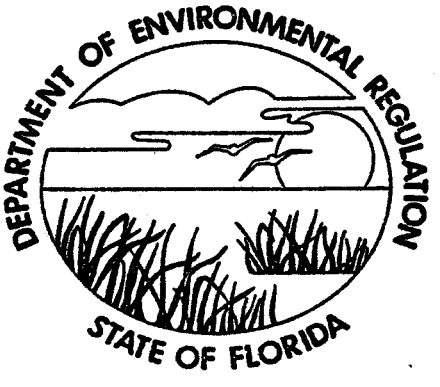


BIOASSAYS OF THE MAJETTE LANDFILL

SLUDGE POND AND POND OVERFLOW SAMPLES

PANAMA CITY, BAY COUNTY, FLORIDA

NO NPDES PERMIT

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December 19, 1984

Biology Section
Division of Environmental Programs

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EXECUTIVE SUMMARY

Majette Landfill, Panama City, on 2 to 5 October, 1984.

The sample collected from the sludge pond of this municipal and industrial landfill proved to be acutely toxic to Notropis leedsi, producing a 72 hour LC_{50} of 85.3%. This sample, however, was not acutely toxic to Daphnia pulex. The sample from the Pond C outfall was not acutely toxic to Daphnia pulex, Simocephalus serulatus, or Heterandria formosa, even though significant concentrations of total ammonia (24.1 mg/l) and unionized ammonia (0.98 mg/l) were detected in this sample. The S. serrulatus assay was characterized by random mortality and excessive control mortality. Significantly lower concentrations of ammonia were detected in the sludge pond sample. Results of the chemical analyses indicate the presence of small concentrations of mercury and zinc in both the Sludge Pond sample and the Pond C effluent sample. No organic compounds were detected in either sample. Although the Sludge Pond sample had slightly higher levels of mercury and zinc than did the Pond C sample, they were still well below the concentrations required to produce a lethal response for freshwater organisms. Therefore, the toxic properties of the Sludge Pond sample can probably be attributed to other unknown compounds or components not adequately tested for during chemical analysis.

INTRODUCTION

The Majette Landfill is located in Panama City, Bay County, Florida (Fig. 1). According to district personnel, this site has received sludge from municipal and industrial waste facilities for decades. Leachate from this source may enter groundwater. Additionally, there is at least one pond on site which overflows to surface waters which flow to Deer Point Lake.

To evaluate the potential toxicity of these pond and pond overflow waters, Mr. Robert Brazzell of DER's Northwest District requested that toxicity bioassays be performed. The Biology Section received a sample from each of these sources and performed a total of five 72 hour definitive and screening bioassays on 2 to 5 October, 1984. The results of these tests are presented in this report. Those involved in performing these tests and in preparation of this report were: C. W. Dye, R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.

