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24. The size of the sulfuric acid aerosols is crucial for determining their net radiation effect, since their impact on the troposphere through their effect on solar radiation is one of cooling while in the thermal infrared they cause a greenhouse warming, as pointed out by Pollack *et al.* (*7*). For the size distribution we have assumed, which is representative of the normal stratospheric aerosol (*15*), the thermal warming effect is only about one-fourth as large as the solar cooling. For a larger mean size, with the shape of the size distribution held fixed, the relative contribution of the greenhouse warming increases. On Venus the aerosols are large enough to yield a net warming and contribute substantially to the strong greenhouse effect on that planet (*31*). On the other hand, if the size distribution falls off toward larger particles faster than the log-normal distribution used in (*15*), the greenhouse warming will be decreased. In future measurements special efforts should be made to obtain not only the mean particle size, but also accurate data on the large-particle tail of the size spectrum.
25. On the basis of the graphs of K. H. Bathen [*J. Geophys. Res.* **77**, 7138 (1972)], we estimate the annual average global mixed-layer depth as about 70 m. The value 100 m is approximately the global average for the maximum mixed-layer depth during the year.
26. For the global average case we use an aerosol optical depth 0.625 times as large as that for the tropical case; that is, the maximum optical depth is 0.125 instead of 0.2. Observations (*8*) indicate that the maximum of 0.2 was applicable to about half the globe, with the maximum in the Northern Hemisphere about four times smaller. The temperature change ΔT in the troposphere for the global average case is reduced by more than the factor 0.625 for several reasons: (i) the average solar insolation is about 20 percent larger for the tropics than for the global average; (ii) the water vapor feedback ΔT is larger in the tropics than for the global average; and (iii) the inclusion of horizontal energy tranports out of the region of interest, with the absolute value of the transports kept fixed, slightly increases ΔT over that in the case of no transports.
27. Because of the difficulty in measuring the small climatic signal in the presence of substantial noise, or natural climatic fluctuations, a valuable complementary approach to the one we have taken is to statistically analyze the observed temperature changes afer all the large eruptions in recent historical times [C. Mass and S. H. Schneider, *J. Atmos. Sci.* **34**, 1995 (1977)]. Although the aerosol properties are not well known for the older eruptions, it is clear from Table 1 that a tropospheric cooling should be expected. Mass and Schneider do find statistical evidence for a posteruption cooling.
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Anilines: Selective Toxicity to Blue-Green Algae

Abstract. *The blue-green alga* Agmenellum quadruplicatum *(strain PR6) was very sensitive to aniline and* p*-toluidine (potential environmental toxicants) in an algal lawn assay (the growth of the algal lawn was inhibited with as little as 1 microgram of* p*-toluidine per disk). Assays with seven other species of blue-green algae showed that they had varying sensitivities ranging from 1 to 100 micrograms of* p*-toluidine. Under comparable conditions, 0.5 milligram or more of* p*-toluidine was needed to inhibit a green alga, a diatom, or two species of bacteria.* p*-Toluidine had no immediate effect on the photosynthesis or respiration of* A. quadruplicatum, *although growth was arrested and viability declined.*

Aniline and its methylated derivatives are commonly used in the manufacture of dyes and other organic chemicals. Although these types of compounds are known to be toxic to humans (*1*), very little is known about whether they are toxic to other living things. Thus, it is of interest that the toxicity of water-soluble extracts of fuel oils to blue-green algae is traceable to their content of toluidines (methylanilines) (*2*). In this report we further characterize the response of blue-green algae to toluidines and other methylated anilines.

