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Use of Diatom Distributions to Monitor Environmental Health

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Summary: *A variety of approaches has been used in the past to assess the environmental impact of anthropogenic contaminants. One reliable index for aquatic environments is the analysis of diatom species distribution; the focus in this case being on the Savannah River. The completed objectives of this study were: A) the development and use of procedures for measuring diatom distribution in the water column and B) the development and evaluation of sediment sampling methods for retrospective analysis.*

Introduction

In the early 1950's, the Savannah River Site (SRS) was established on the bank of the Savannah River, near Aiken South Carolina, to begin production of nuclear materials. Over the years, various radiaoactive substances such as plutonium, tritium, cesium and strontium have been released into the ponds and creeks within the SRS plant (SRS 1991). Because of the continuous exchange of water between the Savannah River and its tributaries, much of the toxic waste has become dispersed throughout the approximately 160 miles of river between SRS and the river's mouth.

For the 40 years that SRS has been in operation, there exist only irregular records of both the amounts and types of waste materials released into the environment and their effects on the ecological systems of the Savannah River. In order to initiate a comprehensive history of the river's exposure to heavy metals and radionuclides, an analysis of diatoms was begun. It is possible that this approach will help in retrospective assessment of environmental contamination.

Diatoms are single-celled plants that are found both as phytoplankton and periphyton. Usually brown in color, and exhibiting a large range of shapes and sizes, diatoms exist in almost every aquatic habitat world-wide. Diatoms are unique in that they secrete silicon oxide, forming symmetrical shells, or frustules, which earn them the name "glass-houses." Along with the silicon

oxide, however, diatoms also incorporate into their frustules any elements accumulated from their aquatic surroundings. Materials enter the cell by diffusion (Rand and Petrocelli 1985), where they are packaged by various organelles and then secreted (Robinson and Sullivan 1987). The relative amounts of different elements in the frustule should therefore reflect the ratios of various materials in the water at the time of frustule formation.

When the diatom dies, the frustules sink and become part of the sediment. The cell decomposes, but there is little or no depuration from the frustule. All elements in the molecular structure of the shell remain intact and, by analyzing diatoms in the river sediment, it is possible to determine the ratio of toxic materials that were present in the water column over a long period of time. In this study, the initial aspects of that approach have been explored. Existing water column samples were analyzed, focusing on species composition to discover any present differences between the Cooper and the Savannah Rivers. Diatom distribution patterns in the water column will be used to initiate a database comparing past and present species composition as well as the amounts of different heavy metals and radionuclides that are suspected components of SRS effluent.

Materials and Methods

Field Procedures:

To collect field samples, both water column and sediment, from the Savannah River, two separate trips were made. The first, on July 1, 1993, began approximately at mile marker 10, north of the river's mouth, and continued upriver to mile 33, over which distance three sample sites were chosen (see figure 1). The second, on July 21, 1993, covered approximately 30 miles of river and 4 different sample sites, beginning 7 miles above SRS and ending close to the southern border of SRS territory (see figure 2). Three samples were also collected on each of two different days, June 25 and June 29, 1993, from the Cooper River as a control (see figures 3 and 4).

Sediment sample sites along both rivers were chosen where there was neither a strong current nor visible eddies and there appeared to be a lot of deposition, such as the inner curve of a bend in the river bed. A Wildco lexan hand corer (Wildlife Supply Co., Saginaw, MI), 2 inches in diameter, was used to take one core sample per site, core length ranging from 10 to 16

inches. Each core was then divided into three sections and placed into ziploc baggies.

Water column samples from both the Savannah and Cooper Rivers were taken in the same area as the sediment cores but in water at least 2 meters deep to avoid collecting bottom material. A submersible pump (Atwood 800 gpm, Attwood Corp.) attached to tygon tubing was lowered halfway into the water column and run for approximately 5 minutes per site (pump time was either increased or decreased depending on the amount of material collected). The water was run through a stack of 5 8-inch diameter sieves, with mesh sizes of 800, 270, 120, 70, and 37 microns (stacked downward from 800 to 37 microns) (Carolina Biological Supply Co.). The material from the 37, 70, and 120 micron sieves was then washed with river water from the mesh into separate collection bottles. Water column samples from the Cooper River, however, were combined by sieve size, i.e. all material collected in the 37 and 70 micron sieves from each site on the same day were washed into the same jar. A Hydrolab was used to measure the salinity/conductivity, pH, dissolved oxygen (DO), temperature, and depth of each water column site. Water column samples were maintained at 4 C during transportation to the lab.

Both water column and sediment samples were stored in a refrigerator when brought back to the lab without any preservation techniques, although 10% formalin was added to some of the water column samples a few days after collection in an attempt to preserve some of the organic material.

Laboratory Procedures: Water Column samples

In order to reduce the volume and concentrate the water column samples from both the Savannah River and the Cooper River, each sample was put into a 15 ml centrifuge tube and spun down at approximately 1000 rpm for 5 minutes. The supernatant was decanted, a few drops of distilled water was added, and the pellet was dispersed. Wet mounts were prepared from a few different samples and examined under a light microscope (Zeiss ICM-405 Inverted microscope equipped with video capabilities) both at 400x and at 1000x. Diatoms were visible at both magnifications and video thermal prints were made of some species to aid in classification (see figure 5).

Because of the ease with which diatoms were found under the scope, permanent slides were made. Three slides from each of the three sieve sizes collected (37, 70 and 120 microns) from all 7 sites sampled on the Savannah

River, and 4 slides from each sample size (37/70 and 120/270 microns) from each of the two Cooper River trips were prepared. A few drops of concentrated sample were placed on a glass slide and heated over an electric hot plate to dry. First Permount and then a coverslip were added to the dry samples before leaving them in a hood overnight for the Permount to harden. Completed slides were examined under the microscope at 400x, and the first twenty diatoms seen on each slide were classified by genus and tallied (see table 1A). Percentages were then calculated of each genus at each sample site (see table 2B).

Laboratory procedures: Sediment samples

Because of the density of the sediment cores and the large amount of material from each site, aliquots of each section of each core were filtered similar to the water column samples. Approximately 75ml of sediment from each sample was placed on the 800 micron sieve and washed through the 4 smaller sieves. Any material remaining on the 37, 70, and 120 micron sieves was then washed into separate sample bottles. After 3-5 spins at approximately 1000 rpm (beginning with 15 ml each time and then decanting the supernatant) for 5 minutes per spin, the samples were completely concentrated. A few drops of distilled water were added and the pellets were dispersed, then allowed to settle naturally.

