

BIOASSAY OF SOUTHERN WOOD PIEDMONT DISCHARGE

(NPDES # FL0000221)

Duval Co., Florida

Biological Section
Bureau of Water Analysis
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INTRODUCTION

Southern Wood Piedmont Company is a wood treating operation located in Baldwin, Duval County, Florida, 10 miles west of Jacksonville (Figures 1 and 2). The plant pressure-treats a minimum of 50,000 cubic feet of wood per year with creosote, pentachlorophenol, and a solution of heavy metals to produce utility poles and crossties that are protected against weathering and decay. Wastewater consists of boiler blowdown, water softener backwash, and storm water run-off contaminated with the aforementioned chemicals. Aside from two small, shallow retention ponds formed by a widening in the effluent ditch, no special wastewater treatment system is used. Figure 3 illustrates the sources and flow pattern of the wastewater into Baldwin Bay Swamp, which eventually drains into the St. Mary's River.

NPDES limitations at outfall #002 are listed below:

CHARACTERISTIC	DAILY AVERAGE	DAILY MAXIMUM
Flow	-	12,000 gpd.
Temperature	35°C	38°C
Phenols	1.0 mg/l	1.5 mg/l
Chromium	1.0 mg/l	1.0 mg/l
Arsenic	0.25 mg/l	0.25 mg/l
Zinc	0.5 mg/l	1 mg/l
Copper	1 mg/l	1 mg/l
Oil and Grease	10 mg/l	15 mg/l
Total Suspended Solids	30 mg/l	50 mg/l
pH	6.0-9.0	6.0-9.0

Southern Wood Piedmont had been found in violation of many of these limitations in the past, and DER's Biological Section was requested to perform a toxicity test on the effluent. Results of several bioassays on this effluent, including a range finding test, a definitive flow-through assay, and several on-site tests are reported here.

