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Effects of Monomethylhydrazine on Selected Species of Marine Diatoms[1]

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Monomethylhydrazine (MMH) is an important reactant in high energy propellant systems and is almost exclusively used as a rocket fuel. It serves as the propellant for the orbital maneuvering and reaction control subsystems of the Space Shuttle Orbitor and is also used in military launches. The major use of MMH as rocket fuel dictates its consumption which fluctuates with its usage in the U.S. space program (Koller 1979). Since MMH is potentially hazardous to the environment (Slonim 1977), there is considerable interest in the effects of low level release as well as spill conditions on the environment.

Scherfig et al. (1977) determined safe concentration (SC) and the mean effective concentration (EC) for hydrazine, MMH, and unsymmetrical dimethylhydrazine on species of green algae. The EC was determined when a concentration of MMH inhibited an algal population to a level of 50% of the control population. Marine cultures in general had lower SC and EC levels than freshwater cultures. Dixon et al. (1979) also studied the response of a number of marine and freshwater green algae. They found that the sensitivity of an algal species seemed to be dependent on the presence or absence of a true cell wall. Bacterized cultures were not significantly different in sensitivity from axenic algal cultures and MMH seemed to cause enlargement of the algal cells.

The aqueous degradation of MMH is relatively rapid compared to the time green algae require to reach maximum standing crop. The diatoms Thalassiosira pseudonana and Skeletonema costatum reach maximum standing crop in 6 to 7 days in culture which makes them ideal for testing short-term effects of MMH. The objectives of this study were to determine the relative sensitivity of selected marine diatoms to MMH and whether species composition would be affected by MMH.

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MATERIALS AND METHODS

Unialgal, axenic cultures of T. pseudonana 3H (estuarine), T. pseudonana 13-1 (oceanic), S. costatum Skel (coastal), and S. costatum G44S (oceanic) were grown in f/2 medium (Guillard 1975). Seawater, collected from 1 m below the surface, was aged, filtered (0.45 um), enriched, and autoclaved for 15 minutes at 120°C and 15 psi. Cultures were maintained at 22°C ± 2°C with a light:dark cycle of 14:10 h and placed 20 cm from two cool-white fluorescent lamps producing an intensity of 1400 uW cm^{-2} at the surface of the culture vessels. Concentrations of MMH were prepared from a primary stock solution of 100 ppm.

Triplicate, sterile 250 ml Erlenmeyer flasks containing 100 ml of f/2 medium were dosed with the desired, freshly prepared concentrations of MMH ranging from controls to 0.5 ppm. Stock solutions of diatoms were slowly stirred with a sterile, magnetic stir bar to reduce clumping of cells and to insure homogeneous mixtures. At the point in time when the primary stock of the diatom reached a concentration of approximately 10 cells/ml, 1 or 2 ml of these exponentially growing cells were introduced into triplicate Erlenmeyer flasks. This inoculation began Day 0. Five concentrations and a control were examined for T. pseudonana 3H and 13-1, and S. costatum Skel. A mixed culture containing T. pseudonana 13-1 and S. costatum GSS4 was also examined. Two samples from each experimental culture were removed every 24 hours and transfered to a hemacytometer (American Optical Bright-Line improved Neubauers) for counting. Six day growth response curves were observed in order to determine the SC and EC_{50} of MMH on each diatom (APHA 1976). The mean number of cells per volume averaged from three flasks is plotted against time for each concentration. A single classification analysis of variance was performed on these curves to determine whether cell division is inhibited at the various concentrations (Sokal and Rohlf 1981). Growth curves were also generated for mixed cultures of S. costatum GSS4 and T. pseudonana 13-1 to determine if MMH effects differ when interspecific competition occurs.

