

BIOASSAYS OF THE SANFORD WASTEWATER TREATMENT PLANT

SEMINOLE COUNTY, FLORIDA

NPDES #FL0020141

NOVEMBER 29, 1984

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE SANFORD WASTEWATER TREATMENT PLANT SEMINOLE COUNTY, FLORIDA NPDES #FL0020141

Biology Section
Bureau of Laboratories
November 29, 1984

EXECUTIVE SUMMARY

Sanford WWTP, NPDES #FL0020141, on 28 to 30 August, 1984

The samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from the municipal waste treatment facility exhibited differing degrees of toxicity to the test organism, Daphnia pulex. The pre-chlorinated effluent was lethal to sufficient numbers of D. pulex to calculate a 48 hour LC_{50} of 3906.2%, however, this mortality is neither acutely nor chronically toxic according to state water quality criteria definitions. The chlorinated effluent, however, proved to be extremely toxic to D. pulex, producing a 48 hour LC_{50} of 7.2%. This toxicity was partly due to the total residual chlorine (0.5 mg/l) measured in the sample. However, this moderate concentration of chlorine could not have been the sole cause of the extreme toxicity noted. The de-chlorinated sample also proved to be acutely toxic to D. pulex (48 hour LC_{50} = 78.7%), suggesting that some toxic components were carried over from the chlorination process. The toxicity could not have been caused by ammonia, since only modest concentration of total (maximum = 1.1 mg/l) and unionized (maximum = 0.01 mg/l) ammonia were measured in the samples. Additional bioassays performed on the pre-chlorinated and de-chlorinated effluents, using Notropis leedsi as test organism, resulted in no mortality to the fish.

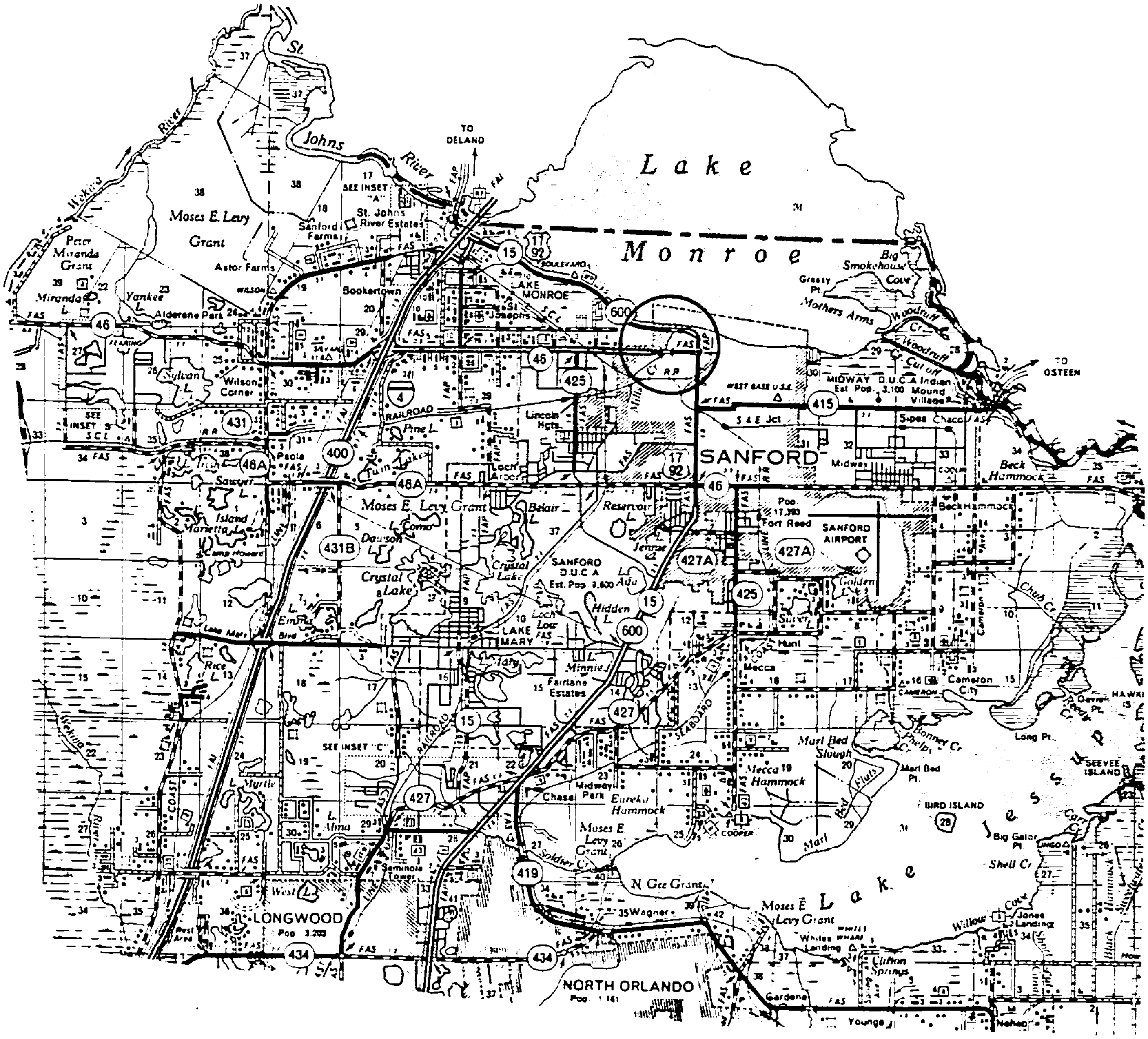
Chemical analyses indicated the presence of only moderate concentrations of lead (11.2 ug/l) and zinc (28 ug/l), and several unidentified organic compounds in this effluent. Therefore, the toxicity exhibited in these bioassays may have been partly due to the organic compounds, but, more likely due to the presence of other constituents or components not adequately tested for in chemical analyses.

