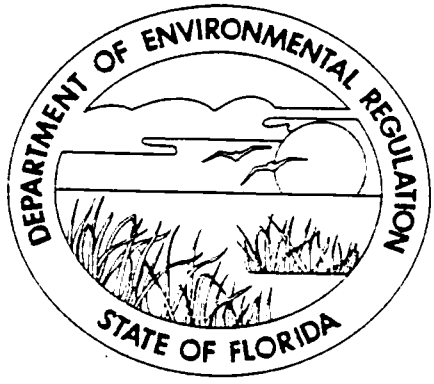


BIOASSAYS OF THE LOXAHATCHEE RIVER REGIONAL WASTEWATER TREATMENT PLANT

JUPITER, PALM BEACH COUNTY, FLORIDA

NPDES #FL0034649

Biological Section
Bureau of Water Analysis
August 11, 1981

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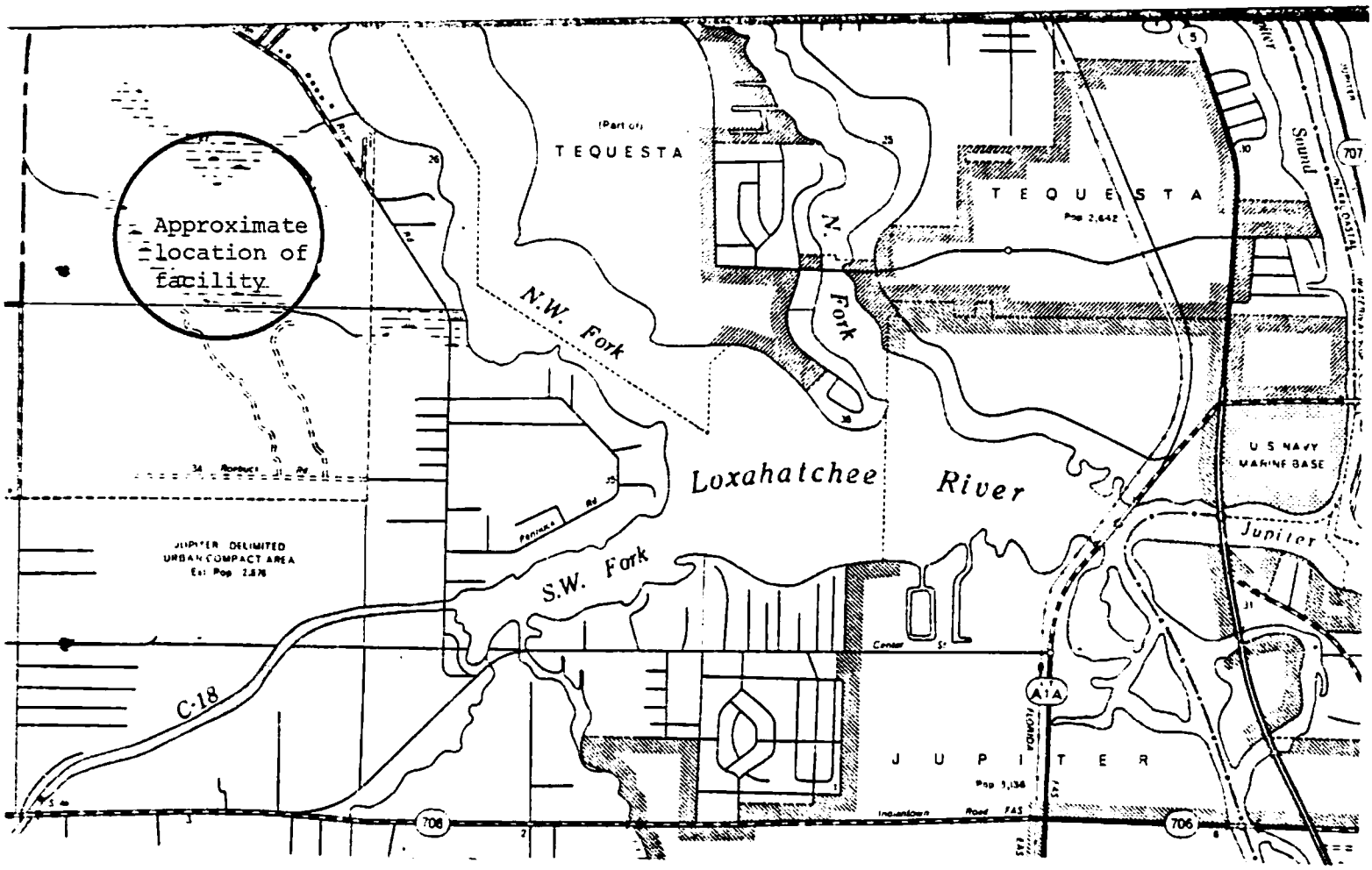
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INTRODUCTION

