BIOASSAYS OF THE CAPE CORAL SEWAGE TREATMENT PLANT LEE COUNTY, FLORIDA NPDES #FL0030007

Biology Section
Bureau of Laboratories and
Special Programs
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EXECUTIVE SUMMARY

Cape Coral STP, Lee County, NPDES #FL0030007, on 24 to 26 June, 1986.

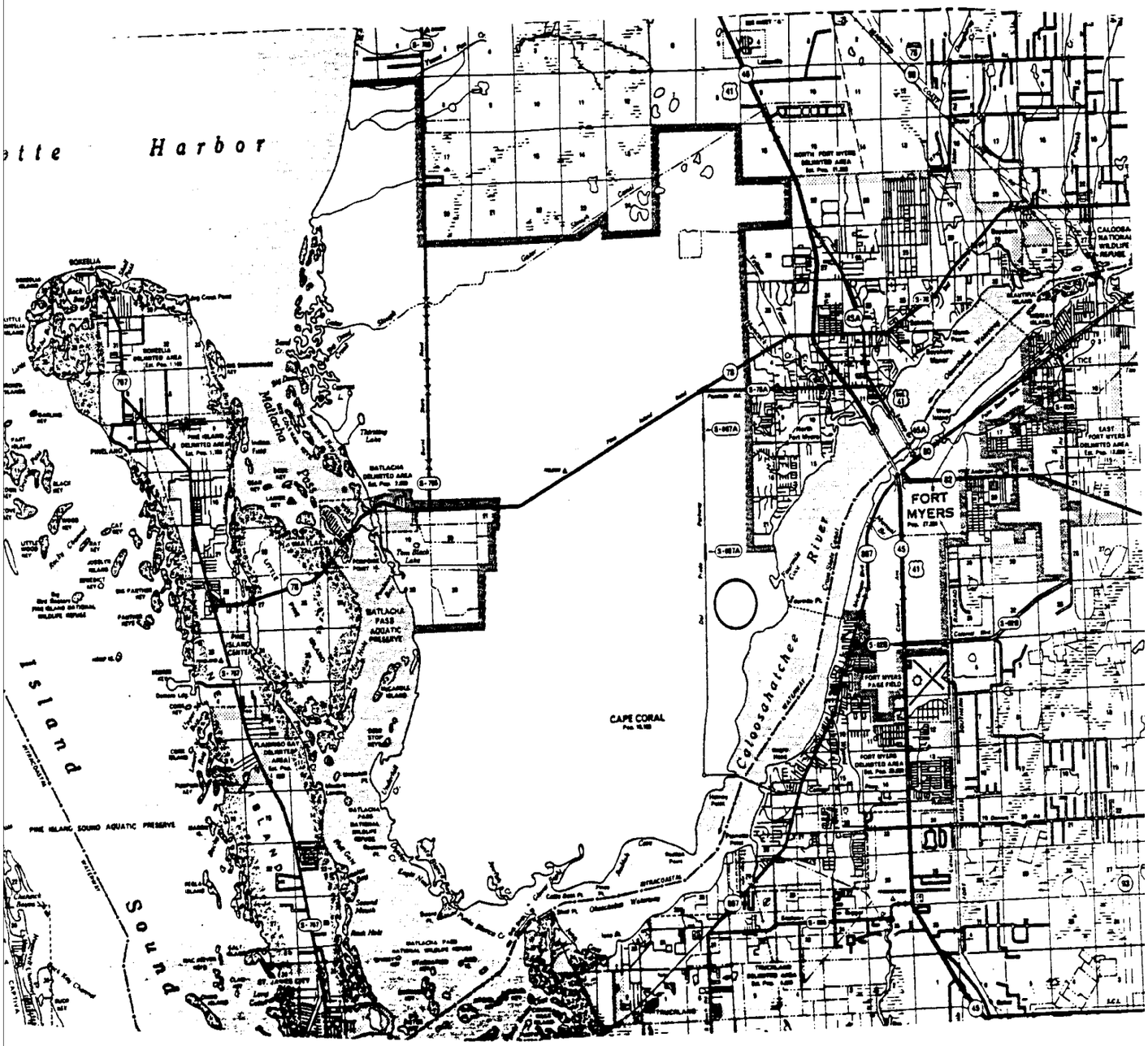
Four 48 hour static acute toxicity bioassays were performed on samples of final chlorinated and de-chlorinated effluent from this domestic waste treatment facility which discharges to the Caloosahatchee River. The samples were not acutely toxic to Notropis leedsi. No mortality was noted during the tests. The remaining two bioassays using Daphnia pulex were, unfortunately, invalidated at 24 hours due to excessive control mortality, and no data from these tests are given. The residual chlorine measured in the final effluent (0.3 mg/l) was apparently insufficient to have caused any toxicity to the N. leedsi during the test period. Since the sample was not acutely toxic, further chemical analyses were not requested.

