Concentration of dissolved organic copper in relation to other chemical and biological parameters in coastal Baltic waters

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ABSTRACT: During 4 different seasons dissolved organic copper was determined by absorption onto silanized porous glass with subsequent elution. Additionally, the following parameters were measured: total dissolved copper, chlorophyll a, phytoplankton composition, phytoplankton carbon, total cell numbers, dissolved organic carbon (DOC), and microbial activity. The biological and chemical situation during each sampling period was characterized by these parameters and was found to be dependent upon phytoplanktonic activity/inactivity. On the other hand, the organic copper values showed no direct response to phytoplankton blooms. A strong correlation could only be established between the concentrations of organic copper and the eluate extinction at 400 nm, which is suggested to be a measure for humic and fulvic acids in this eluate. It is concluded, that in regions with a high content of DOC and total dissolved copper we encounter a constant background level of organic copper. Assuming that there are small and/or fast changes as a response to biological events this could not be detected by the methods applied.

INTRODUCTION

Laboratory experiments have demonstrated that algae excrete substances to render metals in a complexed form, thereby enhancing either their uptake or detoxifying them. The algae *Anabaena cylindrica*, *Navicula pelliculosa* and *Scenedesmus quadricauda* excreted 6.73, 2.86 and 0.66 μmol dm^{-3} of complexing ligands respectively (determined by ion exchange MnO_2 method) to reduce the free cupric ion activity to $10^{-10.3}$ mol dm^{-3} and which were able to ameliorate the growth rate of *Chlorella* sp., when poisoned with copper sulfate (Van den Berg et al. 1979). McKnight & Morel (1979) found in 21 species of algae that blue-green algae released strong copper complexing agents ($^cK = 10^8$ to 10^{12}) in an amount of 5×10^{-7} mol dm^{-3}, and that eucaryotic algae released mostly weak ones ($^cK = 10^{0.5}$) in an amount of 10^{-6} mol dm^{-3}. This occured mainly during the stationary phase when significant amounts of dissolved organic matter were accumulated anyway. Ragan et al. (1979) showed that high-molecular-weight, non-dialyzable polyphloroglucinols from brown algae *Ascophyllum nodosum* and *Fucus vesiculosus* chelated copper 150 times stronger than manganese or cadmium. However, the

