

**BORON AND ARSENIC STUDIES IN FLORIDA WATERS**

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## ABSTRACT

Levels of arsenic and boron in Florida natural waters have been measured and found generally to be low and not of toxic concern. Boron levels in municipal sewage effluent are high enough to cause phytotoxicity, at least to sensitive plants such as citrus. The source of the elevated boron levels in sewage evidently is detergent use of borates as softeners and perborates as bleaching agents. Analysis of boron in rainfall found increasing concentrations near the coasts, indicating marine-derived aerosols are an important source of boron in the natural cycle of boron in Florida. Boron is a relatively conservative substance and model ecosystem studies indicate little toxic hazard and little bioaccumulation at the boron levels expected in natural waters.

Arsenic levels in Florida surface and ground waters were found to be normally quite low (a few ppb) to undetectable (less than 1 ppb). Canals in South Florida around Lake Okeechobee had higher levels than other parts of the state, with detectable levels in almost all samples and a mean of 5 ppb. The arsenic cycle in aquatic systems appears to be sediment dominated. In citrus grove soils arsenic was associated mainly with the iron fraction, but in anaerobic Lake Apopka sediments, arsenic was present primarily in a leachable "water soluble" form and was present as As III. Catfish from Lake Apopka do not appear to concentrate arsenic, but waterhyacinths from Lake Apopka had about 1 ppm (dry wt).

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## CHAPTER 1. INTRODUCTION

Far too frequently, environmental problems arise as a result of man's lack of foresight to investigate the consequences of adding to the environment in copious quantities a chemical or class of chemicals that seem of great importance to his culture. The recent concern over mercury pollution and the cultural eutrophication of many lakes from sewage effluents rich in nutrients, especially phosphates from detergents, are two prime examples of problems which might well have been avoided. If the effects of these chemicals on the environment had been understood before their widespread use and discharge into lakes and streams, there likely would have been no reason for concern now about mercury levels in fish, and perhaps the eutrophication of some of our lakes and rivers would have been avoided.

It is not possible to investigate all consequences of discharging into the environment every chemical beneficial to man. The task would simply be too large and complex and likely would yield far more irrelevant results than information pertinent to potential problems. However, there are criteria which can aid in setting priorities on potential areas of research. Three questions which, while not all-encompassing, help identify elements and chemicals worthy of investigating in this context are the following:

1. How much do we know about the chemical or element, its natural abundance, and its reactions in the environment?
2. Is it likely that man will add the chemical or element to the environment, or is he already adding it, and if so, how much?
3. Is this chemical or element known to be toxic or growth-promoting to plants or animals?

Obviously, the smaller our understanding of an element's cycle or a chemical's role in the environment, the greater the possibility of overlooking a potential problem. However, unless it is likely that man will be enriching the environment with this element or chemical, there probably will be no problem. If the substance under consideration is known to be toxic or growth-promoting, the significance of investigating the consequences of its discharge into streams is accentuated.

Applying these questions to boron and arsenic compounds, it is apparent that concentrations of both elements in streams and lakes are potentially significant problems and deserve investigation. There is little information currently available on typical levels of either element in Florida natural waters or on their sources, sinks, or role in plant nutrition. However, uses of boron in agriculture, industry, and the home are increasing daily. Arsenic has long been used as a pesticide; recently it has been found as a contaminant in detergents,

owing apparently to its association with mineral phosphates. Phosphate deposits are common in Florida and the widespread natural occurrence of relatively high arsenic levels was a distinct possibility. In addition, boron is known to be toxic to certain plants at relatively low concentrations, and arsenic is toxic to man and other animals. Arsenic is a known carcinogen, and recently it has been found that arsenic can be methylated under anoxic conditions to form volatile methyl arsines. All these factors combine to make the distribution of boron and arsenic in Florida waters worthy of investigation.

This research was undertaken in an effort to augment the meager supply of information on boron and arsenic concentrations in various Florida waters and to increase our understanding of their cycling in aquatic environments. The work necessary to achieve this was accomplished in three phases for each element. In the first phase the literature was reviewed, and a sensitive reliable analytical method was developed which requires no pretreatment of samples and is suitable for analysis of large numbers of samples. The second phase consisted of developing a sampling program and collecting and analyzing water samples from a variety of sources throughout Florida. The final phase of the work involved designing and conducting laboratory experiments to evaluate possible sinks for boron and arsenic in aquatic environments. Because work on the two elements was conducted independently, and in fact it is unlikely that significant interactions occur between the sources and cycles of the two elements in the environment, we have treated them separately in this report. The report is thus divided into two parts, the first dealing with boron studies, the second with arsenic studies. In each part a background chapter on the element's chemistry, sources, environmental levels, toxicity and analytical methods is presented, followed by chapters on the scope of the present investigation and analytical methods used in this study, results for the present study and discussion of the results, and finally a summary and conclusions chapter.

## PART 1. BORON

### CHAPTER 2. BACKGROUND INFORMATION ON BORON

#### A. OCCURRENCE, CHARACTERISTICS, AND USES OF BORON

Boron occurs in nature in the form of borax, borates, boric acid, and certain borosilicate minerals, such as tourmaline. The principal U.S. sources of boron are marine evaporite deposits found in the southwest. Boron is also present in the oceans, to the extent of about 4.7 mg/l (world-wide average). Boric acid and borate are similar geochemically to chloride. They are readily leached out of soils, are relatively inert chemically, and tend to be concentrated in the oceans.

Boron has a low toxicity to animals, significant toxic effects occurring only at concentrations exceeding about 30 mg/l, above which it begins interfering with digestive processes. It is an essential micronutrient for plants, but its exact role in plant metabolism is still not clear. Apparently boron is involved in several physiological and biochemical activities of plants. Dugger (1973) states that the "primary" role, if there is one, may be associated with an effect on enzymatic reactions or on nucleic acid biosynthesis. Dugger reviewed recent work on the role of boron in several aspects of plant metabolism, including organic translocation, enzymatic reactions, growth regulator response, cell division, cell maturation, and cell wall metabolism. As is true of many other micronutrients, boron is phytotoxic when present in excess. Boron toxicity is characterized by leaf-yellowing and burning, premature leaf drop, and reduced yields. Bingham (1973) listed various crops according to their tolerances to boron (Table 2-1). Citrus and other fruit trees are the most sensitive crops listed, with threshold concentrations for toxicity ranging from 0.3 to 0.5 mg/l in irrigation waters or soil solution extracts.

Table 2-1. Toxic Boron Concentrations of Saturation Extracts for Sensitive, Semitolerant, and Tolerant Crop Species\*

<u>Saturation-Extract Boron, mg/l</u>		
<u>0.5-1.0</u>	<u>1.0-5.0</u>	<u>5.0-10.0</u>
<u>Sensitive</u>	<u>Semitolerant</u>	<u>Tolerant</u>
Citrus	Lima bean	Carrot
Avocado	Sweet potato	Lettuce
Apricot	Bell pepper	Cabbage
Peach	Oat	Turnip
Cherry	Milo	Onion
Persimmon	Corn	Broad bean
Fig	Wheat	Alfalfa

(Cont. p. 4)

Table 2-1, (cont'd.)

sensitive	semi-tolerant	tolerant
Grape	Barley	Garden beet
Apple	Olive	Mangel
Pear	Field pea	Sugar beet
Plum	Radish	Palm
Navy bean	Tomato	Asparagus
Jerusalem artichoke	Cotton	
Walnut	Potato	

\*Listed in each category according to susceptibility to boron injury (viz., citrus is more sensitive than walnut, lima bean more than potato, etc.).

Since many soils are deficient in boron, inclusion of small amounts of boron in commercial fertilizers recently has become a wide-spread agricultural practice. Industrial uses of boron and boron-containing compounds are varied. Boron is used in the steel industry where it is added to alloys in trace amounts to increase hardness. It is also used extensively in the glass industry in the manufacture of borosilicate glass, but the most important use of boron in terms of impact on water pollution has been in the manufacture and use of household laundry products.

Borax has long been added to detergents for its water softening and buffering properties, but consumption of boron by the detergent industry has been markedly increasing, primarily due to the recent marketing of several dry bleaches using sodium perborate (peroxoborate) as the bleaching agent and the inclusion of this compound in detergent formulations. Until recently, use of perborate bleaches was hindered by their low bleaching efficiencies compared to hypochlorite bleaches at the 120°-140°F maximum temperatures typically produced by hot-water heaters in the United States. However, because of the advantages of cost, ease of manufacture, and ease of handling of these bleaches over hypochlorite bleaches, detergent manufacturers conducted an intensive search for activators to catalyze the perborate bleaching action at lower temperatures. (Anon, 1967). This search resulted in a host of patents for such activators, and typical detergent formulations now often include from 1 to 10 percent sodium perborate, while dry bleach formulations may contain from 40 to 60 percent sodium perborate. In Europe, where some hot-water heaters produce near-boiling temperatures, the relative efficiency of perborate versus hypochlorite has been less of a problem, and perborate bleaches were included in detergent formulations several years ago.

The magnitude of the consumption of boron for bleaching agents included in detergents is illustrated by the following calculations: Based on industry data for 1969 (Anon. 1970a, b), synthetic detergents comprised 84 percent of the total world soap and detergent production, and annual

North American consumption of these products averaged 42.2 lb/cap. Therefore the approximately 200 million people in the United States used some 7.1 billion pounds of detergents in 1969. Assuming that 25 percent of these detergents were compounded with 5 percent sodium perborate containing 8 percent boron by weight, the total boron consumption for bleaching agents in detergents in the United States would be 7 million pounds. Assuming an average daily per capita water consumption of 150 gallons, the boron concentrations in sewage would average about 75  $\mu\text{g}/\text{l}$ . In reality, this is a rather conservative estimate and the actual value could easily be much higher. In addition, these calculations are based only on bleaching agents included in detergents, and do not include boron in dry bleaches, borax included in detergents for softening and buffering, or any industrial sources of boron. Since little, if any, of the boron present in sewage is removed by conventional methods of sewage treatment (Waggott 1969), the boron added to wash water eventually makes its way to surface streams and rivers which receive treated sewage effluent. Bingham (1973) has estimated that perhaps as much as 50 percent of the boron in surface streams is attributable to household uses.

## B. PRIOR INVESTIGATIONS OF BORON IN THE AQUATIC ENVIRONMENT

Despite the role of boron as a micronutrient and its potential hazard to many fruit crops at levels only slightly higher than optimal, investigations of boron in natural waters have been few and infrequent. The small amount of published data is reflected in the limited information on the subject in Hutchinson's (1957) comprehensive work on limnology and in the U.S. Geological Survey's Data of Geochemistry (White et al. 1963; Livingstone 1963).

Although boron is not routinely monitored by the U.S.G.S. in its continuing surveillance of Florida water quality, boron analyses are run when requested by a municipality, or at the discretion of Survey officials. Examination of the published data (U.S.G.S. 1967-1970) revealed that over the period 1967-1970, boron analyses were performed on 134 samples from 32 locations in South Florida, and two samples from South-central Florida. Since 1970 the U.S.G.S. has utilized computer storage of water quality data rather than publication. An information retrieval search in June, 1973, produced no data on boron for the period 1971-1973. The 1967-1970 data apparently were collected to supplement salinity data in monitoring the extent of salt water intrusion in South Florida. Values varied from 0-3700  $\mu\text{g}/\text{l}$ , depending on location and date. While these analyses do not constitute an organized survey of boron levels in Florida waters, they do provide a base of data on selected locations in South Florida.

In the studies reviewed by Livingstone (1963) the average boron content of the major rivers of the USSR was 13  $\mu\text{g}/\text{l}$ , while for several rivers of North America and Norway the average was 11.6  $\mu\text{g}/\text{l}$ . Another study reviewed reported levels of 0.2-1.3 mg/l boron in surface waters of Japan, but Livingstone indicated that vulcanism might be responsible for the high values.

Khachatryan (1970) reported on boron concentrations in waters of the Armenian SSR. In the drinking water supplies from 110 sources in the three hydrogeological regions of the state, average concentrations of 0.39, 0.29 and 0.60 mg/l were observed. In river waters a range of 0.21-0.32 mg/l (average 0.27 mg/l) was found, while artesian wells were considerably higher, ranging from 0.77 to 1.44 mg/l.

Ahl and Jonsson (1972) investigated boron distribution in fresh waters of Sweden and Norway. For 355 samples taken from Swedish and Norwegian rivers they found a range of 0-1500  $\mu\text{g/l}$ , with a median of 12  $\mu\text{g/l}$  and a mean of 31  $\mu\text{g/l}$ . For three sewage treatment plants boron levels ranged from 373 to 620  $\mu\text{g/l}$  in the influent and little or no removal was apparent from grab effluent samples. A range of 2.0-19.0 ppm boron (dry weight) was reported for several aquatic plants, with significant differences evident between species.

In a Canadian study Afghan *et al.* (1972) measured environmental boron concentrations during an evaluation of a proposed fluorometric analytical method. Boron levels in seven Ontario and Saskatchewan lakes ranged from 5 to 3500  $\mu\text{g/l}$  boron; all but one contained less than 750  $\mu\text{g/l}$ , and two contained less than 50  $\mu\text{g/l}$ . Snow samples, collected in the Ottawa area, contained 1 to 12  $\mu\text{g/l}$  boron, and sewage contained 130 to 140  $\mu\text{g/l}$  in the raw waste, and 95  $\mu\text{g/l}$  in the effluents.

In an earlier study of boron in Florida waters, Odum and Parrish (1954) observed ranges of boron of 13-32  $\mu\text{g/l}$  in six freshwater springs, 12-27  $\mu\text{g/l}$  in six streams, and 12-17  $\mu\text{g/l}$  in five North Florida lakes. For two summer thunderstorms in Gainesville, they found 9 and 15  $\mu\text{g/l}$  in the rainwater. Boron concentrations in salty springs were higher, ranging from 24 to 300  $\mu\text{g/l}$ . A positive correlation was observed between boron and chloride, but much higher B:Cl ratios were observed in fresh-water than in salty springs and the ocean.

Boyd and Walley (1972) measured boron in rainfall, plants and small streams in seven states of the southeastern United States, including Florida. The range encountered for 199 stream samples was 0-660  $\mu\text{g/l}$ , with 86 percent less than 100  $\mu\text{g/l}$ , and a mean of 47  $\mu\text{g/l}$ . The distribution was biased geographically, with higher concentrations occurring in the Mississippi River flood plain (mean 138  $\mu\text{g/l}$ ), the flatwoods region near the coast (mean 77  $\mu\text{g/l}$ ), and the Cumberland plateau (mean 106  $\mu\text{g/l}$ ). Samples from the Blue Ridge Mountain region and the sand hills region of South Carolina and Georgia were very low in boron (means 3 and 6  $\mu\text{g/l}$ , respectively). For three rainfall collection stations in Grenada and Jackson, Miss. and Aiken, S.C. Boyd and Walley found mean boron concentrations of 5.3 to 6.7  $\mu\text{g/l}$  over a one year period. Twenty-two species of aquatic plants contained 1.2-11.3 ppm boron (dry weight basis), with significant differences between species, even when they were growing in close proximity to one another.

Waggott (1969) reported measurements of boron in sewage and rivers in England. Flow-weighted concentrations ranged from 1.1 to 3.7 mg/l

in raw sewage entering four different treatment plants, with no discernable removal by conventional treatment. Variations in boron concentration closely followed variations in anionic detergent levels, suggesting that detergents were the primary source of boron in domestic sewage. Boron levels in the River Trent varied from 0.51 to 0.76 mg/l at different locations, while tributaries draining undeveloped country averaged 0.30 mg/l boron. Treated sewage effluent constituted a large proportion of the flow of the River Tame, and the boron levels ranged from 0.9 to 1.4 mg/l boron; in tributary streams levels varied from 0.3 to 0.8 mg/l. Table 2-2 summarizes the literature values for boron in sewage.

Table 2-2. Boron Concentrations in Sewage Reported in the Literature

<u>Location</u>	<u>Boron (<math>\mu\text{g}/\text{l}</math>)</u>		<u>Reference</u>
	<u>Influent</u>	<u>Effluent</u>	
Canada	140	95	Afghan <u>et al.</u> (1973)
	130	95	"
Sweden			
Uppsala	401	436	Ahl and Jönsson (1972)
Orsa	323	340	"
Stockholm	620		"
England			
Bedwell	3070	3070	Waggott (1969)
Rye Meads	1360	1220	"
Preston	1140	1200	"
	1360	1280	"
	1800	1460	"
Luton	3680	4120	"

Banerji et al. (1968) have investigated the toxicity of boron to activated sludge organisms. Significant toxic effects began at about 10 mg/l in the mixed liquor. In the range 0-200 mg/l boron in the raw sewage, the effluent chemical oxygen demand increased nearly exponentially. Prolonged exposures at low levels were more toxic than slug doses of much higher concentrations, leading these workers to suggest that adsorption of boron onto the sludge organisms during prolonged low-level exposure might result in concentrations at the sludge surfaces much higher than in the surrounding solution.

In an effort to determine the significance of sea spray aerosols as a source of boron in rainfall, Nishimura et al. (1970) determined atmospheric boron concentrations over the open ocean and over land. Concentrations over the ocean averaged  $2.1 \times 10^{-4}$  mg/m<sup>3</sup>, about twice the average over land. However, air equilibrated with sea water in the laboratory contained only about  $10^{-5}$  mg/m<sup>3</sup>, leading these workers to conclude that the oceans are a sink, not a source of atmospheric boron. This conclusion is not in agreement with their own measurements of boron levels over land and sea, nor with the data

levels over land and sea, nor with the data in the literature, nor with the results of the present study. Table 2-3 summarizes the results found in the literature for boron concentrations in rainfall. The locations are arranged in order of increasing distance from the coast, and the data indicate a gradient of boron concentration in rainfall versus distance from the coast, as suggested by Boyd and Walley (1972).

Table 2-3. Boron Concentrations in Rainfall Reported in the Literature

<u>Location</u>	<u>Boron (<math>\mu\text{g}/\text{l}</math>)</u>	<u>Reference</u>
Calcutta, India	26	Handa (1969)
Kiriu, Japan	98	Muto (1956)
Gainesville, Fla.	9, 15	Odum and Parrish (1954)
Jackson, Miss.	6.7	Boyd and Walley (1972)
Grenada, Miss.	5.3	"
Aiken, S.C.	5.7	"

In the last 15 years a number of investigators have examined sorption of boron onto various soils and clays. Bingham (1973) has reviewed the principal papers on the subject. The investigators whose work he has reviewed generally agree that boron is sorbed from aqueous solution principally as molecular boric acid rather than borate anion. The sorption process is pH-dependent, with maximum adsorption occurring at about pH 9. If  $\text{B}(\text{OH})_3$  is sorbed preferentially over  $\text{B}(\text{OH})_4^-$ , boron sorption should increase with decreasing pH as molecular  $\text{B}(\text{OH})_3$  becomes the dominant species in solution at pH values lower than the pKa of boric acid ( $\sim 9.3$ ). Bingham believes that the observed maximum boron adsorption near pH 9 results from an increase in the number of available sorption sites with increasing pH. He cites the sharp decrease in boron adsorption which occurs above a pH value of 10 as evidence for the poor sorption of borate anion. Active adsorption sites are thought to be associated with broken Si-O and Al-O bonds exposed at edges of aluminosilicate minerals and also with surfaces of amorphous hydroxide materials present in weathered soils, and hydroxy aluminum and iron compounds. Adsorption has been observed with a number of different soils and clays, but is especially pronounced with illite and amorphous soils derived from volcanic ash. Sorption increases with ionic strength, and adsorption maxima calculated with the Langmuir adsorption equation vary from 10-100  $\mu\text{g}$  boron per gram of soil. The previous investigations were not conducted specifically to determine the role of adsorption in removing boron from natural waters, and the concentrations used were orders of magnitude greater than those normally encountered in natural waters. Nevertheless, the results indicate that clay adsorption is involved in boron removal and could be important in regions rich in certain minerals.

Christman and Ghassemi (1966) found evidence of interactions between boric acid and organic color in natural waters during an investigation of the nature of organic color. While attempting to determine the molecular size distribution of organic color components using gel permeation chromatography, they observed an apparent increase in the molecular size of the organic color eluted from the gel column with a borate buffer,

compared to the same color sample eluted with phosphate buffer. A brief investigation with different pH borate buffers containing varying concentrations of boron revealed that the amount by which the color elution peak was shifted toward higher molecular sizes was directly related to the concentration of boron in the eluant.

### C. ANALYTICAL METHODS FOR BORON

The meager amount of literature available on boron in the aquatic environment has been at least partly due to the lack of a simple, accurate, and reproducible method for analysis at the parts-per-billion level--the level typically encountered in natural waters. A number of methods, including atomic emission spectroscopy, colorimetric methods, and potentiometric titrations yield accurate results for "clean" solutions containing greater than 1 mg/l. However, if concentrations are lower than 1 mg/l, or if certain other species are present in solution, elaborate and time-consuming concentration and extraction procedures must be employed prior to using these methods for boron analysis. Thus, there has been a need for an analysis method for boron that is simple, fast, accurate, and free from interferences.

In recent years a number of workers have engaged this problem, refining earlier methods and utilizing new techniques. The most successful techniques have been use of a hollow cathode source for atomic emission spectroscopy (Daughtry and Harrison 1973), an ion-selective electrode sensitive to the tetrafluoroborate ion (Carlson and Paul 1968), a molecular fluorescence technique (Afghan *et al.* 1972), and modifications of the curcumin colorimetric method (A.P.H.A. 1971) by use of acid anhydrides to eliminate water from the reaction medium (Crawley 1964; Pakalans 1969; Uppstrom 1968).

The technique developed by Daughtry and Harrison (1973) utilizes a commercially available hollow cathode lamp with a demountable cathode as a source for atomic emission spectroscopy. In the procedure the iron or copper cathode is removed from the lamp and sample aliquots are introduced into the cathode cavity. The sample is dried under an infrared lamp, and the procedure repeated until an adequate amount of boron is in the cathode. The cathode is then replaced in the lamp, a suitable vacuum is established, and a current adequate to quickly atomize and excite the boron deposited on the cathode is passed through the lamp. The resulting emission signal is directed into a conventional detector properly adjusted to the maximum emission line of boron, and the intensity of the signal is plotted versus time. The method has several advantages. It is virtually free of interferences and requires very small sample volumes. No sample preparation is required, and the detection limit is low. However, there are some disadvantages. The method is not readily amenable to automation or to simultaneous analyses of several samples. Each sample must be analyzed individually, and the time required per sample prohibits routine analysis of large numbers of samples.

The ion-selective electrode procedure developed by Carlson and Paul (1968) has the advantage that the instrumentation required is found in nearly every laboratory, the only added expense being for the electrode. However, nitrate ion interferes strongly with the electrode response, necessitating a separation step. Carlson and Paul combined the separation of boron from nitrate and the conversion from boric acid to tetrafluoroborate ion by passing the sample through a boron-specific ion-exchange column, then reacting the boric acid with 10% HF before elution from the column. This procedure works well, but is time-consuming, especially for low levels of boron.

Afghan *et al.* (1972) developed an automated fluorometric method for analysis of boron based on its reaction with 4'-chloro-2-hydroxy-4-methoxybenzophenone (CHMB) in 90% sulfuric acid. The only interference with the method is background fluorescence from dissolved organic matter in sewage effluents, polluted streams, and naturally colored waters. This interference was eliminated by adding hydrogen peroxide to the samples and photooxidizing with a 550 watt UV lamp prior to development of the boron fluorescence reaction. The method is reported to have a detection limit of 1  $\mu\text{g/l}$ , and a working range of 1-400  $\mu\text{g/l}$ .

A variety of colorimetric methods have been employed for analysis of boron, but by far the most widely accepted methods have been the carminic acid and curcumin methods recommended by Standard Methods (A. P.H.A. 1971). The carminic acid method has a detection limit of about 1 mg/l, making it of questionable utility in analyzing natural water samples. Under the proper conditions curcumin forms one of two highly colored red complexes with boron: rubrocurcumin, formed in a reaction between curcumin, oxalic acid, and boric acid; and rosocyanin, a more intensely colored complex formed in a strongly acidic, virtually water-free medium. The structures of the two products are depicted in Figure 2-1. Because of its higher absorptivity and its greater stability toward hydrolysis, rosocyanin is preferred over rubrocurcumin as a chromophore for boron. However, formation of either complex is drastically inhibited by trace quantities of water in the reaction medium. Thus, any procedure utilizing curcumin for analysis of boron in aqueous samples must include a step for removal of water. The Standard Methods procedure method (A.P.H.A. 1971) recommends addition of the color-forming reagents, followed by evaporation to dryness on a water bath maintained at 50°C to 55°C -- a time-consuming procedure and one which introduces several possible sources of error.

Recognizing the limitations of such a procedure, several workers have devised alternate procedures for removal of water. The most successful of these have involved reactions between acid anhydrides and water to eliminate water from the medium. Crawley (1964) was the first to report such a procedure, using acetic anhydride and a few drops of HCl to catalyze the dehydration reaction. Since the reaction is highly exothermic, he diluted the reactants with acetic acid, and he carried out the reaction in platinum dishes floating on a water bath at 40°C. In a later paper Pakalans (1969) also used acetic anhydride,

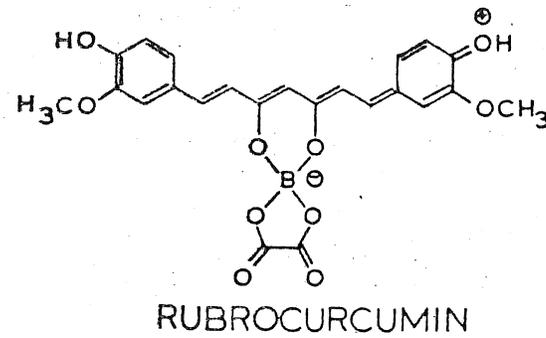
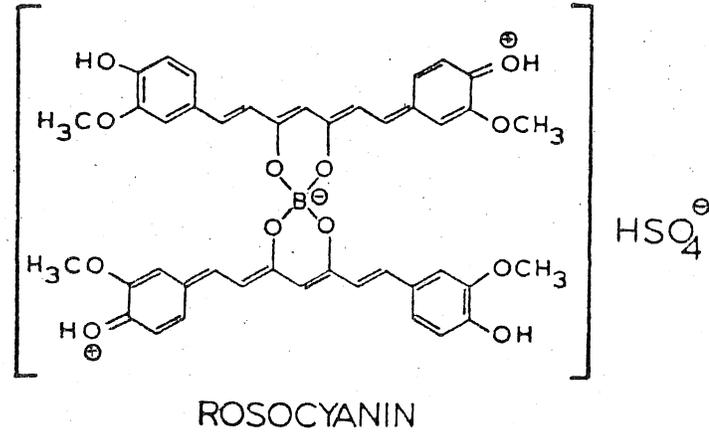


Figure 2-1. Chemical Structures of Rosocyanin and Rubrocurcumin

Reference: Roth and Miller (1964).

but found that adding the anhydride in two aliquots and allowing the mixture to cool between additions resulted in better control of the maximum temperature attained. Since the propionic anhydride-water reaction is somewhat slower and less exothermic, Uppstrom (1968) developed a method using this reagent to eliminate water, catalyzing the reaction with oxalyl chloride. Using propionic anhydride he observed a maximum temperature of about 70°C, compared to about 90°C with acetic anhydride, used as described by Crawley.

Following color development, the absorbance of the rosocyanin is measured in a spectrophotometer at 545 nm. However, the unreacted excess curcumin, which exists in a purple-colored protonated complex in the strongly acidic medium, must be converted to a non-interfering form prior to measurement. The simplest way to achieve this is to adjust the acidity of the medium so that the curcumin reverts to a yellow form stable in less acidic solutions. Crawley accomplished this by diluting the medium to 100 ml with ethanol just prior to measurement, while Pakalans used a 1:1 acetone:water mixture, diluting to the same volume. Uppstrom found that an acetate buffer was more efficient, requiring dilution to only 50 ml to sufficiently lower the acidity, with a consequent increase in the sensitivity of the method.

## CHAPTER 3. EXPERIMENTAL DESIGN AND PROCEDURES FOR BORON

### A. AREAS OF INVESTIGATION

A comprehensive study of the occurrence and distribution of any element in the aquatic environment requires investigation of natural and cultural sources of the element to surface and groundwaters, determination of background levels in waters minimally affected by man, evaluation of chemical and biological reactions which control its distribution, and determination of the ultimate fate of quantities of the element added to natural water bodies as industrial wastes, domestic sewage, and other forms.

In this research samples were collected from natural waters throughout Florida to determine natural background levels and cultural sources of boron and arsenic. Levels in groundwater and surface waters were examined by analyzing "grab" samples collected throughout the state from springs, lakes, rivers, streams, and wells supplying municipal water systems. Several samples for boron were collected from locations previously sampled by Odum and Parrish (1954) to determine the impact of urbanization on boron levels in surface waters. In order to avoid duplication of effort, samples were not collected in regions for which sufficient data had already been accumulated by the U.S.G.S. Several drainage basins were selected for more intensive sampling to locate major points of boron and arsenic addition to the system and to observe if there were significant concentration differences in streams draining urban and rural areas, or in streams draining different areas of the state.

Since domestic sewage has been cited as a major source of boron, several sewage treatment plant effluents were sampled to determine levels of boron in sewage and to compare with average boron levels in municipal water supplies. Land disposal of treated sewage effluents as irrigation waters has been proposed as part of a solution to the culturally accelerated eutrophication of lakes and streams. In view of the toxicity of boron to citrus crops and the potential damage that land disposal might cause to citrus groves, one of the drainage basins selected for intensive sampling was the Oklawaha River drainage basin, originating in the center of a large citrus-producing area. In conjunction with this study several small citrus grove ponds were sampled to further assess the current situation.

Laboratory studies of boron adsorption onto clays and of boron distribution in a series of model aquatic ecosystems were conducted to determine the role of clay adsorption as a boron sink in the environment and to determine the fate of boron added to natural waters. The clay adsorption studies were conducted using attapulgite and kaolinite, representative clay minerals distributed through much of peninsular Florida. For the clay studies, 500 mg of clay was added to each of three series of acid-washed Erlenmeyer flasks, followed by 250 ml of

solution containing 0, 100, 400, or 1000  $\mu\text{g}/\text{l}$  of boron as boric acid. One series received no further additions, while the remaining two received 1-ml and 2-ml aliquots of 0.05 M NaOH solution. The flasks were sealed, and the clays maintained in suspension for 24 hours by stirring with magnetic stirrers. At that point, 10-ml aliquots of each were centrifuged, and the resulting supernatant liquids analyzed.

The model ecosystems consisted of 2.5-gal. (9 $\ell$ ) plexiglass aquaria filled with 6 $\ell$  of a synthetic river water (Freeman 1953) enriched five-fold in nitrate and phosphate, and stocked with one snail (Helisoma trivolvis), three fish (Gambusia affinis), and four species of aquatic plants: duckweed (Lemna minor), floating heart (Nymphoides aquaticum), pennywort (Hydrocotyle umbellata), and coontail (Ceratophyllum demersum) in each aquarium. The fish used in the study were acclimated to the synthetic river water for 10 days in a larger aquarium prior to stocking the test aquaria. Seven model ecosystems used in the study were dosed with boron concentrations ranging from 0 to 50 mg/l. The fish and snails were removed and analyzed after 10 days exposure, and the aquatic plants were analyzed when the experiment was terminated after thirty days.

Because of the chemical similarities between glycols and other polyhydroxy compounds, such as sugars, with which boric acid is known to react, and structures proposed for organic color in natural waters (Black and Christman 1963; Christman and Ghassemi 1966), a series of investigations was directed toward examining possible interactions between boron and organic color and the influence of these interactions on boron sorption.

