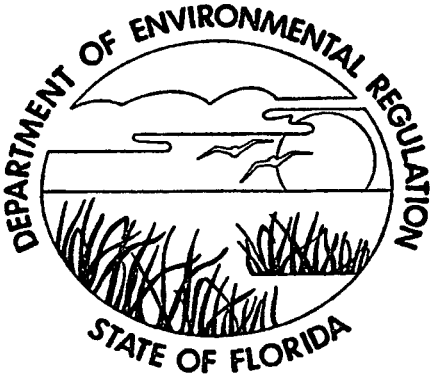


BIOASSAYS OF PANAMA CITY BEACH SEWAGE TREATMENT PLANT

PANAMA CITY BEACH, BAY COUNTY, FLORIDA

NPDES #FL0021512

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April 12, 1985

Biology Section
Division of Environmental Programs

BIOASSAYS OF PANAMA CITY BEACH SEWAGE TREATMENT PLANT

PANAMA CITY BEACH, BAY COUNTY, FLORIDA

NPDES #FL0021512

Biology Section
Bureau of Laboratories
April 12, 1985

EXECUTIVE SUMMARY

Panama City Beach Sewage Treatment Plant, Bay County, Florida, on 19 to 21 February, 1985.

The sample of chlorinated effluent collected from the Panama City Beach Sewage Treatment Plant was acutely toxic to the test organisms Daphnia pulex, Notropis leedsi, and Mysidopsis bahia, producing 48 hour LC_{50}'s of 13.3%, 22.3%, and 17.8%, respectively. A chlorine residual of 1.4 mg/l (HACH and Iodometric methods) was measured in the sample, but, was only partly responsible for this toxicity. In the bioassays of the de-chlorinated effluent, the sample was not toxic to the freshwater test organisms, D. pulex and N. leedsi. However, the sample was toxic to M. bahia, resulting in a 48 hour LC_{50} of 66.1%. The total and unionized ammonia detected in the sample (maximum measured = 11.5 mg/l and .037 mg/l, respectively) may have contributed to the observed mortality. Chemical analyses failed to indicate the presence of any priority pollutant organic compounds. The sample for metal analyses was invalidated.

INTRODUCTION

The Panama City Beach Sewage Treatment Plant is located at 110 South Arnold Road, in Panama City Beach, Bay County, Florida (Fig. 1). According to the NPDES permit, this facility has a design flow capacity of 1.1 MGD, although peak flows of 2.0 MGD have occurred. The facility treats domestic waste from a population which fluctuates seasonally due to tourism from 6,000 to 10,000. Treatment consists of contact stabilization, activated sludge aeration and post-chlorination. Wastewater flows to a polishing pond and is discharged to an unnamed creek which flows to West Bay and eventually to St. Andrews Bay.

At the request of DER's Northwest District office, static acute toxicity bioassays were performed to evaluate the potential aquatic toxicity of this discharge to the biota of the receiving waters. Six bioassays were performed from 19 to 21 February, 1985. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.