INTRODUCTION

The Sanford Wastewater Treatment Plant is located at 300 North Popular Avenue, Sanford, Seminole County, Florida (Fig. 1). According to the NPDES file, this facility treats municipal wastes from a population of approximately 23,000 using a secondary activated sludge process. The plant began operations in 1964 and was last revised in 1973. A schematic of the Sanford WWTP is presented in Figure 2. The plant has a design flow capacity of 6.0 MGD, but currently treats an average of only 3.56 MGD. The final chlorinated effluent is discharged to lake Munroe through a pipe 240 feet long and 7 feet deep.

Additional information in the NPDES file reveals that the Sanford WWTP has not received state certification nor an official wasteload allocation from the Department due to the facility causing water quality standard violations in Lake Munroe. An additional permit limitation was also placed on the Sanford WWTP, requiring that the total chlorine residual not exceed 0.2 mg/l in the effluent. The Department's current position concerning the Sanford WWTP states that the facility cease discharging to lake Munroe in the next few years and investigates alternate means of waste disposal.

To evaluate the potential toxicity of the Sanford WWTP effluent to the biota of the receiving waters, the St. Johns River District requested acute toxicity bioassays be performed. The Biology Section received three samples of effluent and performed a total of five 48 hour static acute toxicity bioassays on 28 to 30 August, 1984. Chemistry samples were also collected and delivered to the Chemistry Section for analyses which may identify toxic components. The results of these tests are presented in this report. Those involved in performing the bioassays and in preparation of this report were: C. W. Dye, R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.



