BIOASSAYS OF CAPE KENNEDY NASA IV WASTEWATER TREATMENT PLANT

J. F. KENNEDY SPACE CENTER
NPDES #FL0022594

Biological Section
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INTRODUCTION

The Cape Kennedy NASA IV Wastewater Treatment Plant is a U.S. government installation located in the Kennedy Space Center, Brevard County, Florida (Fig. 1). The facility serves nearby Air Force and Naval Air Station personnel and their operations, including wastewater from cooling towers, heat plants and vehicle maintenance facilities. The plant has a maximum capacity of 0.108 MGD and discharges from a final polishing pond through Banana Creek into the Indian River.

On 11 to 13 March, 1981, the Biological Section performed static toxicity bioassays on samples of effluent from the Cape Kennedy NASA IV Plant to determine the existence and extent of toxicity to the biota of the receiving waters. The results of these tests are presented in this report.

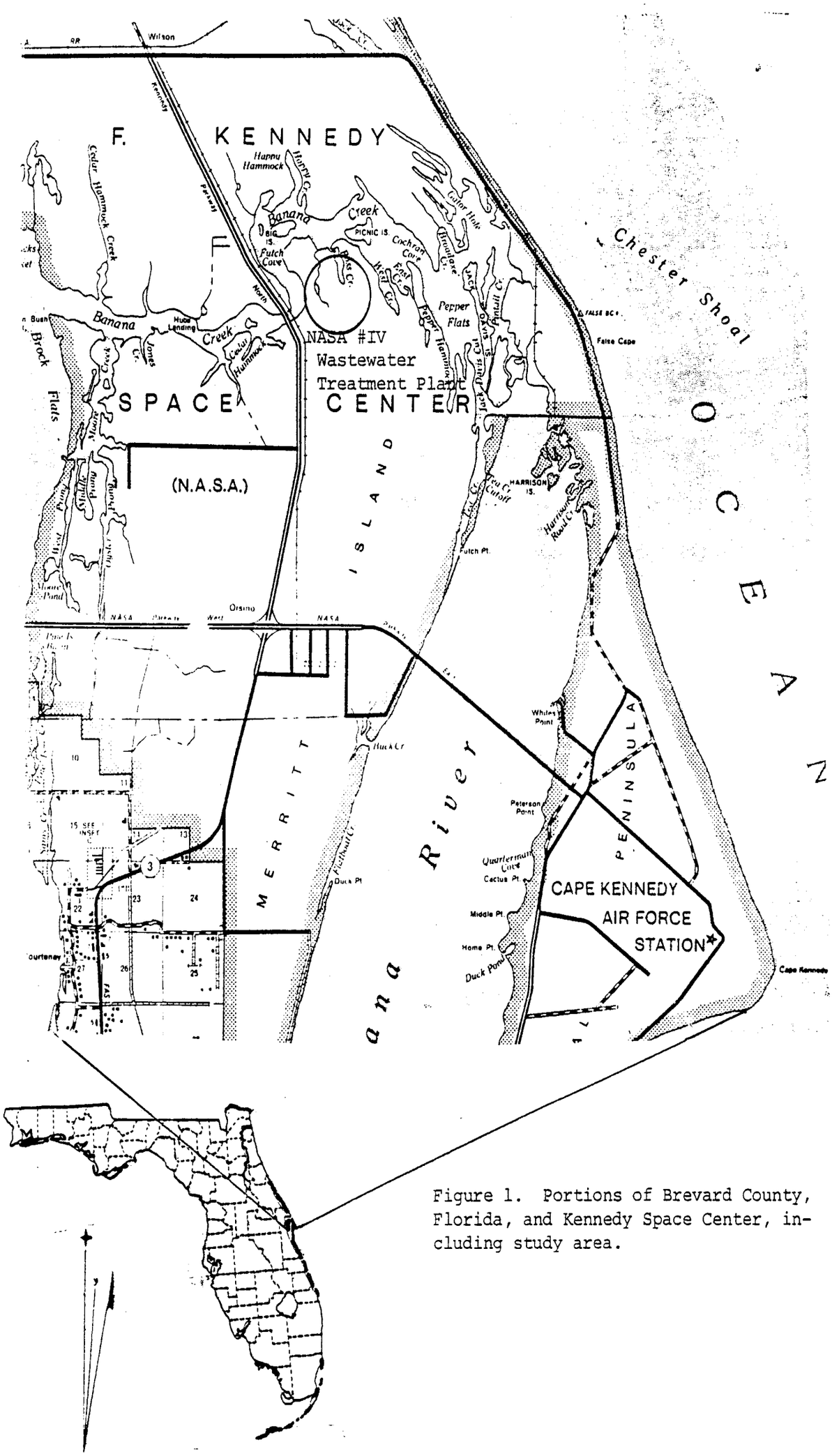


Figure 1. Portions of Brevard County, Florida, and Kennedy Space Center, including study area.

