Bull. Environ. Contam. Toxicol. (1986) 36:114–121

Environmental Contamination and Toxicology

Growth and Photosynthetic Response of a Freshwater Alga, *Selenastrum capricornutum,* to an Oil Shale By-Product Water

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Toxicological research and development of effective control technology are needed to identify and minimize the environmental impact of complex by-product waters generated during in situ recovery of shale oil. The by-product waters, originating primarily from combustion, dehydration of minerals, and groundwater, contain a broad spectrum of inorganic and organic substances (Jackson et al. 1975) which may pose a biological hazard. Separation of by-product water constituents into organophilic and hydrophilic subfractions provides a useful approach for identifying toxic compound classes (Parkhurst et al. 1979), as well as predicting their physiological and environmental fates. For example, the organophilic fraction has the greater bioaccumulation potential, due to its higher lipid affinity. The hydrophilic fraction has greater environmental mobility, owing to its lower soil affinity (Leenheer and Stuber 1981).

Several recent studies have focused on toxic effects of various oil shale waters to freshwater algae (e.g., Cleave et al. 1980; McKnight et al. 1983). Algal bioassays are ecologically significant, since algae are the dominant primary producers in most freshwater environments. Furthermore, algae have been shown to be more sensitive to complex wastes than fish or invertebrates (Miller et al. 1978). Using a standard algal species provides a systematic approach to toxicant evaluation. One such species is Selenastrum capricornutum Printz, a freshwater unicellular green alga (APHA 1981).

A strategy to assess the hazards of complex effluents should include a battery of rapid, easily conducted, predictive tests within the larger framework of tier testing. Combining chemical fractionation with short-term bioassays is a relatively new approach in the screening of complex environmental mixtures (Waters et al. 1983). This approach is used in this study to compare the growth and photosynthetic response of S. capricornutum to an oil shale by-product water and its organophilic and hydrophilic subfractions.

MATERIALS AND METHODS

Test waters included a filtered oil shale by-product water (Whole), designated 150 Ton-R17, and its organophilic (OP) and hydrophilic (HP) subfractions. The by-product water was produced in a simulated in situ retort operated by Western Research Institute in Laramie, Wyoming. Detailed information on its collection, storage, and chemical analysis has been compiled (LETC 1980; Ahern 1982). Selected chemical parameters for the 150 Ton-R17 retort water are listed in Table 1. Separation of the whole oil shale water into its OP and HP subfractions was based on a solvent extraction method (Tobben et al. 1982). The whole water was pressure-filtered through a 0.45-μm nylon membrane, adjusted to pH 7.0, and extracted with methylene chloride (CH_2Cl_2) in a liquid-liquid extractor for 18 hours. The resultant organic and aqueous phases were separated and designated OP and HP, respectively. The OP fraction constituents were transferred into aqueous phase for biological testing by the addition of water and evaporation of CH_2Cl_2. Residual CH_2Cl_2 was removed from both OP and HP fractions by gently purging with nitrogen gas while stirring for 90 minutes. A distilled water control was prepared using the same separation scheme.

A non-axenic unialgal culture of Selenastrum capricornutum was obtained from the American Type Culture Collection (Rockville, MD). Unialgal cultures were grown in filter-sterilized nutrient medium (Miller et al. 1978) in a controlled-environment incubator-shaker (Model G27, New Brunswick Scientific). Light intensity was measured as photosynthetically active radiation (400-700 nm) with a light meter (Model LI-185A, Lambda Instruments), equipped with a quantum sensor. Cultures were transferred weekly to maintain cells in log-phase growth. Cells were counted in a hemacytometer (Bright Line, American Optical Scientific) in order to quantify inoculum density.

Table 1. Selected chemical parameters of a filtered (0.45 μm) oil shale by-product water (150 Ton-R17)[a]

Parameter	Concentration (mg/L)
Sodium	270
Chloride	2260
Zinc	0.16
Copper[b]	0.12
Ammonia-Nitrogen	1250
Phenolic Compounds[b]	26
Inorganic Carbon	1161
Organic Carbon	3268
Total Dissolved Solids	5240
pH (standard units)	8.65

a LETC (1980)
b Ahern (1982)

In growth experiments, algae were grown in a series of paired test and control water concentrations in triplicate. These concentrations included 0.1, 0.5, 1.0, 2.0, 3.0, 5.0, and 10% of either oil shale by-product water (test solution) or distilled water (control solution) with nutrient medium as the diluent in all cases. Erlenmeyer flasks, fit with foam plugs to permit gas exchange (Miller et al. 1978), were filled with 50 mL of test or control solution and inoculated to yield an initial density of 2.0 x 10^4 cells/ mL. Tests were conducted under conditions of constant temperature (24°C), continuous lighting (71 μEinsteins/m^2 sec), and continuous shaking (100 rpm). Flask positions within the growth chamber were randomized. After 120 hours, cells were harvested by vacuum filtration on tared 0.45-μm membranes. Filters were dried overnight at 70°C, cooled in a desiccator, and weighed. Algal dry weight was then determined as the test end point.

