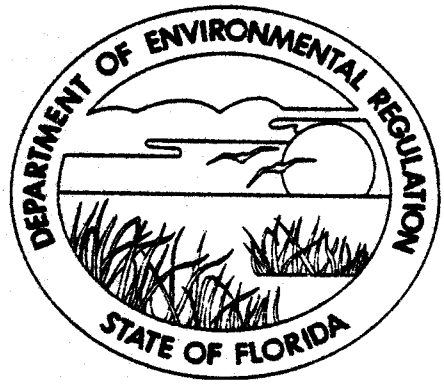


BIOASSAYS OF BUCKMAN STREET SEWAGE TREATMENT PLANT

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0026000

SAMPLED 8/24/81

Biological Section
Bureau of Water Analysis
September 1, 1981

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EXECUTIVE SUMMARY

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To further evaluate the existence and extent of toxicity of effluent samples to the biota of the receiving waters, the Biological Section performed static acute toxicity bioassays on grab samples of chlorinated and de-chlorinated effluent, as well as on a grab sample collected prior to chlorination. These samples were collected from the Buckman Street facility on 24 August, 1981. The bioassays were then performed on 25 to 27 August, 1981, within 24 hours of sample collection. Mysidopsis bahia (mysid shrimp) were used as test organisms.

The pre-chlorinated effluent sample was toxic to mysid shrimp having a 96 hour $LC_{50} \leq 64.4\%$. The chlorinated effluent sample was slightly more toxic with a 96 hour $LC_{50} \leq 56.0\%$. The de-chlorinated effluent sample was also toxic to mysid shrimp in the 100% test concentration. However, the LC_{50} was calculated to be >100%. The greater toxicity (lower LC_{50}) observed in the chlorinated sample may be attributed to its chlorine content (0.55 mg/l). The toxic component(s) in the pre-chlorinated and de-chlorinated samples were not identified.

INTRODUCTION

The Buckman Sewage Treatment Plant is located at 2221 Buckman St., in the center of Jacksonville, Duval County, Florida (Fig. 1). This 35 MGD capacity activated sludge treatment plant serves a population of approximately 200,000, and provides secondary treatment for wastes from a variety of major and minor industrial sources. Treatment consists of primary sedimentation, sludge aeration, secondary clarification and chlorination. Discharge is to the St. John's River, 500 feet from shore. Recent NPDES Compliance Inspection Reports have indicated that this facility has been discharging in excess of its design capacity, and also that, at times, the discharge contains only partially treated wastes.

In October, 1979, the Biological Section performed a static acute toxicity bioassay to determine the toxicity of effluent from this facility. Results indicated a 48 hour LC_{50} of 50%, based on the test organism, Mysidopsis bahia (FDER, 1979). Because of the complex nature of the effluent, toxicity could not be attributed to a single component.

To further evaluate the toxic effects of this discharge to the biota of the receiving waters, the Biological Section performed additional static bioassays on grab samples of effluent, on 25 to 27 August, 1981. The results of these tests are presented in this report.

