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Acute Toxicity to *Selenastrum capricornutum* of Aromatic Compounds from Coal Conversion

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Synthetic fuels from coal conversion processes, and wastewaters produced by these technologies, contain a diverse array of organic compounds. The environmental behavior of some of these compounds, such as the aliphatic hydrocarbons, is fairly well known, but many others, particularly aromatics, have not been tested for their ecological effects (HERBES et al. 1976). Since the potential exists for coal-derived organics to be introduced into aquatic ecosystems, either accidentally or through effluent releases, information about the effects of these compounds on aquatic organisms is needed. This paper presents results of experiments on the effects of aromatic compounds on photosynthesis by a freshwater alga.

The first objective of these experiments was to screen rapidly several classes of common aromatic compounds, to identify those which are most toxic to the alga. The classes tested were unsubstituted aromatic hydrocarbons, aromatic quinones, aromatic amines, methylated aromatics, phenols, azaarenes, and thiophenes. Because aromatics containing 4 or more rings are practically insoluble in water, even solutions saturated with such aromatics are generally not acutely toxic to aquatic organisms (HERBES et al. 1976). For this reason, I tested only 1-, 2-, and 3-ring compounds.

My second objective was to determine ranges of toxic concentrations for the compounds tested, to facilitate further toxicological studies. Each compound was therefore tested over 3-5 orders of magnitude of concentration.

Preliminary screening of potential toxicants requires a simple and rapid means of detecting the response of the test organism. Short-term photosynthetic inhibition was the measure of response in the experiments described here. Compounds found to inhibit algal photosynthesis at the lowest concentrations (relative to the other compounds tested) will be subjected to a battery of toxicity tests, including both short-term (acute) and long-term (chronic) exposures of the compounds to algal cultures and natural algal communities. This battery of tests will form the basis for predicting the effects of each compound on algal communities and, potentially, on the aquatic ecosystem.

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MATERIALS AND METHODS

Algal Cultures. A non-axenic unialgal culture of Selenastrum capricornutum Printz was obtained from the EPA National Environmental Research Laboratory at Corvallis, Oregon. Cultures were maintained in sterile-filtered MBL medium (STEIN 1973) without vitamins. They were grown at 24°C and illuminated by Cool-White fluorescent lights (0.017 ly min^{-1} Photosynthetically Active Radiation) for 16 h per day. Cultures were transferred weekly.

Toxicant Preparation. Dilution series of each toxicant were prepared using fresh, sterile-filtered MBL medium. Soluble compounds were tested at nominal concentrations of 10, 100, and 1000 mg/L. For compounds whose solubility was less than 1000 mg/L, saturated solutions were prepared by adding excess compound to sterile medium and stirring overnight; the solutions were then filtered (Whatman #41 filter paper) and diluted appropriately. These compounds were tested at 1, 10, and 100% saturation. Four compounds (benzoquinone, naphthoquinone, naphthol, and naphthylamine) produced greater than 25% photosynthetic inhibition at the lowest concentrations tested; three of these compounds were re-tested at successively lower concentrations.

Reagent grade chemicals were used except for benzoquinone and naphthoquinone, which were practical grade. Insoluble compounds were washed three times in distilled water before use to remove soluble impurities. For this rapid screening, I did not consider it necessary to measure the actual concentration of each solution or to determine the degree of purity by analysis. Test compounds may have been partially degraded (by UV light, hydrolysis, or microbial activity) during an experiment, but the degradation would probably have been insignificant over a 4-h period. Degradation before the experiment began would also be minimal, since the solutions were sterile and were not exposed to UV light. The possibility of extremely toxic impurities or degradation products influencing the results cannot be discounted, and this possibility will be tested when more detailed toxicological studies are undertaken.

Test Procedure. BOD bottles (125-mL) were filled with toxicant solutions prepared as described above. One mL was then removed from each bottle to leave room for the algal suspension. A portion of a rapidly-growing S. capricornutum culture was concentrated by centrifugation (1500 rpm, 25 min) to approximately 1.25×10^7 cells/mL, and 1 mL of this suspension was added to each test bottle. The bottles were placed on a rotary shaker at 100 rpm in an environmental chamber (24°C, 0.024 ly min^{-1} P.A.R.). After 2 h, 0.2 mL of an $NaH^{14}CO_3$ solution (10 μCi/mL, New England Nuclear) was added to each bottle. After an additional 2 h incubation, biological activity was stopped by the addition of 0.3 mL formalin.

Photosynthesis was determined by measurement of fixed ^{14}C as follows. Two 5-mL samples were taken from each bottle and placed in 20-mL liquid scintillation vials. Each sample was acidified with 0.1 mL of 0.1 N HCl and bubbled with air for 20 min to remove inorganic carbon (SCHINDLER et al. 1972, as modified by THEODORSSON & BJARNASON 1975). Each vial then received 15 mL Aquasol (New England Nuclear), and the activity remaining in the samples was counted.

