BIOASSAYS OF [redacted] ROAD SEWAGE TREATMENT PLANT

ORLANDO, ORANGE COUNTY, FLORIDA

NPDES #FL0022128

SAMPLED 3/17/81

Biological Section
Bureau of Water Analysis

May 4, 1981

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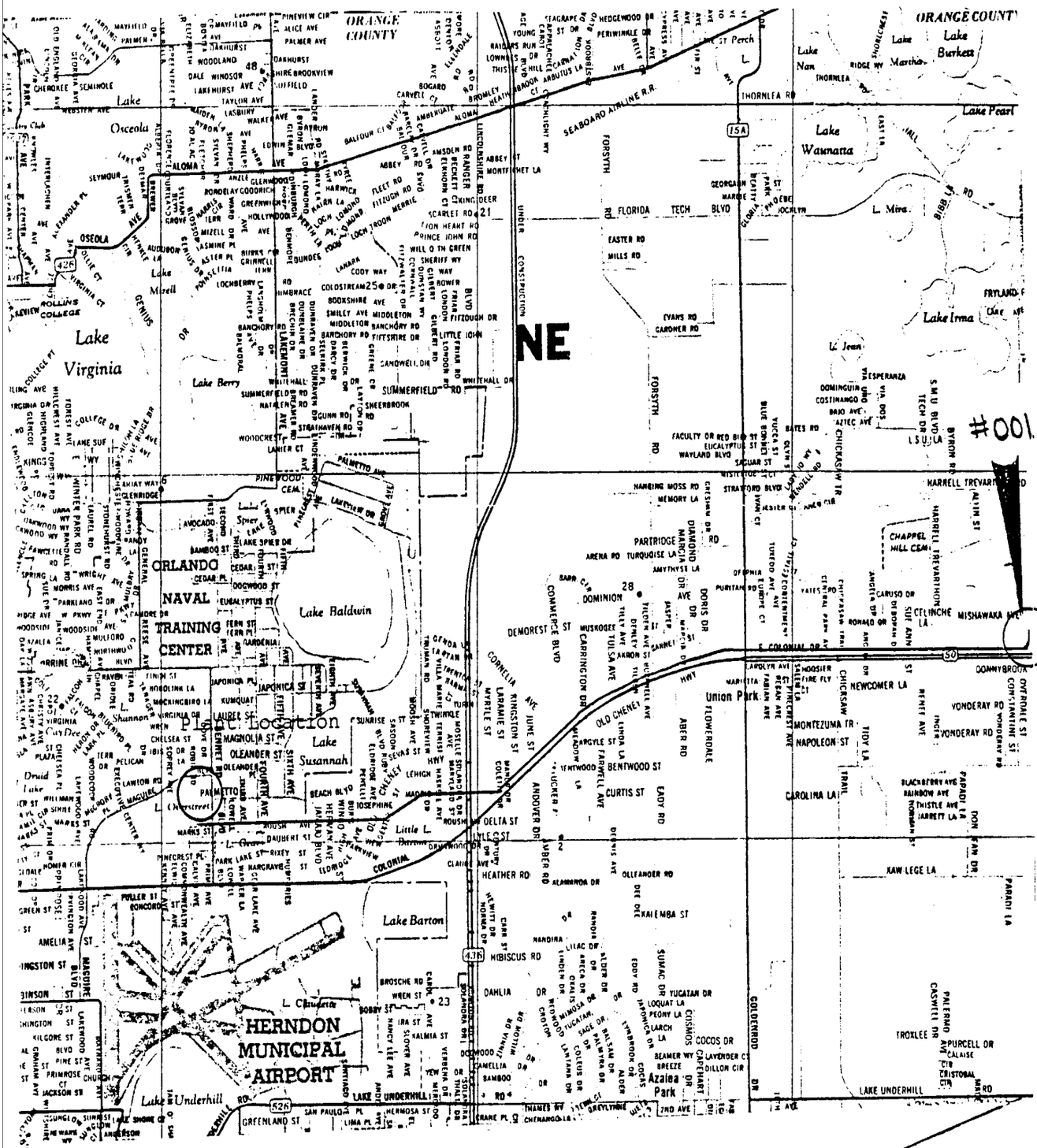
INTRODUCTION

The Bennett Road Sewage Treatment Plant is owned and operated by the City of Orlando, and provides secondary sewage treatment for approximately 96,000 residents and the T. G. Lee Dairy Farm. The facility is located at 900 Bennett Road in northeast Orlando, between the Naval Training Center and Herndon Municipal Airport (Fig. 1). It has a design capacity of 12.0 MGD and a permitted discharge of 10.6 MGD. The NPDES discharge point (Outfall #001) is located several miles east of the treatment plant on Crane Creek (Fig. 1), which ultimately flows into the St. Johns River through the Econlockhatchee River.

