

BIOASSAYS OF ST. CLOUD WASTEWATER TREATMENT PLANT

OSCEOLA COUNTY, FLORIDA

NPDES #FL0028959

Biological Section
Bureau of Water Analysis
October 29, 1981

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EXECUTIVE SUMMARY

Bioassays of St. Cloud Wastewater Treatment Plant (1 to 3 September 1981)

On 23 to 25 January, 1980, the Biological Section determined that pre-chlorinated effluent from this facility was toxic to Daphnia pulex (48 hour LC_{50}=82%). At the request of the DER South Florida Subdistrict, additional static acute toxicity bioassays were performed on pre-chlorinated, chlorinated, and de-chlorinated effluent samples. In these bioassays Daphnia pulex and Heterandria formosa were used as the test organisms.

The grab samples from the St. Cloud Wastewater Treatment Plant were again found to be toxic. The chlorinated sample was the most toxic (48 hour LC_{50}'s were <5.6% for D. pulex and 38% for H. formosa). This toxicity was directly attributed to chlorine. The pre-chlorinated sample resulted in a 48 hour LC_{50} of 88.6% to 89.8% for D. pulex. The toxicity may be the result of the unionized ammonia content of the effluent. The de-chlorinated sample was the least toxic (48 hour LC_{50}=149.2% for D. pulex). Again, unionized ammonia was the suspected toxicant.

INTRODUCTION

The St. Cloud Sanitary Wastewater Treatment Plant is located on West Lakeshore Boulevard in St. Cloud, Osceola County, Florida (Fig. 1). This facility has been in operation since 1953 serving a population of approximately 7500. Secondary sewage treatment at this facility includes primary sedimentation, trickling filter, clarification and chlorination for a design capacity of 2.0 MGD. Halfof the treated effluent (1.0 MGD) is piped to an underdrained spray field with a discharge to an adjoining marsh which flows into Lake Tohopekaliga. The remaining effluent is discharged directly to the St. Cloud Flood Control Canal C-31 which also flows into Lake Tohopekaliga (Fig. 2). The plant is currently under a Temporary Operating Permit pending sprayfield modifications which will accommodate the entire discharge as per 201 Plan requirements.

On 23 to 25 January 1980 the Biological Section performed a static bioassay to determine the existence and extent of toxicity of the pre-chlorinated effluent, from the St. Cloud facility, to the biota of the receiving water. The results of this test (FDER 1980) indicated that this sample was toxic to Daphnia pulex (48 hour LC_{50}=82%). To further evaluate the toxicity of this effluent, additional bioassays were performed on 1 to 3 September 1981. The results of these tests are presented here.