Due to the absence of toxicity to N. leedsi, it is believed that the Cape Coral STP effluent is in compliance with the applicable FAC acute toxicity standards. However, some doubt does remain due to the invalidation of the Daphnia pulex tests since these organisms are more sensitive to chlorine (and to many other potential toxicants) than are Notorpis leedsi. Therefore, it is recommended that these tests be repeated sometime in the future, preferably prior to the renewal of this facility's permit.

INTRODUCTION

The Cape Coral Sewage Treatment Plant is located at 1800 North Everest Parkway, Cape Coral, Lee County, Florida (Fig. 1) The facility has a design capacity of 4.0 MGD, but peak flows have been reported as high as 5.67 MGD (from NPDES file). Treatment of primarily domestic wastes consists of the standard activated sludge process and final chlorination. Treated wastewater is discharged through outfall #001 to the Caloosahatchee River. A schematic of the Cape Coral treatment system is presented in Figure 2.

To evaluate the potential toxicity of this discharge, the South Florida District requested that acute toxicity bioassays be performed. Four such tests were performed on samples of final chlorinated and de-chlorinated effluent from the Cape Coral facility on 24 to 26 June, 1986. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: M. J. Drawdy, R. B. Frydenborg, M. L. Roll, and P. G. Weiland.



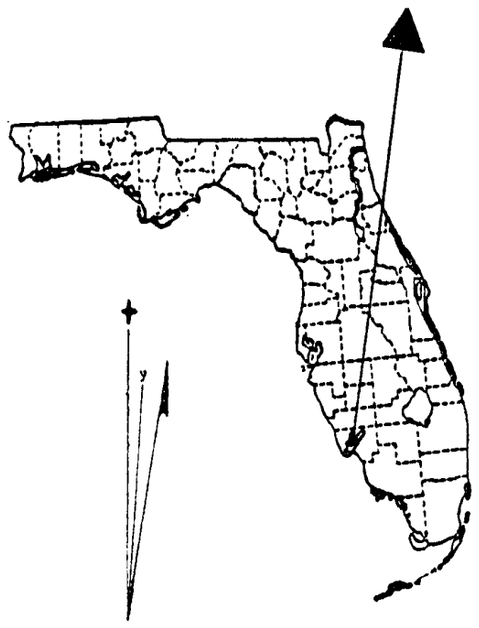
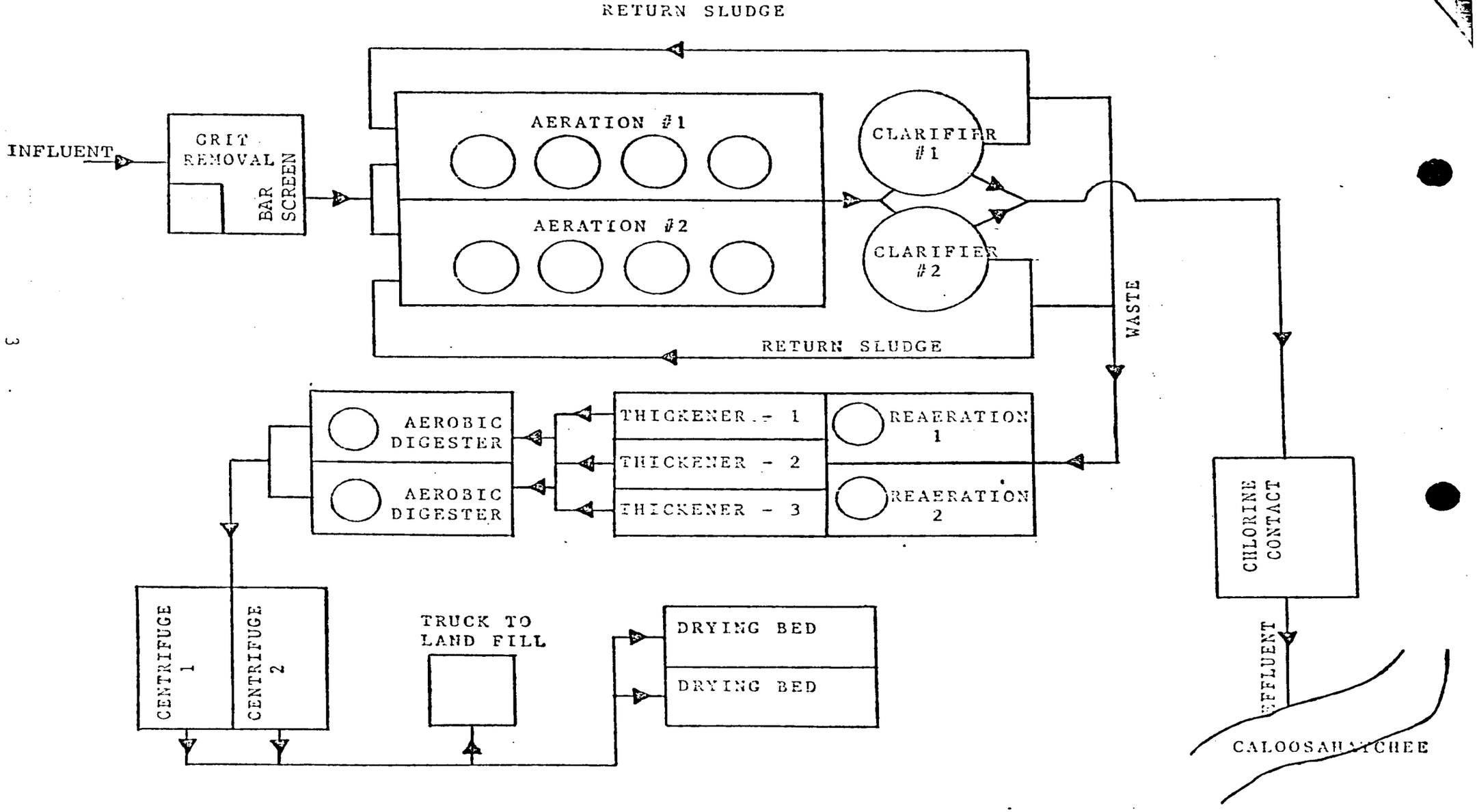


