# BIOASSAYS OF GROUNDWATER SAMPLES

# FLORIDA POWER AND LIGHT FACILITY

# FORT EVERGLADES, BROWARD COUNTY, FLORIDA

SAMPLED 11/14/83

Biology Section
Bureau of Water Analysis
January 9, 1984

## INTRODUCTION

The Florida Power and Light Port Everglades facility is an oil fired electric generating plant located between U.S. 1 and the Intracoastal Waterway in Port Everglades, Broward County, Florida (Fig. 1). The plant has been in operation for approximately 20 years and has cooling water, boiler blowdown, storm water overflow and neutralization basin discharges. The plant also has one or more evaporation/percolation ponds for solids (ash) settling. According to district personnel (Mr. Terry Davis, Port St. Lucie Office) dredged materials from these ash ponds were deposited near the ponds. Subsequent percolation of contaminants from the dredged material, specifically vanadium in concentrations as high as 200 mg/l, have entered the groundwater.

To determine if this contaminated groundwater is toxic to aquatic organisms, Mr. Davis and Mr. Richard Reis of the West Palm Beach Office requested that acute toxicity bioassays be performed. Groundwater samples were collected from two on-site monitoring wells, #OB2A (adjacent to the ash ponds) and #OB4 (a control well some distance away). A diagram of the Port Everglades plant, showing the location of the ash ponds and the monitoring wells, is presented in Figure 2. Three static acute toxicity bioassays were performed on these groundwater samples, on 15 to 17 November, 1983. Those involved in performing the tests and in preparation of this report were: C. W. Dye, R. B. Frydenborg, and M. L. Roll.

