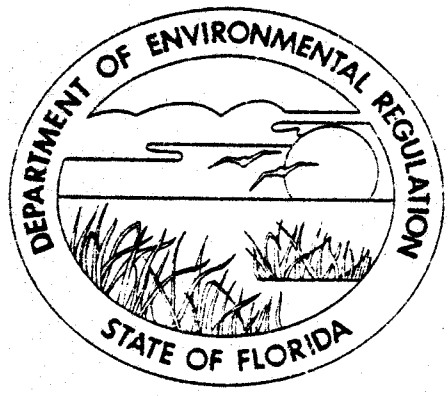


BIOASSAYS OF EASTSIDE WATER COMPANY

WASTEWATER TREATMENT PLANT

TAMPA, HILLSBOROUGH COUNTY, FLORIDA

NPDES # FL0024830

SAMPLED 9/15/80

Biological Section
Bureau of Water Analysis
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INTRODUCTION

The Eastside Water Company Wastewater Treatment Plant is a relatively small sewage treatment facility located at the southwest corner of I-4 and Orient Road, Tampa, Hillsborough County, Florida (Figure 1). This facility treats domestic waste from the town of East Lake Park (population 3000+). Treatment consists of aeration, a primary clarifier, a trickling filter, a secondary clarifier, chlorination, retention in polishing and evaporation ponds, and final discharge. The discharge of 0.3 MGD is to Harney Creek and then to the Palm River, a tributary of McKay and Hillsborough Bays.

Effluent from Eastside Water Company Wastewater Treatment Plant was selected for toxicity testing because of numerous Notices of Violation presented to the facility in the past for exceeding its discharge limitations.

On September 16, 1980, the Biological Section obtained effluent samples from the Eastside Water Company, and performed static bioassays to determine the existence and extent of toxicity to the biota of the receiving waters.

