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Effects of Chlorinated Organics from Wastewater Treatment on Algal Growth

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Chlorinated organic compounds result from chlorination treatment of wastewater. The discharges, containing these compounds, into the aquatic environment have resulted in widespread concern. Investigations have been conducted on the effects of these chloro-organics on aquatic life. Most of the studies, however, have been conducted on zooplankton or higher organisms. Only limited information is available concerning the biotoxic properties of chlorinated effluents on phytoplankton populations (ZILLICH 1972, BRUNGS 1973, GEHRS & JOLLEY 1975). The phytoplankton which constitute the primary trophic level can be important for the early screening of the toxicity of these chlorinated organics to aquatic biota. This study investigates the effects of chlorine-containing organic compounds formed from the chlorination of domestic wastewater of phytoplankton growth.

MATERIALS

Instrumentation and Apparatus

1. Centrifuge, Model PR-6 (International Equipment Company) was used to remove the suspended solids from the samples.
2. Freeze-Concentrator. A cooling solution bath (H_2O/30% ethylene glycol) was stirred by a stainless steel propeller driven at 2000 rpm by a GT21 synchronous motor with controller (G.K. Heller Co.). Stobotach was used to check the speed of the stirrer. The cooling solution was pumped from a LOW Temptrol Unit, Model 154 (Precision Scientific Co.) into a large plastic container where it circulated around a 4-L beaker containing the water sample (HERBES 1974).
3. GME Model VL Linear Fractionator (Gilson Medical Electronics, Middleton, Wisconsin) equipped with a Model DC Drop Counter.
4. Beckman Total Carbon Analyzer, Model 915 with an air purification unit.
5. Sephadex Gels G-10 (MW, 0-700), G-25 (MW, 1000-5000) and G-50 (MW, 5000-30,000), (Pharmacia Fine Chemicals, Inc.).
6. Chromatography columns used were K 25/45 equipped with special flow adaptors.

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Reagents

1. Chlorine Stock Solution. High purity chlorine gas (Union Carbide Corporation, Linde Division) was purged through double distilled water. (6-8 g equivalent Cl/L). The stock solution was stored in a dark glass container at 4C. Working dilutions of this stock solution were prepared and standardized by iodometric titration (APHA 1975) immediately before use.
2. Water Eluant. Glass double-distilled water was sterilized and kept at 4C.
3. Effluent Samples. Chlorinated water from the final effluent and unchlorinated water from the secondary effluent were collected from the Ann Arbor Wastewater Treatment Plant and were stored at 4C. The total residual chlorine was 2.5 mg/L. The same effluent samples were used in all the experiments conducted.

Bioassay Culture Apparatus and Media

1. Bellco Side Arm Flasks, 300-mL (Bellco Biological Glassware) sealed by cotton plugs.
2. G-10 Gyrotory Shakers (New Brunswick Scientific Co., Inc.) and shaking baths (Precision Scientific Co.) provided continuous movement of the algal cultures of 110 cycles per min (cpm).
3. Culture Room. The temperature was kept at 23 $\pm$ 1C. An illumination level of 550 ft-candles was provided by two 40-watt fluorescent lamps (General Electric Co.) and two Sylvania F-40 Gro-lux lights for *Chlorella pyrenoidosa*. Four 40-watt fluorescent lamps (General Electric Co.) provided 160 ft-candles for *Anabaena affinis*. The lamps were suspended 12 in. above the culture flasks.
4. Air-filtration Set-up. Swin-Lok membrane holders and Nucleopore filter paper (Nucleopore Corp.) were used to filter dry air which was provided for *C. pyrenoidosa* only. Dry compressed air was passed through sterile, glass-wool filters, then through sterile, double-distilled water, and finally the air was split and passed through several culture flasks equipped with the Nucleopore filter kits. The air filtration set-up in each flask consisted of 1 mL disposable syringe and #20 needle imbedded in a cotton plug at the mouth of the culture flask. The needles were flame-sterilized before each reinsertion for examination or turbidity measurement.
5. A Binocular Microscope with Whipple ocular micrometer (Zeiss Co.) was used to count cells for standard curves and to determine the bacteria contamination.
6. Palmer-Malony (P-M) Nannoplankton Cell (Model 1EZ) No. 9851, C20, Counting Cell (Arthur H. Thomas Co.) was used for cell counting.
7. Klett-Summerson Photoelectric Colorimeter, Model 900-3 (Klett Mfg. Co., Inc.) was used to measure cell density using the side-arm of the culture flask.
8. Growth Media. Modified Osterlind media was used for *C. pyrenoidosa* [JORGENSON 1962]. The modified ASM-1 media (without the nitrate) was used for *A. affinis* (STEIN 1973). The stock media solutions were kept at 4C.

