

BIOASSAYS OF ST. CLOUD WASTEWATER TREATMENT PLANT

OSCEOLA COUNTY, FLORIDA

NPDES #FL0028959

Biology Section
Bureau of Water Analysis
April 28, 1983

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EXECUTIVE SUMMARY

St. Cloud WWTP, NPDES #FL0028959, on 23 to 25 March, 1983.

The samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this domestic waste treatment facility were all acutely toxic to Daphnia pulex, producing 48 hour LC_{50} values of 78.0%, <5.6% and 100.0%, respectively. An additional bioassay of the pre-chlorinated effluent, using Heterandria formosa as the test organism, showed no mortality. An additional bioassay of the de-chlorinated effluent, however, using Simocephalus serrulatus as the test organism, resulted in a notably low 48 hour LC_{50} of 19.5%. The toxicity of the chlorinated effluent can be attributed to the total residual chlorine (2.35 mg/l Iodometric Method) measured in that sample. The small concentrations of total ammonia (maximum measured = 1.06 mg/l) and of unionized ammonia (maximum = 0.01 mg/l) detected in these samples were insufficient to have contributed significantly to the observed toxicity. Chemical analyses indicated the presence of a variety of chemical constituents in the pre-chlorinated effluent, including 14 unidentified components, five of which were halogenated, and, the pesticide Lindane (0.04 µg/l). Present in the final chlorinated effluent were 16 unidentified components, six of which were halogenated, and, Lindane (0.02 µg/l). Also present in the final effluent were a very significant concentration of copper (440 µg/l), a significant concentration of silver (1.9 µg/l) and some zinc (39 µg/l). The toxicities noted in these effluents, therefore, were probably due to the chemical constituents, specifically the organic components and the copper, and, to total residual chlorine in the chlorinated sample.

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 9000. Secondary sewage treatment at this facility includes primary sedimentation, trickling filter, clarification and chlorination for a design capacity of 2.0 MGD. The effluent is discharged directly to the St. Cloud Flood Control Canal C-31 which flows into Lake Tohopekaliga (Fig. 2).

The Biology Section has previously performed acute toxicity bioassays on effluent from the St. Cloud WWTP, in January, 1980, and again in September, 1981. The results of the 1980 bioassay indicated that pre-chlorinated effluent was acutely toxic to Daphnia pulex, producing a 48 hour LC_{50} of 81.6% (FDER 1980). The results of the 1981 bioassays indicated, again, that pre-chlorinated effluent was acutely toxic to Daphnia pulex (48 hour LC_{50} = 89.8%), and that chlorinated effluent was acutely toxic to both Daphnia pulex and to Heterandria formosa (48 hour LC_{50}'s = <5.6% and 38.2%, respectively) and de-chlorinated effluent was acutely toxic to Daphnia pulex, producing a 48 hour value of 149.2% (FDER 1981).

Since this facility has recently come under the jurisdiction of the St. Johns River District, the Biology Section was requested to again evaluate the toxicity of the discharge. The Biology Section performed five acute toxicity bioassays, on 23 to 25 March, 1983, the results of which are presented in this report.

