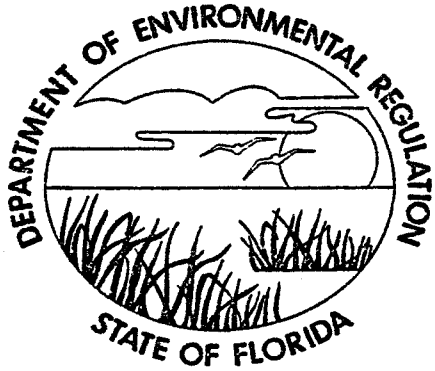


BIOASSAYS OF THE BUCKMAN STREET SEWAGE TREATMENT PLANT JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0026000

SAMPLED 10/29/84

January 15, 1985

Biology Section
Division of Environmental Programs

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EXECUTIVE SUMMARY

Buckman St. STP, Jacksonville, NPDES #FL0026000, on 30 October to 1 November, 1984.

The samples of chlorinated and de-chlorinated effluents from this municipal sewage and industrial waste treatment facility proved to be acutely toxic to Mysidopsis bahia, producing 48 hour LC_{50}'s of 7.0% and 62.5%, respectively. Additional screening tests (100% concentrations only) of these effluents, using the freshwater fish Notropis leedsi as test organism, resulted in the death of all organisms in the chlorinated sample, but no mortality was noted in the de-chlorinated sample. A portion of the toxicity noted in the chlorinated sample can be attributed to the total residual chlorine (2.17 mg/l) measured in that sample. The total and unionized ammonia detected in these samples (maximum measured = 1.48 mg/l and 0.029 mg/l, respectively), however, were insufficient to have contributed substantially to the toxicity. Chemical analyses indicated the presence of several unidentified organic compounds and a small amount of zinc (29 µg/l). The organic compounds may have contributed to the toxicity noted in the samples.

The results of these latest bioassays were consistent with the results of four previous DER bioassays (1979, 1981, 1982, and 1983) in which acute toxicity was determined in one or more effluents from the Buckman St. STP during each series of tests. These latest tests, additionally, may even be considered slightly more toxic, on the average, than the previous tests, indicating no improvement in the quality of this effluent over the years.

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, the discharge contains only partially treated wastes.

In October, 1979, the Biology Section performed a static acute toxicity bioassay on pre-chlorinated effluent collected from the secondary clarifer 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.

In August, 1981, additional bioassays were performed on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this facility, to further evaluate their toxicity. The results of those tests indicated that the pre-chlorinated and chlorinated effluents were acutely toxic to Mysidopsis bahia (approximate LC_{50}'s were 64% and 52%, respectively), while the de-chlorinated effluent was lethal to a number of shrimp (FDER 1981).

In June and July, 1982, five additional bioassays were performed on pre-chlorinated, chlorinated and de-chlorinated effluents from the Buckman St. facility. Those tests were performed using freshwater test organisms, since the salinity of the normally saline St. Johns River at that time had decreased to approximately 1 ppt. The effluents were variously lethal to the test organisms, most notable were the 48 hour LC_{50}'s of 6.3% for Daphnia pulex (chlorinated effluent), and 77.5% for Simocephalus serrulatus (de-chlorinated effluent). The toxicity at that time was attributed to total residual chlorine and to the presence of a variety of chemical constituents (FDER 1982).

In August, 1983, three additional bioassays were performed on samples of pre-chlorinated, chlorinated and de-chlorinated effluents from this facility, using Mysidopsis bahia as the test organism. Acute toxicity was noted in the pre-chlorinated sample (48 hour LC_{50} = 99.3%) and in the chlorinated sample (48 hour LC_{50} = 14.3%), while some mortality was also noted in the de-chlorinated sample (FDER 1983). Four unidentified organic compounds were detected in the effluent at that time.

Since effluent from the Buckman St. STP has been shown to exhibit a continued toxicity problem, additional bioassays were recently performed, on 30 October to 1 November, 1984, on samples of chlorinated and dechlorinated effluents. These latest tests were performed with the saltwater test organism, Mysidopsis bahia. The results of these latest tests are presented in this report. Those involved in performing these tests and in preparation of this report were: C. W. Dye, R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.