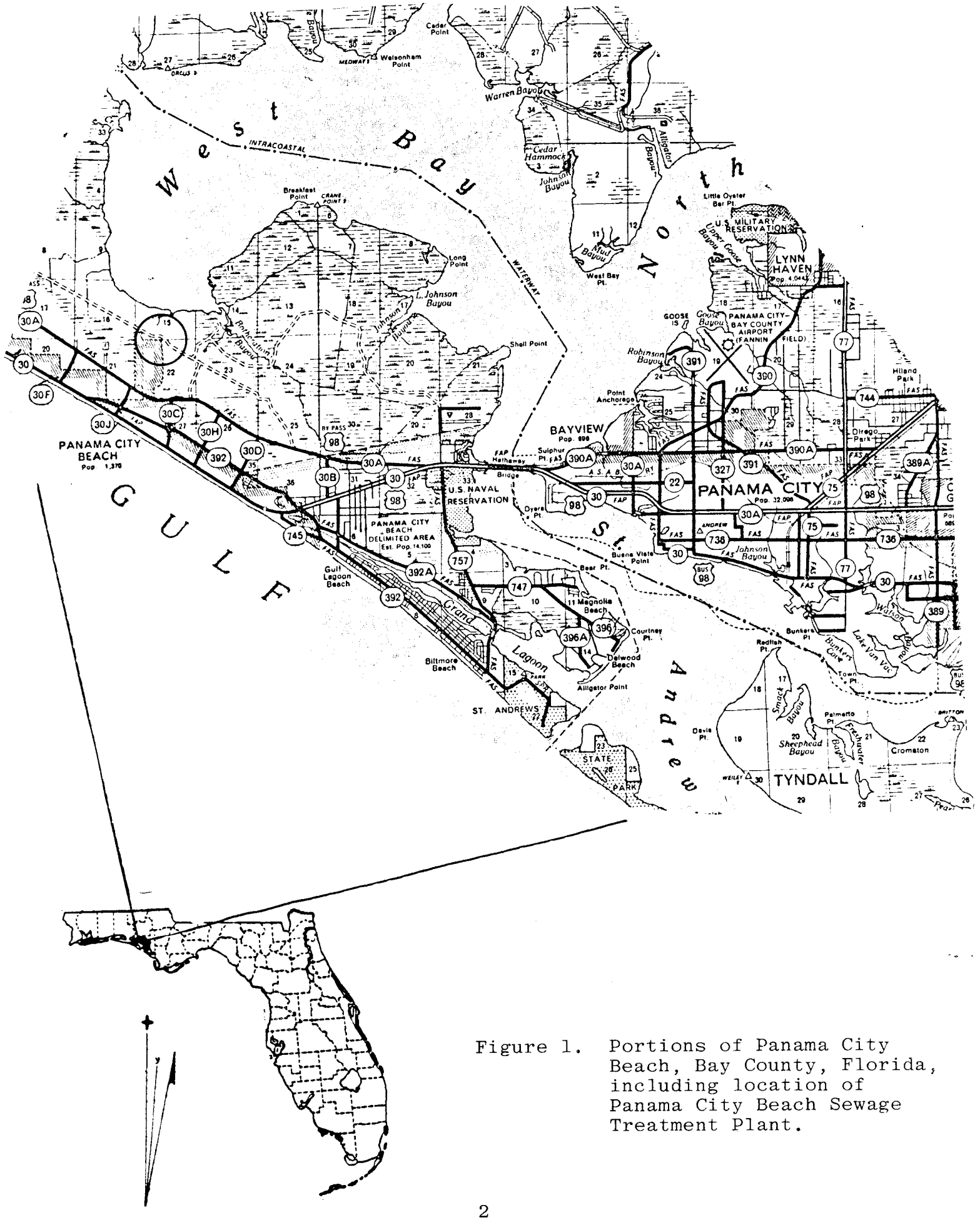


Figure 1. Portions of Panama City Beach, Bay County, Florida, including location of Panama City Beach Sewage Treatment Plant.

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 two 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 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 two weeks old at the time of the test.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. The mysids are cultured under a 16:8 hour light-dark cycle in 10 and 15 gallon all-glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater, used for culturing the test organisms and

as diluent in the bioassays, is prepared by mixing artificial sea salts (Forty Fathoms®) and well water to obtain a final salinity of 20-30 ppt. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily and once daily on weekends with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 23° ± 2°C. Adult or large juvenile mysids were used in this 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

Six separate 48 hour static acute toxicity bioassays were performed on samples of chlorinated and de-chlorinated effluent collected from the Panama City Beach Sewage Treatment Plant. Three bioassays were performed on each sample, consisting of two freshwater tests (*D. pulex* and *N. leedsi*) and one saltwater test (*M. bahia*). The tests were performed from 1045 hours on 19 February to 1120 hours on 21 February, 1985.

The samples were collected by Mr. Richard Sublette of DER's Panama City Branch office at 1427 hours on 18 February, 1985, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples were unpreserved and 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.

