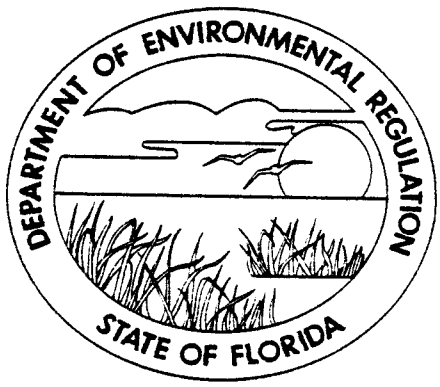


BIOASSAYS OF ASSOCIATED MINERALS (U.S.A.) LTD., INC.

GREEN COVE SPRINGS, CLAY COUNTY, FLORIDA

NPDES #FL0002119

SAMPLED 8/4/81

Biological Section
Bureau of Water Analysis
August 27, 1981

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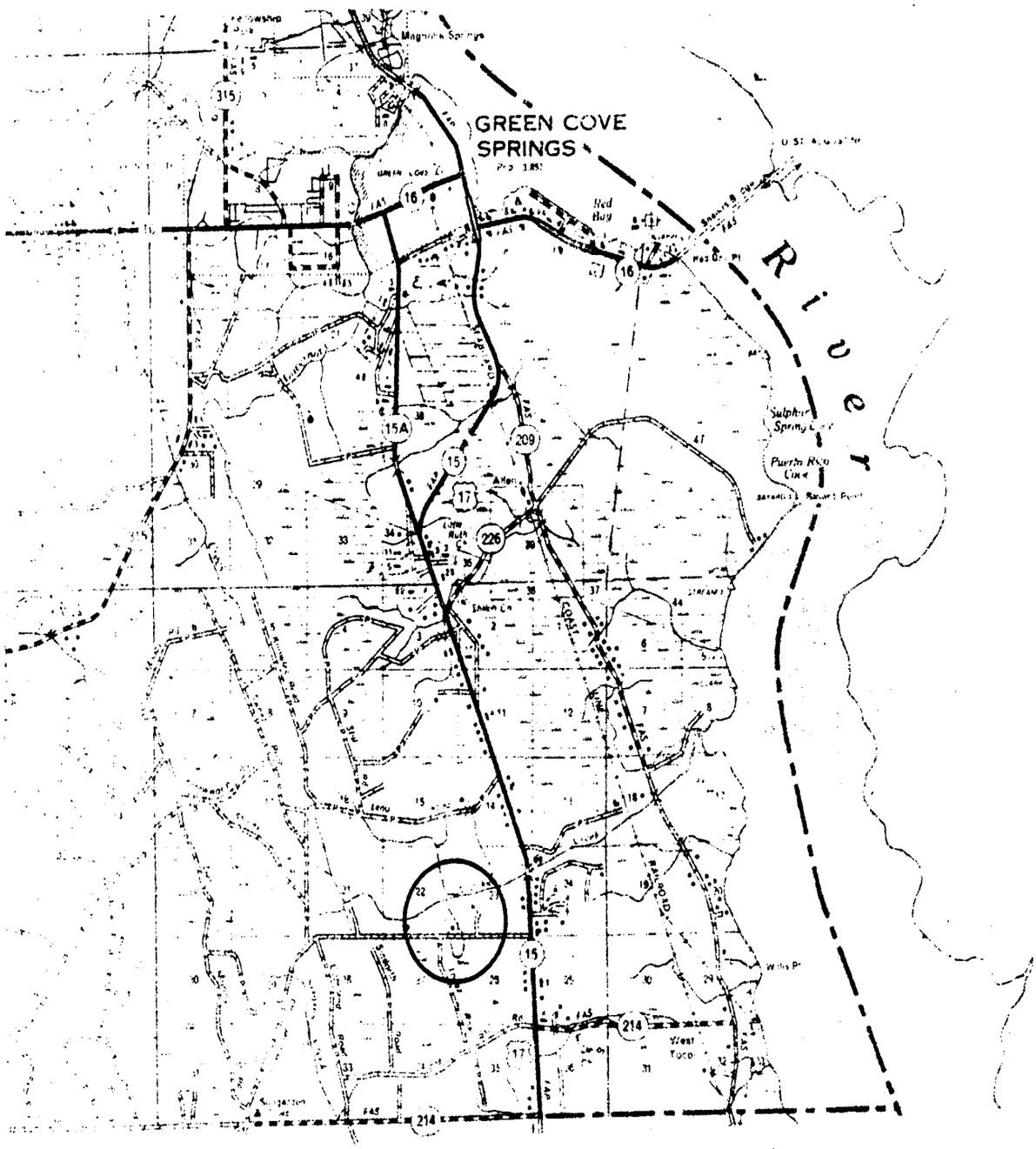
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INTRODUCTION

