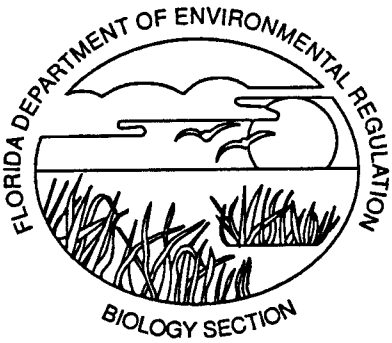


Bioassays of

Belle Glade Sewage Treatment Plant

Belle Glade, Palm Beach County, Florida

NPDES #FL0027740

Sampled 9/18/88

October 12, 1988

Biology Section
Division of Administrative and Technical Services

Bioassays of

Belle Glade Sewage Treatment Plant

Belle Glade, Palm Beach County, Florida

NPDES #FL0027740

Biology Section
Bureau of Laboratories
October 12, 1988

Executive Summary

Belle Glade Sewage Treatment Plant, Belle Glade, Palm Beach County, Florida, NPDES #FL0027740, on 20 to 22 September, 1988.

Three acute toxicity bioassays and one Microtox® assay were performed on a sample of discharge from this facilities percolation pond. The bioassay test organisms used were the water fleas, *Ceriodaphnia dubia* and *Daphnia pulex*, and the fish, *Notropis leedsi*. The sample was not acutely to any of the bioassay test organisms. The sampled proved to be stimulatory to the Microtox® assay organism, *Photobacterium phosphoreum*.

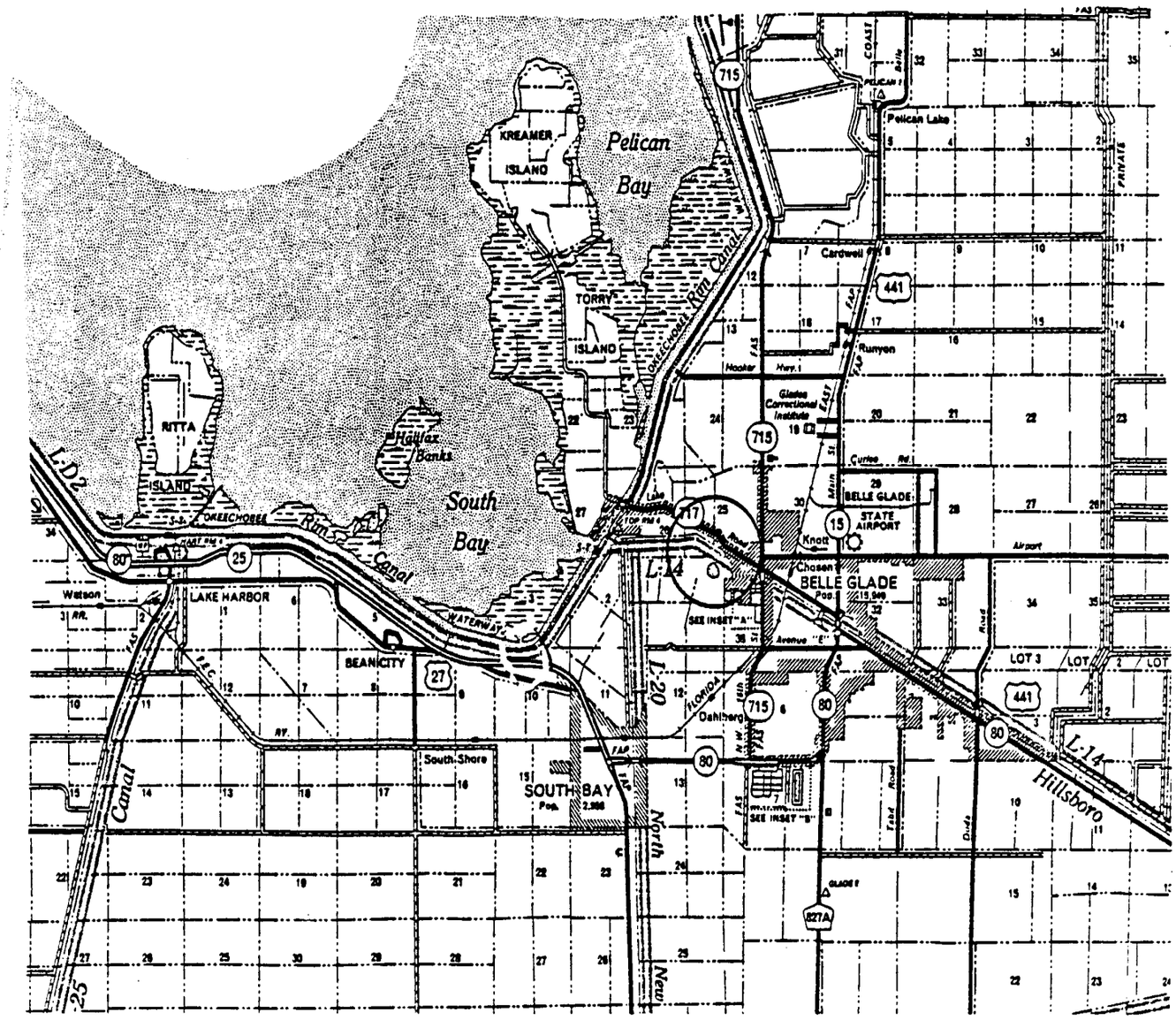
Chemical analyses were not performed on the sample.