Salinity Adjustment

Since this particular bioassay sample eventually enters an estuarine environment, the Biology Section used a saltwater test organism to evaluate toxicity. Therefore, artificial sea salts (Forty Fathoms®) were added to the 100% concentration until a salinity of approximately 7 ppt was obtained. This salinity is well within the salinity tolerance of the test organism. Nevertheless, a Salinity Control was also employed, consisting of artificial seawater diluted with well water until a similar salinity of approximately 8 ppt was obtained. Additional information concerning salinity adjustment and saltwater tests is given below.

pH Adjustment

Since the toxicity, or lack of toxicity, in a bioassay sample may be dependent on the sample's pH, the Biology Section prefers, and recommends, that the pH of a sample at the start of the bioassay be similar to the pH measured during sampling. In this particular case, the sample was

adjusted with an acid (0.1N HCl). This was necessary due to the pH having increased between sampling and initiating the test. This pH increase was likely due to natural biological activity in the sample container, or a result of adjusting the samples (salinity). The pH's of the freshwater and saltwater samples were adjusted to ± 0.3 s.u. of the value measured at sampling. This procedure provides the fairest test possible considering the varied chemical substances in complex wastewaters, the toxicities of which are dependent on pH. Two controls were also employed, consisting of dilution water and artificial seawater in which pH had been adjusted with the same acid and to the same value as the sample. These adjustments are noted on the bioassay data forms.

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 the controls, 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 each concentration 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 these tests.

Saltwater Tests

Saltwater for dilution of the effluent samples was prepared from artificial sea salts (Forty Fathoms®), and adjusted with DER well water until a salinity of approximately 15 ppt was achieved. For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (7.0 ppt to 13.5 ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts were added until salinities of approximately 7 ppt were achieved (within mysid shrimp salinity tolerance). Two control groups (Table 6) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, the pH adjustments, or the salinity range. The control groups included:

1) Diluent Control - Artificial seawater (15.0 ppt). This represents the highest concentration of artificial seawater used in the bioassays, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassays, and therefore tests the tolerance of the organisms to this salinity.

2) Salinity - Acid Control - Artificial seawater (20 ppt) adjusted with well water to a salinity of approximately 7 ppt, with an amount of hydrochloric acid added needed to equal the pH achieved in the pH adjusted effluent.

The concentration series selected for this study (Table 1) is an abbreviated version of that recommended by APHA (1980). Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the appropriate salinity aquaria to culture dishes for observation and selection of individuals for the tests. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

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 or salinity (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 - 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
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

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	------	500 ml
10.0%	450 ml	50 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 Panama City Beach Sewage Treatment Plant, Panama City Beach, Bay County, NPDES #FL0021512, on 19 to 21 February, 1985.

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	10	10	10	7.7	9.0	8.3	7.5	8.0	8.1	21.4	21.0	21.8			Control Water
Control	10	10	9	7.7	9.0	8.3	7.5	8.0	8.1				210	245	NH_3(as NH_3)(mg/l) 0 / Total Alkalinity 125 (mg/l $CaOO_3$)
Acid Control	10	10	10	7.7	9.1	8.2	6.8	7.7	7.9	20.9	19.9	21.5			
Acid Control	10	10	9	7.7	9.1	8.2	6.8	7.7	7.9				225	240	
10.0%	10	10	6	7.7	9.2	8.2	7.5	8.1	8.2	20.8	20.3	21.4			Total ammonia (as N)(mg/l) .01 / Total Hardness 110 (mg/l $CaOO_3$)
10.0%	10	10	7	7.7	9.2	8.2	7.5	8.1	8.2				215	225	
50.0%	10	0	-[b]	7.6	9.2	-[b]	7.1	8.0	-[b]	20.8	20.2	-[b]			
50.0%	10	0	-	7.5	9.2	-	7.1	8.0	-				435	-[b]	
100.0%	10	0	-	7.3	9.2	-	6.8	7.9	-	20.6	20.3	-			
100.0%	10	0	-	7.2	9.2	-	6.8	7.9	-				590	-	100% Effluent
															NH_3(as NH_3)(mg/l) .036 / Total Alkalinity 123 (mg/l $CaOO_3$)
															Total ammonia (as N)(mg/l) 11.5 / Total Hardness 130 (mg/l $CaOO_3$)

[a] Total residual chlorine: 1.4 mg/l (HACH DPD Method and Iodometric Method).