Associated Minerals (U.S.A.) Ltd., Inc., formerly owned by Titanium Inc., is located on Warner Road, off of U.S. Hwy 17, approximately ten miles south of Green Cove Springs in Clay County (Fig. 1). According to the NPDES permit application, this industrial mining company recovers certain minerals (including: ilmenite, leucoxene, rutile, zicron, monazite, and staurolite) from ancient beach sand. During operations, 4.8 MGD wastewater is produced. Treatment of this wastewater, in an 8 MGD design industrial wastewater treatment system, includes: flocculation with alum or sulfuric acid, settling in 180 acres of ponds, and neutralization with caustics. Depending on rainfall, the company discharges some or all of this effluent through a Parshall flume to Clarke's Creek and then to the St. Johns River.

To evaluate the effects of this discharge to the biota of the receiving waters, the Biological Section performed static acute toxicity bioassays on a grab sample of effluent, on 5 to 7 August, 1981. The results of these tests are presented in this report.



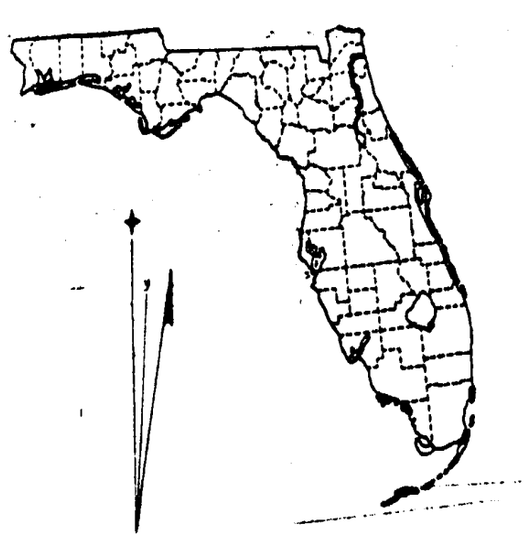


Figure 1. Portions of Clay County, Florida, including Green Cove Springs and approximate location of Associated Minerals.

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° ± 1°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° ± 1°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.

Pimephales promelas (fathead minnows) - A breeding culture of approximately 100 adult fathead minnows was obtained from the EPA laboratory in Cincinnati, Ohio in May, 1979. The fish have been maintained in four 125 gallon all glass aquaria equipped with a flow through water exchange system using unchlorinated well water obtained from a deep well near the DER laboratory. The fish have been reared at a temperature of 22° ± 2°C under a 16:8 hour light-dark cycle. These fish are fed Glencoe Mills Trout Chow® and frozen Artemia (brine shrimp) at a rate of 0.75 g/30 fish, twice daily. Breeding has been encouraged by placing several halved clay pots in each tank to serve as substrates for eggs and to provide cover for the fish. Tanks are examined twice daily (at feeding time) for egg production. Clay pots containing egg clusters are immediately transferred to 15 gallon aerated aquaria for hatching. A new aquarium is prepared for each week's production of eggs, thereby segregating fish into even age groups to be used for testing. After hatching, fathead minnow fry are fed twice daily with freshly hatched Artemia. The fish used in this test were all three weeks old at the time of the test.