METHODS AND MATERIALS

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

History and Maintenance of Bioassays Organisms

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the Gulf Breeze EPA Laboratory in Pensacola, Florida. The mysids have been cultured under continuous lighting in 10 and 15 gallon all glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Sea water is obtained from the FSU Marine Laboratory located at Turkey Point, Florida (salinity range 17-31 ppt) and partial water changes are performed weekly. Fresh water, for dilution of the sea water as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of $22^{o} \pm 1^{o}C$. To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly Standard Reference Toxicant Bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Three separate 48 hour static bioassays were conducted from 1100 hours on 11 March to 1100 hours on 13 March. Grab samples of chlorinated effluent (Outfall #001) and of effluent collected prior to chlorination were obtained by Mr. Louis Ley of DER's St. Johns River District at 1725 hours on 10 March, using standard wastewater sampling procedures. The samples for the bioassays were unpreserved, iced, and were transported to the DER laboratory in Tallahassee within 24 hours of collection.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biological Section adopted the following test procedure:

Test #1. Pre-Chlorinated Effluent (Table 2). This test was performed on an effluent sample collected at the end of the treatment process but prior to chlorination. It was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent (Table 3). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-Chlorinated Effluent (Table 4). This test was performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized[1]. This test was intended to evaluate the combined toxicity of the

[1] De-chlorination was accomplished by titrating with an appropriate amount of sodium thiosulfate.

effluent and the chlorine reaction products.

Saltwater for dilution of the effluent samples was obtained from the FSU Marine Laboratory at Turkey Point (salinity=31.0 ppt). This saltwater diluent was then adjusted to the salinity of the receiving body of water (Banana Creek = 24 ppt) by adding an appropriate amount of de-ionized water. For test concentrations of 56% and below, this adjusted diluent was mixed directly with the effluent, achieving a range of final salinities (11.5 ppt to 23 ppt). For the 100% effluent test concentrations, freshly autoclaved artificial sea salts (Ocean 50®) were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerances). A series of three control groups (Table 5) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included: adjusted Turkey Point seawater (salinity=24 ppt), artificial sea salt (Ocean 50®) adjusted de-ionized water (salinity = 7 ppt), and a salinity control consisting of adjusted Turkey Point seawater and de-ionized water (salinity = 11 ppt).

Two replicates of each test concentration and the controls were used in these static tests. Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the 10 ppt, 20 ppt, and 25 ppt salinity aquaria to culture dishes for observation and selection of individuals for the tests. Individual mysid shrimp were siphoned via glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration the mysid shrimp were pre-acclimated to within 4 ppt salinity. The concentration series selected for this study (Table 1) are those recommended by the APHA (1976).

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

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

Mysidopsis bahia (mysid shrimp)

Concentration	Dilution Water	Effluent	Total Vol.
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%	---	500 ml	500 ml

Table 2. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Kennedy NASA IV Wastewater Treatment Plant, Brevard County, Florida. NPDES #FL0022594, on 11 to 13 March, 1981.

Test #1. Pre-Chlorinated Sample.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 [b] (mg/ℓ)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
5.6%	5	4(5)[a]	4(5)[a]	7.0	7.2	7.2	8.1	7.7	7.7	21.0	19.5	19.4			23.0
5.6%	5	5	5	7.1	7.3	7.4	8.1	7.8	7.8						
10%	5	5	5	7.0	7.5	7.6	8.1	7.8	7.8	20.8	19.5	19.4			22.0
10%	5	5	5	7.1	7.5	7.6	8.1	7.8	7.9						
18%	5	5	5	6.9	7.6	7.7	8.0	7.8	7.8	20.7	19.5	19.3			20.5
18%	5	5	5	7.0	7.6	7.7	8.0	7.8	7.8						
32%	5	5	5	6.8	7.6	7.9	7.7	7.7	7.8	20.6	19.5	19.3			17.0
32%	5	4	4	6.6	7.6	8.0	7.7	7.6	7.8						
56%	5	5	5	6.2	7.6	7.8	7.2	7.5	7.5	20.6	19.5	19.3			11.5
56%	5	5	5	6.0	7.6	7.8	7.2	7.4	7.4						
100%	5	5	5	6.6	7.7	7.7	7.6	7.4	7.4	21.2	19.5	19.3	.03/1.82	.03/1.56	7.5
100%	5	5	5	6.7	7.7	7.6	7.6	7.4	7.3						

Remarks:

[a] Accidental mortality due to cannibalism; therefore, number in parentheses used in calculations.