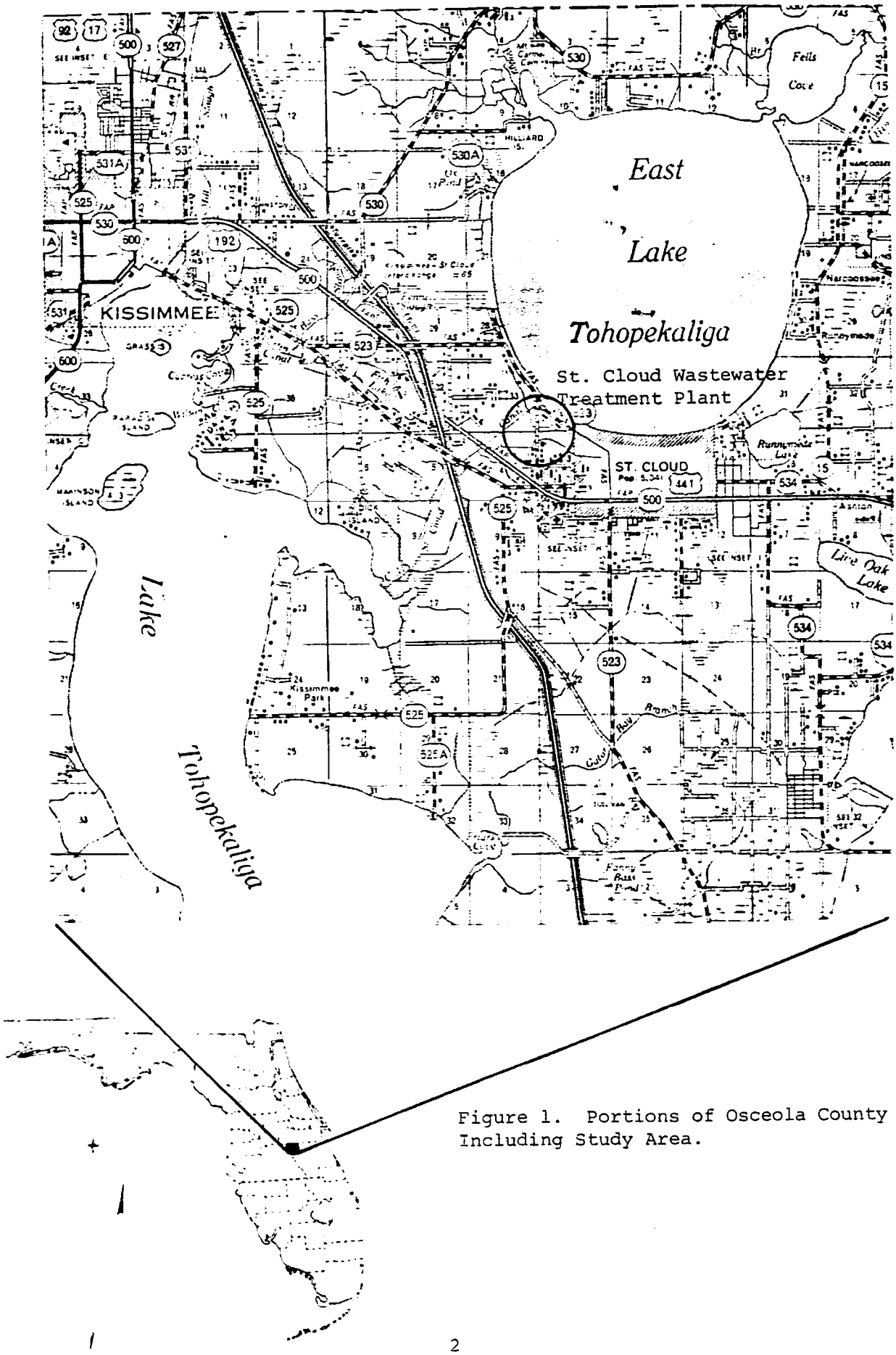


Figure 1. Portions of Osceola County Including Study Area.