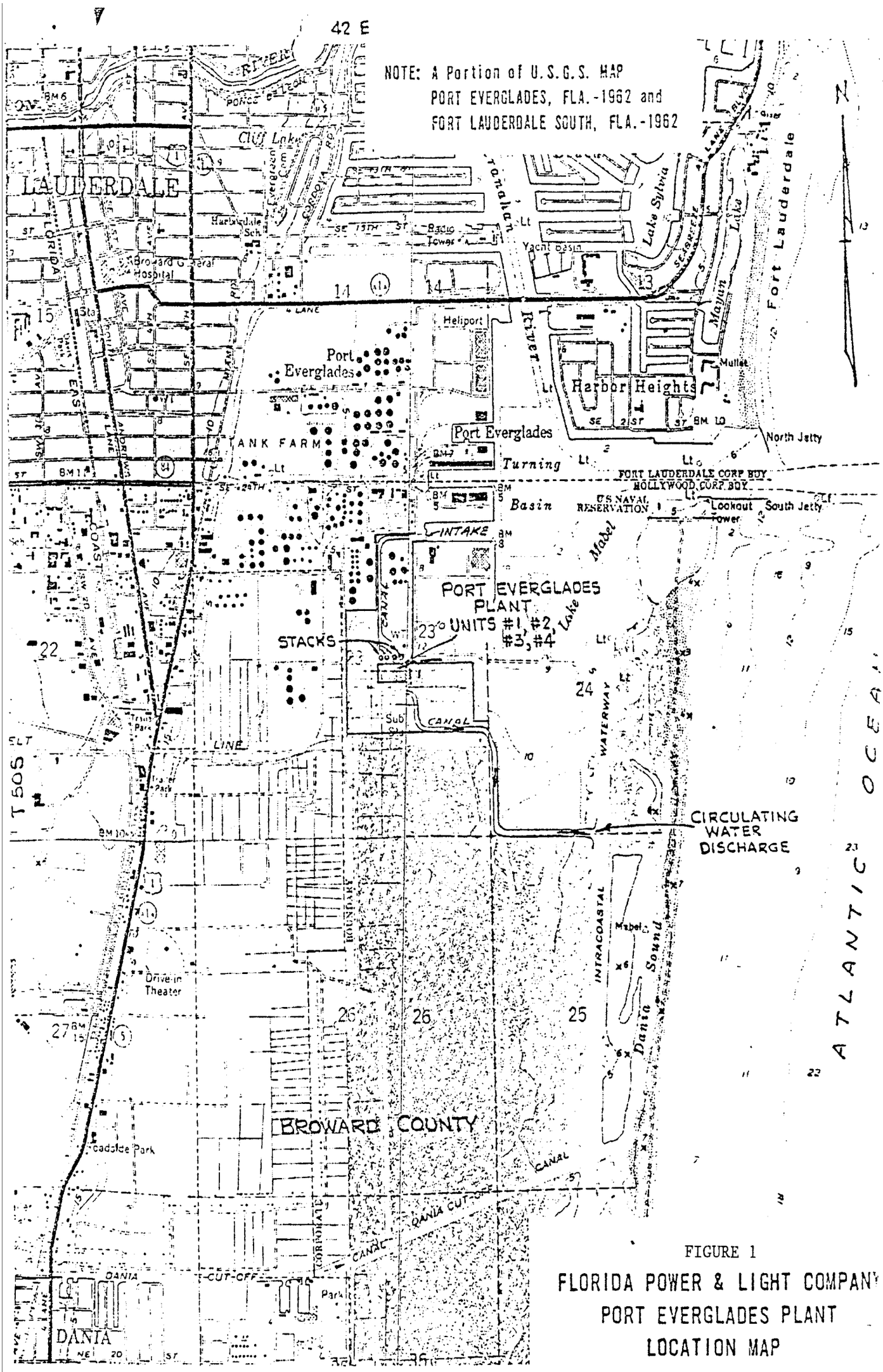
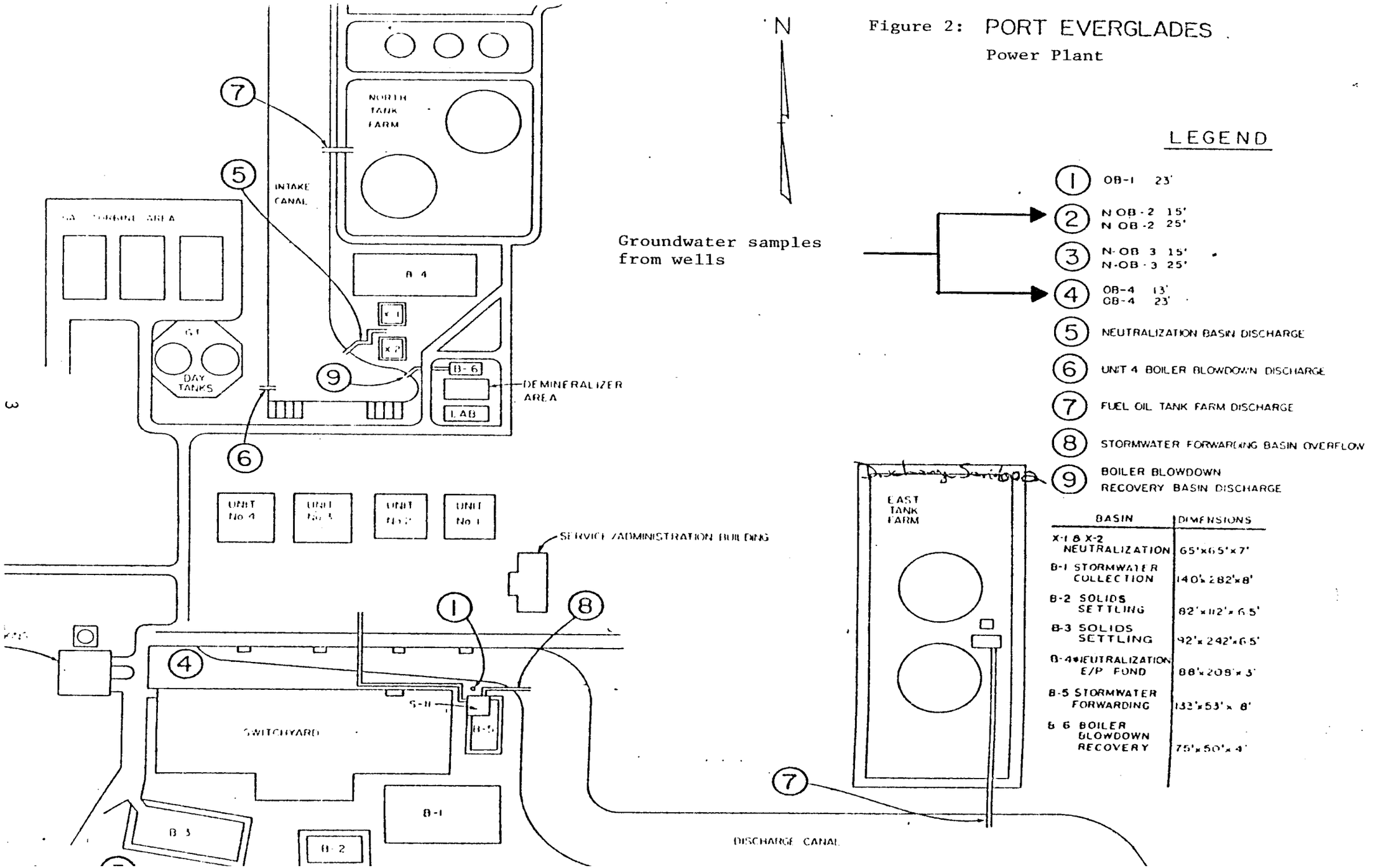