The top layer of material from a few of the samp' s was pipetted onto glass slides and dried on a hot plate. Three slides were made from each of three samples (see table 2). When dry, the slides were examined under a dissecting scope at 50x. Diatoms appeared milky white among the other material on the slides. They were lifted off the slide with an eyebrow hair and a drop of distilled water and placed on a grid, each grid box representing a different shape of diatom.

Electron Microprobe Analysis

Special arrangements were made with Dr. Ann LeFurgey, Director of the Microscope Facility in the Department of Cell Biology at Duke University to use the lab's facilities and scanning electron microscope (SEM) to examine diatoms with electron probe analysis. That analysis would show the relative amounts of all the major elemental components in the diatom frustules.

To prepare the samples for examination in the SEM (JSM-6400), small aliquots of each sample were placed in petri dishes and left overnight under a hood to dry. Under a 10x dissecting scope, diatoms were identified, lifted by a fine brush with a drop of distilled water and placed on carbon stubs (made of spectroscopically pure carbon to avoid a false signal in the microscope) coated with colloidal graphite carbon paste. Approximately 4-10 diatoms were placed on a stub, then the stubs received a top layer of carbon. Completed carbon stubs were placed one by one in the SEM and examined. Three different types of images were used in the analyses: a Secondary Electron Image (SEI), a Backscattered Electron Image (BEI), and an Energy Dispersive X-ray (EDX). Black and white pictures were also taken of each diatom examined with Polaroid 4x5 Type 55 film (see figure 6).

Results

Diatom distributions from water column samples from the Savannah River as well as the Cooper River were calculated (see table 3). The most common diatom genus in the Savannah River is Melosira, comprising an average of 33% of the population. One interesting fact is that Melosira, while still the most abundant genus at each site, shows a definitive decrease in numbers, going from 50% of the diatom population at site 1 (farthest from the river's mouth) to only 22% at site 7 (at mile marker 10). Pleurosigma (also called Gyrosigma) (Heurck 1896) and Coscinodiscus are the next most common diatoms in the Savannah River, averaging 16% and 13% respectively. Except for Synedra (9%) and Navicula (8%), the majority of the other diatoms counted were classified in genera that each accounted for 4% or less of the total population.

The diatom distribution in the Cooper River was found to vary substantially from that of the Savannah. Although the most common diatom there is also Melosira at 32%, next on the list is Cyclotella, composing 21% of the diatom population, and Navicula at 15%. There is also a smaller variety of genera in the Cooper where only 13 genera were identified, compared to 19 in the Savannah River.

In the sediment samples taken from Savannah River sites 1 and 2, a large number of diatoms were found, including a high number of centric diatoms. This corresponds with the large percentage of centric diatoms (Coscinodiscus) found in the water column samples. In the slides from Savannah River site

4, however, insufficient numbers of diatoms were identified to analyze the population. Extracting diatoms from sediment is an exacting process, and considerable work will be required to develop reproducible procedures. Therefore, many more cores will have to be used for developing technical procedures before diatom populations of the past can be calculated on a routine basis.

SEI and BEI taken on the SEM were used for the photographs of the water column diatoms, clearly showing each frustule in detail (see figure 6). EDX data, printed in graph form, showed the relative amounts of each element present in a small section of each frustule. The most common elements were silicon, chlorine, potassium, calcium and barium (see figure 7). The chlorine peak is suspect, however, because there is a chlorine contamination factor from the SEM itself. An EDX was also done on the material inside one of the frustules, and much higher peaks of both aluminum and iron were recorded (see figure 8).

Discussion

The most common diatoms from mile markers 165-135 and 33-10 of the Savannah River are Melosira, Pleurosigma and Coscinodiscus (see table 3). When compared with previous calculations of diatom distributions, some interesting questions are raised.

For the most part, diatom species composition of the Savannah River has changed little over the past 40 years. In a study done by Louis G. Williams from 1960-1962 (Williams 1964), the most common diatoms at Port Wentworth, GA (near Savannah) were Melosira and Coscinodiscus. The Alvin W. Votgle Nuclear Power Plant's report on the algal populations at Port Wentworth from 1951-1968 also listed Melosira and Coscinodiscus as the most prevalent genera (Georgia Power Co. 1972). The results of this study agree with those results in that Melosira and Coscinodiscus comprise a large percentage of the total diatom population in the area; the presence of significant numbers of Pleurosigma is the striking difference to be accounted for.

Further upriver, near North Augusta, SC, Williams (1964) stated that Melosira was the most common diatom genus. The Votgle Nuclear Plant report (1972) concurrred, and also added Navicula to the list. This study has calculated that Melosira and Navicula are still a major portion of the area's

total diatom population. It deviates, however, in that Pleurosigma was also determined to be one of the most common genera, yet it is not prominent in the Savannah River in either of the 2 other studies mentioned above. Not enough information has been uncovered about diatoms as indicator species to be able to determine exactly what the increased appearance of Pleurosigma signifies, but future sediment analyses in areas where Pleurosigma exists in high percentages will hopefully be able to provide a history of the genus' population. That history may follow the rise and fall of the river's toxicity level, or it may show a general adaptation of different diatoms to changing environmental parameters.

On another level, the diatom populations of the Savannah River were compared to those of the Cooper River, and while there were some similarities, other variations were immediately visible (see table 3). For example, Melosira is the most common genus in each of the rivers but, where Pleurosigma and Coscinodiscus are also prominent in the Savannah River, they are not found in great numbers in the Cooper. On the other hand, Fragilaria and Cyclotella, two of the most common diatoms in the Cooper River, are very rare in the Savannah. While it is possible that these differences are due to the increased amount of toxic waste dumped into the Savannah (i.e from SRS) than the Cooper, there are numerous, non-anthropogenic differences between the two watersheds that must be considered.

More research must be completed before firm conclusions of environmental significance can be drawn from this data, although river quality assessment through diatom analysis appears to be a reliable procedure. Results agreed with assessments made previously, and sample sizes proved to be sufficient for statistical analysis. As more information continues to be collected, the database for Savannah River environmental health will be progressively enlarged.

