GEOLOGICA CARPATHICA- SERIES CLAY S, 44, 1, BRATISLAVA, JUNE 1993
49 - 54

INVESTIGATIONS OF Cu-ADSORPTION ON SUSPENDED MATTER FLOCS FORMED BY BACTERIA AND CLAY MINERALS

ANNETTE ERNST[1] and NORBERT GREISER[2]

[1]Department of Geological Sciences, University of Greifswald, Friedrich-Ludwig-Jahn Str. 17a, O-2200 Greifswald, FRG
[2]Department of Botany, University of Hamburg, Ohnhorststr. 18, W-2000 Hamburg 52, FR

(Manuscript received February 5, 1993; accepted in revised form April 26, 1993)

Abstract: In first experiments different model systems with the two basic components, clay minerals and bacteria, were used to study the Cu-adsorption on the organic and inorganic components of river-suspended matter separately and in their combination. The formation of the floc structure, typical of river-suspended matter, developed owing to the bacteria overgrowth on the surface of the clay minerals and the following production of extracellular polymer substances (EPS). Higher protein and carbohydrate contents for the systems with the components of clay minerals and bacteria combined document a higher bioproduction than in the system with only bacteria. After 72 h the Cu quantity of the suspended matter flocs is at least twice as high as in the system only with bacteria. It proves the supporting effect of the clay minerals on the bioproduction as well as the dominant part of the organic matter as main pollutant adsorber in river systems.

Key words: river-suspended matter, model systems, clay minerals, organic matter, bacteria, Cu-adsorption, extracellular polymer substances.

Introduction

When investigating ecological aspects of transport of the suspended matter in rivers and estuaries, the question of the binding, transport and stay of environmental pollutants has to be solved.

It has been known for a long time that the cation exchange capacity (CEC) of organic matter (180 - 300 mval/100 g) (Helling et al. 1964) is essentially higher than the CEC of clay minerals (10 - 180 mval/100 g) (Guy & Chakrabarti 1976). Because of their high weight proportion in the suspended matter the main part of the pollutant binding was considered to be the mineralogical solid pools, above all the clay minerals (grain-size fraction $<2\,\mu m$ up to 50 % of the inorganic ingredients of the suspended matter).

Positive correlations of the pollutant contents of the suspended matter with organic carbon (C_{org}) or ignition loss (Förstner & Müller 1974; Neugebohrn et al. 1982; Brüggmann 1984; Guhr et al. 1986; Wilken & Wirth 1987), the statistical evaluation of chemical analyses (Koopmann 1989) and sorption experiments on river sediments (Tada & Suzuki 1982) as well as experimental studies in multi-chamber devises (Calmano et al. 1988) led to a fresh start in the way of thinking about the importance of the organic component in the suspended matter in relation to the binding of the pollutants. But owing to the complex description of the dynamics of the suspended matter under principal consideration of biological factors by Greiser (1988), it has been shown that organic matter is the dominant solid component (up to 95 vol%). These investigations really challenge the differential quantifying of the pollutant transfer between the organic/inorganic solids and the water to find some information about the remobilization and bioavailability of the pollutants bound to the suspended matter. The separation of the organic components from the inorganic components of the suspended matter without chemical changes is impossible. Therefore an attempt was made to duplicat and quantify the heavy metal transport processes of the river ecological system using models.

The basic idea is to carry out these investigations on "intact" suspended matter, i.e., without destruction of the suspended matter by selective extractions etc.. It will be realized by the specific production of suspended matter flocs with different mineralogical and organic components.

In the first experiment two basic components of the suspended matter of the Elbe river - *clay minerals* and *bacteria* - were combined in various attempts (model systems) with the heavy metal copper.