EXPERIMENTAL PROCEDURES

A. To study the dilution effects of the final effluent on algal growth, final effluent water was centrifuged, filtered and added to the medium in dilutions of 10, 20, 30 and 40%. The resulting total volume of each medium was 150 mL. Pure culture of *A. affinis* was inoculated into each medium and incubated at 23C. The growth rate was photometrically measured and correlated with direct cell counts.

B. Experiments to show the comparison of the chlorinated and unchlorinated effluents on algal growth were conducted. Chlorinated and unchlorinated samples were centrifuged, filtered and treated with sodium thiosulfate to remove residual chlorine. An equivalent amount of sodium thiosulfate was added to the control and unchlorinated samples. The treated samples were concentrated by freeze-concentration methods and added to the growth media using 40, 50 and 60% dilution volumes. A pure culture of *A. affinis* was inoculated in replicate sets.

C. A study of the response of two different algal species on the addition of chlorinated effluents was conducted. The water samples were centrifuged, filtered and treated with sodium thiosulfate. The controls were treated similarly. Different volumes (10, 20, 30, 40 and 50%) of the treated water samples were added to the growth media. Inocula of *A. affinis* and *C. pyrenoidosa* were added separately. Growth rates were determined using methods as described previously.

D. Molecular weight distributions of the unchlorinated and chlorinated effluents were determined and the effects of the fractions on the algal growth were investigated.

Unchlorinated and chlorinated effluents were centrifuged and filtered. Sodium thiosulfate was added to the chlorinated effluents to remove the residual chlorine. The unchlorinated and control samples were also treated with equivalent amounts of sodium thiosulfate. The treated samples were then freeze-concentrated and separated into different molecular weight fractions by Gel-permeation chromatography using Sephadex G-10, G-25 and G-50 (MC DONALD & CLESCERI 1973). The resulting fractions from each resin were pooled and freeze-concentrated. Total organic carbon (TOC) was determined for each molecular weight fraction. Two concentration levels of TOC (0.8 and 2.4 mg/L) for each molecular weight fraction were added into separate media. Pure cultures of both algae *(A. affinis* and *C. pyrenoidosa)* were inoculated in the media. Growth was measured by cell counts and Klett readings for a period of 10 days.

RESULTS AND DISCUSSION

The response of the algae to the chlorinated and unchlorinated sample additions was determined by growth rate calculations. The rates (K, day^{-1}) were calculated by using a linear regression technique of those points of the curve representing

the log phase of growth. Each growth rate experiment was subjected to statistical analysis using the comparison of two slopes. Analysis of co-variance using student's T distribution for small samples was employed. Based on this technique the 90% confidence interval was selected. The data were subjected to the University of Michigan MTS MIDAS Program for measurement of the significance of the difference in growth rates obtained from the controls and the treated cultures.

A. Effect of Dilution of the Final Sewage Treatment Plant Effluent of Algal Growth.

The effects of the different dilutions of the effluents on the growth of *A. affinis* are shown in Table 1. Each dilution shows inhibitory effects which could be associated with the chlorination treatment of the effluent. Mean growth rates were calculated for each dilution covering the 9-15 day period of log phase growth.