Introduction

The Belle Glade Sewage Treatment Plant (NPDES #FL0027740) is located at 2055 West Canal Street South, Belle Glade, Palm Beach County, Florida (Figure 1). The plant has a design flow of 3.0 MGD with one past 30 day average of 3.68 MGD (NPDES files). Treatment at the facility consists of an oxidation ditch, activated sludge process followed by secondary clarification, disinfection by chlorine, and discharge to evaporation/percolation ponds. Discharge from the percolation pond is to the South Florida Conservation District Canal - lateral 1-2N(Hillsboro canal) (Figure 2). Holding time is of sufficiently short duration that the discharge is considered to be a surface water discharge rather than a ground water discharge.

The Biology Section performed a bioassay on samples from the two plants located at this facility in December, 1981. Toxicity was noted in the samples from plant #1 but not from Plant #2. Toxicity was attributed to the total residual chlorine content of the sample [2.9 mg/l (Hach) and 4.08 mg/l (Iodometric)].

To determine the existence and extent of toxicity in the discharge from this facility, the Biology Section, at the request of the FDER Southeast District, performed three (3) acute toxicity bioassays and one (1) Microtox® assay on a sample from this facility. The results of those tests are presented in this report. Those involved in performing the tests and in preparation of this report were: J. M. Faircloth, K. M. Lurding, L. A. Williams, and S. H. Wolfe.



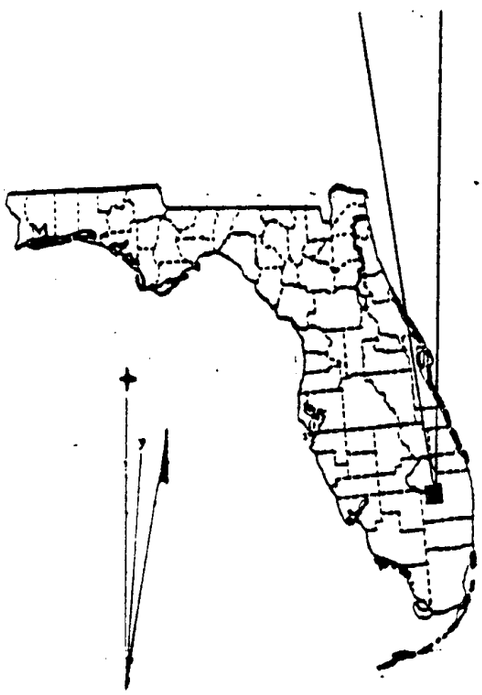


Figure 1. Portions of Palm Beach County Florida, including city of Belle Glade and study area.