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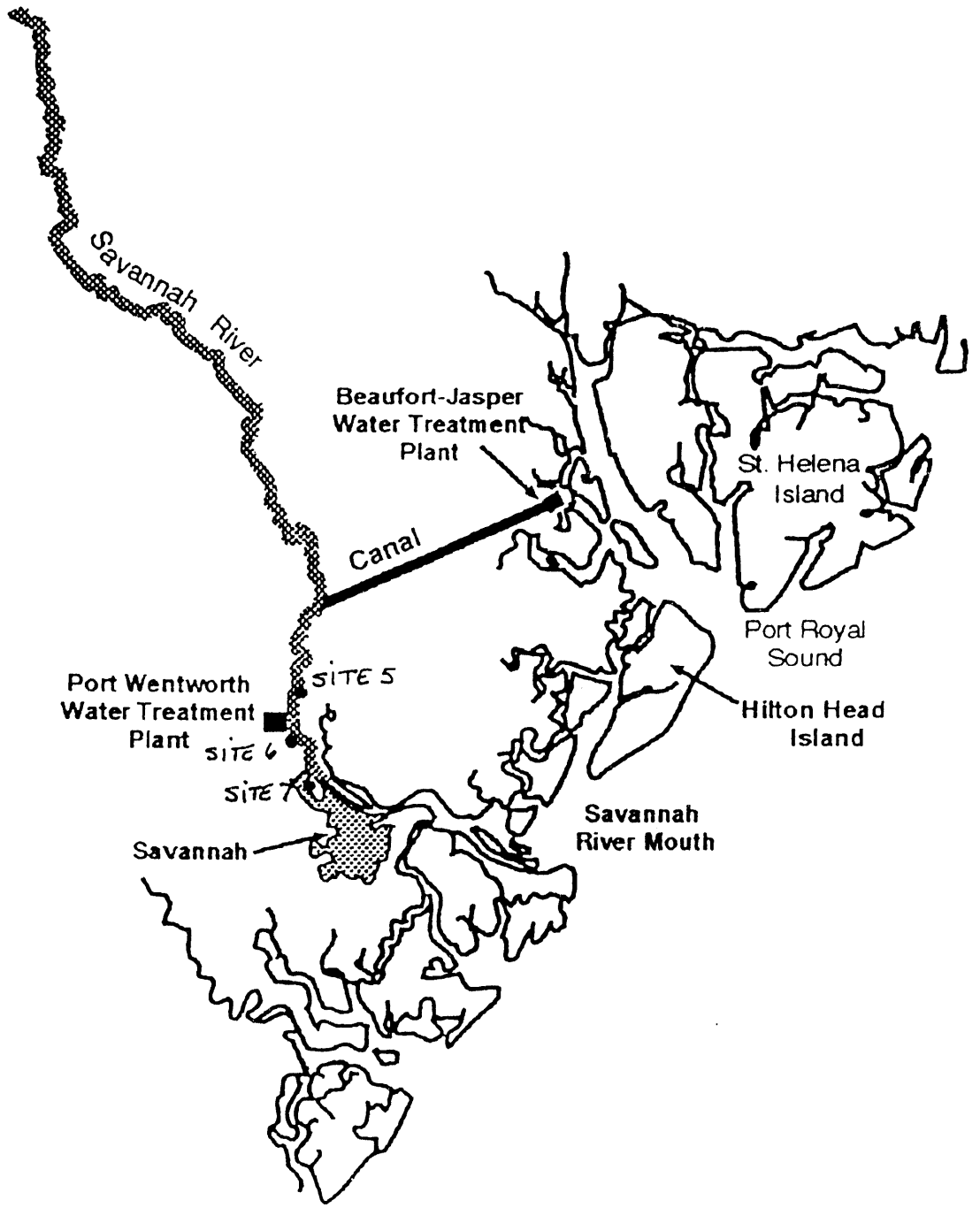


Figure 1. Sites 5-7 sampled on the Savannah River at mile markers 33, 20, and 10 on 7/1/93 are marked with a ●.

