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**Tulane/Xavier University  
Hazardous Materials In  
Aquatic Environments  
Of The Mississippi River Basin**

Annual Technical Report

Project #DE-FG01-93EW53023  
(January 1, 1995 - December 31, 1995)

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## Introduction

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Tulane and Xavier Universities have singled out the environment as a major strategic focus for research and training for now and beyond the year 2000. In 1989, the Tulane/Xavier Center for Bioenvironmental Research (CBR) was established as the umbrella organization which coordinates environmental research at both universities. The CBR is supported by major grants from the Department of Energy, Department of Defense, National Institute of Environmental Health and Safety, National Institute of Health, National Institute of Environmental Health Sciences, and other agencies. This joint venture is truly interdisciplinary, involving faculty and student participation from most schools and divisions at both universities and, thus, presents an integrated approach to environmental problems. Research ranging from creating new technologies for environmental clean-up to understanding the economics that drive environmental policy decisions are coordinated under the CBR auspices.

Founded in 1834, Tulane University is one of the major private research universities in the South. Over 11,000 students are enrolled in its 11 schools and colleges. Undergraduates are enrolled in Tulane's School of Engineering, School of Architecture, A.B. Freeman School of Business, Newcomb College, University College and Paul Tulane College. Over 4,500 graduate students are enrolled in liberal arts and sciences, engineering, public health and tropical medicine, social work, law, business, medicine and architecture. The recently dedicated J. Bennett Johnston Health and Environmental Research building provides state of the art laboratory space for core areas of bioenvironmental research including toxicology and environmental health sciences.

Xavier University, the only historically black Catholic institution in the United States, was founded in 1915 by a religious order dedicated to the education of American minorities. Enrollment is approximately 3,500 and offers preparation in thirty-six undergraduate majors. Xavier ranks first nationally in the number of black undergraduates receiving degrees in the physical sciences. Countering a national trend of declining African American enrollment in advanced degree programs, high numbers of Xavier graduates go on to professional and graduate schools. Tulane and Xavier universities have developed environmental curricula and research programs to help meet the nation's growing demand for environmental scientists. Renovations and a new addition to the College of Pharmacy building have expanded the laboratory facilities for Xavier researchers.

In December, 1992, the Tulane/Xavier CBR was awarded a five year grant to study pollution in the Mississippi River system. The "Hazardous Materials in Aquatic Environments of the Mississippi River Basin" project is a broad research and education program aimed at elucidating the nature and magnitude of toxic materials that contaminate aquatic environments of the Mississippi River Basin. Studies include defining the complex interactions that occur during the transport of contaminants, the actual and potential impact on ecological systems and health, and the mechanisms through which these impacts might be remediated. The Mississippi River Basin represents a model system for analyzing and solving contamination problems that are found in aquatic systems world-wide. These research and education projects are particularly relevant to the U.S. Department of Energy's programs aimed at solving aquatic pollution problems associated with DOE National Laboratories.

The project has completed its third year of funding. This funding has supported twelve collaborative cluster projects (multi-year) and twenty initiation (one year) projects. Over 75 faculty from Xavier University (from the School of Arts and Sciences and College of Pharmacy) and Tulane University (from the Liberal Arts and Sciences, School of Engineering, Medical School, and the School of Public Health and Tropical Medicine) have participated to date. Additionally, more than 50 graduate and numerous undergraduate students have worked on research problems associated with the project.

### Study Sites

Sites in the Mississippi River basin were selected for studying how industrial contaminants enter aquatic ecosystems and how these compounds move through environmental phases and influence different species. The following areas were chosen as the major sampling sites:

- Devils Swamp, a cypress swamp that lies northwest of Baton Rouge, is adjacent to the Mississippi River and includes a man-made lake. The swamp is polluted by a variety of surrounding industrial operations, including an abandoned hazardous waste disposal facility.
- Bayou Trepagnier (designated a "natural and scenic stream" within the Natural and Scenic River Act of 1970) serves as the receiving stream for large volumes of water used in many oil processing activities. The 3 1/2-mile bayou flows in a northeasterly direction through a cypress-tupelo swamp and was selected for study based upon its known contamination by metals, oil and grease.
- Tunica Swamp is a relatively pristine body of water located approximately 20 miles up river from Devils Swamp near St. Francisville. It is the control site for Devils Swamp related studies.
- Bayou Traverse intersects Bayou LaBranche approximately two and half miles from its termination into Lake Pontchartrain. Bayou Traverse is approximately two miles east of Bayou Trepagnier in a relatively pristine marsh with no direct anthropogenic influences to its surrounding environment. Bayou Traverse runs due east into the La Branche wetlands for approximately two and half miles. The control point is at the confluence of Bayou Traverse and Bayou La Branche.

Other sampling sites include: Lake Pontchartrain and Stinking Bayou.

### Methods of Communication

Since this project involves numerous investigators at the two institutions, it is imperative to have well-organized modes of communication among researchers and project administrators. This is facilitated through meetings that rotate among the three participating campuses (Xavier, Tulane uptown and Tulane downtown). All investigators are encouraged to attend. At each meeting, investigators from one or two selected projects present their current research findings. These presentations foster interactions among participants across projects and have resulted in the development of new, interdisciplinary research teams.

Additionally, a poster session was held in December, 1995. Investigators from each project presented their research and answered questions from reviewers, DOE administrators, faculty, students and representatives from state and federal regulatory agencies. This venue provides investigators with important feedback related to their work.

Communications are being improved with the use of electronic mail. Although not all investigators have e-mail capabilities, one of the objectives of this project is to provide all investigators with this essential technology.

Oak Ridge National Laboratory (ORNL) is working closely with project investigators by providing research support and expertise in a variety of areas. Interactions with ORNL have included visits to Oak Ridge by Tulane/Xavier researchers and administrators and student interns. ORNL scientists, particularly, Dr. Michael Maskarinec, have been instrumental in designing the QA/QC protocols related to the sampling and analytical components of the project.

### Technical Highlights

The Biological Fate, and Transport cluster has completed a detailed report concerning the history and present status of heavy metals concentrations in soil collections from Devils Swamp and has prepared an encompassing QA/QC document. Studies to evaluate the physiological and biochemical effects of cadmium in the red swamp crayfish provided the first evidence in a crustacean that cadmium exposure results in hyperglycemia. Preliminary analysis of cypress cores from Bayou Trepagnier for heavy metals suggests cypress is a good indicator of long-term contamination patterns.

The Biomarkers of Exposure and Ecotoxicity cluster has identified and published specific biomarkers of exposure and ecotoxicity in wild fish and frogs exposed to multiple pollutants. Quantifiable histopathological biomarkers in the liver of spotted gar fish from Devils Swamp has been developed and is being verified by studies at Bayou Trepagnier. The black bullhead catfish has been identified as a sentinel species for evaluation of the impact of contaminated sediments. Histopathological biomarkers in the gills of smallmouth and largemouth buffalo fish were identified as indicators of general fish health in contaminated ecosystems. Biomarkers of neuro, immuno and developmental toxicity were identified in frogs exposed to contaminants from Devils Swamp and Bayou Trepagnier. Field and laboratory studies indicate that frogs are excellent sentinel organisms for immune suppression.

The Bioremediation cluster has performed anaerobic serum bottle studies to determine the toxicity of carbon tetrachloride in samples taken from Devils Swamp, Bayou St. John and Lake Pontchartrain. Work has been completed in the development of a method of encapsulating fungi in alginate for delivery to field studies for bioremediation.

The Natural and Active Chemical Remediation cluster has successfully synthesized a new polymer material designed to remove heavy metal ions from waste water. Researchers also investigated the role of sediment acid volatile sulfides (AVS) in limiting the concentration of heavy metals in the water column. Preliminary data suggest that the Barataria estuary, in contrast to Bayou Trepagnier, has a limited capacity to absorb heavy metals via exchange reactions with sediment AVS. AVS does not appear to be ubiquitous in southern Louisiana waterbodies.

The Expert Geographical Information System cluster has created an electronic database of spatial, biotic and abiotic information collected during the course of this project and historically from Devils Swamp and Bayou Trepagnier. A user friendly interface was designed to support and facilitate the decision process for environmental impact assessment. Finally, several thematic maps were generated for the study sites.

The Assessment of Mechanisms of Metal-Induced Reproductive Toxicity Cluster has conducted both laboratory and field studies. Field studies focused on evaluation of water quality, and evaluation of sediments for metals (Lead, Chromium), oil and grease and polyaromatic hydrocarbons in Bayous Trepagnier and Traverse. Evaluation of aquatic species collected in the field study sites demonstrated only very low levels of bioaccumulation of Lead and Chromium. Laboratory studies focused on bioaccumulation of Lead and Chromium in crayfish. These studies demonstrated that crayfish bioaccumulate Lead and Chromium in a time- and dose-dependent manner making them useful as biomarkers for environmental contamination by metals while demonstrating no effects on their reproductive capacity.

The Pore-Level Flow of Microorganisms cluster integrated experimental and computational models of pore-level behavior of microorganisms. Studies included the detailed analysis of convection and diffusion of a contaminant within the pores, and the convection and chemotactic responses of swimming microorganisms to the local contaminant concentration.

The Sensitive Rapid On-Site Immunoassay cluster has shown that monoclonal antibodies to specific metal-chelate complexes may be obtained that permit the quantifications of Indium and Cadmium. This group is working on the development of immunoassays for additional metals as well as the refinement of utilizing this technique to perform quick, cost-effective assessments of metal contamination at field sites.

The Collaborative research with IREP and CREM project is the result of a collaboration between Tulane University personnel and two Institutes in the Republic of Belarus. The project is studying the transport and fate of radionuclides in the Iput River basin including modeling the movement of contaminants.

The Enhancement of Environmental Education cluster is developing a comprehensive education program aimed at producing graduates who can successfully carry out DOE's mission of environmental restoration and waste management. Xavier introduced an Environmental Studies minor and an Environmental Science track within the science disciplines. A B.S. degree in Environmental Engineering has been implemented at Tulane. In addition to the "greening" of several existing courses, new environment-related courses have been added to the curriculum at both universities.

In addition to research cluster activities, initiation project investigators carried out research involving the following: 1) the preparation of a cyclodextrin polymer for pollution-remediation. A variety of methods were explored from this preparation; 2) the use of cytochrome P450 102 isozymes with novel designed catalytic activities as tools to enhance the natural ability of bacteria to remediate recalcitrant organic pollutants in the environment, and the development of genetic tools that will allow us to express P450 102 isozymes that express pyrene hydroxylase and tetrachloethylene dechlorinase activities to be expressed in the bacterium *Caulobacter crescentus* so the investigators can begin to test, develop and optimize their practical use in bioremediation; and 3) to develop new analytical methodology and provide analytical support at the Xavier campus.

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## Administrative Activities

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### Objectives and Goals:

To provide the necessary administrative support to assure that the scientific and educational goals of the project are obtained and to assure that all Department of Energy reporting requirements and requests are fulfilled. The grant reporting is divided into three aspects: Collaborative Cluster projects, Initiation projects and Education projects.

A cluster project is one or more closely related collaborative, multidisciplinary research projects in which a group of investigators employs a synergistic approach to the solution of problems in the same general area of research.

An initiation project typically involves a single investigator. The purpose of the project is to undertake pilot work, lasting no more than one year, which will lead to the successful submission of an externally-funded proposal or the development of a collaborative cluster project.

The education projects are designed to develop courses with emphasis on environmental studies and/or to train students in areas of environmental research.

### Accomplishments:

Administrative personnel interfaced with investigators and DOE personnel regarding the variance between funds approved by DOE and those actually obligated for '94-'95 funding.

E-mail/Internet accessibility for all the project's Principal Investigators has been an objective of this project. This summer investigators were contacted and funds were provided to get 16 additional investigators on-line.

In late July, Dr. JoAnne Jackson, a SAIC consultant, conducted a site visit to assess the on-going research and to assist in better integrating this work into the Office of Technology and Development's plume focus area. Dr. Jackson visited for 3 days. She met with all of the cluster investigators, project directors and the director of the Center for Bioenvironmental Research. Additionally, she toured the Bayou Trepagnier site.

In August, the grant continuation application, project period ending 12-96 budget was prepared and submitted to DOE.

An internal (Tulane/Xavier) request for proposals (RFP) to complete work during the next year of the project was issued in September. Forty-two (42) proposals were received in response to the RFP.

Selected and convened a panel of experts (December 5-6, 1995) to review the proposals and make funding recommendations. (See Appendix A)

The administrative staff interfaced with Oak Ridge National Laboratory regarding QA/QC

issues related to sampling and analysis for the project.

Held numerous phone conversations with DOE representatives regarding funding allocations procedure changes as a result of the delays in the Federal budget and appropriations process this year.

Meetings Held/Attended:

In addition to two project wide meetings, investigators working on data from Devils Swamp and Bayou Trepagnier sites met with GIS investigators to implement a plan for investigators to feed data to the GIS group.

Senior Program Coordinator traveled to Washington D.C. in February to meet with procurement officials regarding equipment purchases for the '94-'95 period as well as other procedural issues.

Verna Lee, Senior Program Coordinator, accompanied four students (2 Tulane and 2 Xavier) to the WERC conference in Las Cruces, New Mexico, April 18-20. As a result of this endeavor the students will form a joint Tulane/Xavier team to enter in the student competition at next year's conference.

Planned and held a project wide retreat on June 20-21. (See Appendix C). The retreat was very well attended with 115 registered participants. In addition to faculty, staff and student participants, Drs. Norman Cutshall and Michael Maskarinac, Oak Ridge National Laboratory, attended the retreat.

In September, Dr. Charles Ide, Project Director, and Verna Lee attended the "Resources Through Technology Conference '95 Solutions to Remediation in Butte, Montana." Verna Lee attended the Weapons Complex Waste Management & Cleanup conference in Florida.

In September, Dr. Charles Ide and Verna Lee met with Jeffrey Walker, the new DOE Program Manager, to discuss how the on-going research in this grant relates to the reorganization of DOE. And more specifically, how this administration should direct this project to meet DOE's objectives and priorities.

Senior Program Coordinator attended Environmental Computing & Information Technology Conference and Exposition in Washington, D.C. on October 11-13, 1995.

CBR Activities:

Developed web page under the Center for Bioenvironmental Research Home Page on the World Wide Web. The CBR is the administrative research center through which the EM grant is administered. The purpose of the Home Page is to provide information on CBR activities and highlight valuable research conducted through such programs as the Hazardous Materials in Aquatic Environments of the Mississippi River Basin project. The information on this Home Page is now available to people all over the world who have Web access.

A two-day meeting was convened to highlight the research and other activities of the CBR to the Tulane University President's Council. This Council serves as a primary advisory group to the University's President, and assists promotion of the University through its community liaison role.

A part-time computing consultant has been hired to develop graphics for CBR presentations, and develop a database to track all CBR-sponsored research. As a follow up to the June retreat, the analytical and sampling processes were evaluated and Dr. Michael Maskarinec has been scheduled to make a consulting visit in October. (See Appendix C for a summary report of the retreat activities.)

### **Publications and Presentations:**

The grant project directors and staff participated in the July 11 DOE/DOD exhibition held at the Hart Senate Building in Washington, DC.

The Project Director, Project Investigators and staff made a presentation at the Innovative Environmental Technologies Demonstration for DOE in Washington, D.C., November 1-2, 1995.

The project's annual poster session was held on December 4, 1995. Sixty-five (65) posters were presented by project participants. (See Appendix D)

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## Collaborative Cluster Projects

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### **Biotic and Abiotic Studies on the Biological Fate, Transport and Ecotoxicity of Toxic and Hazardous Waste in the Mississippi River Basin**

A. Abdelghani, Y. Pramar, T. Mandal

#### **Objectives and Goals:**

The goal of this project is to assess the levels of xenobiotics in Devils Swamp and to study their biological fate, transport and ecotoxicity and ultimately to estimate their potential toxicity to humans.

To accomplish this the project will: 1) assess the acute toxicity of individual xenobiotics and toxicity of organic compound (hexachlorobutadiene (HCB) and hexachlorobenzene (HCBDD)) on juvenile crayfish; 2) determine the biotic influence of temperature, salinity, pH, oxidation-reduction potential, and sediment composition on the migration of xenobiotics; and 3) develop a pharmacokinetics model for xenobiotic absorption and storage, distribution and excretion by fish and crayfish.

#### **Accomplishments:**

All acute toxicity testing on crayfish have been completed.

All water, fish, sediment and vegetation samples collected by Dr. Bart are in the process of being analyzed for two organic compounds (Hexachlorobenzene and Hexachlorobutadiene). Analysis for inorganics: Arsenic, Cadmium, Chromium, Lead, Nickel, Mercury, Copper and Iron have been completed. A partial list of results was submitted to the Biomarkers, Dr. Bart and the GIS clusters (table following). The remaining results are awaiting the completion of the QA/QC procedures.

Screening studies for long term exposure have been completed and bioaccumulation studies on juvenile crayfish is in progress.

**Abdelghani**

Environmental Health Science Laboratories  
School of Public Health and Tropical  
Medicine

Summary of Number of Inorganic Analysis  
Of Samples Collected from Devils' Swamp In 1995

Type	# As	# Cd	# Cr	# Cu	# Fe	# Hg	# Ni	# Pb	Total
fish	510	510	510	111	121	210	510	510	2992
water	19	19	19	12	12	19	19	19	138
sediment	20	20	20	12	12	20	20	20	144
vegetation	12	12	12	12	0	12	12	12	84
crawfish	7	7	7	7	7	7	7	7	56
<b>Total</b>	568	568	568	154	152	268	568	568	3414

Summary of Number of Organic Analysis  
Of Samples Collected from Devils, Swamp In 1995

Type of sample	# of samples received	# of HCB analysis	# of HCBd analysis	Total # of analysis
Fish	510	169	169	238
water	19	19	19	38
sediment	20	20	20	40
<b>Total</b>	<b>549</b>	<b>208</b>	<b>208</b>	<b>416</b>

**Grand Total Number of Organic and Inorganic Analysis = 3830**

## **Publications and Presentations:**

Abdelghani, A.A., Prammar, Y.V., Mandal, T.K., Tchouwou, P.B., and Heyer, L. "Levels and Toxicities of selected Inorganic and Organic Contaminants in a Swamp Environment." *Journal of Environmental Science and Health*, B30(5): 717-731, 1995.

Abdelghani, A.A., et al, "Toxicity of Selected Inorganic and Organic Contaminants to Microorganisms Isolated from Devils Swamp". Submitted to the *Journal of Environmental Toxicology and Water Quality*.

Hartley, W.R., Thiyagarajan, A., Bart, H., Abdelghani, A.A., Anderson, M., Broxon, M., Major, S. "Toxicologic Pathology of the Black Bullhead (*Amerus melas*) from a Contaminated Mississippi River Basin Swamp", Submitted to *Ecotoxicology*

Tchounwou, P.B., Heyer, L., Abdelghani, A.A., Prammar, Y.V., Mandal, T.K., "The Recovery and Analysis of Hexachlorobenzene and Hexachlorobutadiene in Environmental Samples: A Critical Comparison of Two Extraction Techniques". Presented orally at the Thirty-Six DOE-ORNL Conference on Analytical Chemistry in energy Technology, Oct. 10-12, 1995, Gatlinburg, Tennessee.

Abdelghani, A.A., Cooper, B., Acuna, Askar, Pikes and Gaskin, D. "Acute Toxicity of Arsenic, Cadmium, and Mercury to Crayfish and Bluegill Sunfish". Presented at the Tulane/Xavier poster session, 1994.

Heyer, L., Abdelghani, A.A., Prammar, Y.V., Mandal, T.K., and Tchounwou, P.B. "Acute Toxicity of Hexachlorobenzene and Hexachlorobutadiene to Juvenile Crayfish". Presented at the Tulane/Xavier DOE poster session on Dec. 4, 1995.

Regens, J., Hodges, D., Watanabe, K., Hartley, W.R., Abdelghani, A.A., and Thiyagarajah, "A Modeling Bioaccumulation of Pollutant Loading in Aquatic Ecosystem".

## **Assessment of Mechanisms of Metal-Induced Reproductive Toxicity in Aquatic Species as a Biomarker of Exposure**

M. Anderson, W. George, J. Preslan, K. Bundy  
S. Sikka, K. Agrawal, B. Kamath

### **Objectives and Goals:**

- Identification and quantitation of heavy metals and petroleum products (polyaromatic hydrocarbons) present in Bayou Trepagnier relative to remote control sites in the La Branche Wetlands.
- Assessment of the uptake and bioaccumulation of metals and organic contaminants of interest in aquatic species in the field and laboratory.
- Establishment and use of polarographic methods for use in metal speciation studies to identify specific chemical forms present in sediments, waters and organisms.
- Evaluation of these contaminants on reproductive function of aquatic species as potential biomarkers of exposure.

- Periodic evaluation of sediment and water parameters (nutrients, sediment oxygen demand, productivity) to study seasonal changes in Bayou Trepagnier and control sites.
- Measurement of metallothionein and its mRNA in catfish hepatocytes as a biomarker of metal exposure.

## **Accomplishments:**

### Field Studies:

#### Analyses of Waters and Sediments in Bayou Trepagnier and Control Sites

Water Quality Parameters: Water quality parameters have been monitored at five study sites in the LaBranche Wetlands periodically over the course of the past two years. The values measured during 1994 represent conditions existing during the time that the Shell Manufacturing Complex was discharging effluent into Bayou Trepagnier. Those taken in 1995 (after February) reflect conditions in the wetlands since the diversion of the Shell discharge to the Mississippi River. Conditions are noticeably different between the two time periods.

The pH (acidity) of the water declined, the average value for 1994 being pH 8.0 and that for 1995 pH 7.0.

In 1994, conductivity of the water consistently decreased in the downstream direction of Bayou Trepagnier, with average values progressing from 4.2 mS at the headwaters, to 3.5mS downstream. Conductivity in Lake Pontchartrain was 3.15 mS and the value at Bayou Traverse (control site) was the lowest at 2.93 mS. This pattern is consistent with the influx into the headwaters of the Bayou of discharge water containing a high level of dissolved solids which is then diluted as it flows down the waterway by rainwater runoff and Lake water introduced by the tides. In contrast to this situation, conductivity values were lower and more uniform throughout the wetlands waterways during 1995. The 1995 values averaged 1.7 to 2.1 mS for all sites monitored.

The levels of nitrogen-based nutrients (nitrates, nitrites, and ammonia) are also different in 1995 from the levels observed in 1994. The changes which occurred in the concentrations of these nutrients have made Bayou Trepagnier more similar to Lake Pontchartrain and Bayou Traverse.

Ammonia and nitrites are forms of nitrogen which are indicative of the less desirable reducing, or low oxygen conditions in an environment. In high concentrations, they are harmful to aquatic organisms. Levels of these two nutrients were extremely high in Bayou Trepagnier in March, 1994 (greater than 1700 and 1300 ug-N/L for ammonia and nitrites respectively), indicative that perhaps sewerage was being discharged into the Bayou. Even when these March values are not included in the averages, the ammonia concentration in Bayou Trepagnier decreased 3-fold (670 to 206 ug-N/L) between 1994 and 1995. However, the average 1995 value for Bayou Trepagnier was still 3 times higher than the average ammonia concentrations in Bayou Traverse and in Lake Pontchartrain waters ( 70 ug-N/L) during the two years. Nitrite concentration decreased 5-fold between 1994 and 1995, bringing it to about the same level as that found in Bayou Traverse and Lake Pontchartrain (15 ug-N/L) except for occasional high levels which may occur during periods of very low water.

Correspondingly, the concentration of nitrates in Bayou Trepagnier waters increased about 3-fold (122 to 406 ug-N/L) between 1994 and 1995, indicating that conditions were suitable for the conversion of nitrogen to a more oxidized form. The concentration of nitrates in Bayou Trepagnier and Lake Pontchartrain also increased slightly between 1994 and 1995 (206 to 344 ug-N/L). Since high nitrate levels can stimulate aquatic plant growth, two general observations about the study area may be related to the changing nitrate levels. First, an algae bloom which occurred in Lake Pontchartrain (but not in the LaBranche wetlands) during early 1995 was attributed to increased nitrate levels introduced into the Lake by an influx of Mississippi River through the nearby Bonnet Carre Spillway. The higher level of nitrates in the LaBranche wetlands during 1995 may in part be a reflection of this situation. Secondly, in 1994, when nitrate levels were lower, Bayou Trepagnier had a very sparse distribution of aquatic plants. In 1995, the entire length of the Bayou was thickly carpeted with the floating plant *Lemna*, duckweed.

Phosphate concentrations in Bayou Trepagnier decreased 5-fold (1067 to 221 ug P-PO<sub>4</sub>/L) between 1994 and 1995 while phosphates in Bayou Traverse and Lake Pontchartrain decreased only slightly (294 to 179 ug P-PO<sub>4</sub>/L).

Thus, as indicated by the forms of the nitrogenous nutrients and the concentration of phosphates present in Bayou waters, the 1995 conditions in Bayou Trepagnier are improved over what they were in 1994.

Observed water temperatures during the two year study ranged from 7 to 33°C, with the variability in temperatures on any one day varying over a range of 2°C between the five sites. Alkalinity ranged from 68 to 121 ppm CaCO<sub>3</sub> during 1995. Water hardness was characterized as hard to very hard, with values of 244 to 326 ppm CaCO<sub>3</sub>.

Oxygen levels were high in the colder months, 6 to 8 mg O<sub>2</sub>/L, and were generally below 4 mg O<sub>2</sub>/L during the hottest months. The oxygen concentration at one heavily contaminated site fell below 1 mg O<sub>2</sub>/L during July and August and was insufficient to support aquatic life other than fish (like gar) capable of gulping air.

Pipe Study: Evaluation of *in situ* experiments in which 6 foot long pipes were driven into the beds of bayous to isolate areas of sediment and the water column above for evaluation under the imposed "catastrophic" conditions of reduced flow of water, reduced exchange of gases between air and water, and reduced light. Experiments were conducted at a highly contaminated site in Bayou Trepagnier and at the control site in Bayou Traverse in March, 1994 and September, 1995.

The most dramatic difference was that dissolved oxygen in the water column was depleted within 24 hours at the contaminated site in Bayou Trepagnier in September (27°C), whereas it took 5 to 6 days for oxygen to be depleted during the cold weather of March (17°C) and at the control site in Bayou Traverse in September. This again emphasizes that one of the greatest threats to aquatic life in Bayou Trepagnier is the rapidity with which low oxygen conditions can appear during the warm summer months.

Other conditions also deteriorated much more quickly at the contaminated Bayou Trepagnier site than they did at the control Bayou Traverse site. Again, the deterioration was more rapid in the warm month than in the colder ones. In the water column, noxious ammonia accumulated 2 to 20 times faster, phosphate accumulated 3 to 80 times faster, silicates (from dying cells) accumulated more than 100 times faster, and nitrates were depleted 2 to 25 times faster at the Bayou Trepagnier site than at the Bayou Traverse site. Thus, this study demonstrates that the contaminated Bayou Trepagnier environment does

not have the resiliency to withstand severe conditions which the more natural Bayou Traverse is capable.

Lead did not accumulate in the water column and only a trace of Chromium appeared in the water column during the course of the 6 to 7 day experiments. This indicates that metals are strongly bound to the sediments and are not easily converted to their dissolved and more bioavailable form.

Oil and Grease: An assessment of oil and grease amounts in the sediments from core samples of sites in Bayou Trepagnier, Bayou Traverse, and Lake Pontchartrain was initiated in early 1995. The cores were sampled at several depths up to 30 cm. The results from these studies showed that high concentrations (ranging up to 0.5 to 1% dry weight) of these contaminants are present in the upstream sediments of Bayou Trepagnier. The highest oil and grease levels in the cores were typically found between 5 and 10 cm below the surface, revealing that they are being covered by sedimentation. The control site in Bayou Traverse did not demonstrate any such accumulation. After 30 years, oil and grease are still major contaminants in the sediments of Bayou Trepagnier.

Metal Analyses of Sediment Cores: Analyses for Lead and Chromium were conducted on a series of sediment core samples from sites in Bayou Trepagnier, Bayou LaBranche, Bayou Traverse, and Lake Pontchartrain. Included among the samples analyzed were several deep (>60 cm) cores, taken at five study sites. Sediment samples were collected from along the length of these cores at 10 cm intervals, as well as from the surface. Concentrations of Lead in the sediments of the upstream sites of Bayou Trepagnier were found to be extremely high (up to 2% dry weight) in many cases. In the cores from the most contaminated sites, Lead concentrations were highest in the sediments 10-20 cm below the surface. Chromium concentrations, although significant, are considerably lower than those found for Lead. The patterns of accumulation (i.e., in location and core depth) found for Chromium closely parallel those observed for Lead. Both Lake Pontchartrain and Bayou Traverse (control sites) show very little accumulation for both metals.

Polyaromatic Hydrocarbons (PAH) Analyses of Sediment Cores: The protocol for the analysis of 18 PAHs was developed in September, 1995, and samples were collected from the identical sediment cores as were used in the above metal analyses. Extraction and preparation of over 100 samples has been completed, and preliminary results show significant concentrations of certain PAH compounds in the surface sediments of those sites found already to contain high metal concentrations. The method, using gas chromatography/mass spectrometry (GC/MS, Xavier University), has been shown to provide a high degree of differentiation among the various PAH compounds, with sensitivity as low as 100 ng/g (ppb).

#### Collection and Analyses of Tissues from Aquatic Species

Specimens for evaluation of metal and/or PAH bioaccumulation were collected or exposed at an upstream site in Bayou Trepagnier, which is heavily contaminated with metals and hydrocarbons. This is a site which this group has evaluated and continues to monitor as well as the control site in Bayou Traverse.

Crayfish: Red swamp crayfish were placed in cages and exposed to the sediments at the selected study site for a period of one week to evaluate the bioaccumulation of metals in various tissues of the crayfish. This work was done in collaboration with Dr. Milton Fingerman. Tissues were collected from crayfish at time zero for determination of metal concentrations at the beginning of the exposure-period. Of the 5 metals (Lead, Chromium, Arsenic, Cadmium, and Copper) measured, only Lead and Chromium bioaccumulated in

any significant amounts. Lead and Chromium are two metals which contaminate the area. Lead accumulated in significant amounts in the gills (205 ppb) and hepatopancreas (104 ppb) in comparison to time 0, but the concentrations were still below the FDA limit for consumption (300 ppb). The other tissues (muscle and exoskeleton) did not exhibit any significant accumulation of Lead. Significant accumulation of Chromium was seen only in the gills (74 ppb).

Crayfish serve as useful biomarkers of exposure for Lead and Chromium since they do bioaccumulate in crayfish tissues and this accumulation is both time- and concentration-dependent as demonstrated in the laboratory studies described below. Based upon the metal concentrations in the sediment at this site (Lead, 400-6,000 ppm; Chromium, 43-63 ppm) continued exposure in Bayou Trepagnier should result in higher levels of bioaccumulation which would exceed the safe FDA limits for consumption and pose a threat to the food chain.

Fish: The dominant species of the fish collected in Bayou Trepagnier and Bayou Traverse (control site) is the spotted gar. At the heavily contaminated site which was also used for the crayfish studies, spotted gar is the only species which has been caught. Gar are considered to be "trash" fish and can breath air from the surface of the water. The water in Bayou Trepagnier has a low dissolved oxygen concentration except during the winter months. Metal analyses for Chromium and Lead in these fish did not reveal any toxic levels of metal bioaccumulation in the gills, liver, muscle or gonads. Currently tissues taken from fish collected in Bayous Trepagnier and Traverse are being analyzed for PAHs.

The difference in metal bioaccumulation in fish and crayfish is meaningful. Crayfish are bottom feeders and are in direct contact with the sediments, making evaluation of their tissues useful as biomarkers of metal contamination of sediments. On the other hand, only small amounts of metals (Lead and Chromium) are released into the water column which renders pelagic fish species less valuable as biomodels for evaluation of metal contamination of wetland sites. In addition, many fish species, like the gar, are migratory, and therefore, are not subjected to constant exposure.

#### Installation of Tide Gauge in Bayou Trepagnier

Since the LaBranche Wetlands, including Bayou Trepagnier, are influenced by tidal changes and seasonal wind patterns, it is important that water level changes be monitored in Bayou Trepagnier to correlate these with any alterations in parameters under investigation. The gauge was installed in January, 1996. It continuously monitors water levels over a 32 day period.

#### Laboratory Studies:

##### Chromium Exposure Study

Both female and male red swamp crayfish were exposed to three concentrations of Chromium (0.3, 3.0 and 30 ppb) as hexavalent Chromium. Crayfish tissues (gills, hepatopancreas, exoskeleton, muscle, gonads and hemolymph) were collected at time 0 (before exposure), weeks 2, 4 and 7 of exposure, and 3 weeks after completion of exposure (clearance study).

Bioaccumulation in Female Crayfish: Chromium bioaccumulation in all tissues is concentration-dependent and a time-response in accumulation is observed in the gills, hepatopancreas and exoskelton. Chromium clears slowly from all tissues studied. The observation that Chromium accumulation occurs readily but is only slowly removed implies

that some change in the chemical state of the Chromium may have occurred (reduction to the less toxic trivalent state). This reduction in a biological system has been associated with cellular damage and carcinogenesis. The fact that Chromium bioaccumulates and is not readily cleared from tissues makes it a threat to the food chain.

A similar study was previously conducted concerning Lead accumulation. It was found that the pattern of accumulation for the two metals is not the same. Lead accumulation is more rapid and attains higher levels of tissue-concentrations than Chromium. Lead clears faster from the tissues than Chromium. They are similar in that the hepatopancreas and the gills demonstrate a time- and dose-dependent accumulation of the metals. Field studies on the crayfish are in agreement with these laboratory studies. An integration of both laboratory and field studies is critical for a full ecotoxicological evaluation. While the mechanisms of metal accumulation appear to differ, Lead and Chromium accumulations in crayfish tissues are time- and concentration-dependent and thus serve as useful biomarkers of metal contamination of wetland sediments.

Speciation of Chromium: Analytical methodology was developed for analyzing trivalent and hexavalent Chromium content of gills, muscle and hepatopancreas of crayfish exposed to Chromium in the study described above to determine the oxidative state(s) of Chromium in the tissues. Analyses of the gills and muscle have been completed. Speciation studies of the hepatopancreas are still in progress. The results to date indicate that a very significant degree of valent state conversion from the more toxic hexavalent to the much less toxic trivalent form occurs in the gills. While the total Chromium content of the gills increases with both concentration and time, the amount of the more toxic hexavalent form seems limited to a plateau value which is independent of exposure concentration or time. This is a significant and unexpected result. The conversion of Chromium was not evident in the muscle tissue.

Histology: Histopathology of tissues taken from crayfish exposed to Lead or Chromium demonstrated the same type of tissue damage. Histological study of the gills, hepatopancreas and gonads demonstrated tissue damage only in the gills and hepatopancreas. Gill filaments demonstrated the presence of capsules which form within gill filaments to seal off foreign material. The hepatopancreas contained tubules which were disorganized and/or degenerating in response to metal toxicity. This agrees with the bioaccumulation data.

Metal effects on reproductive function: Both Lead and Chromium appear to have no effects on gonadal morphology, weight or function. Very low levels of metals were observed to bioaccumulate in the ovaries. The testes were too small for metal analyses; however, sperm concentration was unaffected by metal treatment. The hemolymph of metal-exposed males demonstrated a significant decrease in superoxide dismutase (SOD), a free radical scavenging enzyme. SOD may prove to be a useful biomarker of metal exposure in crayfish.

It is a significant finding that relatively high concentrations of these metals do not impact on gonadal function of crayfish. This implies that this species may reproduce in metal contaminated environments and perpetuate the movement of metal in the food chain.

#### Metallothionein in catfish hepatocytes

Methods to measure metallothionein (MT) protein and MtmRNA in catfish hepatocytes were investigated, which included ELISA and a quantitative western blot assay.

### Analytical Support:

Analyses were provided by Dr. George's Toxicology Laboratory. Below is a summary of the analyses done to support the research described above:

- 3,103 tissues digested by microwave technique
- 2,304 tissues analyzed by atomic absorption spectrometry
- 923 wet chemistries
- 205 sediment specimens for oil and grease analyses
- 75 sediment specimens for analyses of polyaromatic hydrocarbons
- 16 water proficiencies for five metals

### Catfish Facility:

At Tulane University School of Medicine two circular, 230 gallon fish tanks have been installed. These tanks are designed as continuous flow through units with a Frigid Units Model D1-33, 3950 BTU water chiller-heater-aerator for each tank. This system provides a controlled environment for fish which will allow a continuous dosing exposure of either heavy metals or organics. The water entering and leaving the system is filtered to remove any contaminants in the water. This facility will be used in the future to study what happens when catfish are exposed to controlled levels of the contaminants under investigation.

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## **Hazardous Wastes in Aquatic Environments: Biological Uptake and Metabolism Studies**

J. Barber, A. Apblett, H. Ensley, M. Fingerman, M. Fink, M. Polito

### **Objectives and Goals**

The goals of this cluster were to study the uptake, accumulation, metabolism, toxicity and physiological effects of various environmentally-important contaminants, inorganic and organic, in several wetland species that are interrelated through food webs. Further, it was the goal of this project to investigate the potential for developing and linking chemical and biological methods of remediation so as to encapsulate bioaccumulated ions in stable wasteforms such as ceramics and/or zeolites.

### **Accomplishments:**

Phenol and the chlorinated phenols, as a group, have many commercial uses most of which, in one way or another involve biocidal activities (e.g. disinfectants, fungicides, bactericides, herbicides, etc.). In addition, chlorophenols are a major component of the effluent from paper pulp mills and are by-products from the chlorination of drinking water.

Thus, given the many uses of chlorophenols it is not surprising that they are prominent on lists of priority pollutants. In addition, given the manner in which they are used, they occur very commonly in aquatic ecosystems.

Because plants exist at the base of most food chains they experience the effects of toxic compounds sooner than do organisms that occupy higher trophic levels. Further, the ways in which plants deal with toxic compounds can have very significant effects, positive or negative, on organisms higher up the food chain. Thus, plants may modify the effects of a pollutant by metabolizing it to either a more or less toxic compound or by accumulating it to greater than original concentration. In any event, it is important to know and understand the responses of aquatic plants to chemical challenge so that the potential danger that those chemicals represent to an aquatic ecosystem can be assessed. It is also true that, depending on the nature of their response(s), aquatic plants may provide an *in situ* mechanism for remediation.

Members of the *Lemnaceae* are floating aquatic angiosperms of worldwide distribution. Because of its small size, simple morphology, genetic homogeneity and the ease with which it can be maintained and manipulated (in sterile culture) in the laboratory, duckweed provides an ideal model aquatic higher plant for toxicological studies. In addition, because duckweed is of considerable ecological importance and serves as the base for so many food webs, the information derived from laboratory studies can be readily extrapolated to ecotoxicological situations in the field.

For all of the above reasons, a comprehensive study of the toxicity and metabolism, by *Lemna gibba* (duckweed), of phenol and a series of chlorophenols was conducted. It has been demonstrated that the toxicities of the phenols with regard to vegetative reproduction in *L. gibba*, increased as the number of chlorine substituents in the phenol ring increased. Thus, phenol itself was relatively non-toxic (a concentration of approximately 1 mM was required to reduce the vegetative reproduction by 50%) whereas 4-chlorophenol was more toxic, 2,4-dichlorophenol more toxic yet, etc., up to pentachlorophenol which was approximately 1000-fold more toxic than phenol.

Using radiolabelled substrates it was shown that *Lemna*, during 10 - 14 day incubation periods, metabolized each of the chlorinated phenols, and phenol itself, to the corresponding glucosides. In doing so the plant produced a more polar, more water soluble metabolite that it could dispose of more readily by return to the aqueous growth medium. The metabolites were shown to be less toxic to the plants than were the parent molecules from which they were derived. Therefore, as far as the plants are concerned the problem of phenol and chlorophenol toxicity was solved - toxic molecules were conjugated to glucose molecules to produce less toxic glucosides. However, glucosylation may represent detoxification for the plant but the problem of chlorophenol toxicity remains, albeit in an altered form, since low pH or glucosidase activity cleaves the glycosidic link and returns the molecule to its two components, one of which (the chlorophenol) is as toxic as ever. Thus, it has been shown that phenylglucosides, fed to crayfish, are rapidly (within seconds) hydrolyzed by bacterial glucosidase enzymes, in the animal's gut, to glucose plus chlorophenol which is as toxic as if free chlorophenol had been fed to the crayfish in the first place.