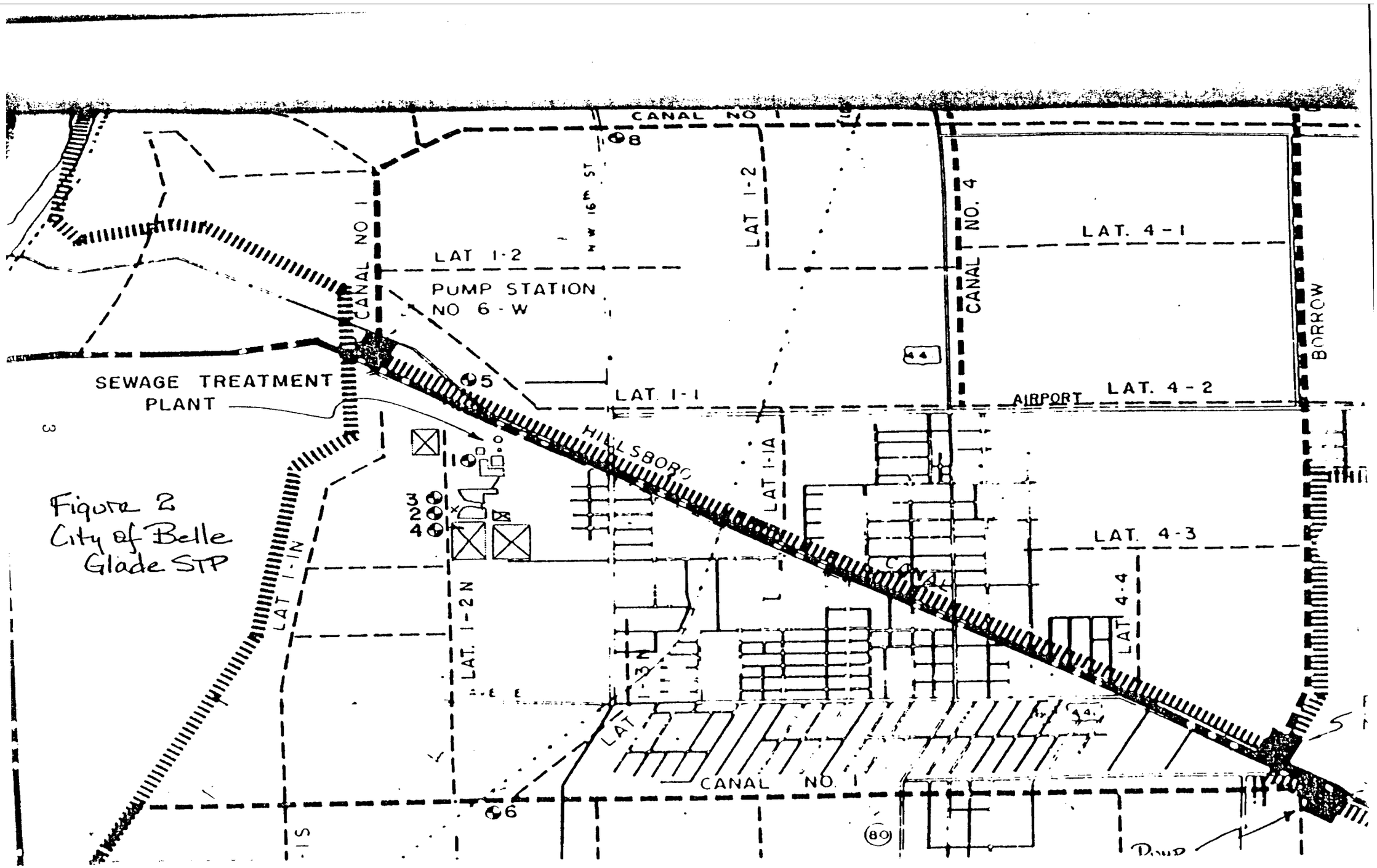


Figure 2
City of Belle Glade STP

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) 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.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman 1982).

1. 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 June, 1987. 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.

Ceriodaphnia dubia (water flea)—A breeding culture of *Ceriodaphnia dubia* was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained 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®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26 ±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 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 Aquarian® flake food 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 Aquarian® flake food. The fish used were 18 days old when the test commenced.

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-606 was used in this assay. Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox Reconstitution Solution).

2. Standard Reference Toxicity Assays

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.

3. 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

Three separate 48 hour acute toxicity bioassays and one Microtox® assay were performed on a sample from the Belle Glade Sewage Treatment Plant facility outfall #001. The tests were performed from 1200 hrs on 20 September, 1988 to 1200 hrs on 22 September, 1988.

Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with $HN0_3$. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were collected by Mr. Paul Strauss of the FDER District Branch office in Port St. Lucie at 1015 hours on 19 September, 1988, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 26 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 ± 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.

