BIOASSAYS OF THE DISTRICT #2

SEWAGE TREATMENT PLANT

JACKSONVILLE, DUVAL COUNTY, FLORIDA

NPDES #FL0026450

SAMPLED 6/13/83

Biology Section
Bureau of Water Analysis
August 19, 1983

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EXECUTIVE SUMMARY

District #2 STP, Jacksonville, NPDES #FL0026450, on 14 to 16 June, 1983.

The pre-chlorinated effluent from this sewage treatment plant, which treats primarily organic brewery wastes, was acutely toxic to Mysidopsis bahia, producing a 24 and 48 hour LC_{50} value of 28.8%. The initial low dissolved oxygen concentration of this effluent (0.75 mg/l) was increased to sufficient levels throughout the test by constant aeration. Aeration was thought unnecessary for the chlorinated and de-chlorinated effluents, since the initial dissolved oxygen concentrations were relatively high (7.5 mg/l and 8.1 mg/l, respectively). However, these effluents exerted a substantial BOD by hour 24 of the test, depleting the dissolved oxygen concentrations to 0.6 mg/l, and most likely accounting for much of the mortality noted in these tests. Since the dissolved oxygen levels in most of the test concentrations in these tests had dropped below 40% saturation, the Biology Section invalidated the test as per standard quality assurance practices (FDER 1983). However, the total residual chlorine measured in the chlorinated sample (1.8 mg/l) would have been sufficient to cause the mortality noted in that test, without the added effects of the depleted oxygen. Small amounts of ammonia were detected in the effluents, which could not have contributed to the toxicity. Since the chlorinated and de-chlorinated tests were invalidated due to depleted dissolved oxygen, data for these tests are not reported.

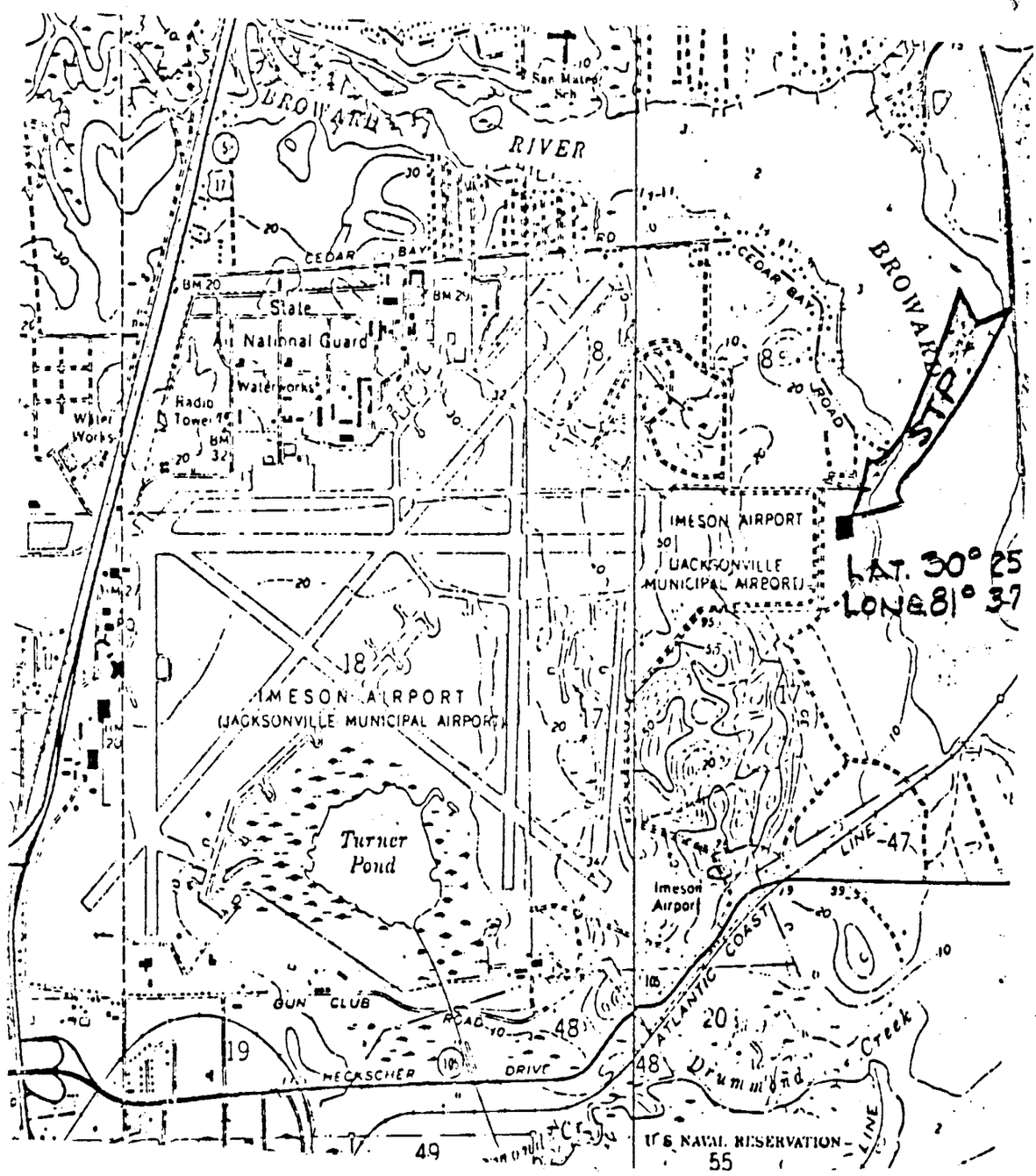
Chemical analyses of these effluents indicate the presence of the organic compound dicylcohexylphthalate, at a concentration of 140 µg/l in the final effluent. Also present were significant concentrations of the heavy metals copper (49 µg/l), and mercury (0.9 µg/l), which most likely contributed to the toxicity noted.