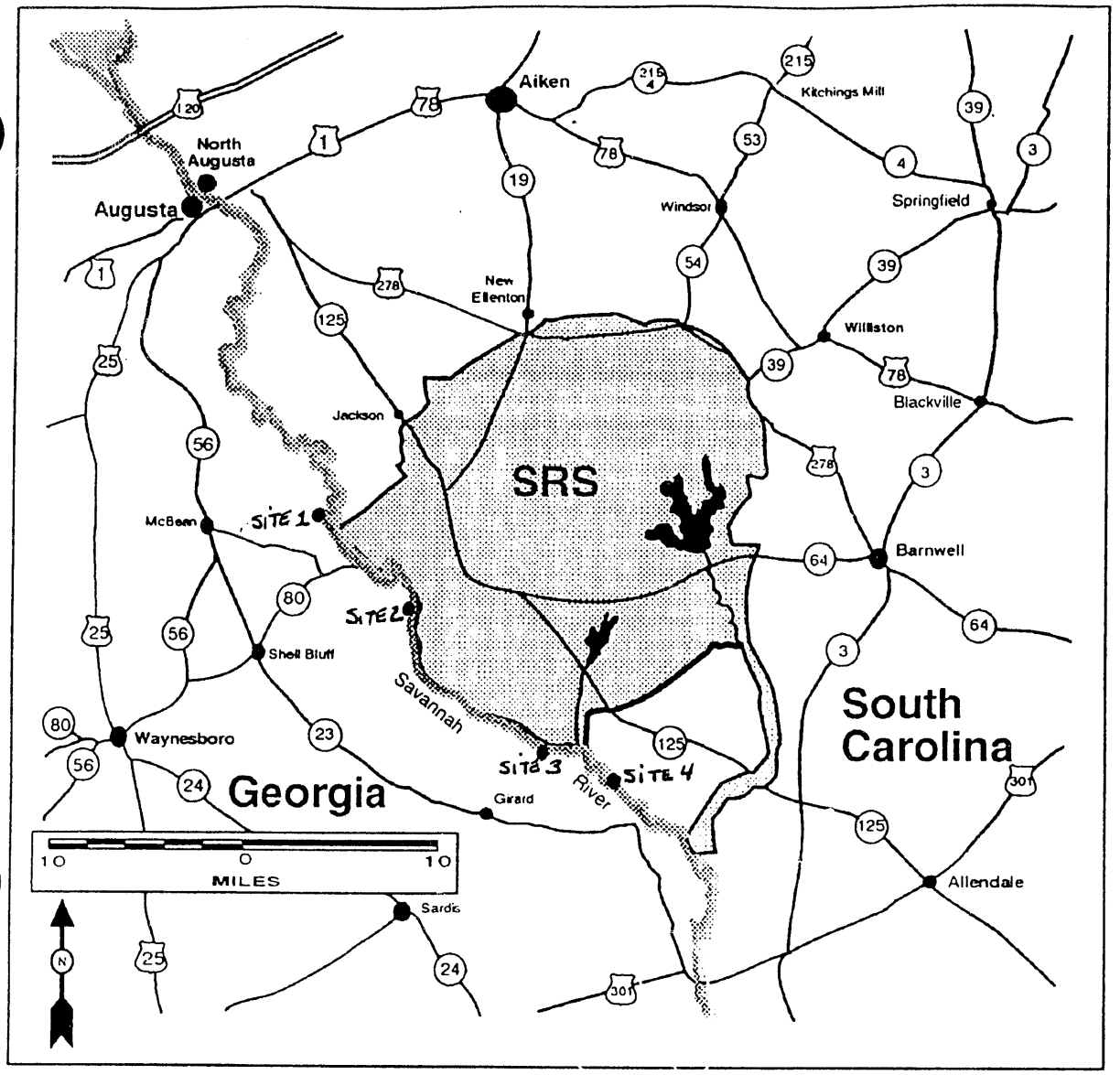


Figure 2. Sites 1-4 sampled on the Savannah River at mile markers 165, 155, 145, and 135 on 7/21/93 are marked with a ●.

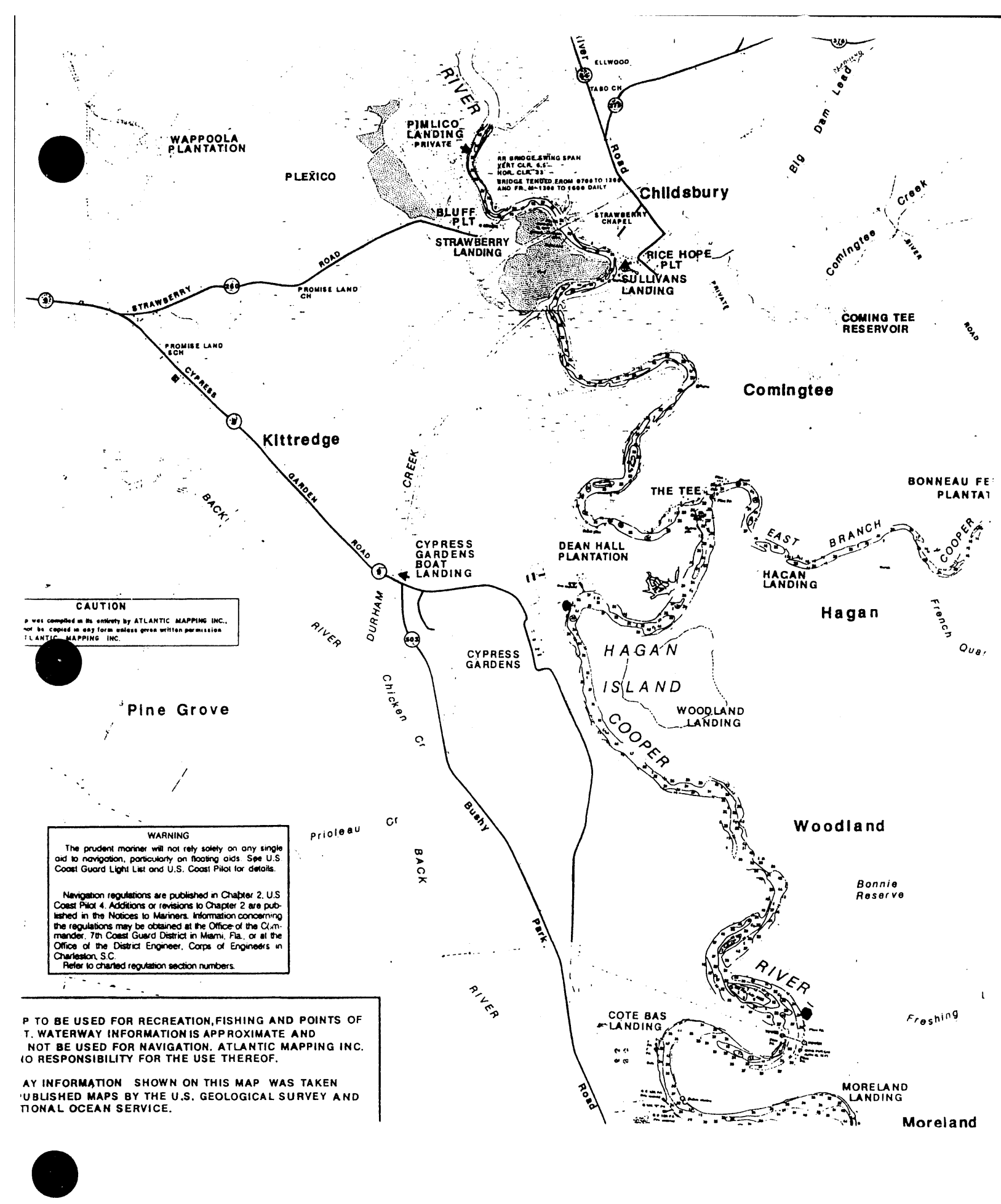


Figure 3. Sites sampled on the Cooper River on 6/25/93 are marked with a ●.

