EFFECTS OF PHARMACEUTICAL WASTES ON GROWTH OF MICROALGAE

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Introduction

The purpose of this work was to assay samples of waste material from Puerto Rican pharmaceutical industries for inhibition of growth of algae. Two samples (noted as I and II) supplied to us were tested for toxicity to six microalgae. The test organisms, two blue-green algae, two green algae, and two diatoms epresent three major divisions of algae.

Methods

Samples of waste material were frozen upon arrival and stored at -10°C. Just before testing, frozen aliquots of each sample were thawed and sterilized by autoclaving (121°C, 15 min). In a few cases, to test for possible sample alteration during autoclaving, the samples were boiled 3 times for 10 minutes each with cooling in between.

The microalgae were grown in the synthetic sea water medium ASP-2 (Provasoli, McLaughlin and Droop 1957; Van Baalen 1962), using the test tube culture techniques of Myers (1950). Green algae used were Chlorella autotrophica, strain 580 and Dunaliella tertiolecta, strain DUN (both obtained from R. R. Guillard); the blue-green algae used were Agmenellum quadruplicatum, strain PR-6, and Coccochloris elabens, strain 17a (isolates of this lab); and the diatoms were Cylindrotheca sp., strain N-1, and Chaetocerous simplex (isolates of this lab). All cultures were pure except possibly C. simplex, the stocks of which were carried in liquid culture. C. simplex cultures were incubated at 27°C, all others

at 30°C. The cultures were illuminated continuously; C. simplex with F20T12D fluorescent lamps and the others with F40/CWX fluorescent lamps. All cultures were continuously aerated with a 1.0 ± 0.1% CO_2-in-air mixture. Growth was followed turbidimetrically using a Lumetron Colorimeter Model 402-E with a red glass gilter (660 mm). For simplicity the data is reported in generations per day.

Autoclaved waste material was added directly to sterile growth medium. No additions were made to control cultures. Duplicate cultures were used in all assays. The culture tubes were inoculated with $\sim 10^5$ cells/ml and incubated immediately. An outline of the liquid culture method is given in Figure 1. Algal lawn assays were done according to the method described previously (Pulich et al. 1974).

Results

Table 1 summarizes the growth rate data for liquid cultures of the six test algae with Samples I and II. All of the tests showed that Sample II was more toxic than Sample I. Sample I was shipped in plastic containers, Sample II was shipped in glass containers. Perhaps the difference in toxicity was due to some interaction of the sample with the plastic.

As a group the green algae were most tolerant of both samples. Sample I showed no toxicity at 5% (v/v) final concentration, and some stimulation of growth was seen at 1% (v/v). Sample II prevented growth at 5% (v/v), and depressed growth rates were noted at 1% (v/v).

The blue-green algae were much more sensitive, growth did not occur at 0.1% (v/v) concentration of either sample, and even 0.025% (v/v) caused depressed growth rates.

Response of the two diatoms was mixed. C. simplex was the most sensitive test alga; growth was completely suppressed by 0.1% (v/v) Sample I, and 0.025% (v/v) Sample II. An estuarine form, Cylindrotheca sp., strain N-1, gave slower growth rates at 0.1% (v/v) of either sample, while growth was completely inhibited at 1.0% (v/v) Sample I, and 0.5% (v/v) Sample II.

Since in this work it was chosen to sterilize the samples by autoclaving, a comparison was made via the algal lawn assay of the toxicity of two aliquots of Sample I, one sterilized by autoclaving, the other "sterilized" by repeated boiling and cooling. As judged by the data of Table 2, the toxicity of Sample I to PR-6 in the algal lawn assay was nearly the same whether it was autoclaved or boiled. This rules out heat-labile (autoclaving) or somewhat volatile compounds as the cause of the toxicity.

For comparative purposes, Table 3 summarizes the growth rate data in liquid culture of the six test algae with Samples II and III of the Shell Chemical industrial wastes. Samples II and III were toxic to the blue-green algae, growth rate decreased about one-half at the highest concentration tested (10% v/v). This pattern of response was also seen with the two green algae and Sample II. Sample III showed little toxicity to the green algae, indeed for reasons unknown, some stimulation of growth was seen. Sample II completely suppressed growth of both diatoms

species. Sample III caused only a partial reduction in growth rate of N-1 at 10% (v/v), but no growth was obtained with C. simplex even after 7 days incubation. Further experiments with C. simplex and Sample III showed growth (4.2 generations/day) would occur at a concentration of 0.5% (v/v) but not at 2.5% (v/v).

Conclusions

This study has shown that both Samples I and II of the pharmaceutical wastes are toxic to algae. The two green algae used as test organisms were most tolerant of both samples, while the blue-green algae were much less resistant. In the diatoms, N-1 was intermediate in response, with C. simplex being the most sensitive of the six test algae.

The results here clearly demonstrate the very toxic nature of these pharmaceutical wastes. Either of these samples may be considered much more toxic to the algae used herein than the Shell Chemical waste samples tested earlier. For example, the blue-green algae were completely inhibited at 0.1% (v/v) in this study, yet tolerated 10% (v/v) of the Shell Chemical wastes. Similarly, the diatom C. simplex did not grow in 0.025% (v/v) Sample II (Puerto Rican) in this study, yet grew near maximally (4.2 generations/day) in 0.5% (v/v) Sample III of the Shell Chemical wastes. As a further comparison, fuel oils have also been shown to inhibit the growth of organisms PR-6, 580, and N-1 at 0.025% - 0.05% (v/v) (Batterton, Winters, and Van Baalen 1978). Thus it is possible to construe from this comparison that the dumping of industrial wastes such as Puerto Rican Sample II

may be akin to a fuel oil spill in terms of toxicity to some of the test algae used in this study.

