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Multiple Bioassays to Assess the Toxicity of a Sanitary Landfill Leachate

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Abstract. Fitchburg, Massachusetts sanitary landfill leachate was subjected to toxicity tests using: fathead minnows (*Pimephales promelas*), zooplankton (*Daphnia magna*), green algae (*Selenastrum capricornutum*) and aerobic luminescent bacteria (*Photobacterium phosphorium*). The leachate was highly toxic to the test bacteria, moderately toxic to daphnids, and slightly toxic to fathead minnows. Algal cells, unable to grow at the 10-percent leachate exposure level, recovered after centrifugation and reinnocuation into algal nutrient medium. Low-flow summer hydrological data indicated that the leachate contributed about 7% to the total flow of the receiving stream, Flagg Brook, and about 0.6% to Sawmill Pond water located further downstream from the leachate outfall. These data, together with observed toxicity values for the test organisms, indicate that the leachate concentration in Flagg Brook impacts the diversity of aquatic life in this system, but may be less severe in Sawmill Pond where increased dilution results in leachate levels below the acutely toxic level. The considerable variation between toxicity test results obtained with the four test organisms, demonstrates the importance of conducting several such toxicity tests using organisms from different trophic levels, to assess the potential impact of a pollutant discharge on an aquatic ecosystem.

Sanitary landfill leachate may cause contamination of ground and surface waters with associated deleterious impacts on aquatic ecosystems. Field studies have provided much information about the chemical content of leachate, but relatively little toxicity data on these complex mixtures are available. McBride *et al.* (1979) and Cameron and Koch (1980) studied the toxicity of landfill leachate to rainbow trout (*Salmon gairdneri*). Other studies such as that characterized by Polprasert and Carlson (1978), have addressed inhibition of sanitary landfill leachate to certain microbially mediated reactions. Further information about the toxicity of sanitary landfill leachate to various test organisms would be of value in determining treatment strategies.

The object of this study was to assess the potentially adverse environmental impact of a sanitary landfill leachate by conducting toxicity tests with several test organisms at different trophic levels; this approach is prudent since the level of toxicity may vary greatly between different test organisms (USEPA 1979). The test organisms included fathead minnows (*Pimephales promelas*), zooplankton (*Daphnia magna*), green algae (*Selenastrum capricornutum*), and bacteria (*Photobacterium phosphorium*).

Leachate was collected from the Fitchburg Sanitary Landfill, located in Westminster, MA. The landfill began operation in 1974, and has an approximate mean loading rate of 1.4×10^5 kg/day of which 40% is household waste products and 60% is attributable to industrial and commercial loading. The categories of industry which contribute to the landfill include construction, electrical and automobile companies, paper mills, tire, plastic, and carpet manufacturers, plumbing suppliers, and tree cutting services. Additionally, general commercial businesses and a hospital use the landfill. The landfill is underlayed with bedrock, and is equipped with a perforated PVC piping collection system, that discharges into two settling ponds which are underlayed with sand. PVC lining beneath the settling ponds collects the leachate which flows by gravity feed to a 15 cm-diameter outfall pipe. The leachate discharges into Flagg Brook which flows

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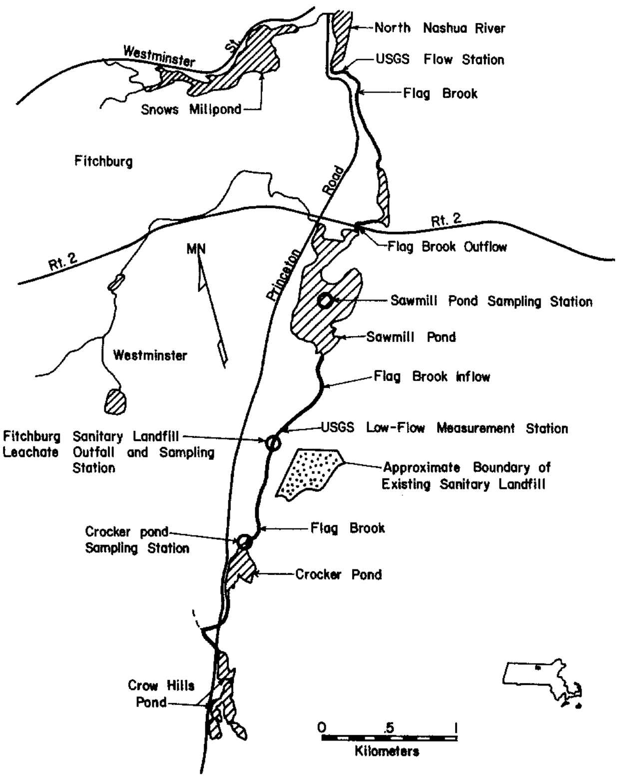

Fig. 1. Map of Sampling Locations for Fitchburg Sanitary Landfill Study

northward approximately one km before discharging into Sawmill Pond. The outflow from Sawmill Pond flows into the continuation of Flagg Brook. A map of the landfill site along with sampling locations is presented in Figure 1. Morphological and hydrological data are presented in Table 1.