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.

To evaluate the effects of this discharge to the biota of the receiving waters, the Biological Section performed static acute toxicity bioassays on grab samples of effluent, on 29 to 31 July, 1981. The results of these tests are presented in this report.



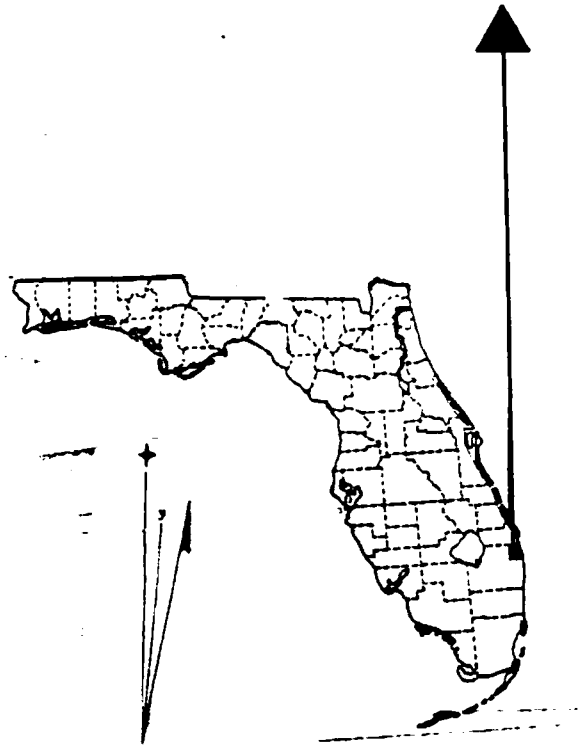


Figure 1. Portions of Palm Beach County, Florida, including approximate location of the Loxahatchee River Regional Wastewater Treatment Plant.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organism 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 less than four 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

Four separate 48 hour static bioassays were conducted from 0930 hours on 29 July, 1981 to 0930 hours on 31 July, 1981. Grab samples of effluent (collected at the recharge pond) and of effluent collected prior to chlorination (collected at the filter box) were obtained by Mr. Kevin Ruddick of DER's Port St. Lucie Office at 1030 hours on 28 July, 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 associated with the sample at the end of the treatment process but prior to chlorination, and the toxicity associated with the sample at the point of discharge, the Biological 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 at the effluent filter box, but prior to chlorination. And, it was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Final Effluent (Tables 3, 4 and 5). These tests were performed on an effluent sample collected at the point of discharge. And, it was intended to evaluate its potential effect on the biota of the receiving waters.

Freshwater for dilution of the effluent sample 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 (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 siphoned into respective large culture dishes through a stainless steel screen 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 5 per chamber resulting in 10 per 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 and total hardness were recorded at the beginning of the test for the controls and the 100% effluent concentrations. Specific conductance was recorded for each

test concentration at the beginning and end of the fathead minnow bioassay (Table 5). Ammonia concentrations, including total (expressed in mg/l as N) and unionized (expressed in mg/l as NH_3) fractions, were recorded in a control and the 100% effluent concentrations at the beginning of the test. Total ammonia measurements were made using an Orion specific ion probe. The unionized fraction was then calculated based on the pH, temperature and salinity of the sample.