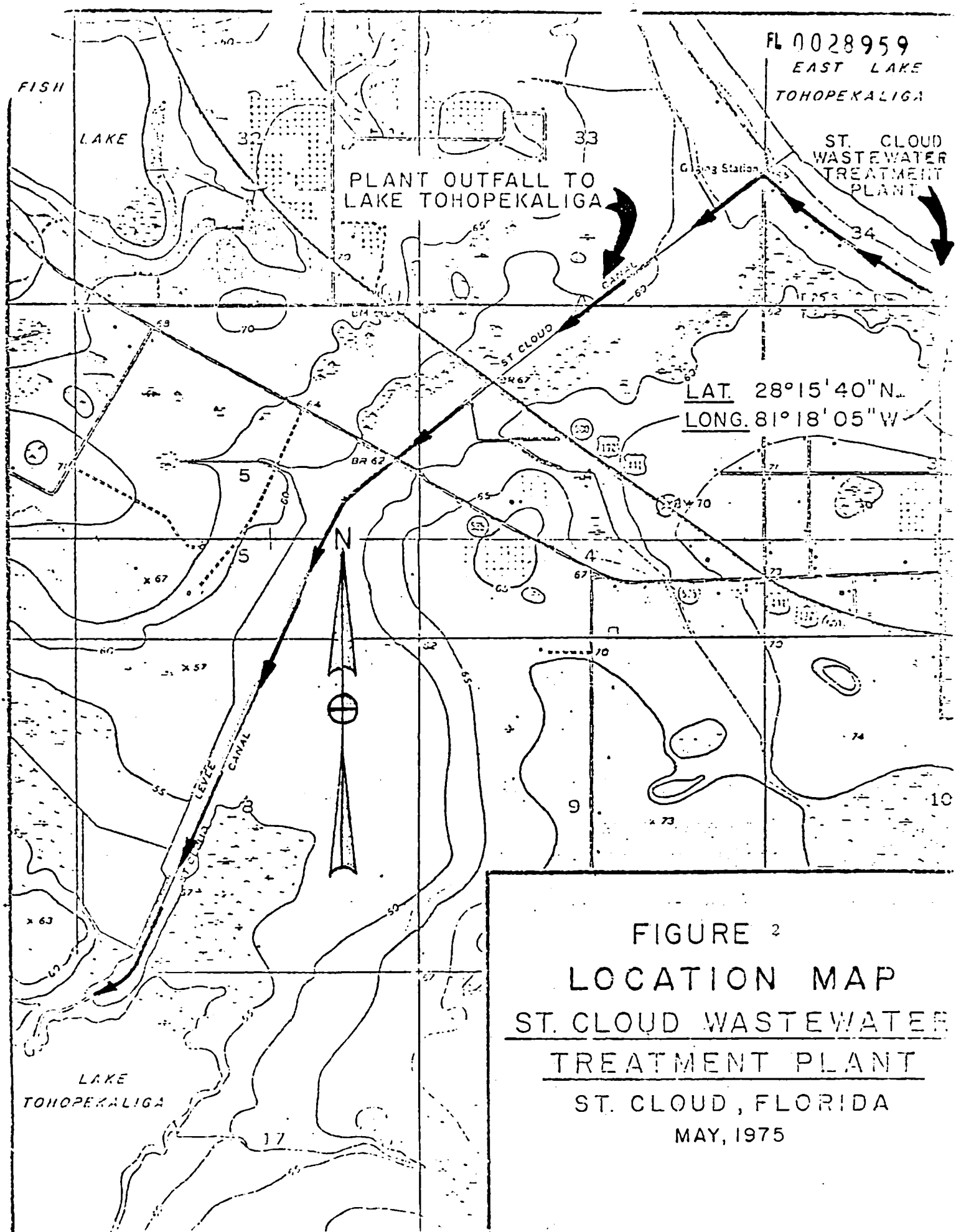


FIGURE 2

LOCATION MAP

ST. CLOUD WASTEWATER TREATMENT PLANT

ST. CLOUD, FLORIDA

MAY, 1975

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° ± 1°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.

Heterandria formosa (least killifish) — Approximately 100 adult killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish have been maintained in 2½ to 5 gallon all glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The fish have been reared at 22° ± 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) twice daily. New born fish are initially maintained in the adult tanks, but are removed at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were 2 to 4 weeks old.

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

Four separate 48 hour static bioassays were conducted from 1015 hours on 1 September, 1981 to 1050 hours on 3 September, 1981. Grab samples of

chlorinated effluent and of effluent collected prior to chlorination were obtained by Mr. Mike Nichols of DER's South Florida Subdistrict Branch Office between 1030 and 1130 hours on 31 August, 1981, using standard wastewater sampling procedures. These unpreserved samples were iced and transported to the DER laboratory in Tallahassee and were tested within 24 hours of their collection.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biological Section adopted the following test procedure:

Test #1. Pre-Chlorinated Effluent (Table 2). This test was performed on an effluent sample collected at the end of the treatment process but prior to alum addition and chlorination, just before the final clarifier. And, it was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent (Table 3). This test was performed on an effluent sample collected at the end of the treatment process, at a manhole approximately 1/8 miles from the NPDES point of discharge.

Test #3. De-Chlorinated Effluent (Table 4). This test was performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized[1]. This test was intended to evaluate the combined toxicity of the effluent and any chlorine reaction products.

