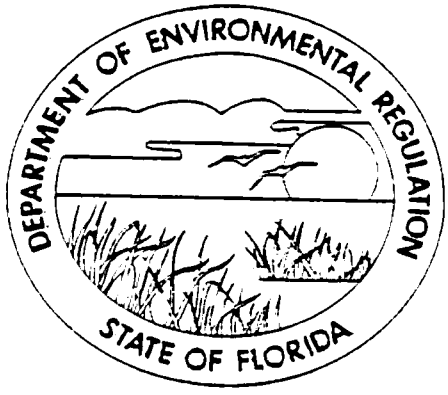


BIOASSAYS OF THE LOXAHATCHEE RIVER REGIONAL WASTEWATER TREATMENT PLANT

JUPITER, PALM BEACH COUNTY, FLORIDA

NPDES #FL0034649

Biology Section
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EXECUTIVE SUMMARY

Loxahatchee WWTP, NPDES #FL0034649, on 27 to 29 April, 1983.

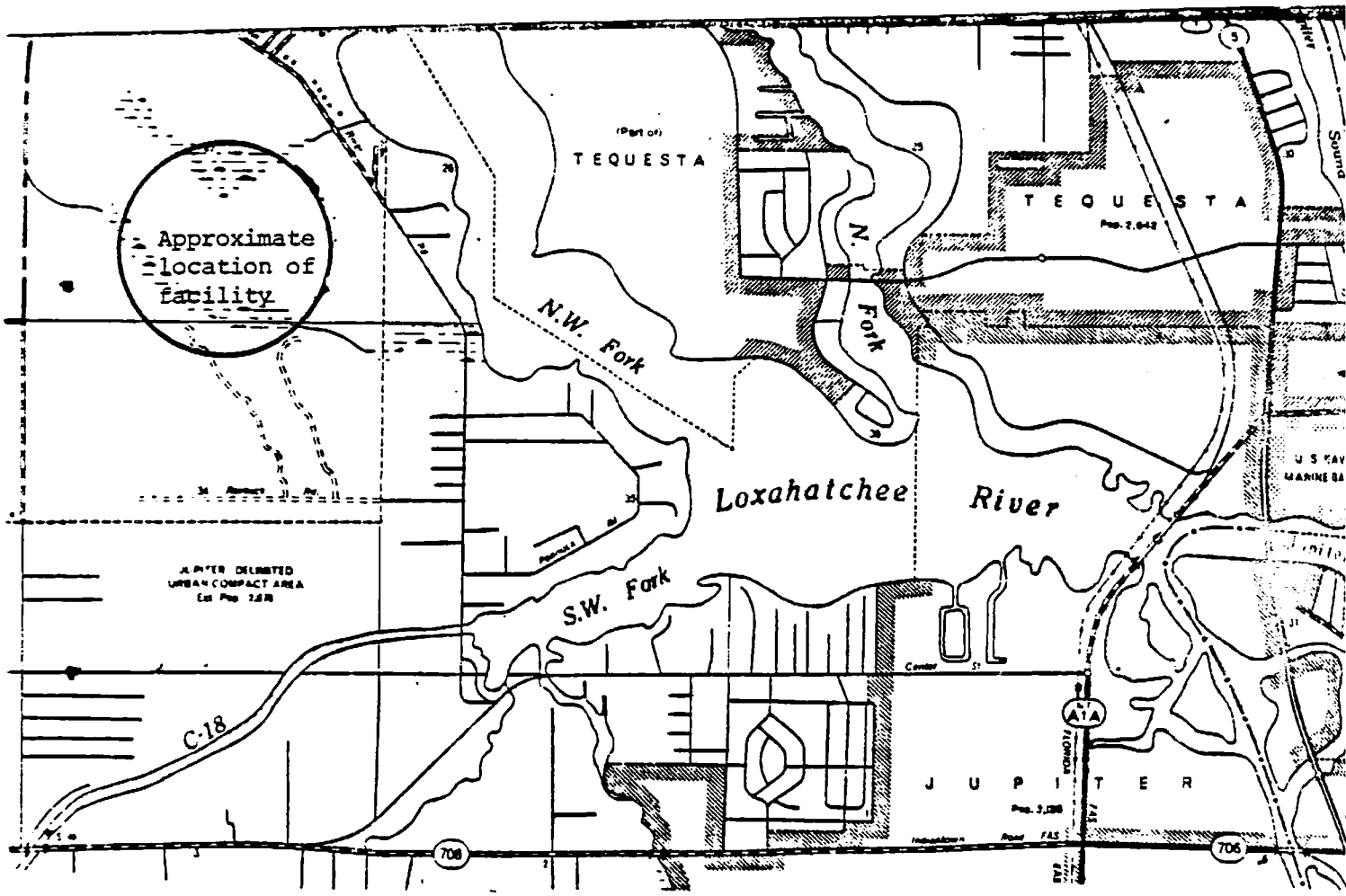
The samples of pre-chlorinated and of final post-chlorinated effluents, in which no total residual chlorine was detected, from this domestic and industrial wastewater treatment plant were not acutely toxic to the test organisms Daphnia pulex, Simocephalus serrulatus and Notropis leedsi. However, in the bioassay of the pre-chlorinated effluent, three of 20 D. pulex were dead in the 100% test concentration by hour 48, and the remaining 17 organisms were effectively immobilized, suggesting some adverse effect caused by the effluent. In the bioassays of the final post-chlorinated effluent, some random mortalities of D. pulex and S. serrulatus were noted, while no N. leedsi died during the 48 hour static tests. Since the effluents were not acutely toxic to the test organisms, no chemical analyses were performed.