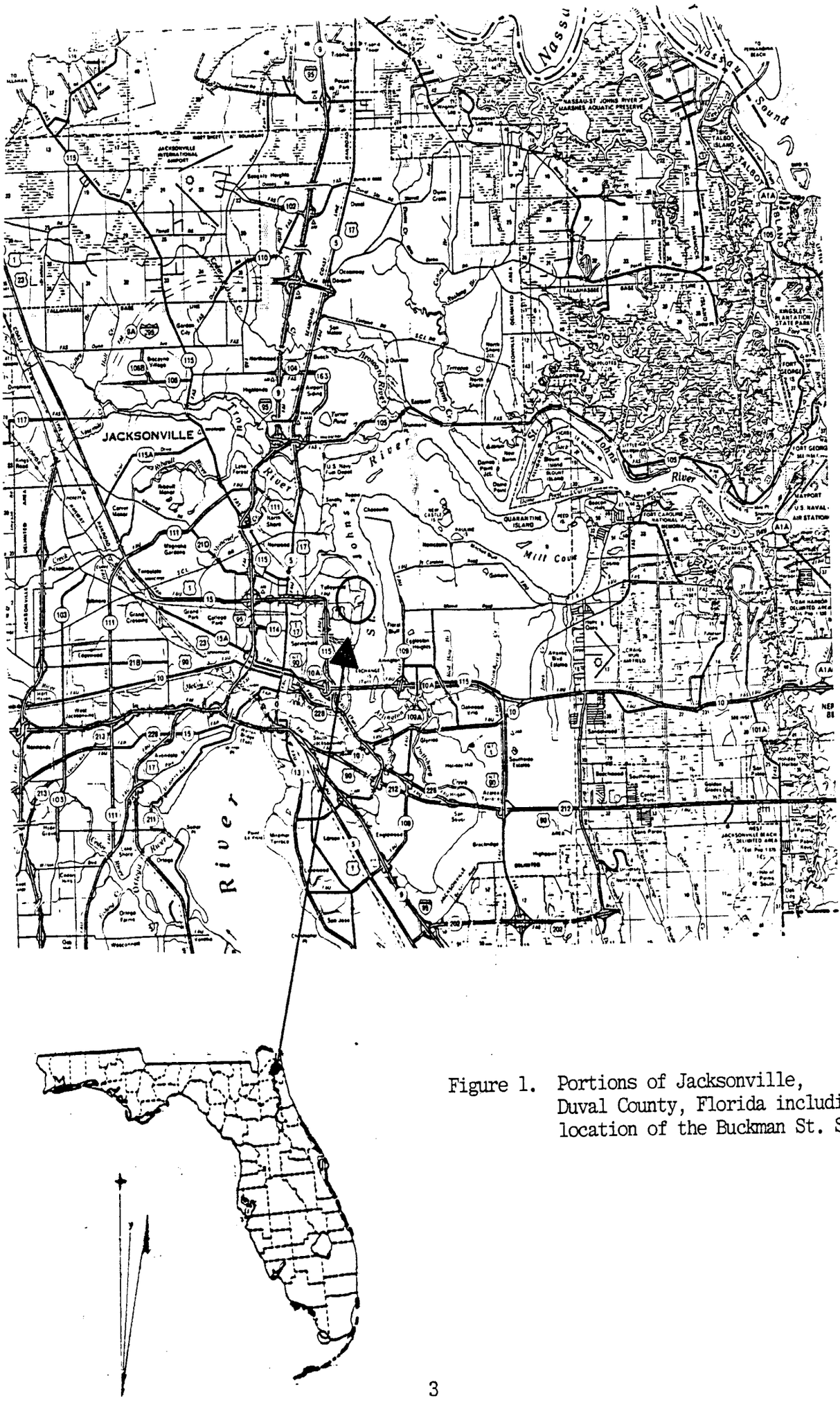


Figure 1. Portions of Jacksonville, Duval County, Florida including location of the Buckman St. STP.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include a species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Notropis leedsi (bannerfin shiner) - Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 30 and 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22° plus or minus 2°C under a 16:8 hour light cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25°C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were approximate three weeks old at the time of the test.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. The mysids are cultured under a 16:8 hour light-dark cycle 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, used for culturing the test organisms and as diluent in the bioassays, is prepared by mixing artificial sea salts (Forty Fathoms®) and well water to obtain a final salinity of 20-30 ppt. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily and once daily on weekends with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 23° plus or minus 3°C. Adult or large juvenile mysids were used in this test.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Four separate 48 hour static acute toxicity bioassays (two definitive and two screening tests) were performed on chlorinated and de-chlorinated effluents from the Buckman St. STP. The tests were performed from 0950 hours on 30 October to 1000 hours on 1 November, 1984.

The samples were collected by Ms. Kathleen Gerald of DER's Northeast District at 1405 hours on 29 October, 1984, 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. Chlorinated Effluent (Tables 2 and 4). These tests were performed on an effluent sample collected at the point of discharge.