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 that recommended by APHA (1976).

Two replicates, each with six concentrations and a control, were used to test with 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 through a stainless steel screen to isolate the proper size and age organisms (neonates). These neonates were then transferred individually by glass pipette, examined for injury and were loaded 10 per chamber, resulting in 20 per test concentration.

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

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[1] De-Chlorination was accomplished by adding a calculated amount of 0.025 N sodium thiosulfate.

Dissolved oxygen, pH, temperature, and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness were recorded at the beginning of the tests for the controls and the 100% effluent concentration. The specific conductance of each test concentration was determined at the beginning and end of the tests. Additionally, ammonia values[2] (including the total expressed in mg/l as N and the unionized fraction expressed in mg/l as NH_3) were determined for the controls and the 100% effluent concentrations at the beginning and end of the bioassays. These values are recorded on the bioassay data forms presented in Tables 2 through 5. The organisms were not fed during these tests.

[2] Total ammonia was determined using an Orion specific ion probe and a Corning Model 10 pH/mv meter. The unionized fraction was calculated based on sample pH and temperature.

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

Daphnia pulex (water fleas)

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

Heterandria formosa (least killifish)

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 the St. Cloud Wastewater Treatment Plant, St. Cloud, Osceola County, Florida, NPDES #FL0028959 on 1 to 3 September, 1981.

Test #1 Pre-Chlorinated Sample

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	10	9.1	8.8	9.0	8.0	8.2	8.3	112		122		21.5	20.1	20.1	.01/.18	.03/.31		
Control	10	10	10	9.1	8.8	9.0	8.0	8.2	8.3										198	210
5.6%	10	10	10	8.9	8.4	8.7	7.9	8.1	8.2					21.5	20.1	20.0				
5.6%	10	10	10	9.0	8.5	8.7	7.9	8.1	8.2										213	220
10%	10	10	10	8.8	8.4	8.6	7.9	8.1	8.1					21.2	20.1	19.9				
10%	10	10	10	8.8	8.4	8.6	7.9	8.1	8.2										220	230
18%	10	10	10	8.6	8.2	8.5	7.8	8.1	8.1					21.2	20.3	20.2				
18%	10	10	10	8.6	8.1	8.5	7.8	8.1	8.1										235	245
32%	10	10	10	8.2	7.9	8.2	7.8	8.0	8.0					21.2	20.3	20.1				
32%	10	10	9	8.2	7.9	8.2	7.8	8.0	8.0										263	270
56%	10	10	9	7.4	7.3	7.5	7.6	7.9	7.9					21.1	20.4	20.2				
56%	10	10	8	7.5	7.3	7.4	7.6	7.9	7.9										309	315
100%	10	10	3	6.3	5.9	6.0	7.4	7.8	7.7	103		112		20.9	20.6	20.4	.10/7.56	.18/7.28		
100%	10	10	5	6.3	5.9	6.0	7.4	7.8	7.7										390	398

Remarks:

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the St. Cloud Wastewater Treatment Plant, St. Cloud, Osceola County, Florida, NPDES #FL0028959 on 1 to 3 September 1981.

Test #2 Chlorinated Sample

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/l)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	10	9.1	8.8	9.0	7.9	8.1	8.2	112		122					.01/.18	.02/.31		
Control	10	10	10	9.1	8.8	9.0	7.9	8.1	8.2					21.5	20.3	20.1			205	222
5.6%	10	0	0	9.1	8.8	--[a]	7.9	7.9	--[a]											
5.6%	10	0	0	9.1	8.8	--	7.9	7.9	--					21.6	20.2	--[a]			219	--[a]
10%	10	0	0	9.0	8.8	--	7.8	8.1	--											
10%	10	0	0	9.0	8.8	--	7.8	8.1	--					21.3	20.2	--			224	--
18%	10	0	0	8.9	8.8	--	7.7	8.1	--											
18%	10	0	0	9.0	8.8	--	7.7	8.1	--					21.3	20.3	--			239	--
32%	10	0	0	8.9	8.8	--	7.6	8.1	--											
32%	10	0	0	8.9	8.8	--	7.6	8.1	--					21.2	20.3	--			264	--
56%	10	0	0	8.7	8.8	--	7.5	8.0	--											
56%	10	0	0	8.7	8.8	--	7.5	8.0	--					19.9	20.4	--			310	--
100%	10	0	0	8.3	8.9	--	7.3	7.9	--	61		113					.08/8.12	.25/6.58		
100%	10	0	0	8.3	8.9	--	7.3	7.9	--					19.9	20.5	--			380	--