Method

Freshwater for diluting the water samples being tested was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is given in Table 1.

Four replicates consisting of 30 ml plastic vials containing 15 mls of the control and each test concentration were used in bioassays with the water flea, *Ceriodaphnia dubia* and *Daphnia pulex*. Adult *Ceriodaphnia* and *Daphnia* were separated using a nylon mesh sieve and placed into large culture dishes 18 hrs before the beginning of the test. Neonates produced during the intervening period (i.e., <18 hr old) were then captured by glass pipette, examined for injury, and transferred into the test chambers. Five individuals (for *C. dubia* and *D. pulex*) were loaded per chamber, resulting in 20 per test concentration. The water fleas were not fed during these tests.

Two replicates of each concentration and a control were used for the *Notropis leedsi* (bannerfin shiner) bioassay. The fish were transferred individually or by twos using a pipette into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Five were loaded per chamber, resulting in 10 per concentration. Fish were not fed during this test.

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% reclaimed water 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. The salinity was measured at the beginning of the test. All instruments were calibrated before use 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% reclaimed water test concentration 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 Table 2.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected for Microtox® analysis. The sample was adjusted to 2% salinity with reagent grade dry sodium chloride to maintain the osmotic balance of the test bacterium during the assay. Four two-fold dilutions were made of the test sample resulting in a test range of 99%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15 °C was maintained throughout the assay. Ten µl of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction, of light output is determined in 15 minutes (EC_{50}). Results are given in Table 3.

Results and Discussion

Three acute toxicity bioassays and one Microtox® assay were performed on a sample from the Belle Glade Sewage Treatment Plant, Belle Glade, Palm Beach County, Florida , NPDES #FL0027740. The sample was not toxic to the bioassay organisms, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Notropis leedsi*. The sample was stimulatory to the Microtox® assay organism, *Photobacterium phosphoreum*.

Chemical analyses were not performed on the sample. In conclusion, the sample from the Belle Glade STP was not toxic on 19 September, 1988.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	15.0 ml	0.0 ml	15 ml
10.0%	13.5 ml	1.5 ml	15 ml
30.0%	10.5 ml	4.5 ml	15 ml
50.0%	7.5 ml	7.5 ml	15 ml
70.0%	4.5 ml	10.5 ml	15 ml
85.0%	2.25 ml	12.75 ml	15 ml
100.0%	0.0 ml	15.0 ml	15 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	100.0 ml	0.0 ml	100 ml
100.0%	0.0 ml	100.0 ml	100 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	0 ml	500 ml
10.0%	450 ml	50 ml	500 ml
30.0%	350 ml	150 ml	500 ml
50.0%	250 ml	250 ml	500 ml
70.0%	150ml	350 ml	500 ml
85.0%	75 ml	425 ml	500 ml
100.0%	0 ml	500 ml	500 ml

Table 2. Data recorded during 48 hour acute toxicity bioassays of a sample from the Belle Glade Sewage Treatment Plant percolation pond discharge, Belle Glade, Palm Beach County, Florida, NPDES #FL0027740, on 20 to 22 September, 1988.

Facility: **Belle Glade STP** NPDES # **FL0027740** Facility Type: **Municipal**
Location: W. Canal St. Belle Glade Contact/District: PS / SE
County: Palm Beach Test type: Static conventional
Sample Collection Date: **9/19/88** Time: 1015 # tests: 3 Receiving Water: Fresh water canal
Test Beginning Date: 9/20/88 Time: 1200 Chlorination Type: **no residual chlorine**
Test Ending Date: 9/22/88 Time: 1200

Total Residual Cl2	mg/l	Method
Field:	0.0	amper. titrat.
Lab:	0.0	Hach
Lab:	-	-

Ammonia	Total (mg/l)	Unionized (mg/l)
Control Water (fish):	0.00	0.00
Cond. Control Water (daphnids):	0.00	0.00
100% Sample:	0.00	0.00

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	120	125
Cond. Control Water (daphnids):	118	135
100% Sample:	175	290

Salinity (ppt)	
Control Water:	0
100% Sample:	0

Organism: ***Ceriodaphnia dubia*** Life stage: neonate
LC50: - Binomial: - Moving Average: - Probit: -