Methods of study

The investigations were carried out in 3 basic model systems:

a - *bacteria*

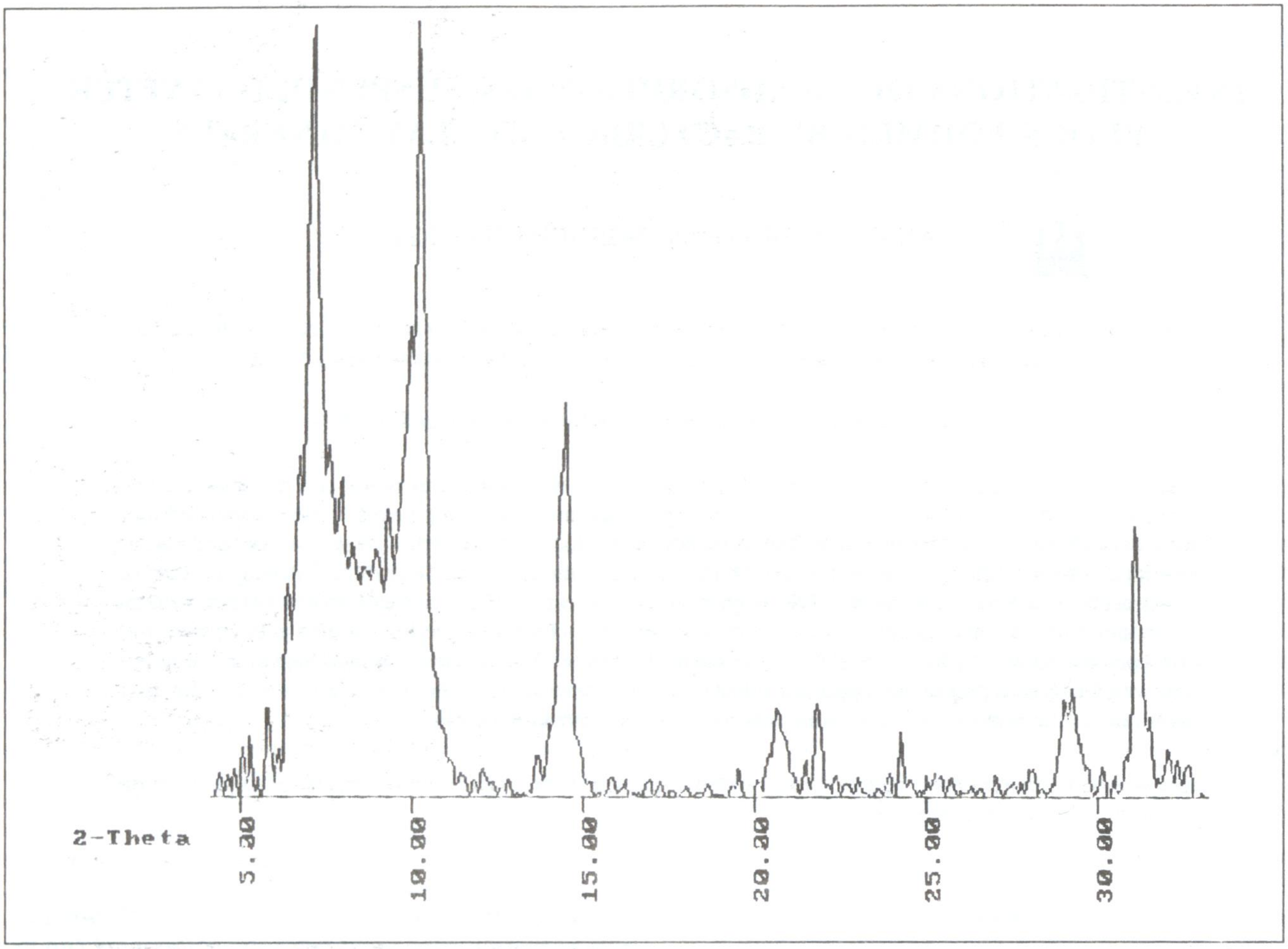

Fig. 1. X-ray diffraction pattern of the banded silt, fraction < 2 μm, air dried (Co-K_{α}1; 2θ step 0.05°; time 5s).

b - *bacteria* + *clay minerals*
c - *clay minerals* + formaldehyde
which were complemented by the systems:
d - *bacteria* + *clay minerals*, added in the stationary phase of bacteria growth
e - *clay minerals* + *bacteria*, added after 24 h
f - *bacteria* + *quartz sand grains*.