Remarks: Total chlorine residual (Hach DPD Method): Control=Not detected,
5.6%=0.10mg/l 100%=1.35mg/l (0.71 mg/l according to the Iodometric Method)
10%=0.15 mg/l
18%=0.24 mg/l
32%=0.40 mg/l
56%=0.60 mg/l

a. Since all the organisms were dead at 24 hours, the 48 hour parameters are not reported.

Table 4 . Data recorded during 48 hour, Heterandria formosa, static acute toxicity bioassay of the St. Cloud Wastewater Treatment Plant, St. Cloud, Osceola County, Florida, NPDES #FL0028959 on 1 to 3 September 1981

Test #2 Chlorinated Sample

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	5	5	5	9.3	7.9	7.7	7.8	8.1	8.0	112		122		21.8	20.4	20.1	.01/.18	.01/.27		
Control	5	5	5	9.3	8.1	7.8	7.8	8.1	8.0										215	220
5.6%	5	5	5	9.3	8.2	8.2	7.8	8.1	8.0					21.7	20.4	20.1				
5.6%	5	5	5	9.3	8.2	8.4	7.8	8.1	8.0										225	222
10%	5	5	5	9.2	8.1	8.3	7.8	8.1	8.0					21.6	20.3	20.1				
10%	5	5	5	9.2	8.0	8.2	7.8	8.1	8.0										232	230
18%	5	5	5	9.2	8.0	8.1	7.7	8.0	8.0					21.5	20.3	20.1				
18%	5	5	5	9.2	8.1	8.1	7.7	8.0	8.0										247	245
32%	5	4	4	9.0	8.2	7.7	7.7	8.0	7.9					21.2	20.4	20.2	---	.09/2.38		
32%	5	4	4	9.0	8.2	7.8	7.7	8.0	7.9										272	270
56%	5	0	-	8.8	8.4	--[a]	7.5	7.9	--[a]					21.0	20.5	--[a]				
56%	5	0	-	8.8	8.4	--	7.5	7.9	--										313	--[a]
100%	5	0	-	8.3	8.6	--	7.3	7.7	--	61		113		20.8	20.6	--	.08/8.12 .17 /7.14[b]			
100%	5	0	-	8.3	8.6	---	7.3	7.7	--										385	--

Remarks:

Total Chlorine Residual (Hach DPD Method):
Control-Not Detected
5.6%=0.10mg/l
10%=0.15mg/l
18%=0.24mg/l
32%=0.40mg/l
56%=0.60mg/l
100%=1.35mg/l (0.71mg/l according to the Iodometric Method)

The fish used in this test weighed an average of 0.017g each.

[a]Since all organisms were dead at 24 hours, the 48 hour parameters are not reported.

[b]Calculations are based on 24 hour pH and temperature.

Table 5. Data recorded during 48 hour, *Daphnia pulex*, static acute toxicity bioassay of the St. Cloud Wastewater Treatment Plant, St. Cloud, Osceola County, Florida, NPDES #FL0028959 on 1 to 3 September 1981.