Table 1. Morphologic and low-flow hydrological data for Sawmill Pond, Flagg Brook, and Fitchburg sanitary landfill leachates

Site	Characteristics	Magnitude
Sawmill Pond[a] Westminster–Fitchburg, MA	Lake area	0.25 Km^2
	Lake volume	3.0×10^5 M^3
	Mean depth[b]	1.2 m
	Retention time	21 days
	Flushing rate	17 times/hr
Flagg Brook[c] Westminster, MA	Inflow to Sawmill Pond	14 L/sec
Flagg Brook[d] Fitchburg, MA	Outflow from Sawmill Pond	164 ± 60 L/sec
Fitchburg Sanitary Landfill leachate Westminster, MA	Outfall flow (Aug. 11, 1981)	0.95 L/sec

[a] Area determined from U.S. Geological Survey Fitchburg Quadrangle map, 1969
[b] Data provided by the Massachusetts Division of Water Pollution Control (unpublished data)
[c] Mean low-flow data from Brackley and Hansen (1977)
[d] Mean ± one standard deviation of low-flow data recorded on 7/30/73, 8/27/73 and 8/29/73 (U.S. Geological Survey 1973).

Materials and Methods

Leachate was grab-sampled from the landfill outfall pipe on July 7, 1982 and July 20, 1982 and subjected to chemical analysis as well as toxicity testing. Most chemical analyses were performed within three days of collection by standard wet chemical analytical techniques. Samples were refrigerated at 4°C in the dark prior to analysis. The leachate and surface water samples were collected in acid washed glass or plastic containers and immediately transported to the laboratory. Leachate portions were filtered within 24 hr of collection through Whatman #4 glass fiber filters. Additional portions to be used for algal assay were filtered through 0.45 μm membrane filters to remove particles and indigenous algae or bacteria. Pond samples were also collected by grab sampling. In an attempt to further categorize the chemical composition of the leachate, a sample was also collected on May 14, 1983 and shipped, on ice, to a commercial laboratory (US Testing Co., Inc., Hoboken, NJ) for determination of 30 purgeable priority organics, 25 pesticides, PCBs, 13 metals, and several miscellaneous constituents. The sample was analyzed in accordance with methods presented in the U.S. Federal Register (12/3/79), USEPA Methods for Chemical Analysis of Water and Wastes (1979) or ASTM Annual Book of Standards (1981). Crocker Pond, which is located upstream of Flagg Brook, was sampled one mile upstream of the lake outfall at approximately a 20 cm depth. Water was collected from Sawmill Pond under completely mixed isothermal conditions at the deepest part of the lake (20 cm depth).

Fish Toxicity Tests

Static 96-hr toxicity tests were conducted in accordance with EPA protocol (Peltier 1978) at 19°C on both filtered and unfiltered leachate using fathead minnows, having a mean wet weight of 0.6 g/fish, as the test organism. The fish were exposed to varying percentages of the leachate diluted with soft reconstituted water as described by Peltier (1978) and shown in Table 2. Two replicates of ten fish per dilution were placed into 15 L of the test solutions contained in five gal glass jars. Since the leachate had appreciable oxygen demand, the test solutions were vigorously aerated throughout the period to maintain a DO level of ≧ 4.0 mg O_2/L. Nylon netting was suspended 3 inches above the bottom of the vessels containing the filtered leachate to preclude fish contact with iron precipitates. A 14-hr light and 10-hr dark cycle was maintained with illumination of about 20 ft-c. Feeding was terminated 48 hr prior to the start of the experiment. Fish mortality was observed at frequent time intervals for later determination of toxicity values. Dead fish were removed immediately from the test vessels.