[b] Total ammonia is expressed as mg/l as N, and unionized ammonia is expressed in mg/l as NH_3.

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Kennedy NASA IV Wastewater Treatment Plant, Brevard County, Florida, NPDES #FL0022594, on 11 to 13 March, 1981.

Test #2. Chlorinated Sample.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 [a] (mg/ℓ)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
5.6%	5	5	5	6.6	7.5	7.5	8.0	8.2	7.5	21.0	19.3	19.3			23.0
5.6%	5	5	5	6.6	7.5	7.5	8.0	8.2	7.6						
10%	5	5	5	7.0	7.5	7.3	8.0	8.0	7.5	20.8	19.3	19.3			22.0
10%	5	5	5	7.1	7.5	7.3	8.0	8.0	7.7						
18%	5	0	---	7.1	8.1	---	7.6	7.8	---	20.6	19.3	19.2			20.0
18%	5	0	---	7.1	8.1	---	7.6	7.8	---						
32%	6	0	---	7.6	8.2	---	7.2	7.7	---	20.8	19.4	19.2			17.0
32%	6	0	---	7.6	8.2	---	7.2	7.6	---						
56%	5	0	---	7.7	8.3	---	6.6	7.4	---	20.6	19.4	19.2			11.5
56%	5	0	---	7.7	8.3	---	6.6	7.3	---						
100%	5	0	---	7.2	8.6	---	6.4	6.9	---	20.8	19.5	19.2	0/.45	.01/1.12	7.0
100%	5	0	---	7.3	8.6	---	6.4	6.9	---						

Remarks: Total chlorine residuals determined by the Hach DPD method:

5.6% = 0.25 mg/l 32% = 1.4 mg/l
10% = 0.4 mg/l 56% = 2.1 mg/l
18% = 0.8 mg/l 100% = 2.8 mg/l

[a] Total ammonia is expressed in mg/l as N, and unionized ammonia is expressed in mg/l as NH_3.

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Kennedy NASA IV, Wastewater Treatment Plant, Brevard County, Florida. NPDES #FL0022594, on 11 to 13 March, 1981.

Test #3. De-Chlorinated Sample.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 [a] (mg/ℓ)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
5.6%	5	5	4	7.3	7.3	7.1	8.0	7.8	7.6	21.0	19.4	19.5			22.5
5.6%	5	5	5	7.3	7.3	7.2	8.0	7.8	7.6						
10%	5	5	5	7.3	7.2	7.3	8.0	7.7	7.6	21.1	19.4	19.5			22.0
10%	5	5	5	7.4	7.2	7.4	8.0	7.7	7.6						
18%	5	5	5	7.2	7.3	7.3	7.8	7.7	7.5	21.3	19.4	19.4			20.0
18%	5	5	5	7.3	7.2	7.3	7.8	7.7	7.5						
32%	5	5	5	7.3	7.3	7.8	7.4	7.6	7.4	21.8	19.5	19.4			17.5
32%	5	5	5	7.3	7.3	7.5	7.4	7.6	7.4						
56%	5	5	5	7.2	7.3	7.6	6.8	7.4	7.4	22.4	19.5	19.5			12.0
56%	5	5	5	7.2	7.3	7.7	6.8	7.4	7.3						
100%	5	5	4	7.5	7.5	7.6	7.1	7.2	7.1	22.6	19.5	19.6	0/.45	.01/1.12	7.5
100%	5	5	5	7.7	7.5	7.6	7.1	7.2	7.1						

Remarks: Total residual chlorine in 100% effluent = 0.03 mg/l (Hach DPD Method).
[a]Total ammonia is expressed as mg/l as N, and unionized ammonia is expressed in mg/l as NH_3.

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Kennedy NASA IV Wastewater Treatment Plant, Brevard County, Florida. NPDES #FL0022594 on 11 to 13 March, 1981.

