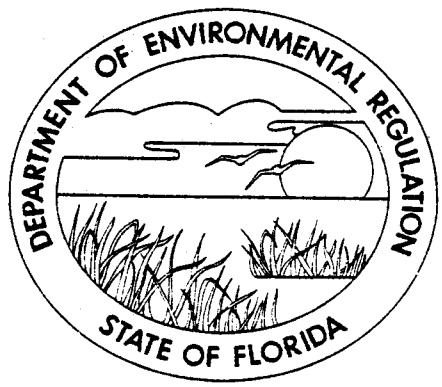


BIOASSAYS OF BORDO CITRUS

PRODUCTS COOPERATIVE

POLK COUNTY, FLORIDA

NPDES #FL0027234

SAMPLED 1/19/81

Biological Section
Bureau of Water Analysis
April 24, 1981

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INTRODUCTION

Bordo Citrus Products Cooperative is located on 7th Street, SW, in Winter Haven, Polk County, Florida (Fig. 1). The facility processes citrus concentrate and has a suspected discharge volume of 1.39 MGD. No calibration information or records are available. Wastewater is generated from October through May and consists of noncontact cooling and process waters. According to the NPDES permit (issued in June 1976) process water treatment includes: shaker and static screening followed by aeration tanks, settling tanks, aeration ponds, settling ponds, and, finally, retention lagoons prior to discharge. Noncontact cooling water joins the treatment process at the aeration ponds. The retention lagoon discharge (Outfall #001) is to the Peace River via Wahneta Farms Drainage Canal (Fig. 2).

Bordo Citrus Products Coop. is currently upgrading the treatment system (DER Construction Permit #IC-29890 expires in August 1981). Presumably, this includes the repair of retention pond dikes, the reduction of excessive BOD and total Kjeldahl nitrogen and the increase of effluent dissolved oxygen content.

To evaluate the existence and extent of toxicity to the biota of the receiving waters, the Biological Section performed several 48 hour static bioassays on effluent samples from Bordo Citrus Products Coop. The results of these tests are reported here.