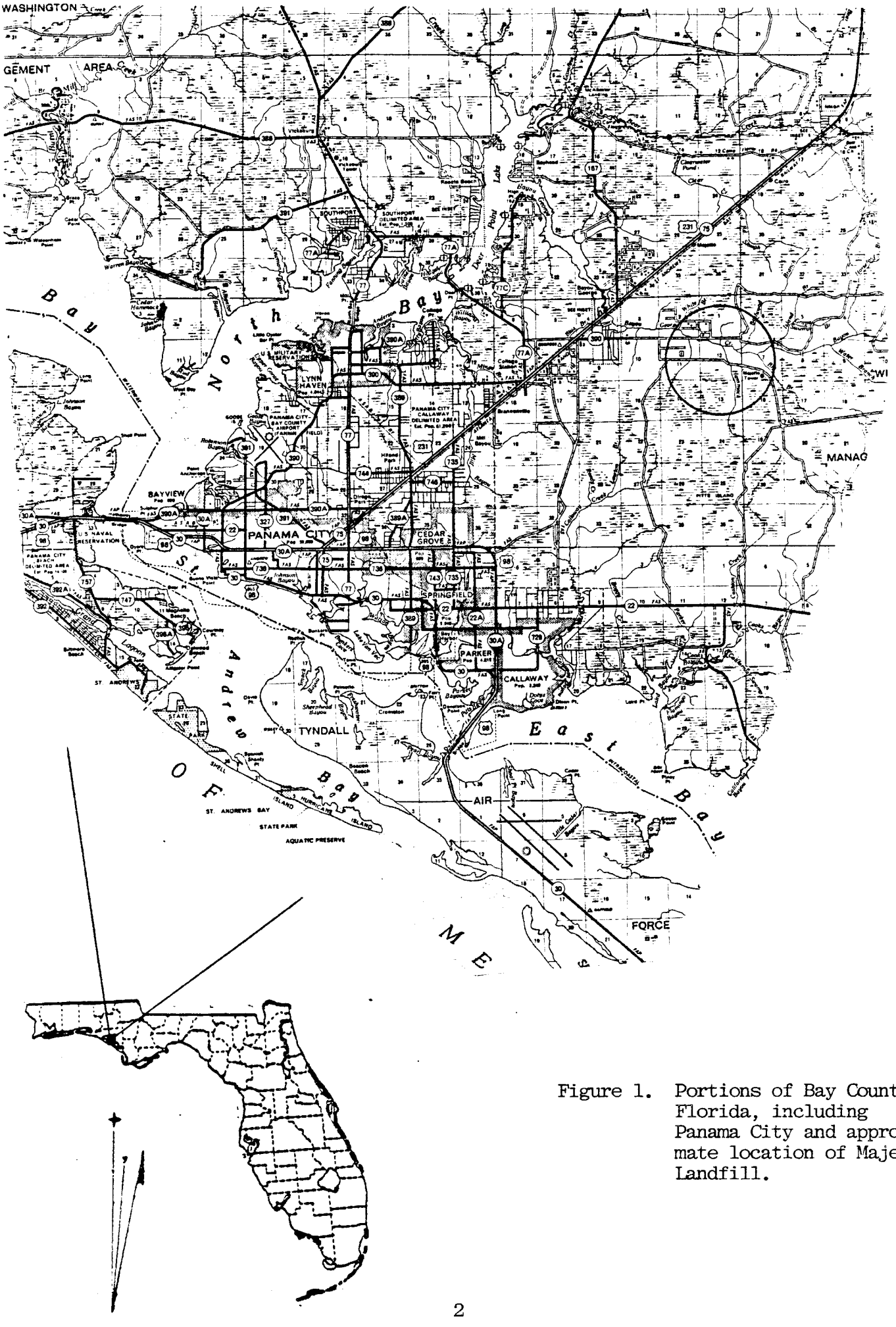


Figure 1. Portions of Bay County, Florida, including Panama City and approximate location of Majette Landfill.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include a species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. Three of these indigenous species were also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of Daphnia pulex was obtained from the EPA laboratory in Athens, Georgia, in March, 1979. This culture has been maintained since its arrival in 2 1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, once daily on weekends, and are kept at a constant temperature of 22° plus or minus 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Simocephalus serrulatus (water flea) - An indigenous breeding population of Simocephalus serrulatus was collected from Lake Jackson, Léon County, Florida, in March, 1980, by personnel from DER's Biology Section. Positive identification of the organisms was made by Mr. Jeff Foran of the University of Florida Department of Environmental Engineering Sciences. The cultures are maintained in two and one-half gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, once daily on weekends, and are kept at a constant temperature of 22° plus or minus 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Heterandria formosa (least killifish) - Approximately 100 adult least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The least killifish are reared at 22° plus or minus 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) and Tetra-Min® twice daily and once daily on weekends. New born fish are initially maintained in the adult tanks, but are transferred to separate aquaria at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were approximately 14 days old.

Notropis leedsi (bannerfin shiner) - Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 30 and 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22° plus or minus 2°C under a 16:8 hour light cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25°C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were approximately 7 days old at the time of the test.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

A total of five separate 72 hour static acute toxicity bioassays (definitive and screening) were performed on samples collected from the Pond-C Outfall and the Sludge Pond of the Majette Landfill. The tests were performed from 1000 hours on 2 October to 0940 hours on 5 October, 1984. The samples were collected by Ms. Lisa Milliman of DER's Northwest District from 1145 to 1205 hours on 1 October, 1984, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Freshwater for dilution of the samples was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1980).