2 ml water of the Elbe river was shaken for 24 h at 25 °C in 250 ml sterilized culture medium (standard I - nutrient broth MERCK) to prepare the bacteria preculture. The grain-size fraction <2 μm of a banded silt (Late Weichsel Glacial Period) was used as the model substance "clay minerals". The clay mineral association of this banded silt is very similar to that of the Middle Elbe river. The respective model substances (2 ml bacteria preculture, 2 ml sterilized clay mineral suspension with concentration 1g/l; 1g quartz sand grains) were mixed with 250 ml culture medium diluted 1 : 10 according to the results of Schultze (1989). The systems were contaminated by addition of copper sulphate (suprapure MERCK) to a final concentration of 2 mg Cu/l. The system (c) *clay minerals* were mixed with formaldehyde to prevent any bacteria growth.

Each model system was probed after 1 h (lag-phase of bacteria growth), 4 h 30'(exponential phase of growth), 8 h 30'(stationary phase of growth), 24 h and 72 h.

The growth of the bacteria was recorded by measuring the:

1 - turbidity (photometric at 578 nm)
2 - protein content (photometric after Bradford 1976) and
3 - carbohydrate content (photometric after Liu et al. 1973).

The structure of the particles was studied by fluorescence or phase contrast microscopy on samples which were conserved with formaldehyde. The concentration of the particularly bound copper was estimated by AAS (PERKIN ELMER).

Results and discussion

The microscopic picture of the system (a) *bacteria* shows that the bacteria added to the systems (Fig. 1) remain free in suspension during the whole experiment (Fig. 2). At the moment of addition of the component clay minerals a flocculation appears (systems b, d, e) (Fig. 3). The formation of this floc structure, typical of river-suspended matter, developed as a result of the bac-

