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

Quarterly Progress Report

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Project #DE-FG01-93EW532023
(January 1, 1995 - March 31, 1995)
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ADMINISTRATIVE ACTIVITIES

- Notified applicants which proposed projects would be funded for 1995. Established internal accounts for these projects.

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

- Began procedures for obtaining e-mail/Internet accessibility for all DOE/EM Principal Investigators.

- Compiled annual technical report for '93-'94 project period.

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

- 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.

- Developed a Center for Bioenvironmental Research (CBR) 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.

- Held a two-day meeting to highlight the research and 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.

- Hired a part-time computing consultant to develop graphics for CBR presentations, and develop a database to track all CBR-sponsored research.
**COLLABORATIVE CLUSTER PROJECTS**

**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

- Toxicity testing of metal mixtures microorganisms, collected from Devils Swamp, have been completed. Data is being analyzed.

- Screening studies for long term exposure have been completed and bioaccumulation studies have been started.

- 80 fish, 32 water and 24 sediment samples were analyzed from Devils Swamp and Tunica Swamp. Samples were analyzed for arsenic, cadmium, chromium, lead, mercury and nickel using ICP equipped with Ultra Sonic Nebulizer.

- Water and sediment samples collected in 1994 were analyzed for hexachlorobutadiene (HCBD).

- HCBD was detected in 4 out of 32 water samples but none exceeded the maximum contaminant limit of 20 ppb.

- HCBD levels in sediment ranged from 0.11 - 5.03 ppb which is below the alert level of 4ppm.

**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

**Meetings**

- One cluster meeting was held including all cluster participants.

- Two meetings were held for the planning and execution of an extensive crayfish hexavalent chromium exposure study.
With completion of the tadpole lead exposure study, Dr. Bundy and D. Berzins have had the work accepted for presentation in May at the V.M. Goldschmidt Conference.

**Laboratory Studies**

The crayfish hexavalent chromium exposure study involves the participation of four principal investigators, a postdoctoral fellow and students in the cluster. The main topics under study are pharmacokinetic uptake and clearance modeling, histological changes and toxicity, and chromium speciation (conversion of valence state due to metabolic action or other causes within the animals).

- This study commenced at the end of this quarter and is currently in progress.

- Crayfish are being exposed to three concentrations of hexavalent chromium (0.3 ppm, 3 ppm, 30 ppm). Control animals are maintained in water with no added chromium.

- Time zero animals were collected to measure endogenous levels of chromium present in the hepatopancreas, gills, ovary, exoskeleton, abdominal muscle and hemolymph.

- Animals are evaluated after 2 days, 2 weeks, 4 weeks and 7 weeks of chromium exposure. After 7 weeks of chromium exposure the animals will be maintained in water with no added chromium for 1 and 3 weeks to monitor clearance of chromium from the tissues.

- At each collection time, the animals are weighed and the above listed tissues are collected for chromium evaluation and/or histological study.

- Development of analytical methodology for conducting tissue chromium speciation studies has continued.

- Ms. L. Millet, a graduate student, has worked out the procedures for determination of total chromium and hexavalent chromium for speciation studies.

- The lead analyses of gills, hepatopancreas, and muscle tissues for the Experiment 3 150 ppb lead exposure study of crayfish were completed. Digestion and analyses of the remaining tissues continues.

- Metal analyses by ICP for water samples collected during the Bonnet Carre Spillway opening and the previous PVC pipe study are near completion.
Field Studies

- As part of the project objective to characterize the waterways of the LaBranche wetlands, water parameters at the study sites (3 sites in Bayou Trepagnier, 1 site in Bayou Traverse and 1 site in Lake Pontchartrain) were again evaluated in February and March.

- In addition to adding information about seasonal variability of parameters, the objective was to see whether or not any effects could be observed which might be related to the cessation of discharge from the Shell chemical complex into the headwaters of Bayou Trepagnier which occurred on February 24, 1995.

- Parameters measured included relative water depth, nutrient content, alkalinity, pH, conductivity, temperature, dissolved oxygen, dissolved metals, and oil and grease. Sediment oxygen demand and nutrient flux from the sediments were also determined.

- Also in March another PVC pipe study was conducted to evaluate the conditions in the wetlands becoming anaerobic. Covered PVC pipes were driven into the bayou sediments which isolated columns of water and sediment. Parameters were measured over a seven day period.

- Drs. Anderson and George are collaborating with Dr. Fingerman in a study involving the exposure of crayfish in Bayou Trepagnier. After sorting crayfish as to gender and size, female crayfish were placed in cages in heavily contaminated areas of Bayou Trepagnier.

- Time zero crayfish were taken for measurement of endogenous concentrations of metals.

- Crayfish were collected after 1, 2, 4 and 7 days of exposure in the field. Work is currently in progress for metal analyses of the various crayfish tissues.

- Dr. Fingerman is measuring neurological parameters and conducting the histological studies.

Hazardous Wastes in Aquatic Environments: Biological Uptake and Metabolism Studies

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

Highlights

- A manuscript describing the detoxification of phenol by *Lemna gibba* was submitted for publication to "Environmental Toxicology and Chemistry".
• Studies on the metabolism of 2,4,5-trichlorophenol to b-trichlorophenylglucoside by *Lemna gibba* were completed and a manuscript is in preparation for submission to "Chemosphere".

• Further work on the reductive dechlorination of 2,4,5-trichlorophenol to b-phenylglucoside, b-chlorophenylglucoside and b-2,4-dichlorophenylglucoside by *Lemna* is in progress.

• An investigation of the metabolism of aniline and n-butanol by *Lemna gibba* is proceeding. The isolation and purification of two aniline metabolites is in progress; structural identification will follow.

• Caged crayfish were placed in Bayou Trepagnier for 0, 1, 2, 4 and 7 days. The pH of the gastric juice, which is secreted by the hepatopancreas, increased from 5.22 to 6.22 during the period of study. Histological studies of the hepatopancreas of the crayfish are in progress.

