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# Effects of Heavy Metals in Combination with NTA, Humic Acid, and Suspended Sediment on Natural Phytoplankton Photosynthesis

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In studies of effects of heavy metals on phytoplankton most experiments are conducted with monocultures in artificial or natural media. The response is measured in growth or in the intensity of biochemical processes. We have adopted the method described by GÄCHTER (1976) using natural phytoplankton communities and measuring the response of heavy metal addition in photosynthesis.

Our aim was to evalute the acute effects of the metals Cu, Cd, Pb, Zn, and Hg separately and in combination on a natural phytoplankton community under different experimental circumstances. These circumstances are thought to simulate various conditions which may occur in a lake. Addition of a dilute suspension of sediment corresponds to resuspension of sediment in shore-near areas during periods of heavy wave action. Addition of bog water increases the content of natural chelators (humic acid). Additions of NTA simulate a situation which may occur if this synthesic chelator is used as a substitute for phosphorus in detergents.

The reported experiment was conducted in coordination with a research program on the eutrophication and contamination of Lake Tyrifjord, Norway.
Lake data: Volume 13.8 x $10^9$ $m^3$, area 136 $km^2$, maximum depth 296 m. The lake is still oligotrophic but significant signs of eutrophication have recently been identified (LANGELAND 1974, ROGNERUD 1975).

Until 1970 the lake was seriously contaminated by mercury from wood products industry.

## MATERIALS AND METHODS

The water used for bioassays: Some biological and chemical data for the used surface water from Lake Tyrifjorden are listed in Table 1.

An enrichment procedure by ion-exchange of the metals (ABDULLAH & ROYLE 1971) was followed by AA for Cu, Cd, Pb, and Zn. Total amounts of Hg were determined by cold vapour flameless AA.

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The phytoplankton was composed mainly of diatoms: *Tabellaria flocculosa* (53% of volume), *Synedra* sp. (13%), and *Asterionella formosa* (7%). The other most important species were *Cryptomonas* spp. *Rhodomonas minuta* var. *lacustris*, *Dinobryon divergens*, small species of chryptomonades, *Gymnodinium* sp:, and *Mallomonas* sp.

TABLE 1

Biological and chemical data for surface water from Lake Tyrifjord August 1979, used for bioassay.

| | | |
|---|---|---|
| Primary production during standard incubation | | 4.1 mg C $m^{-3}$ $h^{-1}$ |
| Chlorophyll a | | 3.8 mg $m^{-3}$ |
| Phytoplankton volume | | 0.22 $mm^3$ $l^{-1}$ |
| pH | | 7.0 - 7.3 |
| Ca | | 4.2 mg $l^{-1}$ |
| Alkalinity | | 0.15 meq $l^{-1}$ |
| Turbidity | | 0.2 F.T.U. |
| Colour | | $<$ 5 mg Pt $l^{-1}$ |
| Particulate carbon | | 235 µg $l^{-1}$ |
| Particulate nitrogen | | 35 µg $l^{-1}$ |
| Particulate phosphorus | | 2.8 µg $l^{-1}$ |
| Dissolved organic carbon | | 4.0 mg $l^{-1}$ |
| Ion-exchangeable metals | Cu | $1.5 \cdot 10^{-8}$ M |
| | Cd | $2 \cdot 10^{-9}$ M |
| | Pb | $5 \cdot 10^{-9}$ M |
| | Zn | $1 \cdot 10^{-7}$ M |
| Total Hg | | $2.5 \cdot 10^{-10}$ M |

Experimental procedure: The procedure was principally the same as described by GÄCHTER (1976). The photosynthetic activity was measured by addition of $^{14}C$-bicarbonate and incubation for 20 hours at *in situ* temperature 16° C and photosynthetic active radiation 200 µE/$cm^{-2}$ $s^{-1}$. Larger forms of zooplankton were removed by filtering through a zooplankton net before incubation. The total volume of the incubation bottles was filtered through 0.45 µm membrane filters, and the radioactivity was measured by liquid scintillation. Added concentrations of heavy metals were the same as used by GÄCHTER (1976, Table 3).

Four experimental series were run:

Exp. I : natural water + metals
Exp. II : natural water + metals + sediment
Exp. III: natural water + metals + humus
Exp. IV : natural water + metals + NTA

In Exp. II a slurry of surface sediments taken from 5 m depth near the shore was added, giving a final concentration of 7.6 mg dry sediment $l^{-1}$ and turbidity 0.64 F.T.U. The sediment was mainly composed of fine clay particles. The loss on ignition was 15%.

In Exp. III the addition of bog water caused an increase in water colour to 12 Pt-units. Dissolved organic carbon increased from 4.0 to 5.6 $mg \cdot l^{-1}$.

In Exp. IV a NTA (nitrilotriacetic acid) solution was added to give a final solution of 0.2 $mg \cdot l^{-1}$.

## RESULTS

A graphical presentation of the results of the bioassays is given in fig.1. In each experiment four reference samples were given the same treatment as the test samples except for the addition of metals. Their mean uptake of $^{14}C$ is set to 100%. The reference values were equal in Exp. I, II, and IV, while sediment addition in Exp. III increased the uptake with 19%.

