Effect of Hexachlorobenzene on Some Growth Parameters of *Chlorella pyrenoidosa*

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Introduction

The extensive distribution of hexachlorobenzene* (HCB) in the environment became evident only recently when high residues of this pollutant were found in raptorial birds (VOS et al. 1968;KOEMAN et al. 1969b), sea birds, fish, and mussels (KOEMAN et al. 1969a). HCB residues were also reported in cereals and other plant material (STIJVE 1971), and recently in human milk and adipose tissue (ACKER and SCHULTE 1970) where the concentrations were considerably higher than those of DDT + DDE.

The origin of these relatively high concentrations of HCB in the environment is not yet well understood. Since 1945, HCB has been used in agriculture for seed dressing against seed born and soil born diseases. For a good protection of the seeds a high concentration of about 500 ppm of this pesticide has to be applied (SCHLÖR 1970). HCB is also used in industry as a plasticiser for PVC as well as a flame retardant. As only little is known about the persistence of HCB, the relatively high concentrations of this pollutant in the environment may be the result of agricultural application as well as of industrial use. Only recently it was shown by FREITAG et al. (1974) that it was not possible to find degradation products of HCB in plants and soil. They concluded that HCB might even be more stable in the environment than dieldrin or DDT

* In the Federal Republik of Germany application of hexachlorobenzene-containing pesticides is no longer allowed but problems may still arise due to contamination of the environment with this compound.

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Although some information about the harmful effects of HCB on animals exists, nothing is known about the action of this pesticide on plants. The present paper describes experiments which were undertaken to study the effect of HCB on some growth parameters of the freshwater alga Chlorella pyrenoidosa.

Materials and Methods

Chlorella pyrenoidosa strain 211-8b was grown in culture tubes with 300 ml of nutrient solution according to KUHL (1962), maintained at 30°C under continuous light (4000 Lux) in a "light-thermostat" (Kniese, Marburg) according to Pirson and Kuhl. The cells were harvested by centrifugation for 10 min at 3000 x g and diluted with fresh nutrient solution to give a cell suspension with a concentration of 0.1 mg chlorophyll/ml. Chlorella equivalent to 1.0 mg chlorophyll was given into culture tubes containing the culture medium after adding the appropriate amount of HCB in acetone solution (1 ml) and was incubated under continuous light and aeration with an air stream of 7.5 l/h for 46 h at 30°C. The controls received the same amount of acetone. The cells were harvested by centrifugation, washed with distilled water, and were made up to 25 ml of suspension from which aliquots were taken for the determination of dry matter, chlorophyll content, carbohydrates, and total nitrogen. Chlorophyll was extracted from the algae with methanol (BÖGER 1964), measured at 652 nm and calculated according to ARNON (1949). For the determination of carbohydrates the anthrone method was used and total nitrogen was determined by the Kjeldahl method.

In an other set of experiments 30 ml of algal suspension equivalent to 1.0 mg chlorophyll were incubated in 100-ml-Erlenmeyer flasks with the appropriate amount of HCB under daylight conditions. After three months, aliquots were analyzed for chlorophyll content, and the remain-

ing algal suspensions were transferred to culture tubes with 270 ml of nutrient solution with the appropriate HCB concentration. These were incubated for another 72 h under continuous light and aeration with 7.5 l/h at 30°C in a "light-thermostat".

Results and Discussion

The effect of HCB application was studied in experiments with 0, 0.001, 0.01, 0.1, 1.0, and 10.0 ppm HCB in the nutrient solution which contained 0.33 % acetone after addition of the pesticide. The data given here are representative values from experiments repeatedly performed with similar results. The results of the short-term experiments with HCB are shown in Fig. 1. An effect of HCB on algal growth is evident. Incubation of the freshwater alga Chlorella pyrenoidosa over a period of 46 h with HCB led to a decrease of all growth parameters studied. This decrease is well correlated with the concentration of the pesticide applied. As can be seen from Fig. 1, HCB affects the chlorophyll content and total nitrogen most strongly whilst dry matter and carbohydrate content were less affected. The increase of dry matter observed in the culture with 10.0 ppm compared with that receiving 1.0 ppm may be due to precipitated HCB, as precipitation of HCB in the cultures containing 10.0 ppm was observed.