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 1000 hours on 5 August, 1981, to 1000 hours on 7 August, 1981, on a grab sample of effluent from Outfall #001. The sample was collected by Mr. Dennis Murrin of DER's St. Johns River District at 1130 hours on 4 August, 1981, using standard wastewater sampling procedures. Mr. Murrin noted that there was very little flow from Outfall #001 at the time of sampling. The 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 deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1976).

Two replicates, each with six concentrations and a control, were used to test with Daphnia pulex and Simocephalus serrulatus. Flint glass jars of 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 large culture dishes through stainless steel screens to isolate the proper size and age organisms (neonates). These neonates were then transferred individually by glass pipette and were loaded 10 per chamber, resulting in 20 per test concentration.

Two replicates, of six concentrations and a control, were used for the fathead minnow bioassay. The fish were transferred individually or by twos, by net, into wide mouth quart jars containing 500 ml each of the appropiate test concentration. Fathead minnows were loaded 5 per chamber resulting in 10 per concentration.

Dissolved oxygen, pH, temperature and the number of live organisms were recorded for each test chamber at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness were recorded at the beginning of the test for the controls and the 100% effluent concentration. Specific conductance was recorded for each test concentration at the beginning and end of the test. Additionally, ammonia concentrations, including total (expressed in mg/l as N) and unionized (expressed in mg/l as NH_3) fractions, were recorded for the controls and 100% effluent concentrations at the beginning of the test. Ammonia measurements were performed using an Orion specific ion probe and a Corning pH/millivolt meter. Unionized ammonia levels were then calculated based on 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. Organisms were not fed during these tests.

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

Daphnia pulex and *Simocephalus serrulatus* (water fleas)

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

Pimephales promelas (fathead minnow)

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, Daphnia pulex, static acute toxicity bioassay of Associated Minerals (U.S.A.) Ltd.,Inc., Green Cove Springs, Clay County, Florida, NPDES #FL0002119, on 5 to 7 August, 1981.

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	48
Control	10	10	9	8.8	8.8	8.6	8.0	8.3	8.1	119		112		21.6	20.4	20.4	.01/.17	---		
Control	10	10	10	8.8	8.7	8.7	8.0	8.3	8.1										210	211
5.6%	10	10	10	8.8	8.7	8.6	8.0	8.2	8.1					21.5	20.3	20.4				
5.6%	10	10	10	8.8	8.7	8.8	8.0	8.2	8.2										216	221
10%	10	10	10	8.8	8.7	8.7	8.0	8.2	8.1					21.4	20.2	20.3				
10%	10	9	8	8.8	8.7	8.7	8.0	8.2	8.1										220	223
18%	10	10	9	8.8	8.7	8.8	7.9	8.2	8.1					21.3	20.3	20.5				
18%	10	9	9	8.8	8.7	8.7	7.9	8.2	8.1										228	230
32%	10	10	10	8.8	8.6	8.7	7.9	8.1	8.0					21.2	20.4	20.4				
32%	10	10	10	8.8	8.6	8.6	7.9	8.1	8.0										240	243
56%	10	10	10	8.8	8.6	8.7	7.8	8.0	8.0					21.1	20.5	20.4				
56%	10	9(10)[a]	8(9)[a]	8.8	8.6	8.7	7.8	8.0	7.9										265	270
100%	10	10	10	8.8	8.6	8.7	6.7	7.2	7.5	18		110		20.9	20.7	20.6	0/.27	---		
100%	10	10	10	8.8	8.6	8.9	6.6	7.2	7.3										310	315

Remarks: Total Residual Chlroine (HACH DPD Method): None detected.

[a]Number in parentheses used in calculations since one organism was stuck to side of glass above the water line between 0 and 24 hours.

Table 3. Data recorded during 48 hour, Simocephalus serrulatus, static acute toxicity bioassay of Associated Minerals (U.S.A.) Ltd., Inc., Green Cove Springs, Clay County, Florida, NPDES #FL0002119, on 5 to 7 August, 1981.

