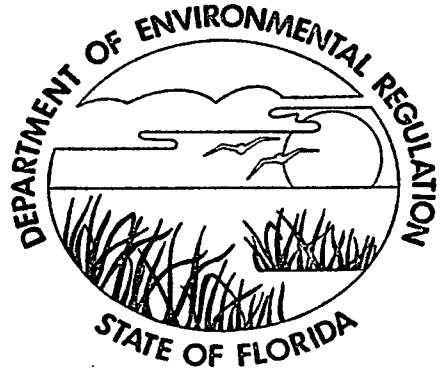


BIOASSAYS OF THE CITY OF DAYTONA BEACH - file

(BETHUNE POINT) SEWAGE TREATMENT PLANT

DAYTONA BEACH, VOLUSIA COUNTY, FLORIDA

NPDES #FL0025984

SAMPLED 10/6/86

NOVEMBER 17, 1986

Biology Section
Division of Environmental Programs

BIOASSAYS OF THE CITY OF DAYTONA BEACH (BETHUNE POINT) SEWAGE TREATMENT PLANT DAYTONA BEACH, VOLUSIA COUNTY, FLORIDA NPDES #FL0025984

Biology Section
Bureau of Laboratories and
Special Programs
November 17, 1986

EXECUTIVE SUMMARY

City of Daytona Beach (Bethune Point) STP, NPDES #FL0025984, on 7 to 9 October, 1986.

The Bethune Point sewage treatment plant is located on the Halifax River at Shady Place, and discharges approximately 15 MGD of treated domestic wastewater through an offshore pipe. The sample of final chlorinated effluent from this facility proved to be acutely toxic to both test organisms, Daphnia pulex and Notropis leedsi, producing 24 hour LC_{50}'s of < 10.0% and 22.2%, respectively. A portion of this toxicity can be attributed to the total residual chlorine (0.7 mg/l) measured in the sample. Moderate concentrations of ammonia, up to 3.8 mg/l total and 0.05 mg/l unionized, were also detected in the wastewater, which may have contributed slightly to the toxicity noted. Conversely, a de-chlorinated sample of effluent was not toxic to either organism. Chemical analyses indicated the presence of numerous unidentified organic compounds, and the metals silver and zinc. These chemical constituents may have contributed to the toxicity noted in the effluent.