• In a laboratory study the DNA content (ng/g) of the ovaries, both immature and mature, increased upon exposure of the crayfish to cadmium.

• The effects of cadmium in a simple aquatic food chain involving duckweed and crayfish have been determined. Bioconcentration and biomagnification were demonstrated and several useful physiological endpoints in the crayfish were identified. The results are currently being written up for submission to "Ecotoxicology and Environmental Safety".

• Experiments have been continued with aniline and 1-butanol to find optimum concentrations of these chemicals in which to grow rice for metabolism studies.

• Established large incubations of germinating rice with 8 mM 1-butanol to analyze, by HPLC, for metabolite production using 14C-labeled 1-butanol as a tracer.

• Demonstrated the efficacy of sodium titanate for the removal of thorium from synthetic thorium-containing duckweed extracts.

• Synthesized Ce(PO₄Na₂)₃, an ion-exchanger for removal of thorium from solution which allows the thorium to be immobilized as monazite, a phosphate rock.

• Tested Ce(PO₄Na₂)₃ as an ion-exchanger for removal of thorium from solution. It did this extremely well but with the unacceptable concomitant release of cerium into solution. The results suggest that FePO₄ might be a more environmentally-acceptable reagent.
- Demonstrated that extraction with 2-ethylhexanoic/ammonium ethylhexanoate mixtures is an excellent solventless method for removal of actinides and lanthanides from aqueous solution.

- Received acceptance for a presentation entitled "Integrated Biological and Chemical Remediation of Environmental Actinide Contamination" to be given at the 78th Canadian Society for Chemistry Conference.

ECOLOGICAL SENTINELS OF AQUATIC CONTAMINATION IN THE LOWER MISSISSIPPI RIVER SYSTEM


Project I: Community and Trophic Responses of Fishes to Aquatic Contamination

- Samples of fish, frogs, crustaceans, invertebrates, water, sediment, soil, and vegetation were taken in Devils and Tunica swamps in January and March.

- The sampling effort in Devils Swamp yielded a total of 29 fish representing 12 species collected in January and March.

- The tag and release effort in Devils Swamp has yielded a total of 62 fish representing 11 species tagged and released in January.

- The sampling effort in Tunica Swamp yielded a total of 72 fish representing 15 species in January and March.

- Fish samples were delivered to Dr. William Hartley for necropsy and histopathological screening.

- Stomachs were removed for contents analysis by co-PI Martinat. The results from the stomach analysis showed that 28, invertebrate taxa were represented in 223 fish stomachs from 22 species of fish.

- Samples of water, sediment, fish, and crayfish are awaiting analysis of heavy metals and selected organic contamination by Dr. Abdelghani and the CIF.
• Dr. Henry Bart traveled to Baton Rouge March 2nd and delivered a presentation at LSU entitled "Ecological Sentinels of Aquatic Contamination in the Lower Mississippi River System."

Project II: Tree Cores as Biomarkers

Collection of a Different Set of Tree Cores From a New Control Site
• A new control site had to be located because analysis of cypress cores from the old control site (Bayou LaBranche) showed contamination by heavy metals.

• The new site is “Stinking Bayou” located on the edge of Lake Pontchartrain (ca 35 miles north of the previous site). This site is also affected by tidal bores from Lake Pontchartrain (as is Bayou LaBranche).

• Ten cypress trees were cored (2 cores from each tree), one tree every .5 km commencing with the edge of the lake.

• The cores were dried, sanded, cross-dated, cut into five year segments, and pressed into wafers for X-ray analysis.

Collection of Tree Cores at Two Points in Bayou Trepagnier (to assess heavy metals in cypress at varying distances from the banks of the bayou)
• In Bayou Trepagnier tree cores were taken at two points (marker 20 and 42).

• Four trees on each bank were taken, i.e., one on the bank another at 50 feet, 100 feet and 400 feet (both sides of bayou).

• A number of years ago spoil from dredging of the bayou was deposited and this set of collections should contrast trees growing on and off the spoil banks.

• Entry of X-ray data into computer for statistical analysis. Data from over 300 tree core sections of cypress are being entered into the computer for statistical analysis of heavy metals.

Project III: Effects of Environmental Contaminants on Colonial Wading Birds
• Construction of channels in Lake Clause in Bueche, Louisiana has been completed to ensure easier passage through the hyacinth-laden lake.
Currently, 29 little blue heron (Egretta caerulea) nests are being monitored twice weekly. This is the same area where white ibis (Eudocimus albus) nested last year and presumably will do so again beginning in late May. Unfortunately, the great egret (Casmerodius albus) nesting area has become inaccessible.

In Bayou Sauvage, 40 nests of great egrets and 50 nests of little blue herons have been tagged and are being visited twice weekly.

The second egg laid in each of five of the great egret nests and 30 of the little blue heron nests (15 in Bueche and 15 in Bayou Sauvage) has been removed, measured, contents frozen pending analysis, and eggshells rinsed and air dried for analysis.

Guano samples collected from adult little blue herons and great egrets in both sites over the last month are currently undergoing analysis.

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


Research investigating anaerobic degradation of carbon tetrachloride by sulfate reducing bacteria has continued over the past quarter.

The following results have been presented at the HMCRI Southeast Conference in Atlanta in March by Vikas Uberoi, Richard Madura and Dr. Sanjoy Bhattacharya. Both propionate utilizing and lactate utilizing sulfate reducing cultures degraded approximately 10 mg/L of carbon tetrachloride to chloroform and subsequently to methylene chloride.

Abiotic controls showed dechlorination of carbon tetrachloride when iron and sulfide were present in the nutrient medium. Only sampling losses were observed when iron was absent from the medium. Degradation of carbon tetrachloride appeared to be a combination of biotic and abiotic processes while degradation of chloroform and methylene chloride appeared to be mediated by bacteria in the presence of substrate.

Degradation of chlorophenols has also continued during the last period. Biodegradation of pentachlorophenol (PCP), trichlorophenols and dichlorophenols by sulfate reducing bacteria and methane producing bacteria is currently being investigated.
Modeling work is being continued by Dr. Law. Additional data regarding degradation of carbon tetrachloride is now being collected using a newly built stainless steel reactor, which should minimize losses due to volatilization.