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

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Panama City Beach Sewage Treatment Plant, Panama City Beach, Bay County, NPDES #FL0021512, on 19 to 21 February, 1985.

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	7.5	9.0	8.2	7.6	8.0	8.2	20.8	20.4	20.9		
Control	5	5	5	7.5	9.0	8.2	7.6	8.0	8.2				215	220
Acid Control	5	5	5	7.6	9.0	8.2	6.8	7.8	8.1	20.9	20.2	20.6		
Acid Control	5	5	5	7.6	9.0	8.2	6.8	7.8	8.1				228	235
10.0%	5	5	5	7.6	9.0	8.2	7.4	7.9	8.1	20.7	20.0	20.5		
10.0%	5	5	5	7.6	9.0	8.2	7.4	7.9	8.1				266	270
50.0%	5	0	–[b]	7.3	9.1	–[b]	7.3	7.9	–[b]	20.6	20.0	–[b]		
50.0%	5	0	–	7.3	9.1	–	7.3	7.9	–				432	–[b]
100.0%	5	0	–	7.2	9.2	–	6.8	7.8	–	20.6	19.9	–		
100.0%	5	0	–	7.2	9.2	–	6.8	7.8	–				610	–

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity 125 (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l) .01

Total Hardness 110 (mg/l $CaOO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .036

Total Alkalinity 123 (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l) 11.5

Total Hardness 130 (mg/l $CaOO_3$)

[a] Total residual chlorine: 1.4 mg/l (HACH DPD Method and Iodometric Method).

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

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Panama City Beach Sewage Treatment Plant, Panama City Beach, Bay County, NPDES #FL0021512, on 19 to 21 February, 1985.

Chlorinated sample[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Salinity (ppt)		
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	
Control	5	5	5	7.5	7.0	4.4	8.7	8.5	8.1	21.2	20.4	20.6			Control Water
Control	5	5	5	7.5	7.0	5.2	8.7	8.5	8.2				15.0	15.0	NH_3(as NH_3)(mg/l) .00 / Total Alkalinity (mg/l $CaOO_3$)
Acid-Salinity Control	5	5	5	7.9	7.2	5.2	6.8	7.6	7.7	21.1	20.2	20.5			
Acid-Salinity Control	5	5	5	7.9	7.2	4.5	6.8	7.6	7.6				9.0	13.5	
10.0%	5	5	5	7.8	7.7	5.6	8.6	8.5	8.3	20.8	20.1	20.5			Total ammonia (as N)(mg/l) .006 / Total Hardness (mg/l $CaOO_3$)
10.0%	5	5	5	7.8	7.7	5.6	8.6	8.5	8.3				13.5	13.0	
32.0%	5	0	-[b]	7.7	8.1	-[b]	8.3	8.4	-[b]	20.8	20.0	-[b]			
32.0%	5	0	-	7.7	8.1	-	8.3	8.4	-				10.5	-[b]	
56.0%	5	0	-	7.3	8.1	-	8.7	8.1	-	20.7	20.0	-			
56.0%	5	0	-	7.3	8.1	-	8.7	8.1	-				7.2	-	100% Effluent
100.0%	5	0	-	7.3	8.3	-	6.8	7.9	-	20.7	20.2	-			NH_3(as NH_3)(mg/l) .029 / Total Alkalinity (mg/l $CaOO_3$)
100.0%	5	0	-	7.3	8.3	-	6.8	7.9	-				7.5	-	
															Total ammonia (as N)(mg/l) 9.8 / Total Hardness (mg/l $CaOO_3$)

[a] Total residual chlorine: 1.4 mg/l (HACH DPD Method and Iodometric Method).