Two replicates, each with five concentrations and a control, were used to test with the water fleas, Daphnia pulex and Simocephalus serrulatus. Flint glass jars of 250 ml capacity were used as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. Daphnia and Simocephalus were siphoned into large culture dishes to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

Two replicates, of five concentrations and a control, were used for the Notropis leedsi (bannerfin shiner) bioassay. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 4 per chamber, resulting in 8 per concentration. For the Heterandria formosa (least killifish) screening bioassay, only one test chamber each of a control and an 100% concentration, each containing 4 fish, was used. Fish were not fed during these tests.

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, 48 hours, and 72 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% sample concentrations. The specific conductance (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% test concentrations at the beginning of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Tables 2 through 5.

Table 1. Concentration Series and Volumes Used for These Static Bioassays

Daphnia pulex and *Simocephalus serrulatus* (water fleas)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	-------	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
18.0%	164.0 ml	36.0 ml	200 ml
32.0%	136.0 ml	64.0 ml	200 ml
56.0%	88.0 ml	112.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	------	500 ml
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100.0%	------	500 ml	500 ml

Heterandria formosa (least killifish)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	------	500 ml
100%	------	500 ml	500 ml

Table 2. Data recorded during 72 hour, Daphnia pulex and Heterandria formosa, static acute toxicity bioassays of Majette Landfill, Panama City, Bay County, Florida, on 2 to 5 October, 1984.

Sample from Pond-C Outfall, D. pulex definitive and H. formosa screening bioassays.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)			NH_3/N (mg/l)	
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	48	72	0	48
Control	10	10	9	8	8.0	8.1	9.0	8.6	7.3	8.2	7.9	8.2	21.5	20.8	19.1	18.9				0.0	
Control	10	10	8	8	8.0	8.1	9.0	8.6	7.4	8.2	8.0	8.2					210	215	210		
10.0%	10	10	10	10	8.1	8.1	9.1	8.6	7.6	8.3	8.2	8.2	21.5	20.5	18.7	18.7					
10.0%	10	10	9	9	8.1	8.1	9.2	8.6	7.6	8.3	8.2	8.2					260	260	255		
18.0%	10	10	10	10	8.2	8.2	9.2	8.7	7.7	8.3	8.2	8.3	21.4	20.3	18.4	18.5					
18.0%	10	10	10	10	8.2	8.2	9.2	8.7	7.7	8.3	8.2	8.3					300	300	292		
32.0%	10	10	9	6	8.2	8.2	9.2	8.7	7.8	8.3	8.3	8.3	21.4	20.3	18.4	18.7					
32.0%	10	10	10	10	8.2	8.2	9.2	8.7	7.8	8.3	8.3	8.3					390	365	355		
56.0%	10	10	10	10	8.3	8.3	9.2	8.7	7.9	8.3	8.2	8.2	21.4	20.4	18.3	18.7					
56.0%	10	10	10	10	8.3	8.3	9.2	8.7	7.9	8.3	8.2	8.2					500	455	440		
100.0%	10	10	10	10	8.3	8.3	9.2	8.7	7.9	8.3	8.3	8.2	21.5	20.4	18.3	18.6				.98/24.1	
100.0%	10	10	10	10	8.3	8.3	9.1	8.7	7.9	8.3	8.3	8.2					750	650	600		
H. formosa screening test																					
Control	4	4	4	4	8.1	7.7	8.5	8.3	7.4	8.0	8.1	8.0	22.1	21.2	19.1	19.2	210	200	210	0/0	
100.0%	4	4	4	4	8.2	7.5	8.5	8.3	7.9	8.1	8.1	8.1	22.1	21.4	19.1	19.1	750	685	600	.98/24.1	

Control Water

Total Alkalinity 96 (mg/l $CaCO_3$)

Total Hardness 111 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 309 (mg/l $CaCO_3$)

Total Hardness 171 (mg/l $CaCO_3$)

Table 3. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Majette Landfill, Panama City, Bay County, Florida, on 2 to 5 October, 1984.