actual metal complexation in sea water, as described by Mantoura et al. (1978), is dependent on the stability constant of the appropriate complex, the pH of sea water, and the major ion concentration. According to their computed speciation model, only about 10% of copper was chelated in sea water, whereas in fresh water it could be more than 90%. The purpose of this investigation is to prove the relevance of phytoplankton blooms and the activity of heterotrophic microorganisms for the formation of organic copper complexes in natural sea water.
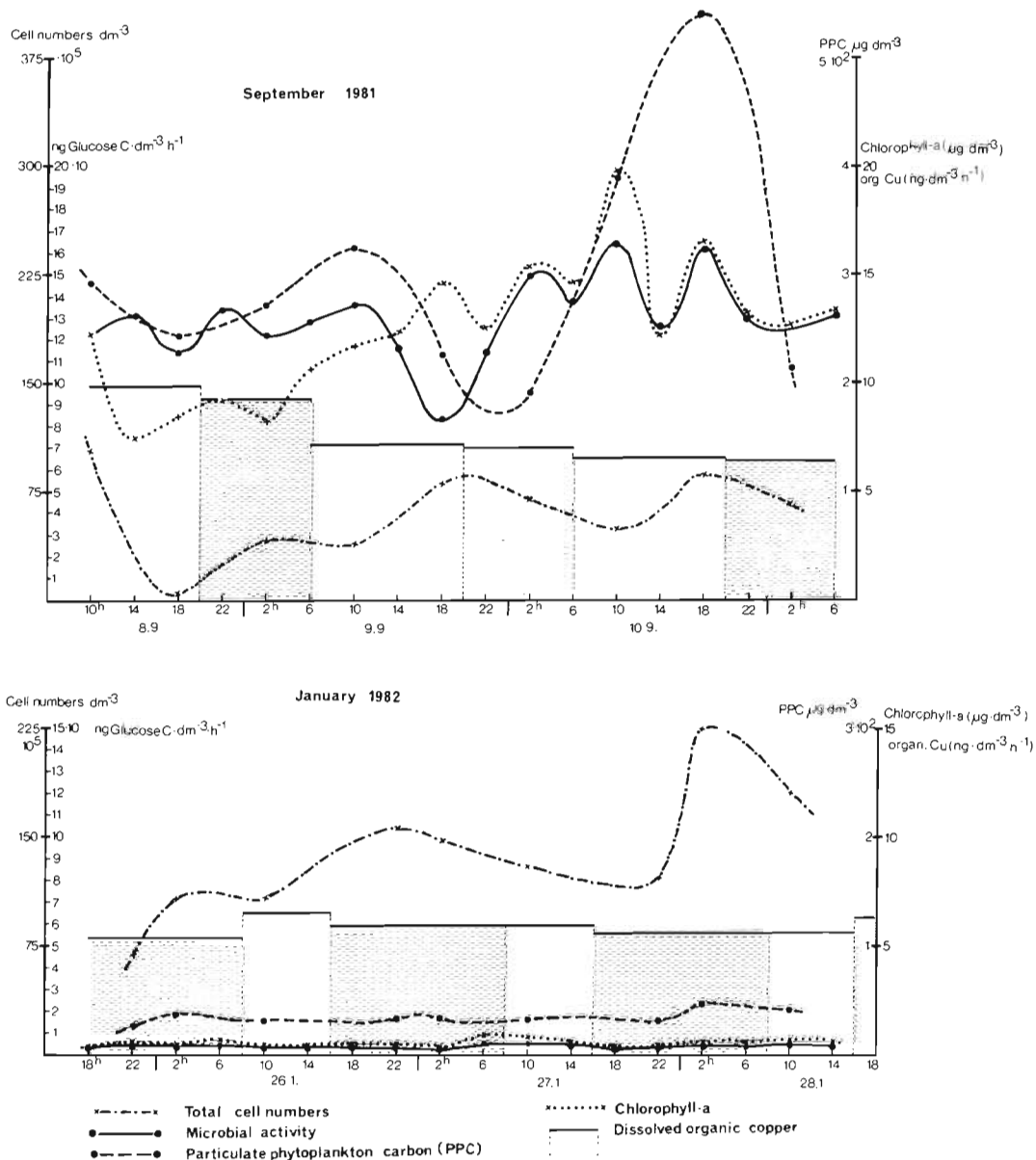
SAMPLING AND ANALYTICAL METHODS

An anchor station, located in coastal waters of the Kiel Bight, was run at 4 different seasons between fall 1981 and summer 1982. Sea water for analysis was pumped continuously aboard ship from a depth of 4 m and extracted by means of silanized porous glass. The extraction column was changed every 4 h. The apparatus as well as the sampling procedure for the determination of organic copper has been described in detail previously (Kremling et al. 1981). Before the sea water was filtered, discrete subsamples were drawn for

determination of chlorophyll *a*, microbial activity and
plankton composition. Subsamples for the determina-
tion of total dissolved copper and DOC were drawn
after filtration. Determination of organic copper in the
eluates and of total dissolved copper was performed by
atomic absorption on a Perkin-Elmer spectrophotome-
ter model 400 or 403, as described by Kremling et al.
(1981, 1983).