Test #2. De-chlorinated Effluent (Tables 3 and 4). These tests were 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.

For the Notropis leedsi (bannerfin shiner) screening bioassays of these effluents, two replicates of one concentration (100%) and a control were used, each containing five fish. Fish were not fed during these tests.

Saltwater for dilution of the effluent samples for the Mysidopsis bahia definitive tests was prepared from artificial sea salts (salinity = 22.5 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (11.0 ppt to 20.5 ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 2) 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:

1) Diluent Control - Artificial seawater (22.5 ppt). This represents the highest concentration of artificial seawater used in the bioassays, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassays, and therefore tests the tolerance of the organisms to this salinity.

2) Salinity Control - Artificial seawater, adjusted with well water to approximately 7 ppt salinity. This tests for tolerance of the organisms to the lowest salinity used in the bioassays.

The concentration series selected for the M. bahia study (Table 1) is that recommended by APHA (1980). Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. 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 using a 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 4 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. The specific conductance or salinity (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% effluent test concentrations at the beginning of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Tables 2, 3 and 4.

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

Mydidopsis bahia (mysid shrimp) Definitive Tests

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	------	500 ml
5.6%[a]	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%[b]	------	500 ml	500 ml

Notropis leedsi (bannerfin shiner) Screening Tests

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

[a] This concentration was not used in the de-chlorinated effluent test.

[b] This concentration was not used in the chlorinated effluent test.

Table 2. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Buckman St. Sewage Treatment Plant, Jacksonville, Duval County, Florida, NPDES #FL002600, on 30 October to 1 November, 1984.

Chlorinated sample[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	5	5	5	7.1	5.9	6.4	8.5	8.4	8.3	22.2	21.8	19.8	22.5	22.5
Control	5	5	5	7.1	5.9	6.4	8.5	8.3	8.3					
Control	5	5	5	7.8	6.8	7.1	8.5	8.2	8.3	22.9	21.8	19.7	7.0	6.8
Control	5	5	5	7.8	6.9	7.3	8.5	8.1	8.3					
5.6%	5	5	5	7.0	6.5	6.7	8.4	8.2	8.3	22.1	21.8	19.6	21.0	21.1
5.6%	5	4	4	7.0	6.6	6.7	8.4	8.3	8.3					
10.0%	5	0	0	6.9	6.9	– [b]	8.4	8.3	– [b]	21.9	21.7	– [b]	20.0	– [b]
10.0%	5	0	0	7.0	6.9	–	8.4	8.3	–					
18.0%	5	0	0	7.0	7.4	–	8.4	8.3	–	21.6	21.6	–	18.5	–
18.0%	5	0	0	7.0	7.4	–	8.4	8.3	–					
32.0%	5	0	0	6.5	7.5	–	8.3	8.2	–	21.3	21.6	–	16.0	–
32.0%	5	0	0	6.6	7.5	–	8.3	8.2	–					
56.0%	5	0	0	6.8	7.9	–	8.0	7.7	–	20.7	21.7	–	11.0	–
56.0%	5	0	0	6.8	7.9	–	8.0	8.0	–					

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0

Total Hardness (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .009

Total Alkalinity (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 1.26

Total Hardness (mg/l $CaCO_3$)

[a] Total residual chlorine = 2.0 mg/l (HACH Method) and 2.17 mg/l (Iodometric Method)

[b] Remaining parameters were not recorded since all organisms were dead by 24 hours.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Buckman St. Sewage Treatment Plant, Jacksonville, Duval County, Florida, NPDES #FL0002600, on 30 October to 1 November, 1984.

De-Chlorinated sample

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
10.0%	5	5	5	7.0	6.6	6.6	8.5	8.2	8.3	21.9	22.1	19.9		
10.0%	5	5	5	7.0	6.7	6.6	8.5	8.2	8.3				20.5	20.5
18.0%	5	5	5	6.7	6.6	6.6	8.4	8.2	8.3	21.8	21.7	19.3		
18.0%	5	5	5	6.9	6.7	6.7	8.4	8.2	8.3				19.0	19.0
32.0%	5	5	5	7.1	6.7	6.1	8.3	8.1	8.3	21.5	21.5	19.3		
32.0%	5	5	5	7.1	6.7	6.2	8.3	8.1	8.3				16.0	16.0
56.0%	5	5	1	7.3	6.9	6.6	8.1	8.1	8.3	21.3	21.6	19.3		
56.0%	5	4	4	7.3	6.9	6.7	8.1	8.0	8.3				11.0	11.0
100.0%	5	5	2	7.5	7.0	6.8	7.6	8.0	8.3	21.4	21.6	19.4		
100.0%	5	4	0	7.5	7.0	7.0	7.6	7.9	8.3				7.5	7.8