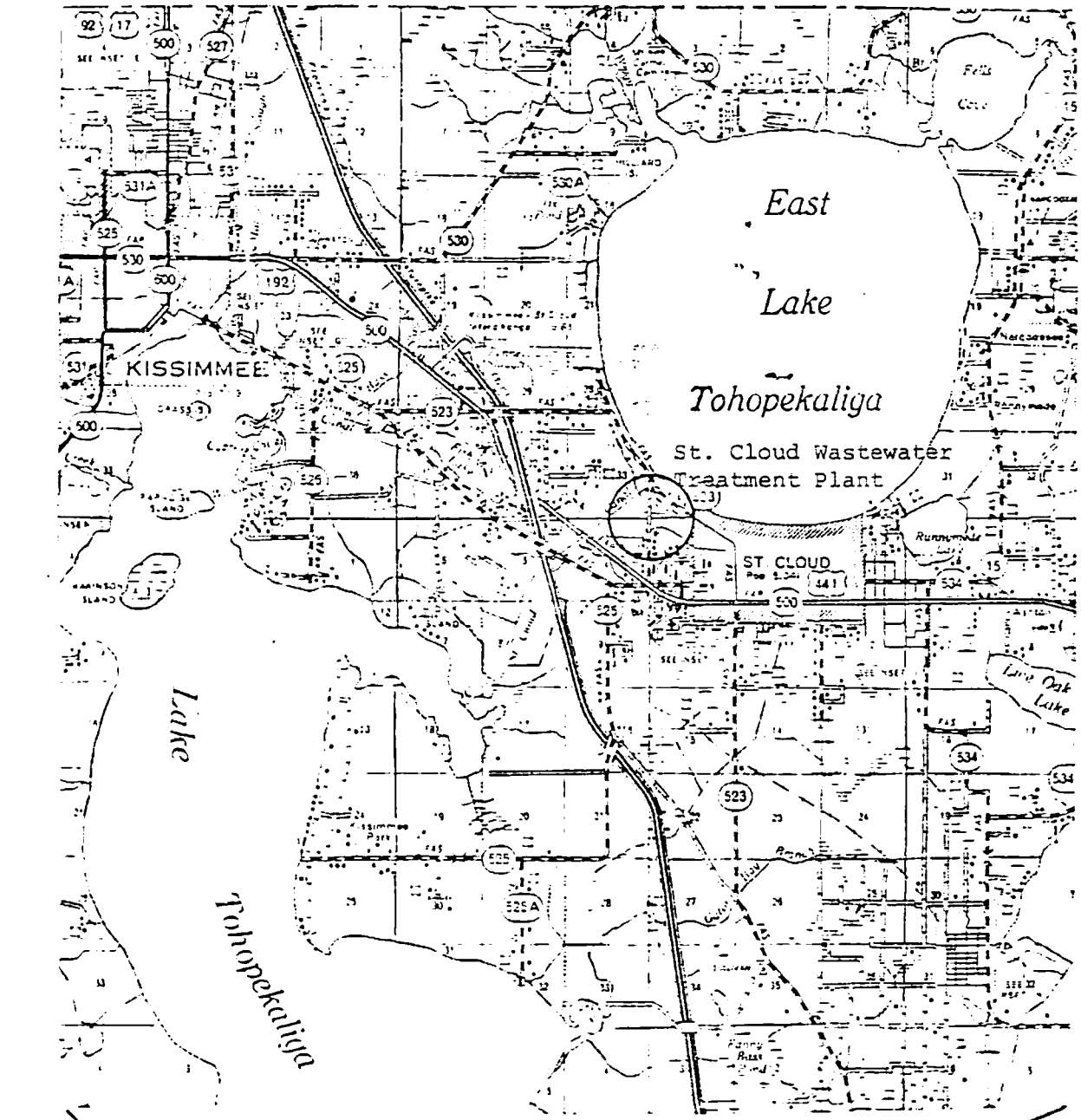


Figure 1. Portions of Osceola County Including Study Area.