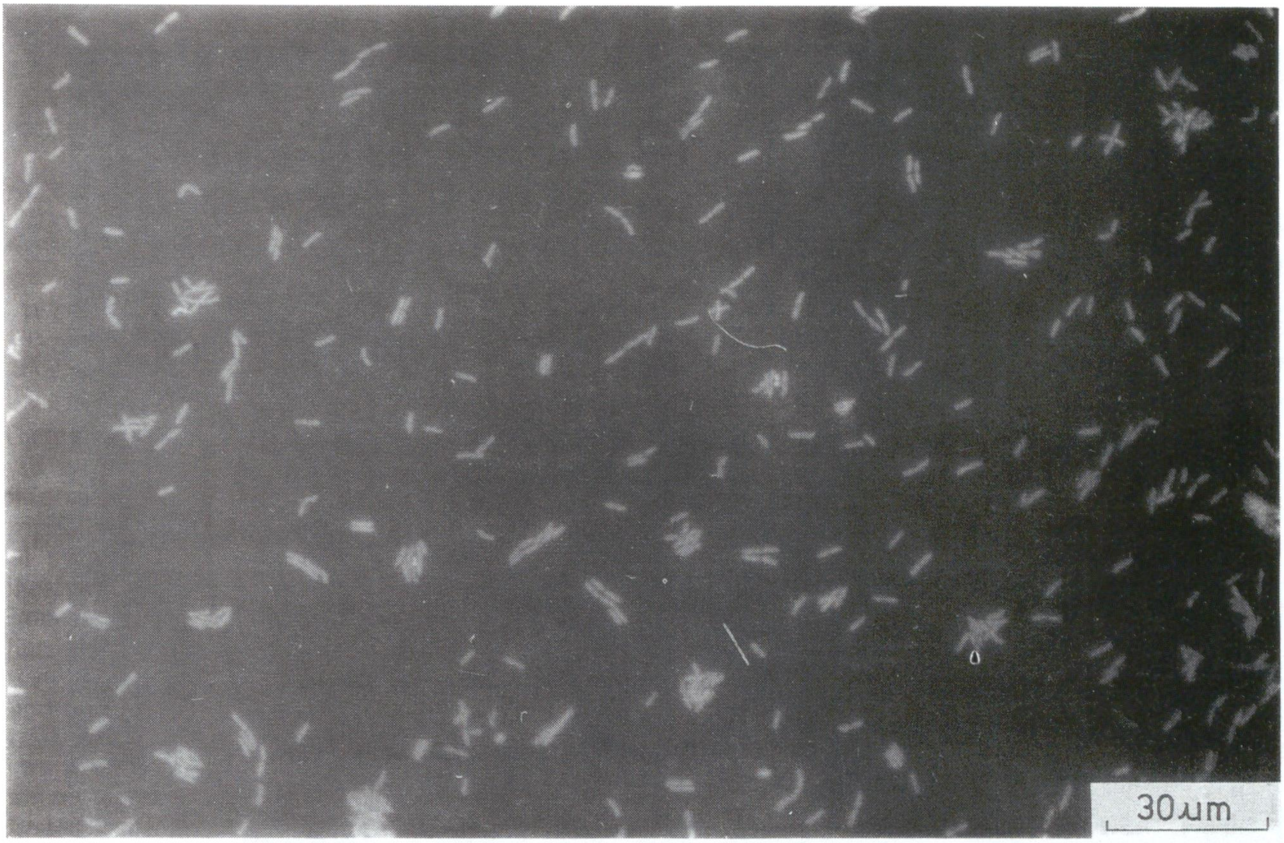

Fig. 2. 1 ml bacteria preculture in 250 ml culture medium coloured with DAPI + AO (DAPI=4'6-Diamidino-2-phenylindol; AO=Acridinorange).

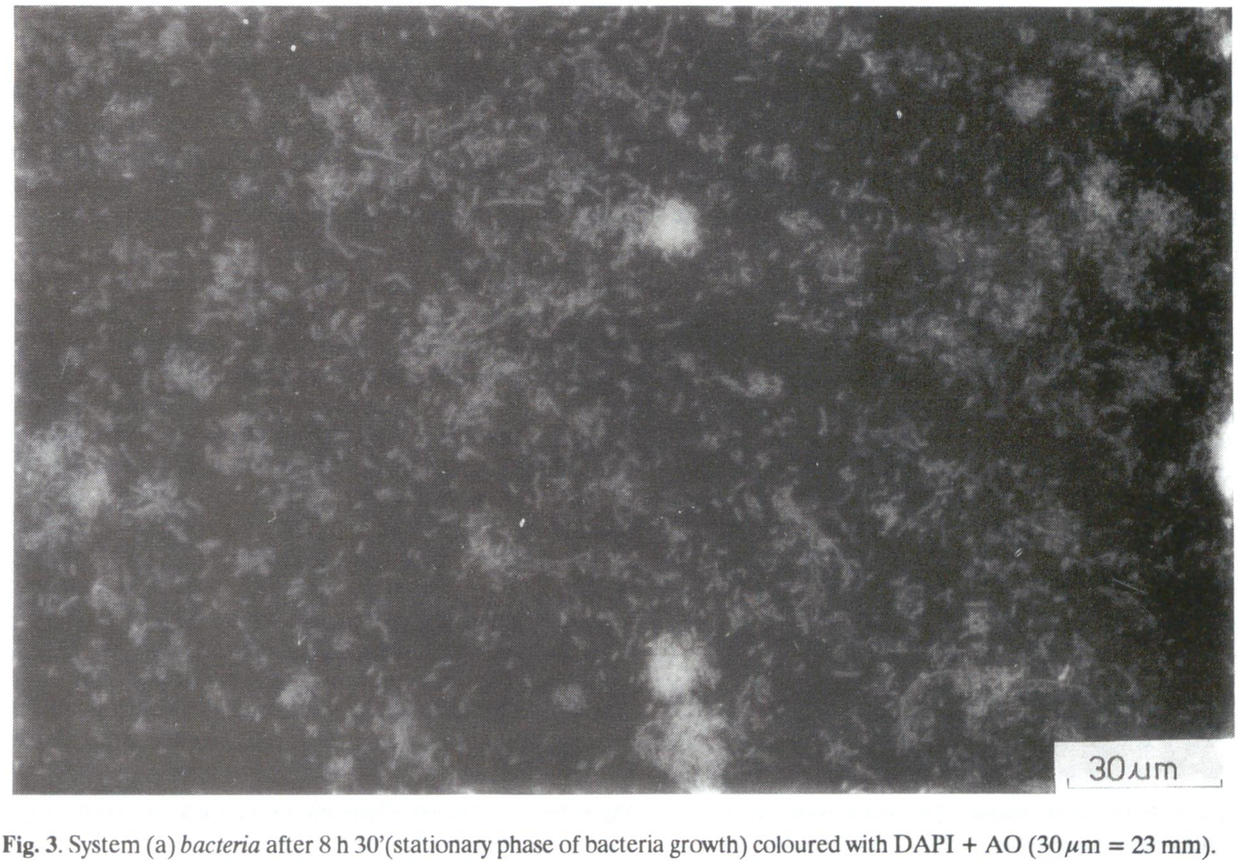

Fig. 3. System (a) *bacteria* after 8 h 30'(stationary phase of bacteria growth) coloured with DAPI + AO (30 μm = 23 mm).