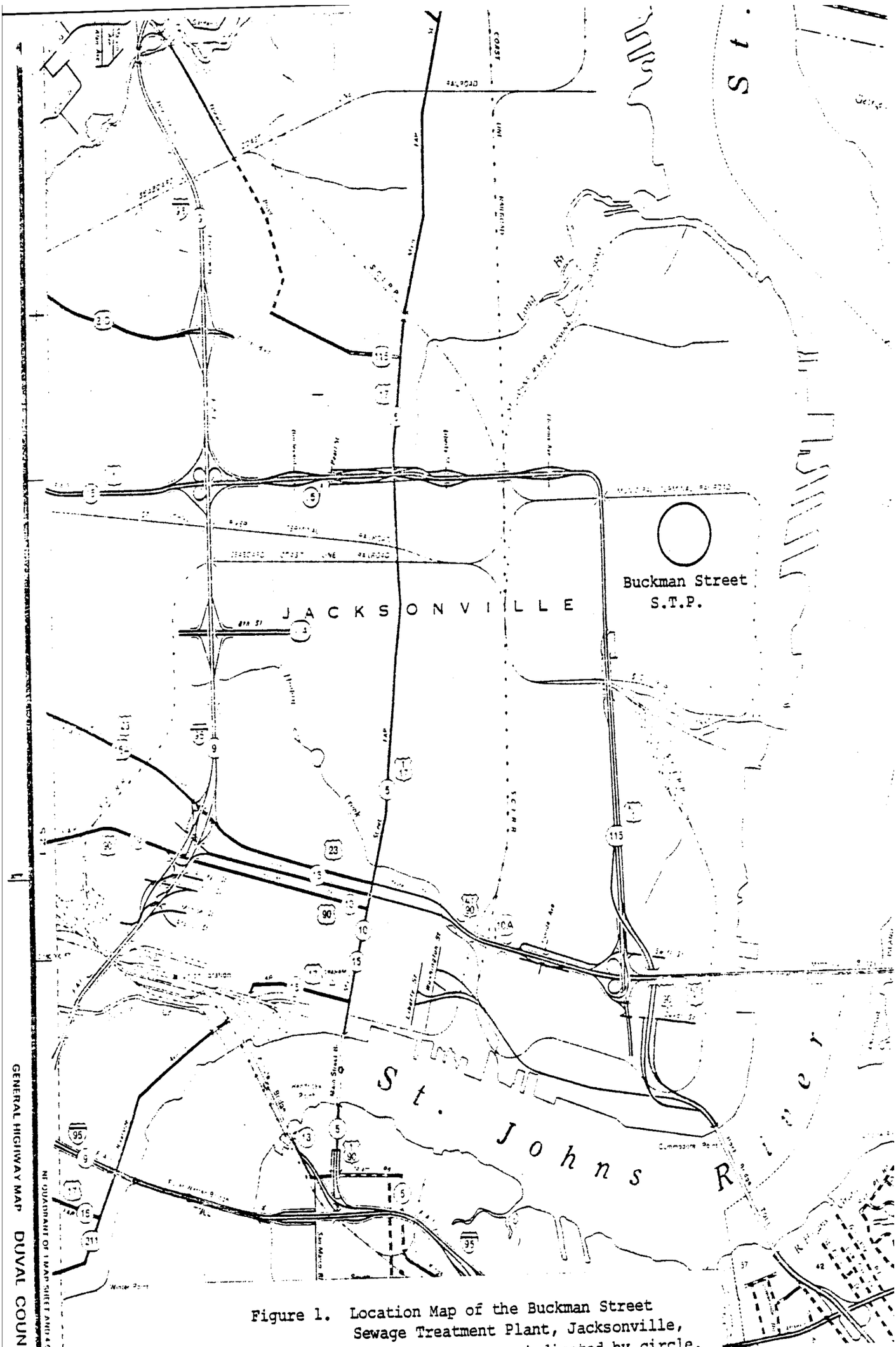


Figure 1. Location Map of the Buckman Street Sewage Treatment Plant, Jacksonville, Florida. Facility indicated by circle.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organism selected for this study is among those recommended by Peltier (1978) and the U.S. EPA (1978). Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the Gulf Breeze EPA Laboratory in Pensacola, Florida. The mysids have been cultured under continuous lighting in 10 and 15 gallon all glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 ppt) and partial water changes are performed weekly. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of $22^{o} \pm 1^{o}C$. Immediately prior to the bioassay, a suitable number of mysids were transferred by net to a culture dish for observation and selection of individuals for the test. Fifteen day old (three-quarter adult size) mysids were used in this test. Organisms selected for testing were transferred individually by glass tube and randomly placed in the test chambers.

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

Three separate 48 hour static bioassays were conducted from 1045 hours on 25 August, 1981, to 1045 hours on 27 August, 1981. Grab samples of chlorinated effluent (Outfall #001) and of effluent collected prior to chlorination were obtained by Mr. John Shailer and Mr. Charles Love of DER's St. Johns River Subdistrict at 1100 hours on 24 August, 1981, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metals analysis were preserved with nitric acid. The samples for organic chemical analysis and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee 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 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 chlorination. 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 NPDES permit sampling location.

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 the chlorine reaction products.