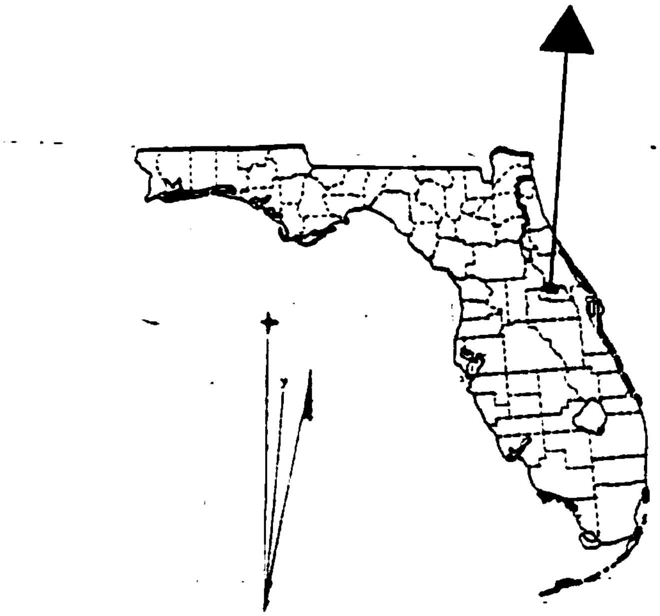


Figure 1. Portions of Seminole County, Florida, including the city of Sanford and the approximate location of the wastewater treatment plant.