TABLE 1

Effect of Final Sewage Effluent on the Growth Rate of *Anabaena Affinis*

Percentage by Volume of Sewage Effluent Added to Modified ASM Medium	Mean Growth Rate K (day^{-1})	Cell Counts/mL x 10^6 at 15th day
10	0.060 ± 0.007	6.2
20	0.074 ± 0.007	5.6
30	0.062 ± 0.006	4.7
40	0.059 ± 0.004	3.8
Control (Modified ASM Medium alone)	0.095 ± 0.005	7.8

The toxic effects could be due to the residual chlorine and other compounds in the effluent. This residual chlorine was not destroyed prior to the bioassay experiments. BROOK & BAKER (1972) reported that 0.32 ml/L Cl_2 depressed photosynthesis and respiration of freshwater phytoplankton by 50%. CARPENTER et al. (1972) found that 0.4 mg/L residual Cl_2 decreased primary productivity by 83%. It is evident from the experiments of TSAI & BRUNGS (1976) that residual chlorine in the chlorinated effluents have effects on aquatic life. Table 1 also shows the effect of the dilutions of chlorinated sewage effluent on the growth of *A. affinis* on the 15th day. The cell concentration decreased as the amount of effluent increased and at 40% dilution, a considerable reduction of cell counts was observed. This indicates that toxicity is directly proportional to the amount of chlorinated effluent in the media.

B. Comparison Study of the Effects on Algal Growth of Chlorinated and Unchlorinated Effluents.

Different dilutions of both chlorinated and unchlorinated effluents were added to the algal media. This time, the residual chlorine in the effluents was destroyed prior to the bioassay experiments. Table 2 shows the results for *A. affinis*.

TABLE 2

Effect of Chlorinated vs. Unchlorinated Effluent on the Growth Rate of *Anabaena Affinis*

Percentage by Volume of Sewage Effluent Added to Modified ASM Medium	Growth Rate K (day^{-1}) Chlorinated Effluent	Growth Rate K (day^{-1}) Unchlorinated Effluent
40	0.064 ± .005	0.074 ± .008
50	0.074 ± .003	0.082 ± .009
60	0.073 ± .004	0.088 + .006
Control	K = 0.060 ± 0.004	

Stimulatory effects of both chlorinated and unchlorinated effluents, which were observed, can be attributed to the presence of nutrients in the effluents (MC DONALD & CLESCERI 1973, SACHDEV & CLESCERI 1978, CLESCERI et al. 1973) not available in the control. The significant difference between the chlorinated and unchlorinated effluents could be associated with the inhibitory effects of organo-chlorine compounds that could be formed during chlorination treatment. KOPPERMAN et al. (1974) and CARLSON & CAPLE (1975) conducted experiments on the effects of chloro compounds on *Daphnia magna* and found that the toxicity generally increased with increasing chlorine concentration associated with organic compounds. SIKKA & BUTLER (1977) stated that chlorine-containing compounds present in sewage effluents may be more toxic to phytoplankton than the parent compounds that would be present in the effluent prior to chlorination.

C. Comparison Study of the Effects of Chlorinated Effluents on Algal Species.

Growth rate curves at 10, 20, 30, 40 and 50% dilution volumes of chlorinated effluents on *C. pyrenoidosa* and *A. affinis* are compared in Table 3.

Statistical analysis shows significant differences between the growth rates of the two algae. This difference is exhibited in all concentrations of the added dilutions. Comparison with the control shows a general enhancement of growth for *A. affinis* but toxic effects on the growth of *C. pyrenoidosa*. There is no immediate explanation for these observations other than to state that the growth of these algae is selective and dependent on their

physiology. SIKKA & BUTLER (1977) stated that the effect of chloro-organic compounds present in the effluent of sewage treatment plants showed different effects on the growth of phytoplankton depending on the species.