Sample from Pond-C Outfall

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)	
	0	24	48a	0	24	48a	0	24	48a	0	24	48a	0	48a	0	48
Control	10	9		8.1	8.2		7.7	8.2		21.5	20.5					
Control	10	9		8.1	8.2		7.7	8.2					210		0/0	
10.0%	10	5		8.0	8.2		7.7	8.3		21.4	20.3					
10.0%	10	6		8.0	8.2		7.7	8.3					255			
18.0%	10	6		8.0	8.3		7.8	8.3		21.4	20.3					
18.0%	10	9		8.0	8.3		7.8	8.3					310			
32.0%	10	7		8.0	8.3		7.8	8.3		21.4	20.4					
32.0%	10	7		8.0	8.3		7.8	8.3					385			
56.0%	10	8		8.0	8.3		7.9	8.3		21.4	20.5					
56.0%	10	6		8.0	8.3		7.9	8.3					500			
100.0%	10	6		8.0	8.3		7.9	8.3		21.4	20.5					
100.0%	10	7		8.0	8.3		7.9	8.3					710		.98/24.1	

Control Water

Total Alkalinity 96 (mg/l $CaCO_3$)

Total Hardness 111 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 309 (mg/l $CaCO_3$)

Total Hardness 171 (mg/l $CaCO_3$)

[a] 48 hour test was invalidated due to excessive control mortality, data not reported.

Table 4. Data recorded during 72 hour, Daphnia pulex, static acute toxicity bioassay of Majette Landfill, Panama City, Bay County, Florida, on 2 to 5 October, 1984.

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)			NH_3/N (mg/l)	
	0	24	48	72	0	24	48	72[b]	0	24	48	72[b]	0	24	48	72[b]	0	48	72[b]	0	48
Control	11 [a]	11	9	8	8.2	8.3	8.8		7.8	8.1	8.2		21.3	20.6	18.3						
Control	11 [a]	11	9	9	8.2	8.3	8.9		7.8	8.1	8.2						210	215		0/0	
10.0%	10	10	10		8.1	8.0	9.0		7.8	8.1	8.1		21.3	20.4	18.2						
10.0%	10	10	10		8.0	8.0	8.9		7.8	8.1	8.1						260	255			
18.0%	10	10	10		8.0	7.7	8.6		7.8	8.1	8.1		21.2	20.3	18.1						
18.0%	10	10	10		7.9	7.7	8.5		7.8	8.1	8.1						320	305			
32.0%	10	9	8		7.7	7.3	8.0		7.8	8.1	8.1		21.1	20.4	18.2						
32.0%	10	10	10		7.5	7.3	8.0		7.8	8.1	8.1						400	370			
56.0%	10	10	10		6.6	6.6	7.9		7.7	8.1	8.1		20.9	20.5	18.4						
56.0%	10	10	10		6.6	6.6	7.8		7.8	8.1	8.2						500	520			
100.0%	10	10	10		5.3	6.0	7.0		7.7	8.2	8.3		20.7	20.7	18.8						
100.0%	10	10	9		5.0	6.0	7.0		7.7	8.2	8.3						750	700		.01/.49	

Control Water

Total Alkalinity 96 (mg/l $CaCO_3$)

Total Hardness 111 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 338 (mg/l $CaCO_3$)

Total Hardness 352 (mg/l $CaCO_3$)

[a] An extra organism was loaded by mistake, allowed to remain.

[b] The 72 hour test is invalidated due to excessive control mortality (>20%). Data not reported.

Table 5. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Majette Landfill, Panama City, Bay County, Florida, on 2 to 5 October, 1984.

