

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

## **Quarterly Progress Report**

**Project #DE-FG01-93EW53023  
(January 1, 1996 - March 31, 1996)**

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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 This Quarter:

- In December the Review Panel recommended that three core groups be set up to support the competitively funded projects: data management, field work, and analyses. In this quarter, several meetings were held with the CBR director, the project's co-directors, and the PI's of the clusters who will be funded this year. In these meetings, and meetings with other investigators, the core support concept was further delineated.
- Investigators were invited to submit applications to participate in the cores. Sixteen applications were received. These applications were reviewed by the projects co-directors who made recommendations to the local members of the Executive Committee of the review panel. This Committee then made recommendations as to which investigators should be solicited and requested that the project's co-directors negotiate with these individuals to perform the work needed.
- Notices were sent out to all of the investigators who submitted proposals to work on the project this year indicating whether or not their proposal was selected for funding and if so at what level. The selection and funding levels were determined at the December, 1995 Review.
- The Program Manager began work on setting up budgets at Tulane and Xavier to support this year's work.

- Reports on the last year's progress were collected from all participants. The administrative staff is assimilating this information to prepare the Annual Technical Report for 1995 which will be submitted next quarter.
- The data management core was established. The main core personnel will be Rong Fan and Stan Dixon, Xavier University and Stephanie Smith and Karen Watanabe, Tulane University. The project's co-directors and a few faculty members will also be actively involved in determining the core's direction.
- The administrative personnel of the project spent several hours communicating with DOE representatives regarding finalizing the Notice of Award for this year's funding. Because of various difficulties the funding was not authorized until the end of February.
- Dr. Charles F. Ide, project co-director and Verna Lee, program manager, as well as several investigators and students attended the annual LEQSF meeting in Baton Rouge.

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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. Prammar, 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 compounds (hexachlorobutadiene and hexachlorobenzene) 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 This Quarter:**

- Bioaccumulation studies on crayfish are in progress.
- Organic and inorganic analysis of water, fish, sediment and vegetation samples collected from both Devils and Tunica swamps have been completed.
- Analytical results were submitted to Drs. Hartley, Bart and Watanabe.

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

Abdelghani, AA., Prammar, YV., Mandal, TK., Tchounwou, 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), (1995).

# **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:**

The objectives are 1) to identify and quantify heavy metals and petroleum products (polyaromatic hydrocarbons) present in Bayou Trepagnier relative to remote control sites in LaBranche Wetlands; 2) to assess the uptake and bioaccumulation of metal and organic contaminants of interest in aquatic species in the field and laboratory; 3) to evaluate these contaminants on reproductive function of aquatic species as potential biomarkers of exposure; and 4) periodic evaluation of sediment and water parameters (nutrients, sediment oxygen demand, productivity) to study seasonal and event related changes in Bayou Trepagnier and control sites.

## **Accomplishments This Quarter:**

### Field Studies

- Spotted gar collected from a heavily contaminated site in Bayou Trepagnier and the control site in Bayou Traverse were evaluated for lead, chromium and iron. Tissue concentrations of lead and chromium were higher in the fish collected in Bayou Trepagnier in comparison to those caught in Bayou Traverse. However, the levels were extremely low. The lead concentrations were below the FDA safe limit of 0.3ppm. Studies are still in progress to determine metal concentrations found in fish caught at other locations in Bayou Trepagnier.
- Dr. James Bollinger is analyzing tissues of fish caught in Bayou Trepagnier and Bayou Traverse to determine the types and quantity of PAHs present in the tissues.
- Dr. Anderson is continuing with the histopathological study of gonads of fish taken from Devils Swamp, Tunica Swamp and the LaBranche Wetlands.
- Tide gauge readings have been recorded continuously over the past two months.

### Laboratory Studies

- Laboratory fish tanks for exposure studies are installed. The exposure of channel catfish to heavy metals is ready to commence.
- Chromium speciation studies in Dr. Bundy's laboratory have been completed for the hepatopancreas and abdominal muscle from chromium exposed crayfish.

- A study concerning the exposure of crayfish to a combination of lead and chromium is being conducted. The 4 week exposure has been completed. Tissues have been collected for metal analyses. The 7 week exposure is still in progress.
- In the area of analytical support, 108 tissues were microwave digested and 365 tissues analyzed for metals.

### **Publications and Presentations:**

Bollinger, JE., Bundy, KJ., Anderson, MB., Millet, L., Jolibois, LS., Chen, H-L., Kamath, B., and George, WJ. Bioaccumulation of Chromium in Red Swamp Crayfish, *Procambarus clarkii*. Journal of Hazardous Materials. (In review)

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

Bollinger, JE., Bundy, KJ., Anderson, MB., Millet, L., Jolibois, LS., Chen, H-L., Kamath, B., and George, WJ. Bioaccumulation of Chromium in Red Swamp Crayfish, *Procambarus clarkii*, supported by DOE funds have been accepted for presentation at the FASEB Meeting in Washington, D.C., in April 1996.

Anderson, MB., Preslan, JE., Jolibois, LS., Bollinger, JE., and George, WJ. Bioaccumulation of Lead in Red Swamp Crayfish, *Procambarus clarkii*, supported by DOE funds have been accepted for presentation at the FASEB Meeting in Washington, D.C., in April 1996.

Bundy, K., Millet, L., Bollinger, JE., and Anderson, M. Speciation of Chromium in Crayfish, supported by DOE funds have been accepted for presentation at the FASEB Meeting in Washington, D.C., in April 1996.

Preslan, JE., Adams, S., Bollinger, J., George, WJ. and Anderson, MB. General Water Quality and Sediment Conditions in the LaBranche Wetlands Assessed Using *in Situ* Testing and Sediment Core Evaluation has been accepted for presentation at "The Basics of the Basin" Research Symposium, May 30 and 31, 1996, at Southeastern Louisiana University.

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

J. Barber, A. Applett, H. Ensley, M. Fingerma, M. Fink, M. Polito

### **Objectives and Goals:**

The goals of this cluster are 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 is 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 This Quarter:

- A study of the passage of 2,4-dichlorophenol (2,4-DCP) through a simple food chain consisting of *Lemna gibba* (duckweed) and *Procambarus clarkii* (crayfish) has been initiated. It has been shown that duckweed converts 2,4-DCP to the corresponding glucoside but that, when consumed by crayfish, the glucoside is hydrolyzed, by bacteria in the crayfish gut, back to glucose and the parent phenol. Crayfish when maintained in water containing labelled 2,4-DCP readily took up the phenol but subsequently, within 24 hours, depurated more than 80% of the label. Strong evidence for metabolism of the 2,4-DCP by the crayfish was obtained but the identity(ies) of the metabolite(s) remains to be elucidated
- The study of the uptake of thorium, a weakly radioactive actinide, by *L. gibba*, was continued and was extended to include *Salvinia minima* (water fern). Effective concentrations (EC<sub>10</sub>'s and EC<sub>50</sub>'s) were determined for both organisms. The potential of these two organisms as agents for the bioremediation of actinide pollution are currently under investigation.
- Studies of the effects of heavy metals on the physiology and biochemistry of *P. clarkii* have continued and been expanded.
- The heavy metals cadmium, lead and mercury had specific effects on the action of serotonin (5-HT), ovary-inhibiting hormone (GIH) and ovary-stimulating hormone (GSH), also upon the incorporation of <sup>14</sup>C-leucine into ovarian proteins. Insofar as ovarian maturation in crayfish is concerned, mercury is far more toxic than either cadmium or lead.
- The results obtained using crayfish illustrate that they can serve as a model in pollution studies but also emphasize the potential dangers of heavy metal pollution particularly in view of the fact that many million pounds of crayfish, sometimes harvested from polluted waters, are eaten annually in Louisiana.
- KFeS<sub>2</sub> was tested as an ion-exchanger for the removal of cadmium, mercury and lead from solution. It did this extremely well and experiments to determine selectivity are currently performing competition.
- The investigation of the removal of thorium from simulated duckweed extract, using a variety of layered titanate materials, has continued.
- Competition studies measuring the relative uptake of the heavy metal ions Cd<sup>2+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup> versus Ca<sup>2+</sup> into several zeolites were performed. In the case of ZeoX and zeolite A-51, an approximate tenfold selectivity of heavy metal cations over Ca<sup>2+</sup> was observed. Zeolite IE-95 was found to be relatively non-selective for heavy metals and further studies on this particular zeolite were discontinued.