Test #3. De-Chlorinated Sample[a]

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Total Alkalinity (mg/l as $CaCO_3$)		Total Hardness (mg/l as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/l)		Conductivity (µmhos)	
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0	48
Control	10	10	9	9.1	8.8	8.9	7.9	8.1	8.2	112		122		21.6	20.2	20.1	.01/.18	.02/.31		
Control	10	10	10	9.1	8.8	8.9	7.9	8.1	8.2										206	210
5.6%	10	10	10	9.1	8.7	8.7	7.9	8.1	8.1					21.7	20.3	20.1				
5.6%	10	10	10	9.1	8.7	8.7	7.9	8.1	8.1										220	222
10%	10	10	10	9.1	8.6	8.7	7.8	8.1	8.1					21.6	20.4	20.1				
10%	10	10	10	9.1	8.7	8.6	7.8	8.1	8.1										220	222
18%	10	10	10	9.1	8.6	8.6	7.8	8.1	8.0					21.5	20.4	20.2				
18%	10	10	10	9.1	8.6	8.6	7.8	8.1	8.1										241	240
32%	10	10	10	9.0	8.5	8.5	7.7	8.1	8.0					21.5	20.6	20.3				
32%	10	10	9	9.0	8.5	8.2	7.7	8.1	8.0										268	260
56%	10	10	7	8.8	8.4	8.1	7.6	8.0	7.9					21.3	20.6	20.7				
56%	10	10	7	8.8	8.5	8.1	7.6	8.0	7.9										309	310
100%	10	8	8	8.5	8.4	7.8	7.4	7.8	7.8	61		110		21.0	20.8	20.7	.11/8.12 .21/6.58			
100%	10	9	7	8.5	8.4	7.8	7.4	7.8	7.8										389	395

Remarks: [a] De-Chlorinated with 1.7ml/l of 0.025N/sodium thiosulfate. Final total chlorine residual of 100% effluent=0.04mg/l (HACH DPD).

RESULTS AND DISCUSSION

These static bioassays indicated that the effluent from the St. Cloud Wastewater Treatment Plant was toxic to the test organisms. The lethal concentrations for these 3 test groups are presented in Table 6. The pre-chlorinated effluent sample exhibited a lethality which was similar to the LC_{50} value (82%) determined in the previous bioassay (FDER 1980). Although the unionized ammonia (the chemical species which is most toxic to aquatic organisms) at 48 hours (.18 mg/l as NH_3) is below the level demonstrated lethal to fish (.2mg/l to 2.0mg/l according to USEPA 1976), it may have caused some of the observed mortality in the generally more sensitive aquatic invertebrate, Daphnia pulex.

The chlorinated effluent sample was toxic to both the organisms tested. Since all of the Daphnia, except the controls, died during this test, the LC_{50} must be reported as less than 5.6%. The cause of this toxicity may be directly attributed to the chlorine content of this sample (USEPA 1976). Heterandria formosa are apparently more tolerant to chlorine as reflected by the greater LC_{50} (38%, Table 6).

The de-chlorinated sample was the least toxic of the samples tested (48 hour LC_{50}=149%, Table 6). Again, unionized ammonia may have been responsible for some of the mortality (.21mg/l as NH_3 at 48 hours, Table 5). Apparently, alum flocculation, and the final clarifier may serve to reduce the toxicity of this effluent.

The 96 hour LC_{50} values for these static bioassays would be less than or equal to the 48 hour values reported in Table 6.

Table 6. LC_{50} Data for Bioassay of the St. Cloud Wastewater Treatment Plant on 1 to 3 September 1981.

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Daphnia pulex Test #1 Pre-chlorinated Sample	Binomial	No mortality	88.6% (56 and ∞),B
	Moving Average		88.6% (73 and 140),B
	Probit		89.8% (73 and 133),B
Daphnia pulex Test #2 Chlorinated Sample	None used	<5.6%, D	<5.6%, D
Heterandria formosa Test #2 Chlorinated Sample	Binomial	38.2% (18 and 56)	38.2% (18 and 56)
	Moving Average	A	A
	Probit	A	A
Daphnia pulex Test #3 De-chlorinated Sample	Binomial	A,B	A,B
	Moving Average	A,B	A,B
	Probit	A,B,C	149.2%(93 and 679),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 distribution of mortality, results using the probit method are not statistically sound.

D. All of the organisms died within 24 hours in all of the test concentrations.

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 "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 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.

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