The blue-green algae tested here can be divided into two groups on the basis of their resistance to *p*-toluidine (Table 1). Four species were completely inhibited by 10 μg of *p*-toluidine per disk. The sensitivity of three of these species persisted to as little as 1 μg of *p*-toluidine per disk, with an evident effect seen as smaller colonies beyond the zone of inhibition. Four other blue-green algae required 100 to 500 μg of *p*-toluidine per disk for complete inhibition. Using a different assay technique, Fitzgerald *et al.* (*3*) reported a 50 percent kill of the blue-green alga *Microcystis aeruginosa* with aniline at 20 parts per million (ppm); a 100 percent kill occurred with *p*-aminodimethylaniline (2 ppm) and *p*-aminodiethylaniline hydrochloride (1 ppm). By comparison, the green alga *Chlorella autotrophica* (strain 580) was not inhibited at 500 μg of *p*-toluidine per disk. Similarly, Smirnova *et al.* reported that *Scenedesmus obliquus*, also a green alga, was not affected by *o*-toluidine (*4*). A diatom, *Cylindrotheca* sp. (strain N1), showed only very slight inhibition at 500 μg of *p*-toluidine per disk. Two test bacteria showed no inhibition, even at 1000 μg per disk.

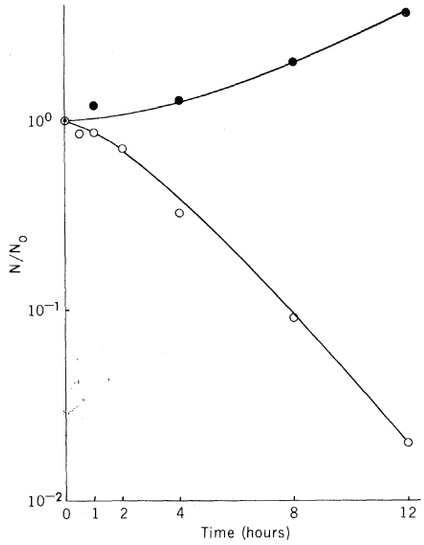

Fig. 1. Viability of *Agmenellum quadruplicatum* (strain PR6) after exposure to *p*-toluidine (500 ppb) in medium ASP-2; N is the cell number in suspension at time t; N_0 is the original cell number in suspension. *p*-Toluidine was added to the culture (1×10^7 cells per milliliter) at zero time; incubation continued at 30°C, with F40CWX lamps, under continuous aeration with 1 percent CO_2 in air. One-tenth of a milliliter of appropriately diluted cell suspensions was plated out on medium ASP-2 plus 1 percent agar and was incubated as described in Table 1. (Closed circles) Control culture; (open circles) culture plus *p*-toluidine.

We tested aniline, toluidines, and other substituted anilines further in order to better establish the spectrum of toxicity of this group of compounds toward a sensitive blue-green alga, *Agmenellum quadruplicatum* (strain PR6), a marine coccoid form. The data (Table 2) show that strain PR6 was about as sensitive to aniline as to *p*-toluidine. Both compounds completely inhibited growth at 10 μg per disk. Of the other compounds tested, complete inhibition of the alga occurred at 100 μg except for those compounds with substituents on the nitrogen atom. *N*-Methylaniline and *N*-ethylaniline were much reduced in toxicity, causing only slight inhibition at 500 μg per disk. *N*,*N*-Dimethylaniline was not toxic even at 1000 μg per disk. This result suggests that the toxicity of the *p*-toluidine and aniline molecules is associated with the amino group. The reduced toxicity of 6-ethyl-*o*-toluidine may simply be due to steric hindrance of the amino group. By comparison *p*-cresol, commonly found in water extracts of fuel oils (*2*), caused little inhibition of strain PR6. Biphenyl, Aroclor 1242 and Aroclor 1254 (Aroclor compounds are polychlorinated biphenyls), and dimethyl-

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phthalate, chemicals whose presence and concentrations in the environment are of such concern, also showed little inhibition of strain PR6 at the initial concentration tested.

The algal lawn response of strain PR6 to *p*-toluidine was closely mimicked in liquid culture. Cultures containing 500 parts per billion (ppb) ($4.7 \times 10^{-6}M$) did not grow. The well-known inhibitor of photosynthesis, dichlorophenyl dimethyl urea (DCMU), when tested under similar conditions, was lethal to strain PR6 at a concentration of $2 \times 10^{-6}M$. Thus *p*-toluidine should be considered a very potent inhibitor for certain blue-green algae.