Chlorophyll *a* was extracted according to the proce-
dure described by Derenbach (1969) and separated

and measured by fluorescence absorption according to
Liebezeit (1980). Plankton cell counts were obtained
with an inverted microscope, and phytoplankton car-
bon (PPC) was derived by multiplying these cell counts
with carbon conversion factors according to Strath-
mann (1967) and Elder (1979). The method for determi-
nation of microbial activity is based on the procedure
described by Wright and Hobbie (1966), but modified
by Gocke (1977). DOC was measured by UV-destruc-
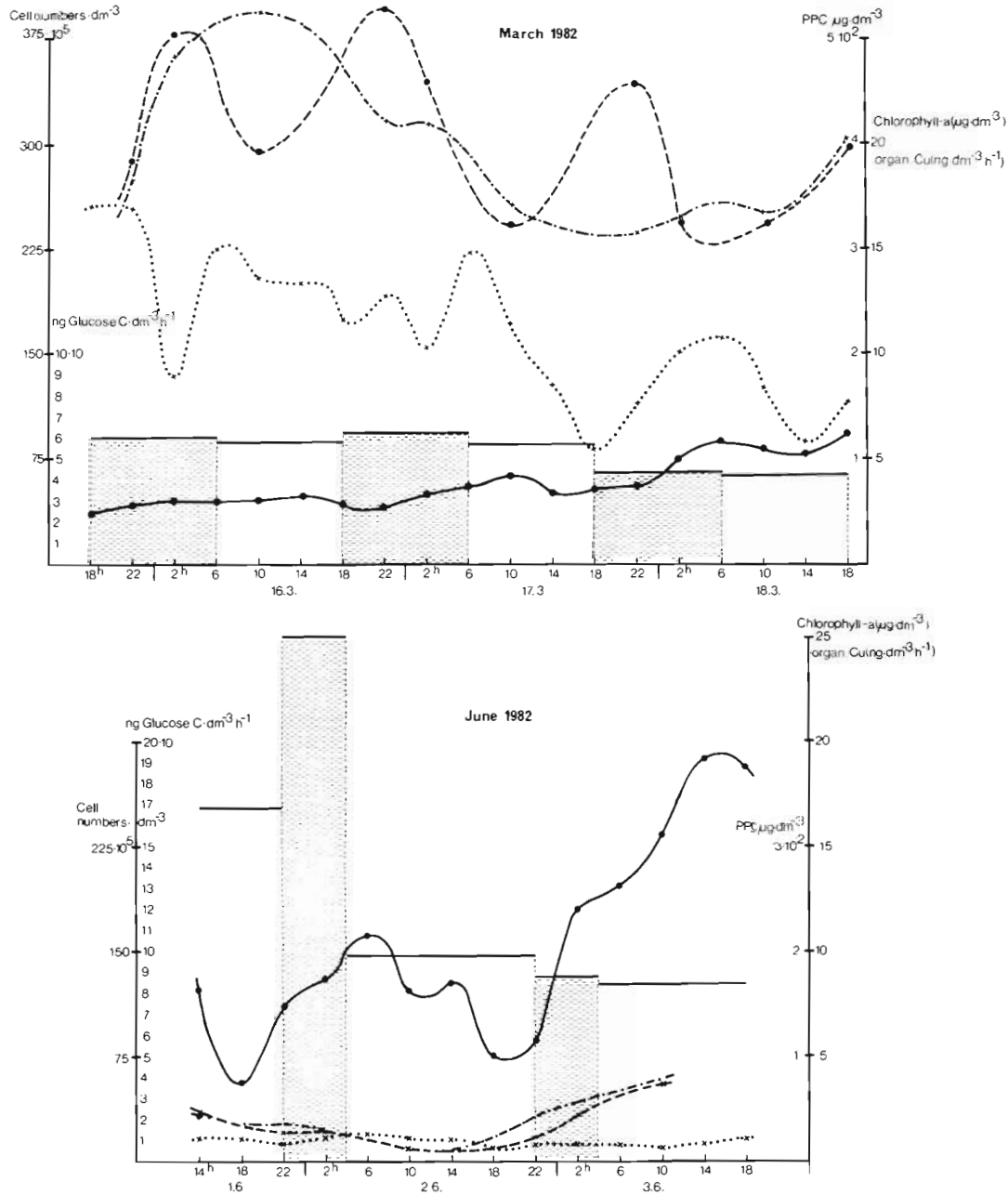tion (Schreurs 1978).