In one investigation, water from a highly colored, oligotrophic lake (Lake Mize, Alachua Co.) was freeze-concentrated to approximately 1/16 its original volume and divided into two portions. One portion was spiked with 32 mg/l boron as boric acid (equivalent to 2 mg/l boron in the original lake water) and both portions were refrigerated. After allowing one week for reaction to take place, gel permeation chromatography was performed on both fractions using Sephadex G-50 (Pharmacia Fine Chemicals, Inc.) fine porosity gel in a 2.5-cm. X 60-cm. column. Elution patterns were determined for spiked and unspiked color concentrate for each of the following solvent systems:

- 1) 0.1 M  $\text{K}_2\text{HPO}_4$  buffer, adjusted to pH 8.0 with HCl;
- 2) 0.1 M  $\text{B}(\text{OH})_3$  buffer, adjusted to pH 8.0 with NaOH;
- 3) distilled, deionized water.

The column eluant was collected in 10-ml. fractions with a Gilson Automatic Fraction Collector and the collected fractions were analyzed for color. Because of the large number of fractions, only selected fractions were analyzed for boron.

In another investigation 100-ml aliquots of water samples from two oligotrophic lakes, one highly colored (Lake Mize, Alachua Co.) and one relatively uncolored (Lake Magnolia, Clay Co.), were added to 125-ml flasks containing 0.2 g. attapulgite clay (Diluex, The Floridan Co.). Half of the flasks were spiked with 1 mg/l boron, and the pH's were adjusted to either pH 4.5 or pH 9.0 so that each lake water was investigated with and without added boron at both pH values. The flasks were sealed and agitated on a mechanical shaker. At the end of four days they were removed from the shaker, allowed to settle, and the supernatants were analyzed for boron.

## B. ANALYTICAL METHODS

A prerequisite for investigation of any chemical or class of chemicals is the availability of a method of analysis sufficiently sensitive, specific, and accurate for the purposes of the investigation. From the previous discussion on analytical methods for boron, several methods would appear to be suitable for determination of low levels of boron in natural waters: the tetrafluoroborate-selective electrode of Carlson and Paul (1968), the hollow cathode atomic emission procedure of Daughtry and Harrison (1973), the fluorometric method of Afghan *et al.* (1972), and variations of the curcumin colorimetric method (Crawley, 1964; Pakalans, 1969; Uppstrom, 1968). Each has advantages and disadvantages, but any of these might be used for such an investigation. However, applying the additional criteria that the method must be fast and easily applicable to analysis of large numbers of samples eliminates the ion-selective electrode and atomic emission procedures, leaving the choice between the fluorometric and colorimetric methods. Of these, the colorimetric method was selected for this research for several reasons. A simple, accurate colorimetric method based on curcumin would likely find wider use than the fluorometric method since reagents are more readily available and the required instrumentation is found in more laboratories. Also, since most of the information available in the literature has been based on analyses using curcumin, the use of a similar method in this study reduces the possibility of differences in analytical methods complicating comparison of results.

In choosing among the variations of the curcumin method, the procedures employing acid anhydrides for water removal have the advantages of requiring less time and allowing the color-forming reaction to proceed in a completely liquid and homogeneous medium. Also, methods using an acetate buffer to convert the unreacted curcumin to a non-interfering form have the advantage of lower detection limits due to the smaller final volume of solution. In view of this, the method developed by Uppstrom, using propionic anhydride and an ammonium acetate buffer, might appear to be the logical choice. However, the large difference in cost of propionic anhydride and oxalyl chloride versus acetic anhydride and HCl would significantly increase the costs of analyzing large numbers of samples. With these considerations in mind, the following procedure was developed in an effort to combine the advantages of the methods of Crawley, Pakalans, and Uppstrom, while minimizing reagent costs.

*Reagents:*

Concentrated HCl, Reagent Grade

Glacial Acetic Acid:Acetic Anhydride, 4:5 (V/V),  
Reagent Grades

Glacial Acetic Acid:Sulfuric Acid, 1:1 (V/V),  
Reagent Grades

Curcumin, 0.125% in Glacial Acetic Acid

Buffer Solution, prepared as follows:

Dissolve 180 g reagent grade ammonium acetate in  
distilled, deionized water.  
Add 35 ml acetic acid, and mix.  
Add 100 ml ethanol, and mix.  
Dilute to 1 liter with distilled, deionized water.  
Filter through 0.45  $\mu$  PVC filter.

*Procedure:*

2 ml sample  
3 drops HCl  
9 ml acetic acid:anhydride reagent  
  
5 ml acetic anhydride  
  
5 ml curcumin solution  
8 ml acetic acid:sulfuric acid reagent  
  
40 ml ammonium acetate buffer

The first three items are added to a plastic beaker and mixed. After a minute or two, a reaction is observed which continues for about 5 minutes. Twenty minutes is allowed for the reaction to subside and the contents of the beaker to cool; then the remainder of the anhydride is added and 15 minutes is allowed for reaction. The curcumin and the acid mixture are then added, mixing well after each addition. The resulting solution is covered and allowed two hours for color development. Finally, 40 ml of the buffer is added and the absorbance is read in a spectrophotometer at 545 nm. Samples should be run in duplicate, and standards should be run with each batch of samples.

Using 4-cm absorbance cells and a Beckman Model DB spectrophotometer for color measurement the observed working range for this procedure is 0-250  $\mu$ g/l. The detection limit is <10  $\mu$ g/l, and the standard deviation,

based on five replicate analyses of a solution containing 40  $\mu\text{g}/\text{l}$  boron, is  $\pm 8 \mu\text{g}/\text{l}$ . The working range may be extended upward by use of shorter absorbance cells, smaller aliquots of sample, or dilution of more concentrated samples. Interferences are the same as for other curcumin methods (A.P.H.A. 1971), with the exception that since the final solution is aqueous instead of alcoholic, the interference of turbidity in the final solution due to  $\text{CaCO}_3$  precipitation is a rare occurrence, even with biological samples such as snails. Using this procedure one worker can conveniently run analyses of up to 40 samples (or 20 samples in duplicate) simultaneously.

### C. COLLECTION AND TREATMENT OF SAMPLES

Stream samples were collected at major points of access, usually by lowering a weighted polyethylene bottle from a highway bridge or culvert into flowing water at mid-stream. In a few instances samples were taken from docks along the river bank, or by wading into the water as far as possible and casting the sampler into the flow. Most lake samples were collected from docks, though a few were collected by wading into the lake, and some were collected from boats. Since most Florida lakes are rather shallow, the waters of small lakes and ponds usually are well-mixed. Therefore, as long as care is taken to avoid sampling near points of discharge of streams or drainage ditches into the lake, a single sample should be fairly representative of the water in the lake as a whole for the lower size range of lakes sampled. As the sizes of the lakes increase there is more likelihood of spatial variation in distribution due to less complete mixing, and thus lower probability that samples collected at a single point are representative of the entire lake.

Rainfall samplers for boron were constructed by fitting polyethylene funnels to polyethylene bottles and covering the tops of the funnels with fine-mesh nylon screen to deny insects access to the collected rainwater. Twelve samplers thus constructed were attached to posts located at approximately 10-mile intervals along a route from Cedar Key to Crescent Beach, Florida. Collection sites were chosen in open areas far enough away from the road to minimize contamination by dust raised by traffic. Samples were collected over the entire route at weekly intervals during September and October, 1973, and from Cedar Key to Gainesville during April, 1974.

After collection, all samples were acidified with a small amount of sulfuric acid to minimize adsorption onto the walls of the polyethylene bottles in which they were stored until analysis. All samples were analyzed promptly after collection, usually within 72 hours. Fish, snail, and plant samples from the model ecosystems study were rinsed thoroughly, dried to constant weight at  $105^\circ\text{C}$ , and then ashed at  $470^\circ\text{C}$ , for 1.5 hours. One milliliter of 0.1%  $\text{Ca}(\text{OH})_2$  solution was added to each of the fish samples to insure basic conditions and prevent loss of boron during ashing. After ashing, boron was extracted from the residue with 2 N  $\text{H}_2\text{SO}_4$ , and the resulting solutions analyzed in the same manner as the water samples.

## CHAPTER 4. BORON IN FLORIDA WATERS: RESULTS AND DISCUSSION

### A. BORON IN RAINFALL

The results of boron analyses performed on weekly rainfall samples collected during September and October, 1973, and April, 1974, are presented in Table 4-1, and Figure 4-1 shows the relationship between boron concentration in rainfall and distance from the coast.

Table 4-1. Boron Concentrations in Rainfall

<u>Station</u>	<u>Distance (km)</u>		<u>Boron (<math>\mu\text{g}/\text{l}</math>)</u>	
	<u>to Atlantic</u>	<u>to Gulf</u>	<u>September and October, 1973</u>	<u>April, 1974</u>
1	195	0.2	90	-
2	180	7.5	36	77
3	150	27.5	33	36
4	130	45	37	29
5	113	65	15	14
6	107	70	29	
7	91	80	43	
8	57	105	24	
9	50	120	27	
10	30	135	30	
11	11	155	29	
12	0.2	166	79	

The maximum concentration observed was 92  $\mu\text{g}/\text{l}$  in one sample collected at Crescent Beach, and the minimum was 12  $\mu\text{g}/\text{l}$  in several samples collected at Station 5 in Gainesville. These results support the supposition of Boyd and Walley (1972) that there is a gradient of atmospheric boron concentration versus distance from the ocean. The results also indicate a significant input from sea spray aerosols to boron levels in fresh waters, contrary to the conclusion of Nishimura *et al.* (1970) that the ocean is a sink, rather than a source of atmospheric boron. The values for sites near the coast are in general agreement with coastal values reported for Calcutta, India and Kiriu, Japan (Table 2-3), and the values for Gainesville rainfall found here are similar to those reported earlier by Odum and Parrish (1954). Boyd and Walley (1972) reported lower boron concentrations in rainfall collected at sites more remote from the coast.

### B. BORON IN MUNICIPAL WATERS AND SEWAGE

Samples of raw and treated water from 18 Florida municipalities were collected by mail through the cooperation of treatment plant operators. Results of analyses of these samples are reported in Table 4-2.

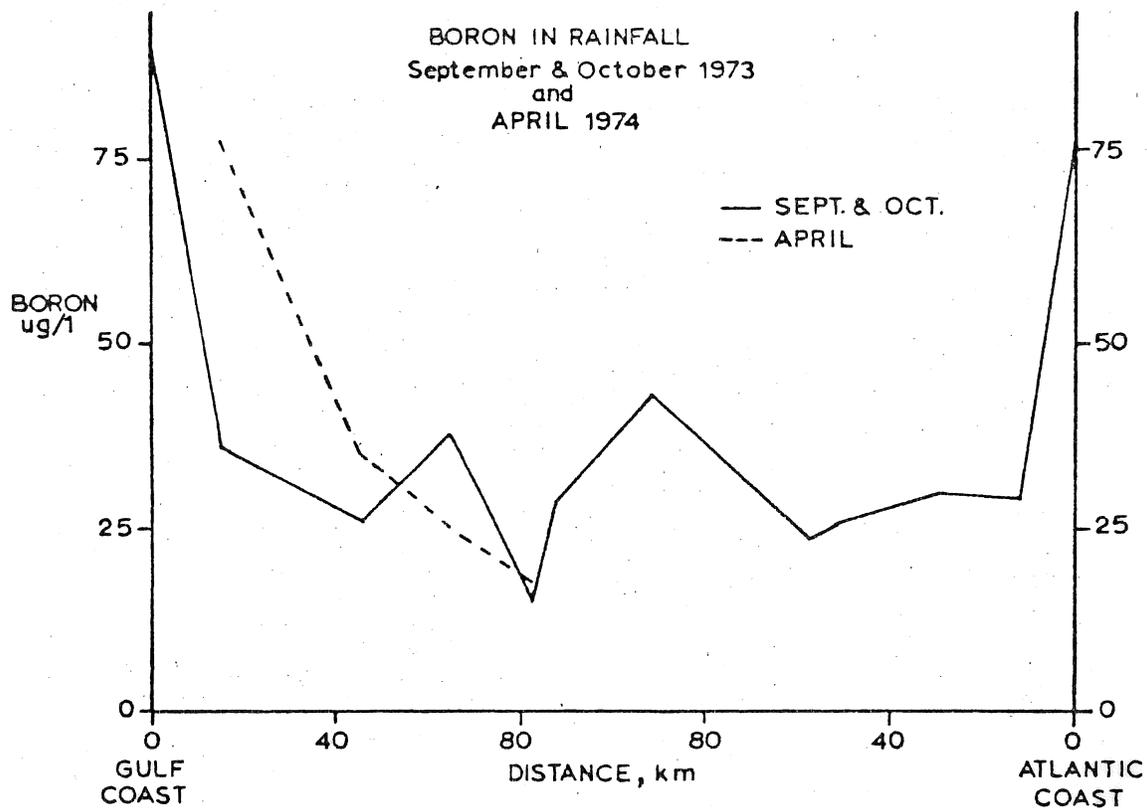


Figure 4-1. Variation of Boron Content of Rainfall with Distance from the Ocean

Table 4-2. Boron Concentrations in Municipal Water Supplies

City	Boron ( $\mu\text{g}/\text{l}$ )	
	Raw Water	Treated Water
Port Charlotte (#1 Plant)	84	53
Port Charlotte (#2 Plant)	46	43
North Port Charlotte	49	39
St. Petersburg	43	36
West Palm Beach	51	51
Ocala	47	43
Ft. Lauderdale	43	33
Pensacola	25	29
Melbourne	41	40
Bradenton	30	32
Lake City	33	36
Jacksonville	48	35
Daytona Beach	21	24
Lakeland	35	25
Tallahassee	29	29
Orlando	41	50
Sebring	25	33
Miami	87	99

Boron concentrations in the raw waters ranged from 21-87  $\mu\text{g}/\text{l}$ , with a mean of 43  $\mu\text{g}/\text{l}$ . Concentrations in the treated waters ranged from 24 to 99  $\mu\text{g}/\text{l}$ , with a mean of 41  $\mu\text{g}/\text{l}$ . These results indicate minimal, if any, boron removal by the water treatment processes used by these municipalities. This is in agreement with Waggott (1969), who observed little or no boron removal by treatment of sewage effluent with lime, alum or ferric sulfate.

Boron levels in treated sewage effluent from the activated sludge plant at the Gainesville, Florida, sewage treatment plant during the week of November 19-26, 1973, are depicted in Figure 4-2, and Figure 4-3 shows boron levels in both raw sewage and treated effluent for the trickling filter units at the same plant during the period June 14-18, 1974. Concentrations fluctuated within the 300-600  $\mu\text{g}/\text{l}$  range during the November, 1973, sampling period and within the 100-500  $\mu\text{g}/\text{l}$  range during the June, 1974 sampling period, with no recognizable daily trends during either period. Concentrations in sewage effluent varied over a narrower range than those in raw sewage during the June, 1974, sampling period, reflecting mixing and hence leveling of variability by the treatment processes. Despite the apparent buffering action, the results indicate little or no removal of boron by conventional sewage treatment processes, in agreement with Waggott (1969). These results are summarized in Table 4-3 along with grab sample data for a smaller Gainesville plant treating residential wastes, and results for sewage effluents from several other Florida municipalities.

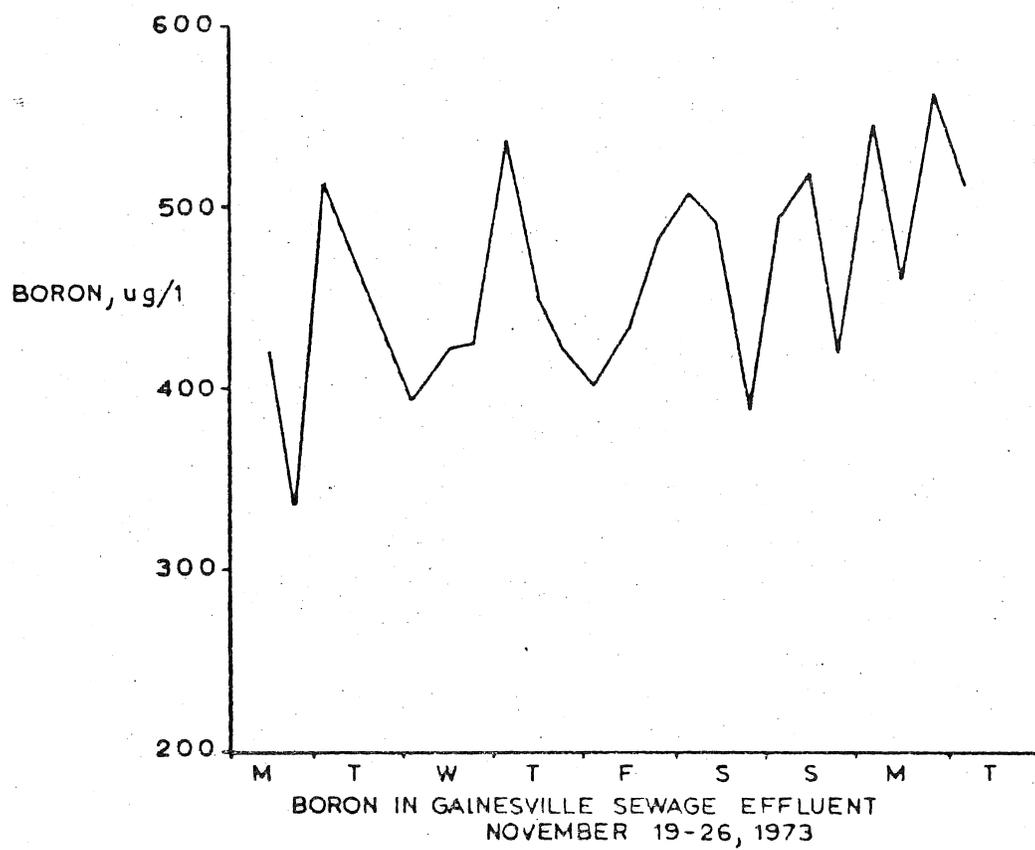


Figure 4-2. Boron in Gainesville Sewage Effluent  
November 19-26, 1973

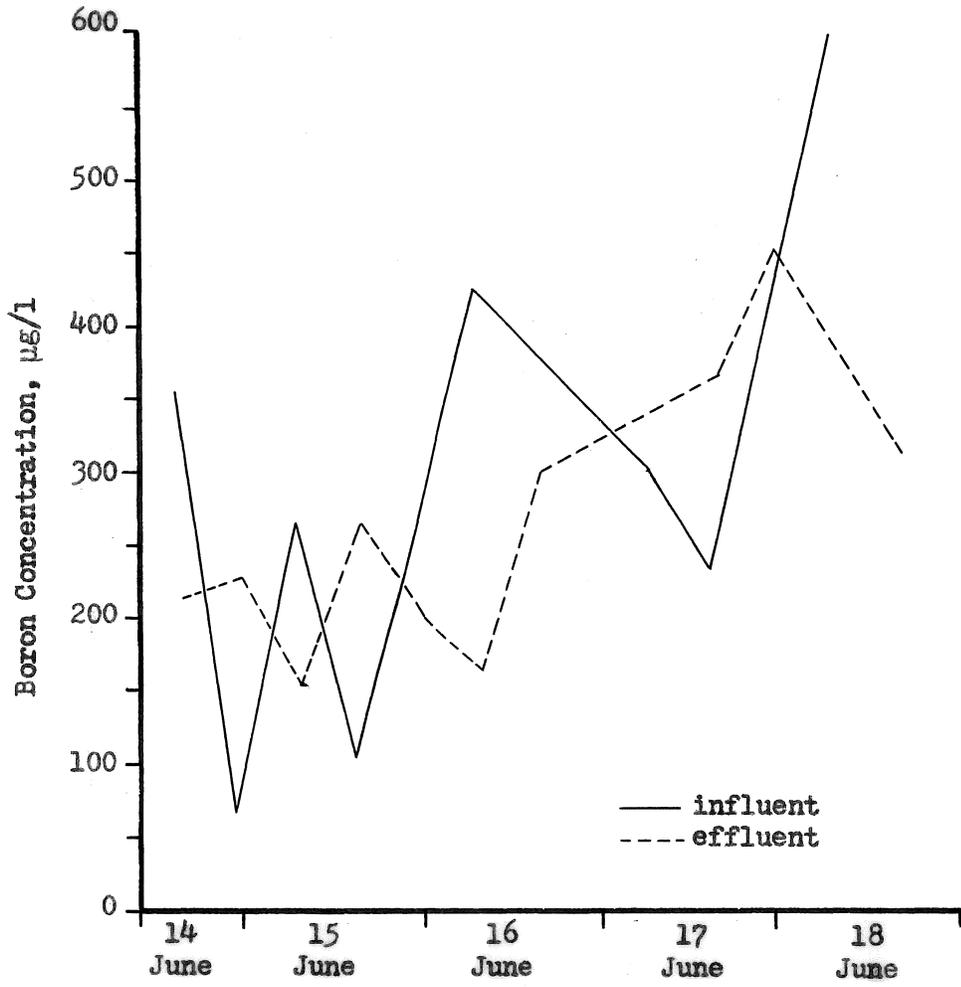


Figure 4-3. Boron in Gainesville Sewage Influent and Effluent June 14-18, 1974

Table 4-3. Boron Concentrations in Florida Sewage

<u>Location</u>	<u>Boron (<math>\mu\text{g}/\text{l}</math>)</u>
Gainesville	
Main Treatment Plant: Activated Sludge Effluent	334-567
Trickling Filter Influent	64-600
Trickling Filter Effluent	156-364
Northwood Treatment Plant: A/S Clarifier Effluent	767
Polishing Pond Effluent	827
Leesburg	268
Mount Dora	816
Winter Garden	368
Eustis	805

Comparison with the literature values (Table 2-2) reveals that boron concentrations in Florida sewage are generally typical, with levels approaching or exceeding the threshold for toxicity to citrus and other fruit trees.

### C. BORON IN FLORIDA SURFACE WATERS AND SPRINGS

The results of analyses on 120 water samples from lakes, ponds, springs, and streams in central and north-central Florida are presented as a histogram in Figure 4-4. A large majority of the samples (80%) contained boron in the range 10-60  $\mu\text{g}/\text{l}$ . Only three samples contained more than 100  $\mu\text{g}/\text{l}$ , and the sampling points for each of these were downstream from sewage treatment plants which contributed significant portions of the total stream flows. The median concentration for the 120 samples was 41.5  $\mu\text{g}/\text{l}$ . These results are similar to those of Boyd and Walley (1972) for a larger area encompassing the southeastern U.S., but somewhat higher than the values reported by Odum and Parrish (1954) for several sites in Florida. The higher values found in this research probably reflect increased cultural influence on many water bodies in the twenty years since their work was published. Results for each of the sites are presented in Tables 4-4 to 4-6 and in the following section on boron distribution in selected watersheds.

Boron concentrations in fourteen springs are listed in Table 4-4. The range observed was 19-90  $\mu\text{g}/\text{l}$ , with a median of 39  $\mu\text{g}/\text{l}$ . Odum and Parrish (1954) found 55  $\mu\text{g}/\text{l}$  in Ponce de Leon Springs, compared to the 46  $\mu\text{g}/\text{l}$  reported here.

The results of boron analyses for 51 lakes and ponds are shown in Table 4-5, and the frequency distribution histogram for these results is depicted in Figure 4-5. In this group of samples the range was 12-89  $\mu\text{g}/\text{l}$ , the median was 46  $\mu\text{g}/\text{l}$ , and mean was 45.5  $\mu\text{g}/\text{l}$ . Odum and Parrish (1954) sampled two of these lakes, reporting 12  $\mu\text{g}/\text{l}$  in Newnans Lake and 17  $\mu\text{g}/\text{l}$  in Lake Lochloosa. These values compare favorably with the 12 and 21  $\mu\text{g}/\text{l}$  concentrations observed for the same lakes in this study. A sub-group of 10 citrus grove lakes and ponds in central Florida had a mean boron content of 46.3  $\mu\text{g}/\text{l}$ .

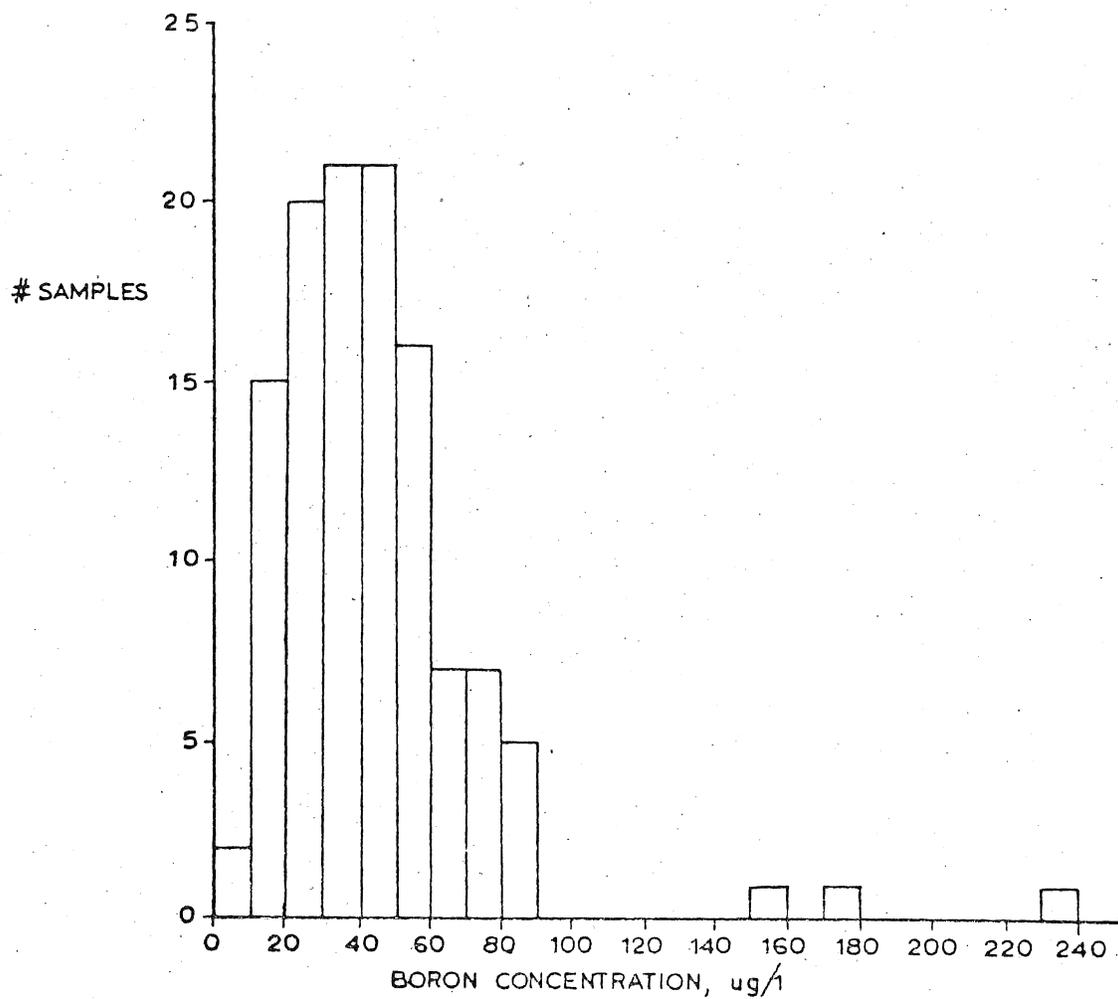


Figure 4-4. Frequency Distribution Histogram for Boron Content of 120 Natural Waters of Florida

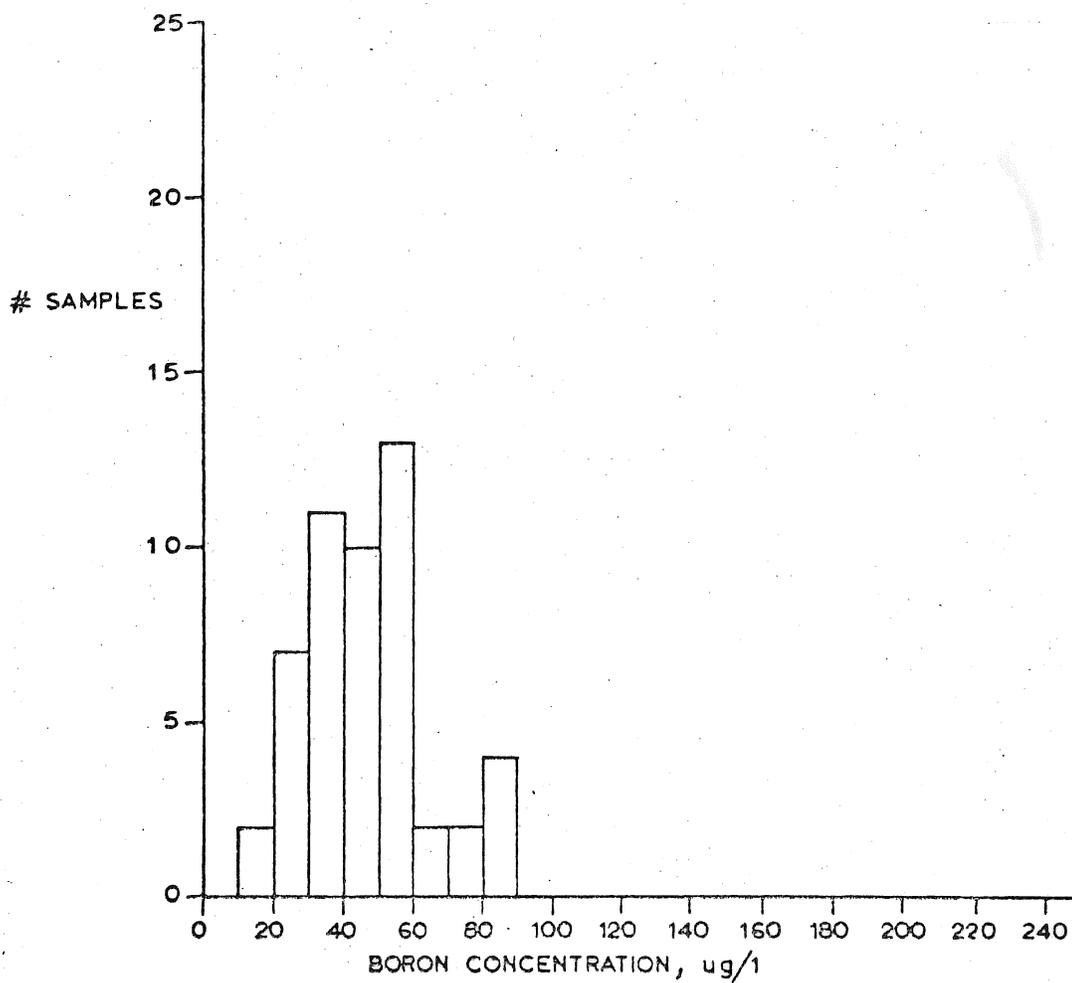


Figure 4-5. Frequency Distribution Histogram for Boron Content of 51 Florida Lakes and Ponds

Table 4-4. Boron Concentrations in Florida Springs

<u>Location</u>	<u>Boron (<math>\mu\text{g}/\text{l}</math>)</u>
Alexander Springs, Ocala National Forest	40
Juniper Springs, Ocala National Forest	20
Ponce de Leon Springs, Volusia Co.	46
Fort Green Springs, Hardee Co.	19
Glen Springs, Gainesville	19
Unnamed spring tributary to Hogtown Creek, Gainesville	33
Springs draining into Devil's Millhopper, Gainesville	
#1	37
#2	46
#3	45
#4	38
#5	38
Magnolia Springs, Alachua Co.	83
Ichnetucknee Springs, Columbia Co.	90
Blue Springs, Gilchrist Co.	70

Results for eight of the 58 stream samples collected are presented in Table 4-6. The remainder of the results for individual stream samples are included in the following section on selected drainage basins.