RESULTS AND DISCUSSION

A run refers to 6 d cell counts. The accuracy and precision of the hemacytometer is better at higher concentrations of cells; the high standard deviations for days 0 and 1 throughout the assays are a reflection of this property. Once a concentration of approximately 100,000 cells/ml is reached, duplicate counts from the same flask may differ by ca. 12%. Data points of all graphs are the means of three replicates.

After an initial run to box in the concentrations of MMH that affect the cell division of S. costatum Skel (Fig. 1a), run 2

further tested this diatom for SC and EC_{50} values at concentrations of 0.2, 0.3, and 0.4 ppm (Fig. 1b). Here, 0.4 ppm did not show any growth and was significantly different ($p < .001$) from the controls from day 2 till the end of the run. The 0.2 ppm cultures never differed significantly from the controls but the 0.3 ppm cultures showed significantly reduced cell counts during days 2, 3, 4, and 5. By day 4 this concentration is significant ($p < 0.05$) to a lesser degree and by day 6 it does not differ significantly from the controls.

Run 3 assayed the concentrations of 0.1, 0.2, 0.3, and 0.4 ppm on the diatom, T. pseudonana 13-1 (Fig. 1c). This open ocean diatom was more sensitive to the MMH than the coastal Skeletonema. The concentrations of 0.3 ppm and 0.4 ppm did not show any growth. Both 0.1 and 0.2 ppm show a reduction in cell division and the 0.1 ppm cultures are significantly reduced ($p < 0.01$) on day 6. The 0.2 ppm cultures become highly significant ($p < 0.001$) beginning on day 2 and remain so throughout the run.

Run 4 tested the concentrations of 0.1, 0.2, 0.3, and 0.4 ppm on the diatom, T. pseudonana 3H (Fig. 1d). This estuarine diatom was the least sensitive to MMH of the three diatoms examined unialgally. Growth occurred at all concentrations, the 0.1, 0.2, and 0.3 ppm cultures did not differ significantly from controls throughout the run. Although some significance was observed (day 2 at 0.1 ppm and day 3 at 0.3 ppm) these isolated points are not overall meaningful. The 0.4 ppm cultures were significantly ($p < 0.001$) reduced on days 2 and 3. By day 6, however, the cultures were no longer different from the controls. Run 5 assayed the concentrations of 0.5 and 0.6 ppm on the same diatom. No growth occurred.

Run 6 is a mixed culture experiment with the diatoms S. costatum GSS4 and T. pseudonana 13-1. The effects of 0.1, 0.2, 0.3, and 0.4 ppm on the dominance of Thalassiosira are shown in Fig. 2. T. pseudonana 13-1 is not affected by the concentrations of 0.1 and 0.2 ppm while the 0.3 ppm cultures show a significant reduction in cell numbers on days 1 to 3 only. The 0.4 ppm cultures have significantly reduced cell populations only on day 3 ($p < 0.05$). The variances for days 4 to 6 are quite high for T. pseudonana 13-1 throughout this run often exceeding the 12% of the mean that could be due just to the inaccurracy of the hemacytometer. S. costatum GSS4 is not affected by the MMH concentration of 0.1 ppm while the 0.2 ppm cultures have significantly lower cell numbers on days 3 and 4 but do not remain so on days 5 and 6. The 0.3 and 0.4 ppm cultures show significantly reduced cell division from day 3 till the end of the run.

The ratios of S. costatum GSS4:T. pseudonana 13-1 also have high variances for this run. The ratios in the 0.1 ppm cultures never significantly differ from the controls. Days 4 and 6 for both the 0.2 and 0.3 ppm cultures have significantly lower ratios ($p < 0.01$)