Concentrations	SURVIVAL # at 0 hr	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	5	5	3*	8.7	-	8.3	8.2	-	8.2	20.1	-	20.5	-	-
Control	5	5	5	8.7	-	8.3	8.2	-	8.2	-	-	-	-	-
Control	5	5	5	8.8	-	8.4	8.2	-	8.2	-	-	-	-	-
Control	5	4	4	8.8	-	8.4	8.2	-	8.2	-	-	-	215	275
10%	5	5	5	8.8	-	8.5	8.2	-	8.2	20.0	-	20.5	-	-
10%	5	5	5	8.8	-	8.5	8.2	-	8.2	-	-	-	-	-
10%	5	5	5	8.8	-	8.5	8.2	-	8.2	-	-	-	-	-
10%	5	5	5	8.8	-	8.5	8.2	-	8.2	-	-	-	305	350
25%	5	5	5	8.8	-	8.6	8.0	-	8.2	19.8	-	20.4	-	-
25%	5	5	5	8.8	-	8.6	8.0	-	8.2	-	-	-	-	-
25%	5	5	5	8.8	-	8.6	8.0	-	8.2	-	-	-	-	-
25%	5	5	5	8.8	-	8.6	8.0	-	8.2	-	-	-	445	510
50%	5	5	5	8.8	-	8.5	8.0	-	8.2	19.8	-	20.4	-	-
50%	5	5	5	8.8	-	8.5	8.0	-	8.2	-	-	-	-	-
50%	5	5	5	8.8	-	8.7	8.0	-	8.2	-	-	-	-	-
50%	5	5	5	8.8	-	8.6	8.0	-	8.2	-	-	-	570	670
70%	5	5	5	8.7	-	8.6	7.9	-	8.2	19.8	-	20.4	-	-
70%	5	5	5	8.7	-	8.6	7.9	-	8.2	-	-	-	-	-
70%	5	5	5	8.7	-	8.6	7.9	-	8.2	-	-	-	-	-
70%	5	5	5	8.7	-	8.6	7.9	-	8.2	-	-	-	715	800
85%	5	5	5	8.7	-	8.6	7.8	-	8.3	19.7	-	20.3	-	-
85%	5	5	5	8.7	-	8.6	7.8	-	8.3	-	-	-	-	-
85%	5	5	5	8.7	-	8.6	7.8	-	8.3	-	-	-	-	-
85%	5	5	5	8.7	-	8.6	7.8	-	8.3	-	-	-	835	940
100%	5	5	5	8.5	-	8.5	7.7	-	8.3	19.5	-	20.3	-	-
100%	5	5	5	8.6	-	8.5	7.7	-	8.3	-	-	-	-	-
100%	5	5	5	8.6	-	8.5	7.7	-	8.3	-	-	-	-	-
100%	5	5	5	8.6	-	8.5	7.7	-	8.3	-	-	-	925	1070

Organism: ***Daphnia pulex*** Life stage: neonate
LC50: - Binomial: - Moving Average: - Probit: -

Concentrations	SURVIVAL # at 0 hr	Live at 24 hr	Live at 48 hr	Dissolved Oxygen 0 hour	24 hour	48 hour	pH 0 hour	24 hour	48 hour	Temperature 0 hour	24 hour	48 hour	Conductivity 0 hour	48 hour
Control	5	5	5	8.9	-	8.6	8.2	-	8.2	19.8	-	20.5	-	-
Control	5	5	5	8.9	-	8.5	8.2	-	8.2	-	-	-	-	-
Control	5	5	5	8.9	-	8.5	8.2	-	8.2	-	-	-	-	-
Control	5	5	5	8.9	-	8.5	8.2	-	8.2	-	-	-	235	275
100%	5	5	5	8.8	-	8.6	7.7	-	8.3	19.5	-	20.5	-	-
100%	5	5	5	8.6	-	8.6	7.7	-	8.3	-	-	-	-	-
100%	5	5	5	8.7	-	8.6	7.7	-	8.3	-	-	-	-	-
100%	5	5	5	8.7	-	8.6	7.7	-	8.3	-	-	-	860	940

Table 2. Continued.