- A CVD system for the chemical derivatization of zeolites has been constructed. This apparatus is being used for encapsulation reactions whereby the metal-loaded zeolite will be chemically sealed. The reaction of phenylsilane at 110°C with Cd<sup>2+</sup> and Pb<sup>2+</sup> loaded ZeoX results in the blockage of 80% of the zeolite pores. The blockage of pores is being optimized as a function of silane exposure and temperature. At high temperatures, significant degradation of the "plugs" is apparent.
- Zeolites are being derivatized for enhanced selectivity towards heavy metal ions (via sulfurization reactions).
- Solid state NMR and FT-IR studies of the reaction between H<sup>+</sup>-ZeoX and phenylsilane were performed. The initial reaction of the zeolite with phenylsilane occurs at 60°C via reaction of an Si-H bond of the silane. Further thermal treatment (200°C) resulted in the loss of all Si-H bonds and finally the loss of benzene. Similar studies with metal-loaded zeolites are planned.

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

Reddy, PS., Katyayani, RV. and Fingerman, M. Cadmium and Naphthalene-Induced Hyperglycemia in the Fiddler Crab, *Uca pugnator*: Differential Modes of Action on the Neuroendocrine System. *Environmental Contamination and Toxicology*, 56: 425 - 431, 1996.

Fingerman, F., Devi, M., Reddy, PS. and Katyayani, RV. Impact of Heavy Metal Exposure on the Nervous System and Endocrine-Mediated Processes in Crustaceans. *Zoological Studies*, 35: 1 - 8, 1996.

Aphlett, AW., Ahmadi, A., and Walker, EH. Ion-Exchangers For Incorporation Of Actinides Into Mineral Phases was presented at the Spring Meeting of the American Chemical Society in New Orleans, Louisiana.

Sharma, HA., Barber, JT., Ensley, HE. and Polito, MA. Toxicity And Metabolism Of Chlorinated Phenols By *Lemna Gibba*, With Special Reference To 2,4,5-Trichlorophenol. *Environmental Toxicology and Chemistry*. (In review)

Aphlett, AW., Ahmadi, A., and Walker, EH. Incorporation Of Actinides Into Titanate-Phases Using Ion-Exchange Materials. *Advances in Ceramics*. (submitted March 1996)

Aphlett, AW., and Georgieva, GD. A Novel Synthetic Route For Synroc. *Advances in Ceramics* (submitted March 1996).

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

H. Bart, Jr., P. Martinat, L. Thien, E. Ellgaard,  
M. Devall, C. Thomas, S. Latimer

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

### **Objectives and Goals:**

The primary objective of this research project is to assess the ecological risk of contaminants from a hazardous waste site on the fish community of a floodplain swamp in the lower Mississippi River system. The ultimate goal of this research is to integrate data on community-level effects of aquatic contamination (population, trophic, and community indicators) with data on effects at the organism level and below (general health and condition indicators, histopathological indicators).

### **Accomplishments This Quarter:**

- No field work was conducted during this quarter.
- Additional analytical results have been received. Organic tissue results from the Environmental Health and Sciences laboratory were received for 112 fish of which 40 fish were collected from Devil's Swamp in 1994, 49 fish were collected from Devils Swamp in 1995, and 23 fish were collected from Tunica Swamp in 1995.
- Organic sediment and water results from the Environmental Health and Sciences laboratory were received for 31 collection site of which 16 sites were collected in 1994 (8 Devils Swamp sites and 8 Tunica Swamp sites) and 15 sites were collected in 1995 (5 Devils Swamp sites and 10 Tunica sites).
- Inorganic tissue results from the Environmental Health and Sciences laboratory were received for 195 of which 13 fish were collected from Devils Swamp in 1994, 58 fish were collected from Devils Swamp in 1995, and 124 fish were collected from Tunica Swamp in 1995.
- Inorganic tissue and skeletal results from the CIF laboratory were received for 40 fish of which 3 fish were collected from Devils Swamp in 1994, 1 fish was collected from Devils Swamp in 1995, and 36 fish were collected from Tunica Swamp in 1995.
- Inorganic sediment and water results from the Environmental Health and Sciences laboratory were received for 22 collection site of which 8 sites were collected in 1994 (5 Devils Swamp sites and 3 Tunica Swamp sites) and 15 sites were collected in 1995 (5 Devils Swamp sites and 10 Tunica sites).

## Sub-Project II: Tree Cores as Biomarkers

### **Objectives and Goals:**

The objective of this section is to utilize the tree-rings of baldcypress (*Taxodium distichum* L.) to record present and past pollution events by heavy metals and document growth trends in the Bayou Trepagnier and Devils Swamp ecosystems in Louisiana.

### **Accomplishments This Quarter:**

- Water levels in the Baldcypress swamp between Bayou Trepagnier and Bayou LaBranche have been extremely low this spring, allowing foot access to the area. At marker 30 on Bayou Trepagnier site tree cores were collected from Baldcypress trees along a transect extending a mile toward Bayou LaBranche. This will enable us to determine if heavy metals in the spoil banks along Bayou Trepagnier have polluted adjoining areas.
- The cores were dated and the trees varied in age from 110 to 145 years in age, which will allow us to determine pre-industrial levels of heavy metals in the area. The cores are now being prepared for heavy metal analysis.

## **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 This Quarter:**

- During this quarter, a continuous column run was conducted to evaluate carbon tetrachloride (CT) biodegradation at retention times of 1 day. The temperature was maintained at approximately 25.5°C. Initial redox potential was about -150 mv. Samples were taken until equilibrium conditions were established as determined by effluent CT concentration. Measurements at various column height were made of CT and chloroform (CF) concentrations, redox potential, biofilm thickness, TOC and

active cell mass concentration as RNA. Cytochrome P450 analysis has also been performed, no substantial Cytochrome P450 activity was observed. All experimental runs have now been completed and final data analysis is underway.

- Statistical analysis (Multiple regression with dummy variables using SAS) was also performed to confirm that the redox potential impact is of statistical importance during *Providencia stuartii* batch studies.
- The parameters analyzed are carbon tetrachloride (ppb), chloroform (CF) (ppb), redox potential, cell mass, RNA, biofilm thickness, TOC, flow rate, QA/QC.
- During this period, the following steps were effected to insure the QA/QC of the data:

In terms of CT and CF measurements: Two calibration curves were prepared for each experiment by using the standards of four concentration of each compound. One QC check standard and one spike sample per ten sample were run to determine accuracy. If EPA criteria were not met, a new calibration curve was prepared. Duplicates were run for each sample.

In terms of other parameter measurements: ORP, pH measurement were calibrated on daily basis. For RNA analysis, every 20 samples, one blank and one spiked samples were analyzed to check recovery and precision. Glassware was cleaned with sodium hypochlorite, rinsed with DI water and acetone.

- The results of studies indicate that the fixed biofilm reactor exhibited an ability to degrade greater than 99% of the CT introduced into the reactor at a retention time of 1 day when the average temperature was 25.5°C. The residue CT concentrations were about 1.4 ppb. Temperature were found very important in terms of the CT removal efficiency. For instance, at column height of 8 inches, residual CT concentration was 51.0 ppb when average temperature was 17.6°C; however, residual CT concentration was 23.7°C when average temperature was 25.5°C. Both biofilm thickness and RNA concentration in the biofilm were substantial higher then those of the run with a lower average temperature. Chloroform was generated as a metabolite at the beginning of the column and then degraded as flow proceeded upwards along the column length. Plate counts indicated that *Pseudomonas cepacia* is the predominant species in the fixed biofilm reactor, which agrees with a previous study indicating that chloroform can be degraded by this bacterial population. Biofilm thickness was greatest at the beginning of the column and decreased along the column length. This agrees with observed total organic carbon decreases with increasing column length.
- In analysis of Cytochrome P450, no substantial Cytochrome P450 activity was observed. Statistical analysis (Multiple regression with dummy variables using SAS) were performed for *Providencia stuartii*. The redox potential impact is of statistical importance if K rate constants are compared at ORP values below zero and above zero. There are no statistical differences in K rate constants for conditions where all ORP values are below zero.

