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Development of Marine Periphyton under Mercury Stress in a Controlled Ecosystem Experiment

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The use of communities of benthic algae in the estimation of biological water quality is well-known (FJERDINGSTAD 1950, SLÄDECKOVA and SLÄDACEK 1963, MCLEAN and BENSON-EVANS 1974, VAN DAM 1974) and has a long history (BUTCHER et al. 1931, BUTCHER 1940). Because of many practical advantages (homogeneity, known age of the algae, ease of handling) the use of artificial substrates in the study of periphyton is widespread (BUTCHER 1940, CASTENHOLZ 1961, SLÄDECKOVA and SLÄDACEK 1963, TIPPETT 1970). Experimental studies to evaluate the impact of various chemicals on the periphyton are rather scarce. Results have been published from freshwater research (EVANS and MARCAN 1976, WILLIAMS and MOUNT 1965, SIGMON et al. 1977) and from a marine environment (VAN RAALTE et al. 1976).

Our laboratory is engaged in studies on the ecotoxicology of chemicals and tries to bridge the gap between laboratory and field conditions by conducting experiments with natural Dutch coastal plankton communities enclosed in large plastic bags, in which the influence of pollutants is investigated (KUIPER 1977a, b, cf. MENZEL and CASE 1977).

In the experiment reported here, in which mercury was added to the bags, substrates of glass and polyethylene were exposed in the water and the development of the biomass and the species composition of the periphyton was measured.

MATERIALS AND METHODS

The construction of the bags and the operation procedures have been described by KUIPER (1977a). The experiment started on 29 March (called day 0) 1976. Six bags (depth 3 m, contents 1400 l) were filled simultaneously. The mercury(II)chloride was added to all but two control bags (bags Ca and Cb) using the same method as KUIPER (1977b). The initial concentration in the treated bags was 0.5 µg $Hg.l^{-1}$ in bag H0.5, 5 µg $.l^{-1}$ in the duplicate bags H5a and H5b, and 50 µg $.l^{-1}$ in bag H50.

Mercury concentrations in the water were measured using atomic absorption spectrometry with an IRDAB 2300 HGM spectrometer. Directly after filling the bags a set of two plexiglass frames each holding twelve glass microscope slides (24x60 mm) were lowered into each bag to a depth of 0.4 m. In two bags (Ca and H5a) slides were also lowered to depths of 0.1, 1.0 and 2.0 m to study the influence of depth. The inner surface of the bags is made of polyethylene. To investigate the differ-

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ences between glass and polyethylene as a substrate for periphyton two sets of slides covered by a polethylene film were lowered into bags Cb and H5b.

Twice a week three slides (both glass and polyethylene) were removed at random from each set for the determination of biomass and species composition of the periphyton. One slide was used for the estimation of biomass by chlorophyll determination according to STRICKLAND and PARSONS (1968) using the formula of LORENZEN (1967) to correct for the presence of phaeopigments. Chlorophyll amounts were expressed as $mg.m^{-2}$.
A second slide was dried in air and mounted with "Aquamount". A Microlab Cell Finder Microgrid was fixed on the slide and 250 random fields of the microgrid, covering a total area of 40 mm^2, were inspected with a Zeiss microscope (magnification 100, 500 and 1000x) for counting and identification. Since identification of all species of diatoms is not possible in these "Aquamount" preparations, the third slide was treated as follows: after drying at 60 °C for 24 hours, the organic contents of the cells were oxidized with 30 % H_2O_2 on a hot-plate and the slide was then washed in 3 % HCl, followed by distilled water. The preparations were dried at 60 °C and mounted with "Aquamount". Organisms were counted and identified in 250 random fields of a microgid as described above.

The average number of cells per mm^2 on the preserved and diatom preparations was used for species which could be easily identified on the preserved slides. For species which could only be identified on the diatom preparation, the number counted on them was used. Diatoms were the most important algae on the slides. They were identified on the basis of the descriptions and the keys published by VAN DER WERFF and HULS (1957), HUSTEDT (1930) and DREBES (1974).

RESULTS

The water that was used to fill the bags contained a considerable amount of silt and detritus. The mercury concentrations during the experiment are shown in Figure 1. The concentrations of mercury in the water decrease rapidly (about one third per day in H0.5, H5a and H5b, somewhat slower in H50).