Saltwater for dilution of the effluent samples was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 30.0 ppt). This saltwater diluent was then adjusted to the salinity of the receiving body of water (St. Johns River = 20.0 ppt) by adding an appropriate amount of DER well water. For test concentrations of 56% and below, this adjusted diluent was mixed directly with the effluent, achieving a range of final salinities (9.2 ppt to 19.2 ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts (Ocean 50®) were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerances). A series of three control groups (Table 5) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included: adjusted Turkey Point seawater (salinity = 21.0 ppt), artificial sea salt (Ocean 50®) adjusted well water (salinity = 7.5 ppt), and a salinity control consisting of Turkey Point seawater and well water (salinity = 9.8 ppt).

Two replicates of each test concentration and the controls were used in these static tests. Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt, and 20 ppt salinity aquaria to culture dishes for observation and selection of individuals for the tests. Individual mysid shrimp were siphoned via glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration the mysid shrimp were pre-acclimated to within 3 ppt salinity. The concentrations series selected for this study (Table 1) are those recommended by the APHA (1976).

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

Five organisms were loaded per test chamber, giving ten per concentration. Temperature, pH, dissolved oxygen, and the number of live organisms were recorded at 0, 24, and 48 hours for each test chamber (Tables 2 through 5). Additionally, ammonia concentrations, including total (expressed in mg/l as N) and unionized (expressed in mg/l as NH_3) fractions, were determined in the 100% test concentrations at the beginning and end of the bioassays and in the controls at the end of the bioassays.[1] According to EPA (1978), "Although it is generally undesirable to feed most fish and macroinvertebrates during static toxicity tests, an exception is necessary for M. bahia". Therefore, the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 per mysid shrimp to minimize cannibalism.

[1] Total ammonia measurements were made with an Orion specific ion probe and a Corning pH/millivolt meter. The unionized ammonia concentration was then calculated based on pH, temperature and salinity.

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

Mysid Shrimp (*Mysidopsis bahia*)

Concentration	Diluent	Effluent	Total Vol.
Control	500 ml	0 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%	0 ml	500 ml	500 ml

Table 2 . Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Buckman Street Sewage Treatment Plant, Jacksonville, Duval County, Florida, NPDES #FL0026000, on 25 to 27 August, 1981.

Test #1. Pre-Chlorinated Sample

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity ppt	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	7.1	7.6	7.0	7.7	7.7	7.7	20.6	20.3	20.1				
5.6%	5	5	5	7.1	7.6	7.0	7.7	7.7	7.7						18.5	19.4
10%	5	5	5	6.9	7.5	6.9	7.6	7.7	7.7	20.6	20.2	20.1				
10%	5	5	5	7.0	7.5	7.0	7.6	7.7	7.7						17.5	18.5
18%	5	5	5	6.7	7.3	6.8	7.6	7.7	7.7	20.7	20.1	20.0				
18%	5	5	5	6.7	7.3	6.9	7.6	7.7	7.7						16.8	17.3
32%	5	5	5	6.4	7.5	6.9	7.5	7.7	7.7	20.8	20.1	20.0				
32%	5	5	4	6.5	7.5	6.9	7.5	7.7	7.7						14.0	14.3
56%	5	5	2	6.1	7.8	6.8	7.5	7.8	7.8	20.8	20.1	20.0				
56%	5	5	3(4)[a]	6.2	7.8	6.8	7.5	7.8	7.8						9.2	9.6
100%	5	5	1(2)[b]	5.8	7.9	8.3	7.9	8.0	7.9	21.1	20.2	20.1	.02/.48	.02/.66		
100%	5	5	0	6.0	8.2	8.3	7.9	8.0	8.0						8.8	9.0

Remarks:
a. Number in parentheses used in calculations since one death between 24 and 48 hours was due to cannibalism.
b. Number in parentheses used in calculations since one organism jumped out between 24 and 48 hours.

Table 3. Data recorded during 48 hour, *Mysidopsis bahia*, static acute toxicity bioassay of Buckman Street Sewage Treatment Plant, Jacksonville, Duval County, Florida, NPDES #FL0026000, on 25 to 27 August, 1981.