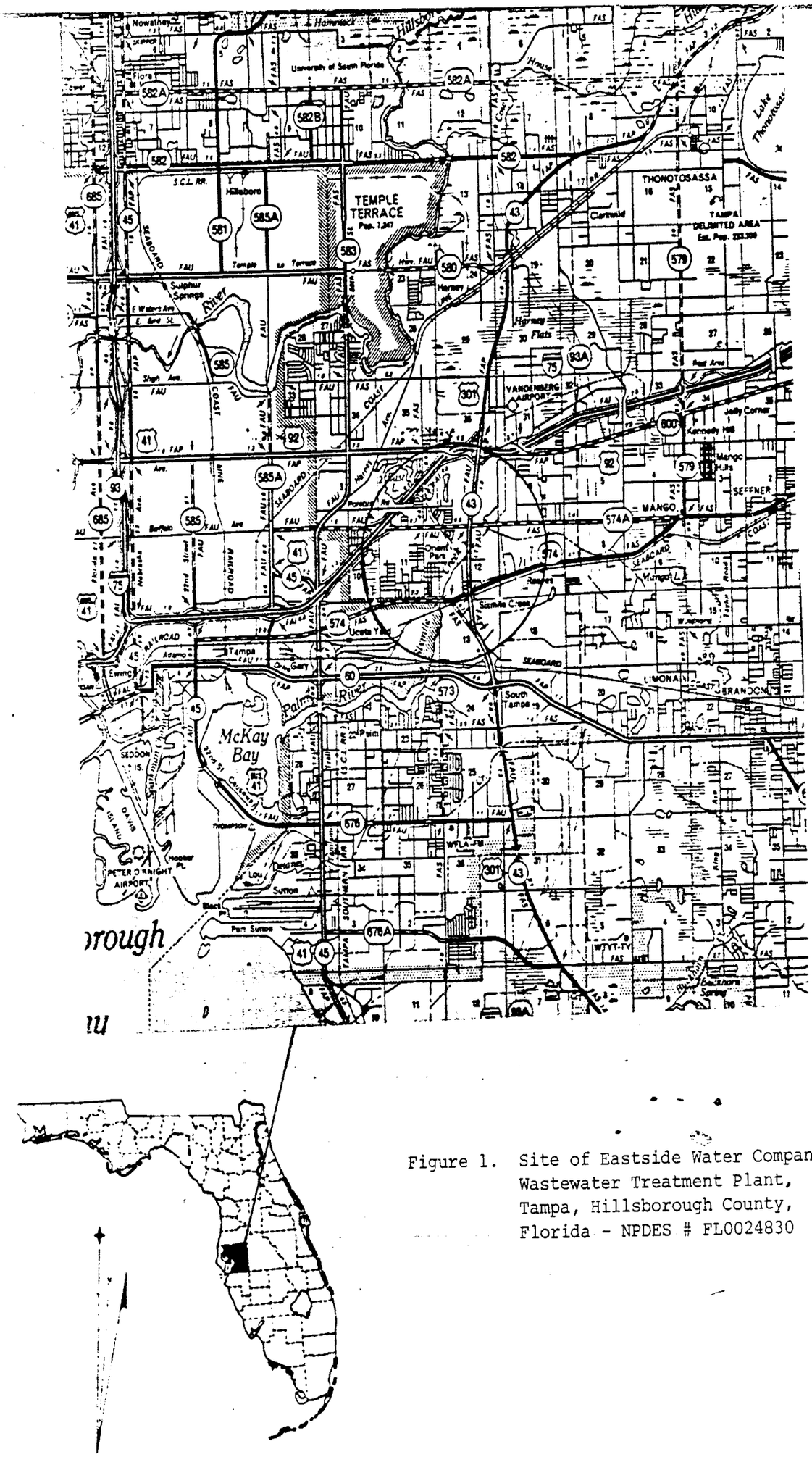


Figure 1. Site of Eastside Water Company Wastewater Treatment Plant, Tampa, Hillsborough County, Florida - NPDES # FL0024830

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organisms selected for this study include species recommended by Peltier (1978) and the U.S. EPA (1978). Additionally, the Biological Section maintains cultures of several species of organisms indigenous to the State of Florida. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of *Daphnia pulex* was obtained from the EPA laboratory in Athens, Georgia in March, 1979. This culture has been maintained since its arrival, in 2½ gallon all glass aquaria filled with unchlorinated DER well water, using a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess daphnids. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Simocephalus serrulatus (water flea) - An indigenous breeding population of *S. serrulatus* was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biological Section. Positive identification of the organism was performed by Mr. Jeff Foran of the Department of Environmental Engineering Sciences, University of Florida in Gainesville. The cultures are maintained in 2½ gallon all glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess population. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances.

Pimephales promelas (fathead minnows) - A breeding culture of approximately 100 adult fathead minnows was obtained from the EPA laboratory in Cincinnati, Ohio in May, 1979. The fish have been maintained in four 125 gallon all glass aquaria equipped with a flow through water exchange system using unchlorinated well water obtained from a deep well near the DER laboratory. The fish have been reared at a temperature of $22^{\circ} \pm 2^{\circ}C$ under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills Trout Chow® and frozen *Artemia* (brine shrimp) at a rate of 0.75 g/30 fish, twice daily. Breeding has been encouraged by placing several halved clay pots in each tank to serve as substrates for eggs and to provide cover for the fish. Tanks are examined twice daily (at feeding time) for egg production. Clay pots containing egg clusters are immediately transferred to 15 gallon aerated aquaria for hatching. A new aquarium is prepared for each week's production of eggs, thereby segregating fish into even age groups to be used for testing. After hatching, fathead minnow fry are fed twice daily with freshly hatched *Artemia*. The fish used in this test were all <3 weeks old at the time of the test.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly Standard Reference Toxicant Bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Five separate 48 hour static bioassays were conducted from 0830 hours, 16 September to 0830 hours, 18 September 1980, on a grab sample of effluent from outfall #001 and on a grab sample from the clarifier before chlorination. The sample was collected by Mr. Eric Klerk of the Hillsborough County Environmental Protection Commission between 0830 and 0930 hours on 15 September 1980, using standard wastewater sampling procedures. The unpreserved samples were iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

Freshwater for dilution of the effluent sample was obtained from the same DER well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1976).