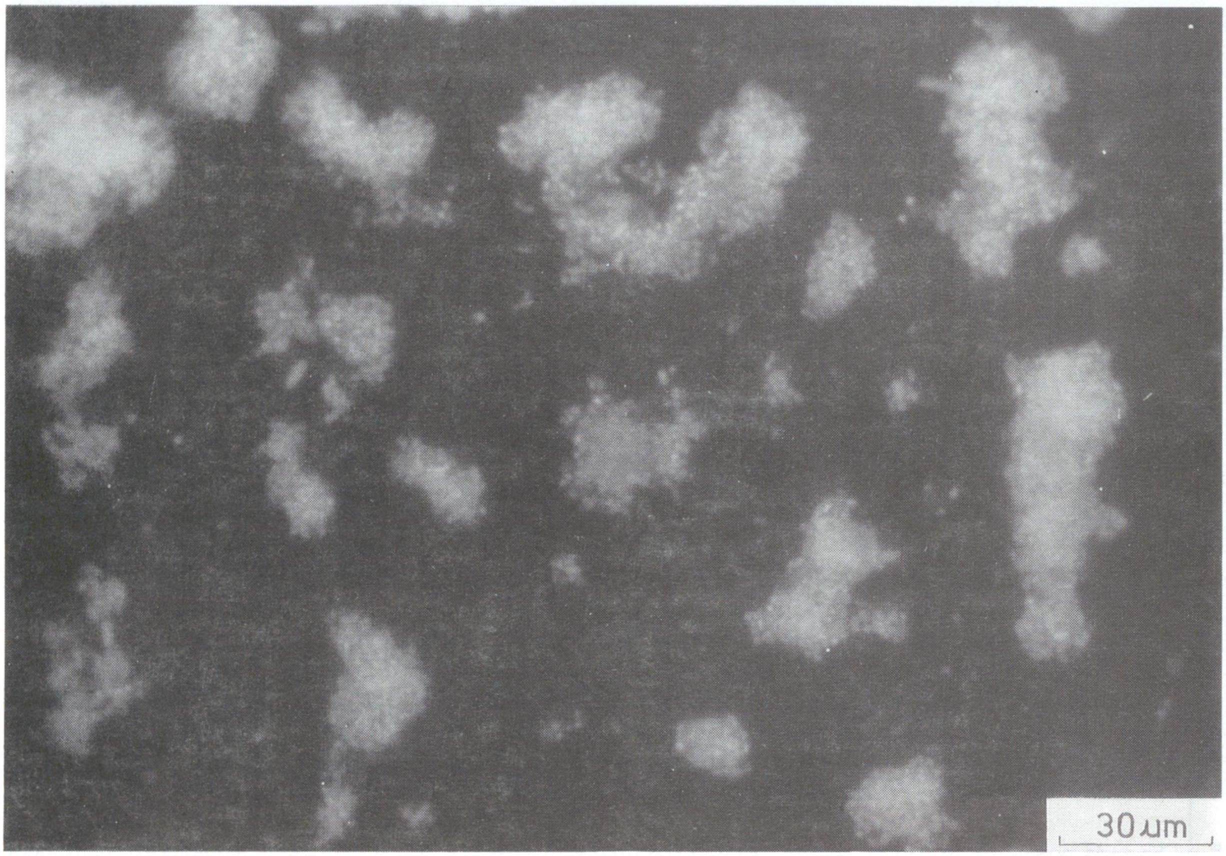

Fig. 4. System (b) *bacteria + clay minerals* after 8 h 30'(stationary phase of bacteria growth) coloured with DAPI + AO.

teria overgrowth on the surface of the clay minerals and the following production of extracellular polymer substances (EPS), which appear as voluminous slimy secretions of the bacteria. The microscopic picture is clearly controlled by the organic substances.