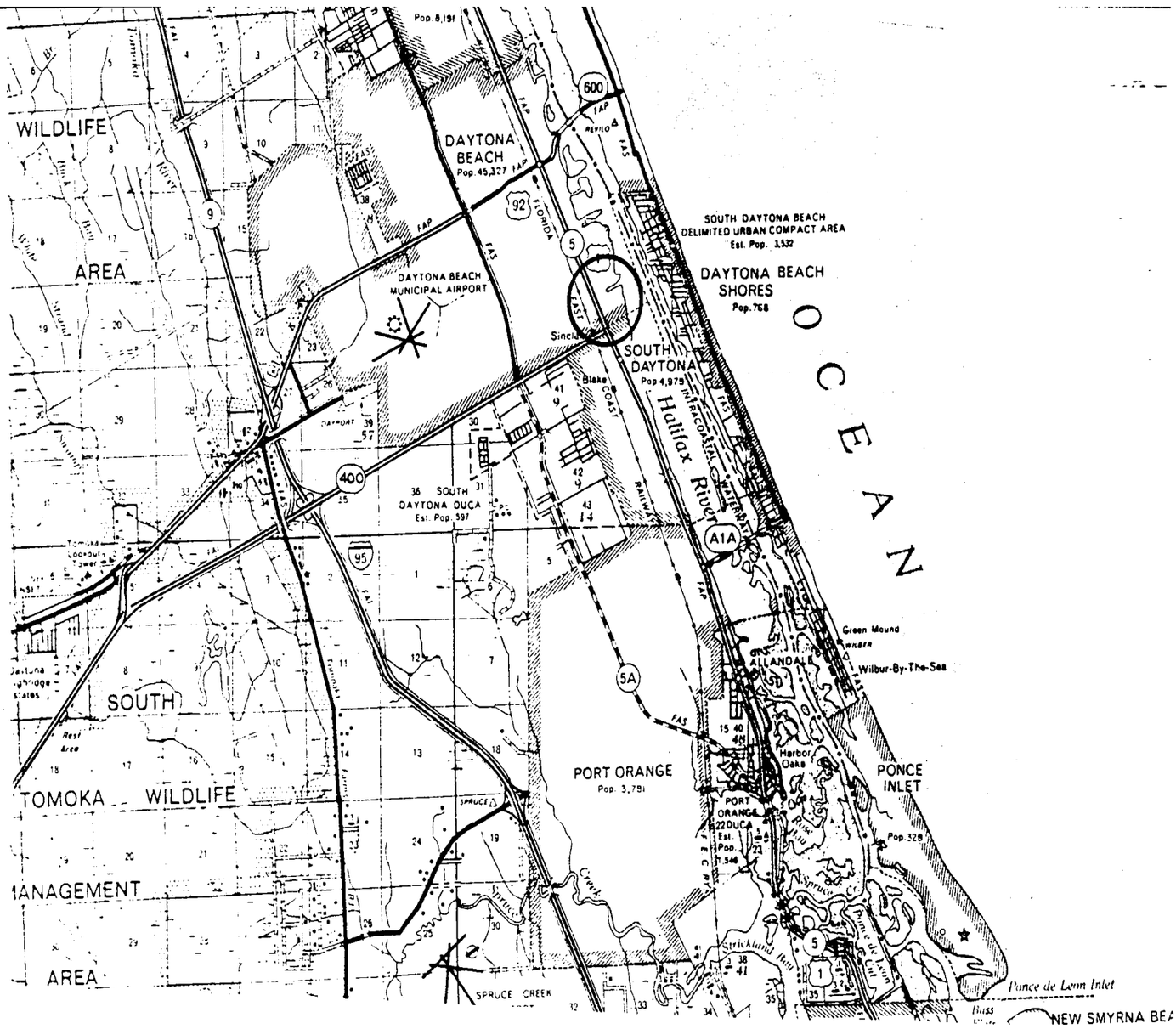
Since most of the toxicity noted in Bethune Point STP's wastewater was likely due to the presence of residual chlorine, we recommend the installation of a de-chlorination unit at this facility. Also, the identity and source of the organic compounds should be investigated, with the eventual goal of eliminating them from the discharge.

INTRODUCTION

The City of Daytona Beach (Bethune Point) Sewage Treatment Plant is located on the Halifax River at Shady Place, in the south portion of Daytona Beach, Volusia County, Florida (Fig. 1). This municipal facility treats domestic wastes from a population of approximately 120,000 using contact stabilization, clarification, filtration, denitification and chlorination. Sludge is disposed of on pasture land. A complete schematic flow diagram from this facility is presented in Figure 2. The average discharge from this facility is 14.8 MGD, resulting in hydraulic overloading of the plant's 12.0 MGD design capacity. Discharge (NPDES Outfall #001) is to the Halifax River through a pipe 84' offshore and 5' deep.

The Biology Section had previously performed bioassays on effluents from the Bethune Point facility on two separate occasions. The first bioassay, performed in March, 1982, on pre-chlorinated, chlorinated and de-chlorinated samples of effluent, produced 48 hour LC_{50}'s of 44.8%, 39.9%, and 46.2%, respectively, using the organism, Mysidopsis bahia. This toxicity may have been enhanced by the presence of the pesticide Lindane in the Bethune Point effluent (FDER 1982). The second bioassay, performed in December, 1982, using pre-chlorinated effluent and the same test organism, produced a 48 hour LC_{50} of 46.4%. Again, this toxicity may have been enhanced by the presence of Lindane, but samll concentrations of various metals also were present in the effluent (FDER 1983).

To further evaluate the extent of toxicity of this discharge, the Biology Section performed four static acute toxicity bioassays on samples of chlorinated and de-chlorinated effluent from the City of Daytona Beach STP (Bethune Point), on 7 to 9 October, 1986. The results of these latest tests are presented in this report. Those involved in performing the test and in preparation of this report were: M. L. Roll and P. G. Weiland.