Development of periphyton biomass

Figure 2 shows the development of the biomass of the periphyton, measured as chlorophyll on the glass slides in the different bags. In the controls chlorophyll increases until a relative maximum on day 13. This maximum coincides with a maximum of the phytoplankton biomass (results not showed). On day 16 a relative minimum is found after which chlorophyll values again increase steadily for the remainder of the experiment. The cell counts show basically the same pattern as the chlorophyll measurements. A linear correlation existed between cell counts per mm^2 (M) and chlorophyll per m^2 (C) ($C = 0.07-0.004M$, $r = 0.85$, $N = 94$).

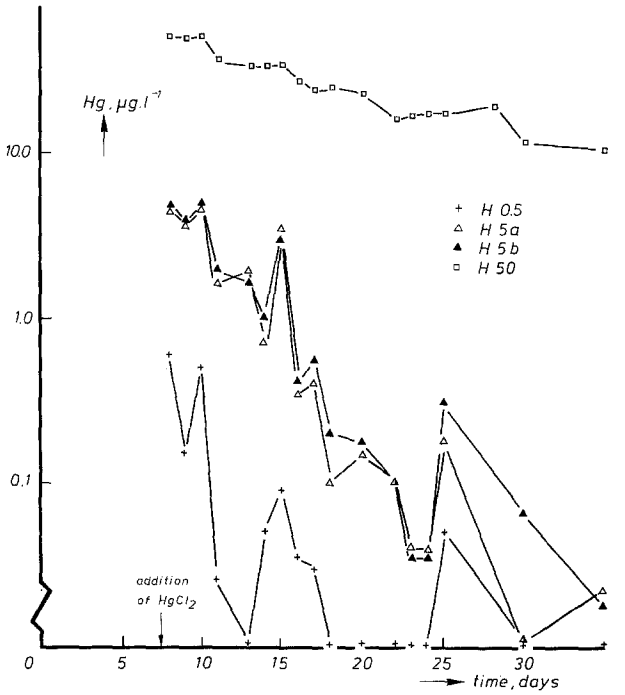

Figure 1: Development of mercury concentrations in the polluted bags (average of samples taken at depths of 0.5 and 2.0 m)

Addition of 0.5 µgHg.l^{-1} does not influence the development of the periphyton biomass. Addition of 5 µgHg.l^{-1} also does not appear to inhibit the growth of periphyton, although on days 10 and 13, when mercury concentrations in the water are still above 2 µgHg.l^{-1}, chlorophyll values in these bags are on the average lower than in the controls and H0.5. These differences were also found in the slides from the other depths in bags Ca and H5a. From day 20 till day 23 periphyton biomass is higher in H5a and H5b than in the controls; thus the growth pattern in H5a and H5b and in the controls is different. Addition of 50 µgHg.l^{-1} completely inhibits the growth of periphyton, only near the end of the experiment low numbers are counted and is chlorophyll again detectable.

In the control, the chlorophyll amount on the polyethylene was on the average 55 % of that on glass. In H5b chlorophyll and cell counts are higher on the polyethylene than on glass in the beginning of the experiment, when mercury concentrations are high. Later on in the experiment the situation in this bag becomes comparable to that in the control, i.e. less biomass on polyethylene compared with glass. On days 20, 23 and 28 the chlorophyll amounts on polyethylene were on the average 63 % from that on glass, cell numbers were 61 % from those on glass.