## **Publications and Presentations:**

Bhattacharya, SK., Leslie, RE. and Madura, RL. Effects of Bioavailable Cadmium on Anaerobic Systems. (1995) Water Environment Research. (Accepted)

Bhattacharya, SK., Sluder, Jr., JL. and Uberoi, V. Effects of 4-Nitrophenol on H<sub>2</sub> and CO Levels in Anaerobic Propionate Systems. (1995) Water Environment Research. (Accepted)

Bhattacharya, SK., Todd, JC., Law, VJ. and Uberoi, V. An Integrated Modeling of Stoichiometry and Biokinetics of Anaerobic Processes. (1994) Environmental Software, 9: 33-45.

Jin, G. and Englande, A., Jr. Kinetic Study of Carbon Tetrachloride Biodegradation by a Mixed Culture in a Fixed-Biofilm Reactor. Water Environmental Federation 69th Annual Conference & Exposition. (Submitted)

Jin, G. and Englande, A., Jr. Biodegradation Kinetics of Carbon Tetrachloride by *Pseudomonas cepacia* Under Varying Redox Potential Conditions. Water Environment Research. (Submitted)

Jin, G. and Englande, A., 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)

## **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. This group seeks to extend these studies 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 This Quarter:

- The following items have been accomplished relating to the preparation of metal-specific antibodies:

Three (3) sets of hybridomas from 3 different mice have been prepared. During the first two screenings, the cells that were producing usable monoclonal antibodies did not survive. A meeting with personnel from the Louisiana State University (LSU) hybridoma facility was held to discuss problems with the isolation and screening procedures. It is anticipated that two modifications in the initial screening procedure (limiting dilutions earlier in the screening process and a more rapid initial screen) will insure a metal specific antibody in the third attempt.

Additional metal-DTPA-protein conjugates have been prepared for the preparation antibodies to copper, chromium, and zinc. No further immunizations will be initialized until LSU has been successful in preparing a monoclonal antibody to lead.

- Accomplishments relating to the characterization of the cadmium-specific monoclonal antibody are listed below:

New binding studies have shown that the metal ion specificity of the monoclonal antibody 2A81G5 can be modulated by changing the format of the immunoassay. Under the proper conditions, 2A81G5 shows a 10-fold greater affinity for mercury than for cadmium. Experiments are underway to construct an immunoassay for mercury in surface and ground water. If successful, two immunoassays will be developed, one for cadmium and one for mercury, from a single monoclonal antibody.

A pH study indicated that the anti-cadmium antibody rapidly loses binding activity at pH values less than 6.5 or greater than 8.0. Care must be taken in the field assay to control the pH of the sample to within these limits.

- The following steps have been taken toward construction of a field-portable immunoassay for cadmium:

The degree of interference that could be expected from other metals in a field-portable assay was determined by running competitive ELISA's for cadmium (1.2-5000 ppb) in the presence of fixed concentrations of other metals. The cadmium immunoassay was insensitive to interference by silver at concentrations less than 1  $\mu$ M, by manganese and indium at concentrations less than 10  $\mu$ M, by zinc and nickel at concentrations less than 100  $\mu$ M, and by lead at concentrations less than 1 mM.

Dr. Flowers obtained 12 different ground water samples from a battery reclamation plant near Ponchatoula, Louisiana that contained varying amounts of cadmium, copper, chromium, lead, nickel, and zinc. Samples were neutralized, made 110 mM in EDTA and analyzed directly in the cadmium immunoassay. Dr. Flowers also analyzed all 12 samples by AA and ICP as an independent determination of cadmium content. The immunoassay overestimated the actual cadmium content in all the samples. This was most likely a problem with interferences from the sample matrix and not from other metals in the sample, since the assay correctly identified samples that were heavily, moderately, or minimally contaminated with cadmium. Dr. Flowers is preparing a less complicated sample matrix from surface water samples from Bayou Trepagnier, Lake

Pontchartrain, and the Mississippi River. These samples will be spiked with cadmium and blind-tested in the immunoassay.

### **Publications and Presentations:**

Blake, DA., Khosraviani, M., and Blake II, RC. Characterization Of A Metal-Specific Monoclonal Antibody was defended at the 211th annual meeting of the American Chemical Society held March 24-28, 1996, in New Orleans, Louisiana.

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

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

### **Objectives and Goals:**

Understanding the detailed pore-level behavior of microorganisms through porous media is essential for the development of effective in situ bio-remediation strategies. Integrated experimental and computational models of the pore-level behavior of microorganisms are being developed. The models include the 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 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:**

- Professor Roseanne Ford, from the Department of Chemical Engineering at University of Virginia visited Tulane as a consultant to the cluster and gave an invited talk. She is an expert on experimental investigations of microbial motility and chemotaxis in porous media.
- Selim Sanin, a student of Dr. Bryers (director of the Center for Biofilm Engineering) visited Tulane and gave a DOE-sponsored presentation on the use of starved bacteria in bioremediation. Selim Sanin is a candidate for a postdoctoral research position in this cluster.
- Robert Dillon, a postdoctoral researcher on this project, has been awarded an NSF postdoctoral fellowship to continue these studies at Tulane. During the most recent quarter, he has completed most of the development of biofilm aggregation models of microorganisms progressing through small pores using the immersed boundary method.
- Stephanie Kute, previously an undergraduate studying basic models of aggregation, was awarded an NSF graduate fellowship to continue these studies at Tulane. Ms. Kute began her Master's degree work this quarter, and has begun to implement

boundary element code into her models of aggregation. This code will permit the analysis of a much larger number of bacteria than the studies by Dr. Dillon, but will not incorporate bacterial swimming behavior into these biofilm studies.

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

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

Dillon, R., Fauci, L., and Gaver, D. A Microscale Model of Bacterial Swimming, Chemotaxis and Substrate Transport. *Journal of Theoretical Biology*. 177:325-340, 1995.

Liu, Z. and Papadopoulos, K. "Chemotaxis In Near-Linear Gradients Of Chemoattractants" *Biotechnology and Bioengineering*. (In review)

Moore, P. and Dillon, R. "A Comparison Of Several Preconditioners In The Solution Of Parabolic Systems In Three Space Dimensions Using DASPK And A High-Order Finite Element Method" *Applied Numerical Mathematics*. (Accepted for publication)

Dillon, R. Modelling Biofilm Processes Using the Immersed Boundary Method. An invited presentation February 26, 1996 at Rice University in the Department of Computational Science, Houston, Texas.

### **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

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

The goals of the project are: 1) to determine which biomarker responses reflect the combined toxicological interactions in frogs and fish exposed to mixtures of environmental contaminants typical of Devils Swamp and Bayou Trepagnier; 2) to determine which biomarker responses integrate exposure over-time. Biomarker responses integrating exposure over-time may indicate subacute, subchronic and chronic adverse ecological effects of mixtures of contaminants; and 3) to identify and characterize the most sensitive sentinel species based on biomarkers.

The specific hypotheses are: 1) histopathological response is a valid biomarker for exposure over time and the toxicological effects of mixtures of contaminants; 2) heavy metal and organic pollutants cause abnormal development and physiology in frogs in controlled laboratory tests; 3) pollutants in water and sediment samples will correlate with accumulations in tissues; 4) pollution levels in tissues will correlate with expression of biomarkers related to altered function in embryonic development, immune responses, and neural function; and 5) esterase enzymes are effective biomarkers of exposure in fish and frogs.

## **Accomplishments This Quarter:**

### Histopathology of Fishes from Devils Swamp and Tunica Swamp

- Histopathological examination of fish species other than gar and buffalo collected from Devils Swamp and Tunica Swamp are currently being evaluated.
- Fish tissues from Bayou Trepagnier and Bayou Traverse are currently being processed for histological evaluation.
- Biomarkers in spotted gar from Devils Swamp and Bayou Trepagnier are similar.
- A manuscript on melanomacrophage centers in the liver of gar fish from Devils Swamp is being prepared.