Test #4. Control Series

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4[b] (mg/ℓ)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
Seawater Control	5	5	5	7.6	7.5	7.5	8.0	7.8	7.6	20.8	19.5	19.4	.01/.142	0/.312	24.0
Seawater Control	5	4	4	7.6	7.4	7.5	8.0	7.8	7.6						
Seasalt Control	5	5	5	6.5	7.8	7.8	8.5[a]	7.6	7.7	22.8	19.5	19.4			7.0
Seasalt Control	5	5	5	6.5	7.8	7.8	8.5[a]	7.5	7.7						
Salinity Control	5	5	5	5.9	7.7	7.8	8.4	7.4	7.4	22.5	19.5	19.8			11.0
Salinity Control	5	4	4	5.8	7.7	7.8	8.4	7.4	7.5						

Remarks:

[a] Initial pH = 10, adjusted to 8.5 with 2N HCl.

[b] Total ammonia is expressed in mg/l as N, and unionized ammonia is expressed in mg/l as NH_3.

RESULTS AND DISCUSSION

Neither the pre-chlorinated nor the de-chlorinated effluent samples from the Cape Kennedy NASA IV Plant exhibited toxicity to the test organism, *Mysidopsis bahia*. However, the chlorinated sample proved to be highly toxic to the mysid shrimp. The 24 and 48 hour LC_{50} for this test was 13.4% (Table 6). The 96 hour LC_{50} is therefore $\leq$13.4%.

The toxicity observed here can be directly attributed to chlorine, as the 2.8 ppm residual chlorine measured in the 100% effluent (Table 3) is well above the tolerance of mysid shrimp (U.S. EPA 1977). The low pH and dissolved oxygen measured at the time of collection may be in violation of the NPDES permit.

Chapter 17-3.051, Florida Administrative Code, indicates that "all waters of the State shall at all places and at all times be free from...components of discharges which...are acutely toxic." "Acute toxicity" is defined in Chapter 17-3.021(1) to mean "the presence of one or more substances...in amounts which are greater than one-third (1/3) of the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50})...". In the absence of a mixing zone, this 1/3 of the 96 hour LC_{50} applies at the point of discharge. Chapter 17-4.244(4) states that "in no event shall the maximum concentration of wastes in the mixing zone exceed the amount lethal to 50% of the test organisms in 96 hours (96 hour LC_{50}) for a species significant to the indigenous aquatic community..." This criterion applies at the point of discharge if there is a mixing zone. The distinction between presence or absence of a mixing zone is not relevant here, however, since the testing procedure used does not involve effluent concentration. The 1/3 of the 96 hour LC_{50} criterion is three times as stringent as the 96 hour LC_{50} criterion, and therefore, in the absence of a mixing zone, the procedure used is very lenient. The procedure followed is also lenient in that testing is only done for 48 hours. The 96 hour LC_{50} derived from this testing is therefore reported as "less than or equal to" the 48 hour measurement. The value found after 48 hours can never be more restrictive than the value found after 96 hours. It must be noted that all organisms used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

Table 6. LC_{50} Data for Bioassay of Cape Kennedy NASA IV Wastewater Treatment Plant, NPDES #FL0022594, on 11 to 13 March, 1981.

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Mysidopsis bahia Test #1 Pre-Chlorinated	Binomial	A	A
	Moving Average	A	A
	Probit	A	A
Mysidopsis bahia Test #2 Chlorinated	Binomial	13.4% (10 and 18)	13.4% (10 and 18)
	Moving Average	A	A
	Probit	A	A
Mysidopsis bahia Test #3 De-Chlorinated	Binomial	A	A
	Moving Average	A	A
	Probit	A	A

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.

Table 7. Field Parameters of Effluent Samples from Cape Kennedy NASA IV Wastewater Treatment Plant, NPDES #FL0022594 on 10 March 1981.

1. Effluent before chlorination:

Temperature :	23°C
Conductivity:	800 µmhos
pH:	4.0
Dissolved Oxygen:	3.75 ppm
Total Residual Chlorine :	0

2. Effluent after chlorination:

Temperature :	23°C
Conductivity:	800 µmhos
pH:	4.0
Dissolved Oxygen:	3.75 ppm
Total Residual Chlorine :	3.0 ppm

Observations: "Floc in effluent"

Remarks: "Plant has been hydraulically overloaded all day".

Measurements, observations and remarks by: Mr. Louis Ley of DER's St. Johns River District

LITERATURE CITED

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