.2/64.2/ 4.3/31.3 (475)
F	0.0/60.7/20.9/18.4 (666)	0.0/35.3/46.9/17.8 (185)	0.0/30.2/61.5/ 8.3 (158)

Table 2.3. Results of the sequential extraction scheme. For steps 3 to 6 the percentages of released copper are shown. Between brackets the sum of the copper concentrations in nmol of copper per gram dry sediment are displayed.

good agreement with the total copper measurements, generally within 10%. Results of duplicate extractions displayed variations of usually less than 10%.

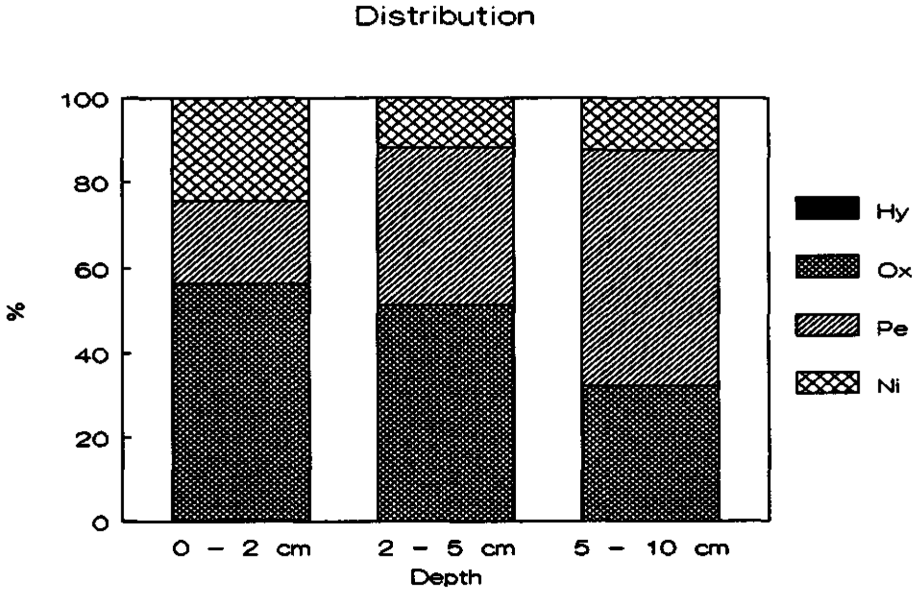

Figure 2.3. Distribution of extracted copper at sampling point A in percents; Hy = hydroxyl amine (hardly visible); Ox = oxalate; Pe = peroxide; Ni = nitric acid.

A typical example of extraction results is displayed in figure 2.3. Table 2.3 contains all the results. The total copper concentrations in the sediments clearly decrease with depth. Extraction with hydroxyl amine hardly releases any copper from the sediments. The fraction of copper released by extraction with oxalate gradually decreases with depth, while the fraction extracted by hydrogen peroxide increases with depth for most samples. The residual fraction displays a varying pattern. The observation that fairly aggressive chemicals are necessary to release any copper at all implies that copper in sediments of Lake Tjeukemeer is strongly bound.

EDTA copper extraction was performed as described by Keizer, Van Riemsdijk & De Haan (unpublished results). To 1 gram of dried sediment 3 ml of a 0.02 M calcium nitrate solution were added. The solution was equilibrated with the sediment by shaking for 80 hours. 1 ml of a solution of a 5 mM Na_2H_2EDTA solution (pH = 7) was added to extract copper; to blanks 1 ml of the calcium nitrate solution was added. After centrifugation, the total copper concentrations were determined using AAS, the amount of copper released from the sediment was calculated after blank correction, and compared to the total concentrations as determined using the mixed acid destruction method. It appeared that only 10% of the copper in the sediment could be extracted this way.

The conclusion drawn from these experiments is that the copper present in sediments of Lake Tjeukemeer was rather strongly bound. Copper (re-)mobilization from the sediments to the water is therefore probably not very important.

Chapter 3

Ultrafiltration

3.1. Discussion of the technique

Ultrafiltration is a technique that may be used to fractionate components of natural waters. Unlike many other speciation techniques, its use is not only limited to a few elements, but it may be applied to determine size fractions of any element provided its total concentration can be determined by some method. Ultrafiltration has been used in for example the characterisation of organic carbon in natural waters (Buffle, Deladoey & Haerdi, 1978), and to gain an insight into the size of copper complexing ligands (Giesy & Alberts, 1989; Alberts, Giesy & Evans, 1984; Giesy, Alberts & Evans, 1986; Benes, Gjessing & Steinnes, 1976; Buffle & Staub, 1984; Giesy & Briese, 1977; Hoffmann et al., 1981). The first three papers in the above list demonstrated that large amounts of organic carbon were small, i.e. not more than a few nm, and may be responsible for complexing copper. Since inorganic ligands were also found in the smallest fraction, these may also complex copper. Buffle & Staub (1984) showed that copper was complexed by small organic ligands.

It is remarkable that only a few workers have investigated the behaviour of metals using ultrafiltration. Benes & Steinnes (1974) and Benes, Gjessing & Steinnes (1976) have: they were able to draw conclusions about which metals were likely to be associated with humus and which were not, but for some reason or another they did not include copper in their research. Giesy & Briese (1977) investigated the size of copper complexes, and found that 70% were smaller than 3.2 nm, but no copper species smaller than 0.9 nm were detected. This is remarkable since more than 70% of organic carbon was smaller than 0.9 nm. The authors state that it is not possible to inorganically complex copper at the pH (3.5) and redox (55 mV) conditions of their sample, thus the copper must be either free or organically complexed or both. The ratio Cu/C was higher in the larger fractions, which can only be explained by a different activity of carbon in the various size fractions. Hoffmann et al. (1981) found that more than half of the copper present was in species with molecular weight of less than 10000, and a small fraction with weights of even less than 1000. Carbon displayed a similar behaviour. Baccini & Suter (1979) found that 30% of Cu had a molecular weight less than 1000 and the remainder less than 10000. Small carbon species could complex more Cu per gram C than larger species.

Since the data obtained by Giesy & Briese are given in nm units, while the others are reported in molecular weights, the sizes obtained by Giesy &

Briese will have to be converted in cut-offs. If copper species behaved like globular proteins, 70% would have a molecular weight less than 50000, while no copper species less than 500 units would be present. The conclusion from the work of Giesy & Briese (1977), Hoffmann et al. (1981) and Baccini & Suter (1979) is that some copper complexes have molecular weights below 1000, while a substantial fraction of copper complexes has weights of less than 50000, and possibly less than 10000.

The concept of ultrafiltration is simple. Nevertheless, some problems may frustrate the application of ultrafiltration. First of all, the relationship between pore size and molecular weight cut-off is not linear. This is because the shape and charge of a molecule determine the actual cut-off, given the pore size and degree of hydrophobicity of the filter (Hoffmann et al., 1981). In addition, the chemical nature of complexes is not simply made clear: Giesy & Alberts (1989) e.g. found copper, organic and inorganic ligands in small fractions. They could not come to any definite conclusions as to the complexation of copper. Statistical relationships between the size fraction and the occurrence can be calculated for copper and ligands, but high correlations do not prove associations; at most, they give strong indications.

Adsorption of all kinds of elements onto the filter may also be a source of error (Benes & Steinnes, 1974; Buffle, Deladoey & Haerdi, 1978). Furthermore, the speciation in the sample may change during the experiments because of the long time periods needed to perform the filtration and the increase in concentration of species too large for the applied pore size (Benes, Gjessing & Steinnes, 1976; Buffle & Staub, 1984; Hoffmann et al., 1981; Sojo & De Haan, 1990).

The greatest risk in using ultrafiltration is probably contamination. Salomons & Förstner (1984), Kramer (1985) and Mart (1979a) warn about the severe risk of contamination of samples when they are filtered. It is evident that at very low copper concentrations ultrafiltration is even more vulnerable to contamination (and in fact becomes extremely difficult), since ultrafiltration requires more time (increasing exchange between solution and vessel), while concentrations are lower. Benes & Steinnes (1974) and Giesy & Briese (1977) point out this risk explicitly.

Since this project focused on free copper, with a size of about 0.6 nm (Morel, 1983), it is obvious that most attention should have been paid to the fraction < 0.6 nm. The smallest pore size used, however, was 5 nm, so the smallest filtrate could possibly contain, besides all of the free copper, considerable concentrations of copper complexes. As a consequence, the copper concentration in the 5 nm filtrate will only allow a limited insight into the concentration and proportion of free copper in Lake Tjeukemeer. It was therefore considered more useful to focus attention on the fractions > 5 nm, since here one could be sure that the copper present in those fractions, is present as some copper complex.

3.2. Materials and methods

Amicon equipment was used in combination with Schleicher & Schüll filters BA 83 (< 200 nm), AC 64 (< 35 nm), AC 63 (< 20 nm), AC 62 (< 10 nm) and AC 61 (< 5 nm). A gauge pressure of 1.75 atm (AC 64) to 5.25 atm (AC 61) was applied using 99.8% nitrogen gas. After sampling, the procedure was as follows: two litres of lake water were filtered over BA 83; the first 100 ml were discarded, 300 ml of the remainder set aside for analyses. The remaining 1600 ml was filtered over AC 64; again the first 100 ml were discarded and 300 ml used for analyses. The remaining 1200 ml were filtered over AC 63 and so forth. During filtration the sample was stirred continuously. A disadvantage of this sequential procedure is that, once contamination has been introduced into the sample, all succeeding filtrates will also be contaminated. The practical consequence of this fact is that once the first ultrafiltrate has a higher concentration than the 0.2 µm filtrate, one can not be sure if this is caused by contamination during the filtration process, or during the (pretreatment for the) total copper determination. Since one must take into account the possibility that the filtration process itself contaminated the sample, measurements in all subsequent filtrates should be rejected. The alternative to the sequential procedure, using raw lake water for all ultrafilters, is however not very practical, since it is excessively time consuming to filter water with an often high particle loading through filters with pores of only a few nm. Besides, filters probably would have to be changed during one filtration session, which implies an extra possible source of contamination.

Copper was measured according to the procedure described in section 2.2, but no duplicate determinations were performed.

In order to eliminate contamination the equipment and filters were thoroughly cleaned with acid, followed by intensive flushing with Milli-Q. Experiments were carried out in a "clean" room (see also section 1.6).

3.3. Results and discussion

Since contamination could be considered the greatest problem in ultrafiltration, results will have to be examined very critically. One way to do this is to compare the starting material for the ultrafiltration, the 0.2 µm filtrate, with the "routine" 0.2 µm filtrate (see section 2.2). These filtrates should contain the same copper concentration, but since they were prepared separately, they can be used to eliminate some unreliable data. At these low concentration levels, an uncertainty of 10% should be accepted for each measurement at the present state of the technique, so a ratio between 0.8 and 1.2 indicates reliable starting material. Table 3.1 shows that six cases

DATE	[Cu] in "routine" samples (nM)	[Cu] in ultrafiltration samples (nM)	ratio
29 Sep 87**	19	16	1.2
8 Dec 87*	30	41	0.7
12 Jan 88	85	134	0.6
8 Mar 88**	36	35	1.0
3 May 88*	54	74	0.7
31 May 88**	38	41	0.9
28 Jun 88	162	42	3.8
6 Sep 88	27	14	1.9
18 Oct 88	42	28	1.5
15 Nov 88	30	20	1.5
8 Feb 89	36	27	1.4
8 Mar 89	36	20	1.8
5 Apr 89**	35	33	1.0
31 May 89**	39	36	1.1
10 Oct 89		14	
15 Nov 89**	9	8	1.2
		average:	1.36
		standard deviation:	0.75
		number:	15

Table 3.1. Comparison of < 200 nm filtrates prepared for ultrafiltration with "routine" filtrates.

satisfied this condition (marked with two asterisks) while two cases almost did so (one asterisk).

Another means of checking for contamination is to see if the copper contents of each ultrafiltrate is lower than or equal to the copper concentration in the filtrate of the next bigger pore size. Since the variation in concentration measurements increases percentually as the concentrations decreased, and approached an absolute level of 3 nM, a difference of 6 nM was considered acceptable. From table 3.2 and figure 3.1 it becomes clear that only five series (marked with a "-") were demonstrably contaminated.

Even after this critical examination five series turned out to satisfy both conditions (marked in table 3.2 with an asterisk). The average copper concentrations of these five gradually decreased down to 19 nM in the 5 nm filtrate, being 56% of the concentration in the 200 nm filtrate.

A quite different approach to judge these results would be to suppose that contamination would be stochastically divided over all samples. If there are enough samples, the average copper concentrations in each size class would be raised by the average contamination, but the absolute decrease in copper concentration, going to a smaller pore size, would not be influenced. Indeed, both series of averages display the same pattern (table 3.2), except for the smallest pore size. This would suggest that the averages of the five selected series were too high for this size class. The percentage of copper in the smallest fraction for all series is 42%, slightly lower than the figure for

DATE	[Cu] < 200 nm	[Cu] < 35 nm	[Cu] < 20 nm	[Cu] < 10 nm	[Cu] < 5 nm
29 Sep 87*	16	16	13	14	14
8 Dec 87*	41	25	25	17	22
12 Jan 88	134	27	25	19	
8 Mar 88*	35	38	22	16	8
3 May 88-	74	132	44	71	31
31 May 88*	41	35	38	17	22
28 Jun 88	42	33	14	6	11
6 Sep 88-	14	16	25	20	16
18 Oct 88	28	28	24	16	19
15 Nov 88	20	20	20	11	11
8 Feb 89-	27	30	28	35	8
8 Mar 89-	20	38	24	8	6
5 Apr 89-	33	35	58	57	6
31 May 89*	36	41	30	30	30
10 Oct 89	14	14	19	11	9
15 Nov 89	8	14	6	5	
average	36	34	26	22	15
st. dev.	29	27	12	18	8
number	16	16	16	16	14
average*	34	31	25	19	19
st. dev.*	9	9	8	6	8
number*	5	5	5	5	5

Table 3.2. Ultrafiltration results in nM; "st. dev." is standard deviation.

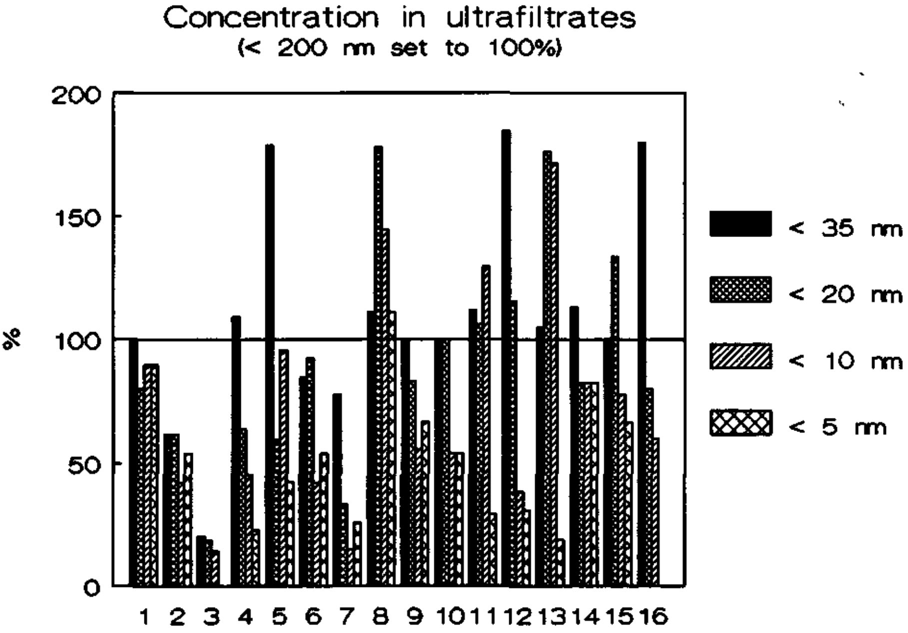

Figure 3.1. Ultrafiltration results. Numbers refer to successive ultrafiltration experiments.

Reference	Result	
Giesy & Briese (1977)	70%	< 3.2 nm
Baccini & Suter (1979)	bulk	< 0.9 to 1.5 nm
Hoffmann et al. (1981)	> 50%	< 1.5 nm
This thesis	50%	< 5 nm

Table 3.3. Comparison of ultrafiltration results.

the selected five series.

Both approaches displayed a consistent pattern: about half of copper complexes smaller than 200 nm were larger than 5 nm, which was quite large, bearing in mind that other workers found most of organic carbon to be very small. If the molecular weight cut-offs of Hoffmann et al. (1981) and Baccini & Suter (1979) are converted to size classes using the conversion factors mentioned by Giesy & Briese (1977) for globular proteins, the results as displayed in table 3.3. are obtained. Values obtained here for Lake Tjeukemeer are comparable to results of other workers who used the same technique.

Chapter 4

Voltammetric techniques

4.1. Anodic stripping voltammetry

4.1.1. Discussion of the technique

Anodic stripping voltammetry (ASV) is probably the most widespread technique in speciation studies (see e.g. Batley (1987), Cleven, Del Castilho & Wolfs (1988), Davison et al. (1987), Florence (1986), Florence, Lumsden & Fardy (1983), Kramer (1986) and Nürnberg (1984)).

The principle of polarography and voltammetry is recording the current at a working electrode as a function of the applied potential.

The theory of ASV and related voltammetric and polarographic techniques will not be treated here, since this is not a methodological thesis, and this theory is described elsewhere (e.g. Bond, 1980; Cleven, 1984). Two items should however be mentioned: the faradaic versus the nonfaradaic processes, and the types of mass transport in the measurement cell.

Nonfaradaic (or "charging") current is caused by changes in the charge of the layer around the electrode without transfer across the electrode-solution interface. If such a transfer does occur, the resulting current is called faradaic. Using modern techniques and instrumentation, it is possible to decrease the influence of undesired nonfaradaic processes, resulting in better achievements.

Provided the transport of metal ions and thus the current is diffusion-controlled *during the measurement, the current is proportional* to the concentration of the species being measured. To reach this situation, an excess of inert electrolyte is added to effectively eliminate migration current.

A clear advantage of ASV is its low detection limit and high sensitivity. Interpretation of ASV-measurements, however, is not easy because of the complexity of the technique (Bond, 1980; Cleven, Del Castilho & Wolfs (1988); Betti & Papoff (1988); Van Leeuwen, Cleven & Buffle (1989)).

The main advantage of ASV, in the context of this thesis, is the suggested relationship of metal detected by ASV with biological availability (Salomons & Förstner, 1984; Florence, 1986).

The most important disadvantage of ASV is that it is only operationally defined: a species which is detected ("labile") under certain circumstances may be not detectable under different conditions. Among the factors controlling the ASV-lability of a species are deposition time and potential, the area of the working electrode, the stirring speed during deposition, the pH of the solution, and complexing characteristics of the buffer (if used).

At a pH of 5, all inorganic copper species are determined, while organic copper species and colloidal copper are not (Batley, 1983, as cited in Salomons & Förstner, 1984; Florence, 1986). Some organic complexes may also dissociate according to Betti & Papoff (1988).

Each ASV-measurement starts with the "deposition step", during which a sufficiently negative potential is applied to reduce the species under study and to concentrate it in the working electrode (usually a mercury drop) where an amalgam is formed. This implies that only metals which react with mercury can be successfully determined. In addition, a kinetic aspect is introduced, since the amount of metal deposited in the mercury drop depends on the reduction rate. If a species is not reduced during the deposition step, it will not be detected, no matter its thermodynamic properties. During the deposition step, the solution is continuously stirred to exclude concentration gradients caused by removal of species at the mercury drop. After deposition, an anodic scan strips the metals out of the mercury, thereby increasing the current. A differential pulse is usually used to reduce the effects of charging currents.

4.1.2. Materials and methods

In this work a 384B polarograph (EG & G Princeton Applied Research) was used with a 303A electrode in the hanging mercury drop mode and a 305 stirrer (400 rpm). Results were displayed on a plotter (model DMP-40 Houston Instruments). Nitrogen gas (99.999%) was used for purging. All potentials were recorded against an Ag/AgCl reference electrode. A platinum wire served as counter electrode.

parameter	ASV	CSV
purge time	240 seconds	240 seconds
drop size	medium	large
deposition time	500 seconds	180 seconds
deposition potential	-0.6 V	0 V
equilibration time	15 seconds	15 seconds
wave form	differential pulse	square wave
pulse height	0.05 V	0.1 V
scan speed	0.004 V/second	0.720 V/second
frequency		90 Hz

Table 4.1. Settings used in voltammetry measurements.

Ion	Concentration
Na^+	0.91 mM
Mg^{2+}	0.26 mM
K^+	0.27 mM
Ca^{2+}	0.95 mM
HCO_3^-	0.91 mM
SO_4^{2-}	0.40 mM
Cl^-	1.91 mM

Table 4.2. Composition of standard solution used for calibration.

A portion of four ml of filtered (< 0.2 µm) lake water was mixed with 1 ml of 0.5 M acetate buffer (0.25 M sodium acetate plus 0.25 M acetic acid; the resulting pH of the sample was 5.0) and equilibrated overnight. Under these conditions, and taking into account ligand concentrations in Lake Tjeukemeer, virtually no inorganic copper species were present. Any inorganic copper present before buffering the sample will have dissociated to form (ASV-labile!) copper acetate complexes, as calculated by the equilibrium model GECHEQ (chapter 7). In fact, a buffer that does not form complexes with copper must be preferred.

The measurements were performed using the settings mentioned in table 4.1. The deposition time was quite long (500 seconds), but shorter times too often resulted in the deposition of copper in the mercury drop in quantities too low for detection. Calibration was carried out in a standard solution, with concentrations of Na^+, Mg^{2+}, K^+, Ca^{2+}, CO_3^{2-}, SO_4^{2-} and Cl^- resembling lake water as closely as possible, as recommended by Florence (1986). Refer to table 4.2 for details. Dissolved Organic Carbon (DOC) was analyzed with a Phase SEP TOCsinII carbon analyser. Optical density (OD) at 250 and 365 nm was measured on a Zeiss PMQII spectrophotometer.

4.1.3. Results and discussion

The results are shown in figures 4.1 and 4.2. It is obvious that ASV-labile copper was low, usually below 2 nM. Expressed as percentage, ASV-labile copper never exceeded 9 percent of the "dissolved" copper (defined as < 0.2 µm).

Quantification of labile copper was very easy in some cases, since the polarograph reports the current if a peak is recognized. In other cases, the peak was too small to be detected by the polarograph, but could be quantified manually. In yet other cases, no peak was observed. In these cases, it was estimated as to how big the peak should have been in order to be quantifiable.

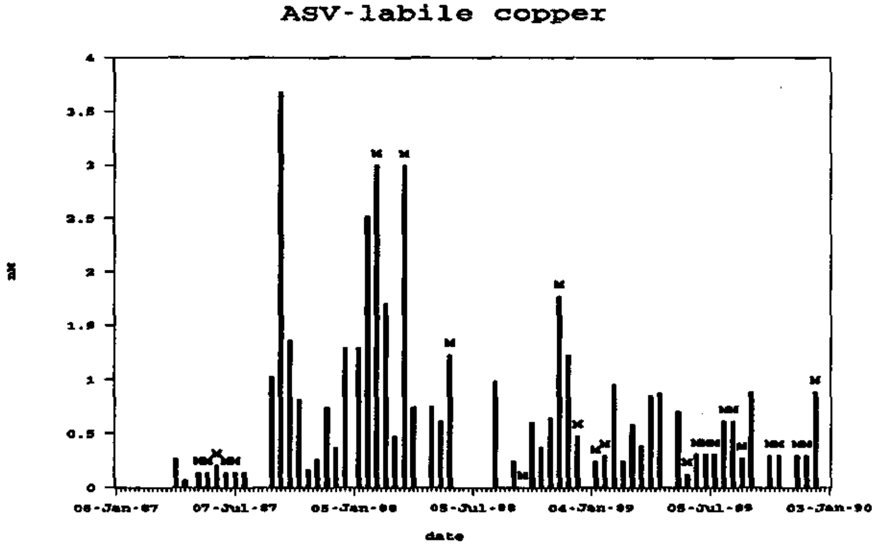

Figure 4.1A. ASV-labile copper in nM; "M" indicates that labile copper was below "lowest quantifiable concentration" which is shown instead.

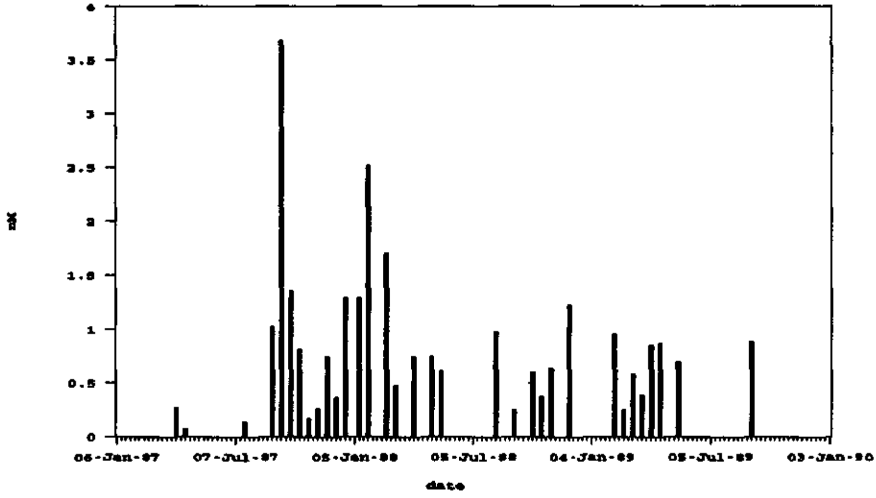

Figure 4.1B. ASV-labile copper in nM; only quantifiable concentrations shown.

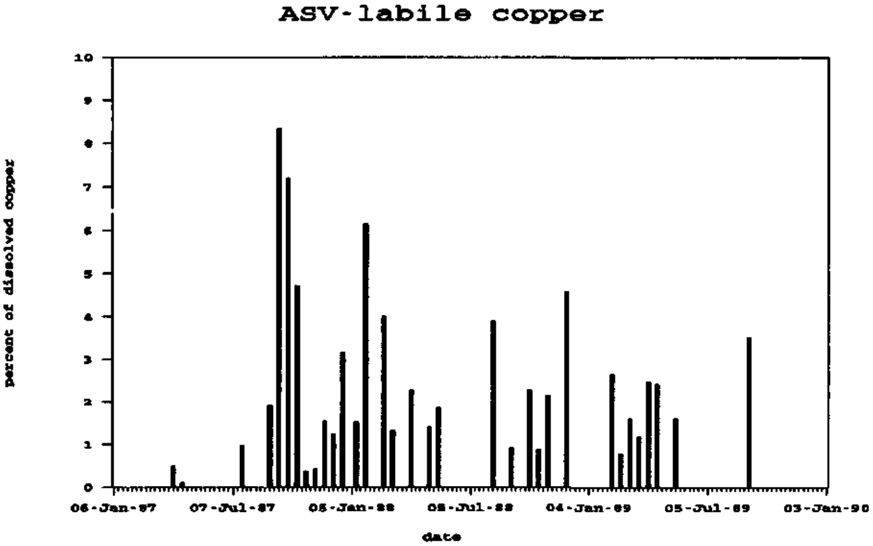

Figure 4.2. ASV-labile copper expressed as percentage of dissolved copper.

Thus a "lowest quantifiable concentration" could be calculated. This concentration varied considerably over the course of the year due to greatly varying background currents. This variation was not caused by the technique as such, since duplicate measurements in the same sample displayed nearly the same background current with only a tiny variation, resulting in small variation for the lowest quantifiable concentration of labile copper, usually less than 10%. So the variation in the course of the year must be caused by variation of concentration and composition of the organic material in the lake water during the year. Indeed, this variation is considerable. For 1989, dissolved organic carbon displayed a standard deviation of 26% (n = 25), OD 250 of 41% (n = 47), and OD 365 of 48% (n = 47). The mechanism by which the organic material influences the background current, and hence the lowest quantifiable concentration labile copper, must be by adsorption to the mercury drop (Buffle, 1980).

The values which could be quantified are shown in figure 4.1B. In 1987, four replicate measurements were performed in each sample. In some cases, in the first sample a significantly higher current was measured, probably due to contamination of the measurement cell. The other three always showed a remarkably small variation (not more than a few percent). Because of this only two samples were measured from 1988 on, and the first measurement was ignored if its current was higher than measured in the second sample. Thus the variation in figure 4.1B is caused by variation in the labile copper concentration and not by contamination.

Inorganic copper species (including free copper) present in lake water samples initially will react to form copper acetate complexes, as already stated in section 4.1.2. Some natural organic copper species may also dissociate as a result of the pH (lower in the buffered solution than in the original), the negative potential (-0.6 Volt), the competition with acetate, or by a combination of these three factors. The rest, 92 - 100%, of copper apparently is present in organic complexes that do not dissociate at all under the applied conditions. Under natural conditions these percentages will be higher. The conclusion is that at least 92 - 100% of copper in Lake Tjeukemeer is organically complexed.

Although ASV has been intensively used in speciation studies, attention has been mostly paid to determining copper complexation parameters, instead of measuring labile copper without adding metal. Van der Werff (1986) did not detect labile copper in filtered samples of Lake IJsselmeer, but after the addition of as much as 400 nM of copper, labile copper was 30 nM. Thus under natural conditions labile copper must have been below 30 nM. Van der Werff performed these measurements at pH = 4.6. Del Castilho & Salomons (1986) measured values between 3 and 9 nM in the same lake at the same pH. Batley & Florence (1976) found 4 nM for seawater at pH = 4.8, using a rotating electrode. Values found here are slightly lower than literature data, probably due to the high concentration of organics in the lake. Kramer (1985) however reports labile copper concentrations of the same order of magnitude, but measured at a quite different pH: 3 to 9 nM labile copper in seawater at a pH of 8.1, and 8 to 19 nM in riverwater pH = 7. Labile copper in these samples was definitely higher than in Lake Tjeukemeer, where none would have been detectable at such pH values.

It is interesting to examine if ASV-labile copper is correlated with the concentration of organics. If this were so, [CuL]/[L] (where L is organic ligands) is proportional to free copper, provided the simple equilibrium relationship $[CuL] = K * [Cu] * [L]$ is valid. If free copper is supposed to control ASV-labile copper, correlations of ASV-labile copper with [CuL]/[L] must be calculated. The [CuL] and [L] are both unknowns, but since the concentration of organic carbon is very high, [CuL]/[L] simplifies to [total copper]/[total organics]. Dissolved organic carbon and optical densities at 250 and 365 nM were used to represent "organics". No significant correlations were found on a $p = 0.1$ level. This implies that although more than 90% of copper is organically complexed, the parameters used to represent organics, are not specific enough to obtain information on organic copper complexation. Obviously one or more functional groups, not strongly correlated with DOC or OD 250 or OD 365, control(s) organic copper complexation.

4.2. Cathodic stripping voltammetry

4.2.1. Discussion of the technique

Cathodic stripping voltammetry (CSV) has only recently been introduced in metal speciation research (Van den Berg, 1984a, 1984b, 1984c, 1986). Until then, CSV was used for determining anions (e.g. Peterson & Wong, 1981) or total metal concentrations (e.g. Wang, 1985). The principle of the method is as follows: a synthetic ligand is added to the sample and forms a complex that specifically adsorbs to the mercury drop during the deposition step. When the potential is lowered the metal in the complex is reduced. The formation of an amalgam is not a necessary step in this procedure, so the use of CSV is not limited to elements reacting with mercury.

Van den Berg proposed catechol (1,2-dihydroxybenzene) as a suitable reagent for copper speciation research (1984a). Catechol is added to the sample in the measurement cell, and the deposition time immediately starts, followed by the equilibration time and scan. Thus a clear kinetic element is introduced in the definition of "CSV-lability": complexes which do not dissociate fast enough will not contribute to the current. Besides, some complexes are so stable that they will not dissociate at all upon catechol addition, and will also not contribute to the current. So the main complexes of interest for CSV are those that dissociate fast enough to form copper catechol complexes.

An advantage of CSV over ASV is that in the case of copper a deposition potential of 0 V (against Ag/AgCl) can be used, so no externally forced redox-reactions occur. In addition, this technique works well at the pH's of 7 and higher, relevant for Lake Tjeukemeer. This was the main reason to use this technique.

4.2.2. Materials and methods

The equipment used is described in section 4.1.2. After Van den Berg, HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid) was used as a buffer. It was chosen because it buffers at a suitable pH ($pK_a = 7.55$; Good et al., 1966) and negligibly acts as a ligand for Mg^{2+}, Ca^{2+}, Mn^{2+} and Cu^{2+} (Good et al., 1966). A stock solution of 1 M HEPES was prepared in 0.5 M NaOH solution, and added to the filtered lake water sample up to a concentration of 7 mM. After equilibration overnight, the sample was purged, spiked with 75 µM of catechol, and the measurement was performed immediately. At this catechol concentration, more than 95% of inorganic copper will dissociate in an equilibrium situation to form copper catechol complexes. It was not very likely that equilibrium was reached, because the deposition time was only three minutes. Catechol solutions were freshly prepared every day because of reported instability of catechol solutions (Van den Berg, 1984a). Calibrations were performed in standard solutions, as for ASV.

A square wave form was used instead of the differential pulse wave form (as Van den Berg did) in order to discriminate against charging current (even more than differential pulse), and because of its high sensitivity and scan speed. Table 4.1 contains the measurement parameters.

4.2.3. Results and discussion

The CSV-results are presented in figures 4.3 and 4.4. The same procedure as used for ASV was used, i.e. if no peak was observed, a "lowest quantifiable concentration" was calculated. As for ASV, background currents varied considerably during the year.

Only a few measurements gave reliable information: usually the CSV-labile copper was not quantifiable or made up more than 100% of "dissolved" copper! However, measuring the same sample twice usually showed good reproducibility.

If labile copper could be quantified, the addition of a small amount of copper often resulted in a smaller current. This incomprehensible phenomenon has also been observed by other workers using a differential pulse wave form (Del Castilho, personal communication).

All these problems make it virtually impossible to draw conclusions from these measurements. At least three explanations can be formulated for the explanation of the observed difficulties:

a. the use of square wave instead of differential pulse;
b. the fact that catechol is not in equilibrium with the solution;
c. the high organic carbon content of Lake Tjeukemeer.

ad a. Square wave.

To test if the problems were caused by the use of square wave, other techniques such as differential pulse, linear sweep, direct current and cyclic voltammetry were applied. For all five techniques the measurement parameters were optimized in lake water samples. For each technique a copper addition series was made to test the current versus addition behaviour. All techniques showed serious shortcomings. However, in seawater the square wave form worked well, while differential pulse gave strange results (Verweij, 1988). The square wave form in Milli-Q water also worked well. The use of the square wave technique therefore seems not to be the cause of the problems.

ad b. Equilibria with catechol.

Although the concentration of catechol was quite high, it could be that

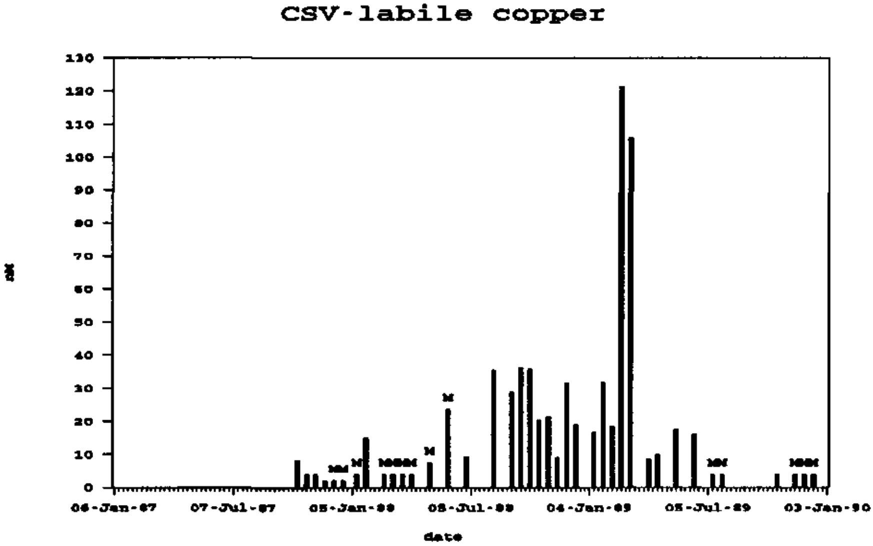

Figure 4.3A. CSV-labile copper in nM; "M" indicates that labile copper was below "lowest quantifiable concentration" which is shown instead.