Fig. 2. Same as Fig. 1, but for anchor stations in March 1982 and June 1982

RESULTS

Biology

The biological situation during the winter station was characterized by a low and equally distributed chlorophyll *a* concentration, PPC content and microbial activity, mainly as a result of the low water temperature and short time of exposure to sunlight (Fig. 1 & 2). In March the high values of cell numbers, PPC content and of chlorophyll *a* concentration, slightly decreasing in the course of the station, probably indicate the logarithmic growth phase of a phytoplankton bloom. The occurrence of biogenic hydrocarbons in the particulate matter from the same station with its overwhelming content of all-cis-heneicosahexaene (HEH) is another good indicator for this phase of the phytoplankton bloom (Osterroht et al. 1983). Due to the continuing low water temperatures, and probably scarce supply of easily degradable compounds, microbial activity increased only to a level intermediate between that encountered in winter and summer.
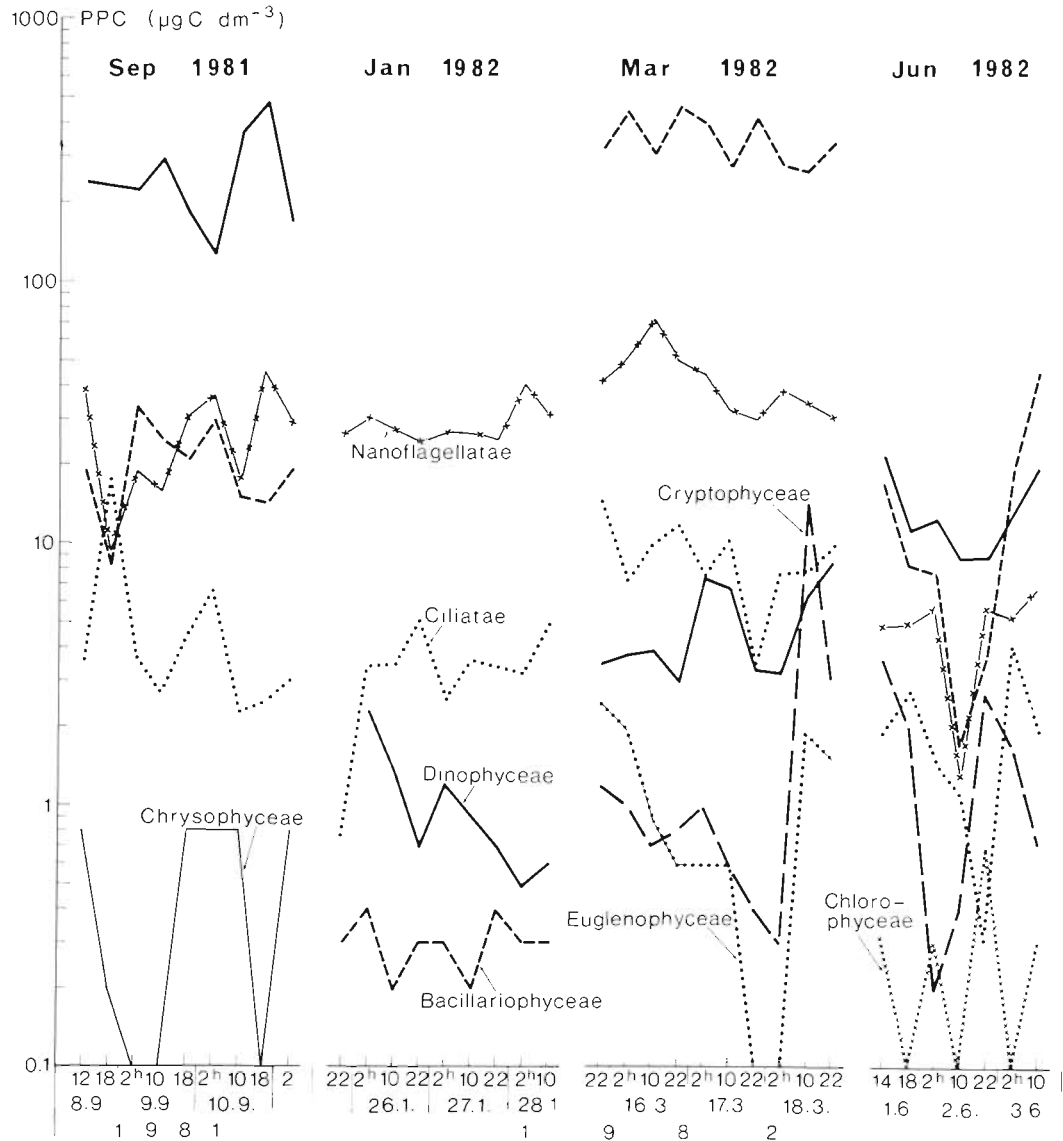
Fig. 3. Distribution of phytoplankton given as amounts of biomass