Daphnia Toxicity Tests

Static 48-hr toxicity tests were conducted in accordance with the methods of Weber (1980). The daphnids were exposed to varying percentages of the leachate, diluted with hard reconstituted water (Table 2). Two replicates of ten daphnids per dilution were

Table 2. Quantities (mg/L) of reagent grade chemicals required to prepare reconstituted fresh waters and the resulting water quantities

Parameter	Water used for fish assays	Water used for *Daphnia magna* assays
Water type	Soft (40–48 mg $CaCO_3$/L)	Hard (160–180 mg $CaCO_3$/L)
Alkalinity	30–35 mg $CaCO_3$/L	110–120 mg $CaCO_3$/L
pH	7.4	7.8
$NaHCO_3$	48	192
$CaSO_4 \cdot 2H_2O$	30	120
$MgSO_4$	30	120
KC1	2.0	8.0

placed into 300 ml of each test solution contained in one L beakers. The solutions were gently aerated to maintain DO levels of 5 mg O_2/L. The tests were conducted under the same temperature and light conditions described for the fish assays.

Algal Assays

Three studies were conducted on the leachate to assess algal nutrient availability and the presence of algal inhibitors using a modified version of the standard *Selenastrum capricornutum* Printz algal assay bottle test (Miller *et al.* 1978). The objective of the first experiment was to determine the lowest level of leachate causing algal growth inhibition. *S. capricornutum* was grown in triplicate flasks containing various proportions of filtered leachate diluted with either algal nutrient medium described by Miller *et al.* (1978) or Crocker Pond water. Nitrogen, phosphorus and $Na_2EDTA \cdot 2H_2O$ were also added to 100% leachate samples singly and in combination at concentrations described by Miller *et al.* (1978) in an attempt to identify possible growth limiting nutrients. The $Na_2EDTA \cdot 2H_2O$ was added in an effort to complex algal toxicants and ensure trace element availability. Algal inoculants for all of the algal assay were made from five- to nine-day old cultures of *S. capricornutum* grown in algal nutrient medium. The cultures were diluted and resuspended in filtered distilled water, and then delivered, in one ml portions, to each flask to give an initial algal concentration of about 1000 cells/ml. All of the algal cultures were incubated at 24°C ± 2°C under continuous cool white fluorescent lighting (400 ft. candles illumination) and kept in suspension by shaking at 100 oscillations per min. The flasks were incubated for 11–21 days until the increase in chlorophyll a concentration or maximum standing crop was less than 5%/day.

Chlorophyll a concentration was measured by the fluorometric method described by Strickland and Parsons (1972). Iron precipitate formed during the incubation period precluded a cell dry weight determination by electronic particle counting techniques. Predicted maximum standing crop was determined from the chemical content of the algal cultures using equation 1, presented by Miller *et al.* (1978) for phosphorous limiting waters.

$$\text{Predicted mass of algae in mg dry wt/L} = [\text{Ortho-P}] \times 430 \pm 20\% \tag{1}$$

The objective of the second experiment was to determine if the algal growth inhibition observed at the lowest level of leachate exposure was an algistatic or cytotoxic response. Following the completion of the first algal growth study, cells were removed from the test solution by centrifugation at 1000 × g for four min., and then resuspended in triplicate flasks containing freshly prepared algal nutrient medium to determine possible cell recovery. Algal nutrient medium containing 1000 fresh *S. capricornutum* cells/ml was additionally centrifuged at 1000-× g and then resuspended into triplicate flasks containing freshly prepared algal nutrient medium to serve as controls.

The third algal experiment was the determination of the nutrient status and/or presence of algal growth inhibitors in Sawmill Pond, located downstream of the leachate effluent. One ml portions of 2.55 mg P/L stock phosphorus solution, 51.00 mg N/L stock nitrate solution, 51.00 mg/L stock $Na_2EDTA \cdot 2H_2O$ solution, and stock micronutrient solution were added to triplicate flasks containing the pond samples, singly and in combination to provide final concentrations described by Miller *et al.* (1978), followed by inoculation with *S. capricornutum* cells. The flasks were incubated until the Maximum Standing Crop (MSC), measured as mg dry weight of algae per L, was obtained. Cell biomass was determined by evaluating mean cell volume, and cell numbers, using a ZBI Coulter Counter (Coulter, Electronics, Healeah, FL) according to equation 2, as described by Miller *et al.* (1978).

$$\begin{array}{c}\text{Total dry}\\ \text{algal weight}\\ \text{(mg/L)}\end{array} = \begin{array}{c}\text{total cell}\\ \text{numbers}\\ \text{(cells/ml)}\end{array} \times \begin{array}{c}\text{mean cell}\\ \text{volume}\\ (\mu m^3/\text{cell})\end{array} \times \begin{array}{c}\text{specific}\\ \text{weight}\\ \text{coefficient}\\ (mg/\mu m^3)\end{array} \times \frac{1000\text{ml}}{1} \tag{2}$$

The specific weight coefficient was determined experimentally, to be equal to 3.6×10^{-10} mg/μm³ (standard deviation = 0.2×10^{-10} mg/μm³).