Leak testing and performance evaluation of the reactor is currently under way using anaerobic cultures. Degradation of carbon tetrachloride and other chlorinated compounds is being continued under microaerophilic conditions and by the use of fungi.

Richard Madura and Vikas Uberoi traveled with two Xavier students to the WERC conference in Las Cruces, New Mexico to attend the conference and student design competition. As a result, they are considering entering the student design competition in the 1996 conference.

A Sensitive Rapid On-Site Immunoassay for Heavy Metal Contamination

R. Blake, D. Blake, G. Flowers

Two Postdoctoral Associate positions were advertised and suitable candidates were selected. The Tulane position has been offered to one candidate, and the Xavier position has been filled by Dr. Michelle Lyles.

An acceptable portable ELISA spectrophotometer has been identified and ordered from Tecan, Inc. This is a battery operated unit that will be tested for its suitability in performing on site immunoassays in the field.

An agreement has been reached with Dr. Martin Breckbiel of the National Institutes of Health to obtain sufficient bifunctional DOTA and DPTA (two "second generation" metal chelators developed by Dr. Breckbiel in response to disadvantages documented for the corresponding EDTA based chelators) for our immediate experimental needs. Protein conjugation activities will be initiated as soon as the reagents are acquired from Dr. Breckbiel.

The effect of incubation time on the extraction of soluble cations from soil samples with EDTA has been investigated. Standard metal contaminated soil samples from the National Institute of Standards and Technology were exposed to EDTA for varying lengths of time. Quantitative extraction of the cation was complete within 15 minutes at ambient temperatures. This is an encouraging result for the development of an eventual field test.

Dr. Flowers has begun to characterize specific field sites for levels of cadmium, lead, zinc, and other metals, in preparation for selecting the most
appropriate site(s) on which to field test the immunoassays under development.

- Preliminary growth experiments on *Xanthomonas maltophilia* strain OR-02 in the presence of divalent lead have been conducted.

- Efforts to replace the tryptone and yeast extract in the rich culture medium with citrate complexed 1:1 with lead have only been partly successful. Although the bacterium appears to grow quite well on the lead amended citrate, we no longer observe the bacterial dependent precipitation of the lead that characterized growth on the lead amended rich broth.

- Efforts to optimize bacterial dependent lead precipitation in the presence of citrate will continue.

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

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

**Goals**

Understanding the detailed pore-level behavior of microorganisms through porous media is essential for the development of effective *in situ* bioremediation strategies. Integrated experimental and computational models of the pore-level behavior of microorganisms are being developed. The models will include detailed analysis of convection and diffusion within the pores and the convection and chemotactic responses of swimming microorganisms to the local concentration gradients. Additionally, these models will include microbial adhesion to each other and the surrounding pore structure, and the reaction kinetics of these organisms to the toxic contaminant.

**Accomplishments this quarter**

- The manuscript "A Microscale Model of Bacterial Swimming, Chemotaxis and Substrate Transport," by Robert Dillon, Lisa Fauci and Donald Gaver has been favorably reviewed by the *Journal of Theoretical Biology*, and is undergoing revision.

- Donald Gaver presented work on interfacial dynamics at the Federation of Societies of Experimental Biology Meeting in Atlanta, GA.

- Professor Bard Ermentrout, from the Department of Mathematics at University of Pittsburgh, served as a consultant for the modeling work from 3/8/95 to 3/10/95.
Robert Dillon, a postdoctoral researcher on this project, has been awarded an NSF postdoctoral fellowship to continue these studies at Tulane.

Stephanie Kute, an undergraduate studying basic models of aggregation, was awarded an NSF graduate fellowship to continue these studies at Tulane.

The computational model of the full coupled bacteria/contaminant/fluid system now includes agglomeration.

Three graduate students, Mr. Dean Bottino (Mathematics), Mr. Zewen Liu (Chemical Engineering) and Mr. Darren Yap (Biomedical Engineering) are working on various aspects of this project.

Biomarkers of Exposure and Ecotoxicity in the Mississippi River Basin

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

Histopathology of Fishes from Devils Swamp and Tunica Swamp

- Histopathological examination of three species of gar fish and three species of buffalo fish collected from Devils swamp and Tunica swamp up to January 1995 have been completed.

Gar Liver

- Metals and organic contaminants from Devils Swamp accumulated in the muscle tissue of all three species of gar. Muscle tissue from gar collected in Tunica Swamp had trace/background levels of metals.

- Levels of HCBD, HCB and arsenic in muscle tissue increased with length and weight.

- Spotted gar from Devils Swamp consistently weighed less per unit length than spotted gar from Tunica Swamp.

- Prominent melano-macrophage centers were present in all sources of gar from Devils Swamp and Tunica Swamp. Spotted gar from Devils Swamp had a greater number, larger and darker melano-macrophage centers than gar from Tunica Swamp.

- The livers of spotted gar from Devils Swamp had generally more hepatocellular iron while the remaining melano-macrophage center pigments (ceroid and melanin) were the same in spotted gar from both sites.
There was an increased prevalence of ductal proliferations in the exocrine pancreas of spotted gar from Devils Swamp. Spotted gar from both Devils Swamp and Tunica Swamp had a high prevalence of pancreatitis and perivascular cuffing. A low prevalence of hepatocytic metaplasia of acinar cells and pancreatic metaplasia of hepatocytes was noted in spotted gar from Devils Swamp but not Tunica Swamp.

Buffalo Gill - Bigmouth Buffalo (BMB)
- The weight-length relationship was the same for fish collected from Devils Swamp and Tunica Swamp.
- Significant levels of mercury, cadmium, and chromium were present in the muscle tissue of BMB from Devils Swamp.
- The prevalence of parasitic infection of the gills of BMB collected from Devils Swamp and Tunica Swamp was the same.
- There were more hyperplastic/proliferative lesions of the gills of fish collected from Devils Swamp.
- Inflammatory (branchitis) lesions occurred in fish from Devils Swamp and Tunica Swamp.