In June we found a typical summer post-bloom phase with low contents of PPC and chlorophyll a as well as low total cell numbers. On the other hand, we believe the high microbial activity to be a consequence of the elevated water temperatures and an accumulation of degradable organic substances, remainders from previously occurred phytoplankton blooms. The microbial activity was still high in autumn, but in contrast to the summer station the high content of PPC and chlorophyll a reflects a phytoplankton growth which is typical for this region in autumn.

The composition of phytoplankton (Fig. 3) is in good agreement with previous data from this area (Smetacek 1975) and shows significantly different phy-

toplankton distributions for every station. Zooplankton played an insignificant role at all times, its biomass being less than 10 % of that of the PPC. Our integrated DOC values showed a basic level throughout the year (Table 1). However, they were elevated during summer and autumn – a fact which could be expected as a consequence of the biological situation outlined above. Von Bröckel (1975) also found a uniform distribution of integrated DOC values in this area, with only small patches of higher amounts, although distinct phytoplankton blooms had occurred during this time. In this coastal area, changes of the generally high background concentrations of DOC as a direct response to biological events are aparently very rare.

Table 1. Concentrations of total dissolved copper, dissolved organic copper and DOC, given as mean values with standard deviation; number of samples in parenthesis

	Sep 1981	Jan 1982	Mar 1982	Jun 1982
Total dissolved copper (ng dm^{-3})	590 ± 51 (9)	650 ± 47 (9)	644 ± 92 (9)	786 ± 190 (6)
Dissolved organic copper (ng dm^{-3})	31 ± 8 (18)	23 ± 3 (17)	22 ± 5 (18)	48 ± 36 (12)
Percentage of organic copper	5.3	3.5	3.4	6.1
DOC (mgC dm^{-3})	2.88 ± 0.11 (17)	2.46 ± 0.13 (19)	2.43 ± 0.22 (19)	2.86 ± 0.15 (14)

Dissolved organic and total copper

During the winter station the concentration of dissolved organic copper was low and equally distributed (Fig. 1 & 2). As no variations in biological parameters were observed, one would not expected variations in the concentration of organic copper. Indeed, these could not be seen. The biological parameters had changed during the spring station, but no immediate change concerning the concentration of organic copper was seen in the course of this phytoplankton growth. The values remained at the same average level as during winter. Contrary to the expected rise, the concentration of the organic copper decreased slightly towards the end of the station, interestingly concomitant with the increase of microbial activity. This inverse variation could be seen more clearly at the summer anchor station. In summer, the concentration of organic copper is higher by a factor of 2 to 3 in comparison to the winter and spring stations. It also varied to a much higher degree. The concentration of organic copper decreased at the same time and to the same extent to which the microbial activity was increasing. In autumn, the concentration of organic copper was lower again than during summer and again diminished in the same way as the microbial activity increased. As already indicated for the spring station, there was no direct response of the organic copper concentration to the phytoplankton bloom.

Total dissolved copper values remained constant within the analytical precision of about ± 10 % at all anchor stations except in June where concentration and variation were slightly elevated (Table 1). These generally high values of total copper, compared to oceanic surface waters, also explain the low percentage of organic copper.