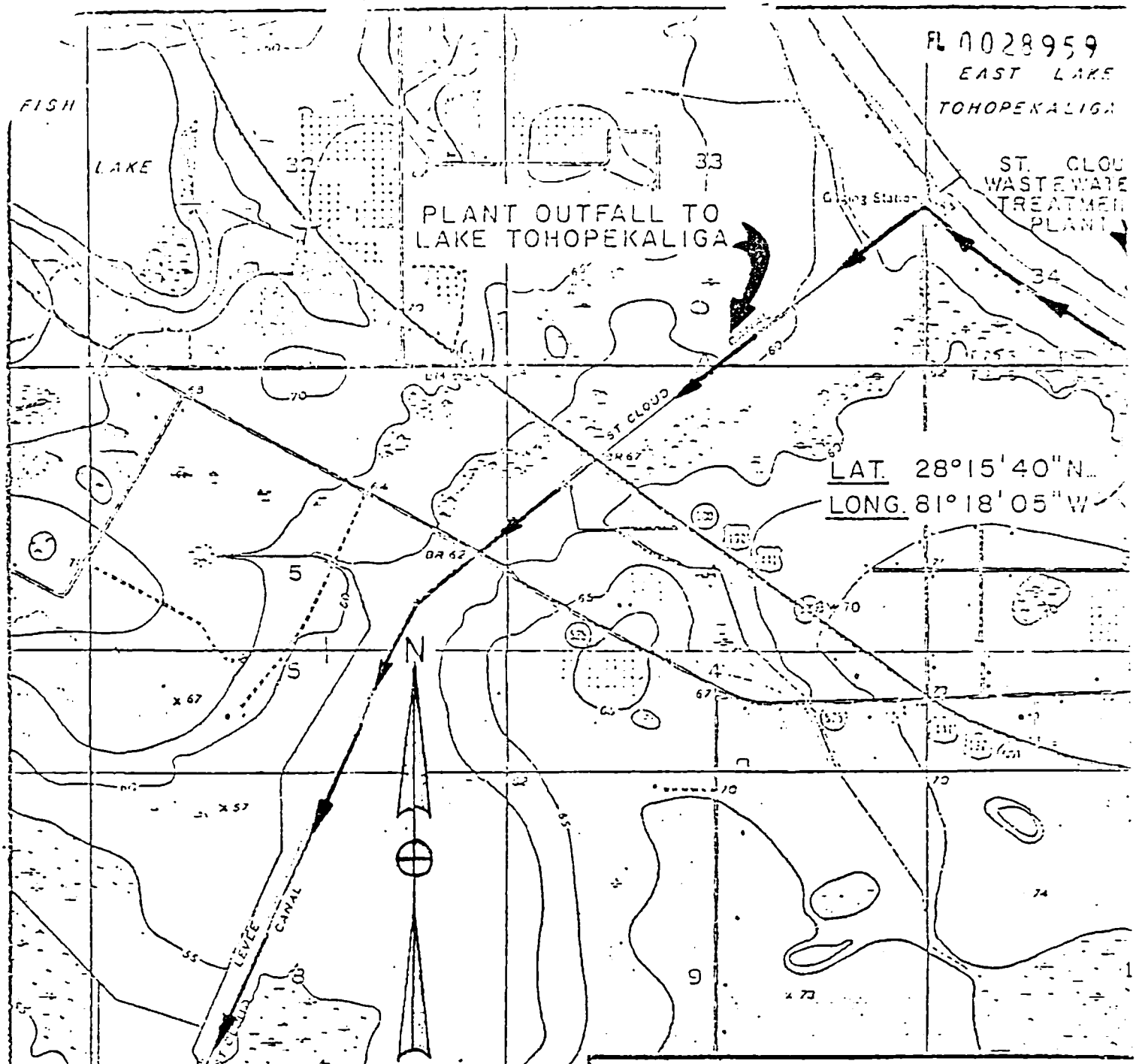


FIGURE 2

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 a species recommended by Peltier (1978) and the U.S. EPA (1978). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. Two of these indigenous species were 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, and are kept at a constant temperature of 22° plus or minus 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. 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 Simocephalus serrulatus was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biology Section. Positive identification of the organisms was made by Mr. Jeff Foran of the University of Florida Department of Environmental Engineering Sciences. The cultures are maintained in two and one-half 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° plus or minus 1°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. 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 least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 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 least killifish are reared at 22° plus or minus 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 3 to 16 days old.

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

Five separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from the St. Cloud WWTP. The tests were performed from 1115 hours on 23 March to 1200 hours on 25 March, 1983. The samples were collected by Mr. Guy Hadley of DER's St. Johns River District at 1500 hours on 22 March, 1983, 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.