Each toxicant was tested at three concentrations, with two replicates per concentration. Three control bottles, without toxicant, were included in each experiment. Carbon-14 fixation in the test solutions was expressed as a percentage of the mean of the controls.

RESULTS AND DISCUSSION

Results are summarized in Table 1. Benzoquinone and naphthoquinone were the most toxic compounds tested. Anthraquinone, presumably due to its insolubility, was essentially non-toxic to S. capricornutum, as were all of the other 3-ring compounds except acridine. Aromatic amines (aniline and naphthylamine) were highly toxic, as was naphthol. Thiophenes and azaarenes were only slightly toxic except at the highest concentrations. The other classes of aromatic compounds were intermediate in toxicity. In all cases, 2-ring compounds were more toxic than related 1-ring compounds. These results are in agreement with HERBES et al. (1976), who reported that within a homologous series of aromatic compounds, toxicity to aquatic organisms generally increased with increased ring number.

My results on the relative toxicities of the compounds tested are in agreement with previously published results, in the few instances where comparisons are possible. BRINGMANN and KUHN (1978) found quinone among the most toxic, and pyridine among the least toxic, of 180 compounds tested with the green alga Scenedesmus quadricauda. Quinone and aniline were reported to be highly toxic to algae by FITZGERALD et al. (1952) and by MCKEE and WOLF (1963). My dose-response data for benzene, toluene, and naphthalene are similar to those obtained by KAUSS et al. (1973) for the growth of Chlorella vulgaris. The slightly greater toxicity of methylnaphthalene compared with naphthalene, and the non-toxicity (to green algae) of phenanthrene, agree with the results of PULICH et al. (1974).

On the basis of these results, quinones and several 2-ring aromatics have been selected for a series of more detailed experiments in which toxicity will be measured under a range of ecological conditions in the laboratory and the field. The results of the preliminary screening and range-finding tests are not sufficient for predicting environmental impacts of coal conversion facilities or for setting water quality standards,

TABLE 1

Carbon-14 fixation by Selenastrum capricornutum in the presence of selected aromatic compounds, expressed as percent of controls (standard deviation in parentheses).

Compound	# of rings	Maximum concentration	Relative concentration[a] 1	10	100
Quinones					
p-benzoquinone	1	10 mg/liter	37(0.7)	17(1.4)	7(0.2)
		1000 mg/liter	13(0.7)	1(0.1)	2(0.0)
1, 4-naphthoquinone	2	1% saturation	83(0.7)	59(0.0)	14(4.2)
		100% saturation	9(0.0)	3(1.8)	2(1.2)
1, 4-anthraquinone	3	100% saturation	97(4.9)	91(2.8)	85(4.2)
Amines					
aniline	1	1000 mg/liter	90(4.9)	34(2.1)	3(0.9)
1-naphthylamine	2	1% saturation	119(4.2)	98(11.3)	63(7.8)
		100% saturation	64(0.7)	16(12.0)	3(2.1)
Phenols					
phenol	1	1000 mg/liter	105(0.7)	92(4.9)	19(3.5)
2-naphthol	2	100% saturation	64(4.2)	12(1.4)	3(0.3)
Methylated Aromatics					
toluene	1	100% saturation	91(8.5)	96(0.7)	3(0.2)
1-methylnaphthalene	2	100% saturation	96(11.3)	71(6.4)	7(6.4)
Hydrocarbons					
benzene	1	1000 mg/liter	95(3.5)	84(10.6)	5(1.2)
naphthalene	2	100% saturation	110(2.1)	89(0.0)	15(2.1)
anthracene	3	100% saturation	99(0.0)	104(1.4)	99(2.1)
phenanthrene	3	100% saturation	96(b)	104(b)	76(1.4)
Thiophenes					
thiophene	1	1000 mg/liter	95(1.4)	97(2.1)	68(12.0)
thianaphthene	2	100% saturation	89(4.9)	82(4.9)	4(1.3)
dibenzothiophene	3	100% saturation	94(11.3)	101(3.5)	80(7.0)
Azaarenes					
pyridine	1	1000 mg/liter	67(0.7)	86(4.2)	80(0.7)
quinoline	2	1000 mg/liter	84(16.3)	73(2.8)	6(2.0)
acridine	3	100% saturation	92(0.7)	75(3.5)	1(0.2)

[a]Percent of maximum concentration.

[b]One replicate lost; no standard deviation.

because the response of natural algal communities to contaminants is determined by many biotic and abiotic factors not considered here. However, the short-term photosynthesis tests did succeed in their objective of revealing broad trends in the toxicity of the aromatics tested.

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