DISCUSSION

Determination of dissolved organic copper by means of adsorption to reversed-phase material has produced reasonable results (Kremling et al. 1981, 1983, Mills & Quinn 1981, Mills et al. 1982). The data of organic copper presented in this paper agree well with those found by Kremling et al. (1981, 1983) for other regions of the Baltic Sea; by Mills et al. (1982), for oceanic waters; and by Mackey (1983), for Port Hacking estuary water. They are, however, lower than the values found in Narragansett Bay by Mills & Quinn (1981).

The concentration of organic copper showed a relative constant background level throughout the year, with exceptions of elevated concentrations in summer and slightly higher ones in autumn. An influence of phytoplankton blooms within hours or days on the concentration of organic copper could not be detected by our type of experiment. No correlations exist between the biological parameters and organic copper concentration during spring and autumn when such biological events occurred. We only found an in general inverse response to the microbial activity in spring, autumn and – most pronounced – in summer.

As demonstrated, we failed to detect interrelations between the concentration of organic copper and phytoplankton activity. We believe that this can be explained as follows: In a previous study (Osterroht & Wenck 1983) we attempted to obtain some insight into the chemical structure of organic copper associates. When using copper as a tracer element in liquid-liquid chromatographic separation of DOC, the fractions with the highest copper content showed UV absorption characteristics quite similar to those reported in the literature for humic and fulvic acids. Besides shoulders in the region between 240 and 270 nm they exhibited a weak absorption band centered near 400 nm. In the present study, the eluates containing the organic copper associates showed also 2 maxima, at 345 nm and 400 nm. The sample-volume-normalized height of
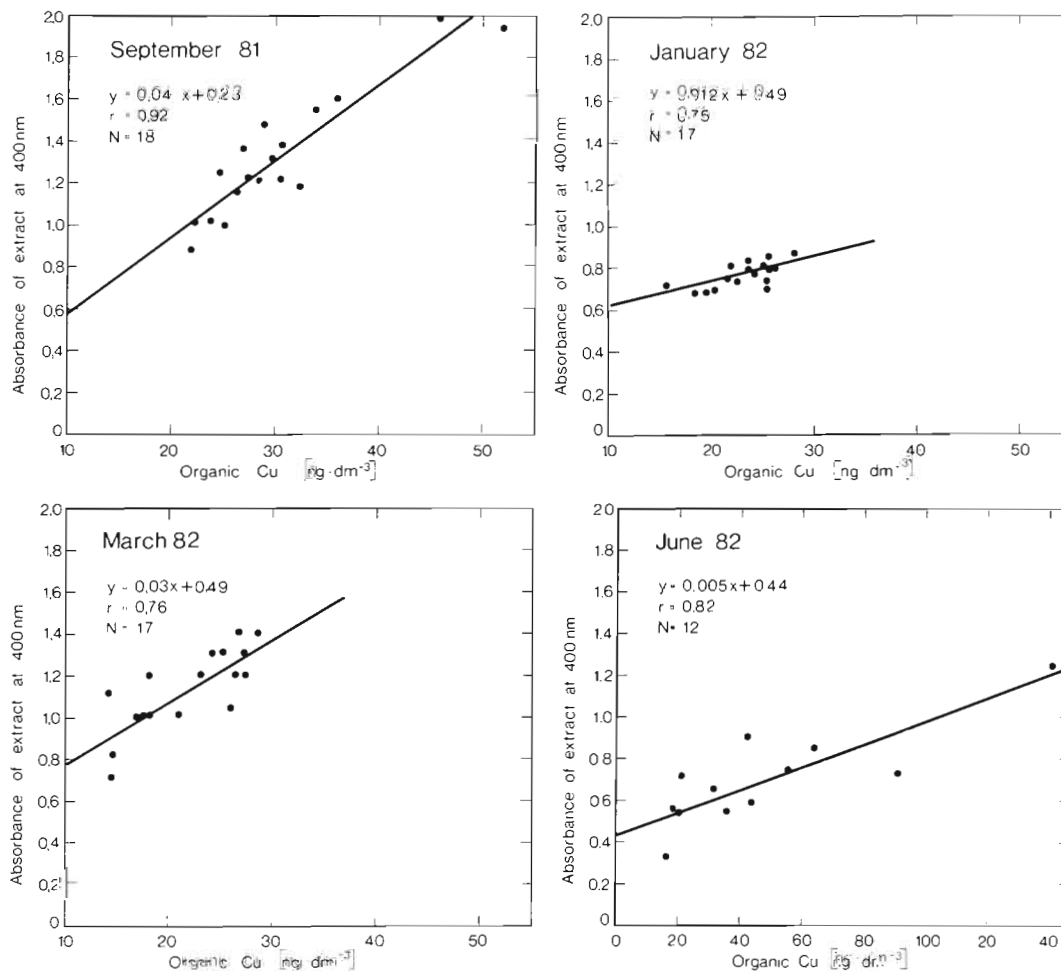
Fig. 4. Results of correlation analyses between sample-volume-normalized concentration of organic copper and absorbance of extract at 400 nm