FIGURE 1

FLORIDA POWER & LIGHT COMPANY
PORT EVERGLADES PLANT
LOCATION MAP

Figure 2: PORT EVERGLADES
Power Plant



## METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organisms selected for this study include species recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. 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 2 1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under 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° plus or minus 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms. 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.

Notropis leedsi (bannerfin shiner) - Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 30 and 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22° plus or minus 2°C under a 16:8 hour light cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily. Breeding and egg hatching are encouraged at 25°C. The eggs are laid under a 1" thick slab of marble raised 3 mm above a removable horizontal pane of glass. Most of the eggs adhere to the glass and are easily removed to an aerated hatching tank. The narrow space between the marble slab and glass eliminates egg predation by adult fish. New hatching tanks are started weekly to segregate fry into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were approximately 10 days old at the time of the test.

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

Two 48 hour static acute toxicity bioasssays were performed on a groundwater sample from well #0B4 (control well), and one bioassay was performed on a groundwater sample from well #0B2A (ash pond well). The tests were performed from 1100 hours on 15 November to 1115 hours on 17 November, 1983.

The samples were collected by Mr. Brad Rieck of DER's Port St. Lucie Office at 1200 and 1250 hours on 14 November, 1983, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with $HNO_3$, while the samples for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Freshwater for dilution of the freshwater sample from well #0B4 (control well) samples 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 (1980).

Two replicates, each with five concentrations and a control, were used to test with the water flea, Daphnia pulex. 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 were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the water fleas were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during this test.

Two replicates, of five concentrations and a control, were used for the Notropis leedsi (bannerfin shiner) bioassay. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 5 per chamber, resulting in 10 per concentration. Fish were not fed during this test.

Saltwater for dilution of the slightly saline (2 ppt) sample from well #0B2A (ash pond well) was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 23.5 ppt). For test concentrations of 56% and below, this diluent was mixed directly with the groundwater sample, achieving a range of final salinities (12.5 ppt to 20.0 ppt). For the 100% test concentration, freshly autoclaved artificial sea salts (Ocean 50®) were added until a salinity of approximately 7 ppt was achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 4) 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 (23.5 ppt). This represents the highest concentration of Turkey Point water used in the bioassay, and tests for toxicity in the diluent. This is also the highest salinity used in the bioassay, 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 bioassay, as well as for tolerance of the organisms to the lowest salin ity used in the bioassay.

The concentration series selected for this study (Table 1) is an abbreviated version of that recommended by APHA (1980). Two replicates of each 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, 20 ppt and 25 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 pre-acclimated 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. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded for the controls and the 100% concentration at the beginning of the freshwater tests. The specific conductance and/or salinity (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the tests. All instruments were calibrated daily according to manufacturer's recommendations. These values were recorded on the bioassay data forms and are presented in Tables 2, 3, and 4.