In May of 1980, the Biological Section performed a static toxicity bioassay on an unchlorinated sample of effluent from the Bennett Road Sewage Treatment Plant. The results indicated that the effluent was toxic to the test organism Daphnia pulex (48 hour LC_{50}=29%). Toxicity may have been caused by the presence of cadmium in the effluent (FDER, 1980).

Since the time of the May 1980 bioassay, DER field inspectors reported deficiencies in records and reports from the Bennett Road Sewage Treatment Plant. Deficiencies included inadequate spill prevention control and sampling techniques. Additionally, this year the U.S. EPA issued a Notice of Violation to the Bennett Road Plant for failure to submit reports. U.S. EPA compliance inspectors were scheduled to visit the Bennett Road Plant in March to inspect monitoring techniques and procedures.

To further evaluate the toxicity of the Bennett Road effluent, the Biological Section performed additional bioassays on samples of effluent on 18 to 20 March, 1981. The results of these tests are presented in this report.



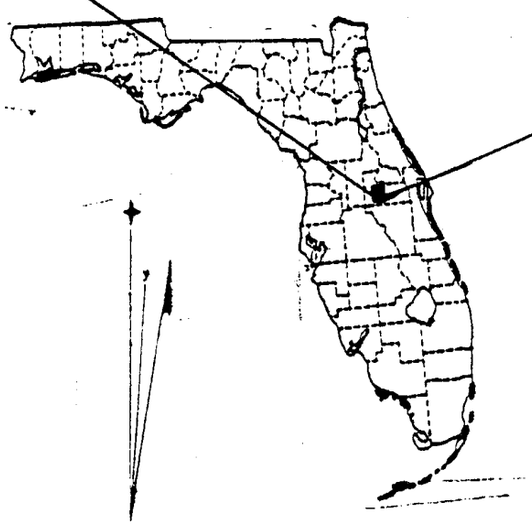


Figure 1. Portions of Orlando and Orange County, including study area with approximate outfall line to Crane Creek.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study include species recommended by Peltier (1978) and the U.S. EPA (1978). Additionally, the Biological Section maintains cultures of several species of organisms indigenous to the State of Florida. 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 2½ gallon all glass aquaria filled with unchlorinated DER well water, using a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of 22° ± 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess daphnids. They are then refilled 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 S. serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biological Section. Positive identification of the organism was performed by Mr. Jeff Foran of the Department of Environmental Engineering Sciences, University of Florida in Gainesville. The cultures are maintained in 2½ 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, and are kept at a constant temperature of 22° ± 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess population. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances.

Pimephales promelas (fathead minnows) - A breeding culture of approximately 100 adult fathead minnows was obtained from the EPA laboratory in Cincinnati, Ohio in May, 1979. The fish have been maintained in four 125 gallon all glass aquaria equipped with a flow through water exchange system using unchlorinated well water obtained from a deep well near the DER laboratory. The fish have been reared at a temperature of 22° ± 2°C under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills Trout Chow® and frozen Artemia (brine shrimp) at a rate of 0.75 g/30 fish, twice daily. Breeding has been encouraged by placing several halved clay pots in each tank to serve as substrates for eggs and to provide cover for the fish. Tanks are examined twice daily (at feeding time) for egg production. Clay pots containing egg clusters are immediately transferred to 15 gallon aerated aquaria for hatching. A new aquarium is prepared for each week's production of eggs, thereby segregating fish into even age groups to be used for testing. After hatching, fathead minnow fry are fed twice daily with freshly hatched Artemia. The fish used in this test were all six weeks old at the time of the test.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly Standard Reference Toxicant Bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Six separate 48 hour static bioassays were conducted from 1030 hours on 18 March to 1130 hours on 20 March, 1981. Grab samples of chlorinated effluent (Outfall #001) and of effluent collected prior to chlorination were obtained by Mr. Louis Ley of DER's St. Johns River District at 1530 hours on 17 March, 1981, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biological Section adopted the following test procedure:

Test #1. Pre-Chlorinated Effluent (Tables 2 and 3). This test was performed on an effluent sample collected at the end of the treatment process but prior to chlorination. And, was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent (Table 4). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-Chlorinated Effluent (Tables 5, 6 and 7). This test was performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized[1]. This test was intended to evaluate the combined toxicity of the effluent and the chlorine reaction products.