The toxicity of *p*-toluidine was not influenced by the addition of complex materials to the medium. Supplements of yeast extract, Soytone, or casein hydrolyzate at 1 g/liter in either liquid or agar cultures had no effect.

The addition of *p*-toluidine (50 ppb) during exponential growth in a liquid culture of strain PR6 (*2*) resulted in a bending of the growth curve to a plateau within 4 hours (about the time needed for the growth of one generation); upon continued incubation (24 to 48 hours) the cultures bleached severely, becoming white.

The viability curve shown in Fig. 1 agrees closely with observations noted in growth curves of liquid cultures. Within a 2- to 4-hour period after the introduction of *p*-toluidine, viability began to drop significantly. This time for the viability decrease corresponds to the period of time during which the growth rate fell to zero.

Experiments in which the rates of photosynthesis and respiration (O_2 electrode) of strain PR6 were monitored revealed only a gradual decline of these processes over the time period coinciding with the loss of viability. Cultures of strain PR6 incubated for 18 hours in darkness with *p*-toluidine showed a similar response (based on decay kinetics) after being returned to the light. We interpret this result to mean that, for an expression of the toxicity of *p*-toluidine, growth must occur. *Nostoc* sp. (strain Mac), which readily shifts from photoautotrophic growth to growth in the dark with glucose, was equally sensitive to *p*-toluidine under either condition. Table 1 shows that the response of *Anabaena* sp. (strain CA) to *p*-toluidine was not changed when grown in a medium with nitrate as the nitrogen source or in a nitrogen-free medium. Likewise, with another nitrogen-fixing blue-green alga, *Anabaena cylindrica*, there was no immediate effect of *p*-toluidine on acetylene reduction. Thus *p*-toluidine does not have an immediate effect on photosynthesis, respiration, or nitrogen-fixation.

Aniline is known to inhibit protein synthesis in vitro by reducing the activity of messenger RNA (*5*). However, protein synthesis of strain PR6 as measured by whole cell uptake of [U-^{14}C]leucine (Amersham/Searle; specific activity of 330 mCi/mmole) was not inhibited by *p*-

Table 1. Response of eight blue-green algae, a green alga, a diatom, and two bacteria to *p*-toluidine. Algal lawns were initially seeded with $1 \pm 0.2 \times 10^5$ cells per milliliter in 1 percent agarized (Difco 0140) medium. *p*-Toluidine (from Chem Service Inc. or MC&B Manufacturing Chemists) in absolute ethanol was absorbed on antibiotic sensitivity disks (12.7 mm; Schleicher and Schuell, No. 740-E; washed by autoclaving in distilled water), which were placed directly on the agar surface. Numbers represent the zone of growth inhibition in millimeters from the edge of the disk: 0 indicates no inhibition, 36 indicates complete inhibition. A number in parentheses indicates a zone of smaller colonies (partial inhibition) this distance from the edge of the disk; NT, not tested. No inhibition was seen in ethanol controls. *Nostoc* sp. (strain Mac), *Fisherella* sp., and *Eucapsis* sp. were grown in medium Cg10 (*12*); all other algae were grown in medium ASP-2 (*2, 13*). *Nostoc* sp. (strain Mac) is an isolate of Bowyer and Skerman (*14*); *Chlorella autotrophica* (strain 580) was obtained from R. L. Guillard. All other algae are isolates of this laboratory. *Escherichia coli* (strain 786) and *Staphylococcus epidermidis* (strain 673), obtained from the Microbiology Department, University of Texas at Austin, were grown in 1.5 percent agarized nutrient broth (Difco). The petri dish cultures were sealed with Scotch Tape and incubated in light from a tungsten lamp for 3 to 7 days at 28° to 30°C. The radius of growth inhibition around the disk was judged visually and microscopically.