INTRODUCTION

The Loxahatchee River Regional Wastewater Treatment Plant is a municipal facility located near Jupiter in northeast Palm Beach County, Florida (Figure 1). It treats waste from both domestic and industrial sources, but is currently operating far below its design capacity. Waste treatment at this facility is supposedly among the most elaborate in the state, including lengthy retention in a series of settling ponds prior to discharge. Discharge is through either a canal to the Northwest Fork of the Loxahatchee River, or through the C-18 Canal to the Southwest Fork of the Loxahatchee River.

The Biology Section has previously performed 48 hour static acute toxicity bioassays on samples of pre-chlorinated and final post-chlorinated effluents from this facility on 29 to 31 July, 1981. The results of those tests indicated that the effluents were not acutely toxic to the test organisms Daphnia pulex, Simocephalus serrulatus and Pimephales promelas (FDER 1981).

To further evaluate the effects of this discharge to the biota of the receiving waters, the Biology Section performed additional static acute toxicity bioassays on grab samples of effluent, on 27 to 29 April, 1983. The results of these latest tests are presented in this report. Those involved in performing these tests and in preparation of the report were: C. W. Dye, M. L. Roll and K. A. Study.



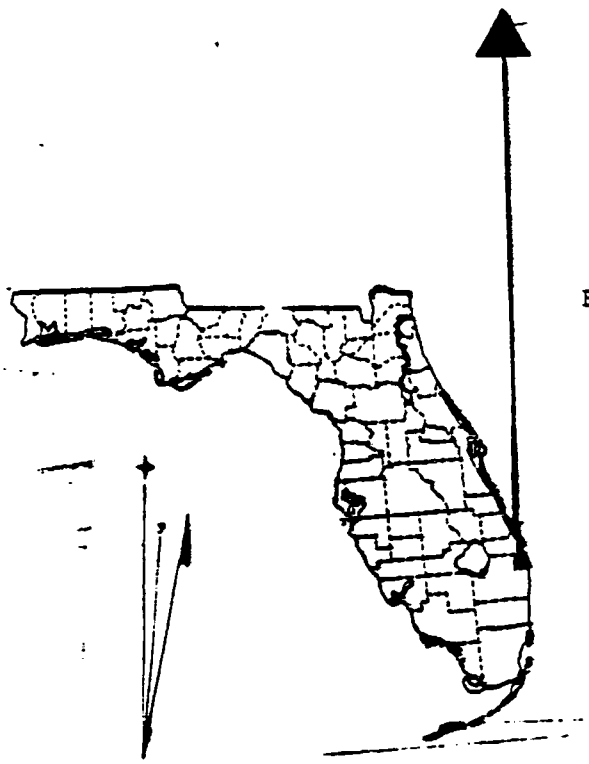


Figure 1. Portions of Palm Beach County, Florida, including approximate location of the Loxahatchee River Regional Wastewater Treatment Plant.

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. Two 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, 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. 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 Simocephalus serrulatus was collected from Lake Jackson, Leon 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, 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. 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.

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. Breeding and egg hatching are encouraged at 25°C. The eggs are laid under a 1" thick slab of marble raised 3 mm above a removable horizontal pane of glass.

Most of the eggs adhere to the glass and are easily removed to an aerated hatching tank. The narrow space between the marble slab and glass eliminates egg predation by adult fish. New hatching tanks are started weekly 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 16 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 lauryl sulfate as the reference toxicant.