The effect of long-term incubation of algae with environmental pollutants is of special interest because very persistent compounds like HCB and DDT can be in contact with the organisms over long periods of time. As long-term treatments cannot be realized in the "light-thermostat", the algae were incubated in Erlenmeyer-flasks under daylight conditions over a period of three months. After this time the chlorophyll content of the algae was used as a measure of algal growth. As

FIG. 1 Effect of HCB on some growth parameters of Chlorella pyrenoidosa.

(1=10^{-3}, 2=10^{-2}, 3=0,1, 4=1,0 and 5=10 ppm; standard deviation calculated from 10 experiments)

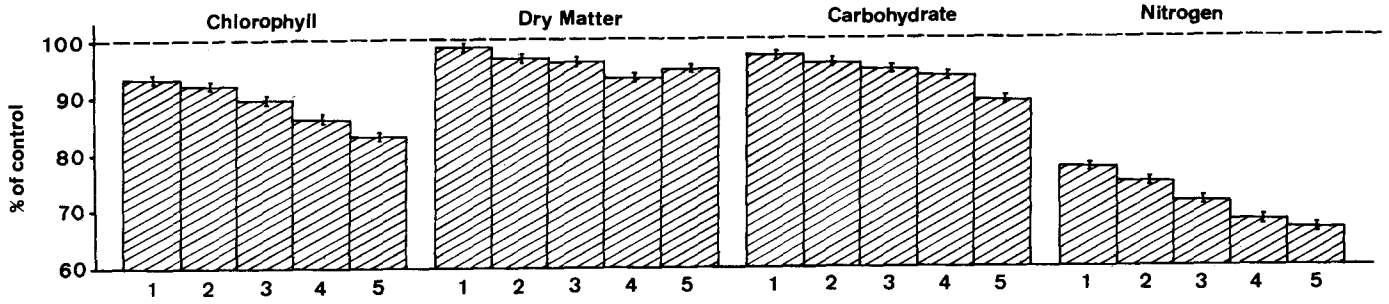
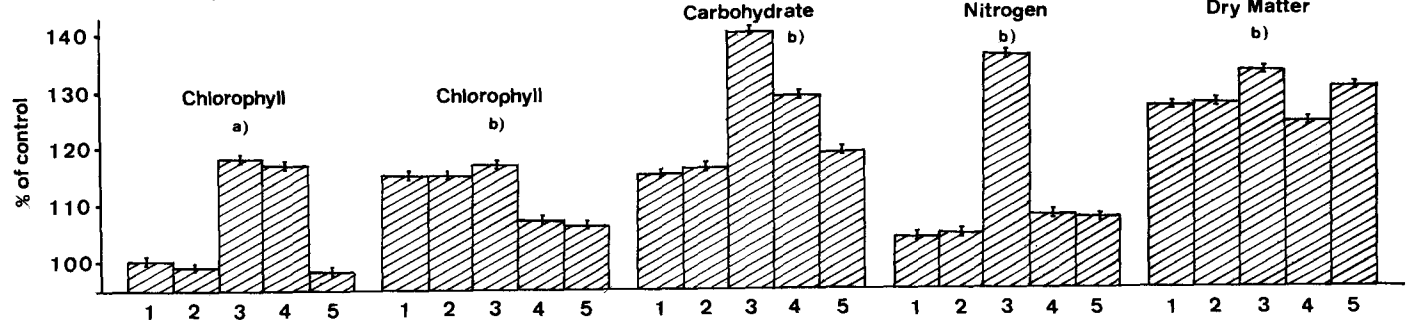


FIG. 2 Effect of a three months HCB treatment on measured growth parameters of Chlorella pyrenoidosa.