Freshwater for dilution of the effluent samples was obtained from the same DER well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1976).

Two replicates, each with six concentrations and a control, were used to test with 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 transferred into large culture dishes for observation and to isolate the proper size and age organisms (neonates). These neonates were then transferred individually by glass pipette and were loaded 10 per chamber, resulting in 20 per test concentration.

Two replicates, of six concentrations and a control, were used for the fathead minnow bioassay. The fish were transferred individually or by twos, by net, into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Fathead minnows were loaded 4 or 5 per chamber resulting in 8 to 10 per concentration. An insufficient number of fish were available to load 10 in all of the concentration.

[1] De-chlorination was accomplished by titrating with an appropriate amount of sodium thiosulfate.

Dissolved oxygen, pH, temperature and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity, total hardness, and conductivity were recorded at the beginning of the test for the controls and the 100% effluent concentration. Additionally, total and unionized ammonia concentrations were recorded at 0 and 48 hours in the controls and 100% effluents in all three tests. These values were recorded on the bioassay data forms and are presented in Tables 2 through 7. Organisms were not fed during these tests.

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

Water fleas (Daphnia pulex and Simocephalus serrulatus)

Concentration	Dilution Water	Effluent	Total Vol.
Control	200 ml	---	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180 ml	20 ml	200 ml
18.0%	164 ml	36 ml	200 ml
32.0%	136 ml	64 ml	200 ml
56.0%	88 ml	112 ml	200 ml
100%	---	200 ml	200 ml

Fathead minnows (Pimephales promelas)

Concentration	Dilution Water	Effluent	Total Vol.
Control	500 ml	---	500 ml
5.6%	472 ml	28 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%	---	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Bennett Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES #FL0022128. 18 to 20 March, 1981.

Test #1. Pre-Chlorinated Sample.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 [b] (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	10	10	8.7	8.9	8.7	7.7	8.2	8.2	116		114		20.4	19.9	19.7	0/<.02	0/.03	
Control	9	9	8	8.8	8.9	8.7	7.7	8.2	8.2										195
5.6%	10	10	10	8.8	8.9	8.3	7.7	8.1	8.2					20.4	19.7	19.7			
5.6%	10	10	10	8.7	8.9	8.1	7.7	8.1	8.2										222
10%	10	10	10	8.3	8.8	8.1	7.6	8.1	8.2					20.5	19.6	19.7			
10%	10	10	10	8.3	8.8	8.0	7.6	8.1	8.2										245
18%	10	10	10	8.2	8.8	8.0	7.5	8.1	8.1					20.7	19.7	19.6			
18%	10	10	10	8.0	8.8	8.0	7.5	8.1	8.1										278
32%	10	10	10	6.9	8.5	7.9	7.4	8.0	8.1					20.7	19.7	19.5			
32%	10	10	8	7.0	8.5	7.9	7.4	8.0	8.1										330
56%	10	8	6	5.9	8.0	7.5	7.3	8.0	8.1					20.9	19.8	19.9			
56%	10	10	3	6.0	8.0	7.3	7.3	8.0	8.0										440
100%	10	8	0	3.4[a]	7.3	6.8	7.1	7.9	8.0	165		129		21.2	20.2	19.9	.05/686	.71/15.4	
100%	10	4	1	3.4[a]	7.1	7.0	7.1	7.9	7.9										690

Remarks: [a] 100% effluent aerated to near saturation prior to loading the test organisms.
[b] Total ammonia is expressed as mg/l as N and unionized ammonia is expressed as mg/l as NH_3.

