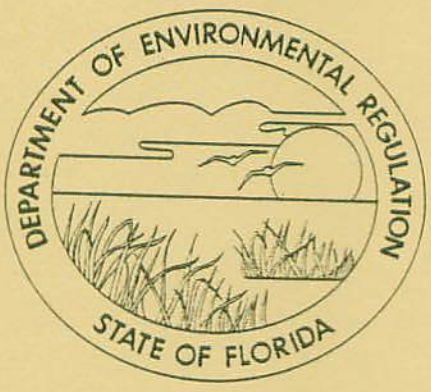


BIOASSAYS OF SAND LAKE ROAD SEWAGE TREATMENT PLANT

ORANGE COUNTY, FLORIDA

NPDES # FL0022110

Biological Section
Bureau of Water Analysis
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INTRODUCTION

The Sand Lake Road Sewage Treatment Plant is located in southwest Orange County, Florida (Figure 1). The facility serves a population of approximately 58,000 including a large part of the City of Orlando, plus 20% of unincorporated Orange County. The plant has a design capacity of 6.0 MGD and an average flow of 5.67 MGD. Secondary sewage treatment consists of contact stabilization, secondary clarification and retention in a polishing pond prior to discharge. The major industrial contributor of the Sand Lake Road Plant is the Martin-Marietta Corporation, producers of guided missile systems and the space shuttle. Effluent discharge is to Shingle Creek, a major tributary to West Lake Tohopekaliga.

The Sand Lake Plant has received numerous notifications of violations, dating back to 1975, usually for exceeding NPDES permit limitations for total suspended solids and fecal coliforms.

In June of 1980, the Biological Section performed a toxicity bioassay on effluent samples from the Sand Lake Road STP. The results of this test indicated that the effluent was toxic to Daphnia pulex (FDER 1980). At the request of DER's St. Johns River District, on 31 March to 2 April, 1981, the Biological Section performed a series of follow up bioassays on effluent samples from this facility to further evaluate the extent of toxicity to the biota of Shingle Creek. The results of these tests are reported here.