### Neurotoxicological and Immunological Studies

- A novel cellular marker was identified in developing frog embryos exposed to methyl mercury. A discrete population of giant neurons were IL-1 $\beta$ -positive during early development.
- IL-1 $\beta$  in these giant neurons was depleted totally at very low levels (10 ppm methyl mercury) of exposure that usually do not cause any gross abnormal developments.
- Adult *Xenopus* frogs are currently being exposed to Bayou Trepagnier sediments in the laboratory. The preliminary results indicate some immunosuppression.

### Metabolism and Neurotoxicology

- Several Devils Swamp contaminants (e.g. HCBD, Cd<sup>+2</sup>, Hg<sup>+2</sup> and Pb<sup>+2</sup>) at varying concentrations (10<sup>-3</sup> to 10<sup>-7</sup> M) were tested on AChE, BuChE and CaE of spotted gar. These contaminants caused significant inhibition of AChE and CaE but were not inhibitory towards BuChE.
- Changes in esterase isozymes are potential biomarkers of exposure to contaminants. Analysis of liver cellular fractions of smallmouth buffalo and bigmouth buffalo from Devils Swamp were completed. Significant differences in esterase isozyme patterns were observed in fish from Tunica Swamp (control site) and Devils Swamp.

## **Publications and Presentations:**

Thiyagarajah, A., Hartley, WR., and Anderson, MB. Histopathology Of A Lung Cyst In A Spotted Gar (*Lepisosteus oculatus*) From A Contaminated Bayou In Louisiana. To be presented at the 15th International Symposium of the Society of Toxicologic Pathologists, St. Louis, Missouri, June 9-13, 1996.

Mizell, M., Romig, E., Hartley, WR., Thiyagarajah, A., Katayani, R., and Smolowitz, R. Comparison of Cardiovascular Defects Induced by Microinjection of the Late Gastrula of the Medaka and Zebrafish Embryo. To be presented at the second biennial meeting on Zebrafish Development and Genetics, Cold Spring Harbor, New York, April 24-28, 1996.

## **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. 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.

### **Accomplishments This Quarter:**

#### Subproject I: Metal-Sediment Interactions at the Fresh-Salt Water Interface

- During the past quarter efforts have been concentrated on studying complexation reactions involving cadmium ( $\text{Cd}^{+2}$ ), lead ( $\text{Pb}^{+2}$ ), and humic acid as a function of pH and ionic strength.
- Ultrafiltration membranes with a molecular weight cutoff of 5,000 Daltons were used to separate free ions from complexed ions. Preliminary results show that metal ion complexation increases with increasing pH until a certain pH value is reached. Further increase in pH have little effect on metal ion complexation.
- Increases in ionic strength tend to decrease metal ion complexation by humic acids.

#### Subproject II: Enzymatic Decontamination of Organic Wastestreams

The research is focused on understanding the removal of phenolic from waste streams through enzyme-catalyzed oxidative coupling. Both pure and mixed streams are being examined. Significant accomplishments this quarter are:

- The finding that trace amounts of anionics significantly modify the molecular weights of the polymer formed. Also, there is evidence for quinonic species in the polymer.
- Characterization of the polymer through IR and NMR and GC/MS.

- Characterization of enzyme viability during the process. Loss of enzyme through adsorption to the polymer.

#### Subproject III: Heavy Metal Chemistry of Bayou Tepagnier

- March, 1996 marked the completion of the first year of water quality monitoring after diversion of the Shell Norco effluent into the Mississippi River. During this period, only one period was observed in which the dissolved lead exceeded EPA water quality criteria. The anomalous lead contents occurred last summer during a prolonged period of low water in the bayou. No exceedances were observed for chromium, zinc, or copper.
- A sampling program to test the hypothesis the sediment erosion from spoil banks is the dominant transport mechanism for contaminants in the bayou has been undertaken. A series of transects were surveyed in crossing the spoil banks and extending up to 1000' into the adjacent marsh on both sides of the bayou. Samples were taken from the upper 2cm of the soil and will be analyzed for metals.

#### Subproject IV Metal-Ion Sequestering Polymers

- Four polyphosphazene polymers: poly[3-methylphenoxy](phenoxy)phosphazene], poly[4-methylphenoxy](phenoxy)phosphazene], poly[(3-ethylphenoxy)(phenoxy)phosphazene], and poly[4-ethylphenoxy](phenoxy)phosphazene] were sulfonated in solution with  $\text{SO}_3$  and cast into membranes from N,N-dimethylacetamide or methyl-2-pyrrolidinone solvents at a temperature of 80°C. The ethylphenoxy polymers underwent severe degradation during sulfonation and were unusable as membranes.
- Depending on the molar ratio of  $\text{SO}_3$  to the polymer, water insoluble membranes from the poly[(methylphenoxy)(phenoxy)polyphosphazenes] had an ion-exchange capacity ranging from near 0 to 2.3 mmol/g, an AC impedance in 0.1 N NaCl between 48 kohm-m and 0.04 ohm-m, and swelling in water ( $\text{SO}_3\text{H}$ -form) from 0.1 to 0.9 g/g. Poly[(3-methylphenoxy)(phenoxy)phosphazene] was found to be the best starting material, in terms of the ease in controlling the degree of sulfonation and the highest polymer ion-exchange capacity for a water insoluble membrane.

#### Subproject V: Role of Dissolved Organic Carbon in Metal Transport

- Ultrafiltration equipment was ordered that will enable us to separate organic carbon in the water column into dissolved, particulate, and colloidal phases. After fractionation, the metal load of each phase will be determined using AAS.
- Water samples were taken from 29 stations in the bayou, filtered through a 0.2 $\mu$  filter, and analyzed using high temperature catalytic oxidation. DOC concentrations ranged from 9 to 26 mg/l with the highest values occurring in the headwaters where wetland drainage is high. High concentrations of DOC are likely to provide an important mechanism for binding of metals.

## **Publications and Presentations:**

McCormick, M., Xu, X., John, VT., McPherson, GL. Coupling Chemical and Enzymatic Oxidations with Immobilized Catalysts. ACS Conference on Emerging Technologies in Hazardous Waste Management, Atlanta, September 1995.

## **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, C. Koutsougeras, S. Ramer, D. Sailor,  
L. Steinberg, E. Eschenazi

## **Objectives and Goals:**

To formulate the problem of aquatic transport in the Iput River, and educate the two Byelorussian students in the numerical methods of transport of radionuclides and apply the knowledge gained from this project to the transport of sediment and pollutants in aquatic environments in Louisiana.

## **Accomplishments This Quarter:**

- Work on the calibration process for the HEC-6 code on the transport of sediment continues and on the work for the transport of simple cases with it have commenced.
- Dr. Olga Zhukova, IREP, Belarus visited Tulane in March. She gave a presentation on the recent monitoring of the Iput River and brought some data which are needed for the efficient running of the HEC-6 code.
- The systematic categorization of the data needed for the Iput River project has been completed.
- This project has begun to work collaboratively with the Steinberg/Luna initiation project on the aquatic transport in the Bayou Trepagnier.

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

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

## **Objectives and Goals:**

The primary objective involves the design and application of 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. More specifically, Dr. Regens' group will construct a chemical and histopathological database, develop coverages, and perform spatial analyses for Devils Swamp. Dr. Bakeer's group will perform parallel tasks to support the primary objective for Bayou Trepagnier.

## **Accomplishments This Quarter:**

- The input of fish tissue chemical analysis continued for newly produced results. The database on Devils Swamp continues to be updated, maintained and backed up.
- A 3-dimensional view of Devils Swamp was created using the USGS elevation data combined with soil permeabilities. Sediment concentrations of arsenic, cadmium, chromium, lead, mercury, hexachlorobenzene, and hexachlorobutadiene at nine sampling sites were displayed to investigate any trends occurring due to soil or elevation differences.
- River flood stage measurements and elevation were integrated to illustrate the seasonal variation in inundation at Devils Swamp. Other layers of data can be added (e.g., fish catch size, concentrations of contaminants in water or sediments) to identify the effect of seasonal flooding on these parameters.
- The Bakeer group's work has included enhancement of the design of the conceptual databases necessary to model various thematic data describing geographical, topographical, and environmental features of Bayou Trepagnier. Improvements were made to the electronic maps and database menus used in the GIS for Bayou Trepagnier. The new menus provide easier access and display of the data contained in the database. Data in the database were collected from different agencies such as LDEQ, USGS, USACOE, private companies, etc.
- 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.
- A graduate student has completed his Master thesis entitled "A Remediation Technologies Selection Program." The program, called RTSP, is an expert system

type software developed to evaluate and recommend a remediation technique for a given contaminated site, specifically in aquatic environments. The software knowledge base contains a large number of remediation techniques suitable currently used to treat soils, water and air contaminations. Ranking of the different technologies is provided based on cost, effectiveness, and applicability to site specific conditions.