INTRODUCTION

The District #2 STP is located at 1840 Cedar Bay Road in Jacksonville, Duval County, Florida (Fig. 1). This 10.0 MGD design capacity Pureox® activated sludge facility treats an average of 3.5 MGD of wastes. The majority of wastes (3.1 MGD) are from the nearby Busch Brewery and have caused frequent upsets at the District #2 facility due to high organic loadings and pH fluctuations (from NPDES file). Improvements at both the brewery and the STP have since been made to minimize these upsets. Treatment of wastes consists of primary and secondary clarification, and two bioreactors (Fig. 2). Sludge is pumped to the Buckman St. STP for incineration. Chlorine is introduced at the discharge pipe to the Broward River, a tributary of the St. Johns River.

As part of routine NPDES and F.A.C. compliance monitoring, the District #2 STP was selected for bioassay analyses, to evaluate the effects that this effluent may have on aquatic organisms. The Biology Section performed three 48 hour static acute toxicity bioassays on grab samples of pre-chlorinated, chlorinated, and de-chlorinated effluents, on 14 to 16 June, 1983. The results of these tests are presented in this report. Those involved in performing these tests and in preparation of this report were: C. W. Dye, J. M. Faircloth, M. L. Roll, and K. A. Study.

The Biology Section later learned that, at the time of sampling, a major upset had occurred at the District #2 STP (Ms. Teri Rhodes, DER Jacksonville Office, personal communication). The upset was apparently caused by a large imput of organic brewery wastes, including yeast, which resulted in high turbidity and an unusually high oxygen demand in the effluent.



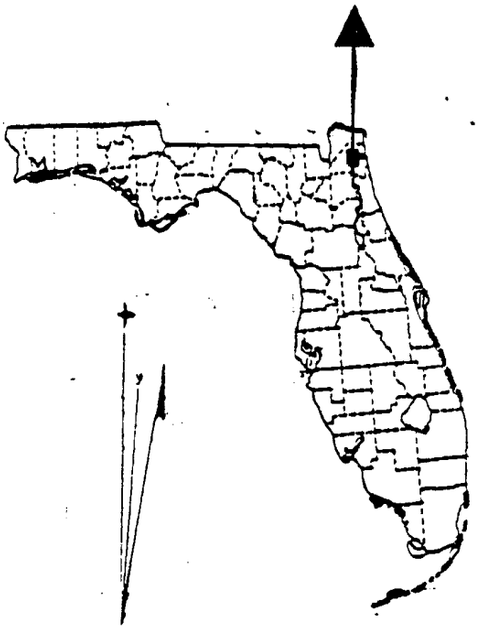


Figure 1. Portions of the City of Jacksonville, Duval County, including the location of the District #2 STP.