Test Methods

Four separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated and on final post-chlorinated effluents from the Loxahatchee WWTP. The tests were performed from 1100 hours on 27 April to 1120 hours on 29 April, 1983. The samples were collected by Mr. Mike Nichols of DER's Port St. Lucie office at 1310 hours on 26 April, 1983, 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 (Table 2). 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 (Tables 3, 4 and 5). This test was performed on an effluent sample collected at the point of discharge.

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 randomly arranged 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 six randomly arranged 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 5 per chamber, resulting in 10 per concentration. Fish were not fed during this test.

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 chamber 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 concentration. 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 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
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 Loxahatchee River WWTP, Jupiter, Palm Beach County, Florida, NPDES #FL0034649, on 27 to 29 April, 1983.

Test #1 Pre-chlorinated Effluent[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	48	0	48
Control	10	10	10	8.9	9.3	9.3	7.9	8.2	8.3	116		114		19.5	19.2	19.9	0/0	--		
Control	10	10	10	8.9	9.3	9.3	7.9	8.2	8.3										203	218
5.6%	10	10	10	8.8	9.4	9.4	7.9	8.2	8.3					19.6	19.1	19.8				
5.6%	10	10	10	8.8	9.4	9.4	7.9	8.2	8.3										227	232
10.0%	10	10	10	8.6	9.4	9.4	7.8	8.2	8.3					19.4	19.0	19.7				
10.0%	10	10	10	8.6	9.4	9.4	7.8	8.2	8.3										242	249
18.0%	10	10	10	8.5	9.4	9.4	7.7	8.2	8.3					20.0	19.0	19.7				
18.0%	10	10	10	8.5	9.4	9.4	7.7	8.2	8.3										271	279
32.0%	10	10	10	8.4	9.4	9.4	7.5	8.2	8.3					19.9	19.0	19.7				
32.0%	10	10	10	8.4	9.4	9.4	7.5	8.2	8.3										319	330
56.0%	10	10	10	8.2	9.4	9.4	7.4	8.1	8.2					19.6	19.0	19.6				
56.0%	10	10	10	8.2	9.4	9.4	7.4	8.1	8.2										400	420
100.0%	10	10	10[b]	7.6	9.1	9.3	7.0	8.0	8.2	106		103		19.3	19.0	19.9	0/0	--		
100.0%	10	10	7[b]	7.6	9.1	9.3	7.0	8.0	8.2										620	680

Remarks: [a] Total Residual Chlorine = None Detected (HACH DPD Method)

[b] Remaining live organisms were immobilized.

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the Loxahatchee River WWTP, Jupiter, Palm Beach County, Florida, NPDES #FL0024649, on 27 to 29 April, 1983.
Test #2 Chlorinated Effluent[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	48	0	48
Control	10	10	10	8.7	9.3	9.2	7.9	8.2	8.3	116		114		19.5	18.8	19.6	0/0	--		
Control	10	10	10	8.7	9.4	9.2	7.9	8.2	8.3										203	211
5.6%	10	10	9(10)[b]	8.6	9.4	9.3	7.9	8.3	8.3					19.4	18.7	19.6				
5.6%	10	10	10	8.6	9.4	9.3	7.9	8.3	8.3										223	225
10.0%	10	10	10	8.6	9.5	9.4	8.0	8.3	8.3					20.0	18.6	19.2				
10.0%	10	9	9	8.6	9.5	9.4	8.0	8.3	8.3										234	238
18.0%	10	10	10	8.6	9.5	9.4	8.0	8.3	8.3					20.0	18.5	19.2				
18.0%	10	10	10	8.6	9.5	9.4	8.0	8.3	8.3										257	260
32.0%	10	10	10	8.7	9.5	9.4	8.1	8.3	8.3					19.9	18.5	19.1				
32.0%	10	10	10	8.7	9.5	9.4	8.1	8.3	8.3										292	299
56.0%	10	10	10	8.8	9.5	9.4	8.2	8.3	8.3					19.6	18.5	19.1				
56.0%	10	10	10	8.8	9.5	9.4	8.2	8.3	8.3										360	370
100.0%	10	10	10	8.9	9.5	9.5	8.5	8.3	8.3	88		95		19.4	18.6	19.3	0/.04	--		
100.0%	10	10	10	8.9	9.5	9.5	8.5	8.3	8.3										470	492

Remarks:

[a] Total Residual Chlorine = None Detected (HACH DPD Method)

[b] Number in parentheses reported since one organism was stuck to side of glass.

Table 4. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of the Loxahatchee River WWTP, Jupiter, Palm Beach County, Florida, NPDES #FL0034649, on 27 to 29 April, 1983.