Sample from Sludge Pond

Concentration or %	Number of Live Organisms				Dissolved Oxygen (mg/l)				pH				Temperature (°C)				Conductivity (umhos)			NH_3/N (mg/l)	
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	0	48	72	0	48
Control	4	4	4	4	7.7	8.0	8.3	8.1	7.8	8.1	8.2	8.1	21.5	20.0	17.9	18.3				0/0	
Control	4	4	4	4	7.8	8.0	8.5	8.1	7.8	8.1	8.2	8.1					215	220	200		
10.0%	4	4	4	4	7.8	7.7	8.4	8.0	7.7	8.1	8.1	8.1	21.0	19.9	17.8	18.0					
10.0%	4	4	4	4	7.9	7.7	8.3	8.0	7.7	8.1	8.1	8.1					275	260	240		
18.0%	4	4	4	4	7.7	6.9	7.8	7.4	7.8	8.0	8.0	8.0	21.2	19.9	17.7	18.0					
18.0%	4	4	4	4	7.7	6.9	7.7	7.4	7.8	8.0	8.1	8.0					330	300	310		
32.0%	4	4	4	4	6.9	6.5	7.1	6.7	7.7	8.0	8.0	8.0	21.0	19.9	17.7	18.0					
32.0%	4	4	4	4	6.9	6.5	7.1	6.7	7.7	8.0	8.0	8.0					410	375	380		
56.0%	4	4	4	4	6.4	5.7	6.6	5.5	7.7	8.0	8.0	8.0	20.8	20.0	17.9	18.2					
56.0%	4	4	4	4	6.4	5.8	6.5	5.5	7.7	8.0	8.1	8.0					550	500	500		
100.0%	4	2	2	2	4.8	5.4	5.7	4.9	7.7	8.0	8.1	8.1	20.2	20.3	18.3	18.5					
100.0%	4	0	0	0	4.8	5.3	5.1	4.9	7.8	8.0	8.1	8.1					790	750	710	.01/.49	

Control Water

Total Alkalinity 96 (mg/l $CaCO_3$)

Total Hardness 111 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 338 (mg/l $CaCO_3$)

Total Hardness 352 (mg/l $CaCO_3$)

Table 6. LC_{50} Data for Bioassays of Majette Landfill, on 2 to 5 October, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals	72 Hour LC_{50} and 95% Confidence Intervals
D. pulex Pond Outfall	Binomial	No Mortality	Random Mortality	Random Mortality
	Moving Average			
	Probit			
S. serrulatus Pond Outfall	Binomial	c	b	b
	Moving Average	c	b	b
	Probit	c	b	b
D. pulex Sludge Pond	Binomial	Random Mortality	Random Mortality	b
	Moving Average			
	Probit			
N. leedsi Sludge Pond	Binomial	85.3% (56 and ∞)	85.3% (56 and ∞)	85.3% (56 and ∞)
	Moving Average	a	a	a
	Probit	a	a	a

a Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

b This test was invalidated due to excessive control mortality.

c The LC_{50}'s calculated for this test are not reported due to severe random mortality of test organisms (Table 3).

Table 7. Results of Chemical Analyses Performed on Samples Collected from the Majette Landfill on 1 October, 1984.

Organics

	Pond C Effluent	Sludge Pond
Acid Extractables:	N.D.	N.D.
Base Neutral Extractables:	N.D.	N.D.
Pesticide Extractables:	N.D.	N.D.

Metals

	Pond C Effluent	Sludge Pond
Antimony	N.D.	N.D.
Arsenic	N.D.	N.D.
Beryllium	N.D.	N.D.
Cadmium	N.D.	N.D.
Chromium	N.D.	N.D.
Copper	N.D.	N.D.
Lead	<5 µg/l	N.D.
Mercury	0.25 µg/l	0.33 µg/l
Nickel	N.D.	N.D.
Selenium	N.D.	N.D.
Silver	N.D.	N.D.
Thallium	N.D.	N.D.
Zinc	11 µg/l	55 µg/l

N.D. = None Detected

RESULTS AND DISCUSSION

The overflow sample collected from the Pond-C Outfall of the Majette Landfill was not toxic to the test organisms Daphnia pulex and Simocephalus serrulatus (definitive tests), or to Heterandria formosa (screening test). This lack of toxicity was not expected since significant concentrations of total ammonia (24.1 mg/l) and unionized ammonia (0.98 mg/l) were detected in this sample. The S. serrulatus test was characterized by severe random mortality at 24 hours, and was invalidated at 48 hours due to excessive control mortality (Table 3).