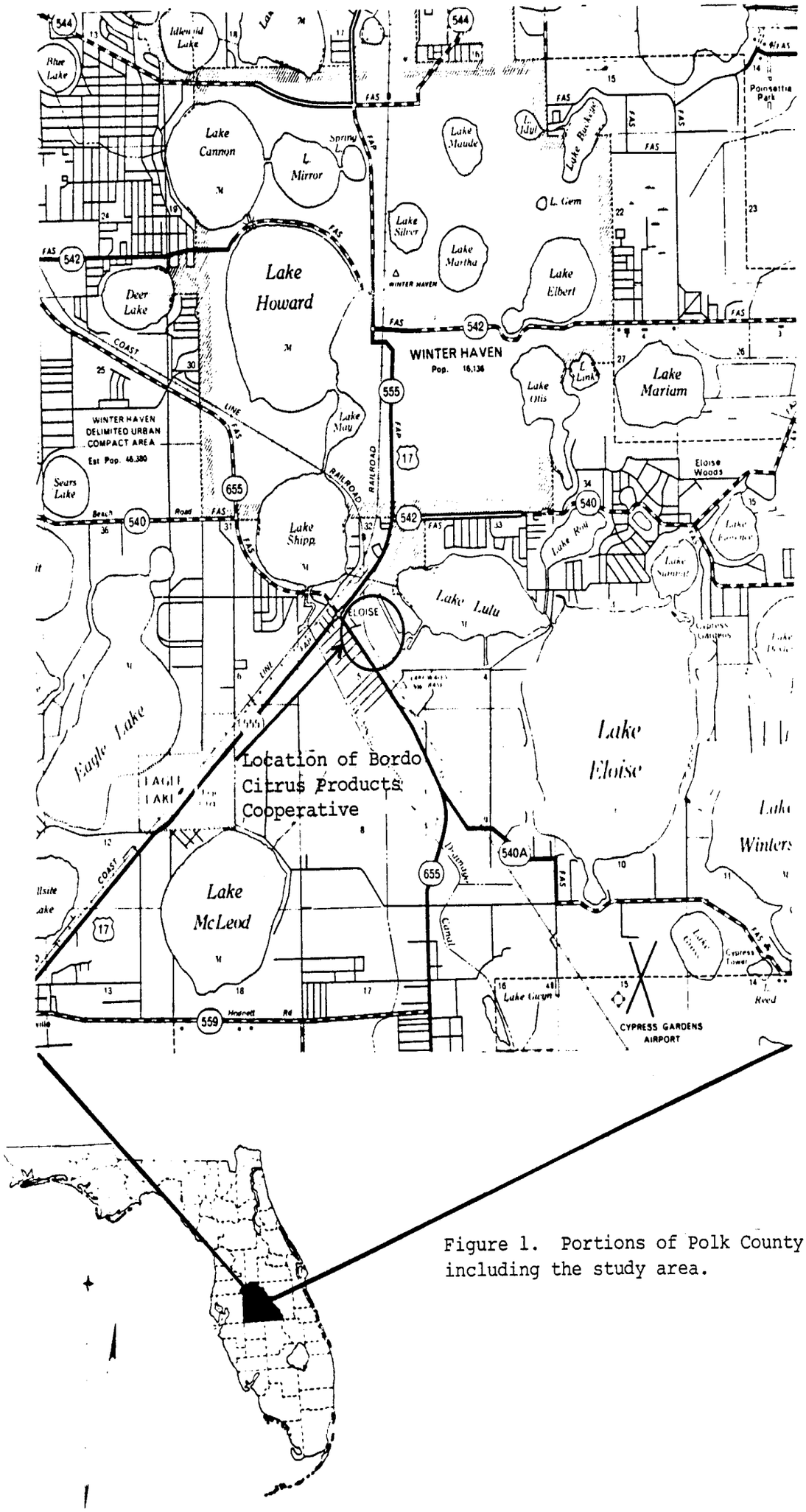
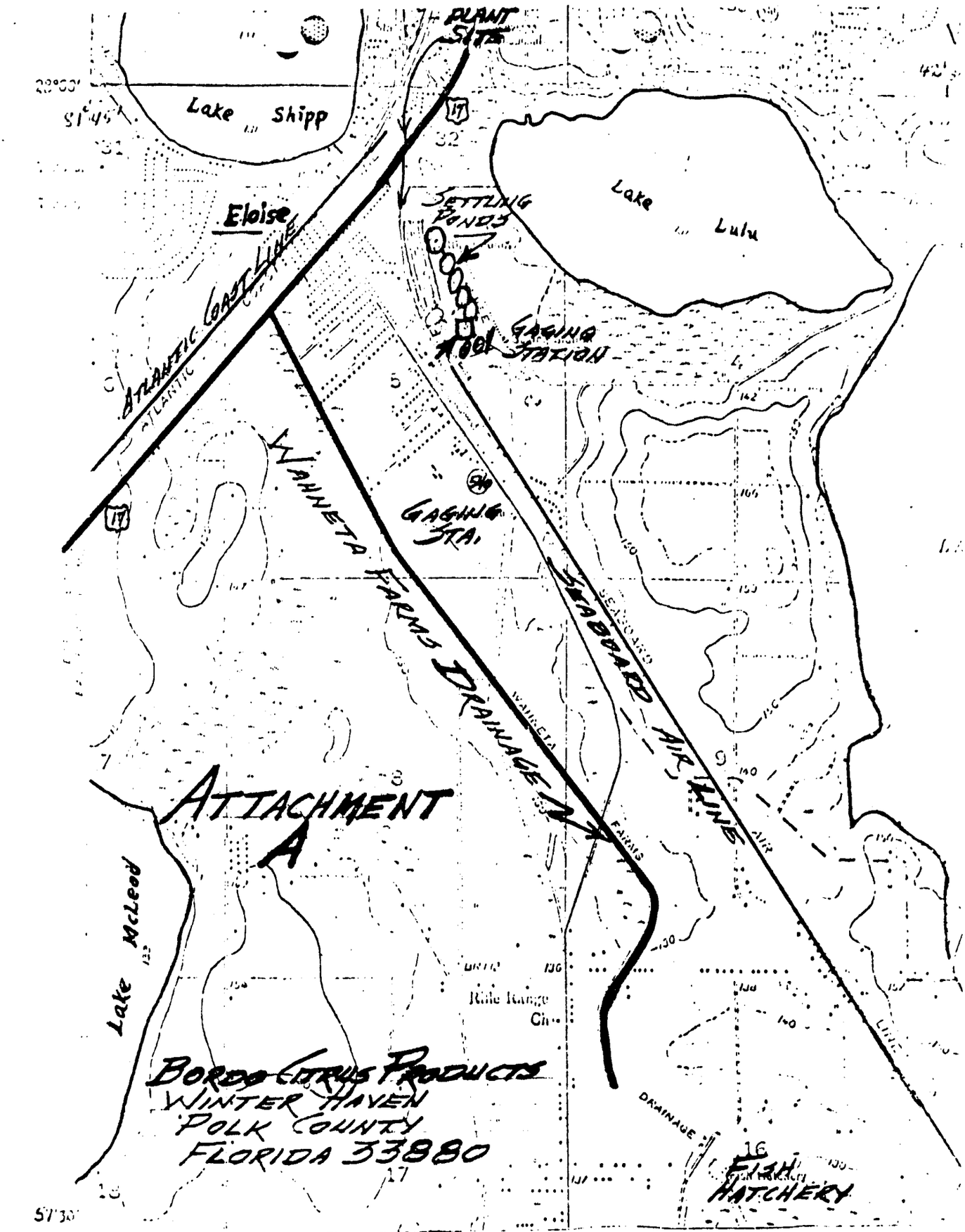