The practical significance of the finding that *Lemna* metabolizes chlorophenols to phenylglucosides is that in doing so the plant masks the pollution problem - the chlorophenol ostensibly disappears. It is therefore, important for those who monitor pollution to be aware of the metabolites that are produced so as to know what compounds to monitor.

While *Lemna*, over short (up to 14 days) exposure periods converts chlorinated phenols to glucosides, over longer (> 3 weeks) periods is able to affect more permanent detoxification of the compounds by reductive dechlorination to the lower chlorinated phenols which, as shown earlier (see above), become progressively less toxic as chlorine atoms are removed from the phenol ring. This interesting and potentially valuable, reductive dechlorination appears to take place in a number of steps, the first of which is conjugation of the chlorinated phenol with glucose. Subsequent, presumably enzymatic, and sequential (seemingly random) removal of the chlorine atoms occurs. Experiments are ongoing to determine conclusively the precise sequence of events in this dechlorination process.

Plants have few alternatives in terms of their possible responses to inorganic pollution. They can exclude the toxic ion(s) or they can take them up and accumulate them often to concentrations that are considerably higher than in their aqueous growth medium. This latter alternative is clearly of interest to remediation efforts. While there are numerous toxic heavy metal ions upon which this study could have been based, the one which was chosen was Thorium, a weakly radioactive actinide that occurs at a number of DOE sites across the country as a result of uranium production and also in certain other situations such as the leachates from the gypsum piles resulting from industrial phosphate fertilizer production. The results, to date, have shown that duckweed, grown in media containing Thorium, at concentrations of 250 mM or less is able to deplete the medium to less than detectable levels, as measured by ICP spectroscopy, within a 7-day growth period. Methodologies to quantitatively determine Thorium in plant samples by ICP spectroscopy have been unreliable therefore techniques have been developed to do these analyses by XRF spectrometry which are proving to be more accurate and dependable. Thus, using XRF measurements it has been shown that the Thorium that disappears from the plant growth media has been accounted for in the plant tissues. These findings open up the possibility of using duckweed as a "bioharvester" of actinides that are present in aqueous media at low concentrations.

However, the ability of plants to bioaccumulate heavy metals also presents a potential danger in that the accumulated metal ions then became available, at greater than original concentrations, to organisms at higher trophic levels. For this reason a simple food chain study was performed. Duckweed were cultured in media containing Cadmium at a sub-lethal concentration (1.81 ppm). After 7 days of growth the media contained 0.032 ppm. The plants, containing the Cadmium they had accumulated, were harvested and fed to crayfish, *Procambarus clarkii*, over a 14-day period. Significant inhibition of acetylcholinesterase activity in the central nervous tissue of the crayfish that were fed Cadmium-grown duckweed was found. The lipid content decreased in the hepatopancreas and was translocated to the ovary of the Cadmium-fed crayfish. The Cadmium concentration in the hepatopancreas of the crayfish fed Cadmium-grown duckweed increased 26-fold and in muscle 7-fold.

This study has shown that the bioaccumulation of Cadmium is deleterious to *P. clarkii*, an ecologically- and economically-important crayfish in Louisiana. Since so many crayfish are sold in Louisiana for consumption by humans it is imperative that the habitats of this commercially-important species be protected and maintained free of pollution.

#### Crayfish: Physiological Effects of Contaminants

Field Studies: Field and laboratory studies were conducted in an effort to better understand the biological effects of pollutants by using the red swamp crayfish, *Procambarus clarkii*. This crayfish is a commercially valuable species in Louisiana. About 150 million pounds of this crayfish are annually harvested and consumed by humans in this state. This

crayfish is also a good indicator organism for monitoring the ecological consequences of pollutants.

A field study was carried out with caged red swamp crayfish to determine the impact of contaminants in Bayou Trepagnier. The crayfish were kept in the bayou for a week. The pH of the gastric juice was determined on Day 0 and after seven days of exposure in the bayou. Similarly, hepatopancreata were fixed for histological study on Day 0 and after 7 days of exposure.

During the exposure period the pH of the gastric juice increased significantly, becoming less acidic. This indicates there was damage to the hepatopancreatic cells which are responsible for the synthesis and release of the digestive juice. Earlier studies in our laboratory showed that exposure of the crayfish to heavy metal ions resulted in an increase in the pH of the gastric juice also and a decrease in the amylase enzyme activity.

The hepatopancreas of the crayfish, an organ comparable to the liver of humans, exhibited extensive cellular damage. Degenerative changes in the hepatopancreatic cells were apparent in the hepatopancreata removed from the crayfish that had been in Bayou Trepagnier for seven days. This observation helps explain the above mentioned increase in the pH of the gastric juice of crayfish exposed in the laboratory and in the field. Thus, the pH of the gastric juice of crayfish can be used as a potential biomarker in monitoring ecological impact.

Laboratory Studies: A study was conducted in the laboratory to determine the effect of sublethal concentrations of Cadmium on ovarian maturation in the crayfish. Ovarian maturation in the crayfish is controlled by a neurotransmitter, 5-hydroxytryptamine (5-HT), whose role is to trigger release of the gonad stimulating hormone from the brain and thoracic ganglia. A group of female crayfish was sacrificed on Day 0 to determine the ovarian index. Four other groups were established. One group received injections of crayfish saline. The second group received injections of 0.1 µg Cadmium/gm body weight. The third group received injections of 15 µg 5-HT/gm body weight, and the fourth group received injections of a combination of Cadmium and 5-HT. After 21 days the crayfish in all of the groups were sacrificed and the mean ovarian index was determined for each group.

The Cadmium injection resulted in a significant reduction of ovarian index as compared with the saline injected group, whereas 5-HT increased the rate of ovarian maturation. The combination of 5-HT and Cadmium also inhibited ovarian growth but to a lesser extent than did Cadmium alone. *In vitro* studies are in progress to further elucidate the effect of Cadmium on the reproductive hormones.

#### Development of Ion-Exchange Materials for Immobilization of Toxic Metals

The problem with the application of constructed wetlands (and the like) to heavy metal remediation is that the generated biomass necessarily contains high concentrations of heavy metals. These will be released back to the environment upon decomposition of the plants. Therefore, methodology must be devised for the removal and/or immobilization of the toxic metals. Some of those metals (e.g. Chromium and cobalt) may be of significant commercial value and "critical" in the sense that the U.S. is dependent on foreign supplies (Clark, J.P., Field, F.R., Busch, J.V., King, T.B., Poggiala, B., and Rothman, E.P. How critical are critical materials? Technology Review: August, 1995). It would be economically sound to recover those metals and, in doing so, prevent further deterioration of the environment. The plants provide the first step in this process by taking up and concentrating the metals. What is then required is a means by which the toxic metal ions

may be separated from the environmentally benign ones. Preferably, the metals should be concentrated to the point where they may be economically disposed of or used as a feedstock in conventional smelting operations.

Ion-exchange materials provide an ideal method for removing and concentrating hazardous metals from aqueous solution. This is particularly true when the toxic ions bind irreversibly to the ion exchanger so that it may be "loaded" to its theoretical limit. This may be accomplished by using either sulfur-containing exchange sites that will bind the heavy metals (which are extremely thiophilic) or phosphate or titanate moieties which could remove multivalent oxophilic metals from solution. Since, the intent is to ultimately incorporate the toxic metals into a ceramic wasteform, ion exchange materials that, once loaded, may be converted directly at low temperature to a ceramic material are being designed.

Phosphate and titanate ceramics have previously been demonstrated to be ideal waste forms for radioactive waste. Therefore, materials were sought which could absorb actinide metal ions and then be converted to a final ceramic repository for them. Ferric phosphate and layered titanate ion exchangers such as  $\text{Na}_2\text{Ti}_3\text{O}_7$  and  $\text{K}_2\text{Ti}_4\text{O}_7$  were investigated as candidates for remediation of actinide pollution. Sodium titanate was found to be an extremely efficient reagent for removal of actinides from solution they may also serve as an excellent barrier material (Apblett, in press). Preparative methods for  $\text{H}_2\text{Ti}_3\text{O}_7$ ,  $\text{H}_2\text{Ti}_4\text{O}_7$ ,  $(\text{NH}_4)_2\text{Ti}_3\text{O}_7$ , and  $(\text{NH}_4)_2\text{Ti}_4\text{O}_7$  which may also serve as effective ion exchangers have been developed as well.

The above ion-exchangers are not expected to be specific for heavy metals but may be used to remove all multivalent metal ions from solution. The separation of heavy metals from other metal ions so that they may be recycled or immobilized individually would be highly beneficial. Fortunately, their thiophilicity (affinity for sulfur) make the heavy metals chemically-distinct. This thiophilicity was used to develop an ion-exchanger that is specific for heavy metals. It was found that  $\text{TiS}_2$ , another layered material, can remove up to an equimolar amount of Cadmium from solution (10 mmol of  $\text{TiS}_2$  reduced the concentration of a 2250 ppm  $\text{Cd}^{2+}$  in 500 ml of water to a level below ICP detection limits).

Presumably, this occurs by solubilization of the oxophilic  $\text{TiO}_2$  + ions. If so, adjustment of the pH of a heavy-metal depleted wastestream would precipitate the oxophilic metals in a form ideal for preparation of a titanate ceramic wasteform. In such a manner, the heavy metals and the lighter metals may be immobilized in separate more-suitable wasteforms that closely resemble their natural repository in the earth's crust.

A similar system based on iron sulfide as an ion exchanger has also been investigated. Commercially-available ferrous sulfide as well as a synthetic high surface-area alumina supported material were used. These were fairly successful for remediating heavy metals, particularly at lower concentrations. It was found that, at higher concentrations of heavy metals, redissolution of the heavy metals as anionic complexes was a problem. Therefore, the porous metal sulfide,  $\text{KFeS}_2$  was synthesized and demonstrated to be particularly useful for remediation of heavy metals.

In conclusion, ion-exchange materials have been developed that are excellent for removal of either actinides or heavy metals from solution. These materials have high selectivity for the toxic ions and are therefore ideal for use in integrated chemical/biological remediation processes which use *L. gibba* as the primary harvester of the pollutant metal ions.

## Encapsulation of Metal Ions in Exchanged Zeolites by Chemical Modification.

One attractive approach for the treatment of contaminated wetland environments is the removal and immobilization of heavy metal and radioactive ions by exchange onto a solid support material. One class of materials which has been used successfully for these purposes are the synthetic and natural zeolites, aluminosilicates having varying ratios of Aluminum (Al) to Silicon (Si). The materials are particularly suitable for wastewater treatment because of (a) their abundance and low cost; (b) their ability to remove picogram quantities of toxic ions from high concentrations of other ions; and (c) their ready incorporation into cements and other solid supports for encapsulation. In addition, zeolites are also resistant to high levels of beta, gamma and neutron radiation which has led to their widespread use in the purification of high level radioactive wastes at a number of sites including the Handford Nuclear Lab, Savannah River and Sellafield in Great Britain.

Zeolite entrapment of toxic ions may be actively employed for *in situ* treatment of contaminated soils. Heavy metals and radionuclides removed from soils by a variety of techniques such as microemulsion flushing or electrokinetic extraction can be concentrated by ion exchange into zeolites prior to disposal. Zeolites used for this purpose may selectively extract a wide range of heavy metal ions including  $Pb^{2+}$ ,  $Hg^{2+}$ , and  $Cd^{2+}$  as well as radionuclides such as  $^{60}Co$ ,  $^{154}Eu$ ,  $^{106}Ru$  and  $^{125}Sb$ .

This research covered two aspects of metal recovery: reversibility and selectivity. The first aspect was concerned with the permanent encapsulation of the offending metal ion in the zeolite pores and channels. The second aspect involved the chemical modification of the zeolite structure such that high specificity for heavy metal ions in the presence of large excesses of innocuous cations was achieved.

The primary emphasis of the research was the development of chemical methods for sealing the zeolite channels and/or surfaces via specifically designed reaction chemistry. This approach involves the incorporation of small reactive molecules either onto the surface or into the cavities of the zeolite followed by their subsequent reaction to form molecular "shells" or "plugs", respectively. Appropriate zeolites must not only have ion exchange selectivity appropriate for sequestering of toxic metal ions but also have channels large enough to allow for small molecule intercalation.

The primary studies focused on the use of volatile compounds of silicon which would react directly with the zeolite to form silicate microstructures either on the surface or in the cavities of the zeolite. Since zeolites are themselves aluminosilicates, the resultant microstructures would closely resemble the overall chemical structure of the zeolite. It was anticipated that subsequent thermal annealing would result in a permanent incorporation of these microstructures into the zeolite by strong covalent bonds.

Typical studies with phenylsilane ( $PhSiH_3$ ) showed the feasibility of this approach. It was found that phenylsilane initially reacts with ZeoX-H at room temperature with loss of hydrogen to yield a chemically modified zeolite. Further heating results in the loss of the phenyl group as benzene and the formation of a silicate "plug".

The interaction of phenylsilane with protio-Zeolite X was examined by solid state  $^{29}Si$  and  $^{27}Al$  NMR spectroscopy, FT-IR, and mass spectrometry.

$^{29}Si$  MAS NMR spectroscopy provides a convenient handle on the interaction of organosilicon species with the zeolite framework since both the framework and the

chemisorbed species display distinctive chemical shifts. In addition, the framework silicons of the zeolite can often be distinguished from those of the chemisorbed phenylsilane by use of high-power proton decoupling which enhances those resonances where the silicon is directly bound to hydrogen.  $^{27}\text{Al}$  MAS NMR spectroscopy provides additional information relating to those sites in the zeolite framework which have been modified by the phenylsilane.

In addition, specular reflectance FT-IR indicates that at  $50^\circ\text{C}$ , the phenyl group and one or more Si-H bonds are retained in this structure. After brief heating of the modified zeolite to  $300^\circ\text{C}$ , the vibrational bands corresponding to the remaining Si-H bonds and the phenyl group were lost indicating the formation of a silicate plug. Mass spectrometry showed that the phenyl group was lost as benzene during the heating process. Further studies of the derivitization event by  $^{29}\text{Si}$  and  $^{27}\text{Al}$  CP-MAS revealed that the modification occurred within the channels and pores and not on the zeolite surface.

Treatment of zeolites which are loaded with  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$  resulted in encapsulation efficiencies of 80%. The encapsulation efficiencies, however, are temperature dependent. This is attributed to the greater channel blocking ability of the initially chemisorbed phenylsilane. At higher temperatures, when the benzene is lost, the remaining plug is smaller and allows metal ion to leach. This problem should be potentially overcome by allowing the phenylsilane to react with the metal loaded zeolites at higher temperatures such that multiple depositions can occur within the pores.

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## **Ecological Sentinels Of Aquatic Contamination In The Lower Mississippi River System**

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### **Sub-Project I: Community and Trophic Responses of Fishes to Aquatic Contamination**

#### **Objectives and Goals:**

Toxic substances have effects on aquatic organisms ranging from acute (lethal) to chronic. The effects of chronic exposure at the population or community level usually are not obvious. Recent studies emphasize the importance of integrating responses of organisms to environmental stresses at all levels of biological organization, and establishing linkages between suborganismal responses and responses at the levels of populations and communities. The purpose of this study is to determine the fate of environmental contaminants in fish from a lateral floodplain swamp in the lower Mississippi River system, and to assess the ecological risks contaminants pose for fish and other aquatic organisms in that system. The specific objectives were:

- Characterize the fish faunas of contaminated and reference swamps in terms of species richness and diversity, and assess what differences, if any, exist at this levels.
- Characterize diets of fish from the two swamps, and classify fish according to diet and feeding habitat (position in the water column).
- Measure levels of environmental and fish contamination in contaminated and reference swamps: Devils Swamp and Tunica respectively, and relate differences in fish contamination between swamps to ecological factors such as fish size, fish condition, diet, habitat and river stage.

#### **Background**

Devils Swamp, is a seasonally flooded swamp situated on the Mississippi River floodplain near Scotlandville, Louisiana. Devils Swamp is drained by Bayou Baton Rouge, a small stream that originates on natural high bluff on the east side of the river and flows through Devils Swamp before entering the Mississippi River. At low river stages much of Devils Swamp sits above the channel of the river. During these times, the natural pattern of water flow is southward through Devils Swamp and Bayou Baton Rouge to the Mississippi River. As the river rises it drowns the lower channel of Bayou Baton Rouge and backs up into Devils Swamp. At very high river stages, the river completely inundates Devils Swamp flooding all of the land between the river and its eastern bluff.

Devils Swamp Lake is a man-made "borrow pit" lake situated at the head of a man-made harbor (Baton Rouge Harbor) which borders the eastern side of the Devils Swamp. Water flowing through Devils Swamp from Bayou Baton Rouge passes through Devils Swamp Lake enroute to the Mississippi River. The lake retains water and fish year-round. At river stages above 20 feet, riverine fishes have access to Devils Swamp Lake through Devils Swamp. As river water recedes and water drains from the swamp, fish and other aquatic life from the swamp retreat to the lake. At river stages below 20 ft, fish and other aquatic organisms become isolated in the lake.

A hazardous waste site, "the Brooklawn site" of Petroleum Processors, Inc., situated on the bluff near the northern end of Devils Swamp, introduced contaminants into the swamp during the 1960's and 1970's. A second hazardous waste site, "the Scenic Highway Site" is situated along Bayou Baton Rouge upstream from the Brooklawn site. Upper portions of Devils Swamp are contaminated with volatile aromatic hydrocarbons, chlorinated hydrocarbons, polyaromatic hydrocarbons and heavy metals. Polychlorinated biphenols (PCB's) - believed to be linked to another hazardous-waste point source near Devils Swamp Lake - were measured at 50 times higher than typical background levels at the northeastern end of Devils Swamp Lake. Lower parts of Devils Swamp are believed to be affected as well because water from the hazardous waste sites, contaminated portions of Bayou Baton Rouge, and upper parts of the swamp drains south through the lower swamp en route to the Mississippi River. One of the aims of this project is to establish the extent to which biota from the lower swamp is affected by contaminants from the hazardous waste site.

The reference site, Tunica Swamp, is a more pristine swamp located approximately 30 miles up river from Devils Swamp near St. Francisville, Louisiana. Like Devils Swamp, Tunica Swamp receives seasonal laminar flow from Mississippi River floodwaters. Tunica Swamp is drained by a system of natural and man-made sloughs. Portions of the swamp under study are managed for wildlife and crop production.

### **Accomplishments:**

Samples of fish, bottom sediments, and water have been taken quarterly from fixed sites in Devils Swamp and Tunica Swamp over the past three years. Sediment and water samples and samples of fish tissue were submitted to the Tulane Environmental Health Laboratory and the Tulane Coordinated Instrumentation Facility for inorganic and organic contaminant analysis. Fish were taken by a combination of active and passive techniques including trammel nets, baited set lines, fishing and electrofishing. The principal means of fish sampling was electrofishing. Captured fish were identified, marked with a coded plastic tag, weighed and measured, and either returned to the laboratory or released alive for possible recapture. All fish removed from the field were transported on ice to the laboratories of investigators of the Biomarkers Cluster for necropsy. Gonads, gills, skin samples, spleen and pieces of liver were removed from the fish and fixed in 10% neutral buffered formalin for histopathological analysis. Brain and remaining liver were delivered to laboratories at Xavier University for neurotoxicology studies.

Stomachs and intestines were removed from selected fish species and fixed in 10% neutral buffered formalin. The GI tracts were later dissected and the contents identified to the lowest possible taxonomic level using a variety of invertebrate identification keys. Fish were categorized as either piscivore vs. invertivore or benthic vs. pelagic based on the percentage of these types of prey or the presence of prey from these habitats in their diets.

An index of relative condition was computed for all fish specimens as:  $\text{weight} / (a \times \text{Length}^b)$ , where  $b$  is a parameter expressing degree of body plumpness and  $a$  is a correction factor to bring the value of  $C$  close to unity. It was expected that this condition would be lower in Devils Swamp than Tunica Swamp if contaminants in Devils Swamp have harmful physiological effects. It was also expected that the condition effect in Devils Swamp Lake would be greatest at low river stage when fish were confined to the lake.

Quarterly sampling in Devils Swamp to date has produced a total of 607 fish representing 33 species. Comparable sampling efforts in Tunica Swamp has produced a total of 285 fish representing 25 species. Fish species encountered in both swamps are characteristic of oxbows, and sloughs associated with the Mississippi River floodplain. Difference in species richness between swamps are probably catch related. Fish diversity in the two swamps, as measured by an information theory-based index, was virtually identical (1.15 in Devils Swamp vs. 1.16 in Tunica Swamp). A greater number of species were caught in both swamps during high river stage, when the swamp was flooded and continuous with the Mississippi River, than during low river stage when fish were confined to permanent water bodies. The species present at low river stage were regarded as year-round residents. It was hypothesized that these species would concentrate contaminants to higher levels than transients which seasonally migrated between the river and the swamp.

A tag and release program was established in 1994 for assessing movements of fishes within Devils Swamp and between Devils Swamp and the river. In addition to tagging, fish are identified, weighed and measured prior to release. The tags bear an identifier number and the address of the Tulane University Museum of Natural History so that captures by fishermen may be reported. Recaptures would not only provide data on movements, but also growth and changes in health/condition since capture. Ninety seven (97) fish representing 14 species were released in Devils Swamp Lake in 1994. Thus far in 1995, 193 fish representing 13 species have been released also in Devils Swamp Lake. To date none of the released fish have been recaptured.

With the exception of Lead, sediment levels of inorganic contaminants in Devils Swamp and Tunica Swamp were similar, generally low parts per billion. Sediment concentrations of Lead in northern most portions of Devils Swamp were hundreds of parts per billion (ppb), decreasing to tens of ppb in southern portions of the swamp. Water samples from Devils Swamp and Tunica Swamp contained only trace amounts of Arsenic, Cadmium, Lead, and Nickel (low ppb). Sediment levels of HCB were tens of ppb in northern portions of Devils Swamp and generally declined to tenths of ppb near the outlet to the Mississippi River. Sediment levels of HCB were much higher but also declined riverward. HCB levels varied from low ppm nearest to the hazardous waste site, to thousandths of ppm in middle portions of the swamp, to tenths of ppb in the lower channel of Bayou Baton Rouge near the Mississippi River outlet. Water samples from Devils Swamp contained no measurable amounts of HCB and only trace amounts of HCB (tenths of parts per billion, ppb). No HCB and only trace levels of HCB (hundredths of ppb) were detected in Tunica Swamp.

Spotted gar from Devils Swamp had significantly higher tissue concentrations of Arsenic, Cadmium and Mercury than gar from Tunica Swamp. Largemouth bass from Devils Swamp had significantly higher levels of HCB and HCB than bass from Tunica Swamp. Common carp and freshwater drum from Devils Swamp had significantly higher concentrations of Nickel than carp and drum from Tunica Swamp. Shad from Devils Swamp had significantly higher Mercury concentrations than shad from Tunica Swamp, but shad from Tunica Swamp had significantly higher Cadmium concentrations. Warmouth from Tunica Swamp had significantly higher Cadmium and Nickel concentrations than warmouth from Devils Swamp. As a group, invertivores from Devils

Swamp had significantly higher tissue concentrations of Chromium and Mercury than invertivores from Tunica Swamp. Pelagic fish had significantly higher Chromium and Pb. Invertivores and pelagic fish from Tunica Swamp had significantly higher Cadmium than Devils Swamp fish. Largemouth bass collected at low river stage in Devils Swamp had significantly higher tissue concentrations of HCBD, Chromium and Mercury than bass collected at high river stage. Black crappie from low river stage in Devils Swamp had significantly higher levels of HCBD, HCB and mercury than fish taken at high river stage. Significant effects of river stage were also noted for warmouth and piscivores in general. Trends of increasing tissue levels of organic and inorganic contaminants with increasing length and weight were noted for black bullhead and largemouth bass.

The only significant differences in fish condition noted between swamps involved striped mullet and black crappie, both of which were in better condition in Tunica Swamp than in Devils Swamp. A number of species from both swamps showed significantly lower condition at low river stage than at high river stage. The condition was significantly lower for benthic fish in Devils Swamp than benthic fish in Tunica Swamp. Trends of decreasing condition factor with increasing tissue concentrations of organic or inorganic contaminants were noted for black bullhead, largemouth bass, gizzard shad and warmouth.

Work completed to date confirms the existence of a plume of organic contaminants in sediments of Devils Swamp. Environmental concentrations generally decrease with distance from two hazardous waste point sources near the swamp. Inorganic contaminants are more uniformly distributed in Devils Swamp and levels are comparable to those in Tunica Swamp, suggesting more diffuse sources. Although environmental levels are low, fish bioaccumulated both classes of contaminants, exhibiting higher concentrations at larger sizes and weights. Heavy metals generally reach higher levels in fish from Devils Swamp than Tunica Swamp. Factors such as where a fish feeds and what it consumes are as important to bioaccumulation as how long it lives and how long it is associated with the swamp. The low environmental levels of contamination coupled with the lack of obvious community-level effects, suggest that the effects of contamination in this system are mainly chronic (sublethal) and manifested mainly at the population and suborganismal level.

## **Sub-project II: Tree Cores as Biomarkers of Pollution**

### **Objectives and Goals:**

The objective of this sub-cluster is to utilize tree-rings of baldcypress (*Taxodium distichum* L.) to record present and past pollution events by heavy metals and also document growth trends of the trees in a heavily polluted ecosystem (Bayou Trepagnier, Louisiana). Baldcypress is a long-lived, deciduous gymnosperm, distributed throughout the southeastern United States, capable of growing in flooded or dry soils. Tree rings can provide a record of the effects of climate on growth and indicate population perturbations in the environment including the effects of pollution. Changes in ring width not only provide information on individual trees, but can be averaged to form indices for a population. In recent years, tree-rings of several species have been used to provide a record of heavy metal uptake.

### **Accomplishments:**

Heavy metals have become widespread in ecosystems as a result of man's activities. Emission of some heavy metals into the atmosphere as a result of human activity now

exceeds natural processes. Heavy metals can enter plants from the soil via roots, through the bark into wood, or can be taken up by leaves and transported via the phloem to xylem. The basic assumption of dendrochemical studies is that the chemical makeup of the tree rings presents a composite picture of a tree's environment through time.

Bayou Trepagnier is located between the Mississippi River and Lake Pontchartrain and merges with Bayou LaBranche a short distance from the lake. These two bayous extend from the backslope of the Mississippi River's natural levees and drain into Lake Pontchartrain which is approximately 2 meters lower than the river. The entire system is just north of New Orleans.

The vegetation between Bayou Trepagnier and Bayou LaBranche consists of widely spaced baldcypress with freshwater marsh and small ponds. The area was selectively logged at the turn of the century, however, many old baldcypress trees still occur along the bayou (oldest 340 years).

In 1916 a petroleum refinery was built near the headwaters of Bayou Trepagnier, and modernized facilities put into operation in 1930. Major dredging of Bayou Trepagnier occurred from 1930 to 1950 to increase stream flow. In the dredging operations, sediments from the bayou were removed and placed at various distances along the bayou resulting in spoil banks high in pollutants; these exposed spoil banks provided new routes for pollutants to enter the ecosystem. Based upon the size of most other trees (general observation), the dumping of spoil from the dredging operations apparently killed most of the vegetation except for the baldcypress trees. Baldcypress trees along the length of the bayou were analyzed to reconstruct past growth patterns and monitor the heavy metals through time.

Bayou Trepagnier was designated a natural and scenic stream in 1971 within the Natural and Scenic River Act. Substantial quantities of pollutants were diverted into Bayou Trepagnier (notably Lead, Zinc, Copper, and Chromium) before enactment of the Clean Water Act of 1972. In early 1995, the refinery ceased discharging all water utilized in the production of petroleum products; it is now diverted into the Mississippi River.

The best predictor of growth in baldcypress was precipitation in February of the previous year and October of the two previous years. The chronology also demonstrated that the observed and predicted growth values for baldcypress in Bayou Trepagnier are similar and run in the same direction most of the time. However, the prediction based on the climate variables is not good during the period 1918-1924, suggesting the growth during this period was influenced by some factor other than rainfall. The oil refinery at the head of the bayou was constructed in 1916 and began operations in 1918. Other important factors that may have affected growth of baldcypress include draining of the land in this area and a hurricane (1916) with winds in excess of 140 mph. The chronology also exhibits decreased growth following the hurricanes of 1947 and 1965. Many large baldcypress trees in Bayou Trepagnier and in the New Orleans area exhibit broken trunks in upper regions of the trees. In these storms tree species often lose large branches and leaves resulting in loss of living biomass which would reduce growth. However, in succeeding years growth tends to increase as nutrients and increased light are available to surviving trees.

The overall historical record for Lead pollution in all baldcypress trees along Bayou Trepagnier is found in the mean concentration in their growth rings. There is a temporal trend with increasing values beginning in the early 1920's (plant opening). The trend continues gradually over the next few decades, peaking in 1969, and then slowly declines in the most recent decades. The decline correlates with the reduction in use of Lead

additive in gasoline, and with the enactment of the Clean Water Act that occurred in the early 1970's. In Bayou Trepagnier it is assumed that the bulk of the Lead pollution in trees in the upper portions of the bayou resulted from uptake of the heavy metal from the spoil banks via the roots and that the spoil banks were originally sediments in the bayou which were contaminated by the refinery. The quantities of Lead in the trees correlates with the contents in the spoil banks.

The geographical distribution profiles of Zinc concentrations in the baldcypress trees along Bayou Trepagnier were not consistent with the profiles observed for Lead. Since Zinc ions are more soluble than Lead ions at the pH levels observed in Bayou Trepagnier, a more uniform distribution of Zinc in trees throughout the bayou may be expected.

An examination of the history of Zinc uptake in the trees by analysis of the mean levels in the growth rings of all trees in the Bayou Trepagnier ecosystem reveals a profile similar to that observed for Lead. An increase in levels begins in the 1920's and peaks in 1969; whether a subsequent decline is in progress in the most recent decades has not yet been answered. The fact that no significant increase in levels of Zinc in trees occurred following dredging of the bayou (1930 to 1950) might be attributed to the greater mobility of Zinc ions in the ecosystem. Thus in contrast to Lead, Zinc might have been rapidly distributed throughout the ecosystem following an initial pollution event rather than being locked up in the sediments until dredging occurred. This might also result from greater translocation of Zinc through the tree rings.

In many woodland ecosystems, heavy metal contamination results in the accumulation of large amounts of litter on the soil surface because the processes of decomposition are altered. In the upper portions of Bayou Trepagnier the soil surface is devoid of litter for frequent heavy rains wash the litter into the bayou or into the abutting baldcypress fresh water marsh. These heavy rains result in flooding that also erodes the spoil banks and spreads the heavy metals over large areas. Studies of other woodland ecosystems show differential movement of various heavy metals in the mineral soil (disregarding litter). In a ten year study of heavy metal movement in Hallam Wood, Lead showed no evidence of mobility in the mineral soil whereas Zinc moved in a progressive wave. This may be an additional factor to account for the more widespread heavy concentrations of Zinc than Lead in the Bayou Trepagnier ecosystem.

Baldcypress is common in large areas of the southeastern United States and one of the few tree species that can grow in permanently flooded swamps or semi-flooded regions. Streams and rivers in many regions carry heavy metals and our work indicates that baldcypress can be used as a historical and current marker of heavy metal pollution in the environment (particularly of Lead).

### **Sub-project III: Effects of Environmental Contamination on Colonial Wading Birds**

#### **Objectives and Goals:**

The goal of this study is to assess the usefulness of colonial wading birds as indicators of environmental contamination. Analyzing pollutant levels in the blood, feathers, eggs, and guano of these birds and comparing these data with levels of pollutants in foods regurgitated by the birds will allow us to evaluate how the birds are effected by food chain contamination. This will also establish whether the birds are accumulating contaminants from their food sources.

### **Accomplishments:**

During the breeding season of 1995 (March - July) thirty (30) nests of great egrets were tagged, fifteen in Bueche, Louisiana, located seven miles northwest of Devils Swamp, and fifteen in Bayou Sauvage National Wildlife Refuge, a control colony. One hundred (100) nest of little blue herons were tagged fifty (50) in each of the colonies named above. Nests of little blue herons were visited twice weekly from egg laying through fledging of young and the number of eggs and young present were noted. Great egret nests were visited as often as they were accessible. A single egg was taken from each of fifteen (15) little blue heron nests in each site and from great egret nests in Bayou Sauvage and seven (7) nests in Bueche. After chicks hatched, weights and measurements were taken twice weekly, feather and blood samples were taken weekly, and guano and regurgitants were samples passively. Additionally, thirty-nine (39) nests of white ibis were visited once in July, fifteen (15) in Bayou Sauvage, fifteen (15) in a colony in the lower Atchafalaya Delta, and nine (9) in a colony in Lake Martin near Lafayette, Louisiana. Feather and blood samples were collected from one chick in each nest. All samples were frozen until analysis.

Since August, more than seven hundred (700) biological samples have been acid digested for metal analysis and three hundred fifty (350) samples were extracted for analysis of organic contaminants.

While not all of the samples have been analyzed, it is clear that Lead and Cadmium are present in the foods collected from little blue herons in Bueche, but not in Bayou Sauvage. This indicates that heavy metals are present in the food chain in the area around (Bueche including Devils Swamp) to a greater degree than they are in the reference colony. The difference does not show up in the eggs collected from both sites. Whatever differences may exist in the prey items of little blue herons in the Bueche and Bayou Sauvage colonies, there is no detectable difference in growth rates of chicks in these two colonies and neither handling nor regular colony disturbance adversely affected

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## Bioremediation of Selected Contaminants in Aquatic Environments of the Mississippi River Basin

S. Bhattacharya, H. Mielke, J. Bennett, J. Eckert, A. Englande, Jr.,  
R. Fulginiti, D. Grimm, V. Law, D. Mullin, J. Ross

### Objectives and Goals:

The objectives of this project are: 1) to determine what environmental conditions are necessary for enhancement of natural biodegradation processes and whether it is feasible to apply the technology developed to field conditions; 2) to develop an user friendly biokinetic model which is applicable to field conditions; 3) to determine if the new fungal isolate *Marasmius* is effective against a variety of contaminants (heavy metals and chlorinated hydrocarbons); 4) to incorporate the inorganic and organic data into the GIS data base to facilitate analysis of the results; and 5) by combining these multidisciplinary efforts, develop an integrated approach to bioremediation.

### Accomplishments:

Following a review of available background information on several contaminated sites around New Orleans, the Devils Swamp (near Baton Rouge) was selected at the beginning of this project. Flows from several Superfund sites enter portions of Devils Swamp. Groundwater sampling has indicated chlorinated solvents, including carbon tetrachloride, to be widely present in unacceptable concentrations. Based on available and/or collected data, a control area, not contaminated with chemicals, was used for comparison purposes. In Years 2 and 3, samples from Bayou Trepagnier were also analyzed for this project.

Dr. Bhattacharaya's lab has studied the interaction between SRB and methanogens has been studied. Additionally anaerobic toxicity assay (ATA) has been completed with PCP.

Dr. Law's work on the carbon tetrachloride degradation by denitrifiers has been completed. One M.S. thesis has been completed by a graduate student.

Dr. Bennett's lab has taxonomically identified twenty-nine species of fungi and then screened in an assay against the polymeric dye R-487, and this screen correlated with ability to degrade benzo[a]pyrene. A *Marasmius* isolate was the best degrader of B[a]P screened, although it did not perform well in the poly R-487 assay. The same 29 species were screened in tannic acid and gallic acid plate assays, which also test presumptive liginase activity. With the exception of *Marasmius*, there was good correlation with the Poly-487 assay.

Mineralization of radiolabeled B[a]P was obtained with both *Marasmius* and *Phanerochaete*. *Aspergillus* and uninoculated controls gave less than 0.2% radiolabeled carbon dioxide.

*Pseudomonas* were tested for growth under starvation conditions. Starved shells showed changes in size and shape as revealed by scanning electron micrograph.

Alginate pellet formulations of *Phanerochaete*, *Marasmius* and *Aspergillus* were tested for viability after storage at three temperatures. Refrigerated formulations yielded close to 100% viability after one year storage.

While carbon tetrachloride was degraded no substantial differences were observed in terms of carbon tetrachloride degradation at different ORP levels. Chloroform production was less at higher ORPs when using glucose as substrate as compared to glycerol as substrate. Preliminary results from the experiments designed to study carbon tetrachloride degradation by using site-isolated culture *Pseudomonas Cepacia* indicated potential degradation. However, further verification is needed. Experiments designed to determine the interaction between sulfate reducing bacteria (SRB) and methane producing bacteria (MPB) showed that both in serum bottle and chemostat studies as the feed acetate (COD) to sulfate ratio decreased, percent substrate utilized by SRB increased. It was also found that as the ORP increased from -200 mV to -50 mV, acetate utilized by SRB increased from 18 to 34 %.

Biodegradation of 2,4,6-trichlorophenol (2,4,6-TCP) was studied using sulfate reducing bacteria, methane producing bacteria and mixed culture of sulfate reducing bacteria and methane producing bacteria. Results showed that removal of 2,4,6-TCP ranged from 67 to 88%. Results of studies on biodegradation of 2-chlorophenol, 2,3-dichlorophenol, 2,5-dichlorophenol and 2,3,5-trichlorophenol using sulfate reducing bacteria, methanogens and mixed culture of sulfate reducing bacteria and methanogens showed that these chlorophenols were removed in the range of 70 to 100%.

From these results it was concluded that both sulfate reducing bacteria and methanogens are effective in the removal of chlorophenols. Studies on HCBP showed that anaerobic degradation of these low solubility compounds is possible

Research in the Bennett group has focused on five main themes which have a common goal of increasing the repertoire of microbial strains which can be applied to "real world" pollution of soils and sludges.

- The isolation and screening of fungi as a novel source of biodegradative germplasm.
- The testing for putative mineralization by selected fungal species in a model system with <sup>14</sup>C-benzo(a)pyrene (BAP).
- The characterization of a species of *Marasmius*, as a possible tool for bioremediation.
- The formulation of fungi for field use in a stable alginate pellet.
- The testing of the bacterial species *Pseudomonas putida* under conditions of nutrient starvation.

To expand upon these specific research programs: Fungi were isolated from Old Inger, Devils Swamp, and other locations in the Gulf South. After extensive screening with the polymeric dye R-481, as well as media containing gallic acid and tannic acid, a strain of the litter mushroom, *Marasmius*, was chosen for further characterization. This species has never been previously used in bioremediation research. It degraded 91% of BAP.

Mineralization tests showed 4.6% <sup>14</sup>CO<sub>2</sub> as compared for 1.2% for the well known "white rot" *Phanerochaete chrysosporium*. Both *Phanerochaete* and *Marasmius* have been formulated in calcium alginate. Stability for both species is best when corn cob grits are incorporated into the formulation and storage is under refrigeration. Surprisingly, mycelia gave greater viability than spores. Attempts to fruit *Marasmius* for taxonomic purposes have to date been unsuccessful.

Investigators screened for biodegradation of carbon tetrachloride employing various site-isolated microorganism in addition to *Pseudomonas Cepacia* which has previously been found effected in carbon tetrachloride degradation earlier in the study. Only one microorganism isolated from the field study sites *Providencia Stuartii* showed substantial degradation of carbon tetrachloride.

The effectiveness of carbon tetrachloride biodegradation by *Providencia Stuartii* was evaluated under various redox potential conditions. Higher percentage removal of carbon tetrachloride was observed under lower redox potential. Pseudo-first-order kinetics model was employed to determine the substrate removal rate constants.

Biodegradation of carbon tetrachloride was evaluated under various redox potentials by mixed culture (50% *Pseudomonas Cepacia* and 50% *Providencia Stuartii*). Results indicated over 99% degradation of CT was effected in 3 days at  $-250\text{mv} < \text{Eh} < -200\text{mv}$ . Less rates were observed at higher Eh values. Plate counts showed that the predominant species in batch-scale mixed culture was *providencia stuartii*. Chloroform accumulated as a metabolite. Kinetic analysis indicated that the overall degradation rate constant increased from  $2.75 \times 10^{-3} \text{ hr}^{-1}$  to  $4.75 \times 10^{-2} \text{ hr}^{-1}$  by controlling Eh at about -200 mv compare to Eh at  $> 0 \text{ mv}$ .