Table 3. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Bennett Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES #FL0022128, 18 to 20 March, 1981. Test #1. Pre-Chlorinated Sample.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4[b] (mg/ℓ)		Conductivity (μmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	7	7	8.9	8.9	8.5	7.6	8.2	8.2	116		114		20.6	19.7	19.9	0/<.02	0/.03	
Control	10	10	10	9.0	8.9	8.5	7.6	8.2	8.2										195
5.6%	10	9	9	8.8	8.9	8.0	7.6	8.2	8.2					20.5	19.7	19.8			
5.6%	10	10	9	8.8	8.9	7.9	7.6	8.2	8.2										222
10%	10	10	9	8.4	8.8	8.0	7.6	8.1	8.1					20.6	19.8	19.8			
10%	10	8	7	8.4	8.7	7.8	7.6	8.1	8.1										245
18%	10	10	4	8.1	8.7	7.7	7.5	8.1	8.1					20.7	19.8	19.8			
18%	10	10	7	8.1	8.6	7.8	7.5	8.1	8.1										278
32%	10	10	2	7.2	8.3	7.5	7.4	8.0	8.0					20.8	19.8	20.0			
32%	10	9	2	7.3	8.3	7.5	7.4	8.0	8.0										330
56%	10	2	0	5.9	8.1	7.2	7.3	8.0	8.0					21.1	19.9	19.9			
56%	10	2	0	6.0	7.9	7.0	7.3	8.0	8.0										440
100%	10	3	0	3.3[a]	7.2	6.3	7.1	7.9	7.9	165		129		21.4	20.2	19.9	.05/6.86	.57/15.4	
100%	10	3	0	3.3[a]	6.9	5.8	7.1	7.9	7.9										690

Remarks: [a] 100% effluent aerated to near saturation prior to loading the test organisms.
[b] Total ammonia is expressed as mg/l as N and unionized ammonia is expressed as mg/l as NH_3.

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Bennett Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES #FL0022128, on 18 to 20 March, 1981.

Test #2. Chlorinated Sample.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4[d] (mg/ℓ)		Conductivity (μmhos)
	0	24	48	0	24	48[a]	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	10	10	8.8	8.9	---	7.6	8.2	8.1	116		114		20.8	20.0	19.8	0/<.02	0/.03	
Control	10	10	10	8.8	8.9	---	7.6	8.2	8.2										203
5.6%	10	10	9	8.8	8.9	---	7.6	8.2	8.2					20.4	19.9	19.5			
5.6%	10	10	7	8.8	8.9	---	7.6	8.2	8.2										230
10%	11[c]	4	0	8.7	9.0	---	7.6	8.2	8.2					20.4	19.9	19.5			
10%	10	2	0	8.7	9.0	---	7.6	8.2	8.2										249
18%	10	0	---	8.7	9.0	---	7.5	8.1	---					20.5	19.8	---			
18%	10	0	---	8.7	9.1	---	7.5	8.1	---										282
32%	10	0	---	8.4	9.1	---	7.4	8.1	---					20.5	19.8	---			
32%	10	0	---	8.4	9.1	---	7.4	8.1	---										338
56%	10	0	---	8.0	9.1	---	7.3	8.0	---					20.5	19.9	---			
56%	10	0	---	8.0	9.1	---	7.3	8.0	---										440
100%[b]	10	0	---	6.9	9.1	---	7.1	8.0	---	155		139		21.1	20.0	---	.04/5.88	---	
100%[b]	10	0	---	6.8	9.1	---	7.1	8.0	---										690

Remarks:
[a] Dissolved oxygen not taken at 48 hours due to high values at 24 hours and to obvious chlorine toxicity.
[b] Total Chlorine residual = 0.80mg/l (Hach DPD), 1.29 (Iodometric Method).
[c] The extra organism was not removed because it would disturb the test concentration.
[d] Total ammonia is expressed as mg/l as N and unionized ammonia is expressed as mg/l as NH_3.

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Bennett Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES #FL0022128, on 18 to 20 March, 1981. Test #3. De-Chlorinated Sample.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4[a] (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	10	10	8.8	9.0	8.8	7.6	8.2	8.2	116		114		20.4	20.1	19.5	0/<.02	0/.03	210
Control	10	10	10	8.8	9.0	8.8	7.7	8.2	8.2										
5.6%	10	10	10	8.7	9.0	8.7	7.7	8.2	8.2					20.4	19.8	19.5			235
5.6%	10	10	10	8.7	9.0	8.7	7.7	8.2	8.2										
10%	10	10	10	8.7	9.0	8.6	7.7	8.2	8.2					20.4	19.7	19.5			259
10%	10	10	10	8.8	9.0	8.8	7.7	8.2	8.2										
18%	10	10	10	8.8	9.0	8.7	7.7	8.1	8.2					20.5	19.8	19.5			290
18%	10	10	10	8.8	9.0	8.7	7.7	8.1	8.2										
32%	10	10	10	8.7	8.6	8.6	7.7	8.1	8.1					20.6	19.7	19.5			350
32%	10	10	10	8.7	8.6	8.6	7.6	8.1	8.1										
56%	10	10	4	8.3	8.5	8.4	7.5	8.1	8.1					21.0	19.7	19.9			450
56%	10	8	5	8.3	8.5	8.4	7.5	8.1	8.1										
100%	10	2	0	7.6	7.8	8.0	7.3	8.0	8.1	155		139		21.1	19.8	20.3	.06/5.32	.39/16.8	700
100%	10	7	2	7.6	7.7	8.0	7.3	8.0	8.1										

Remarks:

[a] Total ammonia is expressed as mg/l as N and unionized ammonia is expressed as mg/l as NH_3.