Table 4-6. Boron Concentrations in Miscellaneous Florida Streams

<u>Location</u>	<u>Boron (<math>\mu\text{g}/\text{l}</math>)</u>
Hatchet Creek at S.R. 24, Gainesville	22
Hogtown Creek at S.R. 26, Gainesville	33
Creek draining into Devil's Millhopper, Gainesville	53
Santa Fe River at U.S. 301, Alachua Co.	27
Palatlahaha Creek, Lake Co.	46
Otter Creek at S.R. 24, Levy Co.	19
Hillsborough River at S.R. 39, Pasco Co.	40
Blackwater Creek at S.R. 39, Pasco Co.	29

Boron concentrations encountered for all stream samples varied from about 4  $\mu\text{g}/\text{l}$  to 236  $\mu\text{g}/\text{l}$ , with a median of 31  $\mu\text{g}/\text{l}$ . The Hatchet and Hogtown Creek sites were sampled by Odum and Parrish (1954), who found 13 and 12  $\mu\text{g}/\text{l}$ , respectively, at those sites, compared with 22 and 33  $\mu\text{g}/\text{l}$  in this research. This increase is probably partly due to increased urbanization of the surrounding area, but may also reflect other factors such as seasonal variations in rainfall. The frequency distribution histogram for boron concentrations in Florida streams is presented in Figure 4-6.

Table 4-5. Boron Concentrations in Florida Lakes and Ponds

	<u>Boron (<math>\mu\text{g}/\text{l}</math>)</u>
Lake Altho, Alachua Co.	23
Lake Mize, Alachua Co.	38
Lake Nauberg, Alachua Co.	26
Lake Santa Fe, Alachua Co.	24
Melrose Bay, Alachua Co.	22
Little Lake Santa Fe, Alachua Co.	30
Hawthorne Lake, Alachua Co.	70
Newnans Lake, Alachua Co.	12
Lake Lochloosa, Alachua Co.	21
Orange Lake, Alachua Co.	30
Little Orange Lake, Alachua Co.	14
Lake Kanapaha, Alachua Co.	28
Cowpen Lake, Putnam Co.	51
Swan Lake, Putnam Co.	45
Rodman Reservoir, Putnam Co.	51
Crescent Lake, Putnam Co.	48
Lake Dorr, Ocala National Forest	32
Lake Kerr, Ocala National Forest	52
Lake Hampton, Bradford Co.	39
Lake Sampson, Bradford Co.	30
Kingsley Lake, Clay Co.	43
Lake Geneva, Clay Co.	38
Lake Magnolia, Clay Co.	20
Lake Brooklyn, Clay Co.	35
Lake Weir, Marion Co.	68
Weir Bay, Marion Co.	76
Lake Poinsett, Brevard Co.	48
Lake Pickett, Orange Co.	57
Lake Apopka, Orange Co.	40
Lake Jessup, Seminole Co.	89
Lake Monroe, Seminole Co.	83
Lake Tohopekaliga, Osceola Co.	68
East Lake Tohopekaliga, Osceola Co.	83
Alligator Lake, Osceola Co.	85
Lake Dora, Lake Co.	47
Lake Harris, Lake Co.	52
Little Lake Harris, Lake Co.	31
Lake Eustis, Lake Co.	53
Lake Griffin, Lake Co.	45
Lake Minneola, Lake Co.	51
Lake Minnehaha, Lake Co.	57
Unnamed Citrus Grove Ponds, Lake Co.	
#1	41
#2	51
#3	49
#4	55
#5	30
#6	46
#7	52
#8	52
#9	36
#10	51

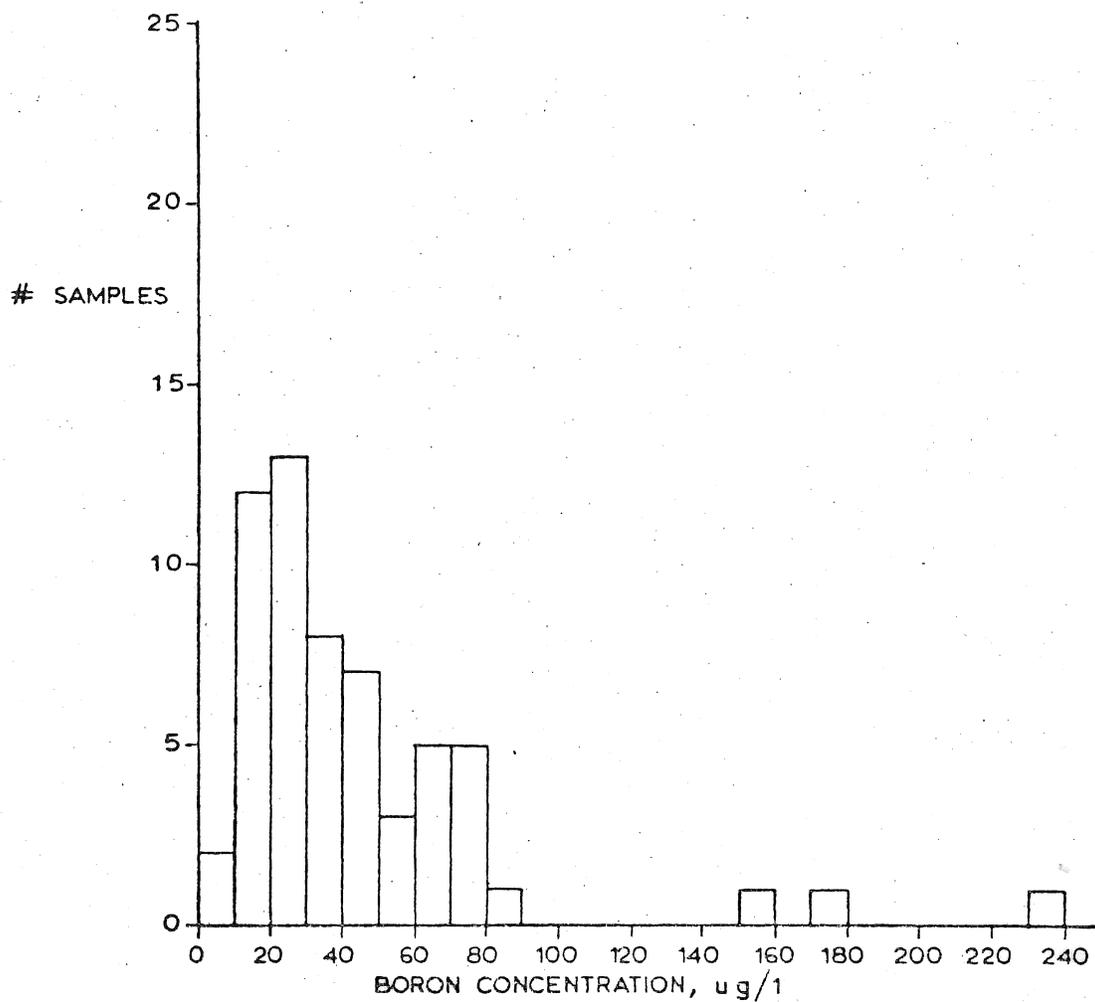


Figure 4-6. Frequency Distribution Histogram for Boron Content of 58 Florida Stream Samples

## D. BORON DISTRIBUTION IN SELECTED DRAINAGE BASINS

### *Hogtown Creek*

Figure 4-7 shows boron concentrations in the Hogtown Creek drainage basin on December 18, 1973. This stream is the principal means of drainage for much of Gainesville, and consists of two branches. The eastern branch drains a combination of industrial, residential, and business areas, while the western branch receives mainly residential drainage. The most prominent feature of the data is the impact of the Northwood sewage treatment plant effluent on boron levels in Possum Branch, the western branch of the creek. Just upstream from the treatment plant the boron concentration in Possum Branch is 24  $\mu\text{g}/\text{l}$ . Disposal of effluent containing about 800  $\mu\text{g}/\text{l}$  raises the level to 232  $\mu\text{g}/\text{l}$  just downstream from the plant. However, the concentration decreases rapidly further downstream from the plant, probably as a result of dilution by groundwater entering the stream bed and tributaries feeding the main stream. Some boron may be sorbed directly onto the sediments and clays or may react with organic color to produce complexes which may be sorbed, but these losses are probably small. In general, in this drainage basin, as in the others investigated, the trend is for the highest boron concentrations to occur in the upper reaches of the stream and gradually decrease further downstream, except where major points of addition of boron occur. Boyd and Walley (1972) observed a similar trend, and attributed it to the greater effect that local variations in geology have on small streams as opposed to the buffering action of larger streams on these effects.

### *River Systems of Southwestern Central Florida*

Figure 4-8 shows the major streams draining the southwestern area of Central Florida and the boron levels observed in them in October, 1973. Boron concentrations in these streams generally were between 10 and 35  $\mu\text{g}/\text{l}$ , with an occasional sample being slightly higher or lower. These values are comparable to those observed in the Hogtown Creek drainage basin. Based on the results for boron concentrations in rainfall, these values seem slightly low given the proximity of the region to the Gulf Coast. However, the samples were taken during the dry season and thus are probably more representative of boron concentrations in the base flow than in surface drainage. In addition, the upper reaches of the streams drain land 30-60 km from the coast, further reducing the contribution of marine-derived rainfall to boron in these streams.

### *St. Johns River*

The St. Johns is unique among the streams investigated in that it flows parallel to the coastline only a short distance from the ocean during its entire course. Because of the higher concentration of boron in rainfall, higher levels of boron were expected in the St. Johns than in streams further inland, and the values shown in Figure 4-9 tend to support this expectation, with concentrations generally in the range of 70-90  $\mu\text{g}/\text{l}$ . Noteworthy is the high boron concentration (154  $\mu\text{g}/\text{l}$ ) south of Lake Harney,

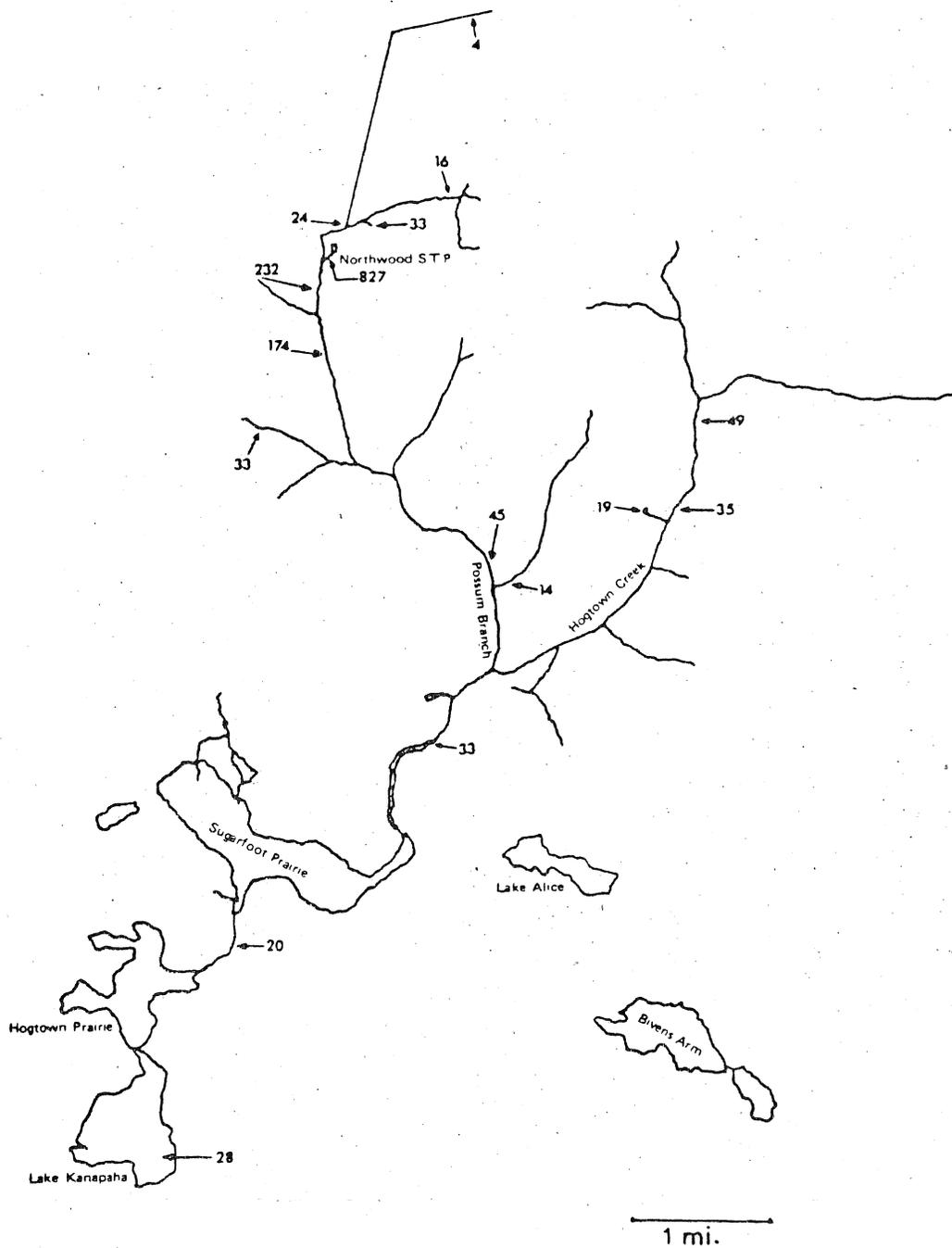


Figure 4-7. Boron Concentrations in the Hogtown Creek Drainage Basin--December 18, 1973

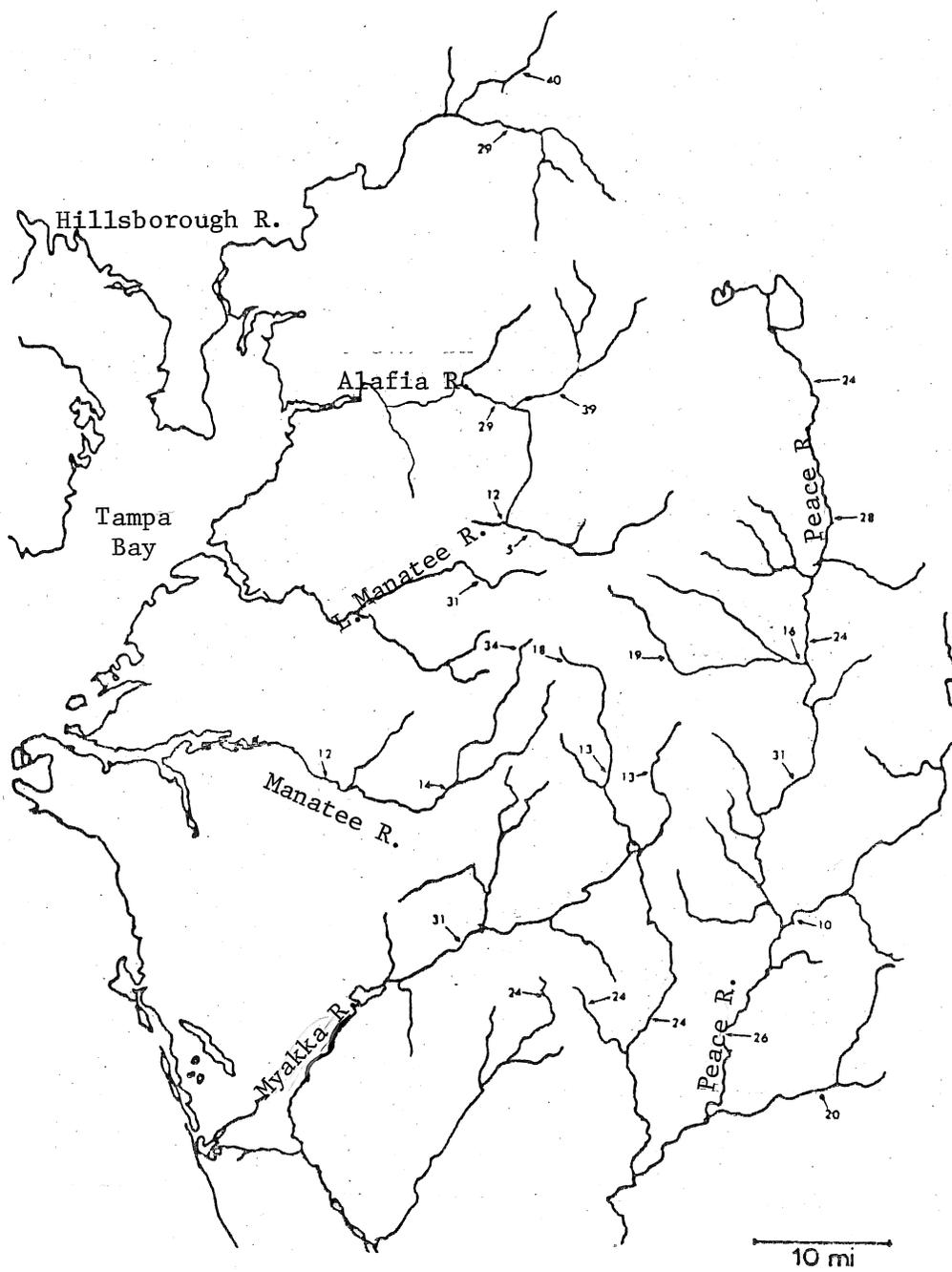


Figure 4-8. Boron Concentrations in the River Systems of Southwestern Central Florida--October, 1973

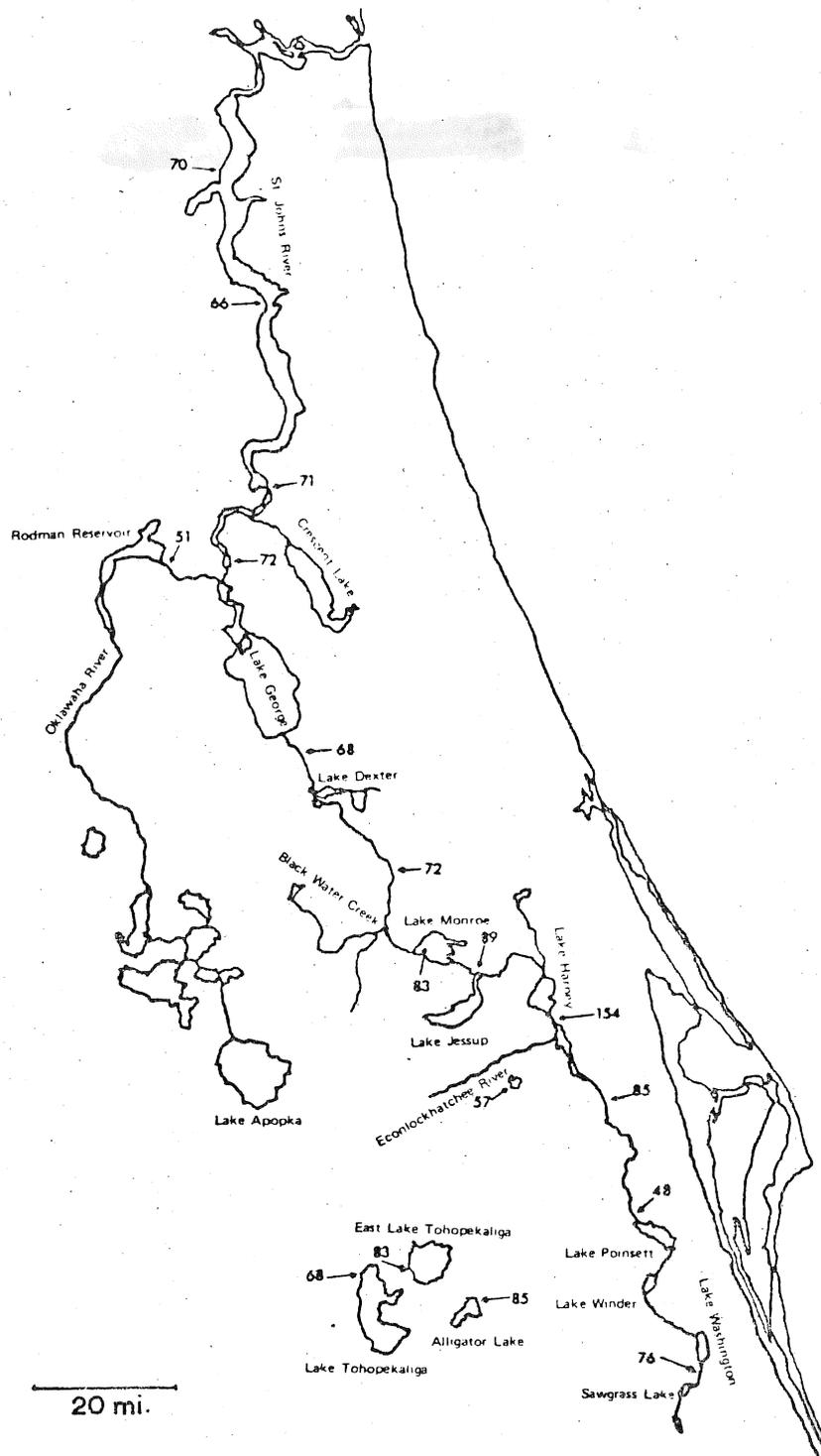


Figure 4-9. Boron Concentrations in the St. Johns River Drainage Basin--January, 1974

immediately downstream from the point where the Econlockhatchee River empties into the St. Johns. The Econlockhatchee River receives a large part of the sewage effluent from Orlando, and apparently has high boron levels though it was not sampled directly. Thus the high concentration in the St. Johns at this sampling point is probably due to the sewage effluent in the Econlockhatchee.

#### *Oklawaha River*

The boron concentrations in the Oklawaha River basin fall between those in the St. Johns and those in the Hogtown Creek and the Southwest-Central Florida river systems as seen in Figure 4-10. Concentrations ranged from 50 to 60  $\mu\text{g}/\text{l}$  in the region of the Oklawaha chain of lakes and decreased further downstream to a concentration of 42  $\mu\text{g}/\text{l}$  just upstream from the Rodman Reservoir. Through the reservoir the level increased to a value of 51  $\mu\text{g}/\text{l}$  at the spillway of the dam at the north end. Sewage effluent, which enters the system at Winter Garden, Mount Dora, Eustis, and Leesburg, raises the boron levels in the chain of lakes region. Drainage water from the muck farms at the north end of Lake Apopka may also contribute to the high levels. The increase through the Rodman Reservoir may be due to decay of organic matter or leaching of boron from the recently inundated soil.

### E. BORON DISTRIBUTION IN MODEL AQUATIC ECOSYSTEMS

Results of boron analyses on the fish, snails, and aquatic plants used in the miniature aquatic ecosystems are presented in Table 4-7. No data are presented for the boron content of three of the plant species in the system containing 0.5 mg/l boron because of their consumption by numerous small snails inadvertently included in the system. Fish in the systems with 0.5 and 5.0 mg/l boron developed disease symptoms (apparently unrelated to boron) after a few days and were removed and not analyzed.

The results indicate pronounced differences between different species of plants both in typical levels and in rates of uptake of boron. All plants used in the study were collected from one location, growing in close proximity to each other, yet the initial boron levels ranged from 30 ppm to 525 ppm on a dry weight basis, depending on the species. Efforts to linearize the data were unproductive. Plots of boron content of plants versus solution concentration, and reciprocal Lineweaver-Burk plots were non-linear. The most useful graphical presentation of the results was a plot of boron content of plants versus the logarithm of solution concentration, depicted in Figure 4-11. From this figure it appears that the rate of boron uptake remains relatively constant up to a concentration of about 1 mg/l boron in solution. Above this concentration the rate of uptake increases, up to the limit of toxicity of boron to the particular species.

Toxic effects were observed for three of the four species of plants used in this study. Hydrocotyle contained the lowest levels of boron and appeared least resistant to high boron concentrations, with leaf

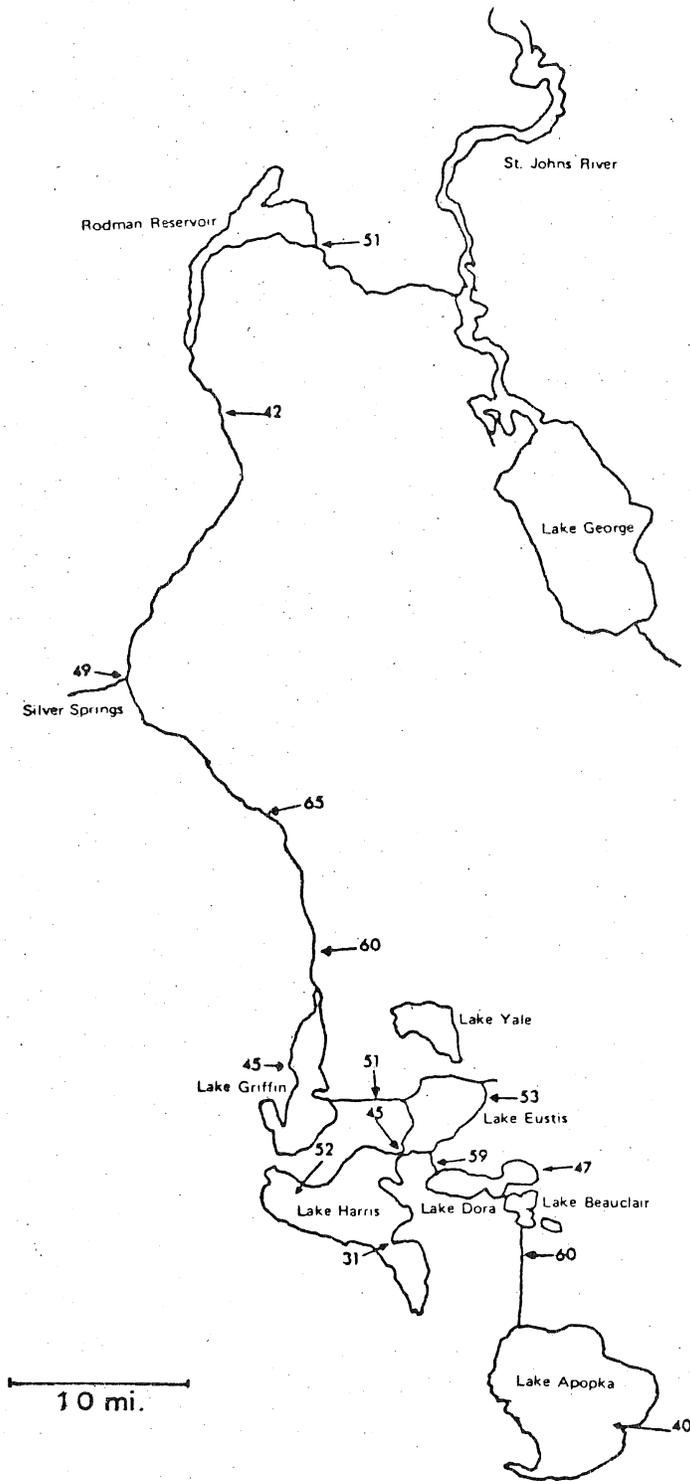


Figure 4-10. Boron Concentrations in the Oklawaha River Drainage Basin--February, 1974

Table 4-7. Boron Content of Gambusia\*, Helisoma\*, and  
Several Species of Aquatic Plants\*\* After Exposure  
to Varying Concentrations of Boron in Solution

Solution Concentration (mg/l)	Boron, ppm dry weight					
	(Duckweed)	(Pennywort)	(Floating heart)	(Coontail)	(Mosquito fish)	(Snail)
	<u>Lemna</u>	<u>Hydrocotyle</u>	<u>Nymphoides</u>	<u>Ceratophyllum</u>	<u>Gambusia</u>	<u>Helisoma</u>
0.03***	525	31.3	78.0	39.0	1.10	2.35
0.1	519	37.5	86.9	50.5	.59	2.16
0.5	--	--	--	43.4	--	1.72
1.0	1132	52.2	71.8	47.6	2.43	1.87
5.0	2700	639	176	129	--	4.15
10.0	2880	237	524	286	5.90	44.9
50.0	2440	--	797	762	23.0	73.6

\*Gambusia and Helisoma analyzed after 10 days' exposure

\*\*Aquatic plants analyzed after 30 days' exposure

\*\*\*The boron concentration in the water used to prepare the synthetic river water was 30  $\mu\text{g/l}$ .

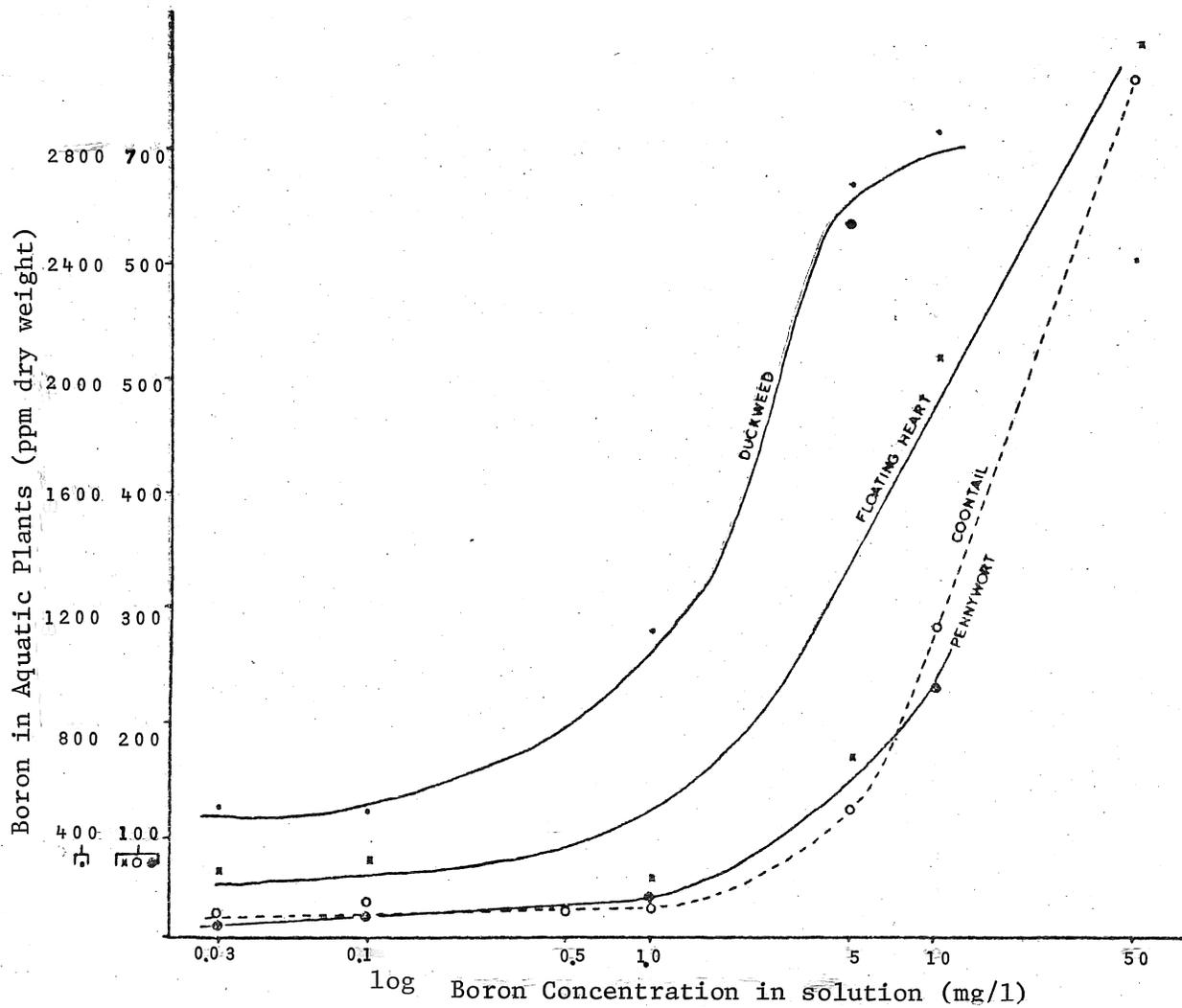


Figure 4-11. Boron Content of Four Species of Aquatic Plants After 30 days' Exposure to Varying Solution Concentrations of Boron Versus log (Solution Concentration)

spotting and yellowing observed in systems containing more than 1 mg/l boron. The specimen in the system containing 50 mg/l died, apparently as a result of boron toxicity. Specimens of Lemna exhibited yellowed leaflets and reduced growth in systems containing more than 5 mg/l boron. Nymphoides and Ceratophyllum appeared more tolerant of high boron concentrations. Neither exhibited leaf-yellowing or reduced growth, but the Nymphoides specimens in the system containing 50 mg/l did show pronounced curling of new leaves. No toxic effects were observed for either Gambusia (mosquito fish) or the snail, Helisoma. Some boron uptake was observed for these organisms in the systems containing 5, 10, and 50 mg/l boron, but the levels were significantly lower than for the plant samples. The total amount of boron assimilated by the plant and animal life in each system averaged less than 1% of that initially added.