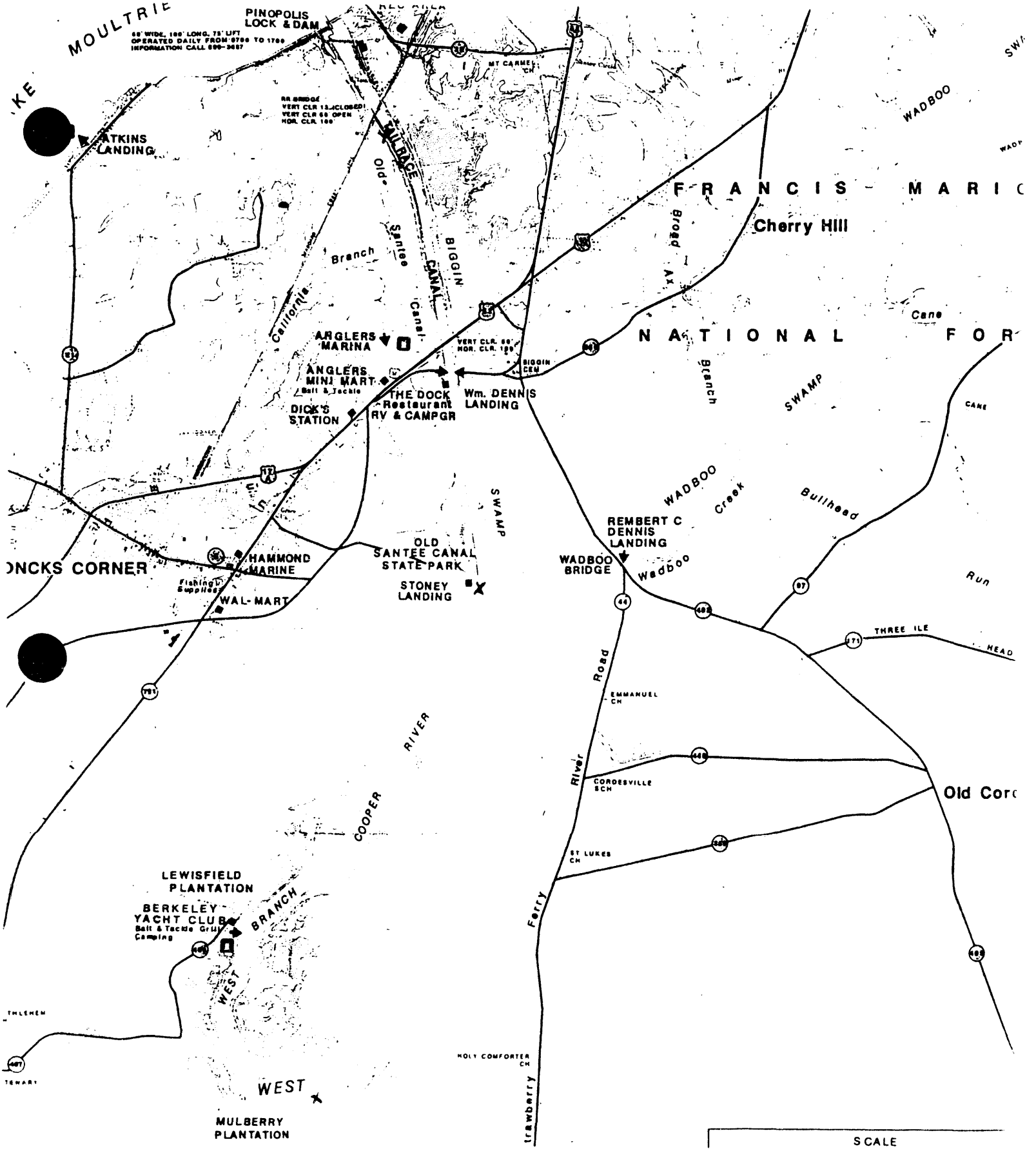


Figure 4. Sites sampled on the Cooper River on 6/29/93 are marked with an X.