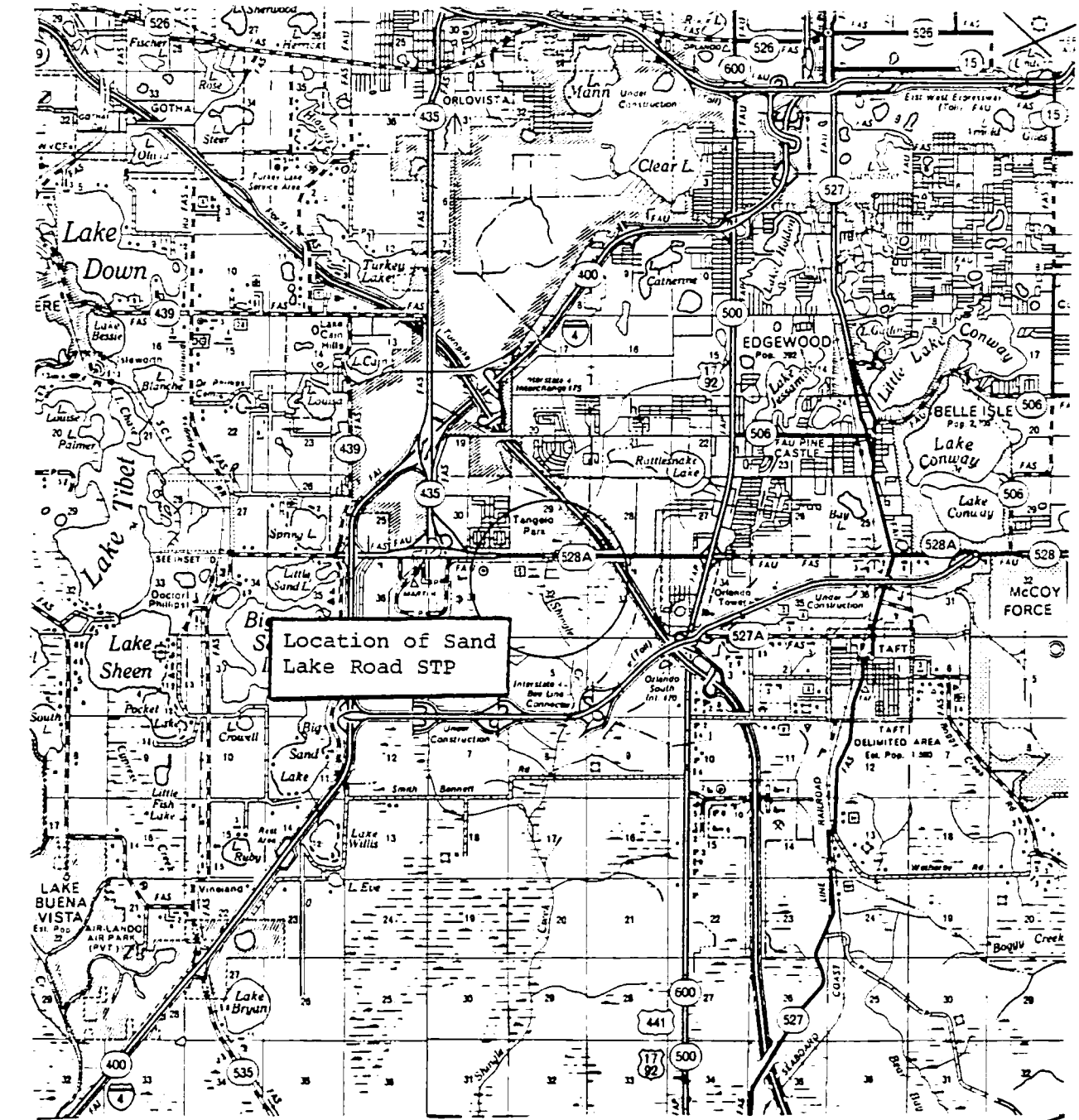


Figure 1. Portions of Orange County, Florida Including Study Area.

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 $22^{o} \pm 1^{o}C$. 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 $22^{o} \pm 1^{o}C$. 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.

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

Five separate 48 hour statis bioassays were conducted from 1030 hours on 31 March to 1245 hours on 2 April 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 Office at 1545 hours on 30 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&3). This test was performed on an effluent sample collected at the end of the treatment process but prior to chlorination. And, it 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). 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 sample 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 respective large culture dishes. Neonates were then transferred individually by glass pipette, examined for injury, and loaded 10 per chamber, resulting in 20 per test concentration.

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, total and un-ionized ammonia, and conductivity were recorded at the beginning of the tests for the controls and the 100% effluent concentrations. These values were recorded on the bioassay data forms and are presented in Tables 2 through 6. Organisms were not fed during these tests.

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

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

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Sand Lake Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES # FL0022110, on 31 March - 2 April, 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 (mg/ℓ)		Conductivity (μmhos)
	0	24	48[a]	0	24	48	0	24	48	0	48	0	48	0	24	48	0[b]	48	0
Control	10	9	2	8.5	9.0	8.6	7.7	8.1	8.1	114		110		20.9	20.5	20.2	0/.147		
Control	10	10	10	8.5	8.9	8.6	7.7	8.1	8.1										222
5.6%	10	10	10	8.5	8.9	8.6	7.7	8.1	8.1					20.7	20.3	20.2			
5.6%	10	10	10	8.4	8.9	8.6	7.7	8.1	8.1										227
10%	10	10	10	8.4	8.9	8.6	7.7	8.1	8.1					20.7	20.2	20.1			
10%	10	10	10	8.4	8.9	8.6	7.7	8.1	8.1										239
18%	10	10	10	8.4	8.9	8.6	7.8	8.1	8.1					20.7	20.2	20.1			
18%	10	10	10	8.3	8.9	8.6	7.7	8.1	8.1										259
32%	10	10	10	8.3	8.9	8.6	7.7	8.1	8.1					20.8	20.2	20.0			
32%	10	10	10	8.3	8.9	8.6	7.7	8.1	8.1										297
56%	10	10	10	8.0	8.9	8.6	7.6	8.1	8.0					21.1	20.1	20.0			
56%	10	10	10	8.0	8.9	8.5	7.6	8.1	8.0										360
100%	10	10	10	7.5	8.8	8.5	7.5	8.0	8.0	85		120		21.4	20.1	20.1	0/.252		
100%	10	10	8	7.5	8.8	8.5	7.5	8.0	8.0										472