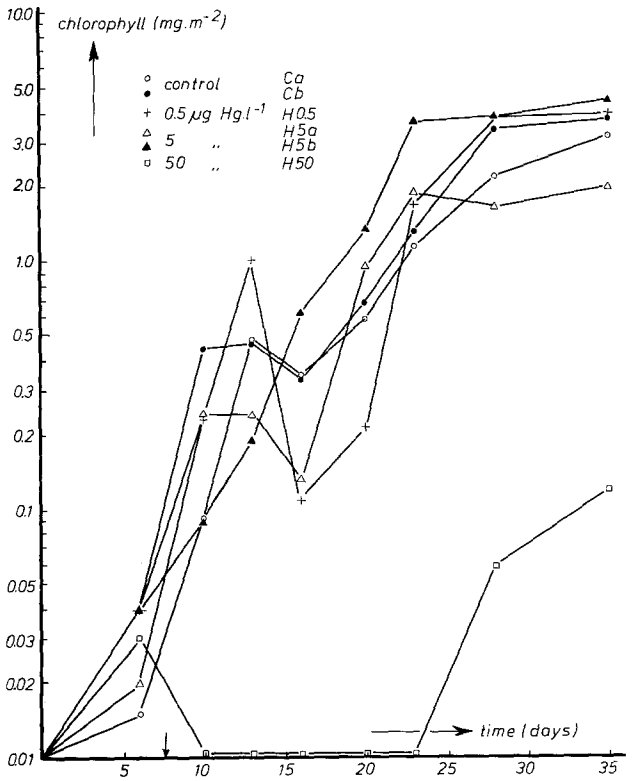

Figure 2: Chlorophyll on glass slides at a depth of 0.4 m as a function of time in the different bags.

Species composition of the periphyton

Figure 3 shows the different species present on the slides in the bags during the experiment (all depths included). The addition of mercury strongly affects the species composition of the periphyton community. In the controls 21 species were found. After addition of 50 µgHg.l^{-1} eleven of these species were not found, while two others only occurred in this bag. After addition of 5 µgHg.l^{-1} five species of the 21 in the controls were not found while three other species were found only in bags to which 5 µgHg.l^{-1} had been added. *Melosira nummuloides* was found only in the controls; in these bags it was found on all slides (15) from days 23-35 at densities of 0.03 - 1.0 per mm^2. This difference between the controls and the polluted bags is significant.

The number of species found on the glass slides in the different bags at a depth of 0.4 m as a function of time is presented in figure 4. It is clear that the number of species found decreases with increasing mercury concentrations. On the polyethylene slides the same species were found as on the corresponding glass slides, so the different substrates were not species selective in this case.

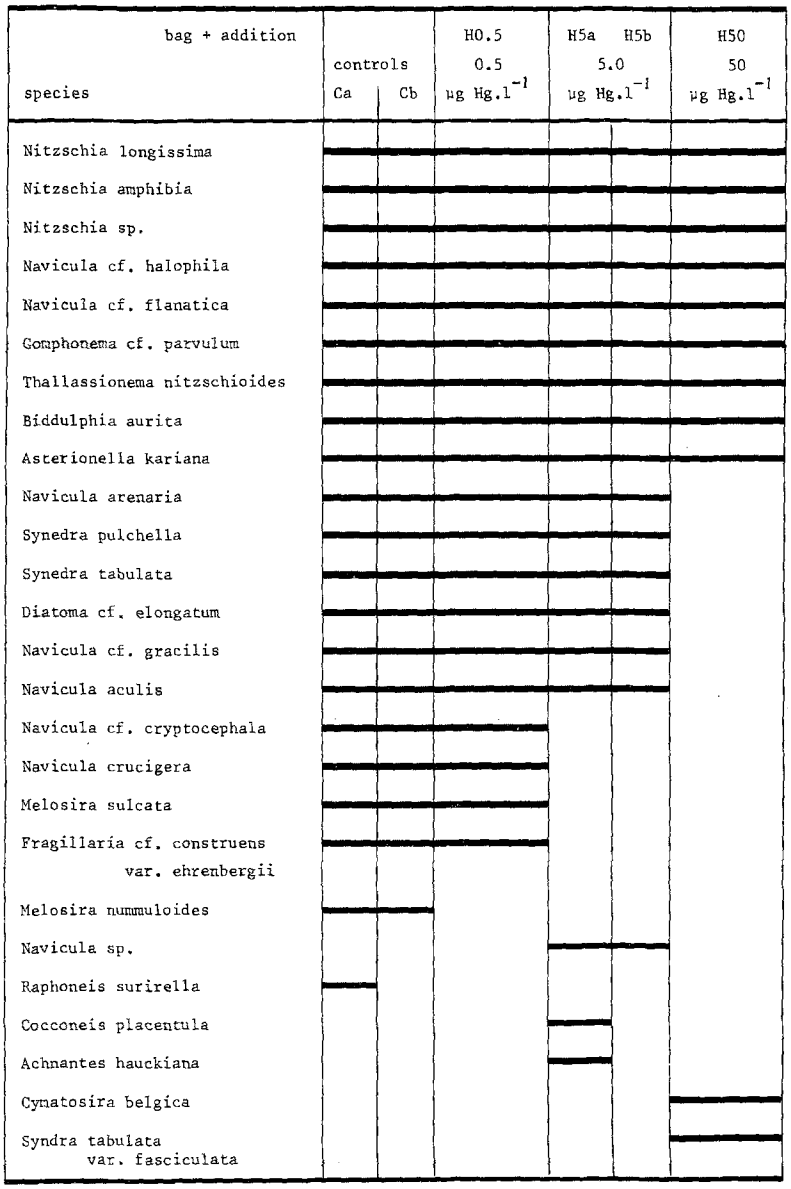

Figure 3 : List of species present during the experiment in the different bags.