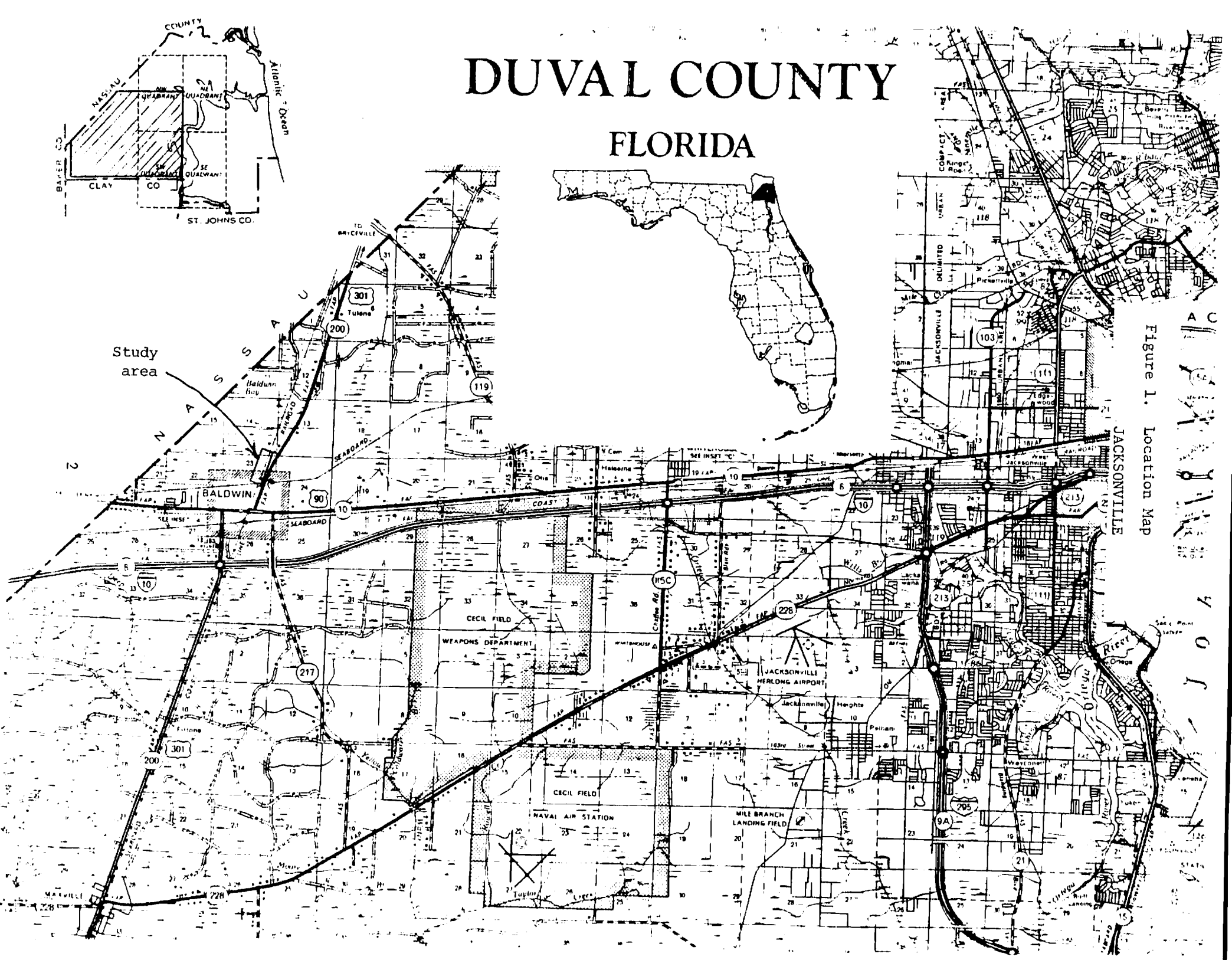


Figure 1. Location Map

Figure 2. Southern Wood Piedmont Facility

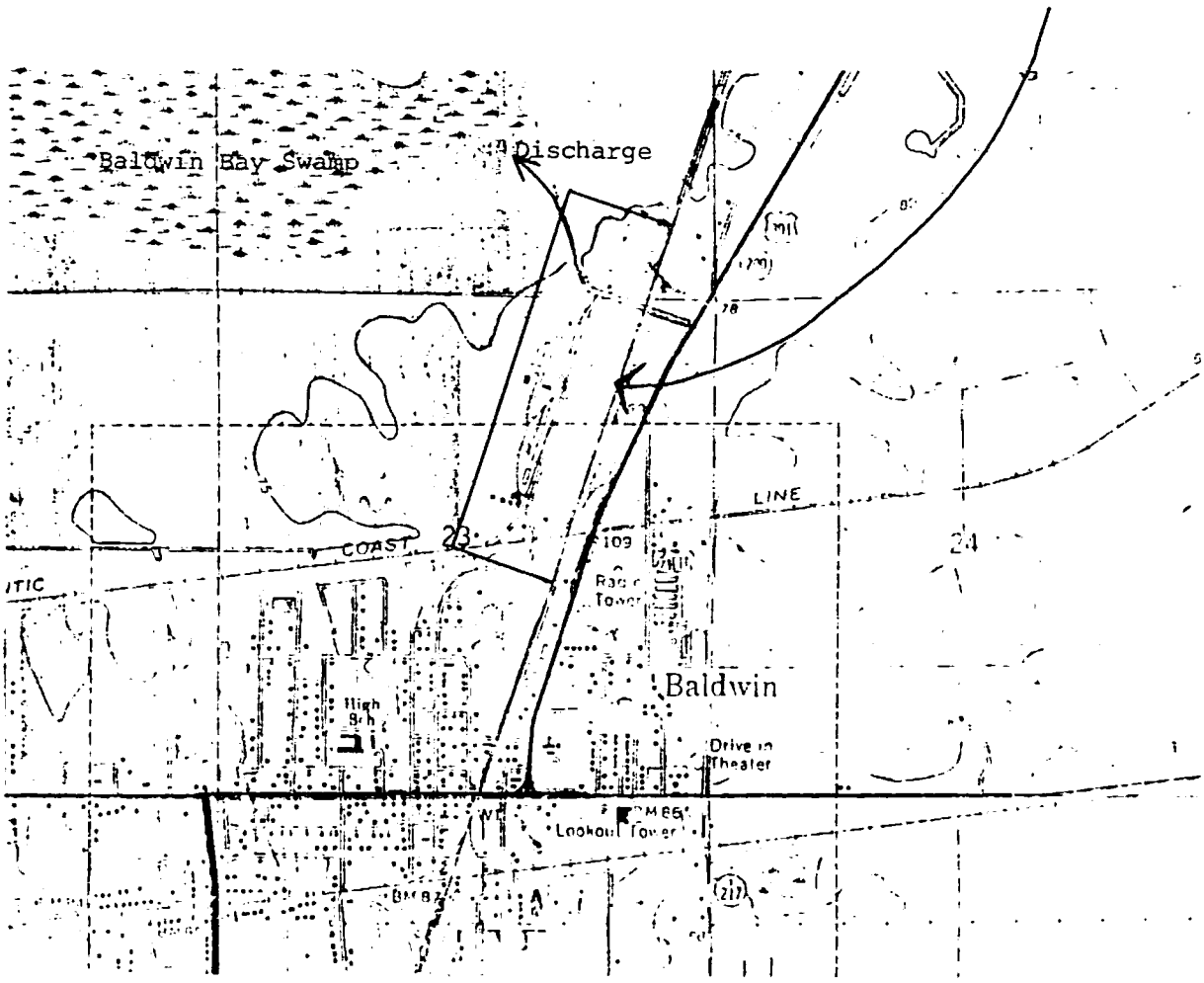
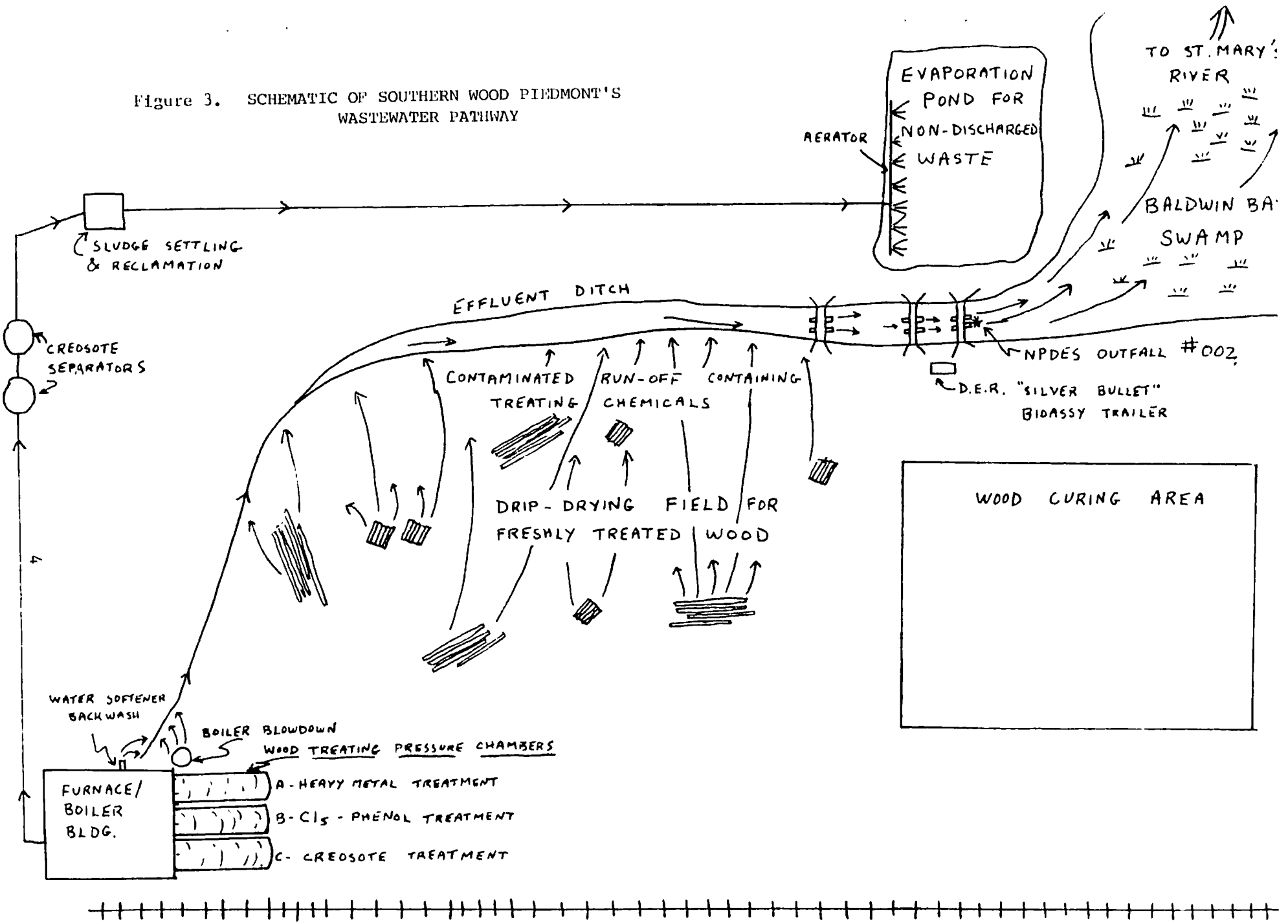


Figure 3. SCHEMATIC OF SOUTHERN WOOD PIEDMONT'S WASTEWATER PATHWAY



METHODS AND MATERIALS

Test methods used to conduct the range-finding, static and flow-through bioassays are extensively described by Peltier (1978). Organisms selected were those recommended by U. S. EPA (1978) and by Peltier (1978). Life histories of test organisms and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Pimephales promelas (Fathead Minnow):

Two hundred, two week old fathead minnow fry were delivered to the DER Biological Section from the Athens, Georgia, EPA lab, 5 days before the flow-through test. These fish were transferred to a 5 gallon aquarium and acclimated to 100% dilution water from the Southern Wood Piedmont well over a 4 day period. Live brine shrimp (Artemia) were fed to the fish until 24 hours before the bioassay began.