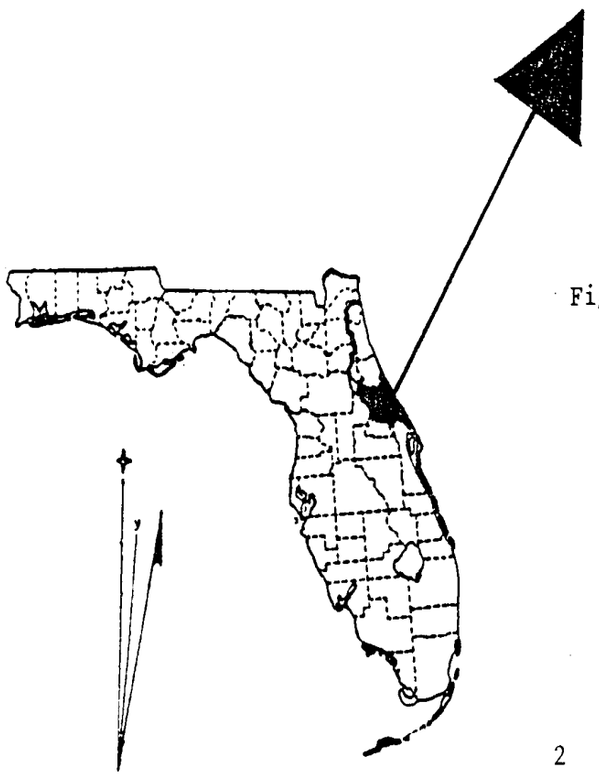


Figure 1. Portions of Volusia County, Florida, including City of Daytona Beach and the approximate location of the Bethune Point STP.