Facility:	**Belle Glade STP**		NPDES #	**FL0027740**	Facility Type: **Municipal**
Location:	W. Canal St. Belle Glade		Contact/District:	PS / SE	
County:	Palm Beach		Test type:	Static conventional	
Sample Collection Date:	**9/19/88**	Time: 1015	# tests:	3	Receiving Water: Fresh water canal
Test Beginning Date:	9/20/88	Time: 1200	Chlorination Type:	**no residual chlorine**	
Test Ending Date:	9/22/88	Time: 1200			**Page 2 of 2**

Total Residual Cl2	mg/l	Method
Field:	0.0	amper. titrat.
Lab:	0.0	Hach
Lab:	-	-

Ammonia	Total (mg/l)	Unionized (mg/l)
Control Water (fish):	0.00	0.00
Cond. Control Water (daphnids):	0.00	0.00
100% Sample:	0.00	0.00

Alk & Hardness	Alkalinity (mg/l)	Hardness (mg/l)
Control Water (fish):	120	125
Cond. Control Water (daphnids):	118	135
100% Sample:	175	290

Salinity (ppt)	
Control Water:	0
100% Sample:	0

Organism: ***Notropis leedsi*** Age: 18 days
LC50: - Binomial: - Moving Average: - Probit: -

	SURVIVAL			Dissolved Oxygen			pH			Temperature			Conductivity	
Concentrations	# at 0 hr	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour
Control	5	5	5	8.6	8.5	7.7	7.8	8.0	8.1	20.7	20.7	20.7	-	-
Control	5	5	5	8.7	8.5	7.7	7.8	8.0	8.1	-	-	-	215	230
10%	5	5	5	8.7	8.5	8.0	7.8	8.0	8.0	20.7	20.5	20.4	-	-
10%	5	5	5	8.7	8.4	8.0	7.8	8.0	8.0	-	-	-	300	305
25%	5	5	5	8.7	8.4	8.0	7.7	7.9	8.0	20.8	20.3	20.2	-	-
25%	5	5	5	8.7	8.4	8.0	7.7	8.0	8.0	-	-	-	455	455
50%	5	5	5	8.4	8.3	8.0	7.7	7.9	8.0	20.7	22.2	20.2	-	-
50%	5	5	5	8.4	8.3	8.0	7.7	7.9	8.0	-	-	-	605	600
70%	5	5	5	8.3	8.3	7.8	7.6	7.9	8.0	20.7	20.1	20.2	-	-
70%	5	5	5	8.2	8.3	7.8	7.6	7.9	8.1	-	-	-	760	765
85%	5	5	5	8.0	8.3	7.9	7.6	7.9	8.0	20.7	20.0	20.2	-	-
85%	5	5	5	8.0	8.3	7.9	7.6	7.9	8.0	-	-	-	860	865
100%	5	5	5	7.8	7.9	7.4	7.5	7.9	8.0	20.6	20.0	20.2	-	-
100%	5	5	5	7.8	7.9	7.6	7.5	7.9	8.0	-	-	-	975	985

Table 3. Microtox test results, Belle Glade STP discharge, Palm Beach County, NPDES #FL0027740, on 20 September, 1988.

Microtox Test Culture Lot # M-606

% Sample	Final Reclaimed Water Nonchlorinated 5-min	15-min
0	98	79
0	90	65
0	92	65
12.38	92	65
12.38	96	67
12.38	92	65
24.75	92	65
24.75	85	59
24.75	100	70
49.5	98	70
49.5	99	70
49.5	106	74
99	108	80
99	107	82
99	108	82

EC_{50} Nonchlorinated (15 minute, 15 °C) = Stimulatory

EC_{50} (15 minute, 15 °C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

Literature Cited

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Florida Department of Environmental Regulation. 1982. Bioassays of Belle Glade Sewage Treatment Plants # 1 and 2, Belle Glade, Palm Beach County, Florida, NPDES #FL0027740. Biology Section, Tallahassee. 16 p.

_____. 1985. Quality assurance manual for performing acute toxicity tests (Second Edition). Biology Section, Tallahassee.

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.