Figure 1. Portions of Lee County, Florida, including Cape Coral and the approximate location of the sewage treatment plant.

Figure 2.

CITY OF CAPE CORAL
WASTEWATER TREATMENT
PLANT



METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include a species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985). 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° ± 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° ± 2°C under a 16:8 hour light-dark 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 approximately 25 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

Methods used to perform acute toxicity bioassays are described by Peltier and Weber (1985) and the FDER Biology Section (1985). Some of the methods described below are more specific than those of Peltier and Weber. These will be noted in more detail.

Sampling Methods and Test Dates

Four separate 48 hour acute toxicity bioassays were performed on samples of final chlorinated and de-chlorinated effluent from the Cape Coral sewage treatment plant. The tests were performed from 1000 hours on 24 June to 1000 hours on 26 June, 1986.

The samples were collected by Mr. Albert S. Walton, Jr. of DER's Punta Gorda Office at 1130 hours on 23 June, 1986, 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.

Test Temperature

Upon arrival of the iced (4°C) bioassay samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples allowed to rise to 20° $\pm$ 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Total Residual Chlorine

Since many wastewaters are chlorinated, especially those originating from sewage treatment facilities, it is often desirable to differentiate between the toxicity resulting from chlorine and the toxicity associated with other components of the wastewater. To accomplish this, the Biology Section performed concurrent bioassays on a sample of chlorinated wastewater in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025N sodium thiosulfate. The total residual chlorine concentrations of the tested effluents are given on the bioassay data forms. A comparison of the results of these tests will distinguish between the toxicity due solely to residual chlorine and the toxicity inherently contained in the wastewater.

Freshwater Tests

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 is presented in (Table 1).

Two replicates, of each concentration 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. Unfortunately, excessive control mortality (> 10%) was observed in these D. pulex tests by 24 hours causing their invalidation. Therefore, no further data from these tests are given.

Two replicates, of each concentration 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.

Test Parameters

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 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. These values were recorded on the bioassay data forms and are presented in Tables 2 and 3.