absorbance at 400 nm correlates with the corresponding concentration of organic copper at all 4 anchor stations (Fig. 4), best so in autumn and summer. According to our previous study we interprete the absorbance as a measure of the concentration of humic acid type material within the DOC and the good correlations with the organic copper as a support for the suggestion, that part of this material is involved in the association with copper. This is in good agreement with results of Piotrowicz et al. (1983).

In regions with high primary productivity organic substances are often accumulated, especially during summer and autumn (Wangersky 1978, Wafar et al. 1984). This is indeed the case in our coastal area as shown by our DOC values. Together with the high and constant concentration of total dissolved copper this creates a background level of organic copper, which in consequence is also elevated during summer and autumn (Table 1). Transitory changes, as they might occur in response to phytoplankton activity, are probably only small compared to this background level.

They are therefore difficult to detect with our method of continuous sampling.

Although Anderson et al. (1984) used a different analytical approach, they too found at a coastal station that the concentration of copper-complexing ligands were not changed by actively growing phytoplankton populations.

Although there still is discussion about the extent, type and sense of extracellular products of phytoplankton as given in the reviews of Sharp (1977) and Fogg (1983), it is generally agreed that exudation accompanies cell growth. Organic ligands directly derived from phytoplankton activity and its resulting exudates are thought to be of low molecular weight and different in their chemical structure from humic acid type material. When looking for this exudate-type of copper associates it seems therefore necessary to separate them first from the bulk of residue organic matter in order to measure their concentrations and changes.

We conclude that humic and fulvic acids are the background material for the formation of organic cop-

per associates in sea water. More sophisticated methods are necessary to detect, in field studies, possible intermediate precursors of this stable metal associates, as they might result, for example, from phytoplankton activity.

LITERATURE CITED

Anderson, D. M., Lively, J. S., Vaccaro, R. F. (1984). Copper complexation during spring phytoplankton blooms in coastal waters. J. mar. Res. 42: 677–695

Berg, Van den, C. M. G., Wong, P. T. S., Chan, Y. K. (1979). Measurement of complexing materials excreted from algae and their ability to ameliorate copper toxicity. J. Fish. Res. Bd Can. 36: 901–905

Bröckel, von, K. (1975). Der Energiefluß im pelagischen Ökosystem vor Boknis Eck (westliche Ostsee). Dissertation, Universität Kiel

Derenbach, J. (1969). Zur Homogenisation des Phytoplanktons für die Chlorophyllbestimmung. Kieler Meeresforsch. 25: 166–171

Elder, L. (1979). Recommendations on methods for marine biological studies in the Baltic Sea, phytoplankton and chlorophyll. The Baltic Marine Biologists, Publication No. 5

Fogg, G. E. (1983). The ecological significance of extracellular products of phytoplankton photosynthesis. Botanica mar. 26: 3–14