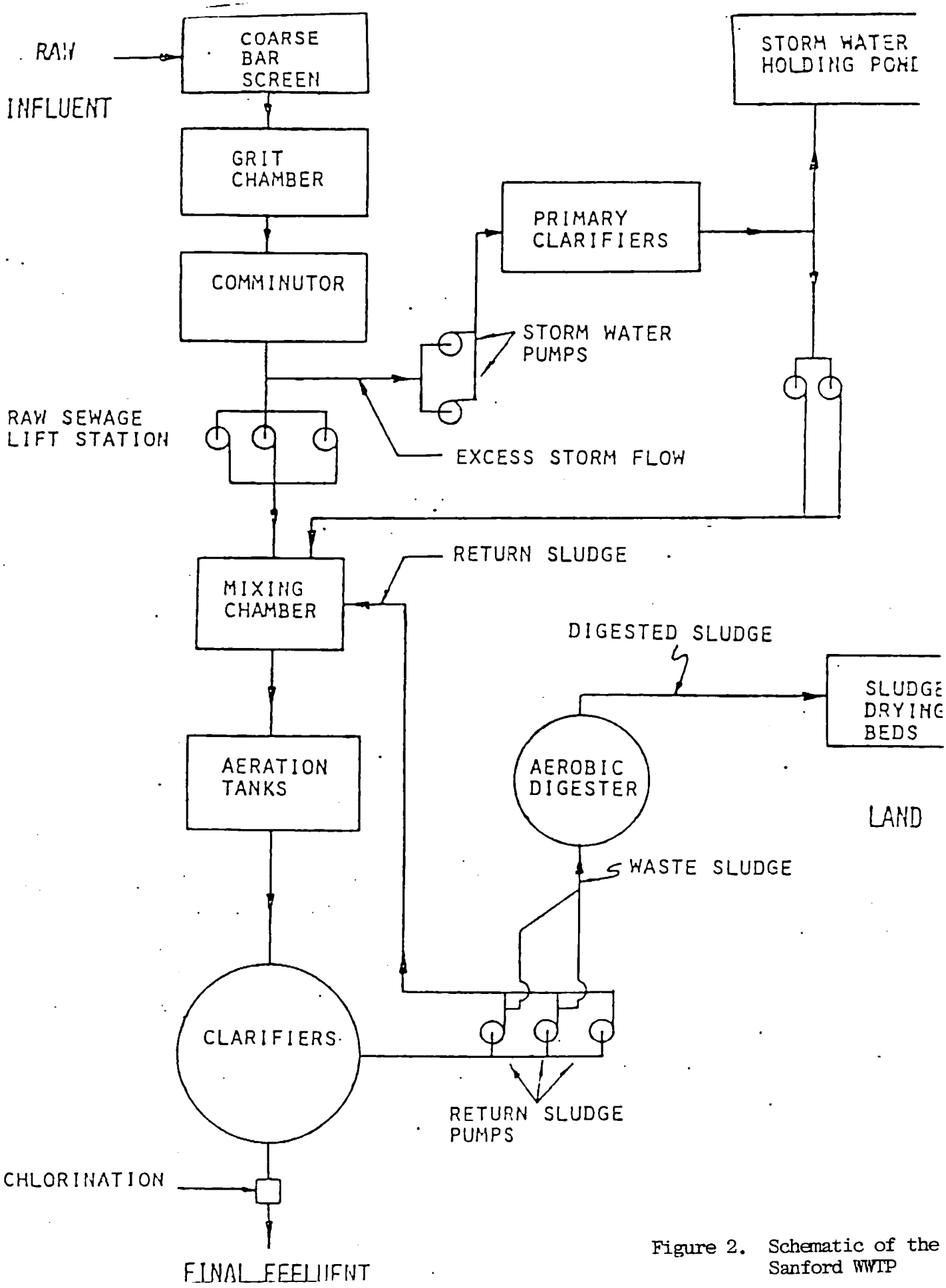


Figure 2. Schematic of the Sanford WWTP

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. One of these indigenous species was 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.

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 14 to 28 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

Five separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from the Sanford WWTP. The tests were performed from 1110 hours on 28 August to 1110 hours on 30 August, 1984. The samples were collected by Mr. Louis Ley of DER's St. John's River District from 1400 to 1415 hours on 27 August, 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.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biology 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 and 6). This test was performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025 N sodium thiosulfate. 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 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 six concentrations and a control, were used to test with the water flea, Daphnia pulex. 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 were siphoned into a large culture dish 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 six concentrations and a control, were used for the Notropis leedsi (bannerfin shiner) bioassays. 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 5 per chamber, resulting in 10 per concentration. 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, and 48 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% effluent 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% effluent 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 6.

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

Daphnia pulex (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	--------	200 ml
5.6%	188.8 ml	11.2 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
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
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Sanford WWTP, Seminole County, Florida, NPDES #FL0020141, on 28 to 30 August, 1984.

Test 1 Pre-chlorinated effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Control	10	10	9	8.7	8.6	7.9	7.7	8.0	7.9	21.4	20.6	20.4	199		.0/.08	
Control	10	10	8	8.7	8.6	7.9	7.7	8.0	7.9					218		
5.6%	10	10	9	8.6	8.5	7.9	7.7	8.0	7.9	21.3	20.2	20.0	217			
5.6%	10	10	10	8.6	8.5	7.9	7.7	8.0	7.9					229		
10%	10	9	7	8.3	8.4	8.0	7.6	8.0	7.9	21.2	20.0	19.9	324			
10%	10	10	10	8.3	8.4	8.0	7.6	8.0	7.9					238		
18%	10	10	9	8.1	8.4	8.0	7.6	8.0	7.9	21.0	20.0	19.9	242			
18%	10	10	10	8.1	8.4	7.9	7.6	8.0	7.9					255		
32%	10	10	10	7.8	8.4	7.6	7.5	7.9	7.9	20.8	20.0	19.9	270			
32%	10	10	10	7.8	8.4	7.6	7.5	7.9	7.9					288		
56%	10	10	10	7.3	8.3	7.4	7.3	7.9	7.8	20.4	20.0	19.9	320			
56%	10	10	7	7.3	8.3	7.4	7.3	7.9	7.8					341		
100%	10	9	7	5.9	8.1	7.3	7.1	7.7	7.7	19.9	19.9	20.3	410		.004/0.73	
100%	10	10	8	5.9	8.1	7.3	7.1	7.7	7.7					441		

Control Water

Total Alkalinity 106 (mg/l $CaCO_3$)

Total Hardness 113 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 100 (mg/l $CaCO_3$)

Total Hardness 99 (mg/l $CaCO_3$)

REMARKS: [a] Total residual chlorine = none detected (HACH method)

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of the Sanford WWTP, Seminole County, Florida, NPDES #FL0020141, on 28 to 30 August, 1984.