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

Test #1. Pre-chlorinated Effluent (Tables 2 and 3). This test was performed on an effluent sample collected at the end of the treatment process but prior to chlorination, and was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent (Table 4). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-chlorinated Effluent (Tables 5 and 6). This test was performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized. De-chlorination was accomplished by adding a calculated amount of 0.025 N sodium thiosulfate. This test was intended to evaluate the combined toxicity of the effluent and the chlorine reaction products.

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 (Table 1) is that recommended by APHA (1980).

Two replicates, each with six concentrations and a control, were used to test with the water fleas, 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. Daphnia and Simocephalus were siphoned into large culture dishes 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 six concentrations and a control, were used for the Heterandria formosa (least killifish) 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 this test.

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 test for the controls and the 100% effluent concentrations. The specific conductance of each test concentration was recorded at the beginning and end of the test. Ammonia concentrations, including total and the unionized fraction (expressed in mg/l as $NH_3 + NH_4$ 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 a Corning pH/millivolt meter. The unionized fraction was then determined 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 6.

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

Daphnia pulex and Simocephalus serrulatus (water fleas)

Concentration	Dilution Water	Effluent	Total Volume
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
100.0%	--------	200 ml	200 ml

Heterandria formosa (least killifish)

Concentration	Dilution Water	Effluent	Total Volume
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
100.0%	--	500 ml	500 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the St. Cloud WWTP, Osceola County, Florida, NPDES #FL0028959, on 23 to 25 March, 1983.

Test #1 Pre-chlorinated Effluent[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	10	9.0	9.2	8.8	7.8	8.2	8.3	103		116		20.0	19.7	19.6	0/.084	--		
Control	10	10	10	9.0	9.2	8.8	7.8	8.2	8.3										190	207
5.6%	10	10	10	8.8	8.9	8.8	7.8	8.2	8.2					20.0	19.6	19.5				
5.6%	10	10	10	8.8	8.9	8.8	7.8	8.2	8.2										210	220
10.0%	10	10	10	8.8	8.7	8.7	7.7	8.1	8.2					19.9	19.5	19.4				
10.0%	10	10	10	8.8	8.8	8.7	7.7	8.1	8.2										225	235
18.0%	10	10	10	8.5	8.6	8.7	7.6	8.1	8.2					19.8	19.5	19.4				
18.0%	10	10	10	8.5	8.6	8.6	7.6	8.1	8.1										250	259
32.0%	10	10	10	7.6	8.2	8.4	7.4	8.0	8.0					19.8	19.5	19.4				
32.0%	10	10	10	7.6	8.2	8.4	7.4	8.0	8.1										290	298
56.0%	10	10	8(10)[b]	6.5	7.5	7.8	7.2	7.8	7.9					19.4	19.5	19.5				
56.0%	10(9)[c]	9	5	6.5	7.5	7.8	7.2	7.8	8.0										355	370
100.0%	10	9	2	4.7	6.0	7.0	7.0	7.6	7.8	108		112		19.2	19.6	19.6	0/1.06	--		
100.0%	10(9)[c]	9	3	4.7	6.0	7.0	7.0	7.6	7.8										480	500

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

[b] Number in parentheses used in calculations since two organisms were not found in the container at 48 hours.

[c] Numbers in parentheses used in calculations since one organism was not found in each container at 24 hours.

Table 3 . Data recorded during 48 hour, Heterandria formosa[a], static acute toxicity bioassay of the St. Cloud WWTP, Osceola County, Florida, NPDES #FL0028959, on 23 to 25 March, 1983.