Test #2. Chlorinated Sample.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity ppt	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	7.2	7.6	7.4	7.6	7.7	7.8	20.8	20.2	20.0				
5.6%	5	5	5	7.2	7.6	7.4	7.6	7.7	7.8						19.2	19.8
10%	5	5	4(5)[a]	7.0	7.9	7.1	7.6	7.8	7.7	20.6	20.1	20.0				
10%	5	5	5	7.0	7.9	7.2	7.6	7.8	7.7						18.2	18.7
18%	5	5	4(5)[b]	7.1	8.2	6.8	7.6	7.8	7.7	20.6	20.2	20.0				
18%	5	5	5	7.1	8.2	6.8	7.6	7.8	7.7						16.8	17.2
32%	5	5	5	7.2	8.4	8.2	7.5	7.8	7.8	20.7	20.2	20.1				
32%	5	4	4	7.1	8.4	8.2	7.5	7.8	7.8						14.0	14.4
56%	5	2	2	7.1	8.8	8.6	7.5	7.8	7.9	20.9	20.3	20.1				
56%	5	5	3	7.1	8.7	8.7	7.5	7.8	7.9						9.2	9.6
100%	5	0	0	7.4	8.9	8.7	7.8	8.0	8.0	20.9	20.4	20.3	.01/.39	.02/.60		
100%	5	0	0	7.5	9.0	8.7	7.8	8.0	8.1						7.1	7.5

Remarks: a. Number in parentheses used in calculations since one organism jumped out between 24 and 48 hours.
b. Number in parentheses used in calculations since one death between 24 and 48 hours was due to cannibalism.
Total Chlorine Residual (Hach DPD Method): 5.6% = 0.05 mg/l 18% = 0.09 mg/l 56% = 0.40 mg/l
10% = 0.07 mg/l 32% = 0.14 mg/l 100% = 0.55 mg/l

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Buckman Street Sewage Treatment Plant, Jacksonville, Duval County, Florida, NPDES #FL0026000, on 25 to 27 August, 1981.

Test #3. De-Chlorinated Sample

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity ppt	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
5.6%	5	5	5	7.3	7.6	7.1	7.6	7.7	7.8	21.0	20.4	20.2				
5.6%	5	5	5	7.3	7.5	7.0	7.7	7.7	7.7						19.1	19.9
10%	5	4	3(4)[a]	7.2	7.2	6.7	7.6	7.7	7.7	20.9	20.4	20.1				
10%	5	5	5	7.2	7.3	6.9	7.6	7.7	7.7						18.2	18.9
18%	5(4)[b]	5(4)[b]	4	7.3	7.5	6.9	7.6	7.7	7.7	20.9	20.4	20.1				
18%	5	5	5	7.4	7.5	6.9	7.6	7.7	7.7						16.5	17.0
32%	5	5	4(5)[a]	7.4	7.5	7.0	7.6	7.7	7.7	20.9	20.4	20.2				
32%	5	5	3(5)[c]	7.4	7.5	7.0	7.6	7.7	7.8						14.0	14.5
56%	5	5	5	7.6	7.9	6.9	7.5	7.8	7.8	20.9	20.5	20.3				
56%	5	5	5	7.5	7.9	7.0	7.5	7.8	7.8						9.2	9.7
100%	5	5	3(4)[a]	7.8	8.3	7.4	8.0	8.0	7.9	21.2	20.6	20.4	.01/.39	.01/.42		
100%	5	5	2(3)[a]	7.8	8.4	7.5	8.0	8.0	8.0						7.1	7.5

Remarks:

a. Number in parentheses used in calculations since one death between 24 and 48 hours was due to cannibalism.
b. Number in parentheses used in calculations since one organism jumped out between 0 and 24 hours.
c. Number in parentheses used in calculations since two organisms jumped out between 24 and 48 hours.

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Buckman Street Sewage Treatment Plant, Jacksonville, Duval County, Florida, NPDES #FL0026000, on 25 to 27 August, 1981.