The entire aquarium full of fish was transported to the Baldwin site, with aeration being provided by a battery operated pump. No mortality was observed in the 24 hour period before the bioassay.

Daphnia pulex (Water flea):

A breeding culture of Daphnia pulex was obtained from the Athens, Georgia, EPA laboratory on March 9, 1979. Daphnia were maintained in 2½ gallon all-glass aquaria under a 16:8 light-dark cycle. Organisms were fed a liquified blend of trout chow and Cerophyl^{R} twice daily. Daphnia used for the bioassay were transported in their culture tank with no aeration. No mortality was observed 24 hours before the test. A series of nested screens were used to isolate the proper sized organisms (neonates), for the bioassay.

Test Methods

Fathead minnows (Pimephales promelas) and water fleas (Daphnia pulex) were used as test organisms in each of two different test methods utilized in this study. The two bioassay methods used were:

1.) Ninety-six hour flow-through acute toxicity bioassays using a solenoid valve operated proportional dilutor system;

2.) Forty-eight hour static acute toxicity bioassays performed on grab samples of effluent.

Preliminary Visit and Range Finding Test

Mr. G. R. Lee and Mr. R. Frydenborg of DER's Biological Section, and Mr. M. Bryant from DER's Office of Enforcement visited Southern Wood Piedmont Co. on July 23, 1979. The facility was toured to observe the operating procedures and to find a suitable location for the mobile bioassay trailer. An effluent sample was obtained from NPDES outfall #002 and two samples of possible diluent water, one from the company well and one from the St. Mary's River near MacClenny were taken. Because of low pH (4.0) in the St. Mary's River water, which provided toxic to the test organism (Daphnia pulex), it was decided to use the well water (pH = 8).

Flint glass jars (250 ml capacity) were used as test containers. One control and six different concentrations of effluent were prepared, as indicated below:

CONCENTRATION (% effluent to diluent)	TIME Total # of Live Organism		
	0	24	48
Control	20	20	20
0.2%	20	17	17
1%	20	20	15
5%	20	19	8
25%	20	20	8
50%	20	19	13
100%	20	17	14

The final volume in each container was 200 ml. Dilutions ranged from 100% to 0.2% effluent. Three replicates of each concentration were tested, bringing the total number of test containers to 28. Five Daphnia pulex were transferred to each jar via micro-pipette at 1030 hours 24 July 1979, to begin the test.

Throughout the bioassay, the animals were maintained in constant fluorescent lighting and temperature (22.0°C). Live animals were counted at 24 and 48 hours. The survival of the Daphnia in each of the different concentrations is recorded above.

An LC_{50} could not be determined for this data set using either the binomial test, the moving average method, or the probit method, due to the abberant distribution of mortality. However, it was evident that the effluent exhibited some toxicity. Therefore, it was decided that a more definitive test would be necessary to evaluate this effluent.

Flow-through Test

DER's mobile bioassay laboratory was constructed following EPA design specifications (Peltier, 1978) and is equipped with two solenoid valve operated, flow-through, proportional dilutor systems. The mobile lab also has suitable working area for performing static tests concurrently with the flow-through tests.

The mobile laboratory was transported by commercial mover from its base in Tallahassee to the Southern Wood Piedmont facility located in Baldwin, Florida on August 3, 1979. The trailer was positioned adjacent to the NPDES discharge site (outfall #002) and power hook-up was then accomplished by plant electricians.

During the flow-through test the effluent sampling pump was submerged just below the outfall pipe (#002) and the effluent conveyed directly and continuously from the source to the proportional dilutor system. A 500 gallon Nalgene® tank was used as a reservoir for the diluent water which was obtained from the well on the plant grounds. To assure an adequate supply of diluent water in the reservoir throughout the test period, 200 gallons of fresh well-water were conveyed, by truck, daily from the well to the reservoir tank. The diluent sampling pump was submerged in the reservoir for a continuous flow to the dilutor system.

Preliminary calibration of the proportional dilutor was performed prior to moving the mobile laboratory to the test site (Table 1). Additionally, calibration was verified at the beginning and end of the 96-hour test. Flow rates were adjusted to operate the dilutor at 8.3 cycles per hour, which provided for a complete volume change in the flow-through aquaria approximately every 80 minutes. The dilutor system was operated for approximately 20 hours before the introduction of the test organisms at the beginning of the test at 8:00 a.m. on August 6, 1979.

Two replicas of six concentrations of effluent and a control were used. Pimephales promelas and Daphnia pulex were tested simultaneously in the same aquaria by segregating them into separate retention chambers within the tanks. The retention chambers consist of a standard 10 cm glass petri dish to which a 25 cm high cylinder of Nitex® screen (243 microns mesh-size) is attached by silicone cement. Ten fathead minnows (Pimephales promelas) were transferred individually, by net, into retention chambers placed in each of the 14 aquaria. Ten water fleas (Daphnia pulex) were transferred individually by glass tube into separate retention chambers in each of the aquaria.