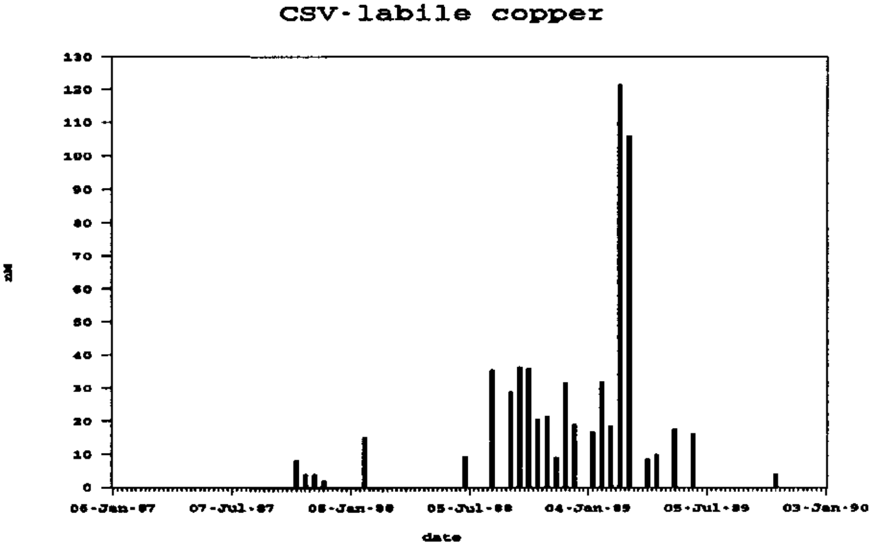

Figure 4.3B. CSV-labile copper in nM; only quantifiable concentrations shown.

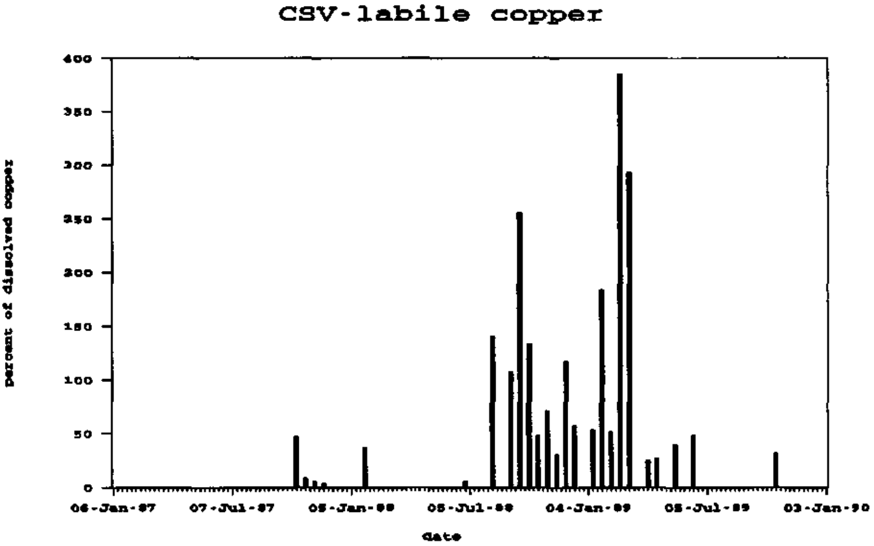

Figure 4.4. CSV-labile copper expressed as percentage of dissolved copper.

equilibria with catechol are not established within three minutes. The technique therefore strongly relies on association rates of catechol complexes and dissociation rates of other complexes. An instable system will result, possibly influencing measurements. In the literature, however, no indications were found that this may cause problems. Therefore, it is assumed that the non equilibrium situation probably does not give rise to problems.

ad c. Organic carbon.

Some organics adsorb onto mercury (Buffle, 1980). In a method depending on adsorption, interferences can be expected in samples with high organic carbon contents, like Lake Tjeukemeer. Gerringa (1990) also had problems measuring labile copper in samples rich in organic carbon. According to Del Castilho (personal communication) problems occur if organic carbon concentrations exceed 0.1 mM. In Lake Tjeukemeer, typical values for dissolved organic carbon are 1.7 mM!

The high organic carbon concentration in Lake Tjeukemeer therefore would seem to make it impossible to apply CSV successfully. The results obtained can not be considered reliable.

Chapter 5

Ligand competition techniques

5.1. MnO_2

5.1.1. Discussion of the technique

Although no techniques are available that measure Cu^{2+}_{aq} directly at concentration levels as low as in Lake Tjeukemeer, a group of techniques uses a combination of measurements and equilibrium calculations to assay Cu^{2+}_{aq} indirectly, i.e. the ligand competition techniques. The principle of these techniques is as follows: two copper addition series of the sample are prepared, one with a synthetic ligand addition. After equilibration, copper is measured using a method which does not detect the complex of copper with the synthetic ligand. By subtraction, the concentration of the complex can be calculated. The equilibrium constant K of the complex must be known, as well as the free ligand concentration. Using appropriate equilibrium relationships $[Cu^{2+}_{aq}]$ can be calculated.

The advantage of these techniques is evident: they give information on the free copper concentration or activity itself, rather than on a group of species more or less proportional to free copper, as described in earlier chapters. Since free copper is assumed to control the copper availability to algae, these techniques provide more directly relevant information than techniques discussed earlier in this thesis. A disadvantage of ligand competition techniques is that they rely on calculations which use, sometimes not too well known, equilibrium constants.

The MnO_2 method has been developed by Van den Berg & Kramer (1979a, 1979b) and Van den Berg (1982a, 1982b, 1983). The competing ligand, MnO_2, is a solid onto which metals can adsorb. The MnO_2 is prepared synthetically by a redox reaction of Mn(II) and Mn(VII). Calibration is performed in standards resembling the sample as closely as possible, or in UV-radiated samples (to destroy organic matter). Using the Langmuir equation

$$\Gamma = \frac{\Gamma_{max} * [Cu^{2+}] * K}{1 + [Cu^{2+}] * K} \qquad (5\text{-}1)$$

(where Γ is the amount of copper adsorbed (mole copper/mole MnO_2), Γ_{max} is the maximum value of Γ, $[Cu^{2+}]$ is the concentration of free copper in equilibrium with MnO_2 and K is the conditional stability constant for the adsorption equilibrium), Γ_{max} and K can be calculated. A copper addition series of the sample is used to determine the conditional stability constant and concentration of the ligands. These data can be used to calculate free copper in the samples.

5.1.2. Materials and methods

MnO_2 was prepared as described by Van den Berg (1982b). Mn(VII) and Mn(II) were mixed in a ratio 1:1.5, $Mn(IV)O_2$ formed and subsequently purified. The MnO_2-"concentration" in this stock was 0.05 M. Up to 0.01 M of NaCl was added. The same batch of MnO_2 was used for all experiments. For calibration a standard solution as described in section 4.2.2 was used: 50 ml of it was buffered with HEPES (4 mM; $pK_a = 7.55$); seven copper additions were made up to 15.7 µM; 50 µL of the MnO_2 stock was added, resulting in a MnO_2-"concentration" of 50 µM. Samples were also buffered with HEPES, but no copper was added. Standards and samples were shaken for one hour on a shaking bath, set aside for a week and shaken again for half an hour. When shorter equilibration times were used, no useful calibration data could be obtained. TEFLON bottles were used for these operations to prevent adsorption. Suspensions were then transferred to centrifugation tubes and centrifugated for half an hour. The efficiency of the centrifugation turned out to be good; no increase in [Mn] could be observed using graphite furnace AAS. The supernatant was decanted and acidified to a pH of about 2, as recommended by the manufacturer of the AAS. Dissolved copper was measured on an AAS, either by graphite furnace or by flame depending on the concentration. After acidification, several hours should have elapsed, since measuring too soon caused inexplicable problems with the graphite furnace. We suspect that HEPES was responsible for some matrix effect. This possibility was not investigated further, as after a few hours no problems were observed any longer. To be on the safe side, after acidification samples were equilibrated at least overnight. Γ was calculated by subtraction. Total carbonate was determined by titrating 0.05 M HCl and turned out to be constant for both the calibration standards and lake water samples. The equilibrium program GECHEQ (chapter 7) was used to calculate free copper in equilibrium with MnO_2 in the standards (table 5.1). The percentage of free copper was independent of the total copper, as the ligands were present in excess. Thus Γ and free copper were known for all standards.

It is not obvious that the Langmuir equation is the most appropriate mathematical description of the adsorption process, since it assumes that the adsorbent forms a monomolecular layer on the adsorption surface, and that the desorption rate is proportional to the extent of adsorption. Changes in the surface charge often spoil this last assumption (Barrow, 1979) and may be the

[Free $Cu(II)^{2+}$]	4.62%
[$Cu(II)(OH)^{+}$]	4.11%
[$Cu(II)(OH)_2$]	0.27%
[$Cu(II)(CO_3$]	78.96%
[$Cu(II)H(CO_3)^{+}$]	0.55%
[$Cu(II)(CO_3)_2^{2-}$]	1.17%
[$Cu(II)(CO_3)(OH)^{-}$]	10.12%
[$Cu(II)(SO_4)$]	0.19%

Table 5.1. Speciation of copper in standards as calculated by GECHEQ.

reason that this equation can not always be used satisfactorily to describe the adsorption of metals onto solids (Verweij, 1986).

Van den Berg (1982a) argued that the Langmuir equation could be applied to his results, since only a small amount of MnO_2 was used; he explicitly did not state that adsorption actually occurs in a monomolecular layer. Stroes-Gascoyne, Kramer & Snodgrass (1986) concluded that a normal Langmuir equation can not be used to describe adsorption of copper onto MnO_2, and proposed an "implicit Langmuir model". Their derivation of that model however contains a few errors and disputable assumptions.

In this thesis, data were processed in three ways. The first and second use the Langmuir equation, but differ in its linearisation. The first linearisation is recommended by Van den Berg (1982a):

$$\frac{[Cu^{2+}]}{\Gamma} = \frac{[Cu^{2+}]}{\Gamma_{max}} + \frac{1}{(K * \Gamma_{max})} \qquad (5\text{-}2)$$

This equation should be used with care, since at low metal concentrations Γ is proportional to [M] (which can easily be seen from equation 5-1) resulting in a constant term for the left part of equation 5-2, making it unsuitable as a dependent variable for linear regression. A second linearisation is suggested here:

$$\frac{1}{\Gamma} = \frac{1}{(K * \Gamma_{max})} * \frac{1}{[Cu^{2+}]} + \frac{1}{\Gamma_{max}} \qquad (5\text{-}3)$$

This linearisation can be used at low metal concentrations, but not at high: in that case Γ is nearly constant (Γ_{max}) because of saturation and is therefore no longer suitable for regression calculations.

It is not clear in advance which linearisation should be used, since the value of Γ_{max} determines if a concentration must be considered low or high, so the combination of metal concentration and adsorption maximum determines which linearisation should be used.

If [M] is relatively low, Γ will be proportional to [M]. So adsorption data are processed in a third way, by supposing adsorption is linearly dependent on free metal concentration:

$$\Gamma = \text{constant} * [Cu^{2+}] \qquad (5\text{-}4)$$

It is obvious that Γ is zero when the [free metal] is zero, so regression lines were forced to have a zero intercept.

The Langmuir linearisation methods yield values for Γ_{max} and K, which can be used to calculate free copper in the samples. The linear model gives a value for free copper by simply dividing Γ by the constant mentioned in equation 5-4.

The thus obtained values for free copper are in fact not representative for lake water samples, because all calculations refer to suspensions containing MnO_2. By adding MnO_2 an extra ligand is introduced in the samples, so the free copper concentration is artificially lowered, so the calculated values are lower than natural values. It is possible, however, to convert [free copper] in the presence of MnO_2 to [free copper] in the absence of MnO_2. This can be done as follows. In the presence of MnO_2 the following mass balance equation holds:

$$\begin{aligned}\text{[total copper]} &= \text{[free copper]} + \text{[copper complexes]} + \\ &+ \text{[adsorbed copper]}\end{aligned} \qquad (5\text{-}5)$$

The sum of inorganic complexes can be described as follows:

$$\text{[inorganic complexes]} = \text{[free copper]} * \sum_{i=1}^{n} (K_i[L_i]) \qquad (5\text{-}6)$$

(where $[L_i]$ is the concentration free ligand, K_i the conditional equilibrium constant for the i-th complex, and the summation occurs for all relevant complexes).

For organic complexes such a formula would probably be an oversimplification because complexation and surface charge influence each other and therefore the conditional stability constant may vary. If this simplification nevertheless is made, equation 5-6 can be extended to all complexes, and insertion into equation 5-5 yields:

$$\begin{aligned}\text{[total copper]} &= \text{[free copper]} + (\text{[free copper]} * \sum_{i=1}^{n} (K_i[L_i])) + \\ &+ \text{[adsorbed copper]}\end{aligned} \qquad (5\text{-}7)$$

In equation 5-7, the summation term $\Sigma (K_i[L_i])$ is the only unknown and can therefore be calculated.
Since addition of MnO_2 will not significantly change K_i or $[L_i]$, the value obtained by equation 5-7 can be used in equation 5-8 and 5-9 which are the same as equation 5-5 to 5-7 but are valid only in the <u>absence</u> of MnO_2 (which is the natural situation):

$$[\text{total copper}] = [\text{free copper}] + [\text{copper complexes}] \qquad (5\text{-}8)$$

and:

$$[\text{total copper}] = [\text{free copper}] + ([\text{free copper}] * \sum_{i=1}^{n} (K_i[L_i])) \qquad (5\text{-}9)$$

It should be noted that [free copper] in equation 5-8 and 5-9 is not the same as in equations 5-5 to 5-7, because equations 5-5 to 5-7 refer to a situation <u>with</u> MnO_2, while equations 5-8 and 5-9 refer to the natural situation. In fact, the summation term as calculated by equation 5-7 can be inserted into equation 5-9, thus yielding [free copper] in lake water samples without added MnO_2.

A complication of the MnO_2 method is the possible competition of organics in natural water samples with copper for the same adsorption sites, because this competition does not occur in the standards. If this phenomenon occurs, Γ will be lowered, resulting in a too low value for free copper. Although Van den Berg (1982b) stated that neither organic ligands nor copper complexes adsorb onto MnO_2, Dempsey & O'Melia (1983) showed that fulvic acids may interact with MnO_x. Van den Berg (1984d) later demonstrated that organic copper complexes are not kinetically inert. The implications of this finding for the MnO_2 method are not clear. Weber (1988) stated that both humic acids and their complexes may adsorb onto MnO_2, but unfortunately he did not give any evidence for this statement.

DOC, OD 250 and OD 365 were measured as described in section 4.2.1.

5.1.3. Results and discussion

5.1.3.1. Calibration

A typical example of a calibration curve is shown in figure 5.1. Its good linearity already suggests that equation 5-2 is not a suitable description in this case. Figure 5.2A confirms that by using the Langmuir linearisation as recommended by Van den Berg (1982a) an average correlation coefficient of only 0.1484 (standard deviation = 0.3903) is achieved. For the

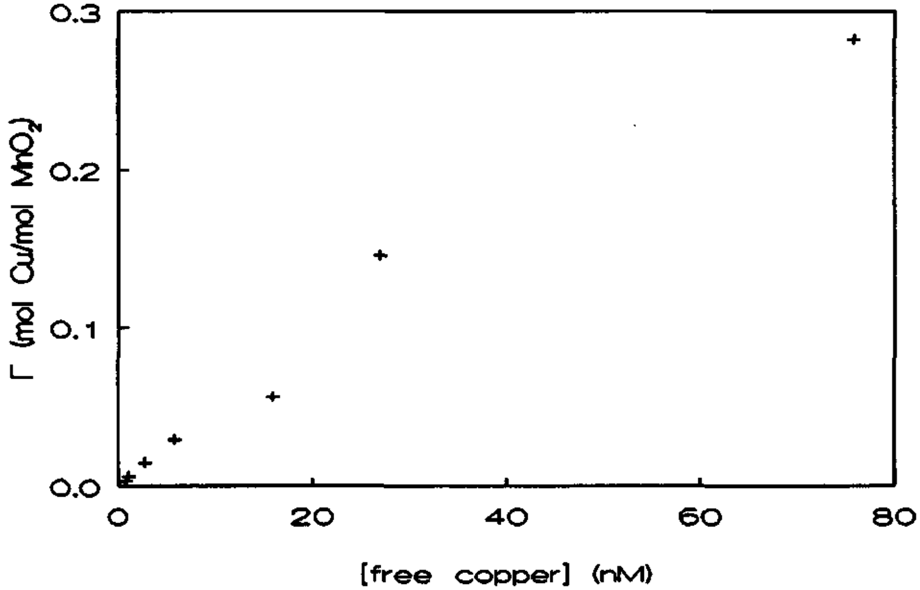

Figure 5.1. Typical example of a calibration curve, showing Γ as a function of [free copper].

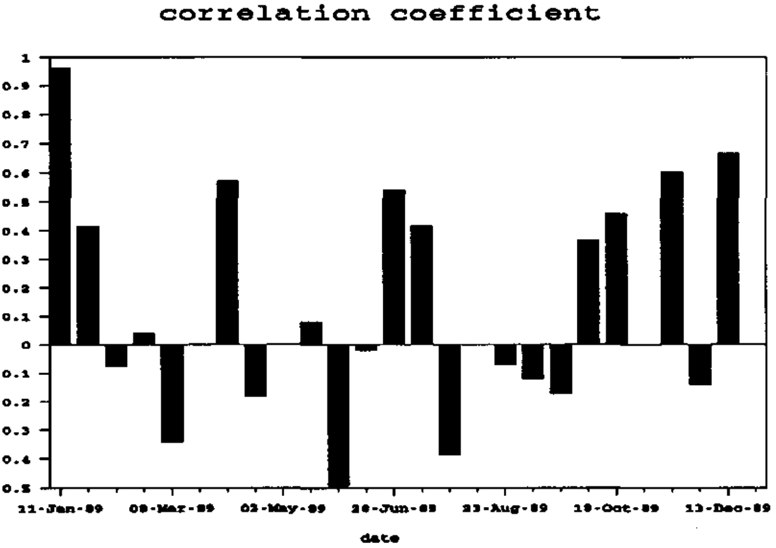

Figure 5.2A. Correlation coefficients linearisation of Van den Berg (1982a).

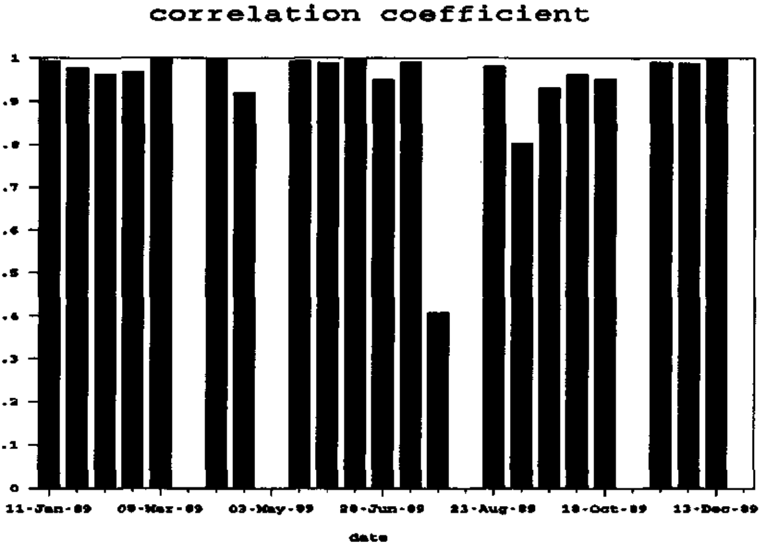

Figure 5.2B. Correlation coefficients linearisation of Verweij (this thesis).

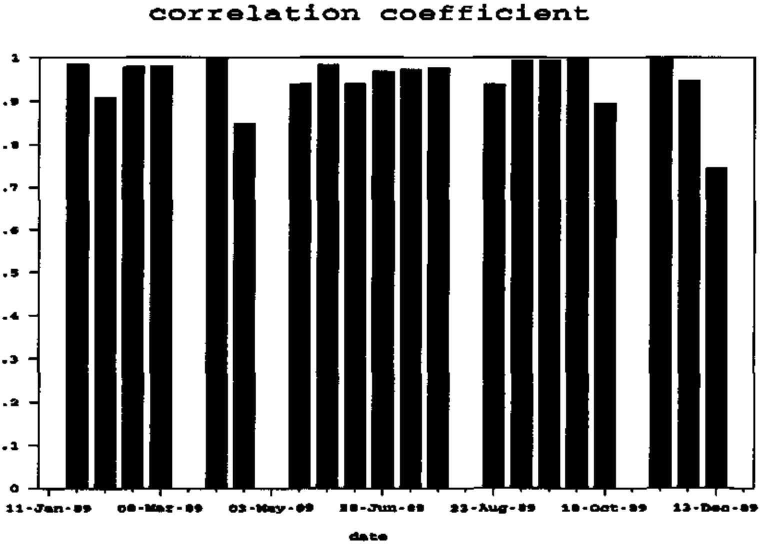

Figure 5.2C. Correlation coefficients linear model.

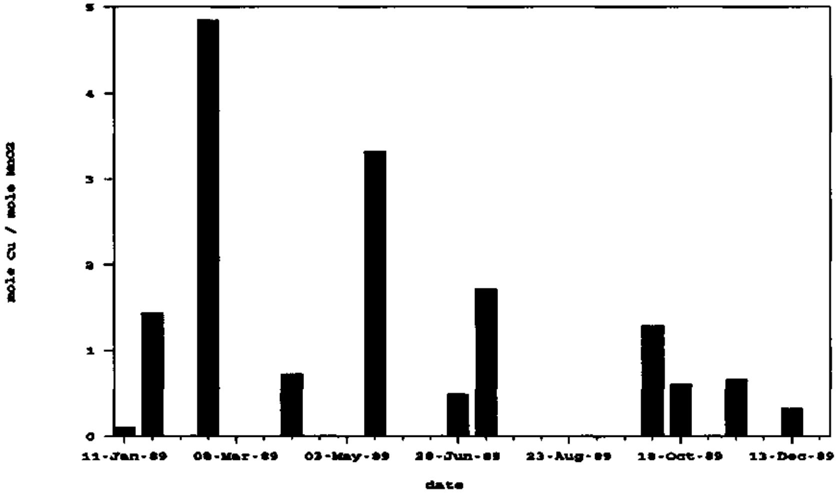

Figure 5.3A. Γ_{max} Langmuir linearisation Van den Berg (1982a).

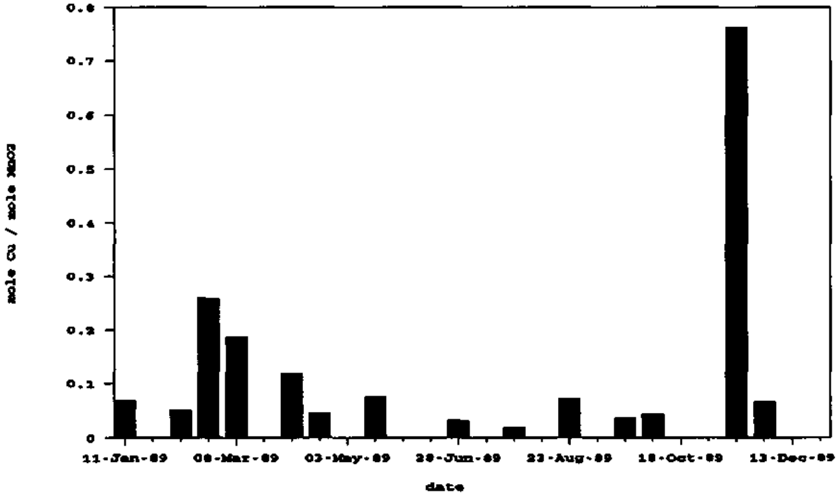

Figure 5.3B. Γ_{max} Langmuir linearisation Verweij (this thesis).

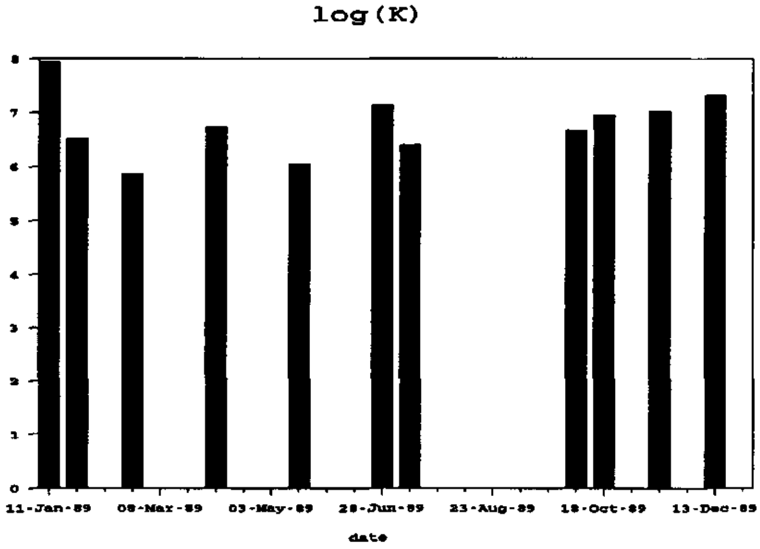

Figure 5.4A. Log(K) Langmuir linearisation Van den Berg (1982a).

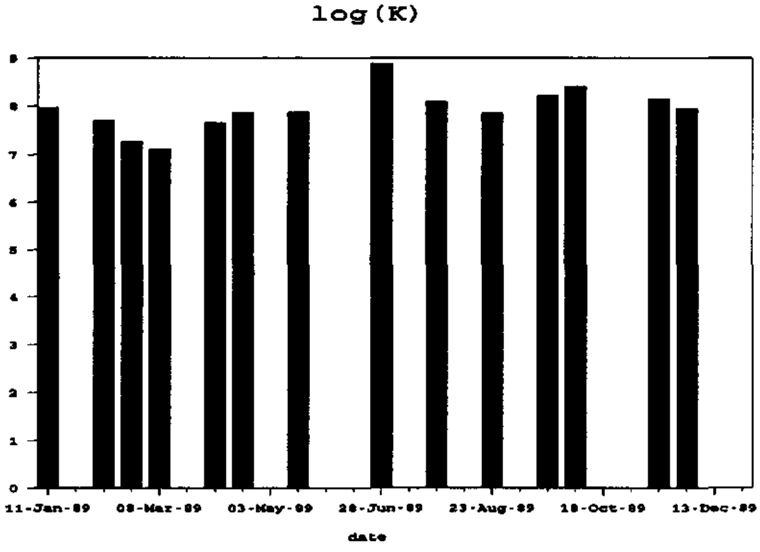

Figure 5.4B. Log(K) Langmuir linearisation Verweij (this thesis).

	Type of linearisation					
	Verweij			Van den Berg		
parameter	Γ_{max}	log(K)	r	Γ_{max}	log(K)	r
average	0.131	7.923	0.9312	1.41	6.781	0.4650
standard deviation	0.187	0.425	0.1471	1.38	0.561	0.2466

Table 5.2. Calibration results for the Langmuir linearisations.

other type of linearisation the average is much higher (0.9401; standard deviation = 0.1265), as can be seen in figure 5.2B, and even increases if calibration obtained on 26th of July (r = 0.4080) is omitted to 0.9667 (standard deviation = 0.0441). Results of the linear model are similar (figure 5.2C): 0.9487 (standard deviation = 0.0608). One calibration line is not included in this average because the square of the correlation coefficient was smaller than zero, making it impossible to calculate the square root. Since data obtained for Lake Tjeukemeer are extrapolations of the calibration curves or line, the linear model should be however preferred.

Both equation 5-2 and 5-3 (the Langmuir linearisations) sometimes give negative intercepts (equation 2 - ten cases, equation 3 - seven), resulting in (negative) values for Γ_{max} and K without physical meaning. Results for good calibration lines are shown in figures 5.3 (Γ_{max}) and 5.4 (log(K)), and table 5.2. Remarkable are the values for Γ_{max} in figure 5.3A (linearisation Van den Berg), i.e. sometimes they are higher than 1 mole Cu/mole MnO_2! For the other linearisation no values higher than one were obtained. Log(K) values were much lower than those reported by Van den Berg (1982b; log(K) = 9 to 9.5) and Gerringa (1990; log(K) = 9.1), while their Γ_{max} values were much lower (0.08 and 0.07, respectively). The reason for this is not clear, but the range of copper concentrations used here was different from the range the cited authors used. Besides, the high standard deviations may indicate that the reproducibility of copper adsorption onto MnO_2 was not too good. Another explanation could be the variation of adsorption characteristics of different batches of MnO_2.

5.1.3.2. Samples

In several cases, copper was not measurably adsorbed or even had increased in concentration during equilibration due to contamination. The other cases are shown in figure 5.5. The correction as described in the previous section (to convert values in the presence of MnO_2 to values in the absence of it) has not been applied to these data. All Langmuir data were processed, to get an idea if this correction was required. For the linearisation

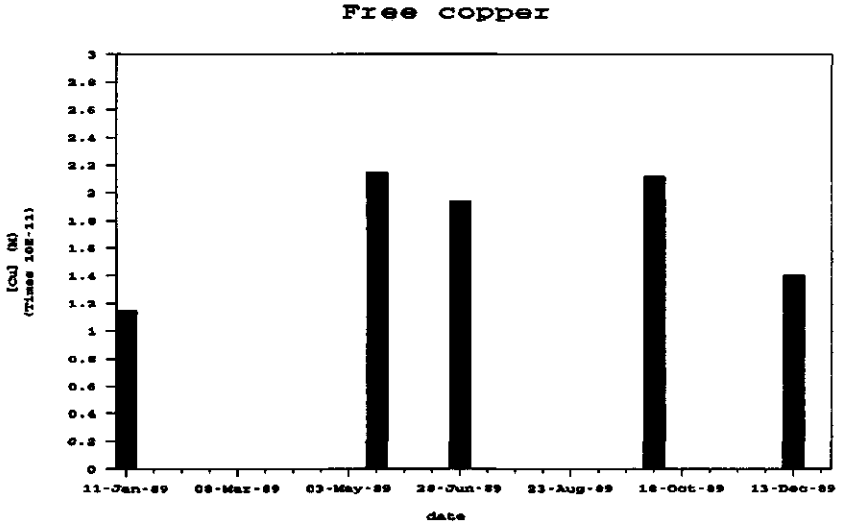

Figure 5.5A. Free copper Langmuir linearisation Van den Berg (1982a). Average is 1.75E-11 M; standard deviation 0.40E-11 M.

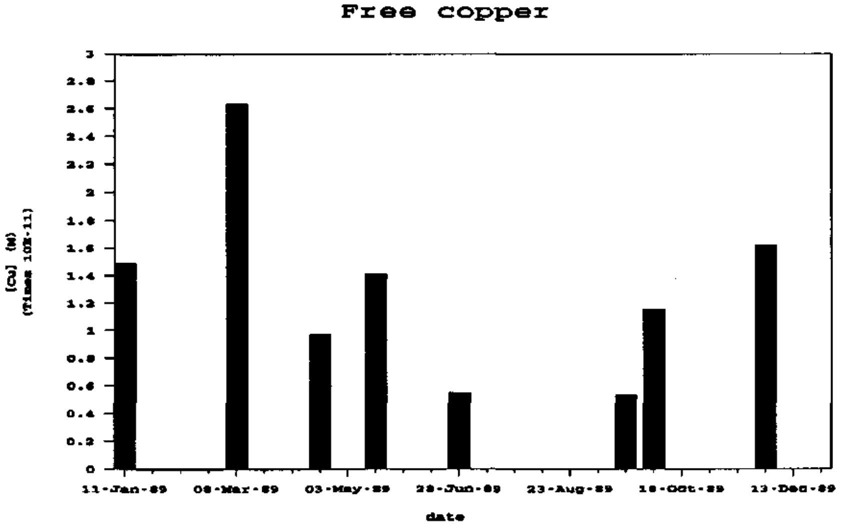

Figure 5.5B. Free copper Langmuir linearisation Verweij (this thesis). Average is 1.29E-11 M; standard deviation 0.64E-11 M.

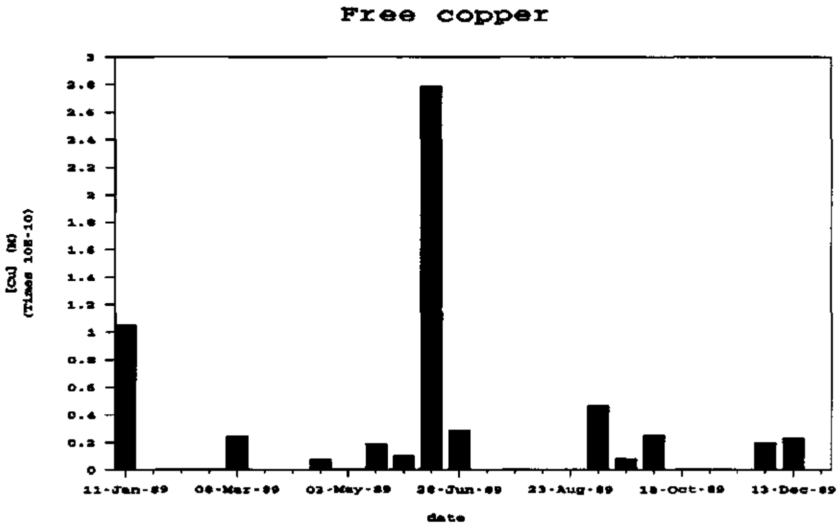

Figure 5.5C. Free copper linear model. Average is 4.98E-11 M; standard deviation 7.35E-11 M. Note the different scale compared to figures 5.5A and 5.5B.

of equation 5-2, free copper increased by 34% (average of five values), and for equation 5-3 by 28% (eight values), so the correction does significantly increase the calculated free copper concentration, but not the order of magnitude. For this reason and to avoid the simplifications of the derivation of equations 5-7 to 5-9, the uncorrected data are displayed in figure 5.5.

Calculated free copper is around 10^{-11} M. It is interesting to compare these results with results obtained using other techniques. It is to be noted that earlier in this thesis no techniques were described that can be expected to detect only free copper. In chapters 3 and 4 it was demonstrated that ultrafiltration and ASV cover more than only free copper. MnO_2-results for free copper concentrations therefore must be lower than the copper concentrations in the 5 nm filtrate and ASV-labile copper. This is indeed the case. MnO_2-results for free copper concentration are two orders of magnitude lower than ASV-labile copper, and three orders lower than the copper concentration in the fraction < 5 nm.

For seawater, Van den Berg (1982b) also found values of around 10^{-11} M. From the data of Gerringa (1990) values ranging from $9 * 10^{-13}$ to $2 * 10^{-11}$ M may be calculated for marine sediment slurries.

Taking into account the results of voltammetric measurements, which point to a high degree of organic copper complexation, it is interesting to investigate possible correlations of organics with free copper. If free copper concentration is primarily controlled by organic ligands, as from the equilibrium reaction

$Cu + L === CuL$

(where L represent organic ligands) the following relationships can be obtained:

a) $[CuL] = K * [Cu] * [L]$

or:

b) $[CuL]/[L] = K * [Cu]$

These imply that the concentration of free copper is proportional to [CuL]/[L]. However, neither [CuL] nor [L] is known, but since there are high concentrations of dissolved organic material in the lake, it can be assumed that virtually all copper is present as [CuL], while organics are hardly present as copper complexes. So the term [CuL]/[L] simplifies to [total copper]/[total organics]. Correlations of [free copper] with [total copper]/[total organics] are shown in table 5.3, where "organics" are represented by dissolved organic carbon and optical density at 250 and 365 nm. Two correlations are significant at a $p < 0.1$ level, indeed indicating copper complexation is controlled by organics. However, seven of the correlations are not significant (five are even negative), suggesting that measuring dissolved organic carbon or the optical density at 250 or 365 nm is not specific enough to investigate organic copper complexation. It indicates that at these extremely low copper concentrations only a few functional groups whose concentrations are not proportional to DOC, OD 250 or 365 are responsible for copper complexation. The two significant correlations are therefore probably coincidence.