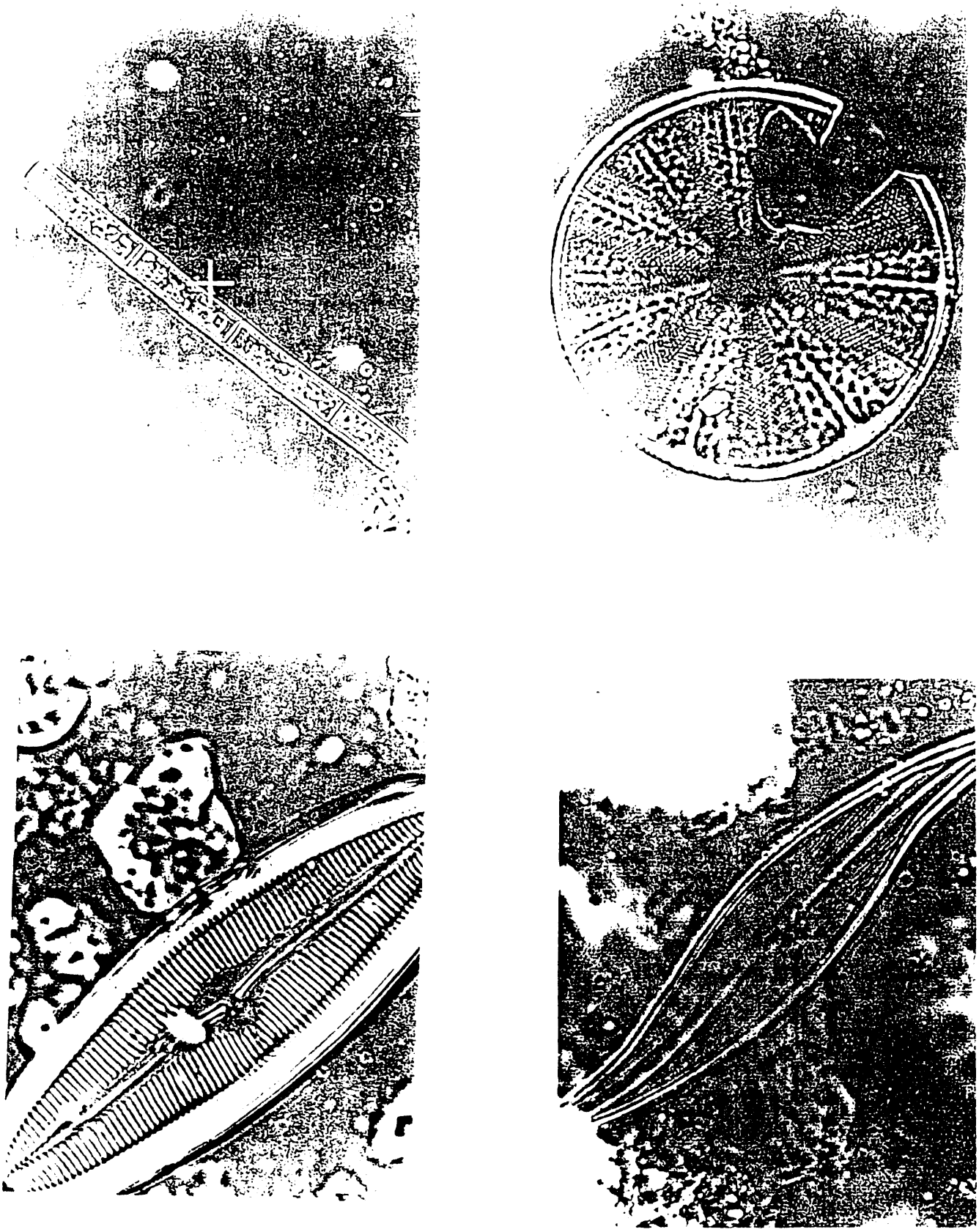
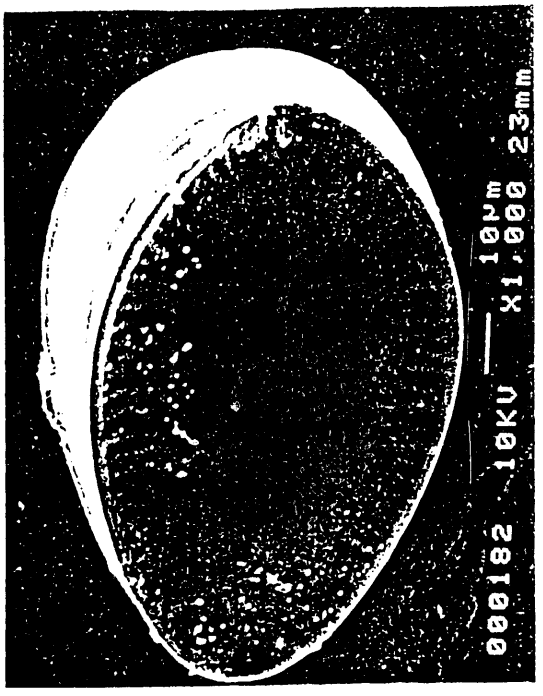
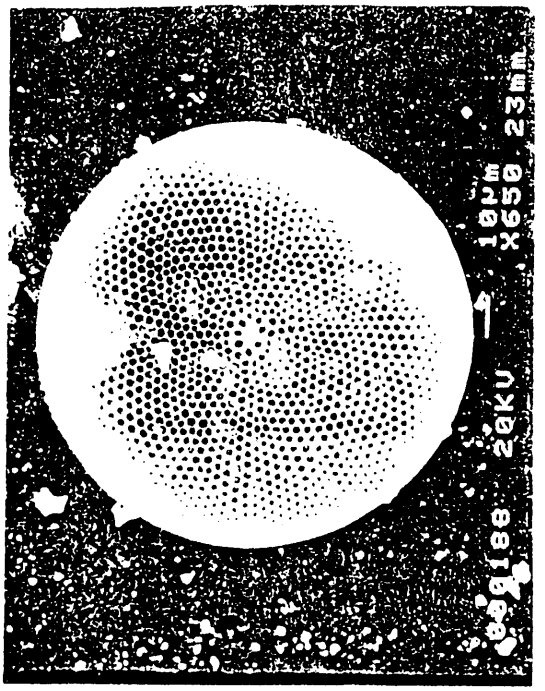
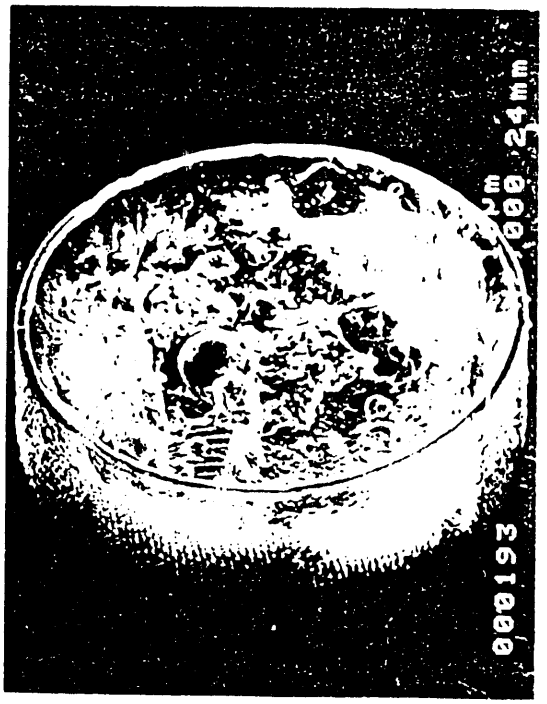


Figure 5 Thermal prints taken with video equipment hooked up to the light microscope.







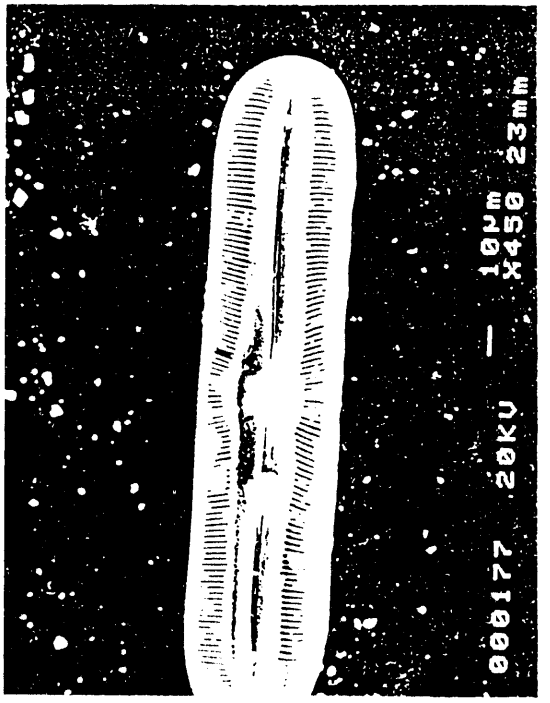


Figure 6. SEM SEI 4x5 photos.