Table 1. Effluent Concentrations (and calibrations) used in bioassay of Southern Wood Piedmont; Baldwin, Florida

Calibration of Flow-through Dilutor Unit A

I. Pre-dilution chamber was not required.

II. Concentrations and volumes delivered to aquaria each cycle Measured 5 August 1979 Values in parentheses indicate verification performed August 10, 1979.

Diluent	Effluent	Total	Final Concentration
0 ml (0)	900 ml (900)	900 ml	100%
398 ml (420)	498 ml (498)	896 ml	55.5%
624 ml (625)	289 ml (287)	913 ml	31.9%
750 ml.(755)	166 ml (165)	916 ml	18.1%
819 ml (818)	92 ml (91)	911 ml	10.0%
860 ml (858)	57 ml (56)	917 ml	6.2%

III. Flow rate adjusted to deliver::

8.3 cycles/hour which provided for a complete volume exchange in the flow-through aquaria approximately every 80 minutes.

Concentrations and Volumes Used in All Static Tests

Concentration	ml Diluent	ml Effluent	Total Volume
100%	-	200 ml	200 ml
55.5%	89 ml	111 ml	200 ml
31.9%	136.2 ml	63.8 ml	200 ml
18.1%	163.8 ml	36.2 ml	200 ml
10.0%	180 ml	20.0 ml	200 ml
6.2%	187.6 ml	12.4 ml	200 ml
Control	200 ml	-	200 ml

This arrangement resulted in twenty organisms of each type per concentration. Organisms were not fed during the test.

Temperature during the flow-through test was maintained within the range of 24.0°C to 24.5°C for the duration of the test by passing both effluent and diluent lines through a circulating temperature-controlled water-bath before passsing to the diluter system. Temperature, dissolved oxygen, total alkalinity, total hardness and pH were measured in each tank. Counts of the numbers of live organisms were made at 0 hours, 24 hours, 48 hours, 72 hours, and 96 hours. These values were recorded daily and appear in this report in Tables 2 and 3.

Static tests

Four 48 hour static acute toxicity bioassays were performed during this study. A grab sample of effluent was taken simultaneously with the beginning of the 96 hour flow-through test, and separate 48 hour static bioassays were conducted with fat-head minnows (Pimephales promelas) and water fleas (Daphnia pulex) using the same concentration series as the flow-through test. At the conclusion of these tests, two similar 48 hour bioassays were initiated with a fresh grab sample of effluent. Well water was used for dilution in all tests. Organisms were transferred (fathead minnows by net, water fleas by glass tube) individually, into 250 ml flint glass jars. Five organisms were placed in each container, ten per concentration. Temperature, dissolved oxygen, pH, and the number of live organisms were recorded for each container at 0 hours, 24 hours, and 48 hours for each test. These values were recorded daily and are presented in Tables 4 through 7. Organisms were not fed during tests.

Table 2. Data recorded during a 96-hour, Pimephales promelas, acute toxicity flow-through test at Southern Wood Piedmont, Baldwin, Florida, August 6 - 10, 1979.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A7F	10	10	10	10	10	7.8	7.4	5.9	6.4	6.8	8.1	8.3	7.8	8.2		250	260	280	275	280	250	220	225	235	245	24.5	24.0	24.0	24.0	24.0
Control	A7B	10	10	10	10	10	7.7	7.2	5.2	6.1	6.3	8.2	8.3	7.7	8.1		250	255	275	280	280	250	210	225	240	250	↓	↓	↓	↓	↓
6.2%	A5F	10	10	10	10	10	7.2	6.6	5.2	5.8	6.1	8.2	8.3	7.8	8.0		240	260	265	265	250	240	240	235	240	245					
6.2%	A5B	10	10	10	10	10	7.1	6.5	4.7	5.6	5.9	8.2	8.3	7.7	8.0		240	250	265	270	250	240	240	235	240	245					
10.0%	A4F	10	8	7	7	7	6.8	6.1	4.6	5.4	5.9	8.0	8.3	7.8	8.0		235	250	260	250	240	230	230	230	225	240					
10.0%	A4B	10	10	10	10	10	6.9	6.3	4.8	5.5	6.0	8.0	8.3	7.8	8.0		235	240	260	255	250	230	225	225	230	240					
18.1%	A3F	10	0	0	0	0	6.3	5.6	4.3	-	-	8.1	8.2	7.9	-	-	220	230	250	240	-	220	220	225	225	-					
18.1%	A3B	10	0	0	0	0	6.0	5.2	3.9	-	-	8.2	8.2	7.8	-	-	220	225	250	-	-	220	220	220	-	-					
31.9%	A2F	10	0	0	0	0	5.5	4.4	-	-	-	7.9	8.2	-	-	-	185	210	235	-	-	190	200	220	-	-					
31.9%	A2B	10	0	0	0	0	5.3	4.3	-	-	-	7.9	8.2	-	-	-	185	205	235	-	-	190	200	220	-	-					
55.5%	A1F	10	0	0	0	0	4.6	3.1	-	-	-	7.9	8.2	-	-	-	155	170	-	-	-	160	180	-	-	-					
55.5%	A1B	10	0	0	0	0	4.5	3.0	-	-	-	7.8	8.1	-	-	-	155	170	190	-	-	160	180	200	-	-					
100%	A6F	10	0	0	0	0	4.1	2.9	-	-	-	7.9	7.9	-	-	-	80	100	-	-	-	100	140	-	-	-					
100%	A6B	10	0	0	0	0	4.3	2.7	-	-	-	7.8	7.9	-	-	-	80	100	-	-	-	100	130	-	-	-	↓	↓	↓	↓	↓