The average concentrations as obtained by the three calculation methods, were used to calculate inorganic copper in the samples under laboratory conditions and to compare those values with dissolved (< 0.2 µm) copper. It

	Langmuir linearisation		Linear model
	Verweij	Van den Berg	
[Cu]/OD 250	-0.2916 (8)	0.8172*(5)	-0.1414 (12)
[Cu]/OD 365	-0.3042 (8)	0.7102 (5)	-0.1831 (12)
[Cu]/DOC	0.6753*(8)	-0.1579 (5)	0.0964 (12)

Table 5.3. Correlations of free copper with [Cu]/OD 250, [Cu]/OD 365 and [Cu]/DOC (between brackets: n); * denotes $p < 0.1$.

appeared that for the Langmuir calculations inorganic copper was slightly more than 1%, while for the linear model a percentage of nearly 5 was found. A large percentage of copper (95 to 99) was therefore organically complexed. Using the data of Van den Berg (1982b) a percentage of 97 organically complexed copper can be calculated for his Irish Sea sample. If the values for log(K) and Γ_{max} of Van den Berg (1982b) and Gerringa (1990) are used, comparable values for free copper are found.

5.2. EDTA at the rotating mercury film electrode

5.2.1. Discussion of the technique

The ligand competition technique used in this study is derived from the SEP-PAK C_{18} adsorption method developed by Sunda & Hanson (1987). The SEP-PAK columns are used to establish a physical separation between hydrophobic components of water samples (which will be retained by the column) and hydrophilic components (which will pass through). Ethylenediaminetetraacetate (EDTA) is used as synthetic ligand. Complexes of metals with EDTA generally have high equilibrium constants, i.e. complex formation is strongly favoured.

The principle of the technique is as follows. Two copper addition series are prepared, one also had EDTA included. Labile copper is measured in all samples by some technique which does not detect the copper-EDTA complex. Because adding EDTA will result in some part of copper being complexed by EDTA, the concentration of labile copper will decrease (figure 5.6). The technique depends on the fact that in all samples a direct relationship exists between copper complexes and free copper if solution parameters such as pH remain constant and the solutions are in equilibrium (Sunda & Hanson, 1987). Thus if two samples which differ only in copper and EDTA concentration have the same free copper concentration, they will also yield the same labile copper concentration, regardless which technique is used to measure "labile copper". As a consequence, two points on the two curves in figure 5.6 with the same labile copper concentration should have the same concentration of natural copper complexes. The difference in total copper can only be explained by the presence of the copper-EDTA complex, which then can be quantified by subtracting total copper. If complexes of EDTA with other metals, which compete with copper for EDTA, are taken into account, the concentration of free EDTA can be calculated, allowing free copper to be calculated.
The formula for free copper can be derived as follows:

$$[Y_{total}] = [CaY] + [MgY] + [CuY] \qquad (5\text{-}10)$$

where Y represents EDTA, and free and protonated EDTA are omitted because they

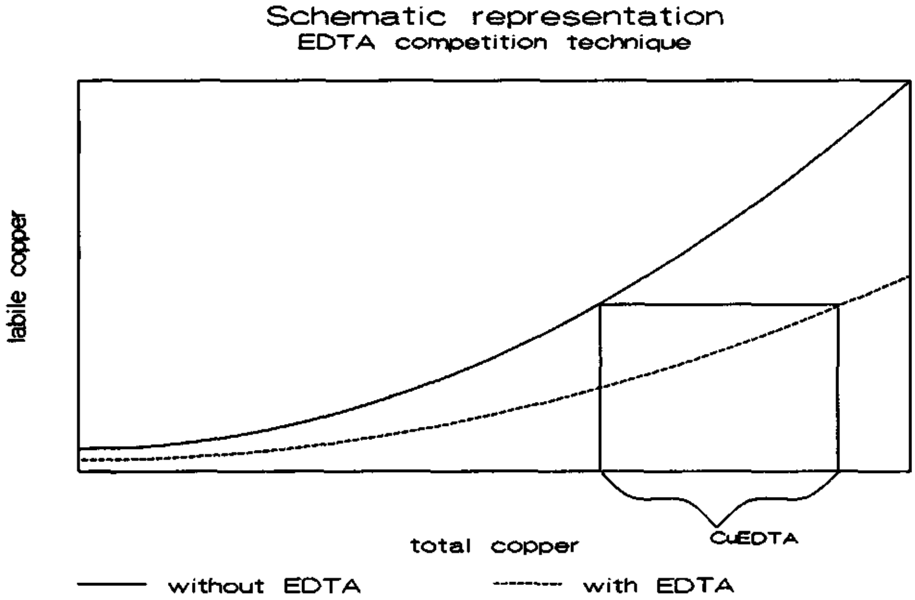

Figure 5.6. Schematic representation of EDTA competition technique.

will be shown to be negligible; other metals can not be neglected a priori, but the derivation can be simply adapted if other metals are taken into account. From equation 5-10 and the general relationship [ML] = K * [M] * [L], equation 5-11 can be obtained:

$$K_{CuY} * [Cu^{2+}] * [Y^{4-}] = [Y_{total}] - K_{CaY} * [Ca^{2+}] * [Y^{4-}] - K_{MgY} * [Mg^{2+}] * [Y^{4-}] \quad (5\text{-}11)$$

Dividing by $[Y^{4-}]$, and inserting $[Y^{4-}] = [CuY^{2-}]/([Cu^{2+}] * K_{CuY})$, yields:

$$K_{CuY}' * [Cu^{2+}] = \frac{[Y_{total}] * [Cu^{2+}] * K_{CuY}}{[CuY^{2-}]} - K_{CaY} * [Ca^{2+}] - K_{MgY} * [Mg^{2+}] \quad (5\text{-}12)$$

All the terms containing $[Cu^{2+}]$ are transported to the left hand of the equation, after which multiplication by $[CuY^{2-}]$ results in:

$$K_{CuY} * [Cu^{2+}] ([Y_{total}] - [CuY^{2-}]) = [CuY^{2-}](K_{CaY} * [Ca^{2+}] + K_{MgY} * [Mg^{2+}]) \quad (5\text{-}13)$$

Isolating $[Cu^{2+}]$ finally yields equation 5-14:

$$[Cu^{2+}] = \frac{[CuY^{2-}]\ (K_{CaY} * [Ca^{2+}] + K_{MgY} * [Mg^{2+}])}{K_{CuY} * ([Y_{total}] - [CuY^{2-}])} \qquad (5\text{-}14)$$

which is also given by Sunda and Hanson (1987), and allows the calculation of free copper from measured values.

It should be noted that in this derivation only mass balance constraints and general equilibrium relationships are used. Equation 5-14 is therefore generally applicable, even if EDTA is (super-)saturated with copper, but such a saturation would influence the relationship between the concentrations of labile and total copper. The schematic representation of the EDTA competition technique as shown in figure 5.6 would look different in such a case.

The SEP-PAK columns used by Sunda & Hanson (1987) have a few disadvantages. First of all, positive ions (e.g. Cu^{2+} or $CuOH^{+}$!) may adsorb to silanol groups that have not reacted with C_{18} chains during the production of these columns (Sunda & Hanson, 1987). Besides, it has turned out to be impossible to clean the SEP-PAK C_{18} columns sufficiently (D. Mackey, personal communication). Because of these reasons, a voltammetric version of the ligand competition technique was developed for this study. Because copper-EDTA-complexes are labile on a mercury drop but not on a rotating mercury film electrode, the latter was used.

5.2.2. Materials and methods

Measurements were performed on a ROTEL (EG & G GmbH) rotating glassy carbon electrode operating at 2000 rpm. For further description of the equipment used please refer to section 4.1.2. A mercury film was created by applying a potential of -0.6 V (versus Ag/AgCl) to a solution of 50 µM Hg^{2+} in 24 mM hydrochloric acid (pH = 1.62) for half an hour. Samples were measured

purge time	600 seconds
condition time	60 seconds
condition potential	0 V
deposition time	900 seconds
deposition potential	-0.6 V
equilibration time	30 seconds
wave form	differential pulse
pulse height	0.05 V
scan speed	0.013 V/second

Table 5.4. Parameters used for EDTA competition measurements.

according to table 5.4.

Samples were prepared one day before the measurements: copper was added to 50 ml of filtered (< 0.2 µm) lake water, HEPES (pK_a = 7.55) and EDTA (one addition series). The concentration of HEPES in the samples was 20 mM, EDTA 0.8 µM. Sensitivity was determined by standard additions, and labile copper was plotted versus total copper for samples with and without EDTA. Labile copper between data points was estimated by linear interpolation, resulting in a value for $[CuEDTA^{2-}]$ for each copper concentration in the applied range. It would have been better to use more and smaller additions, allowing non linear regression (e.g. cubic spline). This was however not possible in this project. Total concentrations of calcium and magnesium were determined by AAS (flame). Sulphate concentrations were measured by adding a known excess of barium, filtering barium sulphate off, and measuring the dissolved barium. GECHEQ (see chapter 7) was used to calculate inorganic speciation in the samples, including the equilibrium constants for the valid ionic strength (I). Other metal-EDTA complexes were taken account of, but found to be unimportant. For pH and total concentrations of calcium, magnesium, copper and sulphate measured values were used as an input for GECHEQ. For sodium, potassium, trace metals, alkalinity, silicon, phosphate and chloride the 1989 average total concentrations of Lake Tjeukemeer were used instead.

5.2.3. Results and discussion

Preliminary experiments had shown that the copper-EDTA complex was not labile at the rotating electrode. The speciation calculations gave 1.16 mM free Ca (90%), 0.42 mM free Mg (92%) and $4 * 10^{-13}$ M free Cu. At the calculated ionic strength of 8.66 mM the conditional $\log(K_{MEDTA})$ for the equilibrium M + EDTA === MEDTA was 11.67 for M = Ca, 9.87 for M = Mg and 19.80 for M = Cu. Free and protonated EDTA was, as expected, negligible compared to total EDTA, as supposed in the derivation of the equations in the previous sections.

The EDTA technique was applied four times in the beginning of 1990. Adding EDTA resulted in a higher ASV-signal (figure 5.7) for the sample (without added copper) of January 10th. The calculation of free copper in the natural sample was therefore not possible. Figure 5.7 shows that in the series with EDTA the concentration of the copper-EDTA complex was about half of total copper concentration. In figure 5.7 it can also be seen that for the series without EDTA the signal for higher additions was lower as expected due to saturation of the mercury film. Figure 5.8 shows that calculated log(free copper) gradually decreases with log(total copper), until log(total copper) is slightly below -6.9. This concentration corresponds to the concentration range where the interpolated lines cross so that the distance approaches zero (see figure 5.7).

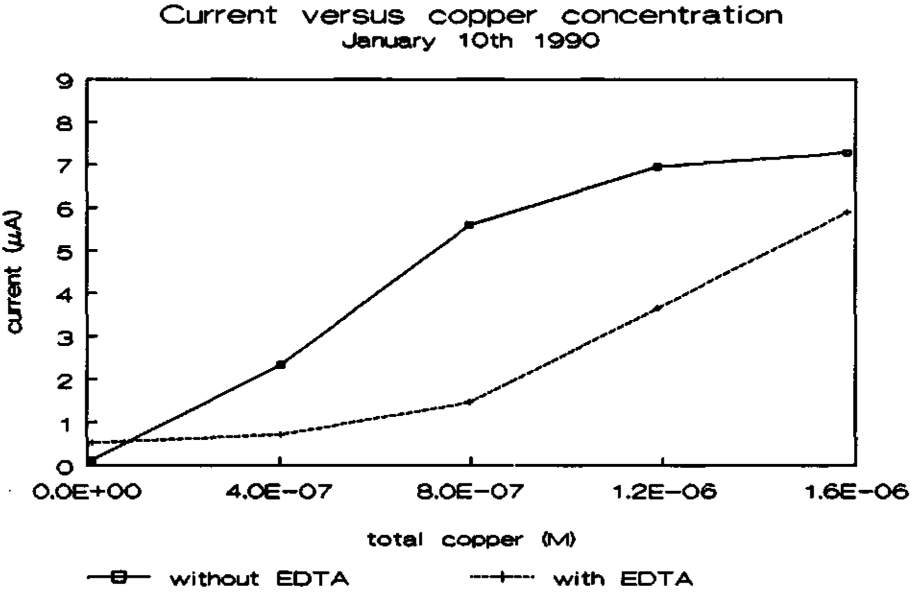

Figure 5.7. Measured currents.

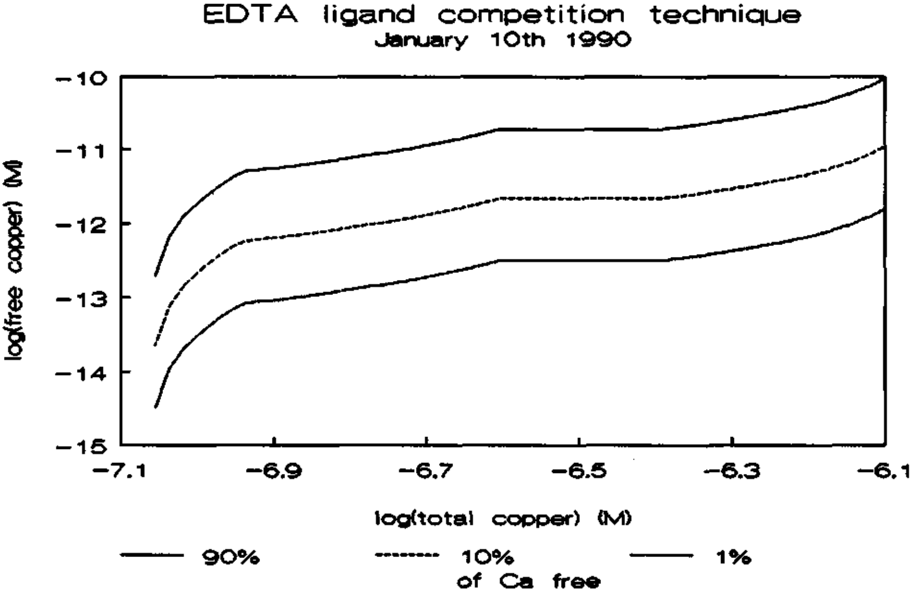

Figure 5.8. Calculated log(free copper) versus log(total copper).

The upper curve in figure 5.8 was constructed using the above-mentioned percentages of free alkaline earth metals (90% for Ca; 92% for Mg). Although Sunda & Hanson (1987) used comparable values for their calculations in seawater (84% for Ca; 90% for Mg), one could argue that in a humus-rich environment like Lake Tjeukemeer lower percentages should be used, since alkaline earth metals also form complexes with humic substances (see e.g. Hering & Morel, 1988). Arbitrary percentages of 10 and 1 for free calcium, being more important than magnesium, were used to calculate the other curves in figure 5.8, thus decreasing free copper by about one or two order(s) of magnitude. Thus in the samples with EDTA the degree of calcium complexation controls free EDTA concentration, which in return controls free and ASV-labile copper. Since there is no information available on calcium-humus interactions in Lake Tjeukemeer, literature data can only be used to estimate free calcium, while keeping in mind that the percentage of 90 is calculated without interactions with organics, and therefore is a maximum. Hering & Morel (1988) reported fractions of free calcium down to about 1% for freshwater (one of the plotted scenarios above). However, the concentration of humic acids they used was much higher than the organic carbon concentrations in Lake Tjeukemeer. If their value for the calcium complexing capacity (2 mmol Ca/gram humic acid) is multiplied by the 1989 average for dissolved organic carbon (19.1 mg $C \cdot L^{-1}$), it can be estimated that Lake Tjeukemeer organics can not complex more than $3.8 * 10^{-5}$ mol $Ca \cdot L^{-1}$, which is 3% of the 1989 average calcium concentration in Lake Tjeukemeer. So in our case free calcium can not be as low as 1%, but calcium will instead be hardly organically complexed. This implies that the calculated value for free Ca (1.16 mM; 90%) will only slightly decrease as a result of organic complexation.

Results of other workers also would indicate that organic complexation may not be very important in the calcium speciation in Lake Tjeukemeer. Schnitzer (1971) reported a total acidity of 12.4 meq/gram fulvic acid. If each meq is supposed to be able to complex half a mmol of Ca, 9% of Ca in Lake Tjeukemeer can at most be organically complexed. Dempsey & O'Melia (1983) found that natural organic matter was able to complex around 2.5 mmol Ca/gram C, corresponding to at most 4% of Ca being organically complexed.

Therefore, one can assume organic complexation to be of minor importance for calcium speciation in Lake Tjeukemeer, so the upper curve in figure 5.8 can be best considered to represent the actual situation.

Graphical extrapolation of the curve in figure 5.8 to the natural copper concentration of 9.4 nM (log = -8.05) is impossible, but some conclusions may still be drawn. The lowest reliable value for the log(free copper concentration) is between -12 and -11. The corresponding value for total copper is around -7, which is about one order of magnitude lower than in the natural situation. In figure 5.8 it can be seen that for total copper going from 10^{-6} to 10^{-7} M, free copper decreases about one order, suggesting a more or less constant ratio of free copper to total copper in this concentration range. If this ratio is indeed assumed to be constant, free copper in the natural situation would be between 10^{-13} and 10^{-12} M. This value is about two orders of magnitude lower than was found using the MnO_2-method. The speciation calculations of Lake Tjeukemeer indicated a concentration of $4 * 10^{-13}$ M for free copper. Complexation of copper by natural organic ligands is not included in these calculations. If additional ligands are present, the free copper concentrations will be lower, so it is likely that the extrapolation yields a reliable value.

If 10^{-11} M was taken as the maximum free copper concentration, total inorganic (including free) copper was calculated to be $2.2 * 10^{-10}$ M, which was 2.4% of total copper. So organic complexation must have accounted for at least 97.6% of total copper. For a free copper concentration of 10^{-12} M these percentages would be 0.24 and 99.76, for 10^{-13} M 0.024 and 99.98.

These values corresponded reasonably well with those reported by Sunda & Hanson (1987) for seawater at pH = 8 as obtained with their C_{18} adsorption version of the EDTA competition technique, i.e. $\log(Cu^{2+})$ between -12.3 and -11.5, resulting in at least 98% of the copper being organically complexed. This was roughly one order of magnitude higher than the estimated value for Lake Tjeukemeer. This difference can probably be explained by the much higher concentration of organic carbon in Lake Tjeukemeer.

The voltammetric measurements can also be interpreted without the ligand competition approach. In that case, for the sample without added copper and EDTA, a value of 10.3 nM of labile copper was found. This was more than 100% of total copper, a result which was probably due to contamination. The results of the ligand competition were not however effected by this contamination, since the labile concentration in this sample was lower than in the corresponding one with EDTA addition.

Samples of other dates were comparable: without copper addition the current was higher with EDTA than without. As a consequence, the curves in figure 5.9 also displayed a sharp decrease in log(free copper) below some log(total copper) value in the region where the two interpolated lines nearly crossed. The steep parts of the graphs therefore had to be considered unreliable. Unfortunately, copper additions for these three sampling dates were chosen lower than for the first sample; in the series containing EDTA these additions turned out to be too low, so that the "level" in figure 5.8 could not be constructed for the other dates.

If the irrelevant parts of the curves were not considered, [free copper] was around 10^{-11} M when [total copper] $\approx 10^{-6.5}$ M and only inorganic calcium complexation was considered. Not much difference was found for the four sampling dates, although it seems that over the course of the year the free copper concentration decreased.

It remained unclear as to why EDTA addition increased the current when no copper was added; as using AAS no significant copper contamination was detected in the EDTA solution. Standard solutions resembling Lake Tjeukemeer for the major cations and ligands (for details please refer to section 4.2.2)

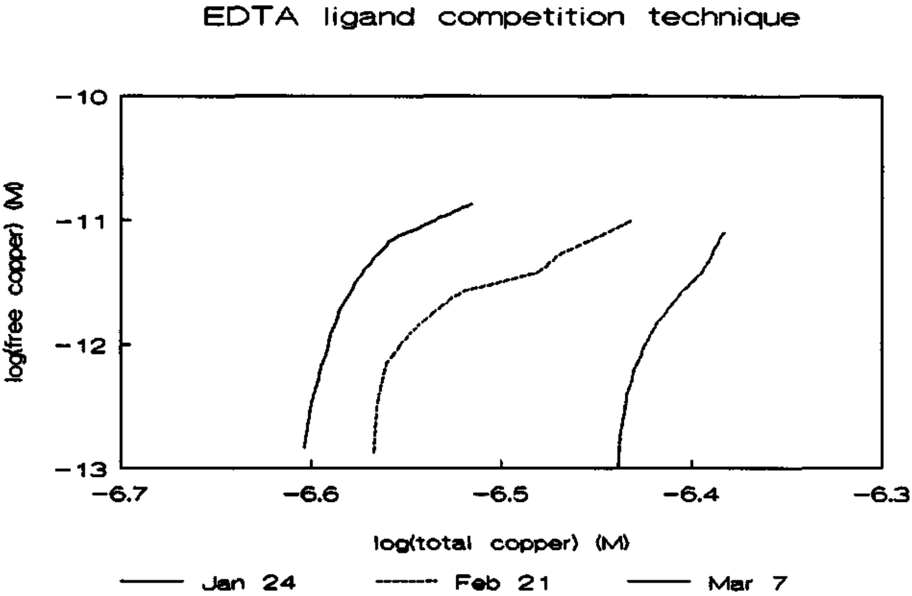

Figure 5.9. Results of EDTA competition technique for three dates.

did not display any measurable current as long as the [EDTA] was higher than the [Cu]. The presence of EDTA is therefore not an adequate explanation of the observed increase in current. One could argue that the current measured in the sample with EDTA and without added copper should be subtracted from the other samples in the EDTA series. However, since it is not understood why the observed phenomenon occurs, the physicochemical implications of such a subtraction cannot be judged. Therefore no attempts were made to process the data this way.

The conclusion of section 5.2 is that the free copper concentration in Lake Tjeukemeer was probably between 10^{-13} and 10^{-12} M. Organic copper complexes represented by far the major part of copper species. Although some problems were encountered when the voltammetric version of this ligand competition technique was first tested, it appears promising.

Chapter 6

Copper titrations

6.1. Discussion of the techniques

The methods described in the chapters 3 to 5 all give an insight into the level of complexation of copper under natural conditions in lake water samples. It was also of interest to obtain information on the copper complexation when copper was added to samples of Lake Tjeukemeer. In an equilibrium situation the fraction of copper present as free copper will increase when copper is added, as in an equilibrium situation the thermodynamically most favoured species will be formed first.

Titrating a sample with copper ions is a generally accepted way to obtain information on copper complexation. Typical parameters which may be obtained from the processing of these titration data are the apparent concentration(s) of binding sites [L] and conditional stability constant(s) K. Much less generally accepted are the methods used in the measurement of free or labile copper (anodic stripping voltammetry (ASV), ion selective electrode (ISE), fixed potential amperometry, column methods) and the methods of processing titration data, i.e. there are numerous models described in literature, and there is much scientific disagreement on which model is most suitable. Two "schools" can be identified. One direction tries primarily to find a mathematical description of metal-organic ligand-interactions which mimic the experimental data optimally; the physico-chemical background is less important for this school. By way of contrast, the second school is mainly interested in unravelling the physico-chemical details of metal-organic ligand-interactions. Typical exponents of the first and second schools are Van den Berg (e.g. Van den Berg & Kramer, 1979a) and Ružić (e.g. Ružić, 1982), and Marinsky and co-workers (e.g. Marinsky & Ephraim, 1986; Ephraim et al., 1986; Ephraim & Marinsky, 1986), Tipping and co-workers (e.g. Tipping, Backes & Hurley, 1988) and Van Riemsdijk and co-workers (e.g. Van Riemsdijk et al., 1987) respectively.

Dzombak, Fish & Morel (1986) and Fish, Dzombak & Morel (1986) compared several empirical models. Their conclusion was that empirical models which propose only a limited number of sites can adequately describe metal-organic ligand-interactions.

The question of whether an empirical model allows a good description of a specific situation can usually be answered relatively simply by reconstructing the titration curve using the results of the model and comparing it with the measured titration curve. A good match might not however mean that the model gives meaningful information from a physico-chemical point of view. In fact, it is very hard to unravel the details of the mechanisms of complexation in natural waters.

In this thesis no attempts will be made to obtain mechanistic information, because the aim of this project excludes this type of research.

Copper titrations may be carried out in two ways: in equilibrium and in non-equilibrium. In the former case copper additions are allowed to equilibrate over a relatively long time period (e.g. overnight) resulting in the formation of the most stable complexes, and in the latter case the free/labile copper measurement is performed immediately or shortly after adding copper, yielding an unpredictable mix of stable (high equilibrium constant) and "fast" (high association rate) complexes. The equilibrium titrations yield higher apparent ligand concentrations and higher (conditional) stability constants (Kramer, 1985). It should be emphasized that in both cases only information is obtained on the complexation of added copper.

This chapter is organized as follows: first of all, results of monitoring the copper complexation capacity during three years using copper titrations in combination with an ion selective electrode are described (section 6.2). A more detailed study of one titration curve using various empirical models is described in section 6.3, the influence of the pH in section 6.4, while section 6.5 deals with copper titrations using ASV. Finally, in section 6.6 the combination of ultrafiltration with copper titrations is dealt with.

The references of the paper in section 6.2 are included in the list of references at the end of this thesis.

6.2. Copper complexation in eutrophic and humic Lake Tjeukemeer, the Netherlands.

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Freshwater Biology (1989) **21**, 427–436

Copper complexation in eutrophic and humic Lake Tjeukemeer, The Netherlands

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SUMMARY. 1. Copper(II) complexation in the eutrophic, humus-rich Lake Tjeukemeer was measured fortnightly for several years by copper titration (Ion Selective Electrode) and by copper solubilization. Additionally, the copper speciation during titration was followed by ultrafiltration.

2. The Tjeukemeer showed high ligand concentrations able to complex up to 8.5×10^{-5} M Cu.

3. Scatchard plots and affinity spectra of the titration data allowed the discrimination of at least three different binding sites. In Scatchard plots log *K* values ranged from 5 to 9, in affinity spectra from 4.5 to 8.

4. The highest log *K* values coincided with relatively low humus concentrations and blooms of algae, mainly Cyanobacteria.

5. The ultrafiltration experiments indicated that relatively small size fractions (<10 nm) have the highest copper binding affinity.

Introduction

Free copper (Cu^{2+}_{aq}) is generally assumed to control bioavailability and toxicity of copper (Canterford & Canterford, 1980; Huntsman & Sunda, 1980; Petersen, 1982). The proportion of free copper is determined by adsorption/desorption, precipitation/dissolution and association/dissociation of inorganic and organic complexes. Because natural organic matter is a very complex and chemically ill-defined mixture, it is still difficult to interpret the interactions between copper and natural organic matter at a molecular level. Therefore, description of these interactions is limited to some global characteristics.

To gain insight into the copper complexing capacity in Lake Tjeukemeer, and to investigate correlations with chemical and/or biological parameters, we studied the copper complexing capacity, fortnightly for 3 years (1984–86), by copper titration and a copper solubilization method. The titration data were processed in three different ways: Van den Berg–Ružić–Lee, Scatchard and affinity spectrum analysis. Titrations were combined with ultrafiltration experiments. The resulting complexation parameters (total ligand concentration L_T and conditional stability constants *K*) were compared with chemical parameters and dominant algal species.

Lake Tjeukemeer is a shallow ($\bar{z}=1.5$ m), alkaline, humus-rich and eutrophic lake in north Netherlands (Fig. 1) with an area of 21.5 km^2 (Leenen, 1982). Its hydrology is typically manmade: in the wet period of the year (October–April) the very eutrophic and humus-rich surplus of water in the surrounding peaty polders is pumped into the lake. In the dry period (April–October) there is usually a lack of water in the polders, and lake water is let in. To

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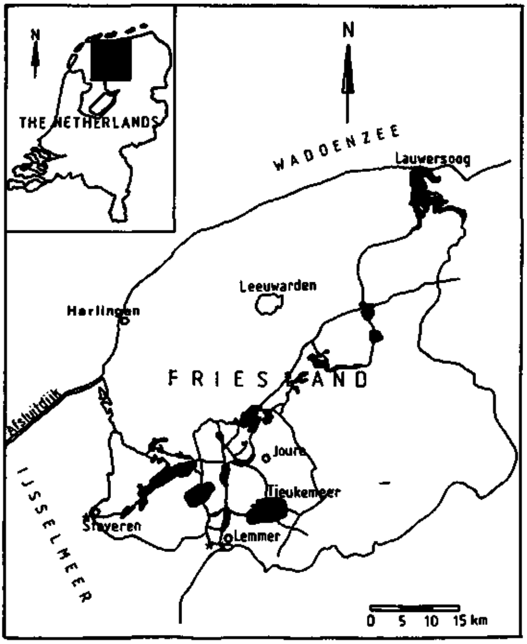

FIG. 1. Map showing the location of Lake Tjeukemeer and Lake IJsselmeer.

offset this loss, the lake is supplied with water from Lake IJsselmeer (Fig. 1), which is mainly fed by the oligohaline river IJssel (a branch of the river Rhine). In the summer the lake chemistry is especially defined by the oligohaline and eutrophic IJsselmeer water. The eutrophication of the lake results in huge algal blooms, usually dominated by diatoms (February–April) and Cyanobacteria (May–October). Cryptophyceae (May) and green algae (May and August) occur also in large amounts, but they seldom dominate the phytoplankton community. During 1984–86 the relative importance of Cyanobacteria decreased.

Materials and Methods

Sampling was carried out every 2 weeks (except when there was ice) at ten locations (upper 1 m of water column). Samples were collected with a perspex tube, then stored in polyethylene bottles and brought to the laboratory as soon as possible. The samples were then mixed, filtered (0.2 μm) and analysed. Acid and distilled, deionized water were used for cleaning and flushing of sampling equipment, respectively, to prevent copper contamination. Total copper in filtrates, ranging from 3×10^{-9} to 7×10^{-8} M, was considered negligible compared to added copper.

The apparent copper complexing capacity was measured in two ways. A copper titration was carried out by placing a calibrated copper ion selective electrode (ISE) (ORION) at constant temperature (20°C) in a sample, brought to a $NaNO_3$-concentration of 0.1 M and kept at pH=6 (to prevent copper precipitation) by addition of hydrochloric acid or a sodium hydroxide solution by a pH-stat. Twenty-five copper additions were made (up to 9.0×10^{-4} M), and after each addition the pH was automatically adjusted. When after a few minutes apparent equilibrium was reached, the potential of the ISE was read. Since 1985 smaller, more frequent additions were used, allowing calculation of affinity spectra. Model calculations indicate that in spite of the high phosphate concentration (up to 10 μM P in 0.2 μm filtrate) copper phosphate complexes or solids do not contribute significantly to the copper speciation, even at the addition of nearly 1 mM Cu. Under equilibrium conditions copper is not soluble to such levels, but our titration curves never indicated that such an equilibrium situation actually occurred, so precipitation is not likely to have affected our results.

The titration data were processed in three ways:

(i) Van den Berg–Ružić–Lee analysis (Van den Berg & Kramer, 1979; Ružić 1982; Lee, 1983). Only 1:1 complexes were assumed. Least squares linear regression was used.

(ii) Scatchard analysis, which assumes a small number of binding sites, resulted in three groups of ligands each with its own concentration and conditional stability constant (Scatchard, 1949). Least squares linear regression was applied to the ranges 3.1×10^{-7} to 3.0×10^{-6} M, 3.7×10^{-6} to 8.7×10^{-6} M and 1.2×10^{-5} to 3.9×10^{-5} M of added copper, because these ranges appeared linear. Although dividing a curve in lines is risky, correlation coefficients usually turned out to be >0.95.

(iii) The affinity spectrum approach, which indicates the probability $N(K)$ of finding a binding site at log K as a continuous function of log K (Hunston, 1975; Thakur *et al.*, 1980). By means of linear interpolation between points in the $\bar{\nu}$ versus log [Cu] plot ($\bar{\nu}$ = [CuL]/[L_T]), and using [L_T] as obtained by Van den Berg–Ružić–Lee analysis $N(K)$ was calculated for the complete range of the titration, corresponding with $4.5<\log K<8$. Because linear interpolation is easier to automate and gives nearly the same results as non-linear interpolation, the former was chosen.

For comparison we used the solubilization method of Borchers (1959) and De Haan, De Boer & Hoogveld (1981). A sample was mixed with a copper suspension, buffered with 0.05 M

TABLE 1. Correlation matrix for apparent copper complexing capacity with chemical and biological parameters (n) (*P <0.05; **P <0.01; Van den B-R-L = Van den Berg–Ružić–Lee)

	Apparent copper complexing capacity according to:					
	Borchers		Van den B-R-L		Scatchard	
1984						
Borchers	1.0000	(20)				
Van den B-R-L	0.5720	(9)	1.0000	(11)		
Scatchard	0.1507	(12)	0.7292*	(11)	1.0000	(14)
Chlorophyll-*a*	−0.2852	(20)	−0.0661	(11)	−0.0302	(14)
OD 250	0.2737	(20)	0.0074	(11)	−0.0583	(14)
OC	−0.0038	(12)	−0.4509	(9)	−0.1831	(10)
Diatoms	0.0877	(17)	−0.3375	(9)	−0.2290	(9)
Cyanobacteria	−0.1446	(20)	−0.0933	(11)	0.0498	(14)
1985						
Borchers	1.0000	(17)				
Van den B-R-L	0.0557	(15)	1.0000	(17)		
Scatchard	0.1828	(14)	0.6569**	(16)	1.0000	(16)
Chlorophyll-*a*	0.3053	(17)	0.1810	(17)	0.1257	(16)
OD 250	0.1494	(17)	0.2424	(17)	−0.3611	(16)
DOC	0.5953*	(14)	0.0130	(12)	0.1359	(11)
Diatoms	−0.4030	(16)	−0.2288	(17)	−0.0016	(16)
Cyanobacteria	0.4199	(16)	0.2526	(17)	0.1310	(16)
1986						
Borchers	1.0000	(19)				
Van den B-R-L	0.3127	(17)	1.0000	(19)		
Scatchard	0.4269	(17)	0.5844**	(19)	1.0000	(19)
Chlorophyll-*a*	−0.1495	(19)	−0.4363	(19)	−0.3596	(19)
OD 250	0.5011*	(19)	0.5778**	(19)	0.5107*	(19)
DOC	−0.0108	(11)	0.1646	(12)	0.0279	(12)
Diatoms	−0.0130	(11)	−0.0080	(11)	0.5358*	(11)
Cyanobacteria	−0.1868	(14)	−0.2410	(12)	−0.3771	(12)

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[1] The paper by Van den Berg & Kramer can be found in the list of references under Van den Berg & Kramer (1979a).

borax (pH=9.1), and, after centrifugation, the copper concentration in the supernatant was estimated spectrophotometrically by measuring the colour after addition of cuprizone. The surplus of copper, compared to a blank, is the apparent copper complexing capacity, and includes both organic and inorganic complexation.

For ultrafiltration experiments, the fraction <0.2 μm was filtered subsequently through filters with nominal pore sizes of 10–20 nm, 5–10 nm and 5 nm (Schleicher & Schüll AC 63, AC 62 and AC 61 filters, respectively). The various filtrates were titrated. Also, titrations in 0.2 μm filtered samples were interrupted at certain stages, and a subsample ultrafiltered.

Dissolved Organic Carbon (DOC) was measured with a Phase Sep TOCsinII carbon analyser. UV absorption at 250 nm (OD 250) was measured on a Zeiss PMQII spectrophotometer. Chlorophyll-*a* was measured by pigment extraction with 80% ethanol, according to Moed (1973). Copper was measured with a Perkin Elmer 2380 Atomic Absorption Spectrophotometer equipped with a deuterium background corrector, with the flame or the graphite furnace, depending on the concentration range.

All reagents were analytical grade (J.T. Baker).

Algae were counted as described by Moed & Hoogveld (1982).