Pahl-Lake FL Palm Bch Co.
P. Strum
FL0027740

1 3

9-20 88 12:00
1-20 83 12:00
Ceriodaphnia dubia
Neonate

												Conductivity	
Control	1/2	5/5	5/5	3/5	8.7/8.7	8.3/8.3	8.2/8.2	8.2/8.2	20.1	20.5			
Control	3/4	5/5	5/4	5/4	8.8/8.8	8.4/8.4	8.2/8.2	8.2/8.2				215	275
10%	5/6	5/5	5/5	5/5	8.8/8.8	8.5/8.5	8.2/8.2	8.2/8.2	20.0	20.5			
10%	7/8	5/5	5/5	5/5	8.8/8.8	8.5/8.5	8.2/8.2	8.2/8.2				305	350
30%	9/10	5/5	5/5	5/5	8.8/8.8	8.6/8.6	8.0/8.0	8.2/8.2	19.8	20.4			
30%	11/12	5/5	5/5	5/5	8.8/8.8	8.6/8.6	8.0/8.0	8.2/8.2				415	510
50%	13/14	5/5	5/5	5/5	8.8/8.8	8.5/8.5	8.0/8.0	8.2/8.2	19.8	20.4			
50%	15/16	5/5	5/5	5/5	8.8/8.8	8.7/8.6	8.0/8.0	8.2/8.2				570	670
70%	17/18	5/5	5/5	5/5	8.7/8.7	8.6/8.5	7.9/7.9	8.2/8.2	19.8	20.4			
70%	19/20	5/5	5/5	5/5	8.7/8.7	8.6/8.6	7.9/7.9	8.2/8.2				715	900
85%	21/22	5/5	5/5	5/5	8.7/8.7	8.6/8.6	7.8/7.8	8.3/8.3	19.7	20.3			
85%	23/24	5/5	5/5	5/5	8.7/8.7	8.6/8.6	7.8/7.8	8.3/8.3				835	940
100%	25/26	5/5	5/5	5/5	8.5/8.6	8.5/8.5	7.7/7.7	8.3/8.3	19.5	20.3			
100%	27/28	5/5	5/5	5/5	8.6/8.6	8.5/8.5	7.7/7.7	8.3/8.3				925	1070
Initial of Investigator		JHF	MF	MF	SHW	MF	SHW	MF	JMF ~~SHW~~	MF		SHW	MF

well 120/125
100% Sample 175/290
Cond. Control 118/135
Measured by: JMF

Ammonia unionized/total (mg/l as NH3/N)
Hour 0
Control Water: 0/0
100% Sample: 0/0
Other:

Hour ____
Control Water:
100% Sample:
Other:
Measured by: JMF

Signatures: Marshall Fairchild
KMLurding
Steven H Wolfe

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	7at7; 9at9	9.0 at 21°C	101 at 100; 11 at 10; 2 at 1; 1000 at 1000
24 Hours			
48 Hours	7.9 @ 9	8.7 @ 23°C	1320 @ 21.7°C (1319)
72 Hours			
96 Hours			

Total Residual Chlorine:
Field data: 0 Method: AMPER. TITRAT.
Lab: 0.0 Method: HACH
Lab: ____ Method: ____

Reviewed by: [signature]
Date: 10/21/88

De-chlorinated:
Measurements by: KML

Remarks: Cond - 0 hours - (21.4) 1313 = 1310
a. 1 missing 1 immobile

Industry/Toxicant Belle Glade STP
Address
Contact/District
Effluent Serial Number
NPDES Permit Number

BIOASSAY DATA FORM

Test Number: 2 of 3
Test Type: (Static) Renewal, (Screening) Conventional
Other:

Sample Collection: Date 9-19-88 Time 1015
Test Beginning: Date 9-20-88 Time 1130
Test Ending: Date 9-22-88 Time 1200
Test Organism Daphnia pulex
Age/Life Stage Neonate

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	29	5	5	5			8.9		8.6			8.2	8.2	8.2			19.8		20.5							
Control	30	5	5	5			8.9		8.5			8.2	8.1	8.2												
Control	31	5	5	5			8.9		8.5			8.2		8.2												
Control	32	5	5	5			8.9		8.5			8.2		8.2								235	–	275		
100%	33	5	5	5			8.8		8.6			7.7		8.3			19.5		20.5							
100%	34	5	5	5			8.6		8.6			7.7		8.3												
100%	35	5	5	5			8.7		8.6			7.7		8.3												
100%	36	5	5	5			8.7		8.6			7.7		8.3								860	–	940		
Initial of Investigator		MHL	MF	MF			SHW		MF			SHW		MF			JMF		MF			SHW		MF		