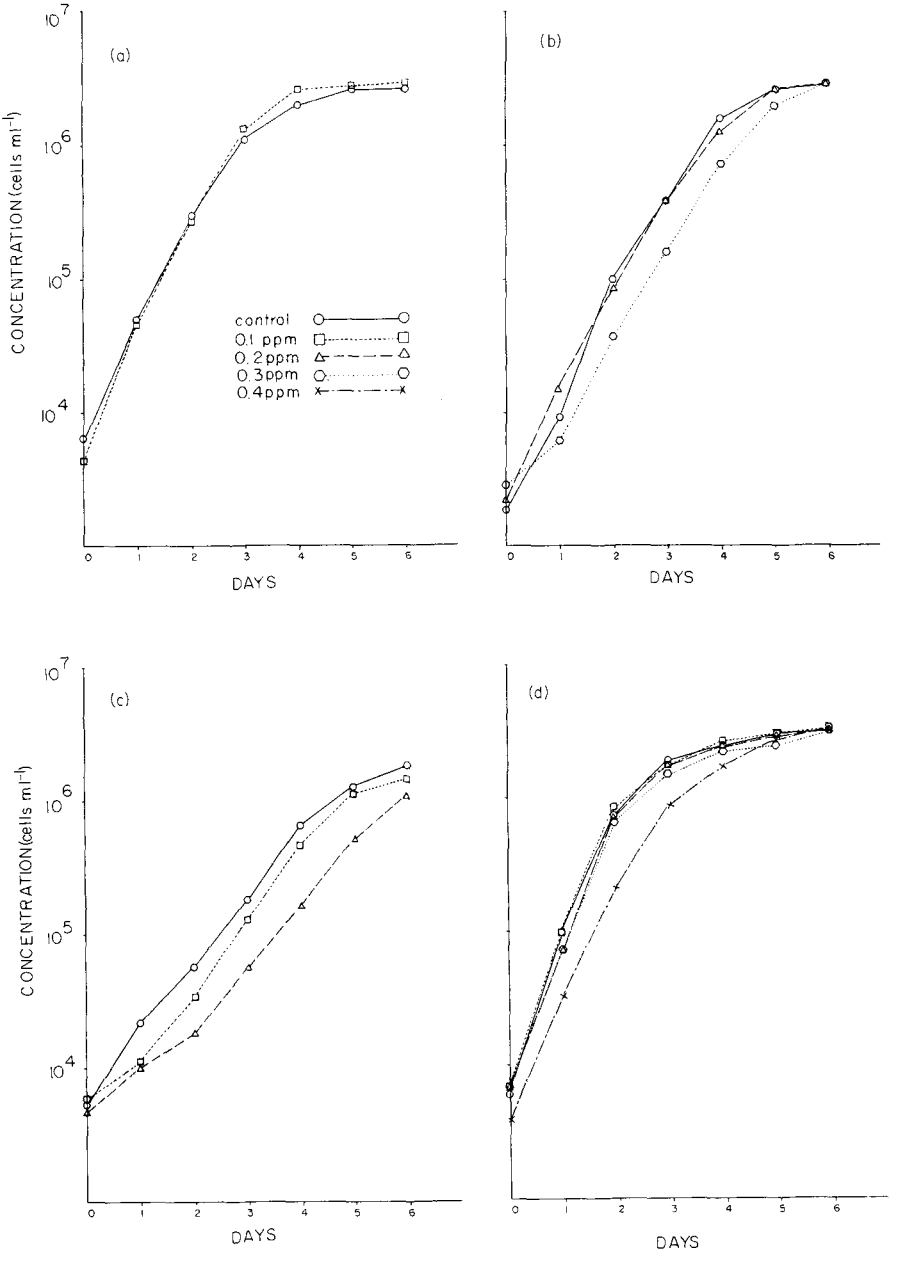

Fig. 1. Six day growth curves of unialgal (a) Skeletonema costatum Skel, (b) Skeletonema costatum Skel, (c) Thalassiosira pseudonana 13-1, and (d) Thalassiosira pseudonana 3H