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

Andrade, FW. "A Remediation Technologies Selection Program," Master Thesis., Tulane University, 1996.

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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 This Quarter:

##### Student Development

- The LIFE Scholars have been assigned Xavier University faculty mentors to supervise them in research projects conducted during the academic year.
- The Environmental Scholars and Interns first monthly meeting for the Spring Semester was held February 27, 1996. The meeting began with the introduction of the new Associate Director of the Center for Environmental Programs, Dr. Bernadette Chachere of understanding environmental problems for minority communities.

Students were encouraged to come to her office and share any problems/concerns/experiences (positive or negative) related to their scholarships, internships, research activities and mentors. Students introduced themselves and summarized their research projects. This provided an opportunity for students to become familiar with each other's projects. Dr. Chachere proposed that future monthly meetings would include greater sharing and interaction among the students.

- Three Brown Bag lunches were held at Xavier this quarter. All have been very well attended with approximately 70 students in each and seem to be accomplishing the objective of stimulating thought about environmental issues.

The first, held February 14, 1996, included an introduction to the series and posed the question "Global Environment: Does It Really Matter?" Discussion of the topic ensued; each group made a presentation of their views. To further initiate critical thinking,

slides of the Freeport McMoran gold mining operations in Indonesia were shown, and the investigative report by the local newspaper on the operations was discussed.

The second, held February 28, 1996, featured a presentation by Dr. Peter Martinat, Assistant Professor of Biology on "The Environment from the Perspective of a Biologist." A lively question/discussion/debate followed Dr. Martinat's presentation.

The third, held March 8, 1996, featured Dr. Howard Mielke, Associate Professor of Pharmacy presentation on "Geochemical Distribution and the Association Between Lead, Zinc and Cadmium in New Orleans Soils." His talk described the activities and findings of his ongoing research project investigating the levels of heavy metals contamination in local sediment.

- The first ACT environmental film, entitled "Ground Zero," was shown on February 27, 1996. This film is about nuclear testing in the outback of Australia. This event was also well attended and students have requested that it be shown again for those who missed it!
- The Xavier University Library put into circulation its new acquisitions in Environmental Toxicology (a total of 69 entries) made possible by funding administered by CEP.
- Xavier scholars and interns submitted 9 abstracts for the Undergraduate Student Science Competition of the Fourth Annual HBCU/Private Sector/DoD Research and Development Technology Transfer Symposium held April 2-4, 1996, in Greensboro, North Carolina. Three of the abstracts have been accepted.
- Guidelines for the Environmental Studies Internship (EVST 456-457) course have been developed. The guidelines will provide information regarding salaries, grading, sponsorships.
- An additional forty-five environmental studies books have been purchased. When the final order has been received, most will be donated to Howard-Tilton Memorial Library (Tulane) to be processed and shelved there for general use. Some of the books, which Howard-Tilton already holds, will be kept in the Environmental Studies office and be available for check-out by students and faculty.
- Christine Murphey's book review and essay on the interdisciplinary nature of Environmental Studies was included in the February, 1996 issue of Environmental Form, a Tulane student publication.
- Enrollment of Environmental Studies Coordinate Majors at Tulane jumped 50% last Fall, from twenty to thirty students.

#### Faculty Development

- The Center for Environmental Programs sponsored the attendance of faculty members at the following scientific conferences:

Dr. Joseph Olubadewo, (Pharmacy), Society of Toxicology, 35th Annual Meeting, Anaheim, California.

Drs. Rosemary Effiong (Chemistry), Howard Mielke (Pharmacy), John Sevenair (Chemistry), Wei Wang (Chemistry), American Chemical Society (ACS) Meeting, New Orleans, Louisiana.

- Environmental Studies co-sponsored the second Brown Bag Lunch/Environmental Speaker's Meeting on February 2, 1996. Stephanie Smith, a Tulane University Research Scientist, spoke on the Potential Environmental Sources for Lead Exposure to Children, Using GIS.

#### Curriculum Development

- Drs. Sally O'Connor and Joan Bennett and program coordinator, C. Murphey, attended a Conference on Interdisciplinary Environmental Education, hosted by the University of Colorado. Discussed at the conference were the differences between science-oriented and policy-oriented environmental studies curricula, the comparative advantages of administering environmental studies degree programs through departments versus centers, the use of industrial/private sector expertise and the importance of student internship in an environmental studies program.
- The LAS Environmental Education Committee Meeting met on February 7, 1996. No additional meeting will be scheduled this semester since a subcommittee consisting of Drs. Joan Bennett and Charles Reith, Christine Murphey and two student assistants (Ridgely Pittmann and Greg Rose) will meet weekly to plan the Intensive Summer Faculty Seminar.
- Michael Zimmerman visited the Environmental Studies Programs at the University of California at Berkeley in February, to establish ties and learn about potential applicability of these programs to Tulane/Xavier projects.
- Dr. Charles Reith was hired as a half-time Coordinator for the Faculty Seminar and the first-year student pilot program. He assumed his duties in mid-February. Dr. Bianchi (EEOB) will teach the Environmental Biology section of the pilot course, which is geared to the experimental team-teaching model. This component will be paired with Molly Rothenberg's (English) writing course, which will fulfill student writing requirements for Liberal Arts and Sciences.
- Christine Murphey, Program Coordinator is developing a Home Page for the to date Environmental Studies program that can be accessed through the Internet.
- Preparations for the Faculty Seminar have begun. Videos have been selected to be shown. Discussions have begun regarding the distance learning to be used, who the speakers will be, possibilities for field trips, room scheduling on campus, and preliminary agendas.

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## INITIATION PROJECTS

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### **Molecular Mechanisms of the Developmental Toxicity Induced by Retinoids and Retinoid-like Molecules**

C.A. Burdsal

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

Retinoids are potent teratogens in vertebrates. Retinoids, like steroids, are small lipophilic molecules that are passively transported through the cell membrane. Retinoid molecules bind to receptors and induce target gene expression. Accordingly, any molecule that can mimic retinoid action may activate target gene expression and be a dangerous environmental teratogen. Recently, the pesticide, methaprene, and its derivatives have been shown to influence transcription in vertebrates by directly activating one class of the retinoid receptors. The aims of this study are 1) to determine if molecules with retinoid activity are present in aquatic environments of the southern Mississippi River basin and; 2) to investigate the mechanism by which retinoids induce teratogenesis during mouse embryonic development.

#### **Accomplishments This Quarter:**

- *RARE-lacZ* indicator cells - These cells will be used to detect molecules that interact with the retinoid pathway in the waters and sediments of Bayou Trepagnier. These cells express a transgenic construct in which *lacZ* expression is coupled to a retinoid response element. Thus, when retinoids or retinoid-like molecules, which are potent teratogens, are encountered by the cells they will express *lacZ* protein which can be easily detected histologically. During this quarter, the conditions and regimen for the *in vitro* culture of the indicator *RARE-lacZ* cells were established in the newly furnished laboratory of Dr. Burdsal at the Cell and Molecular Biology Department at Tulane.
- Experimental samples - The methodology for organic extractions of samples from Bayou Trepagnier and the mechanism by which Dr. Burdsal will obtain extracts were coordinated with Dr. Gary MacPherson and the CIF at Tulane University.

### **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 solids and emulsified oils. Understanding flocculation kinetics in various regimes is crucial to design proper remediation.

### **Accomplishments This Quarter:**

#### Samples Preparation

- Samples of n-hexadecane/water emulsions with and without surfactants were prepared by Dr. R. Effiong and her student, Chris J. Watts. New samples with surfactants have been prepared.
- Two students, Ernest Harris and Jameel Uqdah have been trained to prepare a wide variety of emulsions. The suspensions will be contaminated with metals. The cluster is presently interacting with other investigators in the program to select those metal to contaminants which are particularly abundant in the area under study in this project (Bayou Trepanier and Devil Swamp).