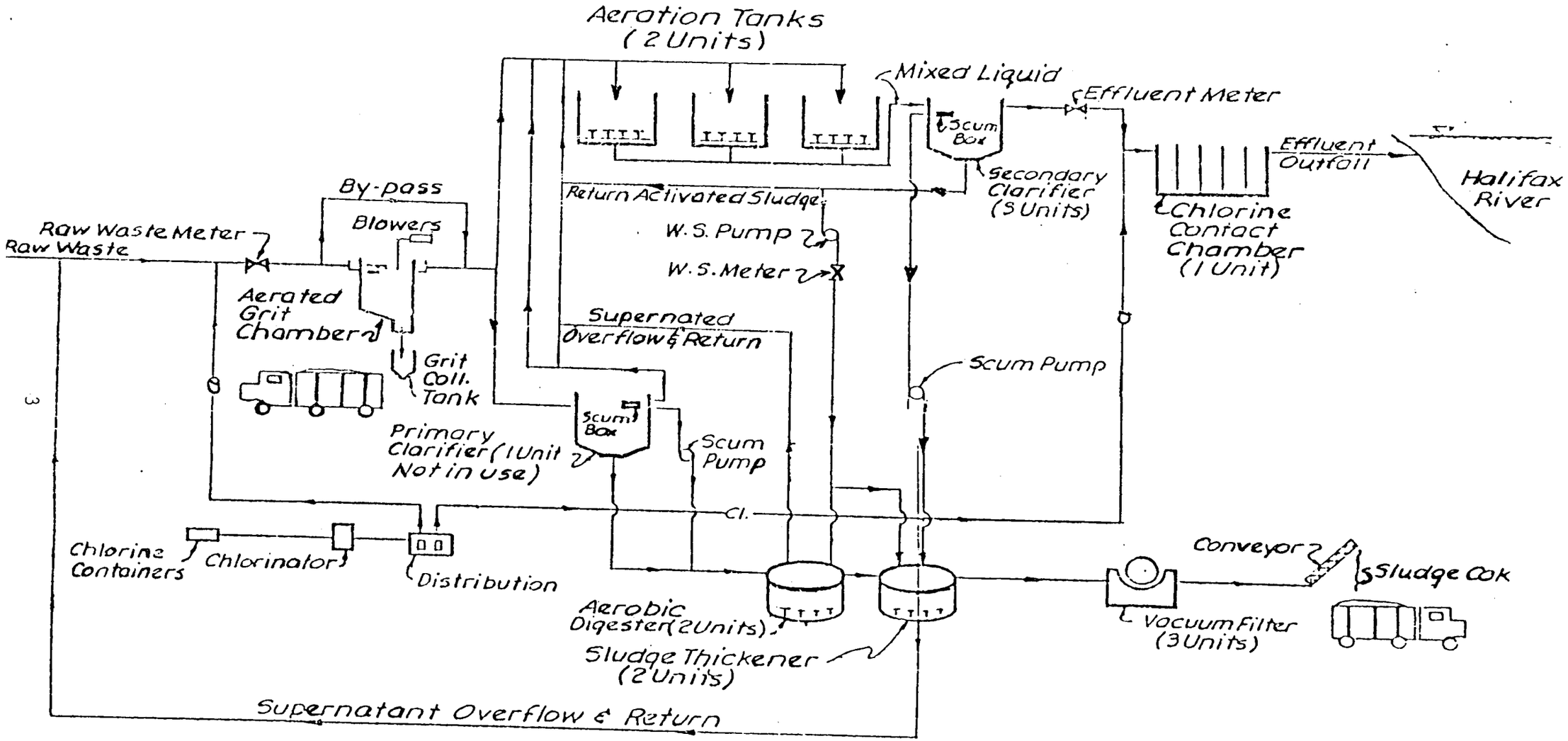


Figure 2

BETHUNE POINT SEWAGE TREATMENT PLANT

SCHEMATIC FLOW DIAGRAM

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 13 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 static acute toxicity bioassays were performed on samples of final chlorinated and de-chlorinated effluent collected from the City of Daytona Beach STP (Bethune Point). The tests were performed from 1030 hours on 7 October to 1030 hours on 10 October, 1986.

The samples were collected by Mr. Louis Ley of DER's St. Johns River District at 1300 hours on 6 October, 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 sample 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 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 an abbreviated version of that recommended by APHA (1980).

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 Daphnia 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.

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. 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 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 Tables 2 through 5.

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Bethune Point Sewage Treatment Plant, NPDES #FL0025984, on 7 to 9 October, 1986.

Chlorinated Effluent[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	8.8	8.6	8.2	8.2	8.0	8.0	21.0	19.1	19.6		
Control	10	10	9	8.8	8.6	8.2	8.2	8.0	8.0				202	218
10.0%	10	2	1	9.1	8.7	8.3	8.1	8.0	8.0	21.0	19.0	19.5		
10.0%	10	0	0	8.1	8.7	-b	8.1	8.0	-b				262	-b
32.0%	10	0	0	8.9	8.8	-	7.8	8.0	-	21.5	19.0	-b		
32.0%	10	0	0	8.9	8.8	-	7.8	8.0	-				397	-
56.0%	10	0	0	8.8	8.9	-	7.6	8.0	-	22.2	19.0	-		
56.0%	10	0	0	8.8	8.9	-	7.6	8.0	-				550	-
100.0%	10	0	0	8.4	8.9	-	7.3	8.0	-	23.2	19.1	-		
100.0%	10	0	0	8.4	8.9	-	7.3	8.0	-				800	-

Control Water

NH_3(as NH_3)(mg/l) .003

Total ammonia (as N)(mg/l) .04

Total Alkalinity 122 (mg/l $CaCO_3$)

Total Hardness 125 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .045

Total ammonia (as N)(mg/l) 3.8

Total Alkalinity 125 (mg/l $CaCO_3$)

Total Hardness 151 (mg/l $CaCO_3$)

[a] Total Residual Chlorine = 0.7 mg/l (HACH DPD Method).

[b] Parameters not measured since all organisms were dead at 24 hours.

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Bethune Point Sewage Treatment Plant, NPDES #FL00025984, on 7 to 9 October, 1986.

Chlorinated Effluent[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	8.8	8.4	8.4	7.6	7.9	7.9	21.6	18.8	19.3		
Control	5	5	5	8.8	8.4	8.4	7.6	7.9	7.9				217	210
10.0%	5	5	5	8.8	8.4	8.2	7.5	7.9	7.9	21.6	18.7	19.2		
10.0%	5	5	5	8.8	8.4	8.2	7.5	7.9	7.9				279	272
32.0%	5	2	2	8.9	8.5	8.0	7.5	7.9	7.8	22.1	18.6	19.2		
32.0%	5	0	0	8.9	8.5	-b	7.5	7.9	-b				409	-b
56.0%	5	0	0	8.7	8.6	-	7.4	7.9	-	23.0	18.6	-b		
56.0%	5	0	0	8.7	8.6	-	7.4	7.9	-				580	-
100.0%	5	0	0	8.5	8.6	-	7.3	7.9	-	23.8	19.7	-		
100.0%	6	0	0	8.5	8.6	-	7.3	7.9	-				830	-

Control Water

NH_3(as NH_3)(mg/l) .001

Total ammonia (as N)(mg/l) .04

Total Alkalinity (mg/l $CaCO_3$) 122

Total Hardness (mg/l $CaCO_3$) 125

100% Effluent

NH_3(as NH_3)(mg/l) .05

Total ammonia (as N)(mg/l) 3.8

Total Alkalinity (mg/l $CaCO_3$) 125

Total Hardness (mg/l $CaCO_3$) 151

[a]Total Residual Chlorine = 0.7 mg/l (HACH DPD Method).