"Microtox" Toxicity Assays

Light diminution of bioluminescent bacterial cells (*Photobacterium phosphorium*) in the presence of varying percentages of the leachate was used as an additional assessment of the leachate toxicity. Bacterial five-minute EC50 values have been previously compared to toxicity results from daphnid and fish bioassays (Curtis *et al.* 1981; US EPA 1979; Lebsack *et al.* 1981). The assays were performed with a Microtox toxicity analyzer (Beckman Instruments, Inc., Carlsbad, CA), according to instructions supplied by the manufacturer (Beckman Instruments 1982). Light diminution was determined after 5- to 30-min. exposure periods for both filtered and unfiltered leachate. All samples were corrected for color interference according to the methods described by the manufacturer.

Results and Discussion

Table 3 presents chemical data for the leachate, algal nutrient medium, and pond waters. Additional chemical data for leachate sampled at other times (MDWPC 1980, 1981) are shown in Table 4. Tables 5 and 6 indicate the concentration of volatile or-

Table 3. Water quality data for Sawmill Pond, Crocker Pond, and the Fitchburg sanitary landfill leachate (mg/L unless otherwise stated)

PARAMETER															
Site	Date	NO_3^--N	NO_2^--N	NH_3-N	TSIN[a]	TON	Ortho-P	Total P	N:P	Alk. mg $CaCO_3$/L	Hard. mg $CaCO_3$/L	*in situ* pH[b]	Temp. C° *in situ*	DO	Conductivity (mhos)
Sawmill Pond unfiltered	7/7/82	—	—	0.277	0.277	0.725		0.033	—	28.2	103.35	6.8	13.5	7.5	84
Sawmill Pond filtered	7/7/82	0.02	0.01	0.228	0.258	0.366	0.002	0.018	129	—	—	—	—	—	—
Sawmill Pond autoclaved & filtered	7/7/82	0.02	0.01	0.238	0.268	0.492	0.005	0.023	54	—	—	—	—	—	—
Crocker Pond unfiltered	7/7/82	—	—	—	—	0.527	—	0.031	—	5.88	74.1	5.4	25.5	8.6	52
Crocker Pond filtered	7/7/82	0.03	0	0.048	0.078	0.352	0.001	0.006	78	—	—	—	—	—	—
Crocker Pond autoclaved & filtered	7/7/82	0.01	0.01	—	0.098	0.378	0.004	0.014	25	—	—	—	—	—	—
Leachate unfiltered	7/7/82	—	—	23.06	23.06	9.57	—	0.293	—	902.4	687.4	5.8	21.5	4.4	920
Leachate filtered	7/7/82	0.03	0.09	22.34	22.46	17.1	0.017	0.151	1321		—	—	—	—	—
Leachate unfiltered	7/20/82	—	—	12.35	12.35	—	—	—	—	720	707	6.5	—	—	900
Algal nutrient medium	7/9/82	3.12	0	0.092	3.212	0.075	0.152	0.162	21		—	7.5	—	—	—

[a] TSIN = total soluble inorganic nitrogen; TON = total organic nitrogen; ortho-P = orthophosphate; total P = total phosphorus; N:P = TSIN/ortho-P DO = dissolved oxygen

[b] pH was determined upon return to the laboratory

Table 4. Concentration (mg/L except where shown) of miscellaneous water quality constituents in Fitchburg leachate[a]

	Date sampled			
	4/29/80	6/3/80	5/26/81	5/14/83
pH	5.9	6.4	5.7	6.4
BOD	—	—	1,200	255
COD	2,210	2,850	1,310	1,787
Turbidity (NTU)	—	—	28	—
Alkalinity (as $CaCO_3$)	1,000	135	417	555
Acidity (as $CaCO_3$)	—	—	—	887
Hardness (as $CaCO_3$)	—	914	—	444
Conductivity (μmhos)	2,800	3,000	1,700	—
Kjeldahl-N	—	31.5	26	—
Nitrate-N	0.0	0.2	0.1	—
Total-P	—	1.5	0.87	—
Chlorides	—	7	—	—

[a] Unpublished data, Massachusetts Division of Water Pollution Control (1980, 1981)

ganics, pesticides, metals, and miscellaneous constituents in leachate sampled on May 14, 1983, and several other dates, along with some reported LC50 values for daphnids and fathead minnows. Values for chemical constituents sampled on April 29, 1982, June 6, 1980, and May 26, 1981 were obtained from unpublished data of the Massachusetts Division of Water Pollution Control (1980, 1981).