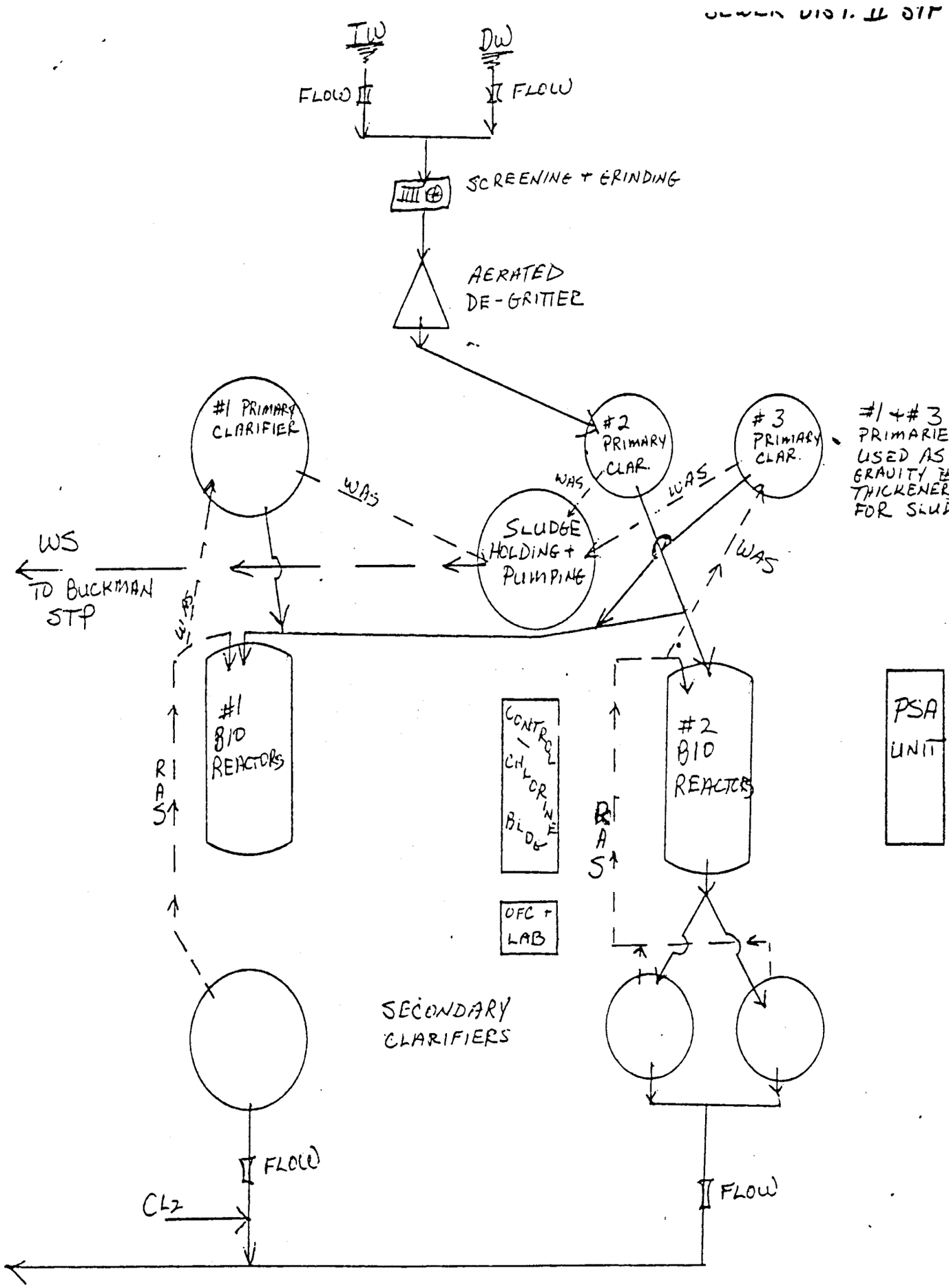


Figure 2. Schematic of Waste Treatment, District #2 STP

METHODS AND MATERIALS

Test methods used to conduct this these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organism selected for this study is among those recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). The life history and maintenance procedures used to culture the organism 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 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 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° plus or minus 2°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

Three separate 48 hour static acute toxicity bioassays were performed on samples of pre-chlorinated, chlorinated, and de-chlorinated effluents from the District #2 STP. The tests were performed from 1510 hours on 14 June to 1520 hours on 16 June, 1983. The samples were collected by Ms. Teri Rhodes of DER's Jacksonville office at 0738 -0746 hours on 14 June, 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 (Table 2). 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[a]. Chlorinated Effluent. This test was performed on an effluent sample collected at the point of discharge.