[b]Parameters not measured since all organisms were dead at 24 hours.

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Bethune Point Sewage Treatment Plant, NPDES #FL0025984, on 7 to 9 October, 1986.

De-chlorinated Effluent[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	8.9	8.7	8.5	8.2	8.1	8.0	21.0	19.1	19.6		
Control	10	10	10	8.9	8.7	8.5	8.2	8.1	8.0				210	219
10.0%	10	10	10	9.0	8.7	8.2	8.1	8.0	7.9	21.1	19.0	19.6		
10.0%	10	10	10	9.0	8.7	8.2	8.1	8.0	7.9				268	270
32.0%	10	10	10	8.8	8.7	7.9	7.9	9.0	7.9	21.7	19.0	19.6		
32.0%	10	10	10	8.8	8.7	7.9	7.8	8.0	7.9				395	390
56.0%	10	10	10	8.7	8.6	7.9	7.6	8.0	7.9	22.2	19.0	19.5		
56.0%	10	10	10	8.7	8.6	7.9	7.6	8.0	7.9				580	550
100.0%	10	10	10	8.4	8.5	7.9	7.3	8.0	7.9	22.7	19.0	19.5		
100.0%	10	10	10	8.4	8.5	7.9	7.3	8.0	7.9				810	800

Control Water

NH_3(as NH_3)(mg/l) .003

Total Alkalinity 122 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) .04

Total Hardness 125 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .04

Total Alkalinity 125 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 3.7

Total Hardness 151 (mg/l $CaCO_3$)

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

Table 5. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Bethune Point Sewage Treatment Plant, NPDES #FL0025984, on 7 to 9 October, 1986.

De-chlorinated Effluent[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	8.8	8.4	8.4	7.6	7.9	7.9	21.6	18.8	19.3			
Control	5	5	5	8.8	8.4	8.4	7.6	7.9	7.9				217	210	
10.0%	5	5	5	8.7	8.2	8.2	7.5	7.9	7.9	21.7	18.9	19.5			
10.0%	5	5	5	8.7	8.2	8.2	7.5	7.9	7.9				272	273	
32.0%	5	5	5	8.8	8.1	8.2	7.5	7.8	7.9	21.9	19.9	19.4			
32.0%	5	5	5	8.8	8.1	8.2	7.5	7.8	7.9				408	392	
56.0%	5	5	5	8.7	8.1	8.1	7.4	7.8	7.8	22.4	19.7	19.2			
56.0%	5	5	5	8.7	8.1	8.1	7.4	7.8	7.8				530	550	
100.0%	5	5	5	8.6	7.9	7.9	7.3	7.8	7.8	23.0	18.7	19.2			
100.0%	5	5	5	8.6	7.9	7.9	7.3	7.8	7.8				820	800	

Control Water

NH_3(as NH_3)(mg/l) .003

Total Alkalinity 122 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) .04

Total Hardness 125 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .04

Total Alkalinity 125 (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 3.7

Total Hardness 151 (mg/l $CaCO_3$)

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

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
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
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 6. LC_{50} Data for Bioassays of City of Daytona Beach STP (Bethune Point) on 7 to 9 October, 1986.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Chlorinated Sample			
Daphnia pulex	Binomial Moving Average Probit	< 10.0%, a	< 10.0%, a
Notropis leedsi	Binomial Moving Average Probit	22.19% (10 and 56) b b	22.19% (10 and 56) b b
De-chlorinated Sample			
D. pulex	Binomial Moving Average Probit	No Mortality	No Mortality
N. leedsi	Binomial Moving Average Probit	No Mortality	No Mortality

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a The LC_{50} is < 10% since almost all the organisms died in this lowest test concentration (10%).

b 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.

Table 7. Results of Chemical Analyses Performed on Final Effluent from the Daytona Beach STP at Bethune Point, NPDES #FL0024984, collected on 6 October, 1986.