## F. BORON ADSORPTION BY KAOLINITE AND ATTAPULGITE

Results of the investigation of boron adsorption onto two clays, kaolinite (EPK/AF, Edgar Plastic Kaolin Co., Edgar, Fl.), and attapulgite (Diluex, The Floridan Co., Quincy, Fl.), are presented in Table 4-8. It is apparent from the data that essentially no sorption occurred for either of these clays, even though the pH values were near the optimum reported by other workers. This seems in disagreement with the work reviewed by Bingham (1973). However, before drawing such a conclusion additional factors must be considered. The studies reported in the literature were conducted using boric acid solutions varying from about 10-400 mg/l boron and allowing sorption to occur over a period of three or four weeks before analysis. In addition, much of that work was conducted using synthetic seawater media. These are quite different conditions from the 0-1 mg/l concentrations, 24-hour reaction time, and distilled water medium used in this study. Interpretation of the results presented here is confounded by the variation in ionic strengths of the solutions used and by the absence of similar trends within each series. The two types of clays investigated are representative of much of the surficial clay deposits in Florida, and these preliminary results suggest that boron in Florida waters is not controlled by direct interaction with soils or mineral sediments. However, attempts to extrapolate these results to boron adsorption by other clay minerals or adsorption of boron-organic complexes would not be warranted.

Comparison of boron sorption rates by samples of attapulgite under similar conditions with highly colored Lake Mize water and clear Lake Magnolia water (Table 4-9) indicates that the organic color present in Lake Mize water has no dramatic effect on boron sorption by this clay.

The observed boron sorption rates were slightly different for the two water samples, but it would be inappropriate to overemphasize these differences for such a brief experiment. Additional experiments with more rigorous control of the variables are necessary to more precisely quantify the boron sorption capacities of clays and the effects of organic color on the sorption process. However, it appears from the results of this preliminary investigation that little boron sorption by

Table 4-8. Boron Concentrations in Solutions After 24 Hours contact with Kaolinite and Attapulgite

Approximate* Concentrations of Boron Initially Added	Volume of 0.05 M NaOH	Concentration of Boron ( $\mu\text{g}/\text{l}$ ) in Solution							
		0 $\mu\text{g}/\text{l}$		100 $\mu\text{g}/\text{l}$		400 $\mu\text{g}/\text{l}$		1000 $\mu\text{g}/\text{l}$	
Type of Clay		pH	Boron	pH	Boron	pH	Boron	pH	Boron
Controls (No Clay Added)	0 ml	4.7	23	5.2	121	5.4	544	5.1	1240
	1 ml	9.8	22	10.2	117	9.7	608	9.2	1250
	2 ml	10.4	55	10.5	136	10.1	524	10.2	1290
Kaolinite	0 ml	5.7	44	6.8	179	6.1	464	5.9	1310
	1 ml	8.6	30	9.2	152	8.7	484	8.8	1240
	2 ml	10.0	35	10.3	153	9.8	460	9.7	1310
Attapulgite	0 ml	9.2	55	9.2	144	8.6	504	8.7	1250
	1 ml	9.8	76	---	---	9.6	584	9.4	1310
	2 ml	10.1	98	9.0	121	9.9	524	10.0	1310

\* Because of the background level of 30  $\mu\text{g}/\text{l}$  boron in the water used to prepare the solutions used in this study, no attempt was made to prepare solutions containing exactly 100, 400, and 1000  $\mu\text{g}/\text{l}$  boron. Instead, 4 liters of each solution was prepared with the approximate concentrations of boron and 250-ml portions of these stock solutions were added to the flasks used in each series, insuring uniformity of added boron concentrations within each series and depending on analysis of the controls to establish the added concentrations more exactly.

clays occurs in natural waters and that if boron-organic color interactions occur, they do not have a major effect on boron uptake by clays.

Table 4-9. Boron sorption by attapulgite clay after 24 hours equilibration with clear and colored oligotrophic lake water.

pH	<u>µg Boron sorbed per gram of clay</u>			
	<u>Lake Mize (colored)</u>		<u>Lake Magnolia (clear)</u>	
	Control (34 µg B/l)	Spiked (1034 µg B/l)	Control (10 µg/l)	Spiked (1010 µg/l)
4.5	17	82	5	105
9.0	12	87	0	110

## G. INVESTIGATIONS OF BORON-ORGANIC COLOR INTERACTIONS

Results of the gel permeation chromatography investigations of boron-organic color interaction are presented in Figures 4-12 to 4-16. Figures 4-12 to 4-14 show results obtained with distilled water as the eluant, Figure 4-15, with pH 8.0 phosphate buffer, and Figure 4-16, with pH 8.0 borate buffer. Figure 4-12 illustrates a typical elution profile for the dyes used to determine the excluded and included volumes of the Sephadex column. The excluded volume corresponds to the peak elution of the high molecular weight dye, Blue Dextran 2000 ( $MW \approx 2 \times 10^6$ ), and the included volume corresponds to the peak elution of the low molecular weight dye, methyl orange ( $MW \approx 700$ ). For the column used in this work the Blue Dextran 2000 was consistently eluted principally in the eleventh fraction; thus the excluded volume was 110 ml. The methyl orange was generally eluted principally in the 32nd to 34th fraction, indicating an included volume of 320-340 ml. Some problems were encountered using methyl orange as the low molecular weight dye with the phosphate buffer eluant. Under these conditions the methyl orange was retained on the column about 15 fractions longer than when either distilled water or borate buffer was employed as the eluant. This problem was circumvented by using a dilute  $K_2CrO_4$  solution for the low molecular weight dye when eluting with phosphate buffer.

Figures 4-13 and 4-14 show the color and boron elution profiles for both spiked and unspiked color concentrate with distilled water eluant. The organic color was eluted in two peaks, one occurring at the high molecular weight end, with the high molecular weight fraction having a slightly more intense color peak under these conditions. The fact that the color elution patterns were identical for both spiked and unspiked samples indicates that either no significant boron-organic color complexes form under these conditions, or that interaction with the Sephadex gel destabilizes any complexes formed. The boron elution profiles also support such an argument. With the unspiked color concentrate the background boron was eluted in two peaks. A small peak occurred at about

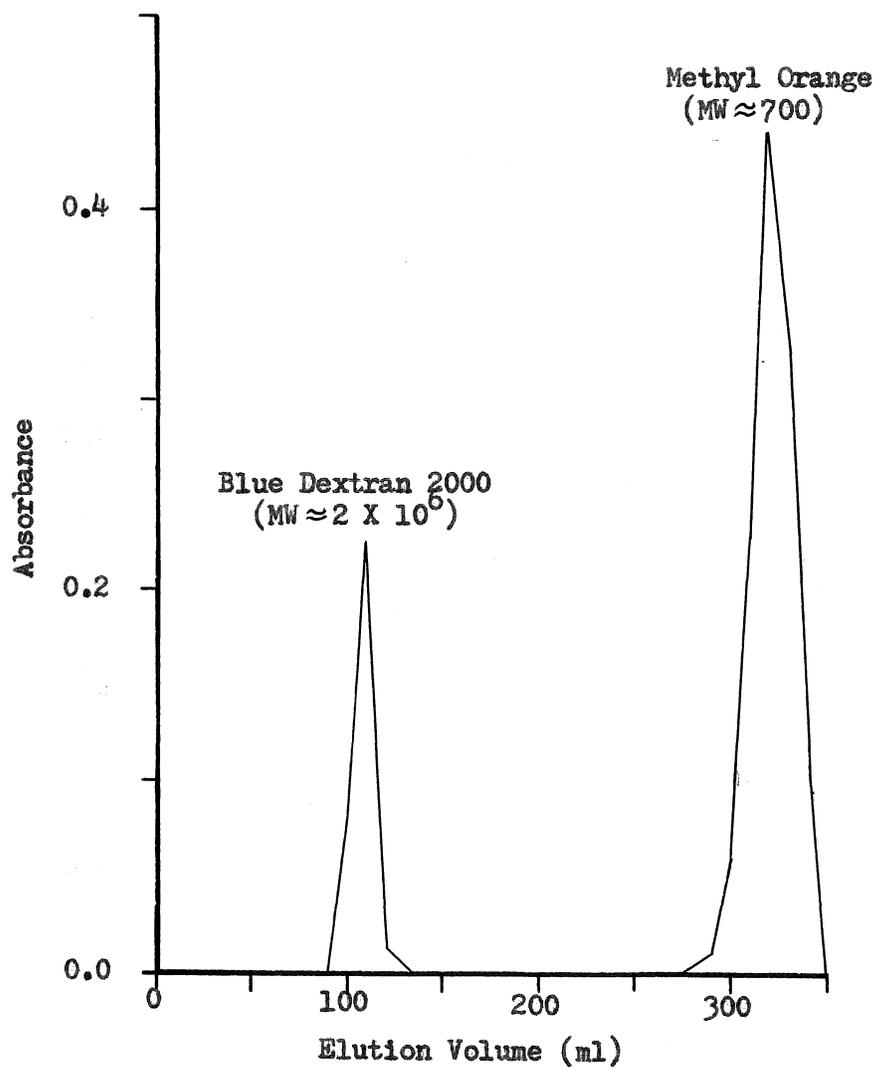


Figure 4-12. Low and High Molecular Weight Dye Elution Profile on Sephadex G-50 Eluted with Distilled Water. (Flow rate = 2ml/min.)

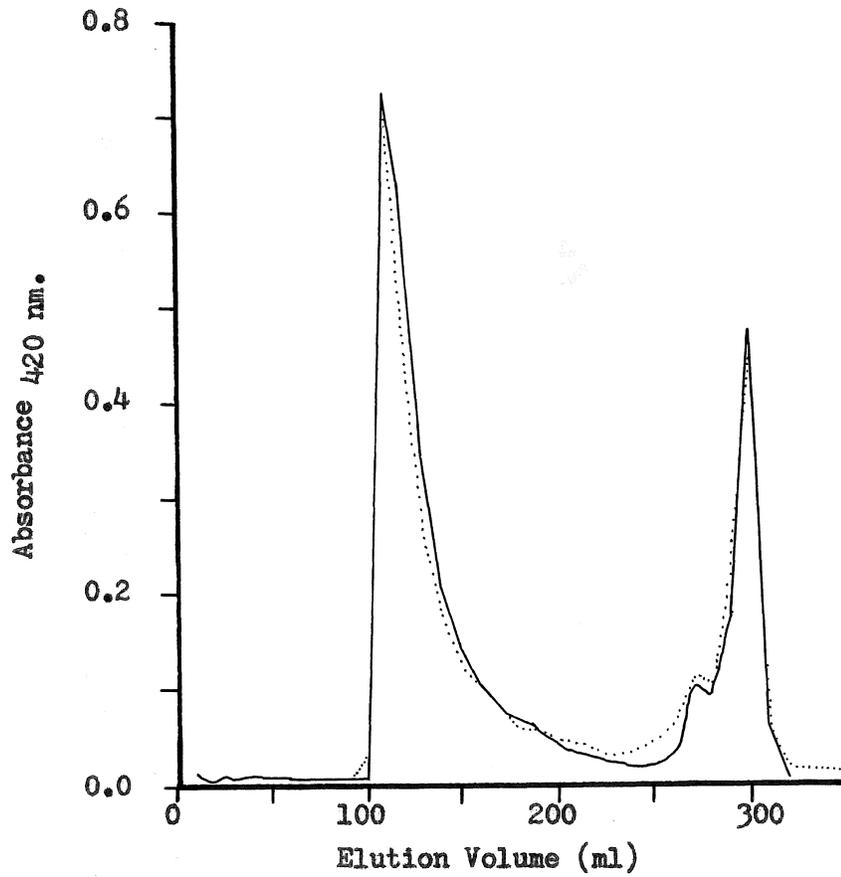


Figure 4-13. Organic Color Elution Profile on Sephadex G-50 Eluted with Distilled Water. (Flow rate = 2 ml/min. Legend: — = unspiked color concentrate, ..... = concentrate spiked with 96 mg/l boron.)

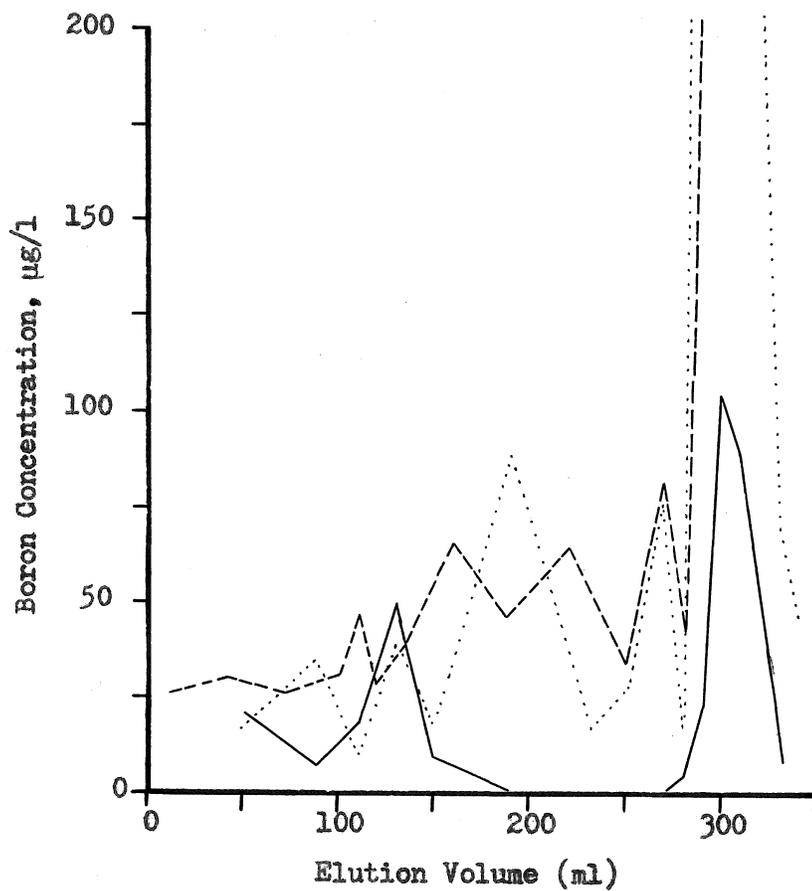


Figure 4-14. Boron Elution Profile for Organic Color Concentrate Eluted from Sephadex G-50 with Distilled Water. (Flow rate = 2 ml/min. Legend: — = unspiked color concentrate, - - - = concentrate spiked with 32 mg/l boron, ..... = concentrate spiked with 96 mg/l boron.)

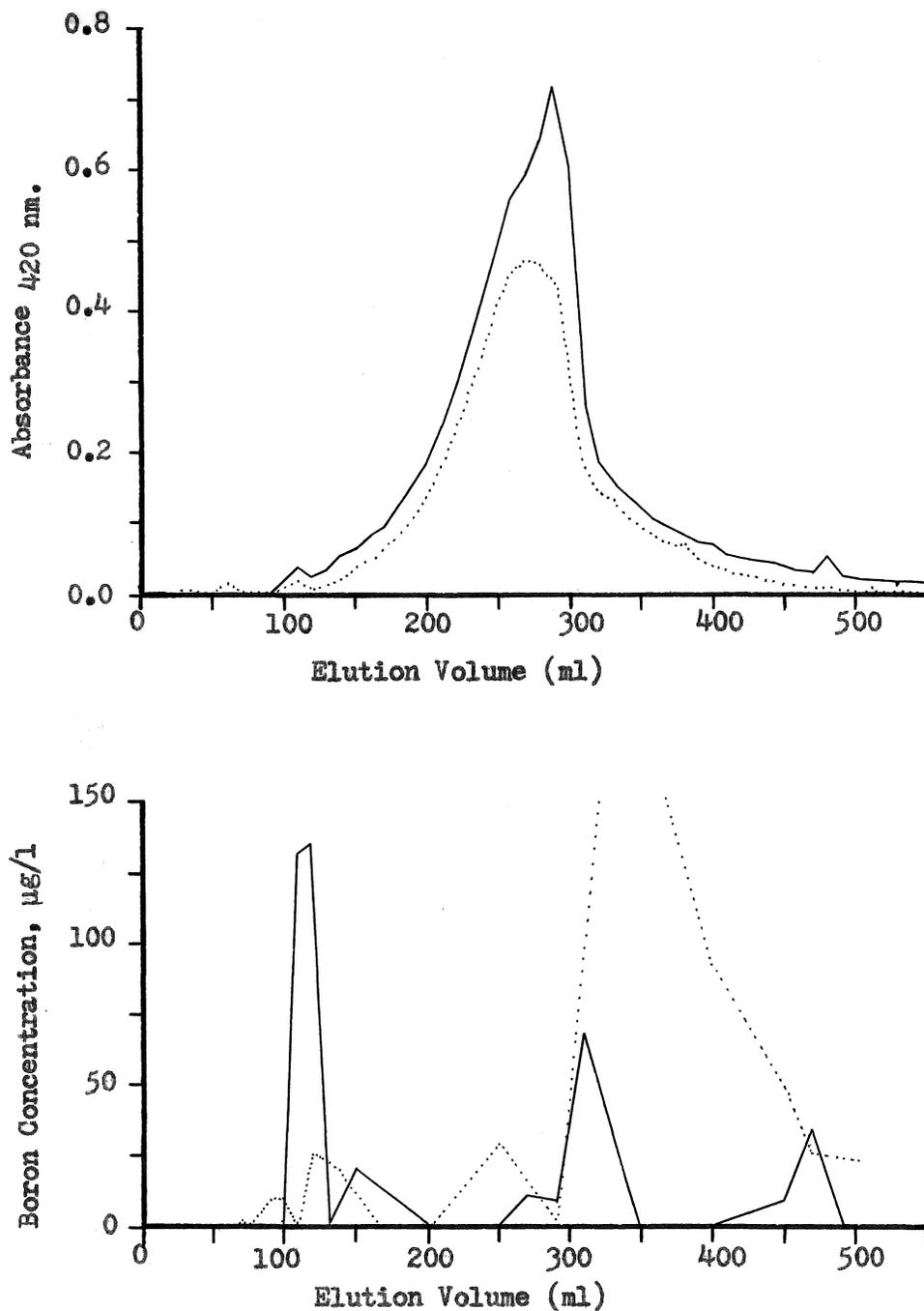


Figure 4-15. Organic Color and Boron Elution Profiles for Color Concentrate Eluted from Sephadex G-50 with pH 8.0 Phosphate Buffer. (Flow rate = 2 ml/min  
 Legend: ————— = unspiked color concentrate  
 ..... = concentrate spiked with 32 mg/l boron.)

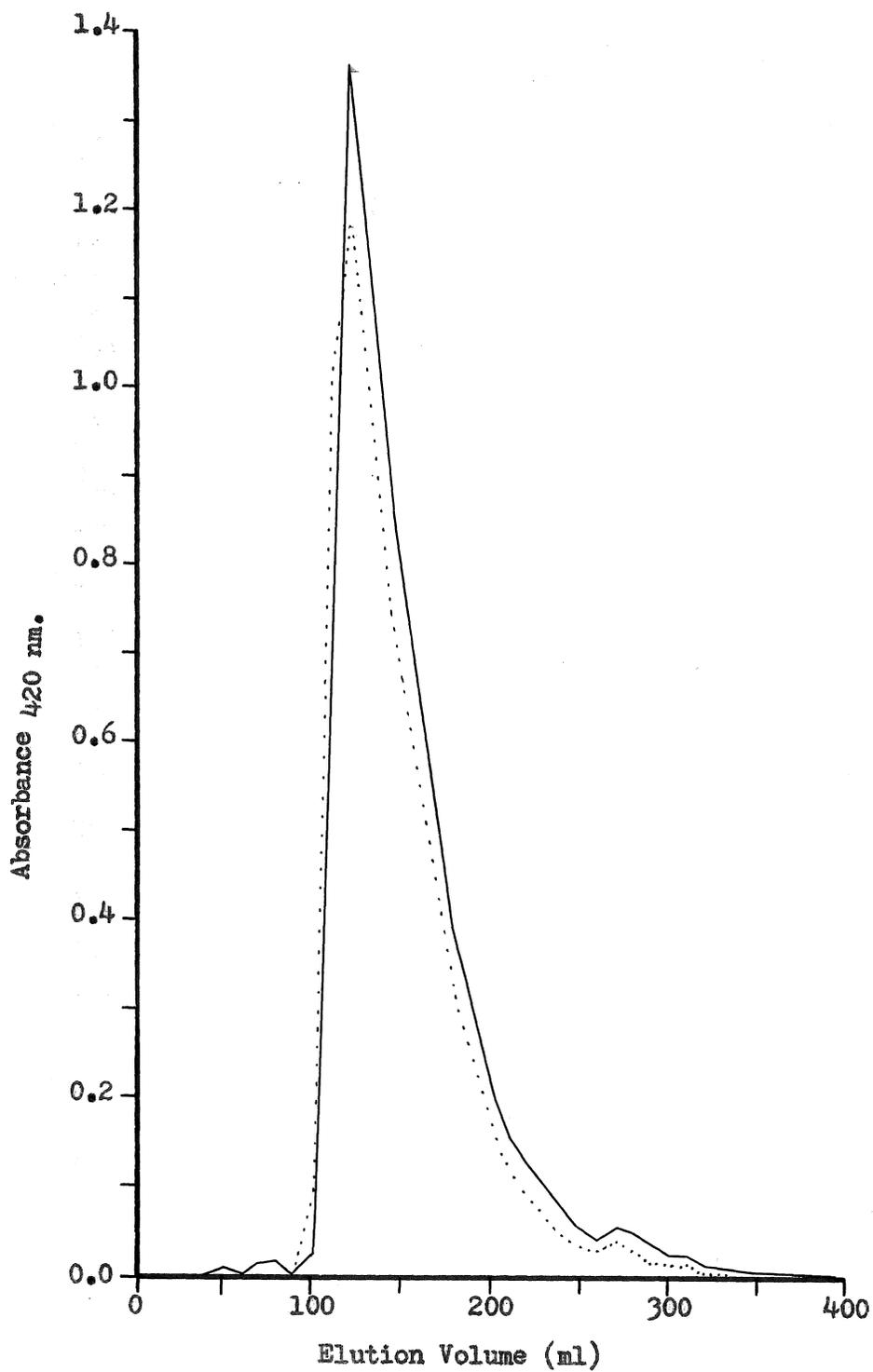


Figure 4-16. Organic Color Elution Profile for Color Concentrate Eluted from Sephadex G-50 with pH 8.0 Borate Buffer. (Flow rate = 2 ml/min. Legend — = unspiked color concentrate, ..... = concentrate spiked with 96 mg/l boron.)

the same elution volume as the high molecular weight color fraction; the other occurred at the low molecular weight end of the chromatogram in the same region as the second color peak, but also the region in which one would expect to find any low molecular weight solute such as  $B(OH)_3$  or  $B(OH)_4^-$  eluted. This suggests that at least part of the boron present in natural waters is associated with the high molecular weight organic fraction. However, the degree of association apparently is not increased by addition of more boron, as the boron elution profiles of the samples spiked with 32 mg/l and 96 mg/l boron show. In these profiles fluctuations in the base line mask any peaks in the medium to high molecular size ranges which might be attributable to boron-organic color association.

The color and boron elution profiles obtained using 0.1 M pH 8.0 phosphate buffer as the eluant are presented in Figure 4-15. With this eluant the organic color was almost totally eluted as a single broad peak near the low molecular weight end of the chromatogram, and the large high molecular weight peak observed with distilled water eluant is present only in vestigial form. Again, there are no substantive differences between the elution profiles for the spiked and unspiked samples. The boron elution patterns are similar to those observed with distilled water eluant. For the unspiked sample two boron peaks are present, occurring at either end of the chromatogram, and for the sample spiked with 32 mg/l boron the high molecular weight peak again becomes indistinguishable from the background. The reason for the apparent decrease in the boron content of the high molecular weight fraction is not clear. Presumably the major portion of the added boron is eluted as molecular boric acid in the low molecular weight peak.

When eluted with 0.1 M pH 8.0 borate buffer, nearly all the organic color came off the column in a peak at the high molecular weight end of the chromatogram (Figure 4-16). In this case, all that remained of the low molecular weight peaks observed with distilled water and phosphate buffer eluants was a small shoulder on the tail of the main peak. As would be expected with borate buffer, no difference was observed between the spiked and unspiked samples. For obvious reasons, boron elution profiles were not determined using this eluant.

These results agree with the observation of Christman and Ghassemi (1966) who observed an apparent increase in molecular weight of organic color eluted with borate buffers as compared with the same color samples eluted with phosphate buffers. Both sets of results are compatible with a scheme in which a small fraction of the boron present in natural waters is strongly bound to a high molecular weight organic fraction, while the major portion is present as molecular boric acid which at most may form weak complexes with organic color. The similarity in color elution patterns for spiked and unspiked color concentrates could be the result of break-up of such loosely bonded boron-color complexes by interactions with the Sephadex gel. Formation of a loose network of boron-color complexes could account for the apparent increase in molecular weight of organic color eluted from Sephadex gel by a relatively concentrated borate

buffer. However, an equally or more likely conclusion is that boron-organic color interactions are small and relatively unimportant, but that boron interacts with the Sephadex gel itself (Sephadex being a dextran or glucose polymer). At low boron concentrations (the unspiked and spiked samples eluted with phosphate buffer and distilled water) the interactions are insignificant and the color elution profiles are unaffected. At high concentrations (0.1 M borate buffer) the borate reacts with the gel and partially excludes the color from the internal volume of the gel, either by electrostatic repulsion or by physically decreasing the pore size. Hence the color remains in the external volume of the column, moves down it more rapidly and consequently only appears to be larger.

## CHAPTER 5. SUMMARY AND CONCLUSIONS FOR BORON STUDIES

Review of the literature revealed few investigations of boron concentrations in the aquatic environment despite its role as a micro-nutrient for plants, its toxicity to many plants at concentrations only slightly above optimal, and its increasing use by man, especially in household laundry products. The limited amount of literature is partly attributable to the lack of a suitable analytical method for direct analysis of the low boron concentrations present in natural waters. The method of analysis presented in this work utilizes the high sensitivity of curcumin for boron and incorporates the work of several different researchers on use of acid anhydrides for removal of water to produce a fast, accurate procedure sensitive to boron in the parts-per billion range. Application of this procedure to analysis of water samples from a variety of sources throughout Florida and to laboratory studies of boron adsorption by clays and boron uptake by components of model ecosystems has led to the following conclusions.

Boron concentrations in natural waters of Florida generally are below 100  $\mu\text{g}/\text{l}$ , and are close to concentrations reported for streams in the southeastern United States (Boyd and Walley 1972), rivers of Norway and Sweden (Ahl and Jonsson 1972), and the major rivers of the U.S.S.R. (Livingston 1963).

Rainfall is a major source of boron to surface waters in Florida. Concentrations in rainfall vary from about 90  $\mu\text{g}/\text{l}$  at the coasts to 10-15  $\mu\text{g}/\text{l}$  inland. The concentration gradient with respect to distance from the coast suggests that the ocean is a source of atmospheric boron via sea spray aerosols.

Sewage effluents are the major cultural sources of boron to Florida lakes and rivers. Domestic sewage in Florida typically contains concentrations of 300-800  $\mu\text{g}/\text{l}$  boron, equalling and exceeding the threshold for toxicity to boron-sensitive plants including citrus and other fruit trees. Present concentrations of boron in citrus grove ponds are well below toxic levels, indicating that concentrations in the soil solution extracts also are below toxic levels. For these reasons, disposal of sewage effluents as irrigation waters should be carefully studied before full-scale implementation occurs.

Boron concentrations in municipal water supplies in Florida also are generally below 100  $\mu\text{g}/\text{l}$ , and no detectable quantities are removed by the conventional treatment processes of lime-soda softening or coagulation with alum or ferric salts. The large differences in boron concentrations between municipal water supplies and sewage and the large-scale use of boron-containing compounds by the detergent industry implicate household laundry products as the primary source of boron in sewage.

Results of investigations of boron uptake by aquatic plants and

animals indicate that although uptake is accelerated in the presence of high concentrations of boron, only a small part of the total boron present is assimilated. The boron concentrations observed in natural waters of Florida present no toxic hazard to aquatic biota. These results support the theory that boron is an essentially conservative element in the environment.

Results of a brief laboratory study of boron adsorption by kaolinite and attapulgite indicate that these clay minerals are ineffective in removing low concentrations of boron from solution and probably are only minor sinks for boron in the environment. A subsequent investigation indicated that while some boron is removed from solution by attapulgite over a period of several days, the amount is small (about 100  $\mu\text{g}$  B/gram clay) and that the sorption process is apparently unaffected by possible boron-organic color interactions.

Results of gel permeation chromatography of organic color concentrates with and without added boron indicate that the dominant forms of boron in natural waters are molecular boric acid and borate anion. However, the results also indicate that at least a small portion of the ambient boron is associated with the high molecular weight component or organic fraction. The possibility that boric acid and borate anions may become loosely associated with organic color cannot be excluded, but it is unlikely that this phenomenon plays a major role in the structure of natural water organic color.

## PART II. ARSENIC

### CHAPTER 6. BACKGROUND INFORMATION ON ARSENIC

#### A. ARSENIC CHEMISTRY AND BIOLOGICAL MEDIATION OF TRANSFORMATIONS

Arsenic is widespread in nature, but it is relatively lower in abundance than phosphorus. The chemistry of arsenic is similar to the other Group V elements (phosphorus, antimony, bismuth and nitrogen); it differs from phosphorus, which is primarily covalent, with its increasing cationic behavior. Two oxides of arsenic exist:  $\text{As}_4\text{O}_6$  and  $\text{As}_2\text{O}_5$ , corresponding to oxidation states +3 and +5 respectively. In acid solutions of  $\text{As}_4\text{O}_6$  (arsenite) the primary species is  $\text{As}(\text{OH})_3$ ; in increasingly basic solutions  $\text{As}(\text{OH})_2\text{O}^-$ ,  $\text{As}(\text{OH})\text{O}_2^-$  and  $\text{AsO}_3^{3-}$  may occur.  $\text{As}_2\text{O}_5$  (arsenate) occurs in water as arsenic acid,  $\text{H}_3\text{AsO}_4$  ( $\text{pK}_1 = 2.3$ ,  $\text{pK}_2 = 7.0$ ). Arsenic is also found in an extensive series of "cacodyl" or dimethyl compounds, such as  $\text{Me}_2\text{AsCl}$ , cacodyl chloride. There are also diarsenic tetraalkyls such as dicacodyl,  $\text{Me}_2\text{AsAsMe}_2$ . With the exception of dimethylarsinic acid, the methylated compounds are generally man made. In highly reducing environments arsenic can also exist in the -3 oxidation state as the volatile arsenic compounds: arsine ( $\text{AsH}_3$ ), methylarsine ( $\text{CH}_3\text{AsH}_2$ ), dimethylarsine ( $(\text{CH}_3)_2\text{AsH}$ ), and trimethylarsine  $(\text{CH}_3)_3\text{As}$ .