The evolution of the turbidity of the suspension in the system (a) *bacteria* is represented in Fig. 4. After 72 h the protein and carbohydrate contents estimated simultaneously with the microscopic investigations show generally higher amounts for the systems (b), (d) and (e) with the components *bacteria and clay minerals* than for the system (a) *bacteria*, indicating a higher bioproduction in the systems (b), (d) and (e) (Figs. 5, 6). The supporting effect of the clay minerals on the bioproduction is em-

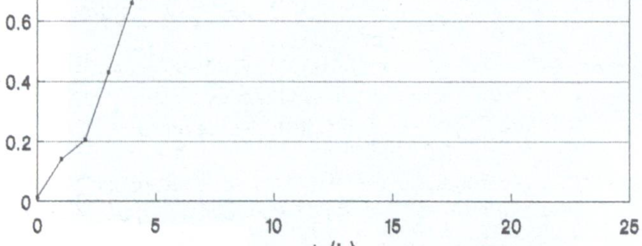

Fig. 5. Evolution of the turbidity of the suspension, system (a) *bacteria*, as a function of time.

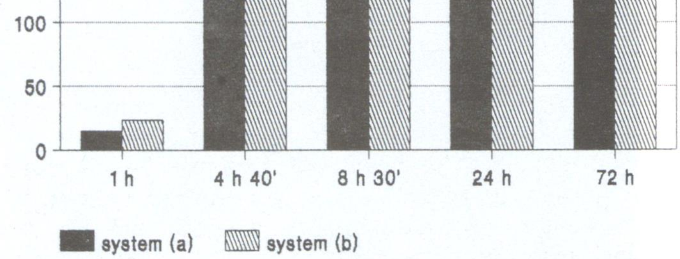

Fig. 6. Protein content, documenting the bacteria growth (mg/l)

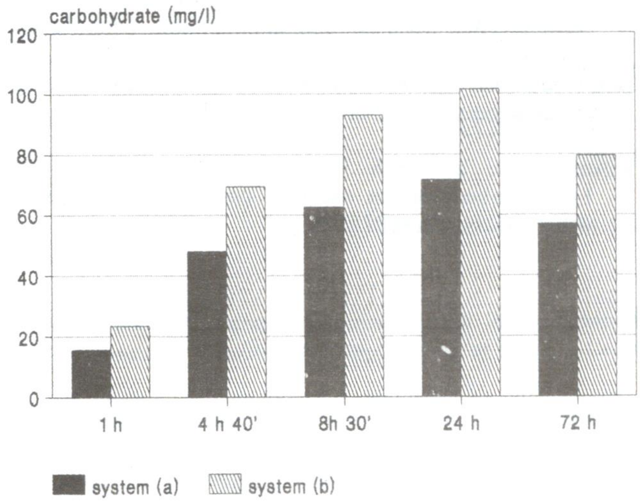

Fig. 7. Carbohydrates content, documenting the bacteria growth (mg/l).

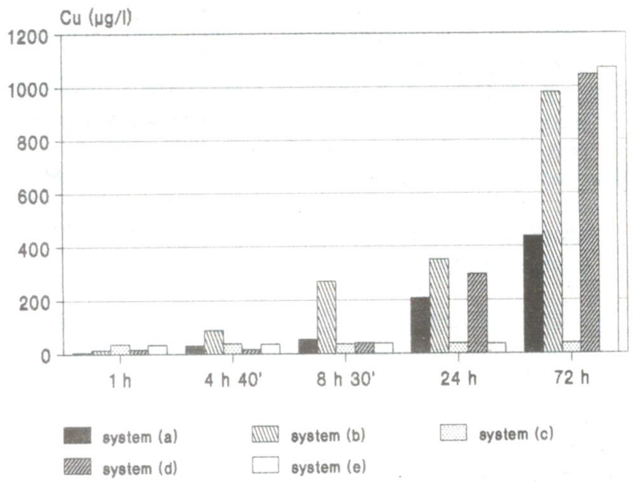

Fig.8. Concentration of particularly bound Cu (µg/l).

phasized by the aspect that no flocs were formed in the system (f) *bacteria + quartz sand grains*.

The particularly bound Cu estimated for the systems (a), (b), (c), (d) and (e) is shown in Fig. 7. It is clearly shown that after 72 h the Cu quantity of the suspended matter in the systems (b), (d) and (e) with the components *bacteria and clay minerals* which produced slimes is at least twice as high as in system (a) with *bacteria* only. No Cu-adsorption could be ascertained in system (c) *clay minerals*.