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

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Panama City Beach Sewage Treatment Plant, Panama City Beach, Bay County, NPDES #FL0021512, on 19 to 21 February, 1985.

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	10	10	10	7.7	9.0	8.3	7.5	8.0	8.1	21.4	21.0	21.8			Control Water
Control	10	10	9	7.7	9.0	8.3	7.5	8.0	8.1				210	245	NH_3(as NH_3)(mg/l) .00 — Total Alkalinity 125 (mg/l $CaOO_3$)
Acid Control	10	10	10	7.7	9.1	8.2	6.8	7.7	7.9	20.9	19.9	21.5			
Acid Control	10	10	9	7.7	9.1	8.2	6.8	7.7	7.9				225	240	
10.0%	10	10	10	7.5	9.1	8.3	7.5	8.1	8.2	20.8	20.2	21.4			Total ammonia (as N)(mg/l) .01 — Total Hardness 110 (mg/l $CaOO_3$)
10.0%	10	10	9	7.5	9.1	8.3	7.5	8.1	8.2				251	260	
50.0%	10	10	10	7.5	9.2	8.2	7.3	8.1	8.2	21.0	20.3	21.3			
50.0%	10	10	10	7.5	9.2	8.2	7.3	8.1	8.2				438	440	
100.0%	10	10	10	7.5	9.0	8.2	6.8	7.9	8.1	21.1	20.3	21.3			
100.0%	10	10	8	7.4	9.0	8.2	6.8	7.9	8.1				690	700	100% Effluent
															NH_3(as NH_3)(mg/l) .037 — Total Alkalinity 123 (mg/l $CaOO_3$)
															Total ammonia (as N)(mg/l) 11.5 — Total Hardness 130 (mg/l $CaOO_3$)

[a] Total residual chlorine: <0.02 mg/l (HACH DPD Method).

Table 6. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Panama City Beach Sewage Treatment Plant, Panama City Beach, Bay County, NPDES #FL0021512, on 19 to 21 February, 1985.

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	7.5	9.0	8.2	7.6	8.0	8.2	20.8	20.4	20.9		
Control	5	5	5	7.5	9.0	8.2	7.6	8.0	8.2				215	220
Acid Control	5	5	5	7.6	9.0	8.2	6.8	7.8	8.1	20.9	20.2	20.6		
Acid Control	5	5	5	7.6	9.0	8.2	6.8	7.8	8.1				228	235
10.0%	5	5	5	7.4	8.9	8.4	7.4	7.9	8.1	20.9	20.2	20.5		
10.0%	5	4	4	7.4	8.9	8.4	7.4	7.9	8.1				270	270
50.0%	5	5	5	7.5	8.9	8.2	7.1	7.8	8.0	21.0	20.2	20.5		
50.0%	5	5	5	7.6	8.9	8.2	7.1	7.8	8.0				480	480
100.0%	6[b]	6	5	7.5	8.6	8.1	6.8	7.8	8.0	21.2	20.3	20.6		
100.0%	5	5	5	7.4	8.6	8.1	6.8	7.8	8.0				710	710

Control Water

NH_3(as NH_3)(mg/l) .00

Total Alkalinity ____ (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l) .01

Total Hardness ____ (mg/l $CaOO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .037

Total Alkalinity ____ (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l) 11.5

Total Hardness ____ (mg/l $CaOO_3$)

[a] Total residual chlorine: <0.02 mg/l (HACH DPD Method).

[b] Extra organism loaded by mistake, allowed to remain.

Table 7. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Panama City Beach Sewage Treatment Plant, Panama City Beach, Bay County, NPDES #FL0021512, on 19 to 21 February, 1985.