Remarks: 6 Aug 79 - heavy rainfall night of 5 Aug
8 Aug 79 - Rainfall @ 3:30 p.m., moderate amount

[940 cycles
113.5 hours]

Table 3. Data recorded during a 96-hour, Daphnia pulex, acute toxicity flow-through test at Southern Wood Piedmont, Baldwin, Florida, August 6 - 10, 1979.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control		5	5	5	4	4																								
Control		5	4	4	4	4																								
6.2%		5	①3	3	3	2																								
6.2%		5	5	4	2	2																								
10.0%		5	5	4	3	2																								
10.0%		5	5	3	1	1																								
18.1%		5	5	1	0	0																								
18.1%		5	5	0	0	0																								
31.9%		5	5	0	0	0																								
41.9%		5	3	0	0	0																								
55.5%		5	0	0	0	0																								
55.5%		5	1	0	0	0																								
100%		5	0	0	0	0																								
100%		5	0	0	0	0																								

Remarks: For p-chem parameters see fathead sheet

7 Aug 79 - ① 2 dead at surface caught on screen

Table 4. Data recorded during a 48-hour, Pimephales promelas, acute toxicity static test at Southern Wood Piedmont, Baldwin, Florida, August 6 - 8, 1979.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control		5	5	5			5.7	7.1	6.8			8.0	8.3														22.0	21.0			
Control		5	5	5			5.4	5.5	7.9			8.0	8.4														↓	↓			
6.2%		5	5	5			5.8	6.6	7.3			8.1	8.3																		
6.2%		5	5	5			5.6	5.2	6.4			8.1	8.3																		
10.0%		5	4	4			5.9	6.2	7.1			8.1	8.3																		
10.0%		5	5	5			5.7	5.2	6.8			8.1	8.3																		
18.1%		5	0	0			5.7	1.1	3.1			8.0	8.0																		
18.1%		5	0	0			5.8	2.6	4.2			8.0	8.0																		
31.9%		5	0	0			5.3	0.6	0.7			8.0	7.9																		
31.9%		5	0	0			5.3	0.6	1.0			8.0	7.9																		
55.5%		5	0	0			5.4	0.6	2.2			8.0	7.8																		
55.5%		5	0	0			5.5	0.6	2.4			8.0	7.7																		
100%		5	0	0			5.0	0.7	3.7			7.9	7.7																		
100%		5	0	0			4.8	0.4	0.7			7.9	7.6																		

Remarks: 8 Aug 79 - Fathead assay accidentally terminated before pH or temperature recorded.

Table 5. Data recorded during a 48-hour, Pimephales promelas, acute toxicity static test at Southern Wood Piedmont, Baldwin, Florida, August 8 - 10, 1979.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control		5	5	5			6.9	5.3	7.1			7.8	8.4	8.2													22.0	21.0	21.0		
Control		5	5	5			6.9	5.7	6.9			7.8	8.3	8.1													↓	↓	↓		
6.2%		5	5	5			7.1	6.3	7.4			7.8	8.3	8.3																	
6.2%		5	5	5			6.9	5.8	6.5			7.8	8.2	8.2																	
10.0%		5	5	5			6.8	5.9	6.8			7.7	8.2	8.2																	
10.0%		5	5	5			6.7	4.9	6.2			7.7	8.2	8.1																	
18.1%		5	5	5			6.7	4.3	4.9			7.7	8.1	8.1																	
18.1%		5	5	5			6.9	4.9	6.5			7.8	8.1	8.1																	
31.9%		5	3	3			6.1	3.7	4.5			7.7	8.1	8.0																	
31.9%		5	3	3			6.2	3.7	4.8			7.7	8.0	8.0																	
55.5%		5	0	0			5.6	0.7	-			7.7	7.8	-																	
55.5%		5	0	0			5.7	0.4	-			7.7	7.8	-																	
100%		5	0	0			4.0	0.5	-			7.5	7.6	-																	
100%		5	0	0			4.1	0.6	-			7.5	7.6	-																	