If one takes into account the high CEC of the organic matter this process can only be explained by:

1 - the higher bioproduction in systems of the components *bacteria and clay minerals* combined and

2 - the produced EPS which contain anionic groups (saccharine acids). The EPS can act as an excellent ion exchanger. In contrast to the particularly bound Cu, which doubled in the systems (b), (d) and (e), the quantity of protein and carbohydrates was not doubled. It can be treated as a sign of the important role of the EPS.

Nelson et al. (1981) and Schultze (1989) found out that this mechanism of binding is only a surface reaction, i.e., a result of physicochemical processes. There are no signs of an active, energy-binding process.

According to the findings of Remacle & Houba (1984) similar results are to expected for the heavy metal Cd.

Conclusion

Even if the clay minerals should prove to be unimportant as direct adsorbers of the pollutants in the rivers, their function as an overgrowth medium for microorganisms and their influence on the growth of microorganisms with subsequent EPS-production must be seriously taken into consideration, with the processes of the interaction between the clay minerals and the microorganisms in fluviatile systems still being unknown.

Acknowledgement: We thank Dr. H. Harms and his colleagues (University of Hamburg, Department of Botany) for giving us the opportunity of conducting these investigations in their laboratories.

References

Bradford M., 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proteine dye binding. *Anal. Biochem.*, 72, 385 - 389.

Brüggmann L., 1984: Bericht über potentielle Schadstoffe im Wasser und Sediment der Elbe vom 27.8.1984. Internal report, Institut für Wasserwirtschaft, Rostock-Warnemünde.

Calmano W., Ahlf W. & Förstner U., 1988: Study of metal sorption/desorption processes on competing sediment components with multi-chamber device. *Environ. Geol. Water Sci.*, 11, 77 - 84.

Förstner U. & Müller G., 1974: Schwermetalle in Flüssen und Seen. *Springer Verlag*, Berlin-Heidelberg-New York.

Greiser N., 1988: Zur Dynamik von Schwebstoffen und ihren biologischen Komponenten in der Elbe bei Hamburg. *Hamb. Küstenforschung*, 45, 1 - 170.

Guhr H., Rudolpf G., Spott D. & Weber E., 1986: Aufklärung der Selbstreinigungsvorgänge in der Elbe. Internal report. Institut für Wasserwirtschaft, Magdeburg.

Guy R. D. & Chakrabarti C. L., 1976: Studies of metal-organic interactions in model systems pertaining to natural waters. *Can. J. Chem.*, 54, 2600 - 2611.

Helling C. S., Chesters G. & Corey R. B., 1964: Contribution of organic matter and clay to soil cation-exchange capacity as affected by pH and the saturating solution. *Soil Sci. Soc. Am. Proc.*, 28, 517 - 520.

Houba C. & Remacle J., 1984: Removal of cadmium by microorganisms in a two-stage chemostat. *Appl. Environ. Microbiol.*, 47, 1158 - 1160.

Koopmann Ch., 1988: Röntgendiffraktometrische und chemische Untersuchungen an Schwebstoffen aus der Unterelbe. Diss. Univ. Hamb., GKSS-report 89/E/15.

Liu D., Wong T. S. & Dutka B. J., 1973: Determination of carbohydrate in lake sediment by modified phenol-sulphuric acid method. *Water Res.*, 7, 741 - 746.

Nelson P. O., Chung A. K. & Hudson M. C., 1981: Factors affecting the fate of heavy metals in the activated sludge process. *J. Water Pollut. Control Fed.*, 53, 1323 - 1333.

Neugebohrn L., Decke U. & Nowak K., 1982: Untersuchungen zur Schwermetallkontamination von Sedimenten einiger ausgewählter Becken des Hamburger Hafens. *Hamb.Küstenforschung*, 41, 21 - 42.

Schultze S., 1989: Kinetik bakterieller Kupferbindung an Schwebstoffen der Elbe und der Nordsee. Dipl. Arbeit Univ. Kiel.

Tada F. & Suzuki S., 1982: Adsorption and desorption of heavy metals in bottom mud of urban rivers. *Water Res.*, 16, 1489 - 1494.

Wilken H.-D. & Wirth H., 1987: The adsorption of Hexachlorbenzene on naturally occuring adsorbents in water. GKSS-report 87/E/26.