Buffalo Gill - Smallmouth Buffalo (SMB)
- The SMB collected from Devils Swamp generally weighed less per unit length than fish collected from Tunica Swamp.
- SMB collected from Devils Swamp had significant levels of lead in muscle tissue while lead levels were below detection in SMB from Tunica Swamp.
- SMB from Devils Swamp had a high prevalence of protozoan infections in the gill.
- The prevalence of hyperplastic/proliferative lesions of the gill was much greater in fish from Devils Swamp. The lesions with higher prevalences were chloride cell hyperplasia and chondroplasia.
- Inflammatory (branchitis) lesions of the gill occurred in most fish collected from Devils swamp.

Microinjection of Fish Embryo
- Histopathological evaluation of 6-month-old medaka (embryo microinjected with hexachlorobenzene) have been completed. A manuscript is currently being prepared for publication.
Neurotoxicological and Immunological Studies

- An in vitro system was used to study the pollutant effects on the immune system and on the nervous system of Xenopus laevis. These results are still being evaluated and additional tests are being prepared.

- Stomach contents of frogs from 1000 m SW of Devils Swamp Lake included undigested crayfish, indicating consumption of detritus feeders (crayfish).

- Frogs with elevated lead levels displayed elevated mitogen reactivity consistent with an irritant effect of the pollutant.

- Frogs from Devils Swamp Lake with elevated lead content also had visual evidence of parasites, which were absent from the other frogs.

Metabolism and Neurotoxicology

- The acetylcholinesterase, butyrylcholinesterase and non specific carboxylesterase activities in the subcellular fractions of brain and liver of smallmouth buffalo, bigmouth buffalo and spotted gar have been characterized.

Publications/Presentations


2. Thiagarajah, A., Hartley, WR., Major, SE., and Broxson, MW. Gill histopathology of two species of buffalo fish from a contaminated swamp. Marine Environmental Research (submitted).


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


Oxidation of Aromatics

- Research over the last quarter involved oxidation of aromatic contaminants using Fe(II) ion-exchanged into Nafion type membranes.

- Research has shown that these systems are effective in catalytic oxidation and operate through Fenton type mechanisms.

- It was found that the iron species gradually leaches out of the membrane.

- Quantifying the loss of iron through controlled studies on leaching has been done. These studies are detailed in the table below.

<table>
<thead>
<tr>
<th>Salt-pH\Time</th>
<th>Percent leached in 3Hrs. at pH 2</th>
<th>Percent leached in 24Hrs. at pH 2</th>
<th>Percent leached in 3Hrs. at pH 5</th>
<th>Percent leached in 24Hrs. at pH 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Salt</td>
<td>28.8 % (1.28 wt%)</td>
<td>28.3 % (1.29 wt%)</td>
<td>0 % (1.80 wt%)</td>
<td>0 % (1.80 wt%)</td>
</tr>
<tr>
<td>NaCl</td>
<td>34.4 % (1.18 wt%)</td>
<td>34.7 % (1.18 wt%)</td>
<td>0.8 % (1.79 wt%)</td>
<td>0 % (1.80 wt%)</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>80.4 % (0.35 wt%)</td>
<td>79.3 % (0.37 wt%)</td>
<td>3.1 % (1.74 wt%)</td>
<td>2.8 % (1.75 wt%)</td>
</tr>
<tr>
<td>KCl</td>
<td>46.8 % (0.96 wt%)</td>
<td>49.1 % (0.92 wt%)</td>
<td>1.1 % (1.78 wt%)</td>
<td>0.8 % (1.79 wt%)</td>
</tr>
</tbody>
</table>

The experiments conducted are those wherein an Fe(II) containing membrane is soaked in salt solution and the exchange characteristics are monitored. The results indicate that considerable stability of Fe(II) in the membrane is achieved at a pH of 5. Most of the leaching takes place within 3 hours. Divalent cations more easily exchange out Fe(II). Of the cations studied, Na⁺ is the least capable of ion-exchanging out Fe(II). Work continues with Fe(II)
catalysis at pH 5 where the iron is relatively stable. To maintain pH during oxidation NaOH is used. These results will be presented in the next report.

Stability of Heavy Metals in Bayou Trepagnier

- Began monitoring Bayou Trepagnier for changes in water chemistry after shutdown of the Shell Norco effluent.

- Shell terminated its discharge into the bayou on Friday, February 24, 1995.

- Over the past two months data has been collected on the dissolved lead, chromium, zinc, and copper content of the water column.

- Preliminary data indicates that the metal content of the water column is not significantly higher now than before shut down.

- The sampling program for the spoil banks adjacent to the bayou has also begun.

- The plan is to determine total lead, zinc, copper, and chromium, as well as acid volatile sulfide (AVS) for these samples.

- Preliminary data indicates that bottom sediments in the spoil banks have been weathered because essentially no AVS is present.

- It has not, as yet, been determined whether or not heavy metals are more mobile in the spoil banks as a result of chemical weathering. Conventional wisdom would suggest that weathering liberates metals from the sediments and possibly makes them more bioavailable.

- Samples have been taken from Lake Pontchartrain to see if contamination from Bayou Trepagnier has been discharged into the lake.

- Preliminary data on samples acquired in the vicinity of the mouth of Bayou LaBranche indicates that the lake has not been significantly contaminated.

- Additional samples will be taken to determine whether or not a contaminant plume exists in the lake.

The Fresh-Salt Water Interface

- The effect of humic acid concentration on the adsorption of Pb on montmorillonite has been studied.