The five separate bioassays were performed on chlorinated (Outfall #001), de-chlorinated (Outfall #001) and un-chlorinated (final clarifier) samples. Dechlorination was achieved by obtaining a subsample of the chlorinated effluent, determining its total chlorine content by the Hach DPD Method and the Iodometric Method I (APHA 1976), and then neutralization of this chlorine with 0.025N sodium thiosulfate. The organisms selected for these bioassays were as follows:

1. Daphnia pulex - un-chlorinated - final clarifier.
2. Simocephalus serrulatus - un-chlorinated - final clarifier
3. Daphnia pulex - chlorinated - Outfall #001
4. Daphnia pulex - dechlorinated - Outfall #001
5. Pimephales promelas - dechlorinated - Outfall #001.

Two replicates, each with six concentrations and a control, were used to test with Daphnia pulex and Simocephalus serrulatus. Flint glass jars of 250 ml capacity were used as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. The Daphnia and Simocephalus were siphoned into large culture dishes through a series of nested stainless steel screens to isolate the proper size and age organisms (neonates). These neonates were then transferred individually by glass pipette and were loaded 10 per chamber, resulting in 20 per test concentration.

Two replicates, of six concentrations and a control, were used for the fathead minnow bioassay. The fish were transferred individually or by twos, by net, into wide mouth quart jars containing 500 ml each of the appropriate test concentration. Fathead minnows were loaded 5 per chamber resulting in 10 per concentration.

Dissolved oxygen, pH, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity, total hardness, and conductivity were recorded at the beginning of the test for the controls and the 100% effluent concentration. These values were recorded on the bioassay data forms and are presented in Tables 2 through 6. Organisms were not fed during these tests. Field parameters of the two effluent samples are presented in Table 7.

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

Water fleas (*Daphnia pulex* and *Semocephalus serrulatus*)

Concentration	Dilution Water	Effluent	Total Vol.
Control	200 ml	---	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180 ml	20 ml	200 ml
18.0%	164 ml	36 ml	200 ml
32.0%	136 ml	64 ml	200 ml
56.0%	88 ml	112 ml	200 ml
100%	----	200 ml	200 ml

Fathead Minnow (*Pimephales promelas*)

Concentration	Dilution Water	Effluent	Total Vol.
Control	500 ml	---	500 ml
5.6%	472 ml	28 ml	500 ml
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100%	---	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of an unchlorinated sample from Eastside Water Company, Wastewater Treatment Plant, Tampa, Hillsborough County, Florida. NPDES # FL0024830 16 to 18 September 1980.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)			Total Hardness (mg/l as $CaCO_3$)			Conductivity (Micros)		
		0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48
Control	A	10	10	8	8.2	8.9	8.5	7.5	7.7	7.7	117			116			202		
Control	B	10	8	8	8.2	8.9	8.4	7.5	7.6	7.7									
5.6%	A	10	9	9	7.8	8.8	8.1	7.5	7.8	7.7									
5.6%	B	10	10	10	7.9	8.8	8.3	7.5	7.8	7.7									
10.0%	A	10	10	10	7.7	8.7	8.2	7.5	7.8	7.7									
10.0%	B	10	10	10	7.7	8.7	8.2	7.5	7.8	7.7									
18.0%	A	10	10	10	7.4	8.5	7.7	7.4	7.8	7.7									
18.0%	B	10	10	9	7.4	8.4	7.8	7.4	7.8	7.7									
32.0%	A	10	9	9	6.5	8.1	7.1	7.3	7.7	7.7									
32.0%	B	10	10	10	6.5	8.0	7.1	7.3	7.7	7.7									
56.0%	A	10	10	7	5.1	7.3	6.5	7.2	7.7	7.6									
56.0%	B	10	10	9	5.0	7.2	6.4	7.2	7.7	7.6									
100%	A	10	9	2	2.4*	6.0	4.3	7.1	7.7	7.6									
100%	B	10	7	0	2.3*	6.1	4.6	7.1	7.7	7.6	175			165			575		

Remarks: 1. Test temperature range 21.5° to 22.0°C.

* chambers aerated to near saturation (8.1 and 8.2 mg/l O_2 respectively) before the organisms were loaded.