Figure 1. Portions of Polk County including the study area.



Mapped, edited, and published by the Geological Survey

Control by USGS, USC&GS, and Florida Geodetic Survey

Culture and drainage in part compiled from aerial photographs taken 1952 at 1:20,000. Topography by plane-table surveys 1956-1959

Polyconic projection. 1927 North American datum

10,000-foot grid based on Florida coordinate system, west zone

1,000-meter Universal Transverse Mercator grid ticks, zone 17, shown in blue

Fine red dashed lines indicate selected fence and field lines visible on aerial photographs. This information is unchecked

Figure 2. Wastewater discharge, Bordo Citrus Products Coop., Winter Haven, Polk County, Florida.

METHODS AND MATERIALS

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

History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of *Daphnia pulex* was obtained from the EPA laboratory in Athens, Georgia in March, 1979. This culture has been maintained since its arrival, in 2½ gallon all glass aquaria filled with unchlorinated DER well water, using a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess daphnids. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Simocephalus serrulatus (water flea) - An indigenous breeding population of *S. serrulatus* was collected from Lake Jackson, Leon County, Florida, in March, 1980, by personnel from DER's Biological Section. Positive identification of the organism was performed by Mr. Jeff Foran of the Department of Environmental Engineering Sciences, University of Florida in Gainesville. The cultures are maintained in 2½ gallon all glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. The organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess population. They are then refilled with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances.

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

Four separate 48 hour static bioassays were conducted from 1025 hours 20 January to 1040 hours 22 January 1981, on a grab sample of effluent from Outfall #001. The sample was collected by J. H. Kroll of the DER Southwest District, at 1350 hours on 19 January 1981, using standard wastewater sampling techniques. The unpreserved sample was iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

Freshwater for dilution of the effluent sample was obtained from the same DER well that is used to culture the test organisms. The concentrations series selected for this study (Table 1) is that recommended by APHA (1976).

Because of the low dissolved oxygen content and the suspected high BOD of this effluent, two duplicate static bioassays each for Daphnia pulex and Simocephalus serrulatus were set up. In the first test both the effluent and the dilution water were aerated to near saturation prior to the bioassay. Aeration was then discontinued for the remainder of the test period. In the duplicate bioassays each concentration was gently aerated throughout the 48 hour test period.

For each of the four bioassays, two replicates of each of the six concentrations and the control, were used. Flint glass jars with a 250 ml capacity were used as test chambers, and 200 ml of the appropriate test concentration was placed in each chamber. Daphnia and Simocephalus were siphoned into respective Carolina® culture dishes through a series of nested stainless steel screens to isolate the proper size and age organisms (neonates). These neonates were then transferred individually, examined for injury, and loaded 10 per chamber, resulting in 20 per test concentration.

Temperature, dissolved oxygen, pH, and the number of live organisms were recorded at 0, 24 and 48 hours. Total alkalinity, total hardness, and conductivity were recorded at the beginning of the test for the controls and the 100% concentration. These values were recorded on the bioassay data forms. Organisms were not fed during these tests.