LITERATURE CITED

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Figure 1. Protocol of testing waste material samples

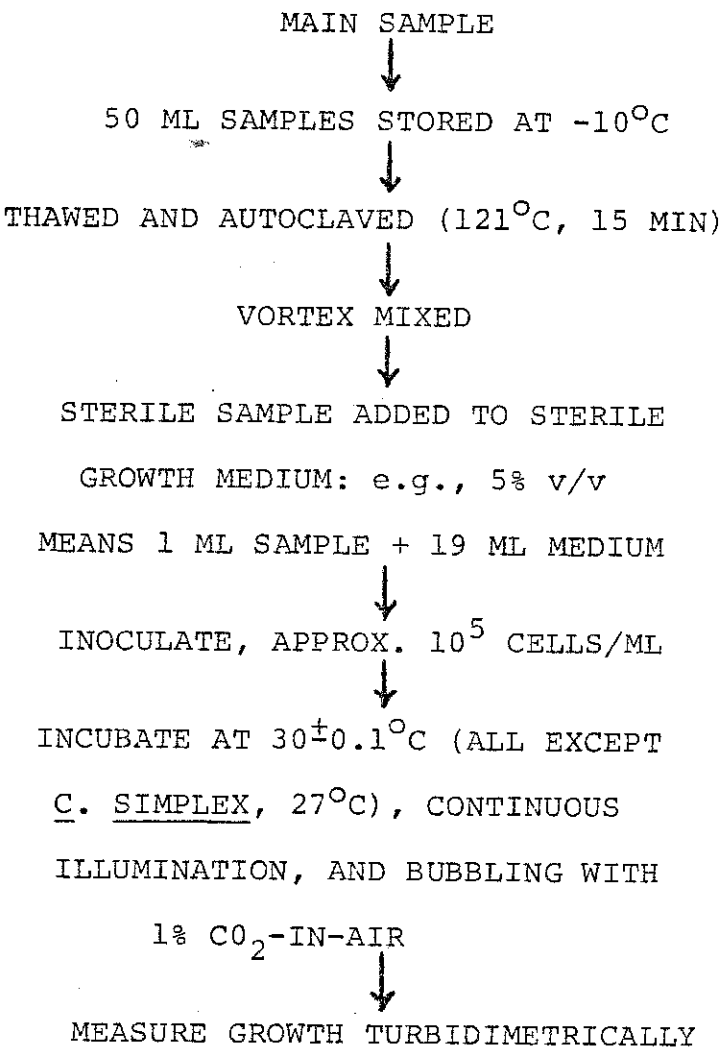

Table 1. Effect of autoclaved Puerto Rico samples I and II added to the medium (v/v) on growth rates of algae.[1]

ALGAE	Sample	Control	0.005%	0.025%	0.1%	0.5%	1.0%	5.0%
Greens								
580	I	2.3±0.2					3.8±0.1	2.5±0.1
	II	2.9±0.2					2.1±0.1	NG-5[3]
Dun	I	2.5±0.1					3.2±0.2	3.4±0.4
	II	2.7±0.1					2.6±0.1	NG-5
Blue-greens								
PR6	I	5.0±0.4	5.0	4.5±0.1	NG-4[2]			
	II	5.6±0.4	5.5±0.1	4.0±0.3	NG-7[3]			
17a	I	3.9±0.4		3.8±0.1	NG-3			
	II	3.7±0.4		3.1±0.1	NG-5			
Diatoms								
N-1	I	4.8±0.3			4.5±0.4	4.0±0.3 (20 h)[4]	NG-2	
	II	4.8±0.3			4.1±0.5	NG-7		
C. simplex	I	4.4±0.3		4.2±0.4	NG-2			
	II	4.8±0.3	4.8±0.2	NG-5				

[1] Growth rates expressed as generations/day, at 30°(C. simplex, 27°) under continuous illumination and aeration with 1% CO_2-in-air

[2] NG-2, etc., means no growth in 2 days after inoculation.

[3] Same results in two different experiments.

[4] Lag in growth for 20 h compared to controls

Table 2. PR-6 Algal Lawn Assay of Pharmaceutical Wastes[1]

Control	Sample I Autoclaved	Sample I Boiled	Sample II Autoclaved
0 mm	23 mm[2]	22 mm	26 mm

[1] 10 µl of each sample was placed on washed (medium ASP-2) antibiotic sensitivity discs (Schleicher and Schuell, No. 740-E).

[2] Radius of growth inhibition zone. A value of 0 mm means no inhibition, 36 mm means no growth on plate.

Shell Chemical Wastes:

Table 3. Effect of autoclaved Samples II and III added to the medium (v/v) on growth rates of algae.[1]

	SAMPLE II			SAMPLE III		
ALGAE	0	5%	10%	0	5%	10%
Blue-greens						
PR6	5.0±0.4	3.0	2.0	5.1±0.4	3.6	1.6
17a	3.6±0.4	2.7	1.9	3.9±0.4	2.6	2.2
Greens						
580	2.6±0.3	1.9	1.2	2.2±0.3	2.9	2.5
Dun	2.6±0.3	1.9	1.6	2.4±0.3	2.4	2.8
Diatoms						
N-1	4.1±0.3	NG-2[2]	NG-2	4.2±0.3	3.3	2.9
C. simplex	5.1±0.5	NG-2	NG-2	5.1±0.5	NG-7	NG-7

[1]Growth rates expressed as generations/day, at 30° (C. simplex, 27°) under continuous illumination and aeration with 1% CO_2-in-air

[2]NG-2 or -7 means no growth in 2 or 7 days after inoculation