Test #1 Pre-chlorinated Effluent[b]

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	9.0	8.1	7.8	7.8	8.0	8.1	103		116		20.3	19.3	19.2	0/.084	--		
Control	5	5	5	9.0	8.1	7.8	7.8	8.1	8.1										200	212
5.6%	5	5	5	8.7	8.0	7.9	7.8	8.0	8.1					20.3	19.0	19.1				
5.6%	5	5	5	8.7	8.1	7.9	7.8	8.0	8.1										220	225
10.0%	5	5	5	8.7	8.1	8.0	7.7	7.9	8.1					20.1	18.9	19.0				
10.0%	5	5	5	8.6	8.1	8.0	7.7	7.9	8.1										235	239
18.0%	5	5	5	7.9	8.0	7.9	7.6	7.9	8.0					20.0	18.9	19.0				
18.0%	5	5	5	7.9	8.0	7.9	7.6	7.9	8.0										260	262
32.0%	5	5	5	7.5	7.5	7.5	7.4	7.7	7.9					19.8	18.9	19.0				
32.0%	5	5	5	7.5	7.5	7.5	7.4	7.7	7.9										300	302
56.0%	5	5	5	5.9	6.7	6.7	7.2	7.6	7.8					19.5	18.9	19.0				
56.0%	5	5	5	6.0	6.7	6.7	7.2	7.6	7.8										365	372
100.0%	5	5	5	3.6	5.6	5.8	7.0	7.5	7.6	108		112		19.2	19.0	19.1	0/1.06	--		
100.0%	5	5	5	3.7	5.6	5.8	7.0	7.4	7.6										490	580

Remarks:

[a] Weight of fish = 8.5 mg each, average of 10 fish.

[b] Total Residual Chlorine = None Detected (HACH DPD Method).

Table 4. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the St. Cloud WWTP, Osceola County, Florida, NPDES #FL0028959, on 23 to 25 March, 1983.

Test #2 Chlorinated Effluent[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	10	8.9	9.0	8.2	7.9	8.1	8.3	103		116		20.0	19.4	19.6	0/.084	--		
Control	10	10	10	8.9	9.0	8.3	7.9	8.1	8.3										200	207
5.6%	10	0	0	8.9	9.1	--[c]	7.8	8.2	--[c]					20.1	19.4	--[c]				
5.6%	10	0	0	8.9	9.1	--	7.8	8.2	--										225	--[c]
10.0%	10	0	0	8.8	9.2	--	7.7	8.2	--					20.1	19.3	--				
10.0%	10	0	0	8.8	9.2	--	7.7	8.2	--										238	--
18.0%	10	0	0	8.8	9.2	--	7.6	8.2	--					20.1	19.3	--				
18.0%	10	0	0	8.8	9.2	--	7.6	8.2	--										260	--
32.0%	10	0	0	8.7	9.2	--	7.4	8.1	--					20.1	19.4	--				
32.0%	11[b]	0	0	8.7	9.2	--	7.4	8.1	--										300	--
56.0%	10	0	0	8.6	9.2	--	7.2	8.0	--					20.1	19.5	--				
56.0%	10	0	0	8.6	9.2	--	7.2	8.0	--										375	--
100.0%	10	0	0	8.5	9.2	--	7.0	7.8	--	113		116		20.1	19.9	--	0/1.06	--		
100.0%	10	0	0	8.5	9.2	--	7.0	7.8	--										500	--

Remarks: [a] Total Residual Chlorine = 1.9 mg/l (HACH DPD Method), 2.35 mg/l (Iodometric Method).

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

[c] Remaining parameters not recorded since all organisms were dead by hour 24.

Table 5. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of the St. Cloud WWTP, Osceola County, Florida, NPDES #FL0028959, on 23 to 25 March, 1983.