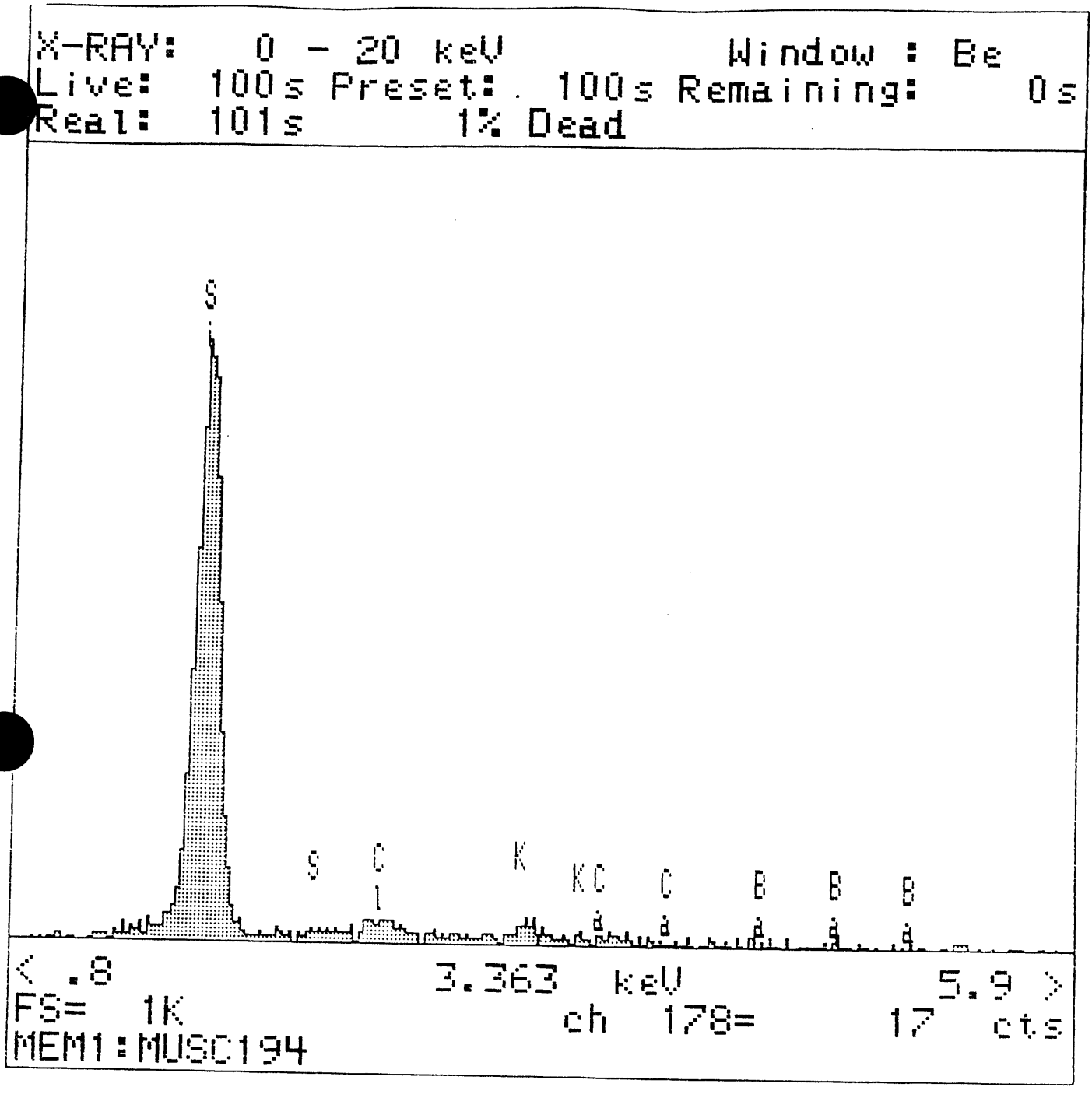


Figure 7. EDX data in graph form showing elemental composition of a diatom frustule. The most common elements are silicon, chlorine, potassium, calcium and barium.