In Exp. I metal addition gave reduction in $^{14}C$ uptake depending on the metal species and concentration. The reduction in uptake with increasing concentration is nearly linear in double log coordinate systems. In Exp. II and III $^{14}C$ uptake at the same metal concentration was greater than in Exp. I. For Zn, Pb, Cu and the metal mixture, addition of humic acid gave the greatest reduction in metal effect, while addition of sediment gave the greatest effect for Hg. For Cd neither humic acid nor sediment had significant effects.

In Exp.IV addition of NTA eliminated the effects of all tested concentrations of Cd, Pb, and Cu, and all but the greatest concentration of Zn. For Hg and the metal mixture a reduction in $^{14}C$ uptake compared with Exp. I was observed.

## DISCUSSION

Exp. I gave this order of metal toxicity towards natural phytoplankton:

$$Hg > Cu > Cd \geqslant Pb > Zn$$

Except for opposite order of Pb and Zn, this result agrees with GÄCHTER (1976) and with the general knowledge of metal toxicity. Reduction in photosynthetic activity was detectable for all concentrations of every metal tested.

The lowest concentrations were only a few times higher than the natural ones for Cu (4 x), Pb (10 x), and Zn (6 x), but considerably higher for Cd (25 x) and Hg (20 x). When all metals were added simultaneously the response was significantly increased in relation to the single metal additions. Metals occurring together in mixtures may have synergistic or antagonistic effects on algal growth (HUTCHINSON and STOKES 1975, CHRISTENSEN and SCHERFIG 1979). WONG et al. (1978) tested metal concentrations recommended as objectives on algae. While the individual metals did not have harmful effects, they became very toxic when added together. The increased toxicity which is obtained when several metals are combined is obviously a result of synergism. At present, not much is known about which metals will act synergistically to different algae.

It seems, however, that there is a great probability, that harmful effects will arise when a lake is exposed to increased concentrations of several heavy metals.

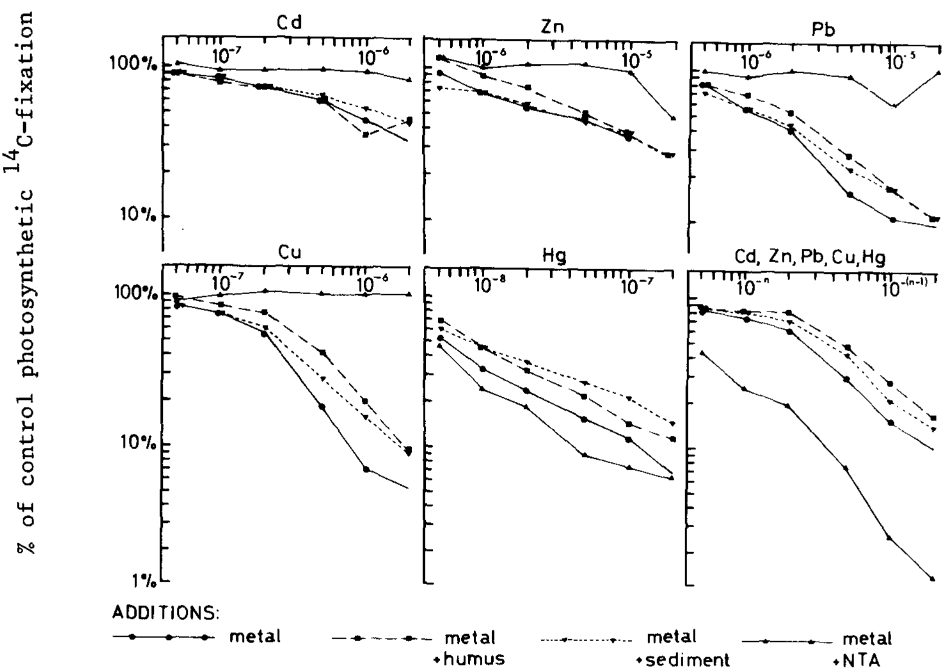


Fig.1. Reduction in photosynthetic $^{14}$C-fixation with various molar concentrations of heavy metals. Metal mixture - Zn, Pb: n = 7, Cd, Cu: n = 8, Hg: n = 9.

Addition of suspended sediment alone to lake water increased the photosynthesis about 19%. This effect may have several causes, including increased level of inorganic and organic nutrients and increased microbiological activity as well as production by benthic algae. The effect of heavy metals in water enriched with natural sediment was to reduce the photosynthesis, but the reduction was less than in the control without sediment.