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
10.0%	180.0 ml	20.0 ml	200 ml
50.0%	100.0 ml	100.0 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
10.0%	450 ml	50 ml	500 ml
50.0%	250 ml	250 ml	500 ml
100.0%	-------	500 ml	500 ml

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Cape Coral Sewage Treatment Plant, NPDES #FL0030007, Lee County, on 24 to 26 June, 1986.

De-chlorinated Sample[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	5	5	5	8.2	8.3	7.7	7.6	7.7	7.7	20.9	18.9	19.0		
Control	5	5	5	7.9	8.3	7.7	7.6	7.7	7.8				208	205
10.0%	5	5	5	7.8	8.5	8.3	7.6	7.8	7.9	20.8	18.8	18.7		
10.0%	5	5	5	7.8	8.5	8.3	7.6	7.8	7.9				362	360
50.0%	5	5	5	8.0	8.5	8.3	7.5	7.8	8.0	20.4	18.7	18.7		
50.0%	5	5	5	8.0	8.5	8.3	7.5	7.8	8.0				1060	1000
100.0%	5	5	5	8.0	8.1	7.8	7.5	7.8	8.0	19.9	18.7	18.7		
100.0%	5	5	5	8.0	8.1	7.8	7.5	7.8	8.0				1800	1700

Control Water

NH_3(as NH_3)(mg/l) _____

Total ammonia (as N)(mg/l) _____

Total Alkalinity (mg/l $CaCO_3$) 115

Total Hardness (mg/l $CaCO_3$) 121

100% Effluent

NH_3(as NH_3)(mg/l) _____

Total ammonia (as N)(mg/l) _____

Total Alkalinity (mg/l $CaCO_3$) 160

Total Hardness (mg/l $CaCO_3$) 348

[a] Total Residual Chlorine: < 0.02 mg/l (HACH DPD Mehtod).

Table 2. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Cape Coral Sewage Treatment Plant, NPDES #FL0030007, Lee County, on 24 to 26 June, 1986.

Chlorinated Sample[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	5	5	5	8.2	8.3	7.7	7.6	7.7	7.7	20.9	18.9	19.0		
Control	5	5	5	7.9	8.3	7.7	7.6	7.7	7.8				208	205
10.0%	5	5	5	8.0	8.4	8.0	7.6	7.7	7.8	20.9	18.8	18.7		
10.0%	5	5	5	7.8	8.4	8.0	7.6	7.7	7.8				370	350
50.0%	5	5	5	8.0	8.4	8.2	7.5	7.8	8.0	20.7	18.7	18.7		
50.0%	5	5	5	8.0	8.4	8.2	7.5	7.8	8.0				1050	950
100.0%	5	5	5	8.2	8.3	8.3	7.5	7.8	8.0	20.2	18.7	18.7		
100.0%	5	5	5	8.2	8.3	8.3	7.5	7.8	8.0				1800	1700

Control Water	
NH_3(as NH_3)(mg/l)	Total Alkalinity 115 (mg/l $CaCO_3$)
Total ammonia (as N)(mg/l)	Total Hardness 121 (mg/l $CaCO_3$)

100% Effluent	
NH_3(as NH_3)(mg/l)	Total Alkalinity 162 (mg/l $CaCO_3$)
Total ammonia (as N)(mg/l)	Total Hardness 352 (mg/l $CaCO_3$)

[a] Total Residual Chlorine: 0.3 mg/l (HACH DPD Method).

RESULTS AND DISCUSSION

The results of 48 hour acute toxicity bioassays using Notropis leedsi on samples of final chlorinated and de-chlorinated effluent from the Cape Coral STP indicated that the samples were not acutely toxic. No mortality was noted during the testing period (Tables 2 and 3), The residual chlorine (0.3 mg/l) measured in the final effluent was apparently insufficient to have caused any toxicity to the fish by 48 hours. Unfortunately, the Daphnia pulex bioassays were both invalidated at 24 hours due to excessive control mortality (> 10%), and no data are given. Due to the absence of toxicity to N. leedsi, it is believed that this effluent is in compliance with the applicable FAC acute toxicity standards. However, some doubt does exist due to the invalidation of the D. pulex tests, since these organisms are more sensitive to chlorine (and to many other potential toxicants) than are N. leedsi.

RECOMMENDATIONS

It is recommeded that toxicity tests of the Cape Coral effluent be repeated sometime in the future, preferably prior to the renewal of the facility's permit.

LITERATURE CITED

Florida Department of Environmental Regulation, Biology Section. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.