Agitation associated with the continuous aeration procedures coupled with air entrapment on the carapace of the test organisms resulted in stress and random mortality in both the Daphnia pulex and Simocephalus serrulatus bioassays. This resulted in the invalidation of the aerated Simocephalus serrulatus bioassay at 24 hours of exposure and these data have not been included in this report. Additionally, the unaerated Simocephalus serrulatus bioassay had to be invalidated at 48 hours due to excessive (i.e. >20%) control mortality.

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

Water fleas (Daphnia pulex and Simocephalus serrulatus)

Concentration	Dilution Water	Effluent	Total Vol.
Control	200 ml	---	200 ml
5.6%	188.8 ml	11.2 ml	200 ml
10.0%	180 ml	20 ml	200 ml
18.0%	164 ml	36 ml	200 ml
32.0%	136 ml	64 ml	200 ml
56.0%	88 ml	112 ml	200 ml
100%	----	200 ml	200 ml

Table 2. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Bordo Citrus Products Cooperative, Winter Haven, Polk County, Florida, NPDES Permit #FL0027243, Outfall #001. From 1025 hours 20 January to 1025 hours 22 January 1981. Unaerated test.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control A	10	10	10	8.9	8.7	8.9	7.7	8.3	8.2	110		113		20.2	20.7	20.1			200
Control B	10	10	10	8.9	8.7	9.1	7.7	8.3	8.3										
5.6% A	10	10	10	8.8	8.5	9.0	7.7	8.3	8.2					20.0	20.3	19.9			
5.6% B	10	10	10	8.8	8.5	8.9	7.7	8.3	8.2										
10.0% A	10	10	10	8.7	8.4	8.9	7.7	8.2	8.2					19.9	20.1	19.7			
10.0% B	10	10	10	8.7	8.3	8.9	7.7	8.2	8.2										
18.0% A	10	10	10	8.7	8.2	8.7	7.7	8.2	8.2					19.9	20.0	19.7			
18.0% B	10	10	10	8.6	8.1	8.7	7.7	8.2	8.2										
32.0% A	10	10	10	8.3	7.6	8.3	7.7	8.2	8.1					19.9	19.9	19.6			
32.0% B	10	10	10	8.3	7.5	8.2	7.7	8.1	8.1										
56.0% A	10	10	10	7.2	6.9	7.8	7.6	8.1	8.1					19.8	19.8	19.7			
56.0% B	10	10	10	7.1	6.9	7.8	7.6	8.1	8.1										
100% A	10	10	10	6.1	5.6	7.0	7.5	8.1	8.1					19.8	20.0	19.8			
100% B	10	10	10	6.2	5.2	6.8	7.5	8.1	8.1	188		130							380

Remarks:

Table 3. Data recorded during 48 hour, Daphnia pulex, static acute toxicity bioassay of Bordo Citrus Products Cooperative, Winter Haven, Polk County, Florida, NPDES #FL0027243, Outfall #001. From 1040 hours 20 January to 1040 hours 22 January 1981. Continuously Aerated Test.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (μmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control A	10	10	10	8.8	8.9	9.1	7.7	8.4	8.2	110		113		20.4	20.1	20.1			200
Control B	10	10	10	8.8	9.0	9.1	7.8	8.3	8.3										
5.6% A	10	9	6	8.7	9.0	9.0	7.8	8.4	8.2					20.2	20.2	20.0			
5.6% B	10	7	7	8.7	9.0	8.8	7.8	8.3	8.2										
10.0% A	10	10	10	8.7	9.0	8.9	7.8	8.3	8.2					20.1	20.4	20.3			
10.0% B	10	7	7	8.7	9.0	8.8	7.8	8.3	8.2										
18.0% A	10	10	6	8.5	9.0	8.7	7.8	8.3	8.2					20.1	20.8	20.3			
18.0% B	10	10	7	8.5	9.0	8.7	7.8	8.3	8.2										
32.0% A	10	7	7	8.1	8.9	8.3	7.7	8.4	8.2					20.9	20.9	20.3			
32.0% B	10	8	7	8.1	8.9	8.3	7.7	8.3	8.2										
56.0% A	10	10	10	7.1	8.9	7.7	7.6	8.4	8.1					19.9	21.0	20.3			
56.0% B	10	9	9	7.1	8.9	7.6	7.6	8.4	8.1										
100% A	10	10	10	5.7	8.4	6.9	7.5	8.4	8.1					20.0	21.4	20.5			
100% B	10	9	8	5.3	8.7	6.9	7.5	8.6	8.1	188		130							380

Remarks:

Table 4. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Bordo Citrus Products Cooperative, Winter Haven, Polk County, Florida, NPDES #FL0027243, Outfall 001. From 1025 hours 20 January to 1025 hours 22 January 1981. Unaerated Test.