Test #3 De-chlorinated Effluent[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_4OH (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	9.0	9.1	8.5	7.9	8.3	8.4	103		116		19.8	19.5	19.3	0/.084	--		
Control	10	10	10	9.0	9.1	8.5	7.9	8.3	8.3										205	203
5.6%	10	10	10	9.0	9.1	8.5	7.8	8.2	8.2					19.8	19.4	19.2				
5.6%	10	10	9	9.0	9.1	8.5	7.8	8.2	8.2										225	215
10.0%	10	10	10	9.0	9.1	8.5	7.8	8.2	8.1					19.9	19.3	19.2				
10.0%	10	10	10	9.0	9.1	8.5	7.8	8.2	8.2										240	229
18.0%	10	10	10	9.0	9.1	8.5	7.7	8.1	8.1					19.8	19.2	19.2				
18.0%	10	10	10	9.0	9.1	8.5	7.7	8.1	8.1										265	254
32.0%	10	10	10	9.0	8.7	8.2	7.5	8.1	8.1					19.8	19.2	19.2				
32.0%	10	10	10	9.0	8.7	8.2	7.5	8.1	8.1										310	298
56.0%	10	10	10	9.0	8.7	8.0	7.3	8.0	8.0					19.7	19.3	19.2				
56.0%	10	10	10	8.9	8.6	8.0	7.3	8.0	8.0										385	372
100.0%	10	10	5	8.8	8.0	7.5	7.1	7.8	7.9	112		119		19.8	19.8	19.5	.01/.98	--		
100.0%	10	10	5	8.8	8.0	7.3	7.1	7.8	7.9										600	590

Remarks: [a] Total Residual Chlorine = <0.02 mg/l (HACH DPD Method).

Table 6. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of the St. Cloud WWTP, Osceola County, Florida, NPDES #FL0028959, on 23 to 25 March, 1983.

Test #3 De-chlorinated Effluent[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	10	9.0	9.2	8.5	7.9	8.3	8.3	103		116		19.8	19.7	19.3	0/.084	--		
Control	10	9	8	9.0	9.2	8.5	7.9	8.3	8.3										200	205
5.6%	10	9	7	9.0	9.2	8.5	7.8	8.2	8.2					19.8	19.6	19.3				
5.6%	10	9	8	9.0	9.2	8.5	7.8	8.2	8.2										225	217
10.0%	10	9	5	9.0	9.2	8.3	7.8	8.2	8.2					19.8	19.6	19.4				
10.0%	10	10	6	9.0	9.2	8.2	7.8	8.2	8.2										240	233
18.0%	10	10	7	9.0	8.9	8.2	7.7	8.1	8.1					19.8	19.7	19.5				
18.0%	10	10	7	9.0	8.9	8.2	7.7	8.1	8.1										265	260
32.0%	10	8	5	9.0	8.2	8.0	7.5	8.0	8.1					19.8	19.8	19.8				
32.0%	10	7	4	9.0	8.3	8.0	7.5	8.0	8.0										310	300
56.0%	10	9	1	9.0	8.0	7.5	7.3	7.9	7.9					19.8	19.8	19.9				
56.0%	10	10	1	9.0	8.0	7.5	7.3	7.9	8.0										385	379
100.0%	10	10	0	8.8	7.4	7.1	7.1	7.8	7.8	112		119		20.1	19.9	20.0	.01/.98	--		
100.0%	10(9)[b]	4	0	8.8	7.3	7.1	7.1	7.8	7.8										600	590

Remarks: [a] Total Residual Chlorine = <0.02 mg/l (HACH DPD Method).

[b] Number in parentheses used in calculations since one organism was not found in the container at 24 hours.

Table 7 . LC_{50} Data for Bioassays of the St. Cloud WWTP, NPDES #FL0028959, on 23 to 25 March, 1983.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: D. pulex	Binomial	a	77.3% (56 and ∞).
Pre-chlorinated	Moving Average	a	77.3% (63 and 98)
Sample	Probit	a	78.0% (66 and 96), d
Test #1: H. formosa	Binomial	No Mortality	No Mortality
Pre-chlorinated	Moving Average		
Sample	Probit		
Test #2: D. pulex		<5.6%, e	<5.6%, e
Chlorinated			
Sample			
Test #3: D. pulex	Binomial	No Mortality	100.0% (56 and ∞), b
De-chlorinated	Moving Average		a
Sample	Probit		185.9% (0 and ∞), c
Test #3: S. serrul.	Binomial	a	28.6% (5.6 and 56)
De-chlorinated	Moving Average	a	19.5% (14 and 29), d
Sample	Probit	2359% (195 and ∞), b	17.1% (4.0 and 41), c

[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 calculated using the probit method are not statistically sound.