Table 3. Data recorded during 48 hour, *Simocephalus serrulatus*, static acute toxicity bioassay of an unchlorinated sample from Eastside Water Company Wastewater Treatment Plant, Tampa, Hillsborough County, Florida. NPDES # FL0024830, 16 to 18 September 1980.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)			Total Hardness (mg/l as $CaCO_3$)			Conductivity (µmhos)		
		0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48
Control	A	10	10	8	8.0	8.7	8.3	7.4	7.8	7.8	117			116			202		
Control	B	10	10	9	8.0	8.8	8.2	7.4	7.8	7.8									
5.6%	A	10	10	10	7.8	8.7	8.2	7.4	7.8	7.8									
5.6%	B	10	10	9	7.8	8.6	8.2	7.4	7.8	7.8									
10.0%	A	10	10	10	7.6	8.6	8.2	7.4	7.8	7.8									
10.0%	B	10	10	10	7.6	8.7	8.1	7.4	7.8	7.8									
18.0%	A	10	9	9	7.3	8.6	7.8	7.3	7.8	7.7									
18.0%	B	10	10	8	7.2	8.5	7.8	7.3	7.8	7.7									
32.0%	A	10	9	3	6.5	8.1	7.2	7.3	7.8	7.7									
32.0%	B	10	10	6	6.5	8.1	7.1	7.3	7.8	7.7									
56.0%	A	10	8	0	5.1	7.2	6.2	7.2	7.7	7.6									
56.0%	B	10	7	0	5.1	7.2	6.1	7.2	7.7	7.6									
100%	A	10	1	0	2.5*	6.3	4.8	7.0	7.7	7.6									
100%	B	10	3	0	2.2*	6.3	4.7	7.0	7.7	7.6	175			165			575		

Remarks: 1. Test temperature range 21.5° to 22.0°C

* chamber aerated to near saturation (7.9 and 8.1 mg/l O_2 respectively) before the organisms were loaded.

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of a chlorinated sample from Eastside Water Company Wastewater Treatment Plant, Tampa, Hillsborough County, Florida. Outfall #001, NPDES #FL0024830, 16 to 18 September 1980.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)			Total Hardness (mg/l as $CaCO_3$)			Conductivity (µmhos)		
		0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48
Control	A	10	10	9	8.2	8.7	8.1	7.5	7.8	7.8	117			116			202		
Control	B	10	10	10	8.2	8.7	8.1	7.5	7.8	7.9									
5.6%	A	10	10	10	8.0	8.7	8.0	7.5	7.9	7.8									
5.6%	B	10	10	10	8.0	8.6	8.0	7.5	7.9	7.8									
10.0%	A	10	10	10	8.0	8.7	7.9	7.5	7.9	7.8									
10.0%	B	10	10	10	8.0	8.7	7.9	7.5	7.9	7.8									
18.0%	A	10	10	10	7.9	8.6	8.0	7.5	7.9	7.8									
18.0%	B	10	10	10	7.9	8.6	7.9	7.5	7.9	7.8									
32.0%	A	11	11	11	7.6	8.6	7.9	7.4	7.9	7.8									
32.0%	B	10	10	10	7.6	8.5	7.9	7.4	7.9	7.8									
56.0%	A	10	10	7	7.1	8.3	7.8	7.3	7.8	7.8									
56.0%	B	10	10	9	7.1	8.3	7.8	7.3	7.8	7.9									
100%	A	10	5	3	6.3	7.0	7.1	7.2	7.8	7.8									
100%	B	10	8	5	6.3	7.0	7.0	7.2	7.7	7.8	175			171			590		

Remarks:
1. Test temperature range 21.5° to 22.0°C.
2. Effluent contains many invertebrates, most of them dead.
3. Detritus observed in effluent.

Table 5 . Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of a dechlorinated sample from Eastside Water Company Wastewater Treatment Plant, Tampa, Hillsborough County, Florida. Outfall #001, NPDES #FL0024830 16 to 18 September 1980.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)			Total Hardness (mg/l as $CaCO_3$)			Conductivity (µmhos)		
		0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48
Control	A	10	10	8*	7.9	8.7	8.0	7.5	7.7	7.8	117			116			202		
Control	B	10	10	10	7.9	8.7	8.3	7.5	7.7	7.9									
5.6%	A	10	10	10	7.9	8.6	8.1	7.5	7.8	7.8									
5.6%	B	10	10	10	7.9	8.6	8.1	7.5	7.8	7.8									
10.0%	A	10	10	10	7.9	8.6	8.0	7.5	7.8	7.8									
10.0%	B	10	10	10	7.9	8.5	8.0	7.5	7.8	7.8									
18.0%	A	10	10	10	7.9	8.4	7.7	7.5	7.8	7.8									
18.0%	B	10	10	10	7.9	8.4	7.8	7.5	7.8	7.8									
32.0%	A	10	10	10	7.7	7.9	7.6	7.5	7.7	7.7									
32.0%	B	10	10	9	7.7	7.9	7.6	7.5	7.7	7.7									
56.0%	A	10	10	8	7.5	7.1	7.5	7.4	7.7	7.7									
56.0%	B	10	9	9	7.4	7.2	7.7	7.4	7.7	7.6									
100%	A	10	7	3**	7.0	6.2	5.1	7.3	7.7	7.6									
100%	B	10	10	3**	7.0	6.2	5.1	7.3	7.7	7.6	175			171			650		