#### Multi-Angle-Light Scattering Experiments

- Experimental studies of flocculation processes, using the computerized laser light scattering apparatus purchased from Brookhaven Instruments (model BI-200SM Goniometer System), continued.
- Further experiments are presently in progress to study more in detail particle size distributions and emulsion stability. The stability of emulsions with and without surfactants is presently being studied as function of the pH. Emulsions contaminated by the presence of metals will be soon analyzed with the light scattering apparatus. Their stability will be studied as function of the pH.

#### Atomic Absorption and HPLC Studies

- Dr. Effiong and her student started a study of the stability of the emulsions using the high performance liquid chromatography (HPLC) apparatus.

#### Monte-Carlo and Numerical Simulations

- Dr. Eschenazi, continued developing and testing a 3D numerical code to investigate the collisional probabilities in concentrated emulsions. The code incorporates Metropolis algorithm.

### **Publications and Presentations:**

Deshiikan, SR., Bush, D., Eschenazi, EV., Papadopoulos, K. Brij58, CTAB and SDS at the Slowly Dilating n-Dodecane-Water Interface. Colloids and Surfaces (Submitted)

# 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 focused on developing pyrene hydroxylases and dehalogenases for use as tools for bioremediation.

## Background

The cytochromes P450 are a class of heme-containing enzymes with diverse biological functions that range from intermediary metabolism and development to roles in detoxifying and eliminating environmental pollutants from biological systems. More than 100 P450 enzymes have been characterized at the molecular level, and these oxidize a wide variety of aliphatic and aromatic compounds. One feature that all P450 enzymes share is the presence of a thiolate coordinated b-type heme which is the reactive center of all such enzymes, and thus, the substrate specificity and reactivity of a particular enzyme results from the unique geometry and chemical character of the substrate binding site which is determined by the amino acid sequence of a particular protein. It therefore seems to be a reasonable assumption that by making appropriate changes to the amino acid sequence of a particular P450 enzyme, one should be able to change its substrate specificity and reactivity.

The experimental approach uses site directed mutagenesis to introduce specific alterations in the amino acid sequence of the cytochrome P450 102 of *Bacillus megaterium* in order to reshape the substrate binding channel and active site. P450 102 is especially useful for our purpose because it has the P450 monooxygenase and heme reductase activities in the same polypeptide chain whereas virtually all other known P450s require a separate P450 reductase protein. The required P450 reductase activity is thus, automatically co-introduced with the oxidase. This last feature should facilitate the expression of P450 102 isozymes in *C. crescentus*.

The investigation of how the natural substrate (long chain fatty acids) binds to wild type and mutant P450 102 isozymes 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 that we should be able to create designed isozymes that have other novel catalytic activities.

## Accomplishments This Quarter:

- Progress continues in the dissection of substrate binding domain of cytochrome P450 102 using site directed mutagenesis. Testing the role of additional amino acid residues in substrate recognition is in process. Using the crystal structure of the P450 102 heme

domain as a starting point, computer-aided molecular modeling is being used to obtain a quantitative understanding of how the mutant isozymes recognize their new substrates. The results of recent genetic analysis of the substrate binding channel will be presented at the National American Society for Microbiology Meeting in May of 1996. In collaboration with Dr. William Alworth's lab, a manuscript describing the new P450 102 polycyclic aromatic hydroxylase was submitted to Archives of Biochem. Biophys.

- A polymerase chain reaction protocol is being developed to facilitate the cloning of DNA fragments that encode mutant P450 102 isozymes of interest so that they can be cloned and expressed in *C. crescentus*. This work will determine if the pyrene hydroxylase and tetrachloroethylene dechlorinase activities function *in vivo*.

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

Xia, Q., Kang, L., Alworth, WL., Liu, HM., Zhao, W., and Mullin, DA. A site specific mutant of the bacterial cytochrome P450 102 9BM-) possessing a new capability to catalyze the hydroxylation of polycyclic aromatic hydrocarbons Pyrene and Benzo[a]pyrene. Archives of Biochem. Biophys. (Submitted)

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

C. Li, H. Ensley

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

To study new methodologies for synthesizing membrane for use in removing aromatic and halogenated organic pollutants from waste water.

#### **Accomplishments This Quarter:**

- During this quarter study has concentrated on a novel carbocycle ring enlargement reaction as a spin-off of the previous research. A variety of carbocycle are enlarged by two-carbon atoms.
- The study has tremendous application in organic synthesis. The protocol of using water as a solvent for organic transformation renders the possibility of developing environmentally friendly processes for chemical synthesis.
- A highlight of this recent research will be documented on the April 8, 1996 C & EN News Broadcast.

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

The study has been accepted for publication in J. Am. Chem.Soc.

# **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 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.

## **Accomplishments This Quarter:**

- The benchmark for determining the rate and degree of progress is the Analytical Products Group, Inc. (APG) laboratory proficiency environmental testing program. In the last proficiency test the results of two elements, copper and selenium were outside the acceptable parameters. A thorough review was undertaken and the manufacturer of the ICP was consulted. The analytical failure appeared to be stemming from a moisture and temperature problem that is causing corrosive deterioration of the argon tube fittings of the ICP. The following corrective actions have been taken for the following problems:
  - The temperature of the back wall of the clean room fluctuates with changes in weather. The clean room was built using the existing block wall as the back wall. The enclosure traps moisture. The wall has been insulated. A dehumidifier was acquired to control humidity in the clean room.
  - Outside air flows through the vent when the equipment is turned off. Humid and polluted air enters the vents and flows over the analytical torch of the ICP. The air flow appears to have changed in the laboratory during the past few months. The laboratory is under negative pressure and air enters through the vents in a way that had not observed before. Vent closing devices that can be shut off were purchased to remedy this problem.
  - A minimum maximum thermometer and hygrometer has been obtained. The temperature in the laboratory undergoes extreme fluctuation. (55-78 F). The analytical equipment is subject to extreme humidity as the temperature drops. Temperature changes also influence the spectrometers. The physical plant was contacted with documentation of the fluctuation and they adjusted the computer program to even out the temperature in the building.
  - Correcting these problems has created a stable environment for the operation of the analytical equipment and the deterioration of the ICP and other analytical equipment in the laboratory has been arrested.
- A data gap for the DOE project is a lack of information on microbiota of Bayou Trepagnier. The laboratory is working with researchers in the biology department on a study of Bayou Trepagnier as a Model System for the Study of Environmental Pollutants and Microbiota. The purpose is to collect sediments and soils of unpolluted

and polluted segments of Bayou Trepagnier, describe the microbiota that were associated with each environment, and publish the results. The work plan is under development.

## **Aryl Hydrocarbon Receptor (AHR) Activation As a General Biomarker of Contamination in Aquatic Systems**

D. Miller

### **Objectives and Goals:**

The objectives of this project are the specific aims at the 1) expression of human AHR and ARNT in yeast and verification of responsiveness to activating-ligands such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), hexachlorobenzene (HCB) and naphthoflavone (NF); 2) To determine the sensitivity and specificity of the yeast system to AHR ligands; and 3) assess the ability of the system to detect AHR transcription activation due to contaminants in samples of fish muscle.

### **Accomplishments This Quarter:**

- Four plasmids needed to perform the work were successfully created. The final 4 plasmids needed to express AHR and ARNT under the control of a constitutively active promoter are in process.
- In the mean time the discovery that exogenous tryptophan in the culture medium acts as a ligand, leading to activation of the AHR have been made. By conducting the assay in tryptophan prototrophic yeast, the background in the system is greatly reduced and the system is now responsive to exogenous ligands. This is an important accomplishment and is probably the greatest hurdle that needed to be cleared for the successful implementation of the project.
- Beta-naphthoflavone and TCDD (dioxin) activate the AHR and stimulate transcription of the lacZ reporter plasmid in the system have been demonstrated. TCDD does not appear to be a "high affinity" against in the system, since it fully activates the receptor at approximately 100 nM. This is about 20x greater than the concentration needed to stimulate activity in mammalian systems. Beta-naphthoflavone is also maximally effective at a concentration of about 100 nM in the system. Thus the engineered yeast is comparable to mammalian cells in sensitivity to this polycyclic aromatic hydrocarbon. Conditions to improve the sensitivity of the system to TCDD and are testing a number of additional ligands for activity are currently being investigated.