Test 1 Pre-chlorinated effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Control	5	5	5	8.3	8.1	7.8	7.7	7.9	7.9	21.7	20.5	20.3			0/.08	
Control	5	5	5	8.3	8.1	7.8	7.7	7.9	7.9				208	229		
5.6%	5	5	5	8.2	8.1	7.9	7.7	7.9	7.9	21.5	20.2	19.9				
5.6%	5	5	5	8.2	8.1	7.9	7.7	7.9	7.9				219	238		
10%	5	5	5	8.2	8.1	7.9	7.6	7.9	7.9	21.4	19.8	19.8				
10%	5	5	5	8.2	8.1	7.9	7.6	7.9	7.9				229	249		
18%	5	5	5	7.9	8.1	7.9	7.5	7.8	7.9	21.2	19.8	19.7				
18%	5	5	5	7.9	8.1	7.9	7.5	7.8	7.9				247	268		
32%	5	5	5	7.6	8.0	7.7	7.4	7.8	7.8	20.9	19.8	19.7				
32%	5	5	5	7.6	8.0	7.7	7.4	7.8	7.8				277	299		
56%	5	5	5	6.9	7.8	7.6	7.3	7.7	7.7	20.4	19.8	19.7				
57%	5	5	5	6.9	7.8	7.6	7.3	7.7	7.7				322	345		
100%	5	5	5	5.8	7.4	7.4	7.1	7.6	7.7	19.7	19.9	19.7			.004/.73	
100%	5	5	5	5.8	7.4	7.4	7.1	7.6	7.7				412	448		

Control Water

Total Alkalinity 106 (mg/l $CaCO_3$)

Total Hardness 113 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 100 (mg/l $CaCO_3$)

Total Hardness 99 (mg/l $CaCO_3$)

REMARKS: [a]Total residual chlorine = none detected (HACH method)

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Sanford WWTP, Seminole County, Florida, NPDES #FL0020141, on 28 to 30 August, 1984.

Test 1 Chlorinated effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Control	10	10	9	8.3	8.7	7.6	7.6	8.0	7.9	21.3	20.1	20.1			0/.08	
Control	10	10	9	8.3	8.7	7.6	7.6	8.0	7.9				199	218		
5.6%	10	8	7	8.3	8.7	7.6	7.6	8.0	7.9	21.2	20.0	20.0				
5.6%	10	9	8	8.3	8.7	7.6	7.6	8.0	7.9				214	229		
10%	10	6	3	8.3	8.7	7.7	7.6	8.0	7.9	21.0	20.3	19.9				
10%	10	6	1	8.3	8.7	7.7	7.6	8.0	7.9				225	239		
18%	10	0	0	8.2	8.7	- [b]	7.6	8.0	- [b]	21.0	20.3	19.9				
18%	10	0	0	8.2	8.7	-	7.6	8.0	-				240	252		
32%	10	0	0	8.1	8.6	-	7.5	8.0	-	20.9	20.4	- [b]				
32%	10	0	0	8.1	8.6	-	7.5	8.0	-				272	- [b]		
56%	10	0	0	8.1	8.5	-	7.4	7.9	-	20.6	20.5	-				
56%	10	0	0	8.1	8.5	-	7.4	7.9	-				328	-		
100%	10	0	0	7.8	8.6	-	7.2	7.9	-	20.2	20.7	-			.008/1.1	
100%	10	0	0	7.8	8.6	-	7.2	7.9	-				420	-		

Control Water

Total Alkalinity 106 (mg/l $CaCO_3$)

Total Hardness 113 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 100 (mg/l $CaCO_3$)

Total Hardness 99 (mg/l $CaCO_3$)

REMARKS: [a]Total residual chlorine = 0.5 mg/l (HACH Method)
0.35 mg/l (Iodometric Method)

[b]Remaining parameters not recorded since all organisms were dead by 24 hours.