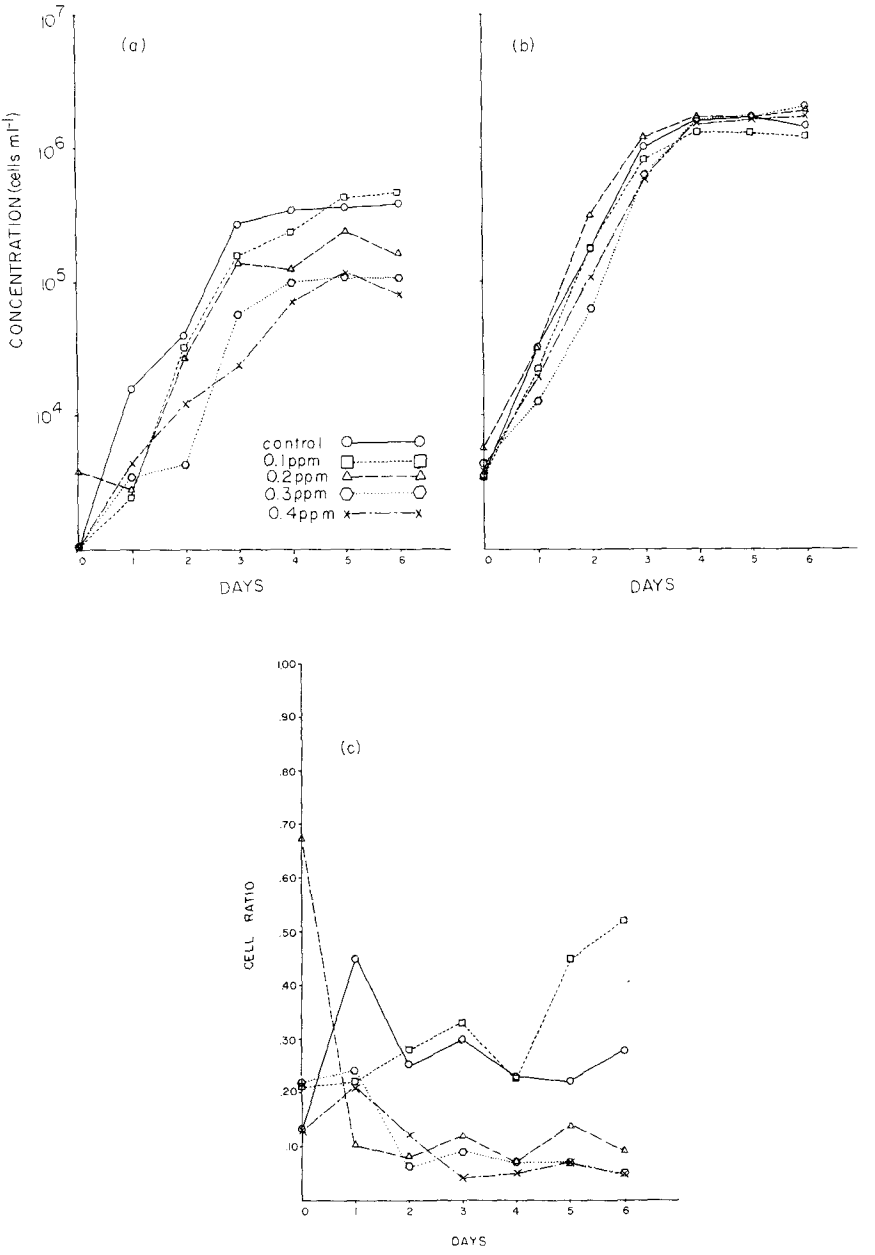

Fig. 2. (a) Six day growth curve of Skeletonema costatum GSS4 in mixed culture; (b) growth curve of Thalassiosira pseudonana 13-1 in mixed culture; (c) the ratio of S. costatum GSS4 to T. pseudonana 13-1

than the controls. The ratios in the 0.4 ppm cultures become significantly less than controls beginning on day 3 ($p<0.05$) and remain so through day 6 ($p<0.01$). The open ocean T. pseudonana 13-1 is clearly dominating S. costatum GSS4 isolated from the same environment and this dominance increases with the increase in MMH concentrations.