Remarks: a. Test invalidated at 48 hours due to excessive control mortality (>20%).
b. Total ammonia is expressed as mg/l as N and unionized ammonia is espressed as mg/l as NH_3.

Table 3. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Sand Lake Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES # FL0022110, on 31 March - 2 April, 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 (mg/l)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0[a]	48	0
Control	10	10	10	8.5	8.9	8.5	7.7	8.1	8.1	114		110		20.9	20.7	19.8	0/.147		
Control	10	9	9	8.5	8.9	8.5	7.7	8.1	8.1										219
5.6%	10	10	9	8.4	8.9	8.5	7.7	8.1	8.1					20.9	20.6	19.8			
5.6	10	10	7	8.4	8.9	8.5	7.8	8.1	8.1										229
10%	10	10	9	8.3	8.9	8.6	7.8	8.1	8.1					20.8	20.4	19.9			
10%	10	10	10	8.3	9.0	8.6	7.8	8.2	8.1										239
18%	10	10	10	8.3	8.9	8.6	7.8	8.1	8.1					20.8	20.4	20.1			
18%	10	10	10	8.3	8.9	8.6	7.8	8.1	8.1										264
32%	10	10	10	8.3	8.8	8.5	7.7	8.1	8.1					20.7	20.5	20.1			
32%	10	10	10	8.2	8.8	8.5	7.7	8.1	8.1										299
56%	10	10	10	7.9	8.8	8.4	7.7	8.1	8.1					20.9	20.5	29.2			
56%	10	10	10	7.9	8.8	8.4	7.7	8.0	8.1										365
100%	10	10	9	7.4	8.8	8.4	7.5	8.0	8.0	85		120		21.2	20.5	20.3	0/.252		
100%	10	9	8	7.2	8.8	8.4	7.5	8.0	8.0										472

Remarks: a. Total ammonia is expressed as mg/l as N and un-ionized ammonia is expressed as mg/l as NH_3.

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Sand Lake Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES # FL0022110, Outfall # 001, on 31 March - 2 April, 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 (mg/l)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0[b]	48	0
Control	10	10	10	8.2	8.9	8.4	7.8	8.1	8.1	114		110		21.0	20.7	20.6	0/.147		
Control	10	10	10	8.2	8.9	8.6	7.8	8.2	8.1										218
5.6%	11[a]	11	11	8.2	8.9	8.5	7.8	8.2	8.1					20.9	20.7	20.6			
5.6%	10	10	10	8.2	8.9	8.7	7.8	8.2	8.1										232
10%	10	10	10	8.2	9.0	8.6	7.8	8.2	8.1					20.8	20.6	20.3			
10%	10	10	10	8.2	9.0	8.6	7.8	8.2	8.1										244
18%	10	10	10	8.2	9.0	8.7	7.8	8.2	8.1					20.9	20.5	20.3			
18%	10	10	10	8.2	9.0	8.7	7.8	8.2	8.1										262
32%	10	10	10	8.1	9.1	8.8	7.7	8.1	8.1					20.8	20.4	20.3			
32%	10	10	10	8.1	9.1	8.8	7.7	8.1	8.1										298
56%	10	10	10	8.1	9.0	8.8	7.6	8.1	8.1					20.8	20.4	20.2			
56%	10	10	10	8.1	9.1	8.8	7.6	8.1	8.1										361
100%	10	9	7	7.7	9.1	8.9	7.4	8.0	8.0	85		118		21.1	20.5	20.3	0/.308		
100%	10	6	3	7.7	9.1	8.8	7.4	8.0	8.0										467