The mean BOD, COD, suspended solids, volatile solids and total iron of the unfiltered leachate sampled on July 7, 1982 and July 20, 1982 was 1,107, 1,631, 343, 207, and 220, mg/L respectively. Both the filtered and unfiltered leachate were colored, having a dominant wavelength of 482 and 587 nm, respectively (pH unadjusted), a luminescence of 89 and 11%, respectively, and a purity of 5 and 78%, respectively. Luminescence and purity were evaluated from a chromaticity diagram. The leachate was nitrogen rich (predominantly ammonia), having an N:P ratio (equal to $[NH_3-N + NO_3^- -N + NO_2^- -N]/[Ortho\text{-}P]$) of 1321. Chemical analysis also indicated that autoclaved and filtered samples of both Sawmill Pond and Crocker Pond were likely phosphorus limiting to algal growth with N:P ratios of 54 and 25, respectively.

Fish Toxicity Test

Fish mortality in the 100% filtered leachate was only 15% after 96-hr exposure. The 100% unfiltered leachate was somewhat more toxic with 55% mortality occurring over the test period. No mortality was observed in any of the controls. The 96LC50 and ILC50 values could not be calculated owing to the observed mortality in only the 100% leachate exposure level.

Daphnia Toxicity Test

Only filtered leachate was tested, since the turbidity of the unfiltered leachate precluded visual confirmation of daphnid mortality. The 48LC50 and ILC50 values were determined graphically to be equal to 62–66% and 54%, respectively. Thus, the filtered-aerated leachate was moderately toxic to *D. magna*.

Algal Assay

Predicted and observed chlorophyll a concentrations after 13 days of incubation in various dilutions of filtered leachate with algal nutrient medium are presented in Figure 2. The data indicated that the leachate was highly inhibitory to algal growth even at the 10% leachate level. Chlorophyll a production in algal nutrient medium containing 1% leachate was equal to 175.07 μg/L while control algal cells grown in 100% nutrient medium produced 188.59 μg chlorophyll a/L. These data indicate that algal growth inhibition was not significant at the 1% toxicant level. The EC50 (leachate concentration resulting in 50% growth inhibition) was, therefore, somewhere between 1 and 10% leachate.

Triplicate flasks containing 100% algal nutrient medium as a control contained a mean MSC of 153 mg dry wt. algae per L; chlorophyll a comprised about 0.12% by weight of the dry algae. Predicted chlorophyll a levels of diluted leachate were estimated by equation 3:

$$\begin{array}{c}\text{Predicted}\\ \text{chlorophyll a}\\ (\mu g/L)\end{array} = \left[\begin{array}{c}\text{Predicted MSC from}\\ \text{equation 1}\\ \text{(mg dry wt/L)}\end{array}\right] \times 1.2 \qquad (3)$$

Chlorophyll a production was not observed in algal cultures containing leachate diluted with 1, 10, 25 and 50% Crocker Pond water. This was attributable to the high level of toxicity at the higher percent leachate dilutions and the very low nutrient levels at leachate concentrations below the observed inhibitory level (less than 10%). The predicted algal growth in 10% leachate diluted with Crocker Pond water in the absence of algal toxicants for example, equaled 1.3 μg chlorophyll a/L, barely a perceivable level. Algal inhibition, in this case, is best demonstrated by using a nutrient-rich

Table 5. Chemical analysis of Fitchburg leachate sampled on 5/14/83 and some reported LC50 values for daphnids (48 hr) and fathead minnows (96 hr)

Chemical	Concentration (mg/L)	LC50 (mg/L)	
		daphnids	fathead minnows
Volatile Organics	0.499	—	—
Vinyl Chloride	0.020	224[a]	193–310 mg/L[d]
Methylene Chloride	0.286	220[b]	—
Trans-1,2- dichlorethylene			
Toluene	0.378	313[c]	20–36[h]
Ethylbenzene	0.114	75[e]	—
Methylethylketone	0.470	>520[b]	—
Methylisobutylketone	0.176	—	—
2-hexanone	0.148	—	—
Pesticides[i]			
Chlordane	not detected	—	—
Toxaphene	not detected	0.010[f]	0.048[g]
All others	<1		

[a] Halomethanes Ambient Water Quality Criteria, USEPA (1978a)
[b] G. LeBlanc (1980)
[c] Toluene Ambient Water Quality Criteria, USEPA (1978b)
[d] Alexander *et. al.* (1978)
[e] Ethylbenzene Ambient Water Quality Criteria, USEPA (1978c)
[f] Toxaphene Ambient Water Quality Criteria, USEPA (1978d)
[g] Mayer *et. al.* (1977) 10 day LC50
[h] Devlin *et. al.* (1982) 1-day-old fish
[i] "<1" = not detected. Compound not present at or above concentration shown. Sample was analyzed for 25 pesticides and/or PCBs

dilution water for the leachate, such as the algal nutrient medium, so that higher chlorophyll a levels can be measured at dilutions containing small percentages of leachate.