In photosynthesis experiments, algae were incubated in a series of paired test and control water concentrations in duplicate. These concentrations included 0.1, 0.5, 1.0, 2.0, 3.0, 5.0, 10, 20, and 40% of either oil shale by-product water (test solution) or distilled water (control solution) with nutrient medium as the diluent in all cases. Glass-stoppered BOD bottles were filled with 20 mL of test or control solution and inoculated to yield a density of 2.0 x 10^5 cells/mL. The test conditions maintained were constant temperature (24°C) and continuous lighting (84 μEinsteins/m^2 sec). Bottles were not shaken, since results are unaffected by agitation during this short-term bioassay (Giddings et al. 1983). Bottle positions within the growth chamber were randomized. After two hours incubation, 50 μL $NaH^{14}CO_3$ (10 μCi/mL, New England Nuclear) were added to each bottle and the contents were swirled to disperse the radioisotope. After another two hours incubation, biological activity was terminated by adding 100 μL formalin. Photosynthetic rate was determined by measurement of fixed C^{14}. A 5-mL aliquot was transferred from each BOD bottle into a glass scintillation vial. The contents of each vial were then acidified (pH<2) with 100 μL concentrated HCl and gently bubbled with air for 15 minutes in order to remove unassimilated ^{14}C. Fixed ^{14}C was determined by adding 10 mL of scintillation fluid (Aquasol, New England Nuclear) to each vial, mixing the contents by shaking, and radioassaying each sample with a liquid scintillation counter (Model LS 2800, Beckman Instruments). Counts per minute, corrected for background, quenching, and total inorganic carbon content of samples (Giddings et al. 1983), served as the test end point.

Significant differences between test-control pairs were evaluated with two-tailed Student's t-tests ($p<0.05$). Results were then converted to percentage of control for each concentration. Median effective concentration (EC50), the toxicant concentration inhibiting growth or photosynthesis by 50%, was determined for each test water (Whole, OP, HP) with logarithmic regressions of test water concentration vs. percent effect. Only percent effects greater than zero and less than 100 were used to construct

regressions. Correlation coefficients, ranging from 0.83 to 0.98, were highly significant ($p<0.001$) for regressions. Ninety-five percent confidence limits for EC50 values were also calculated.

RESULTS AND DISCUSSION

Based on EC50 values in Table 2, toxicity ranked as follows: OP>HP>Whole (growth experiment) and HP>Whole>OP (photosynthesis experiment). Comparison of EC50 data indicates somewhat similar responses for the whole water and HP fraction both within and between experiments. The five-day growth experiment proved to be a much more sensitive measure of OP toxicity than the four-hour photosynthesis test, indicating increased OP toxicity over time.

The finding that the whole water was not the most toxic of the three test waters in either the growth or photosynthesis experiment (Table 2) implies an antagonistic interaction between constituents of the two subfractions. The whole oil shale water contained heavy metals, phenolic compounds, and relatively large amounts of ammonia, ionic salts, inorganic and organic carbon, and total dissolved solids (Table 1). A preliminary characterization of chromatographable carbon in HP and OP fractions has been presented by Delistraty et al. (1983). Approximately 66% of the organic content and all of the inorganic material, including ammonia, heavy metals, and ionic salts, contained in the whole water partitioned to the HP fraction. HP organics were dominated by aliphatic carboxylic acids. The OP fraction contained the remaining organic material, including phenolic compounds.

HP toxicity can be partly attributed to inorganic constituents. Avron (1960) reported that ammonium chloride uncouples photophosphorylation in isolated chloroplasts at an EC50 of 32 mg/L (8.4 mg/L NH_3-N). Based on data in Tables 1 and 2, ammonia-nitrogen was nominally 12.5 mg/L at an HP EC50 of 1.0%. Therefore, ammonia may have been primarily responsible for toxicity. Zinc and copper have been shown to inhibit growth of S.

Table 2. Median-effective concentrations (EC50) with 95% confidence limits (CL) of an oil shale by-product water (Whole) and its organophilic (OP) and hydrophilic (HP) subfractions, as estimated from growth and photosynthesis experiments with Selenastrum capricornutum

Experiment	Test Water	EC50 (%)	(CL)
Growth	Whole	1.7	(1.4 - 2.1)
	OP	0.22	(0.16 - 0.30)
	HP	1.0	(0.79 - 1.3)
Photosynthesis	Whole	2.9	(2.6 - 3.2)
	OP	30	(23 - 38)
	HP	1.9	(1.1 - 3.5)

capricornutum at 0.03 and 0.05 mg/L, respectively (Bartlett et al. 1974). Since zinc and copper levels (Table 1) at the HP EC50 (Table 2) were well below the toxic threshold, these metals could not be directly responsible for the observed toxicity.