Table 6. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Bennett Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES #FL0022128, on 18 to 20 March, 1981.
Test #3 De-Chlorinated Sample.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 [b] (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	9	8	8.7	8.9	8.7	7.7	8.1	8.2	116		114		20.4	20.0	19.6	0/<.02	0/.03	210
Control	10	9	8[a]	8.7	8.9	8.8	7.7	8.1	8.2										
5.6%	10	8	4	8.7	8.9	8.8	7.7	8.1	8.2					20.4	19.8	19.8			235
5.6%	10	8	7	8.7	8.9	8.7	7.7	8.1	8.2										
10%	10	9	7	8.8	8.8	8.6	7.7	8.1	8.1					20.4	19.8	19.6			255
10%	10	7	5	8.8	8.9	8.6	7.7	8.1	8.1										
18%	10	7	3	8.8	8.8	8.4	7.6	8.1	8.1					20.5	19.8	19.6			290
18%	10	9	5	8.8	8.8	8.4	7.6	8.1	8.1										
32%	10	7	1	8.6	8.7	8.4	7.5	8.0	8.1					20.7	19.8	19.7			350
32%	10	8	0	8.6	8.7	8.5	7.5	8.0	8.1										
56%	10	2	0	8.2	8.3	8.3	7.5	8.0	8.0					21.0	19.9	20.1			450
56%	10	4	0	8.2	8.3	8.2	7.5	8.0	8.0										
100%	10	0	---	7.5	7.5	---	7.3	7.9	---	155		139		21.6	20.0	---	.06/5.32	.39/16.8	700
100%	10	0	---	7.5	7.5	---	7.3	7.9	---										

Remarks:

[a] One organism was stuck to side of glass.

[b] Total ammonia is expressed as mg/l as N and unionized ammonia is expressed as mg/l as NH_3.

Table 7 . Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of Bennett Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES #FL0022128, on 18 to 20 March, 1981. Test #3 De-Chlorinated Sample.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4[a] (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	5	5	5	8.9	6.6	7.4	7.7	7.9	7.8	116		114		20.4	19.7	19.5	0/<.02	0/.39	210
Control	5	5	5	8.9	6.1	6.6	7.7	7.9	7.8										
5.6%	4	4	4	8.9	6.2	6.6	7.7	7.9	7.8					20.6	19.6	19.3			235
5.6%	5	5	5	8.9	6.7	6.7	7.7	7.9	7.8										
10%	4	4	4	8.8	6.8	7.0	7.7	7.9	7.8					20.7	19.7	19.3			255
10%	5	5	5	8.8	6.4	7.0	7.7	7.9	7.8										
18%	4	4	4	8.7	6.3	6.9	7.7	7.9	7.8					20.8	19.7	19.3			290
18%	4	4	4	8.7	6.1	6.9	7.7	7.9	7.8										
32%	5	5	5	8.6	6.1	6.8	7.6	7.9	7.8					20.9	19.7	19.3			350
32%	4	4	4	8.6	6.4	6.8	7.6	7.9	7.9										
56%	4	4	4	8.0	6.4	6.8	7.5	7.9	7.9					21.2	19.9	19.4			450
56%	4	4	4	8.0	6.3	6.8	7.5	7.9	7.9										
100%	5	5	5	7.5	5.2	6.1	7.3	7.9	7.8	155		139		21.6	20.1	19.6	.06/5.32	.57/19.6	700
100%	5	5	5	7.5	5.1	6.1	7.3	7.9	7.8										

Remarks: [a] Total ammonia is expressed as mg/l as N and unionized ammonia is expressed as mg/l as NH_3.