Test #3[a]. De-chlorinated Effluent. 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.

Saltwater for dilution of the effluent samples was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 23.0 ppt). This saltwater diluent was then adjusted to 19.5 ppt, a moderate salinity characteristic of the receiving body of water (Broward River), 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 (9.5 ppt to 18.8 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 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 - Turkey Point water (23.0 ppt), adjusted with well water to a salinity of 19.5 ppt. This represents the highest concentrations of both Turkey Point water and well water used in the

[a]These tests were invalidated due to insufficient dissolved oxygen and no data from Tests #2 or #3 are reported.

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) Sea Salts Control - Well water, adjusted with artificial sea salts (Ocean 50®) to approximately 7 ppt salinity. This tests for toxicity of the highest concentration of artificial sea salts used in the bioassays, as well as for tolerance of the organisms to the lowest salinity used in the bioassays.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each randomly arranged 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 preacclimated 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 chamber at 0 hours, 24 hours, and 48 hours. The 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 Sea Salts control 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 form and are presented in Table 2.

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

Mydidopsis bahia (mysid shrimp)

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, Mysidopsis bahia, static acute toxicity bioassay of the District #2 STP, Jacksonville, Duval County, Florida, NPDES #FL0026450, on 14 to 16 June, 1983.

Test #1 Pre-chlorinated Effluent[a]

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/N (mg/l)		Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0	48
Diluent Control	5	5	5	8.2	7.5	7.7	8.2	7.9	7.8	21.2	20.2	20.3				
Diluent Control	5	5	5	8.3	7.4	7.7	8.2	7.9	7.8						19.5	19.3
Sea Salts Control	5	5	5	8.7	8.3	7.5	8.4	8.2	8.0	21.2	20.1	20.0	0/0	--		
Sea Salts Control	5	5	5	8.7	8.3	7.5	8.3	8.2	7.9						7.2	7.2
5.6%	5	5	5	8.1	7.0	7.3	7.9	7.8	7.8	21.0	20.2	20.0				
5.6%	5	5	5	8.1	6.9	7.3	7.9	7.8	7.8						18.8	18.0
10.0%	5	5	5	7.5	6.4	7.2	7.6	7.7	7.8	20.9	20.3	20.0				
10.0%	5	5	5	7.5	6.4	7.2	7.6	7.7	7.8						18.0	17.2
18.0%	5	5	5	6.6	5.5[c]	7.3	7.3	7.6	8.0	20.9	20.2	20.0				
18.0%	5	4	4	6.6	5.5[c]	7.5	7.3	7.6	8.2						16.7	16.0
32.0%	5	0	0	4.2	3.3	--[e]	7.1	7.5	--[e]	20.8	20.2	--[e]				
32.0%	5	0	0	4.2	3.3	--[e]	7.1	7.6	--[e]						14.1	--[e]
56.0%	5	5	5	1.5[b]	7.2[c]	5.9	7.0	8.3	8.5	20.8	20.2	20.1				
56.0%	5	0	0	1.5[b]	7.3[d]	4.3	7.0	8.1	8.1						9.2	9.2
100.0%	5	0	0	0.75[b]	7.2[d]	--[e]	7.1	8.4	--[e]	20.7	20.3	--[e]	0/.02	--		
100.0%	5	0	0	0.75[b]	7.2[d]	--[e]	7.1	8.4	--[e]						7.4	--[e]

Remarks:

[a] Total Residual Chlorine : None Detected (HACH DPD Method).
[b] These test chambers were aerated to 4.2 mg/l, 5.6 mg/l, 4.3 mg/l, and 4.3 mg/l, respectively, prior to loading the organisms.
[c] Aeration started in these test chambers, continued to hour 43 of the test.
[d] Aeration stopped in these test chambers since all organisms were dead.
[e] These parameters not reported since all organisms in the respective test concentration were dead by hour 24.

Table 3. LC_{50} Data for Bioassays of the District #2 STP, Jacksonville, on 14 to 16 June, 1983.