- Humic acid concentration had no effect on the adsorption of Pb as shown in the following table:
<table>
<thead>
<tr>
<th>Sample No</th>
<th>Mont. (g)</th>
<th>Humic acid</th>
<th>Pb adsorbed (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(g)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>.1694</td>
<td>0</td>
<td>1.43</td>
</tr>
<tr>
<td>2</td>
<td>.1694</td>
<td>1.0</td>
<td>1.44</td>
</tr>
<tr>
<td>3</td>
<td>.1694</td>
<td>2.6</td>
<td>1.44</td>
</tr>
<tr>
<td>4</td>
<td>.1694</td>
<td>4.5</td>
<td>1.44</td>
</tr>
<tr>
<td>5</td>
<td>.1694</td>
<td>9.4</td>
<td>1.44</td>
</tr>
<tr>
<td>6</td>
<td>.1262</td>
<td>0</td>
<td>1.939</td>
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<tr>
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<td>1.0</td>
<td>1.939</td>
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<td>2.5</td>
<td>1.919</td>
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<td>5.0</td>
<td>1.898</td>
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<tr>
<td>10</td>
<td>.1262</td>
<td>7.5</td>
<td>1.877</td>
</tr>
</tbody>
</table>

- The above data also shows that a decrease in the weight of montmorillonite results in an increase in the adsorption of Pb (mg/g), which is expected when the concentration of Pb in the starting solution remains constant.

- These results may be due to the fact that humic acid cannot diffuse into the interlayer region due to its very large molecular weight, whereas Pb has no trouble accessing the interlayer region of montmorillonite. Humic acid does adsorb onto montmorillonite as determined spectrophotometrically. Moreover the adsorption is linear in montmorillonite concentration. Presumably, humic acid is adsorbed on the outer surface of the montmorillonite.

- It is generally known that fulvic acid is a smaller molecular weight component of humic acid.

- In order to study the effect of fulvic acid, the humic acid was precipitated out by acidifying the solution.

- The supernatant contains primarily fulvic acid. As shown in the following table, fulvic acid apparently does have an effect on the adsorption of Pb:

<table>
<thead>
<tr>
<th>FA (added)</th>
<th>Pb (ppm) (initial)</th>
<th>pH</th>
<th>Pb (ppm) (sol)</th>
<th>Pb (ads)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>8.3</td>
<td>.209</td>
<td>1.55</td>
</tr>
<tr>
<td>1.0</td>
<td>8</td>
<td>6.9</td>
<td>.458</td>
<td>1.50</td>
</tr>
<tr>
<td>2.0</td>
<td>8</td>
<td>6.82</td>
<td>.623</td>
<td>1.47</td>
</tr>
<tr>
<td>3.0</td>
<td>8</td>
<td>6.7</td>
<td>.623</td>
<td>1.47</td>
</tr>
<tr>
<td>4.0</td>
<td>8</td>
<td>6.63</td>
<td>.789</td>
<td>1.43</td>
</tr>
</tbody>
</table>

These results, although encouraging, need to be duplicated within a more closely controlled pH range.
Polyphosphazene Membrane Fabrication

- A variety of novel polyphosphazenes have been synthesized and purified: poly(3-ethylphenoxy-phenoxy)phosphazene with a 3-ethylphenoxy substituents content of 25%, 75%, and 100%, a poly(4methylphenoxy-phenoxy)phosphazene and a vinyl substituted polyphosphazene.

- Preliminary crosslinking experiments were performed with the polymer containing 75% of 3-ethylphenoxy substituents.

- The results show superior tensile properties of this material. Also the vinyl substituted polyphosphazene showed good mechanical properties.

- Contrary to expectations, however, thermal initiation of crosslinking was not effective in this case. A small degree of crosslinking was obtained by photocrosslinking the vinyl substituted polyphosphazene with UV light of wavelength 365 nm.

- Investigations using the Fourier Transform Infrared device are being carried out to elucidate the crosslinking mechanism of the new polyphosphazene membranes.

- Preliminary sulfonation experiments of poly(3-ethylphenoxy-phenoxy)phosphazene films with the 3-ethylphenoxy substituents content of 0.5 were performed.

- The purpose of this study was to select the most convenient and effective sulfonating agent out of the following three acids: chlorosulfonic acid, oleum, and concentrated sulfuric acid. Chlorosulfonic acid dissolved in dichloroethane appeared to work best.

Polarographic Assays

One of the potential "niche" areas for polarographic analysis in environmental studies is to be able to determine the concentrations of heavy metals that are present in water in ionic and complexed forms in situations where the complexes can not be filtered out mechanically. Polarography measures the electroactive moiety (i.e., the ionic fraction). Hypothetically, the complexed form can be determined by doing a total metal analysis (using ICP or GFAAS for example) and then taking the difference.

- To examine the validity of this approach, experiments have been conducted in which lead solutions have been exposed to EDTA, a chelating agent with known properties. The idea was to get a model system where the amount sequestered in complexed form would be predictable.
• The electroactive lead concentration has been polarographically determined both before acid digestion of the mixture and afterwards. The purpose of the digestion is to free the complexed lead and restore it to electroactive form.

• These experiments have been successful, and the measurements have been in accord with the predicted behavior.

• Researchers have begun to polarographically analyze water samples taken from Bayou Trepagnier for hexavalent chromium. Most of the effort has focused on methodology development, but one sample has yielded data thus far. The concentration measured was 44 ppb, a value that seems consistent with total chromium contents of sediment and pore water measured by other cluster investigators.

• Progress has been made in computerizing the acquisition and analysis of polarographic data. The hope is to have this system completely operational in the next quarter. The analysis of data in the tadpole study (mentioned in the last progress report) has now been completed. The results will be presented at the Goldschmidt Conference in May.

• Dr. Jian Zhang (Chemistry) has begun dialogue with Dr. Suk Lee (Oak Ridge National Laboratory) relative to a collaborative effort on Dr. Zhang's zeolite project. Dr. Zhang will travel to Oak Ridge this summer to spend time in Dr. Lee's laboratory.

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

DEVILS SWAMP AND BAYOU TRAPAGNIER

• Determination of the specific requirements for designing the database model to manage various kinds of environmental data to be handled by this GIS system continues. The first generation of a functional database model was implemented.