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Sanford WWTP, Seminole County, Florida, NPDES #FL0020141, on 28 to 30 August, 1984.

Test 3 De-chlorinated effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Control	10	10	9	8.3	8.7	7.6	7.7	8.0	7.9	21.1	19.9	19.9			0/.08	
Control	10	10	9	8.3	8.7	7.6	7.7	8.0	7.9				199	213		
5.6%	10	9	9	8.2	8.8	7.7	7.7	8.0	7.9	21.2	19.9	19.8				
5.6%	10	10	10	8.2	8.8	7.7	7.7	8.0	7.9				215	228		
10%	10	10	10	8.2	8.8	7.6	7.7	8.1	7.9	21.2	19.8	19.7				
10%	10	10	10	8.2	8.8	7.6	7.7	8.1	7.9				228	238		
18%	10	9	9	8.2	8.7	7.7	7.7	8.0	7.8	21.1	19.8	19.7				
18%	10	10	10	8.2	8.7	7.7	7.7	8.0	7.8				242	253		
32%	10	10	10	8.1	8.7	7.3	7.6	8.0	7.8	21.1	19.8	19.7				
32%	10	10	10	8.1	8.7	7.3	7.6	8.0	7.8				278	290		
56%	10	10	9	8.1	8.5	7.3	7.5	7.9	7.8	21.0	19.9	19.7				
56%	10	10	9	8.1	8.5	7.3	7.5	7.9	7.8				331	342		
100%	10	6	3	8.0	8.4	7.4	7.3	7.8	7.7	20.9	20.2	20.0			.01/.97	
100%	10	7	1	8.0	8.4	7.4	7.3	7.8	7.7				423	449		

Control Water

Total Alkalinity 106 (mg/l $CaCO_3$)

Total Hardness 113 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 95 (mg/l $CaCO_3$)

Total Hardness 103 (mg/l $CaCO_3$)

REMARKS: [a]Total residual chlorine = none detected (HACH method)

Table 6. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of the Sanford WWTP, Seminole County, Florida, NPDES #FL0020141, on 28 to 30 August, 1984.

Test 3 De-chlorinated effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)		NH_3/N (mg/l)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Control	5	5	5	8.3	8.0	7.6	7.7	7.9	7.9	21.4	20.0	19.9			0/.08	
Control	5	5	5	8.3	8.0	7.6	7.7	7.9	7.9				202	227		
5.6%	5	5	5	8.3	8.1	7.6	7.7	7.9	7.9	21.3	19.7	19.7				
5.6%	5	5	5	8.3	8.1	7.6	7.7	7.9	7.9				217	238		
10%	5	5	5	8.3	8.1	7.6	7.7	7.9	7.9	21.2	19.7	19.6				
10%	5	5	5	8.3	8.1	7.6	7.7	7.9	7.9				229	249		
18%	5	5	5	8.2	8.0	7.6	7.6	7.9	7.8	21.1	19.6	19.6				
18%	5	5	5	8.2	8.0	7.6	7.6	7.9	7.8				248	268		
32%	5	4	4	8.2	7.9	7.6	7.6	7.8	7.8	21.0	19.7	19.7				
32%	5	5	5	8.2	7.9	7.6	7.6	7.8	7.8				279	299		
56%	5	5	5	8.1	7.8	7.6	7.5	7.7	7.8	21.1	19.8	19.7				
56%	5	5	5	8.1	7.8	7.6	7.5	7.7	7.8				325	358		
100%	5	5	5	8.0	7.5	7.4	7.3	7.6	7.7	21.0	20.0	19.8			.01/.97	
100%	5	5	5	8.0	7.5	7.4	7.3	7.6	7.7				433	459		

Control Water

Total Alkalinity 106 (mg/l $CaCO_3$)

Total Hardness 113 (mg/l $CaCO_3$)