Conc. or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Total Alkalinity (mg/ℓ as $CaCO_3$)		Total Hardness (mg/ℓ as $CaCO_3$)		Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Conductivity (µmhos)
	0	24	48	0	24	48	0	24	48	0	48	0	48	0	24	48	0	48	0
Control A	10	8	5	8.6	9.0	9.0	7.7	8.3	8.4	110		113		20.3	21.0	20.2			200
Control B	10	8	7	8.8	9.0	8.9	7.8	8.3	8.4										
5.6% A	10	10	10	8.8	9.0	8.9	7.8	8.3	8.4					20.1	20.8	19.9			
5.6% A	10	10	9	8.8	9.0	8.9	7.8	8.3	8.3										
10.0% A	10	9	9	8.6	8.8	8.9	7.8	8.3	8.3					20.0	20.4	19.7			
10.0% B	10	10	10	8.6	8.8	8.9	7.8	8.3	8.3										
18.0% A	10	10	10	8.4	8.5	8.6	7.7	8.2	8.3					20.0	20.4	19.8			
18.0% B	10	10	9	8.4	8.5	8.6	7.7	8.2	8.2										
32.0% A	10	10	9	8.1	7.8	8.2	7.7	8.2	8.3					20.0	21.4	19.7			
32.0% B	10	10	9	8.1	7.8	8.2	7.7	8.2	8.2										
56.0% A	10	8	8	7.7	7.1	7.8	7.6	8.2	8.1					19.8	21.5	19.8			
56.0% B	10	8	8	7.5	7.1	7.8	7.6	8.2	8.1										
100% A	10	6	0	5.9	5.7	6.9	7.5	8.1	8.1					19.7	20.5	20.0			
100% B	10	8	8	5.4	5.4	6.9	7.5	8.1	8.1	188		130							380

Remarks: [a] Test invalidated at 48 hours due to excessive control mortality (>20%).

RESULTS AND DISCUSSION

This particular grab sample from Outfall #001 of Bordo Citrus Products Coop. proved to be non-toxic to Daphnia pulex in the unaerated bioassay. In fact, not a single organism died during the course of this test (Table 2). In the Daphnia pulex aerated bioassay (Table 3) the mortality observed at 24 and 48 hours was attributed either directly to the aeration procedure (by air bubble formation under the carapace or by organisms sticking to the test chamber walls above the water line) or, indirectly by stressing the organisms through continuous agitation. In either case, the distribution of mortality was such that a statistically sound LC_{50} could not be calculated (Table 5).

The aerated Simocephalus serrulatus bioassay was invalidated at 24 hours because of excessive control mortality (>20%) and random mortality throughout the test concentrations. The unaerated bioassay resulted in a 24 hour LC_{50} of >100% (Table 5). At 48 hours, although a statistically sound LC_{50} of < 100% could be calculated, this test was invalidated due to excessive control mortality.

Table 5. LC_{50} Data for Bioassay of Bordo Citrus Products Cooperative, NPDES #FL0027243, Outfall #001, on 20 to 22 January 1981.

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Daphnia pulex	Binomial	A, B	A, B
	Moving Average	A, B	A, B
	Probit	B, C	B, C
Simocephalus serrulatus	Binomial	A, B	D
	Moving Average	A, B	D
	Probit	208.3 (107-2877), B	D

A. Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, and LC_{50} value cannot be calculated using this method of statistical analysis.

B. Due to the distribution of mortality, the LC_{50} value calculated by this method of statistical analysis does not meet the criteria established by the Committee on Methods for Toxicity Tests with Aquatic Organisms.

C. Due to the distribution of mortality, results using the probit method are not statistically sound.

D. Test invalidated due to excessive control mortality (>20%).

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