De-chlorinated sample[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Salinity (ppt)		
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	
Control	5	5	5	7.5	7.0	4.4	8.7	8.5	8.1	21.2	20.4	20.6			Control Water
Control	5	5	5	7.5	7.0	5.2	8.7	8.5	8.2				15.0	15.0	NH_3(as NH_3)(mg/l) 0 — Total Alkalinity (mg/l $CaOO_3$)
Acid-Salinity Control	5	5	5	7.9	7.2	5.2	6.8	7.6	7.7	21.1	20.2	20.5			
Acid-Salinity Control	5	5	5	7.9	7.2	4.5	6.8	7.6	7.6				9.0	13.5	
10.0%	5	5	5	7.6	7.2	4.9	8.6	8.5	8.2	20.9	20.3	20.5			Total ammonia (as N)(mg/l) .006 — Total Hardness (mg/l $CaOO_3$)
10.0%	5	5	5	7.6	7.2	5.5	8.6	8.5	8.3				13.2	13.2	
32.0%	5	4	4	7.6	7.5	5.6	8.3	8.3	8.1	20.9	20.3	20.4			
32.0%	5	5	5	7.6	7.5	5.0	8.3	8.3	8.0				10.5	10.5	
56.0%	5	5	5	7.5	7.6	5.9	7.7	8.1	8.0	21.0	20.3	20.3			
56.0%	5	5	3	7.5	7.6	5.8	7.7	8.1	8.0				7.2	7.0	100% Effluent
100.0%	5	4	1	7.4	7.5	5.9	6.8	7.8	7.9	21.0	20.3	20.4			NH_3(as NH_3)(mg/l) .030 — Total Alkalinity (mg/l $CaOO_3$)
100.0%	5	3	0	7.4	7.5	6.1	6.8	7.8	7.9				7.5	7.1	
															Total ammonia (as N)(mg/l) 9.8 — Total Hardness (mg/l $CaOO_3$)

[a] Total residual chlorine: <0.02 mg/l (HACH DPD Method).

Table 8. LC_{50} Data for Bioassays of Panama City Beach Sewage Treatment Plant, Panama City Beach, Bay County, NPDES #FL0021512, on 19 to 21 February, 1985.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
D. pulex	Binomial	22.3% (10.0 and 50.0)	13.3% (0 and 50.0),[b]
Chlorinated	Moving Average	a	a
Sample	Probit	a	a
N. leedsi	Binomial	22.3% (10.0 and 50.0)	22.3% (10.0 and 50.0)
Chlorinated	Moving Average	a	a
Sample	Probit	a	a
M. bahia	Binomial	17.8% (10.0 and 32.0),[b]	17.8% (10.0 and 32.0)
Chlorinated	Moving Average	a	a
Sample	Probit	a	a
D. pulex	Binomial	a	a
De-chlorinated	Moving Average	a	a
Sample	Probit	a	a
N. leedsi	Binomial	a	a
De-chlorinated	Moving Average	a	a
Sample	Probit	a	a
M. bahia	Binomial	a	71.7% (32.0 and 100.0)
De-chlorinated	Moving Average	a	68.7% (53.2 and 103.2),[c]
Sample	Probit	217.0% (95.4 and ∞)	66.1% (50.5 and 91.4)

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

RESULTS AND DISCUSSION

The sample of chlorinated effluent collected from the Panama City Beach Sewage Treatment Plant was acutely toxic to the test organisms, Daphnia pulex, Notropis leedsi, and Mysidopsis bahia, producing 48 hour LC_{50}'s of 13.3%, 22.3%, and 17.8%, respectively (Table 7). A chlorine residual of 1.4 mg/l (HACH and Iodometric methods) was measured in the sample and was probably, in part, responsible for this toxicity. In the bioassays of the de-chlorinated effluent, the sample was not toxic to the freshwater test organisms, D. pulex and N. leedsi (Tables 5 and 6). However, the sample was toxic to M. bahia (Table 7), resulting in a 48 hour LC_{50} of 68.7%. The total and unionized ammonia detected in the sample (maximum measured = 11.5 mg/l and .037 mg/l, respectively) may have contributed to the observed mortality. Chemical analyses failed to indicate the presence of any priority pollutant organic compounds. The sample for metal analysis was invalidated due to the incorrect use of H_2SO_4 as a preservative.

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