Ferguson and Gavis (1972) constructed an Eh-ph diagram for arsenic in a system including  $\text{H}_2\text{O}$ , oxygen and sulfur, as depicted in Figure 6-1. Under redox conditions where sulfide is stable and at pH values below 5.5, realgar,  $\text{AsS}_3$ , and orpiment,  $\text{As}_2\text{S}_3$ , have low solubilities and occur as stable solids. Aqueous  $\text{HAsS}_2$  is the predominant soluble species at low pH in the presence of sulfide. It has a maximum solubility of 25 ppb.

In the environment organic arsenicals are eventually degraded to inorganic forms; the only exception appears to be dimethylarsinic acid. Braman and Foreback (1973) stated that dimethylarsinic acid is a major and ubiquitous form of arsenic in the environment and is highly resistant to oxidation. Arsenite is 25 times more toxic than dimethylarsinic acid and it is possible that microbial transformation of arsenite into dimethylarsinic acid represents a detoxification mechanism. Crecelius (1975) found that the interstitial water from Lake Washington contained a few percent of the total As as dimethylarsinic acid. The work of Braman and Foreback (1973) indicated that river and lake water in Florida has dimethylarsinic acid in proportions ranging up to 1% of the total arsenic.

These seemingly minute quantities of dimethylarsinic acid increase in significance when the role of dimethylarsinic acid in the volatilization of arsenic is considered. Cox and Alexander (1973) determined that

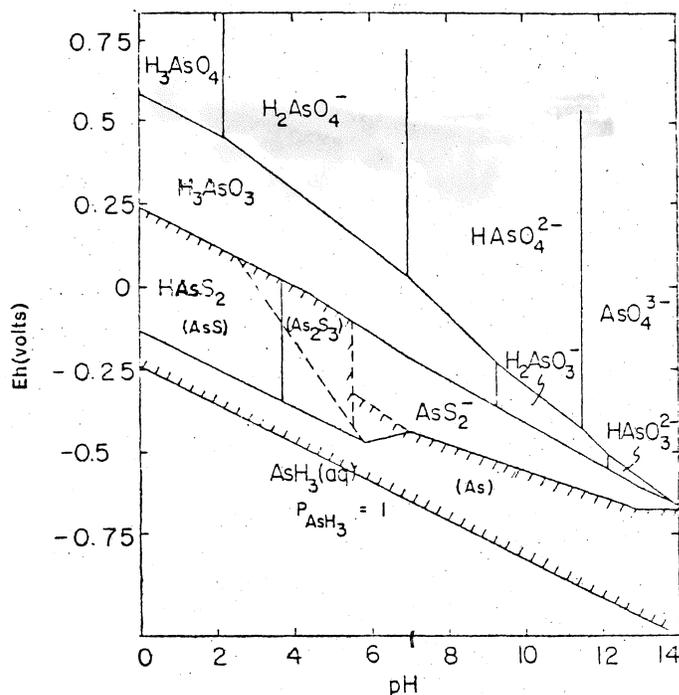
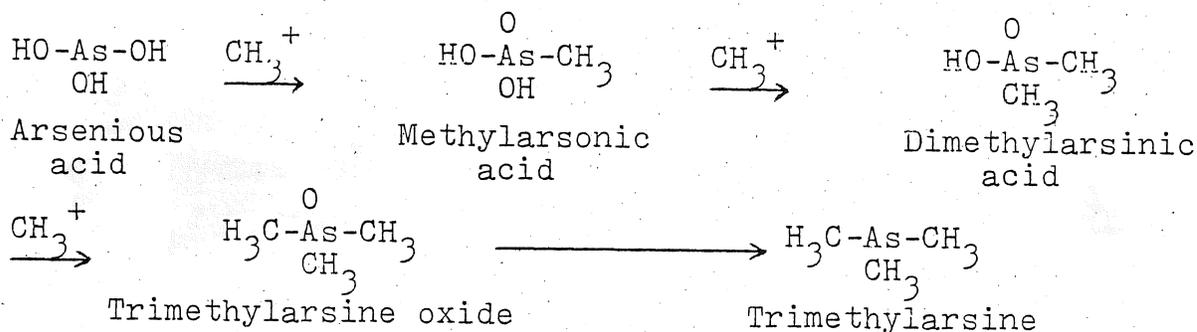


Figure 6-1. The Eh-pH diagram for As at 25°C and one atmosphere with total arsenic  $10^{-5}$  mol  $l^{-1}$  and total sulfur  $10^{-3}$  mol  $l^{-1}$ . Solid species are enclosed in parenthesis in cross-hatched area, which indicates solubility less than  $10^{-5.3}$  mol  $l^{-1}$ . (From Ferguson and Gavis 1972).

methylarsonic acid  $CH_3AsO(OH)_2$  and dimethylarsonic acid  $(CH_3)_2AsO(OH)$  were the optimal sources of arsenic for trimethylarsine production by three sewage fungi. Challenger (1951) proposed the following metabolic pathway for the production of trimethylarsine from arsenite by Scopulariopsis brevicaulis:



From this proposed pathway and from the findings of Cox and Alexander (1973), it appears that methylarsonic acid and dimethylarsonic acid are important steps in the process of arsenic volatilization.

Woolson and Kearney (1973) labeled dimethylarsonic acid with  $^{14}C$  to study its dynamics in soils. At an application rate of 100 ppm of dimethylarsonic acid 35 percent of the arsenic in the soil was volatilized

as arsine, methylarsine, dimethylarsine, trimethylarsine or some other volatile organo-arsenic compound within 24 weeks of application. Under completely anaerobic conditions it was found that 61 percent of the applied dimethylarsinic acid was converted to a volatile form within a 24 week period. Results of Myers *et al.* (1973) suggest that volatilization may occur in sewage treatment plants. These workers found that activated sludge under anaerobic conditions first reduced arsenate to arsenite which then was apparently further reduced and was not detected as arsenate or arsenite.

Microbial mediation is also important in the oxidation-reduction equilibria of arsenite and arsenate. Johnson (1972) reported that at thermodynamic equilibrium the ratio of arsenate to arsenite in oxygenated sea water at pH 8.1 should be about  $10^{26}$ . However, significant quantities of arsenite have been found in all oceanic regions. Johnson also collected a non-colonial culture from Narragansett Bay and added it to an aerobic Sargasso Sea water spiked with arsenate. As the bacterial population entered log phase growth, arsenate was reduced to arsenite. Pilson (1974) determined that a scleractinian coral was capable of removing arsenate from seawater, reducing it to arsenite and then releasing it. This process was postulated as a mechanism to rid the cells of As which is transported into the cells during phosphate accumulation.

Clement and Faust (1973) analyzed several waters with unusually high As contents (Table 6-1) and found that arsenite generally prevailed under anaerobic conditions and arsenate predominated in aerobic conditions. Arsenite in anaerobic water was found to be almost completely oxidized to arsenate within 4 days of aeration of the water (Figure 6-2).

Table 6-1. Oxidation State of Arsenic in Arsenic Rich Natural Waters (From Clement and Faust 1973).

<u>Characteristics</u>	<u>Inorganic Arsenic, ug/l</u>	<u>Percent Arsenite</u>
Well aerated stream	0.059	8
Well aerated stream	0.014	7
Ground water supply	224.	51
Ground water supply	280.	54
Ground water fed stream at flood stage	0.080	26
Anaerobic reservoir	1.320	100
Anaerobic reservoir	0.139	100
Anaerobic reservoir	1.008	98

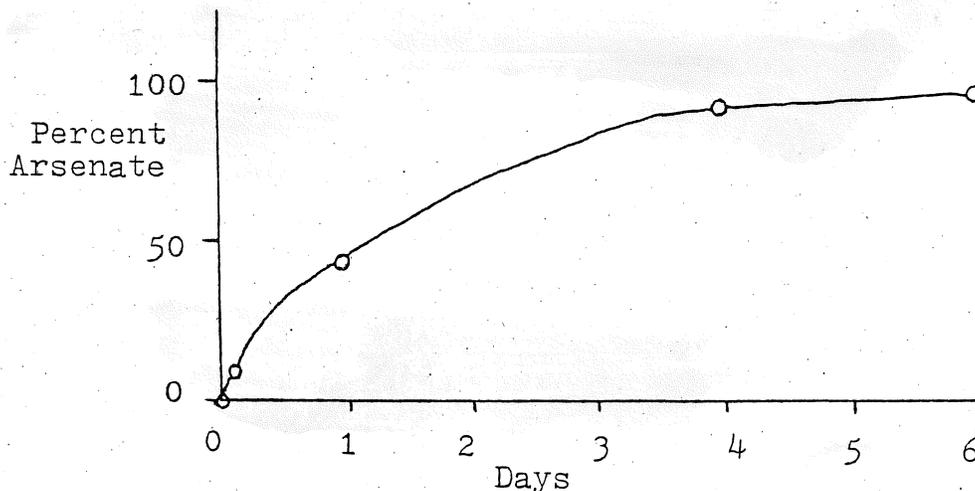


Figure 6-2. Percent Arsenate vs. Time Following Aeration of an Anaerobic Water. At time 0, 0.368 mg/l Arsenite (From Clement and Faust 1973).

## B. OCCURRENCE, USES AND CYCLING OF ARSENIC

Hurlbut (1968) estimated that arsenic comprises approximately  $5.0 \times 10^{-5}$  percent of the earth's crust; or an average of 0.5 ppm. Common mineral forms of arsenic are arsenopyrite ( $\text{FeS}_2 \cdot \text{FeAs}_2$ ), realgar ( $\text{AsS}$ ) and orpiment ( $\text{As}_2\text{S}_3$ ). Cultural sources of arsenic include coal (mean concentration of 14 ppm; Ruch *et al.* 1974), phosphate detergents (concentrations of 10-70 ppm; Angino *et al.* 1970), arsenical pesticides, leaching of mine waste piles, ore smelters and various other industrial activities. Ferguson and Gavis (1972) estimated that U.S. consumption of As is 25,000 metric tons per year and that much of this As is introduced into the environment.

Walsh and Keeney (1975) noted that organic arsenicals are largely replacing inorganic As salts as herbicides and that the organo-arsenicals are applied at lower rates. Registration of inorganic arsenicals for use on nearly all vegetable and agronomic crops was cancelled in 1968. Lead arsenate, calcium arsenate and Paris Green ( $3\text{Cu}(\text{AsO}_2)_2 \cdot \text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2$ ) are still used as insecticides. Lead arsenate is also used to control annual bluegrass on golf greens and fairways. In Florida lead arsenate is used to increase the ratio of solids to acids in grapefruit by application of approximately 6 pounds per acre in late spring or early summer (Florida Citrus Spray and Dust Schedule 1975). Arsenic compounds have been banned for use in aquatic weed control in Florida since 1905.

Calvert (1975) reported that 3-nitro-4-hydroxy-phenylarsonic acid, arsanilic acid, 4-nitrophenylarsine and p-ureidobenzearsonic acid are approved by the U.S. Food and Drug Administration for use in poultry and swine feeds as therapeutic and growth stimulants at levels "low enough not to constitute a human health hazard."

Despite the widespread use of arsenic, concentrations in waters and water supplies remain small. According to McCabe *et al.* (1970), high As concentrations in water supplies in the U.S. are rare and not a threat to public health. Their survey of 969 water supplies found As exceeding 10 ppb in 0.5 percent of the samples and exceeding 50 ppb in only 0.2 percent of the supplies surveyed. The recommended drinking water standard by the U.S. Public Health Service is 10 ppb and the mandatory limit is 50 ppb. Arsenic in naturally arsenic-rich waters is believed to result from water passing through layers of arsenical pyrites. High natural levels of As occur in deep wells and geothermal waters in the western parts of the U.S., the south coast of Taiwan and New Zealand.

In the environment soluble As is readily sorbed by most soils and sediments. Wauchope (1975) found that at low concentrations arsenicals and arsenate had sorption properties similar to phosphate, and at higher concentrations As was actually sorbed better due to a secondary preference of As for some site associated with calcium. Sorption was also found to be highly correlated with the clay fraction and was independent of organic matter.

A modified Chang and Jackson procedure was applied to As contaminated soils by Johnson and Hiltbold (1969). The Chang and Jackson procedure was originally developed to determine the amount of phosphate associated with various soil compounds:  $\text{Al}(\text{OH})_3$ ,  $\text{Fe}(\text{OH})_3$ , calcium compounds, reductant soluble compounds, and water soluble compounds. Johnson and Hiltbold determined that ammonium chloride extracted a large fraction of As in soils indicating that it was loosely bound ("water soluble"). Aluminum bound As was released by complexing the aluminum with fluoride. Arsenic complexed with iron was released by the addition of a sodium hydroxide complex, thus releasing the arsenic. Reductant soluble As (that associated with iron but coated with  $\text{Fe}(\text{OH})_3$ ) was then extracted by reducing the sample with a citrate-dithionate mixture. Calcium bound As was extracted by adding dilute sulfuric acid to dissolve the calcium salts. This fractionation scheme is depicted in Figure 6-3. No arsenic was found combined with the organic fraction. The results of this fractionation procedure when applied to a soil treated with methanearsonate herbicides (Figure 6-4) indicates that As is found in completely different fractions than phosphate.

Woolson *et al.* (1971) used the above fractionation procedure to characterize the mode of inorganic As sorption by several soils. They found that iron-bound As was the predominant form of As in the soils; however, calcium-arsenic and aluminum-arsenic also occasionally predominated. Calcium-arsenic was found to be the largest fraction in Lakeland loamy sand samples. A comparison of these data with the results of Johnson and Hiltbold (1969) (Figure 6-4) indicates that although inorganic and methylated arsenic are sorbed to similar extents (Wauchope 1975), sorption is not by the same processes.

Woolson and Kearney (1973) investigated the sorption properties of cacodylic (dimethylarsinic) acid and arsenate using the modified Chang

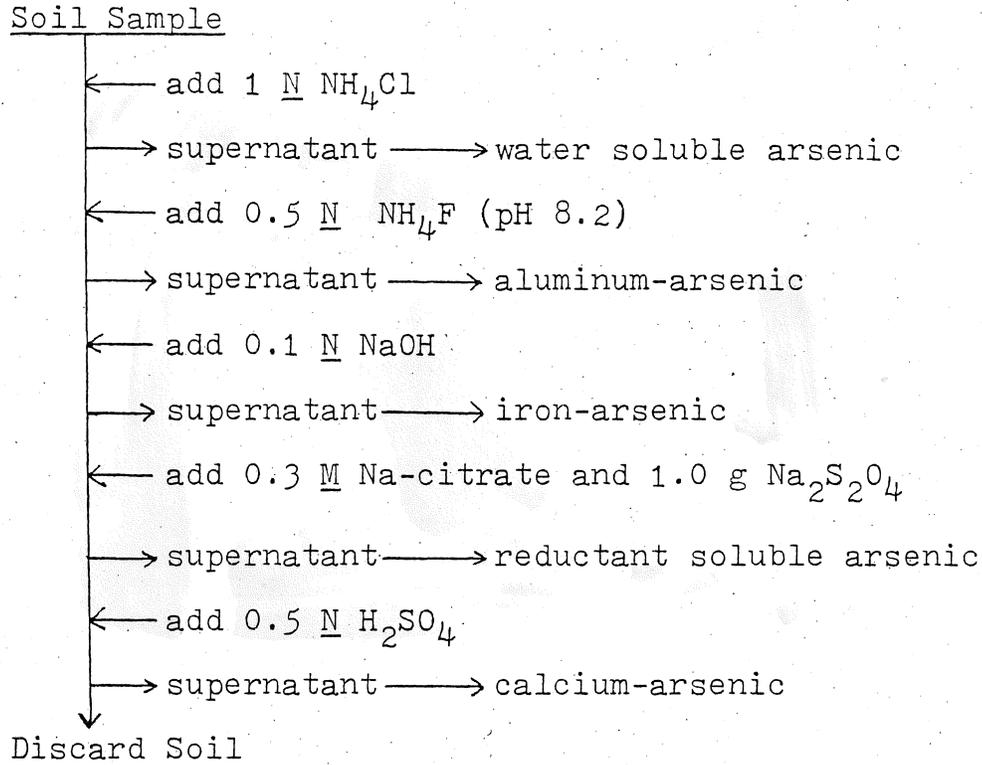


Figure 6-3. A Modified Chang and Jackson Procedure for the Fractionation of Arsenic in Soils (Petersen and Corey 1969)

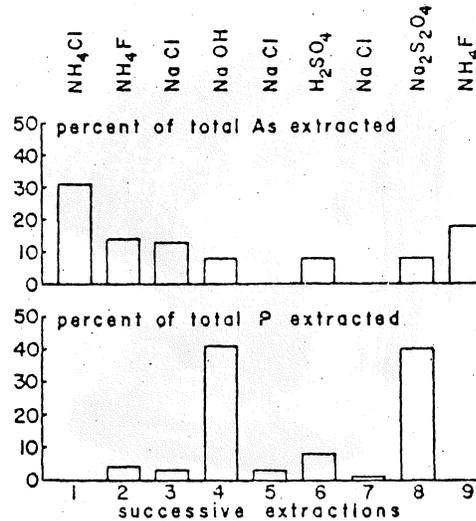


Figure 6-4. Percentage of Total Arsenic and Phosphorous Extracted in Nine Successive Extractions of Chesterfield Sandy Loam (Johnson and Hiltbold 1969).

and Jackson procedure. They found that cacodylic acid was distributed in soils in the decreasing order: water soluble, aluminum fraction, iron fraction, and calcium fraction. In contrast, arsenate was largely present in the iron and aluminum fractions. Jacobs and Keeney (1970) treated soils with oxalate to remove all of the amorphous iron and aluminum components and found that it completely eliminated the arsenic sorption capacity of the soil.

Kanomori (1965) investigated the effects of anaerobic conditions on the solubility of As. Under anaerobic conditions in three Japanese lakes he found values of 10.4, 18.0, and 10.0 ppb, and in aerobic surface waters of the same three lakes he found values of 0.70, 0.38, and 1.9 ppb respectively. When sediments from an oligotrophic lake were incubated under anaerobic conditions maximum dissolved ferrous iron and dissolved As levels, 84 and 80 percent of the total in the sediments, were reached in four days. An incubation experiment for sediments from a eutrophic lake showed similar results, but at maximum values of ferrous iron and dissolved As, black ferrous sulfide precipitated. Arsenic was present as arsenite in the presence of sulfide.

Ferguson and Gavis (1972) presented a conceptual model for the cycling of arsenic in stratified lakes (Figure 6-5). In this model arsenic is oxidized to arsenate in the epilimnion and arsenate is reduced to arsenite in the hypolimnion. Arsenic is removed from solution by precipitation or adsorption with iron hydroxide or some other solid phase compound and is released from sediments by the reduction and resolubilization of iron. A proposed precipitation of arsenite as orpiment is also included.

Several cases of As pollution in the environment have been investigated, and they seem to indicate that As is sorbed and concentrated by the sediments. Wilder (1972) reported that a sewage treatment plant in North Carolina had As concentrations of 1,100 ppb in the effluent, and three months after the source of As was stopped the effluent concentration was still 115 to 260 ppb. Arsenic was found to be concentrated on the sediment materials in the receiving stream in concentrations of 7 to 35 ppm.

Creelius (1975) found As concentrations greater than 200 ppm in the surface sediments of Lake Washington and a high average rainfall As concentration of 16 ppb. The high As concentrations were attributed to the dust from a copper smelter at Tacoma, some 35 miles southwest of the lake. Arsenic levels in Lake Washington water were low, averaging 1.6 ppb. It was determined that 55 percent of the As entering the lake was removed by sediments and 45 percent by outflow.

Seydel (1972) determined that Lake Michigan sediments contained an average of 15.2 ppm As compared to unpolluted Lake Superior which contained only one-fourth as much in its sediments. Lake Michigan had an average of 2.4 ppb in the water. Ruch *et al.* (1970) reported similar values for Lake Michigan sediments and found a correlation ( $r=0.72$ ) between the organic carbon and the As in the sediments. This was attributed to both substances being a product of man's activities.

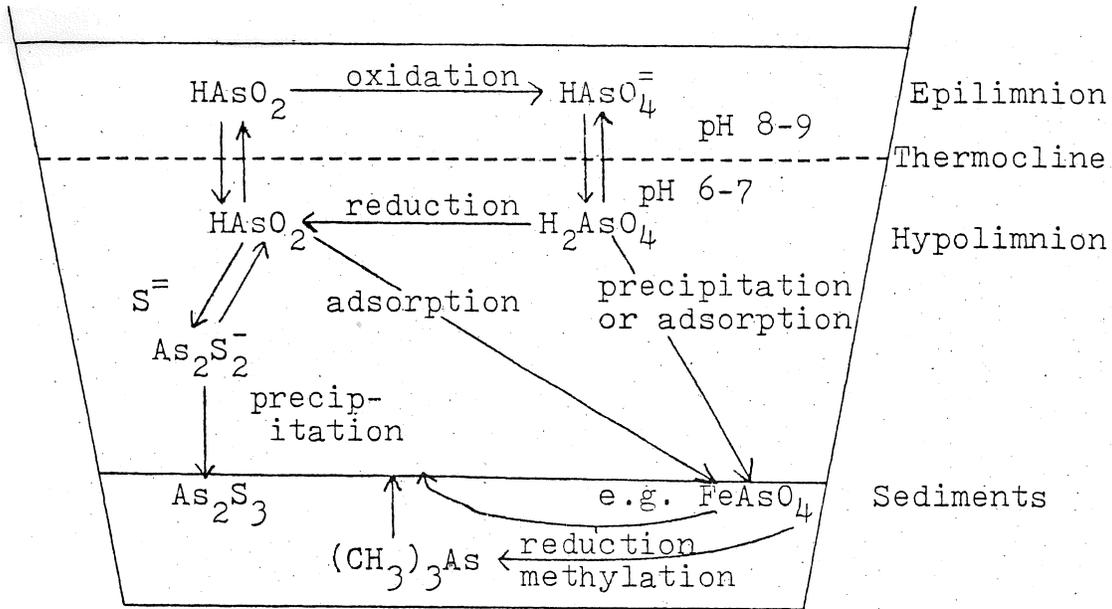


Figure 6-5. Local Cycle of Arsenic in a Stratified Lake (Ferguson and Gavis 1972).

The Water Resources Data for Florida, Part 2 Surface Water Quality Records 1974 indicate that As levels in Florida waters are generally less than 20 ppb and that most waters contain only a few ppb. Chemical analysis of bottom sediments indicated levels less than 10 ppm; with most having less than 5 ppm.

### C. TOXIC EFFECTS AND BIOACCUMULATION OF ARSENIC

There are no current examples of toxic accumulations of As in food organisms, and all reported chronic poisonings are due to the ingestion of water containing harmful levels of As. Zaldivar (1974) suggests that arsenicals may act as pluripotential carcinogens (cause multiple forms of cancer) and cause malignancies of the skin, mouth, esophagus, stomach, liver, nasal sinuses, larynx and lung. Skin carcinoma was the most common delayed effect. Arsenic in drinking water in Taiwan has been related to cutaneous carcinoma. In Torreón, Mexico, drinking water contained 4 to 6 ppm of arsenic and 297 out of 496 persons showed signs of chronic arsenic poisoning. Table 6-2 illustrates the significant decrease in Antofagasta, Chile (200,000 population) after a water treatment plant was built that decreased the arsenic content of the water from 0.58 ppm to 0.08 ppm.

Table 6-2. Decrease in Chronic Arsenic Poisoning in Antofagasta, Chile due to Reduction of Arsenic in Drinking Water (Zaldivar, 1974)

Year	Arsenic ppm	Incidence Rates per 100,000	
		Males	Females
1968-9	0.58	145.5	168.0
1971	0.08	9.1	10.0

The molecular biology of As carcinogenesis involves the human epidermal cells. Inorganic bound arsenate inhibits replication of DNA and interrupts the repair mechanisms. A possible cause is the blockage of DNA polymerase by the linkage of arsenate to sulfide groups (Jung 1970). Arsenic carcinogenesis may also be a function of the ability of arsenic compounds to act as methylating agents, but this hypothesis requires further study. Unfortunately, although it is recognized that arsenite is more toxic than arsenate (N.I.O.S.H. 1975), no distinction has been made in most studies of chronic arsenic poisonings.

Liebig (1966) determined the tolerance of several crops to arsenic as shown in Table 6-3. Woolson (1975) states that aquatic

Table 6-3. Tolerance of several crops to Arsenic (Liebig 1966).

<u>Low or No Tolerance</u>	<u>Fairly Tolerant</u>	<u>Very Tolerant</u>
Snap bean	Strawberries	Asparagus
Lima bean	Sweet corn	Potato
Onion	Beet	Tomato
Pea	Squash	Carrot
Cucumber		Tabacco
Alfalfa		Dewberry
Other Legumes		Grape
		Red Raspberry

plants accumulate much more As than do higher members in the aquatic food chain. Arsenic was found to be bioconcentrated but not bio-magnified. Table 6-4 lists bioaccumulation ratios (concentration in organisms/concentration in water) for several different organisms for arsenate, methylarsonic acid and dimethylarsinic acid.

Reay (1972) found that the amount of arsenic in underwater plants correlated more with the aqueous concentration of arsenic than the arsenic in the sediments. Reay also determined that emergent aquatics contained less arsenic than submerged aquatics.

From the literature it appears that As is not bioaccumulated by animals but is bioconcentrated by plants due to the chemical similarities of arsenate and phosphate. Arsenic has been shown to be carcinogenic at relatively high concentrations, but not enough is known about its effects in trace amounts.

#### D. ANALYTICAL METHODS FOR ARSENIC

Trace level analysis of As has been a popular area for research for the past several years, and the recent literature contains numerous

papers on the topic. Previously only relatively insensitive colorimetric methods were available, and methods to distinguish among the various arsenic species were limited. Today a wide variety of instrumental techniques are capable of analysis at ppb and sub-ppb levels. Several factors must be considered in choosing a method for environmental monitoring of As. First, of course, is sensitivity; the method must be capable of detecting As at levels considerably below 10 ppb (the U.S. P.H.S. recommended drinking water standard). Most natural water samples contain less than 10 ppb of arsenic (Ferguson and Anderson 1974). In most environmental monitoring, a large number of samples must be analyzed to define the system; thus the procedure should not require an unreasonable amount of time per sample. The method should be able to differentiate between the two oxidation states found in environmental samples -- As (III) and As (V). This is particularly important because of the differences in chemical properties of the two species and the greater toxicity of the arsenite species. Finally the use of "exotic" equipment and techniques not common to environmental sciences laboratories should be avoided. With these considerations in mind the methods of analysis of arsenic were reviewed and a procedure developed for this study.

Investigations into the polarographic behavior of As constitute a large body of literature. Arnold and Johnson (1968) compiled a comprehensive review on the polarographic behavior of As in various media and the use of square wave and DC polarography to determine As. Whitnack and Brophy (1969) used a single sweep polarographic technique with a detection limit of 5 ppb. No sample pretreatment was necessary; however, the method requires an oscilloscope readout.

Table 6-4. Bioaccumulation Ratio Values for Arsenic Obtained in Model Ecosystem Studies

Compound	Water Conc. ppm As	ORGANISM							
		Algae	Duck- weed	<u>Daphnia</u>	Snail	<u>Gambusia</u>	Cat- fish	Cray- fish	
$^{74}\text{AsO}_4^{3-}$	1	34	ND*	4	ND	130	ND	5	(1)
$^{14}\text{C-MSMA}$	1	4	ND	8	ND	108	ND	1	(1)
$^{14}\text{C-CA}$	1	3-17	1-3	ND	1-233	-	1	1-16	(2)
	0.1	45	-	39	9-20	1	-	-	(3)
	1.0	17	-	42	2-68	1	-	-	(3)
	10.0	7	-	25	1-7	1	-	-	(3)

References: (1) Woolson, 1975  
 (2) Schuth et al. (1974) \*ND = not determined  
 (3) Isensee et al. (1973)

Myers and Osteryoung (1973) investigated the use of several supporting electrolytes for the determination of As by differential pulse polarography, and 1 M hydrochloric acid was found to give the greatest sensitivity. The detection limit was 0.3 ppb and response was linear up to 60 ppm. This technique is capable of speciating arsenic as it is specific for arsenite. Total As was determined by reducing the arsenate to arsenite by Cu(I) in concentrated HCl followed by extraction of  $\text{AsCl}_3$  into benzene and back extraction into water. They found that this method suffers from interferences by Pb (II), Sn (II) and (IV), and Tl (I) and (III). A deaeration period of 10-15 minutes for each sample is also required.

Neutron activation analysis (NAA) has been used for the analysis of As in many cases, particularly soil samples because it eliminates the digestion step necessary in other methods. Ruch *et al.* (1970) used NAA to investigate the distribution of As in the unconsolidated sediments of southern Lake Michigan. Samples were irradiated for one-half hour in a thermal neutron flux of  $5 \times 10^{12}$  neutrons/cm<sup>2</sup>-sec to convert the As to radioactive arsenic-76. Nonradioactive carrier (32 mg of As (V)) was added to insure isotopic exchange and known recovery of <sup>76</sup>As during subsequent chemical separation. The 0.56 Mev gamma-ray photopeak of <sup>76</sup>As was used for quantitatively measuring arsenic in sediment samples. Counting was performed using a sodium iodide detector. Tanner *et al.* (1973) reported that the use of a Ge(Li) high resolution nuclear diode was required to separate the 0.559 Mev <sup>76</sup>As gamma-ray from the 0.564 Mev gamma-ray of <sup>122</sup>Sb.

The advantages of NAA include a low detection limit (0.1 ng using a thermal neutron flux of  $10^{12}$  neutrons/cm<sup>2</sup>-sec), small sample size, precision and accuracy on the order of 3 to 10 percent, and applicability to a wide variety of samples. Some disadvantages of NAA are the radiochemical separation methods necessary to prevent overlap of the photopeaks or the use of a Ge (Li) detector to separate the <sup>76</sup>As and <sup>122</sup>Sb peaks, and need for a nuclear reactor. Also, the high activity of <sup>24</sup>Na induced in many environmental samples prevents the determination of arsenic below a few ppm. NAA also cannot differentiate between the forms of As without the use of a preliminary separation technique.

Several techniques have been established for the determination of As by molecular absorption spectrophotometry. These methods can be divided into two groups: those that determine As directly and those that require the evolution of arsine gas and its adsorption by another solution containing the chromogenic agent.