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

Daphnia pulex and Simocephalus serrulatus (water fleas)

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

Pimephales promelas (fathead minnow)

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 Loxahatchee Wastewater Treatment Plant, Jupiter, Palm Beach County, Florida, NPDES #FL0034649 on 29 to 31 July, 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	48	0
Control	10	10	8	8.2	8.6	8.4	8.0	8.2	8.2	121		117		21.4	20.4	20.2	.01/.098	---	
Control	10	10	9[a]	8.2	8.6	8.4	8.0	8.2	8.2										
5.6%	10	10	10	8.2	8.6	8.4	8.0	8.2	8.2					21.4	20.3	20.2			
5.6%	10	10	10	8.0	8.6	8.4	8.0	8.2	8.2										
10%	10	10	10	8.0	8.6	8.5	8.0	8.3	8.2					21.4	20.2	20.4			
10%	10	10	10	8.0	8.6	8.5	8.0	8.3	8.2										
18%	10	10	10	7.8	8.6	8.5	7.9	8.3	8.2					21.3	20.3	20.1			
18%	10	10	10	7.8	8.6	8.5	7.9	8.3	8.2										
32%	10	10	10	7.6	8.5	8.5	7.8	8.3	8.2					21.2	20.3	20.1			
32%	10	10	10	7.6	8.5	8.5	7.8	8.2	8.3										
56%	10	10	10	7.1	8.3	8.5	7.7	8.2	8.2					21.2	20.3	20.1			
56%	10	10	10	7.1	8.3	8.5	7.7	8.2	8.3										
100%	10	10	10	6.1	8.2	8.6	7.5	8.2	8.3	181		113		21.6	20.5	20.3	0/.112	---	
100%	10	10	10	6.0	8.2	8.7	7.5	8.2	8.3										

Remarks: a. Dead organism not found.

Total Residual Chlorine (Hach DPD Method): Not detected.

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Loxahatchee Wastewater Treatment Plant, Jupiter, Palm Beach County, Florida NPDES #FL0034649 on 29 to 31 July, 1981.

Test #2. Point of Discharge 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	48	0
Control	10	10	10	8.0	8.6	8.6	7.8	8.2	8.3	121		117		21.4	20.1	20.3	.01/.098	---	
Control	10	10	10	8.0	8.5	8.6	7.9	8.2	8.2										
5.6%	10	10	10	8.0	8.6	8.6	7.9	8.3	8.2					21.3	20.1	20.4			
5.6%	10	10	10	8.1	8.6	8.6	7.9	8.3	8.2										
10%	10	10	10	8.1	8.6	8.6	8.0	8.3	8.2					21.2	20.1	20.4			
10%	10	10	10	8.1	8.6	8.5	8.0	8.3	8.2										
18%	10	10	10	8.1	8.6	8.5	8.0	8.3	8.2					21.1	20.3	20.4			
18%	10	10	10	8.1	8.6	8.5	8.1	8.3	8.3										
32%	10	10	10	8.0	8.6	8.4	8.1	8.3	8.3					21.5	20.3	20.4			
32%	10	10	10	8.0	8.6	8.4	8.2	8.3	8.3										
56%	10	10	10	7.9	8.6	8.4	8.3	8.4	8.3					22.0	20.4	20.4			
56%	10	10	9	7.9	8.6	8.4	8.3	8.4	8.3										
100%	10	10	10	7.4	8.6	8.5	8.5	8.5	8.4	189		121		22.6	20.6	20.6	.01/.08	---	
100%	10	10	10	7.4	8.6	8.5	8.5	8.5	8.4										

Remarks: Total Chlorine residual (Hach DPD Method): Not Detected.

Table 4. Data recorded during 48 hour, *Simocephalus serrulatus*, static acute toxicity bioassay of Loxahatchee Wastewater Treatment Plant, Jupiter, Palm Beach County, Florida, NPDES #FL0034649 on 29 to 31 July, 1981.