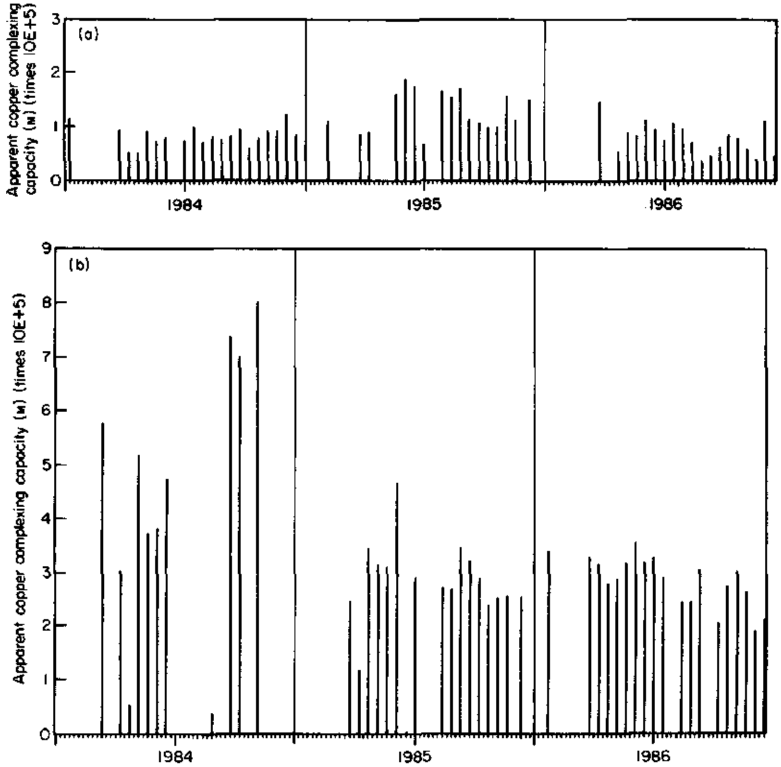
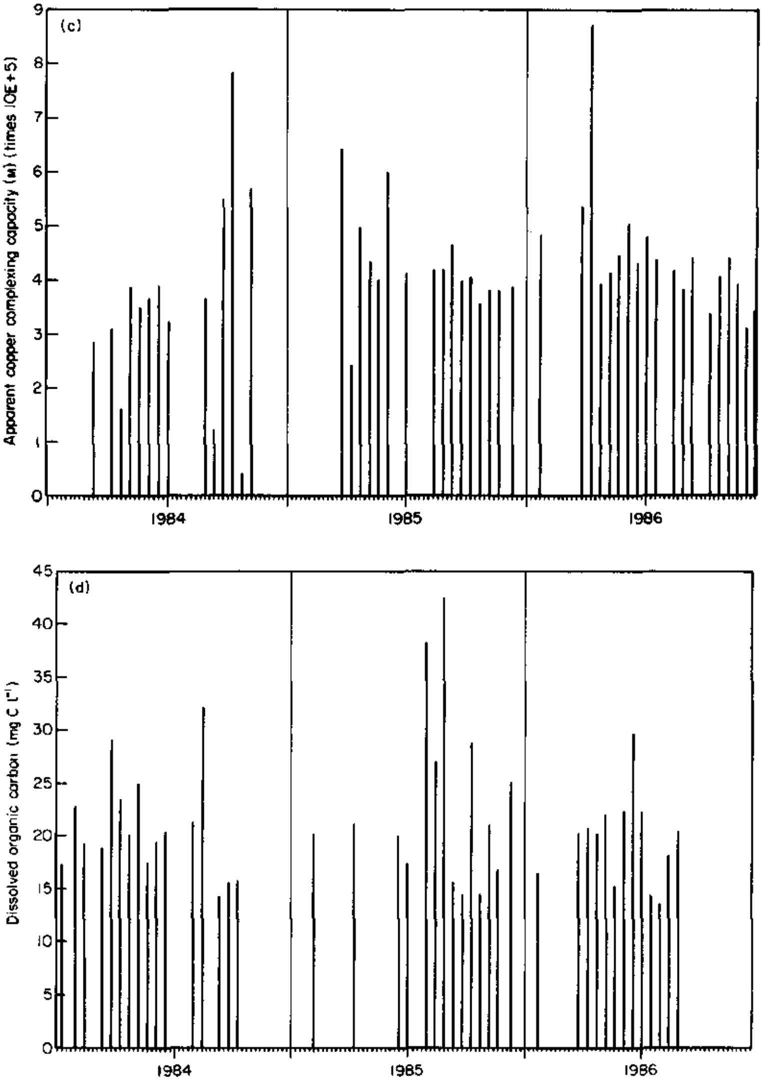


FIG. 2. The copper complexing capacity in Lake Tjeukemeer from 1984 to 1986 according to: (a) the Borchers method, (b) Van den Berg–Ružić–Lee and (c) Scatchard analysis, and (d) the DOC-concentration.



Results

The results of the Borchers method, and the Van den Berg–Ružić–Lee and Scatchard calculations of the ISE-method are quite different (Fig. 2). Throughout the year great fluctuations occur.

In Table 1 the correlation matrices are given for the apparent copper complexing capacity

TABLE 2. Mean ligand concentration ($\bar{x}$) in 10^{-5} M, according to Borchers, Van den Berg–Ružić–Lee and Scatchard

	Borchers			Van den Berg–Ružić–Lee			Scatchard		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
1984	0.84	0.18	20	4.50	2.44	11	3.58	1.83	14
1985	1.31	0.36	17	2.70	0.95	17	4.28	0.90	16
1986	0.78	0.29	19	2.84	0.46	19	4.45	1.14	19

results. Only the Van den Berg–Ružić–Lee and Scatchard calculations correlated significantly in all three years. In 1985 DOC is correlated with Borchers results; in 1986 OD 250 with both Borchers results and Van den Berg–Ružić–Lee and Scatchard calculations. No correlations were found with chlorophyll-*a* or dominant algal species (except 1986: diatoms and Scatchard results), nor between the Borchers results and the Van den Berg–Ružić–Lee or Scatchard calculations (Scatchard calculations being the sum of the three ligand concentrations).

Tables 2 and 3 show the (relative) averages of the total ligand concentrations according to the Borchers method and the Van den Berg–Ružić–Lee and Scatchard calculations. The proportion between Van den Berg–Ružić–Lee and Scatchard calculations in 1984 was quite different from that in 1985 and 1986. The calculations of the different parts of the Scatchard analysis demonstrated that strong complexing material is present in low concentrations, while weaker complexing material was present in much higher concentrations (Table 4).

The highest log K values coincided with maximum numbers of Cyanobacteria (Table 5) in 1984 and 1985. This dependence was less pronounced in 1986, when the bloom was less intense. Table 5 also demonstrates that during the bloom log K increases in *all* groups of binding sites.

Two types of experiments combined ultrafiltration with a copper titration. The first type, in which a titration was carried out in fractions of

TABLE 3(a). The mean ligand concentration relative to Borchers (set to 1)

	Van den Berg–Ružić–Lee	Scatchard
1984	5.39	4.29
1985	2.06	3.27
1986	3.63	5.69

TABLE 3(b). The mean ligand concentration relative to Van den Berg–Ružić–Lee (set to 1)

	Borchers	Scatchard
1984	0.19	0.80
1985	0.49	1.59
1986	0.28	1.57

TABLE 4. Scatchard results: averages (SD) of apparent copper complexing capacity and log conditional stability constants of the three parts of Scatchard analysis; for concentration ranges of the parts see text (experimental methods)

	Ligand concentration		log K	
Part 1				
1984	3.53×10^{-6} M	(2.59×10^{-6} M)	7.09	(0.56)
1985	2.89×10^{-6} M	(0.35×10^{-6} M)	8.30	(0.83)
1986	5.25×10^{-6} M	(0.57×10^{-6} M)	7.91	(0.68)
Part 2				
1984	9.32×10^{-6} M	(2.04×10^{-6} M)	6.14	(0.40)
1985	1.01×10^{-5} M	(0.17×10^{-5} M)	6.92	(0.58)
1986	9.78×10^{-6} M	(0.40×10^{-6} M)	6.84	(0.69)
Part 3				
1984	2.56×10^{-5} M	(1.25×10^{-5} M)	5.28	(0.30)
1985	2.98×10^{-5} M	(0.86×10^{-5} M)	5.69	(0.49)
1986	2.95×10^{-5} M	(0.46×10^{-5} M)	5.43	(0.25)

TABLE 5. Log conditional stability constants, and numbers of Cyanobacteria (ind./ml); VdB-R-L = Van den Berg–Ružić–Lee

	Log K according to:				
		Scatchard			
Date	VdB-R-L	Part 1	Part 2	Part 3	Cyanob
1984					
10.04.84	4.8	7.0	5.8	4.8	880
08.05.84	4.9	7.5	6.0	5.1	109850
22.05.84	5.1	7.2	6.2	5.2	181505
05.06.84	5.3	7.6	6.5	5.4	205615
19.06.84	5.1	7.2	6.3	5.3	161010
1985					
26.03.85	5.4	7.2	6.3	5.5	920
09.04.85	6.1	7.5	6.3	6.7	2040
23.04.85	5.8	7.9	6.6	5.5	4440
07.05.85	6.1	9.1	7.0	5.8	19360
21.05.85	6.2	9.3	7.4	5.9	96480
04.06.85	7.4	9.7	8.2	7.1	44960
02.07.85	6.2	9.2	7.2	5.7	34960
1986					
12.04.86	5.8	7.9	6.7	5.5	240
06.05.86	5.9	8.1	6.8	5.6	1680
20.05.86	5.9	7.8	6.6	5.5	18960
03.06.86	6.0	7.8	6.8	5.5	10560
17.06.86	6.0	8.3	6.9	5.6	30080
01.07.86	5.8	8.3	6.6	5.4	11680

ultrafiltered lakewater, gives information about the size classes of the copper complexing material. The second type, in which titrations were interrupted and subsamples subjected to ultrafiltration, gives information about the size fractions of copper complexes formed upon copper addition.

Table 6(a), presenting the results of titrations in ultrafiltered samples, demonstrates that the L_T hardly decreases with decreasing pore size, until a cut-off of 5 nm. This implies that the major part of L_T is present in the fraction between 5 and 10 nm, and a minor part is still present in the fraction <5 nm. Table 6(b) con-

TABLE 6(a). The ligand concentration (in 10^{-5} M) in ultrafiltrates according to Van den Berg–Ružić–Lee and Scatchard (sum of the three parts), and log conditional stability constant according to Van den Berg–Ružić–Lee

	Van den Berg–Ružić–Lee		Scatchard
Size class	L_T	Log K	L_T
<200 nm	1.94	5.7	3.13
<20 nm	1.98	5.6	3.25
<10 nm	1.77	5.5	3.24
<5 nm	0.36	6.9	0.88

TABLE 6(b). The ligand concentration (in 10^{-5} M) and log conditional stability constants in ultrafiltrates, according to the Scatchard method. For concentration ranges see experimental methods.

	Part 1		Part 2		Part 3	
Size class	L	log K	L	log K	L	log K
<200 nm	0.25	8.2	0.79	6.3	2.09	5.2
<20 nm	0.26	8.2	0.77	6.3	2.22	5.2
<10 nm	0.23	7.8	0.75	6.0	2.26	4.9
<5 nm	0.22	7.6	0.38	6.0	0.28	4.2

firms this picture, also showing that the strongest complexing ligands (part 1) are small (almost entirely <5 nm), while the weaker complexing ligands are for a major part between 5 and 10 nm, and appear in higher concentrations.

This picture was confirmed by three series of ultrafiltration experiments of the second type: in a subsample with little added copper, this extra copper appeared predominantly in the smallest size fractions. Proceeding with the titration, the copper gradually appeared in the larger size class fractions.

Discussion

General

Surprisingly, the Borchers results are much lower than the Van den Berg–Ružić–Lee and Scatchard calculations. One would expect the Borchers method to yield higher values (Campbell & Tessier, 1984). We cannot explain this phenomenon. Standard deviation of several replications was about 10% (see also De Haan *et al.*, 1981).

The lack of correlations clearly indicate that the Borchers method is incompatible with the ISE-titration combined with the Van den Berg–Ružić–Lee or Scatchard analysis. This discrepancy is also clear from Tables 2 and 3 (averages and relative averages): no relationship exists between the Borchers results on one hand, and Van den Berg–Ružić–Lee and Scatchard calculations on the other.

The difference in the proportion of Van den Berg–Ružić–Lee and Scatchard calculations between 1984 on one hand and 1985 and 1986 on the other could be a consequence of the different addition scheme used since 1985. By using more additions, more points for regression analysis became available, structurally influencing the results. The constant ratio of the averages of Van den Berg–Ružić–Lee to Scatchard calculations in 1985 and 1986 is striking and demonstrates good reproducibility.

The maximum apparent copper complexing capacities obtained by the Van den Berg–Ružić–Lee and Scatchard analyses, 4.7×10^{-5} M and 8.5×10^{-5} M respectively, are very high, and must be due to the high concentration of natural organic matter in the lake (Fig. 2d). Comparable values for apparent copper complexing capacity and DOC-concentration, also obtained by an ISE-titration, are reported by Tuschall & Brezonik (1983) for a swamp water sample. However, the value for the apparent copper complexing capacity these workers found for this sample with Anodic Stripping Voltammetry (ASV) was 8.5×10^{-6} M, which is far above the usual ASV-range of 1×10^{-7} to 1×10^{-6} M (Gorman, Skogerboe & Davies, 1986).

The calculations of the different parts of the Scatchard analysis demonstrated that strong complexing material is present in low concentrations, while weaker complexing material was present in much higher concentrations (Table 4). This result agrees with those of earlier reported ISE-titrations (Tuschall & Brezonik, 1983; Turner *et al.*, 1986).

A typical example of an affinity spectrum is given in Fig. 3. The arrows indicate the log *K* values as found by Scatchard analysis. Although the arrows do not point exactly to 'peaks' in the affinity spectrum, in most cases this spectrum indicated binding sites with log *K* values close to those found by Scatchard analysis. This result suggests that separate groups of ligands are inevitably combined by Scatchard analysis. The affinity spectra, just like Scatchard analyses, showed that strong complexing material occurs in low concentrations, while less strong complexing material was present in much higher concentrations.

Correlation with OD 250 and DOC

In 1984 none of the correlations of apparent copper complexing capacity with OD 250 or DOC were significant (Table 1). In 1985 only apparent copper complexing capacity according to the Borchers method was significantly correlated with DOC. These results indicate that in 1984 and 1985 the apparent copper complexing capacity in the lake is not predominantly caused by the DOC concentration, but rather by some unknown component(s) of the DOC pool.

However, in 1986 the apparent copper complexing capacity (according to all three analysis methods) and OD 250 (but not DOC concentrations) were significantly correlated, suggesting that in that year the apparent copper complexing capacity was governed by some class of strongly UV-absorbing organics. Noting the low number of Cyanobacteria this implies that fulvic acids instead of biological C controlled the DOC pool,

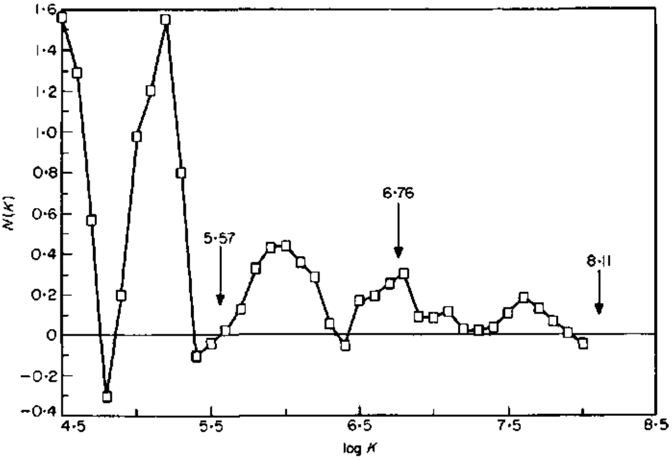


FIG. 3. Affinity spectrum of a 0.2 μm filtrate of Tjeukemeer water. Arrows indicate the log conditional stability constants as found by Scatchard analysis.

possibly pointing to a stronger UV-absorption of fulvic acids than of biological C.

The lack of correlation with DOC suggests that there is no constant ratio of apparent copper complexing capacity to DOC.

Correlation with chlorophyll-a and dominant algal species

The lack of correlation between the apparent copper complexing capacity and the chlorophyll-*a* concentration suggests that the apparent copper complexing capacity in the lake is not predominantly caused by the total algal biomass.

From Table 1 it is also clear that with one exception the apparent copper complexing capacity was not correlated with the number of diatoms and Cyanobacteria, usually the major components of the algal biomass. However, the highest log *K* values coincided with maximum numbers of Cyanobacteria (Table 5) in 1984 and 1985. This dependence was less pronounced in 1986, probably because of the less intense cyanobacterial bloom in that year.

Acknowledgments

We thank Drs J. R. Moed, H. Van Huet and S. Parma for critically reading the manuscript, and Steve Cabaniss for correcting the language.

6.3. A more detailed look at one titration curve

6.3.1. Introduction

In the paper included in this thesis (as section 6.2; Verweij et al., 1989), Scatchard (1949) curves were arbitrarily divided into three parts. In this section, one titration curve will be subjected to a closer look in order to find out if the supposition of three groups of binding sites is really necessary in order to mimic the titration data adequately.

This section is a result of co-operation with Dr. I. Ružić, of the Rudjer Boskovic Institute in Zagreb. It is our intention to publish our results together, but due to communication problems it appeared to be impossible to discuss this section. The author of this thesis is therefore solely responsible for this section.

6.3.2. Materials and methods

Titrations were carried out as described in section 6.2. The titration curve for Lake Tjeukemeer was analyzed using Van den Berg-Ružić-Lee analysis (Van den Berg & Kramer, 1979a; Ružić, 1982; Lee, 1983), "modified" Ružić analysis (Ružić, 1987) and Scatchard analysis (Scatchard, 1949).

6.3.3. Results and discussion

In figure 6.3.1 the titration data are plotted as $[Cu^{2+}]/[CuL]$ versus $[Cu^{2+}]$. The asymptote of this curve can be estimated as:

$$\frac{[Cu^{2+}]}{[CuL]} = 0.1145 * 10^5 * [Cu^{2+}] + 0.3467 \qquad (6.3\text{-}1)$$

which is also shown in figure 6.3.1. From this estimation [L] appears to be 87.3 µM, K 3.3 * 10^4. The difference between the asymptote and the titration data at low values for measured free copper concentration indicates that one group of ligands is not sufficient to describe the titration data adequately. According to the procedure proposed by Ružić (1982) the individual y-values for each point in figure 6.3.1 are subtracted from the estimated asymptote, and the inverse value of this difference is plotted against the measured free copper concentration, resulting in parameters for two groups of ligands. The

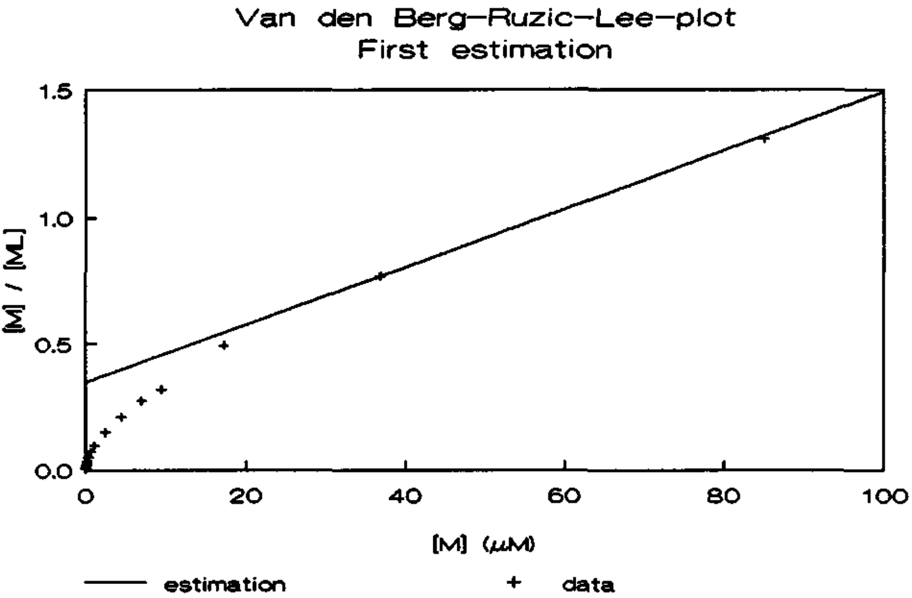


Figure 6.3.1. First estimation of the Van den Berg-Ružić-Lee-plot.

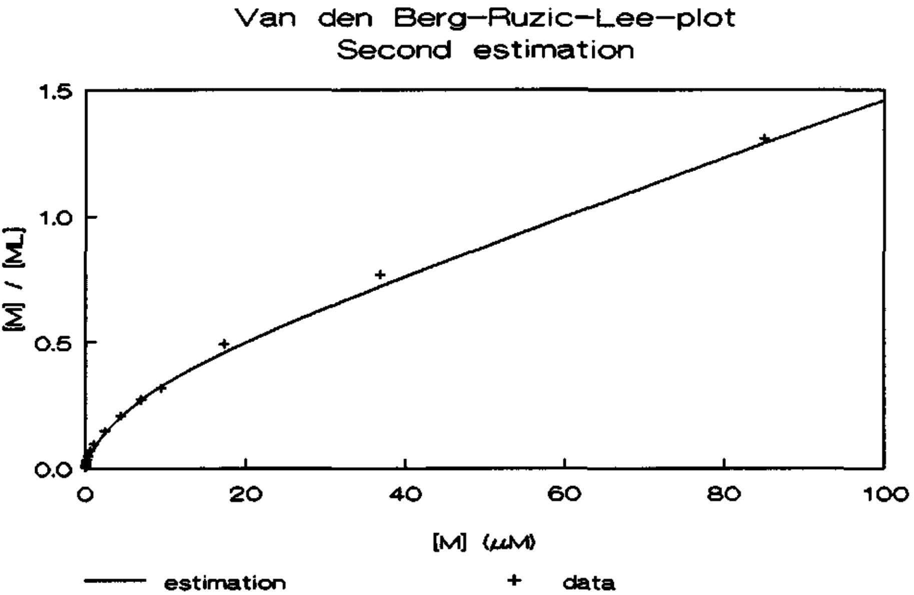


Figure 6.3.2. Second estimation of the Van den Berg-Ružić-Lee-plot.

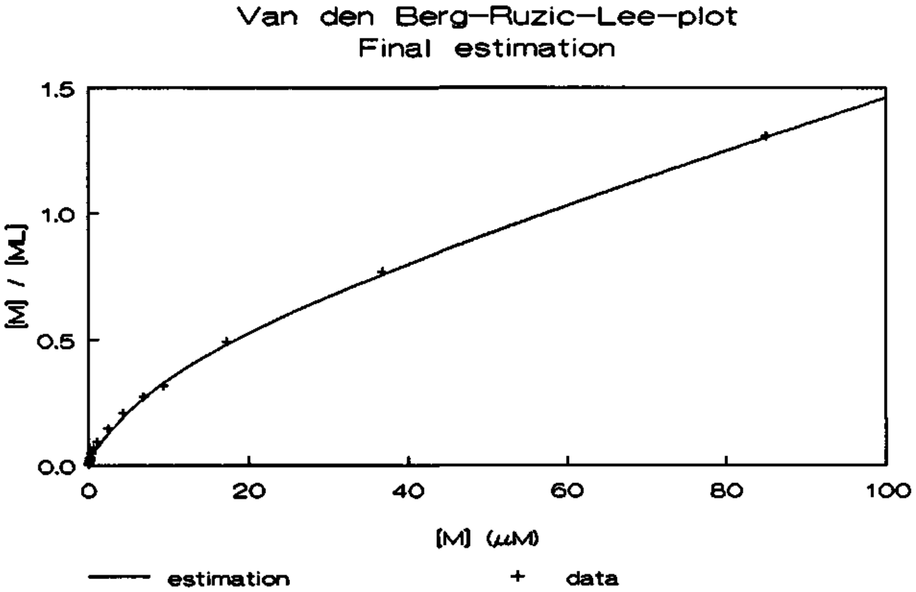


Figure 6.3.3. Final estimation of the Van den Berg-Ružić-Lee-plot.

concentration for the stronger complexing ligand is then 14.9 μM, while K is 3.3 * 10^6; for the other ligand 74.3 μM (K is 2.6 * 10^4). Figure 6.3.2 displays the titration curve that can be reconstructed using these values. The calculated curve obviously is a better approximation than the line in figure 6.3.1. Ružić (1987) described how an iterative procedure can be used to obtain even better approximations of values for ligand concentrations and stability constants. The results of this procedure are a concentration of 19.1 μM for the stronger complexing ligand (K is 2.3 * 10^6), and 80.3 μM for the other ligand (K is 1.6 * 10^4), and are shown in figure 6.3.3.

Considering the procedures used above, the data at higher concentrations of added copper obviously are more important in the fit than data for low concentrations of added copper, which do not contribute very strongly to the results of the fitting. Scatchard analysis on the other hand may give more insight into the complexation behaviour at low concentrations. Not least because in a Scatchard plot points for low additions are clearly separated from other points. It was therefore interesting to investigate if the parameters obtained using Van den Berg-Ružić-Lee-analysis adequately mimic a Scatchard plot of the titration data. Figure 6.3.4 clearly shows that this is not the case. It is evident that strong complexes are not detected by the Van den Berg-Ružić-Lee-analysis. Using an analogous procedure as used to find the data of the second group of ligands, a third group can be introduced in the prediction of the Scatchard plot. The final results, as plotted in figure

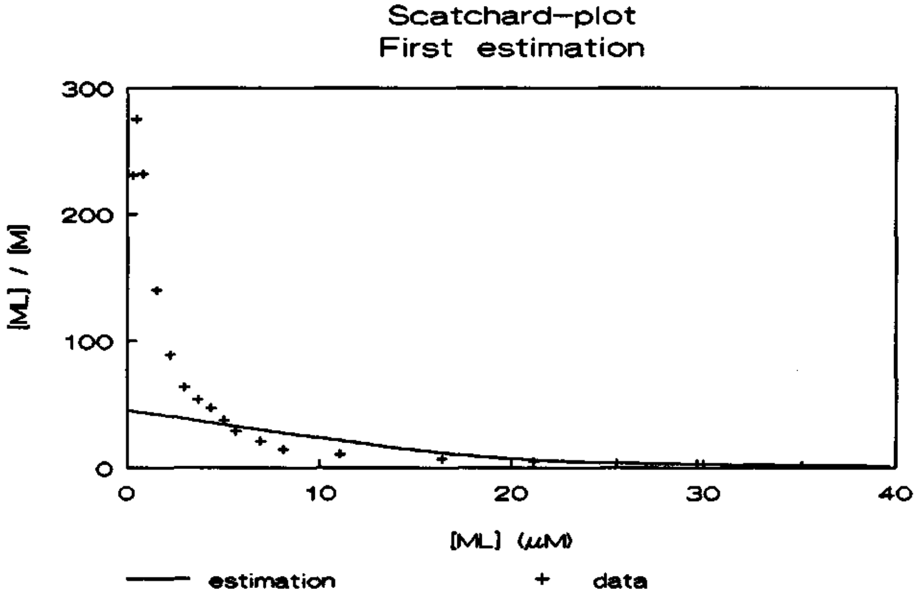


Figure 6.3.4. Estimation of the Scatchard-plot using parameters of figure 6.3.3.

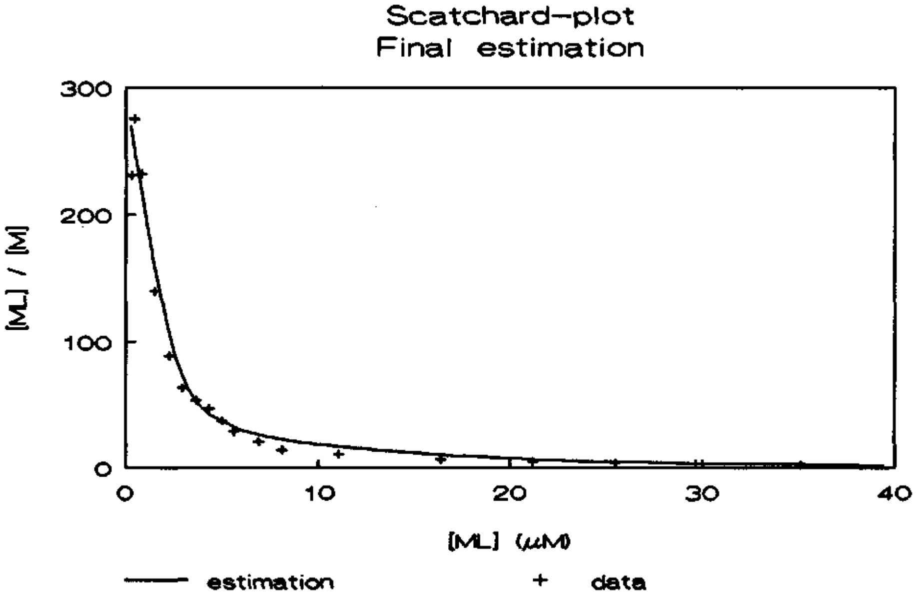


Figure 6.3.5. Final estimation of the Scatchard-plot.

6.3.5, are:
group 1: [L] = 1.1 µM; K = $6.2 * 10^8$;
group 2: [L] = 18.9 µM; K = $2.3 * 10^6$;
group 3: [L] = 79.4 µM; K = $1.6 * 10^4$;
total : [L] = 99.4 µM.

Thus it is shown that three groups of ligands must be supposed in order to mimic the titration data adequately. It is also demonstrated that a combination of analyses is necessary to obtain these parameters. Van den Berg-Ružić-Lee analysis appears not to be an appropriate technique to investigate copper complexation at low copper additions, but Scatchard analysis does.

6.4. Influence of the pH

6.4.1. Introduction

In earlier sections of this chapter, only copper titrations performed at one pH (6) have been discussed. It was considered important to gain an understanding of the copper complexation at higher pH-values, as the pH in Lake Tjeukemeer is usually higher than 6. For that purpose, titrations at pH-values of 3, 4, 5, 6 and 7 were carried out and analyzed by Nederlof and De Wit (Agricultural University of Wageningen, Department of Soil Science and Plant Nutrition). At first sight it may seem odd that titrations were carried out at pH = 3 in order to understand something about copper complexation at pH-values above 6. However, there were two good reasons for doing this. The first reason was a practical one: at high pH-values copper precipitates when added in sufficiently high concentrations. The second reason was a more fundamental one: the most active sites are ecologically most relevant. Unfortunately the detection limit of an Ion Selective Electrode (ISE) is fairly high, so at high pH-values a considerable amount of copper must be added before a reliable ISE-signal can be determined as most of added copper will be complexed by organic ligands. At lower pH, added copper will not so easily form organic complexes due to increased competition with H^+-ions. Those sites however which do react with copper are the most active ones, and thus were the ones of greatest interest to this study. Besides, inorganic complexation is much less favoured at low pH. Consequently, an ISE may only be used at a lower pH to assess the high affinity sites. These high affinity sites are also responsible for copper complexation at a natural pH and copper concentration. A disadvantage of this approach is that it requires extrapolation and therefore supposes copper complexation to have a constant pH-dependence, which is not *a priori* correct.

6.4.2. Materials and methods

Titrations were carried out as described in section 6.2, at pH 3, 4, 5, 6 and 7.

Analysis of pH-dependence was done as follows: if a linear pH-dependence was supposed, complexation of copper by organic ligands would be described by the equilibrium

$$Cu^{2+} + H_xL === CuL + x\,H^+ \qquad (6.4\text{-}1)$$

(where charges of the organic ligand are omitted), for which the equilibrium constant K can be expressed as:

$$K = \frac{[CuL] * [H^+]^x}{[Cu^{2+}] * [H_xL]} \qquad (6.4\text{-}2)$$

The concentration of organic copper may then be expressed as:

$$[CuL] = \frac{K * [Cu^{2+}] * [H_xL]}{[H^+]^x} \qquad (6.4\text{-}3)$$

On the other hand, in the case of chemical heterogeneity, the fraction of occupied binding sites θ may be expressed as

$$\theta_{i,t} = \int \theta_i \, f(\log(K_i)) \, d \log(K_i) \qquad (6.4\text{-}4)$$

after Van Riemsdijk, Bolt & Koopal (1991), where $f(\log(K_i))$ is the distribution function of randomly distributed K values. A possible analytical solution of equation 6.4-4, in the case of complexation of ion B, is the "generalized" Stern-Freundlich equation

$$\theta_{B,t} = \frac{K * [Cu^{2+}] / [H^+]^x}{1 + (K * [Cu^{2+}] / [H^+]^x)} \qquad (6.4\text{-}5)$$

If the term $(K * [Cu^{2+}] / [H^+]^x)$ is much smaller than 1, and $\theta_{B,t}$ is replaced by $[CuL]/Q_{max}$ (where Q_{max} is the number of sites), equation 6.4-5 evaluates to

$$[CuL] = \left(K * \frac{[Cu^{2+}]}{[H^+]^x} \right)^{\beta} \qquad (6.4\text{-}6)$$

This is in fact a Freundlich binding isotherm in which pH is included. The value for K, indicating a weighted average K-value, includes the concentration of free sites; the value for β (ranging from 0 to 1) indicates the width of the distribution around the average (a value of 1 indicates a narrow distribution, a smaller value a wider distribution). An isotherm of this type has been described previously by Lexmond (1980) and De Haan & Van Riemsdijk (1986) to describe pH-dependent adsorption. When converted to a log-scale, data of several pH-values can be fitted using appropriate fitting techniques, resulting in values for x, K and β.

6.4.3. Results and discussion

Data obtained at pH = 7 were not usable. At a certain stage during the titration, the free copper concentration did not increase any more when copper was added. This was probably caused by copper precipitation. Data for pH = 3 and 4 were separated from data for pH = 5 and 6, and analyzed in two groups. In addition, all data were analyzed together (table 6.4.1). It is obvious that there is a huge difference in the results, depending on which pH-values are included in the fit. The fit for all four pH-values is obviously dominated by the higher pH-values, as these results are quite similar. Figure 6.4.1, in which these results are shown, also demonstrates that data for pH = 5 and 6 dominate the fit when all data are included. The much smaller value for β when only the lower values for pH are used indicates a much wider distribution of K-values around the average for the higher affinity sites.

An interesting application of this technique is extrapolation to pH = 7.55, the pH which is used for the experiments with MnO_2 and the EDTA ligand competition (chapter 5). The MnO_2 technique yielded values for the log(free copper concentration) of roughly -11, the EDTA technique -13 to -12. Using the parameters of table 6.4.1, assuming [CuL] equals total copper, and using the 1989 average for dissolved (< 0.2 µm) copper, log(free copper) can be calculated to be -13.9 (only pH of 3 and 4), -10.2 (only pH of 5 and 6) and -10.3 (for all data). Three interesting conclusions can be drawn from these

parameter	pH = 3, 4	pH = 5, 6	pH = 3, 4, 5, 6
x	0.7000	0.4200	0.4657
log K	-13.097	- 5.041	- 5.284
β	0.3500	0.6300	0.6309
sum of squares	$2.7 * 10^{-12}$	$7.9 * 10^{-11}$	$1.4 * 10^{-10}$

Table 6.4.1. Results of the pH-dependent Freundlich fit when only pH = 3 and 4 are included, only pH = 5 and 6 are included, and all pH-values are included.

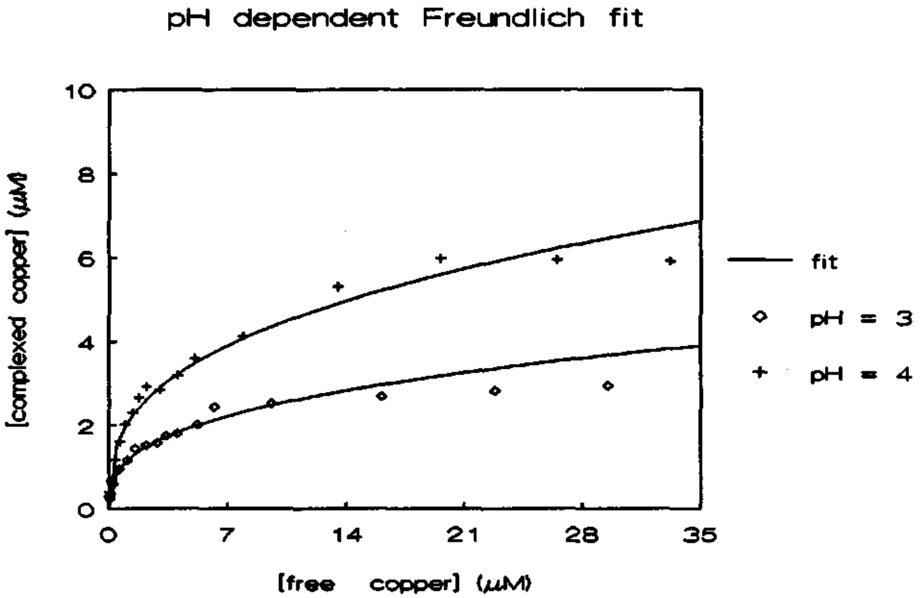


Figure 6.4.1A. pH dependent Freundlich fit for pH = 3 and pH = 4. Lines indicate fit; symbols indicate data points.