Ti (III) citrate toxicity tests were conducted to evaluate its toxicity to *Pesudomonas Cepacia* and *Providencia Stuartii*. The results indicated that Ti(III) citrate did not exhibit any toxic effects to either culture until its concentration exceeds 2mM.

Four continuous column runs were conducted to evaluate CT biodegradation at retention times of 1 day, 2 days, 3 days and 4 days, respectively. Initial redox potential were about -150mV in all columns. Samples were taken until equilibrium conditions were established as determined by effluent carbon tetrachloride concentration. Concentration at equilibrium of CT, chloroform, redox potential, biofilm thickness, TOC and active cell mass concentration as RNA were analyzed at different heights. The fixed biofilm reactor exhibited an ability to degrade greater than 99.9% of the CT introduced into the reactor (at retention time  $> 2$  days).

Investigators effected a mass balance of hexachlorobutadiene in Teflon reactors. Results indicated less than 3% hexachlorobutadiene was adsorbed on the reactor surface and hence Teflon reactors were chosen instead of glass ones.

Biodegradation of hexachlorobutadiene under aerobic, limited aerobic and microaerophilic conditions was evaluated. Complete biodegradation of hexachlorobutadiene was observed under all ORP conditions evaluated with slightly higher removal rates occurring under the most aerobic conditions. Kinetics evaluation indicate that the metabolism of hexachlorobutadiene biodegradation by *Pseudomonas cepacia* is best represented by pseudo-first order relationship.

### **Publications and Presentations:**

Bhattacharya, S.K., Leslie, R.E. and Madura, R.L. "Effects of Bioavailable Cadmium on Anaerobic Systems," *Water Environment Research*, 67,7, Nov./Dec. 1995

Bhattacharya, S.K., Sluder, J.L., Jr. and Uberoi, V., "Effects of 4-Nitrophenol on H<sub>2</sub> and CO Levels in Anaerobic Propionate Systems," *Water Research*, 29, 5, 1995

Bhattacharya, S.K., Todd, J.C., Law, V.J. and Uberoi, V., "An Integrated Modeling of Stoichiometry and Biokinetics of Anaerobic Processes," *Environmental Software*, **9**: 33-45, 1994

Bennett, J.W. and Faison, B., "The Use of Fungi for Biodegradation," Submitted for publication in *Manual of Environmental Microbiology*, 1995

Jin, G., and Englande, A.J., Jr., "Redox Potential As A Controlling Factor To Enhance Carbon Tetrachloride Biodegradation", *Water Science and Technology*, Pergaman Press, London (in press). 1995

Jin, G., and Englande, A.J., Jr., "Biodegradation Kinetics Of Carbon Tetrachloride By *Pseudomonas Cepacia* Under Varying Redox Potential Conditions", *Water Environment Research* (Submitted for publication). 1995

Jin, G., and Englande, A.J., Jr., "Effects Of Electron Donors, Dissolved Oxygen And Redox Potential Conditions On Biodegradation Of Carbon Tetrachloride by *Escherichia Coli* k-12", *Water Environment Research* (Submitted for publication). 1995

Jin, G., and Englande, A.J., Jr., "Redox Potential As A Controlling Factor To Enhance Carbon Tetrachloride Biodegradation", Accepted for presentations at International Association of Water Quality 18th Biennial Conference at Singapore, June 24-28, 1996.

Jin, G., and Englande, A.J., Jr., "Kinetic Study Of Carbon Tetrachloride Biodegradation By A Mixed Culture In A Fixed-Biofilm Reactor", Abstract submitted to the Water Environmental Federation 69th Annual Conference & Exposition, Oct. 5-9, Dallas Convention Center, Dallas, Texas.

Huei-wang Jeng and Englande, A.J., Jr., "Biodegradation of Hexachlorobutadiene by *Pseudomonas Cp*", Abstract submitted to the Water Environmental Federation 69th Annual Conference & Exposition, Oct. 5-9, Dallas Convention Center, Dallas, Texas.

## **A Sensitive Rapid On-Site Immunoassay for Heavy Metal Contamination**

R. Blake, D. Blake, G. Flowers

### **Objectives and Goals:**

The principal goal of this focus group is to develop immunoassays for heavy metals that will permit the rapid on-site analysis of specific heavy metals in water and soil samples. Previous studies have shown that monoclonal antibodies to specific metal-chelate complexes may be obtained that permit the quantification of Indium in the ppb range even in the presence of excess concentrations of other metals. These studies are being extended to develop immunoassays for target metals that include, but are not limited to, Lead and Chromium. Specific aims include: 1) to develop new immunoassays for metals that are priority pollutants; 2) to validate each new metal immunoassay by comparing the results

with those obtained by atomic adsorption spectrometry and inductively coupled plasma spectrometry; 3) to test these new assays on samples collected at previously studied test sites and to initiate the development of field applications for the immunoassay; and 4) to initiate studies on the bacterial bioremediation of Lead and Chromium at contaminated test sites.

### **Accomplishments:**

#### Antibody Characterization And Immunoassay Methodologies Were Developed Using An Anti-Cadmium Monoclonal Antibody

Valuable experience was acquired in heavy metal immunoassay development and in the characterization of monoclonal antibodies using an anti-Cadmium antibody available to our laboratories from previous studies. These activities are summarized in three publications included in section C, (See Presentations and Publications below). Functional and structural studies on the anti-Cadmium monoclonal antibody were included in a manuscript recently submitted to *Biochemistry* that describes the isolation and characterization of the antibody. Results from the latter studies are briefly summarized below.

Binding characteristics of the anti-cadmium monoclonal antibody were quantified using a KinExA™, a new automated immunoassay instrument. The KinExA was used to quantify the extent of antibody binding by capturing the free, unbound antibody remaining in reaction mixtures of free antibody, excess chelated metal, and antibody-metal-chelate complexes. Unbound antibody was removed from each reaction mixture by rapid percolation of the mixture through a packed bed of microbeads charged with the immobilized epitope used to solicit the antibody. Exposure of the antibody-soluble ligand complex to the immobilized ligand was sufficiently brief (240 ms) to insure negligible dissociation of the soluble complex during the time of exposure to the beads. Antibody thus captured on the microbeads was then exposed to a fluorescein-labeled anti-species antibody. The fluorescence signal from the resulting immobilized complex, which was proportional to the quantity of primary monoclonal antibody captured on the beads, was subsequently determined.

Equilibrium binding measurements were conducted with the anti-Cadmium antibody and each of 16 different soluble metal ions chelated with ethylenediaminetetraacetic acid (EDTA). Metals in this survey included those in the same row and column of the periodic chart as Cadmium, those with ionic radii similar to that of Cadmium, and those likely to be present in relatively high concentrations in environmental samples. In all cases the monoclonal antibody, which was directed against an epitope that included ionic cadmium chelated with EDTA, showed the greatest affinity for chelated Cadmium. One unexpected observation from these studies was that the affinity of the antibody for chelated divalent metal ions was clearly correlated with the corresponding ionic volumes of the metals. The closer the ionic volume of the metal was to that of Cadmium, the greater was the affinity of the antibody for the chelated metal, and *vice versa*. It was concluded that ionic volume was an important factor in the complementarity of the binding of this particular antibody to its chelated metal antigen.

The KinExA assays developed over the past year will be applied to all monoclonal antibodies generated as part of this project. The insights into the binding properties of the anti-Cadmium antibody suggested that additional screening activities to minimize the influence of ionic volume on the binding specificity should be devised for the future selection of other monoclonal antibodies with even greater specificity for Cadmium (or, by

analogy, to devise appropriate selection criteria for antibodies directed against any other chelated metal ion).

#### The Development Of An Anti-Lead Monoclonal Antibody Is In Progress

After an extensive literature survey, a backbone-substituted derivative of DTPA, 2-(*p*-isothiocyanatobenzyl)-cyclohexyldiethylenetriaminepentaacetic acid (CHX-DTPA), was chosen as the bifunctional chelating agent to use for the preparation of anti-Lead (II) monoclonal antibodies. This derivative was chosen because its three dimensional structure will be modified when ionic Lead is present as the chelate, and the Lead-chelate complex showed acceptable stability when conjugated to protein and injected into animals. The A-isomer of CHX-DTPA was generously provided by Dr. Martin Brechbeil of the National Cancer Institute, NIH. Two protein conjugates were prepared for this study, a keyhole limpet hemocyanin (KLH) conjugate for injection into mice and a bovine serum albumin (BSA) conjugate for use in screening of the hybridoma cells. The percent substitution of the KLH conjugate (17.1%) was determined by measuring the number of primary amino groups present on the protein before and after conjugation. The percent substitution of the BSA conjugate (7.4%) was determined radioisotopically.

The Lead-loaded protein conjugates were delivered to the LSU Hybridoma Facility on October 16, 1995. After a 10 week series of injections, the sera from five mice injected with protein conjugates bearing complexes of Lead were tested for the presence of anti-Lead antibodies. Each of the 5 sera showed preferential binding to the Lead-CHX-DTPA complex compared with that of the Lead-free chelator. The mouse whose serum showed the greatest degree of preferential binding for Lead was sacrificed to supply the spleen cells for subsequent hybridoma generation. The observation that the sera for all 5 mice showed preferential binding for Lead is a very encouraging preliminary result that anti-Lead antibodies can be identified and isolated.

#### The Groundwork Was Laid For Immunoassay Validation And Field Testing

Bottom sediments water samples were taken on a monthly basis from Bayou Trepagnier and analyzed for dissolved Lead, Zinc, Copper and Chromium among others. Samples were collected using a Van Dorn water sampler at the sediment water interface at selected stations established by Shell Oil Company as part of the remedial investigation of pollution in the bayou. Water samples were filtered through a 0.45m syringe filter and stabilized in the field using concentrated SUPRAPUR hydrochloric acid. Chemical analyses were performed with the Perkin-Elmer atomic absorption spectrometer located in the Tulane CIF inorganic laboratory. Although metals loadings at the sediment-water interface were observed to remain on average below the chronic exposure criteria established by LaDEQ to protect fish, a concentration of dissolved Lead in excess of the chronic criterion was observed in one sample taken in early July after an extended period of dry weather and low water conditions. Although only one sample exceeded the chronic criterion for Lead, all samples taken in July contained elevated Lead levels. A possibility for the increased Lead signal during prolonged periods of low water is the weathering of exposed mudflats in the bayou. Oxidation of bottom sediment sulfides (including Lead sulfide) would generate sulfuric acid that would, in turn, favor metal transport into solution. Regardless of the mechanism responsible, these data clearly emphasize the dynamic nature of the pollution levels at specific sites and within the bayou in general. The availability of a rapid, on-site immunoassay for Lead may be of assistance in determining why the Lead levels periodically fluctuate in Bayou Trepagnier.

In related analytical activities, new methods are under development that will enable investigators to extract heavy metals from soil and sediment samples in the field without

recourse to strong acids, high temperatures, or cumbersome equipment. A 5 minute incubation with EDTA extracted approximately 50% of the Lead from 4 dried samples derived from spoilbanks along Bayou Trepagnier. The concentration of EDTA used in these experiments is compatible with the immunoassay format currently under development.

#### Two Grants Were Secured From External Funding Sources That Complement And Extend The Studies Supported Herein

Dr. Diane Blake was awarded a 3-year grant from the EPA to develop immunoassays for Cadmium (II), Copper (II), and Zinc (II). While the experimental plan is analogous to that presented herein, the focus of the EPA grant is on a different set of heavy metal contaminants.

Dr. Robert Blake received an 18-month grant from the Western Regional Hazardous Research Center of the EPA to study the bioremediation of Lead and Chromium in arid soil (as opposed to the focus on contaminated wet sediments and water column studies presented herein).

#### Two Companies, Perkin-Elmer And Aethena, Have Signed Confidentiality Agreements Prepared By The Tulane Office Of Technology Development With The Aim Of Exploring Issues Related To The Commercialization Of Immunoassays For Heavy Metals

Perkin-Elmer has expressed a formal interest in an exclusive license for the anti-Lead antibody currently under development.

#### **Publications and Presentations:**

Blake, D.A., Wilson, S.E., Chakrabarti, P., and Hatcher, F.M, "Measurement of Heavy Metals in Waste Water by Immunoassay", Adaptation of the Assay for Rapid Field Testing. In *Proceedings of Environmental Laboratories: Testing the Waters*, Water Environment Federation, Alexandria, VA, pp. (6)31-(6)39. 1995.

Blake, D.A., Dawson, G.N., Chakrabarti, P., and Hatcher, F.M, "Assay of Heavy Metals Using Antibodies to Metal-Chelate Complexes", invited chapter for American Chemical Society Symposium Series, in press.

Each of the publications listed above was accompanied by a platform speaking engagement. A platform talk entitled "Isolation and Characterization of Metal-Specific Monoclonal Antibodies" was presented by D. Blake at the National Immunochemistry Summit IV in Las Vegas, NV, in August, 1995.

In response to a personal invitation, an abstract entitled "Characterization of a Metal-Specific Monoclonal Antibody" was submitted for the 211<sup>th</sup> annual meeting of the American Chemical Society to be held March 24-28, 1996, in New Orleans.

## Pore-Level Flow, Transport, Agglomeration and Reaction Kinetics of Microorganisms

L. Fauci, D. Gaver, P. Moore, K. Papadopoulos, B. Sharma

### Objectives and Goals:

Toxic substances released into aquatic environments create plumes of contaminant that extends into porous soil. The development of effective *in situ* bioremediation strategies requires a detailed understanding of the pore-level interaction between these contaminants and microorganisms. Integrated experimental and computational models of the pore-level behavior of microorganisms in such environments are continuing to be developed and refined. These models include the detailed analysis of convection and diffusion within the pores and the convection and chemotactic responses of swimming microorganisms to the local contaminant concentration and its spatial and temporal gradients. Additionally, these models include microbial adhesion to each other and the surrounding pore structure, which creates a biofilm within the porous matrix. Finally, analyses of the reaction kinetics of these organisms to the toxic contaminant are included. Use has been made of state-of-the-art methods of computational fluid dynamics and microscopic visualization of the adhesion/aggregation phenomena to understand this complex system and thus aid the development of improved technologies for bioremediation of aquatic environments.

These studies include theoretical and experimental examinations to determine the influence of physicochemical conditions on the bioavailability of microorganisms to the toxin site.

This research includes:

- Interfacial and transport studies that provide information concerning the binding strength of microorganisms and colloids to the porous media as a function of the fundamental physical characteristics of the microorganisms, toxin and pore structure;
- Microscale computational modeling, wherein the influence of physicochemical conditions on the bioavailability of microorganisms to the toxin site is examined;
- The quantitative determination, from results of transport studies and microscale computational models, of kinetic transport properties that may be used in the analysis of macroscale phenomena;
- Comparison of microscale computational predictions with experimental measurements so as to validate the complex computational procedure. In future years, the computational models will be used to make predictions of systems that are not readily studied in laboratory conditions.

### Interactions with Microbiologists

Since the overall goal is to investigate models of pore-level transport related to natural bioremediation the importance of incorporating 'real-world' understanding of these living systems into these models is understood. To incorporate these facets collaborations with groups at Tulane and the Center for Biofilm Engineering at Montana State University (CBE) have been developed. Strong interactions have been established with the CBE, one of several NSF-supported National Engineering Research Centers. Microbiologists at this center have identified complex dynamic properties of biofilms in microscale environments that are potentially important to bioremediation. Drs. Fauci and Dillon (of this cluster) visited the CBE. Projects of mutual interest were identified.

## Research Goals

The results of the microscale investigations will provide parameter estimates for macroscale models. The microscale investigations involve the study of interfacial forces, which arise from the overlapping of electrical double layers as well as from stearic and van der Waals interactions. These forces are factors that can determine the strength with which bacteria or toxic compounds attach themselves to the soil matrix. If unattached, these substances flow in the porous medium according to hydrodynamic driving forces. In addition, information concerning pore-level activities is also needed since the interaction of bacteria, physicochemical conditions and toxic compounds on this level will determine the bioavailability of microorganisms. The specific goals of the microscale investigation will thus be to: 1) understand and quantify the effects of interfacial forces on the motility and/or adhesion of toxic particles and bacteria in contaminated soils; and 2) elucidate the pore-level transport and bioremediation which occurs.

This work initially focuses on investigations of a single population of bacteria and a single contaminant species. These bacteria and the contaminant are not necessarily from a specific contamination site. However their use allows us to determine experimentally specific binding factors and transport properties that are used in the computational model. Comparison between experimental measurements and computational results will validate this general modeling approach. Once this is accomplished, these models will be used to investigate the behavior of specific single populations of bacteria and a specific contaminant from known contamination sites. This will be accomplished by variation of the physical parameters that are input into the computational model, and the development of scaling relationships that characterize the swimming and aggregation phenomena during different scenarios. Thus, these general models will produce predictions that are relevant to specific pollution sites.

## **Accomplishments:**

The development or improvement of effective strategies for *in situ* bioremediation depends upon understanding the detailed pore-level behavior of contaminants and microorganisms within porous media. This is due to the fact that bioavailability of microorganisms to the toxin site depends upon the local physicochemical conditions (e.g. pH, temperature, concentrations of dissolved gasses). First, these conditions are primary determinants of bioavailability because they influence flocculation, the propensity of microbes to aggregate and adhere to each other and the local pore structure. This creates a biofilm that consists of the adsorbed cells in association with extracellular polymer substances that bind them together. Cells may also be removed from the biofilm by biological processes or flow properties that lead to detachment. This biofilm is the site of bioremediation, and may also hinder microbial migration by lessening forced convection and diffusive transport of the colloidal mixture through small pores. The local physicochemical conditions also influence bioavailability due to chemotaxis, the directional motion induced by variations of chemical concentrations. Thus, if concentration gradients are appropriate, microbes may more readily swim towards contaminated regions and aid in the elimination of toxic waste. Uptake at the contamination site is governed by metabolic kinetics, which is a function of the local physicochemical state. All of the aforementioned processes occur in a moving viscous fluid, and therefore the fluid dynamical events must be included in any realistic model. As is evident by the above description, factors controlling the local environments of microbial communities in the subsurface interstices are critical for *in situ* remediation technology. Unfortunately, knowledge of this small-scale system has yet to be fully investigated, and is extremely complex due to the many components that govern the

physicochemical and flow conditions. The general philosophy of these integrated approaches (both computational and experimental) is describe below. This philosophy is followed by a more detailed description of the accomplishments to date.

Mathematical models of biofilm processes have modeled the biomass as a continuously distributed population. In contrast, our models represent the microbes in the biofilm as discrete entities. Each kind of model has its own advantages. However, by representing cells discretely, it is possible to more accurately model changes in the local geometry brought about by adhesion of cells to a substratum or by cell aggregation. A detailed description of the fluid flow can lead to a greater understanding of the role of hydrodynamic forces on the dynamics of cell-cell and cell-substratum bond formation and destruction. Also, by modeling cells at this level, one has complete control over individual cell specifications such as adhesivity, reactivity to stimuli and consumption of contaminants, rather than only being able to specify these in an average sense at the population level.

Further evidence suggests that physicochemical surface interactions play an enormous role in the transport of colloidal pollutants or microorganisms through subsurface porous media and soils. These forces determine whether suspended particles are transported through or collected by the walls of a porous medium. Preliminary experiments in Dr. Papadopoulos' laboratory have shown that it is possible to visually observe the movement or adhesion behavior of particles and bacteria in straight as well as random pores. Solution parameters such as pH, ionic strength, the presence and nature of surfactants, and the morphology of the medium influence the interfacial forces must be investigated.

#### Computational Investigations

The computational cluster element includes, as primary investigators, Drs. Fauci, Dillon, Gaver, Sharma and Moore. As detailed below, the purpose of this cluster is to develop computational model simulations that will permit estimation of the transport of microbes into porous media, the colonization of these organisms into a biofilm, and the uptake of contaminant. This element works in conjunction with the experimental element described below, and has developed collaboration with the Center for Biofilm Engineering at Montana State University. The fundamental modeling methods used and preliminary results from these studies are described below.

The following two-dimensional model has been developed. State-of-the-art methods in computational fluid dynamics enables the study of flows in simple pore geometries and the interactions of the microbes with the fluid and surrounding pore structure. The dynamic evolution of a single contaminant that is initially deposited within a pore filled with a viscous fluid depends upon the fluid motion induced by motile bacteria, background flow, diffusion and microbial uptake. Moreover, microbes move in direct response to the surrounding contaminant field (chemotaxis). This nonlinear coupled system of equations is described below.

The governing equations describing the fluid dynamics are:

$$\rho (u_t + (u \cdot \nabla)u) = -\nabla p + \mu \nabla^2 u + F \quad (1)$$

and

$$\nabla \cdot u = 0, \quad (2)$$

which are the Navier-Stokes and continuity equations for the incompressible fluid. These equations represent the balance of momentum and conservation of mass, and hold within the fluid domain. Here,  $\rho$  is the fluid density,  $\mathbf{u}$  is the fluid velocity vector,  $p$  is pressure and  $\mu$  is the fluid viscosity. Contributions to  $\mathbf{F}$  arise in several ways: first, the body of each microbe and the walls of the fluid pore are modeled as neutrally buoyant elastic boundaries, and fluid-induced deformation of these boundaries generates restoring forces that act on the fluid to try to reverse the deformation. Secondly,  $\mathbf{F}$  includes forces that model the flagellar beating that propels motile microbes. Lastly,  $\mathbf{F}$  includes forces due to the cell-cell and cell-substratum attraction and repulsion:

$$\mathbf{F} = \sum_{i=1}^N [\mathbf{F}_{microbe(i)} + \mathbf{F}_{swim(i)}] + \mathbf{F}_{walls} + \mathbf{F}_{cell-cell} + \mathbf{F}_{cell-wall} \quad (3)$$

The no-slip/no-penetration boundary conditions are applied to the suspended microorganisms and pore walls.

The equation describing the convection and diffusion of the contaminant species within the fluid-filled pore is

$$c_t + (\mathbf{u} \cdot \nabla)c = D \nabla^2 c - R(c)c, \quad (4)$$

where  $c$  is the concentration,  $D$  is the molecular diffusivity in the liquid phase and  $R(c)$  is a concentration-dependent consumption rate which is non-zero only near the site of each of the  $N$  microbes. Therefore, the microbes act as localized contaminant sinks whose locations evolve with time.

The cell body of a single microorganism in a 2-d representation is modeled as a neutrally buoyant elastic ring, whose configuration is defined by  $\mathbf{X}_i(s,t)$ , where  $s$  is a Lagrangian label,  $t$  is time and  $i$  denotes the  $i$ th microbe. The boundary force per unit length  $f_i(s,t)$  at each point on the ring consists of an elastic spring force that maintains the integrity of the membrane and a bending-resistant force that resists deformation. This elastic force is transmitted directly to the fluid through

$$\mathbf{F}_{microbe(i)}(\mathbf{x},t) = \int f_i(s,t) \delta(\mathbf{x} - \mathbf{X}_i(s,t)) ds. \quad (5)$$

Here, the integration is over the ring structure and  $\delta$  is the two-dimensional Dirac delta function. Note that each organism contributes such forces to the flow field and therefore their interactions, mediated through the fluid, are included in this model.

Bacterial swimming is achieved through the action of one or more flagella. In this model of bacterial motility and chemotaxis, the flagella are not explicitly represented as immersed elastic structures. However, idealized forces of the microbe's locomotory movements are applied to the fluid at specific points 'behind' the cell body. As described in detail in our manuscript published in the *Journal of Theoretical Biology* (177: 325-340, 1995), the placement of the virtual flagellum can be chosen so that, in the absence of boundaries and other microbes, a given mean swimming direction of the microbe is achieved. In the presence of boundaries and other microbes, the swimming direction at the start of a run is the result of a random reorientation during a tumble. Chemotactic bacteria respond to an increase over time in the local concentration of a chemoattractant  $c$  by decreasing the tumbling probability. Thus, individual run times depend upon the history of the chemoattractant concentration at the location of the moving microbe.

When a model microbe is first introduced, its swimming orientation is chosen randomly. During subsequent tumbles, a new orientation is randomly selected. The times at which a cell tumbles are determined randomly with the tumble times drawn from an exponential distribution. The mean of this distribution depends upon the time-history of the chemoattractant concentration near the microbe. The angle by which the swimming orientation changes when a microbe tumbles is chosen randomly from a prescribed distribution.

Cell-cell and cell-wall adhesion are modeled by the creation of elastic links between points on each of the adherent entities. The model for link formation and breaking works as follows: If the distance between the centroids of any given pair of cells is less than a prescribed cohesion distance, an elastic spring is created to link the two cells. The mechanical properties of each link and the cohesion distance are chosen to reflect biological and physicochemical properties of the system. Cell-wall links are created similarly. Detachment of cells from the biofilm is modeled by allowing the links to break when they are stretched beyond a prescribed length.

Finally, the system is closed by requiring microbes to move at the local fluid velocity using

$$\frac{d \mathbf{X}_i(s,t)}{dt} = (\mathbf{u}(\mathbf{X}_i(s,t), t)) \quad (6)$$

The salient feature of this representation is that suspended organisms are replaced by suitable contributions to a force density term in the fluid dynamics equations. A single set of fluid equations holds in the entire domain and there are no internal boundary conditions. Consequently, the fluid dynamics equations may be solved efficiently using finite-difference methods on a uniform computational grid.

The numerical method that was used couples microbial motion with fluid dynamics, and is known as the immersed boundary method. In the computations, the fluid domain is discretized using a uniform rectangular grid fixed in space (Eulerian), and all fluid variables ( $u$ ,  $p$ , and  $F$ ) are defined only at these gridpoints. The immersed boundaries  $X_k$  that represent the cells and walls are represented by discrete Lagrangian points. These immersed boundary points do not coincide with points of the fluid grid, and communication between the immersed boundary points and fluid grid is handled by a discretized representation of the Dirac delta function. The contaminant concentration,  $c$ , is defined on the Eulerian grid.

The algorithm for the numerical solution of the coupled fluid/microbe/contaminant system may be summarized as follows: at the beginning of each timestep  $n$ , is the fluid velocity field  $u_n$ , the configuration of the immersed boundaries, and the chemical concentration field  $c_n$ . In order to update these values to those occurring at the next time step the following processes are completed: 1) calculate the elastic force densities for each immersed boundary; 2) calculate the swimming forces generated by each organism; 3) calculate the cell-cell and cell-wall link forces; 4) spread the force densities to the grid to determine the  $F$  on the fluid; 5) solve the Navier-Stokes equations (equations 1-2) for  $u_{n+1}$ ; 6) interpolate the fluid velocity field to each immersed boundary point and convect that point at the local fluid velocity; 7) using the new positions, alter the chemical concentration to account for microbial consumption, and solve the convection-reaction equation for  $c_{n+1}$  (equation 4).

This model's capacity for simulating the behavior of a coupled fluid/contaminant/microbe system has been demonstrated, as described in *Journal of Theoretical Biology* (177: 325-340, 1995). These models have been extended to include aggregation phenomena in the manner described above. This work has been submitted for publication to the *Journal of Computational Physics* (Dillon, Fauci, Fogelson and Gaver, in review).

The simulations show the influence the swimming forces can have a pronounced effect on the fluid velocity field, making it far from parabolic. This allowed many cells to move close enough to the wall to form cell-wall attachments. This result shows the significance of cell motility in the onset of biofilm formation. In other simulations, the investigators have demonstrated that chemotaxis is instrumental in dictating biofilm structure.

Finally, to investigate the effect of irregular geometries on biofilm formation, a channel with an expansion chamber was simulated. This study was motivated by experiments conducted at the Center for Biofilm Engineering at Montana State University, which study the effects of surface irregularities in the initial deposition of cells. The model predicts that the distribution of cells in the initial deposition will be greatest at the edges of the surface depression, rather than within the depression itself. This correlates well with experimental findings at CBE. In fact, an agreement has been reached to coordinate these computational experiments with the CBE laboratory experiments.

In the laboratory, it is difficult to visualize the fluid velocities and contaminant concentration levels on the length and temporal scales associated with bacterial swimming and biofilm formation. The studies above indicate significant progress in the development of a computational model for a theoretical investigation of how individual cells interact alone and in groups with a complex environment governed by fluid mechanics, the advection, diffusion, and uptake of critical contaminants, and the fine scale microgeometry. The simulations show strong local hydrodynamic interaction between pairs of swimming cells and between an ensemble of motile cells. The nature of this interaction agrees qualitatively with experimental work reported by the experimental component of this cluster.

A controlled environment has been set up in the form of a computational model where microbial motility, contaminant evolution, fluid dynamics and biofilm formation can be measured and visualized. This "experimental apparatus" will be used to systematically evaluate the influence of various physical parameters on contaminant depletion and microbial behavior. This computational work couples with the laboratory experiments in a number of ways. The comparison of simulations and laboratory experiments provides the means for parameter estimation. These estimates will be developed using simple models; and can then be used in computational experiments that are not easily replicated in the laboratory. In addition, once these parameters are known, our computational simulations will provide predictions of system variables (i.e. local concentrations) that are nearly impossible to measure *in situ*. Furthermore, simulation results can be used as a guide to design fruitful laboratory experiments.

### Experimental Investigations

The experimental cluster element is directed by Dr. Papadopoulos, and interacts directly with Drs. Fauci, Dillon and Gaver in the computational element in order to keep a cohesive project. As detailed below, the purpose of this cluster is to develop experiments that are useful for estimation of the parameters used in the simulations. In the first three years of the experimental work, experimental methods to investigate bacterial motility and chemotaxis at the pore-level have been developed. This work is described below.

### Chemotaxis of *E. coli*.

*E. coli* were chosen as the model bacteria to be chemotaxed in the direction of increasing concentration of two different nutrients, glucose and fucose. The strain *E. coli* K12 was obtained from American Type Culture Collection (ATTC Cat No. 10798). These *E. coli* were recovered in Tryptone with NaCl (TNa) medium at 30°C overnight, then transferred to culture plates that contain TNa agar and cultured overnight. Single colonies were selected from the plates, transferred to swarm plates (TNa with 0.3% agar) and grown at 30°C. Motile cells were selected from the edge of the colonies of the swarm plates, and incubated on TN agar slant overnight. The slants were stored at 4°C. For chemotaxis experiments, bacteria were scraped from the slant with an incubating loop, and were incubated in 25ml TNa medium inside a 250 ml PYREX flask. The culture was grown to stationary phase in a rotary shaker at 30°C and 180 rpm. Bacteria were harvested by centrifugation at 3500 rpm for 10 minutes and the supernatant was disposed. The *E. coli* were then resuspended in a 1 lt pH buffer containing 0.029g EDTA, 11.2g K<sub>2</sub>HPO<sub>4</sub>, and 4.8g KH<sub>2</sub>PO<sub>4</sub>. The bacterial suspension was then centrifuged again at 3500 rpm for 10 minutes and the process was repeated three times. Care was taken to ensure that the bacterial culture remain uncontaminated. Also, conditions were set so that cell multiplication was negligible during the course of each experiment.

The novel chemotaxis cell consists of two reservoirs (chambers) on a watchglass, communicating through a narrow capillary. The chambers are made of rubber O-rings of inside diameter ~1.3cm. The connecting capillary has the macroscopic appearance of a thin hair, with a length of 0.8cm and an inside diameter of 60µm. Since the chemotactic movement of *E. coli* is observed inside the capillary, it is advantageous to have as linear a concentration profile (constant gradient) for the nutrient (glucose or fucose) as possible. Nutrient solution was inserted in one chamber and the chemotaxis pH buffer (with no bacteria nor nutrient) in the other, and diffusion was allowed to take place for several hours before experiments commenced. The bacteria were then introduced into the other chamber, termed as the *E. coli* reservoir, and the migration was observed. The results of these experiments have been submitted for publication by Z. Liu and K. Papadopoulos to Biotechnology and Bioengineering.

The video-microscopy/image analysis system was used to capture the bacterial movement in real time, to make video movies of numerous experiments, and to extract qualitative understanding and quantitative measurements from the experiments. This initial experiment focused on visualizing the results of bacterial chemotaxis in a capillary. As described above, the comparison between these laboratory experiments and the computational simulations are useful in determining the validity of our physical assumptions. One quantitative measurement that will be use for this purpose is the chemotactic velocity. The studies showed that in all cases chemotactic velocity increases, not only with increasing concentration gradient, but also with increasing concentration, as the distance from the entrance increases. This strongly suggests that in the computational model described above, the magnitude of the swimming force should depend upon the local concentration, *c*. Presently, more experiments are being conducted to generalize and quantify these observations in ways that can provide laws for the mathematical models.

### Unidirectional Motility of *E. coli* in Restricted Capillaries

Bacterial motility is exhibited not only in bulk phase, but also and more commonly so, in geometrically restrictive environments. In the latter, the "interaction" between bacterial cells and neighboring surfaces may play an important role in bacterial transport. In these studies, the term "interaction" between a cell and a surface or between two cells is not confined to physical contact. It includes mutual proximity that allows the fluid flow field

around a cell, as well as its physicochemical characteristics, to influence (and be influenced by) its proximate surfaces and cells. In restrictive geometries, due to spatial limitations, motile bacteria may be prevented from reversing their swimming direction. Moreover, they may also reach the solid surface before they finish one run. This migration behavior differs significantly from that in unrestricted bulk.

Experiments have been performed that track the movement of individual bacteria inside capillaries with diameters smaller or comparable to the length of the flagella. These studies appear to be the first of their kind. In a 6 mm capillary filled with buffer and in the absence of any chemotactic stimuli, the swimming of individual *E. coli* cells have been tracked. In the absence of any chemotactic stimuli, individual *E. coli* K-12 wild type cells can swim in only one direction. Unidirectional movement of an individual cell is defined as movement along the capillary tube towards one of its ends, while reversal of direction means the switch of bacterial movement towards the other end of the capillary. Movement consists of runs and tumbles and does not imply straight-line motion parallel to the capillary's axis. To explain the cells' failure to reverse their swimming direction, it is hypothesized that their flagella are not short or flexible enough so as to allow them to do so. The finding of this phenomenon is important in understanding flagellated bacterial migration through any porous medium with pores or passages in the range of a few micrometers to a few tens of micrometers. This may be significant in soil bioremediation schemes using flagellated bacteria in soils. Based on these findings, the transport of such bacteria and their access to microscopic sites within a given soil will depend on the soil's porosity in a much greater way than previously thought. Soil micropores with diameters comparable to flagellar length may be inaccessible to swimming bacteria. This work was recently published in *Applied Environmental Microbiology* (Z. Liu and K. D. Papadopoulos, 1995).

#### Bacterial Transport Through Sand-Packed Columns

In order to successfully control the administration of bacterial suspensions in soil clean-up sites, it is imperative to understand how their transport in soil is influenced by easily controlled parameters such as cell starvation and pH, as well as by constraints like pore size and hydrophobicity of the soil. In the course of the last year bacterial-flushing experiments have been conducted in columns packed with two different types of sand in order to reveal the effects of such parameters.

Chromatography columns (15x150 mm) are packed with two sizes of quartz sand: 50 ~ 100 mm and 250 ~ 350 mm. The sand is cleaned using standard procedures and, in order to study the effect of hydrophobicity, it is rendered hydrophobic through a technique that developed by this projects investigators. The pH of the solutions is adjusted with HCl and NaOH and the ionic strength is held constant by addition of 10<sup>-3</sup> M NaCl. In a column-flushing experiment, it is important to pack and wet the columns uniformly. For this reason the column is set up vertically, and it is saturated and balanced with the desired solutions before addition of the bacterial suspension. When the column reaches equilibrium, a pulse of 2 ml of well-dispersed bacterial suspension is added to the column, and the fluid is forced to pass through the column continuously at a constant flow rate via a syringe pump. Effluents collected with test tubes are subjected to UV-Visible Spectrophotometry at 680 nm to determine the concentration of cells in the effluents. It was found that starvation has an effect on cell size and cell surface charge, which in turn influences the elution/retainment of cells in the porous medium. It has been demonstrated that starved cells lose their motility but not their viability, as they can grow upon renewed feeding.

The degree of hydrophobicity of the porous medium is an important factor for bacterial transport that has not been studied in enough detail. The preliminary studies used a high

salinity (0.1M NaCl) solution in bacterial-flushing experiments with both hydrophilic and hydrophobically-treated sand. The studies show that the hydrophilic sand grains entrap practically all bacteria, whereas the hydrophobic sand allows them to flow through. These preliminary studies indicate the potential importance of hydrophobicity on bioremediation.

### Education Contributions

The research team involves six professors from four different departments in two Universities and an NSF Postdoctoral Fellow who began his computational work on this project during the first year of this grant. Faculty investigators are from the departments of Chemical Engineering, and Biomedical Engineering, Mathematics and Cell and Molecular Biology of Tulane University as well as from the Mathematics Department at Xavier University. Four graduate students and one new post-doctoral fellow will be continuously supported from this grant. It is expected that a number of other graduate students (supported by other sources) will also participate in various phases of the project and will benefit from this experience by becoming familiar with the environmental issues pertinent to this project. In addition, five undergraduate students are expected to participate each year (as research assistants during the summer months and during the academic year while preparing undergraduate honors theses). It is anticipated that most of the undergraduate assistants will choose to pursue graduate studies and careers in environmental science or engineering. The following is a list of the student participants in this project.

- Dr. Robert Dillon - Postdoctoral researcher who's main focus includes the detailed simulation of the two-dimensional models. Dr. Dillon's work in this cluster was instrumental in his being chosen as an NSF Postdoctoral Fellow. Dr. Dillon chose to remain at Tulane, and is committed to remaining an active participant in this project.
- Zewen Liu - Chemical Engineering graduate student performing studies of bacteria transport in constant gradient system as part of his PhD research.
- Dean Bottino - Mathematics graduate student performing computational model development of reaction-diffusion equations relevant to contaminant transport in pores as well as developing models of ameboid locomotion.
- Stephanie Kute - Biomedical Engineering undergraduate spent the summer of 1993 developing visualization techniques on a Silicon Graphics workstation for displaying results from simulations. Ms. Kute was awarded an NSF Doctoral Fellowship based on her Honor's Thesis on aggregation models, and is continuing at Tulane.
- Annette Johnson- Mathematics undergraduate from Xavier University collaborated with Dr. Moore in scientific visualization projects related to this work. This work was performed at Tulane on a Mathematics department Silicon Graphics workstation. Presently (Fall 1995), Annette is continuing to develop code for solving reaction-diffusion equations.
- Craig Gates - Chemical Engineering undergraduate worked on two associated studies of latex particle movement in a packed micro-bed, and on the electrokinetic mobilization of particles in a straight capillary.
- Darren Yap - Biomedical Engineering graduate student developing models of interfacial flows and transport.
- Brandon Downey - Mathematics undergraduate spent the summers of 1994 and 1995 developing tools for video presentations of the computational simulations. These videos were used in presentations by Dr. Fauci at the Leeds symposium on Biofluidynamics in July 1994 and by Dr. Dillon at the CBE at Montana State University. Helped develop Internet homepage for better dissemination of results (<http://www.math.tulane.edu>).

- Mark Bray - Biomedical Engineering undergraduate spent the summer of 1994 developing computational tools for analyzing the swimming behavior of microbes based upon the experiments of Zewen Liu.
- Amy McDonald - Mathematics undergraduate completed her Honor's thesis on representing microbe cell bodies in three-dimensions. She was awarded an NSF Doctoral Fellowship and is currently a first-year graduate student at Stanford University in Computational Science.
- Antoinette Diaz - Mathematics Undergraduate from Xavier University spent the summer of 1993 working at Tulane's Mathematics department learning methods for computationally solving diffusion equations.
- Dave Meng - Mathematics graduate student at Tulane University, working on implementing immersed boundary methods in three-dimensions.
- Michael Ostling - Mathematics undergraduate spent the summer of 1995 developing tools for video presentations of the computational simulations. These videos were used in presentations by Dr. Fauci at the Leeds symposium on Biofluidynamics in July 1994 and by Dr. Dillon at the CBE at MSU. Helped develop internet homepage for better dissemination of results (<http://www.math.tulane.edu>).
- Kristin Swanson - Mathematics undergraduate spent the summer of 1995 installing and testing parameter analysis code AUTO94 online to the Mathematics computers used for environmental investigations.