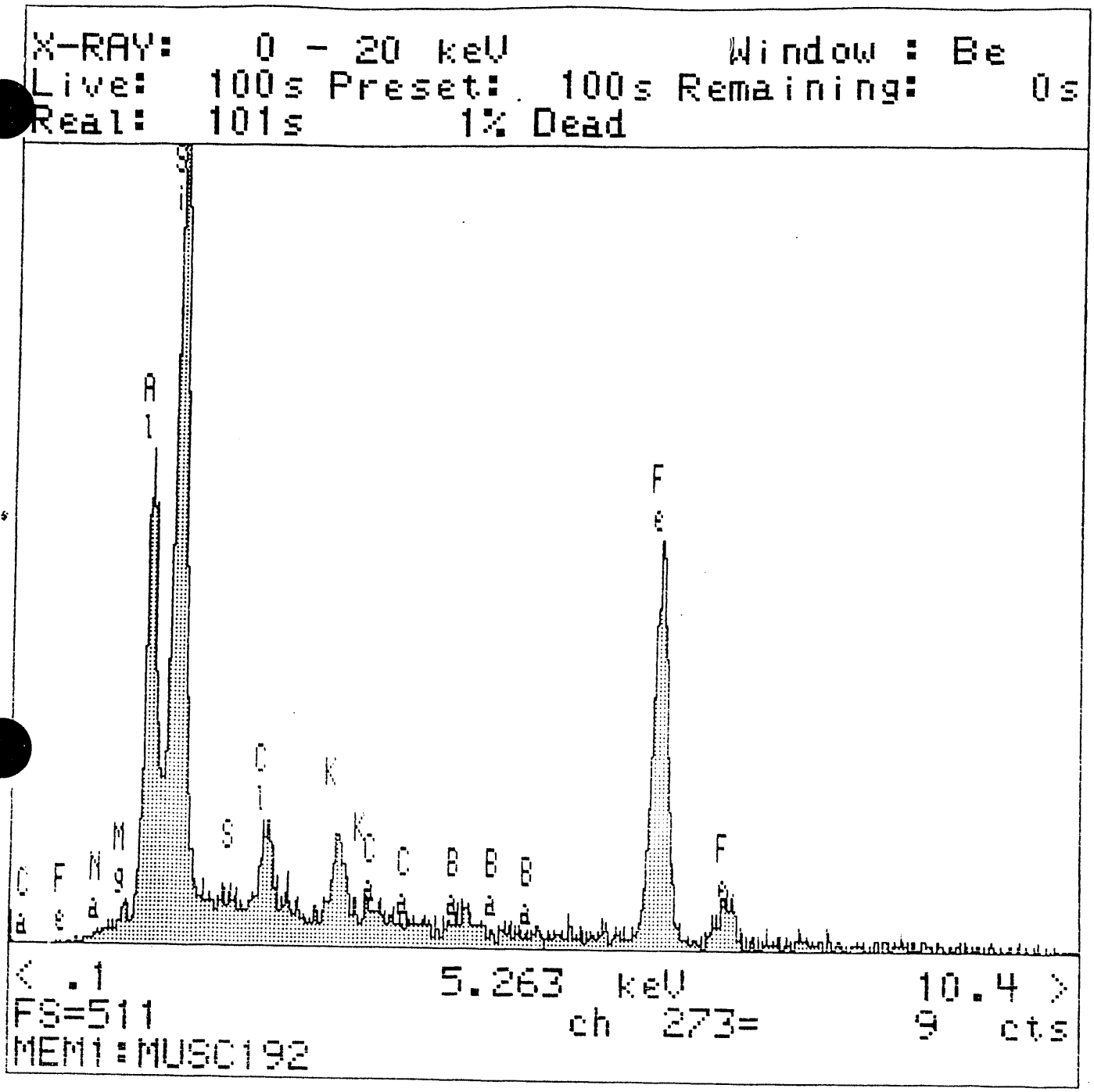


Figure 8. EDX data in graph form showing elemental composition of the material inside part of a diatom frustule. The most common elements are silicon, aluminum, and iron.

	7/21/93	7/21/93	7/21/93	7/21/93	7/21/93	7/21/93	7/21/93	7/21/93	7/21/93	7/21/93
	SR1A 37	SR1B 37	SR1C 37	S1A 70	SR1B 70	SR1C 70	SR1A 120	SR1B 120	SR1C 120	SR1 TOT
MELOSIRA	14	9	10	3	9	10	14	10	11	90
PLEUROSIGMA	0	1	1	1	2	1	1	5	4	16
SURIRELLA	3	4	2	0	0	0	1	0	0	10
COSCINODISCUS	3	3	5	1	0	1	0	1	0	14
SYNEDRA	0	0	0	1	1	1	0	2	1	6
FRAGILARIA	0	2	0	4	0	4	0	1	0	11
CYCLOTELLA	0	1	0	0	1	0	0	0	1	3
TERPSINOE	0	0	2	1	1	0	4	1	0	9
NAVICULA	0	0	0	6	4	0	0	0	0	10
CLAVULARIA	0	0	0	2	1	2	0	0	0	5
NITZSCHIA	0	0	0	1	1	1	0	0	0	3
STARONEIS	0	0	0	0	0	0	0	0	1	1
ASTERIONELLA	0	0	0	0	0	0	0	0	2	2

A. Tallies of diatoms in water column samples from Savannah River sample site 1 were classified by genus.

	7/21/93	7/21/93	7/21/93	7/21/93
	SR1 37	SR1 70	SR1 120	SR1 %
MELOSIRA	55%	37%	58%	50%
PLEUROSIGMA	3%	6%	16%	9%
SURIRELLA	15%	0%	2%	6%
COSCINODISCUS	18%	3%	2%	8%
SYNEDRA	0%	5%	5%	3%
FRAGILARIA	3%	13%	2%	6%
CYCLOTELLA	2%	2%	2%	2%
TERPSINOE	3%	3%	8%	5%
NAVICULA	0%	16%	0%	6%
CLAVULARIA	0%	8%	0%	3%
NITZSCHIA	0%	5%	0%	2%
STARONEIS	0%	0%	2%	1%
ASTERIONELLA	0%	0%	3%	1%

B. The percentage of each diatom genus in Savannah River sample site 1 was calculated from the data in table 1B.

Table 1

Savannah River Sediment Samples

SAMPLE #	RIVER	SITE	CORE LENGTH	SIEVE SIZE
1	Savannah	1	0-6"	120
2	Savannah	2	0-8"	120
3	Savannah	4	0-6"	120

Table 2

Savannah River and Cooper River Diatom % Comparison

	SR1 %	SR2 %	SR3 %	SR4 %	SR5 %	SR6 %	SR7 %	AVG.
MELOSIRA	50%	38%	42%	26%	28%	25%	22%	33%
PLEUROSIGMA	9%	18%	17%	16%	18%	24%	8%	16%
SURIRELLA	6%	3%	2%	6%	4%	3%	7%	4%
COSCINODISCUS	8%	15%	11%	12%	9%	9%	29%	13%
SYNEDRA	3%	9%	12%	9%	14%	11%	8%	9%
FRAGILARIA	6%	4%	1%	3%	0%	0%	1%	2%
CYCLOTELLA	2%	0%	2%	2%	3%	3%	4%	2%
TERPSINOE	5%	2%	1%	2%	1%	4%	0%	2%
NAVICULA	6%	8%	8%	11%	9%	8%	6%	8%
CLAVULARIA	3%	1%	4%	6%	7%	7%	3%	4%
NITZSCHIA	2%	1%	1%	0%	3%	2%	3%	2%
STARONEIS	1%	0%	1%	2%	1%	0%	3%	1%
ASTERIONELLA	1%	3%	0%	3%	1%	0%	1%	1%
CYMBELLA	0%	0%	1%	2%	0%	0%	0%	0%
EPITHEMIA	0%	0%	0%	1%	0%	0%	0%	0%
BIDDULPHIA	0%	0%	0%	0%	1%	2%	3%	1%
CHAETOCEROS	0%	0%	0%	0%	0%	1%	0%	0%
ACHNANTHES	0%	0%	0%	0%	0%	0%	1%	0%
RAPHONEIS	0%	0%	0%	0%	0%	0%	1%	0%
	CR 6/25%	CR 6/29 %						AVG.
MELOSIRA	28%	35%						32%
PLEUROSIGMA	2%	0%						1%
SURIRELLA	1%	0%						1%
COSCINODISCUS	3%	1%						2%
SYNEDRA	3%	3%						3%
FRAGILARIA	9%	18%						14%
CYCLOTELLA	22%	20%						21%
								0%
NAVICULA	15%	15%						15%
CLAVULARIA	2%	0%						1%
NITZSCHIA	10%	5%						8%
								0%
								0%
CYMBELLA	2%	0%						1%
								0%
								0%
CHAETOCEROS	0%	1%						1%
								0%
								0%
ODD	3%	0%						2%

Table 3

END

DATE
FILMED
3/4/94