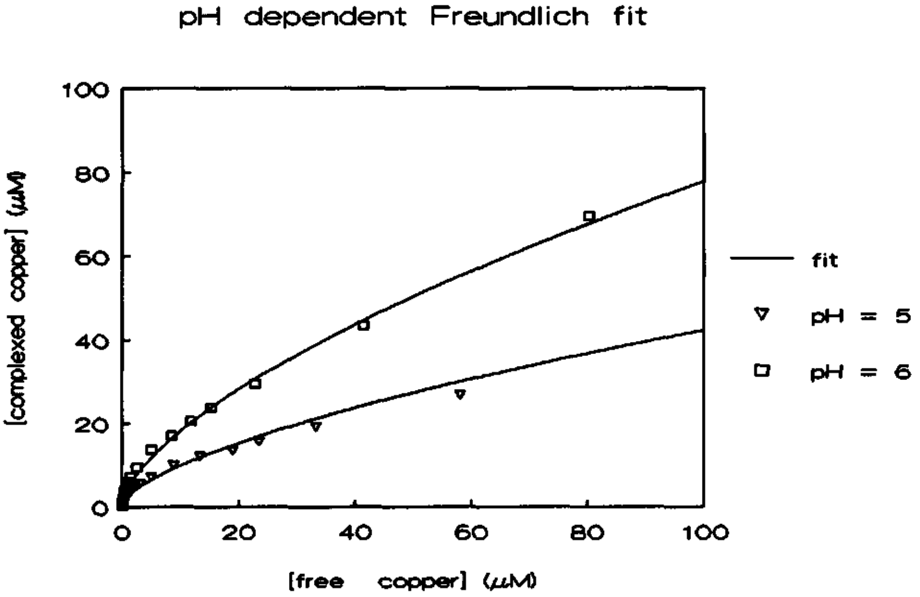


Figure 6.4.1B. pH dependent Freundlich fit for pH = 5 and pH = 6.

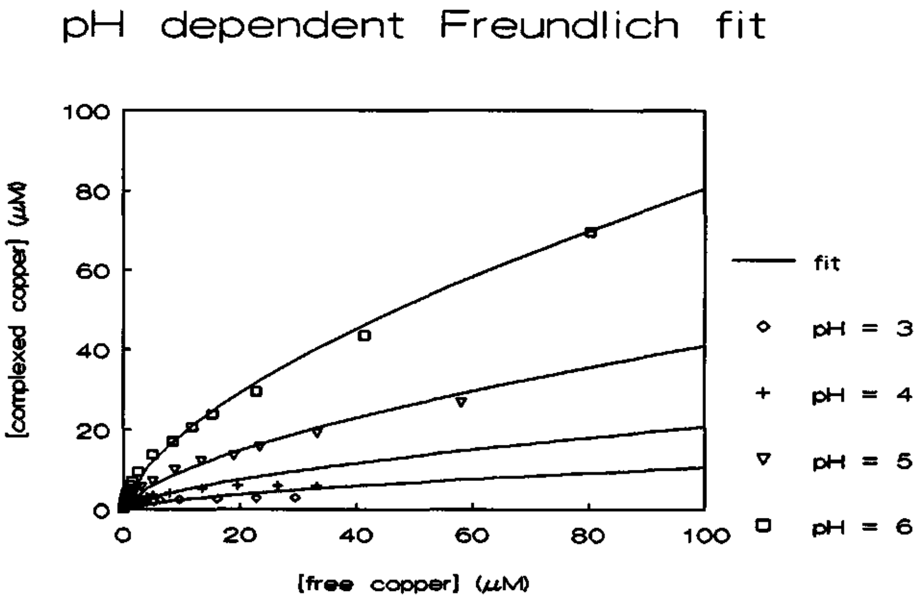


Figure 6.4.1C. pH dependent Freundlich fit for pH = 3, 4, 5, 6.

calculations. First of all, the low value for free copper, obtained when data of pH 3 and 4 are used, clearly indicates that at these pH-values, the most active sites react with copper. Secondly, again the higher pH-values turn out to dominate the fit for all data. Finally, it is interesting to note that the results of this method are not in disagreement with the MnO_2 and EDTA method, in spite of the quite different approach.

6.5. Copper titrations using anodic stripping voltammetry

6.5.1. Introduction

The titration curves described in section 6.2 were analyzed only for [added copper] more than 0.31 μM because of the detection limit of the employed ISE. In fact, under natural conditions the most relevant part of a titration curve is the region where additions are small. Anodic stripping voltammetry (ASV) has a lower detection limit than an ISE and is therefore quite suitable for investigating the behaviour of Cu at low additions.

In section 6.3 it has been demonstrated that Scatchard analysis can conveniently be used for low copper additions. For that reason, this approach was used for the calculations in this section.

6.5.2. Materials and methods

Samples were measured as described in section 4.1.2: 4 ml of lake water, 1 ml of acetate buffer, and the copper additions were equilibrated overnight and measured using differential pulse ASV. Final pH of the sample was 5.0, which is much lower than natural pH. Lowering the pH was however necessary because otherwise the currents would have been too small (i.e. probably not measurable at all).

Seven samples were prepared, two without addition, five with copper additions in steps of 31.5 nM, so that the highest addition was 157.4 nM.

6.5.3. Results and discussion

For three arbitrarily selected dates in 1988, the ASV titration curves were analyzed using the Scatchard method, and the results were compared with the results obtained by ISE titrations. However, ASV titrations were carried out at pH = 5, and ISE titrations at pH = 6. To avoid unnecessary comparison of results obtained at different pH-values, data of an ISE titration carried out at pH = 5 (as described in section 6.4) were used. These measurements were not performed in the same samples as the ASV titrations, but in section 6.2

	Scatchard (ASV)			Scatchard (ISE)	VdB-R-L (ISE)
date	12 Jan 88	4 Oct 88	18 Oct 88	10 Nov 87	10 Nov 87
[L]	192 nM	190 nM	297 nM	6.75 µM	20.3 µM
log K	8.48	8.44	8.13	5.91	5.20
[total copper]	222 nM	184 nM	200 nM	4.44 µM	52.4 µM
correlation coefficient	0.7391	0.8433	0.4901	0.9639	0.9774

Table 6.5.1. Comparison of ISE and ASV complexation parameters. VdB-R-L = Van den Berg-Ružic-Lee. Van den Berg-Ružic-Lee analysis applied to [added copper] from 0.31 to 52.4 µM; ISE Scatchard to [added copper] from 0.82 to 4.43 µM; ASV Scatchard to [added copper] from 0 to 0.16 µM; [total copper] is [natural copper] plus [highest addition]; please note the different unities <u>µ</u>M and <u>n</u>M.

it has been shown that the seasonal variation in copper complexation is relatively small.

Table 6.5.1 contains the results. The ASV titrations clearly yielded lower values for the ligand concentration, which was as expected, since applied copper additions were smaller for ASV titrations (please note the different unities µM and nM). It is remarkable that for all ASV titrations the ligand concentration was higher than the concentration of added copper (157 nM). Only for the last one, however, the ligand concentration was significantly higher than total copper concentration, but for this titration the correlation coefficient of the Scatchard regression analysis was fairly low. It is therefore suggested that all copper is organically complexed (which is to be expected on the basis of earlier chapters), and that this phenomenon can be verified using the Scatchard method.

Conditional stability constants of ASV titrations were clearly higher than of ISE titrations. This is in agreement with expectations for two reasons. First of all, concentrations used in ASV titrations were much lower (up to 157 nM) than in ISE titrations (from 305 nM), so in ASV titrations more stable complexes could be detected, provided the equilibration times were long enough. Secondly, ASV samples were equilibrated overnight, while for ISE titrations not more than a few minutes equilibration time was allowed, so ASV samples were much more likely to have been in equilibrium than during ISE titrations. From these two effects, concentration range and equilibration time, in ASV titrations, the emphasis was laid on the higher activity sites, resulting in higher values for the conditional stability constant.

The conclusion is that for low copper concentrations complexes were formed with log(conditional stability constants) between 8 and 9, and that all copper was organically complexed, according to the Scatchard method.

6.6. Ultrafiltration and copper titrations

6.6.1. Introduction

In chapter 3 it has been described how ultrafiltration was used to gain insight in the size of copper species present in Lake Tjeukemeer. It was found that about half of the copper species was larger than 5 nm. It is interesting to investigate the size of ligands that form complexes with copper when copper is added. This can be done by performing titrations in the several ultrafiltrates. Scatchard analysis can be used to obtain information on the conditional stability constants and the apparent ligand concentrations.

6.6.2. Materials and methods

Lake Tjeukemeer samples from two dates (November 15, 1988 and March 8, 1989) were subjected to ultrafiltration as described in chapter 3. Portions of the five filtrates were equilibrated with acetate buffer and with a copper addition according to the addition scheme mentioned in section 6.5.2. Differential pulse ASV was used to measure labile copper. For samples with additions of 94.4 nM and higher a deposition time of 500 seconds was used, for samples with lower additions the deposition time was 900 seconds.

6.6.3. Results and discussion

In figure 6.6.1A and B the results are depicted. Although some samples must have been contaminated (e.g. the sample < 5 nm for [added Cu] = 94.4 nM in figure 6.6.1B contained more labile copper than total copper), a few interesting conclusions can be drawn. The general trend was that for the same copper addition, labile copper decreased when the pore size increased. This means that after each step in the ultrafiltration process ligands were removed from solution. As in chapter 3, it was proven that some natural copper complexing ligands may be fairly large. On the other hand, in the fraction

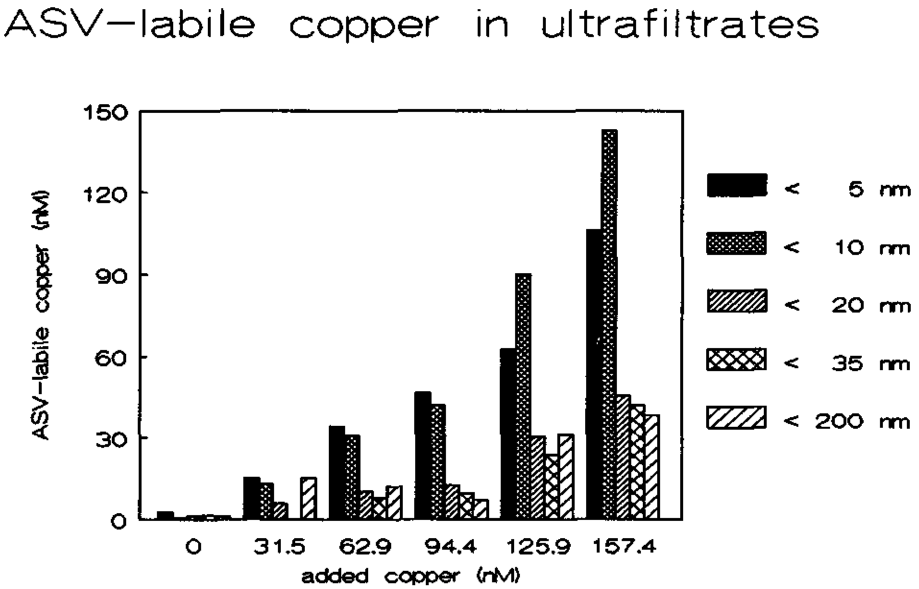


Figure 6.6.1A. ASV-labile copper in ultrafiltrates; sample November 15, 1988.

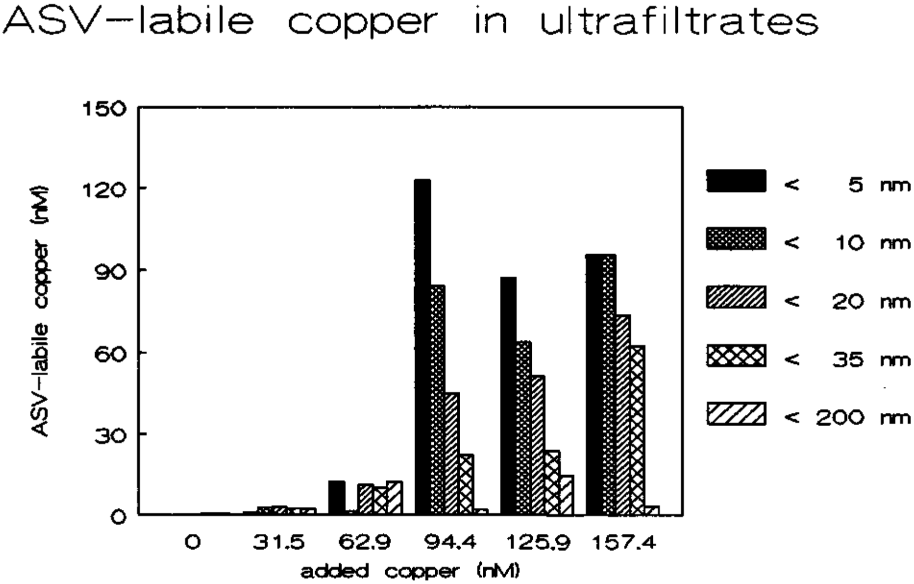


Figure 6.6.1B. ASV-labile copper in ultrafiltrates; sample March 8, 1989.

< 5 nm there was still a considerable part of added copper being complexed, so that it can be concluded that not all ligands were large.

Scatchard analysis of the second dataset was a limited success as a result of the positive slopes of the regression lines. Only for the fractions < 35 nm and < 20 nm useful complexation parameters could be obtained ([L] = 122 nM and 101 nM respectively; log K = 8.43 and 8.37 respectively). For the first data however, Scatchard analysis was performed without problems. Figure 6.6.2 shows that the apparent ligand concentration gradually decreased with pore size, except for the smallest fraction. Log K gradually increased when the pore size became smaller, the only exception being once again the smallest fraction. It is not clear why this fraction displayed a different pattern, but at the extremely low concentration levels used in this study, it is possible that this was caused by unavoidable contamination.

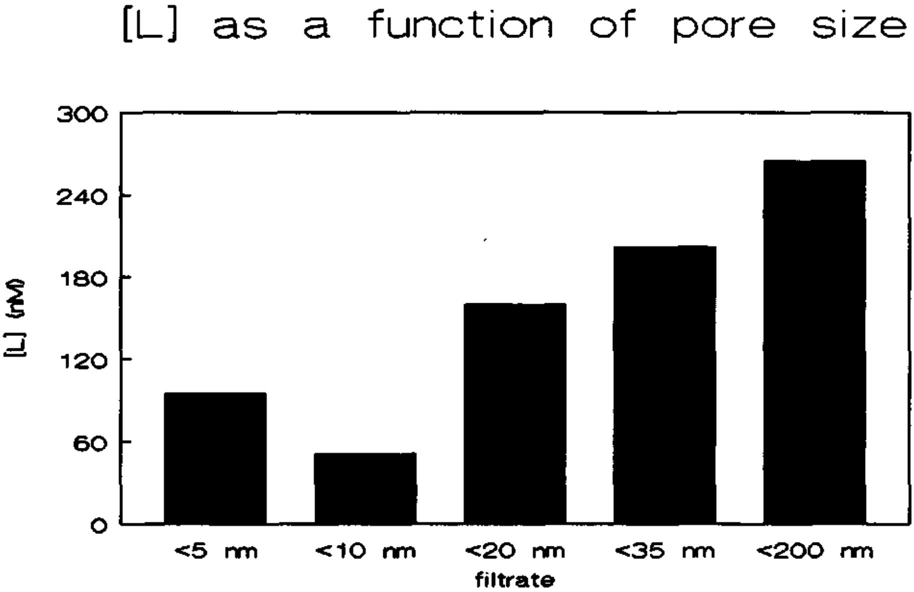


Figure 6.6.2A. Apparent ligand concentration (in nM of Cu) as a function of pore size.

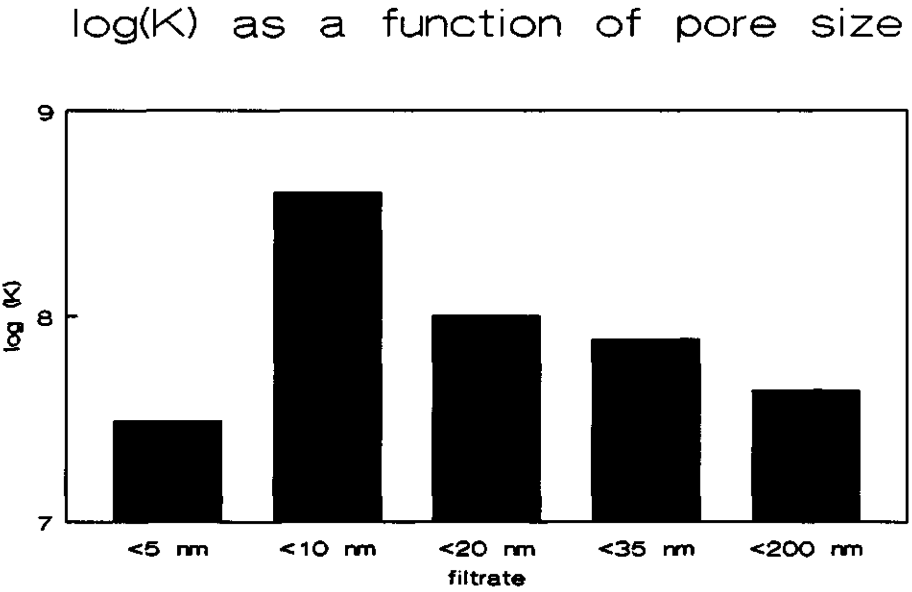


Figure 6.6.2B. Conditional stability constant as a function of pore size.

Chapter 7

Modelling

7.1. Discussion of the technique

In addition to using analytical physical and chemical speciation techniques, speciation can be (partly) calculated by (computer) models. Usually equilibrium is assumed for such calculations, although this assumption is not always valid. Modelling non-equilibrium situations, however, is much more complicated (Kramer, 1985). Two approaches may be used to calculate the equilibrium situation of a system: minimization of the Gibbs free energy or by solving a set of equations describing the system in terms of equilibrium reactions and constants. Since equilibrium constants are considered more reliable than free energy data, the second approach is recommended (Nordstrom et al., 1979; Nordstrom & Ball, 1984). It should be emphasized that from a thermodynamical point of view there is no difference between these approaches, since the free energy and the equilibrium constant of an equilibrium are interrelated according to $\Delta G° = -RT \ln K$; only the calculation methods and the way the problem is mathematically defined are different.

The most striking advantage of modelling is the fast and simple way by which insight can be gained in the speciation of natural or synthetic systems, and the changes in speciation caused by modifications of such systems. A disadvantage is that equilibrium constants reported by different workers may vary by a factor 100 or 1000 (Giesy & Alberts, 1989), obviously yielding very different results. A limitation of the modelling approach is that meaningful calculations can only be done if the chemical species present are relatively well defined and the equilibrium constants known to a sufficiently high degree. This means that, although computers can calculate the concentrations of species with as many digits as desired by the user, the results always have to be examined very critically.

At different ionic strength species have different activities, influencing the concentrations of species on either side of the equilibrium. To be able to calculate equilibrium relationships at ionic strengths different from the ionic strength for which equilibrium constants are obtained, activity coefficients have to be calculated. Several equations are available for this purpose. The one with the most solid theoretical basis is the Debye-Hückel approach.

More empirical descriptions are given by the extended Debye-Hückel, Güntelberg and Davies equation. The Davies equation is probably one of the most applied, mainly because it is valid for ionic strengths up to 0.5 M

(Stumm & Morgan, 1981). Morel (1983) has demonstrated that the Davies-equation yielded different activity coefficients than the Debye-Hückel approach, and that, unfortunately, neither could perfectly describe all situations.

The various programs which have been developed to model speciation are reviewed by Nordstrom et al. (1979) and Nordstrom & Ball (1984). Smith and Martell (in alternating order, 1974, 1975, 1976, 1977, 1982, 1989) made excellent compilations of known equilibrium data.

In principle, it is possible to adapt the conditional stability constants for the current temperature, but such corrections require knowledge of heat capacity data of each equilibrium. However, that knowledge often is not available (Stumm & Morgan, 1981). On the other hand, the uncertainties caused by the lack of knowledge of temperature dependence of equilibria is not so important compared to the uncertainties in the values for the equilibrium constants.

7.2. Materials and methods

For the model calculations used in this thesis, a new program was written; not simply because researchers enjoy programming, as suggested by Nordstrom & Ball (1984), but because writing a program is probably the best way to learn and understand the possibilities and limitations of equilibrium calculations. The program is called GECHEQ (a <u>GE</u>o<u>CH</u>emical <u>EQ</u>uilibrium model) and runs on IBM® and compatible personal computers. More detailed information on the program may be found in Verweij (1990a, 1990b). The intrinsic equilibrium constants were adjusted for the prevailing ionic strengths using activity coefficients calculated with the Davies equation:

$$\log f_i = -z_i^2 * \tfrac{1}{2} * \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3 * I\right) \qquad (7\text{-}1)$$

where f_i is the activity coefficient of the i-th species, z_i its charge, and I the ionic strength. No temperature corrections were performed. Equilibrium constants were taken from Smith & Martell (1989), Sposito & Mattigod (1980), and some other sources (Al Hanson, personal communication; Van Riemsdijk & Keizer (1984); Martell & Smith (1977); Morel (1983); Stumm & Morgan (1981); Turner, Whitfield & Dickson (1981), Weast (1979); West (1969)), but only if they appeared compatible with the former two references. Total concentrations were taken from an internal report (De Boer et al., 1990).

7.3. Results and discussion

Before the calculations can be performed usefully, a critical examination has to be made of the data that are used as input. For carbonate no equilibrium with air was assumed, but the measured values for alkalinity (mainly bicarbonate) were used. Carbonate was higher than in the equilibrium situation, probably due to biological activity.

Oxygen was supposed to be in equilibrium, because measurements have shown complete oxygen saturation of Lake Tjeukemeer (Van Huet, personal communication). For such a situation the relationship $pE = 20.6 - pH$ (Turner, Whitfield & Dickson, 1981) or $pE = 20.75 - pH$ (Stumm & Morgan, 1981) can be derived.

Unfortunately, the analyses performed in Lake Tjeukemeer were not completely compatible, since some methods were applied only to the 0.2 µm filtrate, some only to the raw water, and some to both. To test the sensitivity of the calculations to various inputs, two sets of variables were used, one set contained as many as possible dissolved concentrations, the other set had as many as possible total concentrations. The difference was small, so the dissolved concentrations were used wherever possible, because all methods described in this thesis were applied to filtrates.

It was also tested whether or not precipitation equilibria should be included, by calculating the speciation with and without precipitation equilibria, and comparing the results. Theoretically precipitation equilibria should be included, since in 0.2 µm filtrates some material may be present which is not truly dissolved (Stumm & Morgan, 1981). However, it was not clear if in Lake Tjeukemeer true equilibrium existed between solution and solids. Only small differences were found, and therefore precipitation equilibria were not included for the sake of simplicity.

Special attention has to be paid to copper borate complexes. Using the constants reported by Sposito & Mattigod (1980), calculations had shown that for boron concentrations typically for freshwater (Van der Geugten, 1981) a considerable part of inorganic copper in Lake Tjeukemeer was present as copper borate complexes. Van den Berg (1984d) reported much lower constants for these complexes, and consequently copper borate complexes were calculated to be much less important if these constants were used. We tried to determine total boron concentration in Lake Tjeukemeer to gain some insight in the possible importance of copper borate complexes for inorganic copper speciation, but this caused considerable problems. Because no reliable boron concentrations could be obtained, copper borate complexes ,were not included in the calculations.

Generally, the most complicated problem in modelling natural situations usually is organic complexation, since the organic material will differ depending on the source. In section 6.4 it was described how titrations carried out at several pH-values (3, 4, 5 and 6) can be used to obtain a general relationship between the pH and organic copper complexation. In the samples used for the calculations in this chapter, only titrations at pH = 6 have been carried out. The approach described in section 6.4 will be used here to extrapolate those data, derived from titrations at pH = 6, to natural pH. Two scenarios including organic copper complexation were calculated: one using the fit of only the titration data at pH = 3 and 4, and one using all data (pH = 3, 4, 5 and 6). Since the value for K comprises not only the strength of the complexation, but also a maximum for the number of sites, the value of K was adjusted according to the concentration of dissolved organic carbon in the samples. For comparison the speciation was also calculated supposing only inorganic complexation.

Summarizing, the following assumptions and choices were made:

- for carbonate, alkalinity data were used;
- the water was supposed to be in equilibrium with oxygen; pE was calculated to be (20.6 - pH);
- when possible, concentrations in filtrates were entered; otherwise, concentrations in raw samples were used;
- precipitation equilibria were not calculated;
- boron was not included in the calculations;
- the approach as described in section 6.4 was used to describe organic complexation at natural pH;

Table 7.1 contains the calculation results. If only the inorganic speciation was calculated, copper was mainly present as carbonate complexes. Free copper was already very low (0 - 2% of total copper). As expected, this percentage decreased when organic complexation was included in the calculations. When the data of the fit using all four titrations were used, free copper comprised not more than 0.2% of total copper. Organic copper was calculated to represent more than 90% of the copper concentration, except for the second sample. In this very alkaline sample (pH higher than 9!) carbonates were very important according to the calculations, especially the mixed complex $Cu(CO_3)(OH)^-$. Copper carbonate complexes typically represented a few percent, except for the already mentioned second sample. Copper hydroxide complexes appeared to be negligible. Other copper complexes were calculated to be unimportant.

In section 6.4 it has been explained and demonstrated that using only the titration data of pH = 3 and 4 yielded lower free copper concentrations than using the data at pH = 3, 4, 5 and 6. Indeed, calculating the copper speciation for this scenario resulted in free copper concentrations around 10^{-15} M, which is much less than 0.01% of total copper. Virtually all copper was calculated to be complexed by organic material. The concentrations of both hydroxides and carbonates were found to be negligible.

In chapter 4 (ASV) and 5 (MnO_2 and EDTA) it was estimated that 92 - 100% of the copper in Lake Tjeukemeer was organically complexed. Using speciation

date	8 Mar 89	28 Jun 89	20 Sep 89	13 Dec 89
input				
pH	8.24	9.33	8.32	7.95
alkalin.	1.88 mM	2.11 mM	1.96 mM	2.14 mM
[copper] in 0.2 µm filtrate	36.19 nM	28.33 nM	14.16 nM	15.74 nM
results				
only inorganic				
free copper	0.354 nM (0.98%)	0.005 nM (0.02%)	0.111 nM (0.79%)	0.348 nM (2.21%)
hydroxid.	2.04 nM (5.63%)	1.23 nM (4.34%)	0.76 nM (5.35%)	0.86 nM (5.44%)
carbon.	33.69 nM (93.06%)	27.09 nM (95.62%)	13.25 nM (93.57%)	14.49 nM (92.11%)
also organic (3,4,5,6)				
free copper	0.034 nM (0.10%)	0.003 nM (0.01%)	0.009 nM (0.07%)	0.026 nM (0.17%)
hydroxid.	0.20 nM (0.54%)	0.69 nM (2.45%)	0.06 nM (0.45%)	0.06 nM (0.41%)
carbon.	3.27 nM (9.04%)	15.26 nM (53.88%)	1.12 nM (7.94%)	1.10 nM (6.96%)
organic copper	32.68 nM (90.29%)	12.37 nM (43.65%)	12.96 nM (91.52%)	14.55 nM (92.44%)
also organic (3,4)				
free copper	10.54 fM (0.00%)	1.04 fM (0.00%)	0.82 fM (0.00%)	3.15 fM (0.00%)
hydroxid.	******************	******************	******************	******************
carbon.	0.97 pM (0.00%)	6.02 pM (0.02%)	******************	******************
organic copper	36.19 nM (100.00%)	28.32 nM (99.98%)	14.16 nM (100.00%)	15.74 nM (100.00%)

Table 7.1. Speciation of copper at natural pH as calculated by GECHEQ; "alkalin." = alkalinity; "hydroxid." = sum of copper hydroxides; "carbon." = sum of copper carbonates; pM = 10^{-12} M; fM = 10^{-15} M; ****************** means less than 0.001% of copper. Alkalinity is expressed as carbonate concentration. Three scenarios are displayed: a) only inorganic speciation included in the calculations; b) also organic speciation included using the titration data for pH = 3, 4, 5 and 6; c) idem for pH = 3 and 4.

modelling, copper was calculated to be 90 - 100% organically complexed (except for the second sample), which was in good agreement with values calculated using ASV, MnO_2 and EDTA methods. The free copper concentration also agreed among the four methods, except when the fit of only titrations at pH = 3 and 4 were used. In the latter case, lower free copper concentrations were obtained, which is in agreement with expectations because this way the most active sites were assessed.

It can be concluded that the results obtained using modelling are in good agreement with results of the other methods.

Chapter 8

Speciation of copper in relation to its bioavailability

The various methods used to gain insight into the speciation of copper in Lake Tjeukemeer have been described in chapters 3 to 7 inclusive. The main objective of this project was to relate copper speciation to its bioavailability (section 1.2). Chapter 8 deals with the study of this relationship. Bioassays were performed in lake water and synthetic media. Electrochemical techniques and equilibrium calculations were applied to the same samples and synthetic solutions. The results of these experiments are presented as a paper submitted for publication. As a consequence, some parts of earlier chapters will be repeated.

References are included at the end of this thesis.

Speciation of copper in relation to its bioavailability

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8.1. Abstract

Copper speciation and bioavailability for *Scenedesmus quadricauda* has been studied in natural waters and in synthetic culture media. Other elements were studied simultaneously. When phosphorus and nitrogen limitation were excluded by adding these elements, copper was limiting algal growth in some natural waters. In the toxic range, growth inhibition by copper was highly correlated with copper detected by electrochemical methods and with calculated free copper.

Copper was toxic to *S. quadricauda* when free copper concentrations roughly exceeded $10^{-10.5}$ M, and was limiting for values somewhere lower than $10^{-12.5}$ M. Because we found copper limitation in some natural water samples, free copper concentration in those water samples therefore must have been lower than $10^{-12.5}$ M.

The hypothesis that the free metal concentration rather than the total concentration determines bioavailability was confirmed for copper, cobalt and zinc.

Keywords: bioavailability, speciation, copper.

8.2. Introduction

Copper is known to be an essential metal for algae, but if it is present in too high concentrations, it may be toxic as well. Free copper (Cu^{2+}_{aq}) rather than the total concentration of copper controls its bioavailability to algae (Sunda & Guillard, 1976; Anderson & Morel, 1978; Sunda & Gillespie, 1979; Baccini & Suter, 1979; Petersen, 1982; Sunda & Ferguson, 1983; Sunda, Klaveness & Palumbo, 1984; Anderson, Lively & Vaccaro, 1984). Bioavailability research is therefore only meaningful if the speciation of the metal of interest is also investigated. Information on speciation can be obtained by various techniques: physical, chemical or biological methods, speciation calculations, or a combination of these techniques. Indications that anodic stripping voltammetry (ASV) can be used to obtain correlative information on the fraction of copper that is biologically available can be found in the literature (Young et al., 1979; Florence, 1986). Since ASV-experiments can be performed much easier and faster than bioassays, the possible replacement of bioassays by ASV-measurements would represent an advantage.

The determination of copper speciation and bioavailability can be done in natural water or synthetic media. The former approach has direct ecological importance, but requires knowledge of the extent of organic copper complexation if speciation calculations are to be carried out. In the case of synthetic media most equilibria of interest are known (Giesy & Alberts, 1989) allowing for relatively easy calculation of copper speciation. In this paper it is described how bioassays can be used for obtaining biological information while anodic stripping voltammetry provides chemical measurements. For one sample, these data were compared with calculations from an equilibrium speciation model.

Two types of experiments were performed. The first type (carried out in natural waters) was meant to find out if the three mentioned methods yield compatible information, the other type (performed in synthetic media) was set up to quantify biologically available copper.

8.3. Methodology

Bioassays. A green alga, *Scenedesmus quadricauda*, was used for all bioassays. For bioassays in natural water a stock suspension of algae, grown in batch cultures, was used. Composition of the medium is shown in table 8.1A. *S. quadricauda* was chosen because it is the main green alga in Lake Tjeukemeer.

The stock suspension was used to inoculate the experimental cultures. Preliminary experiments had shown that addition of phosphorus and nitrogen was necessary to observe any increase in number of algae. For this reason, 0.16 µM phosphorus (as K_2HPO_4) and 7.1 µM nitrogen (as $NaNO_3$) was added to the filtered samples. Copper was also added in various concentrations ranging from 0 to 15.7 µM. A solution of 0.2 M of the buffer compound 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES) in 0.1 M NaOH was brought to the pH of the natural sample and added to the sample in a final concentration of 0.02 M. Sample pH was checked each day and adjusted to the original value, if necessary (ranging from 6.83 to 7.87). All cultures were continuously shaken, kept at 23°C in a waterbath and continuously illuminated by two fluorescent tubes. During preliminary experiments, light absorption of

	A	B	C
Na	$1.7 * 10^{-3}$ M	$1.4 * 10^{-2}$ M	$1.5 * 10^{-2}$ M
Mg	$2.1 * 10^{-4}$ M	$1.0 * 10^{-4}$ M	$1.0 * 10^{-4}$ M
K	$2.9 * 10^{-5}$ M	$7.4 * 10^{-5}$ M	$6.5 * 10^{-5}$ M
Ca	$3.0 * 10^{-4}$ M	$3.0 * 10^{-4}$ M	$2.9 * 10^{-4}$ M
Mn	$3.6 * 10^{-6}$ M	$3.6 * 10^{-6}$ M	$5.9 * 10^{-7}$ M
Fe	$8.9 * 10^{-7}$ M	$4.5 * 10^{-7}$ M	$4.0 * 10^{-8}$ M
Co	$2.8 * 10^{-7}$ M	$2.8 * 10^{-6}$ M	$5.1 * 10^{-8}$ M
Cu	$3.2 * 10^{-7}$ M	$7.9 * 10^{-8}$ M	$6.0 * 10^{-9}$ to $1.6 * 10^{-5}$ M
Zn	$1.0 * 10^{-6}$ M	$1.1 * 10^{-6}$ M	$1.7 * 10^{-7}$ M
$B(OH)_4$	$4.9 * 10^{-6}$ M	$4.9 * 10^{-6}$ M	$7.9 * 10^{-7}$ M
CO_3	$5.0 * 10^{-4}$ M	$5.1 * 10^{-4}$ M	$4.8 * 10^{-4}$ M
NH_4	$9.9 * 10^{-7}$ M	$1.4 * 10^{-7}$ M	$1.6 * 10^{-8}$ M
NO_3	$1.2 * 10^{-3}$ M	$6.1 * 10^{-4}$ M	$5.4 * 10^{-5}$ M
PO_4	$1.4 * 10^{-5}$ M	$1.0 * 10^{-5}$ M	$2.5 * 10^{-5}$ M
SO_4	$2.1 * 10^{-4}$ M	$1.0 * 10^{-4}$ M	$1.0 * 10^{-4}$ M
Cl	$6.0 * 10^{-4}$ M	$6.2 * 10^{-4}$ M	$6.4 * 10^{-4}$ M
Mo_7O_{24}	$1.6 * 10^{-8}$ M		
EDTA	$5.4 * 10^{-6}$ M	$5.4 * 10^{-8}$ M	0 to $4.6 * 10^{-5}$ M
HEPES			$3.2 * 10^{-2}$ M

Table 8.1. Composition of the culture media; column A concerns stock for assays in natural waters, B stock for assays in synthetic media, C the synthetic media themselves.

samples with various turbidity was measured. Besides, the number of algae was counted using a microscope. Thus a calibration line of absorption versus number of algae could be constructed. This allowed to calculate the number of algae by simply measuring light absorption. Light absorption was measured at 750 nm using a Zeiss PMQ II spectrophotometer. Experiments with copper additions up to 0.16 µM were carried out in triplicate, with higher additions duplicate experiments were run.

For the bioassays in synthetic media a slightly different setup was used. A stock suspension of algae was grown in a continuous culture at pH = 7.5. A hydrochloric acid solution of 12 mM was added automatically by a pH-stat, if necessary to keep the pH constant. The composition of the stock medium as well as the composition of the medium used in the experiments are shown in table 8.1. Experimental solutions were buffered with HEPES at a pH of 7.55 (the pK_a of HEPES). In order to remove all traces of copper, algae were centrifugated and washed twice before inoculation with the medium used in the experiments. Copper and EDTA additions were equilibrated overnight before algae were inoculated.