Remarks: The total chlorine residual was determined in each concentration as follows:

Control	0.00 mg/l	(Hach DPD)
5.6%	0.01 mg/l	"
10.0%	0.07 mg/l	"
18.0%	0.10 mg/l	"
32.0%	0.20 mg/l	"
56.0%	0.30 mg/l	"
100.0%	0.50 mg/l	"

a. Since it is difficult to remove extra test organisms without disturbing the test container, the extra Daphnia was allowed to remain in the 5.6 concentration.

b. Total ammonia is expressed as mg/l as N and un-ionized ammonia is expressed as mg/l as NH_3

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Sand Lake Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDSS # FL0022110, Outfall # 001, on 31 March - 2 April, 1981.

Test #3. De-Chlorinated Sample [a]

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as Ca^{++})		Temperature (°C)			NH_3/NH_3+NH_4 (mg/l)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0 [b]	48	0
Control	10	10	10	8.4	9.0	8.6	7.8	8.1	8.1	114		110		21.0	20.7	20.6	0./147		
Control	10	10	10	8.4	9.0	8.6	7.8	8.1	8.1										219
5.6%	10	10	10	8.3	9.0	8.6	7.8	8.1	8.1					20.9	20.5	20.4			
5.6%	10	10	10	8.3	9.0	8.6	7.8	8.1	8.1										232
10%	10	10	10	8.3	9.0	8.6	7.8	8.1	8.1					20.8	20.6	20.5			
10%	10	10	10	8.3	9.0	8.6	7.8	8.2	8.1										245
18%	10	10	10	8.3	9.0	8.6	7.8	8.2	8.1					20.8	20.7	20.4			
18%	10	10	10	8.3	9.0	8.5	7.8	8.2	8.1										263
32%	10	10	10	8.3	9.0	8.5	7.8	8.1	8.1					20.9	20.7	20.4			
32%	10	10	10	8.3	9.1	8.5	7.8	8.1	8.1										302
56%	10	10	10	8.3	8.9	8.5	7.7	8.1	8.0					21.0	20.6	20.5			
56%	10	10	10	8.3	9.0	8.4	7.7	8.1	8.0										365
100%	10	10	10	8.1	9.0	8.2	7.5	8.0	8.0	85		118		21.8	20.8	20.6	0/.308		
100%	10	10	10	8.1	9.0	8.2	7.5	8.0	8.0										479

Remarks: a. The total chlorine residual was determined to be 0.55 mg/l (Hach DPD) and 1.13 mg/l (Iodometric Method I). This chlorine concentration was then neutralized with a calculated amount of sodium thiosulfate.

b. Total ammonia is expressed as mg/l as N and un-ionized ammonia is expressed as mg/l as NH_3.

Table 6. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Sand Lake Road Sewage Treatment Plant, Orlando, Orange County, Florida, NPDES # FL0022110, Outfall # 001, on 31 March - 2 April, 1981.

Test # 3. De-Chlorinated Sample[a]