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	48
Control	10	9	8	8.7	8.7	8.8	7.9	8.2	8.1	119		112		21.8	20.4	20.3	.01/.17	---		
Control	10	10	10	8.7	8.7	8.8	7.9	8.2	8.1										212	217
5.6%	10	10	9	8.7	8.6	8.8	7.9	8.2	8.2					21.6	20.3	20.3				
5.6%	10	10	9	8.7	8.6	8.8	7.9	8.2	8.2										219	220
10%	10	9	6	8.7	8.6	8.7	7.9	8.2	8.2					21.5	20.4	20.3				
10%	10	9	9	8.7	8.6	8.8	7.9	8.2	8.2										221	222
18%	10	8	8	8.7	8.6	8.8	7.8	8.2	8.1					21.4	20.4	20.4				
18%	10	10	9	8.7	8.6	8.8	7.8	8.2	8.1										228	233
32%	10	8	8	8.7	8.6	8.8	7.8	8.1	8.1					21.3	20.9	20.5				
32%	10	10	9	8.7	8.6	8.8	7.8	8.1	8.1										241	249
56%	10	10	10	8.7	8.6	8.7	7.6	8.0	8.0					21.1	20.7	20.4				
56%	10	9	8	8.7	8.6	8.8	7.6	8.0	7.9										267	270
100%	10	10	9	8.6	8.6	8.7	6.5	8.0	7.6	18		110		20.9	20.8	20.6	0/.27			
100%	10	9	9	8.7	8.6	8.8	6.5	8.0	7.6										312	327

Remarks: Total Residual Chlorine (HACH DPD Method): None detected.

Table 4. Data recorded during 48 hour, Pimephales promelas, static acute toxicity bioassay of Associated Minerals (U.S.A.) Ltd., Inc., Green Cove Springs, Clay County, Florida, NPDES #FL0002119, on 5 to 7 August, 1981.

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	48
Control	5	5	5	8.8	7.6	7.9	7.8	7.9	7.9	119		112		21.8	20.5	20.5	.01/.17	---		
Control	5	5	5	8.8	7.5	7.8	7.8	8.0	7.9										217	219
5.6%	5	5	5	8.8	7.5	7.6	7.8	8.0	7.9					21.8	20.4	20.4				
5.6%	5	5	5	8.8	7.4	7.6	7.8	8.0	7.9										221	221
10%	5	5	5	8.8	7.6	7.8	7.8	8.0	7.9					21.7	20.4	20.4				
10%	5	5	5	8.8	7.6	7.7	7.8	8.0	7.9										225	228
18%	5	5	5	8.8	7.4	7.6	7.8	7.9	7.9					21.7	20.4	20.4				
18%	5	5	5	8.8	7.2	7.6	7.8	7.9	7.9										233	235
32%	5	5	5	8.8	7.2	7.4	7.8	7.8	7.8					21.6	20.5	20.5				
32%	5	5	5	8.8	7.2	7.4	7.8	7.8	7.8										248	248
56%	5	5	5	8.8	6.9	7.3	7.6	7.7	7.6					21.4	20.6	20.7				
56%	5	5	5	8.8	7.1	7.4	7.6	7.7	7.6										270	271
100%	5	5	5	8.8	6.8	7.5	6.5	7.3	6.6	18		110		21.3	20.8	20.9	0/.27	---		
100%	5	4	4	8.7	6.9	7.5	6.4	7.3	6.5										315	320

Remarks: Total Residual Chlorine (HACH DPD Method): None detected.
Weight of fish: 0.025g each (average of 10).

RESULTS AND DISCUSSION

This particular grab sample of effluent from Associated Minerals was not acutely toxic to the organisms tested. Although a number of water fleas died during the test (Tables 2 and 3), insufficient mortality occurred to calculate statistically sound LC_{50} values. Only one fish died during the test, in the 100% effluent (Table 4). Although this effluent sample was not acutely toxic, it was noted that due to the lack of rainfall this facility's discharge was minimal at the time of sampling.

LITERATURE CITED

American Public Health Association. 1976. Standard Methods for the Examination of Water and Wastewater. 14th ed. 1193pp.

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