Test #2 Chlorinated Effluent[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	48	0	48
Control	10	10	8	8.8	9.4	9.5	7.9	8.2	8.4	116		114		19.5	19.0	19.4	0/0	--		
Control	10	10	9	8.8	9.4	9.5	7.9	8.2	8.4										202	214
5.6%	10	10	10	8.8	9.4	9.5	7.9	8.3	8.4					19.9	19.0	19.4				
5.6%	10	9	9	8.8	9.4	9.5	7.9	8.3	8.4										222	229
10.0%	10	10	10	8.8	9.4	9.5	7.9	8.3	8.4					20.0	19.0	19.4				
10.0%	10	10	8	8.8	9.4	9.5	7.9	8.3	8.4										235	240
18.0%	10	9	9	8.9	9.5	9.5	8.0	8.3	8.3					20.0	18.9	19.5				
18.0%	10	10	10	8.9	9.5	9.5	8.0	8.3	8.3										249	261
32.0%	10	10	9	8.9	9.5	9.5	8.1	8.3	8.3					19.9	18.9	19.5				
32.0%	10	10	10	8.9	9.5	9.5	8.1	8.3	8.3										295	302
56.0%	10	10	10	8.9	9.5	9.5	8.2	8.3	8.3					19.8	18.8	19.4				
56.0%	10	10	10	8.9	9.5	9.5	8.2	8.3	8.3										360	373
100.0%	10	10	9	8.9	9.5	9.6	8.5	8.3	8.3	88		95		19.5	18.9	19.6	0/.04	--		
100.0%	10	10	10	8.9	9.5	9.6	8.5	8.3	8.3										432	497

Remarks: [a] Total Residual Chlorine = None Detected (HACH DPD Method).

Table 5. Data recorded during 48 hour, *Notropis leedsi*[a] static acute toxicity bioassay of the Loxahatchee River WWTP, Jupiter, Palm Beach County, Florida, NPDES #FL0034649, on 27 to 29 April, 1983.

Test #2 Chlorinated Effluent[b]

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	48	0	48
Control	5	5	5	8.6	9.0	8.9	7.9	8.2	8.2	116		114		20.7	18.2	19.1	0/0	--		
Control	5	5	5	8.6	9.0	8.9	7.9	8.2	8.2										198	212
5.6%	5	5	5	8.6	9.0	8.9	7.9	8.2	8.1					20.7	18.1	18.9				
5.6%	5	5	5	8.6	9.0	8.9	7.9	8.2	8.1										218	229
10.0%	5	5	5	8.6	9.0	8.9	7.9	8.2	8.1					20.7	18.1	18.7				
10.0%	5	5	5	8.6	9.0	8.9	7.9	8.2	8.1										230	239
18.0%	6[c]	6	6	8.7	9.0	8.9	7.9	8.2	8.1					20.5	18.1	18.7				
18.0%	5	5	5	8.7	9.0	8.9	7.9	8.2	8.1										250	260
32.0%	5	5	5	8.7	9.0	8.9	8.0	8.2	8.1					20.1	18.5	18.7				
32.0%	5	5	5	8.7	9.0	8.9	8.0	8.2	8.1										291	300
56.0%	5	5	5	9.0	9.0	8.9	8.1	8.2	8.1					19.6	18.2	18.7				
56.0%	5	5	5	9.0	9.0	8.9	8.1	8.2	8.1										350	365
100.0%	5	5	5	9.1	9.0	9.1	8.4	8.2	8.1	88		95		19.2	18.2	18.9	0/.04	--		
100.0%	5	5	5	9.1	9.0	9.1	8.4	8.2	8.1										462	489

Remarks:

[a] Weight of Fish = 6.89 mg each, average of 8 fish.

[b] Total Residual Chlorine = None Detected (HACH DPD Method).

[c] Extra fish loaded by mistake, allowed to remain.

RESULTS AND DISCUSSION

The samples of pre-chlorinated and of final post-chlorinated effluents, in which no total residual chlorine was detected, from the Loxahatchee River Wastewater Treatment Plant were not acutely toxic to the test organisms Daphnia pulex, Simocephalus serrulatus and Notropis leedsi. However, in the bioassay of the pre-chlorinated effluent, three of 20 D. pulex were dead in the 100% test concentration by hour 48, and the remaining 17 organisms were effectively immobilized (Table 2), suggesting some adverse effect caused by the effluent. In the bioassays of the final post-chlorinated effluent, some random mortalities of D. pulex and S. serrulatus were noted, while no N. leedsi died during the 48 hour static tests. Since the effluents were not acutely toxic to the test organisms, no chemical analyses were performed.

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