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 (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0[b]	48	0
Control	10	10	9	8.3	9.0	8.4	7.8	8.2	8.1	114		110		21.0	20.4	20.9	0/.147		
Control	10	9	9	8.3	9.0	8.4	7.8	8.2	8.1										218
5.6%	10	10	10	8.3	9.0	8.5	7.8	8.2	8.1					20.9	20.5	20.9			
5.6%	10	9	8	8.3	9.0	8.5	7.8	8.2	8.1										232
10%	10	10	10	8.3	9.0	8.4	7.8	8.2	8.1					20.8	20.6	20.9			
10%	10	10	10	8.3	9.0	8.4	7.8	8.2	8.1										245
18%	10	10	9	8.3	9.0	8.4	7.8	8.1	8.1					20.9	20.6	20.9			
18%	10	10	8	8.3	9.0	8.4	7.8	8.1	8.1										261
32%	10	10	10	8.3	9.0	8.4	7.8	8.1	8.1					21.0	20.7	20.7			
32%	10	10	10	8.3	9.0	8.4	7.8	8.1	8.1										302
56%	10	10	10	8.3	9.0	8.4	7.7	8.1	8.1					21.3	20.7	20.8			
56%	10	10	10	8.3	9.0	8.3	7.7	8.1	8.1										367
100%	10	10	10	8.1	9.0	8.3	7.5	8.0	8.0	85		118		21.8	21.0	20.9	0/.308		
100%	10	9	9	8.1	9.0	8.3	7.5	8.0	8.0										478

Remarks: a. The total chlorine residual was determined to be 0.55 mg/l (Hach DPD) and 1.13 mg/l (Iodometric Method I). This chlorine concentration was then neutralized with a calculated amount of sodium thiosulfate.

b. Total ammonia is expressed as mg/l as N and un-ionized ammonia is expressed as mg/l as NH_3.

RESULTS AND DISCUSSION

The pre-chlorinated effluent sample from the Sand Lake Road Sewage Treatment Plant caused some mortality to Daphnia and Simocephalus, however, LC_{50} values could not be calculated (Table 7). The 48 hour Daphnia pulex bioassay was invalidated due to excessive control mortality (> 20%). The chlorinated effluent proved to be acutely toxic to Daphnia pulex at 48 hours (48 hours LC_{50} = 100%) (Table 7). The 96 hour LC_{50} would therefore be $\leq$ 100%. The toxicity can be attributed to chlorine since a residual of 0.5 mg/l (Hach DPD) was found in the 100% effluent (Table 4). Additionally, field parameters taken at the time of sampling showed total residual chlorine of 1.3 mg/l (Table 8). These concentrations of chlorine have been found to be toxic to aquatic organisms (U.S. EPA 1976). The dechlorinated effluent was not acutely toxic to Daphnia but did cause some mortality to Simocephalus (Table 7). However, an LC_{50} could not be calculated.

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 (90 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, if no mixing zone is permitted for the subject facility. The toxicity is not in excess of allowable limits if a mixing zone has been established for the discharge.

Table 7. LC_{50} Data for Bioassay of Sand Lake Road Sewage Treatment Plant, NPDES # FL0022110 on 31 March to 2 April, 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 #1. Pre-Chlorinated Sample	Binomial		A,C
	Moving Average	No Mortality	A,C
	Probit		A,C
Simocephalus serrulatus Test #1. Pre-Chlorinated Sample	Binomial	A	A
	Moving Average	A	A
	Probit	A	A
Daphnia pulex Test #2. Chlorinated Sample	Binomial	A	100% (56 and∞),B
	Moving Average	A	A
	Probit	A	A
Daphnia pulex Test #3. De-Chlorinated Sample	Binomial		
	Moving Average	No Mortality	No Mortality
	Probit		
Simocephalus serrulatus Test #3. De-Chlorinated Sample	Binomial	A	A
	Moving Average	A	A
	Probit	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. 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. Test invalidated due to excessive control mortality.

Table 8. Field Parameters of Sand Lake Road Sewage Treatment Plant Effluent Samples Collected on 30 March 1981.

Sample 1. Effluent before chlorination

Temperature:	20°C
Conductivity:	500 umhos
pH:	7. 4
Dissolved Oxygen:	6.7 mg/l
Total Residual Chlorine:	Not Detected

Sample 2. Effluent after chlorination

Temperature:	26°C
Conductivity:	500 umhos
pH:	7.6
Dissolved Oxygen:	7.5 mg/l
Total Residual Chlorine:	1.3 mg/l

Field Measurements by: Guy Hadley

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