The three diatoms S. costatum Skel, T. pseudonana 13-1, and T. pseudonana 3H have SC and EC_{50} values summarized in Table 1. These values follow an expected sensitivity pattern of open ocean to estuarine phytoplankton. The open ocean diatom T. pseudonana 13-1 is the most sensitive to MMH followed by the coastal diatom S. costatum Skel and lastly the estuarine isolate T. pseudonana 3H.

Table 1. SC and EC_{50} values (6 d) for algae examined with MMH

Diatom	SC (ppm)	EC_{50} (ppm)
Skeletonema costatum Skel	0.3	0.3-0.4
Thalassiosira pseudonana 13-1	-	0.2-0.3
Thalassiosira pseudonana 3H	0.4	0.4-0.5
Dunaliella tertiolecta[a]	0.2	0.4
Selenastrum capricornutum[b]	0.2	0.4

[a] From Scherfig et al. (1977). Data is converted to ppm from ul liter^{-1}.

[b] From Dixon et al. (1979). Data is converted to ppm from ul liter^{-1}.

The SC and EC_{50} values for the marine diatoms examined are on the same order of magnitude as that for the marine green alga D. tertiolecta (Scherfig et al. 1977) and the freshwater green alga S. capricornutum (Dixon et al. 1979; Table 1). The open ocean diatom T. pseudonana 13-1 has the lowest EC_{50} value of all algal species tested with MMH. No green algae had a significant change from controls at the 0.1 ppm concentration (Scherfig et al. 1977; Dixon et al. 1979). T. pseudonana 13-1 would therefore be a more sensitive assay for low levels of MMH.

The marine diatoms studied here did not show any obvious increase in cell size as has been observed in green algae (Dixon et al. 1979; Scherfig et al. 1981). However, some cells at the higher

concentrations of MMH did appear to show a disruption of internal structure. The protoplasm in these cells appeared to be shrunken, detached from the cell wall. The addition of MMH has the net effect in temporarily inhibited cultures of causing a lag behind controls because such cultures have fewer viable cells initially than the controls.

Mixed cultures had growth at MMH concentrations which caused permanent inhibition of unialgal cultures. During the preparation of MMH dilutions for this run, the primary stock solution of MMH did not fume greatly under the hood as it did during the unialgal runs. It is strongly suspected that this primary stock solution of MMH was not 100% potent and for this reason it is not appropriate to compare the SC and EC_{50} values found in the mixed cultures to that of the diatoms in the unialgal cultures. Since the primary purpose of this mixed run is to compare species ratios, the results are still interpretable.

The results of the run with the the two Sargasso Sea isolates show that T. pseudonana 13-1 dominates S. costatum GSS4. The end of exponential growth on day 4 comes about when nutrients begin to become limiting to the algal populations or possibly an inhibitor is in affect. Nutrient limitation occurs sooner in mixed cultures than in unialgal cultures due to the greater numbers of cells initially. Since both species are from the same environment, one would expect them to have relatively equal ability to take up nutrients. The stable species ratios with time in this run reflects what one would expect in the environment.

The population of Thalassiosira in the 0.3 and 0.4 ppm cultures show some reduction in cell division until the end of exponential growth. The population of Skeletonema in the same cultures, on the other hand, remains significantly lower than the controls even in the decline of relative growth phase. The species ratio is significantly lower than controls at the higher concentrations of MMH (Fig. 2c). The dominance of Thalassiosira is increased at the higher MMH concentrations due to Skeletonema's apparent greater sensitivity to the rocket fuel. The reduction of the species ratio at the higher concentrations is greater support for the belief that an accidental spill of MMH would change species composition in a closed system for a period of time than is evidence from bioassays of unialgal cultures alone. Due to the rapid degradation of MMH, an accidental spill would probably have only a temporary impact on the area.

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