Organism	Method of Data Analysis	24 LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Test #1: M. bahia Pre-chlorinated Sample	Binomial	22.6% (18 and 100)	22.6% (18 and 100)
	Moving Average	28.8% (21 and 41), a	28.8% (21 and 41), a
	Probit	31.0% (0 and ∞), b	31.0% (0 and ∞), b
Test #2: M. bahia Chlorinated Sample	Binomial	c	c
	Moving Average		
	Probit		
Test #3: M. bahia De-chlorinated Sample	Binomial	c	c
	Moving Average		
	Probit		

[a] This LC50 value is reported in the text since it is the most statistically conservative of the data set.

[b] Due to the distribution of mortality, results calculated using the probit method are not statistically sound.

[c] This test was invalidated, data not reported.

Table 4. Results of Chemical Analyses Performed on Effluents from the District #2 STP, Jacksonville, NPDES #FL0026450, collected 14 June, 1983.

Organics

Pre-chlorinated Effluent	
Acid Extractables:	N.D.
Base Neutral Extractables:	Dicylcohexylphthalate (34.0 µg/l)
Pesticide Extractables:	N.D.
Chlorinated Effluent	
Acid Extractables:	N.D.
Base Neutral Extractables:	Dicylcohexylphthalate (140.0 µg/l)
Pesticide Extractables:	N.D.

Metals

Arsenic	N.D.
Cadmium	N.D.
Chromium	36 µg/l
Copper	49 µg/l
Lead	5 µg/l[a]
Mercury	0.9 µg/l
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Zinc	149 µg/l

N.D. = None Detected

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

RESULTS AND DISCUSSIONS

The pre-chlorinated effluent from the District #2 STP proved to be acutely toxic to Mysidopsis bahia, producing a 24 and 48 hour LC_{50} value of 28.8% (Table 3). The 96 hour LC_{50} would be less than or equal to the 48 hour value. The initial low dissolved oxygen concentration of this effluent (0.75 mg/l, Table 2) was increased to sufficient levels by aeration. It was noted that the test organisms were dead within a few minutes after being introduced into the 100% effluent concentration. Since the dissolved oxygen concentrations had been increased to sufficient levels prior to loading the organisms, and neither residual chlorine nor significant ammonia were present, the toxicity must have been due to other chemical characteristics or components of the effluent.

Aeration was thought to be unnecessary in the chlorinated and de-chlorinated tests, since the initial dissolved oxygen concentrations were relatively high (7.5 mg/l and 8.1 mg/l, respectively). However, these effluents exerted a considerable BOD by hour 24 of the test, depleting the dissolved oxygen concentrations to 0.6 mg/l, most likely accounting for the substantial mortality noted in these tests. As per standard quality assurance practices these tests were invalidated (FDER 1983) and no data are reported here. However, a Total Residual Chlorine concentration of 1.8 mg/l (HACH DPD Method) was detected in the chlorinated effluent, which would have been sufficient to cause the mortality noted in that test, even without the effects of the depleted oxygen.

Since a major upset of this facility had occurred at the time of sampling, the effluents collected for bioassays may not have been characteristic of what is discharged during normal plant operation. Although the chlorinated and de-chlorinated tests were invalidated, the pre-chlorinated results may be used as an indication of the toxicity which may have been expected in the final effluent, since the pre-chlorinated effluent was collected after final treatment, but just prior to chlorination.

The DER Chemistry Section has analyzed samples of effluent from the District #2 STP for certain organics and heavy metals (Table 4). These analyses indicated the presence of the organic compound dicylcohexylphthalate in the final effluent, at a concentration of 140 µg/l. Also present were measurable amounts of the heavy metals chromium (36 µg/l), copper (49 µg/l), mercury (0.9 µg/l), and zinc (149 µg/l). Of these, the copper and mercury are significant based on the water quality criteria in Chapter 17-3 of the F.A.C. Additionally, concentrations of copper and of mercury similar to those detected in the District #2 STP effluent have been shown to be toxic to aquatic life in both acute and chronic tests (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 17-3.021(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 only on a pre-chlorinated effluent sample, which may or may not be representative of the final effluent which enters waters of the state at the point of discharge.

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 bioassay results reported here, for the pre-chlorinated effluent do not necessarily represent the results which might have been obtained from the specific effluent which is discharged by the sewage treatment plant in chlorinated form. However, these results may be used as an indication of the toxicity which may be expected in the final discharged effluent, since the pre-chlorinated effluent was collected after final treatment, but just prior to chlorination.

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