Control Water

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity (mg/l $CaCO_3$)

Total Hardness (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .029

Total ammonia (as N)(mg/l) 1.48

Total Alkalinity (mg/l $CaCO_3$)

Total Hardness (mg/l $CaCO_3$)

Table 4. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Buckman St. Sewage Treatment Plant, Jacksonville, Duval County, Florida, NPDES #FL0002600, on 30 October to 1 November, 1984.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Chlorinated Sample [a]														
Control	5	5	5	8.0	7.9	7.9	7.9	8.1	8.3	22.0	21.8	19.5		
Control	5	5	5	8.0	7.9	7.9	7.9	8.1	8.3				228	225
100.0%	5	0	0	6.9	8.0	- [b]	7.2	7.9	- [b]	19.8	22.1	- [b]		
100.0%	5	0	0	6.9	8.1	-	7.2	7.9	-				790	- [b]
De-Chlorinated Sample														
100.0%	5	5	5	7.4	8.2	7.5	7.4	7.9	8.2	21.1	22.1	19.6	800	790
100.0%	5	5	5	7.4	8.0	7.5	7.4	7.9	8.2					

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 0

Total Hardness (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l)

Total Alkalinity (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l)

Total Hardness (mg/l $CaCO_3$)

[a] Total residual chlorine = 2.0 mg/l (HACH Method) and 2.17 mg/l (Iodometric Method).

[b] Remaining parameters were not recorded since all organisms were dead by 24 hours.

Table 5. LC_{50} Data for the Definitive Bioassays of the Buckman St. STP, NPDES #FL0026000, on 30 October to 1 November, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
M. bahia			
Test #1: Chlorinated Sample	Binomial	7.0% (5.6 and 10)	7.0% (5.6 and 10)
	Moving Average	a	a
	Probit	a	a
Test #2: De-chlorinated Sample	Binomial	a	56.0% (32 and ∞)
	Moving Average	a	62.5% (49.3 and 83.9),[c]
	Probit	327.9% (111.6 and ∞),[b]	64.2% (49.7 and 86.9)

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

Table 6. Results of Chemical Analyses Performed on Effluent from the Buckman St. STP, Jacksonville, NPDES #FL0026000, collected 29 October, 1984.

Organics

Acid Extractables:	Several unidentified compounds (small peaks)
Base Neutral Extractables:	One unidentified compound (large peak)
Pesticide Extractables:	Several unidentified compounds (small to intermediate peaks)

Metals

Antimony	N.D.
Arsenic	N.D.
Beryllium	N.D.
Cadmium	N.D.
Chromium	N.D.
Copper	N.D.
Lead	< 10 µg/l
Mercury	[a]
Nickel	N.D.
Selenium	N.D.
Silver	< 0.5 µg/l
Thallium	N.D.
Zinc	29 µg/l

N.D. = None Detected

[a] Mercury analysis was invalidated.

RESULTS AND DISCUSSION

The samples of chlorinated and de-chlorinated effluents from the Buckman St. STP proved to be acutely toxic to the saltwater test organism, Mysidopsis bahia, producing 48 hour LC_{50}'s of 7.0% and 62.5%, respectively (Table 5). In the screening tests of these effluents, using the freshwater fish Notropis leedsi as test organism, complete mortality of fish was noted in the chlorinated effluent, but no mortality was noted in the de-chlorinated effluent. This increased toxicity of the chlorinated effluent was likely due to the presence of residual chlorine (2.0 mg/l HACH, 2.17 mg/l Iodometric Method, Table 2) in that sample. The ammonia concentrations measured in these effluents (1.48 mg/l total and 0.029 mg/l unionized) were insufficient to have contributed significantly to the observed toxicity.

The results of chemical analyses (Table 6) performed on final effluent from the Buckman St. facility indicated the presence of a variety of unidentified organic compounds (acid, base neutral and pesticide extractables) in various concentrations (peak size). Also present was a small concentration of zinc (29 µg/l). The organic compounds probably contributed to the toxicity noted in these samples.

The results of these latest bioassays were consistent with the results of four previous DER bioassays (1979, 1981, 1982, and 1983) in which acute toxicity was determined in one or more effluents from the Buckman St. STP during each series of tests. These latest tests, additionally, may even be considered slightly more toxic, on the average, than the previous tests, indicating no improvement in the quality of this effluent over the years.

Discussion of the Toxicity Criteria

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

The two basic concepts utilized in the standards are acute and chronic toxicity (defined in 17-3.021(1) and (4), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as 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}. 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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