Because of the chemical similarities of arsenate and phosphate, arsenate can also be determined by the molybdenum blue method (Portmann and Riley 1964). Johnson and Pilson (1972) have developed a method for the determination of phosphate, arsenate and arsenite in natural waters. For the determination of phosphate, the arsenate in the sample is reduced to arsenite and the phosphate determined by

the molybdenum blue method. The arsenate present is determined by analyzing an untreated sample and subtracting the previously determined phosphate absorbance. Arsenite is oxidized in another aliquot, and the absorbance due to arsenite is determined by subtracting the combined absorbance of phosphate and arsenate. Accuracy of the method for arsenite and arsenate is  $\pm 0.017 \mu\text{M}$  ( $1.3 \mu\text{g}/\text{l}$ ) at the 95 percent confidence level.

The group of colorimetric methods requiring the separation of arsenic from the sample by reduction to arsine includes the SDDC method in "Standard Methods" (A.P.H.A. 1971). This technique requires two reduction steps, the first being the reduction of arsenate to arsenite by stannous chloride and potassium iodide. Then the arsenite is reduced to arsine by mossy zinc and hydrochloric acid in a closed system. The arsine is carried by the evolved hydrogen to a silver diethyldithiocarbamate (SDDC) solution and complexes with the SDDC to form a red color with a  $\lambda$  max of 533 nm.

A modification of the SDDC standard method for the determination of As was developed by Clement and Faust (1973). Their method was capable of speciating arsenic by preventing the reduction of arsenate to arsine by the addition of dimethylformamide (DMF). DMF does not inhibit the reduction of arsenite to arsine by the Zn-HCl step. Sensitivity of this method is  $1.0 \mu\text{g}/\text{l}$ .

Whitnack and Martens (1971) compared the SDDC method to a single sweep polarography and a X-ray fluorescence method. They reported the SDDC method may give high values due to the fact that SDDC will chelate with many metals, some of which absorb in the same range as the arsenic diethyldithiocarbamate complex.

Advantages of molecular absorption techniques include the need for only common equipment and the ability to determine the oxidation states. Disadvantages include the time consuming manipulations, interferences from other metal ions, large sample size necessary to obtain desirable sensitivities, and in some cases inadequate detection limits.

Many efforts have been made to improve the detection limit and accuracy of atomic absorption spectrophotometric analysis of As. Early methods using air-acetylene flames were very insensitive, and hollow cathode lamps for As were not reliable till a few years ago. The analytical line for As is at 193 nm, near the limits of the uv region, a fact that also led to early analytical difficulties. An early improvement in AAS analysis of As used an argon-hydrogen-entrained air flame, ~~has been devised~~. Unfortunately, the relatively inefficient atomization and short residence time of arsenic in the absorbing region of the flame still resulted in detection limits of about 0.5 to 1.0 ppm (Kirkbright and Ranson 1971). The determination of As in a cool diffusion flame such as the argon-hydrogen-entrained air flame may also result in interferences from foreign ions, as the cool flame may be unable to vaporize or atomize the other com-

pounds in the solution, resulting in non-atomic absorption. Without some further modification this method thus is not adequate for environmental studies involving trace levels of arsenic.

Madsen (1971) used the Sn(II)-KI and Zn-HCl reduction to produce hydrogen and arsine. He then used a silver nitrate solution to absorb the arsine and determined the As in the silver nitrate solution by atomic absorption spectrophotometry. Recoveries of 96 to 99 percent were reported and the response was linear between 0 and 5 ppm. The method is complicated and suffers from the relatively inefficient atomization of a liquid sample relative to a gas sample.

Use of a carbon rod atomizer can increase the proportion of atomized As in the absorbing region, and an improvement in detection limit to 20 ppb for a 20  $\mu$ l sample has been reported (Varian Instruments Corp. undated). This detection limit is still somewhat above the levels found in the environment and does not provide for determining the oxidation state of arsenic.

Tam (1974) extracted As from 100 ml samples with diethylammonium diethyldithiocarbamate in carbon tetrachloride and analyzed the extracts using a graphite furnace. By use of the chelation-extraction method, interferences are avoided and the sensitivity is increased. Arsenite is the only form of arsenic extracted by this method, and the oxidation state of As in the sample can be determined by extracting an untreated sample and another sample in which the arsenate is first reduced to arsenite. A detection limit of 1  $\mu$ g/l has been obtained. The requirements of manipulating the samples in separatory funnels, evaporating the carbon tetrachloride to a final volume of 0.5 ml, and the hazards associated with carbon tetrachloride make this method unattractive.

The most promising methods for the determination of As combine the arsine generation procedure with the determination of As in the gas phase by atomic absorption spectrophotometry. The generation of arsine separates the As from the aqueous sample, thus reducing molecular absorption interferences from incomplete salt dissociation. Arsine is also more efficiently atomized than is aqueous arsenic. Early adaptations of this technique used the SnCl<sub>2</sub>-KI reduction of arsenate to arsenite and Zn-HCl reduction for the production of arsine (Dalton and Malanoski 1971). This procedure allowed the arsine to be directly introduced into the argon-hydrogen-entrained air flame and had a detection limit of 0.10  $\mu$ g for a 40 ml sample (2.5 ppb).

Fernandez and Manning (1971) included a balloon in this procedure to collect the arsine and then released the arsine spontaneously into the flame. They improved the detection limit to 0.02  $\mu$ g for a 20 ml sample (1 ppb). This method was further refined by Manning (1971) who purged the air from the system prior to the analysis and reduced the detection limit to 10 ng (0.5 ppb). A deuterium background corrector is necessary for these techniques because the surge of

hydrogen into the flame causes a change in flame absorbance (Manning 1971; Pollock and West 1973).

An electrodeless discharge lamp (EDL) has been developed to replace the As hollow cathode lamp in atomic absorption spectrophotometry. The EDL offers an improvement in intensity and stability over the hollow cathode lamp. Fernandez (1973) found that the EDL provides a twofold improvement in sensitivity, and the absolute detection limit is about 3 ng with the EDL versus 10 ng with the hollow cathode lamp. Perkin-Elmer Corp. (1973) stated that their electrodeless discharge lamp has ten times the brightness of hollow cathode lamps for arsenic and are longer lived. They also offer twice the sensitivity and a fivefold improvement in detection limit.

A liquid nitrogen trap was used to collect the generated arsine (boiling temperature  $=-55^{\circ}\text{C}$ ) by Orheim and Bovee (1974). The trap prevented a surge of hydrogen into the flame and concentrated the arsine. The minimum detection level was 5 ng arsenic.

Pollock and West (1973) extended the hydride generation technique to include Bi, Sb, Te and Ge as well as As and Se by the use of  $\text{TiCl}_3$  with Mg metal or sodium borohydride ( $\text{NaBH}_4$ ). The use of sodium borohydride is a significant development which eliminates the difficulties associated with using solid reagents and the necessity of using two reducing reactions ( $\text{SnCl}_2\text{-KI}$  and  $\text{Zn-HCl}$ ).

The sodium borohydride reduction method has simplified automation of As determination, and the slurries of aluminum and complicated reducing reaction reported by Goulden and Brooksbrank (1974) are not necessary. Kan (1973) developed an automated manifold using the sodium borohydride reduction of arsenic.

The use of flameless atomic absorption has been reported to afford an improvement in the determination of arsenic using an arsine production method. Knudson and Christian (1973) volatilized arsine collected in a cold trap into a Perkin-Elmer HGA-2000 graphite furnace for atomic absorption measurement. The graphite furnace was found to have a tenfold lower detection limit than flame atomic absorption. The detection limit for the flameless method is 0.2 ng with a sensitivity of 1.0 ng for a 40 ml sample. Goulden and Brooksbrank (1974) reported an increase in sensitivity of at least two orders of magnitude when a tube furnace was used as a covalent hydride decomposition device rather than the conventional argon-hydrogen-entrained air flame.

Braman and Foreback (1973) found that the reduction of arsenate to arsine was pH dependent and that at pH 4 to 9 only arsenite was reduced to arsine. At pH 1 to 2 arsenate, methylarsonic acid and dimethylarsinic acid are reduced to arsine, methylarsine and dimethylarsine, respectively. These may be separated by trapping them in a liquid nitrogen cooled U-tube and then warming the tube in air and volatilizing them in the order of their boiling points. This

procedure is in effect a simple gas chromatographic analysis.

Talmi and Bostick (1975) have adapted the determination of As to gas chromatography, allowing for the speciation of organo-arsenic compounds. The arsines and alkylarsines are produced by reduction with sodium borohydride and are separated on a column consisting of 5 percent Carbowax 20M on 80/100 mesh Chromosorb 101, using argon as the carrier gas. This system and the system devised by Braman and Foreback (1973) utilize a microwave emission detector which produces arsenic emission lines (234.9 nm or 228.8 nm) by an electrical discharge maintained in the carrier gas. The emission intensities are recorded by a monochromator-photometric readout system. Limits of detection as reported by Braman and Foreback are 0.05 ng for arsenate and arsenite and 0.5 ng for the methylarsonic and dimethylarsinic acids when a liquid nitrogen cooled trap is used. Lichte and Skogerboe (1972) reported a detection limit of 5 ng when arsine is introduced directly into the microwave emission detector.

## CHAPTER 7. EXPERIMENTAL DESIGN AND PROCEDURES FOR ARSENIC STUDIES

### A. AREAS OF INTEREST

Arsenic levels in various surface and ground waters were determined by analyzing "grab" samples collected throughout the state from lakes, streams, rivers, ponds, springs, and wells supplying municipal water systems. Some duplication of effort with data gathered by the United States Geological Survey (1974) was unavoidable. Previous surveys were found to be too imprecise to make quantitative conclusions about the comparability of results. Also, other studies of As in Florida have not differentiated between the oxidation states of arsenic.

In order to determine the best procedure for the preservation of arsenate and arsenite in natural water samples three common preservation techniques were tested. Two sets of samples from the University of Florida sewage treatment plant effluent, Bivens Arm Lake and Gainesville drinking water were spiked with arsenic - one set with arsenite, the other with arsenate. The samples were preserved with 1) refrigeration, 2) adjusting to pH 2.0, 3) addition of  $\text{HgCl}_2$ , 4) or no preservative at all, and the samples were then analyzed periodically.

In an effort to determine the importance of urban runoff as an As source, Hogtown Creek in Gainesville, Florida, was sampled at various points draining from urban and semirural areas during low flow (no land runoff) and high flow (after heavy rain).

Because of the presence of As in laundry detergents and the ultimate inclusion of these products in sewage, the sewage treatment plant effluent and raw sewage for Gainesville, Florida, were monitored by automated sampling equipment for eight days at eight hour intervals to determine As levels in the sewage.

Several drainage basins were selected for investigation to characterize major sources of arsenic and to compare streams draining areas of varying characteristics. These include the Middle St. Johns River Basin, Lake Okeechobee and its associated system of canals in South Florida, and the Peace River and associated southwest Florida rivers.

Early in this research it became apparent that As sorption by sediments and soils was a dominating factor in the cycling of arsenic in the environment, and investigations were directed toward determining the characteristics of As sorbed by sediments and soils and the extent of their sorption of As in the soil of an arsenated grapefruit grove. Soil samples were collected from three depths in the soil and the As was extracted using a modified Chang and Jackson procedure. A nearby non-arsenated grove was sampled and analyzed as a comparison.

Lake Apopka was chosen for a more extensive investigation after it was found to have moderate levels of As in the water. Surface sediment

samples were collected at various distances from the Winter Garden sewage treatment plant and citrus processing plant and from the muck farm pumping station on the opposite side of the lake. The modified Chang and Jackson procedure was also applied to these sediments. The ability of Lake Apopka sediments to sorb and release As was investigated by spiking the sediments with arsenate and arsenite in aquarium simulations. Samples of water hyacinths and catfish were also collected from Lake Apopka and analyzed to determine the degree of bioconcentration.

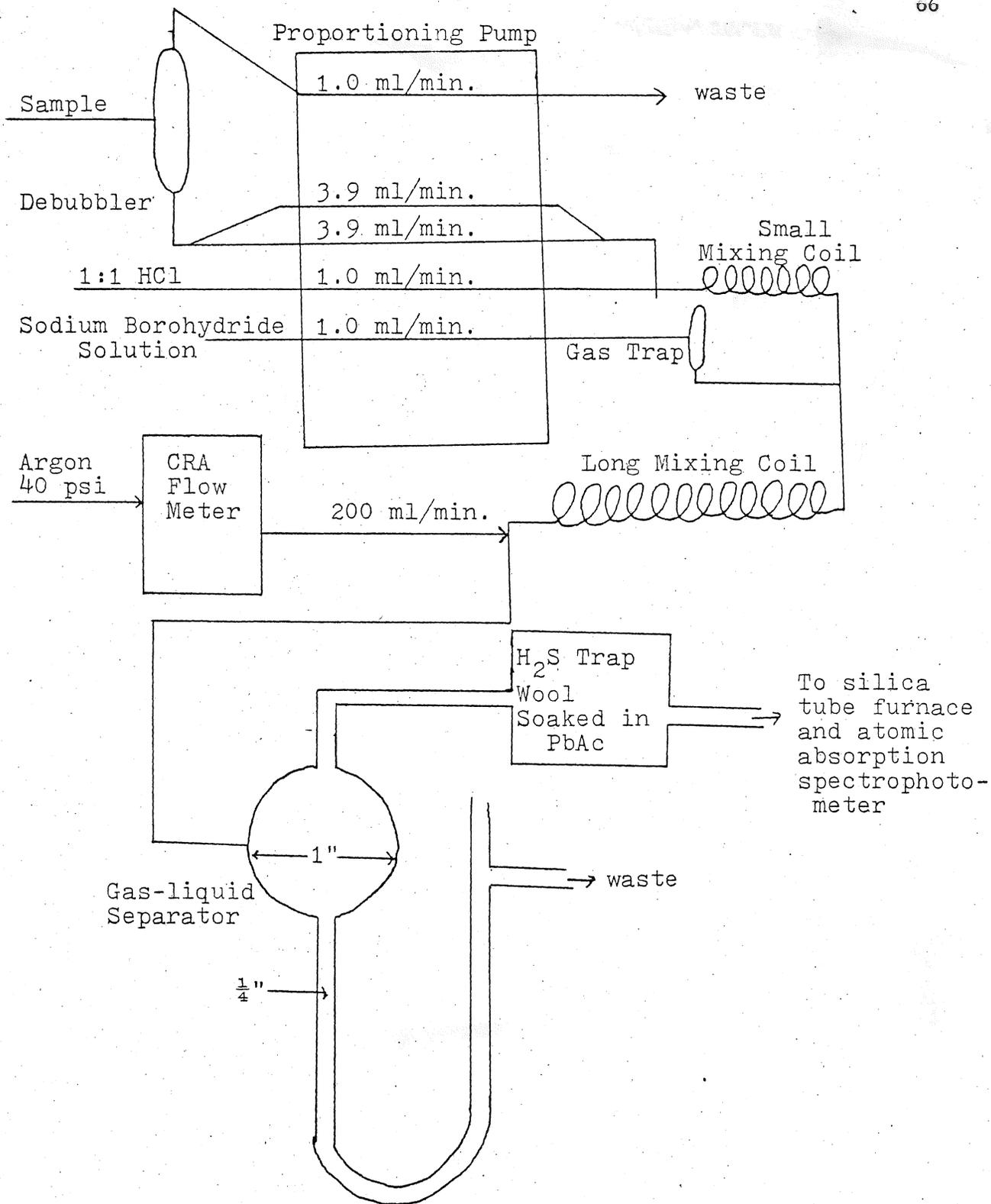
In order to determine the effects of sulfide on As removal from water, another experiment was designed using three 2.5 l plexiglass cylinders in which 1l of a high sulfide anaerobic mud from residential "finger" canals in Port Charlotte, Florida, was placed. The cylinders were then filled with As spiked water from the bay at Port Charlotte, were stirred at different rates, and were sampled periodically.

## B. ANALYTICAL METHODS

A prerequisite for the investigation of arsenic at trace levels in natural waters is the availability of a method sensitive enough to determine the low levels normally found in water with a reasonable degree of accuracy. From the previous literature review it appears that a number of methods are suitable for this investigation: the differential pulse polarography technique of Myers and Osteryoung (1973), the molybdenum blue method of Johnson and Pilson (1972), the silver diethyldithiocarbamate method in Standard Methods (A.P.H.A. 1971) and its modification by Clement and Faust (1973), the extraction and carbon rod atomizer method of Tam (1974), the reduction of As in the sample to arsine and determination by atomic absorption spectrophotometry (Dalton and Malonoski 1971), its automated versions by Goulden and Brooksbank (1974) and by Kan (1973), and the reduction to arsine-microwave emission technique of Braman and Foreback (1973).

All of the above methods are capable of determining the oxidation state of As with some modification of the procedure or sample pretreatment. However, when the additional requirements of speed and applicability to a large number of samples are considered, the automated procedures are preferable. Of the automated procedures, the one developed by Kan (1973) was selected for this research because of its relative simplicity compared to other automated manifolds and because of the widespread acceptance of the sodium borohydride reduction of arsenic to arsine. This manifold is also easily converted from one that reduces the total As to arsine to one that only reduces the arsenite to arsine. This was accomplished by applying the findings of Braman and Foreback (1973) that only arsenite is reduced to arsine by sodium borohydride when potassium biphthalate buffer (pH 4 to 5) is substituted for hydrochloric acid. The manifold (Figure 7-1) is easily transformed to an arsenite-specific manifold by placing the tube for hydrochloric acid into a potassium biphthalate solution.

Determinations of arsine by atomic absorption spectrophotometry can be accomplished by both flame and flameless techniques. Flame techniques are more common and an argon-hydrogen-entrained air flame was used in the



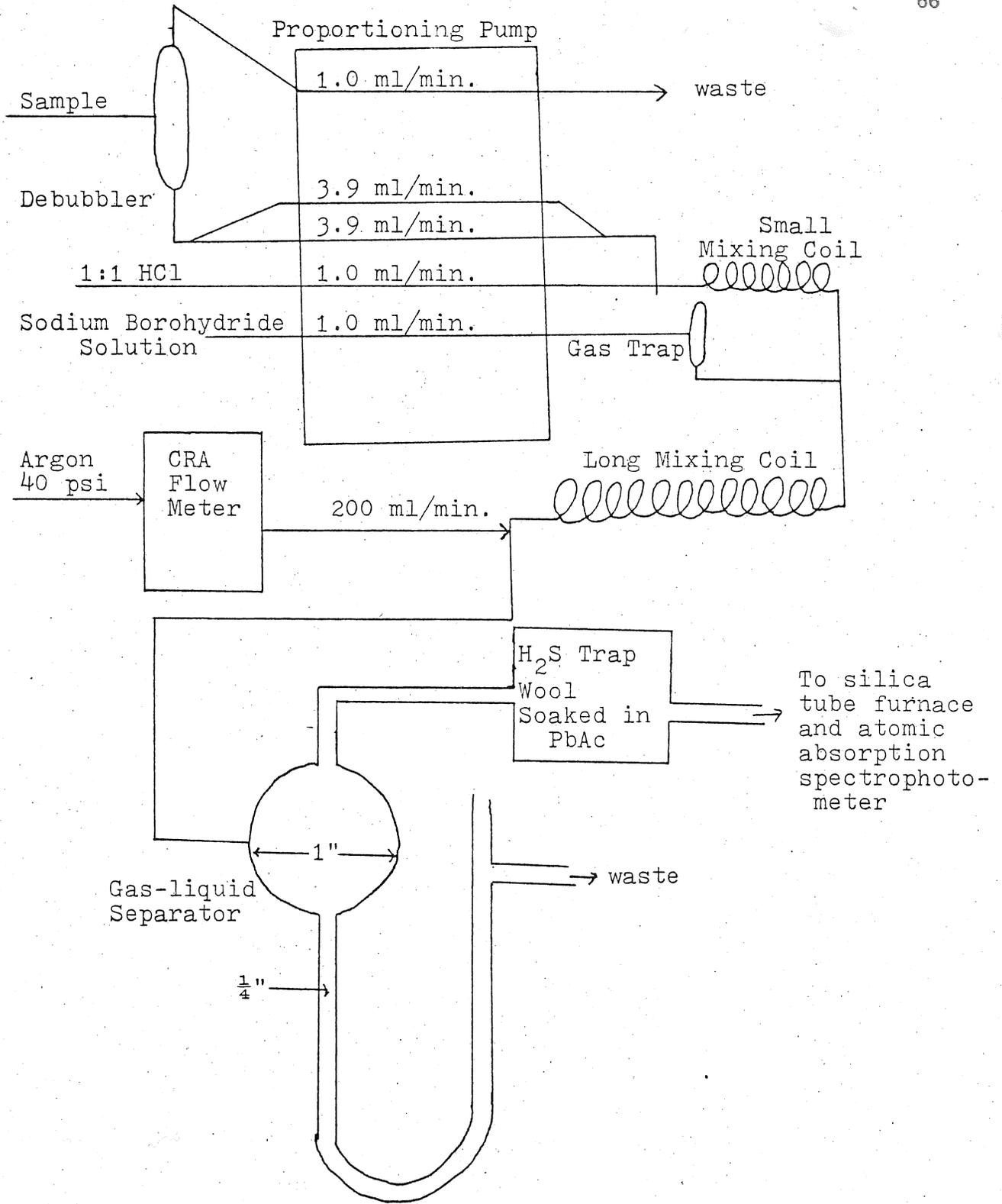


Figure 7-1. Automated Manifold for the Reduction of Trace Levels of Arsenic in Water to Arsine and a Gas-liquid Separator.

early parts of this research. However, non-atomic absorption by the flame and relatively short residence times of the arsenic in the absorbing region of the flame result in this method being less sensitive than flameless techniques. The silica tube furnace developed by Goulden and Brooksbank (1974) was then adopted for this research (Figure 7-2). Along with its increased sensitivity and lower detection limit, it requires no hydrogen other than that produced in the reduction reaction and requires only minimal amounts of argon.

The following reagents were prepared and used in the automated manifold as depicted in Figure 7-1:

Concentrated HCl, Reagent Grade: Distilled  
Deionized Water, 1:1 (V/V)

Potassium Biphthalate Solution, prepare as follows:

Dissolve 20.42 g reagent grade potassium biphthalate  
in distilled deionized water and dilute to 250 ml.

Sodium Borohydride Solution, prepare as follows:

Dissolve 1 g reagent grade sodium hydroxide in  
distilled deionized water, add 5 g reagent grade sodium  
borohydride and dilute to 100 ml.

The procedure observed in setting up and performing the analysis was as follows. The appropriate tubes from the automated manifold are placed in the wash water, sodium borohydride solution, and either 1:1 HCl or potassium biphthalate, depending on whether total arsenic or arsenite is to be determined. The system is allowed to reach a steady state, during which the silica tube furnace is heated to 750°C. After a steady baseline has been achieved the sample tube is transferred from the wash water to the sample for two minutes and then in the wash water for two minutes before proceeding to the next sample.

## C. COLLECTION AND TREATMENT OF SAMPLES

Most of the sampling was accomplished by collecting "grab" samples using a weighted polyethylene bottle on the end of a line cast into the main body of a stream, lake, or river. In some cases sampling was done from a boat; small lakes were sampled from docks or shore. For small, shallow lakes, which are generally well mixed, one sample was regarded as sufficient to represent the lake. Large lakes, such as Apopka and Okeechobee, were sampled in several locations.

After collection, all samples were acidified to approximately pH 2 with hydrochloric acid to preserve the arsenite in the sample. All samples were analyzed as soon as possible after collection, usually within 3 days.

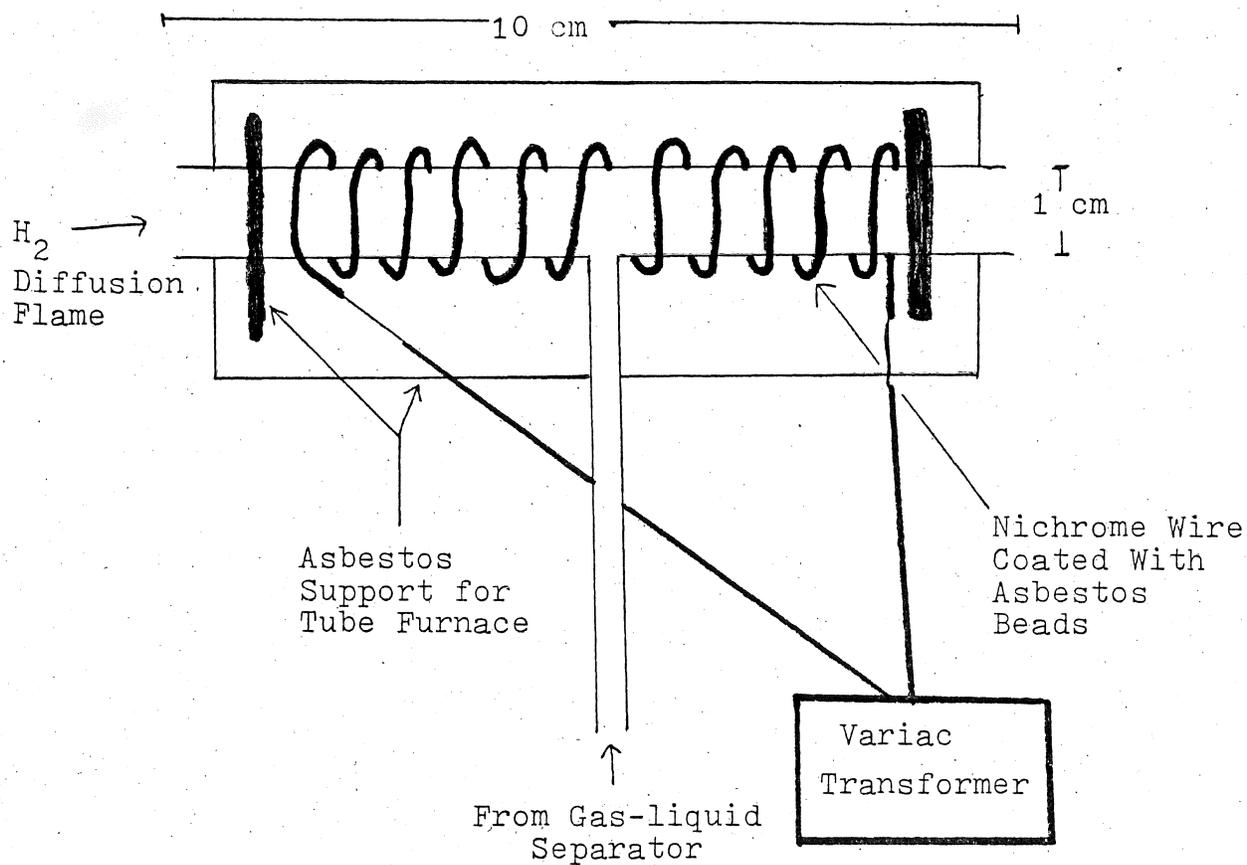


Figure 7-2. Silica Tube Furnace to be Mounted in Atomic Absorption Spectrophotometer in Place of Burner

In addition to the analysis of water samples it was necessary to extract As adsorbed onto soils and sediments. This was accomplished by two methods. The first procedure used was the modified Chang and Jackson procedure used to determine the constituents of the soil or sediment to which the As is adsorbed. A flow diagram for the procedure is given in Figure 7-3. The following reagents were used.

1 N  $\text{NH}_4\text{Cl}$ : dissolve 51.5 g reagent grade ammonium chloride in distilled deionized water and dilute to 1 liter.

0.5 N  $\text{NH}_4\text{F}$ : dissolve 12.5 g reagent grade ammonium fluoride in distilled deionized water and dilute to 1 liter.

Saturated  $\text{NaCl}$ : dissolve reagent grade sodium chloride in distilled deionized water until a solid phase persists.

1.0 N  $\text{NaOH}$ : dissolve 40 g reagent grade sodium hydroxide in distilled deionized water and dilute to 1 liter.

Concentrated  $\text{H}_2\text{SO}_4$

0.3 M Na-citrate: dissolve 104.4 g reagent grade sodium citrate pentahydrate in distilled deionized water and dilute to 1 liter.

$\text{Na}_2\text{S}_2\text{O}_4$ : sodium dithionate

2 N  $\text{H}_2\text{SO}_4$

0.5 N  $\text{H}_2\text{SO}_4$

A simple extraction by acid ammonium oxalate was used to recover arsenic sorbed by Lake Apopka sediments in the sediment sorption experiment. Jacobs *et al.* (1970) found that a single oxalate extraction quantitatively recovered added arsenic from equilibrated soils. The composition of the reagent and procedure are as follows:

Tamm Reagent (acid ammonium oxalate)

Dissolve 24.9 g ammonium oxalate and 12.6 g oxalic acid in distilled deionized water and dilute to 1 liter. (pH=3.25)

Procedure:

Combine 3 g of air dry soil with 100 ml of Tamm Reagent and shake for 1 hour, centrifuge and analyze supernatant.

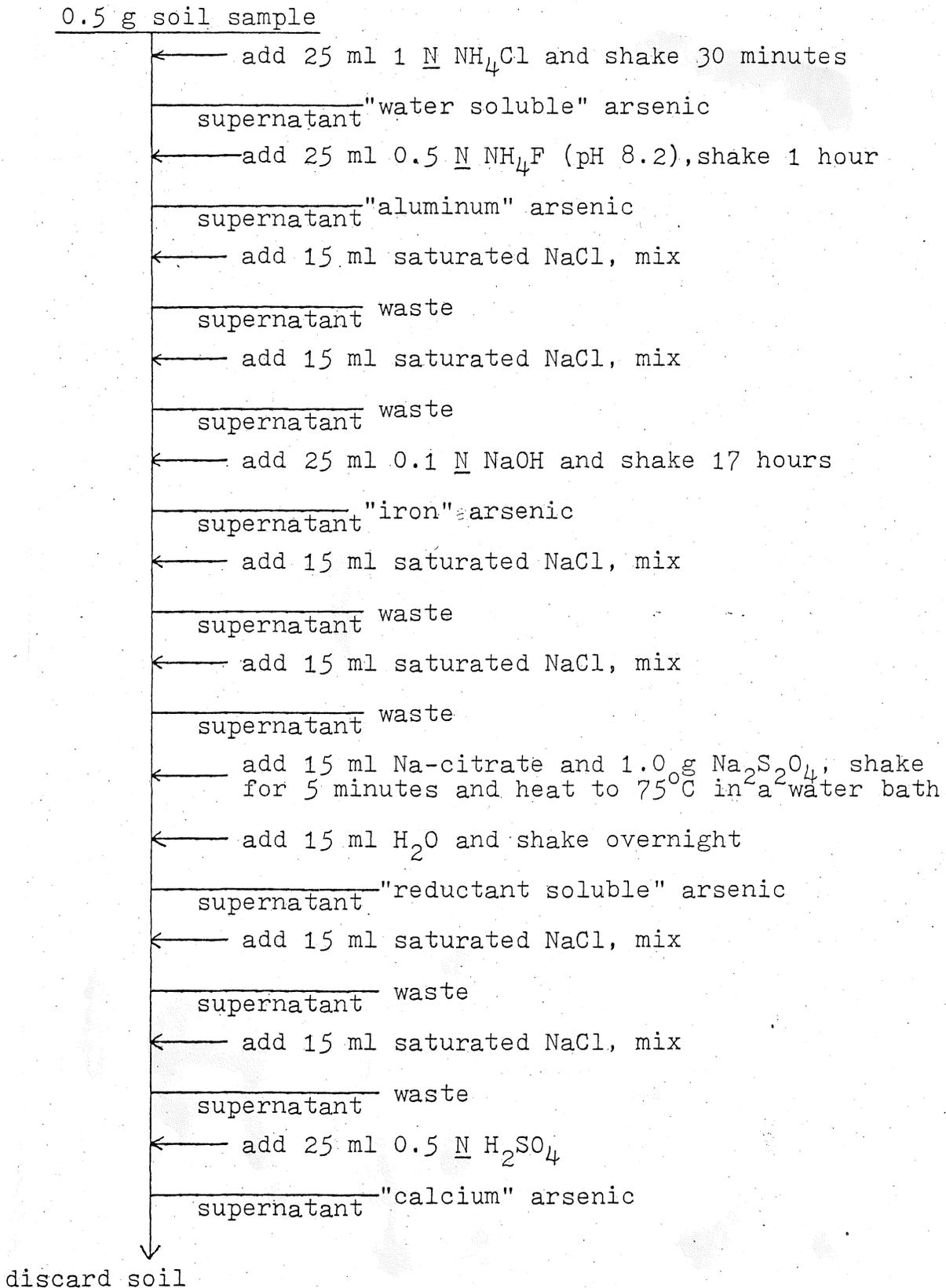


Figure 7-3. A Modified Chang and Jackson Procedure for the Fractionation of Arsenic in Soils and Sediments

The procedure used by George et al. (1973) was adapted for the analysis of water hyacinths and catfish from Lake Apopka. The tissues were blended and 10 g of the tissue placed in a crucible with 3 g MgO. The mixture was charred over a flame until the evolution of smoke ceased, cooled and 3 g  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  added. It was then ashed in a muffle furnace at  $555^\circ\text{C}$  for 2 hours. After cooling, 10 ml distilled deionized water was added to moisten the ash and the ash was dissolved in 6 N HCl and transferred quantitatively to a 100 ml volumetric flask and diluted to the mark with 6 N HCl.