RESULTS AND DISCUSSION

The prechlorinated effluent from the Bennett Road Sewage Treatment Plant demonstrated measurable toxicity to the test organisms Daphnia pulex and Simocephalus serrulatus (48 hour LC_{50}=53% and 17 to 19.5% respectively) (Table 8). The chlorinated effluent from this facility was considerably more toxic to Daphnia pulex (24 and 48 hour LC_{50}=8.7 and 6.7%)(Table 8). The results of the tests with dechlorinated effluent were similar to those with prechlorinated effluent. The 48 hour LC_{50} values for Daphnia pulex were 54 to 58.8%, while Simocephalus serrulatus exhibited 48 hour LC_{50}'s of 9.4 to 13.4% (Table 9). The dechlorinated effluent caused no mortality in the Pimephales promelas bioassay. The 96 hour LC_{50} for these toxicity tests would be less than or equal to the values reported for 48 hours.

That the toxic responses noted in the prechlorinated and dechlorinated bioassays were similar suggests the presence of a toxic substance(s) in the effluent not related to chlorine. The high concentrations of unionized ammonia found in the effluent, particularly at 48 hours (Tables 2-7), may account for the toxicity since values ranging from 0.2 to 2.0 mg/l are known to be lethal to a variety of fish (U.S. EPA 1976). The increase in ammonia noted in all of the effluent samples may be due to the decomposition of organic nitrogen, possibly because of poor or incomplete treatment of the wastewater. The increase in ammonia concentration was not attributed to excretory products from the test organisms since the controls did not exhibit similar increases. In the absence of additional chemical data it is not possible to tell if unionized ammonia was the sole toxic agent in the Bennett Road Sewage Treatment Plant discharge.

Chapter 17-3.051, Florida Administrative Code, indicates that "all waters of the State shall at all places and at all times be free from...components of discharges which...are acutely toxic." "Acute toxicity" is defined in Chapter 17-3.021(1) to mean "the presence of one or more substances...in amounts which are greater than one-third (1/3) of the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50})...". In the absence of a mixing zone, this 1/3 of the 96 hour LC_{50} applies at the point of discharge. Chapter 17-4.244(4) states that "in no event shall the maximum concentration of wastes in the mixing zone exceed the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50}) for a species significant to the indigenous aquatic community..." This criterion applies at the point of discharge if there is a mixing zone. The distinction between presence or absence of a mixing zone is not relevant here, however, since the testing procedure used does not involve effluent concentration. The 1/3 of the 96 hour LC_{50} criterion is three times as stringent as the 96 hour LC_{50} criterion, and therefore, in the absence of a mixing zone, the procedure used is very lenient. The procedure followed is also lenient in that testing is only done for 48 hours. The 96 hour LC_{50} derived from this testing is therefore reported as "less than or equal to" the 48

hour measurement. The value found after 48 hours can never be more restrictive than the value found after 96 hours. It must be noted that all organisms 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.

Table 8 . LC_{50} Data for Bioassay of Bennett Road Sewage Treatment Plant, 11 to 20 March, 1981.

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Daphnia pulex	Binomial	A	53% (32 and 100)
Test #1	Moving Average	A	53% (45 and 65)
Pre-chlorinated Sample	Probit	$\geq$100%, B	53% (45 and 63)
Simocephalus serrulatus	Binomial	45.5% (32 and ∞)	19.5% (10 and 32)
Test #1	Moving Average	50.2% (38 and 62)	17.2% (14 and 21)
Pre-chlorinated Sample	Probit	54.6% (0 and ∞), C	17.0% (14 and 21)
Daphnia pulex	Binomial	8.7% (5.6 and 18)	6.7% (5.6 and 10)
Test #2	Moving Average	A	A
Chlorinated Sample	Probit	A	A

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

B. Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

C. Due to the distribution of mortality, results using the probit method are not statistically sound.

Table 9. LC_{50} Data for Bioassay of Bennett Road Sewage Treatment Plant 18 to 20 March, 1981.

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Daphnia pulex Test #3 De-chlorinated Sample	Binomial	94.4% (56 and ∞),A	54.0% (32 and 100)
	Moving Average	94.4% (56 and ∞),A	58.4% (51 and 68)
	Probit	94.7% (80 and 131),A	58.8% (50 and 69)
Simocephalus serrulatus Test #3. De-chlorinated Sample	Binomial	43.8% (32 and 100)	13.4% (0 and 32),A
	Moving Average	35.3% (28 and 46)	10.5% (6 and 15),A
	Probit	31.2% (6 and 2000),B	9.4% (6 and 12),A
Pimephales promelas Test #3 De-chlorinated sample	Binomial Moving Average Probit	No mortality	No mortality

A. Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

B. Due to the distribution of mortality, results using the probit method are not statistically sound.

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