a) = grown under laboratory conditions, b) = after transfer of these cultures for 72 hr to continuous light and aeration in a light-thermostat. (1,2,3,4 and 5 „see fig. 1")



can be seen from Fig. 2a long-term incubation of Chlorella pyrenoidosa with HCB has a slight negative effect on algal growth measured as chlorophyll content only at the highest concentration (10.0 ppm) while a HCB concentration of 0.1 and 1.0 ppm gave a significant stimulation. After transfer of these cultures to the growth conditions of the "light-thermostat", growth is greatly enhanced by all concentrations of HCB studied with 0.1 ppm being the most effective concentration (Fig. 2b). The chlorophyll content of the algae is greatly increased over control values by HCB concentrations of 0.1 ppm and less, whilst 1.0 and 10.0 ppm were less effective in stimulation. On the other hand, the carbohydrates are greatly increased at a HCB level of 0.1 ppm; the higher concentrations being less effective but showing higher stimulative activity than a concentration of 0.001 and 0.01 ppm. Total nitrogen is also enhanced at a concentration of 0.1 ppm HCB which much exceeds the control values, whilst the other concentrations of HCB give only little stimulation; the higher concentrations being slightly more effective. The yield in algal dry matter was also greatly stimulated by HCB, the higher concentrations being slightly less effective than the lower concentrations. On the basis of dry weight determinations, 10.0 ppm HCB seemed to increase algal growth over the culture receiving 1.0 ppm. These results again seemed to be due to precipitation of HCB in the culture solution.

The results presented here are very surprising because from the data of the short-term experiments one would expect a corresponding decrease in algal growth during long-term incubation. It is known from experiments with chlorinated hydrocarbon insecticides that these compounds inhibit growth of marine phytoplankton and freshwater algae. WURSTER (1968) demonstrated an inhibition of photosynthesis and growth by dieldrin, endrin, and DDT and MENZEL et al. (1970) were also able to show an

inhibition of photosynthesis and growth of marine phytoplankton by chlorinated hydrocarbons. On the other hand different sensitivity of different algal species have been noted (WURSTER 1968; BOWES 1972; VON WITSCH et al. 1974). All of the experiments conducted so far were short-term studies which, in most cases, did not last for more than ten days. BOWES (1972) in his studies noted a lag-phase of nine days and afterwards normal growth when incubating Skeletonema costatum with DDT. These results seem to be in good agreement with the data presented here on the effect of HCB on Chlorella pyrenoidosa in long-term studies.

Only little is known about the mode of action and effect of chlorinated hydrocarbon pesticides in plants. From studies of LAWLER and ROGERS (1969) with susceptible barley it is evident that cyclic photophosphorylation is inhibited by DDT. It was observed that in susceptible algal species a metabolism of DDT to DDE takes place (BOWES 1972) which might result in a decrease of the DDT concentration to nontoxic levels. On the other hand, it is known from studies with animals that chlorinated hydrocarbons do induce several enzymes which in most cases are involved in drug metabolism. HCB is said to be resistant to breakdown in soil and plants (FREITAG et al. 1974) and except for its enhancement of δ-aminolevulinic acid synthetase in liver cells from chick embryo (GRANICK 1963), only little is known about its ability to induce enzymes. Presently nothing can be said about the mode of action of HCB in plants and about possible metabolic degradation.

Summary

Chlorella pyrenoidosa was incubated with HCB (0.001 - 10.0 ppm) in a "light-thermostat" at 30°C for 46 h with continuous light (4000 Lux) and aeration. HCB decreased

growth as deduced from measurements of chlorophyll content, dry matter, carbohydrate content, and total nitrogen. Incubation with HCB for three months in Erlenmeyer-flasks resulted in an increase of chlorophyll over control values in cultures receiving 0.1 and 1.0 ppm HCB. After transfer of the cultures from Erlenmeyer-flasks to growth conditions of a "light-thermostat", growth of the algae was greatly enhanced by all HCB concentrations studied; a concentration of 0.1 ppm being most effective.

Acknowledgment

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