All the student researchers use state-of-the-art equipment for their computational or experimental programs. For this reason, the computational equipment provided by this research grant which is located at Tulane University is available to the Xavier participants in this project as well. The students, graduate and undergraduate, interact with all the investigators of the cluster through regular seminars, workshops and individual consultation. Thus, they receive a truly interdisciplinary education. As an example of this interaction, Zewen Liu and Dean Bottino were students in Dr. Gaver's course in Fluid Mechanics for Biomedical Engineers taught during the Fall of 1993. Dr. Dillon also attended these lectures. A concerted effort is being made to involve women and under-represented minority students in these research projects. Dr. Fauci currently serves as a faculty advisor to Newcomb College's Women in Science Organization, and encourages members of this group to participate in this and other environmental initiatives on campus.

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Pore-level Flow, Transport, Agglomeration and Reaction Kinetics of Microorganisms by Robert Dillon, Lisa Fauci and Donald Gaver was presented at the X International Conference on Computational Methods in Water Resources to be held in Heidelberg, Germany during 7/94.

Dr. Robert Dillon was invited to present our modelling work at the First World Congress on Computational Medicine and Public Health during 4/94 in Austin, Texas.

Dr. Lisa Fauci was invited to present her studies of microorganism locomotion at the Society for Experimental Biology Symposium on Biological Fluid Dynamics at the University of Leeds, England during 7/94.

Zewen Liu and K. Papadopoulos presented a paper describing experimental investigations of bacterial motility and chemotaxis at the annual meeting of the American Society of Chemical Engineers in San Francisco in November, 1994.

Zewen Liu, Wei Chen and K. Papadopoulos presented a paper describing experimental investigations of bacterial transport through sand-packs at the annual meeting of the American Society of Chemical Engineers in Miami in November, 1995.

K. Papadopoulos presented a seminar at Columbia University entitled "Bacterial motility and chemotaxis in restrictive capillaries," September, 1995.

Dr. Robert Dillon presented "Modeling biofilm processes" to the Center for Biofilm Engineering at Montana State University, Bozeman MT, October 1995.

Dr. Robert Dillon presented "Modeling biofilm processes using the immersed boundary method" at the Courant Institute of Mathematical Sciences, October 1995.

Dr. Robert Dillon presented "Modeling biofilm processes using the immersed boundary method" to the Department of Mathematical Sciences at Montana State University, Bozeman MT, October 1995.

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## **Biomarkers of Exposure and Ecotoxicity in the Mississippi River Basin**

W. Hartley, T. Huang, E. Horner, C. Ide, M. Mizell, P. Obih,  
R. Tompkins, A. Thiyagarajah

### **Objectives and Goals:**

General acceptance of biomarkers as a tool for environmental protection is restrained by a lack of clear application methodology. Focused laboratory and field research is needed to validate fundamental understanding of biomarker responses. The biomarkers research was on specific biomarkers of exposure and ecotoxicity in wild fish and frogs from two contaminated Mississippi River Basin sites (Devils Swamp and Bayou Trepagnier) and two control sites (Tunica Swamp and Bayou Traverse). This research focused on cancer and disease, developmental toxicity, neurotoxicity, immunotoxicity, estrogenic effects such as hermaphroditism and enzyme alterations.

The broad objectives of research using wild fish and frogs are: 1) to determine which biomarker responses reflect the combined toxicological interactions in animals exposed to mixtures of environmental contaminants typical of the Mississippi River Basin; 2) to determine which biomarker measurements reflect exposure over-time; 3) to identify and characterize sentinel species; and 4) to quantify the molecular, biochemical and histopathological biomarkers.

### **Accomplishments:**

To date, all objectives have been reasonably well achieved as demonstrated by the identification and publication of specific biomarkers of exposure and ecotoxicity in wild fish and frogs exposed to multiple pollutants.

Five scientific papers on biomarkers in fish from Devils Swamp have been published and one is under review. The primary findings of these papers are as follows:

Ectopic hermaphroditic gonads were found in the laboratory culture of Japanese medaka fish (*Oryzias latipes*) and in a medaka exposed to the Devils Swamp contaminant hexachlorobenzene (HCB). This lesion has never been reported in any vertebrates. The Japanese medaka may be a sensitive animal model for chemicals in the aquatic environment that adversely affect reproduction / development or mimic estrogens.

The exposure of medaka fish to HCB produced a high incidence of heart defects. The Japanese medaka is a sensitive animal model to validate adverse histopathological and developmental effects of an environmental chemical (HCB) found in wild fish from Devils Swamp.

Liver lesions were observed in spotted gar (*Lepisosteus oculatus*), longnose gar (*L. osseus*), and shortnose gar (*L. platostomus*) from Devils Swamp. A quantifiable biomarker of mixed pollution (melano-macrophage centers) was identified in the liver. The identification of this type of quantifiable histopathological biomarker in the liver (the site of xenobiotic metabolism) of the spotted gar provides a potentially powerful tool for assessment of mixed chemical exposure.

Histopathological biomarkers of mixed pollution were identified in the gill of two species of buffalo fish, smallmouth buffalo (*Ictiobus bubalus*) and bigmouth buffalo (*Ictiobus cyprinellus*) from Devils Swamp. These two species of buffalo fish are important commercial and game fish. This gill biomarker will be useful in monitoring general fish health as well as xenobiotic exposure.

The black bullhead (*Ameiurus melas*) from Devils Swamp was identified as a sentinel species for exposure to mixed pollution based on a high incidence of gill lesions typically associated with environmental pollution. The black bullhead is an ideal species for the evaluation of exposure to environmental contaminants bound in sediments.

Significantly lower cholinesterase (AChE and BuChE) and carboxylesterase (CaE) activities were observed in the brain and liver of gar fish from Devils Swamp which correlates well with histopathological liver lesions. Correlation of decreased brain and liver enzyme activities with histopathological biomarkers in the liver of gar fish may provide the basis for a comprehensive mechanistic model for mixed chemical exposure.

Major progress has been made in identifying biomarkers of exposure in frogs. The primary findings are as follows:

Frogs placed in cages in Bayou Trepagnier had levels of interleukin-1 $\beta$  approximately one-half of control site frogs. Interleukin -1 $\beta$  ratios may be a sensitive cell/molecular biomarker in frogs for exposure to mixed pollution. Alterations of interleukin -1 $\beta$  may signal (early warning) these amphibian populations are in danger from exposure to pollutants.

Frogs are a sentinel organism for immune suppression based on both mitogen lymphocyte response and neuroimmune response in frogs exposed to mixed chemical pollution, and to known developmental and neurophysiological toxins.

Frog embryos exposed to the Devils Swamp contaminants HCB had abnormal neurological development including pronounced paralysis in the tail musculature. Frogs may be a sensitive animal model for evaluating the potential of environmental chemicals to cause adverse neurological/developmental effects. They can also be used in laboratory studies to validate field results in wild frogs from Devils Swamp and Bayou Trepagnier.

cDNA probes were developed for components of the interleukin-1 $\beta$  complex which allows a molecular biological analysis of this neuro-immunologic response.

Field data from Devils Swamp and Tunica Swamp biomarkers studies have been entered in to the Geographical Information Systems database. This resulted in spatial correlation of histopathological data in fish with site environmental conditions. The GIS is a powerful tool that will be used for generating hypothesis for further research.

This research cluster will continue to evaluate exposure and ecotoxicity biomarkers in frogs and fish and to confirm field results in laboratory models. The field approach will determine how aquatic organisms (frogs and fish) respond to mixtures of chemicals typical of a contaminated aquatic ecosystem. The laboratory approach will develop predictive *in vivo* and *in vitro* model methods/systems for ecotoxicity evaluation of mixtures. The final phases of the study will involve further field validation of laboratory systems at other contaminated aquatic systems. Biological endpoints that impact on the survival, and growth of fish and frog species will be targeted in the field and laboratory research. These critical biological endpoints include disease and cancer, developmental toxicity, immunotoxicity, neurotoxicity, estrogenic effects and enzyme alterations. Fish and frogs collected in the field will be studied by evaluation of pathological and biochemical analysis of tissues and fluids for abnormalities and compared to a control area. After characterization of site contaminants, novel laboratory methods used to confirm field observations include microinjection of embryos (Japanese medaka fish and frogs) with site contaminant mixtures/extracts, neurotoxicity studies on developing and adult frogs, whole animal exposures (Japanese medaka and frogs) to evaluate cancer potential and developmental effects of mixtures, and cell and molecular endpoints to delineate the mechanism of toxicity.

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## **Natural and Active Chemical Remediation of Toxic Metals, Organics, and Radionuclides in the Aquatic Environment**

G. McPherson, P. Pintauro, S. O'Connor, J. Zhang, R. Gonzales,  
V. John, G. Flowers

### **Objectives and Goals:**

This is a cluster whose research efforts are directed towards studying chemical aspects of remediation. The underlying theme of the research is that chemical remediation does occur naturally, and an understanding of such processes would help both in determining the fate of contaminants and in developing novel remediation strategies. Accordingly, the cluster is loosely divided into two subclusters, one which studies natural remediation and the other active remediation. Among topics in natural remediation are the fate of heavy metal and organic contaminants discharged into aquatic environments, and the development of new technology to accurately assay metal contaminants partitioned into soils, water, and tissue. Among topics in active remediation are the development of novel polymeric membranes and microporous solids for the entrapment of heavy metals, and the development of hybrid chemo-enzymatic oxidative schemes for aromatics decontamination.

Over the past 30 months of the project, the research direction has targeted at a specific site, Bayou Trepagnier. This is a site contaminated with effluent from petroleum/petrochemical plants and has heavy metals and organic contaminants dissolved in pore waters and absorbed to sediments. The site is important to the Shell Oil Company whose refinery at Norco, Louisiana discharged into the bayou for many years. Shell is interested in plume effects as a consequence of discharging into Bayou Trepagnier. Concerns as to whether changes in water chemistry perturb contaminants present in the sediment and release them into the water column are important. As a result, this cluster will be continuing efforts to understand the effects of water chemistry variation on contaminants, as well as developing methods to decontaminate sediments and water. A noteworthy aspect of this focus is that there is now a detailed knowledge of the distribution of heavy metals in Bayou Trepagnier sediments and water column. The understanding of the hydrology and water chemistry of the bayou has progressed as well. As a result investigators are in the position to develop quantitative models for predicting the fate and transport of contaminants in the bayou.

## Accomplishments:

### Natural Remediation Processes

#### Heavy Metal Chemistry of Bayou Trepagnier

On February 24, 1995, Shell Chemical Co., after receiving final regulatory approval from the Louisiana Department of Wildlife and Fisheries, diverted Norco Manufacturing Complex (NMC) effluent into the Mississippi River. Removal of the Shell discharge from Bayou Trepagnier was the source of considerable anxiety for local environmental organizations trying to restore the bayou. In particular, many were concerned that the reduction in flow at the headwaters would result in a stagnant waterbody with a high concentration of dissolved heavy metals. Over the past year, considerable effort was focused on assessing the impact of the diversion on heavy metal stability and water quality in the bayou. In addition, a series of plume characterization studies designed to determine transport mechanisms for heavy metals were carried out. The results of these and other studies carried out with support from the DOE funded Hazardous Materials in Aquatic Environments of the Mississippi River Basin program and Shell Oil Company will be incorporated in a pollution history for the Pontchartrain Basin to be developed in collaboration with the U.S. Geological Survey.

**Stability of Heavy Metals in Bottom Sediments:** Flowers and Koplitz (1994) conducted a study for Shell Oil Company and the DOE funded Hazardous Materials in Aquatic Environments of the Mississippi River Basin program to predict the impact of effluent diversion on heavy metal stability in bayou sediments. After performing a variety of tests designed to determine whether or not changes in bayou water chemistry would release Lead, Zinc, Chromium and Copper from sediments, it was concluded that removal of the Shell discharge would cause no increase in the dissolved metal load of the water column. In order to test this conclusion, sediment samples were taken along the bayou for total metal and pore water metal analysis using procedures summarized in the above-mentioned study, which are consistent with USEPA protocols (see Plumb, 1981; USEPA, 1986). Heavy metal profiles are generally consistent for the two years (Fig. 1; see Fig. 4 for station locations); notable exceptions are "spikes" observed in the heavy metal profiles for Lead and Chromium in both years. Pore water analyses indicate that, with the exception of Chromium the pore water load is less than (for Zinc and Copper) or approximately equal (Lead) to that recorded last year. Low concentrations observed in sediment pore waters indicate that metals remain bound to sediment particles. The observed decrease in the average metal content of sediments, although consistent with a large scale release of metals, probably is due to a paucity of samples at each station. As noted by Flowers and Koplitz (1994), the distribution of heavy metals in the sediment is highly variable and subject to change due to erosion of spoil banks and motor boat activity. It should be noted in this regard that water quality monitoring by Dr. Flowers detected only one exceedance (21 ppb Lead) of chronic water quality criteria since diversion.

**Stability of Heavy Metals in Spoil Banks:** As can be seen in Fig. 4, spoil banks composed of sediment dredged from Bayou Trepagnier occur mainly on the spillway side of the bayou. Preliminary data indicate that spoil bank sediments are contaminated mainly with Lead and to a lesser extent Zinc. Elutriate tests performed with spoil bank sediments and water (pH 6.7) from the bayou indicate that Lead in the spoil banks is unstable and readily dissolves in bayou water (Fig. 2). It is not unreasonable to conclude from this data that rainwater (pH 5) falling on the spoil banks would dissolve and transport Lead into the bayou. Spoil banks are therefore the major source of contaminated water and sediment in the Bayou Trepagnier ecosystem. This assertion is corroborated by Dr. Thein's biomarker

study using trees along the bayou in which he notes an excellent correlation between Lead contamination in tree cores and the spoil banks.

**Metal Contamination in Lake Pontchartrain:** The metal profiles given in Fig. 1 indicate that metal contamination in Bayou Trepagnier sediments decreases toward the confluence with Bayou LaBranche (see Fig. 4). Bottom sediment samples were taken in Lake Pontchartrain near the mouth of Bayou LaBranche to determine whether or not a contaminant plume is present in the lake. For all metals considered, the mean and median concentrations are below the corresponding values for the average shale. This observation is also consistent with the conclusion reached above that metals in the bayou remain bound to sediment particles in Bayou Trepagnier. Although Lead is present in the water column, it is usually present at low concentrations associated with relatively coarse (> 5  $\mu$ ), suspended particulate matter.

**Water Quality in the Bayou:** As mentioned above, environmental groups were concerned that diversion of the Shell discharge would cause Bayou Trepagnier to become a stagnant stream. Environmental assessments completed on Shell's behalf (Suhayda, 1994) indicated that water quality in the bayou probably would not be degraded by removing NMC effluent because it represented a minor part (2% of the discharge caused by tidal variation--the most important source of water level variation) of the total water budget. This conclusion was based, in part, on observations made in the bayou during a three-day shutdown of the discharge in August, 1994. In this experiment, water levels and water quality parameters were measured around the clock every four hours. This study was repeated in August, 1995 in order to begin characterizing seasonal variations in bayou water quality. Although the discharge was a minor component of the bayou's water budget, it significantly affected water chemistry. Comparison of the data for 1994 and 1995 (Fig. 3) reveals that the bayou has changed a lot due to removal of the Shell discharge. The temperature profile for 1994 clearly shows the influence of hot cooling water from the NMC complex on average water temperature. The 1995 profile is more typical of bayous around the lake in that average temperature decreases from the mouth of the lake to the headwaters. Salinity would also be expected typically to decrease upstream as shown in the 1995 profile. Prior to effluent diversion, a salinity minimum existed in the bayou with an increase in average salinity upstream from station 70.

The average pH of bayou water in 1995 was approximately 1 standard pH unit less than average values for 1994, and variability at each station was less in 1995 than in 1994. The higher pH observed in the 1994 data set is probably due to discharge of mildly alkaline effluent from the NMC. NMC effluent is required to have a pH between 6 and 9 standard units. Comparison of dissolved oxygen (DO) profiles shows that DO content increases toward Lake Pontchartrain. The increase in DO at station 60 in both profiles is caused by the inflow of oxygenated water from Engineer's canal (see Fig. 4). In contrast, the oxygen minimum observed at station 90 in 1995 is caused by inflow of oxygen-depleted swamp water. Both profiles show that the DO content of the water column is on average low in the headwaters of the bayou. This contradicts a widely held misconception that Shell's discharge of aerated cooling water kept the bayou from becoming stagnant. Data collected from the reference site, Stinking Bayou, indicates that low DO conditions occur naturally in the headwaters of low-flow delta plain streams. The presence of a canopy over part of Bayou Trepagnier (see Fig. 4) limits *in situ* photosynthetic production of oxygen.

#### Metal-Sediment Interactions at the Fresh-Salt Water Interface

Because the Delta region of the Mississippi River is characterized by a dynamic fresh-salt water interface, changes in the concentration of heavy metals at this interface may occur as a result of the fluctuating pH. Values of the pH at this interface change from approximately

5.0 (fresh water) to about 8.5 (salt water). Seasonal changes due to the rise and fall of the river are reflected in the acidity change of the wetlands in the near vicinity of the river. It is, of course, desirable to keep harmful metals concentrated in the sediments rather than in solution where they are more apt to cause damage to aquatic life that form an important part of the ecosystem. The purpose of this study is to obtain a better understanding of variables that influence the adsorption and desorption of heavy metals at this dynamic fresh-salt water interface, such as exists in Bayou Trepagnier. These variables include pH, salinity, organic acid concentration, and speciation of heavy metal cations in solution.

Studies during the past year were focussed on the adsorption and desorption of Pb and Cd on river sediments. Studies were performed on an Upton, Wyoming montmorillonite,  $M_{0.21}+3(A_{13.06}Fe_{0.32}Mg_{0.66})Al_{10.1}Si_{7.9}(OH)_4$  (Ross and Mortland, 1966). Montmorillonite together with kaolinite and quartz silt are the primary constituents of sediments found in Bayou Trepagnier, which is typical of sediments found throughout the Delta region in Louisiana. In addition to the equilibrium adsorption of Cadmium (Ca) and Lead (Pb) as a function of pH, the salting out effect, i.e., the displacement of heavy metal ions by Sodium ions was also studied. In addition to these studies, the effect of organics such as humic and fulvic acids on the adsorption of  $Pb^{2+}$  and  $Cd^{2+}$  was also studied. Studies on the adsorption  $Cd^{2+}$  appear to be a little better defined than  $Pb^{2+}$ . A paper covering this study has been submitted for publication. The effect of salinity on the adsorption of  $Pb^{2+}$  and  $Cd^{2+}$  at different pH are summarized in Fig. 5. The addition of NaCl clearly resulted in a decrease in the adsorption of both  $Pb^{2+}$  and  $Cd^{2+}$  on montmorillonite. The increase in the adsorption of both  $Pb^{2+}$  and  $Cd^{2+}$  with increasing pH at zero ionic strength is consistent with studies obtained in the initial year of this study. This result suggests that heavy metals will desorb and go into solution when river sediments mix with salt or brackish water at the fresh-salt water interface. This salting out effect is partially offset by the increase in adsorption which will result as a consequence of the higher pH of saltwater.

The effect of both humic and fulvic acid on the adsorption of  $Cd^{2+}$  as a function of both salinity and pH is shown in Fig. 6. A sharp decrease in the adsorption of  $Cd^{2+}$  as a function of either humic or fulvic acid was observed at zero ionic strength. At higher ionic strengths, a decrease in the adsorption of  $Cd^{2+}$  was not observed. Similar trends were observed for  $Pb^{2+}$  adsorption. However, the results were not as well defined as those observed for  $Cd^{2+}$ .

In order to determine whether this decrease in the adsorption of  $Cd^{2+}$  is due to the displacement of  $Cd^{2+}$  from the surface by humic acid or by complexation of the metal by the organic, the adsorption of humic acid on montmorillonite was studied. These experiments show that the adsorption of HA on montmorillonite follows a Langmuir adsorption isotherm. The addition of NaCl appears to enhance the adsorption of humic acid. This result suggests that the increase in humic acid adsorption, as a result of an increase in the ionic strength, may result in the displacement of  $Cd^{2+}$  into solution as shown in Fig.6.

#### Laboratory and Field Polarographic Heavy Metal Analysis

Research to date (Berzins et al., 1994), (Bundy and Berzins, 1994 a & b, 1995) has focused upon development of polarographic analytical techniques to analyze water, sediment, and tissue samples for use in 1) assaying heavy metals of environmental concern for plume characterization studies; and 2) understanding the physical/chemical processes which determine the chemical forms and amounts of pollutants released into the environment and their uptake by biota. Several lines of investigation have been pursued in the past year. Based upon our previous field testing at Devils Swamp, a controlled Lead

exposure study was conducted using *Xenopus laevis* tadpoles. Over the seven week developmental period of the animal to transform to a frog, the tadpoles were exposed to one, five, and ten times the Lead concentrations that had previously been measured in Devils Swamp water and sediment. Kaolin was used as the laboratory sediment analog. This animal model was found to be a sensitive predictor of the effects of pollution. No differences in body weight or developmental retardation were noted for the 1X group compared to control tadpoles, but morbidity was clearly noted at the 5X and 10X concentrations. Heavy metal uptake increased over time, but in terms of tissue concentration, was only increased at the very end of the exposure period (as the animal rate of growth slowed down).

Another main line of investigation area that progressed very well in the past year involved research related to field sensor development to aid in pollutant plume characterization of Bayou Trepagnier and Devils Swamp. In the polarographic method the electrical potential of a substrate is changed, and ions are reduced at its surface. Different chemical species can be identified because each has a unique potential where the reduction current is maximized. Although in a laboratory setting the substrate surface is a drop of Mercury, this is not feasible for a field sensor. A graphite surface was investigated instead, based upon its ready availability, particularly in forms with large surface area per unit volume (an advantage when working at trace level concentrations). Eight different polarographic methods have been investigated for suitability, using test solutions which duplicated those available under field conditions. In all cases the method which performed best was cyclic voltammetry. Initially, Lead was investigated by adding it to a citric acid supporting electrolyte, to assess the basic feasibility of the approach. Next, a method was developed to digest soil that would be suitable for field use. Five minutes of cold digestion in a shaken container containing HCl at room temperature were employed. Afterwards the elutant was filtered (a process easily duplicated in the field with syringe filters) before being added to citric acid. Soil taken from a spoil bank known to be highly polluted was analyzed in this manner. This simulated field test confirmed that the soil was highly polluted with Lead.

In other research in the past year, conducted laboratory tests were conducted to expand the heavy metal detection capability beyond Lead, tri-, and hexavalent Chromium. Analyses for Cadmium(II) and Copper (II) in water have been perfected. As with Chromium, these are heavy metals whose toxicity is very dependent on their oxidation states.

In other work, the utility of polarography to determine the degree to which heavy metals are combined with organic ligands was studied. When the ligand's molecular weight is high enough, the metal partitioning between the free ionic and complexed states can be determined by filtration and separate analysis of each moiety. However, heavy metal pollutants can also combine with low molecular weight organics which can not be filtered out. Analyses using AAS and ICP measure total metal content, but can not reveal the relative partitioning between the two fractions in such a case. Since ionic and complexed fractions can differ widely in toxicity, it is important to know what percentage of metal goes into each. In the example above, the only reducible, and hence polarographically detectable, species would be the free ionic portion. The polarographic metal concentration, subtracted from the total via AAS or ICP, would thus give the amount complexed with organic ligands. This idea was validated in the past year using Lead in solutions containing a chelating agent of well known properties (EDTA). Pilot tests with humic acid and Lead were also conducted.

Further research has been conducted regarding sorption of heavy metals to sediment. Our kaolin model sediment studies this year built on the previous Lead and hexavalent Chromium data. In the present project year, trivalent Chromium sorption was investigated,

emphasizing the influence of pH on adsorption/desorption behavior. Maximum adsorption seemed to occur around pH 4, which was not reversed at pH 6 after 24 hrs. These studies were in part performed to aid in planning the tadpole Chromium exposure study to be conducted next year.

## Chemical Separation and Remediation Processes

### Metal-Ion Sequestering and Organic Species Resins and Polymers

Phosphazene Membranes. Recent developments in the area of phosphazene polymers have aroused considerable interest in their potential technological applications (Allcock et al., 1983a; Allcock et al., 1983b; Blonsky et al., 1984; Shaw et al., 1962; Cohen et al., 1995). Phosphazene polymers, by virtue of their stability to thermal degradation and chemical attack, are particularly suitable for use in the cleanup of wastes containing hazardous metal ions and radionuclides. The properties of the polymers are determined by the types of side groups attached to the phosphorus-nitrogen backbone. Because these side groups can be modified easily by nucleophilic substitution and exchange reactions, phosphazene polymers can be fabricated to yield materials with the targeted desired properties. The goal of this subproject is to develop: 1) ion-exchange membranes made from phosphazene polymers suitable for use in selectively sequestering hazardous metal ions and radionuclides; and 2) neutral polyphosphazene membranes for organic/water and organic/air separations.

To date, significant progress has been made in the synthesis and development of phosphazene-based membranes. Not only have been produced phosphazene materials with metal sequestering side groups (Wang et al., submitted), but an alternate method for producing membranes without the need for the presence of photoinitiators has been devised. This was done by maximizing the crystallinity-property of the materials, or incorporating a side group attached to the phosphazene skeleton that would undergo crosslinking thermally. These are described further below.

One of the goals was to fabricate a polyphosphazene membrane with sulfonic acid ion exchange sites that would not dissolve nor swell excessively in aqueous solutions. To accomplish the latter requirement some degree of crosslinking of the polyphosphazene polymer was required. This can be achieved by a chemical reaction and/or by use of a polymer which is semicrystalline. It was decided to combine both approaches and prepare a semicrystalline membrane of phosphazene polymer with some degree of chemical crosslinking (achieved by photocrosslinking).

Two schemes were adopted for the ion-exchange membrane preparation: Method 1 - Polyphosphazenes containing ethylphenoxy-phenoxy and methylphenoxy-phenoxy substituents are synthesized, e.g., poly[(3-ethylphenoxy)(phenoxy)phosphazene]. The ethylphenoxy or methylphenoxy substituents are used for solid-state photocrosslinking by UV radiation - the hydrogens at benzylic carbons are abstracted by a suitable photoinitiator and the macroradicals that are generated recombine to form crosslinks. In the final step, the phenoxy substituents are sulfonated to obtain the crosslinked ion-exchange membrane. Method 2 - The ethylphenoxy-phenoxy and methylphenoxy-phenoxy polyphosphazenes are first sulfonated in solution, then blended with non-sulfonated polymer and photoinitiator, cast into a dry film, and crosslinked by UV radiation.

The kinetics of the crosslinking of poly[(4-ethylphenoxy)-(phenoxy)phosphazene] (4-EPP; Fig. 7, Structure I) with benzophenone; prepared crosslinked films; performed X-ray, IR and UV studies of the cross-linked films; and measured equilibrium membrane swelling in selected solvents were studied. The results of these studies, which have been accepted for publication (Wycisk et al., 1996), showed that increasing the benzophenone

concentration resulted in a reduction in the degree of crystallinity and in an increase in the film cracking tendency of the membrane when swelled. In order to understand how the pendant side groups affect both the crystallinity properties and the crosslinking behavior of the fabricated membranes, these studies will be extended to a series of polymers: poly[(4-ethylphenoxy)(phenoxy) phosphazene] (4-EPP; Fig. 7, Structure I), poly[(3-ethylphenoxy)(phenoxy) phosphazene] (3-EPP; Fig. 7, II), poly[(4-methylphenoxy)(phenoxy) phosphazene] (4-MPP; Fig. 7, III), and poly[(3-methylphenoxy)(phenoxy) phosphazene] (3-MPP; Fig. 7, IV). Polymers I-IV were synthesized in sufficiently large quantities to allow for their fabrication into membranes.

The following have been accomplished during the past year: 1) performed preliminary kinetic studies of the solid-state photocrosslinking reaction of 3-EPP, 4-MPP, and 3-MPP with benzophenone as the photoinitiator; 2) crosslinked and sulfonated 4-EPP in film form to an ion-exchange capacity of 0.6 meq/g; 3) sulfonated a number of polyphosphazenes in solution prior to crosslinking using SO<sub>3</sub>, acetyl sulfate and chlorosulfonic acid; 4) synthesized a series of poly[(4-ethylphenoxy)(phenoxy) phosphazene] polymers with different ethylphenoxy/phenoxy substitution ratios; and 5) begun to determine the swelling and permeability of organic vapors (benzene and tetrachloroethylene) in crosslinked ethylphenoxy and methylphenoxy polymers.

Based on the results obtained to date it has been concluded that: 1) all of the ethylphenoxy and methylphenoxy substituted polymers examined to date can be crosslinked in the dry-film form; 2) the same polymers can be sulfonated in solution prior to film casting and crosslinking; and 3) to date, 3-MPP appears to be the most promising material for ion-exchange membranes; it is semi-crystalline so it will not require extensive crosslinking for good mechanical strength and it can be sulfonated using SO<sub>3</sub> or acetyl sulfate.

#### Phosphate-Based Inorganic Ion Exchangers

Inorganic ion exchangers or sorbents are attracting more and more attention in environmental protection due to their chemical and thermal stability, as well as high selectivity to certain ions. Examples include the Group IV element phosphates (Bortun and Strelko, 1992), layered titanates (Anthony, Philip and Dosch, 1993), amorphous zirconium phosphate (Zamin, Shaheen and Dyer, 1994), and monophenyldiphosphonate phosphates of zirconium (Clearfield, 1992; Clearfield and Yates, 1995). Most inorganic ion exchange materials have been synthesized with an aim to remove the radioactive Cesium and Strontium from liquid nuclear effluent. The proposed project has a two-fold purpose. One is to synthesize microporous material with large pore sizes and selective cation exchange properties. The other is to evaluate the capability of the synthetic inorganic ion-exchangers to remove heavy metals and radioactive Cesium and Strontium, and study the effects of physical-chemical properties of the materials on ion exchange behavior to provide information for developing new materials.

During the last year the effects of temperature, heating period, composition, and pH of the starting materials, and organic templating agents on crystal growth under hydrothermal conditions have been extensively investigated. A large number of phosphate based complexes have been prepared. Some of them have been characterized by a variety of physical methods. The characterization of the remaining materials is still in progress. Cobalt substituted aluminum phosphate was selected to do ion exchange study. The microporous aluminum phosphate (Clearfield, et al., 1989) has attracted our interest on account of its straight channel system with large pore opening of 8.0Å that allows hydrated metal ions (typically, hydrated monovalent ion radius ranging from 3.0Å to 4.0Å; and divalent ion radius ranging from 4.0Å to 5.0Å,) to move in and out of the channels. In addition, it has high chemical and thermal stability. It is expected that the partial

substitution of divalent metals, such as alkaline earth metals or transition metals, for Al(III) will result in negatively charged absorptive sites, and thus, ion exchange capacity in these porous materials. Little detailed study has been done on their ion exchange properties.

CoAlPO<sub>4</sub> was synthesized under hydrothermal condition using triethylamine as a template agent (Clearfield, et al., 1989), and characterized by X-ray powder diffraction, SEM, FTIR, TGA, and DTA. The CoAlPO<sub>4</sub> was heated up to 480°C in an open furnace to remove the template, and then was treated with 1.0M NH<sub>4</sub>Cl solution at 60-70°C to exchange out whatever species located in the ion-change sites. The selectivity of cation exchange was tested using the AA standard solutions of 100 ppm metal at a ratio of 50 ml solution per 3 grams of the solid material. The cation selectivity was found to increase in the order of Fe(II)<Pb(II)<Cr(II)<Cu(II)<Ni(II)<Mn(II). The XRD shows the same pattern for unsubstituted AlPO<sub>4</sub>, cobalt substituted AlPO<sub>4</sub>, heat-treated, and ion-exchanged material, indicating the material is thermally and chemically stable.

#### Remediation of Aromatics by Chemical and Enzymatic Methods

Aromatics are major pollutants found in aquatic environments and in sediments. While there are many chemical and biochemical processes to remove and/or treat these contaminants, they have to be considered in light of the economics and the time-scales for treatment.

Two separate studies are proposed here based on the research over the last couple of years. In the previous research, attempts were made to couple the chemical oxidation of aromatics through mild Fenton type mechanisms to enzymatic oxidation of phenolic species in order to produce polymeric compounds that are insoluble in solution. In continuing research, these two oxidative mechanisms will be studied separately in order to have more direct relevance to DOE "Hazardous Materials in Aquatic Environments of the Mississippi River Basin objectives.

To, it has been demonstrated that aromatic hydrocarbons can be oxidized using chemical and enzymatic techniques (Xu, et al., 1995). In this reaction scheme, the aromatic is first converted to the corresponding phenol using classical Fenton-type chemistry involving catalysis by ferrous iron. The phenol is subsequently polymerized through an enzymatic mechanism, using horseradish peroxidase as the oxidative enzyme. The polymer is insoluble in water and can be easily recovered. In addition, such polymers are useful products with varied applications in coatings and resins technologies. Aromatic pollutants can thus be treated and recycled.

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Xu, X., John, V.T., McPherson, G.L., D. Grimm, J. Akkara, and Kaplan, D.: "Chemical+Enzymatic Method to Remove Aromatics from Aqueous Streams," ACS Div. Environmental Chemistry, 34, 13, 1994.

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Xu, X., John, V.T., McPherson, G.L., Grimm, D., Akkara, J., and D. Kaplan: "A Combined Chemical-Enzymatic Method to Remove Aromatics from Aqueous Streams," Preprints ACS Div. Environmental Chemistry, 51/52, 649, 1995.

Flowers, G.C., Koplitz, L.V., and McPherson, G.L.: "The Impact of Hurricane Andrew: Changes in the Texture and Chemistry of Barataria Estuary Bottom Sediments," Transactions Gulf Coast Association of Geological Societies, 45, 189, 1995.

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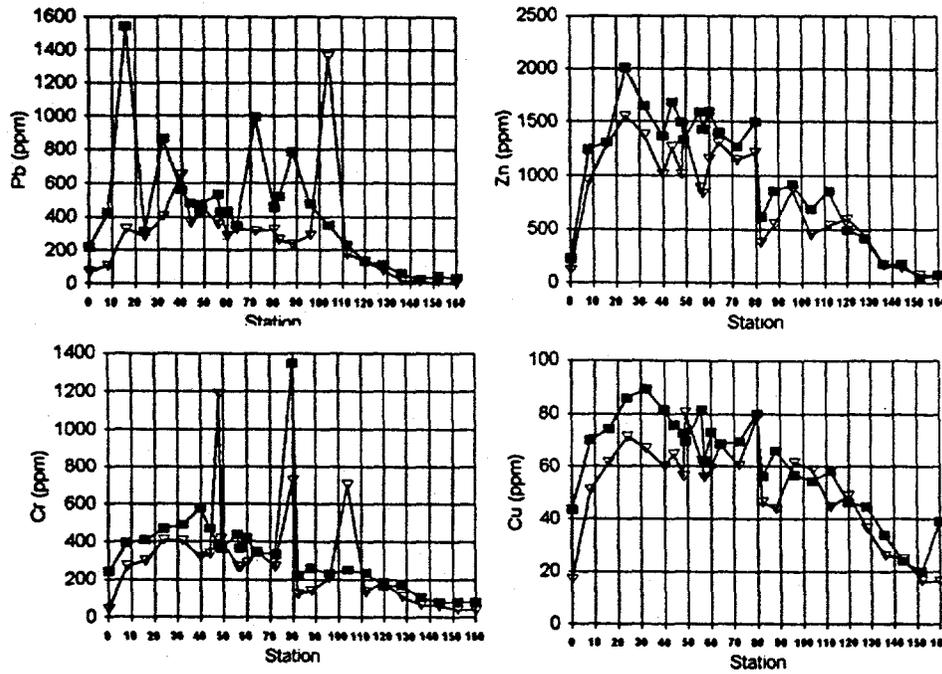


Figure 1. Distribution of Pb, Zn, Cr, and Cu in bottom sediments of Bayou Trepagnier for samples taken in 1994 (squares) and 1995 (triangles).

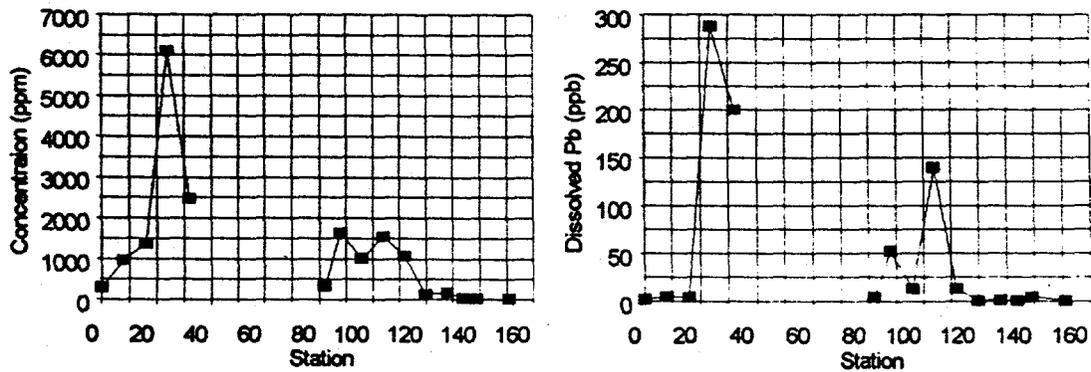


Figure 2. The left-hand graph shows the distribution of Pb in soils taken from spoil banks along Bayou Trepagnier. Results of elutriate tests performed with spoil bank samples and water from the bayou are shown on the right. On both graphs, a data gap occurs because landowner permission for access was not forthcoming along that portion of the spoil banks.

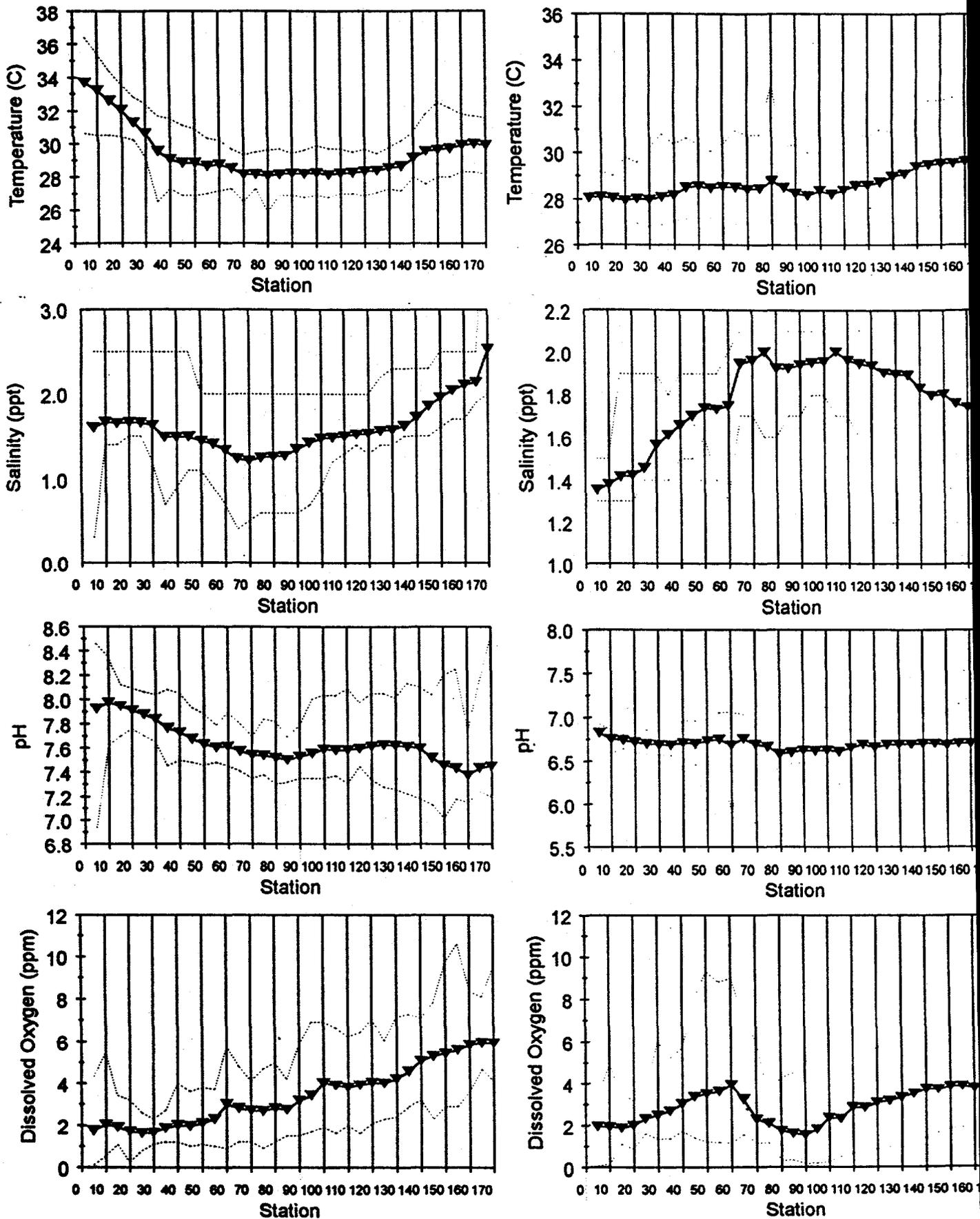


Figure 3. Water quality variations observed in Bayou Trepagneir during the second week of August in 1994 and 1995. For each parameter, the minimum, maximum, and average for a total of 17 and 19 profiles taken in 1994 and 1995, respectively.