## CHAPTER 8. ARSENIC IN FLORIDA WATERS: RESULTS AND DISCUSSION

### A. STORAGE AND PRESERVATION OF SAMPLES

In the initial phases of this study when total arsenic was the only form analyzed, samples were collected and stored in polyethylene bottles with no preservative. Portmann and Riley (1964) reported that equilibrium was attained by samples stored in polyethylene bottles after 10 days with an approximately 6 percent loss of arsenic. An experiment was conducted as outlined in the previous chapter to determine the effectiveness of four preservative methods on arsenic in water samples, and the results are presented in Table 8-1. No significant loss of arsenic occurred with any of these techniques, including no preservation, except for the mercuric chloride treated Bivens Arm Lake water sample. No explanation is given for this result value, but mercuric chloride was not added to any of the samples used in this study.

In the later phases of this study a method for arsenite analysis was developed, and a method of preventing oxidation of arsenite to arsenate was necessary. The same preservation methods examined for total arsenic preservation were tested for arsenite preservation, and the results are tabulated in Table 8-2. The addition of mercuric chloride resulted in the total loss of arsenite from all samples. The reason for this loss was not further investigated; perhaps mercury salts of arsenic are highly insoluble. Unpreserved samples had a rapid loss of arsenite, generally within a few days. Both acidification to pH 2 and simple refrigeration were found to inhibit but not completely stop the oxidation of arsenite. A combination of refrigeration and acidification and analysis within 3 days was used for arsenite determinations. None of the preservation techniques tested was found effective for preserving arsenite in drinking water, undoubtedly due to the oxidation of arsenite to arsenate by chlorine in the water. This also implies that arsenite would not be found in chlorinated drinking water.

### B. ARSENIC IN MUNICIPAL WATERS AND SEWAGE

Samples of raw and treated drinking water from 18 Florida municipalities were collected by mail with the assistance of treatment plant operators. Only 3 out of 18 raw waters and 4 out of 18 treated waters had detectable amounts of As (in this early work the detection limit was 4  $\mu\text{g}/\ell$ ). All values were well below the USPHS drinking water standard of 10  $\mu\text{g}/\ell$ . Because of the low concentrations and inadequate sensitivity of the analytical method, no conclusions could be made regarding the effectiveness of water treatment on arsenic removal from drinking water. The highest value found was 6  $\mu\text{g}/\ell$  in treated water from West Palm Beach.

Table 8-1. Effectiveness of Four Preservation Techniques for Arsenic

Water Samples	Treatment	Arsenate* ( $\mu\text{g}/\text{l}$ ) remaining in sample after:	
		2 Days	2 Weeks
Bivens Arm Lake	Unpreserved	20	19
	HgCl <sub>2</sub>	20	1.5
	pH 2 <sup>2</sup>	20	19
	Refrigerated	20	18
Univ. Florida Sewage Plant Effluent	Unpreserved	22	20
	HgCl <sub>2</sub>	21	20
	pH 2 <sup>2</sup>	24	24
	Refrigerated	22	21
Gainesville, Fla. Drinking Water	Unpreserved	20	21
	HgCl <sub>2</sub>	22	21
	pH 2 <sup>2</sup>	22	22
	Refrigerated	20	22

\* All samples spiked with 20  $\mu\text{g}/\text{l}$  of As V

Table 8-2. Effectiveness of Four Preservation Techniques for Arsenite

Water Samples	Treatment	Arsenite* ( $\mu\text{g}/\text{l}$ ) remaining in sample after:		
		1 Day	3 Days	10 Days
Bivens Arm Lake	Unpreserved	56	ND	ND
	HgCl <sub>2</sub>	ND	ND	ND
	pH 2 <sup>2</sup>	64	51	50
	Refrigerated	52	43	30
Univ. Florida Sewage Plant Effluent	Unpreserved	56	26	ND
	HgCl <sub>2</sub>	ND	ND	ND
	pH 2 <sup>2</sup>	48	32	36
	Refrigerated	50	40	31
Gainesville, Fla. Drinking Water	Unpreserved	3	ND	ND
	HgCl <sub>2</sub>	ND	ND	ND
	pH 2 <sup>2</sup>	5	ND	ND
	Refrigerated	ND	ND	ND

\* All samples spiked with 50  $\mu\text{g}/\text{l}$  of As III

ND= Less than 2  $\mu\text{g}/\text{l}$

Arsenic is present in laundry detergents, apparently as a contaminant of phosphate (Angino *et al.* 1970), and consequently it was thought that significant levels of arsenic might occur in sewage. Arsenic levels were monitored in the raw and treated sewage using automatic samplers to collect samples at eight hour intervals for an 8 day period from the Gainesville, Florida, treatment plant. Contrary to what was anticipated, however, As levels in both the raw and treated sewage rarely exceeded the detection limit of 2  $\mu\text{g}/\text{l}$ , and the maximum concentrations found during the sampling were about 2  $\mu\text{g}/\text{l}$ , in both the raw water and the effluent. Arsenic in the effluent appeared to follow the raw sewage levels with a slight lag indicating little removal of As within the sewage treatment plant.

### C. ARSENIC IN FLORIDA SURFACE WATERS AND SPRINGS

The results of the analysis of 32 lakes and ponds from throughout Florida are presented as a histogram in Figure 8-1. The mean concentration for the samples was 1.25  $\mu\text{g}/\text{l}$ . These results are comparable with those reported by the USGS (1974). Lake Apopka had the highest As concentrations of the sampled lakes and was selected for further investigation. The individual concentrations for the lakes and ponds sampled are listed in Table 8-3. Four springs in north Florida were also sampled for As and all had undetectable amounts ( $<1 \mu\text{g}/\text{l}$ ). These are Alexander Spring (Ocala National Forest), Blue Spring (Volusia Co.), Jenny Spring (Columbia Co.), and a spring draining into the Devils Millhopper sink hole, (Alachua Co.).

The total As and arsenite concentrations of some miscellaneous stream and river samples not included in the following section on selected drainage basins are listed in Table 8-4. A frequency distribution histogram of all of the stream and river sample As concentrations is presented in Figure 8-2. This distribution is similar to that for the lake samples except for the high value for the sample taken from Sweetwater Creek just below the Oviedo (Seminole Co.) sewage treatment plant outfall.

Table 8-4. Arsenic Concentrations in Selected Florida Streams and Rivers ( $\mu\text{g}/\text{l}$ )

<u>Location</u>	<u>Total</u>	
	<u>Arsenic</u>	<u>Arsenite</u>
Caloosahatchee River at Moore Haven, Glade Co.	1	ND
Fisheating Creek, SR 70, Highlands Co.	ND*	ND
Kissimmee River, SR 78, Glade Co.	ND	ND
Loxahatchee River, 2 miles from ocean, Palm Beach Co.	3	ND
Shell Creek, Charlotte Co.	1	ND
Taylor Creek, US 98, Okeechobee Co.	2	ND

\*ND < 1  $\mu\text{g}/\text{l}$

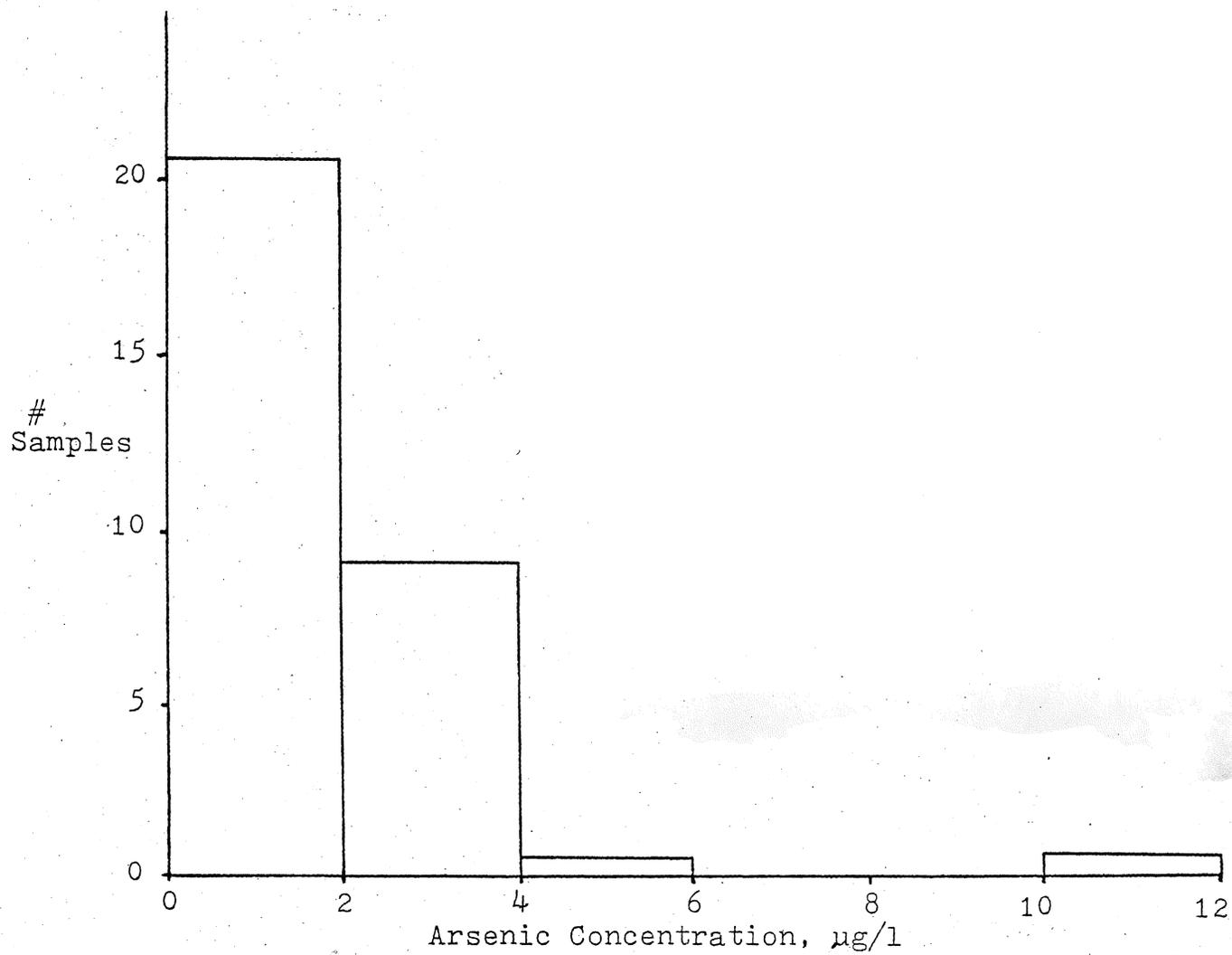


Figure 8-1. Frequency Distribution Histogram for Arsenic Content of 32 Lakes and Ponds in Florida.

Table 8-3. Arsenic Concentrations in Florida Lakes and Ponds

	<u>Arsenic (<math>\mu\text{g}/\text{l}</math>)</u>
Lake Alice, Alachua Co.	ND
Lake Altho, Alachua Co.	ND
Clear Lake, Alachua Co.	ND
Lake Lochloosa, Alachua Co.	ND
Newnans Lake, Alachua Co.	ND
Orange Lake, Alachua Co.	ND
Lake Santa Fe, Alachua Co.	ND
Little Lake Santa Fe, Alachua Co.	4
Lake #20, Alachua Co.	3
Lake Brooklyn, Clay Co.	ND
Lake Geneva, Clay Co.	ND
Lake Okeechobee, Hendry Co.	3
Lake Glanada, Highlands Co.	ND*
Lake Istopoga, Highlands Co.	ND *
Lake Jackson, Highlands Co.	4
Lake Josephine, Highlands Co.	ND*
Lake Lillian, Highlands Co.	1
Lake Placid, Highlands Co.	ND*
Unnamed Citrus Grove Ponds, Lake Co.	
#1 (surrounded by arsenated grove)	3
#2 ( $\frac{1}{2}$ mile from arsenated grove)	2
#3 ( in grove next to U.S. 27)	2
Lake Apopka, Orange Co.	10
Lake Lancaster, Orange Co.	ND*
Blue Lake, Polk Co.	ND*
Lake Clinch, Polk Co.	ND*
Crooked Lake, Polk Co.	ND*
Lake Livingston, Polk Co.	ND *
Lake Moody, Polk Co.	ND*
Lake Reedy, Polk Co.	2
Lake Harney, Seminole Co. (average of 6 samples)	ND
Lake Jessup, Seminole Co. (average of 7 samples)	3.6
Lake Monroe, Seminole Co. (average of 8 samples)	2.1

ND = less than 2  $\mu\text{g}/\text{l}$

ND\*= less than 1  $\mu\text{g}/\text{l}$

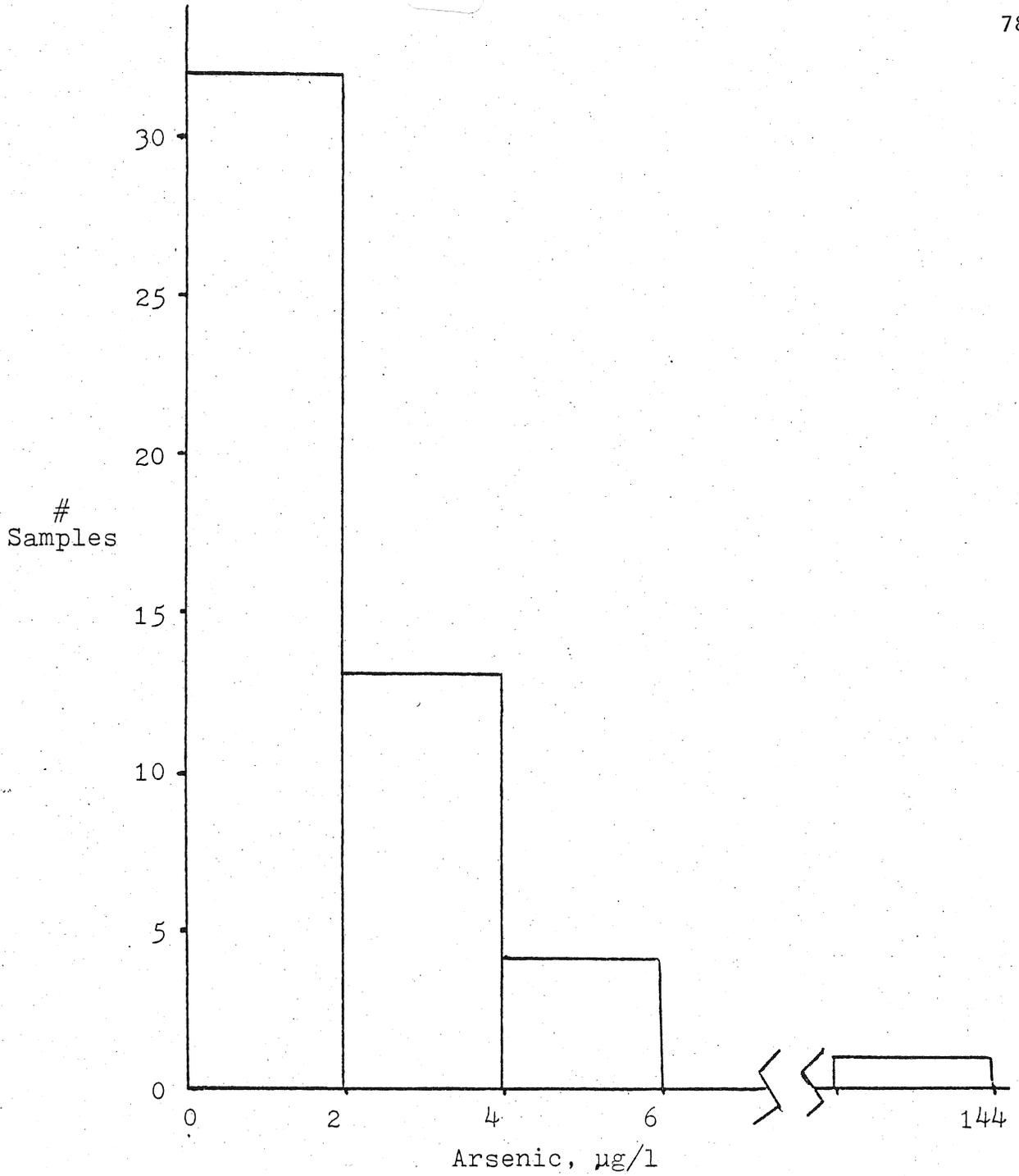


Figure 8-2. Frequency Distribution Histogram for Arsenic Content of 50 Stream and River Samples

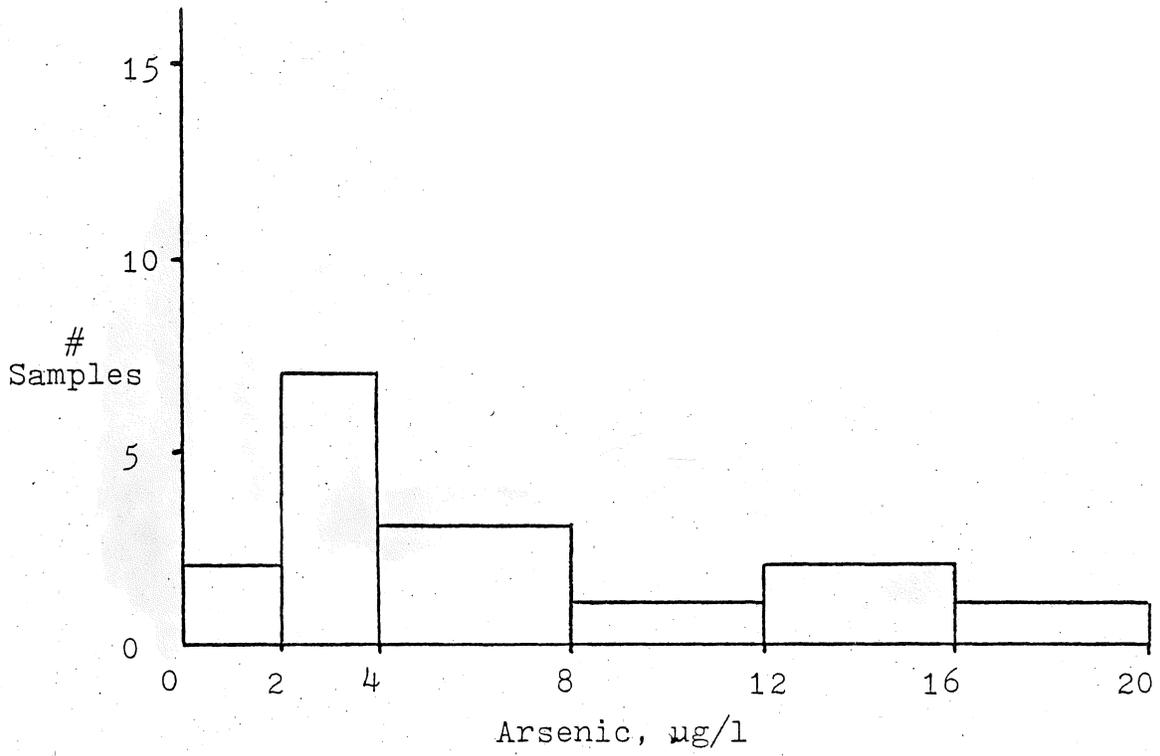


Figure 8-3. Frequency Distribution Histogram for Arsenic Content of 16 South Florida Canals

### *Hogtown Creek, Gainesville, Florida*

Figure 8-4 depicts the As concentrations in the Hogtown Creek drainage basin at low flow during a period of no rain on July 5, 1975, and at high flow after a strong rain on July 8, 1975. The west branch of Hogtown Creek receives run-off from a primarily residential area and the east branch receives run-off from business, residential and industrial areas. Arsenic concentrations at both low and high flow were generally very low with no significant differences between them. Slightly elevated As concentrations were discovered in the north-east branch of the creek; this branch was also polluted with oil and miscellaneous debris which possibly could have been the source of arsenic.

### *Middle St. Johns River*

The Middle St. Johns River receives water from a variety of sources including agricultural runoff, sewage effluents, and urban runoff. The three lakes, Jessup, Monroe, and Harney, and all the major tributaries of this river system were sampled and analyzed for As. The results are presented in Figure 8-5. Due to a poor detection limit of 4  $\mu\text{g}/\text{l}$  in the early phase of the project during which these samples were obtained, the majority of the samples were below the detection limit. Detectable amounts were encountered in Lake Jessup, probably as a result of the high concentration of arsenic in Sweet-water Creek which receives sewage effluent from Oviedo and drains into Lake Jessup. No other detectable amounts were found except for one sample collected near the marina at Sanford.

### *Peace River and Associated Creeks*

The Peace River originates at Lake Hancock and flows southerly to the Gulf of Mexico at Port Charlotte. The Peace River receives wastes from the phosphate mining industry, and for this reason it was expected that significant As concentrations might occur in the river because of the association of arsenic with phosphate. The results from samples taken September 17, 1975, are presented in Figure 8-6 and indicate that the levels of As in the river and its tributaries were low--only a few  $\mu\text{g}/\text{l}$ .

## E. ARSENIC SORPTION BY SOILS AND SEDIMENTS

From the above results indicating low concentrations of arsenic in Florida waters and from the chemical similarities of arsenate and phosphate, it was conjectured that sorption by soils and sediments is the dominant process in the removal of arsenic from natural waters. In order to investigate the mechanisms involved in arsenate sorption a modified Chang and Jackson fractionation procedure described in Chapter 7 was applied to soil samples from an arsenated citrus grove, a non-arsenated citrus grove and sediment samples from Lake Apopka (Table 8-6). Locations of the surface sediment samples taken from

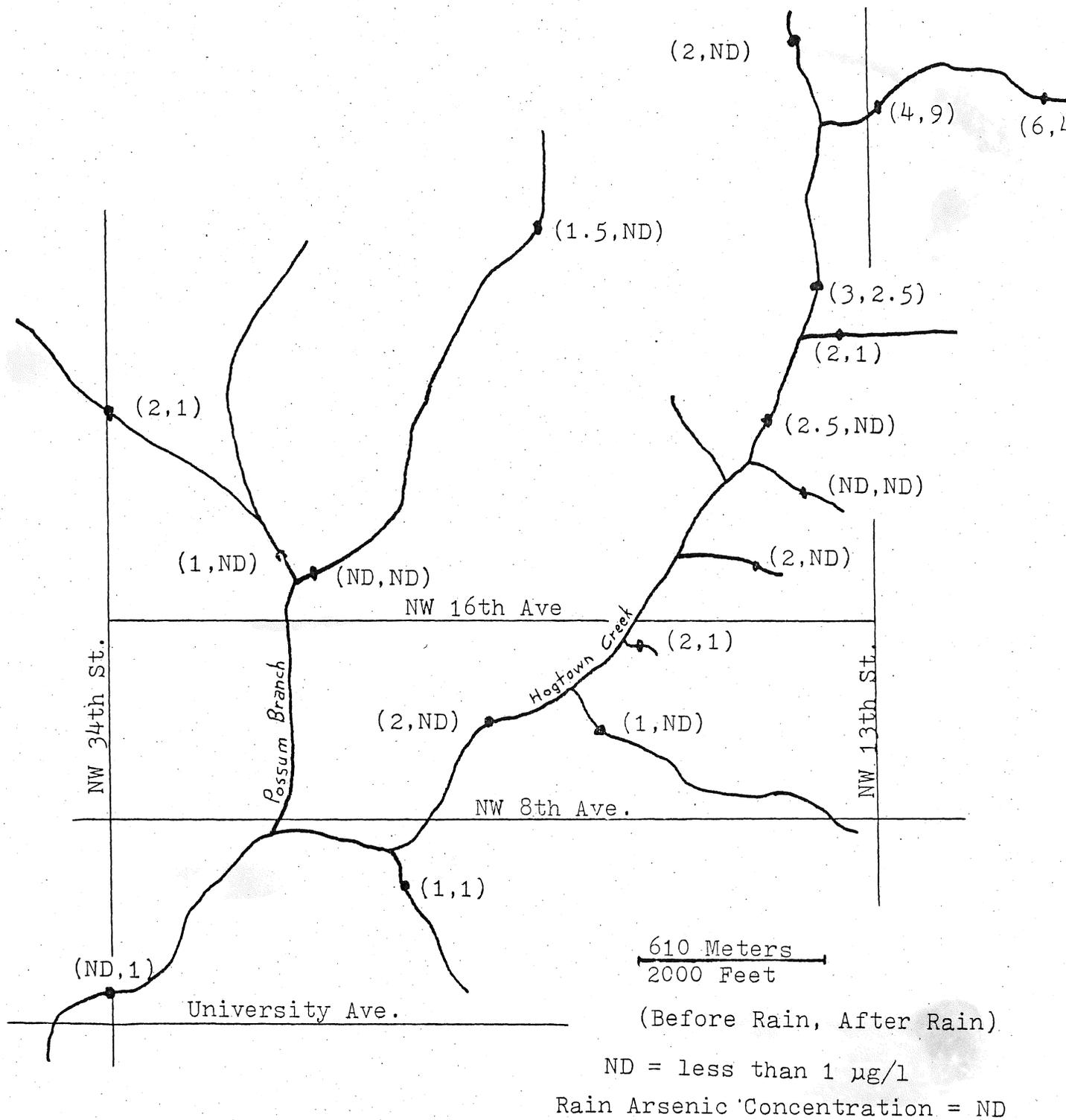
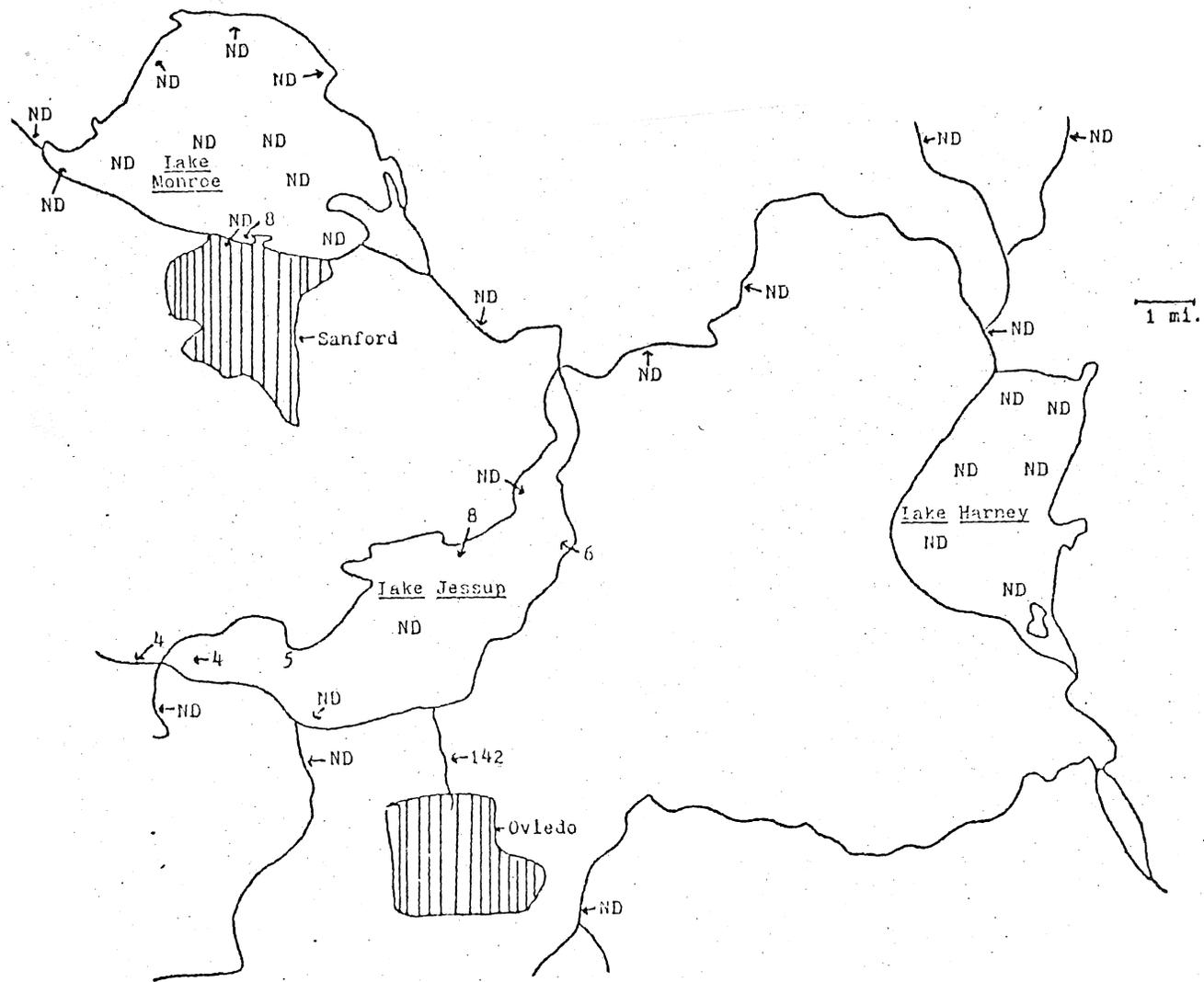


Figure 8-4. Arsenic in Hogtown Creek Drainage Basin Before and After Rainfall - July 5 and 8, 1975



ND < 4 µg/l

Figure 8-5. Arsenic Concentrations in the Middle St. Johns River

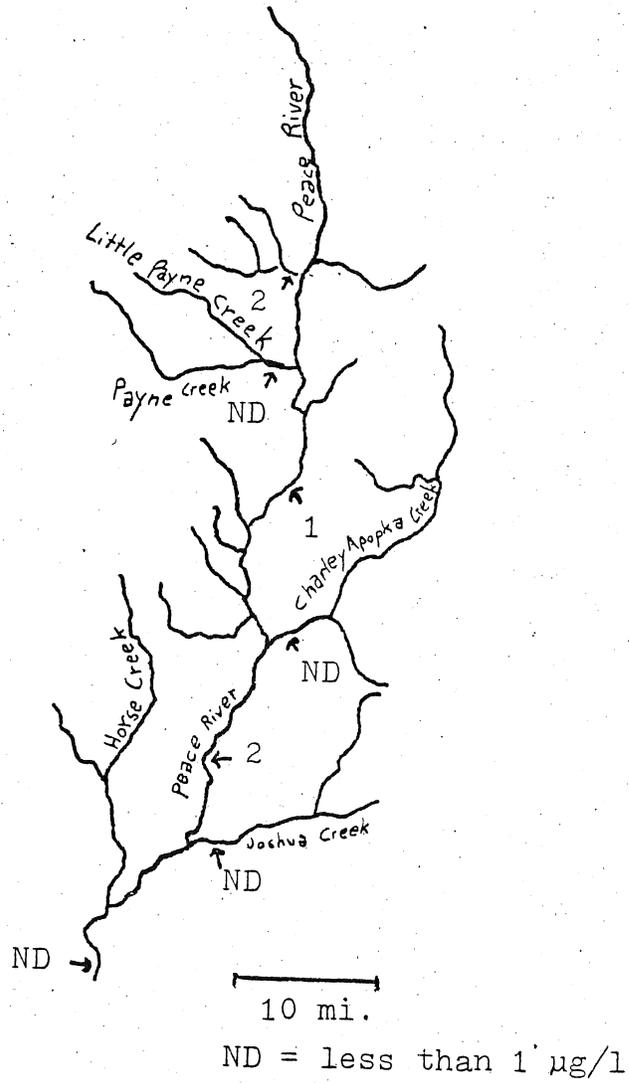


Figure 8-6. Arsenic Concentrations in the Peace River and Associated Creeks - September 17, 1975

Table 8-6. Arsenic Content of Several Sediment and Soil Components  
 From Lake Apopka, a Nonarsenated Citrus Grove and an Arsenated  
 Citrus Grove Utilizing a Modified Chang and Jackson Procedure

Lake Apopka	Extracts*					
	Water Soluble	Aluminum	Iron	Reductant Soluble	Calcium	Total
#1	4.08	0.74	0.92	ND	0.57	6.31
#2	2.82	0.56	2.47	ND	0.36	6.21
#3	0.86	0.19	1.09	ND	1.18	3.32
#4	1.89	0.30	0.91	ND	1.41	4.51
#5	1.62	0.30	1.35	ND	1.03	4.30
#6	2.51	0.49	0.52	ND	1.49	5.01
#7	4.74	0.69	0.73	ND	1.65	7.81
#8	2.58	0.41	1.63	ND	1.28	5.90
<u>Nonarsenated Grove</u>						
average of 3 samples						
0-3"	ND	ND	0.14	0.15	ND	0.29
9-15"	ND	ND	0.09	ND	ND	0.09
21-24"	ND	ND	0.13	ND	ND	0.13
<u>Arsenated Grove</u>						
average of 3 samples						
0-8 cm	0.34	0.71	4.05	0.35	0.44	5.89
23-38 cm	0.13	0.36	1.63	0.20	0.17	2.49
52-61 cm	ND	ND	1.17	0.20	0.14	1.51

\* all concentrations are  $\mu\text{g/g}$  dry weight  
 ND = less than  $0.05 \mu\text{g/g}$  arsenic

Lake Apopka are depicted in Figure 8-7. The results from the arsenated citrus grove samples indicate that As is readily sorbed by the soil. Surface soil samples from the arsenated grove contained approximately four times as much total As as sediments 50-60 cm deep. A nearby non-arsenated grove with similar soil characteristics contained one-twentieth as much As. Nearly all of the As in both soils was associated with iron.