Alkalinity/Hardness (mg/l as $CaCO_3$)

Control Water:
100% Sample:
Other:
Measured by:

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0

Control Water:
100% Sample:
Other:

Hour

Control Water:
100% Sample:
Other:
Measured by:

Signatures:
Marshall Fendott
K M Lurding
Steven W Wolfe

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours			
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:

Field data: Method:
Lab: Method:
Lab: Method:

De-chlorinated:
Measurements by:

Reviewed by:
Date: 10/21/88

Belle Glade STP

Test No.: 3 3

Dates: 9-19-88 1015; 9-20-88 1200; 9-22-88 1200

Test Organism: Nebop.o leeds, 18 days

Concentration	Container Number	Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	48	72	96	pH 0	24	48	72	96	Temperature (°C) 0	24	48	72	96	Conductivity 0	24	48	72	96
Control	67	5	5	5			8.6	8.5	7.7			7.8	8.0	8.1			20.7	20.7	20.1							
"	18	5	5	5			8.7	8.5	7.7			7.8	8.0	8.1								215	–	230		
10%	34	5	5	5			8.7	8.5	8.0			7.8	8.0	8.0			20.7	20.5	20.4							
"	19	5	5	5			8.7	8.4	8.0			7.8	8.0	8.0								300	–	305		
30%	20	5	5	5			8.7	8.4	8.0			7.7	7.9	8.0			20.8	20.3	20.2							
"	21	5	5	5			8.7	8.4	8.0			7.7	8.0	8.0								455	–	455		
50%	72	5	5	5			8.4	8.3	8.0			7.7	7.9	8.0			20.7	22.2	20.2							
"	49	5	5	5			8.4	8.3	8.0			7.7	7.9	8.0								605	–	600		
70%	31	5	5	5			8.3	8.3	7.8			7.6	7.9	8.0			20.7	20.1	20.2							
"	113	5	5	5			8.2	8.3	7.8			7.6	7.9	8.1								760	–	765		
85%	9	5	5	5			8.0	8.3	7.9			7.6	7.9	8.0			20.7	20.0	20.2							
"	44	5	5	5			8.0	8.3	7.9			7.6	7.9	8.0								860	–	865		
100%	16	5	5	5			7.8	7.9	7.4			7.5	7.9	8.0			20.6	20.0	20.2							
"	6	5	5	5			7.8	7.9	7.6			7.5	7.9	8.0								975	–	985		
Initial of Investigator		MF	MF	MF			SHW	JMF	MF			SHW	JMF	MF			JMF	MF	MF			SHW		MF		

Alkalinity/Hardness (mg/l as CaCO3)

Well Water: 120-125
100% Sample: 175-290
Other:
Measured by: MF

Ammonia unionized/total (mg/l as NH3/N)
Hour 0

Control Water:
100% Sample:
Other:

Hour ____

Control Water:
100% Sample:
Other:
Measured by: ______

Signatures:
Marshall Fairchild
[signature]
Steven H Wolfe

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours			
24 Hours	7.07, 9.89	9.0 @ 20C	
48 Hours			
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:

Field data: ______ Method: ______
Lab: ______ Method: ______
Lab: ______ Method: ______

De-chlorinated:
Measurements by: ______

Reviewed by: [signature]

Date: 10/21/88

MICROTOX™ Data Reduction Sheet

SOURCE: Belle Glade STP

GRAPH CALC EC 50 (15 MIN, 15 °C) = 601.3 (%SAMPLE) mg/L

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent-

DATE ASSAYED: 9-20-88 TIME: 9:00Am

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE: none

OPERATOR: Leslie Auerbach REAGENT VIAL LOT NO. M-606

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	98	70	X	X	
B1		90	65			
C1		92	65			
A2	12.38	92	65	.000	.015	a
B2		96	67			
C2		92	65			
A3	24.75	92	65	0.011	.031	b
B3		85	59			
C3		100	70			
A4	49.5	98	70	-.076	-.065	
B4		99	70			
C4		106	74			
A5	99	108	80	-.133	-.180	
B5		107	82			
C5		108	82			
EC_{50} (MIN, °C)				—	681.3	based on a & b only

REVIEWED BY:

DATE: 10/21/88