Test #2: Point of Discharge 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[b]	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control	10	10	5	8.2	8.5	8.4	8.0	8.2	8.3	121		117		21.4	21.1	21.1	.01/.098	---	
Control	10	9	2	8.2	8.5	8.4	8.0	8.2	8.3										
5.6%	10	10	8	8.2	8.5	8.5	8.0	8.3	8.3					21.3	20.9	20.9			
5.6%	10	8	6	8.2	8.5	8.5	8.0	8.3	8.3										
10%	10	9	5	8.1	8.5	8.4	8.1	8.3	8.3					21.2	20.8	20.8			
10%	10	9	7	8.1	8.5	8.4	8.1	8.3	8.3										
18%	10	10	8	8.1	8.6	8.4	8.1	8.3	8.3					21.0	20.6	20.8			
18%	10	8	7	8.1	8.6	8.4	8.1	8.3	8.3										
32%	10	9[a]	8	8.1	8.6	8.3	8.2	8.3	8.3					21.1	21.1	20.7			
32%	10	10	8	8.1	8.6	8.3	8.2	8.3	8.3										
56%	10	10	8	7.9	8.6	8.4	8.3	8.4	8.3					21.6	20.3	20.6			
56%	10	10	7	7.9	8.6	8.3	8.3	8.4	8.3										
100%	10	9	7	7.5	8.6	8.6	8.5	8.5	8.4	189		121		22.1	20.3	20.4	.01/.08	---	
100%	10	10	9	7.5	8.6	8.7	8.6	8.5	8.4										

Remarks:

[a] Dead organism not found.

[b] 48 hour test invalidated due to excessive control mortality (>20%).

Total Residual Chlorine (Hach DPD Method): Not detected.

Table 5. Data recorded during 48 hour, *Pimephales promelas*, static acute toxicity bioassay of Loxahatchee Wastewater Treatment Plant, Jupiter, Palm Beach County, Florida, NPDES #FL0034649 on 29 to 31 July, 1981.

Test #2: Point of Discharge 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	48	0	48
Control	5	5	5	8.2	7.4	7.6	8.0	8.0	8.0	121		117		21.7	20.3	20.4	.01/.098	---	195	198
Control	5	5	5	8.2	7.3	7.5	8.0	8.0	8.0											
5.6%	5	5	5	8.3	7.6	7.7	8.0	8.1	8.0					21.8	20.3	20.5			225	222
5.6%	5	5	5	8.3	7.2	7.3	8.0	8.1	8.0											
10%	5	5	5	8.2	7.2	7.7	8.0	8.0	8.0					21.8	20.3	20.5			250	249
10%	5	5	5	8.2	7.0	7.6	8.0	8.0	7.9											
18%	5	5	5	8.2	7.2	7.9	8.1	8.0	8.0					21.9	20.3	20.5			290	288
18%	5	5	5	8.2	7.2	7.8	8.1	8.0	8.0											
32%	5	5	5	8.2	7.3	7.3	8.2	8.1	8.0					21.5	20.3	20.5			360	367
32%	5	5	5	8.1	7.6	7.7	8.2	8.1	8.0											
56%	5	5	5	7.9	7.2	7.2	8.4	8.1	8.0					22.2	20.5	20.6			480	488
56%	5	5	5	7.9	7.1	7.2	8.4	8.1	8.0											
100%	5	5	5	7.5	7.5	7.8	8.5	8.3	8.2	189		121		22.3	20.7	20.7	.01/.08	---	720	760
100%	5	5	5	7.5	7.8	7.9	8.6	8.3	8.2											

Remarks: Weight of fish: 0.023 g each (average of 10).
Total Chlorine Residual (Hach DPD Method): Not detected.

RESULTS AND DISCUSSION

Both the pre-chlorinated sample and the point of discharge sample from the Loxahatchee Wastewater Treatment Plant were not acutely toxic to the organisms tested. Although a significant number of Simocephalus had died by hour 48 of the test (Table 4), the mortality appeared to be random, and was therefore not attributed to toxicity of the sample. Additionally, the Simocephalus bioassay was invalidated at 48 hours due to excessive (>20%) control mortality. No fish died during the test.

LITERATURE CITED

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