Chlorophyll a production was also not observed in the 100% leachate containing various nutrient additions with or without $Na_2EDTA \cdot 2H_2O$. The predicted chlorophyll a level for 100% leachate in the absence of algal inhibitors was 8.7 μg chlorophyll a/L based upon a predicted MSC of 7.3 mg dry wt/L. Since no chlorophyll a was observed in samples containing 100% leachate plus 1.00 mg $Na_2EDTA \cdot 2H_2O$/L, apparently, the amount of $Na_2EDTA \cdot 2H_2O$ added was insufficient to complex the algal toxicants present in the leachate.

The second algal assay was conducted to determine the level of algal growth, determined by chlorophyll a production, after resuspension of cells previously exposed to 10% leachate (lowest leachate level causing algistatic response) in algal nutrient medium (Table 7). The data demonstrate that the 10% leachate exposure level was only algistatic in that cells were able to recover upon reinoculation into media containing no leachate. The level of chlorophyll a produced, however, was somewhat less than that produced by cells not previously exposed to the leachate. This may be attributable to variability in the chlorophyll a:MSC ratio, or to analytical variability in the determination of chlorophyll a.

The final algal assay on Sawmill Pond water (located downstream of the leachate discharge) confirmed phosphorus limitation in this water as predicted by the N:P ratio (Figure 3). The MSC produced in the control water of 0.82 mg dry wt/L indicates a moderately high productivity based upon guidelines presented by Miller *et al.* (1973), and supported by the observed total phosphorous concentration of 0.03 mg P/L for unfiltered, unautoclaved Sawmill Pond water which may be considered as a lower value for eutrophic waters (Wetzel 1975). $Na_2EDTA \cdot 2H_2O$ additions to Sawmill Pond water did not result in increased MSC indicating that the water was not inhibiting algal growth.

Microtox Assay

Microtox results are shown in Table 8. The five-min EC50 for filtered and unfiltered leachate was about 14 and 17%, respectively. Thus, unfiltered leachate was slightly more toxic to the photobacteria used in this assay. Thirty-min EC50 values were not significantly different from the 5-min results.

Table 6. Chemical analysis of Fitchburg leachate and some reported LC50 values for daphnids (48 hr) and fathead minnows (96 hr)

	Concentration (mg/L)				LC50 Values (mg/L)	
	4/29/80	6/30/80	5/26/81	5/14/83	daphnids	fathead minnows
1. **Metals**						
Lead	0.10	0.00	0.01	0.190	0.450[a]	—
Cadmium	—	0.00	0.00	0.025	0.065[a]	—
Arsenic	—	—	—	0.038	7.400[a]	—
Selenium	—	—	—	0.025	0.43[b]	—
Silver	—	—	—	0.040	0.0015[b]	—
Chromium	—	—	—	0.080	2.000[a]	33.2[h]
Barium	—	—	—	0.540	14.500[a]	—
Berillium	—	—	—	<0.050	1.0[b]	—
Mercury	—	—	—	0.013	0.013[a]	0.168[i]
Nickel	—	0.04	0.00	—	0.130[a]	—
Manganese	—	8.0	3.6	—	9.8[a]	—
Zinc	0.07	0.03	0.01	—	0.10[a]	3.1[g]
Copper	—	0.00	0.01	—	0.06[a]	0.490[e]
2. **Miscellaneous**						
Iron	850	650	270	215	—	—
Un-ionized ammonia[j]	1.14	2.4	0.57	—	1.5 mg/L[c]	1.59[d]
Cyanide	—	—	0.00	—	—	0.117–0.157[f]