Electrochemical measurements. Aliquots of natural water samples were used for electrochemical measurements. A 384B polarograph (EG & G Princeton Applied Research) equipped with a 303A electrode and a 305 stirrer (400 rpm) was used. Results were displayed on a plotter model DMP-40 (Houston Instruments). Nitrogen (99.999%) was used for purging. All potentials were recorded versus an Ag/AgCl reference electrode. A platinum wire served as counter electrode. A four ml sample aliquot was buffered by addition of 1 ml of acetate buffer (final concentration 0.1 M, pH = 5.0) and increasing amounts of copper were added. The pH was much lower than that of the natural samples (varying from 6.83 to 7.87), but acidification was necessary to obtain a measurable ASV-signal. It was calculated that under these conditions all inorganic copper species would dissociate to form copper acetate complexes. Some organic copper complexes may also have dissociated. Samples were equilibrated overnight before being subjected to analysis. Samples with copper additions up to 1.57 µM were measured using differential pulse anodic stripping voltammetry (deposition time 1800 seconds; deposition potential -0.6 V; pulse height 50 mV; scan speed 4 mV/second) while differential pulse polarography (same pulse height and scan speed as for stripping experiments) was used to determine labile copper in samples with higher additions. Calibration was done in a standard solution resembling water samples as closely as possible in sodium, magnesium, potassium, calcium, sulphate and chloride concentration as well as alkalinity, as recommended by Florence (1986).

Equilibrium calculations were carried out using a computer speciation program. The intrinsic equilibrium constants were converted into conditional constants using activity coefficients calculated according to the Davies equation (Stumm & Morgan, 1981):

$$\log f_i = -z_i^2 * \frac{1}{2} * ((\sqrt{I} / (1 + \sqrt{I}) - 0.3 * I) \qquad (8\text{-}1)$$

where I is the ionic strength, z_i and f_i are the charge and the activity coefficient respectively of the i-th species. No temperature corrections were performed. Equilibrium constants were taken from Smith & Martell (1989), Sposito & Mattigod (1980), and some other sources (Al Hanson, personal communication; Van Riemsdijk & Keizer (1984); Martell & Smith (1977); Morel (1983); Stumm & Morgan (1981); Turner, Whitfield & Dickson (1981), Weast (1979); West (1969)), but only if they appeared compatible with the former two references. Total concentrations of sodium, magnesium, potassium, calcium, alkalinity, sulphate and chloride were measured as described in internal reports (De Boer et al., 1987, 1989).

To obtain information on organic copper complexation, we performed copper titrations and measured the free copper concentration using a cupric ion selective electrode (ISE). We would have preferred to carry out these titrations at the pH of the natural sample, but this caused considerable problems. The high pH of the natural samples ($pH \geq 6.83$) may have caused copper precipitation as evidenced by a constant free copper concentration during further copper additions. Because of these problems, we investigated copper complexation as a function of pH at a range of pH-values (3, 4, 5 and 6) lower than the natural value, and used these data to extrapolate to the natural pH. The datasets were split into two parts: data obtained at pH = 3 and 4 on one hand, and data obtained at pH = 5 and 6 on the other. The combined datasets were also analyzed. Since the fit using all data was clearly dominated by the data obtained at pH = 5 and 6, two scenarios were used: one using data at pH = 3 and 4, another using data at pH = 3, 4, 5 and 6. This fitting procedure was done for only one sampling site (Lake Tjeukemeer) because the combination of copper titrations and data analysis is very laborious. Titration data were fitted to a Freundlich type equation of the form:

$$[CuL] = \left(K * \frac{[Cu^{2+}]}{[H^+]^x} \right)^{\beta} \tag{8-2}$$

where [CuL] is the concentration of complexed copper, $[Cu^{2+}]$ the concentration of free copper, K is a mixed constant, expressing both the binding strength and the concentration of binding sites, x a coefficient expressing the effect of the pH, and β a coefficient that is related to the width of the affinity distribution.

Samples were collected at four sites from the upper meter of the water column using a perspex tube, and filtered (0.2 µm) as soon as possible after arrival at the laboratory. The first sampling site was Lake Tjeukemeer (TM), a humus-rich and eutrophic lake in north Netherlands. It is part of a system of interconnected lakes and canals, the Frisian "boezem". The hydrology of the "boezem" is manmade: in summer the usually occurring lack of water is supplemented by letting in water from Lake IJsselmeer (YM), which was chosen as the second sampling site. In winter, the surplus of water in polders around Lake Tjeukemeer is pumped into the lake. A third sampling site was therefore chosen in a polder canal (EB). For comparison, a lake not connected with the "boezem" was also sampled (E9). This lake used to be part of the "boezem", but had been disconnected for about two years prior to sampling. Its main water source is its peaty drainage area.

8.4. Results

Bioassays in natural waters. A typical example of an extinction versus time graph of a bioassay in natural waters is shown in figure 8.1. Since the growth rates were less reproducible than the highest biomass in the stationary phase (measured as extinction), we chose the highest extinction obtained for each bioassay to represent the growth of algae. Figure 8.2 displays the highest extinction in the stationary phase obtained for each bioassay in natural water as a function of total copper concentration (log scale). The points for log(total copper) around -7.5 correspond to the natural samples with only phosphate and nitrate added (no copper addition). In all samples, adding 0.16 µM of copper resulted in a higher biomass. For two cases (Lakes Tjeukemeer and IJsselmeer) this increase was even significant.

The fitting of titration data to equation 8-2 in the pH range from 3 to 6 yielded $5.7 * 10^{-6}$ for K, 0.4657 for x, and 0.6309 for β; for pH 3 and 4 these values were $8.8 * 10^{-14}$, 0.7 and 0.35. These results were used to calculate free copper in the sample from Lake Tjeukemeer. In figure 8.3 these concentrations, together with electrochemically labile copper are shown as a function of total copper (log-log scale). Free copper concentration was also calculated for lower total copper concentrations to find out if the shape of the curves changed when the total copper concentration was lower, but this was not the case. Electrochemically labile copper was much higher than calculated

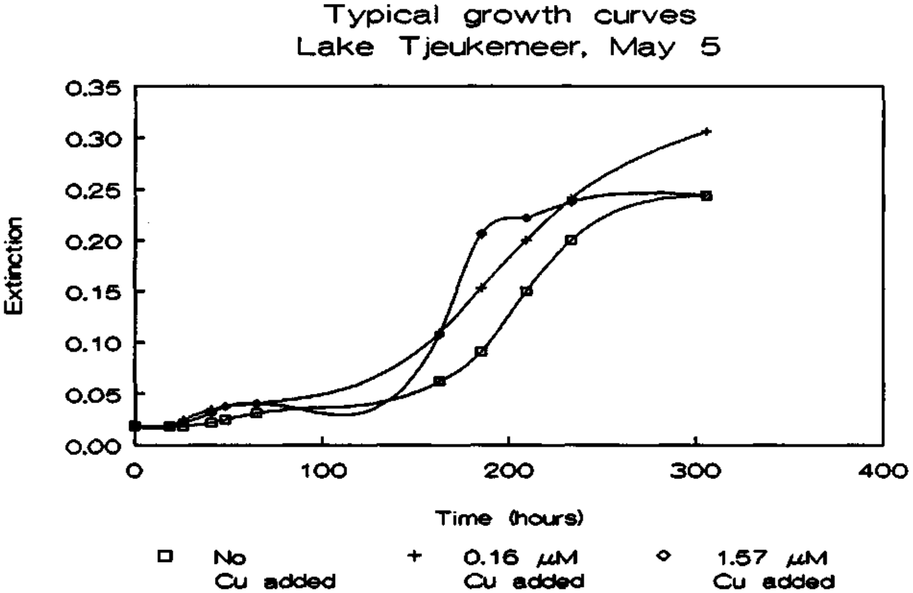

Figure 8.1. Typical example of growth curves. Lines are spline fits. Replicate assays not shown for sake of clarity.

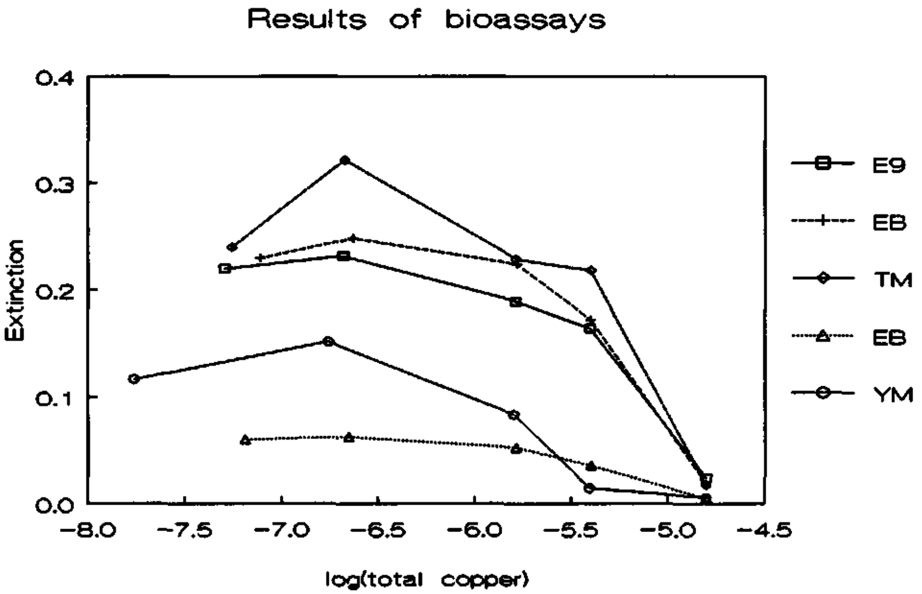

Figure 8.2. Maximum extinction for bioassays. E9 = lake not connected to the "boezem" (sampling date March 14); EB = polder site (crosses March 23; triangles May 31); TM = Lake Tjeukemeer (May 15); YM = Lake IJsselmeer (June 14).

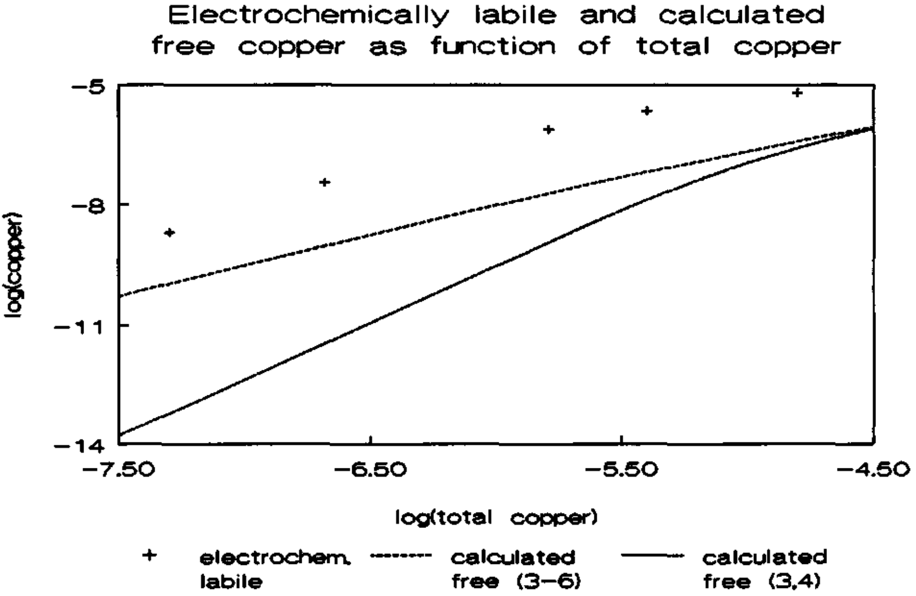

Figure 8.3. Log(electrochemically labile copper) and log(calculated free copper) versus log(total copper) for Lake Tjeukemeer; "3-6": titrations at pH 3, 4, 5 and 6 included in the fit; "3,4": only titrations at pH = 3 and 4 included.

sample	electrochem.-calc.	electrochem.-inh.	calc.-inh.
E9 March 14		0.9910 (<0.01)	
EB March 23		0.9847 (<0.02)	
TM May 15	0.9818 (<0.01) (A) 0.9535 (<0.02) (B)	0.9760 (<0.05)	0.9977 (<0.01) (A) 0.9949 (<0.01) (B)
EB May 31		0.9960 (<0.01)	
YM June 14		0.8251 (>0.1)	

Table 8.2. Correlations between electrochemically labile copper and calculated free copper, electrochemically labile copper and growth inhibition, and calculated free copper and growth inhibition (between brackets: p). A: titrations at pH = 3, 4, 5 and 6 included in the fit; B: only pH = 3 and 4 included. Sample codes are as in figure 8.2.

free copper, but since free copper and all inorganic as well as some organic copper species are known to be electrochemically labile, this was in agreement with expectations. Calculated free copper was much lower when the fit using only the titration data of pH = 3 and 4 was used. Since at these low pH-values the competition of Cu^{2+}- with H^+-ions is more important than at higher pH's, higher affinity sites are assessed. As a result, copper complexation was found to be stronger than when higher pH-values were included as well. Electrochemically labile and calculated free copper displayed a consistent pattern. Therefore, high correlations may be expected between electrochemically labile and calculated free copper. These correlation are shown in table 8.2 for the Lake Tjeukemeer sample.

To calculate correlations between electrochemically labile copper and calculated free copper with bioassay results, the maximum extinction for each bioassay was expressed as the inhibition compared to the maximum extinction at a copper addition of 0.16 µM (the addition that yielded the highest maximum extinction for each sample). Bioassays without added copper were not included in correlation calculations because algae were not copper inhibited under these conditions, but copper limited. Correlations of growth inhibition with electrochemically labile and calculated free copper respectively are also shown in table 8.2.

Correlations were also calculated omitting the data of the highest copper addition, to test if these data dominated the correlations, but this was not the case.

Bioassays in synthetic media. For the bioassays in synthetic media we used EDTA to control free metal activities. Because of reported instability of EDTA to light (Bürgi, 1974; Hill-Cottingham, 1955; Finden et al., 1984) we would have preferred NTA, but in samples of Lake Tjeukemeer that ligand was not able to reduce free metal activities to levels where these activities are limiting for *S. quadricauda*. EDTA (having much higher equilibrium constants for metal complexes than NTA) was able to reduce growth of algae completely, so we chose EDTA as ligand for these experiments.

Adding copper not only increased the free copper concentration, but also other free metal concentrations, by increased competition for common ligands. This effect can be described by the general equilibrium:

$$M1 + M2L === M1L + M2 \qquad (8\text{-}3)$$

(where M1 and M2 are metals and L is a ligand) showing that increasing the concentration of M1 (in this case copper) results in a higher free concentration of M2. Calculations showed that especially free cobalt and zinc concentrations would be quite sensitive for copper additions, while iron and manganese speciation did not significantly change by adding copper. Other metals would also not be significantly influenced. We therefore plotted the maximum extinction not only versus calculated free copper concentration (figure 8.4B), but also versus calculated free cobalt and zinc concentration (figures 8.5B and 8.6B).

In figures 8.4A, 8.5A and 8.6A the maximum extinction is plotted against the total copper, cobalt and zinc concentration.

In synthetic media algae did not reach such high biomass values as in natural samples (cf. figures 8.4 to 8.6 with 8.1 and 8.2).

8.5. Discussion

Bioassays in natural waters. If limitation of phosphorus and nitrogen was prevented by adding these two nutrients, copper turned out to become the limiting element in Lakes Tjeukemeer and IJsselmeer for *S. quadricauda*. The experimental cultures in these two watertypes showed a significantly higher extinction when 0.16 µM of copper was added. The fact that this phenomenon was observed in two lakes, suggested that this was not a single event. To check these results, we repeated these experiments about one year after the first series. Our earlier observations were confirmed: adding 0.05 or 0.16 µM of copper (together with phosphorus and nitrogen) enhanced growth, but 0.47 µM was slightly toxic. As far as we know, potential copper limitation has not been reported earlier for algae in natural waters.

Correlations between electrochemically labile copper, calculated free copper and inhibition of growth were quite high. This is remarkable especially for correlations of electrochemically labile copper, since electrochemical measurements were performed at pH = 5, while the natural pH of the samples was much higher (6.83 to 7.87). The relationship between electrochemically labile and bioavailable copper, as suggested by Young et al. (1979) and Florence (1986) is confirmed by our experiments. However, figure 8.3 already suggests high correlations of total copper concentration with the concentration of

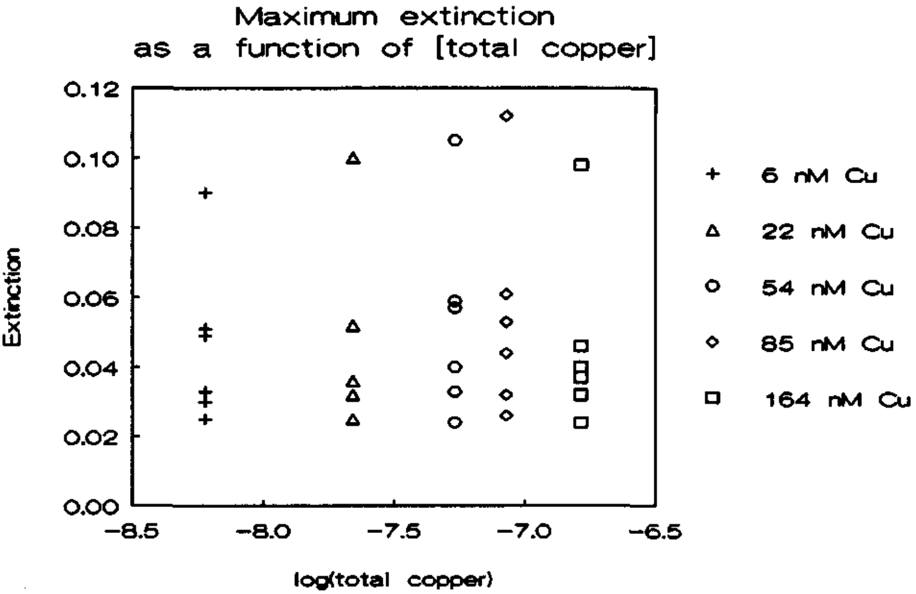

Figure 8.4A. Maximum extinction obtained for bioassays in synthetic media as a function of the total copper concentration.

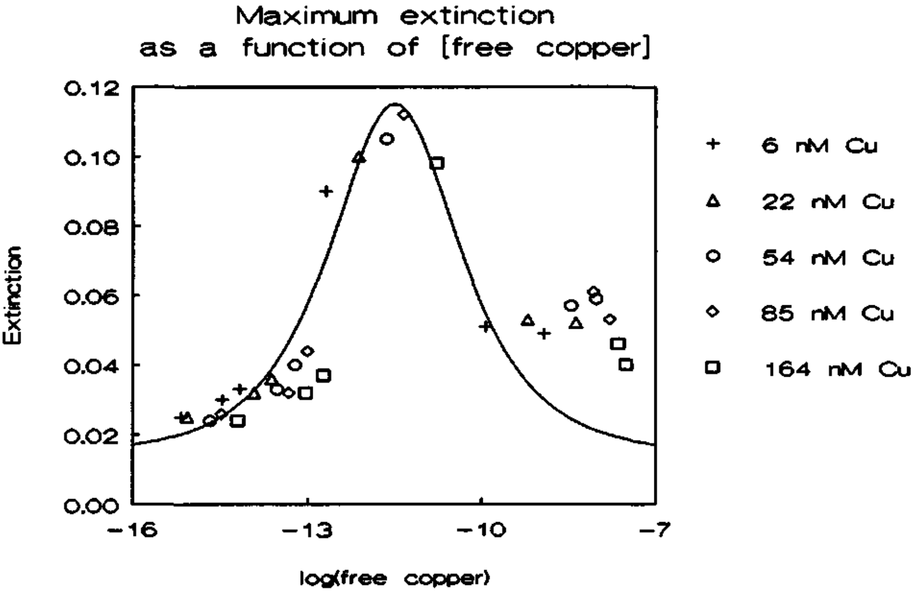

Figure 8.4B. Maximum extinction obtained for bioassays in synthetic media as a function of calculated free copper, for five different total copper concentrations.

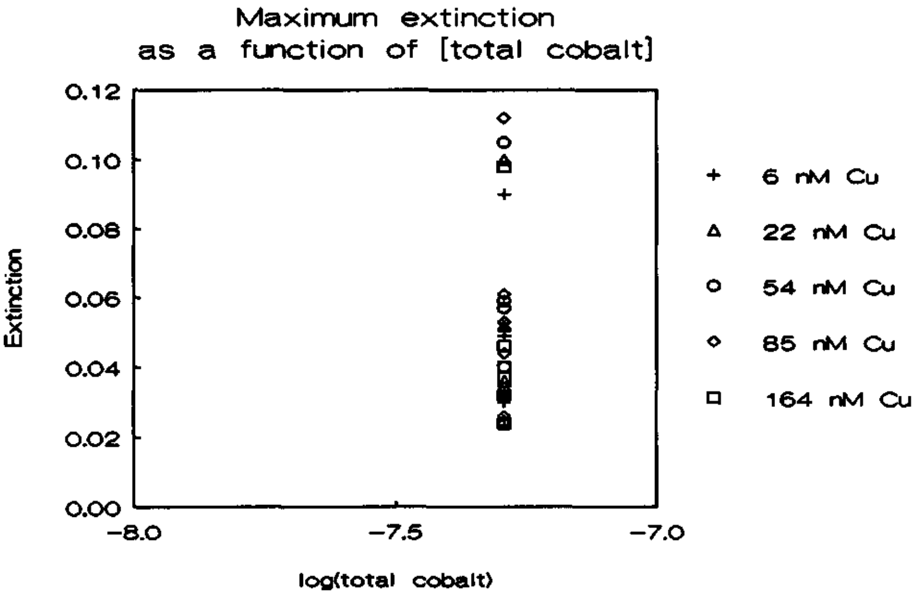

Figure 8.5A. Maximum extinction obtained for bioassays in synthetic media as a function of the total cobalt concentration, for five different total copper concentrations; total cobalt concentration was kept constant at 51 nM.

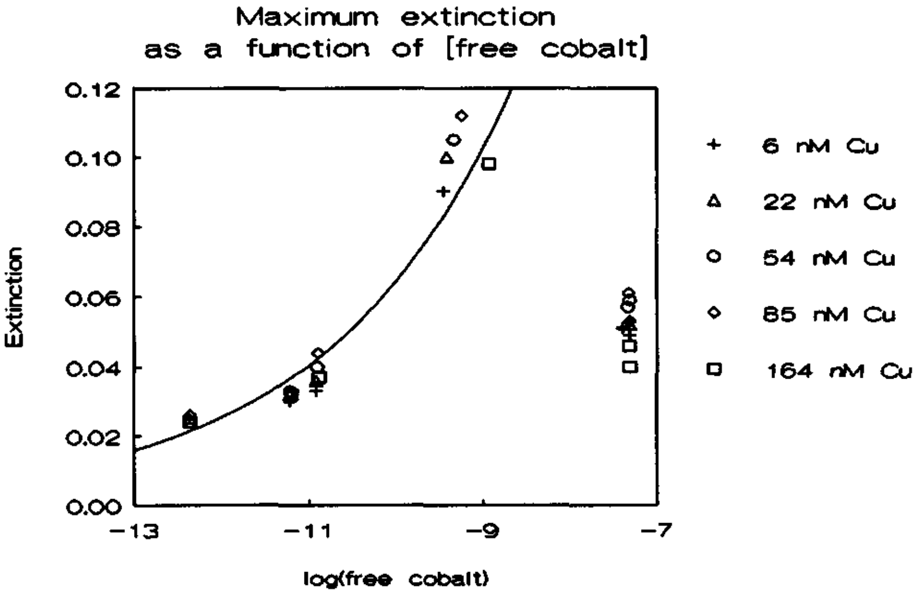

Figure 8.5B. Maximum extinction obtained for bioassays in synthetic media as a function of calculated free cobalt, for five different total copper concentrations; total cobalt concentration was kept constant at 51 nM.

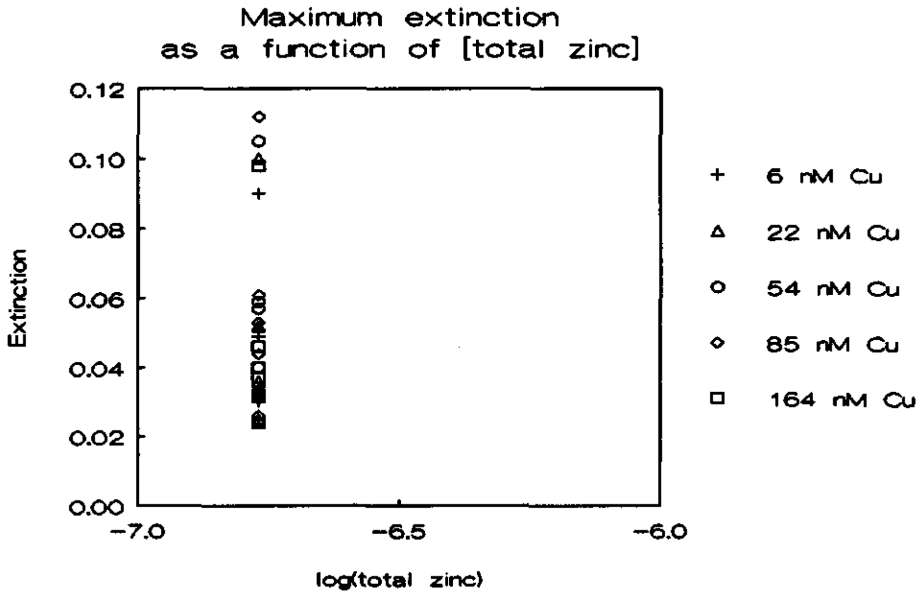

Figure 8.6A. **Maximum extinction obtained for bioassays in *synthetic* media as a function of the total zinc concentration, for five different total copper concentrations; total zinc concentration was kept constant at 170 nM.**

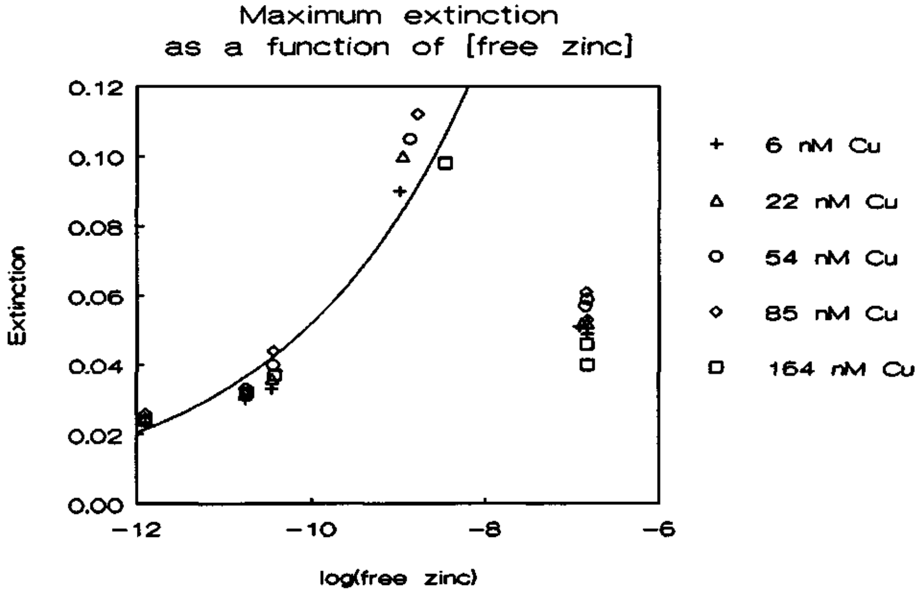

Figure 8.6B. Maximum extinction obtained for bioassays in synthetic media as a function of calculated free zinc, for five different total copper concentrations; total zinc concentration was kept constant at 170 nM.

total copper-electrochem.	total copper-calc.	total copper-inh.
0.9938 (<0.001)	0.9967 (<0.001)	0.9942 (<0.01)

Table 8.3. Correlations of the total copper concentration with electrochemically labile copper, calculated free copper (using titration data of pH = 3, 4, 5 and 6 for the Freundlich fit) and growth inhibition, for the Lake Tjeukemeer sample.

electrochemically labile copper, and of total copper concentration with calculated free copper concentration. This means that bioavailable copper may be also strongly correlated with total copper. Table 8.3 shows that this is indeed the case. In other words, the suggested relationship between electrochemically labile and bioavailable copper was in our case not very meaningful. This does not imply that such high correlations are to be found in other water types; we are not even sure that the high correlations found in the lakes we studied are also found throughout the year. Much more work needs to be done in this area to establish relationships between electrochemically labile copper, calculated free copper and bioavailable copper.

Bioassays in synthetic media. The competition in the synthetic media of added copper with other metals for the same ligands made it difficult to draw unambiguous conclusions. Nevertheless some very interesting information can be obtained from the results.

First of all, figures 8.4 to 8.6 show that the total copper, cobalt and zinc concentrations did not determine bioavailability, but instead the free concentrations did.

Secondly, while copper became toxic at high free copper concentrations, cobalt and zinc were not toxic in our assays. The wide range of extinctions obtained for one single value of free cobalt and free zinc concentrations around 0.1 µM (log = -7) clearly demonstrates this conclusion.

It is a little more complicated to state which metal is limiting for *S. quadricauda* at very low metal concentrations. For the lowest total copper concentration of 6 nM and the highest EDTA concentration an extinction of 0.025 was measured, while the log of free copper concentration was calculated to be -15.6 (most left "+" in figure 8.4B). This low extinction might be due to copper limitation. Slightly lowering EDTA concentration lead to higher free metal concentrations, which stimulated growth, but because the free copper, cobalt and zinc concentration all increased considerably according to speciation calculations, it is not clear which metal was limiting. For the next higher total copper concentration of 22 nM the curve in figure 8.4 was slightly shifted to the right; in other words, the extinction did not increase as much as expected on the basis of only free copper. Since adding copper not only increased free copper concentration, but also free cobalt and zinc

Reference	Organism	Toxic	Limiting
Anderson & Morel (1978)	dinoflagellate	$> 10^{-10}$ M	
Morel, Rueter & Morel (1978)	marine diatom	$> 10^{-8.5}$ M	
Rueter, McCarthy & Carpenter (1978)	marine cyanobacterium	$> 10^{-10.5}$ M	
Sunda & Guillard (1976)	estuarine diatom estuarine green alga	$> 10^{-8.3}$ M $> 10^{-8.7}$ M	$< 10^{-10.5}$ M $< 10^{-10.4}$ M
Sunda & Gillespie (1979)	bacterium	$> 10^{-9.5}$ M	
Petersen (1982)	*S. quadricauda*	$> 10^{-10.5}$ M	
Sunda, Klaveness & Palumbo (1984)	bacterium	$> 10^{-9.5}$ M	
This paper	*S. quadricauda*	$> 10^{-10.5}$ M	$< 10^{-12.5}$ M

Table 8.4. Comparison of toxic and limiting free copper concentrations for several organisms. Values are estimates of point of inflection in the graphs of growth versus free copper concentration.

concentrations, either cobalt or zinc or copper was limiting algal growth. Copper limitation for *S. quadricauda* may therefore only occur at free copper concentrations lower than $10^{-12.5}$ M. Figure 8.4B in combination with figures 8.5B and 8.6B, show that cobalt and zinc did not reach toxic levels in our experiments, while copper did for free copper concentration higher than about $10^{-10.5}$ M.

Although, due to the interference of cobalt and zinc, we could not quantify exactly at which free copper concentrations copper started to get limiting, it must have been for free copper concentration lower than about $10^{-12.5}$ M. Since we found significant growth stimulation when we added copper to samples from Lakes Tjeukemeer and IJsselmeer, we concluded that free copper in these lakes was lower than $10^{-12.5}$ M when we sampled.

The much higher biomass values in natural samples imply that in our synthetic medium some element was either limiting or toxic. We have no idea which element was responsible for hindering growth.

Table 8.4 contains values for toxic and limiting free copper concentrations of earlier studies as well as our values. It should be emphasized that since different organisms are used, different values are expected. It appears that *S. quadricauda* can grow in media containing as little as $10^{-12.5}$ M free copper, which is quite low compared to the values around $10^{-10.5}$ M obtained for two estuarine species. Copper started to get toxic around $10^{-10.5}$ M free copper, which is one of the lowest values found so far.

8.6. Conclusions

It has been established that, when phosphorus and nitrogen were added, copper would limit growth of *S. quadricauda* in Lakes Tjeukemeer and IJsselmeer.

Growth inhibition by toxic free copper concentrations was highly correlated with electrochemically labile copper and calculated free copper. However, correlations with the total copper concentrations were also high.

Copper was toxic for *S. quadricauda* when free copper concentration exceeded $10^{-10.5}$ M; limitation occurred at values lower than $10^{-12.5}$ M, but its onset could not be quantified exactly because of limitation by other elements, most likely cobalt or zinc.

Because of the growth stimulation found in Lakes Tjeukemeer and IJsselmeer we concluded that the free copper concentration was lower than $10^{-12.5}$ M.

Free concentrations of copper, cobalt and zinc determined the bioavailability of these elements rather than the total concentrations, as reported earlier by other workers.

8.7. Acknowledgements

We would like to thank Hans Bouius and Wim van Vilsteren for performing many of the bioassays and electrochemical measurements. Johan Voerman and Thijs de Boer determined many parameters in our samples. Maarten Nederlof and Han de Wit (Agricultural University of Wageningen) determined the fit of the titration data. Frans de Haan and Willem van Riemsdijk (Agricultural University of Wageningen) critically read the manuscript. Finally we thank Anja van der Honig for typing the manuscript and Luis Sojo for correcting the English.

Chapter 9

Looking back and ahead

Taking into account the currently available techniques in the field of metal speciation research, reaching the goals described in chapter 1 was not an easy task to accomplish. Nevertheless lots of interesting and, at least qualitatively, consistent results were obtained. A summary of the applied methods with the corresponding data is given in table 9.1. For the sake of clarity only the rounded averages are included in this table. More detailed information is provided in the previous chapters. Ultrafiltration revealed that about half of the copper species was retained by an ultrafilter with a pore size of 5 nm. Anodic stripping voltammetry showed that only a few percent of the dissolved copper concentration (typically ranging from 10^{-8} to 10^{-7} M) was ASV-labile, in spite of acidification of the samples to pH = 5, long deposition time (500 seconds), and the fact that all inorganic and some organic copper species are labile under the conditions applied (buffer, pH, deposition time). The fraction of free copper therefore must be lower than a few percent. Indeed, the two ligand competition techniques yielded free copper concentrations in the order of 10^{-11} M (MnO_2) or 10^{-13} to 10^{-12} M (EDTA at the rotating mercury film electrode). Using heterogeneity analysis, a free copper concentration around 10^{-14} M was calculated (for pH = 7.55) when only the more active sites were included; if less active sites were included as well, this value became around 10^{-10} M. Equilibrium modelling (using natural pH) also displayed a difference of four orders of magnitude between the two scenarios, but results were one order of magnitude lower (10^{-15} and 10^{-11} M respectively).

Technique	Chapter/ section	Copper fraction	log(Cu)
Ultrafiltration	3	species < 5 nm	- 8
Anodic stripping voltammetry (ASV)	4.1	ASV-labile copper	- 9
MnO_2-adsorption	5.1	free copper	-11
EDTA competition with ASV	5.2	free copper	-13 to -12
Heterogeneity analysis	6.4	free copper	-14 to -10
Equilibrium modelling	7	free copper	-15 to -11
Bioassays/equilibrium modelling	8	free copper	< -13

Table 9.1. Estimates of copper fractions in Lake Tjeukemeer using several techniques. For the sake of clarity only rounded averages are included.

Bioassays in combination with equilibrium modelling clearly led to the conclusion that the free copper concentration in Lake Tjeukemeer was lower than about $10^{-12.5}$ M. Thus five of the techniques shown in table 9.1 claim to determine only free copper. The results range roughly from 10^{-15} to 10^{-10} M of free copper. Unfortunately, it is not possible to state which technique is "right", but it is clear that only a very small fraction of the total copper concentration is present as free copper. In chapter 8 it was demonstrated that copper in Lake Tjeukemeer was limiting growth of *Scenedesmus quadricauda* when phosphorus and nitrogen were added. Based on the results of the present work, it can be stated that the main objective of this project, i.e. establishing the relationship between copper speciation and bioavailability, has been fulfilled in the case of the algal species *Scenedesmus quadricauda*.

In the future more institutes and scientists will work on this type of problems. Their task may be easier when additional relevant knowledge becomes available. One of the topics which requires more information is the interactions between metals and organic matter. Although a tremendous amount of work has already been carried out in this field, more fundamental and generally applicable information would certainly represent an improvement. A critical examination of the results described in this thesis is a convenient way to demonstrate the importance of organic complexation. In some of the previous chapters it has been calculated that by far the largest part of copper occurs as organic complexes. Nevertheless, no direct evidence could be given of such complexes. Most techniques applicable in metal research, treat organic matter as a black box. Heterogeneity analysis (section 6.4) was in fact the approach that did so the least of all used techniques, but even this technique displayed a variation of roughly four orders for the calculated free copper concentration.