• Conceptual design of the database: The environmental data band used are mainly of 3 types: soil and sediment, water, and specimen tissues. For each type, a data series was obtained from Tulane and Xavier researchers, Louisiana's Department of Environmental Quality, and the United States Geological Survey. Each sample is uniquely identified by the source which performed the analysis and identifies contaminant and ecological factors.
From this general data description, a conceptual data model has been developed.

- Implementation of the database: The implementation of all the entities of the database schema was done for Oracle 7 RDBMS. It contains all table definitions necessary to represent the model that has been designed.

- Data entry in the database: A series of programs have been developed to extract and format this information which was provided by DEQ in ASCII files. Additionally, DEQ data for the Devils Swamp site has been manually formatted and entered.

- Training with the expert system shell named Smart Elements continued this quarter. How to set up the knowledge base (rules and facts) for our future user interface, to be able to determine what the user wants to do, and to prepare the data for the GIS product Arc/Info that the user wants to visualize were the major items identified during training this quarter.

- Development of a First Simple Prototype: The last goal is to develop a first simple prototype combining all 3 previous goals and using the data that has been entered in our environmental database model. Work has begun in the design of a user interface with Open Interface that allows the user to select graphically a site of interest, a pollutant of interest, and a date of interest, and then via appropriate rules, the system selects the data and prepares it for the GIS application.

- ARC/INFO upgrades have been fully installed on all workstations and the Oracle converter has been established for both INFO and ARC/Info.

- Two AutoCad digitized maps have been exported to the workstations, the first contains geology information of the Bayou Trepagnier area and the second contains a surface map including the highway, water channel, important structures, oil tanks and geographical locations. Those two maps have been converted to the ARC/INFO coverage file as a directory with all the layers digitized from the AutoCad and can be theme layered by ARCPLOT. More spatial information about Bayou Trepagnier is being compiled from Tulane and Xavier research projects in the area and loaded.

- Four 7.5 minute USGS quad maps have been obtained for both Devils Swamp and three 7.5 minute maps for Bayou Trepagnier. The maps have been transformed into electronic figures for use in the GIS project.

- The groundwater analytical sampling, surface analytical sampling, and fish tissue analyses data, which were provided on PC disk format, have been transformed into INFO and some into Oracle files.
ROCKY FLATS AND SANDIA PROJECT

Summary of Activities

- Existing models continue to be analyzed for use as submodules to build an integrated surface soil particle transport model for Rocky Flats.

- Refinement of the base model for integration with the submodules is being done.

- Emphasis has been on continued analysis of the 1.5 generation flow field, parameter validation and modification in particular topography, restructuring of resuspension component of the model, and deposition models for plutonium contaminated soils.

- Using the EPA Fugitive dust model (FDM), NCAR LocaFLOW, and IEA AIRmod, these models for data pass matches between these and ARC/Info continued to be run. FDM appears to have the best data match and modularity potential.

- Using FLUENT, the model geometry was changed from flatplate to three solid wall objects in a plate field and rebuilt the Computational Mesh for the model flow field in an unstructured cell form and made eight complete runs to validate the mesh integrity. After eight runs, mesh integrity is at 100%.

- Parameter analysis continued and revealed an improvement in the present topological data from EG&G Rocky Flats, a smaller timestep, and particle size validation is needed.

- Seven model runs using a Monte Carlo simulation for particle size have been done producing four Arc/Info based coverages with three month time steps.

- Communication continued with OU3 managers at the Rocky Flats Plant in order to integrate project work with the work of those at Rocky Flats doing similar modeling of air concentrations.

- Data collection activities have continued for the landuse portion of the Sandia Project. After conferring with the counterparts at Sandia, it has been determined that aerial maps are the most consistent and available and thus, best, source of land use data for the downtown area. The major effort during the first quarter has been on collecting and analyzing aerial photos from 1940 - 1990.
EDUCATION PROJECT

Enhancement of Environmental Education at Tulane and Xavier Universities

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

Curriculum Development

- Sr. Stephanie Henry, Assistant Professor of Chemistry, discussed her course Survey of Environmental Chemistry at the February meeting of the Science Education Research Group (SERG). Overall, according to both the student evaluations and her own personal impressions, the course was received well, and the students enjoyed the lab component.

- Dr. Beverly Wright, Associate Director of Environmental Programs, and Croscina O. Crockett, Assistant Director of Environmental Programs, attended the HBCU/MI Consortium Partnership Leaders Workshop II in Miami, Florida. The workshop was designed to encourage and prepare participants to begin environmental education development workshops at consortium member institutions.

Pre-College

- The Internal Grants Committee (IGC) recommended approval of Dr. Noemi Jesalva's final report of her proposal entitled, "Developing a Course in Toxicology. The course will be in Chemical Toxicology, for upper level chemistry majors. The Chemistry Department Curriculum Subcommittee will meet in April to consider recommending this course for approval as part of next year's academic offerings.

- Updated brochures announcing and describing EnviroExplorers '95, a program designed to spark youngster's interest in science and math, were sent to administrators of local area schools to recruit student participants for this summer's session. The director for the summer '95 program is Dr. Etim Eduok. Numerous applications have been received. Details of the program offering are being finalized.

- Xavier University continued its support of the Kumon Program at Xavier Prep High School. The program is designed to strengthen students' math skills through repetitive exercises. Mrs. Olive McCloud, the Xavier Prep math instructor who administers the program, reported that 84% of the class progressed to a higher Kumon level and 68% received first semester grades of "C" of higher.
On March 7, 1995, Mrs. Croscina O. Crockett visited Xavier Preparatory High School to speak to students on environmental careers and the program of studies available at Xavier University. Presentations were made to four sections of students, totaling over 100.

Infusion of Environmental Content Into Courses
- The Internal Grants Committee (IGC) approved the final report submitted by Dr. Stephen Duplantier, Assistant Professor of Communications, on his project entitled, "Instructional Materials for Environmental Courses." This project involved the development of multimedia instructional material for use in several existing courses.