[a] Biesinger and Christensen (1972); in Lake Superior or Lake Erie Water
[b] G. LeBlanc (1980)
[c] Parkhurst et. al. (1979) Unionized ammonia concentration calculated from stated LC50 Value = 25 mg/L total NH_3 at pH = 8.2
[d] DeGraeve, et. al. (1980) Hardness = 655 mg/L as $CaCO_3$
[e] Pickering et. al. (1977) (juvenile)
[f] Broderius et. al. (1977) (juvenile)
[g] Judy and Davies (1979)
[h] Broderius and Smith (1979)
[i] Snarski and Olson (1982)
[j] Un-ionized ammonia = (ammonia-N) (ionization fraction), Peltier (1978)

Conclusions

Table 9 summarizes the LC and EC values for the four toxicity tests. The leachate was highly toxic to the test alga and bacteria, moderately toxic to daphnids, and only slightly toxic to fathead minnows. These results can be further interpreted by examining the concentrations of specific compounds in the leachate with literature values of acute toxicity to daphnids and fathead minnows (Tables 5 and 6). All of the observed concentrations of volatile organic compounds, as well as pesticides, were several orders of magnitude below literature LC50 values. Silver was present at a concentration approximately 27 times greater than one reported 48LC50 value for *Daphnia* in soft water (hardness = 72 mg/L as $CaCO_3$, LeBlanc 1980). However, toxicity generally decreases as hardness increases. Since the leachate hardness was almost an order of magnitude greater than 72 mg/L as $CaCO_3$, the expected silver LC50 value for daphnids in the leachate would likely be greater than the value reported by LeBlanc (1980) for daphnids in soft water. Mercury was present at one reported 48LC50 value for *Daphnia* (Biesinger and Christensen 1972). Once again, the hardness of the leachate was considerably greater than that used by these investigators. Lead, cadmium, and manganese were present in the leachate at 42%, 38%, and 37–81%, respectively, of reported LC50 values for daphnids in soft water (Biesinger and Christensen 1972). All other metals in the leachate were present at levels at least one order of magnitude below literature LC50 values for daphnids and fathead minnows.

Two leachate constituents of particular interest are iron and ammonia. While the laboratory bioassay test reported 100% survival of the fathead minnows in both filtered and unfiltered leachate, iron has been shown to kill rainbow trout larvae at concentrations as low as 1.3 mg/L (Amelung 1981). Thus, while observed iron concentrations (mean approximately = 500 mg/L) were not acutely toxic to fathead minnows in the laboratory study presented here, other sublethal effects to sensitive aquatic or-

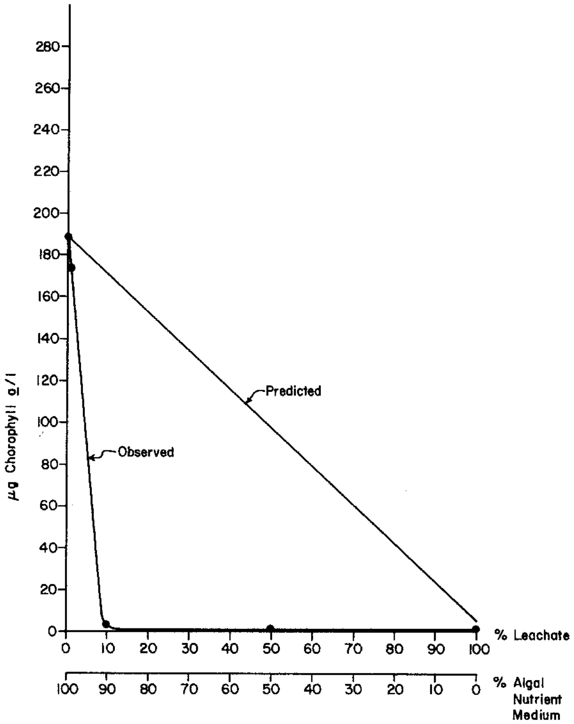

Fig. 2. Predicted and observed chlorophyll a levels in *S. capricornutum* cultures containing varying percentages of Fitchburg, MA sanitary landfill leachate and algal nutrient medium. Predicted chlorophyll a is equal to 0.12% of the MSC predicted from phosphorus concentration in the growth media using equation 1. The 0.12% value was observed in the algal culture containing 100% nutrient medium having a MSC = 188.6 mg.dry wt/L

Table 7. Observed chlorophyll a levels of *S. capricornutum*[a]

Treatment	Chlorophyll a μg/L
1. Control 1: cells grown in 100% ANM[b]	188.6
2. Control 2: 1000 cells/ml suspended in ANM centrifuged and resuspended in freshly prepared ANM	129.0
3. Cells grown in 10% leachate plus 90% ANM[c]	<1.0
4. Cells from treatment 3, centrifuged and resuspended in 100% ANM	99.5