Gocke, G. (1977). Comparison of methods for determining the turnover times of dissolved organic compounds. Mar. Biol. 42: 131–141

Kremling, K., Wenck, A., Osterroht, C. (1981). Investigations on dissolved copper-organic substances in Baltic waters. Mar. Chem. 10: 209–219

Kremling, K., Wenck, A., Osterroht, C. (1983). Variations of dissolved organic copper in marine waters. In: Wong, C.S., Boyle, E., Bruland, K. W., Burton, J. D., Goldberg, E.D. (ed.) Trace metals in sea water Plenum Press, New York and London, p. 609–620

Liebezeit, G. (1980). Chlorophyll *a* in marine phytoplankton: separation by HPLC and specific fluorimetric detection. J. high Resolut. Chromatogr. Chromatogr. Comm. 3: 531–533

Mackey, D. J. (1983) Metal-organic complexes in seawater – an investigation of naturally occuring complexes of Cu, Zn, Fe, Mg, Ni, Cr, Mn and Cd using high-performance liquid chromatography with atomic fluorescence detection. Mar. Chem. 13: 169–180

Mantoura, R. F. C., Dickson, A., Riley, J. P. (1978). The complexation of metals with humic materials in natural waters. Estuar. coast. mar. Sci. 6: 387–408

McKnight, D. M., Morel, F. M. M. (1979). Release of weak and strong copper complexing agents by algae. Limnol. Oceanogr. 24: 823–837

Mills, G. L., Hanson, A. K., Jr., Quinn, J. G., Lammela, W. R., Chasteen, N. D. (1982). Chemical studies of copper-organic complexes isolated from estuarine waters using C_{18} reverse-phase liquid chromatography. Mar. Chem. 11: 355–377

Mills, G. L., Quinn, J. G. (1981). Isolation of dissolved organic matter and copper organic complexes from estuarine waters using reverse-phase liquid chromatography. Mar. Chem. 10: 93–102

Osterroht, C., Petrick, G., Wenck, A. (1983). Seasonal variation of particulate hydrocarbons in relation to biological parameters. Mar. Chem. 14: 175–194

Osterroht, C., Wenck, A. (1983b). Chromatographic and optical properties of organic copper associates from Baltic waters. Fresenius Z. Analyt. Chem. 316: 572–574

Piotrowicz, S. R., Harvey, G. R., Young, M. S., Courant, R. A., Boran, D. A. (1983). Studies of cadmium, copper and zinc complexation by marine fulvic and humic materials in seawater using anodic stripping voltammetry. In: Wong, C. S., Boyle, E., Bruland, K. W., Burton, J. D., Goldberg, E.D. (ed.) Trace metals in sea water. Plenum Press, New York and London, p. 699–717

Ragan, M. A., Smidsrød, O., Larsen, B. (1979). Chelation of divalent metal ions by brown algal polyphenols. Mar. Chem. 7: 265–271

Schreurs, W. (1978). Automated colorimetric method for the determination of dissolved organic carbon in sea water by UV-destruction. Hydrobiol. Bull. 12: 137–142

Sharp, J. H. (1977). Excretion of organic matter by marine phytoplankton: do healthy cells do it? Limnol. Oceanogr. 22: 381–399

Smetacek, V. (1975). Die Sukzession des Phytoplanktons in der westlichen Kieler Bucht. Dissertation, Universität Kiel

Strathmann, R. R. (1967). Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. 12: 411–418

Wafar, M., Le Corre, P., Birrien, J.-L. (1984). Seasonal changes of dissolved organic matter (C, N, P) in permanently well mixed temperate waters. Limnol. Oceanogr. 29: 1127–1131

Wangersky, P. J. (1978). Production of dissolved organic matter. In: Kinne, O. (ed.) Marine ecology, Volume IV, Dynamics. Wiley, Chichester, p. 115–220

Wright, R. T., Hobbie, J. E. (1966). Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47: 447–464