Control Series

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity ppt	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Seawater Control	5	5	5	6.9	7.5	6.9	7.6	7.7	7.7	20.7	20.3	20.6	---	0/.13	21.0	22.5
Seawater Control	5(4)[a]	4	4	7.0	7.5	6.9	7.6	7.7	7.7							
Sea Salts Contrd	5	5	5	8.2	8.4	8.1	8.4	8.2	8.0	21.2	20.3	20.5	---	.01/.24	7.5	8.2
Sea Salts Contrd	5	5	4(5)[b]	8.3	8.5	8.0	8.4	8.2	8.0							
Salinity Control	5	5	5	8.0	7.8	7.1	7.7	7.8	7.8	21.2	20.6	20.6	---	0/.12	9.8	10.0
Salinity Control	5	5	5	8.0	7.8	7.2	7.7	7.8	7.8							

Remarks:
a. Number in parentheses used in calculations since one organism jumped out between 0 and 24 hours.
b. Number in parentheses used in calculations since one organism jumped out between 24 and 48 hours.

RESULTS AND DISCUSSION

The pre-chlorinated effluent (Test #1, Table 2) from the Buckman Street Sewage Treatment Plant demonstrated acute toxicity to the mysid shrimp, with 48 hour LC_{50}'s ranging from 63.7% to 64.4% (Table 6). The 96 hour LC_{50} would therefore be ≤64.4%. The toxicity associated with this sample may be attributed to some component of the effluent unrelated to chlorine, since the sample was collected prior to chlorination. The chlorinated sample (Test #2, Table 3) was also acutely toxic to Mysidopsis bahia, with 24 hour LC_{50}'s of 55.6% to 63.9%, and 48 hour LC_{50}'s of 51.1% to 56.0% (Table 6). The 96 hour LC_{50} would therefore be ≤56%. The toxicity of this sample may be attributed to the effects of chlorine and its reaction byproducts, since the total residual chlorine found in the 100% effluent (0.55 mg/l) is within the range reported to be toxic to certain aquatic organisms (U.S. EPA 1976). The de-chlorinated sample of effluent (Test #3, Table 4) was lethal to a significant number of mysid shrimp, resulting in a 48 hour LC_{50} of >100% (Table 6). This decrease in toxicity may be the result of oxidation of the toxic chemical components in the effluent by chlorine. The subsequent de-chlorination of the sample with sodium thiosulfate may then have eliminated the toxic properties of chlorine. However, since some lethal effects were observed, there is apparently still some toxic component in the effluent which was unaffected by chlorination. The results of these tests are very similar to the results of the bioassays performed on this facility in 1979 (FDER, 1979).

The DER Chemistry Section has chemically characterized a sample of this effluent, the results of which are presented in Table 7. Since this priority pollutant scan did not detect any substance in potentially toxic concentrations, the toxicity observed in this bioassay can not be attributed to a specific chemical other than chlorine (in Test #2).

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.

Table 6. LC_{50} Values for Mysidopsis bahia Bioassays Performed on Buckman Street Sewage Treatment Plant, Jacksonville, Florida, NPDES #FL0026000, on 25 to 27 August, 1981.

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Mysidopsis bahia	Binomial		64.4% (32, and ∞)
Test #1 Pre-Chlorinated	Moving Average	No mortality	63.7% (46 and 102)
	Probit		64.1% (49 and 93)
Mysidopsis bahia Test #2 Chlorinated	Binomial	63.9% (32 and 100)	**56.0%**(32 and 100)
	Moving Average	55.6% (45 and 74)	51.1% (41 and 67)
	Probit	58.1% (46 and 75)	52.4% (41 and 67)
Mysidopsis bahia Test #3 De-Chlorinated	Binomial		A
	Moving Average	No mortality	A
	Probit		>100%, 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.

Table 7. Results of Chemical Analyses Performed on Chlorinated Effluent from Buckman Street Sewage Treatment Plant, Collected on 24 August, 1981.

Parameter	Results (ug/l)
Organic Compounds	
Pesticide Extractables	Not Detected
Base Neutral Extractables	Not Detected
Acid Extractables	Not Detected
Metals	
Antimony	200 k
Arsenic	50 k
Beryllium	10 k
Cadmium	10 k
Chromium	50 k
Copper	25 k
Lead	50 k
Mercury	0.2 k
Nickel	50 k
Selenium	10 k
Silver	15 k
Thallium	100 k
Zinc	25 k

k: Actual value is known to be less than value given.

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