[a] 13-day incubation period except as noted algal inoculum = 1000 cells/ml
[b] ANM: Algal Nutrient Medium
[c] 11 day incubation period

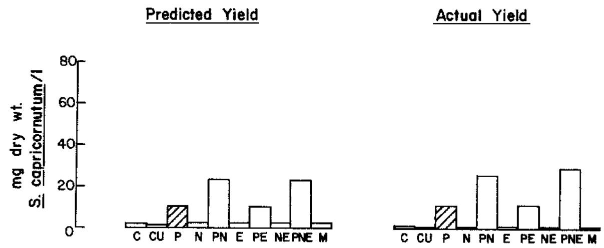

Fig. 3. Predicted and actual yields of *S. capricornutum* grown in Sawmill Pond water sampled on 7/7/82. Algal yields were predicted using the equations:

Predicted Yield = 38 [NH_3-N + NO_2^- $-N$ + NO_3^- $-N$] for N:P < 11.3 (mg dry wt algae/L)

= 430 [Ortho-P] for N:P > 11.3

Cross hatching indicates limiting nutrient

C	= Control	E	= + EDTA
CU	= Control Unautoclaved	PE	= + Phosphorus + EDTA
P	= + Phosphorus	NE	= + Nitrogen + EDTA
N	= + Nitrogen	PNE	= + Phosphorus + Nitrogen + EDTA
PN	+ Phosphorus + Nitrogen	M	= + Micronutrients

Table 8. Microtox data

Sample	EC50 (% leachate) 5 min	10 min	15 min	30 min
Color-corrected unfiltered leachate	14	—	15	14
Color-corrected filtered leachate	17	17.5	—	16

ganisms may be occurring in water receiving the Fitchburg leachate. Un-ionized ammonia was also found to be present near or somewhat above reported LC50 values for both daphnids and fathead minnows (Parkhurst *et al.* 1979; DeGraeve *et al.* 1980); ammonia, and to a lesser extent silver, mercury, lead, cadmium, and manganese, are likely contributing to the observed toxicity of the leachate. Such conclusions are equivocal however, since literature LC50 values were obtained under different experimental conditions (hardness, pH, alkalinity, *etc.*) than those used in this study.

The hydrological data in Table 1 indicates that the flow in the receiving water, Flagg Brook comprises about 7% leachate during the summer. Based on the toxicity data, this dilution level may be inhibitory to some algal and bacterial species, but is not acutely toxic to daphnids and fathead minnows. Flow data also indicate that Sawmill Pond contains approximately 0.6% leachate during the summer. This dilution level was not acutely toxic or growth inhibitory to any of the organisms tested. This is supported by the algal assay results on Sawmill Pond which indicated that this water did not inhibit *S. capricornutum* growth.

These data indicate that the leachate concentration in Flagg Brook impacts the diversity of aquatic life in this system, owing to the varying toxicity of

Table 9. Fitchburg sanitary landfill leachate toxicity test results

Test	Treatment	Toxicity values (% leachate)	Range of dissolved oxygen over test duration (mg/L)
Microtox assay: *Photobacterium phosphoreum*	unfiltered	5 min. EC50 = 14	4.0
	filtered	5 min. EC50 = 17	4.0
Algal assay: *Selenastrum capricornutum*[a]	filtered	1 < EC50 < 10	—
Macroinvertebrate assay: *Daphnia magna*	filtered	48LC50 = 62–66 ILC50 = 37	4.8–7.4
Fish bioassay: *Pimephales promelas*[b]	unfiltered	96LC55 = 100	5.0–8.3
	filtered	96LC15 = 100	4.0–7.9

[a] As determined by chlorophyll a analysis
[b] 96LC values were based solely upon survival in 100 percent leachate after 96 hr of exposure. High survival rate precluded an exact LC 50 value determination

the leachate to different aquatic species. This impact is less severe further downstream, in Sawmill Pond, where increased dilution results in leachate levels below the acutely toxic level. However, further determinations are needed to explore chronic effects from the exposure of aquatic organisms to this level of leachate contamination. The effect of diurnal oxygen variation attributable to the dense macrophytic growth in Sawmill Pond, and the high BOD of the leachate (mean BOD = 1107 mg/L) on the aquatic ecosystem should also be assessed.

The considerable variation between toxicity test results obtained with fish, daphnids, algae, or bacteria as test organisms demonstrates the importance of conducting several such toxicity tests using organisms from several trophic levels, in assessing the potential impact of a pollutant discharge on an aquatic ecosystem.

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