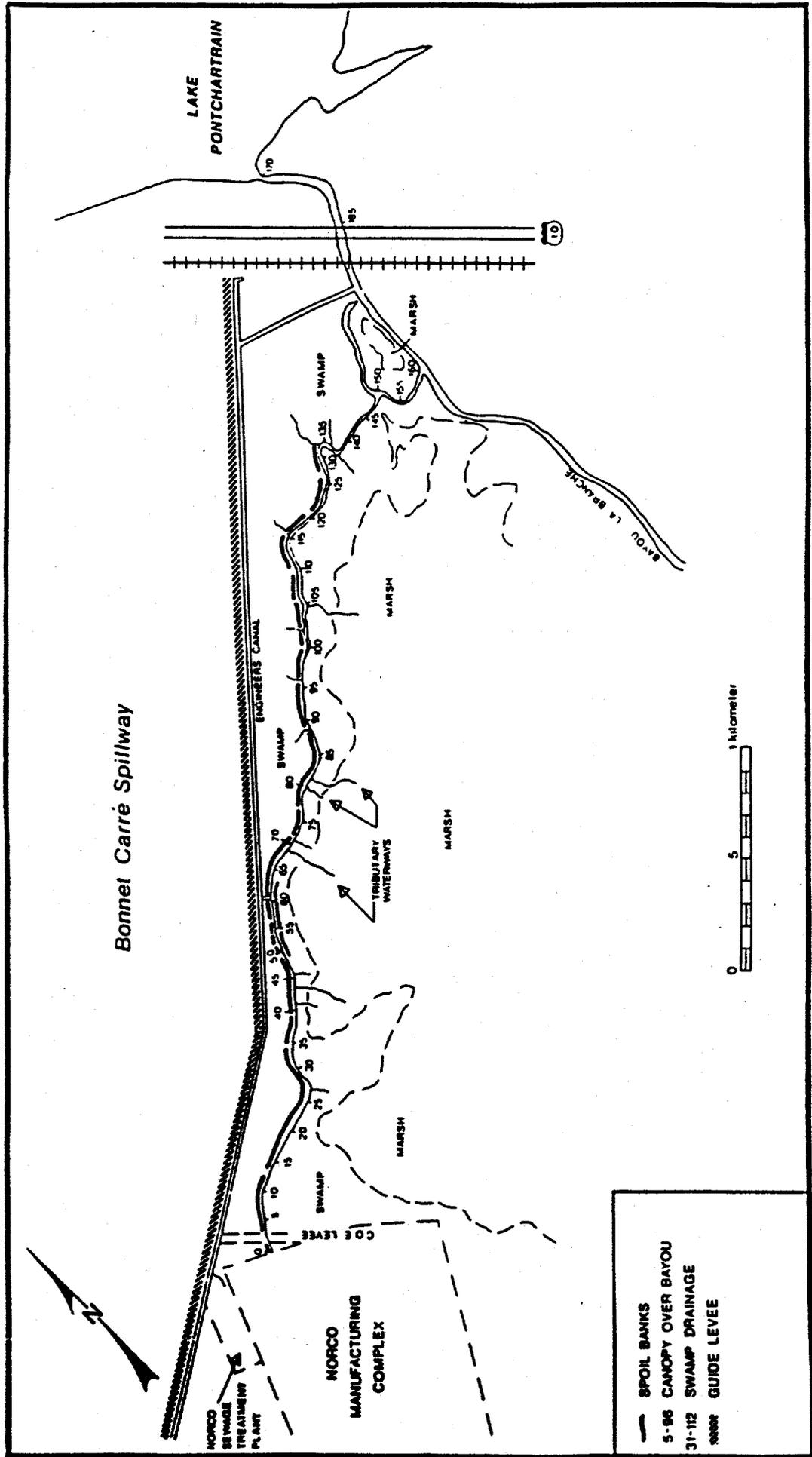


Figure 4. Location map for Bayou Trepagnier showing spoil banks and sample stations.

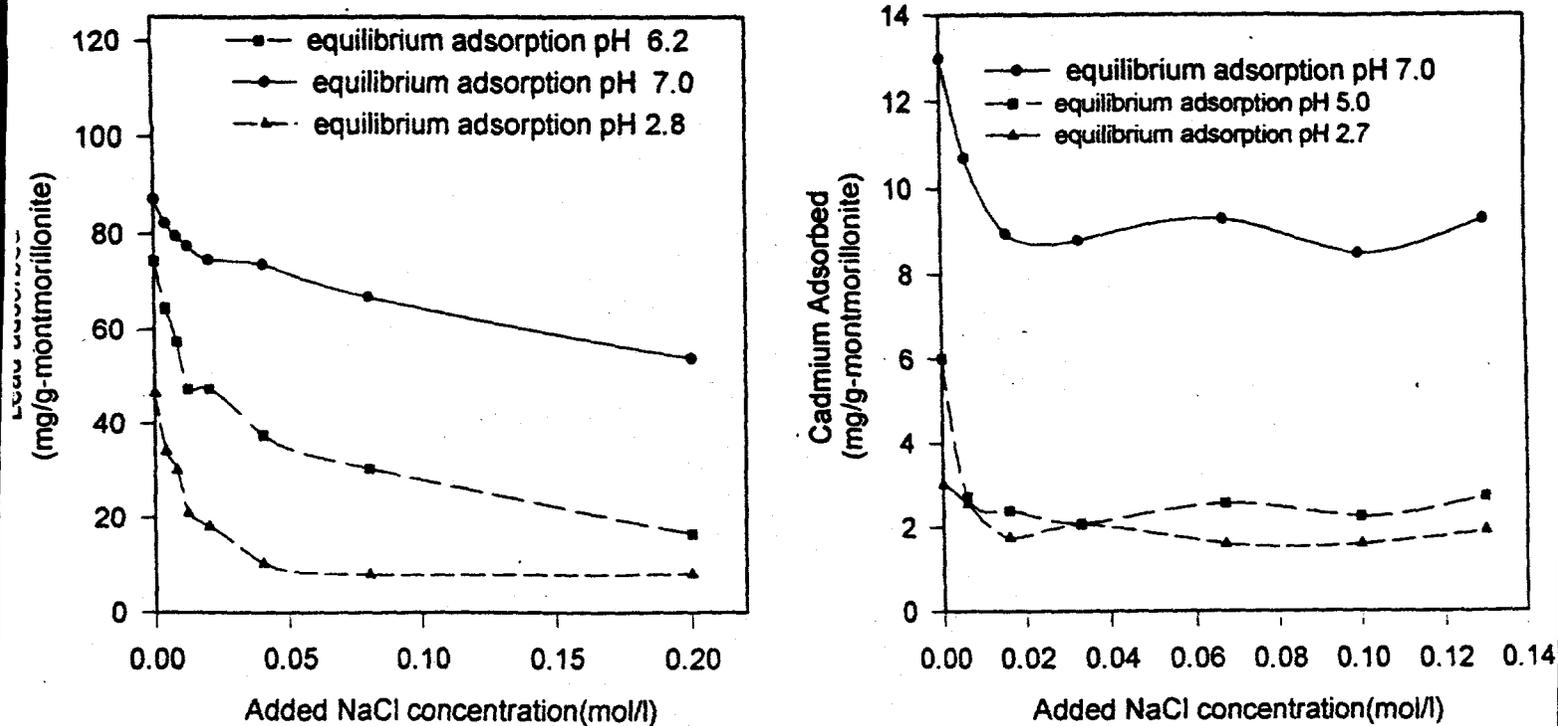


Figure 5. Salinity effect on  $Pb^{2+}$  (left) and  $Cd^{2+}$  (right) adsorption onto montmorillonite as a function of equilibrium pH. In both cases, the initial concentration of the dissolved metal was 8 ppm, and the contact time was 48 hrs.

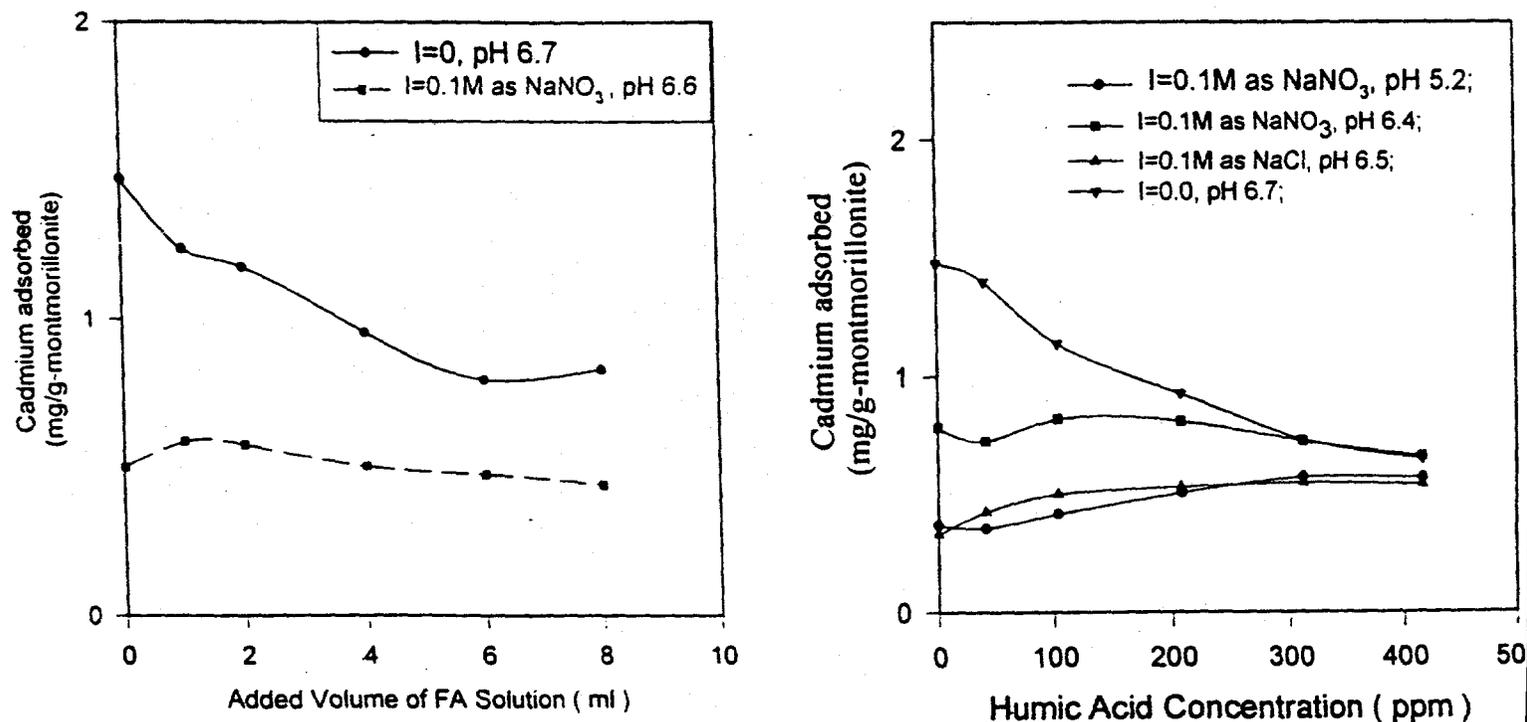


Figure 6. Effect of fulvic (left) and humic (right) on the adsorption of  $Cd^{2+}$  at different ionic strengths and equilibrium pH's.

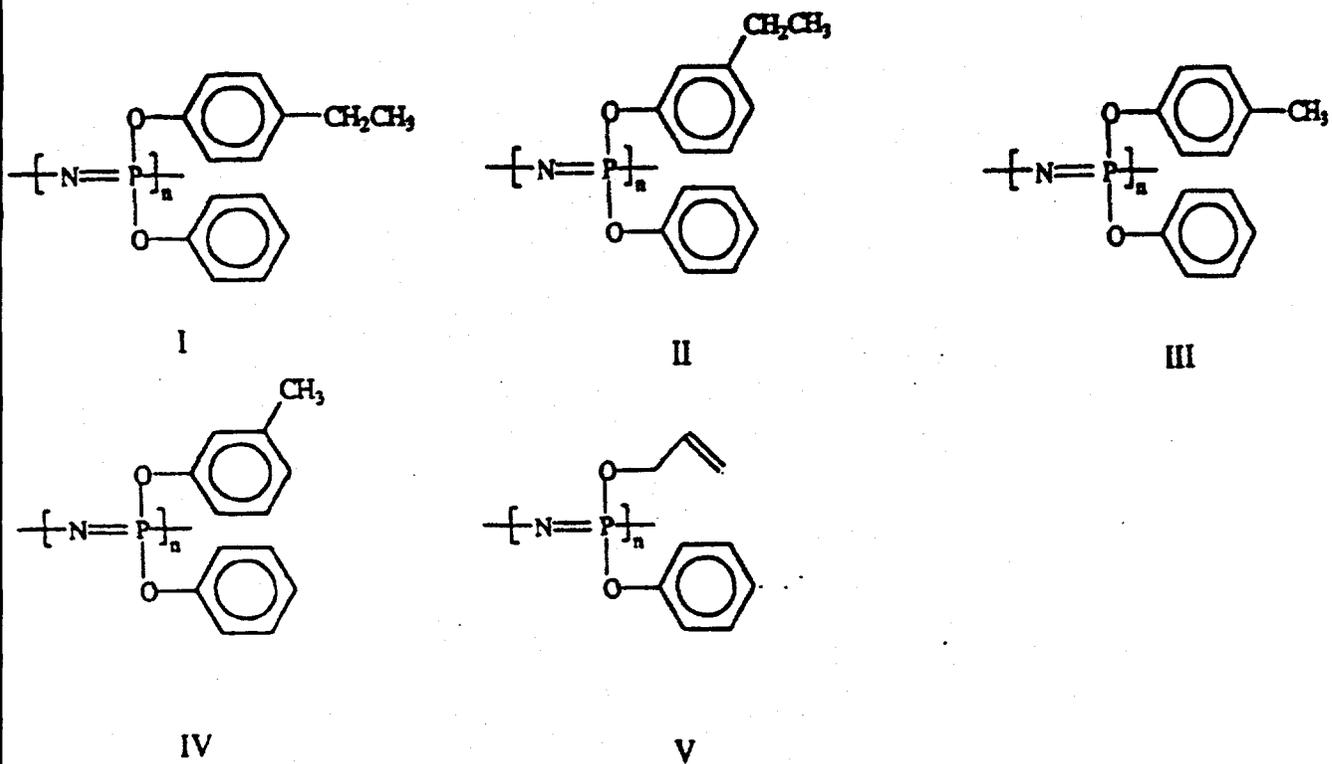


Figure 7. Structural units of polyphosphazene polymers (see text).

## **Expert Geographical Information Systems for Assessing Hazardous Wastes in Aquatic Environments**

J. Regens, R. Bakeer, M. Barber, B. Belkhouche, J. Hughes,  
A. Rene, L. White, J. Wright

### **Objectives and Goals:**

The primary objective involves the design and application of geographic information systems (GIS) to evaluate key indices of heavy metals bioaccumulation and histopathological effects on selected fish species in the Devils Swamp and Bayou Trepagnier ecosystems to integrate data collected by other project clusters.

Specifically, Project Element 1 (Dr. Regens' group) constructed a chemical and histopathological database, developed coverages and performed analyses for Devils Swamp.

Project Element 2 was coordinated by faculty from the Departments of Civil and Environmental Engineering, and Computer Science at Tulane. The research group included two full-time graduate students and part-time undergraduate students. Project Element 2 has focused on: 1) Developing and implementing a smart environmental GIS using the SMART ELEMENTS expert system shell, the ARC/INFO GIS software, the relational database management system ORACLE, and the ground water modelling program MODFLOW; and 2) designing the conceptual databases necessary to model various thematic data describing geographical, topographical, and environmental features of Bayou Trepagnier. Implement and populate the databases using the relational DBMS ORACLE with data obtained from different State and government agencies.

### **Accomplishments:**

#### Project Element 1

Data from several sources were combined to generate the base map of Devils Swamp (Figure 1). Devils Swamp hydrography was digitized from 1:24,000 scale maps of the Scotlandville and Walls quadrangles. The roads and other features of the map were obtained from 1:100,000 scale USGS digital data. The base map is a composite of all these individual elements which can be manipulate as needed (e.g., zoom in or out, add or eliminate features, roads, railroads, hydrography, etc.).

An essential component of any analysis is understanding the system. Toward that end, maps of the physical characteristics of Devils Swamp were generated. Figure 2 is a map of the slopes derived from USGS digital line graph elevation data. The region is relatively flat (slopes range from 0 to 18%) with the highest and steepest areas being near the waterways. The elevation and slope maps provide insight, for example, into how a spill might travel on dry ground since the swamp is only seasonally flooded.

The most recent USDA-Natural Resources Conservation Soils Survey provided the information for creating the soils map (Figure 3). The Crevasse soils have the highest permeability, and the Sharkey-Tunica soil type has the lowest permeability. A map of the

soil permeability has fewer color distinctions since different soil types can have similar permeabilities. The permeability provides some indication of how subsurface contaminants will travel although other mechanisms are involved as well. These maps provide the basis upon which layers of sampling data can be superimposed to investigate the natural mechanisms affecting the transport of contaminants.

As part of the effort to organize the data collected, a database was set up to store and make accessible the data from the Sampling cluster (Dr. Bart et al.), the Analysis cluster (Dr. Abdelghani et al.), and the Histopathology cluster (Dr. Hartley et al.). Electronic data formats were agreed upon to facilitate ongoing transfer between groups. Quality assurance and quality control procedures were established in two parts. A first level check was made by the data entry personnel to confirm that no errors occurred in transporting and manipulating the data sets. Second, the individual investigators cross checked the information in the database with their original data and provided feedback to the database manager.

Using Arc/Info, a graphical user interface was set up to query the database containing field sampling data, environmental variables, histopathological and analytical chemistry results. As an example, the menu in Figure 1 displays the results of querying the samples database. There were a number of fish samples obtained during each collection period which the user can scroll through by clicking on the "next" button to retrieve the characteristics of each sample. Each marker at the sampling sites refers to a collection trip. In this way, one can quickly see how many times a location was sampled in 1994 and 1995. Additional graphical queries can be programmed based on an individual's needs for retrieving and displaying the data.

Historic data from the Environmental Protection Agency's 1992 site investigation were obtained for Devils Swamp Lake and Bayou Baton Rouge from Louisiana's Department of Environmental Quality. A graphic display was produced to display the sediment contamination in Devils Swamp Lake to compare the past presence of metals (Arsenic, Cadmium, Chromium, Lead, Mercury, and Nickel) with current findings. Each metal was detected in one or more sediment samples taken from Devils Swamp Lake during the 1994 and 1995 sampling years.

This study has resulted in the generation of several thematic maps of Devils Swamp providing some insight into the geographical characteristics of the region. More importantly, an infrastructure has been developed to work collaboratively with other investigators collecting data at Devils Swamp. The storage and retrieval of these data has been simplified for the user with the development of a menu driven interface. The geographic information system provides a useful tool for facilitating the exchange of information, displaying spatially distributed data, and elucidating geographical features affecting the transport of contaminants.

### Project Element 2

The GIS facility at the Uptown Campus of Tulane is now fully operational. It consists of the following hardware: a SUN 20 Model 502MP acting as the main server; three stations (SPARC 5 Model 85) providing multi access to the server; four PC's used for data entry and small GIS projects (e.g.: ArcCAD and ARC/INFO PC); two color scanner with OCR; two size E digitizers; two size E plotters; and several other peripherals. In addition, several software packages were installed. These include a C compiler; Email utilities; networking subsystem; SMART ELEMENTS expert system shell and Graphic User Interface (GUI) development system; ORACLE relational database management system (DBMS); ARC/INFO geographic information system (GIS). Other software and hardware were

added to the facility from other external and local resources such as ERDAS and S+. The GIS hardware forms a local area network (LAN), and is a node on the campus wide network.

The design of a friendly user-interface was completed. A high quality human-computer intelligent interface was designed to both support and facilitate the decision process for environmental impact assessment; that is, it helps the user to assess the impact of chemical contaminants in the environment through the analysis of the transport of those agents in the groundwater flow. The major components of the system have been implemented. An interface for MODFLOW to both read and write in the GIS ARC/INFO has been designed.

Data related to Bayou Trepagnier, Devils Swamp and New Orleans were collected from LDEQ, USGS, USACOE, private companies and others and then decoded. Data was collected at two water treatment stations on the Mississippi River, one downstream (St. Francisville), and one upstream (Plaquemine). Data was also collected at nineteen (19) locations in the Devils Swamp Lake Drainage System. These data describe PCB concentrations in soil and sediment samples from the lake and various tributary channels within the lake watershed. Other data acquired are: USGS paper quad maps of Bayou Trepagnier, Devils Swamp and New Orleans, spatial data (geological maps, soil boring location maps, sediment samples location map, push tubes location maps, visible and not visible contamination map, surface water analytical map, multi-layer waste mapping, subsurface geological profiles, vibrocores samples location, ground water analytical sampling location); and thematic data (soil borings, ground water analytical sampling, push tubes, core barrels, and surface water analytical sampling).

A graduate student has completed her Master thesis entitled "Geographical Information System for Devils Swamp and Bayou Trepagnier". Plates 1 through 4 are products of this research. An undergraduate student has completed a senior Honor thesis entitled "A Geographical Information System Application: A Boring Data Retrieval Program for Kenner, Louisiana". Typical screens from this software are shown in Plate 5.

Based on the expanded GIS capabilities of the uptown campus, a research team including some of this project's investigators was awarded a major research grant to conduct a GIS study on the Mississippi River.

The oracle conceptual data model was implemented. This model captures information about Bayou Trepagnier in particular. Spatial data around Bayou Trepagnier was created by AutoCad digitizer, including highway layer, water channel layer, structure layer, oil tank layer, geometry coordinate layer and simple text description layer. The following information was collected:

- Project Completion Report, Sediment Analysis and Sedimentation in Bayou Trepagnier, which contains information about metals, oil and grease and phenols in the samples.
- Lake Pontchartrain, Louisiana, and Vicinity Hurricane Protection Project, Evaluation of Impacts to Scenic Streams, Landfills Oil and Gas Waste Pits and Levee Realignment.
- Benthic and Epibenthic Communities of Bayou Trepagnier and Bayou LaBranche. The report concerns benthic and epibenthic sampling of macrofaunal organisms in Bayou Trepagnier and Bayou LaBranche during August 1987.
- Preliminary Physical Habitat Characterization Bayou Trepagnier/Bayou LaBranche Ecological Reconnaissance. The report contains stream cross section, sediment physical parameters and laboratory test results.

- Impact Assessment of Bayou Trepagnier. This report includes stream profile and flow data, water quality data, sediment data, fish tissue data.
- State of Louisiana Water Quality Management Plan: Water Quality Data Summary. This report includes water quality data for Bayou LaBranche for 1992-1993.
- Remedial Investigation of Bayou Trepagnier. This report covers water quality data, effluent quality data, sediment chemical analysis, spoil banks, surrounding surface soils, background samples, collection of water samples, collection of core samples, collection of spoil bank and soil samples.

#### **Publications and Presentations:**

Andrade, F.W. "A Geographical Information System Application: A Boring Data Retrieval Program for Kenner, Louisiana," Senior Honor Thesis, Dept. of Civil and Env. Eng., Tulane Univ., 1995.

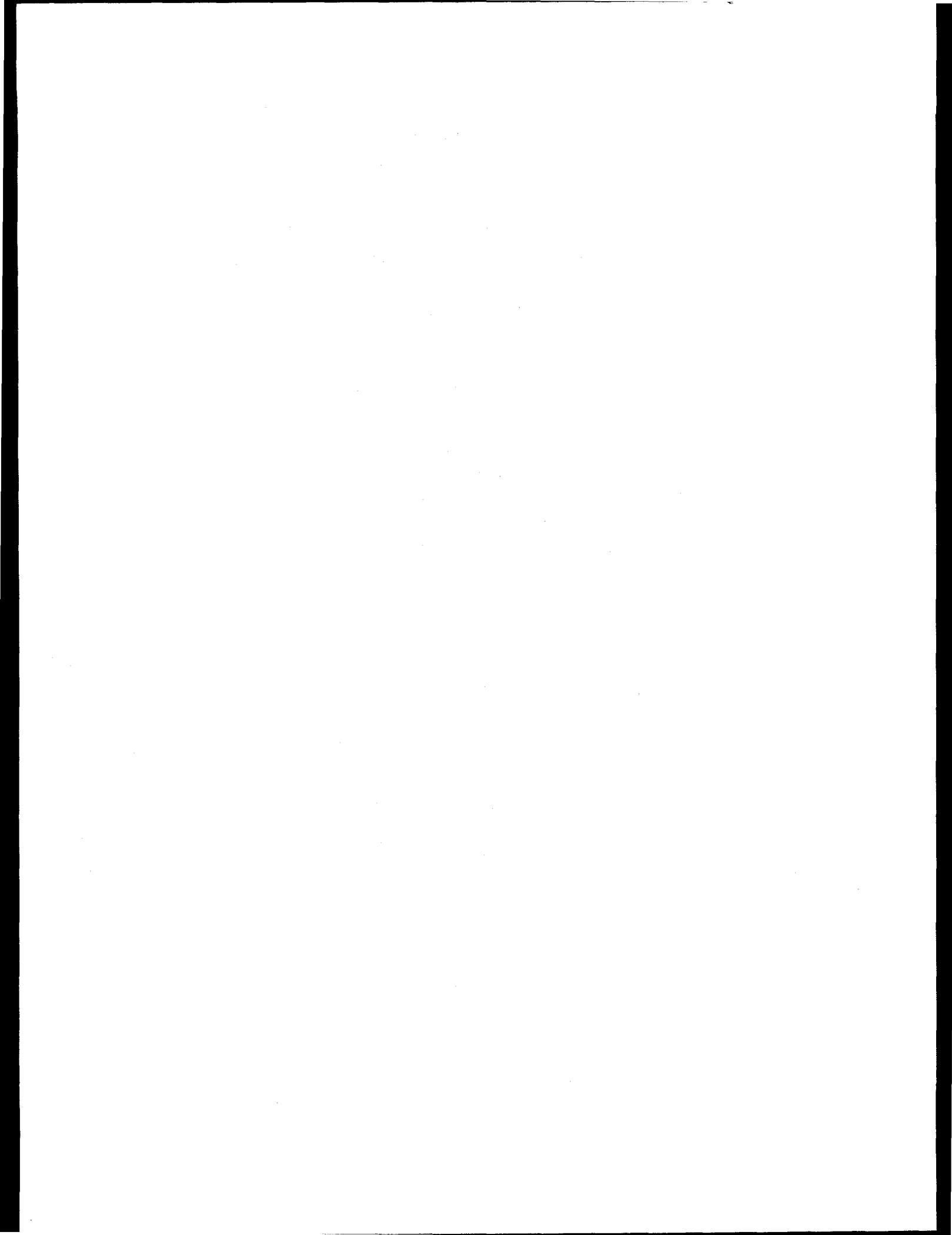
Cui, J. "Geographical Information System for Devils Swamp and Bayou Trepagnier," Master Thesis, Dept. of Civil and Env. Eng., Tulane Univ., 1995.

Regens, J.L., Watanabe, K.H., Hodges, D.G., Johnson, K., and Swalm, C.M., "Applying GIS to Assess the Bioaccumulation of Heavy Metals and Organics in an Aquatic Environment." Poster to be presented at HydroGIS '96, Vienna, Austria. April 16-19, 1996.

Regens, J.L., Watanabe, K.H., Hodges, D.G., Johnson, R., Swalm, C.M., and Davis-Nelson, S.. "Visual Summarization of Data Using GIS for Devils Swamp." Poster to be presented at the 12th Annual Louisiana Remote Sensing and Geographic Information System Workshop, Lafayette, LA. April 16-18, 1996.

Regens, J.L., Watanabe, K.H., Hodges, D.G., Johnson, K., Swalm, C.M.. "Modeling Bioaccumulation of Pollutant Loadings in Aquatic Ecosystems." Poster to be presented at the HSRC/WERC Joint Conference on the Environment, Albuquerque, NM. May 21-23, 1996.

Watanabe, K.H., Regens, J.L., Hodges, D.G., Johnson, K., Swalm, C.M. "The Application of Ecological Modelling to Assess the Bioaccumulation of Contaminants in an Aquatic Ecosystem." Poster to be presented at the Ecological Summit 96, Copenhagen, August 19-23, 1996.



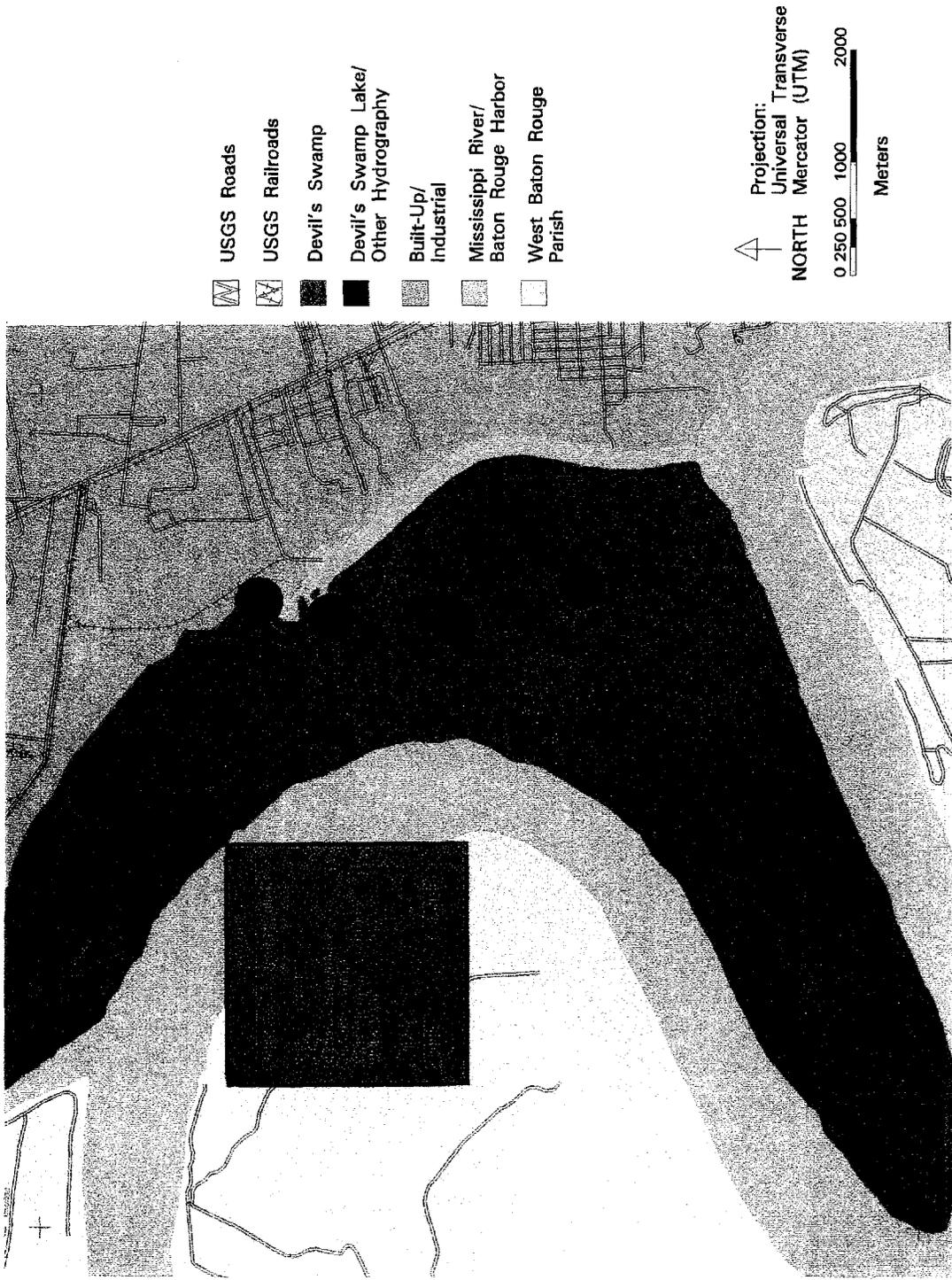
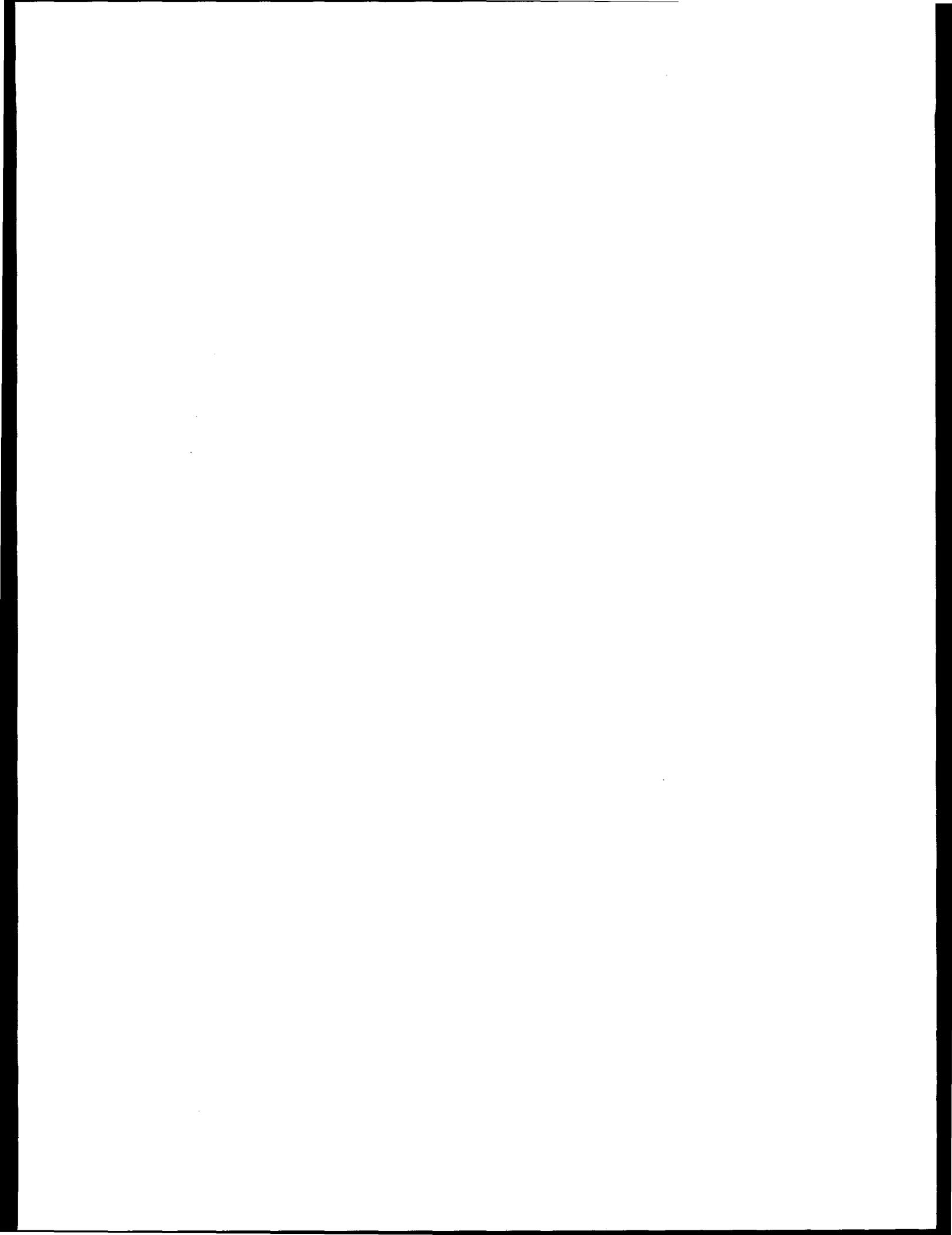


Figure 1: Devil's Swamp base map with Tulane sampling sites labeled. The menu illustrates a graphical method of querying the samples database. Each black point within the red circles represents a collection period. Selection of the point yields a pop-up menu to scroll through the fish samples.