Remarks: 1. Test temperature range: 21.5°C to 22.0°C.
2. Total residual chlorine: 0.25 mg/l, determined by Hach (OPD) and Iodometric Method I (APHA 1976).
3. Dechlorinated with 0.025 N sodium thiosulfate.
* bodies not found
** surviving organisms very stressed

Table 6. Data recorded during 48-hour Pimephales promelas, static acute toxicity bioassay of a dechlorinated sample from Eastside Water Company Wastewater Treatment Plant, Tampa, Hillsborough County, Florida. Outfall #001, NPDES # FL0024830, 16 to 18 September 1980.

Conc. or %	Test Container Number	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)			Total Hardness (mg/l as $CaCO_3$)			Conductivity (µmhos)		
		0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48
Control	A	5	5	5	8.2	5.9	5.8	7.5	7.2	7.1	117			116			202		
Control	B	5	5	5	8.2	6.0	6.3	7.5	7.2	7.2									
5.6%	A	5	5	5	8.1	5.6	6.0	7.5	7.2	7.3									
5.6%	B	4*	4	4	8.1	6.1	6.5	7.5	7.3	7.3									
10.0%	A	5	5	5	8.1	5.5	5.7	7.4	7.3	7.3									
10.0%	B	5	5	5	8.1	5.7	6.0	7.4	7.3	7.4									
18.0%	A	5	4	4	8.0	4.8	5.4	7.4	7.3	7.3									
18.0%	B	5	5	5	8.0	6.0	6.1	7.4	7.4	7.4									
32.0%	A	5	5	5	7.8	5.6	5.6	7.4	7.4	7.4									
32.0%	B	5	5	5	7.8	5.7	5.9	7.4	7.4	7.4									
56.0%	A	4*	4	4	7.5	5.1	5.4	7.3	7.4	7.4									
56.0%	B	5	5	5	7.4	5.1	5.1	7.3	7.4	7.5									
100%	A	5	3	3	7.4	3.5	4.5	7.3	7.4	7.5									
100%	B	5	4	4	7.4	3.7	4.2	7.3	7.5	7.5	175			171			650		

Remarks:
1. Test temperature range: 21.5° to 22.0°C.
2. Total residual chlorine determined from Hach (DPD) and Iodometric Method I (APHA 1976).
3. Dechlorinated with 0.025N sodium thiosulfate.

* not enough fish for full complement

Table 7. Field Parameters of Eastside Water Company's Wastewater Treatment Plant Samples.

Outfall #001*

Temperature:	29°C
Conductivity:	663.9 µmhos
Dissolved Oxygen:	2.8 mg/l
pH:	7.2
Residual Chlorine:	1.0 mg/l total chlorine

Final Clarifier*

Temperature:	29°C
Conductivity:	663.9 µmhos
Dissolved Oxygen:	2.0 mg/l
pH:	7.2
Residual Chlorine:	Not Detectable

* high flows at time of sampling

RESULTS AND DISCUSSION

The results of bioassays performed on unchlorinated effluent (final clarifier) from Eastside Water Company Wastewater Treatment Plant using Daphnia pulex, indicated that toxicity was evident at 24 hours even though an LC_{50} could not be computed (>100%.). However, at 48 hours an LC_{50} of 69 to 71% was calculated (Table 8). A 96 hour LC_{50} would therefore be ≤71%. Bioassays of the same effluent, using the indigenous water flea Simocephalus serrulatus, indicated that the effluent was toxic to this organism at both 24 (LC_{50} 72 to 73%) and 48 hours (LC_{50} 27 to 30%)(Table 8). The 96 hour LC_{50} would therefore be ≤30% for this organism.