Analysis of the fractions from the Lake Apopka sediments indicate larger total As concentrations than those for the arsenated citrus groves and larger than those reported for most Florida lakes by the United States Geological Survey (1974). Total As in the sediments increases in proximity to both the muck farm pumping station and the Winter Garden sewage treatment plant and citrus processing plant. The only exception was a sample taken 50 meters from the muck farm pumping station. This sample had a high sand content, probably caused by rapid settling of the heavy particles close to the pump. Figure 8-8 shows the average distribution of As among the various components for the arsenated citrus grove soil samples and for the Lake Apopka sediments. Significant differences were found between the arsenated grove soil and the sediments from Lake Apopka. Lake Apopka sediments had a majority of its As associated with the "water soluble" fraction and nearly equal amounts of As associated with the "iron" and "calcium" fractions. The arsenated citrus grove soil had almost all of its As associated with the "iron" fraction. The relatively lower concentration of As associated with the "iron" fraction in Lake Apopka sediments may be the result of the reduction and resolubilization of iron in anaerobic bottom sediments.

In order to evaluate the capacity of Lake Apopka sediments for As sorption, experiments were performed in the lab using Lake Apopka sediments. Figure 8-9 shows the results obtained by placing Lake Apopka sediments spiked with As in aerobic and anaerobic aquaria. The sediments were originally spiked with equal amounts of arsenite and arsenate (10  $\mu\text{g/g}$  dry wt.), but after 6 hours no arsenate was detected in the interstitial water, and arsenite was the only form present in the interstitial water for the remainder of the experiment. Differences between the aerobic and anaerobic aquaria were relatively minor indicating that dissolved oxygen in the overlying water was not a controlling factor. This also indicates a relatively small role for iron in the As sorption by these sediments; otherwise the As in the anaerobic aquarium would have been released when the iron was solubilized by reduction to ferrous ion. After 90 hours the interstitial water and the overlying water appeared to achieve steady state levels of approximately 60 and 40  $\mu\text{g/l}$  respectively. The decrease in interstitial water concentrations did not cause a corresponding decrease in the total As content of the sediment, suggesting that sediment binding of As is more or less irreversible.

Results for the anaerobic sorption of arsenite by sulfide-containing sediments are presented in Figure 8-10. The water in the cylinders was spiked with 200  $\mu\text{g}$  of arsenite for an initial aqueous concentration of about 135  $\mu\text{g/l}$ . The quiescent and slow stir cylinders removed only

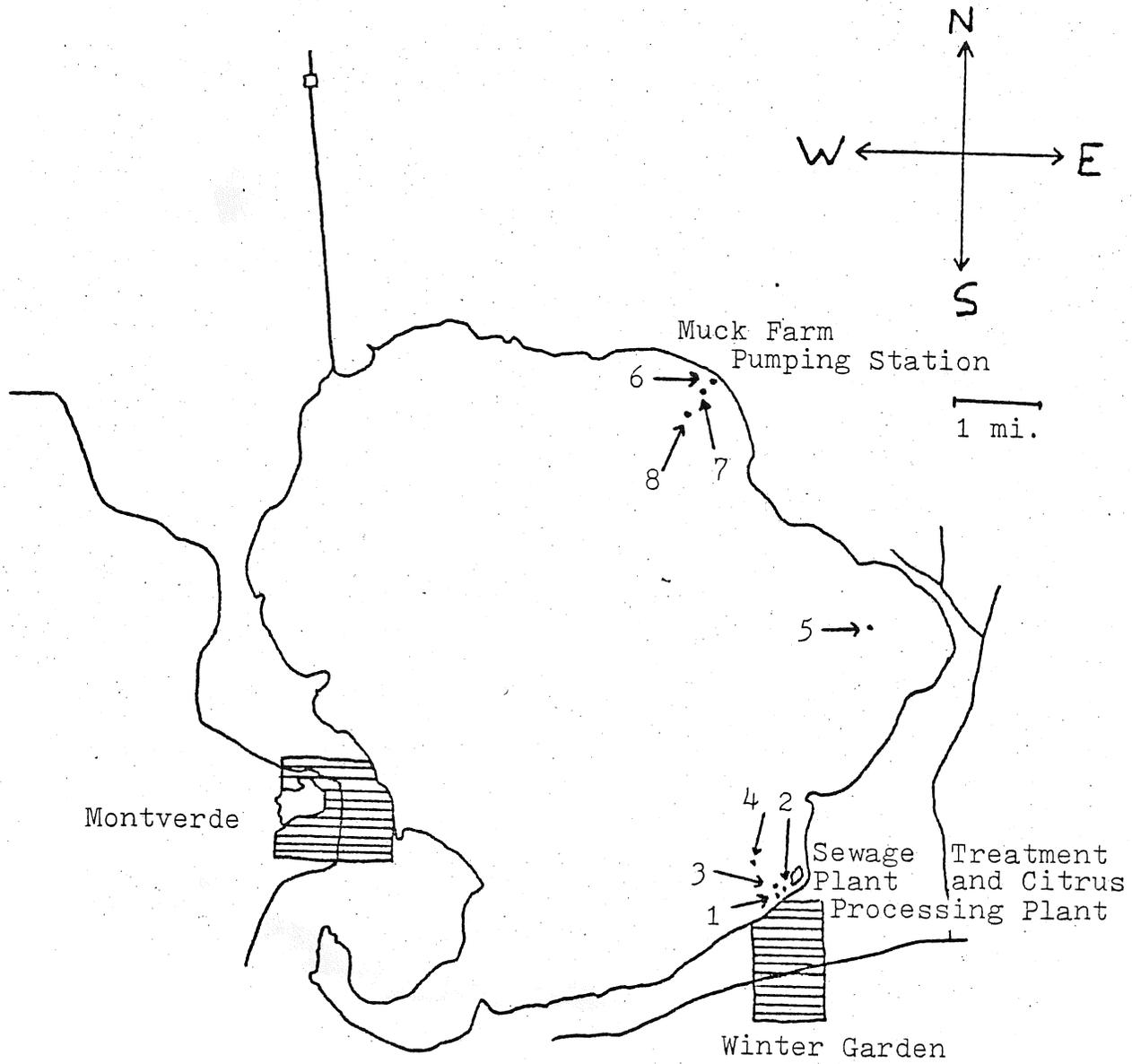
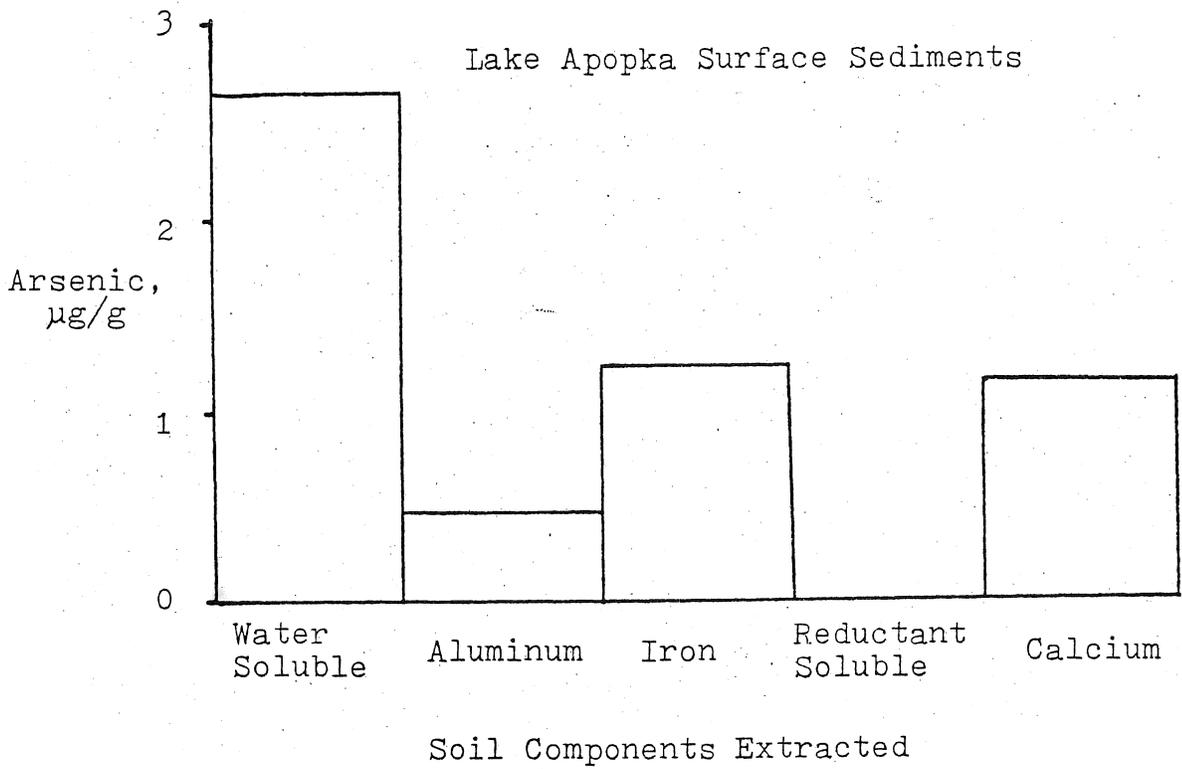
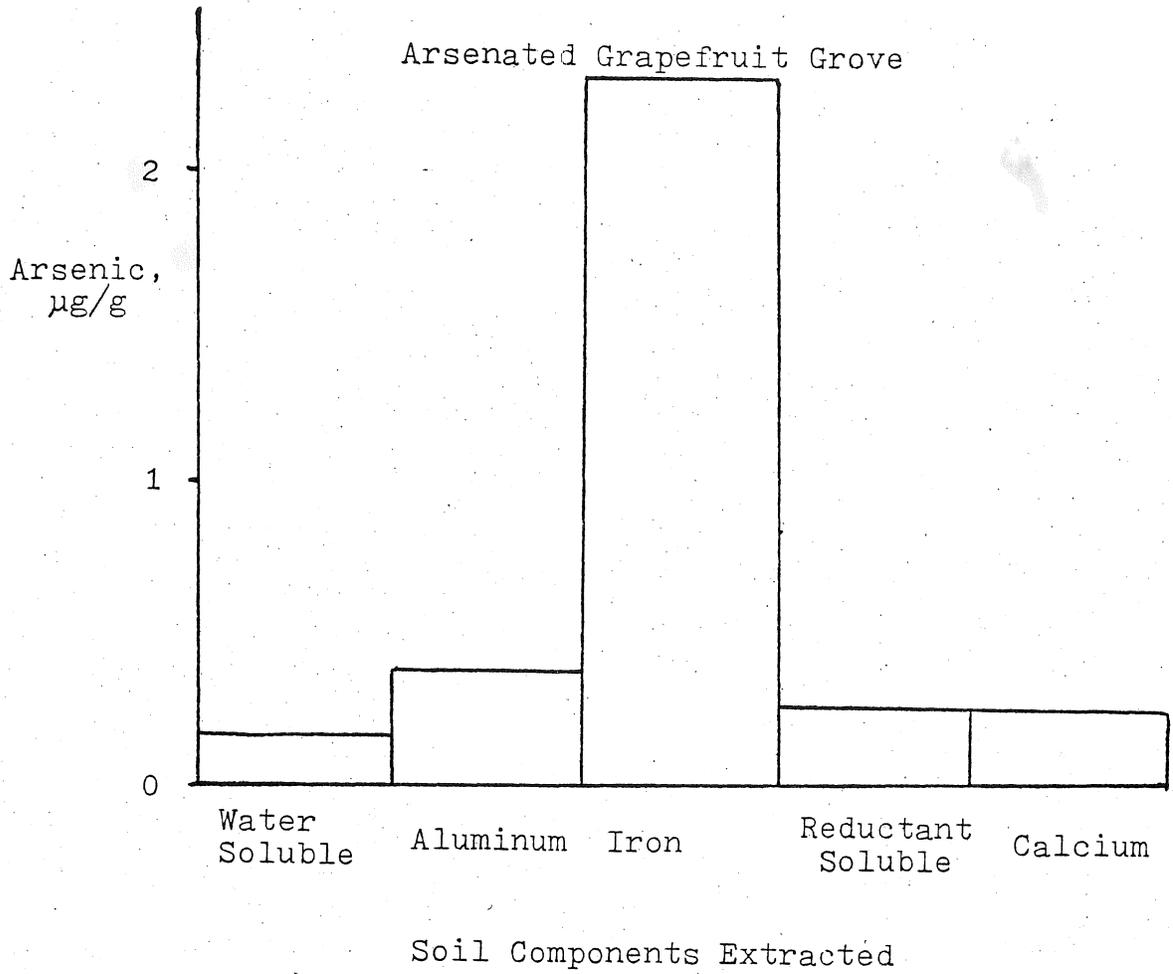


Figure 8-7. Locations of Surface Sediment Samples From Lake Apopka - October 10, 1975



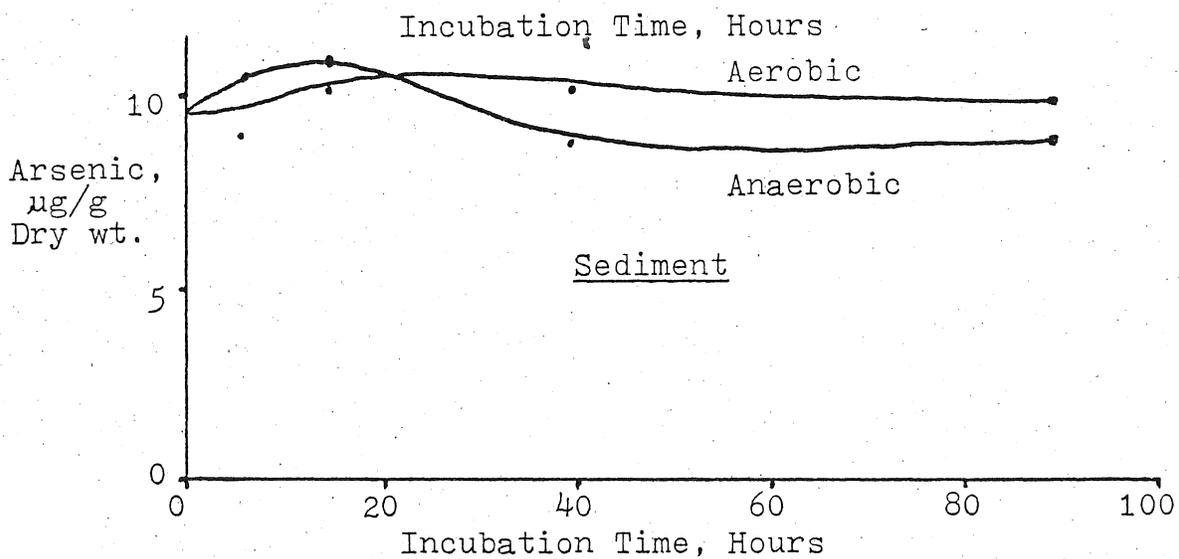
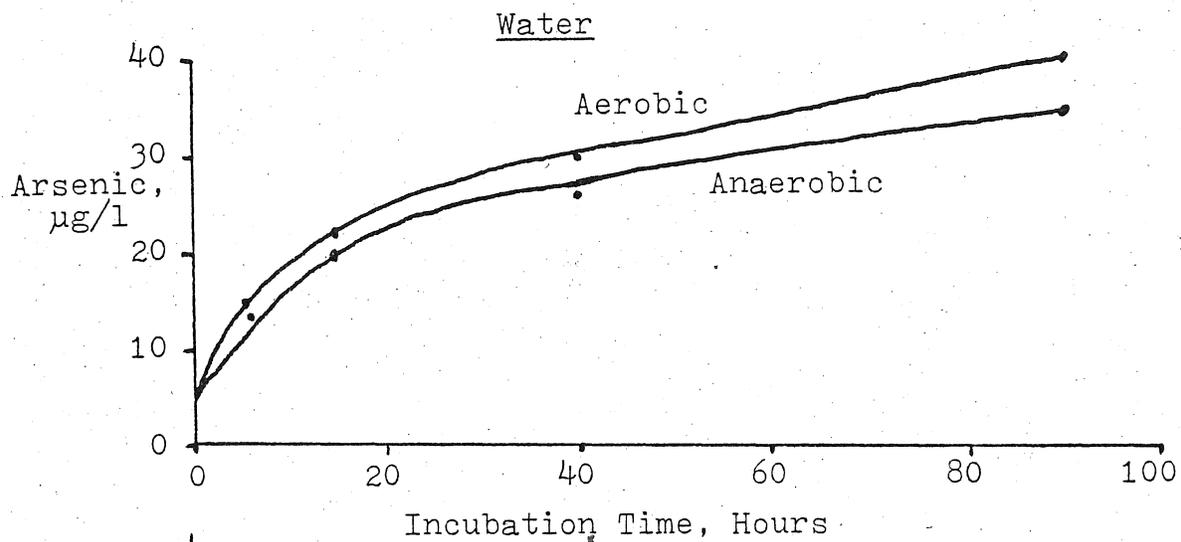
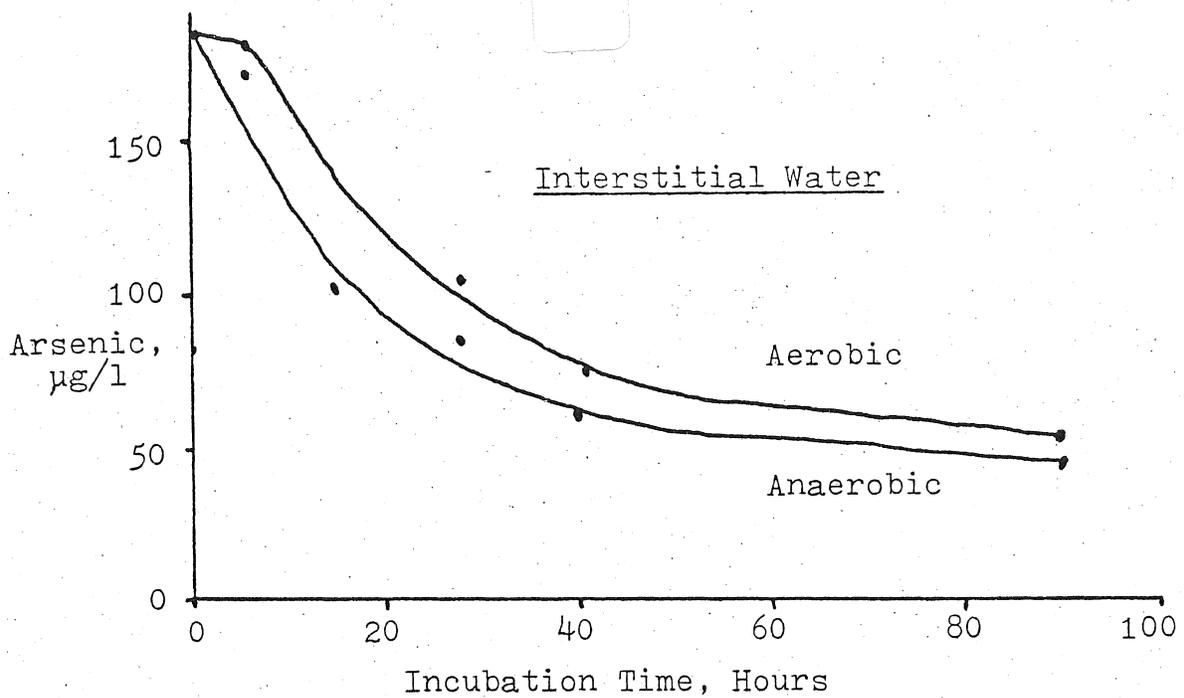


Figure 8-9. Release of Arsenic by Arsenic Spiked Lake Apopka Sediments Under Aerobic and Anaerobic Conditions

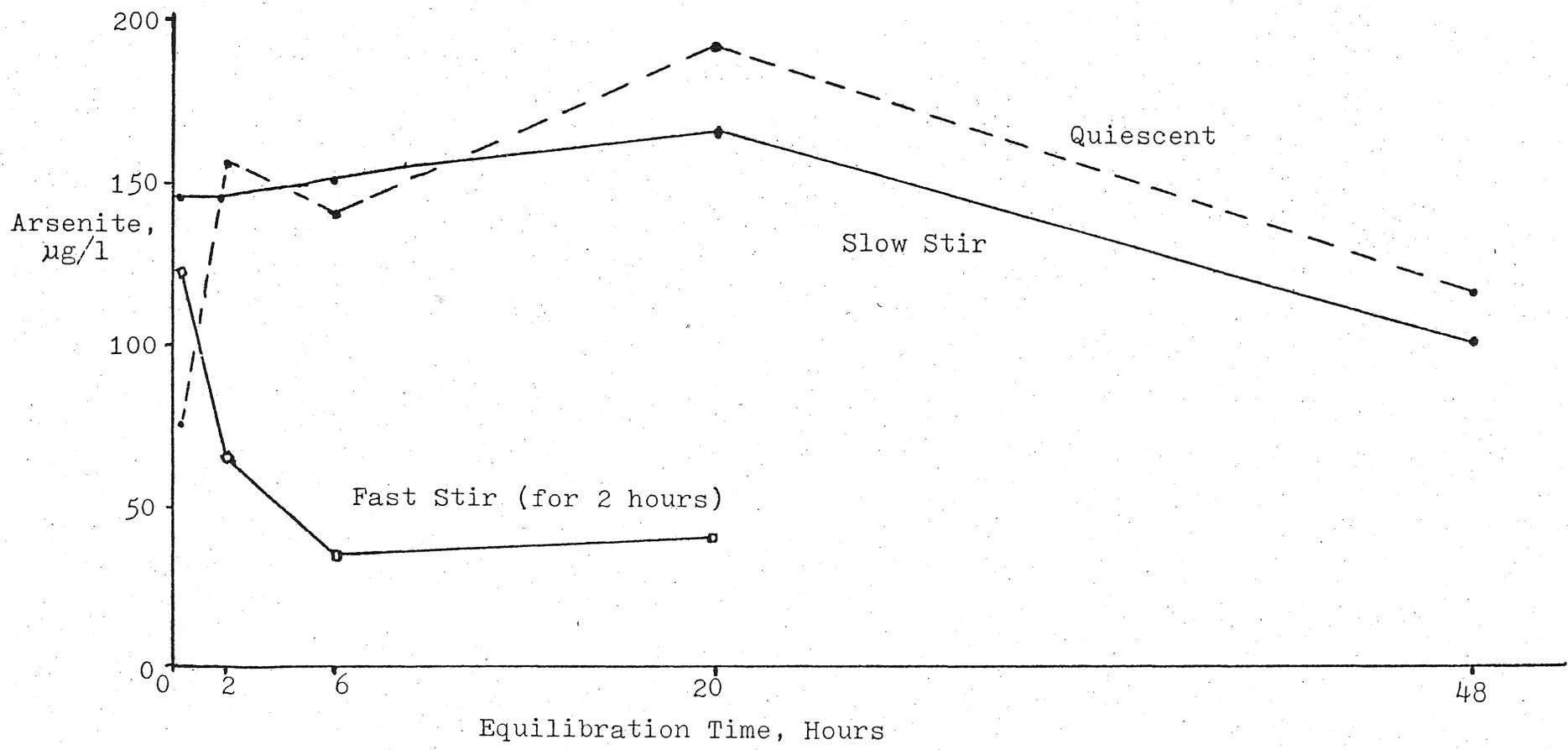


Figure 8-10. Anaerobic Sorption of Arsenic by Sediments Containing Sulfide at Three Rates of Turbulence and pH=7

a small portion of the total arsenite after 48 hours. The cylinder with fast stirring removed the As at a fast rate but a malfunction in the stirring motor occurred after 2 hours. At the fast stirring rate the sediments were suspended, and at the slow stirring rate only the water was mixed. The slower rate of As removal for the quiescent and slow stir cylinders indicates that mass transport (diffusion) of arsenic into the sediment is the controlling factor. The removal of arsenite in these sediments could have been caused by sorption onto the sediments or precipitation of realgar (AsS) or orpiment (AsS<sub>3</sub>). The latter is unlikely since realgar and orpiment normally do not precipitate above pH 5.5, and the pH in these cylinders was 7.0.

#### F. ARSENIC UPTAKE BY WATERHYACINTHS AND CATFISH IN LAKE APOPKA

Arsenic concentrations for two species of catfish and waterhyacinths from Lake Apopka are listed in Table 8-7. Lake Apopka is a major source of commercial catfish in Florida and bioconcentration of As by the catfish could pose a health hazard. Fortunately, both species of catfish contained nondetectable amounts of As. However, the limited sample is insufficient for generalizations, and further work should be undertaken on this topic. The waterhyacinths had relatively high concentrations of As, probably attributable to the chemical similarity of arsenate and phosphate and the uptake of arsenate along with phosphate by the waterhyacinth.

Table 8-7. Arsenic Concentrations for Two Species of Catfish and Waterhyacinths From Lake Apopka

<u>ORGANISM</u>	<u>ARSENIC CONCENTRATION*</u>
Catfish ( <u>Ictalura nebulosus</u> )	less than 0.05 ug/g fresh wt.
Catfish ( <u>Ictaluras natalis</u> )	less than 0.05 ug/g fresh wt.
Waterhyacinth ( <u>Eichhornia crassipes</u> )	0.95 ug/g dry wt.

\* concentrations are the average of two samples

## CHAPTER 9. SUMMARY AND CONCLUSIONS FOR ARSENIC STUDIES

Review of the literature reveals a multitude of investigations of arsenic in aquatic environments. However, the majority of these studies have failed to distinguish the oxidation state of As, despite the greater toxicity of arsenite than arsenate. This failure is attributable to the previous lack of satisfactory analytical techniques. The method of analysis chosen for this study was flameless atomic absorption spectrophotometry using a silica tube furnace with the arsenic forms first reduced to arsine by an automated manifold. The method of Braman and Foreback (1973) for the differentiation of As forms by the pH dependence of arsenate reduction by sodium borohydride was applied to the automated manifold. The procedure was fast and accurate and had a detection limit of 1  $\mu\text{g}/\text{l}$ .

Arsenic concentrations in natural Florida waters are generally less than 10  $\mu\text{g}/\text{l}$ , and the levels found in this study are in close agreement with the values reported by USGS (1974). The USGS survey of 380 Florida waters also substantiates the following conclusions reached in this study: 1) Lake Apopka had relatively high concentrations of As compared to other lakes; 2) the Peace River has low As levels (only a few ppb); and 3) south Florida canals have elevated As concentrations.

Arsenate was found to be the dominant form in surface waters. Arsenite was detected in only one surface water; however, it appears to be an important form in anaerobic lake sediments.

All of the municipal water supplies analyzed had As concentrations well below the recommended drinking water level of 10  $\mu\text{g}/\text{l}$ . Because of the low levels, no conclusions could be made on the effectiveness of water treatment for As removal. The chlorination of drinking water rapidly oxidizes arsenite to the less toxic arsenate. The As levels in sewage from the Gainesville sewage treatment plant exceeded 2  $\mu\text{g}/\text{l}$  in only a few instances during 8 days of monitoring.

The low concentrations found in Florida waters and reports in the literature that As is readily sorbed by soils and sediments indicate that the arsenic cycle in natural waters is sediment oriented. Investigations into the forms of As in soils and sediments revealed that arsenic is held primarily by iron in aerobic soils. In anaerobic sediments from Lake Apopka arsenic was found primarily in a loose "water soluble" phase.

Laboratory studies of As sorption and release by sediments from Lake Apopka showed little difference whether the overlying water was aerobic or anaerobic. This is probably due to relative lack of iron compounds in the sediments. Under both conditions the As was irreversibly bound by the sediments and was not released when interstitial water concentrations decreased. Under turbulent conditions the removal of arsenite by sulfide-containing sediments is excellent. With less turbulence, diffusion of As into the sediment is the rate determining step.

Catfish from Lake Apopka were found not to be bioconcentrating As; however, waterhyacinths from the lake had significant levels of As, probably as a result of the uptake of As along with phosphate.

M Methylated arsenic forms are present in the environment and may represent important intermediates for the volatilization of arsenic. Reported values of methylated As are generally less than 1 percent of the total As in natural waters and methylated As apparently is not biomagnified or bioconcentrated as is methylated mercury. For these reasons methylated arsenic was not investigated in this study. However, under some conditions methylated As may be more important (e.g. in anaerobic sediments and possibly in anoxic lake hypolimnis), and further studies should be undertaken to evaluate the role of methylated arsenic forms in the cycling and transport of arsenic in the environment.

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