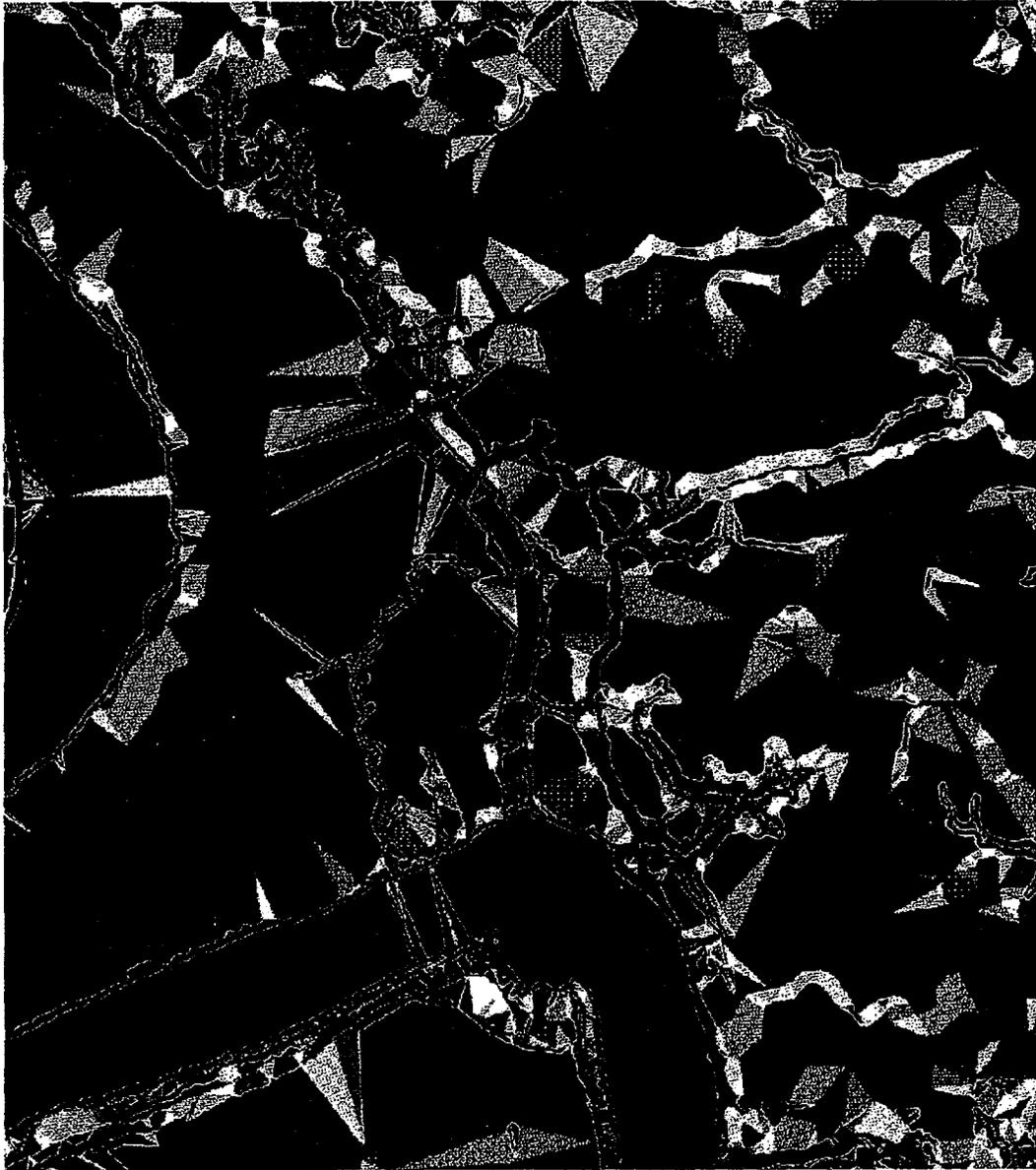
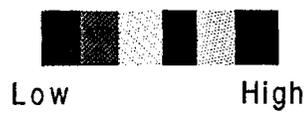


Figure 2: Slope/Aspect Index Derived From Elevation Model



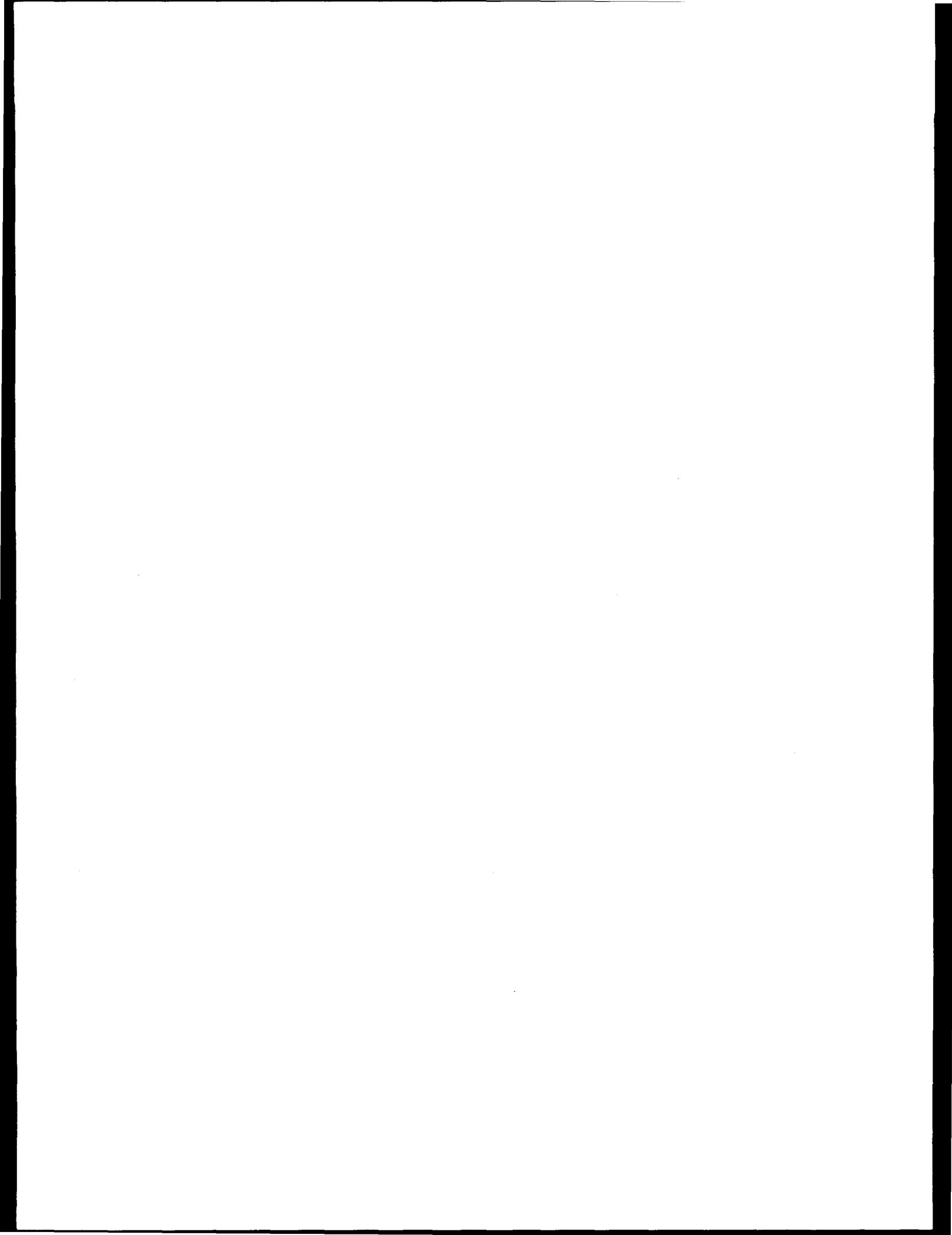




Figure 3: USDA Soils From The Soil Conservation Service Soil Survey  
For East Baton Rouge Parish, Louisiana

- Crevasse soils, overflow
- Loamy alluvial and Mhoon
- Sharkey - Tunica



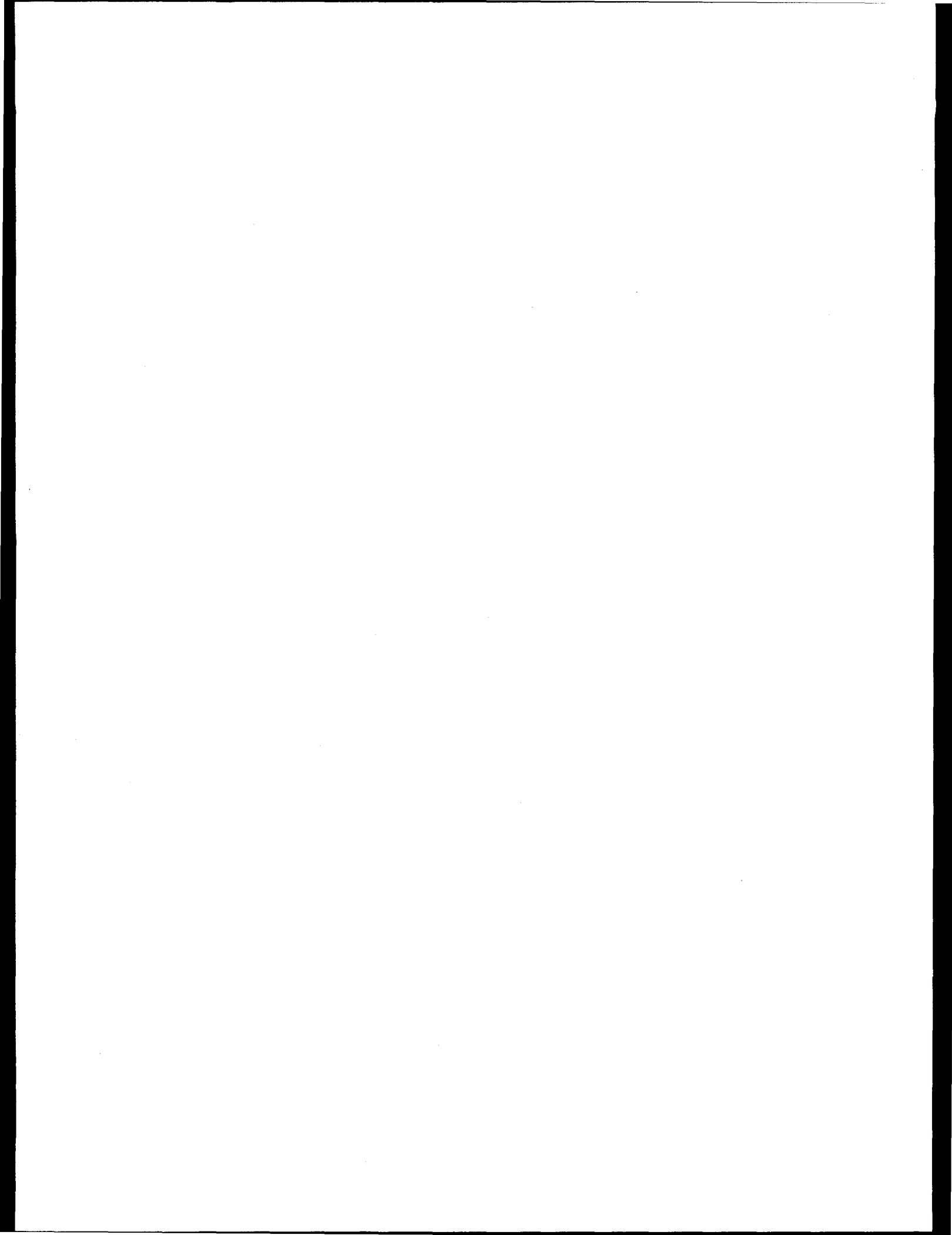
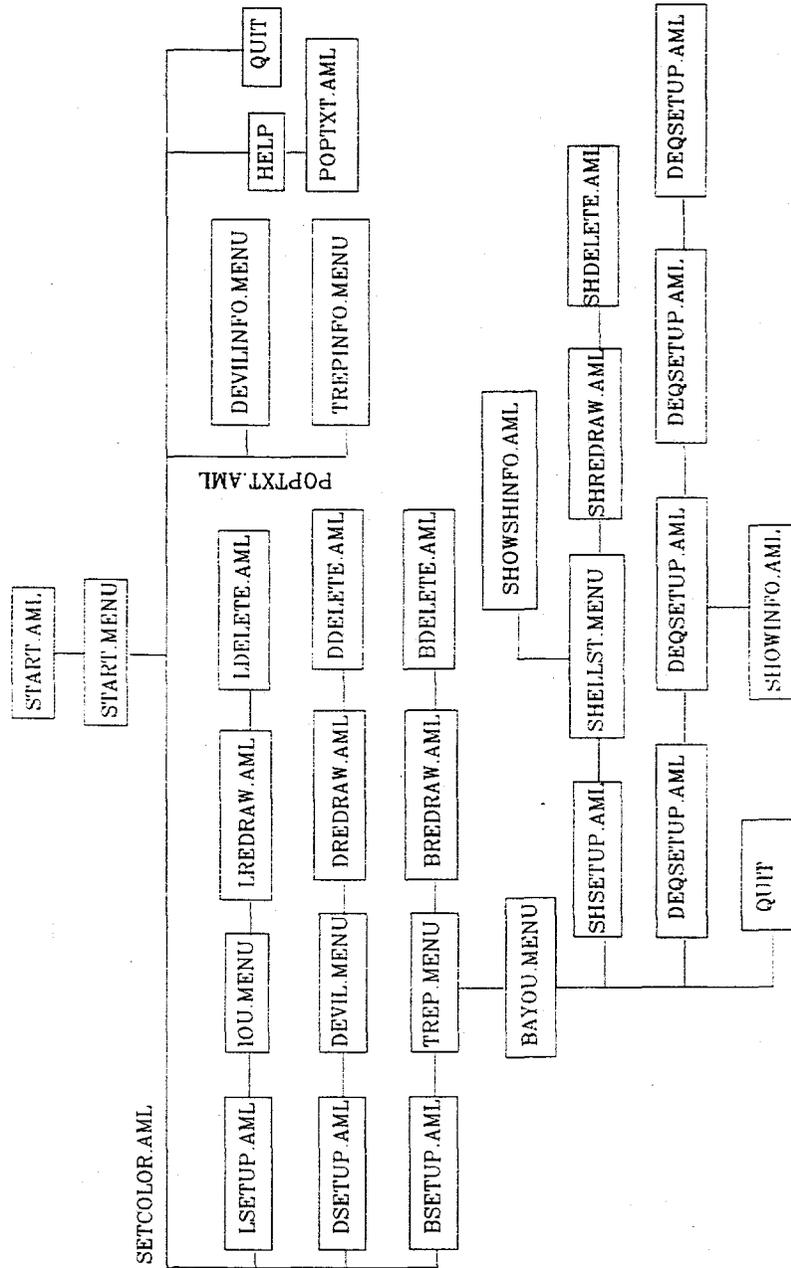
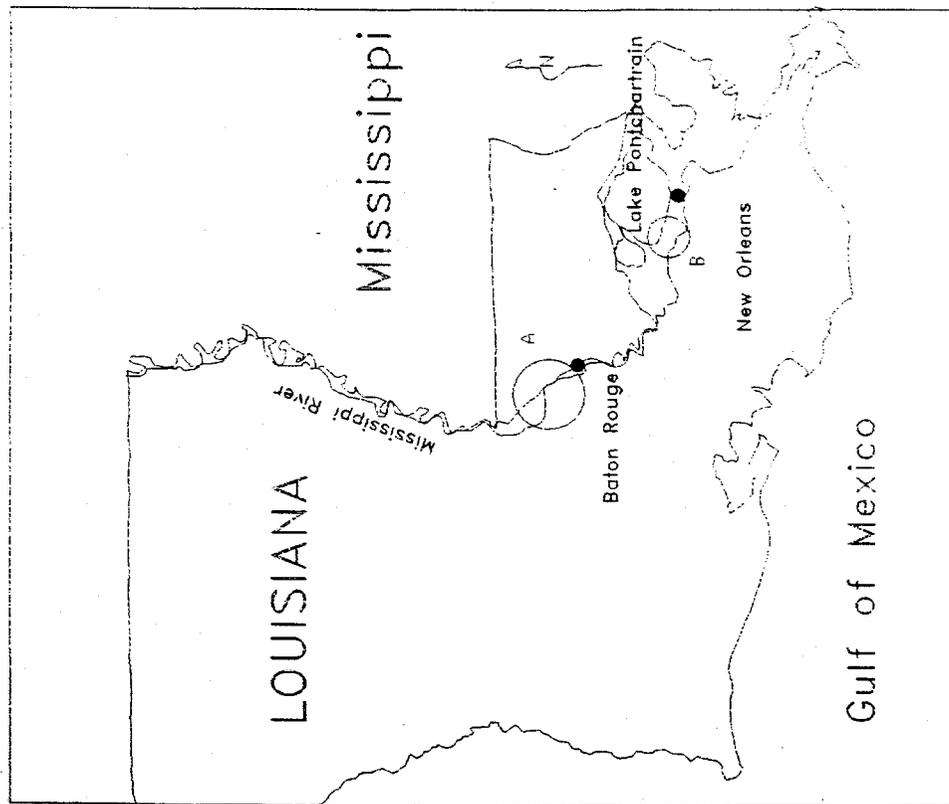


FIGURE 4.1 STRUCTURE OF THE GIS SYSTEM







A: Devil Swamp

Northwest of Baton Rouge

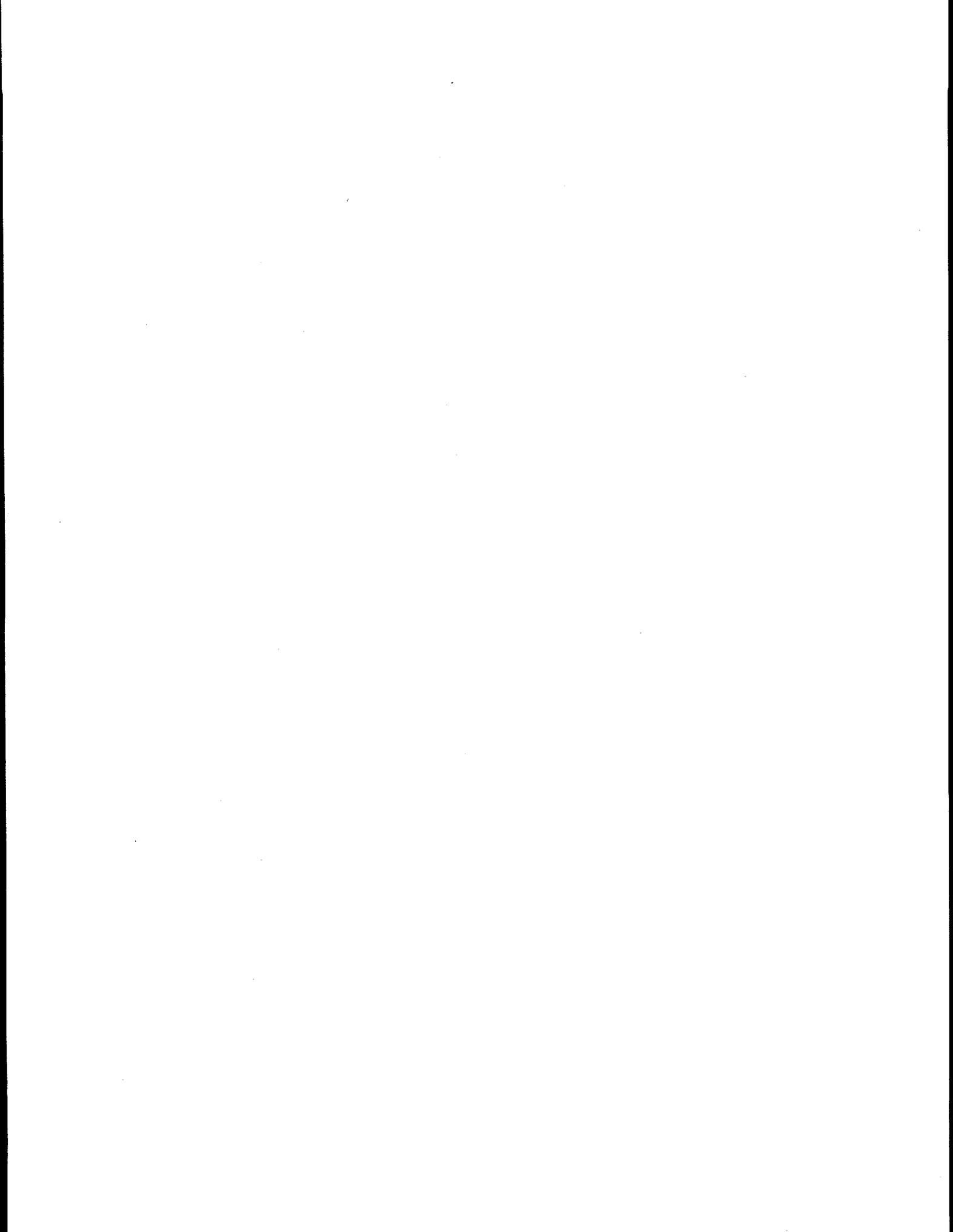
Adjacent to the Mississippi

B Bayou Trepagnier

Southwest of Lake Pontchartrain

Near Norco in St. Charles Parish

Figure 1.1 Devil Swamp and Bayou Trepagnier



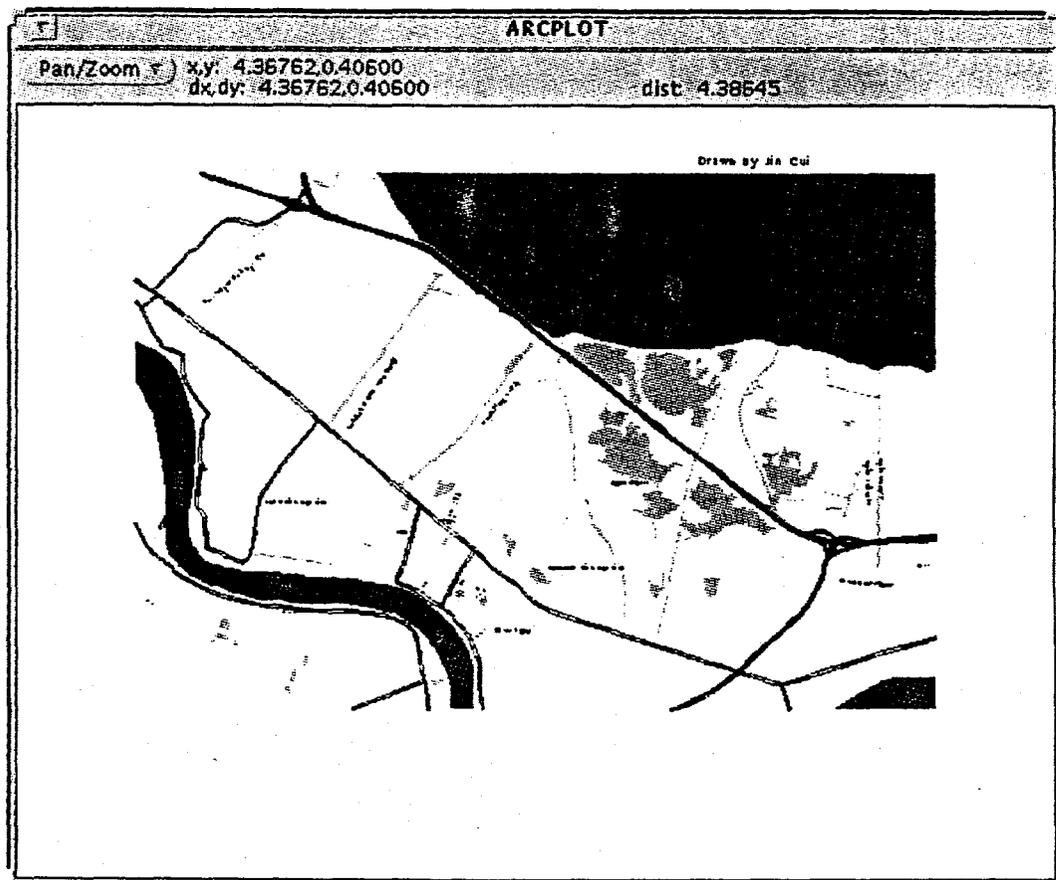
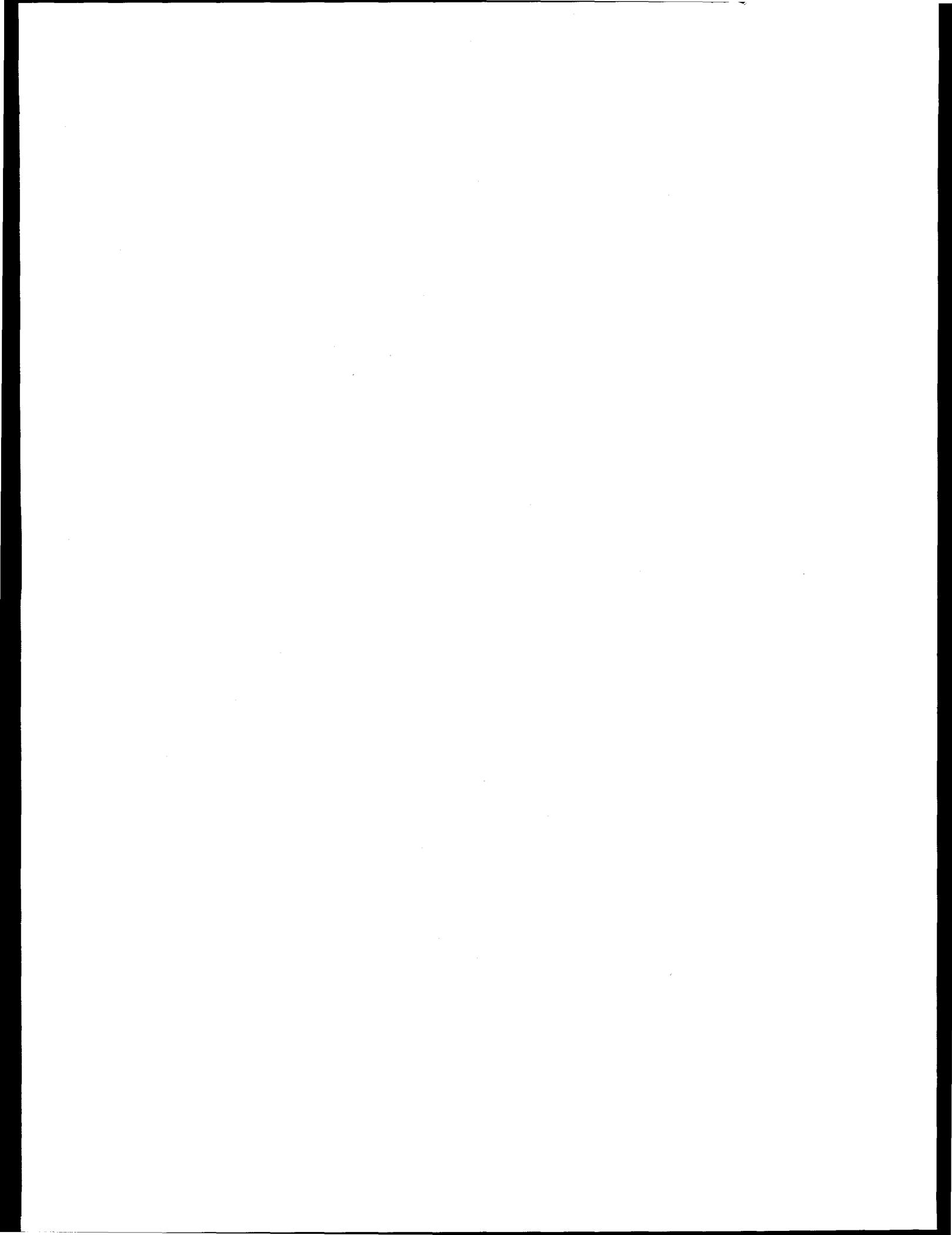


Figure 4.5 Map of Bayou Trepagnier Area



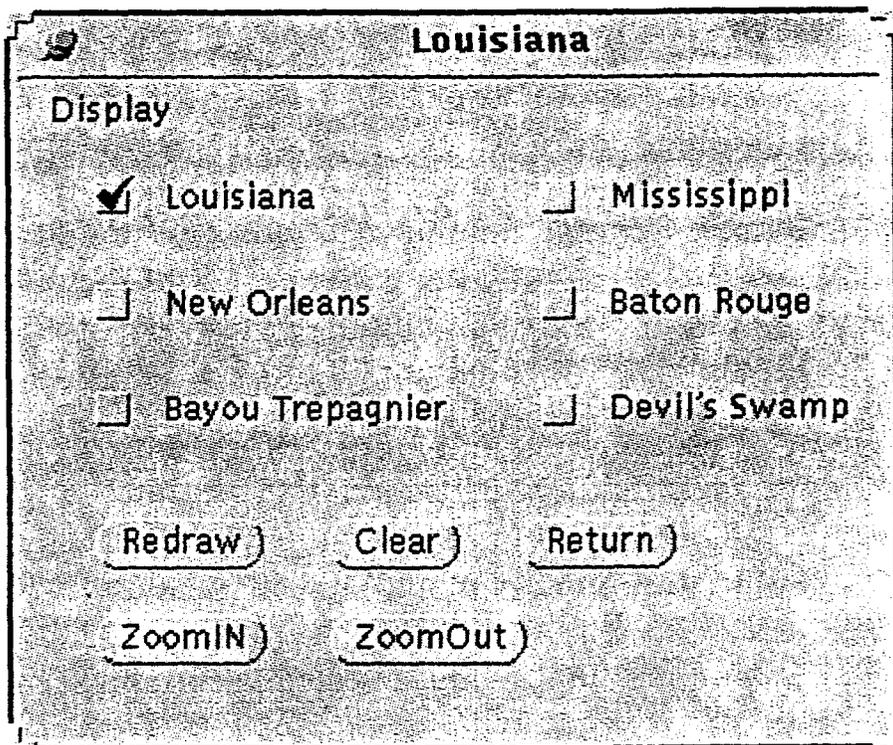
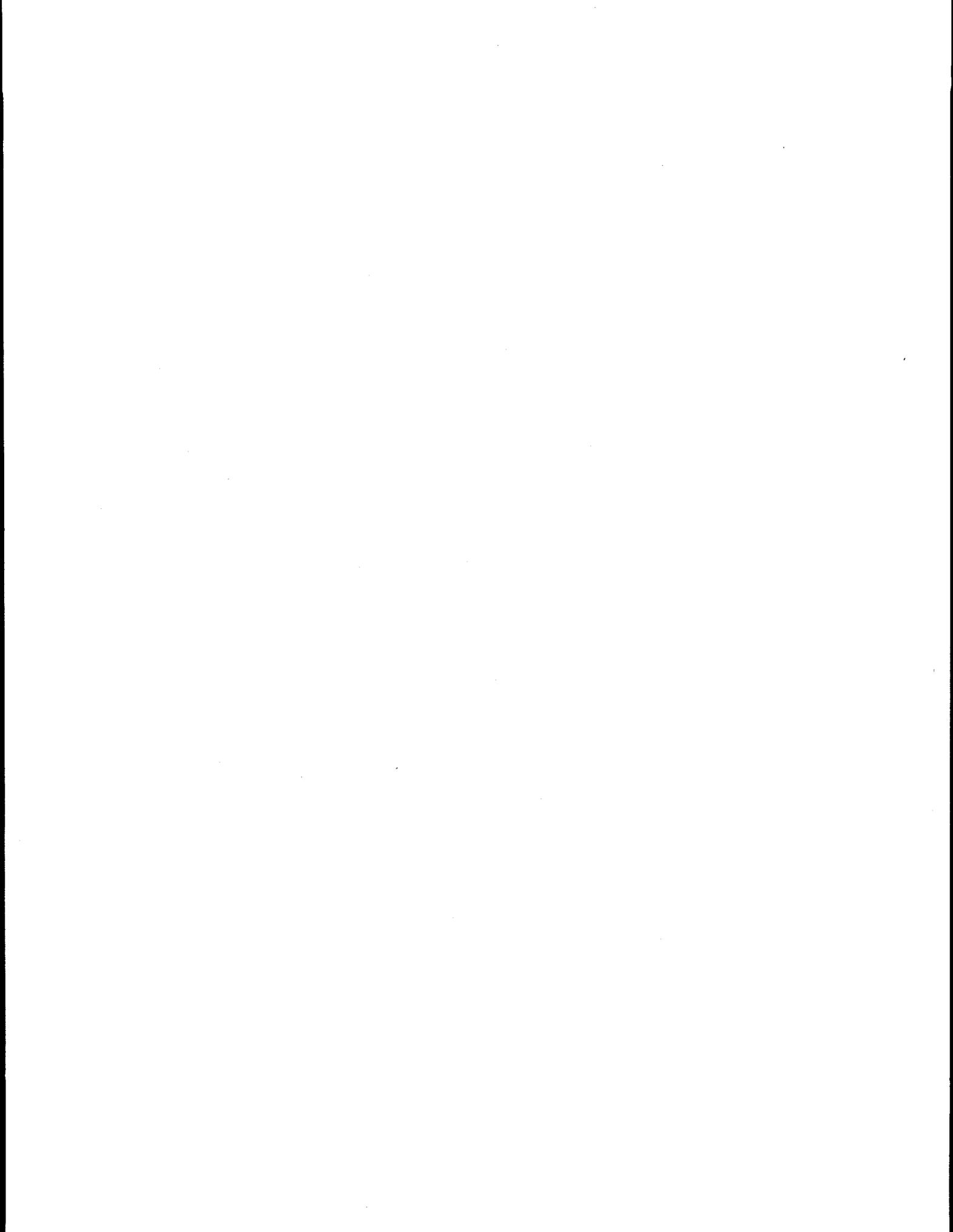


Figure 4.9 Lou.menu



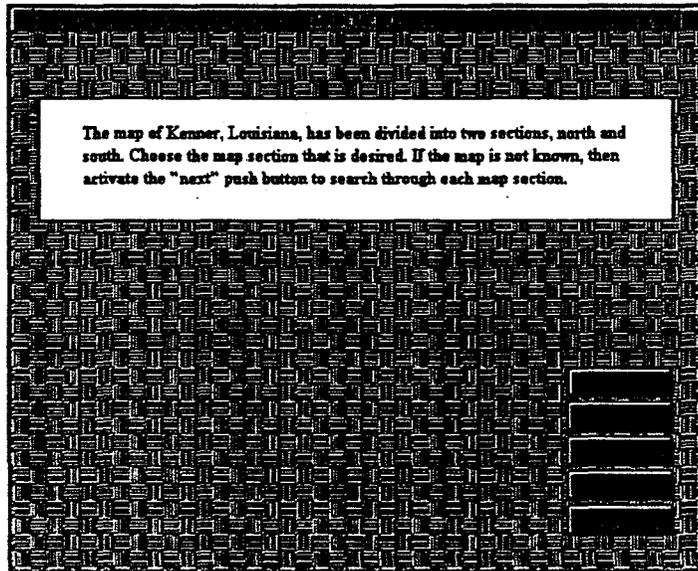


Figure 3.3. The main menu screen.

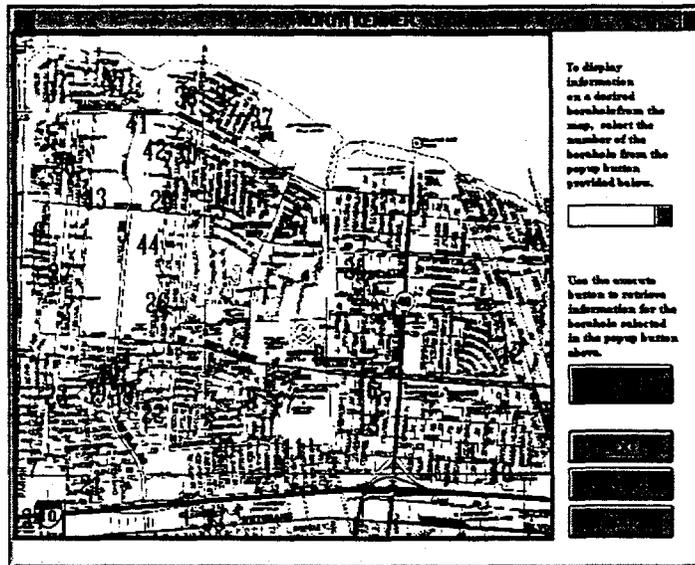
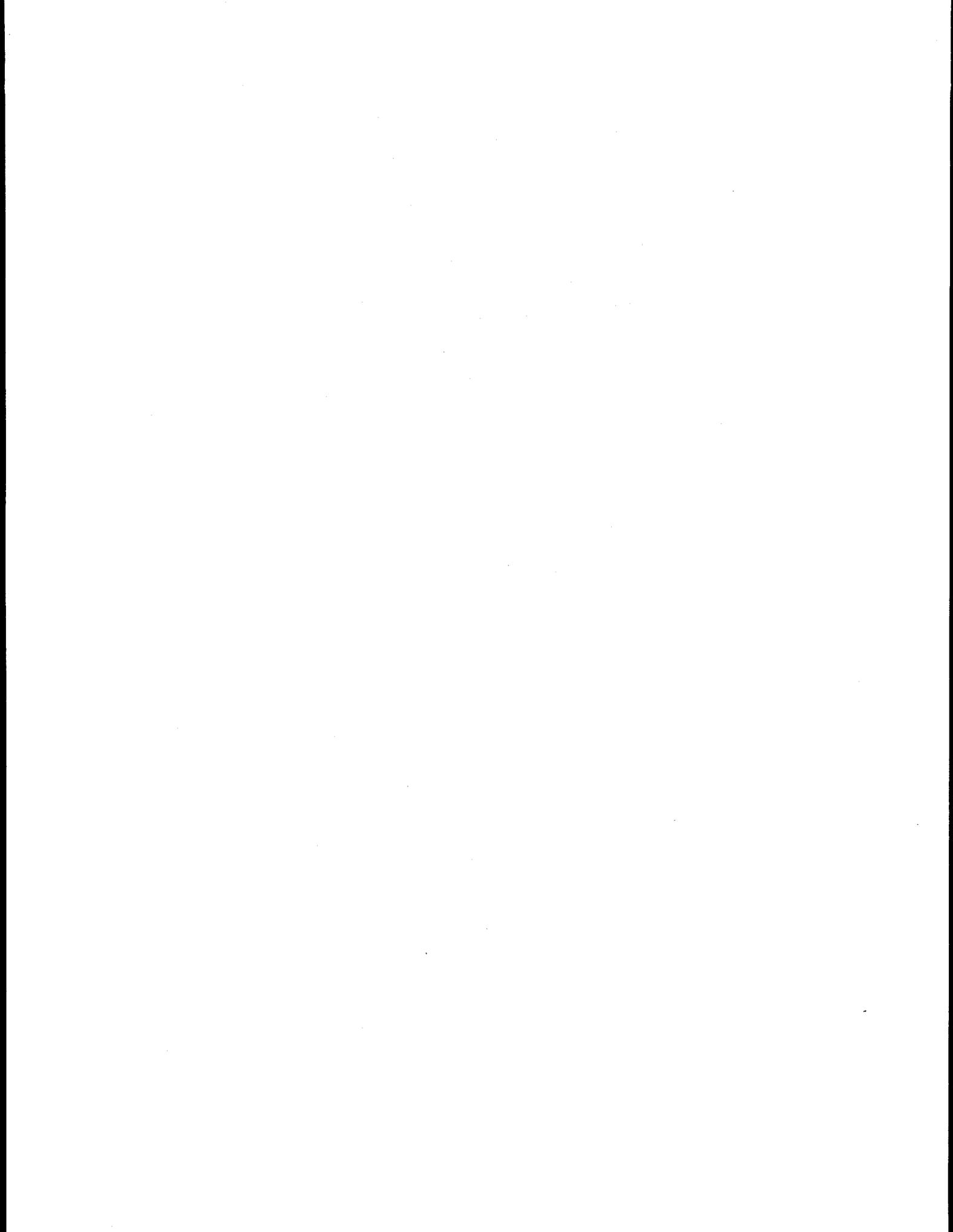


Figure 3.4. The map of northern Kenner.



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## Education Projects

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### Enhancement of Environmental Education at Tulane and Xavier Universities

S. O'Connor, L. White, S. Bhattacharya, J. Bennett, M. Zimmerman

#### Objectives and Goals:

The goal of the environmental education initiative of Tulane and Xavier Universities is to develop a comprehensive environmental education program to produce graduates who can successfully carry out the Department of Energy's mission of environmental restoration and waste management. A major objective of the initiative is to attract minority students into environmental careers. The environmental education programs at the two universities will ultimately range from primary school instruction through graduate level study, and to the continuing education of those in the workplace. The education cluster project supplements this grant's research cluster projects by attracting students into the environmental studies/sciences and providing academic programs relevant to the Department of Energy. The infrastructure for facilitation and coordination of programs is in place, with representatives from each school working together to build on existing strengths and resources within the four schools at the two universities.

#### Accomplishments:

##### Student Development

Two Xavier University faculty members, Drs. Royal Saunders (Biology) and Tujuanda Jordan-Starck (Chemistry), have been appointed to serve as Center for Environmental Programs (CEP) faculty liaisons for their respective departments. The liaisons will assist the CEP in recruiting students for enhanced training in environmental careers.

In May 1995, two of the 1994-95 LIFE scholars were awarded baccalaureate degrees from Xavier University. They were Tamara Mosby (Philosophy, Magna Cum Laude) and Lauren Nicholas (Communications). During the summer, Tamara held an internship position with the Clean Water Act (CWA) in Pittsburgh, Pennsylvania, as a member of their telephone canvassing team. Lauren worked with the Housing Authority of New Orleans on a self-help project for local residents. Currently, Tamara is a law school student at the University of Texas-Austin, and Lauren has accepted a position at the Deep South Center for Environmental Justice at Xavier University.

At Xavier University, seventy (70) completed LIFE scholarship applications were received and evaluated by the Environmental Education Committee. The following students were selected to receive LIFE scholarships for the 1995-96 academic year: Sherika Daniel (Sophomore, Chemistry/ACS), Kefla George (Junior, Biology), Clanford Johnson

(Junior, Chemistry), LáMoyne Williams (Senior, Biology), and Tiffany Zeno (Senior, Communications). LIFE Interns include Monique Davis (3rd Year, Pharmacy), Deshawn Fontenot (2nd year, Pharmacy), James Harry (3rd Year, Pharmacy), Kimberly Houston (3rd Year, Pharmacy), Patricia Kimani (Sophomore, Chemistry), Tracy Lawrence (Sophomore, Biology), Monique Lewis (3rd Year, Pharmacy), and Jacinta Robinet (1st Year, Pharmacy).

Xavier's Annual Environmental Colloquium and Poster Session was held on November 17, 1995 at the Gold/Rush Rooms. Fourteen (14) poster presentations were given by students.

A listing of the titles of the research projects and XU faculty mentors for all continuing LIFE scholars/interns during the 1995-96 academic year follows:

LáMoyne Williams, Project: "The Distribution of Lead, Cadmium and Zinc in Urban Environments," Faculty mentor: Dr. Howard Mielke, Pharmacy  
Tiffany Zeno, Project: "Hilda's Garden: The Impact of Toxic Waste Dumps in an Urban Bayou Environment," Faculty mentor: Dr. Stephen Duplantier, Communications

At Tulane University, the number of undergraduate Environmental Studies Coordinate majors enrolled for the 1996 Spring semester increased by 50% (from 20 to 30).