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

[e] Since every organism was dead in the lowest test concentration (5.6%), the LC_{50} is <5.6%.

Table 8. Results of Chemical Analyses of Effluents from the St. Cloud WWTP, NPDES #FL0028959, collected on 22 March, 1983.

Organics

	Pre-chlorinated Effluent	Chlorinated Effluent
Acid Extractables:	Two unidentified components	Three unidentified components
Base Neutral Extractables:	Seven unidentified components	Seven unidentified components
Pesticide Extractables:	g-BHC (Lindane) 0.04 µg/l Five unidentified halogenated components	g-BHC (Lindane) 0.02 µg/l Six unidentified halogenated components

Metals

Antimony	N.D.
Arsenic	< 2.0 µg/l[a]
Beryllium	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	440 µg/l
Lead	--[b]
Mercury	< 0.2 µg/l[a]
Nickel	N.D.
Selenium	N.D.
Silver	1.9 µg/l
Thallium	N.D.
Zinc	39 µg/l

N.D. = None Detected

[a] Actual value is known to be less than value given.

[b] Lead results were invalidated.

RESULTS AND DISCUSSION

The samples of pre-chlorinated, chlorinated and de-chlorinated effluents from the St. Cloud WWTP proved to be acutely toxic to Daphnia pulex, producing 48 hour LC_{50} values of 78.0%, <5.6% and 100.0%, respectively (Table 7). An additional bioassay of the pre-chlorinated effluent, using Heterandria formosa as the test organism, produced no mortality. An additional bioassay of the de-chlorinated effluent, however, using Simocephalus serrulatus as the test organism, resulted in a notably low 48 hour LC_{50} of 19.5% (Table 7). The 96 hour LC_{50}'s for these effluents would be less than or equal to their 48 hour values. Additionally, the toxicities noted in these bioassays were very similar to those noted in earlier DER bioassays of various effluents from the St. Cloud facility (FDER 1980 and 1981).

The toxicity of the chlorinated effluent can mostly be attributed to the total chlorine residual (1.9 mg/l HACH DPD Method and 2.35 mg/l Iodometric Method, Table 4) detected in that sample. Additionally, small concentrations of total ammonia (maximum measured = 1.06 mg/l) and of unionized ammonia (maximum = 0.01 mg/l) were detected in these effluents, but the amounts measured could not have contributed significantly to the observed toxicity. The toxicities of the pre-chlorinated and de-chlorinated effluents must, therefore, be attributed to other components besides ammonia or total residual chlorine.

The DER Chemistry Section has analyzed samples of pre-chlorinated and chlorinated effluents from this facility for certain organic compounds, and chlorinated effluent for certain metals (Table 8). These analyses indicated the presence of at least fourteen unidentified components in the pre-chlorinated effluent, including two acid extractables, seven base neutral extractables and five halogenated pesticide extractables, and, the pesticide Lindane (0.04 µg/l). The final chlorinated effluent contained a greater number of unidentified components, including three acid extractables, seven base neutral extractables and six halogenated pesticide extractables, and, Lindane (0.02 µg/l). Also present in the final effluent from the St. Cloud facility were a very significant concentration of copper (440 µg/l), a significant amount of silver (1.9 µg/l) and some zinc (39 µg/l).

The toxicities of the St. Cloud WWTP effluents can be attributed to the presence of the wide variety of chemical constituents, with emphasis on the halogenated compounds and the copper and silver. Many halogenated compounds are known to be extremely toxic to aquatic organisms. Concentrations of copper, less than that detected in the St. Cloud effluent (440 µg/l), have been shown to be acutely toxic to certain sensitive freshwater fish (U.S. EPA 1976). A silver concentration as low as 3.2 µg/l was acutely toxic to Daphnia magna (Anderson 1948, in U.S. EPA 1976).

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 17-3-3.021(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 one-twentieth 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 existance of a mixing zone, whether as a specific part of the permit or based on the existance 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} standard. 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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