### **Project milestones**

- Expression: The first step in the establishment of the AHR activation biomarker requires the expression of AHR and ARNT in yeast. A single plasmid with AHR and

ARNT divergently expressed from the galactose-inducible GAL1,10 promoter (C. Miller, unpublished) have been developed. In the system the levels of AHR and ARNT expression are constitutively high, but the proteins are transcriptionally active in the absence of ligand. Several possible explanations for the ligand-independent effect have been ruled out and currently two hypotheses are being investigated: 1) The ligand independent activity of AHR is due to carbon source, i.e., growth on galactose vs. glucose. 2) The ligand independent activity of AHR is due to a failure of the yeast 90-kDa heat shock proteins to retain human AHR in the cytoplasm of the cell prior to ligand binding.

- Thus, to address the problem of ligand independence human AHR and ARNT will be subcloned into a new plasmid. To generate these novel vectors both pcr methodology and standard molecular methods of plasmid manipulation will be used.

#### Determination of Sensitivity and Specificity

- Since samples from the environment often contain low levels of bioactive compounds, the optimal conditions for the detection of AHR activators will be determined. Initially the system will be assessed for the ability to detect specific activators of AHR. TCDD, HCB, and NF will be used as positive compounds and inactive dioxin congeners (i.e., monochlorinated forms) and dexamethazone negative controls.

#### Analysis of Biological Samples

- Exposure of yeast to extracts from environmental samples will be conducted according to the optimized experimental protocols developed in the previous section. Drs. W. Hartley and A. Abdelghani, will provide samples from the contaminated Devils Swamp site and the control Tunica Swamp site. These CBR investigators have made extracted samples from water, sediment and fish tissues available for analysis using the AHR activation assay. These samples have been characterized for hexachlorobenzene (HCB) content. HCB is an activator of the AHR and thus the samples that will be tested are partially characterized by analytical chemistry for a bona fide AHR ligand.

## **Encapsulation and Degradation of Pollutants: Role of Zeolites and Light**

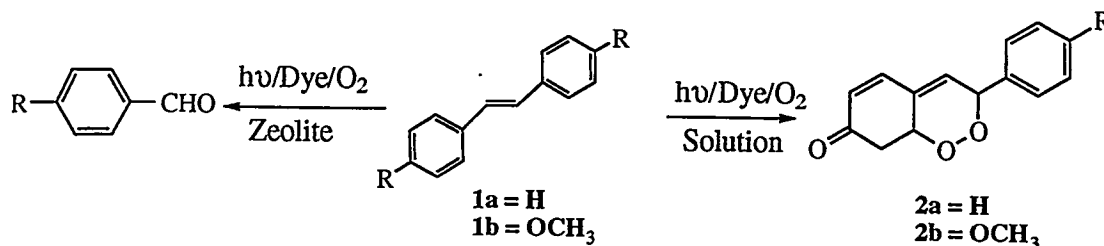
V. Ramamurthy

### **Objectives and Goals:**

During this period the above project has been initiated by Dr. X. Li, a postdoctoral associate in my group. Significant results on the decomposition of diphenyl polyenes has been achieved. The power of the approach in using zeolite-dye composite to decompose organic molecule is summarized in the following paragraph.

### Accomplishments This Quarter:

- A procedure to include organic dye molecules within zeolites has been developed.
- Dyes included within zeolites has been shown to oxidize diphenyl polyenes present in organic solvents (Scheme 1).
- Oxidation is established to proceed by an electron transfer pathway from the substrate to the excited dye molecules.
- Oxidation is more efficient within zeolite than in organic solvents.



**Scheme 1**

### Publications and Presentations:

Ramamurthy, V. Photochemistry of Organic Molecules within Zeolites was presented at the National ACS meeting (March 24-28) at New Orleans and University of Georgia, Athens and Clemson University, Clemson.

## Modeling and Assessment of Environmental Quality of Louisiana Bayous and Swamps

L. Steinberg and R. Luna

### Objectives and Goals:

The goals of this initiation grant are to use available spatial and temporal environmental data on Bayou Trepagnier and Devils Swamp to build a model describing the transport and chemical/ physical/biological processes which affect contaminants in these water bodies. In the process of doing this, the most pressing environmental problems in these water bodies will be identified, and recommendations on additional sampling which would improve model predictions will be provided.

### Accomplishments this Quarter:

- A review of the environmental data which have been collected by DOE investigators and others over the last several years in Bayou Trepagnier has been undertaken.

- All of the environmental data (in electronic form) that is available from the Data Management Core Group has been obtained.
- WASP5 model, EPA software model, for surface water modeling has been installed.
- An undergraduate student has been hired to work on the project recruitment of a graduate student is underway.

## **Effects of Steroid receptor Activation in Neuroendocrine Cells of the Mammalian Hypothalamus: Mechanisms of Action of Hormonally Active Xenobiotics**

J. Tasker

### **Objectives and Goals:**

This study is designed to address the mechanisms of action of hormonally active chemicals in neuroendocrine cells of the mammalian brain. Some chemical contaminants exert their toxic effects on exposed organism by binding with steroid receptors and activating steroid responsive elements within the exposed cells. Several areas of the brain concentrate steroid receptors, and neurally active steroids have been found to alter the gating properties of neuronal ion channels, changing their responsiveness to neurotransmitters. The general objective of this proposal is to determine the effects of ovarian steroid and hormonally active chemicals on the synaptic properties of neuroendocrine cells in the hypothalamus. By combining whole-cell patch-clamp techniques with intracellular dye injection and immunohistochemical labeling, the electrophysiological effects of neurally active steroids on anatomically identified hypothalamic neurons are studied in an *in-vitro* hypothalamic slice preparation.

### **Accomplishments This Quarter:**

- Preliminary experiments have been performed on oxytocin-and vasopressin-secreting cells of the supraoptic nucleus in the rat. The progesterone metabolite, allopregnanolone has been found to have profound effects on inhibitory postsynaptic currents mediated by the amino acid neurotransmitter  $\gamma$ -aminobutyric acid (GABA). Allopregnanolone decreases the size and increases the time constant of these currents, suggesting a modification of the open time of GABA receptor channels. This effect occurs with a relatively short latency and is reversible, suggesting that it is not due to a genomic action of the steroid. Direct intracellular application of the steroid by including it in the patch pipette has no effect, suggesting that the observed effect is mediated by an extracellular membrane receptor. The G-protein dependence of this action is currently being tested.

# 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

## Objectives and Goals:

The objective of this project is to conduct preliminary investigations into the use of *Saururus cernuus* L. (Lizard's tail), a herbaceous plant with extensive rhizomes, in bio-remediation. The study involves documenting the uptake of heavy metals by the plant and the location of the metals in various plant organs plus isolating genes (HSP 70; etc.) involved in heavy metal resistance.

## Accomplishments This Quarter:

- The focus of the initiative proposal on *Saururus cernuus* (Lizard's Tail) is to study the feasibility of using this plant as a biological sentinel in polluted ecosystems. The species grows in Bayou Trepagnier in a variety of localities suggesting high tolerance to heavy metals and also to hydrocarbons. It grows in the bayou itself, i. e., along the margins in shallow water. In addition it grows in the spoil banks which have very high concentrations of heavy metals. It is hypothesized that the clones of this plant (it spreads vegetatively via underground rhizomes and also reproduces by seed) were selected for resistance to the various pollutants over the past 75 years. To study the genetic basis of this tolerance to pollutants we have been trying to isolate heat shock genes via mRNA to make cDNA and sequence these genes for comparison with plants of Lizard's tail that grow in non-polluted areas. In addition, various clones have been grown in pots and exposing them to high metal solutions to determine degrees of tolerance.
- In this quarter three additional different (new) clones were found growing in the heavily polluted areas. For these plants:

These clones are being grown in water (no soil) with major nutrients provided. Once they reach the proper size, approximately 15-20 cm in height and before they flower, a series of growth experiments will be conducted in various concentrations of lead and zinc to determine maximum uptake of heavy metals. This will determine if the levels are high enough for the plant to be used in "harvesting of pollutants".