RAMAMOORTHY and RUST (1978) found the following order of binding strength between river sediment and heavy metals: Hg $>$ Pb $>$ Cu $>$ Cd. Our results are in good agreement with this, since the sediment apparently had greatest effect on the Hg-toxicity and least on Cd- and Zn-toxicity. The metal binding capacity for sediments increases with decreasing grain size and increasing organic content (RAMAMOORTHY and RUST 1978). According to FRENET-ROBIN and BOITEAU (1977), mercury ions are adsorbed at rates near the exchange capacity of clays. When adsorbed to sediment particles, the metals are probably unavailable for algae, although mercury has been shown to become desorbed by bacterial activity (RAMAMOORTHY et al. 1977). The added amount of sediment was however not sufficient for detoxification, not even for the least concentrations.

Increase in the water's concentration of dissolved organic matter in the form of humus had no observable effect on the photosynthesis. In combination with heavy metals it led to partial detoxification of Cu, Zn, Pb, Hg, and the metal mixture, while the effect on Cd was uncertain. The binding mechanisms for humus water are comprehensive and may include adsorption, ion-exchange, and complexation (GJESSING 1976) Interaction between naturally occurring organic substances and copper has been shown to reduce the toxicity of the metal (DAVEY et al. 1973) In brown-water lakes humus may bind $Cu^{++}$ so effectively that addition of $Cu^{++}$ will increase the photosynthetic rate (STEEMANN NIELSEN and BRUUN LARSEN 1976). It is only the ionic form of the metal that is available to the algae and which may be harmful. Similar effects as for copper are to be expected for other metals because of the great complexation capacity of humus and its stability to degradation.

Addition of NTA to natural lake water had no observable effect on the photosynthesis. In water enriched with heavy metals the effect depended on the metal species present. NTA in moderate concentrations is considered as non-toxic (see for example the review by MOTTOLA (1974)). On the other hand, it may affect the availability of metals to the phytoplankton. Reduction in the availability of essential trace metals may thus limit algal growth (BURGI 1974). Like other chelators, NTA may also reduce the toxic effects of metals which are harmful only in their ionic form.

In our experiments, NTA led to detoxification of all tested concentrations of Cu and Cd. This was also the result for Zn and Pb less than $10^{-5}$ M. For the two latter metals, the greatest concentrations exceeded the complexing capacity of the NTA concentration used (ca $10^{-6}$ M).

Therefore, the metal-NTA complexes of Cu, Cd, Zn, and Pb do not affect the algal metabolism in short time experiments.

In water enriched with Hg and the metal mixture the effect of NTA was the opposite. The metal-NTA complex was more toxic than the ionic form of the metal. EISLER et al. (1972) tested the toxicity of NTA-complexes of Cd and Hg on fish. For Cd the toxicity decreased with increasing NTA concentration, while the results for Hg were inconclusive. For mammals the toxicity of mercury compounds is not reduced by NTA, and increase in teratogenous effects are observed (HAMMOND 1971, WRIGHT 1972). In algae, an effect of NTA complexation of $Hg^{++}$ may be that the metal is more easily taken into the cells, and therefore, like other organic mercury compounds, becomes toxic in lower concentrations than in the inorganic form.

The most pronounced effect of NTA was in combination with the metal mixture. This cannot be caused by the Hg-NTA complex alone since Hg was ten times less concentrated than in the assays with only Hg and NTA. A strengthening of the synergistic effects as a result of the increased Hg-toxicity seems to be the explanation.

It should be pointed out that the bioassays give only the acute toxicity which may be different from the results of long term incubations. With time it is probable that natural chelators excreted from the algae will complex the metals and make them unavailable. Natural phytoplankton may also adapt to increased metal concentrations and thrive well after some time. For mercury a difficulty with long-time experiments is that mercury may disappear from the vessels in which the algae are grown (ZINGMARK and MILLER 1972). The apparent adaptation to increased concentrations of mercury which has been reported by several authors may therefore be doubtful.

## CONCLUSIONS

The tested heavy metal concentrations:
Cd, Cu $\geq 5 \cdot 10^{-8}$M, Zn, Pb $\geq 5 \cdot 10^{-7}$M, Hg $\geq 5 \cdot 10^{-9}$M, and a mixture with the concentrations of each metal ten times more dilute, had inhibitory effects on the photosynthesis of phytoplankton from Lake Tyrifjorden. A moderate increase in the water's turbidity or colour reduced the biological availability of the metals and thus their effect on the photosynthetic rate. The increase in anthropogeneous metal load which has taken place in most watercourses may thus be counteracted by the natural influence of dissolved organic matter and by processes in lakes and rivers causing increased turbidity.

NTA led to detoxification of Cd, Zn, Pb, and Cu while Hg and the metal mixture became more toxic. An increase in NTA concentration may be the result if this substance comes into use as a substitute for phosphorus in detergents. In Lake Tyrifjorden this may have some unwanted implications since the sediments in the deeper part of the lake are severely contaminated by mercury (HONGVE and SKOGHEIM unpubl.), and NTA may release this as an NTA-Hg complex to the water (CHAU and SHIOMI 1972). NTA complexations of mercury adsorbed to the sediment may thus give a new and quantitatively important contribution to the internal cycle of mercury in the lake. It will also give directly, without microbiological methylation, a substance which may affect the plankton photosynthesis at a concentration which is only a little higher than the present concentration of mercury in the water.

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