Funds from this project are being used to support printing costs for the student Environmental Forum newsletter. The November and December issues, respectively, contained on articles on the Studies program Christine Murphy, program coordinator, wrote the articles and works closely with publications staff.

#### Seminars Held in 1995

Environmental Remediation: Science and the Superfund Process," presented by Dr. Charles Reith of DynMcDermott (January 17).

Environmental Justice from a Legal Perspective presented by Carlton Waterhouse, EPA Assistant Regional Counsel (February 7).

Environmental Justice: Are There Ethical Dilemmas for the Physician presented by Herman Ellis, MD of the Columbia University School of Public Health and a member of the National Environmental Justice Advisory Council (March 28).

Evaluation of Two New Flowable Grout Technologies for *In situ* Barrier Construction presented by Ms. Eunice Young of Sandia Laboratories, in Albuquerque, NM (April 18).

Development of Biosorption Technology for Treatment of Uranium-Contaminated Wastes , presented by Dr. Brendlyn Faissou, a scientist at Oak Ridge National Laboratory (July 20).

On the Bayou Sauvage and Career Opportunities in the Field of Wildlife Biology, presented by Mr. Pon Dixon, Refuge Manager at Bayou Sauvage National Wildlife Refuge (Sept. 19).

Surviving the Internship: Who's Responsible? presented by Mr. Curtis Nettles, University Programs Specialist with Battelle Pacific Northwest Laboratory in Richland, Washington, (October 10).

Estrogens in the Environment: A Metaphor for the CBR from Turtles to People presented by Dr. John MacLachlan, Director of the Tulane University/Xavier University Center for Bioenvironmental Research, at the Tulane Green Wave Club (student environmental group) meeting (October 30).

Nitrindepine in One-Clip, One-Kidney Hypertension presented by Dr. Edward Smith, Assistant Professor of Molecular Biology at Hampton University, (November 7).

Current Trends in Environmentalism presented by Dr. Michael Zimmerman, Professor of Philosophy at Tulane (December 8).

### Conferences Attended

Three faculty members and ten students, sponsored by the CEP, attended the Pittsburgh Conference '95, held in New Orleans, Louisiana March 5-10, 1995. The major focus for these students was the Environmental Forum held during the conference.

LáMoyne Williams (Biology '96, LIFE Scholar) and his faculty mentor, Dr. Howard Mielke (Associate Professor, Pharmacy) attended an international conference, entitled "Lead - A Community Concern," on July 5 - 7, 1995, in Trail, British Columbia, Canada. LáMoyne participated in panel (round-table) discussions of environmental health hazards to urban communities and attended other seminars given during the conference. Dr. Mielke gave an oral presentation, entitled "Primary Prevention in Lead Dust Contaminated Communities of New Orleans," on his research on incidence of and health risks associated with exposure to high levels of Lead in the New Orleans area.

Five Xavier University students--Clanford Johnson, Chemistry '97 (LIFE Scholar), Richard Forniss, Engineering '97, LáMoyne Williams, Biology '96 (LIFE Scholar), Robert Swayzer, Biochemistry '96 and Raymond Brown, Biology '97--traveled to the Student Environmental Action Coalition (SEAC) Fourth National Conference, held at the University of North Carolina campus in Chapel Hill, October 13-15, 1995. SEAC aims to create a community of young people committed to fighting for environmental and social justice. Among the issues addressed were: environmental racism, immigrant rights, urban communities infrastructure, clean air and clean water, and endangered species. Of particular interest to the Xavier students was a People of Color Caucus in SEAC, created to ensure that the needs of these young people are met in SEAC and in the environmental justice movement.

### Faculty Development

The Tulane Liberal Arts and Sciences (LAS) Environmental Education Committee met October 16 and December 1, 1995. Professor William Balee, of the Anthropology Department, was introduced as the new Committee member. Dr. Balee will serve in place of Dr. Zimmerman who will be on leave this upcoming spring. 27 out of 36 of Tulane faculty with environmental interests have responded to the Environmental Studies questionnaire and all have expressed openness to mentoring students who wish to pursue Special Projects ENST 403,.

### Curriculum Development:

The Xavier Internal Grants Committee (IGC) approved the following faculty mini-grant proposals:

- Participation in the workshop entitled "Metal Speciation and Contamination of Surface Water," Jekyll Island, GA, June 25-28, 1995
- Dr. Elia Eschenazi (Assistant Professor, Physics)
- Attendance and participation in FASEB 1995 Summer Research Conference
- Dr. Joseph Olubadewo (Professor, Pharmacy)
- Release time support for "Ecological Sentinels of Aquatic Contamination in the Lower Mississippi River System"
- Dr. Peter Martinat (Assistant Professor, Biology)
- Creating a course in Environmental Literature
- Dr. Michelle Levy (Chairperson/Professor, English)
- Development of a course on Technical Writing About Environmental Issues
- Ms. Patrice Melnick (Instructor, English)
- "Greening": the Across the Curriculum Thinking (ACT) Program
- Dr. Michelle Levy (Chairperson/Professor, English)

Environmental Studies courses taught in the Spring 1995 Session were:

General Chemistry II and Lab, Environmental Chemistry, and Industrial Toxicology (Chemistry); Diversity of Life and Lab, Biosphere and Society, Vertebrate Morphology, Plant Systematics, and Invertebrate Paleontology (all Ecology, Evolution and Organismal Biology); General Biology (Cell and Molecular Biology); Introduction to Microeconomics (Economics); Politics and Environmental Policies, Risk Communication, and Health Risk Assessment (all Environmental Health Sciences); The Grand Canyon (Colloquium); Nuclear Radiation Physics (Physics); and Environmental Sociology in the Americas (Sociology).

Environmental Studies courses taught in the Summer 1995 semester were:

General Biology, General Biology Lab, and Microbiology (all Cell and Molecular Biology); General Chemistry, General Chemistry Lab, General Chemistry II, and General Chemistry II Lab (all Chemistry); Microeconomics (Economics); Intro Physics I, and Intro Physics II (all Physics); and Global Environmental Politics (Political Science).

A notice was sent by the Center for Environmental Programs to all Xavier University faculty to encourage student enrollment in seven environmental courses offered at Xavier during the Fall Semester '95. The courses are Environmental Biology (Biology), Instrumental Methods of Analysis (Chemistry), Microeconomics (Economics), Environmental Literature (English), Personal and Environmental Health (Health and Physical Education), Calculus III (Mathematics) and Environmental Policy (Political Science).

Three summer course development awards were given to: Tom Bianchi for Biogeochemical Cycling in Ecosystems, Colin MacLachlan for Mississippi Environmental History, and Sam Ramer for The Chernobyl Catastrophe. Professors Bianchi and Ramer are currently teaching these courses.

At Tulane University, the LAS Environmental Education Committee 1) distributed the brochure on the interdisciplinary Environmental Studies program among Tulane students and faculty, distributed questionnaires among Tulane faculty with environmental research interests for possible collaboration with the Environmental Studies program; 2) obtained approval for the establishment of an internship within the Environmental Studies program and developed a list of available internship opportunities for interested students and; 3)

formally recommended the establishment of an Environmental Science Graduate Program to the Tulane Graduate Council. The LAS Environmental Education Committee held a meeting on September 11th and will meet again on October 16th.

The Xavier University Academic Council voted to institute an Environmental Chemistry major in the Chemistry Department. A listing of course requirements has been distributed. Students taking this major will also be eligible for ACS certification. This is the first environmental degree program at Xavier and represents the efforts by the Center for Environmental Programs to include environmental academic programs among the university's curriculum offerings.

Seventy (70) books and 6 videos were added to the Howard Tilton Memorial Library, the main library for Tulane's uptown campus.

## **Graduate Program in Bioenvironmental Studies**

S. Landry, M. Gerber, A. Brody

### **Objectives and Goals:**

The revised goal of this project is to train a Ph.D. scientist for a career in bioenvironmental research using modern molecular and cellular biological techniques. DOE/EM funds support a graduate student assistantship and travel expenses for the student to attend a meeting related to environmental research.

### **Accomplishments:**

During this project period, the Interdisciplinary Program in Molecular and Cellular Biology (MCB Program) recruited an outstanding graduate student, Mr. Brian Foy, to receive the DoE/EM-funded assistantship. Highlights of Mr. Foy's record are provided below. Mr. Foy was selected from among eight entering graduate student candidates on the basis of Graduate Record Exam scores and grade point average (GPA: 3.4 GRE: V: 620, Q: 680, A: 560 (Biology Subject test: 800)

He earned a B.S. in Biology from University of Notre Dame, where he was on the dean's list for four semesters, and received a Hank Fellowship for Undergraduate Research.

Mr. Foy enrolled at Tulane in August, 1995 and has completed his first semester in the MCB Program. He performed well in his coursework, obtaining a 3.6 GPA. Mr. Foy is now rotating in the laboratory of Dr. Krogstad, Professor and Chair, Department of Tropical Medicine.

This project includes funds for the student to attend a national meeting. Mr. Foy will attend a FASEB Summer Conference in 1996.

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## Initiation Projects

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### Flocculation Studies In Environmental Processes

E. Eschenazi , R. Effiong, B. Das

#### Objectives and Goals:

The objective of this project is to study flocculation phenomena in suspensions and emulsions, resulting from the process of aggregation of suspended particles. Flocculation is not only a determining factor in natural environmental processes, but also constitutes a tool for the cleaning of hazardous waste waters which contain suspended solid and emulsified oils. Understanding flocculation kinetics in various regimes is crucial to design proper remediation. The project is a combination of experimental and theoretical studies of two physicists and one chemist from Xavier University.

#### Experimentally

Multi-angle Light Scattering: One of the fundamental experimental techniques which are being used to study flocculation kinetics is light scattering. This technique allow us to measure the Particle Size Distribution (PSD), its temporal evolution as function of heavy metal concentration as well as pH and concentration of indifferent ions (e.g.  $K^+$  and  $Cl^-$ ).

Analysis of metals in the emulsions: Divalent ions specifically adsorb on the surface of suspended particles, thus playing a pivotal role in promoting coalescence. It is therefore important to know how certain metals partition between the bulk aqueous solution and the surface of the emulsion droplets before and after the aggregation. Spectroscopic techniques, such as Atomic Absorption Spectroscopy (AAS), FTIR and ICP are used to trace metals in the solutions.

#### Theoretically

At the theoretical level, the aggregation kinetics by using computer simulation techniques is being studied. In particular Monte-Carlo techniques are used to investigate the collisional probabilities in concentrated emulsions. The experimental results are compared with the calculations to develop a consistent predictive tool for flocculation kinetics in various regimes.

#### Accomplishments:

##### Flocculation Kinetics Modeling: Effect Of Various Surfactants On The Dynamical Surface Tension

Dr. Eschenazi has completed the two dimensional Monte-Carlo simulation to investigate the collisional probabilities in concentrated emulsions. This work has been published in and

presented at a workshop as a poster session. Dr. Eschenazi has developed a formulation for a three dimensional Monte-Carlo simulation. The Metropolis algorithm is going to be fully implemented in the code. This allow us to examine not only the case of rigid spheres in random motions but also to include in the simulations interaction potentials .

Drs. Eschenazi, Papadopoulos and two students, Dwana Bush and S. Deshiikan, studied the effects of Brij58, CTAB and SDS surfactants on the dynamical surface tension at the slowly dilating n-Dodecane-Water Interface. A paper on this study was submitted to Colloids and Surfaces.

Samples preparation: Dr. Effiong and an undergraduate student, Chris J. Watts, prepared emulsions of water/hexadecane using Silverson mixer and microfluidizer at Dr. Papadopoulos' Laboratory at Tulane University. Both surfactant and non surfactant containing samples were prepared. The samples were used by Dr. Effiong and Dr. Das and their students for the amylases.

## II. Multi-angle light scattering

Multi-angle light scattering allow us to measure the Particle Size Distribution (PSD), its temporal evolution as function of heavy metal concentration as well as pH and concentration of indifferent ions. Experimental studies of flocculation processes were initiated in the summer 1995 using the computerized laser light scattering apparatus purchased from Brookhaven Instruments (model BI-200SM Goniometer System). The instrument was installed by a company engineer in July 1995 at the Department of Physics of Xavier University. A sophomore engineering student, Jameel Uqdah, has worked part-time during the summer with Dr. Biman Das on the calibration of the instrument. This student is working in the project this semester. The light scattering apparatus was tested by measuring particle sizes and particle size distributions of known samples such as certified latex from the Duke Scientific, oil-in-water emulsions. After this preliminary testing a systematic study of the emulsions prepared by Dr. Effiong and Mr. Chris J. Watts was initiated. Preliminary measurements indicate particle sizes ranging from 325 to 382 nm for emulsions not containing surfactant and 255 to 272 nm for those with surfactant. The surfactant has a stabilizing effect on the emulsion. Further experiments are presently in progress to study more in detail particle size distributions and emulsion stability. Emulsions contaminated by the presence of metals are being prepared and analyzed with the light scattering apparatus.

## Atomic Absorption Spectroscopy (AAS), ICP And HPCL Analysis To Trace Metals In The Solutions

Dr. Effiong and Chris J. Watts, studied the sample purity using the Gas Chromatography (GC) and Inductively Coupled Plasma (ICP) Techniques. The results indicate that the reagents were of high purity. Further studies of the effect of pH on the stability of the emulsions. Comparative studies of stability of surfactant containing emulsion and without surfactant show tht the former is more stable. Studies have been initiated involving the contamination of emulsions with heavy metals such as Lead, Chromium, Cadmium and Nickel. These metals were chosen since these contaminants occur in the Bayou Trepagnier. High performance liquid chromatography (HPLC) will be used to study the size distribution pattern of metal complexes.

## **Publications and Presentations:**

Eschenazi, E., and Papadopoulos, K., "Collision Probabilities in Concentrated Suspensions: Two Dimensional Case" J. of Col. Int. Sci., (1995), 170, 293-294.

Eschenazi, E.V. and Papadopoulos, K., " Collision Probabilities in Concentrated Suspensions: A 2D Monte Carlo Simulation" - Poster Session at the workshop "Metal Speciation and Contamination of Surface Water" Jekyll Island, GA.

Deshiikan, S.R., Bush, D., Eschenazi, E. V., and Papadopoulos, K., "Nads and BRIJ 58 at the Slowly Dilating Dodecane-Water Interface" , accepted for presentation at the Annual Meeting of the American Institute of Chemical Engineering (AIChE), November 12-17, 1995, Miami Beach, FL.

Deshiikan, S.R., Bush, D., Eschenazi, E. V., and Papadopoulos, K., "Brij58,CTAB and SDS at the Slowly Dilating n -Dodecane-Water Interface", submitted to Colloids and Surfaces.

### **Collaborative Research With the Institute of Radioecological Problems in Minsk: Mathematical Modeling and Computer Simulations on the Fate and Transport of Radionuclides in Belarus Following the Chernobyl Catastrophe**

E. Michaelides, K. Koutsougeras, S. Ramer, D. Sailor

#### **Objectives and Goals:**

To formulate the problem of aquatic transport in the Iput River, and educate the two Byelorussian students (enrolled in Tulane's graduate engineering program) in the numerical methods of transport of radionuclides.

#### **Accomplishments:**

A collaborative project has been undertaken with two Radioecological Institutes in the Republic of Belarus (IREP)(formerly the Byelorussian Soviet Socialist Republic) to investigate two aspects of the transport and fate of radionuclides, which were released during the Chernobyl nuclear power plant accident in 1986. The transport and fate of radionuclides in the Iput River basin and the redistribution of radionuclides resulting from forest or marsh fires is being examined. During extensive discussions and longdistance communications developed with the Byelorussian colleagues at IREP and Committee on Radiation and Environmental Monitoring (CREM) the following common strategy was developed:

The teams at Tulane and IREP will collaborate on the modeling (formulating the equations) effort. IREP and CREM will supply the necessary data. Tulane and CREM will collaborate in the data-collection and monitoring effort. Numerical work by computations will be undertaken at Tulane (which already has a hydrodynamic computer code) with the results being shared among all three Institutions. In addition, the Tulane team will provide expertise in the area of neural networks for pattern identification and better reduction and interpretation of the raw data collected by the Byelorussian scientists. Adapting an existing code (HEX-6) to determine the transport and fate of radionuclides in the Iput River basin is part of

the work in progress. Belarus has already provided several sets of data which have helped our numerical work. Work has been started on the improvement of the HEC-6 code by including a more pragmatic turbulence transport model. This improved code will be applied in order to compute sediment transport in the Bayou Trepagnier. In addition several initiatives in the field of environmental education are being pursued including a new Tulane undergraduate course on the Chernobyl catastrophe which is being offered in the Spring of 1996.

This grant evolved from a 1994 initiation grant entitled "Collaborative Research Relationship between the Tulane/Xavier CBR and the Institute of Radioecological Problems of the Academy of Sciences of Belarus in Minsk, Belarus." In this second initiation grant, the participants began to work more closely with the Byelorussian colleagues to model the fate and transport of the radionuclides that fell on the territory of Belarus as a result of the Chernobyl catastrophe. During the months of the grant period significant progress was made in defining a collaborative project in mathematical modeling and coordinating this project directly with our colleagues at IREP and CREM in Minsk.

Arrangements were made to have a meeting of the Byelorussian and Tulane components to resolve the issues of what would be the specific responsibilities of each set of investigators and to determine how to get needed data on the contamination of the Iput River in southeastern Belarus and to the Tulane component. In May Dr. Georgii A. Sharovarov, the Director of the IREP, and Ivan Matvenko, the director of the CREM the head of the Mathematical Modeling Laboratory at IREP, came to Tulane for ten days. During this time, Professors Michaelides, Koutsougeras, Sailor, Ramer and Bennett held intensive discussions with them on the following topics: 1) the quality and accessibility of the data concerning the transport of radionuclides as a result of forest fires and in river systems; 2) the modeling programs and computer implementation that will be used; 3) the specific tasks that each side would perform, with appropriate deadlines; and 4) the overall importance of such modeling of transport as an essential step in risk analysis and future plans for remediation in Belarus.

There are two main sub-projects that are being investigating in this research effort: 1) the transport and fate of radionuclides in the watershed of the Iput River; and 2) the redistribution of radionuclides following forest and marsh fires. The second part has been completed. In addition to the traditional methods of the transport and fate, which make use of systems of differential equations, the technique of neural networks as a way of looking mechanistically at the problem of distribution of radionuclides is being developed. Additionally the following items have been accomplished.

Several data sets, which are pertinent to the transport of sediment and radionuclides in the Iput River have been received. These sets were obtained from IREP and CREM as well as from the International Atomic Energy Agency (IAEA) in Austria (VAMP, 1994). A compilation of the Byelorussian data sets in the form of a conference paper, has been accepted for presentation and publication in the International Symposium on Environmental Fluid Dynamics, organized by ASME.

The code HEC-6 for the transport of sediment has been acquired. The code was created by the Army Corps of Engineers and further developed in Oak Ridge National Laboratory. The code has been installed in the Risk/6000 cluster and the running simple cases of sediment transport have begun

The model and numerical work on the dispersion of radionuclides from forest fires, which was started two years ago has been completed. The results of the investigation were

recently published in a scientific paper authored by Pimenov, Michaelides, Sailor, Seffal and Sharovarov (1995).

Analytical work has begun on the improvement of the module on turbulent resuspension and transport of the HEC-6 code. A stochastic model (based on the Monte-Carlo technique) for the transport of radionuclide-carrying particles. This is to be used as a subroutine in the turbulent transport of sediment.

An interdisciplinary course on the Chernobyl accident has been developed. The course will be offered in the Spring of 1996 and examines the social and political as well as the technical dimensions of the catastrophe. From the outset it was recognized that it would be of enormous benefit to the project to include the two young Byelorussian investigators as researchers in residence.

The necessary financial support to enable the admission and enrollment of two Byelorussian researchers in the School of Engineering at Tulane was located. As a result, Mr. Oleg Pimenov, a young investigator at IREP, who worked on the physicochemical behavior of radionuclides, has been enrolled in the School of Engineering doctoral program since January 1994 and Ms. Olga Melkozerova, another young investigator at IREP, who worked on the transport modeling of radionuclides, has also been enrolled in the doctoral program of the School of Engineering since August 1994. It is anticipated that the two students will be extremely valuable to this project because of their broad expertise with the transport and physicochemical properties of radionuclides, their familiarity with the research efforts at IREP and more broadly in Belarus and the other States of the Former Soviet Union.

It must be pointed out that modeling of the watershed of the Iput River has been chosen by the Byelorussian Institutes as the main site for the monitoring of the radionuclide contamination in aquatic environments. The river runs in the southeast Belarus region, its length is 427 km and has six tributaries. The area of its watershed covers 10,900 km<sup>2</sup>, comprising 45% arable land and 55% marshes and forests. The river runs through the so-called "Bryansk-caesium spot," whose contamination level ranges from 1 to 60 Ci/km<sup>2</sup> and is considered the highest in the world. The CREM has been monitoring the water quality of the Iput River since the end of 1990 by taking monthly measurements of the radioactivity in the sediment and in the dissolved solids. There are six measuring stations along the river, with each station yielding three measuring points across the river for a total of eighteen measurements per month. The last measuring station is approximately 200m upstream from a dam, near the town of Vilyevo. Caesium-137 concentrations of 83,600 Bq/kg were observed in the sediment near the dam a quantity which is 200,000 times more than the background specific activity of sediments.

The use of neural networks for predictive modeling was also pursued since this is currently the most promising approach in the computer science field when time series and stochastic processes are involved. The method employed was: given a set of finite measurements of the concentration of a certain contaminant in a given large geographical terrain, attempts were made to come up with a method of predicting the distribution of that contaminant's concentration at any desired point of the terrain. In essence the target is to come up with a nonlinear curve fitting scheme, by which to automatically perform interpolation and produce a mapping of the contaminant's distribution. This method would provide a tool to produce an expected map of the concentrations at any given time out of a sample set of measurements at various points of the terrain. It was shown that this is feasible by means of a back-propagation neural network. The required nonlinear curve fitting can be achieved with substantial accuracy but this accuracy depends on the density of the sampling. If the sampling points are sufficient in number then the accuracy is very good. But if the number

of samples is low then the outcome deviates unacceptably. The way the experiments were run was as follows: A set of samples was obtained from a real valued function defined over a two dimensional space. A subset of this sample was selected and removed for later evaluation. The rest of the sample was used for adapting the neural network. Then the neural network was used to predict the sample that was removed and its response was compared to the reserved sample. Arbitrarily generated functions (surfaces) from which to obtain the sample were used because, first, the sample of IREP was very sparse, and second, because it could be determined how well the predictive model was doing as long as the it was known what exactly the surface was that it was trying to approximate.

## **Use Of Protein Engineering To Create Cytochrome P450 102 Isozymes With Novel Catalytic Activities.**

D. Mullin

### **Objectives and Goals:**

The goals of this research project are to use protein engineering methods to construct cytochrome P450 isozymes with designed activities that will enhance the natural ability of bacteria to degrade recalcitrant organic pollutants that are present in the environment. Attention is currently being focus on developing pyrene hydroxylases and dehalogenases for use as tools for bioremediation.

### **Accomplishments:**

The investigation of how the natural substrate (long chain fatty acids) binds to wild type and mutant P450 102 isozymes has helped to guide us in changing it from a fatty acid hydroxylase into a polycyclic aromatic hydroxylase that hydroxylates pyrene and benzo[a]pyrene which are environmental pollutants. These results demonstrate that protein engineering methods can be used to dramatically change the substrate specificity of P450 102, and it suggests this mutagenesis approach can be used to create isozymes that have other novel and useful catalytic activities.

In collaboration with Dr. William Alworth's lab in the Tulane Chemistry Department, a manuscript describing the new P450 102 polycyclic aromatic hydroxylase has been submitted to Archives of Biochem. Biophys. It has received reviews favorable for its publication. A response is being prepared addressing the constructive criticisms of the reviewers.

Steady progress has been made in dissecting the substrate binding domain of the cytochrome P450 102 using site directed mutagenesis. Testing the role of additional amino acid residues in substrate recognition is in process. Computer-aided molecular modeling is being utilized to better visualize and obtain a quantitative understanding of how these mutant isozymes recognize their new substrates. It is hoped that these visualization tools can be used to help design new P450 102 enzyme activities.

A detailed biochemical analysis of several mutant isozymes was finished. F87V, F87Y, F87A, T268S and T268V were overproduced in *Escherichia coli*, and purified to homogeneity by column chromatography. The purified mutant enzymes have been assayed

for their spectral and kinetic properties (specific activity  $V_{max}$ ,  $K_m$ ) and compared to the wild type enzyme. A second manuscript detailing some novel findings concerning substrate binding and electron transfer within two of these mutant isozymes is being prepared. Results that will appear in this second manuscript are scheduled to be presented at the National American Society for Microbiology Meeting in May of 1996.

Work continues on the construction of P450 102 isozymes that have dechlorinase activities. Mutant strain BM312 that expresses a mutant isozyme that actively dechlorinates tetrachloroethylene have been constructed. In a collaboration with Dr. William Alworth's lab (Chemistry Dept., Tulane University) work is being done to identify the product(s) of this dechlorination reaction.

### **Publications and Presentations:**

Zhao, W. and Mullin, D.A.. 1996. Probing the structure and function of cytochrome P450 102 by site directed mutagenesis: and amino acid substitution of valine for threonine at position 268 uncouples hydroxylation of laurate. National American Society for Microbiology

Xia, Q., Kang, L., Alworth, W.L., Liu, H.M., Zhao, W. and Mullin, D.A.. A Site-Specific Mutant of the Bacterial Cytochrome P450 102 (BM-3) Possessing a New Capability to Catalyze the Hydroxylation of Polycyclic Aromatic Hydrocarbons Pyrene and Benzo[a]pyrene. (Submitted to "Archives of Biochem. Biophys").

## **Reusable Synthetic Membranes for the Removal of Aromatic and Halogenated Organic Pollutants from Waste Water**

C. Li, H. Ensley

### **Objectives and Goals:**

Aromatics and chlorinated hydrocarbons constitute the major organic pollutants in waste streams. The objective of this project is to initiate studies on preparing a regenerable, synthetic membrane which consists of polymerized forms of cyclodextrins. When these materials are exposed to aqueous solutions containing suitably small organic contaminants, the organics will be forced into the hydrophobic cavity and immobilized. When the polymer is saturated, it can be regenerated by extraction with a volatile solvent to remove the absorbed organics. The specific way to connect the cyclodextrins in this proposal is to develop novel aqueous synthetic methodology and use the method in the polymer preparation.

### **Accomplishments:**

As the first stage of the project, several synthetically useful reactions have been developed, which are promising for the proposed project. In addition, these reactions have fundamental values and important applications in organic chemistry.

A trimethylmethylen dianion synthon in aqueous media has been developed. With this synthon, have coupled a variety of carbonyl compounds in generating a variety of diols.

Then, to explore alternative ways for the coupling, have discovered a reaction mediated by indium in water generated bis-allylation products with both carbon-carbon bond formations occurring primarily on the same carbon. Such a reaction mode effectively constitutes a gem-allyl dianion equivalent in organic chemistry.

To further explore the synthetic application of these reactions a novel cyclization reaction was developed. Cyclopentanoids constitute one of the most common structural features for many natural and non-natural products. Many research efforts have been devoted to their formations. Among the most useful methods are the [3+2] cyclization approaches. Very often these cyclizations are carried out through transition metal catalysis. A new [3+2] annulation methodology involving a reaction between a trimethylenemethane zwitterion equivalent and a 1,2-zwitterion equivalent was discovered. The cyclization was carried out via the indium mediated allylation reaction in water. A simple and efficient approach towards the carbonyl alkylation of 1,3-dicarbonyl compounds was found. A variety of carbonyl compounds have been nucleophilically allylated in this way. The reaction is based on a Barbier-type reaction in aqueous media by using either tin or indium as the metal mediator. In this reaction, the disfavored effect of enolization of 1,3-dicarbonyl compounds which often competes with the carbonyl alkylation has been prevented.

After these success in developing the reactions for the coupling, corresponding cyclodextrin material was prepared for the polymerization study. A large amount of the cyclodextrin material has been prepared in the laboratory. Unfortunately, due to the low solubility of the cyclodextrin material, the polymerization study gave very complicated results. The product thus generated is difficult to characterize.

To explore novel method that might give better coupling results. Transition metal catalyzed reactions were tested. While explorations to find the best reaction condition continue, an unprecedented functional group reshuffling reaction has been discovered. During this reaction, functionalities of allyl and homoallyl alcohols are completely repositioned. The reaction has fundamental interests in chemistry.

#### **Publications and Presentations:**

Li, CJ.. "Barbier-Grignard Type of Reactions in Aqueous Media: Scope, Mechanism and Synthetic Applications", [Invited Review]. Tetrahedron Report. 1996, in press.

Li; CJ, Wang, D. and Chen, DL. "Reshuffling of Functional Groups in Homoallylic Alcohols Catalyzed by a Ruthenium Complex under a Natural Environment". J. Am. Chem. Soc. 1995, 117, 12867

Lu, YQ., Li, CJ. "Novel [3+2] Annulation via a Trimethylenemethane Zwitterion Equivalent in Water", Tetrahedron Lett. 1996, 36, 471.

Chen, DL., Li, CJ. "A Gem-Allyl Dianion Synthon in Water" Tetrahedron Lett. 1996, 36, 295.

Li, CJ., and Lu, YQ. "Highly Efficient Carbonyl Alkylation of 1,3-Dicarbonyl Compounds in Aqueous Medium", Tetrahedron Lett, 1995, 36, 2721.

## Xavier Analytical Analysis Support

H.W. Mielke

### Objectives and Goals:

The goal of this project is to provide an analytical support laboratory located at the the Xavier campus site which will provide the necessary analyses of inorganics in samples collected for the grant's swamp sites. To attain this goal the lab will work on the following objectives: 1) assess and develop appropriate sample preparation techniques; 2) provide training to improve skills in operating the analytical instruments being used; and 3) run analyses of samples as requested by this grant's investigators.

Three years ago, there was laboratory space in room 210 of the College of Pharmacy at Xavier University that contained a minimum of analytical instruments and supporting research analysis equipment. This grant in combination with several other grants has made it possible to purchase the necessary analytical instruments to develop high quality analytical facilities.

Bayou St. John was used to gain experimental skill in field collection, initial laboratory preparation (drying, sieving, grinding etc.), sample extraction (treatment of samples to dissolve metals), and analysis of sediment and water samples. A total of 303 samples were collected to map the bayou.

The number of samples and sites included in the study from Devil's Swamp were about half the number of samples collected and sites included from Tunica Swamp. The basic lack of samples made it difficult to make conclusions about the quantity of metals in the soils and sediments of Devils Swamp.

Nevertheless, the analytical results provide an indication that the selection of Tunica Swamp as the control site and Devils Swamp as the experimental site is a problem. The control site shows a consistent and significant increase in metal content of sediments compared to the experimental site. These results reinforce the findings first noted and described at the April 1995 cluster meeting. The additional samples collected between then and the end of the year reinforced the same fundamental trend.

It should be noted that there was one sediment site in Devils Swamp (SLT95-18) that yielded 5 samples and measured higher Pb, Cd, Zn, and As than any other site. This finding underscores the basic problem of lack of samples and sites from Devils Swamp. It is possible that Devils Swamp is more polluted with metals than Tunica Swamp. The current sets of samples, however, do not support that conclusion.

Several other characteristics about the correlations between metals of soils and sediments within each swamp have been noted. The metal content of sediments tend to be significantly higher than the metal content of soils. There is a significant association between metals in the same sample. When a sample measures high (or low) for one metal the other metals tend to be high (or low) also.

## **Accomplishments:**

### Laboratory Development

Three years ago, there was laboratory space in room 210 of the College of Pharmacy at Xavier University that contained a minimum of analytical instruments and supporting equipment. This grant in combination with several other grants has made it possible to purchase the necessary analytical instruments and to develop a high quality analytical research facilities at Xavier.

The benchmark for determining the degree of progress is the Analytical Products Group, Inc. (APG) laboratory proficiency environmental testing program. There has been a marked improvement since reporting began in 1990. Initially the lab ranked in the middle of the approximately 100 laboratories who participated in program. This is the result of a rigorous program of laboratory quality control and in which the lab continues to strive for excellence to maintain the highest standards of trace metal analysis.

Another development was an expansion of laboratory capabilities. A Capillary Electrophoresis Instrument was purchased through an ATSDR grant. There are many different compounds of metals, acetates, sulfates, sulfides, carbonates, nitrates, arsenates, chromates, oxides, etc. The species of metallic compound influences reactivity and bioavailability. New capability was required to support the measurement of elements by evaluating their associated anionic portion of the compounds. The Capillary Electrophoresis (CE) was installed during December and is being developed for use on field samples. As a relatively new instrument, any techniques developed with the instrument should be publishable in the scientific literature.

### Research Method Development: Bayou St. John

Bayou St. John is a rich asset as an experimental field site to this laboratory program. Three hundred and three (303) sediment samples have been obtained from the bayou. The distributions of the metals have been mapped for Bayou St. John.

Bayou St. John was used to gain experimental skill in field collection, initial laboratory preparation (drying, sieving, grinding etc.), sample extraction (treatment of samples to dissolve metals), and analysis of sediment and water samples.

The field work from Bayou St. John was written up for publication in two papers "Trace Metals and History: The Sediments of Bayou Saint John, New Orleans, LA. USA," H. W. Mielke, L. D. Williams, L. Hickman, and "Trace Metals in Sediments and Soils of Bayou Saint John" by H.W.Mielke, LaMoyne Williams, Julie Schaeffer, Chris Gonzales and M. Kelley Smith.

### Analytical Results from Devils Swamp and Tunica Swamp

The experience gained from the Bayou St. John field site was transferred to the preparation and analysis of the samples received from Devils Swamp and Tunica Swamp oil. During the course of the study, a total of 176 samples were received for analysis: 22 soil samples from 3 sites and 30 sediment samples from 8 sites for Devils Swamp; and 47 soil samples from 10 sites and 77 sediment sample from 14 sites were obtained.

To date, about half the number of samples and sites were received from Devils Swamp as were received from Tunica Swamp. The basic lack of samples made it difficult to make conclusions about the quality of the samples and sediments of Devils Swamp.

Nevertheless, the analytical results provide an indication that the selection of Tunica Swamp as the control site and Devils Swamp as the experimental site is a problem. The control site shows a consistent and significant increase in metal content of sediments compared to the experimental site. These results reinforce the finding's first noted and described at the April 1995 cluster meeting. The additional samples collected between then and the end of the year reinforce the same trend.

It was noted that there was one sediment site in Devils Swamp (SLT95-18) that yielded 5 samples that measured higher in Lead, Cadmium, Zinc and Arsenic than any other site. This finding underscores the basic problem of lack of samples and sites from Devils Swamp. It is possible that Devils Swamp is more polluted with metals than Tunica Swamp. The current sets of samples, however, do not support that conclusion.

Several other characteristics were noted about the correlations between metals of soils and sediments within each swamp. The metal contents of sediments tend to be significantly higher than the metal content of soils. Also, there is a significant association between metals in the same sample. Thus, when a sample measures high (or low) for one metal the other metals also tend to be high (or low).

### **Analysis of Heat-Shock Protein Genes in Heavy Metal Contaminated Plant Species - *Saururus* (Lizard's-tail)**

L. Thien, E. Ellgaard, D. Hurley, and D. Jobs

#### **Objectives and Goals:**

In recent years, increasingly high levels of heavy metals (e. g., Lead, Zinc, Copper, Chromium, etc.) have become widespread in ecosystems as a result of man's activities. Vegetation is usually one of the first interceptors of heavy metals in the environment, either by direct uptake (leaves and bark) or by transport into the root system. Heavy metal tolerance in plants is well documented and environmental research has begun to focus on efficient methods to remediate contaminated sites to remove pollutants from ecosystems. Phytoremediation utilizes plants, e. g., metal-scavenging plants to restore or stabilize polluted ecosystems. Higher plants have developed different types of mechanisms to adapt to heavy metal contamination. Metal chelating compounds (phytochelatins), compartmentalization within the vacuole, alteration of membrane structure, and induction of heat-shock proteins are the main metabolic modifications that occur during stressful conditions.

To understand the genetics of heavy metal tolerance it is necessary to develop molecular approaches to detect and observe the effects of metals in organisms. Quantitative PCR is a method that rapidly and efficiently amplifies and measures levels of expression of heat-shock genes. The importance of heat shock-shock proteins (also called molecular chaperones) goes beyond their role in protection from temperature stress. Other factors that induce elevated heat shock proteins (HSP) levels include ethanol, arsenate, heavy metals,

and starvation. A rapid rate of expression of HSP is one of the adaptive responses characteristic of plants living in heavy metal contaminated soils.

The objectives of this research was: 1) to collect and grow various clones of Lizard's Tail to test for levels of heavy metals in Bayou Trepagnier; 2) analyze the soil and plants of Lizard's Tail growing in Bayou Trepagnier; 3) attempt to isolate HSP 70 and sequence the gene to determine if it differs in structure; and 4) use quantitative PCR to analyze for heat shock induction as a biomarker.

*Saururus cernuus* L. (Lizard's Tail) is a clonal, herbaceous plant, capable of spreading rapidly by sexual and asexual reproductive; it is widely distributed throughout the eastern United States. Molecular phylogenetic studies and fossils indicate the family (Saururaceae) represents an ancient lineage of plants. The plants are adapted to a wide range of temperatures and are common in the understory of swamp forests, especially in tree gaps; in southern Louisiana the plants form dense stands in ditches and waste sites. In Bayou Trepagnier, Louisiana, Lizard's Tail grow on the heavily polluted spoil banks that line the bayou including the actual margin of the bayou. The spoil banks were created in 1930 - 1950 when the bayou was dredged to increase stream flow for effluents from an oil refinery at its headwaters. Heavy metals in the spoil banks are present at high levels, e. g., Lead (>1600 ppm), Zinc (685 ppm), Copper (50 ppm). It is unusual for a plant to be tolerant to a wide range of heavy metals in addition to the hundreds of organic compounds in the spoil banks.

It is suggested that the clones of Lizard's Tail that grow in the heavily polluted areas of Bayou Trepagnier are the result of a selective process over the past 50 years. Seeds, seedlings, or pieces of mature plants deposited on the spoil banks during local flooding were selected upon by pollutants and only certain genotypes were able to survive. The various clones spread via the underground rhizomes (ca 20 cm in soil).

### **Accomplishments:**

The spoil banks and Bayou Trepagnier itself were surveyed for clones (interconnected plants) of Lizard's Tail. Eight clones were located either growing on the spoil banks or in the water of the bayous. Representative tissue of these clones were transported to the greenhouse and the plants grown to maturity.

At the time of collection, samples of roots, rhizomes, stems, leaves and seeds were also collected and taken to the laboratory (plus soil). The samples were dried and analyzed for heavy metal content (Lead, Copper, Zinc, Chromium, and Nickel) via X-ray diffraction. Roots contained over 1,000 ppm lead, rhizomes, stems, leaves, and seeds ca 50 ppm. The other elements all averaged about 80 ppm. It is obvious that these plants are capable of growing in soil heavily polluted by heavy metals and other by-products of oil refining. The plants are excellent bio-indicators and should be considered as monitors of heavy metals in the southeastern United States. The plants could also serve in bio-remediation and/or in the stabilization of heavily polluted spoil as the plants are capable of growing in water or on dry land.

The genetics of the plant need further study. Some of the first steps in isolating genes that may confer resistance to heavy metals have been taken. HSP 70 genes have been isolated, but as yet the DNA sequences have not been analyzed.

In the process of the molecular component of this work a more efficient method to isolate DNA from plant tissue was developed.

**Publications and Presentations:**

Jobs, DV., Hurley, D.L. and Thien, L.B.. 1995. Plant DNA Isolation: A Method to Efficiently Remove Polyphenolics, Polysaccharides, and RNA. *Taxon* 44: 379- 386.