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

1. Freshwater bioassays of sample from well #0B4 (control well)

Daphnia pulex (water flea)

| Concentration | Dilution Water | Sample | Total Volume |
|---|---|---|---|
| Control | 200.0 ml | -------- | 200 ml |
| 10.0% | 180.0 ml | 20.0 ml | 200 ml |
| 18.0% | 164.0 ml | 36.0 ml | 200 ml |
| 32.0% | 136.0 ml | 64.0 ml | 200 ml |
| 56.0% | 88.0 ml | 112.0 ml | 200 ml |
| 100.0% | -------- | 200.0 ml | 200 ml |

Notropis leedsi (bannerfin shiner)

| Concentration | Dilution Water | Sample | 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 |

2. Saltwater bioassay of sample from well #0B2A (ash pond well)

Mysidopsis bahia (mysid shrimp)

| Concentration | Dilution Water | Sample | Total Volume |
|---|---|---|---|
| Controls | 500 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, Daphnia pulex, static acute toxicity bioassay of groundwater samples from Florida Power and Light, Port Everglades, Broward County, Florida, on 15 to 17 November, 1983.

Freshwater Sample from Well #0B4 (Control well)

| 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 ($^oc$) | | | $NH_3/N$ (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 | 10 | 8.4 | 8.1 | 8.6 | 7.8 | 8.2 | 8.2 | 108 | | 130 | | 21.2 | 20.9 | 20.7 | | | | |
| Control | 10 | 10 | 10 | 8.4 | 8.1 | 8.6 | 7.8 | 8.2 | 8.2 | | | | | | | | | | 210 | 221 |
| 10.0% | 10 | 10 | 10 | 8.2 | 8.3 | 8.6 | 7.8 | 8.3 | 8.3 | | | | | 21.1 | 20.6 | 20.5 | | | | |
| 10.0% | 10 | 10 | 10 | 8.2 | 8.3 | 8.5 | 7.8 | 8.3 | 8.3 | | | | | | | | | | 225 | 232 |
| 18.0% | 10 | 10 | 10 | 8.0 | 8.4 | 8.4 | 7.9 | 8.3 | 8.3 | | | | | 21.0 | 20.6 | 20.4 | | | | |
| 18.0% | 10 | 10 | 10 | 8.0 | 8.3 | 8.4 | 7.9 | 8.3 | 8.3 | | | | | | | | | | 235 | 239 |
| 32.0% | 10 | 9 | 9 | 7.8 | 8.4 | 8.4 | 7.9 | 8.3 | 8.3 | | | | | 20.8 | 20.5 | 20.4 | | | | |
| 32.0% | 10 | 10 | 10 | 7.8 | 8.3 | 8.4 | 7.9 | 8.3 | 8.3 | | | | | | | | | | 250 | 254 |
| 56.0% | 10 | 10 | 10 | 7.2 | 8.4 | 8.4 | 7.9 | 8.3 | 8.3 | | | | | 20.7 | 20.5 | 20.5 | | | | |
| 56.0% | 10 | 10 | 10 | 7.2 | 8.4 | 8.4 | 7.9 | 8.3 | 8.3 | | | | | | | | | | 280 | 288 |
| 100.0% | 10 | 10 | 10 | 6.3 | 8.4 | 8.5 | 7.9 | 8.3 | 8.3 | 111 | | 160 | | 20.4 | 20.5 | 20.8 | | | | |
| 100.0% | 10 | 10 | 10 | 6.3 | 8.4 | 8.5 | 7.9 | 8.3 | 8.3 | | | | | | | | | | 325 | 340 |
| | | | | | | | | | | | | | | | | | | | | |
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Remarks:

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of groundwater samples from Florida Power and Light, Port Everglades, Broward County, Florida, on 15 to 17 November, 1983.
Freshwater Sample from #0B4(Control well)

| 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) | | | $NH_3$/N (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 | 8.1 | 8.2 | 8.4 | 7.8 | 8.2 | 8.3 | 108 | | 130 | | 21.6 | 20.6 | 20.8 | | | | |
| Control | 5 | 4 | 4 | 8.1 | 8.3 | 8.4 | 7.8 | 8.2 | 8.3 | | | | | | | | | | 210 | 218 |
| 10.0% | 5 | 5 | 5 | 8.0 | 8.3 | 8.5 | 7.9 | 8.3 | 8.3 | | | | | 21.5 | 20.3 | 20.7 | | | | |
| 10.0% | 5 | 4 | 4 | 8.0 | 8.4 | 8.5 | 7.9 | 8.3 | 8.3 | | | | | | | | | | 225 | 228 |
| 18.0% | 5 | 5 | 5 | 7.8 | 8.4 | 8.8 | 7.9 | 8.3 | 8.3 | | | | | 21.2 | 20.3 | 20.7 | | | | |
| 18.0% | 5 | 5 | 5 | 7.8 | 8.4 | 8.7 | 7.9 | 8.3 | 8.3 | | | | | | | | | | 235 | 235 |
| 32.0% | 5 | 5 | 5 | 7.6 | 8.4 | 8.8 | 7.9 | 8.3 | 8.3 | | | | | 21.0 | 20.3 | 20.7 | | | | |
| 32.0% | 5 | 5 | 4 | 7.6 | 8.3 | 8.8 | 7.9 | 8.3 | 8.3 | | | | | | | | | | 252 | 252 |
| 56.0% | 5 | 5 | 5 | 7.3 | 8.3 | 8.8 | 7.9 | 8.2 | 8.3 | | | | | 20.8 | 20.3 | 20.8 | | | | |
| 56.0 | 5 | 5 | 5 | 7.3 | 8.2 | 8.8 | 7.9 | 8.2 | 8.3 | | | | | | | | | | 280 | 283 |
| 100.0% | 5 | 5 | 5 | 6.2 | 8.2 | 8.8 | 7.9 | 8.2 | 8.3 | 111 | | 160 | | 20.6 | 20.4 | 21.1 | | | | |
| 100.0% | 5 | 3 | 3 | 6.2 | 8.1 | 8.8 | 7.9 | 8.2 | 8.3 | | | | | | | | | | 333 | 340 |
| | | | | | | | | | | | | | | | | | | | | |
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Remarks:

Table 4. Data recorded during 48 hour, *Mysidopsis bahia*, static acute toxicity bioassay of groundwater samples from Florida Power and Light, Port Everglades, Broward County, Florida, on 15 to 17 November, 1983.

Saltwater sample from well #OB2A(Ash Pond Well)

| 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 | 6.4 | 6.4 | 7.8 | 8.0 | 8.0 | 8.0 | 21.2 | 20.2 | 20.5 | | | | |
| Diluent Control | 5 | 5 | 5 | 6.4 | 6.4 | 7.9 | 8.0 | 8.0 | 8.0 | | | | | | 23.5 | 26.0 |
| Sea Salts Control | 5 | 5 | 5 | 8.0 | 7.4 | 7.8 | 8.2 | 8.3 | 8.2 | 21.4 | 20.1 | 20.3 | | | | |
| Sea Salts Control | 5 | 5 | 5 | 8.0 | 7.5 | 7.9 | 8.2 | 8.3 | 8.2 | | | | | | 7.0 | 7.7 |
| 18.0% | 5 | 3 | 3 | 6.4 | 6.7 | 7.3 | 7.7 | 8.0 | 8.0 | 20.9 | 20.1 | 20.4 | | | | |
| 18.0% | 5 | 5 | 3 | 6.4 | 6.7 | 7.3 | 7.7 | 8.0 | 8.0 | | | | | | 20.0 | 21.4 |
| 32.0% | 5 | 5 | 4 | 6.3 | 6.6 | 7.3 | 7.6 | 7.9 | 8.1 | 20.5 | 20.1 | 20.7 | | | | |
| 32.0% | 5 | 4 | 3 | 6.3 | 6.6 | 7.3 | 7.6 | 7.9 | 8.1 | | | | | | 17.5 | 19.0 |
| 56.0% | 5 | 4 | 3 | 6.3 | 6.9 | 7.4 | 7.5 | 7.9 | 8.1 | 20.3 | 20.3 | 21.0 | | | | |
| 56.0% | 5 | 4 | 4 | 6.3 | 7.0 | 7.4 | 7.5 | 7.9 | 8.1 | | | | | | 12.5 | 13.3 |
| 100.0% | 5 | 2 | 2 | 6.7 | 7.2 | 7.8 | 7.5 | 8.0 | 8.2 | 20.1 | 20.5 | 21.7 | | | | |
| 100.0% | 5 | 4 | 0 | 6.7 | 7.3 | 7.9 | 7.5 | 8.0 | 8.2 | | | | | | 7.0 | 7.5 |
| | | | | | | | | | | | | | | | | |
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Remarks:

Table 5. $LC_{50}$ Data for Bioassays of Groundwater Samples from Florida Power and Light, Port Everglades, on 15 to 17 November, 1983.

| Organism | Method of Data Analysis | 24 Hour $LC_{50}$ and 95% Confidence Intervals | 48 Hour $LC_{50}$ and 95% Confidence Intervals |
|---|---|---|---|
| D. pulex Well #OB4 | Binomial | a | a |
| | Moving Average | a | a |
| | Probit | a | a |
| N. leedsi Well #OB4 | Binomial | a | a |
| | Moving Average | a | a |
| | Probit | a | a |
| M. bahia Well #OB2A | Binomial | a | 70.3% (0 and ∞) |
| | Moving Average | a | 66.1% (37 and 439), c |
| | Probit | 290.4% (75 and ∞), b | 53.6% (0 and ∞) |

[a] Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an $LC_{50}$ 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] This $LC_{50}$ value is reported in the text since it is the most statistically conservative value of the data set.

Table 6. Results of Metal Analyses Performed on Groundwater Samples from well #OB4 and #OB2A, Florida Power and Light, Port Everglades, collected November 14, 1983.

| | Well #OB4 | Well # OB2A |
|---|---|---|
| Copper | N.D. | <25 µg/l |
| Iron | 148 µg/l | 387 µg/l |
| Chromium | N.D. | N.D. |
| Vanadium | >150 µg/l[a] | >150 µg/l[a] |
| Zinc | N.D. | 15.5 µg/l |
| Nickel | N.D. | 388 µg/l |
| Lead | N.D. | <4 µg/l |
| Mercury | <0.2 µg/l | 1.9 µg/l |
| Beryllium | N.D. | N.D. |
| Selenium | N.D. | N.D. |

N.D.=None Detected

[a] Vanadium lamp deteriorated during analysis. Actual value is probably significantly greater than value given.

## RESULTS AND DISCUSSION

The sample of groundwater from the Florida Power and Light Port Everglades' monitoring well #0B4 (control well) was not acutely toxic to Daphnia pulex or to Notropis leedsi, although several N. leedsi mortalities were noted during the test (Table 3). The groundwater sample from monitoring well #0B2A (ash pond well), however, was acutely toxic to Mysidopsis bahia (48 hour $LC_{50}$=66.1%).

The DER Chemistry Section has analyzed samples from these two wells for certain metals (Table 6). These analyses indicated the presence of measurable concentrations of iron (387 µg/l), zinc (15.5 µg/l), nickel (388 µg/l), mercury (1.9 µg/l), and vanadium (>150 µg/l) in the groundwater sample from the ash pond well #0B2A. Vanadium was also detected in the sample from control well #0B4. It was noted on the chemical analysis report that the vanadium lamp deteriorated during analysis and the concentration given is probably significantly less than the actual value. If the concentration of 200 mg/l vanadium (the value provided by Mr. Terry Davis from a previous analysis of groundwater from well #0B2A) can be considered an average value for this groundwater, then the toxicity noted may very well be mostly due to this metal. Tarzell and Henderson (1960) reported that vanadium concentrations as low as 13 mg/l and 55 mg/l were acutely toxic to fathead minnows. Additionally, the mercury present (1.9 µg/l) may have contributed to the toxicity since a concentration of only 3.5 µg/l was found to be acutely toxic to Mysidopsis bahia (Gentile, et al. 1983). Also, the nickel concentration (388 µg/l) may have contributed to the toxicity since a concentration of only 310 µg/l was found to be acutely toxic to hard clam larva (Calabrese and Nelson 1974).

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