Organism	*p*-Toluidine (μg per disk)					
	0	0.1	1	10	100	500
Blue-green algae						
Coccochloris elabens, strain 17a	0	0	13	36	36	36
Eucapsis sp.	0	0	11 (36)	36	36	36
Agmenellum quadruplicatum, strain PR6	0	0	6 (10)	36	36	36
Oscillatoria williamsii, strain Mev	0	0	0	36	36	36
Anabaena sp., strain CA	0	0	0	2	36	36
Anabaena sp., strain CA*	0	0	0	0	36	36
Fisherella sp.	0	0	0	0	8	36
Nostoc sp., strain Mac	0	0	0	0	0 (6)	36
Microcoleus chthonoplastes, strain BA-1	0	0	0	0	0 (10)	36
Diatom						
Cylindrotheca sp., strain N1	0	NT	NT	NT	NT	7
Green alga						
Chlorella autotrophica, strain 580	0	NT	NT	NT	NT	0
Bacteria						
Escherichia coli, strain 786	0	0	0	0	0	NT†
Staphylococcus epidermidis strain 673	0	0	0	0	0	NT†

*Medium ASP-2 minus the nitrogen source, $NaNO_3$. †No inhibition at 1000 μg per disk.

Table 2. Effect of toluidines, aniline, substituted anilines, and other compounds on the growth of the blue-green alga *Agmenellum quadruplicatum* (strain PR6). The algal lawn technique was used as described in Table 1. Other dimethylaniline isomers tested which gave complete inhibition at 100 μg or 500 μg were those with methyl groups at positions 2,3-, 2,5-, 3,4-, and 3,5- on the ring. Data for biphenyl and *p*-cresol were taken from Pulich *et al.* (*15*) and Winters *et al.* (*2*), respectively.

Compound	Concentration (μg per disk)						
	0	0.1	1	10	100	500	1000
p-Toluidine	0	0	6 (10)	36	36	36	36
o-Toluidine	0	0	0	15 (20)	36	36	36
m-Toluidine	0	0	0	0 (3)	36	36	36
Aniline	0	0	0 (8)	36	36	36	36
N,*N*-Dimethylaniline	0	0	0	0	0	0	0
N-Methylaniline	0	0	0	0	0	15	36
N-Ethylaniline	0	0	0	0	0	2	10
o-Ethylaniline	0	0	0	4 (36)	36	36	36
6-Ethyl-*o*-toluidine	0	0	0	0	0	11	20 (36)
2,4-Dimethylaniline	0	0	0	10 (15)	36	36	36
2,4,6-Trimethylaniline	0	0	0	0	0 (36)	36	36
Aroclor 1242	0	NT	NT	NT	2	NT	7
Aroclor 1254	0	NT	NT	NT	0	NT	0
Dimethylphthalate	0	NT	NT	NT	0	NT	11
Biphenyl	0	NT	NT	NT	NT	0	4
p-Cresol	0	NT	NT	NT	NT	0	8

toluidine. Moreover, uptake of [2-^{14}C]uracil (Schwarz/Mann; specific activity of 62 mCi/mmole) (*6*) was blocked by proflavine hemisulfate ($1.9 \times 10^{-5}M$; Sigma), a known inhibitor of RNA synthesis (*7*), but was not blocked by *p*-toluidine ($4.7 \times 10^{-5}M$). Thin-layer chromatography of benzene extracts of supernatants from cultures labeled with *o*-[U-^{14}C]toluidine (California Bionuclear Corporation; specific activity of 13.7 mCi/mmole) showed no formation of other ^{14}C-labeled compounds. Stepwise degradation of cells labeled with *o*-[^{14}C]toluidine according to the method of Pelroy *et al.* (*8*) did not reveal specific labeling of any particular cell fraction.

None of these lines of investigation gave any clear-cut indication of the metabolic process affected by *p*-toluidine. However, lack of any immediate effect and the gradual loss of viability suggest that the metabolic process affected is a slow one approaching the generation time.