Analytical techniques require further development as well. Existing techniques will have to be improved, and perhaps new techniques will be introduced. At present, ligand competition techniques seem to be most promising. Some examples are: a solid ligand (MnO_2; Van den Berg & Kramer, 1979a, 1979b; Van den Berg, 1982a, 1982b, 1983), NTA in combination with a bacterial bioassay (Sunda, Klaveness & Palumbo, 1984), EDTA in combination with adsorption onto SEP-PAK C_{18} columns (Sunda & Hanson, 1987) and EDTA in combination with voltammetric detection (this thesis). It took over a decade for the introduction of these various ligand competition techniques, and it is expected that new methodologies will be developed. To obtain more accurate results using ligand competition techniques, a better knowledge of the composition of inorganic solutions and the (validity of) corresponding equilibrium constants is essential.

Summary

Chapter 1: introduction

In this thesis an account is given of a research project dealing with the chemical speciation[1] and bioavailability of copper in Lake Tjeukemeer, a lake in the north of the Netherlands. The reason for the initiation of this project was a lack of knowledge about the speciation of copper and the influence of copper on the behaviour of algae. This influence may be important, because copper is an essential element for algae, but becomes toxic when it is present at too high concentrations. From a literature search it was concluded that free copper can be assumed to control bioavailability for algae. Therefore, it was decided to focus attention on free copper. Currently, no techniques are available that can measure the concentration of free copper directly at the concentration levels typically found in Lake Tjeukemeer. However, some techniques may give useful information, providing that the results are carefully interpreted. As a consequence of the low concentrations of copper, present in natural waters, determinations of copper concentrations are very prone to interferences due to contamination.

Chapter 2: copper concentration in Lake Tjeukemeer and external influences

Lake Tjeukemeer is a shallow, alkaline and eutrophic lake. It is part of the Frisian "boezem". The hydrology of the lake is man-made: in winter water from the surrounding polders is pumped into the lake and dominates the water composition, while in summer water originating from Lake IJsselmeer is let in. The total "dissolved" (i.e. after filtration through a 0.2 µm pore size filter) copper concentration in Lake Tjeukemeer is typically around 30 nM. Although no evidence could be obtained, it is likely that the copper concentration in the lake is not increased by letting in water from Lake IJsselmeer. However, water originating from the polders probably does increase the copper concentration in Lake Tjeukemeer. It is shown that surface run-off was more likely to cause this elevation than leaching, though during laboratory experiments leaching occurred under certain conditions in the upper layer of the soil column. Sediment samples from the lake were subjected to sequential extractions and to extractions using EDTA. It was concluded that release of copper from the sediment to the water phase is not likely to be an important process.

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[1] "chemical speciation" is defined as the distribution of an element over all possible species of that element.

Chapter 3: ultrafiltration

Ultrafiltration was used to gain an insight into the size of the copper species present in Lake Tjeukemeer. Interpretation of ultrafiltration results is not simple because of several potential artefacts, among which contamination. Free copper, having a size of about 0.6 nm, will be found in the fraction smaller than 5 nm, the smallest pore size which was used, together with other species smaller than 5 nm. Though some samples appeared to have been contaminated, a critical examination allowed drawing of some conclusions. About half of the dissolved copper concentration was found in the fraction smaller than 5 nm (typically a few tens of nM), implying that at least half of the total copper was present in complexes.

Chapter 4: voltammetric techniques

Two voltammetric techniques (anodic and cathodic stripping voltammetry) were applied to obtain information on the fraction of electrochemically labile copper.

Anodic stripping voltammetry (ASV) is an often used technique in speciation studies, but the results are not easy to interpret. Under the conditions applied during the measurements (pH, deposition time, buffer) all inorganic and some organic copper species can be assumed to be detected ("ASV-labile"). The concentration of ASV-labile copper was usually below 2 nM, and often not detectable at all in spite of the long deposition time. This means that at most only a few percent of copper was ASV-labile, and therefore by far the largest part must have been present in organic complexes. At natural pH the concentration of ASV-labile copper must have been even lower. Although most copper was concluded to be organically complexed, no correlations were found with parameters representing organic carbon.

Cathodic stripping voltammetry (CSV), using catechol as synthetic ligand, was applied under non-equilibrium conditions. However, the CSV-labile copper concentration found, was often more than 100% of the dissolved copper concentration. Based on additional experiments, it was concluded that the concentration of organic material in the lake probably was too high to allow useful application of this technique to Lake Tjeukemeer.

Chapter 5: ligand competition techniques

To assess the concentration of free copper itself, two ligand competition techniques were applied.

The *MnO_2 method* was one of these. MnO_2 was added to standards of known composition, allowing the determination of the copper adsorption as a function of the free copper concentration. Three methods were used to process these adsorption data:

1) Langmuir behaviour was assumed and the linearisation method recommended by Van den Berg (1982a) was applied;
2) a different linearisation suggested in this thesis was used to process Langmuir data;
3) finally a linear model supposing copper adsorption (Γ) to be proportional to the free copper concentration; this may be considered an isolation of the first, nearly linear part of a Langmuir curve.

The obtained values for the free copper concentration were valid only in the presence of MnO_2, but they could be converted to values valid in the absence of MnO_2 after a series of assumptions. The Van den Berg linearisation method did not yield satisfactory correlation coefficients; it was reasoned that this linearisation method should be used for higher free metal concentrations than those used here. The second linearisation method and the linear model gave quite satisfactory correlations. Contamination in several cases hindered the calculation of free copper concentration in lake water samples. In other cases, the average of the free copper concentration varied from 0.013 nM (second linearisation) via 0.018 nM (first linearisation) to 0.050 nM (linear model), all in the presence of MnO_2. These values were a few tens of percents higher when converted to the situation without MnO_2. The free copper concentrations found were not systematically correlated with the concentration or UV-absorbance of organic matter. It was calculated that more than 95% of copper in Lake Tjeukemeer was organically complexed when the linear model was used; in the case of Langmuir linearisations this percentage was 99.

A voltammetric version of the SEP-PAK C_{18} adsorption method (Sunda & Hanson, 1987) was developed here. Labile copper was measured at a rotating mercury film electrode using differential pulse ASV in two copper addition series; one also had EDTA included. Plotting the labile versus the total copper concentration allowed quantification of the concentration of the copper-EDTA complex, which in turn allowed the calculation of free copper concentration. Since the ASV-signal in lake water samples without copper addition increased upon addition of EDTA, the free copper concentration could not be determined without adding copper. However, it was argued that extrapolation to these conditions was reasonable. Thus for free copper concentration a value between 10^{-13} and 10^{-12} M was obtained. The corresponding concentration of organically complexed copper was calculated to be more than 99% of the dissolved copper concentration. It was concluded that this new version of a ligand competition technique appears to be promising.

Chapter 6: copper titrations

Copper titrations were used to obtain information on copper complexation in Lake Tjeukemeer. Several type of titrations and data processing were applied.

Using a cupric ion selective electrode (ISE), the copper complexation capacity in Lake Tjeukemeer was monitored fortnightly during three years. Both Van den Berg-Ružić-Lee and Scatchard analysis showed ligand concentrations of several tens of µM. For Van den Berg-Ružić-Lee analysis log(K) varied from about 4.5 to about 7.5, for Scatchard analysis from about 4.5 to about 10. Affinity spectra yielded log(K) values ranging from 4.5 to 8, and suggested at least three groups of binding sites. The highest log(K) values coincided with relatively low humus concentrations and blooms of Cyanobacteria.

One titration curve was subjected to a more detailed look. Using Van den Berg-Ružić-Lee analysis it was shown that the assumption of two groups of ligands is necessary to mimic the titration data adequately. To mimic the Scatchard curve of this titration satisfactory, the presence of a third group of ligands had to be assumed. It was shown that both Van den Berg-Ružić-Lee and Scatchard analysis had to be applied to obtain adequate complexation parameters.

Obtaining information about copper complexation at natural pH could not be done directly, because copper precipitated at high pH-values. Therefore, copper titration data of lower pH-values were fitted using a pH-dependent Freundlich isotherm, and extrapolated to the natural pH. At low pH (3, 4) more active sites were assessed than at higher pH (5, 6). The free copper concentration at pH = 7.55 (the pH used for the experiments described in chapter 5) was calculated to be around 10^{-10} M; when only the more active sites were assessed, this value was about 10^{-14} M.

Because of the relatively high detection limit of an ISE, ASV, in combination with Scatchard analysis, was used to gain insight into the complexation at low copper additions (up to 157 nM). The log(K) at pH = 5 was found to range from 8 to 9. Virtually all copper was organically complexed. If these titrations were performed in ultrafiltrates, it could be shown that the apparent ligand concentration decreased with pore size, while the log(K) increased with pore size, i.e. the smallest ligands form the strongest complexes with copper.

Chapter 7: modelling

An equilibrium model was used to calculate the speciation in four samples. Meaningful calculations can only be done if sufficient knowledge is available about the species which are present and the corresponding equilibrium constants. The data which were used as input for the model were therefore subjected to a critical examination.

When only <u>in</u>organic complexation was included in the calculations, the free copper concentration varied from 10^{-12} to 10^{-10} M, representing at most 2% of the dissolved copper concentration. Copper was mainly present as carbonate complexes, while a few percent of copper was present as copper hydroxide complexes.

When the pH-dependent fit of copper titration data (as described in chapter 6) was used to model complexation of copper by organic ligands, the free copper concentration decreased, ranging from 10^{-12} to 10^{-11} M. More than 90% of copper was calculated to be organically complexed, except for one very alkaline sample which displayed a considerable fraction of carbonates. Hydroxides were of minor importance.

When the fit using only the more active site was used, the free copper concentration ranged from 10^{-16} to 10^{-14} M. All copper (i.e. more than 99.98%) was computed to occur in organic complexes.

Chapter 8: speciation of copper in relation to its bioavailability

Bioassays were performed in water samples from Lake Tjeukemeer, two other lakes and a polder site, to assay the biologically available fraction of copper. A green alga, *Scenedesmus quadricauda*, was grown in batch cultures to which fixed concentrations of phosphorus and nitrogen, and varying concentrations of copper were added. Electrochemically labile copper was determined in samples with the same copper additions. For Lake Tjeukemeer, equilibrium calculations were performed as well, as described in chapter 7. For all samples, adding 0.16 µM of copper resulted in a higher biomass in the stationary phase; for Lakes Tjeukemeer and IJsselmeer this increase was even significant. Copper additions of 1.6 µM and higher displayed growth inhibition. Growth inhibition was well correlated with electrochemically labile and computed free copper, but since these latter two parameters were almost proportional to the total copper concentration, these correlations are not very meaningful.

Scenedesmus quadricauda was also grown in synthetic media of known composition. The copper and EDTA concentrations were varied to obtain a wide range of free copper concentrations. Speciation calculations showed that not only the free copper concentration varied, but also the free cobalt and free zinc concentrations, because of competition of these three metals for EDTA. This competition made it difficult to draw unambiguous conclusions. However, it was clearly shown that the free instead of the total concentrations of copper, cobalt and zinc determined the bioavailability. Cobalt and zinc were not toxic in our assays, but copper became toxic above free copper concentrations of about $10^{-10.5}$ M. At high EDTA concentrations, some element became limiting, probably either copper or cobalt or zinc. The concentration at which copper became limiting could not be quantified exactly because of the competition with cobalt and zinc, but must have been lower than $10^{-12.5}$ M.

The growth stimulation which was found in Lakes Tjeukemeer and IJsselmeer when copper was added, therefore implies that the free copper concentrations in those lake water samples must have been lower than $10^{-12.5}$ M.

In natural samples higher values for the biomass were obtained than in synthetic media. It was not understood why.

Chapter 9: looking back and ahead

Looking back upon this research project, it is concluded that the results obtained by the various methods were at least qualitatively consistent. The techniques which claim to determine only free copper, displayed a wide range of free copper concentrations, but all indicated that only a very small fraction of the dissolved copper concentration was present as free copper. The relation between the copper speciation and bioavailability could be determined for one algal species.

To allow this type of research on a routine basis, more knowledge is needed about the interaction of metals with organic matter. This work clearly demonstrated the importance of those interactions. Ligand competition techniques may be most promising. Better knowledge of inorganic solutions is required to obtain more accurate results.

Samenvatting

Samenvatting van het proefschrift "Speciatie en biologische beschikbaarheid van koper in het Tjeukemeer"

Hoofdstuk 1: inleiding

In dit proefschrift wordt verslag gedaan van een onderzoeksprojekt gericht op de chemische speciatie[1] en biologische beschikbaarheid van koper in het Tjeukemeer, een meer in Friesland. De reden om dit projekt te beginnen was een behoefte aan kennis over de speciatie van koper en de invloed van koper op het gedrag van algen. Die invloed kan belangrijk zijn, omdat koper een essentieel element is voor algen, maar toxisch wordt als het in te hoge koncentraties aanwezig is. Op basis van literatuuronderzoek is gekonkludeerd dat er van uitgegaan mag worden dat vrij koper de biologische beschikbaarheid voor algen reguleert. Daarom is besloten de aandacht te richten op vrij koper. Momenteel zijn geen technieken beschikbaar die de koncentratie vrij koper direkt kunnen meten op voor het Tjeukemeer kenmerkende nivo's. Sommige technieken geven echter zinvolle informatie, mits de resultaten met zorgvuldigheid worden geïnterpreteerd. Omdat in natuurlijke wateren slechts lage koncentraties koper aanwezig zijn worden bepalingen van koperkoncentraties gemakkelijk beïnvloed door verontreiniging.

Hoofdstuk 2: koperkoncentratie in het Tjeukemeer en invloeden van buitenaf

Het Tjeukemeer is een ondiep, alkalisch en eutroof meer. Het maakt deel uit van de Friese boezem. De hydrologie van het meer wordt door de mens bepaald: in de winter wordt water uit de omringende polders in het meer gepompt, en wordt de watersamenstelling daardoor gedomineerd, terwijl in de zomer water afkomstig uit het IJsselmeer wordt ingelaten. De totale "opgeloste" (dat wil zeggen over een filter met poriën van 0.2 µm gefiltreerd) koperkoncentratie in het Tjeukemeer is ongeveer 30 nM. Hoewel het bewijs niet is geleverd, is het aannemelijk dat de koperkoncentratie in het meer niet hoger werd door inlaat uit het IJsselmeer. Water afkomstig uit de polders deed de koperkoncentratie in het Tjeukemeer waarschijnlijk wel toenemen. Er is aangetoond dat afspoeling een waarschijnlijker oorzaak voor deze verhoging is dan uitspoeling, hoewel tijdens laboratoriumproeven onder bepaalde omstandigheden uitspoeling plaatsvond in de bovenste laag van de grondkolom. Sedimentmonsters uit het meer zijn onderworpen aan sekwentiële extrakties en

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[1] "chemische speciatie" is gedefinieerd als de verdeling van een element over alle mogelijke species van dat element.

aan extrakties met EDTA. Er is gekonkludeerd dat afgifte van koper vanuit het sediment aan het water waarschijnlijk niet van belang is.

Hoofdstuk 3: ultrafiltratie

Ultrafiltratie is toegepast om inzicht te verkrijgen in de grootte van koperspecies in het Tjeukemeer. Interpretatie van de resultaten van ultrafiltratie is niet eenvoudig vanwege diverse mogelijke artefakten, waaronder verontreiniging. Vrij koper, met een grootte van ongeveer 0.6 nm, zal aangetroffen worden in de fraktie kleiner dan 5 nm, de kleinste gebruikte poriëngrootte, samen met andere species kleiner dan 5 nm. Hoewel sommige monsters verontreinigd bleken te zijn, kon door een kritische evaluatie van de resultaten een aantal konklusies worden getrokken. Ongeveer de helft van de opgeloste koperkoncentratie is aangetroffen in de fraktie kleiner dan 5 nm (met een karakteristieke waarde van enkele tientallen nM). Dit betekent dat de andere helft aanwezig was in grotere komplexen, met andere woorden op basis van kopermetingen in ultrafiltraten kon gekonkludeerd worden dat meer dan 50% van het totale koper in gekomplexeerde vorm voorkwam.

Hoofdstuk 4: voltammetrische technieken

Twee voltammetrische technieken (anodische en kathodische heroplossings-voltammetrie) zijn toegepast om informatie te verkrijgen over de fraktie elektrochemisch labiel koper.

Anodische heroplossings-voltammetrie (ASV) is een veel gebruikte techniek in speciatie-onderzoek, maar de resultaten ervan zijn niet gemakkelijk te interpreteren. Er mag verondersteld worden dat alle anorganische en een deel van de organische koperspecies worden gedetekteerd ("ASV-labiel" zijn) onder de toegepaste omstandigheden (pH, depositietijd, buffer). De koncentratie ASV-labiel koper was meestal minder dan 2 nM, en vaak helemaal niet aantoonbaar ondanks de lange depositietijd. Dit impliceert dat hooguit enkele procenten van het koper ASV-labiel waren, en dat dus verreweg het grootste gedeelte als organisch komplex aanwezig moet zijn geweest. Bij natuurlijke pH-waarden moet de koncentratie ASV-labiel koper zelfs lager zijn geweest. Hoewel gekonkludeerd is dat het meeste koper organisch gekomplexeerd was, zijn geen korrelaties gevonden met parameters die een maat vormen voor de koncentratie van organische koolstof.

Kathodische heroplossings-voltammetrie (CSV) is in een niet-evenwichtstoestand toegepast. Als synthetisch ligand werd katechol gebruikt. De gemeten CSV-labiele koperkoncentratie bleek vaak meer dan 100% van de opgeloste koperkoncentratie te zijn, wat fysisch onmogelijk is. Op basis van extra proeven is gekonkludeerd dat de koncentratie organisch materiaal in het meer waarschijnlijk te hoog was om deze techniek in het Tjeukemeer zinvol te kunnen toepassen.

Hoofdstuk 5: ligand-kompetitie-technieken

Om de koncentratie van het vrije koper zelf te kunnen bepalen, zijn twee ligand-kompetitie-technieken toegepast.

Eén ervan was de *MnO_2-methode*. MnO_2 is toegevoegd aan standaarden met een bekende samenstelling, waardoor de koperadsorptie als funktie van de koncentratie vrij koper kon worden bepaald. Er zijn drie methoden gebruikt om de adsorptiegegevens te verwerken:

1) Langmuir-gedrag is verondersteld; de linearisatie aanbevolen door Van den Berg (1982a) is toegepast;
2) een andere linearisatie, in dit proefschrift geopperd, is gebruikt om de Langmuir-gegevens te verwerken;
3) tenslotte is een lineair model gebruikt dat veronderstelt dat de koperadsorptie (Γ) evenredig is met de koncentratie vrij koper; dit kan beschouwd worden als een isolatie van het eerste, kwasi-lineaire deel van een Langmuir-kromme.

De verkregen waarden voor de koncentratie vrij koper waren alleen geldig in de aanwezigheid van MnO_2, maar konden, na enkele aannames, omgerekend worden in waarden geldig in de afwezigheid van MnO_2. De Van den Berg-linearisatie gaf geen bevredigende korrelatiekoëfficiënten; er is beredeneerd dat deze linearisatie gebruikt zou moeten worden voor hogere koncentraties vrij metaal dan in dit werk. De tweede linearisatie en het lineaire model gaven heel bevredigende korrelaties. Verontreiniging belette de berekening van de koncentratie vrij koper in verscheidene meerwatermonsters. In de andere gevallen varieerde het gemiddelde van de koncentratie vrij koper van 0.013 nM (tweede linearisatie) via 0.018 nM (eerste linearisatie) tot 0.050 nM (lineaire model), alles in de aanwezigheid van MnO_2. Deze waarden waren enkele tientallen procenten hoger na omrekening naar de situatie zonder MnO_2. De koncentraties vrij koper waren niet systematisch gekorreleerd met de koncentratie of UV-absorptie van organisch materiaal. Er is berekend dat meer dan 95% van het koper in het Tjeukemeer organisch gekomplexeerd was wanneer het lineaire model werd gebruikt; voor de Langmuir-linearisaties was dit percentage 99.

Een *voltammetrische versie van de SEP-PAK-C_{18}-adsorptiemethode* (Sunda & Hanson, 1987) is in dit werk ontwikkeld. Labiel koper werd gemeten met een roterende kwikfilmelektrode met behulp van differentiële-puls-ASV in twee koperadditiereeksen; aan één reeks was ook EDTA toegevoegd. Door de labiele tegen de totale koperkoncentratie uit te zetten kon de koncentratie van het koper-EDTA-komplex worden bepaald, waardoor de koncentratie vrij koper kon worden berekend. Omdat het ASV-signaal in de meerwatermonsters toenam door toevoeging van EDTA, kon de koncentratie vrij koper niet worden bepaald voor de situatie zonder kopertoevoeging. Er is echter beargumenteerd dat extrapolatie naar deze situatie redelijk was. Zo is voor de koncentratie vrij koper een waarde tussen 10^{-13} en 10^{-12} M verkregen. Er is berekend dat de daarmee overeenkomende koncentratie organisch gekomplexeerd koper meer dan 99% van de opgeloste koperkoncentratie bedroeg. De konklusie is getrokken dat deze nieuwe variant van een ligand-kompetitie-techniek veelbelovend lijkt te zijn.

Hoofdstuk 6: kopertitraties

Om gegevens te verkrijgen over koperkomplexering in het Tjeukemeer zijn kopertitraties gebruikt. Verscheidene soorten titraties en gegevensverwerking zijn toegepast.

Met een koper(II)-ion-selektieve elektrode (ISE) is gedurende drie jaar elke twee weken de koperkomplexeringskapaciteit in het Tjeukemeer gemeten. Zowel Van den Berg-Ružić-Lee- als Scatchard-analyse lieten ligandkoncentraties van enkele tientallen µM zien. Voor de Van den Berg-Ružić-Lee-analyse varieerde log(K) van ongeveer 4.5 tot ongeveer 7.5, voor Scatchard-analyse van ongeveer 4.5 tot ongeveer 10. Affiniteitsspektra leverden log(K)-waarden op variërend van 4.5 tot 8, en suggereerden tenminste drie groepen bindingsplaatsen. De hoogste log(K)-waarden vielen samen met relatief lage humuskoncentraties en bloeiperiodes van cyanobacteriën.

Eén titratiekromme is nader bestudeerd. Met behulp van Van den Berg-Ružić-Lee-analyse is aangetoond dat van twee groepen liganden moest worden uitgegaan om de titratiegegevens goed genoeg na te kunnen bootsen. Om ook de Scatchard-kromme van deze titratie bevredigend na te kunnen bootsen moest een derde groep liganden worden verondersteld. Er is aangetoond dat zowel Van den Berg-Ružić-Lee- als Scatchard-analyse toegepast moesten worden om komplexeringsparameters van voldoende kwaliteit te verkrijgen.

Het was niet mogelijk informatie te verkrijgen over koperkomplexering bij de natuurlijke pH, omdat koper neersloeg bij hoge pH-waarden. Daarom zijn kopertitratie-gegevens van lagere pH-waarden gefit met behulp van een pH-afhankelijke Freundlich-isotherm, en geëxtrapoleerd naar de natuurlijke pH. Bij lage pH (3, 4) worden aktievere bindingsplaatsen meegenomen dan bij hogere pH (5, 6). Zo kon er worden berekend dat de koncentratie vrij koper bij pH = 7.55 (de pH die gebruikt werd voor de experimenten beschreven in hoofdstuk 5) ongeveer 10^{-10} M was; wanneer alleen de aktievere bindingsplaatsen werden meegenomen, bedroeg deze waarde ongeveer 10^{-14} M.

Vanwege de relatief hoge detektiegrens van een ISE, is ASV, in kombinatie met Scatchard-analyse, gebruikt om inzicht te verkrijgen in de komplexering bij lage kopertoevoegingen (tot 157 nM). De log(K) bij pH = 5 bleek te variëren van 8 tot 9. Praktisch al het koper was organisch gekomplexeerd. Als deze titraties in ultrafiltraten werden uitgevoerd, kon worden aangetoond dat de schijnbare ligandkoncentratie afnam met de poriëngrootte, terwijl de log(K) toenam met de poriëngrootte; dit betekent dat de kleinste liganden de sterkste komplexen met koper vormden.

Hoofdstuk 7: modelleren

Een evenwichtsmodel is gebruikt om de speciatie in vier monsters te berekenen. Zinvolle berekeningen kunnen alleen worden uitgevoerd indien voldoende kennis beschikbaar is over de aanwezige species en de daarmee korresponderende evenwichtskonstanten. De gegevens die als invoer voor het model zijn gebruikt zijn om die reden kritisch onder de loep genomen.

Als alleen anorganische komplexering in de berekeningen werd meegenomen, varieerde de koncentratie vrij koper van 10^{-12} tot 10^{-10} M, wat overeenkwam met maximaal 2% van de opgeloste koperkoncentratie. Koper was vooral aanwezig in de vorm van karbonaatkomplexen, terwijl enkele procenten van het koper als koper-hydroxide-komplexen aanwezig waren.

Als de pH-afhankelijke fit van kopertitraties (zoals in hoofdstuk 6 beschreven) gebruikt werd om de komplexering van koper door organische liganden te modelleren, nam de koncentratie vrij koper af, variërend van 10^{-12} tot 10^{-11} M. Volgens de berekeningen was meer dan 90% van het koper organisch gekomplexeerd, behalve in één monster met een erg hoge pH dat een aanzienlijke fraktie karbonaten vertoonde. Hydroxides waren van ondergeschikt belang.

Wanneer de fit die alleen de aktievere bindingsplaatsen meenam werd gebruikt, varieerde de koncentratie vrij koper van 10^{-16} tot 10^{-14} M. Volgens de berekeningen kwam al het koper (dat wil zeggen meer dan 99.98%) voor in organische komplexen.

Hoofdstuk 8: koperspeciatie in relatie tot de biologische beschikbaarheid

In watermonsters van het Tjeukemeer, twee andere meren en een polderpunt zijn bioassays uitgevoerd, om de biologisch beschikbare fraktie koper te bepalen. Een groenalg, *Scenedesmus quadricauda*, is gekweekt in batchkulturen waaraan vaste koncentraties fosfor en stikstof, en uiteenlopende koncentraties koper waren toegevoegd. Elektrochemisch labiel koper is bepaald in monsters met dezelfde kopertoevoegingen. Voor het Tjeukemeer zijn bovendien evenwichtsberekeningen uitgevoerd zoals beschreven in hoofdstuk 7. Voor alle monsters resulteerde een kopertoevoeging van 0.16 µM in een hogere biomassa in de stationaire fase; voor het Tjeukemeer en het IJsselmeer was deze toename zelfs signifikant. Kopertoevoegingen van 1.6 µM en meer hadden groeiremming tot gevolg. De groeiremming was hoog gekorreleerd met elektrochemisch labiel en berekend vrij koper, maar omdat die laatste twee parameters bijna evenredig waren aan de totale koperkoncentratie, hadden die korrelaties weinig betekenis.

Scenedesmus quadricauda is ook gekweekt in synthetische media van bekende samenstelling. De koper- en EDTA-koncentraties zijn gevarieerd om een wijd bereik van koncentraties vrij koper te verkrijgen. Speciatieberekeningen toonden aan dat niet alleen de koncentratie vrij koper varieerde, maar ook de koncentratie vrij kobalt en vrij zink, ten gevolge van konkurrentie van deze drie metalen om EDTA. Deze konkurrentie maakte het moeilijk eenduidige konklusies te trekken. Er is echter duidelijk aangetoond dat voor koper, kobalt en zink niet de totale metaalkoncentraties maar de koncentraties vrij metaal de biologische beschikbaarheid bepaalden. Kobalt en zink waren niet toxisch in onze bioassays, maar koper was wel toxisch boven een koncentratie vrij koper van ongeveer $10^{-10.5}$ M. Bij hoge EDTA-koncentraties was een element limiterend, waarschijnlijk hetzij koper, hetzij kobalt, hetzij zink. De koncentratie waarbij koper limiterend werd kon niet exakt worden bepaald vanwege de konkurrentie met kobalt en zink, maar moet lager dan $10^{-12.5}$ M zijn geweest.

De groeistimulering die is aangetroffen in het Tjeukemeer en het IJsselmeer bij toevoeging van koper, impliceert daarom dat de koncentraties vrij koper in die monsters lager moet zijn geweest dan $10^{-12.5}$ M.

In natuurlijke monsters zijn hogere waarden verkregen voor de biomassa dan in synthetische media. Waarom dit verschil optrad bleef onduidelijk.

Hoofdstuk 9: terug- en vooruitkijken

Aan het slot van het onderzoeksprojekt is gekonkludeerd dat de resultaten zoals die met de verschillende methoden zijn verkregen op zijn minst kwalitatief konsistent waren. De technieken die er aanspraak op maken alleen vrij koper te bepalen, vertoonden een wijd bereik van koncentraties vrij koper, maar ze gaven allemaal aan dat slechts een hele kleine fraktie van de opgeloste koperkoncentratie als vrij koper aanwezig was. Het verband tussen de koperspeciatie en biologische beschikbaarheid kon worden bepaald voor één algesoort.

Om dit soort onderzoek beter te kunnen uitvoeren, is meer kennis nodig over de interakties van metalen met organisch materiaal. Dit onderzoek heeft het belang van dergelijke interakties duidelijk aangetoond. Ligand-kompetitietechnieken zijn misschien het meest veelbelovend. Om nauwkeuriger resultaten te verkrijgen is een betere kennis van de samenstelling van anorganische oplossingen nodig.

Gearfetting

Gearfetting fan it proefskrift "Spesjaasje en biologyske beskikberens fan koper yn de Tsjûkemar"

Haadstik 1: ynlieding

Yn dit proefskrift wurdt ferslach dien fan in ûndersyksprojekt rjochte op de gemyske spesjaasje[1] en biologyske beskikberens fan koper yn de Tsjûkemar, in mar tusken De Lemmer en It Hearrenfean. De oanlieding om mei dit projekt útein te setten, wie dat der ferlet fan kennis wie oer de spesjaasje fan koper en de ynfloed fan koper op it hâlden en dragen fan algen. Dy ynfloed kin wichtich wêze, om't koper in ûnmisber elemint is foar algen, mar fergiftich wurdt as it yn te hege konsintraasjes bywêzich is. Op basis fan in literatuerûndersyk is konkludearre dat der fan út gongen wurde mei, dat frij koper de biologyske beskikberens foar algen regulearret. Om dy reden is besletten alle omtinken te jaan oan frij koper. Der besteane op it heden gjin techniken dy't de konsintraasje frij koper mjitte kinne op foar de Tsjûkemar typyske nivo's. Guon techniken kinne brûkbere ynformaasje jaan, mar fereaskje wol in soarchfâldige ynterpretaasje fan de risseltaten. Om't der mar in lyts bytsje koper yn natuerlik wetter foarkomt, wurde kopermjittings maklik troch fersmoarging beynfloede.

Haadstik 2: koperkonsintraasje yn de Tsjûkemar en ynfloeden fan bûtenôf

De Tsjûkemar is in ûndjippe, alkalyske en eutrofe mar. Hy is in ûnderdiel fan it Fryske bûtenwetter. De hydrology fan de mar wurdt troch de minske bepaald: winters wurdt wetter út de polders dy't om de mar hinne lizze, yn de mar pompt en wurdt de gearstalling fan it wetter dêrtroch oerhearske, wylst simmers wetter dat út de Iselmar komt ynlitten wurdt. De totale "oploste" (dat betsjut oer in filter mei poarjes fan 0.2 µm filtere) koperkonsintraasje yn de Tsjûkemar sit sa om de 30 nM hinne. Hoewol't it net bewiisd is, leit it yn de reden dat de koperkonsintraasje yn de mar net heger wurden is troch it ynlitten fan wetter út de Iselmar. Wetter dat út de polders komt, ferheget nei alle gedachten wol de koperkonsintraasje yn de Tsjûkemar. It ûndersyk hat oantoand dat ôfspieling in mear foar de hân lizzende oarsaak is foar dizze ferheging as útspieling, hoewol't by laboratoariumproeven ûnder bepaalde kondysjes him wol útspieling yn de boppelaach fan de boaiemkolom foardien hat. Boaiemmeunsters út de mar binne brûkt foar sekwinsjele

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[1] "gemyske spesjaasje" is definiearre as de ferdieling fan in elemint oer alle mooglike spesiës fan dat elemint.

ekstraksjes en foar ekstraksjes mei EDTA. Der is konkludearre dat it frijkommen fan koper út de boaiem wei nei it wetter nei alle gedachten net in wichtich ferskynsel is.

Haadstik 3: ultrafiltraasje

Ultrafiltraasje is brûkt om ynsjoch te krijen yn de grutte fan koperspesiës yn de Tsjûkemar. Ynterpretaasje fan ultrafiltraasje-risseltaten is dreech yn ferbân mei ferskate mooglike artefakten, wêrûnder fersmoarging. Frij koper, dat ûngefear 0.6 nm grut is, sil fûn wurde yn de fraksje lytser as 5 nm, de lytste poarje-grutte dy't tapast waard, mei oare spesiës dy't lytser as 5 nm binne. Hoewol't it bliken die dat guon meunsters fersmoarge wienen, koenen, troch in krityske skôging, in pear nijsgjirrige konklúzjes lutsen wurde. Likernôch de helte fan de oploste koperkonsintraasjes is fûn yn de fraksje lytser as 5 nm (sa'n pear tsientallen nM), wat betsjut dat mear as de helte fan it totale koper as kompleks foarkaam.

Haadstik 4: foltammetryske techniken

Twa foltammetryske techniken (anodyske en katodyske weroplossings-foltammetry) binne tapast om ynsjoch te krijen yn de fraksje elektrogemysk labyl koper.

Anodyske weroplossings-foltammetry (ASV) is in technyk dy't gauris brûkt wurdt yn spesjaasje-ûndersyk, mar de ynterpretaasje fan de risseltaten is dreech. Der mei fan út gongen wurde dat alle anorganyske en in part fan de organyske koperspesiës mjitten wurde ("ASV-labyl" binne) ûnder de tapaste kondysjes (pH, deposysjetiid, buffer). De konsintraasje ASV-labyl koper wie meastentiids minder as 2 nM en faak hielendal net oantoanber nettsjinsteande de lange deposysjetiid. Dit betsjut dat heechút in pear prosint fan it koper ASV-labyl wie en dêrom moat fierwei it grutste part fan it koper yn de foarm fan organyske kompleksen bywêzich west ha. By natuerlike pH moat de konsintraasje ASV-labyl koper noch leger west ha. Hoewol't de konklúzje lutsen is dat it measte koper organysk kompleksearre wie, binne gjin korrelaasjes fûn mei parameters dy't steane foar organyske koalstof.

Katodyske weroplossings-foltammetry (CSV), is tapast ûnder net-lykwichtskondysjes. Kategol waard as syntetysk ligand brûkt. De metten konsintraasje CSV-labyl koper wie faak mear as 100% fan de oploste koperkonsintraasje, wat fysysk net mooglik is. Op grûn fan ekstra proeven is konkludearre dat de konsintraasje organysk materiaal yn de mar nei alle gedachten te heech wie foar in goede tapassing fan dizze technyk yn de Tsjûkemar.

Om de konsintraasje fan it frije koper sels fêststelle te kinnen, binne twa ligand-kompetysje-techniken tapast.

Ien derfan wie de *MnO_2-metoade*. Standerts mei in bekinde gearstalling is MnO_2 by dien, wêrtroch't de koper-adsorpsje as funksje fan de konsintraasje frij koper fêststeld wurde koe. Trije metoaden binne brûkt om de adsorpje-gegevens te bewurkjen:

1) Langmuir-gedrach is ferûndersteld; de linearisaasje oanrikkemandearre troch Van den Berg (1982a) is tapast;
2) in oare linearisaasje, yn dit proefskrift nei foaren brocht, is brûkt om de Langmuir-gegevens te bewurkjen;
3) as lêste is in lineêr model brûkt dat der fan út giet dat de koper-adsorpsje (Γ) evenredich is mei de konsintraasje frij koper; dit kin opfette wurde as in isolearring fan it earste, sabeare lineêre part fan in Langmuir-kromme.