Guest Lecturers/Seminars/Conferences/Workshops
- The Spring Semester Seminars included the following: "Environmental Remediation: Science and the Superfund Process", presented by Dr. Charles Reith of DynMcDermott on January 17, 1995; Environmental Justice from a Legal Perspective by Carlton Waterhouse, EPA Assistant Regional Counsel on February 7, 1995 and Environmental Justice: Are There Ethical Dilemmas for the Physician by Herman Ellis, MD of the Columbia University School of Public Health and a member of the National Environmental Justice Advisory Council on March 28, 1995.

- On March 9, 1995, Dr. Richard Foust (Chemistry professor at Northern Arizona University) presented a seminar entitled, "Environmental Chemistry: A Global Perspective". He presented an overview of the environmental issues faced by the U.S. and other developed countries, as well those faced by developing countries.

Faculty Development
- Summer Research opportunities for faculty included communications from Battelle Pacific Northwest Laboratory (PNL), an NSF program entitled, "The Center for Applied Aquatic Science and Aquaculture", and the Office of Civilian Radioactive Waste Management/HBCU Faculty Workshop and Faculty Fellowship Program administered through ORISE. Drs. Etim Eduok (Chemistry) and Shubba Kale (Biology) have applied to the fellowship program at Battelle.

- Drs. Qun Lin, Noemi Jesalva, Etim Eduok, Michael Polito and John Sevenair attended the Pittsburgh Conference (PITTCOn) which was held in New Orleans, Louisiana March 5-10, 1995.

Recruitment and Retention
- Three Environmental Scholars Meetings were held this quarter. Students submitted written progress reports. Additionally, information on seminars, conferences and summer internship programs were provided.
The Student Environmental Club had two meetings this quarter. Participation in the Celebration of Earth Day and the continued promotion of recycling efforts directed by the Center for Environmental Programs were discussed.

Three students, Robert Swayzer (Biochemistry), Lauren Nicholas (Communications) and Natasha Crockett (Chemistry), attended the 48th Annual Southern Agribusiness Forum Conference on "Environmental Policy - Eroding Property Rights".

The CEP distributed information regarding upcoming environmental programs to the environmental scholars and the department chairpersons. These programs included: 1) US EPA graduate fellowship program, 2) the University of Michigan's Environmental Studies Program, and 3) the University of North Carolina at Chapel Hill's summer pre-graduate research program.

Ten students, sponsored by the CEP, attended the Pittsburgh Conference '95, held in New Orleans, LA March 5-10,1995. The major focus for these students was the Environmental Forum held during the conference.

Mrs. Crockett coordinated a recruitment visit by Mr. Al Corbin of the Environmental Careers Organization. Mr. Corbin is a representative of the ECO's Diversity Initiative Program which is specifically designed to seek opportunities for minority students.

The March issue of The Communicator, a publication of the Xavier University Communications Department, was dedicated to environmental issues. Lauren Nicholas, a LIFE Intern, authored two articles entitled, "What is Environmental Justice?" and "Sleeping with the Toxic Enemy".

**Tulane - LAS Activities**

The LAS Environmental Education Committee (LASEEC) held two meetings. Specific core requirements for the proposed Environmental Science graduate degree was one of the items discussed.

The student recruitment brochure has been finalized and sent out for printing.

A listing of interdisciplinary environmental courses currently being taught at the graduate level is being prepared for distribution.

The LASEEC has proposed, to the Tulane administration, that the Environmental Science Coordinate Five-Year Graduate Degree be housed in the Geology Department.
Two environmental career speakers were presented on the uptown campus. Chris McDonald of Jacobs Engineering on March 22 spoke on "Career Opportunities in Environmental Consulting". Melissa Smith, Director of Environmental Safety and Health, U.S. Department of Energy on the Strategic Petroleum Reserve addressed "Environmental Career Opportunities in Government" on March 29.

Three summer course development awards were given to: Thomas Bianchi for "Biogeochemical Cycling in Ecosystems", Colin MacLachlan for "Mississippi Environmental History", and Sam Ramer for "Chernobyl".
A proposal was submitted last year to DOE to study the correlation between soil properties and contaminant settling characteristics in the Watts Bar watershed. There is a strong correlation between particle grain size and contaminant absorbance. This site was selected because of a well-documented contaminant release history. Martin-Marietta conducted a study of the entire river system in 1992. Although, well conducted, their study was not intense enough to draw a conclusion about the correlation between grain-size and contaminant fate. For this reason, representatives of Tulane and Washington State University chose a small control volume within which they were able to conduct extensive sampling.

- Both sediment and water samples were taken in a grid with sites spaced approximately thirty meters apart. These samples have been measured for various parameters.

**Soil Analysis**
- The sediment which has been collected is being analyzed for mercury concentration, cesium concentration, grain size, and Atterberg limits.

- The soil was collected primarily by two methods. Along the banks, a hand core was used, while in the river channel, a gravity core was used.

- The soil from the hand core was separated into four inch segments and placed into bags or jars. For this reason, the PVC tubes collected using the gravity core are being cut into four inch segments as well. The four inch samples are homogenized and then processed in a Microwave Digester. Once digested, the samples are analyzed for Mercury using Inductively Coupled Plasma (ICP) with an Ultrasonic Nebulizer.

- Initial findings show an increase of mercury concentration with depth of sample, and with visual inspection of grain size.
There is a greater difference in grain size from top of sample to bottom of sample in the gravity core retrievals than in the hand cores. Most likely, the reason for this is that the water velocity in the channel deposits the larger material while keeping the smaller material in suspension. Along the banks, however, the water velocities slow to nothing so that suspended solids settle out.

**Water Analysis:**
- Water samples were collected at several sites. At each site, samples were taken from the top, middle, and bottom of the water channel.
- The samples were analyzed for hardness, alkalinity, turbidity, dissolved solids, suspended solids, volatile solids, total solids, conductivity, salinity, ammonia, nitrites, and nitrates.
- Several parameters were measured in the field including dissolved oxygen, pH, oxidation-reduction potential (ORP), and temperature.
- From the findings of water analysis, it can be determined that no stratification was occurring at the time of sampling. Further water analysis will include mercury concentration and organic nitrogen.