Organics

Acid Extractables:	N.D.
Base Neutral Extractables:	Numerous unidentified compounds
Pesticide Extractables:	Numerous unidentified compounds

Metals

Antimony	N.D.
Arsenic	N.D.
Beryllium	N.D.
Cadmium	< 0.1 u/gl
Chromium	N.D.
Copper	N.D.
Lead	N.D.
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	0.4 ug/l
Thallium	N.D.
Zinc	13 ug/l

N.D. = None Detected

RESULTS AND DISCUSSION

The sample of final chlorinated effluent from the City of Daytona Beach STP (Bethune Point) proved to be acutely toxic to both test organisms, Daphnia pulex and Notropis leedsi, producing 24 hour LC_{50} values of < 10.0% and 22.19%, respectively (Table 6). A portion of this toxicity can be attributed to the total residual chlorine (0.7 mg/l) measured in the sample. Also, ammonia was detected in this wastewater, up to 3.8 mg/l total and 0.05 mg/l unionized, which may have contributed slightly to the toxicity. Conversely, no mortality occurred in the de-chlorinated sample of effluent.

The results of chemical analyses (Table 7) indicated the presence of numerous unidentified organic compounds, and the metals silver (0.4 ug/l) and zinc (13 ug/l). The chemical constituents may have contributed to the toxicity noted in this effluent.

Recommendation

Since the majority of the toxicity noted in the Bethune Point STP effluent was attributed to the presence of residual chlorine, we recommend the installation of a de-chlorination unit at this facility. Also, the identity and source of the organic compounds should be investigated, with the eventual goal of eliminating them from the discharge. The facility should continue with the biomonitoring program specified in their permit.

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly.

The two basic concepts utilized in the standards are acute and chronic toxicity (defined in 17-3.021(1) and (4), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as onetwentieth of the 96 hour LC_{50}. Therefore, if a test solution kills one- half or more of a group of test organisms in 96 hours, it must contain at least three times the defined "acutely toxic" concentration and at least twenty times the "chronically toxic" concentration of some substance or substances. If a test solution causes that same mortality in less than 96 hours, (for example, in 24 or 48 hours), it is at least as toxic as the example given above, and actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existence of a mixing zone, whetheras a specific part of the permit or based on the existence of a currently valid permit predating the language in Chapter 17-4 requiring mixing zone designation. In the absence of a mixing zone, the general criteria for surface waters [17-3.061] are applicable at all points, beginning at the point of discharge. This section prohibits the discharge of substances in concentrations which are chronically toxic [17-3.061(2)(p)]; that is, the concentrations cannot exceed one-twentieth of the 96 hour LC_{50} as discussed above. If a mixing zone does exist, then this chronic toxicity standard is only applicable from the edge of the mixing zone and beyond. Within the mixing zone, the less stringent acute toxicity standard of the minimum criteria for all waters [17-3.051(1)(d)] applies: one-third of the 96 hour LC_{50}. The acute toxicity standard does not apply, however, to each point within a mixing zone not designated for dredge and fill permitting, but rather to the average of a number of points within such a mixing zone, based on the provisions of the surface water mixing zones section of Chapter 17-4 [17-4.244(4)]. At the same time, though, this same rule prohibits waste concentrations within such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

A number of different dilutions were examined in order to determine by how much, if any, the discharge exceeds the 96 hour LC_{50}. For example, if the sample, after two-fold dilution with diluent (a 33% concentration), kills one-half of the test organisms after 96 hours, then the sample is three times as toxic as is allowed by that standard. Similarly, if a 5% solution of the sample kills 50% of the organisms, the sample is twenty times as toxic as is allowed. Toxicities are reported here in that manner, i.e., in terms of the percentage dilution of the test sample. The smaller the percentage which is reported means a correspondingly greater toxicity.

Note that even if a discharger receives a very large mixing zone not designated for dredge and fill permitting, the regulations require that the maximum toxicity at any point within the mixing zone be restricted to the 96 hour LC_{50}. The results here reported are based on actual effluent samples, representative of the effluent concentration which enters waters of the state at the point of discharge. If it is demonstrated that, under the conditions existing at the point of discharge, effluent concentrations will be less than at the actual sampling point (if the two points are different), the reported bioassay results will reflect the reduced discharge concentration.

With respect to the species of organisms used in these tests, the language of the F.A.C. indicates that they should be "significant to the indigenous aquatic community". All species used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

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