Remarks:

Table 6. Data recorded during a 48-hour, Daphnia pulex, acute toxicity static test at Southern Wood Piedmont, Baldwin, Florida, August 6 - 8, 1979.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control		5	5	4①			5.6	7.8	8.5			8.0	8.5	8.5													22.0	21.0	21.0		
Control		5	5	4①			5.7	7.8	8.6			8.0	8.6	8.5													↓	↓	↓		
6.2%		5	5	5			5.7	8.0	8.4			8.1	8.5	8.4																	
6.2%		5	5	5			5.7	8.0	8.5			8.1	8.5	8.4																	
10.0%		5	5	5			5.8	7.7	8.8			8.1	8.5	8.4																	
10.0%		5	5	2			5.8	7.8	8.7			8.1	8.5	8.4																	
18.1%		5	5	4			5.8	7.4	8.4			8.0	8.2	8.3																	
18.1%		5	5	5			5.8	7.5	8.3			8.0	8.2	8.3																	
31.9%		5	2	0			5.5	7.1	8.1			8.0	8.1	8.2																	
31.9%		5	2	0			5.5	7.2	8.2			8.0	8.1	8.2																	
55.5%		5	0	0			5.3	6.8	7.6			8.0	8.0	8.0																	
55.5%		5	0	0			5.4	6.8	7.6			8.1	7.9	8.0																	
100%		5	0	0			4.8	6.4	6.9			7.9	7.9	7.6																	
100%		5	0	0			4.8	6.5	6.9			7.9	7.8	7.6																	

Remarks: 8 Aug 79 - ① Died - several offspring present

Table 7. Data recorded during a 48-hour, Daphnia pulex, acute toxicity static test at Southern Wood Piedmont, Baldwin, Florida, August 8 - 10, 1979.

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)					Temperature				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control		5	5	5			7.3	8.0	8.4			7.8	8.4	8.4													22.0	21.0	21.0		
Control		5	5	4			7.3	8.3	8.4			7.8	8.4	8.4																	
6.2%		5	5	4			7.2	7.2	8.1			7.9	8.4	8.3																	
6.2%		5	5	4			7.2	7.4	8.2			7.8	8.3	8.3																	
10.0%		5	5	3			6.9	7.4	8.0			7.8	8.3	8.3																	
10.0%		5	5	5			7.0	7.7	8.0			7.8	8.3	8.3																	
18.1%		5	5	3			7.2	7.3	7.9			7.8	8.2	8.3																	
18.1%		5	5	5			7.2	7.5	7.9			7.8	8.2	8.3																	
31.9%		5	4	0			6.5	6.9	7.5			7.7	8.2	8.2																	
31.9%		5	5	2			6.4	7.0	7.4			7.7	8.2	8.3																	
55.5%		5	5	1			5.7	6.6	7.6			7.7	8.1	8.1																	
55.5%		5	3	0			5.8	6.4	7.2			7.7	8.1	8.1																	
100%		5	0	0			3.9	6.1	-			7.5	7.8	-																	
100%		5	2	0			3.8	6.0	7.0			7.5	7.8	7.8													↓	↓	↓		

Remarks:

Table 8. LC_{50} values obtained from bioassay of Southern Wood Piedmont, Baldwin, Florida, 6 - 10 August 1979.

Organism	Type	Data Analysis Method	LC_{50} 24 hour	LC_{50} 48 hour	LC_{50} 72 hour	LC_{50} 96 hour
Pimephales promelas	Flow-Through	Binomial test	12.62	12.32	12.32	12.32
		Moving Average Method	A	A	A	A
		Probit Method	A	A	A	A
Daphnia pulex	Flow-Through	Binomial test	40.1	12.03	6.2[C]	B
		Moving Average Method	40.42	10.19	6.2[C]	B
		Probit Method	34.23[D]	10.04	7.0[C]	5.92[C]
			#1		#2	
			24 hours	48 hours	24 hours	48 hours
Pimephales promelas	Static	Binomial test	12.64	12.64	34.22	34.22
		Moving average method	A	A	A	A
		Probit Method	A	A	A	A
			#1		#2	
			24 hours	48 hours	24 hours	48 hours
Daphnia pulex	Static	Binomial test	29.69	22.64	74.5	24.0
		Moving average method	A	17.83	74.5	20.2
		Probit Method	A	18.41[D]	71.3	19.58

A. When there are less than two concentrations at which the percent dead is between 0 and 100, neither the moving average nor the probit method can give statistically sound results.

B. Neither the Binomial test nor the moving average method can give any results for this data set. Either the highest concentration killed less than 50 percent or the lowest killed more than 50.