De berekkene wearden foar de konsintraasje frij koper wienen allinnich jildich yn de bywêzigens fan MnO_2, mar mei in pear oannames koenen se omrekkene wurde nei wearden jildich yn de ôfwêzigens fan MnO_2. De Van den Berg-linearisaasje joech gjin befredigjende korrelaasje-koëffisjinten; der is beriddenearre dat dizze linearisaasje brûkt wurde moatte soe foar hegere konsintraasjes frij metaal as hjir brûkt binne. De twadde linearisaasje en it lineêre model joegen wol befredigjende korrelaasjes. Troch fersmoarging wie it yn ferskate gefallen net mooglik de konsintraasje frij koper yn meunsters út de mar te berekkenjen. As it wol koe, rûn de trochsneed fan de konsintraasje frij koper útinoar fan 0.013 nM (twadde linearisaasje) fia 0.018 nM (earste linearisaasje) ta 0.050 nM (lineêre model), alles yn de bywêzigens fan MnO_2. Dizze wearden wienen inkelde tsientallen prosinten heger, as se omrekkene waarden nei de sitewaasje sûnder MnO_2. De konsintraasjes frij koper wienen net systematysk korrelearre mei de konsintraasje of UV-absorpsje fan organysk materiaal. Mei de útkomsten fan it lineêre model is berekkene dat mear as 95% fan it koper yn de Tsjûkemar organysk kompleksearre wie; mei de útkomsten fan de Langmuir-linearisaasjes kaam dit persintaazje boppe de 99.

In *foltammetryske ferzje fan de SEP-PAK-C_{18}-adsorpsjemetoade* (Sunda & Hanson, 1987) is yn dit wurk ûntwikkele. Labyl koper waard metten oan in draaiende kwikfilm-elektroade mei differensjele-puls-ASV yn twa koperaddysje-searjes, wêrfan ien mei EDTA. Troch de labile tsjin de totale koperkonsintraasje út te setten koe de konsintraasje fan it koper-EDTA-kompleks berekkene wurde; dêrtroch koe wer de konsintraasje frij koper berekkene wurde. Om't it ASV-sinjaal yn de meunsters sûnder koperaddysje tanaam troch tafoeging fan EDTA, koe de konsintraasje frij koper foar de sitewaasje sûnder tafoege koper net bepaald wurde. Der is beriddenearre dat it reedlik wie om nei dy sitewaasje ta te ekstrapolearjen. Sa is foar de konsintraasje frij koper in wearde tusken de 10^{-13} en 10^{-12} M fûn. De dêrmei oerienstimmende konsintraasje organysk kompleksearre koper is berekkene op mear as 99% fan de oploste koperkonsintraasje. Der is konkludearre dat dizze ferzje fan in ligand-kompetysje-technyk gâns ûnthjit.

Haadstik 6: kopertitraasjes

Kopertitraasjes binne brûkt om ynsjoch te krijen yn koperkompleksearring yn de Tsjûkemar. Ferskate soarten titraasjes en gegevensferwurking binne tapast.

Mei in koper(II)-ion-selektive elektroade (ISE) is alle twa wiken de koperkompleksearringskapasiteit metten yn de Tsjûkemar. Van den Berg-Ružić-Lee- likegoed as Scatchard-analize joegen ligandkonsintraasjes fan in pear tsientallen μM. By Van den Berg-Ružić-Lee-analize rûn de log(K) útinoar fan likernôch 4.5 ta likernôch 7.5, by Scatchard-analize fan likernôch 4.5 ta likernôch 10. Affiniteitsspektra smieten log(K)-wearden op fan likernôch 4.5 ta likernôch 8, en suggerearren op syn minst trije groepen biningsplakken. De heegste log(K)-wearden foelen gear mei betreklik lege humuskonsintraasjes en bloeiperioades fan syanobaktearjes.

Ien titraasjekromme is neier besjoen. Mei Van den Berg-Ružić-Lee-analize is oantoand dat twa groepen liganden ûndersteld wurde moasten om de titraasjegegevens goedernôch rekonstruearje te kinnen. Foar de Scatchard-kromme wie it nedich om trije groepen liganden te ûnderstellen. Der is oantoand dat likegoed Van den Berg-Ružić-Lee- as Scatchard-analize tapast wurde moat om kompleksearringsparameters te krijen dy't goed genôch binne.

It wie net mooglik om rjochtstreeks ynformaasje te krijen oer koperkompleksearring by natuerlike pH, om't koper delsloech by hegere pH. Dêrom binne kopertitraasje-gegevens fan legere pH fitte mei in pH-ôfhinklike Freundlich-isoterm, en ekstrapolearre nei de natuerlike pH. By lege pH (3, 4) wurde aktivere biningsplakken berikt as by hegere pH (5, 6). De konsintraasje frij koper by pH = 7.55 (de pH brûkt foar de eksperiminten beskreaun yn haadstik 5) is berekkene op ûngefear 10^{-10} M; as allinnich de aktivere biningsplakken meinommen waarden, kaam dizze wearde op likernôch 10^{-14} M.

Om't in ISE in betreklik hege deteksjegrins hat, is ASV, yn kombinaasje mei Scatchard-analize, brûkt om ynsjoch te krijen yn de kompleksearring by lege koperaddysjes (oant 157 nM). De log(K) by pH = 5 rûn útinoar fan 8 ta 9. Hast al it koper wie organysk kompleksearre. As dizze titraasjes útfierd waarden yn ultrafiltraten, koe oantoand wurde dat de skynbere ligandkonsintraasje tebekrûn mei de poarjegrutte, wylst de log(K) oprûn mei de poarjegrutte; dat betsjut dat de lytste liganden de sterkste kompleksen mei koper foarmje.

Haadstik 7: modellearjen

In lykwichtsmodel is brûkt om de spesjaasje te berekkenjen yn fjouwer meunsters. Sinfolle berekkenings kinne allinnich útfierd wurde as der genôch bekind is oer de bywêzige spesiës en de dêrby hearrende lykwichtskonstanten. De gegevens dy't brûkt binne as ynfier foar it model, binne dêrom kritysk besjoen.

As allinnich anorganyske kompleksearring meinommen waard yn de berekkenings, rûn de konsintraasje frij koper útinoar fan 10^{-12} ta 10^{-10} M en naam dêrmei net mear as 2% fan de oploste koperkonsintraasje foar syn rekken. Koper wie benammen oanwêzich yn de foarm fan karbonaatkompleksen, wylst in pear prosint oanwêzich wie yn de foarm fan koper-hydrokside-kompleksen.

As de pH-ôfhinklike fit fan kopertitraasje-gegevens (lykas beskreaun yn haadstik 6) brûkt waard om kompleksearring fan koper troch organyske liganden te modellearjen, rûn de konsintraasje fan frij koper tebek, útinoarrinnend fan 10^{-12} ta 10^{-11} M. Neffens de berekkenings wie mear as 90% fan it koper organysk kompleksearre, útsein foar ien tige alkalysk meunster dêr't gâns karbonaten yn sieten. Hydroksides wienen net sa wichtich.

As de fit fan allinne de aktivere biningsplakken brûkt waard, rûn de konsintraasje fan frij koper útinoar fan 10^{-16} ta 10^{-14} M. Alle koper (dat betsjut mear as 99.98%) kaam foar yn organyske kompleksen, neffens de berekkenings.

Haadstik 8: spesjaasje fan koper yn relaasje ta de biologyske beskikberens

Yn wettermeunsters út de Tsjûkemar, de Iselmar, in petgat by Earnewâld en in punt yn de feanpolder fan Ychten, binne bioassays útfierd om de biologysk beskikbere fraksje fan it koper fêst te stellen. In griene alch, *Scenedesmus quadricauda*, is kweke yn batchkultueren der't fêste konsintraasjes fosfor en stikstof en wikseljende konsintraasjes koper by dien wienen. Elektrogemysk labyl koper is metten yn meunsters mei deselde koperaddysjes. Foar de Tsjûkemar binne ek lykwichtsberekkenings dien, lykas beskreaun yn haadstik 7. Foar alle meunsters joech in koperaddysje fan 0.16 µM in hegere biomassa yn de stasjonêre fase; foar de Tsjûkemar en de Iselmar wie dizze tanimming sels signifikant. Koperaddysjes fan 1.6 µM en heger lieten groeiremming sjen. Groeiremming wie goed korrelearre mei elektrogemysk labyl en berekkene frij koper, mar om't dy lêste twa parameters hast evenredich wienen mei de totale koperkonsintraasje, hienen dy korrelaasjes net in soad betsjutting.

Scenedesmus quadricauda is ek kweke yn syntetyske media fan bekinde gearstalling. De koper- en EDTA-konsintraasjes binne fariearre om in grutte fariaasje te krijen yn de konsintraasje frij koper. Spesjaasje-berekkenings toanden oan dat net allinne de konsintraasje frij koper fariearre, mar ek de konsintraasjes frij kobalt en frij sink, troch kompetysje fan dizze trije metalen foar EDTA. Troch dizze kompetysje wie it dreech om ûndûbelsinnige konklúzjes te lûken. Dochs is dúdlik oantoand dat de frije en net de totale konsintraasjes koper, kobalt en sink de biologyske beskikberens bepale. Kobalt en sink wienen net fergiftich yn ús bioassays, mar koper wie al fergiftich by konsintraasjes frij koper boppe ûngefear $10^{-10.5}$ M. By hege konsintraasjes EDTA waard in elemint limitearjend, nei alle gedachten of koper of kobalt of sink. De konsintraasje dêr't koper limitearjend by waard, koe net krekt fêststeld wurde, mar moat leger as $10^{-12.5}$ M west ha.

De groeifuortsterking dy't fûn is yn de Tsjûkemar en de Iselmar by tafoeging fan koper, betsjut dêrom dat de konsintraasje frij koper yn de meunsters leger as $10^{-12.5}$ M west ha moat.

Yn natuerlike meunsters binne hegere wearden berikt foar de biomassa as yn syntetyske media. It bleau ûndúdlik wêrom't dat ferskynsel him foardie.

Haadstik 9: werom- en foarútsjen

Oan de ein fan dit ûndersyksprojekt is konkludearre dat de risseltaten dy't de ferskate metoades oplevere hawwe, op syn minst kwalitatyf konsistint wienen. De techniken dy't harren der op foarstean litte allinnich frij koper te bepalen, lieten in grutte fariaasje yn frij-koper-konsintraasjes sjen, mar allegearre joegen se oan dat mar in hiel lyts bytsje fan de oploste koperkonsintraasjes as frij koper oanwêzich wie. De relaasje tusken de spesjaasje en de biologyske beskikberens fan koper koe foar ien algesoart fêststeld wurde.

Om dit soarte ûndersyk yn de takomst better útfiere te kinnen, is mear kennis nedich fan de ynteraksjes fan metalen mei organysk materiaal. Dit ûndersyk hat it belang fan sokke ynteraksjes dúdlik oantoand. Ligand-kompetysje-techniken ûnthjitte faaks it meast. Om sekuerdere risseltaten te krijen is dan wol mear kennis fan anorganyske oplossings nedich.

(mei tank oan pyt jon foar it neisjen fan de oersetting)

References

Alberts, J.J., J.P. Giesy & D.W. Evans (1984). Distribution of dissolved organic carbon and metal-binding capacity among ultrafilterable fractions isolated from selected surface waters of the southeastern United States. Environmental Geology and Water Sciences 6, 91 - 101.

Allen, H.E., R.H. Hall & T.D. Brisbin (1980). Metal speciation. Effects on aquatic toxicity. Environmental Science & Technology 14, 441 - 443.

Anderson, D.M., J.S. Lively & R.F. Vaccaro (1984). Copper complexation during spring phytoplankton blooms in coastal waters. Journal of Marine Research 42, 677 - 695.

Anderson, D.M. & F.M.M. Morel (1978). Copper sensitivity of *Gonyaulax tamarensis*. Limnology and Oceanography 23, 283 - 295.

Baccini, P. & U. Suter (1979). MELIMEX, an experimental heavy metal pollution study: chemical speciation and biological availability of copper in lake water. Schweizerische Zeitschrift für Hydrologie 41, 291 - 314.

Barrow, G.M. (1979). Physical Chemistry. McGraw-Hill Kogakusha, Tokyo.

Batley, G.E. (1987). Heavy metal speciation in waters, sediments and biota from Lake Macquarie, New South Wales. Australian Journal of Marine and Freshwater Research 38, 591 - 606.

Batley, G.E. & T.M. Florence (1976). Determination of the chemical forms of dissolved cadmium, lead and copper in seawater. Marine Chemistry 4, 347 - 363.

Benes, P., E.T. Gjessing & E. Steinnes (1976). Interactions between humus and trace elements in fresh water. Water Research 10, 711 - 716.

Benes, P. & E. Steinnes (1974). In situ dialysis for the determination of the state of trace elements in natural waters. Water Research 8, 947 - 953.

Bernhard, M., F.E. Brinckman & K.J. Irgolic (1986). Why "speciation"? In: *The importance of chemical "speciation" in environmental processes*, M. Bernhard, F.E. Brinckman & P.J. Sadler (eds.). Springer-Verlag, Berlin.

Betti, M. & P. Papoff (1988). Trace elements: data and information in the characterization of an aqueous ecosystem. CRC Critical Reviews in Analytical Chemistry 19, 271 - 322.

Bond, A.M. (1980). Modern polarographic methods in analytical chemistry. Marcel Dekker, New York.

Borchers, R. (1959). Spectrophotometric determination of amino acids. Alkaline copper salt method using cuprizone, biscyclohexanoneoxalyldihydrazone. Analytical Chemistry 31, 1179 - 1180.

Buffle, J. (1980). A critical comparison of studies of complex formation between copper(II) and fulvic substances of natural waters. Analytica Chimica Acta 118, 29 - 44.

Buffle, J., P. Deladoey & W. Haerdi (1978). The use of ultrafiltration for the separation and fractionation of organic ligands in fresh waters. Analytica Chimica Acta 101, 339 - 357.

Buffle, J. & C. Staub (1984). Measurement of complexation properties of metal ions in natural conditions by ultrafiltration: measurement of equilibrium constants for complexation of zinc by synthetic and natural ligands. Analytical Chemistry 56, 2837 - 2842.

Bürgi, H.R. (1974). Die Wirkung von NTA auf das Wachstum das Phytoplanktons unter besondere Berücksichtung des Eisens als Microelement. Schweizerische Zeitschrift für Hydrologie 36, 1 - 70.

Campbell, P.G.C. & A. Tessier (1984). Determination of the complexation capacity of natural waters using metal solubilization techniques. In: *Complexation of trace metals in natural waters*, C.J.M. Kramer & J.C. Duinker (eds.). Martinus Nijhof/Dr. W. Junk Publishers, The Hague.

Canterford, G.S. & D.R. Canterford (1980). Toxicity of heavy metals to the marine diatom *Ditylum brightwellii* (West) Grunow: correlation between toxicity and metal speciation. Journal of the Marine Biological Association of the United Kingdom 60, 227 - 242.

Cleven, R.F.M.J. (1984). Heavy metal/polyacid interaction. Ph.D. Thesis, Agricultural University of Wageningen.

Cleven, R.F.M.J., P. Del Castilho & P.M. Wolfs (1988). Effects of organic matter on the pulse voltammetric speciation of copper. Environmental Technology Letters 9, 869 - 876.

Davison, W., S.J. De Mora, R.M. Harrison & S. Wilson (1987). pH and ionic strength dependence of the ASV response of cadmium, lead and zinc in solutions which simulate natural waters. The Science of the Total Environment 60, 35 - 44.

De Boer, T., H. Kramer, J. Schrotenboer & J. Voerman (1987). Hydrological, physical and chemical research in Lake Tjeukemeer. Data 1986 (in Dutch). Limnological Institute Oosterzee, the Netherlands, internal report 1987-5.

De Boer, T., H. Kramer, J. Schrotenboer & J. Voerman (1989). Hydrological, physical and chemical research in Lake Tjeukemeer. Data 1988 (in Dutch). Limnological Institute Oosterzee, the Netherlands, internal report 1989-6.

De Boer, T., H. Hoogveld, H. Kramer & J. Voerman (1990). Hydrological, physical and chemical research in Lake Tjeukemeer. Data 1989 (in Dutch). Limnological Institute Oosterzee, the Netherlands, internal report 1990-3.

De Groot, A.J., K.H. Zschuppe & W. Salomons (1982). Standardization of methods of analysis for heavy metals in sediments. Hydrobiologia 92, 689 - 695.

De Haan, F.A.M. & W.H. Van Riemsdijk (1986). Behaviour of inorganic contaminants in soil. In: *Contaminated soil*, J.W. Assink & W.J. Van den Brink (eds.). Martinus Nijhoff, Dordrecht.

De Haan, H., T. De Boer & H.L. Hoogveld (1981). Metal binding capacity in relation to hydrology and algal periodicity in Tjeukemeer, The Netherlands. Archiv für Hydrobiologie 92, 11 - 23.

De Haan, H., J. Voerman, T. De Boer, J.R. Moed, J. Schrotenboer, & H.L. Hoogveld (1990). Trace metal chemistry of a Dutch reservoir, the Tjeukemeer. Freshwater Biology 24, 391 - 400.

Del Castilho, P. & W. Salomons (1986). In situ dialysis of metal species in an eutrophic lake: importance of algal blooms for speciation changes. In: *Proceedings of international conference on chemicals in the environment*, J.N. Lester, R. Perry & R.M. Sterritt (eds.). Selper, London.

Dempsey, B.A. & C.R. O'Melia (1983). Proton and calcium complexation of four fulvic acid fractions. In: *Aquatic and terrestrial humic materials*, R.F. Christman & E.T. Gjessing (eds.). Ann Arbor Science, Michigan.

Dzombak, D.A., W. Fish & F.M.M. Morel (1986). Metal-humate interactions. 1. Discrete ligand and continuous distribution models. Environmental Science & Technology 20, 669 - 675.

Ephraim, J., S. Alegret, A. Mathuthu, M. Bicking, R.L. Malcolm & J.A. Marinsky (1986). A unified physicochemical description of the protonation and metal ion complexation equilibria of natural organic acids (humic and fulvic acids). 2. Influence of polyelectrolyte properties and functional group heterogeneity on the protonation equilibria of fulvic acid. Environmental Science & Technology 20, 354 - 366.

Ephraim, J. & J.A. Marinsky (1986). A unified physicochemical description of the protonation and metal ion complexation equilibria of natural organic acids (humic and fulvic acids). 3. Influence of polyelectrolyte properties and functional heterogeneity on the copper ion binding equilibria in an Armadale Horizon Bh fulvic acid sample. Environmental Science & Technology 20, 367 - 376.

Finden, D.A.S., E. Tipping, G.H.M. Jaworski & C.S. Reynolds (1984). Light-induced reduction of natural iron(III)oxide and its relevance to phytoplankton. Nature 309, 783 - 784.

Fish, W., D.A. Dzombak & F.M.M. Morel (1986). Metal-humate interactions. 2. Application and comparison of models. Environmental Science & Technology 20, 676 - 683.

Florence, T.M. (1986). Electrochemical approaches to trace element speciation in waters. A review. Analyst 111, 489 - 505.

Florence, T.M., B.G. Lumsden & J.J. Fardy (1983). Evaluation of some physico-chemical techniques for the determination of the fraction of dissolved copper toxic to the marine diatom *Nitzschia closterium*. Analytica Chimica Acta 151, 281 - 295.

Gerringa, L.J.A. (1990). Speciation of trace metals in relation to degradation of organic matter in marine sediment slurries. Ph.D. Thesis, State University of Groningen.

Giesy, J.P. & J.J. Alberts (1989). Conditional stability constants and binding capacities for copper(II) by ultrafilterable material isolated from six surface waters of Wyoming, USA. Hydrobiologia 188/189, 659 - 679.

Giesy, J.P., J.J. Alberts & D.W. Evans (1986). Conditional stability constants and binding capacities for copper(II) by dissolved organic carbon isolated from surface waters of the southeastern United States. Environmental Toxicology and Chemistry 5, 139 - 154.

Giesy, J.P. & L.A. Briese (1977). Metals associated with organic carbon extracted from Okefenokee Swamp water. Chemical Geology 20, 109 - 120.

Good, N.E., G.D. Winget, W. Winter, T.N. Connolly, S. Izawa & R.M.M. Singh (1966). Hydrogen ion buffers for biological research. Biochemistry 5, 467 - 477.

Gorman, W.C., R.K. Skogerboe & P.H. Davies (1986). Effects of metal complexation on the accuracy of Anodic Stripping Voltammetry. Analytica Chimica Acta 187, 325 - 331.

Hering, J.G. & F.M.M. Morel (1988). Humic acid complexation of calcium and copper. Environmental Science & Technology 22, 1234 - 1237.

Hill-Cottingham, D.G. (1955). Photosensitivity of iron chelates. Nature 175, 347 - 348.

Hoffmann, M.R., E.C. Yost, S.J. Eisenreich & W.J. Maier (1981). Characterization of soluble and colloidal-phase metal complexes in river water by ultrafiltration. A mass-balance approach. Environmental Science & Technology 15, 655 - 661.

Hunston, D.L. (1975). Two techniques for evaluating small molecule - macromolecule binding in complex system. Analytical Biochemistry 63, 99 - 109.

Huntsman, S.A. & W.G. Sunda (1980). The role of trace metals in regulating phytoplankton growth with emphasis on Fe, Mn and Cu. In: *Studies in ecology volume 7. The physiological ecology of phytoplankton*, I. Morris (ed.). Blackwell Scientific Publications, Oxford.

Kramer, C.J.M. (1985). On the copper complexation capacity in the marine environment. Ph.D. Thesis, State University of Groningen.

Kramer, C.J.M. (1986). Apparent copper complexation capacity and conditional stability constants in north Atlantic waters. Marine Chemistry 18, 335 - 349.

Lee, J. (1983). Complexation analysis of fresh waters by equilibrium diafiltration. Water Research 17, 501 - 510.

Leenen, J.D. (1982). Hydrology of Tjeukemeer. Hydrobiologia 95, 199 - 203.

Lexmond, T.M. (1980). The effect of soil pH on copper toxicity to forage maize grown under field conditions. Netherlands Journal of Agricultural Science 28, 164 - 183.

Marinsky, J.A. & J. Ephraim (1986). A unified physicochemical description of the protonation and metal ion complexation equilibria of natural organic acids (humic and fulvic acids). 1. Analysis of the influence of polyelectrolyte properties on protonation equilibria in ionic media: fundamental concepts. Environmental Science & Technology 20, 349 - 354.

Mart, L. (1979a). Prevention of contamination and other accuracy risks in voltammetric trace metal analysis of natural waters. Part I: preparatory steps. Filtration and storage of water samples. Fresenius Zeitschrift für Analytische Chemie 296, 350 - 357.

Mart, L. (1979b). Prevention of contamination and other accuracy risks in voltammetric trace metal analysis of natural waters. Part II: collection of surface water samples. Fresenius Zeitschrift für Analytische Chemie 299, 97 - 102.

Mart, L., H.W. Nürnberg & P. Valenta (1980). Prevention of contamination and other accuracy risks in voltammetric trace metal analysis of natural waters. Part III: voltammetric ultratrace analysis with a multicell system designed for clean bench working. Fresenius Zeitschrift für Analytische Chemie 300, 350 - 362.

Martell, A.E. & R.M. Smith (1974). Critical stability constants, volume 1: amino acids. Plenum Press, New York.

Martell, A.E. & R.M. Smith (1977). Critical stability constants, volume 3: other organic ligands. Plenum Press, New York.

Martell, A.E. & R.M. Smith (1982). Critical stability constants, volume 5: first supplement. Plenum Press, New York.

Moed, J.R. (1973). Effect of combined action of light and silicon depletion on *Asterionella formosa* Hass. Verhandlungen internationale Vereinigung für Limnologie 18, 1367 - 1374.

Moed, J.R. & H.L. Hoogveld (1982). The algal periodicity in Tjeukemeer during 1968 - 1978. Hydrobiologia 95, 223 - 234.

Morel, F.M.M. (1983). Principles of aquatic chemistry. John Wiley & Sons, New York.

Morel, N.M.L., J.G. Rueter & F.M.M. Morel (1978). Copper toxicity to *Skeletonema costatum* (Bacillariophyceae). Journal of Phycology 14, 43 - 48.

Nordstrom, D.K. & J.W. Ball (1984). Chemical models, computer programs and metal complexation in natural waters. In: *Complexation of trace metals in natural waters*, C.J.M. Kramer & J.C. Duinker (eds.). Nijhoff/Junk Publishers, the Hague.

Nordstrom, D.K., L.N. Plummer, T.M.L. Wigley, T.J. Wolery, J.W. Ball, E.A. Jenne, R.L. Bassett, D.A. Crerar, T.M. Florence, B. Fritz, M. Hoffman, G.R. Holdren Jr., G.M. Lafon, S.V. Mattigod, R.E. McDuff, F. Morel, M.M. Reddy, G. Sposito & J. Thrailkill (1979). In: *Chemical modeling in aqueous systems*, E.A. Jenne (ed.). ACS Symposium Series 93.

Nürnberg, H.W. (1984). The voltammetric approach in trace metal chemistry of natural waters and atmospheric precipitation. Analytica Chimica Acta 164, 1 - 21.

Petersen, R. (1982). Influence of copper and zinc on the growth of a freshwater alga, *Scenedesmus quadricauda*: the significance of chemical speciation. Environmental Science & Technology 16, 443 - 447.

Peterson, W.M. & R.V. Wong (1981). Fundamentals of stripping voltammetry. American Laboratory.

Rapin, F., A. Tessier, P.G.C. Campbell & R. Carignan (1986). Potential artifacts in the determination of metal partitioning in sediments by a sequential extraction procedure. Environmental Science & Technology 20, 836 - 840.

Rueter, J.G., J.J. McCarthy & E.J. Carpenter (1978). The toxic effect of copper on *Oscillatoria (Trichodesmium) theibautti*. Limnology and Oceanography 24, 558 - 562.

Ružić, I. (1982). Theoretical aspects of the direct titration of natural waters and its information yield for trace metal speciation. Analytica Chimica Acta 140, 99 - 113.

Ružić, I. (1987). Comment on "Metal-humate interactions. 1 and 2". Environmental Science & Technology 21, 1132 - 1135.

Salomons, W. & U. Förstner (1984). Metals in the hydrocycle. Springer-Verlag, Berlin.

Salomons, W. & W.G. Mook (1980). Biogeochemical processes affecting metal concentrations in lake sediments (IJsselmeer, The Netherlands). The Science of the Total Environment 16, 217 - 229.

Scatchard, G. (1949). The attractions of proteins for small molecules and ions. Annals New York Academy of Sciences 51, 660 - 672.

Schnitzer, M. (1971). Metal-organic matter interactions in soils and waters. In: *Organic compounds in aquatic environments*, S.J. Faust & J.V. Hunter (eds.). Marcel Dekker, New York.

Smith, R.M. & A.E. Martell (1975). Critical stability constants, volume 2: amines. Plenum Press, New York.

Smith, R.M. & A.E. Martell (1976). Critical stability constants, volume 4: inorganic ligands. Plenum Press, New York.

Smith, R.M. & A.E. Martell (1989). Critical stability constants, volume 6: second supplement. Plenum Press, New York.

Sojo, L.E. & H. De Haan (1991). Multicomponent analysis of iron speciation in Lake Tjeukemeer: comparison with iron speciation in fulvic acid solutions extracted from a peaty polder near Lake Tjeukemeer. Accepted for publication in Environmental Science & Technology.

Sposito, G. & S.V. Mattigod (1980). GEOCHEM, a computer program for the calculation of chemical equilibria. Department of soil science, University of California, Riverside.

Stauber, J.L. & T.M. Florence (1985a). The influence of iron on copper toxicity to the marine diatom, *Nitzschia closterium* (Ehrenberg) W. Smith. Aquatic Toxicology 6, 297 - 305.

Stauber, J.L. & T.M. Florence (1985b). Interactions of copper and manganese: a mechanism by which manganese alleviates copper toxicity to the marine diatom, *Nitzschia closterium* (Ehrenberg) W. Smith. Aquatic Toxicology 7, 241 - 254.

Stauber, J.L. & T.M. Florence (1986). Reversibility of copper-thiol binding in *Nitzschia closterium* and *Chlorella pyrenoidosa*. Aquatic Toxicology 8, 223 - 229.

Stauber, J.L. & T.M. Florence (1987). Mechanism of toxicity of ionic copper and copper complexes to algae. Marine Biology 94, 511 - 519.

Stroes-Gascoyne, S., J.R. Kramer & W.J. Snodgrass (1986). A new model describing the adsorption of copper on MnO_2. Environmental Science & Technology 20, 1047 - 1050.

Stumm, W. & J.J. Morgan (1981). Aquatic chemistry. John Wiley & Sons, New York.

Sunda, W.G. & R.L. Ferguson (1983). Sensitivity of natural bacterial communities to additions of copper and to cupric ion activity: a bioassay of copper complexation in seawater. In: *Trace metals in sea water*, C.S. Wong, E. Boyle, K.W. Bruland, J.D. Burton & E.D. Goldberg (eds.). Plenum Press, New York.

Sunda, W.G. & P.A. Gillespie (1979). The response of a marine bacterium to cupric ion and its use to estimate cupric ion activity. Journal of Marine Research 37, 761 - 777.

Sunda, W. & R.R.L. Guillard (1976). The relationship between cupric ion activity and the toxicity of copper to phytoplankton. Journal of Marine Research 34, 511 - 529.

Sunda, W.G. & A.K. Hanson (1987). Measurement of free cupric ion concentration in seawater by a ligand competition technique involving copper sorption onto C_{18} SEP-PAK cartridges. Limnology and Oceanography 32, 537 - 551.

Sunda, W.G., D. Klaveness & A.V. Palumbo (1984). Bioassays of cupric ion activity and copper complexation. In: *Complexation of trace metals in natural waters*, C.J.M. Kramer & J.C. Duinker (eds.). Nijhoff/Junk Publishers, the Hague.

Tessier, A., P.G.C. Campbell & M. Bisson (1979). Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry 51, 844 - 851.

Thakur, A.K., P.J. Munson, D.L. Hunston & D. Rodbard (1980). Characterization of ligand-binding systems by continuous affinity distributions of arbitrary shape. Analytical Biochemistry 103, 240 - 254.

Tipping, E., C.A. Backes & M.A. Hurley (1988). The complexation of protons, aluminium and calcium by aquatic humic substances: a model incorporating binding-site heterogeneity and macroionic effects. Water Research 22, 597 - 611.

Toledo, A.P.P., V.A. D'Aquino & J.G. Tundisi (1982). Influence of humic acid on growth and tolerance to cupric ions in *Melosira italica* (subsp. *subartica*). Hydrobiologia 87, 247 - 254.

Turner, D.R., M.S. Varney, M. Whitfield, R.F.C. Mantoura & J.P. Riley (1986). Electrochemical studies of copper and lead complexation by fulvic acid. I. Potentiometric measurements and a critical comparison of metal binding models. Geochimica et Cosmochimica Acta 50, 289 - 297.

Turner, D.R., M. Whitfield & A.G. Dickson (1981). The equilibrium speciation of dissolved components in freshwater and seawater at 25° C and 1 atm pressure. Geochimica et Cosmochimica Acta 45, 855 - 881.

Tuschall, J.R. & P.L. Brezonik (1983). Complexation of heavy metals by aquatic humus: a comparative study of five analytical methods. In: *Aquatic and terrestrial humic materials*, R.F. Christman & E.T. Gjessing (eds.). Ann Arbor Science, Michigan.

Van den Berg, C.M.G. (1982a). Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO_2. I. Theory. Marine Chemistry 11, 307 - 322.

Van den Berg, C.M.G. (1982b). Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO_2. II. Experimental procedures and application to surface seawater. Marine Chemistry 11, 323 - 342.

Van den Berg, C.M.G. (1983). Corrections to "Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO_2. I. Theory". Marine Chemistry 13, 83 - 85.

Van den Berg, C.M.G. (1984a). Determining trace concentrations of copper in water by cathodic film stripping voltammetry with adsorptive collection. Analytical Letters 17, 2141 - 2157.

Van den Berg, C.M.G. (1984b). 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. Marine Chemistry 15, 1 - 18.

Van den Berg, C.M.G. (1984c). Determination of copper in sea water by cathodic stripping voltammetry of complexes with catechol. Analytica Chimica Acta 164, 195 - 207.

Van den Berg, C.M.G. (1984d). Organic and inorganic speciation of copper in the Irish Sea. Marine Chemistry 14, 201 - 212.

Van den Berg, C.M.G., P.J.M. Buckley, Z. Qiang Huang & M. Nimmo (1986). An electrochemical study of the speciation of copper, zinc and iron in two estuaries in England. Estuarine, Coastal and Shelf Science 22, 479 - 486.

Van den Berg, C.M.G. & J.R. Kramer (1979a). Determination of complexing capacities of ligands in natural waters and conditional stability constants of the copper complexes by means of manganese dioxide. Analytica Chimica Acta 106, 113 - 120.

Van den Berg, C.M.G. & J.R. Kramer (1979b). Conditional stability constants for copper ions with ligands in natural waters. In: *Chemical modeling in aqueous systems*, E.A. Jenne (ed.). ACS Symposium Series 93.

Van der Geugten, R.P. (1981). Determination of boron in river water with flameless atomic absorption spectrometry (graphite furnace technique). Fresenius Zeitschrift für Analytische Chemie 306, 13 - 14.

Van der Werff, R. (1986). Stripping voltammetry as a tool for speciation research (in Dutch). M.Sc. Thesis, State University of Groningen.

Van Huet, H.J.W.J. (1990). Phosphorus eutrophication research in the lake district of south western Friesland, The Netherlands. Preliminary results of abiotic studies. Hydrobiologia 191, 75 - 85.

Van Huet, H.J.W.J., H. De Haan & T.H.L. Claassen (1987). Phosphorus eutrophication research in some lakes of south western Friesland (in Dutch). H_2O 6, 131 - 135.

Van Leeuwen, H., R. Cleven & J. Buffle (1989). Voltammetric techniques for complexation measurements in natural aquatic media. Pure & Applied Chemistry 61, 255 - 274.

Van Riemsdijk, W.H., G.H. Bolt & L.K. Koopal (1991). The electrified interface of the soil solid phase. B. Effect of surface heterogeneity. In: *Interactions at the soil colloid - soil solution interface*, G.H. Bolt et al. (eds.). Kluwer Academic Publishers.

Van Riemsdijk, W.H., J.C.M. De Wit, L.K. Koopal & G.H. Bolt (1987). Metal ion adsorption on heterogeneous surfaces: adsorption models. Journal of Colloid and Interface Science 116, 511 - 522.

Van Riemsdijk, W.H. & M.G. Keizer (1984). Computer assisted education. Chemical equilibria in soil-water-sediment (in Dutch). Department of soil science and plant nutrition, Agricultural University of Wageningen.

Verweij, W. (1986). Arsenic in the aquatic environment. Part 2. Adsorption of arsenic (in Dutch). M.Sc. Thesis, State University of Groningen/Delft Hydraulics Laboratory.

Verweij, W. (1988). In: *Data report on the international TNO speciation workshop, April 17-30, 1988*, C.J.M. Kramer (ed.). Report R 88/348. TNO, Delft, The Netherlands.

Verweij, W., H. De Haan, T. De Boer & J. Voerman (1989). Copper complexation in eutrophic and humic Lake Tjeukemeer, the Netherlands. Freshwater Biology 21, 427 - 436 (included as section 6.2 in this thesis).

Verweij, W. (1990a). Manual for the geochemical equilibrium model GECHEQ. Limnological Institute Oosterzee / Nieuwersluis, the Netherlands, internal report 1990-9.

Verweij, W. (1990b). GECHEQ: list of constants. Limnological Institute Oosterzee / Nieuwersluis, the Netherlands, internal report 1990-10.

Wagemann, R. & J. Barica (1979). Speciation and rate of loss of copper from lakewater with implications to toxicity. Water Research 13, 515 - 523.

Wang, J. (1985). Adsorptive stripping voltammetry. American Laboratory.

Weast, R.C. (ed.) (1979). CRC handbook of chemistry and physics, 59th edition. CRC Press, Boca Raton, Florida.

Weber, J.H. (1988). Binding and transport of metals by humic materials. In: *Humic substances and their role in the environment*, F.H. Frimmel & R.F. Christman (eds.). John Wiley & Sons, New York.

West, T.S. (1969). Complexometry with EDTA and related agents. BDH Chemicals Ltd.

Young, J.S., J.M. Gurtisen, C.W. Apts & E.A. Crecelius (1979). The relationship between the copper complexing capacity of sea water and copper toxicity in shrimp zoeae. Marine Environmental Research 265 - 273.

Levensloop

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