The sample collected from the Sludge Pond of the Majette Landfill proved to be acutely toxic to Notropis leedsi, producing 24 through 72 hour LC_{50}'s of 85.3% (Table 6). This same sample, however, was not toxic to D. pulex. Only a few random water fleas had died by 72 hours (Table 4). Significantly less total ammonia (0.49 mg/l and unionized ammonia (0.01 mg/l) were detected in this sample. The cause of the toxicity to N. leedsi, therefore, must have been due to other factors.

The results of chemical analyses of these samples (Table 7) indicated the presence of 0.33 µg/l of mercury and 55 µg/l of zinc in the Sludge Pond, and 0.25 µg/l of mercury and 11 µg/l of zinc in the Pond C effluent. No organics were detected in either sample. These rather small concentrations of metals found in the samples are well below the levels required to produce a lethal response for freshwater organisms. Therefore, the toxic properties of the Sludge Pond sample can probably be attributed to other unknown compounds or components not adequately tested for during chemical analyses.

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly.

The two basic concepts utilized in the standards are acute and chronic toxicity (defined in 17-3.021(1) and (4), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as one-twentieth of the 96 hour LC_{50}. Therefore, if a test solution kills one-half or more of a group of test organisms in 96 hours, it must contain at least three times the defined "acutely toxic" concentration and at least twenty times the "chronically toxic" concentration of some substance or substances. If a test solution causes that same mortality in less than 96 hours, (for example, in 24 or 48 hours), it is at least as toxic as the example given above, and actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existance of a mixing zone, whether as a specific part of the permit or based on the existance of a currently valid permit predating the language in Chapter 17-4 requiring mixing zone designation. In the absence of a mixing zone, the general criteria for surface waters (17-3.061) are applicable at all points, beginning at the point of discharge. This section prohibits the discharge of substances in concentrations which are chronically toxic (17-3.061(2)(p)); that is, the concentrations cannot exceed one-twentieth of the 96 hour LC_{50} as discussed above. If a mixing zone does exist, then this chronic toxicity standard is only applicable from the edge of the mixing zone and beyond. Within the mixing zone, the less stringent acute toxicity standard of the minimum criteria for all waters (17-3.051(1)(d)) applies: one-third of the 96 hour LC_{50}. The acute toxicity standard does not apply, however, to each point within a mixing zone not designated for dredge and fill permitting, but rather to the average of a number of points within such a mixing zone, based on the provisions of the surface water mixing zones section of Chapter 17-4 (17-4.244(4)). At the same time, though, this same rule prohibits waste concentrations within such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test

procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

A number of different dilutions were examined in order to determine by how much, if any, the discharge exceeds the 96 hour LC_{50}. For example, if the sample, after two-fold dilution with diluent (a 33% concentration), kills one-half of the test organisms after 96 hours, then the sample is three times as toxic as is allowed by that standard. Similarly, if a 5% solution of the sample kills 50% of the organisms, the sample is twenty times as toxic as is allowed. Toxicities are reported here in that manner, i.e., in terms of the percentage dilution of the test sample. The smaller the percentage which is reported means a correspondingly greater toxicity.

Note that even if a discharger receives a very large mixing zone not designated for dredge and fill permitting, the regulations require that the maximum toxicity at any point within the mixing zone be restricted to the 96 hour LC_{50}. The results here reported are based on actual effluent samples, representative of the effluent concentration which enters waters of the state at the point of discharge. If it is demonstrated that, under the conditions existing at the point of discharge, effluent concentrations will be less than at the actual sampling point (if the two points are different), the reported bioassay results will reflect the reduced discharge concentration.

With respect to the species of organisms used in these tests, the language of the F.A.C. indicates that they should be "significant to the indigenous aquatic community". All species used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

LITERATURE CITED

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