TABLE 3

Comparison of the Effects of Chlorinated Effluents on the Growth of *Anabaena Affinis* and *Chlorella Pyrenoidosa*

Percentage of Sewage Effluent Added to Respective Medium	Growth Rate, K (day $^{-1}$) *Anabaena Affinis*	*Chlorella Pyrenoidosa*
10	0.084 ± .004	0.030 ± .002
20	0.072 ± .004	0.034 ± .002
30	0.078 ± .003	0.037 ± .002
40	0.075 ± .009	0.037 ± .004
50	0.069 ± .012	0.045 ± .003
Control	0.067 ± .005	0.081 ± .007

D. Study of the Effects of Different Molecular Weight Fractions of Chlorinated and Unchlorinated Organics on Algal Growth.

The concentration of carbon in the fractions can be related to growth enhancement when carbon is considered as a nutrient source required for algal production. Two concentration levels of TOC, 0.8 and 2.4 mg/L C, were selected for the experiments. Comparison of the growth response of both algae of different molecular weight groups of chloro-organic compounds are shown in Table 4. Statistical analysis of the data resulted in the following observations:

1. For both species, TOC levels showed a difference in their growth rate only with the G-50 unchlorinated fractions.
2. At both TOC levels a lower growth rate for *C. pyrenoidosa* with the G-50 chlorinated fraction than with the unchlorinated fraction was observed.
3. The *A. affinis* experiments indicated no trends when comparing growth rates among the molecular size fractions in both chlorinated and unchlorinated fractions.
4. In all cases, a general trend is observed with the unchlorinated fractions which exhibited higher growth rates than the chlorinated fractions.
5. Growth rates of *C. pyrenoidosa* increased as the unchlorinated molecular weight fractions increased. This shows a marked difference from the growth rate of *A. affinis* which did not show any trends.
6. No inhibitory effects due to the different molecular weight fractions were observed, suggesting that the toxic

compounds affecting the growth rate of *C. pyrenoidosa* are of higher molecular weights (>30,000).

These observations indicate that fractionation may remove some toxic materials which results in growth enhancement for the algal bioassay experiments. It is also possible that the toxic materials could be those of the higher molecular weight size fractions larger than 30,000, which were not tested in this work.

TABLE 4

Comparison of Algal Growth in Media Containing Different Molecular Weight Fractions of Organic Compounds

Fraction	K (day^{-1}) 0.8 mg/L TOC Chlorinated Fractions		K (day^{-1}) 2.4 mg/L TOC Chlorinated Fractions	
	Anabaena Affinis	*Chlorella Pyrenoidosa*	*Anabaena Affinis*	*Chlorella Pyrenoidosa*
G-10	0.067 ± .003	0.090 ± .011	0.071 ± .006	0.105 ± .014
G-25	0.066 ± .003	0.101 ± .007	0.065 ± .004	0.095 ± .006
G-50	0.064 ± .006	0.109 ± .014	0.065 ± .003	0.110 ± .010
G-10	0.069 ± .005	0.091 ± .006	0.065 ± .003	0.112 ± .011
G-25	0.076 ± .004	0.106 ± .014	0.063 ± .003	0.106 ± .010
G-50	0.069 ± .004	0.123 ± .009	0.080 ± .010	0.145 ± .008
Control:	*Anabaena affinis:* K (day^{-1}) = 0.066 ± .004			
	Chlorella pyrenoidosa: K (day^{-1}) = 0.108 ± .008			

This study has shown that the major contribution for the growth inhibition of algae in the receiving waters of wastewater treatment plants can be attributed to the total residual chlorine in the effluents. It is also suggested that upon removal of the residual chlorine, the inhibitory effects could be due to the chloro-organic compounds which are produced during the chlorination treatment of the effluents. Extrapolations of the results of this study to the natural environment need to consider that the contents of the effluents change during short time intervals and that the natural algal population in the receiving waters consists of diverse species.

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