C. This data set does not meet the criteria established by the committee on methods for toxicity tests with aquatic organisms because no percent dead is less than 35 percent. (Or no percent dead is greater than 65 percent.)

D. Since the probability is less than 0.05, results calculated using the probit method probably should not be used.

RESULTS AND DISCUSSION

Effluent from the Southern Wood Piedmont facility caused significant mortality in all tests performed during this study (Table 8). Results from the 96 hour flow-through bioassays gave a 96 hour LC_{50} of 12% (i.e., approximately 1 part effluent to 8 parts diluent) for the Pimephales promelas and 6% for Daphnia pulex. Forty-eight hour static bioassays performed with a grab sample of effluent taken at the beginning of the 96 hour flow-through test on the 6th of August, resulted in 48 hour LC_{50} values of 13% for Pimephales promelas and 22% for Daphnia pulex. Results from the second set of 48 hour static bioassays performed on a new grab sample taken on the 8th of August, gave 48 hour LC_{50} values of 34% for the Pimephales promelas and 24% for Daphnia pulex, indicating a relative reduction in toxicity of this grab sample in relation to the first.

The cause for this variation in the toxicity of the effluent is believed to be due to runoff contamination from adjacent drying and holding areas where considerable quantities of freshly treated (pressure treated with creosote, pentachlorophenol and other preserving agents) utility poles and railroad crossties are temporarily stored. The ground in this area is contaminated with the drippings from these freshly treated products. Any significant rainfall would incorporate these preserving agents in runoff that would flow to the effluent canal bordering the low side of these drying and holding areas.

A heavy rainfall event on the 5th of August (the day prior to beginning of testing) is likely to have carried significant amounts of preserving agents into the effluent canal by observable runoff from this area. (See sketch of the facility in Figure 3.) A very light rain occurred in the afternoon of 8 August, but was not severe enough to cause noticeable runoff. The remainder of the study period was without rain.

The difference between the values obtained for the fathead minnow and the water flea reflects the relative sensitivity of each species to this effluent. Though none of the organisms were fed during the tests, it is probable that the filter-feeding habits of the Daphnia allowed them to ingest particulate material with adsorbed toxicants, thereby causing increasing mortality through time.

Fathead minnow mortality generally did not increase after 24 hours of exposure in all three tests performed on this fish. This is a common occurrence with this species and is consistent with EPA experience using this organism (Ron Weldon, U.S. EPA, personal communication).

When dissolved oxygen levels were measured in the fathead minnow static tests at 24 hours, values obtained were sharply reduced from those measured at the beginning of the test. This effect was observed only in the highest concentrations, where significant mortality had occurred. Several factors are believed to have contributed to these reduced levels of dissolved oxygen. First, the 200 ml volume used in these static tests was small enough so that any significant oxygen demand present was likely to result in an appreciable decrease in D.O. This volume may have been inadequate for testing fathead minnows. Increased respiration of the test fish was noticeable in the three highest concentrations soon after their introduction to the test chambers. D.O. levels measured at the beginning of these tests were adequate. All of the test fish, in the four highest concentrations of the first fathead static test and the three highest concentrations of the second test, had expired during the first 24 hours. These dead fish were present in the test chambers for at least several hours, before the 24 hour D.O. was measured, allowing decomposition to reduce the recorded dissolved oxygen levels.

Fathead minnows exposed to the same concentration series in the 96 hour flow-through bioassay exhibited a similar reaction to this effluent, in that, they also showed increased respiration at the highest concentrations soon after introduction to the test chambers. In this test, all the fish in the four highest concentrations were also dead within 24 hours. In fact, LC_{50} values calculated for the fathead minnows in both the flow-through bioassay and the first static bioassay were virtually identical at 24 hours. During the static tests performed on Daphnia pulex, dissolved oxygen remained high, and yet, significant mortality was occurring at the same concentrations that were affecting the fathead minnows.

Based on these observations, and on the data from the tests, it is likely that the reduced D.O. levels in the fathead static bioassays at 24 hours were caused by toxicity stress on the fish (i.e., increasing respiration) and decomposition of dead fish in the test chambers. It is possible that reduced D.O. in some test chambers increased mortality to a limited extent, beyond that caused by toxicity of the effluent alone. However, data from the two Daphnia bioassays and from the flow-through fathead minnow bioassay indicate that mortality was most directly related to toxicity, and that this mortality was the primary cause of the low D.O. rather than reduced D.O. causing the death of the fish in these tests. In any case, because of these complications, the results determined from the fathead static bioassays are somewhat dubious, and results from the flow-through tests on both species and from the Daphnia static bioassay should be considered more reliable.

LITERATURE CITED

Peltier, William. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. Environmental Research Laboratory. Athens, Georgia EPA-600/4-78-012 (Revised July 1978) 51 p.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010.