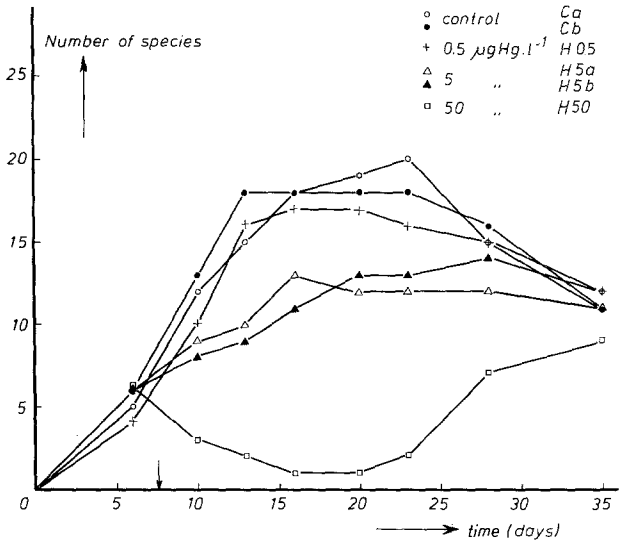

Figure 4: Number of species on glass slides in the different bags at a depth of 0.4 m as a function of time.

DISCUSSION AND CONCLUSIONS

The concentration of the mercury in the water decreases rapidly after the addition. Nevertheless addition of 0.5, 5 and 50 $\mu gHg.l^{-1}$ to the enclosed water columns had a clear influence on the development of the periphyton community in the bags. Addition of 0.5 $\mu gHg.l^{-1}$ effects the species composition of the community. The addition of 5 $\mu gHg.l^{-1}$ leads to a small change in the growth pattern and a reduction in the number of species compared to the controls. Addition of 50 $\mu gHg.l^{-1}$ inhibits the growth of the periphyton completely, growth restarting only at the end of the experiment.

It appears that the species composition of the periphyton community is a useful parameter for estimation of the influence of the added mercury. The lowest mercury concentrations inhibiting phytoplankton growth, range from 1-7 $\mu gHg.l^{-1}$ (HARRISS 1970, KAMP-NIELSEN 1971, NUZZI 1972, KAYSER 1976, BERLAND et al. 1976). BERLAND et al. (1976) also demonstrate large differences in the sensitivity of different species to heavy metals.
KUIPER (1977b) found that mercury concentrations higher than 1.5 $\mu gHg.l^{-1}$ inhibited the growth of North Sea phytoplankton.

VAN RAALTE et al. (1976) studied the effect of fertilization with sewage sludge and urea on the species composition of periphyton grown on glass slides placed on salt marsch mud. They observed a decrease in the diversity of the community in the fertilized areas. WILLIAMS and MOUNT (1965) found that increasing concentrations of zinc sulphate added to experimental channels resulted in a decreasing number of species in the periphyton grown in these channels. Other authors have also reported a decrease of the number of species in the periphyton as a result of pollution

with sewage (cf. EVANS and MARCAN 1976). SIGMON et al. (1977), working with artificial freshwater streams, found reduced periphyphyton diversity after addition of 0.1 and 1.0 µgHg.l^{-1}. Our results are another indication for the usefulness of benthic diatoms in monitoring environmental quality (cf. SLÄDECKOVA and SLÄDACEK 1963, VAN DAM 1974).

We found the same species on glass and polyethylene. CASTENHOLZ (1961) also found no differences between the different substrates he used (glass, plexiglass, rock, wood). The growth on polyethylene and glass was, however, not the same and appeared to be influenced by the mercury addition. This may indicate that one has to be cautious using biomass of the periphyton on artificial substrates as a parameter to evaluate the effect of pollution, since the substrate influences the recorded effects. SLÄDECKOVA (1963, cited by TIPPETT 1970) also found that the development of biomass on different substrates was influenced differently by pollution with organic material.

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