The source of OP toxicity appeared different in growth and photosynthesis tests. Several phenolics have been shown to inhibit algal growth after 80 hours at concentrations as low as 5-10 mg/L (Dedonder and Van Sumere 1971). Based on OP EC50 values (Table 2), phenolic compounds (Table 1) were within this toxic concentration range in the photosynthesis test but were approximately two orders of magnitude below the toxic range in the growth experiment. Although this does not exclude the possibility that low levels of phenolics exerted toxicity in the growth experiment, it suggests that other OP compounds were primarily responsible for the observed toxicity.

Despite differences in bioassay design (i.e., species, endpoint) and test water preparation (i.e., filtration, pH adjustment), several studies have demonstrated that 150 Ton-R17 whole water exerts measurable inhibition in the concentration range of 1 to 5%. In an assay which monitored a reduction in bacterial bioluminescence, the five-minute EC50 was 1.3% for the filtered whole water (Delistraty 1984). Smith-Sonneborn et al. (1983) reported a 24-hour median lethal concentration (LC50) of 5% unfiltered 150 Ton-RI7 water to the ciliated protozoan Paramecium tetraurelia. Reductions in dry weight and leaf area have been observed in five grass plant species grown over a 10-week period in 5% filtered 150 Ton-R17 water (Skinner et al. 1981). These studies support the whole water toxicity evaluation in this study. Since industry plans incorporate provisions for upgrading the quality of oil shale waters (e.g., Ashland Oil 1976), release of raw by-product waters would occur only through accidental spills. The ecological impact of such spills on aquatic systems would, in turn, be largely dependent on dilution with natural waters. Data obtained in this study (Table 3) indicate that concentrations above 2% whole oil shale water are inhibitory to algae. Growth inhibition of indigenous algal populations could have a negative impact on higher trophic levels (Giddings et al. 1983) or possibly shift algal species composition toward dominance by blue-green algae (Cleave et al. 1980).

Stimulation was observed at low levels of the whole water in both growth and photosynthesis experiments and at low levels of the OP fraction in the photosynthesis test (Table 3). Similarly, algal stimulation has been reported at low concentrations of oil shale leachate (Cleave et al. 1980). In the present study, stimulation with the whole water may have been due to assimilation of inorganic (e.g., ammonia) and organic (e.g., acetate) substrates by autotrophic and heterotrophic metabolism. At the stimulatory level of 0.1% whole water (Table 3), ammonia-nitrogen (Table 1) would have been available as a nutrient source at approximately 1 mg/L. The addition of ammonia may stimulate algal growth and

Table 3. Growth and photosynthetic response, expressed as a percentage of controls, of Selenastrum capricornutum to an oil shale by-product water (Whole) and its organophilic (OP) and hydrophilic (HP) subfractions

Experiment	Test Water	Test Water Concentration (%)								
		0.1	0.5	1.0	2.0	3.0	5.0	10	20	40
Growth	Whole	135*(2)[a]	122*(2)	97(2)	43*(1)	27*(1)	4*(1)	0*	-	-
	OP	96(2)	28*(2)	7*(2)	0*	-	-	-	-	-
	HP	90*(1)	75*(2)	64*(2)	40*(2)	30*(1)	27*(1)	0*	-	-
Photosynthesis	Whole	107(6)	113*(3)	96(3)	64*(2)	54*(1)	33*(1)	-	-	-
	OP	133*(1)	123*(1)	-	-	-	83*(1)	73*(1)	60*(3)	44*(1)
	HP	76*(1)	65*(2)	63*(1)	57*(2)	51*(1)	41*(1)	-	-	-

[a] Standard error of the mean is given in parentheses for growth (N=3) and photosynthesis (N=2) percentages.
* Asterisks indicate significant stimulation or inhibition ($p<0.05$).
- Dashes indicate tests not performed.

accelerate the eutrophication process in nitrogen-limited aquatic systems (Horne 1972).

In this study, chemical fractionation provided more detailed toxicity information than would have been obtained by whole water analysis alone. Furthermore, the longer-term growth experiment provided greater sensitivity than the rapid photosynthesis test, most notably for the OP fraction. Therefore, as a screening tool designed to evaluate toxicity of synfuel by-product waters, the five-day algal growth assay was more diagnostic than the four-hour photosynthesis test when combined with chemical fractionation.

Acknowledgements. Work was supported by the U.S. Department of Energy through a postdoctoral appointment with the Associated Western Universities, Inc. Appreciation is expressed to Daniel Caldwell, John Adams, and Sheryl Hill for their technical assistance during this study.

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Received May 8, 1985; accepted July 13, 1985