Bioassays of chlorinated effluent (Outfall #001) using Daphnia pulex, indicated that, as with the unchlorinated effluent, toxicity was evident at 24 hours (7 dead organisms in the 100% effluent) but an LC_{50} was impossible to compute. However, at 48 hours an LC_{50} of 87% was calculated (Table 8). The 96 hour LC_{50} would therefore ≤ 87%.

Bioassay tests on dechlorinated effluent (Outfall #001) using Daphnia pulex, indicated toxicity at 24 hours, though a statistically sound LC_{50} could not be calculated (>100%). However, at 48 hours an LC_{50} of 81 to 82% was computed (Table 9). The 96 hour LC_{50} would therefore be≤ 82%. Tests on the same effluent using fathead minnows (Pimephales promelas) indicated that the effluent was toxic at both 24 and 48 hours (Table 9), although statistically sound LC_{50s} could not be calculated.

The observed toxicity cannot be attributed to chlorine since more organisms died in the unchlorinated and dechlorinated samples than in the chlorinated sample. However, the exact cause of toxicity could not be ascertained, since no chemical analyses were performed.

Chapter 17-3.051, Florida Administrative Code, indicates that "all waters of the State shall at all places and at all times be free from... components of discharges which...are acutely toxic." "Acute toxicity" is defined in Chapter 17-3.021(1) to mean "the presence of one or more substances...in amounts which are greater than one-third (1/3) of the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50})...". In the absence of a mixing zone, this 1/3 of the 96 hour LC_{50}) applies at the point of discharge. Chapter 17-4.244(4) states that "in no event shall the maximum concentration of wastes in the mixing zone exceed the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50}) for a species significant to the indigenous aquatic community..." This criterion applies at the point of discharge if there is a mixing zone. The distinction between the presence or absence of a mixing zone is not relevant here, however, since the testing procedure used does not involve effluent concentration. The 1/3 of the 96 hour LC_{50} criterion is three times as stringent as the 96 hour LC_{50} criterion, and therefore, in the absence of a mixing zone, the procedure used is very lenient. The procedure followed is also lenient in that testing is only done for 48 hours. The 96 hour LC_{50} derived from this testing is therefore reported as "less than or equal to" the 48 hour measurement. The value found after 48 hours can never be more restrictive than the value found after 96 hours. It must be noted that all organisms 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.

Table 8. LC_{50} Data for Bioassay of Eastside Water Company Wastewater Treatment Plant, Tampa, Hillsborough County, Florida. NPDES # FL0024830. 16 to 18 September 1980.

Organism	Method of Data Analysis	24 hr. LC_{50} and 95% confidence intervals	48 hr. LC_{50} and 95% confidence intervals
Daphnia pulex			
Assay #1	Binomial	A	71% (56 and 100)
Final Clarifier	Moving Average	A	69% (59 and 85)
Unchlorinated sample	Probit	>100%	70% C
Simocephalus serrulatus			
Assay #2	Binomial	73% (56 and 100)	30% (18 and 56)
Final Clarifier	Moving Average	72% (60 and 94)	27% (21 and 34)
Unchlorinated sample	Probit	72% (55 and 112)	27% C
Daphnia pulex			
Assay #3	Binomial	A	87% (56 and ∞) B
Outfall #001	Moving Average	A	87% (69 and 167) B
Chlorinated Sample	Probit	A	87% (73 and 117) B

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. Due to the distributation of mortality, results using the probit method are not statistically sound.

Table 9. LC_{50} Data for Bioassay of Eastside Water Company Wastewater Treatment Plant, Tampa, Hillsborough County, Florida. NPDES #FL0024830 16 to 18 September 1980.

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Daphnia pulex	Binomial	A	82% (56 and ∞)
Assay #4	Moving Average	A	82% (69 and 104)
Outfall #001	Probit	> 100%	81% (68 and 108)
Dechlorinated sample			
Pimephales promelas	Binomial	A	A
Assay # 5	Moving Average	A	A
Outfall #001	Probit	>100%	> 100%
Dechlorinated sample			

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.

LITERATURE CITED

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