100% Sample

Total Alkalinity 95 (mg/l $CaCO_3$)

Total Hardness 103 (mg/l $CaCO_3$)

REMARKS: [a]Total residual chlorine = none detected (HACH method)

Table 7. LC_{50} Data for Bioassays of the Sanford WWTP, NPDES #FL0020141, on 28 to 30 August, 1984

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: D. pulex	Binomial	a	a
Pre-chlorinated	Moving Average	a	a
Sample	Probit	a	3906.2%(226 to ∞),b
Test #1: N. leedsi	Binomial		
Pre-chlorinated	Moving Average	No Mortality	No Mortality
Sample	Probit		
Test #2: D. pulex	Binomial	10.8%(5.6 and 18)	7.3%(5.6 and 10)
Chlorinated	Moving Average	9.7%(8.3 and 11),d	7.3%(5.9 and 8.7)
Sample	Probit	9.5%(8.0 and 11)	7.2%(6.0 and 8.5),d
Test #3: D. pulex	Binomial	a	78.7%(56 and 100)
De-chlorinated	Moving Average	a	78.7%(70 and 91),d
Sample	Probit	465.6%(70 and ∞),a,c	89.8%(0 and ∞),c
Test #3: N. leedsi	Binomial		
De-chlorinated	Moving Average	No Mortality	No Mortality
Sample	Probit		

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

[d] This LC_{50} value is reported in the text since it is the most statistically conservative value of the data set.

Table 8. Results of chemical analyses performed on effluents from the Sanford WWTP, NPDES #FL0020141, collected on 27 August, 1984.

Organics

Acid Extractables	N.D.
Base Neutral Extractables	N.D.
Pesticide Extractables	Several unidentified compounds

Metals

Antimony	N.D.
Arsenic	N.D.
Beryllium	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	< .25 ug/l[a]
Lead	11.2 ug/l
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	< 0.5 ug/l
Thallium	N.D.
Zinc	28 ug/l

[a]Actual value is known to be less than value given.

N.D. = None Detected

RESULTS AND DISCUSSION

The samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from the Sanford WWTP exhibited differing degrees of toxicity to the test organism, Daphnia pulex. The pre-chlorinated sample was lethal to a sufficient number of D. pulex to calculate a 48 hour LC_{50} of 3906.2% (Table 7). However, this mortality is neither acutely nor chronically toxic according to state water quality criteria definitions. The chlorinated effluent, however, was extremely toxic to D. pulex, producing a 48 hour LC_{50} of 7.2% (Table 7). This significant increase in toxicity was partly due to the total residual chlorine (0.5 mg/l, Table 4) measured in that sample. However, this extreme toxicity noted in the chlorinated effluent could not have been caused solely by the chlorine since the toxicity of chlorine to aquatic organisms begins at approximately 0.2 mg/l. Since the moderate concentration of 0.5 mg/l chlorine measured in the chlorinated effluent most likely did not cause the LC_{50} of 7.2%, the presence of other toxic components is suggested. The de-chlorinated effluent also proved to be acutely toxic to D. pulex, producing a 48 hour LC_{50} of 78.7% (Table 7). This may suggest that some toxic components were carried over from the chlorination process and were not subsequently detoxified by the sodium thiosulfate used in the de-chlorination procedure. The 96 hour LC_{50}'s for the effluents reported above would be less than or equal to their 48 hour values. The toxicity reported above could not have been due to ammonia since only moderate concentrations of total (maximum = 1.1 mg/l) and unionized (maximum = 0.01 mg/l) ammonia were measured in the samples (Tables 2 through 6).

The results of chemical analyses (Table 8) indicated the presence of only 11.2 ug/l of lead and 28 ug/l of zinc in this effluent. However, several unidentified organic compounds were also detected in this effluent. Therefore, the toxicity exhibited in these bioassays may have been partly due to the organic compounds, but, more likely due to the presence of other constituents or components not adequately tested for in 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.

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