Work continues on trying to isolate heat shock genes via the polymerase chain reaction from plants exposed to high levels of heavy metals. If successful, the genes will be sequenced.

# **Identification of Metal Accumulators Among the Flora of Contaminated Wetlands and Exploration of their Uptake Mechanisms**

J. Whitbeck

## **Objectives and Goals:**

For early the goals of this project are to 1) survey and begin comparing the plant and mycorrhizal fungal communities of known contaminated and relatively pristine bayou sites, and; 2) identify plant hyperaccumulators of heavy metals.

## **Accomplishments this Quarter:**

- Laboratory space, tools and supplies were acquired, and undergraduate research assistants have been hired.
- Research plots in Bayou Trepagnier were selected for the field survey.

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## TECHNICAL SUPPORT ACTIVITIES

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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 This Quarter:

- Inorganic laboratory successfully completed APG PET for arsenic, cadmium, chromium, cobalt, copper, lead, nickel, and zinc.
- Inorganic laboratory used the ICP-AES and AAS to analyze fish, soil, sediments and water for metals and used the ED-XRF to analyze tree cores, soils and sediments for metals for DOE projects.
- Organic laboratory performed GC/MS analyses for semivolatiles on tissues from fish and birds.
- Organic laboratory performed Soxhlet extraction on more than 60 fish.
- Listed below are the instruments used by DOE researchers. The budget for analysis in the CIF was depleted during February. The CIF continues to provide support for the DOE researchers. Therefore, the funded and unfunded hours are listed. The CIF provided an additional \$29,160 in instrument time and an additional \$17,934 in operator/consulting hours above the funding level.
  - ICP - AES 34 hours of funded use and 36 hours of unfunded use.
  - GFAAS 203 hours of funded use and 302 hours of unfunded use.
  - XRF 8 hours of funded use and 18 hours of unfunded use.
  - XRD 12 hours of funded use and 18 hours of unfunded use.
  - GC/MS 334 hours of funded use and 60 hours of unfunded use.
  - CHNS/O 2 hours of funded use and 9 hours of unfunded use.
  - NMR 131 hours of funded use and 232 hours of unfunded use.
- Electronics Shop provided support to several DOE (and CBR) researchers for instrument repair including electronics support to Dr. Bhattacharya at Tulane for repair of an orbital shaker. The electronics shop also assisted Xavier University researchers - Dr. Effiong in repairing an HPLC and Dr. Mielke in repairing his AA spectrometer.

- CIF sent one scientist to the Louisiana Environment '96 Conference in New Orleans in Feb. 1996.
- CIF sent two personnel to the Pittsburgh Conference in Chicago in March 1996.
- CIF sent two personnel and a technical staff to the American Chemical Society National Meeting in New Orleans in March 1996.
- The CIF worked with the CBR and Dr. Mike Maskarinec of DOE-ORNL to make a final decision on GC/MS purchase. It was decided to order Hewlett-Packard GC/MS systems, one for the analysis of volatile organic compounds and one for the analysis of semi-volatile organic compounds.
- Hewlett-Packard GC/MS were delivered to the CIF in late March. System installation is scheduled for April 1996.

#### ACRONYMS

GC/MS - Gas Chromatograph/Mass Spectrometer  
 ICP-AES - Inductively Coupled Plasma - Atomic Emission Spectrometer  
 GFAAS - Graphite Furnace Atomic Absorption Spectrometer  
 AA - Atomic Absorption (Spectrometer)  
 NMR - Nuclear Magnetic Resonance (Spectrometer)  
 XRF - X-ray Fluorescence (Spectrometer)  
 ED-XRF - Energy Dispersive X-ray Fluorescence (Spectrometer)  
 XRD - X-ray Diffractometer  
 CHNOS - Carbon, Hydrogen, Nitrogen, Oxygen and Sulfur Analyzer  
 ACS - American Chemical Society

## **Data Management Core**

C.F. Ide, S. O'Connor, S. Smith, K. Watanabe,  
R. Fan, S. Dixon

### **Objectives and Goals:**

The DOE/EM project has established a Data Management Core (DMC) to modernize and facilitate efficient data communication, sample tracking, QA/QC, and data dissemination.

### **Accomplishments This Quarter:**

The following tasks and coordination has taken place to establish the ground work necessary for implementation of a Center for Bioenvironmental Research DOE/EM data management infrastructure:

- A Relational Laboratory Information Management System (RLIMS) has been acquired and initial system configuration has been developed to begin implementation. The data managed by this system will be stored in a central database maintained by Tulane University Computing Service (TCS).
- Coordination with the Database Administrator at Tulane Computing Services to Interface the R-LIMS sample tracking system to an Oracle central database accessible to all DOE/EM researchers. The database will be maintained on the RS-cluster allowing anyone with a university account the ability to access this data. The data management core will store large amounts of data derived from laboratory results in a consistent, secure way that preserves data integrity.
- Initial configuration of SQL\*Net has begun. SQL\*Net is being used to establish the data communication mechanisms/protocols/platforms between the RLIMS, laboratory instruments, and the Oracle central database.
- The CBR has acquired 4 GB of external SCSI-based hard drive space and will donate it to Tulane Computing Services for the exclusive use of DOE/EM researchers.
- A differentially corrected sub-meter accuracy GPS/GIS unit has been purchased and high accuracy surveying of the study areas has begun. Data generated from this unit can be directly imported into various GIS platforms for improved mapping of the area.
- Development of an efficient database design of environmental information that will be collected has begun. The data generated from the analytical components will be electronically transferred directly into a central database. Security of the data is assured because the TCS DBA can limit access to the data according to CBR specifications, as well as perform regular backups. This will require standardizing the naming conventions for tables and fields to be intuitive to data users; and developing metadata forms that will supply the needed descriptive information about the project's data.

- The analytical equipment that will interface with the central database includes:

Equipment Description	Laboratory Name
GC/MS (volatiles)	CIF Organic Lab
GG/MS(semi volatiles)	CIF Organic Lab
CHNS/O	CIF Organic Lab
ICP-AES	CIF Inorganic Lab
GFAAS	CIF Inorganic Lab
Hg Analyzer	CIF Inorganic Lab
XRF	CIF Inorganic Lab
High resolution MS	CIF Organic Lab
High resolution NMR	CIF Organic Lab
ICP-AES	Dr. Mielke's Lab at Xavier
GFAAS	Dr. Mielke's Lab at Xavier

## Appendix A

### Summary of Publications Resulting from the Hazardous Materials in Aquatic Environments of the Mississippi River Basin Research Project



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

- Abdelghani AA., Prammar 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.
- Abdelghani AA. Toxicity of Five Inorganic and Two Organic Chemicals to Microorganisms Isolated from Devil's Swamp Sediment, Baton Rouge Louisiana *Environmental Toxicology and Water Quality*. (Submitted)
- Ahmadi, A., Apblett, AW. and Walker, EH. (1995). Removal of Thorium from Aqueous Solution Utilizing Layered Titanates. *Environmental Science and Technology*.
- 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. Incorporation Of Actinides Into Titanate-Phases Using Ion-Exchange Materials. *Advances in Ceramics*. (submitted March 1996)
- Apblett, AW., and Georgieva, GD. A Novel Synthetic Route For Synroc. *Advances in Ceramics* (submitted March 1996).
- 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)
- 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.
- Bennett, JW. and Faison, BD. Use of Fungi in Biodegradation. *Manual of Environmental Microbiology: Biotransformation and Biodegradation*. (eds. L. Young, R. Crawford, M. Walter. ASM Press: Washington, DC (Accepted)
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- Berzins, D., Bundy, KJ., and Chan, P. (1994) Polarographic Trace Level Analysis Can Be Applied to the Detection of Environmental Contaminants, in *Trace Substances, Environment, and Health*, 63, Science Reviews, Northwood,
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- Bhattacharya, SK., Sluder, Jr., JL. and Uberoi, V. (1995) Effects of 4-Nitrophenol on H<sub>2</sub> and CO Levels in Anaerobic Propionate Systems. Water Research (Accepted)
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- Blake, DA., Dawson, GN., Chakrabarti, P., and Hatcher, FM (1996) Assay of Heavy Metals Using Antibodies to Metal-Chelate Complexes, invited chapter for American Chemical Society Symposium Series.
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