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## Coordinated Instrumentation Facility

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### Coordinated Instrumentation Facility

T. Lyttle, D. Grimm, P. Burnside, W. Douglas, L. Smith III

#### Objectives and Goals:

The Coordinated Instrumentation Facility (CIF) provides analytical services and equipment maintenance and repair to the researchers involved in the grant. The CIF provides access to and support for state-of-the-art research instrumentation including equipment for analysis of trace metals and organic compounds. The CIF also provides electronics maintenance and repair for equipment used for research. All of these services are available to Tulane and Xavier researchers.

#### Accomplishments:

##### Inorganic Laboratory

The inorganic laboratory was designated a "Laboratory of Excellence" by Analytical Products Group, Inc. In each quarterly evaluation the laboratory was ranked #1 on percent acceptable data. The laboratory reported data on Arsenic (3 quarters), Cadmium (3 quarters), Chromium (3 quarters), Cobalt (3 quarters), Copper (3 quarters), Lead (4 quarters), Mercury (1 quarter), Nickel (3 quarters), and Zinc (3 quarters). Per the request of the QA/QC committee the laboratory now performs the APG evaluation on five elements - Arsenic, Cadmium, Chromium, Cobalt and Lead.

A new Mercury analyzer was installed which provides very low-level analysis for Mercury.

An ultrasonic-nebulizer was installed on the Inductively-Coupled Plasma Atomic Emission Spectrometer (ICP-AES). This sample introduction accessory lowered the detection limit of the spectrometer by about a factor of 2-3 for each element.

A microwave oven for sample digestion was installed. This digester is used to prepare solid samples for analysis by ICP-AES or Graphite Furnance Atomic Absorption Spectrometer (GFAAS).

### Summary of Instrument Use In Inorganic Laboratory for 1995

Instrument	Hours of Instrument Use
Graphite Furnance Atomic Absorption Spectrometer	898 hours
Inductively-Coupled Plasma Atomic Emission Spectrometer	367 hours
X-Ray Fluorescence Spectrometer	361 hours
X-Ray Diffractomer	110 hours
Mercury Analyzer	23 hours
Scanning Electron Microscope	25 hours
Transmission Electron Microscope	3 hours

### Organic Laboratory

The Organic Laboratory established an isolated sample preparation area. A wall was constructed to ensure proper air-handling to isolate the preparatio area from the analysis area. Laboratory benches were installed to provide working space, and two fume hoods were installed to provide room for multiple extractions and concentrations.

Sample preparation equipment was acquired including - Soxhlet extractors, micro-Soxhlet extractors, Kuderna-Danish concentrators, Liquid-Liquid extractors, and an ultra-low freezer.

Upgrades and accessories for the Shimadzu GC-14A were ordered and installed to allow the instrument to perform dual-column methods with ECD detection. This instrument is capable of performing pesticide or PCB analysis.

Carbon - Hydrogen - Nitrogen - Oxygen - Sulfur Analyzer was installed in the laboratory during the summer. The instrument is being used to measure the percent of those elements in sediment samples.

The laboratory established the methodology to detect HCB and HCBd in sediment, water and tissue using SW-846 methods as guidelines.

### Summary of Instrument Use In Organic Laboratory for 1995

Instrument	Hours of Instrument Use
High Resolution Mass Spectrometer	24 hours
Nuclear Magnetic Resonance Spectrometer	463 hours
Gas Chromatogaph/Mass Spectrometer	261 hours
Carbon-Hydrogen-Nitrogen-Oxygen-Sulfur Analyzer	8 hours
Gas Chromatograph	32 hours

### Electronics Laboratory

The electronics laboratory provided service contract support for approximately \$750,000 of Tulane research equipment. The laboratory also provided service contract support for approximately \$75,000 of Xavier research equipment.

Billable maintenance calls were performed on a few pieces of research equipment that were not covered by contract, but were being used for DOE research.

CIF engineers worked closely with Dr. Zhang of Xavier to ensure that proper ventilation was installed for his atomic absorption spectrometer.

### Personnel Changes and Professional Enhancement

Dr. Willard Douglas was hired as manager of the Organic Laboratory. He brings environmental analysis experience to the group from Stennis Space Center.

The CIF as a department participated in the 1995 Pittsburgh Conference. CIF scientists and engineers attended technical sessions and used the exposition to identify potential vendors for environmental - analytical equipment.

Dr. Grimm attended a one-day short course on GC/MS for Environmental Analysis.

Dr. Grimm attended the American Society for Mass Spectrometry conference in May in Atlanta. She attended the environmental analysis sessions.

The CIF hosted a one-day workshop on ICP-AES and GFAAS presented by Perkin-Elmer at Tulane's uptown campus. Complimentary registrations were provided to researchers performing trace metal analysis on this grant. A total of 60 people from the greater New Orleans area attended.

The CIF hires undergraduate students to assist in its laboratories. Typically 6-10 undergraduates are involved in sample preparation, analysis and laboratory record-keeping. These undergraduates provide support to the DOE work as well as learn laboratory protocol.

Drs. Douglas and Grimm attended the DOE-ORNL 36th Chemical and Analytical Sciences Division Conference, "Analytical Chemistry in Energy Technology", in October at Gatlinburg, TN. This conference provided them the opportunity to interact both with ORNL scientists as well as with other Tulane researchers that attended the conference.

An area of concern for the CBR has been the ability to perform volatile and semi-volatile organic compound analyses in a timely and efficient manner. Dr. Mike Maskariniec of ORNL recommended that the CBR purchase two GC/MS systems. Per the CBR's request, the CIF began evaluating such systems. Three vendors were visited. A recommendation for purchase will be made in early 1996.

### **Publications and Presentations:**

Koplitz, L., Urbanik, J., Harris, S. and Mills, O. "Determining Lead in Sediments by X-ray Fluorescence and the Method of Standard Additions". Environmental Science and Technology, v. 28, p. 538, 1994.

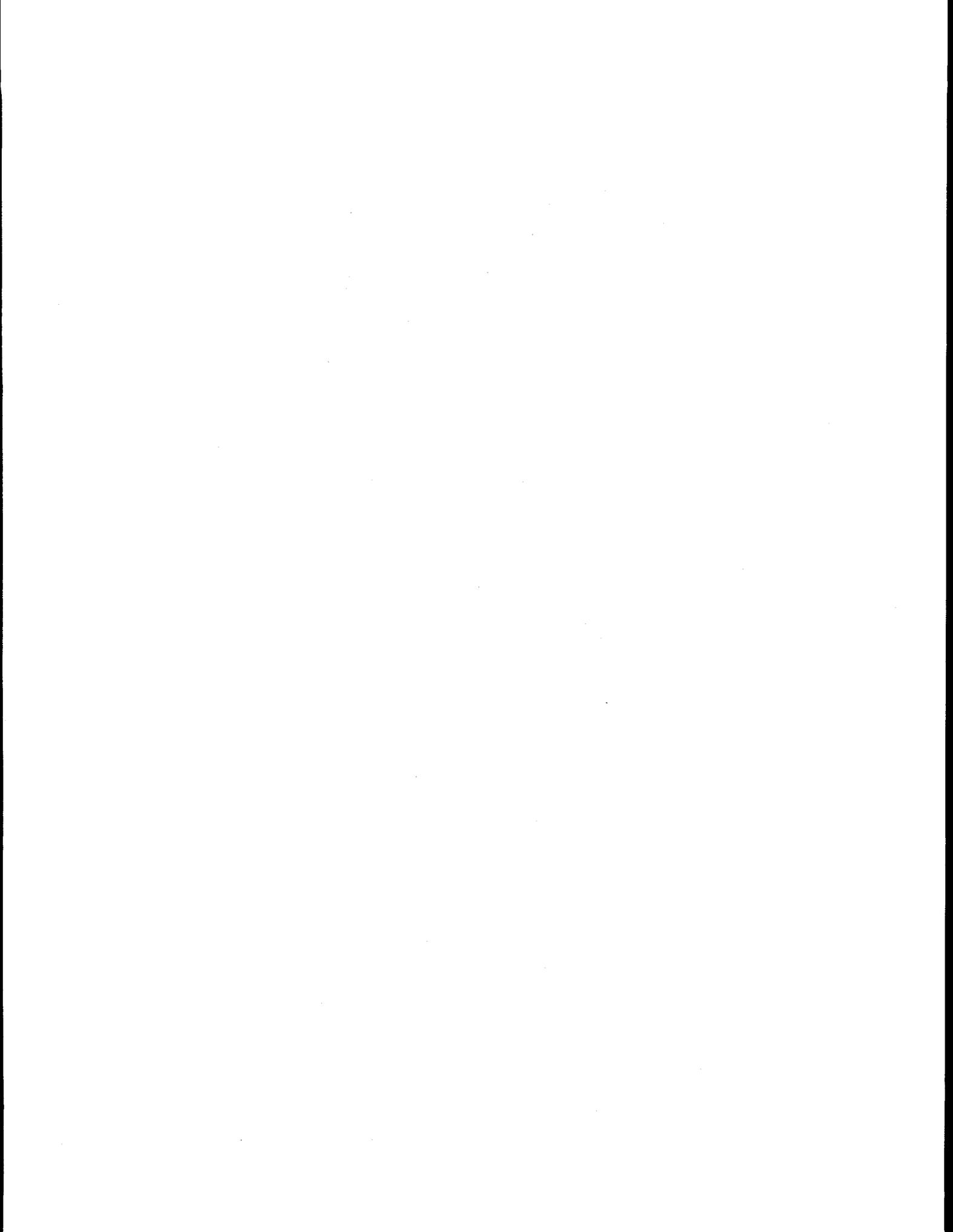
Xu, X., John, V., McPherson, G., Grimm, D., Akkara, J. and Kaplan, D. "A Combined Chemical-Enzymatic Method to Remove Selected Aromatics from Aqueous Streams". *Applied Biochemistry and Biotechnology*, v. 51/52, p. 649, 1995.

Xu, X., John, V., McPherson, G. and Grimm, D. "A Chemical + Enzymatic Method to Remove Aromatics from Aqueous Systems". Division of Environmental Chemistry, 1994 American Chemical Society Meeting. Presented in Aug. 1994, Washington, D.C.

Grimm, D., Clymire, J. and McPherson, G. "Environmental Chemistry of a Historical Fish Collection". 1995 Pittsburgh Conference Abstracts, p. 1164. Presented in March 1995, New Orleans.

## **Appendix A**

### **List of Review Panelist and Consultants**



**Hazardous Materials in Aquatic Environments of the Mississippi River Basin  
Review Panel Listing  
December 4-5, 1995 Review**

**Leroy Folmar (Biological Sciences)**

Fish Biologist/Ecologist

USEPA

Ph.D. - Fisheries - University of Washington

Research areas: Chemically induced Endocrine disruption in fish.

**Carl Gehrs (Biological Sciences)**

Director

Center of Biotechnology

Oak Ridge National Laboratory

Ph.D.- Limnology - University of Oklahoma

Research areas: Population biology, biotechnology, bioremediation and environmental contamination.

**Louis J. Guillette, Jr. (Biological Sciences)**

Professor

Department of Zoology

University of Florida

Ph.D.-Biology-University of Colorado, Boulder

Research areas: Environmental contaminants effects of reproductive biology.

**Gerald LeBlanc (Biological Sciences)**

Associate Professor,

Department of Toxicology

North Carolina State University

Ph.D.-Biology-University of South Florida

Research areas: Endocrine disrupting chemicals, toxicity of environmental pollutants and xenobiotic/steroid metabolism and cellular transport.

**Craig Sullivan (Biological Sciences)**

Professor

Dept. of Zoology

North Carolina State University

Ph.D: -Fisheries Science - University of Washington, Seattle

Research areas: Fish reproductive physiology and endocrinology with emphasis on regulation of gametogenesis and application of findings to aquatic cultures, fisheries management and toxicology.

**Cheryl Walker (Biological Sciences)**

Associate Professor of Carcinogenesis

The University of Texas M.D. Anderson Cancer Center

Ph.D.-Biology-University of Texas Health Sciences Center, Southwestern Medical School, Dallas, Texas.

Research areas: Carcinogenesis

**James Johnson, Jr. (Engineering)**

Professor and Acting Dean

Howard University

Civil Engineering Department

Ph.D.- Applied Sciences, University of Delaware

Research areas: Reuse of wastewater treatment sludge and treatment of hazardous substances.

**Charles Kidd\* (Engineering)**

Associated Vice President for Environmental Programs

Florida A&M University

Ph.D. - Environmental Health Sciences - University of Michigan

M.S. - Sanitary & Industrial Hygiene Engineering, University of Michigan

M.S. - Radiological Health Physics, University of Michigan

B.S. - Civil Engineering, Case Institute of Technology, Cleveland, Ohio

Research areas: Impact of nuclear facilities wastes on terrestrial and aquatic environments including acute and chronic effects of radionuclides on benthic organisms.

**Lovell Jones (Health Effects)**

Professor & Director

Experimental Gynecology-Endocrinology

The University of Texas

M.D. Anderson Cancer Center

Ph.D. - Zoology (Tumor Biology & Endocrinology) - University of California, Berkeley.

Research areas: Hormonal carcinogenesis and public policy issues looking at health care issues as they relate to underserved communities.

**Janice Longstreth (Health Effects)**

Risk Program Director

Waste Policy Institute

Ph.D.-Biomedical Sciences, University of Tennessee-Oak Ridge

Research areas: Health Risk assessment and immunotoxicology and carcinogenesis.

**Norman Cutshall (Physical Science)**

Office Manager

Oak Ridge National Laboratory

Ph.D.-Oceanography - Oregon State University

Research areas: Environmental radioactivity, geochemistry, economic geology.

**Janet S. Herman (Physical Sciences)**

Associate Professor of Environmental Sciences

Dept. of Environmental Sciences

University of Virginia

PhD: - Geochemistry - The Pennsylvania State University

Research areas: Low-temperature aqueous geochemistry, encompassing problems in water-rock interactions, kinetics of geochemical reactions, and evolution of groundwater chemistry in various hydrogeological environments. Understanding the physical, chemical, and biological factors that influence the transport of bacteria and reactive solutes in a sandy aquifer; environmental fate of agricultural chemicals in a karst watershed; and the impact of geological heterogeneities on the transport characteristics of dissolved and suspended constituents in groundwater.

**Consultants**

Cynthia J. Kaleri

US-EPA

Environmental Engineer

Sr. Remedial Project Manager

Mike Maskarinec

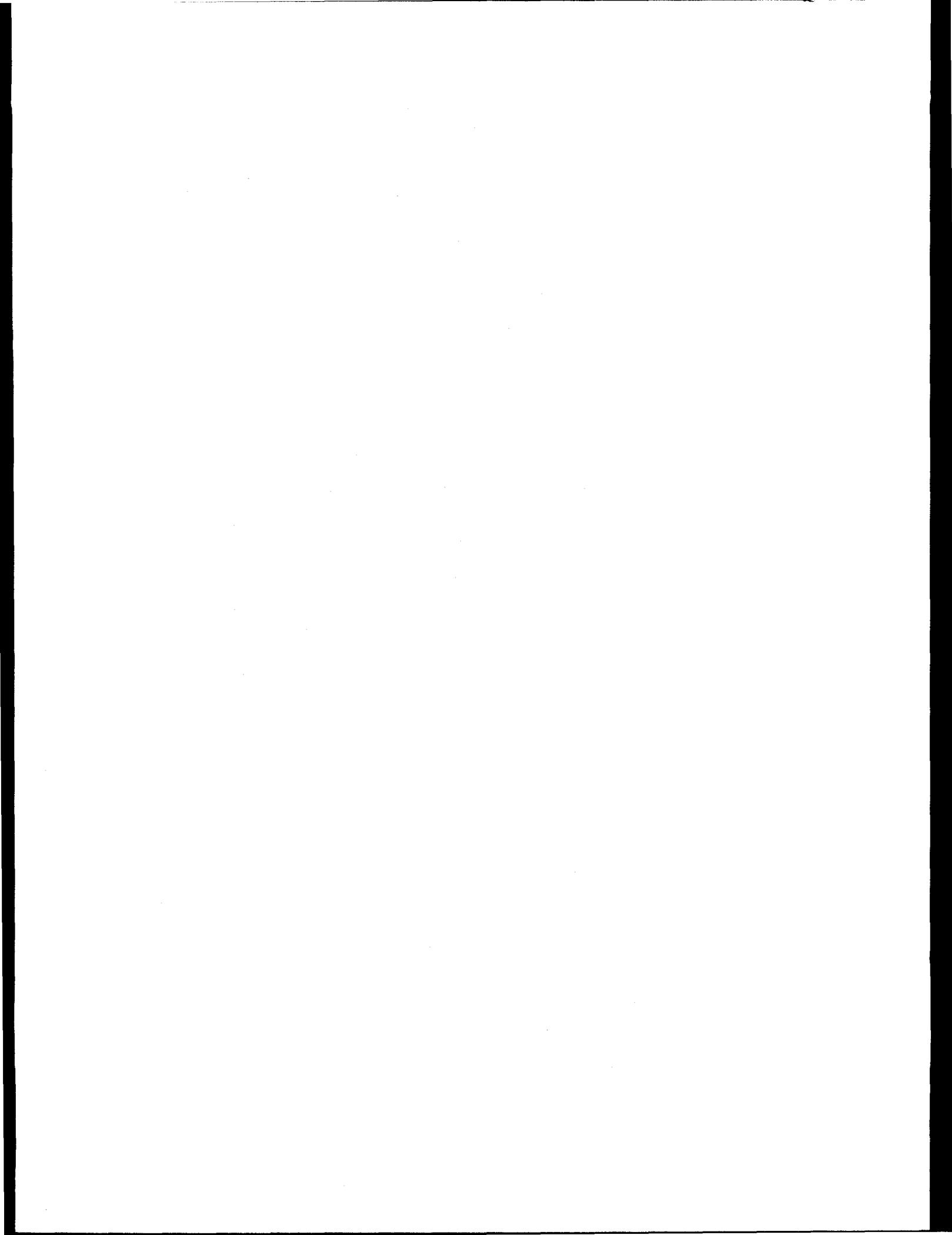
Oak Ridge National Laboratory

Laboratory Director,

Chemical and Analytical Sciences Division

## **Appendix B**

### **July Retreat Program**



**Tulane/Xavier Center for  
Bioenvironmental Research**

**Hazardous Materials In  
Aquatic Environments Of  
The Mississippi River Basin**

**Summer Retreat  
June 19 - 20, 1995  
Hyatt Regency Hotel  
New Orleans, Louisiana**

**Project #DE-FG01-93EW532023  
(a DOE funded project)**

**MONDAY - JUNE 19, 1995**

- 8:00 Registration/Continental Breakfast  
*Cabildo Room B*
- 8:30 Introduction  
Charles Ide, Ph.D.
- 8:50 Bayou Trepagnier Site Overview  
Leonard Thien, Ph.D., Janet Preslan, Ph.D.,  
George Flowers, Ph.D.
- 10:20 Morning Break in *Cabildo Foyer*
- 10:35 Devils Swamp Site Overview  
William Hartley, Ph.D.
- 12:05 Lunch in *Cabildo Room A*
- 12:50 Keynote Presentation  
"Model Ecosystems in Environmental Research"  
John McLachlan, Ph.D.  
*Cabildo Room A*
- 1:30 Break Out Sessions (\* denotes chairperson)
- Analysis - *Delgado Room*  
Panel: G. McPherson D. Grimm  
H. Mielke \* W. George  
A. Abdelghani
- Biomarkers - *Esplanade Room C*  
Panel: M. Anderson W. Hartley  
M. Fingerman \* P. Obih
- Ecology - *Elysian Fields Room*  
Panel: J. Preslan L. Thien \*
- Fate & Transport/Modeling - *Esplanade Room A*  
Panel: R. Baker W. Toscano  
E. Eschenazi L. Fauci \*
- Remediation - *Cabildo Room B*  
Panel: S. Bhattacharya B. Blake  
A. Applett G. Flowers \*

- 3:00 Afternoon Break in *Esplanade Foyer*
- 3:15 Break Out Sessions Continue
- 4:30 Finalize Summary of Break Out Sessions

**TUESDAY - JUNE 20, 1995**

- 8:00 Continental Breakfast  
*Cabildo Room B*
- 8:30 Break Out Session Summary Presentations
- 8:45 Analysis  
Howard Mielke, Ph.D.
- 9:15 Biomarkers  
Milton Fingerman, Ph.D.
- 9:45 Ecology  
Leonard Thien, Ph.D.
- 10:15 Morning Break in *Cabildo Foyer*
- 10:45 Fate & Transport  
Lisa Fauci, Ph.D.
- 11:15 Remediation  
George Flowers, Ph.D.
- 12:00 Lunch in *Cabildo Room A*
- 1:00 A General Discussion of Whether Pollution has Resulted  
in Dysfunction in the Devils Swamp & Bayou Trepagnier  
Ecosystems - *Cabildo Room B*  
Panel: A. Abdelghani S. Bhattacharya  
M. Fingerman W. George  
W. Hartley C. Ide  
G. McPherson H. Mielke  
L. Thien W. Toscano
- 2:00 Environmental Education Cluster Meeting  
*Poydras Room A*
- 3:00 Adjournment

## **Appendix C**

### **Review Procedures**

**HAZARDOUS MATERIALS IN AQUATIC ENVIRONMENTS  
OF THE MISSISSIPPI RIVER BASIN  
TULANE/XAVIER EM PROJECT**

**EXTERNAL REVIEW PANEL PROCEDURE  
Fourth Year Funding Period - 2/1/96-1/31/97**

Proposals will be assigned reviewers based on the focus area(s) designated on the pre-proposal Letter of Intent submitted by each principal investigator. The review areas are 1) Biological Sciences 2) Engineering 3) Health Effects and 4) Physical Sciences. Each research proposal has been assigned a primary and a secondary reader. Larger cluster proposals (6 or more investigators) will be assigned a tertiary reader.

Each panelist will read the full proposal for which he or she has been assigned as a primary; secondary or tertiary reader. Additionally, the panelists will read the executive summaries and review the budgets of the remaining proposals.

The primary and secondary readers will fill out a brief evaluation form (enclosed) for each proposal. These evaluation forms should be completed and signed prior to the panel meeting. Anonymous versions of evaluations will be sent to the proposal PI's after the review process has been completed.

Each panelist will attend the project poster session December 4, 1995 at Tulane University. The session will highlight work from all of the currently funded projects. The session will run from 2:00 p.m. - 5:00 p.m. Following the session, the reviewers will assign a *rating* of either **consider (recommend the proposal be further considered for funding)**, **eliminate (recommend that the proposal not be considered for funding)**, to their proposals. Proposals elected for elimination by all of the primary; secondary and tertiary (when applicable) reviewers will be removed from further discussion, providing that no other panelist presents objections. The panel will review the scientific value of the research and rank the remaining proposals.

This will be accomplished by having the primary and secondary readers make brief presentations on each of the proposals under consideration. The presentations will be followed by a general discussion. Following these discussions, reviewers will give each proposal an overall rating (1-5, 5 being the best). Proposals will be ranked based on these scores.

The panel will adjourn and one panelist will present the results to the Administrative Committee who will determine the funding distribution based on the targeted research needs of the project. The Administrative Committee will consist of the DOE program manager (Jeffrey Walker), the CBR director (John McLachlan), Director of Xavier's Office of Sponsored Programs (Alden Reine), a representative from the review panel (Norm Cutshall), two technical advisors (Cynthia Kaleri, EPA and Michael Maskarinec, ORNL) and William Toscano, Tulane Environmental Health Sciences Department, and Stephen A. Nelson, Tulane Geology Department.

## **Appendix D**

### **Program of the 3rd Annual Poster Session**

**Hazardous Materials In Aquatic Environments Of  
The Mississippi River Basin  
Poster Session - December 4, 1995**

Poster #	Poster Titles - Authors - Affiliations
1	<p>Laser Assisted Particle Removal</p> <p>Allen, S., Lee, J., Zhang, X.</p> <p>TU-Chemistry</p>
2-3	<p>Collaborative Research with IREP and CREM of Belarus on the Contamination of Rivers Following the Chernobyl Disaster</p> <p>Michaelides, E.<sup>1</sup>, Sailor, D.<sup>1</sup>, Ramer, S.<sup>2</sup>, Pimenov, O.<sup>1</sup>, Melkozerova, O.<sup>1</sup></p> <p><sup>1</sup>TU-Mechanical Engineering, <sup>2</sup>TU-History</p>
4	<p>Heavy Metals in Lizard's Tail</p> <p>Thien, L.<sup>1</sup>, Ellgaard, E.<sup>1</sup>, Kumar, S.<sup>1</sup>, Devall, M.<sup>2</sup>, Thomas, C.<sup>2</sup>, Li, M., Jobes, D., Hurley, D.</p> <p><sup>1</sup>TU-Cell &amp; Molecular Biology, <sup>2</sup>US Forestry Service</p>
5	<p>Dendrochronology and Heavy Metals in Tree Rings of Baldcypress III</p> <p>Thien, L.<sup>1</sup>, Ellgaard, E.<sup>1</sup>, Devall, M.<sup>2</sup>, Thomas, C.<sup>2</sup>, Kumar, S.<sup>1</sup>, Latimer, S.<sup>1</sup></p> <p><sup>1</sup>TU-Cell &amp; Molecular Biology, <sup>2</sup>U.S. Forestry Service</p>
6-7	<p>Community, Population, and Trophic Responses to Aquatic Contamination in the Lower Mississippi River System</p> <p>Bart, H.<sup>1</sup>, Martinat, P.<sup>2</sup></p> <p><sup>1</sup>TU-Ecology, Evolution, Organismal Biology, <sup>2</sup>XU-Biology</p>
8	<p>Colonial Wading Birds as Indicators of Heavy Metals in Louisiana Wetlands</p> <p>Spahn, S., Sherry, T.</p> <p>TU-Ecology, Evolution, Organismal Biology</p>
9-10	<p>Levels and Toxicities of Selected Inorganic and Organic Contaminants In a Swamp Environment</p> <p>Abdelghani, A.<sup>1</sup>, Prammar, Y.<sup>2</sup>, Mandal, T.<sup>1</sup>, Tchounwou, P.<sup>2</sup>, Heyer, L.<sup>1</sup></p> <p><sup>1</sup>TSPH-Environmental Health Sciences, <sup>2</sup>XU-Pharmacy</p>
11	<p>Acute Toxicities of Hexachlorobenzene and Hexachlorobutadiene to Juvenile Crawfish Procambarus Species</p> <p>Heyer, L.<sup>1</sup>, Abdelghani, A.<sup>1</sup>, Prammar, Y.<sup>2</sup>, Mandal, T.<sup>2</sup>, Tchounwou, P.<sup>2</sup></p> <p><sup>1</sup>TSPH-Environmental Health Sciences, <sup>2</sup>XU-Pharmacy</p>
12	<p>Recovery and Analysis of Hexachlorobenzene and Hexachlorobutadiene In Environmental Samples: A critical Comparison of Two Extraction Techniques</p> <p>Tchounwou, P.<sup>2</sup>, Heyer, L.<sup>1</sup>, Abdelghani, A.<sup>1</sup>, Prammar, Y.<sup>2</sup>, Mandal, T.<sup>2</sup></p> <p><sup>1</sup>TSPH-Environmental Health Sciences, <sup>2</sup>XU-Pharmacy</p>

13-18	<p>Biomarkers of Exposure and Ecotoxicity in the Mississippi River Basin</p> <p>Hartley, W.<sup>1</sup>, Ide, C.<sup>2</sup>, Mizell, M.<sup>2</sup>, Thiyagarajah, A.<sup>1</sup>, Tompkins, R.<sup>2</sup>, Horner, E.<sup>3</sup>, Huang, T.<sup>4</sup>, Obih, P.<sup>4</sup></p> <p><sup>1</sup>TSPH-Environmental Health Sciences, <sup>2</sup>TU-Cell &amp; Molecular Biology, <sup>3</sup>TUMS-Medicine, <sup>4</sup>XU-Pharmacy</p>
19	<p>Development of Standard Methods to Measure the Effect of Heavy Metals on Sphingomyelin Signal Transduction in Cultured Algae</p> <p>Rosenzweig, N., Clejan, S.</p> <p>TU-Pathology</p>
20	<p>Environmental Spatial Modeling</p> <p>Steinberg, L., Luna, R.</p> <p>TU-Civil &amp; Environmental Engineering</p>
21-22	<p>GIS-Based Modeling of Bayou Trepagnier and Devil's Swamp</p> <p>Bakeer, R.<sup>1</sup>, Belkhouche, B.<sup>2</sup>, Luna, R.<sup>1</sup>, Steinberg, L.<sup>1</sup></p> <p><sup>1</sup>TU-Civil &amp; Environmental Engineering, <sup>2</sup>TU-Computer Science</p>
23	<p>Applying Geographical Information Systems to Devil's Swamp</p> <p>Regens, J.<sup>1</sup>, Watanabe, K.<sup>1</sup>, Hodges, D.<sup>1</sup>, Johnson, R.<sup>1</sup>, Swalm, C.<sup>1</sup>, Davis-Nelson, S.<sup>2</sup>.</p> <p>TSPH-Environmental Health Sciences</p>
24	<p>Modeling Bioaccumulation of Pollutant Loadings in Aquatic Ecosystems</p> <p>Regens, J., Watanabe, K., Hodges, D., Abdelghani, A., Hartley, W., Thiyagarajah, A.</p> <p>TSPH-Environmental Health Sciences</p>
25	<p>Coordinated Instrumentation Facility</p> <p>Lyttle, T., Grimm, D., Burnside, P., Douglas, W., Smith, L.</p> <p>Coordinated Instrumentation Facility</p>
26	<p>Mapping Applications Bayou Trepagnier</p> <p>Smith, S.<sup>1</sup>, Flowers, G.<sup>2</sup>, Means, J.<sup>3</sup></p> <p><sup>1</sup>TU-Center for Bioenvironmental Research, <sup>2</sup>TU-Geology, <sup>3</sup>LSU-Veterinary Medicine</p>
27-28	<p>Enhancement of Environmental Education at Tulane and Xavier Universities</p> <p>Bennett, J.<sup>1</sup>, Bhattacharya, S.<sup>2</sup>, Hassell, J.<sup>3</sup>, O'Connor, S.<sup>4</sup>, White, L.<sup>5</sup>, Zimmerman, M.<sup>6</sup></p> <p><sup>1</sup>TU-Cell &amp; Molecular Biology, <sup>2</sup>TU-Civil &amp; Environmental Engineering, <sup>3</sup>TU-Computer Science, <sup>4</sup>XU-Environmental Programs, <sup>5</sup>TSPH - Tropical Medicine, <sup>6</sup>TU-Philosophy</p>
29	<p>Molecular Mechanisms Regulating Effect of Estrogens on Male Gonadal Axis</p> <p>Sikka, S.<sup>1</sup>, Anderson, M.<sup>2</sup></p> <p><sup>1</sup>TUMS-Urology, <sup>2</sup>TU-Anatomy</p>
30-32	<p>The Mississippi River Basin as a Model System for the Study of Environmental Pollutants and Bicrobiota</p> <p>Mielke, H.<sup>1</sup>, Bennett, J.<sup>2</sup>, Kale, S.<sup>3</sup>, Bell, M.<sup>3</sup>, Buckalew, D.<sup>3</sup>, Wang, G.<sup>4</sup>, Welt, M.<sup>1</sup></p> <p><sup>1</sup>XU-Pharmacy, <sup>2</sup>TU-Cell &amp; Molecular Biology, <sup>3</sup>XU-Biology, <sup>4</sup>XU-Chemistry</p>

33	<p>Similarity of Molecular Regulation of Growth Hormone in Fish and Mammals</p> <p>Hurley, D.<sup>1</sup>, Welt, M.<sup>2</sup></p> <p><sup>1</sup>TU-Cell &amp; Molecular Engineering, <sup>2</sup>XU-Pharmacy</p>
34	<p>Organophosphate Decontamination and Remediation Using Microencapsulated Enzymes</p> <p>John, V.<sup>1</sup>, McPherson, G.<sup>2</sup>, Banerjee, S.</p> <p><sup>1</sup>TU-Chemical Engineering, <sup>2</sup>TU-Chemistry</p>
35-38	<p>Natural and Active Chemical Remediation of Toxic Metals, Organics, and Radionuclides in the Aquatic Environment</p> <p>McPherson, G.<sup>1</sup>, Pintauro, P.<sup>2</sup>, O'Connor, S.<sup>3</sup>, John, V.<sup>2</sup>, Gonzalez, R.<sup>2</sup>, Bianchi, T.<sup>4</sup>, Bundy, K.<sup>5</sup>, Zhang, J.<sup>3</sup>, Flowers, G.<sup>6</sup></p> <p><sup>1</sup>TU-Chemistry, <sup>2</sup>TU-Chemical Engineering, <sup>3</sup>XU-Chemistry, <sup>4</sup>TU-Ecology, Evolution, Organismal Biology, <sup>5</sup>TU-Biomedical Engineering, <sup>6</sup>TU-Geology</p>
39-40	<p>Bayou Trepagnier: Analyses and Characterization of Sediments, Water and Aquatic Species</p> <p>George, W.<sup>1</sup>, Preslan, J.<sup>1</sup>, Anderson, M.<sup>2</sup>, Bollinger, J.<sup>1</sup>, Lee, A.<sup>1</sup>, Adams, S.<sup>2</sup>, St. Philip, J.<sup>1</sup></p> <p><sup>1</sup>TU-Pharmacology, <sup>2</sup>TU-Anatomy</p>
41-43	<p>Laboratory Studies: Metal-Exposure of Crayfish and Biomarkers</p> <p>Anderson, M.<sup>1</sup>, Preslan, J.<sup>2</sup>, George, W.<sup>2</sup>, Sikka, S.<sup>3</sup>, Agrawal, K.<sup>2</sup>, Bundy, K.<sup>4</sup>, Kamath, B.<sup>2</sup>, Bollinger, J.<sup>2</sup>, Rajasekaran, M.<sup>3</sup>, Millet, L.<sup>3</sup>, Jolibois, L.<sup>1</sup>, Chen, H.<sup>2</sup></p> <p><sup>1</sup>TUMS-Anatomy, <sup>2</sup>TU-Pharmacology, <sup>3</sup>TU-Urology, <sup>4</sup>TU-Biomedical Engineering</p>
44	<p>Bioremediation of Petroleum Products</p> <p>Blake, R., Lyles, M.</p> <p>XU-Pharmacy</p>
45-46	<p>A Sensitive Rapid On-Site Immunoassay For Heavy Metal Contamination</p> <p>Blake, R.<sup>1</sup>, Blake, D.<sup>2</sup>, Flowers, G.<sup>3</sup></p> <p><sup>1</sup>XU-Pharmacy, <sup>2</sup>TUMS-Ophthalmology, <sup>3</sup>TU-Geology</p>
47	<p>Characterization of Organics in Sediments by Thermal Analysis</p> <p>Aplett, A., Reinhardt, L., Walker, E.</p> <p>TU-Chemistry</p>
48-49	<p>Biological Uptake and Metabolism Studies</p> <p>Aplett, A.<sup>1</sup>, Barber, J.<sup>2</sup>, Eduok, E.<sup>3</sup>, Ensley, H.<sup>1</sup>, Fingerman, M.<sup>2</sup>, Fink, M.<sup>1</sup></p> <p><sup>1</sup>TU-Chemistry, <sup>2</sup>TU-Ecology, Evolution &amp; Organismal Biology, <sup>3</sup>XU-Chemistry</p>
50	<p>Cytogenetic Monitoring of Crayfish in a Polluted Environment</p> <p>Fingerman, M., Devi, M.</p> <p>TU-Ecology, Evolution, Organismal Biology</p>
51-52	<p>Novel Biomarkers for Heavy Metal Toxicity in Aquatic Species</p> <p>McNamara, D.<sup>1</sup>, Agrawal, K.<sup>1</sup>, Kadowitz, P.<sup>1</sup>, Ochillo, R.<sup>2</sup>, Olubadewo, S.<sup>2</sup></p> <p><sup>1</sup>TU-Pharmacology, <sup>2</sup>XU-Pharmacy</p>

53	- Aryl Hydrocarbon Receptor Activation as a General Biomarker of Contamination in Aquatic Systems Miller, C. TSPH-Environmental Health Sciences
54-56	Flocculation Studies in Environmental Processes Eschenazi, E. <sup>1</sup> , Effiong, R. <sup>2</sup> , Akundi, M. <sup>1</sup> , Kocic, V. <sup>3</sup> , Das, B. <sup>1</sup> , Papadopoulos, K. <sup>4</sup> , Deshiikan, S. <sup>4</sup> <sup>1</sup> TUMS-Anatomy, <sup>2</sup> TU-Pharmacology, <sup>3</sup> XU-Mathematics, <sup>4</sup> TU-Chemical Engineering
57-58	Pore-level flow, Transport, Agglomeration and Reaction Kinetics of Microorganisms Fauci, L. <sup>1</sup> , Dillon, R. <sup>1</sup> , Gaver, D. <sup>2</sup> , Liu, Z. <sup>3</sup> , Moore, P. <sup>1</sup> , Papadopoulos, K. <sup>3</sup> , Sharma, B. <sup>4</sup> <sup>1</sup> TU-Mathematics, <sup>2</sup> TU-Biomedical Engineering, <sup>3</sup> TU-Chemical Engineering, <sup>4</sup> XU-Mathematics
59-62	Bioremediation of Selected Contaminants in Bayou Trapanier Bhattacharya, S. <sup>1</sup> , Law, V. <sup>2</sup> , Mullin, D. <sup>3</sup> , Eckert, J. <sup>4</sup> , Fulginiti, R. <sup>4</sup> , Ross, J. <sup>4</sup> , Medgani, K. <sup>4</sup> , Kamath, B. <sup>5</sup> <sup>1</sup> TU-Civil & Environmental Engineering, <sup>2</sup> TU-Chemical Engineering, <sup>3</sup> TU-Cell & Molecular Biology, <sup>4</sup> XU-Biology, <sup>5</sup> XU-Pharmacy
63	Development of Cytochrome P450 Isozymes with Novel Designed Catalytic Activities for Use in Bioremediation of Recalcitrant Organic Pollutants Mullin, D. TU-Cell & Molecular Biology
64	Encapsulation and Degredation of Pollutants: Role of Zeolites and Light Ramamurthy, V. TU-Chemistry
65	Heavy Metals in Mosquitoes: Toxicity and Bioaccumulation Beier, J., Straif, S., Mazzacano, C. TSPH-Tropical Medicine

TU: Tulane University (Uptown Campus)

TSPH: Tulane University School of Public Health and Tropical Medicine

TUMS: Tulane University Medical School

XU: Xavier University

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## **Appendix E**

### **Summary of Published Articles**

**List of Publications in Peer Reviewed  
Journals Resulting From DOE/EM Funded Work.**

Abdelghani AA., Pramar YV., Mandal TK., Tchouwou PB., and Heyer L. Levels and Toxicities of Selected Inorganic and Organic Contaminants in a Swamp Environment." *Journal of Environmental Science and Health*, B30(5): 717-731, 1995.

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Anderson, MB., Preslan, JE., Jolibois, LS., Bollinger, JE., and George, WJ. "Bioaccumulation of Lead in Red Swamp Crayfish (*Procambarus clarkii*). *Journal of Hazardous Materials*. (Submitted)

Andrade, FW. "A Geographical Information System Application: A Boring Data Retrieval Program for Kenner, Louisiana," Senior Honor Thesis, Dept. of Civil and Env. Eng., Tulane Univ., 1995.

Apblett, AW., Ahmadi, A. and Walker, EH (1996). Layered Titanates as Sinterable Ion-exchangers for Actinides. *Environmental Science Technology*. (Submitted)

Apblett, AW., Ahmadi, A., and Walker, EH. Incorporation Of Actinides Into Titanate-Phases Using Ion-Exchange Materials, *Advances in Ceramics*. (Submitted)

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Barber, JT., Sharma, HA., Ensley, HE., Polito, MA. and Thomas, DA. (1995). Detoxification of Phenol by the Aquatic Angiosperm, *Lemna gibba*. *Chemosphere*, 31 (6): 3567 - 3574.

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Blake, D.A., Chakrabarti, P., Khosraviani, M., Hatcher, F.M., Westhoff, C.M., Goebel, P., Wylie, D. E., and Blake, II, R. C. (1996) "Isolation and Characterization of a Monoclonal Antibody Specific for Cadmium-Chelate Complexes Biochemistry (submitted).

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