**Development of Novel Detoxification of Halocarbons**

L. Byers, A. Apblett

This project is investigating the hydrolysis of environmentally-significant chlorocarbons using novel catalysts based on molybdates and related compounds. The investigation of the reactivity of these molybdate catalysts is progressing very well and in this quarter the hydrolytic dechlorination of several chlorocarbons was examined.

- Completed survey of catalytic dechlorination reactions of 10 environmentally-significant with 15 different molybdate catalysts in water and 95% ethanol.
- Determined the dechlorination rate of CCl₄ in water/ethanol mixtures ranging from 0% water to 100% water and found that maximum dechlorination occurs using a 45:55 mixture.
- Continued search for other co-catalysts (substances that are alkylated by chlorocarbon/molybdate mixtures). Amines and ammonium ions appear promising.
Novel Polymer-Semiconductor Composites for Photocatalytic Destruction of Organic Contaminants in Aqueous Environments

R. Gonzalez, V. John

- Research has continued on the use of Fe(II) exchanged onto Nafion type membranes to serve as catalysts for the oxidation of aromatic contaminants. Detailed studies on chlorophenol oxidation have been completed.

- In recent work, a novel synthesis of enzyme encapsulation in phenolic polymers which have the morphology of submicron spheres and spherical aggregates has been carried out.

- The activity of enzymes encapsulated in this media is being explored. Possible applications include the use of enzymes as contaminant sensors or for enzymatic breakdown of contaminants. The use of these systems for pesticide breakdown is to be studied next.

Heavy Metals Analysis of the Tulane Museum of Natural History Fish Collection: A Feasibility Study

G. McPherson, D. Roundhill, G. Flowers, H. Bart, D. Grimm

- The stand alone mercury analyzer was installed and calibrated.

- The mercury content of two different samples of preserved fish from the Tulane Museum of Natural History Fish Collection were determined. The fish were put into solution by the standard cold digestion procedure.

- Seven small fish (2") collected from the Pearl River showed an average mercury content of 160 ppb (wet weight) with a range from 96 ppb to 282 ppb.

- Twenty-eight larger fish (4.5") collected from Big Creek showed an average mercury content of 120 ppb with a range from 68 ppb to 282 ppb.
These values are typical of baseline values seen in a variety of fish and do not indicate any serious mercury contamination in these particular locations. The analyses were performed by Joe Clymire, a chemistry graduate student.

A paper, "Environmental Chemistry of a Historical Fish Collection", was presented at the Pittsburgh Conference, March 9, 1995, New Orleans, Louisiana by Dr. D. Grimm of the CIF.

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

- Dr. Gordon Blaylock of SENES (formerly of ORNL) made a presentation at Tulane University and has agreed to be a consultant to the project as suggested by the review committee.

- Working with Dr. Blaylock and several researchers of ORNL has expanded project participants knowledge in the aquatic environments and river models and codes.

- Computations on the atmospheric plume and the lift-off of particles has been completed.

- A short paper was written, which was accepted for presentation in the ASME 7th International Symposium on Gas-Solids Flows. The paper will also appear in a volume of papers published for the Symposium.

- Data on the particles lifted in forest fires has been received from the collaborators in Belarus and the results have been incorporated in the study mentioned above.

- A systematic categorization of the data needed for the river project has been started.

- The way of inclusion of the plume data into the CSU mesoscale atmospheric model has been established. Mr. Pimenov is currently working on this model.
Computation of Taylor Dispersion Coefficient

S. Rosencrans

- The quarter was spent doing production runs with the GMRES solver that was implemented earlier. This method was checked by non-sparse methods in a few cases and found to be accurate.

- Ability to extend the range of upper-boundary wavelengths that had been previously computed has been considerable.

- Variations of the Peclet number have been run to see the effect on effective dispersion.

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

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

A new initiative project to use Saururus (Lizard-tail) in heavy metal analysis.

- To commence the project 15 plants were collected from a non-polluted area to grow in pots. Once the plants (rhizomes) commence producing stems and leaves solutions of varying concentrations of heavy metals (lead, cadmium, and zinc) will be applied to induce stress and thus produce mRNA for HSP 70, and other heat shock genes.

- In addition, literature is being accumulated on heat shock proteins in plants and designing primer sequences to use in PCR (polymerase chain reaction) to extract the HSP 70 gene. In about a month the first attempt will be made to isolate the genes. If successful, quantitative PCR will be conducted on treated, potted plants, growing in various concentrations of heavy metals. Some primers have been designed and the heavy metal solutions to be applied soon.
Coordinated Instrumentation Facility
T. Lyttle, O. Mills, D. Grimm

- The Inorganic Laboratory successfully completed and passed APG PET for lead and continues to perform metals analysis for several DOE researchers.

- The Inorganic Laboratory installed a dedicated Hg analyzer and an ultrasonic nebulizer for sample introduction on the ICP-AES. Both instruments provide lower detection limits for metals analysis.

- The Organic Laboratory constructed wall to provide appropriate air-handling to isolate sample prep area from analysis area. Ordered laboratory benches and tables to provide work space for organic sample prep.

- The Organic Laboratory continues to establish environmental analysis capability especially for HCB and HCBD. The methodology has been established for analyzing water samples.

- The Organic Laboratory performed analysis for two DOE projects.

- The Electronics Shop provided support to several DOE researchers for instrument repair.

- Hired new Organic Laboratory Manager - Willard Douglas, Ph.D. Bill has extensive environmental analysis experience and comes to us from Stennis Space Center.

- The CIF participated in the Pittsburgh Conference held in New Orleans in March. D. Grimm presented a paper as part of the Environmental Symposium.

- CIF used PittCon to identify potential vendors for approved DOE shared equipment. Also gathered information concerning sample preparation for environmental, organic analysis.

D. Grimm attended a one-day short course on GC/MS for environmental analysis held in conjunction with PittCon.