In general, the blue-green algae are much more sensitive to aniline and *p*-toluidine than the bacteria and other algae (Table 1). In the case of three blue-green algae the sensitivity to *p*-toluidine was remarkable. The data herein demonstrate the extreme sensitivity of strain PR6 (growth inhibition by *p*-toluidine and aniline at 1 μg per disk in the algal lawn assays and 50 ppb in liquid culture). In view of the lack of information on the concentrations and fate of anilines in the environment, it is of interest to note recent reports of aniline and substituted anilines in water-soluble extracts of fuel oils (*2*), industrial effluents (*9*), and soils as degradation products of herbicides (*10, 11*). Aniline residues are also known to be precursors of azobenzenes (*11*). Games and Hites (*9*) reported that the concentration of aniline in the final effluent of a dye-manufacturing plant was 10 to 96 ppb, well within the toxicity range of organisms such as strain PR6. Clearly, more information is needed about the biological effects of these compounds in order to better understand their impact on the environment.

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Assembly of Greek Marble Inscriptions by Isotopic Methods

Abstract. *Classical Greek inscriptions cut in marble, whose association as original stelai by archeological methods was debatable, were selected for study. Using traditional geological techniques and determinations of the per mil increments in carbon-13 and oxygen-18, it was determined that fragments could be positively assigned to three stelai, but that fragments from three other stelai had been incorrectly associated.*

The provenance of marble used in classical Greece for inscriptions, sculpture, and other monuments is still a leading problem in archeology, despite at least 100 years of research and debate in the field (*1*). In describing inscriptions, archeologists commonly give a statement of provenance along with the dimensions and some physical attributes of the stone. The epigraphic literature, however, is full of cases where different authors describe the same stone in contradictory or confusing terms. For example, sample IG I^2, 732 has been called "weisse," "Hymettian," and "Pentelic" in separate publications (*2*, p. 72). In an attempt to develop a descriptive terminology, without implying provenance, the use of petrographic attributes has been suggested (*2*). These attributes include both the structural elements present—that is, foliation and lineations—as well as other physical properties such as color, grain size, and the presence of accessory minerals.

In addition to these observations, which can be made with only a hand lens, isotopic ratios of oxygen and carbon have been found useful (*3*). Systematic differences in these isotopic ratios were found in samples collected from the principal quarries of classical Greece, and these different values could be seen to characterize each quarry area. Samples from ancient quarries of Paros, Naxos, Penteli, and Hymettus were analyzed and the results plotted as the per mil increment in ^{13}C (δ^{13}C) against the per mil increment in ^{18}O (δ^{18}O) [figure 1 in (*3*)]. Using isotopic ratios, a provenance can be inferred for a particular classical Greek marble; a sample of only 10 to 20 mg is needed for this type of analysis (*4*).

In addition to provenance, isotopic ratios of stone fragments bearing different inscriptions can help decide whether the pieces belonged to the same stele; a small difference in isotopic ratios would suggest that the fragments were originally parts of the same material. Although we have not yet determined the variation within a particular block of marble or the possible effects of slight surficial weathering, our data indicate that surficial samples from the same marble block may have a difference of up to 0.4 per mil in both δ^{18}O and δ^{13}C. If two samples

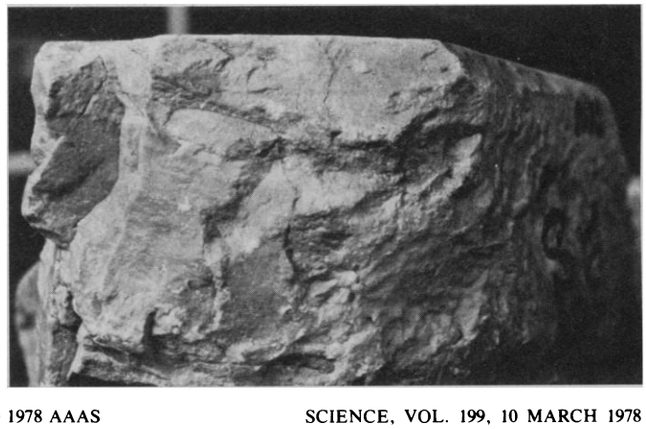

Fig. 1. Sample EM 8682, oriented with the top to the viewer. The stone is 18 cm thick. Color variation in bluish grays makes up the planes of layering, dipping to the right for EM 8682 (shown) and to the left for EM 8680 (not shown).

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